

Zooplankton Community Profiling in a Eutrophic Freshwater Ecosystem-
Lake Tai Basin by DNA Metabarcoding

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Table S1. Latitude/Longitude and group information for each sampling site.

Site	Name	longitude	latitude	Type	Sampling Time
S1	TH.ZSHN.3	120.0364	31.3731	Tai Lake	13.25
S2	TH.ZS.3	120.2675	31.0136	Tai Lake	10.24
S3	TH.XWL.3	120.2294	31.5014	Tai Lake	14.4
S4	TH.XTG.3	119.9947	31.035	Tai Lake	12
S5	TH.XSX.3	120.1495	31.14045	Tai Lake	15
S6	TH.XMK.3	120.1023	30.9697	Tai Lake	8.11
S7	TH.XHX.3	120.4	31.1717	Tai Lake	12.52
S8	TH.XHN.3	120.4213	31.11528	Tai Lake	14.4
S9	TH.WGS.3	120.229	31.3103	Tai Lake	10.15
S10	TH.TS.3	120.1622	31.3919	Tai Lake	11.06
S11	TH.SZ.3	120.2158	31.3992	Tai Lake	11.25
S12	TH.STG.3	120.0375	31.43394	Tai Lake	11.28
S13	TH.SHDB.3	120.1506	31.0628	Tai Lake	11.09
S14	TH.SDG.3	120.4017	31.4422	Tai Lake	13.26
S15	TH.QD.3	120.3811	30.9578	Tai Lake	11.35
S16	TH.PZ.3	120.453	31.1858	Tai Lake	15.3
S17	TH.PTS.3	120.1033	31.2258	Tai Lake	13.34
S18	TH.MS.3	120.2811	31.2639	Tai Lake	10.26
S19	TH.MG.3	120.461	31.0017	Tai Lake	12.39
S20	TH.LJK.3	120.1468	31.49854	Tai Lake	15.2
S21	TH.JSG.3	120.3608	31.3843	Tai Lake	12.3
S22	TH.HX.3	120.2071	31.22508	Tai Lake	14.09
S23	TH.DXTT.3	120.3433	31.08567	Tai Lake	14
S24	TH.DTH.3	120.5067	31.07146	Tai Lake	10.37
S25	TH.DPK.3	119.9381	31.3089	Tai Lake	11.2
S26	TH.DLS.3	120.0119	31.1364	Tai Lake	12.36
S27	TH.BDK.3	120.0447	31.47545	Tai Lake	14.44
S28	SK.LZ.3	119.3006	31.59891	Reservoir	13
S29	SK.LTSK.3	119.3823	32.06801	Reservoir	11.07
S30	RE.ZX.3	119.3601	31.9388	River	12.07
S31	RE.ZJQ.3	120.1626	31.87749	River	
S32	RE.ZGDQ.3	120.721	31.71582	River	9.5
S33	RE.YLQ.3	121.0469	31.50611	River	8.2
S34	RE.XFZ.3	119.5672	32.05335	River	11
S35	RE.WMQ.3	119.947	31.57431	River	13.52
S36	RE.TPZ.3	120.54	31.008	River	12.21
S37	RE.TBQ.3	119.9801	31.87529	River	
S38	RE.SP.3	121.0678	31.2713	River	11.55
S39	RE.ML.3	120.9266	31.60219	River	10.36
S40	RE.LLDQ.3	121.1917	31.46734	River	13.35
S41	RE.JX.3	119.4227	31.8625	River	11.12

S42	RE.JLZ.3	120.874	31.3144	River	10.43
S43	RE.JB.3	120.845	31.017	River	11.02
S44	RE.HSQ2.3	120.3817	31.30113	River	11.45
S45	RE.HNQ.3	119.6028	31.83278	River	14.4
S46	RE.HLJ.3	120.1456	31.67362	River	10.4
S47	RE.HHDQQ.3	119.9565	31.46926	River	10.42
S48	RE.HGZ.3	120.471	31.228	River	14.41
S49	RE.GJK.3	120.659	31.2	River	9.15
S50	RE.FQD.3	121.1121	31.58811	River	9
S51	RE.DZDQ.3	120.5528	31.57972	River	11.03
S52	RE.DPQ.3	119.924	31.97396	River	11.08
S53	RE.BQ.3	119.4615	31.55857	River	15.45
S54	RE.BGDQ.3	120.5455	31.78323	River	8.23
S55	HD.YD.3	120.8693	31.07007	Small lake	14.15
S56	HD.YCZHB.3	120.8057	31.47272	Small lake	13.51
S57	HD.YCXHN.3	120.7169	31.41228	Small lake	10.4
S58	HD.YCDHN.3	120.8308	31.40869	Small lake	9.32
S59	HD.XJDT.3	119.8823	31.53864	Small lake	11.08
S60	HD.KLH.3	120.8604	31.40769	Small lake	13.51
S61	HD.KCH.3	120.7436	31.58195	Small lake	15.2
S62	HD.GD1.3	119.756	31.49916	Small lake	10.15
S63	HD.EZD.3	120.5696	31.51263	Small lake	11.39
S64	HD.DHG.3	119.8734	31.63365	Small lake	13.56
S65	HD.CHN.3	120.8119	31.1973	Small lake	12
S66	HD.CHD.3	120.8544	31.22309	Small lake	11.17
S67	HD.BGHK.3	119.7743	31.57859	Small lake	13.12
S68	HD.GD2.3	119.5936	31.6084	Small lake	10.15
S69	RE.SHQ.3	120.2417	31.73556	River	

Table S2. The body length of each zooplankton species. The zooplankton specimen was stored in 100% alcohol. The body size was measured by microscope.

Category	Species	Mean	SD
Copepod	<i>copepod nauplius*</i>	218.29	83.42445804
Cladocera	<i>Bosmina</i> sp	341.3370833	65.65381948
Copepod	<i>Cyclops</i> larve	723.548	125.333
Copepod	<i>Calanoida</i> larve	806.532	145.658
Copepod	<i>Schmackeria forbesi</i>	997.5571795	167.6123453
Copepod	<i>Sinocalanus dorrii</i>	1275.261622	140.0464081
Rotifer	<i>Keratella cochlearis</i>	103.3071429	35.09603888
Rotifer	<i>Keratella valga</i>	121.3336842	20.90828151
Rotifer	<i>Polyarthra trigla</i>	142.5	#N/A
Rotifer	<i>Keratella quadrata</i>	137.081875	20.70169799
Rotifer	<i>Asplachna</i> sp	527.356	173.9459051
Rotifer	<i>Brachionus angularis</i>	130.5391304	33.13801085
Rotifer	<i>Keratella ticinensis</i>	105	#N/A
Rotifer	<i>Brachionus calyciflorus</i>	258.0832353	61.44659551
Rotifer	<i>Filinia longisela</i>	176.6085185	112.8875276
Rotifer	<i>Synchaeta oblonga</i>	195.6326316	36.78925384
Rotifer	<i>Polyarthra dolichoptera</i>	142.5	#N/A
Cladocera	<i>Ceriodaphnia cornuta</i>	333.899	74.83487996
Rotifer	<i>Conochilus unicornis</i>	102.04	NA
Cladocera	<i>Chydornus</i> sp	257.633	53.02383626
Cladocera	<i>Bosminopsis deitersi</i>	343.4125	125.0807986
Cladocera	<i>Chydornus sphaericus</i>	298.8061765	133.0538081
Copepod	<i>Schmackeria inopinus</i>	1171.148889	135.8830439
Rotifer	<i>Euchlanis dilalata</i>	293.08	49.78065411
Cladocera	<i>Alona guttata</i>	263.8511111	54.08922592
Rotifer	<i>Brachionus caudatus</i>	177.424878	62.29242001
Rotifer	<i>Brachionus urceus</i>	164.64	37.25949901
Rotifer	<i>Ponpholyx sulcata</i>	125	#N/A
Cladocera	<i>Pleuroxus trigonellus</i>	433.0514286	251.1988491
Cladocera	<i>Cyclops vicinus</i>	1706.4875	242.5272825
Copepod	<i>Mesocyclops</i> sp	1035.468636	280.8249261
Rotifer	<i>Brachionus quadridentatus</i>	164.1738462	34.07200447
Rotifer	<i>Notommata</i> sp	120	#N/A
Copepod	<i>Thermocyclops taihokuensis</i>	1042.5445	79.01903831
Cladocera	<i>Moina micrura</i>	587.5685333	162.8728833
Rotifer	<i>Rotaria neptunia</i>	453.7607692	122.2475159
Cladocera	<i>Sida crystallina</i>	881.549375	462.1178685
Cladocera	<i>Alona eximia</i>	334.585	38.84137549
Cladocera	<i>Camptocercus rectirostris</i>	629.74125	72.78557597
Rotifer	<i>Brachionus leydigii</i>	187.8675	29.97819529

Rotifer	<i>Gastropus stylifer</i>	164.195	64.42748762
Copepod	<i>Eucyclops serrulatus</i>	902.8	143.8485695
Rotifer	<i>Lecane bulla</i>	126.674	35.81979816
Copepod	<i>Eucyclops speratus</i>	1160	#N/A
Rotifer	<i>Testudinella patina</i>	176.012	20.40394006
Rotifer	<i>Polyarthra euryptera</i>	150	#N/A
Copepod	<i>Schmackeria</i> sp	1094.595625	208.784569
Rotifer	<i>Polyarthra</i> sp.	130	#N/A
Rotifer	<i>Ploesoma hudsoni</i>	328.5396875	125.2843559
Rotifer	<i>Lecane lunaris</i>	110	#N/A
Rotifer	<i>Lepadella patella</i>	130	#N/A
Rotifer	<i>Trichotria tetractis</i>	78.15	NA
Cladocera	<i>Simocephalus vetulus</i>	825.7209091	223.0956126
Rotifer	<i>Brachionus diversicornis</i>	238.5962	36.61595302
Cladocera	<i>Scapholeberis mucronata</i>	356.425	1.534421715
Rotifer	<i>Lecane buna</i>	123.0092308	18.0483464
Rotifer	<i>Conochilus dossuarius</i>	164.9624324	62.36578284
Rotifer	<i>Trichocerca longiseta</i>	275.66125	34.70184245
Rotifer	<i>Trichocerca stylata</i>	144.202	44.08772301
Copepod	<i>Thermocyclops brevifurcatus</i>	870.7681818	194.4547521
Cladocera	<i>Graptoleberis testudinaria</i>	402.3771429	68.5444709
Cladocera	<i>Daphnia galeata</i>	970.9763158	292.5898683
Cladocera	<i>Alona diaphana</i>	278.6485714	48.70432063
Copepod	<i>Harpacticoida</i> sp	634.7078947	118.1325138
Cladocera	<i>Diaphanosoma dubium</i>	927.536	151.7031014
Copepod	<i>Microcyclops varicans</i>	808.9966667	98.32272694
Cladocera	<i>Pleuroxus laevis</i>	391.555	13.80979544
Cladocera	<i>Ilyocryptus sordidus</i>	335.0875	231.5703712
Cladocera	<i>Diaphanosoma orghidani</i>	629.5741176	121.1526597

* copepod nauplius means the larvae of copepod which cannot be identified by morphological characters

Table S3. The GenBank accession numbers of local reference database.

Species	Genbank	Species	Genbank
<i>Alona diaphana</i>	KY091149	<i>Gastropus stylifer</i>	KY091190
<i>Alona eximia</i>	KY091150	<i>Graptoleberis testudinaria</i>	KY091191
<i>Alona guttata</i>	KY091151	<i>Harpacticoida</i> sp	KY091192
<i>Alona rectangular</i>	KY091152	<i>Ilyocryptus sordidus</i>	KY091193
<i>Alona</i> sp	KY091153	<i>Keratella cochlearis</i>	KY091194
<i>Asplanchna priodonta</i>	KY091154	<i>Keratella quadrata</i>	KY091195
<i>Asplanchna</i> sp	KY091155	<i>Keratella</i> sp	KY091196
<i>Asplanchna girodi</i>	KY091156	<i>Keratella valga</i>	KY091197
<i>Bosmina</i> sp	KY091157	<i>Lecane bulla</i>	KY091198
<i>Bosminopsis deitersi</i>	KY091158	<i>Lecane ungulata</i>	KY091199
<i>Brachionus angularis</i>	KY091159	<i>Limnoithona sinensis</i>	KY091200
<i>Brachionus calyciflorus</i>	KY091160	<i>Macrothrix</i> sp	KY091201
<i>Brachionus caudatus</i>	KY091161	<i>Mesocyclops</i> sp	KY091202
<i>Brachionus diversicornis</i>	KY091162	<i>Microcyclops varicans</i>	KY091203
<i>Brachionus falcatus</i>	KY091163	<i>Moina brachiata</i>	KY091204
<i>Brachionus forficula</i>	KY091164	<i>Moina micrura</i>	KY091205
<i>Brachionus quadridentatus</i>	KY091165	<i>Moina rectirostris</i>	KY091206
<i>Brachionus urceus</i>	KY091166	<i>Mytilina ventralis</i>	KY091207
<i>Brachionus leydigi</i>	KY091167	<i>Neodiaptomus schmackeri</i>	KY091208
<i>Camptocercus rectirostris</i>	KY091168	<i>Notommatidae</i> sp	KY091209
<i>Ceriodaphnia cornuta</i>	KY091169	<i>Pleuroxus aduncus</i>	KY091210
<i>Ceriodaphnia quadrangula</i>	KY091170	<i>Pleuroxus laevis</i>	KY091211
<i>Chydorus</i> sp	KY091171	<i>Pleuroxus trigonellus</i>	KY091212
<i>Chydorus sphaericus</i>	KY091172	<i>Ploesoma hudsoni</i>	KY091213
<i>Conochiloides dossuarius</i>	KY091173	<i>Rotifer</i> sp	KY091214
<i>Conochilus unicornis</i>	KY091174	<i>Scapholeberis mucronata</i>	KY091215
<i>Cyclop</i> sp	KY091175	<i>Schmackeria forbesi</i>	KY091216
<i>Cyclops vicinus</i>	KY091176	<i>Schmackeria inopinus</i>	KY091217
<i>Daphnia galeata</i>	KY091177	<i>Schmackeria</i> sp	KY091218
<i>Daphnia pulex</i>	KY091178	<i>Sida crystallina</i>	KY091219
<i>Daphnia</i> sp	KY091179	<i>Simocephalus vetuloides</i>	KY091220
<i>Diaphanosoma dubium</i>	KY091180	<i>Simocephalus vetulus</i>	KY091221
<i>Diaphanosoma orghidani</i>	KY091181	<i>Sinocalanus dorrii</i>	KY091222
<i>Diaphanosoma</i> sp	KY091182	<i>Synchaeta</i> sp	KY091223
<i>Diaptomidae</i> sp	KY091183	<i>Synchaeta oblonga</i>	KY091224
<i>Disparalona rostrata</i>	KY091184	<i>Testudinella patina</i>	KY091225
<i>Dunhevedia crassa</i>	KY091185	<i>Thermocyclops</i> sp	KY091226
<i>Eodiaptomus sinensis</i>	KY091186	<i>Thermocyclops taihokuensis</i>	KY091227
<i>Euchlanis dilalata</i>	KY091187	<i>Trichocerca elongata</i>	KY091228
<i>Eucyclops serrulatus</i>	KY091188	<i>Trichocerca</i> sp	KY091229
<i>Filinia longiseta</i>	KY091189	<i>Trichocerca stylata</i>	KY091230

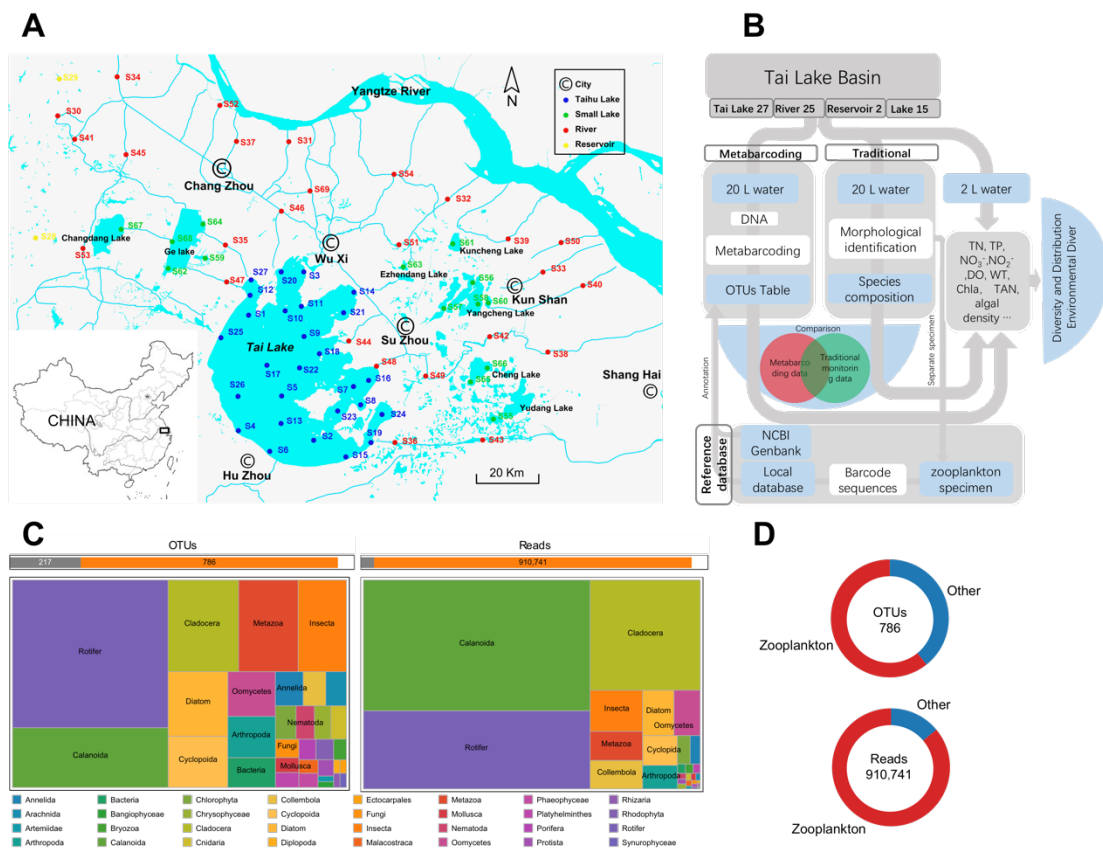


Fig.S1. Samples and methods used in the study and components of eukaryotic plankton biodiversity of the entire mtCO1 metabarcoding data set. (A) Location of sampling sites of the Tai Lake basin of Jiangsu province in China. Sampling sites were grouped into four categories according to the type of water body: 1) Tai Lake, 2) Reservoir, 3) River and 4) Lake. Here, “Lake” means all the relatively smaller lakes around Tai Lake. The map was made by ArcGIS version 10.0 (ESRI Company, USA). (B) Flowchart of methods used in the study. Ion torrent PGM-based sequencing was performed on each sample targeting the CO1 gene region. (C): Components of eukaryotic plankton community of the entire mtCO1 metabarcoding data set. Barcodes were “unassigned” when the maximum sequence similarity to a reference sequence was < 80% and posterior probability > 60% in SAP or match to the “environmental invertebrate sample” in the NCBI database. Diagrams display richness of OTUs (left) and relative abundances of reads (right) of various eukaryotic groups. (D): The pie plot displays the proportion of zooplankton sequences and OTUs in the assigned data.

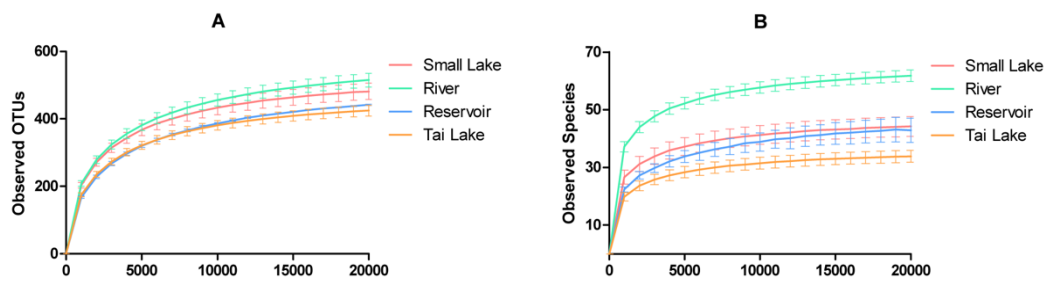


Fig.S2. The observed species/OTUs with the depth of sequencing. (A) number of observed OTUs; (B) number of observed species.

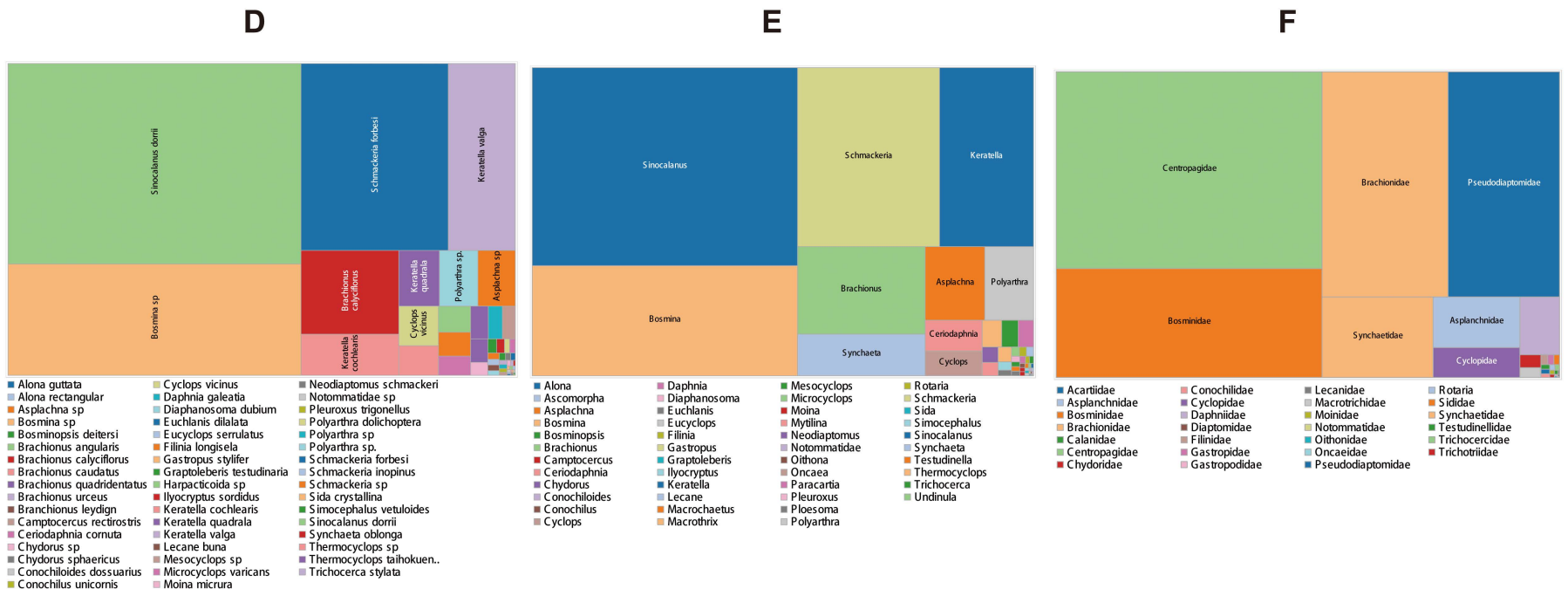
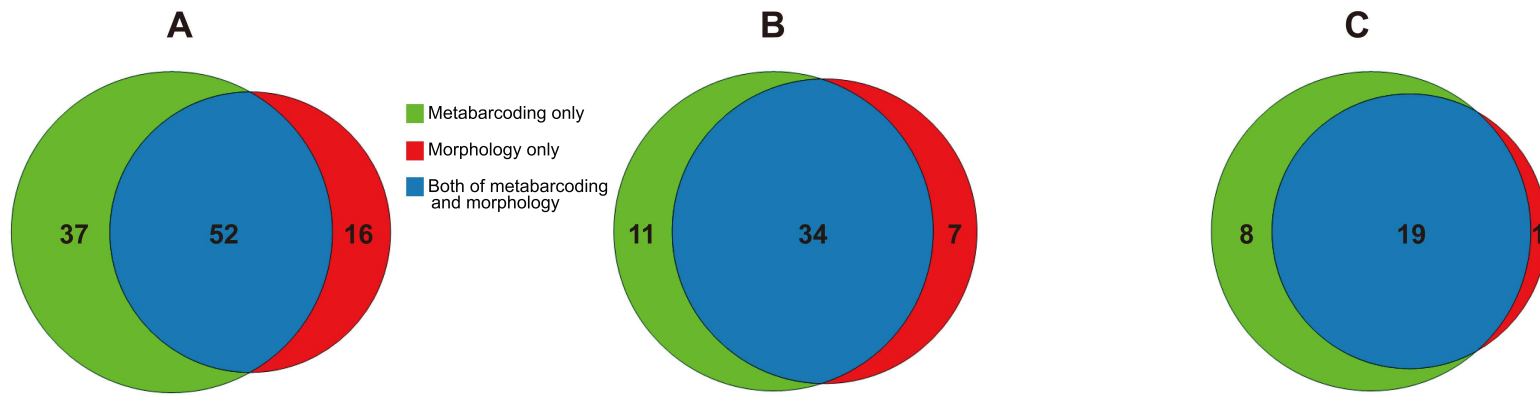


Fig.S3. The comparisons of metabarcoding data with traditional monitoring data in species, genus and family level, respectively. (A) species level; (B) genus level; (C) family level. Diagram D, E & F shows the sequence components of taxon that detected by both metabarcoding and traditional morphological method. (D) species level; (E) genus level; (F) family level.

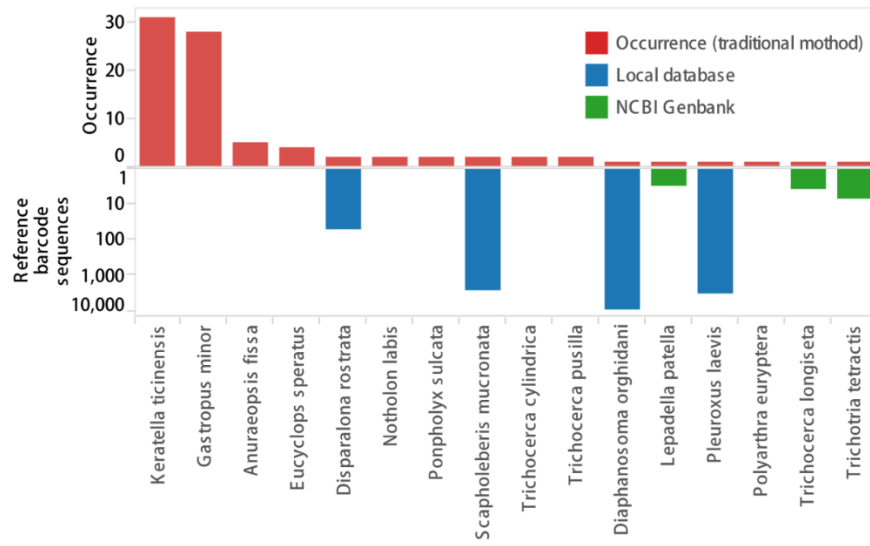


Fig.S4. Number of barcode sequences (CO1) in Genbank and local database of the missing species in metabarcoding data. The red bar represented the frequency of occurrence in traditional monitoring dataset. The green bar indicated the number of CO1 barcode sequence in Genbank.

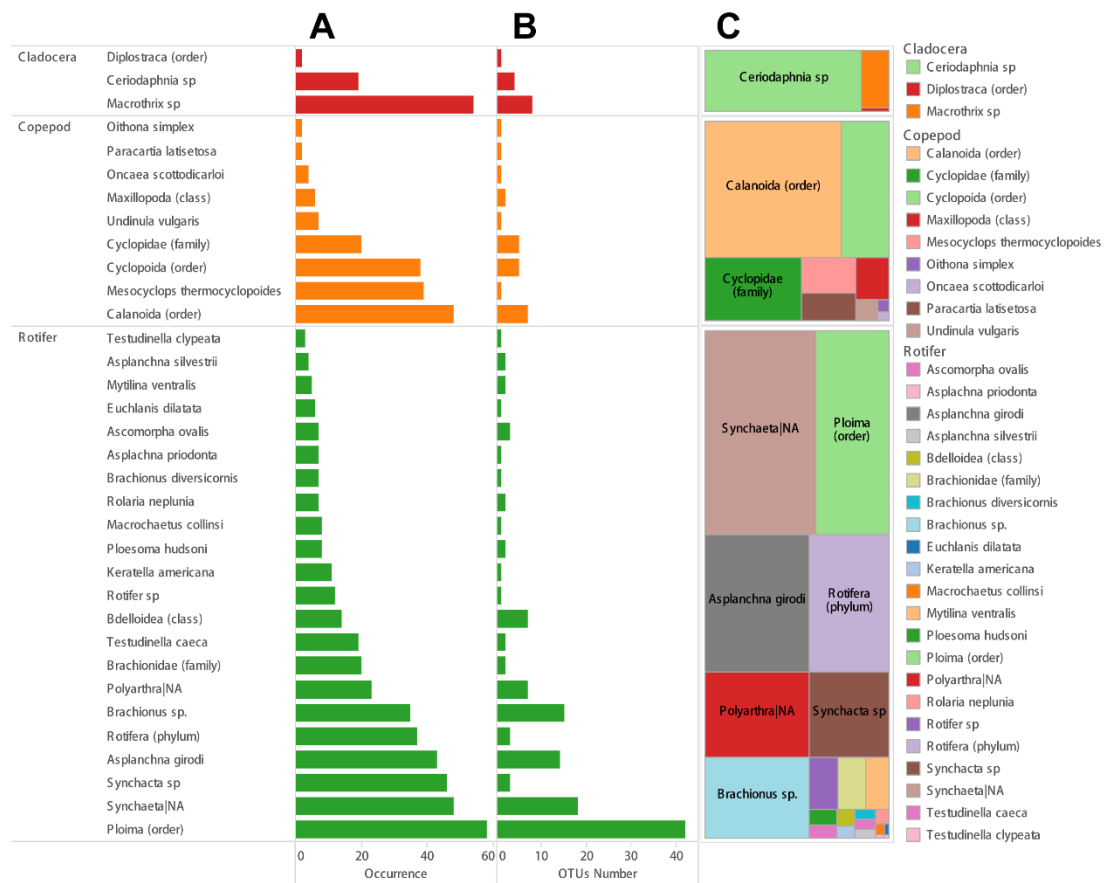


Fig.S5. The novel OTUs clusters/taxa detected by metabarcoding method. (A) the frequency of occurrence of each taxon. (B) the OTUs number of each taxon. (C) the reads composition of rotifer, cladocera and copepod.

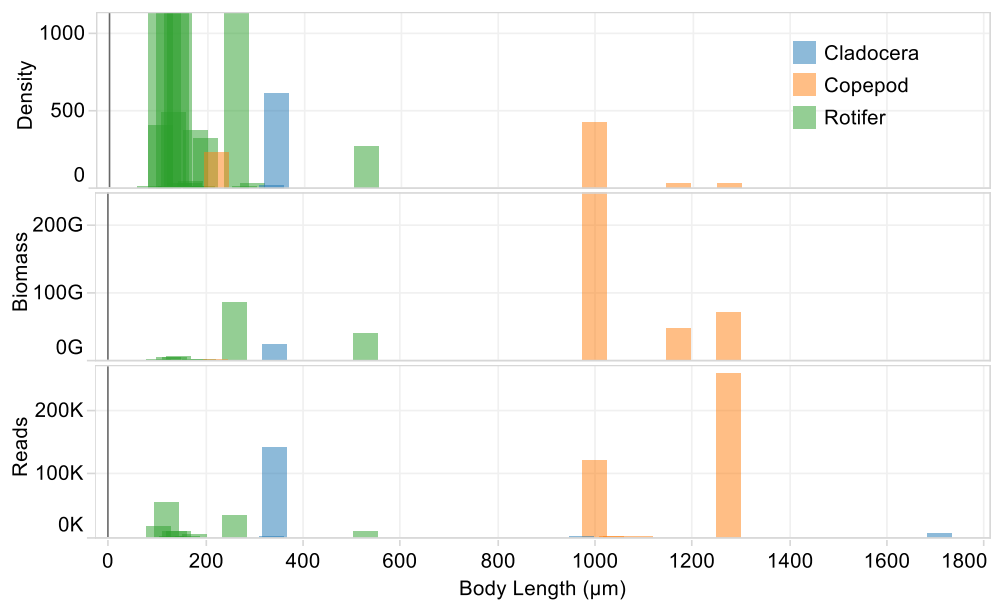


Fig.S6. The comparison results between zooplankton density, inferred biomass and the NGS reads number. The top histogram indicated the relationship between body length and density of zooplankton in traditional monitoring data. The middle histogram shows the relationship between body length and biomass of zooplankton in traditional monitoring data. The bottom histogram shows the relationship between body length and reads number in metabarcoding dataset. The biomass of zooplankton was roughly calculated by the density and body length (biomass = density \times bodylength³).

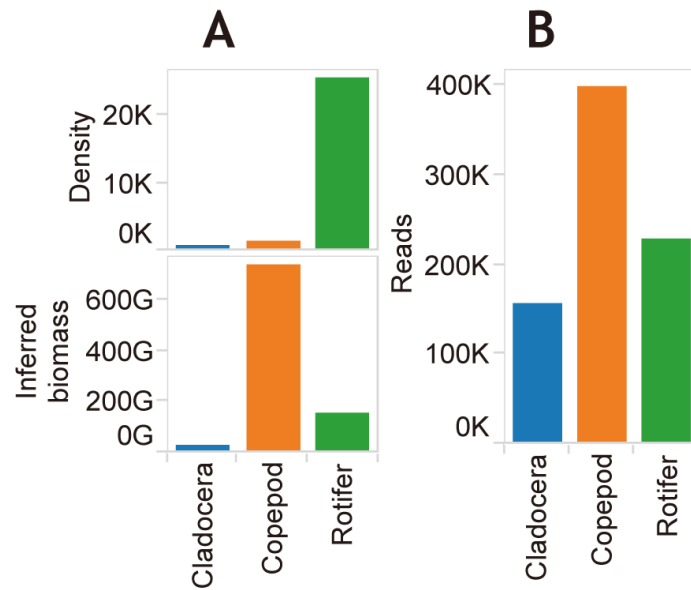


Fig.S7. The comparison results between zooplankton inferred biomass and the NGS reads number. (A) the top panel shows the density among three key group, rotifer, copepod and cladocera. The bottom panel shows the inferred biomass (calculate by bodysize and density) among three group. The inferred biomass of zooplankton was roughly estimated by the density and body length (biomass = density \times bodylength³). (B) The number of reads among rotifer, copepod and cladocera.