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1	Application of environmental DNA metabarcoding in a lake with extensive algal
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18 Abstract

19	Recently, environmental DNA (eDNA) metabarcoding techniques have been applied to
20	biodiversity investigations in aquatic ecosystems. However, no study has yet tested
21	whether this technique is effective for water bodies in which extensive algal blooms
22	break out. In this study, fish DNA metabarcoding was carried out in Lake Taihu, which
23	experiences extensive algal blooms, to confirm whether the technique is also effective
24	for fish diversity research in ecosystems with frequent and extensive blooms. In
25	December 2016, three samples were collected, including one collected in the presence
26	of algal blooms and two collected in the absence of algal blooms. In August 2017, six
27	samples were collected, including three collected in the presence of algal blooms and
28	three in the absence of algal blooms. Equal amount of water samples (1 L) were
29	collected from each site; however, the actual amount of filtrate varied with the site.
30	Twenty-seven freshwater fish species were detected from the water samples collected in
31	Lake Taihu. The results showed that the composition of the detected species did not
32	differ whether or not blooms were present. However, the amount of filtration could
33	influence the number of species detected. The results suggest that future eDNA

- 34 metabarcoding studies under similar water environments should increase the amount of
- 35 filtration to maximize number of species detected.
- 36 Keywords: algal blooms, environmental metabarcoding analysis, fish species
- 37 composition, Lake Taihu

38 Introduction

39	Global biodiversity is decreasing at an unprecedented rate, far beyond people's
40	imagination (Dirzo and Raven 2003). Human activity is one of the main causes of
41	biodiversity loss, which directly threatens natural ecosystems (Butchart et al. 2010).
42	Freshwater vertebrate species populations are declining at twice the rate of decline of
43	land or ocean vertebrates (Grooten and Almond 2018). Among the 29,500 freshwater
44	dependent species so far assessed in the IUCN Red List, 27% are currently at risk of
45	extinction (Tickner et al. 2020). Among aquatic organisms, fish species play an
46	important role in ecosystem linkages as well as commercial fisheries, yet many fish
47	species are in danger of extinction. In fact, in China, approximately 15% of freshwater
48	fish species are listed as endangered or vulnerable (199 out of the 1323 species
49	registered in freshwater systems) (Xing et al. 2016). Therefore, accurate assessment of
50	fish communities is key to maintaining freshwater fish diversity and freshwater
51	ecosystem protection, and it provides essential information for biological protection
52	(Kwak and Peterson 2007).

53	In recent years, environmental DNA (eDNA) analysis and its usage have received
54	widespread attention. Environmental DNA represents all types of DNA found in the
55	environment, including DNA from organisms in the form of metabolic waste, damaged
56	tissue, or sloughed skin cells (Kelly et al. 2014; Barnes and Turner 2016). A method
57	that retrieves DNA from environmental samples has been used to explore aquatic
58	organisms in conservation and ecological studies (Pompanon et al. 2012; Bohmann et
59	al. 2014; Thomsen and Willerslev 2015; Valentini et al. 20016). Two different
60	approaches can be applied. One approach is eDNA analysis, which can detect a single
61	species by using species-specific primers (Sean et al. 2019; Sakata et al. 2017; Wu et al.
62	2018, 2019; Fujiwara et al. 2016), while the other approach promotes diversity
63	assessments of organisms using universal primers, called eDNA metabarcoding (Miya
64	et al. 2015; Komai et al. 2019). The experimental procedure for eDNA metabarcoding
65	includes collecting water samples and PCR amplification of the eDNA from fish species
66	using universal primers (Miya et al. 2015; Deiner et al. 2016). The species composition
67	was estimated by sequencing the PCR amplicons through next-generation sequencing
68	(NGS). This technique is rapid and non-invasive method that is also cost-effective

70	metabarcoding reveals comparable or higher number of fish species, compared to that
71	from traditional surveys (Hänfling et al. 2016; Shaw et al. 2016; Nakagawa et al. 2018).
72	In recent years, eDNA metabarcoding is used increasingly for characterizing the species
73	composition of ecological communities (Evans et al. 2016; Sato et al. 2017). Therefore,
74	we believe it would be suitable for studying fish biodiversity and composition in lakes
75	with serious eutrophication.
76	Eutrophication in reservoirs, estuaries, rivers, and lakes is widespread all over the
77	world, and the severity is increasing, especially in developing countries such as China
78	(Yang et al. 2008). When the nitrogen and phosphorus concentrations in a water body
79	reach certain levels, algal blooms can easily occur (Carpenter 2008; Vadeboncoeur,
80	Lodge and Carpenter 2001). Algal blooms can clog the gills of aquatic organisms
81	(Smith and Schindler 2009), and aquatic organisms such as fish and bottom-dwelling
82	animals cannot survive in oxygen-deprived aquatic environments as those in algal
83	blooms (Ansari, Gill and Khan 2010). Researchers studying such aquatic environments
84	have largely performed biodiversity surveys using traditional methods, and the

69 (Deiner et al. 2017; Yamamoto et al. 2017). In freshwater fish species, eDNA

85	application of eDNA technology has emerged in recent years (Cai et al. 2017; Zhang et
86	al. 2018). In the wake of increased environmental degradation and biodiversity loss,
87	more biodiversity surveys will be required in eutrophic aquatic ecosystems with algal
88	blooms to assess aquatic ecosystem health and the impact of the blooms on the
89	ecosystems. Environmental DNA technology provides information on the presence or
90	absence of organisms based on the genetic information obtained from environmental
91	water samples. Most studies that apply metabarcoding techniques for eDNA analysis
92	have been conducted in oligotrophic or mesotrophic waters. To date, only Handley et al.
93	(2019) has applied the metabarcoding technique to investigate fish composition in
94	eutrophic water. It has been reported that species that were sensitive to the trophic
95	conditions of their habitat were distributed in areas with corresponding trophic
96	conditions, while those that were not were widely distributed regardless of the trophic
97	conditions (Handley et al. 2019). Eutrophic aquatic environments are vulnerable to
98	massive algal blooms, and algae clog filter papers, reducing filtration capacity and
99	organism detection rates in eDNA surveys. Nevertheless, very few studies have been
100	conducted to evaluate the potential application of eDNA technology in aquatic

101	environments with extensive algal blooms on the surfaces. The present study was
102	conducted in Lake Taihu, a representative highly eutrophic lake with extensive algal
103	blooms, and the results of the study could reveal the feasibility of the survey approach
104	in such environments.
105	This study aimed to determine whether the eDNA metabarcoding analysis
106	method could be applied to a highly eutrophicated lake by analyzing the effect of algal
107	blooms. As a test area, we selected Lake Taihu, China, a highly eutrophicated lake with
108	frequent extensive algal blooms, where fish are an important aquatic resource. We
109	compared the number of detected species and the differences in species composition
110	with the presence or absence of algal blooms. Finally, species detection using eDNA
111	metabarcoding was compared with the traditional methods and the use of the illustrated
112	book for the fish of Lake Taihu.
113	

- 114 Materials and Methods
- 115 Study site

116	The study area of Lake Taihu is located in the Yangtze River Delta in eastern China,
117	with a surface area of 2,338 km ² and an average depth of 1.89 m (Sun and Huang 1993).
118	In the 1980s, there were between 72 and 106 different species of fish in the lake (Zhu et
119	al. 2007). During the 2000s, massive blue-green algal blooms broke out in Lake Taihu
120	(Wang and Shi 2008; Zhu 2008). These led to massive mortalities of fish and shrimp
121	and triggered serious drinking water crises (Huang et al. 2014). In addition, due to the
122	multiple impacts of overfishing and degradation of aquatic vegetation, significant
123	changes took place in the fish community structure of the lake, so that now there are
124	only 40 to 50 fish species in Lake Taihu (Zhu 2004).
125	Water samples were collected during summer and winter in the north of Lake
126	Taihu. We surveyed three sites on December 21, 2016 (Fig. 1, blue circle), and six sites
127	on August 27–28, 2017 (Fig. 1, red circle). The different sampling sites were selected as
128	there were relatively few sites in winter with algal blooms present. Consequently,
129	sampling was carried out across four sites with algal blooms and five sites with no algal
130	blooms. We observed that algal blooms appeared in three sampling sites in summer and
131	one site in winter (Table 1). At site T7, we conducted a survey in both summer (referred

132	as T7-S) and winter (T7-W). Two bottles of 500 mL water were collected at each site,
133	and 500 μ L of 10% (mass/volume) benzalkonium chloride solution was added to each
134	sample to prevent DNA degradation (Yamanaka et al. 2017). We transported the water
135	samples at ambient temperature and filtered them in the laboratory at Shanghai Jiao
136	Tong University.
137	
138	eDNA filtration and extraction
139	The water samples were filtered using 47-mm glass-fiber filters (GF/F; GE Healthcare
140	Japan, Tokyo, Japan; nominal pore size = $0.7 \ \mu m$; cf. Minamoto et al. (2016) for
141	selection of filter type for eDNA sampling). In the present study, we unified the
142	filtration labor, and filtration would continue until it could not be further carried out.
143	The amount of sample filtration varied for each water sample because of filter clogging
144	(Table 1). After filtering the samples, we filtered 1 L of ultrapure water to check for
145	unintended cross-contamination in the laboratory (i.e., filtration blank). To prevent
146	contamination, the equipment was cleaned with a bleach solution (diluted household
147	bleach containing 0.1% sodium hypochlorite) for >5 min before reuse to remove

148	residual DNA. After filtration, the filters were stored at -20 °C until DNA extraction.
149	Total eDNA was extracted from the filter using a DNeasy Blood and Tissue Kit
150	(Qiagen, Hilden, Germany) following Minamoto et al. (2019). After DNA extraction,
151	DNA was eluted from the DNeasy spin column with 100 μ L of Buffer AE (Qiagen) and
152	stored at -20 °C until metabarcoding analysis.
153	
154	Amplicon library preparation and MiSeq sequencing
155	Amplicon libraries for fish eDNA metabarcoding were prepared using MiFish-U
156	primers (Miya et al. 2015). The first-round polymerase chain reaction (1 st PCR) was
157	performed in a total reaction volume of 12 μL , containing 6.0 μL 2 \times KAPA HiFi
158	HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA), 3.6 pmol each of
159	MiFish-U primers, 1 μ L eDNA template, and ultrapure water. The thermal cycle profile
160	was 95 °C for 3 min; 40 cycles of 98 °C for 20 s, 65 °C for 15 s, and 72 °C for 15 s; and
161	72 °C for 5 min. A polymerase chain reaction was performed with four repetitions for
162	each DNA sample, to reduce potential bias arising through a stochastic variation during
163	the PCR step. Ultrapure water was used instead of eDNA in four reaction mixtures

164	(non-template negative controls). After four technical replicates of 1st PCR products
165	were pooled into a single tube, unreacted reagents and primer dimers were removed
166	from the 1st PCR products with the SPRI select Reagent Kit (Beckman Coulter, Brea,
167	CA, USA) according to the manufacturer's instructions. The purified 1st PCR product
168	concentration of each sample was measured by Qubit fluorometer 3.0 (Thermo Fisher
169	Scientific, Waltham, MA, USA) using Qubit dsDNA HS assay kit. Then, all samples
170	were diluted to 0.1 ng/ μ L, and all negative controls were diluted using an average
171	dilution ratio.
172	The second-round PCR (2nd PCR) was performed to add MiSeq adapter sequences
173	and 8-bp index sequences to both amplicon ends (Hamady et al. 2008). The total
174	reaction volume of the 2nd PCR was also 12 μL , containing 6.0 μL of 2 \times KAPA HiFi
175	HotStart ReadyMix (KAPA Biosystems), 3.6 pmol each of forward and reverse primers,
176	1 μ L template, and ultrapure water. The thermal cycle profile for the 2nd PCR was
177	95 °C for 3 min; 12 cycles of 98 °C for 20 s, 72 °C for 20 s, and 72 °C for 5 min. The
178	2nd PCR products were pooled in one tube (i.e., one pooled 2nd PCR products that
179	included all samples). The library sample of target size amplicons was obtained by

180	electrophoresis using E-Gel SizeSelect 2% (ThermoFisher Scientific) with the E-Gel
181	Precast Agarose Electrophoresis System (ThermoFisher Scientific). Subsequently, it
182	was confirmed whether only DNA of the target length (approximately 370 bp) was
183	isolated by using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA
184	USA). The concentration of the DNA library was adjusted to 4 nM. Finally, the library
185	was sequenced using an Illumina MiSeq v2 Reagent kit for 2×150 bp PE (Illumina,
186	San Diego, CA, USA).
187	
188	Sequence data analysis
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188 189 190 191 192 193 194	Sequence data analysis The row reads obtained from Miseq sequencing were preprocessed and analyzed using USEARCH v10.0.240 (Edgar 2010). First, R1 and R2 reads were assembled using the command "-fastq_mergepairs". Second, the primer sequence was removed from both edges of the assembled reads by using commands "-fastx_truncate", "-stripleft" and "- stripright". Third, low quality reads with an expected error rate (Edgar and Flyvbjerg 2015) of >1% and too short reads of <140 bp were removed by quality filtering (using

196	"fastx_uniques" command. Fifth, the dereplicated reads were denoised using the
197	"unoise3" command to generate amplicon sequence variants (ASVs) that remove all
198	putatively chimeric, erroneous sequences (Edgar 2016) and partial ASVs with fewer
199	than 10 reads. Finally, ASVs were subjected to taxonomic assignments to species names
200	using the "BLAST" search program to nucleotide database using a nucleotide query
201	$(https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn\&PAGE_TYPE=BlastSearies and and and and and and and and and and$
202	ch&LINK_LOC=blasthome). In the next step, the search was performed with default
203	settings, and ASVs of sequence identity >98.5% (two nucleotide differences allowed)
204	were judged as that species or was judged a species of that genus in the case of no
205	distinction at the species level. When ASVs of sequence identity of <98.5% were
206	assigned to top hit species, and when the sequence identity was >90% to reference
207	sequences, ASVs were classified as belonging to the genus or family level. In addition,
208	the detected reads in the negative control had possibly been contaminated, and the
209	number of Carassius spp. reads detected in the filtration blank of 2016 was removed
210	from reads of all corresponding environmental samples from 2016 (Port et al. 2016;
211	Hayami et al. 2020).

213 Statistical analysis

214	All analyses were performed using R ver. 3.6.0 (R Development Core Team 2019), the
215	vegan package version 2.5-6 (Oksanen et al. 2019), and the lme4 package version 1.1-
216	21 (Bates et al. 2015). The result of the species accumulation curve generated by
217	"rarecurve" function in the vegan package shows that the number of detected species in
218	each sample is saturated, except for the sample T2. In all analyses, the reads data were
219	converted to presence/absence (Jeunen et al. 2019; Fig. S1).
220	A rarefaction analysis was performed to account for the variations in the number
221	of total reads across samples (Fig S2). The following two analyses were performed
222	individually for un-rarefied and rarefied data, which was obtained by unifying the
223	number of total reads to the fewest reads through rarefying, using the R package
224	"vegan" version 2.5-6. To estimate whether algal blooms (presence/absence) affect the
225	number of fish species detected by eDNA metabarcoding, we used a generalized linear
226	mixed model (GLMM) with a poisson distribution. In the model, we assigned the
227	absence of algal blooms = 0 and the presence of algal blooms = 1 and set the

228	presence/absence of algal blooms as explanatory variables, the number of fish species
229	detected by eDNA metabarcoding as a response variable, filtration volume as an offset
230	term, and site IDs as random effects. Regarding the sampling sites in the present
231	analysis, some studies have reported that the fish community in Lake Taihu is evenly
232	distributed throughout the entire lake since being affected by severe environmental
233	change and overfishing (Tao et al. 2010; Mao et al. 2011). Therefore, we assumed that
234	there was no bias in potential fish communities based on sampling location or season,
235	and the site IDs was randomized in the analysis. Non-metric multidimensional scaling
236	(NMDS) was then performed with "Jaccard methods" and 10,000 permutations to
237	visualize the dissimilarity of fish composition by the presence/absence of algal blooms.
238	In addition, to compare fish composition between the presence and absence of algal
239	blooms, we performed PERMANOVA by function "adonis" in the vegan package. In
240	this analysis, all the data were used to conduct community analysis, because similar
241	result was obtained even at site T2, where only one ASV was detected, was removed
242	(Fig. S3). Two mammals (Homo sapiens and Sus scrofa) were detected in the eDNA

243	metabarcoding analysis. Mammals were excluded from the statistical analysis. Parts of
244	graphs were drawn using the ggplot2 package version 3.1.1 (Wickham 2019).
245	Finally, the fish composition obtained from eDNA and the latest traditional
246	survey were compared (eDNA survey: this study, traditional survey and the illustrated
247	handbook for the fish of Lake Taihu: Mao et al. 2011; Li and Shimatani 2016).
248	
249	Results
250	In total, 1,207,279 MiSeq reads were obtained. After bioinformatic filtering (see
251	Materials and Methods), 465,347 reads were retained, corresponding to 38.55% of the
252	total reads. From the filtration blank in 2016, 55 reads of Carassius spp. were detected,
253	while no fish DNA was detected in the filtration blank in 2017. In total, 28 freshwater
254	fish species were detected (Table S1).
255	The results for the un-rarefied data showed that the number of detected fish
256	species was significantly lower in the presence of algal blooms (GLMM, $p = 0.016$; Fig.
257	2). In addition, there was no significant difference in the composition of detected
258	species between the presence/absence of algal blooms ($p = 0.969$; Fig. 3). The results

259	for rarefied data indicated the significantly lower number of detected species in the
260	presence of algal blooms (GLMM, $p=0.014$; Fig. S4) and non-significant difference in
261	the composition of species ($p=0.514$; Fig. S5). Therefore, rarefaction did not alter the
262	results. Additionally, among the 27 freshwater fish species detected by eDNA
263	metabarcoding analysis, 10 species appeared in the traditional survey, and 13 species
264	appeared in the illustrated handbook for the fish of Lake Taihu (Li and Shimatani 2016)
265	(Fig. 4). According to the species accumulation curve, the number of detected species
266	did not reach a saturation by metabarcoding, after sampling nine sites (Fig. S6).
267	
268	Discussion
269	This study attempted to assess the fish diversity in a severely eutrophicated lake with
270	extensive algal blooms using eDNA metabarcoding. Our results showed that fish
271	species could be detected even in eutrophicated areas where extensive algal blooms
272	break out; however, in such an environment, besides the existence of algae blooms,
273	other factors (e.g., filtration volume and PCR inhibitors) could affect the number of

274	species detected. In future research, how the aforementioned factors influence the
275	number of fish species detected should be explored.
276	Although the composition of detected species did not differ by the presence or
277	absence of algal blooms, the number of detected species showed significant differences
278	(Fig. 2, 3). There could be two reasons why the composition of the detected species was
279	not affected. First, since the late 1980s, serious eutrophication has been occurring in
280	Taihu Lake, and fish species sensitive to oxygen-poor environments have been
281	significantly reduced, while those adapted to oxygen-poor environments have survived
282	(Zhu, 2004; Mao et al. 2011). It has also been reported that some fish, such as Coilia
283	and Carassius, can survive by changing their diet (e.g., consume cyanobacteria) (Liu,
284	2009). As a result, only fish species with strong adaptability to oxygen-poor conditions
285	may remain in such an unfavorable environment. Therefore, the presence or absence of
286	algal blooms may not influence species composition in the lake. In the present study,
287	the filtration volume of the water samples at sites without algal blooms was
288	significantly higher than at sites with algal blooms (Table 1; Fig. S7). When
289	investigating fish diversity in water bodies with extensive and frequent algal blooms

290	using eDNA, algae could clog filters, and, in turn, directly influence filtration yield and
291	indirectly influence DNA recovery. Consequently, the number of species detected could
292	be relatively low, and saturation points may not be achieved in study sites. The obtained
293	reads, classified as fish sequences after filtering, were compared; lower reads were
294	obtained for the sites with algal blooms (Table S1). This might be attributed to the
295	limitations in the PCR amplification, such as PCR inhibition in the samples from sites
296	with algal blooms. Although previous studies have shown that sample dilution could
297	circumvent the effects of inhibition (Biggs et al. 2015; Cilleros et al. 2019), no dilution
298	was performed in the present study because the amount of water that could be filtered
299	was very small and fish DNA concentrations in the samples were assumed to be very
300	low. Previous studies have reported positive relationships between the number of
301	detected species and filtered water volume (Mächler et al. 2016; Sakata et al. 2020).
302	Consequently, increasing the amount of filtration is key for improving species detection
303	rates. This is the first investigation in the water environment rich in algae, and the
304	filtration has been performed according to the standard experimental methods stipulated
305	before. In future studies, multiple filter papers, filter with a larger pore size, or pre-

306	filtration to treat water containing more particles are required. Alternatively, the
307	subsurface water under algal blooms could also be collected as opposed to the surface
308	water covered by algal blooms; this could improve the efficiency of eDNA
309	metabarcoding in eutrophic environments. Meanwhile, some studies have demonstrated
310	DNA in sediment has higher concentrations and can be conserved for longer periods
311	than DNA in water (Corinaldesi et al. 2011; Sakata et al. 2021). Therefore, collecting
312	both water samples and sediment samples could enhance detection capacity.
313	MiFish marker is generally useful for identifying fish species in Lake Taihu. A
314	total of 104 fish species were found in Lake Taihu using traditional methods and an
315	illustrated fish handbook in Taihu Lake (Mao et al. 2011; Li and Shimatani 2016).
316	Excluding 17 species that had no reference data, the rest of the 87 species had reference
317	sequences; however, some species were indistinguishable because the sequences in the
318	marker regions were identical among species, so that some species were not easily
319	identifiable in our data (Table S1). Some DNA sequences were not successfully
320	identified because of high similarity within the genus, resulting in the genus-level
321	classification (i.e., Carassius, Hypophthalmichthys). The similarity of DNA sequences

322	of cyprinid fish is high within and between genus, which leads to the subfamily level
323	classification (i.e., Acanthohodeus and Cultrinae). We cannot rule out the possibility
324	that the deposited sequence on NCBI may be wrong or caused by incorrect species
325	identification. Therefore, in future research, it will be essential to establish a perfectly
326	correct database. At the same time, we also found that the sequences of many fishes
327	living in Lake Taihu are very close and cannot be fully identified by using 12S rRNA
328	fragments. It is also suggested that a longer marker for metabarcoding or specific
329	primers for a certain genus is required to solve this identification problem.
330	Environmental DNA metabarcoding analysis has been applied to biomonitoring
331	and has been used to investigate the effect of external factors such as water quality. Our
332	results indicate that the presence of algal blooms did not affect the community structure
333	detected by eDNA metabarcoding, but the number of fish species. A body of essential
334	information of this nature will assist in designing eDNA metabarcoding biomonitoring
335	programs and their application in complex environments, contributing to aquatic
336	biodiversity conservation.

337

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Season	Date	Site	Filtration volume	Algal blooms
	Dec-21, 2016	Т5	400ml	-
Winter	Dec-21, 2016	Т7 - ₩	400ml	-
	Dec-21, 2016	Т8	100mL	+
	Aug-27, 2017	T1	350mL	+
	Aug-27, 2017	T2	155mL	+
Summer	Aug-28, 2017	Т3	1000mL	-
Summer	Aug-28, 2017	T4	22.5mL	+
	Aug-28, 2017	Т6	1000mL	-
	Aug-28, 2017	Т7 <mark>-Ѕ</mark>	560mL	-

Table 1. Filtration volume of each water sample. + means that we observed the presence of algal
blooms at the site and – means that we did not.

538	Figure legends
539	Figure 1.
540	Map of the study sites. Green squares mean that we observed the presence of algal blooms at the
541	site. Red and blue circles show the sampling sites in summer and winter, respectively. A red-edged
542	white circle means no fish ASVs have been detected. Circle size indicates the number of detected
543	species. With regard to T7, we conducted surveys in both summer $(T7-S)$ and winter $(T7-W)$.
544	
545	Figure 2.
546	Number of fish species detected in the presence/absence of algal blooms by environmental DNA
547	metabarcoding. The GLMM result shows that number of species is significant higher when algal
548	blooms are absent than when they are present.
549	
550	Figure 3.
551	Composition of detected fish species in the presence/absence of algal blooms. Circles and grey solid
552	triangle represent the samples with algal blooms ($N = 4$), crosses and white dotted triangle represent
553	the samples without algal blooms (N = 5). No fish species was detected from the sample of T7-S
554	without algal blooms, and therefore, only four points for the latter were plotted.
555	
556	Figure 4.
557	Comparison of the results of detected fish in Lake Taihu confirmed by traditional methods and by
558	the environmental DNA (eDNA) method. The upper-left circle represents the result from eDNA,
559	upper-right represents the fish caught by trawl net (Mao et al. 2011), and the bottom shows the fish
560	species in the illustrated handbook of Lake Taihu (Li and Shimatani 2016).
561	

563 Figures

564 Figure 1.













