

Ecogenomics of Zooplankton Community Reveals Ecological Threshold of Ammonia Nitrogen

Jianghua Yang,[†] Xiaowei Zhang,^{*,†,§,||} Yuwei Xie,[†] Chao Song,[†] Jingying Sun,[†] Yong Zhang,[‡] John P. Giesy,^{†,§,||} and Hongxia Yu[†]

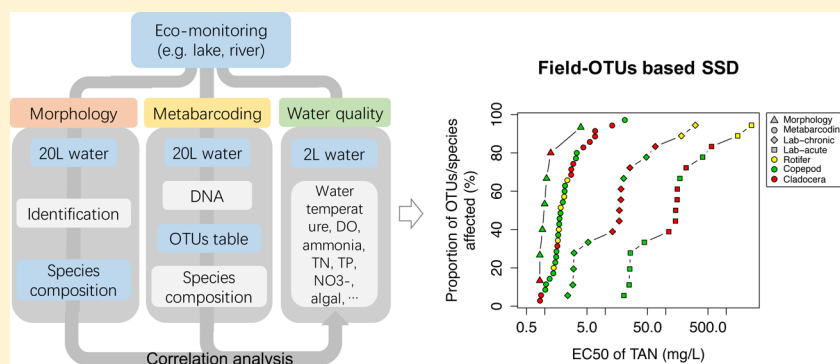
[†]State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, Jiangsu 210023, China

[‡]Jiangsu Environmental Monitoring Center, Nanjing, Jiangsu 210000, China

[§]Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada

^{||}School of Biological Sciences, University of Hong Kong, SAR, China

Supporting Information



ABSTRACT: Communities of zooplankton can be adversely affected by contamination resulting from human activities. Yet understanding the influence of water quality on zooplankton under field-conditions is hindered by traditional labor-intensive approaches that are prone to incomplete or uncertain taxonomic determinations. Here, for the first time, an eco-genomic approach, based on genetic diversity in the mitochondrial cytochrome c oxidase I (COI) region of DNA of zooplankton was used to develop a site-specific, water quality criterion (WQC) for ammonia (NH₃). Ammonia has been recognized as a primary stressor in the catchment of the large, eutrophic Tai Lake, China. Nutrients, especially NH₃ and nitrite (NO₂⁻) had more significant effects on structure of the zooplankton community than did other environmental factors. Abundances of rotifers increased along a gradient of increasing concentrations of total ammonia nitrogen (TAN), while abundances of copepods and cladocera decreased. A novel, rapid, species sensitivity distribution (SSD) approach based on operational taxonomic units (OTUs) was established to develop a WQC for NH₃. The WQC based on OTUs was consistent with the WQC based on the traditional morphology taxonomy approach. This genetics-based SSD approach could be a useful tool for monitoring for status and trends in species composition and deriving ecological criteria and an efficient biomonitoring tool to protect local aquatic ecosystems in virtually any aquatic ecosystem.

INTRODUCTION

One of the major challenges in environmental management is to develop ecological thresholds to protect ecosystem and biodiversity from effects of chemical and physical stressors. Traditional methods of assessment of toxic potency are based on laboratory bioassays with single species,^{1,2} which investigate toxicity of chemical stressors to laboratory-bred, surrogate species. Toxicity data for multiple species can be used in probabilistic approaches to provide a relative ranking of sensitivities of species, by use of species sensitivity distributions (SSDs).^{3,4} The SSD approach, using data from standard laboratory species has been criticized due to misrepresentation

of site-specific biodiversity and failure to acknowledge variations of site-specific conditions.⁵ Even if data derived in laboratory tests are available for multiple species, they might not be representative of species in an environment, especially when they are interacting among species and with the chemical–physical environment.

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As biological science has evolved, one of the major issues has been how to use large amounts of detailed genomic information, available from high through-put analyses in derivation of environmental standards and how to apply them to monitoring programs of status and trends of populations and communities. Ecogenomics technologies, such as metabarcoding which can characterize species composition by DNA, provide a rapid method of assessing biodiversity.⁶ Metabarcoding also provides a semiquantitative estimate of relative abundances because the amount of DNA from a species present in a sample is proportional to abundances of individuals of species.^{7–9} By metabarcoding of the mitochondrial CO1 region of DNA, genetic diversity of zooplankton can be characterized. Species compositions of zooplankton communities, as determined by metabarcoding, were consistent with results based on traditional taxonomy based morphology. Furthermore, metabarcoding significantly improved identification of species, increased the number of zooplankton taxa observed, and successfully identified larvae of copepods.¹⁰ Thus, metabarcoding could be a useful biomonitoring tool to profile zooplankton communities on a large scale.

Ammonia (NH₃), one of several ubiquitous forms of nitrogen (N) that is considered to be one of the most important pollutants in aquatic environments.¹¹ Ammonia can enter aquatic ecosystems from industrial and municipal effluents as well as runoff from agricultural and natural areas. Ammonia, especially in the un-ionized form (NH₃⁰) is very toxic to most aquatic organisms, including zooplankton and fish.¹² Effects of human activities on the global nitrogen cycle makes understanding of toxicity of NH₃ increasingly urgent. Over the past 30 years, the United States Environmental Protection Agency (U.S.EPA) has revised values for the WQC for TAN downward four times, in 1985, 1999, 2009, and 2013.¹²

Ammonia causes both acute and chronic effects on zooplankton at both the individual and community levels.^{13,14} Yet understanding the influence of water quality on zooplankton under field-conditions is hindered by traditional labor-intensive approaches. Herein is presented a proposed method for combining newer genomic techniques with traditional approaches, including probabilistic assessments under field conditions. As an example, the proposed techniques were applied to develop a site-specific, WQC for a globally relevant contaminant, ammonia. First, effects of various environmental factors (e.g., nutrient factors) on the structure of zooplankton community by metabarcoding were statistically assessed in a large area of the catchment of Tai Lake, China. Second, metabarcoding was integrated with field based SSDs for zooplankton to develop a site-specific water quality criterion for TAN.

MATERIALS AND METHODS

Study Area and Water Sampling. The catchment of Tai Lake (Chinese: *Taihu*) is one of the most densely populated and developed areas in China.¹⁵ During the past 20 years, excessive nutrient loading by rapid industrialization and urbanization has caused rapid deterioration of water quality.¹⁶ In the present study, 69 sampling sites across the basin were sampled from 28 November, 2013 to 12 December 2013 (Supporting Information (SI) Figure S1 and Table S1). Zooplankton communities assembled by metabarcoding had been previously compared to results of traditional taxonomic characterization based on morphology.¹⁰ Here, effects of

environmental factors on the zooplankton community were assessed by use of the same data set.

Water Chemistry. The methods for measurements of environmental factors (chemical oxygen demand (CODMn), total phosphorus (TP), phosphate (PO₄⁺), total nitrogen (TN, all forms of nitrogen), nitrate (NO₃⁻), nitrite (NO₂⁻), total ammonia nitrogen (TAN), biochemical oxygen demand (BOD₅) and Chlorophyll a (Chl a), Water temperature (WT), pH, dissolved oxygen (DO), and trophic level index (TLI)) are shown in the SI (Table S3)

Metabarcoding and Morphology Based Zooplankton Biomonitoring. Two samples of zooplankton were collected from each sampling site. One sample was used for metabarcoding analysis while the other was used for identification of zooplankton based on visual morphology.^{17–19}

Details of zooplankton community assembly by metabarcoding protocol have been described previously.¹⁰ Briefly, zooplankton was filtered through a 5- μ m filter (Millipore) and total DNA was isolated by use of the E.Z.N.A. water DNA kit (Omega). The fragment of mitochondria CO1 was used to characterize the zooplankton community.²⁰ Products of polymerase chain reaction (PCR) were sequenced by Ion Torrent PGM (Life Technologies). Metabarcoding data were analyzed according to the UPARSE pipeline,²¹ and OTUs were annotated by the Statistical Assignment Package (SAP).²²

Biodiversity. Shannon, Simpson, and Pielou indices were estimated using the relative abundance of each OTU by use of the “Vegan” package (version 2.2-1) in R software (R version 3.1.2 (2014–10–31)). Beta diversity was estimated by computing unweighted UniFrac distances between samples.²³

Ecotoxicity Data Set. Ecotoxicity data of NH₃ were downloaded from the ECOTOX database (<https://cfpub.epa.gov/ecotox/>) and other studies (see SI, Table S3). Concentrations of TAN associated with specific toxicities were normalized and adjusted to pH 7.0 and temperature = 20 °C (eqs 1 and 2) before the WQC was derived by the SSD model.¹²

$$\log(\text{TAN}_{T=20}) = \log(\text{TAN}_T) - 0.036(20 - T) \quad (1)$$

$$\text{TAN}_{\text{pH}7} = \text{TAN}_{\text{pH}7} \left(\frac{0.0489}{1 + 10^{7.204 - \text{pH}}} + \frac{6.95}{1 + 10^{\text{pH} - 7.204}} \right) \quad (2)$$

Statistical Analyses. Relationships between various environmental factors were analyzed by use of Spearman correlations in the R language. To evaluate their associations with structures of zooplankton communities, environmental factors were transformed ($\ln(x + 1)$) and normalized for use in the Mantel test.²⁴ Redundancy and principal component analyses (PCA) were employed to ordinate samples according to various types of water bodies by use of the “Vegan” package (version 2.2–1) in R. Data on species were transformed to unweighted UniFrac distances between samples by QIIME (version 1.9.1+dfsg-lbiolinux4) before PCA analysis.²³ Relative contributions of environmental factors to explain variance of zooplankton communities were determined using forward selection, distance-based, linear modeling (distLM) in PERMANOVA.²⁵ To identify sensitive OTUs, structures of taxonomic communities were related to each environmental factor by use of quantile regression ($p < 0.1$)²⁶ in R with the package “quantreg”. The 50% effective concentration (EC₅₀) of TAN was calculated by use of quantile regression. Ranking of sensitivities of OTUs to TAN were calculated using the EC₅₀ by

a SSD model developed with a bootstrapping method. The WQC (HC₅ and HC₁₀) for TAN was derived using the normalized TAN by a 3 parameter log–logistic model.

RESULTS

Characteristics of Environmental Factors. Nutrients, including NO₃⁻, NO₂⁻, TAN, TN, PO₄⁺ and TP exhibited significant and positive correlations (Spearman's test ($r^2 > 0.6$, $p < 0.001$)). BOD₅ was strongly correlated ($r^2 = 0.91$, $p < 0.001$) with COD. Transparency of water (a measure of turbidity), numbers of algal cells, DO and pH were weakly ($r^2 < -0.3$, $p < 0.05$) and negatively correlated to concentrations of nutrients. Water temperature was weakly and negatively correlated with DO, algal density, transparency, BOD₅, and COD and positively correlated with concentrations of nutrients (Figure 1).

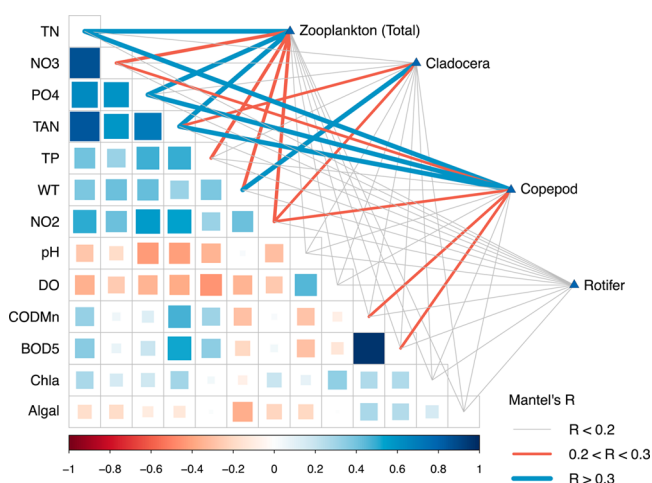


Figure 1. Relationships between environmental variables and composition of the zooplankton community. Pairwise comparisons of environmental factors were displayed with a color gradient denoting Spearman's correlation coefficients. Taxonomic groups were related to each environmental factors by mantel test. TN, all forms of nitrogen; NO₃, nitrate; NO₂, nitrite; TAN, all forms of ammonia; PO₄, orthophosphate; TP, total phosphorus; BOD₅, Five-day Biochemical Oxygen Demand; CODMn, Chemical Oxygen Demand; algal, numbers of algal cells; DO, dissolved oxygen, and WT, water temperature.

Zooplankton Community Structure Altered by Nutrient Factors and TAN. Composition of zooplankton communities, determined by metabarcoding, varied among habitats with distinct nutrient profiles (Figure 2). Redundancy analysis showed that 33.1% of overall variability of zooplankton community composition was explained by the first two principal components (RDA1 and RDA2).

Effects of nutrients and TAN on zooplankton were greater than were other environment factors. RDA1(24.7% variance explained) was mostly explained by nutrient factors and TAN, whereas RDA2 (8.6% variance explained) was mainly explained by other variables. The Mantel test showed most nutrient factors were strongly associated (mantel's $R > 0.2$, $p < 0.05$) with structures of zooplankton communities. TAN, NO₂⁻, and WT had a significant effect on structures of communities of cladocera. Nitrate, NO₂⁻, TN, PO₄⁺, and TAN had a significant effect on structures of copepod communities (Figure 1).

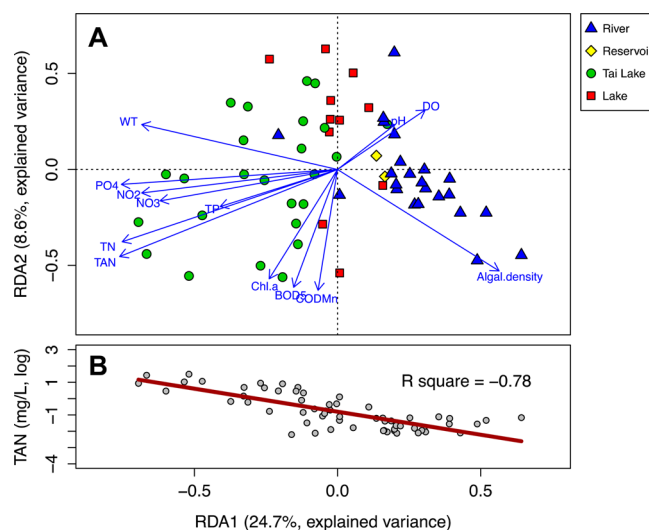


Figure 2. Ordination of the zooplankton community with environmental variables. Blue vectors point to the direction of the increase for a given variable so that water with similar environmental variable profiles or zooplankton community are localized in similar positions in the diagram. A: similarity of samples. B relationship between RDA1 and TAN.

Structures of communities based on either metabarcoding or traditional morphological taxonomy showed that diversity of zooplankton was dependent on comprehensive nutrition level TLI. In locations with higher TLI, diversities of cladocera and copepods were significantly less (Figure 3A), while diversities of rotifers were greater (Figure 3B).

Correlations between Ammonia and the Structure of the Zooplankton Community. Compared to nutrient factors, ammonia was a major determinant of compositions of zooplankton communities in various regions of Tai Lake. Approximately 34% of variation in dissimilarity of composition of zooplankton communities was explained by measured environmental factors (Table 1). In decreasing order of influence, these factors were TAN, numbers of algal cells, and WT, which together explained 25.7% of variation in composition of the zooplankton community (Table 1; Forward selection sequential tests) and TAN was a major contributor explaining approximately 15% of variation. RDA1 was significantly, negatively correlated with TAN ($r^2 = -0.78$, $p < 0.001$).

Ammonia was significantly associated with differences among structures of communities of zooplankton in the catchment of Tai Lake. PCA analysis demonstrated structures of zooplankton communities were distinct between samples of lesser (<0.5 mg/L) and greater concentrations of TAN (>0.5 mg/L) (SI Figure S2). Relative abundances of copepods and cladocera were inversely proportional to concentrations of TAN, whereas total abundance of rotifers increased along a gradient of increasing concentrations of TAN (SI Figure S3). Diversity of the rotifer community was directly proportional to concentration of TAN, whereas diversity of *Bosmina* sp (cladocera) was inversely proportional (see SI, Figure S4). Proportions of sensitive OTUs, those of which abundance was inversely proportional to concentrations of TAN, ($p < 0.1$ in quantile test) were inversely proportional to concentrations of TAN (Figure 4E).

Sensitivities of Various Zooplankton Groups to Total Ammonia Nitrogen. There were thirty-nine zooplankton OTUs classified as being "sensitive" (quantile test, $p < 0.1$) to

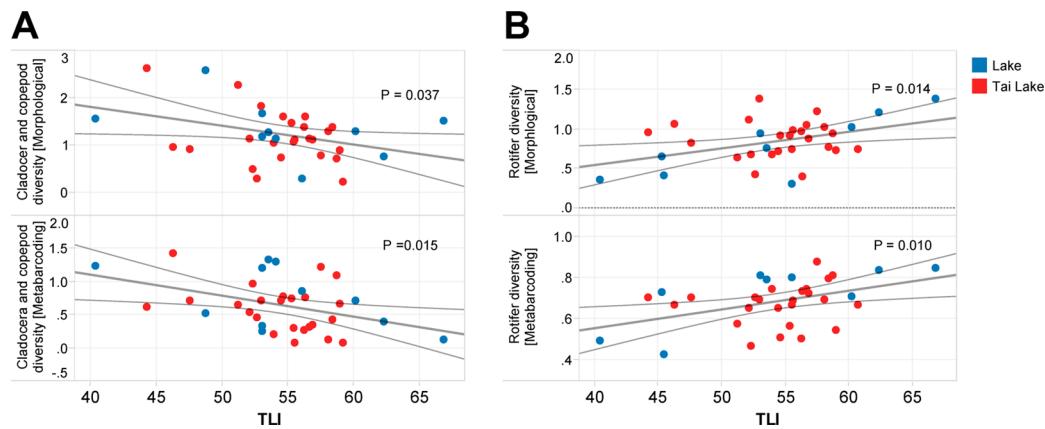


Figure 3. Effects of eutrophication on diversity of zooplankton in the Tai Lake ecosystem: (A) Shannon diversity of cladocera and copepoda; (B) Shannon diversity of rotifer. The upper diagram is based on species determined by use of visual inspection of morphology, whereas the lower diagram is based on OTUs determined by metabarcoding. *p*-values are indicated for each regression axis.

Table 1. Distance-Based, Linear Modelling Results of Zooplankton Community Composition against 13 Predictor Variables in the Full Analysis (9999 Permutations)^a

predictor variable	marginal tests			forward selection sequential tests			
	pseudo-F	variation explained	<i>P</i>	pseudo-F	variation explained	cumulative variation explained	<i>P</i>
+TAN	11.5508	14.955	0.005	11.5508	14.955	14.955	0.005**
+algal.density	7.1576	9.308	0.005	7.0789	7.944	22.899	0.005**
+WT	8.5149	11.131	0.005	3.2025	2.821	25.72	0.005**
+Chl.a	3.4607	3.94	0.01	3	2.518	28.238	0.005**
+PO ₄	10.2423	13.384	0.005	2.539	1.919	30.157	0.01*
+DO	3.049	3.302	0.015	2.1764	1.463	31.62	0.015
+NO ₃	6.9639	9.041	0.005	1.7033	0.879	32.499	0.065
+TN	10.8596	14.113	0.005	1.6569	0.3969	32.8959	0.09
+NO ₂	8.4262	11.014	0.005	1.3922	0.3245	33.2204	0.185
+TP	3.5807	4.124	0.015	1.1126	0.3029	33.5233	0.32
+pH	2.4763	2.401	0.02	1.0588	0.1999	33.7232	0.395
+CODMn	2.5555	2.527	0.02	0.8238	0.1438	33.867	0.65
+BOD ₅	2.6869	2.735	0.03	0.6381	0.1082	33.9752	0.85

^aBold means significantly correlated with community structure at *P* < 0.01.

TAN, which represented 32.1% of CO1 sequences in the metabarcoding data set (Figure 4 and SI Figure S5). The most sensitive OTUs were copepods and cladocera. More than 40% of OTUs classified as cladocera were sensitive to TAN, and they represented two-thirds of the total number of cladocera reads in metabarcoding (Figure 4G, I). For copepods, approximately 17% of copepod OTUs were sensitive to TAN, and they represented 40% of the total number of copepod reads. Only one OTU, representing 2.6% of the rotifer reads was found to be sensitive to TAN, although rotifers represented more than half (55%) of the total number of OTUs in the zooplankton community (Figure 4G, I).

BLAST against the database of local taxa, demonstrated that sensitive OTUs in the metabarcoding data set belong to six species (*Bosmina* sp, *Ceriodaphnia cornuta*, *Schmackeria inpinus*, *Sinocalanus dorrii*, *Macrothrix* sp, and *Keratella quadrata*). *Bosmina* sp and *Sinocalanus dorrii* represented 21 and 10 sensitive OTUs, respectively, that were abundant and exhibited frequent occurrences (Figure 4C, D).

Both metabarcoding data and traditional morphological monitoring showed most of the sensitive taxa were copepoda or cladocera (Figure 4H, G). Laboratory-based toxicity tests showed that copepoda and cladocera were more sensitive to TAN than were species of rotifer (Figure 4F). Seven taxonomic

groups identified by traditional morphological identification, *Bosmina* sp, *Ceriodaphnia cornuta*, *Schmackeria inpinus*, *Sinocalanus dorrii*, *Copepod nauplii*, *Cyclops larvae*, and *Calanoida larvae*, were sensitive to TAN (SI Figure S6). Four species identified by both metabarcoding and morphological monitoring (*Bosmina* sp, *Ceriodaphnia cornuta*, *Schmackeria inpinus*, and *Sinocalanus dorrii*) were sensitive to TAN (Figure 5).

Derivation of a Site-Specific Water Quality Criterion of TAN Based on Sensitive OTUs. The *f*-SSDs for TAN, based on species identified by use of morphological taxonomy or OTUs identified by use of metabarcoding were more sensitive than were laboratory-bred, surrogate zooplankton (Figure 6 and SI Table S5). There were 16 zooplankters for which data on toxicity of NH₃ were available in the ECOTOX database (see SI, Table S3). HC₅ and HC₁₀ derived from the SSD, based on tests done under laboratory conditions, were 2.4 and 2.9 mg/L, respectively. In the field, 7 of the 76 taxonomic groups (9.2%) identified by use of morphology were sensitive to TAN. The HC₅ for concentrations of TAN measured in Tai Lake and the SSD developed for species identified in Tai Lake by morphological taxonomy was 1.1 mg TAN/L. There were 291 zooplankton OTUs detected in more than a third of samples of which 13.4% were sensitive to TAN. Values for HC₅

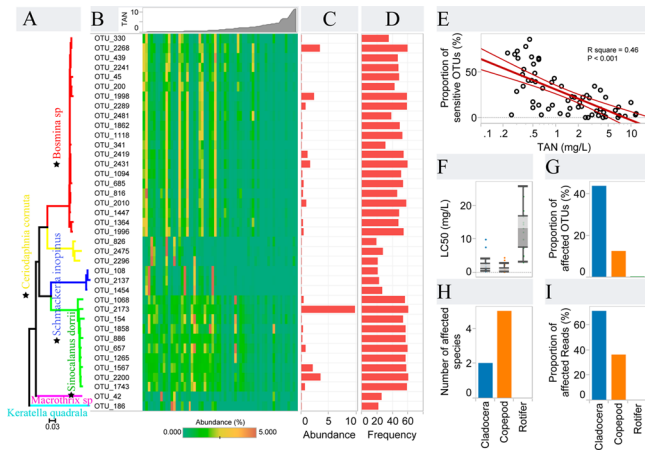


Figure 4. Phylogenetic distribution of assignable components of sensitive OTUs: (A) Tree diagram of representative sequences for each OTU. Distance was measured as number of base substitutions per site, based on the Kimura two-parameter (K2P) method. Asterisks indicate species that were found to be sensitive to TAN based on morphology; (B) Profiles of distribution of each OTU. The color indicates weighting by relative abundances of reads. For instance 0.01 means the number of reads for that taxon accounts for 1% of total number of reads; (C) Relative abundance (%); (D) Frequency of occurrence. (E) Linear regression between TAN and proportion of sensitive OTUs; (F) Sensitivities of copepoda, cladocera, and rotifera to TAN in traditional toxicity test under laboratory conditions; (G) Proportions of OTUs, based on metabarcoding, affected by TAN; (H) Numbers of sensitive species determined by visual inspection of morphology; (I) Proportions of sequences in metabarcoding data affected by TAN.

and HC_{10} derived from the SSD based on OTUs were 1.4 and 2.9 mg TAN/L, respectively. Results of both traditional lab-based toxicity tests and field metabarcoding monitoring data demonstrated that copepods were more susceptible to adverse effects of NH_3 than were cladocera and copepoda.

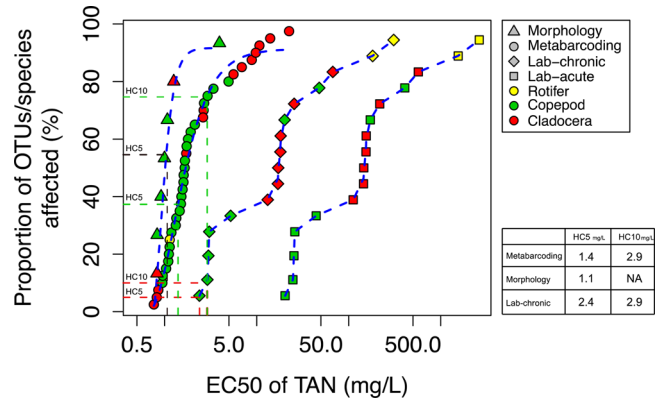


Figure 6. Species sensitivity distribution for TAN based on various evaluation methodologies. HC_{10} for SSD based on species determined by visual morphology was not available because only 9.2% species were found to be sensitive to TAN. For OTUs based on metabarcoding deemed sensitive to TAN were those for which abundances of OTUs decreased along an increasing gradient of concentrations of TAN and the coefficient <0.1 by the quantile test (see SI, Figure S6). Species identified by visual morphology deemed to be sensitive to TAN are those for which the density of species decreased along an increasing gradient of concentrations of TAN and the coefficient <0.1 by the quantile test (see SI, Figure S7). Acute toxicity data of ammonia/TAN were from several previous studies (for details see SI, Table S3). Chronic toxicity data were calculated by the acute to chronic ratio (8.507). In order to better compare the laboratory toxicity data and field monitoring data, all the TAN were normalized and adjusted to pH 7.0 and temperature = 20 °C.

DISCUSSION

Nutrients, especially TN, NO_3^- and NO_2^- , and TAN had more significant effects on communities of zooplankton than did other environmental factors in the Tai Lake basin. The catchment of Tai Lake, situated in the lower reaches of the Yangtze River, is one of the most densely populated and developed areas in China.¹⁵ After decades of intensive input of nutrients from industrial and agricultural sources, most surface

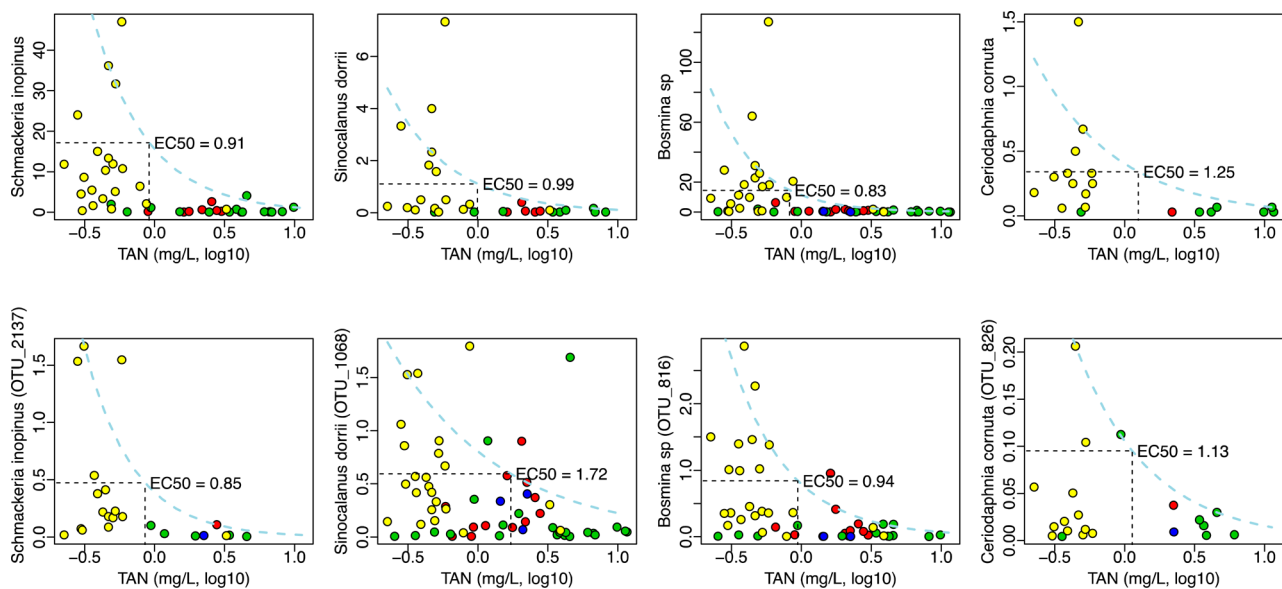


Figure 5. Comparison of metabarcoding and morphological monitoring data in four species sensitive to ammonia. The 50% effective concentration (EC_{50}) for TAN was calculated by use of the quantile regression. Color represents type of water body (yellow, Tai Lake; red, Small Lake; green, River; blue, reservoir).

waters in the Tai Lake basin have become hypereutrophic, which has resulted in numerous large algae blooms in recent years.^{27,28} Eutrophication is still one of the most serious environmental problems confronted by local environmental managers.²⁹ The results reported here demonstrated that TAN and nutrient factors (TN, NO₃, NO₂⁻, and phosphate) had significant effects on compositions of zooplankton communities. As one of the most important pollutants in aquatic environments, TAN was more toxic to and had a greater influence on zooplankton than did other nutrient factors.^{30,31} The results of the variation partitioning analysis suggest TAN is a major stressor on zooplankton in the Tai Lake basin.

Other evidence of TAN being a major stressor for zooplankton is that the diversities and abundances of rotifer were directly proportional to TAN. Some laboratory toxicity studies found some rotifers, such as *Brachionus rubens* and *Brachionus rotundiformis* to be more tolerant of NH₃ than some cladocerans, such as *Moina micrura* and the copepod *Acartia tonsa*.^{32–34} Tolerance of rotifers allows them survive relatively great concentrations of NH₃. In the present study, abundances of copepods and cladocerans were inversely proportional to concentrations of TAN. However, both abundances and diversities of rotifers were directly proportional to concentrations of TAN. Although it is difficult to conclude that TAN was the sole cause of the shift observed in the zooplankton community, effects of NH₃ on zooplankton in Tai Lake basin cannot be ignored. Rotifers exhibited the greatest diversity and accounted for more than half of OTUs observed, of which only one was sensitive to TAN. In contrast, more than 30% of the copepoda and cladocera reads were sensitive to TAN. Identification of species, based on morphological taxonomy, suggested that only copepods and cladocera were sensitive to TAN. So, together ecogenomics and morphology-based monitoring of diversity of zooplankton provided empirical evidence of apparent sensitivities of these species.

Other stressors could potentially affect the response of the zooplankton to nutrient stressors, for example, changes in the food web. This is always a limitation of the apparent effects threshold analysis. Laboratory studies of individual species also lack realism and potential effects of multiple stressors. But application of the two approaches simultaneously, as we have done in this study allows an interpretation of the effects of accessory factors as well as additional stressors on the critical stressor. This approach is analogous to the water-effects ratio approach often applied to individual contaminants.³⁵ This allows a simultaneous correction for mitigating effects of speciation and dissipation as well as potential supra-additive effects of additional stressors. Results of previous studies have indicated that other contaminations, such as metals and pesticides pose hazards to invertebrates benthic, and that eutrophication is still the most serious environmental problem in this area.^{36,37} So, this study assessed whether ammonia was likely to be the critical contaminant by comparing results of laboratory- and field-based assessments. The fact that the results indicated similar thresholds for ammonia based on either the laboratory or field-based results indicates that ammonia was not greatly affected by mitigating or synergistic interactions with other environmental factors or stressors.

Metabarcoding also provides a useful method for determining relative sensitivities of individual species to specific pollutants. Although traditional laboratory toxicity tests allow researchers to control experimental conditions, they are often criticized for lacking ecologically relevance.⁵ Laboratory toxicity

tests are also severely limited by the few surrogate species available for testing.³⁸ Species richness response curves derived from field monitoring data (f-SSDs) are more ecologically relevant, since they assess indigenous species in the presence of relevant accessory, environmental factors.^{38,39} The option of using field data for deriving WQC is permitted by the European Water Framework Directive and has been recommended by the U.S.EPA for suspended sediment benchmarks and nutrient criteria.^{40,41} Although f-SSDs are useful, traditional identifications of species, based on morphology are costly and time-consuming, which limits application of f-SSDs. They are also limited by simultaneous exposures to multiple stressors. Here a novel approach, field based OTUs sensitivity distribution (f-OSD), was demonstrated to derive ecological criteria for pollutants, which combines the advantage of ecologically relevant by f-SSDs and the advantage of species identification by ecogenomics.^{7,42} Which OTUs were sensitive to TAN were determined and used to derive a criterion of TAN in f-OSD. The HC₅ (1.4 mg TAN/L), derived by use of OTUs was consistent with that derived based on field monitoring and identification of species based on morphology (1.1 mg TAN/L). These results provide information for confirming the threshold for TAN in the realistic environment, while integrating all of the conditions and potential effects of other environmental variables.

Metabarcoding provided more comprehensive data on biodiversity than did traditional taxonomy, which allowed detection of slight responses of biota and made development of the site-specific criterion for TAN more accurate and reliable.⁴³ Resolution of thresholds for effects is a function of the number of taxa considered. For instance, if 10 species are included resolution of the assessment would be no better than 10%. Also, selection of species used to generate the SSD has a significant effect on accuracies of assessments. Since it is assumed that the entire range of possible sensitivities is covered, if this is not true, a systematic bias would be introduced. Use of the HC₅ as a measure of de minimus risk is questioned because, based on LC₅₀ data would mean that 50% of individuals of 5% of species would be expected to die. If those species were cornerstone species of ecosystems, that could result in a loss of ecosystem services. This information along with understanding of the structure and functions of ecosystems can then be used to make judgments of the likelihood that critical ecosystem services might be adversely affected.

In this study, 291 OTUs of various species of zooplankton were identified by use of DNA metabarcoding, whereas traditional visual identification of species identified 76 taxa. Furthermore, since derivation of WQC by use of the SSD model depends on number of sensitive species, using OTUs instead of “species” provides more data points than provided by use of the morphological method. Using metabarcoding, 39 OTUs (13.4%) were sensitive to TAN, whereas only seven taxa (9.2%) were identified to be sensitive to TAN by use of visual taxonomy. Furthermore, four species were identified to be sensitive to TAN were also found to be sensitive to TAN by identification as OTUs. EC50s of the four species were consistent between the two approaches. Overall, the f-OSD strategy demonstrated here minimized some of the limitations of identification of species when developing site specific WQC by use of the apparent effect threshold method, which is based on synoptic collection of information on species and concentrations of contaminants in the field.⁴⁴

The threshold for effects of TAN, derived by use of field-OSD was slightly less than that derived by use of the SSD based on laboratory toxicity data. This does not mean the SSD based on results of laboratory bioassays is not acceptable for deriving criteria, but the field-OSD, which is based on the entire assemblage in an ecosystem and includes effects of accessory factors, is more suitable for derivations of site- or regional-specific WQCs.⁴⁵ Most species used to develop current a criterion for NH₃ are based on laboratory toxicity data for non-native species. However, there is insufficient data for toxicity of ammonia to native species for which protection is sought for. The threshold for effects of TAN derived by field-OSD closely matched that derived by field-species method. While the WQC for TAN derived based on OTUs developed by metabarcoding of the zooplankton community during the present study demonstrates a promising approach, since zooplankton might not be the most sensitive taxa, more sensitive species should be considered, such as mollusks^{12,46} and fishes⁴⁷ should also be considered. Balance between f-OSD/SSD and laboratory toxicity testing is also needed in derivation of WQC. The F-OSO/SSD approach is limited because it requires that the contaminant already be in the environment and thus cannot be applied to all compounds.

A novel approach (f-OSDs) for development of ecologically relevant, site-specific, WQC to protect aquatic organisms from effects of TAN based on the quantifiable changes of biodiversity of zooplankton populations was presented. The f-OSDs approach incorporates advantages of environmental relevance from field-based SSD and the advantages of comprehensive, high throughput, high sensitivity, and low cost in species identification from metabarcoding.^{44,48} This method also allowed for rapid assessment of which species/OTUs were more sensitive to a particular contaminant. Once calibrated to a local environment, f-OTUs can be used for rapid screening to understand the status and trend in structures of aquatic ecosystem. While, in this study the ecogenomic approach was used to gain information on effects of TAN on zooplankton, the technology can be expanded to be used to monitor for effects on other groups of organisms, including phytoplankton,^{49,50} insects,⁵¹ and fish.⁵² It might even be possible to monitor for effects on terrestrial animals or aquatic birds.⁵³ This will be particularly important for studying endangered or threatened species. Finally, use of a more focused assessment of abundances of specific genes as indicators of function or general health of species might be possible.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b05606.

Additional information as noted in the text (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (86)-25-89680623; e-mail: zhangxw@nju.edu.cn, howard50003250@yahoo.com.

ORCID

Xiaowei Zhang: 0000-0001-8974-9963

Notes

The authors declare no competing financial interest.

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Ecogenomics of Zooplankton Reveals Ecological Threshold of Ammonia

Nitrogen

Jianghua Yang*, Xiaowei Zhang*, Yuwei Xie*, Chao Song*, Jingying Sun*, Yong Zhang†, John P. Giesy*,‡,§, Hongxia Yu*

* State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, Jiangsu 210023, China

† Jiangsu Environmental Monitoring Center, Nanjing, Jiangsu, 210000, China

‡ Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK S7N 5B3, Canada

§ School of Biological Sciences, University of Hong Kong, SAR, China

Corresponding author: Xiaowei Zhang

School of the Environment, Nanjing University

163 Xianlin Avenue, Nanjing, 210023, China

Tel: (86)-25-89680623

E-mail address: howard50003250@yahoo.com
zhangxw@nju.edu.cn

This file includes:

Methods of water chemistry

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

Table S2. Environmental water variables measured in the study.

Table S3. Acute toxicity of ammonia to zooplankton species.

Fig.S1. Location of sampling sites of the Tai Lake basin of Jiangsu province in China.

Fig.S2. Principal component analysis of zooplankton community components based on COI OTUs.

Fig.S3. Non-linear regression between TAN and relative abundance of cladocera, copepod and rotifer.

Fig.S4. The relationship between diversity of two zooplankton species and total ammonia nitrogen.

Fig.S6. The relationship between diversity of zooplankton and total ammonia nitrogen.

Fig.S6. The ammonia sensitive OTUs in metabarcoding data.

Fig.S7. The ammonia sensitive species/taxon in traditional morphological monitoring data.

Reference in the supporting information

Methods of water chemistry

Samples of water were collected in brown glass bottles by holding them 0.5 m below the surface at each site and then stored at 0-4 °C in the dark. Samples were brought to the laboratory within 12 h and processed immediately. Parameters measured included chemical oxygen demand measured by use of the permanganate index (CODMn), total phosphorus (TP), phosphate (PO₄⁺), total nitrogen (TN, all forms of nitrogen), nitrate (NO₃⁻), nitrite (NO₂⁻), total ammonia nitrogen (TAN), biochemical oxygen demand (BOD5) and Chlorophyll a (Chl a) following standard methods (EPA of china 2002). Water temperature (WT), pH, dissolved oxygen (DO), transparency and algal density were measured using YSI water quality sondes *in situ* (YSI Incorporated, Ohio, USA). The trophic level index (TLI) was used for quantitative evaluation of eutrophication level of lakes (Carlson and Robert 1977), and calculated with monitoring data from three seasons. TLI was calculated (Equations 1-5).

$$TLI(\Sigma) = \sum_{j=1}^m W_j TLI(j) \quad (1)$$

$$TLI_{(Chl-a)} = 10 (2.46 + 1.091 \ln(Chl-a)) \quad (2)$$

$$TLI_{(TP)} = 10 (7.109 + 0.946 \ln(TP)) \quad (3)$$

$$TLI_{(TN)} = 10 (4.934 + 1.310 \ln(TN)) \quad (4)$$

$$TLI_{(SD)} = 10 (4.311 - 2.120 \ln(SD)) \quad (5)$$

Data accessibility: DNA sequences by NGS were uploaded to NCBI Sequence Read Archive (SRA, SRR4241102) and to the dryad database (doi: <http://datadryad.org/review?doi=doi:10.5061/dryad.979cq>).

Table S1. Latitude/Longitude and group information for each sampling site. Sampling sites were grouped according to type of water body into four categories: 1) Tai Lake, 2) Reservoir, 3) River and 4) Lake. Here, “Lake” means all the relatively smaller lakes around Tai Lake.

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. Chemical-physical parameters measured. Chl a: Chlorophyll a, mg/m³; WT: water temperature; AD: algal density, 10k cells/L; TS: transparency, cm; CODMn: measure permanganate index, mg/L; BOD5: five-day biochemical oxygen demand, mg/L; TP: total phosphorus, mg/L; PO₄⁻: phosphate, mg/L; TN: total nitrogen, mg/L; NO₃: nitrate, mg/L; NO₂: nitrite, mg/L; TAN: total ammonia nitrogen, mg/L.

Site	Chl a	WT	pH	DO	AD	TS	CODMn	BOD5	TP	PO4	TN	TAN	NO3	NO2
S1	6.7	9.07	8.53	12.66	2960	35	6.36	7.02	0.05	0.03	1.74	0.039	1.1	0.015
S2	8.6	8.32	7.84	10.5	406	10	2.98	4.02	0.05	0.001	0.52	0.186	0.08	0.005
S3	9.7	9.23	8.52	11.42	2650	35	4.87	5.9	0.18	0.07	0.79	0.051	0.34	0.014
S4	9.1	8.56	8.15	11.45	1570	10	3.25	4.21	0.08	0.04	2.09	0.211	0.95	0.011
S5	8.9	9.1	8.24	11.23	2000	10	8.8	11.3	0.32	0.02	0.81	0.211	0.42	0.005
S6	14.7	8.13	7.68	11.35	2350	10	3.26	2.24	0.09	0.03	1.82	0.312	0.61	0.011
S7	2.1	8.28	7.26	11.41	670	5	3.59	4.33	0.14	0.02	0.62	0.272	0.001	0.008
S8	12.5	8.26	8.29	11.6	630	30	2.83	3.66	0.17	0.03	0.4	0.308	0.001	0.004
S9	6.1	8.35	7.94	8.28	660	25	4.48	5.46	0.06	0.05	1.05	0.254	0.001	0.008
S10	5.3	8.69	8.22	8.63	870	20	5.47	6.97	0.06	0.02	1.07	0.122	0.54	0.009
S11	4.9	8.78	8.25	8.31	540	25	4.83	5.89	0.04	0.02	1.07	0.191	0.15	0.008
S12	18	9.16	8.27	15.72	425.5	40	4.63	5.63	0.06	0.03	3.74	1.11	2.02	0.071
S13	10.7	8.61	8.07	11.48	2200	15	3.34	4.05	0.14	0.08	0.48	0.264	0.14	0.009
S14	7.4	8.68	8.34	7.14	860	25	3.52	4.9	0.03	0.02	0.89	0.163	0.08	0.014
S15	5.5	8.41	8.1	13.31	1380	10	4.95	5.92	0.07	0.03	0.98	0.297	0.16	0.007
S16	2.1	9.99	8.57	11.37	100		2.3	2.78	0.1	0.001	0.48	0.14	0.001	0.004
S17	8.1	9.04	8.33	11.64	1050	20	4.31	5.25	0.06	0.03	1.01	0.143	0.23	0.006
S18	13.7	8.28	7.69	11.41	1150	3	5.12	6.41	0.5	0.02	0.86	0.327	0.17	0.007
S19	5.3	8.18	8.1	13.8	438	30	4.79	5.97	0.07	0.02	0.91	0.153	0.13	0.007
S20	8.2	9.24	8.49	11.74	1327	30	4.03	5	0.06	0.02	0.69	0.024	0.15	0.007
S21	4.8	8.82	8.29	7.05	380	15	4.12	5.3	0.02	0.001	0.77	0.12	0.1	0.008

S22	7.7	9.03	8.32	11.55	1600	15	4.95	6.09	0.08	0.03	1.04	0.122	0.57	0.007
S23	2.9	9.03	8.2	11.44	70	60	2.79	3.54	0.04	0.001	0.41	0.143	0.001	0.005
S24	2.3	8.5	8.39	20.51	40	90	2.28	2.62	0.02	0.001	0.63	0.14	0.349	0.005
S25	7.5	8.85	8.34	11.5	999.5	40	5.34	6.54	0.23	0.04	1.06	0.13	0.87	0.031
S26	6.4	9.68	8.26	11.92	1100	10	3.77	4.65	0.06	0.02	0.71	0.148	0.001	0.007
S27	8	10.37	8.12	14.98	249.7	35	3.97	4.68	0.12	0.07	4.09	1.6	1.81	0.36
S28	6.2	10.03	8.49	10.55	161		2.81	3.47	0.02	0.001	0.56	0.299	0.19	0.008
S29	9.6	9.96	8.83	11.25	310	60	3.1	3.84	0.03	0.001	0.58	0.251	0.14	0.006
S30	1.1	11.9	8.23	10.46	100	45	4.81	5.96	0.05	0.001	1	0.322	0.27	0.028
S31	18.4	15.24	8.21	9.21	300	30	3.45	4.41	0.24	0.14	4.58	1.59	2.29	0.521
S32	3.4	12.3	8.44	10.06	100	30	1.74	2.01	0.18	0.1	1.17	0.12	0.88	0.005
S33	12.7	9.65	8.23	5.26	460	65	5.2	6.96	0.34	0.22	6.15	2.89	2.63	0.394
S34	6	17.25	8.39	8.61	420	15	1.16	2.89	0.13	0.08	2.57	0.839	1.09	0.076
S35	16	9.77	8.18	4.3	407.9	10	6.12	7.33	0.14	0.1	5.05	2.58	0.804	0.099
S36	4	10.84	8.03	9.88	300	50	2.74	3.44	0.41	0.01	1.07	0.168	0.63	0.019
S37	6	14.84	8.33	9.32	270	20	2.98	3.66	0.22	0.15	3.48	0.803	2.54	0.016
S38	20.1	11.1	7.46	4.79	580	10	4.88	6.39	0.29	0.26	9.62	4.19	5	0.234
S39	13.7	10.91	8.07	4.86	340	30	5.34	6.5	0.25	0.05	4.71	0.11	1.85	0.21
S40	23.3	11.81	7.53	7.47	350	25	4.96	6.08	0.21	0.18	5	2.55	1.47	0.764
S41	15.4	10.7	8.43	9.31	660	35	5.83	7.42	0.12	0.04	11	2.48	6.1	0.006
S42	6.3	10.82	7.15	7.89	550	20	3.85	4.71	0.13	0.1	2.59	0.905	1.41	0.227
S43	3.9	11.62	8.12	7.24	270	30	3.83	5.11	0.48	0.02	2.81	0.727	1.54	0.121
S44	5.6	11.23	8.77	9.43	140	50	2.62	3.24	0.08	0.02	4.33	0.429	0.46	0.056
S45	10.5	14.36	8.19	8.99	608	10	1.52	3.89	0.17	0.09	3.07	1.17	1.46	0.259
S46	13.7	14.77	8.21	7.67	400	15	4.88	5.98	0.77	0.17	6.1	2.79	2.91	0.317
S47	18.5	9.45	8.71	15.24	464.5	20	4.61	5.54	0.12	0.07	3.96	1.42	2.03	0.327

S48	1.3	11.48	8.64	10.57	310	80	2.77	3.38	0.27	0.001	0.22	0.13	0.001	0.004
S49	7.9	12.2	8.01	7.04	360	30	3.31	4.22	0.31	0.09	3.96	1.99	1.23	0.453
S50	18.2	10.28	7.59	10.01	600	20	12.5	15.9	0.39	0.15	5.88	4.47	0.3	0.007
S51	4.1	11.46	8.27	8.84	100	35	2.25	2.61	0.12	0.06	1.9	0.401	1.33	0.102
S52	5.5	13.95	8.49	14.2	320.9	15	1.46	1.88	0.12	0.07	2.14	0.226	1.8	0.064
S53	16	10.83	7.97	7.18	870	10	2.15	5.34	0.22	0.16	3.06	1.94	1.01	0.068
S54	9.5	11.09	8.25	5.57	190	20	4.75	5.64	0.1	0.02	3.89	1.99	1.39	0.166
S55	5.2	11.96	8.1	13.62	90	40	3.56	4.31	0.13	0.07	2.52	0.839	1.24	0.062
S56	3.7	8.82	8.13	20.46	90	55	3.98	5	0.24	0.1	2.16	0.426	1.06	0.065
S57	5.9	9.31	8.16	20.34	120	65	3.49	4.64	0.1	0.07	2.26	0.472	1.21	0.068
S58	11.3	8.03	8.32	20.32	280	90	3.84	4.7	0.05	0.03	1.47	0.598	0.51	0.06
S59	51.4	8.57	8.72	21.48	672.1	40	4.48	5.48	0.05	0.03	1.8	0.332	0.54	0.022
S60	4	9.84	8.33	18.61	50	110	4.19	5.14	0.02	0.001	1.2	0.168	0.001	0.009
S61	2.8	11.25	8.69	10.81	336	70	3.59	4.28	0.12	0.07	1.75	0.264	0.75	0.03
S62	27.9	8.34	8.92	21.76	724.6	30	4.45	5.23	0.05	0.03	1.32	0.305	0.24	0.024
S63	3.1	11.59	8.37	9.8	80	50	1.77	2.04	0.13	0.07	1.46	0.15	1.08	0.03
S64	45.1	10.13	8.72	22.43	610.6	35	3.68	4.61	0.05	0.04	1.76	0.342	0.73	0.023
S65	4.8	10.1	8.11	11.07	147	60	3	3.56	0.1	0.07	3.23	0.694	2.39	0.085
S66	4.3	9.6	8.04	11.11	124	100	3.48	4.33	0.27	0.23	3.23	0.459	2.49	0.075
S67	13.7	10.75	8.58	18.91	329.5	40	4.34	5.08	0.07	0.04	2.23	0.494	0.78	0.028
S68	27.9	8.34	8.92	21.76	724.6	30	4.45	5.23	0.05	0.03	1.32	0.305	0.24	0.024
S69	9.7	14.89	8.37	9.29	482	5	2.67	3.79	1.34	0.51	3.16	1.59	1.14	0.108

Table.S3. Acute toxicity data of unionized ammonia for zooplankton by lab-based toxicity test.

Taxa	Species	LC50 (mg/L)	Duration (hour)	PH	Temperature	Reference
Copepod	Nitokra sp	0.35-0.88	96	7.61-8.54	21-22	(Sousa, Zaroni et al. 2011)
Copepod	Nitokra sp	1.32-1.64	96	7.46-8.54	17-23	(Sousa, Zaroni et al. 2011)
Copepod	Nitokra sp	2.27-3.66	96	7.79-8.55	28-29	(Sousa, Zaroni et al. 2011)
Copepod	Nitokra sp	1.29-2.71	96	7.86-8.3	23	(Sousa, Zaroni et al. 2011)
Copepod	Nitocra spinipes	4.5	96	7.84	10	(Linden, Bengtsson et al. 1979)
Copepod	Bryocamptus zschokkei	0.287	96	7.84	10	(Marzio, Castaldo et al. 2008)
Copepod	Bryocamptus minutus	0.281	96	7.84	10	(Marzio, Castaldo et al. 2008)
Copepod	Bryocamptus pygmaeus	0.281	96	7.84	10	(Marzio, Castaldo et al. 2008)
Copepod	Attheyella crassa	0.274	96	7.84	10	(Marzio, Castaldo et al. 2008)
Copepod	Bryocamptus echinatus	0.225	96	7.84	10	(Marzio, Castaldo et al. 2008)
Copepod	Acartia tonsa Dana (larva)	0.22	72	8	17	(Jepsen, Andersen et al. 2013)
Copepod	Acartia tonsa Dana (adult)	0.77	72	8	17	(Jepsen, Andersen et al. 2013)
Copepod	Acartia clausi	0.91	24			(Buttino 1994)
Copepod	Acartia hudsonica	0.18-0.26	48			(Sullivan and Ritacco 1985)
Copepod	Acartia tonsa	0.18-0.224	48			(Sullivan and Ritacco 1985)
						(Venkataramiah, Lakshmi et al. 1982)
Copepod	Eucalanus spp.	0.65-0.92	96			
Cladocera	Ceriodaphnia dubia	1.73	24	8	25	(Andersen and Buckley 1998)
Cladocera	Ceriodaphnia dubia	1.18	48	8	25	(Andersen and Buckley 1998)
Cladocera	Simocephalus vetulus	1.27	96	8.1-8.3	20.4	(Arthur, West et al. 1987)
Cladocera	Duphnia magna	2.94	48	8.4	20.5	(Gersich and Hopkins 1986)
Cladocera	Ceriodaphnia acanthina	0.6	96			(Gersich and Hopkins 1986)
Cladocera	Dupknia pulicuria	1	96			(Gersich and Hopkins 1986)

Cladocera	<i>Simocephalus vetulus</i>	0.5	96			(Gersich and Hopkins 1986)
Cladocera	<i>Ceriodaphnia dubia</i>	1.22	48		25	(Bailey, Elphick et al. 2001)
Cladocera	<i>Ceriodaphnia dubia</i>	1.01	48		25	(Bailey, Elphick et al. 2001)
Cladocera	<i>Ceriodaphnia dubia</i>	1.54	24		25	(Bailey, Elphick et al. 2001)
Cladocera	<i>Ceriodaphnia dubia</i>	1.36	24		25	(Bailey, Elphick et al. 2001)
Cladocera	<i>Moina mongolica</i>	7.52	48	8.48	26	(An 1996)
Cladocera	<i>Moina mongolica</i>	9.89	24	8.48	26	(An 1996)
Cladocera	<i>Moina mongolica</i>	4.17	growth	8.48	26	(An 1996)
Cladocera	<i>Moina mongolica</i>	2.63	reproduction	8.48	26	(An 1996)
Rotifer	<i>Brachionus plicatilis</i>	17	24	7.3-7.8	23	(Yu and Hirayama 2008)
Rotifer	<i>Brachionus plicatilis</i>	13.2	growth	7.3-7.9	24	(Yu and Hirayama 2008)
Rotifer	<i>Brachionus plicatilis</i>	7.8	reproduction	7.3-7.10	25	(Yu and Hirayama 2008)
Rotifer	<i>Brachionus plicatilis</i>	15-25.6	24	7.7	25	(Snell and Persoone 1989)
Rotifer	<i>Brachionus plicatilis</i>	17-18.4	24	7.7	26	(Snell and Persoone 1989)
Rotifer	<i>Brachionus rubens</i>	>5	48			(Schlüter and Groeneweg 1985)
Rotifer	<i>Brachionus rubens</i>	17	24			(Lincoln, Hall et al. 1983)
Rotifer	<i>Brachionus rotundiformis</i>	16.6-22.6	population	7.0-8.0	24-32	(Yoshimura, Iwata et al. 1995)
Rotifer	<i>Brachionus calyciflorus</i>	12	population	7.0-8.1	24-32	(Park, Lee et al. 2001)
Rotifer	<i>Brachionus rotundiformis</i> (Hawaii strain)	3.1	LOEC		24	(de Araujo, Hagiwara et al. 2001)
Rotifer	<i>Brachionus rotundiformis</i> (Langkawi strain)	7.4	LOEC		24	(de Araujo, Hagiwara et al. 2001)
Rotifer	<i>Brachionus plicatilis</i>	>9.8	24	7.3-7.8	24	(de Araujo, Hagiwara et al. 2001)
Rotifer	<i>Brachionus plicatilis</i>	4.9	LOEC	7.3-7.9	24	(de Araujo, Hagiwara et al. 2001)
Rotifer	<i>Brachionus calyciflorus</i>	14.4	96		24	(Moor 1984)

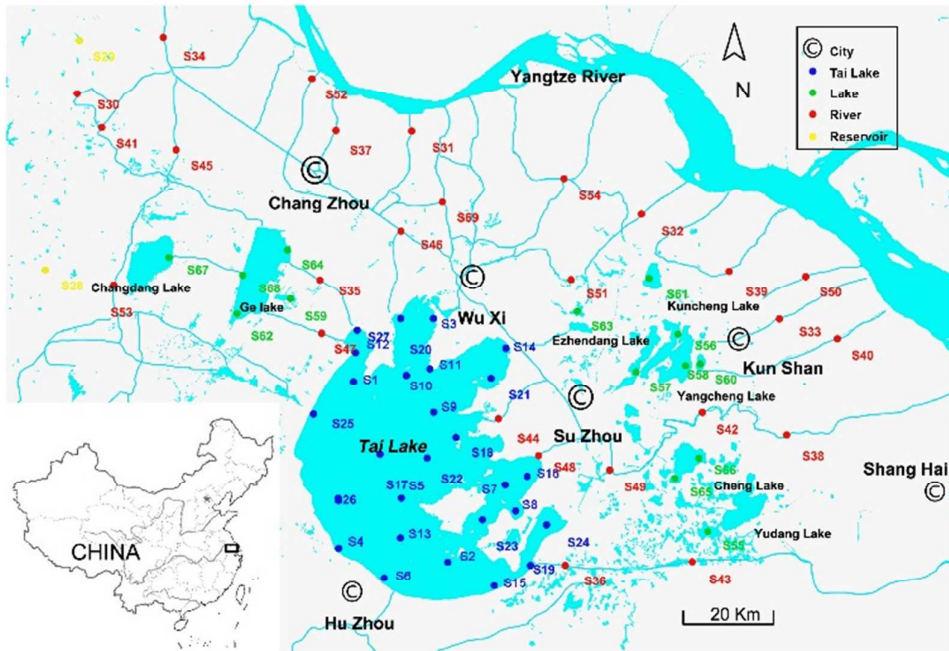


Fig.S1. Location of sampling sites of the Tai Lake basin of Jiangsu province, China. Sampling sites were grouped into four categories according to the type of water body: 1) Tai Lake, 2) Reservoir, 3) River and 4) Lake. Here, “Lake” means all the relatively smaller lakes around Tai Lake.

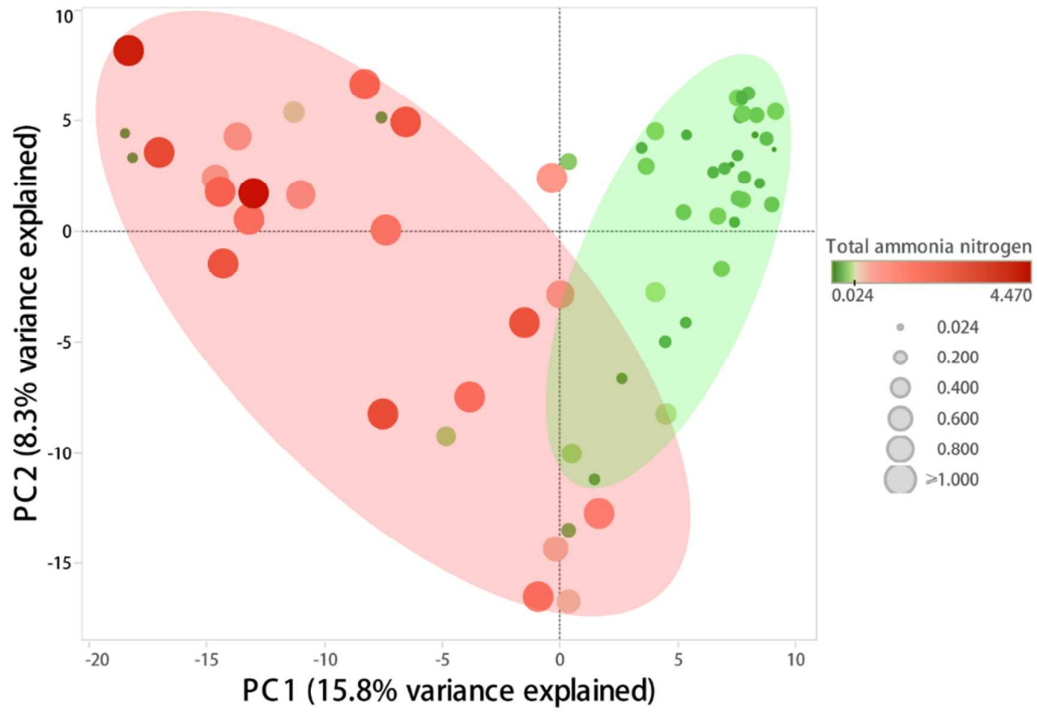
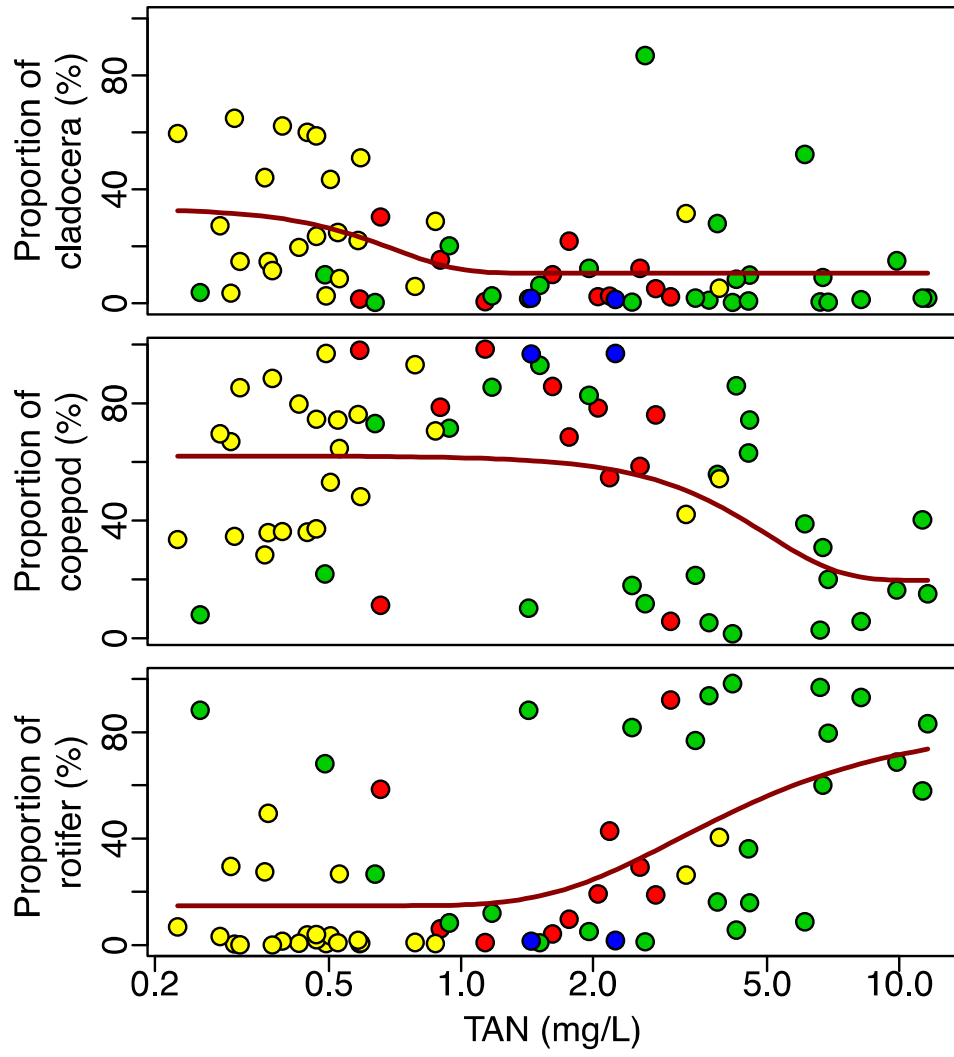


Fig.S2. Principal component analysis (PCA) of zooplankton community components based on CO1 OTUs. The samples were clustered according to the concentration of total ammonia. These high ammonia content (TAN > 0.5 mg/L) samples were shown in red.



rotifer.

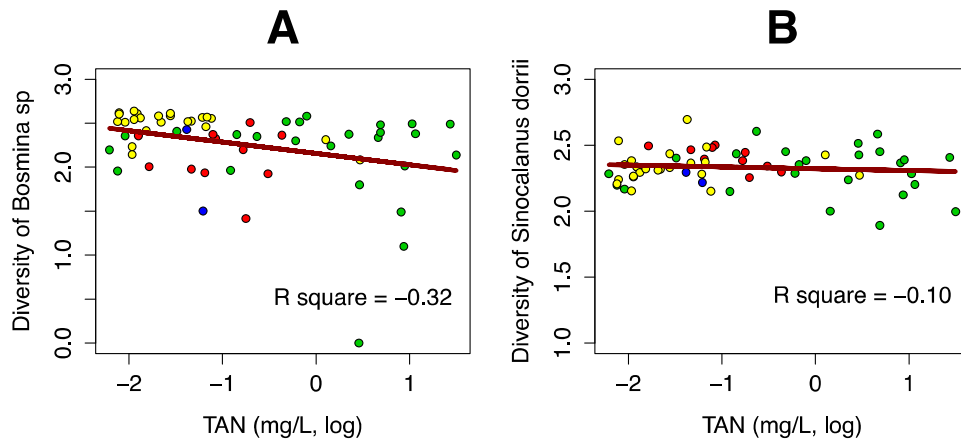


Fig.S4. Relationships between diversity of two zooplankton species and total ammonia nitrogen. (A), *Bosmina* sp. (B), *Sinocalanus dorrii*. Colors designate types of samples; yellow: Tai Lake samples, blue: reservoir samples, green: river sample and red: lake samples.

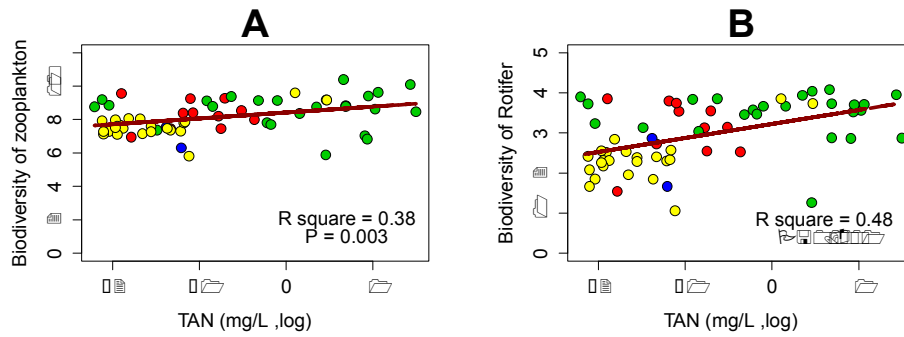
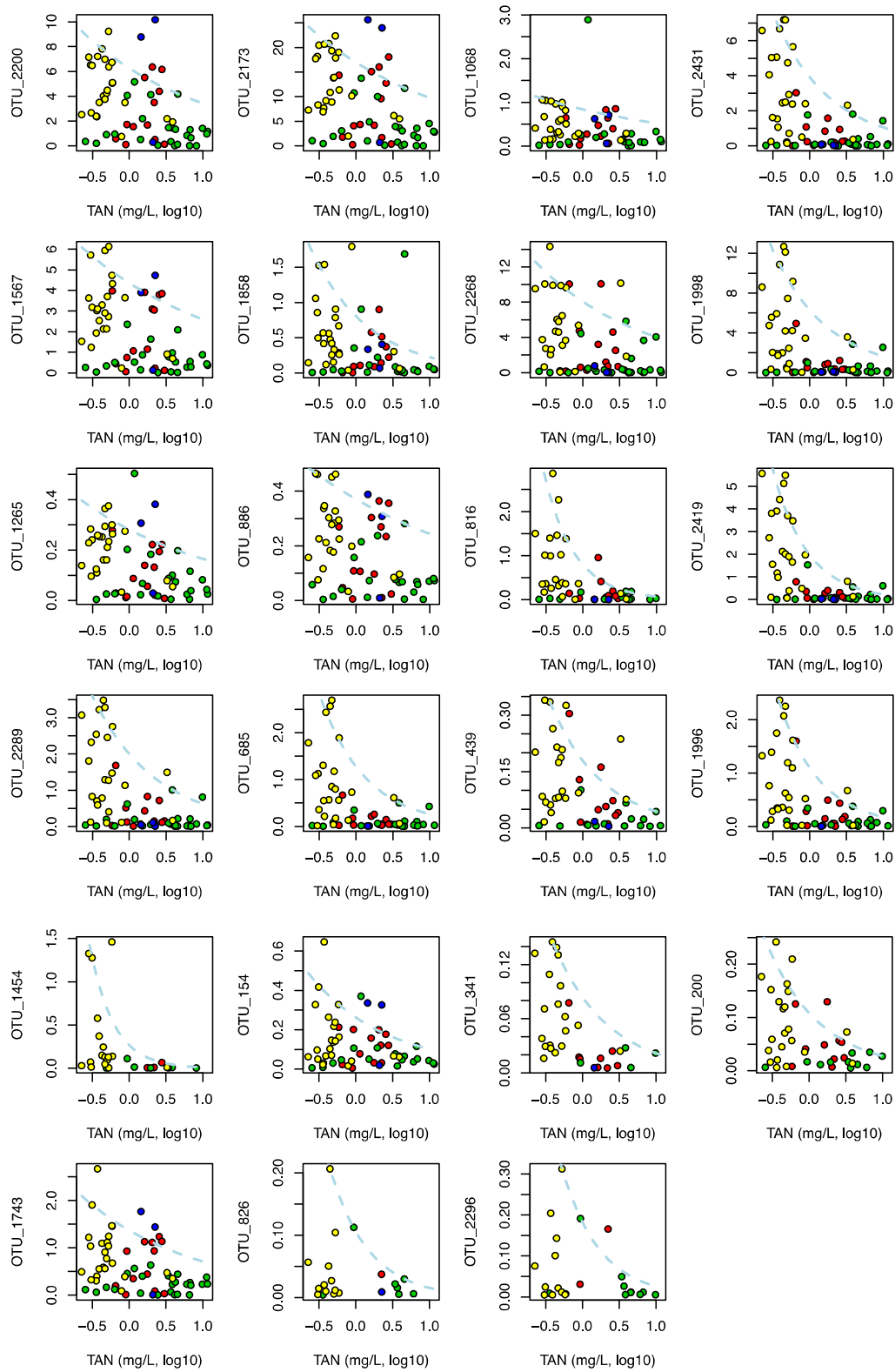


Fig.S5. Relationships between diversity of zooplankton and total ammonia nitrogen. (A), diversity of zooplankton community (including copepod, cladocera and rotifer). (B), the diversity of rotifer community. Colors designate types of samples; yellow: Tai Lake samples, blue: reservoir samples, green: river sample and red: lake samples.



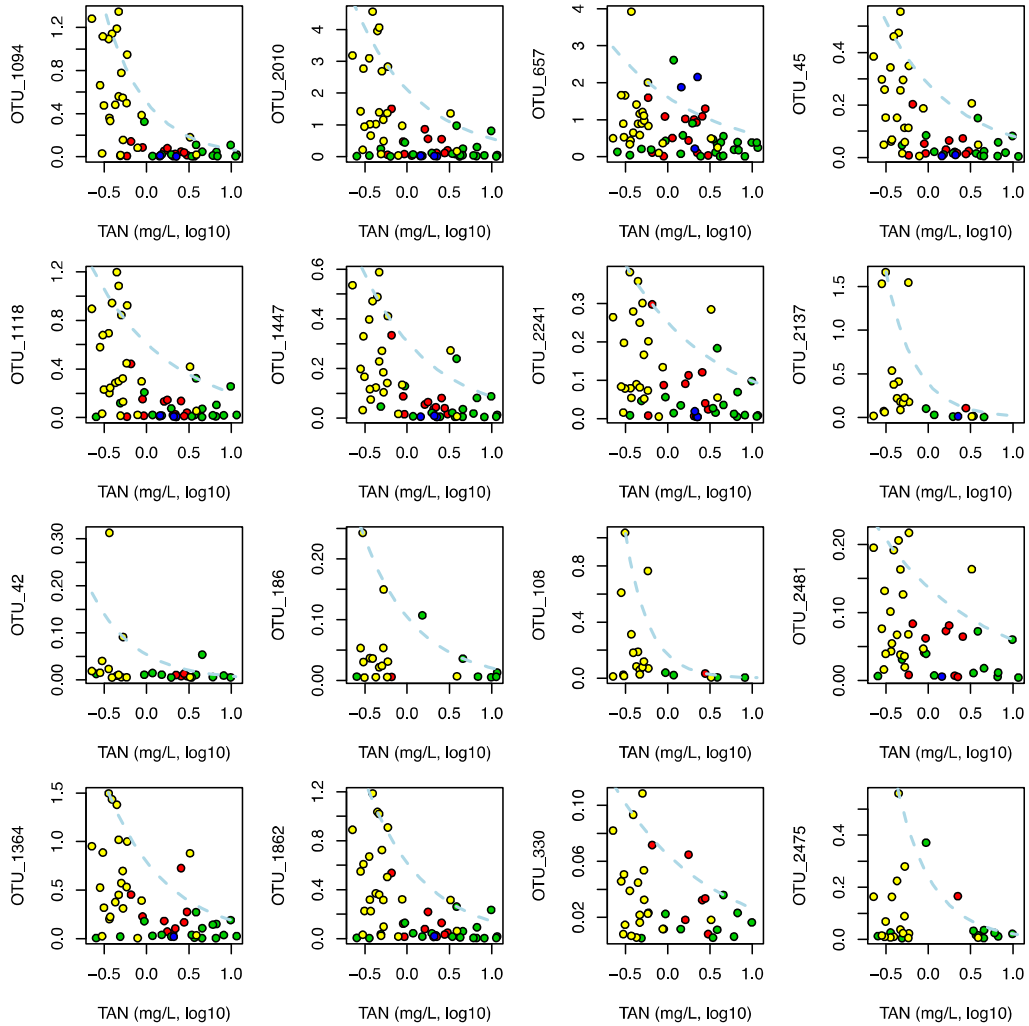


Fig.S6. Ammonia sensitive OTUs in metabarcoding data. Sensitive OTUs were screened out by the quantile regression with the $p < 0.1$. The x-axis is concentration of total ammonia nitrogen, and the y-axis is relative abundance of each OTUs. Colors designate types of samples; yellow: Tai Lake samples, blue: reservoir samples, green: river sample and red: lake samples.

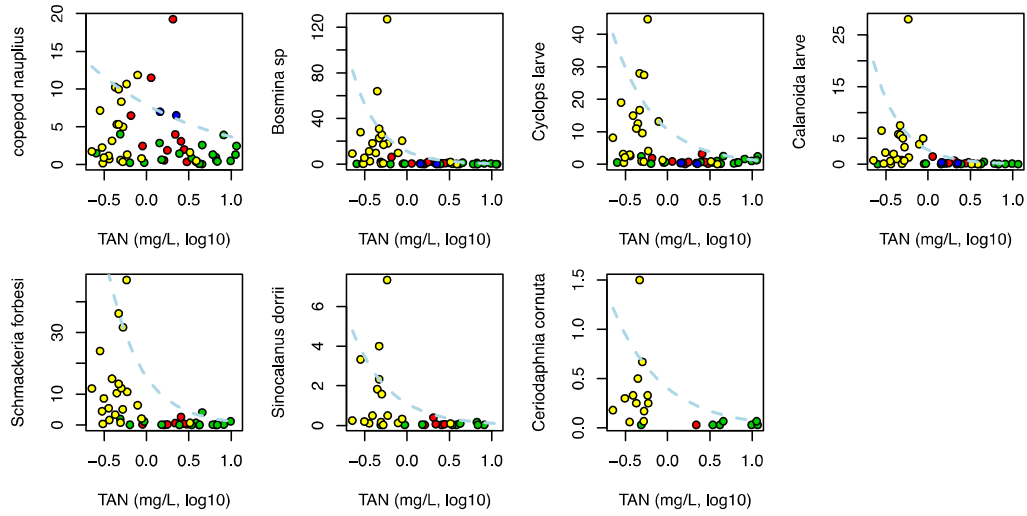


Fig.S7. Ammonia sensitive species/taxon in traditional morphological monitoring data. Sensitive species were screened out by the quantile regression with the $p < 0.1$. The x-axis is concentration of total ammonia nitrogen, and the y-axis is density of each species or taxon. Colors designate types of samples; yellow: Tai Lake samples, blue: reservoir samples, green: river sample and red: lake samples.

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