Ecogenomics of Zooplankton Community Reveals Ecological Threshold of Ammonia Nitrogen

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† 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 (NO₃⁻) 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-throughput 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. 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. 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. 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. 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 (NH₃) is very toxic to most aquatic organisms, including zooplankton and fish. 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.

Ammonia causes both acute and chronic effects on zooplankton at both the individual and community levels. 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 labor-intensive 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: 太湖) is one of the most densely populated and developed areas in China. During the past 20 years, excessive nutrient loading by rapid industrialization and urbanization has caused rapid deterioration of water quality. 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. 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 (PO₄³⁻), 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.

**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.

**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.

\[
\log(TAN_{F=20}) = \log(TAN_T) - 0.036(20 - T) \\
TAN_{pH=7} = \frac{0.0489 \times 10^{-0.0489 \times pH} + 6.95 \times 10^{-6.95 \times pH}}{1 + 10^{3.204-pH}}
\]

**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. 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+digsq-ibinlinux4) before PCA analysis. Relative contributions of environmental factors to explain variance of zooplankton communities were determined using forward selection, distance-based, linear modeling (distLM) in PERMANOVA. To identify sensitive OTUs, structures of taxonomic communities were related to each environmental factor by use of quantile regression (p < 0.1) 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, PO₄³⁻ 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 concentrations of nutrients (Figure 1).

![Figure 1](image1.png)

**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 environmental 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, NO₃⁻, and WT had a significant effect on structures of communities of cladocera. Nitrate, NO₂⁻, TN, PO₄³⁻, and TAN had a significant effect on structures of copepod communities (Figure 1).

![Figure 2](image2.png)

**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
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 quadrala*). *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 copepod or cladocera (Figure 4G, H). Laboratory-based toxicity tests showed that copepod 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 (*Environ. Sci. Technol.* 2017, 51, 3057–3064).

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

<table>
<thead>
<tr>
<th>predictor variable</th>
<th>marginal tests</th>
<th>forward selection sequential tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pseudo-F</td>
<td>variation explained</td>
</tr>
<tr>
<td>+TAN</td>
<td>11.5508</td>
<td>14.955</td>
</tr>
<tr>
<td>+algal.density</td>
<td>7.1576</td>
<td>9.308</td>
</tr>
<tr>
<td>+WT</td>
<td>8.5149</td>
<td>11.131</td>
</tr>
<tr>
<td>+Chl.a</td>
<td>3.4607</td>
<td>3.94</td>
</tr>
<tr>
<td>+PO4</td>
<td>10.2423</td>
<td>13.384</td>
</tr>
<tr>
<td>+DO</td>
<td>3.049</td>
<td>3.302</td>
</tr>
<tr>
<td>+NO3</td>
<td>6.9639</td>
<td>9.041</td>
</tr>
<tr>
<td>+TN</td>
<td>10.8596</td>
<td>14.113</td>
</tr>
<tr>
<td>+NO2</td>
<td>8.4262</td>
<td>11.014</td>
</tr>
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<tr>
<td>+CODMn</td>
<td>2.5555</td>
<td>2.527</td>
</tr>
<tr>
<td>+BOD5</td>
<td>2.6869</td>
<td>2.735</td>
</tr>
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"Bold 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).
and HC10 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 NH3 than were cladocera and copepoda.

**DISCUSSION**

Nutrients, especially TN, NO3− and NO2−, 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. After decades of intensive input of nutrients from industrial and agricultural sources, most surface...
waters in the Tai Lake basin have become hypereutrophic, which has resulted in numerous large algae blooms in recent years. Eutrophication is still one of the most serious environmental problems confronted by local environmental managers. The results reported here demonstrated that TAN and nutrient factors (TN, NO₃-N, NO₂-N, 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. 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 NH₄ than some cladocerans, such as Moina micrura and the copepod Acartia tonsa. Tolerance of rotifers allows them to survive relatively great concentrations of NH₄. In the present study, abundances of copepods and cladocera 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 NH₄ 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 copepods 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. 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. 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. Laboratory toxicity tests are also severely limited by the few surrogate species available for testing. 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. 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. 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. 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. 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. ECSOs 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.
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. Most species used to develop current a criterion for NH4 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 and fishes 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. 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, insects, and fish. It might even be possible to monitor for effects on terrestrial animals or aquatic birds. 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.

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**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 CO1 OTUs.

*Fig.S3.* Non-linear regression between TAN and relative abundance of clodocera, 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 (PO$_4^{3-}$), total nitrogen (TN, all forms of nitrogen), nitrate (NO$_3^-$), nitrite (NO$_2^-$), 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).

\[
\text{TLI}(\sum) = \frac{\sum TLI(j)}{n}
\]

(1)

\[
\text{TLI}_{\text{Chl-a}} = 10 \left(2.46 + 1.091 \ln(\text{Chl-a})\right)
\]

(2)

\[
\text{TLI}_{\text{TP}} = 10 \left(7.109 + 0.946 \ln(\text{TP})\right)
\]

(3)

\[
\text{TLI}_{\text{TN}} = 10 \left(4.934 + 1.310 \ln(\text{TN})\right)
\]

(4)

\[
\text{TLI}_{\text{SD}} = 10 \left(4.311 - 2.120 \ln(\text{SD})\right)
\]

(5)

**Data accessibility:** DNA sequences by NGS were uploaded to NCBI Sequence Read Archieve (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.

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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; PO₄³⁻: phosphate, mg/L; TN: total nitrogen, mg/L; NO₃: nitrate, mg/L; NO₂: nitrite, mg/L; TAN: total ammonia nitrogen, mg/L.

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

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<th>Taxa</th>
<th>Species</th>
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<th>Duration (hour)</th>
<th>PH</th>
<th>Temperature</th>
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<td>0.35-0.88</td>
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<td>7.61-8.54</td>
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<td>(Sousa, Zaroni et al. 2011)</td>
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<td>7.79-8.55</td>
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<td>8</td>
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<td>(Jepsen, Andersen et al. 2013)</td>
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<td>72</td>
<td>8</td>
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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.
Fig. S3. Non-linear regression between TAN and relative abundance of cladocera, copepod and 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.
References:


OF AMMONIA TO DAPHNIA-MAGNA STRAUS." Environmental Toxicology and Chemistry 5(5): 443-447.


