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 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^+), 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).

$$TLI(\Sigma) = {}_{j=1}^{m} WjTLI(j) \quad (1)$$

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

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

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

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

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

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/m^3; 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; PO_4^+: phosphate, mg/L; TN: total nitrogen, mg/L; NO_3: nitrate, mg/L; NO_2: 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)
Copepod	Eucalanus spp.	0.65-0.92	96			(Venkataramiah, Lakshmi et al. 1982)
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	Simocephalus vetulus	0.5	96			(Gersich and Hopkins 1986)
Cladocera	Ceriodaphnia dubia	1.22	48		25	(Bailey, Elphick et al. 2001)
Cladocera	Ceriodaphnia dubia	1.01	48		25	(Bailey, Elphick et al. 2001)
Cladocera	Ceriodaphnia dubia	1.54	24		25	(Bailey, Elphick et al. 2001)
Cladocera	Ceriodaphnia dubia	1.36	24		25	(Bailey, Elphick et al. 2001)
Cladocera	Moina mongolica	7.52	48	8.48	26	(An 1996)
Cladocera	Moina mongolica	9.89	24	8.48	26	(An 1996)
Cladocera	Moina mongolica	4.17	growth	8.48	26	(An 1996)
Cladocera	Moina mongolica	2.63	reproduction	8.48	26	(An 1996)
Rotifer	Brachionus plicatilis	17	24	7.3-7.8	23	(Yu and Hirayama 2008)
Rotifer	Brachionus plicatilis	13.2	growth	7.3-7.9	24	(Yu and Hirayama 2008)
Rotifer	Brachionus plicatilis	7.8	reproduction	7.3-7.10	25	(Yu and Hirayama 2008)
Rotifer	Brachionus plicatilis	15-25.6	24	7.7	25	(Snell and Persoone 1989)
Rotifer	Brachionus plicatilis	17-18.4	24	7.7	26	(Snell and Persoone 1989)
Rotifer	Brachionus rubens	>5	48			(Schlüter and Groeneweg 1985)
Rotifer	Brachionus rubens	17	24			(Lincoln, Hall et al. 1983)
Rotifer	Brachionus rotundiformis	16.6-22.6	population	7.0-8.0	24-32	(Yoshimura, Iwata et al. 1995)
Rotifer	Brachionus calyciflorus	12	population	7.0-8.1	24-32	(Park, Lee et al. 2001)
Rotifer	Brachionus rotundiformis (Hawaii strain)	3.1	LOEC		24	(de Araujo, Hagiwara et al. 2001)
Rotifer	Brachionus rotundiformis (Langkawi strain)	7.4	LOEC		24	(de Araujo, Hagiwara et al. 2001)
Rotifer	Brachionus plicatilis	>9.8	24	7.3-7.8	24	(de Araujo, Hagiwara et al. 2001)
Rotifer	Brachionus plicatilis	4.9	LOEC	7.3-7.9	24	(de Araujo, Hagiwara et al. 2001)
Rotifer	Brachionus calyciflorus	14.4	96		24	(Moor 1984)

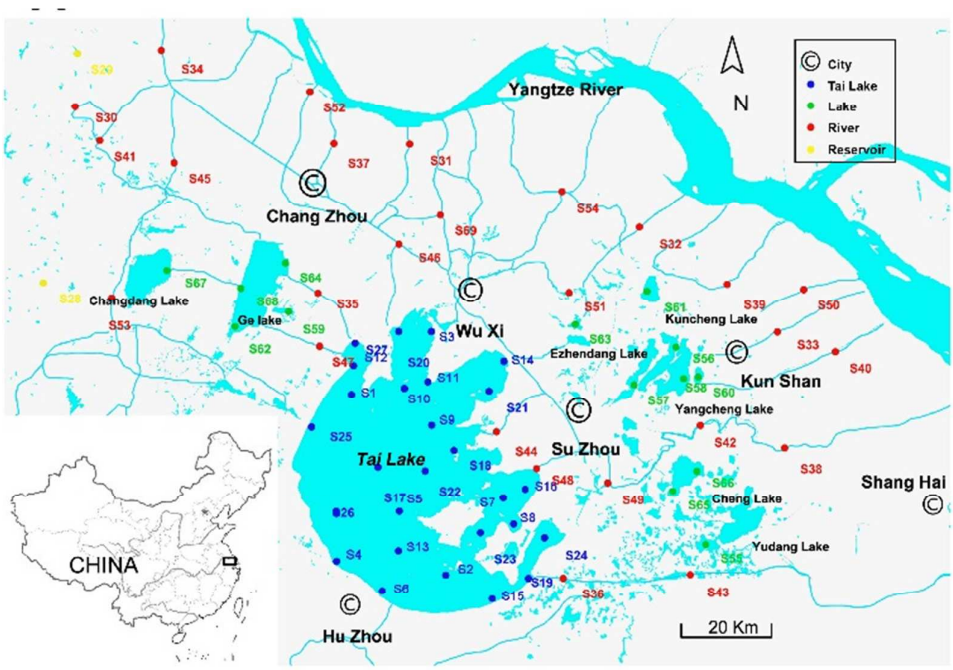


Fig.S1. Location of sampling sites of the Tai Lake basin of Jiangsu province, China. Sampling sites were grouped into four categories according to the type of water body: 1) Tai Lake, 2) Reservoir, 3) River and 4) Lake. Here, "Lake" means all the relatively smaller lakes around Tai Lake.

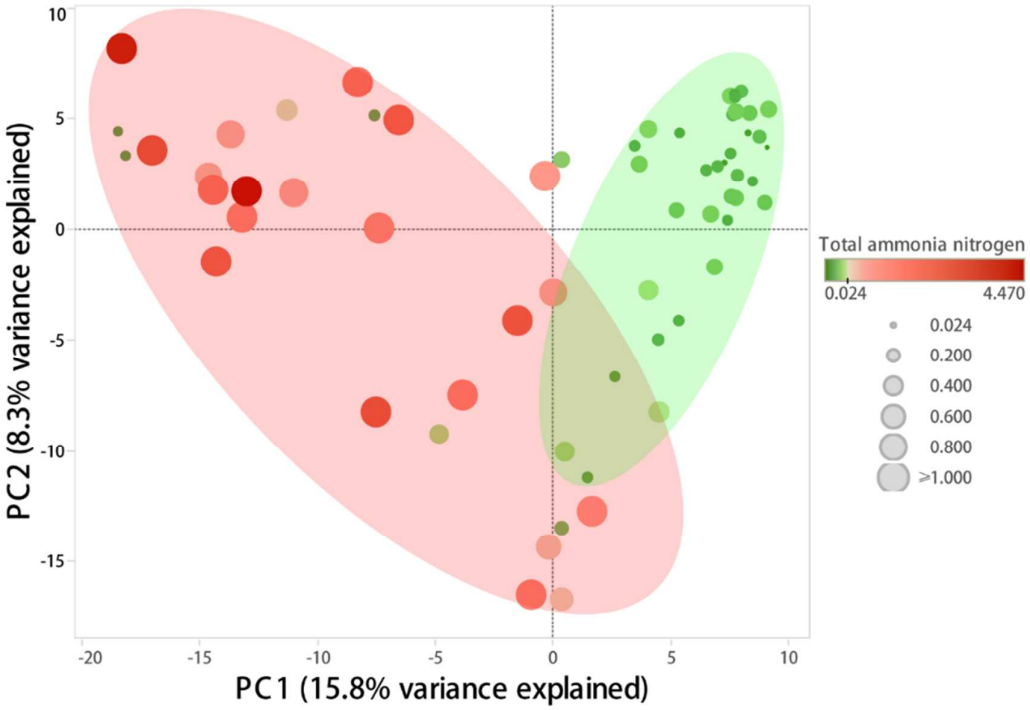
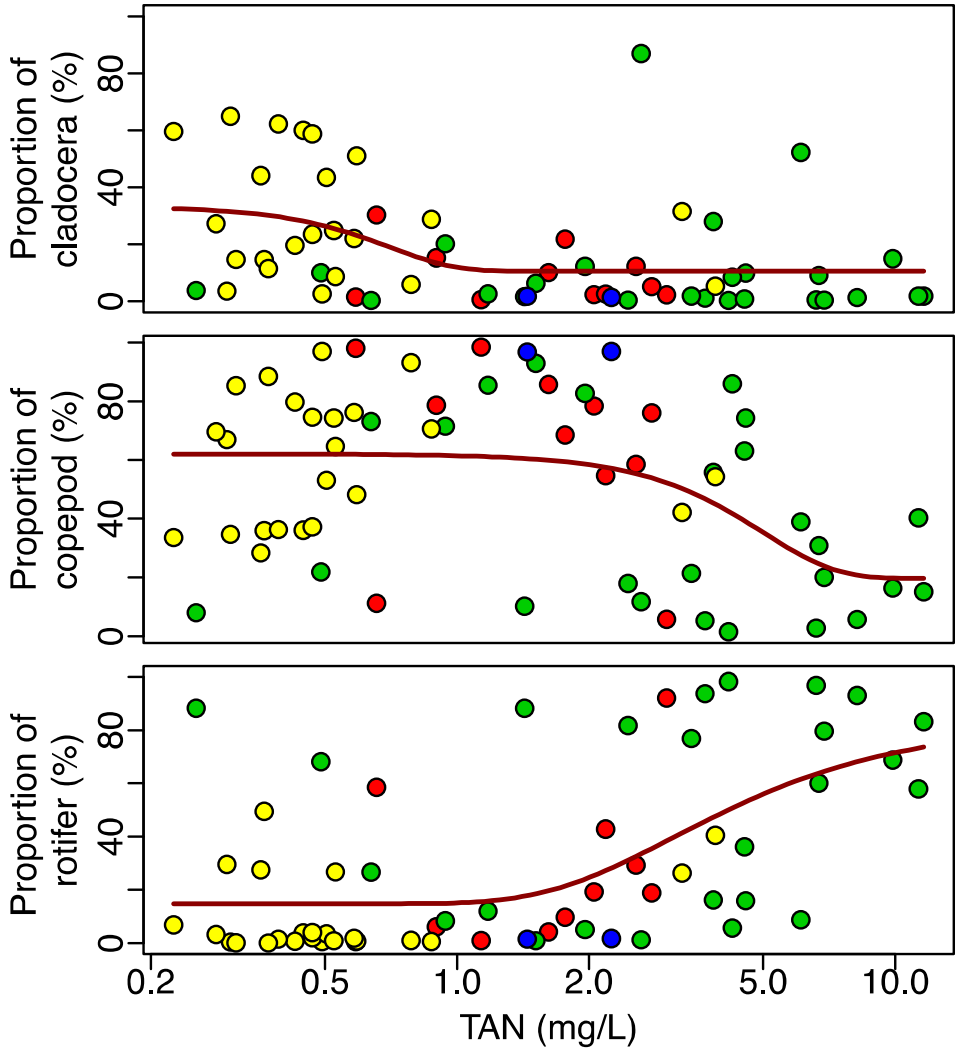


Fig.S2. Principal component analysis (PCA) of zooplankton community components based on CO1 OTUs. The samples were clustered according to the concentration of total ammonia. These high ammonia content (TAN > 0.5 mg/L) samples were shown in red.


Proportion of cladocera (%)
Proportion of copepod (%)
Proportion of rotifer (%)
0
40
80
0.2
0.5
1.0
2.0
5.0
10.0
TAN (mg/L)

rotifer.

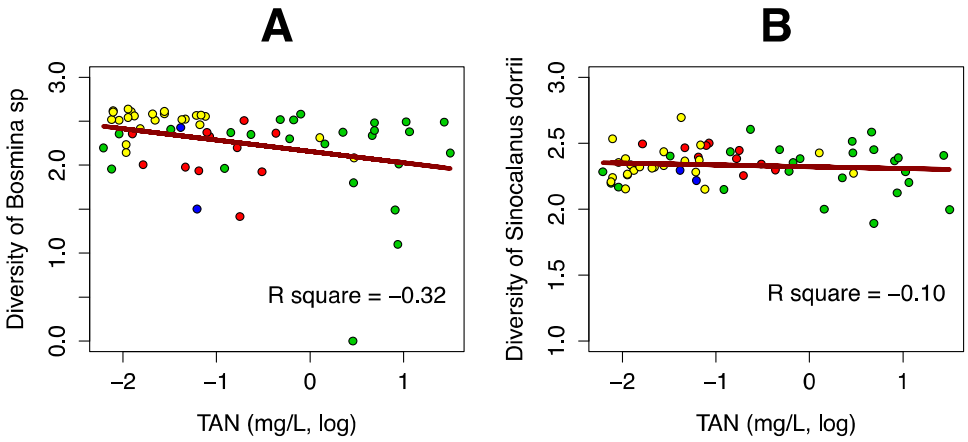


Fig.S4. Relationships between diversity of two zooplankton species and total ammonia nitrogen. (A), Bosmina sp. (B), Sinocalanus dorrii. Colors designate types of samples; yellow: Tai Lake samples, blue: reservoir samples, green: river sample and red: lake samples.

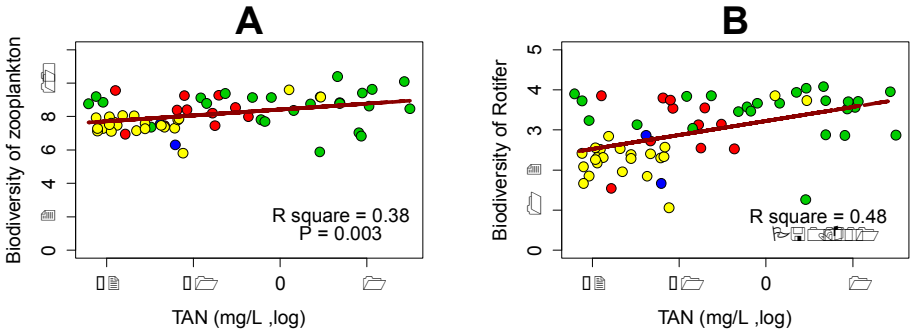


Fig.S5. Relationships between diversity of zooplankton and total ammonia nitrogen. (A), diversity of zooplankton community (including copepod, cladocera and rotifer). (B), the diversity of rotifer community. Colors designate types of samples; yellow: Tai Lake samples, blue: reservoir samples, green: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

OTU_2200
OTU_2173
OTU_1068
OTU_2431
OTU_1567
OTU_1858
OTU_2268
OTU_1998
OTU_1265
OTU_886
OTU_816
OTU_2419
OTU_2289
OTU_685
OTU_439
OTU_1996
OTU_1454
OTU_154
OTU_341
OTU_200
OTU_1743
OTU_826
OTU_2296
TAN (mg/L, log10)

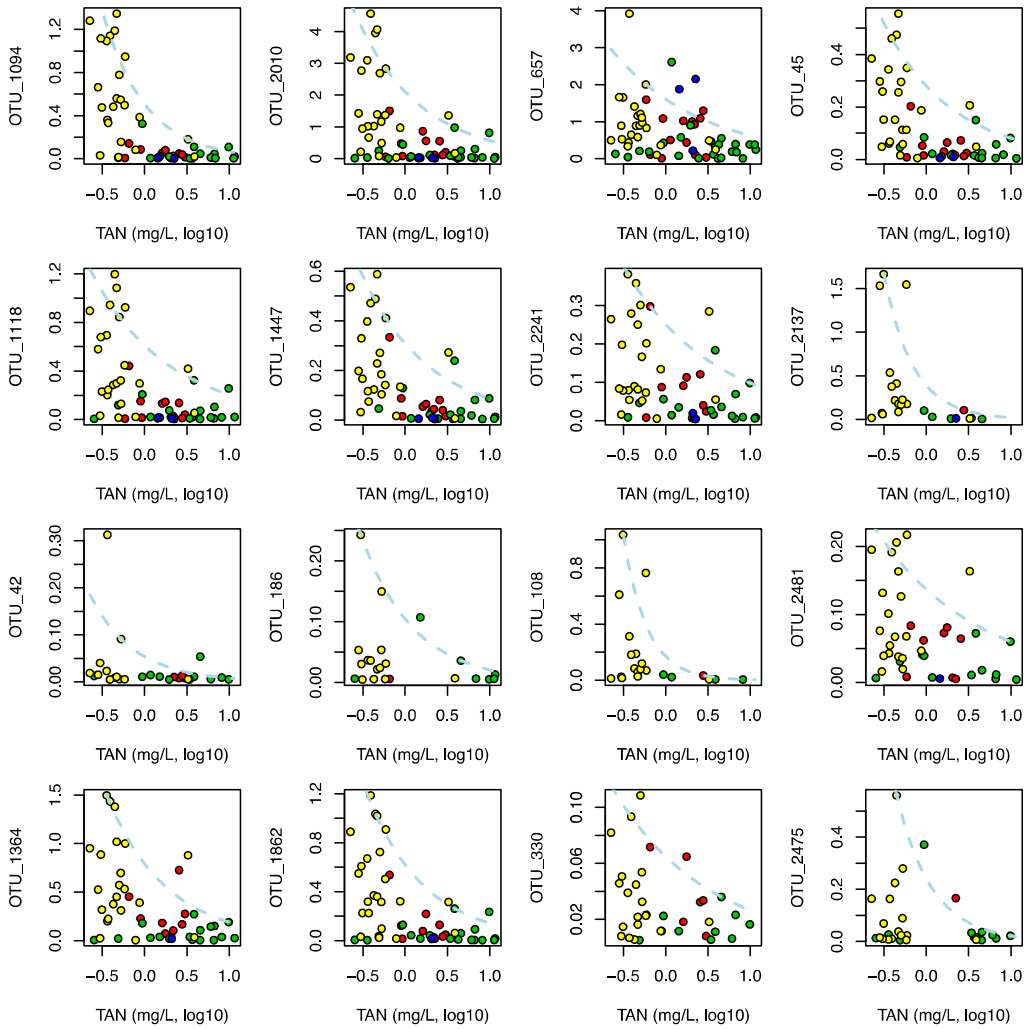


Fig.S6. Ammonia sensitive OTUs in metabarcoding data. Sensitive OTUs were screened out by the quantile regression with the $p < 0.1$. The x-axis is concentration of total ammonia nitrogen, and the y-axis is relative abundance of each OTUs. Colors designate types of samples; yellow: Tai Lake samples,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

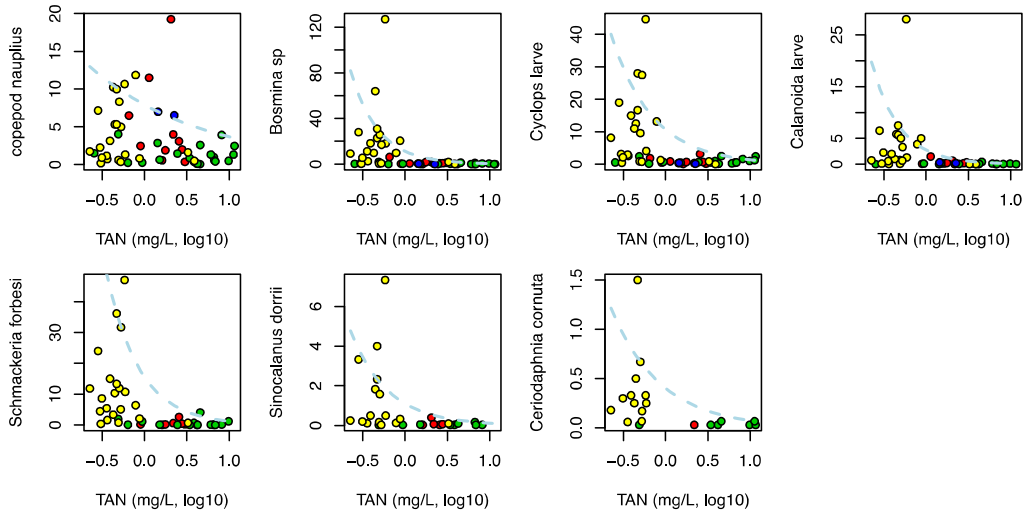


Fig.S7. Ammonia sensitive species/taxon in traditional morphological monitoring data. Sensitive species were screened out by the quantile regression with the $p < 0.1$. The x-axis is concentration of total ammonia nitrogen, and the y-axis is density of each species or taxon. Colors designate types of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

References:

An, Y. (1996). "Toxicity of Ammonia to Moina mongolica Daday." Journal of Dalian Fisheries University.

Andersen, H. B. and J. A. Buckley (1998). "Acute toxicity of ammonia to Ceriodaphnia dubia and a procedure to improve control survival." Bulletin of Environmental Contamination and Toxicology **61**(1): 116-122.

Arthur, J. W., C. W. West, K. N. Allen and S. F. Hedtke (1987). "SEASONAL TOXICITY OF AMMONIA TO 5 FISH AND 9 INVERTEBRATE SPECIES." Bulletin of Environmental Contamination and Toxicology **38**(2): 324-331.

Bailey, H. C., J. R. Elphick, R. Krassoi and A. Lovell (2001). "Joint acute toxicity of diazinon and ammonia to Ceriodaphnia dubia." Environmental Toxicology and Chemistry **20**(12): 2877-2882.

Buttino, I. (1994). "The effect of low concentrations of phenol and ammonia on egg production rates, fecal pellet production and egg viability of the calanoid copepod Acartia clausi." Marine Biology **119**(4): 629-634.

Carlson and E. Robert (1977). "A trophic state index for lakes." Limnology & Oceanography **22**(2): 361-369.

de Araujo, A. B., A. Hagiwara and T. W. Snell (2001). "Effect of unionized ammonia, viscosity and protozoan contamination on reproduction and enzyme activity of the rotifer Brachionus rotundiformis." Hydrobiologia **446**: 363-368.

EPA of china (2002). Water and wastewater monitoring and analysis method. 4th Edition. Beijing, China Environmental Science Press.

Gersich, F. M. and D. L. Hopkins (1986). "SITE-SPECIFIC ACUTE AND CHRONIC TOXICITY

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

Jepsen, P. M., C. V. B. Andersen, J. Schjelde and B. W. Hansen (2013). "Tolerance of un-ionized ammonia in live feed cultures of the calanoid copepod Acartia tonsa Dana." Aquaculture Research **46**(2): 420–431.

Lincoln, E. P., T. W. Hall and B. Koopman (1983). "Zooplankton control in mass algal cultures." Aquaculture **32**(s 3–4): 331-337.

Linden, E., B. E. Bengtsson, O. Svanberg, G. Sundström and B. National Swedish Environment Protection (1979). "The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes." Chemosphere **8**(11): 843-851.

Marzio, W. D. D., D. Castaldo, C. Pantani, A. D. Cioccio, T. D. Lorenzo, M. E. Sáenz and D. M. P. Galassi (2008). "Relative Sensitivity of Hyporheic Copepods to Chemicals." Bulletin of Environmental Contamination & Toxicology **82**(4): 488-491.

Moor, I. J. d. (1984). "TOXIC CONCENTRATION OF FREE AMMONIA TO BRACHIONUS CALYCIFLORUS PALLAS, A ROTIFER PEST SPECIES FOUND IN HIGH RATE ALGAL PONDS (HRAP's)." Journal of the Limnological Society of Southern Africa **10**(2): 33-36.

Park, H. G., K. W. Lee, S. H. Cho, H. S. Kim, M. M. Jung and H. S. Kim (2001). "High density culture of the freshwater rotifer (B. calyciflorus P.)." Hydrobiologia **446-447**(1): 369-374.

Schlüter, M. and J. Groeneweg (1985). "The inhibition by ammonia of population growth of the rotifer, Brachionus rubens , in continuous culture." Aquaculture **46**(3): 215-220.

Snell, T. W. and G. Persoone (1989). "Acute toxicity bioassays using rotifers. I. A test for

brackish and marine environments with Brachionus plicatilis." Aquatic Toxicology **14**(1): 65-80.

Sousa, E. C. P. M. D., L. P. Zaroni, T. U. B. Filho, L. A. Marconato, A. A. Kirschbaum and M. R. Gasparro (2011). "Acute sensitivity to Nitokra sp benthic copepod to potassium dichromate and ammonia chloride." Ecotoxicology & Environmental Contamination **7**(1).

Sullivan, B. K. and P. J. Ritacco (1985). "Ammonia toxicity to larval copepods in eutrophic marine ecosystems: A comparison of results from bioassays and enclosed experimental ecosystems." Aquatic Toxicology **7**(3): 205-217.

Venkataramiah, A., G. J. Lakshmi, C. Best, G. Gunter, E. Hartwig and P. Wilde (1982). "STUDIES ON TOXICITY OF OTEC PLANT-COMPONENTS ON EUCALANUS SP FROM THE GULF OF MEXICO." Ocean Science and Engineering **7**(3): 353-401.

Yoshimura, K., T. Iwata, K. Tanaka, C. Kitajima and F. Isizaki (1995). "A High Density Cultivation of Rotifer in an Acidified Medium for Reducing Undissociated Ammonia." Nihon-suisan-gakkai-shi **61**(4): 602-607.

Yu, J. P. and K. Hirayama (2008). "The effect of un-ionized ammonia on the population growth of the rotifer in mass culture." NIPPON SUISAN GAKKAISHI **52**(9): 1509-1513.