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Author(s): Zou, Wei; Cai, Yongjiu; Tolonen, Kimmo; Zhu, Guangwei; Qin, Boqiang; Peng, Kai; Gong, Zhijun

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Year: 2019

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Please cite the original version:

Zou, W., Cai, Y., Tolonen, K., Zhu, G., Qin, B., Peng, K., & Gong, Z. (2019). The adaptations to tube-dwelling life of Propsilocerus akamusi (Diptera: Chironomidae) larvae and its eutrophication-tolerant mechanisms. Limnologica, 77, Article 125684. https://doi.org/10.1016/j.limno.2019.125684

Accepted Manuscript

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PII: S0075-9511(18)30118-X
DOI: https://doi.org/10.1016/j.limno.2019.125684
Article Number: 125684

Reference: LIMNO 125684

To appear in:

Received date: 5 July 2018
Revised date: 7 May 2019
Accepted date: 8 May 2019

Please cite this article as: Zou W, Cai Y, Tolonen KT, Zhu G, Qin B, Peng K, Gong Z, The adaptations to tube-dwelling life of *Propsilocerus akamusi* (Diptera: Chironomidae) larvae and its eutrophication-tolerant mechanisms, *Limnologica* (2019), https://doi.org/10.1016/j.limno.2019.125684

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The adaptations to tube-dwelling life of *Propsilocerus akamusi* (Diptera: Chironomidae) larvae and its eutrophication-tolerant mechanisms

Wei Zou[1, 2], Yongjiu Cai[1], Kimmo T. Tolonen[3], Guangwei Zhu[1], Boqiang Qin[1], Kai Peng[1, 2], Zhijun Gong[1*]

[1]*State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing* 210008, *P. R. China*

[2]*University of Chinese Academy of Sciences, Beijing 100049, P. R. China*

[3]*Department of Biological and Environmental Sciences, University of Jyväskylä, P.O. Box 35, FI-40014 Jyväskylä, Finland*

[*]**Corresponding author**: Zhijun Gong, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, *P. R.* China. , Tel.: +86 25 86882184; fax: +86 25 57714759. E-mail address: zjgong@niglas.ac.cn

Abstract

Propsilocerus akamusi (Diptera: Chironomidae) is a dominant

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macroinvertebrate species in many eutrophic lakes in subtropical and temperate zones. *P. akamusi* larvae can migrate deep into the sediment (>30 cm) during summer where is no oxygen. However, to our knowledge, the specific adaptive tactics of its tube-dwelling life (>30 cm) and underlying mechanisms why this species is favored by nutrient-rich lakes remain limited. With the understanding above-mentioned issues, we can provide important information for the development of sensitive biomonitoring. We examined monthly morphological dynamics and physiological adaptations of *P. akamusi* to anoxic conditions, and environmental relationships of the species abundance in a subtropical eutrophic lake. Results of our experiments indicated that this species presented extremely low fuel consumption at a state of estivation when the larvae dwelt in deep sediment, and conducted anaerobic respiration, with ethanol as a major metabolite, to withstand long term anoxic conditions. Additionally, in our field study, the abundance of *P. akamusi* correlated positively with most nutrient-related variables, indicating that this species can serve as sentinel organisms of nutrient enrichment. We suggest that the high tolerance to anoxia and detritivorous diet of *P. akamusi* larvae may allow the species to benefit from nutrient enrichment. The vertical niche differentiation and the avoidance of benthivorous predator by vertical sediment migration behavior may also contribute to its high dominance in eutrophic freshwaters.

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1. Introduction

Non-biting midges are the most abundant and ubiquitously distributed group of insects in freshwater ecosystems (Pinder, 2003). Chironomid species usually exhibit a distinct preference for certain type of environmental conditions. The essential factors, such as dissolved oxygen and availability of food, govern the composition of chironomid communities (Armitage et al., 1995), making them widely usable for biomonitoring (Rosenberg, 1992). For example, the genus of *Chironomus* is very tolerant to anoxia (Nagell and Landahl, 1978), making it a good indicator of organic pollution (Armitage et al., 1995; Friberg et al., 2010). Moreover, head capsule of chironomids could be well preserved in sediment, allowing palaeoecologists to reconstruct long term climate changes (Walker, 1995; Brodersen et al., 2008). In addition, Chironomidae are positioned at low trophic levels in the food webs and being abundant, Chironomidae may be capable to control secondary production in freshwaters (Armitage, 1995; Covich et al., 1999), including their riparian systems (Scharnweber et al., 2014). Therefore, Chironomidae may also have a great significance to fishery production (Vander Zanden and Vadeboncoeur, 2002).

Propsilocerus akamusi is a large chironomid midge with the body length of 10-17 mm of 4th instar larvae (Gong et al., 2008). This species is

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known to emerge and oviposit during short period in late-autumn (Hirabayashi et al., 1998). In Chinese lakes, the larvae grow very fast during coldest months of winter (about 5-8 ℃), after about three-month rapid development (from January to March), *P. akamusi* larvae migrate deep into the sediment and dwell in the depth of 40 to 60 cm from April to October (Zou et al., 2018). As we know, there is no oxygen and have little food resources for macroinvertebrates in deep sediment (Zhang et al., 2010). Thus, the tube-dwelling life in deep sediment must be accompanied with adaptions to those environmental stressors. Additionally, *P. akamusi* is a dominant species in many lakes of subtropical and temperate zone and its abundance is related to organic content of the sediment (Iwakuma, 1987; Gong et al., 2008). Therefore, this species is likely to be applied as a good indicator of eutrophication. Hence, by understanding the mechanisms why larvae of *P. akamusi* can withstand long term harsh conditions in deep sediment and by identifying the mechanisms why this species favored by eutrophication can contribute to the development of sensitive biomonitoring protocols. Such important information provides a basis for the macroinvertebrate-based bioassessment approaches in freshwater lake ecosystems.

To our knowledge, few studies have focused on the specific adaptive tactics of the tube-dwelling life of *P. akamusi* larvae in deep sediment (>30 cm). Furthermore, the knowledge of the underlying mechanisms,

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why nutrient-rich conditions of eutrophic lakes are favoring this species, have been limited. In this study, we examined seasonal dynamics in larval morphological characteristics (dry weight and body length), physiological adaptations of the larvae to anoxic conditions and the effects of environmental parameters on the abundance of *P. akamusi* in a subtropical eutrophic lake. Our objectives were (1) to elucidate the species-specific adaptations to long-term harsh conditions by *P. akamusi* during time of deep sediment dwelling of the larvae, and (2) to clarify the underlying mechanisms what make *P. akamusi* well-adapted to eutrophication and further robust indicator of nutrient enrichment. We expected that *P. akamusi* would substantially lower their metabolic rate and conduct anaerobic respiration for fuel supply when they dwell in the deep layers of the sediment. Moreover, the eutrophication-tolerant traits mainly result from the extraordinary ability of anoxia tolerance of the species.

2 Materials and Methods

2.1 Study area

Lake Donghu (30°31′-30°36′ N, 114°21′-114°28′ E) with a total surface area of 32 km^2 is a shallow freshwater lake locating in Wuchang, Wuhan City, China. This lake has multiple ecosystem service functions such as water supply, entertainment and commercial fishery. The

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maximum depth of the lake is 4.5 m with the average value of 2.5 m (Zhou and Zhou, 1997). In the 1960-1970s, the lake was divided into several lake regions by artificial dikes, of which Shuiguohu, Guozhenghu, Tanglinghu are our main study area (Gong and Xie, 2001). With the rapid development of urbanization in China over the past three decades, increasing external nutrient loads have resulted in serious eutrophication and algal bloom of the Lake (Chen et al., 2009).

2.2 Collection of field samples

To clarify underlying mechanisms why the larvae can withstand long term harsh conditions (absence of oxygen and food scarcity) when they dwell in deep layers of sediment, one site (D17) was chosen in Lake Donghu to elaborate the accumulation of dry matter, variations of body length during the larval stage (Figure 1). The larvae were collected monthly from October 2015 to September 2016 using a sediment core sampler (diameter: 90 mm). Ten 100 cm depth cores were collected at each sampling occasion. The cores were divided into 10 cm layers and pre-sieved *in situ* through 250 μm mesh size sieve for subsequent analysis. In the laboratory, the larvae were sorted with a white tray, counted and measured with body length, and then dried to constant weight at 60 ℃ to measure the dry weight with electronic balance.

To elucidate the statistical relationships between the abundance of *P. akamusi* and environment variables, the larvae were collected at 22 sites

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in Lake Donghu (Figure 1). Since the larvae dwell in the sediment deeper than 30 cm from April to October in Lake Donghu (Gong et al., 2008), the sampling was conducted using a 0.025 m^2 Peterson grab in December 2016, with three grabs comprising a pooled sample. The materials collected were pre-sieved *in situ* using a 250 μm mesh size sieve to remove fine sediment and were taken to the laboratory immediately. In the laboratory, *P. akamusi* were picked out under a dissection microscope. After this the larvae were counted and measured with wet weight. As to environmental variables, at each site, water depth (WD) and Secchi depth (SD) were measured in the field with Speedtech SM-5 Portable Depth Sounder and Secchi disk. Water temperature (WT), pH, dissolved oxygen (DO) in sediment water interface were measured *in situ* with the YSI 6600 V2 multi-sensor sonde. Surface water samples within 50 cm were also collected to analyze water chemistry at the study sites. Total nitrogen (TN), total dissolved nitrogen (TDN), ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), total phosphorus (TP), total dissolved phosphorus (TDP), orthophosphate (PO_4^{3-}-P), permanganate index (COD_{Mn}), and chlorophyll *a* (Chl-*a*) were measured in the laboratory based on the standard methods (APHA, 2012). The additional sediment samples were collected at each site to determine total nitrogen (TNs) and total phosphorus (TPs) in the surface sediment. TNs and TPs were determined by subsampling approximately 30 mg of the dried sediment from each

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site, which were ground with a mortar and pestle and weighed. Then 25 ml of distilled water was added, and the samples were analyzed after thawing, using a combined persulphate digestion, followed by spectrophotometric analysis as for phosphate and nitrate. The surface sediment were oven-dried (110 ℃ for 24 hour) and ignited in a muffle furnace (550 ℃ for 4 hour) to determine loss on ignition (LOI).

2.3 Experimental design

The ethanol has been demonstrated as a major metabolic end-product of *Chironomus thummi* larvae under anoxic condition (0 mg/L, >12 hour) (Wilps and Zebe, 1976). Hence, we expect that 4th instar larvae of *P. akamusi* may also conduct anaerobic metabolism with ethanol as major metabolite when they dwell in the deep anoxic sediment. The larvae (4th instar) used to study physiological adaptation to anoxic condition were also sampled with Peterson grab in December 2016 (see 2.2). In our experiments, ten 4th instar larvae (ca.38 mg to 44 mg dry wt.) for each treatment, with the similar body size and high vitality, were selected after acclimatization to 18 ℃ for 24h. Before the incubations, all traces of substratum adhering to the animals required to be removed and the ambient water was changed frequently for emptying gut contents. During the experiment, the head-space bottle was used for incubations of the larvae and the oxygen concentration was continuously monitored using an oxygen microelectrodes system (Presens, Germany). The larvae

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were incubated in 5 ml water for 24, 48 or 72h under total absence of oxygen. Controls with the larvae under aerobic conditions were implemented with each experiment. In controls, the incubations were sealed to prevent volatilization of possible metabolites, and the oxygen concentrations were replenished by injector. At the end of the incubation, the ethanol in ambient water was measured with the enzymatic method kit (Biosentec, Cat.No. 026). The alcohol dehydrogenase method is based on oxidation of alcohol in the presence of ADH as enzyme and NAD^+ as coenzyme and formation of acetaldehyde and NADH that can be measured using spectrophotometer at 334,340 or 365 nm wave lengths (Cornell, 1983).

2.4 Data analysis

In our study lake, the sampling sites were divided into three groups according to the geographic distribution, including the northern sites (D1-D8), the middle sites (D9-D16) and the southern sites (D17-D22). Moreover, the whole year were divided into three periods according the life history of *P. akamusi*, including emergence period, fast-growth period and estivation period (Gong et al., 2008). In our study, the emergence period ranged from October to December 2015 (October 2015, before emergence, BE; November 2015, in emergence, IE; December 2015, after emergence, AE). The fast-growth period was ranged from January to March 2016 (January 2016, early fast-growth, EF; February 2016,

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medium fast-growth, MF; March 2016, late fast-growth, LF). The estivation period ranged from April to September 2016 (April to May 2016, early estivation, EE; June to July 2016, medium estivation, ME; August to September 2016, late estivation, LE).

As to statistical analysis, differences analysis of the morphologic parameters between different life stage were tested by one-way ANOVA following a *post hoc* Tukey test or Kruskal-Wallis test following Mann-Whitney U depending on the normality and homoscedasticity of the data. Moreover, a principal component analysis (PCA) based on a correlation matrix among samples was used to examine the environmental gradients using CANOCO v.4.5 software (Ter Braak and Smilauer, 2002). Spearman correlation analysis was used to examine the relationships between abundance of the larvae and environmental variables. It should be noted that water temperature was greatly influenced by solar radiation and the sampling of different sites in this study was not simultaneous. Hence, the water temperatures of different sampling sites are lack of comparability and this variable was excluded from the above-mentioned statistical analysis.

In addition, multiple linear regression analysis (MLR) was performed in our study. For avoiding high collinearity among variables, environmental parameters that coefficients in Spearman's correlations exceeded 0.6 and $P<0.01$ were exclude from MLR. Three types of

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parameters are used for MLR analysis, including nutrients level of water column, nutrients level in sediment phase and physical condition. The stepwise MLR was used to identify the key parameters that contribute to the variations of *P. akamusi's* abundance. The statistical significance levels were set as 0.05 and 0.10 for variable inclusion and removal. All the variables were standardized by SPSS before data analysis. Difference analysis, correlation analysis and regression analysis were conducted with SPSS 19.0.

3. Results

3.1. Experimental study of larval metabolism under anoxic conditions

Under anoxic conditions, we observed continuously increasing concentration of ethanol in the ambient water of sealed head-space bottles from first to third day of the experiments. The ethanol concentrations differed significantly between the days ($P<0.01$). In contrast, no ethanol were detected in the controls with oxygenated water (Figure 2).

3.2. Temporal variations in larval morphology

In our study, the emergence of *P. akamusi* was observed in November 2015, and the new generation (2016-year-class) was not found in sieved core samples until January, because larvae were too small to remain in the sieve or they were still in the egg stages. Thus, all individuals observed from October to December 2015 were old

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generation (2015-year-class). The body length and dry weight of the larvae in December were significantly (P<0.05) higher than those in November, indicating the rest old generation experienced significant accumulation of dry mater at low temperature (0-10 ℃) when dwelled in the surface layer of sediment (<20 cm). The new generation (2016-years-class) were observed since January 2016 and these two generations could be discerned by body size and color until March 2016. Moreover, the significant increase both of body length and dry weight were tested (Table 1, P<0.05) and the obvious growth trend both of the body length and dry weight were observed (Figure 3). Those results indicated that the fast growth of new generation also occurred in low-temperature when the larvae dwell in surface layer of sediment. From April 2016 to October 2016, all the individuals were distributed in the sediment below 30 cm depth, with little dry weight reduction, and no significant variation were observed during estivation (P=0.134). However, the body length increased significantly (P<0.05) during this period (Table 1, Figure 3).

3.3. The relationships between the abundance of P. akamusi and environmental variables

3.3.1 Environmental characteristics

In general, Lake Donghu exhibited high nutrients content in sediment (LOI 12.05±1.59 %, TNs: 0.30±0.03 %, TPs: 0.05±0.01 %),

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whereas relatively low nutrients concentration were observed in water column in December (TN: 0.98±0.13%, TP: 0.08±0.01 %) (Table 2, Table S1).

As to spatial variations, based on the results from PCA analysis, the first two components accounted for 85.8% and 11.7% of total variance of the environmental variables, respectively. PC1 are loading with SD, pH, NH_4^+-N and Chl-*a* and exhibited most strong positive correlation with SD. PC2 showed a strong negative relationship with other nutrient-related variables and WD, and a positive relationship with DO. The sites in northern part of the lake were characterized by low value of nutrient-related variables, and the values increased from north to south. In contrast, DO show the opposite trend (Figure 4).

3.3.2 Abundance-environment relationships

Correlation analysis showed that the majority of nutrient-related variables were significantly and positively correlated with abundance of *P. akamusi* (Table 3). TP, TDP, TP_S, TDN, NO_3^-, NO_2^- and COD were positively and significantly correlated with the abundance of the larvae ($P<0.05$). However, no significant correlations were observed between the larvae abundance and TN and TN_S. In terms of Chl-*a* and LOI, no statistically significant relationships were found in this study. As to physical variables, only WD showed significant positive correlation with its abundance ($P<0.01$). DO and the abundance of *P. akamusi* larvae

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correlated negatively, but this relationship was not statistically significant (P=0.093).

In the MLR analysis, three environmental parameters (NO_3^-, TPs and WD) were selected as independent variables and natural logarithm of *P. akamusi'* abundance was chosen as dependent variable. The results of stepwise MLR included NO_3^- (P<0.01), but WD and TPs were removed from the model. NO_3^- explained 42% of the variation in the dependent variable (Figure 5).

4. Discussion

4.1. Adaptive tactics of P. akamusi larvae dwelling in deep sediment

For many animals, sufficient fuel reserves and the ability to reduce their metabolic rate are the critical survival strategies to tolerate harsh environmental conditions (Storey, 2000). Our results indicated that *P. akamusi* larvae grew fast and accumulated fuel reserves very efficiently under low temperature when they dwelt in the surface layers of sediment (<30 cm). In contrast, the larvae lose weight only little during estivation in the deep sediment indicating that the larvae conducted extremely slow fuel consumption from April to October (Table 1). Moreover, previous study has found that the guts of the larvae were empty during summer (Yamagishi and Fukuhara, 1972). These results suggest that this species dwell in the deep layer of sediment with a dormancy state during summer

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months (estivation). Adequate body fuel accumulation during low temperature period of fast growth and extremely low metabolic rate during dormancy are allowing the larvae to survive in the harsh environmental conditions.

Tube-dwelling larvae needs also to cope with the deficiency of oxygen during estivation. In our experiments, we observed accumulation of ethanol in the ambient water, when *P. akamusi* larvae were incubated under anoxic condition. Combined the pretreatments and the results of controls, these results suggest that the 4th instar larvae may utilize anaerobic respiration excreting ethanol as major metabolite (see also Wilps and Zebe, 1976) during their deep sediment dwelling stage. Ethanol may be the most suitable anaerobic end-product for aquatic organisms because it rapidly diffuses into surroundings while other products, such as lactate, are easily retained within tissues (Hoback and Stanley, 2001). Different with other common Chironomidae species, the 4th instar larvae of *P. akamusi* have distinctive thick cuticle that enables their high dehydration resistance (Suemoto et al., 2004). On the other hand, this species also benefits from this substantial vertical movements (>50 cm). Obviously, migration into the deep sediment of the larvae play a key role in anti-predator tactics (Kornijów and Pawlikowski, 2016). However, small scale vertical movements in the surface sediment (e.g., 10 cm) rather than larger scale migrations into the deeper sediment

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should be adequate for avoiding predators like benthivorous fish (Persson and Svensson, 2006), implying other mechanisms are also involved. Previous studies indicated that this species favors low temperature (Yamagishi and Fukuhara, 1970; Gong et al., 2002; Gong et al., 2008). Water-sediment interface temperatures varied substantially over the seasons of shallow lakes in these zones, with highest values (about 35 ℃) observed in summer. However, the temperature in the sediment gradually decreased with increasing sediment depth during summer months (Narita, 2006). Thus, the cool temperature preference may derive from the species phylogenetic origin in the Amur region (Sasa, 1978), which may have given rise to an adaptive life history trait of estivation in the deep sediment layers during the hot summer months. The larvae remain in the deep sediment with rather cool and constant temperature in summer (Yamagishi and Fukuhara, 1972), which enable them to avoid the high temperature in upper layer and ensure high survival rates of species during hot summer months (Yamagish and Fukuhara, 1971).

4.2 Eutrophication-tolerant mechanisms

In our field study, the abundance of *P. akamusi* showed strong positive correlation with most nutrient-related variables (Table3), especially for the NO_3^- and TDP (Table 3), indicating that this species is a good indicator of eutrophication. In addition, previous studies also

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indicated that this species favors substrate with high organic content (Iwakuma, 1987). This may arouse from multiple mechanisms. *P. akamusi* is a tube-dwelling species burrowing deep sediment during summer months. As we know, the deep sediment layers are often anoxic and less nutritious (Kornijów, 1997; Kornijów and Pawlikowski, 2016). Under these environmental pressures, *P. akamusi* larvae showed high tolerance to anoxia (Kamimura et al., 2003). With respect to anoxia tolerance, in addition to anaerobic respiration under anoxic condition (see 4.1), the larvae also possess the high-affinity hemoglobin, which can bind oxygen for storage until needed for metabolism (Kamimura et al., 2003). As to diets, organic detritus account for 76 % of its food composition (volume %) (liu et al., 2006). Thus, their ability to withstand considerable oxygen depletion allow it to exist in the deoxygenated zone below organic effluents, and the detritivorous diet make the larvae benefit more from organic matter enrichment in eutrophic freshwaters.

Positive association have been observed between the abundance of *Chironomus* larvae and the maximum burrowing depth (Kornijów and Pawlikowski, 2016), which suggests that the burrowing behavior may intensify due to the intraspecific competition for food and space (Mclachlan and Ladle, 2009). We believe that the vertical niche differentiation may also play a role in our study lakes by intensifying burrowing behavior (Figure6). Moreover, as observed in Lake

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Kasumigaura, predation is responsible for about 50% abundance loss of the larvae during emergence in late-autumn (Iwakuma and Yasuno, 1983). And this high predation mortality may ascribe to its eye-catching red body color, which increase the risk of predation from visual predators when they appeared in sediment-water interface for emergence. Thus, the tube-dwelling behavior will also allow the larvae escape from the top-down effect by benthivorous predator when its abundance reaches extreme high value. In summary, the unavailability to the predator and vertical niche differentiation due to vertical sediment migrations, as well as it sophisticated ability to anoxic tolerance make *P. akamusi* become a dominant species in eutrophic freshwater lakes.

Acknowledgments

This work was supported by Supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA23040203), the National Natural Science Foundation of China (Grants No. 41671110, 31670466, 41471088, 41601203)，Project of Science and Technology of Water Conservancy Department of Jiangsu Province (Grant No. 2017049) and the “One-Three-Five” Strategic Planning of Nanjing Institute of Geography and Limnology of CAS (Grant No. NIGLAS2017GH05).

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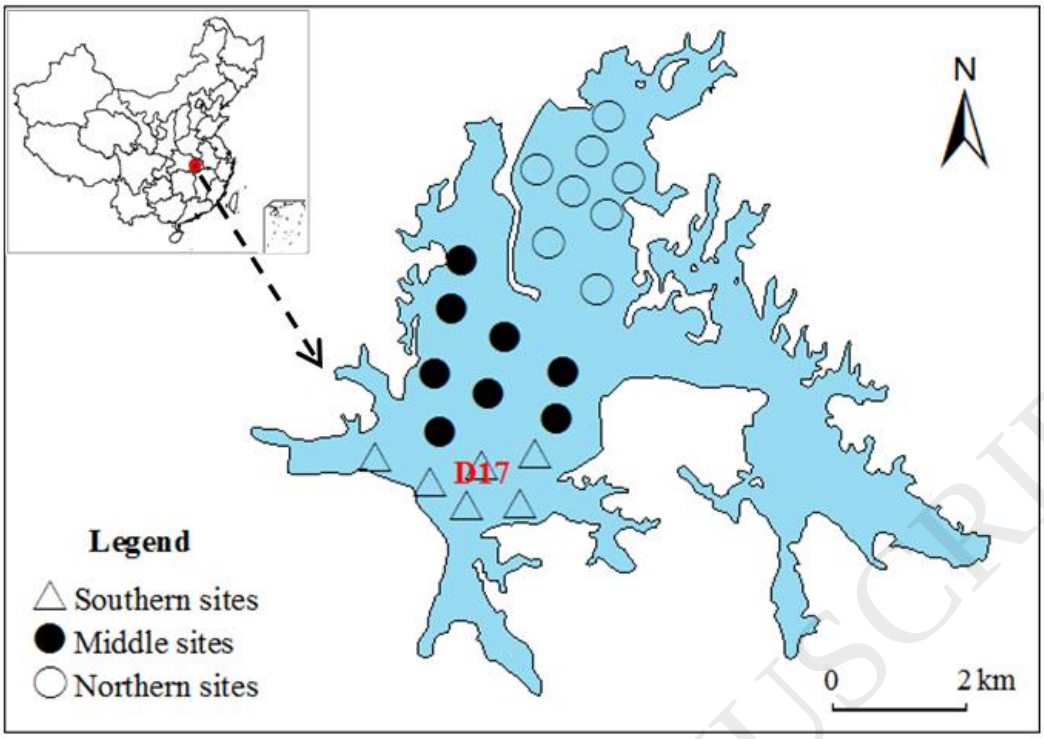

Figure 1 Location of the sampling sites of *P. akamusi* in Lake Donghu. Sites of D1-D21 were collected with Petersen grab in December 2016, and D17 were collected monthly with core sampling from October 2015 to September 2016.

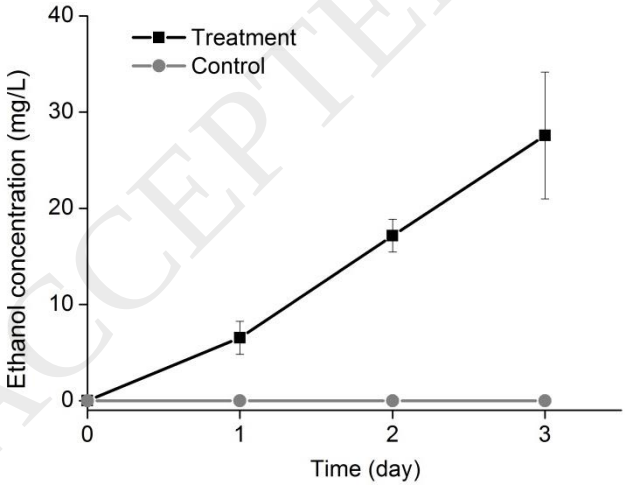

Figure 2 Changes of ethanol concentration in ambient water of treatments and control. The head-space bottle with the volume of 5 ml was used for incubations.

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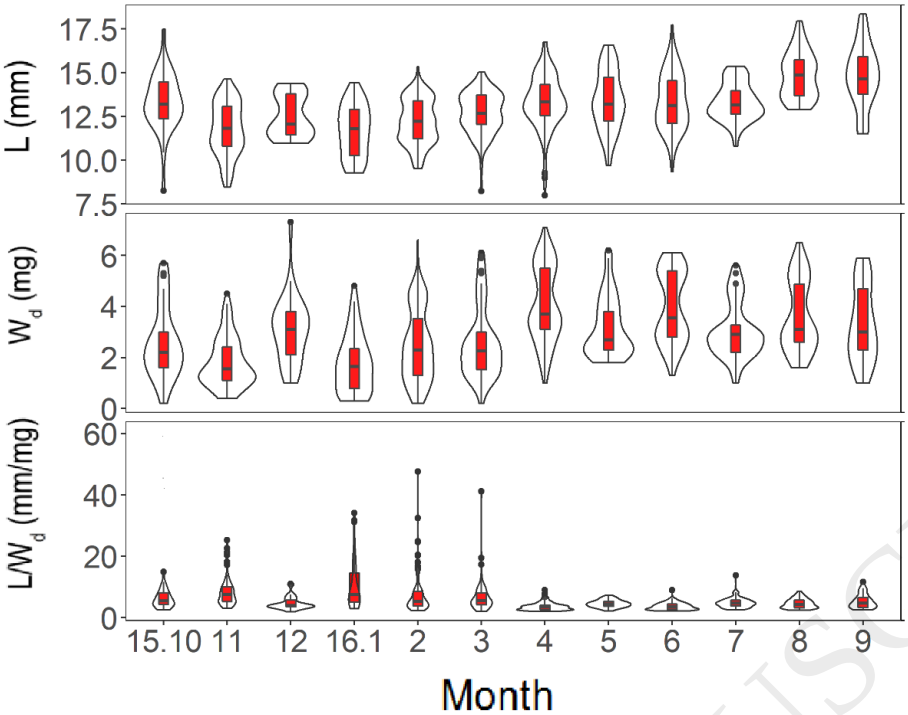

Figure 3 Violin Plot of body length (L), dry weight (W_d) and bodylength/dry weight (L/W_d) of *P. akamusi* in Lake Donghu

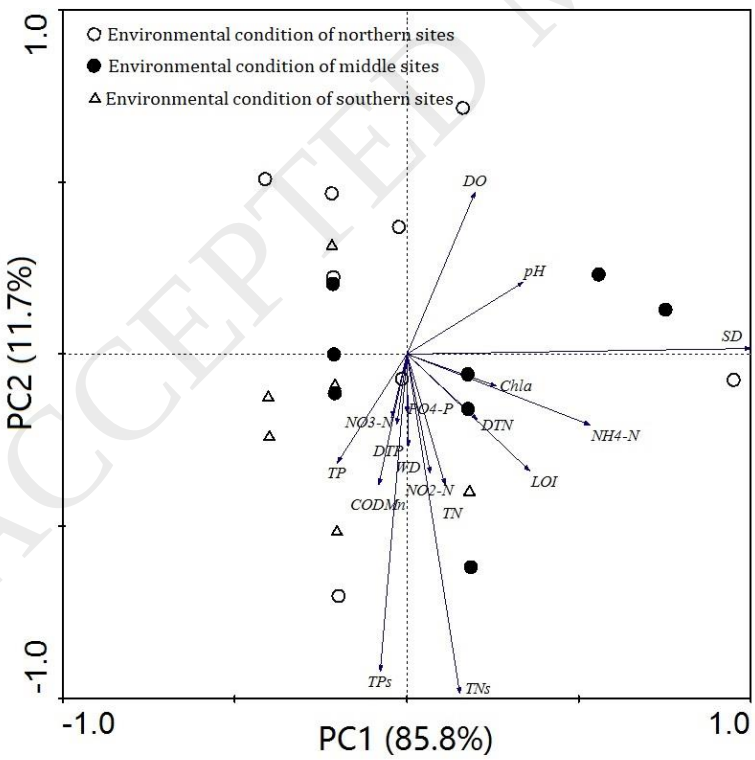

Figure 4 PCA plots of the first 2 principal components of the 17

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environmental variables. The values on the axes indicate the percentage of total variation explained by each axis. The position of sampling site indicated the environmental condition of each site.

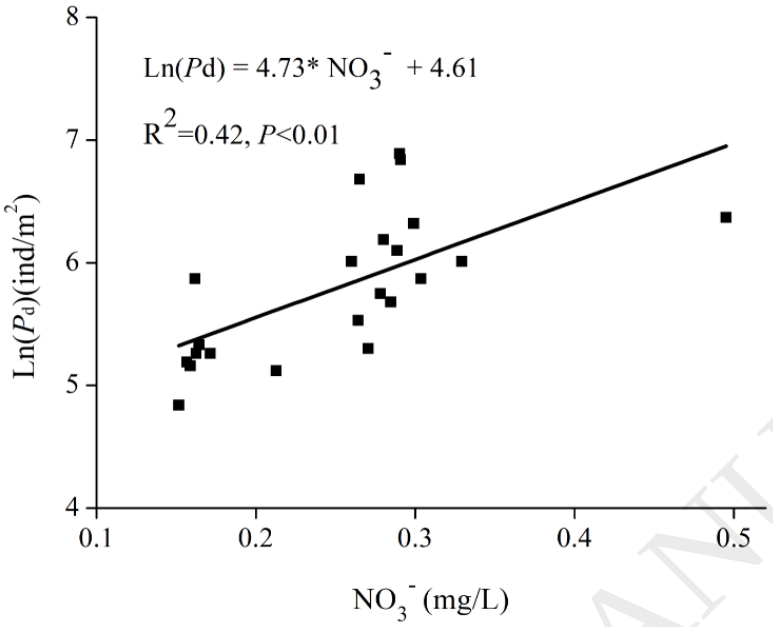

Figure 5 Linear regression between NO_3^- and natural logarithm of *P. akamusi'* abundance (Ln(P_d) ind/m^2) in Lake Donghu.

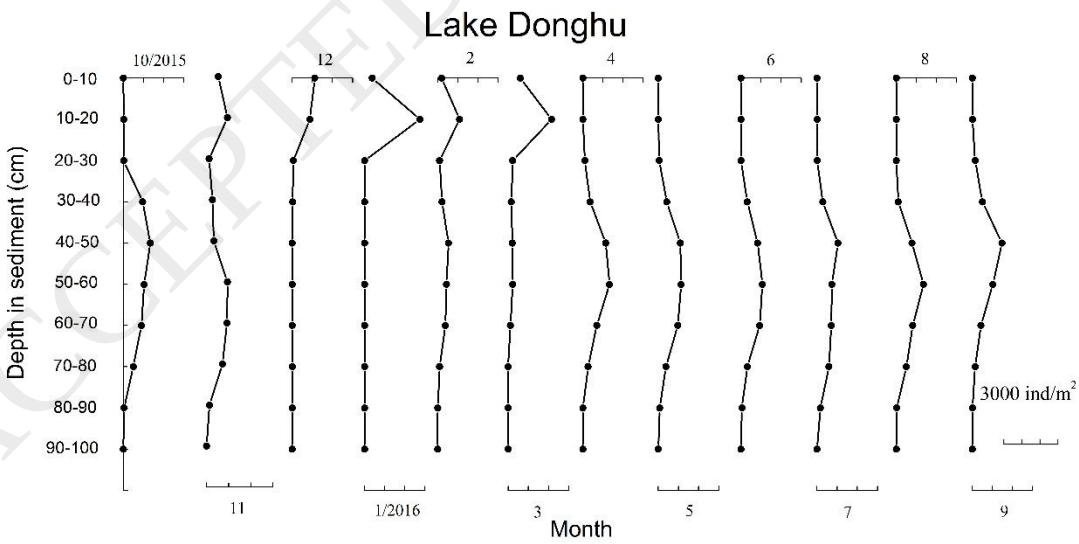

Figure 6 Temporal changes in vertical distribution of *P. akamusi'* abundance from October 2015 to September 2016 in Lake Donghu.

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Table 1 Temporal variations of body length (L), dry weight (W_d) and body length/dry weight ratio (L/W_d) of *P. akamusi* in Lake Donghu. Abbreviations were defined in the text (section 2.4).

Period (Emergence)	Oct. 2015 (BE)	Nov. 2015 (IE)	Dec. 2015 (AE)	*P*
n	33	84	27	
L	13.33±1.63[a]	11.77±1.48[b]	12.5±1.18[a]	<0.001
W_d	2.56±1.37[a]	1.79±0.95[b]	3.09±1.32[a]	<0.001
L/W_d	7.94±9.78[a]	8.51±4.63[b]	7.80±1.67[a]	0.022
Period (Fast-growth)	Jan. 2016 (EF)	Feb. 2016 (MF)	Mar. 2016 (LF)	
n	56	120	78	
L	11.68±1.45[b]	12.19±1.27[b]	12.71±1.19[a]	<0.001
W_d	1.76±1.13[b]	2.45±1.38[a]	2.54±1.37[a]	0.002
L/W_d	10.52±7.67[a]	7.48±6.30[b]	6.80±5.10[b]	0.003
Period (Estivation)	Apr. to May. 2016 (EE)	Jun. to Jul. 2016 (ME)	Aug. to Sep. 2016 (LE)	
n	175	142	107	
L	13.35±1.58[b]	13.35±1.42[b]	14.83±1.46[a]	<0.001
W_d	3.80±1.43[a]	3.6±1.34[a]	3.51±1.39[a]	0.134
L/W_d	3.96±1.31[b]	4.21±1.59[b]	4.87±1.85[a]	<0.001

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Table 2 General description of environmental physicochemical variables of Lake Donghu in December 2016. Abbreviations were defined in the main text (section 2.2). More details of those variables please see Table S1.

Variables	Mean	SD	Max	Min	CV (%)
WT (ºC)	10.04	0.29	10.43	9.36	2.9
pH	8.7	0.3	9.1	8.1	3.3
DO (mg/L)	11.52	0.53	12.39	10.46	4.6
SD (cm)	45	9	70	35	20.4
WD (m)	3.2	0.7	4.1	1.8	23.1
TN (mg/L)	0.98	0.13	1.22	0.72	13.3
TDN (mg/L)	0.64	0.12	0.96	0.46	19.5
TP (mg/L)	0.08	0.01	0.10	0.06	13.8
TDP (mg/L)	0.02	0.01	0.04	0.00	54.1
PO_4^{3-} (μg/L)	2.94	1.19	5.77	1.50	40.5
NH_4^+ (mg/L)	0.06	0.06	0.31	0.02	94.0
NO_3^- (mg/L)	0.25	0.08	0.50	0.15	31.4
NO_2^- (mg/L)	0.02	0.01	0.03	0.00	57.8
Chl-*a*(mg/L)	0.03	0.01	0.05	0.01	24.9
COD (mg/L)	4.03	0.25	4.64	3.67	6.1
LOI (%)	12.05	1.59	15.00	8.79	13.2
TNs (%)	0.30	0.03	0.36	0.24	10.9

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TPs (%)	0.05	0.01	0.08	0.03	23.6

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Table 3 The results of correlation analysis between environmental variables and the abundance of *P. akamusi* in Lake Donghu. Significance of Pearson correlation is marked with * ($P<0.05$) and ** ($P < 0.01$) asterisks.

Variables	Coefficients	*P*
WD	0.555	0.007**
SD	0.012	0.958
pH	-0.392	0.072
DO	-0.367	0.093
TN	0.370	0.09
TDN	0.504	0.017*
TP	0.465	0.029*
TDP	0.532	0.011*
PO_4^{3-}	0.259	0.244
NH_4^+	0.321	0.146
NO_3^-	0.753	<0.001**
NO_2^-	0.661	0.001**
COD	0.522	0.013*
Chl-*a*	0.201	0.369
LOI	-0.187	0.404
TNs	0.085	0.707
TPs	0.425	0.049*

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