Journal of Oceanology and Limnology
Vol. 37 No. 2, P. 665-674, 2019
https://doi.org/10.1007/s00343-019-8025-0

Antioxidant systems of aquatic macrophytes in three life forms: a case study in Lake Erhai, China*

YUAN Changbo[1, 4], ZHU Tianshun[3, 4], CAO Te[1, 2], XI Yilong[2], ZHANG Xiaolin[1, 2, **], NI Leyi[1, 2]

[1] *Donghu Experimental Station of Lake Ecosystems*, *State Key Laboratory of Freshwater Ecology and Biotechnology*, *Institute of Hydrobiology*, *Chinese Academy of Sciences*, *Wuhan 430072*, *China*

[2] *Collaborative Innovation Center of Recovery and Reconstruction of Degraded Ecosystem in Wanjiang City Belt*, *Anhui Province*; *College of Life Sciences*, *Anhui Normal University*, *Wuhu 241000*, *China*

[3] *College of Life Sciences*, *Zaozhuang University*, *Zaozhuang 277160*, *China*

[4] *University of Chinese Academy of Sciences*, *Beijing 100049*, *China*

Received Feb. 9, 2018; accepted in principle Apr. 27, 2018; accepted for publication May 30, 2018

Abstract Antioxidant systems are vital in life activities of macrophytes. Species with different life forms need to cope with distinct environments by modifying physiological characters, especially antioxidant systems. In order to find differences among life forms and consequence of lake eutrophication, we studied three antioxidant enzymes activity (superoxide dismutase (SOD), ascorbate oxidase (APX) and catalase (CAT)) and total soluble phenolics (TP) content in leaves of 26 macrophyte species in September 2013 in Lake Erhai, China. We found that antioxidation varied accordingly with life forms. The activities of SOD and APX in emergent macrophytes (EM) and floating-leaved macrophytes (FM) were much lower than those of submerged macrophytes (SM). On the contrary, TP content was much higher in EM and FM species. There was a negative correlation between TP and antioxidant enzyme activities (CAT and APX). The results suggested that EM and FM species rely on phenolics might to adapt to adverse environments (higher herbivores predation pressure and UV radiation intensity), while SM species more rely on antioxidant enzymes possibly due to lower demand for antioxidation and/or lack of light and inorganic C availability for phenolics synthesis. We also found FM species represent highest fitness in term of antioxidant system, which would lead to overgrowth of FM species and littoral zone bogginess during lake eutrophication. Finally, it is necessary to carry out the verification experiment under the control condition in the later stage, especially for the dominant ones in eutrophic lakes, to understand the exact adaptive mechanisms of them.

Keyword: macrophytes; life forms; phenolics; antioxidant enzymes; eutrophication

1 INTRODUCTION

Aquatic macrophytes are fundamental components in littoral zone and play key roles in ecological functions by means of being highly productive primary producer, providing food (Laguna et al., 2016), shelter and nursery habitats (Wetzel, 2001) for aquatic animals, competing with phytoplankton on nutrients (Ibáñez et al., 2012; Dai et al., 2017) and altering nutrients cycle due to growth and death, etc. They occupy a wide range of different habitats from irregular to permanent waterlogged, and develop mainly three life forms including emergent, floating-leaved and submersed. The different life forms are results due to adaptive evolution in order to cope with various inorganic and biotic environments. For instance, all of the leaves of emergent macrophytes (EM) are exposed in air, the whole plant of submersed macrophyte (SM) is surrounded by water, while the upper leaf surface of floating-leaved macrophytes (FM) open to air and the other side down in water.

* Supported by the State Key Laboratory of Freshwater Ecology and Biotechnology (No. 2016FBZ08), the Natural Science Foundation of Jiangxi Province (No. 2015ZBBF6008), and the National Natural Science Foundation of China (No. 31200356)
** Corresponding author: zhangxl@ihb.ac.cn

The characters of leaves make difference in gas (CO_2 and O_2) exchanges and light availability. Furthermore, EM and FM species generally experience mechanical damages from predation of large animals due to their higher caloric contents (Boyd, 1968), while SM species are damaged by high nutrients and strong wave force (Zhang et al., 2010; Su et al., 2016; Zhu et al., 2018) and suffer from physiological stress induced by toxins of ammonia, sulfide and microcystins, etc. (Cao et al., 2007; Zhang et al., 2011).

Littoral zone is a buffer between land and open water. It is characteristic of high biodiversity and complex environmental conditions. Intensive interactions were found not only among the organisms but also the organisms and their habitat environments, such as high nutrients input and high intensity of human disturbance, etc. Like other plants, macrophytes have to deal with different stresses from external environment and also metabolism in vivo, and some productions of metabolism are harmful to organisms. One of them is the reactive oxygen species (ROS), includes superoxide anion radical ($\cdot O^{2-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$) (Metcalfe and Alonso-Alvarez, 2010). The ROS producing reaction happens rapidly and strongly, and is harmful to macrophytes, for example, ROS usually cause death of biomolecules and cells, lipid membrane overoxidation and protein denaturation (Bowler et al., 1992; Mittler, 2002).

Except for environmental factors, eutrophication in lakes is another strong stress on the survival of macrophytes. During the process of eutrophication, physical and chemical properties of water change dramatically, such as decreasing transparency, dropping dissolved oxygen level, increasing nutrient content (Anderson et al., 2002; Conley et al., 2009; Qin et al., 2015; Watson et al., 2016). These variations may also change some aspects of physiological process of macrophytes, for example the antioxidant system. It has been found that activities of superoxide dismutase (SOD) and ascorbate oxidase (APX) of *Potamogeton crispus* L. fitted a lognormal distribution with increasing ammonium levels in an aquarium experiment (Cao et al., 2004), and potentially significant biochemical damage occurred when NH_4-N≥5 mg/L. A field experiment (Zhang et al., 2011) in an eutrophic lake (Taihu Lake, China) showed that synthetic action of ammonium, microcystins and hypoxia arising from cyanobacteria caused oxidative stress in terms of high SOD activity in FM and SM, and FM were more resistant to the stresses of eutrophication. As a result of eutrophication, submerged macrophytes would finally disappear (Ozimek and Kowalczewski, 1984; Phillips et al., 2016), and the aquatic plant community will succeed to the stage with emerged and floating-leaved macrophytes dominated. Such succession could be related with the antioxidant system, as it is vital to the survival of plants.

Plants develop antioxidant system to remove ROS and lead to a dynamic balance between producing and removing of ROS in the normal growth process (Almeselmani et al., 2006). Antioxidant system in plants contains antioxidant enzymes and antioxygen (Shao et al., 2008), it is generally recognized that they are synergistically working in the process of plants' coping with external adversities (Larson, 1988). However, the mechanisms of oxidation resistance in plants are very distinct in different taxa. Antioxidant enzymes are constructional components in all plant cells, in which SOD is the first defensive line of them changing $\cdot O^{2-}$ into H_2O_2 and then the other enzymes, such as catalase (CAT) and APX, further clearing the H_2O_2 jointly. While phenolics, a class of secondary metabolic compounds in plants, its antioxidant mechanisms are more complex, mainly being summarized for three pathways (Leopoldini et al., 2004; Leopoldini et al., 2006): 1) transformation of phenolic hydroxyl hydrogen atom; 2) get electronics from highly reactive free radicals; 3) complexation with metal ions stopping the Feton reaction ($Fe(II)+H_2O_2$—$Fe(III)\text{-}OH^-+\cdot OH$).

As for the two parts of antioxidant system in plants, former studies have demonstrated that phenolics production concerns with UV radiation (Song et al., 2015), herbivores' predation and pathogens (Lodge, 1991; Dudt and Shure, 1994; Vergeer and Van Der Velde, 1997; Bixenmann et al., 2016; Frew et al., 2016) and are affected by C and N metabolism (Cao et al., 2008), while antioxidant enzymes relate to stresses of nutrient (Cao et al., 2004, 2009; Zhang et al., 2010, 2011), light (Zhang et al., 2010) and heavy metal stresses (Monferrán et al., 2009), etc. But the potential relationship between phenolics and antioxidant enzymes and how the plants evolved different antioxidant system according to different life-forms are still not clear.

In this study, we examined the antioxidant systems of phenolics and enzymes among emergent, floating-leaved and submersed macrophyte, and discuss the relationships between the two antioxidative systems with regard to the plant habitats. Before the study, We

supposed that: 1) total phenolic contents in EM and FM should be higher than SM, while antioxidant enzymes activities could be lower on the contrary; 2) As effects of taxonomy on physiological and chemical characteristics of macrophytes are nonnegligible (Smolders et al., 2000; Su et al., 2016), some interspecific differences within each lifeform would be observed; 3) the EM and FM should represent higher adaptability than SM based on the characters of antioxidant system and natural succession of aquatic macrophytes.

2 MATERIAL AND METHOD

2.1 Study area

The study was carried out in two adjacent bays (Haichao Bay and Shaping Bay) in the northern part of Lake Erhai (25°52′N, 100°06′E) in subtropic Yungui Plateau, China (Fig.1). The lake has a total area of 249.8 km^2, with moderate water depth (max 20.5 m, mean 10.5 m) and a mesotrophic status. These two bays are rich in similar aquatic macrophyte species and share parallel external environments (He et al., 2015).

2.2 Plant material

Mature leaves in good conditions of 26 aquatic macrophyte species (7 EM, 6 FM and 13 SM) were randomly collected with a long-handled scythe-type sampler (0.2 m^2 in sampling area) in the two bays (Haichao Bay and Shaping Bay) in zones with water depth lower than 3 m in September 2013. The distance of each two sampling sites is over 30 m. Leaves were cleaned, sorted by species and put into ziplocked bags with waterproof labels. Then all the samples were put into icebox and taken back to laboratory for further analysis. All species belong to 14 families, and the biggest family is *Potamogetonaceae* (Table 2).

2.3 Measurement

Each plant material in every sampling site was harvested into two replicate samples. One for antioxidant enzymes analysis was ground into fine powder in liquid nitrogen with a mortar and a pestle. 0.5 g fine powder was extracted at 4℃ for 20 min in 5 mL ice-cold buffer (50 mmol/L potassium phosphate, pH 7.8) containing 0.1 mmol/L EDTA, 1 mmol/L PMSF, 1 % (w/v) PVP, then centrifuged at 15 000 r/min for 20 min, at 4℃, and the supernatant was used for antioxidant enzymes examine. Soluble protein content

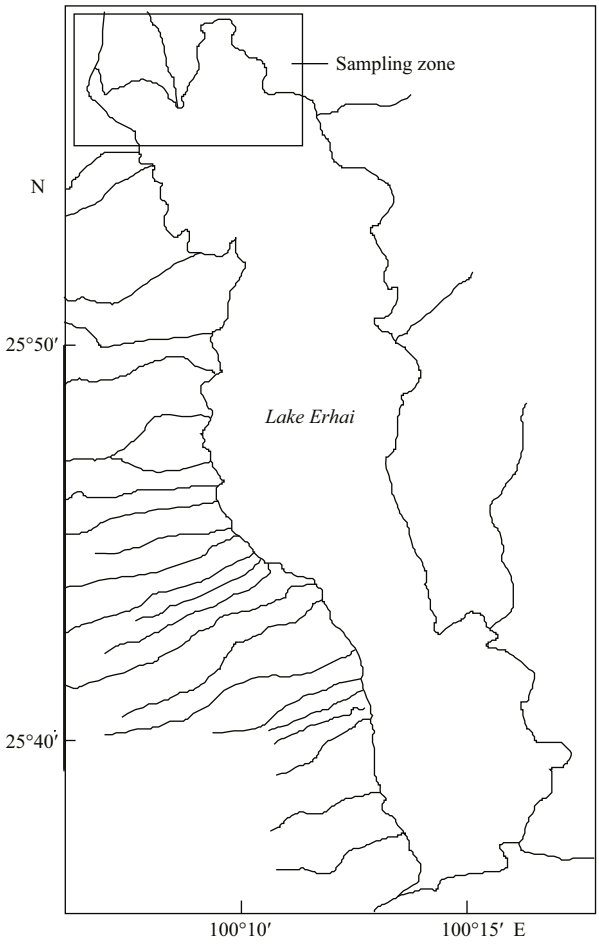

Fig.1 Location of the sampling sites in Sep. 2013

was assayed by the Coomassie blue dyeing method (Bradford, 1976) using bovine serum albumin as a standard. SOD (EC 1.15.1.1) activity was assayed based on a method of T-SOD reagent box from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), one unit of SOD activity is defined as the amount of SOD when its inhibition ratio is 50% of per mg protein in 1 mL reaction liquid. CAT (EC 1.11.1.6) activities was measured according to the modified method (Chance and Maehly, 1955), and one enzyme activity units of CAT is defined as the reduction of A240 by 0.1, respectively. The activity of APX (EC 1.11.1.11) was measured according to (Rout and Shaw, 2001). One enzyme activity unit is defined as oxidation of 1 μmol ascorbate acid of per mg protein per min. The other one for total phenolics (TP) was oven-dried at 80℃ for 48 h to constant weight, and then ground into fine powder with a pestle and mortar. About 50 mg of the powder was extracted with 5 mL of 80 % ethanol at 80℃ for 20 min, then centrifugated for 10 min at 5 000 r/min with supernatant dissociated. The residue was reextracted and recentrifuged, then

Table 1 Basic water environmental information of Haichao Bay and Shaping Bay in September 2013

Bay	Area (km^2)	SD (m)	pH	TON (mg/L)	TOP (mg/L)	NH_4-N (mg/L)	NO_3-N (mg/L)	SRP (mg/L)	Chl-*a* (μg/L)
Hai Chao	8.34	1.34	8.92	0.921	0.046	0.068	0.143	0.004	24.320
Sha Ping	4.81	1.15	8.78	0.781	0.032	0.046	0.117	0.005	29.574

SD: Secchi depth; TON: total nitrogen; TOP: total phosphorus; NH_4-N: ammonium nitrogen; NO_3-N: nitrate nitrogen; SRP: soluble reactive phosphorus; Chl-*a*: chlorophyll *a*. The data were from the National Science Foundation of China (No. 41230853) and the National High Technology Research and Development Program of China (No. 2012ZX07105-004).

merged the supernatant for determination of TP which was determined mainly following the method described by Mole and Waterman (1987). Tannic acid (Sigma Chemical Company) was used as a standard. All measurements were carried out in duplicate using a Shimadzu spectrophotometer (UV-2550).

2.4 Data analysis

Mean values and standard errors of all parameters within each macrophyte were calculated from replicates and one-way ANOVA followed by Tukey test (EM 52 replicates, FM 49 replicates and SM 115 replicates) was used to test differences between individual means. Pearson's correlation analysis (EM 52 replicates, FM 49 replicates and SM 115 replicates) was performed for pairs of the signatures using SPSS 22.0 software (SPSS, Chicago, IL), with significance set at $P<0.05$. Before analysis, all data were test for normality and homogeneity, and non-normal variables were log10-transformed to get normality. Principal component analysis (PCA, 26 samples) was conducted by CANOCO for windows (version 5) to elucidate the relationship between macrophyte life forms and antioxidants. Map location of sampling sites was made by AutoCAD 2015 (Autodesk, Inc).

3 RESULT

3.1 Background environmental factors

Data showed that water nutrient conditions and macrophytes structures (unpublished data) were similar (Table 1), so were the whole external environments, and the dominant macrophytes were *Ceratophyllum demersum* and *Potamogeton maackianus* (He et al., 2015).

3.2 Antioxidant systems

Overall, antioxidants in these three life forms were obviously different and systematic differences were found in them, also prodigious differentiation within each type (Table 2). The average activities of SOD, CAT and APX were 219.4 U/mg protein, 11.4 U/mg protein and 1.0 U/mg protein for EM, and 250.4 U/mg protein, 3.2 U/mg protein and 0.5 U/mg protein for FM, and 411.0 U/mg protein, 2.6 U/mg protein and 1.95 U/mg protein for SM, respectively. The activities of SOD and APX in leaves of SM were much higher than those of EM and FM ($P<0.05$), CAT activity in leaves of FM and SM was much lower than those of EM ($P<0.05$), and the contents of TP in SM and FM were much higher than that of SM ($P<0.05$) (Table 2 and Fig.2). TP contents among all macrophytes were from 2.7 mg/g dry weight (DW) to 69.4 mg/g DW with mean value of 18.2 mg/g DW. EM and FM have significantly higher TP contents than that with submerged leaves ($P<0.05$), which was in accord with previous studies (Smolders et al., 2000; Cao et al., 2008).

In EM, TP were negatively correlated with CAT (R=-0.37, $P<0.05$). SOD was negatively correlated with CAT (R=-0.71, $P<0.001$), and CAT was positively correlated with APX (R=0.42, $P<0.01$) (Table 3). In SM, apparent inhibition among TP and all three enzymes was observed: TP-SOD (R=-0.41, $P<0.001$), -CAT (R=-0.47, $P<0.001$), -APX (R=-0.22, $P<0.05$). While mutual positive correlations between three enzymes were significant: SOD-CAT (R=0.36, $P<0.001$), SOD-APX (R=0.36, $P<0.001$), and CAT-APX (R=0.26, $P<0.01$) (Table 5). However, in FM, there were no obvious correlations among these four antioxidants, except SOD and CAT (R=-0.32, $P<0.05$) (Table 4).

In PCA analysis, most EM and FM species were closed to TP which means high TP contents, while SM ones gathered around SOD indicating that these species were characterized by high antioxidant enzymes (Fig.3).

4 DISCUSSION

In the present study, the activities of antioxidant enzymes (SOD, CAT and APX) and total soluble phenolic content differed greatly among three life forms. The results demonstrated that the aquatic macrophytes developed different antioxidant systems in response to the magnitude of water logging, with the increasing water logging the plants tended to

Table 2 The activities of antioxidant enzymes (U/mg protein) and contents of total phenolics (mg/g DW) in leaves of 26 aquatic and semi-aquatic macrophytes (mean±S.E.)

Species	*N*	LF	SOD (U/mg protein)	CAT (U/mg protein)	APX (U/mg protein)	TP (mg/g DW)
Ceratophyllum demersum L.	9	S	484.35±258.73 (159.98–2 548.62)	2.48±1.03 (0.52–10.46)	1.47±0.51 (0.13–5.10)	5.85±0.64 (3.21–8.95)
Myriophyllun spicatum L.	10	S	476.52±112.81 (171.83–1 377.06)	1.85±0.35 (0.71–3.61)	0.55±0.22 (0.03–1.21)	16.98±2.66 (5.99–30.23)
Trapa bispinosa Roxb.	9	FL	513.31±31.73 (33.62–288.14)	6.40±0.47 (1.45–6.42)	0.17±0.22 (0.11–1.82)	69.44±3.43 (58.1–81.99)
Nymphoides peltata (Gmel.) O. Kuntze	8	FL	130.52±28.50 (62.22–245.97)	3.44±0.46 (0.20–3.81)	0.88±0.04 (0.07–0.47)	14.78±3.15 (8.61–29.98)
Polygonum amphibium L.	8	FL	258.19±44.80 (123.86–448.32)	4.37±0.84 (1.13–7.46)	0.28±0.07 (0.03–0.54)	64.65±3.43 (53.79–78.53)
Polygonum hydropiper L.	6	E	385.41±155.41 (180.44–1 155.73)	7.49±2.11 (2.01–13.07)	0.47±0.10 (0.05–0.78)	53.85±3.70 (42.9–67.47)
Sagittaria sagittifolia L.	4	E	430.36±149.36 (112.81–831.66)	0.76±0.07 (0.70–0.90)	0.37±0.10 (0.07–0.56)	7.34±0.65 (6.41–9.20)
Zizania latifolia (Griseb.) Turcz.ex Stapf	10	E	53.30±12.18 (27.01–141.50)	36.15±5.17 (17.80–71.14)	2.10±0.15 (1.55–2.80)	12.60±0.29 (10.98–13.68)
Phragmites australis (Cav.) Trin.ex Steud.	7	E	148.95±25.75 (58.16–256.20)	5.90±2.75 (0.96–21.80)	2.01±0.63 (0.61–4.72)	15.54±1.68 (8.36–20.86)
Hydrocharis dubia (Blume) Backer	11	FL	178.64±29.50 (68.76–374.59)	2.86±0.91 (0.63–9.43)	0.18±0.04 (0.02–0.38)	5.22±0.25 (4.39–6.58)
Vallisneria natans (Lour.) H. Hara	14	S	910.52±183.17 (214.90–2 493.45)	5.38±0.93 (0.01–10.16)	6.25±3.00 (0.31–41.68)	3.40±0.21 (3.29–5.49)
Hydrilla verticillata (L.f.) Royle	11	S	330.56±74.05 (60.58–903.31)	1.50±0.31 (0.15–3.53)	3.25±1.23 (0.28–11.11)	5.22±1.30 (2.19–14.10)
Najas marina L.	3	S	130.26±3.28 (124.58–135.95)	7.32±1.50 (4.34–9.06)	2.42±0.29 (2.09–3.00)	5.80±0.64 (4.64–6.84)
Eichhornia crassipes (Mart.) Solms	11	FL	182.76±199.49 (50.74–558.78)	1.05±1.19 (2.28–13.80)	0.28±0.04 (0.03–0.39)	6.74±0.70 (4.56–12.45)
Potamogeton acutifolius Link	5	S	253.35±73.39 (103.88–490.77)	1.52±0.41 (0.35–2.72)	2.87±1.15 (0.40–6.71)	8.26±0.92 (6.67–11.65)
Potamogeton maackianus A. Benn.	8	S	89.62±13.64 (53.56–155.15)	0.35±0.08 (0.04–0.72)	1.02±0.16 (0.57–1.82)	20.08±1.85 (13.85–28.29)
Potamogeton perfoliatus L.	11	S	443.79±114.41 (102.44–1 199.19)	1.50±0.63 (0.18–5.69)	2.09±0.73 (0.20–8.33)	15.95±1.37 (9.12–22.71)
Potamogeton lucens L.	10	S	153.86±21.76 (68.09–240.83)	0.37±0.10 (0.08–0.84)	0.49±0.07 (0.21–0.83)	21.88±2.60 (8.10–36.98)
Potamogetn intortifolius J. B. He et al.	11	S	267.20±48.50 (83.90–629.08)	0.47±0.13 (0.11–1.20)	0.93±0.21 (0.20–2.39)	11.67±1.07 (7.93–18.4)
Potamogeton distinctus A. Benn.	4	FL	239.06±58.78 (129.63–403.74)	0.88±0.08 (0.68–1.04)	1.21±0.14 (0.92–1.54)	7.51±0.36 (6.50–8.10)
Potamogeton pectinata L.	10	S	1 262.09±376.09 (220.64–4 170.67)	1.73±0.50 (0.30–5.60)	1.82±0.39 (0.17–3.82)	4.57±0.40 (3.54–7.26)
Potamogeton malaianus Miq.	10	S	200.85±33.79 (84.43–442.27)	0.46±0.10 (0.06–0.86)	0.81±0.16 (0.06–1.95)	10.73±1.24 (5.40–19.50)
Nelumbo nucifera Gaertn.	10	E	148.67±34.70 (58.16–331.54)	4.36±1.03 (1.10–11.10)	0.74±0.11 (0.16–1.21)	49.96±6.07 (33.18–96.10)
Alternanthera philoxeroides (Mart.) Griseb.	9	E	56.17±3.66 (40.98–67.87)	22.07±1.01 (16.81–26.53)	0.48±0.05 (0.28–0.69)	11.31± 1.12 (8.78–16.63)
Acorus calamus L.	6	E	313.00±67.80 (94.30–556.61)	2.78±1.08 (0.91–8.00)	0.84±0.64 (0.04–4.01)	22.28±1.70 (17.06–27.53)
Chara sp.	11	S	340.14±94.21 (91.61–934.45)	8.87±2.42 (1.34–24.61)	1.42±0.45 (0.08–2.66)	2.70±0.20 (1.94–3.88)

LF: life form (S: submersed; FL: floating-leaved; E: emergent); *N*: number of replicate samples; SOD: superoxide dismutase; APX: ascorbate oxidase; CAT: catalase; TP: total phenolics.

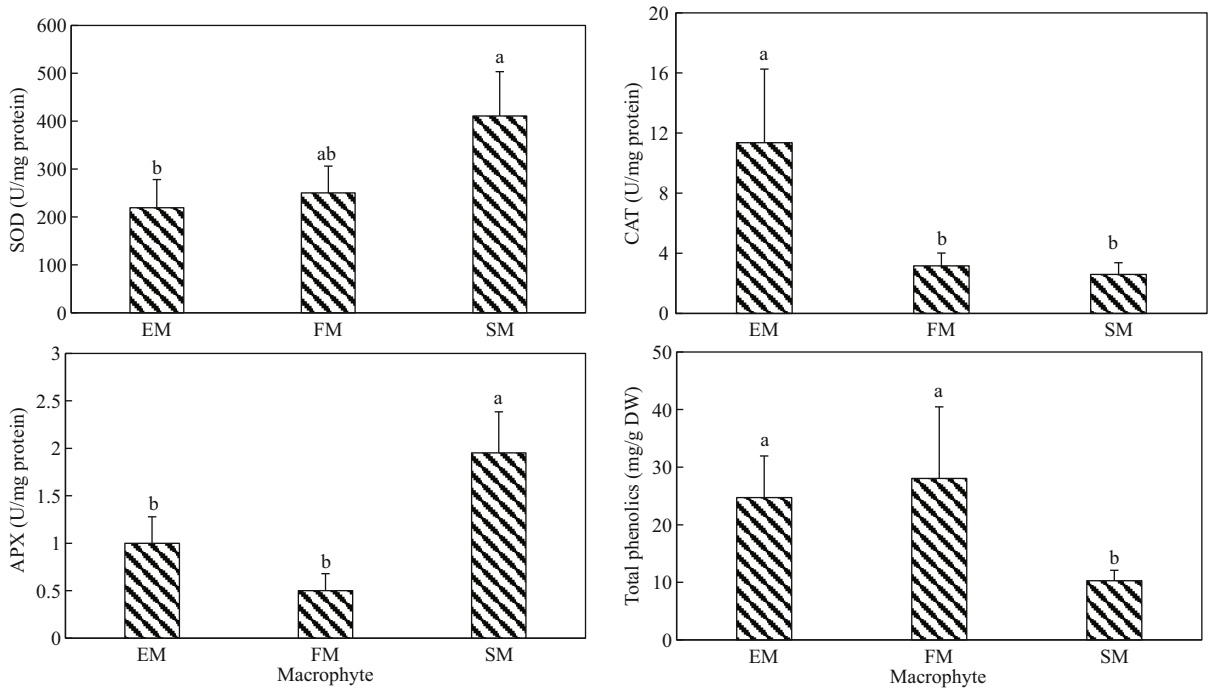

Fig.2 Comparison of antioxidants in macrophytes of three life forms

EM: emergent macrophytes; FM: floating-leaved macrophytes; SM: submerged macrophytes; SOD: superoxide dismutase; APX: ascorbate oxidase; CAT: catalase; DW: dry weight. Values are mean±S.E. Different letters means significant difference.

Table 3 Pearson's correlation analysis among antioxidant enzymes activities and total phenolics content in emergent macrophytes

EM	TP	SOD	CAT	APX
TP	/			
SOD	**0.30***	/		
CAT	**-0.37***	**-0.71*****	/	
APX	-0.23	-0.28	**0.42****	/

SOD: superoxide dismutase; APX: ascorbate oxidase; CAT: catalase; TP: total phenolics. *stands for $P<0.05$, ** stands for $P<0.01$ and *** stands for $P<0.001$. Bold means statistical significance.

Table 4 Pearson's correlation analysis among antioxidant enzymes activities and total phenolics content in floating-leaved macrophytes

FM	TP	SOD	CAT	APX
TP	/			
SOD	-0.26	/		
CAT	0.11	0.08	/	
APX	0.30	-0.32	-0.19	/

SOD: superoxide dismutase; APX: ascorbate oxidase; CAT: catalase; TP: total phenolics.

depend more on antioxidant enzymes than phenolics to cope with oxidative stress. Considering submersed macrophytes are difficult in gaining carbon dioxide and experience low light in water, which make it

Table 5 Pearson's correlation analysis among antioxidant enzymes activities and total phenolics content in submerged macrophytes

SM	TP	SOD	CAT	APX
TP	/			
SOD	**-0.41*****	/		
CAT	**-0.47*****	**0.36*****	/	
APX	**-0.22***	**0.36*****	**0.26****	/

SOD: superoxide dismutase; APX: ascorbate oxidase; CAT: catalase; TP: total phenolics. *stands for $P<0.05$, ** stands for $P<0.01$ and *** stands for $P<0.001$. Bold means statistical significance.

having low photosynthetic carbohydrate production, and thus depend more on antioxidant enzymes—a protein instead of phenolics that consumes much carbohydrates, particularly in eutrophic water where nitrogen and phosphorus are relatively cheap for protein synthesis.

Submersed macrophytes had an obviously higher SOD activity than emergent and floating-leaved macrophytes, this suggested that SM was subjected to more strongly oxidative stress of $\bullet O^{2-}$ than EM and FM on average. EM and FM living in habitats on or above the water surface with high oxygen concentration have relatively more complex organization structures (Wetzel, 2001), and higher photosynthesis rate (Bowes, 1985) combined with strong photoelectron transfer strength, which likely enable them generating

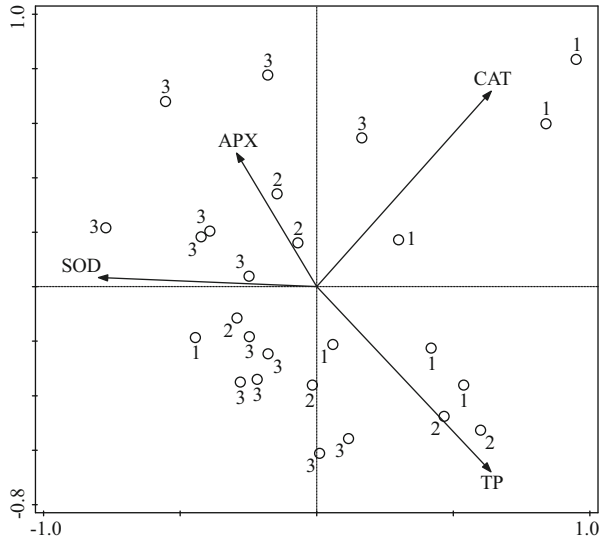

Fig.3 Principal component analysis (PCA) of all macrophytes' antioxidants

The two PCA axes explain 77.47% of total variation (PCA1: 43.76% and PCA2: 33.71%; SOD: superoxide dismutase; APX: ascorbate oxidase; CAT: catalase; TP: total phenolics. 1: emergent; 2: floating-leaved; 3: submerged.

more ROS and consequently showing high SOD activity. While SM under the water surface suffered the lower light intensity due to dense floating macrophyte mats and/or phytoplankton (Barko and Smart, 1981; Klančnik et al., 2018), and excessive absorption of nutrients which is an energy consumption behavior (Cao et al., 2007, 2009). In addition, some studies do have shown that in algal blooming period, FM and SM have a rapid rise in SOD activity, and SM are more vulnerable to stresses of eutrophication than FM (Zhang et al., 2011). Therefore, we speculate that these eutrophic factors might have stronger combined stress on SM than those on EM and FM.

EM and FM have significantly higher caloric contents than SM (Boyd, 1968; Lodge, 1991), thus they might be more attractive to herbivores. They also have high carbon (C) and nutrient contents which effects the synthesis of total phenolics (Cao et al., 2008), therefore they are potential to hold chemical defense against pathogens and herbivore. In addition, stronger intensity of photosynthesis in EM and FM brings higher photoelectronic transfer frequency than in SM, this would consume more energy and generate more ROS. However, in this study, SM produced more ROS which brought more intense oxidative stress reaction under eutrophic water than emergent and floating leaves. This would be a plausible explain for the above results. CAT and APX both take H_2O_2 as catalytic substrate (Mittler, 2002). The reason why CAT activity of SM was lower than those of EM and FM might be linked to the production of H_2O_2.

Phenolics production of plants is affected by environmental factors. For example, high light and low nutrient availability generally increase the production of phenolics by plants (Dudt and Shure, 1994; Vergeer and Van Der Velde, 1997; Cronin and Lodge, 2003). Environments above or on the water and underwater are so distinct that EM and FM are more vulnerable to herbivores and UV radiation than SM. According to Fryer et al. (1970), the number of phytophagous insects was highest for helophytes (semi-submerged) species and much lower for submerged macrophytes, with floating-leaved species having intermediate numbers of phytophagous species. Furthermore, most of EM and FM have a more conservative growth strategy with low turnover rate and underground storage organs such as stolon and rhizomes, which are consistent with a greater investment in chemical defense such as phenolics in these species (Smolders et al., 2000).

Compared to EM and FM, SM are under low predated pressure duo to the low nutrients contents (Yuan et al., 2016) and poor palatability, and low UV radiation which is inhibited by dissolved organic carbon (DOC) in water (Schindler et al., 1996). Hutchinson (1957) found that SM are also less well equipped with alkaloids and other secondary plant compounds than EM and FM. Therefore, we might infer that high content of phenolics is not necessary for SM, otherwise most SM are not well-equipped conditions for synthesis of phenolics. Moreover, some submerged species have low phenolics contents, such as *Chara* spp. (mean total soluble phenolics content 2.70±0.2 mg/g DW), producing allelopathic substances (Wium-Andersen et al., 1982; Ostrofsky and Zettler, 1986; Pakdel et al., 2013), and ones equipped with spines, which protect them from adverse environment.

In addition, high UV radiation in Lake Erhai might be another reason for the variations of TP in this study. Some scholars also believed that the content of phenolics is mainly related to the degree of photodamage (Close and McArthur, 2002), and the light intensity has positive effects on the synthesis of phenolics (Dudt and Shure, 1994). Herbivores and infections by pathogens, for instance, can induce denovo synthesis of phenolics (Hall, 1999), while differences in nutrients status and light intensity also lead to intraspecific variation in TP levels (Dudt and Shure, 1994; Vergeer and Van Der Velde, 1997).

Previous study showed that content of TP was positively correlated with leave C content (R=0.600, P<0.001) (Yuan et al., 2016), therefore we conjectured that high TP contents of EM and FM might partly be related to the strong light intensity and high carbon dioxide content in the air.

Significant negative relationships between TP and antioxidant enzymes were found in EM and SM in this study, representing that the two antioxidant pathways are reciprocally inhibited to a certain extant among them. Carbon-nutrient balance hypothesis (Bryant et al., 1983) suggests that resources in vivo only be used for synthesis of chemical defensive substances after meeting the normal growth need of plants, and their concentrations are limited by environmental carbon and nutrients availability, which indicate that secondary metabolic compounds like phenolics are depending on the internal carbon and nutrients levels. Related study (Cao et al., 2007) showed that a submerged macrophyte, *Vallisneria natans* L., absorbed excessive nutrients to produce more carbohydrates for detoxification of excess NH_4^+ into non-toxic free amino acids. Yuan et al. (2016) found that content of TP was positively correlated with those of carbon (C) and nitrogen (N) in leaves of all these macrophytes in Erhai, and unpublished data showed that activities of antioxidant enzymes also had some correlations with C and N contents. Therefore, these two pathways (antioxidant enzymes and phenolics) might have some competition on resources in vivo, and the low interdependence of them in FM might imply that the resources for antioxidant enzymes and phenolics were sufficient, while those of EM and SM were shortage.

5 CONCLUSION

Antioxidant strategies are diverse among macrophytes with different life forms, emergent and floating-leaved macrophytes both have a strategy with high phenolics might due to their exposure to air where there are high UV radiation and strong predation pressure from herbivores in their habitats. Photosynthetic carbon is relatively cheap in the air compared to the water column, so they might synthesize more phenolics to fight against herbivores, and it might be related to synthesis of lignin which is abundant in emergent macrophytes. While submerged macrophytes were more relying on antioxidant enzymes possibly because they were short of sufficient photosynthetic carbon. Floating-leaved macrophytes have a better balance between phenolics and antioxidant enzymes. However, great interspecific disparities are noticed in the study attributed to the synthetic action of evolution and external environment.

6 DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Almeselmani M, Deshmukh P S, Sairam R K, Kushwaha S R, Singh T P. 2006. Protective role of antioxidant enzymes under high temperature stress. *Plant Science*, **171**(3): 382-388, https://doi.org/10.1016/j.plantsci.2006.04.009.

Anderson D M, Glibert P M, Burkholder J M. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries*, **25**(4): 704-726, https://doi.org/10.1007/bf02804901.

Barko J W, Smart R M. 1981. Comparative influences of light and temperature on the growth and metabolism of selected submersed freshwater macrophytes. *Ecological Monographs*, **51**(2): 219-236, https://doi.org/10.2307/2937264.

Bixenmann C, López-Perea J J, Viñuela J, Florín M, Feliu J, Chicote Á, Cirujano S, Mateo R. 2016. Effects of invasive fish and quality of water and sediment on macrophytes biomass, and their consequences for the waterbird community of a Mediterranean floodplain. *Science of the Total Environment*, **551**: 513-521, https://doi.org/10.1016/j.scitotenv.2016.02.059.

Bowes G. 1985. Pathways of CO_2 fixation by aquatic organisms. *In*: Lucas W J, Berry J A eds. Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms. American Society of Plant Physiologists, Rockville, Maryland. p.187-210.

Bowler C, Van Montagu M, Inzé D. 1992. Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43**: 83-116, https://doi.org/10.1146/annurev.pp.43.060192.000503.

Boyd C E. 1968. Fresh-water plants-a potential source of protein. *Economic Botany*, **22**(4): 359-368, https://doi.org/10.1007/Bf02908132.

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**(1-2): 248-254, https://doi.org/10.1016/0003-2697(76)90527-3.

Bryant J P, Chapin F S, Klein D R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**(3): 357-368, https://doi.org/10.2307/3544308.

Cao T, Ni L Y, Xie P. 2004. Acute biochemical responses of a submersed macrophyte, *Potamogeton crispus* L., to high ammonium in an aquarium experiment. *Journal of Freshwater Ecology*, **19**(2): 279-284, https://doi.org/10.1080/02705060.2004.9664542.

Cao T, Xie P, Li Z Q, Ni L Y, Zhang M, Xu J. 2009. Physiological stress of high NH_4^+ concentration in water column on the submersed macrophyte *Vallisneria natans* L. *Bulletin of Environmental Contamination and Toxicology*, **82**(3): 296-299, https://doi.org/10.1007/s00128-008-9531-5.

Cao T, Xie P, Ni L Y, Wu A P, Zhang M, Wu S K, Smolders A J P. 2007. The role of NH_4^+ toxicity in the decline of the submersed macrophyte *Vallisneria natans* in lakes of the Yangtze River basin, China. *Marine and Freshwater Research*, **58**(6): 581-587, https://doi.org/10.1071/MF06090.

Cao T, Xie P, Ni L Y, Wu A P, Zhang M, Xu J. 2008. Relationships among the contents of total phenolics, soluble carbohydrate, and free amino acids of 15 aquatic macrophytes. *Journal of Freshwater Ecology*, **23**(2): 291-296, https://doi.org/10.1080/02705060.2008.9664201.

Chance B, Maehly A C. 1955. [136] Assay of catalases and peroxidases: Catalase: $2H_2O_2 \rightarrow 2H_2O + O_2$ (1), Catalase and Peroxidase: $ROOH + AH_2 \rightarrow H_2O + ROH + A$ (2). *Methods in Enzymology*, **2**: 764-775, https://doi.org/10.1016/S0076-6879(55)02300-8.

Close D C, McArthur C. 2002. Rethinking the role of many plant phenolics-protection from photodamage not herbivores? *Oikos*, **99**(1): 166-172, https://doi.org/10.1034/j.1600-0706.2002.990117.x.

Conley D J, Paerl H W, Howarth R W, Boesch D F, Seitzinger S P, Havens K E, Lancelot C, Likens G E. 2009. Controlling eutrophication: nitrogen and phosphorus. *Science*, **323**(5917): 1 014-1 015, https://doi.org/10.1126/science.1167755.

Cronin G, Lodge D M. 2003. Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia*, **137**(1): 32-41, https://doi.org/10.1007/s00442-003-1315-3.

Dai Y R, Wu J, Ma X H, Zhong F, Cui N X, Cheng S P. 2017. Increasing phytoplankton-available phosphorus and inhibition of macrophyte on phytoplankton bloom. *Science of the Total Environment*, **579**: 871-880, https://doi.org/10.1016/j.scitotenv.2016.11.002.

Dudt J F, Shure D J. 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, **75**(1): 86-98, https://doi.org/10.2307/1939385.

Frew A, Powell J R, Sallam N, Allsopp P G, Johnson S N. 2016. Trade-offs between silicon and phenolic defenses may explain enhanced performance of root herbivores on phenolic-rich plants. *Journal of Chemical Ecology*, **42**(8): 768-771, https://doi.org/10.1007/s10886-016-0734-7.

Fryer G, Gaevaskaya N S, Muller D G M, Mann K H. 1970. The role of higher aquatic plants in the nutrition of the animals of freshwater basins. *The Journal of Applied Ecology*, **7**(2): 388-389, https://doi.org/10.2307/2401394.

Hall A. 1999. Induced responses to herbivory. *Plant Pathology*, **48**(2): 294, https://doi.org/10.1046/j.1365-3059.1999.00340.x.

He L, Zhu T S, Cao T, Li W, Zhang M, Zhang X L, Ni L Y, Xie P. 2015. Characteristics of early eutrophication encoded in submerged vegetation beyond water quality: a case study in Lake Erhai, China. *Environmental Earth Sciences*, **74**(5): 3 701-3 708, https://doi.org/10.1007/s12665-015-4202-4.

Hutchinson G E. 1957. Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**: 415-427, https://doi.org/10.1101/SQB.1957.022.01.039.

Ibáñez C, Alcaraz C, Caiola N, Rovira A, Trobajo R, Alonso M, Duran C, Jiménez P J, Munné A, Prat N. 2012. Regime shift from phytoplankton to macrophyte dominance in a large river: top-down versus bottom-up effects. *Science of the Total Environment*, **416**: 314-322, https://doi.org/10.1016/j.scitotenv.2011.11.059.

Klančnik K, Iskra I, Gradinjan D, Gaberščik A. 2018. The quality and quantity of light in the water column are altered by the optical properties of natant plant species. *Hydrobiologia*, **812**(1): 203-212, https://doi.org/10.1007/s10750-017-3148-9.

Larson R A. 1988. The antioxidants of higher plants. *Phytochemistry*, **27**(4): 969-978, https://doi.org/10.1016/0031-9422(88)80254-1.

Leopoldini M, Marino T, Russo N, Toscano M. 2004. Density functional computations of the energetic and spectroscopic parameters of quercetin and its radicals in the gas phase and in solvent. *Theoretical Chemistry Accounts*, **111**(2-6): 210-216, https://doi.org/10.1007/s00214-003-0544-1.

Leopoldini M, Russo N, Chiodo S, Toscano M. 2006. Iron chelation by the powerful antioxidant flavonoid quercetin. *Journal of Agricultural and Food Chemistry*, **54**(17): 6 343-6 351, https://doi.org/10.1021/jf060986h.

Lodge D M. 1991. Herbivory on freshwater macrophytes. *Aquatic Botany*, **41**(1-3): 195-224, https://doi.org/10.1016/0304-3770(91)90044-6.

Metcalfe N B, Alonso-Alvarez C. 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology*, **24**(5): 984-996, https://doi.org/10.1111/j.1365-2435.2010.01750.x.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, **7**(9): 405-410, https://doi.org/10.1016/S1360-1385(02)02312-9.

Mole S, Waterman P G. 1987. A critical analysis of techniques for measuring tannins in ecological studies. *Oecologia*, **72**(1): 137-147, https://doi.org/10.1007/bf00385058.

Monferrán M V, Sánchez Agudo J A, Pignata M L, Wunderlin D A. 2009. Copper-induced response of physiological parameters and antioxidant enzymes in the aquatic macrophyte *Potamogeton pusillus*. *Environmental Pollution*, **157**(8-9): 2 570-2 576, https://doi.org/10.1016/j.envpol.2009.02.034.

Ostrofsky M L, Zettler E R. 1986. Chemical defences in aquatic plants. *Journal of Ecology*, **74**(1): 279-287, https://doi.org/10.2307/2260363.

Ozimek T, Kowalczewski A. 1984. Long-term changes of the submerged macrophytes in eutrophic lake Mikołajskie (North Poland). *Aquatic Botany*, **19**(1-2): 1-11, https://doi.org/10.1016/0304-3770(84)90002-0.

Pakdel F M, Sim L, Beardall J, Davis J. 2013. Allelopathic

inhibition of microalgae by the freshwater stonewort, *Chara australis*, and a submerged angiosperm, *Potamogeton crispus*. *Aquatic Botany*, **110**: 24-30, https://doi.org/10.1016/j.aquabot.2013.04.005.

Phillips G, Willby N, Moss B. 2016. Submerged macrophyte decline in shallow lakes: what have we learnt in the last forty years? *Aquatic Botany*, **135**: 37-45, https://doi.org/10.1016/j.aquabot.2016.04.004.

Qin B Q, Li W, Zhu G W, Zhang Y L, Wu T F, Gao G. 2015. Cyanobacterial bloom management through integrated monitoring and forecasting in large shallow eutrophic Lake Taihu (China). *Journal of Hazardous Materials*, **287**: 356-363, https://doi.org/10.1016/j.jhazmat.2015.01.047.

Rout N P, Shaw B P. 2001. Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. *Plant Science*, **160**(3): 415-423, https://doi.org/10.1016/S0168-9452(00)00406-4.

Schindler D W, Curtis P J, Parker B R, Stainton M P. 1996. Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes. *Nature*, **379**(6567): 705-708, https://doi.org/10.1038/379705a0.

Shao H B, Chu L Y, Shao M A, Jaleel C A, Mi H M. 2008. Higher plant antioxidants and redox signaling under environmental stresses. *Comptes Rendus Biologies*, **331**(6): 433-441, https://doi.org/10.1016/j.crvi.2008.03.011.

Smolders A J P, Vergeer L H T, Van Der Velde G, Roelofs J G M. 2000. Phenolic contents of submerged, emergent and floating leaves of aquatic and semi-aquatic macrophyte species: why do they differ? *Oikos*, **91**(2): 307-310, https://doi.org/10.1034/j.1600-0706.2000.910211.x.

Song J Q, Smart R, Wang H, Dambergs B, Sparrow A, Qian M C. 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of *Vitis vinifera* L. cv. Pinot noir wine. *Food Chemistry*, **173**: 424-431, https://doi.org/10.1016/j.foodchem.2014.09.150.

Su H J, Wu Y, Xie P, Chen J F, Cao T, Xia W L. 2016. Effects of taxonomy, sediment, and water column on C:N:P stoichiometry of submerged macrophytes in Yangtze floodplain shallow lakes, China. *Environmental Science and Pollution Research*, **23**(22): 22 577-22 585, https://doi.org/10.1007/s11356-016-7435-1.

Vergeer L H T, Van Der Velde G. 1997. Phenolic content of daylight-exposed and shaded floating leaves of water lilies (Nymphaeaceae) in relation to infection by fungi. *Oecologia*, **112**(4): 481-484, https://doi.org/10.1007/s004420050335.

Watson S B, Miller C, Arhonditsis G, Boyer G L, Carmichael W, Charlton M N, Confesor R,Depew D C, Höök T O, Ludsin S A, Matisoff G, McElmurry S P, Murray M W, Richards R P, Rao Y R, Steffen M M, Wilhelm S W, 2016. The re-eutrophication of Lake Erie: harmful algal blooms and hypoxia. *Harmful Algae*, **56**: 44-66, https://doi.org/10.1016/j.hal.2016.04.010.

Wetzel R G. 2001. Structure and productivity of aquatic ecosystems. *In*: Wetzel R G ed. Limnology. 3rd edn. Academic Press, San Diego, p.129-150.

Wium-Andersen S, Anthoni U, Christophersen C, Houen G. 1982. Allelopathic effects on phytoplankton by substances isolated from aquatic macrophytes (Charales). *Oikos*, **39**(2): 187-190, https://doi.org/10.2307/3544484.

Yuan C B, Cao T, Zhou C Y, Ni L Y, Zhang X L. 2016. Contents of C, N and total phenols in leaves of aquatic macrophytes in lake Erhai, China. *Acta Hydrobiologica Sinica*, **40**(5): 1 025-1 032, https://doi.org/10.7541/2016.133. (in Chinese with English abstract)

Zhang M, Cao T, Ni L Y, Xie P, Li Z Q. 2010. Carbon, nitrogen and antioxidant enzyme responses of *Potamogeton crispus* to both low light and high nutrient stresses. *Environmental and Experimental Botany*, **68**(1): 44-50, https://doi.org/10.1016/j.envexpbot.2009.09.003.

Zhang M, Wang Z Q, Xu J, Liu Y Q, Ni L Y, Cao T, Xie P. 2011. Ammonium, microcystins, and hypoxia of blooms in eutrophic water cause oxidative stress and C-N imbalance in submersed and floating-leaved aquatic plants in Lake Taihu, China. *Chemosphere*, **82**(3): 329-339, https://doi.org/10.1016/j.chemosphere.2010.10.038.

Zhu G R, Yuan C B, Di G L, Zhang M, Ni L Y, Cao T, Fang R T, Wu G G. 2018. Morphological and biomechanical response to eutrophication and hydrodynamic stresses. *Science of the Total Environment*, **622-623**: 421-435, https://doi.org/10.1016/j.scitotenv.2017.11.322.