Knowledge and Management of Aquatic Ecosystems (2013) 410, 07 http://www.kmae-journal.org

DOI: 10.1051/kmae/2013062

Effects of shading on *Vallisneria natans* (Lour.) H. Hara growth

A.D. Fox[(1)], F. Meng[(2)], X. Shen[(2)], X. Yang[(2)], W. Yang[(2)], L. Cao[(2),⋆]

Received May 6, 2013
Revised June 9, 2013
Accepted June 13, 2013

ABSTRACT

Key-words: *China, Macrophyte reproduction, Trapa, Vegetative reproduction, Yangtze River*

Effects of surface shading were measured on above- and below-ground biomass and fruit production of *Vallisneria natans* (Lour.) H. Hara plants grown from seed in replicated microcosm experiments, based on a control (no shading) and four treatments (25%, 50%, 75% and 90% shading). Above- and below-ground biomass was significantly reduced at treatments above 50% shading and first pistillate and staminate florescence dates were significantly delayed above 75% and 50% shading, respectively. Ratios of mature to unripe fruits produced (both in number or dry weight) did not differ between shading treatments, but dry weight fruit production was significantly reduced at 90% shading. We conclude that above 50% surface shading, *V. natans* plants suffer reductions in accumulated biomass and investment in sexual reproduction. We contend that recent expansions in the extent of the native floating water chestnut *Trapa* spp. at seasonally inundated wetlands in the Yangtze River floodplain could, by shading, have contributed to the reduction in annual biomass and seed production of *V. natans*, contributing to declines in distribution and abundance.

RÉSUMÉ

Effets de l'ombrage sur la croissance de *Vallisneria natans* (Lour.) H. Hara

Mots-clés : *Chine, reproduction macrophytes, Trapa, reproduction végétative, Yangtsé*

Les effets de l'ombrage de surface ont été mesurés sur la biomasse au-dessus et en dessous du sol et la production de semences de *Vallisneria natans* (Lour.) H. Hara, plantes issues de semences, dans des expériences en microcosmes répliqués, avec un contrôle (sans ombrage) et quatre traitements (25 %, 50 %, 75 % et 90 % d'ombrage). La biomasse au-dessus et en dessous du sol a été considérablement réduite aux traitements supérieurs à 50 % d'ombrage et les dates de floraison femelle et mâle ont été considérablement retardées au-dessus de 75 % et 50 % d'ombrage, respectivement. Les proportions de fruits produits à maturité ou non (tant en nombre ou qu'en poids sec) ne différaient pas entre les traitements d'ombrage, mais la production de fruits en poids sec a été significativement réduite à 90 % d'ombrage. Nous concluons qu'au-dessus de 50 % d'ombrage de surface, la plante *V. natans* souffre de réductions de sa croissance en biomasse et dans son investissement dans la reproduction sexuée. Nous soutenons que les expansions récentes de la châtaigne d'eau *Trapa spp*. dans les zones humides saisonnièrement inondées de la plaine du fleuve Yangtsé pourrait, par ombrage, avoir contribué à la réduction de la biomasse annuelle et de la production de semences de *V. natans,* contribuant à la baisse dans sa distribution et son abondance.

(1) Department of Bioscience, Aarhus University, Kalø, Grenåvej 14, 8410 Rønde, Denmark
(2) School of Life Sciences, University of Science and Technology of China, 96 Jinzhai Road, Hefei, Anhui 230026, P.R. China
⋆ Corresponding author: caolei@ustc.edu.cn

INTRODUCTION

Light attenuation through the water column is critical to aquatic plant growth, because of effects on photosynthetic efficiency. Reductions in light levels have been shown to have adverse effects on *Zostera marina* L. photosynthesis (Dennison and Alberte, 1982), growth (Dennison and Alberte, 1985; Dennison, 1987), community structure (Backman and Barilotti, 1976) and, ultimately, individual long-term survival (Zimmerman *et al*., 1995; Moore *et al*., 1996). Growth of more vigorous aquatic plants can create competitive interactions through shading effects affecting individual plant fitness (*e.g.* Wang *et al.*, 2008). Some aggressive floating species (such as water chestnuts *Trapa* spp. in Yangtze River floodplain lake systems) can completely cover the water surface, dramatically reducing light penetration to submerged macrophyte species (such as *Vallisneria* spp.).

At Shengjin Lake, Anhui Province (116° 55′ to 117° 15′ E; 30° 15′ to 30° 30′ N), as elsewhere in the Yangtze River basin, recent dramatic reductions in submerged vegetation, particularly the tuber-producing *V. spinulosa* S.Z. Yan, have been cited as the cause of major declines in wintering numbers of Swan Geese *Anser cygnoides* L., Tundra Swans *Cygnus columbianus bewickii* Yarrell and other tuber-feeding birds (Zhang *et al.*, 2011; Fox *et al*., 2011). The causes of general declines in *Vallisneria* spp. are unknown, but summer cover and abundance of *Trapa* spp. in areas formerly dominated by *Vallisneria* spp. suggests that these floating plants may be shading submerged macrophytes by recent aggressive colonization of the surface (see satellite imagery in Zhang *et al.* 2011). Water chestnut plant rosettes typically completely cover the water surface, affecting dissolved oxygen levels in the water column (Goodwin *et al*., 2008) and enabling mapping by remote sensing imagery (Laba *et al*., 2010). Remote sensing of Shengjin Lake showed that the extent of *Trapa* spp. increased from nothing in the early 1990s to 4.2 km^2 in summer 2002 to 9.1 km^2 in summer 2007, covering former dense beds of *Vallisneria* spp. (Zhang *et al.*, 2011). The expansion of newly colonizing floating *Trapa* spp. may therefore have affected growth and productivity of *Vallisneria* spp. in these areas.

To test this hypothesis, we undertook microcosm experiments to determine whether increasing surface shading would affect above- and below-ground biomass production, investment in sexual reproduction and development of mature fruits that could contribute to the explanation of the disappearance of *Vallisneria* spp. at Shengjin Lake.

MATERIAL AND METHODS

> *GENERAL EXPERIMENTAL CONDITIONS*

A microcosm study was carried out on the rooftop of the Life Sciences Building at University of Science and Technology (117° 15′ 3.4″ E; 31° 50′ 26.9″ N), Hefei. Microcosms were exposed to ambient climatic and light conditions and contained substrates which were thoroughly and evenly mixed from one part standard potting compost from Anhui Academy of Agricultural Sciences and two parts campus soil. University tap water was used in all experiments which resulted in average nitrogen and phosphorous levels of 0.95 (±0.145 95% CI) $mg \cdot L^{-1}$ and 0.18 (±0.039 95% CI) $mg \cdot L^{-1}$ within the microcosms. We determined TN and TP using potassium persulphate oxidation and a 721-type spectrophotometer to determine absorption at 200–210 nm and 700 nm wavelengths, following Jin and Tu (1996). Yangtze River lakes containing *Vallisneria* spp. typically have TN varying between 0.05 and 9.00 $mg \cdot L^{-1}$ and TP between 0.004 and 1.000 $mg \cdot L^{-1}$ (Xiong and Li, 2000; Xu and Jiang, 2009). *Vallisneria* spp. has been shown to remove TP and TN at rates above 60% in eutrophicated waters (Zhao *et al*., 2010).

V. natans commences vigorous growth above 20 °C (Li and Cui, 2000), which typifies average temperatures in Hefei from late April to early October (http://www.tutiempo.net/en/Climate/Hefei.htm). We therefore selected 1 April–5 November 2010 as the growth period to ensure that the plants could complete their annual reproductive cycle.

> INITIAL CULTIVATION

On 1 April 2010, 4 g of *V. natans* seeds was placed in substrate in 3 cm of water in each of six plastic bowls (internal dimensions 31 × 21.5 × 12 cm). Seeds germinated after 10 h exposure to sunlight, producing white roots, after which they were transferred to six plastic boxes (46 × 35 × 26.5 cm) with 10 cm substrate under 5 cm water. Phytoplankton and periphyton were removed twice per day to maintain clear water. Water was added as necessary to ensure leaves were submerged at all times. On 15 May, plants were thinned to ensure that inter-plant distances were at least 3 cm.

> REPLANTING

On 28 May, 100 plants of similar height and leaf-state were selected for transplanting from those whose leaves were floating on the water surface; leaf lengths of each plant (*i.e.* initial biomass before transplanting) were measured prior to transplanting. In each of 50 large 80 cm-high microcosms (internal diameters: top 61 cm; bottom 50 cm) containing 30 cm of soil, two plants were transplanted equally spaced in 5 cm holes. After transplanting, water depth was maintained at 50 cm, the maximum possible) and plants were left for one month to recover from transplanting.

> SHADING TREATMENTS

Five shading treatments were each applied to 10 microcosms covering 0% (control), 25%, 50%, 75% and 90% of the surface (representing 100%, 54%, 31%, 20% and 12% of natural light respectively, tested by Digital Lux Meter Tes-1330A at noon) on 27 June, after which leaf lengths of the plants of each container were measured again. Each cover was constructed from two layers of tightly woven fabric (16 rows per cm) sown together: a lower black layer completely restricted light penetration into the microcosm, covered by an upper thick white insulating layer to reduce heating of the microcosm and its contents. The two cloth layers were supported by an iron frame fixed to the top of each microcosm by wire. Different areas of cloth were used to achieve the necessary degree of coverage of the water surface to provide the four shading treatments.

Water was added to the microcosms every two or three days to maintain water depth at 50 cm. Observations were made every day and changes recorded, such as florescence time and fruit maturity; windblown material. phytoplankton and periphyton (which would otherwise differentially shade and introduce a compounding variable) were removed from microcosms to maintain water clarity.

> HARVEST

All microcosms were drained on 5 November and plants washed with running water. Plant parts from each microcosm were cleaned and sorted into leaves, mature fruits, unripe fruits, peduncles, spathes, stolons and roots. Maximum shoot leaf length, the total numbers of shoots, mature fruits and unripe fruits were recorded, as well as the numbers of male and female plants. All parts were weighed after drying for 48 h at 60°.

> DATA ANALYSIS

We calculated the above-ground biomass (AGB dry weight of leaves) and the below-ground biomass (BGB dry weight of stolons and roots) present at the end of the experiment in each container. In addition we recorded the mean dates of first pistillate and staminate florescence weighed the dry mass of mature fruits and determined the ratio of unripe to mature fruits. Data analysis was based on ANOVA tests and Duncan's Multiple Range tests (alpha = 0.05) in SPSS 16.0 (IBM Corporation, Somers, NY 10589).

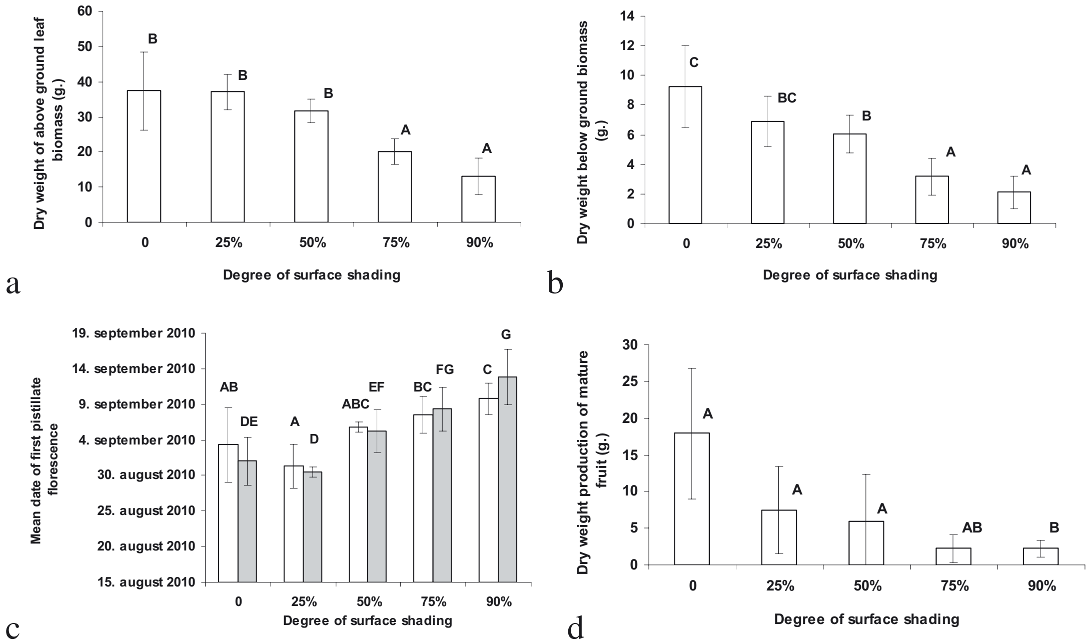

Figure 1

(a) Mean (±95% confidence intervals) above-ground dry-weight Vallisneria natans biomass, (b) below-ground biomass; (c) mean (±95% confidence intervals) date of first pistillate (open histograms) and staminate (shaded) florescence of Vallisneria natans plants under different surface shading treatments and (d) dry weight of fruits accumulated at harvest time under different surface shading treatments in microcosms. Treatments with the same letter indicate no significant difference between these treatment means based on Duncan's Multiple Range tests.

RESULTS

> INITIAL PLANT BIOMASS

There were no significant differences between the total leaf lengths of the plants selected for each of the experimental treatments either at the transplant stage to individual containers (May 28, ANOVA $F_{4,49} = 0.52$, $p = 0.72$) or at the commencement of each shading experimental treatment (June 26/27, ANOVA $F_{4,40} = 0.06$, $p = 0.99$).

> PRODUCTION OF ABOVE-GROUND BIOMASS

There were significant differences between the shading treatments in the mean AGB at harvest (Figure 1a, ANOVA $F_{4,49} = 11.05$, $p < 0.001$). Duncan's Multiple Range tests showed no significant differences between AGB with 75% and 90% shading, which were significantly less (reductions of more than 50% at 90% shading) than the control and other shading treatments, which did not differ significantly from each other (Figure 1a).

> PRODUCTION OF BELOW-GROUND BIOMASS

There were significant differences between the treatment means of BGB at harvest (Figure 1b, ANOVA $F_{4,49} = 10.56$, $p < 0.001$). Duncan's Multiple Range tests showed no significant differences between the control (0% shading) and 25% shading and that there were no significant differences between 75% and 9% shading treatments but that these were significantly less (>50% less) than the control and other treatments (Figure 1b).

> TIMING OF FLORESCENCE

The date of first pistillate florescence was related to the degree of shading, the mean date for 90% shaded plants being significantly later than those of the control and 25% shading treatments (Figure 1c, ANOVA $F_{4,33} = 4.10$, $p = 0.009$). The date of the first staminate florescence was similarly related to degree of shading, the mean date for the 75% and 90% shading treatments being significantly delayed relative to the control (Figure 1c, ANOVA $F_{4,40} = 11.55$, $p < 0.001$).

> PRODUCTION OF MATURE FRUITS

There were no significant differences between the ratio of dry weight production of mature fruits and unripe fruits (ANOVA $F_{4,24} = 0.56$, $p = 0.69$) nor the ratio of mature to unripe fruits (ANOVA $F_{4,24} = 0.49$, $p = 0.74$) between shading treatments. There were significant differences between the shading treatments in the dry weight biomass production of mature fruits (Figure 1d, ANOVA $F_{4,24} = 4.88$, $p = 0.007$); Duncan's Multiple Range tests showed the mean of the 90% shading treatment was significantly less than the control, 25% and 50% treatments, and the 75% shading treatment did not differ from any of the other treatments (Figure 1d).

DISCUSSION

Although the results of the microcosm experiments may not completely reflect conditions in nature under identical growth conditions >50% shading of the water surface was associated with significant reductions in AGB and BGB in *V. natans* replicates compared to controls. Florescence was significantly delayed by 90% surface shading amongst both male and female flowers relative to controls. There were no significant differences in the ratio of numbers and biomass of unripe to mature fruits between the controls and any of the shading treatments, but dry weights of mature fruit production under 90% surface shading were significantly less than controls. Hence, at shading greater than 90% there were adverse effects on AGB and BGB accumulation and reproductive investment of *V. natans* plants, even though the species is adapted to low light environments, especially late in the growth season (Cai *et al.*, 2012) Light has also been shown to affect BGB of the saline *V. americana* Michx. (Boustany *et al*., 2010).

Trapa species of the Yangtze River floodplain are annual species with floating rosette leaves, subsurface feather leaves and a complex system of stems, pseudo roots and roots anchored in the substrate (Tsuchiya and Iwaki, 1984; Hummel and Kiviat, 2004). Dense *Trapa* spp. canopies limit light penetration and promote water column hypoxia (Caraco and Cole, 2002; Bolpagni *et al*., 2006 and 2007; Hummel and Findlay, 2006; Pierobon *et al*., 2007; Goodwin *et al*., 2008). *Trapa* spp. coverage at Shengjin Lake exceeds 95% in denser beds (Cao L. *unpubl. data*), so it is not unreasonable to assume that *V. natans* plants growing below will experience similar or greater shading levels to those of the 90% treatment levels of this microcosm experiment.

The extent of *Vallisneria* spp. at Shengjin Lake declined greatly in recent years, especially in the Upper Lake in areas covered since 2007 with *Trapa* spp. (see map in Fox *et al*., 2011). In 1974, the whole lake was covered by the submerged macrophytes *Vallisneria* spp. and *Potamogeton* spp. (Meng, 1979) and 70% of the Upper Lake in 1996–1998 (Liu *et al*., 2001). Summer surveys in 2008 and 2009 found little *Vallisneria* spp. (Fox *et al*., 2011) and none in 2010 (Cao L. *unpubl., data*). Various environmental factors could have been responsible for this range contraction and ultimate loss of *Vallisneria* spp., which has disappeared in recent years from many lakes in the Yangtze River floodplain (Fang *et al*., 2006). This change may be associated with eutrophication of Yangtze River floodwater that fills Shengjin Lake during summer (*e.g.* Li *et al*., 2007), because it is known that photosynthesis in this species can be inhibited under high nutrient loading (Cai *et al.*, 2012). This could potentially be mediated by the associated enhanced growth of *Trapa* spp., which are known to respond positively to elevated N and P levels in the water column (Li *et al*., 2010), as well as its shading effects on submerged vegetation.

Earlier experiments showed shortening of the inundation period could adversely affect the annual accumulation of biomass and investment in sexual reproduction of *V. natans* (Cao *et al.*, 2011). The microcosm experiment results presented here further confirm that shading levels, similar to those experienced under a floating *Trapa* spp. canopy, caused reductions in AGB ad BGB and reproductive success which could affect the competitive ability of *V. natans,* contributing to declines. We therefore contend that recent expansion in the extent of floating *Trapa* spp. at seasonally inundated Yangtze River floodplain wetlands could have contributed to reductions in annual biomass production and seed production of this important submerged macrophyte and contributed to declines in its distribution and abundance. Such changes have had important knock-on effects for the waterbird and fish communities of such lakes (see for example Fox *et al.* 2011 and Zhang *et al.* 2011) and doubtless had other trophic effects through these aquatic systems. However, proof that *Trapa* spp. is the ultimate cause of the declines in *Vallisneria* spp. must await manipulative field experiments to confirm cause and effect.

ACKNOWLEDGEMENTS

The study was supported by the Chinese Academy of Sciences Innovation Project (Grant No. KZCX2-YW-QN503), National Basic Research Program of China (973 Program, Grant No. 2012CB956104), the National Natural Science Foundation of China (Grant No.31071941), University of Science and Technology of China Graduate School Educational Innovation Base, Plus111 Project, State Administration of Foreign Experts Affairs, PRC and a Danske Universiteter travel grant to one of the authors (ADF). Thanks to Mark Barter for help in preparation of the manuscript, other members of the laboratory for assistance with sorting of plant material and thank the editor and two anonymous referees for improvements to an earlier draft.

REFERENCES

Backman T.W. and Barilotti D.C., 1976. Irradiance reduction: effects on standing crops of the eelgrass *Zostera marina* in a coastal lagoon. *Mar. Biol., 34*, 33–40.

Bolpagni R., Bartoli M. and Viaroli P., 2006. Nitrogen and phosphorus cycling in an oxbow lake dominated by *Trapa natans* L. *Verh. Int. Ver. Theor. Angew. Limnol., 29*, 1981–1988.

Bolpagni R., Pierobon E., Bartoli M., Nizzoli D., Tomaselli M. and Viaroli P., 2007. Methane and carbon dioxide water-atmosphere daily exchanges in an oxbow lake with a *Trapa natans* stand. *Aquat. Bot., 87*, 43–48.

Boustany R.G., Michot T.C. and Moss R.F., 2010. Effects of salinity and light on biomass and growth of *Vallisneria americana* from Lower St. Johns River, FL, USA. *Wetlands Ecol. Manage., 18*, 203–217.

Cai X.L., Gao G., Tang X.M., Dai J.Y. and Chen D. 2012. Photosynthetic response of *Vallisneria natans* (Lour.) Hara (Hydrocharitaceae) to increasing nutrient loadings. *Photosynthetica, 50*, 437–446

Cao L., Meng F.J., Yang W., Shan K.Q., Liu J. and Fox A.D., 2011. Effects of length of growing season on biomass accumulation and reproductive investment of *Vallisneria natans* (Lour.) H. Hara. *Fundam. Appl. Limnol., 179/2*, 115–120.

Caraco N.F. and Cole J.J., 2002. Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. *Ecol. Appl., 12*, 1496–1509.

Dennison W.C., 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquat. Bot., 27*, 15–26.

Dennison W.C. and Alberte R.S., 1982. Photosynthetic responses of *Zostera marina* L. (eelgrass) to *in situ* manipulations of light intensity. *Oecologia, 55*, 137–144.

Dennison W.C. and Alberte R.S., 1985. Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). *Mar. Ecol. Prog. Ser., 25*, 51–61.

Fang J., Wang Z., Zhao S., Li Y., Tang Z., Yu D., Ni L., Liu H., Xie P., Da L., Li Z. and Zheng C., 2006. Biodiversity changes in the lakes of the Central Yangtze. *Front. Ecol. Environ., 4*, 369–377.

Fox A.D., Cao L., Zhang Y., Barter M., Zhao M.J., Meng F.J., and Wang S.L., 2011. Declines in the tuber-feeding waterbird guild at Shengjin Lake National Nature Reserve, China – a barometer of submerged macrophyte collapse. *Aquatic Conserv.*: *Mar. Freshw. Ecosyst., 21*, 82–91.

Goodwin K., Caraco N. and Cole J., 2008. Temporal dynamics of dissolved oxygen in a floating leaved macrophytes bed. *Freshw. Biol., 53*, 1632–1641.

Hummel M. and Findlay S., 2006. Effects of water chestnut (*Trapa natans*) beds on water chemistry in the tidal freshwater Hudson River. *Hydrobiologia, 559*, 169–181.

Hummel M. and Kiviat E., 2004. Review of world literature on water chestnut with implications for management in North America. *J. Aquat. Plant Manag., 42*, 17–28.

Jin X.C. and Tu Q.Y., 1996. Specifications for Lake Eutrophication (Second edition), China Environmental Science Press, Beijing, 162–164 (in Chinese).

Laba M., Blair B., Downs R., Monger B., Philpot W., Smith S., Sullivan P. and Baveye P.C., 2010. Use of textural measurements to map invasive wetland plants in the Hudson River National Estuarine Research Reserve with IKONOS satellite imagery. *Remote Sens. Environ., 114*, 876–886.

Li M., Xu K., Watanabe M. and Chen Z., 2007. Long-term variations in dissolved silicate, nitrogen, and phosphorus flux from the Yangtze River into the East China Sea and impacts on estuarine ecosystem. *Est. Coast. Shelf Sci., 71*, 3–12.

Li K.Y., Liu Z.W. and Guan B.H., 2010. Effects of nutrient levels in surface water and sediment on the growth of the floating-leaved macrophyte *Trapa maximowiczii*: implication for management of macrophytes in East Bay of Lake Taihu, China. *Limnology, 11*, 95–101.

Li Y.D. and Cui Y.Q., 2000. Germination experiment on seeds and stem tubers of *Vallisneria natans* in Lake Donghu of Wuhan. *Acta Hydrobiol. Sin., 24*, 298–300 (in Chinese).

Liu Z.Y., Xu W.B., Wang Q.S., Shi K.C., Xu J.S. and Yu G.Q., 2001. Environmental carrying capacity of over-wintering Hooded Cranes at Shengjin Lake. *Resour. Environ. Yangtze Basin, 10*, 454–459 (in Chinese).

Meng R.X., 1979. The aquatic vegetation of Shengjin Lake. *Anhui Univ. Trans. (Nat. Sci. Sect.), 3*, 71–80 (in Chinese).

Moore K.A., Neckles H.A. and Orth R.J., 1996. *Zostera marina* (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay. *Mar. Ecol. Prog. Ser., 142*, 247–259.

Pierobon E., Bolpagni R., Bartoli M. and Viaroli P., 2007. Net primary production and seasonal CO_2 and CH_4 fluxes in a *Trapa natans* L. meadow. *J. Limnol., 69*, 225–234.

Tsuchiya T. and Iwaki H., 1984. Seasonal changes in photosynthesis and primary production of a floating-leaved plant, *Trapa natans* L., community in Lake Kasumigaura, Japan. *Jpn J. Ecol., 34*, 367–374.

Wang J.W., Dan Y.D. and Xiong W., 2008. Above- and below-ground competition between two submersed macrophytes. *Hydrobiologia, 607*, 113–122.

Xiong B.H. and Li W., 2000. Ecology studies on *Vallisneria* L. in China (in Chinese). *J. Wuhan Bot. Res., 18*, 500–508.

Xu Z.X. and Jiang Y.P., 2009. Lake eutrophication assessment: comprehensive water quality identification index (in Chinese). *J. Tongji Uni. (Nat. Sci.), 37*, 1044–1048.

Zhang Y., Cao L., Barter M., Fox A.D., Zhao M.J., Meng F.J., Shi H.Q., Jiang Y. and Zhu., W., 2011. Changing distribution and abundance of Swan Goose *Anser cygnoides* in the Yangtze River floodplain: the likely loss of a very important wintering site. *Bird Conserv. Int., 20*, 1–13.

Zhao D., Xu W.J., Li Y. and Chen H., 2010. Purification of eutrophicated water by *Vallisneria spinulosa*. *Chin. Agric. Sci. Bull., 26*, 189–192 (in Chinese).

Zimmerman R.C., Reguzzone J.L. and Alberte R.S., 1995. Eelgrass (*Zostera marina* L.) transplants in San Francisco Bay: role of light availability on metabolism, growth and survival. *Aquat. Bot., 51*, 67–86.