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Megalobrama amblycephala grazes preferentially on *Hydrilla verticillata* but makes more efficient use of *Vallisneria denseserrulata*: implications for biological control of submerged macrophytes

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Abstract – Growth of the meadow-forming macrophyte *Vallisneria denseserrulata* is often negatively impacted as result of shading by the canopy-forming *Hydrilla verticillata*. Grazing by the herbivorous cyprinid *Megalobrama amblycephala* is thought to control *H. verticillata.* We hypothesized that *M. amblycephala* would prefer *H. verticillata* over *V. denseserrulata*, and that where the latter is grazed, its growth will not be wholly compromised, due in part to the efficiency with which it is metabolized by the grazer. In a pond experiment, macrophytes were planted with monocultures of *H. verticillata* and *V. denseserrulata*, and in mixed cultures of the two species, with and without grazing by *M. amblycephala*. The results showed that in the absence of fish, the growth rate of *V. denseserrulata* was significantly reduced in the presence of *H. verticillata*. In the mixture, *M. amblycephala* had a significant negative effect on the growth of *H. verticillata* but not on *V. denseserrulata*. Grazing was associated with reductions in plant height, plant fresh weight and leaf fresh weight but the leaf number, maximum root length, maximum blade width, root weight and stem weight in *V. denseserrulata* increased. The food utilization ratio of *M. amblycephala* was significantly higher when grazing solely on *V. denseserrulata than* when grazing on *H. verticillata* or the mixture. Our results imply that *V. denseserrulata* is protected from overgrazing by the ability of *M. amblycephala* to make more effective metabolic use of ingested material than for *H. verticillata*. Furthermore, *M. amblycephala* is beneficial to *V. denseserrulata* in reducing competition from *H. verticillata*.

Keywords: Submerged macrophytes / herbivorous fish / competition / utilization rate / growth morphology

Résumé – La croissance du macrophyte tapissant *Vallisneria denseserrulata* est souvent affectée négativement par l'ombrage causé par *Hydrilla verticillata* qui forme une canopée. Le broutage par le cyprinidé herbivore *Megalobrama amblycephala* est censé contrôler *H. verticillata*. Nous avons émis l'hypothèse que *M. amblycephala* préférerait *H. verticillata* à *V. denseserrulata*, et que lorsque ce dernier est brouté, sa croissance ne sera pas entièrement compromise, en partie à cause de l'efficacité avec laquelle il est métabolisé par le brouteur. Dans une expérience en étang, des macrophytes ont été plantés en monocultures de *H. verticillata* et *V. denseserrulata*, et en cultures mixtes des deux espèces, avec et sans broutage par *M. amblycephala*. Les résultats ont montré qu'en l'absence de poisson, le taux de croissance de *V. denseserrulata* était significativement réduit en présence de *H. verticillata*. Dans le mélange,

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M. amblycephala a eu un effet négatif significatif sur la croissance de *H. verticillata* mais pas sur *V. denseserrulata*. Le broutage a été associé à une réduction de la taille des plantes, du poids frais des plantes et du poids frais des feuilles, mais le nombre de feuilles, la longueur maximale des racines, la largeur maximale du limbe, le poids des racines et le poids des tiges chez *V. denseserrulata* ont augmenté. Le taux d'utilisation de la nourriture de *M. amblycephala* était significativement plus élevé lorsqu'il broutait uniquement sur *V. denseserrulata* que lorsqu'il broutait sur *H. verticillata* ou le mélange. Nos résultats impliquent que *V. denseserrulata* est protégé du surbroutage par la capacité de *M. amblycephala* à faire un usage métabolique plus efficace du matériel ingéré que pour *H. verticillata*. De plus, *M. amblycephala* est bénéfique pour *V. denseserrulata* en réduisant la concurrence de *H. verticillata*.

Mots clés: Macrophytes submergés / poissons herbivores / compétition / taux d'utilisation / morphologie de croissance

1 Introduction

Canopy-forming submerged macrophytes typically outcompete sympatric meadow-forming species for light (Van *et al.*, 1999). *Hydrilla verticillata*, a fast-growing canopy-forming species, thrives in shallow eutrophic lakes and can drive rapid improvements in water transparency (Langeland, 1996; Bianchini *et al.*, 2010). However, massive growth of *H. verticillata* can also limit the availability of light and oxygen in the lower layers of the water column, resulting in an overall adverse effect on water quality (Hilt *et al.*, 2006). Shading by *H. verticillata* can be detrimental to the growth of meadow-forming submerged macrophytes such as *Vallisneria denseserrulata,* whose biomass is concentrated in the lower layers of the water column (Gopal and Goel, 1993). *V. denseserrulata* is also commonly used in lake restoration, since it forms a network of well-developed roots that help to bind the substratum, maintaining stability and reducing the resuspension of sediment (Hayashi *et al.*, 1988; Smith and Adams, 1986; Valk, 2006). Therefore, in order to improve the water quality of eutrophic lakes, it is necessary to inhibit competition from *H. verticillata* in areas successfully colonized by *V. denseserrulata.*

Methods currently employed to control massive overgrowth of canopy-forming submerged macrophytes include physical (artificial and mechanical), chemical and biological measures (Hussner *et al.*, 2017). Among the latter, the use of herbivorous fish is generally considered to be an environmentally friendly, economic and natural choice. The Wuchang bream, *Megalobrama amblycephala*, is a medium-sized cyprinid native to the Yangtze Basin. Its small mouth and throat teeth, modest food intake and relative slow growth allow it to feed on aquatic plants without inflicting serious damage (Cao, 1960) and it is therefore favored over many other herbivorous aquatic organisms as an agent of biological control for submerged macrophytes (Lodge, 1991; Van *et al.*, 1998; Bonar *et al.*, 2002; Ganthy *et al.*, 2015).

Plants of *H. verticillata* are characterized by high water content, soft texture and low concentrations of allelochemicals, making them attractive to herbivorous fish (Leslie *et al.*, 1987). However, while this preference often results in a greater biomass or number of plants being consumed, it doesn't necessarily follow that the food has a higher utilization rate in terms of fish growth. For example, gilthead seabream (*Sparus aurata*) fed on lower protein and low crude protein diets exhibited higher rates of total lipid utilization (Fulvio *et al.*, 2014). When the utilization rate of food is high, grazing rates tend to be low, resulting in less damage to macrophytes.

A study by Zhen *et al.* (2018) suggested that *M. amblycephala* grazing on *H. verticillata* consume greater biomass than conspecifics grazing on *V. denseserrulata*. The same study demonstrated that while *V. denseserrulata* is grazed by *M. amblycephala*, the impact is limited to the extent that total biomass of *V. denseserrulata* in a system can still increase. We therefore hypothesized that despite its apparent predilection for *H. verticillata*, *M. amblycephala* would exhibit a higher food utilization rate when grazing on *V. denseserrulata*. Such a scenario would be highly conducive to the biological control of *H. verticillata* and the protection of *V. denseserrulata*. We designed a pond experiment with three groups: monocultures of *V. denseserrulata* and *H. verticillata* and a mixed culture of both species. The growth of plants in each group was tested with and without the presence of grazing *M. amblycephala*.

2 Materials and methods

The experiments were conducted from March to July 2015 in a former fish pond (30°22′49.84″ N, 114°22′49.84″ E) near Wuhan City, Hubei Province (China). The pond had an area of about 6000 m^2 and no aquatic vegetation prior to the experimental planting. Fish was removed and the pond was drained but not dredged, then refilled with clear water to a depth of 1 m.

The pond was divided into three equal sections (referred to hereafter as the eastern, central and western thirds) using nylon netting. In March, individuals of *V. denseserrulata* were planted evenly in the western third of the pond, clusters of *H. verticillata* with the same fresh weight as individual *V. denseserrulata* were planted in the eastern third of the pond. The central third of the pond was then planted with both *V. denseserrulata* and *H. verticillata*, interspersed at a ratio of 1 individual:1 cluster. All plants were located at 16–18 cm intervals in all three treatments. The macrophytes grew without disturbance for about two months, and by May, both species had achieved full vegetative growth. At that time, 24 nylon mesh enclosures (2.0 m long × 2.0 m wide × 1.5 m height with bore diameter of 1 cm × 1 cm) were used to

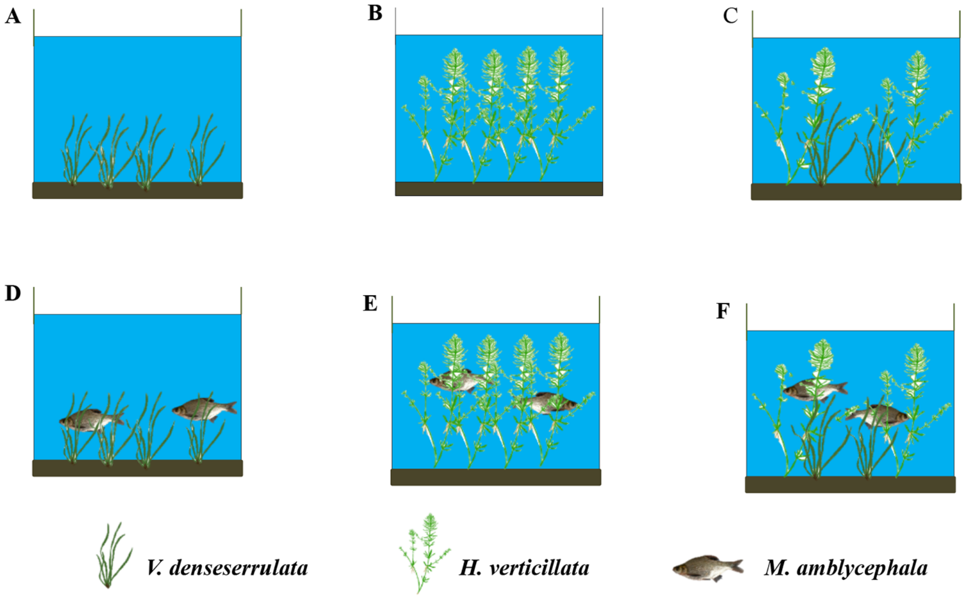

Fig. 1. Diagrammatic representation of the experimental setup (*V. denseserrulata* is *Vallisneria denseserrulata*; *H. verticillata* is *Hydrilla verticillate*; *M. amblycephala* is *Megalobrama amblycephala*).

separate the three groups, referred to hereafter as mono-*H. verticillata*, mono-*V. denseserrulata* and mixed, with eight separately enclosed replicates for each group. Within different plant treatments, four replicates were selected at random to receive two 1-year-old specimens of Wuchang bream, *M. amblycephala*. The nylon nets allowed water to flow freely around the pond, ensuring identical nutrient levels in all treatments, but preventing free movement of introduced fish. At the beginning of the experiment, the average total nitrogen (TN), total phosphorus (TP) and Chlorophyll *a* concentrations in the water were 0.53 mg/L, 0.21 mg/L and 10.57 μg/L, respectively. The enclosures were fixed in the sediment with bamboo poles, the upper parts were open to the air, and the bottom parts were weighted into the sediment with steel bars. The experimental setup is illustrated in Figure 1. *M. amblycephala* were transported from the Wuxi Freshwater Fishery Center to an adjacent pond 15 days before the experiment began and allowed to acclimatize with no feeding. Before being introduced to the experimental enclosures, eight individuals of *M. amblycephala* were selected at random for measurement of initial body length and body weight. The average length of the fish was 22.08 ± 0.60 cm, and the average weight was 117.76 ± 10.68 g.

Macrophyte samples were selected randomly by a 50 cm × 50 cm plot adjacent the experimental enclosures at the beginning of the experiment in order to provide estimates of the biomass of the macrophytes in the enclosures. At the end of the experiment, the plants in all treatment enclosures were collected. All sampling was carried out by hand by divers who uprooted all plants within the sampling frame (50 cm × 50 cm at beginning and 2 m × 2 m at ending). As soon as the macrophyte samples were brought back to the laboratory, they were washed with tap-water and the fresh biomass of submerged macrophytes from each experimental treatment was weighed. Furthermore, in order to determine changes in the growth of individual *V. denseserrulata* after grazing by *M. amblycephala*, we measured biometric indices for 10 plants selected at random from the mono-*V. denseserrulata* groups with fish, both before and after the experiment. Measurements were taken of plant height, plant weight, leaf number, maximum root length, maximum blade width, leaf thickness, root weight, stem weight and leaf weight. At the end of the experiment, all *M. amblycephala* from the fish treatments were recaptured and their body lengths and weights were recorded.

All the data, including the biological indicators from plants and fish were processed and analyzed in Microsoft Office Excel, and curves of changes were plotted. The significance of differences observed in each treatment group was tested by *T*-test after the normality of variable distribution of all data was proven with SPSS 22.

3 Results

3.1 Rate of biomass accumulation in submersed macrophytes

Our results show that in ungrazed (fish free) treatments, the rate of biomass loss in *H. verticillata* was significantly ($P < 0.05$) lower in the mixed culture than that in the monoculture (Fig. 2A). After the addition of *M. amblycephala*, the biomass accumulation rate of *H. verticillata* in both the mono- and the mixed cultures declined significantly faster than in the fish free treatments ($P < 0.05$).

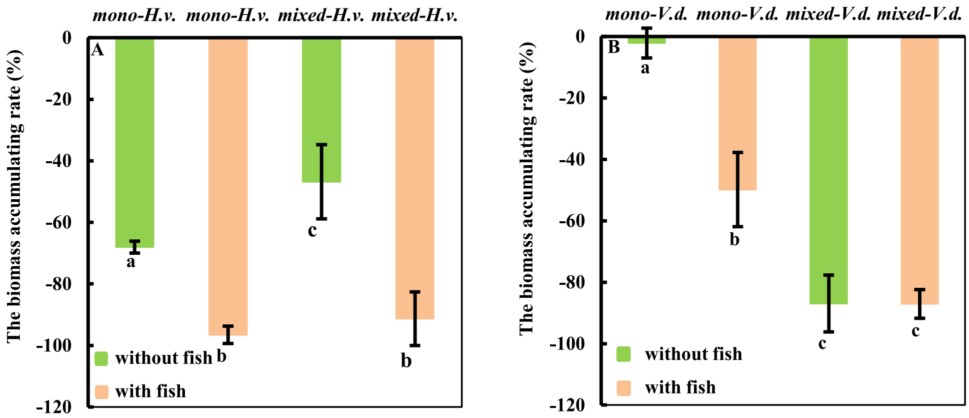

Fig. 2. Biomass accumulation rate of *Hydrilla verticillata* (A) and *Vallisneria denseserrulata* (B) in mono- and mixed culture treatments. Differing letters above the bars indicate statistically significant differences between the four groups ($P < 0.05$). Mean ± SE.

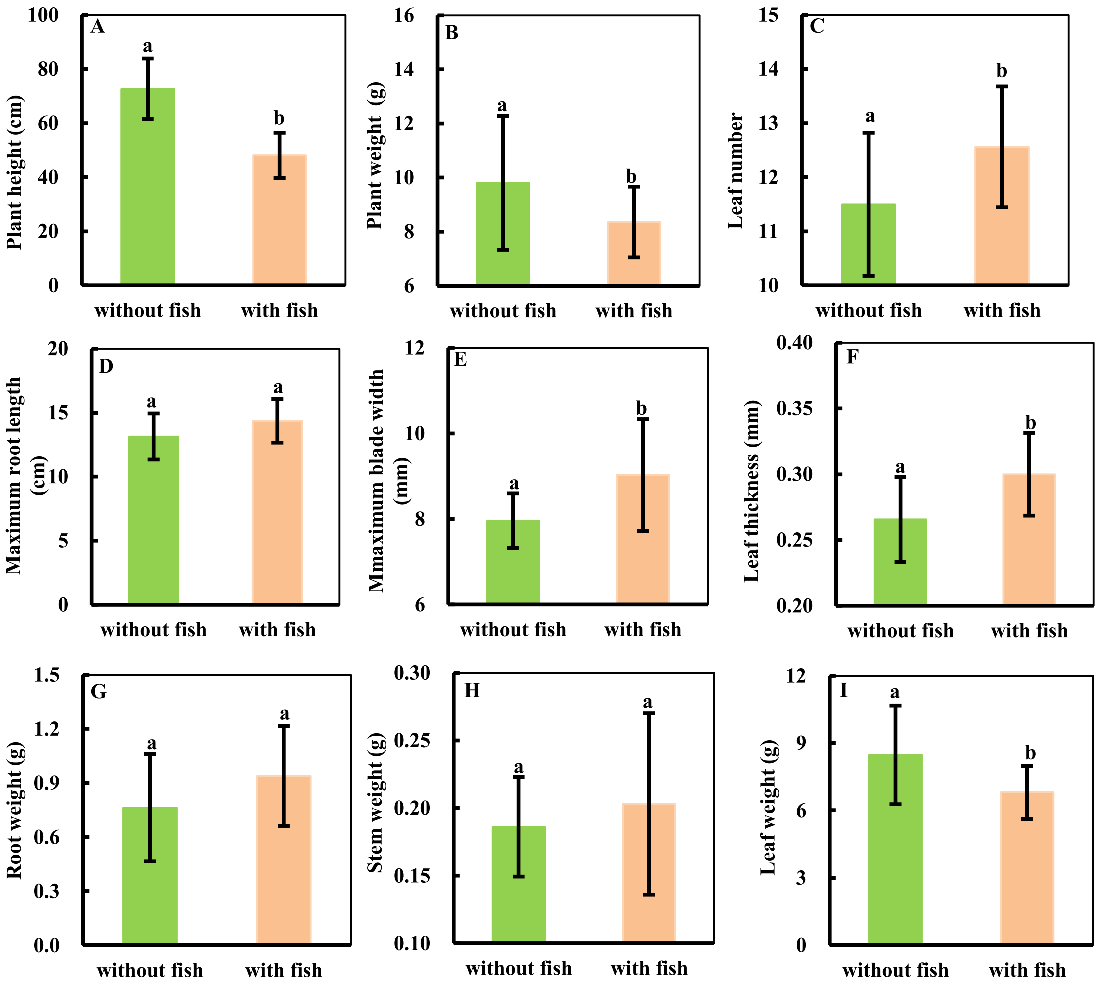

Fig. 3. The effect of *Megalobrama amblycephala* presence on growth morphology of *Vallisneria denseserrulata*. A, plant height; B, plant weight; C, leaf number; D, maximum root length; E, maximum blade width; F, leaf thickness; G, root weight; H, stem weight; I, leaf weight. Differing letters above the bars indicate statistically significant differences between two groups ($P < 0.05$). Mean ± SE.

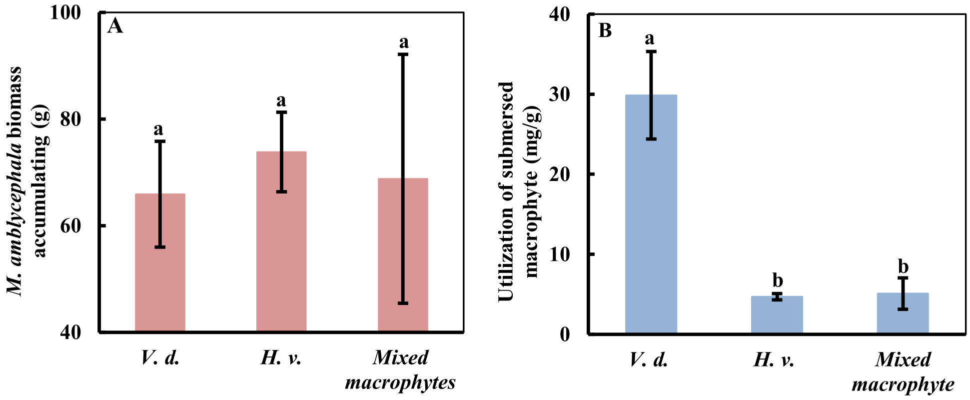

Fig. 4. Fresh biomass gains in *Megalobrama amblycephala* (A) and utilization rates of submersed macrophytes (mg/g) by *M. amblycephala* (B). *V. d.* is *Vallisneria denseserrulata* in the figure. *H. v.* is *Hydrilla verticillata*. Differing letters above the bars indicate statistically significant differences between the three groups ($P < 0.05$). Mean ± SE.

In the absence of fish, the rate of biomass accumulation of *V. denseserrulata* was significantly reduced when it grew alongside *H. verticillata* ($P < 0.05$; Fig. 2B), suggesting that *H. verticillata* has a significant inhibitory effect on the growth of *V. denseserrulata*. Following the introduction of *M. amblycephala*, the biomass accumulation rate of monocultured *V. denseserrulata* declined significantly ($P > 0.05$), but in the mixed culture there was almost no change. Thus, *M. amblycephala* had no significant effect on the growth of *V. denseserrulata* plants interspersed with *H. verticillata*.

3.2 Morphology of *V. denseserrulata*

At the end of the experiment, morphological differences were observed between specimens of monocultured *V. denseserrulata* grown with and without fish. Plant height, plant weight and leaf weight all decreased significantly under grazing pressure from *M. amblycephala*, while leaf number, maximum blade width and leaf thickness all increased significantly ($P < 0.05$). Maximum root length, root weight and stem weight also increased in the presence of fish, but not significantly ($P > 0.05$).

3.3 Fish growth and metabolic utilization of submerged macrophytes

At the end of the experiment, the fresh weight of *M. amblycephala* maintained in the mono-*V. denseserrulata* treatment was smaller than that of fish kept in the mono-*H. verticillata* treatment and the mixed culture, but the difference was not deemed significant ($P > 0.05$; Fig. 4). However, the metabolic utilization of *V. denseserrulata* by fish was significantly greater than that of *H. verticillata* or of a mixed macrophyte diet ($P < 0.05$).

4 Discussion

According to our results, biomasses of *H. verticillata* decreased in both monoculture and mixed treatments, even in the absence of *M. amblycephala*, because the experiment commenced at a season when with the plants in a state of vigorous vegetative growth. The biomass of ungrazed, monocultured *V. denseserrulata* also decreased very slightly. However, in the mixed fish-free treatment, the loss of *V. denseserrulata* biomass was significant. These results indicate that *M. amblycephala* has an obvious inhibitory effect on *H. verticillata* biomass in both mono- and mixed cultures. The results also indicate that in the absence of fish grazing pressure, shading by the canopy-forming *H. verticillata* inhibits the growth of the meadow-forming *V. denseserrulata*, supporting the conclusions of Xiao *et al.* (2007) and Tang *et al.* (2019).

Following the introduction of *M. amblycephala*, the loss of *V. denseserrulata* biomass increased significantly in monoculture, but not in mixed culture. Meanwhile, the biomass losses of *H. verticillata* increased significantly under grazing pressure in both mono- and mixed cultivation. This result suggests a significant preference of *M. amblycephala* for the soft growth of *H. verticillata* over *V. denseserrulata*, such that the latter was only targeted when it was the only food option. Grazing by *M. amblycephala* is also shown to influence the growth morphology of *V. denseserrulata*, the leaves of which became wider, thicker and more numerous in the fish treatments. The results indicate that grazing by *M. amblycephala* results in short, lightweight individuals of *V. denseserrulata* with reduced total leaf biomass but with larger, thicker and more numerous leaves near the sediment. The aboveground biomass of *V. denserrulata* declined in the presence of *M. amblycephala*, but the biomass distribution value per unit height increased (Li *et al.*, 2010). These morphological changes amount to denser plant growth close to the sediment. Previous research suggests that this kind of change can reduce rates of *V. denseserrulata* consumption by small fish and help to slow biomass losses (Valentine *et al.*, 1997; Pípalová, 2006; Planes *et al.*, 2011).

The utilization rate of *V. denseserrulata* by *M. amblycephala* was significantly higher than that of *H. verticillata* or of mixed macrophytes. In other words, *M. amblycephala* consumed less *V. denseserrulata* than *H. verticillata* to achieve the same body weight (Ganga *et al.*, 1985). The results suggest that grazing by *M. amblycephala* can be beneficial in decreasing the biomass of *H. verticillata* without concomitant damage to *V. denseserrulata*.

Overall, the results of the current study suggest that the grazing preference for *H. verticillata* shown by *M. amblycephala* could protect *V. denseserrulata* in the community by reducing shading. Meanwhile, under grazing by *M. amblycephala*, *V. denseserrulata* is likely to adopt a shorter and sturdier growth form, which could also be beneficial in reducing losses to other small herbivorous fish (Gail and Kenneth, 2003; Li *et al.*, 2010). Moreover, the high utilization rate of *V. denseserrulata* by *M. amblycephala* further reduces the damage to the former, shifting competitive advantage further towards the meadow-forming species and suggesting the latter is a highly suitable agent of biological control where the macrophyte community is threatened by overgrowth of *H. verticillata.*

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References

Bianchini I, Cunha-Santino, MB, Milan, JAM, Rodrigues CJ, Dias JHP. 2010. Growth of *Hydrilla verticillata* (Lf) Royle under controlled conditions. *Hydrobiologia* 644: 301–312.

Bonar SA, Bolding B, Divens M. 2002. Effects of triploid grass carp on aquatic plants, water quality, and public satisfaction in Washington State. *North Am J Fish Manag* 22: 96–105.

Cao W. 1960. A biological study of *Megalobrama amblycephala* and *Megalobrama terminalis* of Lake Liangzi. *Acta Hydrobio Sinica (in Chinese)* 1: 57–82.

Fulvio M, Alessio B, Ramon F, Lorenzo M, Anna B, Erika B, Luca P. 2014. Effects of dietary lipid level on growth and feed utilisation of Gilthead Seabream (*Sparus Aurata* L.) reared at mediterranean summer temperature. *Ital J Anim Sci* 13: 29–34.

Gail TF, Kenneth A. 2003. Interactive effects of light and salinity stress on the growth, reproduction, and photosynthetic capabilities of *Vallisneria americana* (wild celery). *Estuar Coast* 26: 1255–1268.

Ganga G, Sulochana CJ, Senthamil SR, Manoharan T. 1985. Influence of food plants on the food utilization and chemical composition of *Henosepilachna septima* (Coleoptera: coccinellidae). *Proc Anim Sci* 94: 161–167.

Ganthy F, Soissons L, Sauriau PG, Verney R, Sottolichio A. 2015. Effects of short flexible seagrass *Zostera noltei* on flow, erosion and deposition processes determined using flume experiments. *Sedimentology* 62: 997–1023.

Gopal B, Goel U. 1993. Competition and allelopathy in aquatic plant communities. *Botan Rev* 59: 155–210.

Hayashi H, Maehara R, Hirabayashi K. 1988. Growth of *Elodea muttallii* and changes of phosphorus in Lake Kizaki (in Japanese). *Bull Limnol Soc Ko-Shin-Etsu District* 13: 27–32.

Hilt S, Gross EM, Hupfer M, Morscheid H, Maehlmann J, Melzer A, Poltz J, Sandrock S, Scharf EM, Schneider S, van de Weyer K. 2006. Restoration of submerged vegetation in shallow eutrophic lakes – a guideline and state of the art in Germany. *Limnologica* 36: 155–171.

Hussner A, Stiers I, Verhofstad MJJM, Bakker ES, Grutters BMC, Haury J, van Valkenburg JLCH, Brundu G, Newman J, Clayton JS, Anderson LWJ, Hofstra D. 2017. Management and control methods of invasive alien freshwater aquatic plants: a review. *Aquat Bot* 136: 112–137.

Langeland KA. 1996. *Hydrilla verticillata* (LF) Royle (Hydrocharitaceae), “the perfect aquatic weed”. *Castanea* 61: 293–304.

Leslie AJ, Dyke JMV, Hestand III RS, Thompson BZ. 1987. Management of aquatic plants in multiuse lakes with grass carp (*Ctenopharyngodon idella*). *Lake Reservoir Manage* 3: 266–276.

Li K, Liu Z, Gu B. 2010. Compensatory growth of a submerged macrophyte (*Vallisneria spiralis*) in response to partial leaf removal: effects of sediment nutrient levels. *Aquat Ecol* 44: 701–707.

Lodge DM. 1991. Herbivory on freshwater macrophytes. *Aquat Bot* 41: 195–224.

Pípalová I. 2006. A review of grass carp use for aquatic weed control and its impact on water bodies. *Aquat Plant Manag* 44: 1–12.

Planes S, Raventos N, Ferrari B, Alcoverro T. 2011. Fish herbivory leads to shifts in seagrass *Posidonia oceanica* investments in sexual reproduction. *Mar Ecol Prog Ser* 431: 205–213.

Smith CS, Adams MS. 1986. Phosphorus transfer from sediment by *Myriophyllum spicatum*. *Limnol Oceanogr* 31: 1312–1321.

Tang Y, Fu B, Zhang X, Liu Z. 2019. Nutrient addition delivers growth advantage to *Hydrilla verticillata* over *Vallisneria natans*: a mesocosm study. *Knowl Manag Aquat Ecosyst* 420: 12.

Valentine JF, Heck KL, Busby J, Web, D. 1997. Experimental evidence that herb-ivory increases shoot density and productivity in a subtropical turtlegrass (*Thalassia testudinum*) meadow. *Oecologia* 112: 193–200.

Valk AGV. 2006. The biology of fresh water wetlands. Oxford: OUP, pp. 113–145.

Van TK, Wheeler GS, Center TD. 1998.Competitive interactions between Hydrilla (Hydrilla verticillata) and Vallisneria (Vallisneria americana) as influenced by insect herbivory. *Biol Control* 11: 185–192.

Van TK, Wheeler GS, Center TD. 1999. Competition between *Hydrilla verticillata* and *Vallisneria americana* as influenced by soil fertility. *Aquat Bot* 62: 225–233.

Xiao K, Yu D, Xu X, Xiong W. 2007. Benefits of clonal integration between interconnected ramets of *Vallisneria spiralis* in heterogeneous light environments. *Aquat Bot* 189: 76–82.

Zhen W, Zhang X, Guan B, Yin C, Yu J, Jeppesen E, Zhao X, Liu Z. 2018. Stocking of herbivorous fish in eutrophic shallow clear-water lakes to reduce standing height of submerged macrophytes while maintaining their biomass. *Ecol Eng* 113: 61–64.

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