Effect of *Potamogeton pusillus* on Water Quality and Plankton Community

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ABSTRACT
In closed aquatic environments like ponds or lakes, water bloom caused by eutrophication has severely damaged aquatic ecosystems. Some previous studies suggested that submerged macrophytes contribute to the development of aquatic ecosystems and water purification. Although water purification and lake ecosystem restoration using submerged macrophytes have been greatly studied, their specific mechanisms remain unclear. We evaluated the effect of a submerged macrophyte *Potamogeton pusillus* on water quality and plankton community using mesocosms with and without the macrophytes. The concentrations of chlorophyll-a, total nitrogen and phosphorus, particulate nitrogen and phosphorus in the mesocosm with *P. pusillus* were lower than those without *P. pusillus* as long as the macrophytes thrived. The cell concentration of cyanobacteria was significantly decreased in the presence of *P. pusillus*, while differences for chlorophytes and diatoms remained minimal. The number of large-sized cladocerans (> 0.1 mm of body length), known as heavy grazers of phytoplankton, was markedly higher in the mesocosm with *P. pusillus*. Our results suggested that *P. pusillus* is potentially useful for water purification and aquatic ecosystem restoration.

Keywords: lake ecosystem restoration, phytoplankton, *Potamogeton pusillus*, water purification, zooplankton

INTRODUCTION
In closed aquatic environments like ponds or lakes, water bloom caused by eutrophication has severely damaged aquatic ecosystems. Some previous studies suggested that submerged macrophytes contribute to the development of aquatic ecosystems (Mastrantuono and Mancinelli, 1999; Ali et al., 2007) and water purification (Schulz et al., 2003; Gao et al., 2009). Therefore, the restoration of submerged macrophytes (Qiu et al., 2001) and environmental restoration using submerged macrophytes (Wheeler and Center, 2001; Chen et al., 2009) have been focused on.

Mastrantuono and Mancinelli (1999) suggested that the decrease of submerged macrophytes is associated with the decrease of species richness and number of
invertebrates in a lake. The results indicated that submerged macrophytes play an important role in a plankton community in a lake. However, most previous studies dealt with the effect of submerged macrophytes on water quality (Qiu et al., 2001; Lau and Lane, 2002; Horppila and Nurminen, 2003; Schulz et al., 2003; Wang et al., 2008; Gao et al., 2009; Bakker et al., 2010), and few reports are found about the effect of the macrophytes on phytoplankton (Chen et al., 2009) and zooplankton (Mastrantuono and Mancinelli, 1999; Ali et al., 2007) community. The basic information on the function of the community development by submerged macrophytes is, thus, required for the understanding of the ecosystem restoration by the macrophytes in a lake. On the other hand, previous studies indicated aquatic macrophytes in lakes seemed ineffective to improve water quality in some cases (Lau and Lane, 2002; Chen et al., 2009). Lau and Lane (2002) obtained water from macrophyte-present (submerged macrophytes like Potamogeton pectinatus or other aquatic macrophytes) and macrophyte-free areas in a lake and analysed some kinds of water qualities, but they showed little or no water purification by the aquatic macrophytes. Chen et al. (2009) cultivated submerged, emergent macrophytes and floating plants in a large mesocosm, but water qualities of the inside and outside of the mesocosm were similar. Although Lau and Lane (2002) calculated the macrophyte coverage ratio [%], Mastrantuono and Mancinelli (1999) and Chen et al. (2009) showed no data about the biomass of aquatic macrophytes. The magnitude of the ability of aquatic macrophytes like the increase of zooplankton biomass or water purification might depend on the biomass of aquatic macrophytes. Therefore, if data about the biomass of aquatic macrophytes are unavailable, it is not clear how much volume of submerged macrophyte is required, or how water quality and plankton community change in response to the biomass of the macrophytes. Not only the evaluation of water quality and plankton community but also the quantitative evaluation of submerged macrophyte biomass is necessary for engineered applications and utilization.

The purpose of this study is to measure the seasonal change in the biomass of a submerged macrophyte, water quality and phyto- and zooplankton community with and without submerged macrophytes. Mesocosms with and without submerge macrophytes were constructed in a pond. Based on the comparison among the mesocosms and the outside of the mesocosms, the effect of submerged macrophytes on water quality and plankton community was discussed.

**MATERIALS AND METHODS**

**Experimental pond**

Bessho Pond (35° 51' 30" N 139° 38' 56" E) in Saitama Prefecture, Japan, was used as a research site. The surface area of the pond is about 2.4 × 10^4 m², and the average depth is about 1.0 m. Its hydraulic retention time is about 46 days. This pond is eutrophic, which might be attributed to ground bait for fishing, and there are fallen leaves from trees around the pond. Few submerged macrophytes seemed to exist in the pond, which would be responsible for low transparency due to eutrophication and feeding damage by fish.
Mesocosm
Mesocosms were constructed in Bessho Pond. Two kinds of mesocosms were prepared: vegetated and unvegetated mesocosms (named VM and UVM, respectively). The mesocosms were made of impermeable liner sheets and metal pipes. The sizes of the mesocosm were 10 m × 10 m = 100 m², and the sheets were put from the bottom of the lake to above the water surface. The bottom of the mesocosms was open, which allowed the flow of water.

In VM, a submerged macrophyte Potamogeton pusillus was cultivated. Pond water from the outside of the mesocosms was continuously supplied by a pump to the two mesocosms for 15 days of hydraulic retention time. This test was conducted from August 23 to December 20, 2007.

Measurement of Potamogeton pusillus biomass
Potamogeton pusillus was classified into two types depending on the shape: Type I is defined as P. pusillus that thrived up to water surface and spread around water surface (Fig. 1 (A)), and Type II is P. pusillus that thrived up to water surface but did not spread around water surface (Fig. 1 (B)). In a preliminary test at Bessho Pond, the biomass of Type I and II was 59.8 and 62.4 g-DW (dry weight)/m², respectively.

The existence of P. pusillus in VM was investigated by visual observation from a boat by 0.1 m × 0.1 m of unit area. When no P. pusillus was found around the water surface of a unit area, the area was regarded as no P. pusillus area. Each unit area was classified into Type I, II and no P. pusillus area as shown in Fig. 1 (C). The biomass of P. pusillus in VM (g-DW/m²) was calculated based on the following equation:

\[
\text{Biomass of } \text{Potamogeton pusillus (g-DW/m}^2\text{)} = \frac{B_I \times A_I + B_{II} \times A_{II}}{A_M}
\]

where,
- \(B_I\) : P. pusillus biomass of Type I (= 59.8 g-DW/m²)
- \(B_{II}\) : P. pusillus biomass of Type II (= 62.4 g-DW/m²)
- \(A_I\) : Total area of Type I (m²)
- \(A_{II}\) : Total area of Type II (m²)
- \(A_M\) : Area of the mesocosm (= 100 m²)

Withered P. pusillus remained in VM during the examination, but the biomass of withered P. pusillus was excluded from the calculation.

Measurement of water quality and plankton
Water between the water surface and 0.9 m of depth was collected using a cylindrical container whose length was 0.9 m at nine sampling points in VM. The mixture of all of the water was used as a water sample (n = 1). Same collection method was also performed in UVM. Water sample was also obtained from the outside of the mesocosms (named OM) around the pump used for the supply of the pond water to the mesocosms. The outside of the mesocosms is located at the inside of Bessho Pond, and the water obtained at OM corresponded to the original water of Bessho Pond without submerged macrophytes. All of the water samples were used for the measurement of water qualities and phyto- and zooplankton observations as shown below.
In this study, chlorophyll \( a \) (Chl.\( a \), \( \mu g/L \), analysed by Holm-Hansen Method), pheophytin (the detritus derived from natural death or the predation by zooplankton, \( \mu g/L \), analysed by Holm-Hansen Method), total nitrogen (TN, mg/L, analysed by peroxysulfate oxidation-ultraviolet spectrophotometry method), total phosphorus (TP, mg/L, analysed by Molybdenum-blue spectrophotometric method), ammonia-nitrogen (NH\(_4\)-N, mg/L, measured by ion chromatography analysis), nitrite-nitrogen (NO\(_2\)-N, mg/L, measured by ion chromatography analysis), nitrate-nitrogen (NO\(_3\)-N, mg/L, measured by ion chromatography analysis), phosphate-phosphorus (PO\(_4\)-P, mg/L, analysed by Molybdenum-blue spectrophotometric method), particulate nitrogen (PN, mg/L, calculated by the deduction of NH\(_4\)-N, NO\(_3\)-N and NO\(_2\)-N from TN), and particulate phosphorus (PP, mg/L, calculated by the deduction of PO\(_4\)-P from TP), were determined. The analytical devices and conditions are summarized in Table 1.

(C) Classification of Type I, II and no \( P. \) pusillus area in vegetated mesocosm
(The data below was obtained in Sep 18, 2007)

![Image](image)

Fig. 1 - Schematic diagram of the measurement of \( Potamogeton \) pusillus biomass in the vegetated mesocosm (VM).
Table 1: Analytical devices and conditions for water quality measurements. (A) analytical device; (B) column and analytical conditions of ion chromatography.

(A) Analytical device

<table>
<thead>
<tr>
<th>Index</th>
<th>Device</th>
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<tbody>
<tr>
<td>Chl. a</td>
<td>UV-2450, SHIMADZU, JAPAN</td>
</tr>
<tr>
<td>Pheophytin</td>
<td>TD-700, Turner Designs, U.S.A</td>
</tr>
<tr>
<td>TN</td>
<td>ICS2000 (anion), ICS1500 (cation),</td>
</tr>
<tr>
<td>TP</td>
<td>Thermo Fisher Scientific, U.S.A</td>
</tr>
<tr>
<td>PO4-P</td>
<td>Ion Chromatography System</td>
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(B) Column and analytical conditions of ion chromatography

ICS2000 (anion)

<table>
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<th>Separation column</th>
<th>Ion Pac AS19</th>
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<tr>
<td>Guard column</td>
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<td>Eluent</td>
<td>KOH</td>
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<td>Flow rate</td>
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<tr>
<td>Gradient</td>
<td>10mmol/L</td>
</tr>
<tr>
<td></td>
<td>0-10min</td>
</tr>
<tr>
<td></td>
<td>10-45mmol/L</td>
</tr>
<tr>
<td></td>
<td>10-25min</td>
</tr>
<tr>
<td></td>
<td>45mmol/L</td>
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<tr>
<td></td>
<td>25-30min</td>
</tr>
<tr>
<td></td>
<td>45-58mmol/L</td>
</tr>
<tr>
<td></td>
<td>30-40min</td>
</tr>
<tr>
<td></td>
<td>58mmol/L</td>
</tr>
<tr>
<td></td>
<td>40-42min</td>
</tr>
<tr>
<td>Suppressor</td>
<td>ASRS-ULTRA II</td>
</tr>
<tr>
<td>Detector</td>
<td>Electric conductivity detector</td>
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</table>

ICS1500 (cation)

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<th>Ion Pac CS12A</th>
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<tbody>
<tr>
<td>Guard column</td>
<td>Ion Pac CG12A</td>
</tr>
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<td>Eluent</td>
<td>20mmol/L Methanesulfonic acid</td>
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<tr>
<td>Flow rate</td>
<td>1.0mL/min</td>
</tr>
<tr>
<td>Gradient</td>
<td>-</td>
</tr>
<tr>
<td>Suppressor</td>
<td>CSRS</td>
</tr>
<tr>
<td>Detector</td>
<td>Electric conductivity detector</td>
</tr>
</tbody>
</table>

Phytoplankton was collected using a plankton net (mesh size: 0.04 mm) at three sampling points in both mesocosms (Ministry of Land, Infrastructure, Transport and Tourism, Japan, 2007). DeMott and Kerfoot (1982) and Vanni (1986) suggested that the decrease in Chl.a accompanied by the feeding of daphnia was more effective than small rotifers and cladocerans. Therefore, some large zooplankton like cladocerans were also
collected using a plankton net (mesh size: 0.1 mm) at three sampling points in both mesocosms. The collection depth of phytoplankton and zooplankton was about 0.7 and 0.8 m. The identification and cell or individual counting of phyto- and zooplankton were conducted using a microscope based on its species group.

The water for the analysis of the water quality and plankton community was collected around 10 or 11 a.m.

RESULTS

Potamogeton pusillus biomass
Figure 2 shows the seasonal variation of *P. pusillus* biomass in VM. The biomass of the submerged macrophytes increased from the start of the examination due to the growth of the macrophytes, and reached the maximum around the end of October. The macrophytes gradually withered around the middle of October and finally almost no *P. pusillus* was found at the end of November.

Water quality
The seasonal variation of Chl.a, pheophytin, TN, TP, PN, PP, DIN (the amount of NH$_4$-N, NO$_3$-N and NO$_2$-N) and PO$_4$-P in VM, UVM and OM are illustrated in Figs. 3 and 4, respectively. While *P. pusillus* existed in VM (Fig. 2), the values of Chl.a, TN, TP, PN and PP in VM were lower than UVM and OM, indicating water purification activity by the submerged macrophytes. The seasonal change in DIN and PO$_4$-P in VM, UVM and OM was similar, which suggested that the decrease in TN and TP was derived from the decrease in PN and PP. The increase of pheophytin in VM was accompanied by the decrease of Chl.a. This result showed that the death of some part of microalgae was accelerated.

Since the middle of November, the values of Chl.a, pheophytin, TN, TP, PN, PP, DIN and PO$_4$-P had been similar among VM, UVM and OM. The result would be due to the disappearance of the water purification activity accompanied by the loss of *P. pusillus*. 
Fig. 2 - Seasonal variation of the biomass of *Potamogeton pusillus* in the vegetated mesocosm (VM).

![Graph of biomass of Potamogeton pusillus](image)

- ▲ Vegetated mesocosm (VM)
- △ Unvegetated mesocosm (UVM)
- × Outside of mesocosm (OM)

Fig. 3 - Seasonal variation of chlorophyll *a* (Chl.a) and pheophytin.

![Graph of chlorophyll a](image)

(A) Chl.a

![Graph of pheophytin](image)

(B) Pheophytin

![Graph of pheophytin](image)
Fig. 4 - Seasonal variation of total nitrogen and phosphorus (TN, TP), particulate nitrogen and phosphorus (PN, PP) and dissolved inorganic nitrogen (DIN) and phosphate phosphorus (PO₄-P).

**Plankton community**

**Phytoplankton**

Figure 5 summarizes the cell concentration of each phytoplankton species in VM, UVM and OM. The cell concentration of cyanobacteria in VM while *P. pusillus* existed was remarkably lower than UVM and OM except at the start of the monitoring. Little change was observed in the concentrations of chlorophytes and diatoms regardless of the existence of *P. pusillus*. The results proposed that the cell concentration of cyanobacteria was specifically reduced by the functions of the submerged macrophytes, suggesting that the decrease in Chl.a (Fig. 3 (A)) was due to the decrease in the biomass of cyanobacteria.
**Zooplankton**

The community structures of zooplankton are summarized in Fig. 6. The number of crustacea in VM was slightly larger than UVM and OM. *Potamogeton pusillus* seemed to have no effect on the number of rotifera and ciliate. On the other hand, rhizopod was observed only in VM except on December 6th, which suggested that *P. pusillus* supported the appearance of the zooplankton.

The total number of the individuals in VM tended to be larger in September and October that was the largest *P. pusillus* community period, than UVM and OM. The results indicated that *P. pusillus* could increase the total number while the community reached the largest.

The number of cladocerans (phytoplankton feeders) was 0 – 1 individual/L in UVM, but the largest number reached 40 individuals/L in VM (Fig. 7). The seasonal variation between the biomass of *P. pusillus* (Fig. 2) and the number of cladocerans was similar. The data indicated that *P. pusillus* contributed to the increase in the number of cladocerans.

Fig. 5 - Cell concentration of each microalgal species in the vegetated, unvegetated mesocosm (VM and UVM) and the outside of the mesocosms (OM). The total includes cyanobacteria, diatoms, chlorophytes and other microalgal species.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Cyanobacteria</th>
<th>Diatoms</th>
<th>Chlorophytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug 23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sep 12</td>
<td></td>
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</tr>
<tr>
<td>Oct 2</td>
<td></td>
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</tr>
<tr>
<td>Oct 22</td>
<td></td>
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<td></td>
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<tr>
<td>Nov 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec 1</td>
<td></td>
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</tr>
</tbody>
</table>

Fig. 6 - The community structures of zooplankton.
Fig. 6 - Structure of zooplankton collected by a plankton net (mesh size: 0.1 mm) in the vegetated, unvegetated mesocosm (VM and UVM) and the outside of the mesocosms (OM). The total includes crustacea, rotifer, ciliate, rhizopod and other zooplankton species.

Fig. 7 - Seasonal change in the number of cladocerans collected by a plankton net (mesh size: 0.1 mm) in the vegetated and unvegetated mesocosm (VM and UVM).
DISCUSSION
We measured the seasonal change in the biomass of \textit{P. pusillus} based on the visual observation. The biomass quantification method applied the characteristic that the stem of well-grown \textit{P. pusillus} reaches the water surface. The existence of \textit{P. pusillus} was neglected in this method when no \textit{P. pusillus} was found around the water surface, therefore, we may have underestimated the biomass while the stem has not reached the water surface. This method would be, thus, applicable from summer to winter in which \textit{P. pusillus} thrive up to the water surface.

The number of large-sized cladocerans became high under the existence of \textit{P. pusillus} (Fig. 7). The small number in UVM would be due to the feeding by fish that stayed in the mesocosms. The large number in VM would be due to \textit{P. pusillus} serving as refuge for cladocerans, and therefore, the cladocerans could avoid the predation of fish. Consequently, the decrease of Chl.a and the increase of pheophytin in VM (Fig. 3) might be the effect of predation by cladocerans with increased number of individuals due to \textit{P. pusillus}.

It should be noted that the cell concentration of cyanobacteria was specifically decreased (Fig. 5). Some previous studies reported that some submerged macrophytes have allelopathic effect inhibiting the growth of microalgae by the algal growth examination of the culture filtrates of the macrophytes (Mulderij \textit{et al.}, 2005; Hilt, 2006). Some previous researches also reported that the allelopathic effect of some aquatic macrophytes suppressed the growth of cyanobacteria by the examination of the culture filtrates (Nakai \textit{et al.}, 2000; Körner and Nicklisch, 2002; Takeda \textit{et al.}, 2009). Takeda \textit{et al.} (2011) examined the water obtained in VM, UVM and OM of this pond and found that the water obtained in VM exhibited allelopathic effect inhibiting the growth of \textit{Microcystis aeruginosa}. This result obtained in this study indicated that allelopathic effect of submerged macrophytes influenced the microalgal structures in lakes.

Consequently, for \textit{P. pusillus}, the increase in the number of cladocerans as phytoplankton feeders and the allelopathic effect seemed the main functions that affected the water quality and plankton community. On the other hand, Bakker \textit{et al.} (2010) examined the effect of submerged macrophytes \textit{Chara globularis, Elodea nuttallii} and \textit{Potamogeton pectinatus} on water quality and zooplankton community. While the macrophytes thrived, the value of ChL.a was low (10 – 50 μg/L) but the number of daphnids (one of cladocerans), which are phytoplankton feeders, was not increased even under the existence of the macrophytes. The results suggested that the submerged macrophytes had little effect on the increase of the number of daphnids, which is different from \textit{P. pusillus}. The decrease in ChL.a by the three kinds of macrophytes might be due not to the increase of the number of daphnids but from other functions, suggesting that the main functions that affect the water quality or plankton community seemed different between \textit{P. pusillus} and the submerged macrophytes.

No increase of DIN concentration was found until the end of October, the period that \textit{P. pusillus} start withering. The result may be because \textit{P. pusillus} absorbed DIN (and PO₄-P) derived from the precipitated phytoplankton and fallen leaves on the bottom of the pond until withering. The decrease of ChL.a, PN and PP was confirmed in a few
days after the start of the monitoring (Fig. 4), and the increase in the number of
cladocerans was observed. The initial biomass of *P. pusillus* was 10 g-DW/m² (Fig. 2),
which might be enough to improve the water purification and the increase in the number
of cladocerans. However, the information on the change in water quality and the
number of cladoceran under different *P. pusillus* biomass, especially the lower biomass
than 10 g-DW/m², is required to understand the minimal biomass for the decrease in
Chl. *a*, PN and PP and increase in the number of cladocerans.

The examinations in this study showed that *P. pusillus* could decrease Chl. *a*, nitrogen
and phosphorus (especially PN and PP) and the cell concentration of cyanobacteria, and
increase the number of cladocerans. However, the effects of other aquatic macrophyte
species on water quality and plankton community are still unclear. The clarification of
aquatic macrophyte functions and their impacts is required for water purification and
lake ecosystem restoration using aquatic macrophytes in further studies.

**CONCLUSIONS**

The monitoring of water quality and plankton community using mesocosms in a pond
showed that a submerged macrophyte *Potamogeton pusillus* had a decrease effect on
chlorophyll *a*, particulate nitrogen and phosphorus. The cell concentration of
cyanobacteria was decreased in the existence of *P. pusillus* despite the almost no effect
observed on chlorophytes and diatoms. In addition, *P. pusillus* had a function that
increased the number of cladocerans (> 0.1 mm) that is one of the main phytoplankton
feeders. Ten g-DW (dry weight)/m² of *P. pusillus* seemed enough to exhibit the above
effects.

**ACKNOWLEDGEMENTS**

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