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Original Article

Impact of Nutrients on Reproduction from Fragments of Invasive Alien Species *Alternanthera philoxeroides*

Longjin Dai [a], Shuhei Tanaka [a], Mayu Kimura [a], Yasuhiro Oshima [a], Yiming Xu [a] Hiroaki Nishikawa [b]

[a] *Graduate School of Global Environmental Studies, Kyoto University, Kyoto, Japan*
[b] *LAGO Co., Ltd., Omihachiman, Japan*

ABSTRACT
Invasive alien species *Alternanthera philoxeroides* was found on Lake Biwa shore for almost two decades and has been causing huge loss for this region. This study aims at investigating three impact factors on reproduction of *A. philoxeroides*, namely nutrients, stem length, and stem diameter. Utilizing a ternary configuration dispersion method on 120 fragment samples collected from Lake Biwa shore after 10-days cultivation, the result indicates that nutrient salts significantly enhance growth ($p < 0.01$, Welch's *t*-test), highlighting the species' preference for eutrophic environments. Smaller stem fragments exhibit reduced growth, emphasizing the importance of thorough removal to minimize its persistence. Comparative analysis with *Ludwigia grandiflora* underscores that the latter exhibited bigger leaves under same experiment condition, and stem diameter significantly influence on its growth (37.7% contribution rate). These findings not only offer valuable references for elimination activities, but also stress the need for appropriate eradication strategies to preserve Lake Biwa's aquatic ecosystem, contributing valuable insights to invasive species management in ecologically sensitive areas.

Keywords: alligator weed, plant invasion, Lake Biwa, regeneration ability, fragmentation

INTRODUCTION

In modern-day global ecological changes, bio-logical invasion has emerged as a crucial factor and poses a significant threat to biodiversity, ecosystem stability, and ecosystem services [1]. According to glossary of phytosanitary terms in the international standards for phytosanitary measures (ISPM 05), invasive alien species refer to a species that colonize or spread to harm plants or that have been shown by risk analysis to have the potential to harm plants. Invasive alien species will destroy the living environment of local species and communities, establish monocultures, cause serious damage to the local ecological balance, and become one of the most important factors for the loss of ecological diversity and species extinction [2,3]. Additionally, the effects of invasive alien species extend to local economic income and pose a threat to human health [4,5].

Alternanthera philoxeroides, also known as alligator weed, is a type of destructive invasive alien species originally from South America that has spread to over 30 countries, including the United States, Australia, and China since the 1930s. The earliest recorded case in Japan was in 1989 in Nizaki City, Hyogo Prefecture [6]. It causes severe negative impacts on biodiversity, ecosystems, and socio-economics. Its invasion results in a reduction of native aquatic vegetation, disappearance of aquatic animals, and significant losses to agriculture, aquaculture, transportation, and tourism. *A. philoxeroides* reproduces rapidly and forms highly dominant communities that cover water surfaces, obstructing channels and reducing oxygen content, leading to the extinction of fish and plant populations. The most typical asexual propagation method of this weed is root propagation, while stem propagation occurs through rooting at stem nodes or germination of single stem segments into plants. These propagation methods facilitate the spread and dispersal of *A. philoxeroides* [7].

In Lake Biwa, *A. philoxeroides* was first found in 2004

Corresponding author: Shuhei Tanaka, E-mail: tanaka.shuhei.3u@kyoto-u.ac.jp
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at Jinjyo-numa pond, Hikone. Since then, a large amount of money has been paid by The Shiga Prefectural Government and other ministries for control and elimination of the overgrown *A. philoxeroides*. However, using heavy machine and other comprehensive weed-cutting operations by local government did not produce significant results, and the number of the species increased exponentially [8]. Physical methods of controlling *A. philoxeroides* infestation are challenging, as each node of the plant is capable of generating a new plant, and mechanical damage can encourage its spread [9].

A. philoxeroides has strong adaptability to the environment, which brings a lot of problems due to its invasion, so many countries have carried out a series of studies on it. At present, most of studies on the invasion of *A. philoxeroides* have focused on the harm of its invasion [10,11], the impact of its invasion on the local ground environment [12], the invasion mechanism of *A. philoxeroides* [13], and how to prevent the invasion of it [14]. It demonstrates that this plant exhibits robust reproductive capabilities, allowing even small fragments to establish themselves and proliferate in new surroundings with ease. However, there has been limited research on its regeneration, while Tanveer *et al*. [15] had suggested that its remarkable vegetative regenerative capacity must be considered to effectively eliminate this species in a certain area.

Furthermore, wetland ecosystems are experiencing both economic and ecological repercussions due to biological invasions and several contributing factors, including nitrogen enrichment. The destructive effects of nitrogen enrichment manifest in the excessive accumulation of biomass in algae and the promotion of specific macrophyte proliferation, ultimately resulting in a reduction in plant species diversity within ecosystems [16]. However, there is limited information regarding the response of *A. philoxeroides* to nitrogen enrichment in wetlands. The study by Sun *et al.* [17] is among the limited research available, demonstrating that elevated nitrogen concentrations in water facilitate the adaptation of *A. philoxeroides* to flooding conditions.

Therefore, this study aims to investigate the impact of nutrients, including nitrogen and phosphorus, on regeneration ability from the fragments of *A. philoxeroides* through laboratory experiments by using specimens collected from Lake Biwa in 2022. In a parallel investigation by Tanaka *et al*. [18], a similar experimental design was employed to examine the impact of nutrients on the regeneration ability of fragments from another invasive alien plant, *Ludwigia grandiflora*, which shares similarities in appearance and habitat with *A. philoxeroides* in Lake Biwa. To effectively manage aquatic ecosystems that are invaded by multiple invasive species, it is essential to gain a deeper understanding of the distinctions among multiple invasive aquatic plants that share common origins and similar characteristics within a community [19]. Consequently, this study aims to compare and contrast our findings with theirs, enabling a comprehensive analysis. The findings of this study can address the research gap and offer valuable insights for local governments in devising effective strategies for the management and eradication of *A. philoxeroides*.

MATERIALS AND METHODS

To discuss the impact of nutrient solution of different concentrations, this paper used *A. philoxeroides* that collected in Lake Biwa in June, 2022 as raw material, as shown in **Fig. 1**, and then brought them to laboratory to carry out the biological experiment.

The following paragraphs are the main steps of the experiment:

The first step in the experiment was to obtain appropriate stem fragments of *A. philoxeroides*. Research has shown that the presence of nodules plays a significant role in regeneration [18]. Therefore, only stem fragments with nodules were selected for this experiment. After that, the stem diameter of all collected *A. philoxeroides* specimens was precisely measured using a Vernier caliper and then classified into two groups: 0.3 cm and 0.5 cm, with each group comprising 60 samples. The groups with a recorded stem diameter of 0.3 cm exhibited a range from 0.28 cm to 0.32 cm, while the groups with a 0.5 cm diameter had a range from 0.48 cm to 0.52 cm.

Fig. 1 *A. philoxeroides* on Lake Biwa shore.

Then, using a Vernier caliper and scissors to measure and cut the selected samples into several pieces that meet the stem length of 0.3 cm, 0.5 cm, 0.7 cm, 1 cm, 3 cm, and 5 cm, and made each length has ten samples, with each sample containing a single nodule. The total number of experimental fragments of *A. philoxeroides* was 120, as shown below.

$$\text{Stem diameter} = \{0.5\ \text{cm}, 0.3\ \text{cm}\} \quad (1)$$

$$\text{Stem length} = \{5\ \text{cm}, 3\ \text{cm}, 1\ \text{cm}, 0.7\text{cm}, 0.5\ \text{cm}, 0.3\ \text{cm}\} \quad (2)$$

The second step involved labeling all of the samples and placing them into separate petri dishes. The samples were then divided into two groups and were treated with different cultivation solutions (Refer to **Table 1**) [18,20]. Each stem diameter and stem length had five samples that were supplemented with nitrogen and phosphorus as nutrients, and the remaining five samples were not.

The third step was to place all the samples under illuminate LED plant cultivation light (HG-LA150, Home Grown, Yokohama, Japan) to cultivate and exposed them to 14 hours of light per day, while maintaining a water temperature of 20 to 25 degrees Celsius for a period of ten days. The process of the experiment is shown in **Fig. 2**.

Throughout the experiment, the samples were photographed every two days (Day 0, Day 2, Day 4, Day 6, Day 8, and Day 10) using a camera (Xperia xz3, 19.2 million pixels, Sony Marketing Inc., Tokyo, Japan). The projected area was calculated by the use of the software (!0_0! Excel length · area measurement), which defines it as the area of the sample obtained by projecting its shape onto an arbitrary plane.

The growth ratio refers to the projected area on day *n* (*n* = 0, 2, 4, 6, 8, and 10) divided by the projected area on day 0, and for our analysis, the growth ratio of *A. philoxeroides* fragments on day *n* served as the objective variable (x_{ijk}). To investigate the impact of three factors, namely nutrient (A_i, with 2 levels), stem length (B_j, with 6 levels), and stem diameter (C_k, with 2 levels), a ternary configuration was employed, and the analysis of variance (ANOVA) was conducted. The equation is as follows.

Table 1 Concentration of elements in nutrient solutions.

Elements	With nutrient (mg/L)	Without nutrient (mg/L)
NH_4+-N	7.00	0.00
NO_3^--N	7.00	0.00
PO_4^{3-}-P	6.00	0.00
K	23.40	23.40
S	9.92	9.92
Ca	12.00	12.00
Mg	14.58	14.58
Fe	2.17	2.17
Si	0.46	0.46
B	0.53	0.53
Mn	0.49	0.49
Cu	0.02	0.02
Zn	0.05	0.05
Mo	0.01	0.01

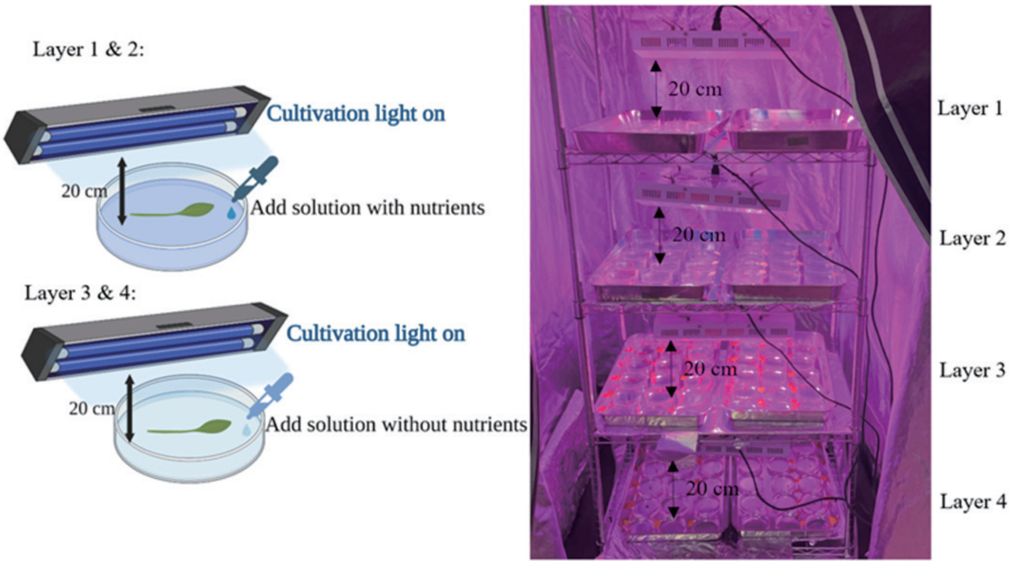

Fig. 2 Samples in cultivation.

$$x_{ijk} = x_0 + A_i + B_j + C_k + (ab)_{ij} + (bc)_{jk} + (ac)_{ik} + e_{ijk} \quad (3)$$

x_{ijk} : the growth ratio on day n (n = 0, 2, 4, 6, 8, and 10)

x_0 : total average of growth ratio

A_i, B_j, C_k : nutrients, stem length, and stem diameter

$(ab)_{ij}, (bc)_{jk}, (ac)_{ik}$: interaction between nutrients and stem length, interaction between stem length and stem diameter, and interaction between nutrients and stem diameter

e_{ijk} : residual error

After the three-way ANOVA, the Welch's t-test was employed to assess the significance of mean differences between two independent groups, providing a more accurate evaluation of group differences.

Besides, differentiation rate represents the number of stem samples within a particular target group that have undergone differentiation (grown new stems from nodules) divided by the total number of stem samples in that group.

RESULTS AND DISCUSSION

Effects of nutrients, stem length, and stem diameter on reproduction

Factors affecting reproduction of stem fragments

Table 2 shows the ANOVA table with the growth ratio at day 10 as the target variable. 72.2% of the total effect could be explained by main effects and interactions between two factors. The contribution rate of nutrients was 29.1% (p < 0.01, Welch's t-test). Nutrients contributed significantly to regeneration from the start of incubation to the tenth day. On the other hand, the contribution rate of stem length was 13.2% and stem diameter was 8.4%. In addition, the contribution rate of the interaction between stem length and stem diameter was 17.1%, which means these two factors also contributed to reproduction of stem fragments.

Effects of nutrients on reproduction

The results of the ANOVA analysis, with the increase in the projected area on day 10 as the target variable, showed a 1.6% contribution rate of nutrients. To explore the effect on the early stages of growth, a ternary distribution ANOVA was conducted with the growth ratio on days 2, 4, 6, 8, and 10 after the start of the experiment as the target variable. **Figure 3** illustrates the contribution rate of nutrients in the days after the start of the experiment. The contribution rates were 8.4% on day 2, 7.7% on day 4, 5.5% on day 6, 3.3% on day 8, and 1.6% on day 10.

The growth ratio with and without nutrients on the experiment is shown in **Fig. 4**. In the system with nutrients, the mean growth ratio was 1.57, higher than 1.38 in the no-added condition, with a p-value of less than 0.001 as determined by Welch's t-test. The results show that the mean value of the growth ratio was higher in the system with nutrients compared to the no-added condition. From the above, it is clear that nutrients have a positive impact on the early growth phase of *A. philoxeroides*.

Effects of stem diameter and stem length on reproduction

The increased projected area on day 10 according to sample's initial stem length is shown in **Fig. 5**. In the com-

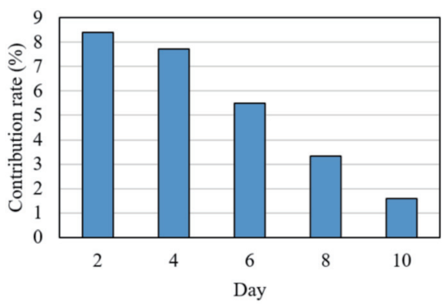

Fig. 3 The contribution rate of nutrients.

Table 2 Reproduction factors and contribution rates of nutrients.

	Sum of squares	Degree of freedom	Unbiased variance	Proportion of variance	p-value	Modified sum of squares	Contribution rate (%)
Nutrient (A)	0.21	1	0.2	25.1	0.004	0.21	29.1
Length (B)	0.14	5	0.0	3.2	0.114	0.09	13.2
Diameter (C)	0.07	1	0.1	8.0	0.037	0.06	8.4
(a*b)	0.16	5	0.0	3.8	0.083	0.12	17.1
(b*c)	0.06	5	0.0	1.5	0.330	0.02	3.1
(a*c)	0.02	1	0.0	2.1	0.211	0.01	1.3
Residual error (e)	0.04	5	0.0			0.20	27.8
Total	0.71	23				0.71	100.0

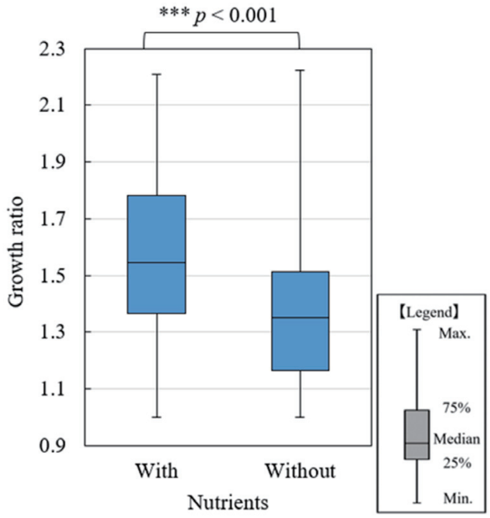

Fig. 4 Growth ratio of samples with different nutrient solutions (Day 10).

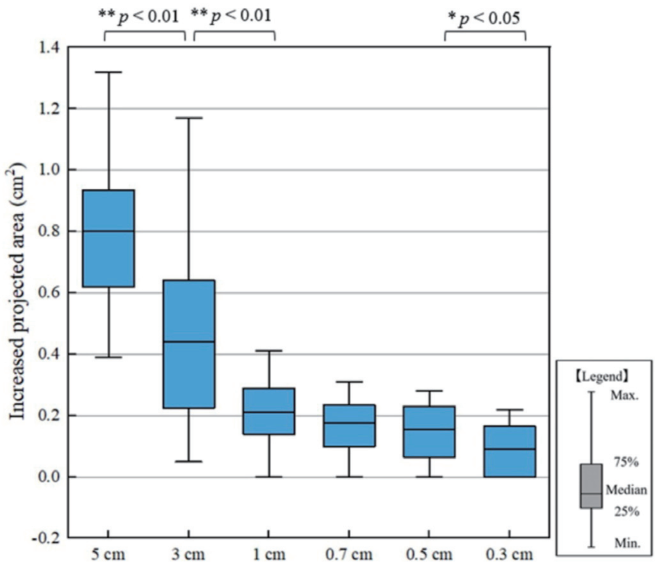

Fig. 5 The increased projected area of samples with different stem lengths (Day10).

parison of the increased projected area based on different stem lengths, statistical analyses revealed highly significant differences between the 5 cm group and the 3 cm group ($p < 0.01$, Welch's t-test), as well as between the 3 cm group and the 1 cm group ($p < 0.01$, Welch's t-test). However, no statistically significant difference was observed between the 1 cm group and the 0.7 cm group, as well as between the 0.7 cm group and the 0.5 cm group. Notably, a significant difference was found between the 0.5 cm group and the 0.3 cm group, with a p-value less than 0.05 by using Welch's t-test.

Besides, the differentiation rates for different initial stem lengths and diameters are shown in **Fig. 6**. The differentiation rate of samples with stem diameter of 0.5 cm had a differentiation rate as high as 93%, and as the initial stem length became smaller, the differentiation rate lightly decreased, although almost every sample still differentiated. However, the differentiation rate of samples with stem diameter of 0.3 cm was 83%, and only half of samples with stem length of 0.3 cm differentiated. These findings indicate that the reproduction capacity of *A. philoxeroides* decreases as the fragment size decreases.

Comparison between the results of *A. philoxeroides* and *L. grandiflora* under same experiment

Appearance of A. philoxeroides and L. grandiflora after the experiment

To investigate the influence of nutrients on the regenerative capacity of fragments from another invasive alien plant, *L. grandiflora*, we utilized the experimental data presented by

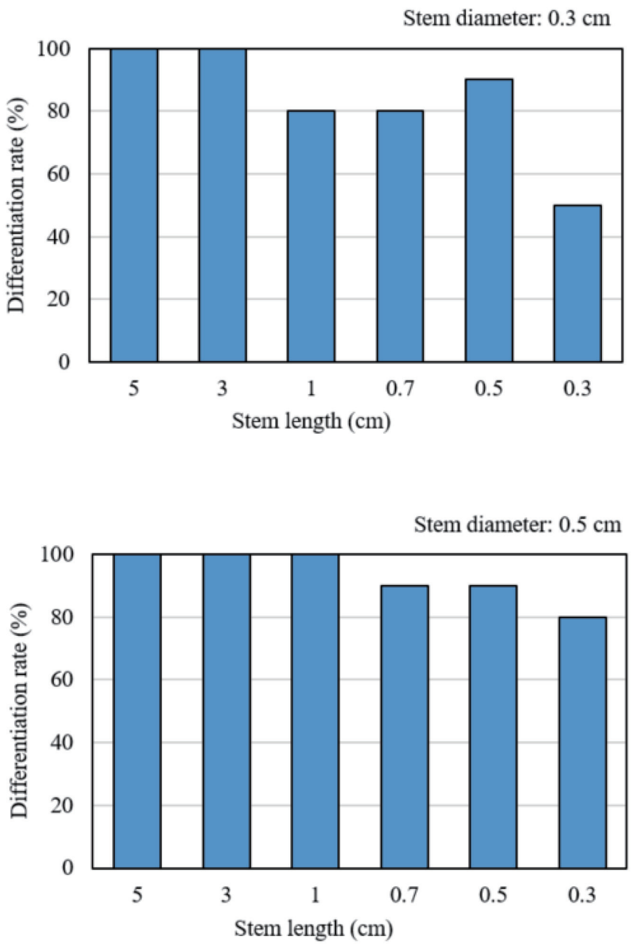

Fig. 6 The differentiation rates of samples with different stem sizes (Day 10).

Tanaka *et al.* [18]. Nevertheless, after conducting the same experiment an incubation period for ten days, the results revealed a significant distinction between *L. grandiflora* and *A. philoxeroides*. Notably, *A. philoxeroides* exhibited a growth pattern characterized by smaller leaves compared to *L. grandiflora*, as shown in **Fig. 7**.

Effects of nutrients on reproduction of these two plants

According to **Fig. 3 and 4**, it can be inferred that nutrients exert a positive influence on the early growth phase of *A. philoxeroides*. Although the contribution rate of nutrients gradually decreased over time, the samples treated with nutrients demonstrated a higher growth rate. The results of contribution rate of the increase in the projected area during cultivation of *L. grandiflora* is shown in **Fig. 8**, revealing that nutrient also contributes during the early stages of its growth.

However, the results of contribution rate of growth ratio on day 10 showed that the stem diameter was the factor significantly affect the growth of fragments, and the contribution rate was as high as 37.7%, as shown in **Table 3**.

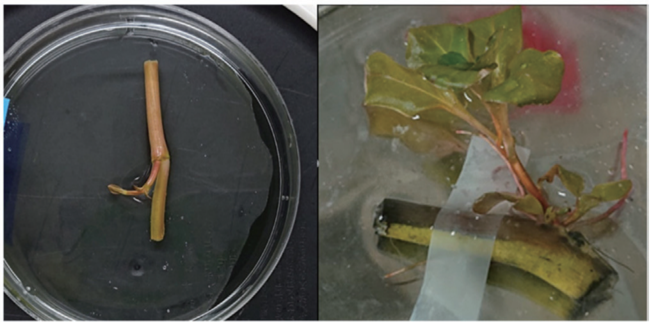

Fig. 7 The images shot on day 10 of same experiment and condition (left: *A. philoxeroides*, right: *L. grandiflora*).

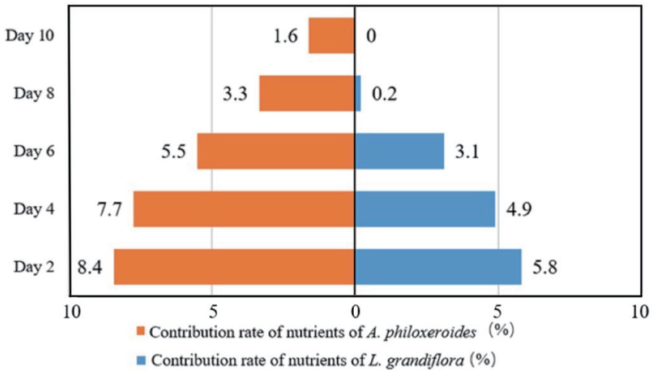

Fig. 8 Comparison between the contribution rate of nutrients of *A. philoxeroides* and *L. grandiflora*.

Table 3 Comparison between the contribution rate of reproduction factors of *A. philoxeroides* and *L. grandiflora*.

	Contribution rate on Day 10 (%)	
	A. philoxeroides	*L. grandiflora*
Nutrient (*A*)	29.1	0.0
Length (*B*)	13.2	12.7
Diameter (*C*)	8.4	37.7
(*a***b*)	17.1	7.8
(*b***c*)	3.1	10.4
(*a***c*)	1.3	0.0
Residual error (*e*)	27.8	31.3
Total	100.0	100.0

CONCLUSIONS

This study explored the impact of nutrient solutions, stem lengths, and stem diameters on *A. philoxeroides* regeneration. Notably, nutrient salts significantly enhanced growth ($p < 0.01$, Welch's *t*-test), emphasizing their role in the early stages. This suggests that *A. philoxeroides* thrives better in contaminated and eutrophicated areas, providing valuable insights for optimizing more effective extermination strategies. Additionally, smaller stem fragments exhibited diminished growth, reinforcing the importance of thorough removal down to the roots to minimize the persistence of fragments and enhance overall control measures. Furthermore, a comparative analysis with *L. grandiflora* revealed that *L. grandiflora* showed larger leaves under the same environmental conditions for 10 days, and stem diameter was the factor significantly influenced its growth, with a contribution rate of 37.7%. These findings not only provide insights for *A. philoxeroides* elimination but also emphasize the importance of selecting optimal eradication strategies for preservation of the aquatic ecosystem on Lake Biwa shore. Looking ahead, crucial discussions on implementing effective eradication strategies are essential for the sustainable management of this region.

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SUPPLEMENTARY MATERIALS

Supplementary Materials file for this article is available at the link below.

https://www.jstage.jst.go.jp/article/jwet/22/2/22_23-091/_supplement/_download/22_23-091_1.pdf

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