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Effect of Herbivore Predation and Water-Wave Disturbance on the Survival of Wild Extinct Aquatic Plant *Eriocaulon heleocharioides* (Eriocaulaceae) in the Reintroduction Site

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Abstract The effects of two factors, an alien herbivore and water-wave disturbance, on the decline of a wild extinct annual amphibious herb, *Eriocaulon heleocharioides* (Eriocaulaceae), was examined in a reintroduction site where the species existed. The numbers of surviving individuals after sowing seeds in three plot types were measured every two weeks. Surviving individuals were recorded only in plots sheltered by a net from all sides (Close). There were no surviving individuals to the reproductive stage in sheltered plots with opened corners (Semi-Close) and the plots without sheltering (Open). The effect of sheltering and vanishing water-wave structure (VWS) revealed that plot closing prevents aquatic organisms and mitigates the water waves. However, Semi-Close allowed aquatic organisms enter and water-wave disturbance as well. Thus, the overall survival is mainly promoted by the prevention of aquatic organisms to enter. The water-wave mitigation could additionally reduce the decline of the plants. To prevent herbivores is most essential for the design and management of the reintroduction site. The mitigation of water-wave disturbance could further help to reduce the decline of this plant.

Key words: Aquatic plant, *Eriocaulon heleocharioides*, extinct in the wild, herbivore, reintroduction.

Introduction

Eriocaulon heleocharioides Satake (Eriocaulaceae) is an annual amphibious herb that is endemic to Japan and extinct in the wild (Ministry of the Environment, 2019). Since 2008, a research project for *ex situ* conservation and reintroduction of the species to its last known habitat in the Sanuma Lake, Ibaraki Prefecture, has been carried out by the Tsukuba Botanical Garden, National Museum of Nature and Science, Japan (Tanaka *et al.*, 2014, 2015, 2018, 2020). Sowing seeds of the species in the reintroduction site produced hundreds to thousands of surviving individuals in the first few years of the project. Then, the number of individuals gradually decreased despite the continued sowing each year (Tanaka *et al.*, 2018).

Various factors – methodological, environmental and biological – are thought to cause the difference between success and failure (Guerrant and Kaye, 2007; Menges, 2008; Godefroid *et al.*, 2011). In the case of *Eriocaulon heleocharioides*, predation by alien aquatic organisms such as bullfrog tadpoles (*Rana catesbeiana*) and red swamp crayfish (*Procambarus clarkii*), is presumed as the leading harmful factors (Tanaka *et al.*, 2018). A net sheltering of the sowing site at the experimental area, located in the last habitat of Sanuma Lake, improved the survival of *E. heleocharioides* seedlings (Tanaka *et al.*, 2018, 2020). The net sheltering could have protected the plants from herbivore predation or water-wave disturbance. Tanaka *et al.* (2018) reported a lower impact of water wave disturbance on the survival than that of herbivores. However, it was an estimate based only on observation and there was no evidence to support it.

These previous researches revealed that *E. heleocharioides* cannot survive to the reproductive stage without protection from the present natural environment of Sanuma Lake. The construction design needs the identification of factors inhibiting plant survival and their degrees of impact. This study aims to examine the degree of impact of two factors, e.g., aquatic herbivore predation and water-wave disturbance, on the survival decline of *E. heleocharioides* in its reintroduction site.

Materials and Methods

Study sites and data measurements

This experiment was conducted within a semi-protected area constructed in Yanagi Wando, Sanuma Lake, Shimotsuma City, Ibaraki Prefecture, Japan. The area was made of timber and waterproof sheets to hold soil and water. It consists of three plots in eight beds in two sites (Fig. 1). Site A was used for the experiment in 2017 (only Bed-1 and -2; Tanaka *et al.*, 2018) and 2018 (Bed-1, -2, -3 and -4; Tanaka *et al.*, 2020). Site B was newly constructed in March, 2019. All beds, except Bed-1 and Bed-5 that were isolated from the water of the lake, were used for this study. Bed-2 and -6, Bed-3 and -7, and Bed-4 and -8 have the same ground levels – 28, 42 and 57 cm below the maximum water levels of the lake, respectively. Each site has nine plots including 25 sub-plots of 0.048 m^2 (0.3 × 0.16 m). All eighteen plots were categorized into three types of treatments, e.g. Open, Semi-Close and Close. The

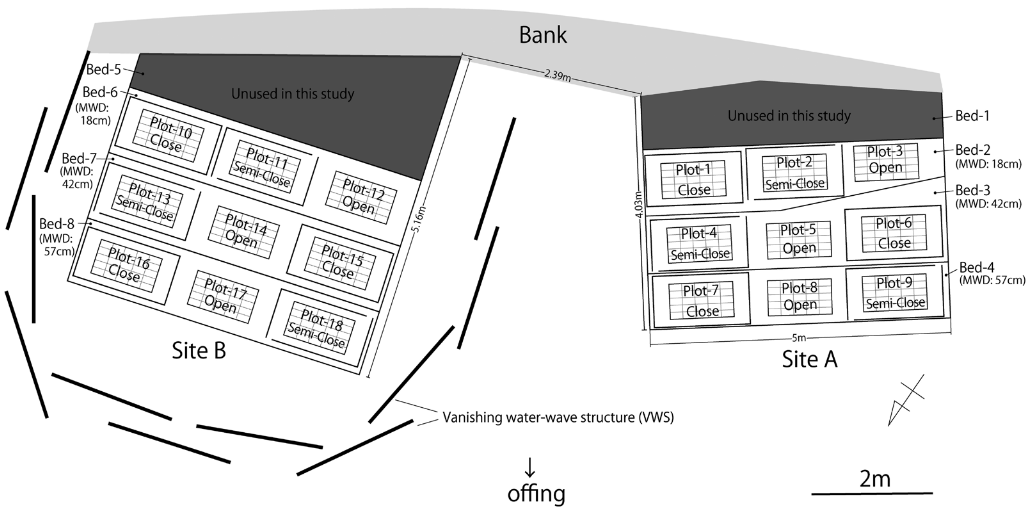

Fig. 1. Schematic layout of the semi-protected area used for this study. MWD means maximum water depth on the soil surface at each bed.

Close treatment (Plots-1, -6, -7, -10, -15 and -16) was fenced all around by nets with 2-mm mesh to prevent herbivores from entering them, while in the Semi-Close treatment, the two diagonal corners were left open (Plots-2, -4, -9, -11, -13 and -18). No fencing was used in the Open treatment (Plots-3, -5, -8, -12, -14 and -17). In each bed, the three treatment types area were randomly arranged.

In March 2018, the soil of Bed-3 and Bed-4 of site A (constructed in March 2017) has been replaced with new soil from a location 10 m offshore. That of Bed-2 had been partly left unchanged, partly replaced with new soil, and partly plowed to replace the surface soil with subsoil. In 2019 (for this study), the surface soil up to 10 cm depth at all beds in site A was replaced with new soil from 10 m offshore. Site B was constructed in March 2019 at about 3 m next to the site A. At the same time, the soils of Bed-6, Bed-7, and Bed-8 in the Site B was newly transported from a location 10 meters offshore. Eleven vanishing water-wave structures (VWS) were constructed to surround site B to mitigate the water-wave disturbance. The VWS is 182 cm wide and 115 cm high and consisted with 13 timbers 7.5 cm high, 182 cm wide and 9 mm thick, boarded with a gap of 5 mm. The mouth of the Yanagi Wando holding the study site directs to west and the winds often blow from west or north west and produces water-waves to the study site, especially in Spring and Autumn (by authors' observation).

On March 29, 2019, 151 seeds of *Eriocaulon heleocharioides* that were collected in the fall of 2018 from individuals raised in the semi-protected area (Bed-1 in Fig. 1) of Sanuma Lake, were sown in each sub-plot (Tanaka *et al.*, 2018, 2020). The numbers of individuals were counted in nine sub-plots, randomly selected from 25 sub-plots per plot, every two weeks from April 15 to October 21, 2019. Surviving individuals until October 21 were considered as final surviving individuals. Most of them flower by October and produce seeds by November.

Statistical Analysis

To quantify the effect of Net-sheltering and VWS on the number of surviving individuals, analysis was performed using a Poisson generalized linear mixed model (GLMM) across the surveyed days. We designated the number of surviving individuals as the response variable, net-sheltering (three category: Open, Semi-Close, and Close), VWS (two category: set and no set) and Bed for experiment (Upper, Middle and Lower) as the predictor and each plot as random effects term using lme4 package (version 1.1-19, Bates *et al.*, 2015). These analyses were performed using R software (version 3.5.3, R Development Core Team, 2019).

Results

Final surviving individuals of *Eriocaulon heleocharioides* were found only in Plot-1 (2.0 individuals/sub-plot), Plot-6 (13.7), Plot-10 (3.6) and Plot-15 (1.6), which are all of the Close

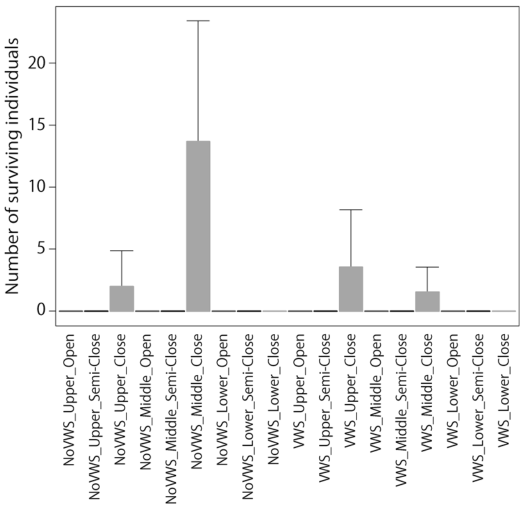

Fig. 2. Mean number of the final surviving individuals for sub-plots in each plot. Bars indicate SD. Each plot includes the combination of three elements: VWS (vanishing water-wave structure) is set or not, the water depth is Upper (Bed-2 and -6), Middle (Bed-3 and -7) or Lower (Bed-4 and -8), and the sheltering type is Open, Semi-Close or Close.

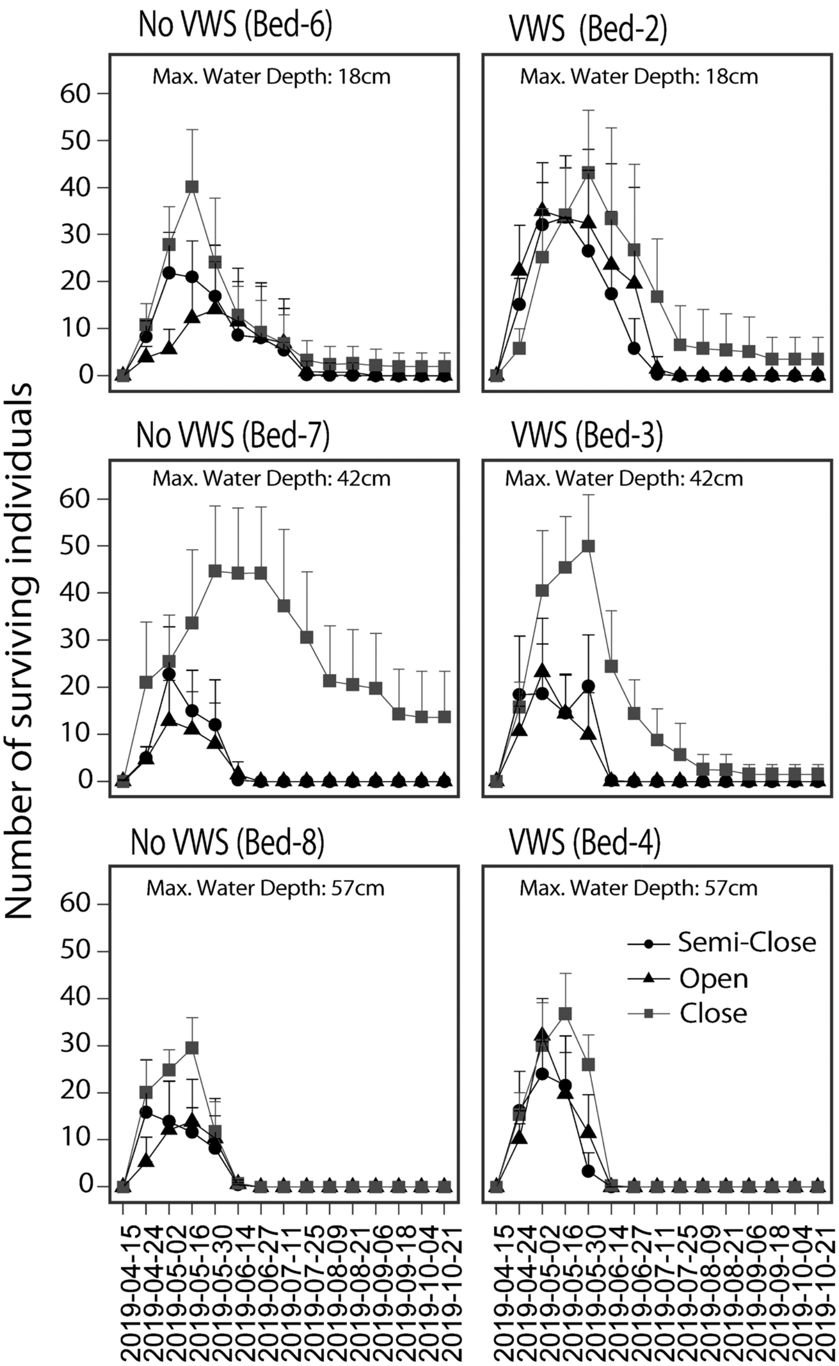

Fig. 3. Temporal change of mean number of surviving individuals for sub-plots in each plot. Bars indicate SD.

treatments in Bed-2 and Bed-3 (Fig. 2). Regardless of the presence or absence of the VWS, no individuals survived until the reproductive stage in any of the Bed-4 and the Open and Semi-Close treatments of Bed-2 and -3.

The temporal change of surviving individual number is shown in Fig. 3. The number of individuals was remarkably changed during the early

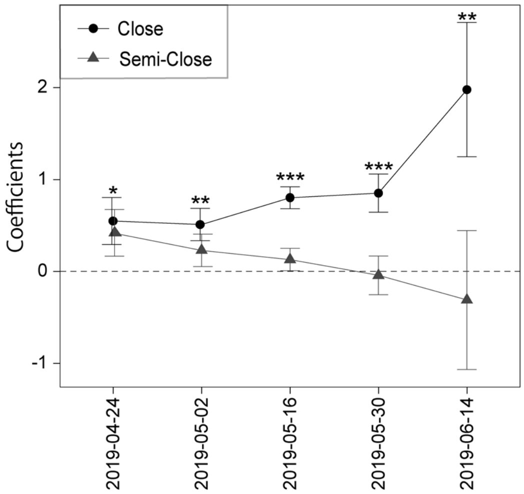

Fig. 4. Temporal change of the effects of net-sheltering in the Close and Semi-Close treatments on the number of surviving individuals. The coefficient values were shown on the vertical axis. Bars indicate SD. Asterisks mean the significant levels (***P＜0.001, **P＜0.01, *P＜0.05). Data after June 14th was excluded from the analysis because the number of surviving individuals became zero at most of plots.

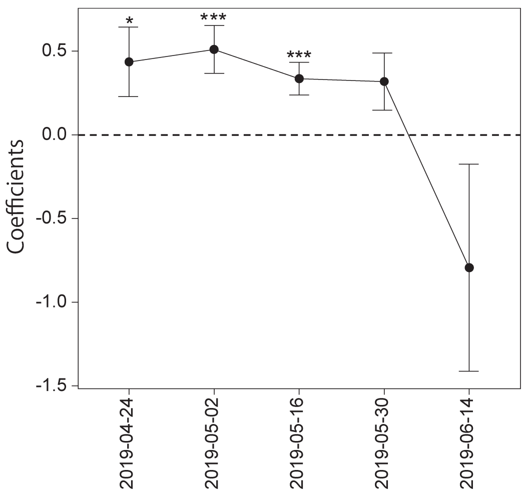

Fig. 5. Temporal change of the effects of VWS on the number of surviving individuals. The coefficient values were shown on the vertical axis. Bars indicate SD. Asterisks mean the significant levels (***P＜0.001, **P＜0.01, *P＜0.05). Data after June 14th was excluded from the analysis because the number of surviving individuals became zero at most of plots.

stage of their life cycle – from April to June. GLMM analysis shows the positive effect of Close treatment throughout the period on the plant survival compared to the Open treatment, especially after the middle of May ($p < 0.01$–0.001, Fig. 4). In contrast, no effect was detected in the Semi-Closed treatment throughout the period.

GLMM analysis showed that the VWS had a positive effect on plant survival in all plots from April to May (Fig. 5). Comparing the types of shelter treatment, definitive effects of the VWS are shown in Open from April to May, while slight effect only in April 15 in Semi-Close, and no effects were detected in Close throughout the period (Fig. 6).

Discussion

The effect of the three sheltering types

The number of the final surviving individuals that can produce seeds for the next generation is an important measure for the suitability of environmental conditions for the reintroduction of an annual species, *Eriocaulon heleocharioides*. In this study, the final surviving individuals were recorded only in four plots. All of them were found in the Close treatments (among all 18 plots) (Fig. 2). The higher survival in the Close treatment compared to the Open treatment agrees with the results of the 2017 and 2018 experiments (Tanaka *et al.*, 2018, 2020).

On the other hand, there was no significant effect in the Semi-Close treatment with two open corners (Fig. 4). Since the lateral side of the semi-closed treatments was about 93% covered by the net, they were set to allow most of the aquatic organisms to enter the plot, but also to mitigate water waves. However, since the strength of water waves was not recorded in each plot, the actual effect of water-wave mitigation was not clearly shown in this experiment. Therefore, it may reflect not only the effect of aquatic organisms but also the effect of being unable to mitigate water waves in the Semi-Close treatment structure. Here, the effect of the VWS in

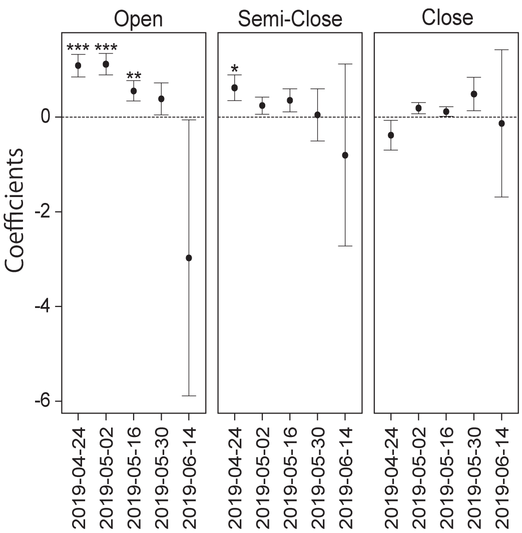

Fig. 6. Difference of the effect of VWS on the number of surviving individuals in the three types of net sheltering: Open, Semi-Close and Close. The coefficient values were shown on the vertical axis. Bars indicate SD. Asterisks mean the significant levels (***$P<0.001$, **$P<0.01$, *$P<0.05$). Data after June 14th was excluded from the analysis because the number of surviving individuals became zero at most of plots.

each treatment (Fig. 5) seems to help this discussion. The clear effect of VWS in the open treatment and the lacking effect in the closed treatment indicate that the closed treatment mitigates the water waves properly. On the other hand, the weak effect observed only in the initial period in the Semi-Close treatment indicates that it has a wave mitigating effect, too. However, it is weaker than that of the closed treatment.

Judging from the above, the positive effect on the survival of individuals in Close treatment, especially the clear effect on the final surviving individual number, is mainly due to the prevention of aquatic organisms' entrance. Also, the water-wave disturbance could additionally inhibit the decline of plants. Water-wave mitigation was shown to improve the survival at least in the early stage since the effect of the VWS was clearly detected (Fig. 5) and the difference between the effects of Semi-Close and that of Open treatments was small in the early stage–until the first half of May (Fig. 6). In general, the seedling stage is the most vulnerable part of the plant life-cycle (Primack and Drayton, 1997), because during that period the seedlings could easily be affected by environmental disturbances such as water waves. Many early seedlings of *E. heleocharioides* were observed by the authors in the Semi Protection area of the Sanuma Lake to detach from the soil and to float on the water surface. The VWS may be effective in the other season as well. However, further discussion is not possible at this time because no plant individuals survive after the early growth stage except those in the closed treatment.

Water depth effect

Regardless of the net-sheltering of the closed treatment, there were no surviving individuals in the lowest bed (Bed-4). Although the previous experiment of 2018 showed that the individuals could survive in the Lower bed (Tanaka *et al.*, 2020), there were no surviving individuals at the same depth in this study. Water depth is generally known to have a strong effect on the survival and growth of aquatic plants, particularly those that are submerged (Havens, 2003; Bornette and Puijjalson, 2011). In the reintroduction site of *E. heleocharioides*, the limitation of water depth on plant growth can be amplified by factors with annual fluctuations, such as changes in water transparency and water level. They can affect the amount of transmitted light to the bottom and water edge position where the physical disturbance is stronger. For reintroduction planning and management, it is necessary to consider the water depth that allows for annual fluctuations.

Design of reintroduction site

Herbivore predation is probably the main factor that inhibits the survival of *E. heleocharioides* in the reintroduction site, while water-wave disturbance is less harmful. In a review paper considering the reintroduction cases of 249 plant species worldwide (Godefroid *et al.*, 2011), the authors have reported that plant reintroduction in protected areas significantly increased the survival rate compared to the reintroduction in unprotected areas. They have also suggested that the management of the out-planting site through either preparation for planting (e.g. fencing) or post-planting management (e.g. fire, reduced competition) increased the probability of reintroduction success. Since it is difficult to remove or mitigate negative factors, such as herbivore predation and water-wave disturbance, throughout the whole Sanuma Lake (having 0.55 km^2 area), the creation of a suitable environment at the reintroduction site can only be an applicable method under the present conditions. So far, the reintroduction site design for this wild extinct plant needs to prevent herbivores from accessing the plants, which is the most essential task. Then, water-wave disturbance mitigation could help to inhibit the decline of the seedlings.

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