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Effect of Net Sheltering on the Survival of a Wild Extinct Aquatic Species *Eriocaulon heleocharioides* in Its Reintroduction Site

Norio Tanaka[1,*], Yuju Horiuchi[2], Yujian Duang[2], Seri Hasegawa[2], Shoh Nagata[3] and Takashi Kamijo[2]

[1] Department of Botany, National Museum of Nature and Science, Amakubo 4–1–1, Tsukuba, Ibaraki 305–0005, Japan
[2] Graduate School of Life and Environmental Sciences, University of Tsukuba, Amakubo 1–1–1, Tsukuba, Ibaraki 305–0005, Japan
[3] Specified Nonprofit Corporation AQUA CAMP, Shimohiro-oka 670–126, Tsukuba, Ibaraki 305–0042, Japan
* E-mail: ntanaka@kahaku.go.jp

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Abstract The effect of net sheltering on the survival and reproduction of a wild extinct aquatic species *Eriocaulon heleocharioides* was examined in its reintroduction site, Sanuma Lake, Japan. The number of surviving individuals and inflorescences between plots with and without a net was compared. Analysis using generalized linear models revealed that net sheltering positively affects the survival and reproductive outcome of this species. Presence of herbivores such as the American bullfrog tadpoles and red swamp crayfish, rather than water waves, may have decreased the number of surviving *E. heleocharioides* individuals in Sanuma Lake.

Kew words : Aquatic plant, *Eriocaulon heleocharioides*, extinct in the wild, herbivory, reintroduction.

Introduction

Eriocaulon heleocharioides Satake (Eriocaulaceae) is an annual aquatic herb endemic to Japan and an extinct species in the wild (Ministry of the Environment, 2017). This species had disappeared from its last habitat, Sanuma Lake, Shimotsuma, Ibaraki Prefecture, central Japan, after a change in the lake's water management plan in 1994 and has only been preserved *ex situ*. In 2008, a project for reintroducing this species in Sanuma Lake was initiated by the Tsukuba Botanical Garden, National Museum of Nature and Science, Japan. Firstly, the project team first persuaded the concerned parties to use the previous method of water management in Sanuma Lake. This was followed by sowing seeds in the lake in 2008. During the same time, their floral visitors and seed storage and germination conditions were revealed for basic information to conduct *ex situ* conservation and reintroduction (Tanaka *et al.*, 2014, 2015). From 2009 onward, although many *E. heleocharioides* individuals survived and bore seeds in Sanuma Lake for the first several years, their number subsequently decreased in spite of continuous sowing (Figs. 1, 2).

Numerous factors affect the success or failure of plant reintroduction, which is defined as that the release and management of a plant into an area in which it has become or is believed to be extinct (Akeroyd and Wise Jackson, 1995; IUCN, 1998). Factors affecting reintroduction are classified into three categories: 1) methodological (site preparation, seedling planting method, protection or no protection, and reintroduced individual number), 2) environmental (predation and habitat change) and 3) biological

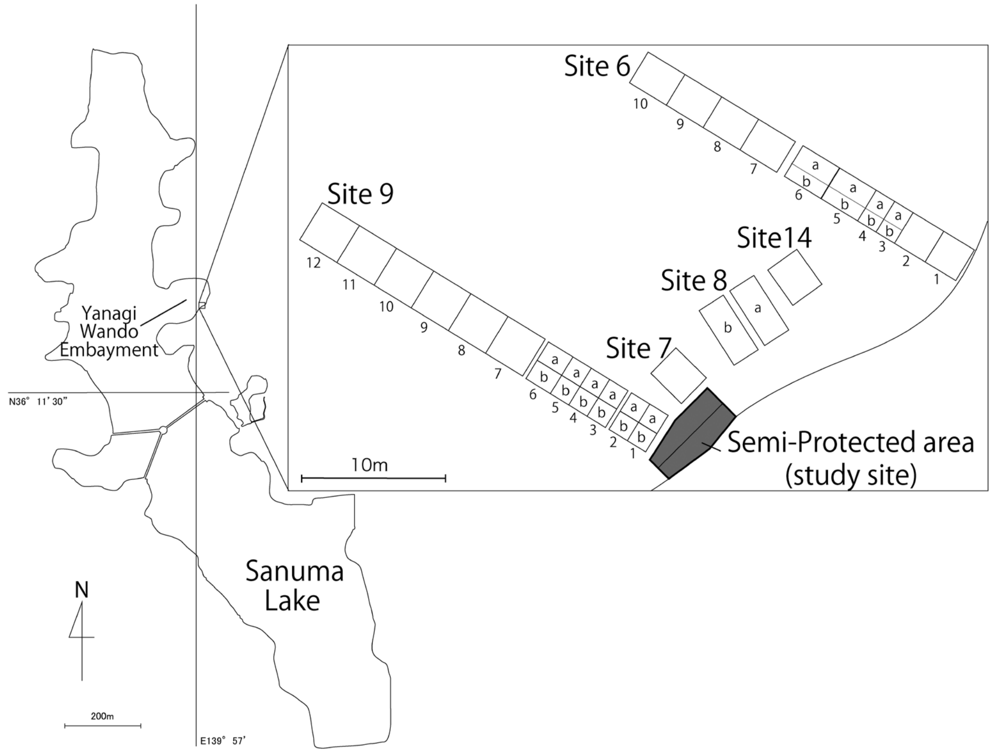

Fig. 1. Reintroduction sites in Sanuma Lake. The positions of site 6, 7, 8, 9 and 14, reintroduction sites from 2011 to 2016, are shown. Semi-protected area is the study site.

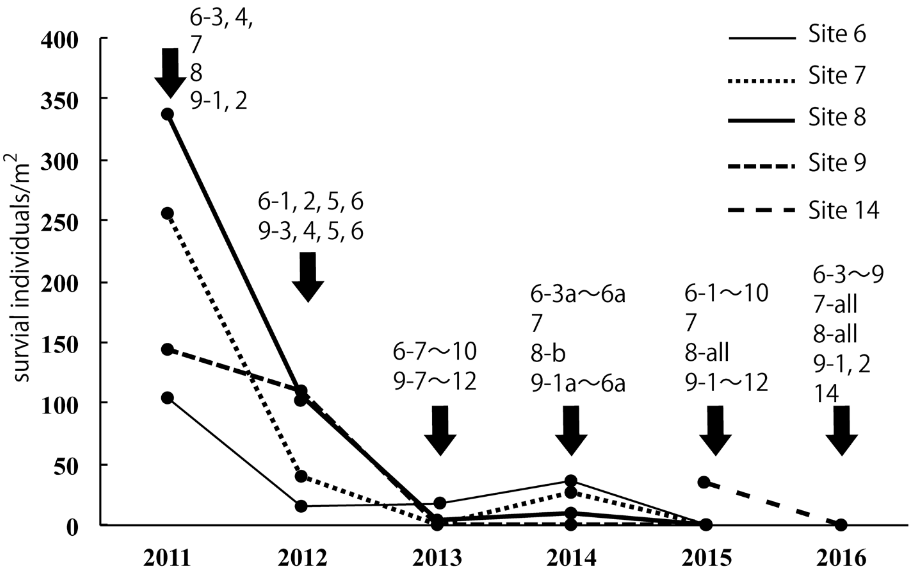

Fig. 2. Change in the survival individuals of *E. heleocharioides* from 2011 to 2016 in the reintroduction sites of Sanuma Lake. The numbers correspond to the site numbers in Fig. 1. Black arrows and numbers show the timing and sites in which seeds were sowed. The number of sowed seeds were 6880/m^2 at all sites.

(propagule types, single or multiple population use, and propagule genetics) factors (Guerrant and Kaye, 2007; Menges, 2008; Godefroid *et al.*, 2011). Godefroid *et al.* (2011) reviewed possible reasons for reintroduction failure, including reinforcement and translocation, and identified predation and habitat change to be the major factors, after wrong habitat use. In a study involving five tree species, the overall survivorship and growth were significantly higher for sheltered than those for unsheltered seedlings (Sweeny *et al.*, 2002). Maschinski *et al.* (2004) concluded that the reintroduction of the endangered shrub *Purshia subintegra* to limestone mesas in Arizona, USA is possible and most promising with the use of cages and provision of supplemental watering.

Candidate herbivores feeding on *E. heleocharioides* in Sanuma Lake include American bullfrog tadpoles (*Rana catesbeiana*) and red swamp crayfish (*Procambarus clarkii*), who are known to consume submerged aquatic plants (Feminella and Resh, 1989; Rodríguez *et al.*, 2003; Pryor and Bjorndal, 2005a, b). Although no quantitative data for the individual numbers of each herbivorous species is available, we sometimes observed these species in Sanuma Lake. In autumn of 2016, numerous American bullfrog tadpoles were observed in shallow water around the reintroduction sites in Sanuma Lake and suspected an increase of their number in the recent years. These herbivores represent a potential risk factor for the successful reintroduction of *E. heleocharioides* in Sanuma Lake.

This study aims to investigate the influence of net sheltering on the survival and reproduction of *E. heleocharioides* in Sanuma Lake by comparing the numbers of surviving individuals and inflorescences between plots with and without net.

Materials and methods

This experiment was conducted within the semi-protected area constructed in Yanagi Wando, Sanuma Lake, Shimotsuma City, Ibaraki Prefecture, Japan (Figs. 1, 3). The area comprised three experimental plots (Plot-1, 2, and 3), each divided into sub-plots of 0.25 m^2 area. Plot-1 was consists of 18 sub-plots and totally 4.5 m^2 area. This plot was structurally isolated from the Lake, and the soil surface is 2 cm below the maximum water level in the lake. The water level is always approximately 5 cm owing to the water supply from a hose by pumping from the lake. In Plot-2 (9 sub-plots, 2.25 m^2 area) and Plot-3 (9 sub-plots, 2.25 m^2 area), the soil surface was 28 cm below the maximum water level of Sanuma Lake, and both the plots were always connected to the lake to maintain an even water level. A net (2-mm mesh) to prevent herbivores was set only for Plot-2. In all plots, the soil was transported from a location 10 meters offshore from these experimental plots.

In March 2017, 1720 seeds of *E. heleocharioides* were sowed in each sub-plot. The seeds were collected in the autumn of 2016 from individuals being cultivated since 2003 in the nursery of Tsukuba Botanical Garden, National Museum of Nature and Science, Japan.

Surviving individuals, defined as the individuals that grew to bear flowers, were counted for each sub-plot on October 1, 2017. The number of inflorescences was evaluated after collecting all individuals from every sub-plot on October 26, 2017.

To quantify the differences in the number of surviving individuals and inflorescences among the three experimental plots, we used generalized linear models (GLM) from the r-package “stat.” The effect of treatments on the number of individuals and inflorescences was analyzed using GLM with families of distributions set to “poisson” and “negative binomial,” respectively. Link function was designated to “log” function. After analyzing using GLM, multiple comparisons among the three plots were performed using r-package’s “multcomp” (Hothorn *et al.*, 2017). These analyses were performed using R software (version 3.51, R Development Core Team, 2018).

Results

The mean number of survival individuals of

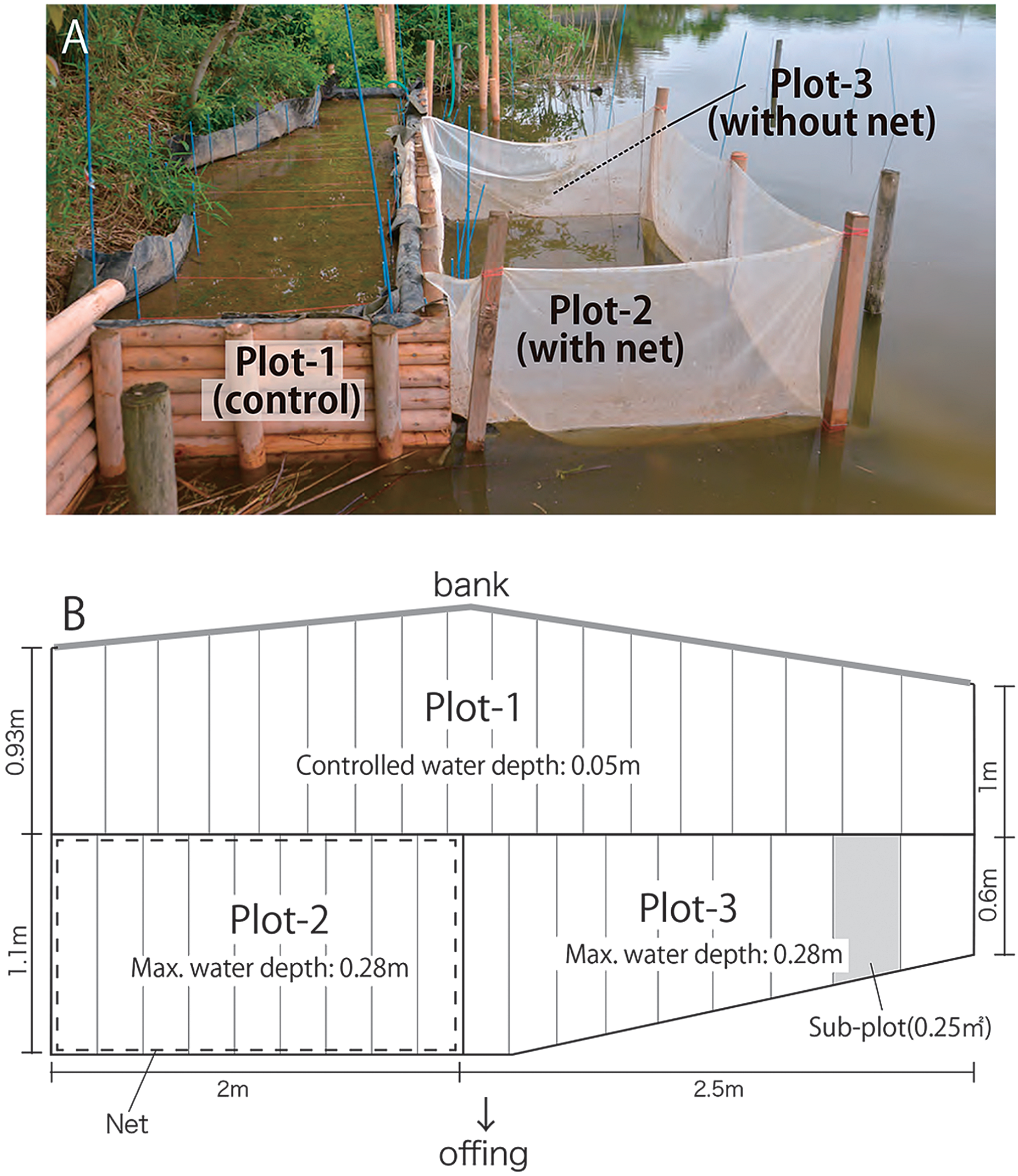

Fig. 3. Semi-protected area. The position in Sanuma is shown in Fig. 1. A. Overall appearance. Plot-2 and 3 are connected to Sanuma Lake but Plot-1 is not. B. Schematic layout of the semi-protected area. The three plots are divided into sub-plots of 0.25 m^2.

Eriocaulon heleocharioides per sub-plot were 162 (individuals/0.25 m^2) in Plot-1 (control), 206 in Plot-2 (with net), and 18 in Plot-3 (without net), and it did not significantly differ between Plot-1 and Plot-2 (GLM, $P = 0.9486$, Fig. 4A). Plot-3 produced a significantly lower number of surviving individuals than the other two plots ($P < 0.001$).

The mean number of inflorescences was 1238 (inflorescences/0.25 m^2) in Plot-1, 1174 in Plot-2, and 330 in Plot-3 and was significantly different among all three plots ($P < 0.001$, Fig. 4B).

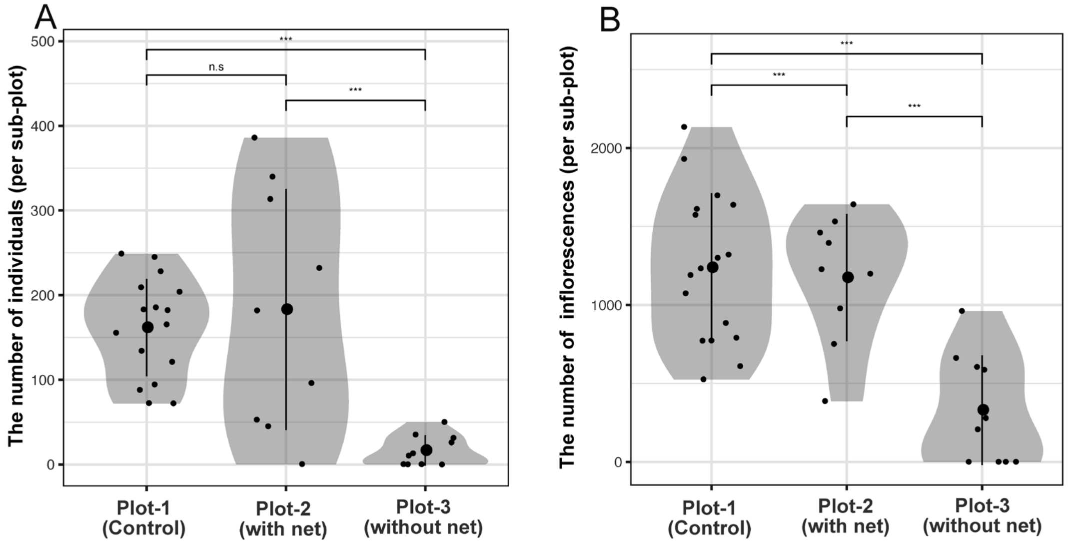

Fig. 4. Comparison of the number of survival individuals and inflorescences in Plot-1, 2 and 3 in the semi-protected area using generalized linear models (GLM). A. number of survival individuals. B. Number of inflorescences. Mean number (big black circle), standard deviation (vertical bar) and distribution pattern of data (grey area) are shown. Significance between three Plots are marked above the data: *** and n.s show significant difference ($p < 0.001$) and not significant, respectively.

Discussion

This study revealed that net sheltering positively affected the survival and reproductive outcome of *Eriocaulon heleocharioides* in its reintroduction sites. Sheltered plots are likely to be protected from predation by herbivores or to eliminate water wave disturbances. Predation by herbivores is an important factor affecting the success or failure of plant species reintroduction (Guerrant and Kaye, 2007; Menges, 2008; Godefroid *et al.*, 2011). In fact, sheltered (caged) areas show higher survival rates of reintroduced plant seedlings than unsheltered areas (Sweeny *et al.*, 2002; Maschinski *et al.*, 2004).

In the Plot-3 (without net), some individuals survived to make a small patch. Most leaves and peduncles of inflorescences of the individuals at the edge of the patch were found to be cut (Fig. 5). The shape of the cuts suggested an attack by an animal and did not seem to result from a disease or physical disturbance. Thus, herbivores are likely to be the cause that resulted in a reduced number of surviving individuals in Plot-3. Although American bullfrog tadpoles and red swamp crayfish are the candidate herbivores, direct evidence on the identity of the herbivore responsible could not be provided.

Water wave disturbances are mainly induced by wind. Wave exposure represents a key factor influencing the abundance, species richness, and depth distribution of submerged macrophytes and emergent vegetation (Spence, 1967; Chambers, 1987; Riis and Hawes, 2003). Wave exposure can directly remove plant biomass by fragmentation, undermining, or uprooting plants and can also prevent the establishment of propagules. In addition, wave-related sediment transport can indirectly affect plants by creating substrate conditions that are unsuitable for plant establishment or by burying established plants (Keddy, 1982). Because the effect of disturbance such as change in soil surface condition and buried individuals were not observed, the possibility of water wave disturbance was considered low.

In this study, we identified the factors that reduce the survival of *E. heleocharioides* and demonstrated that survival can be significantly

Fig. 5. *Eriocaulon heleocharioides* survived at the edge of patch in Plot-3 (without net). White circles show cut leaves and peduncles of inflorescences.

improved by net sheltering. However, the environmental conditions of these experimental plots differ from the previous reintroduction sites in two points. One is using the soil transported from a location 10 meters offshore, which may have different trait from previous soils. The other is that the plots are located in shallower place where the light will much transmit to the bottom compared to most previous reintroduction sites. In addition to net sheltering, verification of the aforementioned two factors will provide important implications for establishing independent population in the reintroduction site.

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