

# Effects of Net Sheltering, Water Depth, and Soil Management on the Survival and Reproduction of the Wild Extinct Aquatic Plant *Eriocaulon heleocharioides* in Sanuma Lake, Japan

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**Abstract** The effects of net sheltering, water depth, and soil management on the survival and reproduction of the aquatic plant *Eriocaulon heleocharioides*, which is currently extinct in the wild, were investigated in Sanuma Lake, Japan. The numbers of surviving individuals and inflorescences per individual were measured in plots with and without nets within three beds that were at different water depths. In addition, the soil in the plots in one bed was subjected to three different management regimes: partial removal and replacement with new soil, plowing to replace the surface soil with subsoil, or remaining undisturbed. It was found that the provision of net sheltering increased the number of surviving *E. heleocharioides* individuals but not inflorescences. Water depth also had some effect on the number of surviving individuals, with the highest survival occurring at the shallowest depth, but *E. heleocharioides* was able to survive at a depth of at least 0.57 m when net sheltering was also provided. In terms of the soil management regime, survival was highest with the soil removal and replacement treatment and lowest with the undisturbed treatment, suggesting that the transport of new soil from the bottom of Sanuma Lake will likely improve the survival of this species.

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

## Introduction

*Eriocaulon heleocharioides* Satake (Eriocaulaceae) is an annual aquatic herb that is endemic to Japan and extinct in the wild (Ministry of the Environment, 2017). Since 2008, a research project has been carried out by the Tsukuba Botanical Garden, National Museum of Nature and Science, Japan with the aim of reintroducing this species to its last known habitat in Sanuma Lake, Ibaraki Prefecture (Tanaka *et al.*, 2014, 2015,

2018). However, although hundreds to thousands of *E. heleocharioides* individuals survived in Sanuma Lake for the first few years of this project, the number of surviving individuals gradually decreased despite continued sowing each year.

Predation is considered to be one of the factors that affects the success or failure of plant reintroductions (Guerrant and Kaye, 2007; Menges, 2008; Godefroid *et al.*, 2011) and has been reported to inhibit the reintroduction of some plant species (Sweeny *et al.*, 2002; Maschinski *et al.*, 2004). Predation by herbivores is also con-

sidered a candidate factor that is inhibiting the reintroduction of *E. heleocharioides* in Sanuma Lake. To examine this effect, Tanaka *et al.* (2018) investigated the influence of net sheltering on the survival and reproduction of *E. heleocharioides* in a semi-protected area in Sanuma Lake and found that the survival of this species was significantly improved by net sheltering. However, the main factor limiting the reintroduction of this species has not yet been identified.

Water depth is an important factor influencing the occurrence and propagation of aquatic plants (Hutchinson, 1975; Havens, 2003; Bornette and Puijgalson, 2011). Although the water depth of the semi-protected area that was constructed in Sanuma Lake in 2017 may have affected the survival and reproduction of *E. heleocharioides* due to differences in the light intensity and wave strength, there was only one water depth in this area, which was lower than the shallowest area in Sanuma Lake (Tanaka *et al.*, 2018). Therefore, experiments using a range of water depths that are similar to those found in Sanuma Lake are required. Soil conditions also have a strong effect on the growth of plants (Passioura, 2002; Bengough *et al.*, 2005), so soil management methods in the semi-protected area of Sanuma Lake, where soil movements are not expected to occur in the same way as on the natural floor of the lake, may greatly affect the growth and survival of *E. heleocharioides*.

This study investigated the effects of net sheltering, water depth, and soil management on the survival and reproduction of *E. heleocharioides* within the semi-protected area in Sanuma Lake, building on the findings of the previous experiment in 2017.

## Materials and Methods

### Study sites and data measurements

This experiment was conducted within the semi-protected area that was constructed in Yanagi Wand, Sanuma Lake, Shimotsuma City, Ibaraki Prefecture, Japan in 2017. This area comprises four beds: Bed-1 [Plot-1 in Tanaka *et al.*

(2018)], which is isolated from the lake and was not used in this study, and Beds-2, -3, and -4, which are located in part of the Sanuma Lake and were used in the present study (Fig. 1). Beds -2, -3, and -4 have the same water levels as the lake, with soil surfaces that are 28, 42, and 57 cm below the maximum water level of the lake, respectively. There were eight experimental plots in each of these three beds (Plots-2A, -2B, -3A, -3B, -4, -5, -6, and -7), which were each divided into 0.25 m<sup>2</sup> sub-plots. Plots-2A, -2B, -3A, -4, and -6 were fenced by nets with 2-mm mesh to prevent herbivores from entering them, while Plots-3B, -5, and -7 were left unfenced. Bed-2 was constructed and filled with soil in 2017, the year before this study, whereas the soil in Beds-3 and -4 was transported from a location 10 meters offshore from these experimental beds. Consequently, the soil in Plot-2A of Bed-2 was removed and replaced with new soil to match the conditions in Beds-3 and -4, while the surface of the soil in Plot-2B in Bed-2 was plowed to replace the surface soil with subsoil as an alternative method of recovering the soil.

In March 2018, 1720 seeds of *Eriocaulon heleocharioides* that had been collected in fall 2017 from individuals being raised in the semi-protected area of Sanuma Lake were sown in each sub-plot within Beds-2, -3, and -4. The numbers of surviving individuals and inflorescences per individual were then counted in each sub-plot in October to November, 2018, where surviving individuals were defined as those that bore flowers.

### Statistical analyses

It is intended that the results that are obtained from this study will be incorporated into guidelines for the preparation of better conditions for *E. heleocharioides* and applied immediately for the conservation management of this species. Therefore, since only small sample sizes were obtained, Bayesian statistical models were used to stabilize the estimates and directly calculate the probabilities of the hypothesized effects.

First, a Poisson hierarchical model was con-

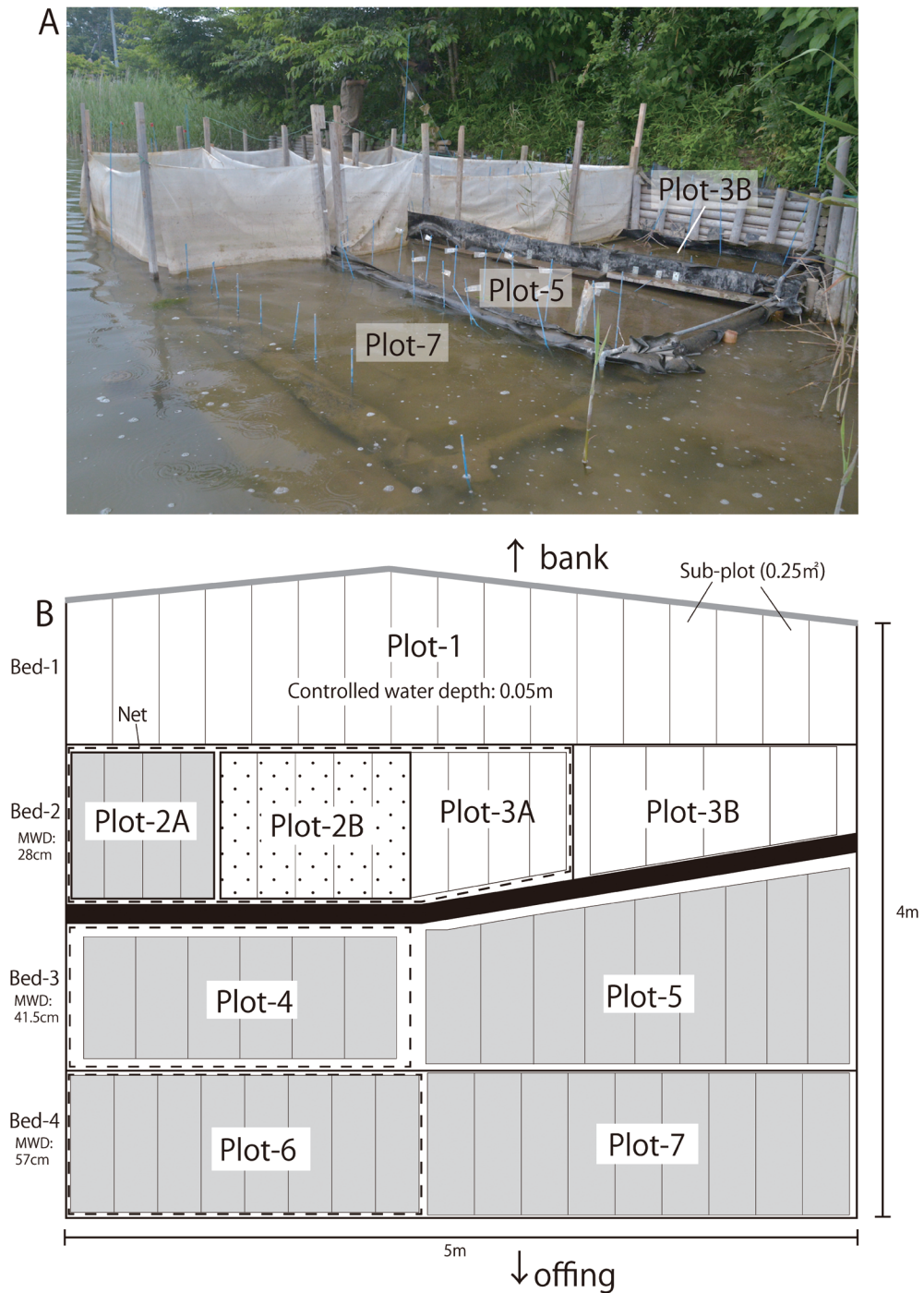


Fig. 1. Semi-protected area in Sanuma Lake. A. Overall appearance. The water level in this photo is around 40 cm lower than the maximum water depth. B. Schematic layout of the area. Maximum water depth (MWD) is 28 cm at Bed-2, 41.5 cm at Bed-3, and 57 cm at Bed-4. All plots consists of 0.25 m<sup>2</sup> sub-plots. Broken lines indicate nets for sheltering. In the light gray plots, the soil was transported from offshore in Spring of 2018. Dotted plot indicates that the soil was plowed to replace surface soil with subsoil. The soil in other plots (white plots) has not been disturbed since 2017.

structed to test the effects of net sheltering (“Net”) and different water levels (“BED”) on the number of surviving individuals of *E. heleocharioides*, as well as the effect of the soil treatment between previous study (Tanaka *et al.*, 2018) and present study, which was the experiment conducted only in BED-2. The effect of the Net treatment between BED lines was estimated as a random effect because it could not be clearly distinguished on some occasions. For instance, experimental plots became same above water condition when the water levels of outside of experimental plots were lower, thus we have the constraints that the effects between BEDs are similar in NET and BED, respectively. In addition, the effects of year and soil treatment were also estimated as random effects. The final model was as follows:

$$\begin{aligned}
 NM_{Indiv} &\sim \text{poisson}(\exp(\lambda_{indiv})) \\
 \lambda_{indiv} &= \begin{cases} r\_year[Year] \\ \quad \text{if } Year = 2017, \text{ Soil} = 0 \\ r\_year[Year] + \\ \quad rn\_net_{without}[BED] + \\ \quad rn\_net_{with}[BED] \times Net \\ \quad \text{if } Year = 2018, \text{ Soil} = 0 \\ r\_year[Year] + \\ \quad rn\_net_{without}[BED] + \\ \quad rn\_net_{with}[BED] \times Net + \\ \quad r\_soil[TR] \\ \quad \text{if } Year = 2018, \text{ Soil} > 0 \end{cases} \\
 rn\_net_{without}[BED] &\sim \text{normal}(\mu_{without}, \sigma_{without}), \\
 rn\_net_{with}[BED] &\sim \text{normal}(\mu_{with}, \sigma_{with}) \\
 r\_year[Year] &\sim \text{normal}(\mu_{year}, \sigma_{year}), \\
 r\_soil[TR] &\sim \text{normal}(\mu_{soil}, \sigma_{soil}) \\
 \mu_{without} &\sim \text{normal}(0, 10^3), \mu_{with} \sim \text{normal}(0, 10^3) \\
 \mu_{year} &\sim \text{normal}(0, 10^3), \mu_{soil} \sim \text{normal}(0, 10^3) \\
 \sigma_{without} &\sim \text{uniform}(0, 10), \sigma_{with} \sim \text{uniform}(0, 10) \\
 \sigma_{year} &\sim \text{uniform}(0, 10), \sigma_{soil} \sim \text{uniform}(0, 10)
 \end{aligned}$$

where  $NM_{indiv}$  is the number of surviving individuals per sub-plot and  $\lambda_{indiv}$  is the linear predictor

for the mean number of surviving individuals. This model also included three random effects terms depending on the condition:  $r\_year[Year]$  for the effect of year, where  $Year$  was an index number (2017 or 2018);  $rn\_net_{without}[BED]$  and  $rn\_net_{with}[BED]$  for the effects of the without-net and with-net treatments, respectively, which varied depending on the  $BED$  to which each observed number of individuals belonged, where  $BED$  was an index number (2, 3, or 4); and  $r\_soil[TR]$  for the effect of the soil treatment (limited to 2018), where  $TR$  was an index number (1, 2, or 3 corresponding to Plot-2A, Plot-2B, and Plot-3A plot treatment, respectively).  $r\_year[Year]$  and  $r\_soil[TR]$  were subjected to the constraint that they follow a normal distribution with means of  $\mu_{year}$  and  $\mu_{soil}$  and standard deviations of  $\sigma_{year}$  and  $\sigma_{soil}$ , respectively. Similarly,  $rn\_net_{without}[BED]$  and  $rn\_net_{with}[BED]$  were subjected to the constraint that they follow a normal distribution with means of  $\mu_{without}$  and  $\mu_{with}$  and standard deviations of  $\sigma_{without}$  and  $\sigma_{with}$ , respectively.  $NET$  was a dummy variable that took a value of 0 for the without-net treatment and 1 for the with-net treatment. Non-informative priors were applied to the prior distributions of all of the parameters (Gelman, 2006; Polson and Scott, 2012). The estimated parameters were used to predict the number of individuals for each of the Net treatments and BED and to calculate the differences between the Net treatments and BED as generated quantities block in Stan.

A second Poisson hierarchical model was then constructed to test the effects of the Net treatment and water level (BED) on the number of inflorescences of *E. heleocharioides* per individual. There were no surviving individuals and thus no inflorescence data for any of the without-net treatments except BED-2. Therefore, the without-net effects were only estimated for BED-2. The effect of the Net treatment between BED lines was estimated as a random effect as same as the model of surviving individuals. In addition, the effect of soil treatment was also included as a random effect. The final model was as follows:

$$\begin{aligned}
 NM_{\text{Inflo}} &\sim \text{poisson}(\exp(\lambda_{\text{Inflo}})) \\
 \lambda_{\text{Inflo}} &= \begin{cases} ri\_net_{\text{with}}[BED] & \text{for } NM_{\text{indiv}} = 0, \text{ Net} = 1 \\ ri\_net_{\text{with}}[BED] + r\_soil[Soil] & \text{for } NM_{\text{indiv}} = 0, \text{ Net} = 1 \\ ri\_net_{\text{with}}[2] + net_{\text{without}} \times Net & \text{for } NM_{\text{indiv}} > 0, \text{ Net} = 0 \end{cases} \\
 ri\_net_{\text{with}}[BED] &\sim \text{normal}(\mu_{\text{with}}, \sigma_{\mu_{\text{with}}}) \\
 r\_soil[TR] &\sim \text{normal}(\mu_{\text{soil}}, \sigma_{\mu_{\text{soil}}}) \\
 \mu_{\text{with}} &\sim \text{normal}(0, 10^3), \sigma_{\mu_{\text{with}}} \sim \text{uniform}(0, 10) \\
 \mu_{\text{soil}} &\sim \text{normal}(0, 10^3), \sigma_{\mu_{\text{soil}}} \sim \text{uniform}(0, 10)
 \end{aligned}$$

where  $NM_{\text{Inflo}}$  is the number of inflorescences of *E. heleocharioides* per mother plant and  $\lambda_{\text{Inflo}}$  is a linear predictor for the mean number of inflorescences. In addition, the model included the random effects term  $ri\_net_{\text{with}}[BED]$  for the effect of the with-net treatment, which varied depending on the BED line to which each observed number of inflorescences belonged, where BED was an index number (1, 2, or 3); the fixed effect  $net_{\text{without}}$  for the effect of the without-net treatment, which was limited to BED-2; and the random effects term  $r\_soil[TR]$  for the soil treatment, where  $TR$  was an index number (1, 2, or 3 corresponding to changed, unchanged, and updown treatment, respectively).  $ri\_net_{\text{with}}[BED]$  and  $r\_soil[TR]$  were subjected to the constraint that they follow a normal distribution with means of  $\mu_{\text{with}}$  and  $\mu_{\text{soil}}$  and standard deviations of  $\sigma_{\mu_{\text{with}}}$  and  $\sigma_{\mu_{\text{soil}}}$ , respectively.  $net_{\text{without}}$  was a coefficient of the with-net condition.  $NET$  was a dummy variable that took a value of 1 for the without-net treatment and 0 for the with-net treatment. Non-informative priors were applied to the prior distributions of all of the parameters. The estimated parameters were used to predict the number of inflorescences for each of the Net treatments and BED and to calculate the differences between the Net treatments and BED as generated quantities block in Stan.

All statistical analyses were conducted using R version 3.2.1 (R Core Team, 2018) and Stan version 2.17.3 (Stan Development Team, 2018a,

2018b), which is a Hamiltonian Monte Carlo sampler for parameter estimation. We ran four parallel chains and retained 21,000 iterations after 11,000 burn-in steps in each chain. The convergence of the Markov chains was checked for each parameter by determining whether the R-hat value was less than 1.1. The estimated parameters were then evaluated using the median and 95% Bayesian credible interval of the Markov Chain Monte Carlo samples.

## Results

The mean number of surviving individuals per sub-plot and the mean number of inflorescences per individual per sub-plot are shown in Fig. 2. At the time of data collection, the soil surfaces of 1st, 5th, and 6th sub-plots in Plot-4 were found to be accidentally covered by the nets that were used in the sheltering experiment. Therefore, because the period of coverage was unknown, the data from these sub-plots were excluded from the following analyses.

Comparison of the numbers of surviving individuals in the plots with and without nets in each bed revealed that *E. heleocharioides* had a higher survival rate in plots with nets than in those without nets (Fig. 3). The probability of a difference between plots with nets and those without nets was 100% in all of the beds, with median differences of 98, 18, and 30 individuals in Beds-2, -3, and -4, respectively (Table 1).

The effect of net sheltering on the number of inflorescences per individual was only analyzed for Bed-2, as this was the only bed in which some individuals survived in plots without nets. In Bed-2, the probability of a difference in the number of inflorescences per individual between plots with and without nets was 57.39%, with a median difference of 0 individuals, indicating that net sheltering had no effect on the number of inflorescences per individual (Fig. 4, Table 1).

For both of the net treatments, the number of surviving individuals was highest in Bed-2, and the probability of there being a difference between these beds within each of the net treat-

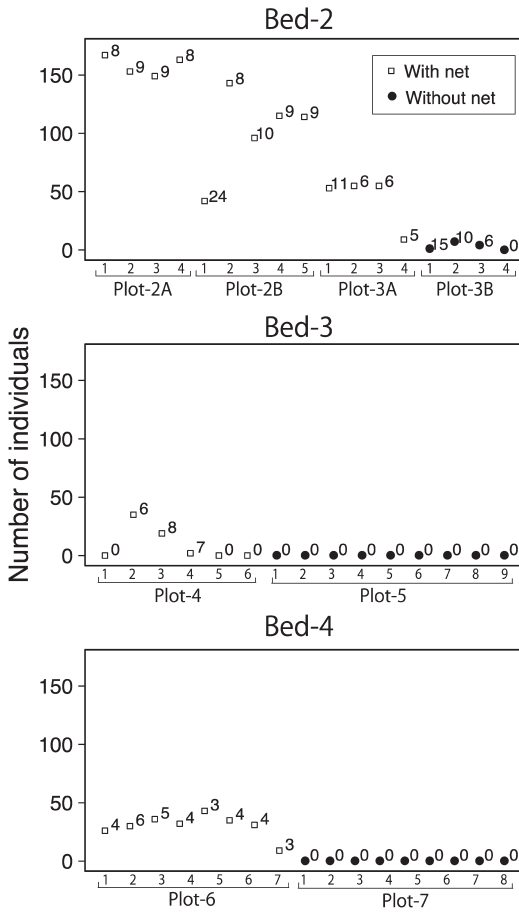


Fig. 2. Mean number of surviving individuals for sub-plots in three beds. Open square and filled circle indicate plots with and without nets, respectively.

ments was mostly high with the exception that there was no difference in the 95% CI between Bed-4 and Bed-3 with the net treatment (95% CI:  $-3$  to  $26$ ; probability of difference:  $94.51\%$ ) or without the net treatment ( $-1$  to  $1$ ,  $95.66\%$ ) (Table 2).

The survival of individuals was compared among the three soil management regimes in the plots in Bed-2 with nets (Plots-2A, -2B, and -3A), as well as with the 2017 result for Bed-2 with nets, which corresponded to the place of Plots-2A and -2B in 2018 (Fig. 5). The highest number of surviving individuals was observed in Plot-2A, in which soil had been removed and

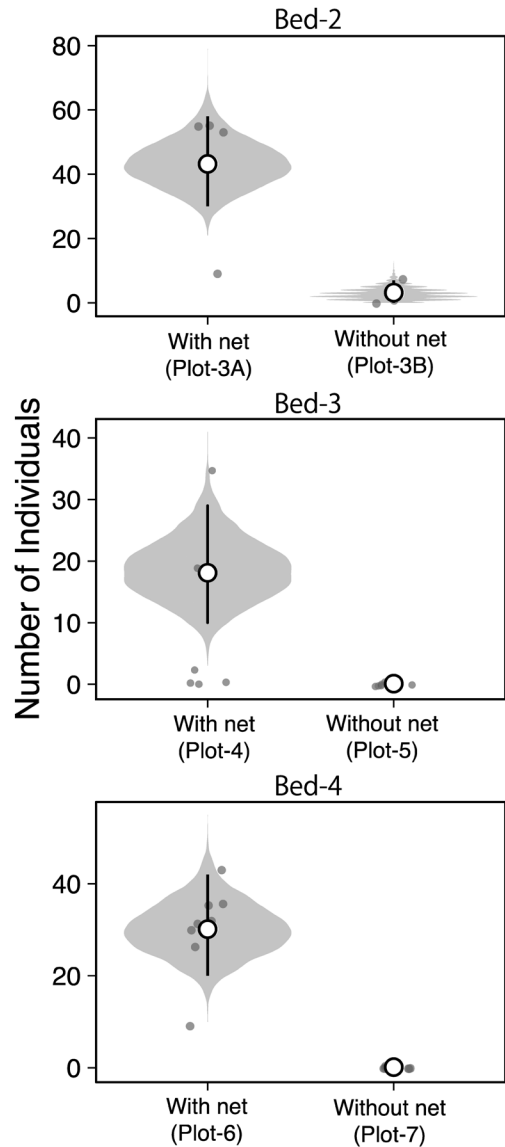


Fig. 3. Number of surviving individuals between plots with and without nets for each bed. Only Plot-3A whose soil has not been changed since 2017 unlike Plot-2A and Plot-2B was used as the treatment with net in Bed-2 to match the soil condition in Plot-3B. Median of predicted posterior distribution (open circle), 95% credible interval of predicted posterior distribution (vertical bar), predicted posterior distribution (grey area), and each data (small grey circle) were shown.

replaced with new soil, followed by Plot-2B, in which the soil had been plowed to replace the surface soil with subsoil, with Plot-3A, in which the soil had been left undisturbed since 2017, having the lowest survival. There was no difference between Plot-2A and the result for Bed-2 in 2017, whereas Plots-2B and -3A clearly differed from this result (Table 3).

## Discussion

### Effects of net sheltering

In this study, it was revealed that the provision of net sheltering increased the number of surviving individuals of *Eriocaulon heleocharioides* in

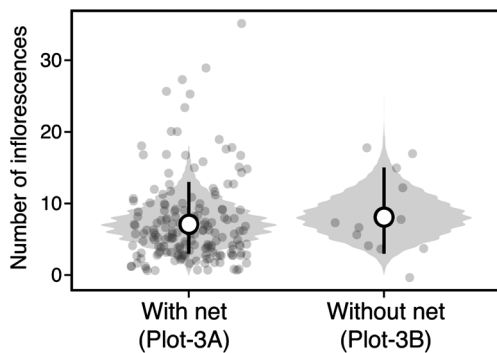


Fig. 4. Number of inflorescences between plots with and without nets. Analysis was conducted only about Bed-2 in which some individuals survived even in without net plots. Median of predicted posterior distribution (open circle), 95% credible interval of predicted posterior distribution (vertical bar), predicted posterior distribution (grey area), and each data (small grey circle) were shown.

each of three beds with different water depths in Sanuma Lake (Fig. 3, Table 1). This improved survival corresponds with the result of the experiment performed in 2017 (Tanaka *et al.*, 2018) that used only a single water depth, which was equivalent to the uppermost bed (Bed-2) in the present study. By contrast, net sheltering had no effect on the number of inflorescences per individual (Fig. 4, Table 1). It is possible that the factors that can be ameliorated by net sheltering directly influence the survival but not the growth and reproduction of *E. heleocharioides*.

Previous studies have shown that net sheltering reduces the negative effects of predators on the reintroductions of aquatic plants (Sweeny *et al.*, 2002; Maschinski *et al.*, 2004; Godefroid *et al.*, 2011). Although the direct cause of the failed reintroduction of *E. heleocharioides* in Sanuma Lake has not yet been clarified, it is probably not a one-off event but rather a constant factor, at least these two years. Therefore, it will be necessary to elucidate this factor and either remove it or protect *E. heleocharioides* from it to make the reintroduction of this species in Sanuma Lake successful.

### Effects of water depth

Water depth is known to have strong effects on the survival and growth of aquatic plants, particularly those that are submerged, as occurs with *E. heleocharioides* during the vegetative growth period in Sanuma Lake (Havens, 2003; Bornette and Puijals, 2011). In the present study, water depth had some effect on the number of surviving individuals of *E. heleocharioides*, with the

Table 1. Difference of the number of survival individuals and inflorescences between plots with net and without net within each bed

| Bed                                 | Comparative plots                           | Probability of difference (%) | Median of difference | 95% credible interval of difference |       |
|-------------------------------------|---------------------------------------------|-------------------------------|----------------------|-------------------------------------|-------|
|                                     |                                             |                               |                      | 2.5%                                | 97.5% |
| The number of surviving individuals |                                             |                               |                      |                                     |       |
| Bed-2                               | Plot-3A (with net) vs Plot-3B (without net) | 100                           | 40                   | 27                                  | 56    |
| Bed-3                               | Plot-4 (with net) vs Plot-5 (without net)   | 100                           | 18                   | 10                                  | 29    |
| Bed-4                               | Plot-6 (with net) vs Plot-7 (without net)   | 100                           | 30                   | 19                                  | 42    |
| The number of inflorescences        |                                             |                               |                      |                                     |       |
| Bed-2                               | Plot-3A (with net) vs Plot-3B (without net) | 57.39                         | 0                    | − 8                                 | 9     |

Table 2. The difference of the number of individuals between the different water depth

| Comparative plots                 | Probability of difference | Median of difference | 95% credible interval of difference |        |
|-----------------------------------|---------------------------|----------------------|-------------------------------------|--------|
|                                   |                           |                      | 2.50%                               | 97.50% |
| The number of individuals         |                           |                      |                                     |        |
| With net treatment                |                           |                      |                                     |        |
| Plot-2A (Bed-2) vs Plot-4 (Bed3)  | 100.00                    | 139                  | 111                                 | 168    |
| Plot-2A (Bed-2) vs Plot-6 (Bed-4) | 100.00                    | 128                  | 98                                  | 158    |
| Plot-6 (Bed-4) vs Plot-4 (Bed-3)  | 94.56                     | 11                   | − 3                                 | 26     |
| Without net treatment             |                           |                      |                                     |        |
| Plot-3B (Bed-2) vs Plot-5 (Bed3)  | 99.55                     | 3                    | 0                                   | 7      |
| Plot-3B (Bed-2) vs Plot-7 (Bed-4) | 99.51                     | 3                    | 0                                   | 7      |
| Plot-7 (Bed-4) vs Plot-5 (Bed-3)  | 96.10                     | 0                    | − 1                                 | 1      |
| The number of inflorescences      |                           |                      |                                     |        |
| With net treatment                |                           |                      |                                     |        |
| Plot-2A (Bed-2) vs Plot-4 (Bed3)  | 80.61                     | 3                    | − 5                                 | 10     |
| Plot-2A (Bed-2) vs Plot-6 (Bed-4) | 92.98                     | 5                    | − 2                                 | 11     |
| Plot-6 (Bed-4) vs Plot-4 (Bed-3)  | 33.16                     | − 2                  | − 8                                 | 4      |

Table 3. Difference of the number of survival individuals in comparison of different soil managements with 2017's result

| Comparative plots             | Probability of difference (%) | Median of difference | 95% credible interval of difference |       |
|-------------------------------|-------------------------------|----------------------|-------------------------------------|-------|
|                               |                               |                      | 2.5%                                | 97.5% |
| 2017 vs Plot-2A (new soil)    | 90.9                          | 25                   | − 12                                | 62    |
| 2017 vs Plot-2B (soil plowed) | 100                           | 140                  | 110                                 | 171   |
| 2017 vs Plot-3A (undisturbed) | 100                           | 81                   | 46                                  | 115   |

highest survival occurring at the shallowest depth (Table 2). Furthermore, similar results were obtained for both the with-net and without-net treatments, indicating that the water depth itself directly affected the number of surviving individuals rather than indirectly promoting the effects of other limiting factors, such as the presence of herbivores and wave action. While further detailed investigation is required to understand the relationship between water depth and the survival of *E. heleocharioides* in Sanuma Lake, our results showed that this species can survive to at least a depth of 0.57 m (the maximum water depth in Bed-4) if net sheltering is installed, which is found approximately 5–10 m from the shore in the last natural habitat of this species in Sanuma Lake. Therefore, these basic data will be important for the future construction of natural habitat in this lake.

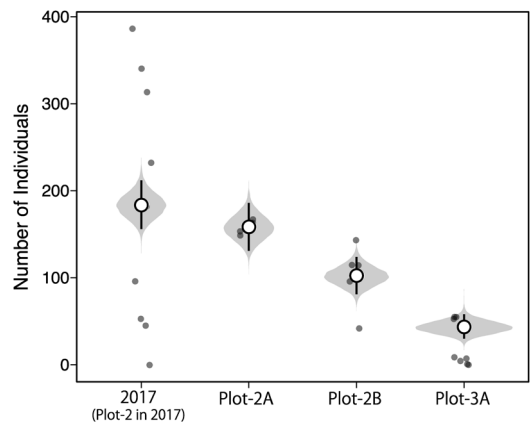


Fig. 5. Number of surviving individuals among three soil management regimes. The 2017 result for Bed-2 with nets was added for comparison. Median of predicted posterior distribution (open circle), 95% credible interval of predicted posterior distribution (vertical bar), predicted posterior distribution (grey area), and each data (small grey circle) were shown.

### Effects of soil management

A number of soil factors have been reported to affect the growth of plants (Passioura, 2002; Bengough *et al.*, 2005; Hayat *et al.*, 2010), including the nutrient content of the soil (Lambers and Poorter, 1992; Aerts and Chapin III, 2000). In the present study, the soil management methods that were applied in the semi-protected area affected the survival of *E. heleocharioides* (Fig. 5, Table 3), with the highest number of individuals surviving in the plot in which soil had been removed and replaced with new soil (Plot-2A) and the lowest number surviving in the plot in which the soil had been left undisturbed (Plot-3A). This finding suggests that the provision of new soil from the bottom of Sanuma Lake will likely improve the survival of individuals. Therefore, if a structure such as the semi-protected area that was constructed for the purposes of this research is used for the future reintroduction of *E. heleocharioides* to Sanuma Lake, periodic replacement of the soil will be required for the maintenance of this species because natural soil movements are unlikely to occur.

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