

Reconstruction of the charophyte community of Lake Shinji by oospore collection

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Abstract – Submerged aquatic vegetation (SAV) aids in maintaining a clear stable state in shallow lakes. However, charophytes are more effective in increasing transparency compared to angiosperms. Lake Shinji was more transparent prior to the beginning of herbicide use for rice weed control in the mid-1950s, because its bottom was covered by SAV up to 3 m depth. Although *Chara braunii* C.C. Gmelin and *Nitella hyalina* (De Candolle) C. Agardh were recorded in the 1960s, there are no reports on SAV in the 1950s. Therefore, in the present study, we aimed to show that the SAV of Lake Shinji was mostly composed of charophytes prior to the 1950s, by conducting a seed analysis. We obtained charophyte oospores from the sediment, but seeds of angiosperms were not identified. In addition to *C. braunii* that was previously recorded in Lake Shinji, we also found two newly identified species, *Chara corallina* Willdenow and *Chara fibrosa* C. Agardh ex Bruzelius. Overall, this study indicates that seed analysis is helpful in reconstructing the former flora of Lake Shinji.

Key-words: Seed analysis / water transparency / *Chara corallina* / submerged aquatic vegetation

Résumé – Reconstruction de la communauté de charophytes du Lac Shinji par collection d'oospores. La végétation aquatique submergée (SAV) aide à maintenir un état clair stable dans les lacs peu profonds. Cependant, les charophytes sont plus efficaces que les angiospermes pour accroître la transparence. Le lac Shinji était plus transparent avant le début de l'utilisation d'herbicides pour le désherbage du riz au milieu des années 1950, parce que son fond était couvert par la SAV jusqu'à 3 m de profondeur. Bien que *Chara braunii* C.C. Gmelin et *Nitella hyalina* (De Candolle) C. Agardh ont été notés dans les années 1960, il n'y a pas de données sur la SAV dans les années 1950. Par conséquent, dans la présente étude, nous avons cherché à montrer que la SAV du Lac Shinji était surtout composée de charophytes avant les années 1950, en procédant à une analyse des semences. Nous avons obtenu des oospores de charophytes du sédiment, mais les graines d'angiospermes n'ont pas été identifiées. En plus de *C. braunii* qui a déjà été enregistré dans le lac Shinji, nous avons aussi trouvé deux espèces nouvellement identifiées, *Chara corallina* Willdenow et *Chara Fibrosa* C. Agardh ex Bruzelius. Dans l'ensemble, cette étude indique que l'analyse des semences est utile dans la reconstruction des anciennes flores du lac Shinji.

Mots-clés : Analyse de semences / transparence de l'eau / *Chara corallina* / végétation aquatique submergée

In shallow lakes, submerged aquatic vegetation (SAV) plays a vital role in maintaining a clear stable state (Scheffer *et al.*, 2001). However, previous studies have shown that angiosperms are not as effective in maintaining a clear stable state as charophytes (Van den Berg *et al.*, 1998; Nöges *et al.*, 2005).

Lake Shinji, Japan, is an oligohaline lagoon with a salinity range of 1–10 psu (Figure 1). The lake area is 79.1 km² and the basin area 1288.4 km². The maximum depth is 6.4 m, while the average depth is 4.5 m. Hiratsuka *et al.* (2006) indicated

that SAV (unknown species) exists up to 2–3 m depth. Additionally, Komuro and Yamamuro (2013) examined an airborne photograph taken in 1947 by the US Army, and showed that creeping type SAV covered the bottom of Lake Shinji up to 3 m depth. SAV was previously used as a fertilizer; however, it almost disappeared from Lake Shinji after the beginning of herbicide use for rice weed control in the mid-1950s (Yamamuro, 2012).

Akiyama and Nishigami (1959) collected *Chara braunii* C.C. Gmelin in 1958, and Kasaki (1964) collected *C. braunii* and *Nitella hyalina* (De Candolle) C. Agardh until the late 1960s. Both studies referred to samples that were collected

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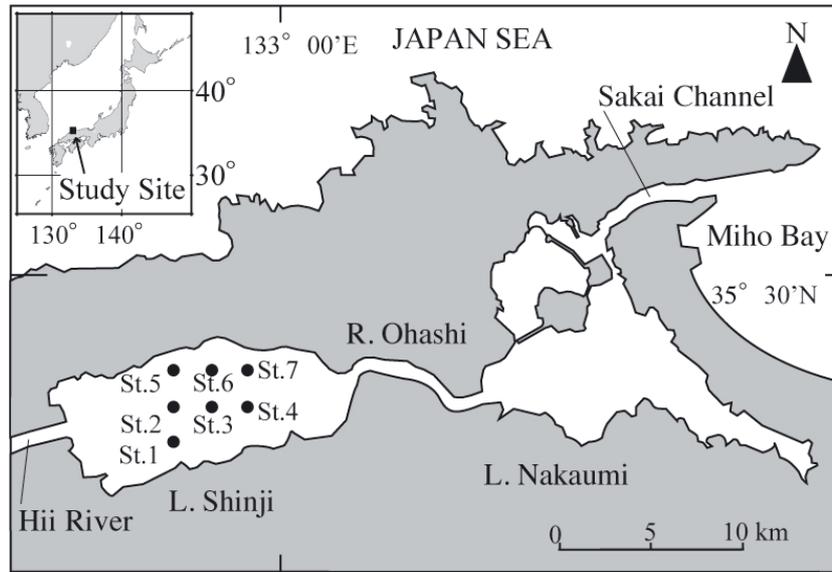


Fig. 1. Location of seven sampling stations (Stations 1–7) in Lake Shinji. Sampling was conducted at Stations 1–7 in 2011, and Stations 2, 4, and 5 in 2013.

from the shoreline of Lake Shinji; thus, it is possible that creeping type SAV at 2–3 m depth comprised other species than those reported in these two studies.

The amount of herbicides used in rice paddies began to decrease after the implementation of a law on the restricted use of herbicides in 2007. As a result, *Potamogeton anguillanus* Koidz. started to grow in the shallow parts of Lake Shinji (Yamamuro *et al.*, 2014), and its canopy reached the water surface. However, transparency did not increase by the mass coverage of SAV (Yamamuro *et al.*, 2014), which is in disagreement with the alternative stable state theory (Scheffer *et al.*, 2001). A possible explanation could be that Lake Shinji was transparent by the 1960s, because SAV was mostly composed of charophytes that are considered more effective in increasing transparency (Blindow *et al.*, 2002; Van Donk *et al.*, 2002). The aim of this study was to investigate the composition of past SAV by collecting sediments and conducting seed analysis, in order to show that charophytes, and not *P. anguillanus*, were dominant when Lake Shinji was transparent before the loss of vegetation due to herbicides.

Sediments were collected from seven stations (Stations 1–7) of Lake Shinji at depths of more than 5 m, where 99% of the sediment is composed of silt and mud (Figure 1). The sedimentation rates of these seven stations were reported by Kanai *et al.* (1997); thus, it was possible to determine the portion of core sediment that accumulated prior to 1960. The sediment core collection was performed in August 2011 and 2013. In 2011, we sampled all seven stations, and in 2013, we sampled three stations (Stations 2, 4, and 5). A professional diver dived to each station, and gently placed an acrylic tube (8.5 cm diameter, 102 cm length) on the sediment surface in order to prevent any disturbance. The top of the tube was closed with a silicon lid, the tube was pulled out of the sediment surface, and then the bottom of the tube was closed with another silicon lid.

The length of cores was approximately 70 cm. Since the sediment accumulation rate of the sampling station is

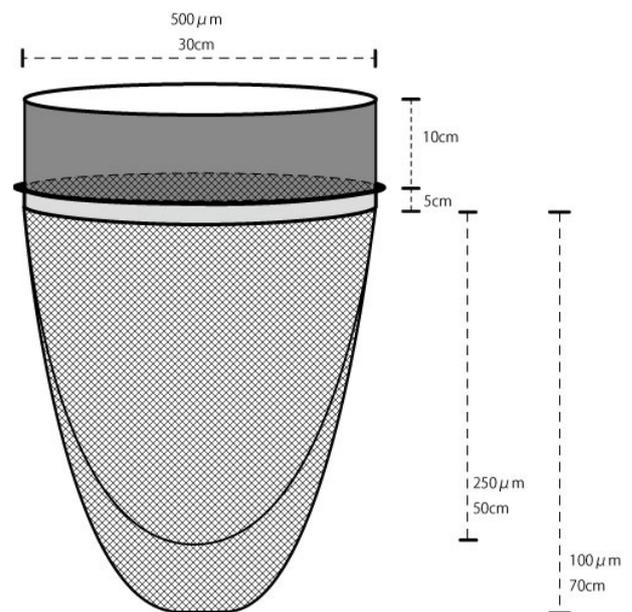


Fig. 2. A representation of the sieve used for sediment sampling in Lake Shinji. Numbers in μm denote the mesh size of the sieve and those in cm show the diameter of the sieve. The top sieve (30-cm diameter) is made of stainless steel, while the remaining part is made of plankton net cloth (Komuro and Yamamuro, 2012).

$0.04\text{--}0.31\text{ cm}\cdot\text{y}^{-1}$ (Kanai *et al.*, 1997), we removed the top 2–15-cm sediment layer to exclude sediment that accumulated after 1960. We also discarded sediment from a depth of approximately 20 cm from the bottom layer, because the mud was tightly fixed by diagenesis and precluded sieving. To reduce the amount of sediment required to obtain seeds and oospores by seed analysis, we used a specially designed net sieve as described by Komuro and Yamamuro (2011) (Figure 2). To collect seeds of angiosperms and

Table 1. Dry weight (DW) of sediments (collected in 2011) on the 250- and 100- μm mesh sieves.

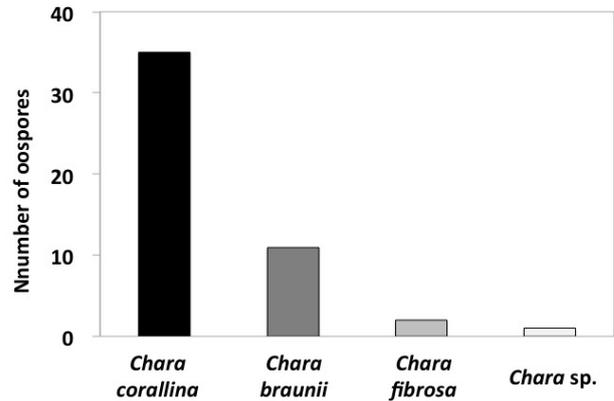
Station	Dry Weight (g)	
	250 μm	100 μm
1	99.4	250.3
2	2.6	6.4
3	0.8	1.9
4	3.5	23.8
5	3.8	10
6	1.7	17
7	2.2	13.4
Total	114.0	322.8

charophyte oospores, the sediment was sieved through 100- μm and 250- μm mesh sieves (Birks, 2001). The particles that remained on the sieves were transferred to the laboratory and immediately sieved through a 100- μm mesh stainless-steel sieve under running water. The particles that remained on the sieve were dried in an oven at 40 °C until constant weight was reached. The dried particles were sieved again through 100- μm and 250- μm mesh stainless steel sieves. Since charophyte oospores are usually 200–900 μm in length and 200–500 μm in width, we expected that most oospores would be retained on the 250- μm mesh sieve. The remaining particles were separately examined using a digital binocular microscope (Keyence, Osaka, Japan) in order to collect seeds and oospores.

Because the length of sediments used for seed analysis in 2011 differed between the sampling stations, the dry weight (DW) of sediments on the 250- μm mesh, as well that on the 100- μm mesh also differed (Table 1). We did not measure the DW of sediments on the 100- μm mesh and 250- μm mesh that were used for seed analysis in 2013. We assumed that coarse particles predominated at Station 1, which is near the river mouth, while mud predominated at all other stations; thus, most sediments were lost during sieving.

Charophyte oospores were observed under a scanning electron microscope (SEM) as described by Sakayama *et al.* (2002) with some modifications. Before observation, oospores were cleaned by incubation in 10% Triton-X 100 at 60 °C for more than 12 h, subjected to acetolysis or sonicated for approximately 2 min, and transferred to centrifuge tubes with distilled water. Acetolysis was not performed in species that had fragile flanges on the surface of oospores. Then, oospores were dehydrated in an ethanol series (50% and 70%), mounted on brass or aluminum stubs with double-side adhesive tape, air-dried, sputter-coated with gold, and viewed under S-2150N and S-4800 scanning electron microscopes (Hitachi, Tokyo, Japan) at 10–20 kV. Oospore identification was performed as described by Kasaki (1964), Wood (1965), Imahori and Kasaki (1977), Casanova (2013), and Casanova and Karol (2014) and maintained at the University of Tokyo, Kashiwa, Japan.

Angiosperm seeds were not obtained in any of the sediments; however, charophyte oospores were identified in sediments sampled in both 2011 and 2013. The total number of

**Fig. 3.** Number of *Chara corallina*, *Chara braunii*, *Chara fibrosa*, and *Chara sp.* oospores collected from Lake Shinji in 2011 and 2013.

oospores obtained in two years was 49 (Figure 3). Detailed information on the number of oospores at each station in 2011 and 2013 is presented in Table 2. Using SEM, we identified four different charophyte species, *C. braunii*, *Chara corallina* Willdenow, *Chara fibrosa* C. Agardh ex Bruzelius, and *Chara sp.* (Figure 4). We obtained 35 oospores of *C. corallina*, 11 of *C. braunii*, two of *C. fibrosa*, and one of *Chara sp.* (Table 2). Overall, *C. corallina* was the most abundant species, in terms of oospore number and the number of stations obtained from. To the best of our knowledge, this is the first record of *C. corallina* and *C. fibrosa* in Lake Shinji.

The number of oospores per DW of sediment (>100- μm) varied from 0.05 to 1.09 among sampling stations (0.05 oospores·g⁻¹ of DW sediment, 0.22 oospores·g⁻¹ of DW sediment, 0.37 oospores·g⁻¹ of DW sediment, 0.92 oospores·g⁻¹ of DW sediment, 1.09 oospores·g⁻¹ of DW sediment, 0.64 oospores·g⁻¹ of DW sediment, and 0.64 oospores·g⁻¹ of DW sediment at Stations 1, 2, 3, 4, 5, 6, and 7, respectively). These densities were much lower than those in previous studies on recent fossil oospores (*i.e.*, Davidson *et al.*, 2005; Rodrigo *et al.*, 2010). For example, the number of oospores and gyrogonites in the sediments of Albufera de València lagoon (area 23 km², mean depth 1–2 m) was more than 30 (Rodrigo *et al.*, 2010). In Lake Shinji, creeping type SAV, possibly charophytes, covered the bottom up to 3 m depth (Komuro and Yamamuro, 2013). Since Lake Shinji is bigger than other studied lakes (*i.e.*, Groby Pool, area 0.12 km²; Davidson *et al.*, 2005), sediments at up to 3 m, composed of sand and organic materials, are removed by strong wave actions and transported to deeper lake basin depths, which may explain the fact that we collected sediment samples from depths exceeding 5 m. In such case, the density of oospores is diluted during transportation and sedimentation.

Before the 1950s, when herbicides were not used in rice paddies, the transparency of Lake Shinji was assumed to be 4 m (Komuro and Yamamuro, 2013) and, based on an airborne photograph, SAV was of creeping type. After 2007 that the use of herbicides decreased, *P. anguillanus* covered the shallows of Lake Shinji; however, transparency remained stable at approximately 1 m (Yamamuro *et al.*, 2014). Our results showed that the sediments of Lake Shinji before 1960 included only

Table 2. Number of *Chara corallina*, *Chara braunii*, *Chara fibrosa*, and *Chara* sp. oospores collected from Lake Shinji (Stations 1–7) in 2011 and 2013.

Station	<i>Chara corallina</i>		<i>Chara braunii</i>		<i>Chara fibrosa</i>		<i>Chara</i> sp.	
	2011	2013	2011	2013	2011	2013	2011	2013
1	1		2	–	0	–	0	–
2	0	0	0	4	2	0	0	0
3	1	–	0	–	0	–	0	–
4	19	1	0	2	0	0	1	0
5	4	1	1	0	0	0	0	0
6	2	–	0	–	0	–	0	–
7	6	–	2	–	0	–	0	–
Total	33	2	5	6	2	0	1	0

–: denotes that no sediment sampling was conducted

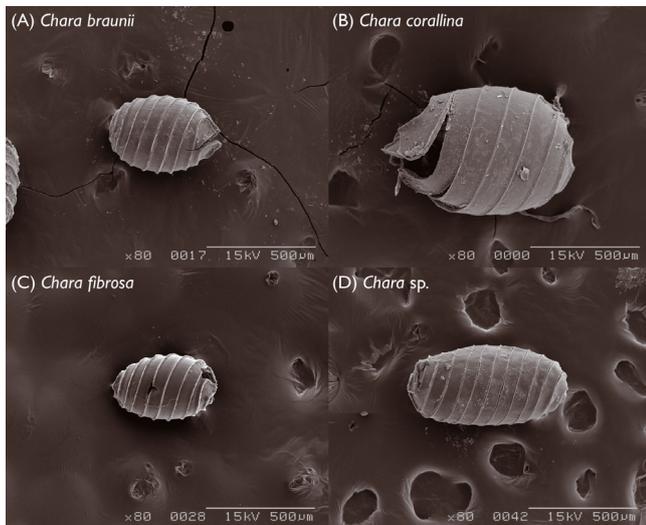


Fig. 4. Scanning electron micrograph of (A) *Chara braunii*, (B) *Chara corallina*, (C) *Chara fibrosa*, and (D) *Chara* sp.

charophytes, which is in accordance with our previous study (Komuro and Yamamuro, 2013).

The present study suggested that charophytes play an important role in maintaining the water transparency in the lake, and thus, invasive angiosperms did not play any role in terms of improving transparency. We found that *C. corallina* was the most abundant charophyte before the reduction in SAV due to herbicide use. Among calcified charophytes, *C. corallina* contains relatively high calcium content (Kawahata et al., 2013). Calcium can induce the clear water state by absorbing the phosphate in the water column (Hilt et al., 2006).

This study indicates that seed analysis is helpful in reconstructing the former flora, which has not been recorded in the literature. The flora before the use of herbicides differed from that which appeared after the reduction in herbicide use, probably because of the invasion of exotic species in the lake. Overall, these results show that the flora appearing after the decrease in human disturbance does not always revive the original flora. Thus, our study contributes important information

that can be useful in conservation and management programs of aquatic ecosystems.

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