



Reconstruction of paleo-environments by two aquatic plants: eDNA analysis in lake sediment cores

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Received: 6 June 2024 / Accepted: 14 January 2025 / Published online: 31 January 2025
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Abstract

Environmental DNA (eDNA) analysis using lake sediment cores can be used to assess populations, dynamics, and historical changes in ecosystems and environments, based on the DNA composition of micro-organisms and plants. While this method has shed light on past environments, a more detailed understanding of past ecosystems requires targeted sediment core eDNA analysis focusing on specific species. Here, we focused on the salt-tolerant aquatic plants *Stuckenia pectinata* and *Potamogeton pusillus*, which are abundant in Lake Shinji and serves as a model system for Japanese brackish lakes, conducted species-specific DNA analysis on nine sediment cores 64–379 cm collected from water depth of 4.6–5.1 m. Additionally, we performed CNS elemental analysis on these sediment cores to reconstruct the paleoenvironment of Lake Shinji. Our findings revealed the presence of *S. pectinata* and *P. pusillus* DNA from approximately 2000 years ago, and a clear relationship was observed between DNA detection and historical salinity levels in the lake. *Stuckenia pectinata* tended to have a higher DNA detection frequency than that of *P. pusillus*, possibly related to the different salinity tolerance levels between the two species. Based on the relationship between the horizontal–vertical distributions of sedimentary eDNA of aquatic plants and their salinity tolerances, the paleoenvironment associated with salinity in Lake Shinji could also be reconstructed. Therefore, it is possible to elucidate the long-term dynamics of these plants associated with past environmental change as water pollution, using the time-series data on eDNA of macro-organisms, such as aquatic plants, in the sediment cores.

Keywords CNS elemental analysis · Lake Shinji · Potamogeton · Salinity · Pondweed

Introduction

Lake and ocean paleoenvironments have been extensively reconstructed by analyzing compositional changes in organisms, including bacteria, plankton, and plants, through

environmental DNA (eDNA) analysis on sediment cores (Ibrahim et al. 2021, 2022; Pedersen et al. 2013). Studies have reconstructed past eutrophic environments based on cyanobacteria DNA composition (Barrenechea Angeles et al. 2023; Mejbel et al. 2023; Zhang et al. 2021) and past temperature changes in cold climates by analyzing plant DNA composition (De Schepper et al. 2019; Voldstad et al. 2020). DNA metabarcoding, a method capable of detecting DNA from various organisms, has become the primary approach for sediment DNA analysis (Holman et al. 2019). However, to achieve a more detailed understanding of paleoenvironments, not only the presence/absence but also quantitative analysis of the specific species abundance, is crucial. While DNA metabarcoding allows for analysis of bio composition with DNA obtained from several amplification steps, challenges persist in conducting quantitative analyses of specific species (Ushio et al. 2018). In contrast, species-specific DNA analysis enables the quantification of a target species DNA using specific primers designed for particular organisms, thus facilitating the estimation of

Communicated by Toshifumi Minamoto.

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the biomass of a target species from DNA in the water or sediment (Hamaguchi et al. 2018; Takahara et al. 2012). For instance, species-specific DNA analysis has elucidated biomass fluctuations in fish species, such as jack mackerel (*Trachurus japonicus*), from approximately 300 years ago to the present in marine sediment cores (Kuwaie et al. 2020). Another study attempted to explain past seagrass (*Zostera marina*) presence and carbon cycling by detecting seagrass DNA for the last 5000 years using marine sediment cores (Hamaguchi et al. 2018). Thus, clarifying the historical presence/absence and biomass of specific macro-organisms like fish and aquatic plants by species-specific DNA analysis may provide an estimation of past environmental changes and ecosystems.

Lake Shinji, located in the northeastern part of Shimane Prefecture, is the seventh largest brackish lake in Japan, with an area of approximately 79 km² and a maximum depth of approximately 6 m (Fig. 1). It is currently in a low-salinity condition because of the inflow of freshwater from the Hii River and high-salinity water from Lake Nakaumi via

the Ohashi River (Fukuoka et al. 2005). Lake Shinji has historically experienced dramatic environmental changes due to natural geomorphic changes. It is thought to have been connected to the open sea approximately 5000 years ago (Tokuoka et al. 1990). However, it is believed to have closed off from the sea over time because of a drop in sea level and riverine sediment influx (Tokuoka 1996; Tokuoka et al. 1990). Approximately 800 years ago, Lake Shinji was thought to have started receiving freshwater from the Hii River and seawater from Lake Nakaumi, causing low-salinity conditions (Seto et al. 2006).

CNS elemental analysis of sediments serves as a valuable tool for understanding environmental changes, particularly salinity variations. This method enables the examination of carbon, nitrogen, and sulfur concentrations in lake sediments, providing insights into past freshwater or saltwater conditions based on carbon and sulfur concentrations and their ratios (Berner and Raiswell 1984). Taking its advantage, paleoenvironmental estimation through CNS elemental analysis using sediment cores has been extensively applied

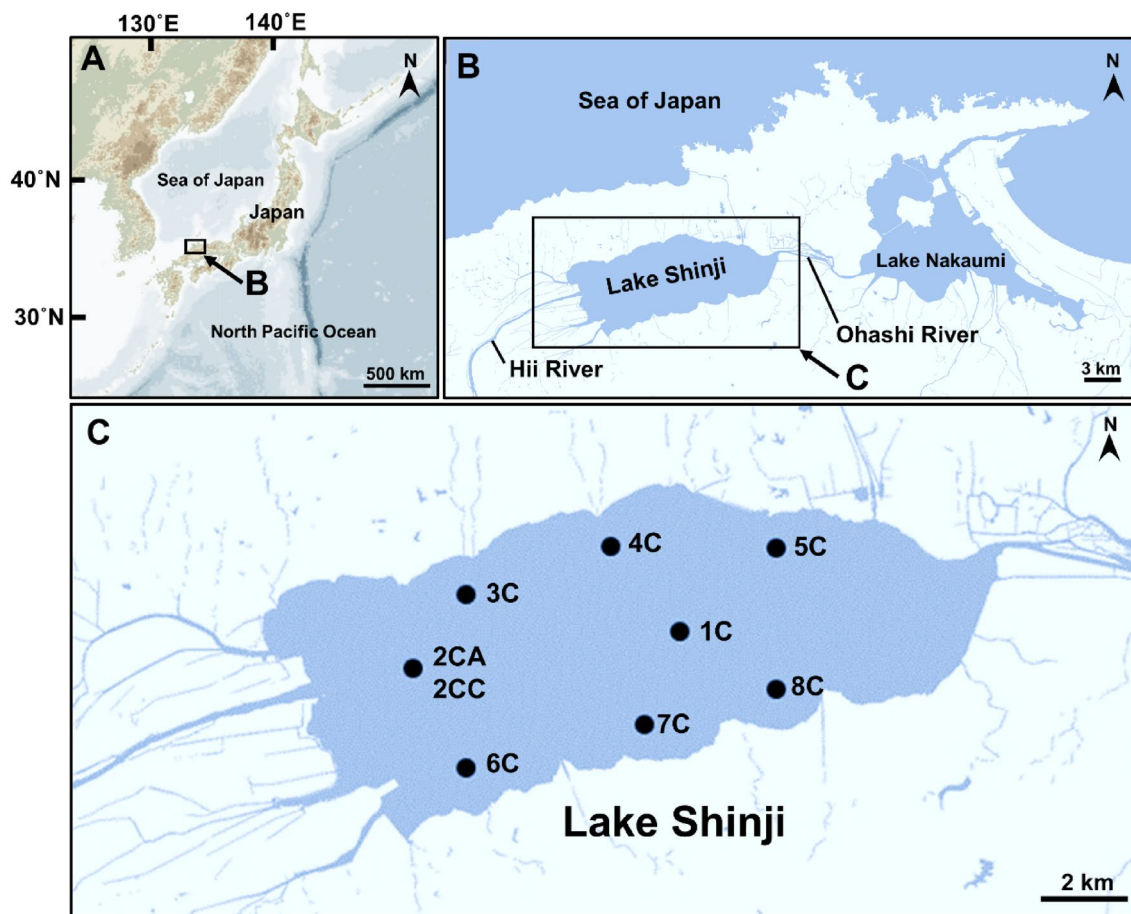


Fig. 1 Locations of study lake and sampling points. **A** Japan and its surroundings. **B** Lake Shinji and its surroundings. **C** Lake Shinji and closed circles indicate the points where 9 sediment cores were col-

lected. The maps were sourced and created by editing the Map of Geospatial Information Authority of Japan (<http://maps.gsi.go.jp>, Accessed 4 Apr 2024)

to Lake Shinji of the brackish environment (Sampei et al. 1997; Seto et al. 2006; Tamura et al. 1996).

Among the primary producers in Lake Shinji, *Stuckenia pectinata* (*Stuckenia pectinata* Linnaeus; Börner 1912) and *Potamogeton pusillus* (*Potamogeton pusillus* Linnaeus; Raunkiaer 1903) are prevalent perennial aquatic plants (Nakagawa and Kunii 2015). Recent environmental changes, such as the anthropogenic increase in salinity caused by the expansion of the Ohashi River approximately 100 years ago (Shimatani and Minagawa 1999) and hypoxic conditions (Fujii et al. 2006; Fukuoka et al. 2005), could have affected the growth of these aquatic plants and other organisms in Lake Shinji (Yamamuro 2012). However, suitable habitats for salt-tolerant aquatic plants, such as *S. pectinata* and *P. pusillus*, have not been thoroughly investigated in Lake Shinji. These salt-tolerant aquatic plants exhibit varying tolerances to salinity, with reported ranges of 0.1–16.7 ‰ for *S. pectinata* (Aston 1973; Brock and Lane 1983; Rodríguez-Gallego et al. 2015) and 0.1–5.9 ‰ for *P. pusillus* (Poirrier et al. 2017; Rodríguez-Gallego et al. 2015). Considering their salinity tolerances, they may respond differently to the environmental changes associated with salinity (Congdon and McComb 1981). Evaluating the relationship between the abundance of these two salt-tolerant aquatic plants and historical changes in anthropogenic and natural salinity could enhance the reconstruction of the paleoenvironment of Lake Shinji.

Using sediment cores, we here analyzed vertical distributions of DNA concentration and abundance (estimated via DNA quantification) of two salt-tolerant aquatic plants (*S. pectinata* and *P. pusillus*) as well as of sedimentary CNS compositions in order to reconstruct the paleoenvironment, especially historical salinity changes, of Lake Shinji. In addition, the relationship between aquatic plant DNA detection and salinity changes from the past to the present was clarified to estimate the relative abundance of these two plants in Lake Shinji.

Materials and methods

Core sampling and chronology

Nine sediment cores (1C, 2CA, 2CC, 3C, 4C, 5C, 6C, 7C, and 8C) of different lengths (64–379 cm) were collected from eight sites in Lake Shinji between 2020 and 2022 (Fig. 1 and Table 1). Upon retrieval, each core was split in half, with one portion designated for eDNA analysis and the other for CNS elemental analysis. However, the upper 85 cm of core sample 2CC was not subjected to CNS analysis in this study due to sediment disturbance during collection. Thus, at Site 2C, the upper portion was treated as 2CA, and the lower portion was treated as 2CC. All sediment cores were moved to a separate clean room after collection, and instruments such as spatulas and tubes were pre-decontaminated with UV irradiation and hypochlorite before subsampling. Sediment samples for eDNA analysis were collected from each layer of the sediment cores and stored at -20°C until DNA extraction. Then, DNA extraction was performed in a clean laboratory, using desks and instruments that had been protected from contamination using UV irradiation and hypochlorite.

Plant fragments picked up from the residue of wet samples washed through a 710 μm sieve were used for dating sediment core 2CC. Dating was performed by converting 14C age to 13C-corrected ages to calendar dates using the Calib 8.20 program, a calendar date calibration program based on the IntCal20 calendar date calibration data set (Reimer et al. 2020). Based on age estimation by accelerator mass spectrometry carbon isotope analysis (AMS ^{14}C ; IntCal20; Paleo Labo Co., and Chikyu Kagaku Kenkyusho Inc., Japan) using plant fragments from the five layers (229.7, 250.5, 266.5, 295.5, and 359.7 cm), the ages of each of the layers were determined to be 1296 CE, 1192 CE, 992 CE, 724 CE, and 279 BCE (Nakamura et al. 2023).

Table 1 Nine sediment cores with different lengths collected from eight sites in Lake Shinji between 2020 and 2022

	Core name	Core length (cm)	Latitude ($^{\circ}\text{N}$)	Longitude ($^{\circ}\text{E}$)	Water depth (m)	Sampling date
1	1C	165	35.450233	132.972850	5.1	2020.07.09
2	2CA	157	35.441867	132.904850	5.0	2021.06.17
3	2CC	379	35.441867	132.904850	5.0	2021.07.01
4	3C	64	35.458158	132.920060	4.6	2021.10.25
5	4C	65	35.468022	132.955238	4.6	2021.10.25
6	5C	79	35.467778	132.997222	4.8	2021.10.25
7	6C	79	35.421370	132.918335	4.7	2022.06.29
8	7C	76	35.430417	132.964187	5.1	2022.06.29
9	8C	70	35.438172	132.997402	4.6	2022.06.29

CNS elemental analysis

The sediment cores, which had been half-split and frozen, were sampled at 1 cm intervals and subsequently subjected to freeze-drying for at least 10 days before being powdered using an agate mortar. To determine the water content, the sediments were weighed before and after freeze-drying (Table S1). Approximately 10 mg of the powdered sediments were then placed in a silver container for solids. Following the addition of an appropriate amount of 1 N HCl to eliminate carbon from the carbonate, the mixture was heated on a hot plate at 110 °C for 2 h to evaporate and solidify it. Subsequently, the sealed containers containing the sediment samples were transferred to tin solid containers, and the total organic carbon (TOC) and total sulfur (TS) were quantified using a CNS elemental analyzer (Flash EA 1112, Thermo Electron Corporation, Milan). Additionally, the carbon–sulfur ratio (C/S) was computed from the TOC and TS measurements. For standardization, 2,5-Bis (5-tert-butyl-2-benzoxazolyl) thiophene (BBOT) was employed, and TOC and TS values were determined based on the calibration curve established for the first five samples, with periodic corrections made by measuring BBOT every 10 samples. Based on the criteria for the classification of saline environments by Nakamura et al. (2023), Seto (2021), Seto et al. (2006), and Tokuoka et al. (1990), the values and trends of TS (%), TOC (%), and C/S in Lake Shinji sediment cores were used to distinguish a low-salinity environment (Unit 1), freshwater environment (Unit 2), a period between the abrupt environmental change indicated by rapid fluctuations in TS (Transitional period), and high-salinity environment (Unit 3). Here, we used a part of CNS elemental analysis results of sediment core 2CC from a previous study (Nakamura et al. 2023).

DNA extraction and quantitative real-time PCR (qPCR)

Environmental DNA was extracted from the sediment using a DNeasy PowerSoil Pro kit (Qiagen, Hilden, Germany). Approximately 250 mg of each sediment was placed in the provided Power Beads Pro tube, to which 800 µL of CD1 was added, vortexed for 10 min, and centrifuged at 15,000 × g for 1 min. After centrifugation, 500–600 µL of the supernatant solution was placed in a 2 mL microcentrifuge tube, to which 200 µL of CD2 was added, vortexed for 5 s, and centrifuged at 15,000 × g for 1 min. After centrifugation, 700 µL of the supernatant solution was added to a new 2 mL microcentrifuge tube, and 600 µL of CD3 was added and vortexed for 5 s. The solution (650 µL) was added to the MB Spin Column and centrifuged at 15,000 × g for 1 min to separate the filtrate, which was then discarded. This process was repeated until the solution was fully processed.

Next, the MB Spin Column was attached to a new 2 mL collection tube, and 500 µL of EA was added before centrifuging again at 15,000 × g for 1 min. After discarding the filtrate, the MB Spin Column was reattached to the same 2 mL collection tube, and 500 µL of C5 was added, followed by another centrifugation at 15,000 × g for 1 min. Subsequently, the MB Spin Column was transferred to a new 2 mL collection tube and centrifuged at 16,000 × g for 2.5 min. Finally, the MB Spin Column was connected to a 1.5 mL elution tube, and after adding 100 µL of C6, it was centrifuged at 15,000 × g for 1 min. The resulting filtrate served as the DNA extraction solution, which was then stored at –30 °C until quantitative real-time PCR (qPCR) analysis could be performed. No negative control was used during DNA extraction; however, we consistently prepared three wells of DW (DNA-free water) as a negative control for each subsequent qPCR operation.

Environmental DNA was quantified using the StepOnePlus Real-Time PCR system (Thermo Fisher Scientific Inc., Carlsbad, CA, USA). Each TaqMan reaction contained 10 µL of TaqPath qPCR master mixes (Thermo Fisher Scientific Inc., Carlsbad, CA, USA), 1 µL of primer (900 nM)/probe (125 nM) mix (final concentration), 7 µL of distilled water, and 2 µL of DNA extraction solution. The primer/probes developed and validated by Takahara et al. (under review) were used as follows:

For *S. pectinata* (97 bp amplified fragment):

Spec18s-F: 5'-GGGCCGAGGGCAAGTCT-3'.

Spec18s-R: 5'-GCTTCGAACACCTTATCCTTGGT-3'.

Spec18s-Pr: 5'-[FAM]-TCAAGTTACGTTGCTCTTTTCA
CCCTCCTCG-[TAMRA]-3'.

For *P. pusillus* (105 bp amplified fragment):

PpusiITS-F: 5'-TTATTTGCCTGCCATGTTATGC-3'.

PpusiITS-R: 5'-CAGTGGCCCTCTCATTTCTCA-3'.

PpusiITS-Pr: 5'-[FAM]-TGTTGTAACAAAAATCCT
G-[NFQ]-[MGB]-3'.

The PCR conditions were as follows: 2 min at 50 °C, 10 min at 95 °C, 55 cycles of 15 s at 95 °C, and 60 s at 60 °C. To produce standard DNA for qPCR, the target amplicon was inserted into the pMD20-T vector (Takara Bio, Shiga, Japan) and digested with BamHI. A dilution series of the plasmid containing 1×10^1 to 1×10^4 copies/template DNA was amplified as the standard in triplicate for all qPCR assays. In each qPCR assay, the DNA extraction solutions were tested in triplicate wells, and the mean value (copies µL⁻¹) was converted to a concentration (copies g⁻¹). Samples yielded a positive result in any of the three replicates, which were classified as containing the targeted DNA. Each qPCR assay included three wells and 2 µL of DNA-free water without the template DNA, as the negative control; in addition, no DNA was detected in any of the PCR assays. The standard curve R² values were 0.987 ± 0.013 (mean ± 1 SD) for *S. pectinata* and 0.963 ± 0.023 for *P. pusillus*.

Statistical analysis

Mann–Whitney U tests were performed to compare whether two aquatic plant DNA detected among the units to identify significant differences in each sediment core. Significant differences were identified between the DNA of the two aquatic plants in the two saline environments (Unit 1 or Unit 3) and the non-saline environment (Unit 2) at a significance level of $\alpha=0.05$ (see the section on CNS elemental analysis for definitions of these sediment core units). Notably, the significance level for 1C and 5C was $\alpha=0.025$ by Bonferroni correction. Additionally, comparisons were not performed for the transitional period due to the small sample size. Moreover, the p -value in 1C could not be calculated because *P. pusillus* DNA was not detected between Unit 2 and Unit 3. Furthermore, Spearman's rank correlation was used to determine whether changes in salinity, indicated by TS value, were correlated with the two aquatic plant DNA concentrations at a significant level of $\alpha=0.05$. However, the correlation coefficient for *P. pusillus* could not be calculated because of its low DNA detection in 2CC. These statistical analyses were performed using R version 4.4.1 (R Core Team 2021).

Results

All sediment cores were distinguished as two to four divisions (i.e., Unit 1, Unit 2, Transitional period, and Unit 3) of saline environments based primarily on TS and C/S values measured by the CNS element analysis (Figs. 2 and 3, Table 2, Tables S2 and S3; Berner and Raiswell 1984;

Ishihara et al. 2000; Sampei et al. 1997). The core length of each environmental category was different due to the effect of different sedimentation rates at each site in Lake Shinji (Table S3; Tamura et al. 1996). Sediment cores 1C and 5C were divided into four categories (Unit 1, Unit 2, Transitional period, and Unit 3). Sediment core 2CC was the longest among the nine sediment cores examined. However, CNS elemental analysis was conducted solely for the layer below 85 cm. The results yielded three distinct divisions: Unit 2, Transitional period, and Unit 3, as illustrated in Fig. 2 and detailed in Table 2, Table S2 and S3. In contrast, the remaining sediment cores, namely 2CA, 3C, 4C, 6C, 7C, and 8C were divided into the upper two sections: Unit 1 and Unit 2, as depicted in Fig. 3 and elaborated in Table 2, Table S2 and S3.

Two aquatic plant DNA samples (*S. pectinata* and *P. pusillus*) were detected in all sediment cores (1C, 2CA, 2CC, 3C, 4C, 5C, 6C, 7C, and 8C; Figs. 2 and 3, Table 2 and Table S2). The DNA concentration of *S. pectinata* and *P. pusillus* tended to be higher in the six sediment cores (3C, 4C, 5C, 6C, 7C, and 8C) collected from the coastal areas than the three sediment cores (1C, 2CA, and 2CC) collected from the central areas in Lake Shinji. In addition, the detection rate (%) of DNA positive results was calculated for the samples assessed from each sediment core (Table 2).

Two sediment cores 2CC and 5C contained DNA of *S. pectinata* and *P. pusillus* in Unit 3 characterized by a high-salinity environment in Lake Shinji; however, core 1C only contained the DNA of *S. pectinata* in Unit 3, in which DNA detection rate was 57.1%. In Unit 3 of 2CC, the DNA detection rates of *S. pectinata* and *P. pusillus* were 70.0% and 5.0%, respectively. In Unit 3 in 2CC, the deepest layers in

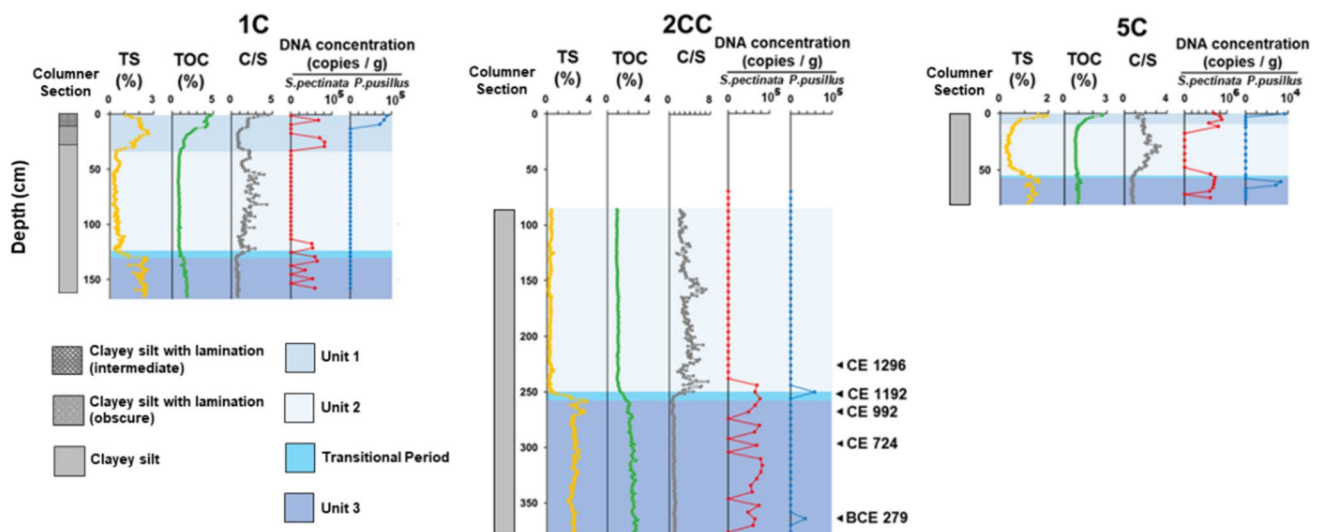


Fig. 2 Aquatic plant DNA concentrations and CNS element analysis for sediment cores 1C, 2CC, and 5C. Each graph represents concentrations of TS (%), TOC (%), C/S (ratio

of TOC to TS, gray line), DNA concentration of *S. pectinata* and *P. pusillus* (copies/g, red line and blue line, respectively)

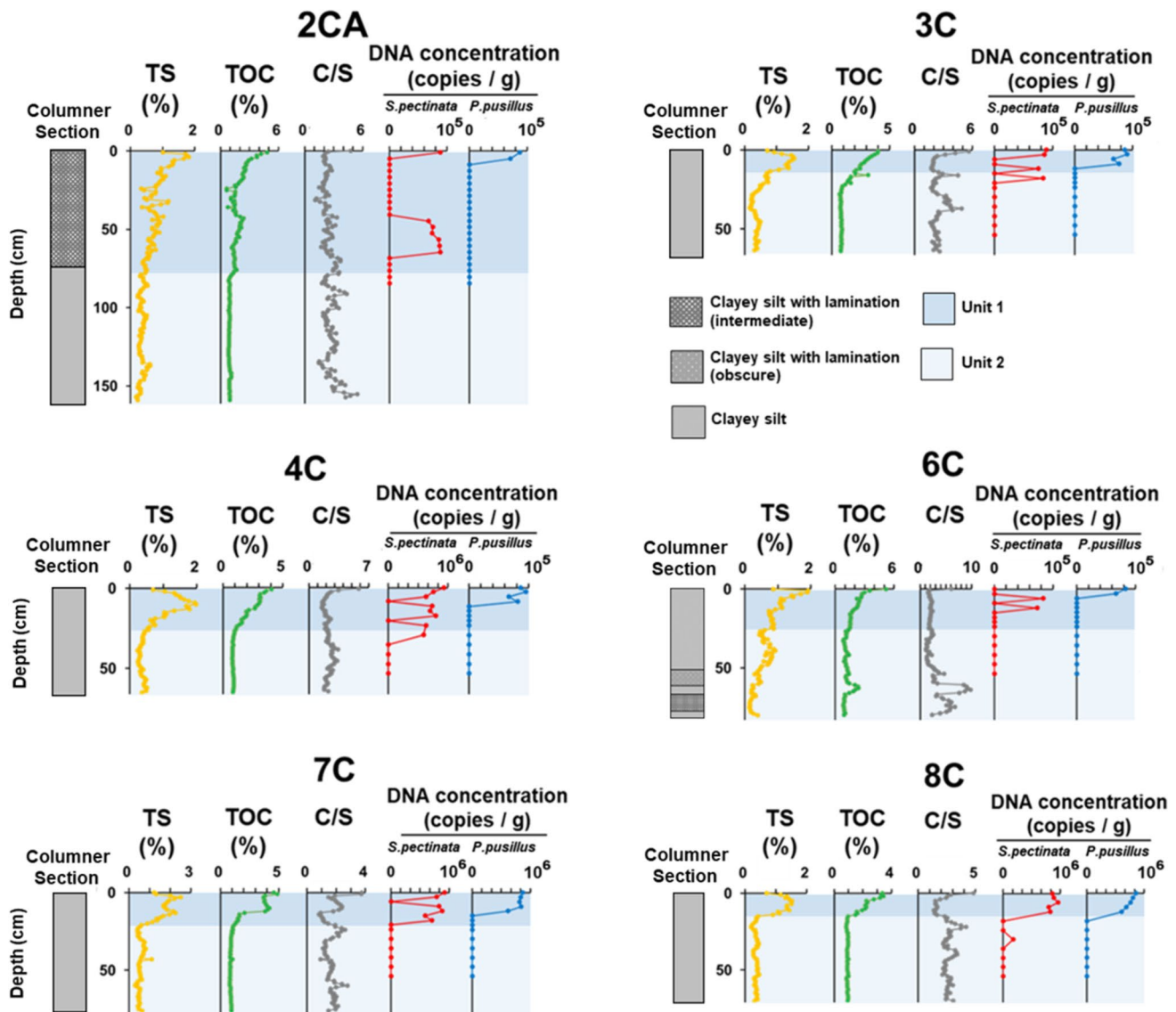


Fig. 3 Aquatic plant DNA concentrations and CNS element analysis for sediment cores 2CA, 3C, 4C, 6C, 7C, and 8C. Each graph represents concentrations of TS (%), TOC (%), C/S

(ratio of TOC to TS, gray line), DNA concentration of *S. pectinata* and *P. pusillus* (copies/g, red line and blue line, respectively)

which *S. pectinata* and *P. pusillus* DNA was detected were 363 cm and 369 cm, respectively, which corresponded to approximately 2000 years ago (Nakamura et al. 2023). The DNA detection rates for *S. pectinata* and *P. pusillus* in Unit 3 of 5C were 83.3% and 33.3%, respectively.

Transitional periods approximately 800 years ago were identified in sediment cores 1C, 2CC, and 5C (Table S3), as reported by Nakamura et al. (2023). In these cores, the *S. pectinata* DNA detection rates were 50.0% (1C), 100% (2CC), and 100% (5C), respectively (Fig. 2 and Table 2). However, during Transitional period, *P. pusillus* DNA detection rates were only 50.0% in core 2CC and 0% (not detected) in cores 1C and 5C, respectively.

In Unit 2, characterized by a mostly freshwater environment, the DNA detection rates of both *S. pectinata* and *P. pusillus* were lower compared to other environmental categories (Figs. 2 and 3, and Table 2). Specifically, DNA detection rates of *S. pectinata* in Unit 2 were 8.7% (1C), 3.7% (2CC), 20.0% (4C), 25.0% (5C), and 14.3% (8C), while all other sediment cores (2CA, 3C, 6C, and 7C) showed 0% detection. Interestingly, the DNA detection rate of *P. pusillus* in Unit 2 was 0% across all sediment cores.

Unit 1 was found to be a low-salinity environment based on the CNS analysis, and DNA for *S. pectinata* and *P. pusillus* was detected in all sediment cores, except for 2CC (Figs. 2 and 3, and Table 2). In Unit 1, the DNA detection

Table 2 Detection of DNA of two aquatic plants (*Stuckenia pectinata* and *Potamogeton pusillus*) and ratios of DNA detection per sample in the environment classifications. Environment classifications (Unit 1, Unit 2, Transitional period, and Unit 3) and core depth (cm) for nine sediment cores

	Depth (cm)	Environment classification	<i>Stuckenia pectinata</i>		<i>Potamogeton pusillus</i>	
			DNA detection rate (%)	DNA detection per samples	DNA detection rate (%)	DNA detection per samples
1C	0–31	Unit1	50.0	4/8	37.5	3/8
	32–122	Unit2	8.7	2/23	0	0/23
	123–130	Transitional period	50.0	1/2	0	0/2
	131–162	Unit3	57.1	4/7	0	0/7
2CA	0–78	Unit1	40.0	8/20	10.0	2/20
	79–157	Unit2	0	0/2	0	0/2
2CC	86–249	Unit2	3.7	1/27	0	0/27
	250–257	Transitional period	100	2/2	50.0	1/2
	258–379	Unit3	70.0	14/20	5.0	1/20
3C	0–22	Unit1	50.0	4/8	50.0	4/8
	23–64	Unit2	0	0/6	0	0/6
4C	0–26	Unit1	77.8	7/9	44.4	4/9
	27–65	Unit2	20.0	1/5	0	0/5
5C	0–10	Unit1	100	4/4	25.0	1/4
	11–54	Unit2	25.0	2/8	0	0/8
	55–57	Transitional period	100	1/1	0	0/1
	58–79	Unit3	83.3	5/6	33.3	2/6
6C	0–26	Unit1	22.2	2/9	22.2	2/9
	27–80	Unit2	0	0/5	0	0/5
7C	0–22	Unit1	75.0	6/8	62.5	5/8
	23–75	Unit2	0	0/6	0	0/6
8C	0–14	Unit1	100	5/5	100	5/5
	15–69	Unit2	14.3	1/7	0	0/7

rate of *S. pectinata* was higher than that of *P. pusillus* in most sediment cores. The DNA detection rates both for *S. pectinata* and *P. pusillus* in Unit 1 were the same: 50.0% (3C), 22.2% (6C), and 100% (8C). The DNA detection rates for *S. pectinata* in Unit 1 of the remaining sediment cores were 50.0% (1C), 40.0% (2CA), 77.8% (4C), 100% (5C), and 75.0% (7C), while that for *P. pusillus* was 37.5% (1C), 10.0% (2CA), 44.4% (4C), 25.0% (5C), and 62.5% (7C).

The detection of *S. pectinata* and *P. pusillus* DNA significantly differed between Unit 2 (i.e., non-saline environment) and Unit 1 (i.e., saline environment) for 1C, 7C, and 8C ($p < 0.05$), and the detection of *S. pectinata* DNA was also significantly different between Unit 2 (i.e., non-saline environment) and Unit 1 or 3 (i.e., saline environment) for 4C ($p < 0.05$) and 5C ($p < 0.025$ after Bonferroni correction; Fig. S1). The detection of *S. pectinata* DNA significantly differed between Unit 2 and Unit 3 for 1C and 2CC ($p < 0.05$). In contrast, *P. pusillus* DNA detection did not significantly differ between Unit 2 and Unit 3 for 2CC and 5C (Fig. S1). Positive correlations were found between the TS and DNA concentration of *S. pectinata* and *P. pusillus* in all sediment cores. Moreover, the correlation coefficients with a significance level of $p < 0.05$ were 0.53 (1C), 0.56

(2CC), 0.53 (5C), 0.68 (7C), and 0.86 (8C) for *S. pectinata* and 0.44 (2CA), 0.61 (3C), 0.62 (4C), 0.68 (7C), and 0.80 (8C) for *P. pusillus* (Table S4).

Discussion

TS values in sediments are known to vary under marine influences and thus can determine whether the sediment is marine or freshwater (Bernier and Raiswell 1984; Sampei et al. 1997); thus, this indicator was also utilized in this study. In Lake Shinji, TS values and plankton DNA compositions in the sediment core indicate that salinity has changed drastically over the past 2000 years (Nakamura et al. 2023). The sedimentary TS also vertically fluctuated in this study, suggesting that Lake Shinji was influenced by historical changes in saline environment. The dredging of the Ohashi River from 1928 to 1929 widened the river course threefold and deepened its water depth (Shimatani and Minagawa 1999). Hence, the salinity of Lake Shinji is thought to have increased due to the increased supply of seawater from Lake Nakaumi to Lake Shinji via Ohashi River (Figs. 2 and 3, Table 2, Tables S2 and S3; also see Seto

2021). We found that TS, the level of which was low in Unit 2, gradually increased toward the present. Therefore, the boundary between Unit 2 and 1 is considered to date back to 1928–1929. Such a vertical variation in the sedimentary TS values was found for all sediment cores through the lake basin.

Both *S. pectinata* and *P. pusillus* DNA were identified in Unit 3 of sediment cores 2CC and 5C. In sediment core 1C, however, *S. pectinata* DNA was present, while *P. pusillus* DNA was absent. Nakamura et al. (2023) estimated that Unit 3 of core 2CC corresponds to an age range of approximately 1000–2000 years ago. Thus, the potential effects of organic matter decomposition, including DNA, over time within the sediment must be considered to discuss the presence/absence of these aquatic plants. Nonetheless, the hypoxic and reducing conditions at the bottom of Lake Shinji, which were reported by Seto et al. (2006), may have inhibited the decomposition of organic matter, such as plant tissues (Corinaldesi et al. 2008; Torti et al. 2015). Moreover, the cell walls of plant tissues may have inhibited decomposition in the sediment (Parducci et al. 2017; Pietramellara et al. 2009). In Unit 3, the TOC of the three sediment cores 1C, 2CC, and 5C was higher than that of the other units, and plankton DNA was detectable in each layer of sediment core 2CC (Nakamura et al. 2023). In addition, the plant fragments used in the dating of sediment core 2CC were taken from the layer corresponding to Unit 3 (Nakamura et al. 2023). Thus, organic matter and DNA derived from aquatic plants may have been preserved in Unit 3.

The three sediment cores, 1C, 2CC, and 5C, showed a higher detection frequency of *S. pectinata* than *P. pusillus* DNA in Unit 3 (Table 2). *Stuckenia pectinata* is more salt-tolerant than *P. pusillus* (Rodríguez-Gallego et al. 2015), and the high-salinity environment of Unit 3 is more suitable for the growth of *S. pectinata* relative to *P. pusillus*, which was reflected in the DNA detection results (Table 2 and Table S4). This is the reason for why *S. pectinata* dominated in Unit 3 of the sediment core in Lake Shinji. In contrast, *P. pusillus* DNA was not detected in Unit 3 of core 1C, suggesting it was not present in this area in the past. Among other sediment cores from which Unit 3 was collected, significant differences were not observed in the detection of *P. pusillus* DNA. Collection of deep sediment cores from much more points within the lake basin could better depict the spatial distribution of these species in Unit 3.

During Transitional period, *S. pectinata* DNA was detected in sediment cores 1C, 2CC, and 5C, while *P. pusillus* DNA was detected only identified in core 2CC. Nakamura et al. (2023) noted that brackish plankton DNA was more prevalent during this Transitional period, although marine plankton was also detected in sediment core 2CC, thus Transitional period was between high and low salinity environment. Hence, it is likely that both *S. pectinata* and *P.*

pusillus DNA coexisted within the Transitional period, with *S. pectinata* exhibiting its dominant presence.

In Unit 2, there was no or little detections of *S. pectinata* DNA in shallow sediment cores and no *P. pusillus* DNA in any sediment cores (Fig. 3). As in Nakamura et al. (2023), the fact that Unit 2 of sediment core 2CC only contained a few plant fragments to calculate ages suggests that the detection of aquatic plant DNA was limited. Another reason for such a low DNA detection is the accelerated degradation of organic matter, including plant fragments, as Unit 2 is a freshwater environment that is assumed to have been an oxidative environment with a high oxygen supply (Nakamura et al. 2023; Seto 2021). In addition, the TOC values obtained from the CNS analysis show lower values in Unit 2 compared to those in other periods (Unit 1, Transitional period, Unit 3; Figs. 2 and 3, Tables S2 and S3). Thus, active decomposition of organic matter, including plant fragments, is likely (Schlumbaum et al. 2008). However, the robust cell walls of these plant species may have hindered their complete degradation even in the freshwater conditions of Unit 2. Moreover, plankton DNA was consistently detected throughout Unit 2 of sediment core 2CC (Nakamura et al. 2023), and *S. pectinata* and *P. pusillus* DNA were found in the Transitional period and Unit 3, which predates Unit 2. This suggests that DNA degradation may have had minimal impact. Since each sediment core was almost entirely composed of clayey silt (Figs. 2 and 3), it is unlikely that the influence of DNA decomposition due to the properties of the sediment core is different between units. Therefore, it is likely that *S. pectinata* and *P. pusillus* were confined to specific areas within Lake Shinji during Unit 2, whose condition was unfavorable for their growth.

In Unit 1, *S. pectinata* and *P. pusillus* DNA were detected in all but 2CC sediment cores, and the detection rates were higher than those in Unit 2, which was a freshwater environment (Table 2). Therefore, the low-salinity environment of Unit 1, which had a higher salt content than the freshwater environment of Unit 2, was considered more suitable for the growth of the salt-tolerant aquatic plants *S. pectinata* and *P. pusillus* in Lake Shinji (Rodríguez-Gallego et al. 2015). These aquatic plants may have inhabited saline areas (e.g., Lake Nakaumi or Ohashi River) and invaded Lake Shinji, and flourished during the Unit 1 period (Nakagawa and Kunii 2015). In Unit 1, the DNA detection rate of *S. pectinata* was higher than that of *P. pusillus* for 1C, 2CA, 4C, 5C, and 7C and the spatial pattern of their DNA detections appeared to be similar to those in other periods, Transitional period and Unit 3, in which *S. pectinata* was more widespread than *P. pusillus* in Lake Shinji. Based on these results, *S. pectinata* may have been the dominant species in Lake Shinji in Unit 1 rather than *P. pusillus*.

The sediment core DNA detection rates of *S. pectinata* and *P. pusillus* collected from the coastal areas (3C, 4C,

5C, 6C, 7C, and 8C) tended to be higher than those collected from the central areas (1C and 2CA; Table 2). This suggests that *S. pectinata* and *P. pusillus* may have preferred the coastal areas rather than the central area of Lake Shinji. In addition, the DNA detection rates for *S. pectinata* and *P. pusillus* in Unit 1 of sediment cores collected from the coastal areas were scattered. The DNA detection rates for both species were lower in the west (3C and 6C) and higher in the east (5C and 8C). However, the detection rate of *P. pusillus* DNA in 5C was lower than that of *S. pectinata*, and a significant difference was not observed between Unit 1 and Unit 2 (Fig. S1). The eastern side of Lake Shinji experiences higher salinity due to the influx of seawater from the Ohashi River, while the western side is less saline under the influence of the Hii River, resulting in a distinct salinity gradient (Fukuoka et al. 2004). Thus, the saline conditions of 5C may have been more suitable for the growth of *S. pectinata* than *P. pusillus* (Rodríguez-Gallego et al. 2015). Interspecific difference in spatial patterns of *S. pectinata* and *P. pusillus* DNA detections in Unit 1 may be attributed to their preferences for areas with higher salinity, especially in the coastal regions of Lake Shinji. Among layers of each sediment core, *S. pectinata* and *P. pusillus* DNA concentrations were positively correlated with salinity, and the range of correlation coefficients for some cores with significant correlation were 0.53–0.86 for *S. pectinata* and 0.44–0.80 for *P. pusillus* (Table S4). Therefore, the change from the freshwater environment of Unit 2 to the brackish environment of Unit 1 owing to the seawater intrusion may have increased the DNA detection of salt-tolerant aquatic plants. In the future, the collection of multiple sediment cores from both high- and low-salinity regions in Lake Shinji and subsequent analysis of the DNA from these aquatic plants could offer deeper insights into their salinity preferences.

In conclusion, we succeeded in reconstructing the Lake Shinji paleoenvironment from approximately 2000 years ago to the present using sediment taken from the lake bottom. Our sediment core survey revealed the spatio-temporal distributions of salt-tolerant aquatic plants through species-specific DNA analysis and the salinity environment through CNS elemental analysis. The detection of eDNA of salt-tolerant aquatic plants and investigation of the eDNA behavior of other macro-organisms affected by the decline in aquatic plant growth may help to elucidate the past environment such as eutrophication and anthropogenic interventions, in the lake ecosystem.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10201-025-00782-z>.

Acknowledgements We would like to thank A. Nagata, Y. Fukatsu, T. Tanaka, H. Sakamoto, and N. Nishikori (Shimane University) for support during the treatment/analysis of samples.

Author contributions SY, YN, KS, and TT designed the research. SY, KS, TA, and KK collected field samples. SY, YN, and TT wrote the manuscript. All authors discussed the results and commented on the manuscript.

Funding Open Access funding provided by Shimane University. This work was supported by JST SPRING, Grant Number JPMJSP2155, by Izumo Office of River, Chugoku Regional Development Bureau, Ministry of Land, Infrastructure and Transport, and by JSP KAKENHI Grant 20K15589, 21H03649, 21H04521, 23H01257, 23K03566, 24K00718.

Data availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Conflict of interests All the data are original results and have not been used or considered for publication before. The authors declare no conflicts of interest.

Ethical standards The experiments comply with the current laws of the country in which they were performed.

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