Sedimentary DNA can reveal past pelagic copepod population dynamics

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Keywords: sedDNA, *Eodiaptomus japonicus*, copepod dynamics, Lake Biwa, quantitative PCR

Abstract

1. Copepods play a key trophic role as secondary producers in transferring primary production to higher trophic levels such as fish. Copepod production significantly contributes to successful fish recruitment. Despite their importance, knowledge of their dynamics over several decades remains limited due to the difficulty to obtain long-term data series with exhaustive sampling and analysis. However, an understanding long-term copepod dynamic is urgently required to head toward better management for sustainable aquatic ecosystems and fish recruitment.

2. Sedimentary DNA (sedDNA) has been developing a useful tool for reconstructing past plankton dynamics. This study evaluates whether sedDNA targeting the pelagic copepod, *Eodiaptomus japonicus*, in Lake Biwa (Japan) can be an effective tool for elucidating its past population dynamics.

3. It was applied a quantitative polymerase chain reaction method targeting the mitochondrial cytochrome c oxidase subunit I gene on sediment cores and compared the detected sedDNA concentrations with the unique long-term dataset of demographic traits, biomass, specific growth rate, production, subitaneous eggs, and resting eggs of *E. japonicus*.

4. The sedDNA concentration of *E. japonicus* recovered from sediment layers did not correlate significantly with its resting eggs and its specific growth rate but with *in situ* production, biomass, and subitaneous egg production. The results provided the evidence for the sedDNA as a tracking tool for assessing copepod past production dynamics.

Introduction

Copepods are the most abundant multicellular organisms on Earth (Schminke, 2007) and are important secondary producers that transfer primary production to higher trophic levels such as fish in aquatic ecosystems (Mauchline, 1998). Copepod production as a food resource is essential for sustainable fish management (Mauchline, 1998). Successful fish recruitment is highly dependent on copepod production because it promotes fish larvae survival (Mauchline, 1998). Copepod population growth is influenced by temperature and food availability (Liu *et al*., 2015; Ban, 1994; Koski & Kuosa, 1999; Hart, 1990). Previous studies have shown that global warming decreases copepod abundance (Garzke *et al*., 2015). Also, large-scale climate events showing oscillation cycles over decades, such as the Pacific Decadal Oscillation and Arctic Oscillation, regulate ocean temperature and consequently affect copepod biomass and production (Peterson & Schwing, 2003; Chiba *et al*., 2006; Liu *et al*., 2021). Information about long-term copepod population dynamics is urgently required to achieve better fish management and to understand how large-scale climatic events influence aquatic ecosystems. Nevertheless, copepod dynamics across decades are rarely investigated due to the cost and time required to obtain long-term data series with exhaustive field sampling and sample analysis.

Sedimentary archives are valuable for gaining information on zooplankton long-term dynamics (Jankowski & Straile, 2003; Brede *et al*., 2009). For specific species, such as the cladoceran zooplankton *Daphnia*, whose remains and resting eggs are well preserved in sediment (Jankowski & Straile, 2003; Tsugeki *et al*., 2009), morphological investigation and genetic analysis are powerful tools to clarify biological responses to anthropogenic perturbations (Jankowski & Straile, 2003; Brede *et al*., 2009; Monchamp *et al*., 2017). However, copepods have been excluded from palaeoecological studies because their remains are not well preserved in sediments (Selden *et al*., 2010). Although copepod resting eggs are preserved in sediments (Hairston *et al*., 1995), they are seldom used in palaeoecological studies because of their poor taxonomic features (Briski *et al*., 2011). Consequently, traditional investigations of sediment archives are insufficient for establishing copepod past dynamics. Alternatively, to overcome such difficulties in reconstructing species lacking remains, sedimentary DNA (sedDNA) could be a useful complementary method (Capo *et al*., 2021). Using the sedDNA approach, scientists could reconstruct plankton community (Monchamp *et al*., 2016; Huang *et al*., 2020; Epp *et al*., 2010) and fish dynamics (Kuwae *et al*., 2020). Combined studies of sedDNA with morphological techniques have been conducted to track the community composition of plankton communities (Monchamp *et al*., 2016; Huang *et al*., 2020). However, most

quantitative studies on sedDNA have focused on phytoplankton; thus, the utility of the sedDNA approach to reconstruct successive zooplankton dynamics remains limited. A previous study on zooplankton sedDNA targeting for *Daphnia galeata* and *Daphnia pulicaria* in Lake Biwa (Japan) using quantitative polymerase chain reaction (qPCR) established successful detection of both *Daphnia* sedDNA concentrations (Tsugeki *et al*., 2022). The temporal variation in *Daphnia* sedDNA concentrations were consistent with their resting egg production, supporting that zooplankton sedDNA could be used as a tool for monitoring spawning activity dating back 100 years (Tsugeki *et al*., 2022). But, continuous and quantitative reconstruction of copepod population dynamics using sedDNA has not been reported (Bissett *et al*., 2005; Xu *et al*., 2011). Therefore, there is an urgent need for additional research to ensure the utility and reliability of sedDNA for reconstructing important zooplankton taxon, including copepods (Mauchline, 1998).

To ensure the reliability of the reconstruction using sedDNA for the past population dynamics, accumulating knowledge about the source of sedDNA is imperative. Without such information, one cannot interpret what is reconstructed. The comparison of historical variation between sedDNA and regular survey data in the water column could confirm the reliability of the information provided by sedDNA. In Lake Biwa (Japan), one of the world's ancient lakes (Hampton *et al*., 2018), regular zooplankton surveys have been

intensively conducted since 1965 (Hsieh *et al*., 2011; Liu *et al*., 2020b). Moreover, the long-term demographic traits of the pelagic dominant copepod *Eodiaptomus japonicus*, such as biomass, population growth rate, and production, have been determined from 1971 to 2010 with experimental and microscopic observations (Liu *et al*., 2015; Liu *et al*., 2021; Liu *et al*., 2020b). *E. japonicus* produces both subitaneous and resting eggs; the historical variation of resting egg concentration was recovered from sediment archives up to 60 years ago (Liu *et al*., 2020a). Lake Biwa sediment has had a continuous record with a high time resolution, such as two or three years per 1-cm layer sample (Tsugeki et al. 2003; Tsugeki *et al*., 2021). Therefore, *E. japonicus* from Lake Biwa is suitable for comparing historical variation between its sedDNA and demographic traits over the past several decades.

We first investigated whether the pelagic copepod *E. japonicus* sedDNA were successively detected from sediment core samples collected in the deep and large northern basin of Lake Biwa via qPCR analysis. We then tested the efficiency of sedDNA as a tool to reconstruct its population dynamics (Fig. 1b) by comparing the detected *E. japonicus* sedDNA and its previously determined demographic traits (Fig. 1g, h). Furthermore, to investigate the potential sources of *E. japonicus* sedDNA, we conducted a sieving experiment to evaluate the inclusion effects of resting eggs (Fig. 1e) and the substances

in pore water to sedDNA concentrations (Fig. 1f). We also evaluated the effects of enzymatic inhibitors in the qPCR process (Fig. 1b) by co-sinking with phytoplankton debris and their aggregates (Fig. 1d). Based on the findings, we discuss what was reconstructed for *E. japonicus* from sedDNA and its reliability as a tool for reconstructing in situ *E. japonicus* population dynamics.

Methods

Sampling site, sediment core, and zooplankton sample collection

We performed a paleogenetic analysis using Lake Biwa sediment core samples to reconstruct temporal variations of *E. japonicus* eDNA concentrations (Fig. 1b). In this study, four 30-cm long gravity core samples (2017LB2, 2017LB5, 2017LB7, and 2019IM1; Fig. 1a-f) were collected on August 17, 2017 (2017LB core) and August 27, 2019 (2019IM1 core) in the northern basin of Lake Biwa (Supplementary Fig. S1). Detailed information of coring sites is shown in Supplementary Fig. S1, and the sampling methods have been previously reported (Tsugeki *et al*., 2022). Briefly, 2017LB2 and 2017LB5 cores were used to establish the variation of *E. japonicus* sedDNA concentrations (Fig. 1b) and to explore potential sources of its sedDNA (Fig. 1e, f).

Additionally, to develop the primer-probe for *E. japonicus,* we collected a total of

95 individuals of *E. japonicus* (including 14 egg-bearing individuals) and 91 individuals of the sympatric copepod *Mesocyclops* spp. (including 17 egg-bearing individuals) from water samples on May 14, 2021, in Lake Biwa. Among these, six individuals of *E. japonicus* (including three egg-bearing individuals) and four individuals of *Mesocyclops* spp. (including two egg-bearing individuals) were randomly selected, stored at –80 °C until DNA extraction, and then stored at –20 °C until *in vitro* validation.

Chronology of sediment cores

Sediment chronology was established for the 2017LB7 core based on the constant rate of supply (CRS) model of ^{210}Pb dating (Appleby & Oldfield, 1978) and verified using the ^{137}Cs peak traced in the period 1962 to 1963 (Appleby, 2001). Details of the chronological method have been reported elsewhere (Tsugeki *et al*., 2021). The ages and age errors of the 2017LB2 and 2017LB5 cores were indirectly estimated using stratigraphic correlations between the cores based on chronological controls in chlorophyll pigments and magnetic susceptibilities of the chronological 2017LB7 core (Tsugeki *et al*., 2021). To compare these proxies, the marked peak or trough layers were used as reference layers (Supplementary Fig. S4). In this study, sedimentary records of resting eggs for *E. japonicus* (Liu *et al*., 2020a) were newly denoted with improved

chronology estimated using CRS-model (Supplementary Fig. S5a, b).

DNA extraction and primer-prove development for *E. japonicus*

DNA extraction of sediment samples followed the methods of previous studies (Kuwae *et al*., 2020; Tsugeki *et al*., 2022; Sakata *et al*., 2020) using the 'Experienced User Protocol 3 to 22' of a fecal-soil DNA extraction kit (Power Soil DNA Isolation Kit, Qiagen, Germany). Finally, 200 μL of the DNA solution was obtained and stored at –20 °C until qPCR analysis. Detailed information on the DNA extraction procedure from 9 g sediment sample has been already reported (Tsugeki *et al*., 2022).

The primer-probe for *E. japonicus* in qPCR analysis was not purchased by a company, thus we exploited it for this study (Supplementary Table S1a). We downloaded the mitochondrial cytochrome c oxidase subunit I gene (COI) from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). We then compared these COI genes among the target species *E. japonicus*, and non-target species of copepod *Mesocyclops thermocyclopoides*, *Cyclops vicinus,* and *Daphnia* in Lake Biwa which is main zooplankton species (Liu *et al*., 2020b). From the preliminary investigation, we identified the variability of sequences among *E. japonicus* and other species, obtained COI sequences of *E. japonicus* and other zooplankton species, and then constructed the

169 primer-probe using Primer3plus (https://www.bioinformatics.nl/cgi-
170 bin/primer3plus/primer3plus.cgi).

171 The reference sequences for the targeted gene regions were queried on possible
172 amplicons between 50–150 bp using NCBI primer blast. The specificities of the primers
173 and probe were then assessed *in silico* with homologous sequences from other copepods
174 and *Daphnia* species in Lake Biwa using NCBI, by targeting 116 bp of the mitochondrial
175 COI gene. Once PCR amplicons were identified for suitable detection, the respective
176 primers and probe were tested against template DNA originating from the *E. japonicus*
177 species to confirm amplification. During the *in silico* screening for specificity, we
178 performed Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). We
179 checked all species from Japan for the order species. Using the *E. japonicus* primer-set,
180 we did not detect any species.

181

182 **qPCR**

183 DNA samples were quantified via real-time TaqMan quantitative PCR using the
184 QuantStudio™ 1 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA,
185 USA) in Center for Marine Environmental Studies, Ehime University. The qPCR method
186 was followed according to a previous study (Tsugeki *et al*., 2022) on the developed

primer-probe set for *E. japonicus* (Supplementary Table S1a). Briefly, the TaqMan reaction contained 900 nM of each forward and reverse primer, 125 nM TaqMan-Probe, 10 µL qPCR master mix (TaqPath; Thermo Fisher Scientific), and 4.0 µL template DNA solution. The final volume of the PCR was 20 µL after adding UltraPure™ DNase/RNase-Free Distilled Water (Thermo Fisher Scientific). The dilution gradient pattern of 20,000, 2,000, 200, and 20 copies per PCR reaction (n = 4) was proposed for the standard curve using the target DNA cloned into a plasmid. The R^2 values of the regression analysis for the standard curves ranged from 0.983 to 0.993 (PCR efficiency = 81.2 to 98.4%). The qPCR data of the DNA copies (copies g^{-1} dry sed.) were shown as mean ± SD of four replicates including zero (i.e., no detection). We also used four replicates for each sample and an NTC (n = 4). No positives were detected from the NTC and the negative control of DNA extraction, confirming that there was no cross-contamination in any of the DNA measurements.

To determine primer-probe specificity, *in vitro* validation was performed using extracted DNA of *E. japonicus* and other copepod *Mesocyclops* spp. collected from a water sample in Lake Biwa (respective 5 pg per PCR reaction, n = 4). Additionally, *in vivo* validation was performed using DNA extracted from the 2017LB5 core samples of 9.5 cm, 19.5 cm, and 29.5 cm depth layers (n = 4 per layer). Positive samples were

randomly collected among the qPCR amplicons of 2017LB2 (n = 12) and were then directly sequenced following ExoSAP-IT (USB Corporation, Cleveland, OH, USA) treatment. Sequences were determined using a commercial sequencing service (Eurofins Genomics, Tokyo, Japan).

Inhibitor test

To test the PCR inhibition effect of substances and minerals included in sediments, spike tests were performed for the 2017LB2 and 2017LB5 core samples (Fig. 1b). Detailed information on the spike test was provided in a previous study (Tsugeki *et al*., 2022) and $\Delta Ct \geq 3$ cycle appeared to be evidence of inhibition (Hartman *et al*., 2005). We used the primer-probe sets for IPC as follows:

IPC1-5′ : CCGAGCTTACAAGGCAGGTT

IPC1-3′: TGGCTCGTACACCAGCATACTAG

IPC1-Taq: [FAM] TAGCTTCAAGCATCTGGCTGTCGGC [TAMRA]

Demographic traits and experimental test to explore potential sources of sedDNA

To determine the source of *E. japonicus* sedDNA, we evaluated the correlation

223 between the sedDNA concentration and demographic traits of this copepod during the
224 past years, including biomass, specific growth rate, population production, and the
225 number of subitaneous and resting eggs (Fig. 1g, h). Long-term monthly data sets of
226 biomass, specific growth rate, and population production in previous studies were
227 referred to (Liu *et al*., 2021; Liu *et al*., 2020b). The calculation methodology is
228 summarized in Supplementary Table S4; detailed descriptions are provided in previous
229 studies (Liu *et al*., 2015; Liu *et al*., 2021; Liu *et al*., 2020b). Briefly, biomass (g dry weight
230 m^{-2}) was calculated as the product of the population density (individuals m^{-2}) with the
231 mean body dry-weight (g $individual^{-1}$). Specific growth rate (g day^{-1}) was estimated using
232 a multiple regression equation with two parameters: mean water temperature (°C) during
233 the copepod development and the food index as a proxy for availability of food required
234 for *E. japonicus* growth (Liu *et al*., 2015). The food index was calculated from the ratio
235 of the prosome length of the females collected from Lake Biwa to the expected prosome
236 length of the females obtained from laboratory experiments under adequate food
237 conditions. Population production (gC m^{-2} $month^{-1}$) was calculated as the product of
238 biomass and specific growth rate. The concentration of subitaneous eggs (eggs m^{-2}) was
239 estimated as the product of proportion of ovigerous female (%), ovigerous female density
240 (individuals m^{-2}), and clutch size (eggs $clutch^{-1}$). Here, all parameters used in the

calculation of subitaneous eggs were based on counts of individuals collected by field survey (Dur *et al*., in press). The concentration of resting eggs (eggs g^{-1} dry sediment) was calculated as the ratio of the number of resting eggs counted by microscopy from each layer of the 2013LB core (eggs $sample^{-1}$) to the dry weight for each layer of sediment (g dry sediment) (Liu *et al*., 2020a).

To determine whether *E. japonicus* sedDNA concentrations were regulated by DNA derived directly from resting eggs included in the analytical sediment, we divided the sediment sample into two fractions using a sieve to exclude the eggs (Fig. 1e). Since the minimum size of the resting egg of this copepod is approximately 80 µm (Liu *et al*., 2020a), the analytical sediments for DNA extraction were divided into two size classes using a 38-µm mesh-sieve on three randomly selected-layer samples (specifically, 2017LB5-2.5 cm, 2017LB5-10.5 cm, and 2017LB5-19.5 cm). Furthermore, to test the possibility of vertical movement of *E. japonicus* sedDNA through pore waters, we examined the sedDNA concentration in pore water and its residual sediment by separating them (Fig. 1f). The DNA extractions of the aforementioned samples were performed according to previous studies (Tsugeki *et al*., 2022).

Possible factors regulating *E. japonicus* sedDNA

To explore potential factors regulating temporal variation in sedDNA concentrations, we analyzed chlorophyll-a pigments and algal remains. Sedimentary pigments of chlorophyll-a were investigated for the 2017LB2 core, and algal remains were investigated for the 2017LB7 core (Fig. 1d). Details of the method used for chlorophyll-a and algal remains are described in previous studies (Tsugeki *et al*., 2022; Tsugeki *et al*., 2021).

Data analysis

All statistical analyses were performed using R ver. 4.1.2 (R Core Team 2021) with the package "smatr" ver. 3.4.8. Type II regression models along with the standardized major axis method were used to determine the relationship between sedDNA concentrations of *E. japonicus* and demographic properties of biomass, specific growth rate, production, and abundance of *E. japonicus* subitaneous, resting eggs, and algal proxies. Since sedDNA and resting egg analysis for *E. japonicus* (Liu *et al*., 2020a) were conducted on different sediment cores (2017LB2, 2017LB5, and 2013LB; Fig. 1b, h), the chronological age of each analytical sample was slightly different. Therefore, prior to statistical analysis, each sedDNA and resting egg concentration in the respective core was

converted to the value at 0.1-year intervals by linear interpolation using the AnalySeries 2.0.3 software package (Paillard *et al*., 1996). We then recalculated the average values within the year corresponding to the sedimentation period in each sample of the chronology 2017LB7 core. The monthly data of demographic traits in *E. japonicus* including biomass, specific growth rate, production, and abundance of subitaneous eggs were annually averaged and converted to the value at 0.1-year intervals by linear interpolation. Thereafter, we calculated the average values in the respective sedimentation period of the 2017LB7 core samples, as mentioned above. These demographic data were limited to the year 2010. However, we estimated that the value until 2011 corresponded to the estimated age at 4.5 cm depth of 2017LB7 core by linear extrapolation using AnalySeries 2.0.3 software package (Paillard *et al*., 1996). Prior to Type II regression analysis, distribution was tested by the Shapiro–Wilk test (significant level $p < 0.05$). Since some data such as sedDNA concentration, subitaneous eggs were identified as non-Gaussian distribution, they were $\log_{10}$-transformed. We then used Type II regression models to determine the relationship between the $\log_{10}$-transformed values of sedDNA concentration and their demographic traits and algal proxies.

Results

sedDNA concentration of *E. japonicus*

The results of qPCR analysis in both cores (2017LB2 and 2017LB5; Fig. 1b) showed that *E. japonicus* sedDNA copies were detected continuously throughout the study period (Fig. 2a, b), except for three missing layers for the 2017LB5 core (5–6 cm, 6–7 cm, 7–8 cm layers; hereafter expressed in middle depth, i.e., 5.5 cm, 6.5 cm, and 7.5 cm, respectively, Supplementary Table S2). The mean DNA copy number of *E. japonicus* varied depending on the core depth and ranged from 6 ± 11 (mean ± SD) copies g^{-1} dry sediment (hereafter copies g^{-1}) in the depth of the 2017LB2-28.5 cm layer (estimated age: 1924.5 ± 39.0) to 290,330 ± 30,848 copies g^{-1} in the 2017LB2-4.5 cm layer (estimated age: 2007.3 ± 0.4). Supplementary Table S2). They were almost abundantly detected in three to four replicates in the upper 8.5 cm layers (estimated age: 1993.8 ± 1.0 and 1999.0 ± 0.7 for 2017LB2 and 2017LB5 cores, respectively; Fig. 2a, Supplementary Table S2). The DNA copy numbers below 8.5 cm layers were relatively low but were detected from all layers in one to four replicates (Fig. 2b, Supplementary Table S2).

No DNA of *E. japonicus* was detected in any of the negative controls for sub-sampling, DNA extraction, or PCR analysis, or the no-template control (NTC), indicating that sample contamination did not occur during these processes. We also confirmed that

311 only *E. japonicus* DNA was amplified through direct sequencing of PCR amplicons, using
312 qPCR analysis of *E. japonicus* DNA.

313 A spike test was performed on both sediment core samples (2017LB2 and 2017LB5)
314 for evaluating the effect of qPCR inhibition (Fig. 1b). All ΔCt values were below 1 (–1.0
315 to 0.87: 2017LB2, –0.49 to 0.21: 2017LB5; Supplementary Table S3), indicating no
316 inhibition.

317

318 **Long-term variation in demographic traits of *E. japonicus***

319 All the mean values of demographic traits such as biomass, specific growth rate, and
320 production for *E. japonicus* were enumerated depending on the time resolution of
321 deposition period on each sample (see details in data analysis). The average biomass of
322 *E. japonicus* till approximately the year 2000 was relatively low with a range of 0.7 to
323 1.2 g dry weight m^{-2}, except for the values measured around 1970 (1.7 g dry weight m^{-2}),
324 but the average biomass rapidly increased approximately two-fold after 2010 compared
325 to that before 2000 (Supplementary Fig. S2a). Likewise, the average mean of their
326 production was stable before approximately 2000, at less than 1.5 g C m^{-2}, and then
327 continuously increased up to 2010 (Supplementary Fig. S2c). Contrarily, the average
328 mean of specific growth rate (*g*) remained relatively constant after 1970 at approximately

329 0.07 day^{-1}. The lowest value of specific growth rate was observed around 1970 and the
330 highest value was observed around 2010 (Supplementary Fig. S2b). The average mean of
331 subitaneous eggs remained constant, being less than 1.5×10^5 eggs m^{-2} up to approximately
332 2000, excluding the marked peak in the late 1990s, and continuously increased 2000
333 onward (Supplementary Fig. S2d). The average mean of resting egg concentration
334 recovered from sediment observation increased markedly from the mid-1980s, peaked
335 around the early 1990s, and then decreased and stabilized (Supplementary Fig. S2e).
336

337 **Correlations of the *E. japonicus* sedDNA and demographic traits with the algal**
338 **remain concentrations**

339 A highly significant correlation was detected between the *E. japonicus* sedDNA
340 concentration and *E. japonicus* production from 1971 to 2010 (Type II regression model:
341 $R^2 = 0.60$, $p = 0.001$, $n = 14$; Fig. 3c), in addition to its biomass concentration ($R^2 = 0.51$,
342 $p = 0.004$, $n = 14$; Fig. 3a). Significant correlation was also detected between the sedDNA
343 concentration and number of subitaneous *E. japonicus* eggs ($R^2 = 0.52$, $p = 0.005$, $n = 13$;
344 Fig. 3d). In contrast, no significant relationship was observed between the sedDNA
345 concentration and the specific growth rate ($R^2 = 0.02$, $p = 0.66$, $n = 14$; Fig. 3b), the
346 number of resting eggs ($R^2 = 0.25$, $p = 0.066$, $n = 14$; Fig. 3e). *E. japonicus* sedDNA

concentrations were positively corelated with diatom valves ($R^2 = 0.65$, $p < 0.001$, $n = 27$; Supplementary Fig. S4a), chlorophyll-a ($R^2 = 0.71$, $p < 0.001$, $n = 29$; Supplementary Fig. S4b) and green algal cells ($R^2 = 0.37$, $p = 0.001$, $n = 25$; Supplementary Fig. S4c), although these long-term trends differed from the trend of variation in *E. japonicus* sedDNA concentrations (Supplementary Fig. S4d, e, f).

Potential sources of *E. japonicus* sedDNA

E. japonicus environmental DNA (eDNA) was not detected from the pore water of all three samples from the 2017LB5 core by qPCR (Fig. 1f, Table 1). In contrast, *E. japonicus* eDNA was detected in the residual sediment samples. *E. japonicus* sedDNA was detected in both sieved and non-sieved samples, except for the sieved sample of 2017LB5-20 (Table 2). High concentrations of sedDNA were detected in the non-sieved samples for two of the three samples, namely 2017LB5-3 (11,630 ± 5,921 copies g^{-1}) 2017LB5-20 (1,449 ± 1,977 copies g^{-1}), compared to those in the sieved samples. However, for 2017LB5-11, the sieved sample (2,630 ± 4,555 copies g^{-1}) was more abundant than the non-sieved sample (1,838 ± 3,184 copies g^{-1}), although the difference between both values was minimal.

365 **Discussion**

366 Several studies have reported the long-term preservation of copepod eDNA in
367 aquatic sediments (Bissett *et al*., 2005; Xu *et al*., 2011; Coolen *et al*., 2013), such as a
368 report by Bissett *et al*. (2005), which detected copepod eDNA from sediment layers as
369 old as 9,950 years in some Antarctic lakes. However, continuous, and quantitative
370 reconstruction of copepod sedDNA is not yet to be reported (Domaizon *et al*., 2017). This
371 study is the first to successively detect copepod eDNA for *E. japonicus* from a sediment
372 core in Lake Biwa over the past 100 years with high-resolution dating.

373 We found that the temporal variation of *E. japonicus* sedDNA concentration
374 significantly correlated with its production and biomass, obtained via microscopic
375 observations of regular field samples. In support of this, the number of reads for copepod
376 obtained by metabarcoding genetic analysis correlated with their biomass in water
377 samples (Yang *et al*., 2017) although a meaningful relationship between eDNA abundance
378 and biomass for some copepod species was not well observed (Djurhuus *et al*., 2018). We
379 also established the temporal increase in *E. japonicus* sedDNA concentration in the 1970s,
380 which is more than 50-fold compared to those before the 1970s when eutrophication
381 occurred in Lake Biwa (Hyodo *et al*., 2008). Concordant with this finding, regular survey
382 reports in Lake Biwa described the density of *E. japonicus* as being temporally and greatly

383 increased in 1975 with densities more than 10-times higher than those in 1974 (Shiga
384 Prefecture, 1964-1979). This coincidence of increased sedDNA concentration and the
385 marked expansion of *E. japonicus* in the water column provides evidence that copepod
386 sedDNA concentration can be a powerful tool to estimate their population biomass over
387 several decades.

388 The temporal variations in the concentration of *E. japonicus* sedDNA were suspected
389 to operate by diagenetic degradation. Leaching from sediments had been mainly observed
390 in coarse-textured sediments that enable fluid flow across layers (Andersen *et al*., 2012;
391 Haile *et al*., 2007). Thus, leaching is generally assumed as a minor process in water-
392 saturated sediments (Giguet-Covex *et al*., 2014) like the fresh sediment investigated in
393 the current study. Since sedimentary compaction formed over an extended period, it is
394 likely that upward fluid flow into the surface layer arises permanently, inducing DNA
395 leaching. However, we did not detect *E. japonicus* eDNA in any of the pore water samples,
396 as previously shown in fish (Kuwae *et al*., 2020) and *Daphnia* (Tsugeki *et al*., 2022)
397 sedDNA analysis in sediment samples. Therefore, it is reasonable to conclude that leached
398 *E. japonicus* eDNA only negligibly influences sedDNA. Moreover, a recent experimental
399 study showed almost no reduction for sedDNA concentration after initial decline within
400 one month (Ogata *et al*., 2021) probably induced by decomposition. The concentrations

of *E. japonicus* sedDNA did not always show increasing trends toward the surface layers. Rather, it decreased toward the surface after the peak observed before 2010, except for the top surface layer. This indicates that the temporal variation in sedDNA of *E. japonicus* cannot be simply explained by time-dependent diagenetic degradation. The sedDNA concentration of *E. japonicus* denoted some correlations with the other proxies, such as pigment concentration and algal diatom remains. However, the long-term trends were different from those of *E. japonicus* sedDNA, indicating that *E. japonicus* sedDNA concentration is not regulated by phytoplankton sinking and/or their debris aggregations.

We detected that the sedDNA concentrations of *E. japonicus* correlated with the concentration of its subitaneous eggs. Moreover, we observed high concentrations of sedDNA from the non-sieved samples likely including resting eggs in two of the three samples compared to those in the sieved samples excluding eggs. However, we did not detect significant correlation with the number of resting eggs, which is likely because in Lake Biwa, subitaneous eggs, rather than the resting eggs, mainly regulate the spawning activity of *E. japonicus* (Liu *et al*., 2020a). The contribution of resting eggs to total *E. japonicus* eggs in the water column was expected to be < 0.3% (Liu *et al*., 2020a). A recent study demonstrated that eDNA were released during spawning periods (Takeuchi *et al*., 2019; Tillotson *et al*., 2018). In addition, in a report of zooplankton *Daphnia*

sedDNA, substances released during spawning activities were assumed to be a significant source of DNA archived in sediments (Tsugeki *et al*., 2022). Therefore, these findings emphasize the importance of sedDNA-sourced material being released during the main spawning period. As shown in the present study, the sedDNA concentrations of *E. japonicus* markedly increased more than 3-fold during the 2000s compared to those before the 1990s, which significantly correlated with their production and biomass. The production of *E. japonicus* greatly increased more than approximately 2-fold in the 2000s, mainly due to the decline of predation effect (Liu *et al*., 2021), which occurred in parallel with their temporal increase of specific growth rate and subitaneous eggs. These facts support that copepod sedDNA can be applied as a powerful tracking tool for reconstruction of their production and biomass related to the intensity of spawning activity.

In conclusion, we successively and qualitatively detected *E. japonicus* sedDNA over a 100-year period, demonstrating the potential utility of sedDNA in evaluating the long-term qualitative dynamics of copepod. The absence of *E. japonicus* sedDNA in the pore water samples suggested that the temporal variation of its sedDNA concentration was not interrupted by leaching reaction after sedimentation. The historical variation in sedDNA concentration was similar to its production, biomass, and subitaneous eggs found in

regular *in situ* survey samples. Therefore, the *E. japonicus* sedDNA concentration is a possible indicator of the magnitude of productive activities in the water. Overall, we confirmed that sedDNA can be used as a proxy for monitoring the production and spawning activity of copepods, which can track 100 years of sedimentary records.

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Acknowledgements

We are grateful to M. Honjo, M. Sakata, S. Goda, T. Akatsuka, and J. Kurata for their assistance with laboratory analysis and field sampling. We also thank Shiga Prefectural Fisheries Experiment Station for providing preserved zooplankton sample collection. This study was supported by Grants-in-Aid for Scientific Research (17K20045, 21K12273, 21H01170, and 18H03961) from the Japan Society for the Promotion of Science (JSPS) and partly supported by a special research grant from Matsuyama University; the Academic Research Organization Joint Usage/Research Grants from Leading Academia in Marine and Environment Pollution Research (LaMer), Ehime University; the Center for Ecological Research (2017–2021 jurc-cer), Kyoto University; the cooperative research program (17A065) of the Center for Advanced Marine Core Research, Kochi University, and the Environment Research and Technology Development Fund (JPMEERF20204004) of the Environmental Restoration and Conservation Agency, Japan; and grants from the Ministry of Agriculture, Forestry and Fisheries, Japan for a research project titled *Development of technologies for mitigation and adaptation to climate change in Agriculture, Forestry and Fisheries*.

Author contributions

N.T. and K.N. conceived the idea and designed the study; K.N., N.T., and M.K. conducted sampling and experiments; X.L., G.D., and S.B. provided data on the target species and contributed to the data analysis; H.D. performed some of the experiments and contributed to primer and probe design; K.N. and N.T. wrote the first draft of the manuscript; All authors revised and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Data Availability Statement

Supplementary information will be available after publication.

Figure legends

Figure 1. Research and experimental design of the study.

(a) Sediment geochronology; (b) Reconstruction of temporal variation in *Eodiaptomus japonicus* sedimentary DNA (sedDNA) concentrations and spike test for inhibition; (c) Checking for contamination; (d) Reconstruction of temporal variation of chlorophyll-a pigment concentration and algal remains abundance; (e) Evaluating potential sources of *E. japonicus* sedDNA between samples with and without sieve; (f) Regulating factors of *E. japonicus* sedDNA; (g) Demographic traits of biomass, specific growth rate, production, and subitaneous eggs of *E. japonicus*; (h) Sedimentary records of resting eggs. The named LB cores were collected in November 2013 (2013LB) and August 2017 (2017LB2, 2017LB5, and 2017LB7), and IM cores (namely 2019IM1) were collected in August 2019 (details are described in the section on sampling site and sediment collection of methods).

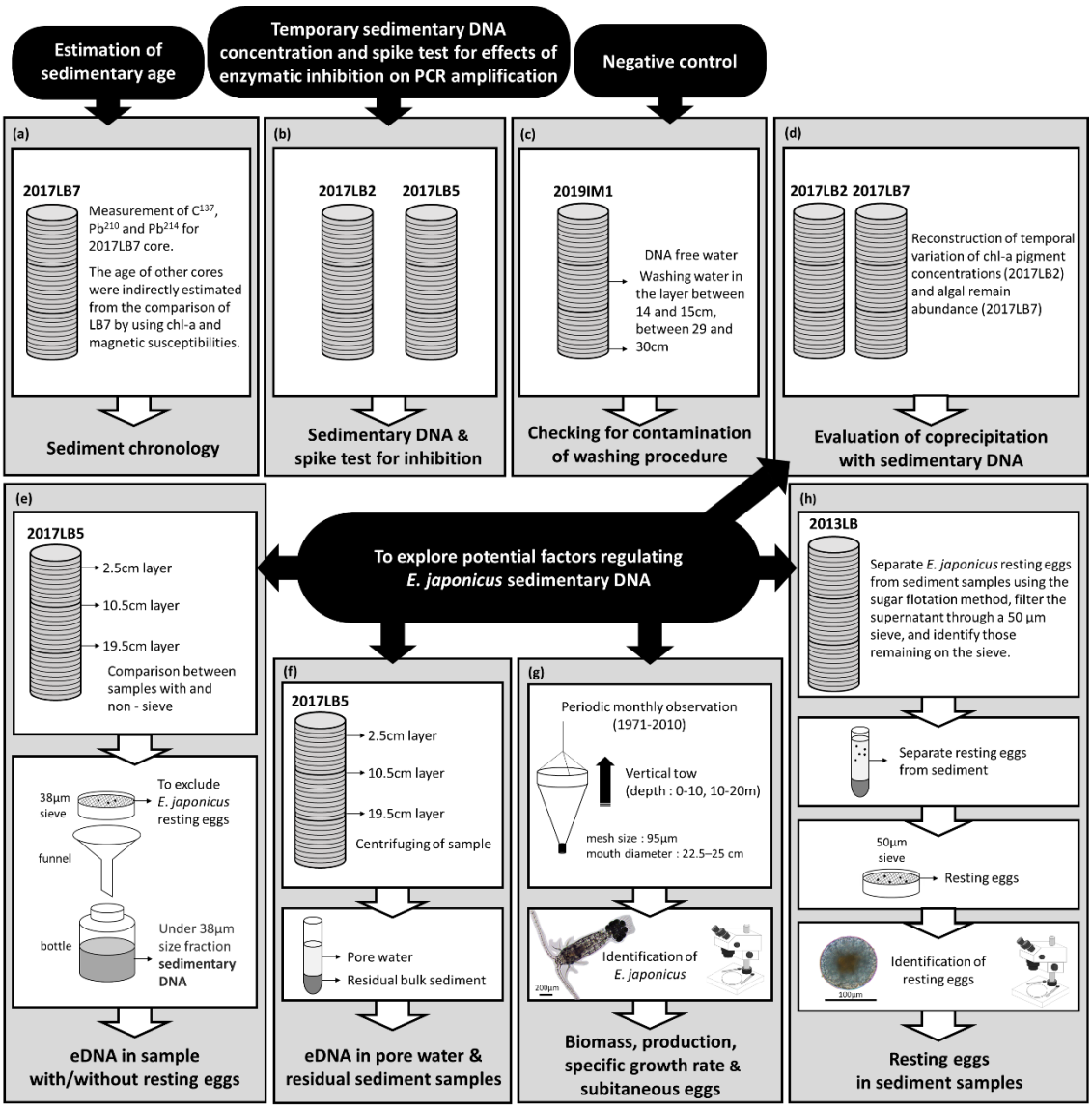

Estimation of sedimentary age
Temporary sedimentary DNA concentration and spike test for effects of enzymatic inhibition on PCR amplification
Negative control
(a)
2017LB7
Measurement of C137, Pb210 and Pb214 for 2017LB7 core.
The age of other cores were indirectly estimated from the comparison of LB7 by using chl-a and magnetic susceptibilities.
Sediment chronology
(b)
2017LB2
2017LB5
Sedimentary DNA & spike test for inhibition
(c)
2019IM1
DNA free water
Washing water in the layer between 14 and 15cm, between 29 and 30cm
Checking for contamination of washing procedure
(d)
2017LB2 2017LB7
Reconstruction of temporal variation of chl-a pigment concentrations (2017LB2) and algal remain abundance (2017LB7)
Evaluation of coprecipitation with sedimentary DNA
To explore potential factors regulating E. japonicus sedimentary DNA
(e)
2017LB5
2.5cm layer
10.5cm layer
19.5cm layer
Comparison between samples with and non - sieve
38µm sieve
To exclude E. japonicus resting eggs
funnel
bottle
Under 38µm size fraction sedimentary DNA
eDNA in sample with/without resting eggs
(f)
2017LB5
2.5cm layer
10.5cm layer
19.5cm layer
Centrifuging of sample
Pore water
Residual bulk sediment
eDNA in pore water & residual sediment samples
(g)
Periodic monthly observation (1971-2010)
Vertical tow (depth : 0-10, 10-20m)
mesh size : 95µm
mouth diameter : 22.5–25 cm
Identification of E. japonicus
200µm
Biomass, production, specific growth rate & subitaneous eggs
(h)
2013LB
Separate E. japonicus resting eggs from sediment samples using the sugar flotation method, filter the supernatant through a 50 µm sieve, and identify those remaining on the sieve.
Separate resting eggs from sediment
50µm sieve
Resting eggs
Identification of resting eggs
100µm
Resting eggs in sediment samples

Figure 2. Temporal changes in *Eodiaptomus japonicus* sedimentary DNA concentration (gene copies of COI, g^{-1} of dry sediments): (a) observed throughout the study period, and (b) limited to the period before 1992. For both panels, the open triangles and squares indicate raw data of the 2017LB2 and 2017LB5 cores, respectively. The gray triangles and squares indicate the average mean (n = 4) of 2017LB2 and 2017LB5 core samples, respectively. For the panel (a) and (b), black circles and thick solid lines indicate the mean (n = 2) of 2017LB2 and 2017LB5 core samples within the year corresponding to the sedimentation period in the chronology 2017LB7 core.

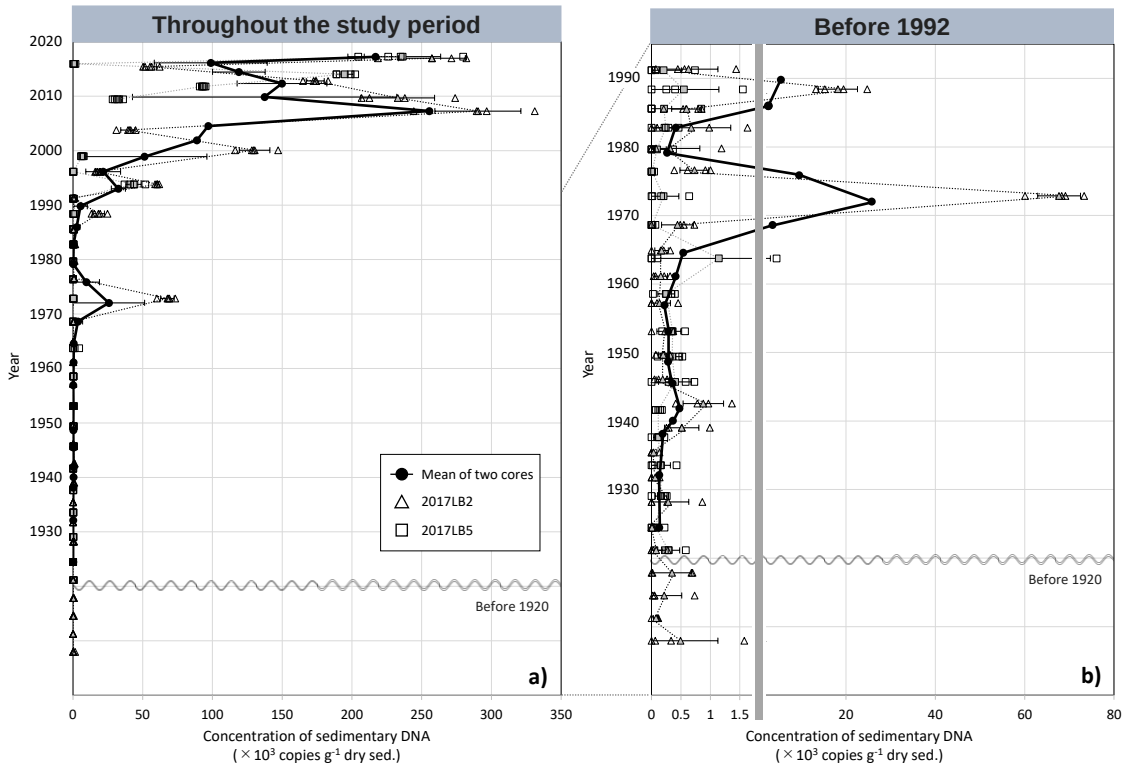
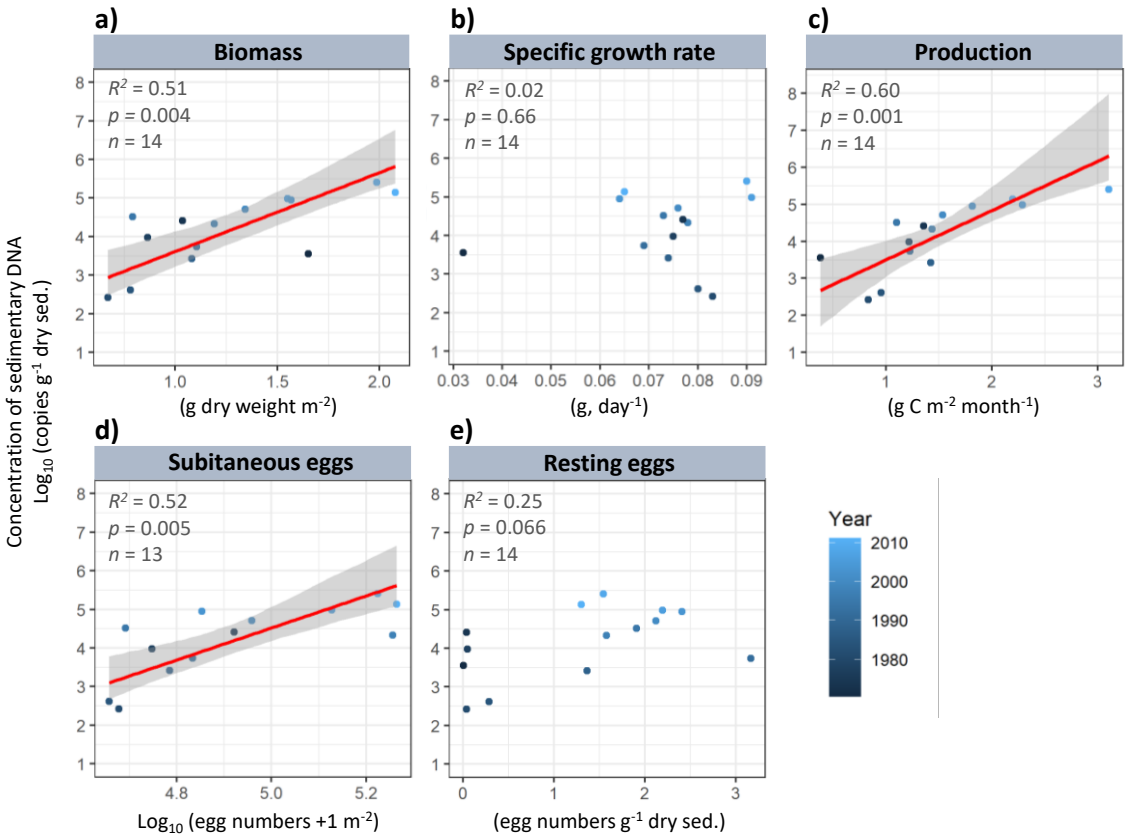


Figure 3. Relationships among log_{10}-transformed sedimentary DNA concentration of *Eodiaptomus japonicus* (gene copies of COI, g^{-1} of dry sediments) and its biomass (g dry weight m^{-2}; a), specific growth rate (day^{-1}; b), production (gC m^{-2} $month^{-1}$; c), log_{10}-transformed subitaneous eggs ($\times 10^3$ egg numbers m^{-2}; d), and resting eggs (egg numbers g^{-1} dry sed.; e). The blue line denotes a Type II regression model with a 95% confidence interval indicated by the gray zone.



Tables

Table 1. The sedimentary DNA concentration of *Eodiaptomus japonicus* (gene copies of COI, g^{-1} of dry sediments) for pore water in sediment and its residual pore water-free sediment samples.

Sample ID (estimated age in sample depth and its age error; yr)	Core depth		Pore water in sediment sample	SD	Detection / total replicates	Pore water - free sediment sample	SD	Detection / total replicates
	Top (cm)	Bottom (cm)	(mean copies g^{-1} dry)			(mean copies g^{-1} dry)		
2017LB5-3 (2014 ± 0.1)	2	3	ND	-	0/4	725,030	98,992	4/4
2017LB5-11 (1994 ± 1.3)	10	11	ND	-	0/4	2,665	608	4/4
2017LB5-20 (1964 ± 9.3)	19	20	ND	-	0/4	12	21	1/4

Table 2. The sedimentary DNA concentration of *Eodiaptomus japonicus* (gene copies of COI, g^{-1} of dry sediments) in sediment samples with and without sieve.

Sample ID (estimated age in sample depth and its age error; yr)	Core depth		Sample with sieve	SD	Detection / total replicates	Sample non - sieve	SD	Detection / total replicates
	Top (cm)	Bottom (cm)	(mean copies g^{-1} dry)			(mean copies g^{-1} dry)		
2017LB5-3 (2014 ± 0.1)	2	3	5,246	6,531	2/4	11,630	5,921	4/4
2017LB5-11 (1994 ± 1.3)	10	11	2,630	4,555	1/4	1,838	3,184	1/4
2017LB5-20 (1964 ± 9.3)	19	20	ND	-	0/4	1,449	1,977	2/4