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Habitat selection and migration of the common shrimp, *Palaemon paucidens* in Lake Biwa, Japan—An eDNA-based study

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Abstract

Palaemon paucidens has a large population and is an important food source for fish in Lake Biwa, Japan. They are abundant in shallow waters from spring to summer, after which most individuals migrate to offshore deep areas where they remain during autumn and winter. However, some individuals are nonmigratory, remaining in shallow waters over winter. It has been reported that *P. paucidens* individuals have declined in recent years; a better understanding of its seasonal distribution is needed to manage this species, and basic information on its seasonal distribution is indispensable. We tracked the environmental DNA (eDNA) distribution of *P. paucidens* in Lake Biwa over a year using a quantitative real-time polymerase chain reaction method. We collected water samples from offshore (both from the surface and from the benthic) and from shallow shore sites adjacent to the shorelines of the main lake and connecting freshwater lagoons. Offshore sampling took place in summer and winter, and shallow shore and lagoon sampling in all four seasons. During summer, eDNA concentrations were significantly higher in shallow and lagoon areas than offshore bottom sites. Conversely, during winter, eDNA concentrations were higher in offshore bottom sites, and relatively high and low eDNA concentrations in lagoons and shallow shore, respectively. These results most likely reflect the spatial and temporal distribution of this species in Lake Biwa. The eDNA concentrations peaked in early August at shallow shore sites in the main lake, with a significant decline in mid-October, while low eDNA concentrations were recorded at offshore bottom sites in late August. These results suggest that *P. paucidens* migrates from shallow waters to offshore bottom sites between early August and mid-October. These results provide important information for the management of this species.

KEYWORDS
environmental DNA (eDNA), Lake Biwa, migration, *Palaemon paucidens*, quantitative real-time PCR, spatial and temporal distribution

1 | INTRODUCTION

Lake Biwa is the largest freshwater lake in Japan, and the third oldest lake in the world (Nishino & Watanabe, 2000). Approximately 2,400 species of animals, including 66 endemics are present in this Lake (Kawanabe, Nishino, & Maehata, 2012). There are four species of shrimp (Nishino, 1978; Nishino & Niwa, 2004), one of which, *Palaemon paucidens*, accounts for most of the biomass, and is an important food source for fish (Harada, 1966; Narita, 2002). It was reported that approximately 1,400 tons per year of catch were recorded in the late 1970s, and that the catch of this species has declined significantly from 90 to 40 tons after 2007 (Shiga Prefecture, 2009).

Most *P. paucidens* has been reported to seasonally migrate from shallow waters to offshore bottom sites where they spend winter season; meanwhile, in spring, they migrate from the offshore bottom to shallow waters for reproduction (Harada, 1966; Nishino, 1983). Wu et al. (2019) found that relatively large individuals started to reproduce between May and July, with a peak detected between August and September. While it has been reported that most individuals move to bottom sites in winter (Harada, 1966; Nishino, 1983), we found that nonmigratory individuals overwinter in shallow waters (Wu et al., 2018). However, the distribution and abundance of this species has not been investigated with respect to these two different movement types.

Shrimp cages and trawl nets are conventionally used to investigate the movements of *P. paucidens*. However, these tools are unsuitable for detecting small populations, and make for time-consuming and labor-intensive monitoring. In recent years, environmental DNA (eDNA) analysis has been widely used for species detection and biodiversity monitoring (Ficetola, Miaud, Pompanon, & Taberlet, 2008; Thomsen et al., 2012). Environmental DNA represents all types of DNA found in the environment, including DNA from organisms in the form of metabolic waste, damaged tissue, or sloughed skin cells (Hambler, Henderson, & Speight, 2011; Lydolph et al., 2005). Environmental DNA analysis has been applied to macroorganisms (Ficetola et al., 2008; Jerde, Mahon, Chadderton, & Lodge, 2011; Minamoto, Yamanaka, Takahara, Honjo, & Kawabata, 2012), and to date, many eDNA analyses have reported successful monitoring of the presence/absence of rare species (Dejean et al., 2011). In combination with quantitative polymerase chain reaction (qPCR), eDNA concentrations can be used as a proxy for it abundance of crustacean species (Carim et al., 2016; Wu et al., 2018) .

The purpose of this study was to use eDNA to examine the spatial and temporal distribution of *P. paucidens* in Lake Biwa. Water samples were collected from offshore (both from the surface and the bottom), and from the shallow shore of the main lake and its connecting freshwater lagoons. Based on our results, we estimated the timing of migration, and considered whether the habitat preferences of *P. paucidens* are related to shoreline landscape types.

2 | MATERIALS AND METHODS

2.1 | Study area

Lake Biwa is located on central Honshu Island (35°01′ N, 136°00′ E, Figure 1). This lake consists of southern and northern basins. The southern basin is small (52.5 km^2) and shallow (average depth, 4 m; maximum depth, <7 m); in contrast, the northern basin is large (617.8 km^2) and deep (average depth, 43 m; maximum depth, 103.6 m) (Okamoto, 1984; Okuda & Kumagai, 1995).

2.2 | Offshore sampling

To examine the offshore distribution of *P. paucidens* water samples were collected from 41 sites (O1–O41) in the summer (19, 25–28 August 2016) and in the winter (18, 19, January and 20 February 2017) (Figure 1, Table S1). The 1-L samples were collected from the surface and 1 m above the bottom using a bucket and van Dorn sampler, respectively. The distance between each sampling site was 1 km. We added 1 ml of 10% (mass/volume) benzalkonium chloride solution to each sample to prevent DNA degradation (Yamanaka et al., 2017). In summer, some of the water samples (samples from sites O27–O32) were filtered on the survey boat when it was not raining; when rain prevented this, then benzalkonium chloride was added to the samples on return to the laboratory. In winter, the same volume of benzalkonium chloride was added to all water samples upon collection. Four sites (O18, O20, O22, and O23; Table S1) were unsampled in January 2017 because of bad weather. We used a field blank on each sampling day. The field blanks contained 1 L of ultrapure water taken from the laboratory and brought to the field site to check for unintended cross-contamination during sampling and transportation. We opened the lid of the blanks in the field, added 1 ml of 10% benzalkonium chloride solution, and brought them back to the laboratory. The water samples collected from the surface and the bottom in summer were designated as Summer-Surface and Summer-Bottom samples, respectively; samples collected from the surface and bottom in winter were designated as Winter-Surface and Winter-Bottom samples, respectively.

The water samples were filtered using 47-mm glass fiber filters (GF/F; GE Healthcare Japan, Tokyo, Japan; nominal pore size = 0.7 μm; cf. Minamoto, Naka, Moji, and Maruyama (2016) for selection of filter type for eDNA sampling). After filtration, filters were stored at −20°C until DNA extraction.

2.3 | Shallow shore sampling

We collected 1 L of water samples from 26 shallow shore sites (S01–S26; Figure 2; Table S2) from spring to winter (23 May, 2 August, and 25 October 2016, and 6 February 2017). We collected water samples from four types of shallow shore sites: sandy beach (S1, S6, S7, S10, S12, S20, S23, S24, and S26), artificial lakeshore (S2, S4, S11, S14, S16, and S22), vegetation (S3, S5, S9, S13, S19, S21, and S25),

and reef (S8, S15, and S18). Benzalkonium chloride solution was added to each sample as described above. Each water sample was collected carefully from the surface water to avoid sediment resuspension. The field blanks were adopted as above, and filtration was carried out under the same conditions as described above. Four samples (S23–S26; Table S2) in May 2016 were not collected because of seasonal change in the water level.

2.4 | Freshwater lagoon sampling

The sampling and DNA extraction methods for lagoon samples slightly differed from those used for the other sample types, because lagoon samples were collected for a separate project. Wu et al. (2018) reported that these differences would not influence the results. We also collected water samples from 32 freshwater lagoon sites (L1–L32, Figure 2; Table S3) from autumn to summer on 16 November 2015, 5 February, 6 May, and 5 August 2016. Once collected, the 500-ml samples were filtered on-site as described by Yamanaka et al. (2016) and filtered samples were placed in a cooler box filled with ice packs precooled in ultralow freezers (NEOICE-16/Hard1250). The cooler box temperature was maintained at approximately −16°C and samples were stored in the laboratory at −20°C. We sampled a field blank at every 10th site, and filtered it as described above.

Samples were collected from offshore and shallow shore sites; all equipment used to collect water was cleaned with bleach solution (diluted household bleach containing 0.1% sodium hypochlorite) for >5 min to remove residual DNA. A disposable, sealed plastic bag (DP16-TN1000; Yanagi, Nagoya, Japan) was used to collect samples from freshwater lagoon sites. To prevent contamination, the equipment was cleaned with a bleach solution before reuse. The decontaminated equipment was rinsed with ultrapure water. Disposable gloves were used in all procedures to minimize the risk of contamination.

2.5 | DNA extraction

For offshore and shallow shore samples, DNA extraction from filter samples followed Uchii et al. (2016). We placed two filters in a single Salivate tube (Sarstedt, Nümbrecht, Germany), and used the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Environmental DNA of freshwater lagoon samples was extracted according to Miya et al. (2015) with a slight modification of the initial lysis step using

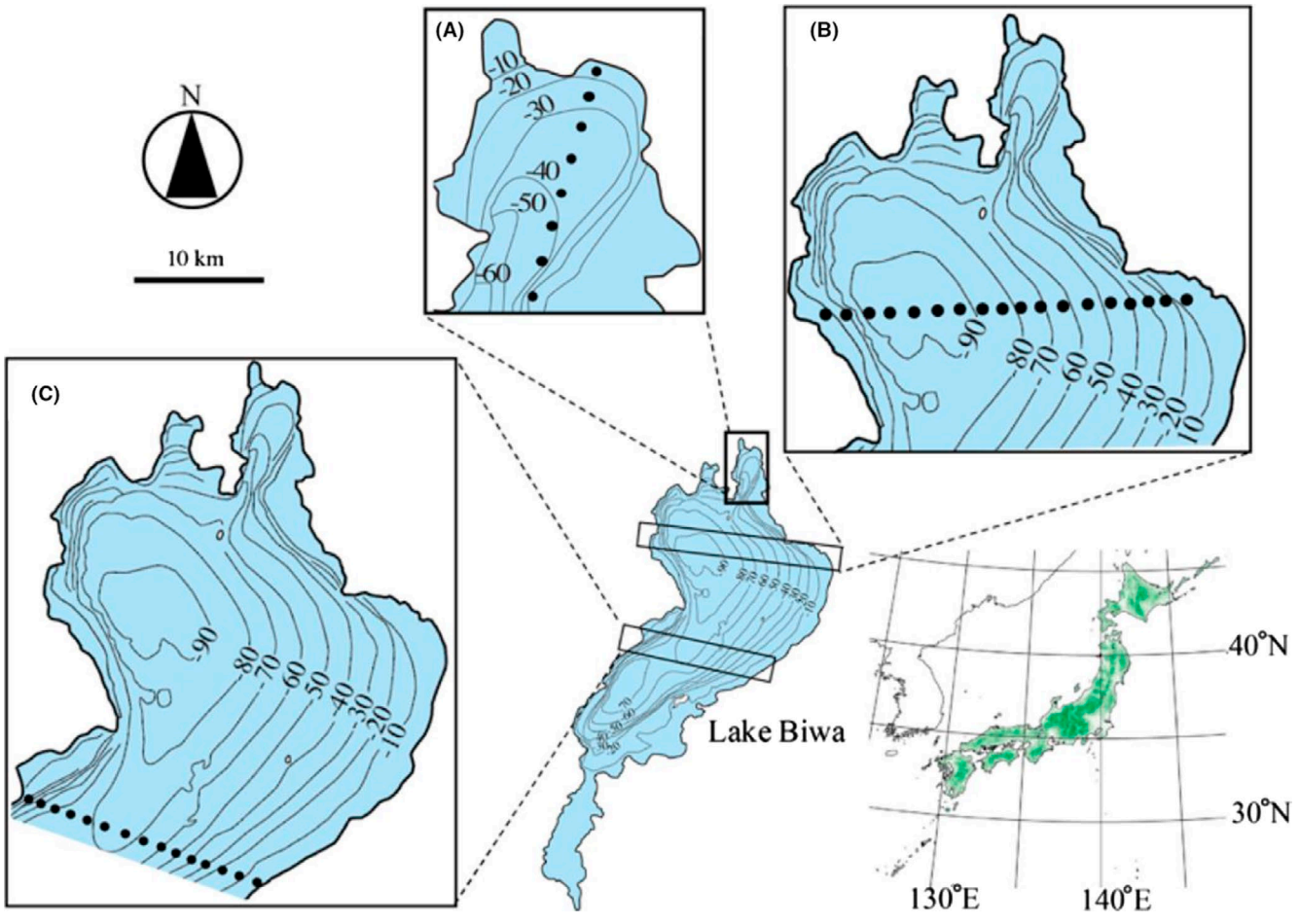

FIGURE 1 Map of the study sites for offshore samples. The water samples were collected from 8 sites from north to south in panel (A) (O1–O8), from 18 sites from west to east in panel (B) (O9–O26), and from 15 sites from west to east in panel (C) (O27–O41). The map of Lake Biwa was modified from Ikeda (2018)

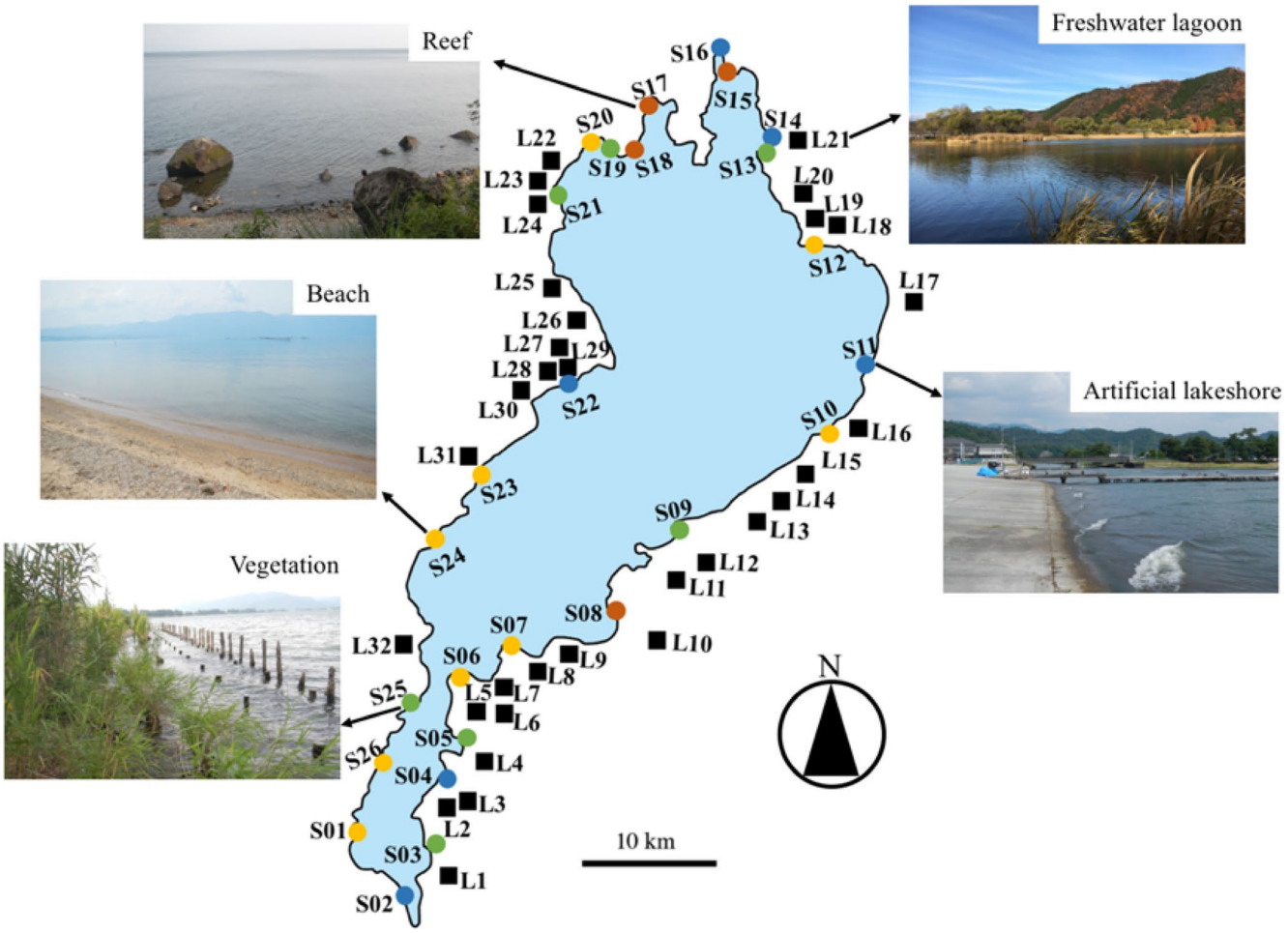

FIGURE 2 Map of the study sites for lakeshore and lagoon sites. Black boxes show the sampling sites within the freshwater lagoon. Circles show the sampling sites along the shallow shore sites. Yellow, blue, green, and brown denotes sandy beach, artificial lakeshore, vegetation, and reef, respectively

the following reagent amounts: 200 µl of ultrapure water, 100 µl of Buffer AL, and 20 µl of proteinase K. After DNA extraction, DNA was eluted from the DNeasy spin column with 100 µL of Buffer AE (Qiagen) and stored at −20°C until qPCR analysis.

2.6 | qPCR quantification of eDNA

To evaluate the amount of eDNA from *P. paucidens*, quantification of the copy number of 16S rRNA genes was performed using real-time TaqMan PCR with the StepOnePlus Real-Time PCR system (Life Technologies, Foster City, CA). Primers and a probe were established in previous study (Wu et al., 2018) that can specifically amplify a 166-bp fragment of 16S rRNA genes of *P. paucidens* (Table 1). The specificity of the qPCR assays has been tested (Wu et al., 2018). Quantitative PCRs were conducted with a 20-µl reaction volume in which there were 900 nM of each primer, 125 nM of TaqMan probe, 1 × Environmental Master Mix 2.0 (Life Technologies), 0.1 µl of AmpErase® Uracil *N*-Glycosylase (Thermo Fisher Scientific), and 5 µl of template DNA. We used a standard dilution series containing 3.0×10^1 to 3.0×10^4 copies of a linearized plasmid containing synthesized artificial DNA fragments of 16S rRNA gene sequence (228-bp) (Table 1) as quantification standards for all real-time PCR assays. The qPCR conditions were 2 min at 50°C followed by 10 min at 95°C and by 55 cycles of 15 s at 95°C and 1 min at 60°C. All PCR runs including samples, quantification standards, and negative controls were performed in triplicate. The average of the three replicates was regarded as the value of the DNA concentration. When a negative detection was obtained for any of the replicates, the DNA concentration of that replicate was assigned a value of zero (Ellison, English, Burns, & Keer, 2006).

2.7 | Statistical analysis

To evaluate the offshore seasonal distribution of *P. paucidens* eDNA concentration (O1–O41), we used a generalized linear mixed model (GLMM) with a normal distribution. In the model, we set collection stations as explanatory variables, log transformed eDNA copy numbers as response variables, and site IDs as random effects. When the effect of collection stations (Summer-Bottom, Summer-Surface, Winter-Bottom, and Winter-Surface) was significant, we performed Tukey multiple comparisons between collection stations.

TABLE 1 Primers, probe, and DNA sequences within the plasmid used in our study

Name	Sequence (5′–3′)
Palaemon paucidens forward primer	AAGTCTAACCTGCCCACTGAGTTA
Palaemon paucidens reverse primer	TTTAAGCCTTTTCACTTAAAGGTCA
Palaemon paucidens probe	FAM-ATGAGGGAAAAACTG-NFQ-MGB
DNA sequences within the plasmid	GAATTCACAT GTCTATATAG ATTCTTAATA TAAGTCTAA CCTGCCACT GAGTTATTAA AGGGCTGCGG TAATTTGACC GTGCAAAGGT AGCATAATCA GTAGTCTTTT AATTGAAGGC TTGAATGAAC GGTTGGATGA GGGAAAAACT GTCTCTCCTA TAAATTGAAA TTTGACCTTT AAGTGAAAAG GCTTAAATTA ACTAAGGGGA CGATAAGACC CTAAGCTT

To examine the distribution of *P. paucidens* eDNA (presence/absence) in the offshore bottom (O1–O41) during summer and winter, we used a GLMM with a binomial distribution. We assigned the absence of *P. paucidens* eDNA = 0 and the presence of *P. paucidens* eDNA = 1, and set these presence/absence values as response variables; depths, sampling season, and their interaction as explanatory variables, and site IDs as random effects.

To compare the relationship between water depth and eDNA concentration (abundance) of *P. paucidens* in the offshore bottom during summer and winter, we used a GLMM with a normal distribution. We used log of *P. paucidens* eDNA concentration for sites where the eDNA was detected. In the model, the log of the eDNA concentration was set as a response variable; depths, sampling season, and their interaction were set as explanatory variables, and site IDs as random effects.

In shallow shore sites (S1–S26), a GLMM with a normal distribution was used to evaluate the seasonal distribution of *P. paucidens* eDNA concentration. In this model, the log of eDNA copy numbers served as the response variables; the sampling seasons and the type of sampling location (sandy beach, artificial lakeshore, vegetation, and reef) as explanatory variables, and site IDs as random effects. When the effects of sampling season and shallow shore type were significant, we performed Tukey multiple comparisons among seasons and types.

In freshwater lagoon sites (L1–L32), a GLMM with a normal distribution was used to evaluate the seasonal distributions of *P. paucidens* eDNA concentration. In the model, we set sampling seasons as explanatory variables; the log of eDNA copy numbers as response variables, and site IDs as random effects. When the effect of sampling season was significant, we performed Tukey multiple comparisons between collection dates.

We ran GLMM using the Automatic Differentiation Model Builder (glmm ADMB) package (Bolker et al., 2012; Fournier et al., 2012). All analyses were performed using R version 3.3.2 (R Core Team, 2016).

3 | RESULTS

3.1 | Quantification of eDNA copy numbers

In all of the PCR runs, R^2 values of calibration curves were >0.985. The range of slopes was between −3.639 and −3.324, the range of intercepts was between 44.868 and 48.485, and PCR efficiencies were between 88.32% and 97.43%. Based on the calibration curve of each run and the Ct value of each sample, we calculated the copy number of the 16S rRNA gene fragment of *P. paucidens* (Tables S1–S3).

In all the experiments, no eDNA of target species were detected from negative controls, including field blanks and PCR blanks.

3.2 | Offshore distribution of eDNA

In summer, eDNA of *P. paucidens* was detected in 4 out of the 41 surface sites and 19 out of 41 bottom sites. The eDNA concentrations at positive sites varied from 4.80×10^1 to 3.58×10^2 and 3.00×10^1 to 5.75×10^2 copies/L (Table S1). In winter, 0 and 21 out of the 38 sites from the surface and bottom, respectively, were positive for *P. paucidens* eDNA, and the bottom site concentrations varied from 2.50×10^1 to 4.53×10^5 copies/L (Table S1). The *P. paucidens* eDNA concentrations differed significantly between surface and bottom sites both in summer and winter (Figure 3a, Table 2a). Water samples from the bottom in winter had the highest concentrations, whereas, in summer and winter, eDNA was hardly detected in surface samples.

The detection of *P. paucidens* eDNA in the offshore bottom samples showed that the interaction between depth and season was significant (GLMM, $p = 0.0069$; Table 2b; Figure 3b). In summer, the detection probability of eDNA was constant within the range of sampling depths (GLMM, $p = 0.3864$). Conversely, in winter, more eDNA was detected in the deep waters.

The concentrations of *P. paucidens* eDNA in the offshore bottom samples also showed a significant interaction between depth and season (GLMM, $p = 0.0036$; Table 2c; Figure 3c). In summer, eDNA concentrations were not significantly related to sampling depth (GLMM, $p = 0.065$), but eDNA concentrations increased as sampling depth increased in winter.

3.3 | Shallow shore distribution of eDNA

Shallow shore eDNA was detected in 15 of the 22 sites, in which concentrations varied from 0.80×10^1 to 1.12×10^3 copies/L in

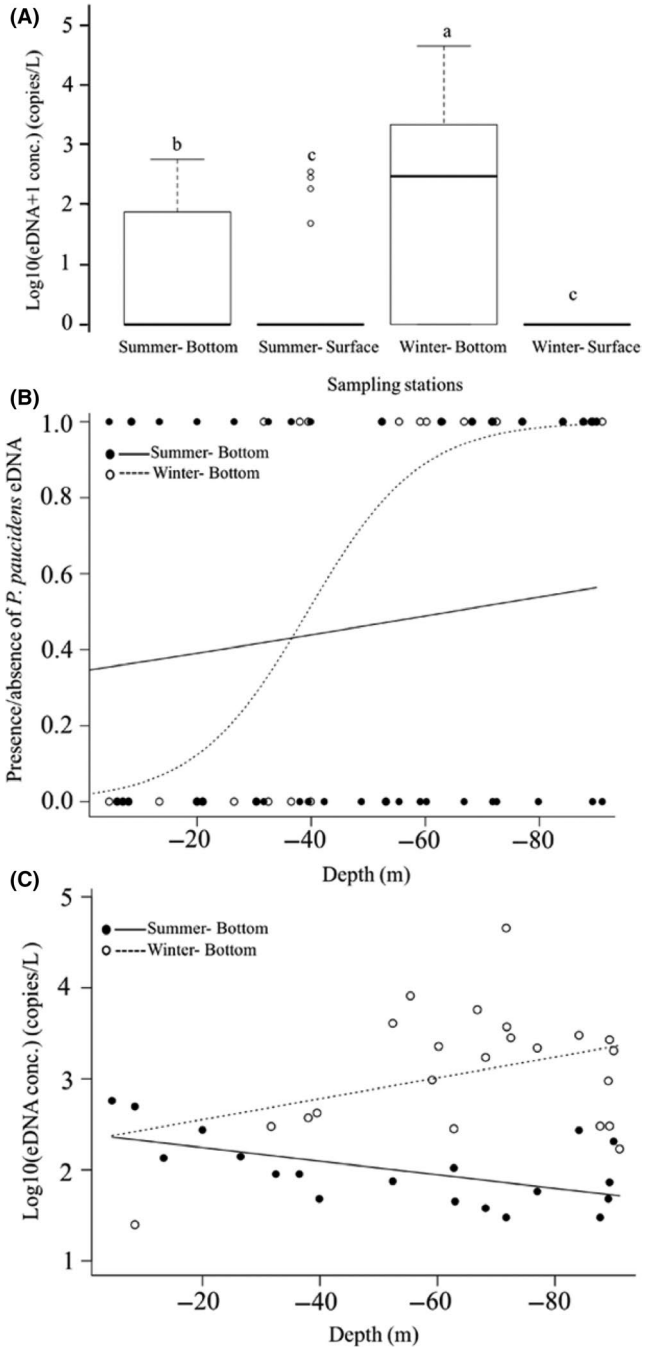

FIGURE 3 *Palaemon paucidens* eDNA concentration offshore. (A) The spatial and temporal variation of *P. paucidens* eDNA concentration offshore. Different letters indicate statistically significant differences. (B) The distribution of *P. paucidens* eDNA; offshore bottom (solid circle, solid line = summer; open circle, dashed line = winter). In this graph, we assigned the presence and absence of *P. paucidens* DNA as 1 and 0, respectively. (C) *P. paucidens* eDNA concentration relative to water depth (solid circle, solid line = summer; open circle, dashed line = winter). Only the positive results of *P. paucidens* eDNA were plotted

spring. In summer, 16 out of the 26 sites were positive, with concentrations ranging from 4.00×10^1 to 3.52×10^5 copies/L. In autumn, 3 of the 26 sites were positive, with concentrations ranging from 2.30×10^1 to 9.50×10^1 copies/L. In winter, 7 out of the 26 sites were positive, with concentrations ranging from 0.90×10^1 to 2.98×10^2 copies/L (Table S2). While eDNA concentrations differed significantly between seasons, this was not associated with the type of shallow shore site (Figure 4; Table 2d). A peak of eDNA concentration was detected in summer, intermediate values in spring, and significantly decreased values in autumn and winter, with the lowest mean concentrations recorded in autumn.

3.4 | Freshwater lagoon distribution of eDNA

In freshwater lagoons, eDNA was detected in 12 of the 32 sites, with concentrations varying from 1.92×10^2 to 2.21×10^3 copies/L in spring. In summer, 9 of the 32 sites were positive, with concentrations ranging from 2.09×10^2 to 1.72×10^5 copies/L. In autumn, 13 of the 32 sites were positive, with concentrations ranging from 1.30×10^1 to 4.56×10^3 copies/L. In winter, 7 of the 32 sites were positive, with concentrations ranging from 1.06×10^2 to 3.64×10^3 copies/L (Table S3). We detected no significant differences between seasons (Figure 5; Table 2e).

4 | DISCUSSION

An understanding of distribution and abundance is a prerequisite for conservation of *P. paucidens*. We tracked distribution of *P. paucidens* eDNA in Lake Biwa and found that it was determined by season and site. Wu et al. (2018) identified a positive correlation between eDNA concentrations and the abundance of shrimps, and we assume this correlation to hold for our research. Our results indicate that the spatial and temporal distribution of *P. paucidens* can be visualized, and its abundance inferred, by checking eDNA concentrations. These results will provide important information for the management of this species.

Previous studies highlighted that most individuals inhabit shallow waters in spring and summer, and migrate to offshore bottom sites where they spend autumn and winter (Harada, 1966; Nishino, 1983). However, some individuals have been reported to overwinter in either the shallow shore or the freshwater lagoons of Lake Biwa (Wu et al., 2018). We found that eDNA concentrations from shallow shore sites and freshwater lagoons in summer were significantly higher than those at offshore bottom sites in the same season (Figures 3a, 4 and 5). During winter, we detected a high concentration of eDNA from the bottom of the offshore (Figure 3a), which increased significantly with water depth (Figure 3c), suggesting the migration to deep offshore in winter. At the same time, relatively high, and low eDNA concentrations were detected in freshwater lagoons and shallow shore sites, respectively (Figures 4 and 5). The eDNA of *P. paucidens* was rarely observed in samples taken from offshore surface areas during summer and winter (Figure 3a). Because *P. paucidens* especially occur in the bottom of lake, sampling water near the bottom may be more accurate for detection. Furthermore, Turner, Uy, and Everhart (2015) reported that the eDNA concentration of bigheaded

TABLE 2 Summary results of generalized linear mixed models

(a) Offshore seasonal distribution of *Palaemon paucidens* eDNA concentration				
Coefficients	**Estimate**	**Std. Error**	***z* value**	**Pr (>|z|)**
(Intercept)	0.746	0.135	5.53	<0.0001***
Summer-Surface	−0.566	0.191	−2.97	0.003**
Winter-Bottom	0.851	0.196	4.35	<0.0001***
Winter-Surface	−0.746	0.196	−3.81	<0.0001***
(b) Seasonal distribution of *P. paucidens* eDNA: offshore bottom				
Coefficients	**Estimate**	**Std. Error**	***z* value**	**Pr (>|z|)**
(Intercept)	−0.646	0.66	−0.98	0.3281
Depth	0.01	0.012	0.87	0.3864
Winter	−3.247	1.474	−2.2	0.0276*
Depth: Winter	0.091	0.034	2.7	0.0069**
(c) Relationship between water depth and *P. paucidens* eDNA concentration, and between sampling seasons for offshore sites				
Coefficients	**Estimate**	**Std. Error**	***z* value**	**Pr (>|z|)**
(Intercept)	2.395	0.2468	9.71	<0.0001***
Depth	−0.007	0.004	−1.84	0.065
Winter	−0.071	0.431	−0.17	0.868
Depth: Winter	0.019	0.007	2.91	0.0036**
(d) Seasonal distribution of *P. paucidens* eDNA concentration: shallow shore sites				
Coefficients	**Estimate**	**Std. Error**	***z* value**	**Pr (>|z|)**
(Intercept)	2.095	0.615	3.41	0.0007***
Artificial lakeshore	−0.331	0.717	−0.46	0.644
Vegetation	0.466	0.69	0.68	0.499
Reef	1.671	0.827	2.02	0.053
Summer	1.895	0.615	3.08	0.002**
Autumn	−2.078	0.615	−3.38	0.0007***
Winter	−1.482	0.615	−2.41	0.016*
(e) Seasonal distribution of *P. paucidens* eDNA concentration: freshwater lagoon				
Coefficients	**Estimate**	**Std. Error**	***z* value**	**Pr (>|z|)**
(Intercept)	1.0554	0.2432	4.34	<0.0001***
Winter	−0.1731	0.2759	−0.63	0.53
Spring	−0.0389	0.2759	−0.14	0.89
Summer	−0.2393	0.2759	−0.87	0.39

Asterisks show the significant effects of each parameter (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$).

Asian carp (*Hypophthalmichthys* spp.) was higher from sediment samples than from water samples. Thus, to study the benthos, sampling sediment instead of water may also help to increase detection rates.

In this research, we found high *P. paucidens* eDNA concentrations in shallow shore areas in summer (early August), followed by a significant decrease in autumn (mid-October) (Figure 4). In addition, low eDNA concentrations were detected at the offshore bottom sites during summer (late August) (Figure 3a). Recent spatiotemporal investigations of *P. paucidens*' distribution showed that some individuals occurred in the offshore bottom areas at the end of August (Idomoto & Hatano, 2015). Thus, the changes in eDNA concentrations between summer and autumn reflected migratory behaviors of *P. paucidens*. We estimate that *P. paucidens* starts migrating from shallow shore sites to the offshore bottom areas between early August and mid-October. The low *P. paucidens* eDNA concentrations at offshore bottom sites in summer reflected the individuals which moved from the bottom earlier. This suggests that not all individuals migrate to the bottom simultaneously. A previous study reported that *P. paucidens* zoea are distributed in the thermocline (10–20 m) of Lake Biwa (Harada,

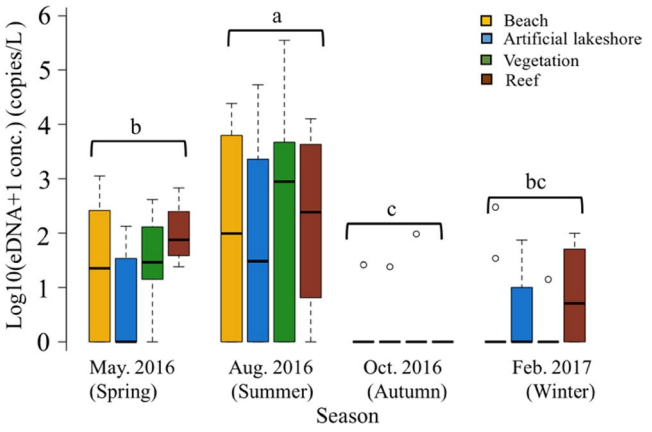

FIGURE 4 Spatial and temporal variation of *Palaemon paucidens* eDNA concentration in the shallow shore sites in Lake Biwa. Yellow, blue, green, and brown denote sandy beach, artificial lakeshore, vegetation, and reef, respectively. Different letters indicate statistically significant differences between seasons. There was no significant difference between types of shores

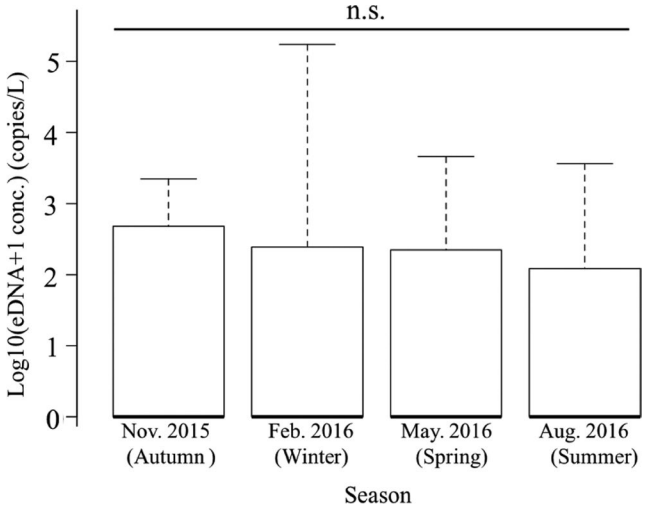

FIGURE 5 Spatial and temporal variation of *Palaemon paucidens* eDNA concentration from freshwater lagoons in Lake Biwa. There was no significant difference between collection seasons (n = 32)

1966). When the zoea die, they would sink to the bottom, and it is possible that the eDNA detected might come from these dead zoea. Jo et al. (2017) pointed out that long DNA fragments had a higher decay rate than short fragments, suggesting that long DNA fragments can remove the effects of the carcasses. Thus, in future studies, long DNA fragments can be used to verify whether or not the detected eDNA originated from dead zoea. Alternatively, it is also possible that some individuals permanently occupy the offshore bottom areas of Lake Biwa. However, no individuals have been found at the offshore bottom areas in July (Idomoto & Hatano, 2015). To clarify this point, we need more intensive (i.e., more frequent and with more sites) sampling and eDNA surveys in offshore bottom areas.

The eDNA concentrations of *P. paucidens* were not significantly different across the four landscape types in the shallow shores shore sites (Figure 4). This suggests that *P. paucidens* does not favor a specific habitat, but that individuals occupied all four shallow shore landscape types.

In addition, we found that the eDNA of *P. paucidens* was detected year-round in freshwater lagoons (Table S3), which suggests that some individuals are nonmigratory, remaining in these lagoons all year. Also, eDNA concentrations in freshwater lagoons were found to be higher than in shallow shore sites between autumn and winter, further suggesting that *P. paucidens* (nonmigratory individuals) favor lagoon habitats for overwintering. With the exception of one site (see Table S2), the eDNA of *P. paucidens* was widely detected in the shallow shore sites and freshwater lagoons of the north basin during winter. Environmental DNA of *P. paucidens* in the samples from freshwater lagoons were mainly detected from the north basin of Lake Biwa. Because the water flow and wind in freshwater lagoons are slow, and the nutrient content is higher (Hamabata, 1999), thereby making the freshwater lagoon a viable habitat for nonmigratory individuals. In a future research, we will focus on the areas in which the eDNA of *P. paucidens* was detected during autumn and winter, and applying DISTLM multivariate regression will help to identify which environmental factors (e.g., water flow, wind, nutrient content) influence habitat choice.

Organism detection using eDNA is a powerful new tool for conservation and management. However, the probability of detecting eDNA is likely influenced by several processes, including production, degradation, adsorption, and transport (Barnes & Turner, 2015), as well as potential life history factors such as death or reproduction (Bylemans et al., 2017; Kamoroff & Goldberg, 2018). In addition, regular monitoring over an extended period of time is necessary to improve the reliability of the results; however, only a few studies have adopted this approach. De Souza, Godwin, Renshaw, and Larson (2016) found that the detection probability was influenced by the seasonal activity of organisms across warm and cool seasons based on a 2-year-long investigation. Bista et al. (2017) examined temporal shifts in the biodiversity of Chironomidae at regular and frequent intervals over a 1-year period. Our study partly clarified the migration ecology of this species despite being a single-year study. With continuous monitoring, we expect to gain a more detailed understanding of this species through longer term surveys and consideration of various environmental factors or life history factors.

Previous studies suggested that *P. paucidens* inhabits the offshore bottom in winter and the plant area (vegetation) for reproduction in spring and summer (Harada, 1966; Nishino, 1983). However, eDNA was detected from not only the offshore bottom, but also from lagoons and shallow shore in winter. In addition, the eDNA was detected regardless of the habitat type of shallow shore sites. These findings update information of the habitat of this species. By further expanding the protection range and analyzing the reasons for the reduction in the number of individuals, future studies will contribute valuable information to guide the conservation and sustainable use of this economically important species.

5 | CONCLUSION

Overall, in this study, we used eDNA analysis to monitor the spatial and temporal distribution of *P. paucidens* in Lake Biwa. In addition, comparing changes in eDNA concentration at different sites allowed us to speculate on the timing of local migrations, and habitat selection. We believe that eDNA analysis is an effective tool which improves upon conventional survey methods. This technology not only allows us to evaluate how organisms are distributed, it can also provide more detailed ecological information; for example, timing of local movements, as in this study. This technology also has wide application across a range of species.

DATA ARCHIVING STATEMENT

Palaemon paucidens 16S rRNA sequence was submitted to GenBank (accession numbers: KM249043-KM249065).

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AUTHOR CONTRIBUTIONS

Q.W. and T.M. conceived and designed the research. Q.W., K.K., T.I., M.K.S., R.N., S.T., H.Y., and T.M. collected the samples. Q.W. and M.K.H. performed the experiments and statistical analysis of the data. Q.W., T.M., S.T., M.K.S., R.N., and H.Y. wrote and edited the manuscript. All authors discussed the results and contributed to development of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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