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The life history with seasonal migration of the lacustrine shrimp *Palaemon paucidens* in an ancient lake in Japan

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Abstract. The lacustrine shrimp *Palaemon paucidens* undertakes seasonal migration between shallow waters in spring and summer to deep waters in autumn and winter in Lake Biwa in Japan. Previous studies hypothesized that the migration to deep waters was for hibernation. The findings of this study oppose this hypothesis. We showed that *P. paucidens* is physiologically active in deep waters during winter; that the carbon stable isotope ratio indicated that the species forages in winter and that there was a difference in food sources between summer and winter; that lipid content was highest in January; and that gene expression activity (as measured by the RNA/DNA ratio) remained constant throughout the year. Thus, *P. paucidens* individuals that migrate to the bottom are likely to forage in winter, but do not hibernate. Nutrients gained in winter were not reflected in individual growth but may have been allocated to gonadal growth and the production of gametes, suggesting that winter foraging by this species in deep sites contributes to obtain resources for reproductive investment. In addition, we found that both small individuals with no reproductive experience and relatively large females with reproductive experience overwintered, indicating that *P. paucidens* has a life-cycle that is longer than 1 yr in Lake Biwa.

Key words: body size; Lake Biwa; lipid content; reproductive condition; RNA/DNA ratio; seasonal migration; stable isotope ratio.

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INTRODUCTION

Migration is defined as synchronized movements of individuals between distinct habitats that occurs at specific life stages (Lucas and Baras 2002), and is common in several animals (Dingle and Drake 2007): mammals (Avgar et al. 2014), birds (Berthold et al. 2003), amphibians (Sinsch 2014), and fish (Secor 2015). Migration studies have described migration trajectories and examined environmental factors that may serve as proximate cues for migratory behavior. Additionally, research efforts have assessed the ultimate causal factors behind migration, that is, those involved in the evolution of different migration strategies (Dodson 1997). Migratory strategies are diverse in nature and are categorized by their purpose and function, that is, (1) migration to a breeding site, (2) seasonal refuge from predators or adverse environmental conditions, and (3) migration to a feeding site (Northcote 1978). These functions are not mutually exclusive and can co-occur in one species, as is seen in many bird species that seasonally migrate between overwintering and breeding sites (King et al. 1965). From an evolutionary point of view, distinguishing these functions as causes or consequences will improve our understanding of the origin of migration. For example, food availability or predation risk may be characterized as driving forces of migration (Brönmark et al. 2008), whereas decreased competition or changes in trophic dynamics may be classified as a consequence of migration (Brodersen et al. 2008, 2011). Therefore, it is important to determine life history traits that are associated with migration to understand the evolution of migratory behavior.

The lacustrine shrimp *Palaemon paucidens* (Crustacea, Decapoda, Palaemonidae) is migratory in its life history. This species has the widest distribution among freshwater shrimps in the Far East Asia (Holthuis 1950, Kim 1977), and is found in southeastern Siberia, Sakhalin Island, the Japanese Archipelago, Korean Peninsula, and mainland China (Kim 1976). In Japan, the distribution of this shrimp ranges widely from Amami Island to the northern limit of Hokkaido Island (Rathbun 1902, Kubo 1942, Shokita 1975, Suzuki et al. 2014). This species occurs in a variety of freshwater habitats, from the mouth to the upper course of rivers, and from small ponds to large, deep lakes (Rathbun 1902, Kubo 1942, Shokita 1975). Based on their migration habits, populations of *P. paucidens* are generally classified as either diadromous or landlocked, which are reported to be genetically different (Chow et al. 1988).

The landlocked population of *P. paucidens* in Lake Biwa in Japan exhibits seasonal migration within the lake (Harada 1966, Nishino 1983, Idomoto and Hatano 2015). They are abundant in shallow lakeshore habitats from spring to summer, but are found in deep parts of the lake (about 90 m in depth) from autumn to winter (Harada 1966, Nishino 1983, Idomoto and Hatano 2015). Recent spatiotemporal investigation on the distribution of *P. paucidens* showed that some individuals began migrating from the end of August and that most individuals were distributed throughout the deeper layers in winter (Fig. 1, modified from Idomoto and Hatano 2015). Recent environmental DNA analysis revealed that some individuals may overwinter in shallow waters of freshwater lagoons, which connect to the main lake (Wu et al. 2018).

The ultimate cause and purpose of the migration of *P. paucidens* within Lake Biwa have not been elucidated. Harada (1966) hypothesized that migration to deep sites in winter was avoiding predation, because lakeshore aquatic

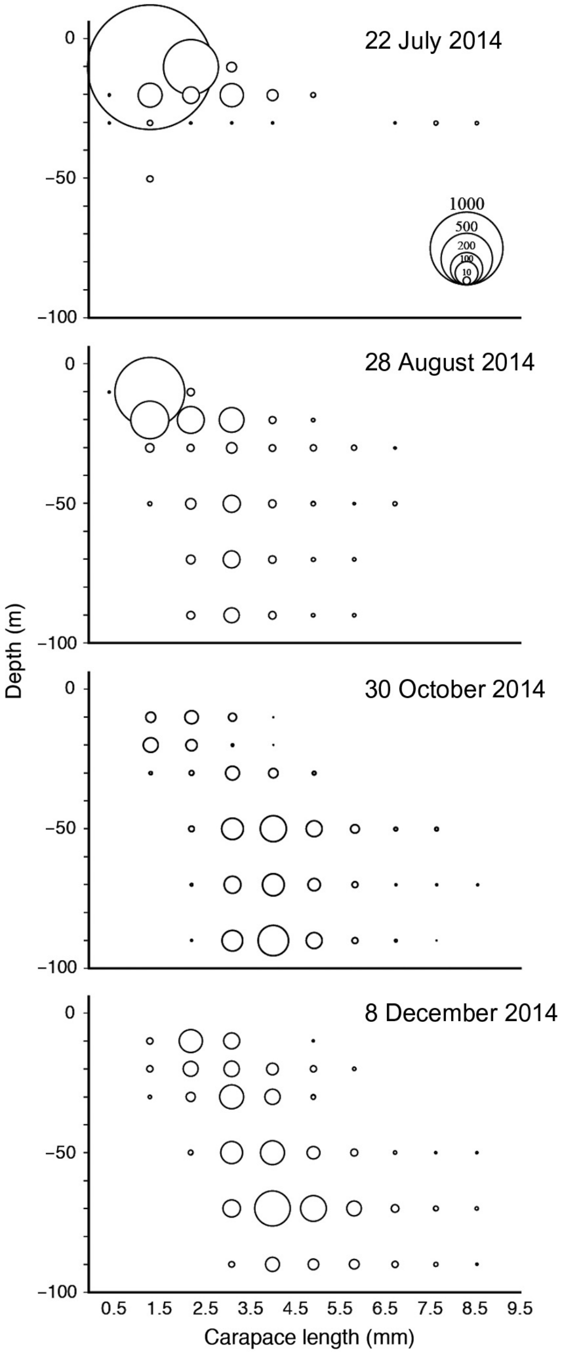

Fig. 1. The spatiotemporal distribution of *Palaemon paucidens* in the north basin of Lake Biwa, indicating seasonal migration between shallow and deep sites. A trawl net (aperture size, 50 × 25 cm) was dragged for 70 m in each site (Idomoto and Hatano 2015). Circle size indicates the number of captured individuals. Plots were modified from Idomoto and Hatano (2015).

plants wither in autumn and shrimps, therefore, lose their refuges from predators (i.e., the predation avoidance hypothesis). However, it is unclear whether migration to a depth of 90 m is necessary to reduce the risk of predation. It has also been hypothesized that *P. paucidens* migrates to deep sites for hibernation without feeding (i.e., the hibernation hypothesis; Harada 1966), because such deep sites are believed to lack food for this species in winter. Although migration may be accompanied by energy loss and predation risk, these costs may be outweighed by benefits from other processes, such as foraging at deep sites. Based on studies of fish migration, Brönmark et al. (2008) suggested that seasonal migration may be adaptive in response to seasonal changes in predation mortality and growth rate, wherein costs due to predation are minimized and benefits from foraging are maximized. However, little is known about the life history of *P. paucidens* in relation to its seasonal migration within Lake Biwa. We hypothesized that *P. paucidens* forages at deeper sites in winter (i.e., the winter foraging hypothesis). We wish to highlight that the hibernation hypothesis and winter foraging hypothesis are mutually exclusive, but that they are not mutually exclusive to the predation avoidance hypothesis.

The aim of the present study was to elucidate the life history traits of *P. paucidens* in relation to its seasonal migration within Lake Biwa, and to test the hibernation and winter foraging hypotheses. The former hypothesis predicts that the species hibernates in the deep parts of the lake in winter, ingests little nutrition, and has low physiological activity levels, while the latter hypothesis predicts that the species migrates to the deeper sites of the lake to forage from a specific nutritional source, and that it exhibits a certain level of physiological activity during this time. To test these hypotheses, the seasonal variation in feeding ecology, individual growth, and reproductive conditions were investigated. We analyzed the spatiotemporal patterns of body size, reproductive condition, lipid content, RNA/DNA ratio, and carbon and nitrogen isotope ratios. Furthermore, we discuss the life history of the species and the importance of feeding in migratory and overwintering individuals.

METHODS

Study area

Lake Biwa (35°01′N, 136°00′E, 669.23 km^2, Fig. 2) is the largest freshwater lake in Japan, and the third oldest lake in the world. This lake harbors over 2400 species of animals, including 66 endemics (Kawanabe et al. 2012). The lake consists of two parts: the small, shallow south basin with an average depth of 3.5 m (Okuda and Kumagai 1995), and the north basin which is the major part of the lake with a maximum depth of 104 m (Okamoto 1984). The water temperature of the surface layer varies seasonally from 7 to 30°C, but hypolimnetic water temperature is stable at 6–7°C (Okamoto 1992). Sampling was conducted in Shiotsu Bay, which is situated in the northernmost part of the north basin.

Sample collection

In the evaluation of seasonal migration and associated changes in body size and reproductive conditions, comparison between samples from different sites at the same time may be useful. To obtain spatiotemporal series of samples, a total of 11 collection trials were performed from shallow and deep sites in warm and cold seasons: 14 November (>-7 and -40 m in depth) in 2014, and 15 January (>-7 and -60 m), 10 April (>-7 and -60 m), 28 May (>-7 and -15 m), 9 July (-7 m), 4 August (-7 m), and 16 September (-7 m) in 2015 (Appendix S1: Table S1). Collections were coordinated with the activity of a fishery, which cooperated in this study (Asahi Fishermen's Cooperative of Lake Biwa). A previous study reported that no or very few individuals were found at the offshore bottom in summer (Fig. 1); as such, we did not collect samples from deep sites in July, August, and September 2015. Trawl nets and basket traps were used to catch shrimps from deep (<-40 m) and shallow (>-15 m) sites, respectively. Sampling effort was similar across collection dates for each method (i.e., trawl net or basket trap), but sampling effort could not be controlled between the methods. Individuals with a carapace length <0.5 mm were excluded from our analysis, because these methods were unable to capture individuals smaller than 0.5 mm.

Shrimps were virtually absent in shallow sites in winter (Appendix S1: Table S1), suggesting

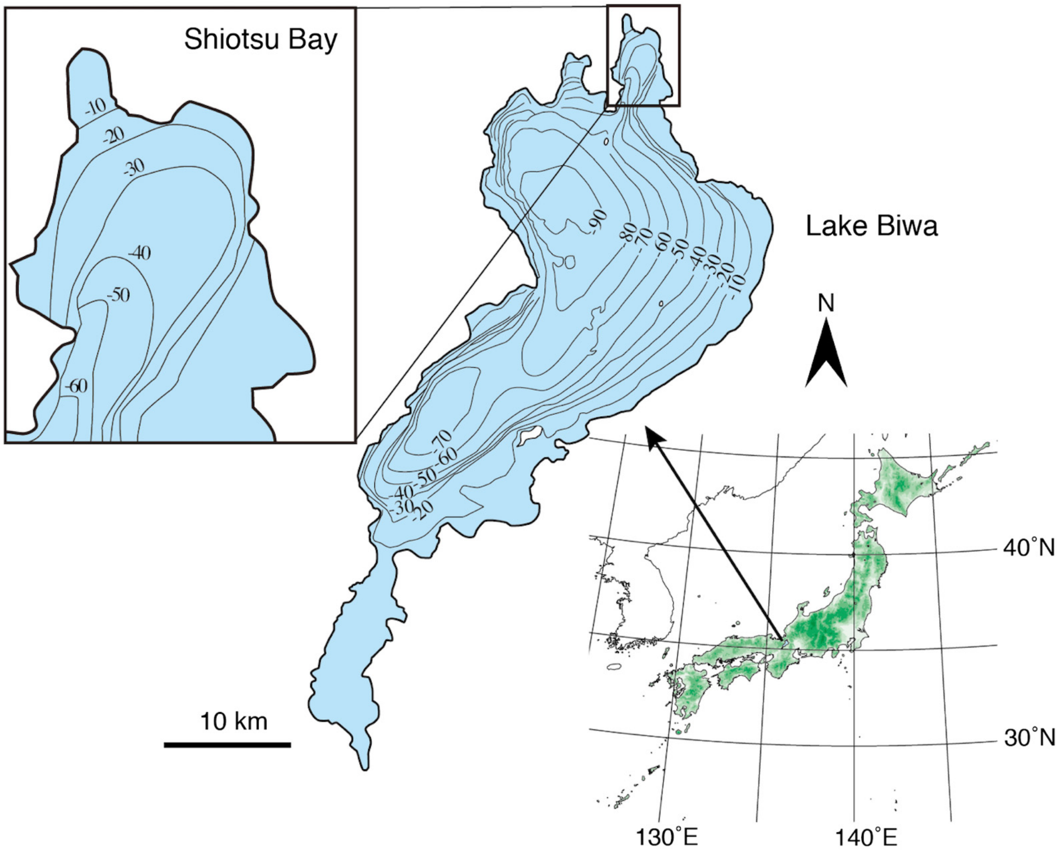

Fig. 2. Map of the study site, Shiotsu Bay, located in the north basin of Lake Biwa, Japan. The map of Lake Biwa was modified from Ikeda (2018).

that they migrate from shallow sites to elsewhere (probably deep sites) in winter. Consequently, we obtained nine samples from nine collection dates from 14 November 2014 to 16 September 2015 without spatial duplication on a collection date. The samples were immediately preserved with ice and transferred to the laboratory in Shiga University. Samples were stored in a freezer (at −20°C) until further analysis.

Body size and reproductive condition

To investigate the sex ratio and sexual difference in body size, 30 individuals were arbitrarily selected from each collection and sexed based on the appendix masculine on the second pleopod of endopod (Bruce 1989). After thawing, the carapace was removed from the cephalothorax and the carapace length was measured to the nearest 0.1 mm using vernier calipers, that is, from the base of the eyestalk to the posterior margin.

To investigate the growth of *P. paucidens*, we measured the body size of the other set of samples. One hundred and fifty individuals were arbitrary selected from every collection, resulting in a total of 1050 individuals throughout the study period. The gender of each individual was not distinguished because of large sample sizes. Instead, we counted individuals that were carrying eggs and those with abdominal exoskeletons that remained deformed after spawning (Fig. 3; hereafter referred to as post-spawning female) to investigate aspects of the species' reproductive ecology. It is unclear whether the distinguishable post-spawning exoskeleton condition is maintained after molting or whether it is limited to a period before the next molt. Little is known about the molting schedule of *P. paucidens*.

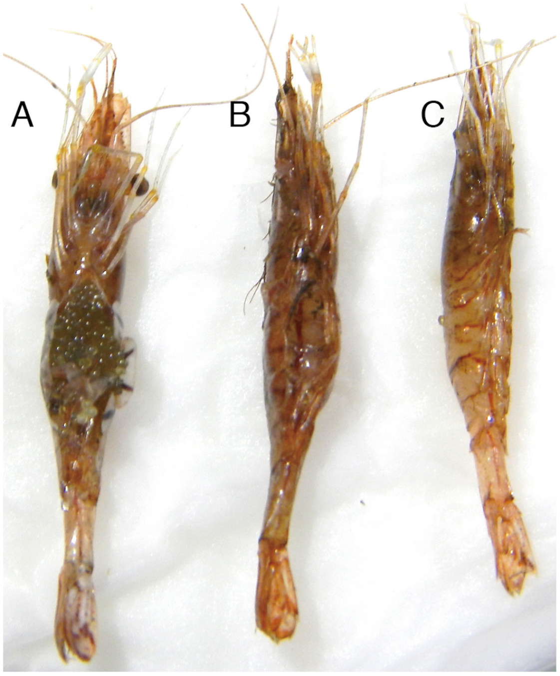

Fig. 3. Variation in the reproductive condition of *Palaemon paucidens*. (A) Female carrying eggs; (B) female with post-spawning exoskeleton condition (i.e., remained deformed); (C) non-reproductive female or male.

Lipid content

Ingested nutritional substances are partly consumed and partly stored (e.g., lipids) for metabolism or growth (Boivin and Power 1990). Individuals that ingest more nutritional substances are able to accumulate a higher amount of lipids in the body. On this basis, we measured the total lipid content in *P. paucidens* samples as a measure of the nutritional condition.

After thawing, individuals were weighed using a top loading electronic balance (Chyo, JS-110) to the nearest 0.0001 g. Specimens weighing ~5.5 g were analyzed: The total lipid content was extracted using methods described by Bligh and Dyer (1959), and the lipid content was measured using a conventional drying method according to Hasegawa (1993). We used the whole body of shrimps in this analysis, but the eggs were removed if the females carried eggs. Arbitrarily chosen nine individuals were measured from each sample collection.

To explore the dependency of lipid content on reproductive condition, we compared the lipid content in three types of shrimps, that were female carrying eggs (excluding eggs), post-spawning females, and others (male or female before reproduction). We used samples from July, August, and September in 2015, because a relatively large number of females carrying eggs were obtained in these months. In addition, we also measured the lipid content in the eggs removed from the females.

RNA/DNA ratio

The RNA/DNA ratio is an index of the gene expression activity of organisms and can be used as another measure of nutritional condition. The rationale for using the RNA concentration as a proxy for nutritional condition and growth rate has been well developed (Buckley 1984, Ferron and Leggett 1994, Westerman and Holt 1994, Bergeron 1997, Buckley et al. 2000). It is expected that well-fed and fast-growing individuals exhibit higher RNA/DNA ratios than those that are starved (Hovenkamp 1990, Hovenkamp and Witte 1991, Wright and Martin 1995).

After measuring the carapace length and removing external skeletons and intestines, the muscles of each sample individual were separated and stored in two experimental tubes for the extraction of RNA and DNA, following the methods described by Buckley (1979). Nine individuals were analyzed from each sample collection. RNA and DNA concentrations were calculated from their absorbance at 260 nm (Buckley 1979). Samples from the 16 September 2015 collection were not analyzed because of a limited opportunity to conduct analyses.

Stable isotope ratio

To estimate the food source and trophic level of *P. paucidens* in Lake Biwa, the carbon and nitrogen stable isotope ratios (δ^{13}C and δ^{15}N) were analyzed, respectively (DeNiro and Epstein 1978, 1981, Minagawa and Wada 1984).

After measuring carapace length, the muscle tissues of specimens (nine individuals per sample collection) were dissected out and dried at 60°C for 24 h. The samples were pulverized and immersed in 2:1 chloroform–methanol solution for 24 h to remove the lipids (Bligh and Dyer 1959). Carbon and nitrogen stable isotope ratios were measured with a mass spectrometer (EA Conflo IV and Delta V Plus; Thermo Fisher Scientific, Waltham, Massachusetts, USA) at the Center for Ecological Research of Kyoto University. Isotopic notations of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were expressed as per mil deviation from the standards (atmospheric N_2 gas for nitrogen and PeeDee belemnite carbonate for carbon) as defined by the following equation:

$$\delta^{13}\mathrm{C}\ \mathrm{or}\ \delta^{15}\mathrm{N} = \frac{(\mathrm{Rsample - Rstandard})}{\mathrm{Rstandard}} \times 1000(‰),$$

where R is $\delta^{15}N/\delta^{14}N$ or $\delta^{13}C/\delta^{12}C$. The analytical errors of $\delta^{13}C$ and $\delta^{15}N$ values were $\pm 0.1‰$. Samples from the 16 September 2015 collection were not analyzed because of a limited opportunity to conduct analyses.

To estimate potential food sources, we used data from Yamada et al. (1998) and Narita (2002), that is, the stable isotopic ratios of organisms (the amphipod *Jesogammarus annandalei* and oligochaetes) and organic matters (detritus and particulate organic matters [POMs]) from the shallow and deep sections of Lake Biwa, respectively. A general food chain study by DeNiro and Epstein (1978) reported that animal bulk $\delta^{13}C$ values are similar to those in their diet (<1‰ difference between the animal and its diet). Nitrogen isotopes increase by ~3.4‰ at each trophic level and can be used to determine trophic position (Minagawa and Wada 1984, Vander Zanden and Rasmussen 2001, Post 2002). We used these criteria to estimate the potential food sources of *P. paucidens* in Lake Biwa.

Statistical analysis

Seasonal variations in body size and lipid content were examined using a one-way analysis of variance, with the collection date as the explanatory variable. Sexual difference in body size was examined using a general linear model with a normal distribution, with the collection date, sex, and their interaction as the explanatory variables. Seasonal variation in the sex ratio (the proportion of females to total individuals) and the proportion of reproductive females to total individuals were examined using generalized linear models with a binomial distribution, in which the sex ratio or the proportion of reproductive females was used as the response variable, and collection date as the explanatory variable. The ratios of RNA/DNA, and carbon and nitrogen stable isotopes were analyzed by general linear models with a normal distribution, with the collection date, body size, and their interaction as the explanatory variables. Non-significant interactions were excluded from the final models. When the effect of collection date was significant, we performed the Tukey multiple comparison tests between collection dates. All analyses were performed using R version 3.3.2 (R Development Core Team 2016).

RESULTS

Body size and reproductive condition

Although the body size significantly varied among collection dates and the ranges of body size variation overlapped between the sexes, the females were significantly larger than males consistently across collections (GLM, sex, $F_{1,\ 208}$ = 50.27, $P < 0.0001$; collection date, $F_{6,\ 208}$ = 53.19, $P < 0.0001$), based on each of 30 individuals from seven collection dates (Appendix S1: Fig. S1). The sex ratio did not differ among collections in deep sites in winter (Fig. 4). In contrast, the sex ratio differed significantly among collections in summer: The proportion of the female was significantly lower in July (−7 m) and higher in September (−7 m), indicating a gradual increase in the proportion of females in shallow sites in summer (Fig. 4).

Based on each of 150 individuals from seven collections, the carapace length varied between 0.5 mm (the lower limit of capture size) and 11 mm throughout the study period from November 2014 to April 2015 (Fig. 4). Carapace length differed significantly between collection dates (ANOVA, $F_{6,\ 1043}$ = 41.17, $P < 0.0001$). Mean carapace length increased significantly from November 2014 to January 2015 and decreased significantly in April 2015 in the deep site. In the shallow site, the mean carapace length was the smallest in collections from May 2015 and differed significantly from samples from other collection dates. The mean carapace length was seen to gradually and significantly increase until September 2015.

The proportion of reproductive females differed significantly (9–42%) between collection dates (GLM, χ^2_6 = 43.5, $P < 0.0001$; Fig. 4), and were significantly higher in January 2015, July 2015, and August 2015 than in April 2015 and

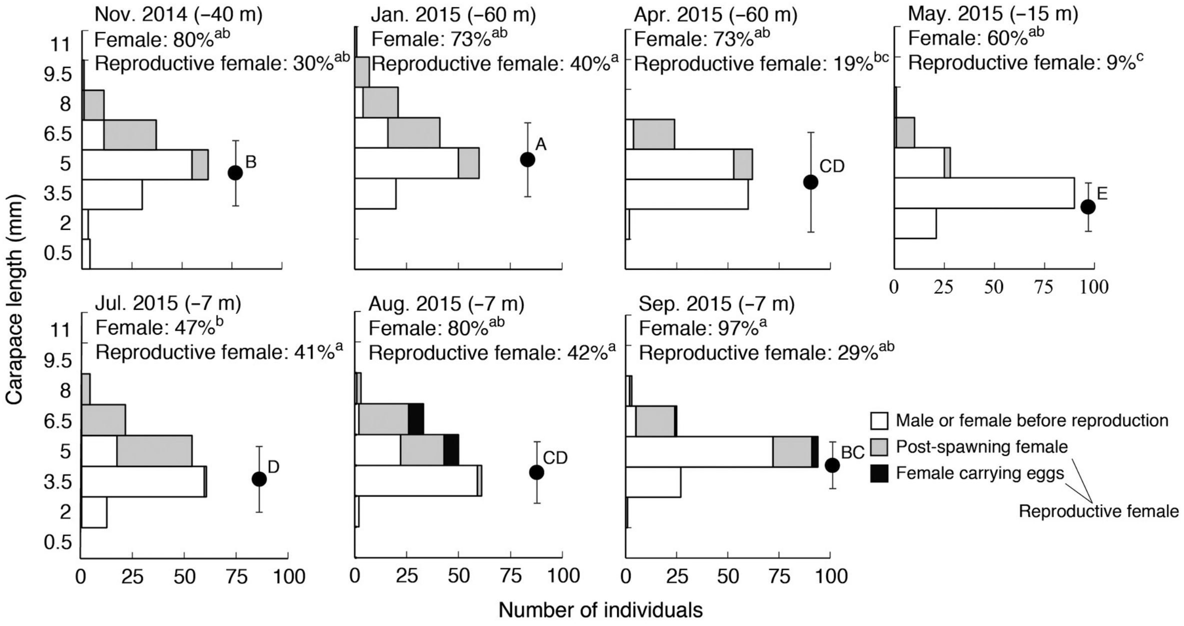

Fig. 4. The seasonal change in the carapace length distribution, sex ratio, and reproductive condition of *Palaemon paucidens* in Lake Biwa. Percentages indicate sex ratio (the proportion of females) and the proportion of reproductive females. Dots and bars indicate the means and standard deviations of carapace length. Lowercase letters indicate statistically significant differences in the sex ratio and the proportion of reproductive females. Uppercase letter indicates statistically significant differences in the mean carapace length.

May 2015. Individuals carrying eggs were found in shallow sites in August and September, and females with post-spawning exoskeleton conditions were found in both summer and winter.

Lipid content

Lipid content showed obvious seasonal variation (Fig. 5A) and differed significantly between collection dates (ANOVA, $F_{6,14} = 12.19$, $P < 0.001$). Lipid content peaked in January in the deep site and in May in the shallow site, with a significant decline in April in the deep site, indicating active feeding in winter as well as in the warm season.

The lipid content in post-spawning females was significantly higher than that in females carrying eggs and in other individuals (GLM, $F_{2,22} = 30.62$, $P < 0.0001$; Fig. 5B). Lipid content in eggs was higher than that in shrimp bodies.

RNA/DNA ratio

The RNA/DNA ratio differed significantly among collection dates and increased with increasing body size (GLM, collection date, $F_{6,47} = 20.73$, $P < 0.0001$; body size, $F_{2,47} = 95.17$, $P < 0.0001$) with a weak but significant interaction between collection date and body size ($F_{5,47} = 2.71$, $P = 0.031$), but post hoc multiple comparisons did not detect significant differences between collection dates ($P > 0.05$). Variation in body size can involve sexual difference, because the male was significantly smaller than the female as shown above. Thus, the RNA/DNA ratio was relatively stable throughout the seasons after controlling the variation in body size and sex together.

For descriptive purposes, individuals were divided into three size categories: large (>6.0 mm), medium (3.0–6.0 mm), and small (<3.0 mm; Fig. 6). The RNA/DNA ratio tended to increase from April to August, especially in smaller individuals, suggesting a relatively high activity of gene expression in the young and growing stages and/or male individuals.

Stable isotope ratio

Variation in the carbon stable isotope ratio (δ^{13}C) involved significant interaction between

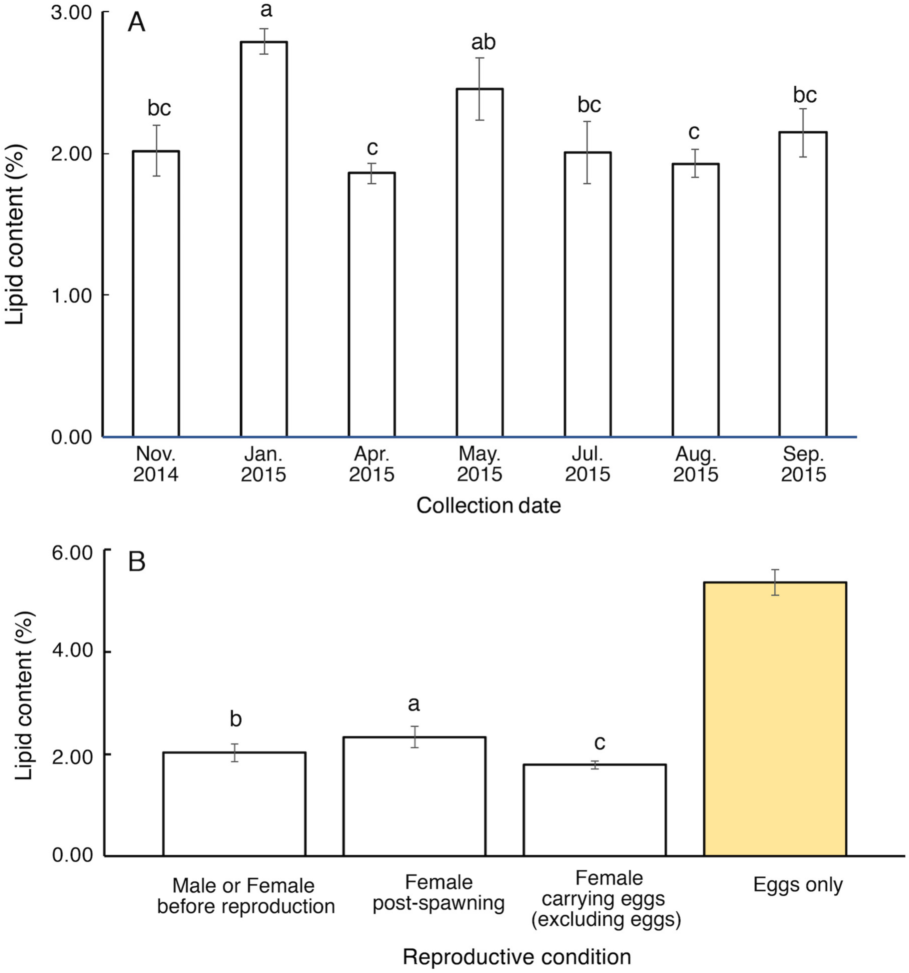

Fig. 5. The lipid content of *Palaemon paucidens*. (A) Temporal variation in the lipid content of *P. paucidens* ($n = 3$ for each sample collection). (B) Different reproductive conditions of *P. paucidens* ($n = 9$ for each reproductive condition; $n = 3$ for eggs). Means and standard deviations are shown. Different letters indicate statistically significant differences.

collection date and body size ($F_{5,47} = 9.72$, $P < 0.0001$), indicating that temporal changes in food sources differed among growth stages and/or sexes. Post hoc tests revealed that $\delta^{13}C$ values did not differ among body size categories in each of collection dates ($P > 0.05$) and differed across the collections (Fig. 7A). This suggests that there was no consistent difference in food sources in relation to body size. Additionally, $\delta^{13}C$ values were significantly higher in July, August, and November than in January and May (Fig. 7A), indicating that food sources differed between summer and winter.

The nitrogen stable isotope ratio ($\delta^{15}N$) did not differ between collection dates and was not associated with body size (collection date, $F_{6,47} = 1.69$, $P = 0.144$; body size, $F_{2,47} = 0.02$, $P = 0.903$; Fig. 7B), suggesting that the trophic level remained unchanged throughout the year.

The C-N map of *P. paucidens* and its potential food sources are shown in Fig. 8. The $\delta^{13}C$ and $\delta^{15}N$ values were −20.8 to −23.8‰ and 11.3 to 13.5‰ in winter, respectively, indicating that the food chain starts from detritus, as well as the amphipod *Jesogammarus annandalei* and oligochaete (Fig. 8). In summer, the $\delta^{13}C$ and $\delta^{15}N$

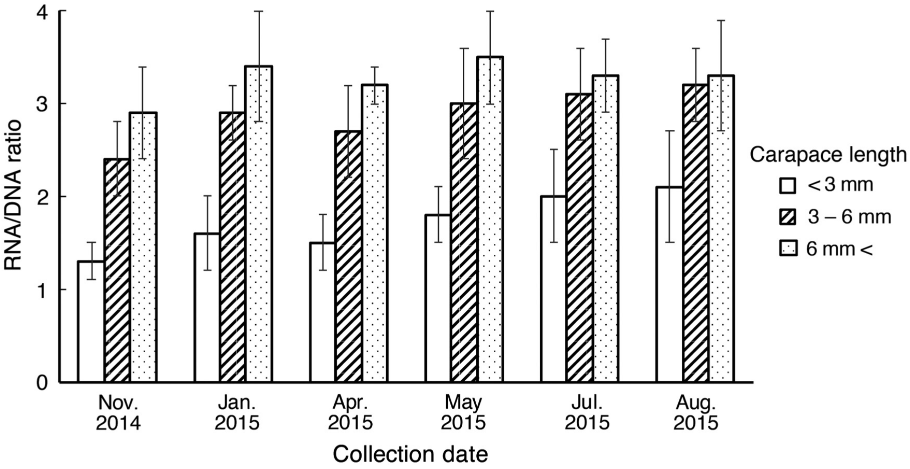

Fig. 6. Temporal variation in the RNA/DNA ratio of *Palaemon paucidens*. Means and standard deviations are shown (n = 9 for each sample collection). RNA/DNA ratio was positively associated with carapace length, but did not differ significantly between collection dates.

values of *P. paucidens* were −20.5 to −24.1‰ and 11.4 to 13.1‰, respectively (Fig. 8), suggesting that this species may consume relatively small particulate organic matter (POM, 2.7–20.0 μm).

DISCUSSION

Observed temporal changes in the body size, sex ratio, and reproductive condition of *P. paucidens* in Lake Biwa offer novel insights into the life history of this species. The mean body size was the smallest in the shallow site in May, at which time the smallest proportion of reproductive female was observed. The body size was seen to gradually increase thereafter (in the shallow site) until September. In this period, the proportion of females (i.e., sex ratio) tended to decrease until July, and then increased until September, and that of reproductive (egg-carrying) females increased from July to August but tended to decrease in September. These results suggest that young (small) shrimps joined the population in the shallow sites in spring and grew up there during summer. As the sex ratio changed during this period and females were larger than males, the observed change in body size may also be influenced by variation in the sex ratio, besides individual growth. Although it is unclear whether the observed variation in sex ratio is due to sex-related ecological difference or sampling error, increase in mean body size from July to September may partly reflect the increased ratio of females (47–97%, Fig. 4). Our findings also indicate that relatively large individuals started to reproduce between May and July, with a peak between August and September, and that a portion of the post-spawning female population disappeared in August to September, probably due to die-offs after reproduction. During this long reproductive period from May to September, a female might reproduce multiple times, but little is known about multiple spawning in a single reproductive season in this species. Relatively small individuals did not exhibit any indicators of spawning during this period. As the females were larger than the males in this species (see also Ogawa and Kakuda 1988) and the females were relatively abundant in our samples (Fig. 4), these small individuals were presumed to include males in addition to small females before reproduction. In November, both relatively large reproductive females and small individuals without indicators of spawning were found in the deep sites, where the body size only slightly increased and the proportion of post-spawning females remained unchanged until January. Then, interestingly, relatively large individuals were not observed in the deep sites until April, which may have been due to die-offs of these large

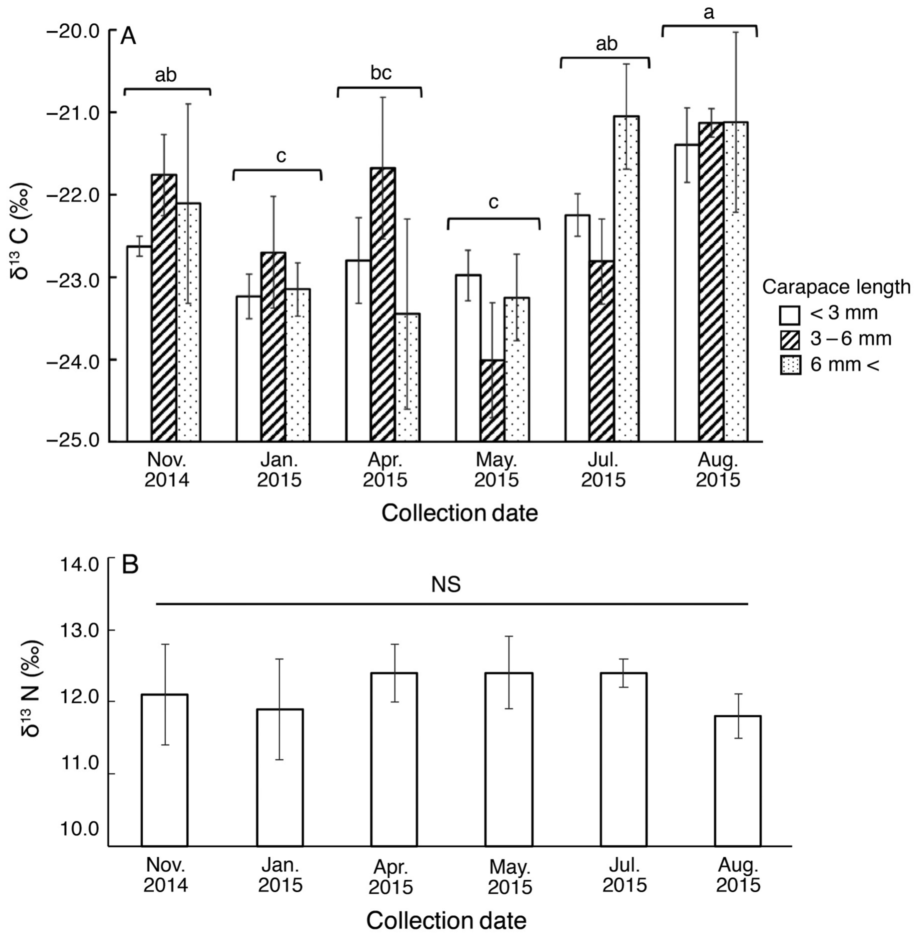

Fig. 7. Temporal variation in (A) carbon stable isotope ratio and (B) nitrogen stable isotope ratio of *Palaemon paucidens*. Means and standard deviations are shown (n = 9 for each sample collection). Different letters indicate statistically significant differences. There was no significant difference in the nitrogen stable isotope ratio between collection dates.

individuals or they may have moved to sites that we did not collect samples from. A decrease in the proportion of large individuals was also observed in the shallow sites in May; however, it is difficult to discriminate whether the large individuals disappeared or the other a large number of small individuals joined, or both. These results suggest that small, non-reproductive individuals can overwinter and may reproduce in their second summer, and that a small proportion of reproductive individuals can overwinter and may survive until the next summer. *Palaemon paucidens* in Lake Biwa may have a life-cycle that spans more than 1 yr, despite previous studies having suggested that this species has an annual life-cycle (Harada 1966, Nishino 1978).

The present study provides novel findings regarding intraspecific variation in the adult lifespan of *P. paucidens*. The lifespan of this species has been reported to differ among regions in Japan. It is assumed to be a univoltine species with the die-off of females occurring after spawning in a pond in Nagano Prefecture (Okubo 1961). In contrast, Handa and Araki (1930) found that a certain proportion of females survive for 2–3 yr in Hokkaido. The findings of the present study support the latter case, wherein we found that the adult lifespan of *P. paucidens* is longer than a year in Lake Biwa. Intraspecific

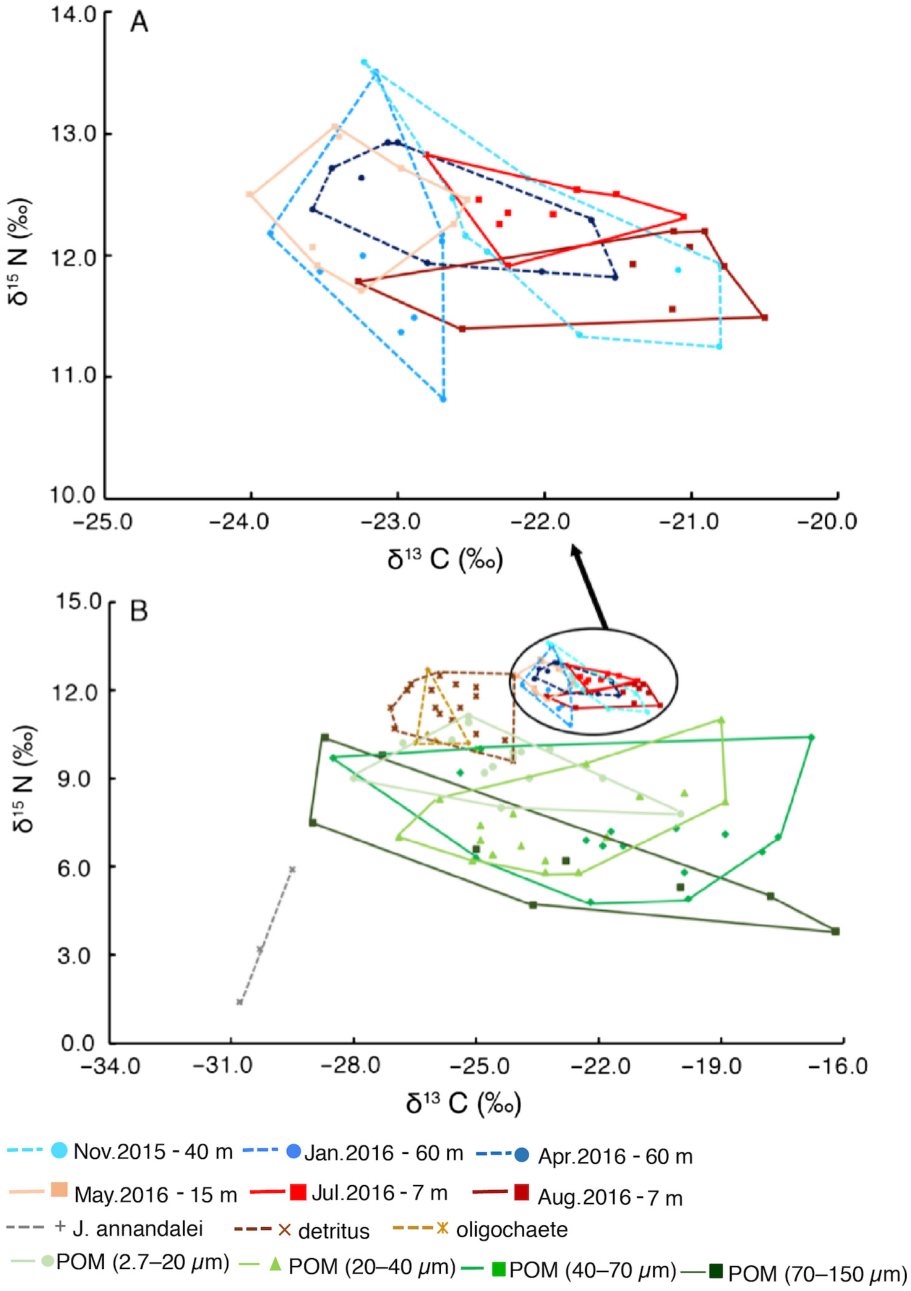

Fig. 8. (A) The C-N maps of *Palaemon paucidens* and (B) its possible food sources in Lake Biwa. The range from sky blue to deep blue within dotted lines indicates the shrimps that were collected in deep sites in winter; the range from flesh color to dark red within solid lines indicates the shrimps that were collected in shallow sites from spring to summer. Data of *Jesogammarus annandalei*, detritus, and oligochaete in deep sites (gray, brown, dark goldenrod; dotted line) are obtained from Narita (2002). Data of particulate organic matter POM (2.7–20.0 μm), POM (20–40 μm), POM (40–70 μm), and POM (70–150 μm) in shallow sites (the range from light green to dark green; solid line) are obtained from Yamada et al. (1998).

variation in lifespan has also been observed in various kinds of fish. Some proportions of reproductive trout survive after the first spawning and return to the sea, and then breed again in the following year (Kiso and Kosaka 1994, Morita 2001). In Lake Biwa, most individuals of the landlocked ayu-fish *Plecoglossus altivelis* exhibit a univoltine life-cycle and die-off after the first spawning; however, a portion of reproductive adults survive after the first spawning and reach sexual maturation in the second year (Matsuyama and Matsuura 1984, Matsuyama 1985). It is worth investigating whether these same factors (or others) can explain the long lifespan of *P. paucidens* in Lake Biwa.

Previous studies hypothesized that *P. paucidens* migrate to deep waters to hibernate (i.e., the hibernation hypothesis; Harada 1966). However, our findings do not support this hypothesis. We found that the *P. paucidens* that collected in deep waters in winter did not hibernate and, instead, were observed to have been physiologically active: Body lipid content was found to be the highest in January (i.e., increased over winter; Fig. 5A), and the RNA/DNA ratio in winter was the same as observed in the warm season (Fig. 6). Additionally, the carbon stable isotope ratio indicated a difference in food sources between summer and winter (Fig. 7B), suggesting that *P. paucidens* foraged in deep sites in winter. Our study, therefore, provides support for the winter foraging hypothesis. Although *P. paucidens* was found to forage in the deep sites in winter, growth rates appeared to be low during this period (November–January, Fig. 4). This suggests that the nutrition gained in deep sites in winter was allocated for processes other than individual growth.

Prosser and Brown (1961) reported that mammals and birds store lipid in their body before overwintering. Fish species inhabiting cold and temperate regions also exhibit this trait (Brown 1957). In contrast, we observed that the lipid content of *P. paucidens* increased during overwintering and was the highest in January, and then decreased until a second peak in lipid content occurred in May. These lipid fluctuations corresponded to the onset of the growing and reproductive seasons (Fig. 5). These results suggest that this species stores lipid for purposes other than overwintering. Atsumi (1990) reported that the ovaries of *P. paucidens* began to grow from November and that the egg-bearing females appeared from mid-March to April in a reservoir in Hyogo Prefecture, Japan. Although we did not find egg-bearing females in winter to spring (but found post-spawning females; Fig. 4), the temporal correspondence between the amount of stored lipid and ovary growth and egg production suggests that *P. paucidens* stored lipids during winter by foraging at deep sites (resulting in the highest amount of lipid in the body in January), and then used these reserves for reproductive purposes (resulting in a decrease in stored lipid in April). This is congruent with our observation that egg-carrying females stored very low levels of lipid in their bodies, while eggs were highly lipid-rich (Fig. 5B). The second peak of lipid content in May might be attributed to subsequent foraging in warm shallow sites. Thus, the purpose of migration and winter foraging by this species in deep sites may be to gain nutrients for reproductive purposes. In addition, foraging in deep sites may also reduce the risk of starvation and compensate for the energy costs of the migration behavior. The observed fluctuation in lipid content could also be explained by fluctuation in the sex ratio and/or composition of reproductive individuals, because we did not discriminate individual sex and reproductive conditions in the analysis of seasonal change in lipid contents (Fig. 5A). The lipid content was significantly higher in post-spawning females than in other individuals, but the proportion of female was unchanged, or rather decreased, during this period (i.e., January–May; Fig. 4). Thus, the observed peaks in lipid content are unlikely to be attributed to the increase in the number of females.

In conclusion, we revealed a novel aspect of the life history of *P. paucidens* with regard to its seasonal migration in Lake Biwa. Our findings opposed the hibernation hypothesis and were in support of the winter foraging hypothesis, in that *P. paucidens* migrated to feeding sites in winter probably to obtain resources for reproductive purposes. In this study, we did not examine the seasonal migration–predator avoidance hypothesis and further investigations are needed in this regard. The present study provides a good example for understanding the life history traits and ecology of the seasonal migration of animals.

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