Limnology (2003) 4:35–41
DOI 10.1007/s10201-002-0087-5

RESEARCH PAPER

Jotaro Urabe · Seiji Ishida · Mayuko Nishimoto
Lawrence J. Weider

Daphnia pulicaria, a zooplankton species that suddenly appeared in 1999 in the offshore zone of Lake Biwa

Received: August 21, 2002 / Accepted: October 26, 2002

Abstract *Daphnia galeata* has been the sole *Daphnia* species and one of the dominant zooplankton species in Lake Biwa for the past 30 years. In 1999, however, another *Daphnia* species suddenly appeared in the lake. Based on morphological characteristics, the species was initially identified as *Daphnia pulicaria*. This identification was supported by mitochondrial (mt)DNA sequences of the 12S rRNA and NADH-5 (ND5) genes. In addition, comparison of the latter sequences showed that *D. pulicaria* in Lake Biwa is genetically very similar to populations distributed across the western regions of North America rather than those in other regions, including Europe. Thus, it is likely that *D. pulicaria* in Lake Biwa is a zooplankton invader from North America. Through routine sampling, we first detected *D. pulicaria* in winter 1999. This species gradually increased in abundance and reached a maximum density of 10000 individuals m^{-2} in mid-May 1999. Thereafter, it decreased in abundance and almost disappeared from the water column after August 1999. *D. pulicaria* are much larger than *D. galeata* and are distributed in deeper water. They showed a marked diel vertical migration, although substantial numbers of individuals stayed above and within the thermocline, both during the day and at night. Based on seasonal and vertical distributions, we discuss the ecological implications of the sudden appearance of *D. pulicaria* in Lake Biwa.

Key words Cladocera · Genetic analysis · Lake Biwa · Taxonomy · Zooplankton invader

Introduction

Lake Biwa is one of the ancient lakes of the world and is the most species-rich freshwater habitat in Japan (Rossiter 2000). In this lake, *Daphnia* is a conspicuous member of the zooplankton fauna. The genus *Daphnia* is classified into three subgenera, *Daphnia* (*pulex* group), *Hyalodaphnia* (*longispina* group), and *Ctenodaphnia* (*magna* group) (Colbourne and Hebert 1996). Among these groups, species of the subgenera *Daphnia* and *Hyalodaphnia* have been recorded in Lake Biwa. In a paleolimnological study, Kadota (1984) found fragmentary remains of individuals belonging to the *pulex* group and those belonging to the *longispina* group in core sediment samples dated at 5–8 × 10^4 years BP. Ueno (1934) collected individuals of the *D. pulex* group in 1926 and 1932 at offshore sites in Lake Biwa. Later, he identified these individuals as an endemic species named *D. biwaensis* (Ueno 1972). For the last 30 years, however, although a number of intensive studies focusing on zooplankton have been conducted (e.g., Okamoto 1984; Kawabata 1989; Miura and Cai 1990; Urabe et al. 1995; Yoshida et al. 2001), no individual of the subgenus *Daphnia* has been found in Lake Biwa. Instead, these studies have shown that members of the subgenus *Hyalodaphnia* have been abundant and have sometimes dominated the zooplankton community. By careful analysis of the morphology, Tanaka (1992) concluded that the *Hyalodaphnia* in this lake is monospecific, consisting of *D. galeata*. Thus, for the last 30 years, *D. galeata* has been the sole *Daphnia* species collected in Lake Biwa.

In 1999, another *Daphnia* species appeared suddenly and abundantly. The characters of the antennule and the comb on the postabdominal claw indicate that this species is a member of the subgenus *Daphnia*. However, it is difficult to identify *Daphnia* at the species level based on morphological examination alone, because of the large intraspecific variation in the phenotype, the similarity of morphological features among species, and the occurrence of interspecific hybrids (Brooks 1957; Dodson 1981; Hebert 1985; Hrbáček 1987; Schwenk et al. 2000). To overcome limitations of the

J. Urabe (✉) · S. Ishida · M. Nishimoto
Center for Ecological Research, Kyoto University, 509-3 Kamitanakami Hirano-cho, Otsu 520-2113, Japan
Tel. +81-77-549-8254; Fax +81-77-549-8201
e-mail: urabe@ecology.kyoto-u.ac.jp

L.J. Weider
University of Oklahoma Biological Station, Kingston, OK, USA

conventional morphological approach, genetic analysis has recently been employed to determine species affinities within the genus *Daphnia*. Indeed, although the taxonomy and phylogeny of the genus *Daphnia* have long been confused, such problems are being resolved through the use of genetic information (Lehman et al. 1995; Colbourne and Hebert 1996; Hebert and Finston 1996; Weider et al. 1999; Schwenk et al. 2000).

In the present study, therefore, we performed both morphological and genetic analyses to identify a species of the subgenus *Daphnia* that appeared in 1999 in the offshore zone of Lake Biwa. Based on these analyses, we have identified this species as *Daphnia pulicaria* Forbes, 1893, emend. Hrbáček, 1959. Although this species is commonly found in North America and Europe, recent studies have shown a large genetic divergence between these continents (Colbourne et al. 1998; Černy and Hebert 1999; Weider et al. 1999). Colbourne et al. (1998) and Weider et al. (1999) found that sequences of the NADH-5 (ND5) mitochondrial gene of *D. pulicaria* differ greatly across large geographic scales. In the present study, we first examined the genetic sequence of the 12S ribosomal RNA mtDNA gene (12S rDNA) to place Lake Biwa *Daphnia* in the known phylogeny of the genus *Daphnia*, and then analyzed sequences of the ND5 gene to assess genetic similarity to *D. pulicaria* in some European and North American lakes. Because *Daphnia* is a key species in lake ecosystems worldwide (Brooks and Dodson 1965; Carpenter and Kitchell 1993; Vanni et al. 1991), we also examined the temporal and spatial dynamics of the *D. pulicaria* population, and we discuss the ecological implications of their appearance in Lake Biwa.

Materials and methods

Sampling and enumeration

Since 1968, Kyoto University has collected monthly zooplankton samples at Station Ie-1 (70–75 m deep), which is situated between Omatsu and Oki Islands (Miura and Cai 1990). In the present study, we analyzed the monthly samples collected from March 1998 to October 1999. Details of the sampling procedure have been described elsewhere (Miura and Cai 1990). In short, the zooplankton were collected with a 15-cm-diameter closing net (70-μm mesh) by vertical hauls in six different water layers: 0–5, 5–10, 10–20, 20–30, 30–50, and 50–70 m. The samples were fixed with 5% formalin solution, concentrated to 20 ml, and preserved in 100% ethanol. In each sample, *Daphnia* individuals were counted according to species and size class (at 0.1-mm intervals) under a dissecting microscope at 40× magnification. The average distribution depth (d) was calculated as

$$d = \Sigma(D_i \times N_i)/\Sigma N_i$$

where D is the mean depth of the sampling layer i and N is the number of individuals within layer i. The dry weight biomass was calculated from body size structure and length – mass equations established for *D. galeata* (Kawabata and Urabe 1998) and for *D. pulicaria* (Urabe, unpublished).

To examine differences in vertical distributions between day and night, additional samples were collected at noon (12:00) and midnight (24:00) on 26 May 1999 at a pelagic site 3 km from Wani (55-m deep). In this sampling, lake water was collected using a 10-l modified Van Dorn sampler at nine depths (0, 3, 6, 9, 12, 15, 18, 25, and 50 m). For each depth, the zooplankton in 10 l of lake water were concentrated using a 20-μm mesh net and fixed with 2% sugar buffered-formalin (Haney and Hall 1973). Thermal profiles were obtained with a CTD profiler (SBE-25, Sea-Bird Electronics, Bellevue, WA, USA) at 1-m intervals. *Daphnia* specimens collected on this date were used for morphological examination. Live *Daphnia* individuals were also collected and brought to the laboratory. These individuals were cultured and used for the genetic analyses.

Genetic analyses

Total genomic DNA was extracted from single animals from cultures, using Chelex 100 (Bio-Rad, Hercules, CA, USA) according to the method of Colbourne and Hebert (1996) for analyzing the mitochondrial (mt)DNA 12S rDNA gene. For analysis of the NADH-5 (ND5) gene, we used the IsoQuick kit (Orca Corp., Bothell, WA, USA) following the manufacturer's protocols for tissue extractions. For the 12S rDNA, polymerase chain reaction (PCR) amplifications were conducted according to the method of Colbourne and Hebert (1996) with the primers (5′-ATGCACTTTCCAGTACATCTAC-3′ and 5′-AAATCG TGCCAGCCGTCGC-3′) designed by the same authors. PCR products were purified using Qiaquick (Qiagen, Hilden, Germany), cycle-sequenced using Big Dye terminator chemistry and AmpliTaq-FS DNA polymerase (Perkin Elmer/Applied Biosystems, Forster City, CA, USA), and run on an ABI-373 DNA sequencer (Perkin Elmer/Applied Biosystems). For the ND5 analyses, PCR amplifications followed the conditions outlined by Colbourne et al. (1998) using the DpuND5a forward primer (5′-ATAAAACTCC AATCAACCTTG-3′) and the DpuND5b reverse primer (5′-GGGGTGTATCTATTAATTCG-3′). The following PCR thermal cycling parameters were used: one cycle of initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 1 min, annealing at 50°C for 1 min, and extension for 1 min at 72°C. PCR products were purified using the QiaQuick (Qiagen) kit, cycle-sequenced using Big Dye terminator chemistry and AmpliTaq-FS DNA polymerase (Perkin Elmer/Applied Biosystems), and run on an ABI-377 DNA sequencer (Perkin Elmer/Applied Biosystems).

To examine the genetic distance of Lake Biwa *Daphnia* from related taxa, published DNA sequences were obtained from the GenBank/EMBL databases. These sequences included 12S rDNA reported by Lehman et al. (1995) for *D. pulex* (accession number U13902), *D. pulicaria* (U13905), *D. middendorffiana* (U13909), *D. obtusa* (U13910), and *D. magna* (U13908), and by Schwenk et al. (2000) for *D. pulex* (AF277284); and those of ND5 reported by Crease (1999)

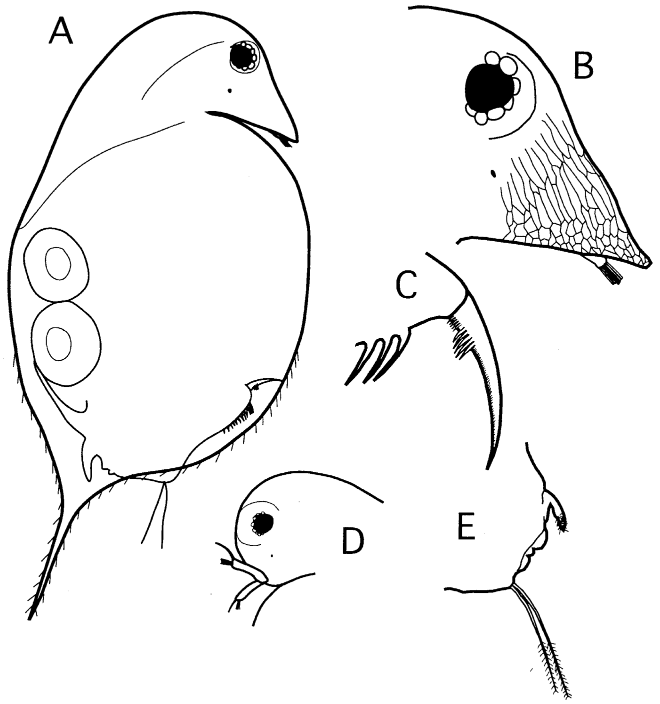

Fig. 1. *Daphnia pulicaria* in Lake Biwa. **A**, Parthenogenetic female; **B** and **C**, head and postabdomen of female; **D** and **E**, head and abdominal process of male

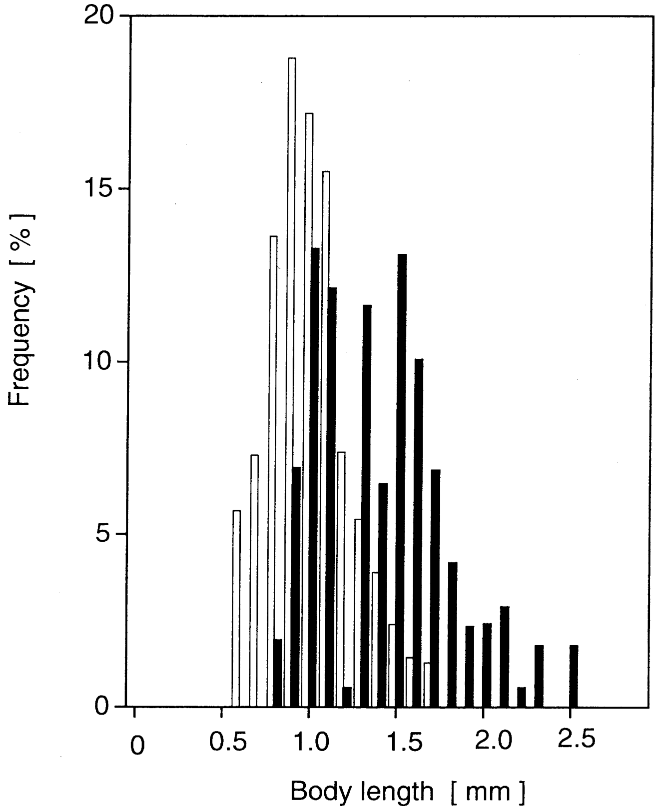

Fig. 2. Size structures of *D. pulicaria* (*filled bars*) and *D. galeata* populations (*open bars*) in Lake Biwa in May 1999

for *D. pulex* (AF117817) and by Adamowicz et al. (2002) for the *Daphnia pulex* complex (AF489527, AF489526). Apart from these, J. Colbourne provided ND5 sequences of *Daphnia* species from various locations. We used these sequences to place Lake Biwa *Daphnia* into the ND5 tree established by Colbourne et al. (1998).

The DNA sequences were aligned using Clustal X (Thompson et al. 1997) and adjusted by eye. Pairwise genetic distances were calculated using Kimura's (1980) two-parameter model (K2P) in MEGA 2.1 (Kumar et al. 2001) and used to construct phenograms by the neighbor-joining (N-J) algorithm (Saitou and Nei 1987). In all cases, trees were constructed using pairwise deletion of missing sites, and bootstrap values were based on 1000 pseudo-replicates.

Results

Morphological characteristics

Female: Body length from the anteriormost margin of the head to the base of the tail spine varies from 0.6 to 2.4 mm (Figs. 1A and 2). In egg-carrying females, body length is greater than 1.6 mm. The head is relatively short, never produces any helmet or projection, and is broadly rounded. In mature females, its length is 1/4 to 1/5 of the valve length. The posterior margin of the head is shallowly concave and gently sinuate near the rostrum. The rostrum is long and extends over the setae hairs of the antennule. Reticulation between the ocellus and the ventral margin of the rostrum contains elongated polygons (Fig. 1B).

The valves are broadly oval: in egg-carrying females, the lateral length is 3/4 of the longitudinal length. The dorsal margin over-junction of the head shield and valve is not concave and is nearly straight. The posterior part of the ventral and dorsal margins of the valves is covered by small spinules. The shell spine is stout and varies depending on the size of the animals: in mature females, it is usually 1/3 as long as the valves. The dorsal margin of the post-abdomen is slightly convex and is armed with 10 to 16 large anal teeth. Among the four postabdominal processes, the first one is the largest, and, in mature females, it curves upward to protect eggs in the brood pouch. The postabdominal claw has five stout teeth in the middle pecten. The teeth of the proximal pecten are short and slender. The distal pecten forms a comb with very fine teeth (Fig. 1C).

Male: The body length of the male is less than 1.3 mm. The head is round and 1/4 as long as the valves. The antennule is about 1/3 the length of the head, and the flagellum is shorter than the antennule (Fig. 1D). The valves are oval and elongated. The dorsal abdominal process is short and never reaches to the abdominal setae (Fig. 1E).

Genetic analyses

Approximately 530 base pairs (bp) of the 12S rDNA were sequenced for *Daphnia* individuals in Lake Biwa, and alignment of the sequences yielded 290 bp for comparisons with related taxa. The N-J analysis based on the 12S rDNA

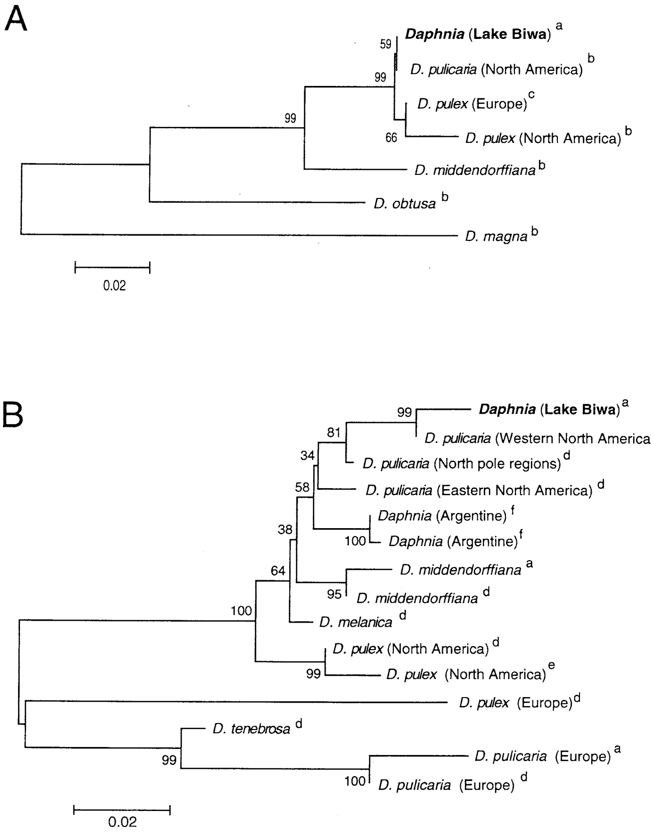

Fig. 3. Neighbor-joining trees of 12S rDNA (**A**) and ND5 (**B**) sequence variations of members of the subgenus *Daphnia*. *Scale bar* indicates Kimura two-parameter genetic distance. Bootstrap values based on 1000 pseudoreplicates are presented for clusters. Data sources: **a** this study; **b** Lehman et al. (1995); **c** Schwenk et al. (2000); **d** Colbourne et al. (1998); **e** Crease (1999); **f** Adamowicz et al. (2002)

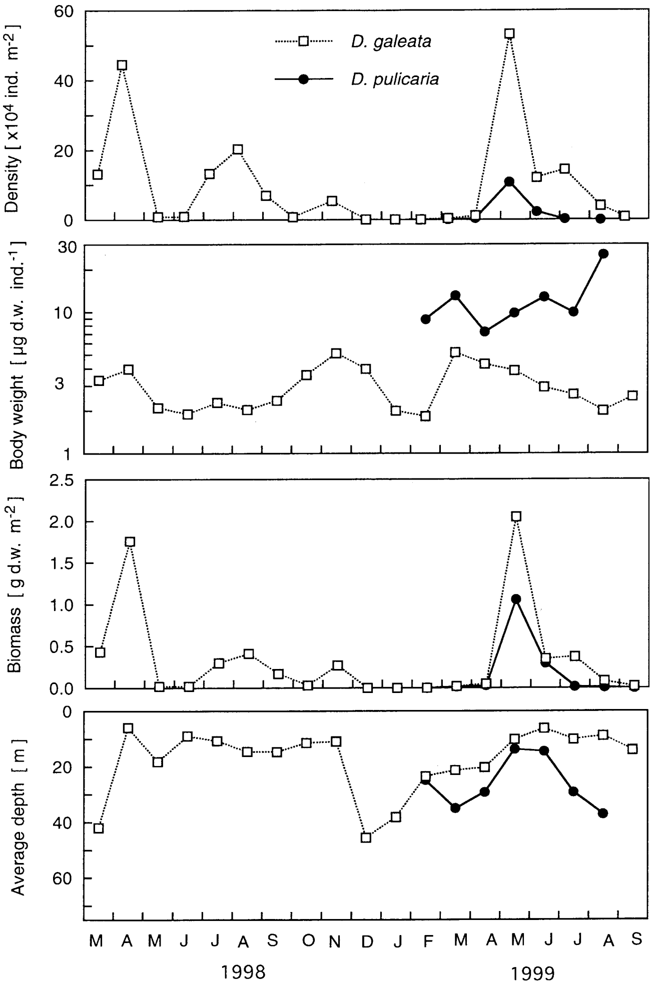

Fig. 4. Individual number, mean body weight, dry weight biomass, and average distributional depth of *D. pulicaria* (●) and *D. galeata* (□)

sequence indicated that the Lake Biwa population under consideration here was a member of the *D. pulex* complex (Fig. 3A). Indeed, the sequence of Lake Biwa *Daphnia* was very close to that of *D. pulicaria* that were collected from Lake Mendota (Lehman et al. 1995), but it largely differed from those of *D. middendorffiana* and *D. obtusa*.

Since divergence of the 12S rDNA gene is very limited between *D. pulex* and *D. pulicaria* (Lehman et al. 1995; Colbourne and Hebert 1996), the same analysis was performed using the sequence of the more rapidly evolving ND5 gene. We sequenced approximately 750 bp of the ND5 gene for Lake Biwa *Daphnia* and aligned 498 bp of the sequence for comparison. The analysis showed that Lake Biwa *Daphnia* is more closely allied to *D. pulicaria* collected from North America than to *D. pulicaria* from Europe (Fig. 3B). Based on ND5 sequence variation, Colbourne et al. (1998) showed that North American *D. pulicaria* consists of three distinct clades. Among these clades, they labeled populations collected in Lake Washington and lakes in Saskatchewan (Canada) as the western clade (Western North America in Fig. 3B). The ND5 sequence variation for the Lake Biwa population was genetically most closely related to *D. pulicaria* from this western (North American) clade.

Seasonal and vertical changes in population abundance

In our monthly samples, *D. pulicaria* first appeared in February 1999 (Fig. 4). At that time, the population was distributed more or less homogeneously in the water column. With the development of the thermocline in spring 1999, the average distributional depth for *D. pulicaria* moved upwards, and the population reached a maximum density of 1×10^4 individuals m^{-2} in May 1999. Most *D. pulicaria* individuals were distributed at greater depths than *D. galeata* and were found just above the thermocline that was formed around 10 m. At night, some individuals ascended to the surface, although a substantial number of *D. pulicaria* stayed at the thermocline (Fig. 5). In May 1999, males were found and several mature females carried resting eggs. After May 1999, *D. pulicaria* gradually disappeared above the thermocline, and the population moved to lower depths. In August 1999, a small number of large adult individuals were collected at depths below the thermocline. Thereafter, no *D. pulicaria* individuals were collected in our routine samplings. Although the individual abundance of *D. pulicaria* was lower than that of *D. galeata* during the

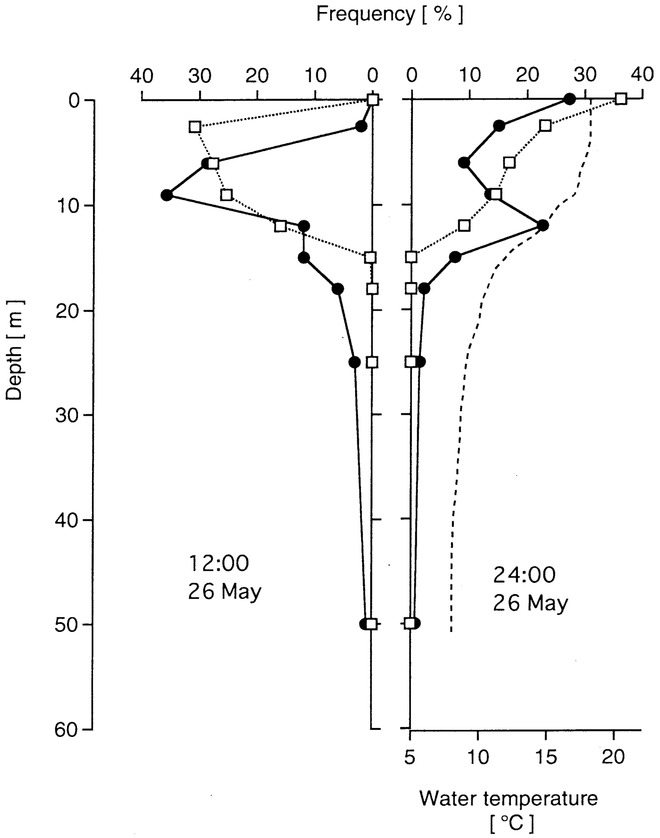

Fig. 5. Vertical distribution of *D. pulicaria* (●) and *D. galeata* (□) at 1200 and 2400 h on 26 May 1999. Vertical profile of water temperatures is shown

present study, their biomass in spring 1999 was comparable to that of *D. galeata* because of their large body size. Thus, the total *Daphnia* biomass in spring 1999 was 60% higher than in spring 1998.

Discussion

Morphological and genetic analyses indicate that the large-bodied *Daphnia* species that appeared in 1999 in Lake Biwa is *D. pulicaria*. Indeed, the morphological features of these daphniids are very close to those of *D. pulicaria* described from North America and Europe (Hrbáček 1959; Brandlova et al. 1972). *D. pulicaria* was originally described by Forbes (1893) from Yellowstone Lake, a large, stratified lake in North America. However, based on re-examination of the specimens collected by Forbes (Brooks 1953), Brooks (1957) placed this species under *D. pulex*. Later, Hrbáček (1959) distinguished *D. pulicaria* from *D. pulex* because the dorsal abdominal process of adult males is short in the former species, whereas it extends beyond the base of the abdominal setae in the latter species. In addition, reticulation between the rostrum and the ocellus consists of very prolonged polygons in *D. pulicaria*, whereas polygons in this region are not prolonged and are roughly equal in size in *D. pulex* (Hrbáček 1959; Brandlova et al. 1972), although there are populations with polygon patterns intermediate between these (Dodson 1981). Another key feature differentiating these two species is the shape of the ventral margin of the head: it is concave and gently sinuate just near the rostrum in *D. pulicaria* but is never sinuate in *D. pulex*. These key characteristics of morphology were also found in the Lake Biwa *D. pulicaria*.

Immature individuals of *D. pulicaria* are sometimes confused with *D. schoedleri* (Evans 1985), which was originally described from Norway. In North America, Brooks (1957) and Brandlova et al. (1972) separated *D. schoedleri* from *D. pulex* on the basis of several characters, including head shape and shell spine length. Since immature individuals of *D. pulicaria* have relatively long shell spines, their body shape and proportions are similar to those described for *D. schoedleri* (Evans 1985). In contrast to *D. pulicaria*, however, *D. schoedleri* produces a rounded helmet or wide crest on the head. Thus, *Daphnia* individuals found in 1999 in Lake Biwa are clearly not what is called *D. schoedleri*. Note: based on genetic analyses, *D. schoedleri* has recently been placed under either *D. pulicaria* or *D. pileata* (Hebert and Finston 1996), and thus *D. schoedleri* is no longer recognized as a member of the daphniid fauna from North America (Colbourne and Hebert 1996).

In Lake Biwa, Kadota (1984) found remains of the postabdominal claws of members of the subgenus *Daphnia* (*D. pulex* group) from a deep sediment core. Ueno (1934), who intensively studied the taxonomy of *Daphnia* in East Asia, also found a species from the subgenus *Daphnia* in plankton samples collected in 1924 and 1932 in Lake Biwa. Based on morphological comparisons with various forms or species in the subgenus *Daphnia*, including *D. pulicaria*, he concluded that the species was peculiar to Lake Biwa (Ueno 1934) and named it *D. biwaensis* (Ueno 1972). Unfortunately, he did not leave any type specimen of this species. However, according to his illustrations (Ueno 1972), the overall body proportions of *D. biwaensis* are indeed much different from those of *D. pulicaria*. Although it is unclear what *D. biwaensis* really is, species of the subgenus *Daphnia* have inhabited Lake Biwa during at least some periods in the past (Ueno 1934; Kadota 1984). However, members of the subgenus *Daphnia* have not been recorded in Lake Biwa for at least 30 years. In the present study, we found *D. pulicaria* individuals carrying resting eggs. In addition, we found resting eggs (ephippia) in the surficial layers of the bottom sediments, whereas these were never detected in the sediment core samples dated from about 1900 to 1998 (Ishida et al., in preparation). Thus, *D. pulicaria* seems to be a recent invader to Lake Biwa.

To our knowledge, however, no studies have recorded *D. pulicaria* from East Asia except that of Sakai (1995), who described species morphologically similar to *D. pulicaria* from some lakes on Hokkaido Island (Japan). *D. pulicaria* is a common limnetic species in North America and Europe. The present study indicates that the *D. pulicaria* population, which appeared in Lake Biwa, is genetically most closely related to populations found in lakes from the western region of North America, such as Lake Washington and lakes in Saskatchewan, Canada. If the Lake Biwa *D. pulicaria* had originated from sources in East Asia, its genetic distance from populations from western North America would probably be much greater than the genetic distance between populations from eastern and western North America. However, this is not the case. Considering the fact that several fish species have invaded Lake Biwa from North America (Yuma et al. 1998), *D. pulicaria* in Lake Biwa most likely represents a zooplankton invader also from North America. However, the small number of records of *D. pulicaria* in East Asia may be due to taxonomic confusion stemming from phenotypic plasticity. Indeed, Ueno (1939) reported a species morphologically similar to *D. pulicaria* from north China. To clarify the origin of *D. pulicaria* in Lake Biwa, therefore, further biogeographical studies with genetic analyses are needed.

D. pulicaria and *D. galeata* often co-occur in the open waters of North American lakes (e.g., Threlkeld 1979; Edmondson and Litt 1982; Tessier and Welser 1991; Liebold 1991; Vanni et al. 1991). Some researchers have shown that, as in the present study, *D. pulicaria* are generally found in deeper water layers than *D. galeata* (Tessier and Welser 1991; Liebold 1991). Since the growth rates of *D. pulicaria* individuals decrease dramatically at 28°C compared with 24°C (Brandlova et al. 1972), it is likely that this species avoids warm surface water layers. However, the difference in vertical distribution between *D. galeata* and *D. pulicaria* found in Lake Biwa cannot be explained by species-specific responses to water temperature, because the water temperatures in May are usually below 20°C. Alternatively, differences in the vertical distribution of *D. pulicaria* and *D. galeata* seem to be related to differential susceptibility to fish predation (Tessier and Welser 1991). In the present study, some individuals of *D. pulicaria* ascended to the surface at night. Such diurnal changes in vertical distribution are known to relate to predation pressure (see review in Lampert 1993). In general, most visual predators, such as planktivorous fish, prey preferentially on larger zooplankton (O'Brien 1979). Thus, it is advantageous for large-bodied zooplankton species such as *D. pulicaria* to use the deeper, darker water layers as a daytime habitat, where they are less conspicuous to size-selective planktivorous fish, and to ascend to the shallow layer only at night (Lampert 1993). Indeed, Liebold (1991) and Liebold and Tessier (1991) have provided definitive evidence that *D. pulicaria* prefers deeper water layers to reduce predation risk in daytime.

This argument provides insight as to why *D. pulicaria* appeared abundantly in 1999 in Lake Biwa. Sudden increases in *D. pulicaria* abundance have also been reported in other lakes, such as Lake Washington (Edmondson and Litt 1982), Lake Michigan (Evans 1985), and Lake Mendota (Vanni et al. 1991). In Lake Michigan and Lake Mendota, *D. pulicaria* appeared abundantly after the decline in planktivorous fish populations (Evans 1985; Vanni et al. 1991). In the case of Lake Washington, Edmondson and Litt (1982) suggested that the predominance of *D. pulicaria* was related to decreases in the density of the invertebrate (opossum shrimp) predator *Neomysis mercedis*. Thus, temporal and seasonal increases in *D. pulicaria* abundance are thought to be a sign of temporal decreases in the standing stocks of planktivorous fish (Vanni et al. 1991). In Lake Biwa, zooplankton community structure and the composition of the fish harvest have changed over the past several decades. Prior to 1980, *Daphnia* were a minor component of the spring zooplankton community (Miura and Cai 1990), whereas they have occurred more abundantly in the spring in recent years (Yoshida et al. 2001). According to Yuma et al. (1998), commercial harvests of cyprinid fishes decreased dramatically in the 1990s, although that of Ayu (*Plecoglossus altivelis*) increased. Ayu prey selectively on large zooplankton such as *Daphnia* (Kawabata et al. 2002) but migrate to the offshore in late spring (Azuma 1970). Thus, a major component of the planktivorous fish fauna in early spring in Lake Biwa is primarily made up of cyprinids, some of which prey selectively on *Daphnia* (Sunaga 1970; Urabe and Maruyama 1986). Considering this fact, it is likely that predation pressure by planktivorous fish had decreased, especially in early spring 1999. This may have allowed for the development of the *D. pulicaria* population until late spring 1999. Unfortunately, we have no definite evidence to confirm this speculation, simply because no quantitative data on temporal changes in abundance of the planktivorous fish are available.

Because *D. pulicaria* have not been detected in our routine samples since fall 1999, their appearance seems to be temporally limited. It should be noted, however, that *D. pulicaria* produced resting eggs that were stored in the lake sediments (Ishida and Urabe, personal observation). Thus, if the present argument is correct and if the resting eggs hatch, *D. pulicaria* may again occur abundantly when the population densities of planktivorous fish decline. To confirm this possibility, further monitoring studies are necessary.

Acknowledgments We thank M. Nakanishi, T. Ueda, T. Koitabashi, T. Narita, and the members of CER Kyoto University for samples, and D.J. Taylor and W. Makino for comments on the manuscript. We thank K.L. Glenn and N.A. Zehrbach for laboratory assistance and J. Colbourne for providing sequence data. This work was supported by a Grant-in Aid for Scientific Research B (No. 12440218) from the MEXT, Japan, and from NSF-IRCEB (9977047).

References

Adamowicz SJ, Gregory R, Marinone MC, Hebert PDN (2002) New insights into the distribution of polyploid *Daphnia*: the holarctic revisited and Argentina explored. Molec Ecol 11:1209–1217

Azuma M (1970) Studies on the variability of the landlocked ayu-fish, *Plecoglossus altivelis* T. et S., in Lake Biwa. I. On the mode of distribution and some body form variation at early phases of development. Jpn J Ecol 20:63–76 (in Japanese)

Brandlova J, Brandl Z, Fernand CH (1972) The Cladocera of Ontario with remarks on some species and distribution. Can J Zool 50:1373–1403

Brooks JL (1953) Re-description of *Daphnia pulex* var. *pulicaria* Forbes, *D. thorata* F. and *D. dentifera* F. Am Midl Nat 49:772–800

Brooks JL (1957) The systematics of North American *Daphnia*. Mem Conn Acad Arts Sci 13:1–180

Brooks JL, Dodson SI (1965) Predation, body size and composition of plankton. Science 150:28–35

Carpenter S, Kitchell JF (1993) The trophic cascade in lakes. Cambridge University Press, Cambridge

Cěrný M, Hebert PDN (1999) Intercontinental allozyme differentiation among four holarctic *Daphnia* species. Limnol Oceanogr 44:1381–1387

Colbourne JK, Hebert PDN (1996) The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. Phil Trans R Soc Lond B 351:349–360

Colbourne JK, Crease TJ, Weider LJ, Hebert PDN, Dufresne F, Hobaek A (1998) Phylogenetics and evolution of a circumarctic species complex (Cladocera: *Daphnia pulex*). Biol J Linnean Soc 65:347–365

Crease TJ (1999) The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). Gene 233:89–99

Dodson SI (1981) Morphological variation of *Daphnia pulex* Leydig (Crustacea: Cladocera) and related species from North America. Hydrobiologia 83:101–114

Edmondson WT, Litt AH (1982) *Daphnia* in Lake Washington. Limnol Oceanogr 27:272–293

Evans MS (1985) The morphology of *Daphnia pulicaria*, a species newly dominating the offshore Southeastern Lake Michigan summer daphnid community. Trans Am Microsc Soc 104:223–231

Forbes SA (1893) A preliminary report on the aquatic invertebrate fauna of the Yellowstone National Park, Wyoming, and the Flathead Region of Montana. US Fish Comm Bull 11:207–256

Haney JF, Hall DJ (1973) Sugar-coated *Daphnia*: a preservation technique for cladocera. Limnol Oceanogr 19:331–333

Hebert PDN (1985) Interspecific hybridization between cyclic parthenogens. Evolution 39:216–220

Hebert PDN, Finston TL (1996) A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). II. New species in the *Daphnia pulex* group from the south-central United States and Mexico. Can J Zool 74:632–653

Hrbáček J (1959) Üeber die angebliche Variabilität von *Daphnia pulex* Leydig. Zool Anz 162:118–125

Hrbáček J (1987) Systematics and biogeography of *Daphnia* species in the northern temperate region. Mem Ist Ital Idorobiol 45:37–76

Kadota S (1984) Animal microfossils. In: Horie S (ed) Lake Biwa. Junk Publisher, Dordrecht, pp 545–555

Kawabata K (1989) Seasonal changes in abundance and vertical distribution of *Mesocyclops thermocyclopoides*, *Cyclops vicinus* and *Daphnia longispina* in Lake Biwa. Jpn J Limnol 50:9–13

Kawabata K, Urabe J (1998) Length-weight relationships of eight freshwater planktonic crustacean species in Japan. Freshwat Biol 39:199–206

Kawabata K, Narita T, Nagoshi M, Nishino M (2002) Stomach contents of the landlocked dwarf ayu in Lake Biwa, Japan. Limnology 3:135–142.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Molec Evol 16:111–120

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: Molecular Evolutionary Genetic Analysis software, Version 2.1. Arizona State University, Tempe, Arizona, USA. Distributed over the Internet by the authors at www.megasoftware.net

Lampert W (1993) Ultimate causes of diel vertical migration of zooplankton: new evidence for the predator-avoidance hypothesis. Arch Hydrobiol Beih Ergeb Limnol 39:79–88

Lehman N, Pfrender ME, Morin PA, Crease TJ, Lynch M (1995) A hierarchical molecular phylogeny within the genus *Daphnia*. Molec Phylogenet Evol 4:395–407

Liebold M (1991) Trophic interactions and habitat segregation between competing *Daphnia* species. Oecologia 86:510–520

Liebold M, Tessier A (1991) Contrasting patterns of body size for *Daphnia* species that segregate by habitat. Oecologia 86:342–348

Miura T, Cai QH (1990) Annual and seasonal occurrences of the zooplankters observed in the North basin of Lake Biwa from 1965 to 1979. Lake Biwa Study Monographs 5, Lake Biwa Research Institute, Otsu, pp 1–35

O'Brien WJ (1979) The predator-prey interaction of planktivorous fish and zooplankton. Am Sci 67:572–581

Okamoto K (1984) Size-selective feeding of *Daphnia longispina hyalina* and *Eudiaptomus japonicus* on a natural phytoplankton assemblage with the fractionizing method. Mem Fac Sci Kyoto Univ (Ser Biol) 9:23–40

Rossiter A (2000) Lake Biwa as a typical ancient lake. Adv Ecol Res 31:571–598

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molec Biol Evol 4:406–425

Sakai S (1995) Revision of taxonomy and distribution of subgenus *Daphnia* in Hokkaido. M.S. Thesis, Hokkaido University, Japan, 35 pp (in Japanese)

Schwenk K, Posada D, Hebert PDN (2000) Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. Proc R Soc Lond B 267:1833–1842

Sunaga T (1970) Relation between food consumption and food selectivity in fishes I. Food selectivity of juvenile Hasu (*Opsarichthys uncirostris* Teminck et Schlegel), in Lake Biwa. Jpn J Ecol 20:129–137

Tanaka S (1992) Morphology and variation of *Daphnia galeata* Sars from Lake Biwa and Lake Kizaki in Japan. Jpn J Limnol 53:47–54

Tessier AJ, Welser J (1991) Cladoceran assemblages, seasonal succession and the importance of a hypolimnetic refuge. Freshwat Biol 25:85–93

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Threlkeld ST (1979) The midsummer dynamics of two *Daphnia* species in Wintergreen Lake, Michigan. Ecology 60:165–179

Ueno M (1934) The freshwater Branchiopoda of Japan. IV genus *Daphnia* of Japan 2. local races of Japanese *Daphnia*. Mem Coll Sci, Kyoto Imperial Univ, Ser B 9:321–349

Ueno M (1939) Manchurian freshwater Cladocera. Annot Zool Japon 18:219–231

Ueno M (1972) Branchiopoda. In: Ueno M (ed) The late Tamiji Kawamura freshwater biology of Japan. Enlarged and revised edition. Hokuryukan, Tokyo, pp 405–430 (in Japanese)

Urabe J, Maruyama T (1986) Prey selectivity of two cyprinid fishes in Ogochi Reservoir. Bull Jpn Soc Sci Fish 52:2045–2054

Urabe J, Nakanishi M, Kawabata K (1995) Contribution of metazoan plankton to the cycling of nitrogen and phosphorus in Lake Biwa. Limnol Oceanogr 40:232–241

Vanni MJ, Lueke C, Kitchel JF, Allen Y, Temte J, Magnuson JJ (1991) Effects on lower trophic levels of massive fish mortality. Nature 344:333–335

Weider LJ, Hob KA, Colbourne JK, Crease TJ, Dufresne F, Hebert PDH (1999) Holarctic phylogeography of an asexual species complex I. Mitochondrial DNA variation in arctic *Daphnia*. Evolution 53:777–792

Yoshida T, Kagami M, Grung TB, Urabe J (2001) Seasonal succession of zooplankton in the north basin of Lake Biwa. Aquat Ecol 35:19–29

Yuma M, Hosoya K, Nagata Y (1998) Distribution of the freshwater fishes of Japan: an historical overview. Environ Biol Fishes 52:97–124