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ABSTRACT -- A limnological assessment project by the University of Nebraska at Kearney and the Nebraska Game and Parks Commission collected weekly vertical zooplankton tows during May through September 2002 at Harlan County Reservoir in Nebraska. Beginning on 5 August 2002, the exotic *Daphnia lumholtzi* (Cladocera: Daphniidae) appeared at a density of 0.04 l^{-1} in one of fifteen standardized sampling stations. By 6 September 2002, *D. lumholtzi* was found in all fifteen stations at an average density of 2.17 ± 3.10 l^{-1} with a site maximum density of 11.43 l^{-1}. Length measurements of *D. lumholtzi* ranged from 0.80 mm to 5.66 mm with a mean length of 2.38 ± 1.107 mm. During sampling, the abundance of *D. lumholtzi* increased relative to the native *Daphnia retrocurva* from less than 1% to greater than 45% of all zooplankton collected. Our finding represents the first account in a Nebraska water system of *D. lumholtzi*, a native of Africa, Asia, and Australia, and shows a northern expansion in the Great Plains of this exotic species.

Key words: Cladocera, *Daphnia lumholtzi*, first account, Harlan County Reservoir, Nebraska, non-indigenous, zooplankton.

North American ecosystems have been invaded by many species of plants and animals, which become established either intentionally or by accident. Once established, these species often spread and in some cases cause significant harm to the environment, existing food webs, native species, and exotic commercial

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species (Pimentel 2002). Several traits, including high reproductive rates, high dispersal rates, and broad environmental tolerances (Mooney and Drake 1986), are common to successful invasive species. However, predicting rates of spread and invader success in new habitats remains problematic (Williamson and Fitter 1996). Invasive species face significant abiotic and biotic challenges in new environments and the most successful invaders often possess adaptations to disturbed habitats and protection from generalist predators (Mooney and Drake 1986).

Water fleas (Cladocera) possess life history characteristics that might make them successful invaders of new habitats. These characteristics include rapid life cycles, the ability to reproduce parthanogenetically, the production of resistant resting stages, and the production of defensive morphology, including the formation of spines, in the presence of vertebrate and invertebrate predators (Work and Gophen 1999). The recent invader *Daphnia lumholtzi* possesses defensive spines that are larger than any native daphnid species. Because of this morphology, *D. lumholtzi* might pose a significant risk to North American aquatic ecosystems by disrupting food chains and reducing feeding efficiency of planktiverous fish (Swaffer and O'Brien 1996, Kolar et al. 1997).

Daphnia lumholtzi is a native to Australia, southern Asia, and eastern Africa (Gophen 1979, Benzie 1988). It was first discovered in North America in 1991 in a small Texas reservoir (Sorensen and Sterner 1992). Since its initial discovery, *D. lumholtzi* has been found in reservoirs, rivers, and lakes in Alabama, Arkansas, Florida, Illinois, Kansas, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Ohio, Oklahoma, South Carolina, Tennessee, Texas, Utah, and recently in the Great Lakes (Sorensen and Sterner 1992, Havel and Herbert 1993, Stoeckel et al. 1996, Dzialowski et al. 2000, Muzinic 2000, USGS 2003). In field and laboratory experiments, Lennon et al. (2001) showed that *D. lumholtzi* becomes abundant in late season when water temperatures are above 25° C and that this increase often corresponds with a decline in native species. The authors suggested that water temperature is a factor in *D. lumholtzi* distribution, limiting it to areas where water temperatures remain above 10° C.

As part of a limnological monitoring project in Harlan County Reservoir in south-central Nebraska, we collected zooplankton and water quality data during 2002. Analysis of collections revealed *D. lumholtzi*, representing the first record of this species in Nebraska.

METHODS

Zooplankton samples were collected weekly from Harlan County Reservoir starting 9 May 2002 and ending 6 September 2002. The reservoir is located in south-central Nebraska between Republican City and Alma (Fig. 1) and covers more than 52 km^2 (13,000 surface acres) at conservation pool. The primary purpose

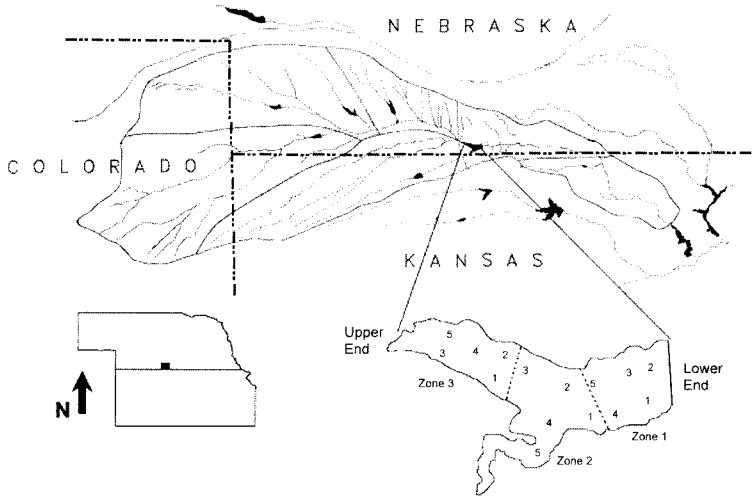

Figure 1. Map of Harlan County Reservoir showing locations of the three zones and five sample stations per zone used to collect zooplankton and limnological data.

of this reservoir is flood control, but it also is used heavily for recreation including fishing and boating. In addition, Harlan County Reservoir is operated for irrigation. Irrigation withdrawals from the reservoir often exceed 3.5 m vertically each year and reservoir filling is dependent on Republican River inflows. Consequently, reservoir elevations are highly variable.

For our study, the reservoir was divided into three zones with five stations in each zone (Fig. 1). At each station an 80-µm Wisconsin plankton net (0.5 m^2 opening) was towed vertically from the substrate to the surface. Samples were preserved in a 4% formalin and sucrose solution to prevent osmotic distortion (Haney and Hall 1973).

Zooplankton, including *D. lumholtzi*, were counted and identified to lowest possible taxon under 20-25X magnification with a Leica Stereomicroscope. Each station sample was diluted to 200 ml, from which four 1 ml subsamples were drawn with a Hensen-Stempel pipette. These samples were placed within the channel of a Ward counting wheel. Each 1 ml subsample was counted and identified individually and a mean was calculated for zooplankton per liter towed.

All observed *D. lumholtzi* from each station sample were measured with an American Optical compound light microscope under 40X power. The number

measured increased from 3 across all samples to more than 340 individuals (Table 2). Measurements were then converted to millimeters by using a Wards stage micrometer. *Daphnia lumholtzi* were measured by standard length (SL), body length (BL), and total length (TL) (Fig. 2). Water temperature and dissolved oxygen concentration were taken at 1 m depth intervals at all sampling stations on each date by using a YSI Model 55 dissolved oxygen meter. Means and standard deviations are given for body measurements and means and standard errors are given for water variables.

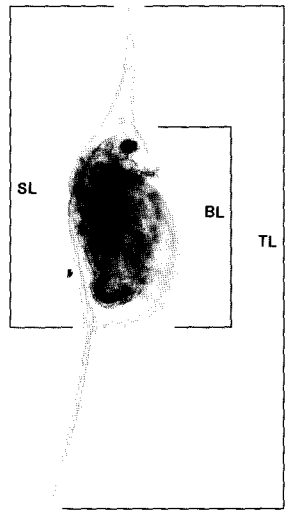

Figure 2. Measurements (mm) taken on *Daphnia lumholtzi*. SL = standard length, BL = body length, and TL = total length.

RESULTS

Daphnia lumholtzi was discovered in one station on 5 August 2002 at a density of 0.04 l^{-1}. The number of stations with *D. lumholtzi* increased until September 2002, when *D. lumholtzi* was found at all fifteen sampling stations at a mean density of 2.17 ± 3.10 l^{-1} and an individual station maximum density of 11.43 l^{-1} (Table 1). Native *Daphnia* (*D. pulicaria* and *D. retrocurva*) also were present in samples that included *D. lumholtzi*. *Daphnia pulicaria* constituted a low proportion of the total cladocerans and was found at only two stations from 5 August 2002 to 6 September 2002 (Fig. 3). *Daphnia retrocurva* was the most

Table 1. Number of sampling stations out of 15 and mean (± 1 S. D.) density of *Daphnia lumholtzi* per liter during 2002 for Harlan County Reservoir, Nebraska. Mean (± 1 S. E.) water temperature (°C) and dissolved oxygen concentration (ppm) of all sampling stations in Harlan County Reservoir for dates containing *D. lumholtzi*.

Date	# of Stations	Density (l^{-1})	Water temperature (°C)	Dissolved Oxygen (ppm)
August 5	1	0.04	25.1 ± 0.46	6.74 ± 0.29
August 19	6	0.04 ± 0.009	24.8 ± 0.55	
August 23	4	0.05 ± 0.019	24.5 ± 0.29	8.75 ± 0.27
August 28	10	0.20 ± 0.158	23.9 ± 0.49	6.57 ± 0.34
September 6	15	2.17 ± 3.104	22.9 ± 0.09	7.21 ± 0.11

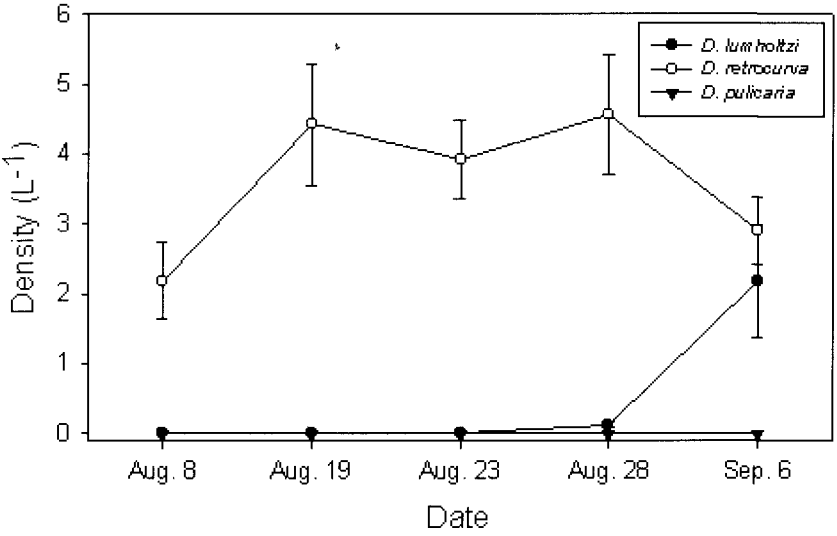

Figure 3. Mean (± 1 S. E.) density of zooplankton per liter from Harlan County Reservoir for five sampling dates in 2002 (15 samples per date). *Daphnia lumholtzi* is exotic while *D. pulicaria* and *D. retrocurva* are native species.

abundant cladoceran and was found at all fifteen stations during the time that *D. lumholtzi* was present. The proportion of *D. retrocurva* declined sharply between 28 August and 6 September at the same time that *D. lumholtzi* increased from about

3% to about 43% of the total cladocerans (Fig. 3). Mean water temperature declined between 8 August and 6 September. Mean dissolved oxygen fluctuated but remained between 75 and 95% of air saturation (Table 1).

The total length of *D. lumholtzi* ranged from 0.80 mm to 5.66 mm with a mean total length of 2.38 ± 1.11 mm per individual during the period when *D. lumholtzi* was most abundant on 6 September 2002 (Table 2). Body lengths were similar among sample periods except for 19 August when individuals tended to be smaller than in other samples (Table 2). The spine lengths accounted for between 57 and 65% of the total length of *D. lumholtzi*.

Table 2. Mean standard length, body length and total length (± 1 S. D.) of the exotic *Daphnia lumholtzi* and the native *Daphnia pulicaria* collected from Harlan County Reservoir, Nebraska during 2002.

	Daphnia lumholtzi				*Daphnia pulicaria*	
Date	Standard Length (mm)	Body Length (mm)	Total Length (mm)	Number measured	Standard Length (mm)	Number measured
August 5	1.50 ± 0.624	0.95 ± 0.401	2.16 ± 0.684	3	0.83 ± 0.316	350
August 19	1.22 ± 0.400	0.70 ± 0.196	1.96 ± 0.644	6	0.74 ± 0.222	350
August 23	1.74 ± 0.608	1.01 ± 0.346	2.91 ± 1.160	18	0.83 ± 0.261	350
August 28	1.64 ± 0.523	1.03 ± 0.307	2.81 ± 1.025	51	0.74 ± 0.200	350
September 6	1.40 ± 0.563	0.95 ± 0.390	2.38 ± 1.107	343	0.79 ± 0.253	350

DISCUSSION

Sampling results from May 2002 to September 2002 revealed that *D. lumholtzi* was only present during August and September samples collected from Harlan County Reservoir. Our sampling was terminated after 6 September 2002; thus, information on persistence of *D. lumholtzi* in the assemblage through the fall is not available. Studies conducted in Missouri and Kansas found similar results with *D. lumholtzi* only occurring during August through October (Havel and Herbert 1993, Dzialowski et al. 2000). In Illinois, *D. lumholtzi* populations peaked in June and July with a small peak in August and no *D. lumholtzi* observed in September or October (Kolar et al. 1997). On the border of Oklahoma and Texas *D. lumholtzi* populations peaked in early July with small numbers present through October (Work and Gophen 1999). These differences between studies might be explained by water temperature because *D. lumholtzi* reproduces more quickly at warmer

temperatures (Work and Gophen 1999, Lennon et al. 2001). Water temperatures during the period when *D. lumholtzi* was present ranged between 22.9 and 25.1° C for all stations (Table 2).

Our samples revealed increasing relative abundance of *D. lumholtzi* with the highest population density (2.17 ± 3.104 l^{-1}) found on the last sampling date. During the station samples of 5 August 2002, *D. lumholtzi* made up 0.1% of the total sampled *Daphnia* population with the native *D. retrocurva* accounting for 99.7%. However, a shift in *Daphnia* community structure was observed during the month of September as *D. lumholtzi* increased to 42.7% of the total *Daphnia* community (Table 1; Fig. 3). Based on this community trend, *D. lumholtzi* was probably the most abundant *Daphnia* species in late September in Harlan County Reservoir. This change in the composition of the *Daphnia* assemblage in Harlan County Reservoir could be the result of competition between species, selective fish predation on native species, or natural population declines in native species from abiotic factors (Dzialowski et al. 2000).

Daphnia lumholtzi is larger than most native species throughout its North American range (Lennon et al. 2001). Measurement of the Harlan County Reservoir population indicated that there were substantial differences in *D. lumholtzi* lengths during 5 August 2002 through 6 September 2002. During the period when *D. lumholtzi* was most abundant (6 September 2002), the smallest *D. lumholtzi* had a total length of 0.80 mm and the largest length was 5.66 mm. The only native species, which occurred in large numbers, was *D. pulicaria* that was approximately half the size of *D. lumholtzi* (Table 2). Our results for *D. lumholtzi* coincided with a study by Sorensen and Sterner (1992), who found maximum total lengths of 5.6 mm. Muzinic (2000) found total lengths of *D. lumholtzi* from the Great Lakes to range between 4.9 and 5.7 mm in length during August.

In our study, body lengths also were taken and results showed that body length ranged from 0.7 mm to 1.03 mm with a mean of 0.95 ± 0.39 mm during September. These results are different than the results found by Sorensen and Sterner (1992), who found a greater maximum body length of 1.8 mm. These measurements suggested that in Harlan County Reservoir the *D. lumholtzi* have relatively larger spines and smaller bodies than those observed by Sorensen and Sterner (1992) for populations from Texas. In our study, standard length measurement results ranged from 1.21 mm to 1.73 mm with mean of 1.40 ± 0.56 mm during September. These standard length measurements were similar to a study conducted by Swar and Fernando (1979), who found standard length measurements of *D. lumholtzi* ranging from 0.7 mm to 1.75 mm for *D. lumholtzi* within its native range in Nepal. Differences among populations should be examined to determine if they are caused by founder effects from establishment in new water bodies, or if the differences are caused by biotic and abiotic factors.

Daphnia lumholtzi's dispersal within North America reservoirs might occur through recreational boating from initially infested reservoirs to nearby reservoirs

(Havel and Hebert, 1993), or by non-human dispersal mechanisms such as waterfowl, wind, flowing water, and fish dispersing the resistant eggs (Dodson, 1992). A study conducted by Stoeckel et al. (1996) concluded that Midwestern river systems might serve as "dispersal highways" for *D. lumholtzi*, allowing them to drift or be transported to uninhabited areas throughout North America. While all the above methods are possible, the mechanism for *D. lumholtzi*'s establishment into Harlan County Reservoir is still uncertain. Dzialowski et al. (2000) concluded that most dispersal events in Kansas were attributed to recreational boating. Research currently is being conducted to determine *D. lumholtzi* presence in other Nebraska reservoirs on the Republican River Drainage (B. Peterson, unpublished data).

The impact of *D. lumholtzi* on native zooplankton communities is not known. However, *D. lumholtzi* has the potential to disrupt the structure of native zooplankton communities (Havel et al., 1995). As part of an ongoing study of Harlan County Reservoir, the feeding preference of gizzard shad (*Dorosoma cepedianum*), a food fish for walleye (*Stizostedion vitreum*) and white bass (*Morone chrysops*), is being examined to determine if their diet contains *D. lumholtzi*. One possibility is the large size of *D. lumholtzi* spines prevents small planktivores from eating it (Havel et al. 1995). Other studies should be implemented to determine the impacts *D. lumholtzi* might have on zooplankton and fish communities in Harlan County Reservoir and other irrigation reservoirs.

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