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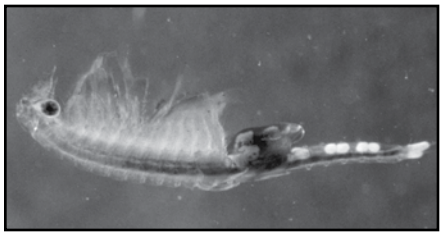
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Determining the Presence of Fairy Shrimps (Crustacea: Anostraca) at Ephemeral Pond Sites in Wisconsin

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Abstract

A spring sampling program for adult anostracans documented that the chirocephalid species *Eubranchipus bundyi* Forbes 1876 has been present at two adjacent vernal ponds in Waukesha County, Wisconsin, since 1994. A monthly photomonitoring program begun in 1998 recorded the pond-to-pond and year-to-year differences in the annual seasonal cycle at this ephemeral wetland site. Core samples taken in November 2004 from the dried sediments in these two pond basins and processed in the laboratory yielded anostracan cysts, confirming a reserve capability of these wetlands to produce fairy shrimps from the underlying cyst banks when future conditions favor their hatching.

Front cover photo: An adult Eubranchipus bundyi*, Wisconsin's most frequently collected fairy shrimp.*

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Introduction

Northern temperate forest fairy shrimps (Crustacea: Anostraca) have life cycles whose seasonal stages are inextricably tied to vernal pools (Broch 1965). Within a few weeks after mating in spring, females release encysted eggs, which sink to the bottom of the pond. The drying of the pool and the progression of the seasons provide the correct sequence of physicochemical conditions that cause the encysted eggs to undergo a pre-hatch in the months prior to snowmelt and spring rain, which are the final stimulus for hatching.

Among a vernal pool's many inhabitants, fairy shrimps are unique in that their life cycle is strictly dependent on the drying of the pool. This is so because the physiological development of the resting egg or cyst has been adapted to periodic dryness (Mossin 1986). While adults of these species may be present for only a few weeks out of the year, it is the cysts that are the persistent element at the pond site from one season to the next (possibly even over multiple years), and in that way they are analogous to the seed bank that exists in the soil for an ephemeral pond's vegetation.

Recently Beladjal and Mertens (2003) showed how identification of the anostracan fauna of ephemeral pools in the Sahara was possible using the method of retrieval and identification of cysts preserved in the sediments from these pools. Their research was based on prior studies that had determined species-specific traits of the cysts produced by the fairy shrimps of that region.

In a review based largely on specimens in the collections of the Milwaukee Public Museum (MPM) and the Science Museum of Minnesota, Jass and Klausmeier (2002) reported three anostracans from Wisconsin: *Eubranchipus bundyi* Forbes 1876, *Eubranchipus ornatus* Holmes 1910, and *Eubranchipus serratus* Forbes 1876, all in the family Chirocephalidae. Belk, et al. (1998) reviewed the cyst traits for members of the genus *Eubranchipus* and showed that, at least for some of them, sufficient differences exist to make distinctions at the species level. Therefore, this study was conducted to test whether retrospective identification of the anostracan fauna that had been present in vernal ponds in this region might also be achieved, since the members of the Wisconsin fauna have been determined and their species-specific cyst traits are known.

Mura (2004) showed that cyst banks played a fundamental role in the survival strategy of a temporary pool fairy shrimp in Italy and detailed a procedure for sampling the bank and retrieving the cysts. This process allowed the presence of the species to be determined, even after the habitat had dried out, and offered the potential of an additional much needed tool for identification of those sites whose conservation is crucial to the survival of the obligate fauna of ephemeral wetlands.

Relatively undisturbed ephemeral ponds have become rare in Wisconsin woodlands because of extensive disturbances to, and loss of, the forests, especially in the most populated regions of the state (Reinartz 2003). Because of some tendency to overlook the importance of these small wetlands in the face of increasing land use demands, ephemeral ponds may be disappearing from the landscape at a faster rate than other habitat types. So there may be some increased urgency in the need for identifying such sites and setting priorities for conserving those of greatest significance.

Wisconsin has recently drafted a list of species of greatest conservation need (Wisconsin DNR 2005) and the three members of the genus *Eubranchipus* reported from the state have been included in this listing. Many small intermittent wetlands may dot the landscape in spring, with a wide variety of aquatic species making use of them, but only some of them harbor fairy shrimps. Due to the short life span of the adults, it is possible to sample Wisconsin ponds for fairy shrimps during only a few weeks of the year. This study, however, was done to demonstrate that the suitability of a particular site for anostracans may be determined even when the pond has dried completely. Because they combine a dependency on a pool's ephemeral nature with the production

of cysts which show species-specific traits at the ultrastructural level, the anostracans themselves can offer the means of identifying sites of greatest conservation need. In situations where research has been able to link these components together, it has facilitated the setting of priorities for conservation of a habitat type which is increasingly coming under pressure in Wisconsin due to expansion of agriculture or urbanization.

Since prior researchers (Belk, et al. 1998) had identified distinctive differences in the ultrastructural characteristics of cysts taken from females of each of these *Eubranchipus* species in the lab, our goal was to test this process in the field by combining springtime collection of anostracan adults with cyst bank sampling of the pond sediments at the same ephemeral wetland locality later in the year when the ponds had dried. By using this combination of adult morphology and cyst traits, our test goal was to confirm that cyst bank sampling is a realistic technique for identifying those ephemeral wetland sites that have harbored fairy shrimp populations in the springtime, even during that 80% of the year when no adult anostracans are present.

Methods and Materials

Cyst Bank Sampling

The presence of *Eubranchipus bundyi* has been documented at two adjacent Waukesha County ephemeral ponds (T07N, R20E, Sec.25 SW) since 1994 by means of dipnet sampling in spring. Adult fairy shrimps were not present in samples from these ponds in each year of the past decade, but immatures were collected at the site as recently as March 2003. In November 2004, we took sediment samples from the dried pond centers by means of a cylindrical 10 cm x 6 cm core sampler. Cores were wrapped in aluminum foil and placed in labeled plastic bags on site and later stored in the dark at 40° F to minimize alteration from initial conditions. Following the procedures of Mura (2004), in the laboratory we sliced each core into 2.5 cm thick sections and retrieved intact cysts by rinsing the sediments through a 150 micron mesh size sieve. Cysts were then dried and mounted on stubs for scanning with MPM's Zeiss EVO 40 scanning electron microscope (SEM). For comparison with material retrieved from the site sediments, we removed encysted eggs from adult female specimens of other members of the genus *Eubranchipus* in the MPM Invertebrate Zoology collection and scanned them using the same SEM.

Ponds Study

Since the beginning of 1998, we recorded the water level status of these two ephemeral ponds on a monthly basis. The larger (south) pond of the two covered only about $^2/_3$ of 1 ha, even at its greatest extent, and the smaller (north) pond was slightly less than a third of that size.

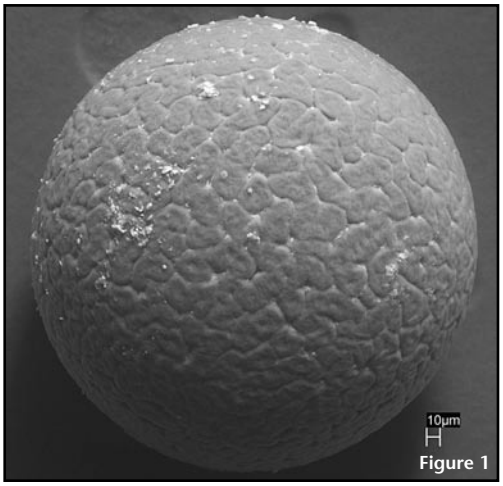
Results and Discussion

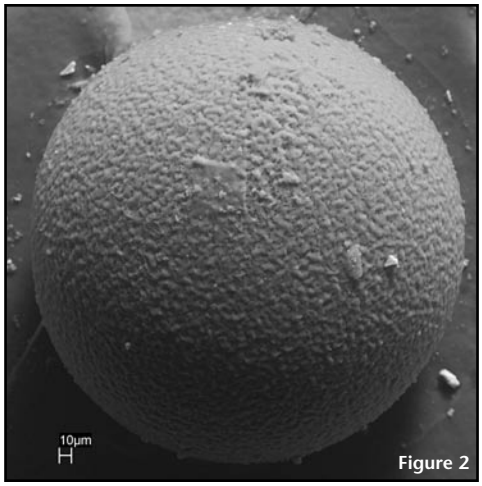
Cyst Bank Sampling

Cores from both north and south pond sediments yielded encysted anostracan eggs. We retrieved intact and broken cysts from the 3 vertical strata sampled: 0-2.5, 2.5-5.0, and 5.0-7.5 cm deep.

Figure 1 presents a scanning electron micrograph of one of the intact cysts. Looking much like that of a brain coral, the surface patterning on these retrieved cysts most closely corresponds to that for *Eubranchipus bundyi* (Belk, et al. 1998), whose existence at this ponds site has been documented by prior spring collecting of adults. Jass and Klausmeier (2002) report this as the most frequently collected anostracan species in Wisconsin, with records from almost every county.

Comparison to cyst surface patterns for other commonly reported Midwestern anostracans (Belk, et al. 1998 and Figures 2-4) confirms that the cysts from these pond core sediments are indeed from *Eubranchipus bundyi*





PHOTOS: MILWAUKEE PUBLIC MUSEUM

Figure 1. *Scanning electron micrograph of encysted* Eubranchipus bundyi *egg collected November 2004 by Barbara Klausmeier and Joan Jass from ephemeral pond sediments in Waukesha County, Wisconsin.*

Figure 2. *Scanning electron micrograph of encysted egg from mature* Eubranchipus ornatus *collected May 1997 by Dale Chelberg from an ephemeral pond in Renville County, Minnesota.*

and not other members of the genus. The fairy shrimp reported as being the next most frequently collected, both in Minnesota and Wisconsin, is *E. ornatus* (Jass and Klausmeier 2002). The cysts retrieved from the sediments do not match that species' cyst wall, which exhibits a granular unevenness (Figure 2) instead of brain-coral-like appearance. A third species, that we have collected from only a very few Wisconsin counties, is *Eubranchipus serratus*. Figure 3 shows the filaments projecting above the cyst surface of this species, which are not part of the ultrastructural sculpturing seen in *E. bundyi*.

Older records in the literature such as Creaser (1930) and Dexter (1946) have referred to the most common fairy shrimp of the Midwest (but not reported from Wisconsin) as being the species *Eubranchipus vernalis*. Belk, et al. (1998) demonstrated that this common Midwestern anostracan is actually *E. neglectus*. The scanning electron micrograph (Figure 4) of an encysted egg removed from a mature female of this species showed the deeper surface grooving in comparison to that of *E. bundyi*, a distinctive contrast also referred to by Belk, et al. (1998).

This process of retrieving and identifying cysts from the dried sediments of these pond basins supports the usefulness of cyst bank sampling as a tool for confirming the presence of fairy shrimps at ephemeral pond sites. Brendonck and De Meester (2003) advocated using the long-term persistence of the cyst bank as a source of information pertinent to the process of wetland conservation and restoration.

Ponds Study

Table 1 presents 6 years (1998-2003) of monthly monitoring data from the two ponds. In spite of the proximity of the ponds to each other (actually joining in flood years), minor hydrological differences between them result in their generally having different water fill patterns during the same annual precipitation cycle. Only in 2002 did the ponds have identical water fill patterns.

Over this entire span of time, the ponds have been dry (D) 53.4% of the time. The annual pattern of dryness, however, has varied greatly from year to year, from a high of 79.2% in the drought year of 2003 to a low of 37.5% in the high precipitation year of 1999, when the

Table 1. Annual cycle at two adjacent vernal pond sites. D = Dry, I = Ice, W = Water, 0 = no available data.

		Month											
Year	**Pond**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**June**	**July**	**Aug.**	**Sept.**	**Oct.**	**Nov.**	**Dec**
1998	South	D	W	I	W	W	W	D	W	W	W	W	D
	North	D	W	I	W	W	D	D	W	W	D	D	D
1999	South	I	W	W	W	W	W	W	W	W	D	0	0
	North	I	W	W	W	W	W	W	W	D	D	0	0
2000	South	D	W	D	D	W	W	W	W	W	W	I	I
	North	D	D	D	D	W	W	W	W	W	W	I	I
2001	South	I	I	I	W	W	W	W	D	D	D	D	D
	North	I	I	W	W	W	W	D	D	D	D	D	D
2002	South	D	D	W	W	W	W	D	W	D	D	D	D
	North	D	D	W	W	W	W	D	W	D	D	D	D
2003	South	D	D	D	D	W	W	D	D	D	D	D	D
	North	D	D	W	W	W	D	D	D	D	D	D	D

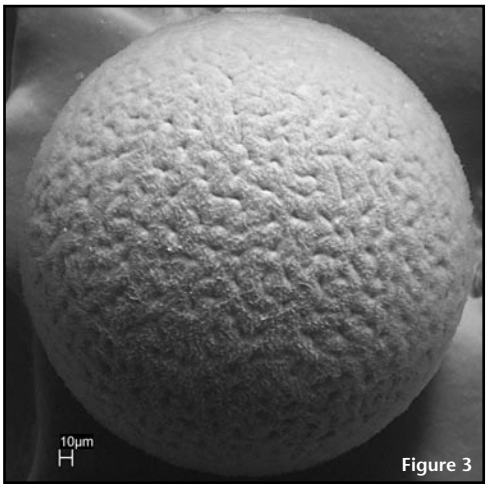


Figure 3

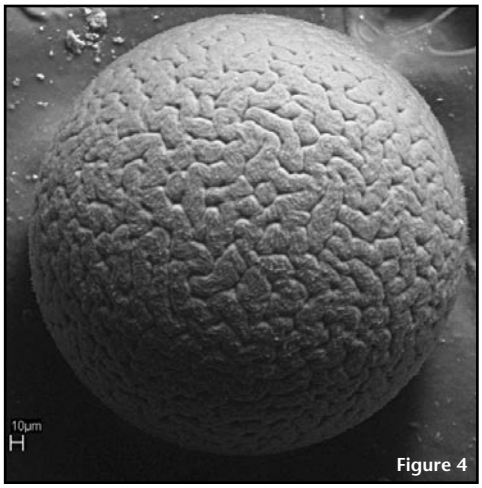


Figure 4

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Figure 3. *Scanning electron micrograph of encysted egg from mature* Eubranchipus serratus *collected April 1993 by Joan Jass from an ephemeral pond in Racine County, Wisconsin.*

Figure 4. *Scanning electron micrograph of encysted egg from mature* Eubranchipus neglectus *collected March 1995 by Barbara Klausmeier and Joan Jass from an ephemeral pond in Switzerland County, Indiana.*

longest continuously wet period occurred. That year the south pond was ice-covered in January and water-filled through September. Each pond experienced one exceptionally long (8 months) dry period, but in different years. From July 2001 through February 2002, the north pond was dry. But for the south pond, a similar 8-month span occurred between September 2002 and April 2003.

Colburn (2004) has proposed a hydrological classification of vernal pools based on their average duration of flooding. The year-to-year variability of the ponds at our site precludes an easy fit into his proposed categories. In 1998 and 2002, both ponds had a mid-season dry period. In all other years, however, no such interruption occurred. The only simple generalization that can be made is that the ponds are truly ephemeral in nature, never failing to fill with water for at least a portion of the year but never remaining water-filled for longer than an 8-month span, during this monitoring period. For anostracans to survive in a habitat like this test site, where there is such high variability in the water fill pattern from year to year, requires not only the production of encysted eggs whose hatching is correlated to the usual annual cycle of changes in the physical and chemical conditions in the pond sediments but also the ability for those cysts to persist over a longer term when necessary, forming a cyst bank in the pond basin. One study that demonstrated how this might occur was made by comparing differences in hatching patterns of the anostracan populations in 5 Minnesota ponds (Jass and Klausmeier 2002). This research showed that cysts in a portion of a pond basin that had been protected from experiencing the factors triggering the final phases of hatching were capable of remaining viable in the sediments until a time when the proper sequence of conditions did occur to provide that trigger.

We hope this current demonstration will offer an additional tool facilitating the identification of similar sites throughout the state that harbor these Wisconsin crustacean species of greatest conservation need. As Belk (1998) emphasized when reviewing approaches to conserving the invertebrate fauna of ephemeral wetlands, the focus "should be directed at actions threatening the diapausing population in the cyst bank."

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About the Authors

Joan Jass studied zoology at the University of Wisconsin-Milwaukee and is MPM's Curator of Non-insect Invertebrates, overseeing a collection of over 200,000 specimens. Her current research interests center on the natural history of crustaceans and mollusks in Wisconsin. Additional information on these groups may be found on the MPM website (**www.mpm.edu/collections/invertebrates/**) or by contacting her at jass@mpm.edu or (414) 278-2761. Barbara Klausmeier is a research assistant with MPM's Invertebrate Zoology section and in that capacity has coauthored dozens of publications, focused chiefly on crustaceans.

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