

## INVERTEBRATE ASSEMBLAGES AND TRACE ELEMENT BIOACCUMULATION ASSOCIATED WITH CONSTRUCTED WETLANDS

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**Abstract:** Invertebrate assemblages were studied in eight monoculture wetland mesocosms constructed for wastewater treatment. Low concentrations of dissolved oxygen (D.O.) were measured in bulrush mesocosms while higher concentrations of D.O. were measured in open water mesocosms containing submerged pondweeds. Invertebrate taxa richness was positively related to D.O. concentrations that were, in turn, related to vegetation communities. Reference wetland sites contained a variety of plant species along with extensive open water areas. Invertebrate taxa richness was greater at reference sites than in any wastewater mesocosm. Invertebrate samples from the wastewater mesocosms and reference sites were analyzed for five trace elements. While the concentrations of aluminum, arsenic, mercury, and silver were below values harmful to wildlife, the concentrations of selenium reached levels of moderate concern on one occasion. Data from this study suggest that selenium bioaccumulation by invertebrates may be related to the type of vegetation community or detrital habitat type. Wetlands designed for invertebrate production for waterfowl should take into account the potential for low D.O. concentrations and trace element bioaccumulation associated with vegetation community types.

**Key Words:** constructed wetlands, bioaccumulation, invertebrates, trace elements, wastewater

### INTRODUCTION

The recent national and worldwide awareness of the value of wetland systems offers opportunities to improve wetland understanding and design. Both natural and constructed wetlands are important for providing habitat and food for many types of wildlife, fish, waterfowl, shorebirds, and migrating neotropical birds. Of the approximately 600 bird species in North America, about 200 species are either partially or wholly dependent on wetlands for some part of their life history (Kroodsma 1978).

Along with treating wastewater or water of poor quality, many constructed wetlands are built for restoration of lost or degraded habitat for fish, waterfowl, and wildlife. Characterizing the success of these constructed wetlands has been difficult, with ambiguous terms such as “equivalent, high quality, or successful” used in describing endpoints. Some characterizations have been more quantitative and have used parameters such as site morphology, water depth, soil properties, water quality, and vegetation type and structure

(Brown 1991). Invertebrate importance in wetland functioning and as a food resource suggests that this community should also play a role in wetland comparisons (e.g., Streever et al. 1996).

Macroinvertebrates aid in the transfer of nutrients from the sediments, detritus, and the water column to higher level organisms. Invertebrates are critical to energy dynamics and functions of wetlands and form the foundation of wetland food chains (Hammer 1989). Aquatic invertebrates, particularly insects, are important in the diets of ducks and many other bird species (e.g., Schroeder 1973). Ducklings may particularly rely upon insects as food during early life stages (Bé langer and Couture 1988, King and Wrubleski 1998). However, little is known about macroinvertebrate communities in natural wetlands, and less is known about those in constructed wetlands.

Because of the ecological importance of aquatic invertebrates, their habitat requirements should be given consideration in the design of wetlands constructed for wastewater treatment. It is well known that excessive nutrient loading and anaerobic conditions often limit

the success of wetlands as invertebrate habitat (Brightman 1984, Schwartz and Gruendling 1985, Best 1994, Frydenborg *et al.* 1994). Types of sediment and vegetation may also affect invertebrate communities (Voights 1976, deSzalay and Resh 1996, Scatolini and Zedler 1996).

Wetlands receiving wastewater may also be prone to the bioaccumulation of toxic materials by food web organisms, with subsequent magnification in higher trophic levels. Changes in reproductive activity, including decreased egg hatching success, are often associated with bioaccumulation of hydrocarbons and heavy metals in aquatic organisms (see review by Dillon and Gibson 1985). Changes in community structure may be caused by bioaccumulation of substances within wetland food webs (Godfrey *et al.* 1985).

The purpose of this study was to analyze physical and chemical factors in constructed wetlands receiving treated municipal wastewater and relate them to invertebrate assemblages. Trace element concentrations found in invertebrates in wastewater wetlands, important but under-reported data, were also documented. These data may provide guidance for designing constructed wetlands for invertebrate production and waterfowl use.

## METHODS

### Study Sites

Studies were conducted in August 1997, May 1998, and August 1998 in eight constructed wetland mesocosms (31.2-m  $\times$  12.1-m) with four duplicate planting schemes at the Southside Water Reclamation Plant (SWRP) in Albuquerque, New Mexico, USA (see Glass *et al.* 1996 for a detailed description of the mesocosms). This allowed for study of invertebrates associated with open water (planted with sago pondweed, *Potamogeton pectinatus* L., and colonized by duckweed, *Lemna* spp.), creeping spikerush (*Eleocharis macrostachya* Britt.), three-square bulrush (*Schoenoplectus pungens* Vahl.), and softstem bulrush (*Schoenoplectus tabernaemontani* Gmel.). Treated SWRP effluent was used as the water source, and equivalent hydraulic residence times were provided to the wetland mesocosms. Nutrient concentrations entering the mesocosms were generally moderate with mean total Kjeldahl nitrogen of 6.9 mg L<sup>-1</sup> for 1997 (n = 19) and 6.1 mg L<sup>-1</sup> for 1998 (n = 10). Mean ortho-phosphate concentrations of 2.9 mg L<sup>-1</sup> for 1997 (n = 10) and 3.3 mg L<sup>-1</sup> for 1998 (n = 10) were also moderate (McCulley, Frick, and Gilman, Inc., unpublished data). Mesocosm construction was of concrete, as was the ~1-m-high perimeter wall surrounding the mesocosms. Individual mesocosms enclosed an area of

378 m<sup>2</sup> and were provided with separate inlets and outlets. Water from softstem bulrush mesocosms, however, flowed into the open water mesocosms to produce the desired residence time. Mesocosms were lined with geomembrane material and backfilled with a local soil amended with 25% organic material. Plant species was the primary variable among mesocosms. Water depth varied according to plant requirements. Average height of spikerush plants ranged from 13 to 45 cm, water depth 10 cm; three-square bulrush from 72 to 80 cm, water depth 20 cm; and softstem bulrush from 87 to 171 cm, water depth 35 cm. Open water mesocosm depth was about 76 cm. Vegetation was planted in 1995 with greenhouse reared wetland plants from local sources.

Wetlands supplied by ground water were constructed and used as reference wetlands for comparing constructed wetlands with and without treated wastewater. Reference wetlands were similar to wastewater wetlands in landscape setting, plant species, water source, and hydrodynamics. Reference wetlands were a few kilometers north of the SWRP site at the Rio Grande Nature Center. Both wastewater and reference wetlands were next to the Rio Grande River, which served as an invertebrate colonization source for both wetland areas. Reference sites contained mixed emergent plant species and large areas of open water containing submerged vegetation. Plants came directly from a local donor wetland and included species used in the wastewater wetlands at the SWRP. Both reference wetlands were lined with geomembrane material; one wetland was built in 1982 and the other in 1995. Water levels in reference wetlands were relatively constant and maintained with water inlets and outlets. Ground water is used by the city of Albuquerque for its water supply and therefore was the fundamental water source for all the wetlands in this study. Reference wetlands were stocked with mosquito fish (*Gambusia*) for mosquito control.

### Sampling Methods

Aquatic invertebrate samples were collected with a D-frame sweep net (700  $\mu$ m mesh). A 1-minute timed sweep through the habitat was used to collect organisms for the community evaluation. During August 1997, invertebrates from duplicate plant mesocosms were combined for 1-minute collections; collections were from individual mesocosms on other dates, producing a total of 22 samples. Sweep nets were also used throughout the mesocosms in the collection of 5-g (wet weight) samples of macroinvertebrate organisms for analysis of trace element content. Materials collected for trace element analyses were from combined duplicate mesocosms because of the difficulty in

collecting adequate biomass. Collection of sufficient invertebrate mass took an average of 1.8 hr per sample.

Organisms collected for community evaluation were preserved in a 10% formalin solution and processed and identified to the lowest practical taxon in the laboratory. Macroinvertebrates collected for analysis of trace element content were picked from material on site, rinsed with deionized water, and frozen in ziplock plastic bags on dry ice for shipment to the laboratory. Trace elements selected for analysis were of local concern [silver (Ag), arsenic (As), and aluminum (Al)] and those with food-web biomagnification potential [mercury (Hg) and selenium (Se)]. Silver and Al were analyzed by Inductively Coupled Plasma/Emission Spectrometer, As and Se with graphite furnace, and Hg with cold vapor atomic absorption. Dissolved oxygen and pH were measured routinely at mid-depth between 10 a.m. and 2 p.m. by on-site personnel or with data-logging probes during site visits. Plant height and area occupied by plants at the water surface were measured in five randomly selected 0.06-m<sup>2</sup> grids in wastewater mesocosms. Open water area covered by duckweed was visually estimated. Height and area of plants in reference wetlands were estimated with maps of vegetation distribution and data from corresponding wastewater mesocosm plants.

We used canonical correspondence analysis (CCA) and biotic indices of abundance and taxa richness to compare invertebrate assemblages. CCA was used to explore relationships between invertebrate assemblages and their environment (ter Braak and Verdonschot 1995). CCA axes are selected as linear combinations of environmental variables that maximize dispersion of species scores. First axis scores are produced by iterations between site scores based on weighted species scores and species scores based on weighted site scores (Gauch 1982). Multiple axes may be extracted using the same iterative method but correcting for the first and succeeding axes. Infrequent taxa (taxa contributing  $\leq 0.4\%$  of total numbers counted) were deleted and data transformed [square root] before analysis. Partial CCA was used to eliminate effects of covariables that expressed year-to-year and May-to-August differences and to relate variation to water quality and vegetation characteristics. Forward selection of environmental variables and Monte Carlo permutations were used to determine whether variables exerted a significant effect on invertebrate distributions. We also examined the trophic structure of the macroinvertebrate assemblages by grouping taxa into three categories (detritivores, predators, and herbivores) using information in Merritt and Cummins (1984), Pennak (1989), and deSzalay and Resh (1996). Spearman rank correlation was used to test for relationships between

D.O. and invertebrate (uncensored data) taxa richness and abundance.

## RESULTS

### Environmental Data

Dissolved oxygen measurements at reference wetlands ranged from 7.5 to 9.4 mg L<sup>-1</sup> during the study. Dissolved oxygen averaged from single measurements taken over 1-month periods in wastewater wetlands were typically lower (Table 1). Water quality at wastewater wetlands was affected in July–August 1997 when SWRP malfunctions resulted in anoxic conditions in wastewater mesocosms (McCulley, Frick, and Gilman, Inc., unpublished data).

The pH parameter was eliminated before CCA analysis because of a high positive correlation ( $r = 0.75$ ,  $P = 0.0001$ ) with D.O. It is unlikely that pH would have any affect on invertebrate distributions in this study since values were typically circumneutral (pH 6–8, Table 1). Forward selection of variables indicated that D.O. ( $P = 0.001$ ) and area occupied by plants at the water surface ( $P = 0.026$ ) (Table 1) were highly significant variables in the CCA model (Figure 1). Plant height (Table 1) was also significant at the 10% level ( $P = 0.097$ ) and therefore retained in the model.

### Invertebrate Data

Fifty-four invertebrate taxa were identified and 12,284 individuals counted from collected samples. Results of CCA for the wetland fauna had eigenvalues of 0.39 and 0.14 for the first two axes and explained 33.4% of the variation. Monte Carlo tests were significant for the first and succeeding canonical axes ( $P \leq 0.002$ ). A CCA triplot of invertebrates, sites, and environmental data showed a distinction between reference site assemblages and most of the wastewater wetland mesocosms (Figure 1).

Taxa loading negatively on Axis I and common at reference sites included amphipods, Chironomini midges, and *Callibaetis* mayflies. These taxa were likely responsible for the ordination discrimination along the first axis. Physidae snails, Erpodellidae leeches (*Mooreobdella* sp. was identified from August 1998 samples), and *Culex* were weighted toward the positive end of Axis I and were common in wastewater mesocosms. Differences in taxa richness and abundance between reference and wastewater sites were apparent (Figure 2), with higher values found at the reference site. Taxa richness and abundance were lower in wastewater wetland mesocosms in August of 1997 (Figure 2) perhaps related to the SWRP malfunction.

The percent detritivores at the reference site in May

Table 1. Measured environmental parameters at wetland sites (mean and standard error except pH, for which range is reported). Values are from data collected up to 1-month before invertebrate sampling. The designation A and B refers to duplicate wastewater wetland mesocosms.

Site	Vegetation Height (cm)			Area of Vegetation (m <sup>2</sup> ) per m <sup>2</sup> of Surface Area			pH (S.U.)			Dissolved Oxygen (mg L <sup>-1</sup> )		
	8/97	5/98	8/98	8/97	5/98	8/98	8/97	5/98	8/98	8/97	5/98	8/98
Reference	38.7	12.0	15.0	0.0073	0.0067	0.0025	8.0–8.5	9.0–9.4	7.5–9.0	8.2 (0.2)	9.1 (0.1)	8.2 (0.7)
Inflow							7.1–7.2	7.0–7.6	6.9–7.7	3.7 (0.1)	4.4 (0.1)	4.8 (0.2)
Spikerush A	33.3 (13.4)	17.4 (5.3)	43.0 (4.7)	0.0005 (0.0002)	0.0021 (0.0011)	0.0018 (0.0007)	6.9–7.5	7.1–8.3	7.0–7.3	0.3 (0.0)	12.2 (2.2)	5.4 (0.7)
Spikerush B	39.3 (6.2)	8.9 (2.4)	47.5 (5.8)	0.0015 (0.0006)	0.0013 (0.0006)	0.0037 (0.0018)	6.9–7.6	7.5–8.8	7.0–7.3	0.4 (0.1)	5.8 (0.4)	6.8 (1.3)
Three-square A	84.2 (6.0)	88.6 (3.4)	71.0 (9.9)	0.0148 (0.0038)	0.0560 (0.0085)	0.0111 (0.0032)	6.9–7.5	7.3–7.4	7.2–7.3	0.3 (0.0)	3.6 (0.6)	0.4 (0.0)
Three-square B	73.2 (13.1)	70.4 (7.7)	73.8 (7.0)	0.0065 (0.0017)	0.0399 (0.0080)	0.0129 (0.0050)	6.9–7.4	7.1–7.3	7.1–7.2	0.3 (0.0)	0.9 (0.4)	0.3 (0.0)
Open water A	0	0	— <sup>a</sup>	1	1	— <sup>a</sup>	7.0–7.5	7.2–7.4	— <sup>a</sup>	0.3 (0.1)	0.3 (0.1)	— <sup>a</sup>
Open water B	0	0	0	1	0.2	1	7.0–7.6	7.8–8.2	7.2–7.3	0.3 (0.0)	8.4 (2.4)	0.3 (0.0)
Soft-stem A	130.0 (12.7)	117.3 (14.7)	167.6 (8.7)	0.0399 (0.0216)	0.1774 (0.0591)	0.0204 (0.0047)	6.8–7.4	7.1–7.6	6.8–7.1	0.3 (0.0)	0.4 (0.1)	0.2 (0.0)
Soft-stem B	102.0 (14.3)	66.7 (17.2)	164.4 (4.4)	0.0199 (0.0092)	0.0198 (0.0127)	0.0528 (0.0183)	6.8–7.3	7.0–7.7	6.9–7.1	0.3 (0.0)	0.3 (0.1)	0.2 (0.0)

<sup>a</sup> Mesocosm was dry on this sampling date.

and August of 1998 ranged from 88.8 to 88.9%. Variation at the wastewater wetlands was greater, with mean percent detritivores in May of  $94.2 \pm 1.6\%$ , decreasing to  $71.6 \pm 5.4\%$  by August. The differences between reference and wastewater wetland assemblages suggested by CCA (Figure 1) were evident in taxa making up the different trophic groups. Detritivores at reference sites were numerically dominated by amphipods, while those at wastewater wetland sites were often oligochaetes or pulmonate snails. Likewise, predators differed, with diverse assemblages of dytiscid beetles and odonates common at reference sites and leeches found only at wastewater wetlands. Herbivores were rare at all sites, making up less than 3% of organism numbers at all sites.

#### Relationships Between Wetlands and Invertebrate Assemblages

Variations in D.O. at the wastewater wetlands seemed to affect macroinvertebrates; D.O. was positively correlated with taxa richness (2-tailed test,  $r = 0.51$ ,  $P < 0.05$ ,  $n = 19$ ) but not with abundance ( $r = 0.13$ ). Sometimes mesocosms with low taxa richness had high abundance of a particular organism. Softstem bulrush mesocosms, for example, often had low taxa richness but high numbers of oligochaetes. Dissolved

oxygen was significantly correlated with taxa richness even when low D.O. data recorded during the wastewater plant upset in 1997 were dropped from the analysis ( $r = 0.45$ ,  $P < 0.10$ ,  $n = 15$ ). Influent D.O. concentrations were typically  $\sim 4.0$  mg L<sup>-1</sup> and seemed to be differentially modified by mesocosm plantings (Table 1). This relationship between D.O. and mesocosm plantings was also suggested by the CCA analysis (Figure 1), where plant height was almost directly opposed to D.O. The negative relationship of plant height to D.O. may be related to shading of oxygen-producing submerged plants and production of oxygen-consuming biomass. In spikerush mesocosms, D.O. usually increased after passage through the mesocosms, while it decreased in three-square, softstem, and the open water mesocosms on most occasions (Table 1).

The single open water mesocosm data point near reference sites on the CCA graph was unique in May 1998, with many Chironomini, *Ephydra*, and Cladocera present, along with the mayfly *Callibaetis*. Except for this single mesocosm in May, open water sites were typically covered by dense growth of duckweed. In May, large open areas with abundant submerged *Potamogeton* were present along with higher D.O.s (Table 1). Spikerush mesocosms also usually maintained higher D.O. concentrations and often contained

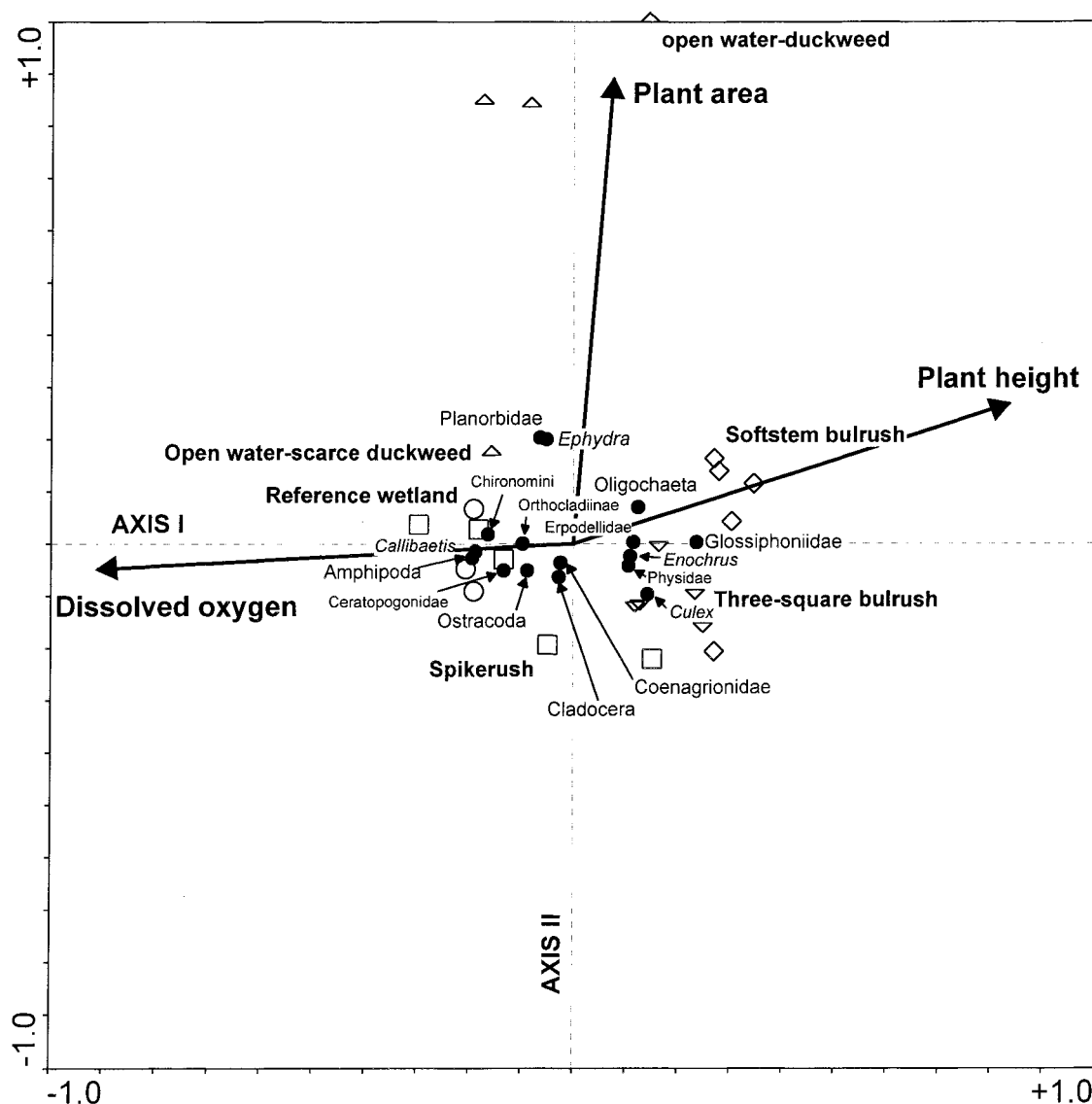


Figure 1. Species conditional triplot based on a canonical correspondence analysis (CCA) of wetland macroinvertebrate data with respect to environmental variables. The eigenvalues of axis 1 (horizontally) and axis 2 (vertically) are 0.39 and 0.14, respectively. Sites are labeled as open water ( $\Delta$ ), softstem ( $\diamond$ ), three-square ( $\nabla$ ), spikerush ( $\square$ ), and reference ( $\circ$ ). Species are represented as small filled circles and only those species that occurred 10 or more times and whose fit to the diagram is  $>5\%$  are shown. Partial CCA was used to eliminate effects of covariables that expressed year-to-year and May-to-August differences and to relate variation to water quality and vegetation characteristics. Concentrations of D.O., plant surface area, and height of vegetation were related ( $P < 0.10$ ) to community attributes as shown by the environmental arrows.

taxa (e.g., cladocera, ostracoda, and Planorbidae snails) that were found in reference wetlands.

Largely ubiquitous taxa such as Physidae snails and Erpodeiidae leeches were associated with many of the wastewater mesocosms (Figure 1). Some intra-site scatter in the CCA (Figure 1) may have occurred because of differences in mesocosm treatments. One spikerush mesocosm was burned in the spring of 1998 while the other was not, and one spikerush mesocosm was host to a family of ducks. Drain repairs in the

Spring of 1998 required water drawdown in a single open water mesocosm.

The invertebrate assemblage associated with open water mesocosms covered with duckweed appeared to form a distinctive group, high on Axis II (Figure 1). Water surfaces of these mesocosms were covered with plant material and low in D.O. (Table 1). Taxa richness and organism abundance were also low (Figure 2).

Invertebrates associated with softstem bulrush were also unique. This group contained high numbers of

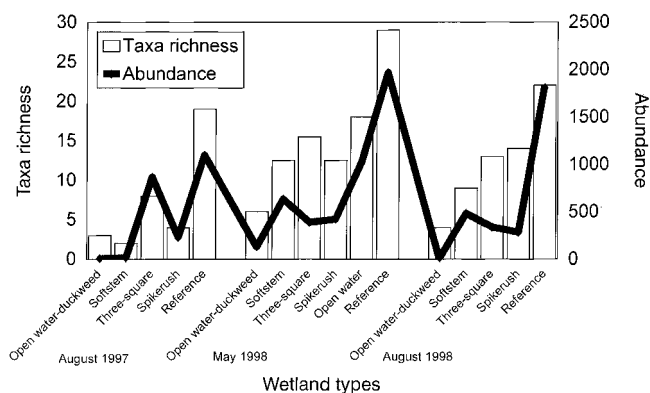


Figure 2. Mean taxa richness and abundance collected in 1-minute timed sweeps at wastewater mesocosms and the reference site.

oligochaetes (Lumbriculidae) and members of the leech family Glossiphoniidae (*Helobdella stagnalis* L. was identified from August 1998 samples) (Figure 1). Unique and uncommon taxa found in softstem bulrush mesocosms included rat-tailed maggots (*Eristalis*) and the dragonfly family Gomphidae. The softstem bulrush plant type was the tallest plant in the study (Table 1) and produced large amounts of organic debris. Plants also floated above the substrate and debris due to heavy loading of the mesocosms in July 1997. This growth form and the underlying debris may have played a role in structuring the community.

Three-square bulrush invertebrate assemblages had some similarities with softstem assemblages but fewer oligochaetes, more damselflies and *Culex*, and greater taxa richness. These differences may have contributed to the CCA discrimination between these mesocosms.

#### Invertebrate Trace Element Bioaccumulation

Mean concentrations of trace elements found in invertebrates from the various sites were relatively similar (Table 2). Arsenic and Ag, however, were higher in the spikerush mesocosms, whereas Hg and Se were slightly higher in the bulrush mesocosms (Table 2).

### DISCUSSION

#### Aquatic Invertebrate Assemblages

Specific invertebrate assemblages in natural systems are often associated with plant morphology or architecture related to particular plant communities (Rooke 1984, de Szalay and Resh 1996). Plants with more complex structure often harbor increased invertebrate taxa richness (Schramm *et al.* 1987, de Szalay and Resh 1996). This study likewise found distinctive invertebrate assemblages associated with specific plants in wastewater wetland mesocosms. Data from this

study, however, suggest that although plant architecture may be important, the effect of this architecture on D.O. may be equally important (*sensu* Sculthorpe 1967). The growth form of some plants may either mitigate or exacerbate problems associated with low D.O. Spikerush plantings with short culms (stems) and limited lodging allow for light penetration to the water surface. Both the limited oxygen demand and allowance for growth of algae result in relatively high D.O. concentrations. Decaying foliage and shade-limited production of benthic algae and plankton were associated with dense bulrush communities and appeared to cause decreased D.O. (*sensu* Rose and Crumpton 1996), as did excessive growth of duckweed on open water mesocosms. The negative effect of duckweed on invertebrates seems to be contrary to Harper and Bolen (1996), who found a positive correlation between duckweed and invertebrate biomass. The effect of duckweed on D.O. in our study may have been similar to that described by Moore *et al.* (1994) for floating-leaved macrophytes. They suggested that low D.O. in their study was from reduced oxygen exchange with the atmosphere and inhibition of wind energy transfer to the water column. The floating mats also curtail penetration of light that would allow oxygenation via photosynthesis by submerged plants, benthic algae, and plankton. The small size and high surrounding walls of the pilot mesocosms probably inhibited the drifting of duckweed and mixing of water that would likely occur in a larger, more open, natural system, thus resulting in the low observed D.O. This anoxia, in turn, produced an invertebrate community low in richness and abundance. In our study, D.O. was relatively high on the single sampling occasion in an open water mesocosm when it was not covered with duckweed. Associated with this increased D.O. were increased abundances of water-column cladocera, epiphytic mayflies, and benthic midges. Midges may have special importance for wildlife, since chironomids in open water habitats are important foods for young ducklings (King and Wrubleski 1998). The presence of the submerged *Potamogeton* and perhaps algae seems to have increased oxygen in the open water mesocosm in May, benefitting aquatic invertebrates. Low invertebrate diversity has been related to limited submergent vegetation in other wastewater wetlands (Schwartz and Gruendling 1985).

For wastewater mesocosms containing softstem bulrush, the low D.O. and large amounts of organic material resulted in a unique but taxon-depauperate invertebrate community. Although D.O. was low, a large detrital food source was present. Many oligochaetes, along with organisms tolerant to organic enrichment such as rat-tailed maggots, were apparently using this resource.

Table 2. Trace elements found in invertebrates from Albuquerque wetlands. Samples were collected in August 1997, May 1998, and August 1998. Mean values are from these three sampling periods.

Mesocosm Type	Mean <sup>a</sup> , Standard Error (in parentheses), and Maximum (in <b>bold</b> ) Trace Element Concentrations (mg kg <sup>-1</sup> , dry weight).				
	Ag	Al	As	Hg	Se
Reference	0.79 (0.21) <b>1.0</b>	584 (318) <b>1220</b>	2.43 (0.42) <b>3.25</b>	0.08 (0.06) <b>0.20</b>	1.94 (0.62) <b>3.12</b>
Spikerush	1.45 (0.23) <b>1.71</b>	681 (402) <b>1470</b>	7.23 (4.69) <b>16.6</b>	0.09 (0.07) <b>0.23</b>	1.14 (0.12) <b>1.39</b>
Three-square	1.14 (0.16) <b>1.46</b>	411 (169) <b>730</b>	2.71 (0.43) <b>3.57</b>	0.11 (0.08) <b>0.28</b>	2.28 (1.33) <b>4.93</b>
Open water	0.74 (0.13) <b>1.0</b>	803 (275) <b>1140</b>	2.78 (0.63) <b>4.04</b>	0.08 (0.06) <b>0.20</b>	1.74 (0.73) <b>3.21</b>
Soft-stem	0.64 (0.18) <b>1.0</b>	557 (384) <b>1300</b>	3.03 (1.72) <b>6.39</b>	0.28 (0.20) <b>0.68</b>	2.42 (1.09) <b>4.56</b>

<sup>a</sup> Values equal to the detection limit were used in calculation of the mean when analytical results were reported as less than detectable.

Overall, the low D.O. of wastewater wetlands appeared to decrease taxa richness relative to reference wetlands. Occasional loading of highly oxygen-demanding material into the wastewater wetlands may also have reduced invertebrate diversity and abundance, particularly during mid-summer of 1997. The diverse and abundant invertebrate assemblage at the reference wetlands may indicate what could be achieved at wastewater wetlands if D.O. can be augmented. Important waterfowl foods such as midges were always found at reference wetlands, perhaps because of the consistently high D.O.s. The only wastewater wetlands that clustered near the reference sites in the CCA were those containing high D.O. (e.g., spikerush and open water when there was little duckweed). Some differences between the reference and wastewater wetlands were likely due to dissimilar vegetation sources and associated invertebrates. This was especially true for those organisms that lack an aerial stage (e.g., amphipods and leeches). Most invertebrates in this study were detritivores, consistent with the large amounts of organic material produced by plants. Emergent plants that produce less detritus may support more diverse trophic levels (deSzalay and Resh 1996).

Associations of invertebrates with plants in the wastewater wetland mesocosms may be affected by vegetation type, maintenance procedures, treatment plant upsets, predation by waterfowl, and quantity of plant-produced organic matter. Concentrations of D.O., however, appeared to play a determining role in invertebrate community development. Data collected from wastewater mesocosms suggest that the quality of habitat (as measured by invertebrate taxa richness and abundance) for invertebrates increases from open water with low D.O. under a continuous cover of duckweed, to tall emergents with low D.O. but large

amounts of detrital food, to short emergents that provide some detrital food but also allow for light penetration to water, and finally to open water with oxygen-producing submerged macrophytes.

#### Trace Element Bioaccumulation

We found no published information on Ag in wetland invertebrates. Eisler (1996), however, indicated that normal or reference Ag concentrations from field collections of aquatic organisms throughout the world were often near or below 1 mg kg<sup>-1</sup> dry weight. The highest Ag concentration that we encountered was 1.71 mg kg<sup>-1</sup> (Table 2) and would probably be considered a normal or baseline value. Arsenic concentrations were less than concentrations considered harmful to ducks (*Anas* spp.) [120 mg As kg<sup>-1</sup> of food (Eisler 1988); oral LD-50 of 186 mg As kg<sup>-1</sup> food (calculated from NaAsO<sub>2</sub> data in Hudson et al. 1984)]. The highest As concentration in invertebrates at reference sites in a lotic study was 16 mg kg<sup>-1</sup> (Cain et al. 1992) and comparable to the high value (16.6 mg kg<sup>-1</sup>) in our study. Aluminum concentrations in invertebrates were similar to those found at other uncontaminated wetlands (Albers and Camardese 1993) and lower than the 5,000 mg kg<sup>-1</sup> found to affect ducks (Sparling 1990). Mean total Hg concentrations were highest in the softstem and three-square bulrush mesocosms. Nevertheless, these values were similar to those found in invertebrates by Albers and Camardese (1993) and less than the 2.2–23.5 mg methyl Hg kg<sup>-1</sup> food LD-50 for methyl mercury for ducks (calculated from Panogen toxicity data from Hudson et al. 1984; in Eisler 1987). Our total Hg concentrations were also typically lower than the 0.5 mg methyl Hg kg<sup>-1</sup> food found to have chronic reproductive effects on mallard ducks (Heinz 1979).

Potential for food web bioaccumulation of Se (Lemly 1995) was moderate ( $4\text{--}5\text{ mg kg}^{-1}$ ) in softstem and three-square bulrush mesocosms on a single sampling occasion, low ( $3\text{--}4\text{ mg kg}^{-1}$ ) in the reference and open water mesocosms, and of no hazard ( $<2\text{ mg kg}^{-1}$ ) in the spikerush mesocosm. The increased flows needed to maintain an equivalent hydraulic residence time in these deeper mesocosms may have also provided increased loading of Se. Other trace elements, however, did not show similar increased concentrations (Table 2), weakening this explanation. It is also possible that bioaccumulation potential varied with vegetation type. The large amounts of organic material found in bulrush mesocosms may produce organic forms of Se that may be more bioavailable to aquatic organisms (e.g., Masscheleyn and Patrick 1993). Detrital food webs may be especially susceptible to bioconcentration of Se (Lemly 1996). Habitat type is recognized as an important aspect in Se cycling (Lemly and Smith 1987). Alternatively, the trace element differences could be related to differences in invertebrate assemblages between the mesocosms and may represent differences in taxon abilities to take up Se.

The moderate concentrations of Se found in invertebrates at bulrush sites may be mitigated in a natural wetland by predator-prey selectivity, habitat usage, and seasons. The invertebrates that we sampled may not necessarily be those selected by waterfowl. Because ducklings often feed in open water rather than bulrushes (King and Wrubleski 1998), they would likely be exposed to the lower levels of Se found at open water wastewater and reference sites. Concentrations of Se were highest in the August 1998 sample, and this seasonal difference in trace element concentration may decrease exposure (e.g., Scheuhammer 1987) because ducklings would be older, less dependent on invertebrate food sources, and perhaps more tolerant of Se.

### Management Implications

Results of this study support conclusions by others (Schwartz and Gruendling 1985, Batzer and Resh 1992) about the importance to wetland macroinvertebrates of shallow ( $\sim 0.5\text{m}$  depth) open water and submerged macrophytes. Submerged plants benefit invertebrates by releasing oxygen into the water column, whereas emergent plants lose oxygen to the atmosphere (Sculthorpe 1967) and shade benthic algae and plankton communities (Crumpton 1989). Plants also contribute large amounts of oxygen-demanding detritus in the water column upon death or senescence, and the humic substances produced may inhibit algae growth (Vymazal 1995). While emergents do provide important cover for waterfowl, the importance of open

water for wildlife should not be underestimated. Chironomids, crucial for waterfowl food, were largely absent from the heavily vegetated wastewater wetland mesocosms. Often treatment wetlands are designed with limited open water areas and dense coverage of emergent vegetation to maximize nutrient removal capacity. Low D.O. caused by external and internal organic loading, however, may limit the usefulness of some constructed wetlands as invertebrate habitat and therefore limit their value to waterfowl.

The design of wastewater treatment wetlands for wildlife habitat may not necessarily preclude use for treatment functions. Sartoris and Thullen (1998) suggested that interspersed emergent vegetation and open water habitats would create alternating aerobic and anoxic environments and allow for nitrogen treatment and degradation of dissolved organic matter, along with providing a mosaic of habitat types for wildlife use. Our studies suggest that some combination of oxygen-producing open water and detritus-producing emergent plant zones maximizes invertebrate biodiversity. This sort of habitat mosaic may also result in benefits to human health because of the increased numbers of predatory macroinvertebrates that decrease populations of mosquitoes (Walton and Workman 1998). Results also suggest that habitat types should be considered in the design of constructed wetlands because of important differences in invertebrate uptake of trace elements important to waterfowl health. Further research on impacts of wetland plant types on D.O. and on trace element bioaccumulation in wetland plants and invertebrates is needed. Constructed wetlands, along with providing water treatment, may be managed to enhance waterfowl production by providing environmental attributes appropriate for invertebrate production.

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