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Monitoring Potential Prey of the Whooping Crane: Evaluating Gear Types and Spatial-Temporal Variation in Aquatic Fauna

by

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ABSTRACT

I assessed methods to sample potential aquatic prey of Whooping Cranes (*Grus americana*) in their remote breeding ponds in Wood Buffalo National Park using activity traps, dip-nets and minnow traps. More taxa were collected, and in greater abundance, with timed dip-net sweeps, but because this technique misses fish, minnow traps are also required. I visited 30 ponds across areas of high and low densities of cranes (high-use and low-use, respectively), as well as more accessible ponds lacking cranes (no-use), during the summer of 2005. Multivariate analyses indicated that assemblages in high-use ponds differed from no-use and low-use ponds, which did not differ from each other. Fish were indicators of high-use ponds, whereas beetles (Dytiscidae) and dragonflies (Libellulidae) were indicators of no-/low-use ponds. Further analyses of the easily reached no-use ponds suggested that they could act as surrogates of the more isolated low-use ponds in an aquatic prey monitoring program.

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CHAPTER I: Introduction

With anthropogenic influences currently affecting most parts of the globe, biological monitoring has become increasingly important for protecting resources and assessing the state of ecosystems (Karr and Chu 1999, Downes et al. 2002, Cayrou and Cereghino 2005, Spellerberg 2005). Monitoring can be defined as the measurement or assessment, over time, of particular features and/or processes (Spellerberg 2005). This can include describing a system of which little is known, detecting anthropogenic impacts on an ecosystem, determining conservation values of an area, and evaluating the status of endangered species (Downes et al. 2002, Spellerberg 2005).

Monitoring can enhance our understanding of populations and/or ecosystems. For example, several international volunteer-based avian monitoring programs have been established for that purpose. The North American Breeding Bird Survey, established in the mid 1960s, is conducted yearly in southern Canada and the United States, with the main objective to estimate population changes in breeding birds (Sauer et al. 2007). The Christmas Bird Count, established in 1900, is also conducted annually in North and South America, and the resulting data have been used in a wide range of studies, including those examining avian distribution and abundance (Dunn et al. 2005). Projects such as these, as well as those conducted on smaller scales, and on other taxa (e.g., FrogWatch (NatureWatch 2006)), are important for collecting baseline data needed for monitoring impacts of development. This type of monitoring is becoming increasingly common, with projects examining, e.g., effects of wind turbines (Reynolds 2006), pipeline construction (Levesque and Dube 2007), rural development (Steiner et al. 2000), and tourism (Warnken and Buckley 2000) on biological populations and communities.

Monitoring of aquatic systems has focused on evaluating anthropogenic influences (Rosenberg and Resh 1993) or determining the conservation value of an area (e.g., Painter 1999), often with the use of indicator taxa (e.g., Fore et al. 1996, Dufrene and Legendre 1997, Sahlen and Ekestubbe 2001, Cao and Hawkins 2005, Bilton et al. 2006). Several countries have established national protocols that employ the use of indicator taxa for rapid assessment of the status (i.e. 'health') of an area. For example, Australia has developed the Australian River Assessment System (AUSRIVAS; Simpson et al. 1997 *in* Downes et al. 2002), and the United Kingdom has developed the River Invertebrate Prediction and Classification System (RIVPACS; Wright 1995). These two protocols were established for stream and river assessments. Studies have also been conducted to examine the use of indicator taxa in specific lentic water bodies (e.g., Sahlen and Ekestubbe 2001, Bilton et al. 2006), however, monitoring protocols are less well developed for lakes, ponds and wetlands (Bilton et al. 2006).

The lack of broadly applicable monitoring programs for ponds and wetlands may often result from a more limited knowledge about the ecology of these systems (Batzer et al. 2004, Nicolet et al. 2004, Della Bella et al. 2005). An example of such a poorly known system, despite its high conservation importance, is the wetland complex in which the endangered Whooping Crane (*Grus americana*) breeds. This complex is located in the northern portion of Wood Buffalo National Park (WBNP), along the Northwest Territories-Alberta border (Figure 1-1), and comprises thousands of small, shallow ponds with diatom substrates (Timoney 1997). Until relatively recently, very

little was known about the ecology of these wetlands, which are unique with respect to historic breeding habitats of the cranes (Timoney 1997), and there was also little information about how cranes use these ponds during the breeding season. Given results of recent studies describing the traits of ponds most prefered by the cranes (Timoney et al. 1997), and their potential diet (Bergeson et al. 2001, Sotiropoulos 2002), efforts can now be directed towards developing a prey monitoring program in the Whooping Crane breeding grounds.

The Whooping Crane is one of the most well recognized endangered species in North America, and has been designated as endangered in both Canada and the United States since the 1970s (Canadian Wildlife Service & U.S. Fish and Wildlife Service (CWS & USFWS) 2007). Historically, the summer breeding grounds of Whooping Cranes extended from central Alberta and southern Saskatchewan and Manitoba, to central Illinois, northeastern Minnesota and Iowa, and northeastern North Dakota. Wintering grounds extended along the Gulf of Mexico, from Florida to north-eastern Mexico, as well as part of the Atlantic coast and New Mexico (Figure 1-2) (CWS & USFWS 2007). The Whooping Crane reached a population low in the 1940s with < 20 birds remaining in the migratory flock that overwintered in Aransas National Wildlife Refuge (ANWR) in Texas, which was later discovered to breed in WBNP (the AWB flock). In 2006, the population of the AWB flock was 262 birds (T. Stehn, USFWS, *in* Unrau 2007). Today, three flocks of Whooping Cranes exist in the wild: the self-sustaining AWB flock, and two recently established flocks - a non-migratory one in Florida, and a migratory flock with wintering grounds in Florida and summer grounds in Wisconsin (CWS & USFWS 2007).

Within ANWR there is little room for expanding the wintering habitat as the refuge is surrounded by development, however, similar habitat does exist east of Houston, Texas, and south to Tampico, Mexico (G. Holroyd, CWS, *pers comm.*, *in* Sotiropoulos 2002). Nonetheless, limited wintering habitat is likely not an immediate concern as evidence suggests that the existing winter territory is not at carrying capacity (Stehn and Johnson 1987, *in* CWS & USFWS 2007). Spatial expansion of the breeding grounds in WBNP, on the other hand, may be possible due to its undisturbed nature and large extent. Currently, major threats to the Whooping Crane include risks along its migratory pathway, such as illegal poaching and deaths resulting from powerline collisions (CWS & USFWS 2007). On the wintering grounds, cranes are susceptible to potential spills from on the adjacent the Gulf Intracoastal Waterway (CWS & USFWS 2007).

The Canadian Wildlife Service, in partnership with the U.S. Fish and Wildlife Service, has developed an International Recovery Plan for the Whooping Crane that will not only help limit risks described above, but will also be used to expand the current AWB population. To meet these objectives, the Recovery Plan outlines many recovery actions, several of which focus on the importance of assessing potential prey in the Whooping Crane breeding grounds. Specifically, potential prey must be measured in summer (and winter) habitat including in areas that are both used and not currently used by cranes (CWS & USFWS 2007). Desirable food sources should be identified and this information should be used to evalute whether both breeding and wintering grounds can support 1000 Whooping Cranes, one of the criteria that will allow downlisting from the current endangered status (CWS & USFWS 2007).

Research was conducted in the late 1990s to identify potential prey of canes in WBNP, based on recommendations in the National Recovery Plan for the Whooping Crane (Edwards et al. 1994), a predecesor to the International Recovery Plan for the Whooping Crane. Initial observations identified dragonfly larvae and other large macroinvertebrates as important food sources for Whooping Cranes immediately after chicks hatched (Bergeson et al. 2001), whereas small-bodied fishes were identified as likely important food sources once chicks were mobile and able to forage on their own (Sotiropoulos 2002). This information provides a foundation for a program to monitor the potential prey within the Whooping Crane breeding grounds. Development and long-term implementation of such a program will be required to achieve the actions of the recovery plan.

The three objectives of this project, therefore, are to 1) evaluate sampling approaches most appropriate for use in the Whooping Crane breeding grounds, 2) assess spatial and temporal patterns of potential prey assemblages, and based on the results of 1) and 2), 3) develop a long-term program to monitor and assess Whooping Crane prey in the summer breeding grounds, to be implemented by Wood Buffalo National Park. Given that there are thousands of ponds in crane nesting areas that could be monitored, I focused on efficient sampling methods and allocation of effort both within and among years.

Chapters II and III of this thesis focus on sampling aquatic fauna and assessing their distribution across the breeding grounds and throughout the breeding season. A protocol for monitoring potential Whooping Crane prey has been developed and is available from Parks Canada. The limited accessibility (only via helicopter) of these ponds makes it imperative to minimize time, effort and cost in developing an effective sampling program. Due to the unique nature of these ponds (i.e., very shallow with a diatom substrate) (Timoney et al. 1997), few sampling methods are appropriate for the aquatic fauna (Sotiropoulos 2002). Three methods are evaluated in Chapter II to determine their potential for inclusion in an effective monitoring protocol. Due to the large number of ponds and their extremely isolated location, it is impractical to sample more than a few of them. Chapter III focuses on assessing the faunal patterns both across the breeding grounds and across a breeding season to determine the spatial and temporal scales appropriate for monitoring.

Not only does this thesis provide information on the selection of sampling strategies for these unique aquatic systems and an assessment of their communities' spatial and temporal variability, it provides data necessary to develop the prey-monitoring program. With long-term implementation of the program, Parks Canada will ideally determine if there is a relationship between yearly prey abundance and chick survival, and identify areas within the breeding grounds that are potentially suitable for introduction of captive-bred cranes. These should assist the recovery team with management of the endangered Whooping Crane for future de-listing from its current endangered status.

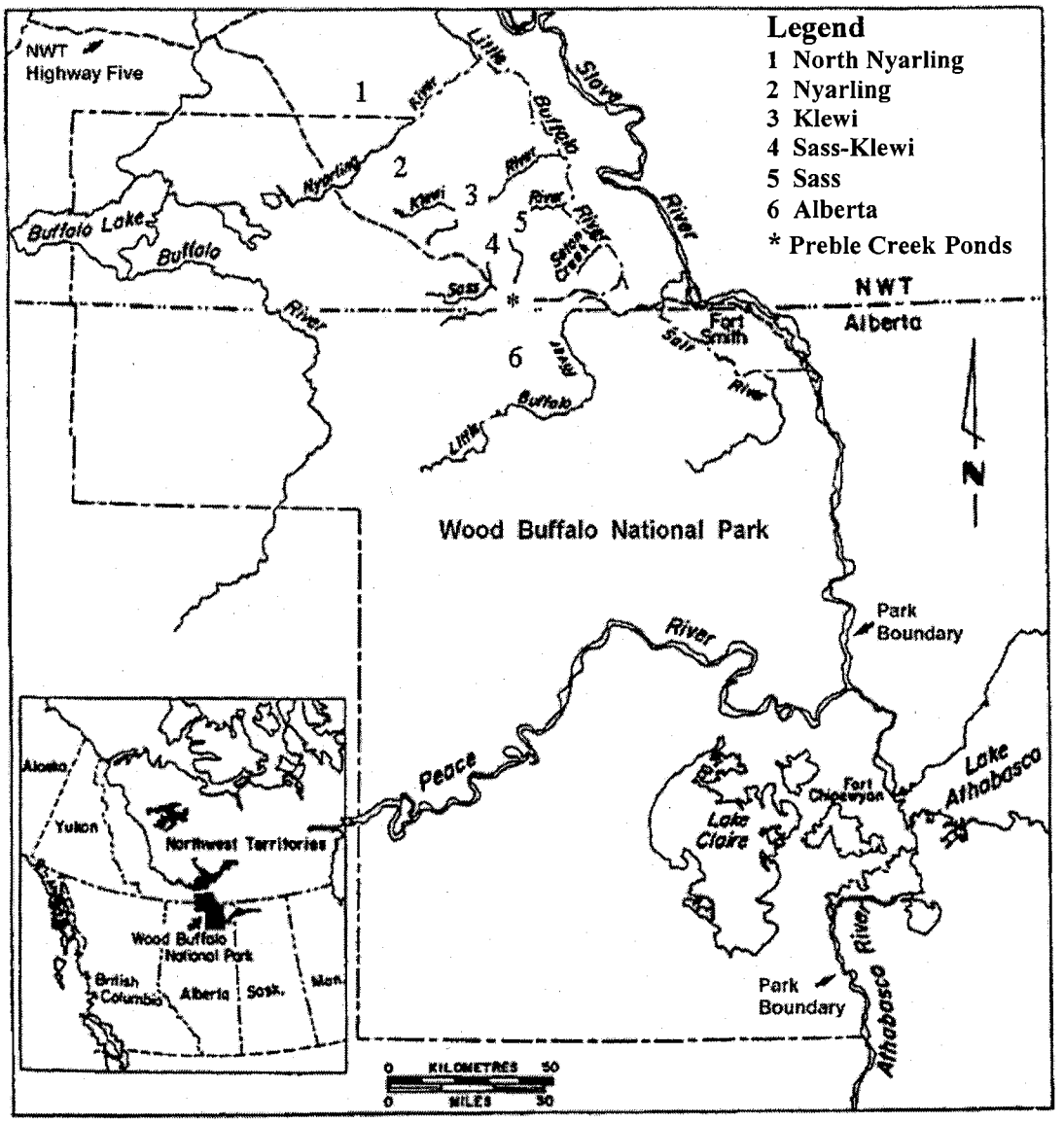

Figure 1-1. Map of six Whooping Crane nesting areas located in the breeding grounds in Wood Buffalo National Park. Map inset is the location of Wood Buffalo National Park in Alberta and the Northwest Territories. Modifed from the International Recovery Plan for the Whooping Crane (CWS & USFWS 2007).

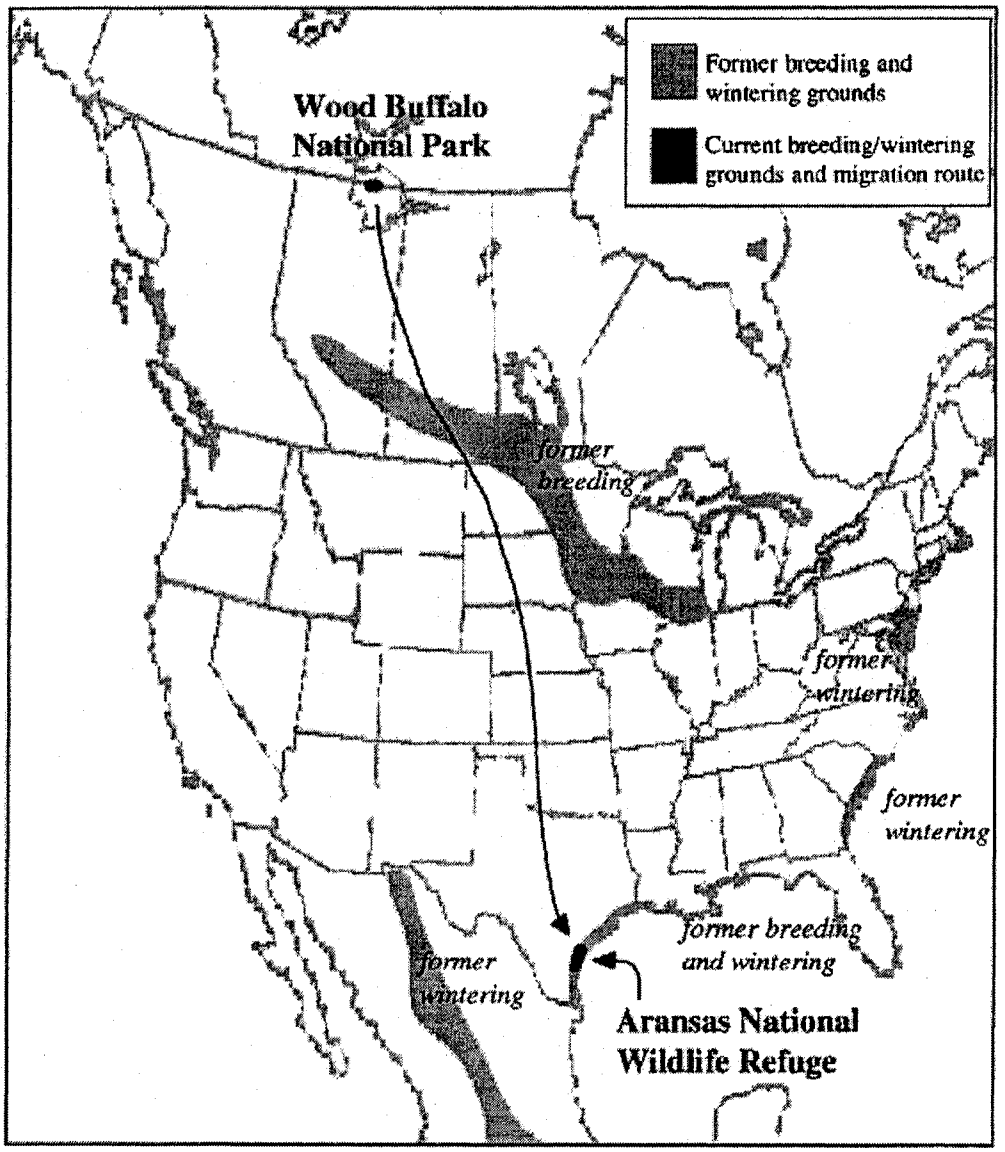

Figure 1-2. Map of North America showing the Whooping Crane's current and former breeding and wintering areas. Modified from Sotiropoulos (2002).

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Chapter II: Evaluation of Three Techniques for Sampling Aquatic Fauna in Whooping Crane Nesting Area Ponds: Implications for Biomonitoring

INTRODUCTION

A wide variety of methods exist for sampling aquatic fauna, particularly aquatic invertebrates (e.g., Hellawell 1978, Merritt et al. 1996, Turner and Trexler 1997, Resh and McElravy 2001), and many factors must be considered when deciding which is most appropriate for a specific study. In general, the habitat and community being sampled will dictate the sampling strategy of sampler that should be used. For example, streams will require different gear than shallow ponds (Merritt et al. 1996).

More than one method, however, may be suitable for a given situation. Many studies have evaluated different sampling strategies for collecting aquatic invertebrates, including some that have assessed the complementarity of different sampling methods (Hyvonen and Nummi 2000), different ways to deploy the same sampler (e.g., vertical vs. horizontal alignment of traps) (Muscha et al. 2001), qualitative vs. quantitative methods (Garcia-Criado and Trigal 2005), or have compared abundance and/or number of taxa collected by various sampling techniques (Murkin et al. 1983, Mackey et al. 1984, Storey et al. 1991, Turner and Trexler 1997, Muzaffar and Colbo 2002, O Connor et al. 2004).

Other factors to consider are the number of sampling units or replicates required for accurate and precise estimates of both species (or taxon) richness and abundance (Merritt et al. 1996). Taxon richness is a very important property of biotic assemblages, and numerous methods have been identified and examined to estimate richness (Colwell and Coddington 1994, Foggo et al. 2003a, b, and Cao 2004). Taxon-accumulation curves are commonly used to assess richness (Lyons 1992, Colwell and Coddington 1994, and Foggo et al. 2003a, b), sample size, and efficiency of sampling strategies (Mackey et al. 1984). Several methods have been developed to determine when a taxon-accumulation curve has reached an asymptote (Foggo et al. 2003a, b), at which point the vast majority of taxa that are vulnerable to a given method have presumably been collected. To properly assess abundance of fauna at a site, a common approach is to calculate the number of samples needed to achieve a given level of precision (Southwood 1978, Pringle 1984).

The purpose of this study was to evaluate the effectiveness of three gear types for sampling potential prey of Whooping Cranes in their nesting-area ponds, with respect to use in a future monitoring program. Previous research in the breeding ponds has identified fish (Sotiropoulos 2002) and aquatic macroinvertebrates, especially dragonfly larvae (Bergeson et al. 2001), as food sources for the Whooping Crane. Minnow traps, activity traps, and dip-net samples have also been identified as the most appropriate sampling gear for collecting the specific taxa found in these extremely shallow, soft-bottom ponds (Timoney et al. 1997, Sotiropoulos 2002). I sampled ponds with these three gear types to answer the following questions:

1. How many sample units are needed to reach a taxon accumulation asymptote, for each of the three sampling methods?

2. Which gear collect the most similar faunal assemblages? Which collect the most complementary assemblages?
3. Does total abundance of fish and invertebrates collected by each gear differ? What sample size is needed to assess abundance at various pre-determined levels of precision?
4. How much time is required to set and collect sampling device, and to process samples?

The results of this study will be used in combination with those from Chapter III to develop a Whooping Crane prey-monitoring program, which will be submitted to Parks Canada.

DESCRIPTION OF STUDY AREA

The Whooping Crane breeding grounds of northern Wood Buffalo National Park, Canada, approximately 59°45' – 60°30' N, and 112°45' – 114°00' W (Sotiropoulos 2002), are located in the Subhumid Mid-boreal ecoclimatic region of Canada (Timoney 1997). Most of the Whooping Cranes nest in remote wetlands around the Sass and Klewi rivers, with fewer birds clustered mostly on the Alberta side of the Alberta/Northwest Territories border, and fewer still near the Nyarling River (Fig. 1-1, Chapter I) and Lobstick Creek (east of the eastern park border, not shown on map).

In addition to the nesting area ponds, there is a suite of ponds located immediately west of Preble Creek (PC), just outside the eastern border of the breeding grounds adjacent to Northwest Territories Highway 5 (Figure 1-1, Chapter I). Although Whooping Cranes have not nested at these ponds, they are visually similar to ponds in the breeding grounds. In contrast to the nesting area ponds, however, they are easily accessible and thus suitable for repeat visits.

The vast majority of the breeding ground landscape is covered by a unique wetland complex, dominated by diatom ponds (sensu Timoney et al. 1997), i.e., ponds containing relatively few plankton, instead, algae is dominated by benthic diatoms; bogs, marshes, and wet meadows are also present (Timoney et al. 1997). The ponds are primarily fed by ground-water discharge from the Caribou Hills, located approximately 80km south-west of the nesting areas (McNaughton 1991). For the most part, the ponds are isolated hydrologically from streams in the area (Timoney et al. 1997), except during spring flooding (D. Bergeson, Parks Canada, *in* Sotiropoulos 2002). Ponds are generally quite small and shallow (10-1000 m diameter, <50 cm deep), and often dry up by the end of the summer. Aquatic macrophytes, largely restricted to the pond fringes, include sedge (*Carex* sp.) and bulrush (*Scirpus* sp.), and shoreline vegetation includes willow (*Salix* sp.), Labrador tea (*Ledum groenlandicum*), and dwarf birch (*Betula glandulosa*) (Timoney et al. 1997).

The dominant bedrock in the area is gypsum karst, the dissolution of which influences the water chemistry of these ponds (Timoney et al. 1997), resulting in alkaline, subsaline conditions (Timoney et al. 1997; pH ranging from 7.6-9.1, conductivity ranging from 1280-1990 uS/cm; M. Classen unpublished data). The

dominant benthic diatoms in these ponds produce a distinctive yellow/pink color during times of higher water levels and a whitish color when ponds are drying out.

METHODS

Field Methods

Sampling was conducted in 2004 from August 11 – August 23 (referred to as August 2004), and during three periods in 2005, from May 25 – June 21, June 24 – July 13, and July 23 - August 12 (referred to as June 2005, July 2005 and August 2005, respectively). I employed two sampling designs, one referred to as 'taxon accumulation' (in 2004) and the other referred to as 'gear effectiveness' (in 2005). Gee minnow traps, activity traps and timed dip-net samples were used in each design. Minnow traps were 42 x 23 cm with a 6 mm square mesh size, activity traps consisted of a 2 L plastic pop bottle with the top ¼ cut off and inverted into the bottle, and dip-nets were 15 cm x 12.5 cm standard aquarium dip net (Hagen Easy Catch Nets) with a 1 mm square mesh. Both minnow traps and activity traps were set horizontally, resting on top of the substrate, overnight for approximately 18-20 hours. Dip-net samples were conducted for 8 minutes, during which time I stood in one location in the pond and collected all of the invertebrates that I saw (and could reach).

For the taxon accumulation study, I sampled nine nesting area (NA) ponds, in three sets of three, located throughout the Sass and Klewi nesting areas (Figure 1-1, Chapter I). Within each set of three ponds, one pond received 24 minnow traps, one received 24 activity traps, and one received 24 timed dip-net samples. Invertebrates from dip-net samples were put into 120 mL plastic vials (one sample/vial) with a small amount of pond water and taken to the laboratory for preservation 2-5 hours later. I returned to retrieve the minnow and activity traps 18-20 hours later, at which time all collected fish were identified, counted and subsequently released. Invertebrates from activity and minnow traps were emptied into 120 mL plastic vials containing pond water and also taken to the laboratory for preservation in 80% ethanol.

For the 'gear effectiveness' study, the Preble Creek (PC) ponds were sampled. Four ponds were sampled in June, and an additional two ponds in July and August. Each pond received 15 overnight minnow traps, 15 overnight activity traps, and 15 8-minute dip-net samples. Samples were handled in the field and laboratory as described above.

Laboratory Procedures

Invertebrate samples were taken to the laboratory within 2-5 hours of being collected where they were drained of pond water and preserved in 80% ethanol. Prior to processing, samples were washed through a 2 mm sieve to remove the majority of the background sediment and smaller organisms, e.g., microcrustaceans, as I was interested in potential Whooping Crane prey of larger sizes. Invertebrates were then picked from the remaining sediment with the aid of a dissecting microscope. For the most part, insects were identified to genus unless they were early instars, missing key body parts necessary for identification, pupae, or of the family Corixidae. Morpho-taxa were used for some Limnephilidae genera (e.g.,

Asynarchus/Philarctus/Limnephilus), due to the difficulties in identifying these individuals to genus (Wiggins 1996). Most of the remaining invertebrates were identified to family or order level, and for all identifications I used keys from Clifford (1991), Merritt and Cummins (1996), Wiggins (1996) and Larson et al. (2000). Invertebrate taxa were then counted, recorded and stored in scintillation vials with 80% ethanol.

Statistical Analyses

Taxon accumulation Ponds

I used EstimateS 8.0.0 (Colwell 2007) to create cumulative taxa curves from 1000 iterations for each of the nine nesting area ponds, with one curve for each of three taxonomic levels (order, family, and lowest feasible taxonomic group (LFTG)). I then calculated an average curve for each gear type at each taxonomic level. Following criteria outlined in Foggo et al. (2003a, b), I determined whether the curves reached an asymptote. The criteria required that the percentage of new taxa collected in the last sample be less than one percent of the number that had accumulated in the previous samples, and percentage of new taxa collected in the last 20 percent of samples be less than five percent of the number that had accumulated in the previous samples (Foggo et al. 2003a, b). Using criteria from Mackey et al. (1984), I also determined the number of samples needed to collect 70 percent of the total taxa, and the sample number where the percentage of new taxa is less than five percent of previously accumulated taxa. I also examined the number of traps needed to collect 80 percent of the taxa, criteria not specific to Mackey et al. (1984), to evaluate the number of traps needed to collect an additional 10 percent.

Gear Effectiveness Ponds

For a qualitative assessment of taxa collected by each gear type, I listed taxa collected across all PC pond-visits, at the three taxonomic levels. I determined for each sampling method the total number of taxa collected, the number of unique taxa collected only by one method, and the number of missing taxa (but collected by the other gear types). I performed a one-way analysis of variance (ANOVA) for each taxonomic level to determine if the average number of taxa collected by each gear type differed. Post-hoc pairwise t-tests were conducted as necessary, with sequentially adjusted Bonferroni p-values (Holm 1979). All taxa were included except for individuals that were found as early instars, missing key body parts necessary for identification, or were in the pupal stage.

To quantitatively assess similarity of taxa collected by each gear type, I calculated the Sorensen similarity index (using all PC pond-visits) for each pairwise gear comparison, at each taxonomic level, using presence/absence (PA) and $\log_{10}$(abundance+1) data using the program EstimateS 8.0.0 (Colwell 2007).

I also used multivariate ordination for graphical evaluation of similarity among gear types based on taxonomic composition. Ordination allows complex multivariate data sets to be summarized relatively simply to help with detection and assessment of patterns in composition among sample units (McCune and Grace 2002). In the simplest sense, ordination arranges sample units along an axis (or axes) in such a manner that sample units that are similar (e.g., in assemblage composition) are placed

closer together in ordination space (McCune and Grace 2002). Indirect ordination is based on the community data only, and arranges sample units based on covariation and association among the taxa (McCune and Grace 2002).

Non-metric multidimensional scaling (NMS) ordination is becoming more widely used in community ecology because of its suitability for non-normal data (McCune and Grace 2002). NMS ordinations are constructed iteratively to minimize stress of the best positions of *n* attributes on *k* axes. Iterations are performed with real data and stress is reduced if distance in ordination space stays the same (or increases) as the real distances (dissimilarity) between sites increase (McCune and Grace 2002). More details are provided in McCune and Grace (2002). I conducted NMS ordinations using the program PC-ORD 5 for Windows (McCune and Mefford 1999). For all ordinations, I used a random starting configuration and the Sorensen (Bray-Curtis) distance measure, and the parameter setup included 1000 runs with real data, 4 axes, and 15 iterations to determine stability. Monte Carlo permutations were conducted with 999 randomized runs to determine the proportion of randomized runs with stress values that were less than or equal to stress in the real data.

To conduct the NMS ordinations, data from the fifteen units of each gear type were combined to produce three assemblages for each pond: one from minnow traps, one from activity traps, and one from dip-net samples. Taxa present in only one pond-visit (out of 16 pond-visits) were removed prior to the NMS analyses. Corresponding pairwise multi-response permutation procedure (MRPP) analyses (McCune and Grace 2002) were also conducted to determine whether gear types collected different faunal assemblages, using sequentially adjusted Bonferroni p-values. MRPP is a non-parametric procedure that tests the null hypothesis of no difference between two or more groups, and is appropriate for non-normal data. Rank-transformed abundance data and Sorensen (Bray-Curtis) distance measure were used because they generate results more similar to NMS (McCune and Grace 2002).

To determine whether samples from the different gear types differed in total abundance, I compared the sum of all individuals collected by each sampling device and the sum of only potential prey taxa (dragonflies, beetles, fish) by one-way ANOVAs. Post-hoc t-tests were performed as needed using sequentially adjusted Bonferroni p-values.

To determine the number of traps needed to detect faunal abundance at various levels of precision, I used the following formula:

$$n = \frac{s^2}{(Ex)^2}$$

where n = number of samples, s = standard deviation, x = mean and E = a pre-determined standard error (i.e., precision), expressed as a proportion of the mean (Southwood 1978). Precision values closer to zero are more precise than values closer to one. I calculated sample sizes needed to determine potential prey taxa (dragonflies, beetles and fish) abundance at three levels of precision (0.2, 0.4, and 0.5). This was performed for each gear type averaging all Preble Creek pond visits across all three sampling periods, at the family and order levels. The same calculations were also conducted using the sum of all potential prey individuals.

Finally, to incorporate a measure of effort required by these different sampling methods, I recorded times required to travel to sites, perform field and lab work, and

enter data for each gear type. From this I determined the approximate time required to sample a 'gear effectiveness pond', as well as a pond sampled using the method outlined in Chapter III.

RESULTS

Taxon accumulation Ponds: Sample Size

Based on the nine taxon accumulation curves for the nesting area ponds (Figures 2-1), 24 samples were sufficient for all curves to reach an asymptote following the criteria outlined above (from Foggo et al. 2003a, b), with the exception of the LFTG dip-nets and activity traps (Table 2-1). Even these two exceptions only missed the asymptote criteria by a small percentage (0.1-0.3%), so I still used these curves to assess the number of samples needed to collect a certain percentage of taxa.

To collect 70% of the total taxa collected in 24 samples, 5-10 dip-net samples, 4-9 minnow traps, and 5-8 activity traps were needed, depending on the taxonomic level to which organisms were identified (Table 2-1). Between two and four additional samples were needed to collect 80% of the taxa. Depending on the taxonomic level, 7-11 dip-net samples, 6-9 activity traps, and 7-8 minnow traps were needed to meet the criterion that the percentage of new taxa is less than five percent of previously accumulated taxa. On average, this sampling effort resulted in collection of 77.4% of dip-net taxa, 76.5% of activity traps taxa, and 74.9% of minnow traps taxa. The sample effort identified by using these criteria will likely miss rare (and/or very cryptic) taxa because a relatively large percentage of taxa was collected in only one of 24 samples/pond (LFTG: depending on the sampling method, 16.7-42.9% of taxa were only collected in one sample per pond; for family: 10.0-42.9%; for order: 0-50%).

Gear Effectiveness Ponds: Similarity of Taxa

Across all pond visits, I collected more taxa using dip-nets than minnow traps and activity traps, which collected a similar number of taxa (±1) when organisms were identified to the LFTG and family levels (Table 2-2; n=16 pond-visits). At the LFTG, dip-nets collected approximately 30% more taxa than activity traps and minnow traps and had 5-10 times more unique taxa. At the family level, dip-nets performed even better relative to the other two gear types (approximately 40% more taxa), with 4-7 times more unique taxa. Dip-nets were also missing the fewest taxa at the LFTG and family levels. At the order level, all gear types performed similarly, the main difference was that minnow traps collected 1-2 fish taxa not caught by activity traps or dip-nets, respectively. Lists of taxa collected by each gear type, at each taxonomic level, are in Appendix B.

As expected, numbers of taxa collected per pond by the three gear types differed, at each taxonomic level (LFTG: $F_{2,45}=12.64$, $p<0.0001$; Family: $F_{2,45}=13.22$, $p<0.0001$; Order: $F_{2,45}=16.43$, $p<0.0001$) (Figure 2-2); all post-hoc pairwise comparisons were significant (MT<AT<DN). At both the LFTG and family level, activity traps and minnow traps had the highest Sorensen similarity indices, indicating that these methods collect the most similar taxa (Table 2-3). At the order level, however, dip-nets and activity traps were most similar.

For LFTG log(abundance+1) data, the NMS ordination showed overlap among the collections from dip-nets and activity traps, with minnow traps more distinct (Figure 2-3). Minnow traps were less distinct for the rest of the comparisons (Figures 2-4, 2-5, 2-6, only LFTG and family ordinations are provided). Corresponding MRPP tests indicated, however, that all pairwise comparisons differed (sequentially adjusted Bonferroni p-values, $p<0.05$) except for one (order, PA data, AT vs. DN, $p=0.071$). Stress values were similar (~16-18) for all ordinations. Finally, all ordination analyses arrived at three-dimensional solutions; however, for simplicity I present the two-dimensional solutions that best exemplify the patterns.

Numbers of individuals collected by each gear type differed ($F_{2,45}=13.3$, $p<0.0001$), with dip-nets collecting more individuals than both minnow traps and activity traps (sequentially adjusted Bonferroni p-value, $p<0.0012$). Total abundances of potential prey taxa only (fish, dragonflies, beetles), however, did not differ among gear types (ANOVA, $F_{2,45}=1.32$, $p>0.26$).

More samples are needed when aiming for a precise estimate of abundance for each taxon of interest, compared to the number of samples needed to collect/detect a given percentage of taxa. At the family level, only the following insect taxa and associated gear require fewer than 30 samples: Dytiscidae-dip-nets at 0.4 precision, and all three gear types at 0.5 precision; Aeshnidae-minnow traps at 0.5 precision (Table 2-4). At the order level, Coleoptera required fewer than 30 samples for dip-nets at 0.4 precision and all gear types at 0.5 precision, and Odonata requires fewer than 30 samples for both minnow and activity traps at 0.5 precision (Table 2-5). Overall, dip-nets performed the best (i.e., require fewer samples) for Dytiscidae (and Coleoptera), while minnow traps perform best for all Anisoptera and Zygoptera (and Odonata). Minnow traps performed better than activity traps for collecting fish taxa (Tables 2-4, 2-5). Results for 0.2 level of precision are not provided, as the minimum number of traps needed (>100 for most estimates) was not practical.

Fewer samples are required to reach precision levels of 0.2, 0.4 and 0.5 when all potential prey taxa (fish, odonates, coleopterans) are summed (Table 2-6). Dip-nets required the fewest samples whereas minnow traps required the most. Fish, however, were missing entirely from the former samples but not from the latter. Reaching a high level of precision (0.2) requires too many samples than are feasible in the Whooping Crane ponds, however, the number of samples required to reach a precision level of 0.4 is much more feasible.

Sampling a pond using the gear effectiveness method (all three gear types; minnow and activity traps set overnight) was estimated to take approximately 10 more hours than sampling a pond with only dip-nets and 2-hr baited minnow traps (Tables 2-7 and 2-8). This was due to increased time needed to travel to the pond twice, to set and collect activity traps, and to preserve and process activity trap samples. Estimates are conservative, and thus provide a maximum estimate of time needed to perform both of the methods.

DISCUSSION

Taxon accumulation Ponds: Sample Size

It is relatively common for cumulative taxon curves not to reach a plateau, indicating that not all taxa have been collected. In a study of ephemeral aquatic systems in Ireland, 15-40 units of each gear type were not sufficient to collect all of the taxa at each site (O Connor et al. 2004). Foggo et al. (2003a) evaluated 32 marine datasets and found that only three met their asymptotic criteria. I applied a more liberal approach to determine whether curves were asymptotic by omitting the criterion that the two final values in a curve be the same (Foggo et al. 2003a, b). Consequently, of the nine taxon accumulation curves I constructed, only two did not meet my asymptote criteria. Had I employed the omitted criterion, only a few of the single-pond order curves (prior to constructing the average curves presented in the figure) would have met the asymptote criteria.

Criteria outlined by Mackey et al. (1984) to determine sample size based on total taxa collected are arbitrary, but are the only criteria explicitly outlined in the literature and have been employed by Bradley and Ormerod (2002) to determine the sampling effort required to collect rare taxa. The sample number for which the percentage of new taxa collected is less than five percent of previously accumulated taxa was always 1-2 greater than the number of samples needed to collect 70 percent of taxa. This difference in number of samples represented $\leq 8\%$ difference in the total taxa collected, depending on the criteria used. This is comparable to what Mackey et al. (1984) found for these criteria. To collect 80 percent of taxa, 2-4 more samples are needed than for the 70 percent collection, and 0-4 more samples are needed compared to the < 5 percent accumulation criteria.

The purpose behind sampling, whether to determine presence/absence or abundance of all taxa or solely to monitor specific taxa, is important in deciding if these criteria are appropriate for use in the Whooping Crane breeding ponds. Setting fewer than 24 traps in each pond, as required to meet the 70 or 80% criteria, will collect fewer rare taxa. If the purpose of monitoring, however, is to assess abundance of only potential Whooping Crane prey, collecting rare taxa will not be important as they likely do not contribute a substantial amount to the diet of the birds. It is important to keep in mind that while infrequently collected taxa may indeed be rare, they may also be abundant but have cryptic behavior, making it difficult to obtain representative samples.

Gear Effectiveness Similarity of Taxa

Selecting appropriate gear for aquatic sampling is extremely important when planning a sampling program because the right gear will not only provide the most representative samples at a site, but can significantly reduce the time and cost of a study (Brinkman and Duffy 1996; Merritt et al. 1996). Suitability of gear types depends in part on the habitat that is being sampled (Muzaffar and Colbo 2002). Surber samplers, for example, are not suitable for lentic habitats because invertebrates may not drift into the net, while Ekman grabs will not close properly if used in rocky substrate (Merritt et al. 1996). Using inappropriate gear will result in collecting unrepresentative samples (Merritt et al. 1996). Seemingly appropriate gear, however,

can also yield samples that are not fully representative. Muzaffar and Colbo (2002) sampled two ponds with qualitative (sweep net) and quantitative (rock-bag) gear and found the two types yielded different estimates of macroinvertebrate diversity and abundance. Differences in taxa collected have even been documented between horizontally and vertically deployed activity traps (Muscha et al. 2001).

Different sampling gear have been compared to determine which is most effective for sampling ponds or wetlands (Murkin et al. 1983; Mackey et al. 1984; Storey et al. 1991; Cheal et al. 1993; Brinkman and Duffy 1996; Turner and Trexler 1997; Hanson et al. 2000; Hyvonen and Nummi 2000; Muscha et al. 2001; Muzaffar and Colbo 2002; O'Connor et al. 2004; Garcia-Criado and Trigal 2005). Each of these studies examined either activity traps or pond-net samples in addition to at least one other sampling device, but only Turner and Trexler (1997) included minnow traps in their comparison. Most of the studies examined have only one gear type in common with each other, so comparing these studies in an effort to determine which gear is most effective is not appropriate, regardless of whether objectives and habitats sampled are similar. What is common among these studies, however, is the suggestion of using a combination of gear to obtain fully representative samples of communities.

Time and funding permitting, a preliminary study can help determine the best methods to meet the objectives of a study (Brinkman and Duffy 1996). The purpose of my study was to compare minnow traps, activity traps and dip-nets to determine which is most appropriate for sampling in the Whooping Crane breeding ponds, to be used in a future prey monitoring program. I found that dip-nets performed better than activity traps and minnow traps, in terms of both numbers of taxa collected and total abundance. This is similar to results found by Mackey et al. (1984), Turner and Trexler (1997) and Muzaffar and Colbo (2002), where more taxa, but not necessarily more individuals, were collected with dip-net samples, and Cheal et al. (1993) where pond-nets collected the most taxa at four out of five lakes. O Connor et al. (2004), however, found that of their two sampling gears, pond-nets consistently collected fewer taxa than box sampling (where an open-bottomed box is affixed to the substrate and all organisms are removed).

Although dip-nets collected the most taxa and had the most unique taxa, at the lowest feasible taxonomic group and family levels, none of the methods collected samples that were fully representative of the prey assemblages. Similar results were obtained by Muzaffar and Colbo (2002), where two gear types collected different taxonomic groups and yielded different abundance estimates. Each of my three gear types missed a moderate percentage (~12-34%) of taxa that was collected by one or both other samplers at the LFTG and family levels, although dip-nets missed the fewest taxa. Furthermore, of the 11 unique taxa collected by dip-nets in the PC ponds, eight were rare (present in 2/48 pond-visits) indicating that dip-nets may also be appropriate for studies where collecting rare taxa is a priority. Dip-nets out-performed minnow traps and activity traps at the family level as well, but all traps performed similarly at the order level.

The conclusions made from this chapter will be incorporated into a prey-monitoring program for the breeding grounds; therefore, methods that best monitor potential food sources (in terms of both presence and abundance) for the Whooping Crane, rather than collect the largest number of rare taxa, are of greatest interest.

Previous research (Bergeson et al. 2001; Sotiropoulos 2002) identified fish, dragonfly larvae, and dytiscid beetles as potential food sources. As just discussed, dip-nets collected the most unique taxa but of those, only one is a potential food source (a dragonfly, *Somatochlora* sp.), and it is rare in the Preble Creek ponds. Only activity traps and minnow traps, however, consistently collected fish, and only minnow traps collected all three genera of fish. All three samplers collected four (of five) dragonfly genera at the LFTG, while only dip-nets collected all dragonfly families. Activity traps out-performed dip-nets and minnow traps for collecting dytiscid beetles at the LFTG; activity traps collected eight genera whereas dip-nets and minnow traps collected five each. Total abundance was higher in dip-net collections than activity traps and minnow traps; however, all three gear types performed similarly when only abundance of potential prey taxa was examined.

Pairwise similarity was consistently lowest for minnow traps and dip-nets, and highest for activity traps and minnow traps, likely because traps collected fish whereas dip-nets did not. Only Olson et al. (1995) employed both activity traps and dip-net samples at the same sites, but they did not compare taxa collected by each gear (Olson et al. 1995). Turner and Trexler (1997) used both minnow traps and dip-nets, but found that minnow traps contained so few taxa that they were omitted from the paper. Thus, it was difficult to compare my results to published data.

While the NMS ordinations did not usually show distinct separation of pond-visits according to gear type, the representative faunal communities collected by sampling method (presence/absence and abundance data) differed according to MRPP results. Thus there is a large enough taxonomic difference to separate out the sites according to the gear with which they were sampled, as was seen by O Connor et al. (2004). Minnow traps and dip-nets, in particular, overlapped very little in ordination space, consistent with their lower Sorensen similarity index. The high number of unique taxa collected by dip-nets contributed to the lowest Sorensen similarity indices for both dip-net/minnow trap and dip-net/activity trap comparisons at the LFTG and family levels. At the order level, the minnow trap/dip-net and minnow trap/activity trap similarities were lower due to the absence of small-bodied taxa (Pelecypoda and Ostracoda) in the minnow traps. The lowest Sorensen similarity value, however, was still quite high (0.721: minnow trap/dip-net comparison at LFTG). Nevertheless, this broad grouping of ponds according to gear type indicates that, on their own, each method does not collect fully representative samples of prey assemblages in the Whooping Crane ponds.

The number of traps required to obtain a certain level of precision (standard error) for the estimates of faunal abundance must also be considered. Obtaining precise estimates of invertebrate abundance often requires a large number of samples, making sampling for the purpose of assessing abundance extremely time-consuming and costly (Bartsch et al. 1998). Sample size, therefore, should never be determined without considering cost. For the most part, far too many samples (43-64) are required to obtain even moderate levels of precision (0.2 – 0.4) in the Whooping Crane ponds. Not only would collecting the required number of samples result in a considerable amount of laboratory work and be extremely costly, it may also be impossible to fit such a large number of traps in a pond without among trap interference and to conduct the necessary number of dip-nets samples at a pond in one day. At the order level,

however, the number of dip net samples needed to detect Coleoptera abundance at 0.4 and 0.5 precision levels (8-33) is feasible. The lower confidence limit of the required sample size interval (i.e., mean – 1SE) for collecting Odonata and Coleoptera with minnow traps is also feasible at 0.5 precision. Gasterosteidae also requires a very feasible sample size to obtain a 0.4 level of precision using minnow traps.

The large sample sizes needed to estimate abundance of individual taxa at moderate levels of precision could be a result of the somewhat clumped spatial distribution of the invertebrates (*pers. obs.*). Additionally, activity traps perform better when deployed vertically, with the opening facing the substrate (Muscha et al. 2001). Vertical deployment, however, is not feasible in these extremely shallow diatom ponds. Surprisingly, fewer minnow traps than activity traps are needed to yield the same precision. This is not only due to the horizontal deployment of activity traps, but also because minnow traps are only efficient at collecting the largest macroinvertebrate individuals (Turner and Trexler 1997), and only large macroinvertebrates were included in the analyses. In fact, minnow traps require even fewer samples than dip-nets for collecting Odonata.

In addition to performance in terms of taxa and abundance, other strengths and weaknesses must be considered when choosing the most appropriate sampling method (Murkin et al. 1983). Neither activity traps nor minnow traps collect a substantial amount of background sediment/substrate or macrophytes, yielding samples that can be processed relatively quickly. For long-term monitoring programs, types can be easily standardized because they are so simple to use (Murkin et al. 1983). All that is required by the sampler is submersion of the trap and retrieval at a set time.

Dip-net sampling, however, was the only method that consistently collected the least mobile taxa, such as Gastropoda. In contrast to activity traps and minnow traps, dip-nets are an active form of sampling (Hellawell 1978). They allow the sampler to collect individuals that are not very mobile and may not enter the passive sampler. Additionally, they only require one visit to a site for complete sampling, an important consideration when sampling sites are only accessible by helicopter. While dip-nets may require less time in the field, they can be time consuming to process, as they often collect a substantial amount of macrophytes and sediment (Murkin et al. 1983; Muzaffar and Colbo 2002; Garcia-Criado and Trigal 2005). Additionally, with numerous people sampling, as is often the case for long-term monitoring programs, it is more difficult to standardize the protocol and may result in less consistent collection of samples (Murkin et al. 1983). Fortunately, it was possible to minimize the amount of macrophytes and sediment we collected in the Whooping Crane ponds. Macrophytes were not abundant and were limited to the periphery of ponds where it was difficult to set traps and perform dip-net samples, thus the plants were easily avoided. In locations where they could not be avoided, the small dip-nets allowed me to avoid or minimize collection of both macrophytes and sediments.

Although traps are simple to use and standardize, they may not provide an accurate estimate of invertebrates. Presence of prey within the traps can act as an attractant for predaceous invertebrates (Murkin et al. 1983), whereas the presence of fish or amphibians in the traps may deter their invertebrate prey from going near the traps and being collected. Predatory taxa may also prey upon other organisms while in

the traps. Furthermore, to collect similar potential prey abundance as dip-net samples, activity traps would likely have to be set overnight.

As far as I know, the use of short-term minnow traps has not been evaluated in the literature as this gear type is commonly set overnight for accurate estimates of abundance (e.g., Jackson and Harvey 1997). Short-term sampling might only be sufficient for the assessment of fish presence (Sotiropoulos 2002). Additionally, minnow traps are obviously not capable of collecting representative invertebrate samples because of the large mesh size (Turner and Trexler 1997). They should be used in conjunction with an appropriate invertebrate trap if collecting invertebrates is an objective.

CONCLUSIONS

Using a combination of gear types to effectively sample aquatic systems is often recommended (Turner and Trexler 1997, Hyvonen and Nummi 2000, Bradley and Ormerod 2002). Such an approach should also maximize the number of potential prey taxa caught at a given sampling occasion in the Whooping Crane breeding ponds and should also provide a relatively precise estimate of relative prey abundance. Considering the performance of the gear types in the Whooping Crane ponds, as well as other strengths and weaknesses discussed in literature, I suggest that a combination of dip-net samples and short-term minnow traps is most suitable (logistics of this methodology are discussed in Chapter III). This combination will collect the majority of invertebrate taxa known to be present, assess fish presence, and greatly reduce the time (and cost) of sampling a pond. Regardless of the taxonomic level of identification desired, 15 minnow traps and 15 dip-net samples should be sufficient to obtain an estimate of abundance at a precision level of 0.5 for some taxa. For summed taxa (i.e., all potential prey), this sampling should be sufficient to obtain a precision level close to of 0.4. Fifteen samples of each method should also collect >80% of the taxa in each pond.

Ideally, time and money saved by using this gear-type combination would be put towards sampling a greater number of ponds when a prey monitoring program is implemented in WBNP. As prey fauna vary both spatially and temporally (discussed in Chapter III), costs saved by using a one-visit sampling regime can be invested in more detailed assessment of spatial and temporal variation of prey

Table 2-1. Summary of taxon accumulation analyses based on criteria outlined in Foggo et al. (2003a, b) and Mackey et al. (1984). For each average accumulation curve (Figure 2-1), I determined the percentage of new taxa collected in the last (24th) trap and the last 20% of traps, the trap number where 70% and 80% of taxa are collected, and trap number where the percentage of new taxa is < 5% of those previously accumulated. *Asymptote criteria of Foggo et al. (2003a, b) (see text for details) were not met. LFTG=Lowest feasible taxonomic group.

Gear Type/ **Taxonomic Level**	**% new taxa collected in last trap**	**% new taxa collected in last 20% of traps**	**Trap # where ≥ X% of taxa is collected (actual %)**		**Trap # where % of new taxa is < 5% of previously accumulated taxa (actual %)**
			70%	*80%*	
Dip-nets					
LFTG	1.11*	5.1*	10 (72.5)	13 (81.3)	11 (75.8)
Family	0.92	4.0	9 (73.7)	12 (82.3)	10 (76.9)
Order	0.67	2.8	5 (71.6)	8 (81.9)	7 (79.4)
Activity Traps					
LFTG	1.21*	5.3*	9 (71.7)	13 (82.3)	9 (71.7)
Family	0.72	3.0	7 (73.4)	10 (82.4)	8 (76.9)
Order	0.27	1.5	4 (72.5)	6 (80.8)	6 (80.8)
Minnow Traps					
LFTG	0.94	4.3	8 (71.6)	12 (82.2)	8 (71.6)
Family	0.93	3.9	7 (70.9)	11 (81.6)	8 (74.1)
Order	0.30	1.9	5 (72.0)	8 (81.8)	7 (79.1)

Table 2-2. Total number of taxa, number of unique taxa, and number (and percentage) of missing taxa for each gear type at each taxonomic level, for all 2005 gear effectiveness pond-visits combined (n=16 pond-visits). For each pond-visit, 15 samples of each gear type were collected. Total taxa collected by all three gear types across all pond-visits were, at the lowest feasible taxonomic group=56, family=35, and order=14. Missing taxa are taxa that were collected by one or both of the other two gear types. % of grand total is the percentage of total taxa across all three gear types that is not collected by one gear type.

	Activity Traps	**Minnow Traps**	**Dip-nets**
	Lowest Feasible Taxonomic Group		
Total taxa	38	37	49
# unique taxa	1	2	11
# missing taxa (% of grand total)	18 (32.1)	19 (33.9)	7 (12.5)
	Family		
Total taxa	24	23	33
# unique taxa	0	1	7
# missing taxa (% of grand total)	11 (31.4)	12 (34.3)	2 (5.7)
	Order		
Total taxa	13	11	12
# unique taxa	0	1	0
# missing taxa (% of grand total)	1 (7.1)	3 (21.4)	2 (14.3)

LFTG		
	DN	**AT**
AT	0.759	---
MT	0.721	0.800

Family		
	DN	**AT**
AT	0.807	---
MT	0.750	0.851

Order		
	DN	**AT**
AT	0.960	---
MT	0.833	0.880

Table 2-3. Sorensen similarity indices for pairwise comparisons of all three gear types, at each of three taxonomic levels. Data from all 2005 Preble Creek pond-visits (n=16), each involving 15 samples per gear type per pond-visit, were included. LFTG= lowest feasible taxonomic group, DN=dip-net, AT=activity trap, MT=minnow trap.

Table 2-4. Average sample sizes (± 1SE) required to achieve two levels of precision (0.4, 0.5; standard error as proportion of the mean) for estimates of potential prey taxa abundance collected by activity traps (AT), minnow traps (MT) and dip-net samples (DN). Samples were collected from the Preble Creek ponds (n=16 pond-visits) during three sampling periods. All taxa are identified to the family level and abbreviations are presented in Appendix B. Estimates that lack a standard error indicate taxa collected in only one pond.

Precision/	**Average Sample Size (±1SE) for Potential Prey Taxa**								
Gear	**Gast**	**Cypr**	**Aesh**	**Cord**	**Libe**	**Coen**	**Dyti**	**Gyri**	**Hydr**
0.4									
AT	26.0	-	65.4 ± 14.3	-	86.7 ± 7.3	75.0 ± 19	33.0 ± 7.7	-	-
MT	6.0	29.0	45.0 ± 10.0	-	55.6 ± 13.1	47.3 ± 14.8	30.3 ± 6.3	-	-
DN	-	-	68.9 ± 12.4	94.0	67.2 ± 16.4	82.8 ± 11.2	14.2 ± 3.1	77.3 ± 16.8	73.0 ± 21.0
0.5									
AT	17.0	-	41.7 ± 9.1	-	55.4 ± 4.6	48.0 ± 12.0	21.0 ± 4.9.0	-	-
MT	4.0	18.0	29.0 ± 6.4	-	35.4 ± 8.4	30.0 ± 9.0	19.5 ± 4.0	-	-
DN	-	-	44.0 ± 8.0	60.0	42.8 ± 10.5	52.8 ± 7.2	9.1 ± 1.9	49.3 ± 10.8	46.5 ± 13.5

Table 2-5. Average sample sizes (± 1SE) required to achieve two levels of precision (0.4, 0.5; standard error as proportion of the mean) for estimates of potential prey taxa abundance collected by activity traps (AT), minnow traps (MT) and dip-net samples (DN). Samples were collected in the Preble Creek ponds (n=16 pond-visits) during three sampling periods. All taxa were identified to the order level and abbreviations are presented in Appendix B. Estimates that lack a standard error indicate taxa were collected in only one pond.

Precision/ **Gear**	**Average Sample Size (±1SE) for Potential Prey Taxa** **Gast**	**Cypr**	**Odon**	**Cole**
0.4				
AT	26.0	-	44.3 ± 12.4	33.0 ± 8.5
MT	6.0	29.0	32.1 ± 9.4	30.1 ± 6.7
DN	-	-	50.6 ± 13.8	13.2 ± 2.9
0.5				
AT	17.0	-	28.3 ± 7.9	21.0 ± 5.4
MT	4.0	18.0	20.4 ± 6.0	19.3 ± 4.2
DN	-	-	32.4 ± 8.8	8.4 ± 1.8

Table 2-6. Average sample size (± 1SE) required to achieve three levels of precision (0.2, 0.4, 0.5; standard error as proportion of the mean) for activity traps (AT), minnow traps (MT) and dip-net samples (DN). Samples were collected in the Preble Creek ponds (n=16 pond-visits) during three sampling periods. Calculations were based on the sum of all potential prey taxa.

	Average Sample Size (±1SE) for each Level of Precision		
Gear	**0.2**	**0.4**	**0.5**
AT	61.8 ± 6.1	15.4 ± 1.5	9.9 ± 1.0
MT	63.9 ± 22.1	16.0 ± 5.5	10.2 ± 3.5
DN	42.7 ± 8.9	10.7 ± 2.2	6.8 ± 1.4

Table 2-7. Breakdown of approximate times required to sample one 'gear effectiveness' pond (15 overnight minnow traps, 15 overnight activity traps and 15 8-minute dip-net samples), with two crew members. All time (except travel) is doubled if only one crew member performs all tasks.

Task	*Approximate time*
Travel to Nesting Area ponds (via helicopter)	~1hr x 4 return trips (pilot usually dropped off the crew and then returned to the base, and then flew back to the site to pick up the crew at a pre-arranged time)
Travel to Preble Creek ponds (via car)	~2hr x 2 return trips
Setting minnow traps and activity traps	1 hour
Performing dip-net samples	1hr 50 min
Collecting minnow traps and activity traps, and recording data	1hr 30min
Extra time to organize, pack up gear, etc. in field on day one	30 min
Extra time to organize, pack up gear, etc. in field on day two	45 min
Lab work following field work (preserving samples, getting gear ready for next day)	45 min
Processing 15 dip-net samples, 15 activity traps, 15 minnow traps	19hr 5 min
Entering data for all 45 samples	3hr
Total	32 hr 25min

Table 2-8. Breakdown of approximate time required to sample one pond with 15 2-hr minnow trap sets and 15 8-min dip-net samples (explained in Chapter III), with two crew members. All time (except travel) is doubled if only one crew member performs all tasks.

Task	**Approximate time**
Travel to Nesting Area ponds (via helicopter)	~1hr x 2 return trips (pilot usually dropped off the crew and then returned to the base, and then flew back to the site to pick up the crew at a pre-arranged time)
Travel to Preble Creek ponds (via car)	~2hr x 1 return trip
Setting minnow traps	25 min
Performing dip-net samples	1 hr 50 min
Collecting minnow traps and recording data	1 hr
Extra time to organize, pack up gear, etc. in field	45 min
Lab work following field work (preserving samples, getting gear ready for next day)	30 min
Processing 15 dip-net samples, and a maximum of 8 minnow traps	11hr 8 min
Entering data for all 30 samples	2 hr
Total	21 hr 38 min

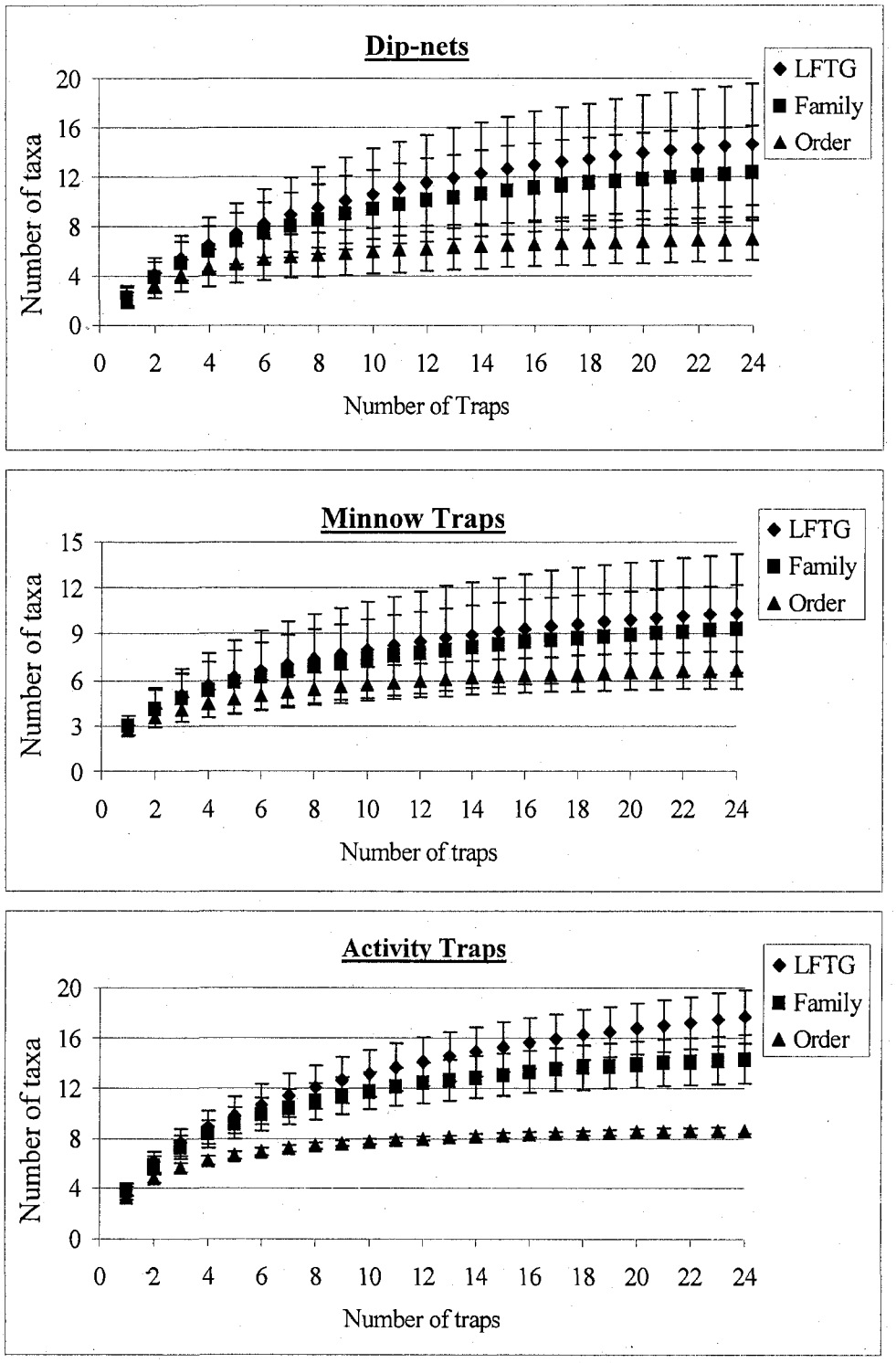

Figure 2-1. Average EstimateS (Colwell 2007) taxon accumulation curves for 2004 nesting area ponds (n=9). Three ponds were sampled with 24 dip-net samples, three with 24 minnow traps, and three with 24 activity traps. One curve for each level of taxonomic identification is provided (LFTG=lowest feasible taxonomic group) for each gear type. Error bars represent ± 1 standard error.

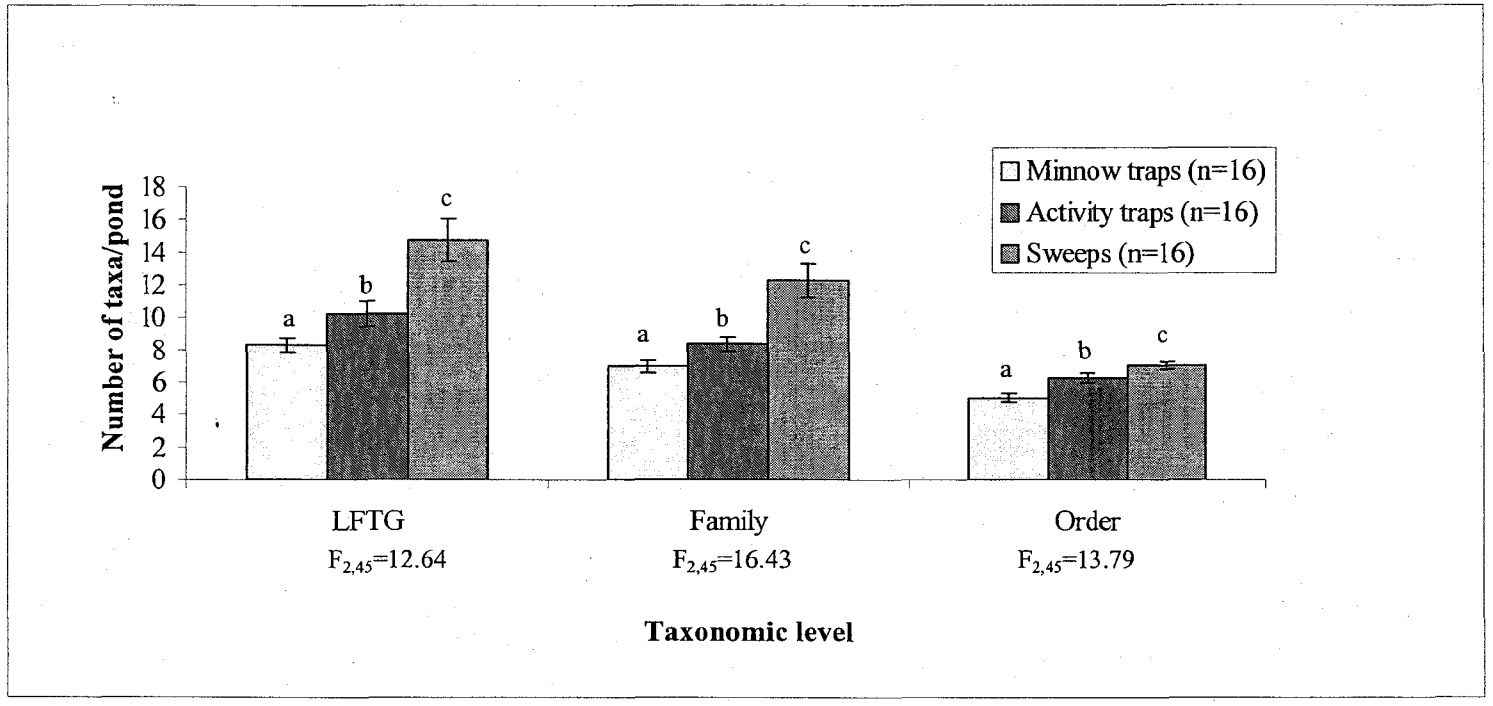

Figure 2-2. Average number of taxa collected per pond in each of three gear types, at three taxonomic levels. At the lowest feasible taxonomic group (LFTG) and family levels, all pairwise comparisons are significant (one-way ANOVA for each taxonomic level, *post hoc* t-tests with sequentially adjusted Bonferroni p-values; calculated p-values were <0.047, <0.01, and <0.0001). For each ANOVA, different letters indicate that groups differed. All 2005 PC pond-visits are included. Error bars are ± 1 standard error.

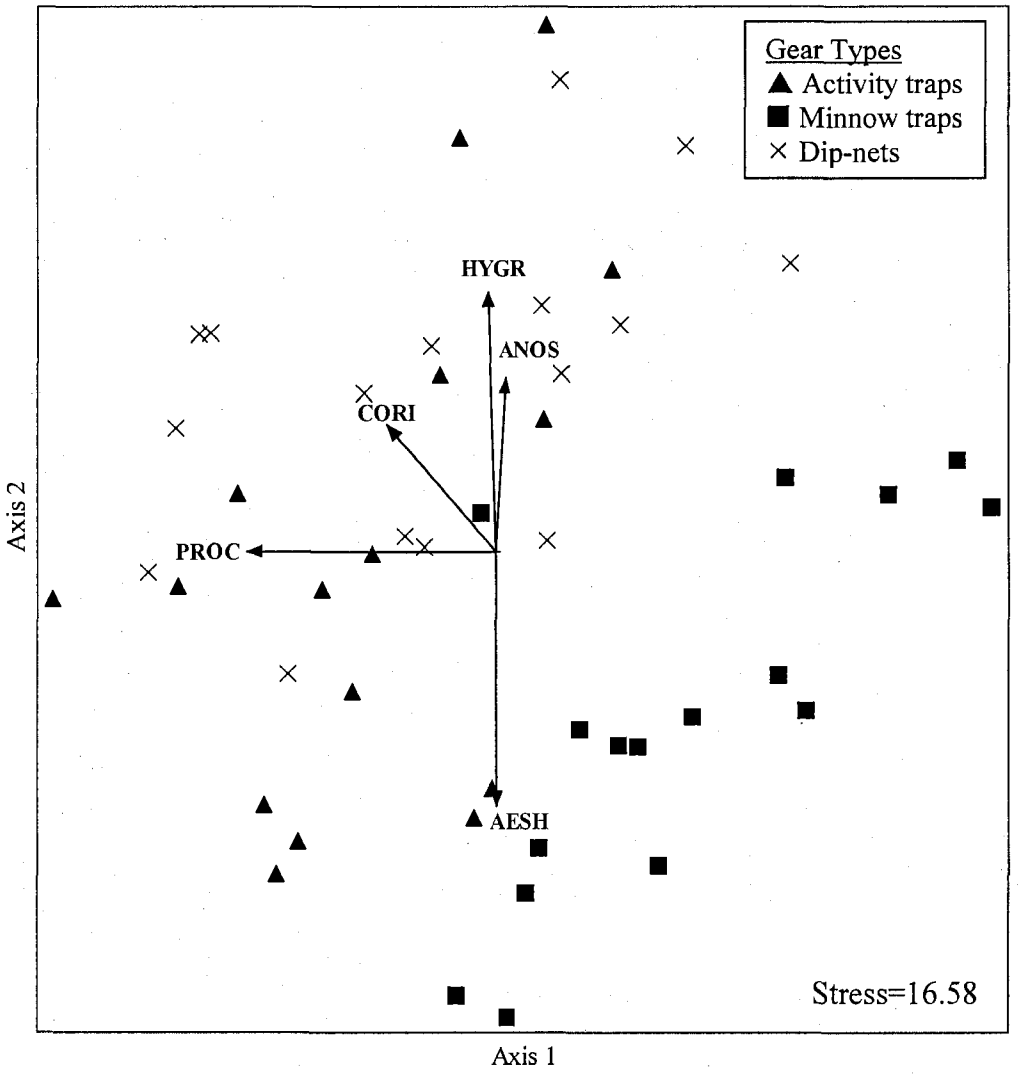

Figure 2-3. Non-metric multidimensional scaling (NMS) joint plot of pond visits based on macroinvertebrate assemblages identified to the lowest feasible taxonomic group (LFTG) and data as log(abund+1) for all 2005 Preble Creek ponds, sampled with the three gear types. Data from all samples of the same gear are combined into one entry for each pond; three sampling periods are not combined; n=48 (n=16 for each gear type). Vectors (arrows and corresponding taxa) point in the direction of increasing values, and a longer line indicates a stronger relationship between the vector and the joint plot axes. Only vectors with an R^2 of 0.30 or greater are plotted. Abbreviations for invertebrate taxa are presented in Appendix B.

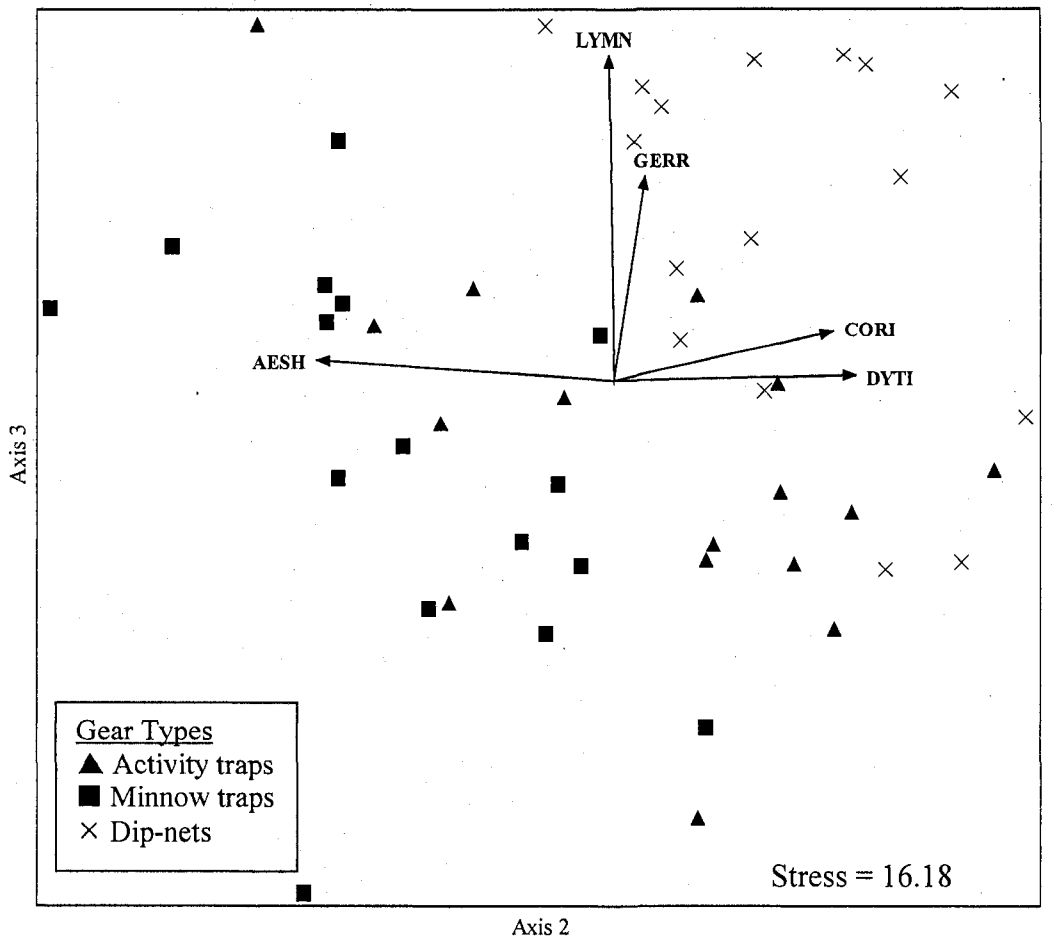

Figure 2-4. NMS joint plot of pond visits based on macroinvertebrate assemblages identified to family and data as log(abund+1) for all 2005 Preble Creek ponds, sampled with the three gear types. Data from all samples of the same gear are combined into one entry for each pond; three sampling periods are not combined; n=48 (n=16 for each gear type). Vectors (arrows and corresponding taxa) point in the direction of increasing values, and a longer line indicates a stronger relationship between the vector and the joint plot axes. Only vectors with an R^2 of 0.30 or greater are plotted. Abbreviations for invertebrate taxa are presented in Appendix B.

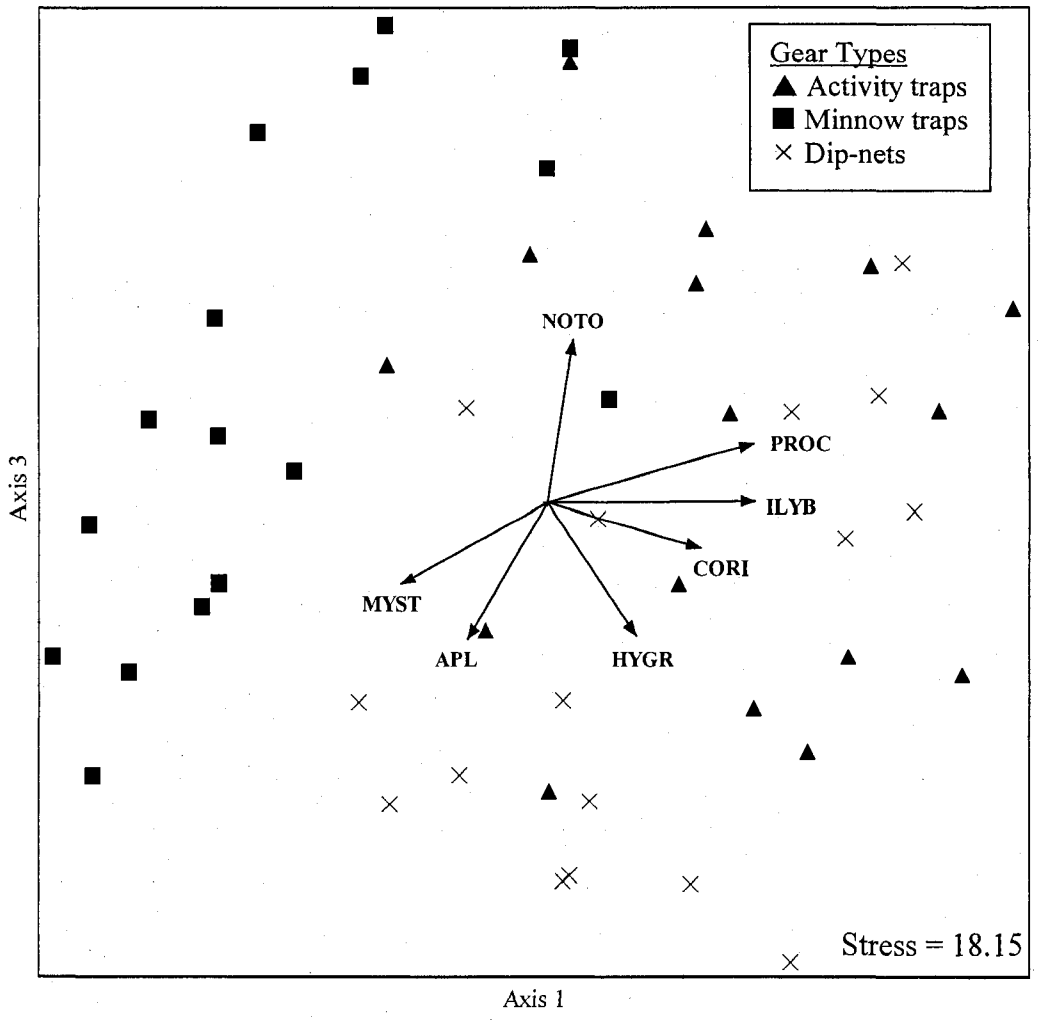

Figure 2-5. NMS joint plot of pond visits based on macroinvertebrate assemblages identified to the LFTG and data as presence/absence for all 2005 Preble Creek ponds, sampled with the three gear types. Data from all samples of the same gear are combined into one entry for each pond; three sampling periods are not combined; n=48 (n=16 for each gear type). Vectors (arrows and corresponding taxa) point in the direction of increasing values, and a longer line indicates a stronger relationship between the vector and the joint plot axes. Only vectors with an R^2 of 0.30 or greater are plotted. Abbreviations for invertebrate taxa are presented in Appendix B.

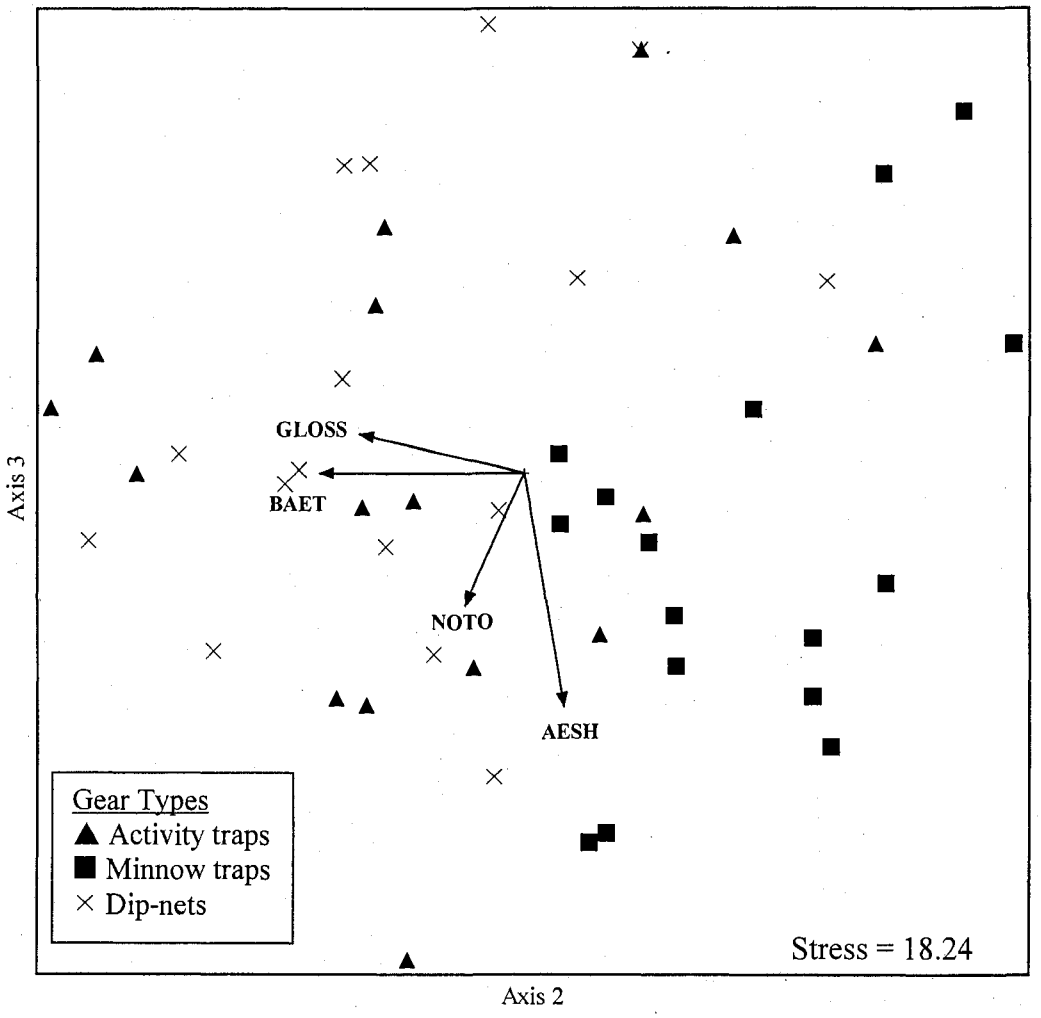

Figure 2-6. NMS joint plot of pond visits based on macroinvertebrate assemblages identified to the family and data as presence/absence for all 2005 Preble Creek ponds, sampled with the three gear types. Data from all samples of the same gear are combined into one entry for each pond; three sampling periods are not combined; n=48 (n=16 for each gear type). Vectors (arrows and corresponding taxa) point in the direction of increasing values, and a longer line indicates a stronger relationship between the vector and the joint plot axes. Only vectors with an R^2 of 0.30 or greater are plotted. Abbreviations for invertebrate taxa are presented in Appendix B.

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Chapter III: Spatial and Temporal Variability of Faunal Assemblages in Whooping Crane Nesting Area Ponds: Relevance to Biomonitoring

INTRODUCTION

The Whooping Crane (*Grus americana*) is a critically endangered species that breeds in a vast wetland complex in Wood Buffalo National Park. Although the nesting area habitat was discovered in the mid 1950s (Canadian Wildlife Service & Environment Canada 1993), only during the past ten years has research begun to focus on the breeding-season habitat. Such research includes the identification of the crane's preference for rare 'diatom ponds' that are characteristically clear and shallow with a benthic diatom substrate (Timoney 1997). Timoney (1997) identified the need to focus on this isolated wetland complex, particularly pond characteristics and the crane's summer diet. Recently, both Bergeson et al. (2001) and Sotiropoulos (2002) have made progress on these recommendations.

In late spring, immediately after young cranes have hatched, large invertebrates, such as dragonflies (sub-order: Anisoptera), are the predominant prey fed to chicks (Bergeson et al. 2001). When young cranes are mobile and able to feed on their own, however, family groups are often observed feeding in ponds that consistently contained fish (Sotiropoulos 2002). Thus, both fish and large invertebrates appear to be important food sources in the Whooping Crane diet at different times in the breeding season.

Annual monitoring of Whooping Crane breeding pairs (Brian Johns, Canadian Wildlife Service, *pers comm.*) has identified six distinct nesting areas (Figure 1-1, Chapter I) within the breeding grounds, all of which are isolated and accessible only via helicopter. Three nesting areas are located in the center of the breeding grounds (Klewi, Sass, and Sass-Klewi; 'high-use' nesting areas) and contain a large number of breeding pairs, while the other three (Alberta, Nyarling and North Nyarling; 'low-use' nesting areas) are located peripheral to high-use nesting areas and contain fewer breeding pairs.

With the above research and monitoring, Parks Canada is in position to develop a long-term Whooping Crane prey-monitoring program, as outlined by the International Recovery Plan for the Whooping Crane (Canadian Wildlife Service & U.S. Fish and Wildlife Service (CWS & USFWS) 2007). The Recovery Plan identifies the need to monitor potential food sources in areas that are both used and not used by the birds (CWS & USFWS 2007). Long-term implementation of this program will help determine if there is a relationship between yearly food availability, Whooping Crane chick survival and, if possible, inform the Recovery Team where captive-bred birds might be introduced into the breeding grounds.

Both spatial and temporal variability of the faunal assemblages in the nesting area ponds must be examined to determine how both types of variability should be incorporated into a prey monitoring program. This will help determine where in the breeding grounds sampling should occur, as well as when it should occur in the breeding season. Numerous studies have examined both spatial and/or temporal variation of freshwater invertebrate assemblages, both seasonally and yearly. For

example, studies have shown that invertebrate diversity increases with increasing hydroperiod (Brooks 2000), spatial differences in invertebrate communities are most pronounced for flightless invertebrates (Batzer et al. 2005), and in inter-connected systems, local environmental conditions can strongly influence invertebrate communities (Cottenie et al. 2003). For this study, spatial variability refers to the variation in aquatic fauna among the different nesting areas, and temporal variability refers to seasonal variation, e.g., immediately after hatching when chicks are not mobile vs. later in the season when chicks are able to feed on their own.

I sampled aquatic fauna in diatom ponds of the Whooping Crane breeding area during the summer of 2005 to examine a) how macroinvertebrate (>2 mm) and fish assemblages change both spatially and temporally, as previously defined, and b) how spatial and/or temporal patterns are affected by changing the taxonomic resolution used in the analyses. Specific questions are as follows:

1. Are distinct faunal communities present in ponds in the different nesting areas and does this vary temporally across the summer months?
    a. How do faunal assemblages change temporally during the breeding season and spatially among crane nesting areas?
    b. Do indicator taxa (sensu Dufrene and Legendre 1997) for the different nesting areas change through the breeding season?
    c. Are particular environmental variables associated with spatial and/or temporal changes in prey assemblages?
    d. Does changing the taxonomic resolution alter the conclusions drawn regarding the spatial and/or temporal variation?
2. Are temporal changes in taxon composition at ponds that are not used by cranes indicative of the temporal changes in nesting area ponds?

An investigation of these questions should help to determine the spatial and temporal scales appropriate for a prey-monitoring program and to what level aquatic taxa should be identified, in addition to increasing the basic understanding of this breeding habitat.

STUDY AREA

For a description of the Whooping Crane breeding grounds in Wood Buffalo National Park, see Chapter II: Study Area.

METHODS

Field Methods

Sampling was conducted during three periods, May 25 – June 21, June 24 – July 13, and July 23 - August 12, 2005 (referred to as June, July and August, respectively). Sampling began in late May when the first crane hatchlings were mobile and moving on their own, making them less vulnerable to disturbance and potential abandonment by parents. The other two periods occurred one and two

months after the first period, with the final month approximately one month prior to the departure of the first birds from WBNP (CWS & USFWS 2007).

In each period a suite of Preble Creek (PC) ponds was sampled, two ponds in June and four additional ponds in July and August. During each sampling period, two ponds were also visited in each of the six nesting areas, with the exception of the Alberta and Sass nesting areas in June. Nesting areas are separated from one another by a portion of breeding grounds not currently used by any cranes. One pond from each nesting area was repeatedly visited in all three sampling periods, whereas the second pond was visited just once. Thus, by the end of the season a total of four ponds were sampled in each nesting area (with the aforementioned exceptions), with one pond receiving three visits and three ponds receiving one visit each. A total of 50 'pond-visits' were conducted over the course of the summer. See Figure 3-1 for a schematic diagram of the nesting area sampling protocol.

This sampling design allowed me to evaluate both temporal and spatial variability within Whooping Crane ponds, with limited funds and resources. With ponds that were sampled repeatedly, I could assess how the invertebrate assemblages changed throughout the summer, across all nesting areas. The single-visit ponds were sampled to better incorporate the variability within each nesting area into the overall among-nesting-area variability. Limited helicopter availability, however, restricted sampling to ponds of one breeding territory per nesting area for the entire summer, in all but one nesting area. Furthermore, to maximize efficiency of helicopter use, I used sampling methods (see below) that did not require a return visit to collect traps.

I collected aquatic invertebrates from each pond using 15 timed dip-net samples with a 15 cm x 12.5 cm standard aquarium dip net (Hagen Easy Catch Net) with a 1 mm square mesh size. Sotiropoulos (2002) found aquarium dip-net samples to be very effective because standard D-nets are too large and cumbersome for the majority of these shallow ponds. I conducted timed dip-net samples, each from a fixed location, as the soft substrate in these ponds made movement along a transect impractical (*pers. obs.*). Dip-net samples were eight minutes long, during which time I collected all invertebrates that I saw, and could reach. The organisms collected from each sample were placed into 120 mL plastic vials (one sample/vial) for transport to the laboratory. To assess fish presence and abundance, I used Gee minnow traps (42 x 23 cm, with a 6 mm mesh). Fifteen minnow traps (baited with half a piece of white bread) were set for approximately 1-2-hours, as Sotiropoulos (2002) found that this length of time was sufficient to assess fish presence. Fish were identified, counted in the field, and subsequently released. I also collected invertebrate taxa from eight (randomly chosen) of the 15 minnow traps to supplement the dip-net samples, because some taxa collected in minnow traps were not collected by dip-nets (see Chapter II). Locations for dip-net samples and minnow traps were chosen in a stratified random design to provide representative sampling in each of the habitats, based on substrate and/or macrophyte type(s) in each pond. For each pond, conductivity was measured with a Waterproof ECTestr Low Microprocessor Series meter, pH was measured with a Waterproof pH Testr10 meter, water temperature was measured with a Thermo-Sensor aquarium thermometer, and water depth to the top of the diatom layer was measured with a standard ruler.

Laboratory Procedures

Invertebrate samples were taken to the laboratory within 3-6 hours of being collected where they were drained of pond water and preserved in 80% ethanol. Prior to processing, samples were washed through a 2mm sieve to remove the majority of the background sediment and smaller organisms, e.g. microcrustaceans, that were probably not potential Whooping Crane prey. Invertebrates were then picked from the remaining sediment with the aid of a dissecting microscope. For the most part, insects were identified to genus unless they were in early larval instars, missing key body parts, pupae, or of the family Corixidae. Morpho-taxa were used for some Limnephilidae genera (e.g., *Asynarchus/ Philarctus/Limnephilus*), due to difficulties in identifying these individuals to genus (Wiggins 1996). Most of the remaining invertebrates were identified to family or order level, and for all identifications I used keys from Clifford (1991), Merritt and Cummins (1996), Wiggins (1996) and Larson et al. (2000). Invertebrate taxa were then counted, recorded and stored in scintillation vials with 80% ethanol.

Statistical Analyses

Depending on the analyses, pond-visits were grouped *a priori* by crane-use groups and/or sampling period. Classifying nesting areas into crane-use groups based on the number of breeding Whooping Crane pairs using the areas (Brian Johns, CWS, *pers comm.*).

1. Preble Creek ponds were classified as 'no-use' because use by Whooping Cranes has never been observed, and they are located outside, albeit adjacent to, the current breeding grounds,
2. Alberta, Nyarling and North Nyarling were called 'low-use' areas because they are used by low numbers of breeding pairs, and
3. Klewi, Sass, and Sass-Klewi were called 'high-use' areas because they are used by a higher number of breeding pairs.

To evaluate environmental differences among these crane-use groups, I conducted single-factor ANOVAs for each of the environmental and landscape characteristics that were measured: pH, conductivity, water depth, distance to nearest creek, and distance to nearest nest. Distance to nearest creek and distance to nearest nest were obtained from Brian Johns (Canadian Wildlife Service, *pers. comm.*). ANOVAs were also conducted for pH, conductivity and water depth, grouped according to sampling period, and post-hoc comparisons were made using sequentially adjusted Bonferroni p-values (Holm 1979).

I used non-metric multidimensional scaling (NMS) ordination to evaluate the spatial and temporal variation of faunal community composition (macroinvertebrates and fish) in the nesting area ponds. See Chapter II for an explanation of this analysis. For all ordinations, I used a random starting configuration, and the parameter setup included 1000 runs with real data, 4 axes, and 15 iterations to determine stability. Monte Carlo permutations were conducted with 999 randomized runs to determine the proportion of randomized runs with stress values less than or equal to stress in the real data. Sorensen (Bray-Curtis) distance measure was used. All multivariate analyses

were conducted using the program PC-ORD 5 for Windows (McCune and Mefford 1999).

Multi-response permutation procedure (MRPP) (McCune and Grace 2002) is a non-parametric analysis that tests the null hypothesis of no difference between two or more groups, and is appropriate for non-normal data. I used MRPP to assess whether the faunal composition in ponds differed among the crane-use groups defined *a priori*. Rank-transformed abundance data and Sorensen (Bray-Curtis) distance measure were used because they generate results more similar to NMS (McCune and Grace 2002).

If the MRPP suggested that pond groups were different, indicator species analyses (Dufrene and Legendre 1997) were then conducted to identify taxa that were common, and relatively exclusive, to each pond-group. To be an indicator, the taxon must have at least 50% of its total abundance (i.e., across all groups) present in the pond-group it indicates (relative abundance), and at least 50% of the ponds within that group should have the indicator taxon present (relative frequency) (Dufrene and Legendre 1997).

I conducted NMS ordinations and MRPP analyses to assess spatial variability among crane-use categories. I examined data from all 50 pond-visits both with and without fish data to determine if fish affected the groupings of pond-visits in ordination space. I also conducted analyses using all ponds visited once (n=18) plus a subset of ponds visited repeatedly (n=12; total n=30), as well as analyses involving only ponds visited repeatedly. Additionally, I examined each sampling period separately (ponds still grouped according to crane-use) to assess how crane-use groups differed within each period. Indicator species were determined for all groups. All of the analyses were conducted at the lowest feasible taxonomic group (LFTG), as well as the family and order levels, using both $\log_{10}$(abundance+1) and presence/absence data.

I also conducted analyses of specific potential prey taxa, i.e., fish, dragonflies, and predaceous diving beetles, as identified by Sotiropoulos (2002) and Bergeson et al. (2001). I performed a two-way ANOVA using the program SPSS Version 15 (©SPSS Inc., 1989-2006) to determine if there was an interaction between the potential prey taxa and ponds grouped according to crane-use.

I conducted variance partitioning analysis (VPA) (see Borcard et al. 1992; Rodriguez and Magnan 1995; Hall et al. 1999; Beisner et al. 2006) using the program CANOCO for Windows Version 4.5 (ter Braak and Smilauer 1999). Variance in the prey assemblage data is broken down into variation that is both unique to and shared by three sets of variables (spatial, environmental, and fish) (Borcard et al. 1992). First, a preliminary detrended correspondence analysis (DCA) was performed to determine whether a linear model (redundancy analysis - RDA) or unimodal model (canonical correspondence analysis - CCA) would be more appropriate. Forward stepwise selection determined which environmental variables were significantly associated with variation in the assemblage data matrix ($p<0.05$) (ter Braak and Smilauer 1999), and should be included in the VPA. The three-way VPA was then conducted to examine the relationship between the invertebrate assemblages and three sets of explanatory variables associated with the environment (E), fish taxa (F), and the crane-use areas (C). The following steps were conducted to complete the three-way VPA, as explained in Hall et al. (1999):

1) one canonical ordination with all explanatory variables constrained (E+F+C), and no covariables, to determine the proportion of variation in the taxa that is not explained by the variables measured;
2) partial canonical ordinations to determine the unique effects of each class of explanatory variables – one for each of the three groups of explanatory variables, with the two remaining variable types as covariables (e.g., E constrained, F+C as covariables);
3) partial canonical ordinations to determine effects of each class of explanatory variables plus two-way interactions – ordinations with one explanatory variable class constrained, and one of the remaining two classes as a covariable (e.g., E, F as covariable; E, C as covariable; and F, E as covariable)
4) appropriate terms from step 2 were subtracted from step 3 to determine the proportion of explained variation shared by two variables (i.e., two-way interactions);
5) terms from steps 1, 2 and 4 were subtracted from 100% to determine the percent of explained variation shared by all three variables (i.e., the three-way interaction).

I conducted NMS ordinations and MRPP analyses to assess temporal variability among sampling periods. As described above, I examined all 50 pond-visits both with and without fish data, all single-visit ponds plus a subset of repeat-visit ponds, and all repeat-visit ponds. Ponds were grouped according to sampling period for each analysis. Indicator species analyses (Dufrene and Legendre 1997) were performed for all data sets except repeat-visit ponds. Additionally, I examined each crane-use group separately (still grouped according to period) to assess how sampling periods differ within each crane-use group. All of the analyses were conducted at the LFTG, family and order levels, using both $\log_{10}$(abundance+1) and presence/absence data. Three-way variance partitioning analysis was conducted as described above, using sampling period, environmental variables, and fish taxa as the three sets of explanatory variables.

Temporal variability of Preble Creek (no-use) ponds was assessed separately using NMS, MRPP, and indicator species analyses. The same analyses were then conducted on the remaining ponds (low- and high-use ponds grouped together). Results for both the Preble Creek and the low/high analyses were compared to determine if similar patterns were seen in ordination space (i.e., were ponds grouped according to sampling period in both analyses) and if the same indicator taxa were identified.

RESULTS

Spatial Variability

Among the ponds defined by the crane-use groups, distance to nearest creek ($F_{2,26}$=5.6, P<0.01) and distance to nearest nest ($F_{2,26}$=184.6, P<0.0001) differed. No-use ponds were closer to the nearest creek than low-use ponds, but not high-use ponds (Table 3-1). All three crane-use comparisons differed for distance to nearest nest; as

expected, high-use ponds were closest to nests whereas no-use ponds were furthest from nests (Table 3-1). Conductivity, pH and water depth did not differ (Table 3-1).

Unless otherwise noted, all NMS analyses recommended three dimensions. For simplicity, however, I present only the two-dimensional graphs using the axes that best illustrate patterns in the data. With only three pond-visit exceptions, the high-use nesting area ponds clustered together in the ordination, distinct from the no- and low-use area ponds, which overlapped almost completely (Figure 3-2; LFTG and log(abund+1)). Other taxonomic levels and presence/absence data showed a similar pattern. High crane-use ponds that overlapped with the no- and low-use ponds were generally consistent across taxonomic levels and, together with no- and low-use ponds, were characterized by an absence of fish. The stress values for presence/absence and abundance data were similar for LFTG and family, but were 3-6 units lower at the order level. Outlier analysis identified all three visits to one Sass-Klewi pond as outliers; their removal, however, reduced overall stress only marginally and did not alter ordination patterns, so they were included in all of the analyses.

As suggested by the ordinations, high-use ponds differed from no- and low-use ponds (MRPP; $p<0.0001$) regardless of taxonomic level and whether presence/absence or abundance data were used. As expected, no- and low-use ponds did not differ ($p>0.14$) for either abundance ($p=0.14$-0.19, depending on taxonomic level) or presence/absence data ($p=0.38$-0.83). These patterns were generally similar, though not identical to MRPP analyses on a month-by-month basis, e.g., not all of the comparisons involving high-use ponds differed (Table 3-2).

For the most part, results of indicator taxon analyses were consistent across identification levels, i.e., indicator taxa at the LFTG were members of indicator families and orders (Table 3-3; no- and low-use ponds grouped together because they do not differ). For example, with abundance data, *Graphoderus* spp. and *Hygrotus* spp. were indicators of the no/low-use group at the LFTG, and Dytiscidae and Coleoptera were indicators of the same group at the family and order levels, respectively.

I repeated the above spatial-pattern analyses after excluding fish from the matrices to assess the influence fish have on the spatial patterns. NMS ordinations showed a similar stress value for the analyses without fish (e.g. abundance, LFTG stress=16.57). With only two exceptions (involving presence/absence and coarser taxonomic levels), the same pairwise MRPP comparisons were significant, using sequentially adjusted Bonferroni p-values ($p<0.016$). With a few exceptions, invertebrate indicator taxa for the fishless data were the same as when fish were included in the analyses.

High-use ponds still generally clustered together, largely distinct from the other groups in spatial analyses conducted on the single-visits data set. The following pairwise comparisons (MRPP) were different using sequentially adjusted Bonferroni p-values: low- vs. high-use, presence/absence and abundance, LFTG and order; no- vs. high-use, presence/absence and abundance, order ($p<0.014$). Analysis of only repeat-visit ponds (presence/absence, LFTG data) indicated that all pairwise comparisons were significant.

There was a strong interaction between the abundance of potential prey and pond-type defined by crane-use group (Figure 3-3). Abundance of Dytiscidae was

highest in the no-use ponds and lowest in the high-use ponds, whereas fish taxa were absent from the no- and low-use ponds but caught in abundance in the high-use ponds. Anisoptera abundance, in contrast, stayed relatively constant across all crane-use groups.

Prior to conducting the VPAs, a preliminary DCA suggested that a linear model was appropriate for these pond assemblages (gradient length=2.4; ter Braak and Smilauer 1999), therefore, I conducted the VPA using RDA. Forward stepwise selection pointed to water depth and pH as the only significant environmental variables ($p<0.05$; ter Braak and Smilauer 1999). Three-way VPAs of abundance and PA data, identified to the lowest feasible taxonomic group, yielded the following results (Figure 3-4), after the appropriate calculations (see Statistical Analyses section):

- Abundance Data:

1) 14.3% of the variance was explained by the environmental parameters alone;
2) 57.3% of the variance was explained by the environment x fish x nesting area interaction; and
3) 28.4% of the variance was unexplained.

Fish and nesting area on their own, as well as all two-way interactions, explained none of the variation (0%).

- Presence/Absence Data:

1) 11.1% of the variance was explained by the environmental variables;
2) 8.8% of the variance was explained by the fish variables;
3) 2.1% of the variance was explained by the fish x nesting area interaction;
4) 0.1% of the variance was explained by the environment x fish interaction;
5) 49.7% of the variance was explained by the environment x fish x nesting area interaction; and
6) 28.2% of the variance was unexplained.

Nesting area alone, plus the nesting area x environment interaction, explained none of the variation (0%).

Temporal Variability

Conductivity, pH and water depth of the ponds did not differ among pond groups defined by sampling period (ANOVAs, $p>0.1$).

Using abundance data and the lowest feasible taxonomic groups, ponds from the June and July sampling periods overlapped across both NMS axes, while ponds from the August sampling period overlapped only slightly with a few July ponds (Fig. 3-5). MRPP results, however, indicated that all sampling periods differed ($p<0.016$) (also for presence/absence data, at the LFTG and family level). As the summer progressed, the general trend among indicator taxa was a shift from Trichoptera and Dytiscidae taxa in June, to Odonata and different Dytiscidae taxa in July, to Corixidae, Ephemeroptera, and different Trichoptera taxa in August (Table 3-5). Patterns were less distinct at the order level; 3 of 6 comparisons among months were not significant ($p>0.057$) and June and July sampling periods lacked indicator taxa (Table 3-5). Analyses without fish yielded the same patterns in the ordinations, and MRPP and indicator taxa results were identical.

MRPP assessments across sampling periods within each crane-use group showed similar results for the LFTG and family level analyses, with 3 of 9 comparisons not significant. At the order level, however, 7 of 9 comparisons were not significant. Temporal differences within the high-use ponds were rarely significant (Table 3-6), and then only between June and August.

For the single-visit data set, NMS ordinations showed weak sampling-period clusters, but the same significant pairwise comparisons between sampling periods were revealed by MRPP (significant June vs. August comparisons, significant July vs. August LFTG and family comparisons, and significant June vs. July LFTG comparison). Similar shifts in indicator taxa occurred, with Trichoptera and Dytiscidae indicators for June, Odonata and different Dytiscidae for July, and Ephemeroptera and different Trichoptera for August. Analyses of only the repeat-visit ponds (presence/absence, LFTG data) indicated that only June differed from August (p-value <0.001).

In the VPAs, identified to the lowest feasible taxonomic group, the percentage of unexplained variation was higher than in the nesting area analyses (Figure 3-6).

- Abundance Data:

1) 16.7% of the variance was explained by sampling period;
2) 10.3% of the variance was explained by environmental parameters;
3) 2.9% of the variance was explained by the environment x sampling period interaction;
4) 32.3% of the variance was explained by the environment x fish x sampling period interaction; and
5) 37.8% of the variance was unexplained.

Fish, fish x period and fish x environment interactions explained none of the variation (0%).

- Presence/Absence Data:

1) 11.8% of the variance was explained by sampling period;
2) 10.9% of the variance was explained by the environment x sampling period interaction;
3) 10.9% of the variance was explained by the fish x sampling period interaction;
4) 9.5% of the variance was explained by the environment x fish interaction;
5) 22.9% of the variance was explained by the environment x fish x sampling period interaction; and
6) 34.0% of the variance was unexplained.

Fish and environment variables explained none of the variation (0%).

Preble Creek Temporal Variability

The Preble Creek (no-use) ponds (abundance data; LFTG) showed little or no overlap among sampling periods, (Fig 3-7; abundance data, LFTG; a similar result was obtained for presence/absence data). All pairwise comparisons were significant (MRPP, $p<0.008$). Nesting area ponds (low- and high-use combined) appeared less distinct across the three sampling periods (Fig 3-8). Nevertheless, all pairwise comparisons were different (MRPP, $p<0.014$). For the most part, indicator taxa were similar for PC ponds and nesting are ponds, although more taxa were significant indicators for the latter (Table 3-7).

DISCUSSION

Spatial Variability

Distance to nearest nest differed across crane-use groups, as expected. Preble Creek ponds were furthest away from nests because cranes do not nest in that area, whereas high-use ponds were closes to nests. Also as expected, Preble Creek (no-use) ponds were closest to a creek. However, high-use ponds were also fairly close to a creek, so only the no-/low-use comparison differed. Lack of difference in water depth was surprising, because Sotiropoulos (2002) found that ponds containing fish (i.e., most of the high-use ponds) were deeper than fishless ponds. In 2005, however, conditions were drier than normal in the Sass and Klewi (high-use) nesting areas, (B. Johns, CWS, *pers comm.*), which likely contributed to my results. Additionally, a few ponds were too deep to safely obtain a water depth measurement. Lack of significance in conductivity may have been partly because the conductivity meter could not measure higher than 1990 µS/cm, and previous work yielded conductivity values as high as 5620 µS/cm (Sotiropoulos 2002). Minimum values in high-use ponds, however, were approximately 300 µS/cm higher than minimum values of any pond in the low- or no-use areas.

Ponds in the highly-used Whooping Crane nesting areas consistently contained relatively discrete faunal communities, whereas both no- and low-use area ponds overlapped extensively. Indeed, most assemblages in high-use area ponds included fish whereas all but one of the no- and low-use area ponds lacked fish, which likely contributed to this pattern. Interestingly, at a much smaller spatial scale, Sotiropoulos (2002) found that ponds in which cranes were observed feeding always contained fish, whereas adjacent ponds did not.

Sotiropoulos (2002) also found that ponds lacking fish formed two distinct groups: one with Dytiscidae genera (predaceous diving beetles) as indicator taxa, and the other with Anisoptera genera (dragonflies) as indicators. While my NMS ordination suggested a distinction between ponds that contained fish (mostly high-use) and ponds that did not (mostly no-/low-use), no-/low-use ponds could not be split into sub-groups with distinct indicator taxa. This combined group of ponds, however, did have both Dytiscidae and Anisoptera taxa as indicators at various taxonomic levels for both presence/absence and abundance data.

Excluding fish from the analyses did not alter the NMS results substantially; there was only slightly more overlap of high-use area ponds with the no-/low-use cluster. The distinctness of high-use ponds broke down, however, in the PA family- and order-level analyses. Because removing fish from the analyses affected the results very little, I suggest that, statistically, fish are not the sole drivers of the community patterns, consistent with Paukert and Willis (2003). Biologically, however, the invertebrate taxa in fish and fishless ponds could result from fish presence or absence, respectively (Hanson and Riggs 1995; Zimmer et al. 2000, 2002; Tonn et al. 2004; Venturelli and Tonn 2005). Broad taxonomic groups, such as Dytiscidae, Diptera and Trichoptera, all of which have representatives as indicators of the no-/low-use group, have been recorded in higher abundance in fishless wetlands than in wetlands with fathead minnows (Zimmer et al. 2000).

Dytiscid beetle abundance was indeed much lower in ponds with fish present, as alluded to by the absence of dytiscid indicators in the high-use ponds. Because large dytiscid larvae or adults have only a limited period of vulnerability as prey of small-bodied fish, low dytiscid abundance in symmetry with fish could partly result from competition with fish for similar prey (Zimmer et al. 2000). Anisopteran abundance, however, stayed relatively constant (and low) across all pond-groups. Different vulnerabilities to fish effects among taxa could contribute to the literature's conflicting results in regards to the response of invertebrates to fish presence and predation (e.g., compare Blumenshine et al. 2000 and Venturelli and Tonn 2005 to Michaletz et al. 2005). Analysis of size class or biomass could have provided more insights into the interactions.

As the taxonomic identification level became finer, the indicator taxa for each crane-use group did not change qualitatively, but simply were components of the indicator taxa at coarser levels. For example, *Graphoderus* spp. was an indicator at the lowest feasible taxonomic group level, Dytiscidae was an indicator at the family level, and Coleoptera was an indicator at the order level. This is consistent with studies advocating the use of higher taxonomic levels, such as family, in monitoring programs (e.g. Bowman and Bailey 1997; Marshall et al. 2006).

Based on the formulae for calculating indicator taxa (Dufrene and Legendre 1997), caution must be taken when there are a small number of groups for which indicators are sought. In these cases, it is possible that an indicator taxon for one group will barely meet the 50% relative abundance cutoff, meaning there is a possibility that the taxon will be close to meeting the cutoff for the other group(s). The taxon under question could also have a high frequency of occurrence for all groups. For example, Corixidae was an indicator for the no-/low-use group (LFTG abundance data) with 58% relative abundance and 100% frequency. For the high-use group, however, Corixidae had 42% relative abundance and 74% relative frequency. When sampling a pond to determine whether it is similar to a high-use or no-/low-use pond, it would possible to mis-identify the pond if only relying on Corixidae as an indicator. Relative abundance and relative frequency for all indicator taxa, and taxa that are almost significant, should be examined to identify taxa that behave similarly to Corixidae. It is rare for a single taxon to consistently and accurately characterize a water body and/or its level of biodiversity (Heino et al. 2003a, b), so I recommend use of as many indicators as possible to avoid potential misclassification, especially when examining a small number of groups. Fish are an exception, however, as they were consistently absent from no- and low-use ponds and present in almost all high-use ponds.

Some between-group comparisons that had been significantly different using the complete 50 pond-visit data set were no longer significant in the single pond-visit dataset. This was likely a result of excluding the repeat-visit ponds, rather than that of small sample size. At least half of the ponds in each pond-group were visited three times, resulting in considerable over-representation of these ponds in the analyses.

Variance partitioning indicated that environmental data, fish composition, and nesting areas combined explained a substantial portion of the variance in the invertebrate communities (~72% for both abundance and presence/absence data). The high percentage of variance explained by the environment x fish x nesting area

interaction clearly shows that these three sets of explanatory variables have similar spatial structuring (Borcard et al. 1992). Lack of significant two-way interactions indicates that all of the overlap among variables was accounted for in the three-way interaction, e.g., the fish x nesting area interaction did not explain any variation that was not already explained by the fish x nesting area x environment interaction. Similarly, both fish and nesting area variables explained very little, if any, variation in the invertebrate community on their own as it was accounted for in the three-way interaction.

For both data sets (log(abund+1) and presence/absence), environmental variables on their own explained a relatively small percentage, suggesting that there may have been more appropriate environmental variables to measure to increase the percent variation explained. Other variables could have included dissolved oxygen, pond area, and total phosphorus, all of which can strongly influence distribution of invertebrates and fish (Kalff 2002). Total phosphorus has been shown to be a predictor of zooplankton community structure (Beisner et al. 2006) and, in WBNP, Sotiropoulos (2002) found that an increase in total phosphorus towards the end of the summer coincided with an increase in invertebrate diversity. Nevertheless, this small percentage accounted for by environmental variables was comparable to that found by Beisner et al. (2006) in their study of zooplankton communities.

Temporal Variability

Although nesting area ponds experience annual spring flooding, summers are generally warm and dry (Environment Canada 1993, *in* Moser et al. 1998) and some ponds often dry up completely by late August/early September (Timoney 1997). Because of this, I expected that water depths would have decreased towards the end of the summer, and conductivity concomitantly increased (Kalff 2002). The 2005 summer started out drier than normal but above average rain in June (B. Johns, CWS, *pers comm.*) may have helped to maintain the water levels, at least in early/mid summer. As a result, the average water depth did not differ among sampling periods and, perhaps as a result, neither did conductivity (Kalff 2002) or pH.

Throughout the summer months, the faunal assemblages within the Whooping Crane ponds changed gradually; although differences in assemblages from adjacent months did not achieve significance, the contrast between June and August was significant. The temporal patterns were broadly similar to those observed in 1999 (Sotiropoulos 2002). My indicator species analysis identified various dytiscid genera as indicators at the beginning and middle of the summer, and *Caenis* spp. and Corixidae as indicators towards the end (abundance LFTG data). In 1999, Coleoptera and Gerridae were indicators in the early summer, whereas a more diverse community, characterized by Corixidae, *Caenis* spp., and *Lethocerus* spp., was observed towards the end of the summer (Sotiropoulos 2002). Those more years of data is required, this similarity does indicate that seasonal trends may be somewhat predictable.

Temporal patterns of the environmental variables may also help to explain why we did not see identical temporal patterns in invertebrate communities in 1999 and 2005. When ponds dry up, mobile taxa that are not tolerant to drying conditions, such as beetles, move to nearby ponds that still contain water (Schell et al. 2001), possibly explaining the absence of beetles towards the end of the summer in 1999 (Sotiropoulos

2002). In the 2005 season, these drought sensitive invertebrates (e.g., beetles) may have experienced less pressure to leave ponds because water permanence was not a critical factor, resulting in their presence throughout the entire 2005 season.

Assemblage seasonality within each crane-use group was similar to the patterns observed in all 50 pond-visits. The general lack of difference among adjacent months in high-use ponds was likely due to the presence of fish throughout the summer, which also contributed to a lower turnover among invertebrates. Though the influence of fish can vary, they tend to reduce abundance and diversity of invertebrates (e.g., Hanson and Riggs 1995; Zimmer et al. 2000, 2001). Similar results were found for the single-visit dataset while the repeat-visit pond analyses only showed significant results for June vs. August. As before, differences among the sampling periods weakened in order level analyses.

As discussed above, not all indicator taxa will meet the 50% relative abundance and relative frequency criteria when the number of groups is low. While *Mystacides* spp. was an indicator for the June sampling period (55% relative abundance and 79% relative frequency), but also had a 41% relative abundance and 78% frequency for July. Once again, if indicator taxa are used to determine suitability of ponds, it will be important to use more than one indicator whenever possible and to examine abundance and frequency of all indicators (and 'near' indicators) in all groups.

Variance partitioning using environmental data, fish taxa, and sampling period explained a substantial amount of variation in the invertebrate communities, only 5-10% less than the environment-fish-nesting area analyses. Similar to the latter spatial analyses, the large percentage of variation explained by the environment x fish x period interaction indicates that these variables have similar temporal structuring (Borcard et al. 1992). At least part of this lack of separate effects is likely attributable to the relative consistency of fish presence or absence across sampling periods. Environmental variables helped explain almost half of the variation, but mostly in conjunction with other explanatory variables, further indicating that environment-fish-sampling period influences cannot be easily separated.

Preble Creek Temporal Variability

Overall, seasonal variation in Preble Creek pond assemblages was similar to that documented in the nesting area ponds (low- and high-use ponds grouped together). Month-by-month, indicator taxa were similar for both groups. There were, however, two cases of a one-month time lag between a taxon's indicator status in the nesting area ponds and the same taxon's indicator status in Preble Creek (no-use) ponds. Recall, however, that no- and low-use ponds did not differ in composition, suggesting that low-use ponds could be driving the similar Preble Creek/nesting area seasonality.

Analysis of the Preble Creek ponds also allows a more direct comparison with Sotiropoulos' (2002) analysis. The surprising lack of similarity between my indicator taxa and Sotiropoulos' (2002) could partly be a result of sampling only two of the same ponds, as well as the previously discussed differences between indicator taxa and the different environmental conditions in 1999 vs 2005. Other temporal studies examining non-permanent water bodies, however, have found that macroinvertebrate

communities vary among years (Boulton et al. 1992; Jeffries 1994; Brooks 2000; Jeffries 2005; Beche et al. 2006).

With this limited data, I tentatively conclude that the Preble Creek ponds can be indicative of the low-use nesting area ponds, until future results indicate otherwise. Additional years of sampling are needed, however, before definitive conclusions can be made.

CONCLUSIONS

Overall, fish played a very important role in community-level patterns for the aquatic faunal of the Whooping Crane breeding ponds. Fish were indicators of the high-use group. Undoubtedly, fish presence or absence in a pond influenced the invertebrate community structure, as seen in other studies (e.g., Zimmer et al. 2000; Venturelli and Tonn 2005). Conversely, Dytiscidae were identified as indicators of the fish-free no-/low-use pond group. Should Parks Canada choose to identify potential areas for introduction of captive-bred cranes, I suggest that presence of fish could indicate a potentially suitable location for introduction. Continual monitoring/sampling, however, should occur throughout the breeding grounds (low- and high-use) to evaluate if the aquatic faunal assemblages change over time.

Faunal communities of all ponds differed across sampling periods with indicator taxa shifting from Dytiscidae at the beginning of the summer to Ephemeroptera and Corixidae towards the end. This is similar to temporal changes in invertebrate communities observed in 1999 (Sotiropoulos 2002). Because Preble Creek (no-use) ponds revealed seasonal patterns similar to those in the nesting area ponds (especially low-use ponds), sampling the easily accessible Preble Creek ponds as surrogates for a low-use nesting area ponds would help decrease the cost of sampling in the remote Whooping Crane breeding grounds.

Though not explicitly addressed in this chapter, the issue of taxonomic resolution in aquatic bioassessments has been widely debated (e.g., Bailey et al. 2001; Arscott et al. 2006). Many studies have examined how various levels of taxonomic resolution affect the detection of patterns and variability in data sets (Marchant et al. 1995; Bowman and Bailey 1997; Doledec et al. 2000; Lenat and Resh 2001; Arscott et al. 2006; Marshall et al. 2006; Metzeling et al. 2006; Heino and Soininen 2007). For the most part, my analyses led to similar conclusions when taxa were identified to the lowest feasible taxonomic group and the family level, but not always consistent at the order level.

Caution must be taken when extrapolating these trends to the entire breeding grounds. Due to limited helicopter availability, sampling generally occurred within only one breeding pair's territory per nesting area. Expanded monitoring is needed to determine whether conclusions made herein are indeed general. Results from this chapter, and Chapter II, have been used to develop a long-term Whooping Crane prey-monitoring program. If sampling continues over the long-term, the protocol can be adjusted to incorporate within-nesting-area variability.

Table 3-1. Means and ranges of environmental characteristics of ponds in the Whooping Crane breeding area, Wood Buffalo National Park. Ponds are grouped according to crane-use (see text). Asterisks denote an overall difference among crane groups (ANOVA; *p<0.05; **p<0.0001). Letter superscripts indicate that groups differ. Post-hoc comparisons were made using sequentially adjusted Bonferroni p-values.

Environment parameter	No-use ponds (n=6)		Low-use ponds (n=11)		High-use ponds (n=12)	
	Mean	Range	Mean	Range	Mean	Range
pH	8.17	7.7 - 8.7	8.43	7.4 - 9.1	8.30	7.6 - 8.7
Conductivity (μS/cm)	1730	1280 - >1990	1854	1350 - >1990	1929	1630 - >1990
Water depth (cm)	33.7	22.3 – 45.0	29.91	22.5- 36.4	36.22	8.6 - 69.5
Distance to nearest creek (km)*	0.26[a]	0.15 - 0.37	2.24[b]	0.40 - 4.2	0.90[a,b]	0 - 2.3
Distance to nearest nest (km)**	5.7[a]	5.6 - 5.8	2.46[b]	0.61 - 3.1	0.80[c]	0.62 - 0.98

Table 3-2. Results of Multi-Response Permutation Procedures (MRPP) examining the taxonomic composition among crane-use groups within each sampling period, for each taxonomic level. LFTG=lowest feasible taxonomic group. Post-hoc comparisons were made using sequentially adjusted Bonferroni p-values. *** $p<0.017$ ** $p<0.025$ * $p<0.05$ NS: $p>0.05$. Sample sizes indicate number of pond-visits.

Month – Taxonomic level	No- vs. low-use	Low- vs. high-use	No- vs. high-use
	Abundance data		
June – LFTG	NS (n=7)	*** (n=12)	** (n=9)
July – LFTG	NS (n=12)	NS (n=12)	*** (n=12)
August – LFTG	NS (n=12)	** (n=12)	*** (n=12)
June – family	NS	***	NS
July – family	NS	NS	NS
August – family	NS	**	***
June – order	NS	***	NS
July – order	NS	NS	***
August – order	NS	**	***
	Presence/absence data		
June – LFTG	NS	***	NS
July – LFTG	***	*	**
August – LFTG	NS	**	***
June – family	NS	NS	NS
July – family	NS	NS	NS
August – family	NS	NS	NS
June – order	NS	***	NS
July – order	NS	NS	NS
August – order	NS	***	**

Table 3-3. Indicator taxa (Dufrene and Legendre 1997) for no/low-use (combined) and high-use pond groups, using abundance and presence/absence data, and analyzed at the three taxonomic levels: lowest feasible taxonomic group (LFTG), family and order. P-values for each taxa are presented in brackets. *Asynarchus/Grammotalius/Limnephilus* and *Asynarchus/Philarctus/Limnephilus* are two morphological Limnephilidae groups that are extremely different in appearance, however, cannot be identified further than these groupings.

No/Low-use group (n=31)	High-use (n=19)
Abundance – LFTG	
Corixidae (p=0.0001)	Dace spp. (p=0.0001)
Graphoderus (p=0.0001)	*C. inconstans* (p=0.0001)
Hygrotus (p=0.0139)	*Limnephilus* (p=0.0018)
Diptera pupa (p=0.0144)	*Agrypnia* (p=0.0215)
Asynarchus/Philarctus/Limnephilus (p=0.0266)	*Hyalella azteca* (p=0.0352)
Abundance – family	
Corixidae (p=0.0002)	Dace spp. (p=0.0001)
Dytiscidae (p=0.0002)	*C. inconstans* (p=0.0002)
Diptera pupa (p=0.0144)	*Hyalella azteca* (p=0.0381)
Libellulidae (p=0.0326)	
Abundance – order	
Hemiptera (p=0.0001)	Dace spp. (p=0.0001)
Coleoptera (p=0.0013)	*C. inconstans* (p=0.0001)
Diptera (p=0.0226)	*Hyalella azteca* (p=0.0353)
Odonata (p=0.0419)	
Presence/absence – LFTG	
Graphoderus (p=0.0003)	Dace spp. (p=0.0001)
Corixidae (p=0.0048)	*C. inconstans* (p=0.0001)
Diptera pupa (p=0.0189)	*Limnephilus* (p=0.0045)
Asynarchus/Philarctus/Limnephilus (p=0.0258)	*Agrypnia* (p=0.0109)
Chaoborus (p=0.0300)	*Hyalella azteca* (p=0.0356)
Procloeon (p=0.0302)	
Hygrotus (p=0.0374)	
Presence/absence – family	
Corixidae (p=0.0057)	Dace spp. (p=0.0001)
Diptera pupa (p=0.0195)	*C. inconstans* (p=0.0001)
Chaoboridae (p=0.0281)	*Hyalella azteca* (p=0.0377)
Baetidae (p=0.0309)	
Presence/absence – order	
Diptera (p=0.0440)	Dace spp. (p=0.0001)
	C. inconstans (p=0.0001)
	Hyalella azteca (p=0.0350)

Table 3-4. Means and ranges of environmental characteristics of ponds in the Whooping Crane breeding area, Wood Buffalo National Park. Ponds are grouped according to sampling period. ANOVAs did not yield any significant comparisons (all p-values >0.10).

	June (n=10)		July (n=10)		August (n=9)	
Environment parameter	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
pH	8.4	7.4 – 9	8.32	7.9 - 9.1	8.22	7.7 - 8.8
Conductivity (μS/cm)	1744	1280 - >1990	1916	1350 - >1990	1926	1480 - >1990
Water depth (cm)	33.3	8.6 – 69.5	30.5	23.2 - 41.0	36.46	28.0- 45.0

Table 3-5. Indicator taxa (Dufrene and Legendre 1997) for June, July and August sampling periods using abundance and presence/absence data, and analyzed at the three taxonomic levels: lowest feasible taxonomic group (LFTG), family and order. P-values for each taxa are presented in brackets.
Asynarchus/Grammotalius/Limnephilus and *Asynarchus/Philarctus/Limnephilus* are two morphological Limnephilidae groups that are extremely different in appearance, however, cannot be identified further than these groupings.

June (n=14)	July (n=18)	August (n=18)
	Abundance - LFTG	
Asynarchus/Grammotalius/ Limnephilus (p=0.0001)	*Hygrotus* (p=0.0010)	*Caenis* (p=0.0001)
Rhantus (p=0.0018)	*Lestes* (p=0.0035)	Phryganeidae 'juv' (p=0.0001)
Mystacides (p=0.0096)	Trichoptera pupa (p=0.0049)	Corixidae (p=0.0195)
Asynarchus/Philarctus/ Limnephilus (p=0.0101)	*Sympetrum* (p=0.0068)	
Acilius (p=0.0320)	*Siphlonurus* (p=0.0092)	
	Ilybius (p=0.0490)	
	Abundance - family	
Limnephilidae (p=0.0001)	Lestidae (p=0.0026)	Phryganeidae (p=0.0001)
Leptoceridae (p=0.0163)	Trichoptera pupa (p=0.0064)	Caenidae (p=0.0002)
Hydrophilidae (p=0.0287)	Siphlonuridae (p=0.0104)	Corixidae (p=0.0204)
	Libellulidae (p=0.0358)	
	Abundance - order	
No taxa	No taxa	Ephemeroptera (p=0.0006)
		Hemiptera (p=0.0180)
	Presence/Absence - LFTG	
Asynarchus/Grammotalius/ Limnephilus (p=0.0001)	*Lestes* (p=0.0019)	Phryganeidae 'juv' (p=0.0001)
Rhantus (p=0.0018)	*Sympetrum* (p=0.0065)	*Caenis* (p=0.0001)
Acilius (p=0.0476)	*Siphlonurus* (p=0.0103)	
Asynarchus/Philarctus/ Limnephilus (p=0.0364)	Trichoptera pupa (p=0.0153)	
	Hygrotus (p=0.0202)	
	Ostracoda (p=0.0443)	
	Presence/Absence - family	
Limnephilidae (p=0.0011)	Lestidae (p=0.0023)	Caenidae (p=0.0001)
Hydrophilidae (p=0.0356)	Siphlonuridae (p=0.0083)	Phryganeidae (p=0.0117)
	Trichoptera pupa (p=0.0144)	
	Ostracoda (p=0.0448)	
	Presence/Absence - order	
No taxa	No taxa	Ephemeroptera (p=0.0004)

Table 3-6. Results of MRPP examining the taxonomic composition among sampling periods within each crane-use group, for each taxonomic group. LFTG=lowest feasible taxonomic level. Post-hoc comparisons were made using sequentially adjusted Bonferroni p-values. *** $p<0.017$ ** $p<0.025$ * $p<0.05$ NS: $p>0.05$. Sample sizes indicate number of pond-visits.

Crane-use – Taxonomic level	**June vs July**	**July vs August**	**June vs August**
	Abundance data		
No-use – LFTG	** (n=8)	*** (n=12)	* (n=8)
Low-use – LFTG	NS (n=11)	** (n=12)	*** (n=11)
High-use – LFTG	NS (n=13)	NS (n=12)	*** (n=13)
No-use – family	*	***	**
Low-use – family	NS	**	***
High-use – family	NS	NS	***
No-use – order	NS	NS	NS
Low-use – order	NS	NS	***
High-use – order	NS	NS	***
	Presence Absence data		
No-use – LFTG	**	***	*
Low-use – LFTG	NS	**	***
High-use – LFTG	NS	NS	***
No-use – family	*	***	**
Low-use – family	NS	***	**
High-use – family	NS	NS	NS
No-use – order	NS	NS	NS
Low-use – order	NS	***	**
High-use – order	NS	NS	NS

Table 3-7. Indicator taxa for each sampling period, using abundance data at the lowest feasible taxonomic group. P-values are presented in brackets. (n=50) *Asynarchus/Grammotalius/Limnephilus* and *Asynarchus/Philarctus/Limnephilus* are two morphological Limnephilidae groups that are extremely different in appearance, however, cannot be identified further than these groupings.

June	July	August
	Preble Creek/no-use ponds	
Asynarchus/Grammotalius/ Limnephilus (p=0.0116)	*Oecetis* (p=0.0120)	*Caenis* (p=0.0005)
Asynarchus/Philarctus/ Limnephilus (p=0.0152)	*Hygrotus* (p=0.0399)	Phryganeidae juv. (p=0.0010)
Culicidae (p=0.0116)		*Procloeon* (p=0.0287)
		Graphoderus (p=0.0399)
	Low- and high-use ponds grouped	
Asynarchus/Grammotalius/ Limnephilus (p=0.0001)	*Hygrotus* (p=0.0236)	*Caenis* (p=0.0152)
Asynarchus/Philarctus/ Limnephilus (p=0.0126)	*Sympetrum* (p=0.0015)	Phryganeidae juv. (p=0.0038)
Rhantus (p=0.0069)	*Siphlonurus* (p=0.0073)	*Agrypnia* (p=0.0332)
Oecetis (p=0.0319)	*Lestes* (p=0.0126)	
Mystacides (p=0.0492)	*Procloeon* (p=0.0243)	

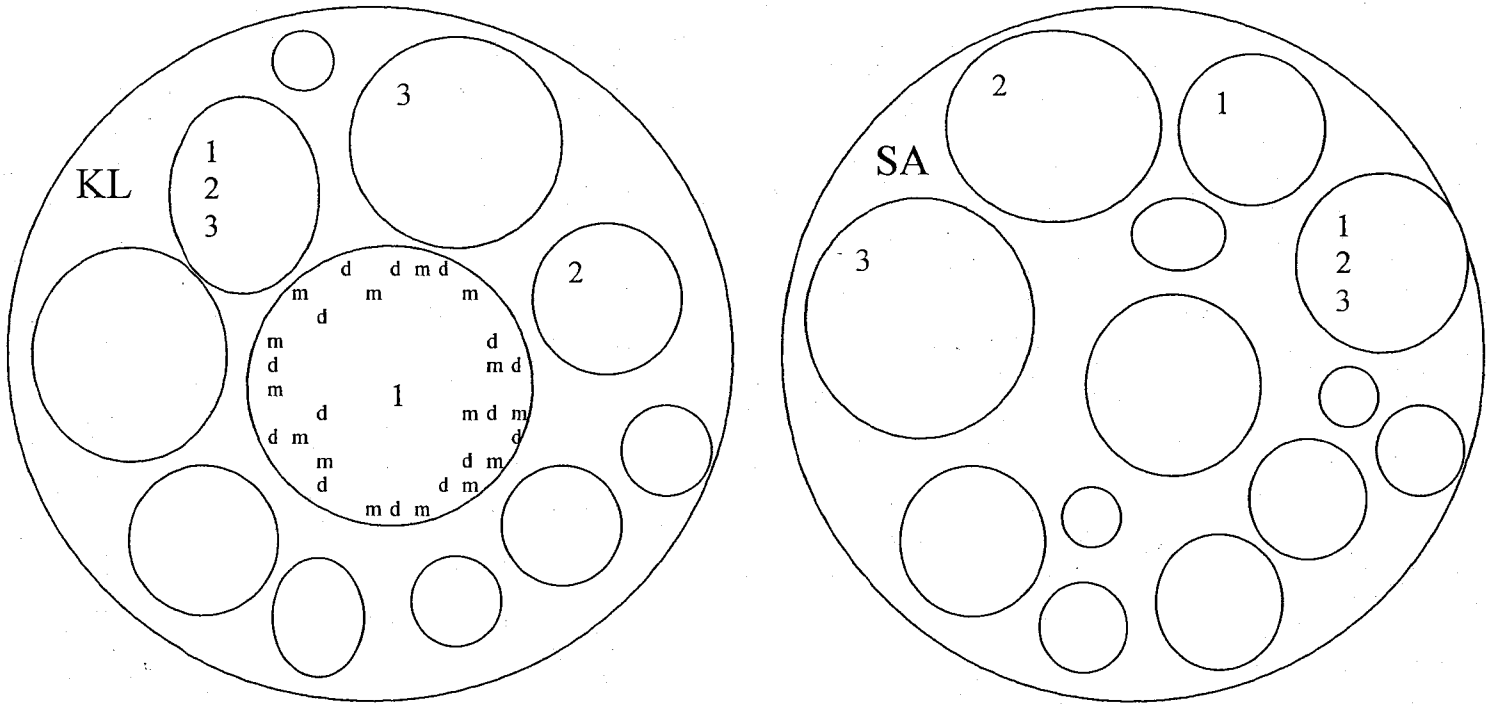


Figure 3-1. Schematic diagram of two nesting areas (KL=Klewi, SA=Sass) sampled in the 2005 field season. Each of the large circles represents one nesting area and small circles within represent ponds within the nesting area. The numbers in the 'ponds' indicate the sampling period during which ponds were sampled. Ponds with 1, 2, 3, were sampled in all three periods. Ponds without numbers were not sampled. Pond labeled 1 in the KL nesting area shows locations of 15 minnow traps (m) and 15 dip-net samples (s), used to sample each pond. The sampling regime was also used for all nesting areas.

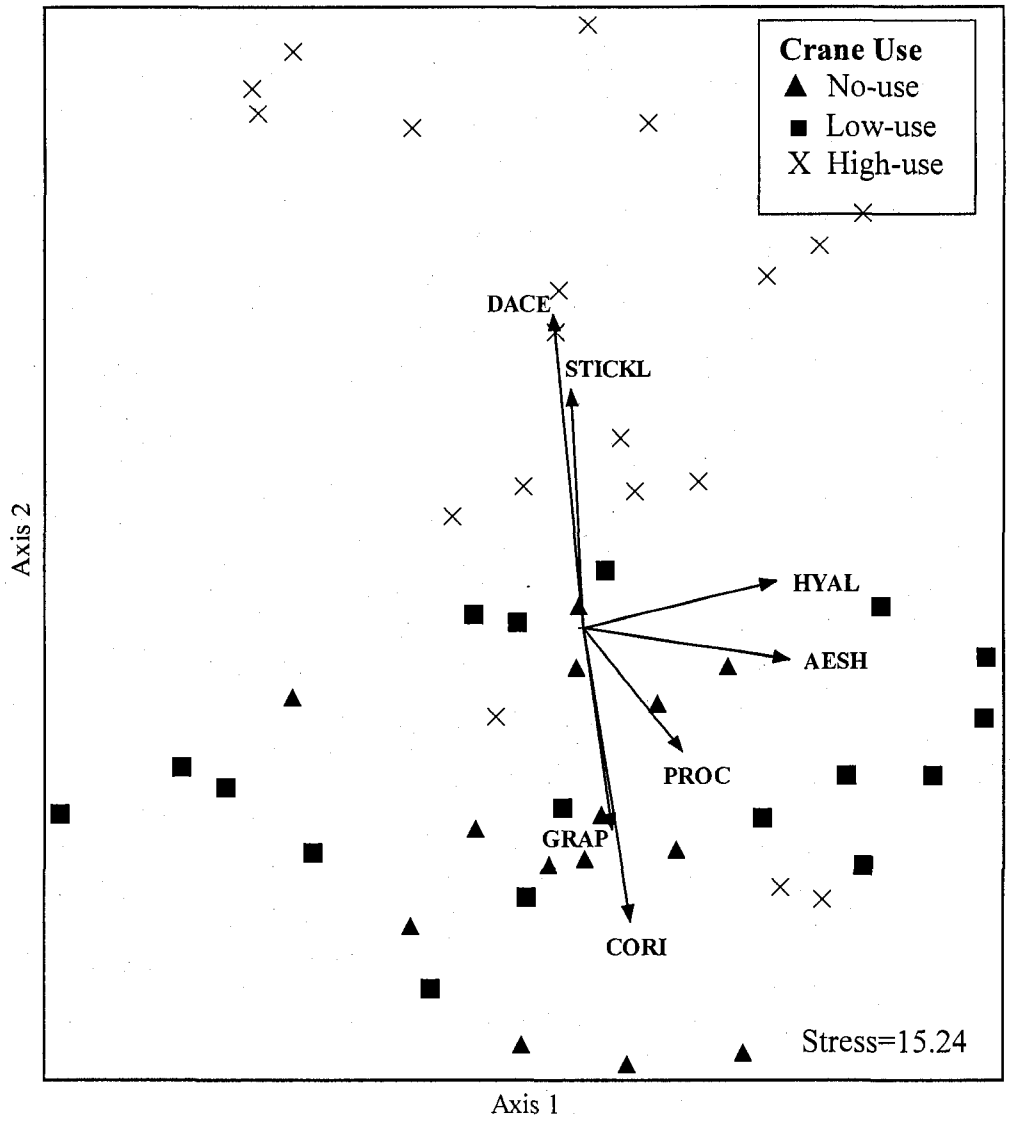


Figure 3-2. Spatial patterns in the pond communities in Wood Buffalo National Park (WBNP). Non-metric multidimensional scaling (NMS) joint plot of pond visits based on macroinvertebrate assemblages identified to the lowest feasible taxonomic group (LFTG) and data as log(abund+1) for all no-, low- and high-use ponds (n=50), sampled with dip-net samples and 2hr minnow traps. Ponds are grouped according to crane-use. Vectors (arrows and corresponding taxa) point in the direction of increasing values, and a longer line indicates a stronger relationship between the vector and the joint plot axes. Only vectors with an R^2 of 0.30 or greater are plotted. Abbreviations for fish and invertebrate taxa are presented in Appendix B.

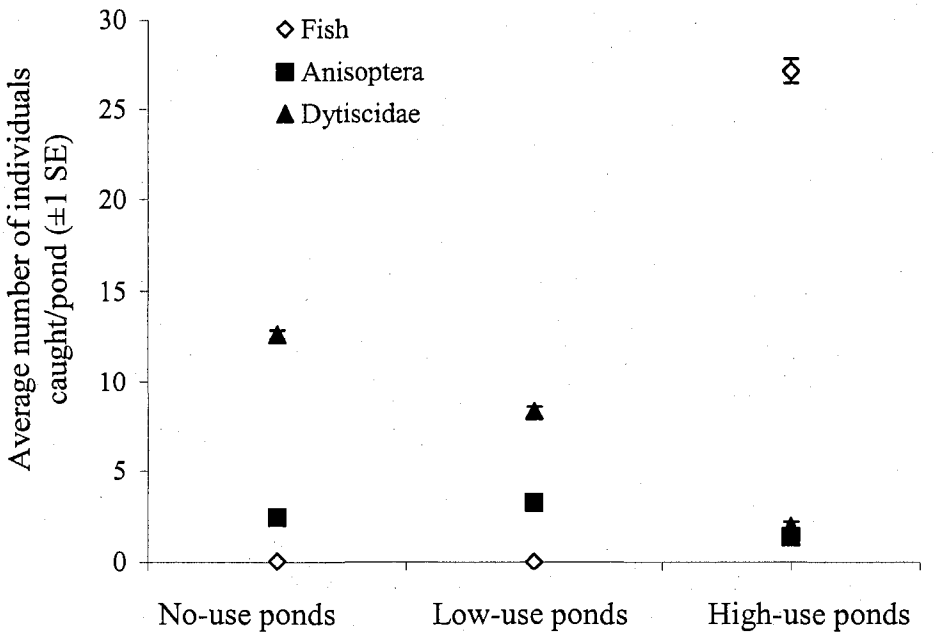
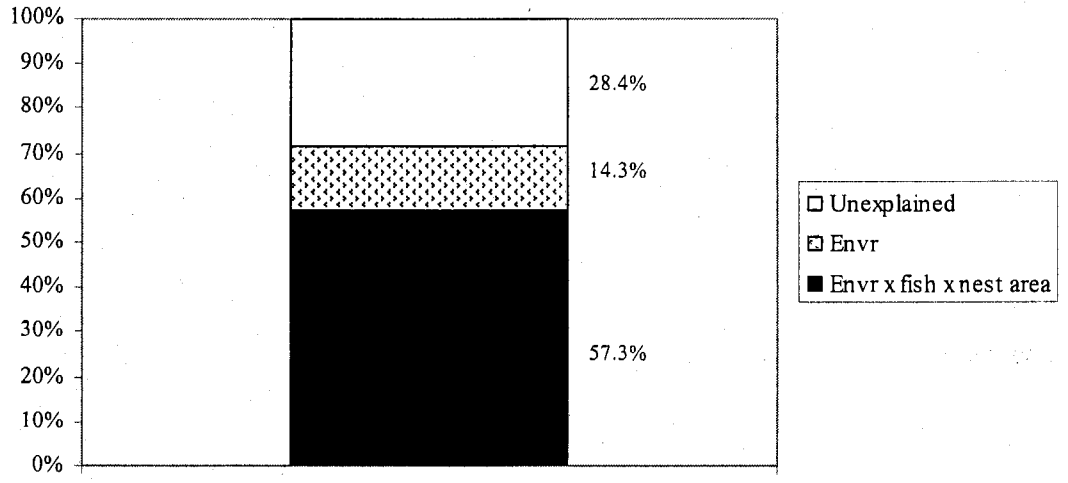
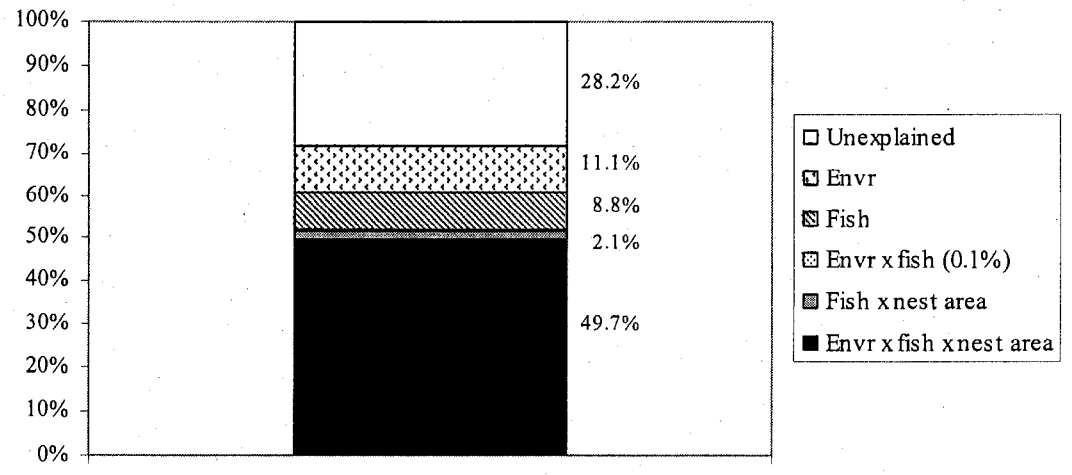


Figure 3-3. Number of potential prey individuals caught/pond for each crane-use group (no-use, low-use and high-use). A two-way ANOVA between potential prey and crane-use group yielded a significant interaction ($F_{4,117}$=30.45, $p<0.001$), and significant main effects (potential prey: $F_{2,117}$=9.92, $p<0.001$; crane-use: $F_{2,117}$=3.72, $p=0.027$).



a) abundance data



b) presence/absence data

Figure 3-4. Results of three-way variance partitioning analyses, indicating the percentage of variation in invertebrate taxa collected in 30 ponds that is explained by environment variables (water depth and pH), fish (Dace sp. and *Culaea inconstans*), nesting areas (no-, low- and high-use groups), and the two- and three-way interactions of these sets of variables.

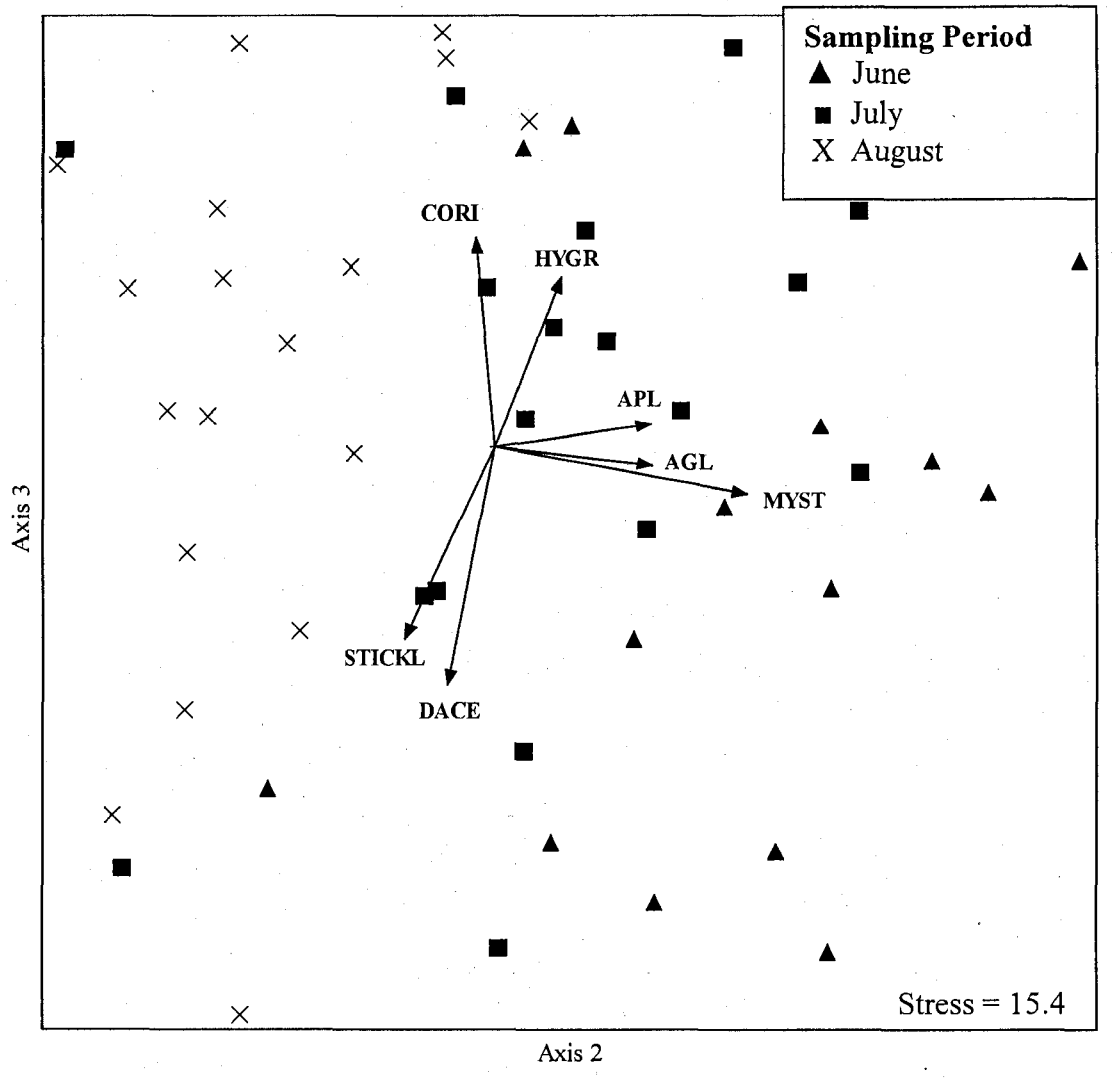


Figure 3-5. Temporal patterns in the pond communities in WBNP. NMS joint plot of pond visits based on macroinvertebrate assemblages identified to the LFTG and data as log(abund+1) for all no-, low- and high-use ponds (n=50), sampled with dip-nets and 2hr minnow traps. Ponds are grouped according to sampling period. Vectors (arrows and corresponding taxa) point in the direction of increasing values, and a longer line indicates a stronger relationship between the vector and the joint plot axes. Only vectors with an R^2 of 0.30 or greater are plotted. Abbreviations for fish and invertebrate taxa are presented in Appendix B.

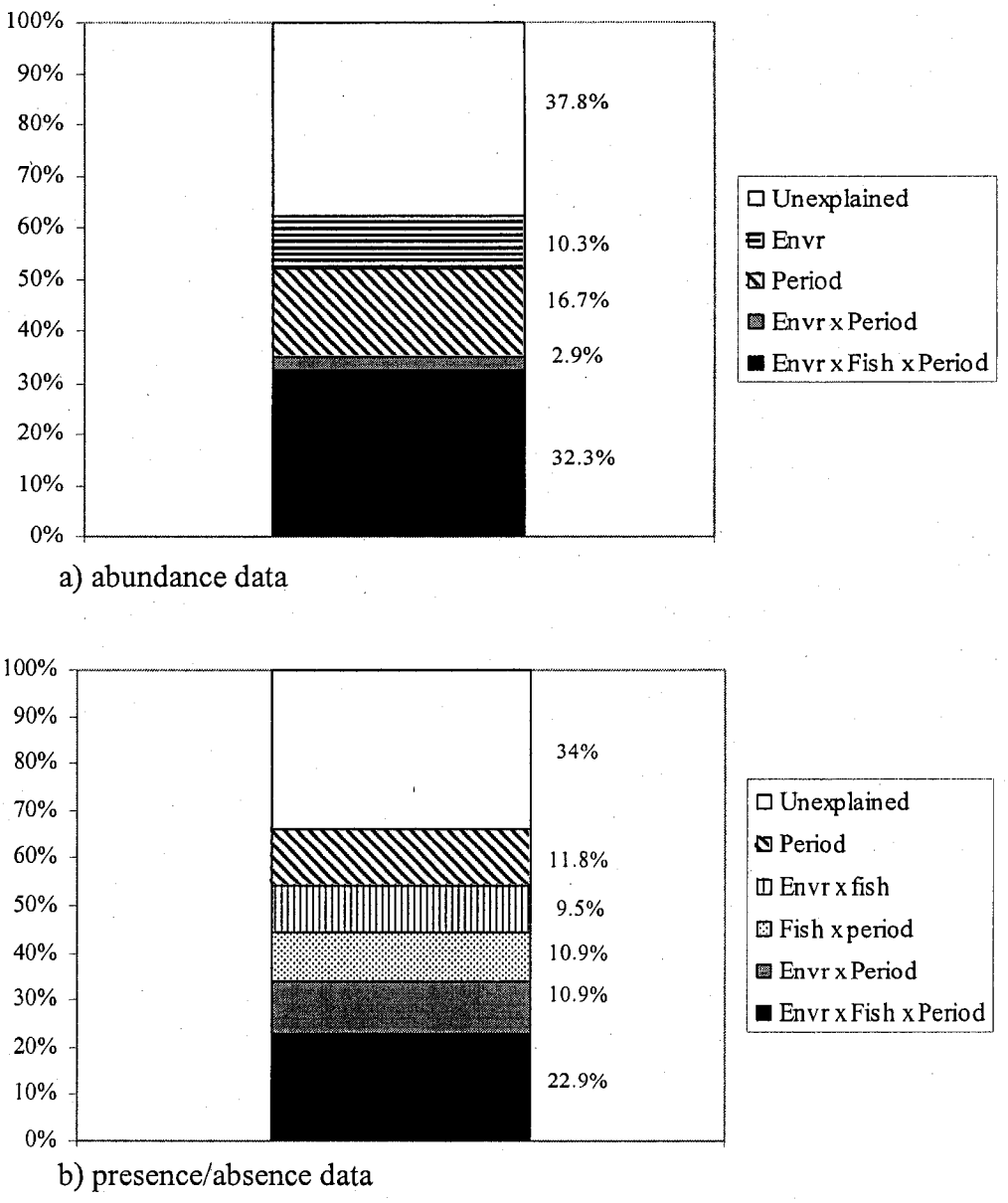


Figure 3-6. Results of three-way variance partitioning analyses, indicating the percentage of variation in invertebrate taxa collected in 30 ponds that is explained by environment variables (water depth and pH), fish (Dace sp. and *Culaea inconstans*), three sampling periods, and the two- and three-way interactions of these sets of variables.

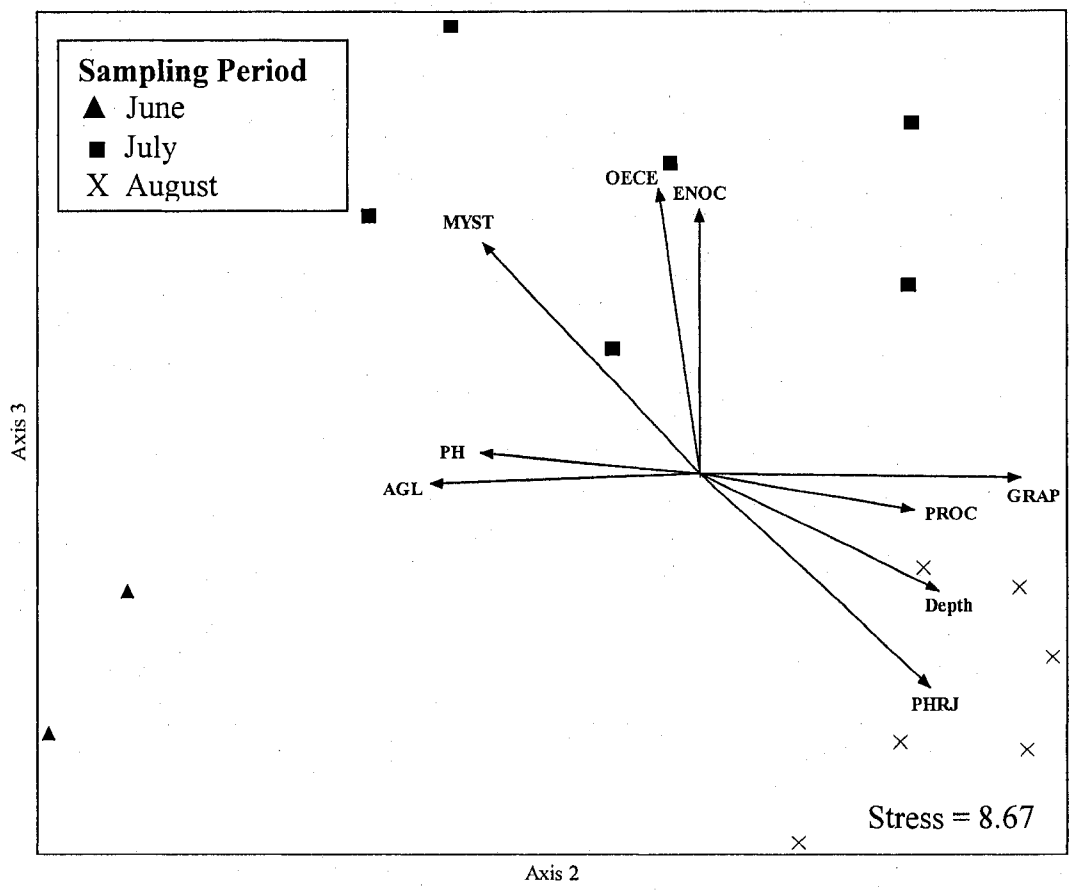


Figure 3-7. Temporal patterns of the Preble Creek (no-use) ponds (n=14). NMS joint plot of pond visits based on macroinvertebrate assemblages identified to the LFTG and data as log(abund+1), sampled with dip-nets and 2hr minnow traps. Ponds are grouped according to sampling period. Vectors (arrows and corresponding taxa) point in the direction of increasing values, and a longer line indicates a stronger relationship between the vector and the joint plot axes. Only vectors with an R^2 of 0.40 or greater are plotted (0.30 cut-off was used for other ordinations, but provided too many taxa in this joint plot). Abbreviations for invertebrate taxa are presented in Appendix B.

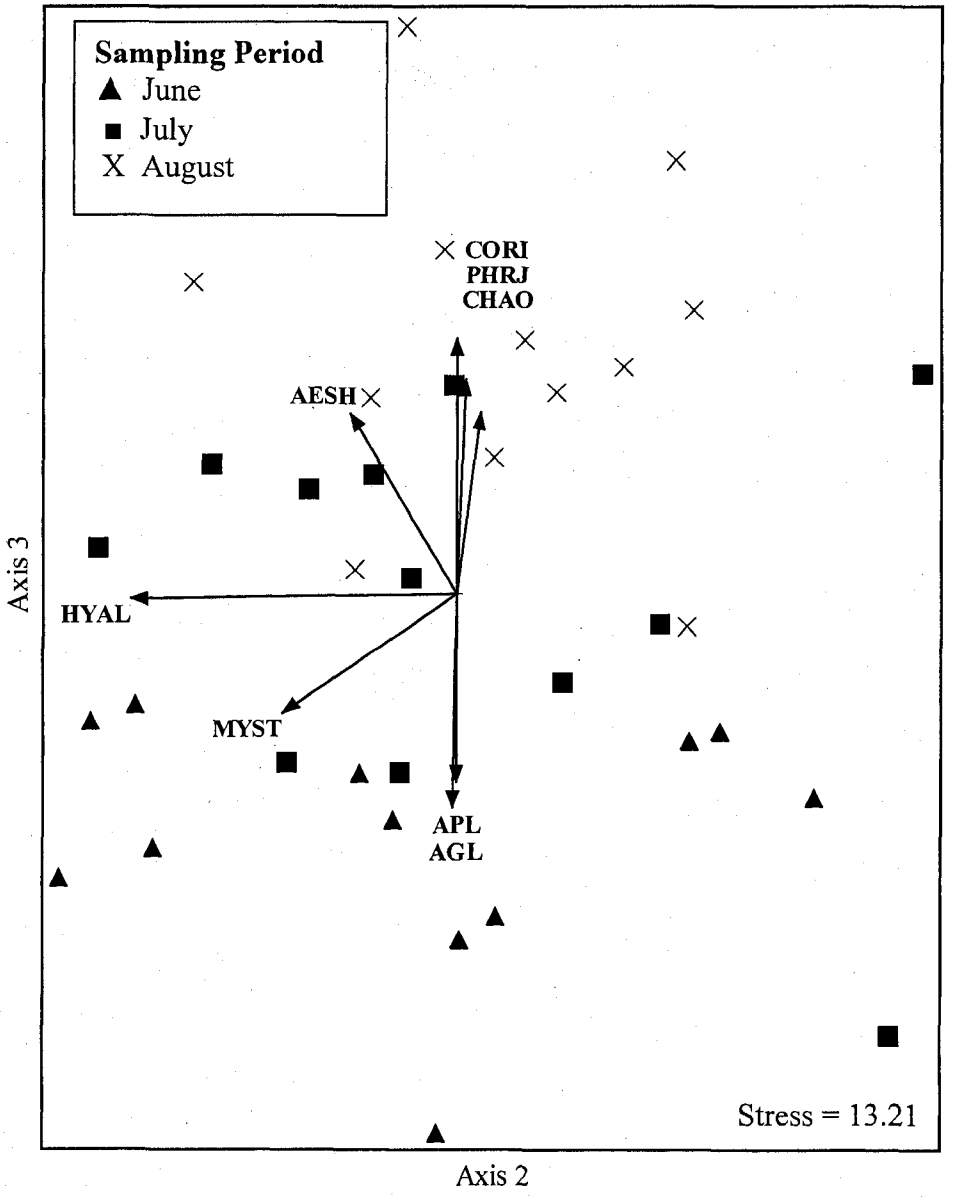


Figure 3-8. Temporal patterns of low-use and high-use nesting area ponds in WBNP (n=36). NMS joint plot of pond visits based on macroinvertebrate assemblages identified to the LFTG and data as log(abund+1), sampled with dip-nets and 2hr minnow traps. Ponds are grouped according to sampling period. Vectors (arrows and corresponding taxa) point in the direction of increasing values, and a longer line indicates a stronger relationship between the vector and the joint plot axes. Only vectors with an R^2 of 0.30 or greater are plotted. Abbreviations for fish and invertebrate taxa are presented in Appendix B.

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Chapter IV. GENERAL DISCUSSION AND CONCLUSIONS

The Whooping Crane is an endangered species that breeds in a rare and isolated wetland complex in Wood Buffalo National Park, Canada (WBNP). The birds reached a population low of <20 individuals in the 1940s (Canadian Wildlife Service & U.S. Fish and Wildlife Service (CWS & USFWS) 2007), and conservation efforts have since increased the crane's population to 237 in 2006 (B. Johns, Canadian Wildlife Service, *pers. comm.*). The International Recovery Plan for the Whooping Crane (CWS & USFWS 2007) outlines the need to identify and monitor potential food sources in the summer breeding grounds. Similar research is to be conducted in the wintering grounds, and information from both areas will be used to determine whether the Aransas and Wood Buffalo habitats would be able to support 1000 Whooping Cranes, a criterion that, if met, would allow down-listing of the crane from its current endangered status. Preliminary research that was conducted in the 1990s has paved the way for the development of a prey monitoring program to be implemented over the long-term in the Whooping Crane breeding grounds in WBNP.

Given the unique nature of the ponds, and their relative inaccessibility, effort must be invested into determining which sampling gear can collect the taxa of interest (i.e., large macroinvertebrates and fish) most efficiently, to minimize time and monetary investment (Brinkman and Duffy 1996; Merritt et al. 1996). Dip-nets were found to collect more taxa than both activity and minnow traps, and missed relatively few taxa collected by the other gear types. While minnow traps are least appropriate for collecting invertebrate taxa (see also Turner and Trexler 1997), they are necessary to collect fish taxa. Minnow traps and dip-nets collected the least similar taxa, suggesting that the two gear are complementary and that using both could collect representative samples.

I suggest a combination of timed dip-net samples and 1-2hr baited minnow trap sets as the most appropriate combination of gears for sampling in the Whooping Crane breeding ponds. To assess potential-prey abundance, 15 units of both minnow traps and dip-nets would be sufficient to yield abundance estimates at a precision of 0.5 for a number of potential prey taxa. For summed taxa (i.e., all potential prey), this is enough to yield estimates at a precision of 0.4 and 0.5 for dip-nets and minnow traps, respectively. This 'one-stop visit' would require significantly less monetary investment than setting overnight minnow traps, which requires a second visit to retrieve traps, allowing efforts to be directed towards sampling a larger number of ponds in the breeding grounds.

Focusing efforts towards sampling more ponds will allow a detailed assessment of both the spatial and temporal variability of Whooping Crane prey. Whooping Cranes nest in six distinct areas within WBNP and, based on the concentration of nesting pairs, these areas can be identified as low-use or high-use (B. Johns, CWS, *pers. comm.*). Additionally, there is a suite of historically unused ponds (no-use ponds) located on the outskirts of the breeding grounds that are easily accessible and suitable for frequent sampling. I found that no- and low-use ponds did not differ in terms of fauna composition, but both groups differed from high-use ponds. High-use ponds were characterized by a combination of fish and Trichoptera taxa, whereas a more diverse invertebrate community, including Corixidae and Dytiscidae genera,

characterized the no-/low-use ponds. Though statistical analyses without fish data yielded results similar to those with fish data, presence/absence of fish in a pond may have a biological influence on the invertebrate community (Zimmer et al. 2000). Previous work in WBNP has also identified the importance of fish and beetles when distinguishing between ponds where cranes were and were not observed feeding (Sotiropoulos 2002). I conclude that fish could potentially be used as indicators of locations suitable for introduction of captive-bred Whooping Cranes.

Temporally, faunal communities differed throughout the summer: early summer was characterized by a variety of Dytiscidae genera, shifting to *Caenis* spp. and Corixidae towards the end of the summer. Analysis of no-use ponds revealed temporal changes in indicator taxa similar to that of low-/high-use ponds grouped together; therefore, no-use ponds could potentially be used as indicators of community changes in the nesting area ponds, especially low-use ponds.

While it is possible to use no-use ponds as less-expensive substitutes of low-use ponds, I recommend that Parks Canada continue monitoring all three pond-use groups (no-, low-, and high-use) if funding permits. Ideally, this will occur for a few years to obtain baseline data that will be used to determine if year-to-year changes in no-use faunal assemblages are also similar to those of the low-use group. To help keep annual costs relatively low and allow sampling of all three pond-use groups, I suggest that sampling occur only twice throughout the breeding season. Because the June and August sampling periods differed most, sampling should occur during those periods. This will allow assessment of prey when Whooping Crane chicks are able to feed on their own, and once again near the end of the summer before the birds leave the breeding grounds.

The International Recovery Plan for the Whooping Crane (CWS & USFWS 2007) outlines a long list of recovery actions necessary for down-listing of the crane, including continued monitoring of the birds in their summer and winter habitats, managing potential threats, establishing populations from captive-bred birds, and monitoring potential food sources. While only one part of an extensive list of recovery actions, long-term implementation of a monitoring program should reveal patterns in faunal communities that will 1) help determine whether potential prey abundance is linked to yearly Whooping Crane chick survival, and 2) identify unused areas in the breeding grounds suitable for introduction of captive-bred Whooping Cranes. Information from this study can be added to that of Timoney (1997), Bergeson et al. (2001) and Sotiropoulos (2002), not only to broaden the knowledge base of this unique wetland but also to help with the eventual recovery of the Whooping Crane.

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Appendix A.

Table A-1. List of taxa collected in the Preble Creek (PC) ponds ('gear effectiveness' study) by activity traps, minnow traps and dip-nets. All pond-visits from each of three sampling periods are included (n=16). Fish are identified to species, except for Dace sp. (*Margariscus margarita* and *Phoxinus* sp.). Invertebrate taxa are identified to the lowest feasible taxonomic group (LFTG) (genus for most taxa in the Class Insecta, family for most taxa not in the Class Insecta). r=rare taxa, only present in 2 of 48 pond-visits.

Lowest Feasible Taxonomic Group		
Activity Traps	**Minnow Traps**	**Dip-nets**
Culaea inconstans (r)	*Culaea inconstans* (r)	---
---	*Pimephales promelas* (r)	---
---	Dace sp. (r)	---
Glossiphoniidae	Glossiphoniidae	Glossiphoniidae
Planorbidae	Planorbidae	Planorbidae
Lymnaeidae	Lymnaeidae	Lymnaeidae
Physidae	---	Physidae
Pisidium	---	*Pisidium*
Araneae	Araneae	Araneae
Anostraca	Anostraca	Anostraca
Ostracoda	---	Ostracoda
Caenis sp.	*Caenis* sp.	*Caenis* sp.
Procloeon sp.	*Procloeon* sp.	*Procloeon* sp.
---	---	*Siphlonurus* sp. (r)
Aeshna sp.	*Aeshna* sp.	*Aeshna* sp.
---	---	*Somatochlora* sp. (r)
Libellula sp.	*Libellula* sp.	---
Leucorrhinia sp.	*Leucorrhinia* sp.	*Leucorrhinia* sp.
Sympetrum sp.	*Sympetrum* sp.	*Sympetrum* sp.
Isch/Coen/Enal sp.	*Isch/Coen/Enal* sp.	*Isch/Coen/Enal* sp.
Lestes sp.	*Lestes* sp.	*Lestes* sp.
Notonecta sp.	*Notonecta* sp.	*Notonecta* sp.
Corixidae	Corixidae	Corixidae
---	*Gerris* sp.	*Gerris* sp.
---	---	*Limnoporus* sp. (r)
---	---	*Polycentropus* sp. (r)
---	*Agrypnia* sp.	*Agrypnia* sp.
Mystacides sp.	*Mystacides* sp.	*Mystacides* sp.
Oecetis sp.	*Oecetis* sp.	*Oecetis* sp.
---	*Limnephilus* sp.	*Limnephilus* sp.
Asyn/Gram/Limn sp.	*Asyn/Gram/Limn* sp.	*Asyn/Gram/Limn* sp.
Asyn/Phil/Limn sp.	*Asyn/Phil/Limn* sp.	*Asyn/Phil/Limn* sp.
Phryganeidae juvenile	Phryganeidae juvenile	Phryganeidae juvenile

Lowest Feasible Taxonomic Group cont.		
Activity Traps	**Minnow Traps**	**Dip-nets**
Acilius sp.	*Acilius* sp.	---
Agabus sp.	---	*Agabus* sp.
Colymbetes sp.	*Colymbetes* sp.	---
Dytiscus sp.	*Dytiscus* sp.	*Dytiscus* sp.
Graphoderus sp.	*Graphoderus* sp.	*Graphoderus* sp.
Hygrotus sp.	*Hygrotus* sp.	*Hygrotus* sp.
---	*Hydroporus* sp. (r)	*Hydroporus* sp. (r)
Hygrotus/Hydroporus sp.		*Hygrotus/Hydroporus* sp.
larva (r)	---	larva (r)
Ilybius sp.	---	*Ilybius* sp.
Laccophilus sp.	*Laccophilus* sp.	*Laccophilus* sp.
Rhantus sp.	*Rhantus* sp.	*Rhantus* sp.
Carrhydrus sp. (r)	---	---
---	---	*Gyrinus sp.*
---	*Enochrus* sp.	*Enochrus* sp.
---	---	*Laccobius* sp. (r)
Chaoborus sp.	*Chaoborus* sp.	*Chaoborus* sp.
---	---	Chironomidae
---	---	Culicidae
Aedes sp.	---	*Aedes* sp. (r)
---	---	*Dixella* sp. (r)
---	---	*Eristalis* sp. (r)
---	---	Stratiomyidae (r)
Diptera adult/pupa	Diptera adult/pupa	Diptera adult/pupa

Table A-2. List of taxa collected in the Preble Creek (PC) ponds ('gear effectiveness' study) by activity traps, minnow traps and dip-nets. All pond-visits from each of three sampling periods are included (n=16). Taxa are identified to the family level, except for the orders Araneae, Anostraca and Ostracoda.

Family		
Activity Traps	**Minnow Traps**	**Dip-nets**
Gasterosteidae	Gasterosteidae	---
---	Cyprinidae	---
Glossiphoniidae	Glossiphoniidae	Glossiphoniidae
Planorbidae	Planorbidae	Planorbidae
Lymnaeidae	Lymnaeidae	Lymnaeidae
Physidae	---	Physidae
Sphaeriidae	---	Sphaeriidae
Araneae	Araneae	Araneae
Anostraca	Anostraca	Anostraca
Ostracoda	---	Ostracoda
Caenidae	Caenidae	Caenidae
Baetidae	Baetidae	Baetidae
---	---	Siphlonuridae
Aeshnidae	Aeshnidae	Aeshnidae
---	---	Corduliidae
Lilbellulidae	Libellulidae	Libellulidae
Coenagrionidae	Coenagrionidae	Coenagrionidae
Lestidae	Lestidae	Lestidae
Notonectidae	Notonectidae	Notonectidae
Corixidae	Corixidae	Corixidae
---	Gerridae	Gerridae
---	---	Polycentropodidae
Phryganeidae	Phryganeidae	Phryganeidae
Leptoceridae	Leptoceridae	Leptoceridae
Limnephilidae	Limnephilidae	Limnephilidae
Dytiscidae	Dytiscidae	Dytiscidae
---	---	Gyrinidae
---	Hydrophilidae	Hydrophilidae
Chaoboridae	Chaoboridae	Chaoboridae
---	---	Chironomidae
Culicidae	---	Culicidae
---	---	Dixidae
---	---	Syrphidae
---	---	Stratiomyidae
Diptera adult/pupa	Diptera adult/pupa	Diptera adult/pupa

Table A-3. List of taxa collected in the Preble Creek (PC) ponds ('gear effectiveness' study) by activity traps, minnow traps and dip-nets. All pond-visits from each of three sampling periods are included (n=16). Taxa are identified to the order level.

	Order	
Activity Traps	**Minnow Traps**	**Dip-nets**
Gasterosteiformes	Gasterosteiformes	---
---	Cypriniformes	---
Glossiphoniidae	Glossiphoniidae	Glossiphoniidae
Gastropoda	Gastropoda	Gastropoda
Pelecypoda	---	Pelecypoda
Araneae	Araneae	Araneae
Anostraca	Anostraca	Anostraca
Ostracoda	---	Ostracoda
Ephemeroptera	Ephemeroptera	Ephemeroptera
Odonata	Odonata	Odonata
Hemiptera	Hemiptera	Hemiptera
Trichoptera	Trichoptera	Trichoptera
Coleoptera	Coleoptera	Coleoptera
Diptera	Diptera	Diptera

Appendix B. List of invertebrates found in 39 ponds in the Whooping Crane breeding grounds during the 2004 and 2005 field seasons. Codes are those used in the statistical analyses. Note that many taxonomic groups have the same code because their genus, family and/or order names are similar. For example, AESH was the code used for *Aeshna* sp. in lowest feasible taxonomic group analyses as well as for Aeshnidae in family analyses. *rare taxa, present in <5% of ponds.

Phylum	Class	Order	Family	Genus	Code
	Subclass: Hirudinea				
		Rhynchobdellidae			RHYN
			Glossiphoniidae		GLOS
		Gnathobdellidae			GNAT
			Hirudinidae*		HIRU
Mollusca					
	Gastropoda				GAST
	Subclass: Pulmonata		Physidae		PHYS
			Lymnaeidae		LYMN
			Planorbidae		PLAN
	Pelecypoda				
			Sphaeriidae		SPHA
				Pisidium sp.	PISI
Arthropoda					
	Arachnida				
		Araneae			ARAN
	Crustacea				
	Subclass: Branchiopoda				
		Anostraca			ANOS
	Subclass: Ostracoda				OSTR
	Subclass: Malacostraca				
		Amphipoda			AMPH
				Hyalella azteca	HYAL

Appendix B. continued

Phylum	Class	Order	Family	Genus	Code
	Insecta				
		Ephemeroptera			EPHE
			Baetidae		BAET
				Procloeon sp.	PROC
			Caenidae		CAEN
				Caenis sp.	CAEN
			Siphlonuridae		SIPH
				Siphlonurus sp.	SIPH
		Odonata			ODON
		Subclass: Anisoptera			
			Aeshnidae		AESH
				Aeshna sp.	AESH
			Corduliidae		CORD
				Somatochlora sp.*	SOMA
			Libellulidae		LIBE
				Libellula sp.	LIBE
				Leuchorrhinia sp.	LEUC
				Sympetrum sp.	SYMP
		Subclass: Zygoptera			
			Coenagrionidae		COEN
				Ischnura/ Coenagrion/ Enallegma sp.	ICE
			Lestidae		LEST
				Lestes sp.	LEST
		Hemiptera			HEMI
			Belostomatidae		BELO

Appendix B. continued

Phylum	Class	Order	Family	Genus	Code
		Hemiptera cont.			
				Lethocerus sp.*	LETH
			Notonectidae		NOTO
				Buenoa sp.	BUEN
				Notonecta sp.	NOTO
			Gerridae		GERR
				Limnoporus sp.*	LIMP
				Gerris sp.	GERR
			Corixidae		CORI
		Trichoptera			TRIC
			Polycentropodidae		POLY
				Polycentropus sp.*	POLY
			Leptoceridae		LEPT
				Oecetis sp.	OECE
				Triaenodes sp.*	TRIA
				Mystacides sp.	MYST
			Phryganeidae		PHRY
				Agrypnia sp.	AGRY
			young Phryganeidae		PHRYJ
			Limnephilidae		LIMN
				Glyphosyche sp.*	GLYP
				Asynarchus/ Philarctus/ Limnephilus sp.	APL
				Limnephilus sp.	LIMN

Appendix B. continued

Phylum	Class	Order	Family	Genus	Code
		Trichoptera cont.			
				Asynarchus/ Grammotalius/ Limnephilus sp.	AGL
		Coleoptera			COLE
			Gyrinidae		GYRI
				Gyrinus sp.	GYRI
			Dytiscidae		DYTI
				Laccophilus sp.	LACP
				Hygrotus sp.	HYGR
				Hygrotus/Hydroporus sp.*	HYHY
				Stictotarsus sp.*	STIC
				Nebrioporus sp.*	NEBR
				Carrhydrus sp.*	CARR
				Agabus sp.	AGAB
				Ilybius sp.	ILYB
				Colymbetes sp.	COLY
				Rhantus sp.	RHAN
				Dytiscus sp.	DYTI
				Graphoderus sp.	GRAP
				Acilius sp.	ACIL
			Hydrophilidae		HYDR
				Enochrus sp.	ENOC
				Laccobius sp.*	LACC
			Haliplidae		HALI
				Haliplus sp.*	HALI

Appendix B. continued

Phylum	Class	Order	Family	Genus	Code
		Diptera			DIPT
		Suborder: Nematocera			
			Chironomidae		CHIR
			Culicidae		CULI
				Aedes sp.*	AEDE
			Chaoboridae		CHAO
				Chaoborus sp.	CHAO
			Dixidae		DIXE
				Dixella sp.*	DIXE
		Suborder: Brachycera			
			Syrphidae		SYRP
				Eristalis sp.*	ERIS
			Dolichopodidae*		DOLI
			Stratiomyidae*		STRA
Chordata	Actinopterygii				
		Gasterosteiformes			GAST
			Gasterosteidae		GAST
				Culaea inconstans	STICKL
		Cypriniformes			CYPR
			Cyprinidae		CYPR
				Pimephales promelas	FATH
				Margariscus margarita and *Phoxinus* sp.	DACE