U.S. FISH AND WILDLIFE SERVICE
DIVISION OF ENVIRONMENTAL QUALITY
REGION 6

EVALUATION OF WETLANDS CREATED WITH EFFLUENT FROM A SWINE CONCENTRATED ANIMAL FEEDING OPERATION USING MALLARD SENTINELS: IMPLICATIONS FOR MCMURTREY NATIONAL WILDLIFE REFUGE.

U.S. Fish and Wildlife Service
Nebraska Field Office
203 West Second Street
Grand Island, Nebraska 68801
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ENVIRONMENTAL CONTAMINANTS ASSOCIATED WITH A SWINE CONCENTRATED ANIMAL FEEDING OPERATION AND IMPLICATIONS FOR MCMURTREY NATIONAL WILDLIFE REFUGE.

Prepared by
Matthew S. Schwarz
Christina D. Lydick

U.S. Fish and Wildlife Service
Division of Environmental Quality
Nebraska Field Office
Grand Island, Nebraska 68801

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ABSTRACT

Previous work by the U.S. Fish and Wildlife Service identified nutrients, elemental contaminants, algal toxins, bacterial pathogens, and hormones as contaminants of concern (COCs) associated with wetlands created from the secondary effluent of a large swine concentrated animal feeding operation. In this follow-up study, COC exposure and effects to waterfowl were evaluated using game farm mallards. Mallards were kept in enclosures built on two created wetlands (treatment sites) and two reference wetlands that are federally managed for waterfowl habitat. Water quality in the created wetland enclosures had higher specific conductivity, BOD, turbidity, pH, and nutrients than reference wetlands. Algal blooms frequently occurred in the created wetlands and included *Microcystis* spp. All sentinel mallards tested negative for duck plague and avian influenza; however, an avian botulism outbreak on the created wetlands occurred in August of 2003 after the study was completed. Cumulative stress from poor water quality and reproduction may have caused hen mortality in the created wetland enclosures, resulting in a greater survival to hatch in the reference wetlands compared to created wetlands. However, wild brood production was observed in the created wetlands. There were no significant differences in sentinel mallard blood plasma chemistry and histology biomarkers between reference and created wetland enclosures. Known toxicity thresholds were only exceeded for selenium concentrations in liver, and included sentinel mallards on reference and created wetland enclosures. It is recommended that a constructed wetland system is developed to further treat Hastings Pork secondary swine wastewater before it is delivered to habitat wetlands for migratory waterfowl.

Keywords: Concentrated Animal Feeding Operation, CAFO, swine, Nebraska, Rainwater Basin, constructed wetlands, game farm mallard, waterfowl, elemental contaminants, metals, pathogens, nutrients, eutrophication.

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ACRONYMS AND ABBREVIATIONS

<	less than	McMurtrey	McMurtrey National Wildlife Refuge
µS/cm	microsiemens per centimeter	MC	Microcystin
µg/g	micrograms per gram	MC-LR	Microcystin-LR
µg/L	micrograms per liter	Mg	magnesium
Al	aluminum	mg/dL	milligrams per deciliter
As	arsenic	mg/kg	milligrams per kilogram
ACF	Analytical Control Facility	mg/L	milligrams per liter
ALP	alkaline phosphatase	ml	milliliter
ALT	alanine aminotransferase	Mn	manganese
AST	aspartate amino-transferase	Mo	molybdenum
B	boron	n	sample size
Ba	barium	NDEQ	Nebraska Department of Environmental Quality
Be	beryllium	NEFO	Nebraska Ecological Services Field Office
BOD	Biological oxygen demand	ng/g	nanograms per gram
^{o}C	degrees Celsius	ng/L	nanograms per liter
CAFO	concentrated animal feeding operation	NGPC	Nebraska Game and Parks Commission
Cd	cadmium	Ni	nickel
CDC	Centers for Disease Control	NRC	National Research Council
CERC	Columbia Environmental Research Center	NTUs	nephelometric turbidity units
cfu	colony forming units	NWHC	National Wildlife Health Center
CK	creatine kinase	p	level of significance
cm	centimeter	*P.*	*Pasteurella*
CO_2	Carbon dioxide	PACF	Patuxent Analytical Control Facility
COCs	Contaminants of concern	Pb	lead
Cr	chromium	pers. comm.	personal commentary
Cu	copper	RWB	Rainwater Basin
CW4	Created Wetland #4	RWBJV	Rainwater Basin Joint Venture
CW6	Created Wetland #6	*S*	*Streptococcus*
CW7	Created Wetland #7	SAS	Statistical Analysis System
dw	dry weight	SE	standard error
E.	*Escherichia*	Se	selenium
e.g.,	example given	Service	U.S. Fish and Wildlife Service
ELISA	enzyme-linked immunosorbent assay	SOPs	standard operating procedures
et al.	and others	Sr	strontium
ft	feet	ssp	species (plural)
Fe	iron	TKN	total kjeldahl nitrogen
g	gram	U/L	Units per liter
GGT	gamma glutamyl transpeptidase	USDA	U.S. Department of Agriculture
Harvard	Harvard Waterfowl Production Area	USDOI	U.S. Department of the Interior
Hg	mercury	USEPA	U.S. Environmental Protection Agency
HPLC	high-pressure liquid chromatography	USGS	U.S. Geological Survey
i.e.	in explanation	V	vanadium
ID	identification	WHO	World Health Organization
Inc.	incorporated	WPA	Waterfowl Production Areas
ISIS	International Species Inventory System	ww	wet weight
kg	kilograms	Zn	zinc
LDH	lactate dehydrogenase		

INTRODUCTION

Contaminants associated with livestock waste generated by concentrated animal feeding operations (CAFOs) include nutrients, pathogens, trace elements, antibiotics, and hormones. These pollutants can enter rivers, streams, and wetlands by spills, lagoon ruptures, field run-off, and contaminated groundwater. In 2000, the U.S. Fish and Wildlife Service (Service) began a contaminants investigation aimed at characterizing CAFO contaminants in lagoons, canals, and created wetlands operated by Hastings Pork, a large swine CAFO in Clay County, Nebraska (Schwarz et al., 2004). The focus of this study was on created wetlands that received lagoon-treated wastewater as their primary water source. These created wetlands were specifically designed to attract waterfowl. Study results indicated that created wetland water had increased pH and specific conductivity as well as high nutrients (phosphorus, ammonia, and organic nitrogen) compared to reference wetlands managed for waterfowl. The study concluded that waterfowl using the created wetlands were potentially at risk to disease pathogens, natural hormones, and cyanobacterial toxins. In 2002, the present study was initiated to further evaluate these concerns and determine whether or not the habitat created from swine waste for waterfowl is actually detrimental to waterfowl.

Study Description and Objectives

The purpose of this research was to use sentinel mallards to further evaluate effects of waterfowl exposure to CAFO contaminants previously characterized at Hastings Pork (Schwarz et al., 2004). Enclosures were built on two created wetlands (treatment sites) and two control sites, -McMurtrey National Wildlife Refuge (McMurtrey) and Harvard Waterfowl Production Area (Harvard). Game farm mallards were kept in the enclosures during the breeding season, allowing for comparisons in hatching success and brood production. Adult mallard health was evaluated by performing a necropsy-based health assessment that included external and internal observations, blood plasma chemistry, histology (liver, kidney, spleen, and gonads), and comparisons of body mass.

Pathogens in mallards were screened by analyzing cloaca swabs for viruses and gastrointestinal contents for bacteria. Analytical analyses of contaminants in mallard tissues included microcystin toxins in liver and elemental contaminants in stomach contents, eggs, and liver. In addition to the mallard health and exposure assessment, water quality was compared between the treatment and control sites. The water quality assessment evaluated bacterial pathogens, nutrients, algal toxins (microcystins), and other water quality parameters (pH, specific conductance, and turbidity). Our hypothesis was that sentinel mallard health assessment endpoints (e.g., blood plasma chemistry, tissue histology, survival and reproduction) would be related to water quality degradation, presence of pathogens in waterfowl, and/or the degree of contamination in mallard stomach contents and tissues.

Site Description and History

The study site was located in Clay County, Nebraska, and included enclosures at Hastings Pork, McMurtrey, and Harvard (Figure 1). Clay County lies within Nebraska's Rainwater Basin (RWB), a 4,200 square mile area that contained nearly 100,000 acres of wetlands before European settlement; however, by 1982, ninety percent of those wetlands were destroyed or altered by draining and filling (McMurtrey et al. 1972; Schildman and Hurt, 1984; Gabig, 2000). To reverse the trend of wetland loss in the area, the Rainwater Basin Joint Venture (RWBJV) was established in 1991 to provide a partnership structure for private landowners, organizations, and government agencies to restore and maintain RWB wetland habitat (Gersib et al., 1992).

Hastings Pork is located on what was formerly a Naval Ammunition Depot. Since the 1960s, Hastings Pork has utilized the area for livestock and crop production. About 260 bunkers that were formerly used by the Navy to store munitions are now used for swine production. These bunkers house approximately 64,000 swine that generate an estimated 325,000 liters of swine urine and manure slurry each day (based on calculations for swine between 36-55 kilograms (kg) of body weight; Fraser, 1991). Approximately 1.5 million liters of water per day is used to rinse the bunkers out, generating a total of

1.8 million liters of wastewater per day. In an effort to utilize this wastewater for the benefit of waterfowl, a partnership between Hastings Pork and the Rainwater Basin Joint Venture (RWBJV) resulted in the creation of seven wetlands (known as the Hayden Thompson wetlands and referred to herein as the created wetlands) totaling 17 acres on Hastings Pork property. These wetlands receive swine wastewater effluent from lagoons by a canal system, with a distance of delivery ranging from less than a mile to five miles. The created wetlands were designed to provide waterfowl habitat and were not intended to treat swine-waste effluent; therefore, the Service and Hastings Pork formed a partnership to evaluate whether migratory birds attracted to the created wetlands are exposed to contaminants and disease pathogens. The Service also was concerned that waterfowl may transmit disease pathogens from Hastings Pork to nearby habitats. McMurtrey is located approximately 1 mile east of the created wetlands and Harvard is within three miles of the created wetlands (Figure 2). McMurtrey contains 650 acres of wetland and 400 acres of upland. Harvard contains 760 acres of wetland and 724 acres of upland habitat. Both basins are managed primarily for migratory waterfowl and receive extensive use by snow geese (*Chen caerulescens*) during the spring migration.

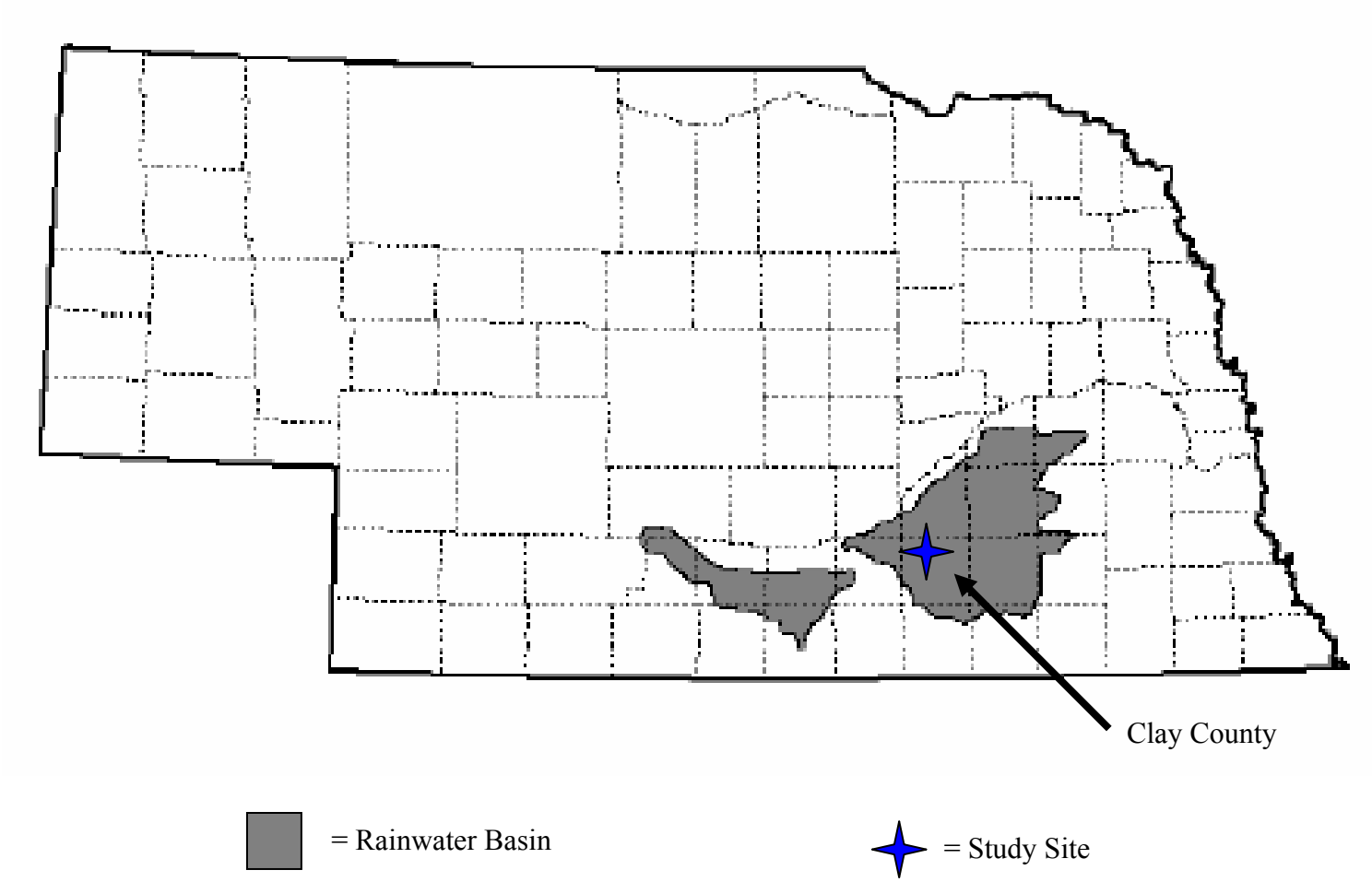

Figure 1. Location of the Nebraska Rainwater Basin and the study site in Clay County, Nebraska.

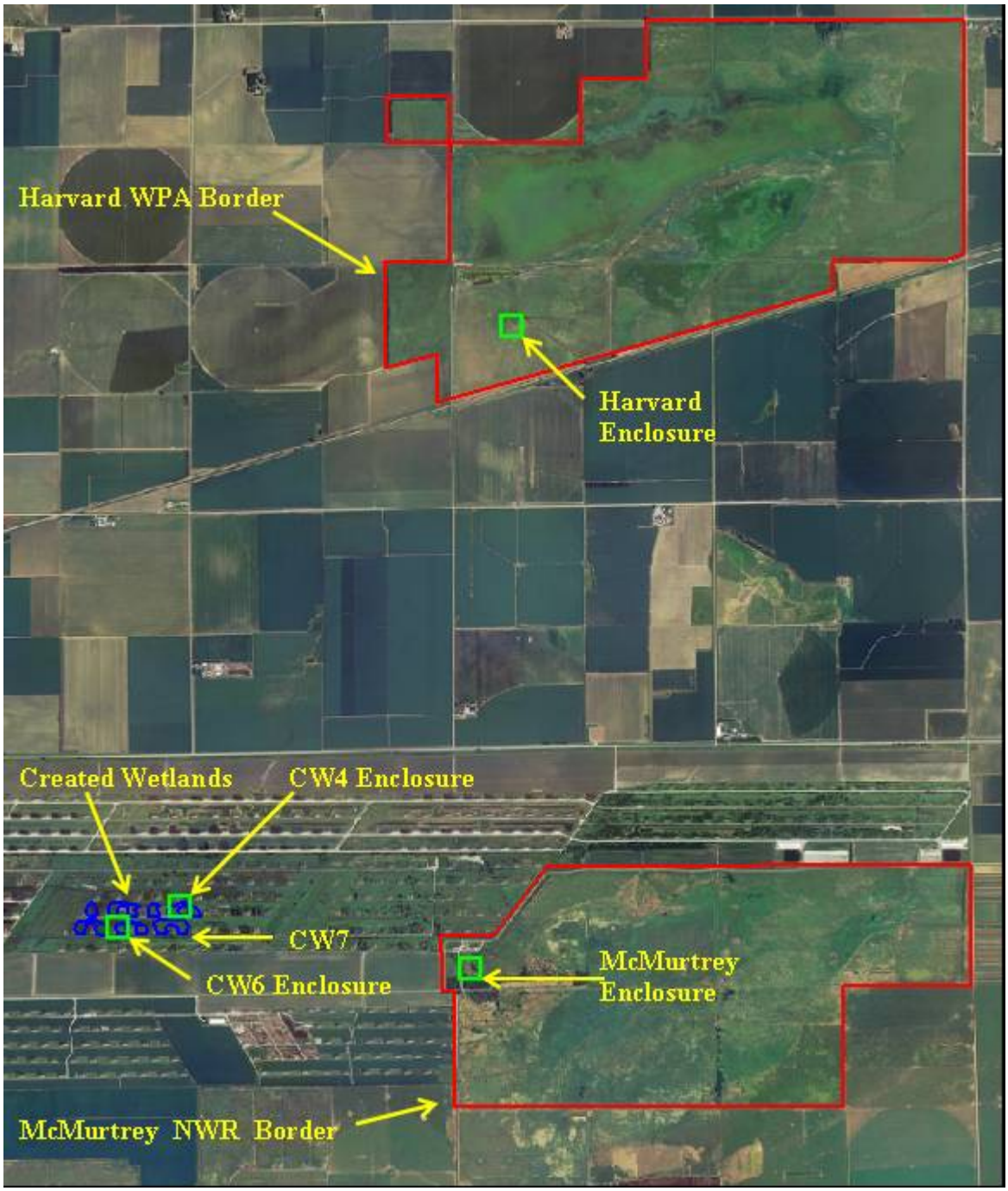

Figure 2. Map of the study site including the location of mallard enclosures at the created wetlands, Harvard Waterfowl Production Area and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002 and 2003.

METHODS

Mallard Enclosures

Enclosures were constructed at McMurtrey, Harvard, and Created Wetlands 4 (CW4) and 6 (CW6) during the early spring of 2002 (Figure 2). Each enclosure consisted of a proportionally similar wetland-to-upland habitat area contained within a 225 foot (ft) by 100 ft perimeter fence and nylon-net cover (Appendix Figures A.1 - A.4). The perimeter fence on dry land consisted of a four ft high apron fence set to T-posts spaced 15 to 25 ft apart. Apron wire fence (4 ft high with a 12-inch horizontal apron) was used on the perimeters with the apron on the outside of the enclosure to prevent animals from entering by burrowing under the fence. Wet areas were fenced in with plastic snow fence set to T-posts spaced 15 ft apart. Nylon netting was hog-ringed to 9-gauge wire that ran above the outside edge of the perimeter fence and was supported by 9-gauge wire fastened to eight interior columns. Columns consisted of an eight ft T-post shaft that supported a hollow 12 ft PVC slat (slats were previously used for flooring on the hog farm). The top of each column had a 1 ft^2 rubber mat fastened to a wood shaft with an eyebolt for the wire grid that was used to support the nylon netting above the enclosure. Electric wire was used along the dry land perimeter to discourage predators from entering the enclosure. Three artificial nest structures, designed to sit on poles for geese, were setup at ground level within each enclosure.

Mallard Sentinels

Adult game farm mallards of breeding plumage were obtained from Whistling Wings, Inc. (Hanover, Illinois) in March of 2002 and 2003. Each mallard was uniquely marked with a numbered patagial tag and leg ban. Mallards were temporally held in an indoor pen before being released to outdoor enclosures in May and April of 2002 and 2003, respectively. Prior to release, mallards were weighed to the nearest 0.05 kg using a Pesola® scale. Mallards were released a month earlier in 2003 than 2002 in an effort to

improve nesting. In 2003, female mallards in all enclosures initiated nests and were kept in enclosures for 79-80 days to allow for brood production.

Each year on the day before sentinel mallards were released to outdoor enclosures, three male and three female sentinel mallards were necropsied as zero-controls (procedures described below). Mallards were initially contained in the enclosures without supplemental feeding; however, in 2002, mallards at the Harvard enclosure became emaciated and had to be removed from the study. For the remainder of 2002, a corn ration of approximately 0.07 kg per bird per day was provided for mallards at McMurtrey and Harvard enclosures but not for those on the created wetland enclosures. A corn ration was provided at all four enclosures in 2003. Adult sentinel mallard survival was recorded in 2002 and 2003. Sentinel mallard reproduction was evaluated for 2003 by recording nest initiation attempts, number of eggs laid, and number of hatchlings.

Mallard Necropsy and Sample Collection

After a minimum 60 day exposure period in the enclosures, mallards were netted inside the enclosure and immediately taken to a lab at McMurtrey for necropsy. A health-based necropsy was performed according to standard operating procedures (SOPs) developed from a U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC) protocol for diagnosing wildlife mortalities. Mallards were weighed to the nearest 0.05 kg using a Pesola® scale. A virology analysis for avian influenza, Newcastle's disease, and duck plague was performed by sampling the cloaca with a sterilized swab that was then used to inoculate a broth prepared by NWHC. Three to six milliliter (ml) blood samples were obtained with a 3 ml syringe and a hepranized needle. Blood was either collected from the jugular vein using a 21 gauge needle or from the brachial vein using a 25 gauge needle. All blood samples were transferred from syringe to lithium heparinized Vacutainer® tubes (Beckton Dickinson, Franklin Lakes, NJ) and stored on ice.

After blood sample collection, mallards were euthanized by carbon dioxide asphyxiation. This was followed by an external examination for parasites, lesions or other anomalies on the body surface. An internal necropsy-based health assessment was used to evaluate the condition of the liver, gall bladder, kidneys, spleen, gonads, and mesenteric fat. Digital photographs of the ventral side of each mallard were taken before and after the opening incision. Liver, gonad, and spleen tissues were weighed to the nearest 0.01 gram (g). Spleen, liver, kidney, and gonad tissues were collected for histology analysis. Breast muscle, skin, brain, proventriculus/gizzard, lung, heart, tibia, and caecum tissue samples also were collected and archived for a potential future histology examination separate from this study. All tissues collected for histology were cut less than 1 cm^3 thick and stored in 10 percent buffered formalin at a ratio of 1 part tissue to 10 parts fixative. The right liver lobe, minus one third of the distal end for histology, was collected for microcystin analysis. The left liver lobe and gizzard contents were collected into certified clean glass containers for elemental contaminant analysis. An approximately 3 inch long section of the large intestine immediately above the anus was collected for the bacterial screen. The ends of the intestine sample were tied shut with monofiliment fishing line soaked in 100 percent ethanol. The intestine sample was placed in a Whirlpack® plastic bag. Inoculated broth and intestine samples were kept cool, but not frozen, and shipped by overnight delivery to NWHC on the day of collection.

Blood samples were centrifuged for 10 minutes at 3500 revolutions per minute to form a plasma fraction. The plasma fraction was aspirated into cryogenic vials, flash frozen in liquid nitrogen, and stored at -80 degrees Celsius (^oC) at the Service's Nebraska Ecological Services Field Office (NEFO). Tissues for histology were shipped to NWHC by overnight delivery at the end of each field season.

Histopathology

Tissue samples delivered to NWHC were sent to the School of Veterinary Medicine, University of Wisconsin, for histological processing. Sections stained with

hematoxylin and eosin were examined with an Olympus B-max microscope. Presence and severity of lesions were qualitatively described by the pathologist and then scored by NEFO as follows: 0 = no lesions, 2 = minimal, 3 = minimal to mild, 4 = mild, 5 = mild to moderate, 6 = moderate, 7 = moderate to severe, 9 = severe, 10 = extremely severe. For each year, the total score for liver, gonad, spleen, kidney and all 4 tissues combined were compared among sites by a Kruskal-Wallis rank sums test.

Blood Chemistry

Blood plasma samples were shipped on dry ice by overnight delivery to Marshfield Laboratories in Marshfield, WI. These samples were submitted for the "avian profile" analysis which includes glucose, aspartate amino-transferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), cholesterol, total protein, phosphorus, calcium, sodium, potassium, chloride, bicarbonate, uric acid and anion gap. These parameters were quantified using a Boehringer Mannheim Hitachi 911 automated chemistry analyzer according to SOPs developed by Marshfield Laboratories. A two-way analysis-of-variance followed by a Tukey's honest significance difference test was used to compare blood chemistry components among enclosure sites and zero-controls.

Water Quality

Water quality was sampled by NEFO personnel at each mallard enclosure from March to November of 2002 and 2003. Temperature, dissolved oxygen, specific conductivity (YSI® model 85), pH (Accument® AP61), and turbidity (LaMotte® 2008) were measured every two weeks. Water samples were collected monthly for nutrient analysis including total kjeldahl nitrogen (TKN), total ammonia, and total phosphorus. Biological oxygen demand (BOD) in water at the enclosure sites was measured in June and July of 2002 and March and April of both 2002 and 2003. Samples for nutrient and BOD analysis were kept on blue ice until transferred later that day to Servi-Tech Laboratories Inc, in Hastings, Nebraska, for analysis. During water quality sampling

events, enclosures were examined for algal blooms. When present, algal blooms were checked for *Microcystis* by collecting a water sample and examining it with an Olympus BX40FU compound light microscope at NEFO.

Water grab samples for microcystin (MC) toxin analysis were collected into 125 ml brown plastic containers and immediately placed on ice. Samples were frozen at -20 ^{o}C and then shipped on dry ice to the USGS Columbia Environmental Research Center (CERC). Concentrations of microcystins were determined by enzyme linked immunosorbent assay (ELISA), which measures total microcystins as the microcystin-LR variant. High-pressure liquid chromatography (HPLC) also was used to specifically measure four microcystin variants (MC-LR, MC-RR, MC-YR, and MC-LA). More detailed descriptions of the ELISA and HPLC methods used to analyze samples collected for this study is available in Echols (2001) and Echols and Feltz (2004, 2006).

Disease Pathogens

Cloacal swabs received by NWHC were stored frozen at -80 ^{o}C until tested for viruses that cause avian influenza, Newcastle's disease, and duck plague. Viral testing followed SOPs developed by NWHC and are described only briefly here. For avian influenza and Newcastle's disease, 0.2 ml of each sample was injected into four chicken eggs. Eggs were then incubated at 98 degrees Fahrenheit for four days. On day 5 of incubation, eggs were chilled and then read for hemagglutination (i.e., the physical binding of virus with red blood cells). Duck plague was tested by inoculating 0.5 ml of viral transport media into a cell culture flask (Muscovy Duck Embryo Fibroblasts). Inoculated cells were then incubated (37 ^{o}C and 2 percent CO_2) for 3 - 7 days before they were observed for cytopathic effects. If no effects were observed, the flask was frozen for blind passage (i.e., a re-inoculation) seven days after the original inoculation.

For analysis of bacterial pathogens, contents of the intestine samples received by NWHC were immediately transferred to the appropriate media and incubated according to NWHC approved SOPs. Samples were tested for bacterial pathogens likely to occur in swine waste, including *Salmonella* spp., *Pasteurella multocida*, *Yersinia* spp.,

Erysipelothrix spp., fecal coliforms, and fecal streptococci. The media and incubation conditions varied for each bacterial pathogen of interest (Appendix Table A.1). Fecal coliform and fecal streptococci counts were quantified by membrane filtration (Clesceri et al., 1998). The presence of *P. multocida*, *Salmonella*, *Yersinia* and *Erysipelothrix* was verified biochemically by either the API-20E or Vitek® systems (bioMerieux, St. Louis, Missouri).

Elemental Contaminants

Elemental contaminants were measured in mallard liver, stomach contents, and egg samples. Samples were collected into U.S. Environmental Protection Agency (USEPA) certified clean glass containers and sampling equipment was decontaminated between sites by following Service SOPs. All samples were collected by Service personnel and submitted to the Patuxent Analytical Control Facility (PACF), since renamed the Analytical Control Facility (ACF) (Appendix Table A.2). Detailed descriptions of lab methods including sample preparation, sample digestion, Quality Assurance/Quality Control results, and detection limits are provided in PACF catalogs which are available upon request (http://chemistry.fws.gov/). In brief, the analysis of duplicate samples, spiked samples, and standard reference materials indicated acceptable levels of precision and accuracy, and limits of detection were within ACF contract requirements (ACF, 2005). For elemental contaminants analyses, all samples were freeze dried, percent moisture was determined, and results were provided as wet weight (ww) and dry weight (dw) concentrations. Inductively coupled plasma atomic emission spectrometry was used to determine concentrations of aluminum (Al), boron (B), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), strontium (Sr), vanadium (V), and zinc (Zn). Mercury (Hg) concentrations were determined by cold vapor atomic absorption, and graphite furnace atomic absorption was used to measure arsenic (As), selenium (Se), and small concentrations of Pb and Cd.

Statistical Analyses

All statistical calculations were performed with JMP® Version 5 software (SAS Institute, 2002). Where means are provided, the "±" refers to a standard error (SE) unless otherwise noted. Data were typically nonparametric; therefore, a Kruskal-Wallis nonparametric one-way analysis of variance was used to test significance among three groups and a Wilcoxon rank sums test was used to test significance between groups. If more than 50 percent of the sample size (n) was above the detection limit for a particular contaminant, then half the detection limit was used in place of those below the detection limit for statistical analyses, unless otherwise noted. If 50 percent or more of the samples were below the detection limit, then results were not analyzed statistically. Use of the term "significant" in this report indicates statistical analysis using a p-value of 0.05.

RESULTS AND DISCUSSION

Enclosure Habitat and Water Quality

Water in the created wetland enclosures had higher specific conductivity, BOD, turbidity, pH, and nutrients than water in the reference enclosures. Specific conductance within site groups was similar for both years and averaged 1,754 ± 63 microsiemens per centimeter (μS/cm) and 511 ± 44 μS/cm in the created wetland (n = 55) and reference enclosures (n = 29), respectively. Specific conductance in the created wetlands appears to be increasing over time, as measurements made in 2000 (Schwarz et al., 2004) were significantly lower than in 2003 (Figure 3). Particulates and salts from swine manure are the likely source for increased specific conductance in the created wetlands. Water pH in created wetland enclosures from 2002 and 2003 (mean = 9.1 ± 0.1, range = 7.7 – 10.3, n = 58) was significantly greater than in reference enclosures (mean = 8.1 ± 0.1, range = 7.4 – 8.2, n = 29). Monthly pH averages from measurements made in 2000 (Schwarz et al., 2004), and 2002-2003 (current study) were higher in created wetlands sites than reference wetlands, especially during the summer months (Figure 4). Algal blooms can cause diurnal shifts in water pH by using carbon dioxide during the day (increasing pH) and adding carbon dioxide at night (decreasing pH). BOD averaged 108 ± 21 milligrams per liter (mg/L) in ten samples from the created wetlands and was much lower in the reference wetlands (mean = 20 ± 14 mg/L, n = 7). In the created wetland enclosures, BOD was higher in summer than spring (Figure 5); whereas in the reference enclosures, a seasonal difference in BOD concentrations was not evident. Turbidity averaged 145 ± 43 nephelometric turbidity units (NTUs) in created wetland enclosures from 2002 and 2003 (n = 47) and was significantly lower in reference enclosures (mean = 40 ± 12 NTUs, n = 20) (Figure 6).

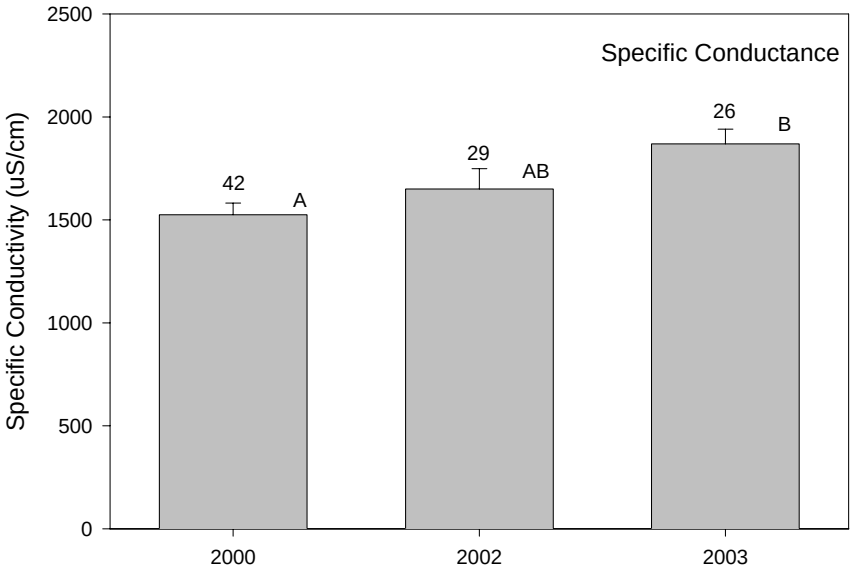

Figure 3. Mean (± SE) annual concentrations of specific conductance in created wetlands, Clay County, Nebraska. Sample size is given above each standard error bar. Letters indicate significance ($p < 0.05$) as determined by a Kruskal-Wallis test followed by pairwise Wilcoxon rank sums tests.

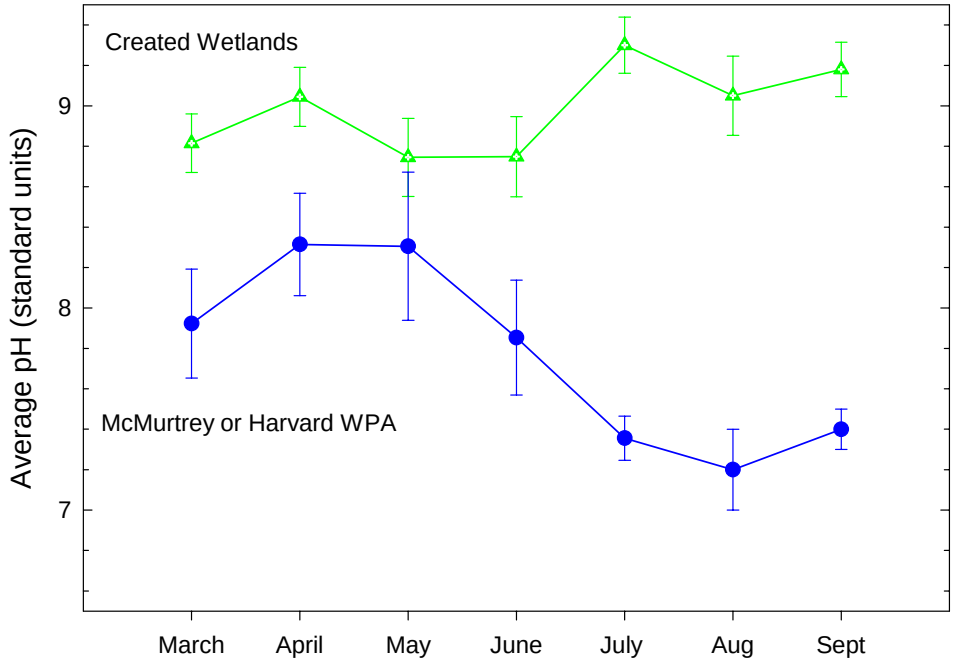

Figure 4. Mean (± SE) monthly concentrations of pH in created wetlands and reference wetlands, Clay County, Nebraska, for 2000, 2002, and 2003.

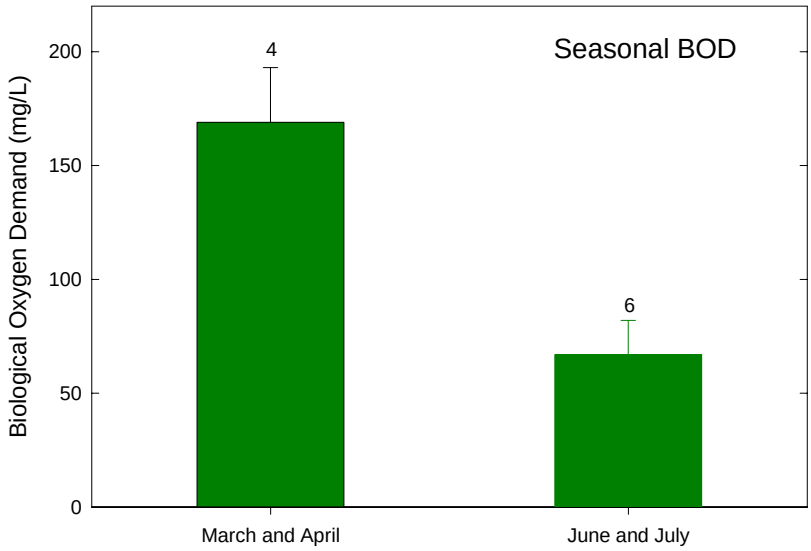

Figure 5. Mean (± SE) Biological Oxygen Demand (BOD) concentrations in the created wetlands during spring and summer months, Clay County, Nebraska, 2002 and 2003. The sample size is given above each standard error bar. Letters indicate significance ($p < 0.05$) as determined by a Wilcoxon rank sums test.

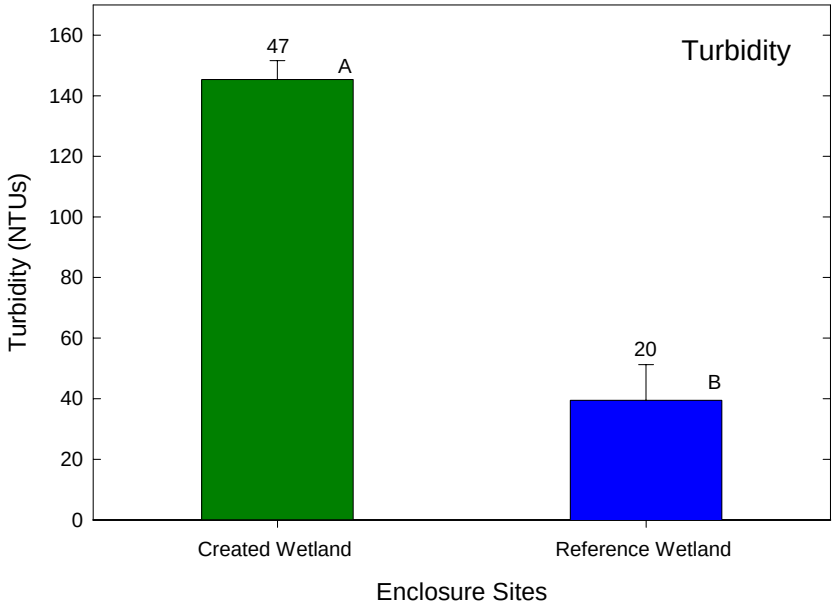

Figure 6. Mean (± SE) concentrations of turbidity in the created wetland and reference enclosures, Clay County, Nebraska, 2002 and 2003. The sample size is given above each standard error bar. Letters indicate significance ($p < 0.05$) as determined by a Wilcoxon rank sums test.

Concentrations of TKN, total phosphorus, and total ammonia were significantly greater in created wetland enclosures than reference enclosures (Figure 7). Total phosphorus and TKN in created wetland enclosures ranged from 0.3 – 21.1 mg/L and 11.3 – 296.4 mg/L, respectively. Total ammonia concentrations in the created wetlands ranged from 0.05 – 208.5 mg/L and exceeded Nebraska Department of Environmental Quality (NDEQ) acute aquatic life water quality standard for ammonia in 25 of 34 samples (NDEQ, 2006)(Table 1). In comparison, ammonia concentrations in reference enclosures ranged from 0.05 – 6.7 mg/L and exceeded the ammonia standard once at Harvard.

Table 1. Waterborne ammonia concentrations in created wetland and reference enclosures that exceeded Nebraska's pH dependent water quality standards for ammonia, Clay County Nebraska, 2002 and 2003.

				Ammonia Concentration (mg/L)		
Year	Month/Day	Site	pH	Measured	Standard	Exceedance
2002	March	CW4	8.5	95.4	3.8	91.6
	March	CW6	9.1	44.0	1.3	42.7
	April	CW6	9.5	2.6	0.8	1.8
	April	CW4	10.0	4.9	0.6	4.3
	May	CW4	9.3	3.3	1.0	2.3
	May	CW6	9.4	23.3	0.9	22.4
	June	Harvard	8.7	3.7	2.6	1.1
	June	CW6	9.7	2.7	0.7	2.0
	July	CW4	9.7	3.0	0.7	2.3
	August	CW6	9.2	4.8	1.2	3.6
	August	CW4	9.4	8.4	0.9	7.5
2003	March	CW6	8.4	111.5	4.6	106.9
	March	CW4	8.4	102.3	4.6	97.7
	April	CW6	8.2	208.5	6.8	201.8
	April	CW4	8.8	63.3	2.2	61.1
	May	CW6	8.2	109.4	6.8	102.7
	May	CW4	8.4	91.1	4.6	86.5
	June	CW6	7.9	60.0	12.0	48.1
	July	CW6	9.3	2.8	1.0	1.8
	August	CW6	8.9	9.0	1.8	7.2
	August	CW4	9.5	4.8	0.8	4.0
	Sept	CW6	8.4	45.6	4.6	41.0
	Sept	CW4	9.6	5.2	0.8	4.4
	Oct	CW6	8.6	19.1	3.1	16.0
	Oct	CW4	8.8	6.4	2.2	4.2

Note: CW = created wetland, Harvard = Harvard Waterfowl Production Area, Standard = Nebraska's pH dependent acute Class B warmwater aquatic life water quality standard (NDEQ, 2006), Exceedence = Difference between measured concentration and ammonia standard.

High ammonia concentrations in the created wetlands are likely harmful to aquatic invertebrates, amphibians and plants. Amphipods exposed to total ammonia concentrations greater than 1.45 mg/L for 10 weeks experienced decreased reproduction in 50 percent of the population tested (Borgmann, 1994). Concentrations of un-ionized ammonia greater than 1.5 mg/L can result in decreased leopard frog (*Rana pipiens*) embryo survival and increased prevalence of deformities in leopard frogs (Jofre and Karasov, 1999). Un-ionized ammonia concentrations greater than 3.0 mg/L can depress duckweed (*Lemna minor*) growth by twenty percent or more (Wang, 1991).

Vegetation differences between the created wetland and reference enclosures were apparent but not quantified. The Harvard enclosure was dominated by smartweed and provided less open water habitat than the other enclosures. Open water habitat at the McMurtrey enclosure was similar to that found on the created wetland enclosures. Upland areas in the reference enclosures were dominated by native grasses and had fewer undesirable plants, including infestations of musk thistle, than created wetland enclosures. Created wetland enclosures had less smartweed (Polygonum spp) than reference enclosures. However, they did contain plants beneficial as a food source to waterfowl including barnyard grass (*Echinochloa muricata*), curly dock (*Rumex crispus*), common sunflower (*Helianthus annuus*), and Lamb's quarters (*Chenopodium album*) (NGPC, 1999). Vegetation may have provided different foraging opportunities but did not appear to affect nesting. Sentinel mallards in all enclosures used natural vegetation to hide their nests and did not use the artificial nesting structures.

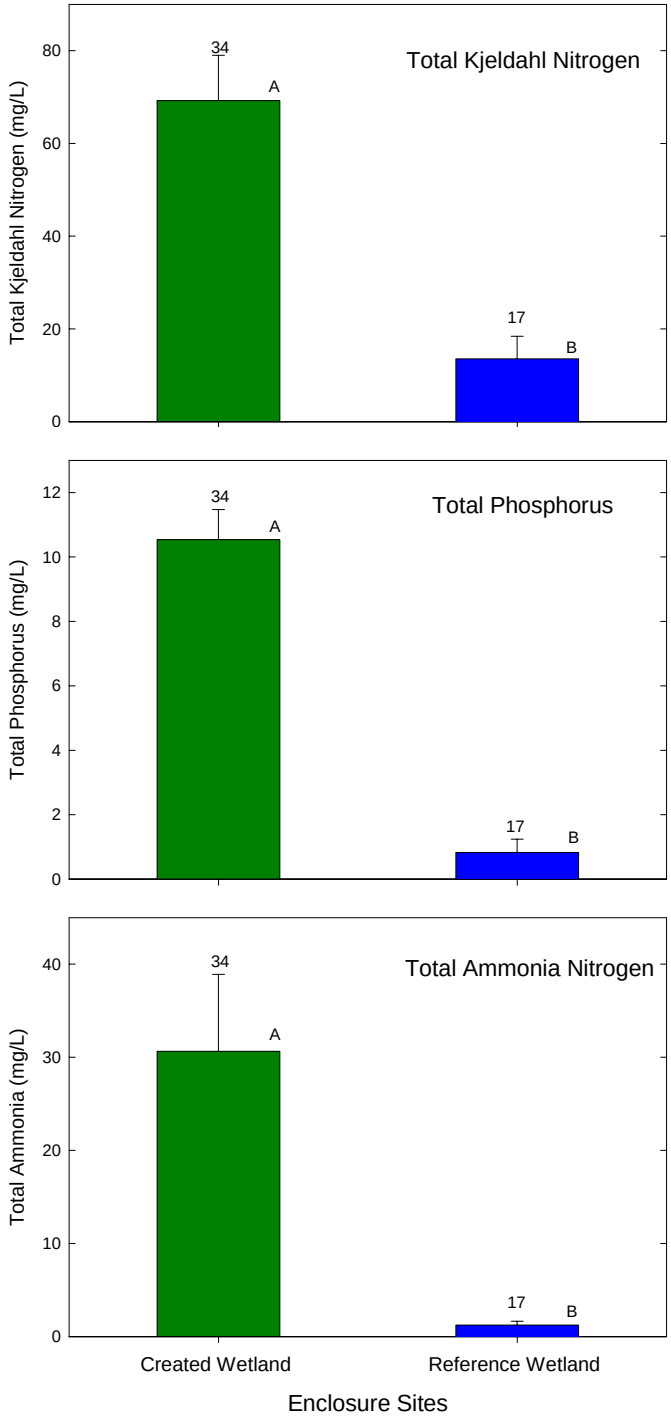

Figure 7. Mean (± SE) concentrations of total kjeldahl nitrogen, total phosphorus, and total ammonia in the created wetland and reference enclosures, Clay County, Nebraska, 2002 and 2003. The sample size is given above each standard error bar. Letters indicate significance ($p < 0.05$) as determined by a Wilcoxon rank sums test.

Sentinel Mallard Exposures

The number of sentinel mallards were kept in each enclosure and available for necropsy varied between years (Table 2). To increase breeding success by decreasing competition between males, the male to female ratio for mallards released to enclosures was changed from 1:1 in 2002, to 3:5 in 2003. Funding was targeted to analyze 8 and 4 mallards each year from created wetland and reference sites, respectively. This was accomplished for reference sites but escapes and mortality events in the created wetland enclosures resulted in an uneven analysis of males and females.

Table 2. Sentinel mallards kept in the McMurtrey, Harvard, Created Wetland 4 (CW4), and Created Wetland 6 (CW6) enclosures, Clay County Nebraska, 2002 and 2003.

	Released to Enclosures				Health Assessment Based Necropsy			
Date obtained	Date released	Enclosure	No. of Males	No. of Females	Date Females	Date Males	No. of Males	No. of Females
020307	020516	CW4	5	5	020715	020715	5	3
	020516	CW6	5	5	020716	020716	5	3
	020517	McMurtrey	5	5	030813	030813	2	2
	020517	Harvard	5	5	020717	020717	2	2
030311	030416	CW4	3	5	030618	030804	3	2
	030417	CW6	3	5	030618	030805	3	4
	030416	McMurtrey	3	5	030617	030804	2	2
	030417	Harvard	3	5	030617	030805	2	2

Sentinel Mallard Mortality

Morality in Enclosures. There were no known mortalities to mallards in the created wetlands in 2002 when no food was provided; whereas, mallards on the reference enclosures were not able to sustain themselves without a ration of corn. Mallards in the created wetland enclosures may have been able to sustain themselves by foraging on algae and microinvertebrates. Wetland water at the reference sites was supplied by a groundwater well and did not appear to contain as much biota.

In both 2002 and 2003, one female mallard from each created wetland enclosure was never found and probably died after escaping the enclosure. Mallards were able to leave the created wetland enclosures by swimming under the fence in submerged areas,

as it was difficult to pin the plastic fencing to the wetland sediments. Mallards were not able to leave the reference enclosures in 2002, but high winds damaged the McMurtrey netting in 2003 and two mallards were never found.

There was one known predation incident at an enclosure. On July 31, 2003, two dead sentinel adult hens and two ducklings were found partly consumed at Harvard. It is believed that a small mammalian predator (weasel or mink) was responsible for the predation as there was no apparent damage to the enclosure fence or netting. The number of ducklings lost to predation at Harvard is unknown, but it is probably why few ducklings were ever counted at the site. Besides the predation incident at Harvard, the only other site where sentinel mallards died while in the enclosure was CW4. In 2003 after nest initiation, two sentinel hen mallards were found decomposed but intact and well hidden by vegetation.

Avian Botulism Die-off. On August 26, 2003, the 17 remaining sentinel mallards that were on the created wetlands were either found dead (n = 10) as a result of an avian botulism outbreak, or were presumed dead and never found (n = 7). These sentinel mallards were kept indoors as extras in 2002, released onto Created Wetland 7 (CW7) on September 11, 2002, captured and over-wintered indoors, and then released back to CW7 in April, 2003. Six dead sentinel mallards were sent to NWHC for testing and avian botulism was determined as the cause of death.

The created wetlands provide habitat that is conducive to avian botulism outbreaks. Botulism spores and phages that produce botulism toxins are prevalent in most wetlands and are not considered to be a limiting factor in the occurrence of outbreaks in waterfowl (USGS, 1999). Instead, botulism outbreaks are largely controlled by ecological factors including environmental conditions that favor spore germination and bacteria growth (USGS, 1999). Such environmental conditions include those found in nutrient rich wetland environments such as bare mud substrates with anoxic sediments (Crowder and Bristow, 1988). Created wetland water is frequently between a pH of 8 to 10, greater than 20^{o}C, and less than 2 parts per thousand salinity; all conditions that tend to favor avian botulism outbreaks (Rocke and Samuel, 1999). Furthermore, dissolved

oxygen concentrations measured in created wetland enclosures during 2002 and 2003 ranged from 0.03 – 1,993 mg/L (n = 60) and indicate a potential for anoxic sediments.

Sentinel Mallard Reproduction

All sentinel mallards were fed a ration of corn from Hastings Pork in 2003 and this resulted in nesting and egg production in all enclosures. Eggs were discovered in mid-June on all sites. Hatches occurred first at McMurtrey and Harvard by end of June, whereas ducklings from sentinel mallards were not observed on created wetlands until mid-July. Counting ducklings that hatched was difficult, especially at the Harvard where vegetation was thick and there was no elevated ground to use as a vantage point. Therefore, the number of hatchings at all sites was evaluated by both actual counts and by the number of non-addled eggs believed to have hatched.

Number of total nests initiated was similar between reference sites and created wetlands (eight for each category); however, number of addled eggs collected was greater on created wetland sites than reference sites and more ducklings hatched at the reference sites than the created wetlands (Table 3). Although hatching success was lower on the created wetland enclosures compared to the reference enclosures, wild duck broods were observed on the created wetlands near the enclosures. One wild brood of mallards was observed on CW4 on June 27. Broods of mallards and teal (2 each) also were observed on CW6 on July 8. Wild hens that successfully hatched ducklings near the created wetlands are able to leave the created wetlands to forage, although it is unclear if this actually occurred.

Table 3. Reproductive endpoints evaluated for sentinel mallards in enclosures at two created wetlands and two reference sites, Clay County, Nebraska, 2003.

Enclosure(s)	Nests initiated	Nests hatched	Eggs laid	Addled Eggs				*Number of Ducklings
				Collected	Fertile	Infertile	Fertility Unknown	
Harvard	5	4	67	22	3	15	4	8 - 40
McMurtrey	3	3	39	3	0	2	1	24 - 31
Created Wetland 6	3	1	51	28	6	21	1	2 - 22
Created Wetland 4	5	1	41	32	20	12	0	8
Total Reference Sites	8	7	106	25	3	17	5	32 - 71
Total Created Wetlands	8	2	92	60	26	33	1	10 - 30

Note: * Number of ducklings is presented as actual number counted (the minimum) and the number estimated by calculating the number of eggs laid minus the number known not to hatch (includes damaged eggs not collected).

Poor hatching success on CW4 was probably a result of poor hen survival. Although all five hens on CW4 nested and produced eggs, only two hens survived to August (time of necropsy) and all but one nest was abandoned. Many of the addled eggs collected from CW4 were fertile and may have hatched had they been incubated longer.

Cumulative stress from poor water quality and reproduction may have caused hen mortality at CW4. Water quality conditions in the created wetlands were similar between 2002 and 2003 but there was a difference between years in hen survival. Hens in the created wetland enclosures survived in 2002, when there was no nesting attempts. The two dead hens found at CW4 were believed to have died soon after eggs were laid and one was found next to an abandoned nest. Female mallards experience substantial weight loss and lipid depletion between prelaying and late egg incubation stages during the nesting cycle (Krapu, 1981).

Average weight loss for mallards kept in enclosures was 169 ± 19 grams and 53 ± 41 grams for the created wetlands and reference wetlands, respectively. All mallards kept in created wetland enclosures lost body mass between the time of release and necropsy, whereas, 6 of 15 mallards kept in reference enclosures maintained or gained body mass (Figure 8). Initial body mass was only measured in zero-controls for 2003, but all of them gained weight between the date they were obtained and time of necropsy. Weight loss in mallards kept in the enclosures was the norm and indicates more stressful living conditions compared to the indoor pen where food was provided *ad lib*. Differences in weight loss between mallards kept on created wetlands and reference wetlands is likely attributed, in part, to reference mallards receiving a corn ration both years. However, there was no significant difference in weight loss between mallards on the created wetlands that were not provided corn in 2002 and those that received corn in 2003.

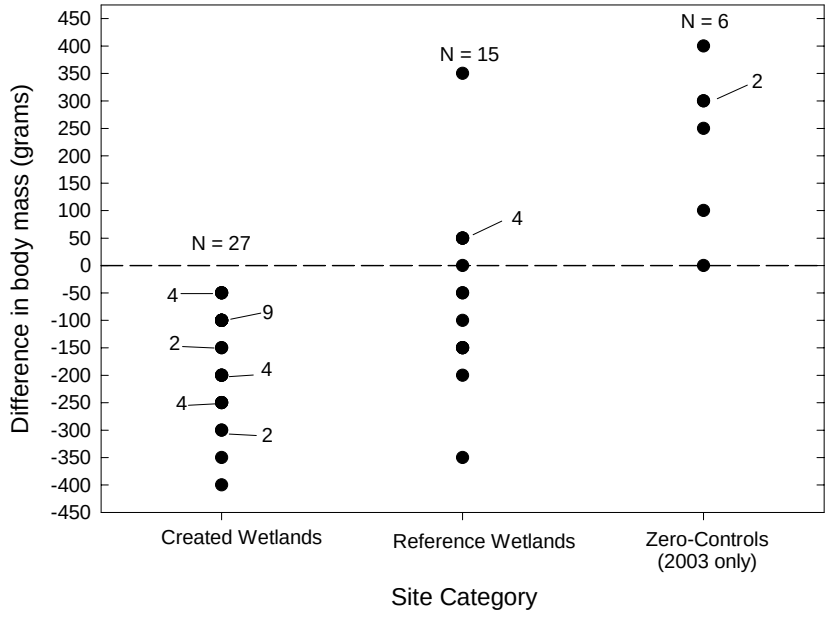

Figure 8. Differences in body mass between time of release and time of necropsy for mallard sentinels from created wetland and reference enclosures, Clay County, Nebraska, 2003. Note: N = sample size and the number near each point indicates the number of samples represented by the point.

Blood Plasma Chemistry

A total of 55 mallard blood plasma samples was analyzed in 2002 and 2003 (Appendix Tables A.3 and A.4). These samples were from day zero-controls (n = 12), and mallards kept in enclosures as follows: CW4 (n = 13), CW6 (n = 15), McMurtrey (n = 7) and Harvard (n = 8).

There were significant differences detected in blood plasma components between years and among sites (Table 4). Cholesterol was significantly greater in mallards from Harvard, where it averaged 215 ± 9 milligrams per deciliter (mg/dL) (n = 8), than CW6 (150 ± 7 mg/dL, n = 15); otherwise, there were no significant differences in blood plasma chemistry among outdoor enclosure sites. However, blood plasma from mallards kept on both created wetland sites had significantly greater chloride, greater ALT, and less phosphorus than day zero-controls. Mallards from this study also had mean concentrations of ALT, APT, and phosphorus outside the reference range reported in the literature (Table 5). Concentrations of ALT were higher in this study than previously reported reference values, whereas APT and phosphorus concentrations were lower.

The ALP enzyme is a mainly a biomarker for liver and bone disease but is also associated with other organs and glands including the adrenals, uterus, prostate, and intestine. Low ALP levels indicate malnutrition and/or protein deficiency (MedlinePlus, 2007). Low phosphorus levels in blood can also indicate a poor diet. Differences in ALP and phosphorus between day zero-controls and enclosed mallards was likely due to nutrition (i.e, zero-control mallards were fed corn *ad lib*, whereas those in the enclosures received no corn or a corn ration).

ALT is an enzyme that is more specific to the liver than ALT for liver stress. Low concentrations of ALT are normally found in the blood but when the liver is damaged or diseased, ALT is released into the blood stream. The higher concentrations of ALT and AST in mallards from the created wetlands indicate that they may have had increased liver stress from exposure to degraded water quality.

Few studies have compared blood chemistry components in mallards to contaminant exposure. However, increased serum AST concentrations have been identified as a biomarker of Se exposure in mallards (Fairbrother and Fowles, 1990).

AST was greater in 2002 and 2003 mallards (n = 28) from the created wetlands, where it averaged 31 ± 5 units per liter (U/L), than reference enclosures (21 ± 6 U/L, n = 14); however, the difference was not significant.

Table 4. Results from Tukey's least significant difference test for blood plasma components in sentinel mallards from zero-control groups and treatment groups at Hastings Pork created wetlands, McMurtrey National Wildlife Refuge, and Harvard Waterfowl Production Area, Clay County, Nebraska, 2002 and 2003.

Analyte	p values Site	Year	Site*Year	Results of Tukey's Least Significant Difference Test				
Glucose	0.4281	0.2207	0.0458	CW4	MM	CW6	HM	Control
AST	0.2780	0.1340	0.3680	CW4	CW6	Control	MM	HM
ALT	0.0002	0.0429	0.1667	CW4^{A}	CW6^{A}	MM^{AB}	HM^{AB}	Control^{B}
ALP	0.9546	0.0980	0.4155	CW6	CW4	Control	MM	HM
CK	0.2200	0.8516	0.9600	CW4	CW6	Control	HM	MM
LDH	0.2867	0.1265	0.0824	CW6	CW4	Control	MM	HM
Cholesterol	0.0154	0.0083	0.2876	HM^{A}	MM^{AB}	Control^{AB}	CW4^{AB}	CW6^{B}
Total Protein	0.1359	0.1928	0.1218	Control	MM	CW6	CW4	HM
Phosphorus	0.0021	0.0693	0.0691	Control^{A}	MM^{AB}	HM^{AB}	CW4^{B}	CW6^{B}
Calcium	0.0697	0.1013	0.1549	Control	MM	CW6	HM	CW4
Sodium	0.3744	0.3962	0.5818	CW6	MM	CW4	HM	Control
Potassium	0.1336	0.0001	0.8638	MM	HM	CW4	CW6	Control
Chloride	0.0021	0.4757	0.5174	CW6^{A}	CW4^{A}	HM^{AB}	MM^{AB}	Control^{B}
Uric acid	0.4616	0.1332	0.0103	HM	CW4	Control	CW6	MM
Anion gap	0.5005	0.0003	0.3824	MM	Control	HM	CW6	CW4

Note: AST = aspartate amino-transferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, CK = creatine kinase, LDH =lactate dehydrogenase, CW4 = Created Wetland 4, CW6 = Created Wetland 6, HM = Harvard, MM = McMurtrey. Different superscript letters indicate significant differences among sites. Sites are listed from left to right in decreasing order of mean concentration for each analyte.

Table 5. Mean concentrations of mallard blood plasma chemistry components from this study compared to those reported in the International Species Inventory System.

	Alanine Aminotransferase (U/L)			Alkaline Phosphatase (U/L)			Phosphorus (mg/dL)		
Site	N	Mean ± SE	Range	N	Mean ± SE	Range	N	Mean ± SE	Range
CW4	13	62 ± 6	27 - 98	12	117 ± 10	63 - 181	12	3 ± 0.2	1 - 4
CW6	15	61 ± 5	31 - 100	13	120 ± 18	51 - 339	13	2 ± 0.2	1 - 3
HM	8	42 ± 4	31 - 58	15	99 ± 22	48 - 241	15	3 ± 0.3	2 - 4
MM	7	47 ± 8	19 - 77	8	108 ± 24	55 - 208	8	3 ± 1.3	1 - 11
Day 0 Control	12	32 ± 4	19 - 58	7	116 ± 27	60 - 402	7	5 ± 0.8	2 - 10
Reference*	19	23 ± 2	6 - 37	22	527 ± 55	126 - 1097	16	7 ± 0.2	6 - 9

Note: * = physiological data reference values from the International Species Inventory System (ISIS, 1996). U/L = units per liter, mg/dL = milligrams per deciliter.

Histopathology

Histopathology conditions commonly found in mallards from all sites included hepatitis (liver inflammation), splenitis (spleen inflammation), and hemosiderosis (excessive iron deposition) in liver and spleen. Lesions in kidneys and gonads were less common (Appendix Tables A.5 – A.8). There were no significant differences, as determined by a Kruskal-Wallis test, in total lesion scores among mallards from created wetland enclosures, reference enclosures, and zero-controls. There also were no significant differences between mallards from created wetlands and reference sites in number of lesions in kidney, spleen, liver, or gonad. However, average number of kidney lesions in mallards from created wetland enclosures and reference enclosures were greater than zero-controls (Figure 9). Liver hemosisdosis also was more prevalent in mallards from created wetland enclosures (85 percent) and reference enclosures (81 percent) than zero-controls (33 percent) (Figure 10). Histopathology results did not indicate that water quality in the created wetlands was more harmful to sentinel mallards than water quality of the reference sites. Stress from an outdoor environment and lack of nutrition probably accounted for the higher prevalence and number of tissue lesions in mallards from the enclosure sites than zero-controls.

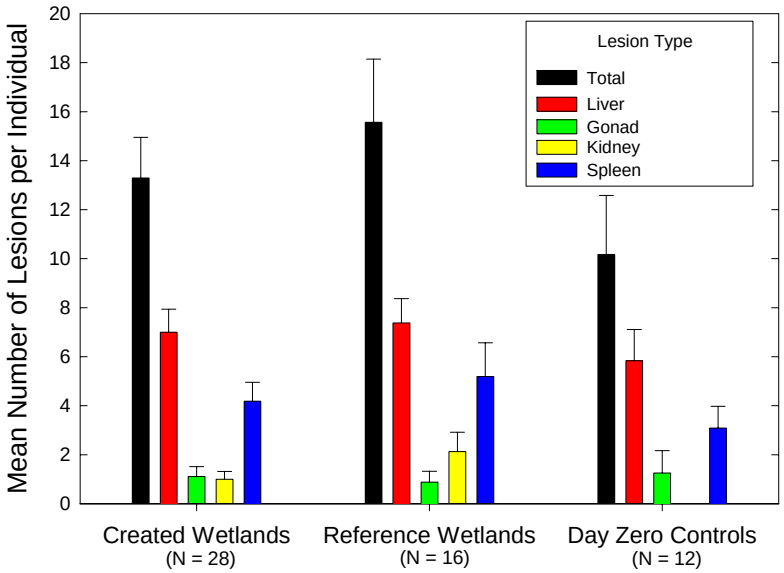

Figure 9. Mean number of tissue lesions per sentinel mallard from created wetland enclosures, reference enclosures, and zero-controls, Clay County, Nebraska, 2002 and 2003. Note: N = sample size.

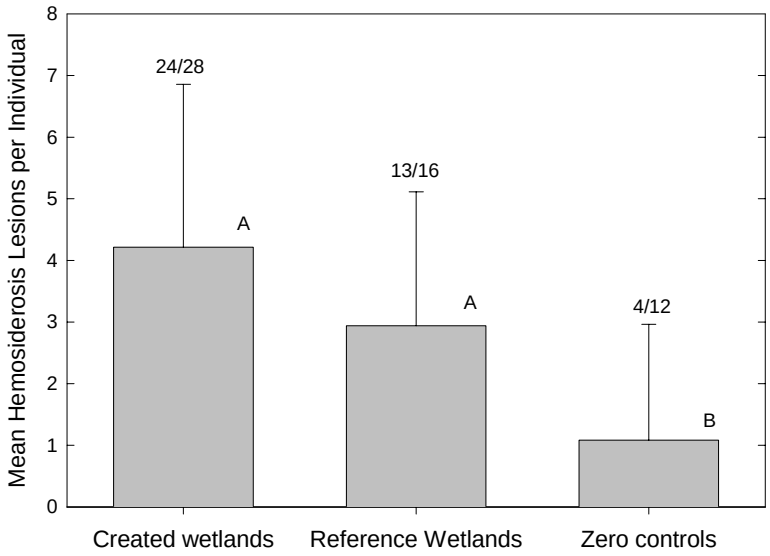

Figure 10. Liver hemosiderosis in sentinel mallards from created wetlands, reference wetland enclosures, and zero-controls, Clay County, Nebraska, 2002 and 2003. Note: the fraction above each standard error bar indicates the number of individuals with hemosiderosis over the number tested. Letters indicate significance ($p < 0.05$) as determined by a Wilcoxon rank sums test.

Algal Toxins in Water and Mallard Tissues

Water. *Microcystis* spp. were not observed in created wetland water samples from March, April, or June of 2002 and 2003. Presence of Microcystis spp. was observed in created wetland enclosures in May and July, 2003, and August and September, 2002 and 2003. Created wetlands frequently appeared eutrophic and full of phytoplankton (Figure 11). Algal blooms were not observed at McMurtrey or Harvard enclosures.

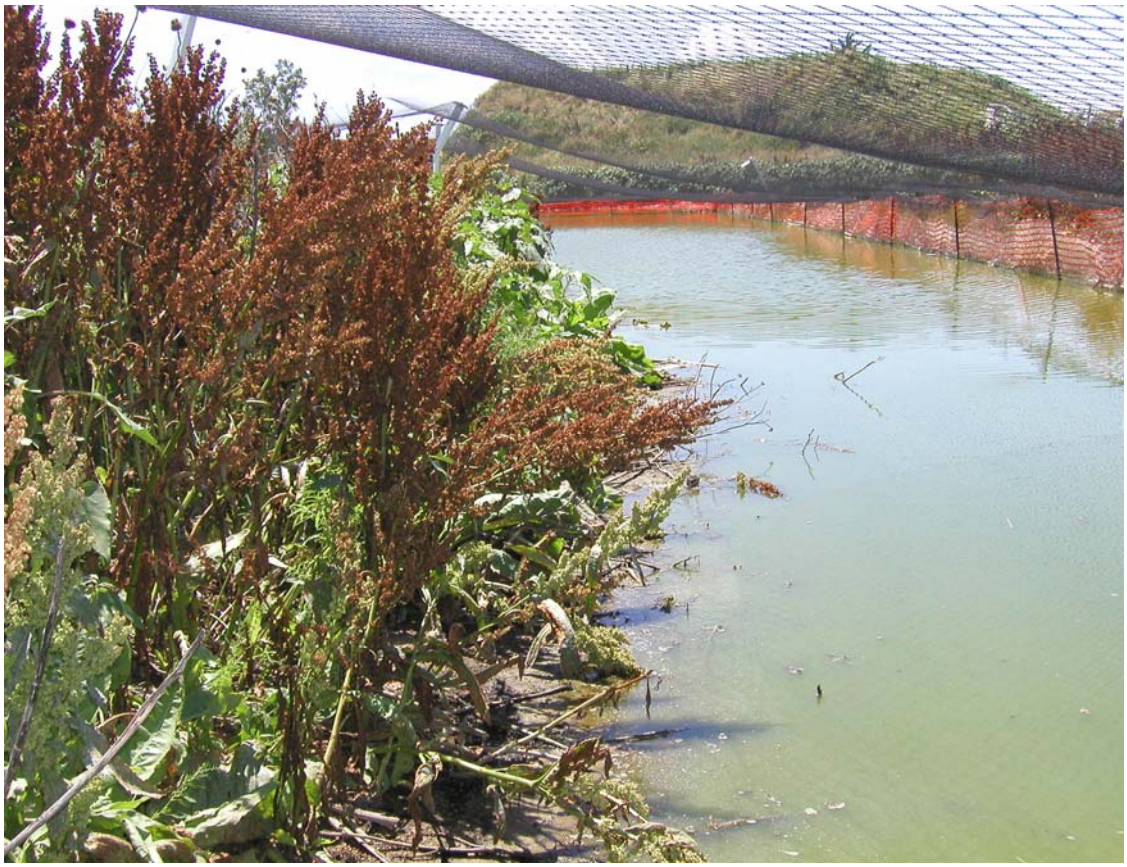

Figure 11. Effects of nutrient enrichment on vegetation and water quality inside the Created Wetland 6 enclosure. Water quality conditions on the other created wetlands were generally similar.

Twelve water samples from the created wetlands were analyzed for microcystins by HPLC to evaluate the mallard die-off on August 26, 2003. Concentrations of microcystins in all of the samples (six filtered and six unfiltered) were below the detection limit of 50 micrograms per liter (μg/L) (Echols and Feltz, 2004). Four more water samples from created wetlands were analyzed for microcystins by ELISA. These samples were collected in 2003 on September 10, September 24, October 22, and November 12. Concentrations of microcystins in these samples averaged 7.0 ± 1.5 μg/ and ranged from 2.8 - 9.1 μg/L (Echols and Feltz, 2006). The effects of chronic microcystin exposure to waterfowl is unknown and toxicity guidelines for protecting waterfowl from harmful concentration of microcystins in water have not been developed. A provisional guideline value of 1.0 μg/L microcystin-LR is recommended by the World Health Organization (WHO) to protect humans from liver damage induced by chronic exposure (WHO, 1998). Health Alert declarations in Nebraska are issued if microcystin concentrations in recreational surface waters exceed 20μg/L (NDEQ, 2007).

Liver. Microcystins were detected in 2 of 15 mallard liver samples tested by HPLC in 2002 (Echols and Feltz, 2004). The MC-YR toxin variant was detected at 0.13 and 0.17 micrograms per gram (μg/g) in samples from CW6 and Harvard, respectively. Concentrations of microcystins in six liver samples collected during a die-off in CW7 in August of 2003 were below a 0.03 μg/g detection limit (Echols and Feltz, 2004).

Stomach Contents. Concentrations of microcystins in stomach contents from sentinel mallards in 2002 and 2003 were analyzed by ELISA (Echols and Feltz, 2006). Microcystins were detected at all sites for both years with the exception of CW4 in 2002 (Table 6). The highest mean concentration of microcystins was from created wetlands for both years. In 2002, CW6 had the highest mean concentration of microcystins at 27.35 ± 0.25 nanograms per gram (ng/g). The highest mean concentration of microcystins in 2003 was at CW4 (18.67 ± 13.35 ng/g). Mallards from CW6 in 2003 had a lower mean concentration of microcystins compared to zero-controls or mallards from Harvard. The detection of microcystins in 3 of 4 zero-controls may indicate that drinking water provided in the indoor pen via a groundwater well at Hastings Pork, may have been a source for microcystins. Algal toxins that originate in surface waters can contaminate

groundwater (Eynard et al., 2000). Concentrations of microcystins also were measured in six gizzard samples from sentinel mallards that died from avian botulism in August of 2003. These samples were analyzed by HPLC and all were below a 0.03 μg/g detection limit (Echols and Feltz, 2004).

Study results indicate that mallards on the created wetlands are exposed to microcystin toxins, although concentrations in tissues were generally not high enough to be detected by HPLC. Microcystins bind irreversibly to protein phosphatase in the liver (Carmichael, 1997), and in the current study, only unbound microcystin was measured. Phosphatase bound microcystin concentrations are typically 5 - 10 times that of unbound microcystins (Echols and Feltz, 2004); therefore, concentrations of bound microcystins in mallards from the created wetlands was probably low.

Table 6. Mean concentrations of microcystins in stomach contents from sentinel mallards on created wetlands, McMurtrey, and Harvard compared to zero-controls, Clay County, Nebraska, 2002 and 2003.

			Concentration (nanograms per gram)	
Year	Group	N_D/N_A	Mean ± S.E.	Range
2002	Created Wetland 4	0/5	NA	NA
	Created Wetland 6	2/2	27.4 ± 0.3	27.1 - 27.6
	Harvard	2/2	1.0 ± 0.3	0.74 - 1.3
	McMurtrey	1/1	NA	NA
2003	Day Zero Control	3/4	3.2 ± 0.8	1.9 - 4.5
	Created Wetland 4	4/4	18.7 ± 13.3	0.67 - 58.2
	Created Wetland 6	4/4	2.8 ± 0.4	1.9 - 3.7
	Harvard	2/2	4.0 ± 0.1	3.9 - 4.1
	McMurtrey	2/2	2.3 ± 1.3	1 - 3.6

Note: N_D = number detected, N_A = number analyzed, S.E. = standard error, NA = not applicable.

Although *Microcystis* spp. were observed in water samples collected from the created wetlands, results indicate that microcystin exposure to sentinel mallards on the created wetlands was low and not associated with adverse effects to the liver as evaluated by histology. Although *Microcystis* spp. were present in the created wetlands, microcystin toxins were only detected at low concentrations indicating that environmental factors during sampling may have not favored toxin production. Presence of *Microcystis* spp. in created wetland algal blooms should warrant concern because environmental stress to cyanobacteria from factors such as pH, temperature, iron limitation, and increased concentrations of phosphorus and nitrogen can increase toxin production (Sivonen, 1996). Concentrations of microcystins measured previously at the site (Schwarz et al., 2004) for algae concentrated water samples (i.e., samples collected by filtering algae with a phytoplankton tow net) ranged from 41,000 – 210,000 ng/g and 700 – 120,000 ng/g as measured by ELISA and HPLC methods, respectively (Echols, 2001). In addition, waterfowl die-offs attributed to microcystins have been reported in Nebraska and Japan (NGPC, 1992; Matsunaga, 1999).

Pathogens in Mallards

A total of 57 gastrointestinal contents (31 in 2002 and 26 in 2003) were tested for bacterial pathogens (Appendix tables A.9 – A.10). There was no bacterial growth in five samples, which included one each from CW6 and CW4 in 2002, and one each from McMurtrey, Harvard, and zero-controls in 2003. Only three samples (two from CW6 and 1 from CW4) had too much bacterial growth to obtain isolates.

Enterococcus spp. and *E. coli* were detected in the majority of mallards from all sites (40 and 46 of 57 mallards tested, respectively), including zero-controls. *Streptococci* spp. were more rarely detected (9 of 57 mallards tested) and only detected in 2 mallards from the created wetland enclosures. *Pasteurella* spp., *Eysiplothrix* spp., and *Yersinia* spp. isolates were not found. *Salmonella bareill*y was only detected in mallards from CW6 (4 of 16 birds tested) in 2002.

Five different species of *Streptococcus* were detected and none were unique to the created wetlands. *Streptococcus equine, S. bovis,* and *S. salivarius* were isolated in zero-

controls and therefore not believed to be associated with either the created wetlands or the reference sites. *Streptococcus uberis* was isolated from 3 mallards (two on CW6 and one on McMurtrey) and *S. constellatus* was only isolated twice (1 each on Harvard and McMurtrey). *Streptococcus uberis* is an important environmental pathogen associated with bovine mastitis in dairy cows and is rarely associated with diseases outside the mammary gland (Leigh, 2003; Coffey et al., 2006). *Streptococcus canstellatus* has a human origin and causes pharyngitis (Facklam, 2002).

The only bacteria pathogen found solely in mallards from a created wetland enclosure was *Salmonella bareilly*. Previously, water sampling from Hastings Pork and McMurtrey found that *Salmonella* spp. (Newport, Typhimunium, Derby, Infantis, and Muenchen) were associated with swine waste, but *S. bareilly* was not reported (Schwarz et al., 2004). Mallards on the created wetlands may have been initially exposed to *S. bareilly* in created wetland water as it can survive in harsh surface water environments (Thomason et al., 1977). Swine are a known reservoir for *S. bareilly* (Porcurull et al., 1971), and it can be transmitted from water to humans (Mendis et al 1976). It is unclear what effects *S. bareilly* has on mallards. However, the prevalence of *Salmonella* spp. in captive-reared and wild ducks is well known and its effects on duck populations appears to be minimal (Henry, 2000).

All 56 cloaca swab inoculates (30 in 2002 and 26 in 2003) that were tested for duck plague and avian influenza were negative. The absence of avian influenza in sentinel mallards may be a result of enclosures limiting contact with migrating waterfowl. Wild birds are the primary natural reservoir for all subtypes of influenza A viruses and are thought to be the source of influenza A viruses in all other animals (CDC, 2006). Prevalence of avian influenza in wild mallards captured in Minnesota was highest in juveniles and ranged from 11 – 23 percent (Hanson et al., 2003). Avian influenza virus has been reported from 12 orders and 88 species of free-living birds, mostly from species in the orders Anseriformes (ducks, geese, and swans) and Charadriiformes (shorebirds and allies) with peak infection occurring in late summer and early fall (Stallknecht and Shane, 2004). Transmission of avian influenza from waterfowl to swine has been documented (Pensaert et al., 1981), and the incidence of influenza A in swine can be

higher than 50 percent (Chambers et al., 1991). Transmission of influenza A from swine to turkeys has been reported (Choi et al., 2004); however, there are no known reports of transmission from swine to wild ducks.

Duck plague mortality events mainly involve captive or captive-reared muscovey (*Cairina moschata*) and mallard ducks (Converse and Kidd, 2001). However, in North America between 1976 and 1995, there have been three duck plague cases resulting in the loss of 100 or more migratory waterfowl (Converse and Kidd, 2001). The largest die-off occurred at Lake Andes National Wildlife Refuge, South Dakota, in January of 1973. The birds were concentrated during severe weather conditions and an estimated 43,000 waterfowl, primarily mallard ducks, died from an estimated population of 163,500 migratory waterfowl (Converse and Kidd, 2001). Duck plague mortality in waterfowl has not been reported in Nebraska. However, the potential for duck plague to cause mortality in resident and migratory waterfowl indicates a need for surveillance of waterfowl flocks to detect early signs of the disease (Converse and Kidd, 2001).

Escherichia coli and *Streptococci* in Water Samples

Annual mean concentrations of fecal *E. coli* and streptococci in water from enclosure sites were highly variable for all sites. Concentrations of *E. coli* were greatest in CW6 and lowest in CW4, but differences were not significant (Table 7). Streptococci concentrations were significantly greater in McMurtrey and CW6 compared to CW4 and Harvard (Table 7).

Table 7. Mean concentrations of *E. coli* and Streptococci in created wetland and reference enclosures, Clay County, Nebraska, 2002 and 2003.

Pathogen	Site	n	Concentration (cfu/100 ml) Mean ± S.E.	Range	Significance
E. Coli	CW6	29	7,843 ± 6,692	10 - 195,000	A
	McMurtrey	13	6,318 ± 3,430	9 - 34,000	A
	Harvard	14	1,267 ± 446	9 - 4,500	A
	CW4	29	585 ± 183	9 - 4,000	A
Streptococci	McMurtrey	13	4,852 ± 1,153	700 - 14,000	A
	CW6	27	3,857 ± 1,026	37 - 23,500	A
	CW4	27	1,650 ± 418	20 - 7,700	B
	Harvard	14	1,100 ± 461	10 - 6,400	B

Note: CW6 = created wetland 6, CW4 = created wetland 4, S.E. = standard error. Letters indicate significance ($p < 0.05$) as determined by a Kruskal-Wallis test followed by pairwise Wilcoxon rank sums tests.

Previous monitoring of fecal coliforms and streptococci at Hastings Pork also found high variability within sites, but the greatest concentrations of these pathogens were consistently found in the lagoons (Schwarz et al., 2004). Streptococci counts measured in 2000 at the created wetlands were significantly greater than those at McMurtrey (Schwarz et al., 2004). Sources of *E. coli* and Streptococci at the Harvard and McMurtrey enclosures include the sentinel mallards and cattle grazing the upland areas near the enclosures. The high counts of Streptococci and E coli measured at McMurtrey and Harvard are not necessarily a waterfowl health concern. Both pathogens are part of the natural microflora in ducks and generally do not cause disease unless the immune system is stressed from poor environmental conditions (e.g., over crowding in a game farm) and/ or infection by other pathogens (Sandhu, 1988; USGS, 1999; Miller et al., 2004). Concern for public human health from exposure to these pathogens also is probably minimal. McMurtrey is closed to the public and Harvard does not provide primary contact recreation (e.g., swimming or water skiing).

Elemental Contaminants

Mallard Liver. A total of 56 mallard liver samples were analyzed in 2002 and 2003 (Appendix Tables A.11 - A.12). Concentrations of barium, iron, molybdenum, selenium, and strontium were highest in sentinel mallards from created wetlands (Table 8); however, only selenium exceeded any known toxicity thresholds for waterfowl. Concentrations of selenium in created wetland mallards averaged 11.1 ± 1.0 (n = 28) and exceeded a 10 milligrams per kilogram (mg/kg) dw toxicity threshold that may be associated with reproductive impairment in laying females (Hamilton, 2004; Heinz, 1996). Concentrations of selenium in reference mallards averaged 9.2 ± 1.4 mg/kg dw, which is above a 7.5 mg/kg dw hepatic background concentration for omnivorous species (USDOI, 1998), and exceeded 10 mg/kg in 4 of 16 samples. Reference wetlands had significantly greater concentrations of cadmium (mean = 1.2 ± 0.2 mg/kg dw, n =16) than created wetlands (mean = 0.82 ± 0.07 mg/kg dw, n =28), but concentrations were still within a 1 – 5 mg/kg ww background for waterfowl (Furness, 1996).

Table 8. Summary statistics for concentrations of elemental contaminants in sentinel mallard liver samples from day zero controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002 and 2003.

Elemental Contaminant	MDL	Created Wetlands			Harvard and McMurtrey			Day Zero Controls		
		N_D/N_A	Mean ± S.E.	Range	N_D/N_A	Mean ± S.E.	Range	N_D/N_A	Mean ± S.E.	Range
As	0.18	18/28	0.25 ± 0.03	0.2 - 1.0	6/16	NA	0.2 - 0.4	5/12	NA	0.2 - 0.4
B	0.90	4/28	NA	1.7 - 3.0	0/16	NA	NA	0/12	NA	NA
Ba	0.09	24/28	3.1 ± 2.8	0.1 - 77.9	13/16	0.7 ± 0.1	0.1 - 0.8	4/12	NA	0.1 - 0.4
Cd	0.04	28/28	0.8 ± 0.1	0.3 - 1.7	16/16	1.2 ± 0.2	0.2 - 2.8	12/12	0.5 ± 0.1	0.2 - 0.9
Cr	0.45	8/28	NA	0.5 - 1.3	4/16	NA	0.8 - 1.6	2/12	NA	0.6 - 1.0
Cu	0.30	28/28	199 ± 24	33 - 465	16/16	467 ± 185	9 - 2870	12/12	402 ± 115	27 - 1370
Fe	0.90	28/28	4945 ± 540	1550 - ####	16/16	3489 ± 546	1080 - 9450	12/12	1638 ± 224	864 - 3210
Hg	0.04	15/28	0.13 ± 0.03	0.05 - 0.40	11/16	0.12 ± 0.02	0.05 - 0.22	0/12	NA	NA
Mg	1.79	28/28	687.1 ± 15.7	426.0 - 942.0	16/16	672.3 ± 23.9	494.0 - 775.0	12/12	611.4 ± 31.6	347 - 800
Mn	0.18	28/28	17.7 ± 1.1	7.0 - 39.9	16/16	16.4 ± 1.2	8.6 - 26.0	12/12	14.3 ± 0.8	9.5 - 18.4
Mo	0.90	28/28	5.8 ± 0.5	2.9 - 17.0	15/16	4.1 ± 0.5	2.0 - 7.0	11/12	3.5 ± 0.4	2.9 - 6.4
Ni	0.45	3/28	NA	0.5 - 0.9	1/16	NA	0.50	1/12	NA	0.67
Pb	0.04	19/28	0.6 ± 0.2	0.1 - 6.4	10/16	0.3 ± 0.1	0.1 - 2.0	0/12	NA	NA
Se	0.02	28/28	11.1 ± 1.0	3.7 - 22.0	16/16	9.2 ± 1.4	4.8 - 23.7	12/12	4.2 ± 0.4	1.9 - 6.3
Sr	0.04	15/28	0.8 ± 0.6	0.1 - 15.8	10/16	0.13 ± 0.02	0.1 - 0.4	7/12	0.1 ± 0.02	0.1 - 0.3
V	0.50	7/28	NA	0.5 - 0.8	1/16	NA	1.11	0/12	NA	NA
Zn	0.45	28/28	159.5 ± 6.3	69.9 - 241.0	16/16	153.2 ± 10.9	61.3 - 214.0	12/12	134.7 ± 13.5	59 - 219

Note: All concentrations are in mg/kg dry weight. MDL = minimum detection limit, N_D/N_A = the number of samples with detected concentrations over the number of samples analyzed, NA = not applicable, S.E. = standard error. The range only includes samples above the detection limit. If 50 percent or more of the samples were above the detection limit then half the detection limit was substituted for the detection limit to calculate the mean.

Elemental contaminant concentrations in liver samples from McMurtrey and Harvard were not significantly different, as determined by a Wilcoxon test, with the exception of higher mercury at Harvard (mean = 0.17 ± 0.02 mg/kg dw, n = 8) than McMurtrey (mean = 0.07 ± 0.02 mg/kg dw, n = 8). Mallards in CW4 had higher concentrations of copper, mercury, selenium, and zinc than those in CW6. A possible explanation for the higher concentrations of metals at CW4 is that it is at the end of the created wetland chain (i.e, CW4 does not drain into any other waterbody), whereas CW6 drains into CW7.

Stomach Contents. Concentrations of elemental contaminants were measured in six stomach content samples collected from sentinel mallards on created wetland enclosures in 2002 (Appendix Table A.13). Concentrations of selenium, boron, and zinc did not exceed any known dietary toxicity thresholds for waterfowl (USDOI, 1998). A stomach content sample from CW6 had a high concentration of lead (430 mg/kg dw). The high lead concentration from CW6 may be due to contamination by lead shot as the created wetlands are located within a game farm for hunting upland birds. Lead toxicity thresholds have been based on tissue concentrations (Pain et al., 1996) and the presence or absence of lead particulates in the gizzard does not confirm lead toxicosis (USGS, 1999). This is because diet is a major modifier of lead absorption and of toxic effects in many species of waterfowl (Eisler, 1988). Mallards on a diet of corn have died within 10 to 14 days after ingesting a single lead shot; whereas, those fed a balanced commercial duck ration may appeared normal after ingesting as many as 32 pellets of the same size (Wobeser, 1981 as cited by Eisler, 1988).

Mallard Eggs. A total of 30 mallard eggs (ten each from CW4, CW6, and Harvard) were collected and analyzed for elemental contaminants in 2003 (Appendix Table A.14). These eggs did not have concentrations of elemental contaminants above any known toxicity thresholds. Some metals previously determined to be associated with swine waste (e.g., boron, barium, iron, strontium, and zinc) were detected more frequently or at significantly greater concentrations in eggs from the created wetlands than those from Harvard (Table 9).

Table 9. Summary statistics for concentrations of elemental contaminants in sentinel mallard egg samples from mallards kept in enclosures at Hastings Pork created wetlands and Harvard Waterfowl Production Area, Clay County, Nebraska, 2003.

Trace Element	MDL	Created Wetlands			Harvard Waterfowl Production Area		
		N_D/N_A	Mean ± S.E.	Range	N_D/N_A	Mean ± S.E.	Range
As	0.18	18/20	0.29 ± 0.02	0.19 - 0.46	9/10	0.27 ± 0.03	0.193 - 0.425
B	0.91	8/20	NA	1.0 - 1.4	0/10	NA	NA
*Ba	0.09	20/20	12.9 ± 1.7	4.4 - 34.8	10/10	5.2 ± 0.7	2.87 - 9.06
Cr	0.45	1/20	NA	0.6 - 0.6	2/10	NA	0.794 - 0.961
Cu	0.45	20/20	4.4 ± 0.3	3.0 - 7.6	10/10	4.6 ± 0.2	3.79 - 5.92
*Fe	0.91	20/20	112 ± 4	82 - 142	10/10	87 ± 7	48 - 115
Mg	1.81	20/20	461 ± 23	312 - 654	10/10	398 ± 30	281 - 642
Mn	0.18	20/20	1.9 ± 0.1	1.1 - 3.4	10/10	1.8 ± 0.3	0.5 - 3.7
Ni	0.45	6/20	NA	0.5 - 2.0	2/10	NA	1.04 - 1.71
Pb	0.05	2/20	NA	0.1 - 0.1	5/10	NA	0.162 - 0.446
*Se	0.02	20/20	2.8 ± 0.1	1.9 - 3.5	10/10	3.3 ± 0.2	2.5 - 4.0
*Sr	0.05	20/20	8.3 ± 0.9	3.8 - 16.5	10/10	4.9 ± 1.1	2.9 - 14.8
*Zn	0.45	20/20	55 ± 2	44 - 68	10/10	47 ± 3	32 - 58

Note: * = significant difference ($p < 0.05$) between created wetlands and Harvard WPA as determined by a Wilcoxon rank sums test. All concentrations are in mg/kg dry weight. MDL = minimum detection limit, N_D/N_A = the number of samples with detected concentrations over the number of samples analyzed, NA = not applicable, S.E. = standard error. The range only includes samples above the detection limit. If 50 percent or more of the samples were above the detection limit, then half the detection limit was substituted for those samples below the detection limit to calculate the mean.

Selenium concentrations in eggs from Harvard (mean = 3.3 ± 0.2 mg/kg dw, range = 2.5 – 4.0 mg/kg dw) were significantly greater than those detected in eggs from created wetlands (mean = 2.8 ± 0.1, range = 1.9 – 3.5). Concentrations of selenium in eggs from all sites were below a 10 mg/kg dw threshold for reduced egg hatchability and within a 3 - 5 mg/kg dw natural background range (USDOI, 1998). Concentrations of elemental contaminants were generally similar between CW4 and CW6; however, eggs from CW4 had significantly greater concentrations of iron, strontium, and zinc (Figure 12).

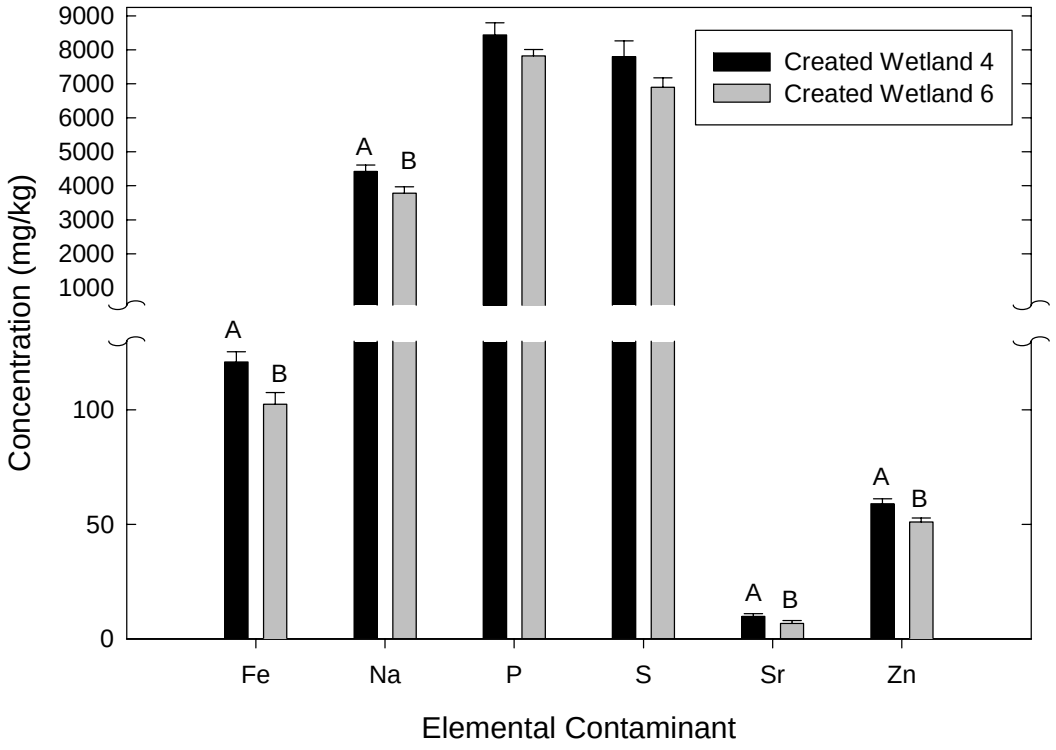

Figure 12. Mean (± SE) concentration of select elemental contaminants in eggs of mallards exposed to swine wastewater effluent in the Hastings Pork created wetlands, Clay County, Nebraska, 2003.

Management Actions

In October of 2004, NDEQ ordered Hastings Pork to discontinue their discharge of swine effluent to the created wetlands. NDEQ determined that the created wetlands were not part of the previously approved livestock waste control facility and did not meet holding pond requirements for compaction, dimensions, and carrying capacity. There has been some discussion between Hastings Pork management and NEFO about the possibility of further treating secondary swine wastewater before delivering to the created wetlands for waterfowl habitat. Treatment options discussed include the use of dilution ponds and constructed wetlands. Any such treatment options could be part of an approved livestock waste control facility provided that the design and monitoring plan are approved by NDEQ, USEPA, and the Service.

Recommendations

Given the current status of wetlands in Nebraska and the high demand on its limited water resources, treatment of secondary swine CAFO effluent to an acceptable level of water quality for creating migratory bird habitat is a desirable goal. It is recommended that Hastings Pork, NGPC, Ducks Unlimited, and the Service form a partnership coordinated by RWBJV to design and implement a strategy to further improve water quality of secondary effluent from Hastings Pork to a level sufficient for use in creating waterfowl habitat.

Constructed Wetlands. In recent years, a number of studies have been published on developing and evaluating constructed wetlands for treating swine wastewater (Stone et al., 2002; Poach et al., 2003; Sezerino et al., 2003; Lee et al., 2004; Szogi et al., 2004; He et al., 2006 Hunt et al., 2002, 2003, and 2006). These studies and more indicate that constructed wetlands can remove nutrients, bacteria, hormones, odorous gases, and elemental contaminants. A surface flow two-cell constructed wetland for a 2,600 head swine nursery in North Carolina reduced overall *Salmonella* and *E. coli* bacteria by 98 and 99 percent, respectively (Hill and Sobsey, 2001). A constructed wetland in North Carolina reduced estrogens in a swine farrowing facility from 200 nanograms per liter

(ng/L) to 3 ng/L (Shappell et al., 2007). Dimethyl disulfide and p-cresol, two of the most troublesome malodorous gases associated with swine waste, were reduced by 80 percent or more after swine wastewater was treated by a constructed wetland (Wood et al., 2000). Dissolved metals, including copper, zinc, and nickel, were reduced by a surface flow constructed wetland designed to treat runoff from residential and agricultural land (Goulet et al, 2001).

Nutrients removed by constructed wetlands include organic nitrogen, phosphorus, and ammonia. Constructed wetlands can reduce nitrogen concentrations from anaerobic lagoon-treated swine wastewater by 85 – 90 percent (Phillips et al., 2000; Stone et al., 2002). Phosphorus removal from swine wastewater by constructed wetlands is more limited than nitrogen removal. Although phosphorus mass load reductions can average 48 percent (Phillips et al., 2000), it is recommended that constructed wetlands be combined with enhanced phosphorus removal strategies for effective nitrogen and phosphorus removal at high loading rates (Hunt et al., 2002). This is especially true given that phosphorus removal by constructed wetlands can decrease over time as sediment binding sites become saturated with phosphorus (Phillips et al., 2000).

Ammonia emissions are a major air quality concern at regional, national, and global levels (NRC, 2002). Therefore, a constructed wetland system at Hastings Pork should be designed to remove ammonia by denitrification as opposed to volatilization. Constructed wetland design features that address this issue include nitrification of wastewater prior to wetland application (Poach et al., 2003), having continuous vegetation as opposed to open areas (Poach et al., 2004), and application of Reciprocating Wastewater Treatment Technology (Rice et al., 2005).

Habitat Wetland Project Plan. Steps needed to establish waterfowl habitat with constructed wetland treated wastewater from Hastings Pork include: 1) secure project funding, 2) design and develop a constructed wetland based treatment system, and 3) perform monitoring.

Funding requests could be submitted through a RWBJV partnership. Potential funding sources include the Nebraska Environmental Trust, USEPA, U.S. Department of

Agriculture (USDA), and the Service. The Nebraska Environmental Trust is a state program dedicated to the preservation of Nebraska's natural resources and has previously funded wetland development projects, including the project that resulted in the created wetlands at Hastings Pork. Section 319 grants from USEPA support a wide variety of activities including technical assistance, financial assistance, demonstration projects, and monitoring to assess the success of specific nonpoint source pollution reduction. USDA has previously funded constructed wetland demonstration projects for treatment of agricultural wastewater. Programs for restoring wetlands for wildlife include the Service's Partners for Fish and Wildlife Program and the Natural Resources Conservation Service's Wetland Reserve Program.

There are no known constructed wetland systems that have been designed for an operation the size of Hastings Pork, and it may not be feasible to design a system that could effectively treat all of the secondary effluent generated by Hastings Pork. However, a portion of Hastings Pork wastewater could be adequately treated through a constructed wetland system for the purpose of providing waterfowl habitat. The University of Nebraska has expertise in designing constructed wetlands for treating municipal and agricultural waste (Dr. John Stansbury, Associate Professor, Environmental/Water Resources Engineering, University of Nebraska, pers. comm., 2007) and could be instrumental in designing an effective system at Hastings Pork.

A monitoring program would be necessary to evaluate the effectiveness of treatment and the quality of wetland habitat produced. Such a plan would need to include chemical, physical, and biological measurements and account for seasonal differences in treatment efficiency. Examples of physical and chemical measurements to evaluate constructed wetlands include hydraulic residence time necessary to remove nutrients, suspended solids, and biochemical oxygen demand. Biological measurements could include invertebrate and plant community assessments and toxicity tests to evaluate constructed wetland effluent. Toxicity tests with *Ceriodaphnia dubia* have been used to show significant toxicity abatement of swine wastewater as it progressed through a constructed wetland system (Belin et al., 2000).

Conclusions

This study evaluated the exposure and effects of contaminants associated with swine wastewater to sentinel game farm mallards. Sentinel mallards were kept in outdoor enclosures at two wetlands created with swine secondary wastewater and two reference wetlands managed by the Service.

Water quality in the created wetland enclosures had higher specific conductivity, BOD, turbidity, pH, and nutrients than reference wetlands. Algal blooms frequently occurred in the created wetlands and included *Microcystis* spp. Although results from this study indicate that microcystin exposure to sentinel mallards on the created wetlands was low, presence of *Microcystis* spp. in created wetlands warrant concern given that environmental conditions can dictate microcystin production and microcystins can cause waterfowl die-offs (NGPC, 1992; Matsunaga et al., 1999).

Water quality in the created wetlands are conducive to avian botulism outbreaks, and the remaining sentinel mallard population on the created wetlands died of avian botulism in August of 2003.

Although wild brood production has been observed at the created wetlands, sentinel mallard reproduction was more successful on the reference wetlands than created wetlands. Number of total nests initiated and number of total eggs laid were similar between reference and created wetland enclosures. However, cumulative stress from reproduction and poor water quality may have caused hen mortality at CW4, resulting in a greater survival to hatch in the reference wetlands compared to created wetlands.

There were no significant differences in mallard blood plasma chemistry and histology biomarkers between reference and created wetland enclosures. Mallards kept in outdoor enclosures tended to lose more weight than zero-controls and biomarker results (i.e., histology and blood chemistry) indicate that they were more stressed than zero-controls.

All sentinel mallards tested negative for duck plague and avian influenza. Bacterial pathogens isolated from sentinel mallards at all sites included *Enterococcus* spp and *E. coli. Salmonella bareilly* was isolated only from mallards kept on created

wetlands; however, prevalence of Salmonella spp. in captive reared and wild duck is well known and its affects on duck populations appears to be minimal

Concentrations of *E. coli* and streptococci in water from enclosure sites were highly variable for all sites. There were no significant differences in waterborne *E. coli* concentrations between reference and created wetland enclosures. Streptococci concentrations were significantly greater in McMurtrey and CW6 than Harvard and CW4. Elevated concentrations of *E. coli* and streptococci at the Harvard and McMurtrey enclosures may have resulted from cattle grazing in the upland areas near the enclosures, wild waterfowl, or the sentinel mallards themselves.

Metals previously determined to be associated with swine waste (barium, boron, iron, molybdenum, selenium, strontium, and zinc) were detected more frequently or at greater concentrations in eggs and/or liver samples from the created wetlands than reference wetlands. Sentinel mallards from reference and created wetland enclosures had selenium concentrations in liver that exceeded 10 mg/kg, a threshold that may be associated with reproductive impairment in laying females.

Hastings Pork ceased wastewater delivery to the created wetlands in October of 2004 as a result of an inspection by NDEQ. It is recommended that Hastings Pork, NGPC, Ducks Unlimited, and the Service form a partnership coordinated by RWBJV to design and implement a strategy to further treat secondary effluent from Hastings Pork before using it to create waterfowl habitat. Constructed wetlands designed to treat secondary swine wastewater are effective in removing nutrients, bacteria, and hormones. Development and monitoring of a constructed wetland system at Hastings Pork is the desired approach for creating migratory bird habitat from swine CAFO wastewater.

REFERENCES

Analytical Control Facility (ACF). 2005. Quality control. U.S. Fish and Wildlife Service Division of Environmental Quality, Shepherdstown, West Virginia. http://chemistry.fws.gov/

Belin JI, McCaskey TA, Black MC. 2000. Evaluating the efficiency of toxicity abatement in a constructed wetland with *Ceriodaphnia dubia*. *Journal of Toxicology and Environmental Health Part A* 60(2):137-151.

Borgmann U. 1994. Chronic toxicity of ammonia to the amphipod *Hyalella azteca*: importance of ammonium ion and water hardness. *Environmental Pollution* 86:329-335.

Carmichael WW. 1997. The cyanotoxins. *Advances in Botanical Research* 27:211-240.

Centers for Disease Control and Prevention (CDC). 2006. The influenza (flu) viruses. http://www.cdc.gov/flu/about/fluviruses.htm

Clesceri LS, Greenberg EA, Eaton AD. 1998. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C.

Chambers TM, Virginia S, Hinshaw Y, Kawaoka B, Easterday C, Webster RG. 1991. Influenza viral infection of swine in the United States 1988–1989. *Journal Archives of Virology* 116(1-4):261-265.

Choi YK, Lee JH, Erickson G, Goyal SM, Joo HS, Webster RG. 2004. H3N2 influenza virus transmission from swine to turkeys, United States. *Emerging Infectious Diseases* 10(12):2156-2160 http://www.cdc.gov/ncidod/EID/vol10no12/04-0581.htm

Coffey TJ, Pullinger GD, Urwin R, Jolley KA, Wilson SM, Maiden MC, Leigh JA. 2006. First insights into the evolution of *Streptococcus uberis*: a multilocus sequence typing scheme that enables investigation of its population biology. *Applied and Environmental Microbiology* 72(2):1420-1428.

Converse KA, Kidd GA. 2001. Duck plague epizootics in the United States, 1967-1995. *Journal of Wildlife Diseases* 37(2):347–357.

Crowder AA, Bristow JM. 1988. The future of waterfowl habitats in the Canadian lower Great Lakes wetlands. *Journal of Great Lakes Research* 14(1):115-127.

Echols KR. 2001. Microcystin algal toxins in algal bloom samples from Nebraska wetlands. U.S. Geological Survey Columbia Environmental Research Center Final Report, Columbia, Missouri. 8 pp.

Echols KR, Feltz K. 2004. Algal toxins in water, algae, livers and gizzards from mallard ducks on Nebraska wetlands. U.S. Geological Survey Columbia Environmental Research Center Final Report # CERC-8335-FY04-31-01, Columbia, Missouri. 10 pp.

Echols KR, Feltz K. 2006. Algal toxins in water and gizzard contents from mallard ducks on Nebraska wetlands. U.S. Geological Survey Columbia Environmental Research Center Final Report # CERC-8335-FY06-31-02, Columbia, Missouri. 8 pp.

Eisler R. 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service Biological Report 85 (1.14), Contaminant Hazard Reviews Report 14. 134 pp.

Eynard F, Mez K, Walther JL. 2000. Risk of cyanobacterial toxins in Riga waters (Latvia). *Water Research* 34:2979-2988.

Facklam R. 2002. What happened to the Streptococci: overview of taxonomic and nomenclature changes. *Clinical Microbiology Reviews* 15(4):613-630.

Fraser H. 1991. Manure characteristics fact sheet. Ontario Ministry of Agriculture, Food, and Rural Affairs, Ontario, Canada. Agdex # 538.

Furness RW. 1996. Cadmium in birds. In Beyer WN, Heinz GH, and Redmon-Norwood AW, eds, Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. CRC Press, Lewis Publishers, Boca Raton, Florida. pp 389-405.

Fairbrother A, Fowles J. 1990. Subchronic effects of sodium selenite and selenomethionine on several immune functions in mallards. *Archives of Environmental Contamination and Toxicology*. 19(6):836-844.

Gabig PJ. 2000. The Rainwater Basin Joint Venture Evaluation Plan. Rainwater Basin Joint Venture, Grand Island, NE. 55 pp.

Gersib RA, Dinan KF, Kauffeld JD, Onnen MD, Gabig PJ, Cornely JE, Jasmer GE, Hyland JM, Strom KJ. 1992. Rainwater Basin Joint Venture Implementation Plan. Nebraska Game and Parks Commission, Lincoln, NE. 56 pp.

Goulet RR, Leclair EN, Pick FR. 2001. The evaluation of metal retention by a constructed wetland using the pulmonate gastropod *Helisoma trivolvis* (Say). *Archives of Environmental Contamination and Toxicology* 40(3):303-310.

Hanson BA, Stallknecht DE, Swayne DE, Lewis LA, Senne DA. 2003. Avian influenza viruses in Minnesota ducks during 1998–2000. *Avian Diseases* 47(3):867-71.

Hamilton, SJ. 2004. Review of selenium toxicity in the aquatic food chain. *Science of the Total Environment* 326(1-3):1-31.

He L, Liu H, Xi B, Zhu Y. 2006. Enhancing treatment efficiency of swine wastewater by effluent recirculation in vertical-flow constructed wetland. *Journal of Environmental Science* (China) 18(2):221-6.

Heinz GH. 1996. Selenium in birds. In Beyer WN, Heinz GH, Redmon AW, eds, Interpreting Environmental Contaminants in Animal Tissues. Lewis Publishers, Boca Raton, Florida. pp 453-464.

Henry RR. 2000. *Salmonella* infection in ducks. In Wray C, ed, *Salmonella* in Domestic Animals. Bacteriology Department, Veterinary Laboratory Agency, Addlestone, Surrey, UK. pp 157 – 158.

Hill VR, Sobsey MD. 2001. Removal of *Salmonella* and microbial indicators in constructed wetlands treating swine wastewater. *Water Science & Technology* 44(11-12):215-222.

Hunt PG, Poach ME, Matheny TA, Reddy GB, Stone KC. 2006. Denitrification in marsh-pond-marsh constructed wetlands treating swine wastewater at different loading rates. *Soil Science Society of American Journal* 70(2):487-93.

Hunt PG, Matheny TA, Szögi AA. 2003. Denitrification in constructed wetlands used for treatment of swine wastewater. *Journal of Environmental Quality* 32(2):727-35.

Hunt PG, Szogi AA, Humenik FJ, Rice JM, Matheny TA, Stone KC. 2002. Constructed wetlands for treatment of swine wastewater from an anaerobic lagoon. *Transactions of the American Society of Agricultural Engineers* 45(3):639-47.

International Species Information System (ISIS). 1996. Physiological data reference values, American Units. Web-based global database. http://www.isis.org/CMSHOME/

Jofre M, Karasov WH. 1999. Direct effect of ammonia on three species of north American anuran amphibians. *Environmental Toxicology and Chemistry* 18(8):1806-1812.

Krapu GL. 1981. The role of nutrient reserves in Mallard reproduction. *Auk* 98:29-38.

Lee C, Lee C, Lee F, Tseng S, Liao C. 2004. Performance of subsurface flow constructed wetland taking pretreated swine effluent under heavy loads. *Bioresource Technology* 92(2):173-9.

Leigh J. 2003. Exploiting the genome in the control of *Streptococcus uberis*. Proceedings of the British Mastitis Conference, Garstang, UK. pp 15-22. http://www.iah.bbsrc.ac.uk/BMC/2003/Papers%20in%20word%20&%20pdf/Word%20papers%202003/Programme%202003.htm

Matsunaga H, Harada KI, Senma M, Ito Y, Yasuda N, Ushida S, Kimura Y. 1999. Possible cause of unnatural mass death of wild birds in a pond in Nishinomiya, Japan: sudden appearance of toxic cyanobacteria. *Natural Toxins* 7(2):81-84.

McMurtrey MD, Craig R, Schildman G. 1972. Nebraska wetland survey. Habitat Work Plan K-71, Nebraska Game and Parks Commission. 78pp.

MedlinePlus. 2007. A service of the U.S. National Library of Medicine and the National Institutes of Health. Medical Encyclopedia topic for ALT. http://medlineplus.gov/

Mendis NM, De La Motte PU, Gunatillaka PD, Nagaratnam W. 1976. Protracted infection with *Salmonella bareilly* in a maternity hospital. *Journal of Tropical Medicine and Hygiene* 79(7):142-150.

Miller DL, Hatkin J, Radi ZA, Mauel MJ. 2004. An *Escherichia Coli* epizootic in captive mallards (*Anas platyrhynchos*). *International Journal of Poultry Science* 3(3):206-210.

Nebraska Department of Environmental Quality (NDEQ). 2006. Title 117 Nebraska Water Quality Standards. NDEQ, Lincoln, Nebraska. http://www.deq.state.ne.us/

Nebraska Department of Environmental Quality (NDEQ). 2007. Precautions and facts regarding toxic algae at Nebraska Lakes. NDEQ, Lincoln, Nebraska. http://www.deq.state.ne.us/

Nebraska Game and Parks Commission (NGPC). 1992. Wildlife disease and mortality investigations. Pittman-Robertson Project W-15-R-49. NGPC, Lincoln, Nebraska. 19 pp.

Nebraska Game and Parks Commission (NGPC). 1999. Waterfowl food values (range of 1-10, with 10 being the highest) for Nebraska Rainwater Basin Wetland Plant List. Unpublished data.

National Research Council (NRC). 2003. Air emissions from animal feeding operations: current knowledge, future needs. Ad Hoc Committee on Air Emissions from Animal Feeding Operations, Committee on Animal Nutrition. The National Academies Press, Washington, D.C. http://www.nap.edu/catalog.php?record_id=10586

Pain DJ. 1996. Lead in waterfowl. In Beyer NW, Heinz GH, Redmon-Norwood AW, eds, Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations, pp 251-264.

Pensaert M, Ottis, K, Vandeputte J, Kaplan MM, Bachmann PA. 1981. Evidence for the natural transmission of influenza A virus from wild ducks to swine and its potential importance for man. *Bulletin World Health Organization* 59(1):75–78.

Phillips BJ, Moore PA, Jr, Daniel TC, Wolf DC, Edwards DR. 2000. Using constructed wetlands for remediation of wastewater originating on swine farms, geological society of America, south-central section, 34th annual meeting. *Geological Society of America* 32(3):38.

Poach ME, Hunt PG, Vanotti MB, Stone KC, Matheny TA, Johnson MH, Sadler EJ. 2003. Improved nitrogen treatment by constructed wetlands receiving partially nitrified liquid swine manure. *Ecological Engineering* 20(2):183-97.

Poach ME, Hunt PG, Reddy GB, Stone KC, Matheny TA, Johnson MH, Sadler EJ. 2004. Ammonia volatilization from marsh-pond-marsh constructed wetlands treating swine wastewater. *Journal of Environmental Quality* 33(3):844-851.

Pocurull DW, Gaines SA, Mercer HD. 1971. Survey of infectious multiple drug resistance among salmonella isolated from animals in the United States. *Applied Microbiology 21(2):358–362.*

Rice M, Behrends L, Privette A, Hodges R, Humenik F. 2005. Reciprocating water treatment technology: An environmentally superior technology candidate. Havenstein GB, ed, North Carolina State University College of Agriculture and Life Sciences, Raleigh, North Carolina. http://www.cals.ncsu.edu/waste_mgt/05wastesymposium/PDFS/Rice(recip).pdf

Rocke TE, Samuel MD. 1999. Water and sediment characteristics associated with avian botulism outbreaks in wetlands. *Journal of Wildlife Management* 63(4):1249-1260.

Sandhu TS. 1988. Fecal streptococcal infection of commercial White Penkin ducklings. *Avian Diseases* 32(3):570-573.

SAS Institute. 2002. JMP user's guide (Version 5). SAS Inst., Cary, NC.

Schildman G, Hurt J. 1984. Update of Rainwater Basin wetland survey. Survey of habitat work plan K-83-W-15-R-40. Nebraska Game and Parks Commission, Lincoln, Nebraska. 13pp.

Schwarz MS, Echols KR, Wolcott MJ, Nelson KJ. 2004. Environmental contaminants associated with swine concentrated animal feeding operation and implications for McMurtrey National Wildlife Refuge. U.S. Fish and Wildlife Service Contaminant Report, Grand Island, Nebraska.

Sezerino P, Reginatto V, Santos M, Kayser K, Kunst S, Philippi L, Soares H. 2003. Nutrient removal from piggery effluent using vertical flow constructed wetlands in southern Brazil. In Shilton AN, Craggs RJ, Walmsley N, eds, Alliance House 12 Caxton Street, London SW1H 0QS, UK: IWA Publishing. 129 p.

Shappell NW, Billey LO, Forbes D, Matheny TA, Poach ME, Reddy GP, Hunt PG. 2007. Estrogenic activity and steroid hormones in swine wastewater through a lagoon constructed-wetland system. *Environmental Science and Technology* 41(2):444 – 450.

Sivonen, K. 1996. Cyanobacterial toxins and toxin production. *Phycologia* 36(6):12-24.

Stallknecht DE, Shane SM. 1988. Host range of avian influenza virus in free-living birds. *Veterinary Research Communications* 12(2-3):125-141.

Stone KC, Hunt PG, Szogi AA, Humenik FJ, Rice JM. 2002. Constructed wetland design and performance for swine lagoon wastewater treatment. *Transactions of the American Society of Agricultural Engineers* 45(3):723-30.

Szogi AA, Hunt PG, Sadler EJ, Evans DE. 2004. Characterization of oxidation-reduction processes in constructed wetlands for swine wastewater treatment. *Applied Engineering in Agriculture* 20(2):189-200.

Thomason BM, Dodd DJ, Cherry WB. 1977. Increased recovery of salmonellae from environmental samples enriched with buffered peptone water. *Applied and Environmental Microbiology* 34(3):270-273.

U.S. Geological Survey (USGS). 1999. Field manual of wildlife diseases: general field procedures and diseases in birds. Friend M, Franson JC, eds, Information and Technology Report 1999-001, Madison, Wisconsin. 426 pp. http://www.nwhc.usgs.gov/pub_metadata/field_manual/field_manual.html.

U.S. Department of the Interior (USDOI). 1998. Guidelines for interpretation of the biological effects of selected constituents in biota, water and sediment. U.S. Department of Interior, National Irrigation Water Quality Program Information Report No. 3, Denver, CO, 198 pp. http://www.usbr.gov/niwqp

Wang W. 1991. Ammonia toxicity to macrophytes (common duckweed and rice) using static and renewal methods. *Environmental Toxicology and Chemistry* 10(9): 1173-1177.

World Health Organization (WHO). 1998. Guidelines for drinking-water quality: Second edition, addendum to volume 2, Health Criteria and Other Supporting Information. World Health Organization, Geneva.

Wobeser, G.A. 1981. Diseases of wild waterfowl. Plenum Press, New York. 300 pp.

Wood SL, Wheeler EF, Berghage RD. 2000. Removal of dimethyl disulfide and p-cresol from swine facility wastewater using constructed subsurface-flow wetlands. *Transactions of the American Society of Agricultural Engineers* 43(4):973-9.

APPENDIX A: ADDITIONAL FIGURES AND TABLES

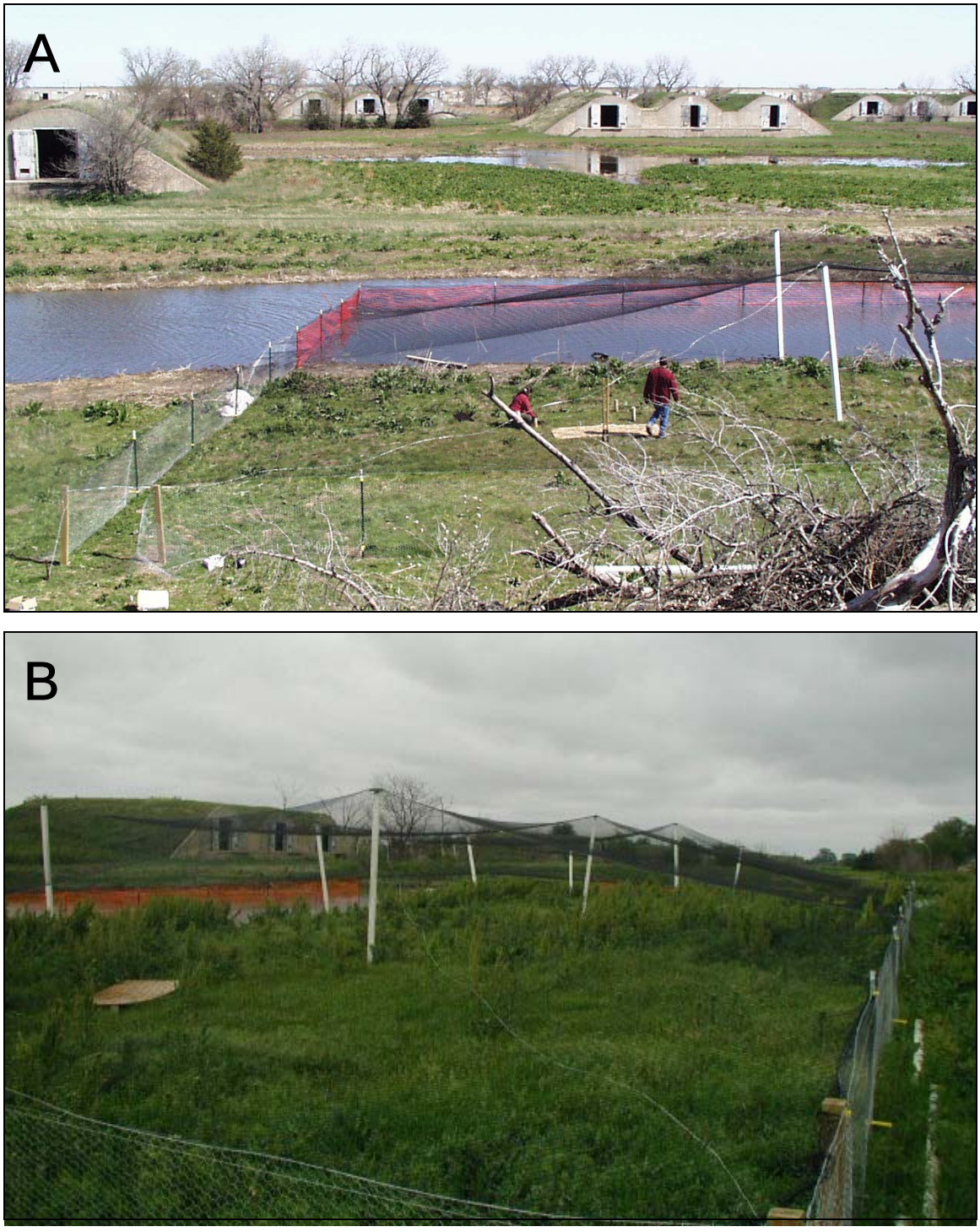

Figure A.1. Pictures of the mallard enclosure at Created Wetland 4, Hastings Pork, Clay County, Nebraska. Note: Picture A was taken facing north from on top of another bunker and picture B was taken later in the spring facing northeast.

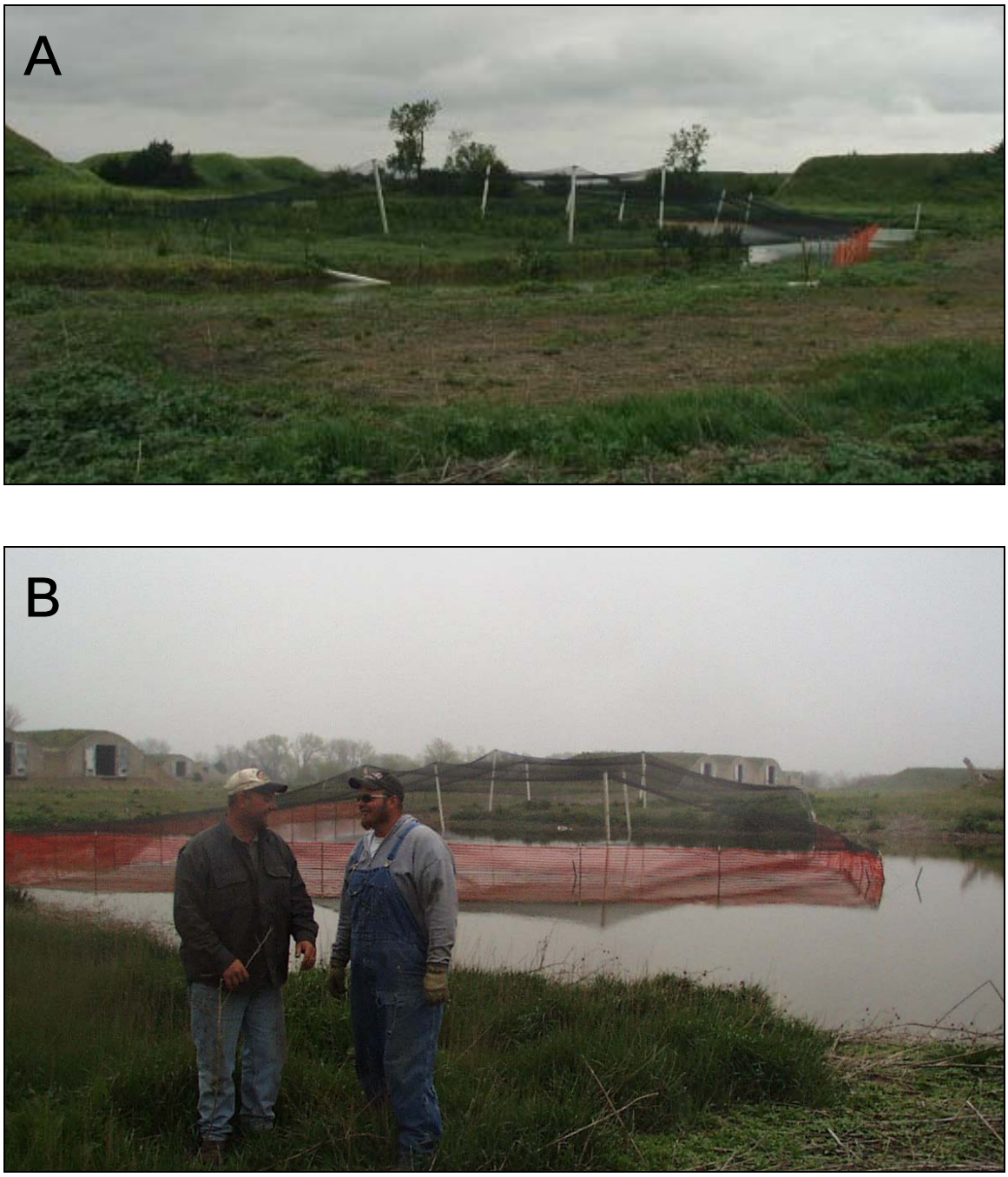

Figure A.2. Pictures of the mallard enclosure at Created Wetland 6, Hastings Pork, Clay County, Nebraska. Note: Picture A was taken facing southwest and picture B was taken facing east.

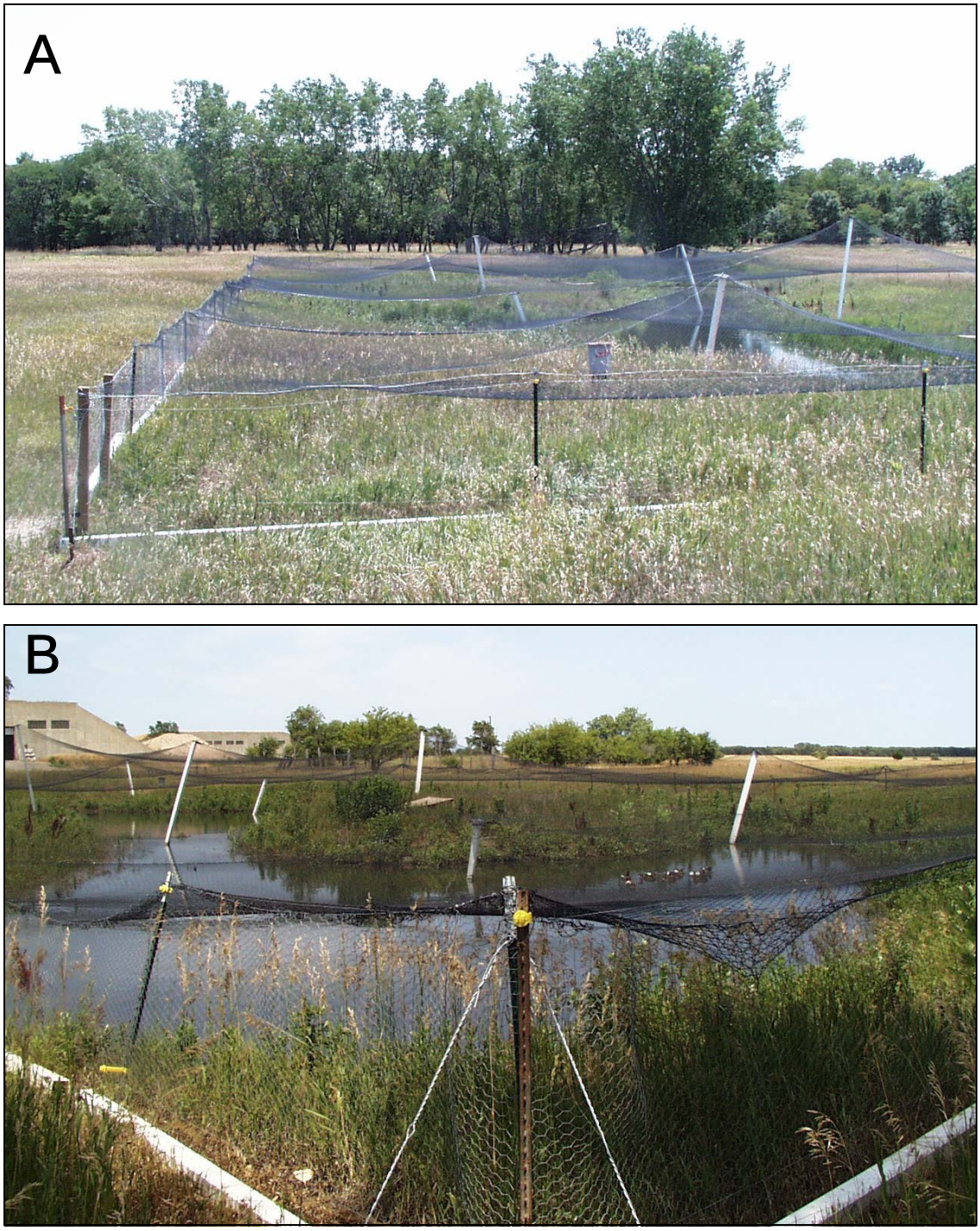

Figure A.3. Pictures of mallard enclosure at McMurtrey National Wildlife Refuge, Clay County, Nebraska. Note: Picture A is from the northeast corner of enclosure and picture B is from the southeast corner.

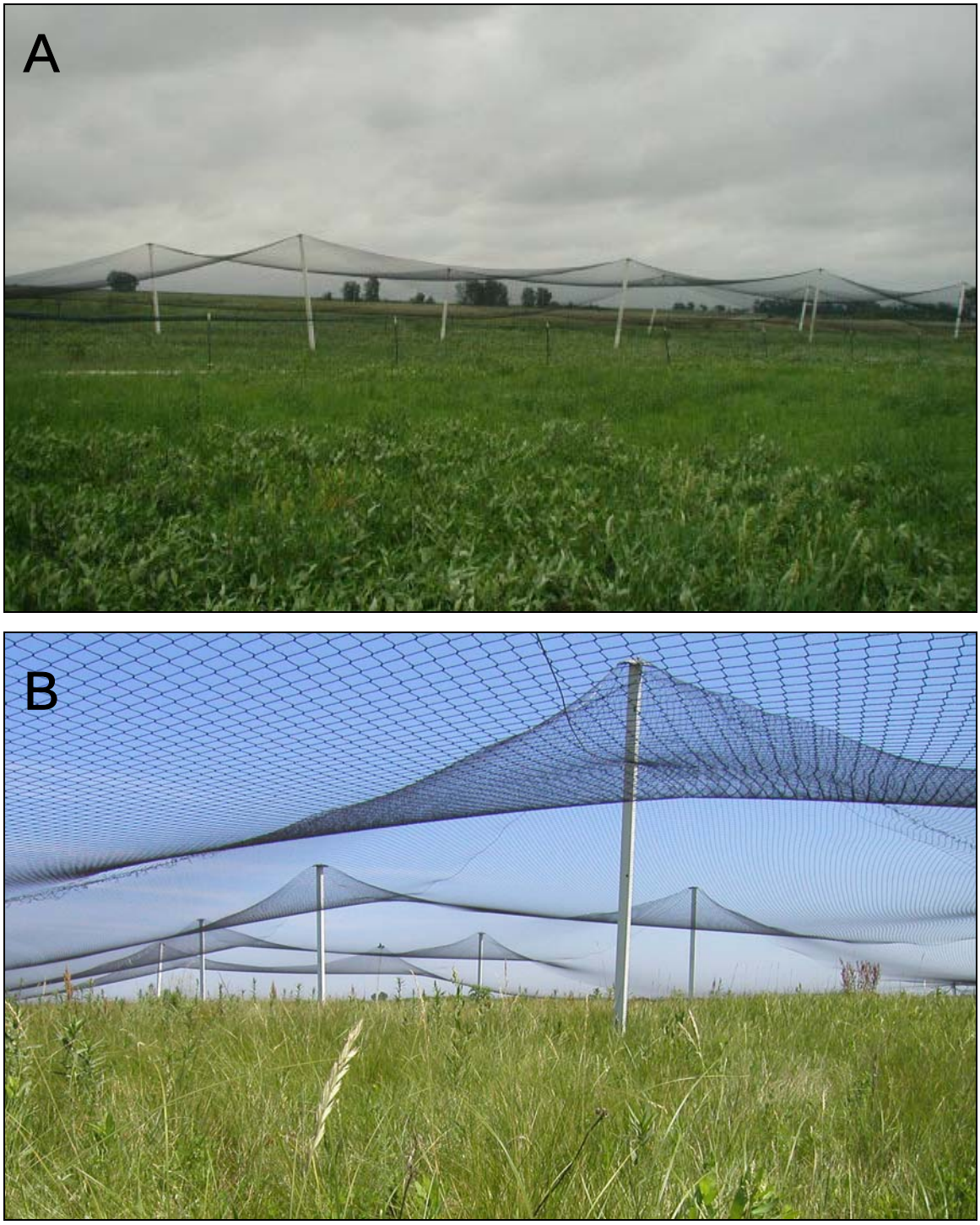

Figure A.4. Pictures of the mallard enclosure at Harvard Waterfowl Production Area, Clay County, Nebraska. Note picture A was taken facing west and picture B was taken from inside the enclosure facing northeast.

Table A.1. Culture media and incubation conditions for bacterial pathogen screen of sentinel mallard intestine contents, Clay County, Nebraska, 2002 and 2003.

Bacteria	Media	Incubation	
		Temperature	Time
Escherichia coli	E. coli Enrichment Broth, CPS agar, and MacConkey's with Sorbitol.	35 - 37 ° C	46 - 48 hrs
Erysipelothrix spp.	Packer's and Brain Heart Infusion Agar.	35 - 37 ° C	18 - 24, 48 hrs
P. multocida	Pasteurella Multocida Selective Broth and Blood Agar Plate.	35 - 37 ° C	18 - 24 hrs
Salmonella spp.	Rappaport-Vassiliadis and Dulcitol-selinite.	41.5 ° C	18 - 24 hrs
	Xylose Lysine Tergitol 4 and Brilliant Green Agar.	35 - 37 ° C	18 - 24 hrs
Yersinia spp	Bile Oxalate Sorbose.	21 - 25 ° C	48 hrs and 5 days
	Cefsulodin Irgasan Novobiocin Agar.	32 - 35 ° C	18 - 24 hrs
Enterococci	CPS agar	35 - 37 ° C	22 - 24 hrs

Table A.2. Sentinel mallard samples analyzed for elemental contaminants through the U.S. Fish and Wildlife Service's Analytical Control Facility.

Analysis	Sample ID	Sample Matrix	Sample Mass (grams)	Percent moisture
Catalog 6050095	002-L-Tm	Liver	9.5	74.2
Elemental Contaminants	004-L-Tm	Liver	8.2	72.3
Mallard Liver and Stomach	008-L-Tm	Liver	6.3	68.4
Contents	013-L-Tm	Liver	6.1	67.5
	017-L-Tm	Liver	6.4	69.5
	018-L-Tm	Liver	8.1	69.3
	020-L-Tm	Liver	6.6	69.1
	021-L-Tm	Liver	6.6	71.4
	056-L-Tm	Liver	11.7	66.8
	062-L-Tm	Liver	27.9	68.2
	063-L-Tm	Liver	7.6	69.0
	064-L-Tm	Liver	8.9	74.1
	067-L-Tm	Liver	18.8	62.5
	069-L-Tm	Liver	5.4	69.8
	070-L-Tm	Liver	7.8	68.4
	071-L-Tm	Liver	6.7	68.9
	077-L-Tm	Liver	5.2	70.4
	080-L-Tm	Liver	6.8	71.0
	081-L-Tm	Liver	8.3	69.2
	086-L-Tm	Liver	7.4	69.4
	089-L-Tm	Liver	8.8	68.9
	090-L-Tm	Liver	5.6	69.1
	091-L-Tm	Liver	6.1	67.7
	098-L-Tm	Liver	6.8	71.9
	188-L-Tm	Liver	8.8	66.5
	194-L-Tm	Liver	10.7	70.2
	198-L-Tm	Liver	10.6	68.2
	200-L-Tm	Liver	13.9	51.5
	201-L-Tm	Liver	6.0	65.5
	203-L-Tm	Liver	13.3	66.1
	004-Sc-R	Stomach Contents	5.4	18.8
	017-Sc-R	Stomach Contents	3.7	27.7
	063-Sc-R	Stomach Contents	8.8	13.4
	086-Sc-R	Stomach Contents	4.1	43.0
	089-Sc-R	Stomach Contents	4.4	34.7
	013-Sc-R	Stomach Contents	3.9	20.5
Catalog 6050112	4C24	Avian Egg	27.2	72.5
Elemental Contaminants	4C47	Avian Egg	35.9	71.5
Mallard Liver and Egg	4C48	Avian Egg	30.9	72.5
	4C68	Avian Egg	35.3	70.1
	4C78	Avian Egg	31.1	71.7
	4X52	Avian Egg	27.7	70.2
	4X53	Avian Egg	29.3	70.3
	4X54	Avian Egg	29.9	70.8
	4X55	Avian Egg	33.4	70.4
	4X56	Avian Egg	25.0	66.3
	6A33	Avian Egg	31.8	70.9
	6A34	Avian Egg	42.5	67.7
	6A43	Avian Egg	34.7	69.7
	6A79	Avian Egg	44.9	68.2
	6A80	Avian Egg	42.2	69.7
	6B10	Avian Egg	21.2	69.7
	6B12	Avian Egg	31.7	67.7
	6B13	Avian Egg	30.7	67.0
	6B15	Avian Egg	24.0	65.0
	6B4	Avian Egg	31.2	62.5
	HC18	Avian Egg	24.5	64.6

Table A.2. Continued.

Analysis	Sample ID	Sample Matrix	Sample Mass (grams)	Percent moisture
Catalog 6050112	HC30	Avian Egg	19.7	66.6
Elemental Contaminants	HC61	Avian Egg	28.3	62.5
Mallard Liver and Egg	HC63	Avian Egg	16.3	71.4
	HC71	Avian Egg	24.6	64.0
	HD22	Avian Egg	19.2	65.8
	HD23	Avian Egg	22.9	65.0
	HD31	Avian Egg	24.7	61.7
	HD70	Avian Egg	33.3	68.9
	HD77	Avian Egg	24.2	68.1
	103-L-Tm	Liver	9.9	68.6
	105-L-Tm	Liver	3.9	68.4
	108-L-Tm	Liver	10.3	70.9
	110-L-Tm	Liver	6.3	70.4
	111-L-Tm	Liver	5.5	74.4
	112-L-Tm	Liver	8.7	73.0
	113-L-Tm	Liver	8.1	71.5
	117-L-Tm	Liver	1.2	61.6
	118-L-Tm	Liver	12.0	68.5
	119-L-Tm	Liver	6.8	71.2
	123-L-Tm	Liver	6.9	68.3
	126-L-Tm	Liver	11.3	67.2
	128-L-Tm	Liver	6.1	69.7
	129-L-Tm	Liver	4.8	68.9
	130-L-Tm	Liver	9.2	67.4
	132-L-Tm	Liver	6.7	73.0
	134-L-Tm	Liver	8.8	73.2
	135-L-Tm	Liver	6.0	72.8
	137-L-Tm	Liver	7.7	70.2
	138-L-Tm	Liver	6.2	66.1
	140-L-Tm	Liver	5.9	65.6
	141-L-Tm	Liver	5.7	69.8
	142-L-Tm	Liver	7.6	68.6
	144-L-Tm	Liver	5.1	69.5
	145-L-Tm	Liver	8.9	68.6
	146-L-Tm	Liver	11.3	65.0

Table A.3. Blood plasma chemistry results for day zero-control mallards and mallards kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002.

				Units																	
				U/L						mg/dL					mmol/L					g/dL	
Date	Site	Mallard ID	Gender	AST	ALT	GGT	AP	CK	LDH	Glucose	Choles.	UA	P	Ca	Na	K	Cl	HCO_3	AG	TP	
020717	Harvard	2	m	7	45	10	90	146	244	242	220	3	4	10	146	5	110	19	22	4	
020716	CW6	4	m	12	52	7	90	237	359	236	126	6	2	10	152	3	112	22	21	4	
020715	CW4	8	m	13	74	3	129	214	268	267	136	10	2	10	148	2	112	22	16	4	
020716	CW6	13	f	24	67	5	100	302	502	243	161	3	2	10	150	4	114	22	18	4	
020716	CW6	17	m	14	69	4	111	370	452	243	155	4	3	9	153	5	117	20	21	4	
020813	McMurtrey	18	m	14	43	1	64	458	364	221	214	3	2	10	152	6	115	19	24	4	
020715	CW4	20	m	19	77	10	113	792	530	210	141	3	4	10	158	4	121	20	21	4	
020715	CW4	21	f	22	59	8	181	797	125	200	119	8	3	9	148	3	112	17	22	4	
020813	McMurtrey	56	f	17	55	20	90	268	311	294	218	4	2	10	150	3	112	21	20	4	
020813	McMurtrey	62	m	14	45	9	208	360	350	311	231	4	2	11	146	3	108	24	17	5	
020715	CW4	63	m	12	80	3	80	403	393	224	140	8	2	9	154	4	116	23	19	4	
020715	CW4	64	f	22	72	7	108	1141	167	194	134	6	4	9	153	2	109	21	25	4	
020813	McMurtrey	67	f	93	77	12	195	395	595	283	75	4	11	34	145	4	109	16	24	6	
020717	Harvard	69	f	<5	38	14	70	292	233	279	212	3	2	10	145	3	111	19	18	4	
020715	CW4	70	m	64	75	3	106	3645	268	237	160	5	3	10	150	3	115	19	19	4	
020717	Harvard	71	f	7	31	17	80	251	270	272	208	4	3	10	147	4	109	22	20	4	
020715	CW4	77	m	12	27	<3	132	160	68	154	86	6	4	8	135	4	100	18	21	3	
020715	CW4	80	f	20	64	2	104	668	225	255	119	7	1	9	148	4	111	24	17	3	
020716	CW6	81	m	38	100	10	78	1182	730	231	139	6	3	10	150	4	114	18	22	4	
020716	CW6	86	m	11	79	12	57	366	307	229	185	7	2	11	151	3	112	18	24	4	
020716	CW6	89	m	11	74	9	131	241	224	218	163	4	2	10	151	4	116	18	21	4	
020716	CW6	90	f	52	82	6	339	3185	446	258	104	3	3	9	149	4	109	25	19	4	
020716	CW6	91	f	12	55	4	167	299	389	346	112	8	1	10	147	3	114	17	19	4	
020717	Harvard	98	m	7	32	4	104	202	196	237	206	3	3	10	149	4	113	20	20	3	
020520	Control	188	m	51	30	4	62	1876	349	252	185	5	2	10	142	4	105	23	18	4	
020520	Control	194	m	17	25	10	124	491	325	213	166	5	4	11	148	3	109	19	23	4	
020520	Control	198	m	23	24	15	124	937	230	279	194	5	6	11	147	3	105	16	29	4	
020520	Control	200	f	13	38	86	116	199	237	184	92	8	9	30	145	3	104	18	26	6	
020520	Control	201	f	12	38	7	96	300	245	259	215	9	7	12	154	3	110	17	30	4	
020520	Control	203	f	23	58	52	402	366	310	208	62	8	10	31	131	3	100	17	17	6	

Note: CW = Hastings Pork created wetland, AST = aspartate amino-transferase, ALT = alanine aminotransferase, GGT = gamma glutamyl transpeptidase, AP = alkaline phosphatase, CK = creatine kinase, LDH = lactate dehydrogenase, Choles. = cholesterol, UA = uric acid, P = phosphorus, Ca = calcium, Na = sodium, K = potassium, Cl = chloride, HCO_3 = bicarbonate AG = anion gap, and TP = total protein.

Table A.4. Mallard blood plasma chemistry for day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2003.

				Units																
				U/L						mg/dL					mmol/L					g/dL
Date	Site	Mallard ID	Gender	AST	ALT	GGT	AP	CK	LDH	Glucose	Choles.	UA	P	Ca	Na	K	Cl	HCO_3	AG	TP
030415	Control	103	f	13	29	<3	71	399	257	251	209	10	4	10	147	1	106	27	15	4
030804	Harvard	108	f	13	35	<3	108	209	208	327	260	3	2	11	152	2	114	25	15	5
030415	Control	110	m	18	19	<3	62	323	285	228	198	6	3	10	150	3	107	25	21	3
030616	Harvard	111	m	20	55	<3	48	212	251	188	187	11	4	9	151	4	113	24	18	3
030805	CW4	112	f	36	37	<3	76	427	629	242	183	4	2	10	149	2	111	21	19	4
030805	CW4	113	f	21	27	<3	174	563	298	299	120	5	2	10	145	3	107	23	18	4
030618	CW4	117	m	21	51	<3	138	396	285	242	276	4	3	12	156	2	113	23	22	5
030618	CW4	118	m	129	98	<3	111	2126	876	657	135	6	3	8	142	3	109	25	11	3
030618	CW4	119	m	56	62	<3	63	764	428	313	289	10	2	10	149	2	110	28	13	4
030415	Control	123	f	54	21	<3	76	855	902	200	190	2	3	11	145	2	106	27	14	4
030415	Control	126	m	62	57	<3	109	339	625	236	236	3	5	11	146	2	103	26	19	4
030805	CW6	128	f	19	57	<3	95	489	297	274	118	7	3	11	151	2	111	22	20	5
030805	CW6	129	f	13	31	<3	77	798	253	254	151	8	3	11	149	3	113	21	18	4
030618	CW6	130	m	56	53	<3	100	1026	549	226	177	4	2	10	149	2	109	27	15	3
030804	McMurtrey	132	f	19	28	<3	73	228	255	323	176	5	1	10	149	2	111	26	14	4
030804	McMurtrey	134	f	23	19	<3	73	326	319	227	172	4	2	12	147	4	107	18	26	4
030616	McMurtrey	135	m	17	63	<3	55	310	256	199	248	5	3	10	152	2	112	23	19	3
030804	Harvard	137	f	20	58	<3	241	678	391	318	245	7	3	10	145	3	107	24	17	4
030415	Control	138	f	21	19	<3	87	324	395	254	205	4	2	11	150	2	112	24	16	4
030415	Control	140	m	21	22	<3	60	552	277	226	231	4	3	11	151	2	110	29	14	4
030616	Harvard	141	m	25	201	<3	54	665	351	186	183	20	4	10	150	3	110	23	20	4
030805	CW6	142	f	36	39	<3	122	988	576	269	153	4	1	11	149	2	111	24	16	4
030805	CW6	144	f	19	61	<3	165	584	305	242	150	8	3	11	151	2	114	21	18	5
020618	CW6	145	m	38	44	<3	51	895	356	321	201	5	2	10	148	2	109	27	14	4
030618	CW6	146	m	71	48	<3	120	861	568	248	161	6	3	10	148	2	109	26	15	4

Note: CW = Hastings Pork created wetland, AST = aspartate amino-transferase, ALT = alanine aminotransferase, GGT = gamma glutamyl transpeptidase, AP = alkaline phosphatase, CK = creatine kinase, LDH = lactate dehydrogenase, Choles. = cholesterol, UA = uric acid, P = phosphorus, Ca = calcium, Na = sodium, K = potassium, Cl = chloride, HCO_3 = bicarbonate, AG = anion gap, and TP = total protein.

Table A.5. Mallard spleen histology scoring for day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002 and 2003.

			Spleen						
Year	Mallard ID	Site	hemosiderosis	splenitis	lymphoid necrosis	lymphoid hyperplasia	follicular sclerosis	hemosiderophages	granulocytosis
2002	188	Control	-	2	4	-	-	-	-
2002	194	Control	-	2	-	-	-	-	-
2002	198	Control	4	4	-	-	-	-	-
2002	200	Control	-	5	-	-	-	-	-
2002	201	Control	2	2	-	-	-	-	-
2002	203	Control	5	2	-	-	-	-	-
2003	103	Control	-	-	-	-	-	-	-
2003	110	Control	-	-	-	-	-	-	-
2003	123	Control	-	-	-	-	-	-	-
2003	126	Control	-	-	-	-	-	-	-
2003	138	Control	-	-	-	-	-	-	-
2003	140	Control	-	-	-	-	-	-	5
2002	008	CW4	5	4	-	-	-	-	-
2002	020	CW4	2	-	-	-	-	-	-
2002	021	CW4	-	6	2	4	-	-	-
2002	063	CW4	7	-	-	-	-	-	-
2002	064	CW4	-	-	-	-	-	-	-
2002	070	CW4	7	-	-	-	-	-	-
2002	077	CW4	2	4	-	-	-	-	-
2002	080	CW4	-	-	6	-	-	-	-
2003	112	CW4	-	-	-	-	-	-	-
2003	113	CW4	-	-	-	-	-	-	-
2003	118	CW4	-	-	-	-	-	-	-
2003	119	CW4	-	-	-	-	-	-	-
2002	004	CW6	4	2	2	-	-	-	-
2002	013	CW6	7	7	-	-	-	-	-
2002	017	CW6	-	6	-	-	-	-	-
2002	081	CW6	-	4	-	-	-	-	-
2002	086	CW6	-	6	4	-	-	-	-
2002	089	CW6	-	6	-	-	-	-	-
2002	090	CW6	2	4	-	-	-	-	-
2002	091	CW6	2	-	2	2	-	-	-

Table A.5. Continued.

			Spleen						
Year	Mallard ID	Site	hemosiderosis	splenitis	lymphoid necrosis	lymphoid hyperplasia	follicular sclerosis	hemosiderophages	granulocytosis
2003	128	CW6	-	-	-	-	2	-	-
2003	129	CW6	-	-	-	-	-	2	-
2003	130	CW6	-	-	-	-	-	-	-
2003	142	CW6	-	-	-	-	-	-	-
2003	144	CW6	-	-	-	-	-	-	4
2003	145	CW6	-	-	-	-	-	-	-
2003	146	CW6	-	-	-	-	-	-	-
2002	002	Harvard	4	2	4	-	-	-	-
2002	069	Harvard	6	4	-	-	-	-	-
2002	071	Harvard	7	5	-	4	-	-	-
2002	098	Harvard	4	5	4	-	-	-	-
2003	108	Harvard	-	-	-	-	-	-	-
2003	111	Harvard	-	-	-	-	-	-	-
2003	137	Harvard	-	-	-	-	-	-	-
2003	141	Harvard	-	-	-	-	-	-	-
2002	018	McMurtrey	2	2	-	-	-	-	-
2002	056	McMurtrey	2	4	-	-	-	-	-
2002	062	McMurtrey	2	7	-	-	-	-	-
2002	067	McMurtrey	4	6	-	-	-	-	-
2003	105	McMurtrey	-	-	-	-	-	-	-
2003	132	McMurtrey	-	-	-	-	-	-	-
2003	134	McMurtrey	-	-	-	-	-	-	5
2003	135	McMurtrey	-	-	-	-	-	-	-

Note: CW = Hastings Pork created wetland. Presence and severity of lesions were qualitatively described by the pathologist and then scored by NEFO as follows: 0 = no lesions, 2 = minimal, 3 = minimal to mild, 4 = mild, 5 = mild to moderate, 6 = moderate, 7 = moderate to severe.

Table A.6. Mallard liver histology scoring for day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands , Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002 and 2003.

			Liver						
Year	Mallard ID	Site	hepatitis	hemosiderosis	hyperplasia	hematopoiesis	vacuolization	lipidosis	lymphocytes
2002	188	Control	4	-	4	-	-	-	-
2002	194	Control	2	-	-	-	-	-	-
2002	198	Control	-	6	-	-	-	-	-
2002	200	Control	-	-	-	-	-	9	-
2002	201	Control	4	-	4	-	-	6	-
2002	203	Control	2	2	-	-	-	6	-
2003	103	Control	4	-	-	-	-	-	-
2003	110	Control	-	2	-	-	4	-	4
2003	123	Control	-	-	-	-	-	-	-
2003	126	Control	-	-	-	-	-	-	-
2003	138	Control	-	-	-	-	4	-	-
2003	140	Control	-	3	-	-	-	-	-
2002	008	CW4	-	6	-	-	-	-	-
2002	020	CW4	-	2	2	-	-	-	-
2002	021	CW4	4	4	-	-	-	-	-
2002	063	CW4	-	9	-	-	-	-	-
2002	064	CW4	2	4	2	-	-	-	-
2002	070	CW4	4	5	4	-	-	-	-
2002	077	CW4	-	2	-	-	-	-	-
2002	080	CW4	6	9	6	-	-	-	-
2003	112	CW4	-	4	-	-	-	-	-
2003	113	CW4	-	-	-	-	-	-	-
2003	118	CW4	-	-	-	-	-	-	-
2003	119	CW4	-	3	-	-	-	-	-
2002	004	CW6	2	4	-	-	-	-	-
2002	013	CW6	4	6	4	-	-	-	-
2002	017	CW6	4	6	4	-	-	-	-
2002	081	CW6	-	-	4	-	-	-	-
2002	086	CW6	-	7	-	-	-	-	-
2002	089	CW6	2	6	-	-	-	-	-
2002	090	CW6	2	4	-	-	-	-	-
2002	091	CW6	-	9	-	-	-	-	-
2003	128	CW6	-	3	-	-	-	-	-
2003	129	CW6	-	2	-	-	-	-	-
2003	130	CW6	-	3	-	4	6	-	-
2003	142	CW6	-	-	-	-	-	-	-
2003	144	CW6	-	5	-	-	-	-	-
2003	145	CW6	-	4	-	-	5	-	-
2003	146	CW6	-	4	-	-	7	-	-
2002	002	Harvard	4	4	-	-	-	-	-
2002	069	Harvard	4	4	-	-	-	-	-
2002	071	Harvard	2	7	-	-	-	-	-
2002	098	Harvard	2	6	-	-	-	-	-
2003	108	Harvard	-	-	-	-	-	-	-
2003	111	Harvard	2	4	-	-	-	-	-
2003	137	Harvard	-	2	-	4	4	-	-
2003	141	Harvard	-	2	-	4	-	-	-
2002	018	McMurtrey	2	-	2	-	-	-	-
2002	056	McMurtrey	2	6	-	-	9	-	-
2002	062	McMurtrey	4	2	-	-	-	-	-
2002	067	McMurtrey	5	-	-	-	-	9	-
2003	105	McMurtrey	-	4	-	-	-	-	-
2003	132	McMurtrey	-	2	-	4	-	-	-
2003	134	McMurtrey	-	2	-	4	-	-	-
2003	135	McMurtrey	-	2	-	4	-	-	-

Note: CW = Hastings Pork created wetland. Presence and severity of lesions were qualitatively described by the pathologist and then scored by NEFO as follows: 0 = no lesions, 2 = minimal, 3 = minimal to mild, 4 = mild, 5 = mild to moderate, 6 = moderate, 7 = moderate to severe, 9 = severe, 10 = extremely severe.

Table A.7. Mallard kidney histology scoring for day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002 and 2003.

			Kidney				
Year	Mallard ID	Site	ureteritis	nephritis	hemorrhage	hemosiderosis	tubular nephorosis
2002	188	Control	-	-	-	-	-
2002	194	Control	-	-	-	-	-
2002	198	Control	-	-	-	-	-
2002	200	Control	-	-	-	-	-
2002	201	Control	-	-	-	-	-
2002	203	Control	-	-	-	-	-
2003	103	Control	-	-	-	-	-
2003	110	Control	-	-	-	-	-
2003	123	Control	-	-	-	-	-
2003	126	Control	-	-	-	-	-
2003	138	Control	-	-	-	-	-
2003	140	Control	-	-	-	-	-
2002	008	CW4	-	-	-	-	-
2002	020	CW4	4	-	-	-	-
2002	021	CW4	-	-	-	-	-
2002	063	CW4	-	-	-	-	-
2002	064	CW4	-	-	-	-	-
2002	070	CW4	-	-	-	-	-
2002	077	CW4	-	-	-	-	-
2002	080	CW4	2	2	-	-	-
2003	112	CW4	-	-	-	-	4
2003	113	CW4	-	-	-	-	-
2003	118	CW4	-	-	-	-	-
2003	119	CW4	-	-	-	-	-
2002	004	CW6	-	-	-	-	-
2002	013	CW6	4	-	-	-	-
2002	017	CW6	-	-	-	-	-
2002	081	CW6	-	-	-	-	-
2002	086	CW6	-	-	-	2	-
2002	089	CW6	-	-	-	-	-
2002	090	CW6	4	-	-	-	-
2002	091	CW6	-	2	-	-	-
2003	128	CW6	-	2	-	2	-
2003	129	CW6	-	-	-	-	-
2003	130	CW6	-	-	-	-	-
2003	142	CW6	-	-	-	-	-
2003	144	CW6	-	-	-	-	-
2003	145	CW6	-	-	-	-	-
2003	146	CW6	-	-	-	-	-
2002	002	Harvard	4	4	-	-	-
2002	069	Harvard	5	5	-	-	-
2002	071	Harvard	-	-	-	-	-
2002	098	Harvard	4	-	-	-	-
2003	108	Harvard	-	4	-	-	-
2003	111	Harvard	-	2	-	-	-
2003	137	Harvard	-	-	-	-	4
2003	141	Harvard	-	-	-	-	-
2002	018	McMurtrey	-	-	-	-	-
2002	056	McMurtrey	-	-	-	-	-
2002	062	McMurtrey	-	-	-	-	-
2002	067	McMurtrey	-	-	2	-	-
2003	105	McMurtrey	-	-	-	-	-
2003	132	McMurtrey	-	-	-	-	-
2003	134	McMurtrey	-	-	-	-	-
2003	135	McMurtrey	-	-	-	-	-

Note: CW = Hastings Pork created wetland. Presence and severity of lesions were qualitatively described by the pathologist and then scored by NEFO as follows: 0 = no lesions, 2 = minimal, 3 = minimal to mild, 4 = mild, 5 = mild to moderate, 6 = moderate, 7 = moderate to severe, 9 = severe, 10 = extremely severe.

Table A.8. Mallard gonad histology scoring for day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002 and 2003.

			Gonads			
Year	Mallard ID	Site	hemosiderosis	granulomatous	seminiferous degeneration	follicular rupture
2002	188	Control	-	-	-	-
2002	194	Control	2	-	-	-
2002	198	Control	2	-	-	-
2002	200	Control	-	-	-	-
2002	201	Control	-	9	-	2
2002	203	Control	-	-	-	-
2003	103	Control	-	-	-	-
2003	110	Control	-	-	-	-
2003	123	Control	-	-	-	-
2003	126	Control	-	-	-	-
2003	138	Control	-	-	-	-
2003	140	Control	-	-	-	-
2002	008	CW4	-	-	-	-
2002	020	CW4	2	-	-	-
2002	021	CW4	-	-	-	-
2002	063	CW4	-	-	-	-
2002	064	CW4	-	-	-	-
2002	070	CW4	5	-	-	-
2002	077	CW4	-	-	4	-
2002	080	CW4	-	-	-	-
2003	112	CW4	-	-	-	-
2003	113	CW4	-	-	-	-
2003	118	CW4	-	-	-	-
2003	119	CW4	-	-	-	-
2002	004	CW6	-	-	-	-
2002	013	CW6	-	-	-	-
2002	017	CW6	-	-	-	-
2002	081	CW6	-	-	-	-
2002	086	CW6	5	-	-	-
2002	089	CW6	7	-	-	-
2002	090	CW6	2	-	-	-
2002	091	CW6	6	-	-	-
2003	128	CW6	-	-	-	-
2003	129	CW6	-	-	-	-
2003	130	CW6	-	-	-	-
2003	142	CW6	-	-	-	-
2003	144	CW6	-	-	-	-
2003	145	CW6	-	-	-	-
2003	146	CW6	-	-	-	-
2002	002	Harvard	-	-	-	-
2002	069	Harvard	-	-	-	-
2002	071	Harvard	2	-	-	-
2002	098	Harvard	6	-	-	-
2003	108	Harvard	-	-	-	-
2003	111	Harvard	-	-	-	-
2003	137	Harvard	-	-	-	-
2003	141	Harvard	-	-	-	-
2002	018	McMurtrey	-	-	-	-
2002	056	McMurtrey	4	-	-	-
2002	062	McMurtrey	-	-	-	-
2002	067	McMurtrey	-	2	-	-
2003	105	McMurtrey	-	-	-	-
2003	132	McMurtrey	-	-	-	-
2003	134	McMurtrey	-	-	-	-
2003	135	McMurtrey	-	-	-	-

Note: CW = Hastings Pork created wetland. Note: CW = Hastings Pork created wetland. Presence and severity of lesions were qualitatively described by the pathologist and then scored by NEFO as follows: 0 = no lesions, 2 = minimal, 3 = minimal to mild, 4 = mild, 5 = mild to moderate, 6 = moderate, 7 = moderate to severe, 9 = severe, 10 = extremely severe.

Table A.9. Bacterial pathogens in sentinel mallards from day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002.

		Number of Isolates															
				Streptococcus					*Enterococcus*								
Site	Mallard ID	*E. Coli*	*Salmonella bareilly*	*constellatus*	*uberis*	*equinus*	*bovis*	*Other species*	*casseliflavus*	*durans*	*hirae*	*faecalis*	*faecium*	*gallinarum*	*TOTAL ENTEROCOCCI*	*TOTAL STREP*	*No. different bacteria*
HE	2	5		1									1		1	1	3
CW6	4	1	2									1	2		3		4
CW4	8	1								1		3			4		3
CW6	13	5	1									2	1		3		4
CW6	17	5													0		1
ME	18	0							2	3					5		2
CW4	20	0								1					1		1
CW4	21	5													0		1
ME	56	0		1	1	1							2		2	3	4
ME	62	5				4						1			1	4	3
CW4	63	0													0		0
CW4	64	0											5		5		1
ME	67	5				1						4			4	1	3
HE	69	5													0		1
CW4	70	2													0		1
HE	71	5											4	1	5		3
CW4	77	5										1	2		3		3
CW4	80	0											4		4		1
CW6	81	5	1										2		2		3
CW6	86	5													0		1
CW6	89	4	1					1					5		5		3
CW6	90	4											5		5		2
CW6	91	0													0		0
HE	98	5					2			1					1	2	3
HE	184	1						2							0		2
BC	188	5												3	3		2
BC	194	5											3		3		2
BC	198	6								2		3			5		3
BC	200	6													0		1
BC	201	5					2					1			1	2	3
BC	203	5											1		1		2

Note: HE = Harvard enclosure, CW = Hastings Pork created wetland, ME = McMurtrey enclosure.

Table A.10. Bacterial pathogens in sentinel mallards from day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2003.

		Number of Isolates															
				Streptococcus					*Enterococcus*								
Site	Mallard ID	*E. Coli*	*Salmonella bareilly*	*constellatus*	*uberis*	*equinus*	*bovis*	*Unknown species*	*durans*	*hirae*	*faecalis*	*faecium*	*gallinarum*	*TOTAL ENTEROCOCCI*	*TOTAL STREP*	*Total Isolates*	*No. different bactria*
BC	103	5								1		3		4		9	3
ME	105	6												0		6	1
HE	108	5							4			1		5		1	3
BC	110	4								2		3		5		9	3
HE	111	5										5		5		1	2
CW4	112	4						1	5					5		1	3
CW4	113	5									1			1		6	2
CW4	117	5										5		5		1	2
CW4	118	5								3				3		8	2
CW4	119	4	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	4	NA
BC	123	0												0			0
BC	126	2				3						1		1	4	7	4
CW6	128	4	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	4	NA
CW6	129	5	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	HG	5	NA
CW6	130	5			1							4		4	1	1	3
ME	132	0												0			0
ME	134	5							3					3		8	2
ME	135	5										5		5		1	2
HE	137	0												0			0
BC	138	5								5				5		1	2
BC	140	0						5						0		5	1
HE	141	5							2	3				5		1	3
CW6	142	5									2	1		3		8	3
CW6	144	4									1			1		5	2
CW6	145	5			1							4		4	1	1	3
CW6	146	5							1			2		3		8	3

Note: HE = Harvard enclosure, CW = Hastings Pork created wetland, ME = McMurtrey enclosure, HG = growth too heavy to determine colony isolates, NA = not applicable

Table A.11. Concentrations of elemental contaminants in sentinel mallard liver samples from day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2002.

		Element Concentration in mg/kg dry weight														
Sample ID	Site	As	B	Ba	Cd	Cu	Fe	Hg	Mg	Mn	Mo	Pb	Se	Sr	V	Zn
002-L-Tm	Harvard	< 0.2	< 2	0.3	1.60	114	4620	0.20	764	23	5.0	< 0.2	6.8	0.2	< 0.5	183
004-L-Tm	CW6	0.3	< 2	0.3	0.76	49	4450	< 0.1	690	15	5.0	< 0.2	9.5	0.2	< 0.5	163
008-L-Tm	CW4	0.2	< 2	0.2	1.30	343	5830	0.40	683	17	6.3	0.81	17.0	< 0.2	< 0.5	183
013-L-Tm	CW6	< 0.2	< 2	0.4	1.30	134	6650	< 0.1	654	21	7.7	0.20	11.0	< 0.2	< 0.5	188
017-L-Tm	CW6	0.3	< 2	1.5	1.40	90	5670	< 0.1	739	17	6.6	0.50	9.0	1.4	0.8	141
018-L-Tm	McMurtrey	< 0.2	< 2	< 0.2	0.46	258	1560	0.20	607	13	3.0	< 0.2	8.8	< 0.2	< 0.5	162
020-L-Tm	CW4	< 0.2	< 2	< 0.2	1.20	381	3550	0.36	695	22	5.0	< 0.2	17.0	< 0.2	< 0.5	185
021-L-Tm	CW4	< 0.2	2.0	0.2	1.70	103	5240	0.32	679	19	6.0	0.50	18.0	< 0.2	< 0.5	147
056-L-Tm	McMurtrey	< 0.2	< 2	0.5	0.71	111	5060	< 0.1	538	8.6	3.0	< 0.2	6.9	< 0.2	< 0.5	109
062-L-Tm	McMurtrey	< 0.2	< 2	< 0.2	0.20	92	1480	< 0.1	568	11	2.0	< 0.2	6.2	< 0.2	< 0.5	126
063-L-Tm	CW4	0.2	< 2	< 0.2	0.75	275	8100	0.35	655	16	7.8	< 0.2	18.0	< 0.2	0.6	165
064-L-Tm	CW4	< 0.2	< 2	0.2	0.81	136	3970	0.39	637	16	5.0	< 0.2	18.0	0.2	< 0.5	147
067-L-Tm	McMurtrey	< 0.2	< 2	0.79	0.55	9	1080	< 0.1	494	12	< 2	< 0.2	6.2	0.4	< 0.5	61.3
069-L-Tm	Harvard	< 0.2	< 2	< 0.2	0.80	189	3770	0.20	741	19	6.5	< 0.2	6.8	< 0.2	< 0.5	214
070-L-Tm	CW4	< 0.2	< 2	0.4	0.98	80	5120	0.30	721	22	5.0	0.30	22.0	< 0.2	0.5	170
071-L-Tm	Harvard	< 0.2	< 2	0.3	1.40	63	6100	0.10	775	19	4.0	0.20	5.1	< 0.2	< 0.5	182
077-L-Tm	CW4	< 0.2	< 2	0.2	1.30	272	4060	0.36	695	26	4.0	< 0.2	21.0	< 0.2	< 0.5	201
080-L-Tm	CW4	< 0.2	2.0	0.4	0.95	82	10000	0.31	695	19	7.4	0.50	17.0	< 0.2	0.6	148
081-L-Tm	CW6	< 0.2	< 2	< 0.2	0.30	93	2360	< 0.1	704	18	4.0	< 0.2	12.0	< 0.2	< 0.5	153
086-L-Tm	CW6	0.4	< 2	0.4	0.70	34	8860	< 0.1	714	20	10.0	< 0.2	11.0	0.2	0.8	144
089-L-Tm	CW6	0.2	< 2	0.2	0.85	335	6930	< 0.1	694	17	7.8	< 0.2	10.0	< 0.2	0.8	186
090-L-Tm	CW6	< 0.2	< 2	0.3	0.47	33	3770	< 0.1	676	17	4.0	< 0.2	13.0	< 0.2	< 0.5	181
091-L-Tm	CW6	< 0.2	3.0	0.5	0.83	61	14400	< 0.1	661	18	17.0	0.30	9.2	< 0.2	0.6	149
098-L-Tm	Harvard	< 0.2	< 2	0.71	0.53	151	9450	0.20	769	26	7.0	0.30	5.8	< 0.2	< 0.5	198
188-L-Tm	Zero-Control	< 0.2	< 2	< 0.2	0.41	798	1980	< 0.1	557	10	3.0	< 0.2	6.3	< 0.2	< 0.5	125
194-L-Tm	Zero-Control	< 0.2	< 2	< 0.2	0.58	424	1410	< 0.1	630	15	3.0	< 0.2	4.6	< 0.2	< 0.5	166
198-L-Tm	Zero-Control	< 0.2	< 2	< 0.2	0.86	285	3210	< 0.1	617	17	4.0	< 0.2	3.4	< 0.2	< 0.5	170
200-L-Tm	Zero-Control	< 0.2	< 2	< 0.2	0.20	27	864	< 0.1	347	9.5	< 2	< 0.2	1.9	< 0.2	< 0.5	58.7
201-L-Tm	Zero-Control	< 0.2	< 2	< 0.2	0.83	300	2470	< 0.1	571	15	5.0	< 0.2	4.3	< 0.2	< 0.5	190
203-L-Tm	Zero-Control	< 0.2	< 2	< 0.2	0.65	48	2690	< 0.1	571	11	3.0	< 0.2	2.3	0.3	< 0.5	89.2

Note: < indicates the sample was below the detection limit (value = detection limit). CW4 = Created Wetland 4, CW6 = Created Wetland 6.

Table A.12. Concentrations of elemental contaminants in sentinel mallard liver samples from day zero-controls and treatment birds kept in enclosures at Hastings Pork created wetlands, Harvard Waterfowl Production Area, and McMurtrey National Wildlife Refuge, Clay County, Nebraska, 2003.

		Trace Element Concentration in mg/kg dry weight										
Sample ID	**Site**	**Al**	**As**	**Ba**	**B**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**K**
103-L-Tm	Zero-Control	< 5.0	< 0.2	< 0.1	< 1.0	0.24	< 0.479	< 0.5	87	1160	< 0.050	8280
105-L-Tm	McMurtrey	< 5.0	0.30	0.22	< 1.0	1.81	< 0.477	1.64	2870	3880	0.083	6910
108-L-Tm	Harvard	< 5.0	< 0.2	0.18	< 1.0	1.12	0.55	< 0.5	93	1590	0.147	9260
110-L-Tm	Zero-Control	< 5.0	0.33	0.11	< 1.0	0.38	< 0.466	0.59	794	1590	< 0.050	9220
111-L-Tm	Harvard	< 5.0	< 0.2	0.19	< 1.0	1.48	< 0.461	0.88	946	4230	0.115	7510
112-L-Tm	CW4	< 5.0	0.32	0.32	< 1.0	0.55	< 0.465	0.88	232	6460	< 0.050	8400
113-L-Tm	CW4	< 5.0	0.29	0.12	< 1.0	0.52	< 0.459	< 0.5	223	2540	< 0.050	8660
117-L-Tm	CW4	7.1	0.28	1.63	< 1.0	0.52	< 0.463	1.27	379	3760	0.121	7390
118-L-Tm	CW4	< 5.0	0.26	0.54	< 1.0	0.57	< 0.473	0.56	421	2260	0.049	8160
119-L-Tm	CW4	< 5.0	0.24	0.34	< 1.0	0.84	< 0.457	0.72	465	3340	0.065	8100
123-L-Tm	Zero-Control	< 5.0	0.38	< 0.1	< 1.0	0.34	< 0.479	< 0.5	153	1220	< 0.050	8460
126-L-Tm	Zero-Control	< 5.0	0.23	0.13	< 1.0	0.29	< 0.487	< 0.5	155	1120	< 0.050	8570
128-L-Tm	CW6	70.7	1.00	77.90	1.71	0.72	< 0.482	0.76	109	1550	< 0.050	8040
129-L-Tm	CW6	< 5.0	0.33	0.16	< 1.0	0.59	< 0.456	0.48	331	2730	< 0.050	8320
130-L-Tm	CW6	< 5.0	0.39	0.22	< 1.0	0.42	< 0.447	0.58	189	2130	0.052	7800
132-L-Tm	McMurtrey	< 5.0	0.35	0.10	< 1.0	2.34	< 0.469	< 0.5	195	2300	< 0.050	9060
134-L-Tm	McMurtrey	< 5.0	0.31	0.17	< 1.0	2.22	< 0.471	< 0.5	160	3500	0.050	7770
135-L-Tm	McMurtrey	< 5.0	0.40	0.18	< 1.0	1.00	< 0.46	0.79	902	2340	< 0.050	8170
137-L-Tm	Harvard	< 5.0	0.24	0.12	< 1.0	0.52	< 0.468	< 0.5	90	1280	0.222	8140
138-L-Tm	Zero-Control	< 5.0	0.42	0.11	< 1.0	0.53	< 0.481	< 0.5	387	897	< 0.050	8490
140-L-Tm	Zero-Control	< 5.0	0.30	0.35	< 1.0	0.39	< 0.478	0.97	1370	1040	< 0.050	9010
141-L-Tm	Harvard	< 5.0	0.31	0.30	< 1.0	2.75	1.16	1.18	1230	3590	0.142	7890
142-L-Tm	CW6	< 5.0	0.32	0.11	< 1.0	0.60	< 0.463	< 0.5	196	4010	0.049	8150
144-L-Tm	CW6	< 5.0	0.39	0.29	< 1.0	1.08	< 0.455	0.69	179	6140	0.066	8300
145-L-Tm	CW6	< 5.0	0.30	< 0.1	< 1.0	0.53	< 0.447	< 0.5	128	2690	0.056	8580
146-L-Tm	CW6	< 5.0	0.29	0.48	< 1.0	0.30	< 0.46	< 0.5	205	1900	< 0.050	7240
		Mg	**Mn**	**Mo**	**Na**	**Ni**	**P**	**Pb**	**S**	**Se**	**Sr**	**Zn**
103-L-Tm	Zero-Control	615	15.4	2.9	3230	< 0.5	9050	< 0.05	7150	3.74	0.08	117
105-L-Tm	McMurtrey	716	15.6	5.3	4880	0.49	11100	0.20	10400	21.90	0.11	162
108-L-Tm	Harvard	627	14.9	2.5	4160	< 0.5	9210	2.02	7040	4.80	0.09	104
110-L-Tm	Zero-Control	711	14.6	3.6	3870	< 0.5	9830	< 0.05	8560	5.33	0.07	131
111-L-Tm	Harvard	728	15.2	5.8	6010	< 0.5	10700	0.12	8730	10.20	0.14	179
112-L-Tm	CW4	764	16.9	6.4	4970	< 0.5	11100	6.39	8810	5.72	0.09	185
113-L-Tm	CW4	730	15.7	2.9	4190	< 0.5	10300	0.13	8390	7.15	0.11	182
117-L-Tm	CW4	715	12.8	5.9	4590	0.69	10800	0.14	7790	5.70	2.22	241
118-L-Tm	CW4	607	13.4	3.8	3680	< 0.5	9330	0.24	6740	5.42	0.09	159
119-L-Tm	CW4	705	15.7	5.6	4870	0.47	10500	2.68	8060	5.69	0.18	124
123-L-Tm	Zero-Control	680	18.4	3.4	2810	< 0.5	9450	< 0.05	7210	3.55	0.08	145
126-L-Tm	Zero-Control	571	12.8	2.9	2770	0.67	8040	< 0.05	6350	3.63	0.06	78
128-L-Tm	CW6	942	39.9	3.3	4000	0.89	9730	1.59	7070	3.71	15.80	145
129-L-Tm	CW6	749	17.9	3.9	4230	< 0.5	11400	0.15	8190	7.07	0.14	181
130-L-Tm	CW6	546	9.4	3.4	3370	< 0.5	7920	0.08	6680	7.99	0.06	94
132-L-Tm	McMurtrey	750	18.2	3.0	5720	< 0.5	11100	0.10	8050	7.24	0.12	165
134-L-Tm	McMurtrey	752	16.0	3.5	6050	< 0.5	11100	0.63	8450	8.45	0.13	168
135-L-Tm	McMurtrey	702	16.4	6.3	5480	< 0.5	10600	0.12	8620	23.70	0.12	144
137-L-Tm	Harvard	535	12.3	2.8	4230	< 0.5	8390	0.25	6290	7.21	0.09	92
138-L-Tm	Zero-Control	667	15.4	4.3	2930	< 0.5	9200	< 0.05	7390	5.47	0.08	127
140-L-Tm	Zero-Control	800	17.6	6.4	3750	< 0.5	11100	< 0.05	9510	5.99	0.07	219
141-L-Tm	Harvard	691	22.3	5.6	4960	< 0.5	10400	0.19	9090	11.30	0.13	202
142-L-Tm	CW6	695	18.9	4.1	3740	< 0.5	10400	0.20	7400	7.36	0.09	129
144-L-Tm	CW6	730	14.8	6.5	4800	< 0.5	11000	0.24	8400	6.44	0.19	174
145-L-Tm	CW6	637	13.7	3.7	4300	< 0.5	8950	0.92	7380	7.85	0.12	132
146-L-Tm	CW6	426	7.0	3.0	2290	< 0.5	6250	0.08	5320	8.33	< 0.05	70

Note: < indicates the sample was below the detection limit (value = detection limit), CW4 = Created Wetland 4, CW6 = Created Wetland 6.

Table A.13. Concentrations of elemental contaminants in stomach content samples from sentinel mallards kept in enclosures at Hastings Pork created wetlands, Clay County, Nebraska, 2002.

		Trace Element Concentration in mg/kg dry weight								
Sample ID	Site	Al	As	B	Ba	Be	Cd	Cr	Cu	Fe
004-Sc-R	CW6	9590	6	15	230	0.4	0.5	10	18	9220
013-Sc-R	CW6	5040	4	10	257	0.3	0.7	6	13	5210
017-Sc-R	CW6	5560	4	6	1990	0.2	< 0.1	26	51	87100
063-Sc-R	CW4	7400	3	11	452	0.3	0.5	8	15	6360
086-Sc-R	CW6	1770	2	6	88	0.1	0.5	2	7	3510
089-Sc-R	CW6	5210	4	6	39	0.2	0.2	6	12	3790
		Mg	Mn	Mo	Ni	Pb	Se	Sr	V	Zn
004-Sc-R	CW6	2430	605	< 2	12	20.0	0.8	61	30	50
013-Sc-R	CW6	1460	402	< 2	8	26.0	< 0.5	50	17	86
017-Sc-R	CW6	1060	973	6	47	6.2	1.0	60	18	59
063-Sc-R	CW4	1460	195	< 2	7	430.0	0.9	38	21	36
086-Sc-R	CW6	937	122	< 2	5	4.2	< 0.5	24	7	26
089-Sc-R	CW6	927	44	< 2	4	6.8	1.0	6	14	24

Note: < indicates the sample was below the detection limit (value = detection limit), CW4 = Created Wetland 4, CW6 = Created Wetland 6.

Table A.14. Concentrations of elemental contaminants in sentinel mallard egg samples from mallards kept in enclosures at Hastings Pork created wetlands and Harvard Waterfowl Production Area, Clay County, Nebraska, 2003.

		Trace Element Concentration in mg/kg dry weight																
Sample ID	**Site**	**As**	**B**	**Ba**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Ni**	**P**	**Pb**	**S**	**Se**	**Sr**	**Zn**
4C24	CW4	0.45	1.23	4.4	< 0.5	7.59	141.0	5950	585	2.4	5460	< 0.05	10300	< 0.05	9950	3.38	16.5	68
4C47	CW4	0.37	1.07	9.0	0.58	5.86	112.0	5170	654	2.5	4680	< 0.05	8880	< 0.05	8710	3.16	9.7	56
4C48	CW4	0.46	1.37	13.4	< 0.5	6.06	130.0	5210	630	2.3	5220	< 0.05	9110	< 0.05	9100	2.64	15.9	67
4C68	CW4	0.45	1.28	7.9	< 0.5	5.33	120.0	5350	640	2.8	4520	< 0.05	9550	< 0.05	8560	3.48	11.5	64
4C78	CW4	0.37	1.40	8.9	< 0.50	5.67	125.0	4920	488	2.2	4700	< 0.05	8980	< 0.05	9120	2.67	9.3	64
4X52	CW4	< 0.2	< 1.0	14.9	< 0.5	3.22	104.0	4290	340	1.3	4020	0.59	7110	< 0.05	6620	2.51	5.7	54
4X53	CW4	0.30	1.04	34.8	< 0.5	3.01	121.0	4840	410	2.2	3920	0.76	7650	0.06	6550	2.30	7.4	51
4X54	CW4	0.21	< 1.0	7.7	< 0.5	3.33	114.0	4290	462	1.5	3990	< 0.05	7140	< 0.05	6970	2.27	7.2	49
4X55	CW4	0.21	< 1.0	16.3	< 0.5	3.15	100.0	4240	509	1.1	4110	< 0.05	7190	< 0.05	7050	2.44	6.4	54
4X56	CW4	0.30	< 1.0	14.6	< 0.5	3.43	142.0	3720	356	1.8	3600	0.54	8490	< 0.05	5330	1.92	8.6	64
6A33	CW6	0.33	< 1.0	9.3	< 0.5	3.83	81.6	4740	401	1.6	3990	1.40	7530	< 0.05	7250	2.80	4.7	44
6A34	CW6	0.32	< 1.0	7.3	< 0.5	3.64	103.0	3780	376	1.9	3030	< 0.05	8170	< 0.05	6030	2.90	4.0	51
6A43	CW6	0.24	< 1.0	7.1	< 0.5	3.66	92.3	4230	388	2.0	3610	< 0.05	7980	< 0.05	6910	2.88	3.8	48
6A79	CW6	0.30	< 1.0	11.9	< 0.5	3.64	102.0	4150	365	2.0	3760	< 0.05	7720	< 0.05	6720	2.49	4.3	53
6A80	CW6	0.28	< 1.0	22.8	< 0.5	3.57	113.0	4160	312	1.8	3450	< 0.05	7930	< 0.05	6170	2.73	5.7	59
6B10	CW6	< 0.2	< 1.0	20.4	< 0.5	4.11	93.8	5190	452	1.3	3660	1.99	7100	0.08	6630	3.05	5.6	46
6B12	CW6	0.29	< 1.0	10.9	< 0.5	4.45	133.0	5470	532	3.4	4320	< 0.05	8990	< 0.05	7910	3.52	10.9	61
6B13	CW6	0.27	1.00	8.3	< 0.5	5.18	120.0	5730	523	1.7	5080	< 0.05	8210	< 0.05	8520	3.01	16.3	52
6B15	CW6	0.19	0.99	21.8	< 0.5	4.27	104.0	3820	417	1.5	3150	< 0.05	7720	< 0.05	5630	2.59	7.6	52
6B4	CW6	0.34	< 1.0	6.8	< 0.5	4.49	81.8	4800	385	1.7	3770	0.57	6910	< 0.05	7230	2.79	4.9	45
HC18	Harvard	0.35	< 1.0	3.1	< 0.5	5.40	48.1	4120	395	0.5	3570	< 0.05	5860	0.27	6480	3.18	4.0	43
HC30	Harvard	0.22	< 1.0	3.0	< 0.5	5.92	55.7	4570	364	0.8	4060	< 0.05	6060	0.18	6970	3.56	3.9	39
HC61	Harvard	0.27	< 1.0	4.1	< 0.5	4.14	90.1	4010	364	1.4	3270	< 0.05	8070	0.16	5900	3.05	3.5	44
HC63	Harvard	0.31	< 1.0	3.3	0.79	4.70	65.4	4880	368	0.6	4810	1.04	5600	0.28	7660	3.68	3.1	32
HC71	Harvard	0.27	< 1.0	6.6	< 0.5	3.84	104.0	3790	371	1.4	3260	< 0.05	7880	0.45	5240	2.45	5.4	47
HD22	Harvard	0.28	< 1.0	7.1	< 0.5	5.20	97.4	5170	428	3.7	4010	< 0.05	8400	< 0.05	7030	3.27	3.5	58
HD23	Harvard	0.25	< 1.0	6.3	< 0.5	3.88	84.2	4420	281	3.0	3660	< 0.05	8110	< 0.046	6050	3.01	3.4	47
HD31	Harvard	< 0.2	< 1.0	2.9	0.96	3.79	115.0	4100	391	2.5	3640	< 0.05	8550	< 0.05	6090	2.70	2.9	50
HD70	Harvard	0.43	< 1.0	9.1	< 0.5	4.06	103.0	5090	642	1.8	4410	1.71	10100	< 0.05	7930	4.02	14.8	55
HD77	Harvard	0.19	< 1.0	6.3	< 0.5	5.02	112.0	4840	377	2.6	3990	< 0.05	7320	< 0.05	6860	3.62	4.3	54

Note: < indicates the sample was below the detection limit (value = detection limit), CW4 = Created Wetland 4, CW6 = Created Wetland 6.