INVESTIGATION OF MIGRATORY BIRD MORTALITY ASSOCIATED WITH EXPOSURE TO SODA ASH MINE TAILINGS WATER IN SOUTHWESTERN WYOMING

Final Report – September 2004

Eared Grebe (Podiceps nigricollis)

BIOLOGICAL RESOURCES DIVISION
United States Geological Survey

National Wildlife Health Center

University of Wisconsin
School of Veterinary Medicine

FINAL REPORT AUTHORS

NATIONAL WILDLIFE HEALTH CENTER

Kurt K. Sladky, MS, DVM
Project Scientist
Department of Surgical Sciences
School of Veterinary Medicine and
Department of Wildlife Ecology
Cooperative Wildlife Research Unit
University of Wisconsin
Madison, Wisconsin

F. Joshua Dein, VMD, MS
Project Coordinator
USGS-National Wildlife Health Center
Madison, Wisconsin

UNITED STATES FISH AND WILDLIFE SERVICE

Pedro Ramirez, Environmental Contaminants Specialist
Ecological Services Field Office
Cheyenne, Wyoming

WILDLIFE HEALTH ASSOCIATES, INC.

Charlotte F. Quist, DVM, PhD, DACVP
Dillon, Montana

We gratefully acknowledge the cooperation and support from the following:

The soda ash mines and their employees in Green River, Wyoming: FMC Corporation (Carl Demshar, Julie Lutz, Mike Wendorf, Simon Lee, and Ron McNeely), OCI Corporation (Terrell Johnson and Larry Cherny), and Solvay Minerals (Dottie Potter, Tim Brown, and Stan Cook). FMC and OCI were instrumental in our ability to collect samples for this project, and we would not have been able to accomplish our goals without their help.

The professional advice of Dr. Stewart Hurlburt, San Diego State University and Dr. Joseph Jehl, Hubbs-Sea World Research Institute, San Diego, Drs. Sean Boyd and Andre Breault, Canadian Wildlife Service, and the technical assistance of David Hill (North Carolina Zoo), Laurie Baeten (NWHC), Peter Vertz (NWHC), Nathan Ramsey (NWHC), and Daniel Findley (NWHC).

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Soda ash is a pulverized mineral, commonly referred to as "trona", and harvested from underground deposits in southwestern Wyoming. Four companies (FMC-Westvaco/FMC-Grainger, OCI, Solvay Minerals, and General Chemical) own 5 mining operations in the vicinity of Green River, Wyoming, and produce 90 percent of the soda ash in the United States, which accounts for more than 30 percent of the world supply. Trona refining produces "tailings" water that is either discharged into open evaporation ponds or recycled back into the mining operation and used to facilitate trona ore extraction. The evaporation ponds range in size from approximately 225 to 1100 acres. As water evaporates from the ponds, chemical residues become concentrated. At cooler temperatures (generally <40˚F) sodium decahydrate precipitates out of the water and crystallizes on solid objects in the ponds or on the water surface.

Migratory birds landing on the evaporation ponds and being exposed to the water are affected by the sodium decahydrate and other chemical precipitates are affected in one or more of the following ways:

1) salts crystallizing on feathers may cause disruption of barbule/hooklet morphology, allowing water to penetrate the protective insulating function of the feathers, and compromising thermoregulation, leading to hypothermia and death;
2) significant accumulation of sodium decahydrate crystals on the feathers will add excessive weight to the bird, and potentially cause drowning; and
3) oral ingestion of the pond water could potentially cause physiologic toxicity (e.g., sodium or other chemicals).

Bird mortality recorded at all of the mines since 1975 is greater than 2000 individuals, but this only includes those species identifiable, which are primarily aquatic birds. The highest mortality is associated with grebe species, particularly the eared grebe (*Podiceps nigricollis*).

Water samples collected from multiple evaporation ponds had similar physical and chemical composition with the exception of phytoplankton species present. Representative total dissolved solids (TDS) ranged from approximately 40,000 ppm (parts per million) to greater than 300,000 ppm depending on the pond and time of year. Typically, the highest TDS values occur during the fall of the year. The pH generally ranged from 10-11, and salinities were in excess of 174 ppt (parts per thousand). Predominant chemicals included sodium decahydrate, sodium bicarbonate, sodium sulfate, and sodium chloride. Protozoan and metazoan zooplankton were not present in any of the sampled ponds.

Eared grebe (N = 63) mean serum sodium (Na), chloride (Cl), bicarbonate (HCO_3), aspartate aminotransferase (AST), creatine kinase (CK), and uric acid (UA) were elevated immediately after capture compared to serum biochemical parameters from eared grebes collected from a reference site in British Columbia, Canada. Eared grebe (N = 11) brain biochemical data indicated that mean brain sodium, zinc and sulfur concentrations were elevated compared to eared grebes collected from the reference site. Brain sodium concentrations were consistently above 2000 ppm, which is considered in a toxic range in domestic mammals and birds.

Histopathologic evidence consistent with drowning was observed in all grebes, but it is not known whether this was a direct mortality factor or a late terminal event. In concordance with the sodium intoxication hypothesis, drowning secondary to sodium intoxication in an aquatic bird species such as the grebe provides a viable explanation for grebe mortality.

Although all of the necessary components of this study were not completed due to funding issues, the working hypothesis associated with eared grebe morbidity and mortality on soda ash evaporation ponds in southwest Wyoming is acute sodium toxicity with secondary drowning. However, it is possible that during brain collection, contamination of brain tissue occurred from residual sodium on skin and feathers. Alternative hypotheses for avian mortality include: 1) hypothermia due to loss of feather integrity and loss of waterproofing, and secondary drowning; 2) physical weight of the crystals on feathers leads directly to drowning; and 3) chemical intoxication other than sodium (e.g., sulfur, zinc), or other chemicals not evaluated (e.g., selenium, fluoride, etc.), and secondary drowning.

Based on our inability to draw conclusions from limited data, we suggest support and continuation of further research.

BACKGROUND

HISTORY

Soda ash is a pulverized mineral, commonly referred to as "trona", and harvested from underground deposits in southwestern Wyoming. Four companies (FMC-Westvaco/FMC-Grainger, OCI, Solvay Minerals, and General Chemical) own 5 mining operations in the vicinity of Green River, Wyoming, and produce 90 percent of the soda ash in the United States, which accounts for more than 30 percent of the world supply. Trona, sodium sesquicarbonate ($Na_2CO_3 \bullet NaHCO_3 \bullet 2H_2O$), is a natural mineral created over millions of years from evaporation cycles causing precipitation of salts in an ancient lake, Lake Gosiute, which covered 8,000 sq. mile, or an area about six times the present size of the Great Salt Lake (Garrett 1992; Phelps 1990). Trona deposits near Green River, Wyoming range from 3-38 feet in thickness, and occur at depths ranging from 1,500-3,000 feet below the surface. Soda ash derived from trona ore is used in a variety of manufacturing processes, including: glass products; paper products; detergents; caustic soda; solar panels; baking soda; sodium cyanide; and as a reagent for SO2 scrubber systems of power plants. Current trona mining operations in the United States include the following: Green River, Wyoming; Searles Lake, Trona, California; and Owens Lake, Lone Pine, California.

Trona refining produces "tailings" water, which is either discharged into open evaporation ponds or recycled back into the mining operation and used to facilitate trona ore extraction. The evaporation ponds range in size from approximately 225 to 1100 acres, and most are immediately adjacent to the refinery buildings, with the exception of the FMC-Grainger pond, which exists 10 miles from the main refinery. The tailings water primarily contains sodium decahydrate ($Na_3CO_2 \bullet 10H_2O$) and is highly alkaline (pH>10), but other chemicals present in the water include, sulfate (as Na2SO4), bicarbonate (as NaHCO3), chloride (as NaCl), calcium (as CaCO3), aluminum, arsenic, boron, cadmium, and magnesium. As water evaporates from the ponds, chemical residues become concentrated. At cooler temperatures (generally <40˚F) sodium decahydrate precipitates out of the water and crystallizes on solid objects in the ponds or on the water surface.

Migratory birds landing on the evaporation ponds and exposed to the water may be affected by the sodium decahydrate and other chemical precipitates in one or more of the following ways:
1) salts crystallizing on feathers may cause disruption of barbule/hooklet morphology, allowing water to penetrate the protective insulation of the feathers, and compromising thermoregulation, leading to hypothermia and death; 2) significant accumulation of sodium decahydrate crystals on the feathers will add excessive weight to the bird, and potentially cause drowning; and 3) oral ingestion of the pond water could potentially cause physiologic toxicity (e.g., sodium or other chemicals). Similar effects have been observed in birds utilizing potash tailings ponds in southeastern New Mexico (Meteyer et al., 1997). The trona mines have reported migratory bird mortalities to the U.S. Fish and Wildlife Service (USFWS) since 1975 as a requirement of their Migratory Bird Treaty Act (MBTA) permits to capture and rehabilitate migratory birds from the evaporation ponds.

Prior to 1973, there were no reported bird mortalities on the evaporation ponds. However, this was possibly a function of human observer unawareness, rather than lack of birds landing on the ponds. During the fall of 1973, agents from the USFWS and Wyoming Fish and Game searched the ponds (FMC and Stauffer Chemical Companies at the time) and found 74 dead migratory birds (Table 1). Because death of migratory bird species on the evaporation ponds could be a violation of the MBTA, the mining operations sought to mitigate the situation by hazing birds during 1974 migration season.
Zon exploder guns were installed at several of the ponds, and mine personnel instructed to collect live

and dead birds on a daily basis. Many of the live birds were released on the Green River, although some were taken to Jim June of Wyoming Fish and Game for rehabilitation. Species of birds recovered from the evaporation ponds were recorded for the fall 1974 migration season (Table 2). According to biologists, the feathers of the birds were coated with sodium decahydrate crystals, which disrupted feather integrity, causing hypothermia, possible shock, and/or drowning due to the weight of the crystals. Although hazing was not entirely effective, these activities continued for the next several years. The mine employees consulted with biologists from the Seedskadee National Wildlife Refuge and local bird rehabilitators in order to train mine personnel in bird rehabilitation methods. By 1975, FMC and Stauffer had purchased airboats for use on the evaporation ponds, both as instruments of hazing, and for collecting live and dead birds from the ponds. In addition, both companies had constructed and implemented heated facilities for use in rehabilitation of live birds recovered from the ponds. In 1975, FMC Corporation retained a commercial waterfowl producer as a rehabilitation consultant, Truslow Farms, Inc., Maryland, in order to develop on-site rehabilitation facilities and train mine personnel in rehabilitation techniques. Birds incapable of flight were captured by the airboat crews and held for treatment. Rehabilitation procedures consisted of washing the salt crystals off the birds with warm water and rehydrating the birds with an orally administered electrolyte solution (e.g. Pedialyte® or similar fluid). Birds were held for 4-24 hours, and then released at the Green River or the Blacks Fork River, which were 1-10 miles from each mine.

Table 1.: Historical Data: deceased migratory birds recovered from trona mine evaporation ponds during the fall of 1973 (Report prepared by M. Bennett, Refuge Manager, Seedskadee National Wildlife Refuge, 1975)**.**

Species	FMC Corp.	Stauffer Chem.	Total
Common Loon	0	3	3
Eared Grebe	1	2	3
Western Grebe	0	5	5
Mallard Duck	1	1	2
Pintail	2	0	2
American Widgeon	0	3	3
Northern Shoveler	2	1	3
Lesser Scaup	2	5	7
Bufflehead	2	0	2
Ruddy Duck	0	6	6
Common Merganser	0	23	23
American Coot	2	7	9
Unidentified	0	6	6
Total	**12**	**62**	**74**

Table 2.: Historical Data: live and deceased migratory birds recovered from trona mine evaporation ponds during the fall of 1974 (Report prepared by M. Bennett, Refuge Manager, Seedskadee National Wildlife Refuge, 1975)**.**

Species	FMC Corp. Live/Dead	Stauffer Chem. Live/Dead	Total
Common Loon	0/1	0/2	3
Eared Grebe	4/10	24/3	41
Horned Grebe	0	0/1	1
Western Grebe	0	0/2	2
Pied-billed Grebe	0	0/1	1
Mallard Duck	0	0/3	2
Redhead	0	2/0	2
Lesser Scaup	0	2/0	3
Bufflehead	0	0/1	3
Ruddy Duck	2/10	31/16	7
Common Goldeneye	0	0/1	2
Common Merganser	0	28/8	23
American Coot	1/8	0/2	9
Unidentified	0/14	0	6
Total	**7/43**	**87/40**	**177**

After discussions between the mine employees and biologists, it was hypothesized that lights from the mine buildings were visually highlighting the ponds and attracting migratory birds to land on them. Based on this hypothesis, Stauffer Company set-up artificial lights on a portion of the Green River along with automatically broadcast water bird calls to attempt to attract the migrating birds away from the evaporation ponds. Interestingly, according to a report generated by Truslow Farms, Inc. (1975), waterfowl rehabilitation consultants to the FMC Corporation, some live captured "waterfowl" (no species given) were transported back to Maryland and force fed 2% body weight evaporation pond liquor. The report indicated that the birds suffered no ill effects, although serum chemistry results were consistent with elevated sodium and bicarbonate. In conjunction with USFWS and Wyoming Fish and Game, and the Seedskadee National Wildlife Refuge, a banding program was initiated, in which an identification band was placed on all live-caught and rehabilitated birds. Due to lack of success of the hazing and banding activities, these programs were discontinued by the late 1990s; however, the rehabilitation programs continue today and have been effective at reducing overall bird mortality (Table 3). Bird mortality recorded at all of the mines since 1975 is greater than 2000 individuals, but this only includes those species identifiable, which are primarily aquatic birds. It is assumed that many thousands of small passerine species are also dying, but these species are more difficult to identify. The highest mortality is associated with grebe species, particularly the eared grebe (*Podiceps nigricollis*). The natural history and behavior of this species appears to make them particularly vulnerable, as they dive when hazed or are approached by predators. This causes more significant exposure time to the pond water. Additionally, grebes attempt to remove the crystals from their feathers using their beak, and this behavior may contribute to greater potential oral ingestion. In addition, many bird carcasses or moribund birds are likely taken by native carnivore predators, and many dead birds may sink in the ponds and never be recorded. According to data maintained at the FMC and OCI mines, other species dying on the evaporation ponds include raptors (golden eagles, owls) and bats.

Table 3.: Representative sample of live and deceased migratory birds recovered from FMC Corporation trona mine evaporation ponds during the fall of 1999 (Data provided by FMC Corp. to USFWS-Wyoming, 1999)**.**

Species	Rehabilitated/ Released	Mortalities	Total
Eared Grebe	622	12	634
Western Grebe	68	1	69
Horned Grebe	0	0	0
Pied-billed Grebe	2	0	2
Ruddy Duck	23	0	23
Common Loon	2	1	3
American Coot	15	0	15
Green-winged Teal	1	0	1
Blue-winged Teal	8	0	8
Mallard Duck	1	0	1
Northern Pintail	1	1	2
Merganser	6	0	6
Northern Shoveler	13	0	13
Gadwall	3	0	3
Total	**7/43**	**87/40**	**780**

The USFWS, Region 6, Cheyenne, Wyoming, Ecological Services Office requested assistance from the United States Geological Survey-National Wildlife Health Center (NWHC) in order to evaluate the effectiveness of bird rehabilitation efforts by the soda ash mining operations in southwestern Wyoming, and to evaluate causes of bird morbidity and mortality after exposure to "tailings" pond water associated with these mines. Once data were collected and analyzed, the information would be provided to the Environmental Specialists at the soda ash mines, as well as USFWS Special Agents in order to assist in the development and/or implementation of procedures to eliminate or further reduce bird morbidity and mortality.

This study was originally designed to: 1) determine the physical effects of sodium decahydrate crystallization on birds; 2) determine the physiological effects of trona wastewater ingestion by birds; 3) estimate the time required for birds using the evaporation ponds to succumb to sodium toxicity by ingesting the wastewater; and 4) estimate the effectiveness of bird carcass search and retrieval as conducted by the mine company rehabilitation personnel.

I. OVERVIEW

This Final Report is a review of cooperative research conducted by the NWHC and the USFWS-Ecological Services Field Office, Cheyenne, Wyoming from August 2000 through August 2003. The study was initiated at the request of the USFWS. The initial study plan, developed prior to August 2000, is outlined in Appendix 1. Based on this initial plan, a meeting was conducted in August 2000 with participants representing the NWHC (Dr. F. Joshua Dein, Dr. Kurt Sladky) and the 4 mining companies (Terrell Johnson & Larry Cherny from OCI Corp.; Carl Demshar, Julie Lutz and Mike Wendorf from FMC Corp.; Stan Cook from Solvay Minerals, LLC; and Jerry Justis from General Chemical Corp.). During this meeting, various evaporation ponds were visited, avian rehabilitation programs reviewed, past hazing and bird-banding activities discussed, and field research plans debated. Based on these discussions, an alternative research study plan was developed (see Appendix 2). This plan was a descriptive research study undertaken in order to initiate understanding of causes of mortality and morbidity of aquatic birds landing on the soda ash mine evaporation ponds in Wyoming. Data were collected within the confines of available funding resources. Interim Reports were submitted to the NWHC, USFWS, and the Environmental Divisions of the 5 soda ash mines in the vicinity of Green River, Wyoming (4 individual companies: FMC, OCI, Solvay Minerals, and General Chemical) in July 2001, June 2002, and June 2003. In this report the terms soda ash and trona are used interchangeably.

FIRST PHASE – October 2000

The first phase of the project included development of a clear study plan. A meeting was conducted in August 2000 between soda ash mine representatives, Dr. F. J. Dein and Dr. K. K. Sladky of the USGS-NWHC. At this meeting, it was determined that previous bird hazing and banding efforts were relatively unsuccessful, and that the eared grebe was the species with the highest morbidity and mortality on the evaporation ponds. Because eared grebes are very difficult to maintain in captivity, it was deemed impractical to consider a laboratory-based component to the study plan. The focus was, therefore, on studying eared grebes in the field. This first phase involved included the following: 1) an evaluation of pond water quality by Dr. Stuart Hurlburt, limnologist with San Diego State University; 2) an evaluation of the natural history and behavior of the eared grebe by Dr. Joseph Jehl, biologist at Hubbs-Sea World Research Institute in San Diego; 3) the opportunistic collection of blood samples for evaluation of serum biochemistry data on surviving grebes captured by mine employees for rehabilitation; 4) the opportunistic collection of dead grebes for complete necropsies and brain biochemical analysis; and 5) further review of avian rehabilitation procedures at the 5 mining operations.

Dr. Hurlburt analyzed pond water samples for total dissolved solids (TDS), pH, salinity and presence of living organisms. Dr. Jehl toured a variety of ponds and evaluated the physical characteristics of the ponds from a “grebes-eye view”. All serum samples collected during 2000 were sent directly to the Wyoming Veterinary Diagnostic Laboratory for analysis of biochemical parameters. Unfortunately, all samples were evaluated as mammalian samples, rather than avian samples, and important avian-specific serum biochemical parameters were not evaluated. Therefore, serum biochemical data from October 2000 were considered non-diagnostic and were not included in this report. Decapitated heads from dead grebes were sent frozen to the NWHC, and preserved in frozen condition until brains could be removed and biochemically evaluated at a later date. Drs. Dein and Sladky evaluated the avian rehabilitation facilities and programs, although General Chemical did not permit access to their rehabilitation program and facilities. FMC Westvaco, FMC Grainger, OCI, and Solvay Minerals granted full access to their rehabilitation programs and facilities.

SECOND PHASE – August 2001

The second phase was an expansion of the first phase, and was undertaken in order to increase our number of blood and brain samples collected. Blood samples were collected opportunistically from grebes captured on the evaporation ponds, and affected birds were kept on fresh water for 24 hours. Blood samples were collected a second time from these same birds at 24 hours in order to evaluate any biochemical changes that may have taken place while kept on fresh water and allowed to reestablish feather water-proofing. Grebes were released onto the Green River after the 24-hour blood sample collection. Serum biochemical data were evaluated at Marshfield Veterinary Diagnostic Laboratories in Marshfield, Wisconsin. Heads from dead birds were sent frozen to the NWHC for biochemical analysis at a later date. During the 2001 fall migration season, eared grebes began migrating earlier in the season than in previous years. This was evidenced by the soda ash mines recording greatest numbers of birds landing on the evaporation ponds in late July and early August, rather than September. Therefore, our field operations missed the greatest number of migrating grebes, and, therefore, our number of blood and brain samples was relatively low.

THIRD PHASE – July-August 2002

Due to low numbers of birds collected during the 2001 field season, and in anticipation of another earlier eared grebe migration, we scheduled our two-week Wyoming field visit for late July and early August. The objective of the third phase of the project was to further increase our sample numbers. The identical procedures used in phase 2 were applied in phase 3.

FOURTH PHASE – August 2003

With adequate sample numbers, we decided to collect data on “normal”, pre-migration eared grebes in order to have comparative data on reference blood and brain biochemical values. Biologists from the Canadian Wildlife Service, Drs. Sean Boyd and Andre Breault, were contacted and arrangements made for a field visit to a breeding/nesting site of the eared grebes in British Columbia, Canada. With the help of the Canadian Wildlife Service, eared grebes were captured on breeding/nesting ponds in the vicinity of Riske Creek, British Columbia, Canada. Blood samples were collected from this population of eared grebes for serum biochemical analysis (N = 20), and a subset were humanely euthanized for complete necropsies and brain biochemical analysis (N = 5).

II. FIELD-BASED STUDIES ON AVIAN MORBIDITY AND MORTALITY

A. LIMNOLOGY

METHODS

Each mining company provided historical data detailing water chemistry parameters. Total dissolved solids varied widely depending on the time of year, with cooler ambient temperatures correlating positively with markedly elevated total dissolved solids. This is typical during the fall and winter. Dr. Stuart Hurlburt collected water samples from several soda ash mine evaporation ponds and evaluated for the following parameters: total dissolved solids (TDS), pH, salinity and presence of phytoplankton and zooplankton (Figs. 1 & 2). The salinity was measured with a hand refractometer with a range of 0 – 160 ppt. As the results from all samples were initially off-scale, the evaporation pond water samples were diluted 1:1 with tap water, and the salinity value for the diluted sample was doubled to provide an estimate of true salinity.

RESULTS & DISCUSSION

Water quality analysis demonstrated that the average trona mine evaporation pond has a ph>10.2 (range 10.25 – 10.66), and total salinities in excess of 174 ppt (range 174 – 240). Incorporating several years of water quality data from the mining operations, total dissolved solids ranged from 80,000-300,000+ ppm depending on the time of year (highest in the fall) (Table 4). To put these numbers in perspective, the total dissolved solids and total sodium concentrations in the tailings ponds in the fall are comparable to, and in some cases greater than, total dissolved solids in the playa lakes of southeastern New Mexico (**REFERENCE**). The range of total dissolved solids in seawater ranges approximately from 30,000-35,000 ppm. These extreme conditions account for the complete absence of both protozoan and metazoan zooplankton. Phytoplankton was exceptionally dense in all ponds evaluated, but contained very few species. No zooplankton were observed in water samples from any evaporation pond, but tiny flagellated chlorophytes were observed in water from all ponds sampled. These chlorophytes, along with dissolved organic matter, presumably interacted to account for the differential coloration of the ponds. On several occasions flying invertebrates were observed around the ponds or dead on the surface of ponds, which may explain why passerine species and occasionally bats were attracted to the ponds. The complete absence of both protozoan and metazoan zooplankton in the evaporation ponds confirmed the fact that the water contained no food source suitable for aquatic avian species, and there is evidence that eared grebes rarely, if ever, attempt to feed during the fall migration (Jehl & McKernan, 2002).

Table 4. Characteristics of soda ash mine evaporation ponds in southwestern Wyoming, sampled in October 2000.

Company Pond	Sampled Pond Area (acres)	pH	Salinity (ppt)	Phytoplankton	Zooplankton
OCI Corp-1[a]	350	10.51	210	Flagellated Chlorophytes (7-12µm)	None
OCI Corp-4[b]	75	10.66	174	"	"
FMC Grainger[c]	630	10.48	180	"	"
FMC Westvaco[d]	950	10.25	240	"	"
Solvay Minerals[e]	60	10.36	230	"	"

[a] OCI Corporation indicates that pond is only several inches deep, but 95% of birds utilize this pond. Phytoplankton dense with olive-green coloration.

[b] Water color is chartreuse green.

[c] Water color is olive green.

[d] Water color is dark brown, apparently reflecting dissolved organic matter, rather then phytoplankton.

[e] Water color is olive green

Figure 1. Photo demonstrating the brown coloration specific to one soda ash mine evaporation pond. The color of the pond water varied from light green to dark purple depending on algal species present. Note the salt crystal formation along the shoreline.

Figure 2. Dr. S. Hurlburt collecting water samples from a soda ash mine evaporation pond. Samples were analyzed for chemicals as well as living biological matter.

B. EVALUATION OF EARED GREBE BEHAVIOR AND NATURAL HISTORY

METHODS

Dr. Joseph Jehl evaluated physical characteristics of the ponds, which may contribute to their attractiveness to the grebes (Figs. 3). During migratory seasons, grebes fly at night, and typically land on water in the early morning hours just prior to sunrise. Only 3-4 evaporation ponds attract large numbers of birds, with most of these ponds in close proximity to the mine extraction/processing plants. The exception is a pond associated with the FMC Grainger plant, with the pond located approximately 10 miles from the main plant. Although this pond attracts many birds during the fall migration period, eared grebe morbidity and mortality is relatively low. The shorelines of most evaporation ponds have the appearance similar to hypersaline lakes, with white, salt precipitates covering the shore (Fig. 4).

RESULTS & DISCUSSION

Dr. Jehl reiterated a hypothesis made in the 1970s that the light illumination onto the ponds from the mine buildings at night makes them easily detectable by migrating birds. This may be the primary factor underlying the attraction of birds. One scenario would be that birds seeking to rest, land on the ponds, and by morning, their feathers are coated with sodium decahydrate crystals making them unable to fly (Figs. 5 & 6). There is a positive correlation between solubility of sodium decahydrate and water temperature, with sodium decahydrate crystallizing on substrate (in this case birds) more rapidly as water temperature decreases. We originally hypothesized that there may have been an aquatic invertebrate food source, such as brine shrimp, in the ponds, but Dr. Jehl indicated that grebes do not eat during migration (Jehl & McKernan, 2002). In fact the gastrointestinal tract decreases dramatically in size during migration. However, the flying invertebrates closely associated with the ponds may play a role in attracting other avian and mammalian species to the ponds. Additional mortality possibilities include: a) the physical weight of the crystals on the feathers, causing exhaustion and drowning; b) hypothermia secondary to skin wetting due to the crystals decreasing the insulation properties of the feathers; and/or c) physiological changes, such as serum electrolyte abnormalities.

Figure 3. OCI soda ash mine near Green River, Wyoming. One of the larger evaporation ponds associated with this mine is visible to the right of the mine plant, and has a typical salt encrusted shoreline.

Figure 4. A closer shoreline view of one of the evaporation ponds associated with the OCI soda ash mine. The white, salt encrusted shoreline appears similar to the shoreline of a typical hypersaline lake (e.g., Great Salt Lake in Utah or Mono Lake in California).

Figure 5. An eared grebe recently captured from the surface of a soda ash mine evaporation pond. The crystallization of the feathers is a typical manifestation of pond water exposure, which is exacerbated by cool water temperatures and duration of exposure. Many grebes attempt to remove the crystals with their beaks, causing self-trauma and bleeding.

Figure 6. A Northern Shoveler with salt encrustation on the ventral body feathers after exposure to the soda ash mine evaporation pond water.

C. CLINICAL PATHOLOGY

METHODS

Blood samples were collected opportunistically from eared grebes captured from soda ash mine evaporation ponds. For comparison, blood samples were also collected from "normal", pre-migratory eared grebes captured on breeding/nesting ponds in British Columbia, Canada. All blood samples were collected from the right jugular vein (Fig. 12) within 2 hours of capture and again at approximately 24 hours post-capture after the birds had been kept on fresh water. Approximately 1 ml of blood was collected from each bird at each of 2 time points. We hypothesized that any electrolyte imbalances (e.g. sodium) might begin to correct toward normal within 24 hours of the grebes being exposed to fresh water. All blood samples were placed in serum separator tubes and microhematocrit tubes and kept cool until processing. Within 60 minutes of blood collection, packed cell volume (PCV) by the microhematocrit technique and total protein by refractometer were determined, blood samples were centrifuged, and serum was refrigerated and shipped overnight to Marshfield Veterinary Laboratories (Marshfield, Wisconsin) for serum biochemical analysis. In addition, eared grebe body weights were determined during the 2002 field season. Data were statistically compared using the Student's T-Test, with statistical significance represented by $p < 0.05$.

RESULTS & DISCUSSION

The objective of collecting blood samples from eared grebes for serum biochemical analysis was to determine whether exposure to soda ash mine evaporation pond water caused physiologic perturbations indicative of organ dysfunction, electrolyte imbalances, or dehydration. A total of 12 serum and hematologic tests were evaluated (Appendix 4), but only a subset was considered biologically significant. Serum biochemical data from the 2001 and 2002 field seasons were combined and are presented in Table 5 and Figure 7. "Normal" eared grebe serum biochemical data are included and compared with those data illustrated in Table 5, and are presented in Table 6 and Figure 7. Exposure to the evaporation pond water caused a variety of physiologic changes in the eared grebes. Compared to the control grebes, most of the physiologic parameters of the eared grebes sampled immediately after capture from the evaporation pond water ("Pre-rehab") were elevated and there was a trend toward normal ranges after 24 hours of fresh water exposure ("Post-rehab"). Although many changes are apparent, the most interesting data include serum aspartate aminotransferase (AST), creatine kinase (CK), sodium (Na), and bicarbonate HCO_3 (see subsections below for detailed discussion).

Mean body weights of eared grebes are also presented in Table 4. Mean body weights decreased by approximately 11 grams for birds kept in rehabilitation facilities for 24 hours. Since the birds are in a catabolic state during migration (Jehl and Johansson, 2002; Jehl and McKernan, 2002), this weight loss may be significant, especially for juvenile birds and those at lighter weights at the onset of migration. Jehl has hypothesized that should the body weight of eared grebes decrease below 260 grams during migration, this may leave the birds with insufficient physiologic reserves to complete migration through the Great Salt Lake and on to Mono Lake, California (pers. comm.). Therefore, it is advised that all birds be rehabilitated as quickly as possible, so that they can be released and continue their migration without further compromise. Those birds unable to recover within 24 hours on fresh water, and dropping below 260 grams body weight should be considered poor candidates for continued successful migration.

Table 5. Mean (± SD) serum biochemical (combined 2001/2002) and body weight (2002 only) data collected from eared grebes at soda ash mine tailings ponds in Wyoming. The "pre" and "post" refer to pre-rehabilitation and post-rehabilitation of the birds at the soda ash mine evaporation ponds. Statistical significance between groups is represented by ***** ($p < 0.05$).

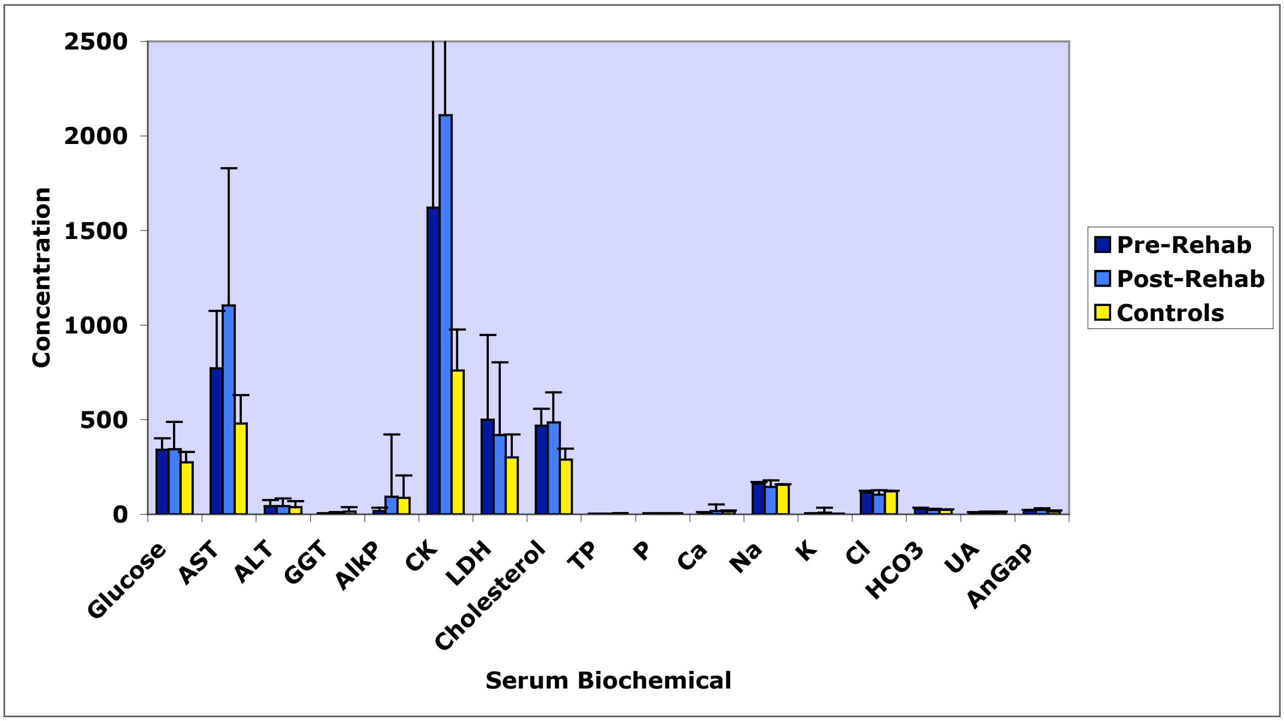
Serum Biochemical	Pre-Rehab (N=63)		Post-Rehab (N=63)
Glucose (mg/dl)	341.4 ± 60.4	*	312.7 ± 37.0
AST (U/L)	772.4 ± 301.9	*	1153.4 ± 677.4
ALT (U/L)	42.5 ± 33.4		44.9 ± 40.4
GGT (U/L)	3.5 ± 3.4	*	7.4 ± 5.2
Alkaline Phos. (U/L)	18.2 ± 16.0		17.6 ±15.4
Creatine Kinase (U/L)	1622.1 ± 1164.0		2166.6 ± 3809.4
LDH (U/L)	500.7 ± 447.2		408.7 ± 386.5
Cholesterol (mg/dl)	469.2 ± 89.5		510.8 ± 105.4
Total Protein (g/dl)	3.4 ± 0.6		3.6 ± 0.6
Phosphorus (mEq/L)	3.5 ± 1.3	*	2.7 ± 0.9
Calcium (mg/dl)	9.3 ± 0.8	*	9.9 ± 0.8
Sodium (mEq/L)	162.5 ± 8.8	*	151.9 ± 4.6
Potassium (mEq/L)	3.0± 1.6		2.7 ± 1.4
Chloride (mEq/L)	116.8 ± 7.2	*	109.4 ± 6.8
Bicarbonate (mmol/L)	29.8 ± 4.6	*	22.4 ± 4.7
Uric Acid (mg/dl)	7.2 ± 3.2	*	8.1 ± 3.0
Anion Gap	18.9 ± 3.5	*	22.6 ± 4.8
PCV (%)	53.6 ± 4.6		50.9 ± 4.8

Body Weight (grams)	293.5 ± 35.6		282.7 ± 31.4

Table 6.: Mean (± SD) serum biochemical (combined 2001/2002) data from eared grebes captured from soda ash mine evaporation ponds in Green River, Wyoming. The "pre" and "post" refer to pre-rehabilitation and post-rehabilitation of the birds at the trona tailings ponds. The "normals" are data collected from eared grebes in British Columbia, Canada. Statistical significance between groups is represented by * ($p < 0.05$).

Serum Biochemical	Pre-Rehab (N=63)		"Normals" (N=20)		Post-Rehab (N=63)
Glucose (mg/dl)	341.4 ± 60.4	*	274.45 ± 55.2	*	312.7 ± 37.0
AST (U/L)	772.4 ± 301.9	*	480.15 ± 150.0	*	1153.4 ± 677.4
ALT (U/L)	42.5 ± 33.4		37.05 ± 31.0		44.9 ± 40.4
GGT (U/L)	3.5 ± 3.4		14.4 ± 23.6		7.4 ± 5.2
Alkaline Phos. (U/L)	18.2 ± 16.0	*	86.7 ± 117.7		17.6 ±15.4
Creatine Kinase (U/L)	1622.1 ± 1164.0	*	759.15 ± 218.7	*	2166.6 ± 3809.4
LDH (U/L)	500.7 ± 447.2	*	301.2 ± 119.4		408.7 ± 386.5
Cholesterol (mg/dl)	469.2 ± 89.5	*	288.7 ± 56.7	*	510.8 ± 105.4
Total Protein (g/dl)	3.4 ± 0.6	*	4.85 ± 0.60	*	3.6 ± 0.6
Phosphorus (mEq/L)	3.5 ± 1.3		3.54 ± 2.4		2.7 ± 0.9
Calcium (mg/dl)	9.3 ± 0.8	*	15.07 ± 6.5		9.9 ± 0.8
Sodium (mEq/L)	162.5 ± 8.8	*	155.85 ± 2.6	*	151.9 ± 4.6
Potassium (mEq/L)	3.0± 1.6	*	2.19 ± 0.6		2.7 ± 1.4
Chloride (mEq/L)	116.8 ± 7.2	*	120.2 ± 3.8	*	109.4 ± 6.8
Bicarbonate (mmol/L)	29.8 ± 4.6	*	22.55 ± 3.3		22.4 ± 4.7
Uric Acid (mg/dl)	7.2 ± 3.2	*	11.54 ± 3.2	*	8.1 ± 3.0
Anion Gap	18.9 ± 3.5	*	15.35 ± 4.5	*	22.6 ± 4.8

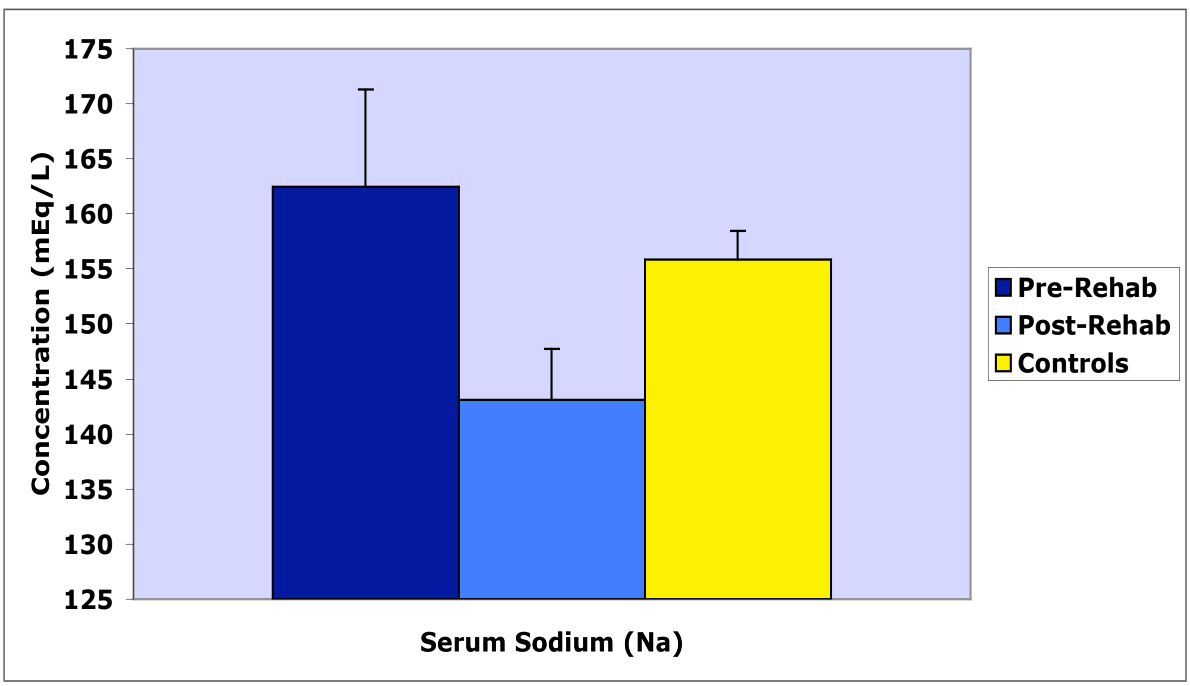
Figure 7. Mean (± SD) serum biochemical data (combined 2001/2002) from eared grebes captured from soda ash mine evaporation ponds in Green River, Wyoming and "normal" eared grebes captured on nesting ponds British Columbia, Canada in August 2003. The "pre" and "post" refer to pre-rehabilitation and post-rehabilitation of the birds at the soda ash mine evaporation ponds.



Serum Sodium

Serum sodium was one of the more compelling biochemical parameters. Serum sodium concentrations were elevated in the pre-rehabilitation sample (mean = 162.5 mEq/L), and decreased toward a more normal range (mean = 151.9 mEq/L) after the birds had been maintained on fresh water for approximately 24 hours (Table 5; Figure 8). Mean serum sodium concentrations from the post-rehabilitation samples were in the range of those collected from the control grebes. Sodium concentrations are maintained physiologically within very narrow limits in birds and mammals, despite wide fluctuations in intake. Elevated serum sodium can result from increased sodium intake, excessive water loss or markedly decreased water intake. In mammalian species studied, serum sodium in excess of 160 mEq/L is frequently considered to be consistent with sodium toxicity. Most studies of sodium toxicity in avian species do not report serum sodium concentrations, but only focus on brain sodium. Free-ranging mallards exposed to hypersaline lakes in New Mexico, and captive mallards experimentally exposed to the same hypersaline water had elevated plasma sodium concentrations of > 160 mEq/L (Meteyer et al., 1997). Northern shoveler ducks exposed to hypersaline lakes in Saskatchewan had serum sodium concentrations that ranged from 154 – 169 mmol/L, which was significantly higher than control birds of the same species (Wobeser & Howard, 1987). In addition, in a species highly adapted to hypersaline environments, it remains unclear whether eared grebes are able to tolerate higher serum sodium concentrations than other avian species.

Figure 8. Mean (± SD) serum sodium (combined 2001/2002) collected from eared grebes at soda ash mine tailings ponds in Green River, Wyoming and "normal" eared grebes captured on nesting ponds British Columbia, Canada in August 2003. The "pre" and "post" refer to pre-rehabilitation and post-rehabilitation of the birds at the trona tailings ponds.



Liver/Muscle Enzymes

Two serum enzymes commonly used to evaluate and differentiate liver cell and muscle cell damage are aspartate aminotransferase (AST) and creatine kinase (CK) (Figs. 9 & 10). Elevated AST concentrations in avian species are frequently associated with liver cell damage, but can be elevated with muscle breakdown as well. Elevated CK is strictly associated with muscle cell damage. Muscle cell damage is associated with weight loss and consequent muscle breakdown. With simultaneous elevation of both AST and CK, it is common to assume that it is muscle breakdown that is significantly contributing to these changes. These grebes are not eating during this migration period and continue to rapidly lose weight during the 24-hour rehabilitation period (Table 5). The CK continues to increase during the rehabilitation period, which would be predicted and associated with the continued weight loss (Fig. 10). The AST concentrations also continue to increase during the 24-hour rehabilitation period, which may also be a function of muscle breakdown (Fig. 9). However, histopathologic data confirm that the livers of all examined eared grebes have vacuolar changes of unknown etiology, so there are clearly pathologic liver changes in these birds as well. No livers from control birds had abnormalities. These data would suggest that liver cell damage couldn't be differentiated from muscle cell breakdown using AST and CK concentrations alone, as the migration-associated muscle breakdown is significant enough to potentially mask evidence of liver-specific cell damage. Other possible contributing factors to elevated CK and AST include muscle cell breakdown from struggling on the surface of the ponds, capture myopathy, and manual handling, although these are less likely as netting and handling control birds in British Columbia did not appear to induce significant increases in CK and AST.

Figure 9. Mean (± SD) serum aspartate aminotransferase (combined 2001/2002) collected from eared grebes at soda ash mine tailings ponds in Green River, Wyoming and "normal" eared grebes captured on nesting ponds British Columbia, Canada in August 2003. The "pre" and "post" refer to pre-rehabilitation and post-rehabilitation of the birds at the trona tailings ponds.

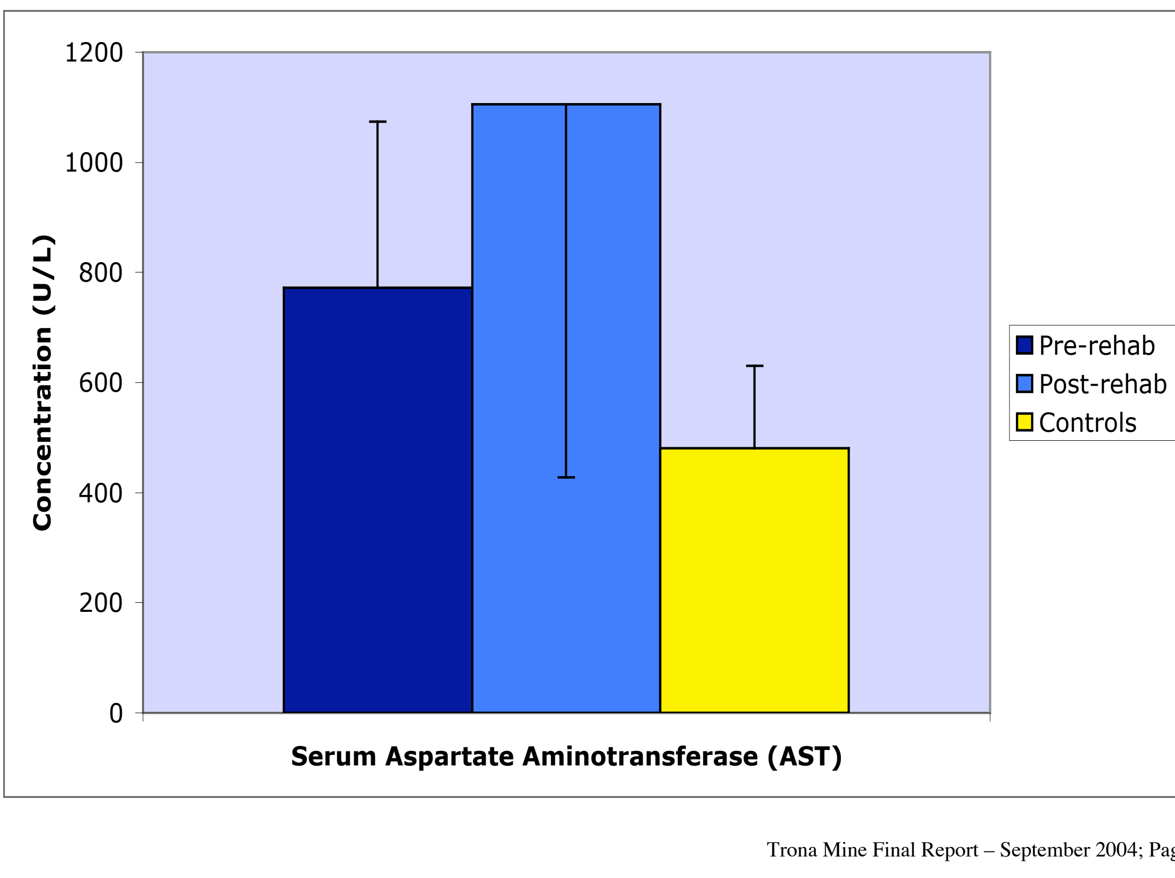
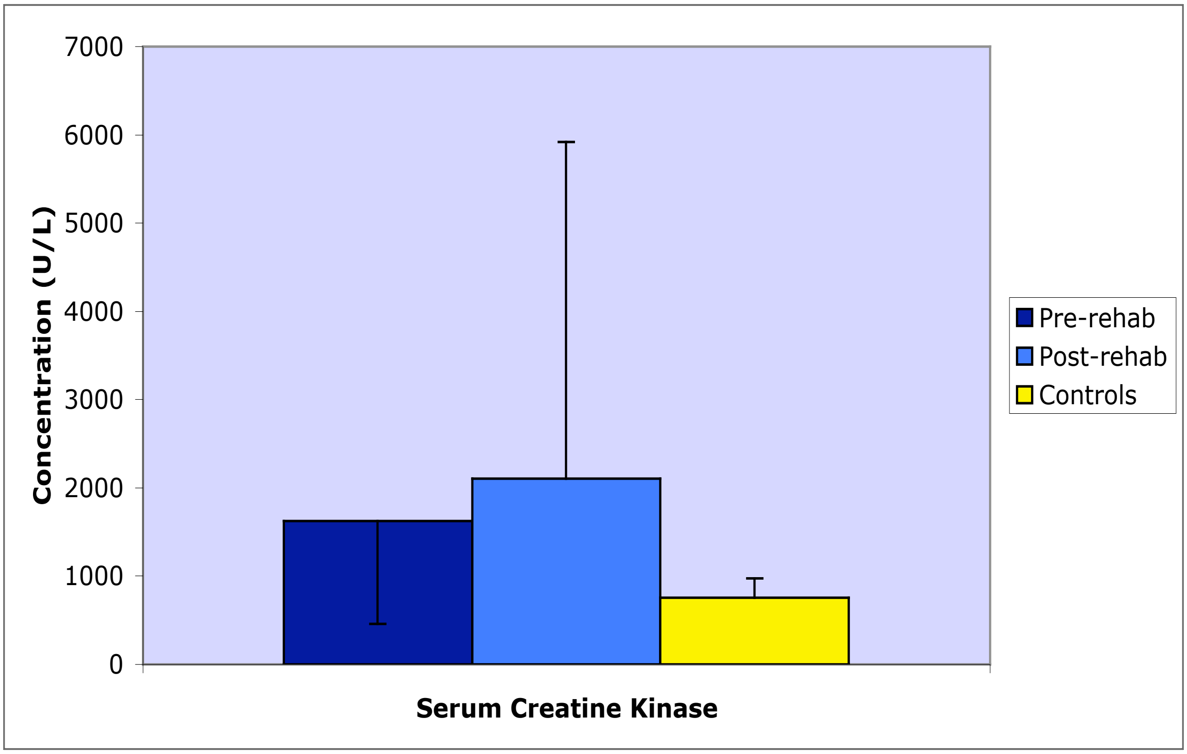


Figure 10. Mean (± SD) serum creatine kinase (combined 2001/2002) collected from eared grebes at soda ash mine tailings ponds in Green River, Wyoming and "normal" eared grebes captured on nesting ponds British Columbia, Canada in August 2003. The "pre" and "post" refer to pre-rehabilitation and post-rehabilitation of the birds at the trona tailings ponds.



Bicarbonate

Elevated serum bicarbonate is used as an indicator of metabolic alkalosis. The acid-base status of the birds tends toward alkaline, rather than neutral or acidic. In the eared grebes captured from soda ash evaporation ponds, serum bicarbonate was significantly elevated during the pre-rehabilitation period compared to both post-rehabilitation samples and controls (Fig. 11). The 24-hour rehabilitation period contributed to a significant decrease in serum bicarbonate to a concentration statistically indistinguishable from controls. Reference values have not been established for most avian species, although the reference range in adult budgerigars is 21 – 26 mmol/l. The marked alkalinity of the evaporation pond water, and the possible ingestion of the primary pond chemical compound, sodium sesquicarbonate, which has sodium bicarbonate in its chemical structure, could be the major contributing factor to the elevated serum bicarbonate.

Figure 11. Mean (± SD) serum bicarbonate (combined 2001/2002) collected from eared grebes at soda ash mine tailings ponds in Green River, Wyoming and "normal" eared grebes captured on nesting ponds British Columbia, Canada in August 2003. The "pre" and "post" refer to pre-rehabilitation and post-rehabilitation of the birds at the trona tailings ponds.

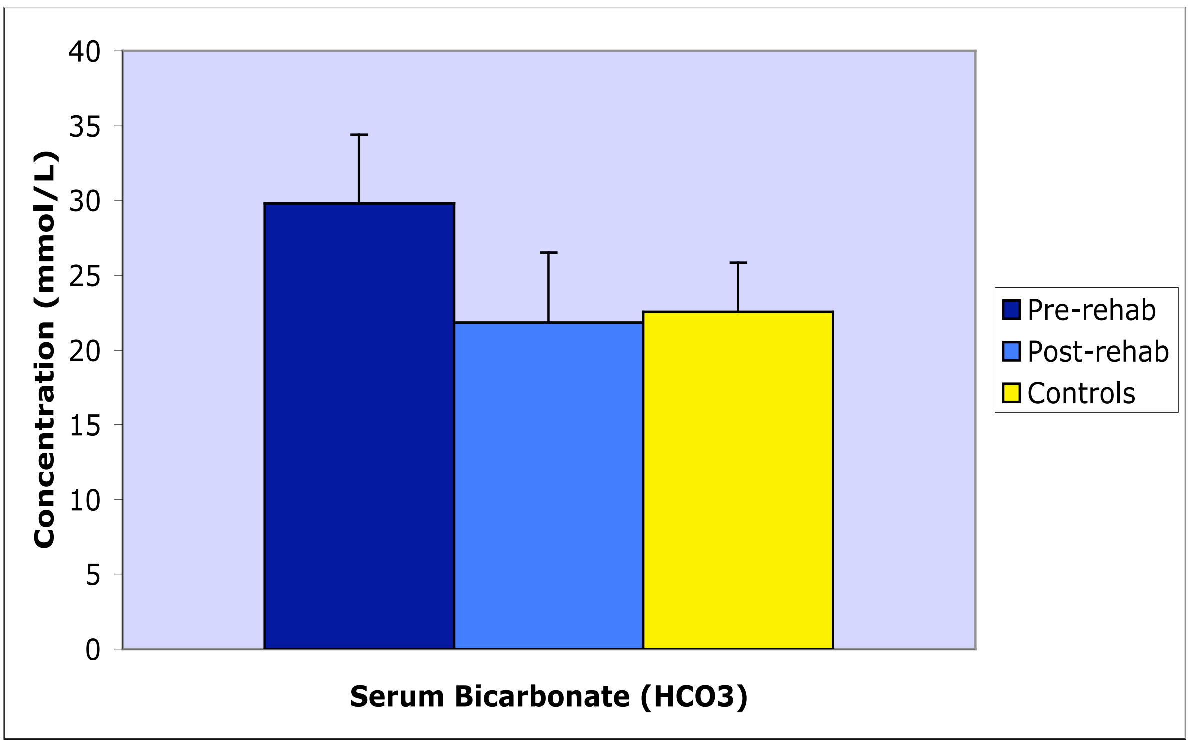


Figure 12. Blood sample collected from the right jugular of an eared grebe recently captured from a soda ash mine evaporation pond.

D. GROSS AND HISTOLOGIC PATHOLOGY

METHODS

All deceased eared grebes (N = 13) in good post-mortem condition found on the surfaces of the soda ash mine evaporation ponds were subjected to full necropsies (Fig. 13). Gross and histologic examinations were completed on 7 eared grebes, while gross examinations have been completed on 6 additional eared grebes (histologic examination is pending). Most of the brains (N = 11) were sent for biochemical analysis, and therefore, were not evaluated grossly or histopathologically. In addition, normal grebes from British Columbia (N = 5) were humanely euthanized and subjected to complete necropsies. All tissues, except most brains, were sent to Dr. Charlotte Quist for histopathologic evaluation. All normal grebe brains (N = 5) were frozen and submitted to the NWHC for biochemical analysis. All original necropsy reports are included in Appendix 5.

RESULTS & DISCUSSION

Birds from the soda ash mine ponds, submitted unwashed, frequently had extensive crystal formation associated with feathers, particularly wings and head, which could have contributed to drowning. However, the degree of crystal accumulation during the ante-mortem versus post-mortem period has not been determined. Body condition was considered good for all grebes examined. All birds examined from the evaporation ponds had significant pulmonary vascular congestion with occasional mild pulmonary hemorrhage (Fig. 14). Histologic evidence was considered consistent with drowning or other event that caused antemortem or terminal pulmonary distress. However, it remains unclear whether pond water passively flowed into the trachea and lungs after death, or whether aspiration of pond water contributed directly to death. None of the control grebes exhibited these pathologic lung changes. Vacuolar hepatopathy was another consistent histopathologic finding from eared grebes exposed to the evaporation pond water. None of the control grebes from British Columbia had evidence of this lesion. This would suggest that the hepatopathy is associated with pond water exposure. On the other hand, physiologic alterations associated with migration or stress cannot be completely ruled out.

One hypothesis proposed to explain grebe mortality on the ponds is hypothermia secondary to excessive loss of feather insulation and wetting of the skin. Although degree of feather wetting was not or could not be evaluated for every bird, there was evidence in 2 of the deceased grebes that the deeper layers of feathers and down feathers were dry. Some of the other birds had been washed of crystals prior to necropsy evaluation, so it was impossible to determine degree of feather wetting. This hypothesis remains viable, and should be tested in future experiments.

Another consistent finding in all affected birds found dead on the soda ash mine evaporation ponds is that they have elevated brain sodium concentrations compared to normal eared grebes (see Brain Biochemical Analysis section below). Most of the affected birds had brain sodium concentrations > 2000 ppm, which is considered toxic in most mammalian species and in domestic chickens (National Research Council, 1980; Julian & Brown, 1997). However, we were unable to document typical antemortem clinical signs that have been reported to be associated with sodium intoxication in other species, such as ataxia, paresis, paralysis, seizures, and coma. Post-mortem changes were also not consistent with previous reports of changes associated with sodium intoxication in birds. For example, brain lesions, other than vasocongestion, were not observed in grebes from which brains were collected for histopathology (as well as a single merganser), despite previously reported lesions observed in the brains of salt-intoxicated pigs (e.g., moderate to severe vasocongestion and menigoencephalitis). In addition, most reports of avian salt intoxication include consistent cerebral lesions including moderate to severe meningeal vasocongestion. Severe vasocongestion associated with any part of the brains was not observed. However, mild cerebral and cerebellar vasocongestion were consistent with similar findings in other wild bird species (Trainer & Karstad, 1960; Windingstad et al., 1987; Meteyer et al., 1997; Gordus et al., 2002). It may be that avian species do not develop classic clinical signs and pathologic lesions (e.g., eosinophilic meningoencephalitis) observed in swine with salt intoxication. Alternative explanations for inconsistencies between studies include; different avian species with different biological and physiological mechanisms, and the exposure to, or accumulation of other chemical substances in tissues.

Previous reports of salt intoxication in avian species have described pathologic skeletal muscle and myocardial lesions associated with dehydration, but the grebes from the soda ash evaporation ponds had no such lesions.

Several grebes from soda ash mine ponds and all normal grebes caught in British Columbia had evidence of cestodes in their gastrointestinal tracts, frequently associated with mild parasitic enteritis. This was considered non-significant and is likely considered representative of most wild-caught eared grebes. Figure 15 demonstrates feather material in the ventriculus of a normal eared grebe captured in British Columbia, and confirms that this species consumes feather material regularly during preening. This may be an important method for chemical ingestion as eared grebes consistently and aggressively preen their feathers during sodium decahydrate encrustation on the soda ash mine evaporation ponds.

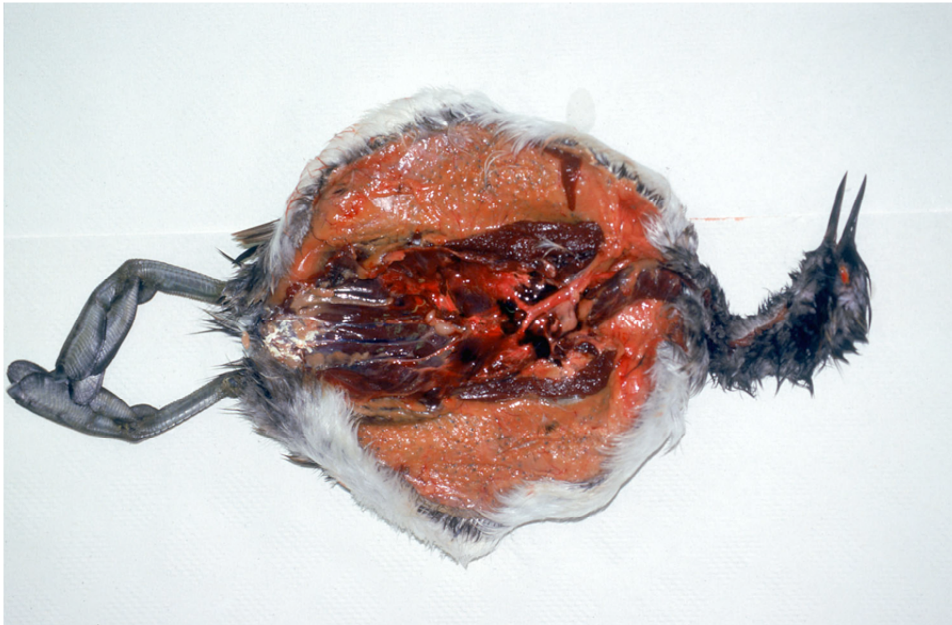


Figure 13. Gross necropsy of an eared grebe found dead on the surface of a soda ash mine evaporation pond.

Figure 14. Lungs from the same deceased eared grebe in Figure 13, demonstrating the marked vascular congestion and hemorrhage typically observed during post-mortem examination of the birds collected from the evaporation pond water.

Figure 15. Proventriculus of a “normal” eared grebe collected from a nesting pond in British Columbia. Note that the proventricular contents consist of food material admixed with many feathers. This ingestion of feathers as a normal behavior may contribute to ingestion of chemicals in soda ash mine evaporation ponds when the birds aggressively preen the salt

E. BRAIN BIOCHEMICAL ANALYSIS

METHODS

Brains from all eared grebes collected from the soda ash mine evaporation ponds (N = 11) and those collected from euthanized eared grebes captured on breeding/nesting in British Columbia, Canada (N = 5) were removed using a standard protocol (Appendix 3). All extracted brains were processed for analysis at the NWHC and tissue extracts submitted to the Soils Analysis Laboratory at the University of Wisconsin for inductively coupled analysis of the following chemicals: phosphorus, potassium, calcium, magnesium, sulfur, zinc, manganese, iron, copper, aluminum, and sodium. Brain biochemical concentrations were statistically compared using the Student’s T-Test, with statistical significance represented by $p < 0.05$.

RESULTS AND DISCUSSION

Eared grebe brain biochemical data from the 2001 and 2002 field seasons, along with control brains samples in British Columbia, Canada are presented in Table 7 and Fig. 16, and brain sodium concentrations are presented in Table 8 and Fig. 17. Brain sodium concentrations from the 3 passerine species (5 birds) opportunistically collected for the soda ash mine evaporation ponds are presented in

Table 9. The brain sodium data from eared grebes collected from soda ash mine evaporation ponds are elevated compared to the control grebes. The toxic range for brain sodium concentrations in domestic species is considered to be greater than 2000 ppm, which is considered toxic in most mammalian species and in domestic chickens (National Research Council, 1980; Julian & Brown, 1997). (Girdus et al., 2002; Meteyer et al., 1997; Windingstad et al., 1987). Eight of 11 grebes from the soda ash evaporation ponds have brain sodium concentrations exceeding 2000 ppm. Two of these grebes died after being kept on fresh water for greater than 24 hours, so it is possible that brain sodium concentrations in these 2 birds had decreased below the range considered toxic. The third bird had a brain sodium concentration of 1991 ppm, which is indistinguishable from 2000 ppm. This is in contrast to the control grebes in which brain sodium concentrations were consistently less than 2000 ppm. Brain sulfur and zinc concentrations are also significantly elevated in the grebes exposed to soda ash mine pond water compared to control grebes.

One hypothesis is that grebes on the soda ash ponds are ingesting the pond water or sodium decahydrate crystals from feathers during preening. Ingesting a small quantity of crystals could be considered the equivalent of ingesting a larger quantity of water, since the crystals are a concentrated form of sodium. This could significantly increase the likelihood of systemic absorption. With sodium intoxication, the birds should become neurologically impaired, which could alter their normal swimming posture and ability to hold their head in an upright position, and they could eventually drown. With feather encrustation by sodium decahydrate crystals, this could exacerbate the physical impairment ultimately leading to drowning as well. This might explain why all birds had lung pathology consistent with drowning. However, sodium toxicity in birds has been described clinically as being consistent with weakness or neurological impairment (Meteyer et al., 1997). However, the eared grebes captured on soda ash mine evaporation ponds in this study did not demonstrate clinical signs consistent with neurological impairment. In fact, most grebes were alert and responsive during the blood sample collection immediately after capture. In other studies, waterfowl with suspected sodium intoxication have not been observed with obvious neurologic impairment or weakness (Windingstad et al., 1987; Wobeser & Howard, 1987; Meteyer et al., 1997). One alternative explanation is that eared grebes as a species may normally have elevated brain sodium concentrations compared to other bird or mammal species, and that they have evolved to physiologically compensate for such differences. Another explanation may be that other chemicals in the evaporation ponds are contributing to physiologic impairments, and therefore, clinical signs of sodium intoxication alone are not observed. For example, fluoride, concentrations are typically high in trona mineral, but we did not include this chemical element in our analysis. A third alternative explanation, which is less likely, is that human error during brain removal was contributing to sodium contamination. Although extreme care was taken to avoid contamination during brain extraction, it remains possible that some contamination occurred.

It is difficult to explain why so many passerine species die acutely and are frequently found on the surfaces of the ponds, but determining mortality factors in other avian species may contribute to our understanding of eared grebe mortality as well. Perhaps, passerines are drinking the evaporation pond water and succumbing to high chemical concentrations, which cause rapid death due to their high metabolic rate. It is also difficult to explain why groups of bats, and occasional large raptor species have been found dead on the pond surfaces. These issues must be addressed in future research.

Table 7.: Mean (± SD) brain biochemical concentrations in parts-per-million (ppm) from eared grebes found dead on Wyoming soda ash mine evaporation ponds, and "normal" grebes captured on nesting ponds in British Columbia, Canada. Statistical significance between groups is represented by ***** ($p < 0.05$).

Brain Chemical	Trona Grebes (N=11)	"Normal" Grebes (N=5)	
Phosphorus	3143.6 ± 406.7	3088.0 ± 71.6	
Potassium	2110.9 ± 511.3	3212.0 ± 144.6	
Calcium	236.6 ± 66.3	74.0 ± 20.7	
Magnesium	151.1 ± 36.2	154.0 ± 5.5	
Sulfur	1928.3 ± 257.2	1554.0 ± 43.9	*
Zinc	15.6 ± 2.9	10.9 ± 0.3	*
Manganese	1.2 ± 1.3	1.9 ± 1.0	
Iron	103.0 ± 115.5	35.22 ± 3.8	
Copper	2.6 ± 0.8	2.31 ± 0.4	
Aluminum	45.8 ± 128.8	7.5 ± 1.0	
Sodium	3338.7 ± 1833.2	1355 ± 59.8	*

Table 8.: Summary of brain sodium results from eared grebes found dead on soda ash mine evaporation ponds in southwestern Wyoming during the 2001 and 2002 migratory season, and control grebes captured on nesting ponds in British Columbia, Canada.

Wyoming Grebes	Brain Na (ppm)	Control Grebes	Brain Na (ppm)
1	4023	1	1449
2	4364	2	1354
3	2288	3	1298
4	7018	4	1309
5	2515	5	1365
6	1149[a]		
7	977 [a]		
8	5459		
9	3317		
10	3627		
11	1991		

[a] Birds rehabilitated on fresh water for ≥ 24 hours prior to death

Table 9.: Summary of brain biochemical results on dead passerines opportunistically collected from one tailings pond in southwestern Wyoming during the 2002 migratory season.

Bird Species	Brain Na (ppm)
Cowbird	4268.82
Cowbird	3226.04
Cowbird	6524.44
Townsend's Warbler	3420.90
House Wren	1764.73

Figure 16.: Mean (± SD) brain biochemical concentrations in parts-per-million (ppm) from eared grebes found dead on Wyoming soda ash mine evaporation ponds, and "normal" grebes captured on nesting ponds in British Columbia, Canada.

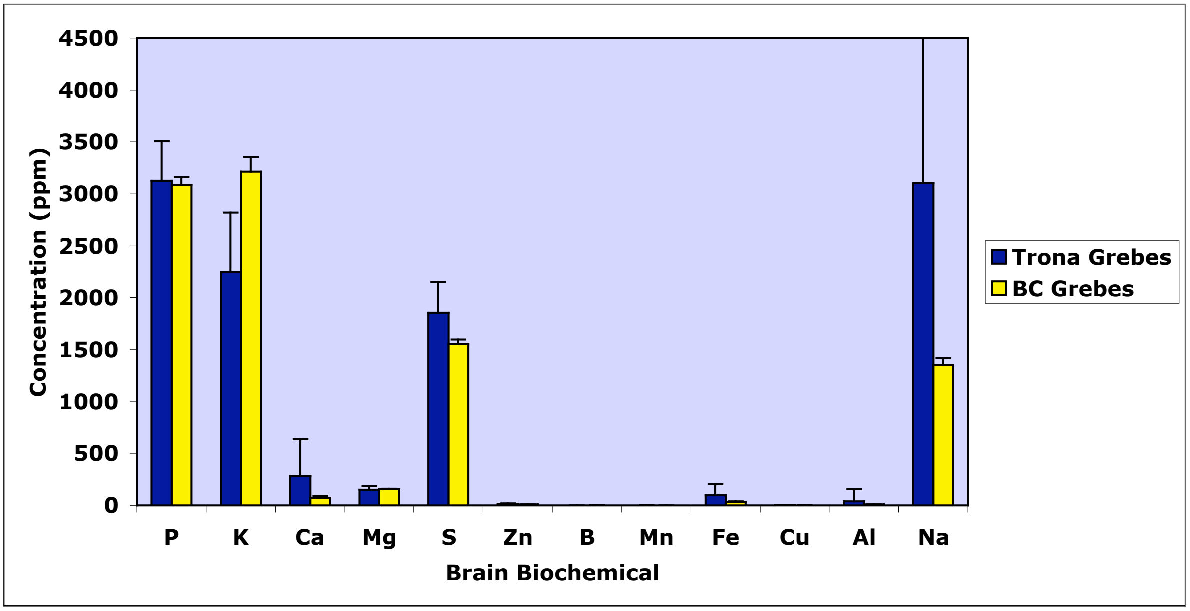
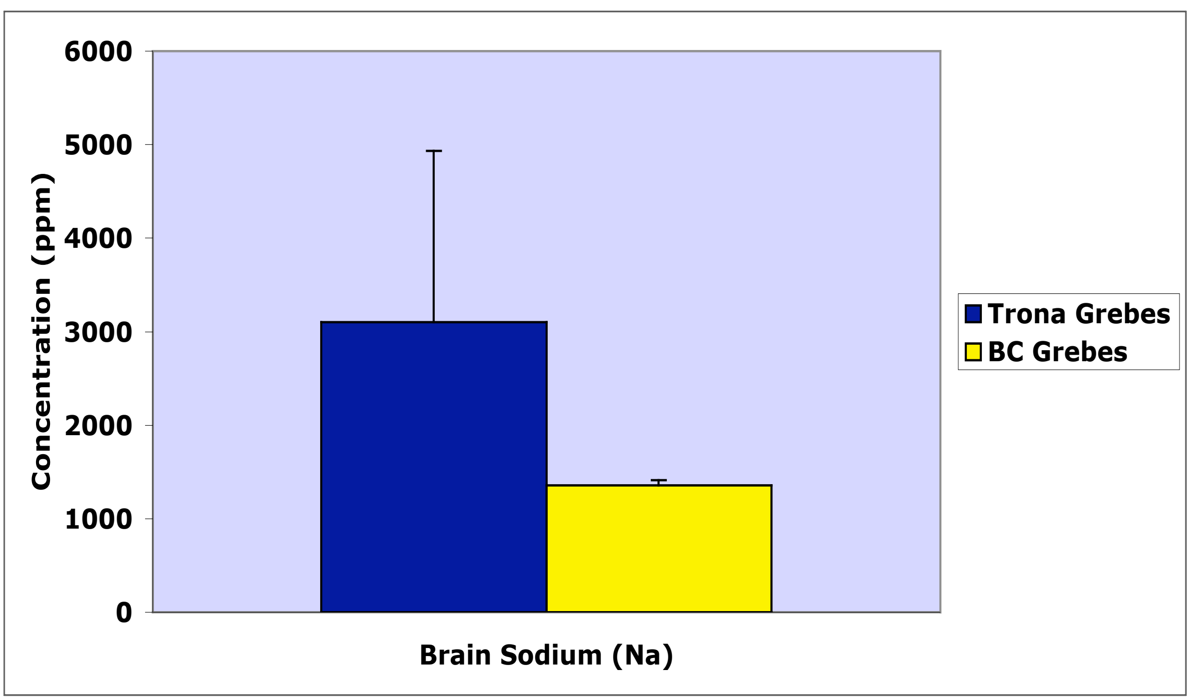


Figure 17.: Comparison of mean brain sodium concentration from eared grebes found dead on soda ash mine evaporation ponds in southwestern Wyoming during the 2001 and 2002 migratory season, and control grebes captured on nesting ponds in British Columbia, Canada.



F. REVIEW OF AVIAN REHABILITATION EFFORTS

In 1975, faced with massive migratory bird mortalities on the soda ash mine evaporation ponds, 2 of the mining companies (FMC and Stauffer at the time) established bird rehabilitation programs. The objective was to retrieve all birds physically/physiologically affected by exposure to evaporation pond water, and rehabilitate them for return to the wild. Airboats were purchased for use on the evaporation ponds for collecting live and dead birds from the pond water. In addition, both companies had constructed and implemented heated facilities for use in bird rehabilitation. In 1975, FMC Corporation retained a waterfowl rehabilitation consultant, Truslow Farms, Inc., Maryland, in order to develop on-site rehabilitation facilities and train mine personnel in rehabilitation techniques. Most of the techniques remain in use today at FMC Corp., OCI Corp., and Solvay Minerals. We have no information on the rehabilitation program at the General Chemical Corporation.

In August 2000, we were shown the rehabilitation facilities at FMC-Westvaco, FMC-Grainger, Solvay and OCI. In general, the mines are doing an excellent job of rehabilitating birds and decreasing overall aquatic bird mortality on the ponds. However, we do not know the long-term consequences of soda ash mine evaporation pond water exposure, nor do we know how many of the birds successfully complete their fall migration. A brief description of the bird rehabilitation procedures is as follows:

> A shore patrol consisting of 1 - 2 individuals is sent out in the early morning in order to determine whether birds are on the pond, and, if so, whether they can be collected from the shore. If not, an airboat is launched, and nets are used to catch the affected birds, which may be covered in sodium decahydrate crystals. and unable to rapidly escape. The degree to which the feathers of a bird are covered with crystals is dependent on a number of factors, including total alkalinity of pond water, ambient temperature, and bird activity. The result of crystals and alkali deposition on feathers is frequently manifested as the bird's inability to fly or dive. Once caught in a net, the birds are placed in holding boxes until the crew can return with all affected birds to the indoor rehabilitation facilities. On any given morning, the crew may collect no birds or have collected close to 200 birds. All captured birds are brought into an indoor holding facility where the alkali deposition and/or sodium decahydrate crystals are washed from the feathers and skin using warm, fresh tap water. In cases of severe weakness, birds are wrapped in towels and warmed. If needed, each bird is syringe-fed approximately 5-10 cc of Pedialyte depending on the size of the bird. All birds are put in an indoor fresh-water holding tank and held until their feather water repellency returns (usually within 24 hours). Once it has been determined by the rehabilitation crew that each bird has regained water repellency, the birds are put into a carrying box and released outside of the mine property, which is frequently on the Green River in the vicinity of Green River, Wyoming. The same procedure is employed in the late afternoon at most of the mines as well.

We were impressed with the rehabilitation facilities at the mines, particularly the indoor holding tanks at FMC-Westvaco. The FMC Westvaco facility is a small building dedicated solely to bird rehabilitation efforts. It has fresh, running water in the large holding tanks, and it is totally enclosed so that it stays warm and decreases exposure to environmental changes. Although we originally recommended that all of the mine bird rehabilitation facilities use the FMC-Westvaco facility as a model, this was not to denigrate the efficacy of the rehabilitation efforts at the other mines. In fact, all 4 mines evaluated had similar success at rehabilitating birds, with greater than 90% of birds captured surviving and being

released. We merely used the FMC-Westvaco rehabilitation facility as an ideal model for others to incorporate into their own facilities if lacking different components.
It was clear that past hazing efforts were futile, other than chasing birds from the ponds using the airboats during routine collection of affected birds. Most species did not leave the ponds after zon guns were used. The only effective method for reducing bird use on the evaporation ponds is to reduce the size of the ponds through active evaporation processes or recycling of the pond water for use in the mining operations. This is being accomplished successfully at Solvay Minerals, and has led to a reduction in overall bird use and numbers of affected individuals. Unfortunately, we have been unable to accurately assess the impact on small avian species, particularly passerines, but we suspect that passerine mortality is high and easily overlooked due to their small size.

III. CONCLUSIONS AND FURTHER QUESTIONS

CONCLUSIONS

Although we had hoped to complete this study within a 2-year period, we were unable to complete all of our objectives due to complications regarding species studied, difficulties associated with laboratory-based experiments, and limited funding. While firm conclusions cannot be made, the following is a list of directions toward progress in understanding the issue of avian mortality associated with soda ash mine evaporation pond water exposure:

1. Soda ash mine evaporation ponds have very high salinities and total dissolved solids, which become concentrated during cool weather in the fall, coincident with the autumnal migration of many avian species. The only viable organisms residing in these ponds are phytoplankton species.
2. Eared grebes were the focus of our study because this is the species with the highest morbidity and mortality at all of the evaporation ponds. Eared grebes generally migrate at night, and typically land on the soda ash mine ponds early in the morning prior to sunrise. It is hypothesized that lights from the mine buildings highlight the evaporation ponds for the night-migrating grebes, and the appearance of the ponds resembles the hypersaline lakes that are part of this species' preferred habitat.
3. Most live eared grebes captured from the soda ash mine evaporation ponds in Wyoming had multiple serum abnormalities compared to control grebes. There is concern that elevated serum sodium concentrations may be contributing to sodium toxicity in these birds. Serum AST and CK were also elevated, which may indicate liver and/or muscle degeneration as well.
4. Most deceased eared grebes found on the evaporation ponds had elevated brain sodium, sulfur and zinc concentrations compared to control grebes. Elevated brain sodium in conjunction with elevated serum sodium concentrations has led us to hypothesize that sodium toxicity may be playing a key role in morbidity and mortality of the grebes. However, the grebes do not demonstrate observable clinical signs typical of sodium toxicity in other avian species. In addition, eared grebes do not demonstrate pathologic brain lesions typical of birds succumbing to sodium intoxication. All deceased grebes had pathologic pulmonary lesions consistent with drowning, but we were unable to determine whether this was the ultimate cause of death or a post-mortem phenomenon. All affected eared grebes evaluated had pathologic vacuolar lesions in the liver (vacuolar hepatopathy), which were not present in the control grebes. A similar lesion has been observed previously in sodium-intoxicated

waterfowl (Windingstad et al., 1987). It is not clear whether this lesion is directly associated with exposure to the chemicals in the evaporation ponds, or if it is associated with the physiologic stress of migration.

5. The rehabilitation programs appear to be working relatively well, and have significantly decreased aquatic bird mortality on the ponds. However, we have not been able to quantify the morbidity and mortality of other avian (e.g., passerines, raptors) and mammalian (e.g., bats) species. Based on anecdotal evidence and nonsystematic observations during our trips to Wyoming, we suspect that many passerines are being affected, and are easily unaccounted for because of small size. It is possible that the small avian species either die acutely on the surface of the ponds, or fly off to die elsewhere. It is possible that some of the small species dying on the pond surface will crystallize rapidly and sink, making quantification impossible.

FURTHER QUESTIONS

1. Is the apparent sodium intoxication a real phenomenon and/or are other chemicals from the evaporation ponds involved in bird deaths, such as sulfur, zinc, fluoride, etc.? Perhaps biochemical evaluation of gut contents would be helpful to determine whether the grebes are ingesting high concentrations of the sodium decahydrate crystals. Biochemical evaluation of liver samples from deceased birds would also be useful in future studies.
2. Could sodium contamination during brain removal for biochemical analysis be responsible for elevated brain sodium concentrations?
3. To what degree does antemortem versus post-mortem salt crystal formation occur, and how does this impact the working hypothesis of drowning as a primary mortality factor?
4. Do affected birds exhibit neurologic signs that are attributable to sodium toxicity as seen in other avian or mammalian species, and to what degree do these clinical signs affect flight ability? This brings into question whether the grebes are succumbing to sodium toxicity without expected neurologic signs, or whether the grebes are able to physiologically compensate with elevated brain sodium concentrations. Grebes are uniquely suited to adaptation to hypersaline environments, and compensatory mechanisms for dealing with elevated brain and serum sodium concentrations would be unique among bird species studied. Further studies, such as histopathologic evaluation of salt glands, and experimental exposure to sodium decahydrate crystals from Wyoming trona ponds may be of benefit.
5. What is the significance of vacuolar hepatopathy and elevated AST concentrations? Are these pathologic lesions associated with toxicity or part of the normal migratory process?
6. Although laboratory-based experiments were not deemed feasible due to the difficulty of maintaining eared grebes in captivity, incomplete data may necessitate developing methods to maintain this species in the laboratory. An alternative would be to use a “surrogate” species of aquatic bird that would provide comparable results to the grebes. This may be very difficult due to the natural history of the eared grebe and their affinity for hypersaline environments. It may be possible to conduct a controlled experiment on eared grebes housed in a pen on Mono Lake or a similar environment. Possible laboratory-based experiments might include:
    a. Determination of physiologic and clinical effects of forced oral ingestion of pond water versus forced oral ingestion of sodium decahydrate crystals. This study would help determine whether oral ingestion of pond water versus concentrated sodium decahydrate crystals causes physiologic perturbations (specifically elevated concentrations of serum and brain sodium). In addition, determination of lethal doses/concentrations of sodium in eared grebes would be possible by controlling dilutions of pond water.

b. Determination of physical, physiologic and clinical effects of exposure to pond water at varying water temperatures. This study would confirm physiologic effects of the tailings pond water as described above, but also would provide temporal information regarding sodium decahydrate crystal production, decline in feather waterproofing, and affect of crystal weight on morbidity/mortality.
c. Evaluation of other avian and/or mammalian species dying on the Wyoming trona ponds. It remains unclear as to why large raptor species, passerine species and bats are found deceased in the middle of these ponds. Are these animals consuming prey items saturated in pond chemicals and dying acutely? Or is it possible that physically contacting the pond water causes entrapment and prevents these animals from escaping?
d. Further characterization of eared grebe physiology and behavior both pre- and post-migration. The study undertaken thus far has provided new information on eared grebe physiology. However, very little has been published on clinically-relevant information of free-ranging eared grebes. Having access to grebes in British Columbia, Wyoming and Mono Lake, we can better to understand the different physiological states associated with pre-migration, peri-migration, and post-migration

REFERENCES

Bennett, DC, Bowes, VA, Hughes MR and LE Hart. 1992. Suspected sodium toxicity in hand-reared great blue heron (*Ardea herodias*) chicks. Avian Dis. 36: 743-748.

Gordus, AG, Shivaprasad HL and PK Swift. 2002. Salt toxicosis in ruddy ducks that winter on an agricultural evaporation basin in California. J. Wildl. Dis. 38; 124-131.

Jehl, JR Jr and C Johansson. 2002. The autumnal migration of eared grebes through southwestern Wyoming: a key to assessing the size of the North American population. Western N. Amer. Nat. 62: 335-340.

Jehl, JR Jr and R McKernan. 2002. Biology and migration of eared grebes at the Salton Sea. Hydrobiologia. 473: 245-253.

Julian RJ and TP Brown. 1997. Other toxins and poisons. In BC Calnek, HJ Barnes, CW Beard, LR McDougald and YM Saif (Eds.), *Diseases of Poultry, 10th edition.* Iowa State University Press, Ames, Iowa. Pp. 985-986

Meteyer CU, Dubielzig RR, Dein FJ, Baeten LA, Moore MK, Jehl JR Jr and K Wesenberg. 1997. Sodium toxicity and pathology associated with exposure of waterfowl to hypersaline playa lakes of southeast New Mexico. J. Vet. Diagn. Invest. 9: 269-280.

National Research Council (U.S.), Subcommittee on Mineral Toxicity in Animals. 1980. Sodium Chloride. In, *Mineral Tolerance of Domestic Animals.* National Academy of Sciences, Washington, D.C. Pp. 441-458.

Riggert TL. 1977. The biology of the mountain duck on Rottnest Island, Western Australia. Wildlife Monographs, No. 52.

Trainer DO and L Karstad. 1960. Salt poisoning in Wisconsin wildlife. J. Amer. Vet. Med. Assoc. 136: 14-17.

Wobeser G and J Howard. 1987. Mortality of waterfowl on a hypersaline wetland as a result of salt encrustation. J. Wildl. Dis. 23; 127-134.

Windingstad, RM, Kartch, FX, Stroud RK and MR Smith. 1987. Salt toxicosis in waterfowl in North Dakota. J. Wildl. Dis. 23: 443-446.

APPENDIX 1: INITIAL STUDY PLAN (Pedro Ramirez, USFWS Wyoming, 2000)

Migratory bird mortality in trona mine wastewater ponds

STUDY DESIGN

<u>Data Collection and Analysis</u>

Bird Use: A trona mine evaporation pond will be selected as a study site based on access provided by the mine. The evaporation pond will be monitored from March until December of each study season. Monitoring will be conducted for one year. The evaporation pond will be observed throughout each study season for two mornings, two evening, and one midday period each week. Morning observations will begin 30 minutes before sunrise and continue for 3.5 hours after sunrise. Evening observations will begin 3.5 hours prior to sunset and continue for 30 minutes after sunset. The midday observations will begin 1.75 hours before noon and end 1.75 hours after noon. The evaporation pond will be scanned with a spotting scope at 15-minute intervals. All migratory aquatic birds on the evaporation pond will be counted and identified by species and location on the pond. The relative use of the evaporation pond by each species will be determined from the length of time individual birds are on the pond.

Weather conditions (e.g., relative humidity, ambient temperature, and wind speed and direction) will be recorded automatically each hour. Conductivity and water temperature will be measured during the morning observation period approximately 5 meters from shore at a depth of 10 cm.

Bird Hazing Effectiveness: The response of birds on evaporation ponds to the airboat and the hazing crews will be recorded. Distance from the airboat to the bird(s) when they flush will be estimated and recorded. Flushed birds will be observed to determine if they land elsewhere on the evaporation pond or whether they leave the area. The behavioral response of birds unable to fly due to salt encrustation will also be recorded. Data on frequency and duration of hazing and recovery efforts for each season will be obtained from the mine.

Physical effects of sodium decahydrate crystallization on birds: All carcasses collected during the study will be frozen on site. At the end of the study season, 15 carcasses from the total collected will be randomly selected from those judged to be in suitable post-mortem condition and submitted to the National Wildlife Health Center (NWHC) for necropsy to determine cause(s) of death.

Attempts will be made to capture eared grebes to conduct the following pen study. An alternative option will consist of obtaining 6-month-old mallards from a commercial supplier and shipping them to Green River, Wyoming. Cloacal swabs will be collected from all mallards immediately after arrival and evaluated for parasites and selected infectious diseases. The mallards will be acclimatized to on ground with pools in plastic netted pens at Green River, fed a standard maintenance waterfowl diet, and provided fresh water through multiple ball-bearing waterers. After 2 weeks, pens and mallards will be moved to trona wastewater ponds. Five birds will be randomly assigned to each of three treatment groups as follows:

1. Pen located in trona wastewater pond with pond water provided in waterers.
2. Pen located on land with pond water provided in waterers.
3. Pen located on land with fresh water provided in waterers.

A 3 cc blood samples will be taken from each bird before placement in pens, and at 3, 7, 24 and 48 hours after being placed in pens at the study site. Birds found in distress at any sampling point will be euthanized, and all survivors will be euthanized at 48 hours. Hematology, electrolytes (Na, K, CL) and chemistries (total protein, osmolality, glucose, uric acid, and BUN) will be analyzed on each bird. Bird bodies will be shipped frozen to NWHC for necropsy and diagnostic testing as described above.

Estimated time to toxicity: To determine whether salt poisoning is due to exposure to hypersaline water (salt precipitation) or due to ingestion of water, grebes or captive-reared mallards will be allowed to drink trona water in a controlled environment without physical exposure to the evaporation pond water.

Effectiveness of bird carcass and retrieval: Twenty bird carcasses (archived from previous collections during other studies) will be tagged and weighed. Ten carcasses will be placed at various locations on the evaporation pond at the end of the day after the hazing/recovery crew has left the pond. Ten additional tagged carcasses will be placed at various locations on the pond the next morning before the hazing/recovery crew begins their work. The hazing/recovery crew will not be informed of the day or location of carcass placement. Tags will contain the date and approximate time the carcass was placed on the pond. The number of tagged carcasses recovered will be compared to the number placed on the pond, and this will be used to determine retrieval effectiveness. This test will be conducted in August and repeated in October and November to cover varying weather conditions.

Dead or moribund birds will be observed to determine the direction and distance of drift. The time and distance from when the bird was first observed until the bird sinks below the surface or reaches shore will be recorded. Following the morning observation period, attempts will be made to recover the tagged carcasses. Collection procedures will follow the U. S. Fish and Wildlife Service's standard operating procedures for environmental contaminant operations (Division of Environmental Contaminants, Quality Assurance Task Force, 1996).

Statistical Analysis: The number of bird-use days will be calculated for each pond by species and used to estimate the number of migatory birds at risk and the mean duration of stay on the ponds. Combining this information with mortality estimates would allow waterfowl mortality to be estimated with a risk function. The number of waterbird mortalities will be estimated by pond, species, age, sex, and season. This estimate will incorporate an estimated disappearance rate for all carcasses. Environmental data and water chemistry data will be plotted and used in ANOVA to identify environmental factors related to bird use and mortality on the intensively monitored ponds. Clinical pathologic data and necropsy diagnostic data will be compared between treatment groups on each pond. A chi-square analysis will be used for the mortality surveys to test the hypothesis that there are no differences in causes of mortality between the ponds.

Investigation Schedule:

Year	**Activity**
2000	Collect field data
2001	Interpret field and analytical data and prepare interim report
	Collect field data
2002	Interpret field and analytical data and prepare final report

APPENDIX 2: REVISED STUDY PLAN

Based on a information generated and discussed at a meeting conducted in August 2000 with participants representing the NWHC (Dr. F. Joshua Dein, Dr. Kurt Sladky) and the 4 mining companies (Terrel Johnson & Larry Cherny from OCI Corp.; Carl Demshar, Julie Lutz and Mike Wendorf from FMC Corp.; Stan Cook from Solvay Minerals, LLC; and Jerry Justis from General Chemical Corp.), the initial study plan was significantly revised. During this meeting, various evaporation ponds were evaluated, avian rehabilitation programs reviewed, past hazing and bird-banding activities discussed, and field research plans debated. Based on these discussions, an alternative research study plan was developed. Because the mining companies had attempted several methods of bird "hazing" utilizing both people and zon guns, with little success (especially against the most commonly affected species, grebes, which dive when hazed), it was mutually determined that pursuing the study of hazing activities would not be effective. Based on the inadequacy of hazing efforts, at least 3 of the mining companies initiated active bird rehabilitation programs during the migration seasons, focusing employee efforts during the fall migration when bird mortality is highest on the evaporation ponds. These rehabilitation programs have been, and continue to be, highly effective at reducing waterfowl mortality at the mines that have active rehabilitation programs. In fact, the environmental staff at FMC Corporation, OCI Corporation, and Solvay Minerals are able to rehabilitate greater than 90% of all birds captured on the evaporation ponds, based on records to which we had access. We believed that the active rehabilitation programs were effective, and our only request was that all 4 mining companies comply and follow a similar regimen. Having watched live bird and carcass retrieval at all of the mines, we agreed that studying this aspect of the bird rehabilitation efforts would not necessarily be cost or time effective. The only ponds in which bird carcasses were visible were associated with the General Chemical Corporation. All other ponds observed were remarkably void of carcasses. Based on previously conducted bird banding activities during the late 1970's and early 1980's, and a fortuitous data set from Susan Halvorson, a biologist at the Seedskadee National Wildlife Refuge, we determined that banding more birds would not be an effective strategy. Although recovery of banded birds was minimal (35 of > 700 banded), the majority of birds did not return to the trona ponds immediately after release. Most of the recovered birds were shot or found dead, and locations were wide ranging. Several birds were shot in the Green River, Wyoming region (N = 11), but many others were located in Mexico (N = 8), Nevada (N = 1), Texas (N = 2), Utah (N = 6), California (N = 4), Oregon (N = 1), Washington (N = 1), and Edmonton Alberta (N = 1).

It was also clear that the highest mortalities were associated with grebe species, and predominantly eared grebes. Because eared grebes have unique behavior and natural history, it would not have been possible to keep individuals of this species successfully in captivity. In addition, a laboratory surrogate species was not a viable option. Eared grebes have adapted to natural hypersaline environments, dive when threatened increasing their exposure to soda ash evaporation pond water, are unable fly without a prolonged water surface running, and cease food consumption during their migration (the gastrointestinal tract naturally shrinks in size and they use fat reserves to successfully migrate). Therefore, mallard ducks would not serve as an ecologically relevant surrogate species.

With all of this information, we decided to focus on the eared grebes in the field and eliminate the laboratory experiments.

Data Collection and Analysis

A. Limnology

Based on our observations during August, 2000, we questioned whether aquatic invertebrate species could survive and thrive in the ponds, and whether such organisms may be providing an attractive food source for the grebes. We decided that it was important to involve a limnologist, Dr. Stuart Hurlburt from San Diego State University, in order to determine if aquatic invertebrates survived in the ponds, and whether different algal organisms contribute to the pond color differences. The objective of this part of the project was to determine whether invertebrate and algal organisms were present in the trona waste water ponds, providing a potential food source, and incentive, for birds landing on the ponds, and to determine basic water chemistry parameters associated with the ponds.

B. Eared Grebe Attractivity to Evaporation Ponds

Based on our observations during August 2000, we thought it useful to have a grebe biologist, Dr. Joseph Jehl Jr. an expert in grebe biology from Hubbs-Sea World Research Institute, evaluate the physical and geographical characteristics of the ponds, and to attempt to determine from a grebe's perspective, what characteristics might be attractive to the birds. The objective of this part of the project was to attempt to evaluate physical characteristics of the ponds, which may be attracting migrating waterfowl to land on the ponds.

C. Pathology

1. Clinical Pathology

The objective in this part of the project was to determine whether affected waterfowl, particularly eared grebes, developed measurable physiological changes associated with exposure (<24 hours) to soda ash mine evaporation pond water. Blood samples (approximately 1cc/sample) from all eared grebes captured from the evaporation ponds were to be collected opportunistically immediately after removal from the ponds, and again at approximately 24 hours after each bird had been kept on freshwater in the rehabilitation facilities. These samples were to be centrifuged immediately and the serum shipped to a laboratory for analysis of standard avian biochemicals. The idea for sampling twice, 24 hours apart, was to determine whether access to freshwater would alter serum biochemical parameters, especially electrolytes (e.g., sodium). If so, this would provide evidence that physical pond water exposure and/or ingestion was contributing to physiologic changes.

2. Gross and Histopathologic Pathology

The objective in this part of the project was to determine the primary or secondary cause(s) of death of the grebes. All recovered carcasses in good post-mortem condition were to be subjected to gross necropsies and tissues preserved in buffered formalin for future histopathologic evaluation. All heads were to be removed at the distal cervical vertebrae, frozen, and shipped to the National Wildlife Health Center, Madison, Wisconsin, for brain extraction and biochemical analysis (see below). Dr. Charlotte Quist, an experienced wildlife pathologist with her own company, Wildlife Health Associates, Inc. in Montana, was recruited for all of the gross and histologic pathology.

3. Brain Biochemistry

The objective in this part of the project was to determine whether exposure to soda ash mine evaporation pond water contributed to changes in chemical in the brains of the grebes. All grebe heads were shipped frozen to the NWHC, and later processed according to standard protocol (see Appendix 2A). We were especially interested in brain sodium, but the following were to be analyzed: phosphorus, potassium, calcium, magnesium, sulfur, zinc, manganese, iron, copper, aluminum, and sodium.

APPENDIX 3: BRAIN SODIUM ANALYSIS PROTOCOL

National Wildlife Health Center
Diagnostic Pathology
Title: Protocol for Removal of Brains for Sodium Analysis
SOP No. 001
Written by: Carol Meteyer, DVM Date: 06/08/92
Approved by: J. Christian Franson, DVM

EQUIPMENT NEEDED:

New scalpel blade for each bird

Two pairs of scissors or poultry shears soaked overnight in 5% nitric acid and triple rinsed in double distilled water. The scissors will be rinsed with tap water, soaked in 5% nitric acid and triple rinsed in double distilled water between each accession during necropsy.

Four 80 ml beakers; one for 5% nitric acid and 3 containing DI water.

Clean plastic spoons soaked overnight in 5% nitric acid and rinsed in double distilled water will be used to remove the brain from the cranium making sure to prevent the brain from touching skull, feathers or gloves. A new spoon will be used for each accession.

PROCEDURE:

1. Give Diagnostic Chemistry more than 24 hours notice to prepare the 5% nitric acid and distilled water. Chemistry will soak scissors and plastic spoons overnight in 5% nitric acid, rinse in distilled water and have utensils dried for necropsy when needed.

2. Rinse all obvious salt from feathers (separate person at separate station).

3. Insert scapel blade onto handle without touching blade.

4. Incise skin with the tip of the scalpel blade and reflect skin with fingers making sure not to touch the skull with fingers. Cut the atlanto-axial joint with the clean distal portion of the scalpel blade.

5. Change gloves.

6. Take scissors soaked in acid and triple rinsed in distilled water out of the third rinse jar and empty and replace the water in the rinse jar with fresh, stock double distilled water. Use these scissors to make a cut in the skull. Keep the lower blade of the scissors just beneath the skull and do not remove when cutting around the cranium. Reflect the cut portion of the cranium to expose the brain without touching the brain. Rinse the scissors thoroughly with tap water. Place the scissors currently in the jar 5% nitric acid into the first "rinse jar". Discard the 5 % nitric acid and replace with fresh nitric acid. Place the thoroughly rinsed scissors just used for cutting the cranium into the clean nitric acid. Move the rinsing scissors to the second and third rinse jars and leave in the third rinse jar to soak while the remainder of the brain removal is performed. Empty rinse jars one and two and replace with clean double distilled water.

7. Remove the brain from the cranium with the plastic spoon making sure to prevent brain from touching skull, feathers or gloves.

8. Send brain in acid-washed jar to Chemistry for biochemical analysis.

APPENDIX 4: SERUM BIOCHEMICAL PARAMETERS DEFINED

Glucose: A quantitative measure of plasma glucose concentration. Birds have higher plasma glucose concentrations than mammals. Transient hyperglycemia can be associated with stress, and persistent hyperglycemia can be associated with other metabolic derangements, such as diabetes mellitus and chronic kidney disease.
ALT (Alanine aminotransferase): A quantitative measure of liver disease in mammals, but not a sensitive indicator of liver cell damage in birds.
AST (Aspartate Transaminase): Nonspecific for liver disease in birds, but frequently used clinically as a measure of liver status. Tissue sources include liver, and skeletal and cardiac muscle. Frequently elevated with liver or muscle cell damage.
GGT (Gamma glutamyl transferase): A quantitative measure of liver disease in mammals, but not a sensitive indicator of liver cell damage in birds.
Alk. Phos. (Alkaline Phosphatase): Elevations are associated with bone damage and infection, but not an organ-specific enzyme.
CK (Creatine Kinase): A quantitative measure of muscle (skeletal or cardiac) damage. Muscle wasting, physical activity or handling can all contribute to serum concentration elevations in CK.
LDH (Lactate Dehydrogenase): Lactate dehydrogenase activity occurs in liver, skeletal muscle, cardiac muscle, kidney and red blood cells. Therefore, many cellular changes can cause an elevation in LDH, including artifactual increase with rupture of red blood cells during blood sampling or handling.
Cholesterol: Elevated cholesterol concentrations can be observed in obese birds and birds with hypothyroidism.
TP (Total Protein): Total protein concentration can be elevated with dehydration or inflammation/infection, and decreased with chronic disease or malnutrition.
Phosphorus: Decreased phosphorus concentrations are associated with malnutrition. Elevated concentrations are associated with kidney damage, nutritional secondary hyperparathyroidism, and hemolysis during sampling or handling.
Calcium: A measure of plasma calcium concentration. Elevated calcium concentration can be associated with normal ovulation and egg production in females, and hemolysis (lysing red blood cells). Decreased calcium concentration can be associated with seizures and malnutrition.
Sodium: A measure of plasma sodium concentration.
Potassium: A measure of plasma potassium concentration. Elevated plasma or serum samples are commonly associated with hemolysis of red blood cells during blood handling and storage.
Chloride: A measure of plasma chloride concentration. Can be elevated with dehydration. Chloride concentrations may vary with acid-base status in mammals, but this has not been systematically evaluated in birds.
Bicarbonate: Elevation of bicarbonate concentrations are associated with metabolic alkalosis, while decreases are associated with metabolic acidosis.
Uric Acid: Elevation in uric acid concentrations is typically associated with kidney cellular damage, although slight to moderate elevations can be observed with dehydration.
Anion Gap: A measure of acid-base balance in mammals, but AG has not been systematically evaluated in birds.
PCV (Packed Cell Volume): A measure of total red blood cell volume.

APPENDIX 5: NECROPSY REPORTS
(Submitted by Dr. Charlotte Quist, see attached)