INVESTIGATION INTO AVIAN MORTALITY

IN

THE PLAYA LAKES REGION

OF

SOUTHEASTERN NEW MEXICO

Final Report - June 1997

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EXECUTIVE SUMMARY

HISTORY

In April 1994, the National Wildlife Health Center (NWHC) and the National Wetlands Research Center (NWRC; formerly the Southern Science Center) received directives to initiate a collaborative study investigating bird mortality in the playa lakes of southeastern New Mexico. The NWHC was assigned lead responsibility for the project in cooperation with the NWRC. The New Mexico Cooperative Fish and Wildlife Research Unit was also an original cooperator but withdrew due to administrative problems. The NWHC and NWRC had previously been involved in diagnostic and research activities in the region, and could now expand these efforts in a more concentrated and directed fashion. A literature survey on related topics of historic and current land use, hydrology, geology, water quality, playa ecology, and bird use and mortality was also requested; this report was completed in September of 1995.

Using the Southeastern New Mexico Playa Lakes Coordinating Committee (SENMPLCC) Mitigation Action Plan as a guide, a Study Plan was developed, sent for peer review and to the Committee; the Committee met and approved the plan in June, 1994. The plan for the first year called for field work assessing bird use to begin in October, 1994, continuing through April, 1995, and experimental studies on determining the cause of death occurring in November, 1994. Security and subsequent administrative problems suspended the fall field season, but the experimental studies were accomplished. An additional cooperator, Hubbs-Sea World Research Institute, was added to assist with the Spring 1995 season. A second fall field season was planned in 1995 but was terminated after one week of execution due to the government shut-down.

An Interim Report was submitted to SENMPLCC in August, 1995 and an oral demonstration of the findings was presented in April, 1996. On SENMPLCC recommendations from this meeting, the NWHC developed a detailed list of potential questions that were raised from the previous work, and could be pursued in the future. NWHC and NWRC were directed by the BLM to proceed on the top three items and present the findings in a final report.

FINDINGS

Water samples from Laguna Toston (LT) and Williams Sink (WS) were taken throughout the experimental period and the Spring 1995 field period. LT was found to contain about 310,000 parts per million (ppm) dissolved ions and WS about 290,000 ppm. This compares to sea water at about 30,000 ppm. Sodium, potassium and chloride were found to be the strongest ions in concentration. There are differences in the ionic composition of LT and WS water, and there is little change in composition of each lake throughout the sampling periods.

Investigations to determine the proximate and ultimate causes of death of birds using the playa lakes were conducted during the week of November 13, 1994. Pens housing captive-bred mallards were constructed at LT and WS, one exposing the birds to direct contact with lake water, another on land with lake water available to drink. All birds in the water pens were adversely affected, some showing abnormal behavior within 3 hours, and leading to death or euthanasia by 35 hours. Blood samples taken at intervals throughout the trial showed a significant elevation of sodium and potassium. Examination of these birds after death showed that there were severe changes in their eyes, and some areas of inflammation in other internal organs, but no evidence of any other infectious or toxic disease. The amount of sodium found in the brains of these birds was consistent with a diagnosis of salt poisoning. Birds in pens on land, but drinking lake water, also had elevations of sodium in the blood and brain, but not to a toxic level.

Other preliminary studies examined the effects of lake water on feathers, body heat loss, body water loss, and flight capabilities. It was determined that birds in the water accumulate salt crystals within the microstructure of their feathers and this may cause a change in the water repellency and aerodynamic form. Practically, this was demonstrated by a decrease in a bird's ability to fly after exposure. Infrared imaging suggested increase in body heat loss after exposure, and measures of body weight and water content of muscle contributed to the conclusion that birds were losing body water. The lack of access to fresh drinking water to combat water loss is frequently indicated as an important feature contributing to salt poisoning in domestic species.

Eight lakes in the area were observed for bird use for 272 hours in March and April, 1995, with the majority of time spent at LT and WS. A total of 1572 individual waterfowl were observed (44% on LT), and Northern Shovelers were the most common species. There was no clear determination as to the pattern of arrivals and departures throughout the day.

Surveys were conducted at the lakes every three days to find sick and dead birds. Blood samples were taken from sick birds, and all carcasses were sent to the NWHC for examination. From the 67 dead birds and 25 sick wild birds found, similar elevations were seen in blood and brain sodium to those from experimental birds. This confirmed that the most likely cause of death was salt poisoning/dehydration(fresh water deprivation). Comparisons of mortality and observational data identified inconsistencies in extent of species use and mortality of that species. This finding, along with climatic observations, suggests that mortality may be significantly related to environmental conditions.

Studies performed under environmentally controlled conditions at the NWHC in 1996 demonstrated that the water from Laguna Toston is directly toxic to mallards, and verified the findings of the 1994 field experimental trials, that "the ultimate cause of death on the subject lakes to be a combination of salt toxicity and water loss." Climatic factors still may play an important role in the timing and extent of mortality and could be better defined through additional field studies. It was also determined that the cataracts discussed in the Interim Report were most likely post-mortem artifact, and are not now considered a significant factor in the process. The most critical research need at this point is additional observational information to document extent and species breadth of mortality over multiple migratory periods.

I. OVERVIEW

This Final Report is a review of work on a cooperative study undertaken by the USGS Biological Resources Division's National Wildlife Health Center (NWHC) and National Wetlands Research Center (NWRC; formerly the Southern Science Center) from 1994 through 1997. The study was initiated at the request of the Bureau of Land Management (BLM), through a request to the former National Biological Service. The Southeastern New Mexico Playa Lakes Coordinating Committee (SENMPLCC) played an important role in outlining the research needs. The initial Study Plan document, which outlines the background, objectives and methods for the first two years is available as Appendix 1. A letter indicating modifications to the Study Plan was sent to the SENMPLCC chair on April 25,1995, and is Appendix 2. An Interim Report, covering this work was completed and submitted in September 1995. The Literature Review section of the study was completed and presented to SENMPLCC in August, 1995. Following SENMPLCC review, NWHC was asked to develop a series of questions that could be posed from information gained in the initial phase (Appendix 3). The NWHC and NWRC were then directed to begin work to answer the top three questions, within the available fiscal resources. NWRC could continue with work outlined under the original Study Plan (Appendix 1), however an additional Study Plan for experiments performed by NWHC and collaborators and is available as Appendix 4.

FIRST PHASE - Fall 1994 and Spring 1995

The initial phase was divided into two broad sections, one covering the extent of bird use and mortality with the NWRC as lead investigator, and the second, a determination of the cause of death of birds found dead in the study area with the NWHC taking the lead. The NWHC served as the project coordinator. Cooperators from the BLM, Fish and Wildlife Service, Hubbs-Sea World Research Institute, IMC Global, Florida Institute for Environmental Studies and the New Mexico Cooperative Fish and Wildlife Research Unit also participated in this phase.

The study sites were identified as Laguna Toston (LT) located in Lea County, New Mexico and Williams Sink (WS) located in Eddy County. The sites were chosen to represent lakes that were currently receiving potash manufacturing discharge LT, and lakes that had received discharge in the past WS. During the spring survey it was decided to identify WS as composed of a North and South part. When this distinction is not present, WS South is assumed. The terminology describing the lake water can be confusing. Salt/salty, brine, saline, and hypersaline have all been used. For ease of communication we are considering all these terms as interchangeable, even though some may feel there are specific differences.

The first field observation period was to begin in October 1994. However, this season was quickly interrupted by a threat to the security of the field personnel. Administrative difficulties then made it difficult to continue the work during that time. A spring field season was organized and occurred from March 9 to April 25, 1995.

An experimental investigation into the causes of death was conducted from November 13 through

18 at LT and WS (Field Experiment). The study design, detailed in the study plan, was to place captive raised mallards into pens at each of the study lakes. The ducks (6 month old males) were divided into treatment groups of 5 and allowed to acclimate for 3 weeks in identical pens. The ducks were then transferred, remaining in the same treatment groups, to the test lakes. Four treatment groups were evaluated at each lake. The ducks in treatment group 1 were placed in a pen in the lake; treatment group 2 in a pen on land with lake water for drinking; treatment group 3 in a pen on land with distilled water for drinking (Figures 1 - 2). These groups will be referred to by number throughout the report. Birds in Group 1 began showing neurologic signs and conjunctivitis within 3 hours on LT (Figure 3) and 24 hours on WS. The first bird was found dead on LT at 11 hours, and 22 hours on WS. Birds salted over quickly after death (Figure 4).

Figure 1: Ducks in treatment group 1; LT - Field Experiment

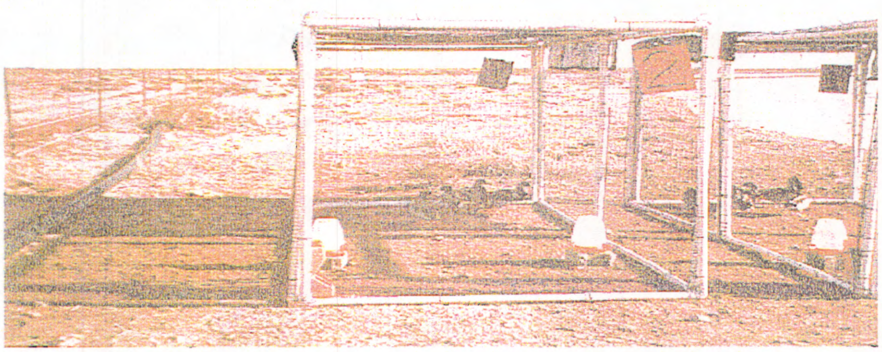


Figure 2: Ducks in treatment groups 2 and 3 pens; LT-Field Experiment

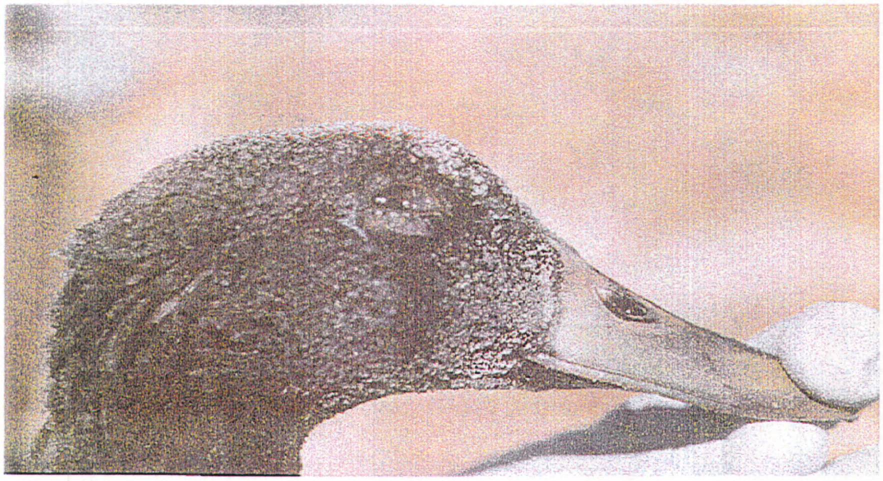


Figure 3: Duck with conjunctivitis.

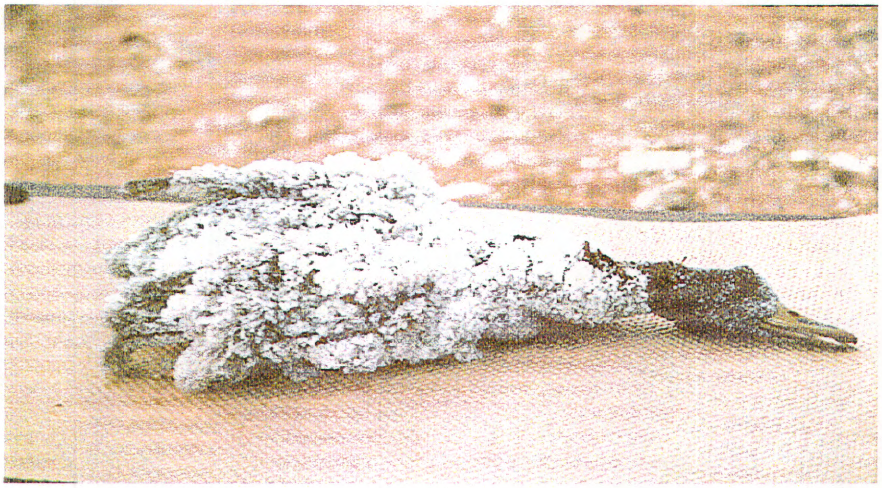


Figure 4: Salted over duck from group 1 (LT).

SECOND PHASE - Fall 1995 and Fall 1996

An attempt was made to continue with the goal of the Study Plan to obtain population, behavioral and mortality data during a fall migratory season in 1995, however this effort was quickly halted by the Federal government shut-down. Observations were only conducted during 13 days between 5-20 November when 504 individual birds were observed using Laguna Toston and 97 carcasses were found. Resources were not available to mount a field season in Fall 1996.

An additional Study Plan (Appendix 4) was developed to determine the morbidity and mortality of ducks exposed to hyper saline water in a controlled environment (Controlled Environment

Study; CES), determine the dilution required to prevent morbidity/mortality, and assess the ophthalmologic changes in the exposed birds. A collaborator, the University of Wisconsin Comparative Ophthalmology Research Laboratory, independently pursued additional studies to determine the nature of cataract formation in exposed birds.

Four groups of mallards were placed in plastic pools at the NWHC. One group was in 100% Laguna Toston water, and the other two in 80% and 70% LT, diluted with tap water. No significant differences were found between the LT groups, although the birds showed similar signs and lesions to those in the field experiments. Ophthalmologic examinations revealed mild to severe conjunctival edema, conjunctival vascular injection, and edema of the eyelids within 1 hour. No cataract development was observed. Cataracts were found to appear both post-mortem, and experimentally exposing the eyes of dead and anesthetized ducks to direct contact with LT water. This condition could be reversed by emersion in fresh water.

This Final Report is divided into sections detailing the separate inquiries involved in the project. In reality, many of these activities occurred simultaneously, and they are separated to enable the reader to focus on specific aspects and questions. An attempt has been made not to duplicate descriptions of methods and results, but place this information in the most direct section with references to other relevant segments.

II. LABORATORY EXPERIMENTS ON MORTALITY IN A CONTROLLED ENVIRONMENT

In response to the clinical data and necropsy findings from the field investigations, experimental trials were conceived to determine if the playa lake waters were toxic themselves, or does environmental conditions play a primary role in mortality (see Study Plan, Appendix 4). An attempt was also made to assess whether dilution of LT water would diminish the reported effects. Ophthalmic examinations were incorporated into the mortality investigation to delineate the development of the ocular lesions noted in the previous experiments; these will be discussed in Section XX. The experiments were performed in a isolation facility located at the National Wildlife Health Center using playa lake water that had been collected at Laguna Toston. The Center offered a situation where birds could be exposed in a controlled environment with respect to temperature, humidity and wind velocity.

METHODS

In August 1996, captive-bred mallards (Whistling Wings, Hanover, Illinois) were obtained. The ducks (5 month old, males) were divided into treatment groups of five and were allowed to acclimate for one week in identical cages. Fresh water was provided in gravity waterers and food (Purina duck grower) was available ad libitum in hanging gravity feeders. Upon receipt of the birds, health screens were performed as described in Appendix 4. The ducks were then transferred, remaining in the same treatment groups, to the test pools, 4' x 4'x 4' in size, with hanging gravity feeders (Figures 5 and 6). The pools were filled with approximately 110 gallons of tap water. The pool levels were varied to allow the ducks to develop adequate waterproofing. After one week of acclimation the pools were drained and filled with water according to the treatment group. The ducks in treatment group 1 (TG1) were placed in a pool with 100% tap water; treatment group 2 (TG2) in a pool with 100% Laguna Toston water; treatment group 3 (TG3) in a pool with 10% tap water and 90% Laguna Toston water; treatment group 4 (TG4) in a pool with 20% tap water and 80% Laguna Toston water.

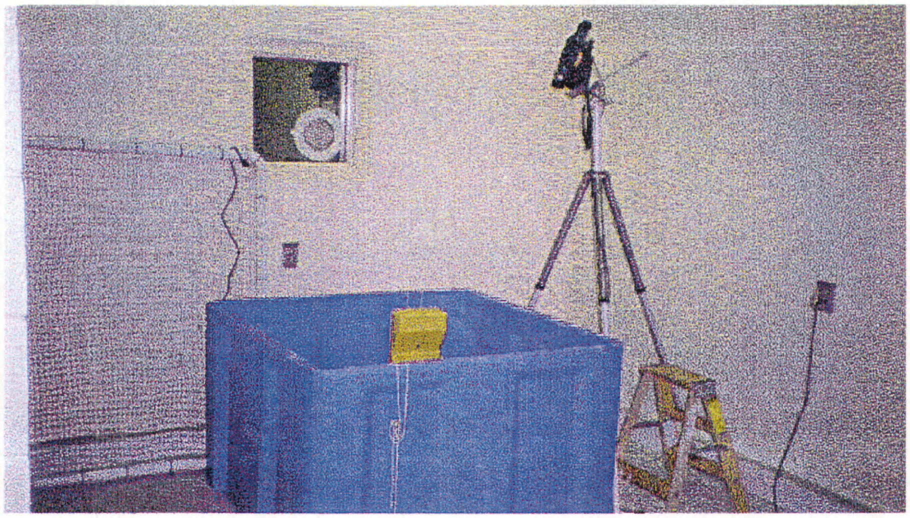


Figure 5: Experimental pool set-up at NWHC

Figure 6: Ducks in experimental pools at NWHC

The ducks in treatment groups A-D were placed in their pools in the morning (8:00 to 10:00 AM) on two consecutive days, two groups per day. Blood, and feathers were collected from each bird at 0, 3, 7, 24, 48 and 72 hours. Body weights were determined at 0 hour. The ducks were observed continuously throughout the first 10 hours (8 AM to 6 PM) and the periodic observations were made every 3 hours (6 PM to 6 AM). Activity levels, behavior, clinical signs, drinking and eating were documented during the observational periods.

Blood collection and analysis was performed as described in Appendix 4, with the following exceptions: packed cell volumes were only analyzed on TG1 and TG2 at 0 and 24 hours, and clinical chemistry testing were sent to the University of Wisconsin Veterinary Medical Teaching Hospital and analyzed using a Kodak Ektachem Analyser.

Routine necropsy was similar to the procedure described in Section XII, with the exception that carcasses were processed within 1 to 12 hours after death. No samples were submitted for cholinesterase, lead or botulism analysis. Other diagnostic tests were performed as warranted by gross necropsy. Necropsy results are in Section XII.

RESULTS AND CONCLUSIONS

The feather condition of the experimental animals upon arrival was inferior to those of the field experiments. The preconditioning period did not result in a complete restoration of natural structure and function. Due to poor waterproofing, some (6) of the birds became quickly waterlogged (both control and experimental groups) and potentially hypothermic (Figure 7). To reduce this effect but continue the experiment and water contact, the pool water levels were dropped to approximately 50 gallons after three hours of exposure. The water level was increased at 24 and 48 hours to 110 gallons and decreased again at 27 and 51 hours. All treatment groups were handled in the same manner.

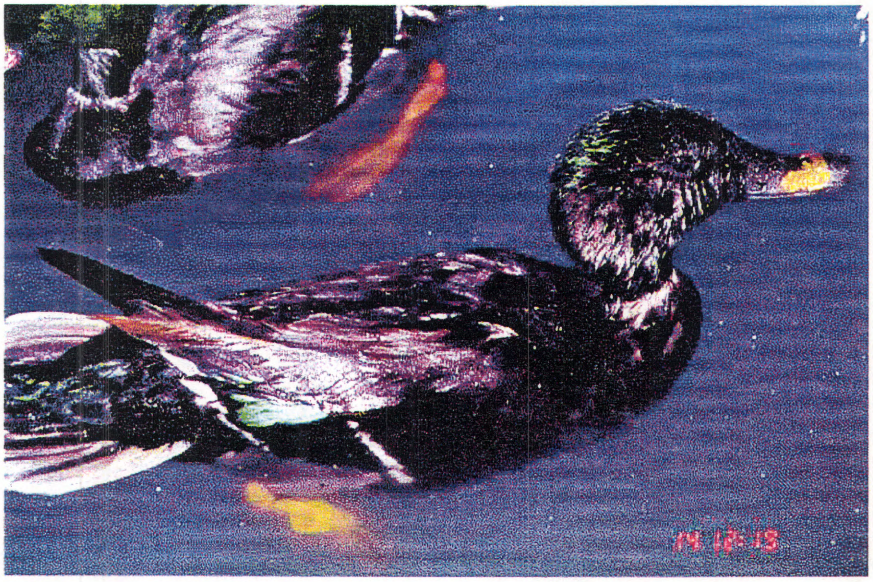


Figure 7: Duck with poor waterproofing, riding low in the water

The first mortality occurred at approximately 10-12 hours in TG3, and all birds had died within 51 hours of exposure. Birds were affected most rapidly in TG3. Two of the most severely waterlogged birds were in TG1 and were euthanized at about 27 hours (Figure 8). There was little significant external salt accumulation on any birds, but there was obvious light precipitate. Until clinical signs became apparent, the birds were eating and drinking normally. The waterlogged birds paddled vigorously to remain afloat. The severe neurologic signs as seen the field experiments were first noticed in one bird in TG4 at 9 hours, and all birds developed similar signs before death.

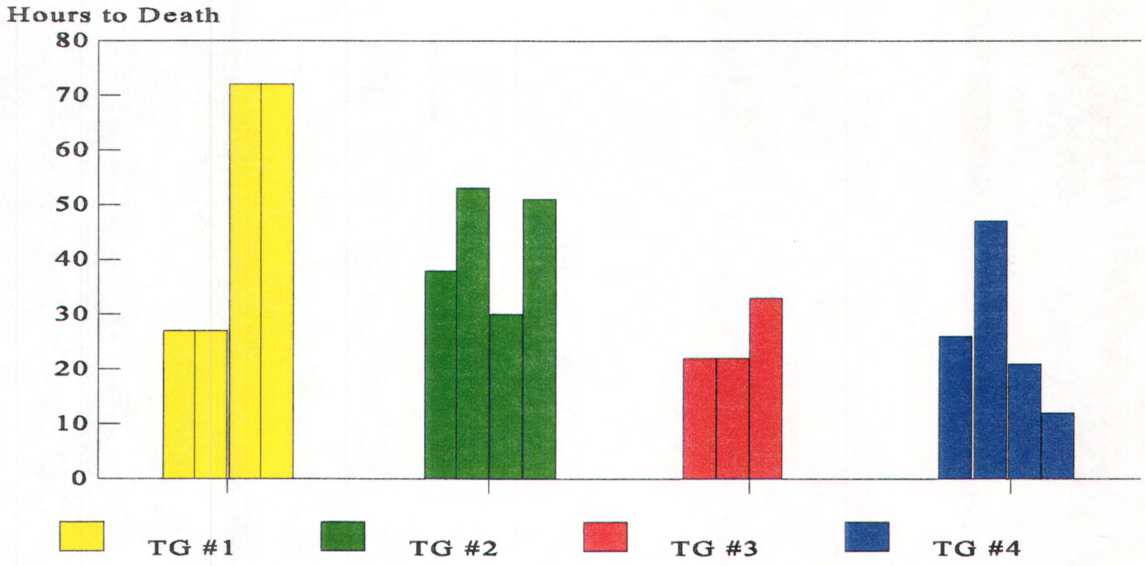


Figure 8: Time to death for birds in each treatment group

In general, the patterns of development of clinical signs and mortality were similar to those seen in the field experiments conducted on site at LT and WS.

An analysis of the survival times (or time to death) was conducted using the non-parametric Kaplan-Meier product-limit method. Number of hours survived was used for all ducks that died as a result of the experimental treatment. However, 6 ducks died from complications with feather conditions; 2 ducks in the control group, 2 in treatment group 3, and 2 in treatment group 4. These ducks were censored from the survival data. In addition, 2 ducks in the control group were alive at the conclusion of the experiment (at 72 hours) these ducks were censored at that time, indicating that they had not died when the experiment ended. This approach assumes that mortality of the 6 ducks with feather complications were independent of the effect of the treatment. This assumption may not be completely correct because some ducks (especially in the treatment groups) may have died from a combination of feather problems (leading to exposure) and effects of the treatment. If the 2 censored ducks from the control group were only affected by exposure, then censoring the ducks with feather problems in all treatment groups should provide a conservative statistical conclusion (less likely to find a significant difference between control and treatment groups). In the censoring process, ducks are considered at risk of mortality until they are removed from the experiment by censoring (or they are alive when the study ends) and their death is not considered in determining survival rates or the survival distribution. There was concern about the effects that the poor feather condition might have on time to mortality. Therefore a statistical tool, the Kaplan-Meier method was used to examine possible effects. The Kaplan-Meier method compares the survival distribution (changes in survival pattern through time) among 1 or more groups. This procedure found that there were no significant differences between treatment groups, although there were differences between pooled treatment groups and the control group. Figure 9 is a plot of these findings. This method projects a conservative picture, so we feel comfortable that the feather condition did not have an appreciable effect on outcome.

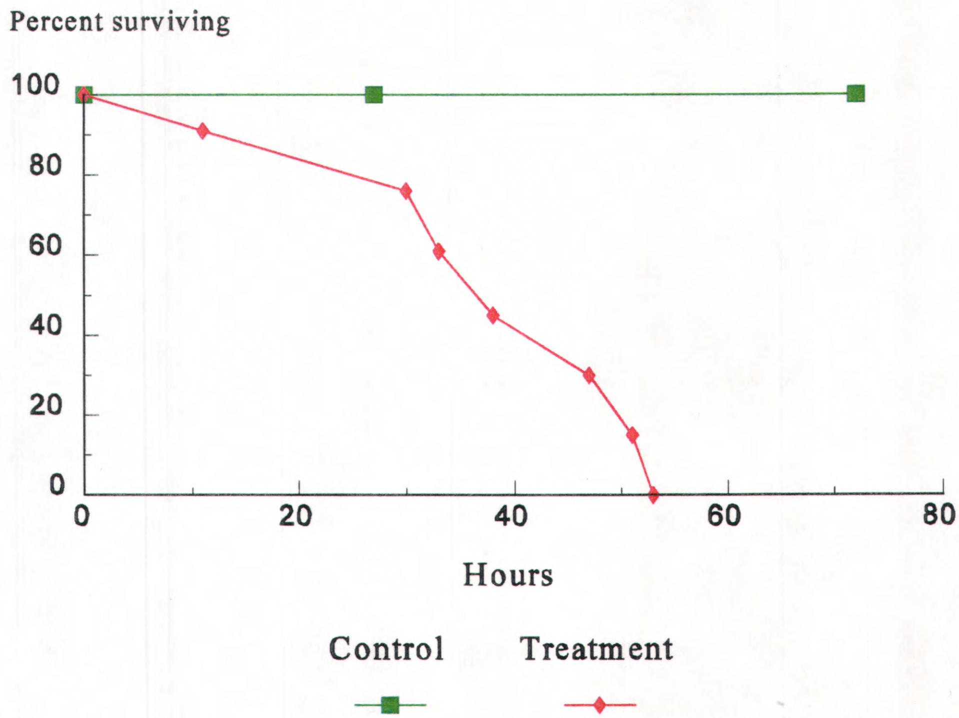


Figure 9: Plot of Kaplan-Meier analysis of mortality in CES.

III. WATER CHEMISTRY - *General and Field Experiment*

Water analysis of hypersaline waters is a very difficult process because of the often supersaturated solutions encountered. Most of the work in this area has been done with sea water, which has a fairly consistent composition. Inland saline waters, such as the Playa Lakes, vary considerably in composition and so analyses that are used for sea water (often conductivity) are not useful for these waters. The best measure of salinity for these waters is the measurement used in fresh waters - total ion concentration. This may be determined by evaporation of a sample (weighing before and after drying) or by direct calculation (adding together all of the major ions). The second method has been found to be more accurate with hypersaline waters. Total ion concentration is roughly equivalent to total dissolved solids, depending on various factors including amount of organic matter.

LT is currently receiving potash effluent; WS received discharge during 1957-1982 from National Potash Company at the rate of 3,200 tons/day to the tailings pile. Some of this then drained into the lake. In 1975, BLM did water sampling of WS and the New Mexico Environmental Department did sampling in 1992 with the following results shown in Table 1.

Table 1: Water chemistries from WS, 1975, 1992, and 1994.

Ion	1975	1992	Fall 1994 (NWHC)
Na	12,000	37,000	80,125
K	3,500	7,000	16,850
Cl	22,000	88,000	157,625
TOTAL	not done	169,000	289,463

The 1992 results were considerably higher than the 1975 records (2-4X) and the values are even higher in 1994 (5-7X). However, we do not know if lake levels were equivalent at the time of sampling, if sampling sites were the same, or whether samples were analyzed in the same manner. It may also be that WS is still receiving some inflow of high salt concentrations - either from the tailings pile or from underground connection with LT.

METHODS

Samples were collected in duplicate in sterile plastic jars. Fall samples were taken during the experimental trials. Samples were taken in the same area each time, about 5-10 feet outside the penned area housing the experimental ducks. Spring samples were taken between March 21 and April 23; locations are noted in the spring field sampling data. All samples were analyzed by IMC Global, Inc. Fall samples were not analyzed until March of 1995 and the spring samples were analyzed within a few weeks of collection. LT and WS are both supersaturated salt solutions and the measures are of the amount of the ion that is in solution at the time of analysis. More salts may settle out of solution after collection, with the amount of precipitation affected by temperature.

RESULTS AND DISCUSSION

Summary of fall water analysis

Five samples from LT and 8 samples from WS were taken over a 6 day period. Since there was little variation, only the sample means are presented. Results are presented in both ppm and mole % of total cations or anions present (Table 2). The mole % represents the total number of ions available in the water whereas the ppm represents the amount of the ion on a total weight basis.

Table 2: Means of major cations and ions in LT and WS, November 1994. PPM or Mole % of total cations or anions. n = 5 (LT); n = 8 (WS)

Ion	PPM		Mole %	
	LT	WS	LT	WS
K	35,900	16,850	17.72	8.78
Mg	20,300	12,038	32.22	20.14
Na	59,640	80,125	50.06	70.84
Ca	0	238	0	0.24
Cl	152,820	157,675	83.20	90.44
SO_4	41,780	22,562	16.80	9.56
TOTAL	310,380	289,463		

Statistical analysis was performed on the data from the fall water samples to detect any lake differences or differences in the ion composition over time. Significant, but minor time trends were found at LT for sodium and potassium. No time trends were noted for WS. These time trends were judged to be of minor importance. All chemistry parameters were found to be different between the two lakes (Figure 10). Potassium, magnesium, sulfate, and total ions were higher at LT. In contrast, sodium, calcium, and chloride were higher at WS.

Summary of spring 1995 water analysis

There were nine sites sampled during the spring field season (March 15-April 23, 1995): LT, WS North, WS South, Laguna Uno, Laguna Dos, Laguna Tres, Laguna Quatro, SENW19 (near Laguna Uno), and LT effluent.

Statistical analysis of the data from the spring water samples were performed to detect differences among the lakes or differences over time within lakes. The LT effluent samples were not included in this analysis. The results showed significant differences among the lakes, but not for the weekly periods. There were significant differences among the lakes for all chemistry parameters (Table 3; Figure 10). Multiple comparisons indicated a variety of differences and similarities among lakes depending on the specific chemistry parameter being evaluated.

LT is very high in potassium for both ppm and mole %, while SENW19 and WS South are the lowest. LT and WS South are highest in magnesium in both ppm and mole %, with SENW19 and WS North lowest. For sodium, SENW19 has the lowest ppm with the highest mole %. This indicates that the total salinity is low but of that total, sodium contributes the largest percent to the total cations. In contrast, LT has the lowest mole % and one of the lowest ppm as well. Calcium is highest in both ppm and mole % at SENW19 and LT is the lowest in both. LT has a high ppm for chloride but the lowest mole %, indicating high concentrations but a lower contribution of the chloride ion on a percent basis as compared to sulfate, the other anion. SENW19 is low in chloride ppm but high in mole %, indicating a low total concentration, with a major portion contributed by chloride ions. Sulfate is highest in both ppm and mole % at LT and WS North is very low in sulfate compared to the other lakes sampled.

In general, results for LT and WS South followed the same patterns as the fall sampling (Figure 11 - 12). There is however, a major difference between WS North and WS South. The experimental trials were performed at WS South. At the time of the experiments it was not known that WS North existed. It is unclear whether previous water chemistry data is from WS South or North. The present data makes it appear that these 2 sites are receiving different waters.

PPM and (mole % of total cations or anions)									
	Laguna Uno	Laguna Dos	Laguna Tres	Laguna Quatro	LT	LT Effluent	WS North	WS South	Pond 19
K	26617 (13.98)	24000 (12.40)	32467 (16.73)	23883 (12.17)	39850 (19.43)	18186 (10.54)	32883 (25.97)	16,520 (8.70)	5317 (6.37)
Mg	7983 (13.48)	7300 (12.12)	11167 (18.52)	7183 (11.77)	17775 (27.90)	3929 (7.34)	1467 (3.72)	11480 (19.40)	1983 (7.62)
Na	80883 (72.35)	85283 (75.10)	73033 (64.58)	87800 (75.80)	63308 (52.60)	80914 (80.51)	50550 (68.22)	79940 (71.52)	39403 (80.43)
Ca	183 (0.20)	250 (0.25)	150 (0.17)	250 (0.25)	38 0.06)	1357 (1.57)	850 (1.53)	300 (0.30)	2366 (5.55)
Cl	151917 (88.18)	157850 (90.17)	155967 (89.23)	161617 (90.72)	159042 (85.62)	142400 (91.91)	110383 (96.97)	157020 (91.12)	72217 (95.63)
SO_4	27833 (11.70)	23317 (9.83)	25583 (10.75)	22767 (9.43)	36075 (14.36)	17171 (8.10)	4667 (3.03)	20720 (8.86)	10917 (4.37)
TOTAL	295383	298033	298333	303483	316142	263928	200967	286000	125717

Table 3: Means of major cations and anions - Spring 1995

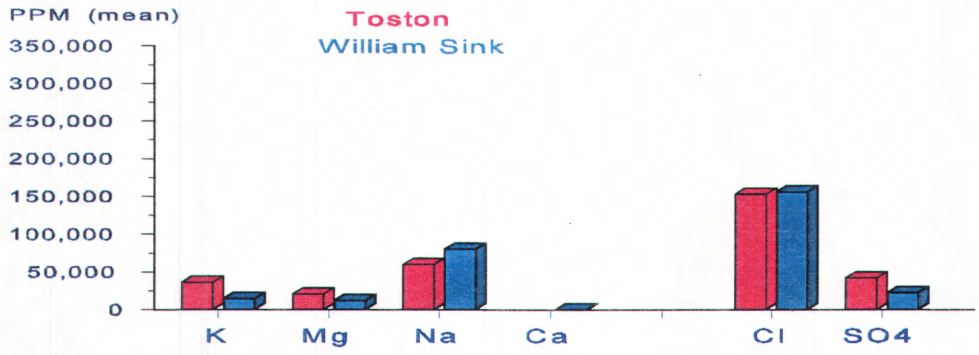


Figure 10: Comparison of ionic concentrations between Laguna Toston and Willams Sink, Fall, 1994

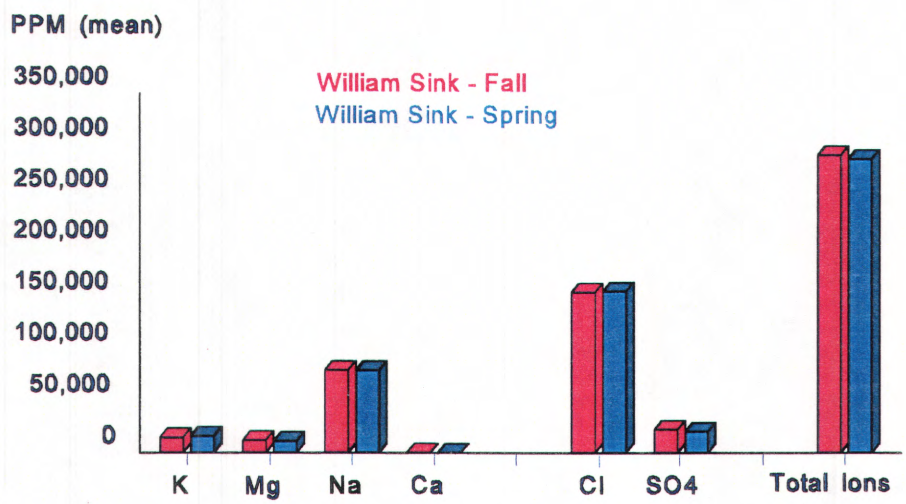


Figure 11: Ionic concentrations from Williams Sink during two seasons, Fall 1994 and Spring 1995.

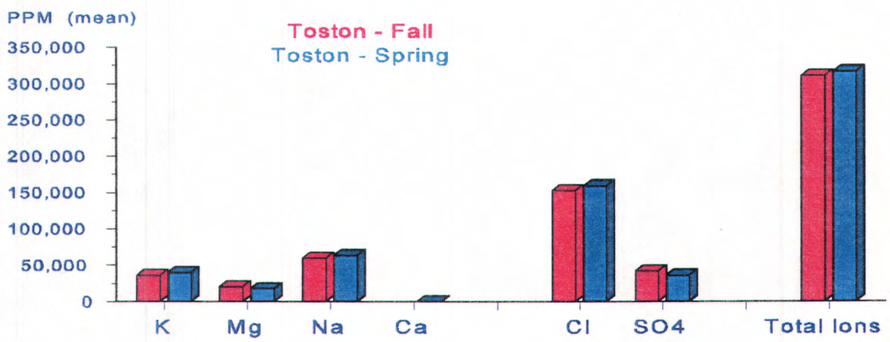


Figure 12: Ionic concentrations from Laguna Toston during two seasons, Fall, 1994 and Spring, 1995

During both sampling periods, levels of sodium are considerably higher at WS than LT. It may be that if a total analysis of both the water and precipitate could be done that LT may have as high or higher levels of sodium, but a lot of sodium chloride may have already precipitated out. The major anion present is chloride, leading to the conclusion that the major salts precipitating out would be chlorides. The Merck Index lists the specific gravity for various salts (Table 4).

In general, the lower the specific gravity the quicker the salt will precipitate out. Since there is little calcium present in solution in either lake as well as fairly low levels of sulfate, the major salts in the precipitate are probably sodium chloride and potassium chloride or related complexes. Analysis of the precipitate on the birds was attempted but problems were encountered with this analysis because of the high concentrations.

Table 4: Specific gravity of selected salts

Salt	Specific gravity
CaS04.2H20	0.999
K2S04	1.086
KCl	1.178
NaCl	1.198
Na2S04	1.208
MgCl2.6H20	1.26
MgS04.7H20	1.3
CaCl2.6H20	1.47

IV. WATER CHEMISTRY - *Controlled Environment Study*

One of the objectives of the NWHC laboratory experiments was to attempt to determine the dilution required to prevent morbidity/mortality of ducks exposed to hyper saline water in a controlled environment. Our original planning, based on previous LT water chemistries, proposed that dilutions from 20 to 30 percent be trialed. A dilution of 20% would put the TDS at a level below the saturation point, 30 % would be in the range suggested to potentially below the toxicity level, 50% in the range of Great Salt Lake, and 80 % in the range of Mono Lake, the latter two hypersaline bodies without significant reported mortality.

RESULTS AND CONCLUSIONS

Logistical constraints limitations precluded performing all trials together, and it was planned to continue with the remaining dilutions based on results of the first trials. Subsequently, limitations of resources did not permit completing the series. Unfortunately, the process of collecting shipping and remixing water from LT did not achieve the same TDS as measured directly from the lake. In addition, with the measurement systems available, we did not achieve the dilutions predicted(Table 5; Figure 13). The restricted amount of LT water available, and the time lag needed to obtain the TDS levels did not allow pre-testing of the system.

Expected Dilution (%)	Expected TDS (PPM)	Actual TDS (PPM)	Actual Dilution (%)
0	316,000	298,500	0
20	252,800	266,360	11
30	221,000	243,640	18
50	158,000	-------	------
80	63,000	-------	------

Table 5: Expected and actual TDS levels for controlled environment studies.

Using these data, it can be seen that TG4 (expected 30%) was at least close to the 20 % dilution expected for TG3. With this experience, additional studies could be performed that could better attain the expected dilutions.

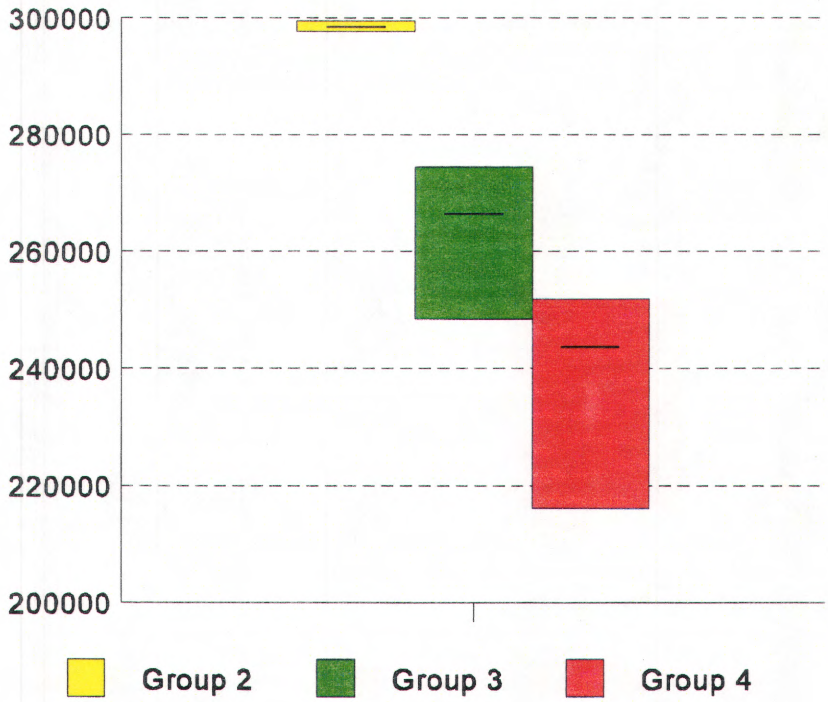


Figure 13: Mean, minimum and maximum values in TDS(PPM) for dilutions in CES experimental groups.

V. OBSERVATIONS ON BIRD USE AND BEHAVIOR- *Spring, 1995*

To answer questions concerning the extent of bird use in the region, extensive observations and surveys are required. Although LT and WS (north lakes) were the focus of the field studies, Lagunas Uno, Dos, Tres, and Quatro, and a small spring fed pond adjacent to the north shore of Laguna Uno named SENW19 (south lakes) were less frequently visited to gain an overall picture of bird use in the region.

METHODS

Playas were visited regularly but opportunistically, emphasizing lakes with the greatest usage. They were scanned at maximum intervals of 15 minutes with binoculars and/or a spotting scope. All migratory birds present were counted and identified to species, and sex if possible; also recorded were their location on the lake, general behavior and condition. Lakes were visited at all times of the day to try to determine patterns of bird use.

Effort was made to visit the north lakes at least four times a week and south lakes twice a week. Typically, observation periods at the north lakes were scheduled for a minimum of four hours, from late afternoon to one half hour after sunset. If ducks were on the water at the end of this period, a return visit was made the following morning.

Behavioral observations on the south lakes were more opportunistic. They were conducted when live ducks were observed during the mortality surveys and continued for a minimum of two hours, daylight permitting.

RESULTS

The presence of 1919 individual waterfowl were documented (Table 6; Figure 14). LT accounted for 706 (44%) of the total sightings. Despite prolonged observations at WS, only 50 waterfowl were noted. Northern shovelers, which made up 53% of all the sightings, were more than four times as common as any other waterfowl species (Figure 14).

The total number of observation hours per lake is shown in Figure 15. The number of waterfowl seen per hour of observation at each lake (Figure 16) where calculated; these ranged from 14.85 at Laguna Tres to <1 at WS and Laguna Quatro. Values at the south lakes may be slightly inflated due to the more opportunistic nature of observations there, rather than the set time of observations at the north lakes.

Table 6: Number of birds observed and found dead (in parentheses) using Playa Lakes in SE New Mexico 9 March - 25 April 1995

	Laguna Toston	Williams Sink No.	Williams Sink So.	Laguna Uno	Laguna Dos	Laguna Tres	Laguna Quatro	SENW19	Total	% Total
WATERFOWL (#Dead)										
Western Grebe				1					1	0.06
Eared Grebe	5		6	5	1				17	1.08
Pied-billed Grebe	1								1	0.06
Wood Duck	2					(1)			2	0.13
Northern Pintail						50			50	3.17
American Wigeon	45	4		4		9			62	3.93
Northern Shoveler	242(22)	12	3	181(3)	20	392	(1)		850	53.83
Green-Winged Teal	161(4)	3			2				166	10.51
Cinnamon Teal	1								1	0.60
Blue-winged Teal	25(2)			8					33	2.09
Mallard								2	2	0.13
Gadwall	56(2)	2			1				59	3.74
Lesser Scaup	108(4)			2(5)	74(2)	8	2	6	200	12.67
Redhead	2			3					5	0.32
Ring-necked Duck	1								1	0.06
Bufflehead	1			6				6	13	0.82
Common Goldeneye				4				20	24	1.52
Snow Goose	3								3	0.19

Ruddy Duck	50(8)			10(1)					60	3.80
Unidentified Duck	3(2)	11	9	6(7)			(1)		29	1.84
DUCKS SEEN	706	32	18	230	98	459	2	34	1579	
%	44.71	2.03	1.14	14.57	6.21	29.07	0.13	2.15		
# DEAD	44	0	0	16	2	1	2	0	65	
OTHER BIRDS										
American Coot	(1)								0	0.00
Black-necked Stilt						3			3	0.88
American Avocet	8					60	2	7	77	22.65
Wilson's Phalarope	54		2			3		151	210	61.76
Ring-billed Gull	17	1	1	13	1			4	37	10.88
Bonaparte's Gull	4								4	1.18
Franklin's Gull	5					2			7	2.06
Unidentified Gull				2					2	0.59
OTHER SEEN	88	1	3	15	1	68	2	162	340	
%	25.81	0.29	0.88	4.40	0.29	19.94	0.59	47.51		
# DEAD	1	0	0	0	0	0	0	0	1	
TOTAL (ALL SPECIES)	794	33	21	245	99	527	4	196	1919	
%	41.31	1.72	1.09	12.75	5.15	27.42	0.21	10.20		

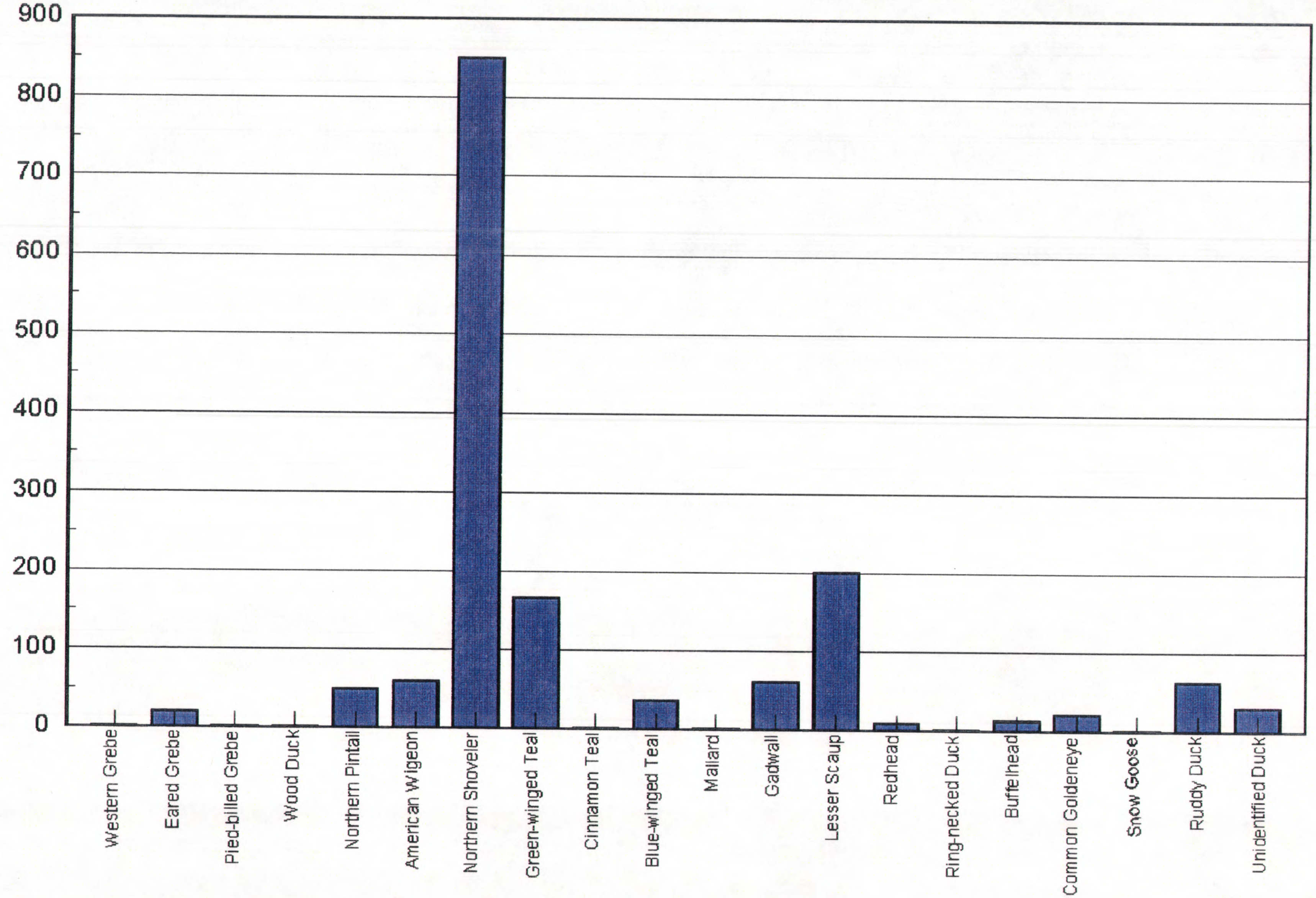


Figure 14: Number of waterfowl observed using saline playas in SE New Mexico 9 March-25 April 1995.

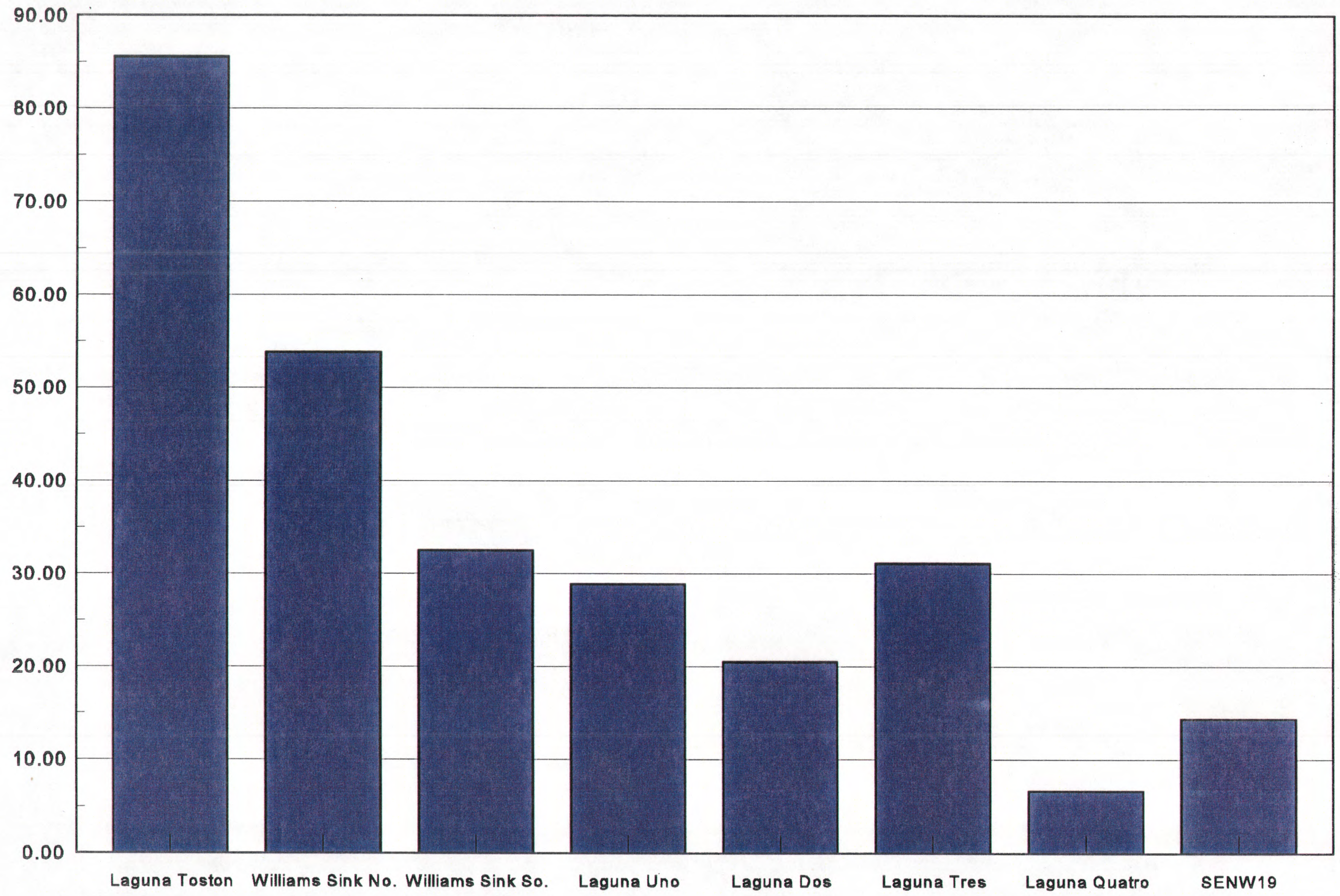


Figure 15: Total hours of observation at playa lakes in SE New Mexico 9 March - 25 April 1995.

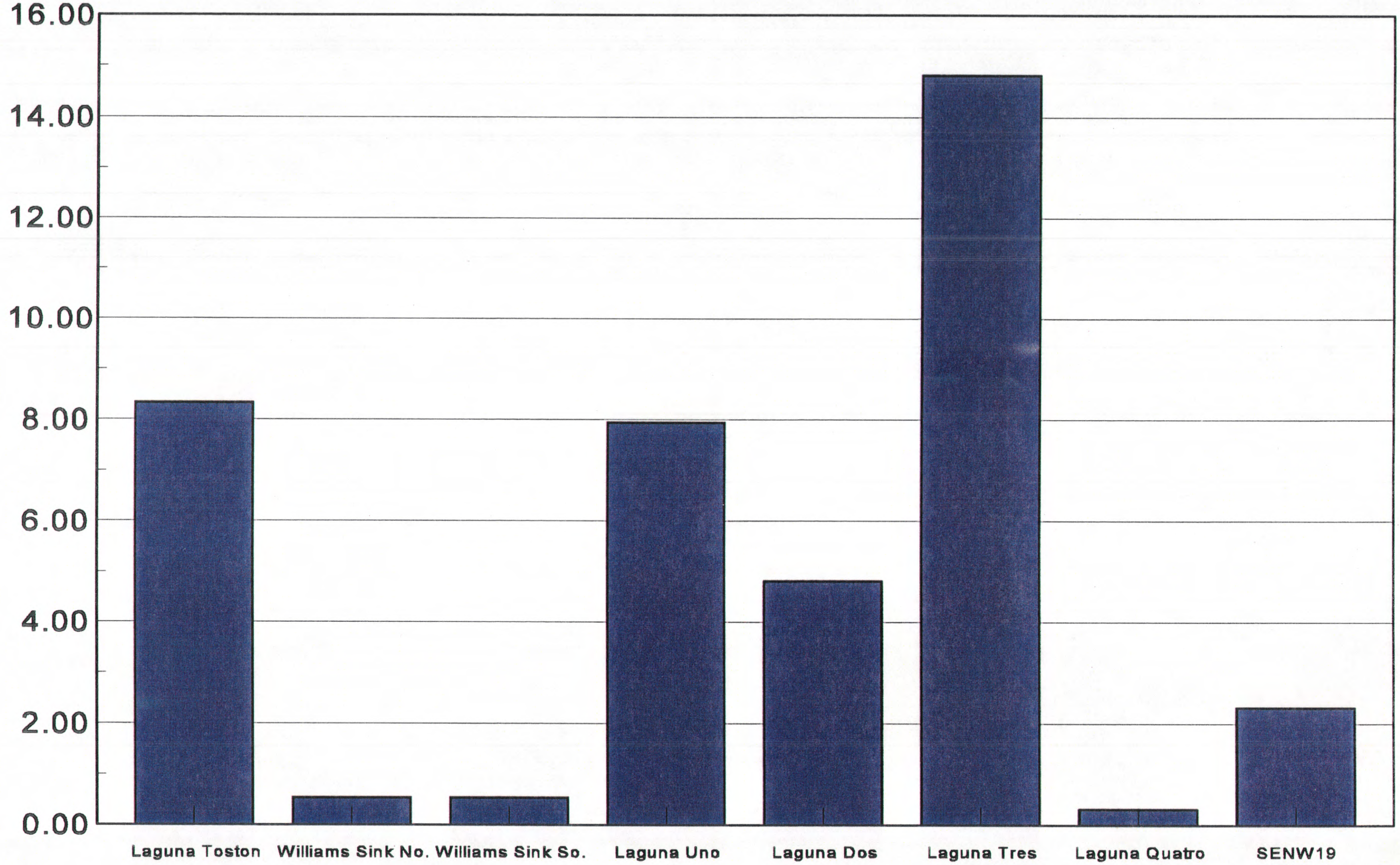


Figure 16: Number of waterfowl seen per hour of observation at playa lakes in SE New Mexico 9 March - 25 April 1995.

Arrival and Use Patterns

In general there was little correspondence between the number of waterfowl seen on censuses in the evening and on the following morning. It appeared that any ducks that remained on the playas through the day would depart after dark and that new arrivals appeared in the morning hours, mostly before noon. However, ducks could appear at any time of the day. It was not determined how many were migrants just ending their flight or were birds shifting between local playas.

Shorebirds using the edges of the lakes tended to be present for most of the day. Migrating flocks were typically observed in the pre-noon hours. Gulls could arrive at any time of the day. They typically would land on the water for several minutes and then either stand on shore or depart the area altogether. Phalaropes would stay only briefly at LT, departing the area in less than five minutes.

The greatest number of waterfowl tended to occur on the largest playas, but the difference was not significant. The reasons remain to be determined, but probably involve greater visibility and protection from predators. There was no indication that salinities affected bird numbers, except at the relatively fresh SENW19, which was sufficiently fresh to provide some food to diving ducks such as Bufflehead and Common Goldeneye.

Behavior

The condition of newly arrived waterfowl was ostensibly good, but deteriorated as time on the playa increased. Most of their time was spent resting, with preening becoming more prevalent the longer they stayed. After 2-3 hours on the water, a thin white layer of salt could often be seen on the breasts and flanks of the darker birds. Several behaviors suggesting irritation were observed in the ducks on the playas including: bill flipping, dolphin swimming, circling flights, searching behavior or leaving the water. The behaviors will each be discussed below.

Bill flipping.-- Most birds, after landing, almost immediately dipped their bills in the water, as if attempting to drink. They then quickly raised their heads and shook them side to side several times, flipping their bill to remove the water. This bill flipping was observed almost consistently for all birds including; ducks, gulls, avocets, phalaropes, and Long-billed Curlews. The rate of bill flipping seemed to be influenced by the birds' activity and possibly by the length of stay on the water. Loafing birds, when they had not preened for a while, may not flip their bills at all, or as infrequently as once a minute. Preening ducks flipped their bills constantly, presumably due to salt ingestion from the feathers. After preening, bill flipping may occur every 5-10 seconds for quite some time.

Dolphin swim.-- Preening behaviors appeared to become more vigorous as the salt encrustation increased. The casual nibbling and bill manipulations of feathers became aggressive tugging.

Irritated birds also appeared to attempt to wash the salt off by dunking their heads under the water and splashing it across their back and wings. This motion almost submerges the birds and they often made consecutive dunks, in a motion that resembles a porpoising dolphin. One female Lesser Scaup did a "dolphin swim" for about 45 seconds after attempting to fly, but apparently was unable to lift off the water. This behavior seems to indicate high irritation.

Circling flights.-- The dolphin swim behavior appeared to be associated with short flights of single birds. Single ducks were observed to leave a flock, fly around the playa once or twice, then land, often away from the flock. Again on the water, they would preen vigorously and/or dolphin swim briefly, then fly again.

An example: A female Green-winged Teal was observed flapping, preening and dolphin swimming for several minutes before she and four other teal flew one lap around LT. When they landed she began to dolphin swim until they all lifted off again a minute later. This occurred four more times with intervals of 50 seconds and a 1:20 between flights. The fourth and fifth flights around the lake were solely by the female. After the fifth flight, she landed on the ground approximately 70 meters from shore.

Searching behavior.-- Flocks of varying sizes from two to over 50 ducks, would occasionally make short flights for up to 2-5 minutes, often flying out of sight only to return several minutes later. The close proximity of the southern lakes to one another occasionally allowed us to make further observations of ducks that had departed. For example, flocks were observed descending over other waters, as if to land, only to abort and return to the original playa from which they left. In another case, after spending four hours and five minutes on LT's water, a male and a female American Widgeon flew north out of sight. Immediately before their departure, the male appeared quite irritated as he was preening vigorously and dolphin diving. Within 10 minutes, a male and female widgeon landed on WS South.

Leaving the water.-- Many birds exited the water, either to stand in shallow water or to walk on shore. The extensive salt shelf at LT afforded a protective overhang, as well as a means to escape the water. Ducks were occasionally flushed out from under this shelf during mortality surveys, and eight of the 45 birds found dead at LT were under the shelf. Birds on shore spent most of their time loafing, with an occasional preening bout and associated bill flipping. On 10 April at LT, at least 21 ducks, predominately Green-winged Teal, left a mixed flock of over 200 ducks (mostly Green-winged Teal and Northern Shoveler) exited the water, walked up on shore, and sat on the salt shelf and hill above it. These teal, which spent approximately the same amount of time on the water as the rest of the flock, exited the water more readily and in greater numbers than other species.

This apparent disposition to leave the water may reduce mortality among teal. As previously mentioned, Green-winged Teal and Northern Shovelers each made up approximately 50% of a flock of over 200 ducks. On the following day, however, teal comprised only 17% of deaths, whereas shovelers made up 60%. Lesser Scaup also represented 17% of mortality for the day, but constituted only 7% of the flock the previous evening.

DISCUSSION

During the spring observation period only 1579 waterfowl (about 4 birds/lake/day) and 340 other water-associated birds were observed on the playas. In general, migrating ducks seemed to arrive at the lake in the early morning hours, often before dawn. They usually test the water by dipping their bills, and then refrain from further drinking efforts. They quickly show signs of irritability, such as bill shaking, which can be followed by other behaviors such as leaving the water and flying off the pond repeatedly. Within several hours a salt crust can form on the dorsal surface; they make efforts to remove it by preening or diving, in some cases, but this effort only leads to further encrustation and, presumably, ingestion of salt. Ducks that remained on the playas for the entire day typically spent their time sleeping and preening offshore. Most migrants probably depart shortly after dark, as there is little night-morning correspondence between species presence and abundance on individual lakes. In periods of bad weather, however, the birds may become heavily salted before they can depart, and it is at these times that mortality is highest. Note, however, that it is not inevitable: some ducks can remain and survive all day under horrible conditions and still have left by the following morning.

VI. MORTALITY SURVEYS - *Spring, 1995*

Assessing the extent and timing of mortality events is often a difficult process. Under normal circumstances, mortality is rarely observed as scavengers and decomposition often remove the carcasses before they are noticed. Consistent repeated surveys must be completed over a defined area to determine the incidence of mortality.

METHODS

To locate and collect dead and dying birds, standard shore surveys were established and walked at maximum intervals of every three days for the north lakes (excluding WS South), and every four days for the south lakes. Some shoreline areas could not be reached due to impassible mud. At the larger playas, 2-4 km stretches were selected for their accessibility and the location on the downwind side of prevailing winds (to which dead or dying waterfowl would be blown). At the smaller playas, the entire shoreline was surveyed. On the first survey any carcasses present were documented or removed, creating a baseline against which to measure further mortality on each playa.

An attempt was made to obtain a blood sample from any bird captured alive; sick birds were then euthanized with CO_2. Carcasses and any blood collected were stored on ice as quickly as possible, and shipped overnight to the NWHC.

RESULTS

Species and Location

The frequency of mortality surveys is given in Table 7. Eighty-two old waterfowl carcasses were found on the initial (early March) mortality survey, (77, 93%) on LT and Laguna Uno. The mortality incidence on these lakes remained consistent through the study with both of these lakes receiving a great deal of waterfowl usage during the study. Laguna Tres had only one death but was second only to LT in number of waterfowl observed (Figure 14). SENW19 was used frequently, despite its small size. Further, because of its much lower salinity, it was the only lake at which ducks were observed feeding. No waterfowl were found dead there. All other lakes were barely used, with little or no mortality noted.

Sixty-five waterfowl, one American Coot, and one passerine were found dead or dying during 75 mortality surveys. Forty of these were relatively intact ducks, which were shipped to the NWHC. The remaining 26 waterfowl were heavily scavenged, and only six had enough remains to send to NWHC. Twenty-five ducks were found alive, were encrusted with salt and unable to fly or escape capture.

Table 7: Dates of beached bird surveys at playa lakes in SE New Mexico 9 March - 25 April 1995.

Laguna Toston	Williams Sink No	Williams Sink So	Laguna Uno	Laguna Dos	Laguna Tres	Laguna Quatro	SENW19
14-Mar	21-Mar	14-Mar	15-Mar	13-Mar	12-Mar	17-Mar	10-Mar
23-Mar	22-Mar	23-Apr	23-Mar	22-Mar	22-Mar	22-Mar	15-Mar
26-Mar	23-Mar		31-Mar	31-Mar	27-Mar	27-Mar	23-Mar
27-Mar	26-Mar		4-Apr	4-Apr	31-Mar	31-Mar	31-Mar
29-Mar	3-Apr		7-Apr	6-Apr	6-Apr	12-Apr	4-Apr
31-Mar	5-Apr		12-Apr	12-Apr	12-Apr	21-Apr	7-Apr
3-Apr	11-Apr		19-Apr	16-Apr	16-Apr		8-Apr
8-Apr	13-Apr		21-Apr	21-Apr	21-Apr		12-Apr
11-Apr	17-Apr		23-Apr	23-Apr	23-Ap		16-Apr
14-Apr	18-Apr						20-Apr
17-Apr	22-Apr						23-Apr
18-Apr	23-Apr						
19-Apr							
20-Apr							
22-Apr							
23-Apr							
25-Apr							

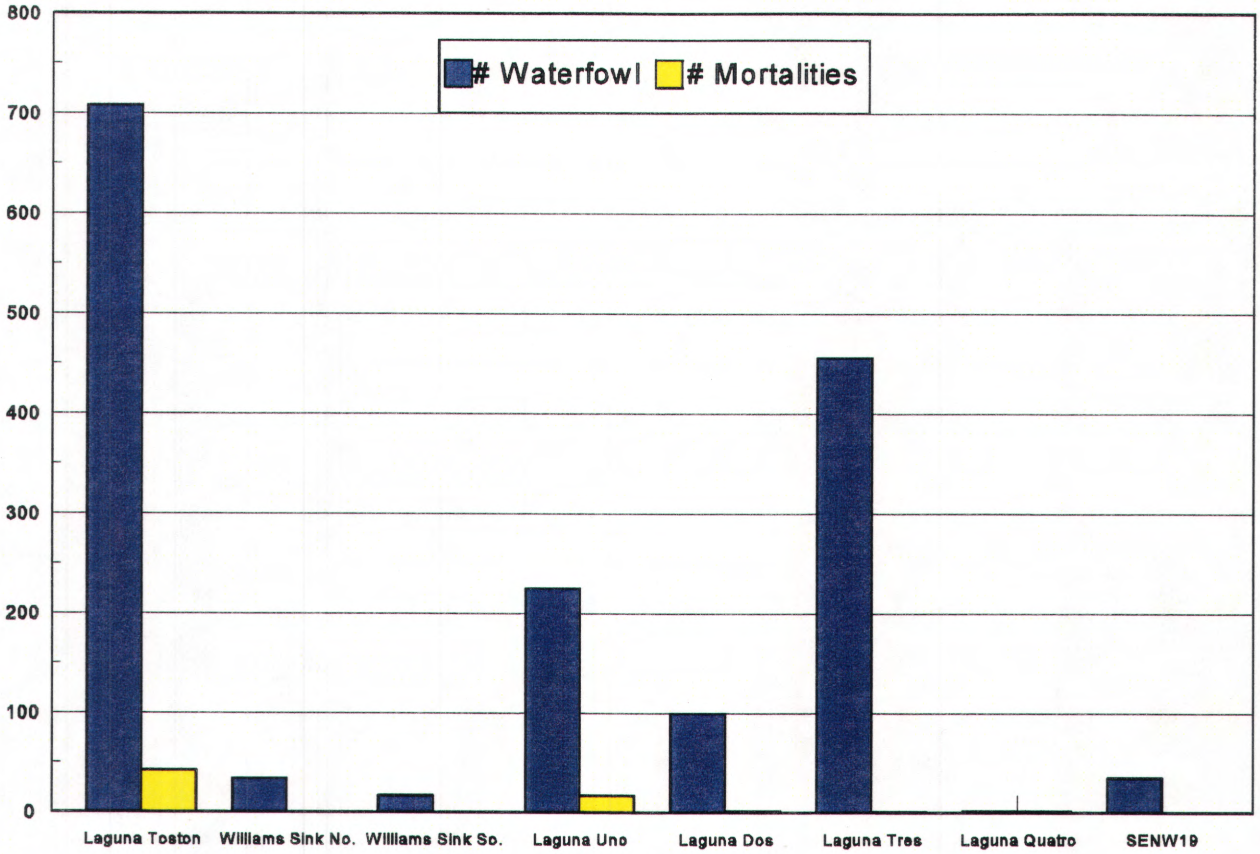


Figure 17: Number of waterfowl observed alive and mortalities discovered at Playa Lakes in SE New Mexico 9 March - 25 April 1995.

The distribution of the dead and dying ducks is shown by lake in Figure 17. LT had greater than twice the mortality of any other lake, with LT and Laguna Uno accounting for 92% of the total. Mortality and morbidity by species is shown in Figure 18. Thirty-nine percent of all birds found dead or dying were Northern Shoveler. This was twice as many as for any other species, but is not surprising as it is proportional to their overall abundance (59%).

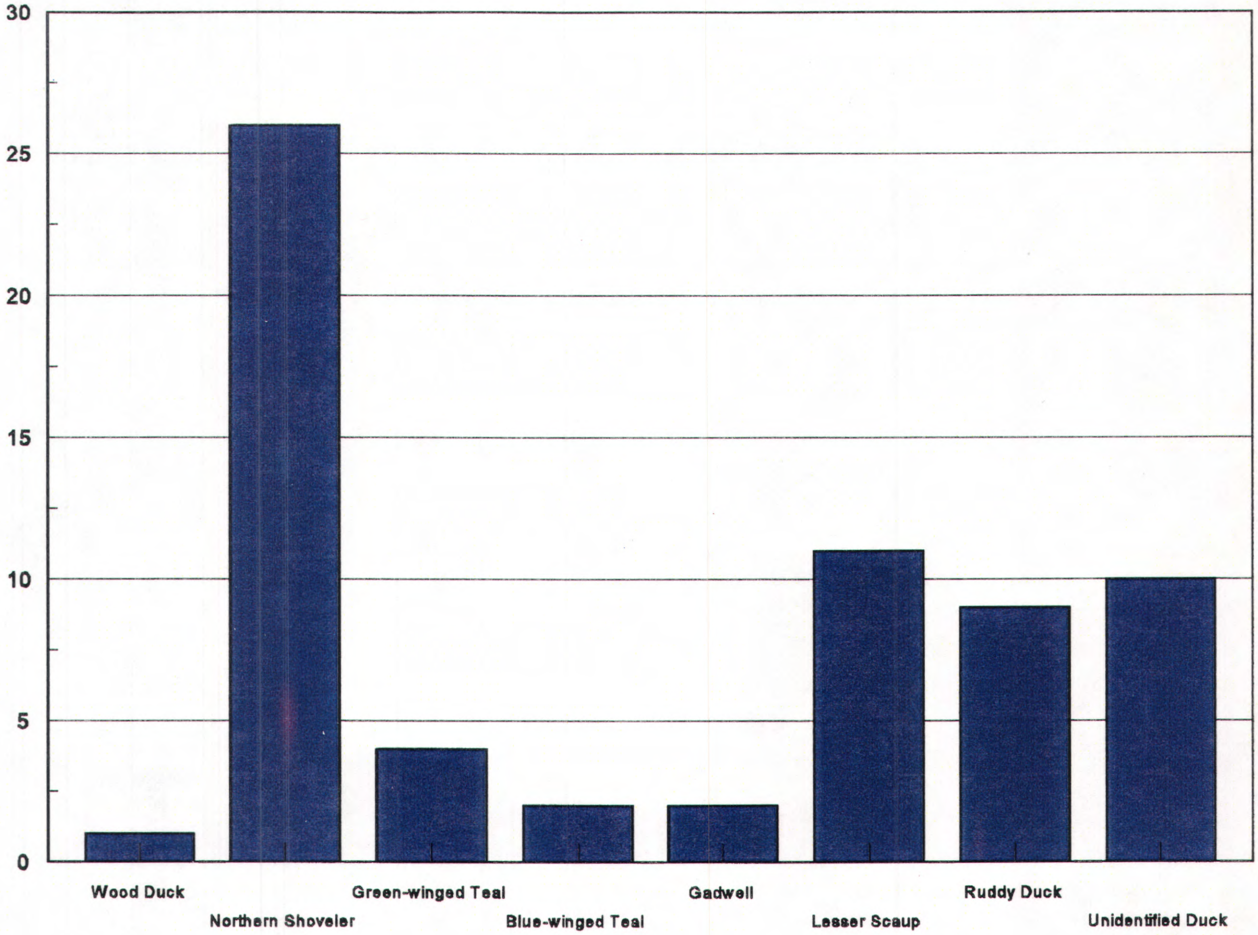


Figure 18: Dead and dying waterfowl collected at Playa Lakes in SE New Mexico 9 March - 25 April 1995.

Timing

Four days accounted for 51 of the 65 (78%) waterfowl deaths:

1. At LT on 11 April, 23 dead and dying waterfowl were found. The previous day there were more than 200 ducks, nearly all being Northern Shovelers and Green-winged Teal. That night was unseasonably cold and strong winds occurred into the following morning. These dead were of the same species makeup, as those on the previous day, and it is inferred they were derived from that flock. Their proportions differed,

however, as Northern Shoveler and Green-wing Teals had been equally represented in live birds, whereas three times as many shovelers as teal were found dead. Only 16 Lesser Scaup were seen alive the day prior, but four were found dead.

2. At LT on 17 April, 11 dead and dying birds were found. No observations had been made the previous day, but the weather pattern was similar to the day noted above; a cold night and windy morning.

3. At LT on 19 April a group of 18 Ruddy Ducks was observed sitting out very high winds (50-70 mph) on choppy water. Seven were found dead and heavily scavenged the following morning. All carcasses were on the downwind shore and were probably birds that were unable to depart because of the weather, long resident time, and subsequent encrustation.

4. The only large number of dead waterfowl on Laguna Uno was discovered on 16 April, when ten highly scavenged remains were found along the shoreline. Observations had not been made there for four days, so when the birds died and the length of time they had been on the water cannot be known.

The sporadic nature of the mortality events, and their association with harsh weather, suggests that these events are environmentally induced. It appears that most mortality occurs after weather conditions that prevent birds from leaving, and the resulting protracted stay causes them to be sprayed with noxious water. One would expect that the most numerous birds would have the highest probability of being caught in such events and our results support this. Northern Shovelers and Lesser Scaup were the most numerous ducks (53% and 12% of total ducks observed) and showed 40% and 16%, respectively, of the total mortality. A disproportionate number of Ruddy Ducks died (9 of 60), but seven of these (of 18 alive the previous day) died in one event, subsequent to some of the strongest winds (50-70 mph) encountered.

One might hypothesize that diving ducks would be more susceptible to salt encrustation than dabbling ducks because their diving results in greater wetting. While mortality was greater among divers (6.2%) than among dabblers (2.8%), this is not biologically significant for several reasons. First, only two of the nine diving species had mortalities. Most importantly, the majority of deaths (77%) occurred on only four days, three of which were known to be associated with bad weather.

No shorebirds or gulls were found dead, probably because only their feet or legs were exposed to the water. A few shorebirds (American Avocets, Wilson's Phalaropes, Black-necked Stilts) were seen swimming, but they mostly stood in the shallows (with the exception of Wilson's Phalaropes). Most phalaropes were observed at the less saline SENW19. When found on other lakes, their time on the water was very brief. Most gulls spent the majority of their time standing in the shallows and generally left the lake within a few hours. A few passerines were found dead during the initial mortality survey, but most were so old they were unidentifiable. No passerines were observed drinking or bathing in the lakes.

Mortality surveys were made regularly, either around the entire lake (small lakes) or for several km on the downwind side of larger lakes. These surveys are assumed to be reliable, because of the results of experiments described in the Carcass Effects and Disposition (Section V).

On the initial mortality survey in March we found 82 ducks, many of which had been dead for months. On the ensuing March-April surveys, conducted at the height of the waterbird migration, we found only 65 more carcasses. Mortality surveys that include the fall migration may offer a better indication of annual mortality.

Two large playas, LT and Uno, accounted for 92% of the deaths. Both received discharge from potash mines, but that may or may not be significant, in that the lake may have other common attributes (e.g., large size, open shores) that differentially attract ducks. Deaths occurred in rough proportionality to the abundance of individual species, with Northern Shovelers predominating (44% of casualties). There was no evidence that dabbling ducks were less vulnerable than divers as a group; however, the propensity for teal to leave the water and come ashore readily suggests that they may be better able to avoid the harmful effects of the water.

Mortality of the ducks observed totaled 4.1%, occurred sporadically, and appeared to be highly influenced by harsh weather, with 77% of the deaths being recorded on four days. Deaths occurred only among species that swam and spent long periods on the water. No species that primarily used the water edge habitats (gulls, shorebirds) was found dead.

VII. CARCASS EFFECTS AND DISPOSITION - *Spring, 1995*

These uncontrolled studies were preliminary attempts to assess the fate of wild birds after exposure to playa waters and their eventual disposition after death. Aspects considered were the effect of prolonged exposure on plumage, the amount of time that carcasses could stay afloat, the rate and persistence of salt deposition and the actions of predators. All these may affect the survival of birds and the reliability of mortality survey investigations. The results cannot be considered definitive because of the limited nature of the studies.

METHODS

Twenty-two dry carcasses (from frozen specimens) of 18 species of birds, ranging in body mass from 5.7 to 1059 g, were utilized in assessing feather wettability and water accumulation. Although not all were waterbirds, birds were selected primarily on the basis of representing a range of body sizes and plumages, as well as their availability. Eleven carcasses covering the range of available body sizes were used as test animals at each of the two playas (LT and WS). The dry carcasses were weighed to the nearest 0.1 g using a portable Ohaus electronic balance. Each bird was submerged for 1 minute in lake water and then reweighed. Carcasses were then tethered to a vertical submerged pole, using monofilament line, for periods ranging from minutes to hours to days and were periodically reweighed. The tether lines allowed the carcasses to float freely; at WS South they were approximately 6 ft. in length, whereas those at LT were 2-3 ft. in length. Carcasses at WS South were removed after two days, but at LT they remained in the lake for more than five days.

Disposition of carcasses after death was investigated in two ways. First, to determine the length of time needed for a bird to become unrecognizable and therefore difficult to locate, weights were attached to a road-killed male Bufflehead and submerged in approximately eight inches of water at LT on 23 March. Second, six heavily encrusted carcasses on WS South and five on LT were allowed to drift ashore, to determine if wave action would result in their encrustation.

The role of scavengers in removing carcasses from the shoreline was also examined. Six heavily encrusted carcasses were dropped approximately 10 meters from shore on WS. Of specific interest was the time needed for scavengers to locate carcasses, and whether they would remove them or eat them on the spot.

RESULTS

All carcasses gained weight after being submerged for one minute; mean weight gain was 12.2% at WS and 16.5% at LT and ranged from 3.9% (Mourning Dove) to 43.7% (Blackcapped Chickadee). After 10 minutes in the lake, the weights continued to increase (means = 26.3% and 38.3%, respectively, range = 9.6% to 76%), and by the end of nearly two days submergence, the mean weight gains were 94% and 269%, respectively, and ranged from 17% to 560%. At WS salt encrustation was plentiful on the backs of the floating carcasses when they were weighed at 29 hours, with the exception of the three smallest carcasses (kinglet, chickadee, and towhee), which had no salt crystals whatsoever on their plumage. At the end of nearly two days submergence, all birds were at least partially floating, even those with heavy salt encrustations. However, the three smallest birds; kinglet, chickadee and towhee, had only tiny salt crystals on their plumage at this time, unlike the heavy encrustations on all of the larger birds.

The carcasses at LT also continuously gained weight. All were still floating, despite heavy salt encrustation, after six hours. However, by 21 hours, some carcasses were neutrally buoyant, although not on the lake bottom. Wave action was more intense at LT, presumably due to its larger size and greater fetch than WS. As a result, waves washed over the backs of the LT birds, whereas at WS, birds tended to float on the relatively calmer water. The greater weight gains of the LT birds over the same 21-22 hour period is presumably due to this effect. However, even after 30 and 43.5 hours submergence, the heads of these birds were still above water at both sites. By 128 hours, all of the LT birds were beneath the surface, although not at the bottom, and had gained from 436% to 1164% of their body weight in salt and water.

Body size appears to have an effect on plumage weight gain. For unknown reasons, birds of increasing body size usually gained relatively more weight than smaller birds, irrespective of experimental submergence time. In reverse fashion, relative weight gain of birds weighing more than 45 g decreased with increasing body size.

A crust began to form on the submerged Bufflehead within two days, and within eight days the bird began to lose its dark coloration and resembled the substrate, although it would still have been easily identified as a duck by its shape alone. Not until 22 April (after 30 days) had it lost enough of its shape that it could have been overlooked. This process was documented on videotape.

The 5-8 mph winds blew the carcasses to shore within five minutes. By early next morning, the carcasses had become free of encrustation; presumably wave action was sufficient to break down the crystallization. All but one of the carcasses was easily found for the next month. The missing carcass was presumed to have been scavenged, as a few feathers were located approximately 30 meters from the water's edge.

At LT on 29 March, two unencrusted carcasses were placed on the salt shelf. They were undisturbed two days later, but were gone on 3 April. No signs of scavenging were observed.

On 30 March at LT, five heavily encrusted birds (gull, 2 Eared Grebes, shearwater, and a

Northern Cardinal) were allowed to drift ashore from 15 meters. As at WS, the carcasses were free of salt encrustation by the next morning. Only one bird (cardinal) remained in the water until the end of the project; the rest were removed by scavengers within 2-3 nights, but their remains were easily seen nearby. The cardinal became heavily encrusted, and might have been overlooked on a mortality survey within eight days. The gull was removed from the water and carried 40 meters inland, where it was partially scavenged. Before it was removed from the water, approximately half of the gull's body had been under the salt ledge, reducing its visibility and ease of access. The two grebes were plucked from the water and scavenged on the salt shelf. The shearwater disappeared.

Nine heavily encrusted carcasses were placed side by side on LT's salt ledge on 3 April to see whether scavengers would remove them. Five days later on 8 April, one carcass was located approximately 40 meters away, slightly torn apart but not necessarily scavenged. A second bird was missing eight days later and was located approximately 15 meters from its original location. It also had been slightly chewed on but not substantially eaten.

Although not a planned experiment, the Ruddy Ducks that died on April 19 were heavily scavenged overnight, but still easy to locate the next morning. Some of the carcasses were dragged up to 20 meters from the shore and were all plainly visible, even though most were just a few scraps of skin and feathers.

Turkey Vultures, Chihuahuan Ravens, Swainson's Hawks, and a Peregrine Falcon were observed feeding on dead waterfowl. Coyotes were frequently seen around the playas and their track and scat could be found along most shorelines.

DISCUSSION

Several preliminary conclusions may be drawn from this study. The plumage of birds that land on these hypersaline playas begins to gain weight immediately and becomes encrusted with salt within minutes to hours. Birds that remain at the playas more than a day or so, and are unable to preen off adhering salt, may not be able to leave. Once a bird dies, the carcass can float for several days, and presumably would easily be blown to shore by the generally high and constant winds. Therefore, beach counts of carcasses presumably yield an accurate assessment of mortality at these playas.

Although this study does suggest some answers on carcass disposition, it also raises questions about plumage wettability and species variation. In some respects, these are similar to the findings and questions from the feather analysis component (Section X). Additional work using a larger sample size, more appropriate species and combined with a more definitive feather analysis, might yield additional answers. Scavenging, while evident, does not appear so intense as to significantly skew the findings from the mortality surveys.

VIII. FIELD OBSERVATIONS - *Fall, 1995*

METHODS

Starting in November 1995, two biologists from the National Wetlands Research Center observed waterbird use and mortality at Laguna Toston. Laguna Toston was visited immediately after sunrise, at noon, or immediately preceding sunset and numbers of waterbirds on Laguna Toston recorded. On all observation days, shorelines were searched for carcasses after the noon observation. Birds found *in extremis* had blood samples collected and were euthanized.

RESULTS AND DISCUSSION

Observations were only conducted during 13 days between 5-20 November. The Federal furlough required that contract employees also be furloughed so observations were limited. During those 13 days of observation, 504 individual birds were observed using Laguna Toston, with lesser scaup (*Aythya affinis*), American wigeon (*Anas americana*), and northern shovelers (*Anas clypeata*) being the most frequently observed (Figure 19). Similarly, lesser scaup was the most common species found dead, although ruddy ducks (*Oxyura jamaicensis*), gadwall (*Anas strepara*) , and common loon (*Gavia immer*) appeared to be more vulnerable to exposure than other species. For ruddy ducks, only 10 individuals were observed, but 17 carcasses were recovered. For gadwalls and loons, the number of carcasses recovered was approximately half of the total number of birds observed alive (Table 8). In almost all cases, significant mortality was observed after inclement weather conditions, particularly high winds and low ambient temperatures.

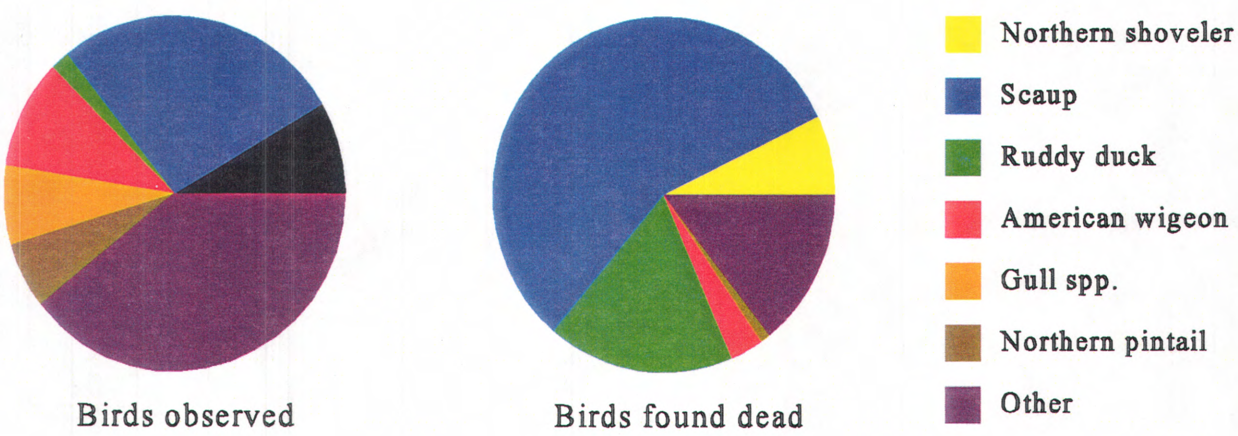


Figure 19: Birds observed and birds found dead at Laguna Toston, Fall 1995

Table 8. Numbers of individual birds observed, carcasses collected, and percent of carcasses recovered related to birds observed on Laguna Toston, 5-20 November 1995.

Species	Number observed	Number dead	Percent found dead
Northern shoveler	46	7	15
Lesser scaup	148	55	37
Gadwall	11	6	55
Ruddy duck	10	17	170
American wigeon	54	3	1
American coot	0	5	*
Northern pintail	33	1	3
Grebe spp.	5	0	0
Canvasback	33	0	0
Ring-necked duck	6	1	17
Green-winged teal	6	0	0
Mallard	2	0	0
Redhead	1	0	0
Lesser snow goose	1	0	0
Loon spp.	4	2	50
Sandhill crane	13	0	0
Gull spp.	39	0	0
Unidentified	92	0	0

* Because no live birds were observed, the value can not be calculated.

XI. A CLINICAL PATHOLOGY - *Field Experiment*

Examination of a blood sample can be considered a window on the physiological status of an organism at the sampling point. Historically, useful as an indicator of salt poisoning/water deprivation in domestic species has been plasma/serum sodium levels. Other chemicals are indicative of organ integrity and function. The emphasis during the experimental trials was evaluation of sodium and other physiologically important indicators of dehydration, electrolyte imbalance, stress and organ function in the sentinel ducks. Appendix 4 lists the tests that were run and their common uses.

METHODS

Blood samples were collected from the ducks at each time period: 0, 3, 7, 24, and 48 hours. Some of the ducks had blood drawn prior to euthanasia. Blood was obtained via needle and syringe primarily from the right jugular vein. In some of the birds, it was difficult to obtain blood due to their moribund state. In these incidences, blood was collected from the medial tarsal vein. In most cases, 3 cc of blood was collected and placed into tubes containing heparin as the anticoagulant.

The packed cell volume (PCV) was determined in the field via the microhematocrit technique. The blood was then centrifuged and plasma separated as a treatment group. Collection time, centrifuge time and separation time were documented during sample processing. The plasma samples were frozen and shipped to Southwest Veterinary Diagnostics where the chemistries were performed on a Hiatchi 736 chemistry analyzer. Osmolalities were determined by calculation.

Blood was collected from 16 moribund wild ducks found during the mortality survey. These samples were submitted as plasma or serum, frozen and shipped to NWHC. The chemistries were run at the University of Wisconsin Veterinary Medical Teaching Hospital using a Kodak Ektachem analyzer.

RESULTS AND DISCUSSION

A total of 14 clinical pathology tests were performed. Only a few of them were determined to be statistically (Appendix 3) or biologically significant during this experiment. Therefore, only the following will be discussed: blood urea nitrogen (BUN), chloride (Cl), creatinine phosphokinase (CPK), glucose (GLU), potassium (K+), sodium (Na), packed cell volume (PCV), and uric acid (UrA). (Table 9)

Sodium

The mean plasma Na levels were different among all three groups but no lake differences were noted (Table 9). Figure 20 shows a dramatic increase in Na for treatment group 1 within 3 hours of exposure. Shortly thereafter, the Na levels reached into the toxic range (above 160 mEq/L). Treatment group 2 shows a more gradual increase in Na levels between 3 and 48 hours, which approach the toxic range. The elevation in group 2 may be a result of dehydration alone, or a combination of dehydration and sodium intake. Treatment group 3 showed no significant change in Na from baseline levels.

Table 9: Clinical Pathology results (group mean values - at 48 hr for both lakes)

Group	Na mEq/L	BUN mg/dl	PCV %	UrA mg/dl	CPK IU/L	Cl mEq/L	AST IU/L	GLU mg/dl
1	163.1	2.1	. 38.5	7.5	1071	128.2	20.7	292.4
2	153.7	2.4	43.4	5.2	496	113.4	16.6	226.9
3	147.3	0.2	. 45.0	3.5	488	107.0	13.4	217.5

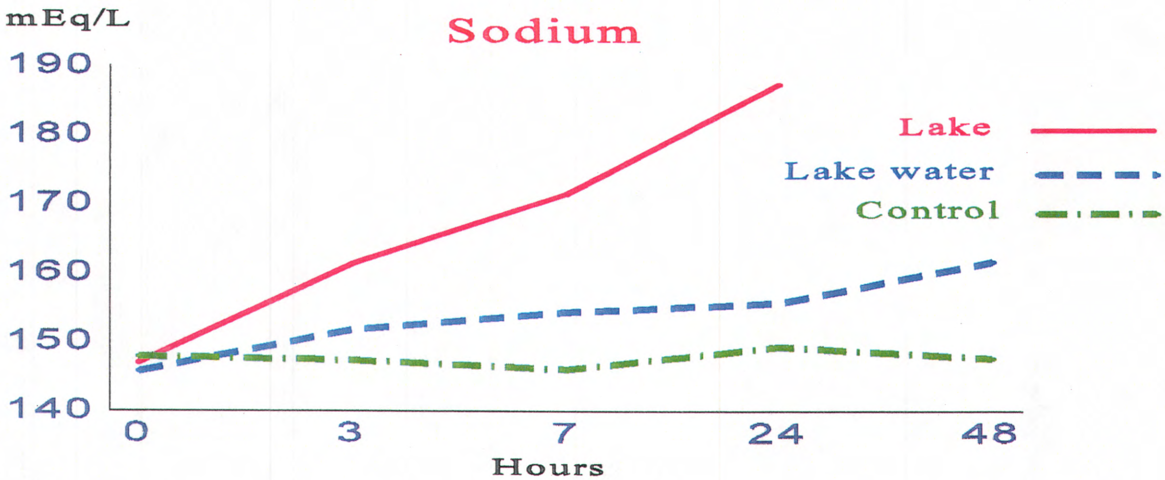


Figure 20: Mean plasma sodium levels for each treatment group in field experiment

Dehydration

Statistically, the mean BUN values showed differences among groups, over time and group by time interactions but there were no lake differences (Table 9). Treatment group 1 showed a rapid increase in BUN over time, reaching abnormal levels within 7 hours (Figure 21). Treatment group 2 levels did not show an increase in BUN until after 7 hours, with the incline mimicking that noted in group 1. The levels for treatment group 3 remained at baseline values throughout the study period. BUN has been demonstrated to rise in dehydrated chickens.

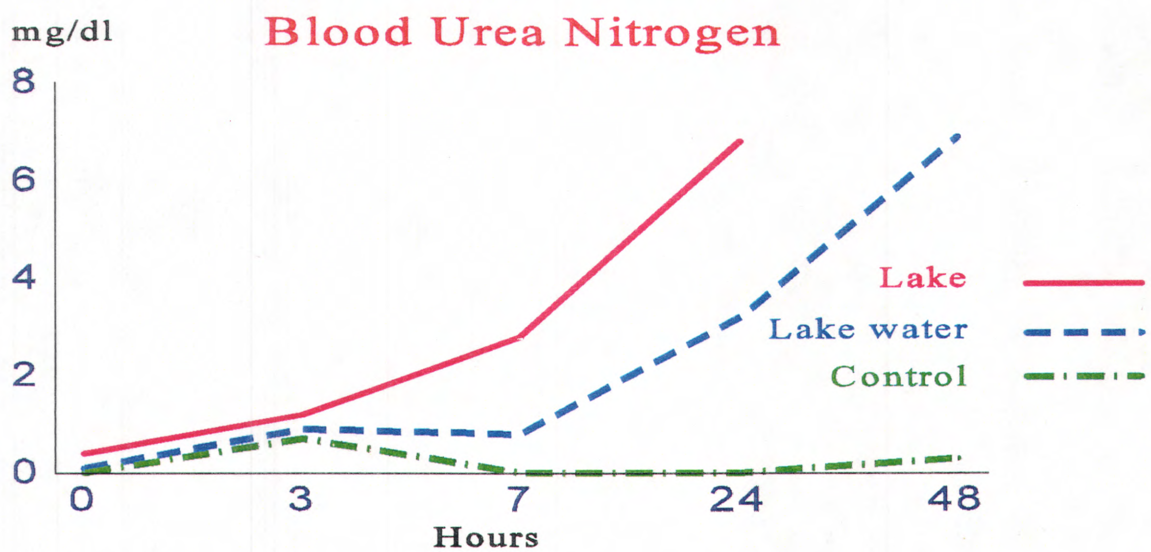


Figure 21: Mean plasma blood area nitrogen levels for all treatment groups.

The PCV values for all three treatment groups showed no consistent pattern throughout the study period. The mean values for treatment group 1 were slightly lower than the other two groups but these differences were not supported by statistical analysis (Table 9). Other investigators have found PCV to be a poor indicator of dehydration for avian species. It may be related to the fact that certain species of migratory birds have been shown to have mechanisms to conserve plasma volumes in the early stages of dehydration.

Historically, TP has been suggested to be a useful indicator of dehydration. The TP values in the experimental birds showed no consistent pattern biologically or statistically. The patterns appeared to show the same inconsistencies as were noted for PCV.

Renal Function

Kidney function is most commonly evaluated in avian species by measuring UrA levels. Mean UrA levels were different among groups with no lake differences noted (Table 9). There was a dramatic increase in UrA in treatment group 1 within the first 3 hours. The levels of UrA remained fairly stable in treatment group 2 until 7 hours, after which there was a gradual increase until the 48 hour time point (Figure 22). The UrA levels for treatment group 3 remained fairly stable over time.

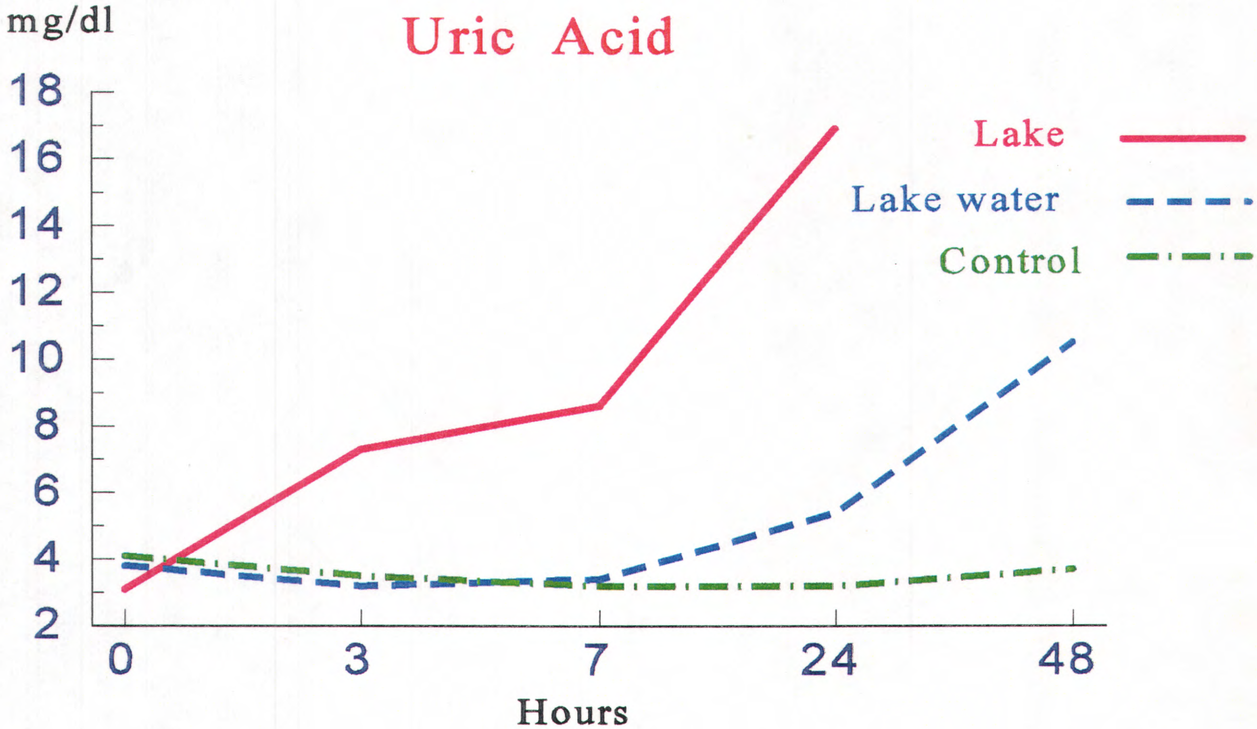


Figure 22: Mean plasma uric acid levels for all treatment groups.

Other Electrolytes

The K+ levels were found to be increase with the duration of time that the plasma was allowed to stay in contact with the red blood cells. All statistical analyses of K+ values were corrected for this variation.

Statistically the K+ levels were found to be different between lakes and among groups, but not for lake by group interactions (Table 10). The K+ levels also changed over time for the lake by time interactions and for group by time interactions. The ducks from treatment group 1 on LT have a dramatic increase in K+ levels whereas the ducks on WS showed a slower gradual incline until after 7 hours (Figure 23- 24). The final K+ levels were higher on WS but these birds also lived longer therefore had longer exposure time. Ducks in treatment group 2 showed an increase in K+ levels between 7 and 24 hours. This elevation may be related to dehydration. The ducks in treatment group 3 had K+ levels that were more stable, but there was an elevation after 24 hours.

Table 10: Potassium (mEq/L) (group mean values at latest sampling before death)

Group	Toston	W. Sink
1	5.0	6.0
2	3.5	3.8
3	3.8	4.1

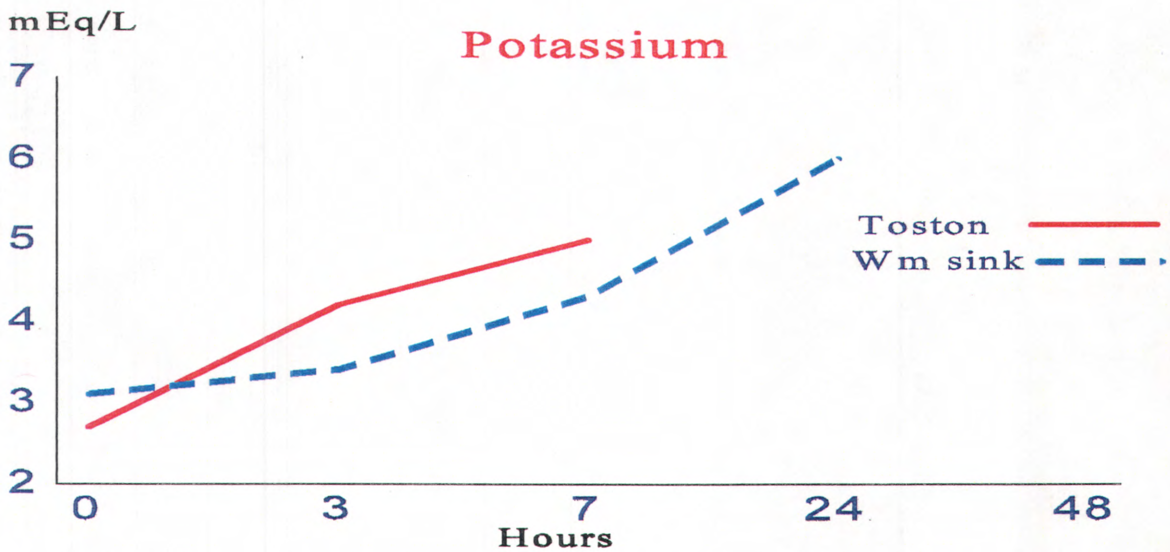


Figure 23: Mean plasma potassium in lake group between LT and WS

The lake differences in plasma K+ levels may be related to the differences noted in the water chemistries between the two lakes (K+ levels were two times higher at LT compared to WS). There is evidence that high oral doses of potassium can be toxic to domestic species. It is hypothesized that potassium may have a synergistic effect resulting in the more rapid death of ducks on LT compared to WS.

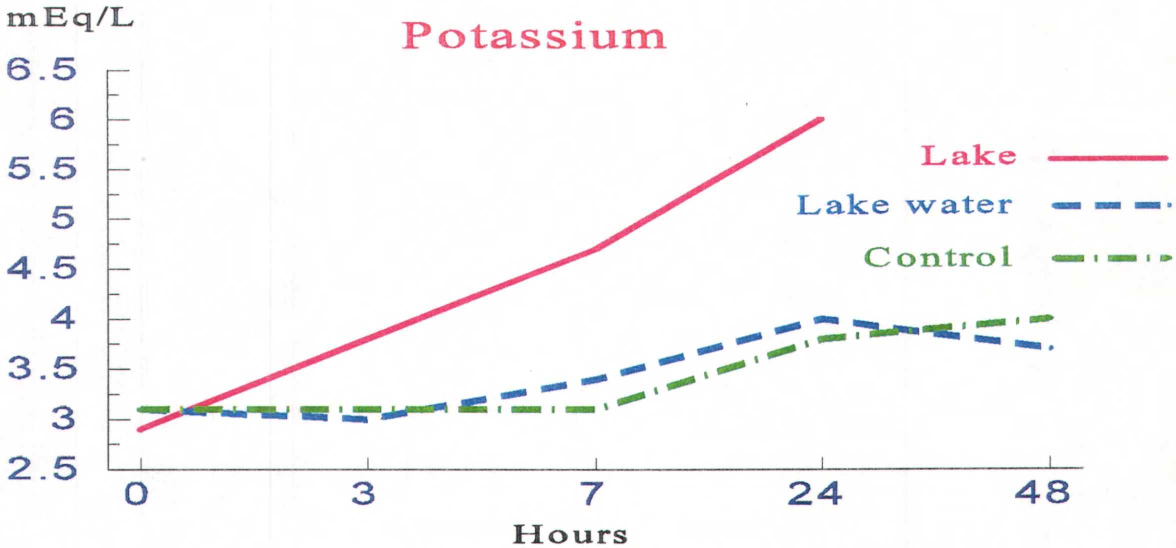


Figure 24: Mean plasma potassium levels for each treatment group in field experiment.

There is very little information available concerning chloride toxicity. Most of the literature relates to sodium chloride as a whole entity. The plasma Cl levels were found to be statistically different between groups with no lake differences noted (Table 9). Treatment group 1 has the highest values but the mean Cl levels for treatment groups 2 and 3 are fairly similar and are not considered to be biologically significant. There is a rapid rise in chloride levels in treatment group 1 (Figure 25). There appears to be a slight increase in group 2 after 24 hours with group 3 Cl levels remaining fairly stable throughout the study period.

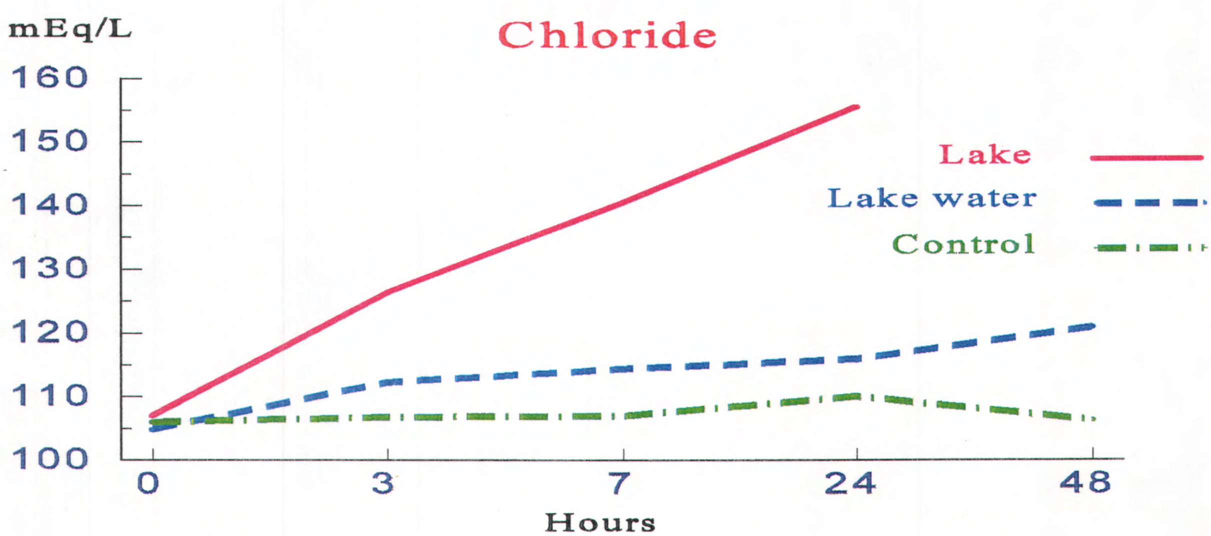


Figure 25: Mean plasma chloride levels for each treatment group in field experiment

Glucose

Blood glucose levels have been noted to be elevated in avian species in response to stress. Elevations in blood glucose has also been implicated in incidences of long term fasting (72 hours) in avian species. The mean GLU levels were different among groups with no lake differences noted (Table 9). Figure 26 shows that there was a dramatic increase in GLU levels for treatment group 1 whereas the values for the other two groups remained fairly stable and remaining within the normal range for Mallards.

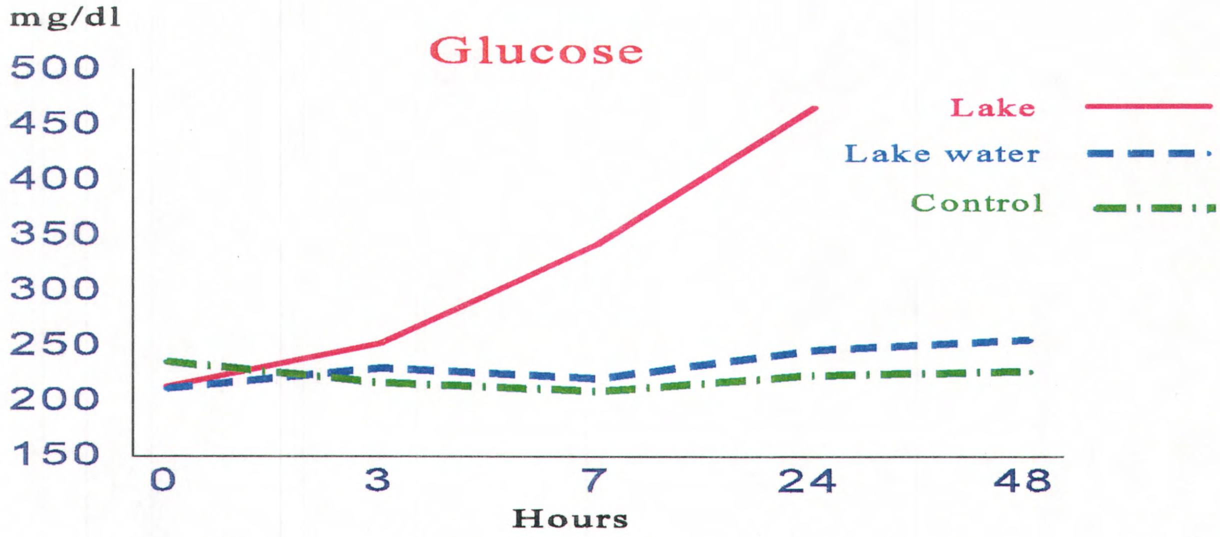


Figure 26: Mean plasma glucose levels for all treatment groups.

Enzymes

Liver and muscle cell damage was evaluated and differentiated using CPK and AST levels. No lake differences were noted but there were group differences for CPK (Table 9). There appeared to be a gradual increase in CPK levels over the first 7 hours in all three groups but was most rapid in treatment group 1 (Figure 16). Treatment groups 2 and 3 showed a decline in CPK values between 7 and 24 hours, reaching near baseline levels at 48 hours. As CPK is a measure of muscle cell damage, these elevations may be related to initial handling and transport of birds at 0 hr. The ducks in treatment group 2 and 3 had minimal handling there after, whereas the ducks in treatment group 1 were within the water for the duration of the experiment requiring more activity and possibly causing an increase in leakage from muscle cells. Elevations in CPK have also been noted in incidences of dehydration.

The elevations in AST were more likely to be related to muscle rather than liver cell damage. No lesions were noted on gross or histological evaluation of the liver of the experimental ducks. Mean AST levels showed statistical differences between groups but no lake differences (Table 7).

The AST levels were fairly stable in all 3 treatment groups until 7 hours. Then there was a dramatic increase noted in AST for treatment group 1 on WS (Figure 17). The ducks on LT died prior to the 24 hour sampling period and it is speculated that there was probably a similar pattern for these birds. The gradual onset of neurological signs could have resulted in increase muscle stimulation and therefore increase in enzyme leakage.

There was a dramatic increase in AST in the ducks from treatment group 2 on LT between 24 and 48 hours. The ducks in the same group on WS started to show a more gradual increase in AST during the same time frame. This is difficult to explain but may be related to dehydration.

Mortality Survey

The clinical chemistries from the mortality survey samples were compared to the ducks in treatment group 1 (Table 9). In this comparison, only Na, BUN and UrA were evaluated. The clinical chemistry values are all considerably higher in the mortality survey samples compared to the mean values from treatment group 1. It is felt that this information substantiates that our experimental trials do mimic what is happening in the wild birds.

X. CLINICAL PATHOLOGY - *Controlled Environmental Studies*

Methodology for this section was similar to that described in Section XI. and Appendix 1, although samples were analyzed by a different laboratory. It is accepted that, while variation does occur between laboratories, these variations should not be different enough to change the interpretation of results.

RESULTS AND CONCLUSIONS

No significant differences were found between treatment groups, so all values were pooled and compared to the TG1 control. Many of the chemistry parameters followed the course as discussed in the preceding section.

The most significant ion, sodium, had values moving out of the normal reference range within 3 hours and into the toxic range within 7 hours (Figure 27).

The other major electrolytes, potassium and chloride rose over time in the treatment groups (Figures 28 - 29).

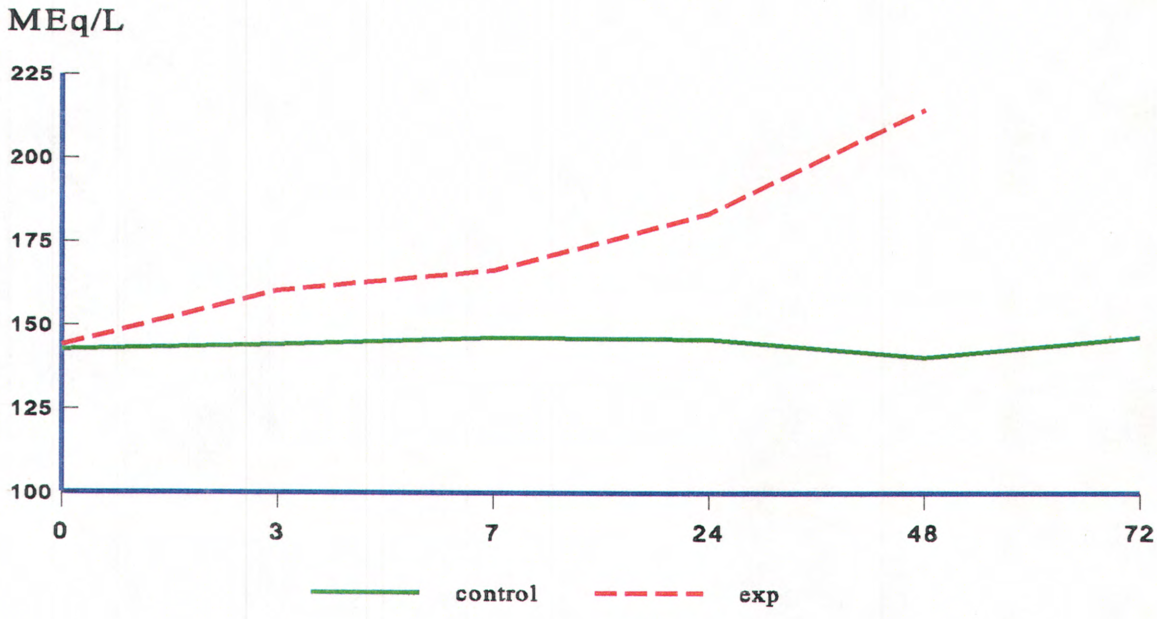


Figure 27: Mean plasma sodium levels in control and pooled experimental groups.

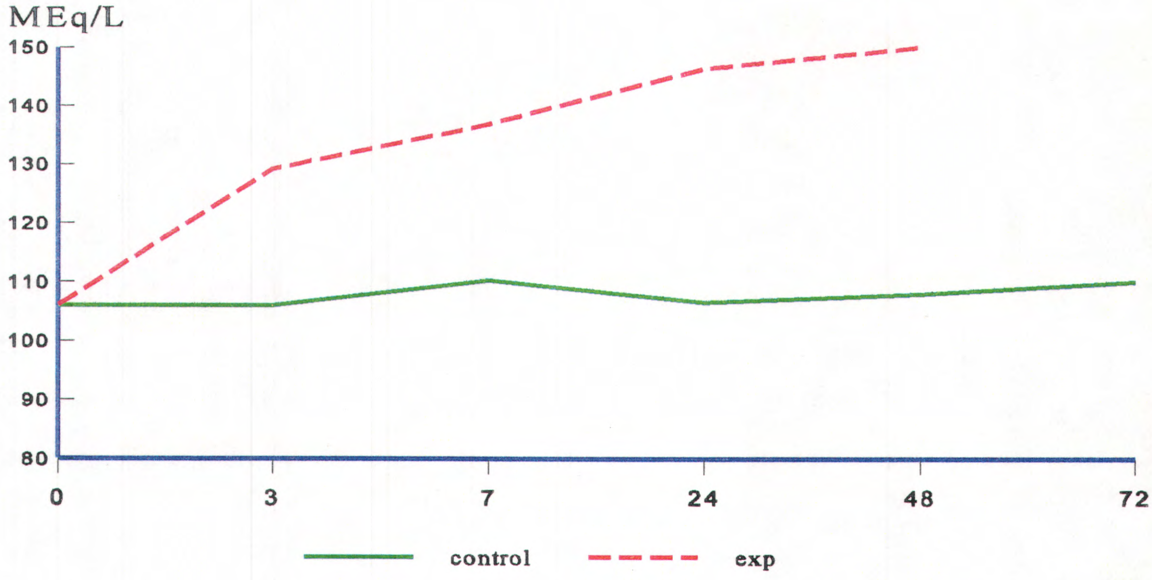


Figure 28: Mean plasma chloride in control and pooled experimental groups.

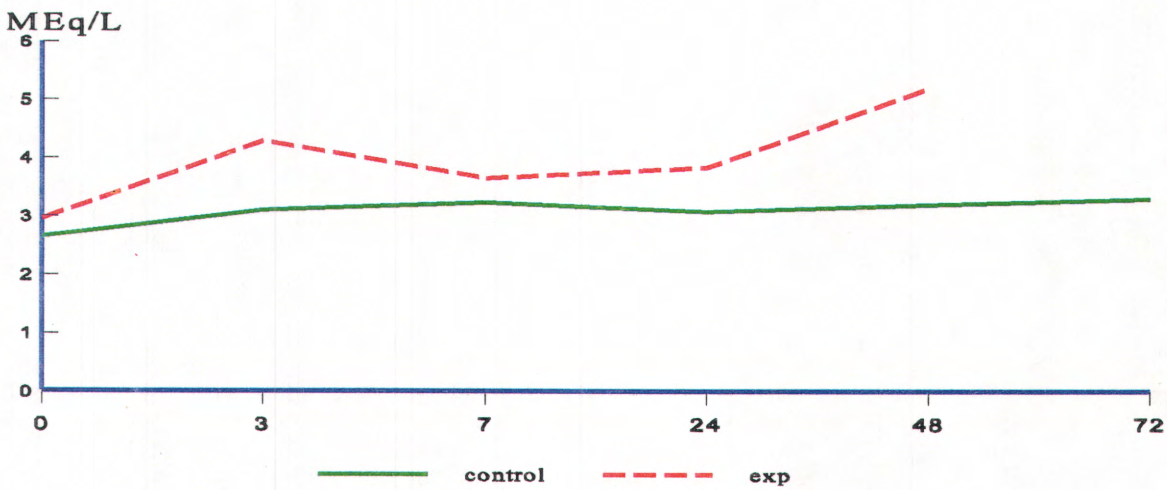


Figure 29: Mean plasma potassium levels in control and pooled experimental groups.

Unexpected results were the elevation of BUN and uric acid in the control groups, along with the treatment groups (Figures 30 - 31). As both of these analytes can be related to kidney function there may be some concern that some renal damage occurred in this experiment. This in some way may also be related to the inconsistent changes in CPK in both groups (Figure 32). CPK is a leakage enzyme that enters the blood due to muscle damage. Myoglobin would also be released at this time, and this substance can have an interfering effect on renal excretion, and in some way effected urea and uric acid processing. However, osmolality, a good indicator of fluid homeostasis maintained by adequate renal function, remained level in the control group, but increased in the treatment groups (Figure 33). Because of these results, a confirmation of dehydration, based on plasma analysis, could not be made from this experiment, as before.

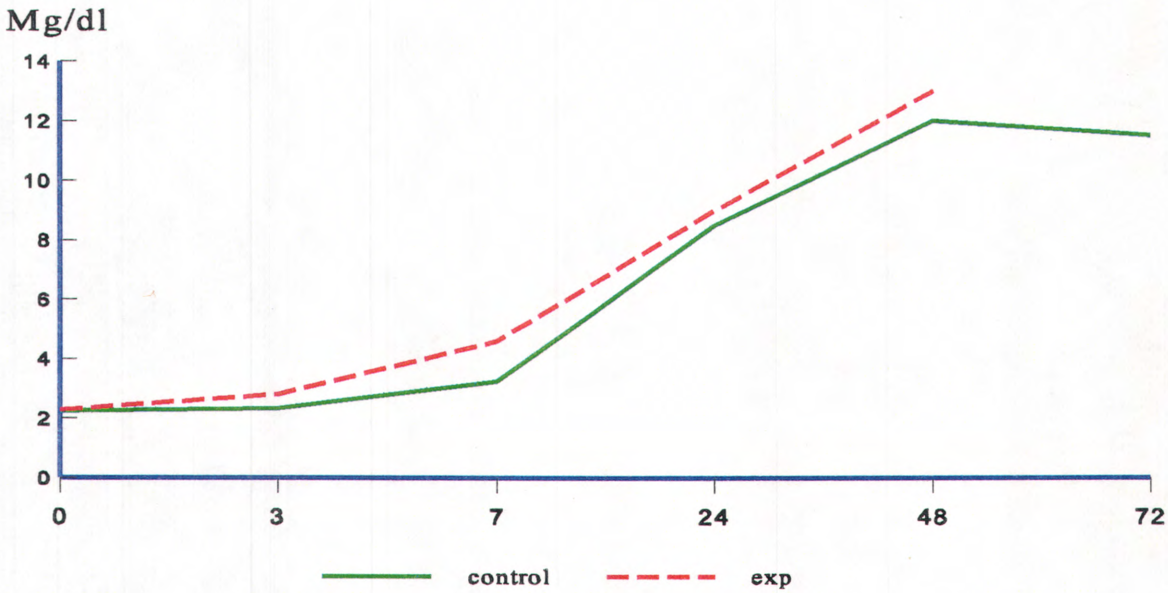


Figure 30: Mean plasma BUN levels in control and pooled experimental groups.

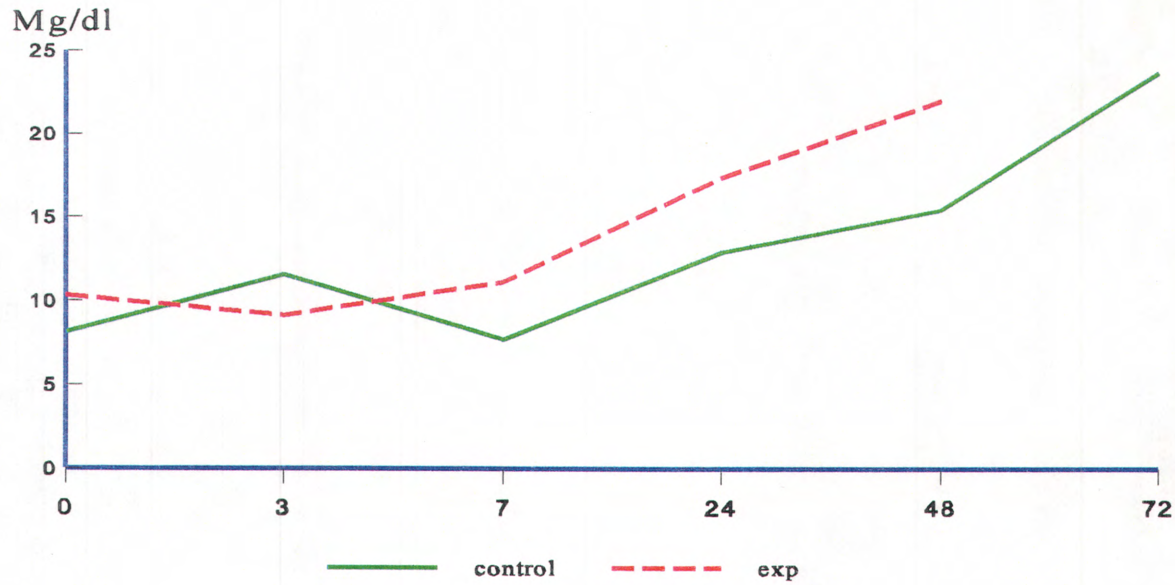


Figure 31: Mean plasma uric acid levels in control and pooled experimental groups.

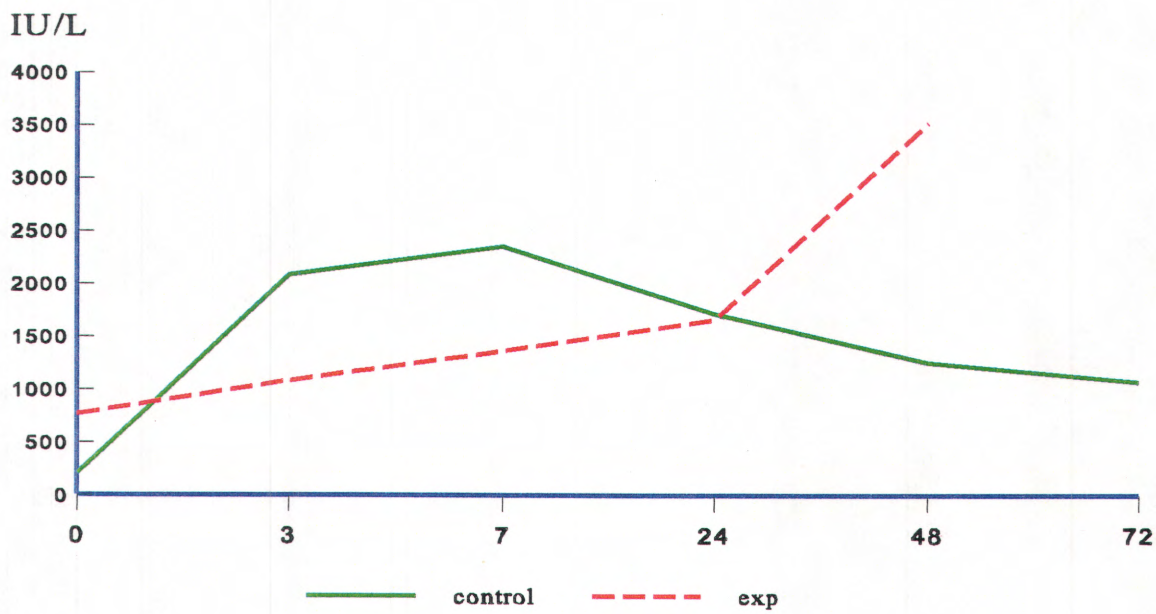


Figure 32: Mean plasma CPK levels in control and pooled experimental groups.

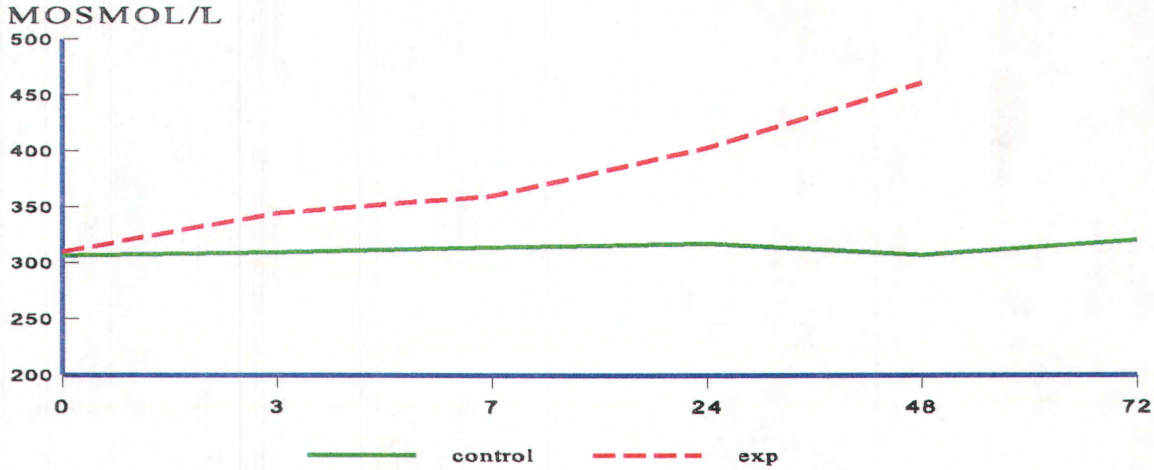


Figure 33: Mean plasma osmolality levels in control and pooled experimental groups.

Glucose values increased in the treatment groups, but not the control(Figure 34). As previously noted, glucose can rise as an indicator of excitement or stress, as well as fasting. A concern was that because the waterlogged birds paddled vigorously, this might elevate the glucose levels in TG1. However, as this is not the case, a more likely explanation, as in the field study, would be related to osmotic stress or inappetance.

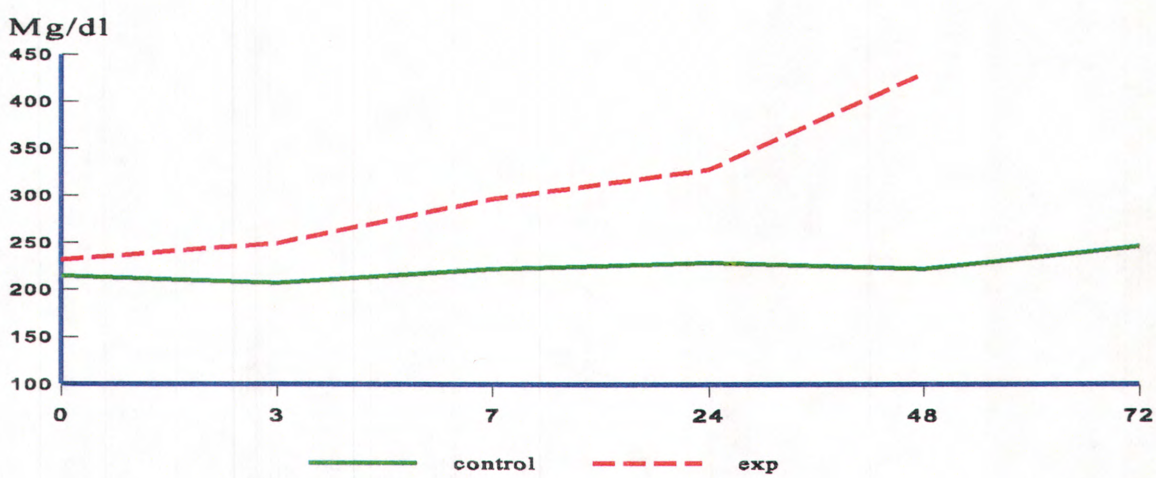


Figure 34: Mean plasma glucose levels in control and pooled experimental groups.

As the conditions affecting the field and controlled environment studies were very different, it was not appropriate to statistically compare the clinical chemistry values obtained. However, visually inspecting the changes and trends for each analyte involved reveals striking similarities.

XI. NECROPSY AND HISTOPATHOLOGY - *General and Field Experiment*

A thorough post-mortem examination will uncover most significant features that contribute to the death of an animal. The animal is examined first externally, then internally, with each organ inspected in a systematic fashion. Samples for further analysis are routinely taken, including bacteriology, virology, parasitology, chemistry and specimens in formalin that will be mounted on slides for microscopic examination (histopathology).

METHODS

Necropsy

Necropsies were performed using a standard protocol on 70 ducks from LT, WS, Uno, Dos, Tres and Quatro. All mallards (n=34) were experimental birds from November 1994. All of the other ducks were from the Spring 1995 mortality survey.

Photographs of 50 carcasses were taken before necropsy to detail the pattern and degree of salt precipitation on the carcasses. The weight of each carcass was taken before and after rinsing (as described in the Appendix 1) to get an estimate of salt precipitation and body weight. Brains were removed from all birds and analyzed as described in the Section XV on Brain Chemistries.

Other testing

Histopathology was performed on approximately 1260 tissues representing samples from all carcasses. Other tests performed for experimental birds and mortality survey samples included: 84 mineral scans and brain chloride concentrations, 24 brain cholinesterase tests, 9 liver lead analyses, 56 bacterial cultures, 30 avian botulism tests, and 13 virology cultures.

RESULTS AND DISCUSSION:

All birds examined were in good to excellent body condition with the exception of the first 4 mallards used for a preliminary trial August 1994, which were in fair body condition.

Salt precipitation

Both experimental and mortality survey samples from LT had abundant salt precipitation on the carcass with as much as 1955 gm of salt on the feathers. The precipitated salt was more than twice the weight of the duck in some cases. Average salt precipitation on duck carcasses from Lagunas Uno, Dos, Tres, Quatro and WS were less than LT (Table 11), however, size of bird, time of euthanasia, weather conditions and the fewer number of birds from these sites may have influenced this data.

Table 11: Mean salt precipitation (gm), experimental trials and mortality survey birds

Lake	LT	WS	Uno	Dos	Tres	Quatro
Precipitation	516	21	133	47	372	115

Necropsy

Very congested, deep red brains (commonly seen in salt poisoned domestic birds) were seen in the experimental mallards housed in the water on LT and WS. The mortality survey samples most likely to have congested brains were those that were collected dead rather than euthanized. The lenses of the eyes of all experimental mallards (10/10) housed on playa water were opaque, suggesting cataracts, all of these mallards had toxic levels of brain sodium. Opaque lenses correlated with salt poisoning (based on brain sodium concentration) in the ducks found dead from Laguna Dos and Tres. Mortality survey samples were often euthanized from LT and it was more difficult to determine lens opacity in these birds.

Other tests

Table 12 summarizes the histopathological lesions noted in the experimental birds. Histopathology performed on the experimental mallards showed high correlation between eye lesions and placement on playa water (those with salt poisoning). Cataracts and ulceration with severe inflammation of eyelids were seen in all mallards on playa water. Ulceration and inflammation was seen less consistently within the lining of the nasal cavities, mouth, at the back of the throat and occasionally within the opening of the cloaca (anus). These changes were not seen in any of the mallards housed on land.

Table 12: Number of birds with histopathologic lesions from each treatment group.

Lesions	Lake	Lake water	Control
Severe conjunctivitis	10	0	0
Cataracts	10	0	0
Turbinate inflammation	10	0	0
Granulocytic bursitis	4	0	0
Granulocytic bronchiolitis	3	8	4

The only mortality survey samples without cataract formation were two euthanized birds; one scaup from Laguna Uno with severe ulcerative conjunctivitis and one gadwall from LT, as well as one coot from LT that was found dead from trauma. None of these ducks had brain sodium in the toxic range. Ulceration of the eyelids, nasal cavities, throat and cloaca were not seen as consistently in the mortality survey samples. This inconsistency may again have been due to euthanasia halting progression of the lesions. It is interesting that early cataract formation was seen in some euthanized ducks before lethal levels of brain sodium were reached.

Brain moisture content was determined in 3 shovelers and a ruddy duck from LT. It is interesting that the two ducks with salt poisoning had an average brain moisture content of 77% and the two ducks with brain sodium in the normal range had an average of 82% moisture again suggesting dehydration of approximately 5%, consistent with the findings in the skeletal muscle moisture analysis (Section XIII).

No significant bacteria were isolated from any of the experimental or mortality survey samples. No viruses were isolated. No lead was detected in the liver and brain cholinesterase activity was normal.

In summary, cause of mortality in experimental ducks at LT and WS and mortality survey samples from LT, Dos and Tres was salt poisoning. Changes seen at death, or euthanasia (salt precipitation, cataracts and inflammation of the internal lining of the eyelid, nose, mouth, throat and cloaca) were identified in both experimental and mortality survey samples but were most pronounced and consistent in the experimental mallards. It would appear that environmental conditions (high winds and cold temperatures) were associated with heavier salt precipitation of the mortality survey carcasses. Behavior of the birds may have accelerated clinical signs and sodium intake in the experimental mallards by excessive preening and therefore ingesting salt from feathers. Ducks were often seen rubbing their heads on their bodies. This may have rubbed salt into their eyes and nose, and could have resulted in the irritation and inflammation seen microscopically.

XII. NECROPSY - *Controlled Environment Study*

METHODS

Necropsy of 15 experimental animals was performed from August 14 to 16 1996. All of the birds from TG1 were euthanized with carbon dioxide. All of the birds from TG2, TG3, and TG4 were found dead. The brains and eyes were extracted as soon as possible after death. Some of the birds were refrigerated prior to finishing the remainder of the necropsy. None of the birds were refrigerated for longer than 15 hours. The majority of the necropsies were performed within 2-5 hours after death.

RESULTS AND DISCUSSION

In general, the carcasses were in good body condition, with scant to no subcutaneous or abdominal fat noted. The feather condition appeared to be good but the majority of the ducks were fairly saturated, only five of the ducks having dry down at the level of the skin. Several of the ducks were noted to have bruising on the webs of their feet.

The birds from TG2, TG3, and TG4 were coated with only a fine layer of salt precipitate that was generally noted on the head and neck. Some of the birds had salt distributed over the back and breast regions.

There were no gross lesions noted on the adnexal tissues of the eye with the exception of #188 which showed 1+ chemosis at the medial canthus of the conjunctiva. Cataract development was variable, with some ducks showing bilateral variation. The birds found dead had collapsed anterior chambers, while those specimens found freshly dead still had intact anterior chambers.

The gross lesions of the oral cavity varied. Most of them contained small to moderate amounts of clear mucoid material. A large mass of feather material was noted under the tongue of #183. A few of the birds did have food particles within the oral cavity. The turbinate and epiglottis scores varied considerably. There appeared to be some epithelial sloughing and plaque formation around the epiglottis of #200.

The salt glands were generally small in size with scores of 1 to 2. The glands measured 1.2 x 0.3 cm to 2.2 x 0.6 cm.

The brains were severely congested in all of the ducks except the four ducks from TG1 (#190, 192, 193, 194).

The tracheal tissues appeared normal. A few of the birds had mild congestion at the syrinx. The majority of the lungs were bright pink in color, floated in formalin and exuded small amounts of tan fluid on cut surface. Three of the birds had serosanguinous fluid in the cranial and caudal air sacs. The lungs of these birds exuded large amounts of tan, frothy fluid on cut surface. The serosanguinous fluid varied in volume from 2 to 25 cc (#183, 185, 184, 188, 196, 198, 199).

Most of the intestinal loops were contracted and/or gas filled with the exception of #192,184 and 194, which were filled with food/fecal material. The ventriculi were filled with sand and pebbles. One contained a small piece of metal (#) The ventriculi of #192, 198 and 199 contained feather material. The esophagus of #184 and 194 were dilated and food filled. Several of the proventriculi were filled with yellow mucoid material (#188, 193, 195), or brown mucoid material (#180, 187, 200). The cecum of #196 was filled with very hard fecal material and petechial hemorrhages were noted along portions of the small intestine. The livers were generally contracted with slightly distended gall bladders.

An enlarged spleen was noted in #200. Small bursa were noted in most birds with the exception of #184, 185, 198 and 200. The bursa were excised with the cloaca. The remaining endocrine/lymphatic tissues were unremarkable.

The testes were small to very large in size. The kidneys were unremarkable with the exception of #188 and 196 which were congested with urates, #185 showed severe congestion.

The external diameter of the cloaca averaged 1 cm. One cloaca showed some sloughing of the superficial layers (#195), another showed an area of ulceration on the external surface (#188). The internal diameter of the cloaca ranged from 1.3cm to 3.0cm. There was only slight dilation of the most distal portion of the internal cloaca (except #188 with no dilation noted). Most were filled with yellow/white or green urates. Some of the urates contained gritty material (#187, 198, 184, 196). Fecal material was noted in the cloaca of #193 and 194.

In general, gross necropsy findings were similar to those found in the field experiment and those in wild birds. Combined with the clinical pathology evidence, there is support for a similar diagnosis of salt toxicity.

XIII. BODY WEIGHT AND MUSCLE MOISTURE CONTENT
General and Field Experiment

Previous investigators have noted significant weight loss when ducks were exposed to brine water (270,000 ppm). The weight loss was suggested to be attributable to cutaneous water loss from the legs, feet and cloaca due to the dramatic osmotic gradient between the brine water and the exposed tissues resulting in dehydration. Other areas that were found to contribute to the dehydration included: respiratory water loss, diarrhea, urine and salt gland excretions.

Visual evaluation of breast muscle tissue is used by the poultry industry as a crude measurement of dehydration. In an attempt to evaluate hydration status in carcasses objectively, a uniform section of breast muscle was sampled from the experimental ducks and analyzed for moisture content.

METHODS

The ducks from the experimental trials were weighed at 0 hour in the field. Weights were not determined again until necropsy (see below) due to the salt and water accumulation that occurred during the experimental trials.

To evaluate the degree of salt precipitation on the carcass exposed to the lake water (treatment group 1) as well as to get an accurate body weight, the ducks from all of the experimental groups were weighed before rinsing at necropsy. All ducks were then rinsed with tap water in a consistent manner and then weighed again. The accumulation of water due to rinsing was estimated from the ducks in treatment group 3 (no salt precipitation). The average weight gain noted in group 3 (due to water accumulation during rinsing) was used to correct the weights of ducks from all three groups. This is a somewhat crude measurement, but the best that could be devised for the circumstances. It was not possible to completely remove all of the salt precipitation without wetting the feathers.

At necropsy, a similar piece of breast muscle was sampled from each bird to determine the moisture content. The pieces of muscle were weighed, desiccated by dry heat, and weighed at periodical intervals until there was no change detected. The percent moisture was calculated from the differences in weight before and after desiccation. This was used as a crude measurement of tissue hydration of each individual bird.

RESULTS AND DISCUSSION:

The percent reduction in body weight and the percent weight loss per hour were calculated for all three groups as an estimate of dehydration. Comparisons were made between groups and between lakes. No lake differences were noted in the total percent weight loss. Treatment group 2 had the highest total weight loss (Figure 35). Treatment group 1 had a lower percent reduction than group 2. There was a minimal change in weight loss in treatment group 3.

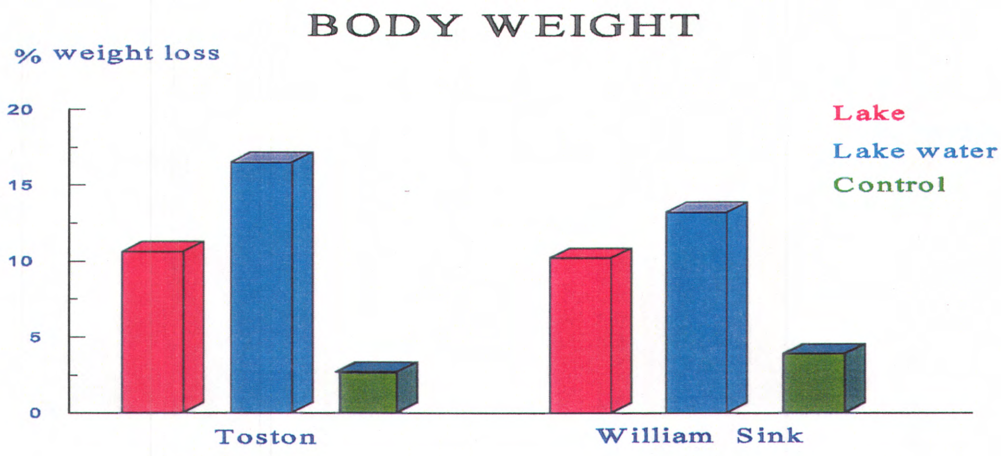


Figure 35: Percent total weight loss between the two experimental sites

When the time to death was taken into consideration, there were lake differences noted in treatment group 1. Ducks in treatment group 1 on LT had the highest weight loss per hour (Figure 36). This loss was two times that of the ducks on WS. Treatment group 2 percent loss per hour was similar to group 1 on WS. Treatment group 3 had a minimal weight loss per hour.

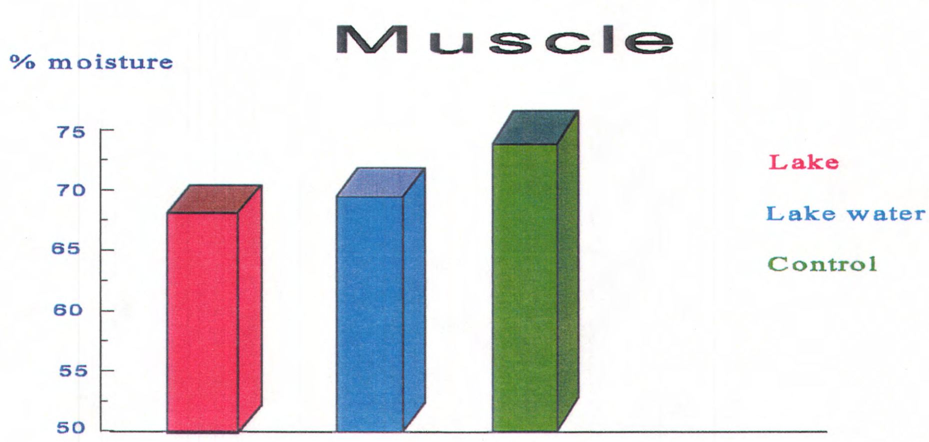


Figure 36: Percent moisture loss amount treatment groups

The moisture content of breast muscle was different among groups but not between lakes. Mean percent moisture was different among all groups, the highest was noted in treatment group 3, with a slight decrease in group 2. Group 1 had the lowest moisture content suggesting the greatest dehydration. (Figure 37). These results follow the pattern expected for dehydration.

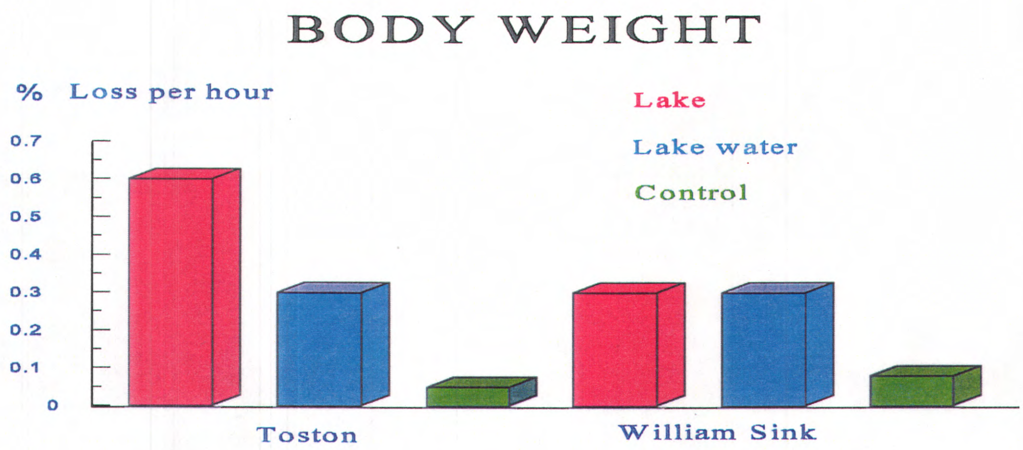


Figure 37: Percent body loss per hour between the two experimental sites

XIV. BODY WEIGHT AND MUSCLE MOISTURE CONTENT
Controlled Environment Study

METHODS

The methods for body weight and muscle moisture content were the same as those described in Section XIII, except for statistical analysis, which will be described below.

RESULTS AND DISCUSSION

Initial data analysis showed that there were no differences between treatment groups, so these data were pooled and compared with the control group(TG1). Previous observations based on relating body weight and muscle moisture content to time to death(ttd) figures were not deemed appropriate in this experiment due to the potential confounding between treatment effects and feather condition. A second analysis was performed, however the ttd data was not used because 6 birds died early in the experiment with complications that could be related to feather maintenance and exposure, and may have affected the ttd data. There were significant differences between the pooled treatment group and control groups for body weight change, and for % body moisture. Body weight for control ducks increased by an average of 59.25 (SD=25.76) grams, but ducks in the treatment groups lost an average of 69.36 (SD=60.93) grams(Figure 38) . Percent muscle moisture was higher for control ducks (mean=73.65, SD=0.39) than for ducks in the treatment groups (69.24, SD=2.37)(Figure 39).

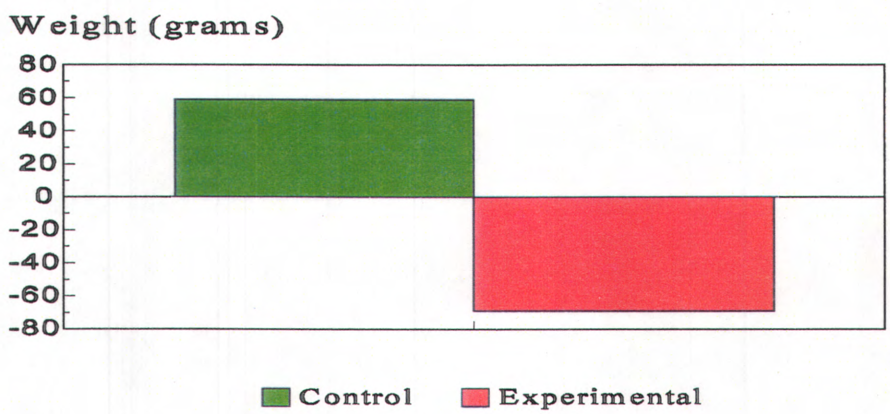


Figure 38: Percent weight gain/loss between control and experimental groups.

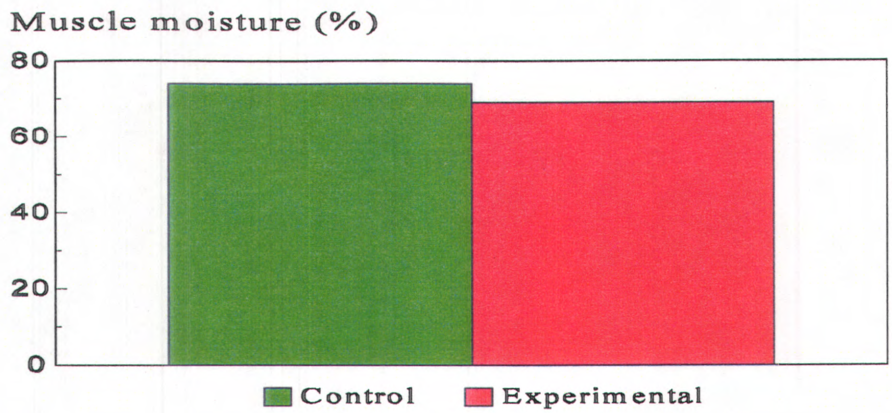


Figure 39: Differences in muscle moisture content at necropsy between control and experimental groups.

XV. BRAIN CHEMISTRY - *General and Field Experiment*

Salt toxicity/water deprivation in domestic species has traditionally been diagnosed by combining a history of limited water intake, clinical signs, and detecting elevations in the sodium levels in plasma and brain tissue. The toxic range for sodium in domestic species is considered to be greater than 160 mEq/L in plasma and greater than 2000 ppm in the brain. The diagnosis of salt toxicosis in wildlife species has been extrapolated from this information. Toxic levels of chloride in dogs has been reported to be 1400-1910 ppm and in pigs as 1400-2700 ppm. No levels are available for birds.

METHODS

Brains from all birds necropsied were removed in a consistent manner following a detailed protocol to prevent tissue contamination (Appendix 4-2;Figure 40). The brains were processed for analysis at the NWHC and tissue extracts were submitted for inductively coupled plasma (ICP) analysis of the following elements: calcium, chloride, copper, iron, manganese, magnesium, phosphorus, potassium, sulfur, sodium and zinc. This testing was performed by the Soils Analysis Laboratory at the University of Wisconsin.

Figure 40: Removal of brain for chemical analysis.

RESULTS AND DISCUSSION

Statistical analysis of element concentrations determined that there were significant differences between the treatment groups for sodium, potassium, sulfur, magnesium and chloride. All other elements did not show differences between treatment groups. Lake differences were noted for sodium, magnesium and sulfur.

Brain sodium levels were different between lakes, among treatment groups and for the lake by group interactions. The ducks in treatment group 3 had similar brain sodium levels (Table 13). These levels were similar to the brain sodium levels from domestic turkeys, that were used as a method control. The brain sodium levels determined for treatment group 2 were similar between the two lakes; the levels were elevated compared to treatment group 3, but were not above the level considered toxic (2000 ppm). The brain sodium levels for treatment group 1 had the highest levels. The levels were different between lakes and well within the toxic range. The brain sodium levels at WS were higher than those at LT. This is most likely due to the fact that the ducks on WS lived longer than those on LT and therefore had an increased exposure. It may also be related to the fact that the sodium concentration is higher in WS than in LT.

Table 13: Mean (ppm) brain levels sodium, magnesium, sulfur, chloride, and potassium PPM (group mean values).

GROUP	SODIUM		MAGNESIUM		SULFUR	
	Toston	W. sink	Toston	W. sink	Toston	W.sink
1	2307	2832	179	152	1429	1346
2	1622	1660	127	128	1199	1191
3	1305	1414	126	130	1175	1198

Brain sodium levels were determined for the mortality survey samples collected in April, 1995. Brain tissue was removed using the protocol mentioned above. The brain sodium levels for this group were similar to those in treatment group 1 (Figure 41). The range for this group (1623-3511 ppm) varied considerably because these birds were euthanized at varied states of morbidity or were found dead.

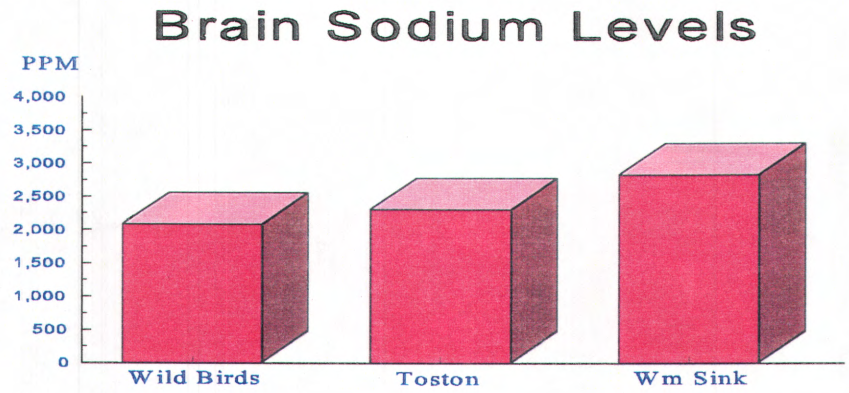


Figure 41: Comparison of brain sodium between experimental sites and wild birds.

The elevations in brain potassium and chloride levels follow a pattern similar to sodium but without lake differences (Table 14). The treatment groups varied with group 1 being the highest, followed by group 2 and group 3 (Figure 42 - 43). The clinical significance of these values has not yet been determined. Brain chloride levels have not been found to be of diagnostic value in cases of salt toxicity (Wells, 1984). Potassium has been implicated as a toxic agent in incidences of high oral doses, mostly observed in anuric patients. Brain levels have never been evaluated in these cases.

Table 14: Mean brain levels of chloride and potassium (mean values, both lakes combined) PPM

GROUP	CHLORIDE	POTASSIUM
1	3790	3940
2	2050	3526
3	1610	3208

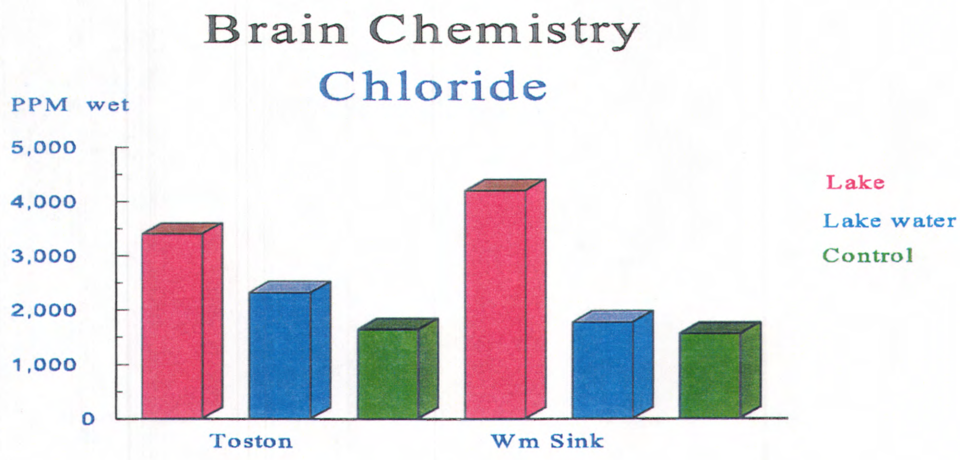


Figure 42: Brain chloride levels between experimental sites.

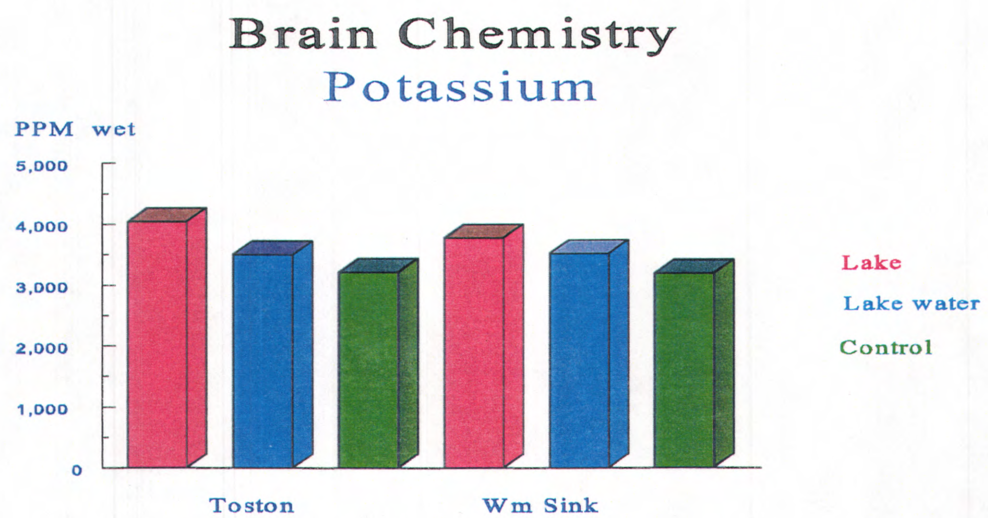


Figure 43: Brain potassium level between experimental sites.

The elevations in brain magnesium and sulfur levels follows a different pattern (Figure 44 - 45). The treatment groups were found to be different with the levels for group 1 being the highest and group 2 and group 3 being similar. Lake difference were noted for group 1, with levels being higher on LT for both elements. The clinical significance of this is unclear. It may be related to the variations of these ions in the lake water. Whether they play a role in the toxic effects is uncertain.

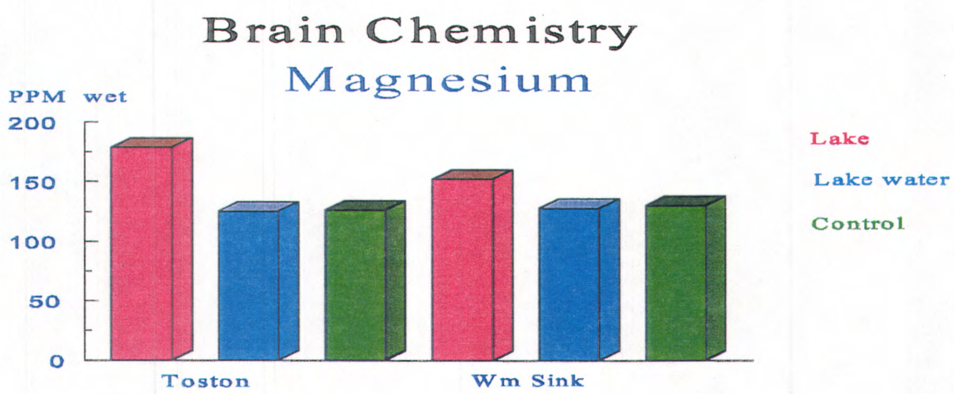


Figure 44: Brain magnesium between experimental sites.

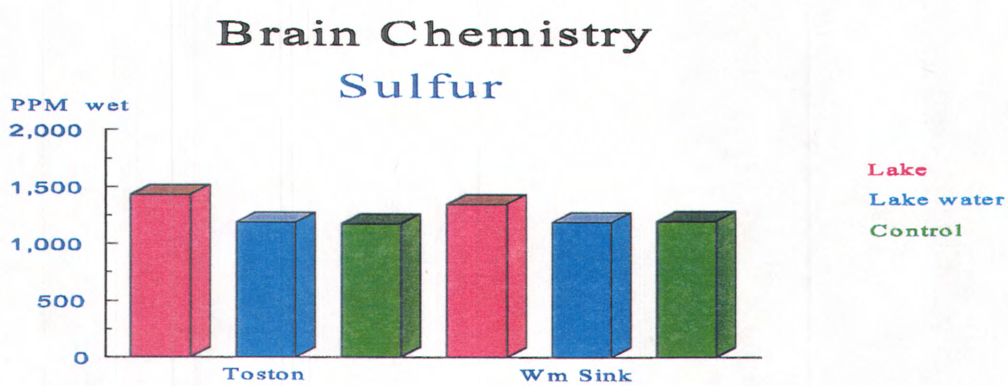


Figure 45: Brain sulfur levels between experimental sites.

XVI. BRAIN CHEMISTRY - *Controlled Environment Study*

METHODS, RESULTS AND DISCUSSION

Brains from this experiment were handled as for the field studied and removed as described in Appendix 5. No significant differences were found between treatment groups, except for boron, which is of unknown and most likely irrelevant significance. The most important ion, sodium, was elevated in all of the treatment birds, and close to, or above the arbitrary toxic level of 2000 ppm in 9 of 11 (Figure 46). This is reasonable confirmation of sodium toxicity/water deprivation as the cause of death in the treatment group animals.

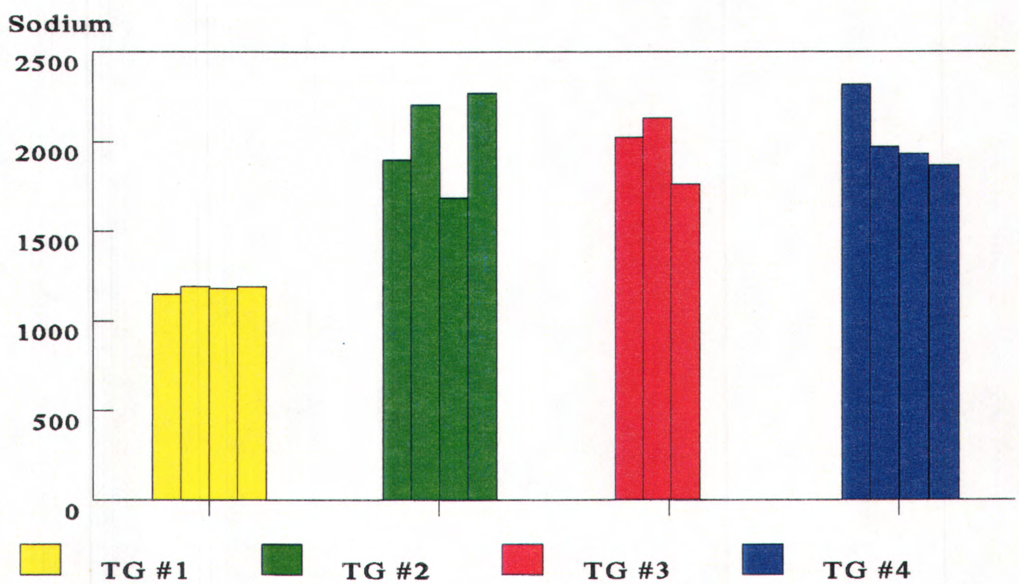


Figure 46: Brain sodium levels for all birds in CES.

XVII. FEATHERS

Initial field trials performed in March and August, 1994 on LT showed that the ducks became encrusted with salt precipitation after being exposed to the lake water. In the current field experiments, feathers were obtained from all of the ducks in an attempt to evaluate the changes that occur to the feathers as a result of the crystallization. The areas of most interest were feather structure, insulation, wettability, as well as a determination if any changes were reversible.

METHODS

Feathers were collected during the November 1995 experimental trials. One remige (flight) and two contour feathers were removed at each collection period (0, 3, 7, 24, 48 hours). The contour feathers were removed from the upper breast region, alternating sides. Primary feathers were collected from alternating sides (right then left) with every other feather removed, starting with the second primary. Multiple remige and contour feathers were removed after euthanasia.
The contour feathers were examined to ascertain if there were any effects on the insulatory properties. The feathers were examined and evaluated by electron and light microscopy (Carla Dove, Smithsonian Institution). The areas of the feather that were of most interest were the pennaceous region and the node structures.

Selected remige feathers from the experimental birds were processed by electron and light microscopy (Heidi Barnhill, UW-Madison). Examination of the flight feathers was focused on the distal end of the outer vane of the feather. Microscopic evaluation was concentrated on the barb/barbule/hooklet region to survey the interactions at this region.

Wings of several species were obtained from the annual Fish and Wildlife Service Wing Bee. The primary feathers from these specimens were evaluated via electron microscopy to detect any microscopic differences in structure between species. The following species were examined: Common Eider, Common Merganser, Lesser Scaup, Mallard, Northern Shoveler and Ruddy duck.

RESULTS AND DISCUSSION

No abnormalities in microscopic structure of the contour feathers were noted. The nodes did not appear to be disrupted as is usually seen with damage to the insulatory properties. The contour feathers were slightly crystallized at the distal portions (pennaceous area) of the feather. The downy region and proximal pennaceous region, the areas closest to the body, did not appear to be altered. Therefore it is concluded that the feathers appear to retain their insulating properties.

In general, the remige feathers appeared to crystalize most heavily on the distal end with heavier precipitation on the outer vane compared to the inner vane. This is probably related to the area that received the most exposure. Feathers with poor structure quality at time 0 hour appeared to crystalize more readily than feathers with good structure quality. Feathers exposed to water on LT appeared to accumulate crystals to a greater degree than feathers exposed to water on WS (Figure 47; A-E).

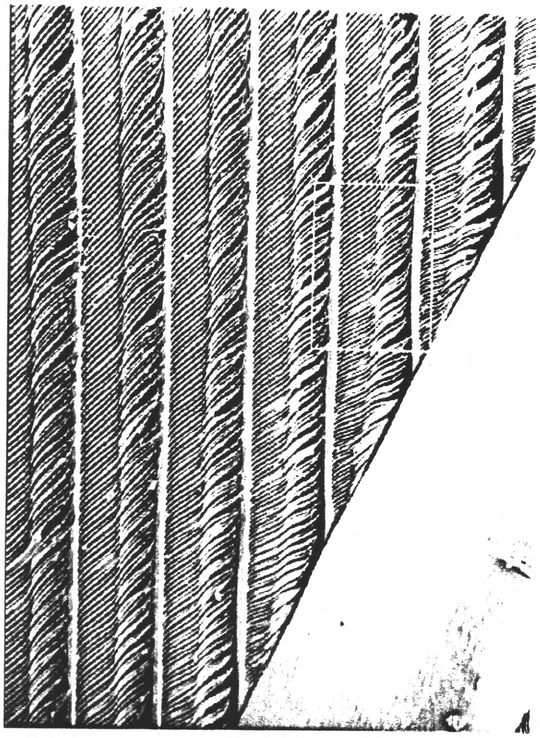
One set of feathers was examined after 40 minutes of exposure to water on LT . Salt precipitation was noted at the microscopic level (Figure 47; F-G). After three hours of exposure the salt accumulation could be observed grossly without microscopic aid. The accumulation was significant enough that it is possible to interfere with the ducks ability to lift off. At which point, or at what degree of weight accumulation this occurs has yet to be determined.

There did not appear to be any major disruption of the barb and barbule interactions in the primary feather microscopic structure. Some of the hooklet interactions were disrupted due to the salt crystallization. This disruption became more severe with increased time of exposure and increase in size of crystals.

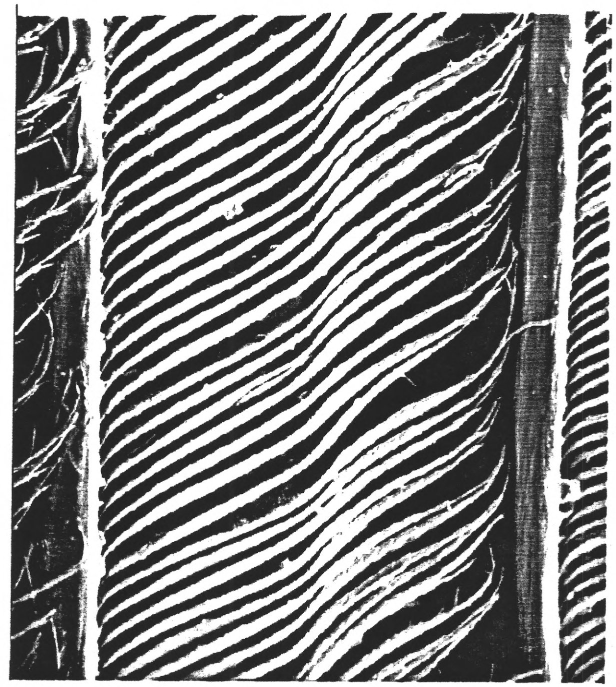
As salt accumulation occurs there is disruption of the barbule/hooklet interactions. The resulting increase in contact angles and space causes an increase in the wettability of the feathers. This change in wettability in turn contributes to the increase in heat loss, resulting in hypothermia. Considerable wetting of the neck and head feathers was noted early on in the experimental trials. The crystallization in this area could also be contributing to the heat loss.

In looking for species variations, it was noted that the Common Eider and Common Merganser (Figure 48; A-B) appeared to have longer barbules that extended over the barbs, all of the other species did not extended past the barbs (Figure 48; C-D). In addition the barbules appeared to be thicker in diameter. All of the other species showed the same basic structure as the Mallards used in the experimental trials. The wild Mallards did not appear to differ from the game farm mallards. Primary feathers from a Northern Shoveler were obtained from a mortality survey sample collected in April 1995. These feathers appeared to have the same pattern of salt accumulation as that noted in the experimental birds (Figure 48; E).

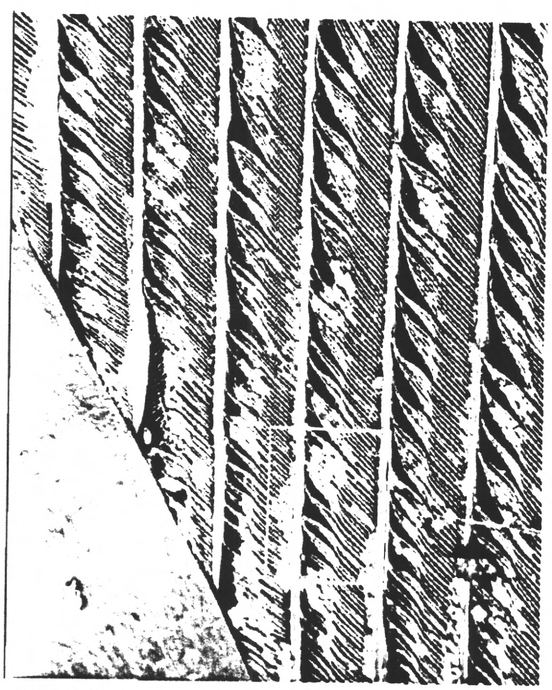
The changes noted on the feather structure appears to be reversible. There was no structural damage to the barb/barbule/hooklet interactions noted, just disruption of this interaction. Once the salt precipitate is dissolved the duck should be able to preen the feathers back to their original structure.



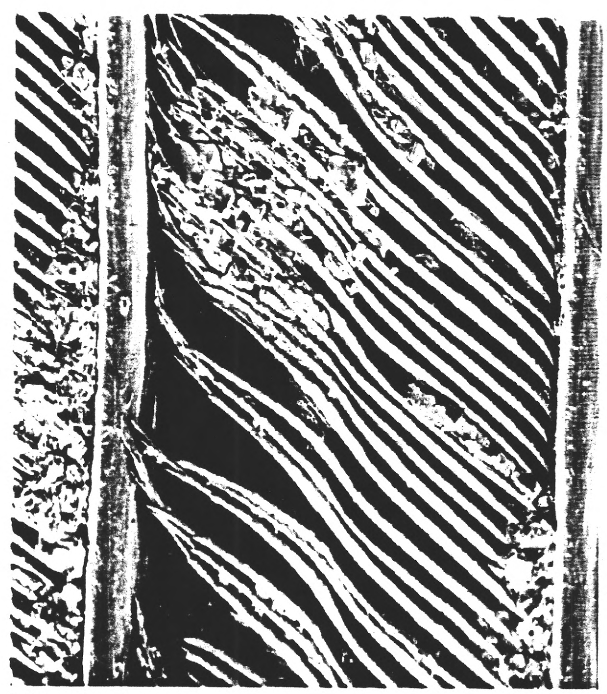
A) #6 0 hrs. x 50



B) #6 0 hrs. x 250

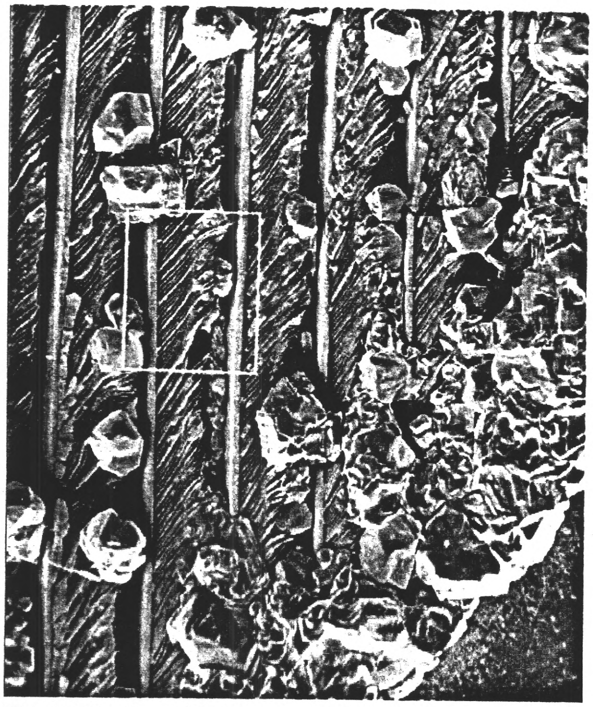


C) #6 3 hrs. x 50

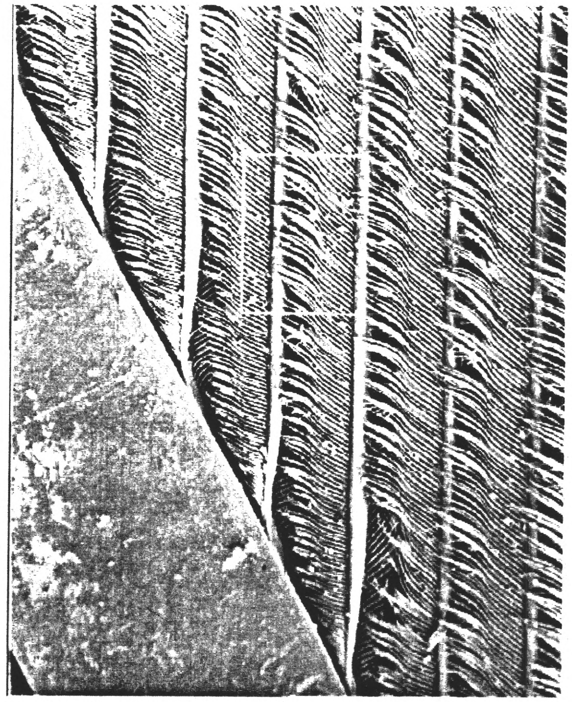


D) #6 3 hrs x 250

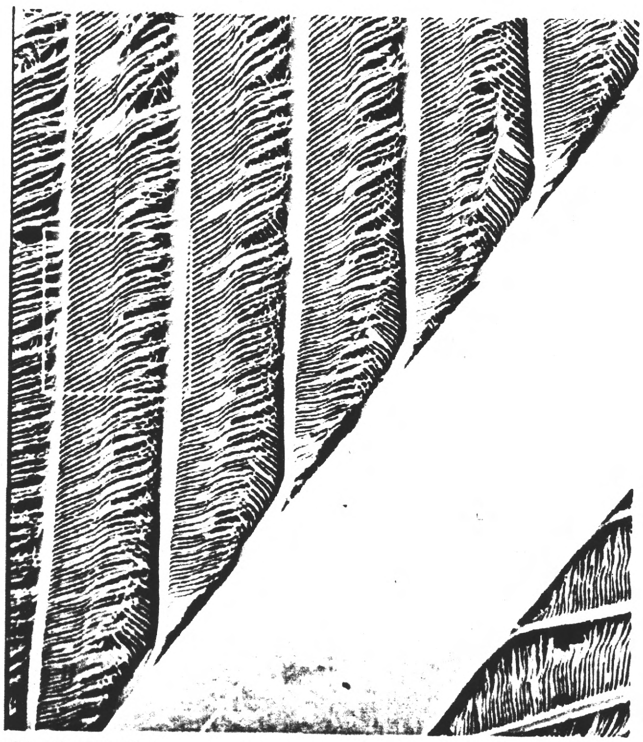
Figure 47: Feather scanning electron micrograph from experimental mallards.



E) #6 20 hrs. X 50

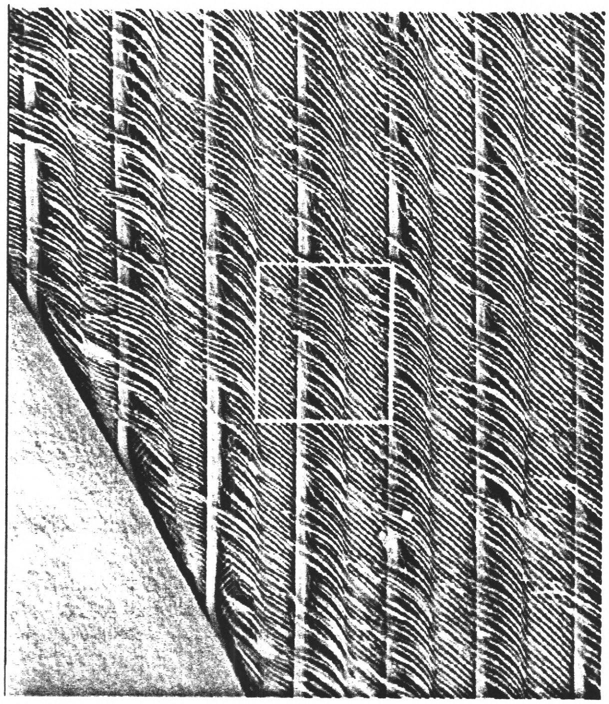


F) #184 40 min x



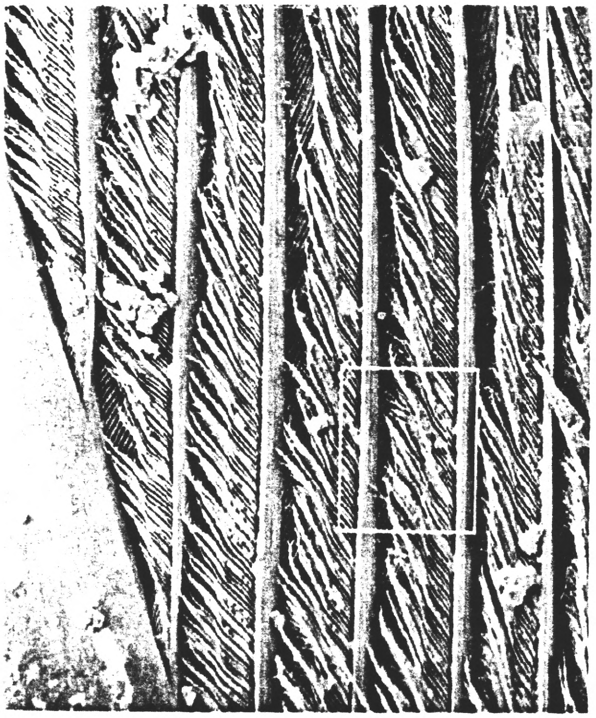
G) #184 0 hrs. x 250

Figure 47 (Cont)



A)Common eider x 250

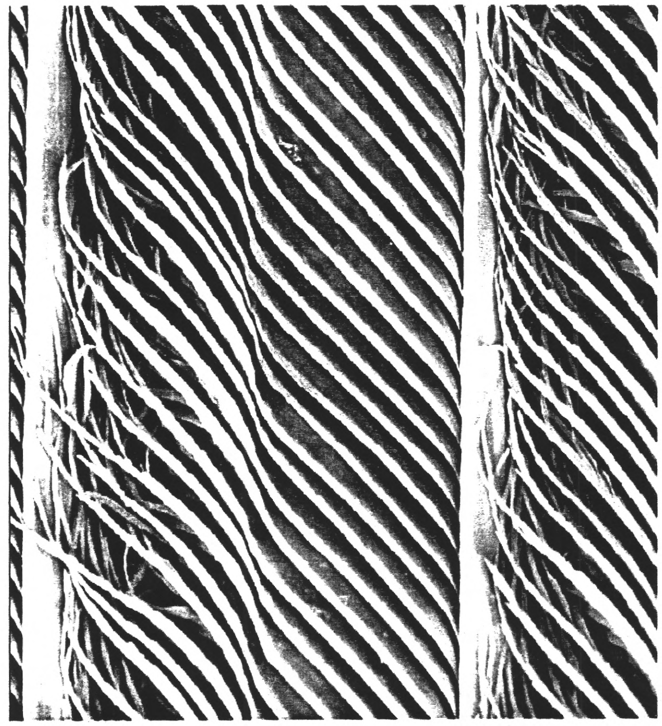
B)Common merganser x 250



C) Northern Shoveler x 250

Figure 48: Feather scanning electron micrographs from wing bee samples (A-D) and wild bird (E)

D) Mallard x 250



E) Ruddy Duck x 250

Figure 48 (Cont)

XVIII. FLIGHT TESTING

During the pilot studies, it was noted that the ducks exposed to the hypersaline water became salt encrusted. Questions were raised concerning the potential effects that the salt crystallization would have on the feathers. Areas of concern were effects on insulation, buoyancy, structure and flight. Previous reports from other locations noted a reluctance to "lift off" (these ducks were flushed from the surface of the water) (Fountain, 1979). The ducks preferred to swim away rather than take off in flight. We devised a simple experiment to evaluate flight capabilities of the ducks after being exposed to the hypersaline water. This study was not designed to provide statistically reliable results, but to test possible methods for future studies.

METHODS

The ducks were housed and acclimated similar to the previous groups. There were five ducks per group at both LT and WS. Blood and feathers were collected from the ducks at 0 hour. The ducks were then placed as a group into each lake. One bird was removed from the pen at each time point (20, 40, 60, 120 and 180 minutes) for flight testing.

For the flight test, each duck was attached to fishing line (8 lb. test) with a velcro strap fastened around its right tarsal region. The duck was held in the air at approximately 8 feet above the ground. A 6 foot square screen net panel was placed approximately 4 feet above the group to catch the ducks if they did not fly (Figure 49).

Two of the ducks from LT were rinsed after the original flight test. These ducks were immersed in fresh water with several changes, allowed access to fresh water and a quiet warm environment for 30 to 60 minutes and then tested again.

RESULTS AND DISCUSSION

In the first trial flight test, a duck that had not been exposed to water flew approximately 200 feet into the wind. With exposure to water this distance decreased significantly. Lake differences were noted in the flight capabilities. The ducks from WS were able to fly a distance of 45 feet after 40 minutes, 42 feet after 60 minutes and at the 120 minute flight trials the distances decreased to 27 feet.

The ducks that were exposed to LT did not fly more than 5 feet at the 40 minute flight test. The birds that were exposed longer did not even attempt to fly and dropped directly into the net below. The duck that was exposed for 180 minutes could not even hold its head up during the flight test.

The duck that was exposed to LT for 60 minutes and then washed was given another flight test and was able to fly about 5 feet. The duck that was exposed for 120 minutes could not fly initially but after it was washed and rested for 60 minutes it flew with some hesitation (17.5 to 45 feet).

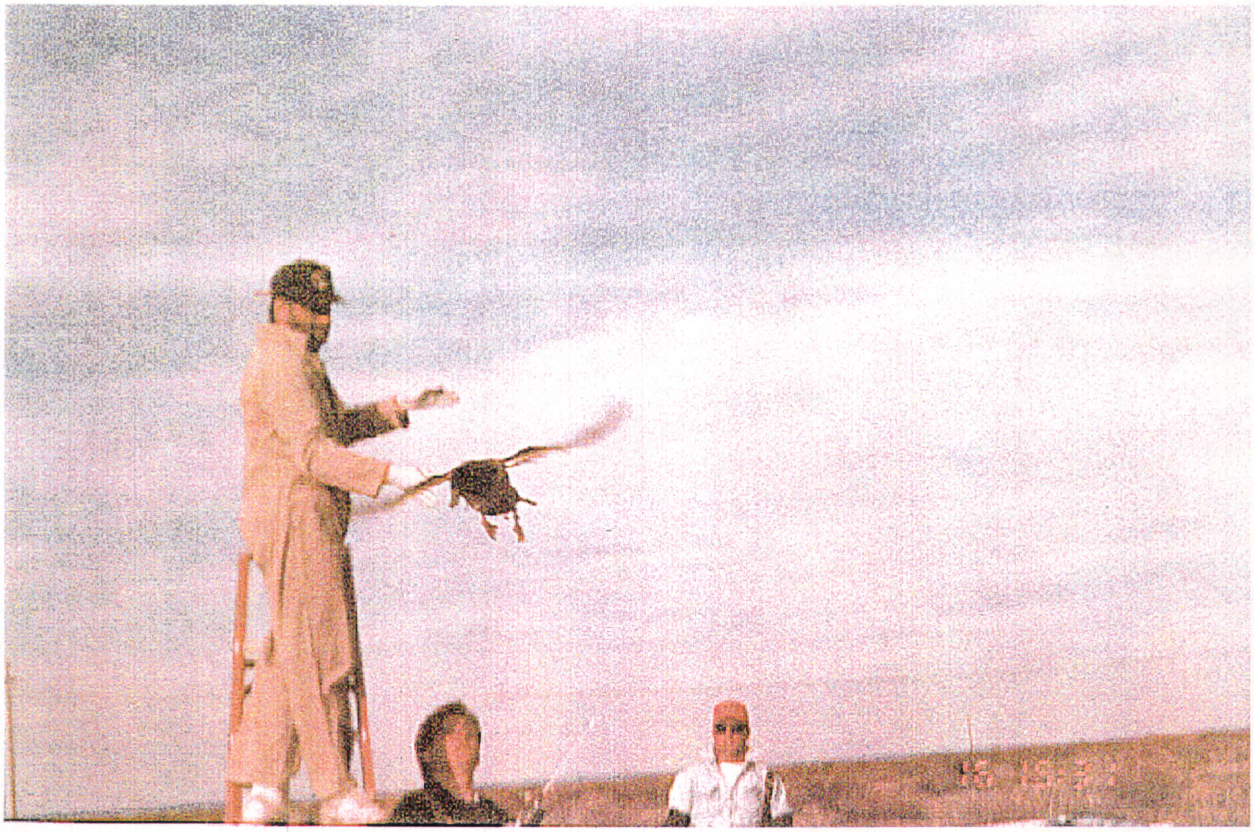


Figure 49: Flight testing procedure.

This method of flight testing was crudely designed but it allowed for a brief evaluation of flight capabilities. The feathers do not appear to be structurally damaged (Section XVII), and the birds are able to improve their flight distance after washing. Therefore the effects of hypersaline water on the feathers appears to be reversible. The decreased flight ability may simply be related to total weight accumulation from the salt crystallization or increased wettability secondary to the salt precipitation.

XIX. INFRARED THERMAL IMAGERY

Previous investigators noted a decrease in body temperature when ducks were exposed to brine water. Therefore waterfowl that die as a result of spending time on hypersaline lakes may be experiencing hypothermia due to damaged feathers or changes in insulation ability. An infrared camera, featuring stationary image display, and based on pyroelectric vidicon technology, was used in an attempt to document these changes.

METHODS

A VideoTherm380 camera, equipped with a spot temperature readout was rented from the ISI Group, Inc. in Albuquerque, New Mexico for this study. This camera detects thermal energy at the surface of an object (indicating heat loss) and transforms it into an image that can be recorded onto a standard video tape. The resulting image is black and white with white images being the coldest, black the hottest, with shades of gray in between.

All thermal images were recorded with an emissivity setting of .95 with the camera iris totally open. Birds were imaged at each sampling time by placing them in a small cage in the shade so that sunlight would not interfere with the readings. A full image of each bird was recorded as well as spot readings for the feet, head, bill, breast, and back. An attempt was also made to take spot readings by pulling back the feathers in certain areas (cloaca, under the wings, neck, and breast) to get a better indication of skin temperature.

RESULTS AND DISCUSSION

The first step in analysis of the thermal images was to convert them to color images using the Infrasoft Computer package, also obtained from ISI Group, Inc. This program allows for presetting color palettes to represent different temperatures within the image, determining average temperature of shapes that the operator delineates, and zooming in on specific areas to observe individual pixels. The resulting images can then be printed on a color printer as a permanent record of the data. Figures 50 and 51 are examples of the final product achieved by this process. The temperature scale ranges from 30 to 110 degrees F with yellow/white being hottest and blue/black being coldest. Figure 50 is duck #035 (housed on land, drinking lake water) from WS, recorded at 3 hours. Notice that the bird is fairly uniformly blue, indicating little heat loss. Figure 51 is a comparison of 3 ducks, one from each treatment group, at WS at 3 hr. Notice that the group 1 bird (kept in the water) shows considerably more heat loss. The yellow areas are the legs, eye, bill/head junction, and the lateral chest area, as a result of considerable heat loss. We attempted to measure temperature of specific areas on each bird (head, breast, cloaca, neck, back) by using the feature of the computer package that determines the average temperature of a delineated shape. Unfortunately, results were too inconsistent to be meaningful.

Most infrared cameras are used under considerably more favorable conditions than we experienced during this study. There were large changes in air temperature (45F-75F) with high winds much of the time. The camera did not function very well in the colder temperatures and it was extremely difficult to find a place out of the wind and sun to do the imaging. As a result of these problems, it was not possible to compare the same bird at different sampling times or to use some of the features of the camera and accompanying software. In spite of these difficulties, the video images achieved by use of the infrared camera are useful to view overall changes in surface temperature of the ducks and to compare the differences between treatment groups at a given time. We feel that the images do indicate more heat loss as a result of exposure to the hypersaline water, particularly in the head and neck areas. Even though it may be possible to refine techniques and conditions so that better results could be achieved, we do not feel that these possible results warrant the time and expense which would be involved.

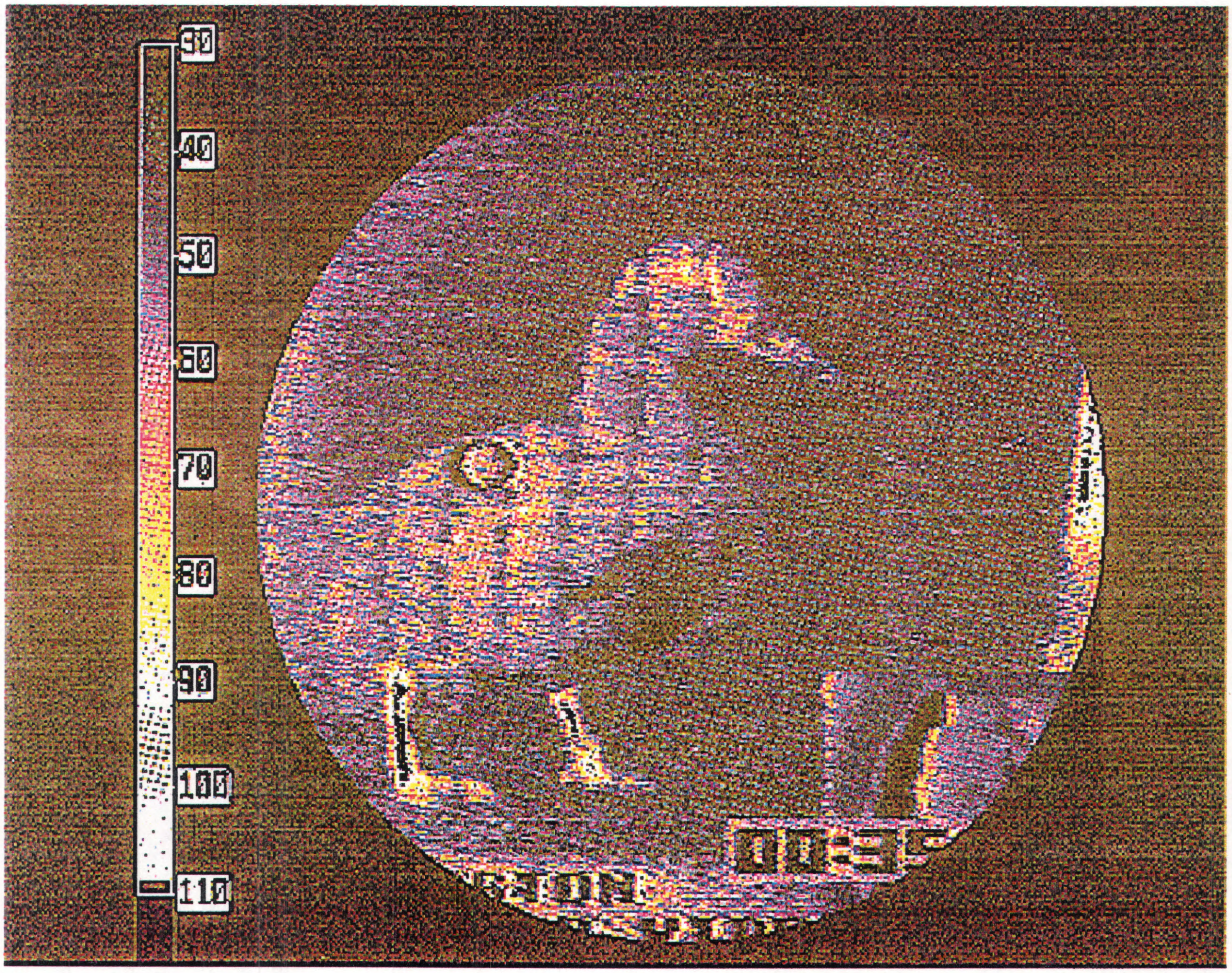


Figure 50: Thermal image of duck from TG2 at 3 hours, Williams Sink.

Figure 51: Comparison of thermal images from all treatment groups, Williams Sink, 3 hours.

XX. OPHTHALMOLOGIC EVALUATIONS

Controlled Environmental Study

The ophthalmic investigations characterized the development and progression of ocular lesions in the laboratory ducks. Because the previous post-mortem field investigation findings of severe ocular surface irritation and cataract formation suggested that affected birds may be visually impaired and therefore reluctant to fly off. It was unclear, however, whether the cataracts occurred ante- or post-mortem, and what role environmental influences played in their formation.

METHODS

Detailed, serial slit-lamp biomicroscopic (hand-held Kowa SL-II biomicroscope) (Figure 52) and indirect ophthalmoscopic examinations (Figure 53) of living birds were performed at 0,2,3,,7,24, and 48 hours, and opportunistically before death or euthanasia. Evaluations were made of the ocular adnexae, and the anterior segment of the eye, including the lens. A Nikor photomicroscope and a Kowa fundus camera was used to document examination findings. Pictures were taken of all birds at almost every examination. A consistent 1-4 scoring scheme was used throughout, grading chemosis, erythema, blepharedema, corneal edema, corneal epithelial defects, aqueous flare, and aqueous cells. Line drawings of significant retinal morphology, and text descriptions of findings were made as needed.

On necropsy samples of fluid from the vitreous and aqueous humors of the eye were obtained and to the UWSVM for analysis of osmolality, sodium and potassium.

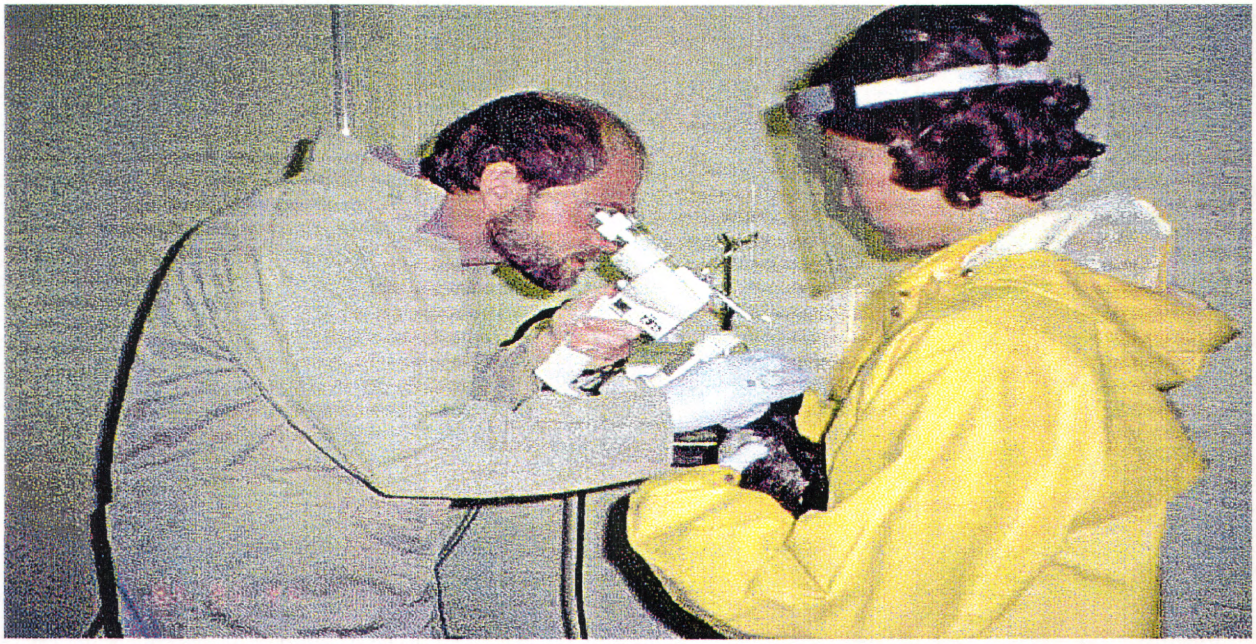


Figure 52: Examination by slit-lamp biomicroscopy.

Figure 53: Examination by indirect ophthalmoscopy.

RESULTS AND DISCUSSION

None of the birds in the Group 1 developed ophthalmic disease, whereas birds exposed to undiluted hypersaline water(Groups 2-4) developed mild to severe conjunctival edema, conjunctival vascular injection, and edema of the eyelids within 1 hour (Figures 54 - 56). Over the ensuing hours the conjunctival surface often partially sloughed and substantial periocular salt encrustation developed. The degree of conjunctival and eyelid edema tended to diminish with time - presumably as a result of tissue desiccation. Cataracts were not seen ante-mortem in any bird in any group. A mature cataract reminiscent of those seen in rodents with anterior chamber dehydration and secondary cataract was noted post-mortem in one eye of one bird exposed to undiluted hypersaline water. It was believed that the lens opacity developed when the bird was moribund or dead and could no longer raise its head, thereby resulting in immersion of this eye in hypersaline water. Clinically significant ophthalmic lesions were not consistently seen in birds exposed to either the 70% or 80% dilutions. All birds exposed to hypersaline water maintained functional vision until severe neurologic disease precluded meaningful assessment of vision.

Exposure to hypersaline water significantly increased the OSMOLALITY, sodium, and potassium in the aqueous and vitreous humors of the eye (Figures 57 - 59). This alone, however, at least in the short term, is insufficient to result in cataract formation, as such opacities were noted to develop only when the anterior has become completely dehydrated and totally collapsed.

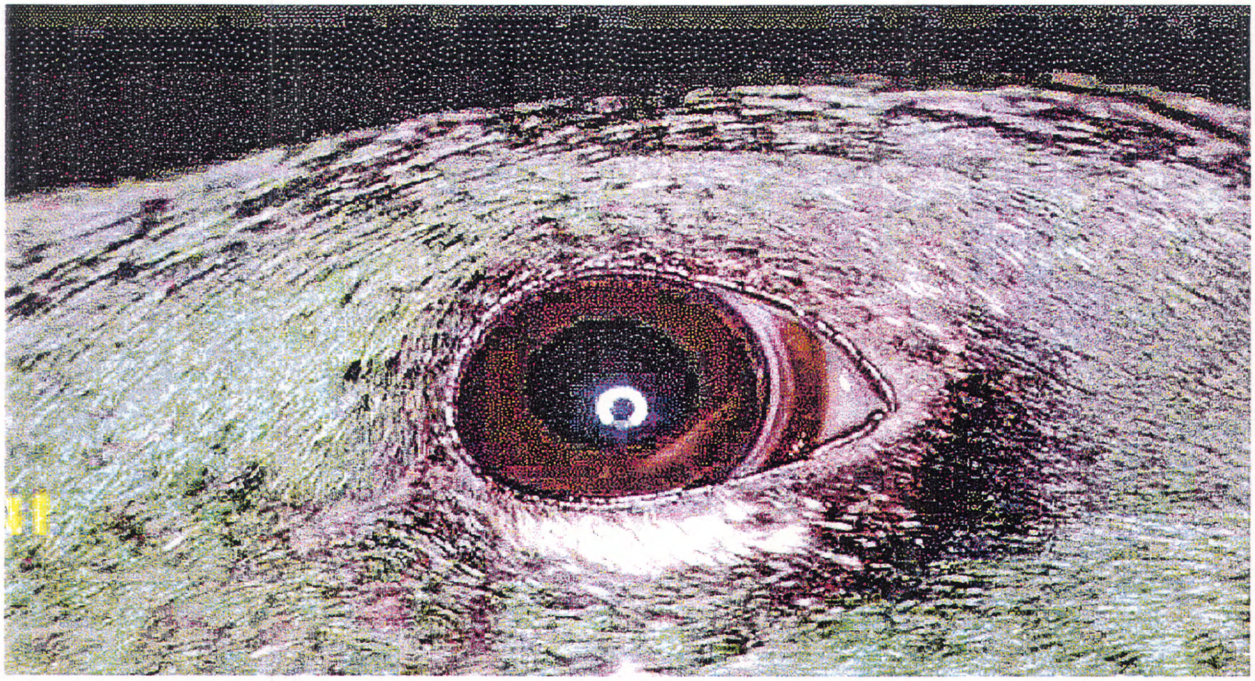


Figure 54: Photograph of normal left eye, bird 195, TG2, 0 hours

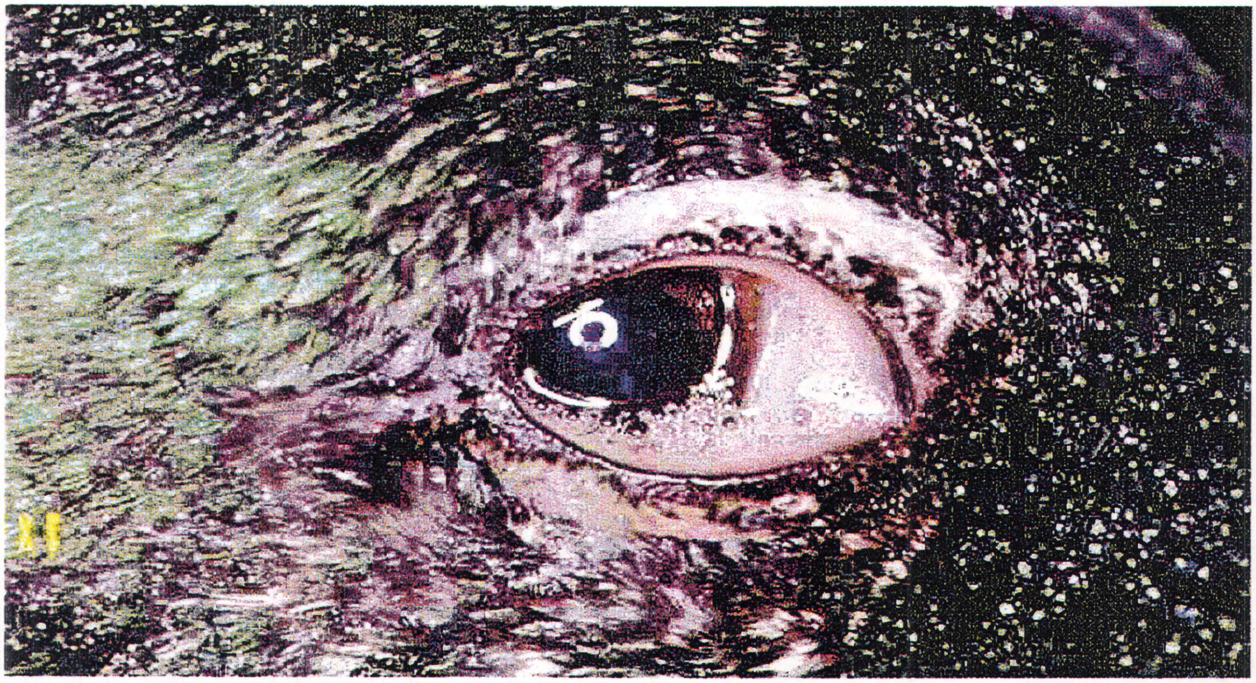


Figure 55: Photograph of effected left eye, bird 195, TG2, 24 hours.

The findings indicate that, at least in a controlled laboratory environment, visual impairment cannot explain the reluctance of the birds to leave the hazardous water. Extensive periocular salt encrustation was insufficient in and of itself to generate lens opacification in living birds, and cataracts result either from immersion of the eye in a hypertonic solution when the bird is moribund/dead or from variations in the natural environment which we controlled for in this study. Dilution of the water to 70 to 80% of its natural concentration virtually eliminated ocular

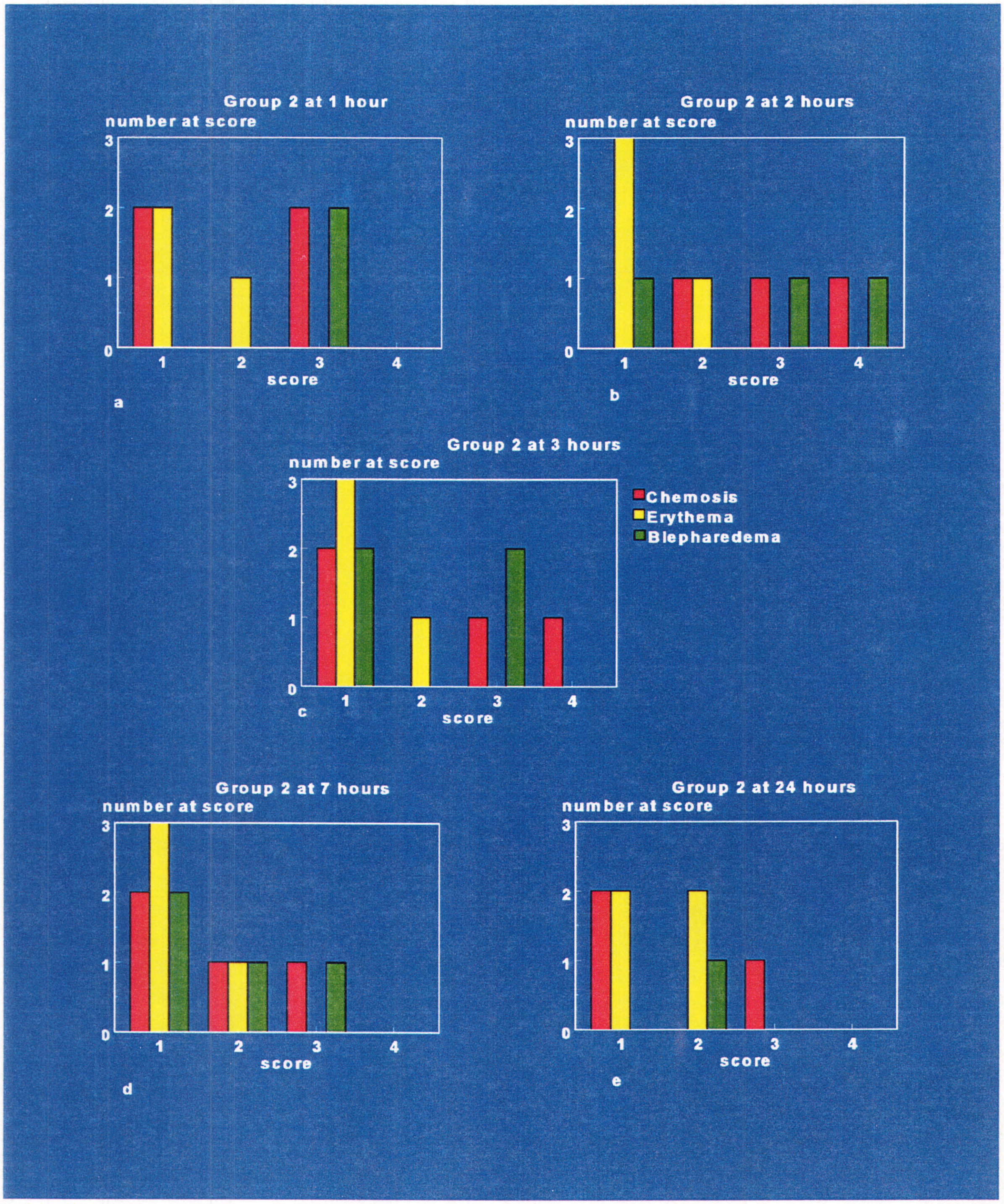


Figure 56: Ophthalmic disease recorded for each bird in TG2 at a)1, b)2, c)3, d)7 and e)24 hours.

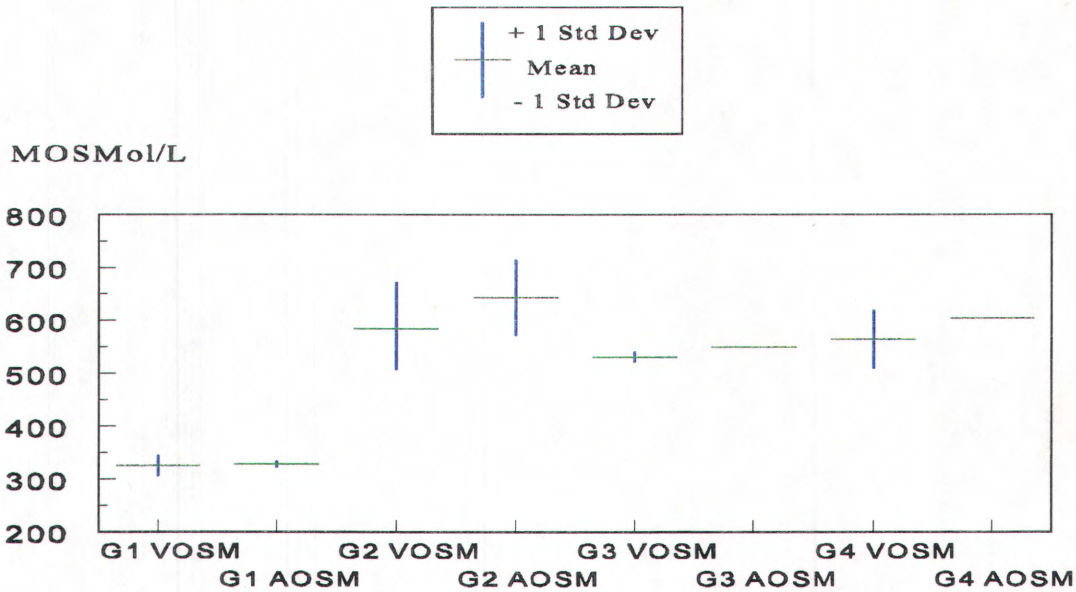


Figure 57: Mean and standard deviations for vitreous (VOSM) and aqueous (AOSM) osmolalities for each treatment group.

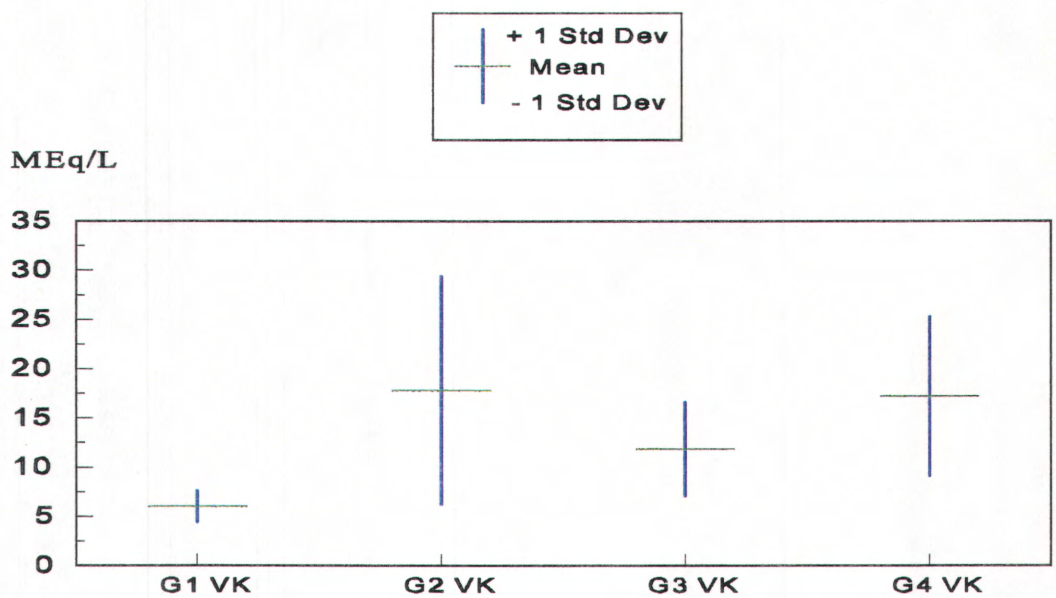


Figure 58: Mean and standard deviations for potassium levels in the vitreous humor for all treatment groups.

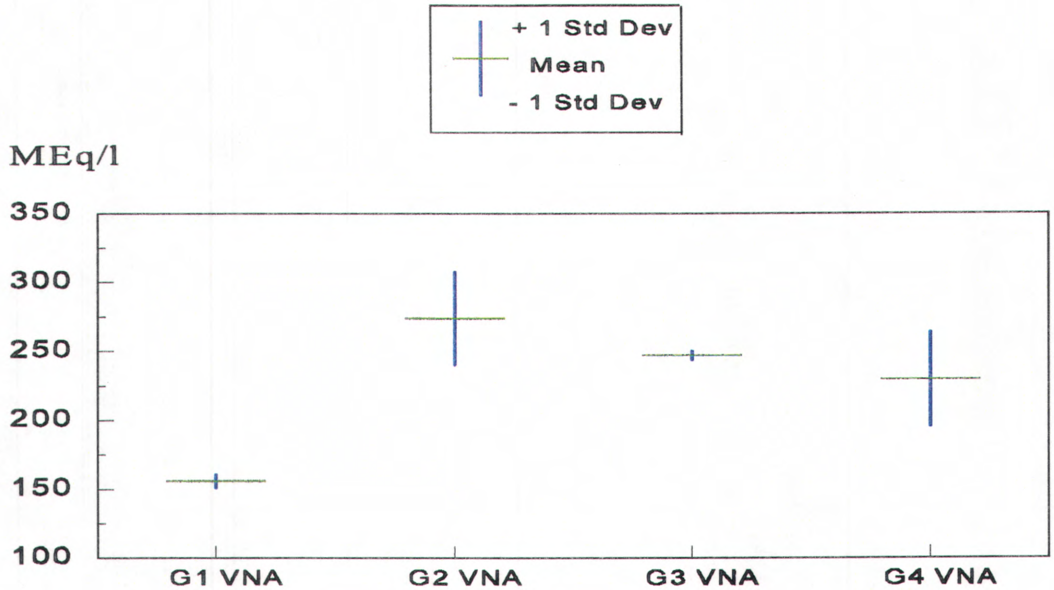


Figure 59: Mean and standard deviations for sodium levels in the vitreous humor for each treatment group.

XXI. INVESTIGATIONS OF CATARACT FORMATION

Further investigations were carried out at the University of Wisconsin School of Veterinary Medicine Comparative Ophthalmic Research Laboratories to determine the mechanism of cataract formation in birds exposed to hypersaline water. Determining this mechanism was important because it may help better understand why cataracts are prevalent in post-mortem samples from birds found at Playa Lakes and yet are uncommon in birds in a controlled laboratory environment exposed to the same water. The mechanism of cataractogenesis was also of interest because few reports exist of spontaneous cataracts after exposure to a natural hypertonic environment, and, if the cataracts are indeed osmotic in nature they are potentially reversible thereby permitting affected birds to be visually rehabilitated. We also sought to confirm the theory that lens opacities could occur with topical exposure to the hypersaline water alone and that the systemic electrolyte and osmotic disturbances noted in the field investigation were not an essential component of cataractogenesis.

METHODS

In this study, 20 Mallard ducks were divided into 4 groups comprised of 5 ducks/group. Ducks in groups I-III were euthanized and both eyes were then immersed in undiluted water from Laguna Toston for 15 (group I), 30 (group II) and 60 minutes (group III) and frequently monitored by slit-lamp biomicroscopy for cataract formation. These groups mimicked the post-mortem samples collected in the field investigation at Laguna Toston and Williams Sink and also precluded the systemic electrolyte and osmotic disorders seen in those birds. At the end of the immersion period one eye was removed for histologic examination and the remaining eye was placed in tap water for 120 minutes and again examined by slit-lamp biomicroscopy at 30 minute intervals. The 5 ducks in group IV were anesthetized and 1 eye was immersed in undiluted hypersaline water for 120 minutes and examined by slit-lamp biomicroscopy every 15 minutes. After 120 minutes, 3 ducks in this group were euthanized and the eyes prepared for histopathology. The hypersaline-exposed eyes from the 2 remaining birds in Group IV were placed in tap water for 120 minutes and monitored closely to assess the reversibility of the lens changes.

RESULTS AND DISCUSSION

In general the severity of the cataracts in groups I-III varied with the duration of exposure to the hypersaline water. Mild to moderate cataracts developed after 15 minutes, most of the lens was involved after 30 minutes, and complete cataract formation occurred by 60 minutes. The reversibility of these changes after immersion in tap water also depended on the degree of exposure to the hypersaline water as cataracts in group I were completely reversible. Cataracts in group II were completely reversed in 3 birds and substantially improved in the remaining 2 birds after tap water immersion. In group III the cataracts significantly diminished after exposure to tap water but persisted at a mild level. Living, but anesthetized ducks developed cataracts at a much slower rate, taking up to 2 hours to reach a level comparable to that achieved within 30 minutes in dead ducks. Cataracts were also completely or partially reversible after exposure to tap water in living birds. Histopathologic changes were found to not be as sensitive in detecting cataracts as slit-lamp biomicroscopy. This is most likely because the slit-lamp biomicroscope easily permits the examiner to observe the entire lens and to determine its physiologic clarity, whereas traditional histopathologic methods detect only gross anatomic changes and examine only a small fraction of the lens.

In general this study supports the theory of an osmotic mechanism of cataractogenesis in which hypersaline water pulls fluid out of the eye thereby disrupting the regular arrangement of lens fibers needed to maintain lens clarity. Systemic exposure to hypersaline water is not necessary for cataracts to form and lens changes identical to those seen in post-mortem specimens from the field investigation can be produced solely by immersing the eyes in hypersaline water after death. The post-mortem nature of the cataracts as suggested by the above 2 studies would, however, need to be confirmed by a detailed ophthalmic examination of birds in the field both before and after death. The lens opacities can be relatively easily reversed, at least in the early stages, by restoring the proper fluid balance to the eye and the animal could be visually rehabilitated provided its systemic metabolic abnormalities were corrected. The living ducks in group IV presumably are more resistant to cataract formation and resolve more readily after exposure to tap water than dead ducks because they have intact mechanisms for maintaining intraocular fluid balance.

XII: CONCLUSIONS

Because of the lack of observational and mortality figures available for the fall migratory period due to circumstances outside the control of the NWHC and NWRC, the collection of these data should be the first priority for any future work. Because of this void, we have not been able to meet two of the five objectives of the first Study plan, a) Estimate weekly migratory waterfowl use and mean duration of stay on selected playa lakes in the Potash Area, and b) Estimate weekly migratory waterfowl mortality on the selected playas. It will be important to know the magnitude and extent of the problem if mitigation procedures are to be considered. Additional questions concerning the degree to which species are differentially effected should also be answered.

Although the study plan called for a second year of both experimental and field research, that enough data was gained in 1994-5 so that a second repeat season of experimental studies was not warranted. Combined with the information from the Controlled Environment Study, we can confidently conclude that the cause of death of the birds found in the study area is salt/sodium toxicity - water deprivation/dehydration, and this is a direct toxic physiological effect. It is also very likely that environmental factors play an important role in influencing mortality.

Physical data shows that birds are also externally effected by the water, causing both a conjunctivitis and feather abnormalities. It is also suggested that the feather damage may lead to increased heat loss. We have also demonstrated a decreased ability/reluctance to fly after exposure to water, but it is not clear if this is due to the feather or eye changes, or both. In depth studies show that ocular irritation is severe, although cataracts do not in all likelihood form before death.

Analysis of the water chemistries, and comparison with reports from other hypersaline environments suggests that the problem with the study sites may not rest entirely with the nature of the potash discharge, but that creating any permanent hypersaline environment, no matter what the source may present a risk to wildlife. Studies on hypersaline lakes other than those containing potash discharge would address this question. Additional trials, continuing the range of dilutions, would contribute information regarding the ability to render the water less harmful.

APPENDIX 1: STUDY PLAN - *First phase*

National Biological Survey

National Wildlife Health Center
Madison, Wisconsin

Southern Ecological Science Center
Lafayette, Louisiana

STUDY PLAN

NWHC Work Unit: 00220 - Investigation of avian mortality in the playa lakes of southeastern New Mexico

NWHC Study Plan: 00220.01. Determination of causes of mortality

SESC Work Unit: 00618 - Investigation of avian use and mortality in the playa lakes of southeastern New Mexico

SESC Study Plan: 00618.01. Bird use estimates and mortality surveys

Background and Justification:

Playa lakes are shallow basins that are scattered through 140,000 square miles in Texas, New Mexico, Oklahoma, Kansas, and Colorado. They are characterized as water catchments that are most often ephemeral, drain internally, accumulate sediment, and serve as recharge points to underground aquifers. An estimated 31% (2,460) of these playas occur in New Mexico. The playas are important wintering areas for many species of waterfowl in the Central Flyway, and provide refuge for many birds along this migratory pathway (Playa Lakes Joint Venture, 1994).

In the early 1990's the deaths of hundreds of migratory birds were documented along the shorelines of several playas in southeastern New Mexico in an area known as the Secretary's Potash Area. This area, including parts of Lea and Eddy Counties, has been the subject of mining and mineral development for 60-70 years. Potash extraction began in the early 1930's following the development of oil and gas resources in the late 1920's, and continues today as a major industry. The playa basins in this area have served as convenient discharge sites for waters produced by these mining and refining operations.

The frequency, magnitude, and geographic extent of migratory bird mortality in this region has not been assessed since there have been no extensive surveys of historical and current bird use or mortality on these playas. Likewise, the cause of death of birds dying during the early 1990's has not been adequately evaluated. Necropsy of a small number of birds found dead during 1993 suggest that physical and physiological effects of high salt concentrations in certain playas caused or contributed to their deaths. Concern has been raised that constituents of discharge water from potash mining and/or oil and gas development into playas may also be involved in causing mortality. Water quality surveys performed on several of the playas experiencing mortality in 1992 showed that there were significant differences in many chemical and biological properties between playas that have received discharges and those without a similar history (Davis and Hopkins, 1993). Questions also have been raised concerning the hydrologic and geologic relationships amongst the playas involved in avian mortality. Information may exist on many of these issues, but pertinent sources have yet to be collected and analyzed.

In April 1994, the National Wildlife Health Center (NWHC) and the Southern Ecological Science Center (SESC) received directives to initiate a collaborative study investigating bird mortality in the playa lakes of southeastern New Mexico. The NWHC was assigned lead responsibility for the project in cooperation with the SESC and the New Mexico Cooperative Fish and Wildlife Research Unit (NMCFWRU). A literature survey on topics related to historic and current land use, hydrology, geology, water quality, playa ecology, and bird use and mortality were also to be included in the study.

Objectives:

1. Estimate weekly migratory waterfowl use and mean duration of stay on selected playa lakes in the Potash Area,

2. Estimate weekly migratory waterfowl mortality on the selected playas,

3. Determine the cause(s) of death of birds found on and around the selected playas,

4. Determine if the ingestion of waters of selected playa lakes in southwestern New

Mexico causes deleterious physiologic changes in mallards, with and without direct contact with the water.

5. Prepare an annotated bibliography of the existing literature on the geology, hydrology, water quality, playa ecology, and historic and current land uses in the Secretary's Potash Region in relation to potential causes or contributing factors to migratory bird mortality and the suitability of playas as wildlife habitat.

Methods and Approach:

General Approach

Two playas, one currently receiving potash mine discharge and one without any history of receiving discharge will be selected in early fall 1994 as study sites on the basis of size, location, and presence of water. These playas will be intensively monitored to determine bird use and mortality rates during October 1994 - March 1995 and October 1995 - March 1996. Carcasses of migratory birds found sick or dead will be necropsied to determine causes of death. In addition, experimental studies conducted involving sentinel mallards will be done during the two fall migration in 1994 and 1995 to determine the physical and physiological effects of exposure to water in the selected playas. A comprehensive collection and annotation of literature related to the geology, hydrology, water quality, playa ecology, and historic and current land uses of the Potash Region will also be conducted.

Methods

a. Bird Use and Mortality

Each playa will be observed throughout each study season for two morning, two evening and one midday periods each week. Morning observation periods will run 30 minutes prior to sunrise through 3.5 hours after sunrise, and evening observation periods will be from 3.5 hours prior to sunset to 30 minutes past sunset. Evening observations at a playa will be followed by morning observations of the same playa. Mid-day observation periods begin 1.75 hours before noon and end 1.75 hours after noon once a week. Observers will be assigned five periods per week. Playa visitation order will be rotated each week. No observations will be made during the periods that the experimental trials are ongoing.

At the beginning of each observation period, and at fifteen minute intervals, the playa will be scanned with a 20-60X spotting scope. All migratory waterfowl present will be counted and identified by species, sex, and location on the playa, and subjective assessment of condition (appears healthy, sick or dead) will be noted. Times of arrival and departure of all migratory birds will also be recorded. Relative use by species will be calculated from length of time individual birds are on the playas.

Environmental parameters (ambient temperature, relative humidity, wind velocity, and wind direction) will be measured automatically each hour. Conductivity and water temperature will be measured at the end of the morning observation period, 5m from shore at a depth of 10 cm.

Any dead or sick bird observed will be monitored to determine the direction and distance of drift. Elapsed time and distance will be recorded from the time it was first observed until the bird sinks below the surface or reaches shore. At the termination of the morning observation period, attempts will be made to recover all waterfowl that drift to shore for post-mortem evaluation. Sick birds that have floated to shore and are immobile will be humanely euthanized and frozen for later necropsy.

During the periods that the experimental bird portion of the project are in progress, 10 waterfowl carcasses will be placed on the water surface near the center of the two playas. Disposition of each carcass will be monitored for four hours or until it either reaches the shore or sinks to the bottom. Those carcasses that reach the shore will be recovered. Water profiles for temperature, conductivity, sodium, potassium, and chloride levels will be developed at the experimental study sites, and water samples will be collected for additional chemical analysis using atomic absorption spectrometry.

b. Causes of Death

1) Free-ranging migratory birds

All carcasses found during mortality monitoring (referenced above) will be frozen on site. At the end of each study period, 15 carcasses from each playa will be randomly selected from those judged to be in suitable postmortem condition, and necropsied to determine cause(s) of death and other pathological conditions. Diagnostic testing on each carcass will include a brain mineral scan for 12 elements, including sodium and potassium; brain chloride; brain cholinesterase level; and avian botulism. In addition, sections of pectoral muscle will be analyzed from each carcass for water content to evaluate dehydration. Additional diagnostic assays including routine bacterial culture, specific bacterial culture (such as for *Salmonella spp.*), virus isolation, parasitology, toxicology, and histopathology will be done as determined by necropsy observations.

2) Sentinel mallards

Six-month old male mallards will be obtained from a commercial supplier and shipped to Carlsbad, NM. Cloacal swabs will be taken from all birds and tested on arrival for parasites and selected bacterial and viral diseases. Birds will be acclimatized on ground with pools in plastic netted pens at Carlsbad, fed a standard waterfowl maintenance diet, and provided water through multiple ball-bearing waterers. After two weeks, the pens and birds will be moved to the study playas. Five birds will be randomly assigned to each of three treatment groups at each playa as follows:

1. Pen located in playa, playa water provided in waterers

2. Pen located on land, playa water provided in waterers

3. Pen located on land, fresh water provided in waterers

A 3 cc blood sample 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 run on each sample.

Birds will be shipped frozen to NWHC for necropsy and diagnostic testing as described above.

c. Literature Review

1) Geographic Review: Because the boundaries and names of the area that now comprise Lea and Eddy counties have undergone extensive changes between 1880 and 1917, a review of the geographic area is crucial to correlate other pertinent information to a specific area. Resources required to accomplish this include:

U.S.G.S. topographic maps,
Historical Atlas of New Mexico,
New Mexico in Maps,
New Mexico Place Names,
Aerial Maps
Expert Contacts- Professor Jerry Williams, Southwest Institute, Univ. of New Mexico, Albuquerque.

2) Historic Land Use: Beginning with agricultural and grazing usage, Eddy and Lea counties have a varied land usage. Stamp mills, reduction works, mines, stock raising, gypsum, sulfur and potash refineries, oil and gas production all have utilized this area. Resources identified to research land usage:

Official Reports of New Mexico 1882-present
Reports of the Highway Engineer
Report of Appraisal of Mines of New Mexico

New Mexico in Maps pp.258-288
Eddy County, New Mexico to 1981
Carlsbad Irrigation Project reports
Atlas of New Mexico
Bibliography of New Mexico Publications

Historical Records for various companies including U.S. Potash, Potash Company of America, New Mexico Potash, Duvall Potash, National Potash, Atesia refinery, Hobbs refinery, Empire Gypsum.

Locations to be visited:
Institute of Technology- Socorro
University of Texas- El Paso
Historical societies in Hobbs, Lovington, Carlsbad
Cowboy Hall of Fame
Expert contacts- Harvey Hicks- President, Northeastern Historical Society;
Dr. Douglas Dinwitte - New Mexico State University

3) Historic and current bird Use: Long term projects by governmental and private groups provide an existing database to analyze historical changes in local and regional bird abundance. Resources to be consulted are:

Christmas Bird Counts
Banding Bird Survey
Breeding Bird Survey
Birds of New Mexico
New Mexico State bird counts
National Audubon Society records
Expert Contacts - Steve West, Carlsbad, NM

4) Hydrological and Geological Use: Many documents exist in this area and are readily retrievable through electronic databases, and depository libraries. Resources identified are:

Online searches of GeoArvhive, GEOBASE, Georef, Water Resources Abstracts, Waternet, Enviroline, Environmental Bibliography
USGS
Water Resources Library - U.W.
Expert contacts - Danny Davis and Scott Hopkins, NM Environmental Dept.

Critical Data:

a. Species and number of individual waterfowl on the playas observed during spring and fall migration periods. Environmental data, water chemistry measures from the transects. Species and number of identified water birds that die on the playa observed during spring and fall migration periods. Distance of drift and sinking rates relative to environmental data.

b Necropsy and diagnostic data from mortality surveys. Clinical pathology data, necropsy and diagnostic data from experimental trials.

c. Documents required to answer questions posed by research staff, and for analyses of project data.

Statistical Treatment:

a. The number of bird-use days will be calculated for each playa by species and used to estimate the number of migratory birds at risk and mean duration of stay on the playas. Combining this information with mortality estimates would allow waterfowl mortality to be estimated with a risk function.

The number of waterfowl mortalities will be estimated by playa, species, age, sex and season. This estimate will incorporate an estimated disappearance rate for all carcasses. Mortality rates will be captured per playa, species, age, sex and season of use observed.

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 playas.

b. Clinical pathology data and necropsy diagnostic data will be compared between treatment groups on each playa and between the same treatment group on different playas using ANOVA.

A chi-square analysis will be used for the mortality surveys to test the hypothesis that there are no differences in the causes of mortality between the playas.

c. See assessment criteria below

Assessment Criteria:

a.& b. The finding of significant differences in natural and experimental mortality between birds exposed to playa waters that have received recent potash discharge and those that have not will suggest that the discharge water is the cause of the recorded mortality.

c. Accumulation of a well defined, organized, annotated resource that the research group can find answers to questions posed during the execution of the project will constitute acceptable completion of this section.

Special Safety Requirements:

All personnel involved in the project will have physical exam, clinical testing and health assessment at beginning of study, after one year and at conclusion of study. Protective clothing and gloves will be worn when in contact with playa waters. All necropsies will be performed in a biocontained facility.

Completion Date: September 30, 1996

Schedule and Milestones:

Research study plan completed for NBS/Outside review	June 15, 1994
Revised study plan available for SENMPLCC review	July 11, 1994
Public meeting and review	August 24, 1994
First study season	Oct. 1994 - Mar. 1995
Interim report	July 30, 1995
Literature review completion and report	September 30, 1995
Second study season	Oct. 1995 - Mar. 1996
Final report and manuscript submissions	September 30, 1996

Data Storage:

a) Data sheets and computer data in SESC and NMCU files. Archive copies at NWHC.

b) Necropsy reports and diagnostic data in NHWC diagnostic files and database. Tissues, blocks, and slides in NWHC archives.

- Clinical pathology reports in NWHC diagnostic files.

Relationship to Other Work:

The NWHC has received three submissions (cases 10688, 10775, and 11955) for mortality investigation since 1992. Possible/probable salt toxicity was the general diagnosis in each case.

In March 1994, the SESC performed a pilot study using captive mallards at Laguna Toston.

References:

Altmann, J. 1974. Observational study of behavior: sampling methods. Behaviour 49:227-267.

Buck, WB, GD Osweiler, and GA Van Gelder. 1976. *Clinical and diagnostic veterinary toxicology*. Kendall/Hunt Pub., Dubuque, IA. 380pp.

Davis, DR and JS Hopkins. 1993. Lake water quality assessment surveys; Playa Lakes. New Mexico Environ. Dept., Sante Fe. 190pp.

Franson, JC, L Sileo, and WJ Fleming. 1981. Iatrogenic salt poisoning in captive sandhill cranes. JAVMA 179:1211-13.

Hatch, RC. 1977. Poisons causing nervous stimulation or depression. In, Jones, LM, NH Booth, and LE McDonald (eds.), *Veterinary pharmacology and therapeutics*, 4th ed. Iowa State University Press, Ames.

Playa Lakes Joint Venture. 1994. Draft management plan. USFWS Region 2, Albuquerque. 28pp.

Riggert, TL. 1977. The biology of the mountain duck on Rottnest Island, Western Australia, Wildl. Monogr. 52:1.

Shaw, PA. 1930. Recent progress in duck disease studies. JAVMA 30:561-567.

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

Wobeser, GA. 1981. *Diseases of wild waterfowl.* Plenum Press, New York. 300pp.

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.

APPENDIX 2: STUDY PLAN MODIFICATIONS

Ms. Leslie Cone
SENMPLCC Chair
Bureau of Land Management
1717 W. Second Street
Roswell, NM 88201-2019

Dear Leslie:

Below are the changes to the formal Playa Lakes investigation study plan. They are keyed to the study plan sections. Most of the changes were necessitated by logistical problems, new/revised information available, and availability of additional resources.

Bird Use and Mortality

- The first study period was from March 13 to April 28, rather than October to March.

- The observational schedule has not been followed exactly as written. The total number of observational hours has exceeded the number planned, but these periods have been varied in a regular and opportunistic manner; observational periods have varied up to 10 hours in duration, depending on bird activity. This will enable us to determine bird use by species per observational hour. In addition, observations have also been made at five additional playas in the Nash Draw area.

- Environmental parameters have not been measured as stated. This data will be available from permanent BLM weather stations.

- Water parameters have not been directly measured due to unavailability of appropriate instrumentation. Duplicate rather than triplicate water samples have been collected at weekly, rather than monthly, intervals at Laguna Toston and William Sink. Duplicate samples have been taken opportunistically at the other Nash Draw playas. Water samples have been analyzed by IMC Global as well as the Southern Science Center.

- Sick birds found alive have had blood samples taken prior to euthanasia. These samples will be analyzed for chemistry parameters as stated in the experimental study.

- The carcass disposition study was not completed as stated and will need to be completed. An additional pilot study on rate of disappearance of submerged birds was done.

Causes of Death

- Carcasses found at sites were not frozen but shipped immediately and processed the day after recovery. Sick birds and carcasses found at the Nash Draw playas have also been recovered for necropsy.

- Birds on experimental study used gravity rather than ball-bearing waterers.

- Additional chemistry parameters (albumin, alkaline phosphatase, AST/SGOT, ALT/SGPT, LDH) were run on experimental birds.

- Samples of feathers from various regions (wing, breast, neck,) were collected at each sampling time.

- Infrared video photography was taken of each bird at each sampling period to assess heat loss.

- A pilot study on flight ability was conducted during the experimental trials. Five birds were placed in each of the study lakes and removed at various time intervals. These birds were allowed to fly from a person's hands and length of flight was recorded.

Literature Review

- In addition to the annotated bibliography, we will be preparing interpretive summaries on some of the review sections.

- A review section on salt toxicity in wildlife has been added.

All of the critical data specified has or will be collected. Most of the changes have been relatively minor and were internally reviewed as needed. On the whole, these changes have allowed more rather than less information to be collected from this study period, despite its shorter course. Data analysis is ongoing and we will have proposals for additional changes and studies included in the interim report.

I hope that this information is useful for SENMPLCC planning. I will be happy to provide additional details on any of the items.

Sincerely,

F. Joshua Dein, VMD,MS
Playa Lakes Project Coordinator

APPENDIX 3:
POTENTIAL FUTURE QUESTIONS
FOR PLAYA LAKES MORTALITY INVESTIGATIONS

QUESTION:

1. Is waterfowl mortality related to environmental conditions?

   STUDY:

   Conduct exposure studies in a controlled environment (constant temperature and no wind velocity)

   MITIGATION POSSIBILITIES:

   - Concentrate hazing techniques during periods of low temperatures or high winds.
   - Provide alternate shelter and/or fresh water during periods of low temperatures or high winds.

2. What is the extent of mortality during the fall migration period? Are there any differences in species, sex or age related mortality as compared to spring migration?

   Continue playa surveys and carcass pick-up/necropsy this fall.

   Determine at what mortality level mitigation efforts are warranted.

3. Is waterfowl mortality related to saline concentration of playa lakes?

   Conduct controlled environment studies with decreasing dilutions of hypersaline water to determine minimal toxic concentration.

   - Dilution of playa lakes may make the environment less toxic.

4. Is dehydration a direct effect of exposure to playa water? Does dehydration have a significant effect on measured clinical parameters?

   Conduct a water deprivation study to evaluate the physical and physiological effects of dehydration. Compare these results to the birds exposed to hypersaline water.

- Provide a fresh water source to counteract the dehydration.

5. Are the physical and physiological effects of exposure to playa lakes reversible?

Conduct controlled environment studies with varied times of exposure, then remove the ducks and offer fresh water.

- Provide alternate shelter and water source at playa lake.

6. Is waterfowl mortality effected by the ionic composition of playa lake water?

Conduct controlled environment studies with controlled ionic compositions to compare rates of morbidity/mortality.

- Alter the ionic composition of discharge by addition or deletion of salts.

7. Is cataract development due to a physiological or contact reaction? Does cataract development effect vision?

Conduct controlled environment studies with in vivo ophthalmic examinations.

- Mitigation not yet determined.

8. Does the hydration status have a direct effect on the susceptibility to salt poisoning?

Conduct controlled environment studies with ducks that have been deprived of water prior to exposure compared to hydrated ducks.

- Availability of fresh water may prevent or decrease morbidity/mortality.

9. Is salt poisoning due to exposure to hypersaline water or due to ingestion of hypersaline water (salt precipitation)?

Gavage ducks with hypersaline solution in a controlled environment without physical exposure to hypersaline water.

- Mitigation not yet determined.

10. Is decrease in flight capability due to the increase in weight secondary to salt precipitation?

    Repeat flight testing with ducks monitoring weight increases with exposure to hypersaline water.

    - Provide fresh water for bathing and preening of salt precipitate.

11. Is the mechanism of dehydration a result of cutaneous water loss through the exposed areas (e.g. legs, feet, and cloaca)?

    Conduct exposure studies using ducks pre-treated with tritiated water to evaluate water loss from the ducks.

    - Coupled with the dilution investigations, this may aid in methods to stratify the hypersaline environment thus making it less toxic.

APPENDIX 4: STUDY PLAN FOR CONTROLLED ENVIRONMENT STUDY

National Biological Service

National Wildlife Health Center
Madison, Wisconsin

STUDY PLAN

NWHC Work Unit: 00220 - Investigation of avian mortality in the playa lakes of southeastern New Mexico.

NWHC Study Plan: 00220.02 - Effects of exposure to playa lakes water in a controlled environment

Background and Justification:

In April 1994, the National Wildlife Health Center (NWHC) and the Southern Science Center (SSC) received directives to initiate a collaborative study investigating bird mortality in the playa lakes of southeastern New Mexico. The NWHC was assigned the lead responsibility for the project in cooperation with the SSC. Studies, covered under study plan 00220.01, were carried out in the Fall of 1994 and Spring of 1995.

A sentinel bird study at Laguna Toston and William sink (Study Plan 00220.01) determined that the most likely cause of death was salt poisoning/water deprivation. The physiological and physical effects of birds exposed to the hyper saline water were consistent with those noted in domestic species. In addition, other factors were noted that may be associated with the mortalities. Ducks that were directly exposed to hyper saline water developed cataracts and became salt encrusted. The birds were also found to be significantly dehydrated. Ducks that were given hyper saline water to drink did not develop cataracts or encrustation but did show evidence of dehydration.

Histopathological results indicated that there was inflammation of the conjunctiva, upper respiratory tracts, bursa, and cloaca of the birds exposed to hyper saline water. Reports of salt poisoning in domestic species have not noted these changes. These changes may be a result of direct contact with the hyper saline water not secondary to ingestion.

Bird use and mortality surveys performed in Spring 1995 suggested that mortalities may be related to temperatures and wind velocities. Carcasses of migratory birds found dead or moribund showed biological and clinical changes similar to those of the experimental birds. In birds from both experimental studies and mortality surveys, it could not be determined how much dehydration, water toxicity or environmental factors contributed to the study. This plan seeks to investigate these questions.

Objectives:

1. Determine the morbidity and mortality of ducks exposed to hyper saline water in a controlled environment.

2. Determine the dilution required to prevent morbidity/mortality of ducks exposed to hyper saline water in a controlled environment.

Methods and Approach:

Twenty six-month-old mallards (males) will be obtained from Whistling Wings (commercial supplier) and transported to NWHC. The mallards will be given a physical examination and weighed upon arrival. Cloacal swabs will be taken from all of the birds and screened for parasites, Salmonella, and viral agents. Blood samples will be taken for clinical pathology to assess the general health status (complete blood cell counts and avian chemistry panel).

Sixteen mallards will be randomly assigned to four treatment groups and placed by group into plastic pools (See Appendix 1) containing fresh tap water, and fed a standard waterfowl maintenance diet in hanging gravity feeders. After one week of acclimation and negative findings regarding pathogens the experimental trials will be started.

The birds will be transferred into pools filled with tap water, hyper saline water from Laguna Toston or a combination of both, to a depth of 1 foot, depending upon the treatment group that they are assigned to (see below).

Treatment Group	Tap water	Hyper saline Water
1	100%	0%
2	0%	100%
3	20%	80%
4	30%	70%

A 2 cc blood sample will be taken from each bird at 0, 3, 7, 24, and 48 hours (and 72 hours for TG1). The following clinical pathology tests will be run on each sample: packed cell volume, electrolytes (sodium, potassium, chloride) and chemistries (albumin, aspartate aminotransferase, urea nitrogen, calcium, creatine phosphokinase, creatinine, globulin, glucose, lactate dehydrogenase, total protein and uric acid). Hematology (hematocrit and WBC) will be performed on 0, 24, and 48 hour samples. Complete ophthalmic examinations will be performed (see Appendix 4) at 0, 1, 2, and 3 hours, looking for the development of cataracts. Any bird found in distress at a sampling period will be euthanized with carbon dioxide. All survivors will be euthanized at 48 hours (TG1 and TG2 at 72 hours). The birds will be weighed at 0, 24, 48 and 72 hours and at necropsy.

Water samples will be collected from the pools at 0, 3, 7, 24, and 48 (72) hours. These will be submitted to IMC Global for ion analysis.

The ducks will be monitored with a time-lapse video camera throughout the experimental trial. This will allow for documentation of behavior and development of clinical signs during periods when there will be no human contact.

All 16 carcasses will be submitted fresh for gross necropsy, and the following tissues will be collected and submitted for testing:

1. Breast muscle for determination of muscle moisture content. (See Appendix 2)
2. Brain tissue for sodium concentration. (See Appendix 3)
3. Tissues for histopathology.

Critical Data:

a. Clinical pathology data, ophthalmic observations, necropsy and histopathology findings.

Statistical Treatment:

a. Clinical pathology data and necropsy diagnostic data will be compared between treatment groups using ANOVA.

b. Clinical pathology data and necropsy diagnostic data will be compared between current experimental trials and previous experimental field trials using ANOVA.

Assessment Criteria:

a. The finding of significant differences between natural environment conditions (previous study) and controlled environment mortality will suggest that temperature and wind may play a role in mortality.

b. The finding of significant differences in mortalities between birds exposed to playa water that has been diluted and that which has not will suggest that mortality is related to the concentration (total dissolved solids) of the hyper saline water.

Funding and FTEs:

Salaries & benefits	FTE	$
Animal Caretaker (GS-05)	.03	
Travel		200
Equipment and supplies		4,200
Experimental animals		438
Animal husbandry		200
Water shipment		2,000
Laboratory diagnostics		5,468
Ophthalmologic examinations		1,080
		13,586

Special Safety Requirements:

All personnel involved in the project will have a physical exam, clinical testing and health assessment within the guidelines of the Center's occupational health program. Protective clothing and gloves will be worn when in contact with playa waters. All necropsies will be performed in an appropriate biocontainment facility.

Completion Date: September 30, 1996

Schedule and Milestones:

Research Study Plan completed for NBS/Outside review - November 15, 1995

Study Season - July 1 - August 30, 1996

Final Report and manuscript submissions - September 30, 1996

Data Storage:

a. Data sheets and computer data in NWHC files.

b. Necropsy reports and diagnostic data in NWHC diagnostic files and database. Tissues, blocks, and slides in NWHC archives.

c. Clinical pathology reports in NWHC diagnostic files.

Relationship to Other Work:

In a previous study plan submitted to SENMPLCC, sentinel birds studies were performed at Laguna Toston and William Sink. These experimental trial indicated that the ducks exposed to playa water died from salt poisoning/water deprivation.

References:

Calderone, L., P. Grimes, M. Shalev. 1986. Acute reversible cataract induced by xylazine and by ketamine-xylazine anesthesia in rats and mice. Exp. Eye Res. 42: 331-337.

Fountain, Jr., E.A. 1979. The physiological and behavioral aspects to swimming in salt water. Thesis. Dept of Physiology and Biophysics. Colorado State University, Fort Collins.

Mahoney, S.A., J.R. Jehl. 1984. Body water content in marine birds. Condor 86: 208-209.

Shaw, P.A., 1930. Recent progress in Duck Disease studies. J. Am. Vet. Med. Assoc. LXXVII(5): 561-567.

Shaw, P.A., 1929. Duck Disease studies: I. Blood analysis in diseased birds. Proc. Soc. Exp. Biol. Med. XXVII: 6-7.

Shaw, P.A., 1929. Duck Disease studies: II. Feeding of single and mixed salts. Proc. Soc. Exp. Biol. Med. XXVII: 120-122.

Will, D.H., J.H. Abel,Jr., B.E. Russell, K.E. Levine. 1977. Colorado River desalinization, wild duck study: Final project report. Dept. of Physiology and Biophysics, Colorado State University, Fort Collins.

APPENDIX 1

POOL DESIGN:

Plastic tubs with the following dimensions = 4' x 4' x 30"

Total square footage = 16 sq. ft.

A spigot or plug will be added to the bottom of the tub to facilitate drainage.

A plastic netting will be stretched across a PVC tubing framework to act as a lit for the fiberglass tubs. This will prevent the ducks from escaping from the tub.

APPENDIX 2

Moisture Analysis Protocol

National Wildlife Health Center
Diagnostic Pathology
Title: Protocol for percent muscle moisture analysis

Determination of % moisture is based on the AOAC method 24.002 found in the Official Methods of Analysis, 14th edition, 1984.

1. A sample of breast tissue will be removed at necropsy from each bird and frozen for analysis in batch fashion. All samples will be treated in the same manner so that freezing would not be a factor.

2. The tissues will be partially thawed and diced into small chunks, removing the outer tissue. A representative sample from each bird of about 5 g will be weighed in a tared, porcelain crucible to determine wet weight. One set of duplicates or 5% of the sample size, whichever is greater, will be prepared as a quality control.

3. Samples will be dried overnight (16-18 hr) at 105-110 degrees C to a constant weight in a convection drying oven. Crucibles will be removed from the drying oven and cooled to room temperature, and will be again weighed to determine dry weight.

4. After subtracting the crucible weight, percent moisture will be recorded as:

$$\frac{\text{wet weight - dry weight}}{\text{wet weight}} \times 100.$$

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 U. 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 beaker; one for 5% nitric acid soak and three containing DI water.

Clean plastic spoon 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 brain from touching skull, feathers or gloves. A new spoon will be used for each accession.

PROCEDURE:

1. Give Diagnostic Chemistry Department more than 24 hours notice so they can prepare stock solutions of dilute acid and distilled water. Chemistry will soak scissors and plastic spoons overnight in 5% nitric acid, rinse in distilled water and have the utensils dried for necropsy when needed.

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

3. Insert scalpel blade onto handle without touching the blade.

4. Incise the skin with the tip of the scalpel blade and reflect the 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 double distilled water out of the third rinse jar and empty and replace the water in this 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 jar with 5% nitric acid in the first "rinse jar". Discard 5% nitric acid and replace with fresh nitric acid. Place the thoroughly rinsed scissors just used for cutting the cranium in 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 brain from cranium with plastic spoon making sure to prevent brain from touching skull, feathers or gloves.

8. Send brain in acid-washed jar to chemistry for sodium analysis.

APPENDIX 4

<u>Ophthalmic Examinations</u>

Birds will be hand-held for examination at 0, 1, 2 and 3 hours. Slit-lamp biomicroscopy will be performed using a hand-held Kowa SL-II biomicroscope. Evaluations will be made of the ocular adnexae, and the anterior segment of the eye, including the lens. A Nikor photomicroscope and a Kowa fundus camera will be used to document examination findings.

APPENDIX 5: TESTS PERFORMED AND COMMON USAGE

ALB Albumin	Measure of plasma albumin
ALT Alanine Aminotransferase	Measure of non-specific cell damage (high levels found in mallard kidney)
ALKPHOS Alkaline Phosphatase	Measure of increased cellular activity irritation of cell rather than damage
AST Aspartate Aminotransferase	Measure of liver or muscle damage
BUN Blood Urea Nitrogen	Measure of glomerular filtration (sensitive indicator of dehydration)
Cl Chloride	Measure of plasma chloride
CPK Creatinine Phosphokinase	Measure of muscle cell damage
GLU Glucose	Measure of plasma glucose (evaluation of stress in avian species)
K+ Potassium	Measure of plasma potassium levels (major intracellular ion)
LDH Lactate Dehydrogenase	Measure of liver and muscle damage (elevations occur more rapidly than AST)
Na Sodium	Measure of plasma sodium levels (major extracellular ion)
PCV Packed Cell Volume	Measure of red blood cell volume
TP Total Protein	Measure of total plasma proteins
UrA Uric Acid	Measure of renal function