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## EFFECTS OF SODIUM AND MAGNESIUM SULFATE IN DRINKING WATER ON MALLARD DUCKLINGS

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**ABSTRACT:** One-day-old mallard (*Anas platyrhynchos*) ducklings were given drinking water for up to 28 days that contained concentrations of sodium and/or magnesium similar to those found in saline wetlands. Growth, tissue development, and biochemical characteristics of these ducklings were compared to those reared on fresh water. Much of the ingested salt was excreted by passage of voluminous fluid excreta. This effect occurred in birds given water with as little as 500 ppm Mg or 1,000 ppm Na. The supraorbital salt gland was active within 4 days in ducklings drinking water containing  $\geq 1,500$  ppm of Na. Feather growth was decreased in ducklings drinking water with  $\geq 1,500$  ppm of either Na or Mg. Ducklings drinking water with 3,000 ppm of either ion, or 1,500 ppm of each, grew more slowly than control birds. Ducklings drinking water with 3,000 ppm of either Na or Mg had reduced thymus size and bone breaking strength. Those drinking water with 3,000 ppm of Mg, or 3,100 ppm Na and 1,300 ppm Mg also had less trabecular bone and enlarged adrenals. Birds drinking the latter water had an elevated concentration of Na and calcium, and a decreased concentration of phosphorus and chloride in their serum, and elevated plasma protein levels. Ducklings reared on fresh or slightly saline water adapted very poorly to an abrupt change to more saline water (specific conductivity = 15,250  $\mu\text{mhos/cm}$ ) at 14 days of age. These birds stopped eating, became inactive and some died within 3 days; survivors had many tissue and biochemical abnormalities at 20 days of age. The level of salinity in these trials was similar to that in "brackish" or "moderately saline" wetlands and lower than that previously found to have effects on growth and feathering of ducklings. Many of the sublethal effects were subtle and non-specific manifestations of stress, and would be difficult to detect in wild ducklings on saline wetlands.

**Key words:** *Anas platyrhynchos*, duckling, wetland, salinity, toxicity, growth, feathering, biochemistry, pathology, experimental.

### INTRODUCTION

The prairie pothole region is the major area of duck production in North America. The low precipitation and high evaporation rate in the region have resulted in accumulation of salts within wetland systems that lack external drainage. In Saskatchewan the major cations in such wetlands are Na and Mg, with sulfate being the common anion (Rawson and Moore, 1944; Hammer, 1978). Waterfowl use saline wetlands for feeding (Serie and Swanson, 1976; Krapu and Swanson, 1978) and molting. Islands in some saline wetlands are used intensively by nesting ducks (Duebbert et al., 1983), but the fate of broods produced on these wetlands is unknown (Swanson et al., 1984).

When exposed to hypertonic saline drinking water, waterfowl osmoregulate

mainly by excreting excess Na via the supraorbital salt gland (Peaker and Linzell, 1975). Adult mallards (*Anas platyrhynchos*) can tolerate water containing about 20 parts per thousand of NaCl but cannot survive on seawater (Holmes et al., 1978). Ducklings are reported to be much less tolerant of salt (Ellis et al., 1963; Schmidt-Nielsen and Kim, 1964; Riggert, 1977) and "salt poisoning" has been suspected in Australian shelducks (*Tadorna tadornoides*) (Riggert, 1977). Swanson et al. (1984) found that ducklings were consistently absent from some saline wetlands in North Dakota, while ducklings on other wetlands were closely associated with local freshwater inflows. Ducklings <1–3 days of age were unable to survive when provided only with saline water from most of the latter wetlands.

Information on the suitability of saline wetlands for waterfowl may become increasingly important. Because drainage is usually directed at fresh rather than saline wetlands, and because current agricultural practices result in increased leaching of salt from the soil with deposition of salt in low-lying areas (Standing Committee on Agriculture, Fisheries, and Forestry, 1984), saline wetlands will likely comprise an increasing proportion of remaining wetland area in the future.

This study was conducted to determine the effects of sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and magnesium sulfate ( $\text{MgSO}_4$ ) singly, and in combination, on the growth, tissue development, hematology and clinical pathology of mallard ducklings.

## MATERIALS AND METHODS

### Experimental design

Four trials were conducted. On day 0 of each trial mallard ducklings obtained on the day after hatching from Whistling Wings (Hanover, Illinois 61041, USA) were marked with web tags, weighed and assigned randomly to groups of 10 ducklings which were placed in individual indoor pens equipped with heat lamps. In Trials I and II each pen had an area of 4.4 m<sup>2</sup>, whereas in Trials III and IIIA each pen had an area of 3.3 m<sup>2</sup>. There was no significant difference in average weight of ducklings on day 0 among groups in any of the trials. Ducklings had access to commercial duck and goose starter (Table 1) and either Saskatoon tap water (controls; Table 2) or salt solution (Table 3) ad libitum. The salt solutions represented a range of concentrations of Na and Mg similar to that found in saline wetlands during June in southern Saskatchewan (Table 2), and were prepared by adding anhydrous  $\text{Na}_2\text{SO}_4$  and/or anhydrous  $\text{MgSO}_4$  on a weight/volume basis to Saskatoon tap water. The conductivity of these solutions measured with a specific conductivity meter was within the low to intermediate range as defined for wetlands by various authors (Fig. 1). Water was supplied in 10-liter double wall vacuum poultry founts. Food was supplied in trough type chicken feeders equipped with an anti-roost reel. Food consumption was measured by weighing food supplied and food remaining each day; no attempt was made to collect spilled food, which appeared to be similar in amount among groups.

Trials I and II lasted 28 days. Ducklings received the same salt solution for the duration of

TABLE 1. Content of selected components in commercial duck and goose starter used in all trials.

Component	Manufacturer's guaranteed analysis (%) <sup>a</sup>	Independent analysis (%) <sup>b</sup>
Protein (minimum)	20.0	21.1
Fat (minimum)	2.0	NT <sup>c</sup>
Fiber (maximum)	6.0	NT
Salt	0.4	NT
Sodium		0.14
Calcium	0.9	0.82
Phosphorus	0.7	0.80
Magnesium		0.14

<sup>a</sup> From label of feed bag (source: Federated Cooperatives Ltd., Saskatoon, Saskatchewan, Canada S7N 1Z3).

<sup>b</sup> Feed Testing Service, College of Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

<sup>c</sup> Not tested.

the trial (Table 3). Trials III and IIIA were of 20 days duration; on day 15 of these trials the original salt solution was discontinued and ducklings in Groups 1A, 2A and 3A of Trial III and Groups 1A, 2 and 3 of Trial IIIA were given water containing 3,100 ppm Na and 1,300 ppm Mg (Table 3). This was an attempt to mimic a situation in which young ducklings move onto a permanent saline wetland when ephemeral freshwater wetlands dry during summer. The concentration of Na and Mg given on day 15 of these trials was similar to that found during June in a wetland (Porter Lake) near Saskatoon (Table 2).

Clinical signs such as nasal gland discharge and condition of droppings were monitored daily. Ducklings were weighed on alternate days to day 16 and then every fourth day until the completion of the trial.

### Sample collection and analysis

On day 14 of each trial 1 ml of blood was collected from the metatarsal vein of each duckling. Osmolality of the serum was measured by freezing point depression (Advanced Wide Range Osmometer, Advanced Instruments, Inc., Needham Heights, Massachusetts 02194, USA). Concentration of Na, potassium and chloride in serum was measured by ion selective electrodes with a Beckman E4A analyzer (Beckman Instruments, Mississauga, Ontario, Canada L5T 1W5); Mg, calcium and inorganic phosphorus concentration in serum was measured with a Bichromatic Analyzer 100 (Abbot Laboratories, Mississauga, Ontario, Canada L5N 3R7).

Blood collected into heparinized capillary tubes from each duckling was used for deter-

TABLE 2. Selected chemical and physical characteristics of water collected in June 1983 from wetlands in Saskatchewan and of Saskatoon tap water.\*

Wetland	Latitude	Longitude	Na	Mg	K	Ca	SO <sub>4</sub>	Cl	CO <sub>3</sub>	Conductivity (μmhos/cm)
Axe Lake	51°31'N	105°13'W	460 <sup>b</sup>	231	39	132	1,710	132	nil	4,500
Little Quill Lake	51°55'N	104°14'W	700	460	84	100	2,770	341	nil	7,000
Middle Quill Lake	51°56'N	104°12'W	920	530	100	200	3,780	466	nil	8,500
Rice Lake	52°03'N	107°08'W	1,100	400	92	140	3,470	383	nil	8,500
Goose Lake Slough	51°46'N	107°19'W	1,700	240	130	60	3,010	348	42	10,000
Porter Lake	52°11'N	106°17'W	3,150	1,270	180	496	9,570	2,130	nil	23,500
Buffer Lake	52°23'N	106°00'W	9,500	2,300	290	520	21,300	6,960	nil	48,500
Saskatoon tap water			22	14	3	32	75	7	nil	357

\* Analyses performed by Analytic Services, Saskatchewan Research Council, Saskatoon, Saskatchewan, Canada S7N 0W0.

<sup>b</sup> ppm.

mination of packed cell volume (PCV), total plasma protein (TP) and preparation of blood smears. Total white blood cell count was estimated from the peripheral blood smear (Lynch et al., 1969). Only heterophils and lymphocytes were counted in the differential white blood cell count.

Approximately 10 samples of excreta collected and pooled from each pen on days 13 and 27 in Trials I and II and on day 13 of Trials III and IIIA were dried, reconstituted as a 5% solution in distilled water, and analyzed for Na and Mg content.

On the last day of each trial, ducklings were weighed, 5–10 ml of blood were collected by cardiac puncture, and the birds were killed by carbon dioxide overdose. Culmen length, total length and quill length of the ninth primary feather, middle retriex length and leg length from the hock to the longest claw tip were measured. Salt glands, right femur, liver, spleen, thymus, bursa of Fabricius, left kidney, adrenals and gonads were fixed in neutral buffered 10% formalin. The left femur was frozen.

After fixation, salt glands, liver (without the gall bladder), spleen, thymic lobes, bursa of Fabricius, left kidney and one adrenal gland were weighed and organ weights were calculated as a percent of body weight. One salt gland, adrenal, gonad and a portion of liver, spleen, thymus, bursa and left kidney were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (H&E). After decalcification in 20% formic acid for 48 hr, two sections were cut from the right femur, processed routinely and stained with H&E. One section was taken longitudinally through the center of the femoral intercondyloid fossa and the other transversely through the midshaft.

Stained slides of tissues were examined with-

out knowledge of the group of origin. Selected variables were ranked subjectively on a scale of 0 to 6, with 0 being a marked decrease in the variable being measured, 3 being normal, and 6 ranked as a marked increase. Tissue characteristics that were ranked included the amount of bursa, splenic and thymic lymphoid tissue, vacuolation of hepatocytes, and salt gland and adrenal cortical hypertrophy. Histologic sections of liver that had been ranked from 3 to 6 for vacuolation were stained with periodic acid-Schiff (PAS) and PAS after treatment with diastase. Vacuolated hepatocytes stained positively for glycogen with PAS and were negative for glycogen when stained with PAS after diastase digestion, indicating that vacuoles within hepatocytes were glycogen.

Characteristics examined on the longitudinal section of the distal femur included width of the zone of proliferation and of the zone of hypertrophy, extent of vascular invasion of cartilage, number of osteoblasts and osteoclasts in the epiphysis and metaphysis and the amount of trabecular bone. Midshaft thickness and number of osteoblasts were ranked on the cross section of the right femur. The frozen left femurs were thawed and femoral bone breaking strength was determined by measuring the force applied perpendicular to the long axis of the bone that resulted in breakage (Cashin and Lewis, 1984).

#### Statistical analysis

Parametric variables were analyzed by a one-way analysis of variance (ANOVA); for variables measured over time, two-way ANOVA was used. When ANOVA showed a significant difference among treatment groups, a Student-Newman-Keuls procedure was performed to detect which groups were significantly different ( $\alpha = <0.05$ ). Non-parametric ordinal scale

TABLE 3. Concentration (parts per million) of Na ( $\text{Na}_2\text{SO}_4 \cdot 0\text{H}_2\text{O}$ ) and Mg ( $\text{MgSO}_4 \cdot 0\text{H}_2\text{O}$ ) added to Saskatoon tap water to prepare saline solutions used in Trials I–IIIA.

Trial	Group(s)	Na	Mg	Approximate specific conductivity ( $\mu\text{mhos/cm}$ )
<b>Days 0–28</b>				
I	1	tap water	—	350
	2	500	—	2,000
	3	1,000	—	4,500
	4	1,500	—	5,900
	5	—	500	3,000
	6	—	1,000	5,100
	7	—	1,500	7,000
<b>Days 0–28</b>				
II	1	tap water	—	350
	2	3,000	—	11,000
	3	—	3,000	12,000
	4	1,500	1,500	11,500
	5	1,500	500	8,000
	6	500	1,500	8,300
	7	500	500	4,600
	8	3,100	1,300	15,250
<b>Days 0–14</b>				
III	1, 1A	tap water	—	350
	2, 2A	500	250	3,650
	3, 3A	1,000	500	6,250
	1, 2, 3	as above	—	—
	1A, 2A, 3A	3,100	1,300	15,250
<b>Days 0–14</b>				
IIIA	1, 1A	tap water	350	—
	2	500	250	3,650
	3	1,000	500	6,250
	1, 2, 3	3,100	1,300	15,250

data was analyzed by the Kruskal-Wallis ANOVA and a multiple comparison procedure (Daniel, 1978) was used to determine which groups were significantly different. Statistical significance was accepted at the 0.05 probability level. Values are reported as means (standard deviation). Detailed numerical data are available in Mitcham (1985).

## RESULTS

### Trial I

Control ducklings and those receiving water with 500 ppm Na added had normal excreta. All other ducklings in the trial had

fluid excreta, beginning on day 1 or 2 with the degree of change directly related to the amount of either  $\text{Na}_2\text{SO}_4$  or  $\text{MgSO}_4$  added to the drinking water. Ducklings drinking solutions containing added  $\text{Na}_2\text{SO}_4$  generally had more Na and less Mg in their excreta, and ducklings receiving  $\text{MgSO}_4$  had more Mg and less Na in their excreta than did control birds (Table 4).

At the conclusion of the trial, there were few differences among groups. Ducklings in the control group were larger than those

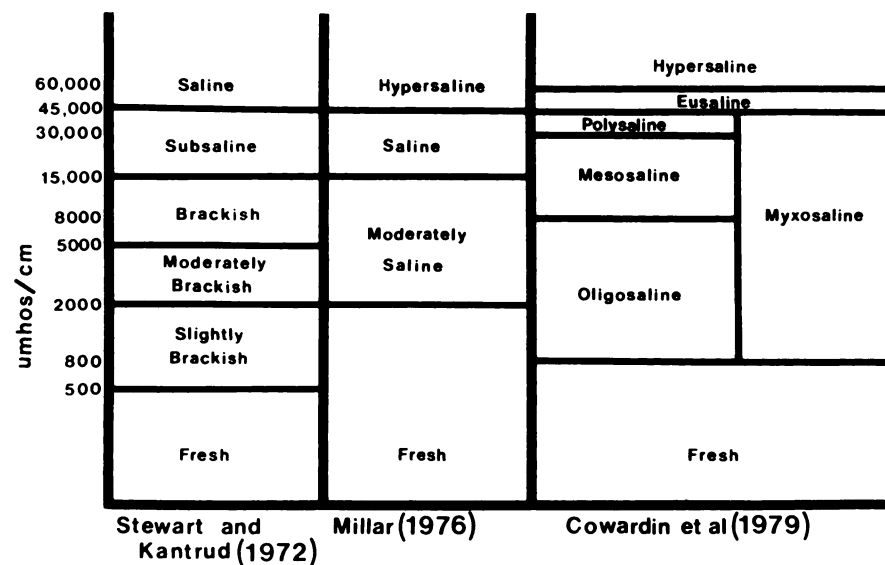


FIGURE 1. Classification systems for natural wetlands based on specific conductivity of the water.

in any of the groups that received saline water, but the difference among groups was not significant (Table 4). Control birds consumed more feed during the trial than did other groups. The only significant difference found in body measurements was in the length of the ninth primary quill, with control birds having significantly longer quills than those in the groups that received the highest concentration of Na and Mg, respectively. Ducklings in all groups were apparently able to compensate for the increased Na and Mg intake, since there was no evidence of hypernatremia or hypermagnesemia, and serum osmolality did not differ significantly among groups.

On day 14 the concentration of calcium in the serum was decreased significantly in Group 6 ( $\bar{x}$  = 2.62, SD = 0.14 mmol/liter) and phosphorus was decreased significantly in Groups 3 ( $\bar{x}$  = 2.45, SD = 0.16 mmol/liter) and 4 ( $\bar{x}$  = 2.60, SD = 0.19 mmol/liter) compared to the control group ( $\bar{x}$  = 2.84, SD = 0.13 and  $\bar{x}$  = 2.91, SD = 0.34 mmol/liter, respectively). The concentration of magnesium in the serum was significantly elevated in Group 7 ( $\bar{x}$  = 2.35, SD = 0.66 mmol/liter) on day 14 and in

Group 6 ( $\bar{x}$  = 2.64, SD = 0.34 mmol/liter) on day 28 compared to that of control ducklings at the same time ( $\bar{x}$  = 1.39, SD = 0.30 and  $\bar{x}$  = 1.96, SD = 0.31 mmol/liter, respectively).

#### Trial II

Ducklings in Groups 2, 4, 5 and 8 were excreting fluid via the nasal glands by day 4 of the trial, as evidenced by droplets around the nares and occasional head shaking. Later in the trial, ducklings in Group 7 occasionally had fluid around the nares.

Ducklings in Groups 2, 3, 4, 5, 6 and 8 all had excreta that appeared more fluid than that of the control group. Ducklings drinking solutions containing the higher concentrations of Na<sub>2</sub>SO<sub>4</sub> and/or MgSO<sub>4</sub> had more Na and/or more Mg in their excreta than did control birds.

Ducklings in Groups 2, 3, 4 and 8 grew poorly, with Groups 3 and 8 most severely affected; food consumption followed a similar pattern (Table 5). Ducklings in Groups 3 and 8 had the shortest ninth primary quills, middle retrices and legs and Group 3 had the shortest culmen (Mitcham, 1985). Groups 2 and 4 were less, but

TABLE 4. Selected variables measured in mallard ducklings given various types of saline water as their only source of drinking water for 28 days (Trial 1).

Variable	Group													
	1		2		3		4		5		6		7	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Body weight (g) day 28	682.7	59.3	609.7	101.6	611.2	43.0	618.9	70.2	660.8	82.7	638.4	85.2	603.4	61.6
Total food consumed (kg)	14.3		12.8		12.9		12.0		13.8		12.2		12.2	
Ninth primary quill length (cm)	2.1 <sup>a</sup>	0.5	1.5 <sup>ab</sup>	0.5	1.7 <sup>ab</sup>	0.5	1.3 <sup>b</sup>	0.3	1.9 <sup>ab</sup>	0.6	1.6 <sup>ab</sup>	0.5	1.4 <sup>b</sup>	0.4
Excreta														
Day 13														
Na (mmol/liter)	10		15.6		29.8		59.1		4.7		10		10	
Mg (mmol/liter)	9.6		7.6		8.4		6.9		21.3		11.6		48.3	
Day 27														
Na (mmol/liter)	10		25		42		29		<10		10		<10	
Mg (mmol/liter)	11.9		3.4		6.1		4.4		10.0		29.6		24.8	

<sup>a,b</sup> Values within rows followed by different superscripts are significantly different ( $P \leq 0.05$ ).

TABLE 5. Selected variables measured in mallard ducklings given various types of saline water as their only source of drinking water for 28 days (Trial II).

Variable	Group															
	1 (control)		2 (3,000 ppm Na)		3 (3,000 ppm Mg)		4 (1,500 ppm Na, 1,500 ppm Mg)		5 (1,500 ppm Na, 500 ppm Mg)		6 (500 ppm Na, 1,500 ppm Mg)		7 (500 ppm Na, 500 ppm Mg)		8 (3,100 ppm Na, 1,300 ppm Mg)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Body weight (g)	633.7 <sup>a</sup>	63.8 <sup>a</sup>	527.0 <sup>a</sup>	64.3	391.1 <sup>1d</sup>	101.6	522.6 <sup>c</sup>	60.7	617.0 <sup>ab</sup>	49.2	605.3 <sup>ab</sup>	67.9	582.1 <sup>1b</sup>	47.4	400.2 <sup>d</sup>	73.9
Total food consumed (kg)	14.2		9.7		6.8		10.6		12.6		12.3		11.4		7.7	
Serum osmolality (mmol/kg)	315.6 <sup>ab</sup>	6.3	310.9 <sup>b</sup>	8.0	318.9 <sup>ab</sup>	16.8	322.9 <sup>ab</sup>	38	317.2 <sup>ab</sup>	8.2	323.0 <sup>ab</sup>	7.3	323.0 <sup>ab</sup>	13.7	327.3 <sup>a</sup>	14.7
Serum electrolyte (mmol/liter)																
Sodium	148.1 <sup>b</sup>	2.7	146.8 <sup>b</sup>	2.7	144.9 <sup>b</sup>	2.7	144.8 <sup>b</sup>	2.9	147.6 <sup>b</sup>	1.2	148.7 <sup>b</sup>	2.9	149.4 <sup>b</sup>	1.9	155.5 <sup>a</sup>	7.0
Magnesium	1.77 <sup>ab</sup>	0.5	1.59 <sup>b</sup>	0.5	2.47 <sup>a</sup>	0.8	2.11 <sup>ab</sup>	0.4	2.1 <sup>ab</sup>	0.5 <sup>ab</sup>	2.1 <sup>ab</sup>	0.4	1.7 <sup>b</sup>	0.3 <sup>b</sup>	2.3 <sup>ab</sup>	0.08
Calcium	3.0 <sup>ac</sup>	0.1	3.2 <sup>b</sup>	0.2	2.8 <sup>c</sup>	0.1	2.9 <sup>c</sup>	0.3 <sup>c</sup>	2.9 <sup>c</sup>	0.2	2.9 <sup>c</sup>	0.1	3.0 <sup>ac</sup>	0.1	3.4 <sup>a</sup>	0.2 <sup>a</sup>
Chloride	106.3 <sup>a</sup>	1.6	102.4 <sup>a</sup>	4.9	104.1 <sup>a</sup>	3.0	103.2 <sup>a</sup>	3.1	103.1 <sup>a</sup>	2.3	104.1 <sup>a</sup>	3.4	103.3 <sup>ab</sup>	4.0	98.0 <sup>b</sup>	6.7
Phosphorus	2.9 <sup>a</sup>	0.4	2.8 <sup>ab</sup>	0.3 <sup>ab</sup>	3.1 <sup>a</sup>	0.6 <sup>a</sup>	3.0 <sup>a</sup>	0.4 <sup>a</sup>	3.1 <sup>a</sup>	0.3	2.7 <sup>ab</sup>	0.3	2.4 <sup>b</sup>	0.3	1.9 <sup>c</sup>	0.4

<sup>a, b, c, d</sup> Values within rows followed by different superscripts are significantly different ( $P \leq 0.05$ ).



still significantly affected. By the end of the trial, ducklings in the control group had molted most of their primary wing feathers, whereas molt had not occurred in any of the other groups.

Ducklings in Groups 2 and 8 had significantly heavier salt glands than those in other groups. The liver of ducklings in Groups 3, 4 and 8 was significantly heavier than that of all other groups. Thymic weight was reduced significantly in Groups 2, 3 and 8, as was weight of the bursa of Fabricius in Group 8. The average kidney weight of Group 3 was significantly heavier than that of all other groups and Groups 3 and 8 had larger adrenals than the other groups (Table 6). The force required to break the left femur of ducklings from Groups 2, 3, 4, 6 and 8 was significantly less than that needed to break the femur of control birds (Table 7).

Group 8 had significantly less thymic and splenic lymphoid tissue than other groups. With splenic lymphoid depletion, a corresponding hyperplasia in reticuloendothelial cells was apparent. Vacuolation of hepatocytes was increased in Groups 4, 6 and 8 and salt glands were hypertrophied in Group 8. Ducklings in Groups 3, 4 and 8 had significantly less trabecular bone in the right femur than did control birds (Mitcham, 1985).

The concentration of Na and calcium in the serum was significantly increased and that of chloride and phosphorus decreased in Group 8 as compared to Group 1 on day 28 (Table 5). The concentration of phosphorus in the serum was reduced in Group 7 on day 28 (Table 5). The PCV of Group 4 on day 28 ( $\bar{x} = 0.465$ ,  $SD = 0.146$  liter/liter) and the TP of Group 8 on days 14 ( $\bar{x} = 51.1$ ,  $SD = 5.4$  g/liter) and 28 ( $\bar{x} = 57.1$ ,  $SD = 4.1$  g/liter) were significantly elevated compared to controls ( $\bar{x} = 0.363$ ,  $SD = 0.104$  liter/liter;  $\bar{x} = 44.7$ ,  $SD = 2.6$  g/liter;  $\bar{x} = 41.2$ ,  $SD = 2.1$  g/liter, respectively).

### **Trial III**

During the first 2 wk of the trial, excreta from ducklings in Groups 3 and 3A was

moderately fluid. Those in Groups 2 and 2A had slightly fluid excreta.

On day 14 the concentration of Na in the serum of Groups 3, 3A, 2A and 1A was significantly elevated and the serum chloride concentration of Group 3A ( $\bar{x} = 112.6$ ,  $SD = 3.6$  mmol/liter), was significantly increased, when compared to Group 1 ( $\bar{x} = 107.4$ ,  $SD = 2.3$  mmol/liter). All groups, except Group 1, had significantly elevated levels of serum Mg on day 14.

Groups 1A, 2A and 3A were started on water containing 3,100 ppm Na and 1,300 ppm Mg on day 15. Within 6 hr ducklings in Groups 2A and 3A had extremely fluid excreta, while those in Group 1A had slightly fluid excreta. On day 16 ducklings in Group 3A were inactive. By day 18 ducklings in all three groups huddled together and those in Groups 1A and 3A were severely depressed. On day 19 three ducklings in Group 3A and one in Group 1A died; the surviving ducklings in all three groups were severely depressed, huddled together and shivered. All remaining ducklings were killed and necropsied on day 20.

There was no significant difference in body weight among groups to day 12, but on day 14 ducklings receiving the highest concentrations of salts (Groups 3 and 3A) were lighter than those in other groups (Table 6). On day 20, Group 2 ducklings were significantly larger than ducklings from all of the other groups (Table 8). Groups 1A, 2A and 3A, given the highly saline water starting on day 15, had either grown very little or lost weight and were significantly lighter than all other groups by day 20 (Table 8). Serum Na, osmolality and plasma TP, as well as salt gland and adrenal weight and extent of vascular invasion of the zone of hypertrophy of the femoral epiphyseal cartilage were all increased significantly in Groups 1A, 2A and 3A when compared to control birds. As well, there was hypertrophy of the salt gland and adrenal. Leg length, serum potassium, and weight of the bursa of Fabricius and thymus were reduced, as was the amount of lymphoid tissue in the bursa

TABLE 6. Organ weight, as a percent of total body weight, of mallard ducklings given various types of saline water as their only source of drinking water for 28 days (Trial II).

Group	Organ weight as % of body weight											
	Salt glands		Liver		Thymus		Bursa of Fabricius		Kidney		Adrenal	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1 (control)	0.018 <sup>c</sup>	0.003	3.37 <sup>d</sup>	0.34	0.54 <sup>ab</sup>	0.11	0.16 <sup>a</sup>	0.04	0.58 <sup>b</sup>	0.10	0.005 <sup>b</sup>	0.002
2 (3,000 ppm Na)	0.025 <sup>b</sup>	0.005	3.86 <sup>bcd</sup>	0.55	0.39 <sup>c</sup>	0.09	0.15 <sup>a</sup>	0.05	0.55 <sup>b</sup>	0.06	0.006 <sup>b</sup>	0.001
3 (3,000 ppm Mg)	0.023 <sup>bc</sup>	0.005	4.11 <sup>bc</sup>	0.58	0.37 <sup>c</sup>	0.12	0.10 <sup>ab</sup>	0.03	0.67 <sup>a</sup>	0.11	0.009 <sup>a</sup>	0.003
4 (1,500 ppm Na, 1,500 ppm Mg)	0.021 <sup>bc</sup>	0.002	4.28 <sup>b</sup>	0.52	0.50 <sup>ac</sup>	0.15	0.13 <sup>a</sup>	0.04	0.57 <sup>b</sup>	0.05	0.005 <sup>b</sup>	0.002
5 (1,500 ppm Na, 500 ppm Mg)	0.021 <sup>c</sup>	0.003	3.49 <sup>d</sup>	0.38	0.60 <sup>ab</sup>	0.09	0.15 <sup>a</sup>	0.04	0.55 <sup>b</sup>	0.04	0.005 <sup>b</sup>	0.001
6 (500 ppm Na, 1,500 ppm Mg)	0.019 <sup>c</sup>	0.003	3.38 <sup>bcd</sup>	0.23	0.47 <sup>bc</sup>	0.16	0.15 <sup>a</sup>	0.07	0.56 <sup>b</sup>	0.05	0.005 <sup>b</sup>	0.001
7 (500 ppm Na, 500 ppm Mg)	0.019 <sup>c</sup>	0.003	3.67 <sup>cd</sup>	0.50	0.67 <sup>a</sup>	0.13	0.16 <sup>a</sup>	0.05	0.57 <sup>b</sup>	0.08	0.007 <sup>b</sup>	0.002
8 (3,100 ppm Na, 1,300 ppm Mg)	0.032 <sup>a</sup>	0.005	4.97 <sup>a</sup>	0.45	0.24 <sup>d</sup>	0.05	0.07 <sup>b</sup>	0.02	0.64 <sup>ab</sup>	0.05	0.010 <sup>a</sup>	0.003

<sup>a,b,c,d</sup> Values within columns followed by different superscripts are significantly different ( $P \leq 0.05$ ).

and thymus, and trabecular bone in the femur.

Groups 1A and 3A differed significantly from the control in having elevated concentrations of Mg in the serum (Table 9), together with lower total white blood cell count as a result of a decrease in absolute lymphocyte count. These birds also had less splenic lymphoid tissue and hepatocytic vacuolation, reduced zone of hypertrophy of femoral epiphyseal cartilage and fewer osteoblasts (Mitcham, 1985). Groups 1A and 2A had decreased concentration of phosphorus in the serum (Table 8). Changes found in only one of these three groups included reduced splenic weight and number of osteoblasts (Group 1A) and reduced force required to break the femur (Group 3A). Group 3 differed from the control group in having an increased concentration of calcium in the serum and reduced bursal lymphoid tissue. Group 2 had increased calcium concentration in the serum, and a lower thymic weight.

#### Trial IIIA

The results from this trial were similar to those obtained from the corresponding portion of Trial III and most variables measured showed the same general trends (Mitcham, 1985). However, the ducklings in this trial were not as severely affected, particularly clinically, by the change to the more saline water on day 15 of the trial. Group 3 ducklings adapted least well.

#### DISCUSSION

Mallard ducklings can survive when their only source of water contains significant amounts of Na, Mg and sulfate ions. The solutions used in these trials had specific conductivity within the low to intermediate range of salinity for wetlands (Fig. 1); and were less saline than natural waters found to be lethal for ducklings (Swanson et al., 1984). Much of the salt apparently was excreted through passage of voluminous fluid excreta. This effect occurred rapidly among ducklings given water containing as little as 500 ppm Mg or 1,000

TABLE 7. Force required to break isolated femurs of mallard ducklings given various types of saline water as their only source of drinking water for 28 days (Trial II).

Group	n	Force at breaking point (kg)	
		$\bar{x}$	SD
1 (control)	9	15.1 <sup>a</sup>	2.1
2 (3,000 ppm Na)	9	12.3 <sup>bc</sup>	1.6
3 (3,000 ppm Mg)	8	7.6 <sup>c</sup>	2.0
4 (1,500 ppm Na, 1,500 ppm Mg)	10	11.2 <sup>c</sup>	1.8
5 (1,500 ppm Na, 500 ppm Mg)	10	14.1 <sup>ab</sup>	1.6
6 (500 ppm Na, 1,500 ppm Mg)	10	12.7 <sup>bc</sup>	1.3
7 (500 ppm Na, 500 ppm Mg)	9	13.9 <sup>ab</sup>	1.9
8 (3,100 ppm Na, 1,300 ppm Mg)	9	9.5 <sup>d</sup>	2.0

<sup>a,b,c,d</sup> Values within columns followed by different superscripts are significantly different ( $P \leq 0.05$ ).

ppm Na, and was more prominent in birds drinking water containing  $\text{MgSO}_4$  than in those given  $\text{Na}_2\text{SO}_4$  solutions. The relative contribution of urinary and fecal excretion to this process was not measured, but both were probably active in excretion. Both salts are cathartic (Fingl, 1980) and the Mg ion is largely responsible for this effect in the case of  $\text{MgSO}_4$  (Reichelderfer et al., 1984). The group of ducklings receiving the highest concentration of Mg (Group 3, Trial II) had enlarged kidneys, as did all of the groups given highly saline water at 14 days of age (Trials III and IIIA) suggesting that increased renal excretion occurred. The salt glands became noticeably active within 4 days in ducklings given water containing  $\geq 1,500$  ppm Na. Despite these mechanisms for salt excretion, water containing concentrations of these ions similar to that found in many prairie wetlands had sublethal effects on ducklings under laboratory conditions. The most obvious effects, other than the passage of fluid excreta, were on growth and food consumption. The mechanisms responsible for decreased food consumption and the relationship between food consumption and

TABLE 8. Selected variables measured in mallard ducklings given various types of saline water as their only source of drinking water for 14 days. Some groups were placed on more highly saline water during days 15–20. All variables measured on day 20 except for body weight.

	Group											
	1		1A		2		2A		3		3A	
	control		control		(500 ppm Na, 250 ppm Mg)		(500 ppm Na, 250 ppm Mg)		(1,000 ppm Na, 500 ppm Mg)		(1,000 ppm Na, 500 ppm Mg)	
Salt solution days 0–14			(3,100 ppm Na, 1,300 ppm Mg)		(500 ppm Na, 250 ppm Mg)		(3,100 ppm Na, 1,300 ppm Mg)		(1,000 ppm Na, 500 ppm Mg)		(3,100 ppm Na, 1,300 ppm Mg)	
Salt solution days 15–20												
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Body weight (g)												
Day 14	278 <sup>a</sup>	38 <sup>a</sup>	277 <sup>a</sup>	26	260 <sup>a</sup>	36	294 <sup>a</sup>	35 <sup>a</sup>	261 <sup>ab</sup>	18	244 <sup>b</sup>	53
Day 20	403 <sup>b</sup>	86	262 <sup>d</sup>	31	467 <sup>c</sup>	58	317 <sup>c</sup>	36.9 <sup>c</sup>	407 <sup>b</sup>	32	244 <sup>d</sup>	38
Serum osmolality (mmol/kg)	322 <sup>c</sup>	6	347 <sup>a</sup>	15	319 <sup>c</sup>	4	332 <sup>c</sup>	10	319 <sup>c</sup>	6	353 <sup>a</sup>	13
Total plasma protein (g/liter)	40.4 <sup>c</sup>	2.2	55.4 <sup>ab</sup>	8.6	43 <sup>c</sup>	1.5	53.9 <sup>b</sup>	3.7	42.4	2.5 <sup>c</sup>	58.8 <sup>a</sup>	6.5
Serum electrolytes (mmol/liter)												
Sodium	148.8 <sup>c</sup>	4.8	171.6 <sup>a</sup>	9.2	151.5 <sup>c</sup>	1.4	162.2 <sup>b</sup>	4.3	153.5 <sup>c</sup>	2.3	172.3 <sup>a</sup>	7.8
Magnesium	2.4 <sup>b</sup>	0.4	3.2 <sup>a</sup>	0.9	2.4 <sup>b</sup>	0.6	2.4 <sup>b</sup>	0.6	2.3 <sup>b</sup>	0.7	3.1 <sup>a</sup>	0.5
Potassium	8.2	4.2	5.47	2.0	6.9	1.7	4.6	1.5	6.0	1.7	6.5	2.9
Phosphorus	5.2 <sup>a</sup>	0.9	4.1 <sup>c</sup>	1.4	5.7 <sup>a</sup>	0.8	3.4	0.7	4.9 <sup>ab</sup>	1.4	4.8 <sup>ab</sup>	0.8

<sup>a,b,c,d</sup> Values within rows followed by different superscripts are significantly different ( $P \leq 0.05$ ).

TABLE 9. Concentration of sodium, chloride and magnesium in the serum of mallard ducklings given various types of saline water for 14 days (Trial III).

Group	n	Concentration of element in serum (mmol/liter)					
		Na		Cl		Mg	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1 (control)	8	144.4 <sup>b</sup>	3.2	107.4 <sup>b</sup>	2.3	0.88 <sup>c</sup>	0.15
1A (control)	9	149.9 <sup>a</sup>	2.8	110.6 <sup>ab</sup>	3.6	2.05 <sup>ab</sup>	0.33
2 (500 ppm Na, 250 ppm Mg)	8	148.0 <sup>ab</sup>	1.7	109.8 <sup>ab</sup>	1.1	2.15 <sup>a</sup>	0.43
2A (500 ppm Na, 250 ppm Mg)	10	150.1 <sup>a</sup>	2.1	109.4 <sup>ab</sup>	2.2	1.93 <sup>ab</sup>	0.40
3 (1,000 ppm Na, 500 ppm Mg)	10	150.0 <sup>a</sup>	4.4	107.6 <sup>b</sup>	5.2	2.38 <sup>a</sup>	0.35
3A (1,000 ppm Na, 500 ppm Mg)	10	150.6 <sup>a</sup>	1.7	112.6 <sup>a</sup>	3.6	1.55 <sup>b</sup>	0.35

<sup>a,b,c</sup> Values within columns followed by different superscripts are significantly different ( $P \leq 0.05$ ).

growth are unknown. Paired feeding trials would be required to determine the proportion of the growth depression that was a direct result of inadequate food intake and alternatively, decreased food consumption may have resulted partially from smaller body size in certain groups. Growth depression has been observed in ducklings given solutions of NaCl to drink (Holmes et al., 1961; Ellis et al., 1963; Riggert, 1977; Wink, 1980); in ducklings exposed to natural saline lake water (Swanson et al., 1984); and in chickens given excess dietary Mg (Lee and Britton, 1980; Lee et al., 1980). Feather growth was decreased in ducklings given water containing 1,500 ppm or more of either Na or Mg, and molt was retarded in birds drinking water containing 3,000 ppm of either ion. Swanson et al. (1984) noted retention of down in ducklings exposed to saline lake water. Consumption of water containing 3,000 ppm of Mg resulted in decreased growth of several body parts, reduction in the amount of trabecular bone in the femur, and the bones broke more easily. These birds also had enlarged adrenals and the thymus was decreased in size. The latter changes were probably the result of stress (Freeman, 1971) with corticosteroid-induced lymphoid depletion (Glick, 1957; Claman, 1972; Glick, 1972). Ducklings receiving water containing 3,000 ppm Na had reduced culmen length, thymus weight and bone breaking strength.

The combination of the two salts pro-

duced more complex effects. The group of ducklings receiving the lowest concentration of the two salts (Trial III, Group 2 with 500 ppm Na + 250 ppm Mg) were heavier than control birds. Those receiving 1,500 ppm of each ion were significantly smaller than control birds and had reduced feather growth and bone breaking strength.

Day-old ducklings were able to survive on the most highly saline water tested (3,100 ppm Na, 1,300 ppm Mg, conductivity 15,250  $\mu\text{mhos/cm}$ ), but at the end of the trial weighed less than two-thirds as much as controls. Also, they had reduced feather growth, retarded molting, enlarged adrenals, small lymphoid organs and reduced trabecular bone in their femurs. Additionally, these birds also had several chemical abnormalities (elevated serum Na and calcium, and plasma TP; decreased serum phosphorus and chloride). The elevation in Na may have been a direct result of inability to excrete sufficient Na. Elevated plasma calcium levels have been documented in cortisol treated birds (Siegel, 1968); thus the elevated calcium concentration could be stress-induced. Renal excretion of chloride is increased when excess Mg is being excreted by the kidney (Mordes and Wacker, 1978), which might explain the low chloride in these birds. Phosphorus depletion can occur also when high levels of Mg are being consumed (Lotz et al., 1968).

Harvey et al. (1981) found that salt-stressed birds had decreased hepatic weight

in proportion to body weight. Salt-stressed birds that were consuming less food should have depleted hepatic glycogen stores as a result of attempting to maintain glucose homeostasis (Davison and Langslow, 1975). Ducklings in Groups 4, 6 and 8 had increased hepatic weight and vacuolation, which is an index of hepatic glycogen as compared with the control group. Holmes et al. (1963) reported enlargement of the liver with increased hepatic glycogen in domestic ducks given hypertonic NaCl, and interpreted this to be the result of enhanced glucocorticoid activity.

The gross and histologic bone lesions reported by Lee et al. (1980) in chicks fed high levels of Mg (0.3, 0.5 and 0.9% Mg added to the ration) were not observed in ducklings given the highest concentration of Mg. However, a decrease in femoral trabecular bone was apparent in Groups 3, 4 and 8 in Trial II. This may have been the result of inadequate food consumption, malabsorption, or decreased retention of nutrients essential for bone growth because of catharsis (Lee et al., 1980), corticosteroid-induced bone loss (Peck et al., 1984), or a combination of these factors. In these trials the force required to break femurs of ducklings may be only another measure of duckling growth. It is not surprising that the smaller femurs from lighter ducklings broke with the application of less force than that required to break femurs from the heavier control ducks. For bone breaking strength to be a true measure of the integrity of bone, it would have to be considered in conjunction with other variables such as bone ash, bone calcium, phosphorus and Mg, and bone length, weight and volume.

The ducklings in Trials III and IIIA did not adapt well to a change to more highly saline water at 2 wk of age. The birds stopped growing, became depressed and several in Trial III died. This was unexpected since day-old ducklings (Group 8, Trial II) survived on the same water, although they grew poorly. Schmidt-Nielsen and Kim (1964) suggested that ducklings

can better adapt to saline water if the concentration of salt is increased gradually, and that exposure to low levels of salt "prime" the salt glands. This effect was not evident in Trials III and IIIA. However, the concentration of Na (500, 1,000 ppm) given may have been inadequate to have a "priming" effect, because it did not cause obvious salt gland activity in earlier trials and the salt glands of Groups 2 and 3 in Trial III were not significantly larger than those of control birds.

Ducklings placed on the highly saline water had enlarged salt glands, with microscopic evidence of hypertrophy as well as enlarged kidneys at day 20, but it appeared that they were unable to compensate completely for the increased ion intake. All of the groups had increased serum osmolality, and five of the six groups had elevated serum concentrations of either Na and/or Mg. Less consistent hematologic changes were decreased serum phosphorus, chloride, and potassium, and increased TP and PCV, which occurred in three groups each. Ducklings in Trial IIIA were not affected as severely as those in Trial III. However, most measured variables showed the same general trend. The increased concentration of calcium in serum and the decreased size of lymphoid organs seen in all the groups, plus the increased adrenal weight and adrenal hypertrophy seen in birds in Trial III, are expected changes in stressed birds. The decreased total white blood cell and lymphocyte count in Groups 1A and 3A of Trial III were consistent with corticosteroid-induced leukopenia and lymphopenia (Siegel, 1968). The bone lesions in ducklings in Groups 1A, 2A and 3A in Trial III resembled changes described in starved or protein-deficient animals (Himes, 1978) and/or in corticosteroid-induced bone loss (Peck et al., 1984), and may have resulted from combined acute stress and malnutrition.

Swanson et al. (1984) found that saline lake water diluted to a conductivity of 17,000  $\mu$ mhos/cm caused growth depres-

sion in mallard ducklings. Our results indicate that much lower concentrations of the salts commonly found in prairie wetlands have sublethal effects on ducklings. These effects would be very difficult to detect in the field in birds of unknown age. Many of the changes are non-specific, could result from other stressors, and may not be present in ducklings exposed to low concentrations of salt.

Several factors need to be considered in extrapolating our results to waterfowl in natural habitat. Wild ducklings may be able to avoid toxic effects of saline water through selection of less saline areas or freshwater sources within a saline marsh (Swanson et al., 1984). In a natural saline environment the food consumed by ducklings might contain a higher concentration of salt than that used in the laboratory, so the overall salt intake could be greater in the wild. Ducklings in the laboratory are exposed to a different range of stressors than are wild ducklings. Thus, it is impossible to predict how saline stressed ducklings would respond to various combinations of environmental factors such as severe weather, food shortage, predator activity and parasitism.

The information from this study and that of Swanson et al. (1984) indicate that saline wetlands have marked limitations for duckling production and may require special management techniques such as provision of or enhancement of freshwater drinking areas to achieve maximal waterfowl productivity.

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