DRINKING RESPONSES OF THE RED CROSSBILL (*LOXIA CURVIROSTRA*) TO SOLUTIONS OF NaCl, $MgCl_2$, AND $CaCl_2$

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The abilities of various land birds to drink saline solutions and obtain physiologically useful water from them have been studied in the past two decades. These studies have contributed to an understanding of water and salt metabolism of birds from diverse habitats, and in particular they have contributed to an understanding of the adaptive specializations of birds that inhabit salt marshes and desertic regions (for reviews see Bartholomew and Cade, 1963; Cade, 1964). Until now these studies have dealt only with metabolism of NaCl and sea water (for example see Bartholomew and Cade, 1963; Cade, 1964; Dawson et al., 1965; Harriman and Kare, 1966; MacMillen and Snelling, 1966; MacMillen and Trost, 1966; Smyth and Bartholomew, 1966; Greenwald et al., 1967; Harriman and Nance, 1968; Poulson, 1969). The results with sea water have been interpreted in terms of the osmotic effects of NaCl alone. Sea water contains significant concentrations of Ca^{++} and Mg^{++}—10.4 mM/l and 54.7 mM/l respectively (Barnes, 1954)—and saline desertic waters frequently contain appreciable amounts of Ca^{++} and Mg^{++} (Margat, 1961; Williams and Siebert, 1963). It is important to know how birds drinking such waters may be affected by these biologically active metals, and what their tolerance limits are for utilizing waters containing them. Therefore I studied the abilities of the Red Crossbill, *Loxia curvirostra*, to drink various concentrations of NaCl, $CaCl_2$, and $MgCl_2$, and to discriminate between different concentrations of these salts.

The Red Crossbill inhabits coniferous forests and does not normally encounter highly saline waters in the wild. It was chosen for study because it was available and easily maintained in captivity, its metabolism of NaCl has already been studied (Dawson et al., 1965), and because its habit of eating salt in nature has raised the question of whether in so doing it obtains some element that it is unable to obtain sufficiently from its food (Dawson et al., 1965).

Methods

The 33 Red Crossbills that were employed in this study were derived partly from a breeding colony maintained at the University of Michigan which originated from birds of the subspecies *L. c. sitkensis*. Seven of the birds were wild birds captured in the Upper Peninsula of Michigan in February 1968 and were also *L. c. sitkensis*.

For measuring rates of fluid consumption, birds were held singly in wire cages measuring 30.5 cm (12 inches) on a side, equipped with a drinker consisting of a graduated cylinder with an L-shaped spout similar to the one described by Bartholomew and Dawson (1954). Fluid levels were read to the nearest 0.5 ml at 24- to 48-hour intervals, and the differences were corrected for evaporation by a similar drinker mounted near the cages. Some crossbills had a tendency to scatter fluid around the cage by shaking their heads after taking fluid into the beak; this was most marked in birds subjected to the higher concentrations of salts. In these instances consumption of fluid could not be measured.

In tests of preference between two different salt concentrations, crossbills were housed in groups of 6 to 8 in wire cages measuring 122 by 61 by 61 cm. Four drinkers designated A, B, C, and D arranged radially around a single ring-stand were placed in the center of the cage. Two of the drinkers contained one of the solutions being tested, and the other two drinkers contained the alternative test solution. The fluid levels in all the drinkers were recorded at 24-hour intervals. After the fluid levels were recorded, the drinkers were removed from all the cages, emptied, cleaned, and intermixed. Letters A, B, C, and D were then drawn blindly from a bag. The order in which these letters were drawn from the bag determined the order in which drinkers bearing the corresponding letters were replaced on the ring-stand. Cards bearing the designations of the respective test solutions to be used were drawn at random and aligned with the previously drawn letters according to the sequence in which they were drawn. This determined which drinkers held which solution. This procedure was an attempt to randomize the distribution of the drinkers with respect to their contents and their positions in the cages in order to eliminate the effects of crossbills that might prefer to drink at a particular drinker or position in the cage regardless of the contents of the drinker, as Dawson et al. (1965) noted.

During experimental runs, crossbills were fed shelled raw pine nuts and whole sunflower seeds, or pine nuts alone. Pine nuts as fed to the crossbills contained the following quantities of metals: Mg^{++}, 0.39 mEq/g; Ca^{++}, 0.0064 mEq/g; Na^{+}, 0.0321 mEq/g; K^{+}, 0.205 mEq/g. Sunflower seed kernels (the birds discarded the seed shell when eating) contained the following quantities of metals: Mg^{++}, 0.33 mEq/g; Ca^{++}, 0.0117 mEq/g; Na^{+}, $<$0.0106 mEq/g; K^{+}, 0.170 mEq/g. These values were determined by digesting a ground sample of food in hot concentrated nitric acid and determining ion content of the filtrate by flame photometry. At other times the birds were maintained on a diet of mixed birdseed, pine nuts, and sunflower seeds, and were supplied with cuttlebone, rock salt, or both.

Temperatures and relative humidities were recorded continuously during experiments with a Bendix recording hygro-thermograph. The birds were subjected to the normal photoperiod of Ann Arbor, Michigan, latitude 42°.

Results

Drinking of salt solutions.—The initial body weight of crossbills during measurements of consumption of salt solutions was 30.4 ± 3.6 g ($\overline{X}$ ± SD). Table 1 summarizes experimental conditions. The ambient temperature varied between a daily maximum of 22.0 ± 1.3°C and a daily minimum of 20.5 ± 0.9°C ($\overline{X}$ ± SD). Figure 1 presents the data on consumption of various concentrations of NaCl and accompanying

TABLE 1
SUMMARY OF EXPERIMENTAL CONDITIONS FOR RED CROSSBILLS DRINKING SALT SOLUTIONS

Freezing point depression of solution[1], °C	Date of experiments	Relative humidity, per cent	
		Daily maximum $\bar{X} \pm SD$	Daily minimum $\bar{X} \pm SD$
0.09	14–22 Sep.	63 ± 13	45 ± 6
0.18	27 Sep.–6 Oct.	64 ± 10	49 ± 4
0.26	20–27 Oct.	51 ± 6	38 ± 2
0.35	9–17 Nov.	37 ± 11	26 ± 9
0.43	6–14 Dec.	44 ± 8	34 ± 5
0.52	8–17 Jan.	16 ± 6	11 ± 4
0.61	30 Jan.–5 Feb.	39 ± 17	26 ± 7

[1] Solutions of NaCl, $CaCl_2$, and $MgCl_2$.

changes in body weight. Birds maintained body weight when drinking all the NaCl solutions presented them. At concentrations of 100 mM and higher, some crossbills scattered fluid around the cage, making determination of their consumption impossible. With each increase of concentration, more birds tended to throw the fluid about; and in the group subjected to 175 mM NaCl, one of the six birds did not drink at all. This bird lost weight at a rate of 2.5 g/day. Those birds that did drink and that yielded reliable figures for consumption showed a tendency to drink less fluid as the concentration increased.

Figure 2 presents comparable data on consumption of $CaCl_2$ solutions. At a concentration of 90 mM, 2 of 6 birds did not drink at all; and at 107 mM, 4 of 7 birds did not drink. At 125 mM, 3 of 7 birds did not drink, while 2 of those that did drink scattered fluid around the cage. The birds that drank were able to maintain their weight drinking concentrations up to 107 mM $CaCl_2$, but at 125 mM, all the birds lost weight. (Weight losses of the few birds drinking 90 and 107 mM $CaCl_2$ are not statistically significant.)

Figure 3 presents data on consumption of $MgCl_2$. Crossbills were unable to maintain body weight when drinking 99 mM and 117 mM $MgCl_2$. At 99 mM and higher, many of the birds scattered fluid, but all appeared to drink at least some of the fluid provided them, unlike the birds that did not drink when given osmotically comparable concentrations of $CaCl_2$. Those crossbills that gave reliable data showed a tendency to reduce fluid intake with increasing concentrations above 68 mM $MgCl_2$.

Discrimination between distilled water and salt solutions.—Table 2 shows the results of preference tests on crossbills given a choice between distilled water and different concentrations of various salt solu-

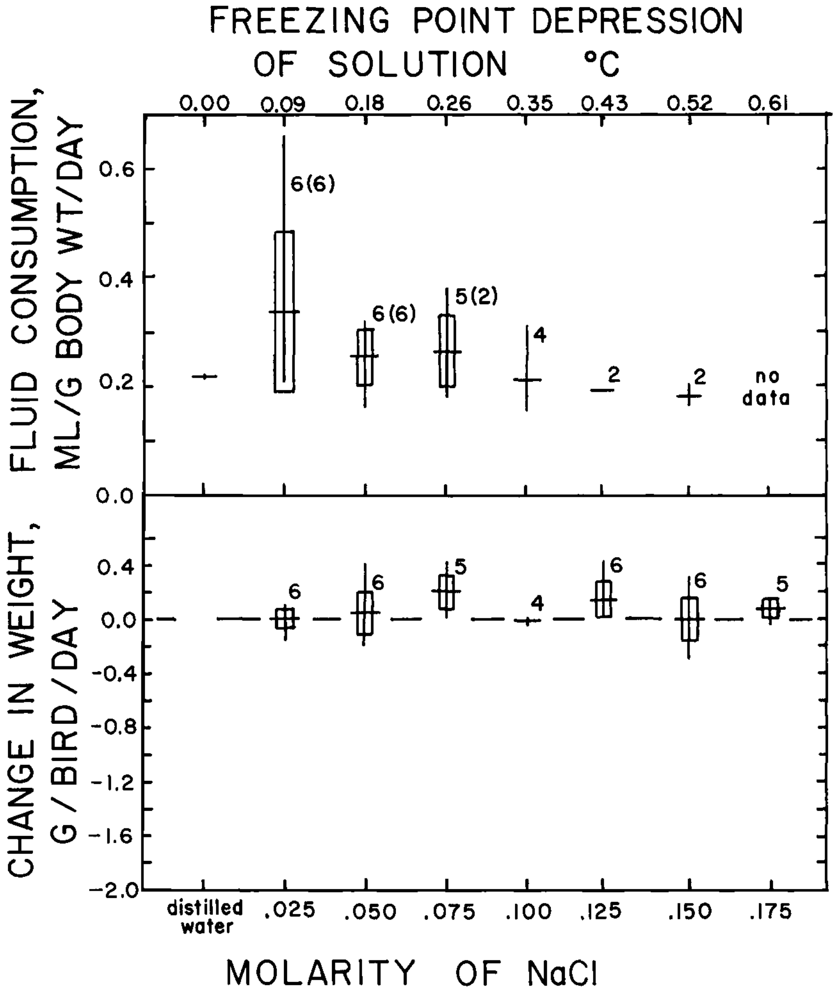

Figure 1. Fluid consumption and attendant changes of body weight of Red Crossbills drinking sodium chloride solutions. The vertical line is the range, horizontal line the mean, and the rectangle encloses the mean ± 2 SE. The numeral is sample size (number of birds), and the number in parentheses indicates the number of birds in the sample that were molting. The value for consumption of distilled water is from Dawson et al. (1965).

tions. In every test but the one involving 10 mM NaCl birds drank more of the distilled water than of the salt solution. (The crossbills did not show a statistically significant preference for the 10 mM NaCl.) This tendency to reject the salt solution in favor of the distilled water is undoubtedly a significant one, although the difference from the

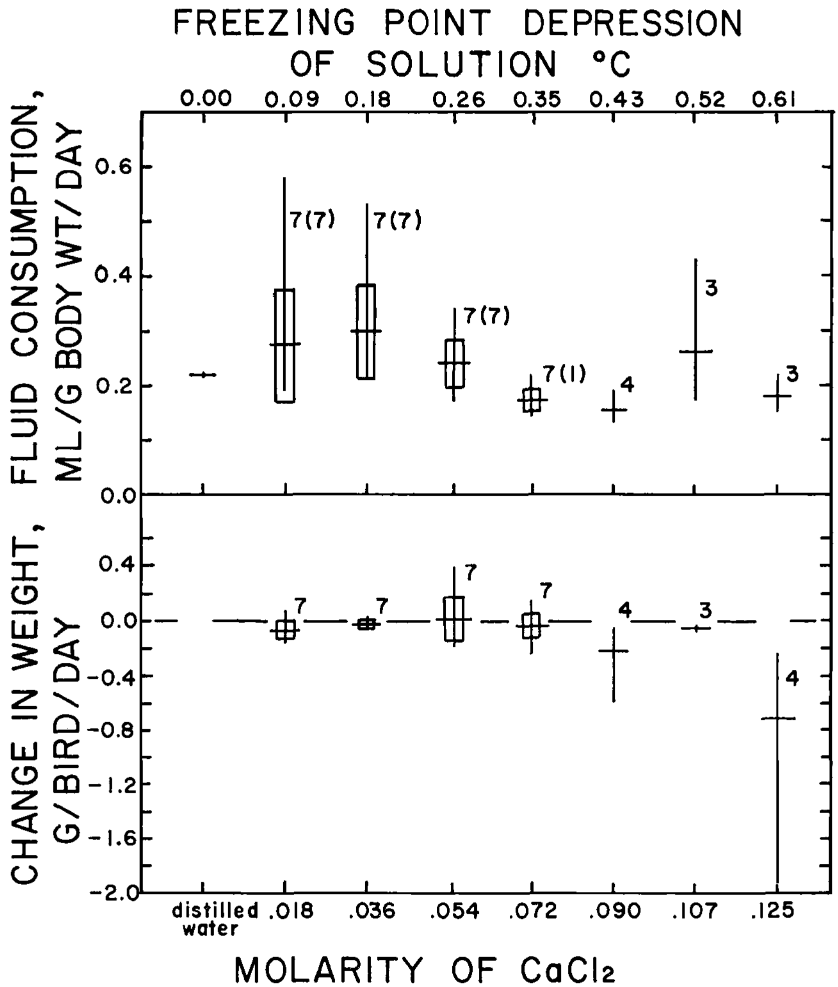

Figure 2. Fluid consumption and attendant changes of body weight of Red Crossbills drinking calcium chloride solutions. Symbolism same as in Figure 1.

expected value under the null hypothesis in many instances is not statistically significant. Solutions of $CaCl_2$ were rejected more readily in lower concentration than were the other salt solutions. Solutions of $MgCl_2$ were more strongly rejected than solutions of NaCl. The three separate runs involving 17 mM $MgCl_2$ appear to show varying degrees of preference for distilled water among the three groups of crossbills.

Discrimination between salt solutions of different concentration.—Three tests were performed on the ability of the crossbills to discriminate

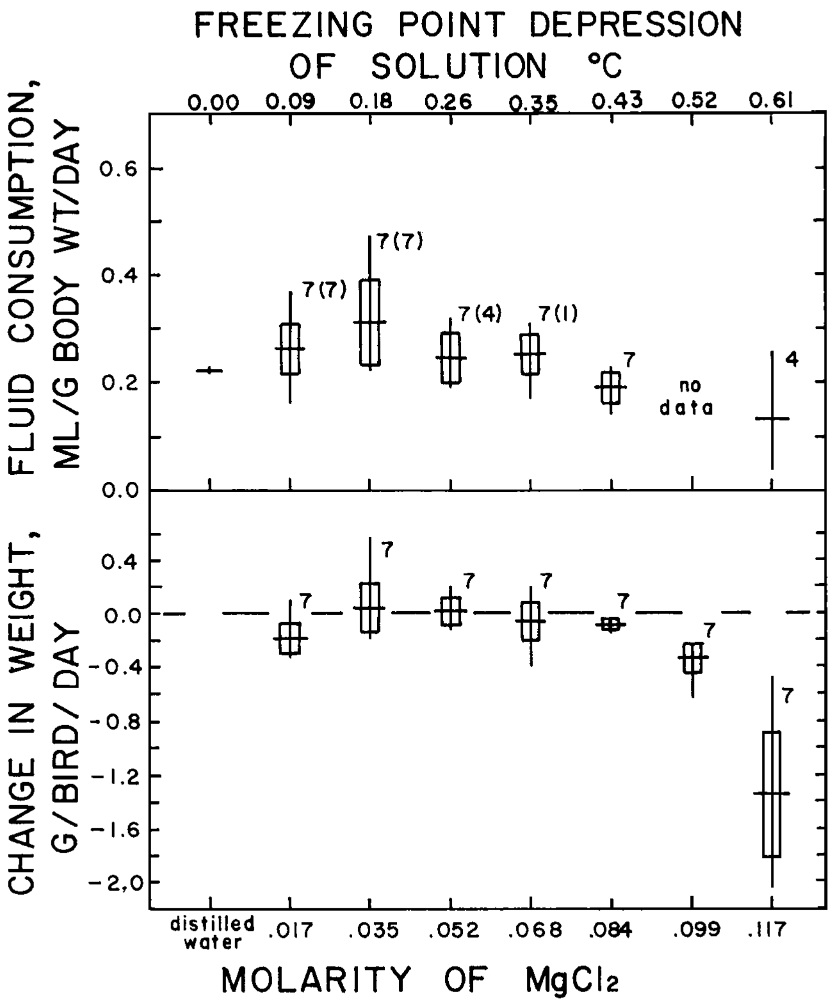

Figure 3. Fluid consumption and attendant changes of body weight of Red Crossbills drinking magnesium chloride solutions. Symbolism same as in Figure 1.

between two slightly different concentrations of $MgCl_2$ or NaCl (Table 2). The results indicate that the crossbills drank less of the higher salt concentration in each test, although the differences are not statistically significant.

Discrimination between solutions of NaCl and of mixtures of salts.—Because the saline waters that wild birds usually encounter contain varying amounts of Mg^{++} or Ca^{++} or both together with Na^{+}, I tested the responses of crossbills given choices between pure solutions of NaCl and solutions containing varying amounts of $CaCl_2$ or $MgCl_2$ with NaCl. The results are shown in Table 2. There were no marked preferences except when 25 mM NaCl was tested against a mixture of 25 mM NaCl and 17 mM $MgCl_2$. In this test, the birds drank significantly more of the pure NaCl solution. In four of the five instances in which the mixture had the same or higher osmosity than the pure NaCl solution, the birds drank slightly more of the pure NaCl solution. The exception was the test comparing 25 mM NaCl against 13 mM NaCl with 8 mM $MgCl_2$, where no difference in consumption occurred. Mixing of salts thus had no marked effect on the performance of the birds.

DISCUSSION

Drinking of salt solutions.—My data on consumption of NaCl solutions supplement the findings of Dawson et al (1965), who reported that the highest concentration of NaCl Red Crossbills could tolerate was near 200 mM. They also noted that some of the crossbills reduced their fluid intake, or did not drink, when the NaCl solutions exceeded about 250 mM. Some of the crossbills in my study scattered fluid about the cage by shaking the head after dipping the beak in the drinker, seemingly as a rejection response to the solution. This tendency first appeared at 100 mM NaCl, and became more intense with increasing concentration. Nevertheless, all the birds that continued to drink maintained body weight over the period of 6 to 8 days of each test.

The data on consumption of $CaCl_2$ and $MgCl_2$ indicate important qualitative differences. The tolerance limit for $CaCl_2$ is near 125 mM (osmotically equal to 175 mM NaCl), whereas for $MgCl_2$ it is between 99 and 117 mM (osmotically equal to 150 and 175 mM NaCl). Both groups showed a tendency to reduce intake of $CaCl_2$ and $MgCl_2$ as concentration increased, and at 107 mM $CaCl_2$ (osmotically equal to 150 mM NaCl) more than half of the birds did not drink, though none of those given $MgCl_2$ definitely stopped drinking.

The tolerance limits for $CaCl_2$ and $MgCl_2$ as determined in these experiments do not necessarily reflect limits of physiological tolerance of the Red Crossbill to these solutions were the birds to consume larger volumes. But as mean consumptions of osmotically equal solutions were nearly the same for all three salts, whereas mean weight losses varied significantly, apparently the three salts have different physiological effects other than those associated with osmotic concentration alone. The crossbills may be responding to differences of taste. In the case of Mg^{++}, perhaps absorption of water and nutrients in the gut is hindered by a purgative action. The droppings of the birds subjected to the higher concentrations of $MgCl_2$ were often watery, and it has been

TABLE 2
RESULT OF DRINKING PREFERENCE TESTS

Regimen[1]	No. of trials	No. of birds per trial	Dates of trials	Mean consumption of solution with lesser osmotic concentration ml/(bird day)	Mean consumption of solution with greater osmotic concentration ml/(bird day)	P, two-tailed *t*-test
Dist. H_2O vs. 10 mM NaCl	9	7	9 Dec.–10 Jan.	3.24	3.91	>0.40
Dist. H_2O vs. 7 mM $CaCl_2$	7	7	26 Sep.–6 Oct.	5.60	3.50	<0.05
Dist. H_2O vs. 7 mM $MgCl_2$	8	7 or 8	9–20 Aug.	5.14	3.67	>0.20
Dist. H_2O vs. 12 mM $CaCl_2$	8	8	26 Sep.–6 Oct.	6.59	1.43	<0.02
Dist. H_2O vs. 12 mM $MgCl_2$	8	8 or 9	9–20 Aug.	4.59	4.20	>0.60
Dist. H_2O vs. 25 mM NaCl	6	7	9 Mar.–7 May	3.53	2.93	>0.20
Dist. H_2O vs. 18 mM $CaCl_2$	10	7	26 Sep.–6 Oct.	5.54	2.34	<0.02
Dist. H_2O vs. 17 mM $MgCl_2$	6	8	22–28 July	5.33	3.03	>0.10
"	8	7	9–20 Aug.	3.55	3.09	>0.40
"	5	7	3–11 Sep.	5.86	3.66	<0.05
Dist. H_2O vs. 35 mM $MgCl_2$	6	9	22–28 July	5.40	1.28	<0.01
Dist. H_2O vs. 52 mM $MgCl_2$	6	7	22–28 July	4.00	1.75	<0.05
12 mM $MgCl_2$ vs. 17 mM $MgCl_2$	6	8	3–11 Sep.	4.48	4.22	>0.60
17 mM $MgCl_2$ vs. 22 mM $MgCl_2$	9	7	3–11 Sep.	3.84	3.50	>0.20
25 mM NaCl vs. 50 mM NaCl	9	7	4–17 Nov.	5.22	2.96	>0.05
25 mM NaCl vs. 13 mM NaCl and 7 mM $CaCl_2$[2]	5	7	8–15 May	3.82	2.98[3]	>0.10
25 mM NaCl vs. 13 mM NaCl and 8 mM $MgCl_2$[2]	9	7	9 Dec.–10 Jan.	3.24	3.30[3]	>0.80
25 mM NaCl vs. 25 mM NaCl and 7 mM $CaCl_2$[4]	6	7	9 Mar.–7 May	3.77	3.33	>0.20
25 mM NaCl vs. 25 mM NaCl and 8 mM $MgCl_2$[4]	9	7	9 Dec.–10 Jan.	3.74	3.00	>0.10
25 mM NaCl vs. 25 mM NaCl and 17 mM $MgCl_2$[4]	10	8	4–17 Nov.	4.86	2.35	<0.01
50 mM NaCl vs. 13 mM NaCl and 8 mM $MgCl_2$[5]	9	7	4–17 Nov.	4.52	3.23	>0.10

[1] Tests involving solutions of similar osmosity are grouped together.
[2] Mixture of salts isosmotic to NaCl solution.
[3] Consumption of the mixture of salts.
[4] Mixture of salts hyperosmotic to NaCl solution.
[5] Mixture of salts hypoosmotic to NaCl solution.

noted that high Mg levels in the food of laboratory rats resulted in diarrhea (Cunningham, 1933). It is also conceivable that the crossbills are unable to produce urine concentrated enough in Mg to permit neutral water balance when drinking solutions of $MgCl_2$ of 99 mM or higher.

The loss of weight by crossbills drinking solutions of $CaCl_2$ may have resulted from inadequate fluid consumption owing to the rejection response of the birds, or from some other physiological disturbance.

Salinity discrimination.—The results for NaCl indicate no significant preference for distilled water or 10 mM or 25 mM NaCl. Dawson et al. (1965) noted no preference in Red Crossbills for either distilled water or 100 mM NaCl, but they found that the crossbills would drink distilled water to the virtual exclusion of 200 mM NaCl. In contrast, the crossbills in my study preferred distilled water to $CaCl_2$ solutions of 7 mM and higher, and to $MgCl_2$ solutions of 17 mM and higher. These qualitative differences in preference for distilled water over the different salt solutions of similar osmosity clearly indicate that the birds are not responding to osmotic effects alone. It is noteworthy that crossbills showed no statistically significant preference for any of the salt solutions over distilled water.

Mixing $CaCl_2$ and $MgCl_2$ into solutions of NaCl had no marked effect on the preference of crossbills for the solutions. On the average, the crossbills drank more of the solution that was osmotically less concentrated, whether or not it contained $CaCl_2$ or $MgCl_2$.

The pattern of discrimination of the Red Crossbill between salt solutions and distilled water is qualitatively similar to that of other avian species that have been tested on solutions of NaCl and various dilutions of sea water. These show indifference between distilled water and low salt concentrations changing to preference for distilled water as the salinity increases (Bartholomew and Cade, 1958; Bartholomew and MacMillen, 1960; Cade et al., 1965; Harriman and Kare, 1966; MacMillen and Snelling, 1966; MacMillen and Trost, 1966; Harriman, 1967; Harriman and Nance, 1968). A possible exception is the Budgerigar *Melopsittacus undulatus* that prefers 50 mM NaCl to distilled water (Cade, 1964). Because the Red Crossbill did not differ markedly from species lacking the habit of eating salt, it appears that the salt-eating habit of this bird is not manifested in its drinking of salt solutions.

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Summary

The drinking responses of 33 Red Crossbills provided with various concentrations of NaCl, $CaCl_2$, and $MgCl_2$ were monitored to understand better how birds in general might be affected by Ca^{++} and Mg^{++} in saline desertic waters and brackish waters utilized for drinking, and how the Red Crossbill in particular might respond in light of its well known habit of eating salt.

Birds that were given solutions of NaCl, $CaCl_2$, or $MgCl_2$ as drinking water tended to reduce their fluid intake as the concentrations of the salt increased. The highest concentrations of $CaCl_2$ and $MgCl_2$ which crossbills could drink and still maintain weight were between 107 and 125 mM, and about 99 mM, respectively. Crossbills can maintain weight when drinking NaCl concentrations in excess of 175 mM, osmotically more concentrated than the highest concentrations of $CaCl_2$ and $MgCl_2$ tolerated.

Crossbills given distilled water and solutions of NaCl, $CaCl_2$, or $MgCl_2$ tended to drink more of the distilled water. They drank about equal amount of distilled water and 10 or 25 mM NaCl. Crossbills drank significantly more distilled water than $CaCl_2$ or $MgCl_2$ when the concentration equalled or exceeded 7 mM and 17 mM, respectively.

Crossbills did not prefer any salt solution over distilled water. When given two different concentrations of salt, crossbills tended to drink more of the less concentrated solution; and mixing NaCl with $CaCl_2$ or $MgCl_2$ did not markedly affect this response.

The results indicate the Na^+, Mg^{++}, and Ca^{++} have qualitatively different physiological effects on the crossbills, and that the birds respond to the different chloride salts of these metals in a way which indicates that they perceive a qualitative difference independent of the osmotic concentration of the salts.

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