

# SALT TOLERANCE OF *RUPPIA MARITIMA* IN LAKES OF HIGH MAGNESIUM SULPHATE CONTENT<sup>1</sup>

ELIZABETH MCKAY

## Introduction

The ability of halophytes to develop under conditions which would mean death to most vegetation is well known. Salt marsh plants, growing where sodium chloride is the chief constituent of the soil solution and where salt concentrations usually vary from 1 to 6 per cent., are the most common halophytes. Much less numerous than the salt marshes are the lakes whose chief constituent is magnesium sulphate, a compound toxic to many plants in any but very dilute concentrations. Although the plants of the two types of lakes are to some extent the same, very little work has been done on those of the magnesium sulphate lakes. In view of the common toxicity of magnesium salts to plants and the scarcity of information concerning the physiological relations of halophytes, it has seemed worth while to investigate the vegetation of certain lakes lying in north central Washington and adjacent British Columbia which are unusual in having a very high proportion of magnesium sulphate in the salt content and in supporting seed plants at salt concentrations as high as 25 or 26 per cent. Studies were made on three lakes lying in the eastern foothills of the Cascade Mountains in the northern part of Okanogan County, Washington, and British Columbia, in one of which *Ruppia maritima* L. grows abundantly, completely submerged in the salty water of the lake.

The first of these, known locally as Epsom Lake and designated in this paper as no. 1, lies about 4 miles northwest of the town of Oroville, in northern Washington, about midway between Lake Osoyoos and the Similkameen River. The geology of the region was described by JENKINS (10). ST. JOHN and COURTNEY (35) listed the phanerogams growing in the lake and on the walls of the surrounding basin. They report the banks to be of practically pure salt, in which the plants were apparently rooted. Analyses of salts taken from the surface of the water gave a content of 99.64 per cent. of  $\text{MgSO}_4$ .

The lake lies at an elevation of about 2000 feet, with walls of the basin reaching several hundred feet higher. The lake itself, which drains an area of about one square mile, is, when full, approximately four acres in extent and 30 feet deep. There is no outlet and drainage waters remain until evaporated during the dry summer season, when the lake is reduced to a series of small

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pools and the bed becomes white and crystalline in appearance. The surrounding vegetation is sparse.

The second lake studied, designated no. 2, lies several miles north of no. 1, in British Columbia, on a ridge of Kruger Mountain, about 8 miles from the town of Osoyoos. It is known locally as Spotted Lake, owing probably to its spotted appearance when summer evaporation has reduced it to a series of round pools. Like no. 1, Spotted Lake is the drainage basin for the surrounding hills and has no outlet. It is considerably larger than no. 1, being about one-half mile long and 300 yards wide. The elevation is somewhat greater than that of lake no. 1 but the walls of the basin are not so steep. Vegetation is even more sparse than around lake no. 1 and no sign of plant life is found in the waters of the lake except a few algae. The lake contracts to a white bed with round pools of saturated water after a dry period (10).

Lake no. 3 lies a few miles to the south of no. 2 and at a greater elevation on Kruger Mountain. It is much larger and considerably deeper than the others. Although it is much less concentrated than the others there is no evidence of plant or animal life and no growth of plants for some distance from the water's edge. The soil of the banks is colored and sandy, and is not so white and crystalline in appearance as that of the other two lakes.

#### Literature review

Until recently work on halophytes has been concerned chiefly with their ecological relations. SCHIMPER (34) described halophytes as those plants which are capable, owing to xeromorphic adaptations of physiological and anatomical characters, of living under conditions of physiological dryness where water, although present, is not readily available to the plant. FITTING (7) found halophytes able to develop osmotic pressures of sufficient value to overcome the resistance to absorption of greatly concentrated soil solutions. DELF (4) found the transpiration rates of *Salicornia annua* and *Suaeda maritima* comparable to mesophytes such as *Vicia faba*, the halophytes having no stomatal modifications to reduce transpiration. YAPP (40) described the xeromorphic character of *Spiraea ulmaria*, showing the development of characters usual in xerophytes. Apparently halophytism may be expressed in either physiological or morphological adaptations or both.

OSTERHOUT (15), in working with marine plants, found that each of the salts of sea water is poisonous when alone but that the mixture, as found in sea water, gives a "physiologically balanced" solution which the plant is capable of using. The idea of balance of solution was soon extended to include fresh water and terrestrial plants (16). Since then extensive investigations by OSTERHOUT (17, 18, 19, 20, 21, 22, 23, 24, 25), TRUE and

BARTLETT (37, 38), RABER (27, 28, 29, 30, 31), SCARTH (33), and many others have laid the basis for the theory of the antagonism of ions, have secured much information concerning the antagonistic relations of various ions, and have added greatly to our knowledge of the effects of acids, alkalies, and anions and cations of different valences on the permeability of plant membranes. OSTERHOUT (26) and HOAGLAND and DAVIS (9) have given analyses of the cell sap of *Valonia* and *Nitella* respectively, as compared with the water in which they were growing.

Recently consideration has been given to the salt requirements of salt marsh plants. Analyses made by ZELLNER (41) of *Salicornia herbacea*, *Suaeda salsa*, *Scorzonera parviflora*, *Plantago maritima*, *Aster tripolium*, and *Erythraea tinariaefolia* growing on soils containing about 3 per cent. of soluble salts showed all of the plants to contain large amounts of Na, Cl, and  $\text{SO}_4$ . Over 20 per cent. of the dry weight was found to be ash in *S. herbacea* and *S. salsa*. These results are in accord with those of KILLIAN (11), who concluded, after studying plants growing in soils rich in salt, that NaCl may be in even greater concentration in the plants than in the soil, and that different species growing in the same soil may show great variation in the salt accumulated in the plant. MONTFORT and BRANDRUP (12, 13, 14) concluded that the resistance of plants to high salt content varies at different stages of development of the plant as well as with different concentrations of salt. The pH of the soil solution plays a minor rôle, but the proportion of Cl and  $\text{SO}_4$  is important in determining plant distribution. RIKHTER (32) likewise found balance important, in that plants can tolerate much greater concentrations of balanced solutions than of pure salts. He groups plants able to tolerate high content of salt into two types with regard to the method of developing the internal osmotic pressures necessary to overcome the external pressures. Such plants as *Helianthus*, *Triticum*, *Amaranthus*, and *Artemisia* increase their own supply of osmotically active materials, while halophytes such as *Salsola*, *Suaeda*, and *Salicornia* can accumulate salts from the outside medium without harm to themselves. Plants of the first group are characterized by a high impermeability of the roots, so that the power to exclude the salts determines the limit of concentration which the plants can endure. These workers were interested in plants growing in salt concentrations close to that of sea water. BRIGHENTI (2) describes salt marshes of the province of Ferrara, Italy, near the Adriatic Sea. These have a depth of 50–90 cm. In summer the NaCl reaches 41 per cent. concentration, although this is diluted in the winter by rain and by the Po di Volano. No phanerogams grow in the marshes but some diatoms are found, and the algae *Ulva latissima* and *Chaetomorpha crassa* are abundant.

FLOWERS (8) describes the vegetation of the Great Salt Lake region, listing the plant associations of the lake, the dunes, salt marshes, etc., and giving ecological data including the salt content of soils and soil waters. He compiles tables of analyses made of Great Salt Lake water at different periods from 1877 to 1910. The salt content varied from 13.79 to 27.72 per cent. during the period. NaCl is the principal constituent. The Cl analyses of the total salt vary from 55.1 to 56.54 per cent. Na varies from 32.81 to 34.65 per cent.;  $\text{SO}_4$  from 5.95 to 6.82 per cent.; and Mg from 0.57 to 3.18 per cent. The main body of the lake supports no vegetation except blue-green algae, of which *Aphanothece utahensis* is at present dominant. Three species of animals are listed by FLOWERS, of which one is *Artemia gracilis*. *Ruppia maritima* is reported as growing in Willard Spur, a part of Bear River Bay, where the strong brines of the main body of the lake are considerably diluted.

While magnesium in minute amounts is essential for all plants, to many it is toxic in very slightly increased amounts. Considerable variation is shown by different plants. EILERS (5), investigating the physiology of nutrition of the alga *Stichococcus bacillaris*, found that this was susceptible to relatively small amounts of magnesium salts. CARMIN (3) grew very young wheat seedlings in solutions of 0.0005 to 0.0150 M solutions of  $\text{MgSO}_4$ . When growth of the roots and tops was plotted against the concentration of  $\text{MgSO}_4$ , the curves showed lessening of the growth with greater concentration. The salt was more toxic to the roots than to the tops. TRELEASE and TRELEASE (36) detected injury due to magnesium toxicity in seedlings of wheat, oats, rye, and barley. The severity of injury increased as the Mg/Ca ratio increased from 1 to 50, showing the injury to be the result of disturbance of balance among the ions.

Very different results were obtained by ESPINO and PALISOC (6), who found that young rice plants grow well in a culture solution containing one part  $\text{KH}_2\text{PO}_4$ , one part  $(\text{NH}_4)_2\text{SO}_4$ , one part  $\text{Ca}(\text{NO}_3)_2$ , and thirty-two parts  $\text{MgSO}_4$  with a total concentration of 0.00875 mols of salt per liter.  $\text{MgSO}_4$  was necessary, apparently, as  $\text{Mg}(\text{NO}_3)_2$  and  $\text{MgHPO}_4$  produced plants with dry and chlorotic tips on the leaves.

WORONICHIN (39) reports a preliminary investigation of the relation of  $\text{MgSO}_4$  deposit on the bottoms of lakes near Pjatigorsk to the plant growth in the lake. Three periods of development are described. In the first period, one of low and medium amounts of salt content, there is a growth of *Ruppia*. In the second period there is a decline in the *Ruppia* and vegetative growth of *Vaucheria dichotoma*. In the third period there is a rapid salting, decline of *Vaucheria* and the building of felt. Several new Myxophyceae are described: *Synechocystis minuscula*, *Oscillatoria*

*tambi*, and *Oscillatoria kützingiana* var. *crassa*. A green alga, *Carteria flos aquae*, is also listed.

### Collection of material

Collecting trips were made to lake no. 1 at three different times. The first was in May of 1931, after several weeks of dry weather. The lake was reduced to a series of pools in which the water was greatly concentrated. The banks were brilliantly white, appearing to be practically pure salt deposits, although digging below the surface showed the mud underneath to be black in appearance and very ill-smelling. At the time of the first visit there was an abundant growth of seed-bearing *Ruppia maritima* and blue-green and green algae in the water. Great numbers of a small red crustacean, the "brine shrimp," *Artemia salina*, were swimming in the water. No plant collections were made, but several liters of water were taken and some of the mud was collected. When dried this was white and crystalline, being nearly identical in appearance with the salts obtained by evaporation of the water. It is in this mud, with its dry, white top layer, that the plants root. As the season advances, progressively deeper layers dry and become white, finally leaving the root system of *Salicornia* and other plants in what appears to be pure salt.

The second trip was on May 19, 1933. The season had been much rainier than that of two years earlier, and a fairly heavy rain had fallen the day before the trip was made. The pools of the lake were connected, the salt concentration in the water was much less than at the previous visit, and the banks, instead of being brilliant and white, were dull gray in appearance, giving evidence of their being composed of more than pure salt. *Ruppia maritima* was growing in even greater abundance than previously and was flowering, but there was little algal growth except in a small pool at one side, where the concentration was greater. *Artemia salina* was abundant in the lake, and a small red Cyclops was also present. Collections were made both of *Ruppia* and of the water in which it was growing.

The third collection was made July 22, 1933. Again the lake had evaporated to a series of pools, the banks around were white, and the water concentrated, although not so greatly as at the visit in 1931. *Ruppia* was growing somewhat less luxuriantly than in May and with a greater admixture of blue-green and green algae. The *Ruppia* had developed seeds about 1-2 mm. in diameter, black and shiny in appearance. Collections were made of the plant material and of the water.

Lake no. 2 was visited in May but no collections were made. The lake was continuous at that time, with grayish white banks. Occasional piles of salt on the bank were snowy in appearance. Except for a few algae, there was no sign of plant life in the waters of the lake.

The lake was visited again in July, 1933. The bed was an expanse of white salt with numerous round pools of saturated water, many of which were nearly covered with layers of salt which had crystallized out on top of the water. No phanerogams were found in the lake, but small numbers of a minute, unicellular, motile, yellowish green alga were seen, probably accounting for the yellowish green appearance of the pools from the hill above. Collections were made of the water and the alga, but the alga was not determined.

On the July trip to lakes no. 1 and no. 2 a stop was made at lake no. 3 and water was collected.

All three of the lakes are highly poisonous to the cattle which graze in the region and are tightly fenced to protect stock from access to them. Several carcasses lying within a few hundred feet of the lakes were testimony to the rapidity with which death followed when an animal did drink the water.

That the accumulation of salts is due to the lack of outlets usual to lakes is evidenced by the fact that some of the lakes lying between no. 2 and no. 3 were supporting an abundant growth of plants usual to lake waters and were left accessible to the stock in the region.

#### Material and methods

Data were obtained on the water and plant material from the three collections of May, 1931, May, 1933, and July, 1933. This included five samples of water, three from lake no. 1, one from lake no. 2, and one from lake no. 3. The plant collections included those of May, 1933, from lake no. 1 and those of July from lake no. 1. The specific gravity of the water was measured at 26° C. The pH was determined by means of a quinhydrone electrode. Concentration of dissolved substances was obtained by evaporation and drying at 100° C. The osmotic pressure was determined by measurement of the freezing point with a Beckmann thermometer. Ash analyses were made, using the methods of the Official Association of Agricultural Chemists, for Mg, Na, K, Ca, Fe and Al, SO<sub>4</sub>, Cl, PO<sub>4</sub>, and NO<sub>3</sub>. No analyses were made for sulphide or sulphite, although traces of sulphide were undoubtedly present.

The plant material was washed thoroughly with tap water, dried at 63° C., and the moisture content determined.

The soluble portion of the plant was extracted by boiling and expressing the sap by pressure. The sample was weighed, distilled water was added and boiled, and the sap was pressed out. Then the whole extract was evaporated to the volume of the original sap as determined by weight of the original sample and weight of the insoluble material. Measurements were then made of the specific gravity, pH, and osmotic pressure of the sap.

Determinations were made for soluble sugars in the sap by the Shaffer-Hartmann method and for protein N by the Kjeldahl method. Ash analyses were made on both the sap and the insoluble residue for Mg, Fe and Al, Na, K, Ca, Cl,  $\text{SO}_4$ ,  $\text{PO}_4$ , and  $\text{NO}_3$ .

As it was thought possible that arsenic might be found in some of the lakes, qualitative analyses were made for it using Marsh's test. No traces were found in any of the five samples analyzed.

In addition to these data, some observations were made on the growth of *Ruppia* and of *Artemia* from the May, 1933, collections in different culture solutions. The solutions were made up as follows: lake water, from the May, 1933, collection from lake no. 1; equal parts of lake water and distilled water; two-thirds lake water and one-third 2 per cent.  $\text{MgCl}_2$  solution; one-third lake water and two-thirds 2 per cent.  $\text{MgCl}_2$  solution; Knop's solution; and Knop's solution with additional  $\text{MgCl}_2$  to bring the osmotic pressure up to that of the lake water. About a quart of each solution was placed in a 2-quart glass jar and a few plants of *Ruppia* were placed in each. Records were made of the vigor of the plants as well as this could be determined by observation.

Three specimens of *Artemia*, a small amount of algal material from lake no. 1, and some culture solution were placed in each of a group of small beakers, and observations were made of the behavior of the crustacean. The culture solutions were as follows: lake water, from the May collection of 1933; one-half lake water and one-half 4 per cent.  $\text{MgCl}_2$  solution; 2 per cent.  $\text{MgCl}_2$  solution; 4 per cent.  $\text{MgCl}_2$  solution; 12 per cent.  $\text{MgCl}_2$  solution; distilled water; and 22 per cent. solution of salts obtained from evaporation of the water collected in May, 1931.

### Data

Analyses of the water samples for  $\text{NH}_3$  showed traces in the water. Data accumulated in measurements and analyses of the samples of water and of plant material are listed in tables I to IX.

Figures obtained for the moisture content of the plants were: May collection, 92.2 per cent.; July collection, 90.8 per cent.

The cultures of *Ruppia* showed varying results. None of the plants remained alive after 45 days. Death at that time was probably due to their being placed in rather narrow-mouthed jars with no special care taken to provide for aeration. Those in Knop's solution were the first to succumb, being dead and decomposing within 15 days. Those in Knop's solution and  $\text{MgCl}_2$  with an osmotic pressure equal to that of the lake water survived only a few days longer than the plants in Knop's solution alone. The plants in two-thirds lake water and one-third 2 per cent.  $\text{MgCl}_2$  behaved much the same as those in one-third lake water and two-thirds 2 per cent.  $\text{MgCl}_2$ .

TABLE I

WATER COLLECTED FROM LAKE NO. 1, MAY, 1931. APPROXIMATE CONCENTRATION, 26%;  
SPECIFIC GRAVITY AT 23° C., 1.21; OSMOTIC PRESSURE, 58.0387 ATM.; PH, 7.4

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	<i>gm.</i>			<i>%</i>	
Mg .....	36.6145	1.5055	3.0110	40.1	30250
Na .....	8.60	0.3739	0.3739	9.96	7100
K .....	1.6651	0.0426	0.0426	1.13	1380
Ca .....	0.0912	0.0023	.0046	0.06	75
Fe and Al .....	Trace				
Total .....	46.9708	1.9243	3.4321	51.25	38805
SO <sub>4</sub> .....	169.7694	1.7684	3.5368	47.16	140305
Cl .....	1.935	0.0546	0.0546	1.45	1600
NO <sub>3</sub> .....	0.1429	0.0023	0.0023	0.06	120
PO <sub>4</sub> .....	0.0131	0.0001	0.0003	.....	10
Total .....	171.8604	1.8254	3.5940	48.67	142035

Total molar concentration, 3.7497 mols per liter.

TABLE II

WATER COLLECTED FROM LAKE NO. 1, MAY, 1933. APPROXIMATE CONCENTRATION, 1.6%;  
SPECIFIC GRAVITY AT 26° C., 1.012; OSMOTIC PRESSURE, 3.797 ATM.; PH, 8.4

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	<i>gm.</i>			<i>%</i>	
Mg .....	2.1207	0.0872	0.1744	38.98	2100
Na .....	0.6161	0.0268	0.0268	11.98	610
K .....	0.0981	0.0025	0.0025	1.12	97
Ca .....	0.0084	0.0002	0.0004	0.08	8
Fe and Al .....	Trace				
Total .....	2.8433	0.1167	0.2041	52.16	2815
SO <sub>4</sub> .....	9.6902	0.1009	0.2018	45.10	9575
Cl .....	0.2070	0.0058	0.0058	2.59	200
NO <sub>3</sub> .....	0.0142	0.0002	0.0002	0.08	15
PO <sub>4</sub> .....	0.0007	.....	.....	.....	.....
Total .....	9.9121	0.1069	0.2078	47.77	9790

Total molar concentration, 0.2236 mols per liter.



TABLE III

WATER COLLECTED FROM LAKE NO. 1, JULY, 1933. APPROXIMATE CONCENTRATION, 6%;  
SPECIFIC GRAVITY AT 26° C., 1.045; OSMOTIC PRESSURE, 12.28 ATM.; PH, ABOVE 8.5

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	<i>gm.</i>			<i>%</i>	
Mg. ....	10.3757	0.4266	0.8532	39.89	9930
Na ....	2.3050	0.1002	0.1002	9.37	2200
K ....	0.4557	0.0116	0.0116	1.08	440
Ca ....	0.0027	0.0001	0.0002	0.01	3
Fe and Al ....	Trace				
Total .....	13.1391	0.5385	0.9652	50.35	12573
SO <sub>4</sub> .....	49.4723	0.5153	1.0306	48.18	47340
Cl .....	0.544	0.0153	0.0153	1.43	520
NO <sub>3</sub> .....	0.0196	0.0003	0.0003	0.03	20
PO <sub>4</sub> .....	0.0026				2
Total .....	50.0385	0.5309	1.0462	49.64	47882

Total molar concentration, 1.0694 mols per liter.

TABLE IV

WATER COLLECTED FROM LAKE NO. 2, JULY, 1933. APPROXIMATE CONCENTRATION, 48%;  
SPECIFIC GRAVITY AT 26° C., 1.380; OSMOTIC PRESSURE,\* 125.80 ATM.; PH, 7.28

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	<i>gm.</i>			<i>%</i>	
Mg .....	46.565	1.9146	3.8292	24.45	33740
Na .....	51.5239	2.2402	2.2402	28.61	37330
K .....	11.3754	0.2909	0.2909	3.71	8240
Ca .....	0.0009				
Fe and Al ....					
Total .....	109.4652	4.4457	6.3603	56.77	79310
SO .....	314.15	3.2724	6.5448	41.79	227644
Cl <sup>4</sup> .....	3.42	0.1123	0.1123	1.43	2480
NO <sub>3</sub> .....	0.0047	0.0001	0.0001		3
PO <sub>4</sub> .....	0.0247	0.0003	0.0009		18
Total .....	317.5994	3.3851	6.6581	43.22	230145

Total molar concentration, 7.8308 mols per liter.

\* To measure the freezing point depression it was found necessary to dilute the water to one-fourth the original concentration. The pressure given is calculated on that basis and is somewhat high.

TABLE V

WATER COLLECTED FROM LAKE NO. 3, JULY, 1933. APPROXIMATE CONCENTRATION, 2.4%;  
SPECIFIC GRAVITY AT 26° C., 1.020; OSMOTIC PRESSURE, 6.506 ATM.; PH, ABOVE 8.5

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	<i>gm.</i>			<i>%</i>	
Mg .....	2.8152	0.1158	0.2316	32.53	2760
Na .....	1.312	0.0570	0.0570	16.01	1290
K .....	0.7704	0.0197	0.0197	5.53	755
Ca .....	0.0124	0.0031	0.0062	0.87	12
Fe and Al .....	Trace	.....	.....	.....	.....
Total .....	4.9100	0.1956	0.3145	54.94	4817
SO <sub>4</sub> .....	14.6053	0.1503	0.3006	42.22	14320
Cl .....	0.3472	0.0098	0.0098	2.75	340
NO <sub>3</sub> .....	0.0106	0.0002	0.0002	0.05	10
PO <sub>4</sub> .....	0.0128	0.0001	0.0003	0.03	12
Total .....	14.9759	0.1604	0.3109	45.05	14682

Total molar concentration, 0.3560 mols per liter.

TABLE VI

PLANT SAP FROM COLLECTION OF MAY, 1933, LAKE NO. 1. SPECIFIC GRAVITY AT 26° C.,  
1.020; SOLUBLE SUGARS, 1.109 MG. GLUCOSE PER CC.; OSMOTIC PRESSURE,  
6.637 ATM.; PH, 6.12; SOLUBLE PROTEIN NITROGEN, 0.27%

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	<i>gm.</i>			<i>%</i>	
Mg .....	1.876	0.0771	0.1542	31.40	1840
Na .....	0.4746	0.0206	0.0206	8.39	465
K .....	0.2800	0.0072	0.0072	2.93	275
Ca .....	0.3494	0.0087	0.0174	3.54	340
Fe and Al .....	Trace	.....	.....	.....	.....
Total .....	2.9800	0.1136	0.1994	46.26	2920
SO <sub>4</sub> .....	6.693	0.0697	0.1394	28.39	6560
Cl .....	1.595	0.045	0.045	18.33	1560
NO <sub>3</sub> .....	0.9876	0.016	0.016	6.51	970
PO <sub>4</sub> .....	0.1135	0.0012	0.0036	0.48	110
Total .....	9.3891	0.1319	0.2040	53.71	9200

Total molar concentration, 0.2455 mols per liter.

TABLE VII

INSOLUBLE PLANT MATERIAL FROM COLLECTION OF MAY, 1933, LAKE NO. 1

	ASH ANALYSIS	
	GRAMS PER 100 GRAMS DRIED INSOLUBLE RESIDUE	INSOLUBLE MATERIAL
	<i>gm.</i>	<i>%</i>
Mg .....	1.8404	1.84
Na .....	0.3600	0.36
K .....	0.2124	0.21
Ca .....	1.1369	1.14
Fe and Al .....	Trace	
Total .....	3.5497	3.55
SO <sub>4</sub> .....	2.36	2.36
Cl .....	0.6588	0.66
NO <sub>3</sub> .....	0.7088	0.71
PO <sub>4</sub> .....	0.0011	.....
Total .....	3.7287	3.73

TABLE VIII

PLANT SAP FROM COLLECTION OF JULY, 1933, LAKE NO. 1. SPECIFIC GRAVITY AT 26° C.,  
1.025; SOLUBLE SUGARS, 1.06 MG. GLUCOSE PER CC.; OSMOTIC PRESSURE, 9.755 ATM.;  
SOLUBLE PROTEIN NITROGEN, 0.51%

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	<i>gm.</i>			<i>%</i>	
Mg .....	2.936	0.1207	0.2414	27.48	2865
Na .....	1.2668	0.0551	0.0551	12.54	1235
K .....	0.8817	0.0225	0.0225	5.12	860
Ca .....	0.4237	0.0106	0.0212	2.41	415
Fe and Al .....	None				
Total .....	6.5082	0.2089	0.3402	47.55	5375
SO <sub>4</sub> .....	15.120	0.1575	0.3150	35.86	14750
Cl .....	2.0409	0.0576	0.0576	13.11	1990
NO <sub>3</sub> .....	0.5128	0.0054	0.0054	1.22	500
PO <sub>4</sub> .....	0.9356	0.0098	0.0294	2.23	910
Total .....	18.6093	0.2303	0.4074	52.42	18150

Total molar concentration, 0.4392 mols per liter.

TABLE IX  
INSOLUBLE PLANT MATERIAL FROM COLLECTION OF JULY, 1933, LAKE NO. 1

	ASH ANALYSIS	
	GRAMS PER 100 GRAMS DRIED INSOLUBLE RESIDUE	INSOLUBLE MATERIAL
	<i>gm.</i>	<i>%</i>
Mg .....	0.619	0.62
Na .....	0.8108	0.81
K .....	0.4783	0.48
Ca .....	0.6151	0.62
Fe and Al .....	.....	
Total .....	2.5232	2.53
SO <sub>4</sub> .....	5.845	5.84
Cl .....	0.1093	0.11
NO <sub>3</sub> .....	0.3544	0.35
PO <sub>4</sub> .....	0.6128	0.62
Total .....	6.9215	6.92

solution, showing a slight browning and a lessening in oxygen given off within two days. Death was gradual, however. The brown color increased slowly and at the end of 40 days some of the plants were still green. Likewise, little difference was shown between the plants grown in normal lake water and those grown in half lake water and half distilled water. These groups survived the best, being apparently healthy at the end of 16 days. By the end of 40 days they were showing some brown color, although somewhat less than the others, and shortly after they were also dead. It is interesting to note that a heavy growth of algae took place in the jars upon death of the *Ruppia*. No attempt was made to identify these, but the jars which contained Knop's solution and no lake water showed what appeared to be primarily a Chlorophycean growth, while those with the lake water appeared to have a Myxophycean growth.

*Artemia* was much quicker in responding to differences of environment. Those allowed to remain in lake water were not affected, being alive and showing no evidence of sluggishness at the end of 15 days, when the observations were discontinued. Those in half lake water and half 4 per cent. MgCl<sub>2</sub> solution were less affected than most of the others. At the end of 24 hours they were somewhat sluggish but recovered from this and survived for some days. At the end of six days one had died, and in four days more all were dead. The specimens placed in 22 per cent. salt from lake no. 1 survived for four days, when two died. The remaining one lived two days longer. Distilled water was the most unfavorable medium. Two of

the specimens were dead within five hours; the remaining one was sluggish and died within another three hours. Those in 2 per cent.  $\text{MgCl}_2$  solution survived slightly better but were slow in movement at the end of eight hours and dead within 24 hours. Two of the *Artemia* in 4 per cent.  $\text{MgCl}_2$  solution died within five hours; the other, although not active, was alive at the end of 24 hours but died a few hours later. Those in 12 per cent.  $\text{MgCl}_2$  did not succumb until nearly 48 hours had passed. Those of the organisms which had died in solutions containing no  $\text{MgCl}_2$  lost the red color but remained colorless after death. Those in the solutions containing  $\text{MgCl}_2$  not only lost the red pigment upon dying but turned black. The black color appeared first along the mid part of the dorsal side and gradually spread out through the rest of the crustacean.

### Analysis of data and conclusions

Comparison of the samples collected from lake no. 1 (tables I, II, III) shows variation in concentration from 1.6 to 26 per cent. at different seasons. *Ruppia maritima* is able to grow well under these concentrations, although the most luxuriant vegetative growth was at the lower concentration. The fact that the *Ruppia* had already produced seeds in May, 1931, when the concentration was very high, while the plant was flowering in May, 1933, at a concentration of slightly more than 1.5 per cent., indicates that fruiting is more dependent upon concentration than upon the season. The increase in concentration from 1.5 per cent. in May to 6 per cent. in July was accompanied by the development of the seeds to maturity. The algal growth, particularly of blue-green algae, was more abundant in the solutions of greater concentration.

Comparison of the salt contents of the three collections shows only small variations in the relative proportions of ions present. The Mg content is almost constant while that of the  $\text{SO}_4$ , although somewhat higher than the Mg, is only slightly more variable. These total between 84 and 88 per cent. of the total salt analyzed. While the K content remains practically the same for the two May collections and drops slightly for the July sample, the Na and Cl are both slightly greater for the dilute waters of the May, 1933, sample. Ca, at all times scant in amount, drops in the July collection.  $\text{NO}_3$  behaves much as Ca; and Fe, Al, and  $\text{PO}_4$  are at all times negligible in amount.

Table IV shows the analysis of lake no. 2. Here the concentration has reached the saturation point, so that, even on the hot July day when the collection was made, crystallization of salts had partially covered the water with a layer of salt. In this lake Ca, Fe, Al,  $\text{NO}_3$ , and  $\text{PO}_4$  are practically lacking. The amount of Cl is about the same as that in tables I and III. Although the amount of  $\text{SO}_4$  is somewhat less than that in lake no. 1, the

greatest difference lies in the amounts of Mg, Na, and K. The Mg content is about three-fifths that of lake no. 1 while the Na is almost three times as great. And the K is slightly more than three times as great as in lake no. 1. As lake no. 2 showed no organic life except the unidentified alga, the failure of plant life to grow there, when it is abundant in lake no. 1, might be due to the extreme concentration of total salt, to the lack of the essential Ca,  $\text{PO}_4$ ,  $\text{NO}_3$ , and Fe, or to the greater proportion of K and Na in the solution.

The analyses of the third lake are given in table V. The concentration (2.4 per cent.) is less than in either of the other lakes in July, although greater than lake no. 1 in May, 1933. There are traces of Fe and Al.  $\text{NO}_3$ ,  $\text{PO}_4$ , and Ca especially are more abundant than in lake no. 1.  $\text{SO}_4$  is present in nearly the same amount (42.22 per cent.) as in lake no. 2. The proportions of Mg, Na, and K are again different. There is about four-fifths as much Mg as in lake no. 1 in July, somewhat less than twice as much Na, and about five times as much K. With comparisons of the actual amounts in the water from lake no. 1 in May, 1933, and lake no. 3 (which are not greatly different in concentration), it is seen that slightly more Mg and much more Na and K are present in lake no. 3. The amounts are nevertheless much smaller in either of these samples than in the water of lake no. 1 in May, 1931, or July, 1933, when plants were growing abundantly. Comparison of the three lakes suggests that the toxicity of lakes 2 and 3 to plant life lies neither in the high concentration nor in the lack of Ca,  $\text{NO}_3$ , and  $\text{PO}_4$ , but rather in the relatively high proportion of Na and K in the total salt content.

This conclusion is supported by the distribution of plant life in Great Salt Lake, as reported by FLOWERS (8). The plant life of the lake is entirely algal, although the concentration reaches no greater value than that of the magnesium sulphate lake no. 1 described here. The chief constituent, however, is NaCl rather than  $\text{MgSO}_4$ . The growth of *Ruppia* in less concentrated bays of Great Salt Lake shows that concentration of NaCl is evidently the limiting factor.

Tables VI and VII show the results of analyses on the soluble sap and the insoluble residue of the plant material collected in May, 1933. Tables VIII and IX show the results of analyses of the July collection. These show a high content of both Mg and  $\text{SO}_4$ . This may be due in part to failure to remove all of the external  $\text{MgSO}_4$  in washing preparatory to extracting the sap, but the content is high in the July collection as well as the May. As the former was washed even more thoroughly than the latter, the amount of salt due to external  $\text{MgSO}_4$  is probably not great.

Comparison of the sap of *Ruppia* from the May collection with the July collection shows a slight decrease of soluble sugars and an increase of soluble

protein nitrogen. The total molar concentration of the salts for which analyses were made has increased from 0.2455 to 0.4392 mols per liter. Tables VI and VIII show the actual amount of salt to have increased during the period from May to July in all cases except that of  $\text{NO}_3$ . This loss of  $\text{NO}_3$  is probably due to the increase in protein nitrogen. The relative molar concentrations have changed considerably. There is a decrease in the Mg and Ca and an increase of Na and K. Likewise the  $\text{SO}_4$  and  $\text{PO}_4$  have increased, the latter greatly, while the Cl and  $\text{NO}_3$  have decreased.

Selective absorption of ions by the plant is shown by the concentrations of different ions in the plant sap and the water in which it is growing. Tables II and VI give these values for the May samples. Mg and  $\text{SO}_4$  are high in both, showing the plant able to tolerate these internally, but the amount is greater in the water than in the sap. The water contains 2.1207 gm. of Mg and 9.6902 gm. of  $\text{SO}_4$  per liter while the sap contains 1.876 gm. of Mg and 6.693 gm. of  $\text{SO}_4$  per liter. The water contains 0.6161 gm. of Na per liter while the sap contains 0.4746 gm. per liter. The remainder of the ions, especially Ca,  $\text{NO}_3$ , and  $\text{PO}_4$ , are more abundant in the plant sap than in the water. While there are 0.0981 gm. per liter of K and 0.2070 gm. per liter of Cl in the water, the sap contains 0.2800 gm. of K and 1.595 gm. of Cl. The water contains 0.0084 gm. and the sap contains 0.3494 gm. of Ca per liter; the water contains 0.0142 gm. and the sap 0.9876 gm. of  $\text{NO}_3$  per liter; the water contains 0.0007 gm. and the sap 0.1135 gm. of  $\text{PO}_4$  per liter.

Similar results are shown by comparison of the sap of the July collection and the water (tables III, VIII), although the concentrations are greater in both sap and water than in the May material. The water contains 10.3757 gm. of Mg, 49.4723 gm. of  $\text{SO}_4$ , and 2.3050 gm. of Na per liter while the plant contains 2.936 gm. of Mg, 15.120 gm. of  $\text{SO}_4$ , and 1.2668 gm. of Na per liter. As in the May material, the other ions are more abundant in the plant than in the water. The water contains 0.4557 gm. of K, 0.0027 gm. of Ca, 0.544 gm. of Cl, 0.0196 gm. of  $\text{NO}_3$ , and 0.0026 gm. of  $\text{PO}_4$  while the plant sap contains 0.8817 gm. of K, 0.4237 gm. of Ca, 2.0409 gm. of Cl, 0.5128 gm. of  $\text{NO}_3$ , and 0.9356 gm. of  $\text{PO}_4$ . There has been considerable change in the ratios of material in the plant to material in the water from those shown in the May material. While the ratios of K, of Cl, and of  $\text{NO}_3$  in the sap to that in the water have decreased from May to July, the ratios of Ca and of  $\text{PO}_4$  in the sap to that in the water have increased greatly.

The contents of both plant sap and water may be compared with those of plants growing in sea water and in pond water. OSTERHOUT (26) reports the concentration of *Valonia* and of the sea water in which it grows. His results, converted from parts per thousand to parts per million for purposes of comparison, are given in table X.

TABLE X

	CONC. IN SEA WATER	CONC. IN CELL SAP
	<i>p.p.m.</i>	<i>p.p.m.</i>
Mg .....	1310	Trace
Na .....	10900	2100
K .....	500	20100
Ca .....	450	700
SO <sub>4</sub> .....	3300	5
Cl .....	19600	21200

With regard to the water, the difference lies chiefly in high NaCl in the sea water and high MgSO<sub>4</sub> in the lakes. The plant sap analyses are interesting. *Valonia* excludes Mg and SO<sub>4</sub> almost entirely and allows little Na to enter, although the concentration is high in the water. Cl and Ca are absorbed in somewhat greater concentration, and K is many times more concentrated than in the water.

In *Ruppia* the sap shows many times as much PO<sub>4</sub>, NO<sub>3</sub>, Ca, and K as the water. Cl is likewise selectively absorbed by the plant and Na is excluded to some extent. Unlike *Valonia*, *Ruppia* is able to tolerate Mg and SO<sub>4</sub> in large quantities, allowing a greater amount of these than any of the other ions to enter. HOAGLAND and DAVIS (9) made analyses of *Nitella clavata* which was growing in pond water (table XI).

TABLE XI

	WEIGHT	
	WATER	CELL SAP
	<i>p.p.m.</i>	<i>p.p.m.</i>
Mg .....	41	430
Na .....	5	230
K .....	Trace	2120
Ca .....	31	410
SO <sub>4</sub> .....	31	800
Cl .....	32	3220
NO <sub>3</sub> .....	34	0
PO <sub>4</sub> .....	0.4	350

In this case the amounts of Mg, SO<sub>4</sub>, and Cl are not greatly different from one another and approximate the amounts of Ca and NO<sub>3</sub> in the water. Na is small in amount. The greatest difference from the water of lake no. 1 is in the much smaller concentration of total salts. While some Mg and SO<sub>4</sub> have been taken up by *Nitella*, the amount is much less than that tolerated by *Ruppia*, while the amounts of K and Cl are much greater.



Determinations of the pH of the five samples of lake water show all of them to be alkaline, three of them being 8.4 or above. Apparently the pH of the lakes has little influence on plant growth, as one of the lower readings (7.4) was obtained from the May, 1931, collection from lake no. 1 where plants were abundant, and the other (7.28) from the highly concentrated water of lake no. 2 where very little life was found.

Osmotic concentration determinations show the water samples to vary from 3.797 atmospheres in lake no. 1 in May, 1933, to nearly 125 atmospheres in lake no. 2 in July, 1933. In July the osmotic pressure of lake no. 1 was 12.28 atmospheres. The osmotic concentration of lake no. 1 in May of 1931, the most concentrated sample in which *Ruppia* was growing, was 58.0387 atmospheres.

The plant sap determined in May, 1933, had an osmotic concentration equal to 6.637 atmospheres, or 2.84 atmospheres more than the water surrounding it. By July the concentration of the plant sap had increased to 9.755 atmospheres. In the meantime, however, the concentration of the water had increased until the equivalent osmotic pressure was 12.28 atmospheres, or 2.525 atmospheres above that of the plant. It was not possible to determine, from the collections made, the stage of development of plants of *Ruppia* when the osmotic pressures shifted to make the external greater than the internal pressure.

The responses of *Ruppia* and *Artemia* to different culture conditions indicate that  $\text{SO}_4$  is necessary when Mg is present, as the substitution of Cl for  $\text{SO}_4$  resulted in rather quick death for *Artemia* and somewhat slower death for *Ruppia*. Distilled water was quickly fatal for *Artemia*; and Knop's solution, a satisfactory culture solution for most plants, caused or allowed *Ruppia* to die within a rather short period. Apparently these organisms are growing in unusual conditions, not only because they survive where other plants and animals cannot but also because they themselves require unusual conditions for growth. It is of interest, in connection with the study of *Artemia* in different culture solutions, to review the work of BOONE and BAAS-BECKING (1). They report KCl as being very toxic to *Artemia salina* although the adult *Artemia* survives in solutions of  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ , HCl, and NaCl. Only NaCl and NaBr solutions are found to cause normal ecdysis, even the chlorides of Ca and Mg being toxic after a few days.

The range of concentrations reported as being suitable for life in *Artemia* is 0 to 3.5 mols. BOONE and BAAS-BECKING (1) state that increasing Mg content of the brine is probably a factor in the disappearance of *Artemia*. These conclusions agree with those arrived at by growing *Artemia* in so far as the eventual toxicity of  $\text{MgCl}_2$  is concerned. However, in view of the fact that *Artemia* was growing in the water collected in May, 1931,

which had a concentration of not less than 3.75 mols, of which 40 per cent. was Mg, it seems necessary to extend the range of suitable concentrations somewhat and to look for some factor other than the increase of Mg as cause for the disappearance of *Artemia*.

The halophyte *Ruppia maritima* presents an example of a plant growing directly in water but under conditions of great "physiological dryness," as the concentration of salts in the water produces osmotic pressure of 58 atmospheres or more without causing the death of the plant. *Ruppia* is able to increase the internal osmotic pressure, by utilization of salts from the water surrounding it, to greater values than the external pressure. At some stage between the external pressures of 3.797 and 12.28 atmospheres, the internal osmotic pressure of *Ruppia* fails to increase as rapidly as the external pressure, resulting in greater external than internal pressure. Further study might show this shift of balance in external and internal pressures to coincide with maturation of the seeds and cessation of vegetative growth in *Ruppia*.

*Ruppia* is unusual in being able to tolerate magnesium sulphate in high concentrations. The percentage of Mg and  $\text{SO}_4$  in the water surrounding *Ruppia* was 84 to 88 per cent. of the total salt content. The plant does not exclude either Mg or  $\text{SO}_4$ . On the contrary, both are found in the plant sap in greater proportionate amounts than any other ions for which analyses were made, although not in so great concentration, compared with other ions, as in the water.

*Ruppia maritima*, although able to develop successfully in lakes of high magnesium sulphate content, does not tolerate high concentration of NaCl. It does not grow in Great Salt Lake, where the concentration is comparable with that of lake no. 1 but where the chief constituent is NaCl. *Ruppia* is likewise not found in either of two lakes near lake no. 1, one of which shows very high concentration and the other low concentration but both of which have relatively more Na and K. Na is evidently much more toxic to *Ruppia* than is Mg. Cultures of *Ruppia* in  $\text{MgCl}_2$  solutions indicate the necessity of  $\text{SO}_4$  with Mg, suggesting an antagonism of  $\text{SO}_4$  and Mg ions.

### Summary

1. Collections were made from a lake supporting *Ruppia* in May, 1931, May, 1933, and July, 1933.
2. At the time of the first collection, the concentration of the lake water was about 26 per cent., the pH was 7.4, the specific gravity was 1.21 at 23° C., and the osmotic equivalent was 58.0387 atmospheres. *Ruppia* was abundant, there was a heavy growth of blue-green and green algae, and the crustacean *Artemia salina* was abundant. The banks of the lake were dry, crystalline, and white on top with black mud underlying. Collections were

made of the water and of the mud. Upon drying, this mud appeared white and crystalline like the layer above.

3. At the time of the second collection, May, 1933, after a rainy season, the concentration of the lake was about 1.6 per cent., the pH was 8.4, the specific gravity was 1.012 at 26° C., and the osmotic pressure was 3.797 atmospheres. *Ruppia* showed a heavy vegetative growth and was flowering. *Artemia* was abundant, but fewer algae were found than at other visits. The banks were gray and dull in appearance. Collections were made of water, of *Ruppia*, and of *Artemia*.

4. At the time of the third collection, July, 1933, after dry weather, the concentration was about 6 per cent., the pH was above 8.5, the specific gravity was 1.045 at 26° C., and the osmotic pressure was 12.28 atmospheres. Algae, *Artemia*, and seed-bearing *Ruppia* were abundant. The banks were white and crystalline in appearance. Collections were made of *Ruppia* and of water.

5. Collections of water were made at the other two lakes in July, 1933. The concentration of one lake was about 48 per cent., the pH was 7.28, the specific gravity was 1.380 at 26° C., and the osmotic pressure was about 125 atmospheres. The lake had been reduced by evaporation to many small round pools with salt crystallized on top, and the lake bed was white and crystalline with black mud under the surface layer. The concentration of the other lake was about 2.4 per cent., the pH was above 8.5, the specific gravity was 1.020 at 26° C., and the osmotic pressure was 6.506 atmospheres.

6. Sap from plants of the May collection showed a specific gravity of 1.020 at 26° C. and an osmotic pressure of 6.637 atmospheres; from the July collection, a specific gravity of 1.025 at 26° C. and an osmotic pressure of 9.755 atmospheres.

7. Quantitative analyses were made for Mg, Na, K, Ca, Fe and Al, SO<sub>4</sub>, Cl, PO<sub>4</sub>, and NO<sub>3</sub> in the sap and insoluble material of the plant collections and in each of the five water samples. Analyses were made of the water samples for NH<sub>3</sub>, showing traces present, and for As, showing no trace of any present.

8. The analyses show 40 per cent. of the total molar concentration of the salt in the lake supporting *Ruppia* to be due to Mg; 45 to 48 per cent. to be due to SO<sub>4</sub>; and 9 to 11 per cent. to be due to Na.

9. The pH is apparently not a factor in plant distribution in these lakes.

10. *Ruppia* is able to tolerate Mg and SO<sub>4</sub> in high concentrations internally as well as externally, evidently using these in developing its own osmotic pressure.

11. The limiting factor in the development of *Ruppia* in lakes not supporting it is evidently a greater proportion of Na<sub>2</sub>SO<sub>4</sub>.

12. Cultures of *Artemia* and *Ruppia* indicate that  $\text{MgCl}_2$  cannot replace  $\text{MgSO}_4$ .

13. Time of flowering and seed development of *Ruppia* are evidently influenced by the concentration of the lake in which it grows.

14. *Ruppia* was growing in external osmotic pressures of 3.797 atmospheres with an internal pressure of 6.637 atmospheres, and 12.28 atmospheres with an internal pressure of 9.755 atmospheres.

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STATE COLLEGE OF WASHINGTON  
PULLMAN, WASHINGTON

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