THE CHEMISTRY OF *ARTEMIA* HABITATS[1]

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Abstract. Some populations of *Artemia salina* (L.) occur in high-carbonate waters and potassium-rich media where Na/K ratios are low. These habitats previously have been thought to exclude this crustacean.

INTRODUCTION

Thriving populations of the brine shrimp, *Artemia salina* (L.), were discovered recently in two saline ponds about 32 km north of Saint Johns, Apache Co., Arizona, at the Long-H Ranch (Koehn and Cole 1964). These pools, Red Pond and Green Pond, figure in Zuñi mythology as Kiatuthlanna, "Place of the Big Water," and are at an old archaeological site (Roberts 1931). Some limnological features of the ponds have been discussed by Cole and Whiteside (1965a, b). Discovery of *Artemia* in the Long-H ponds represented the first record of this crustacean in Arizona and revealed some erroneous ideas that have prevailed about the chemistry of *Artemia* habitats. It is the purpose of this paper to draw attention to the occurrence of *Artemia* in types of water that previously have been thought to preclude its survival.

Many saline waters have been listed in Table 1 for comparison with the Kiatuthlanna ponds and plotted on triangular coordinate paper (Fig. 1) to show relative anionic composition, by weight, of *Artemia* habitats. Bicarbonate data and total alkalinity values have been converted to carbonate in all plots and in Table 1. All the *Artemia* habitats in Figure 1 have total salinities of at least 30 g/liter (Table 1). Changing concentration in any such saline water probably results in only slight change in the relative composition of the three principal anions, carbonate, sulfate, and chloride (Langbein 1961; Bayly and Williams 1966). This is shown in the case of Red Pond (Fig. 1, RP) and Green Pond (Fig. 1, GP) where concentration increases of almost two-fold (Table 1) result in a very small shift toward the chloride corner of the plot.

ARTEMIA IN CHLORIDE AND SULFATE WATERS

Most of the *Artemia* waters in Figure 1 lie near the left arm of the triangle and are characterized by carbonate making up less than 5% by weight of the three

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TABLE 1. Some chemical features of waters containing *Artemia salina* compared to the Long-H Ponds, Apache Co., Arizona. Amounts in g/liter

LAKE	CO_3^{--}	SO_4^{--}	Cl^-	Na:K Ratio by weight	Total Salinity	Reference
Green Pond, Arizona	20.68–46.16	0.30–0.47	20.2–42.8	104.8	61.3–112.0	Cole and Whiteside 1965b
Red Pond, Arizona	15.82–39.78	0.52–1.22	51.4–106.8	101.6	120.0–220.0	Cole and Whiteside 1965b
Mono Lake, California	12.12	6.93	12.5	15.4	54.1	Dunn 1953
Chloride Lakes						
Leslie Salt Works, Cal.	tr	29.53	179.2	8.6	318.2	Clarke 1924
Grable's Salt Works, Tex.	0.05	1.84	39.8	—	71.2	Deevey 1957
La Sal del Rey, Tex.	0.11	1.10	106.9	—	177.9	Deevey 1957
Ouargla Chott, Algeria	0.13	10.04	34.6	19.6	67.8	Beadle 1943
Zuñi Salt Lake, N. M.	0.18	7.00	126.0	173.0	210.5	Cole, unpubl. data
Great Salt Lake, Utah	0.18	14.85	111.1	17.9	203.5	Adams 1964
Lake Urmia, Iran	0.18	11.70	146.0	63.4	258.0	Löffler 1961
Schor-gōl, Iran	0.79	1.23	17.6	86.3	31.3	Löffler 1961
Sambhar Salt Lake, India	2.52	6.73	60.8	432.0	115.0	Clarke 1924; Baid 1958
Sulfate Lakes						
Little Manitou, Saskatchewan	0.51	51.72	23.3	17.6	106.9	Rawson and Moore 1944
Tso Kar, Tibet	1.05	35.07	11.7	2.9	72.8	Hutchinson 1937
Beadle's Station # 61, Algeria	0.38	40.03	12.5	12.9	69.6	Beadle 1943
Hot Lake, Washington	1.50	243.55	1.9	10.7	161.2	Anderson 1958
Nebraska Potash Ponds						McCarraher, pers. comm.
Jesse Lake, Sheridan Co.	34.38	13.80	4.1	12.5	86.8	
Cook Lake, Sheridan Co.	46.58	8.00	4.3	306.9	80.8	
Lily Lake, Sheridan Co.	4.49	—	0.1	—	—	
Pond # 1, Sec. 29, Sheridan Co.	—	—	—	1.1–2.5	—	
Lake # 1, Sheridan Co.	—	—	—	3.4	—	
Lake # 2, Sheridan Co.	—	—	—	1.2	—	
Lake # 3, Sheridan Co.	—	—	—	0.9	—	
Johnson Lake, Garden Co.	12.44	—	—	—	—	
Ryan Lake, Garden Co.	21.76	—	1.2	—	—	
Richardson Lake, Garden Co.	30.83	—	1.6	1.4	—	

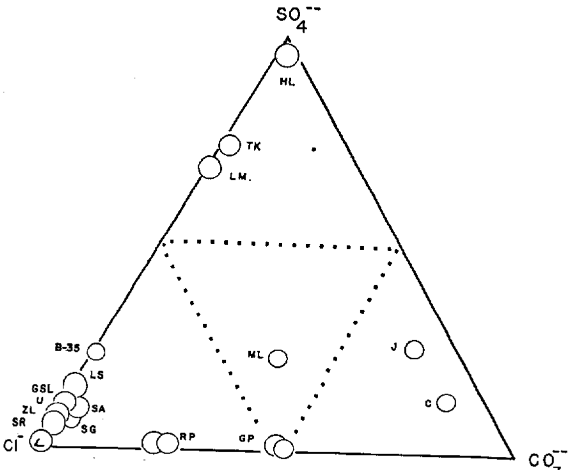

FIG. 1. Relative composition of the three principal anions in the waters of the Long-H Ranch, Ariz., and other *Artemia* habitats plotted on triangular coordinate paper. Sum of SO_4^{--}, CO_3^{--} and Cl^- by weight considered 100%. Fifty per cent lines shown for each anion by dotted line. Diameters of circles proportional to logarithms of total salinities by weight.
Symbols: **GP**, Green Pond, and **RP**, Red Pond, Ariz., March 1964 and more concentrated in August 64 (Cole and Whiteside 1965b); **ML**, Mono Lake, Cal. (Dunn 1953); **SR**, La Sal del Rey, Texas (Deevey 1957); **U**, Lake of Urmia and **SG**, Schor-göl, Iran (Löffler 1961); **GSL**, Great Salt Lake, Utah (Adams 1964); **SA**, Sambhar Salt Lake, India (Clarke 1924, Baid 1958); **LS**, Leslie Salt Refining Works, San Mateo, Cal. (Clarke 1924); **LM**, Little Manitou Lake, Saskatchewan (Rawson and Moore 1944); **TK**, Tso Kar, Tibet (Hutchinson 1937); **HL**, Hot Lake, Washington (Anderson 1958); **J**, Jesse Lake, and **C**, Cook Lake, Sheridan Co., Nebraska (McCarraher, personal communication); **B-35**, station #35, Ouargla Chott, Algeria (Beadle 1943); **ZL**, Zuñi Salt Lake, Quemado, New Mexico (Cole, unpubl. data).

principal anions; from Table 1 it can be seen that these are the sulfate and chloride lakes, maximum concentrations of carbonate being relatively low. They range from the extreme chloride type represented by La Sal del Rey, Texas (Fig. 1, SR; Deevey 1957), to the remarkable sulfate water of Hot Lake, Washington (Fig. 1, HL; Anderson 1958). Furthermore, there is little doubt that the Alviso salt ponds, California (Carpelan 1957), salterns in Puerto Rico and similar brine pools elsewhere that contain *Artemia* (Dexter 1956; Bowen 1964) are also chloride waters with relatively little carbonate and would fall on the plot close to La Sal del Rey or the Leslie Salt Refining Works in California. (Fig. 1, LS); probably the modal *Artemia* habitat is characterized by a predominance of NaCl and could be termed thalassohaline (Bond 1935), being much like concentrated sea water.

The occasional occurrence of *Artemia* populations in athalassohaline sulfate lakes has been pointed out by Macan (1963) and Hedgpeth (1959). Three such lakes are plotted in Figure 1 and all have relatively little carbonate (Table 1). In Hot Lake, magnesium and sulfate are the principal ions (Anderson 1958), and Na_2SO_4 is remarkably abundant in Tso Kar, Tibet (Fig. 1, TK; Hutchinson 1937), and Little Manitou Lake, Saskatchewan (Fig. 1, LM; Rawson and Moore 1944). In addition, the Algerian pool called Station #61 by Beadle (1943) contained *Artemia* and would fall on the Figure-1 plot very near Tso Kar, although with slightly more relative sulfate (Table 1).

Croghan (1958a) showed that pure solutions of 0.25 M Na_2SO_4 and $MgCl_2$ are slightly toxic to *Artemia*, although survival time is several days in 0.5 M solutions of NaCl. The concentration of sulfate in the four sulfate lakes (Table 1) surpasses the concentration used by Croghan, and in Hot Lake the magnesium also is well above the toxic threshold (Anderson 1958). Also, the *Artemia* of the Kalia potash works on the Dead Sea shore are living in a magnesium chloride solution greater than 0.25 molarity (Goldschmidt 1952). Various other ions in these waters may play a role in reducing the toxicity of sulfate and magnesium compounds.

ARTEMIA IN HIGH-CARBONATE WATERS

It has been the general opinion that high concentrations of carbonate and bicarbonate are lethal to *Artemia*. Croghan (1958a) reported that a molar solution of $NaHCO_3$ is rapidly toxic, bringing about loss of mobility and apparent death within 5 min. He suggested the lethal factor to be either high pH or the narcotic effects of bicarbonate. Macan (1963) summarized many data from saline lakes in Africa, Asia and North America, emphasizing that the brine shrimp occurred in no lakes of the soda type. Hedgpeth (1959) stated that *Artemia* seems to be generally absent from carbonate water and pointed out that it may be raised in the absence of carbonate.

Baid (1958) stated that *Artemia* in the Sambhar Salt Lake, India (Fig. 1, SA), begin to die in summer; he attributed this to increasing carbonate concentration although the values presented are puzzlingly low. As total salinity increased from 3.64 to 19.43%, the concentration of Na_2CO_3 ranged from 18.3–39.4 mg/liter and $NaHCO_3$ increased from 4.8–12.3 mg/liter. From these data a final carbonate figure of only 30.8 mg/liter can be inferred. Clarke (1924:175) presented results of analyses made in 1909 showing carbonate to be 2.19% of the total ions of the Sambhar Salt Lake, although total salinity was not given. If Baid's highest salinity figure is used to compute the carbonate ion from Clarke's data, the result is 4.27 g/liter, far below the lethal concentration of 60 g/liter to which Croghan (1958a) subjected *Artemia*. The mean of Baid's extremes for total salinity (115 g/liter) was used to calculate the amount of each anion shown in Table 1. This method yields a carbonate value of 2.52 g/liter. In 1909 there was only a trace of calcium and 0.01% magnesium in the lake water; it seems reasonable, therefore, to assume that most of the carbonate is associated with sodium. The lake is not a soda lake, however; it belongs in the chloride series, although it has the highest absolute and relative carbonate concentration of the group (Fig. 1, Table 1).

Löffler (1961) explained the absence of living *Artemia* in the Iranian Schor-göl on the basis of high carbonate; the sediments contained many recent exuvia. Schor-göl is plotted in Figure 1, SG, where it can be seen that carbonate is only about 4% by weight of the principal anions. The absolute carbonate value, derived from Löffler's alkalinity meq datum, is 790 mg/liter. This is the second highest amount in the chloride lakes of Table 1.

In light of the above, it is noteworthy that *Artemia* does occur in high-carbonate waters. Mono Lake, California (Fig. 1, ML; Table 1) contains about 12,125 mg/liter of carbonate and has a high pH of 10 or more

(Dunn 1953). Carbonate is about 38% of the three principal anions and Mono Lake is a triple water lake, employing Clarke's (1924) terminology to describe fairly concentrated water in which sulfate, chloride and carbonate are sub-equal. *Artemia* has been noted in Mono Lake since the time of Mark Twain (Clemens 1872).

The Kiatuthlanna waters, especially Green Pond with carbonate making up 50% of the major anions and ranging up to 46.16 g/liter (Table 1) and with pH values of 9.0–10.0, show that high carbonate and low hydrogen-ion concentrations are not lethal to some populations of brine shrimp.

Also, the mention of *Artemia* occurring in Soda Lake Washington (Whittaker and Fairbanks 1958; W. T. Edmondson, pers. comm.) led us to investigate what is known about the chemistry of its water. Whittaker and Fairbanks presented only the total salinity, about 10%, but the following information supplied by the U.S. Bureau of Reclamation (in litt.) shows that this was indeed a soda lake before it became inundated as part of the Columbia Basin Irrigation Project. The lake covered about 0.7 ha and its bed contained a conspicuous deposit of $Na_2CO_3 \cdot 10\ H_2O$ crystals; during late summer and early autumn the water was in saturated equilibrium. In spite of this, Soda Lake supported a large population of *Artemia* that imparted a red hue to the water.

Perhaps the most remarkable brine shrimp habitats, however, are the potash ponds of Sheridan and Garden Co., Nebraska, which are extremely high in carbonate and characterized by pH values of 10 (D. B. McCarraher, pers. comm.). Two of these, Jesse Lake (Fig. 1, J) and Cook Lake (Fig. 1, C) have high relative carbonate concentrations, therefore falling far to the right on the triangular coordinate plot. The absolute carbonate for these two lakes (Table 1) implies 0.57–0.78 M solutions of sodium carbonate. Carbonate data from one other lake in Sheridan Co., and three in Garden Co. also are high (Table 1). These data are all from McCarraher at the State Limnology Laboratory of Nebraska.

A final note on the toxic properties of soda water is based on our preliminary experiments with *Artemia*. We have found that placing *Artemia* in a molar solution of $NaHCO_3$ results in distress and almost immediate immobility—the condition described by Croghan (1958a). Within 20–30 min, however, the animals recover and swim about normally for 3 or 4 days in the medium. This is the case using either *Artemia* from the high-carbonate Long-H ponds or those from the Zuñi Salt Lake near Quemado, N.M. (Fig. 1, ZL). This lake is a good example of chloride water with a low carbonate content, only 0.2% of the principal anions, and an absolute value of but 186 mg/liter (Table 1).

ARTEMIA IN POTASSIUM-RICH WATERS

Croghan (1958a) confirmed the observations of earlier workers (Martin and Wilbur 1921; Boone and Baas-Becking 1931) that potassium salts are highly toxic to *Artemia*, and showed (Croghan 1958b) that the rapidity of death in a potassium-rich medium is caused by a fast exchange of potassium for sodium with a concurrent rise in the haemolymph potassium concentration. Croghan's (1958a) experiments also confirmed previous reports that sodium exerts an "antagonistic" effect, and protects against potassium. Using KCl and NaCl mixtures, he showed that 100 mM of KCl was lethal unless the Na/K ratio by weight was 10 or greater; prolonged survival occurred in a mixture where the ratio was 10.6 (100 mM KCl, and 1,800 mM NaCl), but the crustaceans began to die after 6 hr in a mixture of 100 mM KCl and 1,000 mM NaCl where the Na/K ratio by weight would be 5.9. In mixtures where the ratio was smaller, almost all *Artemia* had died by 3 or 4 hr. The reason for this is not clear, but Holmes and McBean (1964) suggest that sodium from sea water ingested by the marine green turtle may act as a "vehicle" for the excretion of excess potassium derived from its food.

Most brine-shrimp waters, including those plotted in Figure 1, have a marked excess of sodium over potassium. An exception is Tso Kar, where the relative concentrations were considered critical by Hutchinson (1937); his data reveal 16.3 and 5.5 g/liter of Na and K, respectively, a ratio of about 3. Also, the occurrence of *Artemia* in pools of the Kalia potash works by the Dead Sea (Goldschmidt 1952) has ecological implications that have been overlooked. Goldschmidt's chemical data are incomplete, but the water contains at least 2.2 g/liter of potassium and the Na/K ratio is about 3.9 by weight.

The lowest Na/K ratios and the highest absolute potassium values, however, come from *Artemia* sites in Nebraska, although at present Jesse Lake and Cook Lake are not particularly unusual (Table 1) and there are discrepancies in the data. The ratio for the former reported by Hicks (1920) was 0.85, but recently McCarraher (pers. comm.) reported 12.5 and a potassium value of 1.6 g/liter. In Cook Lake, with 26.4 g/liter of sodium, the ratio from McCarraher's data is 307. In five other ponds in southern Sheridan and northern Garden counties, where McCarraher finds brine shrimp, the Na/K ratios range from 0.9–3.4 (Table 1) and potassium occurs in amounts from 5.2–16.0 g/liter, well above Croghan's lethal value of 3.9 g/liter (computed from 100 mM of KCl).

DISCUSSION AND CONCLUSIONS

Probably most brine-shrimp localities are thalassohaline, with ionic proportions much like sea water. Thus, many other brines in which *Artemia* occurs are similar to those waters listed as chloride lakes in Table 1 and would fall near the lower left corner in the triangular plot, Figure 1. There are a few examples in the literature of brine shrimp in athalassohaline waters of the sulfate type, but it has been believed generally that *Artemia* is absent from athalassohaline ponds of the carbonate type, and this crustacean has never been included in lists of typical natrophils (see Löffler 1961; Hedgpeth 1959). In spite of this, populations in Mono Lake, the Long-H ponds of Arizona, and especially in the Nebraska lakes studied by McCarraher show that high absolute and relative carbonate does not preclude establishment and survival *Artemia* in some instances. Moreover, the occurrence of the brine shrimp in several Nebraska potash ponds with low Na/K ratios belies earlier conclusions regarding the toxicity of potassium-rich media with relatively low accompanying sodium concentrations.

Discrepancies and contradictions concerning the effects of various ions and their relative concentrations may have a simple explanation, however. The taxonomic status of *Artemia* is not settled. Cytogenetic work has shown various chromosome complexes (Goldschmidt 1952), and certain parthenogenetic races are reproductively isolated from normal bisexual populations (see Bowen 1964). In addition, there is evidence for physiological races which are reproductively isolated by ecological barriers, probably of a chemical nature. Bowen (1964) reported that *Artemia* from Mono Lake will not live in a standard culture medium made by adding 50 g of NaCl to 1 liter of sea water. Moreover, she found that Mono Lake water was lethal to *Artemia* from the Great

Salt Lake and commercial salterns on the California coast. She was unable to develop a compatible medium that would permit crossing the Mono Lake brine shrimps with the others. Mono Lake is one of several California lakes characterized by unusually high boron content (Wetzel 1964) in addition to its remarkable carbonate and high pH. These data emphasize the importance of stating the locality from which experimental *Artemia* are derived; there could be a host of sibling species and the older concepts concerning ionic limiting factors may not apply to all of them.

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CONSTRUCTION AND PERFORMANCE OF A TEMPERATURE-GRADIENT BAR AND CHAMBER

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Abstract. An inexpensive temperature-gradient bar and chamber for use in studies of seed germination and root growth is described. Aluminum troughs and a bar, surrounded by insulation and heated and cooled at their ends by water baths, form the floor of a chamber. A plexiglass top permits illumination by overhead light banks. The temperature gradient may be adjusted to any range within the limits of 10° and 50°C. The linearity and stability of the bar gradient, and the presence of temperature and humidity gradients in the chamber air above the bar, are discussed.