# THE OSMOTIC ADAPTATION OF THE FAIRY SHRIMP *BRANCHINECTA CAMPESTRIS* LYNCH TO SALINE ASTATIC WATERS[1]

*Edmund S. Broch*
Department of Zoology, Washington State University, Pullman 99163

## ABSTRACT

The fairy shrimp *Branchinecta campestris* was found in association with *Artemia salina* (L.) in four saline astatic lakes in Okanogan County, Washington, and in British Columbia. In all four lakes sulfate was the predominant ion making up in milliequivalence 48% of the total ions. The predominant cations sodium and magnesium occurred in varying proportions. The osmotic concentration of Penley Lake from hatching to death of the adult population of *B. campestris* increased progressively from 36 to 660 mOsm/liter. At concentrations of 286 mOsm/liter and less, *B. campestris* regulates hyperosmotically. However, from 451 to 660 mOsm/liter *B. campestris* is poikiloosmotic and not a hypoosmotic regulator like *A. salina*. Therefore, its ability to live in saline astatic waters can be partly attributed to tolerance of a high hemolymph concentration.

Coexistence between *A. salina* and *B. campestris* is brief. *B. campestris* is restricted to the early phase of the saline astatic lake, characterized by low but increasing salinity and temperature, whereas *A. salina* appears a month later and is restricted to the high salinity-temperature phase of the lake. Factors responsible for temporal separation of these two species appear to be differences in osmoregulation and in factors regulating development and hatching.

## INTRODUCTION

The occurrence of the fairy shrimp *Branchinecta campestris* Lynch with the brine shrimp *Artemia salina* (L.) in saline astatic waters raises the question of the type of osmoregulation of *B. campestris.* Lynch (1960) reported that often both species occurred simultaneously and at other times only one or the other species was present. Hartland-Rowe (1966) found *B. campestris* with *A. salina* in waters ranging in specific conductance from 30,000 to 80,000 μmhos/cm (approximately 600 to 1,600 mOsm/liter). However, he gives no empirical data of the osmotic concentration of these waters. He classified *B. campestris* with *A. salina* as a high salinity stenohaline species.

Hypoosmotic regulation in *A. salina* is well known from the work of Croghan (1958*a, b, c, d*). Whether this adaptation is unique within the Anostraca or whether other groups have adapted to saline waters by evolving the same osmoregulatory mechanism is not known. However, of the species of Anostraca, *B. campestris* by reason of its association with *A. salina* seemed most likely to be osmotically similar to *A. salina.*

Investigations of osmoregulation in other species of Anostraca have not only been few, but so far restricted to freshwater forms. Published work by Krogh (1939) on *Branchipus* sp., Panikkar (1941) on *Chirocephalus diaphanus* Prevost, and Ralph (1967) on *Branchinecta gaini* Daday establishes these species as typical hyperosmotic regulators. Further study of the physiological adaptation of fairy shrimp to saline astatic waters should be of interest since the ionic composition of these waters differs from both marine and brackish waters, and their great seasonal fluctuations in salinity set them apart from fresh waters.

From an ecological view, the temporary saline pond represents an extreme environment with a unique fauna. Furthermore, because of its spatial-temporal restrictions and its relatively few numbers of species

---

[1] Preliminary research was supported in part by a Washington State University Medical and Biological grant; subsequent research was supported by National Science Foundation Grant GB-5052.

TABLE 1. *Ionic composition of* Branchinecta campestris *waters*

| Ionic species | Penley Lake |  | Basque No. 5 |  | Hot Lake |  |
|---|---|---|---|---|---|---|
|  | mM/liter | % meq/liter | mM/liter | % meq/liter | mM/liter | % meq/liter |
| $Na^+$ | 321.7 | 44.60 | 360.8 | 30.56 | 452.1 | 7.3 |
| $K^+$ | 0.3 | 0.04 | 1.7 | 0.17 | 25.6 | 0.41 |
| $Mg^{2+}$ | 8.1 | 2.21 | 111.2 | 18.67 | 1,295.8 | 41.8 |
| $Ca^{2+}$ | 4.5 | 1.20 | 5.2 | 0.85 | 2.0 | 0.06 |
| $CO_3^{2-}$ | — | — | — | — | — | 0.13 |
| $HCO_3^-$ | 2.8 | 0.0028 | 7.8 | 0.67 | 33.8 | 0.53 |
| $SO_4^{2-}$ | 173.4 | 48.11 | 286.4 | 48.55 | 1,510.4 | 48.8 |
| $Cl^-$ | 24.4 | 3.38 | 6.3 | 0.51 | 52.9 | 0.85 |

it offers many advantages pursuant to ecological problems.

Multispecies populations of fairy shrimp are well known. However, little is known concerning the physiological differences or the degree of competition between coexisting species. A portion of this paper is concerned with problems of coexistence between *A. salina* and *B. campestris.*

## METHODS

After collection, the animals were kept as close as possible to the temperature of the water. Hemolymph samples were taken approximately 48 hr after collection, using a procedure similar to that described by Croghan (1958*b*). After rinsing with deionized water and blotting with tissue (Kimwipes), the animal was submersed in mineral oil to prevent evaporation. A dorsal abdominal puncture was made and the hemolymph pipetted into microquartz tubing. A Nanoliter osmometer (Advanced Instruments, Inc.) was used for all freezing-point determinations of both hemolymph and medium. Each hemolymph value is the mean of single samples from 10 individuals. Each value for the concentration of the medium is the mean of 10 samples.

To determine the upper limit of concentration endured by *B. campestris,* groups of 50 adult animals were placed in Penley Lake water of 60, 600, 700, and 850 mOsm/liter and maintained at 18C. Different concentrations were made by evaporation or dilution of the lake water. The animals used were collected on 20 April 1968. Survival percentage was determined over 48 hr.

Ecological data and surface water samples were taken weekly, and plankton samples were taken to determine levels and structure of *B. campestris* and *A. salina* populations. Only the appearance and disappearance of each population will be reported here. Water was analyzed according to the Am. Public Health Assoc. (1965).

## OBSERVATIONS

### *Habitats of* B. campestris

Populations of *B. campestris* were found in four saline lakes, in each of which *A. salina* also occurred.

Analyses for three *B. campestris* habitats are presented in Table 1. The data are based on samples from Penley Lake and Basque No. 5 taken on 6 April and 9 May 1968 respectively. Both lakes contained populations of both animals on these dates. However, the data for Hot Lake are based on a sample taken after the disappearance of *B. campestris* when only *A. salina* was present. In spite of the higher concentration of the water at this time, the relative composition of the principal ions remained relatively constant (Langbein 1961).

Penley Lake is located in the Omak Basin, a portion of the Okanogan Plateau, in Okanogan County, Washington. It lies in an undrained depression at an altitude of between 719 and 732 m (Bennett 1962). At its maximum depth (32.5 cm) the lake covers approximately 4.4 ha. Deposits of permanently crystallized salt, predominantly mirabilite ($Na_2SO_4 \cdot 10\ H_2O$), occur

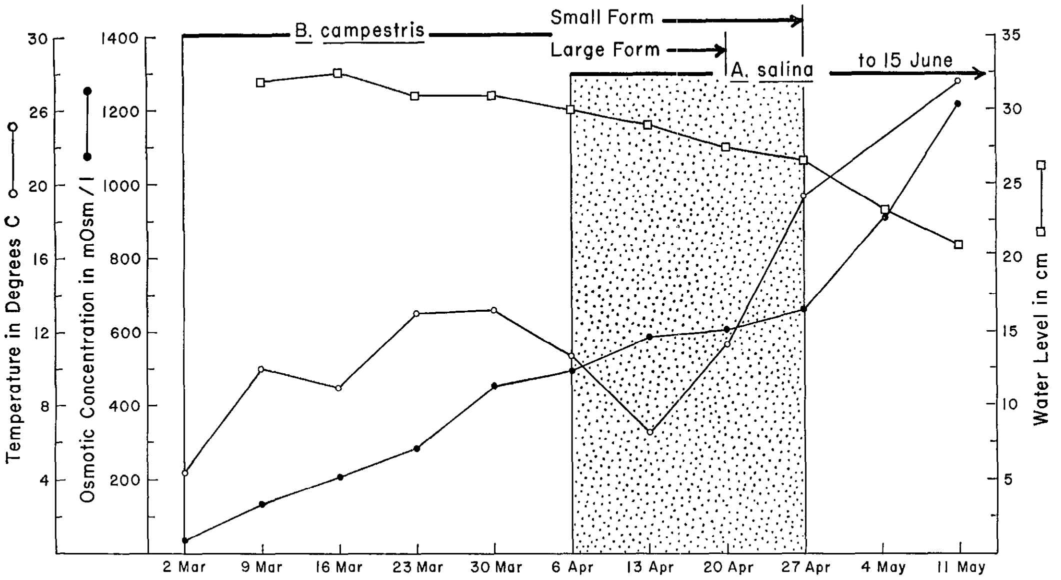

FIG. 1. Changes in water level, temperature, and osmotic concentration in Penley Lake as a function of time. Horizontal arrows at the top of the graph represent population phases of the large and small forms of *Branchinecta campestris* and of *Artemia salina*. The period of coexistence is indicated by the shaded area.

as circular pans at the surface of the lake beds and are separated by lake-bed mud. The arrangement of these pans gives the lake the appearance that led Jenkins (1918) to refer to such lakes as 'spotted lakes'.

The fauna of Penley Lake consists of three species of macroscopic animals, *A. salina*, *B. campestris*, and a brine fly: *Ephydra pectinulata* Cresson. The latter was identified by Dr. M. James of Washington State University.

Penley Lake is a sodium sulfate lake of the periodic astatic type. It dries up in late June and does not refill until the late winter or spring thaw. Sodium and sulfate ions constitute 92.4% of the total principal ions in terms of mM/liter (Table 1). Cameron Lake, located about 1.6 km from Penley Lake, is similar in ionic composition and fauna, though no data are presented for it.

Basque No. 5 is a sodium sulfate lake about 61 m in diameter (Goudge 1926). On 6 April 1968 the lake depth was 12 cm. Like Penley Lake, it is a periodic astatic lake that had, in addition to populations of *A. salina*, *B. campestris*, and *Ephydra* species, larvae of the mosquito, *Aedes dorsalis* (Meigen). Sodium and sulfate make up 82.8% of the total concentration in terms of mM/liter. It has a lower percentage of sodium and sulfate ions than Penley Lake due to a higher magnesium concentration.

Anderson (1958) reported an unknown species of *Branchinecta* occurring with *A. salina* in Hot Lake, Okanogan County, Washington. Lynch (1960) later described it as *B. campestris*. Hot Lake differs from the other three waters in the predominance of magnesium over sodium. Magnesium and sulfate made up 83.2% of the total principal ions in terms of mM/liter, whereas sodium makes up only 13.4% (Table 1). It also differs in that it is a perennial astatic lake with a pronounced meromixis accompanied during summer by an extreme temperature inversion (Anderson 1958). The depth on 14 July 1968 was 2.7 m.

### *Life cycle and coexistence*

Figure 1 shows the changes in lake level, temperature, osmotic concentration in milliosmoles, and population phases in Penley Lake.

There was no standing water until 3 February 1968. For three weeks after this, conditions varied from standing water to frozen slush and ice. From 9 to 16 March the osmotic concentration of the lake increased in spite of an increase in water level (Fig. 1), evidently because of gradual solution of salts and mixing. Conductivity was measured in different parts of the lake and at different depths on each collection date. However, no vertical or horizontal salinity or temperature gradients were detected in the lake.

On 2 March 1968, *B. campestris* metanauplii appeared in great number. But *A. salina* did not appear until 6 April at which time *B. campestris* was mature and females contained eggs in the ovisac. The period of coexistence, 6 to 27 April, was characterized by increasing numbers and rapid growth of *A. salina* and decreasing numbers of *B. campestris*. The population of the latter disappeared by 27 April 1968, whereas the *A. salina* population continued until the middle of June.

The data show that coexistence between these two species is brief—21 days (Fig. 1). Each species occupies the habitat at a different time and under different ecological conditions. The *B. campestris* is restricted to the early phase of the saline astatic lake, characterized by low but increasing salinity and temperature. In contrast, *A. salina* hatches a month later and is restricted to the high salinity-temperature phase of the lake. These different life cycles occurred in all four of the lakes studied.

By 6 April 1968, it became apparent that there were two different morphological forms of *B. campestris* in Penley Lake. A form closely resembling Lynch's (1960) original description of the species is here referred to as the large form; a second, much smaller, morphologically distinct form is referred to as the small form. Although both forms were sexually mature on 13 April 1968, the large form reached sexual maturity at least a week earlier than did the small form. The large form was present on 20 April but disappeared by 27 April, whereas the small form was present on 27 April and gone by 4 May. During this period the concentration of the pond increased from 604 to 732 mOsm/liter. Therefore, in nature, the small form survived longer under higher salinities than the large form. This might be attributed to environmentally induced modifications, as a consequence of the later hatch, resulting in physiological as well as morphological differences within the population. However, the causal factors and the salinity tolerance levels of both forms will have to be investigated in the laboratory.

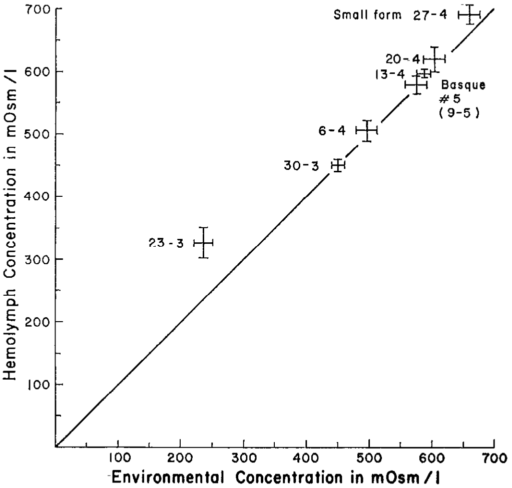

FIG. 2. Hemolymph concentration of *Branchinecta campestris* as a function of environmental concentration. Vertical lines indicate plus or minus one standard deviation from the means of 10 hemolymph samples. Horizontal lines indicate the same for water samples. Sampling dates are to the left of each value. All data are from Penley Lake and the large form *B. campestris* except as otherwise designated.

### *Osmoregulation*

Hemolymph concentration for *B. campestris* is plotted as a function of environmental concentration in Fig. 2 for each collection date beginning with 23 March. The hemolymph value for the 27 April collection is of the small form as no large form animals were present at that time.

Included with the Penley Lake data are data from a collection made at Basque No. 5 in British Columbia. In waters as saline as 320 mOsm/liter, but not as saline as 450 mOsm/liter, *B. campestris* is a hyperosmotic regulator, while at higher concentrations it is isoosmotic (i.e., the hemolymph concentration follows the environmental concentration). Thus *B. campestris* is not a hypoosmotic regulator like *A. salina.* Its ability to live in saline astatic waters can be partly attributed to its tolerance of a high internal ionic concentration.

The percentage survival of the large form after 48 hr at different salinities was as follows:

| Concn (mOsm/liter) | Survival (%) |
|---|---|
| 60 | 78 |
| 600 | 84 |
| 700 | 57 |
| 850 | 0 |

At 850 mOsm/liter, all animals died within 24 hr. Thus, the upper limit of concentration endured by adult *B. campestris* was above 600 but less than 850 mOsm/liter. This compares closely with the disappearance of the population in nature (*see* Fig. 1).

## DISCUSSION

Classification of inland temporary saline waters appears to have defied limnologists. According to the classification of Decksbach (1929), Hot Lake is a perennial astatic lake whereas the other three are periodic astatic lakes. Although this classification does emphasize the dominant feature of these waters, namely their unstable water level and volume, it does not give an indication of their variable or seasonable high salinity. However, in the absence of suitable terminology the lakes are referred to simply as saline astatic waters.

The environments of *B. campestris* were predominantly sodium sulfate and magnesium sulfate waters. The ponds are remarkably similar in their sulfate level, sulfate making up in milliequivalence 48% of the total principal ions (Table 1). Whether this is coincidental or an actual chemical requirement for *B. campestris* is not known; further information on *B. campestris* waters and laboratory experiments on survival in media varying in ionic ratios are needed. Of the anions, chloride was third in concentration making up 0.5% to 4.5% of the total ions in terms of milliequivalence. Finally, the low carbonate and bicarbonate values of these waters seems to be generally characteristic of *A. salina* habitats, though Cole and Brown (1967) point out some exceptions.

In contrast to the essentially similar anion ratios of these waters, the predominant cations, sodium and magnesium, occur in varying proportions. Thus, in Penley Lake the ratio of sodium to magnesium in terms of % meq is 20:1; in Basque No. 5, 1.6:1; and Hot Lake, 1:5.7.

Hartland-Rowe (1966) found *B. campestris* in waters ranging in conductivity from 30,000 to 80,000 $\mu$mhos/cm. He equates 100 units of conductivity to 60 mg/liter sodium chloride. Therefore in milliosmoles these waters range from approximately 600 to 1,600 mOsm/liter. The lower value is comparable to the findings of this study, but the upper value is much higher. However, the upper limit of concentration would depend on the time that samples were taken. If sampling followed a rapid increase in concentration by evaporation some individuals might still be alive though death would occur shortly thereafter. This might explain reports of *B. campestris* in more saline waters than those reported here. If one considers the range of salinity from hatching to death of the adult population, it is apparent that *B. campestris* is euryhaline, since it was found in nature at concentrations from 36 to 651 mOsm/liter.

The adaptation of species to their environments has involved the evolution of mechanisms that synchronize each phase of the life cycle with the environmental conditions most favorable for survival. Broch (1965) has pointed out that the synchronization of hatching to pond formation constitutes an important adaptation of many species of fairy shrimp to temporary waters. The adaptation of *B. campestris* to saline astatic waters involves both the tolerance of a high blood concentration and the synchronization of the active portion of the life cycle to the low salinity phase of the pond. The low initial concentration of the pond (36 mOsm/liter) and the hyperosmotic condition on 23 March 1968 suggest an initial hyperosmotic regulation followed by isoosmotic condition as the salinity increases with evaporation of water. However, the data on hemolymph concentration were obtained for the higher salinity phase of the pond to determine whether this species is similar osmotically to *A. salina*. The latter is a hypoosmotic regulator at the range of salinities investigated, though laboratory investigations have shown that it regulates hyperosmotically in media of less than 25% seawater (Croghan 1958*c*).

The osmotic behavior of *B. campestris* is similar to the freshwater *Gammarus lacustris*. Sutcliffe and Shaw (1967) were able to acclimate this species to a medium of 650 mOsm/liter, though the animals gradually died off. They also report that above 100 mOsm/liter the osmotic concentration of the blood approached the concentration of the environment but remained slightly hypertonic to it.

A further consequence of the differences in life cycles of *B. campestris* and *A. salina* is that the coexistence of these two species is minimized. The inability of *B. campestris* to hypoosmoregulate restricts it to the low salinity phase of the pond. On the other hand, the evolution of hypoosmotic regulation enables *A. salina* to occupy the high salinity phase without competition from other species of Anostraca and essentially free from predators. However, the primary mechanism of the separation of life cycles resides in the embryonated egg in which development leading to hatching is regulated by seasonally induced environmental factors ensuring hatching at the proper ecological moment (Broch 1965).

The factors regulating development and hatching of *B. campestris* have not been investigated, but many investigations of hatching of *Artemia* eggs suggest that hatching of *A. salina* is regulated primarily by temperature. According to Dutrieu (1960), low temperatures inhibit development of *A. salina* and nauplii begin to appear in nature when the temperature reaches 10C. Hatching of *A. salina* in Penley Lake began at temperatures from 10 to 13C. In contrast low temperatures did not inhibit development and hatching of *B. campestris*, since the initial hatch in Penley Lake took place at 4C. Moore (1963) reported on some interspecies relationships of Anostraca populations of certain Louisiana ponds. He described a seasonal separation of populations of *Eubranchipus holmani* and *Streptocephalus seali* in the same temporary pond, which he attributes to difference in hatching temperatures.

Dutrieu (1960) reported that hatching of *A. salina* occurs in concentrations from distilled water to a maximum of 1,367 mOsm/liter sodium chloride. Hatching of *A. salina* in Penley Lake occurred at approximately 451 to 495 mOsm/liter; thus salinity *per se* does not appear to be a factor in its delayed hatch. Hatching of *B. campestris* coincided with the low salinity period, beginning at 36 mOsm/liter. Since no metanauplii of this species were seen during the *A. salina* phase, it is possible that high salinities or high temperatures or both inhibit hatching of *B. campestris*. Given the osmotic limits of the animals and the nature of the environment, this could be an important adaptation for increasing the chance of attaining the reproductive stage. However this remains to be tested.

Adaptation to high salinities necessitates other physiological adaptations besides those of osmoregulation. The decrease in oxygen levels with increase in salinity requires an effective mode of oxygen uptake. Gilchrist (1954) has shown that in *A. salina* there is an increase in blood hemoglobin in response to a decrease of oxygen level as salinity increases. The increased blood hemoglobin is readily observed in the animal. No such phenomenon was visible in *B. campestris*. Therefore, it is possible that reduced oxygen as a result of increased salinity may be a factor limiting *B. campestris* to a concentration below 650 mOsm/liter, rather than simply its inability to hypoosmoregulate.

The presence of two distinct morphological forms of *B. campestris* raises an interesting question. One can conjecture that the small form is environmentally induced. Individuals hatching at a later time would develop under higher salinities and temperature, lower oxygen levels, and different competitive circumstances. Studies on the effect of salinity on growth and form of *A. salina* provide a working hypothesis. Baid (1963) reported that female *A. salina* reared in 12.5% salinity have a higher growth rate than those reared in 6.5% salinity, but the size of the adult varies inversely with the salinity of the medium. Weisz (1947) showed that sexual maturity was reached sooner at high salinities than at low salinities. Possibly the small form of *B. campestris* hatched later, developed more rapidly due to the higher salinities, and reached sexual maturity only slightly later than the large form. Such a control mechanism would constitute an important adaptation to saline astatic waters by increasing the probability of reproduction in the event of a shortened seasonal succession of the temporary pond.

Associated with the morphological divergence within the population there appears to be a distinct physiological difference, evidenced by the ability of the small form to survive longer at increased salinity. According to Smith (1959), owing to the discontinuous and extreme nature of the brackish-water habitat, adult populations may be produced that are genetically alike but differ physiologically as a result of environmental conditions during ontogeny. Population declines in fairy shrimp have often been attributed to senility (*see* Moore 1963). Whether the earlier disappearance of the large form is due to senility, or whether the small form manifests physiological differences, has not been investigated.

Finally, the presence of *A. salina* and *B. campestris* in waters so different ionically from seawater and freshwater raises some interesting questions concerning ionic regulation. Croghan (1958*b*) found that Na and Cl account for almost all the osmotic pressure of the hemolymph of *A. salina*. It would appear that the increase in the osmotic concentration of the hemolymph with the environment is due to increase in sodium chloride. The mechanisms by which these species regulate ionically in view of the high Mg : Na and $SO_4$ : Cl ratios merit further investigation.

## REFERENCES

American Public Health Association. 1965. Standard methods for the examination of water and wastewater, 12th ed. APHA, New York. 592 p.

Anderson, G. C. 1958. Some limnological features of a shallow saline meromictic lake. Limnol. Oceanog., **3**: 259–270.

Baid, I. C. 1963. The effect of salinity on growth and form of *Artemia salina* (L). J. Exptl. Zool., **153**: 279–284.

Bennett, W. A. G. 1962. Saline lake deposits in Washington. Wash. State Dept. Conserv. Bull. 49. 129 p.

Broch, E. S. 1965. Mechanism of adaptation of the fairy shrimp *Chirocephalopsis bundyi* Forbes to the temporary pond. Cornell Univ. Agr. Expt. Sta. Mem. 392. 48 p.

Cole, G. A., and R. J. Brown. 1967. The chemistry of *Artemia* habitats. Ecology, **48**: 858–861.

Croghan, P. C. 1958*a*. The survival of *Artemia salina* (L.) in various media. J. Exptl. Biol., **35**: 213–218.

———. 1958*b*. The osmotic and ionic regulation of *Artemia salina* (L.). J. Exptl. Biol., **35**: 219–233.

———. 1958*c*. The mechanism of osmotic regulation in *Artemia salina* (L.): the physiology of the branchiae. J. Exptl. Biol., **35**: 234–242.

———. 1958*d*. The mechanism of osmotic regulation in *Artemia salina* (L.): the physiology of the gut. J. Exptl. Biol., **35**: 243–249.

Decksbach, N. K. 1929. Sur klassifikation der gewasser vam astatischen typus. Arch. Hydrobiol., **20**: 399–406.

Dutrieu, J. 1960. Observations biochimiques et physiologiques sur le developpement d'*Artemia salina* Leach. Arch. Zool. Exptl. Gen., **99**: 1–133.

GILCHRIST, B. 1954. Hemoglobin in *Artemia.* Proc. Roy. Soc. (London), Ser. B, **143:** 136–146.

GOUDGE, M. F. 1926. Magnesium sulphate in British Columbia, p. 62–80. *In* Investigations of mineral resources and the mining industry, 1924. Can., Dept. Mines Tech. Surveys. Geol. Survey Canada, Ottawa.

HARTLAND-ROWE, R. 1966. The fauna and ecology of temporary pools in Western Canada. Intern. Ver. Theoret. Angew. Limnol. Verhand., **16:** 577–584.

JENKINS, O. P. 1918. Spotted lakes of epsomite in Washington and British Columbia. Am. J. Sci., **196:** 638–644.

KROGH, A. 1939. Osmotic regulation in aquatic animals. Cambridge. 241 p.

LANGBEIN, W. V. 1961. Salinity and hydrology of closed lakes. U.S. Geol. Surv. Profess. Papers. No. 412, p. 1–20.

LYNCH, J. E. 1960. The fairy shrimp *Branchinecta campestris* from Northwestern United States. Proc. U.S. Natl. Museum. No. 112, p. 549–561.

MOORE, W. G. 1963. Some interspecies relationships in Anostraca populations of certain Louisiana ponds. Ecology, **44:** 131–139.

PANIKKAR, N. K. 1941. Osmotic behaviour of the fairy shrimp, *Chirocephalus diaphanus.* J. Exptl. Biol., **18:** 110–114.

RALPH, R. 1967. The osmotic and ionic regulation of *Branchinecta gaini* Daday. Phil. Trans. Roy. Soc. (London), Ser. B, **252:** 339–341.

SMITH, R. I. 1959. Physiological and ecological problems of brackish waters. Oregon State Coll. Biol. Colloq., **20:** 59–69.

SUTCLIFFE, D. W., AND J. SHAW. 1967. The sodium balance mechanism in the fresh-water Amphipod, *Gammarus lacustris* Sars. J. Exptl. Biol., **46:** 519–538.

WEISZ, P. B. 1947. The histological pattern of metameric development in *Artemia salina.* J. Morphol., **81:** 45–89.