J. Insect Physiol. Vol. 34, No. 10, pp. 903–909, 1988
Printed in Great Britain.

0022-1910/88 $3.00 + 0.00

OSMOREGULATION IN AN ALKALINE SALT LAKE INSECT, *EPHYDRA (HYDROPYRUS) HIANS* SAY (DIPTERA: EPHYDRIDAE) IN RELATION TO WATER CHEMISTRY

DAVID B. HERBST†, FRANK P. CONTE and VICTOR J. BROOKES*
Department of Zoology and *Department of Entomology, Oregon State University, Corvallis, OR 97331, U.S.A.

(*Received* 24 *September* 1987; *revised* 9 *February* 1988)

Abstract—Larvae of the alkali fly *Ephydra hians*, from Mono Lake (California), were exposed to osmotic concentrations ranging from distilled water to over 6000 mOsm of either alkaline Mono Lake water, or non-alkaline sea water/sodium chloride solutions. Larvae were capable of both hyper- and hypo-osmotic regulation of haemolymph osmolality (at around 300 mOsm) in Mono Lake water, but this ability was less effective in sea water/sodium chloride. In addition, higher mortality rates in sea water/sodium chloride suggest larvae are less tolerant of this chloride-dominated water chemistry. Pre-acclimation of larvae in sea water/sodium chloride did not improve survival in higher concentrations of this solution relative to larvae acclimated to Mono Lake water. At and above 200 g/l total dissolved solids (about 4800 mOsm) of Mono Lake water, larval survival is severely reduced, in association with osmotic dehydration. Sodium and chloride are the major haemolymph osmolytes, comprising about 70% of the total osmotic concentration. The results suggest that this species is alkali-adapted, and that restriction in habitat distribution from non-alkaline chloride waters and salinities above 200 g/l has a physiological basis.

Key Word Index: Osmoregulation, Ephydridae, saline lake, Mono Lake, *Ephydra*

INTRODUCTION

Among aquatic insects, members of the shore fly family Ephydridae are well-known for their tolerance of severe environmental conditions. High temperatures and salinities, acid and alkaline pH, anoxia and ephemeral waters are among the factors to which a variety of ephydrids have become adapted. Hot springs, tidal splash pools, salt evaporation ponds, hypersaline desert lakes, and even crude petroleum have been described as larval habitats (Wirth, 1971).

Collection records for species in the genus *Ephydra*, which are common in saline waters, indicate a wide range in chemical composition and salinity is tolerated by this group, but that any one species tends to be restricted to a particular type of habitat water chemistry (Simpson, 1979). Although surveys of plant and animal distribution in relation to the physical and chemical characteristics of saline habitats outline the potential range of different species (e.g. Bayly and Williams, 1966), such surveys do not indicate the influence of salinity and water chemistry on the relative abundance and viability of populations in variable environments. What is needed to identify the limitations of salinity on growth and survival, or specializations within populations to local chemical conditions, are studies of physiological adaptations to variation in osmotic and ionic conditions. These adaptations can restrict species distributions to a narrow range of habitats, and may account in part for the high proportions of endemic faunas in desert aquatic ecosystems (Collins, 1977; Smith, 1978; Hendrickson and Kubly, 1984).

First described as the alkali fly by Aldrich (1912), *Ephydra (Hydropyrus) hians* characteristically inhabits alkaline salt lakes in western North America, from Mexico to British Columbia, with its distribution centred in the Great Basin. Eggs, 3 larval instars, and pupae of the alkali fly are entirely aquatic, and are usually found in lentic waters containing moderate to high concentrations of carbonates, and to a lesser extent, chloride and sometimes sulphate, salts of sodium (pH normally >9.0). The salinity range of *E. hians* habitats, though difficult to reconstruct because of incomplete collection records in the literature and museums, appears to be from <5 g/l to about 200 g/l total dissolved solids. Larvae and adults feed on algae (primarily diatoms) and detritus.

The objective of the present study is to examine whether a physiological basis exists for the apparent alkaline water chemistry preference of *E. hians*, in terms of salt tolerance, and osmoregulatory ability, upon exposure to either natural alkaline lake water, or sea water/sodium chloride solutions. The results of this study, for *E. hians* larvae from Mono Lake (California), demonstrate an expected lower salt tolerance and poor osmoregulatory ability in sea water/chloride brines compared to alkaline lake water. In addition, above 150–200 g/l total dissolved solids of Mono Lake water, larval survival is substantially reduced while haemolymph osmotic concentration increases, and blood volume and body water decrease.

†Present address: Sierra Nevada Aquatic Research Laboratory, University of California, Star Route 1, Box 198, Mammoth Lakes, CA 93546, U.S.A.

IP 34/10—A

MATERIALS AND METHODS

Collections

The third (final)-instar larva of *E. hians* is the longest-lived stage of the larval life cycle, and was the subject of the physiological studies reported here. As in other salt lake invertebrates such as the brine shrimp (Conte *et al.*, 1972) and brine mosquito (Bradley, 1976), early-instar larvae of the alkali fly are considerably more intolerant of salinity increases than later instars (Herbst, unpublished). However, due to the difficulty in obtaining enough haemolymph from early instars, and the emphasis in this study on comparisons of physiological responses, only final-instar larvae were used in studies of osmoregulation.

Larvae were collected from shallow tufa (limestone rock that forms in some alkaline lakes) substrates at Mono Lake (California) during summer months (June–September 1978–87). During this period of time the salinity of Mono Lake varied between approx 80–100 g/l total dissolved solids (Herbst, 1986; unpublished data). Larvae were transported in cooled containers on moist paper towels, and used in the experiments outlined below within 72 h of collection.

Salt solutions

Concentrated salinities of Mono Lake water, up to 300 g/l, were prepared either by boiling, or by evaporation at low temperatures (35–40°C) under vacuum in a rotary evaporator. Neither method produced precipitation of salts at the acclimation temperatures used in experiments. Salinities lower than that of natural Mono Lake water were prepared by dilution with distilled water. In all solutions the pH was around 9.5 (+/− 0.3). These solutions will be referred to as Mono Lake water. Details on the chemisry of Mono Lake may be found in Mason (1967).

Sea water/NaCl solutions were prepared by addition of 0.25, 0.5, 1, 2 or 3 molar NaCl to half-strength Instant Ocean (trademark) sea salts (= 19 g/l), so that minor ions and trace minerals were present. These solutions ranged from around 25–200 g/l, and the pH of each was adjusted to 7.5. These solutions will be referred to as sea water/sodium chloride.

An empirically determined relationship between total dissolved solids and osmotic concentration (as milliosmoles/kg water = milliosmolal = mOsm) was established both for Mono Lake water, and sea water/sodium chloride solutions, allowing haemolymph osmolality to be compared directly to external osmolality.

Salt-tolerance bioassays

Field-collected groups of alkali fly larvae were exposed for at least a week (or until no survivors remained) to a range of salinities of both Mono Lake water and sea water/sodium chloride. Temperature was maintained at 20°C, on a photoperiod of 16 h light–8 h dark. Food was provided in the form of mixed filamentous green algae (*Ctenocladus circinnatus*), and epiphytic diatoms (*Nitzschia frustulum*), a natural larval food source that was both collected in the field and cultured in the laboratory. The deaths of larvae were distinguished by an absence of response to being handled with forceps or a probe. Dead larvae also often had a darkened, and shrunken, or flaccid appearance. Cumulative mortality plots were converted to probits, and LC_{50} and LT_{50} values (salt concentration for 50% mortality, and time for 50% mortality, respectively) calculated from the transformed data.

The influence of pre-acclimation in sea water/sodium chloride vs Mono Lake water on subsequent survival in high salinities of sea water/sodium chloride was investigated in laboratory reared groups of third-instar larvae. Second-instar larvae were collected in the field and exposed for 11 days to either 1000 mOsm Mono Lake water, or 1000 mOsm seawater/sodium chloride. Larvae that had developed into third instars at the end of this acclimation period were then randomly assigned to mortality bioassays in either 135 g/l or 195 g/l sea water/sodium chloride (=4200 and 6110 mOsm, respectively). All other conditions were maintained as above.

Haemolymph osmotic concentration

Larvae were acclimated to each test salinity of Mono Lake water or sea water/sodium chloride for 48 h, at 15°C, without food. Preliminary studies showed that in larvae exposed for 24 h to a salinity elevated above natural lake water, the haemolymph had reached essentially the same concentration as that found at 48 h, indicating haemolymph rapidly reaches osmotic equilibrium. After the acclimation period, larvae were removed from the treatment salinity, rinsed briefly in distilled water, blotted dry on tissue paper, and placed on a square of Parafilm. Using fine-tipped dissecting forceps, the cuticle was carefully torn open so that haemolymph flowed out onto the Parafilm (without rupturing the gut). A five microliter haemolymph sample was immediately taken up in a Clay-Adams micropipette, and the sample introduced into a pre-calibrated vapour-pressure osmometer (Wescot model 5100). Additional samples were diluted into distilled water and used for ion analysis. Chloride was determined in aliquots of the diluted haemolymph by the microtitration method of Schales and Schales (1941). Sodium was determined by further dilution of blood samples to a total volume of 5.0 ml, with a 0.02% solution of Alconox detergent. These samples were then vaporized in a Coleman Model 21 flame photometer, and compared to a standard sodium curve prepared on each day of analysis.

RESULTS

Salt tolerance

Mortality curves (Fig. 1a and b) show that death occurred in dose-dependent response to increased salinity in both Mono Lake water and seawater/sodium chloride. Equivalent osmotic concentrations of the two types of water chemistry produced higher mortality rates in sea water/sodium chloride than in Mono Lake water (comparing either 75 g/l sea water/sodium chloride and 100 g/l Mono Lake water, or 195 g/l sea water/sodium chloride and 250 g/l Mono Lake water between which there is less than 5% difference in osmotic concentration). This results in lower LC_{50} and LT_{50} values for larvae in sea

water/sodium chloride than for those exposed to Mono Lake water salinities (Table 1). Only at 96 h is the LC_{50} marginally lower in Mono Lake water than in sea water/sodium chloride. The greater toxicity of sea water/sodium chloride was also apparent in higher mortality at the lowest comparable salinities, with 22% of larvae dying after 1 week in 75 g/l sodium chloride (2320 mOsm) compared to only about 4% in 100 g/l Mono Lake water (2350 mOsm) (Figs 1a and b). Comparing Mono Lake water to the sea water–NaCl solutions, an equivalent osmotic concentration occurs at a lower total dissolved solids in sea water/sodium chloride, due to the higher proportion of low molecular weight chloride relative to the carbonates of Mono Lake water (and the tendency for greater dissociation/solubility of these ions). This documents the importance of comparing osmotic concentrations rather than total dissolved solids for waters of dissimilar chemical composition.

Pre-acclimation of larvae to either sea water/sodium chloride or Mono Lake water prior to exposure to hypersaline solutions of sea water/sodium chloride resulted in matching mortality curves at the highest salinity (195 g/l), and somewhat higher survival rates among Mono Lake water acclimated larvae at 135 g/l (Fig. 2).

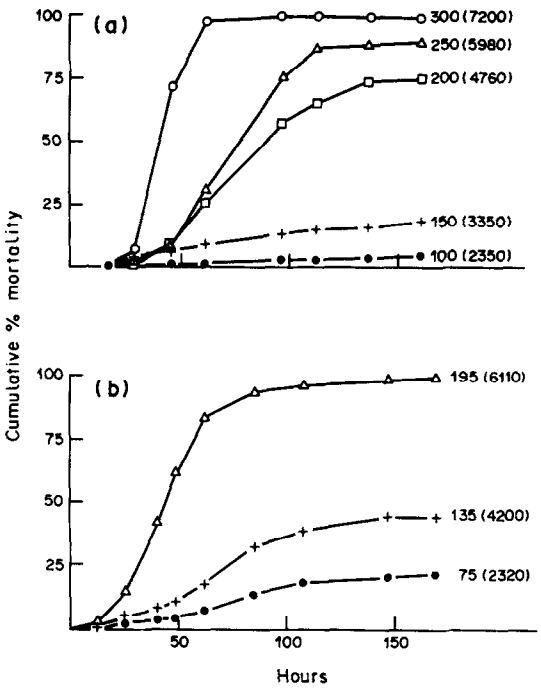

Fig. 1. a. Larval mortality curves of salinity tolerance bioassays in varied salinities of Mono Lake water. Number to right of each curve indicates the salinity (as g/l), and the osmolality (in parentheses, as mOsm). Initial sample size of larvae in each bioassay is as follows: salinity (number of larvae): 100 (115), 150 (116), 200 (113), 250 (119), 300 (121). b. Same as in 1a but for salinity tolerance bioassays in varied salinities of sea water/sodium chloride solutions (half-strength Instant Ocean with sodium chloride added). Initial sample size of larvae in each bioassay: 75 (150), 135 (150), 195 (158).

Osmoregulation

E. hians larvae were capable of both hypo- and hyperosmotic regulation of haemolymph osmolality over a wide range of salinities in Mono Lake water (Fig. 3). At salinities where survival rates were high (up to and including 150 g/l total dissolved solids), haemolymph osmolality was maintained between 250–320 mOsm. More than half of the larvae exposed to the highest salinity of Mono Lake water (300 g/l) were dead after the 48-h acclimation period (Table 1), and many were dead or moribund at salinities of 200 and 250 g/l. However, the survivors showed hypo-osmotic regulation of the haemolymph between 300 and 400 mOsm, against external concentrations over an order of magnitude higher. At these high salinities, blood samples usually had to be pooled to obtain the 5 μl required for consistent operation of the osmometer.

Although *E. hians* larvae were capable of osmoregulation in sea water/sodium chloride (Fig. 4), the osmolality of the haemolymph was higher and more variable than among larvae at comparable concentrations of Mono Lake water. Over the entire salinity range for hypoosmotic regulation in sea water/sodium chloride, haemolymph osmolality ranged from no less than 300 mOsm, to over 500 mOsm.

Sodium and chloride regulation

Sodium and chloride concentrations in the haemolymph of *E. hians* larvae are regulated at around 120–150 mEq/l. Assuming mEq/l will approximate mOsm at low ionic concentrations, sodium and chloride constitute about 70% of the osmotic solutes in the haemolymph at all salinities of Mono Lake water where both ions were assayed.

Body water content

As the salinity of Mono Lake water was increased, the per cent of the total body weight contributed by water declined from 90% at an external salinity of 600 mOsm to less than 70% at almost 7000 mOsm (after 3–4 days exposure) [Fig. 6]. These changes in body water were accompanied by body shrinkage and decreased blood sample volume.

DISCUSSION

Maintaining a constant osmotic concentration in extracellular fluids is a characteristic of many halotolerant metazoans, and permits cell volume regulation in environments of varying salinities (Prosser, 1973). The osmoregulatory ability demonstrated for *Ephydra hians* larvae in this study is an important component of the tolerance this insect shows to highly saline, alkaline waters. Gainey (1984) used the slope of hypoosmotic regulation lines to compare the effectiveness of osmoregulation in various insects, and found dipterans to be the best regulators. A slope of 0.02 can be derived from the hypoosmotic line of *E. hians* in Mono Lake water (Fig. 3), equalling that of the most constant regulators on his list. However, poor survival, and debilitation of larvae at and above 200 g/l, suggests that even though survivors show good osmoregulatory ability after 48 h, the long-term

Table 1. Lethal salinity tolerance concentrations and exposure times for Mono Lake *E. hians* larvae

	LC_{50}			
	Mono Lake water		Sea water/sodium chloride	
Time (h)	TDS	mOsm	TDS	mOsm
48	273	6550	183	5780
72	252	6030	158	4970
96	190	4520	144	4550

	LT_{50}		
Mono Lake water		Sea water/sodium chloride	
Salinity [g/l (mOsm)]	LT_{50} (hours)	Salinity [g/l (mOsm)]	LT_{50} (hours)
200 (4760)	88.5	—	
250 (5980)	76.0	193 (6110)	43.5
300 (7200)	40.5	—	

Values of lethal tolerances for 50% of the test populations are calculated by interpolating between probit-transformed mortality curves at the 50% level for specified times (LC_{50}s), or by the time when the curve at a specified concentration crosses 50% (LT_{50}s). The 48–96 h period is in general the linear portion of the transformed curves (exponential mortality rates). Values are rounded to the nearest 10 mOsm, and 1 g/l.
TDS = total dissolved solids.

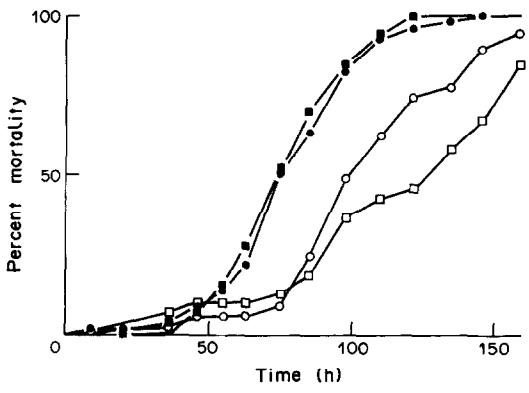

Fig. 2. Mortality curves in seawater/sodium chloride for larvae pre-acclimated to sea water/sodium chloride vs Mono Lake water. Squares represent larvae acclimated to 1000 mOsm Mono Lake water and circles for larvae acclimated to 1000 mOsm sea water/sodium chloride. Filled symbols are exposures to 195 g/l sea water/sodium chloride, and open symbols are exposures to 135 g/l sea water/sodium chloride. Initial sample size for filled squares = 33, filled circles = 60, open squares = 33, and open circles = 58.

effects of these high salinities are costly. Exposures of a week or more usually result in death (Fig. 1a). In surviving larvae blood volume is decreased and numbers and size of pupae formed are reduced as salinity is increased (Herbst, 1986). Early instars do not survive at all beyond a few days of acute exposure (Herbst, unpublished).

In sea water/NaCl solutions, larval mortality rates are higher yet (Table 1 and Fig. 1b), and haemolymph osmotic concentrations are elevated and more variable (Fig. 4), compared to similar Mono Lake water salinities (Fig. 3). This indicates that *E. hians* larvae from Mono Lake are less tolerant of the ionic composition of sodium chloride-type brines than of carbonate-rich alkaline waters and possess less effective osmoregulatory mechanisms for this mineral chemistry. In addition, development studies have also shown that larval growth is impaired in sea water relative to osmotically equivalent salinities of Mono Lake water (Herbst, unpublished). However, the few larvae surviving in high salinities over the 48-h acclimation in either medium have about the same blood osmolality, thus for brief exposures, regulation may be accomplished in both types of hypersaline water.

If acclimation permits an induced physiological adaptation, then larval survival in sea water/sodium chloride should be improved among larvae acclimated to sea water/sodium chloride relative to those acclimated to Mono Lake water. The mortality curves of Fig. 2 indicate no difference in survival between acclimation groups exposed to 195 g/l sea water/sodium chloride, and higher survival rates among Mono Lake water-acclimated animals in 135 g/l sea water/sodium chloride. This suggests that, if anything, pre-acclimation to seawater/sodium chloride is debilitating to the salt tolerance of *E. hians* larvae.

Other Ephydrids have also been shown to be osmoregulators, but have not been studied with regard to physiological adaptation to waters of varied chemistry, as done in this study. Nemenz (1960) showed that *E. cinerea* from Great Salt Lake (Utah) regulates haemolymph osmolality at the equivalent of about 900 mOsm (converted from freezing-point depression measures) at external concentrations in excess of 10,000 mOsm. Nemenz proposed that this unusually high osmotic content of the blood permitted *E. cinerea* to tolerate the very high salinities of Great Salt Lake (>260 g/l, around 1957). Increased osmotic concentration in the blood should in theory reduce the effective gradient against which osmoregulation must work, reducing metabolic costs and thereby improving survival at higher salinities. The lower relative tolerance to high salinity shown by *E. hians* (especially in sea water/sodium chloride), and its lower haemolymph osmolality is consistent with this proposal. Since the water chemistry of Great Salt Lake is comparable to the sea water/sodium chloride solutions used in this study, it would be of interest to examine whether the salt tolerance and osmoregulatory ability in *E. cinerea* (which is found mainly in chloride-waters) are the reverse of those observed here for *E. hians*.

There is, however, an ecological basis for believing that *E. hians* might naturally enter non-alkaline, chloride-dominated waters, because of its recent colonization of the Great Salt Lake (Herbst, personal collection). Prior to the recent (post-1982) rise in lake level, the only benthic metazoan observed in Great Salt Lake had been *E. cinerea* (Collins, 1980). Over the past several years, salinity has decreased from about 160 to 50 g/l in locations, and during this period, *E. hians* appeared in the lake (it was probably restricted to peripheral estuaries or ponds before this time; N. Collins, pers. commun.). This recent finding of *E. hians* in the diluted waters of Great Salt Lake suggests that either larvae do not survive when in high salinities having this type of chemical composition (a conclusion of this study), and/or that females avoid ovipositing in such habitats. It is not known whether adult female ephydrids are able to distinguish between habitats of differing chemistry or salinity when selecting a place to oviposit.

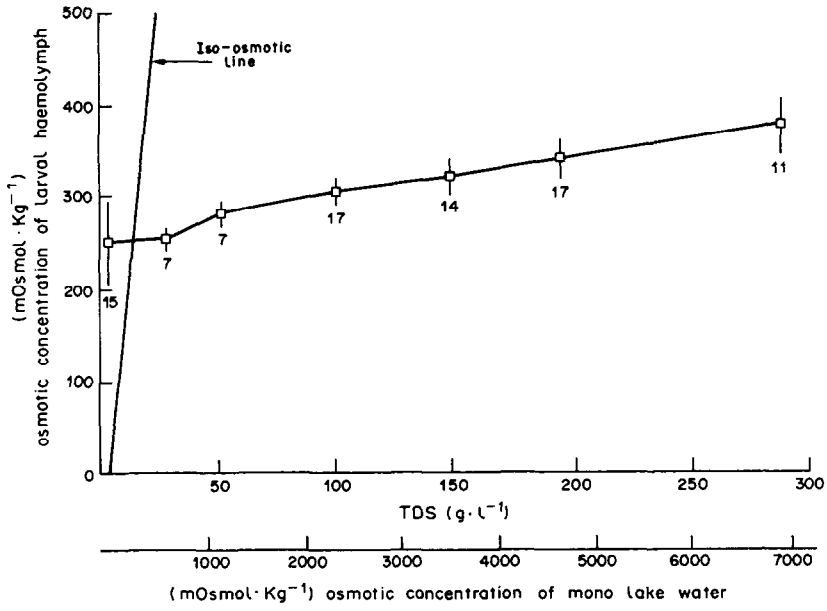

Fig. 3. Osmotic concentration of *Ephydra hians* larval haemolymph in relation to the osmotic concentration of Mono Lake water. Vertical lines through mean values are standard deviation, and sample sizes are indicated below each mean. Mono Lake water scaled both as total dissolved solids (in g/l), and as mOsm/kg water (mOsm).

Ion toxicity studies in brine mosquito larvae have shown that although waters of varied ion composition are tolerated, some ions are more toxic, and some species more tolerant than others. Bradley and Perkins (1975) found that *Culex pipiens* larvae were more tolerant of mixed solutions of salts containing both mono- and divalent cations than of solutions containing single cations only (monovalent cations being most toxic). Bradley and Phillips (1977) further showed that the inland species *Aedes campestris* (often inhabiting magnesium sulphate lakes) was more tolerant of magnesium and sulphate than the coastal species *A. taeniorhynchus*. However, *A. campestris* is considerably less tolerant of magnesium or sulphate than of other major ions. *A. dorsalis* larvae are commonly found in alkaline waters of the Great Basin, and though they are not as tolerant of magnesium sulphate waters or sea water as the above

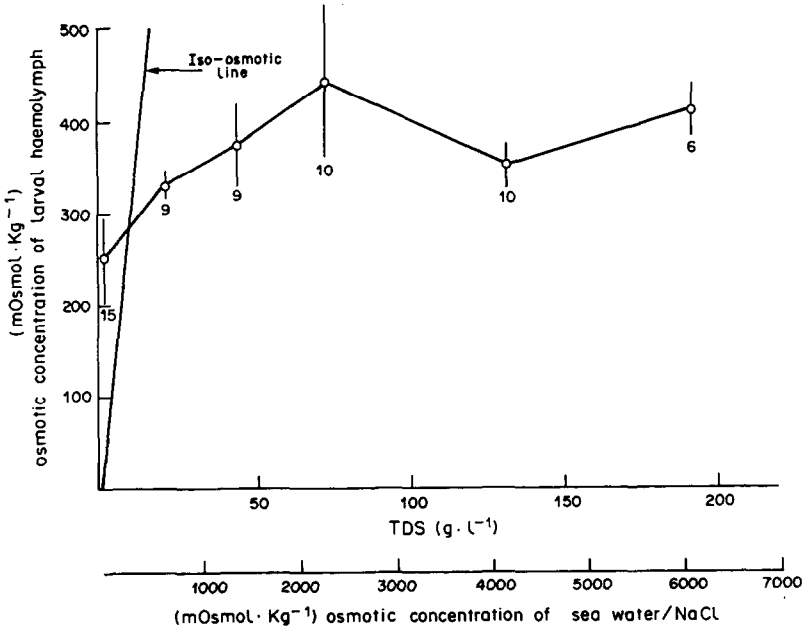

Fig. 4. Osmotic concentration of *Ephydra hians* larval haemolymph in relation to the osmotic concentration of sea water/sodium chloride solutions. Vertical lines through mean values are standard deviation, and sample sizes are indicated below each mean. Sea water/sodium chloride scaled both as total dissolved solids (in g/l) and as mOsm/kg water (mOsm).

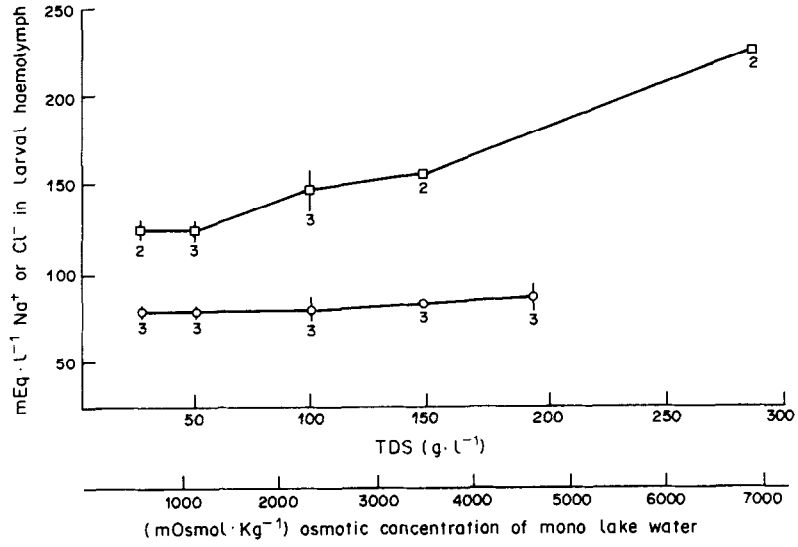

Fig. 5. Sodium and chloride ion regulation in *E. hians* larval haemolymph in relation to the salinity and osmolality of Mono Lake water. Squares = sodium, circles = chloride. Vertical lines through mean values are standard deviations, and numbers below means are sample sizes.

congeners (Sheplay and Bradley, 1982), this species is able to survive in high concentrations of bicarbonate and carbonate by secreting a concentrated rectal fluid containing these ions (Strange *et al.*, 1982).

Carbonate/bicarbonate are also major ionic components of Mono Lake water. *E. hians* larvae may possess a physiological specialization in osmoregulatory mechanism for the excretion of bicarbonate/carbonate. The Malpighian tubules on one side of the gut are modified into a pair of large glands containing a particulate white substance. This substance is insoluble in sodium hydroxide, but dissolves readily in acid, with the release of gas bubbles. The contents of these glands appear to be stored during larval life and are discharged at pupariation. These modified Malpighian tubules may store excreted bicarbonate/carbonate as an insoluble salt (evolving carbon dioxide in acid), and thus serve as a crucial ion-regulating organ. The possible storage–excretion role of these Malpighian tubules contrasts with the structural modifications of the ileum and rectum that have been implicated in ion regulation in other ephydrids (Marshall and Wright, 1974; Eichelberg, 1976). Detailed structural, chemical, and physiological descriptions of these glands will be reported elsewhere.

Although the primary difference in major ion chemistry between Mono Lake water and sea water/sodium chloride is bicarbonate/carbonate versus chloride as the dominant anions, it is possible that

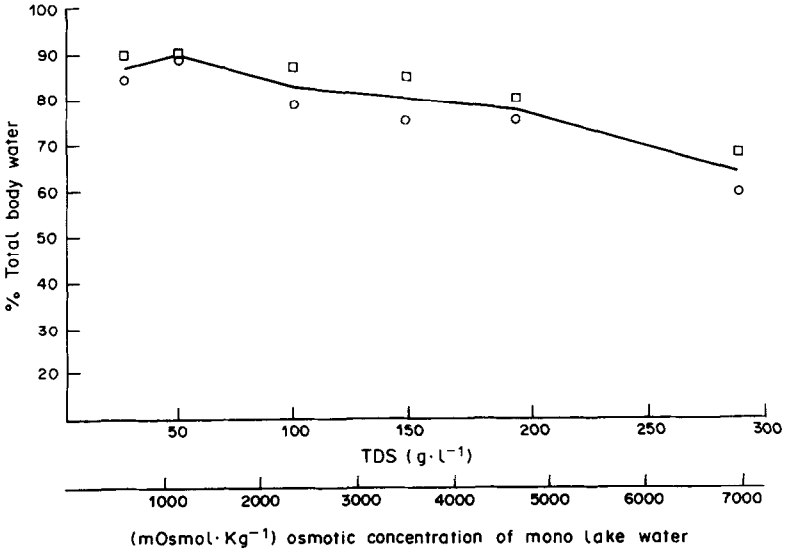

Fig. 6. Total body water of *E. hians* larvae as per cent of wet (live) weight, in relation to salinity and osmolality of Mono Lake water. Circles and squares denote the results of two separate experiments, and the line is drawn through the median between these experiments. 20–30 larvae pooled for each determination of water content.

the physiological responses observed are also a result of differences in magnesium, calcium, potassium or sulphate content. However, the concentrations of these ions are low compared to those of sodium, chloride, and bicarbonate/carbonate, and are unlikely to be toxic. In any case, the results demonstrate that larvae possess superior physiological adaptation to natural alkaline-type waters than for sea water/chloride-type water chemistry.

Haemolymph osmolytes of *E. hians* consist mainly of sodium and chloride (Fig. 5), and are in the same range and relative proportion as found in other aquatic insects (Sutcliffe, 1962). Consistently higher concentrations of sodium over chloride results in an anion deficit, probably made up in part by bicarbonate in *E. hians* larvae. Sutcliffe (1962) has suggested that although amino acids may be important non-electrolyte osmotic solutes in insects, it is non-amino carboxylic acids that balance the anion deficit. Further investigation of the solute composition of *E. hians* haemolymph is needed to identify other components of osmotic regulation in this species.

The small volumes of blood samples yielded from larvae exposed to higher salinities suggests that the observed decline in per cent body water with higher salinity (Fig. 6) reflects a loss of blood volume through osmotic dehydration. However, blood osmolality is regulated, permitting homeostasis in the extracellular fluids bathing tissues, despite large losses in plasma volume. This is also a common observation among terrestrial insects faced with a desiccating environment (Woodring, 1985).

To define the mechanisms of osmoregulation in *Ephydra hians*, further studies of cuticular permeability, drinking rates, and excretion processes are needed to construct a budget for water and solute balance. From this information it will be possible to evaluate how physiological limitations on salt tolerance may be related to the ecological distribution and population biology of this insect.

REFERENCES

Aldrich J. M. (1912) The biology of some Western species of the dipterous genus *Ephydra*. *J. N.Y. ent. Soc.* **20,** 77–99.

Bayley I. A. E. and Williams W. D. (1966) Chemical and biological studies on some saline lakes of south-east Australia. *Aust. J. Mar. Freshwater Res.* **17,** 177–228.

Bradley T. J. (1976) The mechanism of hyperosmotic urine formation in the recta of saline-water mosquito larvae. Ph.D. thesis, University of British Columbia, Vancouver. 135 pp.

Bradley T. J. and Perkins D. L. (1975) Ionic antagonism in mosquito larvae, *Culex pipiens*. *Comp. Biochem. Physiol.* **52A,** 403–407.

Bradley T. J. and Phillips J. E. (1977) Regulation of rectal secretion in saline-water mosquito larvae living in waters of diverse ionic composition. *J. exp. Biol.* **66,** 83–96.

Collins N. C. (1977) Mechanisms determining the relative abundance of brine flies (Diptera: Ephydridae) in Yellowstone thermal spring effluents. *Can. Ent.* **109,** 415–422.

Collins N. C. (1980) Population ecology of *Ephydra cinerea* Jones (Diptera: Ephydridae), the only benthic metazoan of the Great Salt Lake, U.S.A. *Hydrobiologia* **68,** 99–112.

Conte F. P., Hootman S. R. and Harris P. J. (1972) Neck organ of *Artemia salina* Nauplii. *J. comp. Physiol.* **80,** 239–246.

Eichelberg D. (1976) Feinstruktur und funktion der rektalpapillen der salinenfliege *Ephydra riparia* (Diptera: Ephydridae). *Ent. Germ.* **3,** 173–184.

Gainey L. F. (1984) Osmoregulation in the larvae of *Odontomyia cincta* (Diptera: Stratiomyidae). *Physiol. Zool.* **57,** 663–672.

Hendrickson D. A. and Kubly D. W. (1984) Desert waters: past, present and future. *Nature Conservancy News* **34,** 6–12.

Herbst D. B. (1986) Comparative studies of the population ecology and life history patterns of an alkaline salt lake insect: *Ephydra* (*Hydropyrus*) *hians* (Diptera: Ephydridae). Ph.D. thesis, Oregon State University, Corvallis. 206 pp.

Marshall A. T. and Wright A. (1974) Ultrastructure changes associated with osmoregulation in the hindgut cells of a saltwater insect, *Ephydrella* sp. (Ephydridae: Diptera). *Tissue Cell* **6,** 301–318.

Mason D. T. (1967) Limnology of Mono Lake, California. University of California Publications in Zoology 83.

Nemenz H. (1960) On the osmotic regulation of the larvae of *Ephydra cinerea*. *J. Insect Physiol.* **4,** 38–44.

Prosser C. L. (1973) *Comparative Animal Physiology*, 3rd edn. W. B. Saunders Co., Philadelphia.

Schales O. and Schales S. S. (1941) A simple and accurate method for the determination of chloride in biological fluids. *J. biol. Chem.* **140,** 879–884.

Sheplay A. W. and Bradley T. J. (1982) A comparative study of magnesium sulphate tolerance in saline-water mosquito larvae. *J. Insect Physiol.* **28,** 641–646.

Simpson K. W. (1979) Evolution of life histories in the Ephydrini. *First Symposium on the Systematics and Ecology of Ephydridae* (*Diptera*) (Edited by Doenier D. L.), pp. 99–109. North American Benthological Society Publication.

Smith G. R. (1978) Biogeography of intermountain fishes. *Great Basin Nat. Memoirs* **26,** 17–42.

Strange K., Phillips J. E. and Quamme G. A. (1982) Active HCO_3 secretion in the rectal salt gland of a mosquito larva inhabiting $NaHCO_3$–CO_3 lakes. *J. exp. Biol.* **101,** 171–186.

Sutcliffe D. W. (1962) The composition of hemolymph in aquatic insects. *J. exp. Biol.* **39,** 3325–343.

Wirth W. W. (1971) The brine flies of the genus *Ephydra* in North America (Diptera: Ephydridae). *Ann. ent. Soc. Am.* **64,** 357–377.

Woodring J. P. (1985) Circulatory systems. *Fundamentals of Insect Physiology* (Ed. by Blun M. S.), pp. 5–57. John Wiley and Sons, New York.