

AN ABSTRACT OF THE THESIS OF

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Title: Ecological Physiology of the Larval Brine Fly *Ephydra*
(*Hydropyrus*) *hians*, an Alkaline-Salt Lake Inhabiting Ephydrid
(Diptera).

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Dipterans of the family Ephydridae (shore and brine flies) are well known for their ability to tolerate and thrive in a wide variety of physically harsh circumstances. The immature stages of one such species of this family, *Ephydra hians*, are characteristically limited in distribution as benthic inhabitants of highly alkaline salt lakes in the arid regions of western North America. In order to examine the nature of this habitat specificity, the intraspecific comparative physiology of osmotic and ionic regulation, energy metabolism and survival was examined in two populations of brine fly larvae which differ in the historical-environmental stability of their native habitats. The population at Mono Lake, California, has, until recent times, been evolving under relatively stable conditions of water chemistry and habitat availability (i.e., little fluctuation in salinity or littoral benthic habitat due to stable lake level). Since 1940 however, the lake level has been steadily dropping and the dissolved salt content increasing due to diversion of tributary water. The population at Abert Lake, Oregon, has by contrast been subject to severe short-term changes

in salinity and habitat availability throughout its history. Larvae were exposed to both alkaline and non-alkaline brines. While both populations displayed hyposmotic regulation in alkaline salt media, Mono Lake larvae consistently showed more effective maintenance of homeostasis. However, in non-alkaline salt water, Mono Lake larvae show poor ability to acclimate to these conditions while Abert Lake larvae are capable of limited physiological adaptation. The general pattern of response to increased salinity or "foreign" water chemistry seems to be an attempt to physiologically accommodate on the part of Abert larvae while Mono larvae become behaviorally and metabolically inactive. The results indicate a physiological basis for biogeographic restriction, with the degree of specificity and efficiency of physiological adjustment in this species being apparently related to the historical experience of the particular population. In high salinities of their native lake water, the poor survival and reduced developmental/physiological activity of Mono Lake larvae suggest that a reduction in abundance is likely to accompany further increases in the salinity of the lake.

Ecological Physiology of the Larval Brine Fly
Ephydra (Hydropyrus) hians,
an Alkaline-Salt Lake Inhabiting Ephydrid (Diptera)

by

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Typed by Linda B. Moak for

David Bruce Herbst

This work and the insights
gained are dedicated to
the animals sacrificed...

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ECOLOGICAL PHYSIOLOGY OF THE LARVAL BRINE FLY EPHYDRA (HYDROPYRUS)
HIANS, AN ALKALINE-SALINE LAKE INHABITING EPHYDRID (DIPTERA).

INTRODUCTION

Background and Natural History.

There are great inland seas in the high desert from which no water flows. These closed basins, like the oceans, retain all the dissolved constituents of the water they receive. Because of the heat and dry winds of such arid regions, a great evaporative loss of water occurs, resulting in salt enriched brine lakes. The underlying geochemistry of local drainage basins give rise to a variety of inorganic and organic solutes in different combinations and concentrations from one lake to another. Aquatic environments created under such extreme and variable chemical conditions exert strong selection for adaptations optimizing the cost of survival with reproduction, foremost among which would seem to be osmoregulatory ability.

One inhabitant of saline desert lakes is the primitive branchiopod crustacean Artemia, commonly known as brine shrimp. These animals have been studied extensively with regard to the physiology of salt water adaptation (Croghan 1958a,b,c,d; Gilchrist, 1958; Conte et al., 1972, Conte 1977). Few other metazoans are as tolerant of extreme osmotic conditions, but among them are the aquatic larvae of the brine fly Ephydra hians (Diptera: Ephydridae). Aldrich (1912) first described the

biology of this species from several salt lakes in the Great Basin. Adults, gathering in tremendous densities around the margins of these lakes, feed on algae and detritus washed up along the shore. Females have the unusual reproductive behavior of walking down partially exposed objects in the water and submerging themselves, enclosed in a physical-gill air bubble, oviposit on porous rock substrate or in detrital lake sediments. The aquatic larvae develop through 4 instars and then pupate after securing themselves to some submerged hard substratum by means of the enlarged terminal pair of clawed prolegs. The requirement of the larvae for a rocky substrate on which to attach prior to pupation may be responsible for the patchiness in local spatial distribution of both the larvae and shore-dwelling adults (Herbst, 1977). Furthermore, this type of larval behavior could possibly limit occurrence to salt lake basins where this substrate type is common. The range of distribution of E. hians is restricted to alkaline salt lakes of western North America, from Mexico to British Columbia.

Brine fly populations commonly reach high levels of abundance where they occur. Such high productivity is probably attributable to the lack of interspecific competition (since few other benthic herbivores are physiologically adapted to highly alkaline salt water) and absence of intense predation. At several locations, most notably Mono Lake, California, these dense gatherings of adult flies are taken advantage of as a food source by the migratory birds of the Pacific flyway (e.g. Phalaropes, Avocets). In addition, washed up windrows of pupae have been gathered as food from the shores of several Great Basin lakes by Paiute Indians (Williston, 1883). Dried in the sun and "husked" of the puparium, these pupae were a rich food source and trade item to this culture.

Physiology of Salt Balance in Euryhaline Diptera Larvae.

A great deal of study has been devoted to elucidation of the physiological mechanisms underlying water conservation in terrestrial insects (see reviews by Phillips, 1970; 1977; Wall, 1971). Insects are subject to desiccation if their environment, such as is found in a salt lake, has a higher osmotic pressure than their haemolymph. The physiology of saltwater mosquito larvae of the genus Aedes has been investigated with respect to osmotic-ionic regulation by Beadle (1939), Ramsay (1950) and Phillips and co-workers (Meredith and Phillips, 1973; Kiceniuk and Phillips, 1974; Bradley and Phillips, 1975, 1976). The anatomical features of this particular regulatory system include the renal malpighian tubules and an extra-renal organ known as the anal papilla. The latter is concerned with the active absorption of Na^+ and Cl^- from dilute waters. Phillips has reported that the highest salinity tolerated is 300% (3x) sea water, by Aedes campestris. The behavioral response of this species to a hypersaline stress is to increase its drinking rate, which acts to effectively replace the water lost by osmosis. The midgut appears to be freely permeable to both water and ions ingested, while the body wall is less permeable than that reported for freshwater insects. As salinity increases, mosquito larvae react physiologically through increased secretions of sodium and chloride ions into the posterior portion of the rectum, followed by excretion of this fluid.

In contrast to the information available on the brine mosquito (Aedes spp.), study of osmoregulation in the brine fly genus Ephydra has been incomplete. Nemenz (1960) has studied E. cinerea from Utah's Great Salt Lake and found that haemolymph osmotic pressure is regulated in both hypotonic as well as hypertonic conditions, though this regulatory system falters in diluted lake water at higher temperatures (21°C, regulation remains effective at 3°C). Larvae which have the gastrointestinal tract ligatured at both ends cannot regulate internal fluid osmotic pressure at any temperature. This suggests the gut and perhaps epithelial secretions within the midgut or rectum as the site of osmotic and ionic regulatory action. In addition, the cuticle appears readily permeable to water in either direction as ligatured larvae swell in dilute media and shrink in concentrated media. Ultrastructural evidence relating osmotically induced changes in the epithelia lining the ileum of an Australina ephydrid larva Ephydrella sp., supports the idea of hindgut localization for water uptake and electrolyte regulation (Marshall and Wright, 1974).

Sutcliffe (1960) reported on E. riparia from coastal salt marsh habitat as being capable of regulation over a wide range of sea water dilutions and evaporations, holding blood concentrations at less than one percent NaCl. Contrasted with E. cinerea, E. riparia maintains a low osmotic pressure while the former possesses the highest known osmotic blood pressure among the insects (20.4 atm. or 2.5% NaCl). These differences may be attributed to selection for different ion regulation optima in habitats of differing chemical composition and concentration.

Ecology and Evolution of Ephydra in Saline Lakes.

Ecological information on salt water Ephydra is limited primarily to descriptive accounts of their habits, biogeography and systematic relationships (Ping, 1921; Wirth, 1970; Scheiring, 1974 and 1975; Simpson 1976a,b). However, the recent quantitative studies of Collins (1980) provide new insights to the importance of habitat quality and salinity in shaping patterns of distribution and abundance of E. cinerea in the Great Salt Lake. The larvae of this species show a distinct preference for rocky substrate or shallow mud regions, avoiding areas of unstable sandy bottom or deep anaerobic sediments. There is a marked tendency for larvae to disperse from sandy sites and to accumulate on rock substrate. Population regulation appears to be density-dependent as high larval densities on rock substrate result in reductions in larval growth, pupation size and success, and adult fecundities. However, observations over a period of years during which salinity of surface waters has varied suggests that the abundance decreases as salinity increases, in density-independent fashion. Furthermore, the isolated north arm of the lake, at salt saturated salinities of around 325 g/l, supports no appreciable fly production.

The Halephydra and Hydropyrus subgenera occur in more highly saline conditions than any of the other species in the genus Ephydra. E. cinerea (Halephydra) is common in waters of high chloride content like the Great Salt Lake, whereas E. hians (Hydropyrus) thrives in highly alkaline-saline lakes. The differing physical and biological structure of the saline habitats where brine flies occur may have led to unique physiological and ecological adaptations. For example, at Mono Lake

(California), E. hians is the dominant benthic invertebrate while Abert Lake (Oregon), with a lower salinity at present (though pH is comparable), has a relatively diverse shore and littoral community, including an abundant E. hians population (Herbst, unpub. obs.). In a comparative study, Scudder et al. (1972) has shown that the distribution of saltwater corixids (Hemiptera) is limited by differing osmoregulatory capabilities of each species.

The purpose of the present study is to investigate the intraspecific comparative physiology of osmotic and ionic regulation in two populations of the brine fly species Ephydra hians from different salt lakes of the western Great Basin. One population, at Mono Lake, California, has experienced an evolutionary history of relative stability with regard to salinity and aquatic habitat permanence. By contrast, the population at Abert Lake, Oregon, has experienced recent and frequent periods of salinity variation including the complete disappearance (drying) of the aquatic habitat. The results derived from this study may provide information on the evolutionary consequences of salinity variation and habitat stability to osmotic and ionic regulation abilities and life history adaptations of saltwater insects.

Chemical and Hydrological History of the Lake Study Sites.

Mono Lake is one of the oldest continuously standing lakes in North America. Derived from Pleistocene Lake Russell, which once covered a large region of the high desert, water has stood in the Mono Basin for at least a half million years. Mono has existed as a closed basin since

the last period of overflow (about 22,000 years ago, at an elevation of 7,180') and has gradually become enriched in salts since this time. The high alkalinity of Mono Lake is imparted by carbonates and borates which buffer the lake water at a pH of around 10. In addition, this is a rare "triple-water" lake, having as its major anionic constituents Cl^-

$\text{HCO}_3^-/\text{CO}_3^{=}$ and $\text{SO}_4^{=}$, combined as mixed sodium salts to approximately 1.3 M. The current total dissolved solids content of the lake (July, 1979) is about 96.4 grams per liter (refer to Table 1 for a comparison of the water chemistry at Mono and Abert Lakes).

Lake levels fell rapidly beginning at the end of the last glacial advance (Wisconsin) approximately 12,500 years ago and eventually came to a minimum level at an elevation near 6365' during a warm period some four to seven thousand years ago. Since then the lake has come into balance between this low value of 6365' and a higher elevation of 6480' (Loeffler, 1977). Recent evidence (Stine, 1979) suggests that during the past thousand years, the lake level has been consistently stable around 6400'. Figure 1 depicts geologically recent changes in the elevation of Mono Lake. During the past 40 years, the lake has been rapidly and consistently dropping due to the diversion of approximately 75% of the freshwater inflow (Rush and Lee Vining Creeks) into the Los Angeles aqueduct of the Owens Valley. Such diversion of freshwater has caused an attendant increase in salinity and imposes a prolonged and directional selection on the larval population of E. hians for increased osmoregulatory ability. The limitations of such adaptation are a further objective of the present research in that a basis for predicting the ability of the population at Mono Lake to persist in an environment subject to increasing salinity would be provided.

TABLE 1: Water Chemistry

| | <u>MONO LAKE (year, reference)</u> | | <u>ABERT LAKE (year, reference)</u> | |
|--|------------------------------------|--|-------------------------------------|--|
| TDS | >94.6 84.3 | (1979, Herbst) (1974, LADWP) | >54.4 40.8 | (1979, Herbst) (1961, Phillips & Van Denburgh) |
| pH | 9.95 10.15 | (1978, Herbst) (1979, Melack) | 9.95 9.7 | (1978, Herbst) (1961, Phillips & Van Denburgh) |
| Osmolality (mOsmoles/Kg H ₂ O) | 2350 | (1978, Herbst) | 1100 | (1978, Herbst) |
| Specific Gravity (density in g/ml) | 1.074 1.080 1.084 | (1976, MBRG) (1978, Herbst) (1979, Herbst) | 1.077 1.032 1.035 | (1939, Phillips & Van Denburgh) (1961, Phillips & Van Denburgh) (1978, Herbst) |
| Na ⁺ | 29.5 | (1974, LADWP) | 16.3 | (1961, Phillips & Van Denburgh) |
| Cl ⁻ | 21.3 17.6 | (1978, Herbst) (1974, LADWP) | 15.1 14.3 | (1978, Herbst) (1961, Phillips & Van Denburgh) |
| HCO ₃ ⁻ | 11.2 | (1974, LADWP) | 2.4 | (1961, Phillips & Van Denburgh) |
| CO ₃ ⁼ | 18.9 | (1974, LADWP) | 6.5 | (1961, Phillips & Van Denburgh) |
| SO ₄ ⁼ | 10.3 | (1974, LADWP) | .73 | (1961, Phillips & Van Denburgh) |
| K ⁺ | 1.5 | (1974, LADWP) | .53 | (1961, Phillips & Van Denburgh) |
| B | .33 | (1974, LADWP) | .06 | (1961, Phillips & Van Denburgh) |

note: all ions reported as grams per liter

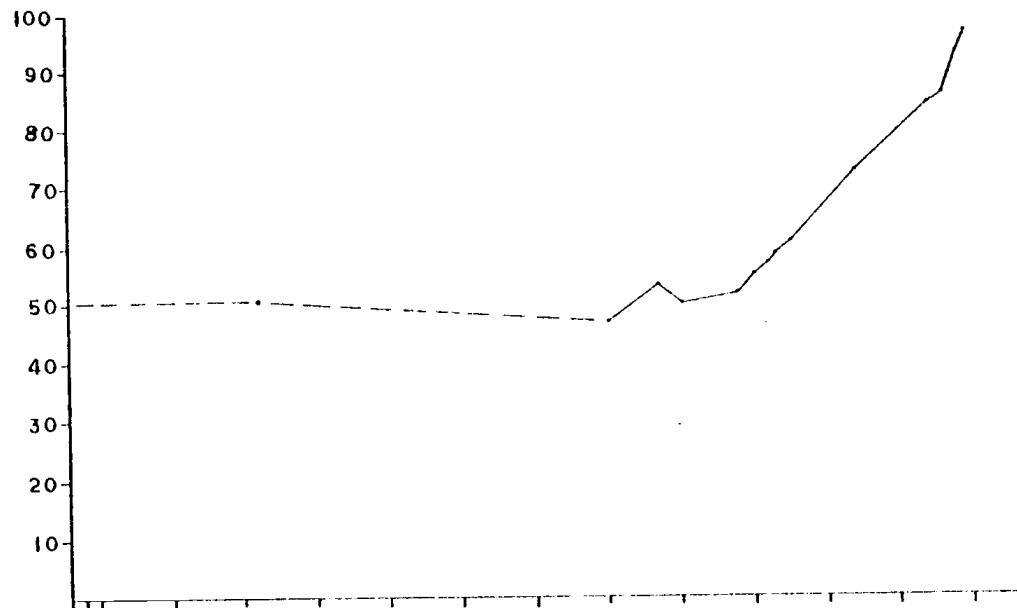
References:

- Herbst, D.B. (1978-79) unpublished observations
- Melack, J. (1979) pers. communication
- Phillips, K.N. and A.S. Van Denburgh (1961) in: Hydrology and Geochemistry of Abert, Summer and Goose Lakes, and Other Closed-Basin Lakes in South-Central Oregon. 1971 U.S. Geol. Surv. Prof. paper #502-B.
- (also see Van Denburgh, A.S. (1975) Solute Balance at Abert and Summer Lakes, South-Central Oregon. U.S. Geol. Surv. Prof. paper #502-C.
- LADWP - analyses of the Los Angeles Department of Water and Power, done in 1974; these data taken from averages compiled by the Mono Basin Research Group (MBRG) in 1976 and reported in: An Ecological Study of Mono Lake, California. Ed. D.W. Winkler. Institute of Ecology publication #12 (June, 1977). U.C. Davis

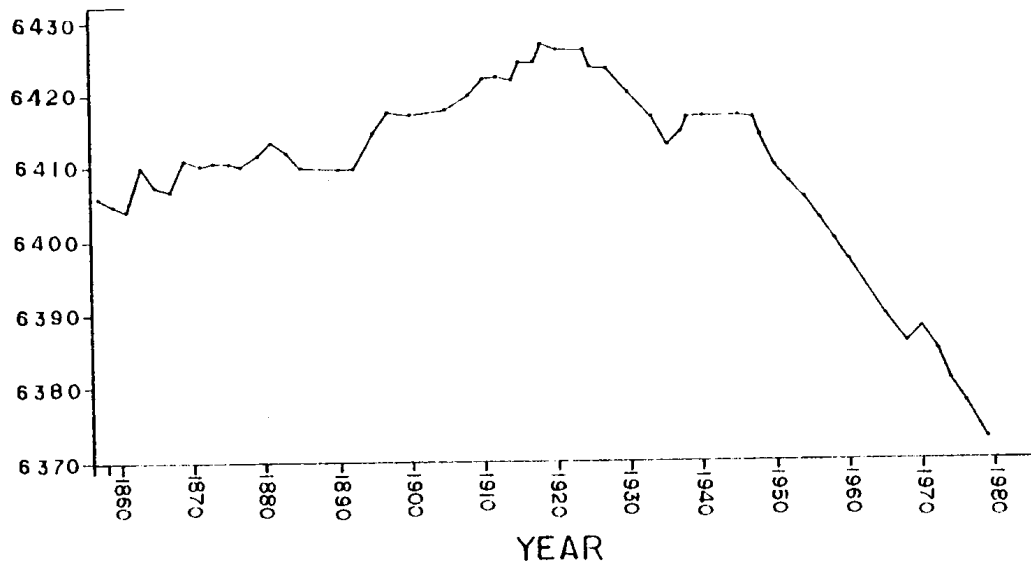
Figure 1. Recent Historical Elevations and Salinity of Mono Lake:

These elevation-salinity profiles are reconstructed from the following sources of data: Stine, 1979 (elevation between 1857-1885); Harding, 1965 (elevation between 1885-1962); Black, 1958 (salinity from 1882-1955); MBGR, 1977 (salinity from 1940-1976) [see References for all citations]. Elevation is plotted in feet above 6350' elevation. Salinity is total dissolved solids (grams/liter).

SALINITY OF MONO LAKE
(grams/liter TDS)



ELEVATION OF MONO LAKE
(feet above mean sea level)



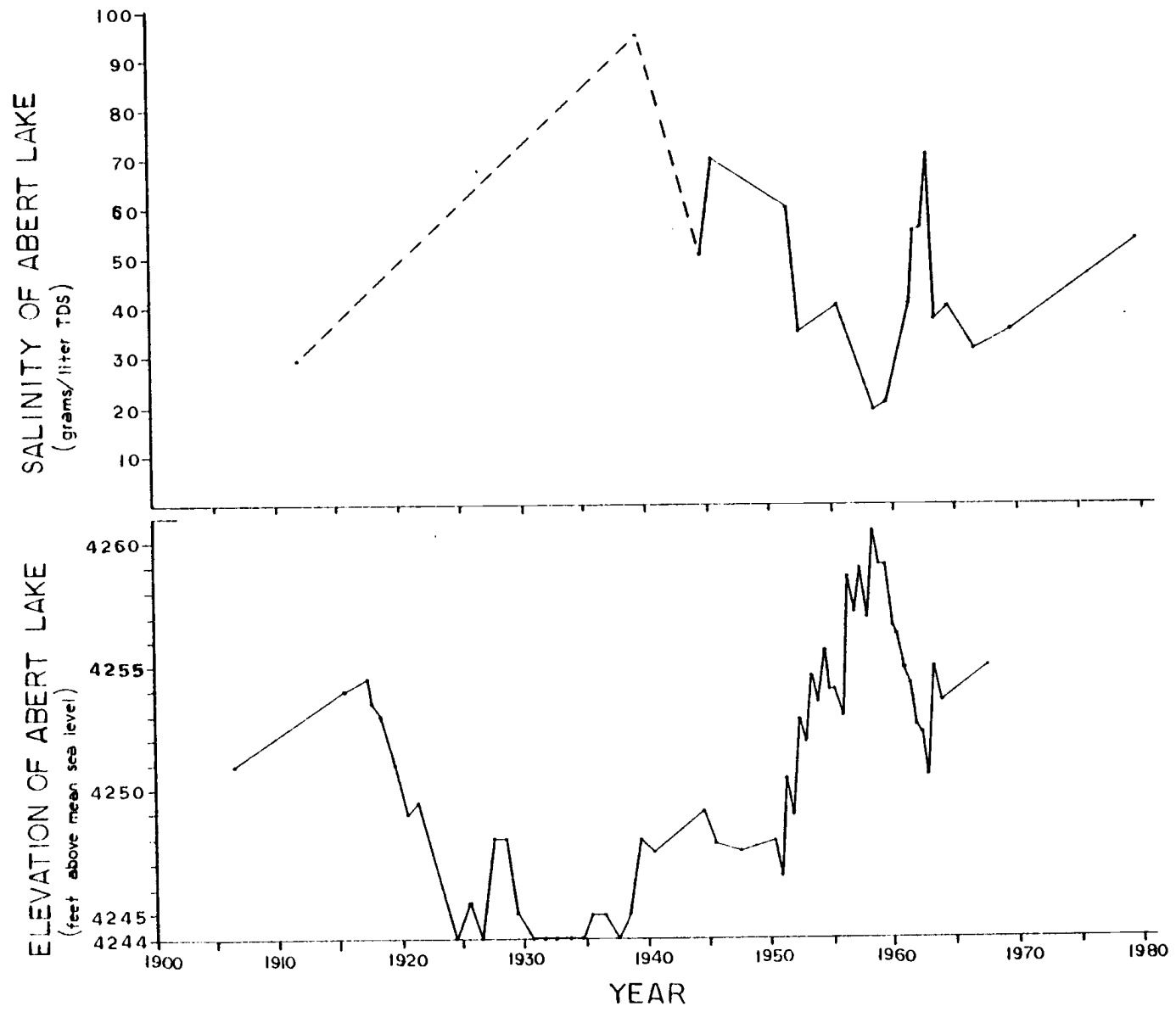
Abert Lake, along with several other shallow saltwater lakes in south-central Oregon, is a remnant of pleistocene Lake Chewaucan (encompassing the present Abert and Summer Lake basins) which left shoreline deposits more than 200' above the present day lake bed (Phillips and Van Denburgh, 1971). Abert Lake has been cited as a classic example of a fault scarp lake (Hutchinson, 1957), with Abert Rim, a 3000' fault block rising abruptly from its eastern shore.

The dry conditions characteristic of the Great Basin from seven to four thousand years ago decreased freshwater inflows to Abert Lake, with the result that the lake may have dried frequently during this period of time. Records of tree-ring growth (Antevs, 1938) indicate that for the past 650 years, periods of maximum wetness (rapid-tree growth) and thus high lake stage occurred in 1790-92, 1805-25 and 1907-09 (the 1950's were also quite wet). Extremely dry periods occurred from 1842-49 and particularly 1918-34. During this latter period Abert was frequently dry or near dryness. Thus, with possible exception of small spring-fed salt pools remaining as breeding site refuges, benthic larval habitat would have been greatly reduced and of uncertain suitability. Diversion of water for agricultural irrigation from the Chewaucan River, the lake's only major tributary, has been practiced since around the turn of the century. In periods of drought this diversion makes the lake more susceptible to drying. Recent fluctuations in the stage and salinity of Abert Lake are presented in Figure 2.

Langbein (1961), using hydrologic and salt budget characteristics of various closed basin lakes has derived parameters of lake stability and time element required for accumulation of salts in solution (i.e. age as a saline water body). These calculations, pertinent to

Figure 2. Recent Historical Elevation and Salinity of Abert Lake:

These elevation-salinity profiles based on data in Phillips and Van Denburgh, 1971 and Van Denburgh, 1975. Elevation is plotted in feet above 4200' elevation. Salinity is in total dissolved solids (grams/liter).



consideration of the evolutionary history of Mono versus Abert Lake, are presented in Table 2. Since there is greater variation in the surface area of Abert Lake, exposing alkali-salt deposits along the shoreline more often than at Mono Lake, this instable body of water tends to lose salts by aerial transport to the wind and thus does not accumulate as large a salt mass in solution. It may be expected that Abert Lake becomes highly saline only after recession to near-dryness or in early refilling stages after having disappeared. Due to this solute-water budget, Abert Lake's shallow waters have gone through frequent and rapid changes in salinity, though seldom reach salinities as high as found at present in Mono Lake. By comparison, Mono is a deep-basined lake, less prone to surface area variation that depletes salt and consequently increases substantially in its total dissolved solutes content as it continues to recede. As evidenced by its greater long-term stability (P, Table 2) and age (T, Table 2), Mono also has a long history of relatively constant conditions. With such a history, and recently increasing salinity, it would be expected that the homeostatic mechanisms developed by the population at Mono Lake is capable of more efficient hyposmotic regulation at the higher range of salinities (>100 g/l). Furthermore, this population should be closely adapted to these alkaline waters as non-migratory residents of this permanent habitat. In contrast, Abert Lake brine fly larvae, being subject to more variable conditions and lower salinities (usually well below 100 g/l) would not be expected to regulate as effectively at higher salinities (with the highest known salinity recorded as 95 g/l in 1939, see Figure 2). In addition, these larvae should in general show greater phenotypic flexibility, due to the more transient and fluctuating nature of biotic

TABLE 2: Parameters Characterizing Hydrological Stability and Age at Mono and Abert Lakes

| <u>Parameter</u> | <u>MONO LAKE</u> | <u>ABERT LAKE</u> |
|--|-----------------------|----------------------|
| Coefficient of Surface Variation (V) | .043 [†] (a) | .083* (b-1) |
| | .05* (a) | .23* (b-2) |
| Response Time (k, in years) | 35 [†] (a) | .5 [†] (a) |
| | | 6.9* (b-2) |
| Long-term Stability | 200 [†] (a) | 11.5* (b-1) |
| | | 3.5* (b-2) |
| Age of Salt Accumulation (T, in years) | 5.6* (b-1) | 500 [†] (a) |
| | 11 [†] (a) | |
| | 220,000 (c-1) | |
| | 130,000 (c-2) | |
| | 32,000 (c-3) | |
| | 27,000 (c-4) | |
| | 31,000 (d-1) | |

note: for detailed discussion of the meaning, problems and variability associated with the above parameters see: Langbein, W.B. 1961. Salinity and Hydrology of Closed Lakes. U.S. Geol. Surv. Prof. paper #412.

* calculated from hydrological data

† theoretical

Sources:

- a) Langbein, W.B. 1961. Salinity and Hydrology of Closed Lakes. U.S. Geol. Survey Prof. paper #412.
- b) Van Denburgh, A.S. 1975. Solute Balance at Abert and Summer Lakes, South-Central Oregon. U.S. Geol. Survey Prof. paper #502-C.
 - 1 = based on period 1952-65 (particularly high lake stage, wet period)
 - 2 = based on period 1916-65 (includes both wet and dry years)
- c) Mason, D.T. 1967. Limnology of Mono Lake, California. University of California Publications in Zoology. 83.
 - 1 = based on Na budget
 - 2 = based on Cl budget
 - 3 = based on SO₄ budget
 - 4 = based on K budget
- d) Scholl, D.W. et al. 1967. Age and Origin of Topography Beneath Mono Lake, a Remnant Pleistocene Lake, California. Bull. Geol. Soc. Amer. 78: 583-600.
 - 1 = based on Cl budget

and abiotic conditions within the habitat and the shorter period available for evolutionary change. Enhanced migratory activity might also be characteristic of the Abert population as this would enable escape from a drying lake to alternate habitat by adult emigration. Flight immigration or emergence from prolonged quiescence are potential sources of colonization when increased water input rejuvenates the lake.

METHODS

Collection and Holding of Animals.

All larvae used in the experiments outlined below were collected from late June to late August 1978 and transported from field sites to the laboratory within 10-48 hours in paper towels dampened with lake water and kept cool in styrofoam containers. Last larval instars (4th, recognizable by black dorsal pigmentation pattern and a prominent patch of darkened cuticle on the ventral side of the 3rd segment) were used exclusively in these studies. All holding aquaria and acclimations were held at 15°C on a 16:8 light:dark photoperiod. The holding aquaria were provided with rock substrate and food in the form of natural algae or detritus. Evaporation of the filtered/aerated lake water from these aquaria was replaced regularly with an equal volume of distilled water.

Exposure Conditions: Mortality and Pupariation During Acclimation.

Salinity exposure conditions were selected so as to provide information on survival and osmoregulatory compensation in larvae acclimated to: (1) dilutions and evaporations of filtered natural lake water¹ (Mono Lake larvae in Mono Lake water = MLW; and Abert Lake larvae

¹Studies of the effect of boiling on ionic proportions in solution show no enrichment or depletion of chloride and insignificant change in pH (little change in carbonate to bicarbonate ratio), though the effect on sulphate and borate is unknown (Herbst, unpub. data).

in Abert Lake water = ALW), (2) half-strength sea water (instant ocean sea salts - to avoid trace mineral deficiency) fortified with NaCl only (at an unbuffered pH of 6-7) to examine the influence of non-alkaline hypersaline conditions, and (3) simulated lake water (SLW), prepared from the sodium salts of bicarbonate, chloride and carbonate in the ratio of 3:2:1 respectively (pH 9.1-9.3), the typical composition of alkaline sodium chloro-carbonate lakes, of which both Mono and Abert are representatives (Wetzel, 1975)². As both populations were exposed to this latter series of SLW media, this provides a standard of comparison for the two populations in this type of saline water.

Salinity was varied in each of the three types of media described above and animals were transferred directly from holding aquaria into 100 ml of the aerated test medium (10-30 larvae each). During the following 48 hour acclimation period, survival and moult development of larvae into pupae were noted each 24 hours. The animals were not fed over these two days. Mortality was scored as a failure to respond to tactile stimulus, though a moribund state was easily recognized (shrunken, discolored). The development of larvae into pupae was determined by the event of pupariation which, distinct from pupation, involves the contraction, hardening and tanning of the final larval cuticle to form the puparium within which the pupa develops (typical coarctate form of cyclorrhaphous flies). The pupa is formed only many hours after pupariation, following tissue reorganization during the metamorphic process (Fraenkel and Bhaskaran, 1973).

²Wetzel, R.G. 1975 Limnology - indicates on p. 169 that at pH 10 (near the pH of both lakes at present) the carbonate buffering system consists of HCO_3/CO_3 ratio of about 3:1.

Hemolymph Osmolality at Different Salinities.

Previous studies by Phillips and Meredith (1969) on larvae of the saltwater mosquito and preliminary work done here, show that a steady-state osmotic equilibrium is reached within 24-48 hours of exposure. Two days should thus be an adequate time interval in which to expect homeostatic responses to occur and be maintained. After 48 hours of acclimation in the various experimental media, larvae were rinsed in distilled water, gently rolled dry on tissue paper and placed on squares of parafilm. The cuticle was carefully torn open with fine-tip forceps (damage to gut or internal organs being avoided) and haemolymph flowing out onto the parafilm was quickly taken up in a 5 microliter Yankee capillary-tube micropet pipettor (Clay-Adams) and delivered onto the filter paper discs of a Model 5100 Wescor vapor-pressure osmometer which had been pre-calibrated in the same range as expected haemolymph concentrations. For larvae acclimated at higher salinities, pooling of blood samples was necessary in order to obtain the 5 microliters required for proper saturation of the filter paper discs. The values of hemolymph osmolality were compared directly with measurements of osmolality obtained for the external medium.

Hemolymph Sodium and Chloride Content at Different Salinities.

In order to gain some insight as to the mechanism underlying osmotic and ionic regulation, measurements of sodium and chloride

concentration were selected for examination as osmotic solute constituents of the haemolymph.

As much blood as possible (10-50 microliters) was obtained from larvae acclimated for 48 hours in each series of native lake water salinities and stored in 100 microliters of 10 mM DTT (dithrothrietol, a commonly used anti-oxidant that protects sulfhydryl groups of proteins) and stored at 0-5°C until analysis. This method of storage presented a problem in that a dark precipitant (protein?) formed within a few months, as the DTT became oxidized, losing its protective ability. Since unknown amounts of protein may have become degraded, this precluded analysis of the haemolymph amino acid pool. Residual DTT interfered with the chloride assay by the electrometric-chloridometer method, but the interference was alleviated following complete oxidation with hydrogen peroxide. Stored samples were brought up to 1.0 ml total volume with distilled water and three drops of 30% H₂O₂ added to completely oxidize DTT. After 24 hours, 200 microliter aliquots of this solution were assayed by the Schales and Schales (1941) method of chloride determination using a Beckman/Spinco microtitrator.

Sodium determination was accomplished by diluting 200 microliters of the peroxide-treated haemolymph to 5.0 ml total, containing .02% Alconox detergent. The .02% detergent served as the reagent blank and sodium standards containing .25, .50 and .75 mEq/l Na⁺ in .02% Alconox solution were used for a calibration curve. Sodium measurements were made in a Coleman Model 21 flame photometer.

Hemolymph Bicarbonate

Inorganic bicarbonate content of the haemolymph was determined by assay for uptake of radioisotope ($\text{NaH}^{14}\text{CO}_3$) from both low and high salinity media. Abert Lake larvae were acclimated for a 20 hour period to either 100 mM NaCl or 2000 mM NaCl aqueous media containing 50 mM NaHCO_3 in pH 7.75 phosphate buffer. Acclimated animals were transferred with 10 ml of medium to a reaction flask and 5 microCuries of $\text{NaH}^{14}\text{CO}_3$ added and allowed to equilibrate for 45, 90 and 150 minute periods. Control (heat killed) and live animals (pre-weighed) were removed from the radioactive solution after this equilibration period, washed and homogenized in buffer. The homogenate was transferred to a Kontes metabolic reaction flask containing an alkaline CO_2 -trap, and free $\text{H}^{14}\text{CO}_3^-$, representing the unincorporated endogenous concentration, was then released as $^{14}\text{CO}_2$ by injection of 1.0 ml 2M HCl and aliquots of the trap counted on a liquid scintillation counter.

Metabolism of Hemolymph Bicarbonate.

The fate of bicarbonate was further investigated to determine if CO_2 is fixed into organic acids for utilization as a supplementary energy source as has been shown to occur in brine shrimp (Conte, 1977).

Following acid hydrolysis of the homogenate (see section E), $\text{H}^{14}\text{CO}_3^-$ was purged by heating to 60°C for 30 minutes. Aliquots of the cooled acid-soluble homogenate were counted to measure any non-specific incorporation of $\text{H}^{14}\text{CO}_3^-$. To oxidize all organic acids, 1.0 ml of 30%

H₂O₂ was injected into reaction flasks containing new alkaline CO₂ traps. Subsequent counts of these traps were used to measure specific incorporation into this organic acid fraction.

Total Body Water at Different Salinities.

Brine fly larvae were acclimated for 48 hours to a range of salinities of their native lake waters, then rinsed and carefully rolled dry on tissue paper to absorb adhering water. After recording wet weights, these animals were dried to constant weight at 70°C and the percent total body water calculated as the difference between wet and dry weights. Partitioning of total body water into extracellular vs intracellular water volume may then be calculated using the chloride space method.

Respiration at Different Salinities.

Brine fly larvae were acclimated to the full range of native lake water salinities (MLW and ALW) for 48 hours at 15°C. Those animals which survived were placed in 15 ml of the same medium to which they had been acclimated but which had been aerated to saturate with oxygen, and brought to 15°C constant temperature and pressure equilibration in a Gilson Differential Respirometer. Recordings of oxygen consumption were continued for 2 1/2 hours with readings made at 10 minute intervals. Control flasks contained heat-killed larvae at three intermediate salinities of each series.

Population Ecology Studies

In order to assess the dynamics of abundance and age structure of the benthic dwelling larval population, a standard sampling technique employing the introduction and periodic removal of artificial substrates was used. In addition to counts of larval density, attachment of puparia to the artificial substrate and scoring of emergence (empty puparia = adults) and intact puparia (pupa stage within) would enable estimation of adult production and duration of the pupal stage. Morphometric (body length, excluding respiratory siphon) and pigmentation characteristics of the larvae were used as criteria in age grouping of stage-specific abundance for each instar. Periodic observations and non-quantitative collections at both lakes provided information revealing general life cycle patterns and ecological interactions.

RESULTS

Mortality and Pupariation During Acclimation

The frequency of mortality and pupariation are presented as cumulative percentage histograms for the 48 hour acclimation period at each salinity (Figures 3, 4, 5). As sample size of these acute toxicity bioassays was often low, with single deaths accounting for a large part of observed mortality, the data should be regarded as preliminary and noted only for the general trends that they suggest.

An acute stress response to sudden change in osmotic conditions can be inferred from an increased death rate and/or reduced pupariation (i.e. inhibition of development to pupa stage). Levels of mortality and pupal development near those of control (100% salinity, untreated 1978 lake water), which are assumed to be optimized, indicate a successful adjustment to those conditions.

In waters of their native habitats, both Abert and Mono Lake populations show diminished pupariation at dilute as well as at high osmotic concentrations (Figure 3). In addition, mortality in evaporated lake water from either source increases gradually with increased salinity. While the frequency of pupariation is reduced at higher salinities of Mono Lake water, the response of Mono Lake larvae to comparable osmotic concentrations of the non-alkaline salt water is an increased occurrence of pupariation (Figure 5A). Abert Lake larvae, on the other hand, show virtually no pupariation in either simulated alkaline lake water or non-alkaline salt water (Figure 4B, 5B,C). Higher concentrations of these media do, however, produce substantial

Figure 3. Mortality and Pupariation in Acute Exposures of Brine Fly Larvae to Different Concentrations of Native Lake Water. A. Mono Lake, B. Abert Lake. Numbers in parentheses indicate first, the number of replicate groups tested at each salinity and second, the total number of larvae examined. Cumulative mortality reported is the mean of all replicate groups, with each group weighted by the number of animals in that replicate. M = mortality, P = pupariation. Star indicates control bioassay in untreated 1978 lake water. (mOsM = milliosmoles osmotic solutes/kg water).

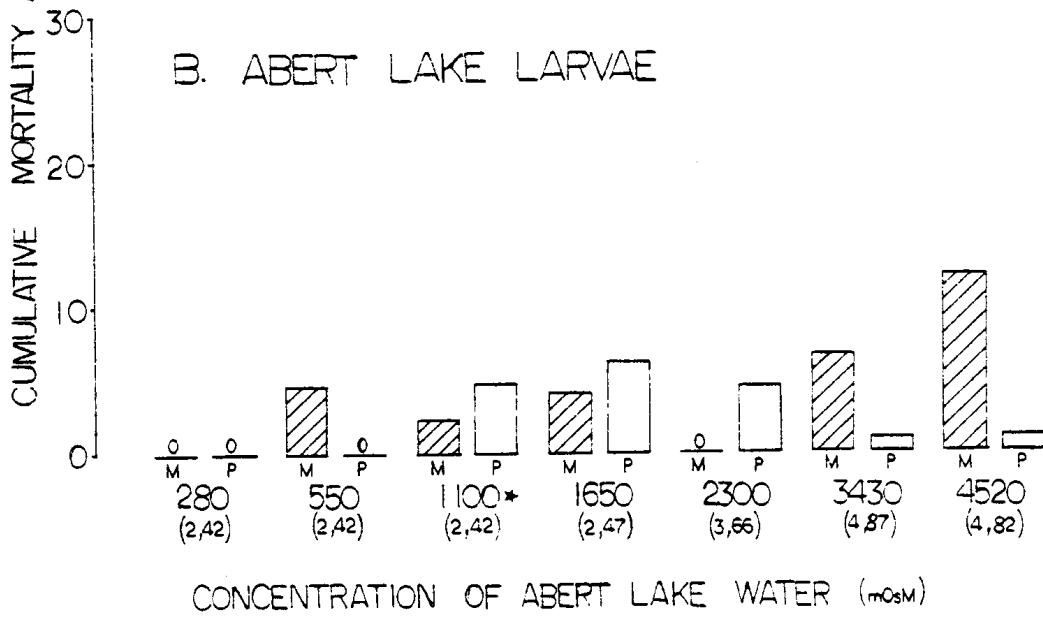
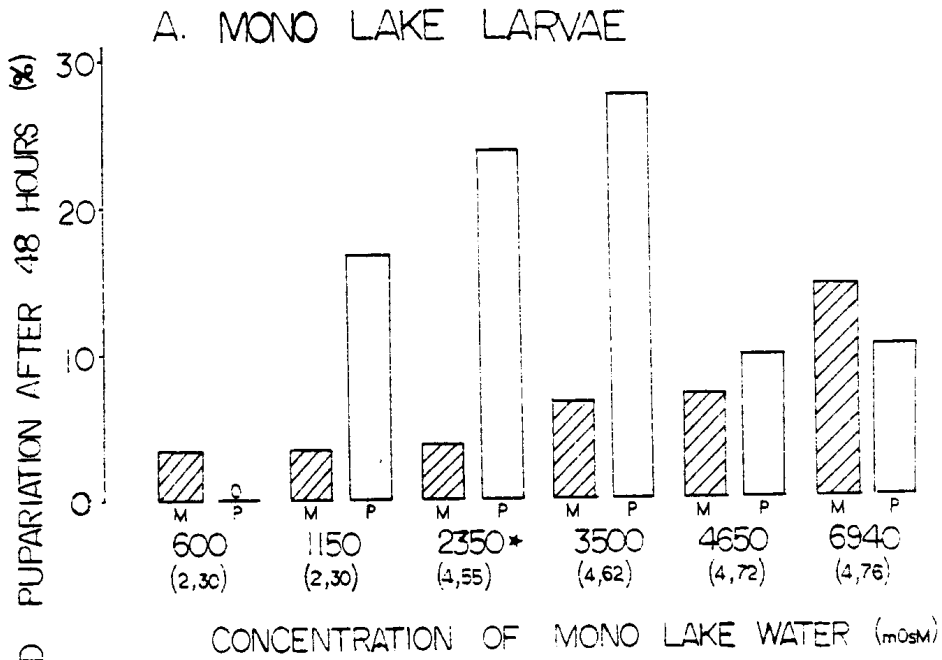


Figure 4. Mortality and Pupariation in Acute Exposures to Simulated Lake Water. A. Mono Lake, B. Abert Lake. Data computed, and notation as in Figure 3. The osmotic concentrations of this alkaline (pH = 9.1-9.3) salt water correspond to solutions of the sodium salts of $\text{HCO}_3^-/\text{Cl}^-/\text{CO}_3^{=}$ in the molar proportions of .15/.10/.05, .3/.2/.1, .45/.3/.15, .6/.4/.2 and .9/.6/.3 from low to high salinity respectively.

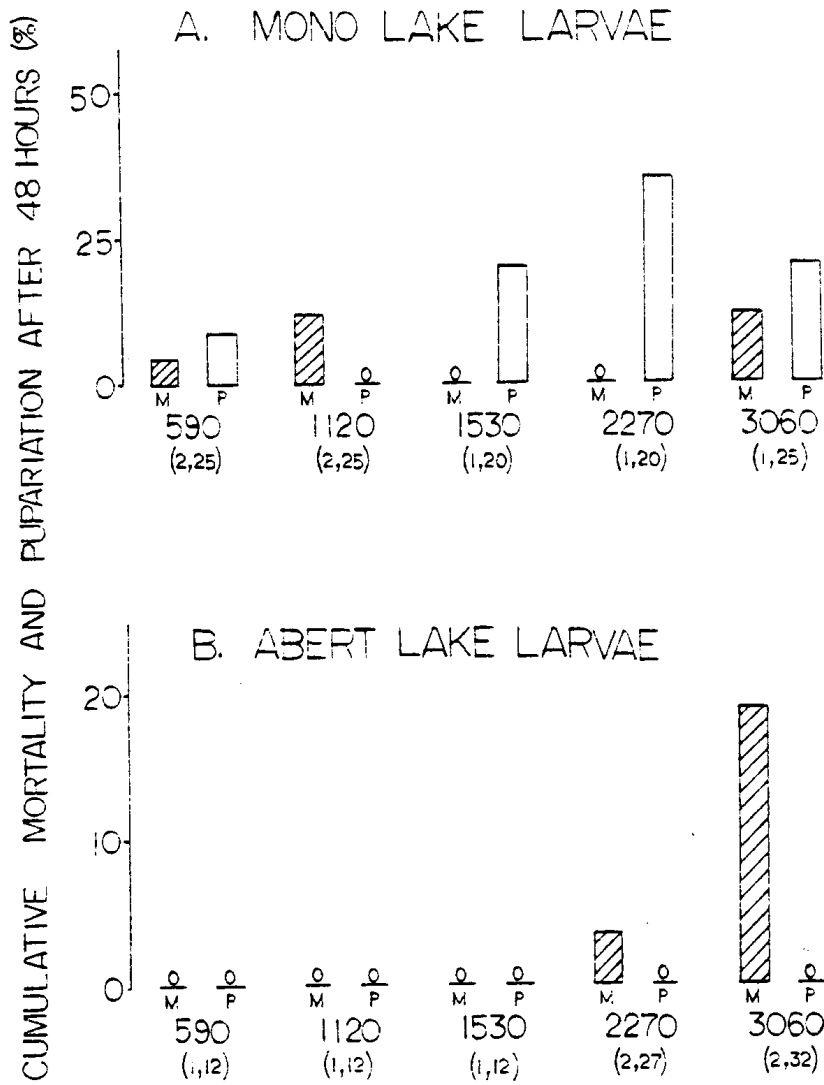
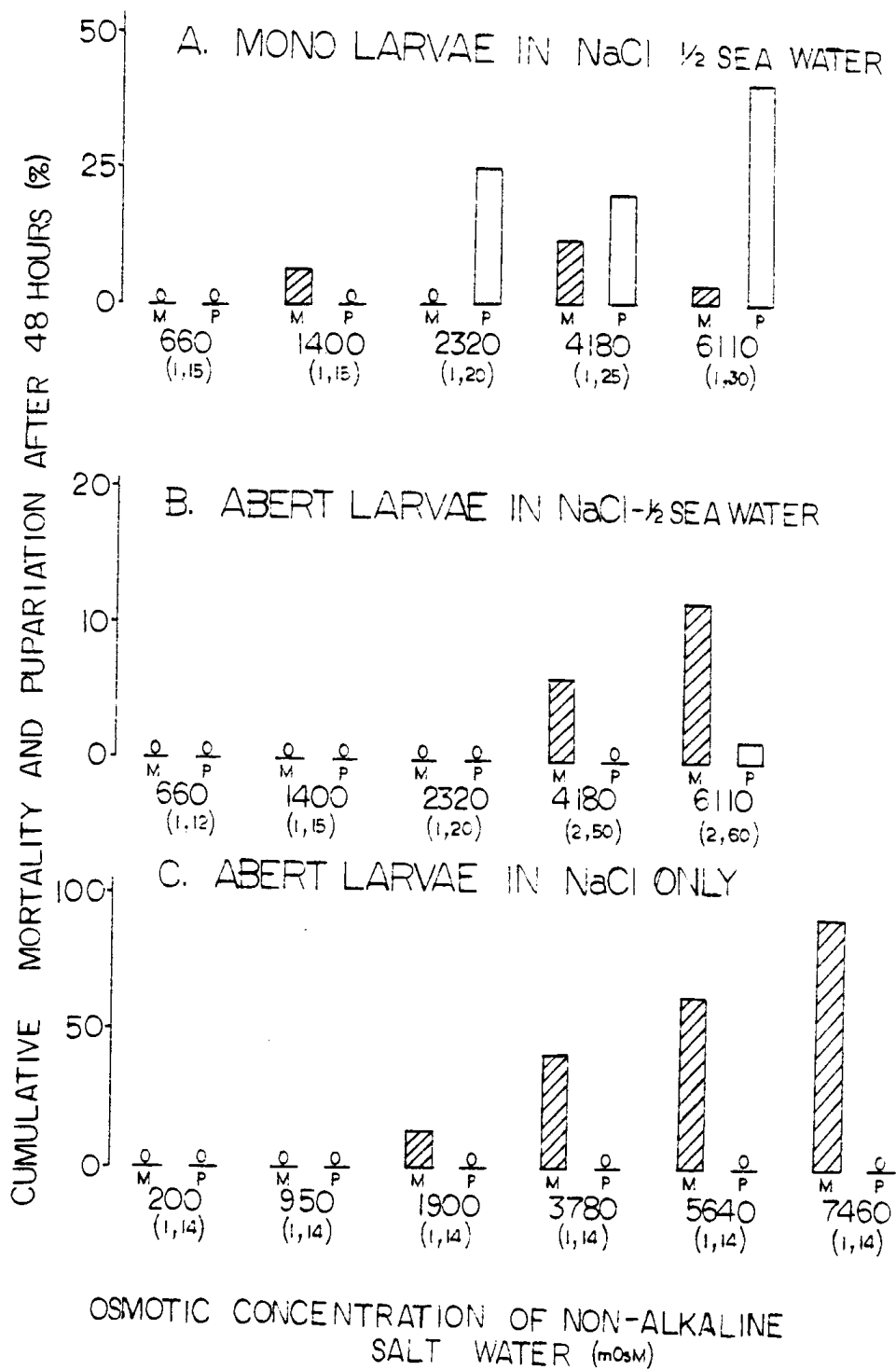


Figure 5. Mortality and Pupariation in Acute Exposures to Non-alkaline Salt Water. A. Mono Lake larvae in NaCl/1/2 sea water, B. Abert Lake larvae in NaCl/1/2 sea water, C. Abert lake larvae in NaCl alone. Osmotic concentrations equivalent to .1M, .5M, 1M, 2M, 3M in NaCl (with 1/2 strength sea water in A and B, without in C) at each salinity from low to high respectively (except in C, where highest is 4M NaCl). Note differences in scale of vertical axis in each group. Notation as in Figure 3. The pH of these sodium chloride solutions is approximately 6-7, unbuffered.



increases in mortality, particularly in those enriched with NaCl. A consistent trend seen in all media is a higher incidence of pupariation among Mono Lake larvae than in Abert Lake larvae.

Osmoregulation

The data show that brine fly larvae from Mono Lake and Abert Lake are hyposmotic regulators in their native or simulated lake water, but that osmotic regulation operates poorly in non-alkaline salt water (Figures 6, 7, 8). In alkaline media (native and simulated lake water), a uniform result was that Mono Lake larvae regulate haemolymph osmotic concentrations at somewhat lower levels than Abert Lake larvae over the entire range of external salinities. Moreover, Abert Lake larvae begin losing osmoregulatory ability at a lower salinity in comparison to Mono Lake animals at the equivalent concentration (though the reverse is true in non-alkaline media).

Figure 6 compares osmotic regulation between larval populations in their native lake waters. Mono Lake brine fly larvae maintain a relatively constant haemolymph osmolality over the range of 250-300 mOsm when exposed to external salinities between 1000-3500 mOsm. In Abert Lake larvae, regulation extends only between 1000-2500 mOsm external concentration, a somewhat smaller range than that observed for Mono Lake larvae. In distilled water, osmolality of the blood decreases markedly, as would be expected due to the influx of water across the semi-permeable integument. Even under these diluting conditions, however, hyperosmotic regulation is observed (at least for a 48 hour exposure).

Figure 6. Osmoregulation in Native Lake Water. Δ and dashed lines for Abert lake larvae, \circ and solid lines for Mono Lake larvae. DW = distilled water, AL 1978 = concentration of Abert Lake brine at time of collection (untreated except for filtering), ML 1978 = concentration of Mono Lake brine at time of collection. Error bars indicate standard deviation for 5-10 separate determinations of haemolymph osmolality on either individual or pooled blood samples (pooled samples weighted by number of larvae used to obtain sample).

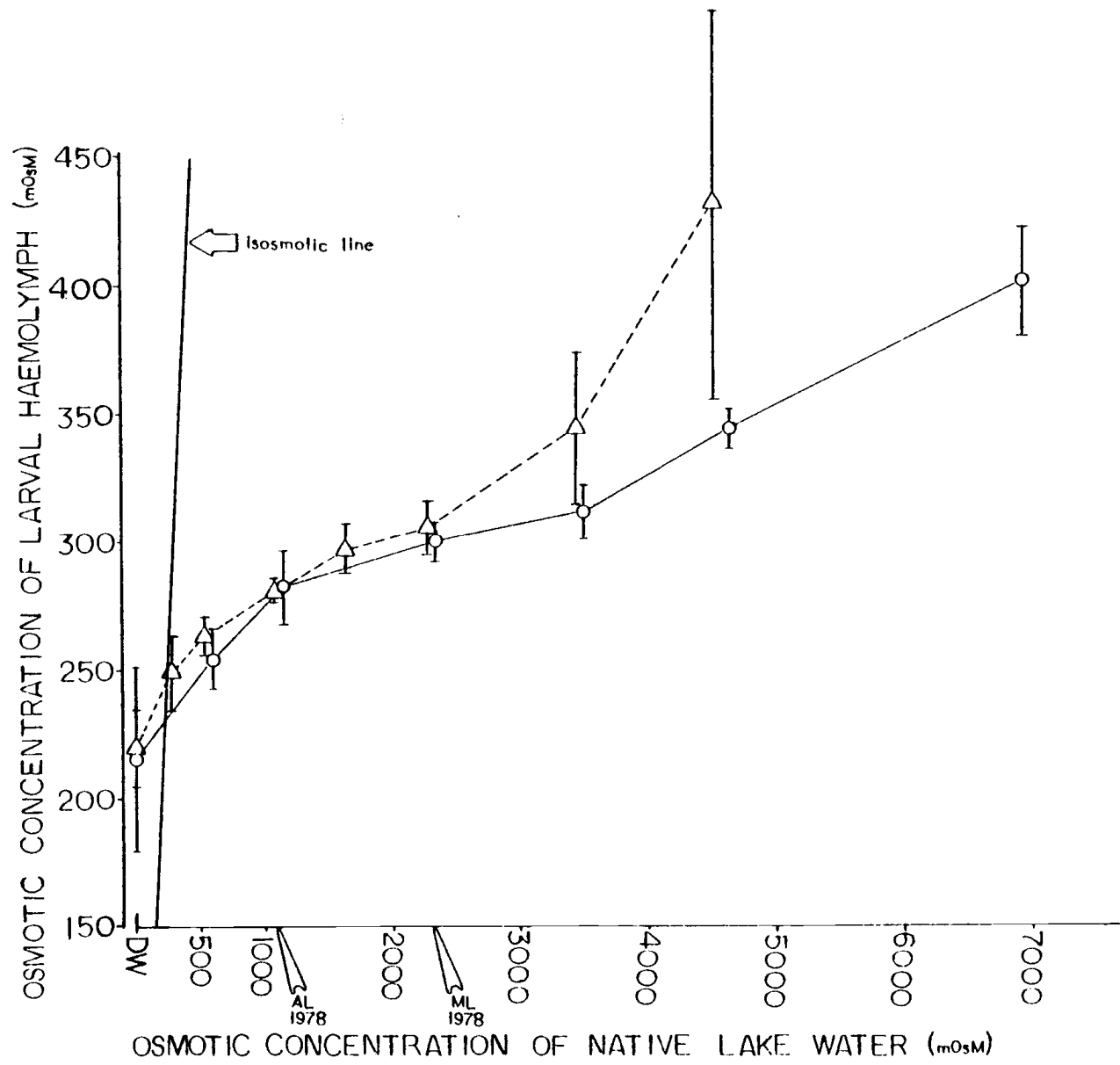


Figure 7. Osmoregulation in Simulated (Alkaline) Lake Water. Δ and dashed lines for Abert Lake larvae, 0 and solid lines for Mono Lake larvae, DW = distilled water. The pH of this artificial media is 9.1-9.3 and contains the sodium salts of $\text{HCO}_3^-/\text{Cl}^-/\text{CO}_3^{=}$ in the ration of 3:2:1 respectively. Note that the range of exposure salinities is approximately only half that in Figure 6 and 8. Error bars as in Figure 6.

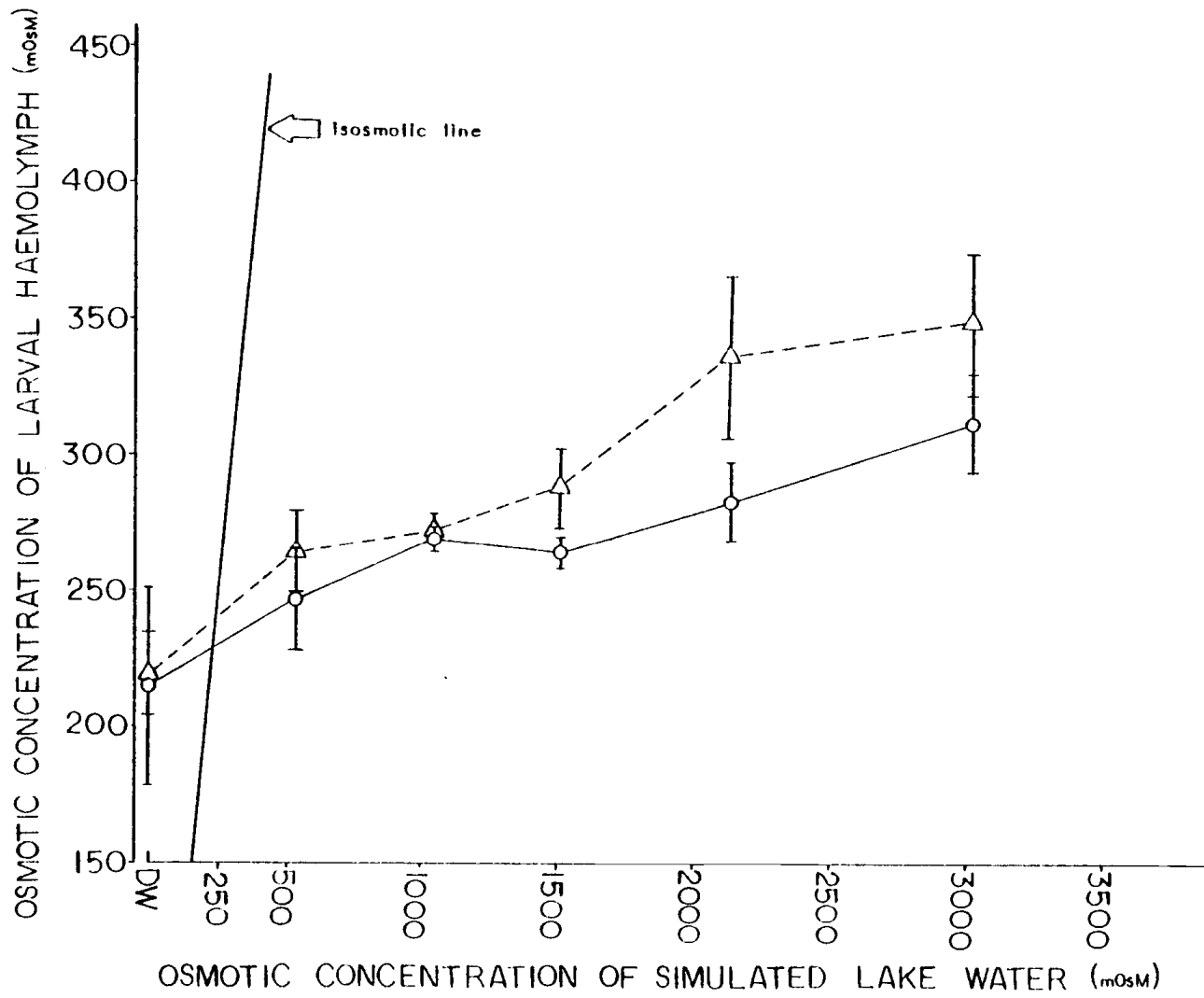
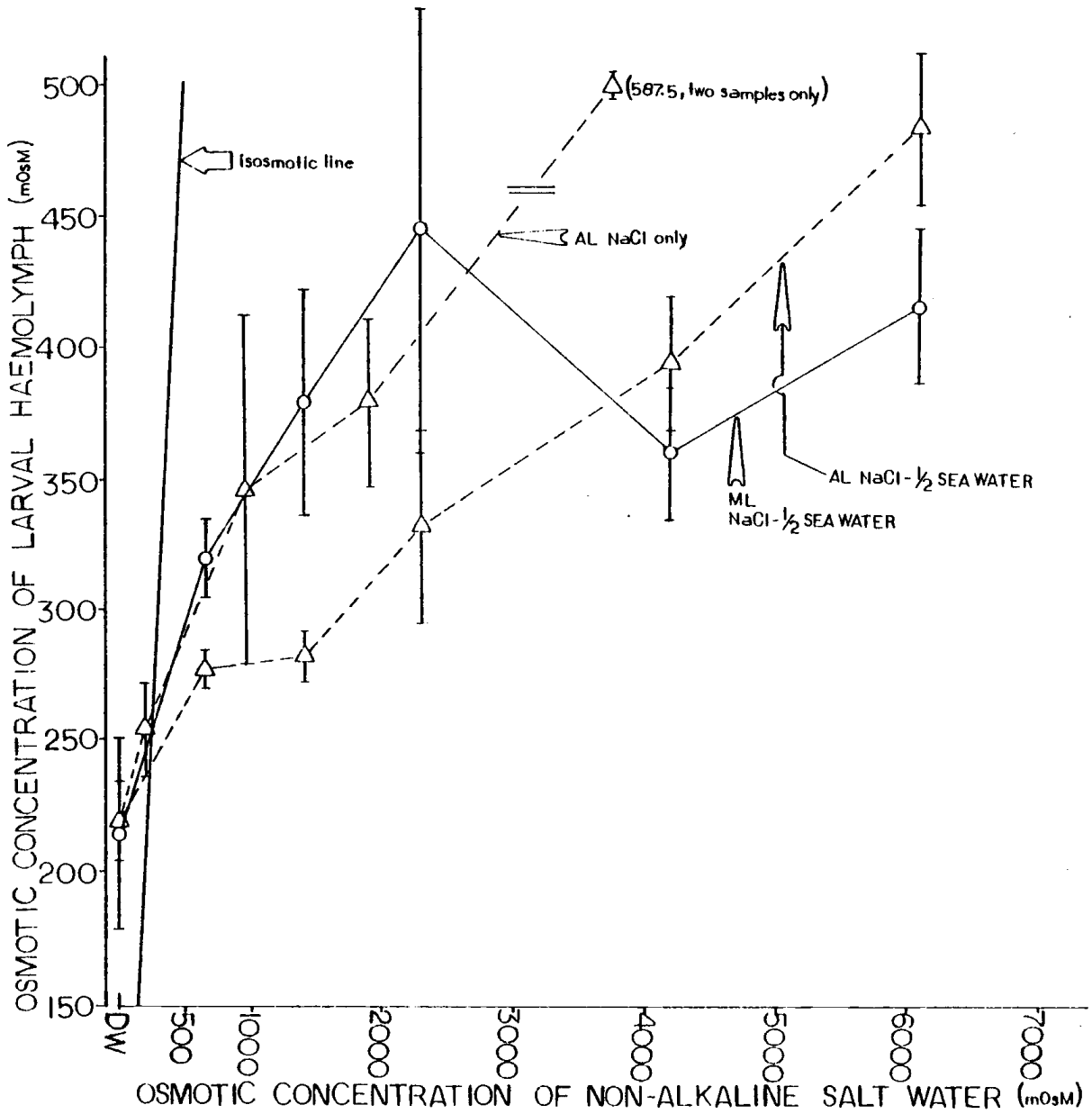


Figure 8. Osmoregulation in Non-alkaline Salt Water. Δ and dashed lines for Abert Lake larvae, 0 and solid lines for Mono Lake larvae. This artificial medium was prepared by fortifying half-strength sea salts (instant ocean) with NaCl, such that trace minerals were available but the solution was predominated by sodium chloride (pH = 6-7). Abert Lake larvae also exposed to NaCl alone. Error bars as in Figure 6.



At the higher external salinities (> 3500 mOsM for Mono larvae and > 2500 mOsM for Abert larvae), osmotic equilibria are established only at substantially higher, but still hyposmotic, internal osmolalities. Greater variability in haemolymph osmolality may also be discerned at these higher salinities, with this effect being more pronounced among Abert Lake larvae. This observation may be attributed in part to a mixed physiological response in the population, where the pooled blood samples of shrunken, moribund animals usually show a higher osmotic concentration than larger larvae, which are clearly more physiologically resilient. It should be noted that for standard deviation data reported on osmotic concentration values (error bars on Figures 6-8), pooled blood samples (necessary at higher salinities) are weighted by the number of larvae used to obtain the minimum blood sample volume required for each osmolality determination ($X = \Sigma[(mOsM)_i (n)_i] / \Sigma(n)_i$, for i replicates with n larvae per replicate). This will have the statistical effect of reducing the dispersion shown between individuals. For this reason, standard deviations reported for high salinity acclimations underestimate variability in the sample population.

Osmotic regulation is demonstrated by Mono Lake larvae throughout the range of salinities of simulated (alkaline) lake water tested (Figure 7). As SLW concentrations reach only to about 3000 mOsM, all salinities are within the natural regulatory range of Mono Lake larvae (see Figure 6). Abert Lake larvae, though also showing hyposmotic regulation, achieve only higher and more variable osmotic values at salinities in excess of 1500 mOsM. These patterns are consistent with those displayed in native lake water.

An inability to osmoregulate effectively in non-alkaline salt water is exhibited by both Mono and Abert Lake larvae (Figure 8). While Mono Lake larvae showed no stable range over which homeostasis occurred, Abert Lake larvae appear to maintain osmotic constancy over a narrower range at the lower salinities (500-1500 mOsm). In NaCl solution alone however, Abert Lake larvae show no sustained osmoregulation and high mortality (Figure 5C).

Water and Solute Regulation

Chloride regulation in the haemolymph is illustrated in Figure 9 and shows that this solute is maintained at a constant level of about 80 mEq/lCl⁻ in Mono Lake larvae. The data for Abert Lake larvae more adequately reflect the variability of these measurements as replicate samples are represented. Even so, Abert Lake larvae show similar levels of chloride regulation (though slightly elevated at the highest salinity tested), with deviations of ± 10 mEq/l at low salinity and somewhat greater at high salinity.

Sodium regulation in larval blood is depicted in Figure 10 and show a steady-state concentration of 125 mEq/l for a range between 500-2500 mOsm. In contrast to data on haemolymph chloride concentration, sodium regulation is not maintained at external salinities above 2500 mOsm in Abert Lake larvae, or above 3500 mOsm in Mono Lake larvae. The results of ¹⁴C-bicarbonate studies indicate that haemolymph concentrations of this solute are regulated at fairly low levels, calculated to be around 3 mM³ (Table 3), in a medium that is 50 mM in NaHCO₃. Although this

Figure 9. Chloride Regulation. Δ = Abert Lake larvae, O = Mono Lake larvae. Chloride content of haemolymph at different salinities of native lake water. Each value is the mean result of several determinations on a single pooled and diluted blood sample (see text).

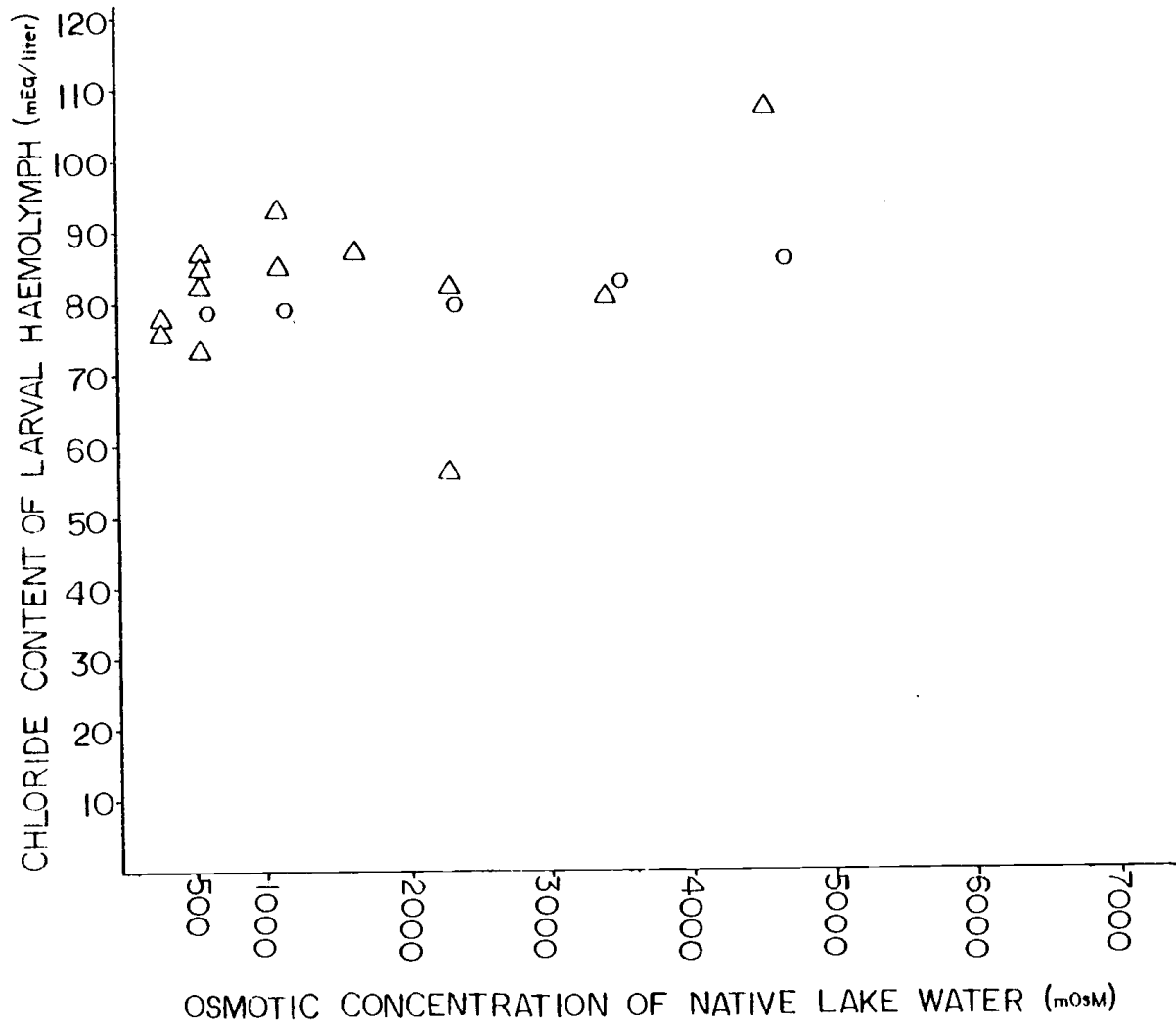


Figure 10. Sodium Regulation. Δ = Abert Lake larvae, O = Mono Lake larvae. Sodium content of haemolymph at different salinities of native lake water. Each value is the mean result of several determinations on a single pooled and diluted blood sample (see text).

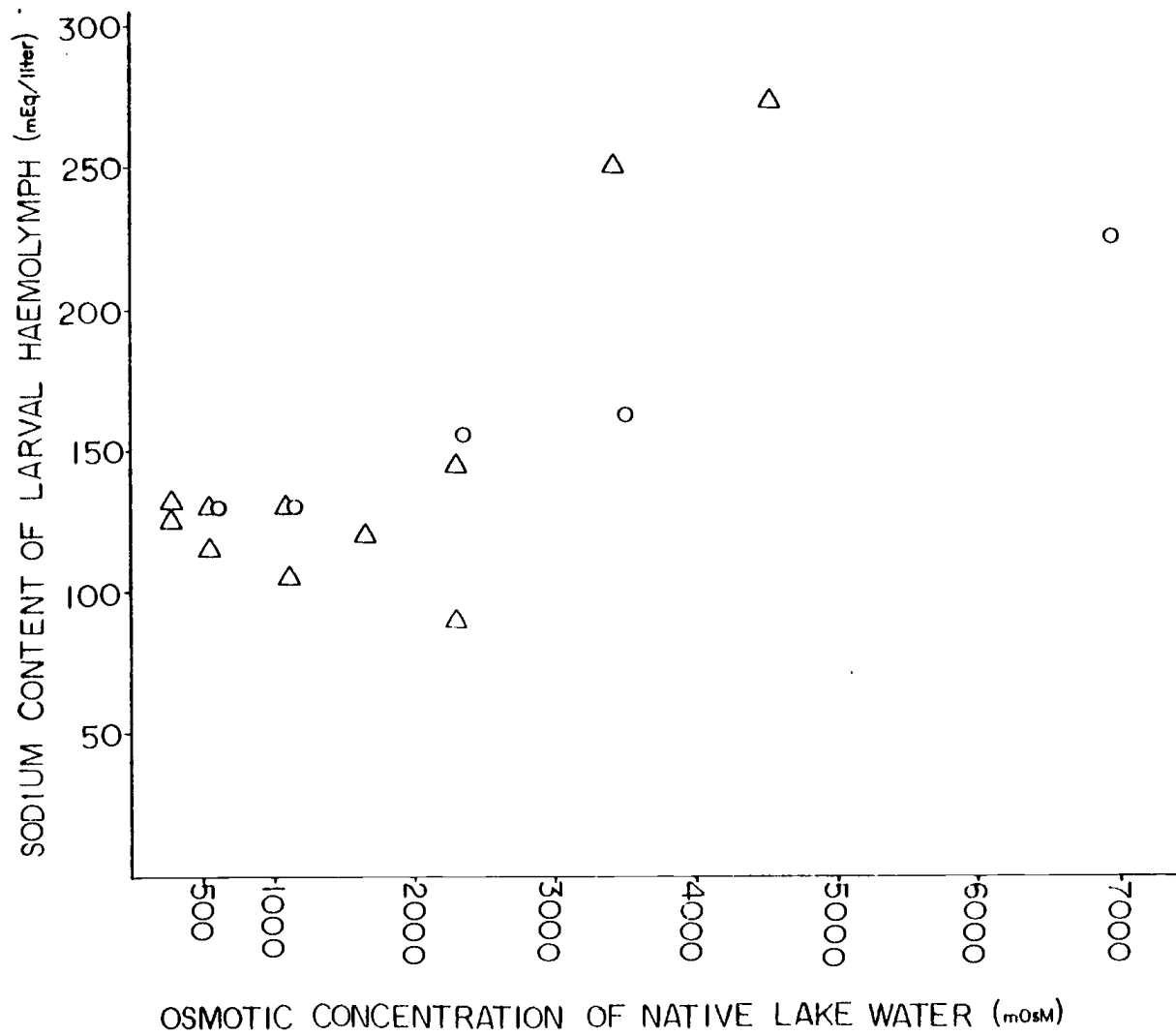


TABLE 3: Bicarbonate Metabolism and Osmotic Equilibria

| | $\mu\text{moles HCO}_3^-/\text{g}$ wet tissue | average haemolymph concentration (mM) | non-specific incorporation ($\mu\text{moles HCO}_3^-/\text{g}$ wet weight) | incorporation into α -Keto acids ($\mu\text{moles HCO}_3^-/\text{g}$ wet weight) |
|------------|--|--|--|---|
| LS exp. | 1.5 - 2.1 | 3 mM | 1.9 | .14 |
| LS control | 5.9 - 12.2 | 15 mM | 2.3 | .69 |
| HS exp. | 1.3 - 2.4 | 3 mM | 2.0 | .14 |
| HS control | 6.1 - 11.9 | 15 mM | 1.7 | .43 |

LS = Low salinity; see text for composition

HS = High salinity; see text for composition

Control experiments are heat-killed animals exposed to radioisotope.
Haemolymph concentration is based on calculations assuming 60% wet weight
as equivalent extracellular fluid volume. All data are for a 90 minute
exposure to $^{14}\text{CO}_3^-$ in the Abert Lake brine fly population only.

TABLE 4: Total Proportion Body Water at Different Salinities

| <u>Salinity of Native Lake Water</u> <u>(mOsM)</u> | | <u>Percent Total Weight</u> <u>as Water</u> |
|---|------|--|
| AL | 550 | 85.9 |
| AL | 1650 | 83.8 |
| AL | 3430 | 82.4 |
| ML | 600 | 90.0 |
| ML | 1150 | 90.0 |
| ML | 2350 | 87.2 |
| ML | 3500 | 85.0 |
| ML | 4650 | 80.0 |
| ML | 6940 | 68.2 |

AL = Abert Lake larvae in native lake waters

ML = Mono Lake larvae in native lake waters

These data calculated from a single determination of total body water as the percent weight change from wet weight to dry weight (after drying to constant weight) for groups of larvae exposed to the specified salinity for 48 hours. See text for further discussion.

value may be different in native lake water, where bicarbonate concentrations are several times higher than in these experiments, the similarity of low and high salinity acclimations demonstrates that these low haemolymph bicarbonate levels are retained regardless of the total salinity. Note that at a pH of 7.75 in the incubation medium, CO_2 is partitioned as ca. 98% HCO_3^- and 2% H_2CO_3 . In the haemolymph, at a physiological pH estimated at 7.0 (checked with pH indicator papers), bicarbonate represents ca. 91% and 9% H_2CO_3 . Since the calculations assumed all CO_2 present as HCO_3^- , the reported values of this haemolymph solute are slightly high. Table 3 also indicates that carboxylation of inorganic bicarbonate into organic compounds, which may be important in intermediary energy metabolism, does not occur. The quantities of $^{14}\text{C}^- \text{HCO}_3^-$ incorporated are not significantly different from residual control levels at either salinity.

The water budget of brine fly larvae is presented in Table 4 and reflects the percent of the body mass that is composed of water (both extracellular and intracellular) at different salinities. The results show that an osmotic desiccation of the body tissues occurs as salinity increases (particularly striking among Mono Lake larvae at salinities > 3500 mOsM), while hydration proceeds at dilute salinities. These findings are consistent with observed changes in blood (extracellular fluid) volume, necessitating pooling of samples at high salinity, while a copious flow of haemolymph occurs at low salinity.

Respiration

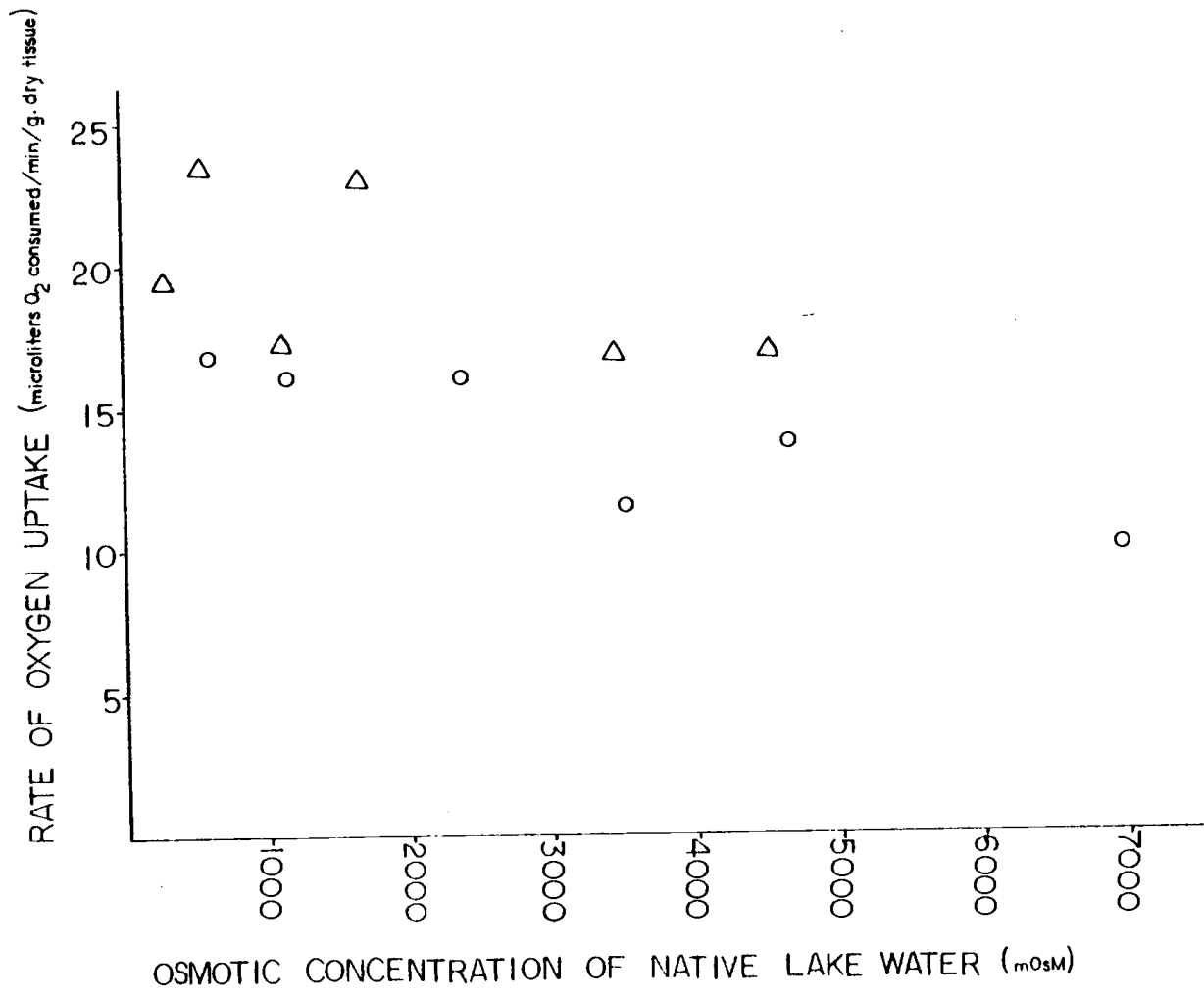
Oxygen consumption was calculated over a constant rate portion of the volume change curve (after approximately 1 hour) and corrected for control levels. The results of these experiments, graphed in Figure 11, resemble closely the rates of respiration obtained in a previous study (Herbst and Dana, 1977) when $V-O_2$ respiratory activities (based on wet weight of tissue) are converted to $Q-O_2$ values (dry weight), as used in the present study. The data in each study reveal that gradually decreasing rates of oxygen consumption are occurring among Mono Lake larvae as salinity increases. Although respiration rates among Abert Lake larvae are variable, they show consistently higher aerobic activity than Mono Lake larvae at comparable salinities. Moreover, there is no pronounced decline in oxygen consumption at high salinity, as is observed in Mono Lake larvae.

Population Ecology

Comments in this section will be limited primarily to qualitative observations since artificial substrate sampling was not complete enough to obtain reliable information on life history or population dynamics.

The active season in both lakes begins in early spring when overwintering pupae at Abert Lake emerge as adults, and diapausing larvae at Mono Lake continue development to pupae and adults (though some pupae may overwinter at Mono). Late summer to early fall marks the onset of developmental quiescence and diapause. During the summer,

Figure 11. Oxygen Consumption ($Q-O_2$) at Different Salinities. Δ = Abert Lake larvae, 0 = Mono Lake larvae. Following acclimation to the appropriate salinities of native lake water, each respiratory rate value (for groups of larvae) was determined only once (however, for comparison to $V-O_2$ trend in aerobic activity, see Herbst and Dana, 1977).



reproduction and oviposition are continuous and several overlapping generations occur.

Size and pigmentation characteristics of the larvae collected from artificial substrate in Abert Lake suggest that there are four larval instars. Developmental time appears to be dependent on temperature and possibly food quality (Collins, 1980). Cumulative duration of the larval stage is variable, but probably averages about a month. The pupa stage also has a variable development time, typically about 2 weeks long (15°C). Duration of the adult stage is unknown.

The primary food source of both larvae and adults is algae. At Abert Lake a luxuriant growth of the filamentous green alga Ctenocladus circinnatus occurs on submerged rock. Adult females of this population feed and oviposit in this algae, while the developing larvae crawl amongst and graze on this abundant periphyton. In contrast, this alga does not occur at Mono Lake, the epilithic forms which are present being far less abundant than at Abert Lake. Therefore, a relatively food limited situation may exist at Mono Lake for developing brine fly larvae, although benthic detrital feeding could supplement algal scraping. Adults at both lakes also feed on decomposing material along the shore which possibly includes epipellic (detritus surface) algae and bacteria.

Being a less saline body of water at present than Mono Lake, Abert Lake has a more diverse community in both the plankton and benthos. While the only other benthic metazoan at Mono lake is the larval biting midge Culicoides varipennis (Diptera: Ceratopogonidae), Abert Lake brine fly larvae are joined in the benthos by large populations of dolichopodid larvae (Diptera) and oligochaetes, with smaller numbers of

tabanid larvae (Diptera), ostracods (Crustacea) and aquatic beetles. Potential competition in the aquatic habitat as well as the terrestrial shore habitat of the adults may thus be a more important factor in shaping population dynamics and abundance at Abert Lake than at Mono Lake. Predation has been observed on adult brine flies by shorebirds, robber flies and tiger beetles at both lakes. The influence of competition and predation on the population ecology of Ephydra hians, as opposed to strictly abiotic factors, may be studied through experimental manipulation of densities and long-term monitoring of change in the abundance and life history features of compared populations (e.g. Mono and Abert).

DISCUSSION

Results obtained in preliminary work with the brine fly populations at Mono Lake (Herbst and Dana, 1977) showed a low incidence of mortality with moderate increases in salinity, while further increases produce somewhat higher rates of mortality, reduced pupariation and a dehydration of body tissues. Larvae appear able to tolerate moderate increases in salinity by establishing what appears to be a behaviorally and metabolically quiescent state. At high salinity, the larvae become bouyant and coil up on themselves or in groups at the water surface and remain largely inactive. Concomitantly, rates of respiration have declined to levels which would seem to prohibit active development and limit metabolic energy available for basal maintenance costs. The findings of the present study corroborate these preliminary observations.

In external salinities that range from distilled water to 260 g/l in dissolved salts (about 8000 mOsm of principally NaCl) of the Great Salt Lake, Ephydra cinerea can maintain a constant haemolymph osmotic equilibrium at approximately 2.5% NaCl or 800-900 mOsm (Nemanz, 1960). In contrast, E. hians is not as effective an osmoregulator, maintaining osmotic homeostasis over a narrower range of external salinity - although at much lower blood osmotic concentrations (one-third of that found in E. cinerea). While the larvae of E. cinerea appear physiologically tolerant of salinity variation, it may be inferred from observations of decreasing abundance of this benthic fly population during years when lake level is low (Collins, 1980), that increases in dissolved solute concentration suppress reproductive and/or developmental activity.

While larvae from Mono Lake show an inhibition of development to the pupa stage (pupariation) in both dilute and concentrated salinities of their native lake waters, exposure to high salinities of non-alkaline salt water produce a relative increase in pupariation. Inhibition of pupariation in high salinities of Mono Lake water is probably associated with the salt stressed physiological condition of brine fly larvae (regulatory failure and decreased metabolic activity). In dilute salinities, the reduced osmotic turgor pressure of the larval haemolymph may lead to an inhibition of pupariation since muscular contraction and increased internal pressure are prerequisite changes to proper puparium formation and cuticular hardening (Zdarek and Fraenkel, 1972). It seems reasonable to assume that pupariation by enclosing the presumptive pupa tissues within an impermeable, sclerotized cuticular case, isolate the animal from the external environment. The elevated levels of pupariation noted among Mono Lake brine fly larvae in high salinities of non-alkaline salt water might therefore be interpreted as a response which enables an escape from physiologically intolerable conditions. In this context it is important to note that osmotic regulation is absent among Mono Lake larvae in non-alkaline salt water - indicative of their nature as a more obligate alkaline water adapted population. It remains to be established what proportion of these early pupariations in non-alkaline salt water reach successful emergence (i.e. does rapid functional isolation result in a higher number of successful emergences than do delayed pupariations?).

Generally, higher levels of pupariation among Mono Lake larvae than Abert Lake larvae (Figures 3-5) suggest basic differences in developmental timing between these populations. Possible explanations

include an intrinsically more rapid developmental rate among Mono Lake larvae or a stress reaction to acclimation conditions (e.g. no food, 15°C, 16:8 light cycle). As the former explanation seems more likely, it would be of interest to examine the consequences to population ecology at each lake.

Abert Lake larvae do not show the increased levels of pupariation seen in Mono Lake larvae in non-alkaline salt water, though high concentrations of this medium produce high mortality. Although unable to osmoregulate in NaCl solution alone, when provided with additional small quantities of other solutes such as Mg⁺⁺, Ca⁺⁺, SO₄⁻ and HCO₃⁻ (those present in the half-strength sea water medium), Abert Lake larvae become capable of osmotic regulation over at least a narrow range of salinity (ca. 500-1500 mOsM, Figure 8). Thus these trace minerals seem to be essential to the survival and physiological adaptation of the Abert Lake larval population to non-alkaline salt water - Mono Lake larvae demonstrate no such flexibility. These patterns suggest that Abert Lake larvae, originating in the more geographically common chloro-carbonate type lake, possess greater physiological plasticity in adapting to diverse chemical environments. Rather than escaping through a pupariation reaction, these larvae remain active in the changing aquatic environments to which they are introduced, until the onset of death in salt-stressed situations. Additional evidence of such sustained activity are the relatively high levels of respiratory metabolism maintained by Abert Lake larvae over a wide range of salinity (Figure 11).

In addition to the fluctuating nature of Abert Lake salinity, and its more typical salt lake chemistry, the probable necessity of

migratory flight from this relatively instable habitat to alternate breeding sites may account in part for the wider habitat tolerance of this population. Although migratory colonization by Mono Lake brine flies may by comparison be of somewhat limited success in non-alkaline salt lakes, they do survive in low to intermediate salinities of this type of water. While it is shown that in either population of E. hians studied, survival is possible in salinities well below those encountered in their native environment, there are probably biotic influences such as competition and predation which determine their presence or absence in less saline habitats.

The variation in osmoregulation data at higher salt concentrations is indicative of phenotypic variability in the sample population. Under these extreme conditions it can be seen that stressed Abert lake larvae show wide variation in blood osmolality and lose regulatory ability rapidly at concentrations above 2500 mOsm (Figure 6), which is nearly equal to the salinity of the lowest lake level in recent times (approx. 95 ppt, see Introduction and Figure 2).

Considering the relative salinity of each native habitat at present and the steadily increasing salt content of Mono Lake over the past 40 years, the more restricted variance in blood osmolality figures for Mono Lake larvae, and the observed difference in the onset of osmoregulatory failure (> 2500 mOsm for Abert larvae vs > 3500 mOsm for Mono larvae) may be attributed to directional selection for intensified solute regulating ability in the Mono Lake population. The large variability in osmotic equilibria of Mono larvae in all concentrations of non-alkaline salt water (Figure 8) is indicative of evolutionary inexperience in this type of solute composition.

It is clear from inspection of Figures 9 and 10 that sodium and chloride are the major haemolymph osmotic solutes, together accounting for about 70% of the total osmolality at most salinities. Although measurements of other blood constituents were not undertaken, it is likely that a significant amount of the remaining osmotic solutes are negatively charged amino acids (common in most insects; Wyatt, 1961).

The failure of osmotic regulation at high salinities in both Mono and Abert Lake larvae occurs coincident with the appearance of excessive sodium in the haemolymph (Figure 10). This is also accompanied by a pronounced loss of water balance as shown by the decreases in total body water (Table 4). These observations imply that the problems of maintaining osmotic regulation of the extracellular fluids during salt stress are associated with both an overburdening of sodium secretion and water conservation mechanisms.

Water loading in dilute salinities and dehydration in high salinities (Table 4) suggest that the cuticle is fairly permeable to water. However, experiments using ligatured animals (both ends of alimentary tract tied off) to examine the extent of cuticular permeability independent of the gut were not performed. Such studies, in combination with experiments measuring drinking rates, rectal secretions and intra/extracellular volume at different salinities would permit resolution of the site of salt secretion and assessment of overall water and electrolyte flux in brine fly larvae.

Preliminary studies with Abert Lake larvae, on haemolymph bicarbonate content shows that it occurs in very low concentrations in larval extracellular fluid (Table 3). This suggests a strong regulatory mechanism for this ion since bicarbonate is a major solute in alkaline

salt lakes (see Table 1). The lack of fixation of bicarbonate into the organic substrates of intermediary metabolism is not surprising considering the importance of lipid catabolism rather than carbohydrate as the primary energy source of insect metabolism (Wigglesworth, 1972). It might be argued however, that since a reduction in oxygen consumption with increased salinity is seen only in Mono Lake larvae, supplementary energy from a CO₂-fixation source is more likely to be needed by these larvae than the Abert Lake animals. Alternatively, adjustment to increased energy costs of osmoregulation, while maintaining only the same or decreasing rates of oxygen consumption, may occur through the reapportioning of energy according to metabolic demands. Experiments are needed to distinguish the relative contribution of each possibility. The ultimate loss of osmotic regulation in high salinity, oxygen depleted water, does however suggest that neither additional energy supplies, nor a shift in the economy of energy flow are sufficient to maintain osmotic homeostasis.

Further aspects of the biology of E. hians should be examined from an ecological perspective. In particular, the budgeting of energy between the physiological costs of homeostasis, growth, reproduction, fat accumulation and the developmental physiology of earlier larval instars, may give insights to the life history characteristics of this species under different environmental conditions. In addition, lab and field manipulations should be done to establish the relationship of salinity, substrate type, food quality and quantity, competitors and parasites to patterns of abundance and distribution. As there appears to be a difference in the diapausing stage of the life cycle between the populations at Abert and Mono Lake, studies on genetic and environmental

induction of diapause along with further comparative information on diapause of E. hians in other aquatic situations, may provide clues as to how this variable feature of life history is adaptive.

In conclusion, the issue of physiological adaptation and brine fly population persistence in an increasingly saline Mono Lake will be considered. The following changes among Mono Lake brine fly larvae at high salinity are suggested by the present study:

- i) increased mortality
- ii) decreased pupariation
- iii) decreased metabolic energy production
- iv) loss of osmotic homeostasis
- v) physiological quiescence

Such constraints are likely to result in decreased population productivity through a reduction of the intrinsic rate of growth (since development time would be longer and less successful) and/or abundance. Decrease in metabolic activity may reflect either a moribund state in these larvae or perhaps a state of quiescence which enables them to survive under a prolonged developmental schedule, thus avoiding unfavorable environmental conditions.

The results contained in this study provide a basis for assessing the present adaptive capacity of the E. hians population at Mono Lake with respect to increasing salinity. Although the possibility of evolved genetic adaptation to increasing salinity exists, it is problematical and may occur only within the limits imposed by bioenergetic costs necessary to maintain reproduction, growth and other physiological energy requiring processes.

If, under a program of continued water diversion, the lake stabilizes at a salinity some three and one-half times its present salt content (Loeffler, 1977) - far above the zone of physiological tolerance as defined in this study - the brine fly population is likely to decline substantially and possibly be eliminated altogether.

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