EGG HATCHING AND LIFE HISTORY OF A FAIRY SHRIMP *BRANCHINECTA MACKINI* DEXTER (CRUSTACEA: ANOSTRACA) IN A MOHAVE DESERT PLAYA (RABBIT DRY LAKE)[1]

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Abstract. Observations on populations of *Branchinecta mackini* in temporary ponds, which last for as little as 3 days to as long as 4 months, on a desert playa showed that hatching of dehydrated cysts (eggs) follows initial entry of water into the basin if salinity remains low. Salinity ranges from an initial 0.5‰ to as much as 34‰ as the pond evaporates. Hatching is continuous at constant low salinity, but since salinity generally increases rapidly, initial hatch is usually of short duration. Additional periods of hatching follow further inflows of water or after melting of ice, that is, after reductions in salinity. The duration of hatching is inversely proportional to rate of increase in salinity. When salinity of small-volume summer ponds increases at rates above 500 ppm (1,000 μmhos) per day, there is virtual inhibition of hatching.

Laboratory studies showed that egg hatching was controlled by both salinity and oxygen operating in various combinations to inhibit or stimulate hatch. The hatching characteristics of desiccated eggs collected from the dried mud of the lake basin differed from non-dried eggs obtained from laboratory cultures after ejection from ovisacs of living females.

The finding that a salinity-oxygen complex regulates hatching in a desert pond permits tentative explanation of a difference between branchiopods of humid and arid regions. In both cases the branchiopods are characteristic of astatic waters, and stimulation of the egg to hatch must be by some factor that changes at the time of origin of the temporary pond. In humid regions the factors of concern are temperature and oxygen. If the water undergoes significant changes in salinity, as it does in arid regions, control of egg hatch may be by both salinity and oxygen, with temperature limited to control of rate of development.

INTRODUCTION

The Anostraca (fairy shrimps) are branchiopod crustaceans whose usual habitats according to Dexter (1959) are "rain pools and temporary ponds that form from melting snow and ice." The dormant stage, which is usually an embryonated egg or cyst, permits survival during periods when the ponds are dry. Because mere wetting often is not sufficient to bring about resumption of development, various factors or combinations of factors, reviewed by Hutchinson (1967, p. 562), have been suggested as stimuli for hatching. Failure to develop has sometimes been attributed to an obligate diapause, but it is more likely an adaptation to stimuli other than wetting, thereby inhibiting development under certain conditions unfavorable to completion of life cycle.

In the study reported below it was found that hatching of eggs of *Brachinecta mackini* (Dexter) in a Mohave desert pool was regulated mainly by changes in salinity, with involvement of oxygen tensions, and without influence of temperature. The life cycle is so well synchronized with environment that there is little wastage of eggs.

Jennings and Whitaker (1941) reported that salinity affects the rate of excystment of *Artemia salina,* a specialized Anostracan, exceptional for its adaptations to hypersaline waters. The control of emergence from its dormant stage by external osmotic pressure has been clarified by Clegg (1964). Although there are reports of osmotic and ionic adaptations of active stages of other branchiopods (e.g., Broch 1969, Cole and Brown 1967), Horne (1967) seems to have written the only paper reporting that salinity inhibits the hatching of freshwater Anostraca in natural waters. Most studies of freshwater Anostraca have been made not in arid but in humid regions, where changes in salinity are negligible and where regulation of hatch is predominantly by oxygen concentration. Thus Broch (1965) found that stimulation of the dormant stage of *Chirocephalopsis bundyi* (Forbes) is by low oxygen concentration. Low oxygen tension can also inhibit excystment as reported by Moore (1963), who found that eggs of two species of Anostraca—*Streptocephalus seali* Ryder and *Eubranchipus holmani* (Ryder)—would hatch only in well-aerated water. Bishop (1967) found that low oxygen concentrations inhibit hatching of the conchostracan, *Limnadia stanleyana* King. Brewer (1964) reported that lowering of oxygen tension is the stimulus for hatching of a calanoid copepod, *Diaptomus stagnalis* Forbes. Sussman and Halvorson (1966,

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p. 201) give even greater generality to oxygen as a stimulus in their discussion of presence and absence of oxygen as an environmental influence on germination of spores.

Need for combinations of factors has been reported. It has been known since 1941 that low oxygen stimulates hatching of aedine mosquito eggs (Gjullin, Hegarty, and Bollen 1941). But Horsfall (1956 & 1958) showed that additional factors, such as time for development and proper temperature, are needed to "condition" *Aedes* eggs before they can be stimulated to hatch by low oxygen concentrations. Interactions of two or more factors are thus possible, and Weissman-Strum and Kindler (1963) have postulated a two-stage process of hatching of *Aedes* eggs that requires low oxygen to stimulate breakdown of a water-permeable barrier, which then permits the entry of water, an osmotic phenomenon.

It thus seems that apparently conflicting reports of what stimulates and what inhibits arthropod excystment are due to specific differences, due to involvement of more than one factor, or due to all eggs of one species not being in the same "condition." The differences in stimuli required by humid region (freshwater) anostraca such as *Chirocephalopsis,* which responds to low oxygen and temperature, and the arid region (hypersaline) *Artemia,* which responds largely to osmotic changes, suggested that study of a species inhabiting waters of intermediate salinity might contribute to understanding the requirements for resumption of development and the broader problem of adaptation to environment.

To put the Rabbit Dry Lake waters into perspective, we can compare them with conditions reported elsewhere. In the tropical desert of northern Sudan, Rzoska (1961) reported conductance at the end of the existence of a temporary pond to be 1,130 μmhos (about 550 ppm). This terminal concentration is about equal to the minimum initial salinity of the Mohave desert pond, and not much greater than salinities reported by Prophet (1963) in studies in Kansas and Oklahoma, where no pond had a conductivity greater than 555 μmhos (about 275 ppm). The waters of Rabbit Dry Lake increase in salinity from about 0.5‰ to terminal salinities about equal to sea water (34‰) during the final stages of summer ponds. Horne (1967) found that the salinity in several Wyoming ponds increased to a maximum of from 0.5 to 1‰, except for one pond in which *Branchinecta lindahli* and *Streptocephalus texanus* occurred in which the salinity increased to 16.5‰ The latter concentration is comparable to Rabbit Dry Lake.

Field Studies

Introduction

This report is restricted to ponds appearing in one basin on one dry lake bed on the Mohave Desert. Rabbit Dry Lake is in the Lucerne Valley at an altitude of 2,950 ft (950 m) in the southeastern corner of the Mohave Desert in San Bernardino County, California (34°27′ N, 117° W). Although other California desert ponds have been studied, this pond is chosen for report because it was the most intensively studied (data are available from six different fillings, during three seasons over a two-year period) and also because it is the simplest biologically. One species of branchiopod—*B. mackini* Dexter—predominates almost to the exclusion of other macroscopic animals. *B. mackini* was first reported from brackish lakes in the state of Washington by Dexter (1956) and has since been reported in California and Nevada (Dexter 1959).

In nearby Mohave waters there are other branchiopods, including another *Branchinecta* sp. (*B. lindahli*) as well as *Thamnocephalus platyurus* and *Triops longicaudatus* (which are often associated), together with complex communities of Cladocera, Ostracoda, Conchostraca, and Insecta.

In Rabbit Dry Lake, the only macroscopic invertebrate in addition to *B. mackini* is *Branchinecta gigas* Lynch. Unlike *mackini* which is present in all ponds formed—winter, spring and summer—*B. gigas* is present in winter and spring only. It apparently is intolerant of summer heat. *B. gigas* was first reported from Washington in 1937 and since then from Montana, Nevada, and Utah (Dexter 1959); it has not previously been reported from California. Despite its large size (50–90 mm) it may be missed in sampling because of its relatively small numbers. It is a predator on *mackini,* which outnumber it in a ratio of one *gigas* to 40,000 *mackini* in some collections. *Gigas* was observed to consume about 35 young *mackini* per day. Prey smaller than 1.5 cm are eaten whole, larger ones are nipped in two and only the abdomen eaten. Both prey and predator hatch at the start of the pond, so predation on *mackini* occurs from the very beginning. *B. gigas* is mentioned here only incidentally, but it obviously is not unimportant to the general ecology of the pond and to the population dynamics of *B. mackini. Gigas* is ignored in the following account in order to focus attention on the egg-hatching of *B. mackini.*

Field methods

Instruments.—Changes in temperature, specific conductance, oxygen concentration, and pH were

measured with the following battery-powered instruments: *Temperature:* Yellow Springs Instrument Company, Tele-Thermometer, Model 43TD; *Specific conductance:* Industrial Instruments, Solu Bridge, RB3-338, using conductivity cells G-2 and G-20; *Oxygen concentration:* Yellow Springs Instrument Company, Oxygen Meter, Model 51; *pH:* Beckman Instruments, portable pH meter, Model N; *Radiation:* Instrument Specialities Company, Radiometer, ISCO Model SR.

Sampling.—Sampling of the shrimp population was accomplished from shore either with a triangular net (80-mesh nylon), mounted at right angles on the end of a 10-ft aluminum pole, or by suction. The shrimp were filtered into a small 80-mesh nylon bag placed over the inlet of the sampling vessel. The vacuum method was used for sampling ponds whose depths were 1–2 cm or less. The samples were preserved in 4% formaldehyde for measuring and counting. The sizes reported are lengths of preserved individuals from anterior end to anus as measured under a stereoscopic microscope with a calibrated eyepiece-micrometer.

Estimation of date of hatching.—Because the ponds were not under daily observation, the date of hatching was not always known for the shrimp that hatched subsequent to the initial hatching period. An estimate of subsequent hatches was made from the growth rate of the initial hatch, using the largest immature individual collected on a given day, taking 10 mm as the usual size at first maturity. Growth rates of older mature individuals (longer than 10 mm) could not be used, because growth almost ceases during maturation of the gonads. After maturation, growth resumes and then slows as maximum size is reached, according to studies of Dexter and Ferguson (1943), Coopey (1950), and Moore (1963). The maximum size of three-month-old *B. mackini* adults does not greatly exceed 30 mm. Collections showed that hatching in Rabbit Dry Lake is seldom continuous. Though there may be a continuous size distribution in the population, frequency peaks indicate separate bursts of hatching.

Results

Climate.—The study pond is in an arid region. According to U. S. Weather Bureau records, rainfall in Lucerne Valley during calendar years 1965, 1966, and 1967 totaled 18.2, 6.1, and 11.1 cm, respectively, for an average of 11.8 cm (4.65 in.). Most rain falls in November and December. Rains in spring can be negligible, but rains in April 1967 amounted to a third of that year's total. May and June are generally rainless as are September and October, but the summer rains can extend into September. Summer rains, mostly in July and August, usually amount to a relatively small fraction (1/10 to 1/4) of the annual total.

Standard air temperatures in July and August average 28°C, with maximum of 41° and minimum of 12°C. In winter air temperatures range from a minimum of −8°C to a maximum of 20° for an average of 9°C. Temperatures below freezing can occur from November through April on this high desert.

General environment.—

a. Morphometry.—Rabbit Dry Lake is a playa (a "flat-floored bottom of an undrained desert basin") about 3 km long and 1 km wide lying in a valley between the Granite and San Bernardino Mountains. The lake bed is bisected by a highway running east/west, which dams off the southern quarter of the playa. The study pond is the deepest part in this south end of the lake. Water flows into it from the northern slopes of the San Bernardino Mountains, via ravines and canyons, but the valley is essentially arheic, and little rain falls on the valley itself.

It is seldom that the entire playa is covered with water. In winter the study pond usually is about 120 m long by 24 m wide, with a maximum depth of 20 cm, which is not at the center but near the northern edge of the long dimension, which parallels the highway. A volume equal to that of the winter pond is seldom attained in summer. Due to the shallow profile of the basin, a large fraction (up to ¾) of the area covered by a large-volume winter pond may be covered by a small-volume summer pond, but with a very shallow sheet of water, most of which is only 1–2 cm deep. Because of the extreme shallowness, the ponds do not last long; the surface-to-volume ratio favors rapid evaporation.

b. Light.—The pond water is opaque with suspended soil particles so fine they do not settle even during windless periods or when ice covers the pond. The colloidal-sized particles do precipitate near the end of a pond's existence when the salinity increases rapidly (see below).

Data on light penetration taken on a clear windless day between 1200 and 1225 on April 23, 1967, are representative of data gathered at other seasons. Columns 1, 2, and 3 of Table 1 show the energy falling on the pond's surface and penetrating to depths of 4 and 10 mm. The 4-mm depth was selected because the remote sensor used with the radiometer was just visible at this depth. Columns 4 and 5 show the energy at 4 and 10 mm relative to that at the surface. The amount reflected and re-radiated was not determined. It is obvious from these data that longer wave lengths

TABLE 1. Energy transmitted to 4 and 10 mm depths, Spring Pond, Rabbit Dry Lake, April 23, 1967

	(1)	(2)	(3)	(4)	(5)
	Energy-μw/cm² per mμ			Percent of energy at pond surface	
mμ	At pond surface	At 4 mm	At 10 mm	(2)/(1)	(3)/(1)
380	29.1	0.110	0.036	0.38	0.12
400	50.5	0.000	0.002	0.00	0.00
425	58.9	0.000	0.000	0.00	0.00
450	81.6	0.000	0.000	0.00	0.00
475	90.0	0.005	0.001	<0.01	<0.01
500	86.1	0.038	0.063	0.04	0.07
525	83.9	0.160	0.230	0.19	0.27
550	72.9	0.530	0.660	0.73	0.91
575	76.2	1.600	0.730	2.10	0.96
600	73.3	4.700	2.300	6.41	3.12
626	66.6	5.300	2.800	7.96	4.21
650	66.5	5.300	4.000	8.00	6.02
675	62.0	11.200	4.900	18.20	7.91
700	51.1	10.900	5.700	21.30	11.10
725	45.6	7.800	6.000	17.10	13.12
750	40.9	8.200	3.700	20.00	9.05

make up most of the energy penetrating into the water. At no wave length does light penetrate below 20-25 mm.

One effect of this turbidity is that the bottom is dark, and hence photosynthesis is restricted to the uppermost layer of water or to the substratum at the shallow edges of the pond. Another effect is that it is impossible to observe the life in the pond except as organisms surface or become visible in the uppermost 4 mm.

c. Salinity.—Estimates of salinity are based on determinations of conductivity. A graph for the interconversion of specific conductance in micromhos (μmhos) of the pond water to osmolality in milliosmols (mOs) and to salinity in parts per thousand (‰) is presented in Figure 1. The osmolality of seven samples of water from Rabbit Dry Lake (Spring Pond, 1967) and of a standard NaCl solution were determined with a line-operated freezing point osmometer (Advanced Instruments Co., Series 63).

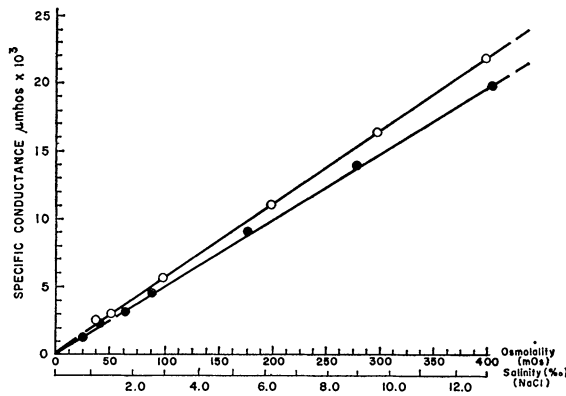

FIG. 1. Relation of specific conductance to osmolality and salinity, based on standard NaCl solutions (o) and samples from the Spring Pond, 1967 (•).

Because all the ponds discussed are within the same basin, the waters have the same ionic composition. Notations of salinity (‰) shown along the curves of Figures 3–6 are expressed as NaCl, based on conductivity measured in the field converted to salinity by the graph in Figure 1. In Table 2 are shown the ionic constituents of the water in Rabbit Dry Lake in the range of salinity of concern to the egg-hatching observations. It is a chloride water but with fairly high proportion of bicarbonate, which accounts for deviation of its curve from that of the chloride standard in Figure 1.

d. Dissolved Oxygen.—Oxygen was usually near saturation at all seasons and at all times of day. In the shallow waters concentrations were the same at the surface and just above the bottom especially under windy conditions. Occasionally, on windless days, supersaturation was observed in restricted areas when the planktonic algae, which are predominately flagellated single cells, rose to the surface where they formed visible green streaks. Within such a streak the oxygen could be above saturation (e.g., 112%), while just out-

TABLE 2. Chemical analyses of three samples of Rabbit Dry Lake water, at 1.4 ‰ (2,200 μmhos), at 3.5 ‰ (5,600 μmhos), and at 6.0 ‰ (10,000 μmhos)

	Analysis at 1.4 ‰			Analysis at 3.5 ‰			Analysis at 6.0 ‰		
Ion	Meq/l	‰	‰ ratio ion/total	Meq/l	‰	‰ ratio ion/total	Meq/l	‰	‰ ratio ion/total
Na	22.0	0.506	0.374	52.5	1.210	0.347	95.0	2.200	0.369
K	0.15	0.006	0.004	0.14	0.005	0.001	0.26	0.010	0.002
Ca	0.38	0.008	0.006	0.65	0.010	0.003	1.10	0.020	0.003
Mg	0.02	0.002	0.0001	0.14	0.002	0.0006	0.31	0.004	0.0007
Cl	14.60	0.520	0.384	38.69	1.380	0.396	68.03	2.400	0.403
SO_4	1.30	0.062	0.046	3.33	0.160	0.046	6.04	0.290	0.048
CO_3	0.8	0.050	0.037	2.96	0.170	0.049	5.36	0.320	0.054
HCO_3	4.50	0.202	0.149	12.30	0.550	0.158	15.90	0.710	0.119

side and below the green area the concentration would be at saturation against air.

During windless periods and when salinity was greater than 10‰, the algae settled out together with the suspended sediments. Bubbles of oxygen then appeared on the bottom in localized areas of algal concentration, and the clear overlying water showed oxygen supersaturation up to 166%. Oxygen concentrations as low as 65% were observed under ice. Still lower values occurred at the mud/water interface and in the bottom mud. Conditions at the bottom will be discussed in the section below on the microenvironment of the egg.

e. Hydrogen ion concentration (pH).—The pH was usually 8.8 to 9.2 during the entire existence of a pond. Often there was no change greater than 0.1 pH unit during any given day. Only within localized areas of intense photosynthetic activity, as along the edge of the pond in a mat of algae or within a streak of suspended algae, were higher pH values observed. Above actively photosynthesizing algae the pH at times was 10.2, while values an entire pH unit lower (in the usual range from 8.8 to 9.2) were present 1–2 cm from the algal concentration.

Existence of such localized conditions was dependent upon lack of winds. Stirring by winds rapidly reduced extremes of pH to the usual value near 9.0. At the mud/water interface pH was 8.6, while the pH of the mud itself was 8.3.

f. Temperature.—During the summer (August 11 to 29, 1965) the minimum temperature of the water was 12° and the maximum 32° (maximum 39° recorded in summer 1966). The diurnal range varied from 12° to 19°. In the winter pond (November 25, 1965 to March 15, 1966) the minimum temperature (under ice) was 1° and the maximum was 14°C. The smallest diurnal change in winter was 3° and the greatest was 12°. The minimum and maximum temperatures recorded for the spring pond (April 11 to May 9, 1967) were 3° and 22°, with diurnal fluctuations as great as 17° and as small as 7°.

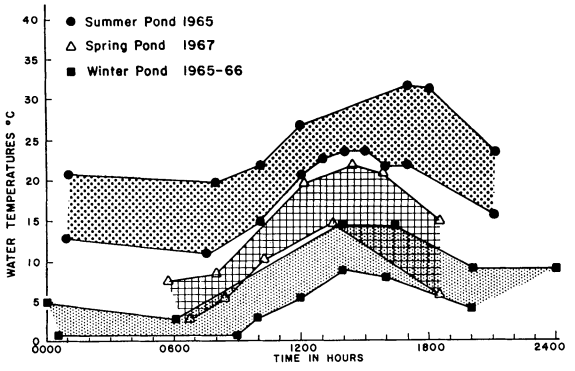

FIG. 2. Ranges of diurnal water temperatures in summer, winter, and spring.

Figure 2 indicates that temperatures in spring were generally intermediate between winter and summer values. Smallest ranges occurred in winter, with more variable conditions in spring and summer. *B. mackini,* present in all these conditions, is obviously eurythermal, swimming under ice in winter and also active at temperatures greater than 30° in summer; it was observed to withstand daily changes as great as 19°.

Microenvironment of the egg.—

a. Dried state.—When the pond basin is dry the eggs (cysts) are in the dried mud, not layered at the surface but distributed through the top 5–10 mm. Two layers can be differentiated: an uppermost darker brown layer (A), a lighter brown layer (B), and the soil beneath (C). The surface shrinks on drying, showing polygonal desiccation cracks that extend down into C. The eggs are predominantly in layers A and B, which remain together and separate from C when completely dry. The thickness of combined layers A and B varies from 5 to 10 mm. On addition of water, layers A and B swell to a jelly-like consistency; it is from these layers that the suspended sediments (from layer A) and the slightly denser slurry (from layer B) are derived. The eggs within these layers are from two sources: those ejected during the life of the pond by living females, and those remaining in the brood pouches of females dying at the end of a pond.

b. Changes at pond origin.—The pond can fill rapidly with runoff from cloudburst-type rains in the area. On one occasion it was observed to fill within an hour by inflow from the south and east. Many eggs are initially in the topmost portion of the gelatinous slurry as the soil surrounding them goes into suspension. Currents can carry the eggs about until the pond is established. Then the eggs settle into the bottom mud. The eggs do not float, either in the desiccated or in the hydrated condition. No eggs can be collected by net after a pond is established unless the net dips into the mud. Hence, the eggs must be stimulated to hatch or inhibited from hatching by bottom conditions, which may be different from the open water above, and are not homogeneous with respect to oxygen concentrations nor, initially at least, to salinity.

As water flows into the dry basin, the eggs begin to absorb water. The desiccated eggs are deeply indented on one side and become spherical only after taking up water, a process requiring time. Laboratory observations show that at a constant salinity of 0.5‰ (1,000 μmhos), the first egg rounds up at the end of 50 min; at 10,000 and

20,000 μmhos it takes 60 and 120 min, respectively. The time for half the sample of eggs (50 of 100) to swell (at room temperature of 20–25°C) was 88, 117, and 172 min at the three concentrations mentioned.

c. Salinity.—The salinity at the egg surface while in the bottom mud is difficult to determine. It has been assumed, because of their proximity to the soil/water interface, that the eggs are surrounded by the same salinity as in the open water of the overlying pond after equilibrium has been attained. However, as in the case of the oxygen concentrations discussed below, a salinity gradient may exist from the interface downward. The most deeply-lying eggs may therefore be at a salinity greater than that determined in the water above. The existence of these gradients, which results in the eggs being in a heterogeneous environment, helps account for all eggs not hatching at once, though variability of eggs is also involved.

d. Oxygen.—It proved difficult to obtain satisfactory measurements in the microenvironment surrounding the eggs. Because of the sediments and the difficulty of obtaining samples from definite depths below the interface, the Winkler method for oxygen could not be used. Better results were obtained with a polarographic oxygen probe (Yellow Springs Instrument Co.), but because it consumes oxygen, it must be kept moving to obtain a steady reading. The probe can be positioned at desired depths, but the movement required during measurement may stir in better-aerated water from above and mud of lower oxygen content from below.

A sharp reduction in oxygen was found just beneath the soil/water interface. While the open water of the pond had oxygen at saturation against air (e.g., 8 mg O_2/l), the concentration at the interface (with the membrane of the oxygen probe resting on the soft mud surface) was 4–5 mg/l. In the uppermost layer of mud (the top 3–5 mm) the oxygen concentration was 2–3 mg/l; in the layer 5–10 mm below the surface there was less than 1 mg/l. This finding is in keeping with the expectation according to the description of Bouldin (1968) of an aerobic layer of mud above an anaerobic layer.

Observations on individual ponds

Each pond that occurred in the study basin from the summer of 1965 to the summer of 1967 is described below, but with emphasis on one pond for each season. Summaries of the findings pertinent to egg hatching are presented in Figures 3–6. Two short-duration summer ponds are discussed, but findings are not figured.

Summer Pond, August 11 to 29, 1965. (Fig. 3).—The slower increase in salinity from the second to fourth days was due to rain on the second and third days. After the rains salinity increased rapidly, reaching 2.5‰ on the ninth day. On the final (18th) day salinity was about 34‰ as indicated by a specific conductance of 55,000 μmhos in the remaining 5 mm film of water.

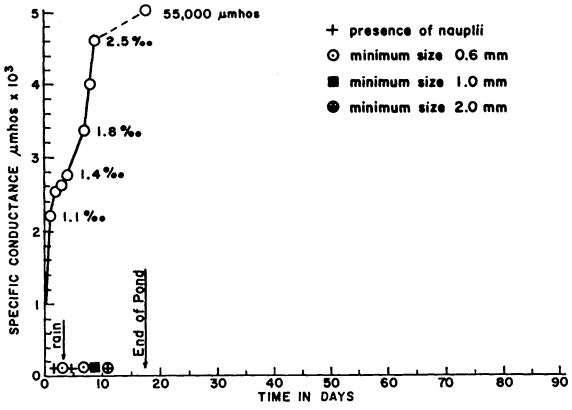

FIG. 3. Changes in salinity and presence of young, Summer 1965 pond.

From maximum sizes collected on the second, third, and fourth days (2.5, 4, and 6 mm) the growth rate of early stages is 1.25 mm/day. This rate is minimal, for by the tenth day 17% of the population was 19 mm long, and the longest individual was 21 mm, indicating that 2 mm per day is about the maximum rate in summer.

Nauplii were present up to the second day, absent on the third, reappeared on the fourth, and young immature stages were present until the 11th day (Fig. 3). Those longer than 10 mm on the tenth day (80% of the population) probably hatched during the first two days (the initial hatch); a second hatch accounts for those shorter than 10 mm. The reappearance of nauplii coincided with dilution and a slowed rate of salinity increase.

The hatching in the summer pond of 1965 occurred in two bursts, each following inflow of water and ending during rapid increase in salinity in a shallow pond.

Winter Pond, November 25, 1965 to March 15, 1966. (Fig. 4).—This pond lasted 111 days, which was the longest duration of any pond observed. The initial salinity of 1,000 μmhos (the dotted line of Fig. 4) was estimated from the runoff that was filling other ponds. There was reduction in salinity when more water entered on the 14th day; then a series of light rains kept salinity low until the 44th day. On the last day the remaining film of water had a conductance of 30,000 μmhos (about 18‰).

On the 15th day (the first sampling of the pop-

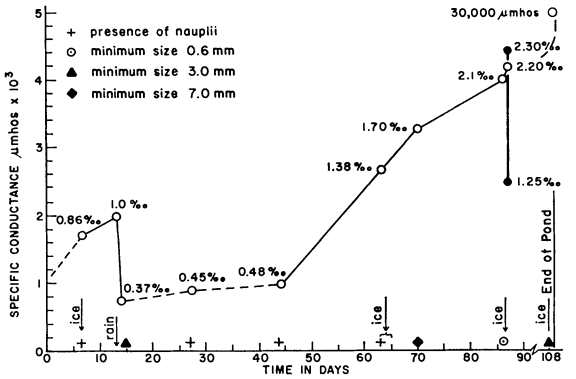

FIG. 4. Changes in salinity and presence of young, Winter 1965–66 pond.

ulation) 41% of the shrimp were mature (10 mm or greater), 38% were 7–10 mm long, 21% 3–6.9 mm long. The growth rate of the 10-mm individuals was 0.66 mm/day, assuming that they hatched 15 days previously. This is ½ the growth rate estimated for immatures of the same species in summer. *B. mackini* apparently takes two weeks to reach maturity in winter. On the 86th day of the pond the largest individual in the population was 32 mm long, and a third of the population was longer than 25 mm.

Because nauplii were still present on the second of December, continuous hatching can be assumed during at least the first seven days of the pond. It probably ceased after another five days, because no shrimp less than 3 mm long were present on the 15th day (Fig. 4). An initial hatching period of 12 days can therefore be postulated, during a period of salinity below 1‰ and with slight increase in salinity. Nauplii collected on the 27th and 44th days showed a long second hatching period, which probably started with the rain on the 14th day and continued from the 15th to the 48th day. Salinity remained below 1‰, and increase in salinity was slight. Under these conditions hatching was continuous, in contrast to periodic hatching observed following reductions from higher salinities during periods of increasing salinity. It cannot be determined whether the hatching was of dried eggs from previous fillings of the pond or of eggs freshly ejected by living females. From observations in the laboratory (described below) of differences in behavior of ejected and dried eggs, the eggs most likely to hatch under conditions of constant low salinity are the ejected eggs—the ones that have never been dried nor subjected to low oxygen concentrations.

Despite the overall increase in salinity after the 45th day of the pond, nauplii reappeared on the 63rd day, amounting to 6.2% of total population, and very small stages were present on the 86th day, when 5.6% were in the range from 0.6 to 4.5 mm. None less than 7 mm were found on the 70th day, when 97% were 10 mm or longer. On the 108th day 3.6% were 3.0–3.9 mm long.

The stimulus for hatching after the 44th day is not known, but each of the hatchings coincided with melting of ice. The pond does not freeze over for any great length of time, but during cold periods it is covered in the morning by a thin sheet of ice, which usually melts by noon. In Figure 4 are shown the salinity changes observed on the 86th day. The salinity was 2.1‰ at 1400 on February 19th. At 0700 on the 20th, when the pond was completely covered with ice, the salinity of the unfrozen water beneath was 2.3‰. At 1030, just as all the ice had melted, the surface salinity was 1.25‰. Stratification continued until a wind came up at noon. By 1400 the salinity throughout the pond was 2.5‰. In studies by others (e.g., Coopey 1950), snow melt has been reported as the stimulus for hatching of branchiopod eggs, though in these cases the stimulus was liquid water after its absence. In Rabbit Dry Lake liquid water was continually present, but the ice-melt water could subject eggs to reduction in salinity.

In this winter pond hatching was continuous while nearly constant salinities prevailed, but ceased when salinity increase. At salinities above 1‰ hatching occurred only coincident with dilution by rainwater inflow or ice-melt.

Summer Pond, August 2 to 5, 1966.—This was a pond of short duration (66 hrs), of small initial volume, and with a continuous and rapid increase in salinity. At the end of 42 hours specific conductance was 5,850 µmhos.

At 1800 on August 4th, 54 hours from the estimated time of origin, as much as possible of the remaining water was removed. The three liters collected contained a total of 74 individuals with a size range of 0.5–1.5 mm. The temperature range and the oxygen values in the open water of this pond were similar to those found in the summer pond of 1965. And the initial salinity of ca. 1,000 µmhos was the same. Yet in this pond, a negligible number of nauplii appeared, considering that about a quarter of the basin was initially covered and that this included the lowest point in the basin where most of the shrimp collect at the end of each pond, die and decompose and leave their eggs. The obvious environmental differences between this and the pond of the previous summer are the smaller volume and the continuous and very rapid increase in specific conductance immediately following formation of the pond. In the 1965 summer pond in which many eggs hatched, it took ten days to reach a salinity corresponding to 5,800 µmhos. In this 1966 pond a specific con-

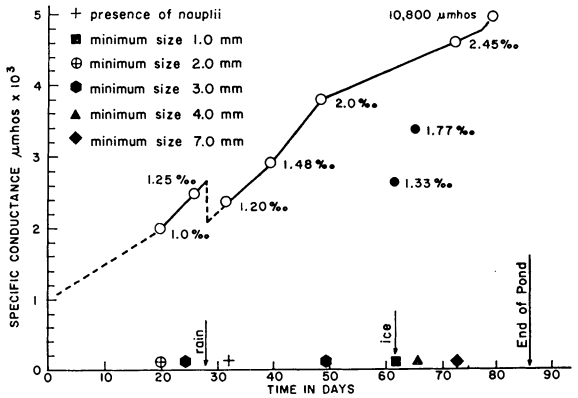

FIG. 5. Changes in salinity and presence of young, Winter 1966–67 pond.

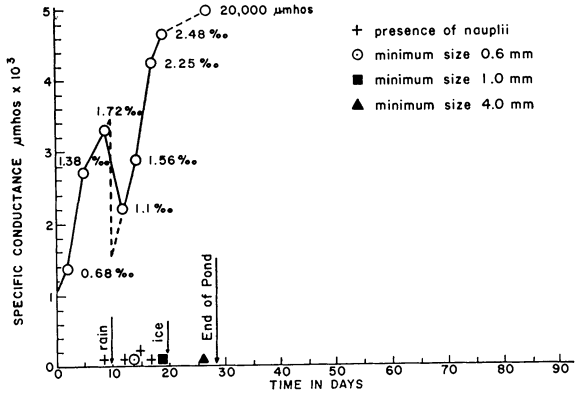

FIG. 6. Changes in salinity and presence of young, Spring 1967 pond.

ductance of 5,880 was reached in 42 hours, a rate of increase of more than 2,400 µmhos per day (see Table 3).

Winter Pond, November 7, 1966 to February 2, 1967. (Fig. 5).—This pond, which lasted 87 days, differed from the winter pond of the previous year in that there was continuous increase in salinity with only one interruption by additional water. On the 29th day the estimated decrease was from 2,630 to 2,100 µmhos. On the 80th day the specific conductance was 10,800 µmhos, a salinity of about 6‰.

There were two major periods of hatching—an initial hatch during the first 15 days of the pond, and a second hatch after the rains on the 29th day. On the 26th day the smallest individual was 3 mm long, and 96% of the population was over 5 mm long. In contrast, on the 32nd day, 92% of the population was less than 3 mm long. The second hatch was essentially over by the 35th day, with some hatching up to the 40th day. 6.6% were 3.0–4.9 mm long on the 49th day. A third hatch during the first week in January (62nd day) correlated with a period of ice melt.

There was no dilution by increase in volume after the 32nd day, as shown by consistent decrease in depth of the pond. The depths recorded were: 20th day, 5 cm; 29th day, rain; 32nd day, 15 cm; 40th day, 9.5 cm; 49th day, 9 cm; 62nd day, 5 cm; 66th day, 4 cm; 73rd day, 3 cm; 87th day, moist mud. Despite decreasing depth, which is usually indicative of evaporation and increasing salinity, there were local decreases in salinity on the 8th and 12th of January (62nd and 66th days). The salinities of samples taken just after the ice had melted were 1.33 and 1.77‰ (Fig. 5). The occurrence of small immatures at times of ice-melt suggests, as in the pond of the previous winter, a relation between lowered salinity and hatching.

Spring Pond, April 11 to May 9, 1967. (Fig. 6).—The only spring pond that occurred during the study lasted for 28 days. Toward the end of the pond the increase in salinity was very rapid. On the 23rd, 27th, and 28th days the specific conductance values were 9,200, 14,000, and 20,000 µmhos, corresponding to salinities of about 5, 8, and 11.5‰. At the last determination the depth of the remaining water was less than 5 mm.

The longest individual (5.6 mm) on the ninth day had a growth rate of 0.6 mm per day, assuming it hatched on the first day. This rate of growth of immatures is about the same as in winter ponds. Less than 1% of the population on the ninth day was shorter than 2 mm, which suggests that for 99% of the population hatching had ceased 3–4 days previously. According to the growth rate, it would be expected that 99% of the population would be at least 3.8 mm long on the 12th day; however, on that day 28% of the population was less than 3.0 mm long. This change in population structure between the 9th and 12th days indicates resumption of hatching after inflow of more water on the 10th day. The presence of nauplii and young stages on the 15th to the 19th days coincides with a cold period when ice was on the ponds in the mornings.

Summer Pond, September 1 to 6, 1967.—This pond lasted 127 hr. During the first 28 hr salinity increased from 1,100 to 2,300 µmhos. On September 2 as a result of a second rain the salinity decreased to 1,300 µmhos in a matter of minutes. About 70% of the basin was covered with water. The maximum depth (in the northeast corner) was 4–5 cm, but most of the water was only 1–2 cm deep. Evaporation was rapid, and during the next 75 hours the salinity increased from 1,300 to 5,000 µmhos. On September 5 the water that remained in the basin was siphoned out, yielding 24 shrimp ranging in size from 0.5 to 5.2 mm. The water was replaced and when resiphoned on

TABLE 3. Summary of field hatching periods

Date of pond	Range of salinity increase (μmhos)	Salinity increase during hatching (μmhos)	Duration of hatching (days)	Rate of salinity increase (μmhos/day)
Winter 65-66.....	730–960	230	38	6
Winter 66-67.....	1000–1795	795	15	53
Winter 66-67.....	2100–2990	890	12	74
Winter 65-66.....	1000–1800	800	10	80
Spring 1967.....	1000–3450	2450	11–12	213
Spring 1967.....	1500–4250	2750	7	397
Summer 1965.....	2600–4000	1400	4–5	315
Summer 1965.....	1000–3000	2000	2	1000
Summer 1966.....	1000–5850	4850	2	2425
Summer 1967.....	1000–2300	1200	1	1200
Summer 1967.....	1300–5000	3700	3	1230

the sixth (final) day, eight shrimp, the largest 8 mm long, the smallest 1.4 mm, were collected in the remaining water.

Obviously some environmental factor inhibited the hatching, for only 32 eggs hatched despite inundation of 70% of a basin, which at times supports 400,000 shrimp. Neither temperature nor light nor pH nor initial salinity were different from conditions in the summer pond of 1965, which supported a large population. But relative to the 1965 summer pond, the ponds of 1966 and 1967 had smaller volumes and shorter duration—too short to allow completion of life cycle. And in these short-lived ponds few eggs hatched.

Duration of hatching

Duration of hatching is inversely related to rate of increase in salinity (Table 3). As the rate of increase in specific conductance increased from 6 to 1,000 μmhos per day, the duration of hatching decreased from 38 days to 2. When salinity increased at rates above 1,000 μmhos per day, as in the summer ponds of 1966 and 1967 there was virtual inhibition of hatching.

LABORATORY STUDIES OF EGG HATCH

Field observations reported in the previous section suggested that of the obvious environmental variables immediately surrounding the egg, salinity is the most likely to affect hatching. Temperature, the most studied of variables, seemed unimportant in this case. For though it was observed that temperature influenced growth rate, egg hatching of *B. mackini* was not inhibited in the range of temperature observed. Large hatches were observed in winter ponds when temperatures were 1–14°C and in summer ponds in the range from 12–32°C. There are desert species of Branchiopoda that are seasonal (e.g., *B. gigas*) similar to what has been reported by Dexter (1953), Prophet (1963), Moore (1959), and Horne (1967) for some of the species they studied. But *B. mackini* is not a seasonal species. It seems to be temperature-independent in the range occurring on the Mohave desert, appearing whenever there is sufficient water. But periods of egg hatch took place during lowered salinity, and inhibition of hatch occurred during increasing salinity. There was no field evidence that oxygen had a role in regulating the hatch of *B. mackini* eggs, but the implication of oxygen as a stimulus and inhibitor of hatch of eggs of other species and the presence of a gradient of oxygen in the mud surrounding the eggs in Rabbit Dry Lake made oxygen of interest. Hence, preliminary laboratory tests were made of the effects of salinity and oxygen on egg hatch.

Egg collecting and handling

Dried eggs.—Fifty-gram samples of dry soil from the top 5–10 mm of the study basin were placed on 80-mesh nylon netting. The eggs were washed free of the fine silt, by a stream of tap water, and immediately dried at room temperature. A 50-gram sample of the soil contained between 110 and 200 eggs, which at the end of the washing still had their dehydrated, indented appearance. It takes the eggs an hour or more to hydrate, and the washing took only 3–5 min. Freeing the eggs from the mud by washing them even briefly was not desirable, for it leaves the experiments open to the objection that as a result of the wetting, the eggs may have been stimulated to develop. But the washing proved to be the only way to get eggs in known number, uninjured and free of mud. Although the hatch was the same if the eggs were only blotted, they were allowed to redry for at least 24 hr before being placed in plastic petri dishes (Falcon Plastics, B-D Laboratories, Los Angeles), which were kept in high humidity chambers to minimize evaporation of the test media in which salinity was the variable. The petri dishes were used when oxygen concentration was not controlled.

Ejected eggs.—Eggs collected from dry soil in the manner described above are referred to as "dried" eggs in contrast to eggs which are referred to as "ejected," which were collected directly from females that had matured in the field.

Females with full egg sacs were placed in vials (Thorton Plastic Co., Salt Lake City) containing the test medium. After the eggs were ejected, the females were removed from the 40- or 3.5-dram vial. (Vials of different size were used depending on whether a single brood or multiple broods were being tested.) Eggs obtained in this way were never dried and never subjected to any salt concentration other than that of the test medium into which they were ejected. Because the vials are porous to oxygen, tests could be made in them with

oxygen at equilibrium with the atmosphere, but without bubbling with air. In other tests oxygen concentrations were controlled by bubbling through the medium either air, an air-nitrogen mixture, or nitrogen alone.

Experimental conditions.—In the non-air-bubbled vials and petri dishes, the oxygen concentration in the "macroenvironment" determined by polarographic probe was always above 7 mg/l. "Macroenvironment" is emphasized, because there is, by inference from other studies (Table 5), a lower oxygen concentration at the bottom of the dish or vial in the immediate vicinity of the egg surface. The concentrations at the egg surface ("microenvironment") become of concern when eggs are allowed to rest at the bottom of a vessel without agitation, simulating the natural conditions in which eggs lie at the bottom of a pond. In other tests, under less natural conditions, the eggs were kept in constant motion by bubbling with gas. In these tests the oxygen concentration at the egg surface could be assumed to be at the concentration measured in the "macroenvironment."

Results

General hatching characteristics.—Hatching of dried eggs begins within 24 to 36 hr after wetting or after a substantial reduction in salinity. After termination of a hatch, the remaining unhatched eggs do not hatch unless there is another reduction in salinity. In contrast, the response of ejected eggs is less predictable, liable to either bursts of hatching or continuous hatching at all salinities and apparently independent of changes in salinity, perhaps more dependent upon oxygen concentrations (see below).

Effect of salinity on hatching.—In the following experiments the eggs were on the bottom of plastic vessels in which the oxygen concentration of the medium was at saturation with air but without the agitation provided by bubbling. Salinity was estimated from specific conductance of 2-ml samples drawn into a pipette cell. Salinity at the egg surface was assumed to be the same as that of the medium.

a. Dried eggs.—With increase in salinity progressively fewer eggs hatch when they go from dry to the wet state (Table 4a). Whereas more than 80% of eggs hatch at 360 and 1,000 μmhos, only half of them hatch at 5,000 μmhos. At still higher concentrations (10,000 and 20,000 μmhos) less than 1% hatch. In only one of seven trials at 20,000 was there any hatch at all; in that sample two of 149 eggs hatched.

Upon dilution, by addition of water to the medium, response depends on the relative reduction in salinity as well as upon the initial salinity from which the dilution was made (Fig. 7). There is no absolute threshold below which hatching occurs but with inhibition above that salinity. Rather it is a reduction, a change relative to the initial concentration, that serves as stimulus. At 3,000 μmhos a 10% dilution results in about 50% hatch. At salinities above 3,000 μmhos a greater dilution is required to obtain 50% hatch. Thus at 6,000 and 9,000 μmhos at 50% dilution results in slightly more than a 50% hatch; at 70,000 μmhos an 80% dilution produces 10% hatch. The resulting dilutions, the concentrations at which the eggs hatched, were 2,000, 3,000, 4,500 and 14,000 μmhos. So the eggs are inhibited from hatching at constant salinities, which may be lower than concentrations at which eggs will hatch, providing that the higher salinity is a reduction from a still higher concentration. That is, hatching results from a change, a stimulus.

TABLE 4. Percentage hatch within 120 hr of dry eggs placed in test solutions

Specific conductance (μmhos)	No. of eggs tested	Total no. hatched	Total hatch (%)
a. Without air bubbling (4 or 7 replicates)			
360	699	619	88.4
1,000	649	528	81.4
5,000	897	406[1]	45.3
10,000	827	7[1]	0.86
20,000	842	2[1]	0.24
b With air bubbling (2 or 5 replicates)			
360	352	233	66.2
1,000	368	160	43.5
2,000	280	109	39.0
3,000	234	80	34.2
4,000	279	77	27.6
5,000	705	23[2]	3.3

[1] 7 replicates
[2] 5 replicates

b. Ejected eggs.—In contrast to dried eggs, some ejected eggs after ejection into water from the egg sac will hatch though they have never been subjected to reduction in salinity and have never been dried. The proportion that hatches depends on the salinity, though only on the average because of great variability among the eggs, even those of a single brood. Eggs ejected into water at 1,000 μmhos began to hatch two days after ejection, with 17–73% of the eggs in each brood hatching during a 5-day period. In a mixture of broods (eggs from several females), 585 of a total of 1,742 (33%) hatched during a 9-day period after hatching began four days after ejection. At this salinity 80% of dried eggs hatch. At 2,600 μmhos

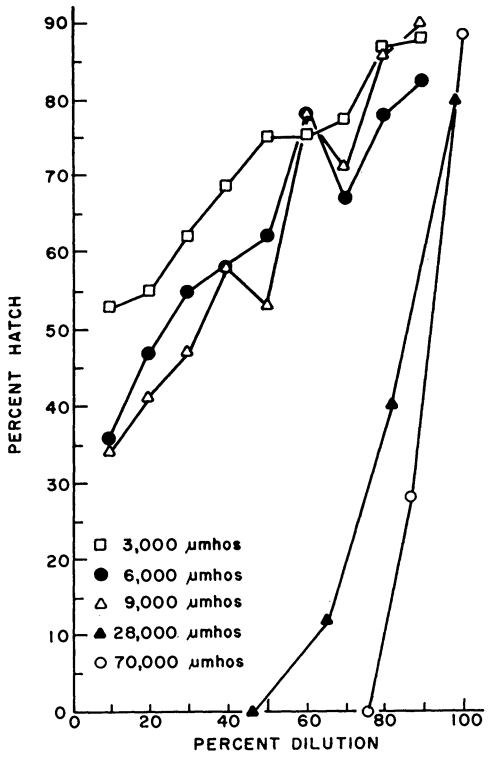

FIG. 7. Percentage hatch as a function of dilution from initial salinity indicated.

ejected eggs began to hatch after three days, and 365 of 1,573 (23%) hatched during the next seven days. In a single brood study at 10,000 µmhos hatching begain four days after ejection, and the average hatch was 13% during a 12-day period. In contrast, dried eggs are inhibited from hatching at 10,000 µmhos.

The tests showed that ejected eggs take longer than dried eggs before beginning to hatch but then continue to hatch for longer periods. Thus in a pair of tests at 360 µmhos (a salinity lower than ever found in the study pond), 82 and 91% of dried eggs hatched within two days. A slightly smaller proportion (67–75%) of eggs ejected into water of this low salinity hatched also during a 2-day period, but hatching did not begin until five days after ejection of eggs from the ovisac.

It was also found that salinities high enough to inhibit hatch of dried eggs do not inhibit hatching of ejected eggs, which are stimulated to hatch by some other factor under conditions (constant salinity) that preclude possibility that change in salinity (osmotic shock) is the stimulus.

Effects of oxygen on hatching.—

a. Dried eggs.—When placed in water of low salinity (360 µmhos), in which the eggs are free from inhibiting effects of salinity, there is 80–90% hatch when the oxygen of the medium is in equilibrium with the air. Four replicates of dried eggs (387, 178, 144, and 164) totaling 873 eggs were tested in water of this salinity (360 µmhos), but with oxygen maintained at less than 1 mg/1 by an air-nitrogen mixture. There was complete inhibition: at the end of 120 hr no eggs had hatched. To verify viability, three of the four vials were then bubbled with air. In them 60–70% of the eggs hatched during 120 hr. In the fourth vial, which was continued as a control with oxygen at 0.1–1.0 mg/1, inhibition of hatching continued.

Similar tests were made with eggs that had been stored wet at 20,000 µmhos with oxygen in equilibrium with air. On transfer to 360 µmhos water (also with oxygen at saturation) there was 80–90% hatch during a 120-hr period. On transfer from 20,000 µmhos (and with oxygen in equilibrium with air) to 360 µmhos but with oxygen at 0.1–1.0 mg/1, there was complete inhibition of hatching. Subsequent air-bubbling produced a 60–70% hatch in 120 hr.

In these tests in waters of low salinity, eggs were inhibited from hatching at oxygen concentrations below 1 mg/1. It seems that at low salinity nearly complete oxygen depletion is needed for inhibition: slightly higher concentrations of 1–2 mg/1 did not inhibit hatching. The observation that only 60–70% hatch in agitated solutions, as compared to an 80–90% hatch in nonagitated solutions, showed the possibility that noninhibiting intermediate concentrations of oxygen at the bottom of nonagitated vials might enhance hatching and be responsible for the differences in hatch observed in agitated and nonagitated test solutions.

Tests were therefore made with agitation provided by bubbling with air. Comparison of results shown in Table 4b with those in Table 4a, shows that at all salinities tested fewer eggs hatched when kept in motion in air-bubbled solutions than when the eggs lay quietly at the bottom. At 5,000 µmhos very few eggs hatched while air-bubbled. This was surprising, because 40–50% of the eggs had hatched within 120 hr in the tests tabulated in Table 4a. In five trials with air-bubbling at 5,000 µmhos the average hatch was only 3.3%: (6/118; 2/116; 2/141; 0/163; 13/167).

These data suggest that a transitory period of low oxygen at the egg surface may have been present in the nonagitated tests and that this previous exposure might account for the enhanced hatch in nonagitated test media, especially at 5,000 µmhos. Therefore another test was made at 5,000 µmhos with oxygen maintained below 2 mg/1 for 120 hr. There was no hatching. The test medium was then bubbled with air, and during the next 120 hr 47–68% of the eggs hatched in three repli-

cates totaling 544 eggs. This is far greater than the 3% hatch observed with air-bubbling without a prior period of low oxygen and is in the range found for nonagitated solutions (Table 4a).

These results show that a period of low oxygen tension does enhance hatch. However, since a good proportion of eggs will hatch without it, exposure to low oxygen is not mandatory, as has been found for some species of arthropod eggs (see Discussion). Enhancement of hatch by previous exposure to low oxygen tension is less noticeable at low than at high salinities (Table 4b), and the effect of oxygen as an inhibitor of hatch also varies with salinity. At 360 and 1,000 μmhos, hatching is inhibited only by concentrations below 1 mg/1, and there is no inhibition at high concentrations. At 5,000 μmhos there is inhibition both below 2 mg/1, and above 7 mg/1, provided that no exposure to low oxygen concentrations has occurred while eggs were hydrated.

b. Ejected eggs.—When eggs were ejected from the ovisac of living females into a constantly air-bubbled solution of low salinity (360 μmhos), hatching began in 36–48 hr instead of the 120 hr that was characteristic of nonbubbled media, indicating delay of hatching by the lower oxygen surrounding the ejected egg at the bottom of the nonagitated medium. There was delay, rather than inhibition, because eventually the same proportion of eggs hatched in the nonagitated solution (67–75%) as in the agitated solution (60–66%). In contrast dried eggs at 360 μmhos did not show a time difference in initiation of hatching, but the final hatch was 60–70% in the air-agitated medium versus 80–90% in the nonagitated medium.

The delay of hatching of ejected eggs by lowered oxygen concentration offered an additional explanation for apparent inhibition of hatching of ejected eggs in the long-lasting winter ponds. In the laboratory cultures, once a female ejects eggs, she produces another clutch, is inseminated and again ejects eggs in 2- to 4-day cycles until she dies or the pond dries up. At constant salinity in laboratory cultures non-dried eggs seem to hatch without stimulation by reduction in salinity. It seems likely that continuous hatching probably does not occur in Rabbit Dry Lake, because salinity there is seldom constant, especially during later stages when salinity is high. Arrest of development of both non-dried and dried eggs is probably the result of increasing salinity (Table 3). But there is the possibility that the non-dried eggs are also inhibited by low oxygen at the bottom, as reported by Moore (1963) in waters without marked salinity changes.

The effect of bottom sediments on their hatching was investigated in tests on eggs ejected into salinities from 1,000 to 10,000 μmhos. At each salinity about 10,000 eggs were collected (from 100 mature females each with about 100 eggs). When egg collection was complete, the eggs were transferred to water of the same salinity that had been centrifuged to get rid of the suspended soil. Following removal of sediments, the oxygen concentration was above 7 mg/1 in all the solutions. Within 1–5 days hatching began at all salinities.

About 1,000 eggs were removed as controls and kept in sediment-free (centrifuged) water, and then, to simulate field conditions, just enough sediment was added to the vial to cover the remainder of the eggs. Results in Table 5 show that inhibition of hatching could occur, except during the first 24 hr, even though the eggs were covered by no more than 1–2 mm of sediment. Hatching continued in the vials without sediment. The inhibition of hatching occurred at intermediate oxygen tensions (2.7 mg/1 and less) as measured with the oxygen probe in the bottom 5 mm of the vials. Although the nauplii present at 1,000 and 2,000 μmhos may be too few to be significant, it could be that at low salinities only very low oxygen concentrations (near depletion) will inhibit the hatch of ejected eggs, as was found for dried eggs.

In the centrifuged water, where oxygen was not reduced below 5.6 mg/1, the proportion hatching (2–10%) seems low, but this does not include

TABLE 5. Inhibition of hatching by natural sediments

Salinity (μmhos)		Oxygen tension after 6 days (mg/1)		No. hatched in 6 days	
Sediment-free	With sediments	Sediment-free top/bottom	With sediments top/bottom	No./ca. 1000 eggs sediment-free	No./ca. 8000 eggs with sediments
500	500	7.0/6.5	6.1/2.7	100	0
1000	1000	6.3/6.0	6.1/2.0	72	4
2000	2000	7.0/6.6	6.6/2.0	65	12
3000	3000	7.1/6.9	6.5/1.9	63	0
4000	4000	7.4/7.0	6.5/1.5	59	0
5000	5000	7.5/7.5	6.4/2.7	38	0
6000	6000	7.4/7.3	5.4/2.3	74	0
7000	7000	7.0/5.6	6.2/1.4	19	0

those that hatched in the first part of the experiment.

The oxygen concentrations measured at the bottom of the vials are in the range surrounding the eggs at the bottom of the pond. It is thus possible that low oxygen may prevent continuous hatching of ejected eggs except at low salinities, and that oxygen plays a role in inhibiting hatch as well as in conditioning eggs to respond to salinity changes.

Discussion

In reviewing life histories of freshwater Branchiopoda, Hutchinson (1967, p. 557) states that they "live in waters that are subject to periodic drying, or extensive freezing, or, as in the habitats of *Artemia,* are excessively saline." This report concerns a species living in temporary ponds in an arid region where all three factors—drying, freezing, and salinity changes—are involved, though the freezing in not extensive and the salinity is not excessive. Hutchinson further suggests (p. 566) that "the main difference between species of humid and arid regions, if there is such a difference, is most likely to lie in the facultative nature of the diapause in those species . . . that inhabit humid regions." This implication that an obligate diapause exists in desert species is most unlikely because of the year-to-year variability in time and amount of rain. It seems more likely that the dormant stage always responds to an outside stimulus and that the diapause therefore is facultative.

In his review of the various factors and combinations of factors that have been reported to stimulate and inhibit hatching, Hutchinson states (p. 562) "it is quite likely that the fundamental change needed to produce hatching is always a lowering of osmotic pressure at the egg surface." This opinion of Hutchinson is essentially corroborated by observations in the desert pond, and verified in the laboratory, but the regulation of development is not that simple, and it was found that dried and non-dried eggs behaved differently.

Hatching of dried eggs of *B. mackini* begins within 24–36 hr after wetting or following a substantial reduction in salinity. In going from the dry to the wet state, progressively fewer eggs hatch at higher salinities. Thus, whereas 80% of dried eggs hatched when placed in waters at concentrations below 1,000 µmhos, only 50% hatch at 5,000 µmhos, and fewer than 1% hatch when placed in waters more concentrated than 10,000 µmhos. After the initial hatch, response to dilution depends both upon the relative reduction in salinity and upon the salinity prior to dilution. At the relatively low salinity of 3,000 µmhos, a 10% dilution results in a 50% hatch, while at 6,000 and 9,000 µmhos a 50% hatch requires a 35–40% dilution. Eggs will hatch at high salinities, but a great dilution is required for a small proportion of the eggs to hatch. There seems to be no threshold value below which hatching always occurs, with absolute inhibition above that salinity; rather it is a change, a reduction in concentration, that serves as stimulus.

The response of ejected eggs—those never dried—is less predictable, and some hatch without reduction in salinity. Rather than needing a stimulus to hatch, non-dried eggs seem to require an inhibitor to prevent hatching. Low concentrations of oxygen in the bottom mud seem to provide such inhibition.

Though the "fundamental" stimulus for hatching may, indeed, be lowered osmotic pressure, the regulation of development is complex. In a humid-region study Broch (1965) found that hatching of *Chirocephalopsis bundyi* was initiated by low oxygen concentrations, but that development could be arrested by other factors at any one of four stages if conditions were unfavorable for continued development. For example, one of the phases could be stopped from developing by low temperature and/or anaerobic conditions. The latter is similar to control of development of *B. mackini* by low oxygen in the bottom mud. Ability to stop development is an important adaptation, because if development, once started, were irrevocable, synchronization of development with conditions favorable for completion of life cycle would not be possible.

Experiments on the effect of oxygen concentrations on the dried eggs of *B. mackini* showed that the effects varied with salinity. The two factors interact. Inhibition of hatching occurs at all salinities when oxygen tension is less than 1 mg/l (at 20–25°C). The oxygen concentration required for hatching of previously dry eggs increases from 1 mg/l at 360 µmhos, to 2 mg/l at 5,000 µmhos; at 10,000 µmhos and above few eggs hatch regardless of oxygen tension. With increase in salinity, high oxygen concentrations (7–9 mg/l) become increasingly inhibitory to dried eggs, unless the eggs, after hydration, were previously "conditioned" by low oxygen concentration. With constantly high oxygen concentrations there is a sharp rise in inhibition of hatching at salinities above 4,000 µmhos.

Dried and non-dried eggs differ in response to oxygen when in higher salinities (10,000 µmhos). Non-dried eggs are stimulated to hatch by changes in oxygen concentration, a response shown by dried eggs only at low salinities. With increase in salinity, oxygen has a decreasing role in the

regulation of hatching of dried eggs. The delayed hatch and prolonged period of hatch of ejected (non-dried) eggs, is eliminated by preconditioning at low oxygen concentration. After such treatment, dried and non-dried eggs respond alike.

It seems probable therefore that the differences between species of humid and arid regions lie not in different types of diapause but in different responses to changes in salinity and oxygen concentrations and to the degree of such changes. The osmotic changes in freshwaters of humid regions are so slight that students of freshwater branchiopods generally disregard salinity, justifiably considering it to be a factor of no concern. Thus neither Broch (1965) in his study of *Chirocephalopsis bundyi,* nor Moore (1963) working with *Streptocephalus seali* and *Eubranchipus holmani,* nor Bishop (1967) with *Limnadia stanleyana* even mention salinity. Their studies showed low oxygen concentrations to be the factor stimulating hatching of some species and inhibiting hatching of others. It seems logical that in humid regions some factor other than water would stimulate hatching of eggs that are constantly moist. Or looking at it from another point of view, some factor must change to remove an inhibition of further development. It is not unexpected that the relatively great changes in salinity that occur in arid regions can serve to correlate hatching with the likelihood of completing a life cycle. Osmotic pressure could act to inhibit hatching (at high salinity) and to stimulate hatching (at low salinity).

In both humid and arid regions the stimulation of the egg to hatch must be by some factor that changes at the time of origin of the pond. Temperature can serve this function in ponds originating from snow melt. As Hutchinson (p. 565) says of Conchostraca, "those species that inhabit humid regions generally have life cycles determined mainly by temperature." But if the water in the basin undergoes significant changes in salinity, as it does in arid regions, control of egg hatch may be independent of temperature and dependent upon both salinity and oxygen. The different effects of various combinations of these two factors provide means for stimulating and inhibiting development. The eggs themselves are highly variable in response, and the presence of at least two types of eggs, capable of responding differently as the result of different histories (conditioning) increases variability. The heterogeneous environment provided by an oxygen gradient in the mud surrounding the eggs provides added insurance that all eggs will not hatch at once under any single set of conditions.

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