The Marine Rotifer Brachionus Mulleri Subjected to Salinity Changes
Author(s): Leonard G. Worley
Source: *Ecology*, Vol. 10, No. 4 (Oct., 1929), pp. 420-426
Published by: Ecological Society of America
Stable URL: http://www.jstor.org/stable/1931152
Accessed: 03/06/2011 02:20

THE MARINE ROTIFER *BRACHIONUS MULLERI* SUBJECTED TO SALINITY CHANGES [1]

LEONARD G. WORLEY
University of Nebraska

The rotifer, *Brachionus mulleri,* was described by Ehrenberg, in 1833, from the shores of England, and since that time it has been found to be rather widely distributed in salt waters. Its wide distribution probably results from the ease with which its fertilized eggs may be carried in mud on the feet of water birds or blown by the wind. At Lincoln, Nebraska, there is a strongly alkaline salt water pond in which this rotifer occurs in large numbers. Due, however, to the irregular rainfall and to the long periods of sunshine the quantity of water in the pond varies greatly, and consequently the salinity changes must be considerable in extent. As this rotifer has been able to maintain itself in this pond for several years it was considered of interest to determine what ranges of salinity it could endure and still be able to survive.

MATERIALS AND METHODS

Brachionus mulleri occurs in abundance in this pond in the summer, while during the colder months of the year only the resistant fertilized eggs are found in the mud at the sides and bottom of the pond.

In January of 1928 the hydrogen-ion concentration in the pond was about 8.8, and the sodium chloride content of the water was approximately one-third greater than that of sea water. Determination of NaCl by the chlorine method gave 25,000 parts chlorine per million of water, or a salinity of about 4.5 per cent, while sea water contains approximately 18,000 to 20,000 parts of chlorine per million of water, which is equal to a salinity of 3.2–3.6 per cent of NaCl.

All of the experiments in which the rotifers were transferred from one solution to another were carried out in exactly the same manner. They were strained out of one solution by using a cloth strainer, and then liberated into the new solution. The first visible effect invariably noted upon individuals thus transferred was shown in a cessation of swimming. This rotifer is a free swimming one and is usually very active and a fast swimmer. Transference was always followed by a settling to the bottom of the jar in which the individuals were placed and this was followed either by death or a renewal of activity after a few days. Usually the individuals that settled to the bottom, although failing to swim, would continue to feed

[1] Studies from the Zoological Laboratory of the University of Nebraska, No. 164.

and were never seen to cease movement of the mastax until death. Dried yellow split peas were used as food media for the various protozoa in the cultures upon which the rotifers fed, and the experimental jars were placed in strong south light but not in direct sunlight.

Mud containing fertilized eggs of *Brachionus mulleri* was taken from the edge of the alkaline pond on January 28, 1928, and 20 cc. of it placed in each of sixteen 3,000 cc. battery jars, and the jars placed in a warm room. Some of these battery jars contained varying amounts of the alkaline pond water diluted with rain water, while others contained undiluted alkaline pond water. As would be expected, the rotifers appeared first in the jars where there was no dilution of the alkaline pond water. Rotifers were noted, however, actively swimming on the third day in the jars containing 3 parts rain water and 1 part alkaline pond water, and soon were as abundant in this low concentration of salt water as in any of the other jars.

TRANSFERENCE FROM ALKALINE POND WATER TO ARTIFICIAL SEA WATER

Artificial sea water was prepared in the laboratory according to the formula of McClendon ('17). The rotifers were collected by means of a cloth strainer from the jars of alkaline pond water, and transferred to several 200 cc. jars containing this artificial sea water. Rotifers thus transferred immediately showed ill effects by ceasing to swim and settling to the bottom of the jars.

On February 13, eight 200 cc. jars were filled with artificial sea water having the normal concentration of NaCl. One-half of a pea was dropped into each of four of these, and two pea halves into each of the others. Eight other 200 cc. jars were filled with the alkaline pond water, and into each of four of these a single pea half was dropped and into each of the other four jars two pea halves were dropped. These eight jars of alkaline pond water served as controls. Two days were allowed for these 16 jars to develop protozoa and then they were inoculated with rotifers from the alkaline pond water jars.

On March 30 the experiments were concluded. Active rotifers were found in all of the 16 jars. The controls contained active rotifers in abundance throughout this period, while the rotifers in the artificial sea water were inactive and sluggish for several days but later became more active and began to reproduce at the normal rate. These observations seem to show that the rotifers after becoming adjusted to the artificial sea water can live and thrive in it as well as in the alkaline pond water, although the NaCl content is much lower in the artificial sea water than in the alkaline pond water.

TRANSFERENCE OF ROTIFERS FROM ALKALINE POND WATER TO ARTIFICIAL SEA WATER OF THE SAME SODIUM CHLORIDE CONTENT

On February 25, six 200 cc. jars were filled with artificial hypertonic sea water having a concentration of about 25,000 parts of chlorine per million parts of water (4.54 per cent of NaCl) and a dried pea added to each. Six other jars were filled with the alkaline pond water (4.5 per cent of NaCl) to serve as controls. On February 27 the jars were inoculated with rotifers.

On March 30 this experiment was concluded. All of the 6 alkaline pond water jars contained many rotifers. In the artificial hypertonic sea water, although most of the 6 jars contained living rotifers, the numbers were smaller, thus showing that the 4.5 per cent of NaCl in the alkaline pond water does not in itself fully produce normal environmental conditions for the rotifers.

THE MAXIMUM CONCENTRATION OF SODIUM CHLORIDE IN THE ALKALINE POND WATER THAT *BRACHIONUS MULLERI* CAN ENDURE

Eight jars containing rotifers in the undiluted alkaline pond water of 4.5 per cent NaCl were allowed to stand in the sunshine in a warm room and the water allowed to slowly evaporate and thus gradually to raise the salinity of the water that remained. Daily measurements of the salinity were made. Table I shows these experiments in a condensed form, and one may see that *Brachionus mulleri* can endure alkaline water of a salinity of about 9 per cent of NaCl. An acclimatization process was carried on

TABLE I. *Experiments showing the maximum concentration of sodium chloride that the rotifers can endure when in the presence of other salts contained in the alkaline pond water*

Experiment	Time	Parts Chlorine per Million Parts Water	Salinity % NaCl	Condition of Rotifers
1	Mar. 3	24,800	4.5	Normal
	Mar. 9	37,000	6.7	Normal
	Mar. 12	48,200	8.73	Very sluggish
	Mar. 13	52,800	9.56	All dead
2	Mar. 21	24,300	4.41	Normal
	Mar. 28	35,600	6.45	Normal
	Apr. 2	44,000	7.97	Sluggish
	Apr. 4	49,000	8.87	Dying
	Apr. 5	52,000	9.41	All dead
3	Apr. 9	24,400	4.43	Normal
	Apr. 22	40,200	7.28	Normal
	Apr. 24	44,500	8.06	Sluggish
	Apr. 25	46,600	8.44	Dying
	Apr. 26	50,000	9.05	Few living
	Apr. 27	53,700	9.72	All dead

for 10 days in Experiment I, for 15 days in Experiment II and for 16 days in Experiment III, but in all cases the rotifers died at about the same degree of salinity of the alkaline water. They apparently suffered no ill effects until the salinity reached about 7 to 8 per cent of NaCl, but after that point they became more and more sluggish until death. This lethal per cent of NaCl is nearly three times the NaCl content of ordinary sea water.

THE MINIMUM CONCENTRATION OF SODIUM CHLORIDE IN THE ALKALINE POND WATER THAT *BRACHIONUS MULLERI* CAN ENDURE

Experiments to determine how low a salinity the rotifers could endure were carried on in very much the same manner as were the experiments to determine the maximum concentration of NaCl that these animals could endure. Rotifers were placed in four 200 cc. jars, each containing 150 cc. of the alkaline pond water of one-fourth concentration of NaCl. Rotifers were used that had developed in this low concentration from general cultures. The salinity of the water in these 200 cc. jars was reduced daily by about 100 parts chlorine per million parts of water by further dilution with rain water. Alkaline pond water of the same concentration of NaCl was used in a set of four jars to serve as controls. Rotifers were also placed in these, but the water was not further diluted. At the beginning of the experiment all jars had the same degree of salinity of .9920 per cent (5,200 parts chlorine per million parts of water).

Table II is a general summary of these 4 jars, and shows that in these experiments *Brachionus mulleri* lived in water with a salinity as low as .5715 per cent of NaCl (3,000 parts chlorine per million parts of water) when 28 days were allowed for it to become acclimatized. The rotifers appeared active until the salinity dropped below .5956 per cent, and were always inactive when it was as low as .5776 per cent. They usually were killed at a salinity of about .5715 per cent, although one of the cultures contained a few very sluggish living individuals at that salinity. The control jars on March 31 contained vigorous cultures of the rotifers.

TABLE II. *Showing the minimum concentration of sodium chloride that the rotifers can endure when in the presence of other salts contained in the alkaline pond water*

Time	Parts Chlorine per Million Parts Water	Salinity % NaCl	Condition of Rotifers
March 3	5200	.9920	Normal
March 15	4500	.8422	Normal
March 24	3700	.6970	Normal
March 29	3200	.6076	Sluggish
March 30	3100	.5896	Sluggish
March 31	3000	.5715	Very sluggish and dying

EFFECT OF CALCIUM-FREE ARTIFICIAL SEA WATER

This experiment was made in order to find out the effect of the omission of one of the major salts from a solution of artificial sea water. The sea water was prepared in the usual manner excepting that the $CaCl_2$ was omitted. Rotifers that had been acclimatized to artificial sea water of the normal salinity were transferred to ten jars containing the calcium-free solution. Eight other jars containing sea water with calcium chloride were used as controls. All jars were examined at intervals of 2–3 days for about three weeks. At the end of this time the rotifers that were in the calcium-free artificial sea water showed no more ill effects than the rotifers that were in the controls containing calcium. In fact, after eleven days the rotifers were equally abundant in both lots of jars, and appeared to be in normal condition as well as multiplying at the normal rate.

ROTIFERS IN SOLUTIONS OF SODIUM CHLORIDE

Four jars were filled with a solution of NaCl and distilled water in the same proportion as found in sea water, but without any of the other salts that are found in sea water. The per cent was 3.213 of NaCl (17,800 parts chlorine per million parts of water). One pea half was placed in each of the jars. As controls, four other jars were filled with artificial sea water containing all the salts that occur in sea water, and each of these jars was furnished with one pea half to serve as food for the protozoa upon which the rotifers fed.

These jars were kept for about three weeks, and examinations were made every 2–3 days. The rotifers seemed to be affected adversely soon after they were transferred into the solutions of NaCl. Only a few rotifers lived in these NaCl solutions more than three days after transference, while those in the control jars thrived and multiplied. In some subsequent experiments, however, the rotifers were found to live and reproduce readily in rain water containing 0.5–1 per cent of table NaCl. In fact, flourishing cultures may be easily maintained for several weeks in this low per cent of NaCl if a half pea is added weekly. In these experiments, however, additional salts present in table salt were introduced, making the solution more like dilute sea water.

Thus it would seem that the rotifers could withstand the influence of 3–4 per cent of NaCl when in the presence of the several other salts in sea water, but NaCl alone at this per cent is fatal.

DISCUSSION

Examples of complete acclimatization of higher marine animals to fresh water are abundant in nature, and Semper (1880) has called attention to the cases of the salmon, eel, herring and plaice which spend part of their life in the sea and part of it in fresh water streams and lakes. Many marine molluscs and crustaceans, particularly littoral forms, are subjected constantly to the effects produced by a dilution of the water in which they live.

Plateau (1871), working over long periods of time, was successful in acclimatizing various species of marine Crustacea, Acari and Insecta to fresh water, which died within a few hours when transferred directly. He also found that these forms lived much longer when transferred directly to a solution containing pure NaCl than when transferred directly to fresh water, and concluded that NaCl was the only indispensable salt necessary to the life of these organisms.

Paul Bert (1871–73) attempted to learn why these animals died when transferred from one medium to another, and he found (1883) that the poisonous effect was usually not entirely due to a dilution of the quantities of dissolved salts in the water, but to some other factor in addition.

Ringer (1882), in his work on the physiology of the contraction of the ventricle of the heart, and Loeb ('00, '03, '06) discovered that toxicity was due to a disturbance in the balance of salts dissolved in the water. Loeb found that *Gammarus* lived only a few hours in a solution composed of only two of the more important salts contained in sea water; NaCl, KCl, $CaCl_2$, $MgCl_2$ and $MgSO_4$. He also discovered that a lack of Mg was not so fatal as a lack of one of the other metals, Cl, K and Ca. Animals that died within a few hours in a solution containing only two of the salts named lived as long as two or three days in a solution of NaCl, KCl and $CaCl_2$, and for an indefinite period of time when $MgCl_2$ was added. He also noted that Tubularian polyps had to have all four salts in the medium in which they were living in order to reproduce, and the eggs of the sea urchin, *Strongylocentrotus purpuratus,* failed to develop unless all four salts were present in their proper proportions.

Adolph more recently ('25) found that various marine species were killed in distilled water, but survived in a mixture of 98 or 99 per cent of distilled water with only 2 or 1 per cent of sea water. "In other words," he says, "most marine animals are able to live after abrupt change to almost pure fresh water provided the remaining salts are present in physiological proportions." Gradual dilution of the sea water over several days, in Adolph's experiments, did not materially help marine animals to endure pure water.

The experiments with *Brachionus mulleri* are quite in harmony with those of Adolph, and in addition cover a wider range of salinity changes in that higher concentrations of sodium chloride were used than are found in normal sea water.

SUMMARY

1. *Brachionus mulleri,* transferred from alkaline pond water with a salinity of 4.5 per cent to artificial sea water with a salinity of 3.20, soon became active and thrived and reproduced at a normal rate.

2. They did not thrive as well in artificial sea water with a salinity as great as that of the alkaline pond water in which they normally live.

3. *Brachionus mulleri* can endure alkaline pond water varying in salinity from 9.05 per cent to about .5715 per cent of NaCl.

4. The rotifers were scarcely effected when transferred to solutions of calcium-free artificial sea water.

5. *Brachionus mulleri* was unable to live in culture solutions having only the one salt NaCl present, although in the same proportions as is found in sea water or in the alkaline pond water.

The experiments and observations were made at the suggestion and under the supervision of Professor D. D. Whitney, to whom the writer expresses his appreciation.

Literature Cited

Adolph, Edward F. 1925. Some physiological distinctions between fresh-water and marine organisms. Biol. Bull., **48.**

Adolph, Edward F., and Adolph, P. E. 1925. Regulation of body volume in aquatic organisms. Biol. Bull., **48.**

Bert, Paul. 1871. Sur les phenomènes et les causes de la mort des animaux d'eau douce que l'on plonge dans l'eau de mer. Compt. Rend. Acad. Sci., **73:** 382–385; 464–467.

——. 1873. La mort des animaux d'eau douce que l'on immerge dans l'eau de mer. Compt. Rend. Soc. Biol., **23:** 59–61.

——. 1883. Sur la cause de la mort des animaux d'eau douce qu'on plonge dans l'eau de mer et reciproquement. Compt. Rend. Acad. Sci., **97:** 133–136.

Beudant, F. S. 1916. Memoire sur le possibilité de faire vivre des mollusques flurratiles dans les eaux salenes, etc. Jour. de Phys., **83:** 268–284.

Loeb, Jacques. 1900. On ion-proteid compounds and their rôle in the mechanics of life phenomena. I. The poisonous character of a pure NaCl solution. Jour. Physiol., **3:** 327–338.

——. 1903. On the relative toxicity of distilled water, sugar solutions and solutions of the various constituents of the sea water for marine animals. Univ. Col. Pub. Physiol., **1:** 56–69.

——. 1906. The dynamics of living matter. New York, pp. 71–105.

McClendon, J. F. 1917. Standardization of a new colorimetric method for the determination of CO_2 tension and CO_2 and O_2 of sea water. Jour. Biol. Chem., **30:** 265–288.

Plateau, F. 1871. Recherches physio-chemique sur les articules aquatique. Mem.-Cour. Royal Acad., Belg., **36:** 68–99.

Ringer, S. 1882. Concerning the influence exerted by each of the constituents of the blood in the contraction of the ventricle. Jour. Physiol., **3:** 380–393.

Semper, Karl. 1880. Animal life as affected by the natural conditions of existance. London.

Sumner, F. B. 1906. The physiological effect upon fishes of changes in the density and salinity of water. Bull. U. S. Bur. Fish., **25:** 53–108.