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No. 340

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A REPORT ON GAMMARUS INVESTIGATION IN THE SUMMER OF 1931.

by

Ernest S. Pentland.

Biological Board of Canada.

Introduction

In the fall of 1929 an investigation was begun by the writer into the life history and ecology of the two species of fresh-water shrimp, Gammarus fasciatus and G. limnaeus to determine the natural distribution of the two species as found in Ontario, the factors which limit this distribution, and the possibility of introducing them into waters in which they do not now occur to augment the food supply of the brook trout.

The preliminary step in this investigation was a physical, chemical, and biological study of various localities from which Gammarus of either species had been reported, with a similar study of habitats in the same regions in which they did not occur. It was hoped that some constant differences might be discovered which would throw some light on the factors controlling distribution. This work was continued until freezing weather rendered further work of this nature impractical. During the winter of 1929-30 experimental work was carried on and a thorough literature review was made at the Department of Biology of of the University of Toronto. In May 1930 field work was again begun, and during the summer of 1930 a general survey was made of a considerable part of southern Ontario. In addition to this survey work, some field experiments were carried on at Glen Major, Ontario, during this time.

Both Gammarus limnaeus and G. fasciatus were found to be distributed rather generally, though irregularily, throughout southern Ontario, but occurred in very small numbers in the soft water areas of central and northern Ontario, and were entirely absent from most of the habitats of this region.

In southern Ontario the only factors which appeared to exert a significant controlling effect were temperature, vegetation, and the presence of natural enemies (e.g. trout). The chemical composition of the water in the various habitats in which Gammarus of either species were found and in habitats in which Gammarus did not occur, did not show a significant variation and did not appear to be a limiting factor.

Temperature appeared to be the most important single factor limiting distribution, no specimens of either species being taken from water above 20 deg. C. Temperature also appeared to be the main factor in determining specific distribution, G. limnaeus being taken only in those waters which would remain cold throughout the year, such as spring fed streams and ponds, while G. fasciatus also occurred in ponds and streams which reached a much higher summer temperature. In this connection, however, it is interesting to note that G. fasciatus disappeared from these habitats as the temperature rose in the summer.

A preliminary experimental introduction of G. limnaeus into the trout ponds at Glen Major, in which Gammarus did not occur naturally, showed that conditions there were entirely satisfactory when the Gammarus were given protection from the trout. Full details of the results of this work up to September 1930 may be found in previous reports (Pentland, 1929, 1930a, 1930b, 1930c).

The investigation was discontinued during the winter of 1930-31 owing to the resumption of academic work by the writer, but was again taken up in May 1931. At this time the work planned for the summer was an experimental laboratory study of the effect of temperature, pH, hardness of the water, and food on the growth of Gammarus, and the probable value of each of these as a limiting factor in their distribution. This report presents an account of the work done and the results obtained between May 7th and August 15th, 1931.

ACKNOWLEDGEMENTS.

The writer is indebted to the University of Toronto for the use of laboratory space and equipment and to the Biological Board of Canada for financial assistance. The Glen Major Angling Company have cooperated by providing facilities for field experimental work. Professor W.J.K. Harkness of the Department of Biology of the University of Toronto has given frequent advice, helpful criticism, and encouragement. To all of these I desire to express my appreciation.

SOURCE OF MATERIAL.

Early in May Gammarus fasciatus were fairly abundant in a small stream in High Park and were present in apparently sufficient numbers to afford a continuous supply for the summer's work. With the approach of warm weather, however, they disappeared rapidly from this stream, their place being taken by large numbers of isopods which were present in only small numbers before the stream became warm. For the remainder of the summer the only source of supply was a small spring stream near Dagmar, Ontario, about forty miles from Toronto. The only form taken at either of these places in 1931 was G. fasciatus, although G. limnaeus had been taken at Dagmar on other visits.

This scarcity of material within reach of the laboratory would seem to indicate that for satisfactory results from an investigation of this kind it will be advisable to grow the experimental stock in the laboratory under standard conditions since results obtained from Gammarus taken from habitats differing widely in water temperature, pH, rate of flow, vegetation, etc. can scarcely be considered comparable. The determination of the proper conditions under which Gammarus could be raised in the laboratory thus constitued a large part of the summer's investigation.

Until this information was available satisfactory controls for the experimental work could not be maintained, and the value of any results obtained from the experiments could not be decided.

Of the various factors which had been considered in the natural habitats, temperature, food, and rate of flow of the water, and in addition the size of the aquaria, seem to be the most important in the propagation of Gammarus under laboratory conditions. Hardness and pH are dependent upon the water supply and will be relatively constant in any laboratory.

METHODS.

The first trials were made using ordinary wide mouthed pint fruit jars containing about 350-400 c.c. of water. Each of these jars contained some green aquatic plants, and the jars were kept in the laboratory at a temperature of 20 to 25 deg. C. No satisfactory results were obtained and it was found impossible to keep Gammarus alive under these conditions for any length of time. Death in a few cases was due to a deficiency of oxygen, but even when care was taken to avoid this, death ensued in a short time varying from several hours to several days. Slightly better results were obtained when larger jars holding five or six litres were used and changing the water frequently was advantageous, but no combinations of conditions were found which gave satisfactory results over long periods at laboratory temperature.

One large jar holding about five litres was arranged as a balanced aquarium containing sand, Myriophyllum, and a few dead leaves. Five Gammarus were placed in this jar on July 13th. Death occurred in at least two cases in about two weeks, two were not seen again, and one was still alive on August 14th. This was the most satisfactory attempt at keeping Gammarus alive at room temperature, which at times was as high as 26 deg. C. but still it did not throw any light on methods of keeping large numbers alive

On the other hand practically no difficulty was experienced in keeping moderately large numbers of Gammarus alive in standing water in jars of various sized at temperatures of 10 to 15 deg. C. in the dark in the refrigerator.

Under these conditions the mortality was no higher than could be expected under natural conditions. On July 6th about fifty Gammarus were placed in a large jar in the refrigerator at a temperature of about 15 deg. C. On July 23rd the jar was examined and no dead Gammarus were found. They were then transferred to a large dish pan of water which was kept in the laboratory. Efforts were made to maintain the temperature as close to 20 deg. C. as possible by the addition of ice, but variations between 18 and 23 deg. C. were noticed. The change to a higher temperature resulted in a great increase in activity and many moulted, but by July 28th five had died, and by July 29th only twelve or fifteen were left alive.

Later in the season thirty-five were left in a small dish at ten degrees C. from August 15th to September 19th with only a very small number of fatalities.

As low temperature appeared to be essential to success several attempts were made to utilize running water instead of standing water in the laboratory. These did not give satisfactory results. During the summer the normal temperature of the tap water varies from about 14 to 16 deg. C. At 16 deg. C. the content of dissolved oxygen was found to be at or slightly above saturation and a slight increase in temperature resulted in the liberation of the excess gas. This immediately formed bubbles on the sides of the jar, and more important, on the appendages of the amphipods. As soon as this happened they were carried to the surface as the result of their decreased specific gravity and death was the invariable result. Several females which died in this manner were found to have the entire brood pouch filled with gas.

In an effort to overcome this condition several methods of deoxygenating the water to a certain extent were tried. The most successful of these was an arrangement by which the water collected slowly in a carboy, and when the carboy was completely filled, was carried over to the stock jar by means of an intermittent siphon. When the rate of flow was carefully regulated the water in the carboy rose gradually to room temperature, the excess of gas was liberated, and the water was finally carried over to the aquarium. The size of the siphon was such that the arrangement was automatic. While this system reduced the oxygen content so that the water was no longer supersaturated, it also raised the temperature so that the final result was really standing water which was automatically changed about once an hour. The arrangement used is shown in the accompanying diagram.

TEMPERATURE.

Embody (1912) has reported G. fasciatus occurring naturally at a temperature of 30 deg. C. So far in the course of this investigation this fact has not been verified. Gammarus have not been taken by the writer in habitats even approaching this temperature and the results of this summer's work already presented have indicated that high temperatures are detrimental under laboratory conditions.

Huntsman and Sparks (1924) found that the lethal temperature of marine forms is only slightly higher than the highest temperature at which they are taken. A series of lethal temperature experiments on G. fasciatus bears out this statement if we consider the value of 30 deg. C. as given by Embody, as the normal maximum. Table I gives the results of this lethal temperature series. The lethal temperature was taken as that temperature at which movement ceased and there was no response to stimuli, the temperature being raised slowly from 20 deg. C. at the uniform rate of one deg. C. in five minutes. Miscroscopic examination showed that this was not the true death point as the heart beat may still be observed, but there were no recoveries under these conditions.

TABLE I. Lethal Temperatures at a Uniform Rate of Heating.

No.	Date	Species.	Locality	Age	Lethal Temp.
1.	May 25th	G. fasciatus	High Park	Adult	34.8-35.0
2.	May 26th	G. fasciatus	High Park	Adult	33.5
3.	May 26th	G. fasciatus	High Park	Adult	34.5
4.	June 15th	G. fasciatus	High Park	Adult	35.5
5.	June 18th	G. fasciatus	High Park	Juvenile	35.0
6.	June 23rd	G. fasciatus	Dagmar.	Adult.	33.0

It is of interest to note that the lethal temperature of the juvenile was approximately the same as that of the adults. The specimen from Dagmar, (No. 6) was taken from a spring stream at a temperature of 10 deg. C. while those from High Park were from water showing a wide daily and seasonal variation in temperature. The lethal temperature variation was slight.

An experiment was carried out to determine the effect of temperature on the rate of heart beat. A specimen was confined in a small cell mounted on a microscope slide immersed in a water bath, which was placed on the stage of a binocular microscope. The water was heated by means of an electric lamp. The heart rate was determined by timing to 1/5 of a second the time required for ten beats, and from this number of beats per minute were calculated. The results are given in Table II. In each case the figure in column (a) is the time required for ten beats and that in column (b) the number of beats per minute.

Before much confidence could be placed in results of this nature it would be necessary to have a large number of determinations as other factors, expecially muscular activity, have a great influence on the heart rate. This is clearly shown in the results for 10 deg. C. where the rate

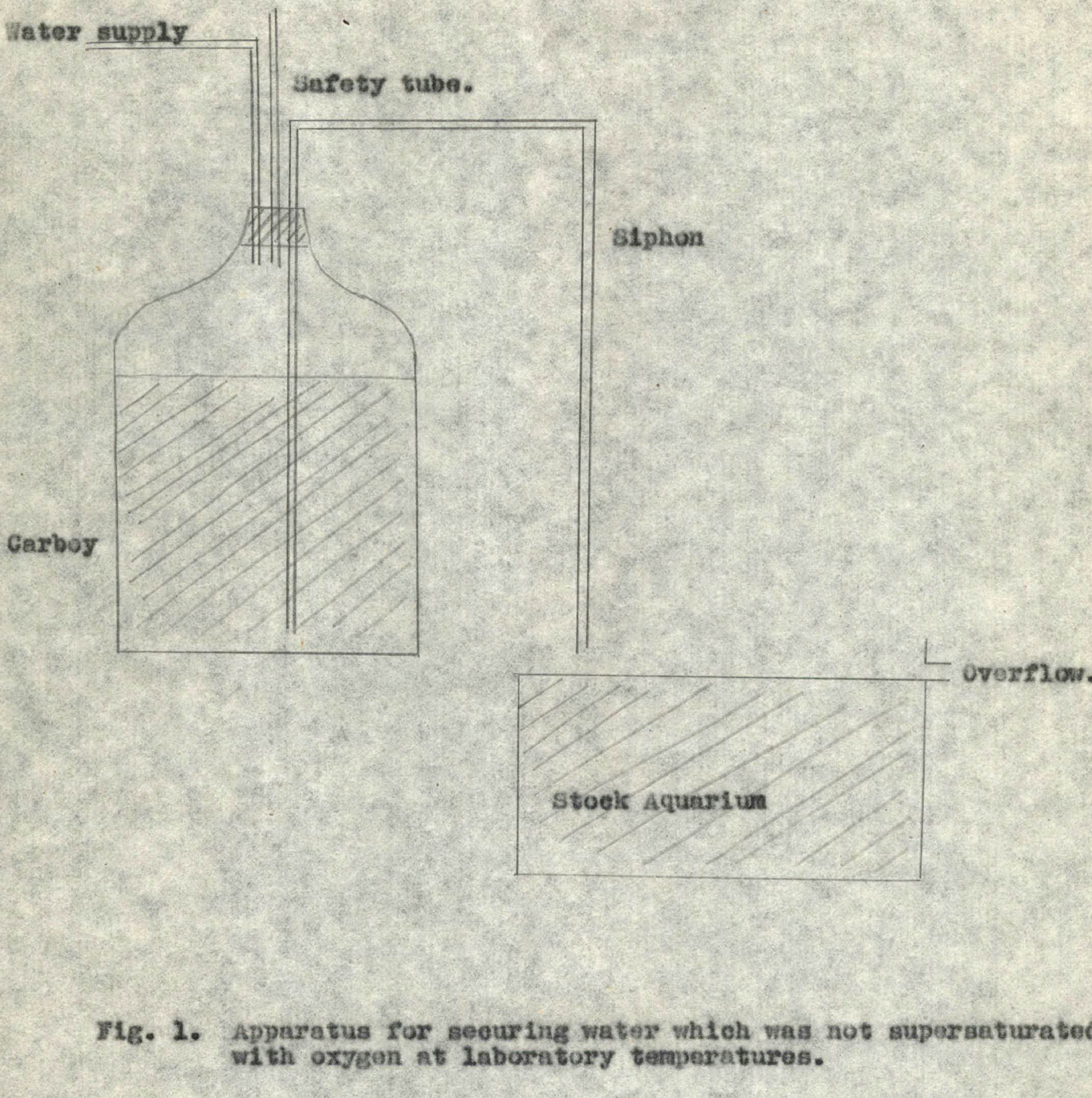

Fig. 1. Apparatus for securing water which was not supersaturated with oxygen at laboratory temperatures.

varied from 103 to 142 per minute.

When the movement of the pleopods is strictly a repiratory movement as opposed to a locomotory movement, the rate of movement is practically the same as the heart rate.

TABLE II. The Influence of Temperature on the Heart Rate.

	8 deg. C		10 deg. C		11 deg. C		15 deg. C	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
	5.0	120	5.8	103	4.8	125	6.0	100
	5.4	111	5.8	103	4.2	142	5.4	111
	5.0	120	5.4	111	5.0	120	5.4	111
	5.0	120	5.0	120	4.8	125	5.6	107
	5.0	120	4.4	136	4.8	125	5.4	111
	5.4	111	5.2	115	4.8	125	5.8	103
	4.8	125	5.4	111	4.8	125	5.8	103
	5.0	120	5.0	120			5.8	103
	5.0	120	4.2	142			5.0	120
	5.0	120	4.2	142				
	5.0	120						
Average		116		120		127		107.

pH

Only a small amount of work was done along this line, chiefly as the result of the impossibility of maintaining satisfactory controls. During the early part of the summer pH standards were not available and while the buffers used were carefully prepared to the theoretical values they were not checked. When the pH series was prepared on July 23rd, colorimetric standards were available and the various solutions were checked. The standards themselves, however, showed considerable variation and when checked against an old LaMotte set, and a new B.D.H.Capillator did not give good agreement. For this reason only slight value may be attached to the results. If they are considered at all they tend to support the conclusions resulting from field work as to the influence of pH. The accompanying graphs show the results obtained.

BREEDING.

Part of the work planned for the summer was a study of the effect of temperature on the rate of breeding. Owing to the complete failure of all breeding experiments, both at laboratory temperature and in the refrigerator, definite data on this problem cannot be presented at this time. General observations have shown, however, that breeding activities are greatly slowed down by low temperatures. In the stock jar which was kept in the refrigerator at ten to fifteen degrees only a few mated pairs were found while many mated within a few days when the jar was brought into the laboratory.

One female which had just extruded a batch of eggs was isolated and placed in the refrigerator at 10 deg. C. She lived for two weeks and at the end of that time the eggs were still in the brood pouch and showed very little sign of development.

While development proceeded much more rapidly at laboratory temperature all attempts to secure complete development from eggs to hatching met with failure. Under laboratory conditions development apparently proceeded normally but was always halted before completion by the death of the adult. The number of eggs produced by the individual female varied from 3 to 31.

FOOD.

Various foods were tried including aquatic plants, organic debris, and various kinds of leaves. The most satisfactory food used was dead elm leaves, well soaked. These were readily taken and are eaten until only the stems and veins are left. Harder leaves of oak and maple are not taken.

SUMMARY.

During the summer of 1931 the writer has continued the study of Gammarus begun in 1929 with special attention to the various factors which appear to be the most important under laboratory conditions.

Temperature was the most important single factor, either alone as a limiting factor at high temperatures, or, at medium temperatures, by reducing the resistance of the Gammarus to other unfavorable conditions. At temperatures above 15 deg. C. considerable difficulty was experienced in all attempts to keep Gammarus alive in the laboratory while below this temperature no excessive mortality occurred.

The lethal temperature was found to be relatively constant for various individuals between 33 and 35.5 deg. C. and did not show any pronounced variation which could be associated with age or temperature of the habitat. The temperature appeared to be correlated with the rate of heart beat to a certain extent, although the heart rate was also closely connected with muscular activity.

Some pH work was carried out which tended to confirm the conclusion from field work that pH is a factor of minor importance in controlling the distribution of Gammarus in southern Ontario.

No breeding experiments were successful, and definite data on the relation of temperature and reproductive rate were not obtained.

The most satisfactory food found was dead elm leaves, well washed and soaked. Harder leaves of oak and maple were not taken.

RECOMMENDATIONS FOR FUTURE WORK.

All of the work to date, laboratory, field survey, and field experimental, has supported the conclusion that, in southern Ontario at least, the chemical factors are of minor importance and that the chief controlling factor is temperature. In northern Ontario the occurrence of Gammarus is so rare that definite conclusions can scarcely be drawn. There is, however, a distinct change in the character of the water from hard and alkaline in the south to soft and acid in the north. This difference has been considered (Titcomb 1927) to account for the distribution of the two species, but his conclusions have not been substantiated in the present study.

In the laboratory work of the past summer the chief difficulty which has been encountered is that of maintaining satisfactory controls at temperatures at or above 20 deg. C. Since G. fasciatus occurs at temperatures up to 20 deg. C. in Ontario and has been reported by Titcomb and Embody at 30 deg. C. this difficulty may be overcome when the proper conditions have been determined. The writer hopes to be able to continue this part of the problem during the winter and spring of 1931-32.

A considerable amount of experimental work is still necessary to determine the maximum and minimum values of pH which may occur and the part played by the hardness of the water in the development of the two species. Since temperature appears to play such an important part in the ecology of Gammarus it would seem strongly advisable to determine these values at a series of constant temperatures. This would practically necessitate the use of thermostatically controlled constant temperature boxes and standing water which could be changed regularily if necessary.

The importance of dissolved gases, particularily carbon dioxide and oxygen, in nature is questionable. Free carbon dioxide occurs but rarely in natural waters, while a concentration of dissolved oxygen which is below the amount necessary for the life of Gammarus is seldom found in waters which are otherwise suitable. The lower limit of oxygen could easily be determined and the presence of dissolved gases above saturation has already been shown to be harmful. The problem of carbon dioxide is closely connected with carbonate, bicarbonate, and pH, and would require special apparatus and methods for its consideration.

On the basis of the data already available from previous work it would seem advisable in future laboratory work to concentrate chiefly on the effect and interrelations of temperature, pH, and the salt content of the water.

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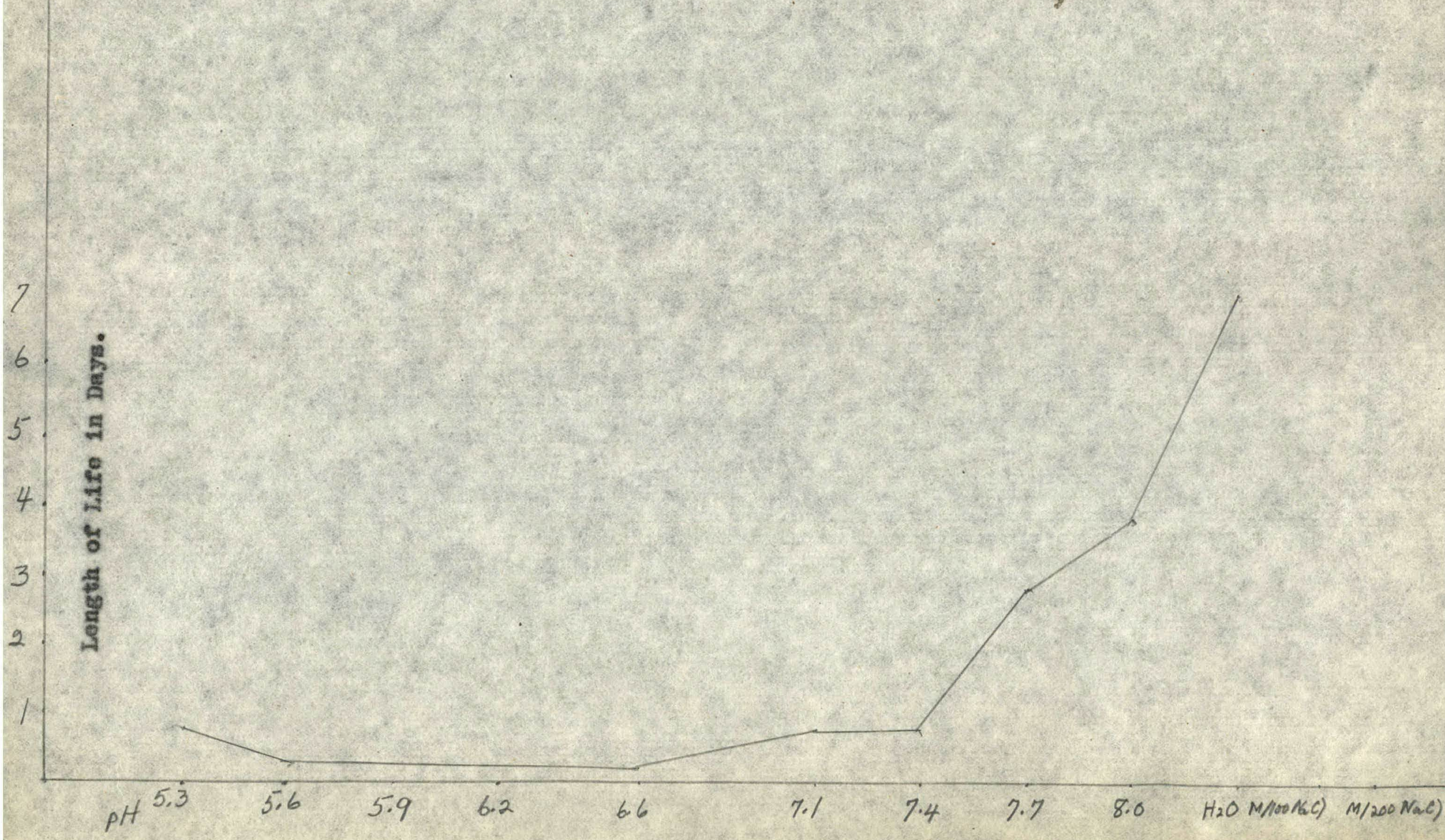
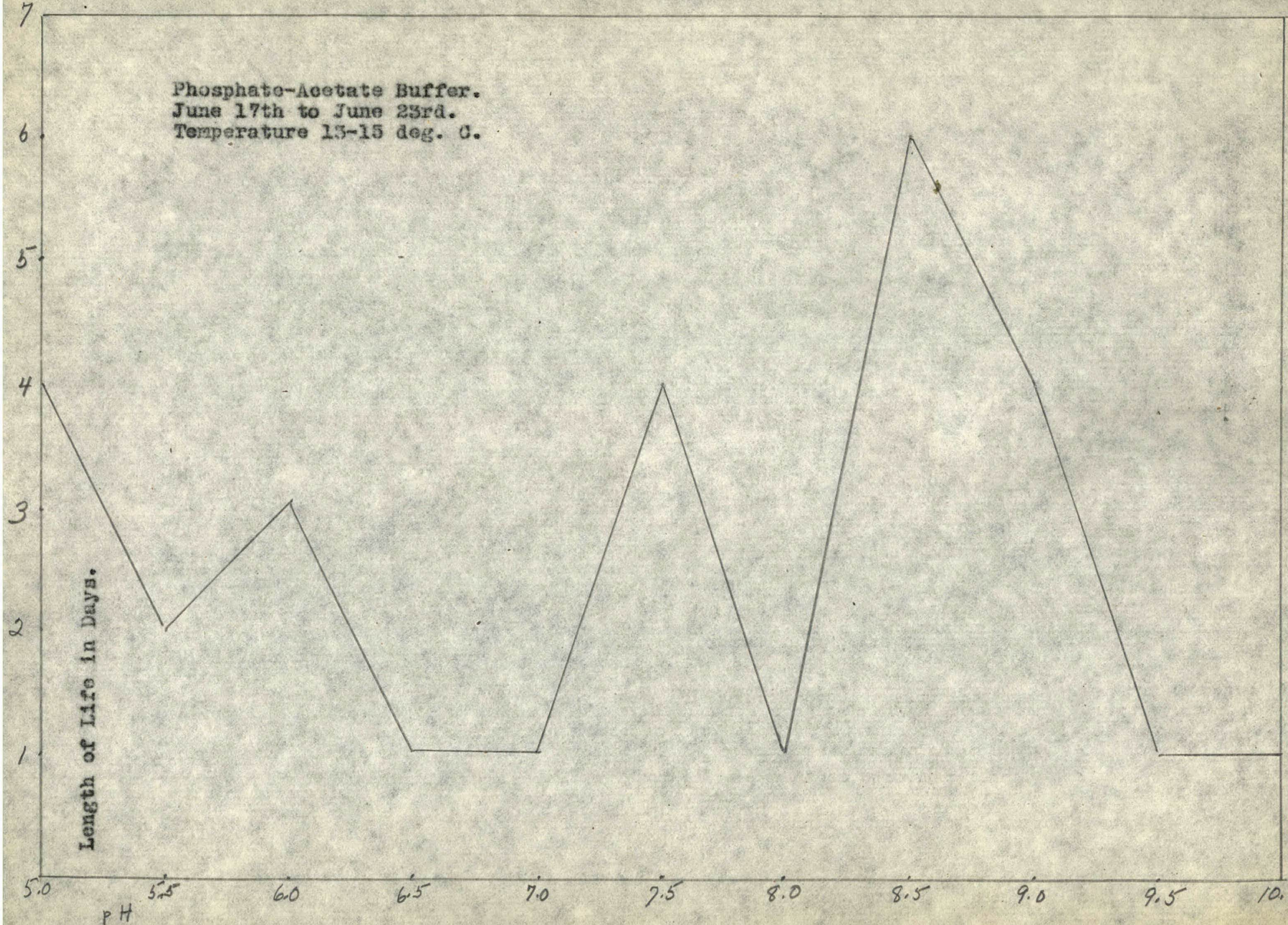
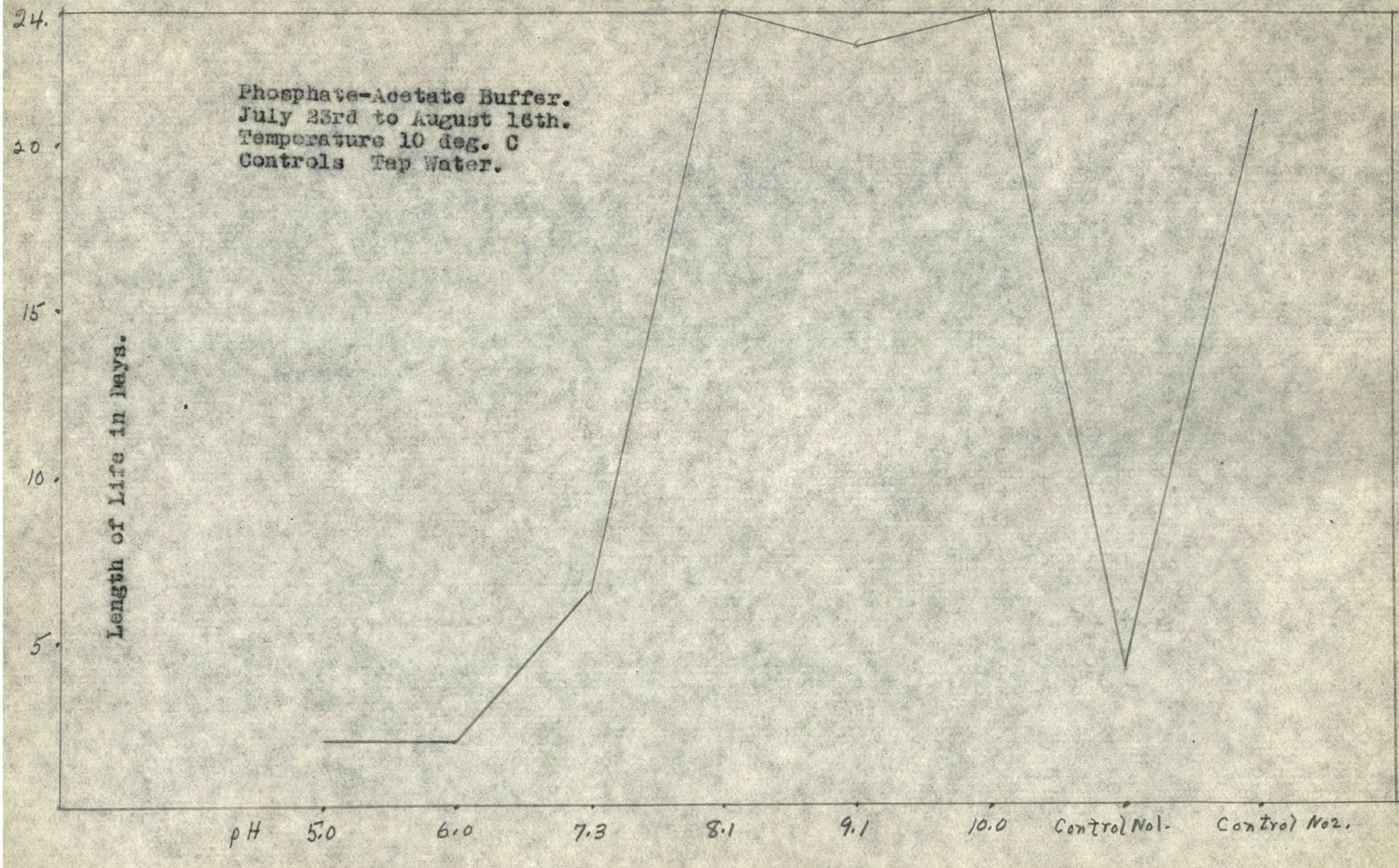
Trans. Amer. Fish. Soc. Volume 57

pp 150-156.

Sorensen's Phosphate Buffer.
Duration of Experiment June 9th to June 13th.
Temperature approximately constant at 15 deg.
Three controls used, one distilled water, one M 100 NaCl, one M 200 NaCl.
All controls still alive at termination of experiment.




Phosphate-Acetate Buffer.
June 17th to June 23rd.
Temperature 13-15 deg. C.
Length of Life in Days.
7
6
5
4
3
2
1
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
pH


Phosphate-Acetate Buffer.
July 23rd to August 16th.
Temperature 10 deg. C
Controls Tap Water.
Length of Life in Days.
24.
20
15
10
5
pH
5.0
6.0
7.3
8.1
9.1
10.0
Control No1.
Control No2.