DISTRIBUTION, GROWTH, FEEDING HABITS, ABUNDANCE, THERMAL, AND SALINITY RELATIONS OF NEOMYSIS MERCEDIS (HOLMES) FROM THE NICOMEKL AND SERPENTINE RIVERS, BRITISH COLUMBIA.

by

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ABSTRACT

A study was made of the distribution, feeding habits, growth, temperature tolerance and salinity relations of *Neomysis mercedis*.

It was found to exist in salt, brackish and fresh water where it feeds on diatoms, algae, vascular plant material, animal material and possibly detritus.

Growth to maturity appears to take one year with reproduction occurring in the fall and possibly the spring. There is evidence of two populations, one produced in the fall and the other in the spring.

Temperature tolerance was determined by subjecting animals from various acclimation temperatures to a range of temperatures and noting the times to death. The tolerance was determined, in units of square degrees centrigrade, to be 491 units, with the lower and upper lethal temperatures being 0^{o}C. and 23^{o}C.

An attempt was made to determine the rate of acclimation to increasing temperature by raising the temperature of separate groups of animals at different rates. Indications were that *Neomysis* acclimate thermally at a rate faster than 3^{o}C. per day (1^{o}C. per 8 hours).

Salinity relations were tested by subjecting animals from a constant salinity to various lower salinities; by gradually reducing the salinity of the environment; by

subjecting animals from various salinities to fresh water; and by setting up a salinity or fresh water preference gradient.

About 1 o/oo chlorinity was found to be lethal for Neomysis maintained in an environment of 10.33 o/oo chlorinity. Gradually decreasing the salinity over a 6-day period indicated no increased ability by the animals to withstand lower salinities. There is a temporal order in the times to death of animals from various locations up the river (i.e. animals from different salinities) when placed in fresh water with those from regions of highest salinity dying first. In some of the lower reaches of the river surface chlorinity was negligible yet Neomysis taken from these regions existed only for a limited time in fresh water. Those from upper reaches (10--14 miles upstream) survived well in fresh water.

The crustaceans exhibited no ability to distinguish fresh from salt water. They did however exhibit a rheotaxic tendency. It is suggested that the rheotaxic response, plus the animal's ability to osmoregulate account for their distribution into fresh water.

Indications are that Neomysis mercedis may be suitable for transplantation into some lakes as a supplement to the fish food there.

TABLE OF CONTENTS

INTRODUCTION

The Fisheries Research Branch of the British Columbia Game Department was interested in obtaining a type of plankton organism which would be suitable for introduction into lakes as a supplement to the existing trout food. Particularly were they interested in oligotrophic lakes such as Kootenay lake where the Kamloops trout (S. gairdnerii) grow quickly to about 8 inches in length then slow in growth till reaching 16 inches (two pounds approximately) after which they are large enough to feed on kokanee and consequently speed their growth until they reach fifteen to twenty pounds. The slow growth period was thought to be caused by a lack of suitable plankters.

Mysis relicta and Pontoporeia affinis were suggested as supplements for lakes with sparse bottom fuana (Larkin, 1948) and consequently several thousand of these animals from Waterton lakes, Alta. were transplanted into Kootenay lake, B. C. Subsequently some preliminary experimental work was carried out on these crustaceans in the laboratory but it was found impractical to maintain a sufficiently large stock. In September, 1950, a Mysidacean (Neomysis mercedis) was found in the brackish water estuary of the Nicomekl river. Since it had also been reported in Lakelse lake it might be suitable for introduction into fresh water. Accordingly collections were made with a view to determining some of the salient characteristics of these crustaceans in relation to their possible transplantation.

If introductions are to be made, insight into feeding habits, growth rate, times of reproduction, abundance, thermal tolerance and salinity tolerance might be of primary importance. The following, therefore, is an endeavor to throw some light on these factors.

DESCRIPTION

Specimens have been identified (A. H. Banner, 1951) as Neomysis mercedis Holmes of the family Mysidae, order Mysidacea, sub class Malacostraca, class Crustacea.

The organism has been adequately described by Tattersal (1932). For the present study a short review of the development and a description of its secondary sex characteristics are sufficient.

The animal has no free living larval stages; eggs and young develop in a brood pouch formed by plates (oostegites) on the last two thoracic segments of the adult female. Young are liberated when 3--4 mm. long and are then replicas of the adult. Mature adults are 9--10 mm. in length (from eye to base of telson), but growth may proceed to 13 mm. in some individuals.

In females the brood pouch is characteristic of the adult. The genital pores are on the sixth thoracic segment at the base of the appendages. The pleopods are all uniform in size.

In males the genital pores are on the last thoracic segment. The fourth pleopods of the adult are biramous and have extended exopods, presumably for sperm transfer.

It might be noted here that in the February and March collections the animals were infested with Zoothamnion *arbuscula*, a member of the family *Vorticellidae*. The protozoan is not parasitic and is believed to be harmless to the crustaceans.

GENERAL DISTRIBUTION

N. mercedis was first reported from lake Merced, a fresh water lake on the San Francisco peninsula, California, (Tattersal, 1932). Since then it has been collected in San Francisco bay (Tattersal, 1932); Nanaimo and Departure bay, and Quatsino sound, Vancouver island; Lost Lagoon, Vancouver, (Tattersal, 1933). It has also been found in Lakelse lake and was identified from there by A. H. Banner (Chace, June 3,1949). Dr. S. C. Carl (letter to author) tentatively identified a mutilated specimen from St. Mary's lake, Salt Spring island, as Mysis relicta and mentions a record of Mysis from lake Washington near Seattle.

Since then Banner (1951) has reported that Neomysis mercedis occurs in lake Washington. It might be, as Dr. Carl did suggest, that this is actually the species in St. Mary's lake.

The animals used in the present work were discovered in the estuaries of the Nicomekl and Serpentine rivers. So far as can be ascertained there is no previous record of them from this locality.

Neomysis mercedis is predominantly a brackish water species, although it has been taken in regions of relatively high salinity and also in fresh water. In San Francisco bay it is limited largely to the upper part of the bay; Tattersal

(1932) believes the relatively high salinity of the lower bay to be most important in effecting this restricted distribution. In the Serpentine and Nicomekl rivers the species occurs from the mouths of the rivers to fourteen miles upstream where the water is fresh.

DISTRIBUTION AND ABUNDANCE OF N. MERCEDIS IN THE NICOMEKL AND SERPENTINE RIVERS.

A brief account of the physical characteristics of the rivers is applicable to this section of the work.

Both rivers drain low lying land in a valley running easterly from Mud bay (fig. 1). They are slow flowing, winding and fairly muddy.

Since about 3/5 of each river is below the 25 foot contour line a large portion is affected by the tides. The tidal influence causes large daily variations in the river levels but the amount of sea water in the rivers is limited by flood gates about 3 miles from the mouths. These gates are constructed so that they are open when the force of the river current is greater than that of the incoming tide and closed when the tidal flow equals or exceeds that of the river. Thus at high tide the gates act as a dam causing the rivers to back up. The effect of this has been noted 9¾ miles upstream.

Although most of the sea water is kept out, some does flow up the rivers past the flood gates. On January 27, surface and bottom water samples were taken a quarter of a mile above the flood gates on the Nicomekl. The surface sample gave a negligible chlorinity while the bottom sample showed a chlorinity of 6 o/oo. Surface and bottom samples both gave negligible chlorinities 8 miles upstream, indicating the absence of an underlying layer of sea water this far up the river.

Salient features of the abundance and distribution of Neomysis are given in Tables I and II. These may be used in conjunction with the map (fig. 1.) which indicates the exact location of the collections.

In the tables the abundance listed as good, fair or poor, is based on the number of individuals taken per sweep of the net. "Good" indicates 30 to 40 individuals, "fair"--10 to 15, and "poor"--1 or 2.

The animals were collected by the use of a dip net which had a 9" diameter mouth, was made of cheese cloth and was attached to the end of a 6' wooden handle. Collections were made from the banks of the rivers. Most crustacea were found in reedy or grassy locations where the bank sloped gradually into the river. Where the river bank was vertical or overhanging, Neomysis were taken close against the bank, or under the overhang, although in such locations collections were never large.

At the river mouths where the shore of Mud bay slopes very gradually, Neomysis were collected in about 18" of water 10' off shore when the tide was low (about 6'). The bottom here is a mixture of mud, sand and gravel. Reeds and grasses grow farther back on the shore and are under water at high tide.

As shown in fig. 1, Neomysis were taken from the mouth upstream 10$\frac{3}{4}$ miles in the Serpentine, and 14 miles in the Nicomekl. The latter river is more like a creek in this location, being small and fairly fast flowing.

The greatest abundance of crustacea occurred in the fall collections of October and November (Table I). At this time the animals taken were nearly all females of which many were carrying eggs or young. Compared with these catches the numbers of animals in succeeding collections was very much reduced.

TABLE I -- COLLECTIONS IN NICOMEKL

DATE	TIME	LOCATION -- Mi. From Mouth	ABUNDANCE & REMARKS	TIDE (Approx. Height)	SURFACE CHLORINITY 0/00	SURFACE TEMP. °C.
Oct.9/50	1200	4¼	Very Good-Nearly all females many with brood pouches.	9'	3.1	12.0
Oct.22/50	1400	4¼	Good-Nearly all females. Many brood pouches.	12'		9.5
Nov.5/50	1200--1400	3	Good-Nearly all female.	13'		9.0
	1130	Drainage ditch along H'wy.	None.		Negligible	11.0
Dec.12/50	1330	3	None.	12'	.54	7.0
	1400	4¼	Very poor.	12'		7.0
	1600	9¾	Fair--Males and females taken.	12'	Negligible	6.5
Dec.17/50	1430	3	None by scoop net. Few by bottom drag net.	13'	.50	6.5
	1500	9¾	Fair	12'	Negligible	5.9
Dec.19/50	2100	3	1 only seen	4'	0.4	6.0
	2130	2	None			
	2200	9¾	None	5'	Negligible	5.8
Jan.12/51	1000	3	None	15'	.07	6.0
	1200	2½	Fair. Very few males.	12'	.07	

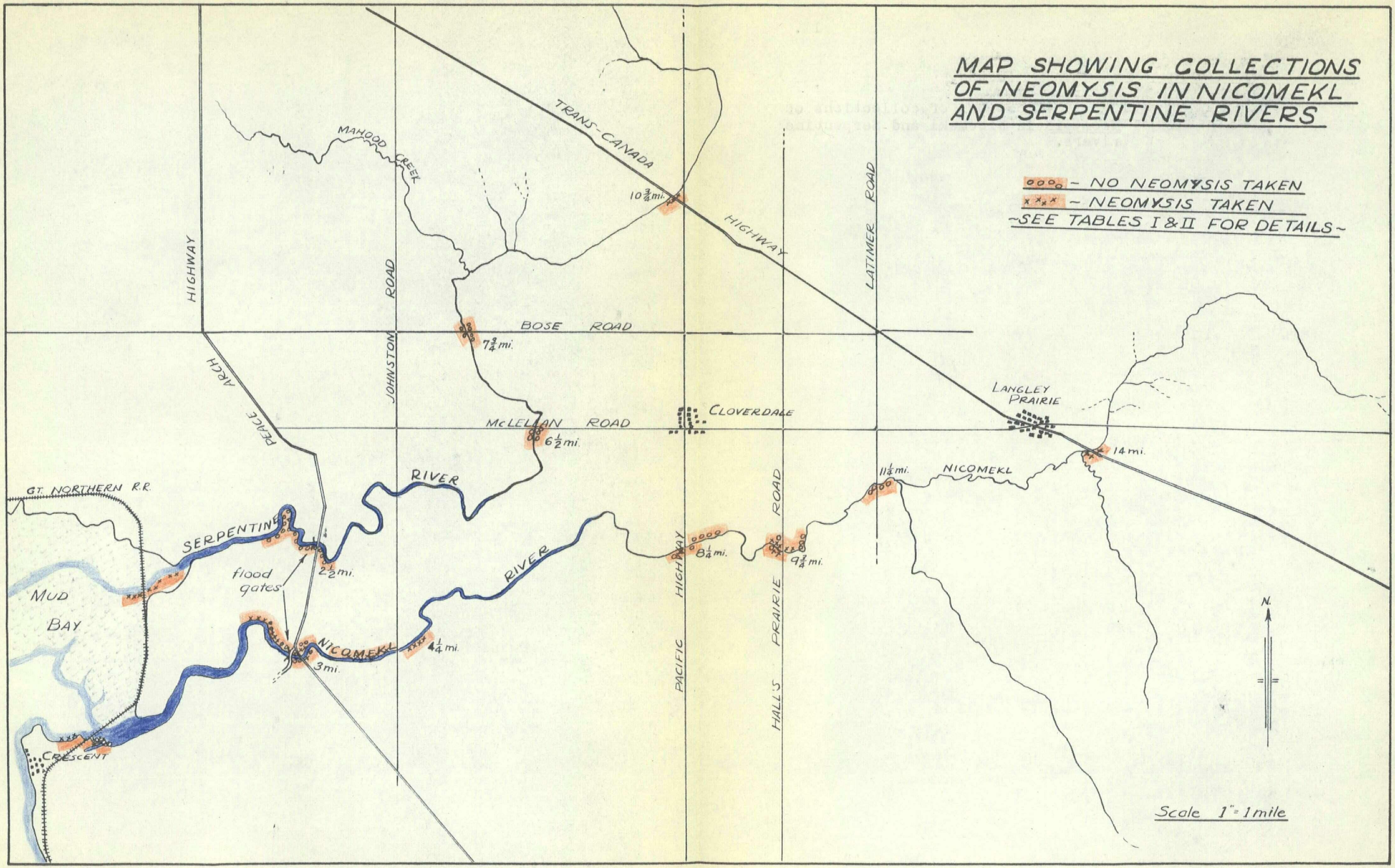
TABLE I -- CONTINUED.

DATE	TIME	LOCATION Mi.From Mouth	ABUNDANCE & REMARKS	TIDE (Approx. Height)	SURFACE CHLORINITY 0/00	SURFACE TEMP. °C.
Jan.21/51	1300	$2\frac{1}{2}$	Poor.	12'	.523	6.5
		2	Fair. Very few males		.523	6.5
	1500	3	None.	13'	Negligible	6.5
	1600	$9\frac{3}{4}$	5 taken.	12'	Negligible	6.2
Jan.27/51	1430	Mouth	None.	8'	3.66	5.0
	1600	3	None.	9'	Negligible	4.5
	1630	$8\frac{1}{4}$	1 only.	10'	Negligible	4.5
Feb.3/51	1400	$9\frac{3}{4}$	Poor.	14'	Negligible	3.0
	1500	$8\frac{1}{4}$--$8\frac{3}{4}$	None.	13'	Negligible	3.0
	1600	2	Very Poor.	12'	.4	3.0
		3	None.		Negligible	3.0
Feb.17/51	1600	Mouth (Actually Mud Bay)	Fair.	8'	7.42	5.0
	1645	$4\frac{1}{4}$	Poor.	7'	Negligible	4.0
Feb.24/51	1400	14 (L.Prairie) River small here.	Poor.	7'	Negligible	4.0
	1430	$11\frac{1}{4}$	None.	7'	Negligible	4.0
	1500	$4\frac{1}{4}$	Poor.	8'	Negligible	4.0
Mar.17/51	1430	Mouth-Mud bay	Fair.	8'	3.63	6.0
	1500	Short Dist. up river.	Fair.	8'	1.34	5.0
	1430	$3\frac{1}{2}$	Poor.	8'	Negligible	4.0
	1530	$4\frac{1}{4}$	Poor.	8'	Negligible	4.0
	1630	$9\frac{3}{4}$	Fair.	7'	Negligible	4.0

TABLE II -- COLLECTIONS IN SERPENTINE

DATE	TIME	LOCATION Mi.from Mouth	ABUNDANCE & REMARKS	TIDE (Approx. Height)	SURFACE CHLORINITY 0/00	SURFACE TEMP. °C.
Dec.12/50	1500	$7\frac{3}{4}$	None	12'	Negligible	6.5
Dec.17/50	1400	$2\frac{1}{2}$	None	12'	.7	5.0
Dec.19/50	2145	$7\frac{3}{4}$	None	4'	Negligible	6.0
Jan.12/51	1300	$1\frac{1}{2}$--$2\frac{1}{2}$	None	11'	.20	6.0
Jan.21/51	1430	Mouth	Good	13'	7.57	5.0
Jan.27/51	1600	$6\frac{1}{2}$	None	9'	Negligible	4.5
Feb.3/51	1500	Mouth	Good	13'	10.33	3.0
Feb.24/51	1330	$10\frac{3}{4}$	Fair. River had been high.	7'	Negligible	4.0
Feb.24/51	1445	$6\frac{1}{2}$	None	8'	Negligible	4.0

fig. 1. Locations of collections of *Neomysis* in Nicomekl and Serpentine rivers.


MAP SHOWING COLLECTIONS OF NEOMYSIS IN NICOMEKL AND SERPENTINE RIVERS
oooo - NO NEOMYSIS TAKEN
xxxx - NEOMYSIS TAKEN
~SEE TABLES I & II FOR DETAILS~
TRANS-CANADA HIGHWAY
MAHOOD CREEK
HIGHWAY
JOHNSTON ROAD
LATIMER ROAD
BOSE ROAD
McLELLAN ROAD
PEACE ARCH
CLOVERDALE
LANGLEY PRAIRIE
PACIFIC HIGHWAY
HALL'S PRAIRIE ROAD
GT. NORTHERN R.R.
SERPENTINE
RIVER
NICOMEKL
RIVER
NICOMEKL
MUD BAY
CRESCENT
flood gates
10 3/4 mi.
7 3/4 mi.
6 1/2 mi.
2 1/2 mi.
3 mi.
4 1/4 mi.
8 1/4 mi.
9 3/4 mi.
11 1/4 mi.
14 mi.
N.
Scale 1" = 1 mile

GROWTH

Method of Analysis

The growth analysis is based on measurements for females collected during the months of October, November, February and March. The collections in these months were made in the same general location in the river whereas collections in December and January were made in different areas. It was considered that the animals obtained up the river where the salinity is low might possibly grow at a different rate from those nearer the mouth, consequently the results of the December and January samplings were omitted from the calculations. Only females were numerous enough for consideration in the October and November collections.

Crustaceans, having exoskeletons, grow only during the moulting period. Growth may be followed by determining the intervals between moults from the modes in length frequency polygons. Some difficulty was experienced in determining the modal peak for size classes by inspection. Therefore a method described by Harding (1949) has been used to establish the modal peaks. His method consists of plotting accumulated percentages on probability paper. In a normal distribution, if the variates be taken as percentages of the total number of variates and these percentages then be accumulated and plotted on probability paper the resulting graph is a straight line. Thus if, from a sample, two straight lines appear when using this method the inference is that the sample is comprised of two populations. The calculations (Table III), and graph (fig. 2.), for the collections of February--March follow as an example of the use of the method.

TABLE III -- DATA FROM FEBRUARY--MARCH COLLECTIONS USED TO PLOT GRAPH IN FIG. 2.

Size mm. Eyes-Base Telson	Frequency	% Frequency	% Accumulated
6	9	6.72	6.72
7	16	11.94	18.66
8	17	12.69	31.35
9	15	11.20	42.55
10	23	17.16	59.71
11	33	24.61	84.32
12	15	11.20	95.52
13	5	3.73	99.25
14	1	0.75	100.00
	134	100.00	

The plots of the accumulated percentages (dotted line in fig. 2. result in two straight lines representing two populations or age classes, the limits of which are indicated by the point of inflection in the curve joining the straight lines.

The smaller size class is taken to be 39% (point of inflection) and the larger size class is taken to be 61% of the whole sample. Each of these classes is treated independently and plotted on the probability paper as though it

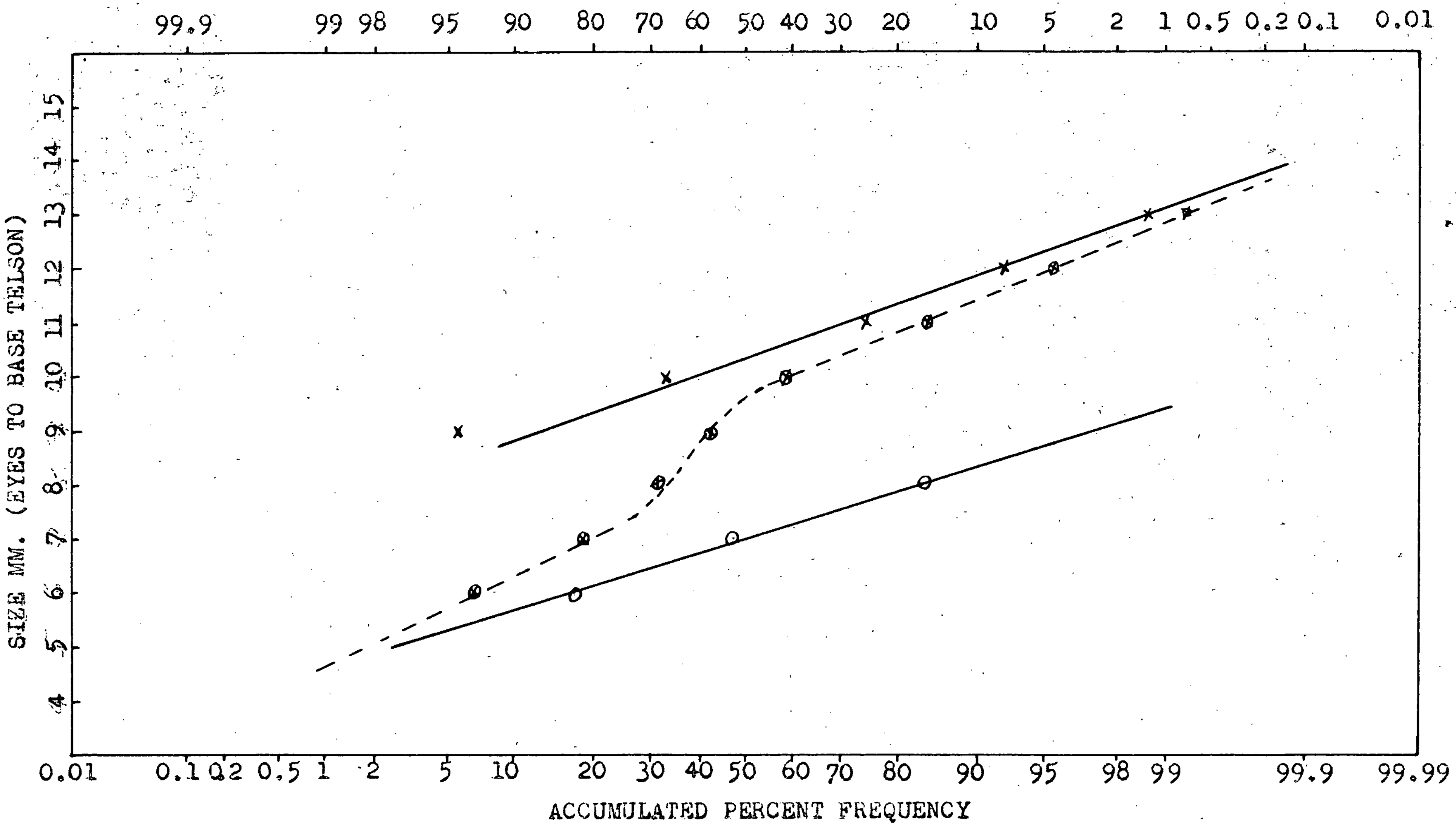


fig. 2. Length of Neomysis plotted against accumulated percentage frequency.

occupied the whole range of 100%. Using the class of smaller individuals (occupying 39% of the sample) as an example, this is done by multiplying the accumulated percentages of the group by $\frac{100}{39}$. These values produce a straight line when plotted (lower solid line in fig. 2.). The point where it cuts the 50% vertical is taken to be the mean size of the class read off on the ordinate. The standard deviation of this mean is determined by projecting verticals 34.13% each side of the 50% vertical. These verticals intersect the line representing the age class. The values of the points of intersection read off on the ordinate give the standard deviation each side of the mean. Essentially the same procedure is adopted for the other age class so that the mean and standard deviation for each can be determined.

Results

Since the October and November collections were but two weeks apart and the February--March collections three weeks apart, the data of the spring samples were combined and so were those of the fall samples.

Table IV lists the frequency of appearance of the various size classes for the combined collections. These data are graphed in fig. 3. Treating it by Harding's method gives averages for size classes as follows:--

October--November collections--5.8mm.(S.D.±1.0) and
9.3 mm. (S.D.±1.2)

February--March collections --7.1mm.(S.D.±1.0) and
10.4 mm. (S.D.±1.2)

TABLE IV -- COMBINED COLLECTIONS OF OCTOBER--NOVEMBER AND FEBRUARY--MARCH.

Size mm. Eyes-Base Telson	Frequency (No. Individuals Collected)	
	October--November	February--March
4	1	
5	4	
6	15	9
7	10	16
8	21	17
9	32	15
10	39	23
11	23	33
12	10	15
13	2	5
14		1
	157	134

It would seem that there were two periods of reproduction each year. One period is known to occur in September and October (observations in the field). This would produce the 5.8 mm. class of the October--November sample, with 7.2 mm. representing the size of this class in the spring. Growth would likely bring this class to about 10 or 11 mm. by August with reproduction occurring in the fall. It is suggested that after releasing the young the majority of females perish, while a few survive to reach a

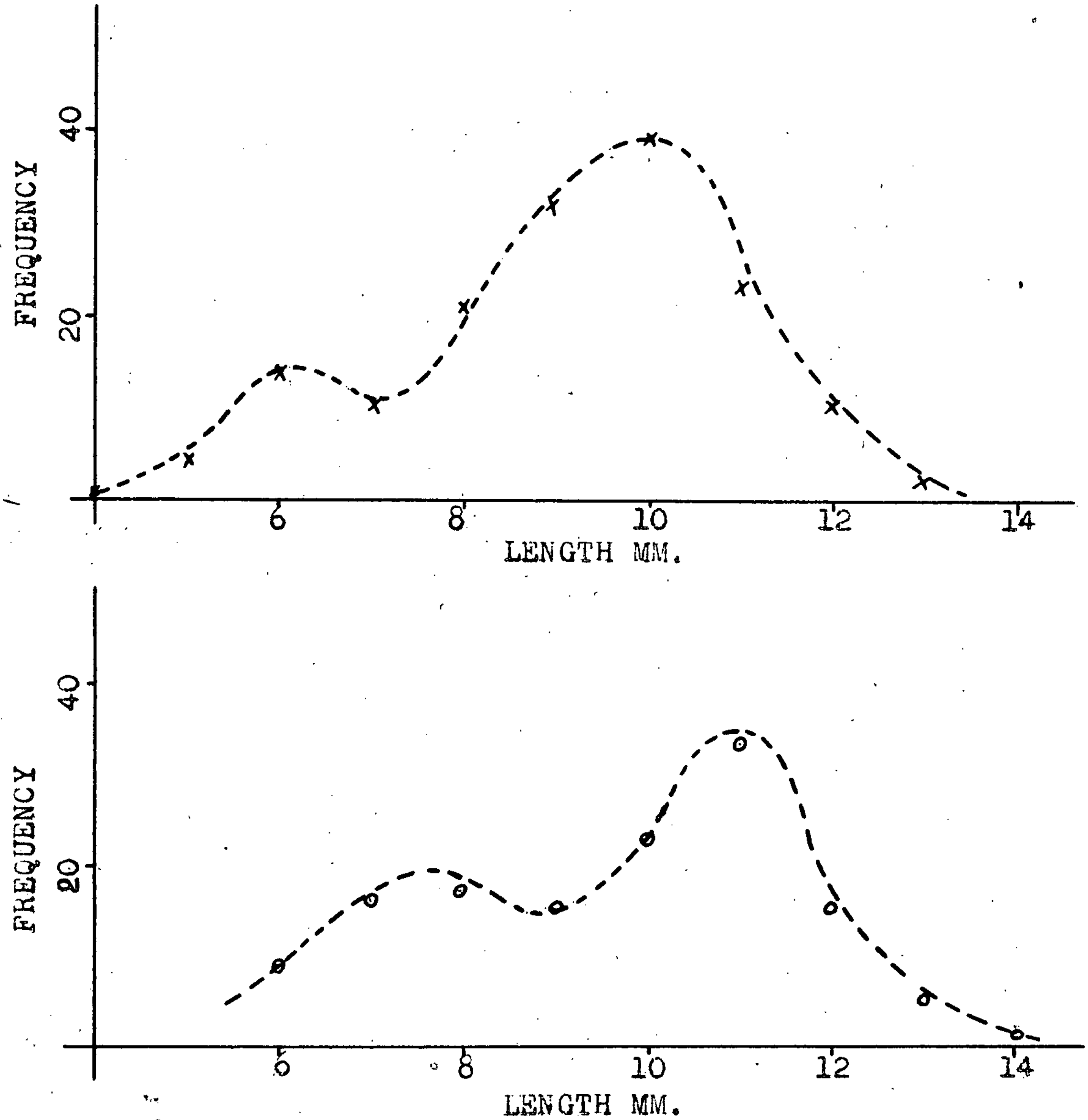


Fig. 3. Length--frequency polygons for <u>Neomysis mercedis</u>.

Upper diagram October--November collections.

Lower diagram February--March collections.

size of 12-13 mm. in the spring when they again reproduce.

The presence of the 9.3 mm. group in the October--November collections can be accounted for by supposing a reproductive period in the spring possibly around May--June. The fact that egg bearing females (12--13 mm.) were taken in the February--March collections lends some support to this suggestion.

The pattern of growth is indicated diagrammatically in fig. 4. The cross hatched areas represent the suggested growth of the animals throughout the year.

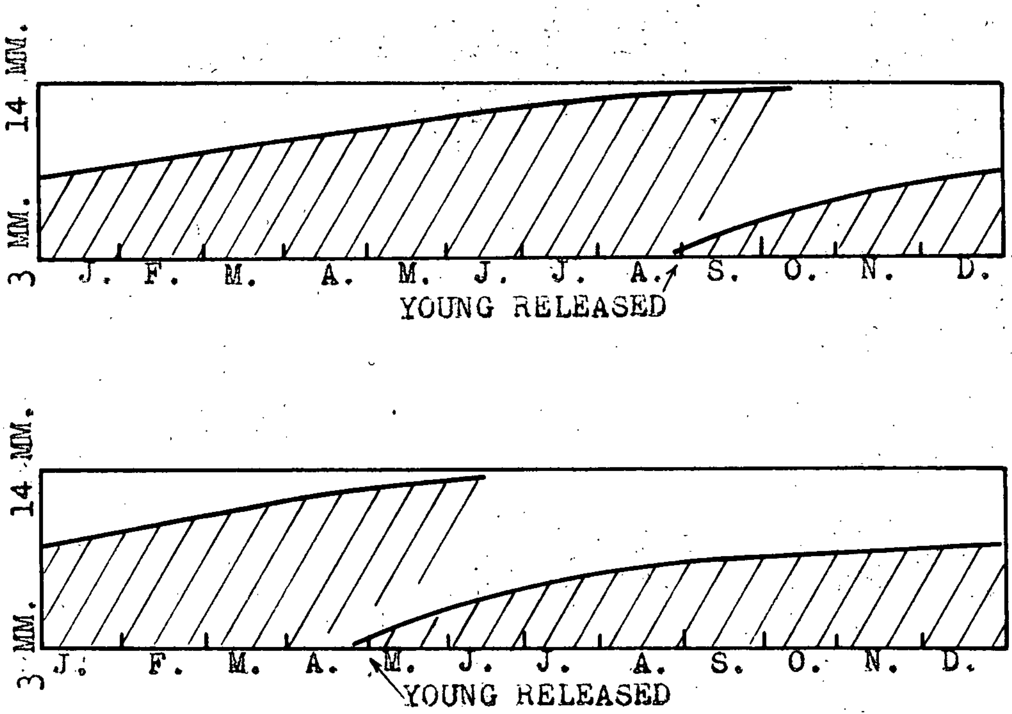


fig. 4. Diagrammatic representation of suggested growth of N. mercedis.

These conclusions may be presumptuous considering the supporting data. Some reproduction may occur during the whole year. However, field observations indicate a heavy breeding period in the fall, and collections (small as they are) indicate a period in the spring as well. It remains for 12 monthly collections to be taken before definite conclusions can be reached.

FEEDING HABITS

Borradaile (1935) describes the feeding mechanism of Mysidaceans. Food particles are carried to the mouth in a current of water set up by rapid movements of the maxillae. Large pieces of material are held by the endopodites of the thoracic limbs while being eaten.

In order to determine the type of food eaten the stomachs of 33 freshly caught animals were examined. In most cases much of the material was unidentifiable, suggesting detritus; however, it was found that both plant and animal organisms were consumed. Diatoms, dinoflagellates, blue green algae, vascular plant and animal material were observed. Copepod and mysidacean remains seemed to comprise the greatest part of the animal matter. Diatoms and animal remains were part of all contents examined. Very often the diatoms had been well digested so that only the shells were left. Recognizable vascular plant material occurred in 85%, dinoflagellates in 35%, and blue green algae in 35% of the stomachs. The figures for dinoflagellates and blue green algae may be considered as approximate.

While some of the diatoms in the stomach contents were decomposed suggesting they had been taken up by other crustacea which had fallen prey to the Neomysis, others were in remarkably good condition. It appears that N. mercedis feeds on such plankters as well as on larger organisms.

The animals in captivity are cannabilistic but incapacitated individuals are most often attacked. They were also observed to feed on dead sticklebacks in one of the aquaria. In the laboratory they obtained food from detritus material on the bottom of the aquaria. A supplemental food was supplied consisting of a ground-up mixture of dried plankton, dried daphnia eggs, and dried canned salmon. Micro-examination of the stomach contents of animals held in detritus free water for 21 days showed that this mixture of food was eaten.

TEMPERATURE RELATIONS

INTRODUCTORY REMARKS

The effects of temperature on animal and plant organisms were studied early in the annals of physiology. This was perhaps due to the fact that temperature experiments were relatively simple to carry out. Davenport (1908) has tabled the results of temperature experiments performed on some 85 types of tissue and animals by early workers. The striking thing about this table is the fact that time and again the upper lethal temperature of an organism is cited with no mention of the method used to determine this temperature. Jacobs (1918) states that the earlier workers were interested chiefly in the so-called upper thermal death point, and that they overlooked the time factor in their work. The ability of animals to acclimatize themselves to temperature changes causes a difficulty in the determination of their thermal tolerance which was overlooked by these workers.

It is now generally realized that the thermal history of most organisms is of the utmost importance with regard to their tolerance to heat and/or cold. This fact is exemplified by Davenport's experiment with Bufo tadpoles (Davenport, 1908). Those tadpoles maintained at 15°C went into heat rigor at 40.3°C. If kept at 25°C for 28 days heat rigor was not produced until the temperature had been raised to 43.5°C. Loeb and Wastenays (1912) found that Fundulus maintained at

10°C. were killed in a few minutes at 35°C., whereas *Fundulus* kept at 27°C. for 40 hours were able to endure 35°C. temperatures indefinitely. Results of experiments of this nature are given by Hathaway (1927) or Behr (1918).

To overcome the effects of time and acclimation a method may be employed wherein the animals being tested are subjected suddenly to higher or lower temperatures and the time to death noted.

The method used here is of this nature, being patterned after that used by Brett (1941) and Fry, Brett and Clawson (1942).

EFFECT OF ABRUPT TEMPERATURE CHANGE

Method

The *Neomysis* used in the temperature experiments were taken from the Nicomekl and Serpentine rivers. Individuals of both sexes and of all sizes were used.

The crustaceans, on being brought to the laboratory, were placed in constant temperature (±0.5°C.) aquaria. In these aquaria they obtained food from a small amount of river bottom sediment, by feeding on dead and dying *Neomysis* or from a mixture of daphnia eggs ground up with dried canned salmon.

From the water at constant temperature (acclimation temperature) groups of shrimps were transferred to 3000 ml. beakers containing 1000 ml. water. The water was maintained at various test temperatures by placing the beakers in constant temperature aquaria. Thermoregulators maintained the water temperatures constant within ± 0.5°C. The water in the beakers and in the aquaria was agitated by using air-breakers connected to the compressed air line. This served to keep the temperature throughout the aquaria and beakers uniform. For temperatures below that of running tap water three refrigeration units were available. Mortalities were checked, when possible, every 2 hours during the first two days of the experiments. Where several mortalities occurred overnight the times to death were interpolated, consideration being taken of the condition of the animals at the last observation.

Results

Tables V, VI, and VII present the results for the temperature experiments. In the left hand column are listed the test temperatures to which the organisms were subjected. The vertical lines to the right of this column, numbered 0 to 100 from left to right indicate percent of the individuals dying at the various test temperatures; (reading from right to left indicates percent survival). The numbers in the columns represent the hours elapsed from the start of the experiment to the time of death of that percentage of individuals as indicated by the column. For example, in Table V at a test temperature of 1.8°C. the number 21 is seen in the first column with a small vertical red mark about 2/3 of the way across the column. This indicates that for Neomysis acclimated to 6°C., about 6% (indicated by small vertical red mark) of those subjected to a test temperature of 1.8°C. died or 94%

were surviving in 21 hours.

This type of table records the whole picture regarding times to death in various temperatures. It will be noted that in the tables the percent of animals dead or surviving was not always recorded at 48 hours, this occurred because it was not always possible to take readings at these times. In such cases the percent survival was interpolated. Figure 5 represents these data graphically for the survival at 48 hours.

Figure 6 was drawn from the same data as fig. 5. Fry, Brett and Clawson (1942) used this method in describing the thermal tolerance of goldfish. For trout Brett concluded that if the fish could stand a certain temperature for 14 hours they could tolerate it "indefinitely". Temperatures which he called lethal were those which killed 50% of the fish during a period of 14 hours.

This same general procedure was adopted for Neomysis although it was found that there was no time beyond which the crustaceans would live "indefinitely". It was difficult to maintain the animals in the laboratory and even in the controls there was considerable mortality. (Tables V, VI and VII)

In the Neomysis experiments, 48 hours was arbitrarily chosen as the time period upon which to base the lethal temperature determinations. Thus the temperature at which 50% of the Neomysis died at 48 hours after being acclimated to a given temperature is taken to be the "lethal temperature" for

TABLE V -- TIMES OF SURVIVAL OF NEOMYSIS FROM 6°C. ACCLIMATION IN VARIOUS TEST TEMPERATURES. (DESCRIPTION IN TEXT.)

Test Temp. °C.	% DEAD 0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	
	% ALIVE 100–90	90–80	80–70	70–60	60–50	50–40	40–30	30–20	20–10	10–0	
1.8	21		48	64		87		192			
		27	48	65		83		185			
3.0	50	61		95							
10.0	49	59	70								
13.0	40	65		94							
19.8	1	3	46	73					192		
	3	22	46		73					192	
21.0	21	25½	45	51					72	192	
23.0		21	25		33		45	51	72	192	
Controls	45	72	100		126	142	192				
	71	100		142		192					

TABLE VI -- TIMES OF SURVIVAL OF <u>NEOMYSIS</u> FROM 14°C. ACCLIMATION IN VARIOUS TEST TEMPERATURES. (DESCRIPTION IN TEXT.)

Test Temp. °C.	% DEAD 0–10 / % ALIVE 100–90	10–20 / 90–80	20–30 / 80–70	30–40 / 70–60	40–50 / 60–50	50–60 / 50–40	60–70 / 40–30	70–80 / 30–20	80–90 / 20–10	90–100 / 10–0
2 *[1]		½	21	30, 46						
		½	1½	21	33	73				
5	51	70		105						
3.6	45		70		105					
18		54		101	119					
	61		93							
22	6½	30½		47						
23	4	6	24	31	53			70	93	117
	2		20		47			73		93, 111
24 *[2]	1		22		58	70				
*[2]		22	25	33		46				70
	6		17	21	44	64	87		124	
	5	14½	26		48	60	87			124
25					20			30	40	68
	3		16	17	20½	24		40		
		20		30				40	50	68
26	1	1½	14		27	43				
	16	18	20	24				40		

TABLE VI -- CONTINUED

Test Temp. °C.	% DEAD 0–10 / % ALIVE 100–90	10–20 / 90–80	20–30 / 80–70	30–40 / 70–60	40–50 / 60–50	50–60 / 50–40	60–70 / 40–30	70–80 / 30–20	80–90 / 20–10	90–100 / 10–0
27	1½	2 2½	4	4½	5					16
Controls	54	74		95	124					
	60	81		103						
	61	76		120	131					

*[1] all were extremely inactive

*[2] temperature went up to 26°C. for several hours. These results ignored when plotting graph.

TABLE VII -- TIMES OF SURVIVAL OF NEOMYSIS FROM 20°C. ACCLIMATION IN VARIOUS TEST TEMPERATURES. (DESCRIPTION IN TEXT.)

Test Temp. °C.	% DEAD 0–10 / % ALIVE 100–90	10–20 / 90–80	20–30 / 80–70	30–40 / 70–60	40–50 / 60–50	50–60 / 50–40	60–70 / 40–30	70–80 / 30–20	80–90 / 20–10	90–100 / 10–0
1.8									½	
3.0	10		24		48		71			
4.7		24	48	57			93	117	165	
		124	48	51	71	80	112			
7.6	53	71		112						
14.0	53		74	87						
21.3	27	45		71						
24.0		6		21		47	51	70		144
24.8	10			24				48		60
24.8	7	9			12	24	31	48 60		120
25.6		1			21				48	57
25.6	1		21			29			48	57
27.2		¼								18
Controls	63		79 110		129	165				
	60	70	81	114	140					

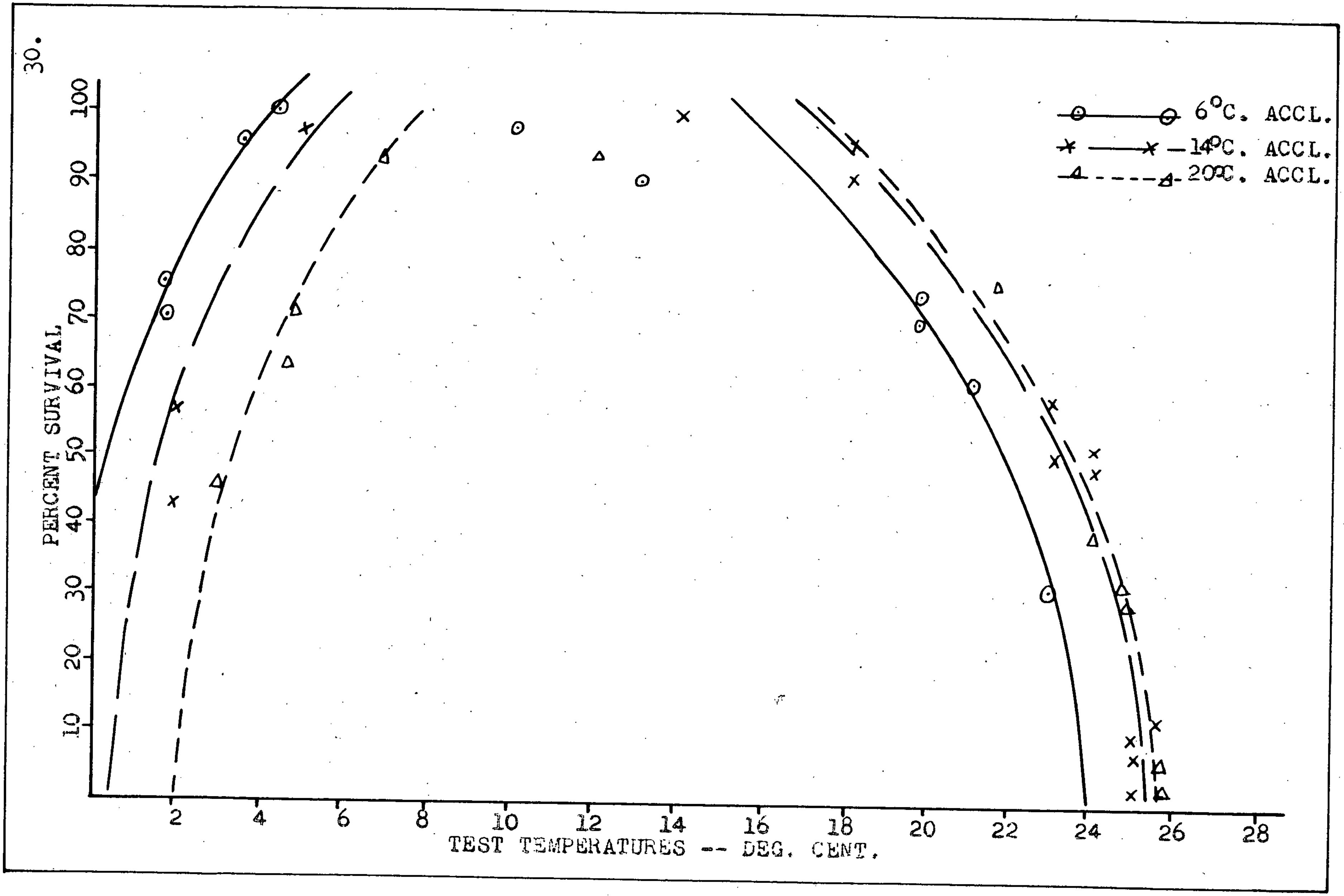


fig. 5. Percent survival in various temperatures of <u>Neomysis</u> acclimated to 6°C., 14°C., and 20°C.

30.

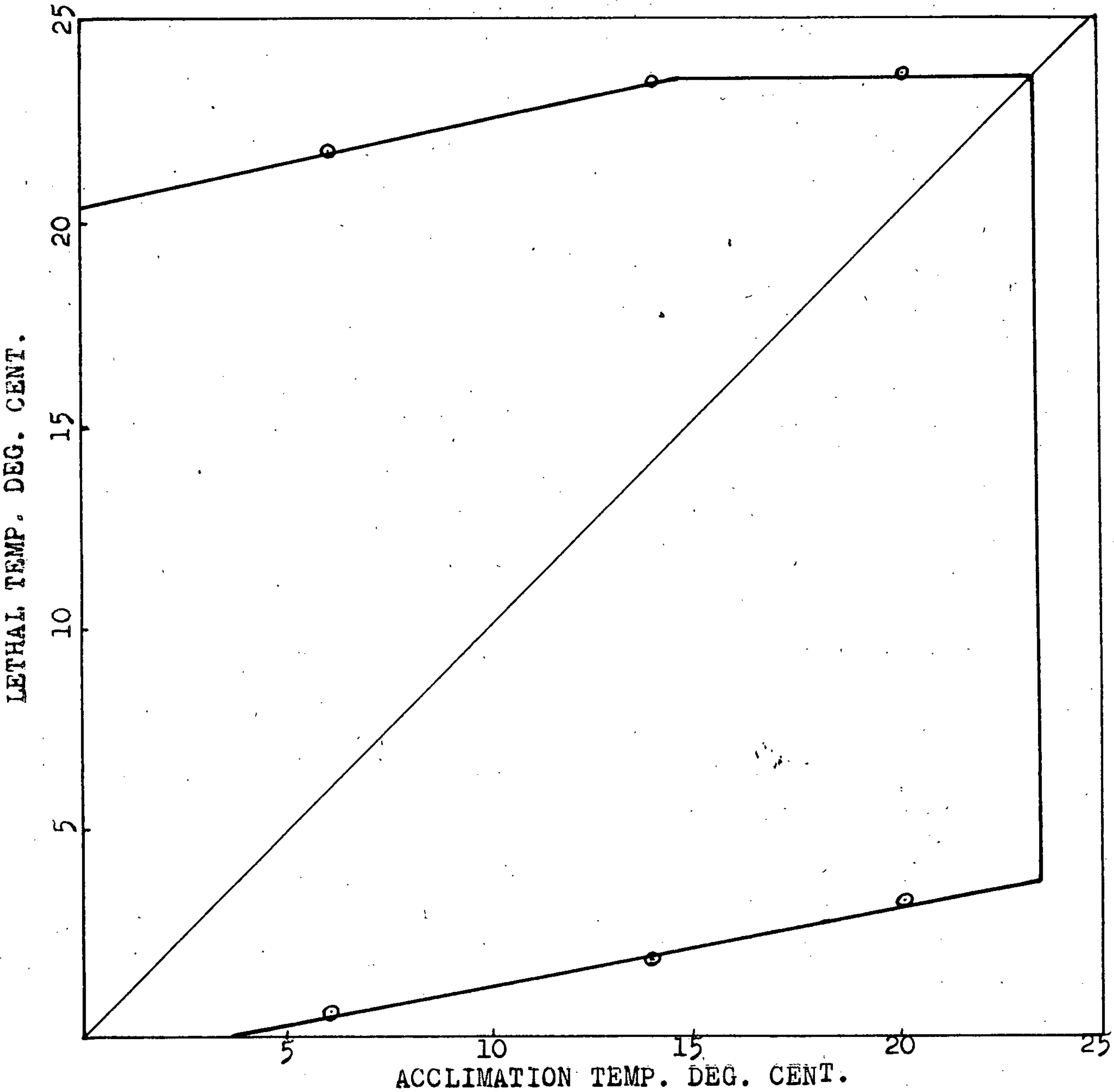


fig. 6. Thermal tolerance of Neomysis. (Description in text.)

these animals at this particular acclimation temperature. The period of 48 hours was considered ample since these animals characteristically exhibit diurnal migrations and even though they inhabited the upper warmer strata of lakes or rivers periodically, the length of time spent there would be but a fraction of 48 hours.

In fig. 6. lethal temperatures are plotted against the acclimation temperatures. According to Fry (1947) the diagonal line represents the point where both the lethal and acclimation temperatures are the same. The place where the upper lethal temperature line crosses this diagonal indicates the limit of acclimation to the higher temperatures. Since acclimation can proceed no further in this direction a perpendicular may be dropped from this point to the lower lethal temperature line. Had the lower lethal line run into the diagonal then a perpendicular could be raised from the intersection to the upper lethal line. However, water becomes ice at a temperature above the theoretical ultimate lower lethal level so that the actual lower level is at 0°C. or just slightly above. The resultant area, enclosed by the axes, the upper and lower lethal lines, and the perpendicular on the right, indicates the range of temperatures that Neomysis can tolerate. The area may be measured in Centigrade units of one square degree. Thus the thermal tolerance of these crustaceans can be stated as being 491 Centigrade units (or square degrees C.), with the upper lethal temperature limit 23.6°C. and the lower lethal temperature limit of 0°C. Fry, Hart and

Walker (1946) found a value of 625 units for yearling speckled trout, *Salvelinus fontinalis*, and Brett (1944) found 1160 units to be the thermal tolerance of the bullhead, *Ameirus nebulosus*.

It is realized that the thermal tolerance of *Neomysis* may vary with sex but it is considered that such deviations would not be of sufficient magnitude to affect the value of this figure.

EFFECT OF GRADUAL TEMPERATURE CHANGE

Method

Fifteen to twenty *Neomysis* were placed in 1000 cc. water in 3000 cc. beakers which were kept in water baths. The low temperatures were maintained by refrigerator units and the upper temperatures by immersion coils--thermoregulated to ±0.5°C. Three tests were carried out. In one the temperature was raised 1.0°C. per day, in another 1.5°C. per day, and in the third 3.0°C. per day. Figure 7 illustrates the results in presenting the time and temperature for 50% survival at the various rates of temperature increase.

Results

The graph indicates that individuals subjected to a rising temperature of 3°C. per day survived a higher temperature than those subjected to 1°C. per day increase. This would suggest that the rate of acclimation was greater than 3°C. per day for individuals maintained at approximately 7.5°C. If acclimation had proceeded at a rate slower than this then animals with environmental temperatures rising at 1°C. or 1.5°C. per day should have survived higher temperatures than those whose temperature was raised more quickly.

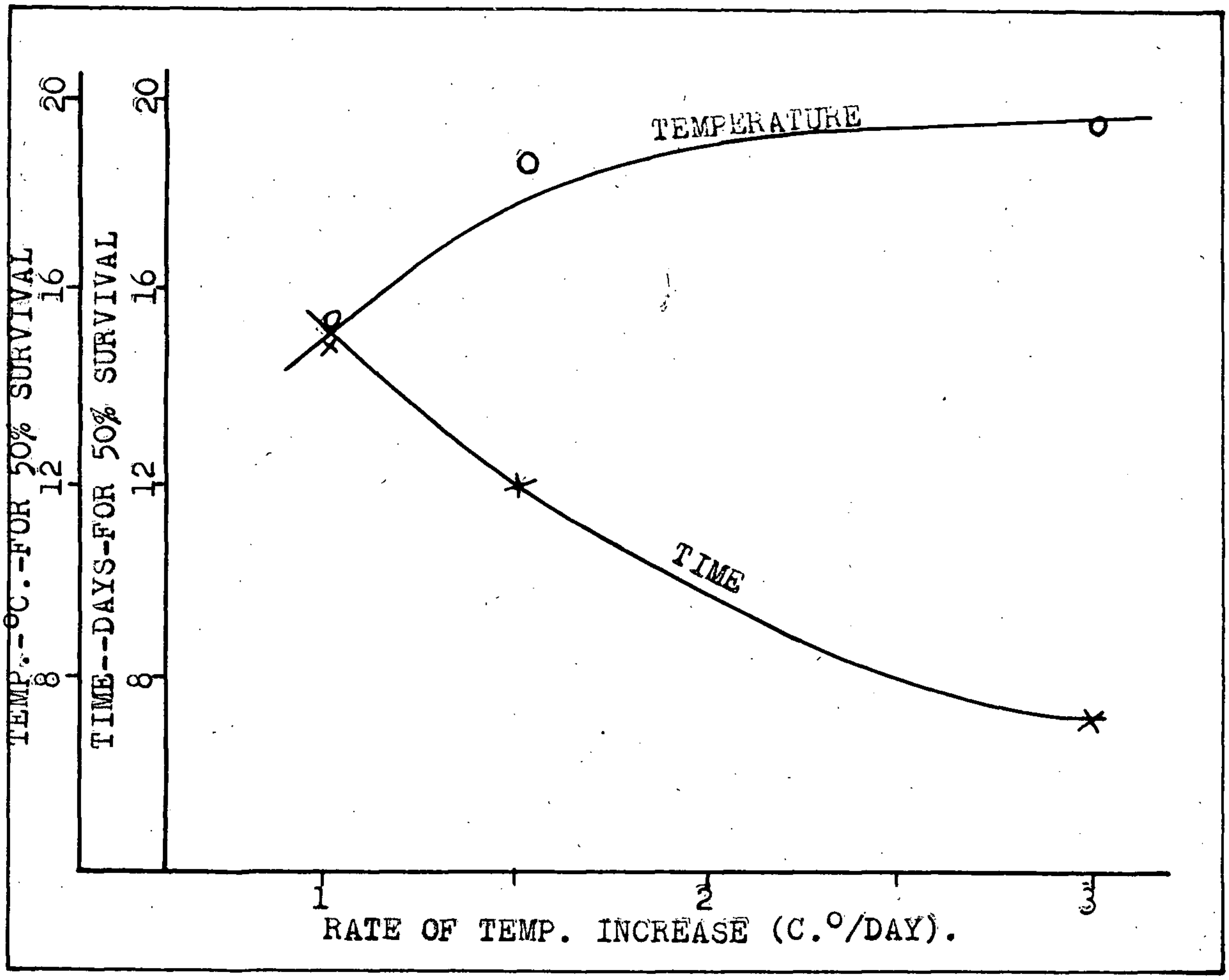


fig. 7. Time and temperature for 50% survival of Neomysis undergoing various rates of temperature increase.

EFFECT OF SIZE ON THERMAL TOLERANCE

In order to determine whether age affected the survival ability in various temperatures, note was taken during several of the tests of the size of the animals and the order in which they perished. The data collected are represented in Table VIII. From these data the average sizes of the first 50% and the last 50% dying were determined and the standard error of the difference of the means of these two was calculated.

Results

The results indicate that there is a significant difference (at the .05 probability level) between the sizes of Neomysis dying first and last in high temperatures. The indications are that smaller animals are more resistant; however, significance is not shown at the .01 probability level which indicates the necessity of a repetition of this experiment.

TABLE VIII -- DATA AND CALCULATIONS FOR DETERMINING EFFECT OF AGE OF NEOMYSIS ON UPPER THERMAL TOLERANCE.

TEMP. °C.		SIZE (MM.) IN ORDER OF DEATH			
ACCL.	TEST	1st DYING		LAST DYING	
			AVERAGE		AVERAGE
6	21.8	9.0,11.0,9.0 10.0	9.75	11.0,9.0,8.0,6.0	8.50
14	23	9.0,9.0,10.0	9.33	8.0,6.0,8.0	7.33
	23	6.0,10.0	8.00	9.0,11.0	10.00
	24	11.5,11.0, 9.5,10.0	10.50	6.0,9.5,9.5,7.0	8.00
	24	10.0,11.5	10.75	10.0,9.5	9.75
	24	10.0,7.0, 10.0,10.0	9.25	9.0,9.0,10.0, 6.0	8.50
	25	12.0,9.0,11.0 10.0, 11.0	10.60	8.0, 10.5, 9.0, 10.0, 10.0	9.50
	25	8.0, 9.0, 11.0, 8.0	9.0	7.5, 8.0, 10.0, 10.0	8.88
20	24.8	10.5, 11.0	10.75	8.0, 6.5	7.25
		Av. Total	87.93		77.71
		$\bar{x}$ =	9.77	$\bar{y}$ =	8.63

S.D.$\bar{x}$ = .9023

S.D.$\bar{y}$ = .8676

Dm. = 1.14

S.E.$\bar{x}$ = .3007

S.E.$\bar{y}$ = .2892

S.E.D. = .417

t = 2.733

SALINITY RELATIONS

INTRODUCTORY REMARKS

In order to exist, aquatic animals must maintain their body fluids at the same concentration as the external environment or else utilize some means of osmoregulation. Many forms have very limited powers of osmotic control. Others, those which move from salt to fresh or brackish waters, have well developed means for maintaining body salt balance in changing environments. For example the crayfish, which can tolerate a range of salinities, does so by producing large volumes of hypotonic urine, and by active salt absorption through the gills. The marine crab, *Eriocheir*, which can exist in fresh water produces isotonic or hypertonic urine but has a very low salt and water permeability. It can also absorb chloride ions through the gills (Prosser, 1950). Between these two methods there is a range of specialization from high to low impermeability, from isotonic to hypotonic urine production, and from small to large powers of salt and/or ion absorbtion or retention.

In conjunction with these diverse methods, various animals employ different organs for osmoregulation. Marshall and Smith (1930) have shown that the kidney of fresh water teleosts is important in osmoregulation. The anal gills of the larvae of the mosquito, *Aedes aegypti*, are claimed by Wigglesworth (1933) to function as osmoregulators by absorbing water. Schlieper and Herrmann (1930) and Schlieper (1930) believe that the body surface of the crabs, Potamon fluviatele and Eriocheir sinensis, is an organ of osmoregulation. These workers have also produced evidence of the importance of the excretory organs in this function. Lienemann (1938) credits the green glands (antennal glands) of Cambarus clarkii with playing a part in osmotic regulation. This same organ is claimed by Samuel (1945) to be the most important structure for osmoregulation in the decapods. This conclusion is opposed by Pannikkar (1941) who claims that in the prawns, at least, the gills bear most of the burden for maintaining osmotic balance.

Although the present study is more concerned with salinity tolerance of Neomysis than with the physiology of the osmoregulatory function some familiarity with the organs supposedly concerned may be desirable.

In general the organs claimed to be active in osmoregulation are gills, excretory organs and skin (external covering). As far as can be determined Neomysis lack gills, which leaves but the skin (chitinous exoskeleton) and the excretory organ (antennal gland) to be considered. This latter structure could not be seen by dissection methods so longitudinal and transverse serial sections were made which were sufficient to provide a brief description of the histology of the organ.

HISTOLOGICAL EXAMINATION

Method

Because the animals possess a chitinous exoskeleton and since the antennal gland is located near the anterior end of the animal only the anterior half of the animal was sectioned. This made for easier and better penetration of fixative and wax. Fixation was in 5% formaldehyde, embedding was carried out by the dioxan wax process, the sections were stained in Harris's haematoxylin and eosin, and mounted in Canada balsam.

A rough reconstruction of the gland was obtained in the following manner. Each of a series of longitudinal sections of the gland was drawn on separate sheets of thin tracing paper. The outline of the gland in each drawing was then inked, the sheets were soaked in xylol and piled up over a lighted tracing table. The xylol made the paper transparent so that the inked outlines of the gland could be oriented in the correct position on top of one another. With the drawings stacked, 3 holes were punched through from top to bottom to serve as points of reference on each sheet of paper. A contour drawing of the gland was then made by tracing the outlines onto a sheet of tracing paper as though the sections of the gland were superimposed on each other. The three holes were used to orient the drawings for this procedure.

Reconstructions of the antennal gland by this method were made for both Neomysis and Mysis relicta.

The reconstruction by the described method of the antennal gland of <u>Mysis relicta</u> gave results closely comparable to those of Vogt (1933) whose work was not obtained until after the above reconstruction was finished. It appears that this simple method is reasonably reliable.

<u>Results</u>.

The antennal glands are paired organs lying laterally in the antero-ventral region of the animal. They are located ventral and lateral to the stomach and extend forward from a position half way along this organ into the bases of the antennae.

The gland was found to be composed of three parts, an end sac, a convoluted tubular portion, and a bladder which opens to the exterior through a pore in the base of the antenna. The parts of the gland can be distinguished by the structure of the walls. In the end sac the walls are thick and infolded, in the tubule they are thin and in the bladder they are thick with no invaginations (fig. 8.). In all parts the nuclei are very prominent, being large, oval and stained deep blue. Cell outlines are not apparent, the walls of the organ appearing to be formed of a homogeneous red staining tissue with nuclei scattered randomly throughout.

Some tissue resembling that of the antennal gland was found just under the outer integument in a region under the carapace (fig. 9.). This tissue may be compared with the section of antennal gland seen in fig. 10.

The antennal glands in M. relicta and Neomysis are similar in arrangement and form. The gland in Neomysis however appears to be somewhat larger and the convoluted tubule more coiled than in M. relicta.

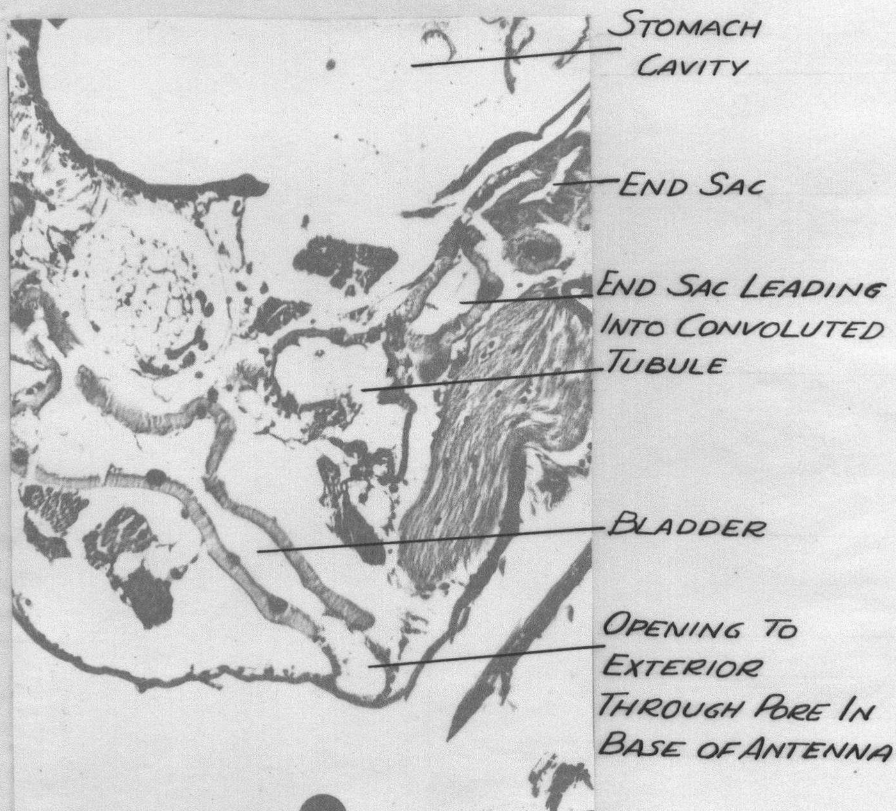


fig. 8. Cross section showing 3 regions of left antennal gland of N. mercedis. X 150.

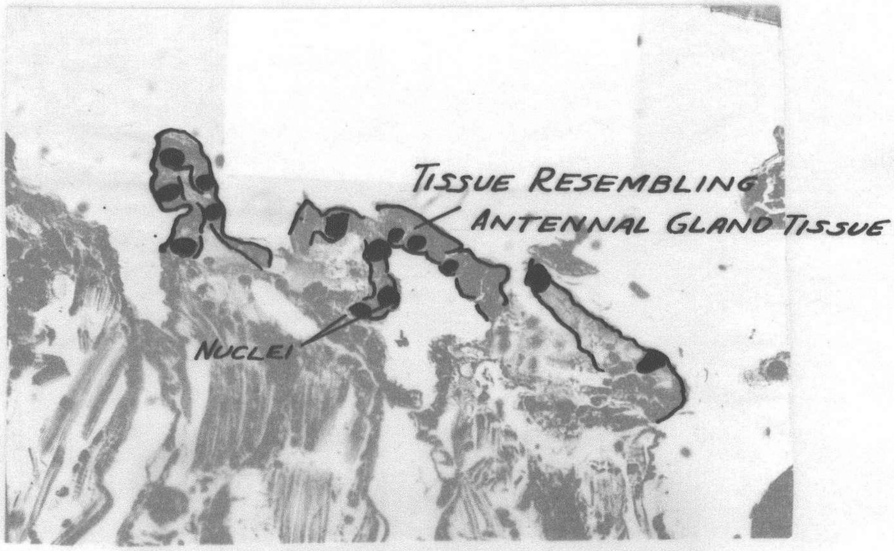


fig. 9. Longitudinal section showing tissue in dorsal region of N. mercedis resembling antennal gland tissue. X 200.

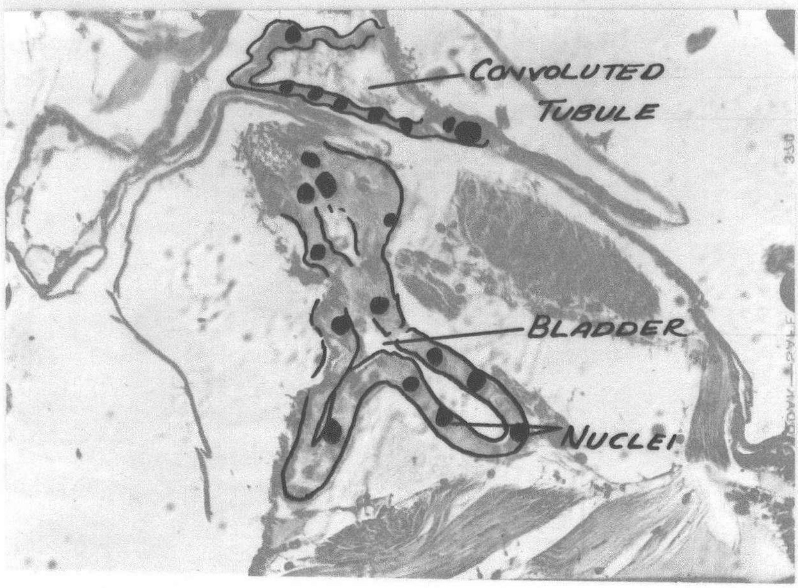


fig. 10. Longitudinal section through bladder and part of convoluted tubule of antennal gland of N. mercedis. X 200

EFFECT OF ABRUPT SALINITY CHANGE

Method

Beakers containing water of chlorinities 0.29 o/oo, 0.70 o/oo, 1.11 o/oo, 3.15 o/oo, 5.19 o/oo, and 10.33 o/oo (control) were set up in a constant temperature water bath, at 5°C. Ten to twelve Neomysis from a chlorinity of 10.33 o/oo were placed in each beaker and the times to death noted. When this experiment was repeated the same chlorinities were not used but a comparable range was established. Essentially similar results were obtained.

Results

Fig. 11 represents graphically the results of this experiment. The survivals at various times (5, 7, 9,and 15 days) are plotted against the test chlorinities. Survival was irregular (note curve for 5 days) till about 7 days. At this time greatly increased mortality occurred in the lower salinities. As time progressed mortalities in higher salinities occurred (curves moving to left). This would seem to indicate that a chlorinity of approximately 1 o/oo (bend of curve for 7 days) exerts a lethal effect on Neomysis from 10.33 o/oo chlorinity. Continued exposure to chlorinities somewhat higher than this will also cause death but because of the time taken for death to occur it is suggested that factors other than low salinity may be contributing to the effect.

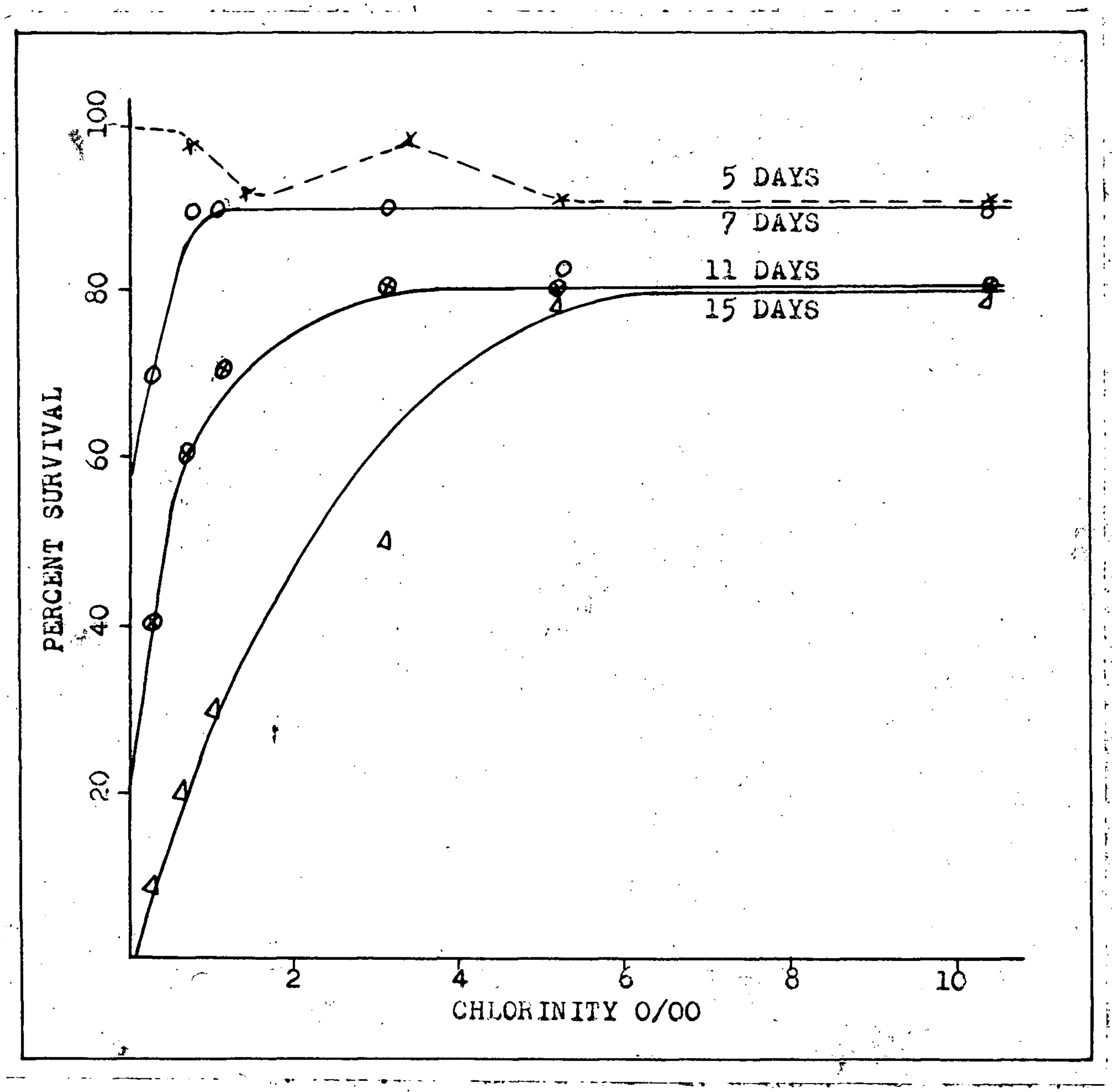


fig. 11. Percent survival of <u>Neomysis</u> (from 10.33 o/oo chlorinity) exposed to a range of chlorinities.

EFFECT OF GRADUAL SALINITY CHANGE

Method

Fresh water was siphoned slowly from a 2000 cc. Erlenmyer flask into a beaker containing 10 to 12 crustacea in environmental water. The flow was regulated by clamping the rubber siphon hose; it could be cut down to about 15 drops per minute. When the Erlenmyer was emptied (it took approximately 12-18 hours) a water sample for chlorinity test was taken from the beaker, the Erlenmyer was refilled and the experiment continued.

Results

Figures 12 and 13 depict the results of the gradual salinity change experiment. Here the rate of decreasing chlorinity and the rate of mortalities of *Neomysis* are shown together. In one case (fig. 13) the initial drop of chlorinity was greater but in both instances greatly increased mortality occurred after the chlorinity had dropped to about 1 o/oo. Fig. 12 indicates some mortality (up to 40%) even in the higher salinities. A suggested explanation of this is that the animals used had been kept in the laboratory about five weeks. The condition of some of them may thus have been affected causing a greater susceptibility to environmental change. Notwithstanding this effect it is noted that a greatly increased rate of mortality occurred after the chlorinity had dropped to about 1 o/oo.

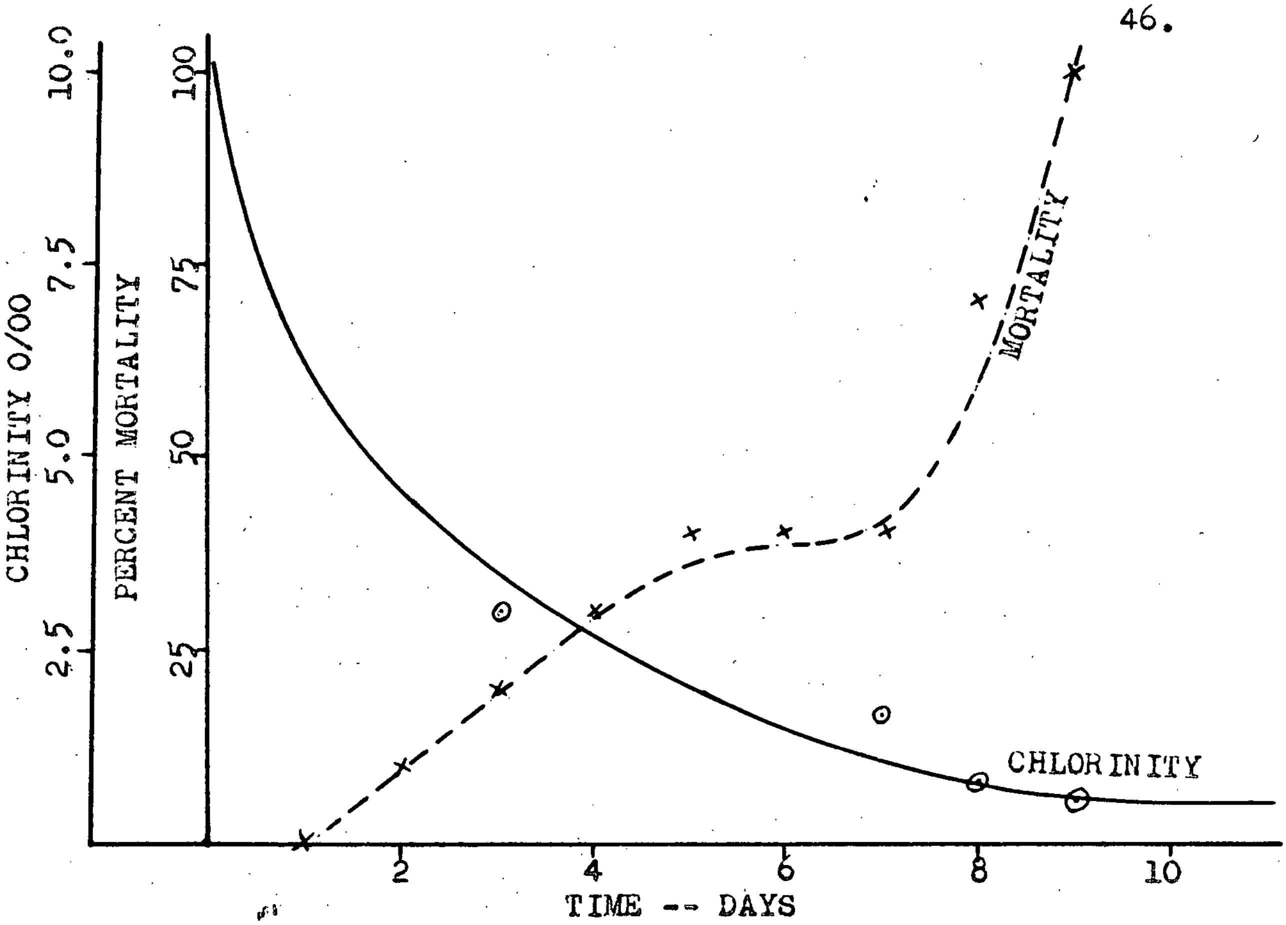


fig. 12. Curves showing rate of mortality increase with rate of chlorinity decrease.

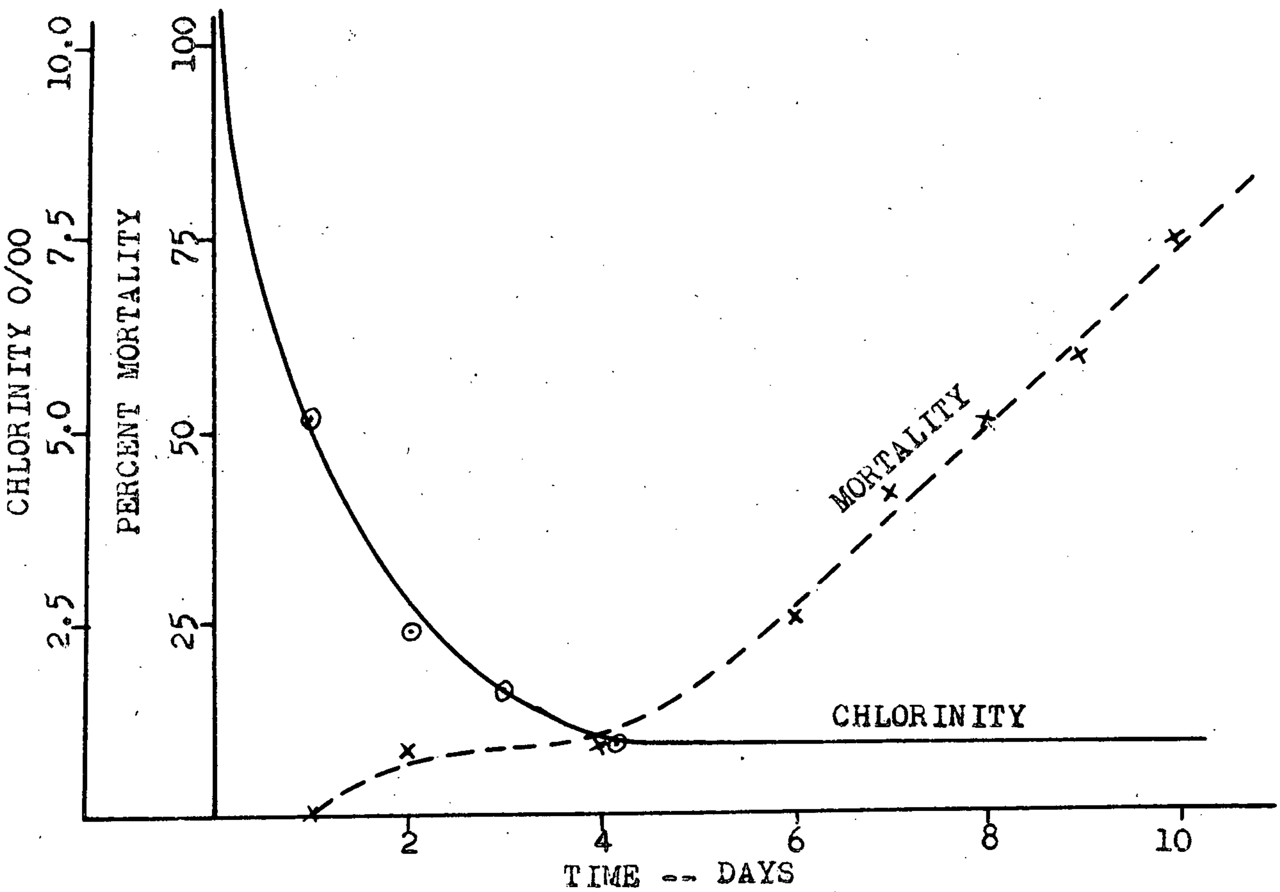


fig. 13. Curves showing rate of mortality increase with rate of chlorinity decrease.

EFFECT OF CHANGE FROM VARIOUS SALINITIES INTO FRESH WATER

Method

Collections on March 17 were made at the mouth of the river (in Mud bay --3.63 o/oo cl.), a short distance upstream (1.69 o/oo cl.), 3 to 3½ miles upstream (chlorinity negligible), 4¼ miles upstream (chlorinity negligible), and 9¾ miles upstream (chlorinity negligible). Individuals from each of these locations were placed in beakers of fresh water and the times to death noted.

Results

The bargraph in fig. 14 indicates the results of the test. Repetition produced similar results.

It is shown that the animals collected farther up the river survived longer in fresh water. This indicates the possibility that these animals may be conditioned to lower salinities. Such conditioning presumably occurs over a fairly long period of time if the results of the other salinity tolerance experiments are accepted. It will be remembered that the crustaceans showed no tolerance to chlorinities lower than 1 o/oo even when the salinity was decreased gradually.

SALINITY PREFERANCE

The experiment is designed to show whether or not the crustaceans show attraction to or avoidance of fresh water.

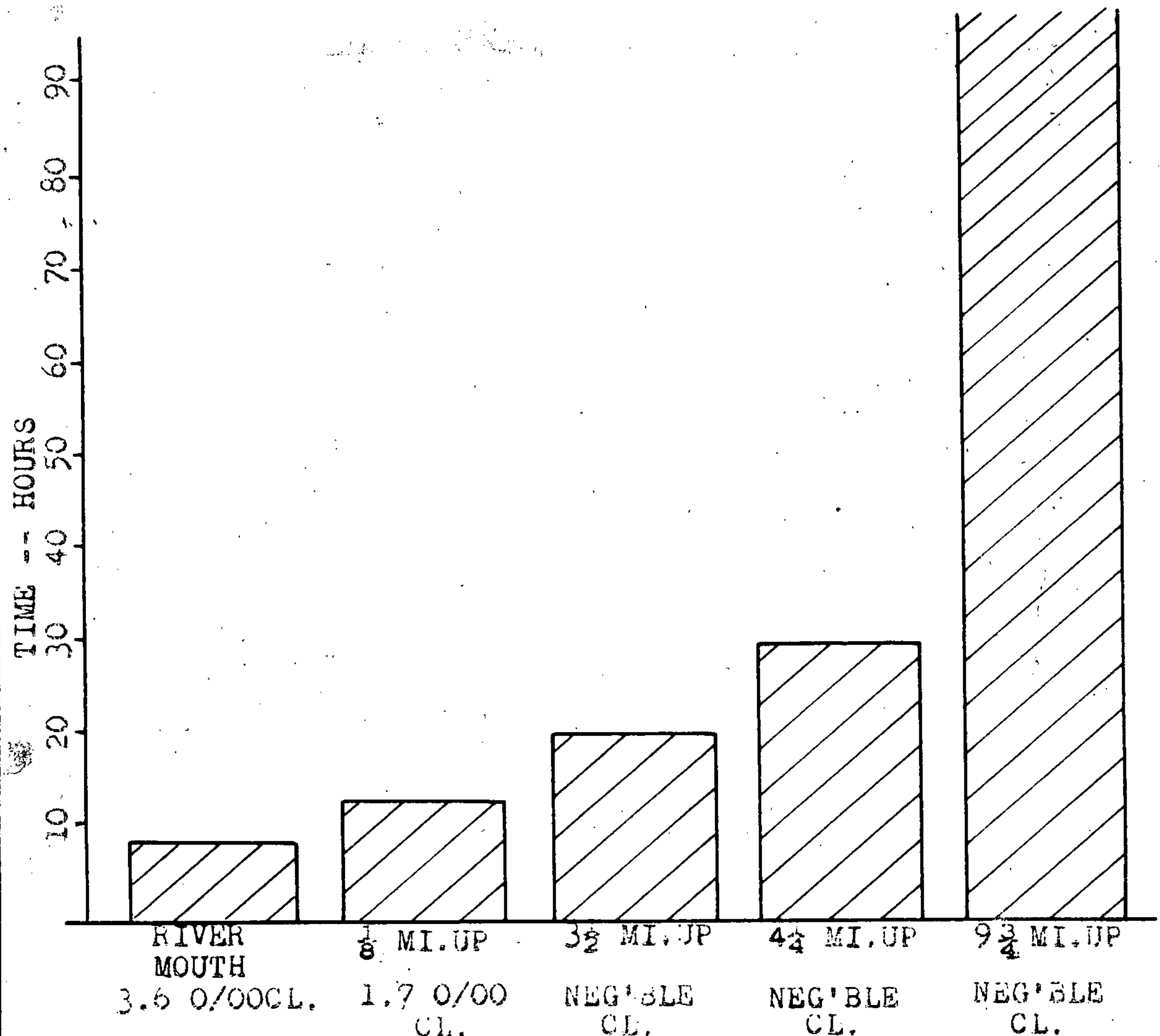


fig. 14. Time to 50% mortality of Neomysis mercedis from various locations when put in fresh water.

Apparatus

The apparatus was similar to that used by Erickson Jones (1949) in his experiments on the reaction of fish to toxic substances. It consists of a glass tube (24" long x 1" dia.) with an inlet in each end and 2 outlets about 2" apart in the middle. Two large jars are used as resevoirs from which the solutions to be used are siphoned. One jar contained environmental salt water and the other fresh water. The salt water ran into one end of the tube, and out one of the outlets in the middle. A similar course was followed by fresh water entering from the other end of the tube. This arrangement served to provide 2 separate types of water with mixing occurring only in the middle of the tube.

Method

Neomysis were introduced into the apparatus and salt water was run in from both ends. Half an hour later observations of the positions of the animals were begun and carried out every 2 minutes for 20 to 30 minutes. The salt water was then shut off from one end and fresh water run in instead. After half an hour observations were again carried out. Finally this arrangement was reversed so that the salt and fresh waters were made to flow in from the other ends. Observations were again made after a period of 30 minutes.

The period of half an hour was allowed before taking observations in order to let the animals settle down. Note was taken of the number of Neomysis in each end and in the middle of the tube. The "middle" comprised that region between the outlets where mixing occurred. By injecting a small amount of methylene blue into the inlets a comparison of the rate of flow from each end was obtained. The dye also served to indicate whether or not layering of fresh on salt water occurred. Since no stratification was observed when the tube was less than half full, all tests were made under this condition.

Results

The results of this experiment indicated that N. mercedis from the mouth of the Nicomekl river (chlorinity 10.33 o/oo) showed no preference for fresh or salt water. At least there was no tendency to move toward or away from fresh water. The experiment did demonstrate that the crustacea are positively rheotaxic. It was noted that when the animals were in a current of water they moved against it, consequently the greatest concentrations of animals were found most often at the extreme ends of the apparatus (the inlets were in the ends). If the flow was cut off, the animals at that end of the tube would gradually spread out so that there would be more or less even distribution throughout a length of the apparatus. Starting the flow from one end caused an eventual concentration of animals at that end. Fig. 15 indicates the altered distribution in the apparatus caused by switching the inflow from one end to the other.

This diagram represents the results of one of the trials. The experiment was repeated four times with comparable results. In the test described animals happened to be almost evenly distributed at the start of the experiment. (In some trials an uneven distribution occurred at the start, it then remained this way until the flow was altered.) The even distribution remained until the 8-minute mark when the flow was stopped at the right end. By 16 minutes the animals were distributed in the middle and left end of the tube. At 18 minutes the flow was started in the right end and stopped in the left. By 26 minutes some of the animals had moved back across the tube to the right end. The trial was discontinued after 26 minutes but the trend towards the right end is evident at this time.

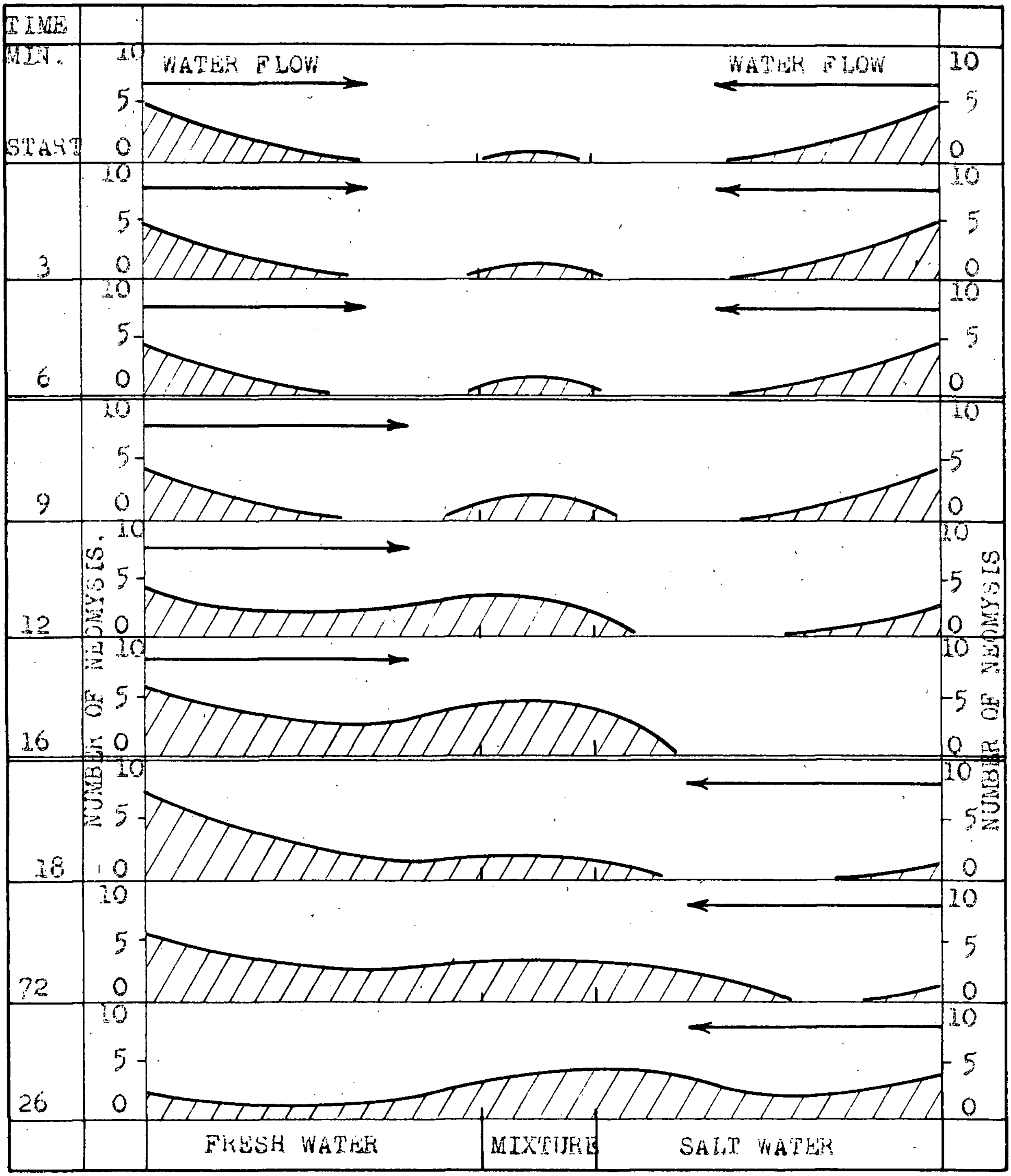


fig. 15. Chart indicating distribution of N. mercedis (cross hatched areas) in apparatus with change of water flow.

Additional description in text.

DISCUSSION

The distributions of *N. mercedis* on the Pacific coast of North America indicates the extent of euryhalinity exhibited by this species. It might be compared with *Neomysis vulgaris* which is also a brackish water mysidacean widely distributed in European waters. This species has been taken in the open sea and Elton (1936) found the species in loch Barvas More, a shallow, fresh water lake close to the sea in the Isle of Lewis, Outer Hebrides. He reports that the crustacea are well established and in sufficient numbers to be an important trout food.

The fact that *N. mercedis* is found in the Nicomekl and Serpentine rivers from the mouths upstream to regions of fresh water raises the question of how and why such a distribution occurs. To adequately answer the question of "how" will require an extensive study of the physiology of the animal's osmoregulatory and locomotory mechanisms. Some contribution to the problem of "why" has been brought out by this study.

Salinity experiments and the distribution of the species show it to be capable of a high degree of osmoregulation, other salinity experiments demonstrate the rheotaxic tendency of the crustaceans, and their inability to distinguish fresh from salt water.

The ability to osmoregulate allows the animals to penetrate waters of low salinity and rheotaxis causes them to move upstream, which movement is not impeded by the presence of fresh water since in experiments they have shown no ability to distinguish it from salt water. The combination of these characteristics probably accounts for the distribution of Neomysis from the salt water of Mud bay to the fresh waters of the Nicomekl and Serpentine rivers.

It was remarked that Neomysis were noticeably more abundant in October and November than in any of the following months in which collections were made. Several explanations may be offered.

The collections of October and November were predominantly of females carrying developing young in their brood pouches. These collections were made in locations sheltered from the direct force of the river current. It is suggested that females congregated in these sheltered areas temporarily to release the young. Tattersal (1938) suggested that in some species of marine Mysidacea, breeding females rose to the surface when young were ready to be liberated.

Another explanation is based on the fact that precipitation and runoff is much greater in the fall and winter. This effect combined with that of high tides during these seasons raises the river level greatly so that collections were hampered. Since collections were made by using a dip net on the end of a six foot pole, high water prevented the collector from reaching areas which had hitherto proven well populated. Although this factor may have contributed somewhat it is concluded that there was an actual decrease in the population either through death or translocation. In support of this view is the fact that a bottom drag net, operated in a location which had previously shown abundance of mysids with the dip net, yielded only a few individuals.

Further, the increased runoff greatly increases the amount of fresh water on top of the underlying layer of sea water above the flood gates. With this increased volume of fresh water there is a greater chance that Neomysis will move into it (experiment has shown they are not repelled by fresh water) where death will occur if they remain long enough. Even if they remain only till losing powers of motility they would then sink and be carried downstream on the ebbing tide.

While summer collections may throw more light on the problem of apparent decrease in abundance, the raising of the river level in the winter undoubtedly has an important effect.

According to the growth analysis the animals mature and breed in a year, some however probably live 1½ years to breed a second time. Indications are that two reproductive periods occur, one in the fall the other in the spring, so that two populations, half a year out of phase, exist together.

Since each female carries only 20 to 30 young and breeds but once a year the reproductive potential of Neomysis is low compared with other crustaceans. (The crayfish, Cambarus affinis, bears 200--400 eggs, Storer, 1943.) Even with this low potential the animals were very abundant in the Nicomekl during the early fall indicating they are particularly suited to their environment in the estuary of this river.

So far as can be discovered, no work has been done on the range of temperature tolerance of invertebrates which would be comparable with the results of this study. There are many records of the lethal temperatures of invertebrates but evaluations of overall thermal tolerance have been evaded. The method of treatment in this study was patterned after that used by Fry and his associates who worked mainly with fish. Comparisons can accordingly be made with their results. The thermal tolerance of Neomysis mercedis was found to be 491 units, and this may be compared with that of the speckled trout, Salvelinus fontinalis,--625 units (Fry, Hart, and Walker, 1946); the bullhead, Ameirus nebulosis,--1160 units (Brett, 1944); the goldfish, Carassius auratus,--1220 units (Fry, Brett and Clawson, 1942). In all cases the lower lethal temperature was 0°C.

By using this method a fairly clear picture of the temperature relations of animals may be presented. For the greatest clarity the upper and lower lethal temperatures should be stated along with the units of thermal tolerance, so that some idea of the animal's ability to acclimate as well as its resistance to temperature change may be obtained.

There was an indication that smaller individuals survived high temperatures better than larger ones. Fry (1946) found no significant difference in the abilities of small and large speckled trout, Salvelinis fontinalis, to withstand high temperatures. He suggests however that the fish he used were nearly of the same age. Gunter (1947) found that small anchovys, Anchoa mitchelli diaphana, and silversides, Minidia berylinna peninsulae, survived cold better than larger animals. Huntsman and Sparks (1924) report that the resistance of fish to temperature extremes diminishes as the size increases.

The reasons for this difference between large and small animals in surviving temperature extremes are as yet unknown, however, it is suggested that in the case of Neomysis the larger individuals are approaching the limits of their life span which may in some way account for their reduced resistance.

There has been a suggestion that Mysis relicta, the fresh water Mysidacean, will breed only at temperatures below 7°C. (Samter and Weltner, 1904). It is possible that a temperature limitation may similarly affect N. mercedis.

The histological examination showed a similarity between some subexoskeletal tissue lying under the carapace and that of the antennal gland. It is considered that the tissue under the carapace may be involved in respiration. Lang (1891) mentions that in some crustacea, including members of the Malacostraca, the inner wall of the carapace, remains soft and is used in the respiratory function. Since gills are reported to be active in osmoregulation, and since this tissue resembles tissue of the antennal gland which also is claimed to have an osmoregulatory function it is possible that both the gland and this suggested respiratory tissue are concerned in the osmotic mechanism of Neomysis.

Although no definite measurements were made, reconstruction of sectioned material and microscopic examination of the slides seemed to indicate the convoluted tubular portion of Neomysis antennal gland to be longer and more coiled than that of Mysis relicta. According to Krogh (1939) the opposite of this might have been expected. He reports, "it seems to be a general rule that the nephridial organs are better developed and have a longer nephridial canal in fresh water Crustacea than in related marine forms". (The terms 'nephridial canal' and 'convoluted tubule' both refer to the same part of the gland.) Perhaps the fact that N. mercedis is a brackish water species and capable of existence in fresh water accounts for its well developed nephridial canal. It is reasonable that in a form inhabiting an environment of wide salinity range the organ of osmoregulation should be fairly complex.

The salinity experiments indicated that Neomysis from a chlorinity of 10.33 o/oo could tolerate chlorinities down to about 1 o/oo while field observations have shown the animals to be present in fresh water. Other salinity experiments show that acclimatization to low salinities and therefore to fresh water must take some time to occur. This is perhaps not surprising considering the fact that the organs concerned in osmoregulation are suggested to be the antennal glands and the body surface, thus an increasing ability to regulate osmotically would likely be dependent on a gradual change in these structures.

This type of acclimatization where an individual can, over a period of time, adjust to environmental changes may be referred to as physiological acclimatization. It would seem that Neomysis can to some extent adjust osmotically in this fashion. However there is also genetic acclimatization which operates by selection and it is likely that this is the mechanism which allows those animals in the upper, fresh water regions of the Nicomekl and Serpentine rivers to survive. This would be associated with the rheotaxic tendencies of the crustaceans which cause them to move up into the regions of fresher water. The contention is that the rheotaxis causes the animals to move into regions of low salinity or fresh water where selection operates, producing a variety capable of existence in this environment.

Transferring Neomysis from various salinities to fresh water showed that even though taken from regions of negligible chlorinity those animals from such regions in the lower reaches were unable to survive in fresh water. Apparently Neomysis are sensitive to salinities lower than can be determined by chemical titration. (Beadle and Cragg, 1940, found that Gammarus duebeni, a brackish water species can survive so long as there is a trace of salt in the water.) This fact is important from a practical point of view. It indicates that <u>Neomysis</u>, although existing in water of salinity too low to be determined chemically, still may be killed in what is known as fresh water. Apparently the only way to determine whether certain water is lethal to the animals is by subjecting them to it; actually this was done with <u>Neomysis</u> from the location 9¾ miles upstream. They were put in a beaker of water from the fish pond on the west side of the University campus. In this environment 71% survived for 4 weeks, at which time the experiment was discontinued. It was considered that mortality after this time would not be due to the inability to adjust osmotically.

The following is a short discussion on the practical application of the findings in this study.

The fact that the crustacea feed on plant and animal material of a type which is available in fresh water indicates that food should be little or no problem to them in this type of environment. There is some indication that they are detritus feeders. If this is so then, could they be established in a lake, they would likely contribute much to the feed available for fish.

It has been suggested that if the animals feed largely on secondary feed (e.g. copepods, cladocerans) then introducing them would serve no useful purpose as far as increasing the food supply in a lake for fish. The argument is that such organisms add but another link to the food chain and actually reduce the weight of food material because of the energy expended in extablishing this extra link. However, from the fishes' viewpoint it would seem that much less energy is expended in obtaining sufficient food of this larger size than in acquiring the smaller organisms. Consequently more energy is available for growth.

Collections from the Nicomekl indicated that, compared with winter and spring, greatest abundance of *Neomysis* occurred in the early fall. Also noted was the fact that females were bearing young at this time of the year. These findings indicate that if collections are to be made for transplanting purposes then September and October likely constitute the best times for the collections.

It was suggested that the majority of animals live for a year and reproduce once during that time. Apparently some individuals survive the following winter (living 1½ years) and reproduce a second time in the spring. The largest number of eggs observed on a female was twenty-six. Indications are that females bear between twenty and thirty young. This appears to be a fairly low reproductive potential, consequently a lake stocking program would necessitate the introduction of large numbers of animals in order that the population may become established.

Temperature experiments indicate the upper lethal temperature of *Neomysis* to be 23°C. This may be compared with that of Kamloops trout fingerlings, (*Salmo gairdneri* *kamloops Jordan*, which Black (1951) found to be 24°C. for animals acclimated to 11°C. Fry, Hart and Walker (1946) found 25.3°C. to be the ultimate upper limit for Speckled trout, *Salvelinus fontinalis*. A comparison with temperatures in two oligotrophic lakes may also be made. Paul lake, a boarderline oligotroph, showed in August a surface temperature of 20°C. with a bottom temperature of 4.4°C., Larkin et al (1951). Kootenay lake, an oligotrophic type had a surface temperature of 19°C. and a bottom temperature of 4°C. in June.

It would appear that although the thermal tolerance of *Neomysis* is somewhat lower than that of trout its upper lethal limit is still above the temperatures of lakes in which it may be introduced.

The suggestion that temperature acclimation is fairly rapid should be considered if transplanting is undertaken. If there is a large difference of temperature (10°C. or more) between the place of introduction and location of collection, holding the crustaceans for a time at an intermediate temperature is advocated.

The work on salinity tolerance showed that *Neomysis* taken in the lower reaches of either the Nicomekl or Serpentine rivers are unsuitable for lake introduction. It was also indicated that conditioning these animals to fresh water would be a lengthy process and not practically feasible. However, it was demonstrated that animals from the upper reaches (8 or 9 miles upstream) could survive in fresh water thus indicating the obvious location for collections.

The foregoing indicates that Neomysis mercedis taken from the upper reaches of the Nicomekl or Serpentine rivers would be able to adapt themselves to conditions and survive in some types of fresh water lakes, and that once established they would add to the type of food sought by fish (mainly trout). It is realized, however, that living organisms do not always react according to the predictions made for them, and that man when tampering with the natural distribution of animals can too easily overlook facts which may prove vital. It is suggested, therefore, that before large numbers of Neomysis are "dumped" in a lake there be a preliminary introduction in an enclosed area so that an estimation of the success or failure of the proposed operation can be made.

SUMMARY

1. *Neomysis mercedis* was found to be distributed from salt to fresh water in two rivers.
2. It feeds on plant, animal and possibly detritus material.
3. Growth to maturity is suggested to take one year with some individuals living one and a half years. Breeding occurs in the fall and possibly the spring.
4. The thermal tolerance of *Neomysis* was found to be 491 units (in square degrees centigrade) with lower and upper lethal temperatures of 0°C. and 23°C. respectively. Temperature acclimatization was found to occur fairly rapidly.
5. Chlorinity of 1 o/oo was observed to be the lower lethal salinity level for *Neomysis* from water of 10.33 chlorinity. Acclimatization to salinity change was suggested to be a slow process.
6. *Neomysis* exhibited no preference for salt or fresh water.
7. *Neomysis* did exhibit a rheotaxic tendency.

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LITERATURE CITED

BANNER, A. H., 1951. Letter to author dated April 2, 1951.

BEADLE, L. C., and J. B. CRAGG, 1940. Studies on adaptation to salinity in _Gammarus_ spp. I Regulation of blood and tissue and the problem of adaptation to fresh water. J. Exp. Biol., _17_: 153--163.

BEHR, E. H., 1918. An experimental study of acclimation to temperature in _Planaria dorotocephala_. Biol. Bull., _35_: 277--317.

BLACK, E. C., and V. S. Black, 1951. Upper lethal temperatures for five species of British Columbia freshwater fishes acclimated at 11°C. Manuscript copy.

BORRADAILE, L. A., and F. A. POTTS, 1935. The Invertebrata. MacMillan Co., New York.

BRETT, J. R., 1941. Tempering versus acclimation in the planting of speckled trout. Trans. Am. Fish. Soc., _70_: 397--403.

1944. Some lethal temperature relations of Algonquin park fishes. Pub. Ont. Fish. Res. Lab., _63_: 1--49.

CHACE, F. A., Jr., 1949. Letter of June 3, 1949, to Dr. P. A. Larkin.

DAVENPORT, C. B., 1908. Experimental morphology. MacMillan Co., New York.

ELTON, C., 1936. Notes on fresh water animals in the Isle of Lewis, Outer Hebrides. Proc. Zool. Soc. London, for 1936, _2_: 939--944.

ERICHSEN JONES, J. R., 1947. The reactions of _Pygosteus pungitius_ L., to toxic solutions. J. Exp. Biol., _24_: 110--122.

FRY, F. E. J., 1947. Effects of the environment on animal activity. No. 55., Publ. Ont. Fish. Res. Lab., Univ. Toronto Press.

FRY, F. E. J., J. R. BRETT, and G. H. CLAWSON, 1942. Lethal limits of temperature for young goldfish (<u>Carrasius auratus</u> L.). Rev. Can. de Biol., <u>1</u>: 50--56.

FRY, F. E. J., J. S. HART, and K. F. WALKER, 1946. Lethal temperature relations for a sample of young speckled trout, <u>Salvelinus fontinalis</u>. No. 54., Publ. Ont. Fish. Res. Lab., Univ. Toronto Press.

GUNTER, G., 1947. Differential rate of death for large and small fishes caused by hard cold waves. Science, <u>106</u>: 472.

HARDING, J. P., 1949. The use of probability paper for the graphical analysis of polymodal frequency distributions. Journ. Mar. Biol. Assoc., <u>28</u>: 141--153.

HATHAWAY, E. S., 1927. Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. Bull. U. S. B. F., <u>43</u>: Part 2: 169--192.

HUNTSMAN, A. G., and M. I. Sparks, 1924. Limiting factors for marine animals. 3. Relative resistance to high temperatures. Contr. Can. Biol. N. S. <u>2</u>: 95--114.

JACOBS, M. H., 1918. Acclimatization as a factor affecting the upper thermal death points of organisms. J. Exp. Zool., <u>27</u>: 427--442.

KROGH, A., 1939. Osmotic regulation in aquatic animals. Cambridge Univ. Press.

LANG, A., 1891. Text book of comparative anatomy. Part 1. MacMillan & Co. London and New York.

LARKIN, P. A., 1948. Pontoporeia and Mysis in Athabaska, Great Bear and Great Slave lakes. Fish. Res. Bd. Can., Bull. 78.

LARKIN, P. A., G. C. ANDERSON, W. A. CLEMENS, and D. C. G. MACKAY, 1950. The production of Kamloops trout (Salmo gairdnerii kamloops, Jordan) in Paul lake, British Columbia. Scientific Publications of B. C. Game Dept. No. 1.

LIENEMANN, L. J., 1938. The green glands as a mechanism for osmotic and ionic regulation in the crayfish (Cambarus clarkii, Girard) J. Cell. and Comp. Physiol., 27: 149--161.

LOEB, J. and H. WASTENEYS, 1912. On the adaptation of fish (Fundulus) to higher temperatures. J. Exp. Zool., 12: 543--557.

MARSHALL, E. K., Jr. and H. W. SMITH, 1930. The glomerular development of the vertebrate kidney in relation to habitat. Biol. Bull., 58--59: 135--153.

PANNIKAR, N. K., 1941. Osmoregulation in some Palaemonid prawns. Journ. Mar. Biol. Assoc., 25: 317--359.

PROSSER, C. L., D. W. BISHOP, F. A. BROWN Jr., T. L. JAHN, and V. J. WULFF, 1950. Comparative animal physiology. W. B. Saunders Co., Philadelphia and London.

SAMTER, M., and W. WELTNER, 1904. Biologische Eigentumlichkeiten der Mysis relicta, Pallasiella quadrispinosa und Pontoporeia affinis erklart aus ihrer eiszeitlichen Entstehung. Zool. Anz., 27: 676--694.

SAMUEL, N., 1945. Some aspects of the morphology and physiology of the antennary glands of three decapod crustaceans. J. Univ. Bombay, 14 (3): 29--41.

SCHLIEPER, C., 1930. Die Osmoregulation wasserlebender Tiere. Biol. Rev., 5: 309--356.

SCHLIEPER, C., and F. HERRMAN,
1930. Cited from Lienemann 1938.

STORER, T. I., 1943. General zoology. McGraw-Hill Book Co., Inc. New York and London.

TATTERSAL, W. M., 1932. Contributions to a knowledge of the Mysidacea of California. II The Mysidacea collected during the survey of San Francisco bay by the U. S. S. Albatross in 1914. Univ. Cal. Publ. Zool., 37: 315--347.

1933. Euphausiacea and Mysidacea from western Canada. Cont. Can. Biol. and Fish. 8: 181--205.

1938. The seasonal occurrence of Mysids off Plymouth. J. Mar. Biol. Ass., Plymouth., 23: 43--56.

VOGT, W., 1933. Über die Antennendrüse von Mysis relicta. Zool. Jahr. Abt. fur Anatomie, 56: 373--386.

WIGGLESWORTH, V. B.,
1933. The function of the anal gills of the mosquito larva. J. Exp. Biol., 10: 16--17.