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A Study of Osmotic Response in Neomysis americana Smith

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A STUDY OF OSMOTIC RESPONSE IN *NEOMYSIS AMERICANA* Smith

by

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ABSTRACT

Three aspects of osmotic behavior were studied in samples of the mysid, Neomysis americana Smith, drawn from populations found in environments with high and low salinity. These were: (1) mortality rates in extreme salinities, (2) blood total osmotic concentration after acclimation to various concentrations of the medium, and (3) oxygen consumption in waters of three different salinities. The samples from the two populations differed in their mortality rates in low extreme salinities but not in their body fluid concentration or respiratory rate in a series of salinities. N. americana was found to regulate its body fluid concentration both hypo- and hyperosmotically to the medium and was estimated to be isosmotic at ca. 27 o/oo. Respiration in a medium of 10 or 30 o/oo salinity was significantly higher than in the acclimation medium of 20 o/oo.

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INTRODUCTION

Neomysis americana Smith, commonly called an opossum shrimp, is a small (up to 14 mm long), shrimp-like crustacean of the subclass Malacostraca and order Mysidacea. It has a reported range extending from the Gulf of Saint Lawrence (Tattersall, 1939) at least as far south as Cape Hatteras (Bigelow and Sears, 1939) in shallow inshore waters. It is abundant and widespread in the Chesapeake (Cowles, 1930), Delaware (Hulbert, 1957), and Narragansett (Herman, 1963) estuaries. It exhibits diurnal vertical migration (Herman, 1963) and is primarily a detritus feeder. Additional information on the life history of the genus may be found in Tattersall and Tattersall (1951).

N. americana has been extensively collected in oceanic salinities by Fish (1925), Cowles (1930), Tattersall (1939), Bigelow and Sears (1939), Cronin *et al.* (1962), and others. The species was consistently found as far up Delaware Bay as the 4 o/oo isohaline (Hulbert, 1957). The same wide salinity distribution was noted from plankton collections of the Ichthyology Department of the Virginia Institute of Marine Science made in the York River and over the continental shelf off the Virginia coast. Comparatively few individuals were found in lower Chesapeake Bay collections of the same series. The wide range of distribution implies strong euryhaline qualities for the species. These observations provoke the questions: (1) What adjustments are made in the species to enable it to survive this wide range of salinities? (2) Does the nature of these adjustments change for populations in

different regions of the salinity range?

Karpevich (1958) appears to have done the only experimentation with salinity variables on any of the Mysidacea. He determined survival and respiration rates of Mesomysis kawalevskyi in waters of different salinities and ionic ratios. Excellent reviews of osmoregulation in Crustacea have been made by Robertson (1960), Prosser and Brown (1961), Lockwood (1962), and Kinne (1963).

Samples of two populations from different salinity regimes were studied in order to find any differences in the adaptation (either genetic or through long-term acclimation) of individuals to different parts of the salinity range. One population was from the lower end of the York River which has mean monthly maximum, minimum surface salinities of 22.6 and 14.9 o/oo and an annual mean surface salinity of 19.4 o/oo (U. S. C. G. S., 1960). The other was from the ocean side of eastern shore of Virginia with a salinity range of 29 to 33 o/oo (Virginia Institute of Marine Science, unpublished records).

Three variables were measured, all of which have been widely used to study osmotic behavior in aquatic animals. These were mortality rates to establish a general working range for subsequent experimentation, blood osmotic concentration to determine whether they were osmoregulators or conformers, and respiratory rate as a clue to the energy requirements for adjustment to their particular salinity regime.

MATERIALS AND METHODS

Collections

York River collections were made at Gloucester Point, Virginia, in the eel grass zone (ca. 2 feet deep at MLW) in the spring, and in the river channel at Gloucester Point (ca. 30 feet deep at MLW) in the summer and fall. Eastern shore collections were made near Wachapreague, Virginia, in the salt marsh channels (2 to 10 feet depths) in the spring and at Wachapreague Inlet (15 to 30 feet depths) in the summer and fall.

Collections were made with a 50 cm semi-circular epifauna net towed slowly (1 to 2 knots) along the bottom behind a skiff. Data on location, water temperature, salinity, time, tide, depth, bottom type, and estimated numbers and size of mysids were recorded for possible correlation with experimental results.

In the laboratory, individuals were sorted by carefully pouring them into a coarse mesh (1mm) net which was immersed to the open end in sea water. Smaller individuals, not restricted by the mesh size, swam to the bottom of the sea water container and were discarded. The remaining, larger, individuals were saved for experiments. There is no gross morphological difference other than size between juveniles and adults, since the adult form is attained before the individuals are released from the brood pouch of the female. An arbitrary minimum length of ca. 8 mm was set for experimental animals. The mean length was 10 to 12 mm throughout the sampling period.

Aliquots of most collections were checked for taxonomic identification. The shape and pigmentation of the telson was found to be a convenient method for distinguishing N. americana from other local species (Hopkins, 1958). Two specimens of Mysidopsis bigelowi from a York River collection in December were the only individuals not identified as N. americana.

Acclimation

Experimental animals were acclimated to the desired temperature and salinity for a minimum of six days. The acclimation temperature was 20 ± 2°C for all experiments. The acclimation salinities were 20 o/oo for the mortality and respiration experiments, and 10, 20, 30, and 36 o/oo for the blood concentration experiments.

Oyster feces were chosen as food; this had the advantage of prolonged suspension, because 80 percent of the particles were less than 2 μ in diameter (Dexter Haven, personal communication). The disadvantage lay in the suitability of oyster feces as a bacterial culture medium; this was counteracted with moderate success by adding dihydrostreptomycin sulfate to the holding containers at a dosage of 50 mg/l (Marshall and Orr, 1958).

Artificial sea water (see Appendix, Table I), mixed to the desired salinity with distilled water, was the acclimation medium. Salinities were determined with a hydrometer to ±0.3 o/oo or by titration (Knudsen method).

The animals were held in either 10 gallon Plexiglass aquaria or in covered, shallow, enamel pans.

Experimental Procedure

All experiments were conducted at the acclimation temperature of 20°C.

Mortality in Extreme Salinities: The experimental containers were rectangular, plastic, disposable rat cages of 5 liters capacity. Four liters of artificial sea water were made for each container at the experimental salinities of 2, 4, 6, 32, 34, 36, and 38 o/oo, with 20 o/oo as the control (acclimation) salinity.

Mysids were transferred from the holding to the experimental containers with a dip net. Subsamples (N=25) from each population sample were tested for relative mortality rate at the above salinities in two to four replicate containers. Experiments were run for 24 hours with the number surviving recorded for each container at one to four hour intervals.

Animals were considered dead when they did not respond to tactile stimulation.

Blood Osmotic Concentration: The blood concentration was determined by the freezing point depression technique modified from Jones (1941).

Eight to 12 lambda of blood were collected by capillary action using a drawn capillary tube (inside diameter ca. 100μ) inserted into the punctured pericardium. The end of the capillary tube containing the sample was sealed by sucking melted paraffin into the tube behind the sample. The other end of the tube was sealed by dipping it into

the melted paraffin. The samples were placed in a calcium chloride solution in a freezer at -25°C at least overnight.

The samples were then removed to a cold room at 4.5°C and allowed to melt slowly in a chilled (-3°C), but slowly warming, brine solution. The temperature of the brine was taken with a stem thermometer to the nearest 0.05°C (thermometer graduated in 0.1°C intervals) at the time the last crystal of a sample melted. This was taken as the freezing point depression of a sample.

Artificial sea water was used for the 10, 20, and 30 o/oo standards. Blood concentration is expressed in parts-per-thousand salinity by converting °C to parts-per-thousand on the standard curve (Fig. 1).

Respiratory Rate: Respiratory rates were determined in a constant volume Warburg respirometer (Umbreit et al., 1957).

Animals were transferred from the 20 o/oo acclimation salinity to the experimental salinities contained in the 100 ml Warburg flasks. An experimental run consisted of two sets of 10, 20, and 30 o/oo flasks, one set for each population sample, and one flask as a thermobarometer (control).

Preliminary experiments showed 35 individuals per flask to be an optimum number. A greater number increased cannibalism and also inhibited O_2 consumption. Fewer mysids gave smaller readings, hence smaller differences between experimental variables.

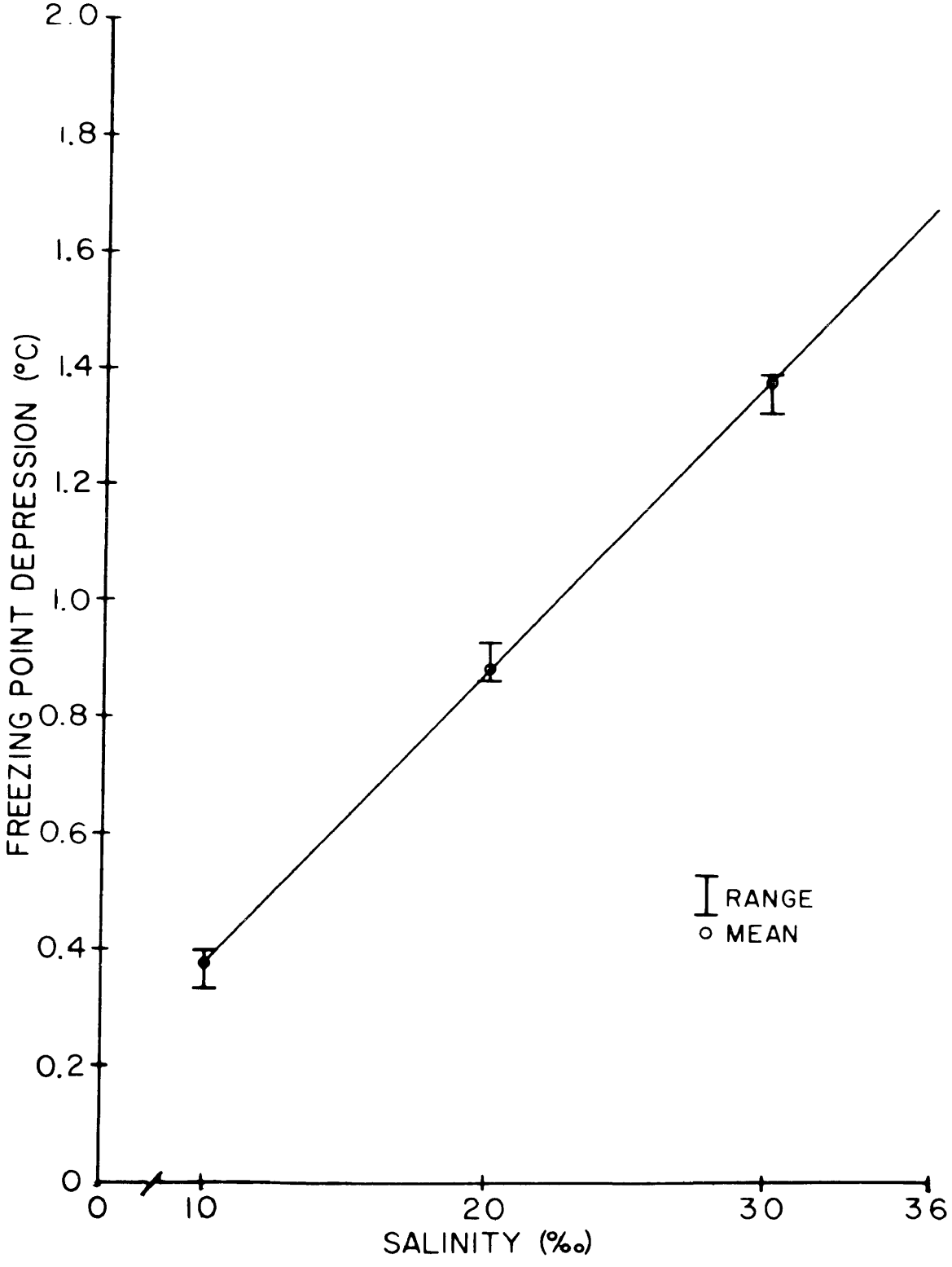

Fig. 1. Freezing point depressions of artificial sea water standards.

Four anesthetics, magnesium chloride, succinyl choline chloride, eserine sulphate, and tricaine methanesulphonate (MS-222), were applied to determine whether differences in the metabolism of animals exposed to different salinities were due to osmoregulation or to an escape reaction as hypothesized by Lofts (1956).

RESULTS

Mortality in Extreme Salinities

The York individuals were more tolerant of the low salinities than the animals from Wachapreague. The two groups were equally tolerant of high salinities (Figs. 2 and 3). The mortalities of the samples from the two populations at 12 hours elapsed time are typical of the pattern of mortality at the other elapsed times (Fig. 2). The curves showing cumulative percent mortality of the samples for each salinity at a series of elapsed times have distinctive features (Fig. 3). They approximate the general shape of the top half of a sigmoid curve with a rapid initial rise giving way to a gradual leveling off. The high initial mortality is where the difference (statistical significance) between the population samples is established. Population sample differences in percent mortality are as great at eight hours as at 24 hours or are greater at 8 than at 24 in all salinities except 6 o/oo. The mortality in the control is considered insignificant.

Chi-square was employed to test for significance of differences in the deaths, per 4-hour interval, of mysid shrimp in samples obtained from the York and Wachapreague areas. Table II of the Appendix summarizes the percent mortality by 4-hour intervals. Significant differences were found at 2, 4, 6, and 38 o/oo.

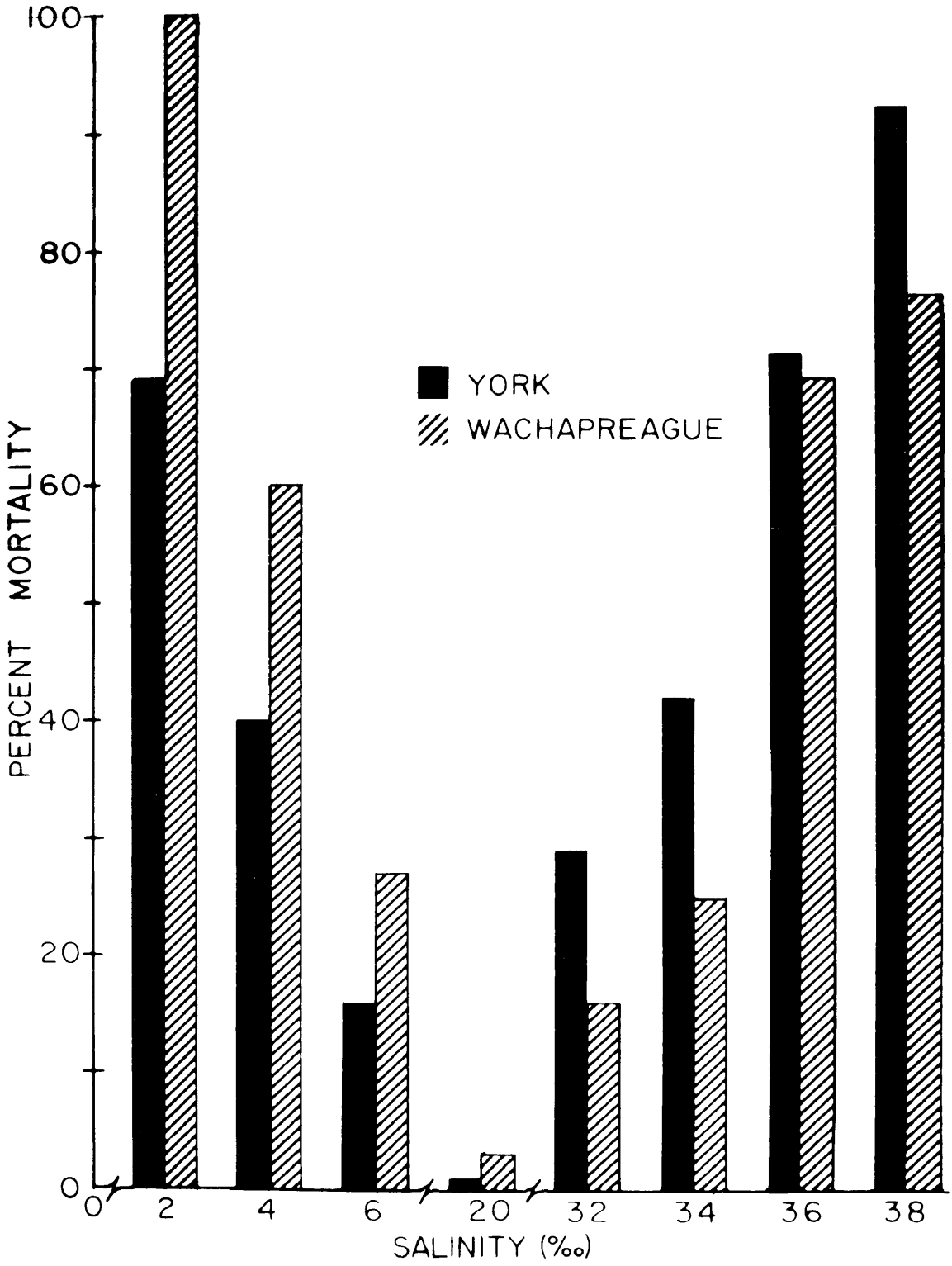

Fig. 2. Average of percent mortality of each population sample in the series of test salinities at 12 hours elapsed time.

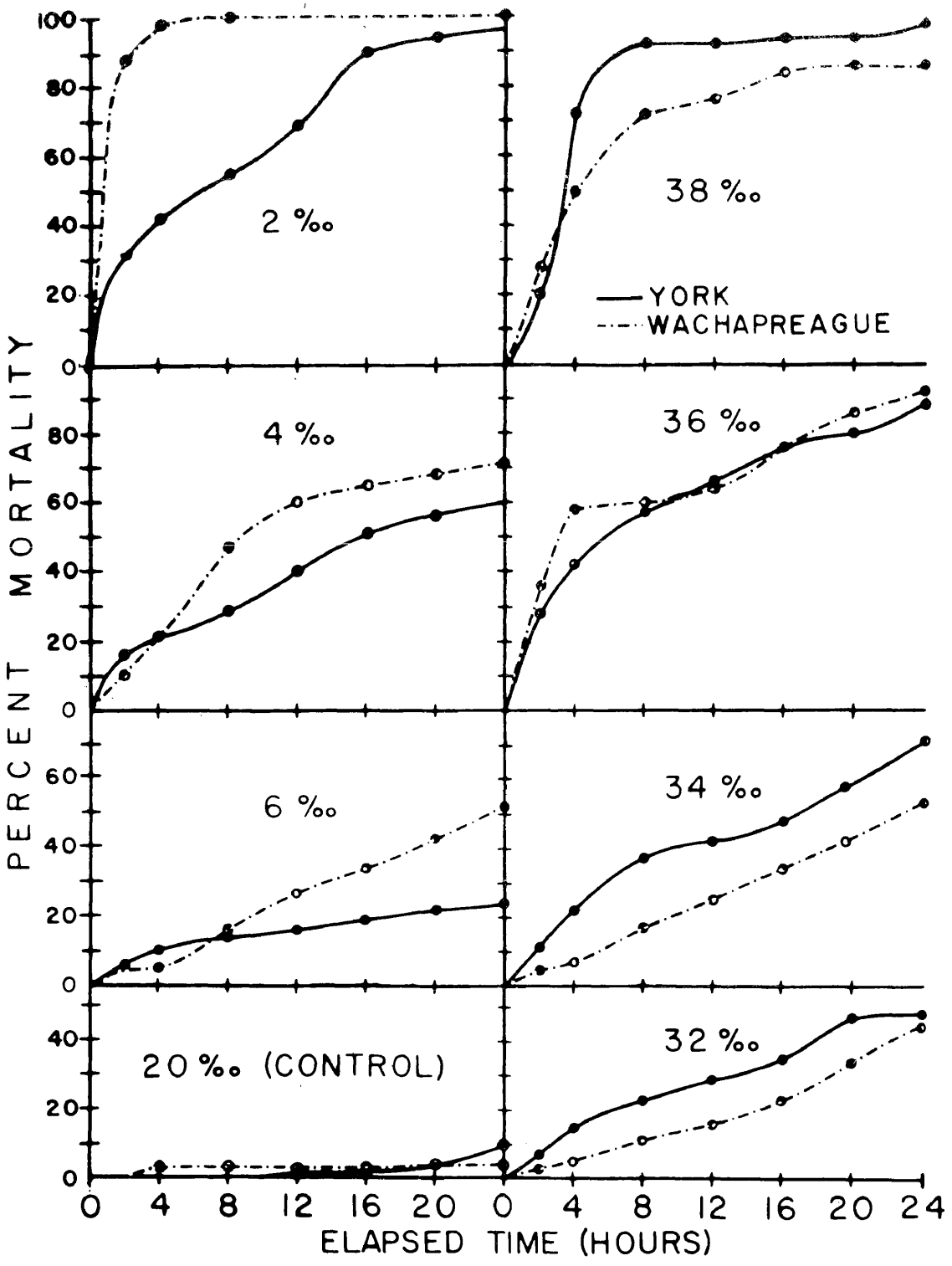

Fig. 3. Average of percent mortality of each population sample in the series of test salinities at given elapsed time.

Blood Osmotic Concentration

The blood freezing point depressions (Appendix, Table III) were analysed for population differences by the "t" test. The means were 0.88, 1.05, 1.30, and 1.47°C at 10, 20, 30, and 36 o/oo, respectively, for the York population and; 0.81, 1.08, 1.37, and 1.38°C at 10, 20, 30, and 36 o/oo, respectively, for the Wachapreague population. No significant differences (at the 5% level of confidence) were found between the population samples at any one of the four test salinities.

It was found that Neomysis regulates both hyperosmotically and hyposmotically and is isosmotic at about 27 o/oo. The isosmotic line, showing theoretically perfect osmotic conformity, and the experimental curve are compared in Fig. 4. The latter is a computed regression line drawn from the combined data from the samples from both populations. There is approximately a 4 o/oo increase in body fluid concentration for every 10 o/oo increase in ambient medium salinity. The data also suggest the possibility that the relationship between the media and blood concentration may not be linear but may follow the isosmotic line for a distance between 20 and 30 o/oo salinity. The means, interval estimates, and ranges of freezing point depressions are also given in this figure and in Table III of the Appendix.

Respiration Data

The respiration data means were 162, 135, and 161 µl O_2/mg N/hr for 10, 20, and 30 o/oo samples, respectively, of the York population and 156, 134, and 150 µl O_2/mg N/hr for 10, 20 and 30 o/oo samples, respectively, of the Wachapreague population (Fig. 5; Appendix, Table IV). No significant mean differences in respiration rates between samples at any of the three salinities could be found with "t" tests. Comparisons

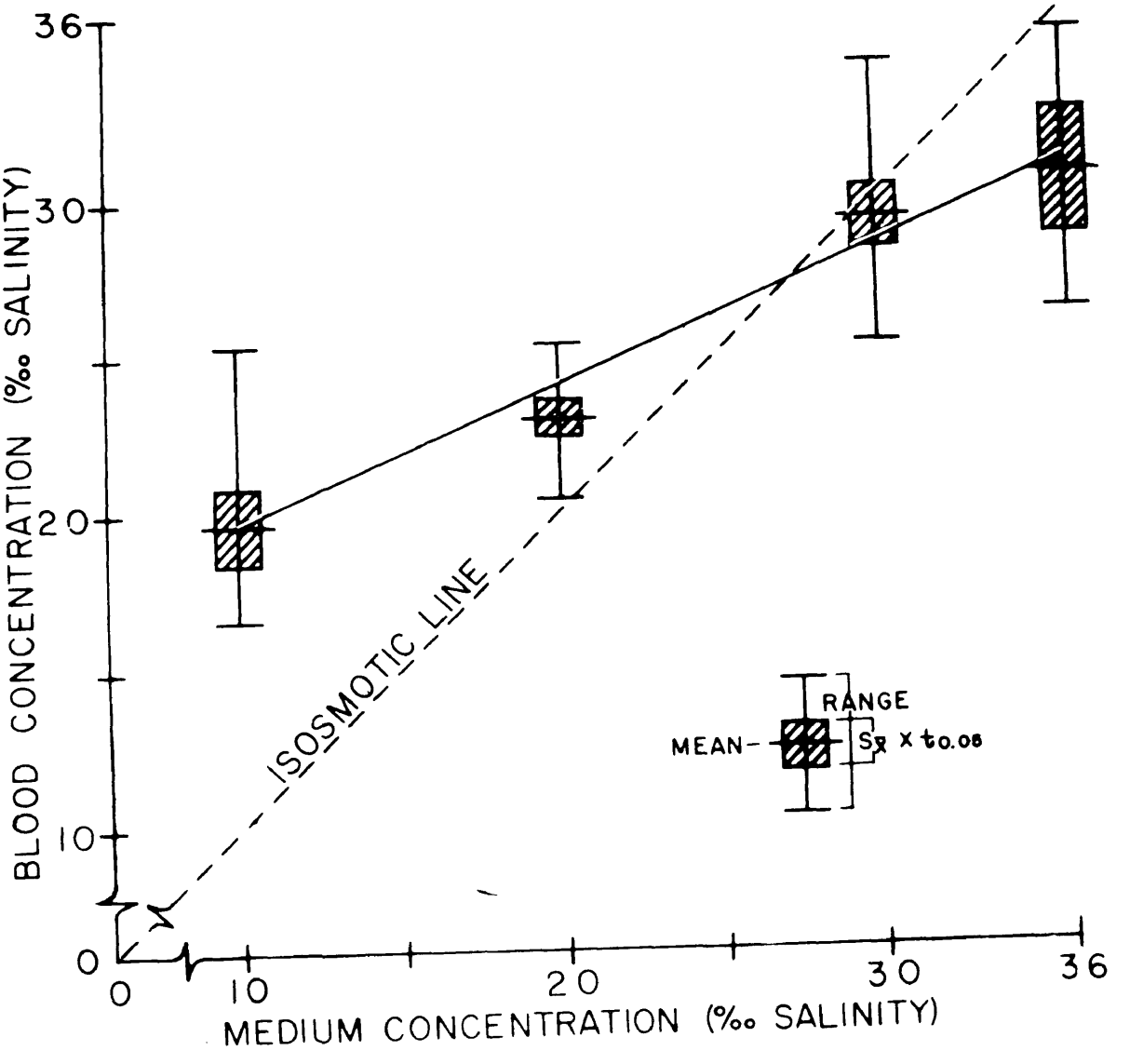

Fig. 4. The means, interval estimates, and ranges of the combined blood concentration data from both population samples at 10, 20, 30, and 36 o/oo salinity. The solid computed regression line is compared to the isosmotic line of theoretical, perfect osmoconformity.

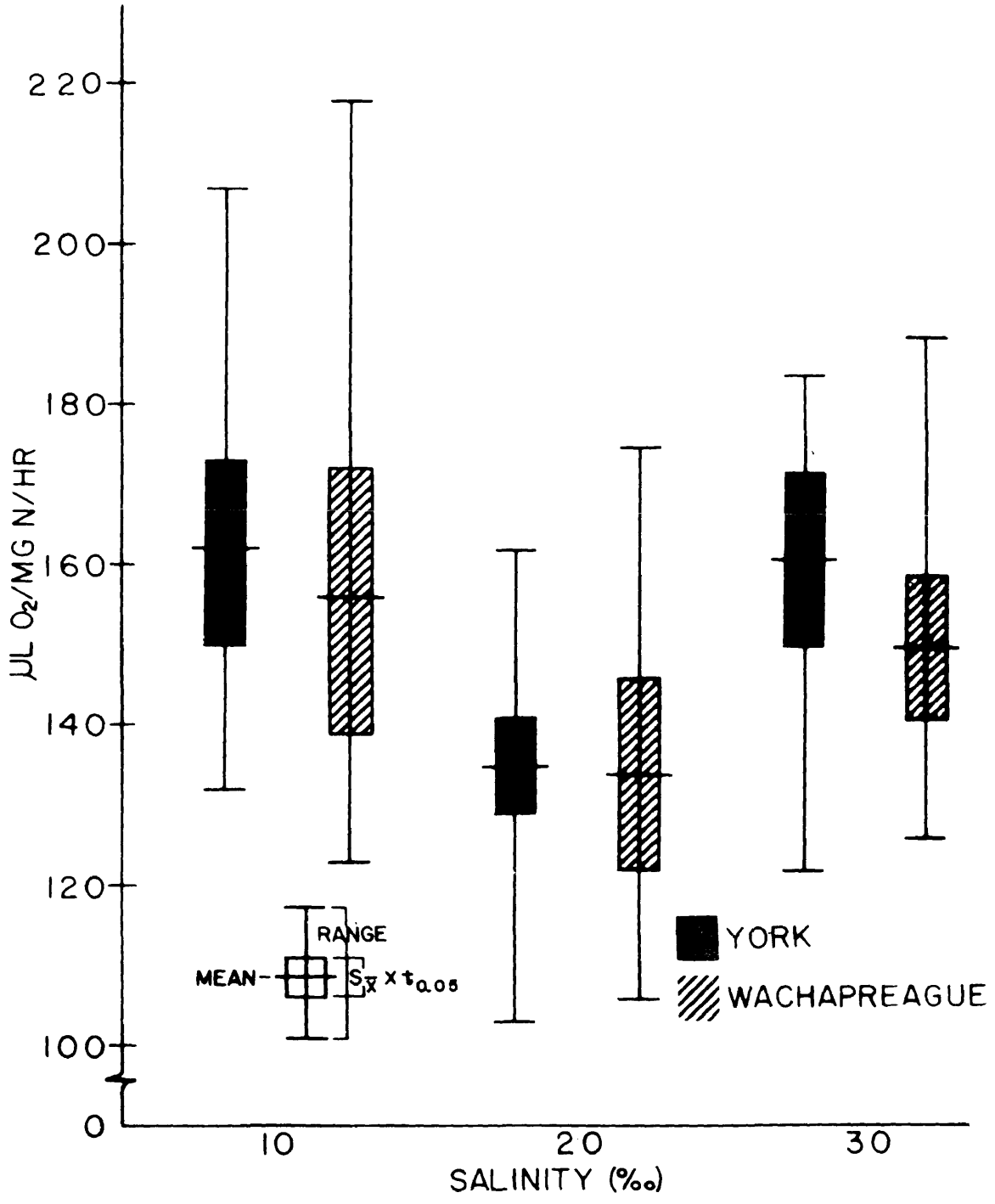

Fig. 5. The means, interval estimates, and ranges of oxygen consumption for samples drawn from the York and Wachapreague populations at three test salinities.

of respiratory rates between the experimental salinities, however, showed significant differences. That is, respiration rates at the extreme test salinities of 10 and 30 o/oo were significantly higher than at the acclimation salinity of 20 o/oo in the samples from both populations. The significance was at the 1% level of confidence for the York samples and at the 5% level of confidence for the Wachapreague samples.

Attempts to narcotize Neomysis were unsuccessful. The animals did not resume normal activity in fresh sea water after being exposed to doses of anesthetic large enough to inhibit motility. Death was not instantaneous, but followed a gradual weakening as evidenced by an obvious decline in the rate of heartbeat.

DISCUSSION

Three aspects of the osmotic behavior of Neomysis americana have been studied: mortality rates in extreme salinities, blood concentration at varying concentrations of the medium, and oxygen consumption in water of three different salinities.

Due to the limitations of time and facilities osmoregulation was studied at a single temperature in this investigation. Temperature is known to have a marked influence on osmoregulation, however. In general, Crustacea are more tolerant of high salinities at low temperatures and low salinities at high temperatures (Verwey, 1957). Panikkar (1940) postulates that invasion of fresh and estuarine waters is easier in warmer climates. Broekema (1941) attributes the fall offshore migrations to high salinity water and the spring inshore migration to lower salinities of the decapod Crangon crangon to temperature-related changes in salinity tolerance.

The mortality data show the relative mortality rates of the samples from the two populations resulting from a direct transfer from acclimation to extreme salinities. A general salinity working range for subsequent experimentation was also based upon the mortality experiments.

Samples from the two populations differed in their mortality rates in the low (2, 4, and 6 o/oo) experimental salinities. There was

also a significant difference at 38 o/oo for which there is no ready explanation. The fact that it is beyond natural environmental salinities is an important consideration in its interpretation and precludes direct attribution to environmental influences. The differences in the low salinities are in the direction expected: that is, the York samples, from a low salinity environment, showed less mortality than the Wachapreague samples. The lack of differences at the high salinities is assumed to be related to the genetic makeup of the species and/or exposure to similar high environmental salinities at some time during their life cycle.

The high initial mortality, revealed in the percent mortality curves (Fig. 3), is where the difference (statistical significance) between population samples is established. Two possible explanations for these differences are indicated. (1) The York individuals are more tolerant of the body fluid dilution that must accompany the transfer to low salinities. Death in low salinities is generally assumed to result from the dilution of body fluids after the saturation of enzyme systems which regulate the concentration of ions in the cytoplasm (Giese, 1962). (2) The York individuals regulate their blood ion concentration more effectively under lethal stress than do the Wachapreague Neomysis.

The above hypothesis could be tested by determining blood concentrations in the lethal salinities at brief intervals after transfer from the acclimation salinity is made.

It was found that Neomysis regulates both hyperosmotically and hyposmotically (Fig. 4). Osmoregulation is an essential adaptation to survival in estuarine environments for nearly all Crustacea. An associated adaptation is reduction of the "preferred" (regulated) body fluid concentration. It is postulated by Potts (1954) that this is the most important adaptation in reducing the work load of hyperosmotic regulation. Other means of facilitating hyperosmotic regulation are reduced ion and water permeability, excretion of urine hypotonic to the blood, urine volume regulation, and efficient absorption of ions from the medium. The body fluid concentration of N. americana at 10 o/oo ambient salinity is ca. 20 o/oo (Fig. 4). The species' acquisition of the ability to regulate hyperosmotically and the reduction of its "preferred" body fluid concentration from marine salinities (35 to 20 o/oo at the 10 o/oo acclimation salinity) shows that the species has made important steps toward colonizing fresh water.

The principal area for active absorption and excretion of salts in Crustacea is the gills (Prosser and Brown, 1961). Because Neomysis respires through its integument and has no gills it would be of interest to determine the organ responsible for this function. There is no information available on urine concentration or volume, or integumentary permeability.

Although the species does not lend itself well to respiratory measurements because of its sensitivity to handling and its habit of cannibalism, an approximation of oxygen requirements has been made. Oxygen consumption was significantly higher in 10 and 30 o/oo than in the acclimation salinity of 20 o/oo (Fig. 5). This differs from the

findings of Karpevich (1958) who states that the respiration of Mesomysis kowalevskyi is higher in water of optimum salinities than in high, sub-lethal salinity waters. These seemingly contradictory findings are unexplained, but the fact that Karpevich worked with a species which had a different salinity range (ca. 0-10 o/oo) provides a possible solution.

Differences in respiration in a series of salinities have classically been attributed to work performed in osmoregulation. The further the concentration of the medium from the animal's isosmotic point, the higher is the respiratory rate within the animal's limits of survival. Recent work by a number of authors on various crustaceans indicates, however, that the correlation of whole animal respiratory rates with osmotic work is uncertain. This subject is discussed by Wolvekamp and Waterman (1960).

It may be that osmoregulation is not the primary cause of salinity-correlated variations in respiration. Gross (1957) proposed that respiration differences in certain decapods were primarily due to an increase in motile activity resulting from an escape reaction when placed in a less favorable salinity. An attempt to test this hypothesis by comparing the respiratory rates of anesthetized and nonanesthetized animals under conditions of osmotic stress failed because an anesthetic suitable for Neomysis was not found.

The mean respiration rate for Neomysis at the 20 o/oo acclimation salinity, 135 µl O_2/mg N/hr for a mean animal weight of 0.02 mg N, compares reasonably well with ca. 186 µl O_2/mg N/hr for an animal weight of 0.02 mg N on a composite curve for Crustacea given by Zeuthen (1947).

It is also close to a measurement made by Grainger (1956), 188 µl O_2/mg N/hr, on a freshwater mysid, Hemimysis lamornae. Karpevich's results, cs. 17 µl O_2/mg N/hr, is not even roughly comparable to those of Zeuthen, Grainger or the author. There is no ready explanation for this difference. Zeuthen's units cc O_2/kg/hr (assumed to be wet weight); Grainger's units µl O_2/gm wet weight/hr; and Karpevich's units ml O_2/ zolotnik live weight/hr (1 zolotnik= 4.266 gm); have been converted to µl O_2/mg N/hr, the units used in this study. In the above conversions one mg nitrogen equals 40 mg dry weight and 170 mg wet, or live, weight.

The failure of the blood concentration and oxygen consumption tests to delineate differences between the two population samples may result from the fact that neither of these two studies was conducted under extreme (i.e., near-lethal) conditions of salinity. Attempts to acclimate mysids to these extreme conditions for the purpose of running the necessary experiments were not successful. The question of whether the two populations show different adaptations, genetic or through long-term acclimation, to their respective salinity regimes must, therefore, be left open.

SUMMARY

1. Three aspects of the osmotic behavior of high and low salinity environment population samples of Neomysis americana have been studied in light of their method(s) of adjustment to the ambient salinity.

2. The two population samples differed in their mortality rates in low extremes of salinity with the sample from low environmental salinities being more tolerant of low test salinities. The two samples did not differ, however, in their blood concentrations or rates of oxygen consumption at various concentrations of the medium. This is possibly due to the fact that the mortality data represent more extreme conditions of salinity than that derived from the other two methods.

3. N. americana regulates its body fluid concentration both hypo- and hyperosmotically over the tested range of 10 to 36 o/oo and is isosmotic at ca. 27 o/oo salinity. Osmoregulation and reduction of the "preferred" body fluid concentration are known to be important crustacean adaptations to the estuarine environment. The acquisition of both of these mechanisms by Neomysis shows that it has made an important step toward the colonization of fresh water.

4. Respiration in media of 10 and 30 o/oo salinity was significantly higher than in the acclimation medium of 20 o/oo. Although

osmotic work is a possible explanation for these differences, they are probably more correctly attributed to other causes, for example, increased motile activity in an unfavorable salinity.

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APPENDIX

Table I. Composition of the artificial sea water, Seven Seas Mix, produced by the Utility Chemical Co., Paterson, N. J. The following list of constituents is from the manufacturer's Technical Bulletin No. 202.

NaCl	1.5	g/100 ml
$MgCl_2 \cdot 6H_2O$	0.25	" "
KCl	0.04	" "
Ca (as Cl^-)	0.001	" "
Ca (as $SO_4^=$)	0.011	" "
BR (as K^+)	2.2	mg/100 ml
Sr (as Cl^-)	0.38	" "
Al (as Cl^-)	0.0028	" "
Rb (as Cl^-)	0.0061	" "
Li (as Cl^-)	0.0006	" "
I (as K^+)	0.002	" "
Na_2EDTA	2.0	" "
Fe (as EDTA)	0.01	" "
Zn (as $SO_4^=$)	0.23	" "
Mn (as $SO_4^=$)	0.065	" "
Mo (as NaMoO)	0.02	" "
Co (as $SO_4^=$)	0.00063	"
Cu (as $SO_4^=$)	0.00013	"
TRIS AMINOHYDROXY METHANE	50	" "
KNO_3	10	" "
K_2HPO_4	1.0	" "
$Na_2SiO_3 \cdot 9H_2O$	10	" "

Table II. Percent mortality in 2, 4, 6, 32, 34, 36, 38, and 20 (control) o/oo salinity at 4, 8, 12, 16, 20, and 24 hours elapsed time.

Salinity (o/oo)	Population	Replication	Elapsed time (hours) 4	8	12	16	20	24
2	Y	I	44	54	70	90	96	100
		II	40	56	68	90	92	92
	W	I	100	100	100	100	100	100
		II	96	100	100	100	100	100
4	Y	I	16	28	48	60	64	64
		II	16	36	48	64	76	80
		III	28	28	36	40	44	52
		IV	24	24	28	40	40	44
	W	I	40	40	64	76	84	84
		II	20	40	48	54	56	68
		III	16	58	64	64	64	64
		IV	8	50	64	64	66	68
6	Y	I	12	16	16	18	20	20
		II	4	6	12	16	22	28
		III	10	16	16	16	16	20
		IV	14	16	18	24	28	28
	W	I	4	12	32	36	44	52
		II	12	20	40	40	46	64
		III	4	20	20	38	48	52
		IV	0	14	16	20	32	40
32	Y	I	12	18	34	40	48	48
		II	20	30	32	42	52	56
		III	14	20	22	24	40	40
	W	I	12	24	28	38	48	56
		II	4	10	12	16	24	32
		III	0	0	8	16	30	44
34	Y	I	12	26	38	48	54	76
		II	36	48	52	60	76	92
		III	18	36	36	36	44	44
	W	I	12	28	40	56	60	80
		II	4	12	24	32	40	40
		III	4	10	12	14	26	40
36	Y	I	36	56	66	80	80	92
		II	48	58	66	72	80	84
	W	I	60	64	72	82	92	100
		II	56	56	56	70	80	84

Y- York population
W- Wachapreague population

Table II continued

Salinity (o/oo)	Population	Replication	Elapsed time (hours) 4	8	12	16	20	24
38	Y	I	36	56	66	80	80	92
		II	48	58	66	72	80	84
	W	I	60	64	72	82	92	100
		II	56	56	56	70	80	84
20	Y	I	0	0	4	4	4	12
		II	0	0	2	4	4	12
		III	0	0	0	4	0	8
		IV	0	0	0	2	0	8
	W	I	0	8	8	8	8	8
		II	0	0	0	4	0	4
		III	0	0	0	4	0	4

Table III. Freezing point depressions in °C at 10, 20, 30, and 36 o/oo salinity.

Population	Salinity 10 o/oo	20 o/oo	30 o/oo	36 o/oo
York	0.75	1.05	1.20	1.25
	1.15	1.00	1.15	1.35
	1.00	1.05	1.30	1.65
	0.95	0.95	1.25	1.60
	1.00	1.10	1.30	1.50
	0.80	1.00	1.60	
	0.80	0.90		
	0.90	0.90		
	0.80	1.10		
	0.85	1.20		
	0.90	1.05		
	0.65	1.10		
		1.10		
		1.15		
Wachapreague	0.90	0.95	1.35	1.50
	0.70	1.00	1.35	1.20
	0.75	1.10	1.40	1.20
	0.80	1.10	1.30	1.40
	0.80	1.10	1.30	1.30
	0.90	1.15	1.40	1.40
	0.80	1.15	1.50	1.65
		1.10	1.40	
		0.95	1.40	
		1.10	1.35	
		1.20	1.20	
		1.15	1.45	
		1.00	1.40	
		1.10	1.35	
		1.00		
N	19	29	20	12
$\bar{X}$	0.850	1.062	1.348	1.417
Interval Estimates	0.057	0.031	0.050	0.103

Table IV. Oxygen consumption in µl O_2/mg N/hr at 10, 20 and 30 o/oo salinity

10 o/oo		20 o/oo		30 o/oo	
Y	W	Y	W	Y	W
147	130	130	214*	184	159
157	132	130	206*	166	152
162	123	103	208*	167	156
194	164	121	148	160	132
207	176	127	175	183	141
201	164	119	137	172	123
149	189	124	144	174	189
145	218	121	154	180	178
132	210	150	175	167	166
186	145	145	123	130	140
182	145	116	131	138	155
157	140	123	106	122	162
150	141	123	124	147	172
156	141	147	124	168	142
130	126	158	108	152	150
147		147	129		126
147		157	125		138
160		162	111		126
		147			
		158			
		112			
		124			
		138			
		146			
		129			
		129			
		152			
		136			
N 18	15	28	15	15	18
$\bar{X}$ 162	156	135	134	161	150
Interval Estimates 11.4	16.6	6.1	11.9	10.6	9.3

Y- York population
W- Wachapreague population

*reading not included in calculations, nitrogen determinations believed to be incorrect