THE INITIAL CHANGES IN OXYGEN CONSUMPTION OF *FUNDULUS HETEROCLITUS* DUE TO COMBINED CHANGES OF TEMPERATURE AND SALINITY

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by

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CHAPTER I

INTRODUCTION

In order to better understand an animal's ability to live in a particular environment, it is necessary to understand the animal's physiological compatibility with the environment and the animal's ability to adjust to changes in the environment. This research was undertaken in order to determine the ability of Fundulus heteroclitus to adjust to combined environmental changes of temperature and salinity.

There are numerous accounts of how an animal reacts physiologically to a single environmental factor. Reviews by Bullock (1955) and Fry (1958) give general accounts of the effects of temperature and temperature adaptation, while the review by Beadle (1957) gives a general account of the problems and mechanisms of osmotic and ionic regulation. Reviews dealing with fish are found in Brett (1956), Brown (1957), Fisher (1958), and Prosser and Brown (1961).

There are several simultaneous mechanisms for temperature compensation. According to Bullock (1955) and Brett (1956), they are biochemical, cellular, organ, system, and behavioral levels. The overall effect of temperature changes on the organism is the stress placed on the balancing of all of the metabolic activities essential to life (Bullock, 1955; Fry, 1956).

The problem of osmotic and ionic change is a problem of balance as is temperature compensation. Here the organism has the problem of maintaining the proper internal water and ionic balance in relation to environmental changes. The mechanisms of osmotic and ionic regulation require the expenditure of energy although not all of the extra respiration recorded in many experiments is concerned with osmotic or ionic regulation. There

is no evidence that active transport of water contributes to the osmoregulation of aquatic animals (Beadle, 1957; Prosser and Brown, 1961).

One major problem with the approach of using a single environmental factor is that the interaction of the many factors that make up the environment is not taken into consideration when the physiological response is measured. Studies employing combinations of controlled environmental factors permit a more intimate understanding of the fine adjustments by the animal in order for it to function in a given location (Kinne, 1951b; Allee et al, 1949).

Fundulus heteroclitus is known for its ability to range throughout the estuarine environment. Therefore, it was decided to test the physiological response of the animal to combinations of temperature and salinity which are considered to be the two most important ecological factors in its environment (Wells, 1961; Kinne, 1961a).

CHAPTER II

MATERIALS AND METHODS

The fish used in this experiment were collected from the same tidal creek approximately two miles east of Beaufort, N. C. All collections were made with minnow traps during the months of October and November, 1963. The identity of the species, Fundulus heteroclitus, was verified by keys in Hildebrand and Schroeder (1928).

During October, November, and December, 1963, the fish were kept in 29°/oo artificial sea water. The sea salt mix was obtained from Utility Chemical Co. The chemical analysis of the artificial sea water is listed in Table 9 (Appendix). The fish were fed a commercial tropical fish food every third day with the water changed every three weeks. No temperature regulation other than room temperature was used. A constant six-hour dark period was maintained.

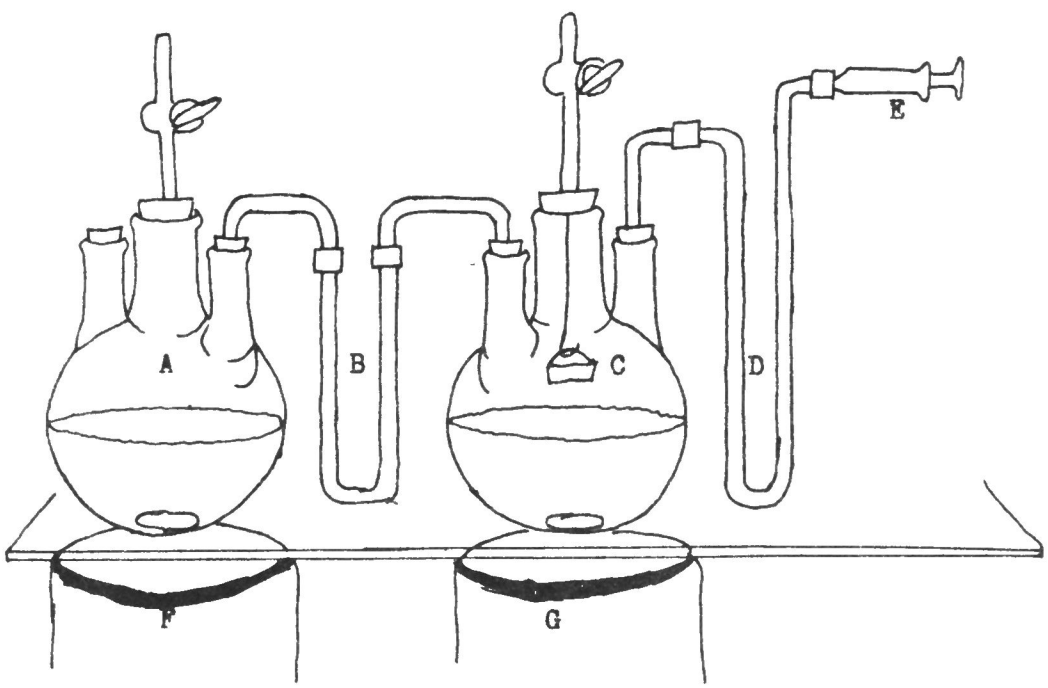
Beginning January first, the fish were kept in aquaria with the water changed every two weeks and checked constantly to ensure the 29°/oo salinity. The temperature was maintained at 20±3°C with the same dark period as before. The fish were fed all that could be eaten in a five-minute period twice daily. The above conditions were maintained for two weeks before the collection of any experimental data and were continued throughout the data collection period.

A problem was encountered with an external parasite identified as belonging to the genus, Gyrodactylus. Approximately one half of the original population died before preventive measures became effective. The most effective treatment found was a modification of the formaldehyde treatment for trout as outlined by Davis (1946). The treatment used consisted of

dipping the fish in a one part 40% formaldehyde to 50 parts sea water. The fish were not used for experimental purposes for one week after this treatment.

The oxygen consumption of each fish was measured with a modified Barcroft respirometer similar to that described by Morris (1963). The apparatus consisted of two 1000 ml, three neck, distilling flasks connected with a manometer. See figure below. Flask A was used as a compensating vessel having the same contents as Flask C, the respiration vessel. Carbon dioxide was absorbed by the use of 10% KOH in the respiration vessel. Readings were made by compressing the liquid in the graduated

Figure I



A Modified Barcroft Respirometer

A - Compensation Vessel
B - Manometer
C - Respiration Vessel
D.- Graduated Pipette
E - Syringe
F & G - Magnetic Stirrers

pipette (D on Figure I) with the syringe (E on Figure I) until the liquid in the manometer was returned to its starting level. The amount of oxygen consumed was then read on the graduated pipette. Constant slow stirring was obtained in each flask by the use of magnetic stirrers.

The constant control and experimental temperatures were obtained by the use of thermostatically controlled heaters and a Blue M constant flow portable cooling coil. A 20 gal. aquarium was used as the water bath. At the control temperature, the heaters and cooling coil were used in opposition to each other in order to maintain the proper test temperature. At the high temperature the heater was used alone and at the low temperature the cooling coil was used alone. The temperature of the respirometer was controlled in the above manner to the test temperature $\pm 0.5^{\circ}C$.

The salinity of the water used in the experimental and control measurements was checked by the method outlined by Welsh and Smith (1960). The procedure is given in Table 10 (Appendix). This method was also used to check the salinity of the water in which the fish were held.

Before a fish was introduced into the respirometer, the apparatus and the contents were allowed to reach equilibrium with the water bath. A single fish was then introduced into the respirometer. Thirty minutes was then allowed for the fish to settle down after the shock of being moved. The system was then closed off and readings made at 15 or 30 minute intervals for 45, 90, or 120 minutes depending upon the experimental temperature used.

The experimental temperatures and salinities used were all possible combinations of 9, 20, 31 degrees Centigrade and 9, 29, and 49 parts per thousand ($^{\circ}/_{oo}$) for salinity. The experimental control was $20^{\circ}C$ and $29^{\circ}/_{oo}$.

CHAPTER III

RESULTS

In view of the relationship between size and metabolic rate in Fundulus (Wells, 1935b), the experimental data is treated in two different groups. The first group consists of the oxygen consumption of all fish regardless of weight while the second group consists of the oxygen consumption from those fish whose weight fell between 3.00gm and 6.00gm. Table 2 (Appendix) shows the oxygen consumption of each fish with the mean weight of the fish for that particular temperature and salinity, the mean oxygen consumption, and the standard deviation of the oxygen consumption. Table 3 (Appendix) shows the same data for the fish in the 3.00-6.00gm group. Table 1 summarizes this data from Tables 2 and 3.

Table 1

Summary of Data for All Fish and Fish Grouped on a Weight Basis

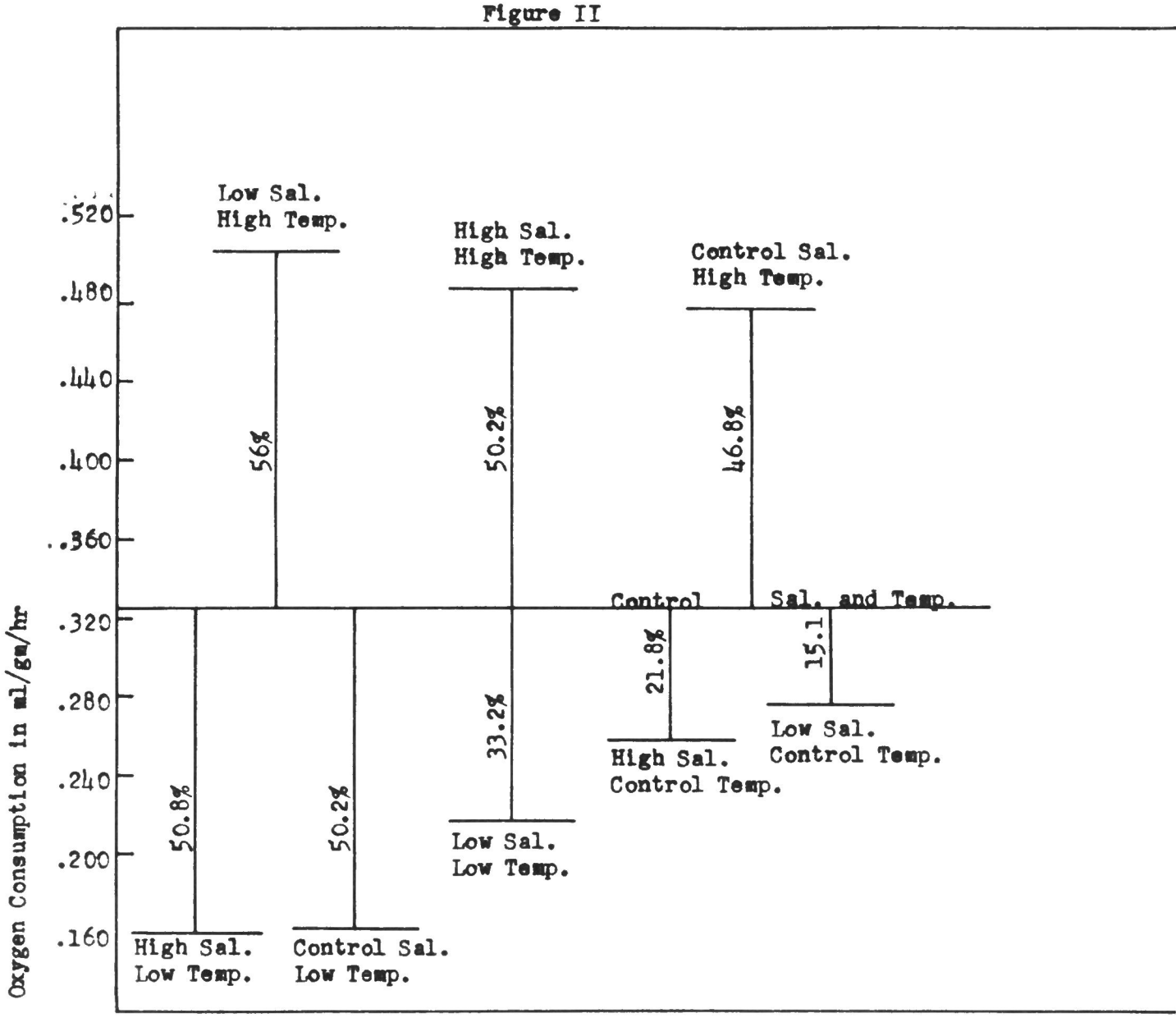
Temperature Salinity	Mean Weight of Fish	Mean O_2 Consumption	S.D. of O_2 Consumption
All Fish			
9°C, 29°/oo	5.09gm	.162ml/gm/hr	.049
20°C, 29°/oo (Control)	4.85	.325	.093
31°C, 29°/oo	4.84	.477	.127
9°C, 49°/oo	4.29	.160	.020
20°C, 49°/oo	4.10	.254	.031
31°C, 49°/oo	4.96	.488	.125
9°C, 9°/oo	4.46	.217	.002
20°C, 9°/oo	4.48	.276	.088
31°C, 9°/oo	5.34	.507	.146
Fish 3.00-6.00gm			
9°C, 29°/oo	3.92gm	.192ml/gm/hr	.049
20°C, 29°/oo	4.32	.349	.089
31°C, 29°/oo	4.30	.496	.155
9°C, 49°/oo	4.29	.160	.020
20°C, 49°/oo	4.24	.254	.039
31°C, 49°/oo	4.54	.538	.077
9°C, 9°/oo	4.46	.217	.002
20°C, 9°/oo	4.03	.291	.090
31°C, 9°/oo	4.81	.531	.196

A standard t-test for small samples was used to determine the level of significance between each of the experimental groups and between the experimental groups and the control. This data was computed by the East Carolina Computer Center. Tables 4 and 5 (Appendix) show the comparisons of levels of significance between each of the experimental groups and the control. Figures II and III summarize this graphically. Tables 6, 7, and 8 (Appendix) show the comparisons between the experimental groups. Figures IV - VII summarize this data graphically.

In comparing all experimental groups with the controls the only experimental group that did not show a significant change was the normal temperature, low salinity group.

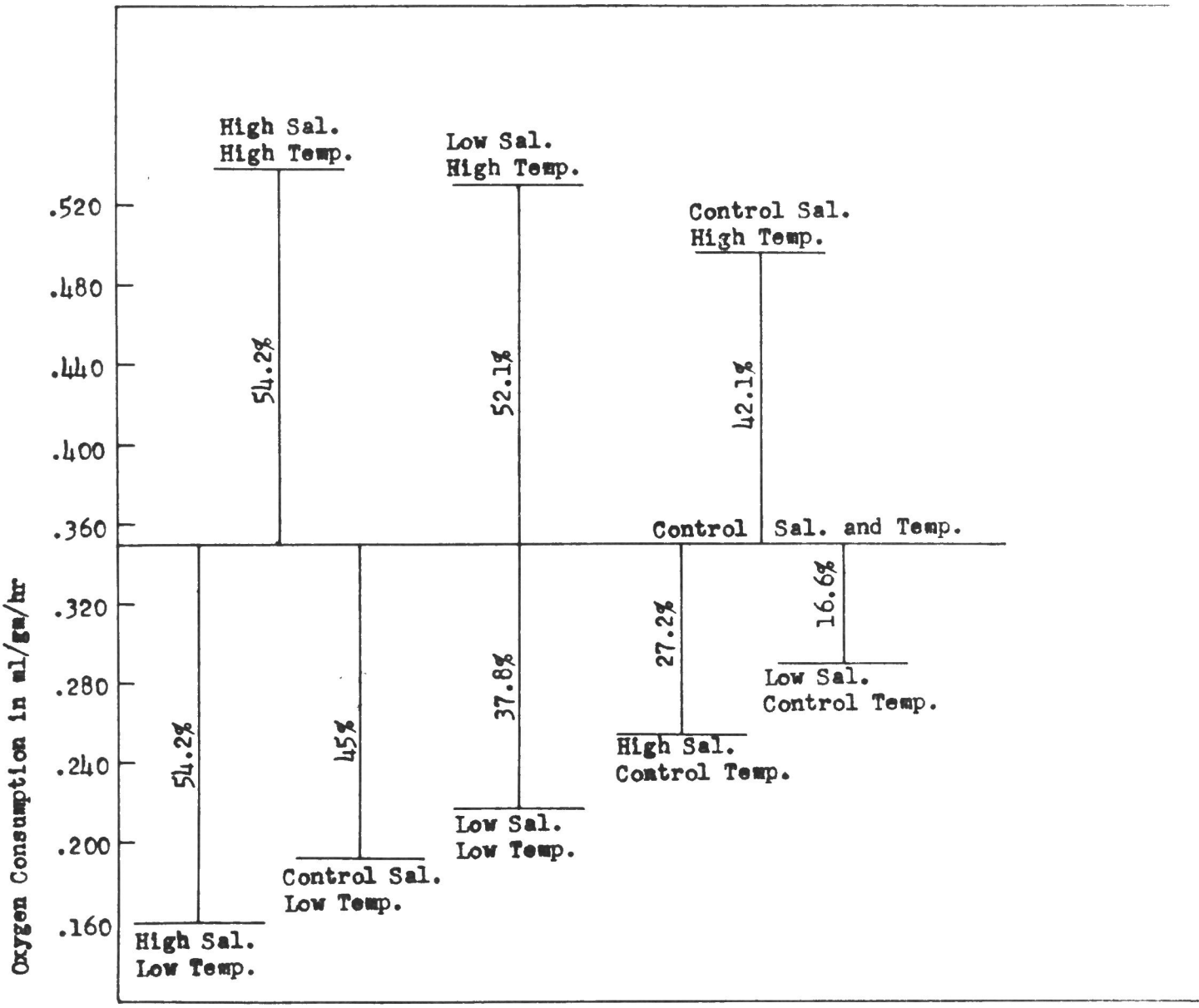
As was expected, the highest levels of significance were found when the experimental groups and the control groups were compared on the basis of temperature. The oxygen consumption of the high temperature groups increased from 46.8% to 56% over the control temperature group. The oxygen consumption of the low temperature groups decreased from 33.2% to 50.8% when compared with the control temperature group. Compared on this basis, the only group that did not show a significant change was the low temperature, low salinity group compared with the control temperature, low salinity group. The 33.2% decrease in the low temperature group was at the low salinity. Some slight modification of the temperature effect was obtained at the experimental salinities. These will be described in the comparisons of the groups on a salinity basis. At the control salinities the amount of change in oxygen consumption was in approximate agreement with that obtained by Wells (1935) for Fundulus parivipinnis.

Figure II



All Fish - Comparison of Experimental Groups and Control Group

Figure III



Fish, 3.00-6.00gm - Comparison of Experimental Groups and Control Group

Figure IV

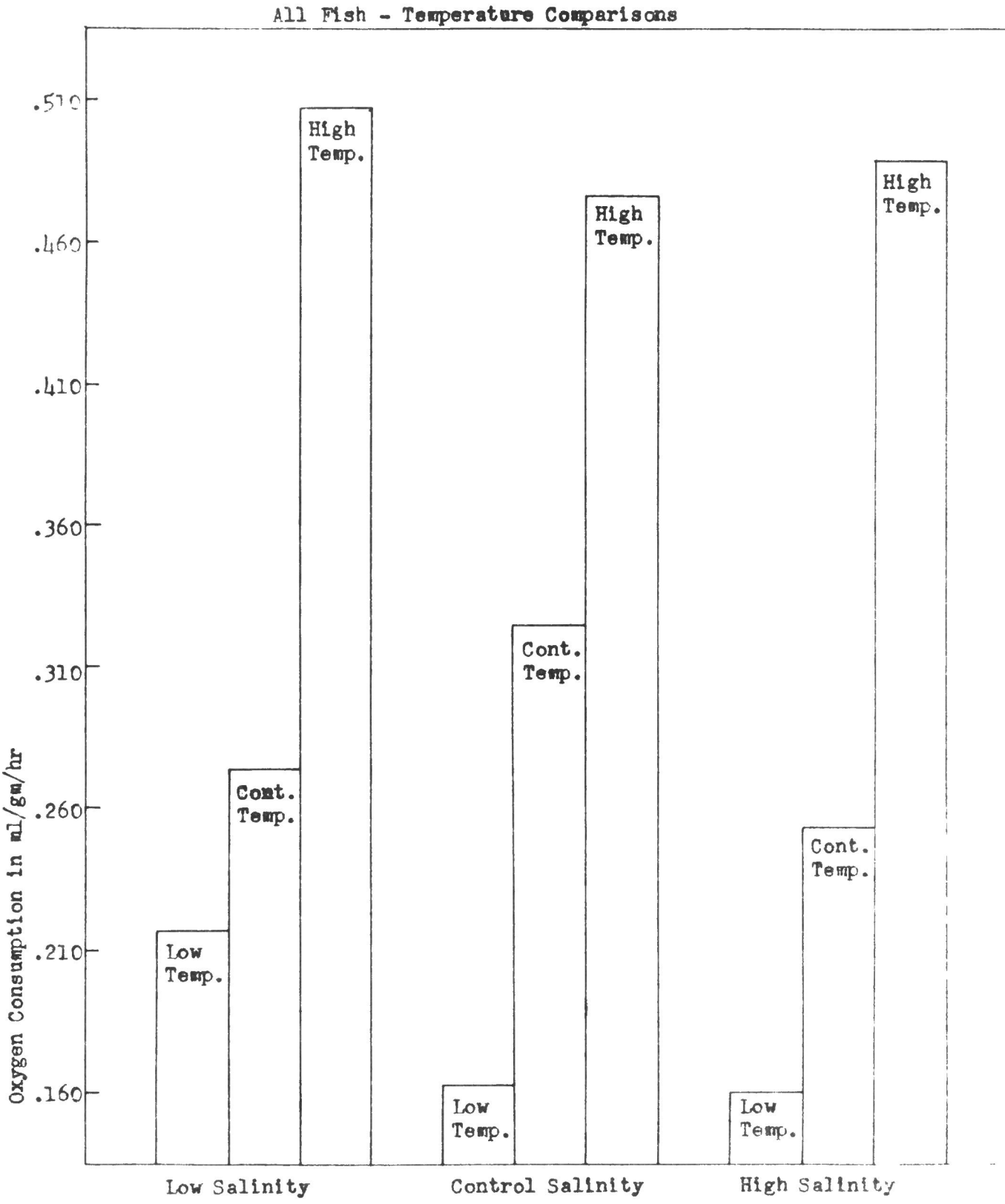

All Fish - Temperature Comparisons
.510
.460
.410
.360
.310
.260
.210
.160
Oxygen Consumption in ml/gm/hr
Low Temp.
Cont. Temp.
High Temp.
Low Temp.
Cont. Temp.
High Temp.
Low Temp.
Cont. Temp.
High Temp.
Low Salinity
Control Salinity
High Salinity

Figure V

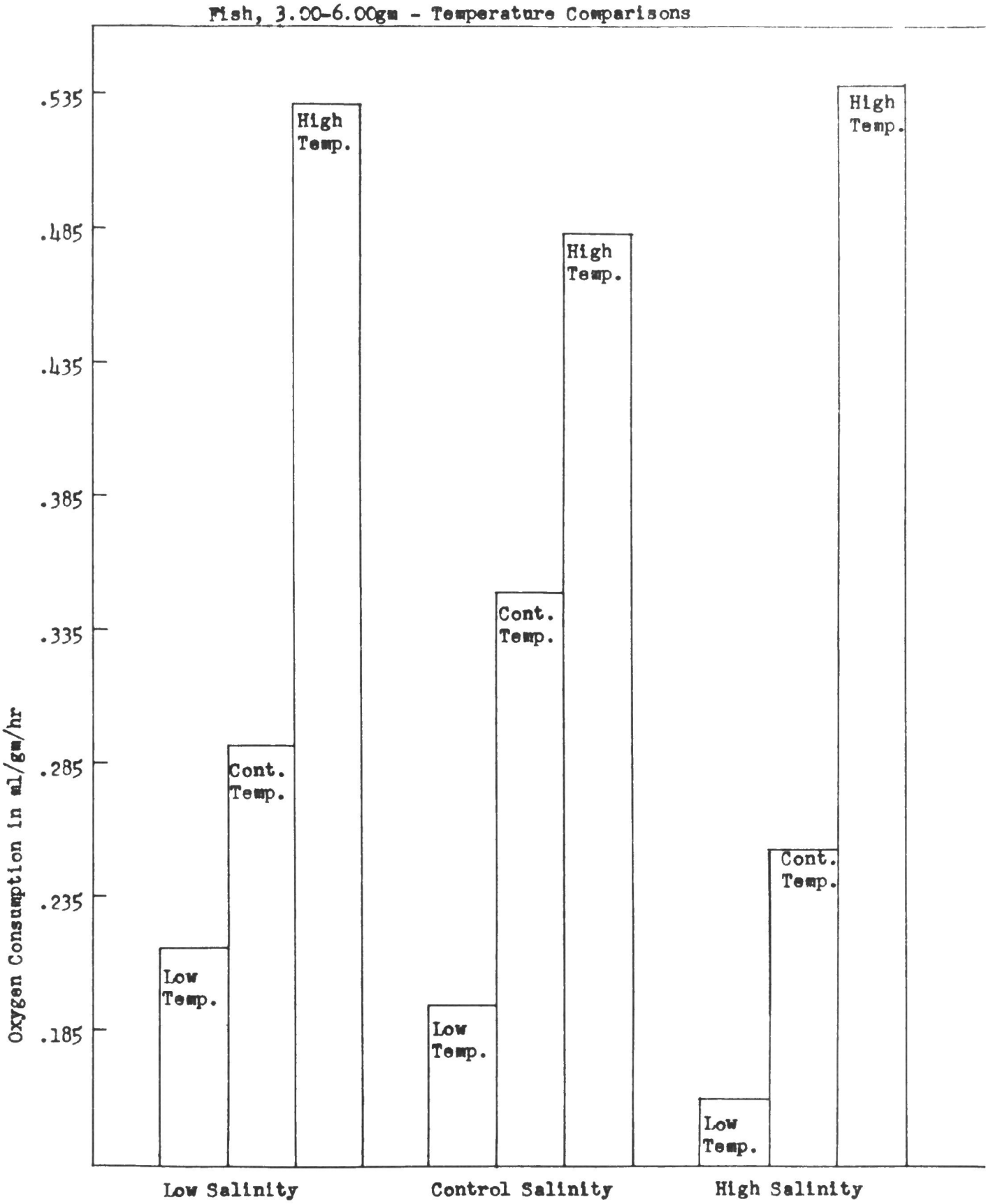

Fish, 3.00-6.00gm - Temperature Comparisons
Oxygen Consumption in ml/gm/hr
.535
.485
.435
.385
.335
.285
.235
.185
Low Temp.
Cont. Temp.
High Temp.
Low Temp.
Cont. Temp.
High Temp.
Low Temp.
Cont. Temp.
High Temp.
Low Salinity
Control Salinity
High Salinity

Figure VI

All Fish - Salinity Comparisons

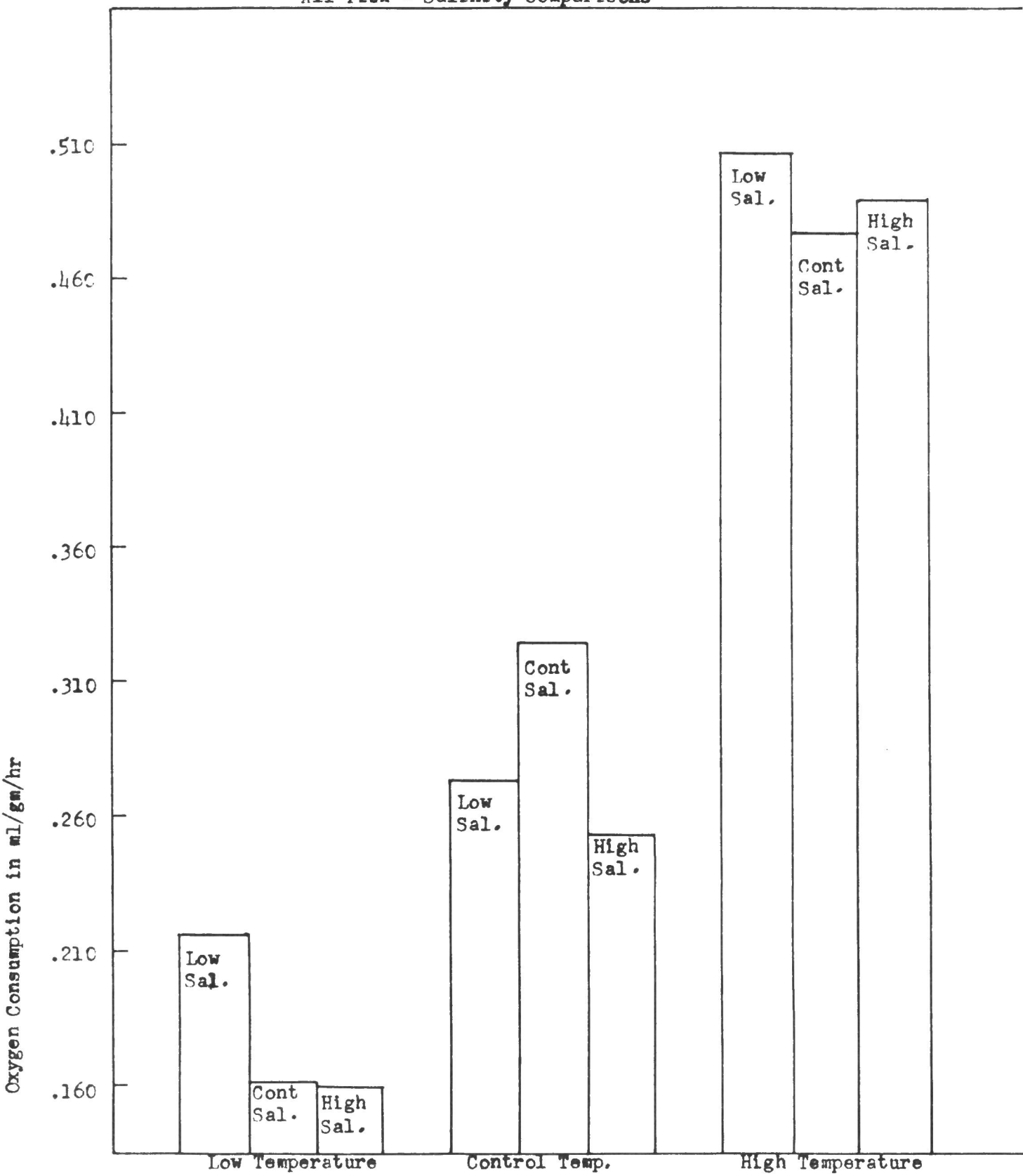
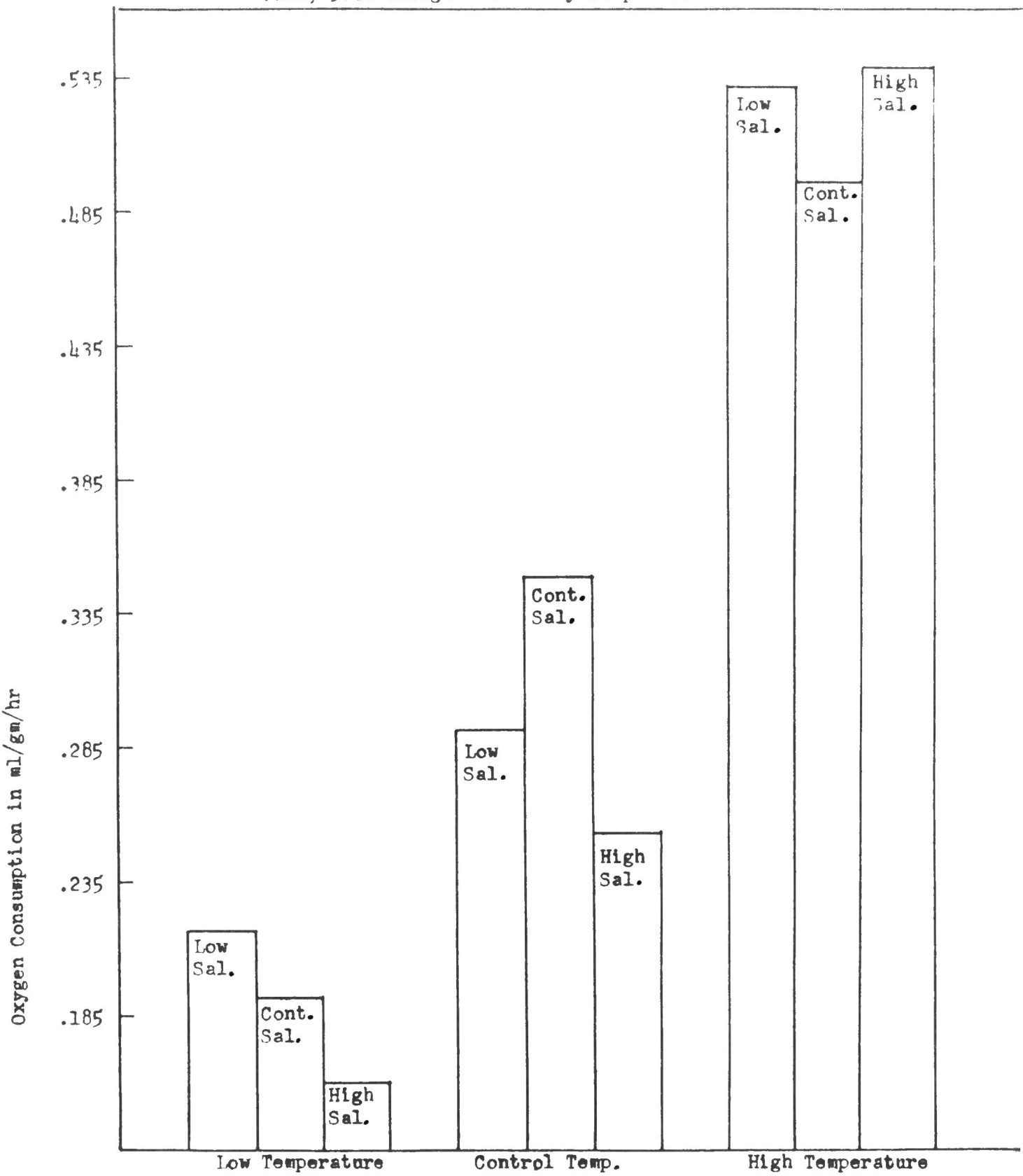


Figure VII

Fish, 3.00-6.00gm - Salinity Comparisons



The modifications of the oxygen consumption due to salinity variations appear to be much more ill-defined than those due to temperature change. No definite pattern could be established for all temperature groups on the basis of salinity change, but within each temperature group a pattern could be established even though some of the changes were not at a level of significance above 95%. At the high and low temperatures, the lowest salinity group always showed the highest mean oxygen consumption (see Figure II). On a weight comparison this pattern did not remain in the high temperature group. At the control temperature the least amount of change occurred in the low salinity group, with the low salinity group having a higher oxygen consumption than the high salinity group.

When the significance levels between the groups on a salinity basis were checked, the only levels found above 90% were the control temperature, control salinity group compared with the control temperature, high salinity group; and the low temperature, low salinity group compared with both the control salinity and high salinity groups. When these same groups were checked in the 3.00-6.00gm weight range, the only levels of significance found to be above 90% were the control temperature, control salinity compared with the control temperature, high salinity group; and the low temperature groups compared at the low salinity and high salinity. This is shown in Tables 6 and 7.

Certain behavioral patterns were noted that seemed to be constant throughout the experiment. They are as follows:

1. high temperature, all salinities - very little activity, increased opercular movements, normal movement of pectoral fins.

2. control temperature, all salinities - normal movement of animal, possibly a little less at experimental salinities.

3. low temperature, all salinities - initial shock shown by loss of equilibrium; after initial shock, activity appeared to be sporatic with violent bursts of energy followed by very little movement. These behavioral patterns correspond to those described by Brett (1956).

CHAPTER IV

DISCUSSION

The changes in oxygen consumption that were recorded in this experiment are basically those that can be attributed to the initial response of any fish to changes in temperature, the oxygen consumption increasing with the increased temperature and decreasing with the lowered temperature. Wells (1935) stated that the oxygen consumption increases with the increasing temperature in Fundulus parvipinnis, with the increase being greater in the small fish than in large ones. Fry (1957) stated that for confined fish the standard metabolism shows an ever-increasing rate with increasing temperature, while in active metabolism the metabolic rate is related to the animal's activity. Fry and Hart (1948) found that in goldfish the standard metabolism increased when the temperature was varied from 5°C to 35°C. Any modifications of this pattern under the conditions of this research would therefore have to be considered to be due to the alteration of the salinity and its effect on the balancing of the metabolic activities necessary for life.

The pattern of the initial response of Fundulus heteroclitus in this experiment follows the individual patterns of adaptation to the factors of temperature and salinity. The difficulty of adaptation to both lowered salinity and temperature is greater than adaptation to increased salinity and temperature. Brett (1956) stated the gain of tolerance to temperatures above 20°C requires less than 24 hours while the gain in resistance to low temperatures can take up to 20 days. Black (1948) found that acclimation to fresh water required at least 24 hours while the chloride content of the blood and body density returned to normal in six hours after the fish were returned to sea water from fresh water.

The relative ease with which eurythermal fish can adapt to elevated temperatures is the increased rate of cellular metabolism caused by the rise in temperature. This increased rate of metabolism accelerates the rate of adaptation as long as the amount of dissolved oxygen available for respiration is not limited (Brett, 1956; Fry, 1958). The increased metabolic rate would also speed up the mechanisms necessary for salinity adaptations as long as the combination of temperature and salinity does not exceed the animal's ability to adjust (Doudoroff, 1945). The difficulty in adaptation to lowered temperatures is shown in Fundulus heteroclitus by the effect of temperature on brain cholinesterase activity. Baslow and Nigrelli (1964) found that lethal high temperatures in Fundulus did not inactivate brain cholinesterase while cold lessened the activity of this enzyme. Since the ability to maintain proper osmotic and ionic balance involves the coordinating mechanism of nervous tissue, this could explain some of the difficulty of adaptation to combinations of salinity and lowered temperature.

Stanly and Fleming (1964) have reported the production of urine hypertonic to the blood in Fundulus kansae during periods of adaptation to increased salinity. Gordon (1963) reported in rainbow trout that a major mechanism responsible for osmoregulation is a change in skin permeability, the permeability decreasing with lowered salinity and increasing with increased salinity. The combination of two factors such as these could account for the relative ease of adaptation to increased salinity.

The difficulty of adaptation to the lowered environmental factors could account for the increase of oxygen consumption of the low salinity groups at the high and low temperatures. The oxygen consumption of certain euryhaline invertebrate species is also greater in dilute brackish water

than in sea water (Rao, 1958). This is the general pattern of oxygen consumption shown in this experiment at the high and low temperatures, but it is not the case at the control temperature. Here the oxygen consumption of both the low salinity and high salinity groups dropped below the control group. This drop in oxygen consumption may be due to a change in the animal's muscular activity. Brett (1956) described the same initial reaction of fish to lowered temperature that was reported in this experiment. Fisher (1958) also reported that any abrupt change in temperature can cause a slowing of muscular activity. From these it can be seen that there is a definite behavioral adaptation to temperature change. Therefore, if similar behavior is present in F. heteroclitus, the animal could be reducing muscular activity in response to an osmotic stress.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. The oxygen consumption of Fundulus heteroclitus was measured under all possible combinations of 9°/oo, 29°/oo, and 49°/oo salinities and 9°C, 20°C, and 31°C temperatures. This was done to establish the initial pattern of the response of the fish to the combined stresses of temperature and salinity.

2. At the temperature and salinity combinations used, the temperature changes caused the greatest effect in oxygen consumption. At the high temperature, the oxygen consumption showed increases of 46.8% to 56% over the oxygen consumption of the animals at the control temperature. The low temperature groups showed a decrease in oxygen consumption of 33.2% to 50.8%.

3. Within each temperature group, the oxygen consumption was related to the salinity even though some of the differences were not significant. At the high and low temperature, the low salinity groups showed the highest oxygen consumption. The only significant differences found that could be caused by a salinity change were found in comparing the low temperature, low salinity group with the low temperature, control salinity group and the low temperature, low salinity group compared with the low temperature, high salinity group.

4. The pattern of response of these fish follows that established by earlier researchers for the individual factors of temperature and salinity. The adaptations to increased temperature and increased salinity require less expenditure of energy than the adaptations to lowered temperature and lowered salinity. In this experiment, temperature appeared to show the greatest overall effect.

5. The increased metabolic rate at increased temperatures speeds the rate of adaptation to the osmotic and ionic changes caused by the salinity changes. The effect of the lowered temperature would cause the reduction in the ability of the animal to osmoregulate. A decrease in the activity of the animal could cause the reduction in oxygen consumption shown by the experimental salinities at the control temperature.

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APPENDIX

Table 2. Oxygen Consumption of All Fish

9°C 29°/oo	
Weight	O_2 Consumption
3.12gm	.188 ml/gm/hr
3.99	.181
4.04	.259
4.52	.142
6.13	.175
6.23	.116
6.24	.132
6.45	.109
mean weight	5.09
mean O_2 cons.	.162
S.D. O_2 cons.	.049

20°C 29°/oo	
Weight	O_2 Consumption
2.43gm	.454 ml/gm/hr
2.75	.286
3.55	.362
3.59	.351
3.68	.532
4.10	.374
4.25	.357
4.39	.251
4.92	.369
4.92	.280
5.51	.258
6.04	.305
6.94	.278
7.26	.183
8.50	.245
mean weight	4.85
mean O_2 cons.	.325
S.D. O_2 cons.	.093

31°C 29°/oo	
Weight	O_2 Consumption
3.53gm	.838 ml/gm/hr
3.64	.456
3.75	.505
4.28	.355
4.34	.359
4.42	.475
4.56	.426
5.88	.397
6.00	.653
8.01	.313
mean weight	4.84
mean O_2 cons.	.477
S.D. O_2 cons.	.127

9°C 49°/oo	
Weight	O_2 Consumption
3.57gm	.157 ml/gm/hr
3.60	.179
3.97	.149
3.98	.179
4.07	.183
4.11	.167
4.27	.165
5.45	.126
5.55	.137
mean weight	4.29
mean O_2 cons.	.160
S.D. O_2 cons.	.020

20°C 49°/oo	
Weight	O_2 Consumption
2.85gm	.257 ml/gm/hr
3.69	.213
4.02	.247
4.03	.298
4.04	.252
4.38	.225
4.45	.211
4.50	.287
4.52	.285
4.54	.273
mean weight	4.10
mean O_2 cons.	.254
S.D. O_2 cons.	.031

31°C 49°/oo	
Weight	O_2 Consumption
4.05gm	.626 ml/gm/hr
4.05	.474
4.11	.409
4.45	.624
4.50	.583
4.82	.537
5.04	.500
5.27	.553
6.56	.256
6.75	.325
mean weight	4.96
mean O_2 cons.	.488
S.D. O_2 cons.	.125

Table 2. (Continued)

9°C 9°/oo		20°C 9°/oo		31°C 9°/oo	
Weight	O_2 Consumption	Weight	O_2 Consumption	Weight	O_2 Consumption
3.10gm	.183 ml/gm/hr	3.55gm	.225 ml/gm/hr	2.97gm	.555 ml/gm/hr
3.49	.149	3.85	.431	3.337	.797
3.78	.284	3.97	.416	4.75	.441
4.25	.212	4.03	.218	4.79	.276
4.32	.325	4.06	.205	5.55	.513
4.58	.220	4.09	.243	5.58	.651
4.64	.164	4.19	.330	7.01	.431
4.78	.272	4.53	.265	7.03	.463
5.67	.138	6.06	.177	7.04	.456
5.95	.224	6.44	.253		
mean weight	4.46	mean weight	4.48	mean weight	5.34
mean O_2 cons.	.217	mean O_2 cons.	.276	mean O_2 cons.	.507
S.D. O_2 cons.	.062	S.D. O_2 cons.	.088	S.D. O_2 cons.	.146

Table 3. Oxygen Consumption of 3.00-6.00gm Fish

9°C 29°/oo

Weight	O_2 Consumption
3.12gm	.188 ml/gm/hr
3.99	.181
4.04	.259
4.52	.142
mean weight	3.92
mean O_2 cons.	.192
S.D. O_2 cons.	.049

20°C 29°/oo

Weight	O_2 Consumption
3.55gm	.302 ml/gm/hr
3.59	.351
3.68	.532
4.10	.374
4.25	.357
4.39	.251
4.92	.369
4.92	.280
5.51	.258
mean weight	4.32
mean O_2 cons.	.349
S.D. O_2 cons.	.089

31°C 29°/oo

Weight	O_2 Consumption
3.50gm	.838 ml/gm/hr
3.64	.456
3.75	.505
4.28	.355
4.34	.359
4.42	.475
4.56	.426
5.88	.397
mean weight	4.30
mean O_2 cons.	.496
S.D. O_2 cons.	.155

9°C 49°/oo

Weight	O_2 Consumption
3.57gm	.157 ml/gm/hr
3.60	.179
3.97	.149
3.98	.179
4.07	.183
4.11	.167
4.27	.165
5.45	.126
5.55	.137
mean weight	4.29
mean O_2 cons.	.160
S.D. O_2 cons.	.020

2 20°C 49°/oo

Weight	O_2 Consumption
3.69gm	.213 ml/gm/hr
4.02	.247
4.03	.298
4.04	.252
4.38	.225
4.45	.211
4.50	.287
4.52	.285
4.54	.273
mean weight	4.24
mean O_2 cons.	.254
S.D. O_2 cons.	.039

31°C 49°/oo

Weight	O_2 Consumption
4.05gm	.626 ml/gm/hr
4.05	.474
4.11	.409
4.45	.624
4.50	.583
4.82	.537
5.04	.500
5.27	.553
mean weight	4.54
mean O_2 cons.	.538
S.D. O_2 cons.	.077

9°C 9°/oo

Weight	O_2 Consumption
3.10gm	.183 ml/gm/hr
3.49	.149
3.78	.284
4.25	.212
4.32	.325
4.58	.220
4.64	.164
4.78	.272
5.67	.138
5.95	.224
mean weight	4.46
mean O_2 cons.	.217
S.D. O_2 cons.	.062

20°C 9°/oo

Weight	O_2 Consumption
3.53gm	.223 ml/gm/hr
3.85	.431
3.97	.416
4.03	.218
4.06	.205
4.09	.243
4.19	.330
4.53	.265
mean weight	4.03
mean O_2 cons.	.291
S.D. O_2 cons.	.090

31°C 9°/oo

Weight	O_2 Consumption
3.37gm	.797 ml/gm/hr
4.75	.441
4.79	.276
5.55	.513
5.58	.631
mean weight	4.81
mean O_2 cons.	.531
S.D. O_2 cons.	.196

Table 4

All Fish - Comparison of Control and Experimental

	Mean O_2 Consumption	T-Score	Level of Significance				
			Below 90%	90-95%	95-98%	98-99.9%	Above 99.9%
29°/oo, 20°C vs 49°/oo, 20°C	.325 .254	2.419			X		
29°/oo, 20°C vs 9°/oo, 20°C	.325 .276	1.376	X				
29°/oo, 20°C vs 9°/oo, 9°C	.325 .217	3.364				X	
29°/oo, 20°C vs 29°/oo, 9°C	.325 .162	4.796					X
29°/oo, 20°C vs 49°/oo, 9°C	.325 .160	5.481					X
29°/oo, 20°C vs 9°/oo, 31°C	.325 .507	-3.816					X
29°/oo, 20°C vs 29°/oo, 31°C	.325 .477	-3.077				X	
29°/oo, 20°C vs 49°/oo, 31°C	.325 .488	-3.832					X

Table 5

Fish, 3.00-6.00gm - Comparison of Control and Experimental

	Mean O2 Consumption	T-Score	Level of Significance				
			Below 90%	90-95%	95-98%	98-99.9%	Above 99.9%
29°/oo, 20°C vs 49°/oo, 20°C	.349 .254	3.110				X	
29°/oo, 20°C vs 9°/oo, 20°C	.349 .291	1.356	X				
29°/oo, 20°C vs 9°/oo, 9°C	.349 .217	3.908				X	
29°/oo, 20°C vs 29°/oo, 9°C	.349 .192	3.394				X	
29°/oo, 20°C vs 49°/oo, 9°C	.349 .160	6.498					X
29°/oo, 20°C vs 9°/oo, 31°C	.349 .531	-2.463			X		
29°/oo, 20°C vs 29°/oo, 31°C	.349 .496	-2.471			X		
29°/oo, 20°C vs 49°/oo, 31°C	.349 .538	-4.838					X

Table 6

All Fish - Comparison of Temperature Effects

Control vs Experimental	Mean O^2 Consumption	T-Score	Level of Significance				
			Below 90%	90-95%	95-98%	98-99.9%	Above 99.9%
20°C, 29°/oo	.325	-3.077				X	
vs 31°C, 29°/oo	.477						
20°C, 29°/oo	.325	4.796					X
vs 9°C, 29°/oo	.162						
20°C, 9°/oo	.276	-4.237					X
vs 31°C, 9°/oo	.507						
20°C, 9°/oo	.276	1.740		X			
vs 9°C, 9°/oo	.217						
20°C, 49°/oo	.254	-5.749					X
vs 31°C 49°/oo	.488						
20°C, 49°/oo	.254	7.775					X
vs 9°C, 49°/oo	.160						

Fish 3.00-6.00gm - Comparison of Temperature Effects

20°C, 29°/oo	.349	-2.471			X		
vs 31°C, 29°/oo	.496						
20°C, 29°/oo	.349	3.394				X	
vs 9°C, 29°/oo	.192						
20°C, 9°/oo	.291	-3.040				X	
vs 31°C, 9°/oo	.531						
20°C, 9°/oo	.291	2.074		X			
vs 9°C, 9°/oo	.217						
20°C, 49°/oo	.254	-10.284					X
vs 31°C, 49°/oo	.538						
20°C, 49°/oo	.254	7.335					X
vs 9°C, 49°/oo	.160						

Table 7

All Fish - Comparison of Salinity Effects

Control vs Experimental	Mean O_2 Consumption	T-Score	Below 90%	Level of Significance			
				90-95%	95-98%	98-99.9%	Above 99.9%
29°/oo, 20°C vs 49°/oo, 20°C	.325 / .254	2.419		X			
29°/oo, 20°C vs 9°/oo, 20°C	.325 / .276	1.376	X				
49°/oo, 20°C vs 9°/oo, 20°C	.254 / .276	- .723	X				
29°/oo, 9°C vs 49°/oo, 9°C	.162 / .160	.142	X				
29°/oo, 9°C vs 9°/oo, 9°C	.162 / .217	-2.030		X			
49°/oo, 9°C vs 9°/oo, 9°C	.160 / .217	-2.645				X	
29°/oo, 31°C vs 49°/oo, 31°C	.477 / .488	- .172	X				
29°/oo, 31°C vs 9°/oo, 31°C	.477 / .507	- .417	X				
49°/oo, 31°C vs 9°/oo, 31°C	.488 / .507	- .294	X				

Table 8

Fish, 3.00-6.00gm - Comparison of Salinity Effects

Control vs Experimental	Mean O_2 Consumption	T-Score	Below 90%	Level of Significance			
				90-95%	95-98%	98-99.9%	Above 99.9%
29°/oo, 20°C vs 49°/oo, 20°C	.349 .254	3.110				X	
29°/oo, 20°C vs 9°/oo, 20°C	.349 .291	1.356	X				
49°/oo, 20°C vs 9°/oo, 20°C	.254 .291	-1.143	X				
29°/oo, 9°C vs 49°/oo, 9°C	.192 .160	1.758	X				
29°/oo, 9°C vs 9°/oo, 9°C	.192 .217	- .709	X				
49°/oo, 9°C vs 9°/oo, 9°C	.160 .217	-2.645				X	
29°/oo, 31°C vs 49°/oo, 31°C	.496 .538	- .692	X				
29°/oo, 31°C vs 9°/oo, 31°C	.496 .531	- .373	X				
49°/oo, 31°C vs 9°/oo, 31°C	.538 .531	.087	X				

Table 9

Average Analysis of Utility Seven-Seas Marine Mix

Sodium Chloride	27.5 gm/l
Magnesium Chloride	5.38
Magnesium Sulfate	6.77
Potassium Chloride	.722
Sodium Bicarbonate	.200
Strontium Chloride	19.7 mg/l
Manganese Sulfate	3.95
Disodium Phosphate	3.29
Lithium Chloride	.987
Sodium Molybdate	.987
Calcium Chloride	1.375gm/l
Calcium Gluconate	.658mg/l
Potassium Iodide	.095
Potassium Bromide	.0285
Aluminum Sulfate	.475
Cobalt Sulfate	.0526
Rubidium Chloride	.157
Copper Sulfate	.448
Zinc Sulfate	.101

Based on 1 lb. Utility Seven-Seas Marine Mix per 3 gallons of water

Data furnished by Utility Chemical Company, 145 Peel Street, Paterson, N. J.

Table 10

Procedure for Salinity Titration

1. Ten drops of 5% KCr_2O_3 is added to a 50ml sample of the water to be tested.

2. The sample is then titrated with a 0.5 molar $AgNO_3$ solution until the appearance of a pink color.

3. Calculations for salinity:

   (a) molarity of Cl = $\frac{(\text{Vol } AgNO_3)\ (\text{molarity of } AgNO_3)}{\text{sample vol in ml}}$

   (b) chlorosity = molarity of Cl X 35.5

   (c) chlorinity = $\frac{\text{chlorosity}}{\text{density of sample at } 20^{\circ}C}$

   (d) salinity in °/oo = 0.03 + (1.805 X chlorinity)