THE EFFECT OF CALCIUM ON THE OSMOTIC TOLERANCE OF JUVENILE STRIPED MULLET (*MUGIL CEPHALUS* L.)[1]

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ABSTRACT

Juvenile striped mullet, ranging in size from 25 mm to 150 mm standard length and acclimated to 10% and 50% seawater, were subjected to direct transfer from acclimation salinity to salinity levels varying from distilled water to 200% seawater. Tests were also made using two commercially prepared, osmotically balanced, synthetic seawater mixes; one containing twice the normal calcium level and the other calcium-free. The presence of calcium significantly increased both survival time and survival rate. Acclimation tests also indicated a beneficial effect from increased calcium levels.

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INTRODUCTION

Mugil cephalus L. is an extremely successful euryhaline teleost of world-wide tropical and sub-tropical distribution. It supports a thriving fishery almost every place it is found and is becoming increasingly more important in culture efforts (Perlmutter *et al.*, 1957; Pownall, 1969) due to its wide physiological tolerances and advantageous position on the food chain (Odum, 1968; 1970).

It has been reported from salinities as high as 113‰ (Zenkevich, 1963) and from freshwaters with chlorinities as low as 3 parts per million (Odum, 1953). The exact limits of its salinity tolerances and their relationship to temperature have never been determined. To maximize the value of this hardy and productive fish in future culture efforts, its physiological tolerances and their effects on production should be investigated.

Ionic and osmotic regulation in teleost fishes has been extensively studied, and several good reviews exist (see Black, 1957; Parry, 1966; Hickman and Trump, 1969; and Conte, 1969). The ability of marine fishes to enter and survive certain freshwaters requires a readjustment in the osmoregulatory process which has long been of interest (Breder, 1934; Gunter, 1956; Odum, 1953) but of which relatively little is actually known. External physical factors may well play a major role in the adaptation of these fishes to a hypoosmotic environment. Odum

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(1953) has attributed the ability of these animals to maintain the osmotic pressure of their body fluids to the relatively high salt content of the freshwaters in which they are found. In contrast to this, other investigators have felt that the high calcium content of the specific waters involved was the prime factor (Breder, 1934; Black, 1957; Huets, 1944; Hulet, *et al.* 1967).

With these questions in mind, investigations were conducted to determine what salinity changes the mullet could tolerate with reasonable survival, and the effects calcium played on this tolerance.

MATERIALS AND METHODS

Juvenile striped mullet, ranging in size from 25 mm to 150 mm were collected from three nursery areas along the Gulf Coast of Florida during the spring and early summer of 1970 and 1971. A description of these three areas is given in Table 1. The fish were collected by beach seine from the sites described and transported to the laboratory in ice chests, where they were placed in 10 gallon aquaria and allowed to adjust to laboratory conditions for a period of three to five days. Fish collected from the high salinity area described in Table 1 were acclimated to 17‰ or 50% seawater, while those fish collected in the low salinity areas were acclimated to 3.4‰ or 10% seawater. All fish were held in a constant temperature chamber at the temperatures recorded in the field at the time of their collection. Temperatures were taken either with a mercury thermometer or with the thermistor of an induction salinometer, approximately one foot below the surface of the water.

Two series of experiments were run. These are summarized in Tables 2A and 2B. Series I consisted of three sets of experiments in which the fish underwent a direct transfer from acclimation salinity to one of the test levels listed in Table 2A. The experimental concentrations were prepared from natural seawater collected from the Gulf of Mexico at Pensacola Beach, Florida and stored under refrigeration in polyethylene carboys until used. A commercial seawater mix was added to prepare those test levels which exceeded 100% seawater.

The second series, Series II, of tests consisted of both direct transfer and acclimation of the juvenile mullet to dilutions of two commercially prepared artificial seawater mixtures. The experimental levels are given in Table 2A. Information concerning specific composition of the seawaters used is presented in Table 3. Basically, one mixture was "free" of calcium and the other mix had twice the calcium level of "normal" oceanic seawater, or approximately 800 ppm. The concentrations in this series of experiments ranged from 50% seawater to distilled water.

After the initial adjustment period, fish were transferred in groups of ten to the different test situations. In the direct transfer tests the fish were herded from the holding tanks into a large glass beaker. The water in the beaker was then adjusted to each desired salinity, and the fish were then placed into the respective experimental tank. In the acclimation tests all tanks contained water of the initial acclimation salinity at the outset. The desired dilution was arrived at by the removal of approximately four liters of water from the tanks and its replacement with the same volume of distilled water every four hours until the final salinity was reached.

All experiments were conducted in 5 gallon aquaria, in which the water was aereated, but not filtered. Wastes were removed daily by means of a commercially available device. Tests were allowed to run for a period of five days. The tanks were checked for mortalities at least once every four hours during the first twenty-four hour period, and once every eight hours during the remainder of the experiment.

RESULTS AND DISCUSSION

There were three experiments in Series I, each using fish which probably had a different history of exposure to dilute salinities. These experiments consisted of direct transfers of the fish from the acclimation levels to the test salinity. In Experiment I-A, fish from the Big Sabine Bay area were used (see Table 1). These were from a high salinity area and had been acclimated to 50% seawater prior to the test. Experiment I-B used fish from Perdido Bay, a moderately dilute environment, which had been acclimated to 10% seawater. Experiment I-C used fish from Mullato Bayou of upper Escambia Bay. These fish had been acclimatized to the most dilute environmental conditions. They also were held at 10% seawater prior to the experiment.

The effect of the different acclimation histories is clearly evident in Table 2A. The percent mortality is higher for those fish from the higher environmental salinity, and higher still for those which were acclimated to 50% seawater prior to the experiment, (Exp. I-A) in contrast to the two others which were both acclimated to 10% seawater.

Acclimation to the new salinity level, at least as far as it affected mortality, was a relatively swift provess. Upon transfer to the more dilute test levels, affected fish evidenced considerable trouble in swimming and would often go into a sort of "spasmodic tetany" when startled. Another common characteristic of stricken fish was its loss of equilibrium. Often fish which were in obvious distress would survive for hours, swimming about upside down, and only in the final minutes show extreme signs of distress such as spasmodic gill movements and hemorraging about the fins and anus. More than ninety-five percent of all mortalities occurred within the first 24 hour period, and approximately one-third of these within the first four hours of the experiment. Significant mortality did not occur at concentrations above 5% seawater, even for those fish acclimated to 50% seawater.

The results of the second series of experiments, those involving experimental calcium levels, are given in Table 2B. These experiments were all conducted using fish from a high salinity area and acclimated to 50% seawater prior to the start of the experiment. Both direct transfer and acclimation experiments were attempted.

The lack of calcium shows no appreciable effect on the direct transfer of mullet to more dilute surroundings (compare Tables 2B and 2A). Increasing the environmental calcium however, beneficially effects both the survival rate and time. It is not known if this is simply due to the effect of the calcium alone or to other factors not taken into account when preparing the experimental media. It will require replication of these results to insure that these results are significant.

Only total mortality is reported for the acclimation test. There was only one experiment performed, II-B, and comparison of survival times with the direct transfer experiments is meaningless due to the slow rate of dilution. In comparing the percentage mortality of the acclimation test with that of the direct transfer tests, the beneficial effect of both increased calcium and gradual acclimation to the more dilute environment is obvious. Again, replicate tests will be necessary to determine to what extent each of these variables is involved.

CONCLUSION

While the results presented here are quite preliminary, they will still serve to give some insight to the plasticity of such a hardy fish as the mullet. In terms of culturing efforts, there is some indication that low level additions of calcium may serve to ameliorate osmotic shock and promote growth by accelerating the acclimation to a more dilute environment.

Table 1. Summary of Characteristics of Collection Areas.

Locality	Salinity Range	Bottom Type	Major Fish Species Captured with Mullet
1. Mulatto Bayou Escambia Bay, Florida	0.3‰ - 8.0‰	Mud & Sandy Silt	*Brevoortia patronus* *Leiostomus xanthurus*
2. Interarity Pt. Perdido Bay, Florida	1.2‰ - 12.4‰	Sand & Mud	*Anchoa mitchilli* *Leiostomus xanthurus*
3. Big Sabine Bay Santa Rosa Sound, Florida	18.1‰ - 31.0‰	Sand & Plant Detritus	*Mollingsia latipinna* *Haemulon sciurus*

Table 2A. Summary of Experimental Procedure and Results.
Series I = Direct Transfer Experiments, 10 Fish Per Test
Total Time Elapsed = 120 Hours

Experiment I-A

Seawater Concentrations (100%SW = 34‰)	Approximate Elapsed Time Initial Mortality	50% Mortality	Final Mortality	Total Mortality (%)
200% SW	10 hrs	-	10 hrs	10%
150% SW	-	-	-	0%
100% SW	-	-	-	0%
50% SW	-	-	-	0%
25% SW	-	-	-	0%
10% SW	8 hrs	-	21 hrs	20%
5% SW	2 hrs	41 hrs	56 hrs	60%
1% SW	0.5 hrs	9 hrs	56 hrs	100%
Freshwater (Chlorinity < 10ppm)	0.5 hrs	3.5 hrs	12 hrs	100%
Distilled H2O	0.3 hrs	2.5 hrs	4 hrs	100%

Experiment I-B

Seawater Concentrations (100% SW = 34‰)	Approximate Elapsed Time Initial Mortality	50% Mortality	Final Mortality	Total Mortality (%)
200% SW	1 hr	-	12 hr	20%
150% SW	-	-	-	0%
100% SW	-	-	-	0%
50% SW	-	-	-	0%
25% SW	96 hr	-	96 hr	10%
10% SW	6 hr	-	8 hr	20%
5% SW	1 hr	-	9 hr	20%
1% SW	0.5 hr	6 hr	80 hr	100%
Freshwater (Chlorinity < 10ppm)	0.5 hr	4 hr	28 hr	90%
Distilled H2O	1 hr	2 hr	4 hr	100%

Table 2A(cont). Summary of Experimental Procedure and Results.

Experiment I-C				
Seawater Concentrations (100%SW = 34‰)	Approximate Elapsed Time Initial Mortality	50% Mortality	Final Mortality	Total Mortality (%)
200% SW	1 hr	-	1 hr	10%
150% SW	-	-	-	0%
100% SW	-	-	-	0%
50% SW	-	-	-	0%
25% SW	-	-	-	0%
10% SW	-	-	-	0%
5% SW	-	-	-	0%
1% SW	2 hr	-	2 hr	20%
Freshwater (Chlorinity < 10ppm)	1 hr	12 hr	24 hr	60%
Distilled H2O	0.5 hr	3 hr	8	100%

Table 2B. Summary of Experimental Procedure and Results

Series II = Direct Transfer and Acclimation Experiments, 10 Fish. Per Test, Total Time = 120 Hrs.

	Experiment II-A Approximate Elapsed Time				
Calcium "Free" Artificial Seawater (100%SW = 34‰)	Initial Mortality	(50%) Mortality	Final Mortality	Total Mortality (%)	Total Mortality (%)
50% SW	-	-	-	0%	0%
25% SW	-	-	-	0%	0%
10% SW	h hr	-	8 hr	20%	0%
5% SW	1 hr	4 hr	50 hr	60%	40%
1% SW	1 hr	4 hr	50 hr	100%	40%
Freshwater	0.5 hr	4 hr	24 hr	100%	60%
"Distilled Water"	0.5 hr	2 hr	4 hr	100%	90%
Twice "Normal" Calcium					
50% SW	-	-	-	0%	0%
25% SW	-	-	-	0%	0%
10% SW	-	-	-	0%	0%
5% SW	4 hr	24 hr	36 hr	60%	10%
1% SW	4 hr	24 hr	36 hr	60%	10%
Freshwater	4 hr	8 hr	36 hr	100%	30%

Table 3. Major Constituents of Seawater Used In This Study.

Constituent	Normal Mix* (ppm)	"Calcium Free" Mix* (ppm)	Twice "Normal Calcium" Mix* (ppm)	Natural Seawater** (ppm)
Cl	18400	18400	18600	18980
Na	10200	10800	10000	10556
SO4	2500	2500	2500	2649
Mg	1300	1300	1100	1272
K	370	370	370	380
Ca	370	0	800	400
HCO3	140	140	140	140
H3BO3	25	25	25	26
Br	20	20	2	

*These figures are for an artificial Seawater with a specific gravity of 1.025 at 15°C. The mixes were prepared by Aquar-Systems, Inc., 33208 Lakeland Blvd., Eastlanke, Ohio 44094.
**From Sverdrup *et al* (1942).

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