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Title of Study: THE REACTION OF GAMBUSIA TO SALT WATER AND METALLIC CHLORIDES

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Scope of Study: The study attempted to determine the toxic thresholds for the *Gambusia* in sea water and in solutions of metallic chlorides. Observations were made to determine if the lethal thresholds were the same for males and females. Concentrations of metallic chlorides from 0.0001 M. to 0.7 M. were employed.

Findings and Conclusions: All specimens were able to survive in 40 percent solutions of sea water. While all specimens were killed in solutions of 70 percent sea water.

The females were much more resistant to the toxic effects of sea water than the males.

All metallic chlorides produced lethal effects if the concentration was greater than 0.7 M. The heavier metallic chlorides were more toxic to the *Gambusia* than the lighter metallic chlorides.

The least toxic chloride was lithium and the most lethal chloride was mercury.

Adviser's Approval Roy W. Jones

THE REACTIONS OF *GAMBUSIA AFFINIS* (GIRARD)
TO SALT WATER AND METALLIC CHLORIDES

By

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Report Approved:

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PREFACE

Gambusia affinis (Girard) is a member of the Family Poeciliidae better known as the "Top-Water Minnows" or "Livebearers", also called the "Live-bearing Tooth Carps" and "Mosquito Fish". The members of this family are small fishes of the southeastern and southwestern areas of the United States. They are different from most other fresh-water fishes of the United States in their method of reproduction. The females give birth to living young.

Gambusia affinis is one of the fresh-water fishes which can adapt themselves to life in water of high salt content. These fishes are found in both the brackish waters of the gulf coast in southeastern United States and in the streams of Oklahoma with large amounts of salt water brine from oil wells and salt deposits. The *Gambusia* are also found in the fresh-water streams and ponds of Texas and Oklahoma.

In the literature there are many references to the toxicity of metallic chlorides to many different fishes; however, there is little literature dealing with the effects of chlorides upon the *Gambusia*. Because of the paucity of the experimental data in many of these papers, they were of little help on this problem.

The purpose of this study was to determine the toxicity of salt water to *Gambusia affinis*. This paper contains a study of the tolerance levels of the *Gambusia* to sodium chloride solutions. A series of experiments was performed to determine the thresholds of toxicity for the chlorides of various metals. The effects of sodium chloride upon the pregnant females and the process of birth, the effects of sodium chloride upon the newly born fish and the study of the susceptibility

of the sexes to toxic salt solutions are reported in this paper.

It is a pleasure to acknowledge indebtedness to Drs. R. W. Jones and L. H. Bruneau for their valuable assistances and guidance, and to Drs. R. L. Chermock and J. C. O'Kelly of the University of Alabama for their many helpful suggestions.

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CHAPTER I

THE PROBLEM

The problem was developed from the observation of the fact that the *Gambusia* can survive in brackish water without injurious effects. There are members of the *Gambusia* genus that live entirely in the salt water along the coast of Cuba. The *Gambusia* can maintain life in fresh water free of all metallic chlorides. The many phases of this problem were developed from the original observation that the *Gambusia* can be found living in water with high salt content. This problem was set up to determine if it is possible that salt water could be a barrier to movements of fresh-water *Gambusia* into the ocean.

The first phase of this problem dealt with determining the amount of sea water that the *Gambusia* could tolerate without injurious effects.

The second phase was set up to determine whether the *Gambusia* could survive in solutions of sodium chloride, and to determine the lethal thresholds. This phase of the problem was set up to determine whether the sodium chloride is the lethal agent in the sea water, or whether it is some other metallic chloride.

The third phase of the problem consisted of determining if the lethal thresholds for sea water were the same for males and females.

The fourth phase of this problem was to determine the toxicity thresholds for the chlorides that are found in sea water. This phase was set up to determine how much of the metallic chlorides is lethal to the *Gambusia*, and to determine if it is possible that one of these is the lethal agent in the sea water.

The final phase of this problem was set up to determine if the effects of sodium chloride would interfere with the normal processes of birth in the pregnant females. The effects of sodium chloride upon newborn young were observed during this phase. This phase was developed after the problem had started, when there were observed a number of premature young in the lethal solutions of sodium chloride.

CHAPTER II

REVIEW OF THE LITERATURE

In the literature there are many studies dealing with the effects of different metallic chlorides and salts upon many kinds of fresh-water fishes. Jones (1954) and Clemens (1954) reported in studies of the median toxicity thresholds for ten species of fish in brine wastes of oil wells a range of the thresholds from 4.3 to 11.2 percent of the original brine by volume. Median toxicity thresholds were determined also for species of fish in tapwater and sodium chloride. A comparison of these thresholds with those in brine water revealed that three species of fish (plain killifish, Gambusia and the black bullhead) could withstand more sodium chloride in brine waste than in a solution of sodium chloride alone. The reverse was true for three other species (green sunfish, red shiner, and the fathead minnow).

Axelrod (1955) reported that many fresh-water fishes can withstand certain amounts of salt in water, because many are collected in brackish water. Otto (1929) reported that there are striking individual variations to resistence to the increased salinity. Many fresh-water fishes are able to adapt themselves to life in water of high salinity. The Gambusia is one example.

Hankinson (1929) found that the most tolerant fresh-water fishes to salty waters were the common sticklebacks (Eucalia inconstone Kirt.) and the blackhead minnow (Pimephales promelas Raf.). Fresh-water fishes were found to be extremely sensitive to salinity changes by Raffy (1932). They would die promptly in salt water solutions if the ratio of salt water to fresh water was greater than one to one.

The brine wastes used by Jones (1954) and Clemens (1954) had a high concentration of chlorides, 119,000 p.p.m. With 59,700 p.p.m. of sodium and 11,000 p.p.m. of calcium, concentrations of the above ions were considerably higher than in fresh-water streams of Oklahoma and about twice as high as in sea water. The Gambusia had a lethal threshold of 7.4 percent over a 96 hour test period.

The threshold for the Gambusia in brine solutions was above 12,690 p.p.m. of sodium chloride, while the threshold for sodium chloride in tap water was above 10,670 p.p.m. The plain killifish was the most resistant of the ten species tested by Jones (1954) and Clemens (1954). The thresholds for the killifish in the brine waste was 18,783 p.p.m. of NaCl and in the sodium chloride solutions it was 16,000 p.p.m. of NaCl.

Sodium Chloride

Ellis (1937) found that goldfish (Carassius auratus) were unharmed by 5,000 p.p.m. (0.085 molar) of sodium chloride in Mississippi River water but that they survived 10 days or less in 10,000 p.p.m. (0.171 molar). Edmister (1948) and Gray (1948) reported that fry of yellow pikeperch were immobilized by 3,859 p.p.m. (0.066 molar) sodium chloride.

Young Sticklebacks (Gasterosteus aculeatus) were placed in solutions of 0.1 N. NaCl and were not killed. They were able to withstand solutions up to 0.25 N. (Ramult, 1929). Nekotyre (1944) reported that the Gambusia affinis could tolerate up to 4.5 percent (0.8 M. or 46,000 p.p.m.) solutions of sodium chloride without injurious effects.

Meyer (1937) working with young salmonoid fry reported that they could tolerate 1.3 percent (0.22 M. or 13,000 p.p.m.) solutions of sodium chloride. Oliphant (1941) working with young carp reported that they were killed by solutions that contained 15 parts per 1,000 (0.25 M. or 15,000 p.p.m.) of sodium chloride.

Egashira (1937) used male and female gold fish (Carassius auratus) which were placed in sodium chloride solutions consisting of 0.32 M. (19,708 p.p.m.), 0.3 M. (17,550 p.p.m.) and 0.25 M. (13,625 p.p.m.). The females were shown to be more resistant than males.

Other Chlorides

Ellis (1937) referred to only one set of experiments on fish with calcium chloride, in which golden shiners (Notemigonus crysoleucas) were killed by 5,000 p.p.m. (0.045 molar) in 143 hours. Edmister (1948) and Gray (1948) reported that fry of yellow pikeperch and whitefish were immobilized by 12,060 p.p.m. (0.118 molar) and 22,080 p.p.m. (0.2 M.) respectively.

Ramult (1929) used solutions of pure calcium chloride in the ratio of one to one, one to two, one to three and one to four parts distilled water. The stickleback minnows placed in these solutions were all killed.

Jones (1939) gave 7,355 p.p.m. (0.0123 molar) as the threshold for magnesium chloride to the three-spine stickleback. Welch (1935) reported that large doses of magnesium chlorides are toxic to the Gambusia.

Jones (1939) found that 2,163 p.p.m. (0.0137 molar) of strontium chloride was the threshold for the three-spine stickleback which is higher than with magnesium chloride and calcium chloride.

The effects of tin upon the fresh-water fish was reported by Finkel (1940). This report showed that the goldfish could survive in a solution of tin with the concentration of 1.3 p.p.m. (0.000005 molar). Hutchinson (1937) reported that fresh-water contains 13 p.p.m. of stanous chloride without harmful effects upon the fish living in it.

Jones (1939) reported that the three-spine stickleback died in all concentrations of manganese dichloride above 0.000728 molar (91.3 p.p.m.).

Ellis (1937) reported that 1,000 p.p.m. (0.0079 molar) ferrous chloride killed goldfish in less than 10 hours but 100 p.p.m. (0.00079 molar) was apparently not harmful in 96 hours.

Ellis (1937) reported that the threshold limits of lithium chloride was very high for the goldfish.

Jones (1939) found that 0.0029 molar (2,456 p.p.m.) solution of barium chloride was the threshold for the three-spine stickleback. In a comparison of metallic chlorides of barium, sodium, calcium, and magnesium it was reported that barium was the most toxic to the blunt-nose minnow (*Pimephales notatus*) (Power, 1937).

Ellis (1937) reported that 1,000 p.p.m. (0.0053 molar) stannous chloride in hard water killed goldfish in 4 to 5 hours.

Ellis (1937) found that 100 p.p.m. (0.00062 molar) ferric chloride kills goldfish in less than 90 minutes. Jones (1939) found that ferric chloride in concentrations of 1.2 p.p.m. (0.000021 molar) killed the three spine stickleback in six days.

Jones (1939) reported that the three-spine stickleback did not survive in concentrations of aluminum chloride greater than 0.0000026 molar (0.24 p.p.m.). Ellis (1937) found that 100 p.p.m. (0.00039 molar) aluminum chloride killed goldfish in 12 to 96 hours. Pulley (1950) working

Carins (1957).

Jones (1939) reported that the three-spine stickleback did not survive in concentrations of cupris chloride above 0.000000236 molar (0.02 p.p.m.). Ellis (1937) carried out a large series of experiments on cupric sulfate with goldfish as test animals. He reported that they survived 1 p.p.m. (0.0000063 molar) for 3 days and longer.

Many investigations have been carried out on mercuric chloride in which relatively high concentrations killed in a short time. Jones (1939) reported that the three-spine stickleback was killed by concentrations above 0.00000004 molar (0.009 p.p.m.) in tap water.

In a comparison of metallic chlorides by Powers (1937), it was reported that the toxicity of the metal chlorides were arranged in the following order of lethality: the most toxic was barium chloride, then sodium chloride, calcium chloride and magnesium chloride. The test animal used in the report was the blunt-nose minnow (Pimephales notatus).

LaRue (1955) working with the Squalas cephalus reported that the following metallic ions will produce toxic effects in these fish: lead, silver, uranium, barium, lithium, iron, aluminum, copper and mercury.

The Gambusia were first placed in a fresh-water aquarium for a period of four days to prevent any loss due to handling. The average length of the females used was 44 to 59 m.m. The average size of the males was 20 to 39 m.m.

An attempt was made to keep the metallic impurities down by using all glass aquaria, plastic filters and plastic connections instead of metal.

In the first phase of the problem three types of salt water solutions were tested with the Gambusia. The first was natural sea water. Using natural sea water required careful collection and storage. The ideal place for the collection of sea water is at a place far enough out to avoid contamination by shore areas. The sea water used in this problem was obtained twenty-two miles out in the Gulf of Mexico near Mobile, Alabama.

An attempt was made to keep the specific gravity of the salt water around 1.020. This must be kept constant because as the water evaporates the salt content increases unless distilled water is returned. An attempt was made to keep the salt water content within a normal deviation of 0.003 with a high specific gravity of 1.025 and a low of 1.016. A careful check of the specific gravity and the pH value of each solution was made before adding the fish (Table I).

The composition of sea water was determined in this problem by referring to the Handbook of Chemistry and Physics 1953-1954 (Table II).

The first series of tests dealing with the effects of sea water upon the Gambusia affinis consisted of setting up eleven three-gallon, all-glass aquaria and adding to each sea water and diluting it by 10 percent with distilled and deionized water.

TABLE I

THE SPECIFIC GRAVITY AND pH VALUES OF THE SEA WATER USED IN THIS PROBLEM

Solutions (Percentage)	Specific Gravity	pH
100 %	1.026	7.72
90 %	1.025	7.71
80 %	1.023	7.82
70 %	1.022	7.89
60 %	1.021	7.77
50 %	1.017	7.85
40 %	1.012	7.88
30 %	1.009	7.92
20 %	1.006	7.96
10 %	1.003	7.34
Control	1.000	7.01

TABLE II

THE CHEMICAL COMPOSITION OF THE SEA WATER

Item	P.P.M.	Molar Value
Chloride	18,980	0.53
Sodium	10,561	0.46
Magnesium	1,272	0.05
Calcium	400	0.01
Potassium	380	0.009
Stronium	13	0.00015
Aluminum	0.16-1.9	0.00007
Lithium	0.1	0.000014
Barium	0.05	0.00000025
Iron	0.002-0.02	0.0000003
Copper	0.001-0.09	0.0000014
Manganese	0.001-0.01	0.00000019
Lead	0.004-0.005	0.000000029
Tin	0.003	0.000000025
Nickel	0.0001-0.0005	0.000000009
Mercury	0.0003	0.0000000015
Silver	0.00016-0.0003	0.000000002
Gold	0.000004-0.0000008	0.000000000002
Cobalt	unknown	unknown
Zinc	0.005-0.014	0.0000002

Another experiment was set up to determine the actual threshold for the Gambusia in sea water diluted by distilled water. The concentrations used in this phase consisted of eight solutions between 50 and 64 percent.

The Gambusia affinis was placed in two other solutions of sea water, which were prepared by two different formulae. The first was one used by the University of Illinois to maintain salt water invertebrates (Axelrod, 1955). The second synthetic sea water solution was a commercial brand sold under the trade mark of "Neptune Sea" on sale at most pet stores (Table III).

TABLE III

CHEMICAL COMPOSITION OF SYNTHETIC SALT WATER SOLUTIONS AND NEPTUNE SEA WATER

Item	Synthetic Salt Water	Neptune Salt Water*
Sodium Chloride	27,500 p.p.m.	x
Magnesium Chloride	3,800 p.p.m.	x
Calcium Sulfate	1,300 p.p.m.	x
Calcium Carbonate	100 p.p.m.	x
Potassium Sulfate	900 p.p.m.	x
Magnesium Carbonate	100 p.p.m.	x
Barium Chloride	X	x
Copper Chloride	X	x
Trace Elements	X	x
Distilled Water	1-1	4.228 1.
Sea Water	3-1.	1-1.

*True composition unknown.
XPresence in solution.

The second phase of the problem was set up using solutions of sodium chloride to determine the threshold of tolerance to this metallic salt without injurious effects (Table IV).

The third phase of tests in this problem was set up to determine the metallic chlorides and their concentrations that are lethal to the Gambusia affinis. There were set up a series of eight different concentrations of the metals listed in Table V.

TABLE IV

SODIUM CHLORIDE CONCENTRATIONS USED DURING THE SECOND PHASE TO DETERMINE THE THRESHOLD FOR THE GAMBUSIA

Percent of Solution	P.P.M.	Molar Conc.
Control	0,000	0.00
0.5	5,020	0.08
1.0	10,050	0.17
1.5	15,070	0.26
2.0	20,250	0.34
2.5	25,320	0.43
3.0	31,010	0.53
3.5	36,130	0.62
4.0	41,070	0.70
4.5	46,210	0.80
5.0	51,350	0.90

Due to the 100 percent lethal effect of most chlorides in phase three another series of test solutions was used consisting of all the metallic chlorides except sodium, lithium, magnesium and calcium. The concentrations used in this phase were 0.01, 0.001 and 0.0001 molar solutions of chlorides.

TABLE V

METALLIC CHLORIDES THAT WERE TESTED UPON THE GAMBUSIA

Ferric	Mercuric	*Calcium
Ferrous	Zinc	Manganese
Aluminum	*Sodium	Barium
Stannic	*Lithium	Cobalt
Stannous	Potassium	Nickel
Mercurous	*Magnesium	Cupric

*Used only during third phase.

Solution concentrations were 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 molar.

The final phase dealing with the effects of sodium chlorides upon the pregnant females consisted of using sodium chloride solutions within the lethal range. The females were placed in breeding cages and kept in the cages until the end of the experiments. A check was made of the young to determine if there were any effects due to the toxicity of the solutions.

All experiments in this problem were over a 24 hour test period. In all phases of this problem except the fourth phase the same numbers of males and females were used in each test solution. The total number of animals used in each solution and the total number of animals used in all solutions are found in Table VI.

TABLE VI

THE NUMBER OF TEST ANIMALS USED IN EACH PHASE OF THIS PROBLEM WITH THE GAMBUSIA

Phase	No. of Solutions	Test Solutions	Gambusia No. in Each Sol.	Gambusia Total Number
First	11	Sea Water	108	1,188
First	11	Synthetic Sea Water	48	528
First	11	Neptune Sea Water	48	528
First	8	Sea Water[1]	24	192
Second	10	Sodium Chloride	24	240
Third	11	Metallic Chlorides	24	264
Fourth	10	Sodium Chloride[2]	3	30

[1]Solutions to determine lethal point for sea water.
[2]Solutions using pregnant females.

CHAPTER FOUR

RESULTS

The reactions of the Gambusia to sea water and metallic chlorides yields many interesting results. The results of this problem will be discussed in several parts.

A comparison of the results obtained with sea water, synthetic sea water and Neptune sea solutions shows that only a few Gambusia can survive in solutions above 60 percent salt water (Table VII). The lethal thresholds for the Gambusia in sea water and Neptune sea solution is 70 percent, while in the synthetic solutions a 60 percent solution was lethal. Gambusia in solutions below 40 percent were unaffected.

TABLE VII

COMPARISONS OF THE SURVIVAL OF THE GAMBUSIA IN SOLUTIONS OF SEA WATER, SYNTHETIC SEA WATER AND NEPTUNE SOLUTION DURING A 24-HOUR TEST PERIOD

Conc. of Sol. in Percentage	Sea Water No. to Sur.	%	Synthetic No. to Sur.	%	Neptune No. to Sur.	%
100 %	0/108	None	0/48	None	0/48	None
90 %	0/108	None	0/48	None	0/48	None
80 %	0/108	None	0/48	None	0/48	None
70 %	7/108	6.6	0/48	None	4/48	8.3
60 %	29/108	26.9	18/48	37.7	14/48	29.1
50 %	89/108	82.4	30/48	62.4	36/48	75.0
40 %	104/108	96.3	47/48	97.9	46/48	92.9
30 %	108/108	100.0	48/48	100.0	48/48	100.0
20 %	108/108	100.0	48/48	100.0	48/48	100.0
10 %	108/108	100.0	48/48	100.0	48/48	100.0
Control	108/108	100.0	48/48	100.0	48/48	100.0

The survival time for the Gambusia in the three salt solutions was found to be longer in the sea water, while the shortest survival times were reported in the synthetic solutions. Most fish placed in solutions of 70 percent or above of salt water were killed in less than eight hours (Table VIII).

TABLE VIII

THE AVERAGE SURVIVAL TIME FOR GAMBUSIA PLACED IN SOLUTIONS OF SEA WATER, SYNTHETIC SEA WATER AND NEPTUNE SEA WATER

(Expressed in Hours)

Percentage Concentration of Solutions	Sea Water	Synthetic Solution	Neptune Solution
100 %	2.25	1.60	2.39
90 %	3.25	2.70	3.60
80 %	3.90	3.30	3.90
70 %	5.60	5.00	5.65
60 %	13.50	12.70	13.80
50 %	20.60	20.10	20.60
40 %	23.60	23.50	22.00
30 %	24.00	24.00	24.00
20 %	24.00	24.00	24.00
10 %	24.00	24.00	24.00
Control	24.00	24.00	24.00

Comparison of the results obtained in this experiment for males and females in the three salt solutions shows several outstanding results. The females as a group did not show the lethal effects of the solutions as quickly as the males. The survival time for females was about twice as long as those of the males in each lethal solution. The females were able to withstand a higher concentration of salt solutions than the males and in most cases up to 10 percent higher.

In all solutions of 70 percent or above all males were dead before any females were affected (Table IX).

The lethal threshold percentage for Gambusia in natural sea water was found to be between 56 and 58 percent for the male *Gambusia* while the lethal percentage for the females fell between 58 and 60 percent. The group threshold for the *Gambusia* was determined to be between 56 and 60 percent. All solutions below 56 percent sea water were not lethal to any of the test animals and all solutions above 60 percent were lethal to most *Gambusia* (Table X). The 56 percent solution of natural sea water was the first solution that failed to show any lethal effects upon both the males and females.

TABLE IX

COMPARISON OF THE TOLERANCE OF THE SEXES TO SEA WATER, SYNTHETIC SEA WATER AND NEPTUNE SOLUTION

	Number of Surviving *Gambusia* and Average Time (Hours)					
	Sea		Synthetic		Neptune	
Solution	108 Test Animals		48 Test Animals		48 Test Animals	
Percentage	Males	Females	Males	Females	Males	Females
100 %	0- 1.5	0- 3.0	0- 1.1	0- 2.1	0- 1.6	0- 3.0
90 %	0- 2.0	0- 4.5	0- 1.9	0- 3.5	0- 3.0	0- 4.2
80 %	0- 2.5	0- 5.3	0- 2.5	0- 4.1	0- 2.5	0- 5.3
70 %	0- 3.1	7- 8.2	0- 3.1	0- 6.9	0- 3.1	4- 8.2
60 %	8- 9.0	21-18.6	4- 8.2	14-15.3	4- 9.0	10-18.6
50 %	40-19.2	49-22.0	8-18.6	22-21.6	16-19.2	20-22.0
40 %	50-23.2	54-24.0	23-23.1	24-24.0	22-20.1	24-24.0
30 %	54-24.0	54-24.0	24-24.0	24-24.0	24-24.0	24-24.0
20 %	54-24.0	54-24.0	24-24.0	24-24.0	24-24.0	24-24.0
10 %	54-24.0	54-24.0	24-24.0	24-24.0	24-24.0	24-24.0
Control	54-24.0	54-24.0	24-24.0	24-24.0	24-24.0	24-24.0

A comparison was made of the lethal effects of sodium chloride in pure solution and the sodium chloride found in sea water. Results showed the sodium chloride did not occur in lethal amounts in sea water. The concentration of sodium chloride in sea water was 29,541 p.p.m. The lethal threshold for sodium chloride in this problem was a 3.5 percent solution or 36,130 p.p.m. (Table XI).

TABLE X

DETERMINATION OF ACTUAL LETHAL PERCENTAGE FOR THE *Gambusia* IN NATURAL SEA WATER SOLUTION

Solution Percent	No. of Test Animals	Surviving Fish		Percentage Surviving	
		Males	Females	Males	Females
64	24	0	0	00.00	00.00
62	24	0	3	00.00	25.00
60	24	2	7	16.60	58.30
58	24	9	10	75.00	83.30
56	24	12	12	100.00	100.00
54	24	12	12	100.00	100.00
52	24	12	12	100.00	100.00
50	24	12	12	100.00	100.00

TABLE XI

DETERMINATION OF THE LETHAL THRESHOLD CONCENTRATIONS OF SODIUM CHLORIDE ON *Gambusia*

Solution Percentage	Solution p.p.m.	No. of Test Animals Used	No. of Animals That Survived
Control	0.00	24	24
0.5	5,020	24	24
1.0	10,050	24	24
1.5	15,070	24	24
2.0	20,250	24	24
2.5	25,320	24	20
3.0	31,010	24	6
3.5	36,136	24	0
4.0	41,070	24	0
4.5	46,210	24	0
5.0	51,350	24	0

The thresholds of toxicity for the chlorides of various metals as determined on the basis of the present experiment are given in Table XII. In Table XII the values preceded by an asterisk (*) are not true threshold concentrations since the value given was 100 percent lethal to the *Gambusia*. The lethal threshold for these solutions is below 0.0001 molar.

A comparison was made of the metallic chlorides threshold in this experiment with the concentration of the same chlorides in sea water. This shows if the concentrations of the metallic chloride in sea water is lethal.

TABLE XII

THRESHOLD CONCENTRATIONS FOR THE METALLIC CHLORIDES AND THE GAMBUSIA

Substances Chlorides of	Formula	Lethal Threshold (Molar)	Lethal Threshold (p.p.m.)	Conc. in Sea Water (p.p.m.)
Ferric	$FeCl_3$	*0.0001	15.2	0.02
Ferrous	$FeCl_2$	*0.0001	12.6	0.02
Copper	$CuCl_2$	*0.0001	13.4	0.09
Aluminum	$AlCl_3$	*0.0001	11.3	0.19
Stannic	$SnCl_4$	*0.0001	26.7	0.003
Stannous	$SnCl_2$	*0.0001	18.9	0.003
Mercuric	$HgCl_2$	*0.0001	27.1	0.0003
Mercurous	HgCl	*0.0001	23.5	0.0003
Zinc	$ZnCl_2$	*0.0001	13.6	0.014
Sodium	NaCl	0.4	23,000	10,561
Lithium	LiCl	0.6	25,500	0.1
Potassium	KCl	0.0001	7.4	380
Magnesium	$MgCl_2$	0.5	47,650	1,272
Calcium	$CaCl_2$	0.2	22,200	400
Manganese	$MnCl_2$	0.0001	12.6	0.01
Barium	$BaCl_2$	0.1	20,800	0.05
Cobalt	$CoCl_2$	0.0001	12.0	unknown
Nickel	$NiCl_3$	0.0001	16.5	0.0005
Strontium	$SrCl_2$	0.2	31,720	13
Lead	$PbCl_2$	*0.0001	27.8	unknown

*The true threshold concentrations is below this concentration.

In earlier observations it was noticed that many times the pregnant females when placed in the sodium chloride solutions would have premature births. As a result it was decided to set up a solution using only females. The lethal sodium chloride caused premature births in solutions when the concentrations were 3.5 percent or higher. Normal birth occured in solutions with concentrations below 2.5 percent sodium chloride. Some of the females placed in 2.5-3.5 percent carried on some normal birth processes while other females were unable to give birth to live young.

The effects of sodium chloride upon the young *Gambusia* showed very quickly after they were placed in 2.5 percent or higher. The results show that the young will be affected by metallic ions much faster than the older fish.

CHAPTER FIVE

INTERPRETATION OF RESULTS

The results of the experiments with natural sea water upon *Gambusia affinis* showed that in most cases that any amount above 60 percent was fatal. Raffy (1932) reported that most fresh-water fish were killed in any solution that was of a greater ratio than one part salt water to one part fresh water. Therefore the brackish water that is inhabited by the *Gambusia* must not have a sea water content higher than 60 percent. This experiment shows that the actual lethal threshold for the sea water was between 56 and 60 percent. Otto (1929) reported that the *Gambusia affinis* could withstand waters of high salinity and in this problem it was showed that they could live in many solutions as high as 60 percent sea water without harmful effects.

In most cases the lethal effects were noticed within a 24-hour period for all solutions that were of a greater concentration than 50 percent.

The results of the synthetic sea water tests proved that the same effects were obtained as in sea water. The Neptune sea solutions showed similar results. The lethal point for the *Gambusia* once again lay between 50 and 60 percent for the above solutions.

A difference in the degree of tolerance of the two sexes to the three salt water solutions was noticed. The results showed that the females were much more tolerant to the effects of sea water than were the males. The females placed in very high solutions of natural sea water could withstand the lethal effects an average of twice as long as the males. There was clear cut evidence that the females could survive in a solution at least 10 percent higher than the lethal solutions for males. Egashira (1937) using females and males of Carassius aurature showed that females were much more resistant than males.

Sodium chloride was one of the least toxic of all chlorides tested. Its toxicity threshold was estimated at 3.5 percent (31,010 p.p.m.) for the females and 2.5 percent (25,250 p.p.m.) for males. This value is considerably lower than that reported by Nekotyre (1944) when the thresholds were calculated on the basis of a 48-hour period. The results of Meyer (1944) working with young salmon showed that they were killed in solutions of 1.2 to 1.3 percent sodium chloride. A solution of 15 parts of sodium chloride per 1,000 parts water is lethal to carp (Cypronius carpio) (Olphant, 1941). Ellis (1937) found that goldfish (Carassius auratus) were unharmed by 5,000 p.p.m. (0.085 molar) but that they survived 10 days or less in 10,000 p.p.m. (0.171 molar).

Calcium chloride was fifth in the scale of increasing toxicity of the chlorides investigated. Its threshold of toxicity was 0.2 molar (22,200 p.p.m.). Ellis (1937) referred to a set of experiments in which golden shiners were killed by 5,000 p.p.m. (0.045 molar) in 143 hours. Powers (1937) placed calcium chloride third but in this study calcium ranks fifth in its lethal effects on the Gambusia.

Magnesium chloride was somewhat less toxic than calcium chloride, having a difference of 0.3 molar. Powers (1937) listed magnesium chloride as less toxic than any other chlorides. In this study only lithium was less toxic to the Gambusia. The threshold was 0.5 molar (47,650 p.p.m.)

for magnesium chloride. Jones (1939) gave 0.0123 molar as the threshold for the stickleback minnow in magnesium chloride. The Gambusia threshold was much higher than that of the stickleback.

Of the chlorides tested, potassium chloride proved to be seventh in the scale of increasing toxicity. Its threshold was 0.0001 molar (7.4 p.p.m.). The lethal effects of potassium chloride were reported by Ramault (1929) showing that a solution of pure potassium chloride was very toxic to the stickleback. Ellis (1937) listed the thresholds for potassium as 1.203 p.p.m. (0.012 molar) for the bluegill (Lepomis macrochirus). Jones (1939) gave 0.00127 molar as the threshold for the stickleback.

Strontium chloride was much less toxic to the Gambusia than potassium chloride. The toxicity threshold for the strontium chloride is 0.2 molar (31,720 p.p.m.). Jones (1939) found that strontium was lethal for the stickleback when the concentration was (0.0137 molar). His results were much lower than the ones obtained in this study.

The threshold concentration for manganese dichloride was (0.0001 molar) 12.0 p.p.m. Jones reported that the three-spine stickleback died in all concentrations of manganese dichloride above (0.000728 molar).

Ferrous chloride immobilized the Gambusia in concentrations above 0.0001 molar (12.6 p.p.m.). Ellis (1939) reported that 1,000 p.p.m. (0.0079 molar) killed goldfish in less than 10 hours but 100 p.p.m. (0.00079 p.p.m.) was not harmful over 96 hours. The ferrous chloride killed the Gambusia in 15 minutes.

Lithium chloride was the least toxic of all chlorides of the alkali metals studied in these experiments. The threshold for lithium was 0.6 molar (25,500 p.p.m.).

The toxicity threshold for barium chloride was 0.1 molar (20,800 p.p.m.). Jones (1939) found that 0.0029 molar barium chloride was the threshold for the three-spine stickleback. Power (1937) reported that the light metallic chlorides were toxic to the fresh-water fish in the following order: barium, sodium, calcium and magnesium. In this problem the barium was the most toxic of the light metals.

Stannous chloride was about twice as lethal as stannic chloride. The threshold for the stannous chloride was 0.0001 molar (26.7 p.p.m.). The survival time in the stannous was cut to one hour. Ellis (1937) reported that 1,000 p.p.m. (0.0053 molar) stannous chloride killed goldfish in 4 to 5 hours.

The ferric chloride threshold was 0.0001 molar (15.2 p.p.m.) which is about the same as the ferrous chloride. The survival time in ferric chloride solution was thirty minutes or less. Ellis (1937) found that 100 p.p.m. (0.00062 molar) ferric chloride will kill goldfish in less than 90 minutes. Jones (1939) found that ferric chloride in a concentration of 1.2 p.p.m. (0.000021 molar) iron will kill stickleback in six days.

Aluminum chloride was toxic in concentrations above 0.0001 molar (11.3 P.p.m.) to the Gambusia. Allele (1946) reported that aluminum chloride was fatal to most minnows when the concentration was greater than 1 p.p.m.

The toxicity threshold of cobalt chloride was 0.0001 molar (12.0 p.p.m.). Ellis (1939) found that 10 p.p.m. (0.00077 molar) cobalt chloride in distilled water was fatal to goldfish in 168 hours. Jones (1939) stated that the lethal concentrations for the three-spine stickleback was 0.000023 molar.

Nickel chloride had the same threshold as the cobalt chloride. Its threshold of toxicity was 0.0001 molar (16.5 p.p.m.). Ellis (1939) pointed out that 10 p.p.m. (0.000077 molar) killed goldfish in 200 to 210 hours.

Lead chloride was somewhat more toxic than nickel chloride. Its threshold was below 0.0001 molar (27.8 p.p.m.). Ellis (1937) cited Carpenter as believing that 0.33 p.p.m. (0.0000016 molar) lead may be lethal to fresh-water fishes. Ellis listed the results of other investigators and pointed out that 10 p.p.m. (0.00003 molar) lead chloride killed young trout. Ellis found that 100 p.p.m. (0.0003 molar) was fatal to goldfish in 80 hours but 10 p.p.m. caused no injury in 96 hours. Both solutions killed the Gambusia in this study in one hour.

The threshold of toxicity for zinc chloride was below 0.0001 molar (13.6 p.p.m.). Abbot (1924) reported that zinc in the amounts of 1.6 p.p.m. will kill many small fish in less than eight hours. Carpenter (1931) reported that zinc in amounts of 3 p.p.m. was lethal to many small fish. All Gambusia in this problem were killed within the hour by the zinc chloride.

The threshold concentration for cupric chloride on the Gambusia is below 0.0001 molar (13.4 p.p.m.). The copper chloride killed all fish within fifteen minutes after they were placed in the solutions. Ellis (1937) reported that goldfish could survive three days in cupric chloride solutions of 1 p.p.m. (0.0000063 molar). Warrack (1944) reported that 3 p.p.m. of copper was lethal to most fish.

In this problem mercury chloride was the most toxic compound tested. The threshold for the Gambusia was below 0.0001 molar (23.5 p.p.m.). The mercury chloride killed the Gambusia in five minutes. Jones (1939) reported that the three-spine stickleback was killed by all concentrations above 0.000000022 molar mercury chloride.

The results reported above show that the metallic ions are arranged

CHAPTER SIX

SUMMARY AND CONCLUSIONS

Gambusia affinis affinis and Gambusia affinis holbrooki both were able to withstand large amounts of natural sea water without suffering lethal effects. The lethal point determined in this study is between 56 and 58 percent. The females were found to be much more resistent to natural sea water than the males. The females were able to withstand solutions that were about ten percent higher than the males. Also the survival time was about twice that of the males.

The two solutions of synthetic sea water and Neptune Sea Water showed that the lethal effects upon the Gambusia occured when the concentrations were between 50 and 60 percent. Once again the female was much more resistent to the toxic effects of the solution.

The Gambusia were unable to survive in solutions of sodium chloride above the concentration of 2.5 to 3.5 percent. Again females were able to withstand the effects much better than the males.

Any concentration of the metallic chlorides greater than 0.6 molar is lethal to the Gambusia. The heavier the metallic chloride the greater is the lethal effects. In general the heavy metallic ions are much more lethal than metallic ions of the lighter metals. The most toxic ions used in this problem was mercury. Death followed submission within minutes.

The strong toxic solutions of sodium chloride were found also to affect the birth processes by producing premature and dead off-spring. The strong solutions were also lethal to newborn young and killing them very quickly after birth.

Implications of the Study

The results of this report show that the natural sea water could act as a barrier to the movement of many fresh-water fish. The fact that the *Gambusia* could withstand certain amounts of salt-water solutions shows that the brackish water must not contain the lethal amounts of natural sea water or the *Gambusia* could not survive in it.

The results also point out that it is not the sodium chloride alone in the natural sea water that is the lethal agent which kills the *Gambusia*. The fact is that sea water contains a great many other metallic chlorides that are lethal to the *Gambusia*. The combination of all metallic ions and chlorides rather than the presence of any one ion causes the sea water to be lethal to fresh-water fishes.

The lethal effect of the metallic chlorides and natural sea water is brought about when these solutions upset the enzyme balance of the body. Death occurs in the protoplasma of the cell. Most salt water animals contain within their bodies a chlorine cell that removes the toxic chlorides from sea water. Fresh-water fish lack this chlorine cell and depend upon the kidneys to remove the metallic chlorides and keep the balance of salts within the body. The higher concentrations then are lethal because the kidneys cannot handle the excess of metallic salts that are found in these solutions.

In general this report shows that the female *Gambusia* were much more tolerant to the concentrations used than were the males. The females

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