THE TOLERANCE OF CERTAIN FRESH WATER FISHES TO A GROUP OF BACTERICIDAL AND PARASITICIDAL COMPOUNDS

by

Henry B. Peters

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ABSTRACT

This investigation records the tolerance of Notropis blennius (Meek), Perca flavescens (Mitch) and Carassius carassius L. to a group of thirty one chemical compounds in various dilutions, from 10,000 parts per 1,000,000 to 1 part per 1,000,000.

As a result of the experiments carried out it was found that except for the dilution of 10,000 parts per 1,000,000 Carassius carassius L. exhibited the highest degree of tolerance, Perca flavescens (Mitch) followed closely and Notropis blennius (Meek) clearly the least tolerant of the three.

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INTRODUCTION

The increasing rate of pollution of fresh water lakes and rivers with chemical wastes from factories, pulp mills and mines, is becoming one of great importance to those concerned with the conservation and propagation of fishes. The combating of bacterial fungus and parasitic diseases of hatchery and aquaria fishes is a problem of long standing. The tolerance of fishes to various chemical substances in different concentrations is practically unknown. In either case, whether the fish comes in contact with the chemical accidentally through the pollution of its natural waters, or in the antiseptic treatment of disease, this factor is rapidly becoming of great importance.

The following data shall represent a preliminary survey of the subject, carried out to determine if the concentration of waste chemicals in some portions of our lakes or rivers is beyond the tolerance of fishes, and also to determine if antiseptic solutions can be tolerated in sufficient concentration to be of value in the treatment of diseases of hatchery fishes. There are several questions which arise in the discussion of this topic and the experiments were carried out with the following points in mind.

1. What is the tolerance of fresh water fishes to a chosen group of chemical compounds, using fish free from any infection?

2. Having found the tolerance in relation to time, what effects have these compounds on fishes exposed to various fungus growths, Saprolegniaceae for example? Here again the question arises, will the tolerance of an infected fish be as great as that of a normal healthy fish? Will the growth of the fungus be inhibited, while the animal makes a complete recovery?

3. What will the effect of the chemical compounds on the spores of a Saprolegniaceae culture in comparison to the fungus growing on the fish itself? In which case will the chemical compounds inhibit growth more readily?

It is not the purpose of this investigation to explore the three suggested problems, but rather to find some information regarding the tolerance of selected fishes to a group of chemical compounds. This information is necessary before carrying forward further experiments which would lead to a more comprehensive treatment of the subject matter in hand.

Powers (1917) has used the Goldfish, Carrasius carassius L. which is the Crucian carp or Goldfish, as a test animal, and subjected it to a series of experiments. He tested the resistance of the Goldfish to various concentrations of certain toxic substances, using Molar solutions for his concentrations, whereas the present experiments made use of percentage solutions ranging from 1% to .0001%.

MATERIAL

The fishes used in these experiments were as follows:

1. Minnow - Notropis blennius (Meek)
2. Fingerling Perch - Perca flavescens (Mitch)
3. Goldfish - Carassius carassius (L.)

The Minnows were obtained from St. Andrews Locks on the Red River, twelve miles below the City of Winnipeg, Manitoba. There are some twenty four species of Notropis in Manitoba and at least thirteen of the species are to be found at St. Andrews. The experimental work was carried out with one species only, the name of which is not available, due to the fact that the genus Notropis is undergoing a complete reorganization, and agreement on this genus with its species is not yet complete, but it is believed to be the species blennius.

Minnows are quite prevalent in these waters during the summer months, until the end of August, after which time their appearance is quite uncommon. It was during this time, July and August, that the investigations with the Minnows took place. The current at the St. Andrews Locks is very fast, there being a drop of some twenty five feet in the water levels. Transferring Minnows from this habitat where there was a large volume of fast water presented a real problem. A number of attempts were made to acclimatize them to life in the aquarium, but without success.

There were several problems which played a large part in making them most difficult to maintain out of their natural environment.

1. The water was still in the aquarium, allowing no current.

2. The still water did not allow a proper aeration of the water in which they were kept.

3. The water did not maintain a constant temperature, rising to a point which was toxic to the Minnow.

The difficulties listed above were overcome to a marked degree by having two holes drilled in the wall of the aquarium. This allowed a steady flow of water into, and drainage out of, the aquarium. In this manner the continual flow developed a current, provided a fresh supply of oxygen and maintained a constant temperature. Prior to this alteration of the aquarium it was impossible to keep a stock of any size more than six hours, but with the continual stream of water flowing the same number could be maintained for an indefinite period without any appreciable mortality rate. Since each experiment required at least ten specimens and in many cases a number in excess of fifteen, it was imperative that a reasonably large stock be kept on hand. The number usually kept in the aquarium was in the neighbourhood of two hundred. It was not of course necessary that they all be kept in the same tank, as long as the conditions under which they existed were uniform.

Minnows are very smart and vigorous swimmers, schooling in the center of the aquarium. The writer has observed them on several occasions jump as much as five inches out of the water, and in some cases out of the aquarium. Their reaction when placed

in a smaller vessel showed very little change so far as their vigor was concerned, save for the first few moments when the transfer was made from the large aquarium to a smaller experimental vessel. The action of the Minnow in the smaller vessel, of course varied to a remarkable degree depending to some extent on the solution, and the dilution of the solution being used. Complete distress was exhibited when the specimen took up a position with its ventral surface upwards.

Fingerling perches make their appearance early in September, almost at the same time as the Minnow disappears. They are found in the same waters as are the Minnows, and in large numbers. The same problems arose in maintaining the Fingerling Perches as in the case of the Minnows and were overcome in a similar manner. Although the mortality rate of the Minnow was not negligible, it was found that the number of Fingerling Perches dying under the new conditions was nil.

The swimming prowess of Fingerling Perches is not nearly so marked as in the case of Minnows and only on rare occasions did they exhibit vigor as displayed by the Minnows. This specimen schooled at the ends of the aquarium. Distress was displayed when the specimen took up a position with the ventral surface upwards.

The Goldfish employed in the following experiments were imported from Eastern Canada, reaching Winnipeg the latter part of December, at which time the investigations into this specimen were made. They were a small sample of their variety ranging from 1.5 gms. to not more than 5 gms. in weight, and were the regular Goldfish sold commercially by dealers. Acclimatization

was easily carried out in a manner similar to the above specimens. Mortality was nil. Goldfish are not active swimmers, schooling at the ends of the aquarium. Complete distress was noted when the Goldfish took up a position on its side with its short axis in a horizontal plane.

The maintainance of the specimens used in these investigations was relatively simple. Ordinary fish food as put on the market by various pet shops served the needs of the Minnows and the Goldfish, whereas earthworms were used as food for the Fingerling Perch.

PROCEDURE

The fishes used in these experiments were subjected to various dilutions of thirty one compounds in solution. These compounds used were as follows:

Aluminium Sulphate	Boric Acid
Ammonium Chloride	Carbolic Acid
Ammonium Hydroxide	Ethyl Alcohol
Copper Sulphate	Formalin
Ferric Chloride	Methyl Alcohol
Hydrochloric Acid	Argyrol
Magnesium Sulphate	Chinosol
Mercuric Chloride	Lunosol
Potassium Hydroxide	Mercurescein
Potassium Permanganate	Merthiolate
Sodium Chloride	Neo Silvol
Sodium Peroxide	Silver Lactate
Sulphuric Acid	Silver Nitrate
Zinc Sulphate	Silver Protein
Acetic Acid	Tr. Iodine
Borax	

The above inorganic and organic compounds were carefully selected before the experiments were carried out. Mellen (1928) used them in the treatment of fish diseases at the New York Aquarium with gratifying results. The other compounds, Argyrol, Chinosol, Lunosol, Merthiolate, Neo Silvol, Silver Lactate and Silver Protein are a group of organo metallic substances, many of which have only recently found their way to the

market. They are commonly known as mucous membrane antiseptics. A short description of them follows:

Argyrol is an organic silver compound, prepared for sale by Burroughs Wellcome and Co., Montreal.

Chinosol is prepared by Burroughs Wellcome and Co., Montreal.

Lunosol is a colloidal silver chloride compound, freely soluble in water. The makers are Hille Laboratories, Philadelphia.

Merthiolate has a chemical constitution known as sodium ethyl mercurithiosalicylate. It is for sale as a biological preservative, made by Eli Lilly and Co., Indianapolis.

Neo Silvol is colloidal silver iodide, containing 20% silver iodide in a colloidal form. It is made by Parke Davis and Co., Walkerville, Ontario.

Silver Lactate is made by Merck and Co., Rahway, N.J.

Silver Protein was formerly on the market as Silver Proteinate. It contains 8.3% of silver and is freely soluble in water. Marketed by March and Co., Rahway, N.J.

The investigations were not carried out with Molar, but rather with percentage solutions, these being used due to their ease of preparation. The compounds listed above were all made up to 1% (10,000 parts in 1,000,000) and from this solution the various dilutions were made. These dilutions ranged from 10,000 parts per 1,000,000 to 1 part per 1,000,000 or .0001%. The dilution 1% was chosen as it was considered to be the maximum fish tolerance of the compounds, while the .0001% dilution was used believing it to be the limit of bactericidal action.

The specimens were kept in the aquarium for a period of at least two days in order that acclimatization might be accomplished. After which time they were subjected to the experiments described below.

The experiments were carried out with single fishes in small glass tanks with a capacity of 300 c.c. containing 250 c.c. of solution. This allowed free movement of the fish and obviated the preparation of large volumes of the solutions. All the water used in making them was taken from the aquarium, thus keeping the temperature of the experiment the same as the stock water.

In finding the tolerance of an animal to a substance it was difficult to determine whether a time limit should or should not be employed. In many of the dilutions which were used, results would have been interesting if the experiment had been allowed to go to completion, or in other words to the point where the fish displayed complete distress. This however was not done. It was decided to employ an arbitrary time limit of sixty minutes, after which, if the specimen survived that time, it was concluded that the dilution used was tolerated for an indefinite period. The sixty minutes was chosen for only one reason. It was felt that if a dilution took any longer than one hour to exhibit bactericidal action, it would be impracticable for use in bacteria treatment. All results embodied in this investigation are based on that factor.

Five dilutions were made in carrying out this work. They were as follows:

1. 1% or 10,000 parts per 1,000,000.
2. .1% or 1000 parts per 1,000,000.
3. .01% or 100 parts per 1,000,000.
4. .001% or 10 parts per 1,000,000.
5. .0001% or 1 part per 1,000,000

The volume of the solution in each case was 250 c.c. The time was noted at the beginning of each experiment and it was carried on until such a time as the specimen displayed complete distress, in the manner discussed above, when the time was again noted, the difference being the number of minutes or seconds, that the fish was able to tolerate a given dilution.

At the end of the experiment, the specimen was placed in a battery jar of stock water for observation. In a number of instances it recovered to a remarkable degree and lived for some time. If however it died before one hour had elapsed, a note to that effect was made. If it died in not more than two hours a similar note was made, but if it carried on for an indefinite period, it was described as having fully survived the effects of the experiment. In the course of a few days these could again be used for further tests. The same process was carried out in the case of the other dilutions.

This experiment was repeated again at a later date and the results obtained checked with the first trial. If agreement was reached the test was completed, and if not, it was repeated the third time, or as many trials were made as were deemed necessary to obtain the required degree of consistency. The number of trials reached as high as six or seven. The average of all the trials was taken and this number is embodied in the

results which are found in the tables. This repetition entailed the use of a large quantity of specimens, the number of individual experiments exceeding three hundred and fifty.

All specimens used were weighed and tabulated. If more than one was used, the average weight was noted. These results are employed in the tables.

In order to check each experiment, an identical vessel was used with stock water, to the required volume, into which was placed a fish of the same species. This was allowed to remain the length of the experiment. A further check was carried out in order to show that the volume of water used did not introduce a problem of lack of oxygen. This was done by allowing the stock water specimen to remain in the vessel for periods up to ten hours without distress.