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Study on induced breeding in ornamental fish,poecilia sphenops

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ABSTRACT

An attempt has been made to determine the effect of Natrum muriaticum, a homeopathic medicine on the weight gain, fecundity, excretion and tissue protein level in Poecilia sphenops (white molly), an ornamental viviparous fish. Natrum muriaticum of 30 centesimal potency of dilution of 0.025% medium was used. The amount of protein was determined in the ovary, liver and muscle of the control and the treated fishes. The amount of protein in the ovary after the treatment of the drug leads to precocious maturation of ovary and release of young ones.

Key words: Induced breeding, *Poecilia sphenops*, Natum muriaticum, 30 Centesimal Potency, Fecundity Rate, Protein Profile.

INTRODUCTION

Ornamental fish keeping is one of the most popular hobbies in the world today. The growing interest in aquarium fishes has resulted in steady increase in aquarium fish trade globally. An estimated trade with a turnover of US$ 5 billion and an annual growth rate of 8 percent offer a lot of scope for its development (Das, 2003). Induced breeding is a technique by which ripe fish breed in confined water when stimulated by an agent. A common method used for induced breeding in fish is the administration of pituitary extract from a mammal or a fish. The hormones that induce breeding are gonadotropins. Chorionic gonadotropin, which is a placental hormone is also found to be equally effective (Yadav, 1995).Reports are there to show that Natrum muriaticum can be used to induce breeding in fish (Visakan, 2005). However, a comprehensive study that explains the principles involved in this phenomenon is lacking. Hence, the present study is aimed to trace some of the principles involved in the use of this homeopathy drug for the induced breeding of the commercially important ornamental fish, white molly, (*Poecilia sphenops*). This work is designed to provide some important baseline information on the use of a natural product for the mass production of commercially important fishes.

MATERIALS AND METHODS

Experimental Animal

Poecilia sphenops (white molly), an omnivorous ornamental fish was chosen for the present study as the experimental animal. It inhabits freshwaters and successfully establishes in a variety of environmental conditions. It can grow to a maximum length of 3 inches. It gives birth to young ones directly. White molly was selected for the present study, because of its commercial importance, easy availability and viviparous nature.

Procurement of fish

The fishes were collected from a fishery form at Theneerpatti, Trichy. The fishes were brought in polythene bags filled with oxygen. *Poecilia sphenops* having a length of 4-5 cm approximately were selected for the study. The female fishes which have completed 10 days after the previous breeding were chosen.

Acclimatization

The chosen fishes were transferred to a plastic water tub and acclimatized to the laboratory conditions for a period of 15 days. The fish were kept in water tub and the water was changed daily in order to maintain sufficient amount of oxygen and to get rid of toxic ammonia in the trough. They were fed with pellet diet regularly.

Procurement and preparation of Natrum muriaticum

Natrum muriaticum is a homeopathic medicine obtained from rocky shore minerals.The naturm muraticum solution of dilution namely 30 centesimal (30C) are brought from Trichy Homeo Medicals. The above mentioned dilution was preferred as it is known as the typical potency with peculiar potenzing effect. From natrum muriaticum 30 centersimal potency 0.025% dilution was prepared. 0.1 ml of natrum muriaticum 30 c potency was diluted to 0.025% by adding 400 ml water.

The fish were grouped into two sets consisting of 24 each. The first set was grouped into 3 with 6 individuals in each trough and treated as control. The second set was grouped into 3 with 6 individual in each trough and treated as experimental. Experimental fishes were left in each trough of 1 litre capacity containing the solution prepared with N.muriaticum of 0.025%. The medium in which the fishes left were fed with pellet diet at the same time. The experiment was carried out for 4 days (0,1st, 2nd, 3rd days).

During the course of the experiment, the animals weighed daily and the ammonia was estimated in the medium. Every day three animals each were sacrificed from the control and the experimental group and the tissues such as ovary, liver and muscle were collected. The tissues were analyzed for proteins. On the third of the experiment, the ovary sample was subjected to SDS polyacrylamide gel analysis to study the protein profile.The fecundity was measured in terms of the number of young ones in the control and the experimental groups. A set of fishes were maintained in the control and the experiment after the spawning to study the fecundity in the next spawning.

RESULTS

The present study deals with the effect of Natrum muriaticum on the duration for spawning, growth, fecundity, excretion and protein level of ovary. The results of this study revealed that 0.025% solution of Natrum muriaticum advanced the process of spawning in *Poecilia sphenops*. The experimental fishes released the young ones on the 4th of their exposure to the salt solution. On the other hand, the control fishes released the young ones only on the 8th day of the experiment. Table I represent the growth pattern shown by the control and the treated fishes. When the data was subjected to student‘t’ test it showed that there was significant difference in the growth rates of the control and the treated ones ($P < 0.05$).

Fecundity

The data on the young ones released by the control and the treated fishes during the first cycle of spawning are presented in Table 2 and Figure 2. Student‘t’ test revealed that there was a significant difference in the number of young ones produced by the control group during the second cycle of spawning.

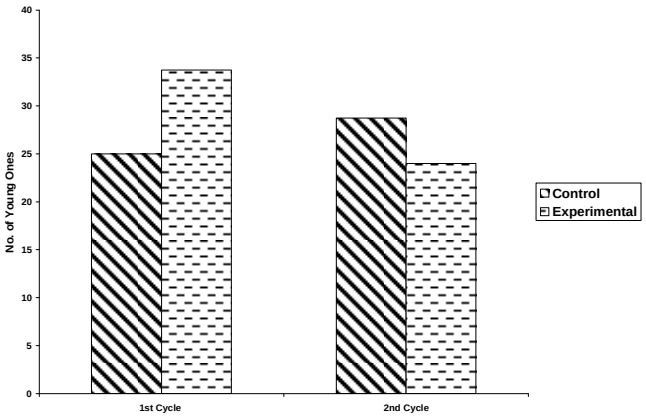
TABLE – 1: Weight in grams in the control and the treated fishes (*Poecilia sphenops)*

Days	Control		Treated	
	Weight (g)	Growth rate g/fish/day	Weight (g)	Growth rate g/fish/day
0 day	1.36 ± 0.057	-	1.36±0.057	-
1st day	1.46 ± 0.152	0.033	1.53 ± 0.152	0.056
2nd day	1.66 ± 0.115	0.066	1.9 ± 0.100	0.123
3rd day	1.93 ± 0.057	0.1	2.3 ± 0.150	0.133

Table 2: Number of young ones released by control and the experimental groups during the 1st & 2nd cycle of spawning.

Groups	Number of young ones	
	1st cycle	2nd cycle
Control	25 ± 1.82	28.75 ± 1.70
Experimental	33.75 ± 2.62	24 ± 1.41

Fig 2



Ammonia

The ammonia content in the control and the treated systems are recorded in Table 3 and Fig 3. When the data was subjected to student 't' test it showed that there was a significant difference in the ammonia levels on both control and the treated systems on 3rd day ($P < 0.05$) (Table 3a).

Table – 3: Ammonia content (mg/fish/h) in the control and the treated fishes (*Poecilia sphenops*)

Days	Control	Experimental
0 day	0.13 ± 0.057	0.13 ± 0.057
1st day	0.08 ± 0.005	0.06 ± 0.005
2nd day	0.07 ± 0.005	0.07 ± 0.005
3rd day	0.06 ± 0.005	0.04 ± 0.005

Protein content

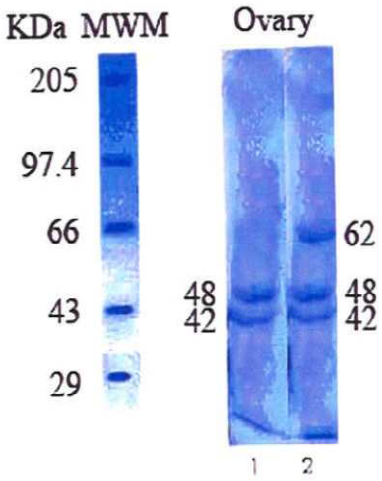
The protein content (mg%) present in ovary, liver and muscle of the control and the treated fishes was tabulated in table 4.The amount of protein content in the liver and muscle of control and experimental fishes was calculated. There is no much difference in the control and experimental group.

Table – 4: Protein content (mg %) present in ovary, liver and muscle of the control and the treated fish, *P.sphenops*

Days	Ovary		Liver		Muscle	
	Control	Experimental	Control	Experimental	Control	Experimental
0	4.6	4.8	1.3	1.8	1.9	2.1
1	5.0	6.3	1.7	2.3	2.4	2.8
2	5.5	7.6	1.8	2.4	2.8	3.2
3	5.9	9.2	2.4	2.8	3.6	3.8

The protein profile of Ovary of the control and Natrum muriaticum treated *Poecilia sphenops* in SDS-PAGE(7%)

Fig: 7



The yolk proteins extracted from the ovary of 3rd day control fishes showed the polypeptides at 42 and 48 KDa regions in 7% SDS-PAGE (Fig ,7 Lane 1).The protein profile of the ovary in the treated fishes at 3rd day showed a more or less similar pattern of polypeptides at 42 and 48 KDa regions but there is an extra polypeptide at 62 kDa region (Fig 7, Lane 2)

DISCUSSION

In the present study Natrum muriaticum 30 centesimal potency of dilution0.025% has advanced the spawning in *P.sphenops*. For instance the time taken for spawning is four days in the ones exposed to this medium compared to the control that took eight days for spawning. Similar results are reported by Vishakan et.al., (2005) in goldfish. Injection of the Natrum muriaticum 1000 centesimal potency into the goldfish (1ml/kg) induced spawning within 22Hours against 5 days in the control. In the same way, Ovaprim induced breeding in Indian major carps at a dosage of 0.4 to 0.5ml per kg body weight (Roy,1996). Similarly, *Ompok bimaculatus*(butterfish)is induced to spawn by a single intramuscular injection of ovaprim0.5ml/kg. Spawning is observed 5-6 hr after the injection(Sridhar and Vijayakumar,1997).In Indian major carp *Cirrhina mrigala*, administration of a single dose of LH-RH analogue(10mg/kg)and pimozide(10mg/kg)causes ovulation(Kaul et al., 1986).

Successful spawning of *C.punctatus* is observed a 0.3and 0.5ml/kg and 3000IU/kg body mass of HCG. For *H.fossilis* successful body spawning is observed at 0.3,0.5 and 0.7ml/kg body mass for ovaprim and1000,2000 and 3000 IU/kg body mass for HCG(Haniffa,2002).The endangered riverine catfish, pangas (*Pangasius pangasius*)administration of10mg/kg body weight of pituitary gland extracts demonstrated the best result in consideration to fertilization and hatching rates of eggs. Hatching of fertilized eggs occurred between 28 and 32 hours (Khan and Mollah, 2004).These reports reveal that comparison of PG, HCG and Ovaprim, the cost and effectiveness of Natrum muriaticum is cheaper and useful one.

Another noteworthy observation in this study is significant increase in the body weight of the experimental fishes prior to the 1st cycle of spawning compared to the control. Similar reports are made by Vishakan and balamurugan (2005) in gold fish. This shows that prior to spawning there may be weight gain in spawning. Natrum muriaticum is reported to increase the production of red blood cells and albumin, a protein found in animal vegetable tissues(Sawyer,2004)It does not cure by supplying the amount of salt that the body needs, but acts to alter and restore the tissues of the body's needs, for salt from food. This may be the reason for the increase in the body weight.

The fecundity rate in P.sphenops 25 ± 1.82 and 2.62 in control and experimental groups respectively during the first cycle of spawning. There was a significant increase in fecundity rate of treated group during the first cycle of spawning .Injection of the homeopathic preparation of N.muriaticum 1000(CM) induces the spawning in gold fish. The experimental animals laid 2856eggs while control laid 209 eggs(Vishakan and Balamurugan,2005)An average of 4012± 100 eggs are spawned by each female by a single intramuscular injection of ovaprim(0.5ml/kg)(Sridhar and Vijaykumar,1997).Fecundity in Chana punctatus is 3273± 75 for ovaprim and 1253± 126 for HCG,whereas in H.fossilis it is 6692 ± 790 for ovaprim 82922 ± 5432 for HCG(Haniffa,2002).

At the same time the number of young ones produced by the experimental group is less compared to the control during the second cycle of spawning the fecundity is 28.75 ± 1.70 and 24 ± 1.41 respectively in controls and experimental group. There is no supporting evidence for this report N. muriaticum seems to have an adverse effect on 2nd cycle of spawning.

In the present study N. muriaticum 30 centesimal potency of dilution of 0.025% has accelerated the protein in the ovary of *P.sphenops* when treated for 4 days. The increase must probably due to the uptake of vitellogenin,the precursor of yolk protein which are taken by oocytes during maturation(Love,1974)

During vitellogenesis, the egg cytoplasm is packaged by reserve food materials such as glycogen, certain other carbohydrate, lipids and proteins which are collectively known as yolk or vitellin (Saidapur,1982).A similar finding is reported by Wallace(1978)who states that Vitolellogenin which is synthesized by liver in response to estradiol is released into the blood and then transported to ovary. Ultrastructural evidence shows that protein yolk precursors are incorporated into oocytes by micropinocytosis(Gupta and Yamamota,1972).

Natrum muriaticum may also cause changes in the biochemical constituents of the eggs, there by influencing fast ovulation(Vishakan et al.,2005).It is well known fact that biochemical changes in various cellular organelles of the oocyte during oogenesis in number of vertebrates(Florkin and Scherr,1974).

The ammonia content in the control and the treated group is the same on 0 day. On the other hand a significant difference in the ammonia excretion is observed in control and the treated systems on 3rd day. Ammonia content for control and experimental fishes is 0.06 ± 0.005 and 0.04 ± 0.005mg/fish/hr (unit) respectively. The ammonia excretion is decreased on 3rd day when compared to 0 day in the experimental group. This shows that protein catabolism is decreased in them and hence the excretion of ammonia. Ammonia in the end product of protein catabolism and it is eliminated from the blood upon passage through the gills (Randall, 2004).

The protein profile as observed in SDS-Polyacrylamide gel electrophoresis of the ovary of the control fish on 3rd day showed two polypeptides at 42 and 48 KDa regions. The treated fishes showed the polypeptides at 42, 48 and 62 KDa regions. Comparison of this shows an extra polypeptide at 62 KDa regions in the experimental animal. Tyler (1988) reports that fish vitellogenin is a complex protein which binds to calcium, iron and carotenoid and has a molecular weight between 33 KDa to 60KDa.

One of the interesting of this work is that though exposure to Natrum muriaticum induces breeding and produces more number of young ones, the fecundity of these fishes is significantly reduced during the subsequent spawn.

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