

Studies on Natrum Muriaticum in Inducing Spawning, Growth and Biochemical Analysis in the Ornamental Fish *Poecilia Sphenops* (White molly)

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Abstract: *Poecilia sphenops* were sampled to ascertain the effect of Natrum muriaticum of 30c potency by two different diets of commercial feed and chicken liver feed. Recent studies reveal that a homeopathic hormone Natrum muriaticum is found to be effective in inducing spawning and growth of the fishes. In the present study, Homeopathic medicine Natrum muriaticum used to induce spawning and growth by two different diets in the ornamental fish *Poecilia sphenops*. The fishes were introduced into water containing Natrum muriaticum 30 centesimal potency of dilution 0.025%. A control was also maintained simultaneously. The time taken for spawning in control and experiment groups was compared. From the result, it is inferred that the homeopathic medicine Natrum muriaticum has advanced spawning in experimental group of fishes. There was a considerable change in weight gain, and ammonia excretion in experimental fishes. We conclude that quality of the water should be maintained for the successful spawning and proper growth of the fishes. The amount of protein, lipid and carbohydrate was determined in the muscle and liver of the fishes. There was a significant difference in the protein, lipid and carbohydrate at 0.025% dilution. There was a significant changes in spawning, growth of *Poecilia sphenops* when it was treated with chicken liver feed.

Key words: Induced spawning, *Poecilia sphenops*, Natrum muriaticum, 30 Centesimal Potency, Fecundity Rate, Biochemical analysis

INTRODUCTION

Ornamental fish keeping is becoming popular as an easy and stress relieving hobby. About 7.2 million houses in the USA and 3.2 million in the European Union have an aquarium and the number is increasing day by day throughout the World. Ornamental fish farming is also growing to meet this demand¹. In order to meet this demand many modern techniques are used. Now- a-days fish culturists who wanted to induce spawning with hormones. The cost of modern medicine which is rising higher on account of progressive specialization, sophistication of diagnostic techniques and apparatus has increased its popularity. Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish internal regulating factors with injected hormones or other substances. The internal mechanisms that regulate spawning are similar for most fishes. It is intended for using as a spawning aid in reproductively mature conditioned fish. Two types of

hormones (Ovatide and Ovapel) are in testing condition (Jose, 2001). But all these hormones are synthetic and highly priced one. The preparation technologies of these hormones are also very tiresome. Natrum muriaticum is one of the oldest medical sciences and utilizes indigenous drugs of either plant or mineral origin for induced spawning, growth and treatment of diseases. Natrum muriaticum is a homeopathic medicine of mineral origin. So the present study is aimed to ascertain the effect of Natrum muriaticum on spawning, growth and biochemical changes in *Poecilia sphenops*.

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 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 Rinodinojudes Pet Shop at Thoothukudi. 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.

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 Natrum muriaticum solution of dilution namely 30 centesimal (30 C) is brought from Saraswathi homeopathi medical centre at Thoothukudi. From Natrum muriaticum 30 centesimal 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.

Experimental procedure

Well acclimatized, healthy fishes were chosen for the experiment. Based on previous literatures on Natrum muriaticum, one concentration was chosen for the present experiment, the concentration namely 0.025% Natrum muriaticum was chosen respectively. Two sets of experiment were carried out to study the effect of Natrum muriaticum. One set of experiment was maintained to analyse the biochemical parameter such as protein, lipid and carbohydrate and another set of experiment was to induce breeding.

In each set of experiment, six cement tanks with 20 L capacity were chosen. Two tanks are for control and other two tanks for commercial feed and other two for liver feed treated with Natrum muriaticum. To study the effect of Natrum muriaticum on biochemical changes 20 individuals were introduced in each tank. Totally 60 fishes were chosen for the experiment. To study the effect of Natrum muriaticum induced breeding, four female fishes and two male fishes were selected for the experiment. Six fishes were introduced in each tank. Totally 18 fishes were introduced for

induced spawning. In control and Natrum muriaticum treated tanks, 10L of water medium was added. The desired concentration of Natrum muriaticum was diluted in test medium. The experiment was conducted for 30 days. The animals were scarified on the 15th and 30th days for the biochemical analysis of the protein, lipid and carbohydrate. The weight of the fishes was taken periodically.

During the course of the experiment, the animals weighed 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 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. Manufactured feeds are an important part of modern commercial aquaculture, providing the balanced nutrition needed by farmed fish. Special feeds can be used to enrich the nutritional of the prey. In the present study Chicken liver used as a feed to enhance the nutritional quality of fish. It increase the weight of the fish. The chicken liver contains most vitamin like vitamin B, E and vitamin C. They contain high amount of iron and they contain low carbohydrate.

RESULT

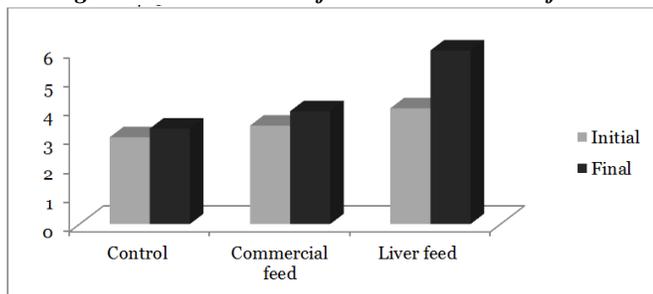
The present study deals with the effect of Natrum muriaticum on the duration for spawning, growth, fecundity, excretion and protein, lipid and carbohydrate level of ovary, muscle and liver. The results of this study revealed that 0.025% solution of Natrum muriaticum advanced the process of spawning in Poecilia sphenops. Table 1 and Figure 1 represent the growth rate shown by the control and the treated fishes. It showed the fishes maintained in experimental set up by feeding chicken liver attain maximum growth rate than that of other fishes which maintained in the control and commercial feed.

Table: 1 Growth rate of control and treated fishes

Rearing days	Control (1)		Treated			
			Commercial feed (2)		Liver feed (3)	
	Weight (g)	Growth	Weight (g)	Growth	Weight (g)	Growth

		rate		rate		Rate
Initial	3±0.22	0.3	3.4±0.16	0.5	4±0.11	1.1
Final	3.3±0.21		3.9±0.14		6±0.28	

Figure : 1 Growth rate of control and treated fishes



Fecundity

The data on the young ones released by the control and the treated fishes during experimental period is presented in Table 2. It revealed that there was a significant difference in the number of young ones produced by the control experimental groups.

Table: 2 Number of young ones released by control and experimental groups

Groups	Number of young ones released
Control	11±0.36
Commercial feed	18±0.25
Liver feed	26±0.32

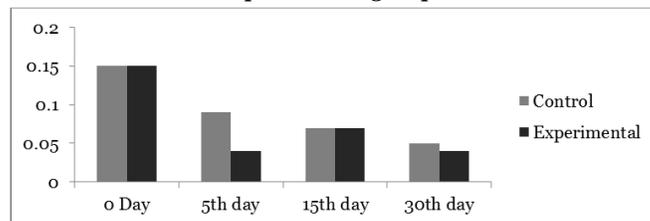
Ammonia

The ammonia content in the control and the treated systems are recorded in Table 3 and Figure 2. It showed that there was a significant difference in the ammonia levels on both control and the treated systems.

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

Days	Control	Experimental
0 Day	0.15±0.061	0.15±0.061
5 th day	0.09±0.004	0.04±0.005
15 th day	0.07±0.003	0.07±0.003
30 th day	0.05±0.004	0.04±0.003

Figure: 2 Number of young ones released by control and experimental groups



Protein, lipid and carbohydrate content

The biochemical analysis of the protein, lipid and carbohydrate of commercial feed and chicken liver feed is tabulated in table 4. It showed that there was increase of protein and lipid content in the chicken liver feed but it showed low carbohydrate level than that of the commercial feed. Two way ANOVA test showed that the protein content was significant levels (P <0.01) of muscle and liver. The lipid was significant levels of (P <0.01) muscle. The carbohydrate was significant levels (P <0.01) of liver (Table 4).

Table: 4 Two way ANOVA Table for biochemical parameters in different tissues of Poecilia sphenops fed with different diets as a function of rearing period

Sources of variation	SS	df	MS	F	P – value
Protein Muscle					
Between different diets	5.79	2	2.89	11.41	P<0.01
Between Rearing period	20.0	2	10.01	39.37	P<0.01
Interaction	3.40	4	0.85	3.35	
Within	72	18	4.0		
Total	4.58	26	0.25		
Protein Liver					
Between different diets	66.2	2	33.15	89.40	P<0.01
Between Rearing period	77.0	2	38.55	103.96	P<0.01
Interaction	33.3	4	8.34	22.49	
Within	7	18	0.37		
Total	183.4	26	7.05		
Lipid Muscle					
Between different	0.4	2	0.2	10.86	P<0.01

diets					
Between Rearing period	1.16	2	0.58	31.36	P<0.01
Interaction	0.28	4	0.07	3.82	
Within	0.33	18	0.019		
Total	2.18	26			
Lipid Liver					
Between different diets	2.02	2	1.011	1.41	P<0.01
Between Rearing period	8.72	2	4.36	6.07	P<0.01
Interaction	1.07	4	0.27	0.37	
Within	12.9	18	0.72		
Total	24.7	26			
	4				
Carbohydrate Muscle					
Between different diets	1.56	2	0.78	0.86	P<0.01
Between rearing period	19.4	2	9.74	10.67	P<0.01
Interaction	1.12	4	0.28	0.31	
Within	16.4	18	0.91		
Total	38.5	26			
	8				
Carbohydrate Liver					
Between different diets	13.5	2	6.76	11.56	P<0.01
Between rearing period	7.8	2	3.9	6.67	P<0.01
Interaction	7.54	4	1.89	3.22	
Within	1053	18	0.59		
Total	39.3	26			
	9				

Before induced breeding there is increased in the amount of protein and lipid content in the ovary of the fishes which is treated with Natrum muriaticum and feed with liver. After induces breeding there is reduced in the amount of protein and lipid in the ovary of the fishes treated with Natrum muriaticum. Two way ANOVA test showed that analyse of the ovary the protein content level was significant ($P < 0.01$) and lipid (Table 5).

Table: 5 Two way ANOVA Table for biochemical parameters in ovary tissues of *Poecilia sphenops* fed with different diets as a function of rearing period

Sources of variation	SS	df	MS	F	P – value
Before breeding ovary protein					
Between different diets	7.79	1	3.89	74.4	P<0.01
Between Rearing period	3.89	2	11.01	11.37	P<0.01
Interaction	5.25	2	0.85	21.35	
Within	3.58	3	0.54		
Total	20.52	8			
Before breeding ovary lipid					
Between different diets	0.29	1	0.15	3.1	P<0.01
Between Rearing period	1.09	2	0.55	0.96	P<0.05
Interaction	0.37	2	0.34	4.49	
Within	0.67	3	0.37		
Total	2.43	8			
After breeding ovary protein					
Between different diets	4.4	1	2.2	26.86	P<0.01
Between Rearing period	2.16	2	9.58	9.36	P<0.01
Interaction	4.28	2	0.07	3.82	
Within	2.33	3	0.019		
Total	10.85	8			
After breeding ovary lipid					
Between different diets	0.02	1	0.011	2.41	P<0.05
Between Rearing period	1.72	2	0.36	0.07	P<0.05
Interaction	0.07	2	0.27	0.37	
Within	0.93	3	0.72		
Total	2.74	8			

DISCUSSION

It is evident from the result that the Natrum muriaticum was useful in induce breeding. In the present study *Poecilia sphenops* fish was treated with Natrum muriaticum that shoes induce breeding and young ones were released earlier than that of the untreated fishes. Vishakan et al., (2005) suggested that the injection of the Natrum muriaticum 1000 centesimal potency in to the gold fish (1ml/Kg) induced spawning g within 22 hours 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/Kg body weight (Roy, 1996). In *H.fossilis* spawning at 0.3, 0.5 and 0.7ml/Kg body mass for ovaprim and 1000, 2000 and 3000 IU/KG body mass for HCG (Haniffa, 2002). So in the present investigation shows that Natrum muriaticum was useful in induced breeding.

The present study shows that high level of body weight and growth rate observed in *Natrum muriaticum* treated fish. Similar result reported in *Natrum muriaticum* increased the production of red blood cells and albumin, a protein found in animal vegetable tissues (Sawyer, 2004). It does not supplied 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.

Natrum muriaticum may also cause changes in the biochemical constituents of the eggs, thereby influenced fast ovulation (Vishakan et al., 2005). The present investigation shows that young ones were produce earlier when they were treated with *Natrum muriaticum*.

In the present study shows that there was increase in the protein, lipid and carbohydrate during maturation and decrease in post-spawning period. The biochemical analysis revealed that the fluctuating trend of proteins and carbohydrates during different stages of maturity. However, total lipid percentage of the ovary showed a gradual increase from stage I to stage IV (Ghulam Nabi hajam et al., 2012). The protein level increased in ovary, muscle and haemolymph. Protein content of the ovary *Epinephelus diacanthus* increased with the maturation (Chandrasekhara Rao Krishnan, 2011). According to Robards, (1999) protein percentage of gonads increased in the pacific sand lance (*Ammodytes hexapterus*) in relation to maturity. The lipid content also increased in tissues and the heamolymph of *Portunus pelagicus* (Raghunath Ravi, 2012). Proteins are of vital utility for fish growth and other metabolic activity.

Among the biochemical components analysed lipid and carbohydrate contents showed significant variations in the different tissues of *Haliotis* sp. during progress of gonad maturation (Najamudeen,2007). Lipid concentration increased from stage 1 through 3 and decreased in stage 4. (previtellogenesis, vitellogenesis, final maturation and post ovulation.) in female *Rachycentron canadum* (Biesiot,1994). It has been demonstrated that vitellogenin which is synthesized by liver in response to estradiol is released into the blood and then transported to ovary (Wallace et al., 1978). It is well known fact that biochemical changes in various cellular organelles of the oocytes occur during oogenesis in number of vertebrates.

The reduction of protein, lipid, carbohydrate suggested that possibility may due to the ovulation of the fish. According to Saidapur (1982) 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.

The present investigation showed the fecundity rate in *Poecilia sphenops* in control 11 ± 0.36 and in the commercial feed 18 ± 0.25 and in the liver feed 26 ± 0.32 . There was significant increase in the fecundity rate in the treated fish with liver. The fecundity rate in *P.sphenops* is 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 *Natrum muriaticum* 1000 (cm) induces the spawning in gold fish. The experimental animals laid 2856 eggs while control laid 209 eggs. (Vishakan et al., 2005). An average of 4012 ± 10 eggs are spawned by each female by a single intramuscular injection of ovaprim (0.5ml/Kg) (Sridhar and Vijayakumar, 1997). Fecundity in *Chana punctatus* is 3273 ± 75 for ovaprim and 1253 ± 126 for HCG, whereas in *H.fossilos* it is 6692 ± 790 for ovaprim 82922 ± 5432 for HCG (Haniffa,2002).

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 treated systems on 30th day. Ammonia content for control and experimental fishes is 0.06 ± 0.005 and 0.04 ± 0.005 mg/fish/hr respectively. The ammonia excretion is decreased on 30th day when compared to o 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 present report shows there was an increase in ammonia content in experiment may due to the fish treated with *Natrum muriaticum*.

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