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LABORATORY INVESTIGATION ON SALINITY TOLERANCE TO *Barbodes gonionotus* (Bleeker)

M. Akther, A. R. Mollah and M. Kadir[1]
Department of Fisheries, Bangladesh, Dhaka

ABSTRACT

Salinity tolerance to Thai silver barb, *Barbodes gonionotus* was investigated under laboratory conditions using common salt (NaCl) and sea water. With a view to determine the median lethal concentrations (LC50 values), the fish were exposed to various concentrations of the salt and sea water for a period of 96. Common salt up to a concentration of 12 ppt and sea water up to a concentration of 10 ppt did not cause mortality to the experimental fish. The LC50 values for 6h, 12h, 24h, 48h, 72h, and 96 hexposure times were 17.91 ppt, 16.91 ppt, 15.35 ppt, 14.51 ppt and 13.92 ppt common salt, respectively. During experiment action with sea water, the LC50 values obtained were 15.02 ppt, 14.18 ppt, 13.48 ppt, 12.61 ppt, 12.35 ppt and 12.25 ppt for 6 h, 12 h, 24 h, 48 h, 72 h and 96 h exposure times, respectively, On the other hand, pre-exposure acclimation in modifying the acute lethal toxicity of the salt showed, a concentration of 18 ppt salt killed all the fish within 4 days, indicating that gradual increases in salinity increases the salt tolerance of *B. gonionotus.*

Key words : Silver barb, Salt tolerance, LC50

INTRODUCTION

In Bangladesh, rajponti or sliver barb, *Barbodes gonionotus* was introduced as exotic species from Thailand in 1987. The fish withstand up to 15 ppt salinity and grow well up to 7 ppt saline water (BFRI, 1993). Therefore the fish has high culture potential under coastal aquaculture practice. Every aquatic organism has a number of limiting factors that influence their abundance in aquatic media, salinity is one of them. Acute salinity tests of aquatic organisms especially in case of fish have been used extensively to determine the effects of salinity during short-exposure. Acute lathality test is used to determine the concentration of a test material or the level of an agent that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled conditions. Therefore, determination of acute lethal salinity values is one of the prerequisite of the testing culture potentialities of rajpunti. Salinity tolerance or adaptation tests are, in many cases, are carried out with common salt (sodium chloride). Since the overall objective of the study was to provide guideline information on salinity tolerance of the freshwater fish *B.gonionotus* for coastal aquaculture, it was decided to carry out tests with common salt in addition to diluted sea water.

[1] Department of Fisheries, Dhaka, Bangladesh

MATERIALS AND METHODS

The experiment was carried out in the out-door laboratory facilities of Department of Zoology, University of Dhaka. Fingerlings of *B. gonionotus* (3.7-4.1cm, 0.5-0.8g) used as test animal were obtained from Tongi Fish Seed Multiplication Farm, Gazipur. The fish were brought to the laboratory in oxygenated polythene bags.

As soon as the fish arrived at the laboratory, immediately they were transferred to a large aquarium containing tap water, essentially similar to that was used in the experimental trials and thereafter maintained there for about 4 – 5 days in static condition. The fish were fed *Tubifex* in live and dried form, twice daily. Oxygenation of water was done by using air pumps. Before transferring the fish to the experimental system only healthy fish of approximate similar size were sorted and measurement of their lengths were taken.

The experiments were conducted in a static bioassay system. The system, consisted of 40 independent small tanks 8' x 7' x 7' during holding the fish. The tank was cleaned filled with tap water. The mouth of each tank was covered with wooden hood, thus prevented the fish jumping out of the tank.

In the present study common table salt (NaCl) and concentrated seawater were used. The concentrated seawater was obtained from salt-bed of Cox's Bazar. This was later diluted with tap water to get the desired concentrations. Salinity was measured by Refractometer. On the basis of the information obtained from the preliminary trial, the different salt levels to be used in the experiment were selected.

Two experiments and an preliminary trial were conducted with each of the table salt and sea salt. Two experiments were conducted to determine the medium lethal concentrations of table salt (NaCl) and sea-water. The preliminary trial (range finding trial) was done in order to find a suitable range of salt concentrations that would cover zero to 100% mortality and to be used in the actual experimental trial, Table 1 shows the concentration of salt used in different experimental trials.

Table 1. The concentrations of salt used in different experimental trials

Experimental trials	Concentration used
Preliminary	5, 10, 15, 20, 25, 30, 35
Actual trial 1 with NacL	8, 10, 12, 14, 16, 18, 20
Actual trial 2 with NaCL	10, 11, 12, 13, 14,15, 16, 17, 18, 20
Actual trial 1 with sea water	8, 10, 12, 14, 16, 18, 20
Actual trail 2 with sea water	10, 11, 12, 13, 14, 15, 16, 17, 18, 20

The actual trial with individual salt was replicated twice in order to demonstrate the reproductibility of results. Before performing any experimental trial, the experimental tanks were washed and cleaned and further dried in the sun light for a day. Two days

before performing the experimentation a batch of 10 fish randomly selected were transferred to each of the experimental tanks and thereafter acclimatized in the system until they were challenged by the salt.

Organism, loading of approximately 1.0 gm/1 of water was maintained in all the tests as recommended by American Society for Testing and Materials (1970) and American Public Health Association (APHA, 1980). Test media was renewed daily, Each test, whether preliminary of actual, trial was conducted for a period of 96 hours to determine the medium lethal concentrations (LC50) as recommended by Sprague (1969, 1973), APHA (1980).

Records of mortality were made at logarithmic time intervals (Sprague,1973; Hasan, 1986) i.e. after 6-, 12-, 24-, 48-, 72 and 96 hours from the starting of the test and once daily thereafter. However, in addition to these fixed time observations, several inspections were made in between these periods and fish were removed from the test tanks as soon as they were found dead to prevent water quality deterioration. The cessation of opercula movements was used as the criterion for death.

The physical and chemical characteristics of the test water before and during bioassay were measured. The temperature, dissolved oxygen content and pH of the test solution were measured every day representing each salt concentration. The temperature was measured by N-filled mercury thermometer in ^{o}C. Dissolve oxygen concentration was measured using oxygen meter (Model – 9070) and pH measured by pH meter (Model-3100) Ammonia-Nitrogen was measured following Nesslerization method (Stirling, 1983) with modifications after Knud-Hansen (1992). Alkalinity, total hardness, carbon-di-oxide & Nitrite-N were measured from the test water once daily during each trial using portable water test Kit (HACH, USA). The median lethal concentration data was obtained by probit analysis.

RESULTS AND DISCUSSION

Behaviour

Various behavioral anomalies were observed in test fish when exposed to the salts. Intoxication and death following treatment with salt and different behaviour associated with them varied with the rate of application and the time of exposure. Behavioral anomalies in fish observed during the exposure are briefly summarized below :

The first visible reaction of salt exposure was observed at the highest concentrations within few minutes of exposure. The fish at these concentrations frequently tried to jump out of the fish holding tanks and showed heightened activity indicated by rapid to and fro movement of the fish. The highest concentrations (20, 25, 30, 35 ppt) induced fish mortality within 15 minutes of exposures. The fish in those concentrations gradually became lethargic and showed erratic movement and developed muscle spasm and body

convulsions. The fish tended to settle down on the bottom of the tank and later the fish was found to swim on their back or lateral side with occasional body jerking.

These sorts of behaviour were more pronounced prior to their death and were exhibited by the fish which died later. The fish at the lower concentrations also showed initially hyperactivity towards the end of the exposure period than control fish. The body of fish appeared to be more slimy due to excessive mucous secretion. Throughout the experimental period the control fish remained active and appeared normal. They also responded well on gentle prodding.

Preliminary trial with common salt

The concentrations up to 10 ppt of salt did not induce any mortality within 96 h of exposure. However a 20% mortality occurred by 24 h in fish group exposed to 15 ppt and by 96 h showed a 100% mortality. Fish exposed to 20 ppt salt recorded a mortality of 50% by 6 h of exposure and a100% mortality was observed in the same group of fish within 12 h of exposure. The fish groups exposed to the rest of concentrations died within 6 h of exposure.

Experimental trial with common salt

The data on cumulative mortality (%) of *B. gonionotus* challenged with different concentration of common salt during a 96 h exposure period are presented in Table 2 There was no death either in control (0 ppt) group of fish or in fish groups exposed up to concentrations of 12 ppt common salt during the experimental period. A10% mortality occurred at 13 ppt salt within 24 h of exposure and the same group of fish showed a 60% mortality by 96 h of exposure. However, among the lower concentrations a complete (100%) mortality was observed in fish group held at 16 ppt salt within 72 h of exposure and a 100% mortality first occurred in fish group exposed to 18 ppt salt by 24 h of exposure.

Table 2. Cumulative mortality (%) of *B. gonionotus* at different concentrations of common salt (NaCl) within 96-hours exposure time

Treatment No	Concentration table salt (NaCl) ppt	No. fish released	Exposure time (hours)					
			Cumulative mortality (%)					
Control	0	20	0	0	0	0	0	0
1	8	20	0	0	0	0	0	0
2	10	20	0	0	0	0	0	0
3	12	20	0	0	0	0	0	0
4	14	20	0	0	30	45	50	60
5	16	20	0	20	55	70	90	100
6	18	20	50	85	100	100	100	100
7	20	20	80	100	100	100	100	100

The median lethal concentrations (with 95% confidence limit) for common salt at various exposure times are presented in Table 3. In this trial 6, 12, 24, 48, 72 and 96 h LC50 values were 18.42, 17.05, 15.19, 14.64, 14.19 ppt of common salts respectively.

Table 3. Median lethal concentrations (LC50) and 95% confidence limit (CL for *B. gonionotus*) exposure to common salt for various time

Exposure time (Hour)	LC50	95% confidents limit (CL*)	
06	17.40	16.99	18.05
12	16.77	16.32	17.30
24	15.51	15.01	15.96
48	14.37	13.85	14.87
72	13.65	--	--
96	13.21	--	--

* Confidents limit could not be calculated

Preliminary trial with sea water

The concentrations up to 10 ppt of salt did not induce any mortality within 96 h of exposure. However, a 20% mortality occurred by 12 h in fish exposed to 15 ppt salt and by 96 h this fish group of fish showed a 100% mortality.

Fish exposed to 20 ppt sea water recorded a mortality of 65% by 6 h of exposure & a 100% mortality was observed in the same group of fish within 12 h of exposure. The fish groups exposed to the rest of the concentrations died within 6 h of exposure.

Experimental trial with sea water

The cumulative mortality (%) of *B. gonionotus* exposed to various concentration of sea water at different time intervals are presented in Table 4. No mortality was found to occur in fish up to the sea water concentration of 10 ppt as well as in control fish. After 12 h of exposure a 100% mortality was observed in fish group at 16 ppt sea water. The lowest mortality (10%) however, occurred at 12 h exposure in fish group maintained at a sea water concentration of 12 ppt.

The median lethal concentrations (with 95% confidence limit) for sea water at various exposure times are presented in Table 5. In this trial 6, 12, 24, 48, 72, and 96 h LC50 values were 15.01, 14.19, 12.74, 12.46, 12.37 and 12.27 ppt of sea water respectively.

Water quality

It is evident from the results that water quality parameters measured during experiments with common salt (NaCI) among the different treatment tanks as well as between the duplicate tanks did not very. Since, except salt levels all other treatments were similar. In

all experimental tanks and since NaCl has no or little influence on water quality (except salinity), variations are not expected. Since, Water quality did not very except salinity the results obtained in the present investigation are attributable to the changes in salinity alone.

Table 4. Cumulative mortality (%) of *B. gonionotus* at different concentrations of Sea water within 96-hours exposure time

Treatment No.	Concentration sea salt (ppt)	No. of fish released	Exposure time hours					
			Cumulative mortality (%)					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	8	20	0	0	0	0	0	0
2	10	20	0	0	0	0	0	0
3	12	20	0	10	30	35	40	40
4	14	20	40	50	80	90	90	95
5	16	20	70	75	100	100	100	100
6	18	20	85	100	100	100	100	100
7	20	20	100	100	100	100	100	100

Table 5. Median lethal concentrations (LC50) and 95% confidence limit (CL for *B. gonionotus*) exposure to sea water for various time

Exposure time (Hour)	LC50	95% confidents limit (CL*)	
6	15.010	14.289	15.689
12	14.186	13.518	14.842
24	12.743	12.177	13.286
48	12.464	11.926	12.973
72	12.369	11.423	12.885
96	12.274	11.756	12.761

Water quality parameters, measured during experiments with sea water, showed some degree of variations increased with increasing salinities. The observed variations in the above parameters were avoidable since sea water concentrations used were prepared by diluted sea water.

The dilution of sea water with freshwater resulted in the variations at in the results obtained for various treatments. However, the observed parameters are within the normal ranges for fish and it does not appear that the slight variations in pH levels had any effect on the fish mortality.

Behaviour

The abnormal behaviour depends on different concentrations of salt such as erratic swimming, hyper-activity, increased opercular activity, jumping out of the test media, violent spasm, convulsions, erratic movement, loss of equilibrium etc. Similar observations were also made with pesticides (Schoetger, 1970; Verma *et al.,* 1977). Hyperactivity of fish under chemical stress, particularly at the initial stage is frequently observed phenomena (Rand and Petrocelli, 1985). This is probably a response to the stressful environment created by chemical that the fish wants to avoid (Rand and Petrocelli, 1985). This may also be due to stimulation of the surfacial perceptive organ caused by the chemical.

Increased opercular activity signify increased activity and oxygen demand caused by the salt and are consistent. However, in the present case hyperactivity was only observed at early stage of exposure only rather the fish became lathargic during the later stage of exposure. Similar observation was also made by Rao *et al.* (1967) for malathion.

Slow movement and loss of equilibrium observed in the present study could be due to the weakness of the fish. It is very likely that the treated fish were unable to carry out their normal physiological functions, particularly which of osmoregulatory function. Secretion of excessive body mucous was observed during present study. Excess mucous secretion may be an adaptive effort to prevent entry of salt through skin and gill also. Irritation to the surfacial tissue by salt may also cause the secretion of mucus.

Mortality and median lethal concentrations

It is evident from the results that mortality of *B. gonionotus* was found to be dose depended. However, up to a certain concentration mortality did not occur, above which mortality response was rapid. This level of concentration may be termed as threshold level and is observed in many fish species (Sprague, 1969). The mortality data observed in the present study allowed the estimation of LC50 levels for all observation times. This was possible, probably, due to use of well thought spaced concentration within the range of 0 to 100% mortality. Khatun and Mollah(1991) observed a complete of mortality of *Clarias gariepinus* at 14% sea water salinity which is less than the present level of salt concentration which caused 100% mortality.

The variance in results could be due to the differences in fish size. In the present study 96 h LC50 values of common salt was 13.21 and that of sea water was 12.27 ppt respectively. A value of 9.1 ppt has been reported for *Perca flavescens* by Wilkerson *et al.* (1992). Begner and Pellentier(1991) recorded a value of 7g/1 for Atlantic cod. A LC50 value of 17.2 ppt was calculated for *Oreochromis niloticus* by Watanabe (1985) *et al*.

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