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Anaesthetic efficacy of table salt on two live fishes *Anabas testudineus* and *Channa punctatus*

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Abstract

Different concentrations of table salt (NaCl) were evaluated for its induction/anaesthetic and physiological effects on two live fish species *viz*., *Anabas testudineus* (Koi) and *Channa punctatus* (Taki). The time taken to immobilize and fully anaesthesize the fishes were quite long, 36-52.83 hours and 37.75-53 hours respectively for Koi, 64.50-71.50 hours and 68.25-71.50 hours respectively for Taki, but thereafter a quick mortality occurred in both species. The induction time was negatively related with the concentrations of salt, and independent of the intrinsic factors (total length and total weight) of the fish. No changes in the colours of skin, eye and gill were observed at lower concentrations were observed in *A. testudineus*, but the eye and gill were found to be affected at low concentrations in *C. punctatus*, irrespective of the exposure time. Table salt produced a bad smell at all concentrations at longer exposure in both species of fish.

Key words: Sodium Chloride, induction, anaesthetic, *A. testudineus*, *C. punctatus*

INTRODUCTION

Fishes are subjected to stress everyday due to changes in the culture system, water quality, environment, and their physiology (Koeypudsa & Jongjareanjai, 2011) and other stress factors regarding harvesting, handling and transportation (Woody *et al*., 2002). Stress disturbs the final internal balance of fish, such as behaviour, growth, reproduction, immune function and disease tolerance (Goos & Consten, 2000; Tanck *et al*., 2000; Chen *et al*., 2004; Davis & Griffin, 2004; Morales *et al*., 2005). To reduce the stress in fishes in culture system, in the research laboratory, handling and transportation several types of anaesthetics have been prescribed by the fisheries scientists. The scientists prefer low euthanization or sedation than full anaesthesia (Wurts, 1995; Davis & Griffin, 2004). However, the degree of anaesthetization varies, for example, for therapeutic use, laboratory research and transportation comparatively longer induction time is preferable, otherwise, a recovery time within 15 minutes in clean water is desirable (Gilderhus & Marking, 1987; Kaiser *et al*., 2006).

In this context a large number of anaesthetic compounds have been screened for use in aquaculture since a long time. The use of chemical fish anaesthetics sometimes showed

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hazardous effect in human and other animals when anaesthetized fish were subsequently eaten (Peake, 1998), and some of these compounds, an example, MS222 (tricaine methane sulphonate) is no longer used in some European countries (Kaiser *et al*., 2006), banned in Canada and restricted in United States (Peake, 1998). As an alternative to these chemical anaesthetics, food salts were introduced in aquaculture, research laboratories, and transportation of fishes and their fingerlings in live condition. Use of sodium bicarbonate was registered in USA as Low Regulatory Priority Compound (Peake, 1998; Bowser, 2001). Along with sodium chloride other food salts like sodium sulfate and sodium bicarbonate have been used to improve the quality of fish during transporting (Wurts, 1995; Gomes *et al*., 2006; Velasco-Santamaria & Cruz-Casallas, 2008; Koeypudsa & Jongjareanjai, 2011). The United States Food and Drug Administration (FDA) approved table salt or sodium chloride for use as anaesthetic on the food fish (Davis & Griffin, 2004). The present research focused on the anaesthetic effect of table salt (sodium chloride) in two live fishes *Anabas testudineus* (Bloch) and *Channa punctatus* (Bloch).

MATERIALS AND METHODS

Selection of fish species: Both *Anabas testudineus* (Koi) and *Channa punctatus* (Taki) are hardy fish and can survive out of water for comparatively longer time hence they are marketed in live condition. These two species can live in any type of container with very small volume of water, and also can withstand a wide range of temperature. Both the fishes are popular for their taste. For these reasons they are selected for the present experiment to observe the anaesthetic effect of table salt and the effect on their morphology and behavior.

Collection of fish and acclimatization: Healthy *A. testudineus* and *C. punctatus* fishes were collected in live condition from the fish markets of Rajshahi city. After collection the fish were immediately placed in plastic buckets with water, and carried to the Fisheries laboratory of the Department of Zoology, University of Rajshahi.

In the laboratory the fishes were released in aerated aquaria. Two species were kept separately. Tap water was used in these aquaria. Five fishes were released in each aquarium containing 10 liter of water. For the first 24 hours no food was given to the fishes, and afterward rice bran and wheat flour in the form of small ball were given once daily. One third of water of the aquaria was removed daily and fresh water was added. The fishes were acclimatized for week. Feeding was withheld 24-h before the commencement of the experiments. Healthy fishes with strong physique were selected for the experiments.

Concentrations of salt used: A pilot experiment was run to find out the sublethal concentrations of salt on the test fishes. *C. punctatus* was found to be more susceptible to salt bath compared to *A. testudineus*. Based on the pilot experiment, two different sets of salt concentrations were chosen for the two species. The concentrations used against *A.*

testudineus were 30, 40, 50 and 60 mg salt/l of water; and those were against *C. punctatus* were 5, 10, 15 and 20 mg salt/l of water.

Experimentation: Experimental fishes were collected individually using a hand net to avoid any damage and released separate aquarium containing five liter of fresh tap water. Before releasing the fish the water was mixed with salt and stirred well with a glass rod. Thus three sets of aquaria were used for each concentration of salt, and each fish species. Control batches of both species were set similarly in untreated water. No food and aeration were allowed in the experimental aquaria. The experiments were conducted at room temperature (17-20^{o}C) for 80 hours.

The time was recorded when each fish was released in the experimental aquarium. The fishes were monitored continuously to observe the effect of salt. First induction to salt was determined as the stage where total loss of mobility became evident (immobilization) and full anaesthetization stage was that when the fish could no longer swim and/or maintain a vertical position in the water. The time taken for immobilization and induction (anaesthetization) of individual fish was recorded. To observe further effect of salt the fishes were kept in the treated water without running the recovery test. The time when death occurred was also recorded.

When the fishes became immobilized, total length (TL) and body weight (TW) of individual fish were measured carefully and quickly then released in the aquarium.

Changes in morphological characters and behavior: Similar sets of experiment were arranged with approximately similar size of fishes as mentioned, to observe the changes of some morphological characters and behavior of each species due to salt bath. Observations were made and the data recorded after 4-, 8-, 12- and 24 hours of exposure. The morphological characters studied included the colors of skin, eye, gills and smell. The swimming behavior of the fishes was observed.

Anaesthetic effect of salt on dissected fish (*C. punctatus*): Total 40 specimens of *C. punctatus* of different sizes (total length ranged from 145-160 mm) were immersed in a solution containing 200 g salt in a small quantity of water in a plastic bucket to induce the fish before and during dissection in a practical class. The observation is described in the result.

RESULTS AND DISCUSSION

Anaesthetic effect of salt: *A. testudineus* was found to be more resistant to the salt induction activity than *C. punctatus*. A concentrations of 40 mg/l salt immobilized *A. testudineus* at 52.83 h, whereas, a concentrations of 30 mg/l did not affect the normal behavior of the fish up to 80-h of exposure. The fish became senseless after 53.00 h and died after 71.00 h at 40 mg/l. At highest concentrations (60 mg/l) the time required to immobilize the fish at 36 h; after 37.75 h the fish were fully induced and died after 40.50 h (Table 1a).

Table 1a. Time (hour) required for inducing anaesthesia to *Anabas testudineus* by different concentrations of table salt

Concentrations (mgL^{-1})	Average total length (mm)	Average total weight (g)	Time for immobilization (h)	Time for induction (h)	Time for total collapse (h)
30	126.67	35.33	-	-	-
40	120.00	31.67	52.83	53.00	71.00
50	117.33	31.67	45.33	52.50	65.50
60	114.00	31.83	36.00	37.75	40.50
Control	120.00	31.00	-	-	-

In case of *C. punctatus* the lowest concentrations (5 mg/l) immobilized the fish at 71.50 h, thereafter became slightly stressed, no lethal effect was observed after 80h exposure. At 10 mg/l the fish became immobilized after 67.50 h, lost sense after 71.50h and lived at a stressed condition up to the last of the experimental time. At highest concentrations (20 mg/l) the fish was motionless at 64.50 h, fully induced at 68.25 h and died at 69.42 h (Table 1b).

Table 1b. Time (hour) required for inducing anaesthesia to *Channa punctatus* by different concentrations of table salt

Concent-rations (mgL^{-1})	Average total length (mm)	Average total weight (g)	Time for immobilization (h)	Time for induction (h)	Time for total collapse (h)
5	158.00	93.33	71.50	-	-
10	151.67	38.00	67.50	71.50	-
15	157.67	40.67	67.83	69.75	70.50
20	153.67	38.67	64.50	68.25	69.42
Control	151.33	37.33	-	-	-

In case of both the species, the time required for induction stages (motionless to death) were positively related with the extrinsic factor (concentrations of salt), but there was no relationship with the intrinsic factors (total length and total weight) of fish. The differences between induction time at different concentrations were insignificant (Tables 1a, b).

Observation on the dissected *Channa punctatus*: The fishes became induced within 10-12 minutes in salt bath. To observe the internal organs the anaesthetized fishes were dissected open from thoracic to abdominal region (up to anus). It took nearly 30-40 minutes to complete the dissection. During this period it was noticed that heart of the dissected fishes were beating rhythmically but at a slight slow rate. Heart beat continued up to the end of the dissection class. Heart beat of these anaesthetized and dissected fishes were observed for 3.30 h (then the specimens were discarded). With all the trauma of incision, clearing some of the body muscles, pinning the abdominal muscles for clear view, the fishes were alive.

Effect of salt on morphological and behavioral characters: Effect of salt on colors of skin, eye and gills and on the swimming behavior of both the species were studied at 4-, 8-, 12- and 24 h after treatment. The normal skin color (greenish-black) of *A. testudineus* remained unchanged at concentrations of 30 and 40mg/l during the total exposure period; slight difference was observed at 40 mg/l after 24h. In concentrations of 50 and 60 mg/l the normal skin color was changed to brownish at12-h exposure. Later the color became light brown (50 mg/l, 24 h) and yellowish (60 mg/l, 24-h) (Table 2a). In *C. punctatus* white spots appeared on the skin at 12 h (5 and 10 mg/l) and 8h (15 and 20 mg/l). The scales became loose after 24 h at 5 and 10 mg/l, and 12 h at 15 and 20 mg/l (Table 2b).

Eye color of *A. testudineus* was quite normal (transparent) throughout 24h exposure at 30 and 40 mg/l, which changed to brownish after 12 to 24 h (50 mg/l), 8 to 12 h (60 mg/l) and then yellowish at 24 h at 60 mg/l (Table 2a). Eye color of *C. punctatus* was affected more at lower concentrations compared to *A. testudineus*. After 12 h eye became yellowish (5 and 10 mg/l). Then the color became cloudy after 24 h (5 mg/l) and whitish (10 mg/l). After 8 h the eye became cloudy in the fishes kept in 15 and 20 mg/l of salt (Table 2b).

Table 2a. Effect of table salt on morphological characters and behavior of *Anabas testudineus* at different exposure time (hour)

Concentrations (mgL^{-1})	Exposure time (h)	Parameters				
		Skin colour	Eye colour	Gill colour	Smell	Movement
00	24	Normal (greenish black)	Normal (transparent/clear)	Deep Red	Normal	Normal (frequent)
30	4	Normal	Normal	Deep Red	Normal	Normal
	8	Normal	Normal	Red	Normal	Rapid
	12	Normal	Normal	Red	Fishy	Slightly slow
	24	Normal	Normal	Pale Red	Fishy	Slow
40	4	Normal	Normal	Red	Normal	Stressing
	8	Normal	Normal	Red	Normal	Stressing
	12	Normal	Normal	Red	Fishy	Slow
	24	Light Green	Normal	Pale Red	Fishy	Slow
50	4	Normal	Normal	Red	Normal	Rapid
	8	Normal	Normal	Red	Fishy	Striving
	12	Brownish	Brownish	Pale Red	Fishy	Slow
	24	Light Brown	Brownish	Brownish	Fishy	Very slow
60	4	Normal	Normal	Pale Red	Normal	Stressing
	8	Normal	Brownish	Pale Red	Fishy	Stressing
	12	Brownish	Brownish	Brownish	Fishy	Slow
	24	Yellowish	Yellowish	Yellowish	Fishy	Very slow

The normal gill color (deep red) was changed to reddish (8-12 h), pale red (24 h) at 30 mg/l; red (4 to 12 h), pale red (24 h) at 40 mg/l; red (4 to 8 h), pale red (12 h), brownish (24 h) at 50 mg/l; pale red (4 to 8 h), brownish (12 h), yellowish (24 h) at 60 mg/l in *A.*

testudineus (Table 2a). Gill color of *C. punctatus* was changed after 8 h in 5 mg/l and after 4h at other higher concentrations (Table 2b).

Fishy smell appeared at all concentrations levels after 8 to 12 h in both the species. The smell was more intensed in *C. punctatus* at all concentrations and exposure times (Tables 2a, b). The odd smell was increased with the concentrations and exposure time.

Table 2b. Effect of table salt on morphological characters and behavior of *Channa punctatus* at different exposure time (hour)

Concentrations (mgL^{-1})	Exposure time (h)	Parameters				
		Skin colour	Eye colour	Gill colour	Smell	Movement
00	24	Normal (yellowish black)	Normal (transparent/ clear)	Deep Red	Normal	Normal (frequent)
5	4	Normal	Normal	Deep Red	Normal	Slow
	8	Normal	Normal	Reddish	Normal	Slow
	12	White spots	Yellowish	Pale Red	Fishy	Slow
	24	Loose scales	Cloudy	Brownish	Fishy	Rapid, unbalanced
10	4	Normal	Normal	Reddish	Normal	Rapid
	8	Normal	Normal	Pale Red	Normal	Slow
	12	White spots	Yellowish	Brownish	Fishy	Slow
	24	Loose scales	Whitish	Brownish	Fishy	Very slow
15	4	Normal	Normal	Reddish	Normal	Rapid
	8	White spots	Cloudy	Brownish	Fishy	Striving
	12	Loose scales	Cloudy	Brownish	Fishy	Slow
	24	Grayish	Cloudy	Yellowish	Fishy	Very slow
20	4	Normal	Normal	Reddish	Normal	Stressing
	8	White or black spots	Cloudy	Brownish	Fishy	Slow
	12	Loose scales	Cloudy	Yellowish	Fishy	Slow
	24	Whitish	Cloudy	Pale yellow	Fishy	Very slow

Salt bath affected the normal swimming movement of both the fishes at all concentrations levels throughout the experimental period. *C. punctatus* showed more stressed behavior (swimming and opercular rate). *A. testudineus* showed rapid swimming at 4h exposure and then moved slowly (8 to 24 h) at 30 mg/l; slow (4 h) then stressed and finally very slow movements at 40 mg/l. At 50 mg/l the fishes rapidly swam (4 h), began to strive (8 h) and then swam slowly. Stressed movement was noticed at 60 mg/l at 4h and then swimming became slow from 12h onward (Table 2a). Movement became slow during 4 to 12h and then rapid and unbalanced swimming was noticed in *C. punctatus* at 5 mg/l. At 10 mg/l the fish swam rapidly during first 4 h then it became slow gradually. Rapid swimming was noticed at 4 h then the fish strived (8 h) and became slow from 12 h at 15 mg/l. At 20 mg/l the fish showed stressed movement from more or less half an hour and

then gradually became slow (Table 2b). *C. punctatus* tried to escape from the salt treated water after 1-2 h at concentrations of 10-20mg/l.

Table salt or sodium chloride is one of the most commonly used drugs in the aquaculture, and fisheries scientists referred it as the 'aspirin of aquaculture' (Swann & Fitzgerald, 1992), as it is a therapeutic against the bacterial diseases in fish (Van Duijn, 1973) and also to treat the ectoparasites of fish, like *Costia, Epistylis, Trichodina, Chilodonella, Dactylogyrus and Gyrodactylus* (Swann & Fitzgerald, 1992). Fashina-Bombata & Busari (2003) reported that salt treatment is potential to control pathogens in developmental stages of African catfish.

During transportation of live fish a higher percentage of mortality occurs due to the activation of latent disease organisms and osmoregulatory problem (Jensen, 1990) and addition of sodium chloride is able to minimize these stresses (Francis-Floyd, 1995). During the transportation of live juvenile fish in tropical countries bacterial proliferation occurs, which can be prevented by adding either sodium chloride or methylene blue in the transporting water (Fajardo, 2002).

The anaesthetic effect of table salt was observed on two hardy fishes, *A. testudineus* and *C. punctatus*, and found that *Anabas* can tolerate comparatively higher concentrations of salt than *Channa*; but the induction time was less in *Anabas* than that of *Channa*. *Anabas* was induced in between 37-53 h, and *Channa* was induced in between 68-72h, depending on the concentrations. The long time taken for induction could be enhanced by using higher concentrations than those were used in this experiment. Toxicity of salt based on induction time was found to be dependent on the concentration of salt, but not with the total length or total weight of the fishes. The survival time for mosquito fish in salt bath was found to be correlated with the extrinsic factor like salt concentration and intrinsic factor like total weight (Newman & Aplin, 1992).

It was noticed that after total induction both the species died quickly. That means the concentrations used affected the salt level of blood and created osmotic imbalance. Wurts (1995) reported that 10% dehydration occurs in live fish when 10g/l salt was added to the transported water, and resulted in lethality. The author also reported that salt tolerance varies among different species and the temperature.

Both the species suffered salt stress at an exposure of 72h at all concentrations levels. *Channa* was more stressed than *Anabas*. The color of skin, eye and gills were affected at higher concentrations and longer exposure in *Anabas*. At all concentrations levels from 5-20mg/l, skin color changed loosing the brightness and slime secretion was enhanced in *C. punctatus*, and at longer exposure the scales became loose. The eye color became dull and cloudy and the gills lost the normal deep red color. Salt affected the eye at exposure more than 10h. Normal blood circulation in the gills was affected severely and respiratory stress was detected by discolored gills and rapid opercular rate, along with abnormal and unbalanced slow swimming. However, *C. punctatus* was alive in anaesthetized condition and tolerated the stress of dissecting trauma for more than three hours.

Besides handling and transportation, other stress factors like chill coma in tropical fish (Sun *et al*., 1995), physical damage and high nitrate level (Andrews *et al*., 2002) can also be prevented by the application of salt. Authors also reported the threat of osmoregulatory and other physiological stress occurred due to higher concentration of salt (Andrews *et al*., 2002; Burgdorf-Moisuk *et al*., 2011). Again it is observed that the anaesthetic stress in fish can be lowered if sodium chloride is applied along with the anaesthetic (Davis & Griffin, 2004).

Table salt was found to be a good anaesthetic agent which affect the heart rate insignificantly in *C. punctatus* for more than 3-h in severe traumatic stage and keep the fish alive. The salt concentrations used in the present experiment did not induce both the species quickly, but higher concentrations would be acted as quick inducing agent. Longer exposure to the salt at higher concentrations than the salt content in the fish blood would be fatal, so it is better to use salt in transporting water for juveniles and live fishes and also as therapeutic purpose in pisciculture, rather to use as an anaesthetic.

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