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CERTAIN BEHAVIORAL RESPONSES IN SELECTED FRESHWATER FISHES EXPOSED TO NITRAZEPAM AS ANAESTHETICS

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Abstract: Use of anaesthetic is aimed at achieving higher survival without affecting. Besides this, it remains established that anaesthetices make the weighing, marking, tagging fin clipping, stripping, transportation, spawning, measuring, photography and other operative procedures much convenient. In response to various doses of nitrazepam, in both the experimental fishes desired level of anaesthesia was obtained. During exposure to anesthetic slight change in body colour of fish was evident (nitrazepam 0.5 to 2.0 mg/l) notable reduction in metabolism of fish was evident.
Keywords: Anaesthetics, Nitrazepam, L. rohita, C. mrigala, Fry, Fingerlings.

Introduction

With the increasing importance of inland fisheries and aquaculture in particular, the need of live-fish transport has become inevitable. Further, the vagaries of monsoon and subsequent failure of fish breeding in nature stresses the need for large scale transport of fish seed in huge quantity in order to cope with increasing demand of fish seed in culture as well as capture fisheries sectors. Thus, any effort to reduce metabolic rate of fish during transportation would facilate transport of higher number of fish for a larger distance. In this context anesthetics are becoming increasingly important.

Anaesthetics are drugs which have ability to produce loss of all sensation and consciousness in the vertebrates exposed to such drugs. The main features of general anesthetics are:

(1) Loss of sensation

(2) Sleep (unconsciousness)

(3) Muscle relaxation and

(4) Abolition of refluxes

The word ‘Anesthesia’ was first introduced in medical science in 1846 through tha anaesthetis agent were known to science earlier. The present day anaesthesiology is a highly developed science both of medical and veterinary use. U.S. Food and Drug Administration (FDA) has set certain requirement for registration of fish anaesthetics. So for only tricaine methane sulfonate (MS-222) and carbon dioxide (Co_2) are registered by FDA for use on food

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fish(schnick et. al.,1979). Carbandioxide has been considered as very effective agents for anaesthetizing fish. Howeor, use of carban dioxide is very limited in live-fish transport. Rodman (1963) used dry ice for the transport of sturgeon while Abbot and swartz (1968) employed chilled water to brieng about sedation in fundulus heteroclitus. Therefore, this study has been conducted on two Indian major Carps viz. L.rohita and C. Mrigala.

Materials and methods

Advance fry of L.rohita and C.mrigala were procured from a fish farm under oxygen packing. The test fishes were feed rice bran and oil-cake in the ratio of 1:1. Feeding was discontinued 24hours prior to initiation of the experiment. In order to assess behavioral responses of fishes preliminary experiments were conducted in glass jar of 15 liters capacity. For this purpose different concentrations of anaesthetics were used. Before experimentation the fishes were acclimatized to laboratory condition for a week. For this study nitrazepam are used. The anesthetics used in the present study have been described here below and summarized in table1.1.

The stages and planes of anesthesia noticed in the present investigation (Durve and Dharma raja 1966) are described below. A summary of changes in experimental fish behaviour with corresponding stimuli imposed at various stages is presented in table 1.2.

Table- 1.1: Properties and uses of anaesthetic drugs

Sr. No.	Anaesthetic	Moleular weight	solubility	Stability	Hazard	Toxicity & behavioural Changes	Mode of action	Users & remarks
1	Nitrazepam	281.3	Very slightly soluble in water & organic solvent	Protect from light	Intact to skin and mucous	Exitment as lrritability and nervousness	Irritability and muscle relaxant	Sedative, hypnotics, sedative in medicines promising for live fish transport and opercular beat recorads

Table – 1.2: Summary of different stages of anesthesia in fishes with corresponding behavioural changes

S. No.	Anaesthetic stages	External vibrational stimuli	Tectile stimuli	Fin movements, dorsal fin (DF), pectoral fin (PF), pelvic fin (PV)	Nature of swimming movements	Respiratory movements
1.	Normal	Reactive	Reaction	Normal, DF raised and Dropped simultaneously (incessantly) PC constantly beats by spreading in and out	Co-ordinated	Normal, opercular beats regular
2.	Light sedation	Slightly reactive	Reactive	Slow down, DF raised only occa-sionally and never fully extended. PF beat with a slow speed, PV close or rarely open.	Slow down, swimming mainly by body move-ments	Slow, opercular beats slow but regular
3.	Deep sedation	No reaction	No reaction	Extremely slow or nil, DF collapsed , PF extremely slow or nil. PV collapsed.	Stationary at the bott-om or slow swimming only by wobbling bidy movements.	Slow, opercular beats slow but regular.
4.	Partial loss of equilibrium	No reaction	No reaction	Absent	Fish rolles in the water	Slow, opercular movemants slow but regular.
5.	Total loss of Equilibrium	No reaction	No reaction	Absent	Nil, Fish lies on the bottom with belly upward.	Slow, opercular movemants slow but regular.
6.	Loss of reflex Activity	No reaction	No reaction	Absent	Nil, Fish lies on the bottom belly upward.	Irregular quivering movements of operculum. Cease
7.	Medullary Collapse	No reaction	No reaction	Absent	Nil, Fish lies on the bottom with belly upward.	

Results & Discussion

In each case 20 fishes exposed to varied concentrations of 0.5 to 6.0 mg/l nitrazepam exhibited different levels of sedation in L. rohita fry (Table 1.3). The lowest concentration of nitrazepam caused light sedation in 60% (stage 1), 20% fish undergone depp sedation (DS) whereas remaining 20% were unaffected at the lowest dose. Partial equilibrium was obtained in L. rohita fry at 2.5 mg/l dose of nitrazepam. The concentrations of 6.0 mg/l proved lethal for 20% fish. The average induction and recovery time for stage 4 at the concentrations of 6.0 mg/l were 70.00 and 52.00 minutes respectively. The time required reach stage 4 decreased as the concentrations increased. During exposure to nitrazepam the fish exhibited a characteristic behaviour where in individual fish showed slimy mucous secretion and changed their body coloration which turned dark blue (especially on gills).

The lowest and highest concentrations were 1.0 and 9.0 mg/l respectively (Table 1.4). At 1.0 majority (60%) of fish reached the stage of light sedation and rest (40%) remain normal (stage 0). At the concentrations of 2.0mg/l fish reached the stage 1 and 40% in stage 2 while 20% remained normal. 80 present C.mrigala fry attained stage 4 at a concentrations of 9.0mg/l. With the average corresponding average induction and recovery times of 68.0 and 48.5 minutes, respectively. At concentration of 5.0 and 7.0 mg/l both recovery as well as induction time were 110, 84 and 24, 430 respectively. During experimental period fish showed jerking movement at the higher concentrations.

Tolerance test

For this, separate tolerance test was performed in which 20 fishes were left in the circular plastic tubs containing 30 liters of water with the desired concentrations of anesthetic. The fish were observed at every 6 hours interval for a period of 24 hours, whenever necessary. At the end of this period, the stage of sedation was noted and the fishes were transferred to another container with running water. The fish died during experimental period were removed. After experiment, fish were kept under observation for a period of 24 hours to find out. The post experimental mortality if any. The results of this experiment are tabulated in table 1.5. It may be seen from these tables that the fish were observed in stage 1 and 2 throughout the experiment. The mortality during and 24 hours of experimentation was also recorded.

Table –1.3 : Response L. rohita fry to prelimsinary doses of nitrazepam

S. No.	Concentration (mg/l)	No. of Fishes	Loss of equilibrium			Partial loss Of equilibrium (PLE) (% Fish)	Deep sedation (DS) (% Fish)	Light Sedation (LS) (% Fish)	Mortality (% Fish)	Normal (% Fish)
			% Fishes	Av. time induction (min.)	Av. time recovery (min)					
1.	0.5	20	-	-	-	-	20	60	-	20
2.	1.00	20	-	-	-	-	40	40	-	20
3.	2.00	20	-	-	-	20	60	20	-	-
4.	3.00	20	-	-	-	40	60	-	-	-
5.	4.00	20	-	-	-	60	40	-	-	-
6.	5.00	20	40	90	65	40	20	-	-	-
7.	6.00	20	60	70	52	-	20	-	20	-

Table – 1.4 : Response C. mrigala fry to preliminary doses of Nitrazepam

S. No.	Concentration (mg/l)	No. of Fishes	Loss of equilibrium			Partial loss of equilibrium (PLE) (%Fish)	Deep Sedation (DS) (% Fish)	Light Sedation (LS) (% Fish)	Mortality (% Fish)	Normal (% Fish)
			% Fishes	Av. time induction (min.)	Av. Time recovery (min.)					
1.	1.00	20	-	-	-	-	-	60	-	40
2.	2.00	20	-	-	-	-	40	40	-	20
3.	3.00	20	-	-	-	20	60	20	-	-
4.	4.00	20	-	-	-	40	40	20	-	-
5.	5.00	20	40	110	24	40	20	-	-	-
6.	7.00	20	110	84	43	-	-	-	-	-
7.	9.00	20	80	68	48.5	-	-	-	20	-

Table 1.5: Tolerance of L. rohita and C. mrigala to different doses of Nitrazepam for inducting various stages of anaesthesia

S. No.	Anaesthetics and Fish	Concentration Mg/l	Average exposure (hr)	Mortality (% fishes)		Stages Reached	Remarks
				During Experiment	Within next 24 hrs.		
	Nitrazepam						
1.	L. rohita	0.5	24	NIL	NIL	LS	-
		1.00	24	NIL	20	DS	-
		2.00	24	NIL	40	DS, PLE	-
		3.00	24	60	NIL	DS, PLE	Mortality after 15
		4.00	24	60	NIL	DS, PLE	hour during exp. & fish lost balance
2.	C.mrigala	1.00	24	NIL	NIL	LS	
		2.00	24	NIL	NIL	LS, DP	
		3.00	24	20	NIL	DS	
		4.00	24	NIL	100	DS, PLE	Fishes lost balance Between 10 to 15 hr

The preliminary dose assessment for nitrazepam in L. rohita and C. mrigala clearly indicated usefulness of this anesthetic in solution from (tables 1.3 to 1.5). The dose of notrazepam @ 1.0 mg/l for inducing light and deep sedation was found effective in L.rohita. This dose is lower than the dose of similar group of anesthetic by Sharma (1992).

Out of seven doses tried on C.mrigala dose of 2.0 mg/l nitrazepam was found effective to induce light and deep sedation. From these observation on L.rohgita and C.mrigala with reference to nitrazepam, it me be seen that this anesthetic turned up as fairly effective anesthetic compared to the other drugs tried on different fishr (chloral hydrate, Mc Far land,(1960).,Sodium barbital., Aitken (1936) and Mc Far land (1994)., Cooke SJ, et al (2004)., Yonar et al (2001)., Janicke nordgreen et al (2014).

The tolerance test conducted on L. rohita and C. mrigala with the use of nitrazepam also justified the dose of 1.0 and 2.0 mg/l., respectively without causing any mortality during 24 hours of exposure. Moreover, at the dose of 2.0 mg/l nitrazepam post-exposure mortality was also nil in C.mrigala where as L.rohita exposure at 1.0 mg/l nitrazepam post-exposure mortality was 20 present. From the behaviour at responses in both these carps with the use of nitrazepam it may be inferred that nitrazepam may be used as general anaesthetic in solution from for inducing light and deep sedation. Need less to state here that this anaesthetic has been tried on fish for the first time and its use is fairly justified because of its low price an easy availability.

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