STUDIES ON RESPONSES DURING TRANSPORTATION OF SELECTED FRESH WATER FISHES WITH NITRAZEPAM AS AN ANAESTHETICS

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Abstract: *The anaesthetics are useful for inducting mild sedation during transport to total anaesthetization required for surgical procedure. Present study was conducted on two economically important major carps I.e, L. rohita and C. mrigala with the use of nitrazepam. The results on live–fish transport of L. rohita and C. mrigala with nitrazepam further revealed that higher fish density correspond with higher dissolved oxygen consumption and thus higher metabolic waste generation. In transportation study, analysis of variance was highly significant (1%) for each of group and density tried. Similarly, for the interactions among groups and densities were highly significant (at 1 or 5 %).*

Keywords: *Anaesthetics, Nitrazepam, L. rohita, C. mrigala, Fry, Fingerlings.*

Introduction

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 the increasing demand of fish seed in culture as well as capture fisheries sectors. In this context anaesthetics are becoming increasingly important. In India notable contribution in this field are from Natrajan (1960), Sree-nivasan (1962), Durve and Dharma Raja (1966), Kewalramani and Gogate (1968), Durve 1970, 1975, Jain (1981) and Sharma (1992), Akbari et al (2010), Husen and Sharma (2014) with the use of anaesthetics for sedation of fish following advantage are evident : (1) Decrease in the rate of oxygen consumption.(2) Controlling the excitability of fish and thereby reducing chances of injury.(3) Reducing the time of handling. A large number of solid and liquid anaesthetics are available for use in fishery science. However, water soluble anaesthetics are more suitable for fish. Therefore, this study has been conducted on two Indian major carps I. e. 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. However, yearling of L. rohita and C. mrigala were collected from the departmental rearing ponds. The test fishes were fed rice bran and oil-cake in the ratio of 1: 1. Feeding was discontinued 24 hour prior to initiation of the experiment. Before experimentation the test fishes were measured length and weighed. For individuals or group tests fishes of more or less same size and weight groups were preferred. The anaesthetics used and stages in present study have been described here below and summarised in table 1.1 and 1.2.

Results and Discussion

With the different densities (D1, D2 and D3) of fry with oxygen packing for transportation of L. rohita and C. mrigala fry using various concentration of anaesthetics, have been shown in table 1.3. Table 1.3 shows a general decline in dissolved oxygen levels of the experimental water. However, in the case of G1 D1 and G2 D2 combination an increase of 0.603-1.055 mg/l of dissolved oxygen was observed.

The increase in CO_2 levels in the medium varied between 109.333 to 143.333 mg/l in the control group. Whereas, the range for experimental water with anesthetic varied between 50.666-84.666 and 59.33-114.00mg/l in G1 and G2 respectively (Table 1.3).

During experimental period of 48 hours of air packing of L. rohita fry, there was a marginal increase of ammonia in the controls as compared to nitrazepam treated medium. The increase in NH3 was 1.555 - 2.378 mg/l in G0 as compared to G1 (1.326 -1.60 mg/l) and G2 (1.532 - 2.104 mg/l).

The decline in pH (1.666-1.333) in G0 was higher a compared to the group G1 (6.633-1.00) and G2 (0.666-1.0666). From these results it would be further clear that higher density of L. rohita fry led to a greater decline in pH.

The per cent mortality of L. rohita fry was 13. 333 – 57. 776 in the control group (Table 1.3) Comparatively, mortality was lower in nitrazepam treated groups, G1 (0.00 – 28.333 %) and G2 (0.00 – 31.06%). Further, mortality was nil in the nitrazepam treated combination G1D1 and G2D1. It was further observed that higher density of L. rohita fry caused increased mortality.

A notable decline in dissolved oxygen was observed during 48 hours. However in nitrazepam treated combination G1D1, G1D2 and G2D1 and G2D2, slight increase in DO_2 was evident (Table 1.4). Similarly, the variance has also been significant (at 1%) for interactions of groups with densities. During the experimental period the increase in ammonia has been 1.463 – 2.355 mg/l in the control as compared to G1 (0.731 – 1.257 mg/l) and G2 (0.914 – 1.852 mg/l). The accumulation of NH3 was greater with higher densities.

The declines in pH (0.60 – 0.733) in G0 was higher as compared to the groups G1 (0.433 – 0.50) and G2 (0.50-0.566). A higher number of C. mrigala fry led to a greater decline in pH. The data obtained on fry mortality revealed higher (6.666-24.883%) mortality in control groups. The mortality was nil in four treatment combination. G1D1, G1D2, G1D3 and G2D1. Aitken (1936) was perhaps the first to recognize the potential use of anaesthetics in live fish transport.

The present observations of fish transport indicate the nitrazepam at correct doses is very useful for the live fish transport of L. rohita and C. mrigala fry (Tables 1.3 and 1.4). The recommended doses range from 0.5 and 1.0 mg/l for L. rohita and 1.0 and 2.0 mg/l for C. mrigala fry. In most of the cases, a notable decline in dissolved oxygen was observed during 48 hours. With increasing time was a notable increase in free CO_2 levels in all the cases (Tables 1.3 and 1.4). Further, higher densities of fish did show direct impact to cause higher free CO_2 levels; Vass (1951) and Sharma (1992) opined that the accumulation of CO_2 may be cause of fish mortality. During experimental period of 48 hours of air packing of fry of both the fishes, there was a marginal increase of NH3, of course, to lesser extent in the control, as compared to nitrazepam treated medium. Moreover, the decline in pH in control was higher as compared to treatments. It was further observed that higher density of both fry caused increased mortality.

Durve (1995) and Sharma (1992) considered light and deep sedation suitable for live-fish transport where as stage of PLE could we considered totally unsuitable for the purpose of live-fish transport. The doses of nitrazepam recommended for L. rohita and C. mrigala range between 0.5- 2.0 mg/l, fairly match the requirements for ideal anaesthetic such as its easy solubility in water low dose, economy and to tolerance of fish.

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Table- 1.1: Properties and uses of anaesthetic drugs

Sr. No.	Anaesthetic	Molecular weight	solubility	Stability	Hazard	Toxicity & behavioral Changes	Mode of action	Users & remarks
1	Nitrazepam	281.3	Very slightly soluble in water & organic sol vent	Protect from light	Irritant to skin and mucous	Excitement as irritability and hallucinations may occur C. N. S. disturbances decrease O2 Consumption	Irritability anxiety and muscle relaxant	Sedative, hypnotics, sedative in medicines promising for live fish transport and opercular beats records

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, swimming mainly by body movements	Slow, opercular beats slow but regular
3.	Deep sedation	No reaction	No reaction	Slow down, DF raised only occa-sionally and never fully extended. PF beat with a slow or nil. PV close or rarely open.	Stationary at the bottom or slow swimming only by wobbling body movements	Slow, opercular beats slow but regular
4.	Partial loss of equilibrium	No reaction	No reaction	Extremely slow or nil, DF collapsed, PF extremely slow or nil. PV collapsed.		Slow, opercular movemants slow but regular
5.	Total loss of Equilibrium	No reaction	No reaction	Absent	Fish rolls in the water	Slow, opercular movemants slow but regular

6.	Loss of reflex Activity	No reaction	No reaction	Absent	Nil, Fish lies on the bottom with belly upward.	Slow, opercular movemants slow but regular.
7.	Medullary Collapse	No reaction	No reaction	Absent Absent	Nil, Fish lies on the bottom belly upward. Nil, Fish lies on the bottom whith belly upward.	Irregular quivering movements of operculum.

Table 1.3: Efficacy of Nitrazepam in air packing of L. rohita fry with effects on selected water quality parameters

S. No.	Treatment	Dose of Nitrazepam mg/l	Density	No. Of fry/bag	Average weight of fry (gm)	Available water/fry (ml)	Increase (±) or Decrease (-) in Do (mg/l)	Increase in		Decrease in pH value	Mortality (%)
								Free co_2 (mg/l)	Ammonia (mg/l)		
1.	T_1	0.00 (G_0)	D_1	5	1.66 ±0.004	100	-2.412 ±0.212	109.333 ±0.942	1.555 ±0.031	1.166 ±0.044	13.333 ±9.427
2.	T_2	0.00 (G_0)	D_2	10	1.63 ±0.047	50	-4.373 ±0.738	136.666 ±0.942	1.807 ±0.063	1.266 ±0.044	26.666 ±4.713
3.	T_3	0.00 (G_0)	D_3	15	1.62 ±0.031	33.33	-6.199 ±0.187	143.333 ±0.042	2.378 ±0.031	1.333 ±0.054	57.776 ±3.139
4.	T_4	0.5 (G_1)	D_1	5	1.86 ±0.047	100	±0.603 ±0.212	50.666 ±1.885	1.326 ±0.031	0.633 ±0.044	0.00 ±0.00
5.	T_5	0.5 (G_1)	D_2	10	1.93 ±0.031	50	±1.055 ±0.212	77.333 ±0.942	1.395 ±0.031	0.933 ±0.044	3.333 ±0.00
6.	T_6	0.5 (G_1)	D_3	15	1.75 ±0.047	33.33	-0.754 ±0.00	84.666 ±0.942	1.601 ±0.063	1.00 ±0.00	28.333 ±3.143
7.	T_7	1.0 (G_2)	D_1	5	1.93 ±0.047	100	-0.452 ±0.214	59.33 ±0.942	1.532 ±0.031	0.666 ±0.044	0.00 ±0.00
8.	T_8	1.0 (G_2)	D_2	10	1.83 ±0.047	50	-1.659 ±0.047	82.666 ±0.942	1.692 ±0.031	0.966 ±0.044	10.00 ±2.120
9.	T_9	1.0 (G_2)	D_3	15	1.75 ±0.031	33.33	-3.317 ±0.031	114.00 ±0.00	2.104 ±0.031	1.066 ±0.054	31.106 ±3.143

Table 1.4: Efficacy of Nitrazepam in air packing of L. rohita fry with effects on selected water quality parameters

S. No.	Treatment	Dose of Nitrazepam mg/l	Density	No. of fry/beg	Average weight of fry 0(gm)	Available water/fry (ml)	Increase (_+) or decrease (-) in DO_2 (mg/l)	Increase in		Decrease in pH value	Mortality (%)
								Free Co_2 (mg/l)	Ammonia (mg/l)		
1.	T1	0.00 (G0)	D1	5	1.33 ±0.004	100	-1.508 ±0.214	67.333 ±8.21	1.463 ±0.031	0.60 ±0.00	6.666 ±2.340
2.	T2	0.00 (G0)	D2	10	1.53 ±0.047	50	-413 ±0.194	98.0 ±1.632	1.898 ±0.031	0.70 ±0.00	16.666 ±4.714
3.	T3	0.00 (G0)	D3	15	1.57 ±0.031	33.33	-4.675 ±0.212	105.333 ±0.942	2.355 ±0.031	0.733 ±0.54	24.883 ±3.143
4.	T1	1.0 (G1)	D1	5	1.46 ±0.094	100	±2.111 ±0.212	47.333 ±0.042	0.731 ±0.00	0.433 ±0.054	0.00 ±0.00
5.	T2	1.0 (G1)	D2	10	1.63 ±0.047	50	±1.2.6 ±0.214	58.0 ±1.632	0.914 ±0.063	0.466 ±0.054	0.00 ±0.00

6.	T3	1.0 (G1)	D3	15	1.60 ±0.00	33.33	-0.597 ±0.214	84.666 ±0.942	1.257 ±0.031	0.50 ±0.00	0.00 ±0.00
7.	T1	2.0(G2)	D1	5	1.70 ±0.081	100	±1.507 ±0.212	51.333 ±0.941	0.914 ±0.031	0.50 ±0.00	0.00 ±0.00
8.	T2	2.0 (G2)	D2	10	1.75 ±0.040	50	±0.602 ±0.214	63.333 ±0.941	1.074 ±0.031	0.533 ±0.044	3.333 ±0.00
9.	T3	2.0 (G2)	D3	15	1.60 ±0.054	33.33	-1.960 ±0.214	92.666 ±0.942	1.852 ±0.00	0.566 ±0.044	6.666 ±0.00