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Simulated Live Transportation of Fishes in Tanks Under Light and Dark Conditions

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Live fish command higher price in the fresh fish market than dead iced fish. Preliminary trials were held to find the effect of light and dark conditions on live fish transportation for the fresh fish market. Sub-adult Rohu (*Labeo rohita)* of 220-250g were held at high density (130 fish.m^{-3}) in aquarium and plastic tank to simulate live fish transportation conditions. The effect of light and dark conditions on oxygen consumption, ammonia build-up, pH and temperature were monitored during the study. Average rates of decrease in dissolved oxygen (DO) were highest under full light conditions (3.2ppm DO.h^{-1}) followed by semi dark and least in fully dark conditions (1.94 ppm DO.h^{-1}). Similar results were seen when fish were held in plastic tanks as well. Increased levels of salinity in water decreased the DO depletion rates (1.5-1.98 ppm DO.h^{-1}). Ammonia concentrations increased rapidly under fully dark conditions in presence of 0.9 and 1.1% salt (0.8-1.59 ppm $NH_3.h^{-1}$). Lowest rates of increase in ammonia concentrations were seen under full light conditions (0.68 ppm $NH_3.h^{-1}$). The pH under all conditions of holding decreased steadily in spite of the increase in ammonia. It is concluded that dark conditions are beneficial for live fish transportation provided the fecal matter could be removed effectively to counter the build-up of ammonia.

Keywords : Live fish, transportation, storage conditions, light & dark, *Labeo rohita*

Live fish trade is a highly lucrative enterprise. The average price of 28 fish species recently (July 17, 2007) traded in the Hong Kong market was HK$30.89/kg, while the average price of another lot of 15 species of live fish traded on the same day was HK$113.66/kg (FMO, 2006). Medina Pizzali (2001) observed that live fish in the Kolkata market was usually sold at higher prices than dead fish and most consumers were prepared to pay premium prices for live fish, which is considered as the the best guarantee of freshness, quality, and intrinsic characteristics of its flesh (better texture and delicate flavour) in comparison with fresh/chilled seafood. All fish have certain minimum requirements for loading density, dissolved oxygen (DO), temperature, pH and toleration limits for ammonia for their normal activities. Optimum DO content of pond waters for aquaculture should be in range of 5mg/l, while ammonia should be 0.02-0.05mg/ml (Adhikari, 2005). These are however not attainable when fish are transported live in confined conditions. Higher concentrations of CO_2 are tolerated by Indian major carp fry and fingerlings in presence of higher concentrations of DO_2, but conversely, at lower concentrations of DO, the tolerance limit for CO_2 is also reduced (Jhingran, 1991). The DO, temperature, pH and ammonia levels are some of the most critical factors in deciding the density and length of storage of fish in limited amounts of water. Hence, it is necessary to understand the factors that can influence the requirements of these factors by fish. This paper reports the effect of light and dark conditions on the water quality parameters of a freshwater fish under simulated conditions of transport.

Materials and Methods

The experiments were conducted in a glass aquarium of 100 x 60 x 60 cm (360 l)

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size provided with a externally mounted 0.2 HP centrifugal monoblock pump in recirculation mode and a 220V AC operated aquarium aerator with one aeration stone. All sides of the aquarium were covered with dark colored 5mm thick black polyurethane sheets to obtain Full dark (FD) conditions, the top sheet having holes for pump, aerator and sampling ports. Semi-dark (SD) conditions were obtained by removing the top sheet, while Full Light (FL) conditions were obtained by removing all the sheets except the bottom. For some of the experiments a black coloured cylindrical plastic tank (150cm dia., 500l capacity) was also used with its lid either closed or open to simulate FD and SD conditions more closely in actual transport conditions.

Rohu (*Labeo rohita*) (Mean length 22-26cm Mean weight 220- 250g) caught by drag netting from the aquaculture ponds of the Division were used for the experiment. Fish were not fed on the day previous to the experiment and confined in 1m^3 cisterns for acclimatization. A loading density of 65 fish.m^{-3} was maintained in the initial period of the experiment during which time the aerator and the pump were operated for 15-20 min to achieve full aeration. Fecal matter if any, was removed by siphoning and the loading density was increased to 130 fish.m^{-3} by removal of required quantity of water just before commencing the experiment. The experiment was begun by switching off the pump and aerator. Samples were drawn by siphoning from the bottom of the aquarium, for analysis of dissolved oxygen (DO), Ammonia and pH. Temperature of the water was also recorded. In case of FD experiments, fish were maintained overnight under FD conditions in the aquarium itself and fecal matter was removed as much as possible without disturbing the FD conditions as described before.

Borewell water stored overnight in an overhead tank was used for all experiments. Temperature was recorded using a mercury in glass thermometer and the pH using a digital pH meter with a combined glass electrode. Dissolved oxygen was determined by the Winkler's method and ammonia by the method of SolarZano (Strickland & Parsons 1972).

Results and Discussion

Temperature of the water varied from 22 to 29.8°C with an average of 25.8°C during the period of study. As the experiments were conducted at different time intervels of the year, temperatures could not be held constant. But temperature during any one experiment did not vary appreciably (Fig.1) from the initial value.

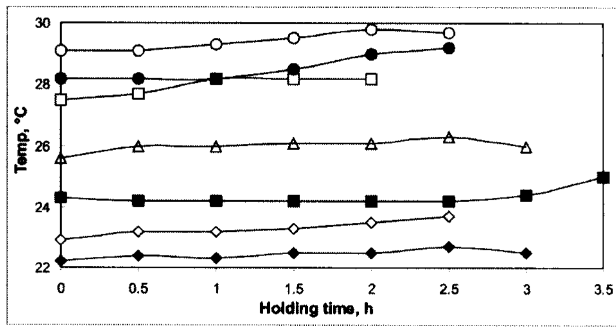

Fig. 1. Changes in temperature of water on holding Rohu (*Labeo rohita*) under simulated conditions of transport. Key to legends: ◇ - Full light ◆ - Semi dark, ■ - Full dark, □ - Full dark 0.9% saline, Δ - Full dark 1.1% saline, ○ - Tank semi dark, ● - Tank full dark.

The prevailing DO levels in water during the confined storage of fishes under various conditions are depicted in Fig.2. Experiments were terminated when the DO reached or was likely to reach levels of 1ppm O_2. The fishes also exhibited signs of distress at this DO level. As aeration / re-circulation was stopped when the experiment began, DO levels decreased during the course of the experiments. It was apparent from the results that Full dark (FD) conditions in the glass tank resulted in the longest holding times of 3.5h, while the shortest holding times were in the case of FL conditions (2.5h, 0.79 ppm DO) and FD with 0.9% saline (2h, 1.61ppm DO). Differences in the DO levels between the storage conditions and time were found to be highly significant (p < 1.16

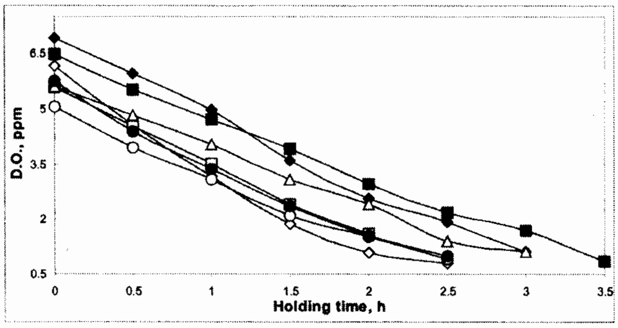

Fig. 2. Changes in dissolved oxygen concentration in water on holding Rohu (*Labeo rohita*) under simulated conditions of transport. For key to legends refer to Fig.1

$\times 10^{-9}$) indicating that it is possible to prolong storage time under confined conditions by maintaining darkness. The benefit of dark conditions however, was found to be not instantaneous and the fish needed to be acclimatized to the dark conditions to take advantage of the longer holding times. As the initial DO levels can influence the maximum possible holding times, a more stringent way of comparing the treatments would be to compare the rates of oxygen consumption / depletion, which indicates the speed of oxygen consumption by the fish. Even when compared in this way (Fig. 3), maximum rate of O_2 depletion was in case of FL conditions (2.61ppm/h) and the least in case of FD with 1.1% saline (1.61ppm/h). Lack of any visual stimuli was also beneficial as O_2 depletion rates were lower under SD conditions compared to FL conditions. However, the differences in depletion rates between the storage conditions were found not statistically significant, as there was large variation in the rates within each storage condition during the course of the experiment (Fig.3). In general, as the DO levels decreased with storage time, the O_2 consumption rates decreased. Experiments conducted in dark coloured cylindrical plastic tanks to simulate possible live-transport conditions showed results similar to those obtained in full and semi-dark conditions in glass tanks in respect of oxygen depletion rates.

Ammonia levels in water increased with storage time in all experiments (Fig.4). In general, NH_3 levels in case of experiments under FD conditions tended to be higher than Light and semi-dark conditions. Higher levels of NH_3 in the former conditions were due to the inability to remove fecal matter from the holding tanks without compromising the dark conditions. Highly significant differences existed between the different storage conditions due to the wide range of initial NH_3 levels (0.87 to 5.08 mg.atm/l). But upon statistical analysis of the rates of ammonia accumulation, no significant differences were discernible, which indicated that NH_3 excretion rates by fish are not much influenced by the light/dark conditions. However, it was observed that under saline conditions (0.9 and 1.1% saline) NH_3 levels, both initial and during the experiment were much higher in water, indicating higher excretion of ammonia by fish stored in saline, perhaps in response to the altered osmotic conditions.

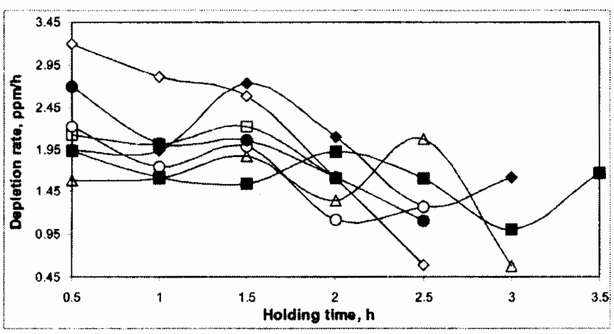

Fig. 3. Rate of depletion of dissolved oxygen concentration in water on holding Rohu (*Labeo rohita*) under simulated conditions of transport. For key to legends refer to Fig.1

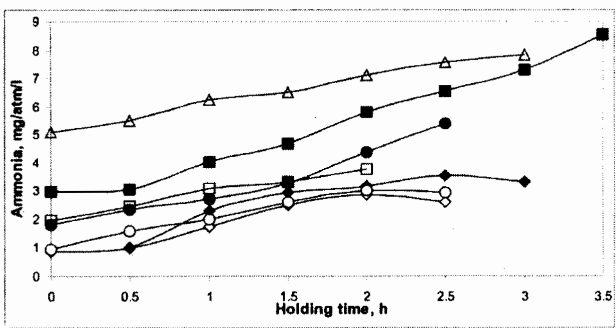

Fig. 4. Changes in ammonia concentration in water on holding Rohu (*Labeo rohita*) under simulated conditions of transport. For key to legends refer to Fig.1

The course of change in the pH of water during the experiments is shown in Fig.5. The average pH of water during the various experiments varied from 7.44 to 7.80 and the differences were found to be highly significant. These differences are however, mostly attributable to the differences in initial pH, caused by the presence of fecal matter in water which could not be removed fully under the FD experimental conditions. It can be seen from the figure that the higher pH was recorded in the darker conditions. However, the general pattern of change in pH during the holding time of fish was same for all experimental conditions and was seen to decrease by an average of 0.25 units. Differences between the rates of decrease or transient increases, when analyzed statistically, were not found to be significant.

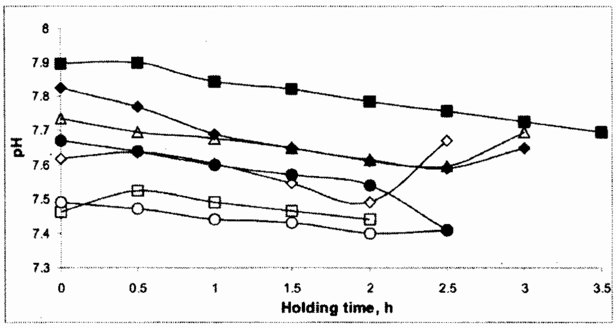

Fig. 5. Changes in pH of water on holding Rohu (*Labeo rohita)* under simulated conditions of transport. For key to legends refer to Fig.1

Distress of the fish which was observed at the end of experimental period when DO levels fell to <1.0 ppm, disappeared almost immediately after the fish were transferred to fresh water with aeration, in the 0.5-1h period before their transfer to holding tanks. The fish retained their natural coloration and exhibited normal swimming activities after the experiments.

As temperature bears an inverse relationship to the DO, the initial temperature played a key role in deciding the initial DO level of water. A negative and highly significant correlation of – 0.8865 was found between the temperature and initial DO level of the water. Lobsters have been anaesthetized by a direct cooling technique for live transport without the benefit of water (Suryaningrum *et al.,* 1997). The near constancy of temperature during the experiment is also important as a relatively constant temperature is a basic requirement of live fish transport (Wedemeyer, 1992). Decreased DO concentration in seawater at higher temperatures during live transport of Plaice (*Paralichthy olivaceus)* has been reported by Cho *et al.* (1994). The effect of DO on survival and transport of Indian major carp seeds of 2.4 to 3.5g size have been investigated by Santhanam *et al.* (1988), but those of bigger sized carps weighing 200-250g as in this study, under high loading density have not been reported. High oxygen concentrations of up to 500% saturation, achieved artificially during transport have not been reported to affect rainbow trout (Winstone & Solomon, 1976). Higher air pressures using a simple bicycle air pump (Selvaraj *et al.,* 1984) can also raise oxygen concentration. Continuous aeration to offset the depleting DO levels during the experiment was not employed in this study as the DO levels will be then subject to aeration efficiency, circulation, turbulence caused by the aeration etc., that are not easily measured / quantified. Turbulence in water, especially splashing that occurs during transportation can be expected to raise DO levels., but it may cause some stress to the fish. Simulation of turbulence caused by transportation is not easily duplicated while at rest and hence was not attempted. Our experience in live transport of both fingerlings and adults peninsular carp (*Puntius pulchellus*) captured from the wild suggest that lining of the transport containers with 10mm thick sponge considerably reduces injuries to the fish (unpublished).

The general decrease in pH upon holding the fish in confined conditions without aeration, can perhaps be attributed to the accumulation of CO_2 in the water due to respiration by the fish. Although the actual concentrations of CO_2 in the water were not measured, it would appear to be considerable, as the decrease in pH took place in spite of the increase in NH_3 concentrations during the same period. Further, CO_2 mitigates the toxicity of ammonia, since only unionized ammonia is toxic and the low pH brought about by accumulation of CO_2, increases degree of ionization of ammonia molecule (Alabaster & Herbert, 1954). However, correlation calculated between pH and ammonia levels (0.1517) was not significant, indicating absence of any relationship between the two under the conditions of this experiment. Carbon dioxide has been successfully used as an anaesthetic by some workers, but has not been found to be practical (Takeda & Itazawa, 1983., Itazawa & Takeda, 1982). The pH during transportation of live fish has been controlled by use of Tris buffer at pH 8.0 by Amend *et al.* (1982), who also used the Zeolite, Clinoptilolite for controlling the buildup of ammonia. Bower & Turner (1982) have also used the latter substance in transportation of ornamental fishes. A period of starvation before moving is recommended to avoid ammonia buildup (Solomon & Taylor 1979). As seen in this study, starvation becomes quite important when fecal matter is to be incompletely removed prior to beginning of simulated transport. Although salt has been used as possible anaesthetic and stress reliever in channel catfish (Wurts, 1995), at the concentrations 0.9 & 1.1% used in this study, it was not found to reduce oxygen consumption. Instead, it increased ammonia buildup.

The study showed that maintenance of dark conditions is necessary to reduce the oxygen depletion in water while attempting to transport fish under confined conditions at high loading density. While the higher NH_3 levels prevailing under dark conditions has to be overcome by designing effective fecal matter removal systems, use of saline as a storage medium was found to lead to higher excretion of NH_3 in the water.

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