Fishery Technology 49 (2012) : 59 - 63

Optimization of Packing Conditions for Live Transportation of *Anabas testudineus* and *Channa punctatus*

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Abstract

The study reports the optimized conditions for the live transportation of two air breathing fishes *viz., Anabas testudineus* and *Channa punctatus.* The requirement of bimodal oxygen, water and commercial oxygen for packing the fishes was determined. The use and suitable dose of MS-222, a standard fish anaesthetic containing ethyl-m-aminobenzoate as an active ingredient during live transportation was also studied. Five specimens of A. *testudineus* (total weight 72.50 g) required 385.880 and 497.977 mg oxygen from water and air respectively for packing and transportation for 72 h. For five specimens of C. *punctatus* (total weight: 57.50 g), 377.182 mg aquatic oxygen and 253.669 mg aerial oxygen was required. The optimum dose of MS-222 for *A. testudineus* (body weight: 14.00 ± 0.5 g) and for *C. punctatus* (body weight: 11.55 ± 0.65 g) was determined to be 70.0 mg l^{-1} and 32.50 mg l^{-1} respectively. The fishes transported in the optimized conditions reached the destination after 72 h without mortality.

Key words: *Anabas testudineus, Channa punctatus,* oxygen uptake, sedative, live transportation

Received 02 March 2009, Revised 23 August 2011, Accepted 20 October 2011

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Introduction

The successful transportation of live fish needs to consider various factors such as optimum number and size of fish, variation in the *en route* temperature, effect of handling, dissipation of end products, duration of transportation and above all, requirement of oxygen. Normally, all packing methods involve use of some device for aeration to ensure an adequate oxygen supply for the fish. The relationship between body weight and oxygen uptake of fishes is an essential parameter to be considered for packing and transportation (Jana & Das, 1996). The concentration of oxygen in water, which depends on water temperature, plays the most important role in the survival of fishes during live transportation. The amount of dissolved oxygen in water at different temperatures has been reported by Pandey & Shukla (2005). Anaesthetics are used to reduce the rate of oxygen uptake (Pandit & Ghosh, 2005), release of carbon dioxide and to control excitability of fishes. Various anaesthetics have been used for packing purposes depending upon the nature of fish (Basavaraju et al., 1998; Venkateshwarlu & Pal, 2003; Pal et al., 2005). Lindstedt (1914) suggested a formula, *viz.*, $R = V/N$ and $V/N \propto W^{2/3}$, where R = rate of oxygen uptake, V = entry of oxygen into a volume of water per hour, N = number of fishes and W = weight of fishes, for calculating the oxygen requirement for the transportation of fish.

Of late, in Indian scenario, attention has been given to improve live transportation of fishes but it is mostly limited to carps. The transportation of air breathing fishes is comparatively easy due to their bimodal oxygen uptake (Ghosh et al., 1994). In India, *Anabas testudineus,* Kawai or Koi and *Channa punctatus,* Garai or Gurai are considered economically important air breathing fishes but their culture in derelict water remained neglected since long. They are treated as excellent table fishes in many states of India but their transportation to distant places in live condition pose several problems such as hypoxia, injury, disease and finally, death. This study attempts to determine the requirements of aerial and aquatic oxygen, water, commercial oxygen and optimum dose of sedative (MS-222) for packing and transportation of live *A. testudineus* and *C. punctatus*.

Materials and Methods

Live specimens of *A. testudineus* (4.5 – 16 g) were procured from Katihar (North Bihar) fish market and *C. punctatus* (1.45 – 11.55 g) were collected from local fishermen of Bhagalpur. The fishes were acclimatized for a fortnight in chlorine free tap water and fed with chopped trash fishes and goat liver on alternate days. A continuous water flow system of cylindrical glass respirometer was used to determine aquatic oxygen uptake (Munshi & Dube, 1973). The respirometer (capacity: 0.5 to 1.0 l) was connected to a water reservoir and uniform water flow was maintained. The rate of water flow through the respirometer was adjusted according to the size of fishes so that they do not get stressed. The fishes were acclimatized in the respirometer for 2 h before the experiment, to reduce stress associated with handling. Each experimental set up was with a single fish and repeated four times.

A closed glass respirometer (Ghosh & Munshi, 1987) of 3 l capacity was used to measure bimodal oxygen uptake. The fishes were kept in respirometer containing 1.5 l tap water. A thermocole float with a semi-circular hole was placed over water to separate the air-water interphase of respirometer. The air phase of respirometer was connected to a kerosene oil manometer. A 'T' shaped glass tube was placed between manometer and the outlet from the respirometer. Another tube was attached to a glass 'T' and the end of the tube was fitted with a plastic stopper. The fishes were allowed to take air-gulps. Pellets of potassium hydroxide, which absorb CO_2 liberated during respiration were kept over the float in a petridish. The aerial oxygen uptake was measured as per Dejours (1975). The amount of dissolved oxygen in inspired and expired water either from aquatic or bimodal sources was calculated by Winkler's volumetric method (Welch, 1948). Variation in aquatic oxygen uptake under sedation was taken into account as suggested by Saha et al. (1956). Following the method of Martyshev (1983), the volume of water was calculated for requisite period of transportation. In this method, aquatic oxygen uptake (mg kg^{-1} h^{-1}) was divided by amount of oxygen (mg l^{-1}) in water. Similarly, volume of oxygen required for aerial respiration was calculated. Atmospheric oxygen diffuses by 1 mg h^{-1}, while commercial oxygen diffuses by about 5 mg h^{-1} under similar conditions (Saha et al., 1956), which enables transportation of five times more fish in the same medium. Due to this benefit, commercial oxygen was used in this experiment.

According to Mc Farland (1960), total loss in reactivity except strong pressure and slight decrease in opercular rate of fishes to external stimuli in anaesthesia is known as (1 [2]) sedation stage. MS-222, a standard fish anaesthetic containing ethyl-m-aminobenzoate as an active ingredient was selected as the anaesthetic. The sedation stage of MS-222 was determined for proper sedation of the fishes by keeping 10 isomorphic individuals of different weight group separately in known volume of tap water and increasing the dose of anaesthetic accordingly. Serrated and spiny structures of *Anabas testudineus* were trimmed out to prevent damage of packing materials. The trimmed *Anabas testudineus* were treated with oxy-tetracycline @ 0.2 mg l^{-1} and left for about a week for wound healing. To prevent any change in pH of water, sodium phosphate @ 2 g l^{-1} was added. Clinoptilolite @ 10 g l^{-1} of water was added to reduce ammonia produced during transit. Before packing, an instant bath in methylene blue @ 0.1 ml^{-1} was done to curb transmission of infections to fishes in the consignment. The treated fishes were acclimatized for 4 to 5 h in chlorine free tap water. Feeding was stopped before 24 h of packing. Fishes of similar size were treated with chloramphenicol @ 10 mg l^{-1} which has a therapeutic effect (Pyatkin & Krivoshein, 1987). Water temperature and oxygen content of water were recorded before introducing the fishes into polythene bags.

The polythene bags (thickness: 0.6 mm, dimensions; 65 x 45 cm and capacity 16 to 18 l) were filled with calculated volume of chlorine free tap water with 500 mg l^{-1} of sodium chloride and calculated MS-222 depending upon the size of the fishes. The five treated fishes were introduced into the bags. The bags were squeezed to expel air and slowly filled with calculated volume of commercial oxygen. The bag was shut, folded over, tied with a rubber band and placed in cardboard cartons with the tied end directing upwards. Pieces of paper were put between the bags so that fish do not get exposed to external disturbances. The bags were meant for transportation to Kolkata by rail and then to Indiana University, Indiana by air. The total time required for transportation was 72 h.

Results and Discussion

The oxygen requirement of *Anabas testudineus* and *Channa punctatus* in relation to their body weight was calculated and presented in Table 1. The amount of dissolved oxygen in the tap water was estimated to be 8.20 ± 0.74 and 7.68 ± 0.48 mg l^{-1} at a temperature of 23.0 ± 1.0°C and 30.0 ± 1.0°C respectively. The observations resemble with the work of Ghosh & Munshi (1987). The dose of MS-222 for proper sedation of *Anabas testudineus* and *Channa punctatus* was determined (Table 2). The optimum dose of MS-222 for *A. testudineus of* body weight 4.4 g was determined as 40.0 mg $l^{-1}$and for 16.0 g weight as 76.0 mg l^{-1}. For *C. punctatus,* the requirement of MS-222 for specimen weighing 1.45 g was 20.0 mg l^{-1} and for 11.55 g specimen it was 32.5 mg l^{-1}. Five specimens of *A. testudineus* (total weight: 72.50 g) and *C. punctatus* (total weight: 57.50 g) were randomly selected. The average oxygen required for *A. testudineus* was found to be 385.880 and 497.977 mg from aquatic and aerial source respectively at 23°C (Table 3) for packing for 72 h. Similarly, the oxygen requirement was 377.182 mg from aquatic and 253.669 mg from aerial source for *C. punctatus.* Singh (1977) observed that 2712.0 mg aquatic oxygen was required for transportation of fifty *Cirrhinus mrigala* (total weight 320 g) at 31.0 ± 0.3°C for 48 h. Being air breathing fishes *A. testudineus* and *C. punctatus* are able to utilize oxygen from both aerial and aquatic sources, hence their aquatic oxygen requirement becomes less.

The water requirement of *A. testudineus* of 1.0 kg body weight (4.506 l) was multiplied by total weight of fish to be packed (0.0725 kg) and duration of packing (72 h). Therefore, a total of 4.506 x 0.0725 x 72= 23.52 l of water was required fo*r A. testudineus* while for *C. punctatus,* water requirement was 5.556 x 0.0575 x 72 = 23.1 l. Under sedation, a 40% reduction in aquatic oxygen uptake was reported (Pandit & Ghosh, 2005). Hence to make the consign-

Table 1. Oxygen requirement for live transportation of *Anabas testudineus* and *Channa punctatus*

Anabas testudineus			*Channa punctatus*		
weight of fish (g)	Oxygen uptake ($mg\ O_2\ h^{-1}$) Aerial	Aquatic	weight of fish (g)	Oxygen uptake ($mg\ O_2\ h^{-1}$) Aerial	Aquatic
2.70	0.254	0.256	1.45	0.095	0.172
4.45	0.420	0.392	2.05	0.132	0.232
5.10	0.482	0.440	3.50	0.221	0.366
7.30	0.692	0.598	4.25	0.266	0.433
8.50	0.807	0.681	5.45	0.337	0.538
10.10	0.960	0.788	7.40	0.452	0.701
12.75	1.215	0.961	11.80	1.705	1.048
14.00	1.335	1.041	-	-	-
16.00	1.528	1.165	-	-	-

Table 2. Concentration of MS-222 required for sedation of *Anabas testudineus* and *Channa punctatus*

Anabas testudineus		*Channa punctatus*	
Body weight (g) (Mean ± SD)	Anaesthetic concentration (mg l^{-1})	Body weight (g) (Mean ± SD)	Anaesthetic concentration (mg l^{-1})
4.45 ± 1.25	40.00	1.45 ± 0.55	20.00
7.30 ± 0.7	54.00	3.5 ± 1.00	24.00
10.10 ± 0.9	62.00	5.45 ± 0.55	27.00
14.0 ± 0.5	70.00	11.55 ± 1.65	32.50
16.0 ± 1.0	76.00		

Table 3. Ingredients required for live transportation of *Anabas testudineus* and *Channa punctatus*

No. of fishes	Fish	Total weight (g)	Total bimodal oxygen uptake (mg O_2) Aerial	Aquatic	water (l)	oxygen (l)	MS-222 (mg l^{-1})	NaCl (mg l^{-1})
5	*Anabas testudineus*	72.50	497.977	385.880	14.113	1.500	990	500
5	*Channa punctatus*	57.50	253.669	377.182	14.161	1.006	460	500

ment lighter, 14.1 and 14.16 l of water was added to the bags of *A. testudineus* and *C. punctatus* respectively instead of their original demand of 23.52 l and 23.1 l. The reduced amount of water was further balanced by giving calculated quantity of commercial oxygen. According to Saunders (1963) gills are able to utilize 80% of oxygen dissolved in water. This may go down to 10% under stressful conditions of dissolved oxygen going below 6 mgl^{-1} (Shepherd & Bromage, 1988). Therefore, the requirement of oxygen from aerial source needs special consideration due to bimodal nature of the fishes. Moreover, utilization of some oxygen by bacteria and other micro-organisms (Saha & Sen, 1958) is also important. It has been reported that for every ml of oxygen that a fish consumes, about 0.9 ml CO_2 is released (Singh, 1977).

A. testudineus and *C. punctatus* of 1.0 kg body weight need 94.79 and 61.08 mg oxygen respectively from air in one hour. Therefore, a total of 494.804 mg and 253.531 mg aerial oxygen was originally required to pack five specimens of *A. testudineus* and *C. punctatus* respectively for 72 h duration. As per the suggestion of Saha et al. (1956), additional 25% as reserve oxygen was added to the bags. Thus, the volume of 772.856 mg and 498.71 mg aerial oxygen was calculated for *A. testudineus* and *C. punctatus* respectively. The commercial oxygen contains 90% oxygen. At the time of filling 870.0 mg (610 ml) and 555.0 mg (390.0 ml) of commercial oxygen was filled in the bags of *A. testudineus* and *C. punctatus* respectively (Table 3). The use of polythene bags reduced the weight of the consignment and the transparent material facilitated in observing the activity of the fish from outside. To avoid external disturbances, the bags were kept in a carton. The bag was filled with 14.113 l of water and 610 ml of commercial oxygen in the ratio of 23.1: 1 along with 28 mg sodium phosphate, 140 mg clinoptilolite and 990 mg of MS-222 to pack five *A. testudineus*. Similarly, 14.161 l of water and 390 ml of commercial oxygen in the ratio of 36.3: 1, 28 mg sodium phosphate, 140 mg clinoptilolite and 460 mg of MS-222 were filled in the bags for *C. punctatus.* The consignments were transported to Kolkata by rail and from Kolkata to Indiana University, School of Medicine, Indiana by air. The bags were received at the destination points in good condition after a total duration of 72 h without any mortality.

The study showed that optimizing the conditions *viz.*, requirements of water, oxygen (bimodal and commercial) and suitable dose of sedative guarantee safe transportation of *Anabas testudineus* and *Channa punctatus* in live condition for 72 h.

Acknowledgements

The authors are grateful to Indian Council of Agricultural Research (ICAR), New Delhi for financial assistance.

References

Basavaraju, Y., Mair, G. C., Mukthyakka, G., Devi, B. S. R. and Penmen, D. I. (1998) The use of Benzocaine as an anaesthetic and sedative for carps. Proc. Indian Natn. Sci. Acad. 68: 231-240

Dejours, P. (1975) Principles of Comparative Respiratory Physiology, pp 252-253, North Holland, Amsterdam

Ghosh, T. K. and Munshi, J. S. D. (1987) Bimodal oxygen uptake in relation to body weight and seasonal temperature of an air breathing climbing perch, *Anabas testudineus* (Bloch). Zool. Beitr. N F. 31: 357-364

Ghosh, T. K., Jha, K. K. and Pandit, D. N. (1994) Effect of group behaviour on the aquatic oxygen uptake in the juveniles of a siluroid fish, *Heteropneustes fossilis* (Bloch), 22 p, VIII Cong. Sod Europaea lchthyologorium, Oviedo, Spain

Jana, B. B. and Das, S. (1996) Influence of ambient oxygen level and fish density on oxygen uptake of Indian major carp *(Labeo rohita)* fingerlings. J. Inland Fish. Soc. India. 28: 1-12

Lindstedt, Ph. (1914) Untersach ungen uber respiration Van Katt bluten. Zeischrifl fur Fisheries. 14: 183

Martyshev, F. G. (1983) Pond Fisheries, pp 432-451, Amerind Publishing Co. Pvt. Ltd., New Delhi, India

Mc Farland, W. N. (1960) The use of anaesthetics for handling and transporting of fish. Calif Fish Game. 66: 408-431

Munshi, J. S. D. and Dube, S. C. (1973) Oxygen uptake capacity of gills in relation to body size of air breathing fish, *Anabas testudineus* (Bloch). Acta. Physiol. Sci. Hungaricae. 44: 113-123

Pal, A. K., Venkateshwarlu, G., Manush, S. M., Chatterjee, N. and Mukherjee, S. C. (2005) Clove oil, A potential anaesthetic for fish and prawn. Fishing Chimes. 23: 27

Pandey, K. and Shukla, J. P. (2005) Fish and Fisheries, pp 262-405, Rastogi Publications, Meerut, India

Pandit, D. N. and Ghosh, T. K. (2005) Effect of Benzocaine on opercular movement and oxygen consumption in *Channa punctatus* (Bloch). J. Indian Fish. Assoc. 32: 149-155

Pyatkin, K. and Krivoshein, Yu. (1987) Microbiology with Virology and Immunology, pp 272-274, Mir Publications, Moscow

Saha, K. C. and Sen, D. P. (1958) Studies on the mortality in spawn and fry of Indian major carps during transport. Part IV. Types of containers used for transporting spawn and fry under oxygen pressure. Indian J. Fish. 5: 362-374

Saha, K. C., Sen, D. P. and Mazumdar, P. (1956) Studies on the mortality in spawn and fry of Indian major carps during tansport. Part - II Effect of oxygen pressure, free surface area, water volume and number of fry in the medium of transport. lndian J. Fish. 3: 127-134

Saunders, R. L. (1963) Respiration in Atlantic cod. J. Fish. Res. Bd Can. 20: 373-386

Shepherd, J. and Bromage, N. (1988) Intensive Fish Farming, 404 p, BSP Professional Books, Oxford

Singh, B. N. (1977) Oxygen consumption and the amount of oxygen required for transport of rohu and mrigal fingerlings. J. Inland. Fish. Soc. India. 9: 98-104

Venkateshwarlu, G. and Pal, A. K. (2003) Anaesthetics and other techniques for handling and transportation of live shell fish. In: Aquaculture Medicine (Bright Singh, I. S., Pai, S. S., Philip. R. and Mahandas., Eds), pp 213-218, CIDDM, SESCUSAT, Kochi

Welch, P. S. (1948) Limnological Methods, pp 207-209, McGraw Hill, New Delhi