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Critical water quality parameters affecting survival of *Labeo rohita* (Hamilton) fry during closed system transportation

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ABSTRACT

Changes in water quality parameters during closed system transport of *Labeo rohita* fry were studied to identify the critical changes responsible for seed mortality. Fry of *L. rohita* (2.38 ± 0.25 g, 44.2 ± 6.6 mm) were packed in 39 polythene bags and placed on a continuously shaking platform. Water quality changes and fish mortality in the packs were studied for 36 h at every 3 h interval. Marked increase in dissolved oxygen (DO), free carbon dioxide (CO_2), total alkalinity, total ammonia nitrogen (TAN) and nitrite-nitrogen (NO_2-N) were observed during initial 12 h. Thereafter, most of these parameters remained either consistent or varied in a narrow range up to 27 h, without any significant increase in mortality of fry. However, subsequently during 27-36 h there was significant reduction in dissolved oxygen (< 3.6 mg l^{-1}), increase in CO_2 (> 60 mg l^{-1}) and total alkalinity (>120 mg as $CaCO_3$ l^{-1}). Mortality of fry increased significantly during this period which can be attributed to increased stress due to changes in the above water quality parameters.

Keywords: *Labeo rohita*, Mortality, Seed packing, Seed transport, Stress, Water quality parameters

Introduction

Fish seed is one of the basic requirement in fish farming and the success of fish culture largely depends on stocking of healthy and quality seeds. In India, about 50% of the 2.4 million ha area of available freshwater pond and tank resources (DAHDF, 2011) are presently utilised for fish culture. These water bodies are stocked every year with fry or fingerlings of various freshwater fish species. Carps are the dominant group cultured comprising Indian major carps (IMC), exotic carps, minor carps and barbs. Carp spawn/fry/fingerlings are supplied by more than 1,700 carp hatcheries distributed throughout the country. The spawn are reared to fry and fingerling stages in seed rearing ponds usually located away from the hatchery. A wide range of techniques are being employed for fish seed (spawn, fry and fingerlings) transportation in India. Open system of seed transport is popular for short distance transport, while closed system with oxygen packaging in polyethylene bags is adopted for long distance transport (Jhingran and Pullin, 1985). However, in both systems seeds are transported in crowded conditions often leading to mortality during transportation due to stress arising out of handling, packing, crowding, physical injury (Kutty, 1987; Singh *et al.*, 2004; Basavaraja, 2007) and changes in ambient water quality inside the seed container (Kutty, 1987). Severity of transport stress depends on duration of transport and the physico-chemical characteristics of the medium. Different processes and materials like pre-conditioning at high density prior to packaging (Kumar, 1992), oxygen packaging (Jhingran and Pullin, 1985; Milwain *et al.,* 2002), use of tranquilizer or anesthetics to reduce metabolic activity and transportation stress (Woynarovich and Horvath, 1980; Mishra *et al.*, 1983; Mohamed and Devraj, 1997; Hasan and Bart, 2007) are some of the effective measures used for reducing stress and mortality during transportation. Singh *et al.* (2004) reported 100% survival of IMC fry after 48 h of transportation with the use of zeolite at 7 g l^{-1}, 0.01 M tris buffer or 0.09 ml l^{-1} of 2-phenoxyethanol while higher dose of these chemicals resulted in fry mortality.

Among the different water parameters, changes in pH, dissolved oxygen (DO), temperature and carbon di oxide (CO_2) regimes in the transporting medium are critical for seed survival. Information on the dynamics of these water parameters inside the seed containers during transportation and their subsequent impact on seed survival, is limited. The present study aimed at assessing changes in the important water quality parameters during transportation of *Labeo rohita* fry, to identify the critical ones affecting seed survival which would further enable to devise suitable measures for reducing seed loss during transportation.

Materials and methods

Fry of *L. rohita* (2.38 ± 0.25 g, 44.2 ± 6.6 mm) kept unfed for one day were netted out from the seed rearing pond in the freshwater aquaculture farm of the Central Institute of Freshwater Aquaculture (CIFA), Odisha, India. These fry were conditioned in the conditioning hapa (2m x 1m x 1m), fixed in the same pond. Fry were kept under crowded condition approximately at 3000 fish m^{-3} for 6 h prior to packing, to facilitate release of faecal matter. Polyethylene bags of 28 l capacity (62 x 46 cm) were used for seed packing. The bags were filled with 4 l of clear water from the same pond and 100 numbers of fry were introduced in each bag. Further, the bags were packed with approximately 8-9 l of medical grade oxygen, tied tightly with jute twine and placed inside another bag provided with polythene materials as cushion. Polythene bags (total 39) were then placed on a gently and continuously shaking platform in order to simulate condetions similar to the splashing movement of water during seed transportation. Three bags each were sampled at 3 h intervals to study the water quality changes. This experiment was continued upto 36 h. During each sampling, the bags were opened, dead fry were counted and water samples were collected for analysis of different parameters. Temperature and pH were measured. pH was determined using pH meter (Orion, Thermo scientific, 2 star), while dissolved oxygen (DO) (Winkler's method), free CO_2, total alkalinity and total hardness were measured by titrimetric methods (APHA, 2005). Total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were measured following standard methods (APHA, 2005). Data on different water quality parameters were analysed using PC-SAS programme for Windows, release v 6.12 (SAS Institute, Cary, NC, UK). Duncan's multiple range test was also performed at 95% significance level to compare the treatment means for different water quality parameters and fry mortality.

Results and discussion

Fish seed survival during transportation is a function of changes and interaction of the various water parameters inside the seed container that affects physiology of the fish. Water temperature and pressure above it greatly influence the diffusion of oxygen into the underlying water (Boyd, 1990). During the present study, water temperature in the seed pack varied between 21.3-23.1°C (Fig. 1). Oxygen packed at high pressure inside the container obviously increased the dissolved oxygen content in water, sharply within 3 h from a level of 4.5 mg l^{-1} to a maximum of 8.5 mg l^{-1} and thereafter, it gradually reduced (Fig. 1). Oxygen solubility range in water is reported to be 8.9-8.6 mg l^{-1} at 21-23°C (Colt, 1984). Probably the 8.5 mg l^{-1} DO level observed in this study might be the saturation point at the prevailing water temperature (21.3-21.6°C) and subsequently, elevated respiration rate due to the hyperactivity of fry during initial phase (Kutty, 1987) might have contributed reduced DO tension in water.

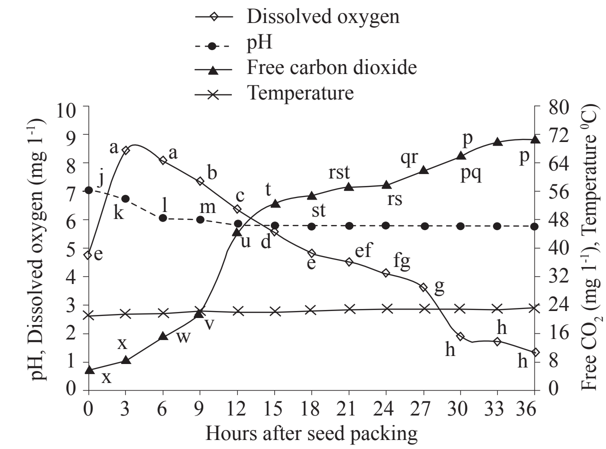

Fig. 1. Changes in pH, dissolved oxygen, free CO_2 and temperature of water in seed pack during fry transportation (Data point with a different font in a data series indicates significant change ($p<0.05$, n=3)

Despite the increase in oxygen concentration of water, pH inside the seed container reduced faster from 7.0 to 6.0 during initial 9 h ($p<0.05$) and thereafter, it remained almost consistent at that level. This may be attributed to the production of more CO_2 from respiration during this phase which led to formation of more carbonic acid and subsequent dissociation yielded more hydrogen ion making water acidic (Boyd, 1990; Wurts, 2003). In fact, the CO_2 content in water showed a gradual increase with time (significant between 9-12 h after seed packing) compared to the later part (Fig. 1) confirming the above phenomenon.

Among the inorganic nitrogen, total ammonia nitrogen (TAN) concentration increased sharply within 3 h of packing from near zero level to 3.6 mg l^{-1} ($p<0.05$) and subsequently reduced to 2.7 mg l^{-1} (Fig. 2). Such initial increase in TAN could be attributed to the release of more nitrogenous metabolites from the fry especially during the initial period. Further, lower water temperature and the

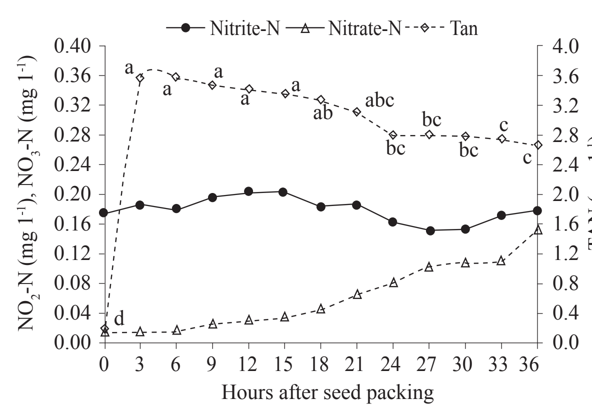

Fig. 2. Changes in total ammonia, nitrite and nitrate-nitrogen concentrations of water in seed pack during fry transportation (Data point with a different font in a data series indicates significant change ($p<0.05$, n=3)

acidic condition inside the seed pack during this period as stated earlier, might have shifted the reaction between nitrogenous metabolites and water towards production of less toxic ammonium ions (Tomasso *et al.,* 1980; Meade, 1985; Boyd, 1990; Hargreaves, 1998; Wurts, 2003). Das *et al.* (2004a, 2005) observed meager signs of stress in fingerlings of IMC after exposing them to sublethal concentrations of TAN up to 8 mg l^{-1}. Since the recorded TAN concentration (2.6-3.6 mg l^{-1}) in this study was much lower compared to the above report, it might not have been a potential threat for the fry inside the closed seed pack.

Temporal changes in NO_2-N levels showed minor variations (0.02 to 0.20 mg l^{-1}) and remained more or less stationary during the observation period (Fig. 2). An increase in NO_2 concentration in water is considered toxic to fish. However, fingerlings of *L. rohita* have shown tolerance to NO_2 exposure levels up to 1 mg l^{-1} with no significant change either in hematology or enzymatic parameters (Das *et al.,* 2004b, c). NO_2 concentration that prevailed in this study was much lower and there was no mortality when similar NO_2 levels prevailed during initial period. This indicated that NO_2 at its recorded concentration could have had the least chance to exert stress on the fry causing increased morality observed during later period.

Ammonia released by fish into water reacts with water molecules to form ammonium (NH_4^+) and hydroxyl (OH^-) ions and further, the hydroxyl ion reacts with CO_2 to produce HCO_3^- which results in alkalinity (Boyd, 1990). Total alkalinity of water in the seed pack increased with progress of time from an initial 48 to 160 mg $CaCO_3$ l^{-1} after 36 h (Fig. 3). The increase was significant after 15 h with rapid increase from 21 h onwards. This corroborates the phenomena explained by Boyd (1990) as there had been continuous addition of ammonia and CO_2 to the ambient water from the excretion and respiration of fry respectively. The faster increase in alkalinity towards the

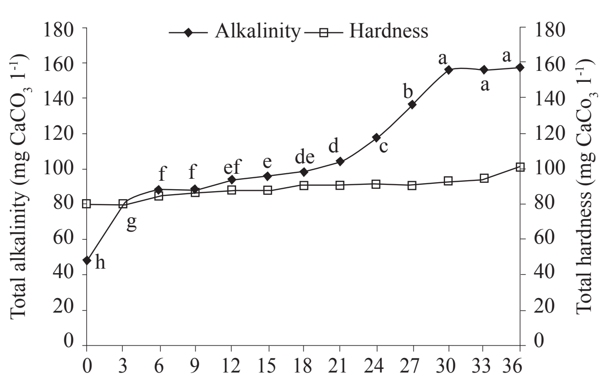

Fig. 3. Changes in total alkalinity and total hardness of water in seed pack during fry transportation (Data point with a different font in a data series indicates significant change (p<0.05, n=3)

later part of the study also indicated increased conversion of ammonia and CO_2 available in the water as there was reduction of TAN level and slower rate of increase in CO_2 (Fig. 1) in the ambient water during this period. Total alkalinity plays a significant role in carp seed survival and water with alkalinity above 100 mg $CaCO_3$ l^{-1} is usually not considered an ideal condition for IMC fry. But in the present study, the alkalinity in water surpassed 120 mg $CaCO_3$ l^{-1} approximately after 24 h which might have exerted certain degree of stress on the fry. Seed mortality also increased significantly during this period of the study indicating probable correlation between seed mortality and increased alkalinity. Therefore, the stress arising from the higher alkalinity level in water inside the seed pack could be a potential factor causing mortality.

Seed mortality during transportation showed a gradual rise with progress of time (Fig. 4). Percentage mortality was low (6%) up to 27 h and increased significantly thereafter with marked increase between 33-36 h ($p<0.05$). Apart from the increased total alkalinity towards later phase (27-36 h), there were significant reductions in DO and increase in free CO_2 ($p<0.05$) while the pH and TAN did not show marked change. This indicated that low DO (<3.6 mg l^{-1}), high free CO_2 (>60 mg l^{-1}) and high total alkalinity (>120 mg as $CaCO_3$ l^{-1}) were the critical factors which were either directly associated with fry mortality or indirectly increased stress to cause mortality. While reduction in DO level inside the seed pack may be controlled through reducing oxygen uptake of fry with use of anesthetic or tranquilizer (Jhingran and Pullin, 1985; Berka, 1986), CO_2 production can be controlled using chemicals like tris buffer (Amend *et al*., 1982). Similar efforts to control total alkalinity inside the seed pack may help ensuring better seed survival during transportation.

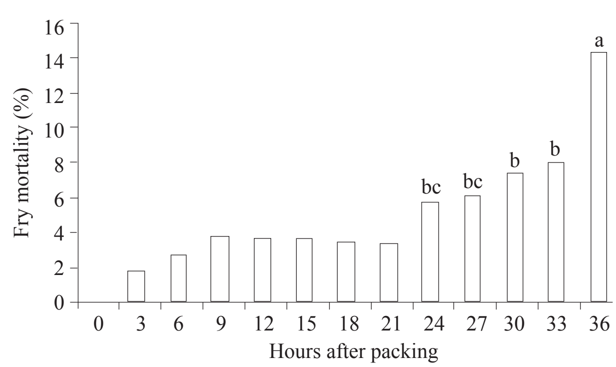

Fig. 4. Fry mortality in the seed container at different hours after packing (Data point with a different font in a data series indicates significant change (p<0.05, n=3)

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