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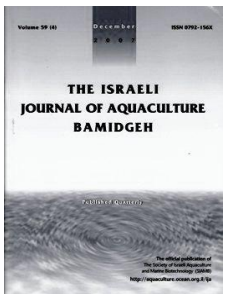

Secondary Stress Response to High Stocking Density in Fry of Gray Mullet (*Mugil cephalus*)

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Abstract

Monoculture and polyculture of gray mullet (*Mugil cephalus*) depends on the supply of wild seed. Heavy mortalities occur because optimum stocking requirements during transportation of fry are unknown. The present experiment was conducted to determine the optimum stocking density of gray mullet fry in closed oxygenated polythene bags during transportation. Glycogen content and activity of metabolic enzymes including lactate dehydrogenase (LDH), malate dehydrogenase (MDH), aspartate amino transferase (AST), and alanine amino transferase (ALT) were investigated to understand the effect of stocking density on metabolism. Fry (1.2±0.48 g, 2.4±0.52 cm) were packed in five densities (80, 160, 240, 320, 400/l). At the end of 18 h of transportation, 24 specimens from each density were randomly sampled. The glycogen content decreased significantly (p<0.05) as the stocking density increased. The activity of enzymes LDH, MDH, AST, and ALT showed a rising trend with increasing stocking density. Dissolved oxygen and pH decreased significantly as the stocking density increased while ammonia nitrogen, nitrite nitrogen, and carbon dioxide increased. Survival at destination significantly decreased as the stocking density rose. The optimum stocking density for gray mullet fry for transportation up to 18 h was 240 fry/l.

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Introduction

Gray mullet (*Mugil cephalus*) is one of the most popular food fishes in India. It is extensively farmed in sea, brackish, and freshwater environments in mono and polyculture systems. Culture depends completely on wild seed collected from brackish waters, transported to farm sites, and stocked in grow-out ponds. Crowding of fish during transportation results in hyperactivity, strain, and the deterioration of water quality caused by the accumulation of harmful gases (ammonia and carbon dioxide) and wastes (fecal matter). In India, stocking density is determined by the size of the fish and the duration of transportation. As it is difficult or impossible to exchange water during transport, conditioning measures (Gomes et al., 2002) and stocking density must be optimized to reduce transportation stress. Understanding the biochemical changes of gray mullet caused by high stocking densities during transportation can reduce losses.

Stress responses in all vertebrates including fish results in activation of the neuro-endocrine system, which brings about changes in metabolism, osmoregulation, and hematology (Barton, 2000). Metabolism shifts from anabolism to catabolism to supply the extra energy needed to combat stress (Pickering, 1992). Many stress studies focus on the primary responses of fish, i.e., cortisol and catecholamine levels (Barton and Iwama, 1991) and secondary stress responses, e.g., blood glucose and lactate levels. Glycogen level is another important secondary stress response while metabolic enzyme activity can be a valuable stress indicator in marine invertebrates and fish where it is difficult to accurately determine metabolic rates (Dahlhoff, 2004).

The present study examined the metabolic responses of gray mullet fry to different stocking densities during transportation in closed oxygenated polythene bags.

Materials and Methods

Experimental fish. Fry of gray mullet, *Mugil cephalus* (1.2±0.48 g, 2.4±0.52 cm), were netted from the brackish water of Puthuyvype (Kochi, Kerla, India) and acclimated in 1000-l tanks for 12 h under showers of water with 2 ppt salinity and 25°C (Mires and Shak, 1974).

Experimental procedure. Plastic bags (100 µm thick and 15 l) were filled with 5 l filtered saline water (25°C, salinity 2 ppt, pH 8, CO_2 9 ppm, ammonia 0.10 ppm, dissolved oxygen 4.5 ppm) and placed in an expanded styrofoam box. Fish were randomly distributed into the bags at densities of 80, 160, 240, 320, or 400 fish/l (total biomass 480, 960, 1440, 1920, and 2400 g/bag, respectively) and the air was squeezed out of the bags. The bags were filled with medical grade oxygen, tied tightly with rubber strings, and the lid of the styrofoam box was taped in place. Each treatment was conducted in four replicates. The packed fish were transported from Puthuyvype, Kochi, Kerla, India, to the Mutukadu Experimental Station of the Central Institute of Brackishwater Aquaculture (CIBA) in Chennai by rail and road. The duration of transportation was 18 h. On arrival at the laboratory, dead fish were counted, twelve fish from each stocking density were used to estimate glycogen, and twelve fish were used for enzyme assay.

Water quality analysis. Temperature was measured with a mercury thermometer with an accuracy of 0.1°C. Salinity was measured with a refractometer (ATAGO) and pH with a digital pH meter (Lab India). Dissolved oxygen was measured by Winkler's method, CO_2 by the titrimetric method, un-ionized ammonia (phenate method) and nitrite were determined spectrophotometrically at wavelengths 635 nm and 543 nm, respectively (APHA-AWWA-WEF, 1998).

Sample preparation and estimation of parameters. Fish were anesthetized with clove oil (50 µl/l), decapitated, dissolved in 30% KOH, and further treated with 95% alcohol to extract glycogen. For enzyme assay, whole-body tissue was homogenized in a chilled 0.25 M sucrose solution using a mechanical tissue homogenizer. The homogenized samples were centrifuged (5000 rpm for 10 min), and the supernatants were collected and stored at -80°C for subsequent enzyme assay.

Muscle glycogen was estimated by the colorimetric method using anthrone reagent (Hassid and Abraham, 1957). Lactate dehydrogenase (LDH, E.C.1.1.1.27) and malate

dehydrogenase (MDH, E.C.1.1.1.37) activity was measured by the change in optical density (OD) at 340 nm for 3 min at 15 s intervals using the methods of Wroblewski and Ladue (1951) and Ochoa (1955), respectively. Aspartate amino transferase (AST, E.C.2.6.1.1) and alanine amino transferase (ALT, E.C.2.6.1.2) were measured by estimating the released oxaloacetate and pyruvate, respectively, after incubation of the reaction mixture at 37°C for 60 min (Wooton, 1964). Enzyme activities were determined using a spectrophotometer. The protein content of the supernatant was analyzed using the method of Lowry (1951).

Statistical analysis. Statistical analysis of water quality parameters, muscle glycogen, LDH, MDH, AST, ALT, and percent survival were made by one-way analysis of variance (ANOVA) using SPSS-16 for Windows software. Duncan's multiple range test was used for post-hoc mean comparisons ($p<0.05$).

Results

Water quality after transportation is given in Table 1. Dissolved oxygen and pH decreased significantly as the stocking density increased while the opposite was observed for carbon dioxide, ammonia nitrogen, and nitrite nitrogen. Survival decreased while muscle glycogen content was significantly lower in treatments with a higher stocking density. Activity of all tested enzymes increased with stocking density.

Table 1. Effect of stocking density on water quality, survival, and secondary stress responses in mullet fry during transportation (mean±SE, n = 4).

	Stocking density (gray mullet fry/l)				
	80	*160*	*240*	*320*	*400*
Dissolved oxygen (ppm)	4.35±0.13^{a}	4.30±0.06^{a}	4.10±0.06^{ab}	4.03±0.02^{b}	3.75±0.05^{c}
Carbon dioxide (CO_2; ppm)	10.25±0.48^{d}	12.25±0.29^{c}	12.55±1.19^{c}	21.24±0.48^{b}	35.14±1.19^{a}
Ammonia (NH_3; ppm)	0.15±0.02^{d}	0.19±0.01^{bc}	0.24±0.01^{b}	0.25±0.25^{b}	0.33±0.01^{a}
Nitrite (NO_2^-; ppm)	0.02±0.00^{c}	0.02±0.00^{c}	0.02±0.00^{c}	0.08±0.00^{b}	0.10±0.00^{a}
pH	7.63±0.13^{a}	7.48±0.03^{ab}	7.30±0.10^{ab}	7.15±0.09^{c}	7.08±0.02^{d}
Survival (%)	100±0.00^{a}	100±0.00^{a}	92±0.00^{a}	80.5±0.50^{b}	75.5±0.44^{c}
Glycogen (mg/g wet tissue)	2.96±0.20^{a}	2.86±0.17^{a}	2.19±0.21^{a}	1.63±0.32^{b}	1.63±0.33^{b}
Aspartate amino transferase	18±1.89^{d}	16±1.99^{d}	23±1.54^{c}	33±0.23^{b}	42±0.87^{a}
Alanine amino transferase	28±2.98^{d}	22±1.23^{d}	24±1.98^{c}	37±2.11^{b}	47±1.54^{a}
Lactate dehydrogenase	65±0.56^{b}	76±0.33^{b}	75±0.43^{b}	106±0.23^{a}	131±0.45^{a}
Malate dehydrogenase	56±0.43^{b}	66±1.09^{b}	98±1.34^{b}	144±1.98^{a}	157±0.45^{a}

Different superscripts in a row indicate significant differences ($p<0.05$) between stocking densities; Duncan multiple range test ($\alpha = 0.05$).

Discussion

In nature, gray mullet migrate to sea where they mature and spawn while juveniles drift back into brackishwater systems. For aquaculture, fry and fingerling are collected from brackish waters, acclimated, and transported to grow-out farms. Beside stress from transportation, stress may be caused by the deterioration of water quality (Erikson et al., 1997), salinity, or temperature fluctuations (Mires and Shak, 1974) that can alter the metabolism of fish. Transportation of gray mullet for stocking is carried out mainly during the fry and fingerling stages. Biochemical parameters are reliable indicators of the physiological status of organisms (Ferry-Graham and Gibb, 2001). The tertiary response is the final stage of stress and leads to disease or exhaustion, growth retardation, and eventually death (Chatterjee et al., 2006). Catecholamine and cortisol induce glycogenolysis and gluconeogenesis, respectively. Together, both processes cause a rise in blood glucose. Blood glucose and hepatic glycogen are therefore commonly measured parameters of stress response (Manush et al., 2005). In the present study, glycogen decreased significantly as the stocking density increased. Glycogen is broken down into glucose in the early stage of stress (Barton and Iwama, 1991), maybe to meet the higher energy requirements needed to combat stress. The present results are consistent with previous reports of glycogen depletion in tilapia after 1 h of transportation (Orji, 1998)

and in Labeo *rohita* after exposure to increased stocking density (Chatterjee et al., 2006).

Lactate dehydrogenase (LDH) helps produce ATP in the muscle under anaerobic conditions by converting pyruvate into lactate. In the present study, LDH activity increased significantly as the stocking density increased as lactate is the preferred substrate for gluconeogenesis in fish. Similar results were reported in Indian major carps at high stocking densities (Chatterjee et al., 2006).

In addition to lactate, amino acids are preferred substrates for gluconeogenesis in fish. Fish utilize protein and lipid sources rather than carbohydrates for energy (Demeal, 1978). Therefore, AST and ALT activity indicates the mobilization of aspartate and alanine via gluconeogenesis to produce glucose to cope with stress. AST and ALT activity rises in Indian major carps transported at high stocking density (Chatterjee et al., 2006). Together with the elevated AST and ALT activity, MDH activity rises because of its role in the conversion of oxaloacetate, the byproduct of AST, to pyruvate in the cytoplasm. Pyruvate is further utilized as a substrate for gluconeogenesis.

Carbon dioxide and total ammonia concentrations increased significantly with the increase in stocking density and biomass whereas dissolved oxygen followed a reverse trend, indicating that the metabolic rate of gray mullet is higher in higher stocking densities, as found by Pavlidis et al. (2003). Ammonia, which increased with the stocking density, is the main waste product of protein metabolism in fish, with un-ionized ammonia being the most toxic (Fivelstad et al., 1993). Survival was affected by crowding stress because of the increased stocking densities, similar to findings of Gomes et al. (2006).

The overall results of the present investigation suggest that stocking density has a marked effect on metabolism in gray mullet. Therefore it is important to optimize the stocking density to improve survival. From the present study, a stocking density of 240 fry/l, which corresponds to approximately 288 g biomass/l, is optimum for 18 h transportation.

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