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Optimization of Seed and Broodstock Transport Densities for Improved Survival of Cultured Nile Tilapia (*Oreochromis niloticus*, L. 1758)

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ABSTRACT
Nile tilapia fingerlings (mean weight 0.2g, 0.5g and 5g) were packed at 35, 60, 90, 120 and 150 fish/bag for simulated 24h transportation. Similarly, brooders (mean weight 65g) were packed at 8, 16 and 24 fish/bag. The bags, each with a capacity of 5 L, were filled with 2 L of water and filled with pressurized oxygen. DO, pH, temperature, total ammonia, non-ionized ammonia and mortality were monitored throughout the experiment. After simulation, mortality was monitored in hapas over 7 days. Combined effect of duration, load densities and weight on DO, ammonia levels and mortalities was significant ($p < 0.05$) with highest fingerling survival (100%) at 120/bag for 0.2g fingerlings and lowest (74.5 %) at 150/bag for 5g fingerlings. Transport duration and DO levels ($p < 0.05$) significantly affected the survival of brooders. Highest brooder survival (96%) was recorded at 8 fish/bag and lowest survival (67%) at 24 fish/bag. Size, number and duration of transport of Nile tilapia fingerlings and brooders need to be considered during transportation by ensuring transportation duration is within 12 hr to avoid low DO and increased ammonia levels.

Keywords: Load density, *Oreochromis niloticus*, survival, transportation, water quality.

1. Introduction

In the new millennium, global aquaculture production has continued to grow leading to technological innovation and adaptation advancement to meet changing requirements [1]. Tilapia is second to Carp as the most important group of farmed fish. Native to Africa, Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) distribution ranges from the upper Nile River south to the equator and west to the Atlantic coast and is a major source of income and animal protein across the globe [2]. Tolerance to a wide range of environmental conditions such as pH, temperature, nitrogen wastes, low dissolved oxygen concentration and handling easiness has led to the expansion of *O. niloticus* farming [3]. Fish movements have been reported across the globe and Nile tilapia is among the exotic aquatic species that have been widely introduced across the world and used for mass production in aquaculture especially in Asian countries [1].

Live fish transportation mainly involves fingerlings for stocking in grow-out ponds and brooders supply to hatcheries from other hatcheries or the wild. They are also brought to the market to be sold live, or delivered to processing plants for slaughter [1]. However, transportation of live fish is one of the most difficult aspects of fish culture due to poor water exchange leading to reduced oxygen and other water quality levels through accumulation of carbon dioxide, ammonia and suspended solids [4, 5]. Other than water quality, transportation success depends on a number of other factors including time, temperature, density and size of fish, fish physical condition, capture stress response, packing, transportation and unpacking for stocking [6, 7, 8, 9]. The most preferred mode of fingerling and broodstock transportation in Kenya is by use of closed polythene bags. The closed mode of transport is challenged by nitrogenous waste build up mainly ammonia and carbon dioxide during transit, a factor closely associated with transportation duration and load density [9, 10]. Fish release ammonia as a major end product of protein break down through gills and fecal matter oxidizing it to nitrite then to nitrate [11]. Ammonia is also likely to buildup in a fish holding water body due to decomposition of organic matter, but the toxic ammonia (non-ionized ammonia) is dependent on pH and temperature [12]. This study was aimed at determining the best size and load density for transportation of *O. niloticus* by evaluating their survival and water quality parameters against transport duration.

2. Materials and methods

Four holding tanks at the National Aquaculture Research Development and Training Centre hatchery were filled with water for conditioning of fingerlings and brooders harvested from broodstock ponds. Fingerlings weighing 0.2 g, 0.5 g and 5 g were conditioned for 24 h and packed in oxygen pressurized 5 L polythene bags filled with 2 L of water at load densities of 150, 120, 90, 60 and 35 per bag. Initial pH, temperature, total ammonia, non-ionized ammonia and DO were determined prior to packaging of fingerlings for transportation. pH, temperature and DO were determined using Hanna Multi-parameter HI 9829, while non-ionized ammonia was calculated upon determining total ammonia nitrogen [13]. The water quality parameters were monitored over a 24-hour period at intervals of 12 h and fingerling total mortality recorded by subtracting mortality from an initial number at close of the transport simulation experiment. Surviving fingerlings were conditioned before introduction in hapas measuring 2 x 2 x 1.5m mounted in a pond with flow through system (each bag in its own hapa) and survival monitored over 7 days.

Brooders weighing 65g were also conditioned for 24 h before packaging for transportation in similar oxygen pressurized 5 L polythene bags filled with 2 L of water at a stocking of 8, 16 and 24 fish per bag. Water quality parameters similar to those of the fingerlings were determined at packaging and at 12 and 24 h. Mortality of brooders in each bag was recorded at each water quality monitoring session and at the end of 24 h; a total count of survival was performed. Nine hapas measuring 2 x 2 x 1.5m were mounted prior to close of brooders experiment and initial pond water quality recorded. The brooders were then conditioned each bag into individual hapa and survival monitored over a period of 7 days. The fingerling experiment used a 5 x 3 x 2 factorial design (5 load densities, 3 fingerling sizes and 2 time durations) with 45 treatments (3 replicates per treatment) and the brooders used a 3 x 2 factorial design (3 load densities and 2 time durations) with 9 treatments (3 replicates per treatment). Data was analyzed using the SAS statistical package (version 12.0 for windows). Simple regression analysis was used to determine relationship between water quality variables, stocking and survival in each treatment. Tukey's HSD test was applied to identify means that were significantly different from each other.

3. Results

Transport duration and weight of fingerlings had a significant effect ($p < 0.05$) on DO levels with 5 g fingerlings recording the lowest DO levels (3.26 - 4.34 mg L^{-1}) across all load densities (Table 1). Non-ionized ammonia levels were not detected at packaging time, but a significant buildup was noted between 12 and 24 hr among 5 g fingerlings ($p < 0.05$). Highest un-ionized ammonia levels (0.06 mg L^{-1}) were recorded in 5 g of 150-load density (Table 2). No significant changes ($p > 0.05$) were recorded for pH during the transportation duration and at different load densities (Table 3). The pH levels ranged from 7.02 to 7.45 indicating that water condition was slightly changing towards basic.

Table 1: DO levels (mg L^{-1}) at different loading densities, transportation duration and weights (g) of *O. niloticus* fingerlings

Duration of transport (h)			**Load density (No./bag)**			
	Wt (g)	35	60	90	120	150
0	0.2	8.51^{aB}	8.51^{aB}	8.51^{aB}	8.51^{Ab}	8.51^{aB}
	0.5	8.51^{aB}	8.51^{aB}	8.51^{aB}	8.51^{aB}	8.51^{aB}
	5	8.51^{aB}	8.51^{aB}	8.51^{aB}	8.51^{aB}	8.51^{aB}
12	0.2	6.95 ± 0.05^{aA}	6.10 ± 0.11^{aA}	5.59 ± 0.29^{aA}	6.74 ± 0.09^{aA}	6.98 ± 0.50^{aA}
	0.5	6.79 ± 0.13^{aA}	7.48 ± 0.17^{bD}	8.16 ± 0.32^{bAD}	6.35 ± 0.12^{aA}	5.71 ± 0.48^{aA}
	5	6.54 ± 0.14^{aA}	6.93 ± 0.03^{aA}	5.84 ± 0.39^{aA}	6.48 ± 0.22^{aA}	5.32 ± 0.21^{aA}
24	0.2	6.20 ± 0.41^{aA}	6.09 ± 0.44^{aA}	5.51 ± 0.24^{aA}	5.74 ± 0.08^{aA}	6.70 ± 0.13^{aA}
	0.5	6.34 ± 0.15^{aA}	7.05 ± 0.10^{aD}	7.67 ± 1.34^{bD}	5.88 ± 0.39^{aA}	4.50 ± 0.21^{cA}
	5	4.12 ± 0.64^{cAB}	4.34 ± 0.19^{cAB}	3.76 ± 0.67^{cAB}	3.99 ± 0.59^{cAB}	3.26 ± 0.15^{cAB}

Values are reported as mean ± S.E.M. Means identified by different capital letters in the columns or small letters in the rows were significantly different ($p < 0.05$) as determined by analysis of variance and tukey's comparison of mean values.

Table 2: Un-ionized ammonia levels (mg L^{-1}) at different loading densities, transportation duration and weights (g) of *O. niloticus* fingerlings

Duration of transport (hr)		**Load density (No. /bag)**				
	Weight (g)	35	60	90	120	150
0	0.2	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
	0.5	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
	5	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
12	0.2	0.01 ± 0.05^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}
	0.5	0.01 ± 0.13^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}
	5	0.01 ± 0.14^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}
24	0.2	0.02 ± 0.41^{aC}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.02 ± 0.00^{aC}
	0.5	0.02 ± 0.00^{aC}	0.03 ± 0.00^{bD}	0.04 ± 0.00^{cAB}	0.03 ± 0.00^{bD}	0.03 ± 0.00^{bD}
	5	0.03 ± 0.00^{bD}	0.04 ± 0.00^{cAB}	0.04 ± 0.00^{cAB}	$0.05\pm0.00^{d\ AC}$	0.06 ± 0.01^{eAD}

Values are reported as mean ± S.E.M. Means identified by different capital letters in the columns or small letters in the rows were significantly different ($P < 0.05$) as determined by analysis of variance and tukey's comparison of mean values.

Table 3: pH levels at different loading densities, transportation duration and weights (g) of *O. niloticus* fingerlings.

Duration of transport (hr)						**Load density (No./bag)**
	Weight (g)	35	60	90	120	150
0	0.2	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}
	0.5	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}
	5	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}
12	0.2	7.45 ± 0.00^{aB}	7.31 ± 0.09^{aB}	7.00 ± 0.01^{aB}	7.04 ± 0.00^{aB}	7.08 ± 0.02^{aB}
	0.5	7.14 ± 0.01^{aB}	7.18 ± 0.01^{aB}	7.22 ± 0.01^{aB}	7.24 ± 0.01^{aB}	7.29 ± 0.02^{aB}
	5	7.33 ± 0.01^{aBB}	7.34 ± 0.00^{aBB}	7.37 ± 0.01^{aBB}	7.40 ± 0.00^{aBB}	7.42 ± 0.01^{aBB}
24	0.2	7.35 ± 0.05^{aC}	7.32 ± 0.18^{aC}	7.16 ± 0.00^{aC}	7.18 ± 0.00^{aC}	7.20 ± 0.01^{aC}
	0.5	7.23 ± 0.01^{aC}	7.25 ± 0.00^{aC}	7.30 ± 0.01^{aC}	7.31 ± 0.01^{aC}	7.36 ± 0.02^{aC}
	5	7.39 ± 0.00^{aCC}	7.41 ± 0.03^{aCC}	7.45 ± 0.04^{aCC}	7.52 ± 0.03^{aCC}	7.60 ± 0.04^{aCC}

Values are reported as mean ± S.E.M. Means identified by different capital letters in the columns or small letters in the rows were significantly different ($P < 0.05$) as determined by analysis of variance and Tukey's comparison of mean values.

There was a significant effect of load density on mortality of the fingerlings ($p < 0.05$) with an exception of fingerlings weighing 0.2g whose load densities experienced zero mortality throughout the transportation duration (Fig. 1). Fingerlings weighing 0.5 g recorded mortalities of 0.3% and 2.9% at loaddensities of 120 and 150/bag while fingerlings weighing 5g experienced mortalities at all load densities. The highest mortality (11.3%) was recorded for fingerlings weighing 5 g at a load density of 150/bag. No mortalities were recorded in hapas for fingerlings of all load densities and sizes.

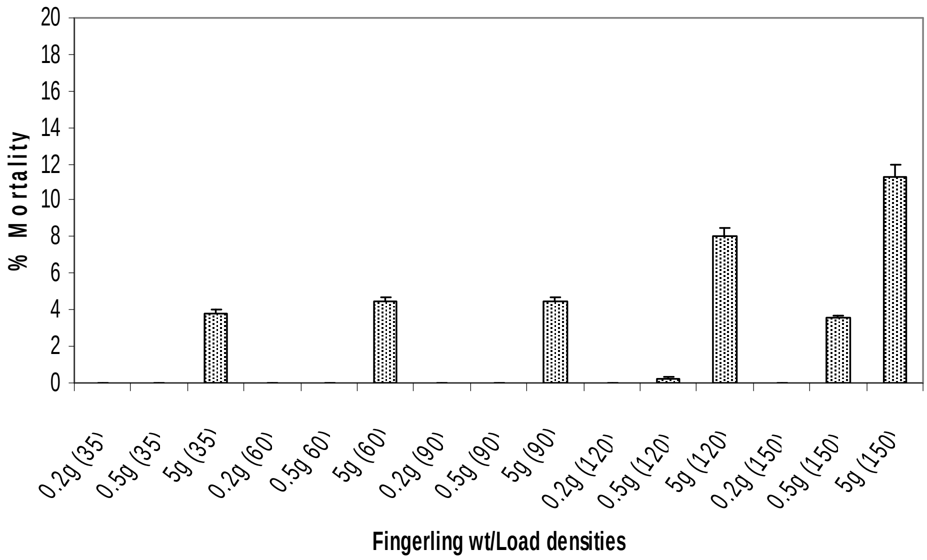

Fig. 1: Accumulated mortality of the Nile tilapia (*O. niloticus*) fingerlings as a function of load density and size (g) at end of 24 hr transportation duration.

There was a significant effect ($p < 0.05$) of broodstock load densities 16 and 24/bag on the DO levels at 12 and 24 h intervals (Table 4). However, no significant difference ($p > 0.05$) in DO levels was recorded at load densities of 8/bag over the 24 h transportation duration. There was a significant effect ($p < 0.05$) of transport duration (24 h) on DO levels at load density of 16/bag and 24/bag with both recording lowest DO levels of 3.46 mg L^{-1} and 2.71 mg L^{-1} (Table 4). There was no significant effect ($p > 0.05$) of load density and transport duration on un-ionized ammonia levels for load densities 8 and 16/bag (Table 5). Load density of 16/bag recorded un-ionized ammonia build up at 0.01mgL^{-1} just like the other two load densities as at 12 h of transportation, but posted a significantly ($p < 0.05$) high un-ionized ammonia increase of 0.03 mg L^{-1} at close of transportation (24 h). There was a significant ($p < 0.05$) effect of load density on the pH levels while no effect was recorded for transport duration (Table 6).

Table 4: DO levels (mg L^{-1}) at different loading densities and transportation duration of *O. niloticus* brooders

Duration of transport (hr)		Load density (No./bag)		
	Wt (g)	8	16	24
0	65	8.21 ± 0.00^{aB}	8.21 ± 0.00^{aA}	8.21 ± 0.00^{aA}
12	65	7.53 ± 0.16^{aB}	5.42 ± 0.16^{bB}	3.67 ± 0.6^{bB}
24	65	7.3 ± 0.84^{aB}	3.46 ± 0.85^{bC}	2.71 ± 1.17^{bB}

Values are reported as mean ± S.E.M. Means identified by different capital letters in the columns or small letters in the rows were significantly different ($P < 0.05$) as determined by analysis of variance and tukey's comparison of mean values.

Table 5: Un-ionized ammonia levels (mg L^{-1}) at different loading densities and transportation duration of *O. niloticus* brooders

Duration of transport (hr)		Load density (No./bag)		
	Wt (g)	8	16	24
0	65	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
12	65	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}	0.01 ± 0.00^{aB}
24	65	0.01 ± 0.00^{aB}	0.01 ± 0.01^{aB}	0.03 ± 0.01^{bC}

Values are reported as mean ± S.E.M. Means identified by different capital letters in the columns or small letters in the rows were significantly different ($P < 0.05$) as determined by analysis of variance and tukey's comparison of mean values.

Table 6: pH levels at different loading densities and transportation duration of *O. niloticus* brooders

Duration of transport (hr)		Load density (No./bag)		
	Wt (g)	8	16	24
0	65	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}	7.02 ± 0.00^{aA}
12	65	7.17 ± 0.05^{aB}	7.36 ± 0.15^{aB}	7.43 ± 0.07^{bBC}
24	65	7.16 ± 0.03^{aB}	7.50 ± 0.17^{aB}	7.85 ± 0.26^{bBC}

Values are reported as mean ± S.E.M. Means identified by different capital letters in the columns or small letters in the rows were significantly different ($P < 0.05$) as determined by analysis of variance and tukey's comparison of mean values.

The pH was high at load density of 24/bag and lowest at 8/bag. Highest mortality (26, n= 72) was recorded at load density of 24/bag and least mortality (1, n= 24) at load density of 8/bag at the close of simulated transportation (Fig. 2). However, all load densities recorded 100% survival within 12 h of transportation. There was no significant change in temperature with all load densities recording temperature ranging between 22.79 and 23.61 ºC.

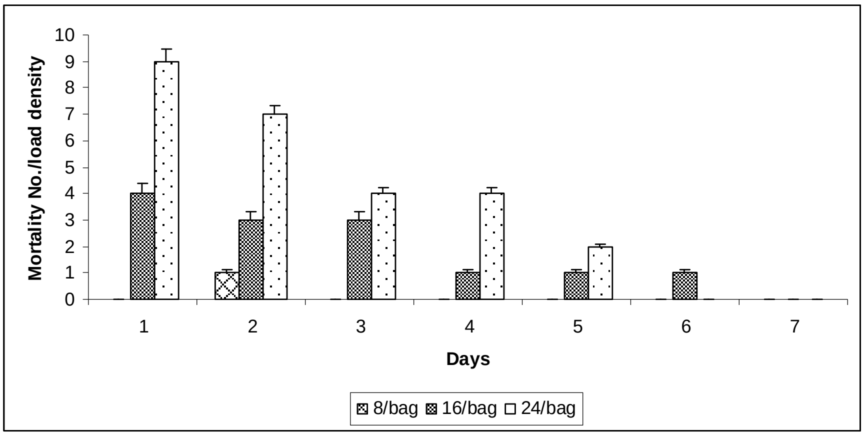

Fig 2: Daily mortality of the Nile tilapia (*O. niloticus*) brooders as a function of load density in hapas over 7 days post transportation.

4. Discussion and Conclusion

Dissolved oxygen (DO) is the most important single factor in live fish transport and should be supplied in adequate concentrations. Levels of dissolved oxygen in fresh water are dependent on temperature. Increased demand for dissolved oxygen is a result of rise in temperature and fish weight [9, 14].

An increase on temperature by 5 ºC results in increased metabolism and therefore increased dissolved oxygen demand resulting in a buildup of ammonia and carbon dioxide and a decrease of the 5 ºC implies lowered metabolism, reduced dissolved oxygen demand and thus low ammonia and carbon dioxide build up [15, 16]. Though there was a marginal change in temperature among the fingerling load densities at 12 h of transportation with an average rise of 1 ºC between transportation duration in all weights and load densities, recorded mortalities may have resulted from competition for oxygen by live and dead fingerlings decomposition process resulting in more toxic metabolites [17, 12].

Study findings indicated that survival of *O. niloticus* fingerlings increased significantly with low fingerlings weight (0.2g) and duration of transportation in polythene bags. All load densities for fingerlings and brooders recorded zero mortality within 12 h of transportation. The mortalities were recorded at end of the transportation duration (24 h), an occurrence associated with buildup of un-ionized ammonia and a slight increase in temperature followed by a decrease in dissolved oxygen. Almost similar observations have been made for transportation of Silver catfish (*Rhamdia quelen)* and Channel catfish [17, 18, 14]. Other than the water quality parameters at the end of the transportation period, bags with mortalities had observable foam accumulation and fouling odor recorded in fingerlings of 5g weight and 120 and 150/bag load densities recording highest un-ionized ammonia buildup at 0.05 and 0.06 mg L^{-1}.

Though the highest recorded un-ionized ammonia for brooders was 0.03 mg L^{-1} which is tolerable by most fish, high mortalities recorded for load density of 24/bag during 7 days hapa observation may have resulted from prolonged periods (24 h) of transportation. This implies that though the brooders were delivered alive, they were affected by prolonged exposure to un-ionized ammonia resulting in recorded mortalities over the 7 days period.

Survival of *O. niloticus* fingerlings transported in plastic bags is related to fingerling size, load density and transport duration while brooders safe transportation is dependent upon load density and transport duration. It is therefore recommended that *O. niloticus* brooders and fingerlings transporters put into consideration fish size, number and duration of transportation to minimize losses. Transportation duration of 12 h is recommended for all sizes of fish and load densities with water quality being maintained within recommended ranges. However, fingerlings weighing 0.2 g at 150/bag are also recommended for transportation period between 12 and 24 h. Brooders of all studied densities are recommended for transportation for up to 12 h but only 8/bag may be transported for up to 24 h without compromising survival.

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