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Optimization of seed and broodstock transport densities for improved survival of African carp (*Labeo victorianus*, Boulenger, 1901)

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Labeo victorianus fingerlings, post-fingerlings and brooders weighing 0.5 g, 5 g and 100 g respectively were harvested and conditioned for 24hrs before packaging for simulated transport. Fingerlings and brooders were packed in oxygen pressurized 5 L polythene bags filled with 1.5 L of water treated with 5 g of salt (NaCl) to reduce ammonia toxicity effect. Fingerling and post-fingerling packaging was done at 12, 15 and 20 g L^{-1} and 120, 150 and 200 g L^{-1}while brooders were packaged in 400, 800 and 1200 g L^{-1}. A 24 hour observation and recording was performed on mortality, pH, temperature, total ammonia, free ammonia, and dissolved oxygen (DO) at 0, 6, 12, 18 and 24 hours intervals. DO, pH and temperature were determined using Hanna Multi-parameter HI 9829. At the end of the transportation, the fish from the three treatments were each conditioned and stocked in hapas and mortality monitored over a period of 7 days. DO levels for all load densities had a significant increase ($P<0.05$) at 6 h due to addition of pressurized oxygen and experience a significant drop at 24 h but all remained within fish transportation recommended ranges. No mortalities were recorded at the end of simulated transportation and seven day observation in hapas mounted in earthen ponds. The study therefore recommends *L. victorianus* transportation load densities of 20 g L^{-1}, 200 g L^{-1} and 1200 g L^{-1} for fingerlings, post-fingerlings and brooders respectively at a temperature range of 18.45±0.42 °C to 22.70±0.00 °C for high survival without compromising water quality.

Keyword: *Labeo victorianus,* free ammonia, load density, transportation, water quality

1. Introduction

African carp (*Labeo victorianus*) commonly known as Ningu, is a fresh water cyprinid endemic to the Lake Victoria basin [1], but just like other members of the genus, it moves into affluent rivers to spawn in vegetated flooded pools [1, 2, 3, 4, 5, 6, 7]. Due to its predictable potamodromy, the Lake Victoria riparian communities have overtime used fish traps to harvest mature males and gravid female *L. victorianus* migrating upstream to breed. The migratory habit coupled with the fish's delicacy has overtime contributed to a great decline of the fish in rivers and lakes. These have resulted in high local market demand prompting fish farmers to create a keen interest in culturing the fish with no immediate source for quality fingerlings. The rising need for *L. victorianus* fingerlings by the traditional African catfish and Nile tilapia fish grow-out farms requires hatchery operators to acquire initial brood-stock from the wild. Brood-stock acquisition of the wild and eventual supply of fingerlings from hatcheries to grow-out farms and other markets will involve live fish transportation [8].

Live fish transportation is one of the most difficult aspects of fish culture due to poor water exchange, reduced dissolved oxygen, increased suspended solids and ammonia and carbon dioxide accumulation [9, 10]. Transport of fish in Kenya is by use of open tanks and oxygenated polythene bags. However, use of oxygenated polythene bags is the most preferred

mode of fingerling and broodstock transportation. Other findings [11, 12, 13, 14] indicate that other than water quality, transportation success depends on density and size of fish, fish physical condition, handling time, temperature, capture stress response, packaging, transport duration and unpacking for stocking. Transport duration and load density are factors that are closely associated with ammonia, carbon dioxide and nitrogenous waste build up during live fish transportation under a closed mode of transportation [14, 15]. Ammonia is a major fish end product of protein breakdown into unionized and ionized ammonia and is likely to build up in a fish holding water facility due to decomposition of organic matter leading to fish mortality. Therefore, the objective of the present study was to determine the best load density and time duration for transport of *L. victorianus* fingerlings, post-fingerlings and brooders without compromising their survival.

2. Methodology

Labeo victorianus fingerlings, post-fingerlings and brooders with average weights of 0.5 g, 5 g and 100 g respectively were harvested from KMFRI Sagana Research ponds and conditioned for 24hrs before packaging for simulated transport. Fingerlings and brooders were packed in oxygen pressurized 5 L polythene bags filled with 1.5 L of water treated with 5 g of salt (NaCl) to reduce ammonia toxicity effect. Fingerling and post-fingerling packaging was done at 12, 15 and 20 g L^{-1} and 120, 150 and 200 g L^{-1} while brooders were packaged in 400, 800 and 1200 g L^{-1}. Brooders were subjected to a 3 x 5 factorial design (3 load densities and 5 time durations) with 15 treatments (3 replicates per treatment), while post fingerlings and fingerlings experiment used a 5 x 3 x 2 factorial design (5 time durations, 3 load densities and 2 fingerling sizes) with 45 treatments (3 replicates per treatment).

During 24 hr transportation period, measurements were taken on mortality, pH, temperature, total ammonia, free ammonia, and dissolved oxygen (DO) at 0, 6, 12, 18 and 24 hrs intervals. DO, pH and temperature were determined using Hanna Multi-parameter HI 9829. Unionized ammonia (NH_3) was calculated upon determining levels of pH, temperature and total ammonia nitrogen (TAN). Total ammonia nitrogen (TAN) was measured by use of ammonia salicylate method as adapted from Clin Chim Acta., 14, 403 (1966). At the end of the transportation, fish from each treatment and stocking densities were conditioned and stocked in hapas and mortality monitored over a period of 7 days.

Data was analyzed using SAS statistical package (12.0 for windows). Simple regression analysis was used to determine relationship between water quality variables, load density, size and survival in each treatment. Tukey's HSD test was applied to identify means that were significantly different from each other.

3. Results

The DO levels for each load densities of brooders under simulated transport experienced a significant ($P<0.05$) increase during the first 6 h of transport but experienced no significant difference ($P> 0.05$) between 6 h and 18 h of transportation. At the close of the simulated transport (24 h), there was a recorded significant decrease across all load densities from between 7.60±1.36 and 11.96±6.44 mg L^{-1} at 18 h to between 6.87±1.21 and 7.52±0.52 mg L^{-1} at 24h (Table 1).

Table 1: Changes in water DO (Dissolved oxygen) during brood-stock transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	400	**800**	**1200**
0	4.03 ± 0.00^{aA}	4.03 ± 0.00^{aA}	4.03 ± 0.00^{aA}
6	13.78 ± 0.84^{bA}	12.31 ± 1.43^{bAB}	10.60 ± 0.99^{bB}
12	11.43 ± 4.97^{bA}	11.28 ± 1.56^{bAB}	12.08 ± 1.8^{bB}
18	11.96 ± 6.44^{bA}	10.29 ± 2.19^{bAB}	7.60 ± 1.36^{bB}
24	7.38 ± 0.87^{cAB}	6.87 ± 1.21^{cAB}	7.52 ± 0.52^{cAB}

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

All load densities displayed a similar trend on pH levels over transportation duration with a significant decrease in the first 6 h but no significant difference between 6 h and 12 h. However, there was a significant rise of pH between 12 h and 18 h but had no significant difference between 18 h and 24 h. All load densities recorded no significant difference ($P > 0.05$) across the load densities over the experiment period (Table 2).

Table 2: Changes in water pH during brood-stock transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	400	**800**	**1200**
0	7.30 ± 0.00^{aA}	7.30 ± 0.00^{aA}	7.30 ± 0.00^{aA}
6	6.87 ± 0.95^{bA}	7.16 ± 0.14^{bA}	6.73 ± 0.27^{bA}
12	6.91 ± 0.1^{bA}	6.87 ± 0.1^{bA}	7.0 ± 0.02^{bA}
18	7.2 ± 0.13^{aA}	7.25 ± 0.12^{aA}	7.34 ± 0.23^{aA}
24	7.13 ± 0.32^{aA}	7.38 ± 0.45^{aA}	7.44 ± 0.25^{aA}

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

Temperature recorded no significant differences ($P > 0.05$) across load densities over the 24 h simulated transport period. However, there was a recorded significant rise in temperature from an overall average of 19.29± 0.23 ºC to 25.59± 2.11 ºC at all load densities between 18 h and 24 h of simulated transportation (Table 3).

Table 3: Changes in water temperature during brood-stock transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	400	**800**	**1200**
0	22.70 ± 0.00^{aA}	22.70 ± 0.00^{aA}	22.70 ± 0.00^{aA}
6	21.67 ± 0.92^{bA}	21.23 ± 0.37^{bA}	22.7 ± 1.46^{bA}
12	20.49 ± 0.76^{cA}	19.78 ± 0.87^{cA}	18.79 ± 0.39^{cA}
18	19.26 ± 0.27^{cA}	19.33 ± 0.28^{cA}	19.29 ± 0.15^{cA}
24	26.39 ± 0.85^{dA}	26.37 ± 0.43^{dA}	25.0 ± 0.83^{dA}

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

There was no significant difference ($P > 0.05$) in NH_3 across treatments throughout the simulated transport duration. During the simulated transport duration, no significant increase was recorded between 6 h and 18 h within load densities. However, at 18 h NH_3 levels of load density 1200 g L^{-1} were significantly higher than load densities 400 and 800 g L^{-1} but experienced no significant difference between 18 h and 24 h. Load density 800 g L^{-1} recorded the highest NH_3 levels (0.12±0.15) in the transportation bags at 24 h close of simulated transport experiment (Table 4).

Post fingerlings under simulated transport at all load densities exhibited a significant increase ($P < 0.05$) in DO levels at 6 h of transportation followed by a decrease at 12 h. Further decrease was recorded at 18 h with no significant difference by end of simulated transport (24 h). Load density 120 mg L^{-1} had a significantly lower DO levels between 6 h and 24 h of simulated transport as compared to load densities 150 and 200 mg L^{-1}. There was a significant difference ($P < 0.05$) across load densities with load density 120 g L^{-1} recording a significantly lower DO level against

load densities 150 and 200 g L^{-1} at 6 to 24 h (Table 5).

Table 4: Changes in water NH_3 during brood-stock transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	400	800	1200
0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
6	0.01 ± 0.00^{aA}	0.02 ± 0.01^{aA}	0.01 ± 0.00^{aA}
12	0.02 ± 0.00^{aA}	0.02 ± 0.00^{aA}	0.02 ± 0.00^{aA}
18	0.03 ± 0.01^{aA}	0.04 ± 0.00^{aA}	0.08 ± 0.021^{bA}
24	0.07 ± 0.04^{bA}	0.12 ± 0.15^{cA}	0.08 ± 0.07^{bA}

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

Table 5: Changes in water DO (Dissolved oxygen) during post-fingerling transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	120	150	200
0	4.03 ± 0.00^{aA}	4.03 ± 0.00^{aA}	4.03 ± 0.00^{aA}
6	8.70 ± 1.45^{bA}	9.75 ± 1.52^{bB}	9.73 ± 0.28^{bB}
12	6.99 ± 1.77^{cA}	8.42 ± 2.13^{cB}	8.74 ± 1.37^{cB}
18	5.81 ± 1.76^{dA}	6.40 ± 0.88^{dB}	6.33 ± 0.89^{dB}
24	5.02 ± 1.54^{dA}	6.87 ± 1.21^{dB}	7.52 ± 0.73^{dB}

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

There was no significant difference ($P > 0.05$) in temperature across and within treatments at 6 h of simulated transportation. However, while load densities 120 and 200 g L^{-1} recorded a significant drop in temperature between 6 h and 12 h, load density 150 g L^{-1} recorded a significant temperature rise from 21.68±0.31 ºC to 22.94±4.59 ºC. There was no significant ($P > 0.05$) difference in temperature across all load densities at 18 h and 24 h (Table 6). A significant ($P < 0.05$) rise in temperature was recorded at close of the simulated transport (24 h) within all load densities but posed no significant difference ($P > 0.05$) across the load densities.

Table 6: Changes in water temperature during post-fingerling transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	120	150	200
0	22.7 ± 0.00^{aA}	22.7 ± 0.00^{aA}	22.7 ± 0.00^{aA}
6	21.68 ± 0.73^{aA}	21.68 ± 0.31^{aA}	21.56 ± 0.28^{aA}
12	19.79 ± 0.20^{abB}	22.94 ± 4.59^{acB}	20.16 ± 0.38^{abB}
18	19.31 ± 0.44^{cA}	18.45 ± 0.42^{cA}	18.81 ± 0.19^{cA}
24	27.96 ± 0.65^{dA}	28.20 ± 0.16^{dA}	28.74 ± 0.22^{dA}

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

There was no significant difference (P>0.05) for pH levels within all post fingerling load densities at 6 h of simulated transport. However, there was a recorded significant difference across load densities with load density 120 mg L^{-1} recording a significantly lower pH levels at 6 h as compared to 150 and 200 mg L^{-1}. At between 12 and 24 h of simulated transportation, all load densities recorded a significant increase with load density 200 mg L^{-1} recording a significantly higher pH levels (7.59±0.31) as compared to 120 and 150 mg L^{-1}(Table 7).

Table 7: Changes in water pH during post-fingerling transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	120	**150**	**200**
0	7.30±0.00^{aA}	7.30±0.00^{aA}	7.30±0.00^{aA}
6	7.15±0.04^{aA}	7.45±0.06^{aAB}	7.53±0.08^{aAB}
12	7.04±0.22^{bA}	7.11±0.25^{bA}	6.91±0.07^{bA}
18	7.21±0.09^{abA}	7.24±0.10^{abA}	7.22±0.08^{abA}
24	7.31±0.09^{aA}	7.36±0.05^{aA}	7.59±0.31^{aAB}

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

There was no significant difference (p> 0.05) in free ammonia (NH_3) across and within load densities between start of transport simulation and 18 h. However, NH_3 increased significantly at close of transport simulation (24 h) within all load densities (Table 8).

Table 8: Changes in water NH_3 during post-fingerling transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	120	**150**	**200**
0	0.00±0.00^{aA}	0.00±0.00^{aA}	0.00±0.00^{aA}
6	0.01±0.01^{aA}	0.03±0.00^{aA}	0.04±0.01^{aA}
12	0.03±0.02^{aA}	0.04±0.04^{aA}	0.02±0.00^{aA}
18	0.02±0.01^{aA}	0.03±0.02^{aA}	0.03±0.01^{aA}
24	0.07±0.02^{bA}	0.08±0.02^{bA}	0.16±0.15^{bA}

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

There was a significant increase in DO levels at 6 h of transportation for all load densities with load density 12 mg L^{-1} having the highest increase from the initial 4.03± 0.00 mg L^{-1} to 10.97± 2.50 mg L^{-1}. However, at the end of the simulated transport period (24 h), load density 120 mg L^{-1} recorded the lowest DO levels compared to load densities 15 and 20 mg L^{-1} (Table 9).

There was no significant ($p < 0.05$) temperature difference across and within all load densities over 6 h of simulated transport period. However there was a significant temperature decrease at 12 h and was maintained over to 18 h except for load density 120 mg L^{-1} which recorded a significant increase. This was followed with a significant rise in temperature at 24 h of simulated transport period across all load densities (Table 10).

Table 9: Changes in water DO (Dissolved oxygen) during fingerling transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	12	15	20
0	4.03±0.00^{aA}	4.03±0.00^{aA}	4.03±0.00^{aA}
6	10.97±2.50^{bA}	10.10±0.77^{bB}	9.86±1.44^{bB}
12	9.07±3.72^{bA}	10.19±1.74^{cA}	8.72±1.91^{cA}
18	10.55±1.44^{cA}	8.02±2.39^{dA}	7.60±2.04^{dA}
24	4.71±0.93^{dA}	7.28±3.04^{dB}	6.62±3.69^{dB}

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

Table 10: Changes in water temperature during fingerling transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	12	15	20
0	22.70± 0.00^{aA}	22.70±0.00^{aA}	22.70±0.00^{aA}
6	21.32±0.28^{aA}	22.48±0.84^{aA}	21.92±0.67^{aA}
12	19.94±0.30^{bA}	20.17±0.19^{bA}	20.38±0.29^{bA}
18	21.11±4.66^{dA}	19.20±0.12^{bA}	18.98±0.23^{bA}
24	28.54±0.04^{cA}	28.42±0.36^{cA}	28.12±0.10^{cA}

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

No significant difference was recorded across and within all load densities throughout the simulated transport period for pH (Table 11).

Table 11: Changes in water pH during fingerling transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	12	15	20
0	7.30±0.00^{aA}	7.30±0.00^{aA}	7.30±0.00^{aA}
6	7.31±0.32^{aA}	7.46±0.19^{aA}	7.39±0.21^{aA}
12	6.97±0.10^{aA}	7.00±0.21^{aA}	6.98±0.07^{aA}
18	7.24±0.25^{aA}	7.38±0.10^{aA}	7.18±0.07^{aA}
24	7.39±0.01^{aA}	6.35±1.64^{aA}	7.40±0.06^{aA}

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

There was no significant ($p > 0.05$) difference in NH_3 levels in fingerling transportation bags across all load densities. However, there was a recorded NH_3 increase within load densities with time over the simulated transport period (Table 12).

Table 12: Changes in water NH_3 during fingerling transportation

Duration of Transport (hours)	Load density (g L^{-1})		
	12	15	20
0	0.00±0.00^{aA}	0.00±0.00^{aA}	0.00±0.00^{aA}
6	0.03±0.01^{bA}	0.04±0.02^{bA}	0.03±0.02^{bA}
12	0.02±0.00^{cA}	0.02±0.01^{cA}	0.02±0.00^{cA}
18	0.03±0.01^{bcA}	0.04±0.02^{bcA}	0.03±0.01^{bcA}
24	0.09±0.00^{dA}	0.08±0.02^{dA}	0.08±0.02^{dA}

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

4. Discussion

Water quality is important in determining survival of fish during transport in closed conditions. Water quality degradation mostly occurs within the first hour or two after packing, and subsequent deterioration in water quality may occur relatively slowly [16]. During live fish transportation, oxygen demand is dependent on water temperature and fish weight [17, 14]. In this study, there was an increase in DO levels with transportation period in most treatments because at the time of sealing the packaging bags, oxygen saturation in the water is related to atmospheric air. The use of pure oxygen in the packaging bags increased the DO levels in the water up to saturation in relation to the partial pressure of oxygen contained in the bags, a trend similar to earlier findings by [18].

All load densities for brooders, post fingerlings and fingerlings recorded zero mortality over the 24 h transportation period. There was a close relationship between temperature and pH increase to DO decrease and inversely NH_3 increase with transportation time. Fish transported in a closed system such as the packaging bags may experience oxygen deficit due to high load density coupled with prolonged transport period. In case there is any dead fish during transportation, they also compete with the living ones for oxygen due to the increased bacterial multiplication leading to further production of toxic metabolites [19]. Similarly, slime produced by the fish is another substrate for bacterial growth, resulting in a decrease of the water oxygen content, a process further intensified with rising water temperature [14]. Transport duration 24 h had the lowest DO levels and highest NH_3 levels since there was increased metabolic activity resulting from increased temperature leading to increased release of ammonia and oxygen escape from the water.

The brooders tended to settle faster thus expressing minimal activity in the packaging bags as compared to post fingerlings and fingerlings. These resulted in the lower DO decline for brooders bags despite their higher load density as compared to post fingerlings and fingerlings an occurrence similar earlier findings [20]. Though there were no mortalities recorded during and after simulated transport, one of the major causes of direct fish mortalities during closed transportation system is due to high concentrations or prolonged durations of exposure to free ammonia [21]. All transportation periods within and across treatments had free ammonia increase with increased temperature and pH a trend similar to earlier findings by [22, 23]. It was however important to note that free ammsonia levels were within recommended ranges for warm water fish transportation. Transfer of treatments from the transport simulation point (hatchery) to the ponds where the final readings were recorded contributed to the increase in temperature due to direct exposure to the sun. However, the increase in temperature had no effect on fish survival since the rise of free ammonia in the various fish sizes and load densities was not prolonged considering that it happened at close of transport simulation.

5. Conclusion and Recommendation

The study findings indicate that water quality used for fish transportation is dependent upon transport duration and load density for *L. victorianus* even though in the current study all water quality parameters were with recommended ranges. Unionized ammonia build up in the packaging bags is dependent on pH and temperature thus a temperature range between 18-23 °C is best for live transportation

of all sizes of *L. victorianus*. The study therefore recommends *L. victorianus* transportation load densities of 20 g L^{-1}, 200 g L^{-1} and 1200 g L^{-1} for fingerlings, post fingerlings and brooders respectively at a temperature range of 18-23 °C without compromising water quality. It is further recommended that any increase in load density should put into consideration the transport duration.

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