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Survival and physiological response of *Labeo victorianus* (Pisces: Cyprinidae, Boulenger 1901) juveniles to transport stress under a salinity gradient

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ABSTRACT

Survival and physiological response of *Labeo victorianus* juveniles under varying salinity gradients were studied during a 6 h transport. Salinity ranges were: 0, 0.25, 0.5, 1, 2, 4, 8 and 10 psu. To each transport bag, 100 juvenile *L. victorianus* (mean weight = 8.0 ± 1.1 g, stocking biomass = 16 kg m^{-3}) were transferred. Water temperature, dissolved oxygen (DO), pH, total ammonia nitrogen (TAN) and carbon dioxide (CO_2) were measured before and after transport. Plasma cortisol, blood glucose, plasma sodium, plasma chloride and blood ammonia were also determined. No juvenile mortalities occurred in salinity ranges of 1 to 4 psu. After transport, survival and parameters of physiological response in the juvenile of *L. victorianus* were significantly different among different salinity treatments ($p<0.05$). Low survival, of less than 70% occurred in control treatments (0 psu) and in salinities 0.25, 0.5 psu and at 10 psu. Increased salinity correlated negatively with TAN and CO_2 in water after transport. Plasma cortisol in salinities of 0.5 to 8 psu, blood glucose and blood ammonia in salinities ranging from 1 to 4 psu as well as plasma sodium and plasma chloride in salinity ranging from 1 to 8 psu were similar before and after transport. This study recommends salinity ranges of 1 to 4 psu for minimizing the physiological effects associated with both the primary and secondary physiological response induced by transport stress in juvenile *L. victorianus*.

1. Introduction

Labeines are economically important food fish throughout the African continent (Reid, 1985). *Labeo victorianus* (Boulenger 1901) is endemic to Lake Victoria basin (Greenwood, 1966). The fish is a potamodromic species which migrates from the lake up rivers to spawn (Cadwalladr, 1965a). The fish was once one of the most important and highly priced commercial fish in the 1930s to mid 1950s around the Lake Victoria region (Cadwalladr, 1965b; Ochumba and Manyala, 1992). The introduction of gill nets, set at the river mouths during spawning migrations, led to the rapid decline of the species since the 1950s (Ogutu-Ohwayo, 1990; Rutaisire, 2003; Seehausen, 1996) from which the stocks are yet to recover. Plans to introduce *L. victorianus* as an aquaculture species in Africa to reduce pressure on and encourage recovery of wild stocks as well as improve aquaculture production is underway and was spearheaded by the World Bank sponsorship of the Lake Victoria Environmental Management Project (LVEMP) since 1999. Broodstocks are obtained from the wild during breeding seasons, which coincides with the long rainy seasons normally between April to July and induced to breed before the fingerlings are transported to the farmers for stocking purposes.

The transport process may induce disturbances, which can cause physiological responses in fish (Gomes et al., 2006). The disturbance process during transportation activates the pituitary–interrenal axis in the neuro-endocrine system resulting in release of hormones cortisol and catecholamines as primary stress response (Barton, 2000). Persistent disturbance results in secondary response in fish, which can be detected by alterations of blood glucose and electrolytes (sodium Na^+ and chlorides Cl^- ions) as well as increase in the blood ammonia levels (Carmichael et al., 1983). Extreme disturbances induce a tertiary response leading to exhaustion and may cause fish death (Urbinati et al., 2003).

The use of salt (sodium chloride) has continued to gain acceptance as fish transport additive to minimize stress (Carneiro and Urbinati, 2001; Carneiro et al., 2007; Gomes et al., 2003; Gomes et al., 2006). The addition of salt reduces the osmotic gradient between the fish internal fluids and external water, stimulates mucous production, reduces fish agitation in the transport units and reduces stress during transportation (Francis-Floyd, 1995; Tsuzuki et al., 2001). However, the concentration of salt to apply in water during transport remains

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highly debatable since most of the recommendations are based on mortality responses of fish. Yet lack of mortality does not imply absence of stress. To complicate matters, the general identification of stress in any fish under aquaculture, field, or laboratory conditions is difficult as the stress responses have been reported to vary with the nature and application rate of the stress stimulus and the species of fish (Barton 2000; Vijayan and Moon 1994; Waring et al., 1992).

Knowledge of physiological response during transport in salt water is necessary to provide aquaculturists with vital information necessary to mitigate stress before death of fish occurs. Lack of such understanding has hindered the protocols for fish seed distribution. Our knowledge also continues to languish on the physiological response of potamodromic fish during transport under varying salinity treatments. The aim of the current study was to determine the survival and physiological response of *Labeo victorianus* juveniles under different salinities after 6 h of transport.

2. Materials and methods

2.1. Experimental procedure

The experiment was performed in between May and December 2009 at Moi University (0°34′13.8″N and 35°18′49.8″E), Kenya. Mature *L. victorianus* were collected from River Mara in Kenya during the breeding season between May to July and reared at Moi University Aquaculture Research Farm hatchery and nursery ponds. Larvae were obtained through induced breeding and semi-natural spawning. The larvae were then cultured for a period of 42 day. A total of 14,500 *L. victorianus* juveniles (mean weight = 8.0 ± 1.1 g) were netted from the nursery ponds and transferred to 24 fiberglass (600 each) open flow-through tanks of capacity 200 L (density = 3 juvenile/L) containing tap water that had been stocked in an intermediate holding tank for at least 48 h to remove chlorine and well aerated by means of electric pump with air stone diffusers. The fish were held in the tanks for a period of six days. No mortality was reported during this initial acclimation period. Apart from three tanks designated as control, 0.25 and 0.5 psu, salinity in all the other tanks was increased by adding 1 g/L salt (NaCl, food grade evaporated granulated salt) after every 6 h to the tanks until the desired salinity values of 1, 2, 4, 8 and 10 psu respectively were obtained. To make 0.25 and 0.5 psu, 0.25 and 0.5 g of NaCl was added per liter of water in the tank. Designated salinities were measured using salinometer (Model IC/SB-1 Salinity Cell). After overnight aeration, salinity was again verified. Osmolality and ions data of these waters are provided in Table 1. Fish were maintained in the acclimation tanks at different salinities for 14 days before transport. Fish were fed commercial diets purchased from Raanan Fish Feed Industries Limited, Israel twice a day at 4% body weight. Feeding was stopped 16 h before transport. To each of the 80 L polythene bags, 50 L of water containing designated salinities (prepared by dissolving designated NaCl in water and verified by salinometer) was added. Each of the eight salinity treatments was executed in triplicate. To each air bag, 100 juvenile *L. victorianus* (stocking biomass = 16 kg/m^3) were carefully transferred from holding water of the same salinity. Medical grade oxygen was then bubbled through the water using oxygen meter to 100% saturation at the local temperature corresponding to 12.2 mg/L. The bags were then sealed. Prior to sealing the bags, water was drawn from the bag using plastic bottles for determination of initial water quality parameters before transport. Further, 10 fish from each transport bag were sampled for determination of pre-transport physiological parameters. The bags were then placed in Styrofoam boxes to provide insulation in the transport truck. The transport truck was lined with insulated materials and fitted with air conditioner to maintain temperature to near room temperature (25 °C). The packed fish were transported for six hours to a designated aquaculture farm (mean speed = 50 ± 10 km/h; 10 min interval record on speedometer) by road to achieve practical transport conditions. Before and at the end of the transport period, temperature, dissolved oxygen and pH were measured using a calibrated JENWAY 3405 electrochemical analyzer (Barloword Scientific Ltd, Essex UK), with independent probes for each variable. Concentration of total ammonia nitrogen (TAN) and carbon dioxide (CO_2) were determined using standard analytical procedures (APHA, 1998).

2.2. Sample preparation and estimation of parameters

At the end of transport period, the bags were opened and 10 fish from each bag were randomly captured with dip nets and quickly anesthetized with benzocaine (5 mg/L) for 2–3 min. Blood was withdrawn from the caudal vein of each sampled fish into 1 mL heparinized insulin syringe. Blood glucose was measured according to King and Garner (1947). Heparinized blood was centrifuged at 3000 r.p.m. for 10 min and plasma frozen at −196 °C in liquid nitrogen for further plasma cortisol (Radioimmunoassay with a Coat-to-Count Kit, Diagnostic Products Corporation, Los Angeles, CA, USA), chloride (SIGMA kit 461, Sigma Diagnostics, USA), blood ammonia (Nessler's method modified by Gentzkow and Masen, 1942) and plasma sodium (flame photometry) analyses. Capillary tubes with blood samples were centrifuged to separate cells from plasma. Plasma osmolality was then determined using Wescor 5520 vapor pressure osmometer. Survival was determined per bag by counting the number of dead fish and subtracting the number from the live juveniles.

2.3. Statistical analysis

Since survival or death is a binary random variable, and it is natural to assume that this follows a binomial distribution. Therefore identification of both the prophylactic and toxic ranges of concentration requires a statistical model that can identify a non-linear response to the concentrations. We fitted a logistic model on the data (Agresti, 1990). We used the logit model, $Log\left[\frac{\rho}{1-\rho}\right] = \beta_0 + \beta_1 C + \beta_2 C^2 + \beta_3 C^3$; where ρ denotes the probability of survival, β_0 is the intercept, β_1 is the coefficient of concentration C, β_2 is the coefficient of quadratic response in C and β_3 is the coefficient of cubic response in C. The study design contained eight levels of primary predictor variables (sodium chloride concentration). Independent sample *t*-test was used to analyze changes in physiological parameters before and after transport while paired

Table 1
Ions and osmolality measured in the sample of the control and experimental waters prepared (n = 8 × 3 replicates).

Water salinity (psu)	Na^+ (mmol/L)	Cl^- (mmol/L)	K^+ (mmol/L)	Mg^{2+} (mmol/L)	Osmolality (mOsm/kgH_2O)
0 (0 g/L NaCl)	0.020	0.011	0.110	0.351	2.61
0.25 (0.25 g/L NaCl)	4.072	3.997	0.112	0.354	7.87
0.5 (0.5 g/L NaCl)	8.193	8.084	0.115	0.356	17.98
1 (1 g/L NaCl)	17.327	17.786	0.119	0.362	36.32
2 (2 g/L NaCl)	34.655	35.935	0.124	0.367	72.61
4 (4 g/L NaCl)	69.311	72.596	0.128	0.588	145.22
8 (8 g/L NaCl)	138.621	157.76	0.133	0.803	290.50
10 (10 g/L NaCl)	173.276	199.71	0.135	0.943	363.11

t-test was used to analyze changes in water quality variables before and after transport in each of the designated salinity treatments. All data on water quality and physiological parameters measured in fish were analyzed by one-way ANOVA: the factor "salinity" had eight levels and was followed by Tukey's post hoc test to localize significant differences. Trends of the biological parameters were fitted using non-linear response models with measured biological parameters being the response variables and sodium chloride concentration the predictor variables. The inter-relationships between salinity, water quality parameters, fish survival and physiological response (blood parameters) were analyzed by Pearson's correlation. Significance of the correlation coefficient was determined by Student Neuman Keuls (SNK) test. Significance was declared at $p \leq 0.05$ unless otherwise stated.

3. Results

Changes in water quality parameters during transport are provided in Table 2. Water temperature was similar in all treatment before and after transport ($p > 0.05$). Dissolved oxygen concentration and pH were significantly ($p < 0.05$) lower in water after transport than pre-transport levels in all the treatments. However, concentration of TAN and CO_2 levels after transport were significantly ($p < 0.05$) higher than the pre-transport levels. After 6 h of transport DO was significantly ($p < 0.05$) higher in transport water having salinity ranging from 1 to 4 psu than in all the other treatments. The highest DO in bags after transport was recorded in 10 psu water salinity. Although pH varied among salinity treatments, the variability was not statistically significant ($p > 0.05$). Concentration of TAN and CO_2 were generally reduced at higher salinity levels.

The survival response model and parameter statistics of juvenile *L. victorianus* in different salinity treatments are given in Fig. 1. Survival response of *L. victorianus* fingerlings after 6 h transport was dependant on water salinities (ANOVA; $p < 0.05$). Lowest survival occurred in juvenile *L. victorianus* transported in 10 psu water salinity (39%), followed by control (52.4%) although transport in salinities 0.25 and 0.5 psu resulted in survival of less than 70%. However, no mortality was reported in juvenile *L. victorianus* transported in 1 to 4 psu water salinity.

Physiological responses in fish before and after transport are provided in Fig. 2. After 6 h transport, plasma cortisol was significantly ($p < 0.05$) higher in fish transported in 0 and 0.25 psu water salinity while at 0.5 to 8 psu water salinities, there was no significant changes in plasma cortisol when compared to levels before transport. Higher plasma cortisol was also established in transport water of salinity 10 psu. Blood glucose levels increased significantly in fish groups in salinities ranging from 0 to 0.5 psu as well as at salinities of 8 and 10 psu but not in salinities of 1 to 4 psu. Plasma sodium and chlorides were lower in the control and in water of salinities 0.25 and 0.5 psu but increased in salinity 10 psu after transport; concentrations of plasma sodium and chloride were similar in juveniles transported in salinities ranging from 1 to 8 psu. Fish in all the treatments produced higher blood ammonia levels after transport, although fish transported in water with 1 to 4 psu salinity presented no significant ($p > 0.05$) alteration in blood ammonia. Plasma osmolality was higher in fish transported at salinities 0, 0.25 and 0.5 psu but increased in fish transported at salinity 10 psu.

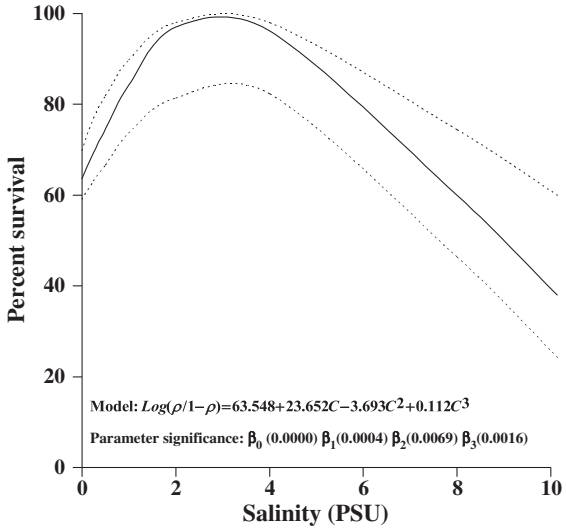

Fig. 1. Predicted modeled percent survival response of *Labeo victorianus* juveniles (solid) and 95% confidence intervals (dashed) from the logistic regression model after 6 h transport under salinity treatments (For each treatment $n = 8 \times 3$ replicates). The survival response model and parameter statics of the values used in the model are also provided.

We also correlated fish survival and blood parameters under different salinities with water quality parameters (Table 3). In the transport water, increased salinities had a positive correlation with fish survival, plasma sodium and plasma chlorides but clearly resulted in a reduction in all other parameters. Fish survival was reduced due to increase in TAN, CO_2 and blood ammonia levels. Concentration of TAN was positively correlated to CO_2 and negatively to DO; CO_2 was negatively correlated to pH and DO while pH was positively correlated to DO. Increase in TAN in water resulted in increased plasma cortisol, blood glucose and blood ammonia but a reduction in the concentration of plasma chloride. CO_2 was positively correlated to blood glucose and blood ammonia. Water pH correlated positively with plasma cortisol, blood glucose and blood ammonia and negatively with plasma sodium. Oxygen content in water was negatively correlated to plasma cortisol, blood glucose and blood ammonia.

4. Discussion

Most freshwater fish have their body fluids hyper-osmotic (osmolality of 260–330 mOsm/kgH_2O) with respect to their external medium and may experience osmoregulatory disturbances in freshwater if external disturbances are introduced. In the current study, up to 48% mortality in juvenile *L. victorianus* was recorded in water of salinity 0 psu after transport, suggesting presence of osmoregulatory disturbances as a

Table 2
Water quality changes in the fish during transport in bags under salinity treatments ($n = 8 \times 3$ replicates).

Variable	Before transport	After 6 h transport							
		0 psu	0.25 psu	0.5 psu	1 psu	2 psu	4 psu	8 psu	10 psu
Temperature (°C)	24.1 ± 2.1	24.1 ± 2.7	24.1 ± 2.1	23.1 ± 2.7	24.3 ± 3.1	23.6 ± 2.8	23.9 ± 2.8	23.5 ± 2.8	23.5 ± 2.2
DO (mg/L)	12.1 ± 0.1^{e}	3.1 ± 0.3^{a}	3.0 ± 0.3^{a}	3.5 ± 0.4^{b}	3.7 ± 0.4^{c}	3.9 ± 0.3^{c}	3.9 ± 0.2^{c}	3.0 ± 0.4^{a}	5.1 ± 0.3^{d}
pH	7.1 ± 0.3^{b}	6.1 ± 0.5^{a}	6.2 ± 0.3^{a}	6.4 ± 0.5^{a}	6.4 ± 0.2^{a}	6.4 ± 0.3^{a}	6.2 ± 0.4^{a}	5.8 ± 0.2^{a}	5.8 ± 0.2^{a}
TAN (mg/L)	0.3 ± 0.2^{a}	15.4 ± 4.7^{d}	16.1 ± 3.2^{d}	14.2 ± 3.7^{d}	13.2 ± 1.8^{d}	7.23 ± 2.1^{c}	8.17 ± 1.5^{c}	7.3 ± 1.1^{c}	4.4 ± 2.0^{b}
CO_2 (ppm)	2.4 ± 0.1^{a}	10.3 ± 1.3^{d}	10.9 ± 1.6^{d}	9.2 ± 2.4^{d}	7.3 ± 1.6^{c}	6.9 ± 1.3^{c}	5.2 ± 1.3^{b}	4.6 ± 1.3^{b}	3.1 ± 1.1^{a}

Means with the same letters as superscripts are not significantly different ($p > 0.05$).
SE: standard error, calculated from the mean-square for error of the ANOVA.
TAN = total ammonia nitrogen.

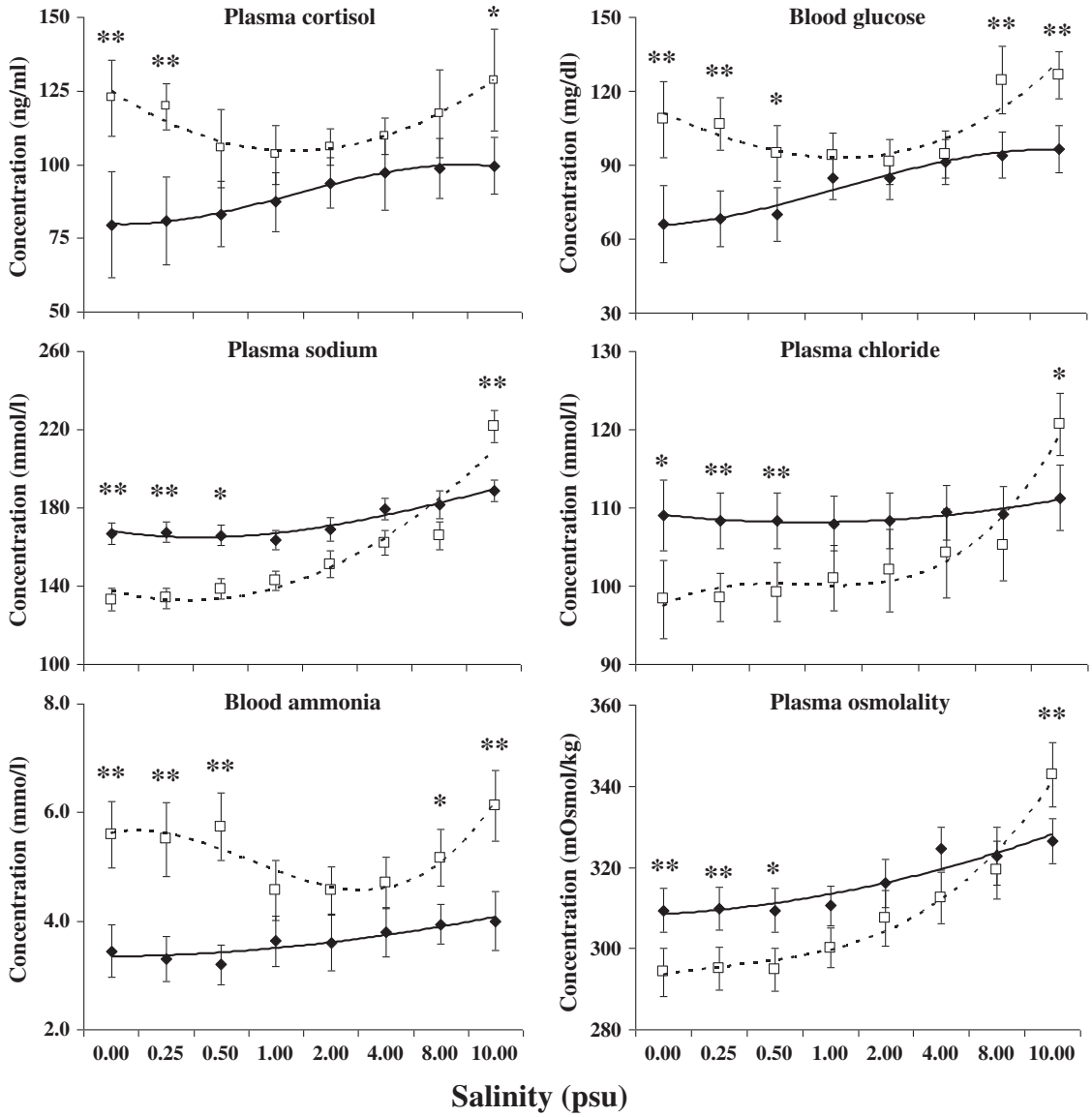

Fig. 2. Mean (±SEM) plasma cortisol, blood glucose, plasma sodium, plasma chloride and blood ammonia levels in *Labeo victorianus* fingerlings transported under various salinity. Black diamonds indicate the concentration before transport while the white squares indicate levels of the measured parameters after transport (for each treatment before and after transport, n = 8 × 3 replicates; * denotes that the treatments are significantly different before and after transport at $p<0.05$ while ** denotes that the treatments are significantly different before and after transport at $p<0.01$).

result of transport disturbance. Previously, this was reported to cause fish mortality due to stress (Breves et al., 2010). Mortalities of 31.4%, 29.8% in transport water of salinities 0.25 psu and 0.5 psu respectively suggested presence of osmoregulatory disturbance, which however reduced with increasing salinity up to 4 psu, which is lower than that reported for channel catfish, *Ictalurus punctatus* (Wurt, 1995). Absence

Table 3
Pearson's correlation coefficients observed between water salinity, fish survival, water quality parameters and physiological parameters measured in fish blood (**Correlation is significant at $p \leq 0.01$; *Correlation is significant at $p \leq 0.05$).

Parameters	Water salinity (psu)	Fish survival	TAN (mg/L)	CO_2 (ppm)	pH	DO (mg/L)
Salinity (psu)	1					
Fish survival	0.61*	1				
[a]TAN (mg/L)	−0.93**	−0.75**	1			
CO_2 (ppm)	−0.92**	−0.84**	0.88**	1		
pH	−0.82**	−0.28	0.33	−0.70*	1	
[b]DO (mg/L)	0.46*	0.22	−0.92**	−0.77**	0.56*	1
Plasma cortisol (ng/mL)	−0.76**	−0.11	0.54*	0.32	0.61*	−0.44*
Blood glucose (mg/dL)	−0.83**	0.18	0.61*	0.92**	0.68**	0.37*
Plasma sodium (mmol/L)	0.85**	0.38*	−0.32	−0.14	−0.48*	0.43*
Plasma chlorides (mmol/L)	0.53*	0.23	−0.53*	0.21	−0.22	0.22
Blood ammonia (mmol/L)	−0.87**	−0.45*	0.63*	0.44*	0.76**	−0.34*

[a] TAN: total ammonia nitrogen.
[b] DO: dissolved oxygen.

of any mortality in juvenile *L. victorianus* transported in water of salinities ranging from 1 to 4 psu are consistent with results for other freshwater fish species such as in anadromous stripped bass, *Morone saxatilis* transported in freshwater, which can adapt to salinity changes in its environment (Grizzle et al., 1992). However, they were inconsistent with results obtained for silver catfish, *Rhamdia quelen* (Gomes et al., 1999) and tambaqui, *Colossoma macropomum* (Gomes et al., 2002) transported in water of salinity <1 psu. This could probably be due to fish being iso-osmotic with the transport media and therefore minimized passive loss or gains of ions resulting to minimal stress. Highest mortality of up to 60% was recorded in water of salinity 10 psu, which could be due to increased ionoregulatory disturbances as a result of increasing water up to the osmolality beyond the salinity adaptation threshold of the fish in presence of transport disturbance.

After transport in 0 psu water salinity, plasma cortisol was higher than pre-transport levels, similar to findings by several workers (Barton and Peter, 1982; Carneiro and Urbinati 2001; Iversen et al., 1998). The high plasma cortisol could therefore be due to mobilization of energy for osmoregulatory purposes. However under 0.5 to 8 psu water salinity, plasma cortisol levels, in fish did not change significantly as compared to the control probably due to the low energy costs in osmotic and ionic regulation that prevent excessive mobilization of energy stores in the blood (Wedemeyer, 1972). The findings however differ from those obtained by Gomes et al. (2006) in tambaqui (*Colossoma macropomum*) who established that after transport disturbance, cortisol levels in salinity treatments of 1 psu increased tremendously.

Transport in water of salinities of 0, 0.25 and 0.5 psu resulted in increased blood glucose levels of the juvenile *L. victorianus* exhibiting similar trends as smallmouth bass, *Micropterus dolomieui* (Carmichael et al., 1983) and *Arapaima gigas* (Gomes et al., 2006). In fish, blood glucose is intermediated by catecholamines that stimulate glycogenolysis in the liver (Mommsen et al., 1999). Transporting fish in salinity ranging from 1 to 4 psu maintained the blood glucose levels to that of the pre-transport levels suggesting that transport under such ranges of salinities does not affect cortisol production. However, salinities of 8 and 10 psu appear to increase physiological activities that resulted in excessive energy production.

Transport in salinities 0, 0.25 and 0.5 psu resulted in reduced plasma sodium and plasma chlorides than the amount physiologically required by the fish probably due to passive water gains resulting in loss of the Na^+ and Cl^- ions similar to mouth bass, *M. dolomieui*, (Carmichael et al., 1983) and hybrid striped bass, *Morone chrysops* (Tomasso et al., 1980) after transport at 0 psu salinity. Significant increase in plasma sodium and plasma chlorides of fish transported in water of salinity 10 psu could be due to gains of Na^+ and Cl^-. The increase in the plasma sodium and plasma chloride is a possible indicator of impairment in ionic (both Na^+ and Cl^-) regulation of fish when transported in water above the fish osmolality.

In water of salinities below or above 1 to 4 psu, blood ammonia levels (measured as TAN) in fish were higher after transport. The high TAN after transport suggests an increased physiological activity of the fish during osmoregulation. The accumulation of ammonia nitrogen due to increased metabolites usually observed in transport water may cause serious problems to the fish, such as increased oxygen consumption and heart rate, decreased plasma sodium and alteration of the acid–base balance (El-Shafey, 1998; Tomasso, 1994). In spite of diffusion of unionized ammonia being the preferential mechanism of ammonia excretion in teleosts, there is a branchial exchange system between NH_4^+ and Na^+ ions that may become more important in an environment with Na^+ ions (Tomasso 1994).

Increased salinity correlated negatively with TAN, CO_2, pH, plasma cortisol, blood glucose and blood ammonia suggesting a possible reduction of fish metabolism. Reduced metabolism could result in a potential decrease in the amounts of unionized ammonia produced by fish thus ameliorating ammonia toxicity. Although our calculation indicated very minimal reduction of un-ionized ammonia with increased salinity, such reductions are beneficial for the juvenile fish to cope up with ammonia toxicity.

In conclusion, transporting juvenile *L. victorianus* did not result in any mortality in salinity ranging from 1 to 4 psu. In salinities 0, 0.25 and 0.5 psu survival of the fish under transport was below 70% while up to 60% mortality occurred in salinity 10 psu. It appears that salinity ranging from 0.5 to 8 psu mitigated production of cortisol as a precursor to primary stress response while 1 to 4 psu water salinities were found suitable for maintaining most of the blood parameters associated with secondary stress response to levels before transport in *L. victorianus*. The present study provides data which should be beneficial for the future transport of this potamodromic fish species. We recommend further studies on the physiological response of this species of fish for periods of transport longer than the current 6 h.

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