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Captive Breeding of Threatened African Carp, *Labeo victorianus*, of Lake Victoria

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Abstract

African carp, *Labeo victorianus*, is one of the threatened endemic fishes of Lake Victoria which requires conservation and has potential for aquaculture. We conducted a series of experiments on *L. victorianus* aiming at producing juveniles for both human consumption and stock enhancement. A total of 79 broodstock (mean body weight = 227.06 g) were collected from the wild; packed at 200, 300, and 500 g/L; and transported by road for 7 h. All fish survived during transportation whereas dissolved oxygen decreased and total ammonia nitrogen increased significantly after transport. Females produced 46,702–86,712 eggs (mean = 68,189 ± 2397) and fecundity was highly correlated with the fish body size ($R^2 = 0.96$). Only males re-spawned in captivity while females did not. At 28 C, females spawned 11–12 h after pairing with males. Larvae can either be fed with formulated diet or natural zooplankton. Juveniles (mean length = 36.7 mm; mean body weight = 0.46 g) can be packed as high as 60 fish/L and transported by road for 12 h with low mortality (2.7–10.3%). These results show that captive breeding of *L. victorianus* can be a good strategy to produce juveniles to boost wild population as well as a source of seeds for culture.

Labeo victorianus is the only labine species (a distinct subfamily of ray-finned fish of Cyprinidae) in Lake Victoria and its catchment. This species displays a potamodromous behavior, thus, moving up to rivers to spawn (Kibaara 1981). Before the 1950s, *L. victorianus* supported a commercial fishery in Lake Victoria, yet, in the late 1950s, its population rapidly declined and the fishery collapsed (Cadwalladr 1965; Ogutu-Ohwayo 1990). Predation by the introduced Nile perch, *Lates niloticus*, competition for the same food resources with other fishes such as Nile tilapia, *Oreochromis niloticus*, environmental pollution, illegal fishing methods, and overfishing were cited as the main causes of disappearance of *L. victorianus* from Lake Victoria (Cadwalladr 1965; Ogutu-Ohwayo 1990; Greboval and Mannini 1992). Currently, *L. victorianus* is on the International Union for Conservation of Nature (IUCN) red list of endangered species of Lake Victoria; therefore, there is an urgent need to preserve the remaining fishes and restock wild population.

In Kenya, government efforts spearheaded by the Kenya Marine Fisheries Research Institute (KMFRI) with the support of Kenyan public universities attempted to breed *L. victorianus* in captivity in order to revive the wild population (Orina et al. 2014). Aside from a captive breeding study, feeding and culture experiments were also conducted, which showed that *L. victorianus* can spawn in captivity (Orina et al. 2014),

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have fast growth rate, can readily accept artificial diets (Magondu et al. 2013; Mokoro et al. 2014) and thus have high aquaculture potential. However, all of these studies are just experimental or small scale and no fish farmer has considered the culture of *L. victorianus* on a larger scale because of lack of comprehensive knowledge of its biology and culture.

Captive breeding has been adopted as one of the global strategies by which economically important and endangered species (including fish) can be effectively managed and restored. Breeding in captivity involves transportation of wild fishes as well as the fingerlings produced in the hatcheries to the culture facilities, managing of broodstock, and understanding its breeding and feeding behavior (De Silva and Anderson 1995; Mylonas et al. 2010). Many of the deficiencies and problems encountered during the early stages of domestication are directly related to broodstock management, nutrition, and feeding which directly affect both reproduction in captivity and the ability to produce high-quality larvae (Izquierdo et al. 2001; Teletchea and Fontaine 2014). Furthermore, successful larval rearing as well as considering the quality and vulnerability of larvae to stress is paramount to the success of a captive breeding program (Rhody et al. 2014).

So far, few studies have been undertaken on *L. victorianus* in Kenya, and only one paper dealt with its captive breeding. Results of this study can be used by researchers and farmers alike who intend to explore the potential of this species for stock enhancement or culture purposes.

Materials and Methods

Transport of Wild Broodstock

A total of 79 wild broodstock (body weight = 97.41–720.11 g; mean = 227.06 g) were caught from the Mara river (1°03′00″S, 35°14′00″E) using an electrofisher and kept in a cage (set within the river) overnight to empty their stomachs. The next day, they were packed in 15-L polythene bags filled with 3 L of river water at 200, 300, and 500 g fish/L. Sodium chloride (NaCl) was added at 2 g/L to reduce ammonia toxicity as recommended by Oyoo-Okoth et al. (2011). The bags were aerated with medical grade oxygen using an oxygen meter to 100% saturation. Prior to sealing the bags, pH, dissolved oxygen (DO), and temperature were measured using a YSI multi-parameter meter (Probe 2030), and some water was drawn using a plastic bottle and stored in ice for total ammonia nitrogen (TAN) analysis.

Three bags were prepared for each packing density; each bag contained 4–10 fish. The bags were packed into an insulated plastic container and loaded inside an air-conditioned car. The fish were transported by road for 7 h to the KMFRI, Sangoro Station (altitude 1230 m, latitude 0°39′S and longitude 37°12′E), which is about 240 km away, at an average speed of 70 km/h with intermittent stops. Upon arrival, pH, DO, and temperature in each bag were determined and water samples for TAN analysis were collected and stored on ice for analysis. TAN was analyzed in the laboratory using standard methods (AOAC 2000).

When the fish reached the laboratory, each bag was stocked in a tank containing water of 2 psu (Practical Salinity Unit) and opened after the fish acclimatized to the outside water temperature. Mortality was determined by counting the number of dead fish in each tank. Mortality in each tank was monitored for 5 d and was then added to the mortality immediately after transport to obtain the cumulative mortality. A day after transportation, the fish were provided with a KMFRI-formulated diet for tilapia containing 30% crude protein (Munguti et al. 2014) at 3% of their average body weight.

Broodstock Management and Spawning

L. victorianus broodstock were sexed and assessed for maturity according to Orina et al. (2014). Mature males produce whitish milt, whereas females produce greenish oocytes. Each mature female was induced to spawn by injecting Ovaprim at 0.2 mL/kg of fish following Orina et al. (2014). After injection, one female was paired with two mature males in a glass tank with controlled thermostat (28 ± 1 C). The number of

spawned eggs was counted and correlated to the size of fish. Fecundity was calculated by multiplying the average weight of one egg against the weight lost after spawning (Orina et al. 2014). After 12 h of spawning, broodstock was removed from the aquaria.

After fecundity of females was assessed, they were weighed again and reared in either a net cage ($1.0 \times 1.0 \times 1.0$ m) suspended into a pond or a concrete raceway ($3.0 \times 0.35 \times 0.35$ m). A total of nine cages were prepared; each was stocked with two fish (one male and one female). A total of 18 fish (nine males and nine females) were tagged and stocked into the raceway. The raceway was supplied with pond water at a rate of 20 L/h. Fish in both cages and raceway were fed daily with a KMFRI-formulated diet for tilapia containing 30% crude protein at 3% body weight. Daily ration was supplied at 0900 and 1500 h. The maturity stage of each fish was monitored once a week by applying gentle pressure on the belly of the fish. Monitoring was done for 10 mo (October 2014 to July 2015).

Incubation and Larval Rearing

Spawned eggs (from the above experiment) were placed in a glass tank with a thermostat heater set at 28 C. Larvae were monitored daily until they absorbed their yolk and started to feed. Green water (containing various species of phytoplankton and zooplankton) were collected from a fishpond, sieved in a 250 µm plankton net, and the supernatant was added into the rearing tank. After 20 d of hatching, larvae were stocked at a density of 20 larvae/L in glass aquaria containing 50 L of rain water (1000 larvae/aquaria). Nine aquaria were prepared; three each were provided with: (1) Japanese commercial diet containing 56% crude protein (Hayashikane Sangyo Co., Ltd), (2) KMFRI-formulated diet for tilapia containing 30% crude protein (Munguti et al. 2014), and (3) natural zooplankton (mainly rotifers, copepods, and cladocerans) collected from a fishpond. The aquaria were placed in a completely randomized design. Feeding experiment was conducted for 45 d. Larvae in treatments 1 and 2 were fed *ad libitum* three times a day (at 0800, 1200, and 1600 h), while larvae in treatment 3 were fed at a density of 3–10 individuals/mL of mixed zooplankton collected from fishponds using a 50 µm plankton net.

Growth rate was monitored every 15 d by taking 15–20 larvae in each tank. Larvae were first held in a freezer for 20 min (to kill the larvae in extended form) before measuring the length under a stereomicroscope (Motic SMZ-168 series) and body weight to the nearest 0.001 g (Metler Toledo AG204). The larvae were subsequently preserved in 4% buffered formalin. Survival was determined on day 45, by counting the live larvae in each aquarium.

Transport of Fingerlings

Hatchery-bred juveniles (mean total length [TL] = 36.7 mm; mean body weight = 0.46 g) produced in KMFRI Sagana Station (altitude 1230 m, latitude 0°39′S and longitude 37°12′E) were used in this experiment. A day before packing, the fish were stocked in a net cage with running water without feeding. Early in the morning, the fish were packed in 15-L polythene bags filled with 3 L of water at 20, 40, and 60 fish/L. All other procedures including addition of NaCl, packing, and water samplings were the same as those for transporting broodstock.

Three bags were prepared for each packing density. The bags were packed into an insulated plastic container and loaded inside an air-conditioned car. The fish were transported by road for 12 h (from KMFRI, Sagana Station KMFRI to KMFRI Sangoro Station, altitude 1134 m, latitude 0°20′N and longitude 31°53′E, which is about 800 km away) at an average speed of 60 km/h with intermittent stops. Water parameters including pH, DO, temperature, and TAN were measured before and after transport as described in the transport of broodstock experiment.

Statistical Analysis

Data were subjected to ANOVA followed by Tukey–Kramer post hoc test when significant at $P < 0.05$. All percentage data were normalized by arcsine transformation prior to statistical analysis (R-Project for Statistical Computing).

TABLE 1. *Survival and mortality rates of* Labeo victorianus *broodstock after 7-h transport (*N = *3).*

Stocking density (g/L)	Mortality on arrival (%)	Mortality in the tank After 5 d (%)	Survival After 5 d (%)
200	0	0	100
300	0	0	100
500	0	0	100

Regression analysis was used to test the relationship between the fecundity and length of female broodstock.

Results

Transport of Broodstock

After 7 h of transport, no mortality was recorded at all packing densities, nor after stocking fish in tanks for 5 d (Table 1). Changes in the water quality (TAN, pH, temperature, and DO) during transport are shown in Table 2. TAN in all packing densities significantly increased after 7 h of transport, while DO significantly decreased. pH remained stable, while temperature increased from 22 to 25 C. Only the temperature at the highest density (500 g/L) was significantly higher after 7 h of transport. All water quality parameters in all packing densities were not significantly different from each other.

Broodstock Management

Male broodstock in cages and raceway rematured every month after stripping of milt, while none of the females respawn after spawning. Around 90% of the mature females that were induced with ovaprim spawned within 11–12 h after injection and pairing with males. Females (TL = 27.23 to 39.45 cm) released eggs ranging from 46,702 to 86,712 (mean = 68,189 ± 2397). Fecundity increased with the increase in length of fish ($R^2 = 0.96$; Fig. 1). Fertilization rate ranged from 92 to 95% (mean = 93 ± 4%), while hatching rate ranged from 94 to 96% (mean = 95 ± 1%).

Incubation and Larval Rearing

Hatching of the spawned eggs in the glass tanks was observed within 10–12 h with a mean of 11 ± 0.6 h. For the larval rearing experiment, larvae fed Japanese formulated diet had the highest weight gain, followed by KMFRI-formulated diet and those fed zooplankton had the lowest ($P < 0.05$; Table 3). Larvae fed zooplankton had the highest survival (51%), followed by Japan-formulated diet (49%) and KMFRI-formulated diet (46%). There was no significant difference in survival of larvae fed on the three diets (ANOVA, $P > 0.05$). However, there was a significant difference among the final mean length and final average weight of larvae fed on the three different feeds (ANOVA, $P < 0.05$).

Transport of Fingerlings

After 12 h of transport, 2.7–10.3% mortality rates were recorded (Table 4). Survival rates ranged from 88.9 to 93.9% with no significant difference among packing densities.

TAN in all packing densities significantly increased ($P < 0.01$) after 12 h of transport, while DO significantly decreased ($P < 0.01$; Table 5). pH slightly decreased, while temperature

TABLE 2. *Water quality changes in the transport bags containing* Labeo victorianus *broodstock (mean body weight = 227.06 g) during transport.*[1]

		After transport (7 h)		
Parameter	Before transport	200 g/L	300 g/L	500 g/L
Temperature (C)	22.2 ± 0.3^a	24.2 ± 0.3^a	24.2 ± 0.3^a	25.0 ± 0.0^b
DO (mg/L)	12.00 ± 0.40^a	5.13 ± 0.47^b	5.09 ± 0.30^b	4.66 ± 0.32^b
pH	6.93 ± 0.05^a	6.80 ± 0.04^a	6.10 ± 0.28^a	6.84 ± 0.28^a
TAN (mg/L)	0.09 ± 0.02^b	1.29 ± 0.02^a	1.55 ± 0.02^a	3.06 ± 0.04^c

DO = dissolved oxygen; TAN = total ammonia nitrogen.

[1]Data are mean ± SD ($n = 3$). Means with the same letter in the row are not significantly different ($P < 0.05$).

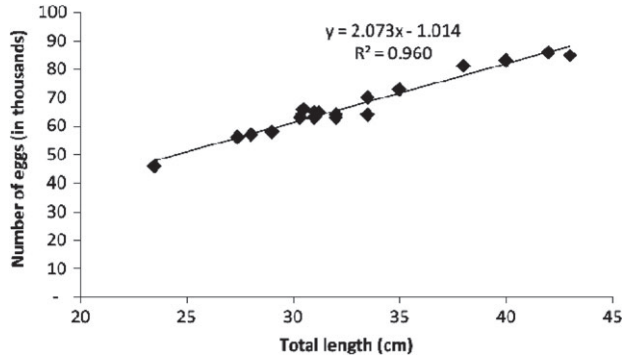

FIGURE 1. *Relationship between the total length of* Labeo victorianus *female broodstock and the number of eggs spawned.* N = *18.*

TABLE 3. *Growth and survival of* Labeo victorianus *larvae fed on different diets.*[1, 2]

Parameter	Japanese-formulated Diet	KMFRI-formulated Diet	Natural Zooplankton
Initial length (cm)	0.43 ± 0.01^a	0.44 ± 0.03^a	0.44 ± 0.02^a
Final length (cm)	3.16 ± 0.05^a	2.96 ± 0.01^a	2.10 ± 0.07^b
Initial weight (mg)	4.03 ± 0.19^a	3.93 ± 0.27^a	4.01 ± 0.67^a
Final weight (mg)	341.06 ± 60.02^a	254.01 ± 42.23^b	119.02 ± 20.03^c
Weight gain (mg)	336.045^a	247.146^b	106.043^c
Survival (%)	49 ± 0.76^a	46 ± 0.81^a	51 ± 0.63^a

KMFRI = Kenya Marine Fisheries Research Institute.

[1]Data are mean ± SD ($n = 3$).

[2]Means with the same letter in the row are not significantly different ($P < 0.05$).

TABLE 4. *Survival and mortality rates of* Labeo victorianus *fingerlings after 12 h transport (*N = *3).*

Stocking density (Fish/L)	Mortality on arrival (%)	Mortality in aquaria After 5 d (%)	Survival After 5 d (%)
20	2.7	3.4	93.9
30	7.5	2.2	90.3
60	10.3	0.8	88.9

increased from 22 to between 25 and 26 C after 12 h of transport. All water quality parameters in all packing densities were not significantly different from each other.

Discussion

Captive breeding programs are extensively applied in advanced countries in Europe and North America for fish species with imminent extinction or those with declining population (Lorenzen et al. 2012). The ultimate goal of such programs is to maintain the genetic diversity and fitness within fish populations (Philippart 1995; Utter and Epifanio 2002; Fraser 2008). This paper describes comprehensive ways to produce *L. victorianus*, an endangered fish of Lake Victoria, in captivity with the hope that it can serve as a basis for future studies on stock enhancement programs for this species not only in Kenya but also in other countries, which share Lake Victoria, as well as for culturists who want to culture this fish for human consumption.

Few studies have been reported on *L. victorianus* despite having been once a favorite fish in riparian communities (Dadebo et al. 2003). Recent publications (e.g., Magondu et al. 2013; Mokoro et al. 2014; Orina et al. 2014) deal with its culture in captivity with the aim of enhancing the wild stocks and potential aquaculture for human consumption.

Most research laboratories, hatcheries, or fish seed production facilities in Kenya are far from fish-rearing ponds or farms; thus, fish have to be transported for long distances (Omasaki et al.

TABLE 5. *Changes in water quality in the transport bags containing* Labeo victorianus *fingerlings (mean total length = 36.7 mm; mean body weight = 0.46 g) during transport.*[1]

Parameter	Before transport	After transport (12 h)		
		20 fish/L	30 fish/L	60 fish/L
Temperature (°C)	22.2 ± 0.5^{a}	25.1 ± 0.3^{a}	26.0 ± 0.5^{a}	25.7 ± 0.6^{a}
DO (mg/L)	12.54 ± 0.40^{a}	4.23 ± 0.49^{b}	4.29 ± 0.30^{b}	3.56 ± 0.31^{b}
pH	7.03 ± 0.05^{a}	6.81 ± 0.04^{a}	6.71 ± 0.28^{a}	6.85 ± 0.25^{a}
TAN (mg/L)	0.05 ± 0.01^{b}	1.75 ± 0.21^{a}	3.08 ± 0.34^{a}	2.93 ± 0.38^{a}

DO = dissolved oxygen; TAN = total ammonia nitrogen.

[1]Data are shown in mean ± SD ($n = 3$). Means with the same letter in the row are not significantly different ($P < 0.05$).

2013). Also, it is usual practice to transport fish using an open tank or polythene bags loaded onto trucks without controlling the temperature. Keeping these usual practices in mind, we developed a cost-effective packaging technology that not only reduces the cost of transport but also reduces the mortality of fish, thus reducing losses associated with transport. The use of anesthetics/sedatives and NaCl (table salt) to lessen mortalities during transport of *L. rohita* and *L. victorianus* was recommended by Hasan and Bart (2007) and Oyoo-Okoth et al. (2011), respectively. While the latter is readily available, the former is quite difficult to obtain by culturists. We, therefore, added table salt during our transport experiment at a concentration recommended by Oyoo-Okoth et al. (2011). Our results showed that *L. victorianus* broodstock and fingerlings can be transported at packing density of up to 500 g/L and 60 fish/L, respectively, for long hours with minimal or no mortalities. Although Oyoo-Okoth et al. (2011) recorded no mortality when they added salt (1–4 psu) during the transport of *L. victorianus* fingerlings (average weight = 8.0 g), their packing density was very low (2 fish/L = 16 g/L) compared to our study; thus, our packing density is more economical. High survival of *L. victorianus* over long-distance transport at high packing densities indicates the fish's ability to quickly adapt to packing condition. This is in contrast to other cyprinid species such as rohu, *L. rohita,* and silver carp, *Hypophthalmichthys molitrix*, which are sensitive to transport stress and whose mortality could be as high as 30 and 14%, respectively, without the use of anesthesia (Hasan and Bart 2007).

In addition, the results of our study showed that the level of TAN after transport significantly increased, while DO significantly decreased, and temperature, although not statistically significant, also increased. It is generally known that temperature is an important environmental factor to be considered in transporting live fish. Cool water generally holds more oxygen compared with warm water and fish consume less oxygen and excrete less toxic metabolites at low temperature due to reduced metabolism (Estudillo and Duray 2003). The temperature was not controlled during this experiment because we wanted to simulate the usual way of transporting fish in Kenya, where lowering of temperature with the use of ice or coolant is not practiced; fish are transported early in the morning or late at night. The fish were also transported in an insulated plastic tank further reducing the effect of temperature. The decrease in DO in the water was due to consumption by fish, while the increase in TAN was attributed to increased metabolic rate resulting from increased physical activity under crowded conditions and the limited supply of DO. Singh et al. (2004) recommended the use of some agents including Zeolite (7 g/L), Tris buffer (0.01 M), and 2-phenoxyethanol (0.09 ml/L) to control the increase of TAN during the transport of Indian carps, *Catla catla*, *L. rohita*, and *Cirrhinus mrigala.* They also recommended the use of Oxyflow at 250–500 mg/L to increase oxygen in the transport bag. In this study, although DO was significantly reduced, it was still within the limit of fish to survive.

The fact that *L. victorianus* can be transported at high packing densities reduces the transport

costs for the farmers or researchers because they can transport many fish at once and with minimal packaging materials.

Spawning season of *L. victorianus* is synchronized with rainfall seasons (Rutaisire and Booth 2005; Orina et al. 2014), and like other potamodromous fishes, they use various environmental cues and internal stimuli for initiating migratory movement prior to spawning. Cadwalladr (1965) indicated that water quality parameters such as conductivity, total suspended solids, and river flow rate were important for the migration of *L. victorianus*. In Uganda, it was reported that the migration of *L. victorianus* upstream to Rivers Kagera and Sio coincided with the onset of rains which occur in two seasons (Rutaisire and Booth 2005). In Kenya, Ochumba and Manyala (1992) observed that the *L. victorianus* migrates upstream to Rivers Mara, Yala, Sondu Miriu, and Migori to spawn. During this study most of the females we obtained during the rainy months (April, May, and September) in Mara River, Kenya, were gravid.

Concurrent to Orina et al. (2014), we were able to induce mature female fish to spawn using Ovaprim. Once spawned, they did not respawn during 10 mo in captivity, whether they were reared in fish pond or raceway. This may be due to the fact that females did not receive the signal for inducement of gonad development. Although we simulated river conditions by rearing some females in a raceway with running water, none of them rematured. Cadwalladr (1965) indicated that food/nutrition, temperature, and other suitable environmental conditions are necessary for the respawning of *L. victorianus* in captivity. In this study, fish were fed a formulated diet containing 30% crude protein as recommended by Magondu et al. (2013) under natural temperature (17–28 C). It is possible that, aside from the water movement, nutrition, and temperature, other environmental and innate factors control the respawning of *L. victorianus*.

L. victorianus is a highly fecund fish producing a mean of $68{,}189 \pm 2397$ eggs per female. The fecundity showed significant and positive correlation with fish TL ($R^2 = 0.96$). This is in agreement with Rutaisire and Booth (2005) on *L. victorianus* from Rivers Sio and Kagera in Uganda. Also, a high mean fertilization rate ($93 \pm 4\%$) and a high hatching rate ($95 \pm 1\%$) were recorded in this study.

Newly hatched *L. victorianus* larvae are transparent, tiny, with a TL = 7.1–9.0 mm, in and needle-like in shape. Hatched larvae successfully survived in filtered fishpond water containing a mixture of small zoo- and phytoplankton. Subsequently, larvae fed on mixed zooplankton had the highest survival, although it had the lowest growth rate compared with those fed on high-protein-content formulated diet. The slow growth rate in mix zooplankton treatment can be attributed to the energy requirements for larvae to chase after live prey or possibly because of the low density of zooplankton (3–10/mL) supplied to the larvae. It is generally known that live prey is an excellent starting food in larviculture of *L. victorianus* (El Moghraby and El Rahman 1984; Owori-Wadunde 2012). The overall larval survival rate (51%) we obtained in this study is lower than those obtained in *L. rohita* larvae (92%) fed on mixed zooplankton (Bakhtiyar et al. 2011). *L. victorianus* might be more sensitive in handling and/or the density of zooplankton given was not enough to the larvae, resulting to higher mortality.

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