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Quality of African Carp Larvae, *Labeo parvus* Boulenger, 1902 (Pisces: Cyprinidae) Obtained in Captivity by Hormonal Induction of the Ovulation

[1, 2]Elie Montchowui, [2]Pamphile Tobada, [3]Florian Dadjo Daga, [2]Hyppolite Agadjihouede, [2]Philippe Laleye and [4]Pascal Poncin
[1]National Higher School of Agricultural Science and Techniques Agronomiques of Ketou,
[2]Laboratory of Hydrobiology and Aquaculture,
University of Abomey-Calavi, 01BP 526 Cotonou, Republic of Benin
[3]Department of Production and Health Animal, Polytechnic School of Abomey-Calavi,
University of Abomey-Calavi, 01BP 2009 Cotonou, Republic of Benin
[4]Behavioural Biology Unity, University of Liege, 22 Quai Van Beneden, 4020 Liege, Belgium

Abstract: The aim of this study was to evaluate the resistance and quality of *Labeo parvus* larvae obtained in captivity by artificial reproduction. The larvae used derive from a stock of larvae obtained by hormonal induction of the ovulation using Ovaprim (0.6 mL kg^{-1} body weight). Larval quality expressed as the time at total mortality and median survival was examined in all crosses under the conditions of osmotic shock (2% NaCl, 26.3°C temperature, 4.4 mg L^{-1} dissolved oxygen, 8.0 pH) and prolonged fasting (25.2°C temperature, 6.5 mg L^{-1} dissolved oxygen, 7.6 pH). Osmotic shock and fasting test were conducted using 30 larvae (9 days old) and 30 embryos, respectively at hatching in two replications per cross. Under stress tests no survival was observed after 30 min for osmotic shock and after 16 days for the prolonged fasting test in *L. parvus* larvae. The median survivals (TL_{50}) were 26.5 min for osmotic shock and 12.5 days for prolonged fasting. There was a statistically significant difference between the median survival of the group exposed for osmotic shock and that the group exposed for prolonged fasting test ($p<0.05$). These results show a great resistance of the larvae to the osmotic shock and prolonged fasting.

Key words: Larval resistance, osmotic shock, fasting test, *Labeo parvus*, prolonged fasting

INTRODUCTION

The Labeines fishes are important ichthyofaunal component of freshwater ecosystems in West Africa. *Labeo parvus* Boulenger, 1902 is one of three common Labeo in Benin waters and the more popular food fish. *L. parvus* is a synchronous spawner (Montchowui *et al*., 2007, 2011a) known to reproduce in river floodplains with unfavourable conditions such as receding water, decreasing oxygen concentrations and rapidly fluctuating temperatures soon after spawning (Albaret, 1982; Montchowui *et al*., 2007). This reproduction strategy includes first a compulsorily whole egg-laying for the majority of fish species and second a migration in group to adjacent flooded areas or in connected water course where they are dependant to rain falls and other environmental conditions (Bowmaker, 1973; Tomasson *et al*., 1984). This strategy makes *L. parvus* particularly vulnerable due to over fishing and environmental condition changes that influence its reproductive strategy (Skelton *et al*., 1991). The increasing intensification of the exploitation of this species by local populations in a permanent increase causes a major risk of demographical collapse. This threat and the high demand of this fish allow to develop its intensive cultured including artificial reproduction and controlled rearing.

Like other cyprinids, the African carp *L. parvus* held in captivity does not spawn easily spontaneously and it is often necessary to use some physical or hormonal treatments to synchronize the final maturation of the oocytes and to induce ovulation. The hormonal induction of the ovulation with Ovaprim (0.6 mL kg^{-1} body weight) was the first technique employed and this technique had given the successful results (Montchowui *et al*., 2011b). According to those researchers this technique is a reliable method to induce breeding in captive *L. parvus*. High fecundity, high fertilisation and hatching rates were obtained with the hormonal induction of the ovulation. The research hypothesis is to know if the *L. parvus* larvae obtained in captivity with this technique are of better quality.

Corresponding Author: Elie Montchowui, Laboratory of Hydrobiology and Aquaculture,
National Higher School of Agricultural Sciences and Techniques of Ketou,
University of Abomey-Calavi, 01BP 526 Cotonou, Republic of Benin

Although, there is no universally accepted method of determining post larvae quality many different criteria have been suggested for the evaluation of post larvae quality (Samocha *et al.*, 1998). According to Kestemont *et al.* (1999) egg and larval quality was assessed by the hatching rate/fertilization rate ratio and the resistance of larvae to stress tests, respectively. Higher survival rates at each developmental stage and when subjected to stress tests can evidence healthier or better quality fish (Samocha *et al.*, 1998; Steer *et al.*, 2004). Nevertheless, quality or survival of larvae can be modified by nutrition and culture conditions. The aim of this study is to evaluate larval resistance to stress tests such as osmotic shocks and prolonged fasting.

MATERIALS AND METHODS

Studies were carried out at the Unit of Training and Research in Fish-farming of Hydrobiology and Aquaculture Laboratory, Benin.

Choice of the method for evaluating *Labeo parvus* larvae quality: Although, there is no universally accepted method of determining post larvae quality many different criteria have been suggested for the evaluation of post larvae quality. Yunker (1989), Bauman and Jamandre (1990), Villalon (1991) and Samocha and Lawrence (1992) report that post larvae activity in the larval rearing tanks, post larvae survival, body pigmentation and muscle development can be used to determine post larvae quality. Other morphological conditions such as integumental fouling, debris accumulation on appendages, body deformities and muscle opaqueness have also been suggested as tools for evaluating post larvae quality (Hirono, 1989; Bauman and Jamandre, 1990; Villalon, 1991; Samocha and Lawrence, 1992). Time needed to complete larval metamorphosis, survival during larval development and post larvae size have been recommended as useful criteria for post larvae quality evaluation (Wilkenfeld *et al.*, 1984; Kuban *et al.*, 1985; Samocha *et al.*, 1989; Smith *et al.*, 1992). Bray and Lawrence (1992a, b) reviewed different tests and standards proposed for seedstock quality evaluation.

Acute stress tests have been described to distinguish between healthy and weak post larvae (Maugle, 1988; Baybay, 1989; Tackaert *et al.*, 1989; Bauman and Jamandre, 1990; Bauman and Scura, 1990; Nietes, 1990; Gomez *et al.*, 1991) and used by commercial hatcheries to evaluate post larvae's hardiness. According to Kestemont *et al.* (1999) egg and larval quality was assessed by the hatching rate/fertilization rate ratio and the resistance of larvae to stress tests, respectively. In the present study, researchers made choice to use the resistance of larvae to stress tests such as osmotic shock and prolonged fasting test for evaluating the quality of *L. parvus* larvae obtained in captivity by hormonal induction of the ovulation.

Fish origin and resistance test: *Labeo parvus* larvae used in this study were obtained by artificial reproduction according the procedure described by Montchowui *et al.* (2011b). Females were hormonally induced to spawn using Ovaprim at 0.6 mL kg^{-1} body weight and stripped 12 h post-injection for egg collection. Males were injected at 0.3 mL kg^{-1} body weight and sperm were obtained by squeezing the testes. Eggs were collected in sterile plastic containers by applying a gentle pressure on the abdomen in a caudal-cranial direction and fertilization was performed. Fertilized eggs were transferred in hatcheries with flowing water (29.7±0.4°C). The hatching was done 11 h after incubation and lasted 5 h (Montchowui *et al.*, 2011b). After hatching, the larvae used in the osmotic shock were fed with Artemia nauplii for 9 days.

Larval quality expressed as the time at total mortality and median survival was examined in all crosses under the conditions of osmotic shock (2% NaCl, 26.3°C temperature, 4.4 mg L^{-1} dissolved oxygen, 8.0 pH) and prolonged fasting (25.2°C temperature, 6.5 mg L^{-1} dissolved oxygen, 7.6 pH). Osmotic shock was conducted using 30 larvae (9 days old) and the fasting test was conducted using 30 embryos at hatching in two replications per cross. Examined larvae were placed in 0.25×0.18 m fish-net and immersed in a 60×30×30 cm aquarium for osmotic shock. For the prolonged fasting test, larvae were placed in a 0.25×0.18 m fish-net installed in an isolated recirculating system. Dead larvae were removed and counted every 5 min for osmotic shock and daily for the fasting test. The ANOVA one factor was used to compare the median of two tests.

RESULTS AND DISCUSSION

Under stress tests no survival was observed after 30 min and 16 days for osmotic shock and prolonged fasting test, respectively, in larvae (Fig. 1 and 2). The median survivals (TL_{50}) were 26.5 min and 12.5 days for osmotic shock and prolonged fasting, respectively (Fig. 1 and 2). There was a statistically significant difference between the median survival of the group exposed for osmotic shock and that the group exposed for prolonged fasting test ($p<0.05$). The best survival rate was observed within the first 11 days in the fasting experiment. Under osmotic shock, the first mortality was recorded after 20 min. These results show a great resistance of the larvae to the osmotic shock and prolonged fasting.

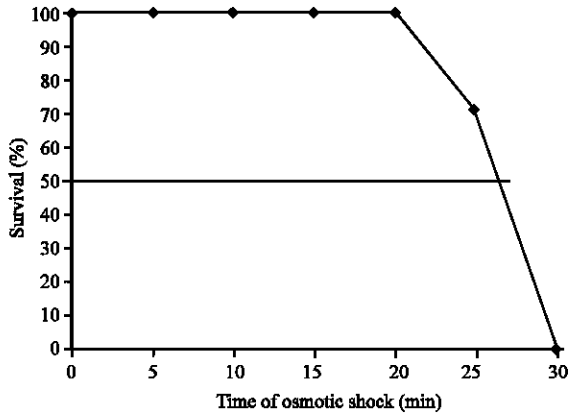

Fig. 1: Survival of *Labeo parvus* larvae upon exposure to osmotic shock

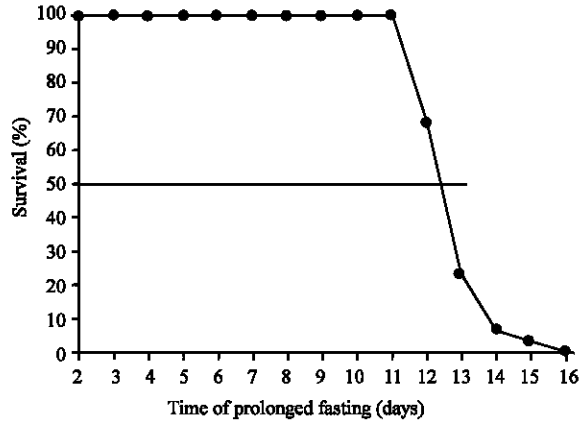

Fig. 2: Survival of *Labeo parvus* larvae upon exposure to prolonged fasting

This study is the first report to examine the resistance and quality of the larvae of *L. parvus* obtained in captivity by artificial reproduction. The higher survival rates were obtained in larvae in two the experiments.

In the prolonged fasting test, the best survival rate was observed within the first 11 days in the experiment. This result is similar to that reported earlier by Nzau-Matondo *et al.* (2007) in hybrids of three common European cyprinid species (*Rutilus rutilus*, *Blicca bjoerkna* and *Abramis brama*). *Labeo parvus* larvae could have developed a better adaptability to prolonged fasting. One could think that the yolk sac played an important role in the best survival of larvae to the prolonged fasting. But this hypothesis does not seem to support the reality of observations because larvae obtained in captivity by artificial reproduction finish completely their yolk sac 24 h after hatching (Montchowui *et al.*, 2011b). So, the best adaptation of larvae to the prolonged fasting could be bound to intrinsic factors of the species.

From an ecology point of view, survival to prolonged fasting could translate into a capacity on the part of *L. parvus* larvae to easily thrive in the living conditions in natural habitats. Indeed in natural habitats, *L. parvus* is a synchronous spawner known to reproduce in river flood plains with unfavourable conditions such as receding water, decreasing oxygen concentrations and rapidly fluctuating temperatures soon after spawning (Albaret, 1982; Montchowui *et al.*, 2007). *Labeo parvus* larvae do not benefit from parental care after spawning and their survival depends greatly on their ability to adapt to the conditions of these environments. These results might indicate that larvae obtained by the artificial reproduction preserve some genetic characteristics of the parents live from the wild as the capacity of resistance on difficult conditions. This is an interesting fact and it shows that larvae obtained in captivity are of excellent quality and good candidate for tropical aquaculture.

Under osmotic shock, total mortality time is identical to that reported for silver bream in an early study (Nzau-Matondo *et al.*, 2007). The median survival (LT_{50}) times obtained (26.5 min) are similar to that reported by Nzau-Matondo *et al.* (2007) in hybrids of common bream (*Abramis brama*) x roach (*Rutilus rutilus*). One notes a great resistance of the *L. parvus* larvae to the osmotic stress. This could possibly be explained by gill surfaces which modulate the osmoregulatory capacity in fish. In addition, the great resistance of larvae to the osmotic stress could be due to the composition (in particular the content of essential fatty acids, minerals and vitamins) of the food used (*Artemia nauplii*) to feed the larvae before the experiment. Hugues observed in the mammals subjected to various agent stressors an increase in the requirements in ascorbic acid and possibly in essential fatty-acids. Similar observations were also made in the larvae of *Clarias gariepinus* (Merchie *et al.*, 1995, 1997) of *Stizostedion vitreum* (Kolkovski *et al.*, 1998; Czesny *et al.*, 1999) and of *Sparus aurata* (Henriquea *et al.*, 1998).

CONCLUSION

These results show a great resistance of the larvae to the osmotic shock and prolonged fasting and demonstrate the best quality of *L. parvus* larvae obtained in captivity. The resistance of these larvae to stress tests would make it possible to confirm if these larvae could generally live longer in hostile environments.

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