OPTIMIZATION OF INDUCED OVULATION AND SPAWNING OF AFRICAN CLARIID CATFISH, *Heterobranchus bidorsalis* (Geoffroy-Saint-Hilaire, 1809) USING SYNTHETIC HORMONE

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ABSTRACT

In this study, induced spawning of *Heterobranchus bidorsalis* was optimized for artificial propagation. A single dose (ml) of synthetic hormone, Ovaprim® (Syndel, Canada), per kg body weight (bw) at 0.5, 0.75, 1 and 1.25 were administered on the broodstock. Ovulation and spermiation were achieved within the latency period of 14±1 h at 27±0.5 °C. Mean spawned eggs for doses of 1 ml/kg (56.77±8.92g) and 1.25 ml/kg (59.93±3.26g) gave significant production compared to 0.5 ml/kg (5.07±2.29g) and 0.75ml/kg (9.13±1.10g) ($p<0.05$). Percentages of fertility (72.33±2.52, 79.33±4.04, 84±4.58, 82±4.36); hatchability (43.47±4.77, 61.73±10.02, 70.4±5.51, 68.57±12.78) and survival (47.37±3.16, 54.07±7.74, 64.67±4.63, 62.4±2.82) for the test doses of 0.5, 0.75, 1, 1.25 ml/kg bw were recorded respectively at 28.5 ± 0.5 °C. The higher doses of 1 and 1.25 ml/kg bw showed significant difference compared to the lowest dose of 0.5ml/kg ($p<0.05$) while 0.75ml/kg was not significantly different to all other doses ($p>0.05$). Hatching occurred at 21 h in all the treatments. This study revealed reproductive dysfunction in *H. bidorsalis* and recommend a dose of 1ml/kg bw for optimal induction using synthetic hormone Ovaprim.

Keywords: *Heterobranchus bidorsalis*; Ovaprim; eclosion

INTRODUCTION

In Nigeria, *Clarias* and *Heterobranchus* are the two most popular and economic important *Clariidae* genera for aquaculture (Fagbenro *et al.*, 1993; Adebayo and Fagbenro, 2004). *Clarias* species have been widely cultured and researched (Bongers *et al.*, 1995; Hecht *et al.*, 1996) due to its hardiness and readily availability compared to *Heterobranchus* species (Fagbenro *et al.*, 1993; Adebayo and Fagbenro, 2004). A *Heterobranchus* species *H. longifilis* has been proven to perform twice better in their growth than *Clarias gariepinus* raised under the same conditions (Legendre et al., 1992; Teugels, 1996). Study established that *H. bidorsalis* mature early by 10-12 months of domestication compared to its wild species that takes 2-3 years (Fagbenro et al., 1993). However, researches are very limited on genus *Heterobranchus* (Anselme et al., 2008) as very few studies are reported on its four valid species viz, *H. bidorsalis* Geoffroy Saint Hilaire, 1809; *H. longifilis* Valenciennes, 1840; *H. isopterus* Bleeker, 1863; and *H. boulengeri* Pellegrin 1922 (Reed et al., 1967; Teugels et al., 1990; Teugels, 1996). Very fewer works on breeding has been conducted of this study species *H. bidorsalis* therefore optimization of the induced breeding, and artificial propagation of this species is essential for its successful aquaculture and biodiversity, hence this study.

MATERIALS AND METHODS

Broodstock collection and management: Pure breed of *H. bidorsalis* of Lake Kainji origin, were acquired from the National Institute for Freshwater and Fisheries Research, NIFFR, New Bussa, Nigeria and transported to the Wet Laboratory, Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. The species were acclimatized, maintained in conditioning systems typical of the normal seasons as experienced by the wild species except that the photothermal conditions were simulated to trigger reproductive responses as desired for better performance (Bromage and Roberts, 1995; Haylor and Mollah, 1995) for 2 years. They were fed with 6.0-9.0 mm imported feed Multifeed Coppens® (Netherlands) pelleted fish feed (45% crude protein) twice daily at 009 hrs and 17.00 hrs.

Induced breeding: Breeding was conducted during the peak of rainy season June - August corresponding to their best breeding period (Fagbenro et al., 1993; Adebayo and Fagbenro, 2004). Sexually matured broodstock were identified and selected based on the external sexual characteristics (Olaniyi and Omitogun, 2013). Four matured male broodstock (1600 ± 100 g) were identified by their pinkish-coloured pointed genital papillae and five female (1380 ± 170 g) with swollen abdomen. In addition to the identification of gravid female broodstock was the swollen and

extended pinkish/reddish vent that freely oozed out eggs upon gentle pressure application on their distended abdomen. Extruded flap of the gravid female broodstock was noticed so that the female was not mistaken for male, and then sacrificed. A single dose of synthetic hormone, Ovaprim® (Syndel, Canada) per kg bw was administered intramuscularly through the dorsal muscle to the broodstock with 0.5, 0.75, 1, 1.25 ml/kg bw. The treated broodstock were kept separately at 27 ± 0.5 °C during the latency period. At ovulation, eggs were stripped from the female broodstock into a dry bowl. The testes were surgically eviscerated laterally from the male, and milt was obtained. "*Wet*" fertilization was carried out whereby eggs were fertilized with diluted milt 1:10 physiological saline solution (0.9 % NaCl). At 7±1 mins thereafter, the fertilized eggs were distributed in single layer on the already set incubation net (mesh size: 1 mm) in water aquaria (2m × 1m × 0.5m). The treatments were in triplicate and incubations were carried out at a controlled temperature of 28.5 ± 0.5 °C. A RESUN LP- 100 low noise air-pump was employed to aerate the set-up. Owing to unique form of eclosion in *H. bidorsalis* flow-through system was set-up to trickle away the polluted incubated water and to maintain optimum dissolved oxygen in addition to the air-pump (Olaniyi and Omitogun, 2014).

Fertility, hatchability and survival: Fertility was evaluated at 2-3 h (morula stage) post-fertilization (Olaniyi and Omitogun, 2013, 2014) with respect to the total number of eggs set (equation 1). The hatchability and survival percentages were then calculated. The percentage hatchability (equation 2) was calculated by the number of hatched larvae (N_H) in relation to the number of fertilized eggs (N_F); while the percentage survival (equation 3) was calculated following Don and Avtalion (1986) that is, relative to defined developmental age, i.e. day 7.

% fertility (c) = Number of fertilized eggs / Number of eggs set ... Equation (1)

% hatchability = $N_H \times 100 / N_F$ ... Equation (2)

% survival = $n \times 100/[i \times (c/100)] = n \times 10^4/(i \times c)$ ... Equation (3)

where; c = the absolute percentage of fertile eggs (% of morula at 2–5 h); i = the initial number of the eggs set; n = the number of eggs which survived up to a given developmental stage (i.e. day 7); N_H = number of larvae hatched; and N_F = number of eggs fertilized.

Data **analysis:** Data recorded for fertility, hatchability and survival were subjected to General Linear Model procedure choosing Duncan option.

RESULTS AND DISCUSSION

Induced spawning: pH, alkalinity, dissolved oxygen and temperature at incubation were maintained at 7.1±1, 112.31±1.14 mg/l, 16.5±0.5 mg/l and 28.5±0.5 °C respectively. Ovulation was achieved within the latency period of 14±1 h at temperature of 27±0.5 °C. Mean spawned unfertilized eggs measured 1±0.1 mm diameter and had ~ 20% increase in size when hydrated (1.2±0.2 mm). The hydration and large yolk reserve are highly essential since they respectively contribute to viable larvae production and their good survival (Mylonas et al., 2010). The mean spawned eggs for doses of 1 ml/kg (56.77±8.92g) and 1.25 ml/kg (59.93±3.26g) were not different from each other ($p>0.05$) but both significantly different from the doses of 0.5 ml/kg (5.07±2.29g) and 0.75 ml/kg (9.13±1.10g) ($p<0.05$) that were in turn not different significantly from each other ($p<0.05$) (Table 1). The quantity and quality of gametes are affected largely by factors such as environment (e.g. temperature, salinity, tank size and volume of water, stocking density etc), hormonal factors (hormone type) and induction processes (dosage administration, time of induction – single or multiple doses, latency period between injection and ovulation etc). This study maintained temperature of 27±0.5 °C during the latency period being that *H. bidorsalis* belongs to freshwater or warmer fish. At this temperature good quality eggs and sperm can be obtained, as it was reported for *C. gariepinus* that 25 °C is best and at 30 °C leads to atresia in the oocyte and regression of the testes. Moreover, low temperature affects the ova and its maturation and the latency period which may be delayed while very high temperature is detrimental e.g. atresia (Legendre et al., 1996; Mylonas et al., 2010). Generally, there was low egg production in *H. bidorsalis* though doses 1 and 1.25 ml/kg gave

significant spawned eggs. In comparison to other African Clariid species at 0.5ml/kg bw, *C. gariepinus* spawned 230.85±22.2 g (Olaniyi, 2013) under same conditions; however, Nwokoye et al., (2007) reported ~ 16.49 g for *H. bidorsalis* species. Few works on *H bidorsalis* reported no actual data of eggs being spawned (Adebayo and Fagbenro, 2004; Agbebi et al., 2005). Despite administration of higher doses of the Ovaprim in this study, it can be deduced that the ovaries of *H. bidorsalis* do not contain abundant postvitellogenic oocytes, hence its artificial propagation is at its best during the peak of rainy season. The mean spawned egg of *H. bidorsalis* was very small in quantity in relation to other catfish species and this revealed that the species exhibits reproductive dysfunction in captivity (Mylonas et al. 2010) like some other teleost species viz *Pseudoplatystoma fasciatum* (Leonardo et al. 2004), *P. corruscans* (Campos 2005), *Rhamdia quelen* (Baldisseroto and Neto 2005), *Steindachneridion melanodermatum* (Ludwig et al. 2005), and *S. parahybae* (Honji et al. 2009, 2012). Low egg production is attributed to reproductive dysfunction in fish species (Mylonas et al., 2010). Other effects of dysfunctions are lower quantity of sperm and failure of oocyte maturation at the completion of vitellogenesis (Mylonas et al., 2010).

Fertility, hatchability and survival: Percent fertility for doses of 0.5, 0.75, 1, 1.25 ml/kg bw recorded 72.33±2.52, 79.33±4.04, 84±4.58, 82±4.36, respectively. Doses (ml/kg) of 1 and 1.25 were not different from each other ($p>0.05$) but both significantly different from 0.5ml/kg dose ($p<0.05$). 0.75ml/kg showed no significant different compared to the rest doses ($p<0.05$). The difference in fertility of the 0.5ml/kg compared to the rest can be attributed to the oocyte quality spawned as a result of the hormone dosage and latency period. The hormonal dosage or level may not be enough to develop the eggs to maturity, therefore leads to low quantity and quality eggs been spawned. Hatchability of dose 0.5ml/kg (43.47±4.77%) was significantly different ($p<0.05$) from 1ml/kg (70.4±5.51%) and 1.25ml/kg (68.57±12.78%), while 0.75ml/kg (61.73±10.02%) ($p>0.05$) was not significantly different from either of the doses. The egg size and quality effects also determine hatchability in species (Bromage and Roberts, 1995), but most importantly the developmental process from fertilized egg to hatching in biological processes is dependent upon water temperature (Olaniyi and Omitogun, 2012, 2014). The higher the water temperature the faster the eggs hatched and the better (De Graaf and Janssen, 1996). More importantly, the unique characteristic mode of hatching in *H. bidorsalis* plays significant effect on its hatchability and survival (Olaniyi and Omitogun, 2014). Percent survival with respect to doses of 1.25ml/kg (62.4±2.82) and 1 ml/kg (64.67±4.63) were both significantly not different from each other ($p>0.05$), but both different from 0.5ml/kg dose (47.37±3.16%) ($p<0.05$). 0.75ml/kg (54.07±7.74%) was not significantly different from all other doses ($p>0.05$). Latency period for all the species were not different from one another (14±1). Constant supply of fresh water in a trickling mode for flow-through system to wash away impurities that can lead to pollution or turbidity of incubation water, and sufficient dissolved oxygen also contribute to high survival.

CONCLUSION

This study revealed reproductive dysfunction in *H. bidorsalis* as expressed in the quantity of spawned eggs, a dose of 1ml/kg bw is recommended for optimal induction using synthetic hormone Ovaprim.

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Table 1: Effect of induced ovulation and spawning on *Heterobranchus bidorsalis* using synthetic Ovaprim

Parameters	Dose (ml/kg)			
	0.5	0.75	1	1.25
Egg spawned (g)	5.07±2.29^{b}	9.13±1.10^{b}	56.77±8.92^{a}	59.93±3.26^{a}
Latency	13 -15	13 -15	13 -15	13 -15
Fertility (%)	72.33±2.52^{b}	79.33±4.04^{ab}	84±4.58^{a}	82±4.36^{a}
Hatching (%)	43.47±4.77^{b}	61.73±10.02^{ab}	70.4±5.51^{a}	68.57±12.78^{a}
Survival (%)	47.37±3.16^{b}	54.07±7.74^{ab}	64.67±4.63^{a}	62.4±2.82^{a}

^{ab}Means within a row with different superscripts are significantly different ($P<0.05$). General Linear Model procedure (SAS®, 2003).