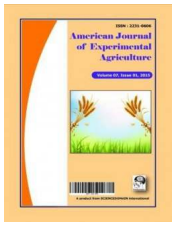

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Effects of Incision Variation Length on *Heterobranchus bidorsalis* Male Spawners to Extract Milt for Induced Breeding

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Authors' contributions

This work was carried out in collaboration between all authors. Author TAY designed the study, wrote the protocol and wrote the first draft of the manuscript. Author SLL reviewed the experimental design and all drafts of the manuscript. Authors SMT and RJK managed the analyses of the study. Authors TAY and SLL performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Matured African catfish (*Heterobranchus bidorsalis*), size ranging from 1.40-2.00 kg average total body weight (TBW) and mean length 44.50 cm were procured from a private fish farm and transported in perforated 50 litre water holding capacity jerry can to the hatchery and maintained for 2 weeks. They were fed with 40% crude protein commercial diet with good water quality management before being used for breeding. Incision at variation length 2.50, 3.40 and 4.00 cm were made on the abdominal region (gonad position) of the male spawners after anaesthetic treatment using sterilized surgical instrument to extract milt to fertilize egg. The incised position was sutured using simple interrupted suture pattern with catgut chromic 2/0 stitch. The spent spawners after operation survived on gradual healing and recuperation within 14 days post- surgery. Fecundity, volume of milt extracted, percentage fertility and hatching differed significantly ($P<0.05$) among treatments. Treatment 3 (3.40 cm) was the most effective incision length that gave best results in term of percentage hatching (92.04^{a}), weight gain (28.37), specific growth rate (2.36) and

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percentage survival (68.77±4.224[a]) of the bred hatchlings as compared to other treatments. The male spawners of *H. bidorsalis* could be incised (cut) on abdominal region (gonad position) at length of 3.40 cm to effectively extract milt to fertilize egg without adverse effect on the fish to enhance fingerling production.

Keywords: Incision; milt; H. bidorsalis; recuperation and induced breeding.

1. INTRODUCTION

Aquaculture presently is one of the fastest growing food production systems in the world with developing countries contributing significantly [1]. Over the years there has been stagnating yields from many capture fisheries due to attendant reasons such as environmental unfriendly practices by man and challenge of climate change. There is increasing demand for fish and fishery products, high expectation for tremendous contribution to the world's aquatic food production. This in no small measure will help in contributing to food security and alleviate poverty particularly in developing countries [2]. Aquaculture practice is significant in nutritional, social, economic and environmental aspects of human endeavour; hence the need for further development and expansion of the sector and effort for sustainable development is important [3].

Fish is known to be one of the cheapest sources of protein for human consumption globally especially in areas where cattle and beef production is expensive [4]. According to [5] the demand for fish protein is rising and there is need to increase production of food including fish to feed human population. Fish protein has many advantages over beef, mutton and pork as it supplies essential nutrients such as omega-3 fatty acids and iodine which are lacking in other animal proteins [6].

One major constraint to aquaculture development in developing countries is inadequate supply of good quality fish seed to stock culture enclosures. Successful aquaculture begins with stocking adequate and good quality fingerlings in ponds with optimum conditions that enhance rapid growth and harvest within short possible time [7]. For maximum production, especially for commercial purpose, the fish farmer has to obtain adequate number of fingerlings to meet the production target.

Pandey [8] stated that fish breeders need to make use of brood fish that is viable with good vigour and disease free and disease resistant strain that produces healthy progeny. In Carp species milt in mature males are released by a slight press on the abdomen and semen hand stripped on to the eggs. This is not possible in African male catfish species hence poses a great challenge to fish breeders because they cannot be handled in like manner. The slight press on the abdomen of male African catfish (*H. bidorsalis*) spawners releases milt from gonads into lobes of the seminal vesicles and not directly through the genital papilla [9].

The conventional practice in artificial breeding of catfish is to sacrifice the male and have their gonads removed to squeeze out milt to fertilize egg. In view of the above, this research was conducted to determine the appropriate length of incision on catfish *H. bidorsalis* male spawners to extract milt to fertilize egg as alternative to sacrifice of male. The spared male can be use for further breeding and genetic improvement especially parent stock that have strong genetic potential.

2. MATERIALS AND METHODS

Thirty-six (36) matured brooders (24 males and 12 females) of *H. bidorsalis* ranging from 1.40-2.00 kg average total body weight (TBW) and mean length 44.50 cm were procured from a private fish farm and transported in perforated 50 litre water holding capacity jerry can to the hatchery. On arrival, they were disinfected with 5% salt bath for 5 minutes as described by [1] and then acclimatized for 2 weeks. They were maintained under optimum temperature, good water quality management at optimum required limit for fresh water fish culture. They were fed adequately with 40% crude protein commercial diet. After acclimatization the brooders were carefully selected and treated with a single dose of hormone (Ovaprim) 0.5 ml/kg body weight according to the method of [10]. Injection was given intraperitoneally. Eggs were stripped into a clean plastic bowl from injected females after latency period of about eleven hours at water temperature of between 25-32℃. Fecundity was determined as thus:

$$\text{Fecundity} = \frac{\text{Total weight of Stripped Eggs}}{\text{Weight of Sub-Sample}} \times \text{Total Number of Eggs in Sub-Sample}$$

About 9.5 g of total stripped eggs was use for fertilization.

Six males were sacrificed in treatment 1 to serve as (control) (conventional method of breeding catfish) to remove testis and squeezed milt to fertilize egg. Surgical operation was carried out on the remaining males using sterilized surgical instruments at the dorso-ventral part of abdominal region, location of the testis. The fish were injected with general anaethesia Ketamine Hcl at dose of 0.1 ml/kg body weight before operation. Transparent metre rule was used to take measurements of 2.50, 3.40 and 4.00 cm long at ventral side of the abdomen to determine the appropriate and exact length to cut or incise that would expose the testes good enough to extract milt. Four treatments were considered and (T1: 0.00 cm) serve as control; T2 (2.50 cm); T3 (3.40 cm and T4 (4.00 cm) and were all replicated three times.

The fish were placed dorsally on a wet disinfected white clean cloth spread on clean table with the head covered with a piece of wet clean towel. The surface of the abdomen was disinfected with methylated spirit (40%) before incision was made on the ventral side. The incision was extended towards the head with sterilized surgical scissors 3-5 cm long for all replicates with the aid of 2 assistants to expose the internal organs including testes which remained intact. The digestive tracts were pushed aside to reveal the testes. Milt was extracted from the testes using sterilized 2 ml size syringe and needle. Milt volume was weighed and then observed under Binocular Olympus Microscope Agary model xSz-107BN at x 40 magnification power to determine their motility before being used to fertilize eggs. The incised spot was sutured using simple interrupted suture pattern with catgut chromic 2/0 stitch. The surgery on each fish lasted for about 30 minutes. Each incised fish was placed in a plastic trough with well aerated fresh water to enable them recover. After recovery, each fish was kept in a separate concrete tank (2 m x 2 m x 1 m) length, width and depth respectively. This contained broad spectrum antibiotic (Dav-Biotic) at a dose of 5 g/L. The fish were there for 8 days without food under intensive care and monitored for healing, recuperation and recovery. This reduces pressure on the incision point and facilitated healing. The surgery and milt collection were performed in two replicates. The milt and eggs were mixed together gently with plastic spoon for 2-3 minutes. Small quantity of saline solution was then poured onto the fertilized eggs to avoid sticking together. The fertilized eggs were rinsed with distilled water and introduced into the incubators. Incubation was monitored for 24-48 hours with gentle water flow through in which aeration was maintained using aquarium electric pump.

Water quality parameters such as Dissolved Oxygen (DO), pH, temperature, conductivity and ammonia were monitored and measured fortnightly during incubation and rearing of the hatchlings. Percentage fertility, hatching and survival were determined according to method described by [11] using the formulae:

$$\text{Percentage fertility} = \frac{\text{Number of fertilized eggs}}{\text{Number of eggs stripped}} \times 100$$

$$\text{Percentage hatching} = \frac{\text{Number of fry hatched}}{\text{Number of fertilized eggs}} \times 100$$

$$\text{Percentage survival} = \frac{\text{Cumulative survival}}{\text{Total number stocked}} \times 100$$

Specific Growth Rate (SGR) was determined using the method as described by [12].

$$SGR = \frac{\text{Log } W_2 - \text{Log } W_1}{T_2 - T_1} \times 100$$

Where W_1 = weight at time 1, W_2 = weight at time 2, T_1 = Time one, T_2 = Time two and Log = Natural logarithm.

Results obtained were analyzed by One Way Analysis of Variance (ANOVA) while means were compared for significant differences at 0.05 significant levels using Duncan's multiple range tests using SPSS version 15.0.

3. RESULTS

The results in Table 1 shows fecundity, volume of milt extracted, percentage fertility and hatching. Treatment 3 had highest fecundity and differed significantly ($P<0.05$) from other treatments. Volume of milt extracted in treatment 1 (control) was higher (0.86^a) and differed

significantly (P<0.05) as compared to other treatments. Similarly, percentage fertility and hatching in treatment 4 and 3 respectively differed significantly (P<0.05) as compared to other treatments. The results in Table 2 shows Incision Variation Lengths (IVL) (2.50, 3.40 and 4.00 cm) long made on the dorsal ventral side of the abdomen on the position of testes. The variation in incision length was to determine the best length that could reveal the testes properly to extract milt without stressing the fish. One mortality each of male spent spawners from T2 and T4 was recorded at 3rd and 6th day post-surgery respectively as shown in Plates 1 and 2. The results of gradual and progressive healing and recuperation process of the incision point at various time intervals are presented in plates 3. The results in Table 3 shows growth performance of *H. bidorsalis* bred fingerlings through induced breeding of incision variation length method for 12 weeks. Treatment 3 had highest mean length gain, mean weight gain and specific growth rate of 6.88, 28.37 and 2.36 respectively. Similarly, the results in Table 4 shows grand cumulative mean mortality/survival percentages of *H. bidorsalis* fingerlings produced through induced breeding methods of incision variation length and reared for 12 weeks. It reveals that treatment 3 had the highest survival of 68.77% bred *H. bidorsalis* fingerlings.

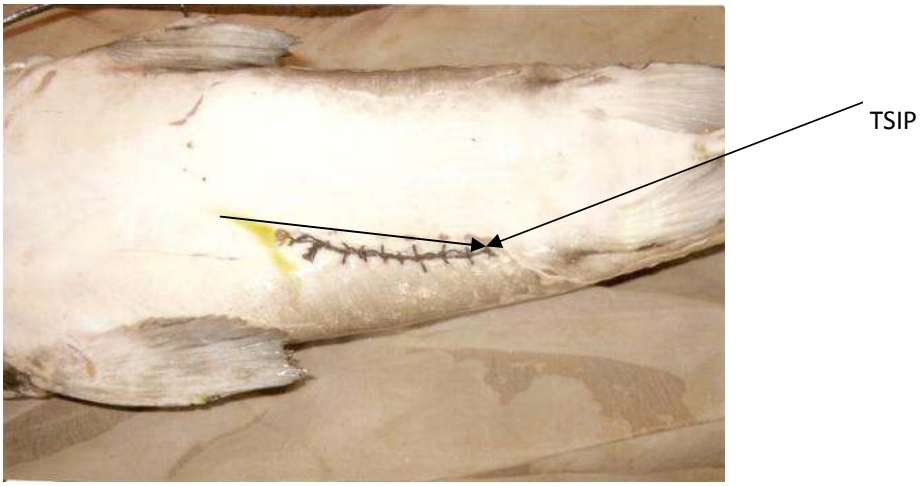

Plate 1. The stitched incision point on the body of dead male broodstock *H. bidorsalis* (Died after 3 days)

Key: TSIP = The Stitched Incision Point

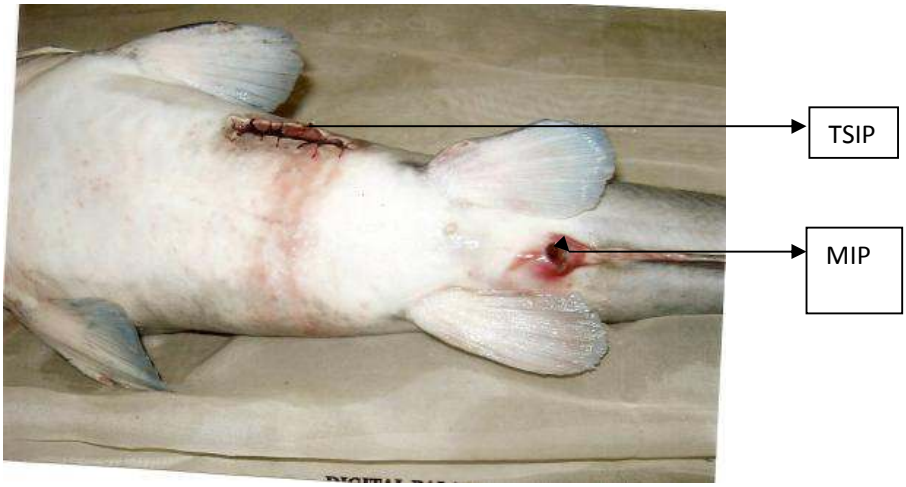

Plate 2. The stitched incision point on the body of dead male broodstock *H. bidorsalis* (Died after 6 days)

Key: TSIP = The Stitched Incision Point

Plate 3. The stitched incision point on the body of survived male broodstock *H. bidorsalis* (8 days after)

Key: TSIP = The Stitched Incision Point

Table 1. Fecundity, volume of milt extracted, percentage fertilization and hatchability from *H. bidorsalis* for induced breeding using incision variation length method (IVLM)

Treatment	IVLM (cm)	Fecundity	Vol. of milt extracted (ml)	Percentage fertility (%)	Percentage hatching (%)
1	0.00	84,924.9^{c}	0.86^{a}	68.22^{c}	72.01^{b}
2	2.50	95,266.7^{b}	0.52^{c}	54.92^{c}	63.23^{c}
3	3.40	102,101.2^{a}	0.81^{b}	72.21^{b}	92.04^{a}
4	4.00	99,686.4^{b}	0.66^{c}	85.93^{a}	69.22^{c}

Table 2. Incision variation lengths on male brood stock of *H. bidorsalis*

Incision length variation (CM)				
Species type	**Length I**	**Length II**	**Length III**	**No. of fish incised**
H. bidorsalis	2.50	3.40	4.00	18

Table 3. Growth Performance of *H. bidorsalis* fingerlings bred through induced breeding of Incision Variation Length Method (IVLM) for 12 Weeks

Treatments	IVLM (cm)	MITL (cm)	MFTL (cm)	MITBW (g)	MFTBW (g)	MLG (cm)	MWG (g)	SGR
1	0.00	1.50±0.10	8.20±1.02	2.68±0.06	28.50±0.66	6.70	25.82	2.15
2	2.50	1.52±0.11	8.30±1.03	2.69±0.06	28.51±0.65	6.78	25.82	2.15
3	3.40	1.77±0.12	8.65±1.21	2.75±0.08	31.12±0.86	6.88	28.37	2.36
4	4.00	1.50±0.10	8.20±1.02	2.65±0.05	26.01±0.52	6.70	23.36	1.95

Key: IVLM= Incision Variation Length Method, MITL= Mean Initial Total Length, MFTL= Mean Final Total Length, MITBW= Mean Initial Total Body Weight, MFTBW= Mean Final Total Body Weight, MLG= Mean Length Gain, MWG= Mean Weight Gain and SGR= Specific Growth Rate

Table 4. Grand cumulative mean moetality/survival percentages for *H. bidorsalis* fingerlings bred through incision variation length method and reared for 12 weeks

Treatments	IVLM (cm)	Mortality (%)	Survival (%)
1	0.00	41.93±5.724[b]	59.06±5.724[b]
2	2.50	42.80±1.803[b]	57.20±1.803[b]
3	3.40	31.23±4.224[a]	68.77±4.224[a]
4	4.00	46.72±8.254[b]	53.28±8.254[b]

Values on the same column for each treatment carrying the same superscript did not differed significantly (p>0.05) from each other

4. DISCUSSION

The highest percentage fertility in treatment 4 (85.93[a]) and hatching in treatment 3 (92.04[a]) might be attributed to egg quality and viability. The eggs were dark brown and light green in colour and were not watery, an indication of good quality and viability. Treatment 1 (control) with the highest volume of milt extracted (0.86[a]) was attributed to the fact that the brood stocks were sacrificed to remove testes to fertilize egg hence much milt was squeezed as compared to treatments 2, 3 and 4 where milt was extracted and obtained in little quantity. [13] made similar observation where milt volume collected through ablation in African catfish (*Clarias anguillaris*) before and after regeneration was 0.67±0.31 and 0.40 ml±0.10 respectively. The dead of spent spawners at 3rd and 6th day post-surgery (plates I and II) could be due to stress and microbial infection on the incision point as a result of opening on the wound. When micro-organisms infected the wound it slows down the healing process because the body immune system (antibody) that fights against germs might be destroyed. The eight days hunger that the fish were subjected was to facilitate the healing process because pressure on the incision point and water pollution was reduced. This however, was at variance to the work of [14] and [13]. Anaesthetic agent used on the fish was to reduce stress and ensured calmness during surgical operation as similarly reported by [13]. Out of the three incision variation length (Table 2) adopted, length 3.40 cm was the best that revealed the testes good enough and properly to extract milt. This method was however at variance with the method adopted by [13], milt was collected from *Clarias anguillaris* through ablation, ¾ of the testes was removed and placed in petri-dish containing saline solution (0.09% NaCl) and preserved in refrigerator at 4℃. Healing of the incision point (cut) occurred within 14 days post-surgery for treatments 2, 3 and 4. This corroborates the work of [13] who reported that healing of the cut occurred within 14 days in male *Clarias anguillaris*. However, this is contrary to the work of [14] who reported that cicatrization (healing) of the cut occurred within 4 weeks in male *H. longifilis* probably due to different environmental conditions and species of fish.

Incision variation length treatment 3 (3.40 cm) made on *H. birdorsalis* brood stocks had positive effect because it had highest survival of hatchlings as compared to other treatments. The growth performance of the bred hatchlings (Table 3) was an indication that the milt extracted were of good quality with viable eggs hence gave best growth performance in terms of body weight gain, length gain and specific growth rates as compared to other treatments. Similarly, survival rate was highest in treatment 3 (68.77±224[a]) as compared to other treatments. This was also attributed to good egg viability and quality milt resulted in vigour hatchlings which increases chances of high survival as similarly observed by [15]. This could be traced to high genetic potential and strength of the parent stock. The variation length on the abdominal region (gonad position) exposes the testes good enough to extract milt using 2 ml size syringe and needle to fertilize egg without sacrificing the male.

5. CONCLUSION

It could be concluded that incision variation length (3.40 cm) made on abdominal region (gonad position) of *H. bidorsalis* brood stocks exposed testes good enough to extract milt to fertilize egg without adverse effect on fish hence enhance fingerling production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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