

Effects Of Incision Variation Length On *Heterobranchus bidorsalis* Male Spawners To Extract Milt For Induced Breeding.

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 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.

Key words: Incision, milt, *H. bidorsalis*, recuperation and induced breeding.

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

37 demand for fish protein is rising and there is need to increase production of food including
38 fish to feed human population. Fish protein has many advantages over beef, mutton and pork
39 as it supplies essential nutrients such as omega-3 fatty acids and iodine which are lacking in
40 other animal proteins [6].

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

47 [8] stated that fish breeders need to make use of brood fish that is viable with good vigour
48 and disease free and disease resistant strain that produces healthy progeny. In Carp species
49 milt in mature males are released by a slight press on the abdomen and semen hand stripped
50 on to the eggs. This is not possible in African male catfish species hence poses a great
51 challenge to fish breeders because they cannot be handled in like manner. The slight press on
52 the abdomen of male African catfish (*H. bidorsalis*) spawners releases milt from gonads into
53 lobes of the seminal vesicles and not directly through the genital papilla [9].

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

60 **Materials and Method**

61 Thirty-six (36) matured brooders (24 males and 12 females) of *H. bidorsalis* ranging from
62 1.40-2.00 kg average total body weight (TBW) and mean length 44.50 cm were procured
63 from a private fish farm and transported in perforated 50 litre water holding capacity jerry
64 can to the hatchery. On arrival, they were disinfected with 5 % salt bath for 5 minutes as
65 described by [1] and then acclimatized for 2 weeks. They were maintained under optimum
66 temperature, good water quality management at optimum required limit for fresh water fish
67 culture. They were fed adequately with 40 % crude protein commercial diet. After
68 acclimatization the brooders were carefully selected and treated with a single dose of
69 hormone (Ovaprim) 0.5 ml/kg body weight according to the method of [10]. Injection was
70 given intraperitoneally. Eggs were stripped into a clean plastic bowl from injected females
71 after latency period of about eleven hours at water temperature of between 25-32°C.
72 Fecundity was determined as thus:

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

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

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

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

109 hatchlings. Percentage fertility, hatching and survival were determined according to method
110 described by [11] using the formulae:

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

112

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

114

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

116

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

118 $\frac{\log W_2 - \log W_1}{T_2 - T_1} \times 100$

119

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

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

125 **Results**

126 The results in Table 1 shows fecundity, volume of milt extracted, percentage fertility and
127 hatching. Treatment 3 had highest fecundity and differed significantly ($P < 0.05$) from other
128 treatments. Volume of milt extracted in treatment 1 (control) was higher (0.86^a) and differed
129 significantly ($P < 0.05$) as compared to other treatments. Similarly, percentage fertility and
130 hatching in treatment 4 and 3 respectively differed significantly ($P < 0.05$) as compared to
131 other treatments. The results in Table 2 shows Incision Variation Lengths (IVL) (2.50, 3.40
132 and 4.00 cm) long made on the dorsal ventral side of the abdomen on the position of testes.
133 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 I and II. The results of gradual and progressive healing and recuperation process of the incision point at various time intervals are presented in plates III. 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.

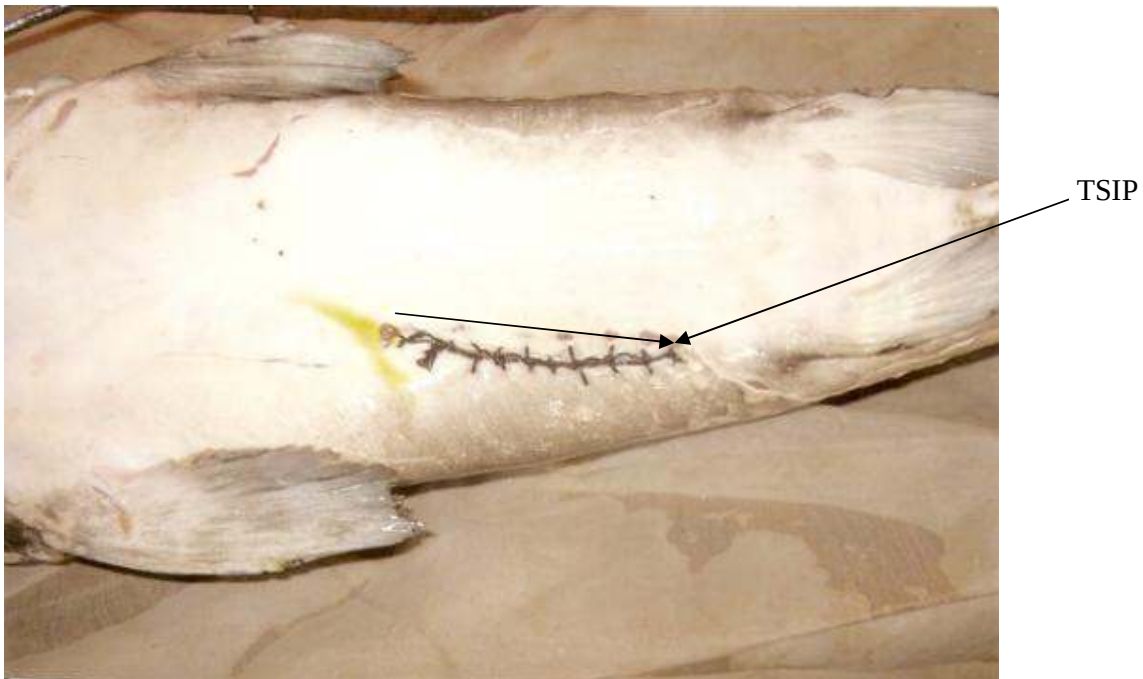
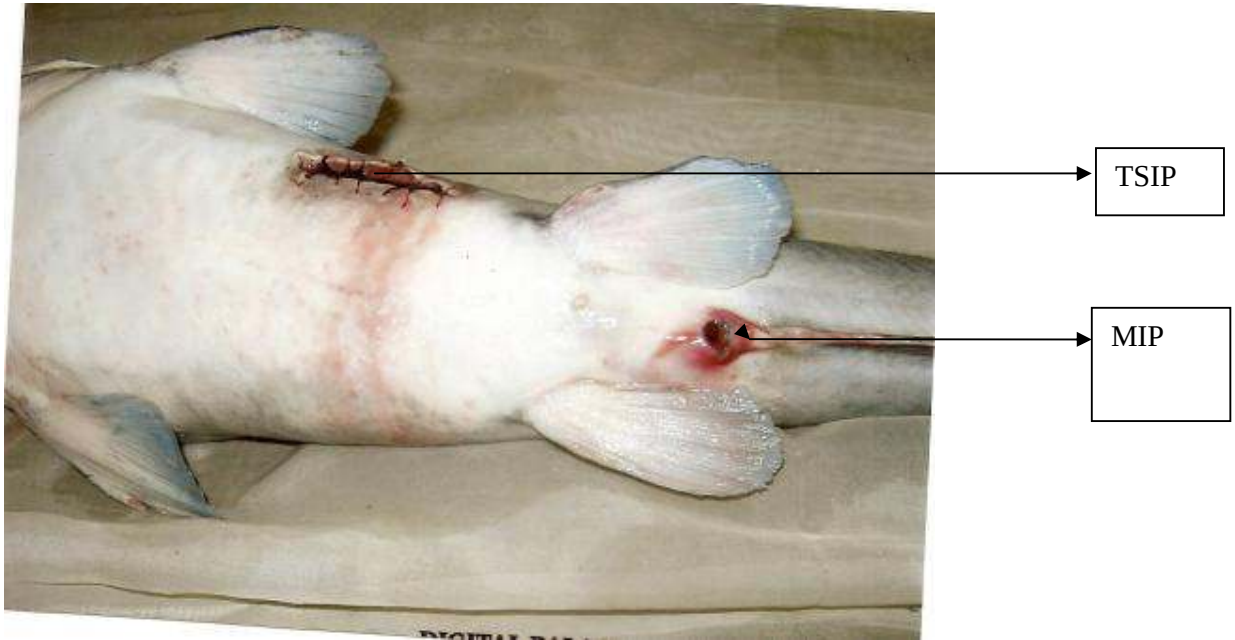


Plate I: The stitched incision point on the body of dead male broodstock *H. bidorsalis* (Died after 3 days)

Key: TSIP = The Stitched Incision Point.



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

Key: TSIP = The Stitched Incision Point.



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

Key: TSIP = The Stitched Incision Point.

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 death 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 to 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 lengths (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^o C. 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.

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.

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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*.

Species type	Incision Length Variation (CM)				No. of fish incised
	Length I	Length II	Length III		
<i>H. bidorsalis</i>	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.

Treatm ents	IVL M	MITL (cm)	MFTL (cm)	MITBW (g)	MFTBW (g)	ML G	MWG (g)	SGR
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	(cm)					(cm)		
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 Moortality/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.