



REGENERATING CAPACITY OF ASIAN CAT FISH, *CLARIAS BATRACHUS* (LINNAEUS, 1758)

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Abstract: In spite of being commercially important and having high market demand, efforts put in for breeding and propagation of the Asian cat fish, *Clarias batrachus* has not gained the desired momentum as the process demands ablation of the male brood fish for collection of testes. The deep wound so created leads to mortality of the fish resulting in negative impact on broodstock population and farmers' income. Attempts made for post-surgical revival of the fish (weight 110.0-190.0 g/fish) by performing the incision with minimum injury revealed that the fish had amazing capacity of wound healing by regeneration of lost tissue with 83.9-92.6 % (average 87.8%) post-surgical recovery. The incision (4.0-4.5 cm in length) had completely healed within 30-35 days without any medication or stitching. There was post-surgical regeneration of the testicular tissue after 190 ± 10 days in 90% of the randomly sampled fish. Fully developed testes were observed in 86.9% of the samples after 280-310 days of post-surgery rearing. These findings pave the way to address the inherent problem of mortality of male broods *vis a vis* to explore possible biomedical application of the regenerating capacity of the fish.

Keywords: Biomedical application, Incision, Regeneration, Self-healing, Skin wound, Testis.

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INTRODUCTION

Skin wounds and diseases are emphasized as one of the primary constraints to the sustainable aquaculture production as a wound in fish often leads to secondary infection and mortality *vis a vis* reduction in market value of the fish (Singh

and Chaube, 2021; Chakraborty *et al.*, 2021a). In general, a fish skin is comprised of the outer cuticle (mucus layer) built from glycoprotein formed by secretions from cells in the epidermal layer, an outer epithelial layer (the epidermis) with live epithelial cells and mucus producing



cells, the intermediate dermal layer (the dermis). The dermis largely contains connective tissue, nerves, blood vessels, pigment cells and adipose tissue and the deeper hypodermis, which is primarily built from adipocytes and also contributes to the vascularization and skin pigmentation (Lene *et al.*, 2020).

A wound in fish is the disruption of the skin integrity and associated mucus layer and may be mechanically induced or caused by skin pathogen or other disease and may be superficial (affecting only epidermis), partially thick (extending up to dermis) or deep (cutting through skin and subcutaneous adipose tissue) as distinguished by Lene *et al.* (2020). Teleost fishes are bony fishes known to have the capacity to regenerate their lost tissue (Verma, 2017; Verma and Prakash, 2020). However, not much work has been done on this aspect except some studies on a few species (Lene *et al.*, 2020).

The Zebra fish (*Danio rerio*) is regarded as a unique model animal for biomedical research including studies on human diseases and has been studied extensively for regeneration of different tissues and organs (Tsegay *et al.*, 2019; Richardson *et al.*, 2013, 2016). However, the systematic study on potential regenerative capacity of other fish species and their application in biomedical studies has not been geared up. The present communication reports the amazing capacity for self healing of mechanically induced deep skin wound and regeneration of mechanically removed male reproductive organ *i.e.*, testes in Asian cat fish, *Clarias batrachus* (Linn).

The Asian cat fish or the walking cat fish, *Clarias batrachus* (Linnaeus, 1758) is one of the most important indigenous food fishes of India. With capacity to breathe atmospheric oxygen with special air breathing organ (Dutta Munshi, 1961), the fish can be marketed alive to the delight of both sellers and consumers. This fish species has very high nutritional value not only as a food commodity with high protein content ($14.87 \pm 1.19\%$), vitamins and minerals (Bogard *et al.*, 2015; Paul *et al.*, 2015; Thorat, 2017), but also known as a rich source for poly unsaturated fatty acids (PUFA) content (Jakhar *et al.*, 2012). The

species is believed to have medicinal/therapeutic value, and have very high market demand, particularly in the North East region of India (Chetia Borah, 2020) and fetches much higher market price in comparison to carps and other commercially important food fishes in India and Bangladesh (Chakraborty *et al.*, 2021b).

The air breathing capacity enabling the species to thrive under adverse conditions like low dissolved oxygen (hypoxic water), high stocking density and low water depth qualifies the fish as a suitable candidate for culture in small, swallow ponds. The skin of this fish, which is devoid of any scale and covered with a layer of mucus, acts as an air breathing organ in addition to the supra branchial chambers, the gill plates and the respiratory tree that act as accessory respiratory organs in the fish (Dutta Munshi, 1961). The testes of *C. batrachus* are paired, elongated structure lying on lateral side to the alimentary tract within the peritoneal cavity and remain attached to the body wall and digestive system by means of connective tissue. The testicular lobes are connected to each other to a common sperm duct that opens through the urino-genital aperture. During the development process, the testes transform from very thin, delicate translucent, off-white structure to enlarged, thick, lobular, highly vascular, pinkish white structure.

The recommended technology for artificial breeding and seed production of *C. batrachus* (Anon, 2005), requires dissecting the male brood fish to collect the testis for preparation of sperm solution, resulting in mortality of the dissected male broods. A considerable number of male brooders need to be sacrificed to carry out artificial breeding by following this method known as dry fertilization method that leads to negative impact on the male broodstock population. Further, the male fishes so sacrificed are not in marketable form (dead with cut and wound), making the technology not economically viable for farmers. In an attempt to address this issue by keeping the fish alive after dissection and removal of the testis, it was observed that the fish has the amazing capacity to heal the incision without any medication or stitching within 30-35 days (Chetia Borah and Gogoi, 2014). The present communication includes the results of the further study on refinement of the technology by

using the self healing and regenerating capacity of the fish without stitching and medication as well as regeneration of lost testicular tissue in due course.

MATERIALS AND METHODS

Location of study

The study was a part of the trial on refinement of breeding technology of *C. batrachus*, being conducted in continuation of the ICAR-DARE Mega Seed Project entitled Seed production of Cat fish Magur, at Fisheries Research Centre, Assam Agricultural University, Jorhat, Assam, India situated in between 26°48' 296" N Latitude and 94°11'961" E Longitude, during 2015-2020 (Fig 1).

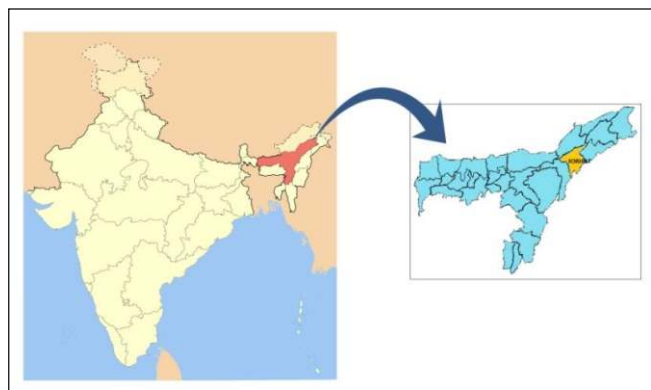


Fig.1: Location of the study.

The study was conducted in two steps. In the first step, mature male magur brooders (weight 110-190 g/fish) were dissected to take out the testes during peak breeding season (May-June) for artificial breeding as per the recommended technology (Anon, 2005). The process of dissection (in straight line beginning with vent, 4.0 - 4.5 cm length) was made with minimum injury to the surrounding tissue, blood vessels and internal organs (Chetia Borah and Gogoi, 2014). To achieve this, a small scissor with pointed edge was taken, gently inserted through the vent, lifted a little to facilitate dissection of the body wall in a smooth straight line to create a slit like opening, without damaging the surrounding tissues (Fig 2 - 4).

After removal of the testes, the fish were released in FRP tanks (1m X 0.75m X 0.75m, filled with 20-25 cm depth of water) at the rate of 5 fish/ tank and reared for 40 days. Fish were fed with formulated feed with 35% protein content from the second

day of surgery. Daily observation on behavior of the fish, mortality, and secondary infection was recorded. Rate of survival and time period required for complete healing were studied up to the 40th day after incision. The physico-chemical parameters of water were recorded at an interval of 8 days during the period by following standard procedure (APHA, 1989).



Fig 2

Fig 3

Fig 4

Fig. (2-4): Refined technique for incision and collection of testes.

In the second step, the recovered fishes were aggregated randomly in 10 groups and reared in cement cisterns (length 3.0m, breadth 2.0m and height 0.75m) for different observation period viz, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 days for studying the post surgery survival of the operated fish over long term and regeneration of the testis. The bottoms of the cisterns were covered with mud (10-15cm thickness) and filled with 60±5cm depth of water supplied from earthen pond. The water of the cisterns was exchanged partially at monthly interval and completely in every six months. The water quality parameters were studied at monthly interval following standard procedure (APHA, 1989). The stocking was done at the rate of 2 fish/sqm (12 fish/cistern).

The recovery percentage was recorded at monthly intervals by partial dewatering of the cistern and counting the number of recovering fishes. Observation on post-surgery regeneration of testes was done by random sampling.

RESULTS AND DISCUSSION

Regeneration is the process of renewal of lost tissue or removed part of a living body and in broad sense is the resultant of vegetative reproduction of a variety of component cells required to recreate that lost tissue or body part. In this study it was observed that without stitching or using any medication, the healing process of the slit like mechanical wound made by incision was distinctly visible after 3-4 days.

No abnormal behavior was observed in the fish during these days except excessive mucus secretion, slow movement and minimum feed intake during initial 2-4 days. In the beginning of the healing process, a thin membrane was observed covering the wound, which became thicker gradually as the days went by and finally the slit was joined. The results of the trials on post surgery regeneration and survival of the test fish are presented in the Table 1. The rate of survival in different trials was found to be 83.9-92.6 % (average 87.8 ± 2.9 %). Within 30-35 days, the process of healing of the mechanical wound was completed and the incision mark was found disappeared indicating complete healing (Fig 5-8). This was in agreement with the preliminary report on the amazing capacity of self healing of mechanically induced wound in the fish (Chetia Borah and Gogoi, 2014).

Earlier study on healing process suggested that the healing process of deep wound is somewhat complex and may take weeks or months, depending on wound severity, the fish species and the rearing environment (Richardson *et al.*, 2016). The epidermis of the skin of magur was reported to be a typical stratified epithelium distinctly divided into three layers of cells epithelial cells, mucous cells and club cells. The epidermis and the mucus layer known to have critical roles in skin repair as the mucus gel secreted by epithelial mucus cells protects the epithelial surface (Dash *et al.*, 2018). In this study, the wounded fishes exhibited excessive mucus secretion when released in water after the dissection. The mucus gel enhanced the wound healing process through its haemolytic activity and promoted vasoconstriction of smooth muscle cells in addition to providing physical and antimicrobial protection of the wounded surface (Al- Hassan *et al.*, 1985 and 1986). Earlier it was reported that different component skin cells of fish response and contribute to wound repair and regeneration in teleost fish (Richardson *et al.*, 2013, 2016).

No secondary infection was recorded in the experimental fishes which may again due to the protective mucus layer over the wound. The mortality recorded (8.0-18.2%) was might be due to handling stress during administration of

hormone and surgical trauma. These findings indicated that this remarkable capacity of self healing of mechanical incision of the fish can be used to address the problem of mortality of the fish during the process of testis collection. An average of 8.0% (3.8-11.1%) of the revived fishes in different trials showed malformation of the regenerated tissue (Table-1), which might be due to improper incision and handling (Fig. 9). Earlier Dutta and Rai (1994) reported healing of mechanically induced skin in *Clarias batrachus* with 5X3mm cut within 35 days. Recently Sanap *et al.* (2018) reported post surgery survival of the male brooders of the species and faster healing process by stitching with absorbable surgical stitching thread and using Mupiban ointment. This process (Sanap *et al.*, 2018) apparently involved handling of the fish for longer time and probable secondary injury and infection. This along with the needed expenses (for procuring absorbable surgical thread and ointment) and requirement of stitching skill might make the technique not economically viable and farmers' friendly. As such the procedure of healing the incision by using the inherent self healing capacity of the fish without any stitching or medication, as followed in the present work, was found to be easier and more convenient for the farmers.

The natural healing process in the fish observed in this study opens up probability of using the skin of *Clarias batrachus* as a scaffold or skin substitutes in treatment of human wound, as fish skin has been identified as a potential biocompatible, biodegradable skin substitute having a high collagen content (Yamada *et al.*, 2014; Tang and Saito, 2015). Earlier report on Tilapia skin when used as scaffolds or skin substitutes indicated acceleration of wound healing process and combat local infections (Zhou *et al.*, 2016). Earlier in a study conducted on Zebra fish, Richardson *et al.* (2013) observed that fish skin recovers from wound faster than human skin and does not result in scarring. Another report suggested application of fish mucus on mammalian wounds may activate healing (Al Hassan, 1990; Al Hassan *et al.*, 1991; Akunne *et al.*, 2016). Pioneering use of fish skin in tissue regeneration for burn victim was done which revealed quick healing without secondary infection after treatment with fish skin graft.

There involves less risk of disease transfer too (Khursid *et al.*, 2019).



Fig 5

Fig 6

Fig 7

Fig. (5-7) Self-healing process of mechanical wound in *Clarias batrachus*.



Fig. 8: Complete healing of the incision 30-35 days. Fig. 9: Malformation of regenerated tissue.

Earlier works on fin regeneration in teleost fishes indicate that the process is extremely sensitive to external physical and chemical factors, such as temperature variation (Johnson and Weston, 1995) and action of some environmental contaminants (Zodrow and Tanguay, 2003). The range of water quality parameters recorded during this study were temperature 22.7-34.7°C, depth 18.8 cm- 25cm, pH 6.5 -8.1 and DO 4.9- 7.1 mg/l. The long-term post-surgery rearing of the

experimental fish revealed 83.3-91.7% survivability in different replication in the first year. In the second year, the rate of survival was higher (94.1-100%). The survival rates indicate the good health status and complete recovery of the fish from the wound and trauma of surgery. The findings also revealed that the prevalent water quality parameters recorded (table 2) during the period of experiment are favorable for the survivability and regeneration process of the fish.

Details of observation recorded on regeneration of testes in the experimental fish are given in table 3. There was distinct sign of regeneration of the testicular tissue after 190 ± 10 days in 90% of the randomly sampled fish. The testis was found to be developed fully in 86.9% of the sample after an average 280 -310 days of post surgery rearing (during the month of May- June) (Fig. 10).

Some species of phylogenetically primitive vertebrates such as amphibians (Urodele amphibians, Salamanders) and fish (Teleost) are capable of regenerating certain organ or part of body but not the entire body (Poss *et al.*, 2000) Zebra fish (*Danio rerio*), a tropical fish belonging to family Cyprinidae, was reported to have the capability of regenerating certain organs of body like heart, limbs, fins, optic nerve scales, muscles and spinal cord (Poss *et al.*, 2000). Similar findings of testis regeneration were recorded in African Cat fish *Clarias anguillaris* after milt collection through ablation (Diyaware *et al.*, 2010). However, there is no report of regenerating

Table 1: Post surgery revival and healing of mechanical wound in *Clarias batrachus*.

Treatment No.	Nos. dissected	Wt. of fish (g)	Nos. recovered	% of recovery	Time required for healing (days)	Number and % Occurrence of malformation
1.	30	138 ± 27	27	90.0	32 ± 1	3(11.1%)
2.	28	160 ± 26	24	85.7	32 ± 2	2 (8.3%)
3.	31	155 ± 35	26	83.9	34 ± 2	2(7.7%)
4	30	142 ± 32	26	86.7	33 ± 2	1(3.8%)
5	27	128 ± 29	25	92.6	33 ± 3	2(9.1%)

the testicular tissue in *Clarias batrachus* (Linn). The findings of this study open a new avenue for reusing the magur male brooders for breeding

purpose after healing and regeneration of testes and hence are useful to address the inherent problems of the technology.

Table 2: Range of water quality parameters recorded during the study.

Expt. No.	Temp (°C)	Depth (cm)	pH	DO (mg/l)
1.	28.5±5.1	22.0±2.0	6.8±0.3	5.8±1.3
2	28.8±5.3	22.3±2.7	6.9±0.5	6.2±1.1
3	28.5±5.8	21.6±2.5	7.3±0.8	6.3±0.6
4	29.2±5.1	22.0±2.5	7.2±0.7	5.6±0.8
5	29.3±5.4	21.4±2.6	7.5±0.6	5.3±0.5

Table 3: Observation on post- surgical regeneration of testis in *C. batrachus*.

Replications	Nos. stocked	Nos. recovered	% of recovery	Average time period for regeneration of testis (days)	% of fish recovery with regenerated testis	
1 st	1	24	21	87.5	287	86.3
	2	25	22	88.0	293	
	3	24	20	83.3	300	
2 nd	1	16	14	87.5	310	87.5
	2	17	15	88.2	298	
	3	15	13	86.7	280	

**Fig. 10: Post- surgical regeneration and development of testes in *C. batrachus*.**

The present findings open a new direction of research towards utilizing the capacity for the benefit of aquaculture sector. Further studies at molecular level are required to identify the responsible component as well as to understand the genetic programming for the self healing and regenerating capacity of Magur and to explore

its potential application in aquaculture as well as in biomedicine.

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