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Effects of temperature on the caudal fin regeneration of Flying Barb, *Esomus danricus* (Hamilton, 1822)

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Abstract
The present investigation was carried out in winter season to investigate the effect of different temperature ranges on the caudal fin regeneration of Flying Barb *Esomus danricus* in glass aquariums (30.0 x 15.0 x 15.0 cm). Hence the fishes were reared at three different temperature treatments *viz*. 22±1°C (T_{22}), 26±1°C (T_{26}), 30±1°C (T_{30}) with three replications of each treatment for 30 days under laboratory condition. Fishes were fed twice daily with mixed zooplankton upto adlibitum. Ten numbers of fish species (n=10, Mean caudal fin length=5.240±0.274mm) were stocked into each glass aquariums following a completely randomized design (CRD) after the amputation of the caudal fin using sterilised scissor. Here the temperature was maintained by using thermostats and aeration was provided. At the end of the study, the higher temperature (T_{30}) treatment group shown the highest ($P < 0.05$) total mean caudal fin length followed by T_{26} treatment group compared to lower temperature (T_{22}) treatment group. The length increment (mm) gain and percent weight gain was found significantly ($P < 0.05$) higher in T_{30} and T_{26} treatment group compared with T_{22} treatment group. It has also observed that there was no mortality during the experimental periods. The results indicated that that high temperature has an overall stimulating impact on the structural recovery caudal fin of *Esomus danricus* may help to consider the fish as model fish for genetics, biotechnology and biomedical studies.

Keywords: Flying Barb; Regeneration; Caudal fin; Temperature changes.

1. Introduction

For more than two centuries, the concept of regeneration has fascinated among the biologists. When amputated, the bony rayed fins of teleosts regenerate quickly to restore the lost part (Morgan, 1900) [16]. This property of fin regeneration in fish may help to judge the fish as model fish for genetics, biotechnology and biomedical studies. The fins have simple architecture, consisting of numerous segmented, bony fin rays composed of concave, facing hemirays that surround connective tissue, nerves and blood vessels, is an excellent model organ for studying regeneration (Ferretti and Géraudie, 1995) [8]. In biology, regeneration is the method of renewal, restoration, and growth that makes genomes, cells, organisms, and ecosystems resilient to natural fluctuations or events that cause disturbance or damage (Birbrair *et al*., 2013) [3]. It is a gene regulation (Himeno *et al*., 1992; Bryant and Fraser, 1988) [12, 7] mainly refers to the morphogenic processes that allowing multi-cellular organisms to repair and maintain the veracity of their physiological and morphological states. The capability to restore organs and appendages is not common among fishes. Fish, being cold-blooded animal is affected by the temperature of the surrounding water which influences the body temperature, growth rate, body development, food consumption, feed conversion and other body functions (Britz *et al*., 1997; Azevedo *et al*., 1998) [5, 2]. Increasing temperature leads to increase in the efficiency of food energy conversion to net energy (Brett and Groves 1979) [4], thus increasing the scope for growth and development. Therefore, water temperature is a dynamic force in the fish growth, development and maintenance since its effects are more than any other single factor. Every fish species have a suitable temperature range within which it grows rapidly.

Flying barb fin regeneration can be emerged as an attractive model system in the study of regenerative biology. The species has several advantages like, rapid regeneration time, relatively simple architecture of the caudal fin and the ability to maintain and study flying barb in large numbers. The flying barb, *Esomus danricus* (Hamilton, 1822) [11] belongs to the family *Cyprinidae*, is a small indigenous economically important fish species mostly inhabits shallow water bodies of Indian sub-continent (Froese and Pauly, 2015) [9]. It is locally known as Darkina, Dadhikha, Danrika, Jongia, Chilwa, Jhai, Astapakke, Messaparavi and Meesa-parava (Talwar and Jhingran, 1991) [18]. The flying barb, *E. danricus* inhabits ponds, weedy ditches, streams, beels and submersed shallow water. Recently, *E. danricus* also considered to a potential indigenous ornamental fish due to their colour, size and playful behaviour (Froese and Pauly, 2015) [9]. However, Broussonet (1786) [6] in his experiment on fin regeneration of gold fish decisively found out that the caudal fin shows experimental advantages over the other fin types as it regenerates more rapidly than the ventral, pectoral, and dorsal appendages. Likewise, Sîrbulescu and Zupanc (2010) [17] also established the significantly re-growth of the tail of weakly electric fish kept at high temperature i.e. at 30°C and importantly, he observed the advantageous effect of increased temperature on both structural and behavioural healing after amputation of the caudal fin of weakly electric fish. In fact, this findings still holds today, and recent research continues to use the tail of fish to study the cellular, molecular mechanisms and also its effects with changed physiological factors underlying organ recovery in vertebrates. Keeping in view the information given above, the research was designed to find out the effect of different temperature ranges on the caudal fin regeneration of flying barb.

2. Materials and Methods

2.1. Experimental animal and design procedure

This experiment was conducted in the wet laboratory under Department of aquaculture, College of Fisheries, Tripura, India to study the effect of different temperature ranges on the caudal fin regeneration of Flying Barb for 30 days. The aquariums (30.0 x 15.0 x 15.0 cm, 9 nos. aquariums) set up, desired temperature maintenance, working space etc. were assured before the experiment program. Three different temperature treatments 22±1°C (T_{22}), 26±1°C (T_{26}), 30±1°C (T_{30}) were tested on flying barb with three replicate for each temperature treatment. Fishes were acclimated to laboratory surroundings for about two week before the experiment started. The fish were not fed for 24 h before they were transferred to experimental aquariums. Prior to the stocking, the fishes were anesthetized using clove oil at a rate of 0.04ppm and then, amputated using sterilised scissor at the base of the caudal fin (little away from caudal fork). Each aquarium tank was stocked with 10 nos. (Mean caudal fin length=5.240±0.274mm) fishes and the temperature of each treatment was maintained to a desirable level by thermostat. Water was changed in every weekly interval at the rate of 30-40% of the total unit volume with water that had similar temperatures and also siphoned to remove the faecal matters. They were fed twice daily at 10.00 AM and at 5.00 PM with mixed zooplanktons upto *ad libitum* in three different temperature conditions. All fish were individually measured using vernier scale in each aquarium on a regular interval of 3 days till their caudal fin fully regenerate. The water quality parameters were analysed at every 4-5 days (APHA, 2005) [1] in the early morning (8:00–9:00 a.m.) hours. Five fishes were taken from each treatment for sampling to record the total caudal length and released back in their respective tanks. In the present study, the growth parameters such as length increment (mm) and length gain percentage undertaken to find out the significant difference among the temperature treatments. At the end of the experiment (day 30), all the survived fishes were counted and recorded.

The experimental data were statistically analyzed by one-way analysis of variance (ANOVA) and the significant difference among the treatments means ($P<0.05$) were determined by Duncan's multiple-range test using SPSS (Version 16.0) statistical software. Results are presented as mean ± SE (standard error).

3. Results

Physio-chemical parameters such as temperature (°C), pH, dissolved oxygen (mg/l), total alkalinity (mg/l), total hardness (mg/l), ammonia nitrogen(NH^{3+}-N, mg/l) were presented in Table 1. These water quality parameters were within the acceptable limits except temperature recommended for the rearing of ornamental fishes (Jain *et al*., 2016) [13], which was possibly due to water exchange (with water that had similar temperatures), aeration and siphoning from the aquariums during the experiment. The total caudal fin length of *Esomus danricus* reared in three different temperature ranges were analyzed and were reflected in Table 2 and figure 1. The total length of the caudal fin immediately after amputation did not have any significant difference ($p>0.05$) among the treatment. Thereafter, the result of the experiment showed that the total caudal fin length was significantly highest ($p<0.05$) in T_3 (6.524±0.150 mm) and lowest in T_1 (4.837±0.251 mm) group of the fishes on 3rd day. Similar trend was found during 6th, 9th and 12th day of observation. It has been found that on 12th day the regeneration was complete in T3 group whereas the progress was little slower in T2 (14th Day) followed by T1 (30th Day). In the present study, length increment (mm) was found slower in T_{22} (1.833±0.331) treatment group while T_{26} (4.118±0.399) and T_{30} (4.728±0.261) shown faster growth and no significance difference ($p>0.05$) is observed in the T_{26} and T_{30} groups. Similar results were also obtained in length gain percentage where T_{22} (41.444±8.414 mm) was shown lower length gain percentage ($p<0.05$) while twofold length gain percentage ($p>0.05$) was achieved with those of the T_{26} (93.649±18.646 mm) and T_{30} (86.314±7.672 mm) groups. This was due to better feed utilisation in the warmer temperature compared to others. No significant response ($P>0.05$) was recorded in survival (%) among the treatment groups.

Table 1: Physico-chemical parameters of water during the rearing of *Esomus danricus* in different temperature ranges

Parameters	22±1°C (T_{22})	26±1°C (T_{26})	30±1°C (T_{30})
Temperature (ºC)	22.33± 0.28	26.37± 0.27	30.31± 0.26
Ph	7.60-7.83	7.58-7.85	7.61-7.80
Dissolved oxygen (mg L^{-1})	7.53-8.83	7.50-8.35	7.61-8.79
Alkalinity (mg L^{-1})	56.4-60.8	54.0-63.6	55.6-63.0
Hardness (mg L^{-1})	35.0-42.2	35.2-43.3	35.0-45.2
Ammonia (mg L^{-1})	0.01-0.025	0.025-0.043	0.04-0.052

'±' indicate standard error (S.E.)

Table 2: Showing details on the effect of temperature on the caudal fin regeneration of *Esomus danricus* during the experiment

Parameters	22±1°C (T_{22})	26±1°C (T_{26})	30±1°C (T_{30})
Before amputation			
Initial caudal fin length (mm)	10.817^a±0.319	10.109^a±0.154	10.405^a±0.210
After amputation			
Initial caudal fin length (mm)	5.031^a±0.286	5.025^a±0.329	5.663^a±0.208
3rd day caudal fin length (mm)	4.837^a±0.251	5.690^b±0.145	6.524^c±0.150
6th day caudal fin length (mm)	5.515^a±0.236	7.536^b±0.175	8.395^c±0.190
9th day caudal fin length (mm)	6.677^a±0.233	8.297^b±0.179	9.156^c±0.269
12th day caudal fin length (mm)	6.863^a±0.104*	9.143^b±0.179**	10.391^c±0.148***
Length increment (mm)[1]	1.833^a±0.331	4.118^b±0.399	4.728^b±0.261
Length gain (%)[2]	41.444^a±8.414	93.649^b±18.646	86.314^b±7.672
Survival (%)[3]	100	100	100

* Treatment group (T_{22}) shown the complete regeneration of caudal fin on 30th day.
** The regeneration progress was little slower in T_{26} treatment (15th-16th days after amputation).
*** Earlier regeneration was observed in T_{30} treatment (12th day after amputation).
**** (a-c) Mean values in the same row with different superscripts are significantly different (P<0.05) (mean ± S.E.).
[1]Length increment (mm) = Final length – initial length;
[2]Length gain (%) = 100 [(final length – initial length)/ initial length];
[3]Survival (%) = 100 [(Number of surviving fish/ Total number of fish stocked).

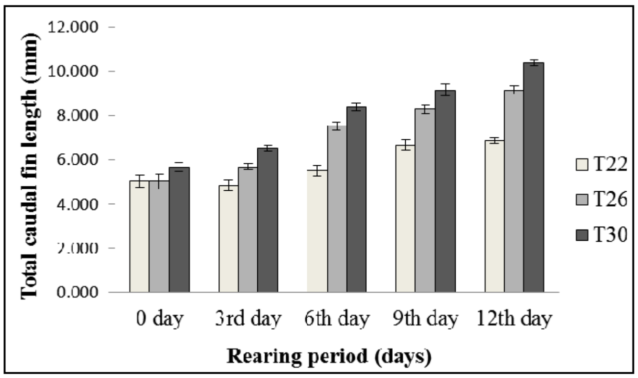

Fig 1: Total caudal fin length (mm) of *Esomus danricus* reared in different temperature range. The data are based on the means (±SE) of triplicate tanks in each group (T_{22}, T_{26} and T_{30})

4. Discussion

Aquaculturists are trying to find the most favourable temperature conditions for each species so that fish will have better growth, developments and organ regeneration. The present study tries to discover the effect of temperature on structural recovery of caudal fin in a regeneration-competent, poikilotherm model organism. In this study *Esomus danricus* were reared in three temperatures range namely 22±1°C (T_{22}), 26±1°C (T_{26}) and 30±1°C (T_{30}) for about 30 days under laboratory condition. The results of the study indicated that there was significantly higher length increment (mm) in T_{26} and T_{30} treatment groups compared with lower (p<0.05) length increment in T_{22} treatment group. Similar observation was also reported in the weakly electric fish, *Apteronotus leptorhynchus* where temperature manipulation has been shown to significantly affect recovery after spinal cord injury and also structural regeneration of caudal fin after amputation (Sîrbulescu and Zupanc, 2010) [17]. Sîrbulescu and Zupanc (2010) [17] found significant improved structural and functional recovery of caudal fin in *Apteronotus leptorhynchus* after amputation kept under warm-water conditions was paralleled, and possibly caused, by a reduced duration of the post-lesion wave of apoptosis and by an increased rate of cell proliferation (Sîrbulescu and Zupanc, 2010) [17]. The results of the present study also found significantly higher structural and functional recovery of caudal fin after amputation with higher temperature where on 12th day the regeneration was complete in T_{30} treatment group whereas the progress was little slower in T_{26} (14th-15th Day) followed by T_{22} (30th Day) treatment group.

Brett and Groves (1979) [4] found that in fishes an increasing in temperature leads to a faster growth rate and developments, in parallel with a higher food intake. Similarly, as a poikilothermic aquatic animal, growth developments and organ regeneration is strongly dependent on water temperature where it lives. It was clear that environmental temperature promotes growth through a direct effect on growth hormone (GH) secretion that leads to an increase of plasma IGF (Insulin-like growth factor) levels in connection with the growth and development (Gabillard, 2005) [10]. In present study, fish kept at 26±1°C and 30±1°C showed an almost twofold increase in length gain (%), as compared to fish kept at 22±1°C. So the present result is in conformity with the theory that high temperature has a stimulating effect on cellular metabolism in poikilotherm organisms. The present study is in agreement with the explanations in goldfish, where with increasing temperature (up to 35°C), the proliferation rate of primary cells also increased proportionally (Kondo and Watabe 2004) [14]. The low recovery of caudal fin in *Esomus danricus* at lower temperature (T_{22}) led to lower metabolism which subsequently to decreased cell proliferation. As results metabolic rate decreases which affect on GH secretion that leads late regeneration of caudal fin.

5. Conclusion

In conclusion present study suggested that the caudal fin of flying barb, *Esomus danricus* regenerates better in high temperature (30±1°C) followed by (26±1°C) compare to low temperature (22±1°C). Hence, the study explores the beneficial effect of ambient temperature on the quick recovery of the tail of *Esomus danricus* than that of the lower temperature. Thus, the study gave baseline information about fin regeneration capacity of fish which will be helpful for considering the species as model fish for genetics, biotechnology and biomedical studies.

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