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Optimizing Acclimatization for *Channa punctatus* in Different Laboratory Environments

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Authors' contributions

This work was carried out in collaboration between both authors. Author DNC designed the work and prepared the manuscript. Author PKA installed and monitored the whole experiment. Both authors read and approved the final manuscript.

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ABSTRACT

The present study was conducted to investigate the optimized acclimatization process of *Channa punctatus,* a freshwater air breathing fish in varying laboratory conditions, using four different tanks setups – glass bottom, sand bottom, sand-soil mix and mud bottom. The study aimed to identify the optimal condition for survival and adaptability. Observation also included mortality rates, changes in pigmentation and behavioral anomalies. Results indicated that tank with mud bottom provided the best environment for acclimatization, highest survival rates and least stress indicators. These findings offered a valuable insight for laboratory studies and conservation efforts involving *Channa punctatus* under controlled conditions.

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Keywords: Acclimatization; Channa punctatus; behavior anomalies; mortality; laboratory condition.

1. INTRODUCTION

Acclimatization is a process in which an individual organism adjust to a change in its environment (such as a change in altitude, temperature, humidity, photoperiod, hardness or pH) allowing it to maintain between a range of environmental conditions (Mathur, 2019).

Experimental Study is an important aspect of biological research. Experimental study using live intact animals (fishes, rats, mice, rabbit etc.) have continued to play essential role in development of new knowledge and better understanding of life process (Ghosh et al., 1990; Akter et al., 2008; Pandey et al., 2011).

The use of intact animal in biological research for efficient and effective design is always required. In this context, different species of fishes (species of *Channa, Clarias, Heteropneustes, Anabas etc*) have been frequently used as biological model in the laboratory conditions (Hughes & Singh, 1970; Munshi et al., 1982; Singh et al., 1982; Patra et al., 1983; Ghosh et al., 1986; Ghosh & Munshi, 1987; Ojha et al., 1988; Okpala et al., 2017).

Fishes form one of the important group of vertebrates influencing the human life in various ways. Fish is a cold blooded animal, which has the ability to adjust with the changed environment (Al-Arabi et al., 1993; Akter et al., 2008). At the same time they are useful indicator of environmental quality and ecological integrity (Roy & Munshi, 1987; Khanna & Singh, 2017). The unique adaptation and physiological specialization of fish made them especially suitable for use as physiological and biological model (Lakra & Nagpure, 2009; Pandey et al., 2011). Fish occupies a peculiar position in the hierarchy of animal life because higher vertebrates evolved from them. Fishes have great importance as a secondary consumer in the food chain. This significance is magnified as it forms a major food for higher trophic levels (Kumar & Tembhre, 2014).

Presently due to rapid industrialization and discharge of pollutant in their aquatic ecosystem, fishes and other aquatic animals are running under threat and ultimately causing higher mortality (Ramona et al., 1979; Kumari & Ram Kumari, 1997). Many laboratory and field studies suggest that aquatic organism may acclimate to some toxicants. It has been hypothesized that animal exposed to sublethal level of a pollutant could become more tolerant or weakened depending upon the mode of action of the poison at which the animal has been acclimated (Sprague et al., 1965). Various acclimatization methods have been applied to overcome the fish mortality.

So far as *Channa* species are concerned it is not easy to keep them in laboratory for longer period as compared to other fishes like species of *Anabas*, *Clarias, Colisa and Heteropneustes.*

Keeping the above facts in mind, the present experiment was conducted to investigate the optimized acclimatization process for *Channa punctatus*, a fresh water air breathing teleost fish in different laboratory conditions.

2. MATERIALS AND METHODS

2.1 Test Animal

The live healthy specimens of fresh water air breathing fish *Channa punctatus* (Total length 15.5 – 17.5cm and body weight 35-45 g) were collected from local fish markets of Bhagalpur, Bihar,and brought to the laboratory. *Channa punctatus*, locally known as Garai, is easily available snake headed air breathing fish belong to the family Channidae and order Channiformes. These fishes are mostly found in the fresh water pools & wetlands like ponds, ditches chaurs and swampy areas of Northern India. These fishes can survive in oxygen deficient water bodies (Munshi et al., 1976; Ojha et al., 1981; Ghosh & Munshi, 1987).

2.2 Experiment Design

The experiment trials were conducted in four glass tanks (aquaria) arranged in a row on a wooden table in the departmental Research Laboratory. Fishes were categorized into four groups each containing 10 live specimens of *Channa punctatus*. These fishes were placed in four glass tanks (size 50cm×30cm×25cm) separately containing 25 liters of water in each tank. The tanks were named as A,B,C & D.

All tanks (A-D) containing 10 live test specimens, had different bottom habitat (glass bottom sand bottom, sand- mud mixture bottom and pure mud bottom). Fishes were kept under observation for the period of 4 weeks for acclimatization to laboratory conditions. Tank A was with only glass bottom, tank B was covered with pure sand (1 inch thick) on the bottom, tank C was having equal proportion of sand and soil of the same thickness, whereas the tank D had mud at the bottom of same thickness.

All the four tanks (A,B,C & D) were kept in the laboratory and the photoperiod was maintained as per the normal day and night around 10 hours with proper optimal condition for fish. Around 50% of water from each tank was replaced with tap water after every week during the experiment period. No mortality occurred during this period. All the effort was made to provide natural environment to the fishes as far as possible in the laboratory. During this period fishes were fed boil chicken eggs and chopped parts of small fishes @ 5% of body weight approximately on alternate days (Pandey et al., 2011).

The experiment was conducted in Biodiversity laboratory of Univ. Dept. of Zoology, TMBU, Bhagalpur, Bihar during the month of October 2023. Fishes were exposed and frequent monitoring was made to observe the behavior pattern and survival of the fishes. A fish was considered dead when no response observed after touching/probing with glass rod. Dead fishes were immediately eliminated from the tank. Total mortality and abnormal behavior (if any) were monitored and recorded regularly between 8a.m. – 5p.m. Every observation and data were collected properly to draw better inferences.

3. RESULTS AND OBSERVATIONS

During the study of four weeks, mainly the mortality observations and behavioral responses were recorded:-

- No any mortality or significant changes in behavior was noticed among the fishes in the four days of experiment in all the tanks (A-D).
- At the beginning, fishes were found to be healthy and very active. But after four days of the experiment, fishes of tanks A, B & C expressed some structural and behavioural abnormalities.
- On fifth day of experiment some changes were noticed among the fishes of tanks A, B & C. Fishes were found less active. Swimming as well as body pigmentation were also found decreased in comparision to fishes of the tank D. Colour of the fishes changed from dark black to pale in colour.
- Feeding attempts and feeding activity were also found decreased in among the fishes in among all the tanks (A-D).
- On day eight, it was noticed that from some fishes of tanks A&B were showing random swimming movement and loss of equilibrium.
- Fishes of tank C were also found in stress, showed increased opercular activities.
- Fishes of tank D were expressing normal swimming movement. No sign of above mention abnormalities were noticed among the fishes of tank D till date.
- Mortality of fishes were recorded after eight days of experiment. 02 fishes from tank A&B and 01 fish from tank C was found dead in the morning on day 9th of the experiment. These dead fishes floating on the water surface keeping their bodies upside down.
- Similarly, on day twelve again 02 fishes of tank A and 01 fishes each from tank B&C died.
- No mortality was recorded among the fishes of tank D. Fishes were normal and these were spending most of the time buried in the mud. They were coming to water surface for engulfing air at regular intervals.
- After 3 weeks (22nd day) of experiment 01 fish died from tank A&B in the afternoon.
- Before death it was noticed that fishes were assigned in vertical position with keeping its mouth near the water surface and tail in downward direction. Thereafter, they settled down passively at the bottom of the tank turning its bellies upward.
- At the completion of four weeks mortality rate was found maximum among the fishes of tank A (50%), 30% among the fishes of tank B, 20% among the fishes tank C and no mortality was recorded up to this date among the fish of tank D.
- Except colour changing and no sign of any further mortality behavioral anomalies were recorded among the fishes of tank D at the completion of this experiment.

Table 1. Mortality and behavioral anomalies among the fishes during acclimatization indifferent laboratory conditions

Fish tanks	No. of fishes	Days of mortality of the fishes					Total mortalities	Behavior observed	
		7th	8th	9th	12th	22nd		Before 1st week	After 1st week
Tank A	10	-	02	-	02	01	05	L.A. L.F.A, L.P	L.E.
Tank B	10	-	02	-	01	01	04	S.F.A.,L.P.	L.E.
Tank C	10	-	-	01	01	-	02	F.O.A.& L.F.A	I.O.A
Tank D	10	-	-	-	-	-	-	N.	N.

Abbreviations :-
L.A- Less Active, S.F.A - Slow Feeding Attempts, F.O.A - Fast Opercular Activity, N.- Normal, L. P- Less Pigmentation, L.S- Less swimming, L.F.A.-Less Feeding Attempts & I.O.A-Increase Opercular Activity, L. E.- Loss of Equilibrium

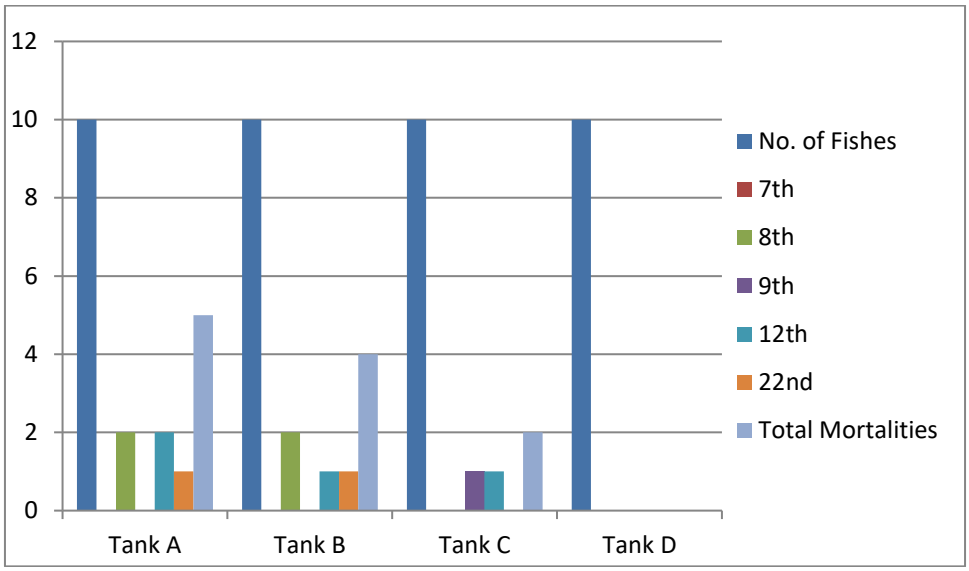

Fig. 1. The number of mortality of fishes in different tanks (habitat) during acclimatization

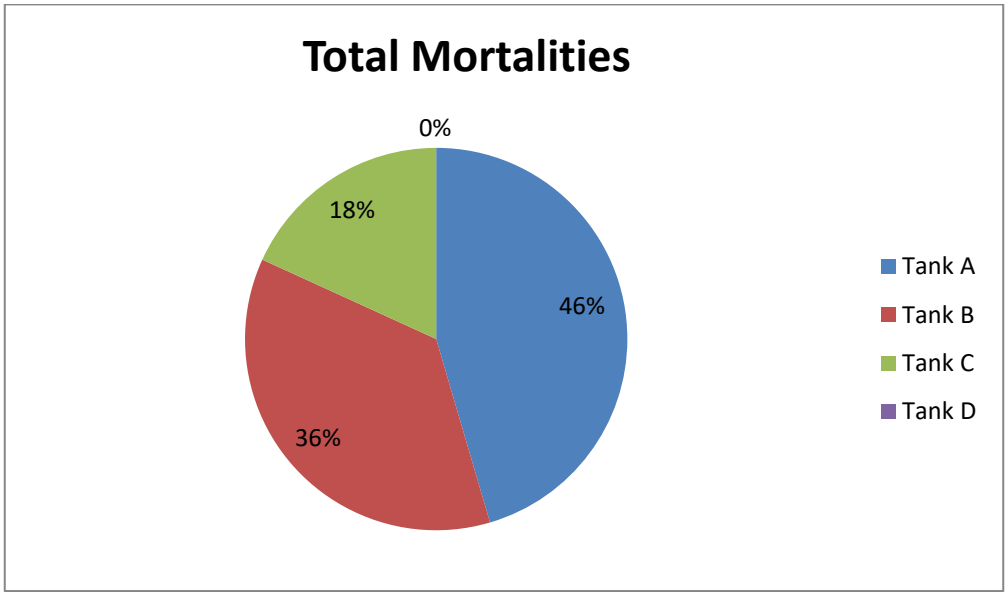

Fig. 2. The % of mortality of the fishes in tanks (A-D) in different habitat conditions

4. DISCUSSION

The present study elucidate the better acclimatization of *Channa punctatus* in different laboratory conditions. The behavioral study gives direct responses of the fish to the ambient environment or chemical present in the water (Pandey et al., 2011). Warner et al. (1966) and Radhaiah et al. (1987) suggested that behavioral anomalies of an organism represent the final integrated result of diversified biochemical and physiological processes.

Most of the fishes under observation were found less active and expressed erratic swimming movement. It may be the effect of stress caused due to sudden change in their habitat condition of their new aquatic environment (Srivastava et al., 2010). Roy & Munshi (1989) also reported that fishes need a stable environment during the acclimatization process in specially in terms of stable pH.

Body pigmentation were also decreasing gradually in almost all the fishes on all the tanks (A-D), may be due to dysfunction of the pituitary gland under stress causing changes in the number and distribution of chromatophores (Pandey et al., 1990; Yadav et al., 2007; Ramesh & Saravanan, 2008).

Behavioral anomalies as a result of stress are further accepted at the most sensitive indication of adverse effect of aquatic environment (Nwani et al., 2010). Koprucu et al. (2006) also observed lightening skin colour in the fingerlings of European cat fish exposed to contaminated water. There are few evidence that alteration of the chemical composition in the natural aquatic environment, usually effect behavioral and physiological system of inhabitants particularly fishes (Radhaiah et al., 1987; Khan & Law, 2005). Bad water quality or changes in physico-chemical properties of water as well as the unfavourable habitat of the fish tank may also cause stress or may influence the behavioral pattern of fishes including loss of equilibrium, increase rate of opercular activity and less feeding attempts, may be the another reasons.

Faster opercular cavity and loss of equilibrium were observed among the fishes of tank A, B, & C Hassanein & Okail (2008) had also observd loss of buoyancy and balance with an initial increase in the opercular ventilation rate which then decreased significantly in *Ctenopharyngodon idella* (grass carp) after exposure to the biopesticide. According to Fulton & Key (2001), the restlessness and hyperactivity in fish may occur due to the stress or inactivation of acetylcholinesterase, enzyme leading to accumulation of acetylcholine at synaptic junctions. Finally, fishes were almost paralysed and settled on the bottom of the tank and died. Mortality of fishes were recorded in tank A, B & C but maximum from tank A (50%) then tank B (30%) and tank C (20%) may be due to above mention reasons.

Survival rate was found maximum among the fishes of tank D (100%) may be due to favorable habitat and aquatic environment as *Channa punctatus* is an air-breathing fish and found in muddy and swampy areas with less oxygen content (Ghosh et al., 1990). It was well acclimatized in mud water, and showed more adaptability in this habitat then other fishes of other tanks, enhancing the implication for laboratory acclimatization.

5. CONCLUSION

Study clearly revealed that the fishes in tank D expressed more adaptability. Survival rate was also found maximum among these fishes in comparision to fishes of other three tanks. Fishes expressed some behavioral anomalies due to stress caused by the sudden change in their natural habitat or aquatic environment. Mudwater and adequate size of the tank /aquarium containing sufficient volume of water is the best habitat for the better acclimatization of *Channa punctatus* in the different laboratory conditions.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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