Research Article

EMERSION TOLERANCE WITH RESPONSE'S TO PHYSIOLOGICAL AND BEHAVIORAL IMPLICATIONS OF THREE COMMONLY AVAILABLE AIR BREATHING FISHES OF DIGBOI REGION, ASSAM

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ABSTRACT

One of the most comprehensive study that needs an attention to supplement fish diversity data is different kind of adaptations the fishes have adapted due to change in environmental conditions like drought and dry weather which ultimately affects the distribution pattern and use of varied habitats. Present study deals with physiological and behavioral implications of three commonly available air breathing fishes found in selected sites of Digboi region, Assam. The study was conducted in Digboi region for 6 months at different rivers and ponds of the study area from November, 2016 to March, 2017. To check the behavioral and emersion tolerance of amphibious fishes, three commonly available air breathing fishes were collected and passed through an experimental series. Gill ventilation rate was also observed during the period and behavioural changes were noted down during different phases. Among the three species of amphibious air-breathers, all showed significant differences in the pattern of refugia use across the different phases of treatment. However, Clarias batrachus showed better emersion tolerance and can adjust their behavior and physiology in response to emersion, more importantly reverse to a normal state after re-emersion much faster than that of Anabas testidineus and Channa punctatus

INTRODUCTION

Fishes are found in almost all aquatic systems with many diverse adaptations related to their living habitats. Amphibious air breathers have an advantage compared to other non-air breathing fishes as they are able to utilize atmospheric oxygen during periods of exposure to air (emersion) and many of them are capable of surviving periods without surface water (Martin et al, 1993; Sayer, 2005; Taylor et al., 2008). In contrast, aquatic air-breathers never leave water, but periodically break the water surface to gulp air (Gonzales et al., 2006). These capabilities confer several advantages, which include avoidance of hypoxic conditions and survival in temporary habitats (Eldon, 1979a; Graham, 1997; McPhail, 1999; Sayer, 2005). So in order to overcome stress and drought related situations, the air breathing fishes are capable of utilizing atmospheric oxygen during periods of exposure to air which in turn facilitates the use of various means of movement on land in search of new water bodies.

Air breathing fishes use a combination of behavioural, structural and physiological adaptations to survive without water on exposure to air (Sayer, 2005). Behavioral adaptations may involve burrowing into subterranean refugia or piles of vegetative debris and logs (Taylor et al., 2008), where cool damp conditions may allow fishes to survive temporary cessation of surface flow as observed in the mangrove killifish *Kryptolebias marmoratus* (Poey) (Taylor et al., 2008) and the Australian salamander fish *Lepidogalaxias salamandroides* Mees (Berra & Allen, 1989). Breathing out of water may be facilitated by possession of accessory respiratory structures, which include specialized lungs in the African lung fish *Protopterus* species (Maina & Maloiy, 1985), pharyngeal organs, such as the heavily vascularized buccopharyngeal cavity in the Gobiidae (Gee & Gee, 1995) and cutaneous respiration in the Canterbury mudfish *Neochanna burrowsius* (Phillipps) (Eldon, 1979b; Meredith et al., 1982). Physiological adaptations to emersion include modification of gill function (Graham et al., 1985; Ong et al., 2007), metabolic adjustments (McPhail, 1999; Richards, 2010) and mechanisms to reduce toxic waste products in air (Ip et al., 2004; Randall et al., 2004). All these studies examined whether a fish species known to be capable of air-breathing possess any certain

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adaptation to breathe out of water. Studies on air-breathing fishes are crucial in the understanding of the evolution of vertebrate air-breathing and the fish-tetrapod transition (Brauner *et al*., 2004; Graham & Lee, 2004). Such knowledge of the patterns of air-breathing capabilities is also important in understanding the factors that shape the biogeography and evolutionary history of freshwater fishes (Main, 1989; Fraser *et al*., 1995). The present study focuses on the amphibious survival capacity of such fishes by assessing some of its behavioural, structural and physiological adaptations for emersion survival.

METHODOLOGY

For assessing emersion tolerance and refugia use behavior exhibited by the amphibious fishes, 3 commonly available amphibious fishes of the study area were identified. Fishes were transported to a nearby field laboratory and kept overnight in aerated holding tanks. All the water used for experiment was collected from same site and kept at ambient temperature (23-27°C). Sand for substratum, rocks and vegetation was also collected from the same sampling site. To determine tolerance for each species, 4 plastic trays (one control and three experimental set-up) were taken and filled with required refugia and water. Three fishes of each species were placed in each tray and allowed to acclimatize for 6hrs. Trays were covered with net to reduce external disturbances. The experiment consisted of five phases: (1) pretreatment (P), (2) recession (R), (3) partial emersion (Pe), (4) emersion (E) and (5) re-immersion (Ri). Phases two (R), three (Pe) and four (E) are dewatering phases. The duration of each phase was 6 hrs.

For the pre-treatment phase (P), the trays were simply monitored to record the behaviour of the fishes before experimental manipulations. For the recession phase (R), half of the water was drained from each of the treatment trays, such that the fishes remained completely immersed in water. It took an average of 5 min. to drain this volume of water from each tray. Another quantity of water were drained from all treatment trays during the partial emersion phase (Pe) leaving only that much water so that the fishes were partially exposed to air. For the emersion phase (E), all the surface water was drained from the treatment trays, leaving only the sand, vegetation and underside of rocks moist and fishes completely exposed to air. Finally, for the re-immersion phase (Ri), water was added to the respective trays at the end of each emersion period. The survival of the fishes was confirmed and the time that the fish took to recover following re-immersion was noted. The fishes were monitored for at least another 12 hr. to assess post-treatment behaviour and survival. The control trays remained with water throughout the experiment. Observations were conducted throughout these phases. The no. of fishes seeking refugia or visible in open areas in each tray were noted down at each phase of treatment for three times viz. at the beginning of each phase (0 hr.), at midpoint (3 hr.) and finally at the end of each phase (6 hr.). Similarly, respiratory physiological responses to emersion was determined by visually quantifying opercular movement and calculating gill ventilation rate (GVR) as the number of opercular movements per minute for the three selected amphibious species for each phase of treatment. Three 1 minute observations viz. at the beginning of each phase (0

hr.), at midpoint (3 hr.) and finally at the end of each phase (6 hr.) were carried out to record the frequency of opercular movements (a proxy for Gill ventilation rate - GVR) for both control and experimental fishes. At the end of the experiment, the fishes were released alive at the site of collection. (Chakona.A *et.al*. 2011). To assess the structural adaptations of the three commonly available amphibious species identified at the sampling sites of the study area, the same species were collected from the market and dissected in the laboratory to carry out the morphometric measurements viz. organ colour, shape, size and weight. The results of these morphometric measurements among the three species were compared. For emersion and behavioral observation, to establish whether there were any differences in the use of refugia, the control and the experimental data were subjected to ANOVA with species as independent variable and again with phases of treatment as the independent variable to assess the differences in refugia use in different phases. Then, to assess whether exposure period affected refugia use, separate Kruskal Wallis for each species were carried out on the data from the emersion and re-immersion phases (four and five, the two phases likely to be affected) with exposure period as the independent variable. For experiment two, the data per phase for each tray were converted into means and ANOVA was carried out with species as independent variable and then again with the five phases as the independent variable to assess the differences in GVR between phases. To find out whether the mean opercular movement differed in Partial emersion and emersion phases of the treatment, Wilcoxon tests were performed.

RESULTS

In the first set of experiment the tolerance of three commonly available amphibious fishes of the study area viz. *Channa punctatus*, *Anabas testidineus* and *Clarias batrachus* to emersion was observed. There was no discernible pattern in the use of refugia among the control fishes across the three species (ANOVA, d.f = 2, *P* >0.05; Table - 1). For the treatment fishes, however the refugia use does show significant differences among the three species (ANOVA, d.f = 2, *P* <0.05; Table - 1).

Table 1 Response of control and experimental fishes to emersion across different species

Species name	Control		Experimental	
	Refugia	Open water	Refugia	Open water
Anabas testidineus	0.33 ± 0.12	2.7±0.13	1.96±0.31	1.04±0.31
Channa punctatus	0.47±0.13	2.6±0.13	2.49±.16	0.6±0.16
Clarius batrachus	0.2±0.107	2.8±0.18	1.07±0.29	2.33±0.43
ANOVA F value	1.183	1.183	7.585	8.471
(*P* value)	(*P*> 0.05)	(*P*> 0.05)	(*P*< 0.05)	(*P*< 0.05)

Table 2 Response of three amphibious species to emersion across the different phase of treatment

Phase of Treatment	Control Refugia	Control Openwater	Experimental Refugia	Experimental open water
Preatreatment	0.22 ± 0.15	2.8 ± 0.15	1.11 ± 0.43	1.9 ± 0.43
Recession	0.43 ± 0.11	2.9 ± 0.11	1.4 ± 0.33	1.4 ± 0.33
Partial emersion	0.44 ± 0.18	2.6 ± 0.18	2.3 ± 0.32	0.8 ± 0.32
Emersion	0.44 ± 0.18	2.6 ± 0.18	2.7 ± 0.23	0.4 ± 0.23
Re-immersion	0.44 ± 0.18	2.6 ± 0.18	1.6 ± 0.42	2.11 ± 0.74
ANOVA F value (*P* value)	0.976 (*P*> 0.05)	0.976 (*P*> 0.05)	2.895 (*P*< 0.05)	2.736 (*P*< 0.05)

Again the use of refugia by the three species also showed significant differences among the different phases of treatment in the treatment fishes (Table - 2, Fig. 1).
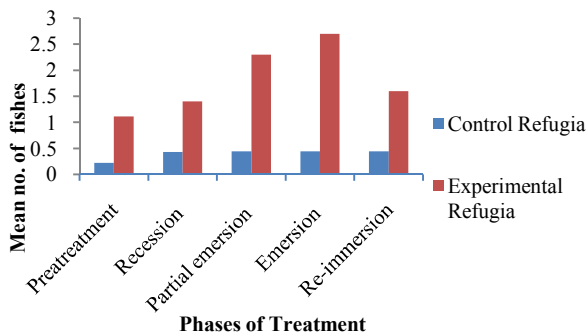

Fig 1 Response of control and experimental fishes of three species to emersion across the different phases of treatment

Similarly to assess whether the exposure period affects the use of refugia by the three species, separate Kruskal Wallis tests were carried out with the data of emersion and re-immersion phases (four and five, the two phases likely to be affected) of the three species. It was found that for *Anabas testidineus* and *Clarias batrachus*, the exposure period affects the refugia use significantly in both emersion and re-immersion period while it is not significant for *Channa punctatus* (Kruskal–Wallis test, $\chi^2 = 0.196$, P>0.05; Table - 3).

Table 3 Effect of exposure period on the refugia use by three amphibious species

Species	Phase of Treatment	χ^2 value	*P* value
Anabas testidineus	Emersion Re-immersion	4.355	0.037*
Channa punctatus	Emersion Re-immersion	0.196	0.69
Clarius batrachus	Emersion Re-immersion	4.5	0.034*

* P< 0.05

All the three species immediately responded to partial exposure (Pe) by moving over the wet sand using serpentine movements which was more conspicuously seen in *Channa punctatus*. Some of them made excavations in the sand while moving, mainly around rocks and clumps of vegetation, but none of the fish burrowed into the substratum. Most fishes became inactive while maintaining a dorso-ventral position within the first 2 h of emersion, but a few individuals occasionally moved laterally or rolled onto their dorsal side. No opercular movements or other visible respiratory behaviour was observed nor was there any visible mucus production in *Anabas* and *Channa* while *Clarias* showed both the opercular movements and mucus production during emersion. All the three fishes responded rapidly to re-immersion. Approximately 25% of the fish were positively buoyant, suggesting that their gas bladders were inflated or they had taken an air bubble into the mouth and gill chamber. All the fishes expelled an air bubble through the mouth immediately following re-immersion. Many of the fishes immediately started active swimming, 50% even swimming hyperactively, but all fishes had calmed within the first 2 hr. of re-immersion.

In the second experiment, there was significant differences in mean opercular movement of the control and experimental

fishes among the three species (ANOVA, d.f = 2, *P* < 0.05) (Table - 4).

Table 4 Mean opercular movement of control and experimental fishes of three amphibious fishes

Species	Control	Experimental
Anabas testidineus	45.9 ± 0.09	31.93 ± 5.16
Channa punctatus	35 ± 0.1	21.98 ± 4.04
Clarius batrachus	44.93 ± 0.11	43.96 ± 1.5
ANOVA; F value (*P* value)	3427.620 (*P*< 0.01)	6.123 (*P*< 0.01)

The treatment fish for all the three species taken together did significantly differ in mean opercular movement or GVR between the five phases (ANOVA, df = 4,*P* <0.001) (Table - 5) but not significant for time within phases.

Table 5 Mean opercular movement of control and experimental fishes of three amphibious fishes across different phases of treatment

Phase of Treatment	Control	Experimental
Preatreatment	42 ± 1.8	42.4 ± 1.9
Recession	41.8 ± 1.8	41 ± 1.63
Partial emersion	41.8 ± 1.8	39.33 ± 1.98
Emersion	41.9 ± 1.73	11.63 ± 5.8
Re-immersion	42.22 ± 1.73	33.74 ± 6.8
ANOVA; F value (p value)	.011(>0.05)	9.085(<0.05)

Mean opercular movement significantly differed between the five phases (ANOVA, d.f = 4, *P* <0.05) for experimental fishes of all the three species while for the control fishes, the mean opercular movement did not significantly varied between the five phases. A significant differences between the partial exposure and emersion phases (Wilcoxon, *n* = 6, *P* <0.05) was observed for *Clarias batrachus*. While the emersion caused an instant cessation of opercular movement in *Anabas* and *Channa* it was exceptionally higher in *Clarias* with an average of 34 beats min−1 and was maintained around this rate throughout this phase (Fig. 2). Though all the fishes switched back to gill breathing when re-immersed, *Clarias* sp. exhibited an initial phase of hyperventilation (56 beats min−1) with GVR declining and approaching pretreatment and control values within the first 2 hr of re-immersion (Fig. 2). However, in *Anabas* and *Channa* sp. the fishes were still dependent on the atmospheric air with no GVR in the first 2 hr. of re-immersion, and rolled back to gill breathing only in the 3 hr. of re-immersion with GVR more or less equal to that of pre-treatment.
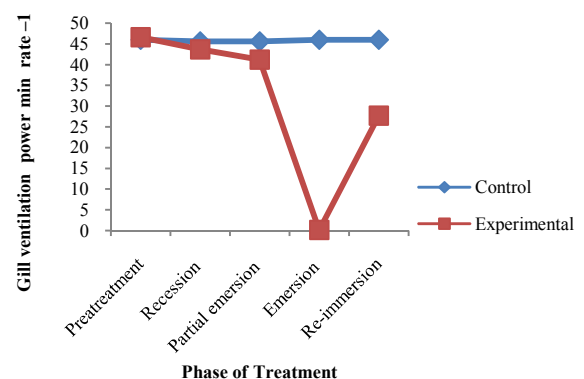

Fig 2 Gill ventilation rates for control and experimental fishes of *Anabas testidineus* across different phases of treatment
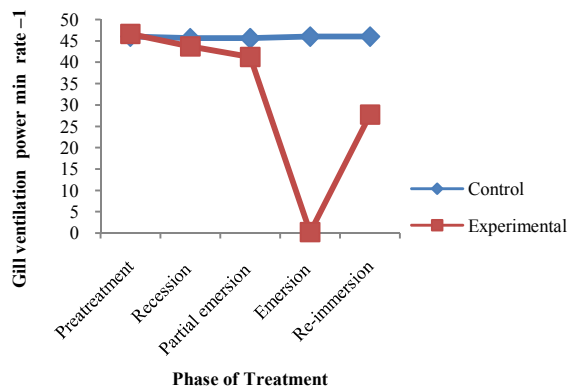
Fig 3 Gill ventilation rates for control and experimental fishes of *Channa punctatus* across different phases of treatment
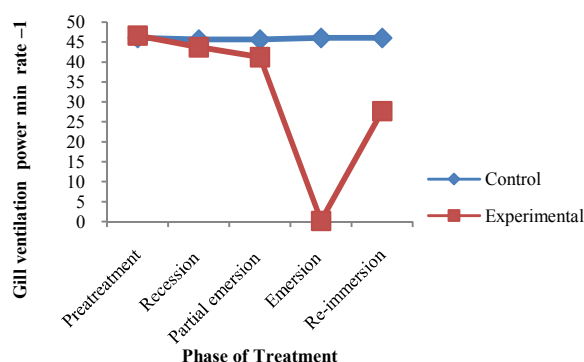

Fig 4 Gill ventilation rates for control and experimental fishes of *Clarius batrachus* across different phases of treatment

DISCUSSION

The transition from aquatic to terrestrial life represents a major step in vertebrate evolution because the physical conditions between these environments are dramatically different. Low concentrations of aquatic O_2, particularly in hypoxic habitats, have often been hypothesized to be one of the driving forces behind the evolution of amphibious or terrestrial life histories because invasion of land would allow animals to exploit the O_2-rich aerial environment (Graham, 1997).

The air breathing fishes exhibits both behavioural and physiological responses consistent with known amphibious adaptations in other fishes (Meredith, 1985; Sayer, 2005). The limited activity in the three air breathing species when immersed probably indicates the suppression of metabolic rate corresponding to reduced energy and oxygen demand when exposed to an environment where respiration is constrained (Crocker & Cech, 1997). Maintenance of a dorso-ventral posture, facilitated by a low anguiliform body shape, is likely to enhance emersion tolerance by reducing pressure on the opercular region, which in turn may enable the fish to inflate their buccal cavities (Meredith, 1985). The occasional rolling observed in these fishes may function to rehydrate the skin and expose different body surfaces, which may facilitate gas exchange, enhance waste excretion and prevent desiccation of gills through rehydration from residual water within the opercular cavity (Meredith, 1985).

Secretion of substantial amounts of mucus is considered to be a critical mechanism that enhances emersion tolerance, because it keeps the gills and skin surfaces moist for efficient gaseous exchange (Eldon, 1978; Meredith, 1985) and also facilitates retention of air bubbles in buccal and opercular cavities (Meredith, 1985). *Anabas* and *Channa* however, did not produce detectable mucus which could have affected their ability to retain air bubbles in the buccal cavity as none of the fish were observed to exhibit distinctly distended jaws or inflated opercular regions. In the case of *Anabas* and *Channa*, it is probable that they cannot withstand considerable dehydration through complete drying of their habitat. In this study, fish were positively buoyant when re-immersed, suggesting that their gas bladders may have been inflated.

Among the three air breathing fishes studied in the study area, *Clarias batrachus* proved to be a better tolerant to emersion and *Clarias batrachus* showed better emersion tolerance and can adjust their behavior and physiology in response to emersion, more importantly reverse to a normal state after re-emersion much faster than that of *Anabas testidineus* and *Channa punctatus*. Different types of accessory respiratory organs were observed that varied in size and colour in the three air breathing fishes. These modified gill organs were the ultimate reason of air breathing fishes to survive in different water conditions. The presence of distinct arborescent organ composed of gill lamellae showed a upper hand in terms of emersion tolerance for *Clariasbatrachus* than that of the other two species.

While this is the first demonstration of emersion tolerance in these three air breathing fishes, the occurrence of air-breathing adaptations in galaxiids is well documented.

CONCLUSION

Fisheries sector occupies a very important place in the socio-economic development of the country. It has been recognized as a powerful income and employment generator as it stimulates growth of a number of subsidiary industries, and is a source of cheap and nutritious food besides being a foreign exchange earner. Most importantly, it is the source of livelihood for a large section of economically backward population of the country.

In Assam, fishery sector has a higher growth rate than other agriculture and allied sectors and could play a pivotal role in the socio-economic development and employment generation in the State. Department of Fishery has been implementing a number of schemes in the State with a view to achieve self-sufficiency in fish production and socio-economic development of poor section of the society. Fish farming is gradually gaining popularity in the State, as farmers have taken up fish seed farming and fish farming on commercial scales as a result of extensive awareness campaign by the Department.

In view of the importance of the sector in providing nutrition to the people, employment generation and livelihood to the rural sector, and in view of the bright prospects for the sector to grow further, such research on the behavioural, structural and physiological adaptations of the fishes would definitely address a better use of these amphibious fishes for commercial

purposes, wherein these fishes could sustain in low level of water successfully.

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