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Acclimation of *Anabas testudineus* (Bloch) to three test temperatures influences thermal tolerance and oxygen consumption

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Abstract Teleost fish have developed their own specific adaptive mechanism, both behavioral and physiological, to maintain homeostasis in response to unfavorable temperatures. Therefore, this study was aimed at assessing the critical thermal maxima (CT_{Max}), critical thermal minima (CT_{Min}), and oxygen consumption rate of *Anabas testudineus* (17.03 ± 1.2 g) after acclimating to three preset temperatures (25, 30, and 35°C) for 30 days. The CT_{Max} and CT_{Min} were 40.15, 41.40, 41.88°C and 12.43, 13.06, 13.94°C, respectively, and were significantly different ($P < 0.05$). The thermal tolerance polygon for the specified temperatures was 278.30°C^2. The oxygen consumption rate (117.03, 125.70, 198.48 mg O_2 kg^{-1} h^{-1}, respectively) increased significantly ($P < 0.05$) with increasing acclimation temperatures. The overall results indicate that the thermal tolerance and oxygen consumption of *A. testudineus* are dependent on acclimation.

Keywords Critical thermal maxima · Critical thermal minima · Thermal tolerance polygon · Oxygen consumption rate · *Anabas testudineus*

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Introduction

As poikilotherms, fishes cannot maintain their body temperatures differently from their surrounding water. So, any alteration in the water temperature affects virtually many physiological and behavioral activities of the fish. Therefore, fisheries researchers have been making continuous attempts to define the thermal tolerance of various species of aquaculture importance. The United States National Council proposed that the global mean air temperature may increase by about 1.5–4.5°C in the next half century (Beitinger et al. 2000). Rising temperature to certain extent is beneficial to the aquaculture production, as it can increase the growth rate of fish and, thereby, reduce the culture period to produce marketable size fish. Moreover, it can also produce more generations per year by reducing the time to attain maturation.

However, temperatures beyond the optimum limit of a particular species can adversely affect its health due to metabolic stress and subsequently increased oxygen demand (Debnath et al. 2006). It also assists in the proliferation, invasiveness, and virulence of various pathogenic micro-organisms that cause a variety of pathophysiological disturbances in the host (Wedemeyer et al. 1999). The ability of the organism to adapt to its altered environment is the key process for its survival. Many teleosts have developed their own specific adaptive mechanism, both behavioral and physiological, to cope with temperature fluctuations (Prosser and Heath 1991).

Climbing perch, *Anabas testudineus*, is an important air-breathing freshwater species widely distributed throughout India and also in other Southeast Asian countries. The species is considered as a valuable dietary item for sick and convalescents, as it contains a high amount of physiologically available iron and copper, which are essentially needed for hemoglobin synthesis. Over the years, owing to their high consumer demand, commercial and medicinal value, *A. testudineus* is becoming a candidate species for aquaculture in India and also in other Southeast Asian countries. Considering the future prospects and needs of culturing this fish, the present study was conducted to investigate the temperature tolerance and oxygen consumption rate of *A. testudineus* at three different acclimation temperatures (25, 30, and 35°C). This study may help in suggesting the species for culture in wide agro-climatic regions of the country of India. In addition, this investigation will highlight the impact of global warming and climatic change on the tolerance capability of *A. testudineus*.

Materials and methods

Experimental fish

A. testudineus (17.03 ± 1.20 g) procured from the local fish market of Mumbai, India, were transported with proper oxygenation to the wet lab of the Central Institute of Fisheries Education, Mumbai, India. They were first given a prophylactic dip in salt solution (2%) and were then held for 15 days at ambient water temperature (30°C). During this period, they were fed with supplementary pelleted feed (35% crude protein). The fish were not fed for 24 h before being subjected to critical thermal methodology (CTM) tests and oxygen consumption experiments.

Acclimation of experimental fish

The acclimation of fish (five per aquarium, each at temperatures of 25, 30, and 35°C) was carried out in a thermostatic aquarium (52-l water capacity, sensitivity ±0.2°C). It was carried out at 1°C per day over ambient water temperature (30°C) to reach the test temperatures (25, 30, and 35°C) and maintained for a period of 30 days. Pretrial acclimation periods and experimental acclimation temperatures suggested for conducting experiments in fish remain as a debatable topic among physiologists across the globe. No reports were available on the thermal acclimation period of adult stages of *A. testudineus*. However, in our previous studies, CTM tests were carried out in Indian Major Carps (*Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala*), *Cyprinus carpio*, and *Pangasius pangasius* after acclimation for 30 days (Chatterjee et al. 2004; Das et al. 2004; Debnath et al. 2006). In another study, Bennett and Beitinger (1997) also found complete acclimation of sheepshead minnows, *Cyprinodon variegates*, to laboratory conditions after 30 days of acclimation. Therefore, in the present study, we followed the previous acclimation procedure and assumed that the test fish were completely acclimated prior to CTM tests.

Thermal tolerance experiment

Fish acclimated to a particular temperature were subjected to a constant rate of increase or decrease at the rate of 0.3°C min^{-1} until loss of equilibrium (LOE) was reached, which were designated as the critical thermal maxima (CT_{Max}) and critical thermal minima (CT_{Min}), respectively (Beitinger et al. 2000; Paladino et al. 1980). This technique has been critically evaluated by numerous workers (Chatterjee et al. 2004; Das et al. 2004, 2005; Debnath et al. 2006; Reynolds and Casterlin 1979) and is well established as a powerful tool for studying the physiology of stress and adaptation in fish (Beitinger and McCauley 1990; Paladino et al. 1980). The fishes used in the thermal tolerance study (CT_{Max} and CT_{Min}) were then subsequently transferred to their respective acclimation temperatures and all of the fishes recovered. Dissolved oxygen concentration was maintained at 5.5 ± 0.5 mg l^{-1} throughout the studies by continuous aeration using a 2-HP centralized air pump. In order to calculate the area of thermal tolerance of this species, the thermal tolerance polygon was generated from CT_{Max} and CT_{Min} data by plotting the acclimation temperature (°C) on the X-axis and the tolerance zone (°C) on the Y-axis. The thermal tolerance area was determined from the polygon (Fig. 1).

Determination of oxygen consumption

Climbing perch is an air-breathing fish. In the present study, the oxygen consumption rate was measured not from the aerial fraction but from the water fraction at the end of acclimation period (30 days) in different acclimation temperatures (25, 30, and 35°C) following the method adopted in our earlier investigation (Debnath et al. 2006) using a separate lot of acclimated fish. Briefly, five acclimated fishes per treatment were placed individually into a sealed glass chamber (5 l) with 6.4-mm-thick glass lid, cut to cover the top portion completely. An opening in the lid fitted with a gasket to ensure an air-tight seal permitted the insertion of a dissolved oxygen probe. A magnetic stir bar was used to maintain constant water circulation. A plastic mesh shield was placed over the stir bar to prevent accidental contact with the fish. The chamber was placed inside the thermostatic aquarium at their respective temperatures for an hour. All four sides of the aquarium were covered with opaque screens to minimize visual disturbances of the experimental fish. The initial and final oxygen levels were measured using a digital oxymeter 330 (E-Merck, Germany, sensitivity 0.01 mg O_2 mg l^{-1}). The temperature quotient (Q_{10}) was calculated to assess the effect of acclimation on the oxygen consumption rate by using the formula: $Q_{10} = (\text{Rate}_2/\text{Rate}_1)^{(10/\text{Temp2}-\text{Temp1})}$.

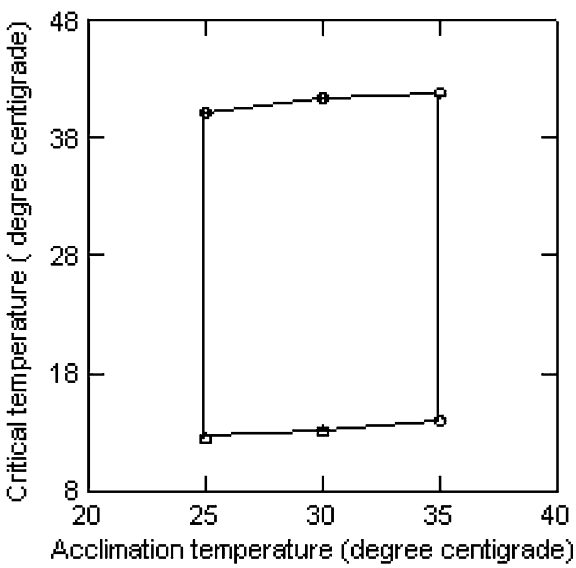

Fig. 1 Thermal tolerance polygon generated from the critical thermal limits (CT_{Max} and CT_{Min}). The thermal tolerance zone of *Anabas testudineus* acclimated at three different temperatures (25, 30, and 35°C) is calculated as 278.30°C^2

Statistical analysis

Statistical analyses of CT_{Max}, CT_{Min}, and the rate of oxygen consumption were carried out using one-way analysis of variance (ANOVA) via SPSS 11.0 for Windows. Duncan's multiple range tests were used to determine the differences among treatment means at $P < 0.05$.

Results

Data pertaining to the thermal tolerance of *A. testudineus* are presented in Table 1. The CT_{Max} (40.15, 41.40, and 41.88°C) increased with increasing acclimation temperatures (25, 30, and 35°C) and were significantly different ($P < 0.05$). Similarly, the CT_{Min} (12.43, 13.06, and 13.94°C) also increased significantly ($P < 0.05$) with increasing acclimation temperatures. However, there was no significant ($P > 0.05$) differences between the CT_{Min} of fish acclimated at 25 and 30°C. The thermal tolerance polygon over the range of temperatures (25–35°C) was generated from the mean CTM values and was calculated as 278.30°C^2 (Fig. 1).

Table 1 Thermal tolerance (CT_{Max} and CT_{Min}) of *Anabas testudineus* acclimated at three different temperatures (25, 30, and 35°C)

Parameters	Acclimation temperatures (°C)		
	25	30	35
CT_{Max}	40.15 ± 0.06^a	41.40 ± 0.17^b	41.88 ± 0.04^c
CT_{Min}	12.43 ± 0.14^a	13.06 ± 0.11^a	13.94 ± 0.35^b

Superscripts with different letters (a, b, c) in a row indicate significant differences ($P < 0.05$). Values are expressed as mean ± SE ($n = 5$)

The rate of oxygen consumption of *A. testudineus* increased significantly ($P < 0.05$) with increasing acclimation temperatures (Fig. 2). However, no significant change in the oxygen consumption rate was observed between 25 and 30°C. The Q_{10} value was calculated as 1.70 (between 25 and 35°C).

Discussion

Temperature influences all biochemical reactions and, therefore, has a significant impact on the physiology of an organism. Therefore, investigation on the thermal tolerance of fish and its influence on fish health has become an important area of research over the years. Various studies had demonstrated that the thermal tolerance of fish largely depends on their prior thermal exposure history or acclimation (Chatterjee et al. 2004; Das et al. 2005; Debnath et al. 2006; Manush et al. 2004). Additionally, a variety of other factors, like size and condition factor of the animals (Baker and Heidinger 1996), nutrient status of the test fishes (Irvin et al. 1957), presence of toxic chemicals (Beitinger et al. 2000), and species (Das et al. 2004), also influenced the thermal tolerance values. In the present study, thermal tolerance (CT_{Max} and CT_{Min}) values increased with increasing acclimation temperatures, i.e., fish exposed to higher acclimation temperatures showed higher CT_{Max} and

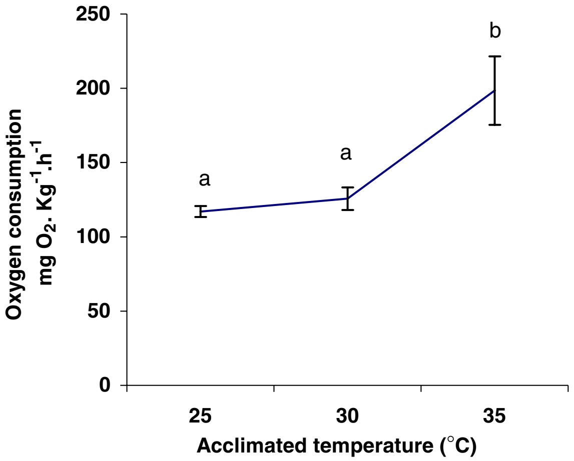

Fig. 2 Rate of oxygen consumption (mg O_2 kg^{-1} h^{-1}) of *A. testudineus* acclimated at three different temperatures (25, 30, and 35°C). Different letters along the line indicate significant differences ($P < 0.05$). A temperature quotient (Q_{10}) of *A. testudineus* between 25 and 35°C was calculated

CT_{Min} values. This indicates that there is a strong relation between acclimation temperature and thermal tolerance (CTM) level. Similar findings were also observed in our earlier studies in carp (Chatterjee et al. 2004; Das et al. 2004, 2005), *Pangasius pangasius* (Debnath et al. 2006), *Macrobrachium rosenbergii* (Manush et al. 2004), and also in other studies in *Lepomis gibbosus* (Becker et al. 1977), *Micropterus salmoides* (Currie et al. 1998), *Pygocentrus nattereri* (Bennett et al. 1997). From the present study results and those of the above-mentioned authors, it can be said that such typical seasonal acclimation may allow fish to be more tolerant to higher temperatures in summer than in winter (Bevelhimer and Bennett 2000).

The area of the tolerance zone is a useful index of thermal tolerance (Elliott 1981). In the present study, the thermal tolerance polygon of climbing perch acclimated to three preset temperatures (25, 30, and 35°C) was found to be 278.30°C^2. There are no parallel reports available on this species to compare with our finding. However, data extracted from our earlier studies on *L. rohita*, *C. carpio* (Chatterjee et al. 2004), and *M. rosenbergii* (Manush et al. 2004) over the same acclimation range (25–35°C) revealed almost similar thermal tolerance polygon zones of 273.50, 311.60, and 255°C^2, respectively. The results indicated that the zone of thermal tolerance of *A. testudineus* over the acclimation range (25–35°C) is almost similar to *L. rohita*. However, their tolerance limit was lower than *C. carpio* and higher than *M. rosenbergii*.

Metabolism is a physiological process reflecting the energy expenditure of living organisms. The metabolic rate of fish is usually indirectly measured as their rate of oxygen consumption (Kutty 1981). In the present investigation, the rate of oxygen consumption increased significantly with increasing acclimation temperatures, suggesting an increase in the total aerobic metabolism with rises in temperature (Kutty and Peer Mohamed 1975). Absolute values of the oxygen consumption rate were similar to our earlier investigation in Indian Major Carps (Das et al. 2004) and in *P. pangasius* (Debnath et al. 2006). However, no significant variation was observed with an initial increase of 5°C (25–30°C), indicating that the rise in acclimation temperature was within the acceptable limits. The temperature quotient (Q_{10}) in the present investigation between 25 and 35°C was

1.7, which was concurrent with our earlier reports in *L. rohita*, *C. catla*, and *C. mrigala* (Das et al. 2004), *C. carpio* (Chatterjee et al. 2004), indicating that climbing perch may also exhibit a similar pattern of energy utilization under thermal acclimation.

Conclusion

The present investigation indicates that thermal tolerance and oxygen consumption are essential physiological phenomena in the life of poikilotherms and are strongly dependent on acclimation temperatures. The test fishes showed an initial adaptive behavior with an initial 5°C rise in temperature. However, with a further increase of 5°C, the test fishes failed to adapt. This preliminary study on thermal tolerance poses new avenues of research, including the induction of stress proteins at different life stages and the importance in cross-protection against several forms of biotic and abiotic stress (DuBeau et al. 1998; Wedemeyer et al. 1999). But there are no reports available about any such mechanism in *Anabas testudineus*. More research on its adaptability to high temperatures and the possibility of culturing this commercially important air-breathing fish in high temperatures is essential in this era of global warming.

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