

Thermal tolerance and oxygen consumption of *Labeo rohita* and *Cyprinus carpio* early fingerlings acclimated to three different temperatures

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Abstract

To compare the species-specific variation in thermal tolerance and metabolic activity, critical thermal maximum (CTMax), critical thermal minimum (CTMin), lethal thermal maximum (LTMax), lethal thermal minimum (LTMin) and oxygen consumption rate of *Labeo rohita* and *Cyprinus carpio* early fingerlings were determined after acclimating at 25°C, 30°C and 35°C for 30 days.

CTMax (40.2 ± 0.04 , 41.6 ± 0.08 , 42.2 ± 0.11); CTMin (12.9 ± 0.04 , 14.2 ± 0.04 , 15.0 ± 0.05); LTMax (40.4 ± 0.05 , 41.9 ± 0.09 , 42.7 ± 0.05); LTMin (11.5 ± 0.03 , 13.5 ± 0.05 , 14.4 ± 0.04) increased significantly ($p < 0.05$) in *L. rohita* with increasing acclimation temperatures. Similarly, CTMax (39.7 ± 0.31 , 40.6 ± 0.07 , 42.9 ± 0.10); CTMin (8.4 ± 0.04 , 8.6 ± 0.04 , 10.2 ± 0.09); LTMax (39.8 ± 0.06 , 40.9 ± 0.07 , 42.9 ± 0.04); LTMin (8.2 ± 0.05 , 8.4 ± 0.04 , 10.1 ± 0.05) increased significantly ($p < 0.05$) in *C. carpio* with increasing acclimation temperatures. Inter-species specific-variation (between *L. rohita* and *C. carpio*) was evident at 30°C and 35°C.

Oxygen consumption rate increased significantly ($p < 0.05$) with increasing temperatures in both the species. However, temperature quotient (Q_{10}) was more in *L. rohita* (1.61), in comparison to *C. carpio* (1.53).

Overall results suggest that *C. carpio* is more thermal-tolerant and show better adaptation in comparison to *L. rohita*.
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Keywords: Thermal tolerance; Oxygen consumption; *Labeo rohita*; *Cyprinus carpio*

1. Introduction

The escalating trends in global temperature are compelling fisheries researchers to make continuous effort to define thermal-tolerance of fishes and their consequences on fish health (Beitinger et al., 2000). Temperature affects virtually all biochemical and physiological activities of an animal. It should be viewed as an environmental resource, which evokes multiple effects on an animal (Magnuson et al., 1979). Long-term

changes in temperature lead ectothermic animals to display acclimatory responses, which may include enzymatic changes thought to mitigate the effect of temperature on metabolism (Hezel and Prosser, 1974). It is reported that acclimation of walleye, *Sander vitreus* fry in hatcheries prior to stocking in open water bodies could be a useful strategy to increase survival post stocking (Clapp et al., 1997). Rising temperature up to a certain limit, favors aquaculture by increasing growth rate and reducing the time to attain maturity. On the contrary, temperature beyond optimum limits of a particular species adversely affects the health of aquatic animal by increasing metabolic rates and subsequent oxygen demand. It also assists proliferation, invasive-

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ness and virulence of bacteria and other pathogens that causes a variety of pathophysiological disturbances in the host (Wedemeyer et al., 1999).

Freshwater aquaculture constitutes one-third of the total fish production in India and carps contribute a major share. Unlike *Labeo rohita*, which is exclusively cultured in the tropical and sub tropical zones of Asian continent, *Cyprinus carpio* culture is well suited in temperate as well as tropical countries of the globe. Considering the culture potential of both *L. rohita* and *C. carpio*, we investigated the temperature tolerance and oxygen consumption of *L. rohita* and *C. carpio* fingerlings at three different acclimation temperatures (25°C, 30°C and 35°C). Lethal temperature methodology is being used in our study to know the thermal limit at which mortality occurs. Routine oxygen consumption rate is used to estimate the metabolic activity of the fishes. Finally, we propose CTM as a reliable method to predict the species-specific variation in thermal adaptation to various agro-climatic regions of the world so as to suggest their suitability for aquaculture.

2. Materials and methods

2.1. Experimental fishes

L. rohita (Mean±SE: 0.7±0.1 g) and *C. carpio* (Mean±SE: 0.8±0.1 g) were brought in separate, aerated open containers from Khopoli fish seed farm, Government of Maharashtra, to wet laboratory, Central Institute of Fisheries Education, Mumbai, and were acclimated for 30 days to laboratory conditions (30°C). During this period, fishes were fed with supplementary feed before thermal tolerance studies.

2.2. Acclimation of experimental fishes

Acclimation of fishes (6 per aquarium, each at temperatures 25°C, 30°C and 35°C) was carried out separately in different thermostatic aquaria (521 water capacity, sensitivity +0.2°C) to determine CTMax, CTMin, LTMax, LTMin and oxygen consumption at the rate one degree/day from ambient temperatures (30°C) to reach test acclimation temperatures (25°C, 30°C and 35°C) and maintained for a period of 30 days prior to the experiment. Pre-trial acclimation periods and experimental acclimation temperatures suggested for conducting experiments in fishes remain as a debatable topic among physiologists across the globe. One of our previous investigations on the effect of acclimation temperatures on thermal tolerance and oxygen consumption suggested that Indian Major Carps were completely acclimated to test temperatures in 30 days (Das et al., 2004). In another study, Sheepshead minnow, *Cyprinodon variegatus* was completely accli-

ated to laboratory conditions after 30 days (Bennett and Beitinger, 1997). Therefore, we assume that the test fishes were completely acclimated prior to CTM tests.

2.3. Thermal tolerance

Acclimated fishes were subjected to constant rate of increase (for CTMax) or decrease (CTMin) at the rate 0.3°C min⁻¹ until loss of equilibrium (LOE) was reached, which was designated as critical thermal maxima (CTMax) and critical thermal minima (CTMin) (Beitinger et al., 2000). This technique has been critically evaluated by numerous workers (Hutchinson, 1976; Reynolds and Casterlin, 1979) and is well established as a powerful tool for studying the physiology of stress and adaptation in fishes (Paladino et al., 1980; Beitinger and McCauley, 1990). Thermal tolerance polygon was generated from these CTMax and CTMin data by plotting acclimation temperatures (°C) on X-axis and tolerance zone (°C) on Y-axis. The area of thermal tolerance for both the species was calculated from the graph (Fig. 1). Dissolved oxygen concentration was maintained at 5.5±0.5 mg l⁻¹ in the aquarium throughout the temperature tolerance studies by continuous aeration using a 2HP centralized air blower. A similar experimental setup was used for performing lethal temperature tests (LTMax and LTMin) to know the lethal tolerance limit in relation to acclimation temperatures (25°C, 30°C and 35°C). LTMax and LTMin were determined by observing the cessation of operculum

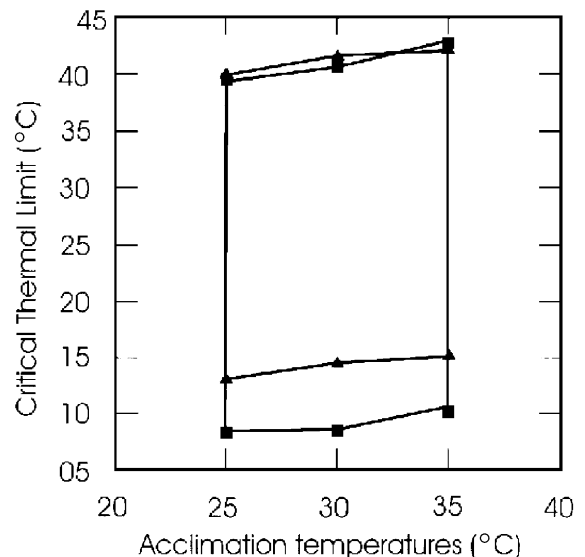


Fig. 1. Thermal tolerance polygon generated from CTM data to indicate thermal tolerance zone of *L. rohita* (—▲—) and *C. carpio* (—■—) acclimated to three different temperatures (25°C, 30°C and 35°C). Thermal tolerance polygon for *C. carpio* (311.6°C²) and *L. rohita* (273.5°C²).

movement (Tsuchida, 1995). Acclimation response ratio was calculated to elucidate the extent of acclimatory adaptation in *L. rohita* and *C. carpio* to different temperatures (Claussen, 1977).

2.4. Oxygen consumption rate

Another set of 18 acclimated fishes (6 per aquarium at 25°C, 30°C and 35°C) of each species (*L. rohita* and *C. carpio*) were kept individually in a sealed glass chamber (5l) 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 airtight seal, permitted the insertion of a dissolved oxygen probe. The chamber was placed inside the thermostatic aquarium at their respective temperatures for an hour. All the four sides of the aquarium were covered with opaque screen to minimize visual disturbances of the experimental fishes (Das et al., 2004). Oxygen consumption was measured at the end of acclimation period (30 days) in different acclimation temperatures (25°C, 30°C and 35°C). The initial and final oxygen content was measured using a digital oxy-meter 330 (sensitivity 0.01 mg O₂ mg l⁻¹, E-Merck, Germany) and their difference were expressed as mg O₂ kg⁻¹ h⁻¹.

Temperature quotients (Q_{10}) in both cases were calculated to assess the acclimatory effect on oxygen consumption rate by using the formulae:

$$Q_{10} = (\text{Rate 2}/\text{Rate 1})^{(10/\text{Temp2}-\text{Temp1})}$$

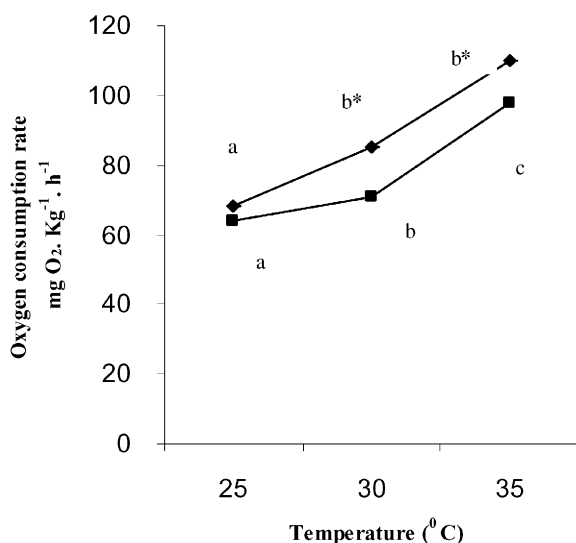


Fig. 2. Rate of oxygen consumption (mg O₂ kg⁻¹ h⁻¹) of *L. rohita* (—◆—) and *C. carpio* (—■—) acclimated to three different temperatures (25°C, 30°C and 35°C). Temperature quotient (Q_{10}) of *L. rohita* (1.61) and *C. carpio* (1.53) between 25°C–35°C.

2.5. Statistical analysis

Statistical analyses of CTMax, CTMin, LTMax, LTMin 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 test was carried out for post hoc mean comparisons ($p < 0.05$). Two species variations (between *L. rohita* and *C. carpio*) were tested using Student's t-test. A linear regression analysis was performed to define the relationship between acclimation temperatures and these test parameters.

3. Results

Data pertaining to thermal tolerance and oxygen consumption of *L. rohita* and *C. carpio* fingerlings are presented in Table 1. In *L. rohita*, CTMax increased significantly ($p < 0.05$, $y = 0.9917x + 39.378$, $r^2 = 0.9583$) with increasing acclimation temperatures (25°C, 30°C and 35°C). Two species variations (between *L. rohita* and *C. carpio*) existed at 30°C and 35°C. In *L. rohita*, CTMin values increased significantly ($p < 0.05$, $y = 1.0583x + 11.928$, $r^2 = 0.9926$) with increasing acclimation temperatures and the trend of increase was similar to CTMax. In *C. carpio*, CTMax increased significantly ($p < 0.05$, $y = 1.5875x + 37.867$, $r^2 = 0.9451$) with increasing acclimation temperatures (25°C, 30°C and 35°C). CTMin values increased significantly ($p < 0.05$, $y = 0.9017x + 7.2478$, $r^2 = 0.8119$) with increasing acclimation temperatures in *C. carpio*. The regression trend indicates a strong relationship between acclimation temperature and CTM values of both *L. rohita* and *C. carpio*. The fishes at CTMax and CTMin were then immediately transferred to the pre-trial acclimation temperatures for rescue and recovery (Beitinger et al., 2000). All the fishes survived the CTM test. Thermal tolerance polygon generated from CTM data (Fig. 1) indicated a larger tolerance zone (318.8°C²) for *C. carpio* than for *L. rohita* (273.5°C²).

LTMax in *L. rohita* were significantly ($p < 0.05$, $y = 1.175x + 39.356$, $r^2 = 0.9799$) different at three different acclimation temperatures (Table 1) as well as in *C. carpio* ($p < 0.05$, $y = 1.55x + 38.089$, $r^2 = 0.9686$). Similarly, LTMin in *L. rohita* were significantly ($p < 0.05$, $y = 1.425x + 10.261$, $r^2 = 0.9513$) different at three different acclimation temperatures (Table 1) as well as in *C. carpio* ($p < 0.05$, $y = 0.9833x + 6.9333$, $r^2 = 0.8376$). The regression trend clearly indicates that LTM values strongly depend on acclimation temperatures of both *L. rohita* and *C. carpio*. However, the t-test comparison indicates that LTMax values were lower in *C. carpio* as compared to *L. rohita* at 30°C and 35°C ($p < 0.05$).

Acclimation response ratio (ARR) of *L. rohita* and *C. carpio* are depicted in Table 2 in order to elucidate the

Table 1

Thermal tolerance (CTMax, CTMin, LTMax, LTMin) and oxygen consumption of *L. rohita* and *C. carpio* acclimated at three different temperatures (25°C, 30°C and 35°C)

Samples	Species	Acclimation temperatures (°C)		
		25	30	35
CTMax (°C)	<i>L. rohita</i>	40.2±0.04 ^a	41.6±0.08 ^{b*}	42.2±0.11 ^{c*}
	<i>C. carpio</i>	39.7±0.31 ^a	40.6±0.07 ^b	42.9±0.10 ^c
CTMin (°C)	<i>L. rohita</i>	12.9±0.04 ^{a*}	14.2±0.04 ^{b*}	15.0±0.05 ^{c*}
	<i>C. carpio</i>	8.4±0.04 ^a	8.6±0.04 ^a	10.2±0.09 ^b
LTMax (°C)	<i>L. rohita</i>	40.4±0.05 ^{a*}	41.9±0.09 ^{b*}	42.7±0.05 ^c
	<i>C. carpio</i>	39.8±0.06 ^a	40.9±0.07 ^b	42.9±0.04 ^c
LTMin (°C)	<i>L. rohita</i>	11.5±0.03 ^{a*}	13.5±0.05 ^{b*}	14.4±0.04 ^{c*}
	<i>C. carpio</i>	8.2±0.05 ^a	8.4±0.04 ^b	10.1±0.05 ^c

Different superscripts in the same row indicate significant ($p < 0.05$) difference (Duncan's multiple range test, $\alpha = 0.05$). *Indicates significant ($p < 0.05$) difference between the species at a particular temperature regime (Student's t-test). Values are expressed as mean ± SE ($n = 6$).

Table 2

Acclimation response ratio of *L. rohita* and *C. carpio* acclimated to three temperatures (25°C, 30°C and 35°C)

Species	Initial Temp (°C)	Final Temp (°C)	ΔT (°C)	ΔCTM (°C)	$\Delta CTM / \Delta T$
<i>L. rohita</i> (CTMax)	25	30	5	1.4	0.28
<i>L. rohita</i> (CTMax)	25	35	10	2.0	0.20
<i>L. rohita</i> (CTMax)	30	35	5	0.6	0.12
<i>L. rohita</i> (LTMax)	25	30	5	1.5	0.3
<i>L. rohita</i> (LTMax)	25	35	10	2.3	0.23
<i>L. rohita</i> (LTMax)	30	35	5	0.80	0.16
<i>L. rohita</i> (CTMin)	30	25	5	1.3	0.26
<i>L. rohita</i> (CTMin)	35	25	10	2.1	0.21
<i>L. rohita</i> (CTMin)	35	30	5	0.8	0.16
<i>L. rohita</i> (LTMin)	30	25	5	2.0	0.4
<i>L. rohita</i> (LTMin)	35	25	10	2.9	0.29
<i>L. rohita</i> (LTMin)	35	30	5	0.9	0.18
<i>C. carpio</i> (CTMax)	25	30	5	0.9	0.18
<i>C. carpio</i> (CTMax)	25	35	10	3.2	0.32
<i>C. carpio</i> (CTMax)	30	35	5	2.3	0.46
<i>C. carpio</i> (LTMax)	25	30	5	1.1	0.22
<i>C. carpio</i> (LTMax)	25	35	10	3.1	0.31
<i>C. carpio</i> (LTMax)	30	35	5	2.0	0.4
<i>C. carpio</i> (CTMin)	30	25	5	0.2	0.04
<i>C. carpio</i> (CTMin)	35	25	10	1.8	0.18
<i>C. carpio</i> (CTMin)	35	30	5	1.6	0.32
<i>C. carpio</i> (LTMin)	30	25	5	0.2	0.04
<i>C. carpio</i> (LTMin)	35	25	10	1.9	0.19
<i>C. carpio</i> (LTMin)	35	30	5	1.7	0.34

rate and magnitude of thermal acclimation achieved during the investigation. In our study, magnitude of thermal acclimation (ΔCTM) increased with ΔT in both the species. In *L. rohita*, the extent of heat acclimation was lower than cold acclimation. However, any such relation could not be established in *C. carpio*.

Rate of oxygen consumption increased concomitantly with the increasing temperatures (Fig. 2). Oxygen consumption increased significantly ($p < 0.05$) till 30°C and stabilized thereafter compared to control in *L. rohita*, whereas it increased significantly ($p < 0.05$) till 35°C in *C. carpio*. Two species variations in the rate of oxygen consumption were significantly different ($p < 0.05$) at 30°C and 35°C. However, rate of increase of oxygen consumption was more pronounced in *L. rohita* fingerlings compared to *C. carpio*, which is evident from the temperature quotient (Q_{10}). Q_{10} was found to be more in *L. rohita* (1.61) in comparison to *C. carpio* (1.53).

4. Discussion

Every fish has a range of temperature that it can tolerate, beyond which it creates thermal stress. Such drastic temperature changes may produce a significant disturbance in the normal functions of survival (Beitinger et al., 2000). In our investigation, CTMax, CTMin, LTMax and LTMin increased significantly with increasing acclimation temperatures in both the species (Table 1). Student's t-test to investigate two species variation in response to acclimation temperatures in our study clearly indicated that *C. carpio* fingerlings are more temperature-tolerant species than *L. rohita* (Table 1). One of our previous investigations in Indian Major Carps, *C. catla*, *L. rohita*, *C. mrigala* (30, 28 and 25 g) at 26°C, 31°C, 33°C and 36°C revealed that CTMax, CTMin, LTMax and LTMin differ significantly with four different acclimation temperatures (Das et al., 2004). However, the data extrapolated were consistent with our present CTM findings (Das et al., 2004). Similar observations have been made for CTMin in *Lepomis gibbosus* (Becker et al., 1977), *Micropterus salmoides* (Currie et al., 1998) and *Pygocentrus natterii*

(Bennett et al., 1997), and for CTMax in *M. salmoides* (Smith and Scott, 1975; Currie et al., 1998). Thus our present investigation indicates that CTM values are species-specific but are independent of the size of fishes.

In the present study, thermal tolerance polygon of *L. rohita* with three preset temperatures was found to be 273.5°C^2 . Interestingly, thermal-tolerance polygon in *C. carpio* (311.6°C^2) is higher than in *L. rohita* by virtue of its cold tolerance (Fig. 1). There are no parallel reports available on *C. carpio* to compare our findings. This *prima facie* study reveals that *C. carpio* is more tolerant than *L. rohita*, which must be the reason why *C. carpio* is showing cosmopolitan distribution and culture potential in comparison to *L. rohita*. From our previous investigations, the complete zone of thermal-tolerance of Indian Major carps were *L. rohita* (744.8°C^2), *C. catla* (728.8°C^2) and *C. mrigala* (801.8°C^2) (Das et al., 2004). However, the report was between 12°C – 40°C based on nine acclimation temperatures. However, the thermal-tolerance polygon data extracted from these investigations in the range of 25°C – 35°C for *L. rohita* (271.7°C^2) were consistent with our findings (273.5°C^2). It indicates that the area of thermal-tolerance polygon is dependent on acclimation temperatures during the experiment. Our investigation on acclimation temperatures (25°C , 30°C and 35°C) in freshwater prawn, *Macrobrachium rosenbergii*, revealed similar findings (Manush et al., 2004).

According to Claussen (1977), ARR is defined as the $\Delta\text{CTM}/\Delta T$ or the change in the CTM per change in acclimation temperatures. It can be considered as a reliable measure to denote the physiological response of the fishes to a given change of temperature. In our study, it was noticed that ΔCTM values have shown a concomitant increase with ΔT in both the species (Table 2) and heat tolerance was found to be lower than cold tolerance in *L. rohita*. However, no such trend was observed in *C. carpio*.

Oxygen consumption is often used as an index of metabolism of freshwater fishes. (Kutty and Peer Mohamed, 1975) and is strongly dependent on acclimation temperatures (Kita et al., 1996). In the present study, rate of oxygen consumption was increased with rising temperature in both species. Rate of oxygen consumption also varied between *L. rohita* and *C. carpio* at three acclimation temperatures. Higher rate of oxygen consumption and Q_{10} value in *L. rohita*, in comparison to *C. carpio*, is an indication of species-variation for energy utilization under thermal acclimation. It indicates that *C. carpio* adapts better to acclimated temperatures (between 25°C and 35°C) than *L. rohita*.

As we have not found any parallel reports, our findings may be a *prima facie* report on differential rate of thermal tolerance and oxygen consumption of *L. rohita* and *C. carpio* early fingerlings at three different acclimation temperatures. From the results, it is evident that *C. carpio* can tolerate more temperature fluctua-

tions than *L. rohita*. It is worth mentioning the cold tolerance ability of *C. carpio* over *L. rohita*. This must be due to the difference in ecological niches (bottom feeding and cosmopolitan distribution) of *C. carpio* in comparison to *L. rohita* (column feeding and tropical distribution). Our hypothesis presumes that *C. carpio* and *L. rohita* have similar adaptive capability to global warming, which is evident from their CTMax results. This hypothesis is valid as we have used CTM for our thermal-tolerance study. CTM requires a constant, linear change of temperature upward or downward with/from acclimation temperature until a disorganization occurs. Thus, CTM approximates natural conditions better than any other methods (Bennett and Judd, 1992). So, it can be extensively used as a comparative method for quantifying the differences in thermal tolerances between different species.

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