EFFECT OF TEMPERATURE ON OXYGEN CONSUMPTION AND AMMONIA EXCRETION IN RED BELLIED PIRANHA *PYGOCENTRUS NATTERERI* KNER, 1858

Divya, P.K. and Ranjeet, K.*

Post Graduate Department of Aquaculture and Fishery Microbiology, MES
Ponnani College, Ponnani South, P.O., Malappuram (Dist), Kerala
* Corresponding author: ranjeet.mes@gmail.com

Abstract: Domestic ornamental fish trade in India is dominated by exotic fishes. Red bellied Piranhas, *Pygocentrus nattereri,* a denizen of Amazon has been a recent introduction to internal trade. Adaptability of any fish to aquarium environments is a prerequisite in success of its trade. Temperature is the most important abiotic parameter affecting the metabolism in fish. Any change in temperature has been directly related to oxygen consumption and ammonia excretion. A study was planned to study the thermal tolerance of *P. nattereri* under different temperature regimes. Results of the present study showed that beyond 30°C *P. nattereri* showed increased metabolic activity, increased oxygen consumption and ammonia excretion. Analysis of mean values for oxygen consumption indicated a temperature range of 28-30°C was suitable rearing temperature for this species. In the present study, least Q_{10} value was observed between 28 and 30°C (1.49), which indicates that within this range *P. nattereri* has a better capacity for maintaining homeostasis. Highest rate of ammonia excretion were recorded at 34°C. As a result at the end of 4 hrs the survival rate recorded was 33.33%. Results of Duncuns multiple range test showed that variation was more pronounced at temperatures beyond 30°C, indicating that this species has a zone of comfort below 30°C. Therefore for increasing the survivability of this fish in aquariums it is advisable to acclimatize them preferably at 30°C.

INTRODUCTION

Aquarium keeping is amongst the most popular of hobbies with millions of enthusiasts worldwide. As an industry, around 2000 species of ornamental fishes are traded annually (Livengood and Chapman, 2009). Among them the bulk is contributed by freshwater species. The domestic freshwater aquarium fish trade in India has been dominated by exotic species. A major impediment in aquarium keeping is rearing and transporting fishes in optimum temperature. Hence, while importing exotic species it is pivotal to assess the potential of the species to survive in tropical environments (Prang, 2001). In the last few years, the ornamental fish exporters have recorded a reduction of 40-50% on their trade (WTO, 2010). Several factors have contributed for this market loss, such as the increasing supply of higher quality species from aquaculture, the high mortality rate of wild fish, and the low quality of the fish exported. Maintaining a stable water quality condition is very important and its poor quality may become one of the main causes for the high fish mortality rates. Among the abiotic parameter water temperature, dissolved oxygen concentration and resulting production of ammonia are the parameters that influence the survival, feed consumption, metabolic rate and thus, growth of fish (Beitinger *et al.*, 2000; Reddy-Lopata *et al.*, 2006).

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). Similarly fish need oxygen to generate energy for body maintenance, locomotion and metabolism. Hence it is essential to keep the dissolved oxygen in the aquarium water up to optimum level for better growth of fishes. Therefore the changes of these factors in aquarium warrant a thorough investigation. Temperature beyond optimum limits for a particular species adversely affects the health of aquatic animal by increasing metabolic rates and subsequent oxygen demand. Small changes in water temperature therefore can have considerable consequences for freshwater fishes (Poole and Berman, 2001, Chatterjee *et al.*, 2004). Another factor that acts as a limiting factor in aquarium tank is accumulation of ammonia. Fish exposed to low levels of ammonia over time are more susceptible to bacterial infections, have poor growth, and will not tolerate routine handling as well as they otherwise would (Wang and Walsh, 2000). Hence ammonia toxicity can be a major issue that leads to mass mortality under unfavorable aquarium condi-tions. Under the above perspective a sound knowledge of the rate of oxygen consumption and ammonia excretion under different temperature regime can provide valuable information on the survivability and potentiality of transportation of any exotic fish species to India.

Red bellied piranha (*Pygocentrus nattereri*) is a freshwater fish, denizen of Amazon, Guiana and Paraguayan basins in the South America. Although fairly good number of these fishes are bred and reared in captivity, most of the demand at present is met through wild caught species acclimatized to aquarium environments. *Pygocentrus nattereri,* although belonging to tropical environment has not succeeded in establishing itself as a suitable aquarium fish. Earlier reports of Bennett *et al.* (1997) have indicated that this species is susceptible to changing temperatures. Introduction of this species in Indian domestic trade is recent and hence there is very little information of the performance of this fish in Indian waters. Earlier reports have shown that this is a sturdy fish however it is susceptible to temperature fluctuations (Bennett *et al.*, 1997). Hence any information on understanding the optimal temperature range for survival of *Pygocentrus nattereri* in aquarium tanks would be beneficial to aquarium keepers as well as traders. Also knowledge on the metabolic activity of this species under different ranges of temperature would enable ornamental fish traders to effectively transport this fish in optimum environmental condition and avoid possible thermal shock or stress during its transportation. Under the above pretext a study was planned with a view to determine the thermal tolerance, rate of oxygen consumption and ammonia excretion of *Pygocentrus nattereri* acclimated to different acclimation temperatures, which in turn would give valuable information in understanding the optimum temperature for rearing and transportation of this species in a tropical environment.

MATERIALS AND METHODS

Determination of Thermal Tolerance

Red bellied piranhas, *Pygocentrus nattereri* (30.25 ± 5.58 g) were procured from local aquarium fish vendor at Ponnani. They were kept in rectangular FRP tanks (1000 L) at ambient temperature (26°C) in the aquaculture wet laboratory, MES Ponnani College for 15 days and weaned to pelleted feed. Prior to commencement of the study, thirty uniform sized adult fishes were taken in a 100 litre rectangular glass tanks and equally distributed between five treatments (26, 28, 30, 32 and 34°C). Hence each tank had six fishes. The fishes were fed with pelleted finisher prawn feed of Higashimaru. The experiment was repeated for two more observations. Uniform rearing conditions were maintained in all the experimental groups except for the water temperatures. Initial water temperature was maintained at 26°C and the temperatures were gradually increased by 1°C/hr to the target temperatures (31, 33 and 36°C) and were

maintained for 30 days. A fixed photoperiod of 12L:12D (Light: Dark) was maintained with light exposure from 6 to 18 h. Aeration was provided in all the experimental containers to maintain the dissolved oxygen level.

Thermal tolerance of *P. nattereri* was evaluated after 30 days of acclimation using the critical thermal methodology (CTM). Because the CTM does not involve death as the experimental end point, it is a useful method for estimating the thermal tolerances of fishes which has narrow temperature tolerance. The CTM tests were conducted in their respective thermostatic aquarium to avoid handling stress in the fish. Fish (6 per treatment for CT_{Max} and CT_{Min} tests, respectively) acclimated to a particular temperature were subjected to constant rate (1 °C per hr) of increase or decrease in the water temperature until loss of equilibrium (LOE) was reached, which were designated as the critical thermal maxima (CT_{Max}) and critical thermal minima (CT_{Min}), respectively. Continuous aeration was provided during each test to maintain adequate DO levels, and the temperature at which LOE occurred was recorded for each fish.

Determination of Oxygen Consumption and Ammonia Excretion

The experimental setup included a rectangular glass tank (15 litre capacity), with a water holding height of 60 cm. This tank was provided with an inlet/outlet tube for drawing water samples for DO and NH_3 estimation and a thermostat with marked temperature ranges from 20-34°C. A series of progressively higher or lower water temperatures was achieved by using thermostat, which controlled the gradual raising or lowering temperature to its desired point. A magnetic stirrer was used to maintain a constant water circulation and for ensuring even distribution of temperature throughout the water column. Once the desired temperature was achieved, two adult fishes were introduced in the tank. A thin film of liquid paraffin was poured on the upper layer of water to avoid any gaseous exchange from the atmosphere. At this point water samples were drawn for estimation of initial DO and NH_3. The

experiment was continued for four hours and water samples were drawn at the end of each hour.

Rate of oxygen consumption was measured under similar conditions at five different acclimation temperatures (26, 28, 30, 32 and 34°C), movement of the fish was not restricted. Hence, any significant differences in oxygen consumption between treatments must be due to the acclimation status. All four sides of the aquarium were covered with opaque screen to minimize the visual disturbances of the experimental animal. The initial and successive oxygen in the water column was measured on an hourly basis following Winklers method. Based on this the oxygen consumption was calculated as

(Final oxygen concentration - Initial oxygen concentration)/Weight of fish (Kg) x Time (Hrs)

The temperature quotients (Q_{10}) were calculated to assess the effect of acclimation on oxygen consumption rate by using the formula:

$$Q_{10} = (Rate2 / Rate1) / {}^{(10 = Temp2 - Temp1)}$$

Simultaneously along with estimation of oxygen consumption, the rate of ammonia excretion was also recorded following hypochlorite method (APHA, 1998).

Statistical analysis

Mean values of all the parameters were analyzed by one way analysis of variance using statistical software (SPSS, version 17.0). Duncan's multiple range test (DMRT) was carried out for post hoc mean comparisons. Regression analysis was carried out to know the relationship between water temperatures and oxygen consumption and ammonia excretion.

RESULTS AND DISCUSSION

Thermal Tolerance

No mortality was recorded during the 24 h observation period subsequent to determination of thermal tolerance in *P. nattereri*. The CT_{Max} and CT_{Min} values increased significantly (P<0.05) with increasing acclimation temperature (Table 1). The result of the present study suggests that beyond acclimation temperature of 30°C there

is a significant difference in the temperature tolerance levels for *P. nattereri.*

Oxygen Consumption

The rate of oxygen depletion under different temperature for a duration of 4 hrs is shown in Figure 1. Results clearly suggest that oxygen consumption is directly related to temperature. The oxygen consumption rates of *P. nattereri* increased significantly (P<0.05) with increasing acclimation temperatures. However, least reduction in residual oxygen was observed at 28 °C (Fig. 1). The Q_{10} calculated from the oxygen consumption rates suggested that values decreased with increasing acclimation temperature, however, beyond 30°C the values increased (Fig. 2). Highest and lowest Q_{10} values of 2.49 and 1.34 were observed between 26–28 and 32–34 °C acclimation temperatures, respectively. Results of Duncuns Multiple Range Test (Table 2) on the rate of residual oxygen under different temperature and time clearly indicated that at 26 and 28°C the oxygen consumption of fishes is rather uniform, which means that at this temperature the thermal stress, is comparatively less. The strong relationship between the acclimation temperatures and the thermal tolerance level (CTM) of *P. nattereri* supports evidence that temperature adaptation is an essential physiological phenomenon in fishes and is dependent on the acclimation temperature (Beitinger and Bennett, 2000). Similar results were observed by Das *et al.* (2004) in common carp and Indian major carps and Debnath *et al.* (2006) in yellowtail catfish. In poikilothermic animals, the metabolic responses that are quantified in terms of oxygen consumption show a linear correlation to temperature due to its direct effect on the kinetics of the enzyme reactions involved (Hazel and Prosser, 1974).

In the present study, lowest Q_{10} value was observed between 28 and 30 °C (1.49), which indicates that within this range *P. nattereri* has a better capacity for maintaining homeostasis. Brett and Zala (1975) have pointed out that the final preferred temperature coincides with optimum temperature for various physiological processes, particularly for growth (Tsuchida, 1995). Kita *et al.* (1996) reported that the final preferred temperature corresponds to the temperature at which increase in oxygen consumption rate with temperature is gradually lessened. The decrease in Q_{10} indicates that the metabolic rate of the fish has decreased and that more energy is potentially available for growth (Díaz *et al.*, 2007). Thus, the final preferred temperature may be estimated indirectly based on the relationship between Q_{10} for oxygen consumption rates and the acclimation temperatures (Das *et al.*, 2004). Therefore, it could be suggested that the final preferred temperature of *P. nattereri* is in the range of 28–30 °C.

Ammonia Excretion

Similar to the result of oxygen consumption, the rate of ammonia excretion was also found to be directly related to rise in temperature. Figure 3 shows the concentration of ammonia accumulated at hourly intervals under different temperatures. Highest rate of ammonia excretion were recorded at 34°C. As a result at the end of 4 hrs the survival rate recorded was 33.33%. In all other treatments there was 100% survival.

Results of multiple regression studies using DMRT (Table 3) showed that variation was more pronounced even at temperatures beyond 30°C. This indicates that *P. nattereri* has a zone of comfort below 30°C. Similar results were obtained in *L. vannamei* by Kita *et al.* (1996), Das *et al.* (2004) and Chatterjee *et al.* (2004). Results of the present study also corroborates with earlier reports of Waichman *et al.* (2001), who while evaluating the water quality used for transportation of cardinal tetra reported a temperature range of 29 to 31°C to be optimum fish transportation. At higher temperature mortality rate is high since fish finds hard to survive at this level. Eurythermal fish such as the gold fish, *Carassiusauratus,* have been reported to survive temperatures between 0 and 41°C and short term exposures to 44°C (Fort and Beitinger, 2005). The fish acclimated to low temperatures are more tolerant to cold and more sensitive to

heat than fish acclimated to warm temperatures (Elliot, 1995). However, *P. nattereri* has a very narrow range of temperature tolerance. Since temperature changes can produce a significant disturbance in the normal functioning of fishes, identifying optimal temperature range will ensure better possibilities for its rearing in aquariums. The present study reveals that the temperature is an important parameter in determining the physiology of *P. nattereri* in tropical environment. A slight increase of temperature has a prolonged influence on the basic metabolism of the fish. In order to increase the survivability of this fish in our aquariums it is advisable to acclimatize them preferably at 30°C. The present study clearly differentiates the optimal rearing temperature of *P. nattereri* to be 28-30°C.

Table 1. Critical thermal maxima (CT_{Max}) and critical thermal minima (CT_{Min}) of *Pygocentrusnattereri* acclimated to five different temperatures

Acclimation temperature (°C)	CT_{Max} (°C)	CT_{Min} (°C)
26	32.62± 0.03[a]	21.96[a]± 0.07[a]
28	33.74 ± 0.05[a,b]	22.15 ± 0.05[b]
30	34.16 ± 0.03[b]	22.79 ± 0.08[b]
32	35.82 ± 0.04[c]	23.26 ± 0.14[c]
34	37.12 ± 0.08[d]	24.52 ± 0.06[d]

Table 2. Comparison of mean residual oxygen during every hour for different temperatures

Temp	Initial	1 hour	2 hour	3 hour	4 hour
34°C	5.803±0.54[a]	2.295 ±1.48[a]	1.290 ±0.51[a]	0.287 ±0.25[a]	0.023±0.01[a]
32 °C	5.646±1.83[a]	2.653 ±0.62[a]	2.395 ±0.58[b]	1.109 ±0.54[b]	0.907±0.39[b]
30 °C	5.601±0.55[a]	4.013±1.63[a]	3.234 ±0.35[b]	2.424 ±0.79[b]	1.338±0.17[b,c]
28°C	5.415±2.34[a]	4.057 ±1.84[a]	3.186 ±1.28[b]	2.742 ±0.92[b]	1.519±0.26[b]
26 °C	5.984±1.42[a]	4.576±0.63[c]	3.431 ±2.18[b]	2.387 ±0.68[b]	1.401 ±0.36[c]

Values are expressed as mean ± SE (n=6). Different superscript letters in the same column indicate significant difference (ANOVA, $P<0.05$).

Table 3. Comparison of mean ammonia excreted every hour under different temperatures

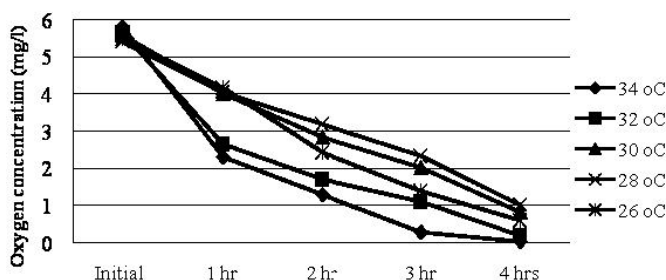| Temp	Initial	1 hour	2 hour	3 hour	4 hour
34°C	0.001±0.00[a]	0.554±0.17[a]	0.703±0.17[a]	1.225±0.16[a]	1.478±0.12[a]
32 °C	0.001±0.00[a]	0.261 ±0.03[b]	0.309±0.07[b]	0.372±0.06[b]	0.378±0.09[b]
30 °C	0.001±0.00[a]	0.132 ±0.03[c]	0.181±0.02[c]	0.188±0.01[c]	0.291±0.05[b,c]
28°C	0.001±0.00[a]	0.151 ±0.04[b]	0.145±0.02[c]	0.179 ±0.01[c]	0.225±0.01[c]
26 °C	0.001±0.00[a]	0.175 ±0.02[b]	0.140±0.05[c]	0.176±0.07[c]	0.200±0.04[c]

Fig. 1. Rate of Oxygen depletion with time under different acclimated temperatures
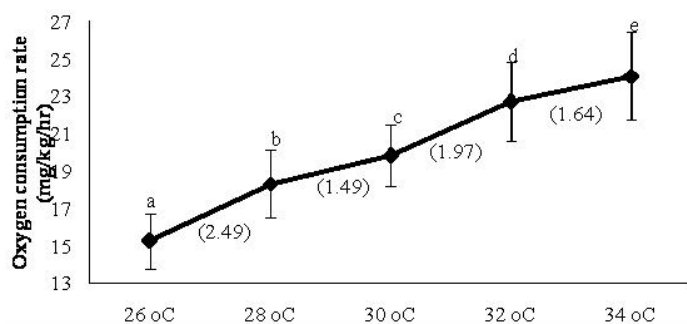
Values are expressed as mean ± SE (n=6). Different superscript letters indicate significant difference (ANOVA, P<0.05). Numbers in parenthesis are Q_{10}s between acclimation temperatures (26–28, 28–30, 30–32, and 32–34 °C).

Fig. 2. Oxygen consumption rates of *Pygocentrus natteri* acclimated to 26, 28, 30, 32, and 34 °C
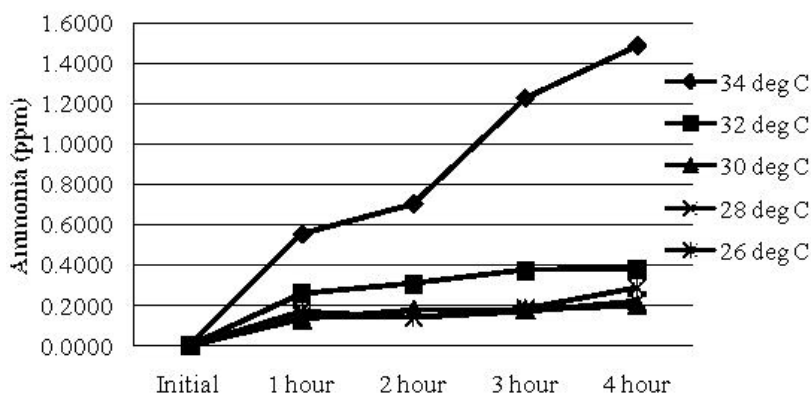
Fig. 3. Concentration of ammonia accumulated under different temperatures

CONCLUSIONS

Maintenance of ornamental fishes in their optimal temperature is a major cause of concern for aquarium keepers. The study clearly showed that *P. nattereri* prefers a narrow temperature range. Results of thermal tolerance observed in the present study suggest that beyond acclimation temperature of 30°C there is a significant difference in the metabolic rate of *P. nattereri*. Similar to the result of oxygen consumption, the rate of ammonia excretion was also found to be directly related to rise in temperature. This indicates that *P. nattereri* has a zone of comfort below 30°C. Hence it could be concluded that the optimum temperature for the survival range of this species in between 28-30°C, beyond which the incidence of mortality increases by 30%. The present study provides valuable information to aquarium keeper, traders and exporters to rear and transport red bellied piranha *Pygocentrus nattereri* at an optimum temperature of 28-30°C in tropical environments.

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