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Thermal Tolerance, Oxygen Consumption and Stress Response in *Danio dangila* and *Brachydanio rerio* (Hamilton, 1822) Acclimated to Four Temperatures

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Abstract

In this study, we determine critical (CTMax) and lethal (LTMax) thermal tolerance, acclimation response ration (ARR), rate of oxygen consumption and stress in two potential ornamental fishes of the North Eastern Hill region of India, *Danio dangila* and *Brachydanio rerio*. The fishes were collected from wild and acclimatized at 18°C for 30 days. The fishes were then constantly reared at temperature regime of 20, 25, 30 and 35°C separately in insulated plastic tank for 45 days. Fishes from each rearing temperature were subjected to constant rate of increase at 1.0°C/min to determine the thermal tolerance. The results implicate significant increase ($p<0.05$) in CTMax (36.2±0.02, 37.7±0.31, 39.6±0.07, 40.9±0.10) and LTMax (38.1±0.08, 39.8±0.06, 40.0±0.07, 41.1±0.04) in *D. dangila* with increasing acclimation temperatures of 20, 25, 30 and 35°C, respectively. Similarly, CTMax (36.4±0.05, 37.2±0.04, 38.7±0.03, 39.8±0.01) and LTMax (39.8±0.03, 40.4±0.02, 41.2±0.06, 42.2±0.03) increased significantly ($p<0.05$) in *B. rerio* with increasing acclimation temperatures. Inter species variation was evident between the temperatures. Oxygen consumption rate increased ($p<0.05$) with increasing temperature in both the species. Overall, our results suggest that *B. rerio* is more thermal tolerant and show better adaptation in comparison to *D. dangila*.

Keywords: Thermal tolerance, oxygen consumption, *Danio dangila*, *Brachydanio rerio*, global warming.

Introduction

The water temperature is known to be an important environmental resource for the aquatic organisms, and fish often compete for favorable temperatures for growth and reproduction (Magnuson *et al.,* 1979). Temperature increase beyond the optimal range for any fish species can influence metabolic activities and have lasting effects on behavior, such as predator avoidance, migration, and spawning (Reynold and Casterlin, 1979). Few reports even suggests that continuous exposure to elevated temperature can make the fish sterile and/or sexually incompetent (Strüssmann *et al.,* 1998; Majhi *et al.,* 2009; Ito *et al.,* 2003) and even causes swimming disability (MacNutt *et al.,* 2004).

The North Eastern Hill (NEH) region of India is considered as hotspot for fish diversity. The region harbors approximately 274 fish species belongs to 114 genera under 37 families and 10 orders inhibiting various rivers and streams (Majhi *et al.,* 2006; Mahapatra *et al.,* 2004). The temperatures in such streams of NEH region are greatly influenced by rainfall (Kumar, 2011). However, over the years decline in rainfall has led to reductions in stream volume, which combined with loss of habitat result in increased daily and annual temperature fluctuations (Kumar, 2011). Consequently, temperatures in degraded streams are now more likely to be influenced by atmospheric temperature and if situation do not improve, the streams temperature might be equal with ambient air temperatures (Kothawale and Kumar, 2005).

Danio dangila and *Brachydanio rerio* (Figure 1) are two potential freshwater ornamental fishes inhabiting streams and rivers of NEH region of India (The Himalayan region). Both belong to order cypriniformes, family cyprinidae and are important model organisms in many fields of research, including genetics, neuroscience, developmental biology, physiology, toxicology and biomedicine, and it is frequently used as a model of many human diseases (Vascotto *et al.*, 1997; Fishman, 2001). Because they evolved in mid to high altitude environment of Himalayan region (500-1000 m above MSL) these species are considered to be less tolerant to high

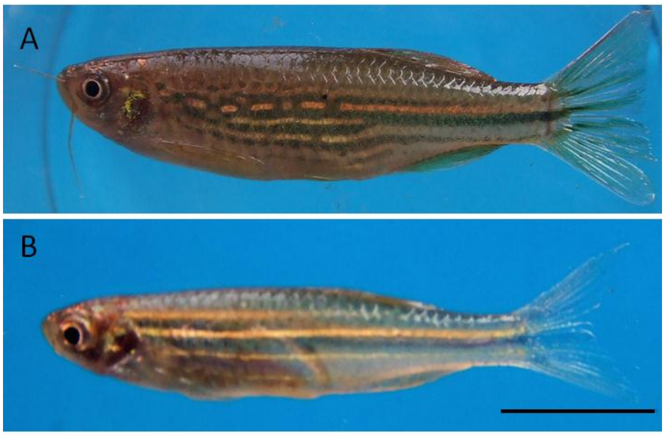

Figure 1. The fish species used in the study: *Danio dangila* (panel A) and *Brachydanio rerio* (panel B). Scale bar indicates 1cm (A and B).

temperature (Mahapatra *et al.*, 2004); however, no data are available to support this assumption. Available information is limited to field observations and a small number of laboratory studies (Mahapatra *et al.*, 2003). Recently, from the annual catch data and discussion with local fishers, we observed that the population of these two fish has sharply declined in natural water bodies. We assume this phenomenal declining trend may be due to environmental changes like habitat loss, aquatic pollution and steadily increasing water temperature in the region. Considering the potential of these two fish species in ornamental fish industry, we investigated the thermal tolerance, stress response and rate of oxygen consumption in these fish species that were experimentally-acclimatized at four different temperatures (20, 25, 30 and 35°C).

Materials and Methods

Fish collection

Danio dangila (n=50; Mean ± SE : 0.83± 0.1 g) and *Brachydanio rerio* (n=60; Mean ± SE : 0.75 ± 0.4 g) were collected from the wild and brought in separate aerated open containers to the Indian Council of Agricultural Research fish farm complex at Barapani, Meghalaya. The fishes were acclimated for 30 days to wet laboratory conditions (water temperature; 18±1.5°C). The choice of acclimation temperature and handling were based on published information (Mahapatra *et al*., 2003). During this period, fishes were fed with natural (planktons) and supplementary (rice polish and mustard oil cake; 1:1 w/w) diets daily up to satiation before subjecting to thermal tolerance studies.

Acclimation of Fishes

Acclimation of fishes (10/tank at each temperature, 20, 25, 30 and 35°C) was carried out separately in different insulated plastic tank (30 liters water capacity and flow through) to determine Critical Temperature Maximum (CTMax), Lethal Temperature Maximum (LTMax) and rate of oxygen consumption. The temperature in experimental tank was gradually increased at 1°C /day from 18°C to reach test acclimation temperatures (20, 25, 30 and 35°C) and maintained for a period of 45 days prior to determine CTMax and LTMax in these animals.

Thermal Tolerance

Acclimated fishes were subjected to constant rate of increase at 1.0°C/ min until loss of equilibrium (LOE) was reached, which was designated as CTMax (Beitinger *et al.*, 2000). A similar experimental set up was used for performing lethal temperature tests (LTMax) to know the lethal tolerance limit in relation to acclimation temperatures (20, 25, 30 and 35°C). LTMax was determined by observing the cessation of operculum movement (Tsuchida *et al.,* 1995). Acclimation response ratio (ARR) was calculated to elucidate the extent of acclamatory adaptation in *D. dangila* and *B. rerio* to different temperatures (Claussen *et al.,* 1977) by taking the difference between the endpoint at each acclimation temperature and then dividing by the difference in the acclimation temperatures.

Oxygen Consumption Rate

The oxygen consumption rate was determined following the protocol prescribed by Das *et al.,* (2004). Briefly, 20 acclimated fishes (5/tank at 20, 25, 30 and 35°C) of each species (*D. dangila* and *B. rerio*) were kept individually in a sealed glass chamber (5 litres) 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. The chamber was placed inside the insulated plastic tank at their respective temperatures for an hour. All necessary measures were taken to minimize visual disturbances of the experimental fishes. Oxygen consumption was measured at the end of acclimation period (45 days) in different acclimation temperatures (20, 25, 30 and 35°C). The initial and final oxygen content was measured using a digital oxy-meter 330 (sensitivity 0.01 mg O_2 mg L^{-1}, E-Merck, Germany) and their difference were expressed as mg O_2. kg^{-1}. h^{-1}.

Blood Collection and Sample Preparation

Five fishes each from thermal stressed and non-stressed (control) group were anesthetized in MS-222 (Tricaine methanesulfanate, Sigma, St. Louis, MO) once they reached CTMax and LTMax. Blood was collected from each fish by cutting the caudal peduncle. The blood samples were left at room temperature for one hour and then stored at 4°C overnight. The blood was centrifuged at 3000 rpm over 10 min for the collection of serum. The aliquots of serum were used for glucose analysis.

Plasma Glucose Estimation

The serum sample (20 µl) was added to 2000 µl glucose reagent in a test tube. The content was mixed and incubated for 10 minutes at 37°C. A quantity (20 µl) of glucose standard solution was also mixed with 200 µl of glucose reagent and incubated for 10 minutes at 37°C. The absorbance of the standard solution and those of the samples were measured against those of the reagent blank in a UV spectrophotometer (Thermo Scientific, NC 28803, USA) at 505 nm (Iwama *et al.,* 1999).

Statistical Analysis

Statistical analyses of CTMax, LTMax, rate of oxygen consumption and blood glucose estimation were carried out using one-way analysis of variance (ANOVA) via GraphPad Software Inc., Ver. 6.0 for Windows (Graphpad software, San Diego, California, USA). Tukey's multiple comparison test was carried out for post hoc mean comparisons (P<0.05).

Results

Thermal Tolerance and ARR

Results pertaining to thermal tolerance of *D. dangila* and *B. rerio* are presented in Table 1. We observed that, in *D. dangila*, CTMax increased significantly (P<0.05) with increasing acclimation temperatures (20, 25, 30 and 35°C). For instance, at acclimation temperature of 20°C the CTMax value was 36.2±0.02°C, whereas at 35°C it significantly increased to 40.9±0.10°C. Similarly, in *B. rerio* CTMax value increased significantly (P<0.05) with increasing acclimation temperatures (Table 1). A variation in thermal tolerance (CTMax) between *D. dangila* and *B. rerio* was observed at 35°C. The regression trend indicates a strong relationship between acclimation temperature and CTMax values of both *D. dangila* (y=1.6x+34.6, r^2=0.995) and *B. rerio* (y=1.17x+35.1, r^2=0.988). Both the species at CTMax when returned to the pretrial acclimation temperatures for recovery, we observed that all the fishes survived the test. Further, LTMax in both *D. dangila* and *B. rerio* was significantly different at different acclimation temperatures (P<0.05; Table 1).

Acclimation response ratio (ARR) of *D. dangila* and *B. rerio* are presented in Table 2. The ARR elucidate the rate and magnitude of thermal acclimation obtained during the experiment. We observed that, magnitude and/or level of thermal acclimation (ΔCTM) increased with ΔT in both the species.

Rate of Oxygen Consumption

In general, rate of oxygen consumption increased concomitantly with the increasing water temperatures. We observed a significantly increasing trend in oxygen consumption in both the species. However, in *B. rerio* the increase in oxygen consumption was more prominent, especially after reaching 30 °C (P<0.05). Further, we observed that inter species variation in rate of oxygen consumption were significantly different (P<0.05) at 35 °C (Figure 2).Overall, rate of increase of oxygen consumption was more pronounced in *B. rerio* compared to *D. dangila*.

Blood Plasma Glucose Assay

We examined the thermal stress response through blood glucose assay in both the species and observed that among the four acclimated temperature regimes, irrespective of species tested, fishes

Table. 1. Thermal tolerance (CTMax and LTMax) of *Danio dangila* and *Brachydanio rerio* acclimated at four different temperatures (20, 25, 30 and 35°C)

Thermal test	Species	Acclimation temperatures (°C)			
		20	25	30	35
CTmax(°C)	*Danio dangila*	36.2±0.02^a	37.7 ±0.31^b	39.6±0.07^c	40.9±0.10^d
	Brachydanio rerio	36.4±0.05^a	37.2±0.04^b	38.7±0.03^c	39.8±0.01^c
LTmax(°C)	*Danio dangila*	38.1±0.08^b	39.8±0.06^c	40.0±0.07^d	41.1±0.04^e
	Brachydanio rerio	39.8±0.03^c	40.4±0.02^d	41.2±0.06^e	42.2±0.03^f

Different superscripts in the same row and same column indicate significant difference (Tukey's multiple comparison test, *P*<0.05). Values are expressed as mean ± SE (n= 6).

Table. 2. Acclimation response ratio (ARR) of *Brachydanio rerio and Danio dangila* acclimated to four temperatures (20, 25, 30 and 35°C)

Species	Initial Temp (°C)	Final Temp (°C)	ΔT(°C)	ΔCTM(°C)	ΔCTM/ ΔT
Brachydanio rerio (CTMax)	20	25	5	0.8	0.16
Brachydanio rerio (CTMax)	20	30	10	2.3	0.23
Brachydanio rerio (CTMax)	20	35	15	3.4	0.22
Brachydanio rerio (CTMax)	25	30	5	1.5	0.3
Brachydanio rerio (CTMax)	25	35	10	2.6	0.26
Brachydanio rerio (CTMax)	30	35	5	1.1	0.22
Brachydanio rerio (LTMax)	20	25	5	1.5	0.3
Brachydanio rerio (LTMax)	20	30	10	3.4	0.34
Brachydanio rerio (LTMax)	20	35	15	4.7	0.31
Brachydanio rerio (LTMax)	25	30	5	1.9	0.38
Brachydanio rerio (LTMax)	25	35	10	3.2	0.32
Brachydanio rerio (LTMax)	30	35	5	1.3	0.26
Danio dangila (CTMax)	20	25	5	0.6	0.12
Danio dangila (CTMax)	20	30	10	1.4	0.14
Danio dangila (CTMax)	20	35	15	2.4	0.16
Danio dangila (CTMax)	25	30	5	0.8	0.16
Danio dangila (CTMax)	25	35	10	1.8	0.18
Danio dangila (CTMax)	30	35	5	1	0.2
Danio dangila (LTMax)	20	25	5	1.7	0.34
Danio dangila (LTMax)	20	30	10	1.9	0.19
Danio dangila (LTMax)	20	35	15	3	0.2
Danio dangila (LTMax)	25	30	5	0.2	0.04
Danio dangila (LTMax)	25	35	10	1.3	0.13
Danio dangila (LTMax)	30	35	5	1.1	0.22

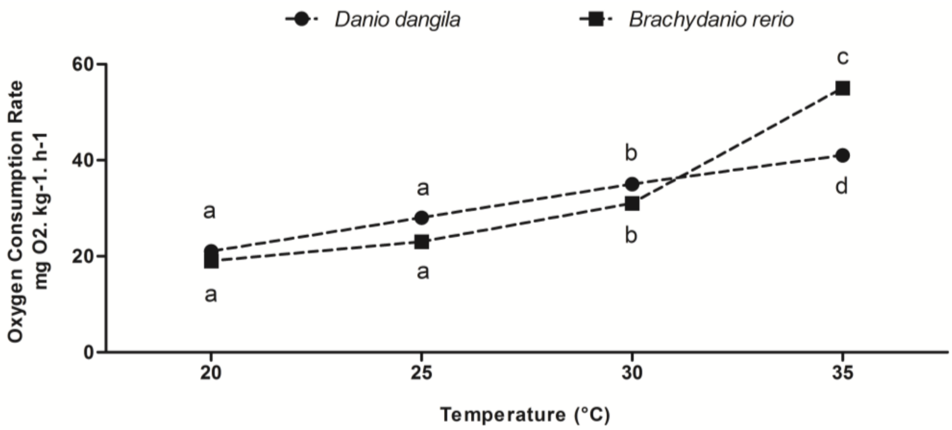

Figure 2. Rate of oxygen consumption (mg O_2. kg^{-1}. h^{-1}) of *Danio dangila* (—•—) and *Brachydanio rerio* (—■—) acclimated to four different temperatures (20, 25, 30 and 35°C). Trends with different letter vary significantly (Tukey's multiple comparison test, P<0.05).

acclimated at lower temperature (20°C) and subjected to thermal tolerance test encounter more stress. For instance, *D. dangila* acclimated at 20°C, the blood glucose level at CTMax was 8.2±0.6 mmol/l, whereas acclimation at 35°C, the glucose level significantly reduced to 4.1±0.1 mmol/l, suggesting that acclimation history is critical in inducing stress in fishes (Figure 3).

Discussion

The *Danio dangila* and *Brachydanio rerio* are two very important native ornamental fish contributing significantly to the ornamental fish trade in India. Both the species are hardy and prolific breeder (Hill *et al.*, 2005). The natural stock of these two fish species were once abundantly found in the streams and natural tanks of the NEH region of India. However, over the years the population in wild has drastically reduced and may be attributed to various environmental factors, including climate change (Kumar, 2011). It is known that each species has a range of temperatures that it prefers, and they cannot survive in temperatures too far out of this range. Some reports even suggest that, fish might also have less offspring as temperatures rise beyond the tolerable range, and some may not be able to reproduce at all and become permanently sterile

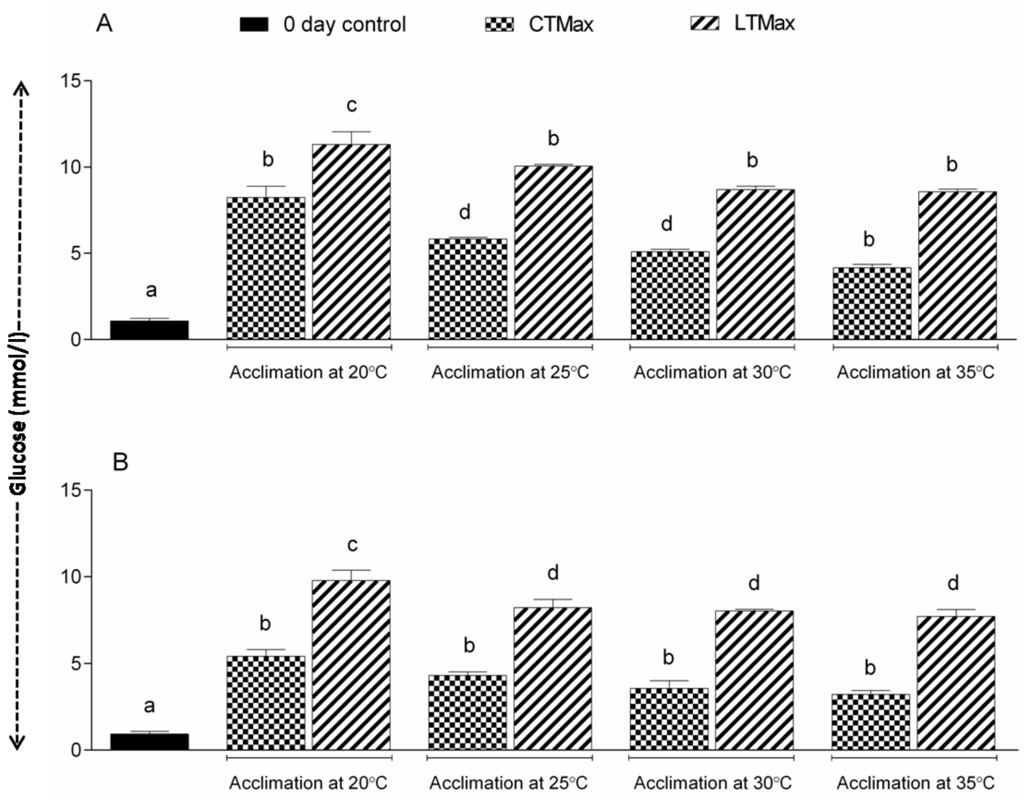

Figure 3. Changes in plasma glucose level in *Danio dangila* (panel A) and *Brachydanio rerio* (panel B) at CTMax and LTMax in various acclimation temperatures. Columns with different letter vary significantly (Tukey's multiple comparison test, P<0.05).

(Strüssmann *et al.,* 1998). This may be particularly true in the case of sub-tropical fishes, like in our case, because of their evolutionary history. Base on the results of this study, we speculate that both the species might be undergoing stress in the nature due to steadily increasing temperature in the region coupled with reducing annual rainfall. This might, probably one of the reasons for declining natural stock of these two species in the nature. Here, we concluded that, thermal tolerance is largely dependent on acclimatization history rather than evolutionary history of fish. Further, among the two species we tested, *B. rerio* is found to be more thermal adaptive than the *D. dangila*.

We observed significant increase in CTMax and LTMax with increasing acclimation temperatures in both the species. This result confirms that, in teleosts, thermal tolerance is largely dependent on acclimation temperatures of fish and are species specific (Dülger *et al.,* 2012), but independent of size or age (Das *et al.,* 2004; Diaz *et al.,* 2007). As a result, typical seasonal acclimation allows fish, irrespective of their size, to become more tolerant to higher temperatures in summer and lower temperature in winter (Bevelhimer and Bennett, 2000). This observation is very much true for the teleost and was yet again proved in our study. For instance, we used sub-adults in this experiment to determine the thermal tolerance limits. We observed, while acclimatizing the fishes at 20-35°C, the CTMax values were in the range of 40-41°C. Contrary, Indian Major Carps fry acclimatized at 26-36°C for 30 days attained CTMax at 42-45°C, respectively (Das *et al.,* 2004). This allows us to confirm that, in addition to size and age, evolutionary history of animal also does not seems to influence the thermal tolerance in fish. However, future studies should explore possibilities of making the fish species from temperate region, that have better growth attributes, to adapt and grow in tropical environment. This might add to species diversification in aquaculture for production enhancement.

The acclimation response ratio (ARR) is considered as a reliable measure to denote the physiological response of the fishes to a given change of temperature (Claussen, 1977; Das *et at.,* 2004; Herrera *et al.,* 1998). Some studies have confirmed that ARR is largely dependent on geographical temperature gradients (Herrera *et al.,* 1998). For instance, species originating from sub tropical and/or tropical region shows higher ARR values than species belonging to cold or temperate regions (Re *et al.,* 2005; Dülger *et al.,* 2012). In our study we observed that, depending on acclimation temperature, the ARR value ranged from 0.12 (*D. dangila*) to 0.38 (*B. rerio*). It is reported that, higher the ARR values, the fish species might be able to increase their upper thermal tolerances even more with increasing acclimation temperature (Carveth *et al.,* 2006).This could be inferred to the adaptation capability of the species to greater fluctuations of temperature over the short period (Re *et al.*, 2005). This allow us to confirm that, *B. rerio* is more thermal adaptive than the *D. dangila*. Nevertheless, future studies should address the reproductive traits of these valuable fish species with increasing water temperature.

Oxygen consumption is often used as an index of metabolism of 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. The rate of oxygen consumption also varied between *D. dangila* and *B. rerio*, especially at 35°C. Higher rate of oxygen consumption value in *B. rerio* in comparison to *D. dangila* is an indication of species variation for energy utilization under thermal acclimation (Das *et al.*, 2004; Eme and Bennett, 2009). These observations along with the results of glucose level in plasma serum confirm that, *B. rerio* adapts better to acclimated temperatures (between 20 and 35°C) than *D. dangila.*

In summary, our results report the relative thermal tolerances of two commercially important native ornamental fish species of Himalayan region. Although fishes from NEH region of India were previously believed to be less tolerant to high temperature owing to their evolution in hilly environments that are characterized by moderate to low temperature. This maiden study suggests that probably many of such fishes from the NEH region of India are comparatively thermo-tolerant than previously thought.

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References

Beitinger, T.L., Bennett, W.A. and McCauley, R.W. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environmental Biology of Fishes, 58(3): 237-275.

Bevelhimer, M. and Bennett, W. 2000. Assessing cumulative thermal stress in fish during chronic intermittent exposure to high temperatures. Environmental Science and Policy, 3(1): 211–216. doi: 10.1016/S1462-9011(00)00056-3

Carveth, C.J., Widmer, A.M. and Bonar, S.A. 2006. Comparison of upper thermal tolerance of native and nonnative fish species in Arizona. Transactions of the American Fisheries Society 135(6): 1433-1440. doi: 10.1577/T05-025.1

Claussen, D.L. 1977. Thermal acclimation in *ambistomatid salamanders*. Comparative Biochemistry and Physiology Part A: Physiology, 58(4): 333-340. doi: 10.1016/0300-9629(77)90150-5

Das, T., Pal, A.K., Chakraborty, S.K., Manush, S.M., Chatterjee, N. and Mukherjee, S.C. 2004. Thermal tolerance and oxygen consumption of Indian Major Carps acclimated to four temperatures. Journal of Thermal Biology, 29(3): 157-163. doi: 10.1016/j.jtherbio.2004.02.001

Diaz, F., Re, A.D., Gonzalez, R.A., Sanchez, L.N., Leyva, G. and Valenzuela, F. 2007. Temperature preference and oxygen consumption of the largemouth bass *Micropterus salmoides* (Lacepede) acclimated to different temperatures. Aquaculture Research, 38(13): 1387–1394. doi: 10.1111/j.1365-2109.2007.01817.x

Dülger, N., Kumlu, M., Türkmen, S., Ölçülü, A., Eroldoĝan, O.T., Yılmaz, H.A. and Öçal, N 2012. Thermal tolerance of European Sea Bass (*Dicentrarchus labrax*) juveniles acclimated to three temperature levels. Journal of Thermal Biology, 37(1): 79-82. doi: 10.1016/j.jtherbio.2011.11.003

Eme, J. and Bennett,W.A. 2009. Critical thermal tolerance polygons of tropical marine fishes from Sulawesi, Indonesia. Journal of Thermal Biology., 34(5): 220-225. doi: 10.1016/j.jtherbio.2009.02.005

Fishman, M.C. 2001. Zebrafish-the canonical vertebrate. Science, 294: 1290–1291. doi: 10.1126/science.1066652

Gerhard, G.S. and Cheng, K.C. 2002. "A call to fins! Zebrafish as a gerontological model". Aging Cell, 1(2): 104–111. PMID: 12882339

Herrera, F.D., Uribe, E.S., Ramirez, L.F.B. and Mora, A.G. 1998. Critical thermal maxima and minima of *Macrobrachium rosenbergii* (Decapoda: Palaemonidae). Journal of Thermal Biology, 23(6): 381–385. doi: 10.1016/S0306-4565(98)00029-1

Hill, A.J., Teraoka, H, Heideman, W. and Peterson, R.E. 2005. "Zebrafish as a Model Vertebrate for Investigating Chemical Toxicity". Toxicological Sciences 86 (1): 6–19. doi: 10.1093/toxsci/kfi110

Ito, L.S., Yamashita, M. and Strüssmann, C.A. 2003. Histological process and dynamics of germ cell degeneration in Pejerrey *Odontesthes bonariensis* larvae and juveniles during exposure to warm water. Journal of Experimental Zoology Part A: Comparative Experimental Biology, 297A(2): 169-179. doi: 10.1002/jez.a.10249

Iwama, G.K., Vijayan, M.M., Forsyth, R.B. and Ackerman, P.A. 1999. Heat shock proteins and physiological stress in fish. Amer. Zool., 39(6): 901-909. doi: 10.1093/icb/39.6.901

Kita, J., Tsuchida, S. and Setoguma, T. 1996. Temperature preference and tolerance, and oxygen consumption of the marbled rock-fish, *Sebastiscus marmoratus*. Marine Biology, 125(3): 467-471.

Kothawale, D.R. and Kumar, K.R. 2005. On the recent changes in surface temperature trends over India, Geophysical Research Letters, 32(15): L18714. doi: 10.1029/2005GL023528

Kumar, M. 2011. Evidences, Projections and Potential Impacts of Climate Change on Food Production in Northeast Indian Journal of Hill Farming, 24(1&2): 1-10.

Kutty, M.N. and Peer Mohamed, M., 1975. Metabolic adaptations of mullet, *Rhinomugil cersula* (Hamilton) with special reference to energy utilization. Aquaculture 5(3): 253-270. doi: 10.1016/0044-8486(75)90003-4

MacNutt, M.J., Hinch, S.G., Farrell, A.P. and Topp, S. 2004. The effect of temperature and acclimation period on repeat swimming performance in cutthroat trout. Journal of Fish Biology, 65(2): 342–353. doi: 10.1111/j.0022-1112.2004.00453.x

Magnuson, J.J., Crowder L.B. and Medwick, P.A. 1979. Temperature as an ecological resource. Amer. Zool., 19(1): 331-343. doi: 10.1093/icb/19.1.331

Mahapatra, B.K., Vinod, K. and Mandal, B.K. 2003. Studies on native ornamental fish of Meghalaya with a note on their cultural prospects. Aquaculture 4 (2): 171-180.

Mahapatra, B.K., Vinod, K. and Mandal, B.K. 2004. Ornamental fish of North Eastern India – Its distribution and conservation status. Environ. Ecol., 22(3): 674-683.

Majhi, S.K., Das, A. and Mandal, B.K. 2006. Growth Performance of Organically Cultured Grass Carp *C. idella* (Val.) Under Mid-hill Conditions of Meghalaya: North Eastern India. Turkish Journal of Fisheries and Aquatic Sciences, 6: 105-108.

Majhi, S.K., Hattori, R.S., Rahman, S.M., Suzuki, T. and Strüssmann, C.A. 2009. Experimentally-induced depletion of germ cells in sub-adult Patagonian pejerrey (*Odontesthes hatcheri*). Theriogenology, 71(7) 1162-1172. doi: 10.1016/j.theriogenology.2008.12.008

Re, A.D., Diaz, F., Sierra, E, Rodriguez, J. and Perez, E. 2005. Effect of salinity and temperature on thermal tolerance of brown shrimp *Farfantepenaeus aztecus* (Ive) (Crustacea, Penaeidae). Journal of Thermal Biology, 30(8): 618-622. doi: 10.1016/j.jtherbio.2005.09.004

Reynolds, W.W. and Casterlin, M.E. 1979. Behavioural thermoregulation and the final preferandum paradigm. Amer. Zool., 19(1): 211-224. doi:10.1093/icb/19.1.193

Strüssmann, C.A., Saito, T. and Takashima, F. 1998. Heat induced germ cell deficiency in the teleosts *Odontesthes bonariensis* and *Patagonina hatcheri*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 119 (2): 637-644. doi: 10.1016/S1095-6433(97)00477-7

Tsuchida, S. 1995. The relationship between upper temperature tolerance and final preferendum of Japanese marine fish. Journal of Thermal Biology, 20(1,2): 35-41. doi: 10.1016/0306-4565(94)00024-D

Vascotto, S.G., Beckham,Y. and Kelly, G.M. 1997. The zebrafish's swim to fame as an experimental model in biology. Biochemistry and Cell Biology, 75(5): 479–485. 10.1139/o97-081