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RESEARCH PAPER

Sex Differences in Thermal Tolerance of Nine Ornamental Fish Species from the Poecilidae, Cichlidae and Cyprinidae Family

Mahmut Yanar[1], Ece Evliyaoğlu[1,*], Bilge Kaan Tekelioğlu[2]

[1]Çukurova University, Faculty of Fisheries, Department of Aquaculture, Adana, Türkiye.
[2]Çukurova University, Faculty of Ceyhan Veterinary Medicine, Adana, Türkiye.

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Corresponding Author
Tel.: +903223386074
E-mail: eevliyaoglu@cu.edu.tr

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Abstract

Thermal tolerance and intersexual differences of nine ornamental fish species (*Chindango demosoni*, *Aulonocara stuartgranti*, *Mylandia lombardoi*; Cichlidae; *Carassius auratus, Pethia conchonius*, *Branchydanio rerio*; Cyprinidae; *Xiphophorus helleri*, *X. maculatus, Poecilia reticulate;* Poecilidae) were determined in this study. CT-min of the species ranged between 9.63 and 11.37 in poecilids, 11.29 and 12.53 in cichlids, 4.67 and 8.46°C in cyprinids, while CT-max ranged between 39.12 and 40.53 in poecilids, 37.82 and 39.37 in cichlids, 36.12 and 40.30°C in cyprinids. While intersexual differences in the CT-min/CT-max values were not evident in cichlids and cyprinids, these values significantly differed across poecilids. The females of poecilids were approximately 0.5 to 1°C more resistant to both low and high temperatures than their males. Among the species included in our study, due to their resistance to low temperature, especially *C. auratus* and a lesser extent *B. rerio* and *P. conchonius* were considered the most suitable ones for outdoor aquaculture in subtropical regions. However, if they are cultured outside of their natural distribution, it should be noted that upon escapement from aquaculture facilities to nature, they may interfere with the ecosystem and compete with native species there due to their wide range of thermal tolerance.

Introduction

The interest and demand for ornamental fish have increased globally, and the ornamental fishery trade has been growing steadily. Together with its sub-sectors, the sector has a global trade value of 15 billion USD (Tlusty *et al*., 2013; FAO, 2014). However, though ornamental fish contribute significantly to the global economy, they are strong potential candidates to become exotic species in new aquatic ecosystems due to their intensive worldwide trade. The most important ecological barrier in the geographical spread of poikilothermic aquatic animals is water temperature. Some species can settle in new habitats by crossing this barrier due to their superior thermal abilities and may cause severe damage to the natural ecosystem there. For example, some exotic fish species entering some streams and lakes of the USA have been reported to replace many native fish species (Crossman, 1991; Ross, 1991; Lever, 1996; USGS, 2005). Therefore, the determination of the thermal tolerance of fish species provides a sound assessment of their adaptation to new habitats in terms of temperature (Bennet and Beitinger, 1997). Temperature is also one of the most important criteria when selecting a species for aquaculture. One of the most important problems encountered when tropical fish species are farmed in subtropical climates is the rise/fall of water temperature to critical lower (<12°C) or upper (>33°C) levels in some seasons. Therefore, the critical temperature levels of each fish species must be well-known for success in production.

It is known that females in some animal species are more resistant than males to some environmental factors. The basis of these phenomena goes back to

Darwin's "sexual selection theory". Since nature is based on reproduction and fertility, life itself is based on a feminine basis. In sexual selection, the one who chooses is female, and the one who chooses is male. While almost all females participate in reproduction, most of the males are disqualified from the race and do not have a chance to mating. That is, nature favoured the female in sexual selection. This is likely due to the unique biological structure of the female and the high energy cost of producing eggs. Therefore, females can be expected to be more resistant to environmental factors than males. There are some examples related to this: If we take people as an example; women are more resistant to Covid 19 than men and have lower mortality rates (Pradhan and Olsson, 2020; Takashi *et al*., 2020). Freshwater amphipod (*Gammarus roeseli)* females have better defence capacities against xenobiotics (Sroda and Cossu-Leguille, 2001), tropical top shell (*Trochus histrio*) females cope better with oxidative stress (Grilo *et al*., 2018), Italian wall lizard (*Podarcis siculus*) females have a wider thermal tolerance range (Liwanag *et al*., 2018), and marine copepod (*Acartia tonsa*) females have a higher thermal tolerance (Sasaki *et al*., 2019) as compared to their male counterparts. There are data on thermal tolerance differences between sexes in fish, limited to only *P. reticulata* (Fuji *et al*., 1990; Kanda ve ark., 1992; Nakajima *et al*., 2009) and *Gambusia affinis* (Britton 2005). Therefore, in this study, we wanted to test whether females are more resistant to extreme water temperatures than males in nine ornamental fish species.

Critical thermal methodology (CTM) is one of the widely used methods for determining the upper and lower temperature tolerances of animals, and it is a laboratory approach that specifically characterizes the relative or comparative temperature tolerances of species. This method which was first described by Cowles and Bogert (1944) to determine the temperature tolerance of desert reptiles was later developed by various researchers and has been applied to other aquatic animals (Cox, 1974; Spotila *et al*., 1979; Lutterschmidt and Hutchison, 1997; Beitinger *et al*., 2000). In this study, the lower (CT-min) and upper (CT-max) temperature tolerances of a total of 9 popular ornamental fish species from three different families and their sexes were determined. This study is also a continuation of our previous research (Yanar *et al*., 2019) on the temperature tolerance of thirteen ornamental fish species.

Materials and Methods

Experimental Fish

Test fish were obtained from local breeders. Nine ornamental fish species belonging to three fish families were used in this study. All fish in the same family used in the experiment are of the same age, but the weight differences are due to gender differences and are as follows;

Cichlidae:

Demosoni cichlid (*Chindango demosoni,* males 8.50±0.30 g, females 6.44±0.21 g)

Peacock cichlid (*Aulonocara stuartgranti*, males 8.89±0.30 g, females 7.47±0.24 g)

Kenyi cichlid (*Mylandia lombardoi*, males 9.34±0.34 g, females 8.13±0.32 g)

Cyprinidae:

Goldfish (*Carassius auratus*, males 16,12±0.64 g, females 16.81±0.66 g)

Zebra fish (*Branchydanio rerio,* males 1.18±0.04 g, females 1.79±0.05 g)

Rosy burb (*Pethia conchonius,* males 2.64±0.06 g, females 2.93±0.09 g)

Poecilidae:

Guppy (*Poecilia reticulata*, males 1.34±0.05 g, females 2.15±0.10 g)

Platy (*Xiphophorus maculatus*, males 2.31±0.08 g, females 2.96±0.12 g)

Swordtail (*X. helleri*, males 2.19±0.85 g, females 3.39±0.17 g)

Experiments were conducted according to the European Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Acclimatization of Fish

The fish brought to the laboratory were separated according to species and sexes and placed in 100 L aquariums, and then acclimated here at 24 °C for one month. During the acclimation period, aquarium water was continuously oxygenated using an air blower, the remaining feed and faces were away from the aquariums by siphoning off and 25% of freshwater was replaced daily. The fish were fed three times daily with a commercial feed (Tetra-Min flake). A 12 hours light and 12 hours dark period was applied during the acclimation period. In this period, the dissolved oxygen level was measured as >6.4 mg/L, pH 7.6-7.8, and hardness as 300 mg/L $CaCO_3$. Fish were individually weighed to determine average weight towards the end of the acclimation period.

CT-min and CT-max Tests

Before thermal tolerance tests (CT-min and CT-max), the fish were starved for 1 day. 5 males and 5 female individuals for each fish species were taken from acclimation aquariums and transferred to 20 L of the CT-min or CT-max test aquariums at the same temperature (24°C). Trials were performed in three replicates (n=5, N=15 for each sex in each species). Thus, a total of 60 individuals for each fish species (30 for CT-min and 30 for CT-max) were used. The oxygen amount was kept above 6.5 mg/L by aeration in the test aquariums, and also, the temperature was ensured to be evenly distributed throughout the aquariums. The regulation of the temperature levels of the aquarium waters was provided by using a water heater (Xilong AT-700/China) and a water cooler (Resin 650-CL/China). In addition, hot water and ice were used when necessary. Lower (CT-min) and upper (CT-max) thermal tolerances of fish were determined using a critical thermal methodology (CTM). Because ornamental fish are small, the 0.3°C/min temperature change rate recommended by Becker and Genewoy (1979) for small fish was preferred to increase or decrease temperature. The water temperature was gradually decreased (CT-min) or increased (CT-max) until the loss of the locomotive activity, coordination and eventually equilibrium (LOE) of fish. LOE was assessed that the fish could not sustain dorso-ventral orientation for more than 1 min (Bennett and Beitinger, 1997). The arithmetic mean of the individual recorded CT-min or CT-max values of the fish was noted as the CT-min or CT-max value of the groups. After the thermal tolerance test, the fish were taken from the test aquariums and transferred back to the acclimation aquariums, and dead fish were recorded for 96 hours.

Statistical Analyses

Data in the text are expressed as mean ± standard error (S.E). Statistical differences in CT-min or CT-max values between the sexes of each fish species were determined using the Student's t-test at a 5% significance level. Statistical procedures were performed by using the SPSS 20.0 software for Windows.

Results and Discussion

In general, as water temperature decreased in CT-min treatments, first the movement of the fish slowed down, and then the coordination and balance were lost. However, temperature increase in CT-max treatments resulted in increased fish excitement and mobility, followed by higher mucus secretion, uncoordinated movements and loss of balance. When fish were taken from test aquariums and transferred back to habituation aquariums, the survival rate was 100% on CT-min treatments, while the recovery rate ranged from 95 to 99% on CT-max treatments. Therefore, it was concluded that extremely high temperatures are more lethal than extremely low temperatures. Overall, mortality was higher in cichlid species during CTM trials.

The hypothesis that females of fish have better thermal tolerance than males occurred in all three members of the poecilids (Table 1) (P<0.05). But this phenomenon was not observed in cichlids (Table 2) or cyprinids (Table 3) (P>0.05). Females of all Poecilid species tested in our study were approximately 0.5 to 1°C more resistant to both low and high temperatures than males. In addition, females of this family had a wider temperature tolerance range or thermal plasticity (CT-max-CT-min) of approximately 1.5-2°C (P<0.05) than males (Figure 1). Similar to our results, females had wider thermal tolerance than males in *Gambusia affinis* (Britton 2005) and *P. reticulata* (Fuji *et al*., 1990; Kanda *et al*., 1992; Nakajima *et al*., 2009). Further studies involving multiple fish families and species are recommended to further elucidate whether there is such a difference in thermal tolerance between the sexes. In addition, this issue should be tested not only in terms of thermal tolerance but also in terms of other environmental factors.

The CT-min of species ranged between 9.63 and 11.37°C in poecilids (Table 1), 11.29 and 12.53°C in cichlids (Table 2), 4.67 and 8.46°C in cyprinids (Table 3), while the CT-max values ranged between 39.12 and 40.53°C in poecilids, 37.82 and 39.37°C in cichlids, 36.12

Table 1. Thermal tolerance and intersexual differences of poecilid species

		Poecilia retuculata	*Xiphophorus helleri*	*X.maculatus*
CT-min (°C)	♂	10.11±0.07*	11.61±0.09*	10.47±0.07*
	♀	9.14±0.10	11.12±0.08	9.93±0.08
	Mean	9.63±0.59	11,37±0.41	10.20±0.41
CT-max (°C)	♂	40.12±0.12	38.77±0.12	39.17±0.07
	♀	40.95±0.11*	39.47±0.11*	39.85±0.08*
	Mean	40.53±0.61	39.12±0.56	39.51±0.46

Values are mean ± SE (3 replications, 5 fish in each replicate). Means marked with an asterisk in the same column for CT-min or CT-max values between sexes of the same fish species are significantly different from each other (*P*<0.05).

Table 2. Thermal tolerance and intersexual differences of cichlid species

		Chindango demosoni	*Aulonocara stuartgranti*	*Maylandia lombardoi*
CT-min (°C)	♂	11.31±0.14	12.48±0.12	12.05±0.13
	♀	11.27±0.13	12.57±0.12	12.28±0.18
	Mean	11.29±0.51	12.53±0.47	12.16±0.60
CT-max (°C)	♂	38.59±0.15	39.42±0.12	37.91±0.13
	♀	38.45±0.18	39.31±0.18	37.72±0.21
	Mean	38.52±0.63	39.37±0.57	37.82±0.66

Values are mean ± SE (3 replications, 5 fish in each replicate). Means marked with an asterisk in the same column for CT-min or CT-max values between sexes of the same fish species are significantly different from each other (*P*<0.05).

and 40.30°C in cyprinids. Thus, the most resistant species to low temperatures tested in our study were cyprinids, poecilids and cichlids, respectively. Except for *Pethia conchonius*, which is a cyprinid, the resistance of all families to high temperature was found to be almost close to each other. As a result of the CT-min and CT-max data mentioned above, the species with the widest thermal tolerance range (CT-max-CT-min) were listed as cyprinids (27.66-35.47°C), followed by poecilids (27.75-30.90°C) and cichlids (25.66 -27.23°C) (Figure 1).

It should be noted that since CTM data are obtained over a limited time interval and rate of temperature change, it gives an approximation of the temperature limits of animals rather than an exact level, and may allow a relative comparison between species in particular. The thermal tolerance values of the cichlids *C. demosoni*, *A. stuartgranti* and *M. lombardoi* and the cyprinids *P. conchonius* were shown for the first time in this study. The thermal tolerance values determined in other species in our study were also determined and published by Yanar *et al*. (2019) for *P. reticulata* and *X. helleri*, Prodocimo and Freire (2001) for *X. maculatus*, Ford and Beitinger (2005) and Yanar *et al*. (2019) for *C. auratus*, and by Cartemeglia and Beitinger (2005) and Yanar *et al*. (2019) for *B. rerio*. It is thought that the existing differences between studies are probably due to differences in acclimation temperatures in the trials.

In subtropical regions, the water temperature drops to 10°C in winter in shallow and small ponds, while it reaches up to 35°C in summer months. These temperature levels are critical for many fish species. For this reason, fish farmers should consider these low and high-temperature tolerances of fish or take the necessary precautions when choosing fish species. From this point of view, *C. auratus* and partially *B. rerio* and *P. conchonius* are considered the most suitable species among the fish species tested in our study for farming in outdoor ponds in subtropical regions, as they are more resistant to low temperatures. On the other hand, since *P. conchonius*, and to a lesser extent, *M. lombardoi* and

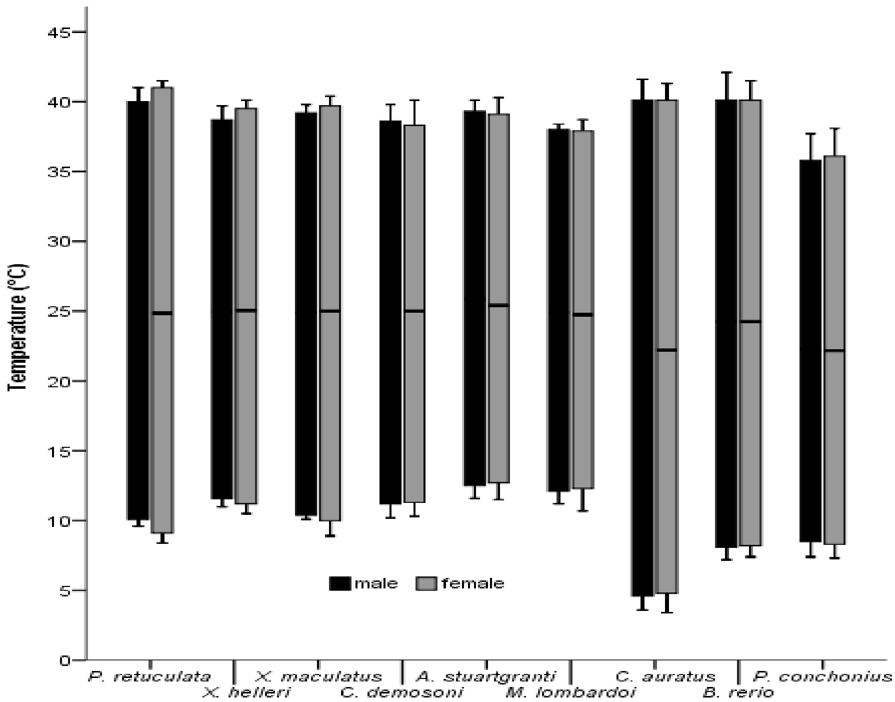

Figure 1. Temperature tolerance range (CT-max-CT-min) of nine fish species based on sex (n=5, N=15 for each sex in each species). The asterisk represents the significant difference between the sexes of each species (*P<0.05*).

Table 3. Thermal tolerance and intersexual differences of cyprinid species

		Carassius auratus	*Brachydanio rerio*	*Pethia conchonius*
CT-min (°C)	♂	4.57±0.20	8.22±0.15	8.58±0.18
	♀	4.77±0.20	8.20±0.12	8.34±0.17
	Mean	4.67±0.76	8.21±0.51	8.46±0.67
CT-max (°C)	♂	40.21±0.18	40.25±0.17	36.01±0.23
	♀	40.07±0.19	40.35±0.18	36.23±0.22
	Mean	40.14±0.71	40.30±0.67	36.12±0.85

Values are mean ± SE (3 replications, 5 fish in each replicate). Means marked with an asterisk in the same column for CT-min or CT-max values between sexes of the same fish species are significantly different from each other (*P*<0.05).

C. demosoni are species that are relatively less resistant to high temperatures, small ponds where these species are grown should be shaded to avoid water overheating. Among the fish, *C. auratus*, which is one of the most suitable ornamental fish species for culture, is very popular because it is extremely resistant to both cold and heat.

Conclusion

The CT-min values of the species ranged from 4.67°C (*C. auratus*) to 12.53°C (*A. stuartgranti*), while CT-Max values ranged from 36.12°C (*P. conchonius*) to 40.53°C (*P. reticulata*). Due to resistance to low temperatures, *C. auratus*, and partially *B. rerio and P. conchonius* are considered more suitable ornamental fish species for farming in outdoor ponds in subtropical regions. However, due to their superior thermal abilities, these species may settle in new habitats by escaping from their breeding areas and cause severe damage to native species there. Since *P. conchonius* and to a lesser extent *M. lombardoi* and *C. demosoni* are less resistant to high temperatures, small ponds where these species are grown should be shaded in hot summer months in tropical or subtropical regions to prevent the water from overheating. Females of the poecilids were found to be more resistant than males to both low and high temperatures, but this phenomenon was not observed in cichlids and cyprinids. This phenomenon needs more attention and clarification with more species belonging to different ornamental fish families.

Ethical Statement

All fish handling procedures complied with Turkish guidelines for animal care (No. 28141) set by the Ministry of Agriculture and Forestry. Tests were conducted according to the guidance of the ethical committee of Cukurova University. The care and use of experimental animals were permitted by the Ethics Committee of Cukurova University.

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Author Contribution

Mahmut YANAR: Conceptualization, Methodology, Experiment performing, Writing- Original draft preparation Ece EVLİYAOĞLU: Experiment performing, Statistical analyses, Writing- Reviewing and Editing Kaan TEKELİOĞLU: Writing- Reviewing and Editing

Conflict of Interest

No conflict of interest exists in the submission of this manuscript. All authors have seen the manuscript and approve it to submit to your journal. It is not being submitted to any other journal.

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