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Influence of water temperature on phenotypic sex ratio of an ornamental fish *Poecilia reticulate*

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Abstract
The effect of water temperature on sex ratio of guppy (*Poecilia reticulate*) was investigated. Treatments of 26, 28, 30, 32°C and control (normal temperature) were used on gravid female and rearing of hatchlings. The highest temperature treatment (32°C) caused the death of gravid females. In this experiment, female offspring production was found increased with decreasing temperature. As such the highest (66.67%) females were observed in control. Whereas the highest male (63.33%) were noticed in 30 ^{0}C treatment. Generally, sex ratio biased to male and female at high and low temperature treatments respectively

Keywords: Gravid female, guppy, offspring, sex ratio**,** temperature

Introduction
The guppy, *Poecilia reticulata* native to fresh and brackish water of north eastern South America and adjacent islands of the Caribbean is one of the famous tropical ornamental fish in the world. Guppies are highly prolific livebearers. The guppy also known as million fish and rainbow fish is one of the world's most widely distributed tropical fish and one of the most popular freshwater aquarium fish species. They are highly adaptable and thrive in many different environmental and ecological conditions. Guppies are used as a model organism in the field of ecology, evolution, and behavioural studies. Live bearing poeciliidae breed easily in captivity [1]. It tolerates comparatively wide ranges of temperature, salinity and crowding. Although culture of the guppy has increased in recent years due to population demand and pressure on wide resources, few studied have been conducted to determine the effects of temperature on the growth and reproductive success of this species [2]. Robinson and Wilson [3] have reported the ability of the guppy to change phenotypes in one generation. The sex differentiation has also been established under controlled thermal conditions using normal and putative monosex progeny of various fish species [4, 5, 6]. Paliwal, *et. al*. [7] have also studied the effect of temperatures on sex ratio of minor carp, *Labeo rajasthanicus*. They reported that in higher temperature during early developmental stages favoured for male production (>26°C), while lower temperature (<20°C) was in favour of female production. Guppy can survive at water temperatures up to 32°C with limited tolerance to higher (36°C) temperatures. The temperature requirement differs among species and various development stages of a given species. The growth, reproduction of fish is also affected by temperature which is the most studied variable. Sex ratio may arise at different stages in the life cycle, including fertilization (Primary sex ratio), birth (secondary sex ratio) and maturity (tertiary sex ratio). Since, male guppies are more colourful and attractive, therefore they are preferred as ornamental fish. Considering this, the present study was conducted to investigate the effect of temperature on the production of male phenotypes.

Materials and Methods
Experimental Fish: The Guppy (*Poecilia reticulata*) with an average weight (2-3 gm.) and average total length (3.4±0.02 cm) were collected from the cemented nursery pond and kept for conditioning in FRP tank (1 m^3) at Aquaculture Research & Seed Unit, Directorate of Research, Maharana Pratap University of Agriculture and Technology, Udaipur. During this period, they were fed on ornamental fish feed.

Experimental setup: This experiment was conducted during Feb.- March, 2016 following CRD under air conditioned environment. For this purpose, 15 aquarium of 30x30x45 cm sized

were used for keeping females and fry rearing. Net cage frames of 25x25x20 cm were placed into the aquarium for keeping gravid female and especially avoiding female guppies to devour newly born babies. The static heat treatment units were used for thermal treatment of gravid females and newly born fry at four (26°, 28°, 30° and 32°C) different temperatures selected through preliminary trials. A control set (mean temperature 19.43°C) was also run simultaneously. The heat-treatment units were filled with clean and aerated tap water. Thermostatically controlled submerged heaters were used to maintain the water temperature at desired levels. For the effect of temperature on phenotypic sex ratio purpose, 50 gravid females distinguishable by their black sacs, denoting their pregnancy were chosen and maintained individually in net cages at 26 ±1° C until the succeeding parturition. Female guppies produce young every 4 weeks or so. At 16th day, the females were introduced to 5 different heat-treatment units {Control (normal temperature), T_1 (26°C), T_2 (28°C), T_3 (30°C) and T_4 (32°C)} containing 10 female for each tank. Thus the progeny produced was reared for a period of 35 days and sex of the fish was assessed based on the presence of the male secondary sex characters and determined by external examination using caudal, dorsal and anal fins as markers. The fishes lacking these characters were classified as the female.

Water Quality Monitoring: The selected water quality parameters such as pH(7.84-8.32, Dissolved Oxygen (4.67-7.09 ppm), Electrical conductivity (0.190.26mMho), hardness (432-473ppm) and alkalinity (208-223 ppm) were analysed following standard methods of APHA [8].

Stastical Analysis: To assess the significance of temperature in relation to sex ratio of guppy, analysis of variance (ANOVA) was applied. A $p<0.05$ was considered as significant [9].

Results and Discussion

In this experiments, the effect of temperate on phenotypic sex ratio of guppy was evaluated for a periods of 30 days. Five different treatments unit such as Control, 26, 28, 30 and 32°C in aquarium (45 cm^3 sized) has significant impact on fish sex ratio. As such the results pertaining to sex ratio are presented in Table 1 and Figs. 1 &2). The highest female population of 66.67% was observed in control which was followed by 63.33(T1), 56.67 (T2), and 36.67 %(T3). Further, the highest male population of 63.33 % was obtained from T3 treatment. The lowest number of male (33.33%) was observed in control. In different treatments, the male: female was noticed in following increasing order: 3.3:6.7(Control)> 3.7:6.3(T1)> 4.3:5.7(T2)> 6.3:3.7(T3)

Generally, sex ratio biased to female and male at lower and higher temperature treatment respectively. The relationship between average percentage of male offspring and average temperature used in this experiment was found to follow a straight line and predicted as follow:

Pm=149.66+4.833T

From this equation, the quantity –a/b of this relationship will define the temperature at which all offspring would be male. Which was found to be 30.96 ^{0}C. While the relationship between percentage of females and variation of water temperature was found to behave similarly and followed the mathematical equation:

Pf= 70.334+4.833T

Again the quantity –a/b here, in this equation will define the temperature at which all offspring would be female; this was found to be 14.55 ºC. The near equal production of males and females by most organisms has fascinated biologists since the days of Darwin. In 1929, Fisher pointed out that, since each individual arising from sexual reproduction, males equal that of parental females. Therefore, if the population sex ratio deviates from equal numbers of males and females, pairs producing the rarer sex will have a selective advantage until the population sex ratio is equalized. This statistical mechanism maintaining equal sex ratios has been later demonstrated [2, 10].

The environmental factors such as pH may affect sex ratio in some Cichlids and Poeciliids. Numerous experimental studies have recently reported, however, that environmental sex determination (ESD) is prevalent among poikilothermic vertebrates. In these vertebrates, incubation temperature determines the course of primary sex differentiation (and thereby the sex ratio of progeny) during a specific period of embryonic or larval development. In some species, lower temperatures produce males and higher temperatures produce females (e.g. Turtles) while in others, the pattern is reversed (Lizards, alligators, fishes [11, 12, 6]

In this study, sex ratio was evaluated at five different treatment units. The sex ratio of *Poecilia reticulata* at different temperature treatments is presented in Table 1. Phenotypic sex ratio was influenced by temperature and highest (63.33%) male offspring at T_3 treatment (30°C) were produced, the lowest (33.33%) being in control. Whereas, the highest (66.67%) and lowest (36.67%) female population was observed in control and 30 ^{0}C respectively. Similar results was also produced in *P.sphenops* [13] and *Labeo rajasthanicus*[7]. The combination of high temperature (32°C) and pH 6 or 7 induced higher male ratio, while the female ratio is increase at 26°C at a pH 7-8. The effect of temperature on sex ratio of *Poecilia reticulata* reported in this study showed similarity with the work of Dzikowski, *et.al*. [14] and the observations of Karayucel, *et.al*. [4] that the proportion of female increase gradually with decreasing temperature and the proportion of male increase gradually with increase temperature rather than having a threshold response in heat treated guppy fry.

In the Atlantic silverside fish *Menidia*, the temperature affects primary sex determination during the larval development. Most offspring produced under lower temperature regimes of the early breeding season become females, while most offspring produced at the higher temperatures prevailing during, the late breeding season become males. However, the fish *Menidia peninsula* showed that the sex is biased towards females at cold temperature (11-19C°), while at warm fluctuating temperature (17-25°C) the ratio is 1:1 or favoured males [15]. Moreover, in the poeciliid, *Xiphophorus helleri* the sex ratio was affected by (pH) and water temperature [16] and the poeciliid fish *Poeciliopsis lucida* [17]. In these species, sex appears to be determined by genetic factors, temperature level and genotype-temperature interaction [18]. The response of sex ratio to temperature was adjusted upward or downward, perhaps by selection of sex-determining genes sensitive to higher or lower temperatures [19, 20]. The hypothesis of environmental sex determination (ESD) suggests environmental determination of sex whenever the environment affects the relative fitness of males and females differentially[21]. In addition, parental adjustment of sex ratio in offspring during the period of care may affect the reproductive success of male and female offspring differently [6]. Adaptive variation in environmental and genetic sex

determination in *Menidia* according to this hypothesis could be demonstrated [22, 23]. Such mechanism is now suspected throughout the fish life as emphasized in this study. Thus, on considering the sex ratio of the offspring, statistical analysis showed significant differences which may throw some light on the effect of temperature on this fish. On the basis of present study and previous works [2, 6, 7, 24] indicated that there may be deferential temperature range for higher survival (< 28 ^{0}C) and production of desired sex (male) offsprings (>28 ^{0}C) in guppy.

Table 1: Phenotypic sex ratio of *Poecilia reticulata* reared at different temperatures under laboratory conditions

Treatment	Observed		Expected		Sex ratio	χ^2
	Male (Nos)	Female (Nos)	Male	Female	(M:F)	
Control	33.33^c	66.67^a	40.25	50.75	3.3:6.7	0.2214**
T_1	36.67^c	63.33^a	40.25	50.75	3.7:6.3	
T_2	43.33^b	56.67^b	40.25	50.75	4.3:5.7	
T_3	63.33^a	36.67^c	40.25	50.75	6.3:3.7	
T_4	+	+	+	+	+	

*Values superscripted with same letter are not significantly different ** Non significant (NS)
+In 32 ^{0}C (T_4) breeding was normal. However, the newly hatched larva did not survive in this treatment

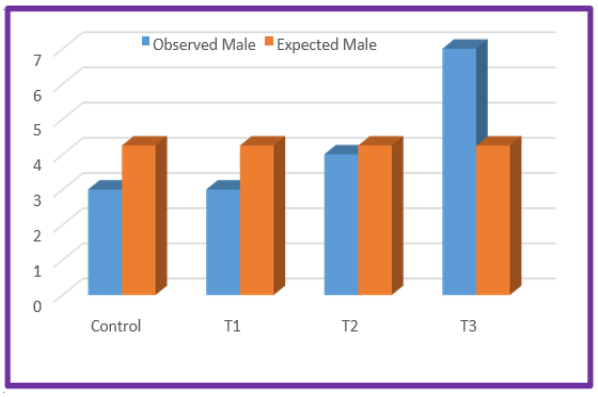

Fig 1: Observed and expected numbers of male guppy reared at different temperatures

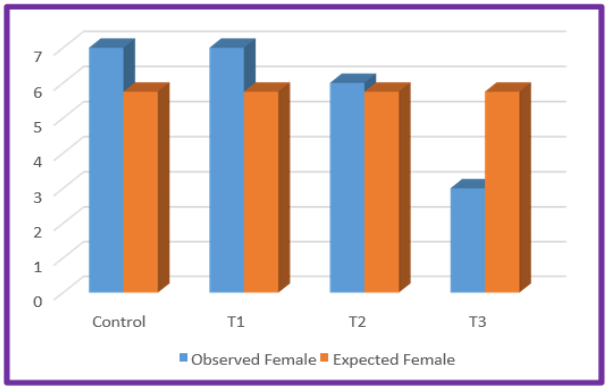

Fig 2: Observed and expected numbers of female guppy reared at different temperatures

Conclusion

The experiment has been carried out to investigate the effect of temperature on phenotypic sex ratio of guppy. A significan increase in male population with increasing temperature treatement was noticed. The potential temperature of 30 ^{0}C yielded the highest male population. This study has shown that the phenotypic sex can be altered by the thermal conditions prevailing during the early larval development period.

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