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Effect of Temperature on Some Reproductive Parameters of Gravid Females and Growth of Newly Hatched Fry in Guppy, *Poecilia reticulta* (Peters, 1860)

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Abstract: In this study, the effect of temperature on growth and some reproductive parameters of guppy *Poecilia reticulata* were investigated. And 19, 21, 22.5, 26, 29, 32 and 35°C water temperature treatments were used on newly hatched fry and gravid females for 11 days after parturitions and several days starting from the 16th day after first parturition until second parturition, respectively. Survival rates of heat treated fry were lower in the high temperature groups of 29°C and over than the low temperature groups of 22.5°C and lower. Same trend was observed for gravid female which all died in the temperature groups of 29°C and over. Guppy fry grown better at temperature between 22.5-26°C and the gravid female guppy produced high number of fry at temperature between 21-26°C may support the hypothesis that an ontogenetic reduction in temperature optima with increasing size for growth and the optimum temperature for growth broadness as body increases. However, size and sex dependent optimum temperature range for optimum growth and reproduction of guppy could be in question.

Key words: Guppy, *Poecilia reticulata*, temperature, growth, survival

INTRODUCTION

All freshwater fish are poikilothermic and their physiological mechanisms are directly or indirectly temperature dependent. The optimal physical and biological ranges of a fish species are determined by temperatures that can affect virtually all aspects of fish's physiological rates and efficiencies and more complex organismal activities such as developmental rates, locomotor performance, reproductive success and larval development including hatching time and success, growth, feeding rate and disease resistance (Blaxter, 1992; Elliott, 1994; Ojanguren *et al*., 2001). Amongst them, growth, resulting from digestion, absorption, assimilation, metabolic expenditure and excretion, is an important factor during early life stages of fish with respect to survival and is controlled by enzymatic activities (Elliott, 1994; Ojanguren *et al*., 2001). Therefore, embryonic development of ectotherms mainly depends on the differential expression of certain genes and temperature. Temperature acts on internal physiological process by affecting the rate at which biochemical reactions occur and are catalysed (Laurence and Howell, 1981). Thus, temperature requirement differs among species and various development stages of a given species (Herzig and Winkler, 1986). Thermal limits are narrower for early stages, in which organogenesis predominates over growth, than for later stages, so that embryo and juvenile survival can be reduced at temperatures within the tolerance range for adults (Cossins and Bowler, 1987).

At any temperature, fish must allocate energy to standard metabolic rate. When the consumption rate exceeds the standard metabolic rate, a fish can allocate energy to other compartments and when there is surplus energy, it can be used for activity or growth. This surplus energy is known as the metabolic scope and this tends to be highest at the fish's metabolic optimum temperature (Ficke, 2005). Fish generally have temperature ranges at which growth and survival are at an optimum (Brett, 1979).

Like growth, reproduction of fish is also affected by temperature which is the most studied variable. Temperature cues to play a role in ovary development and spawning initation and experienced during early phases influences adult size and the balance of trade-offs such as egg size versus fecundity, current versus future reproduction and age at maturity (Ojanguren *et al*., 2001).

The guppy *Poecilia reticulata*, native to fresh and brackish waters of north eastern South America and adjacent islands of the Caribbean, is one of the famous tropical ornamental fish in the world. It tolerates comparatively wide ranges of temperature, salinity and crowding. Although, culture of the guppy has increased

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in recent years due to popular demand and pressure on wild resources, few studies have been conducted to determine the effects of temperature on the growth and reproductive success on this species. The temperature ranges and upper lethal temperature reported for guppy are between 24-30°C and 32°C by Liley and Seghers (1975) cited in Dzikowski *et al.* (2001) and Gibson (1954 cited in Dzikowski *et al.*, 2001), respectively. The highest growth rates of guppy were obtained at 23 and 25°C in the studied temperature range of 20-30°C by Gibson and Hurst (1955) cited in Dzikowski *et al.* (2001). It should be noticed that the original articles of all these aforementioned 3 studies couldn't be found since, the date of the studies were so old. The most recent studies done on the effect of temperature on reproductive success and sex ratio of guppy were reported by Dzikowski *et al.* (2001) and Karayücel *et al.* (2005), respectively. In the former study, the optimal temperature for reproduction as brood size and brood interval was identified at 26-27°C. At 30°C, these fish experienced increased fry and female adult mortality, degeneration of ovaries and reduced brood size. In the later study, a major gene linked to X chromosome suggested for resistance and sensitivity to both high and low temperature since, the proportion of females increased gradually with decreasing temperature and the proportion of males increased gradually with increasing temperatures rather than having a threshold response in low and heat treated guppy fry.

In the light of the sorely needed new further study about temperature effects on guppy, the present study aimed to describe the effect of various temperature regimes on growth of newly hatched fry and some reproductive parameters of adult female guppy.

MATERIALS AND METHODS

Experimental fish stock and their maintenance: The *P. reticulata* used in this study, were obtained from a commercial aquarium fish production facility of Aqua-Mak Company, Ankara, where the experiments were also carried out.

Fish were kept at 26±1°C under a 14/10 h light/dark cycle. They were fed with a commercial diet (Lansy Dynamic 3 with 42% protein for broodstocks and Impact 0820 with 56% protein for fry) 3 times a day *ad libitum*. Water quality parameters (O_2, pH, ammonia, nitrite and nitrate) were monitored every 15 days during the study. A total of 44 aquarium of 60×30×40 cm were used for keeping females, rearing fry and establishing static heat treatment units. Net cages of 18×8×8 cm were placed into the aquarium when necessary for each gravid female ready for birth. Fourteen static heat treatment units were used for thermal treatment of gravid females and newly born fry separately at 7 different temperature (19, 21, 22.5, 26, 29, 32 and 35°C) regimes. In each heat treatment unit, 3 plastic holding tanks of 4.5-5 L were placed into a glass aquarium. The whole heat treatment units were filled with clean and aerated tap water before the experiment started. Thermostatically controlled submerged heaters were used to maintain the water temperature at desired level with a stirrer pump placed in the glass aquarium. The stirrer pump provided the circulation of water at a certain temperature around the plastic holding tanks. Aeration was provided to each aquarium and plastic holding tanks through an air pump. The water temperature was checked with a digital thermometer having 0.1°C division 6 times a day. All aquariums were cleaned daily by siphoning the uneaten food and excreta and water were then topped up with clean aerated tap water at the desired temperature.

Experimental design

Experiment 1 heat treatment to gravid female guppy: Twenty one gravid females (2.49±0.13 g w/w; 5.30±0.09 cm standard length) which are distinguishable by their black sacs, denoting their pregnancy were chosen and maintained individually in net cages at 26°C until the succeeding parturition. The females were kept at 26±1°C for 15 days. Female guppies produce young every 4 weeks or so. At 16th day, the females were then transferred to 7 different heat treatment units containing 3 plastic holding tanks with 1 female to each holding tanks until the next parturition. The brood interval time and the number of the resulting broods from each holding tanks were recorded.

Experiment 2 heat treatment to fry guppy: Twenty one gravid females (2.28±0.12 g w w^{-1}; 5.79±0.13 cm standard length) were chosen and maintained individually in net cages at 26±1°C until the succeeding parturition. After the length and the weight of the resulting broods were recorded, they moved to 7 different heat treatment units containing 3 plastic holding tanks for 11 days by slowly adapting to desired water temperature. At 12th day, the fry were separately transferred to fry rearing tanks at 26±1°C and reared for further 38 days then their length and weight were recorded.

Calculations: Survival was calculated as: (Number of fry at the end of the treatment/Number of fry at the beginning of the treatment)×100. Survival rates were calculated at 11th day and 50th day for experiment 2.

The following equations were used to calculate Specific Growth Rate (SGR), Relative Growth Rate (RGR) and Weight Gain (WG):

$$SGR=[(\ln W_2)- (\ln W_1)/ (t^2-t^1)]\times 100$$
$$RGR= [(W_2- W_1)/ W_1]\times 100$$
$$WG= W_2-W_1$$

where,

W_1, W_2 = The wet-weight of the larvae at the start and the end of the experiment, respectively.

Statistical analysis: All data were expressed as the mean±S.E. Growth parameters and arcsine transformed survival rate data of groups were analysed for significant differences using Analyses of Variance (ANOVA) with a Tukey posthoc test for comparison or Kruskal-Wallis nonparametric test according to the data number and distribution. A $p<0.05$ was considered as significant. Analyses were performed using Minitab 13 software for windows.

RESULTS

The temperature range recorded during the experiments did not differ significantly from the pre-set temperatures for the different treatments.

Survival rates: Eleven gravid females were died during the temperature treatment, in one of 21 and 22.5°C groups and 9 in the groups treated over 29°C so no broods were produced from these females.

While, at the 11th day of the experiment, the mean survival rates of *P. reticulata* fry ranged from 22.70±8.55% to 93.46±1.85% in 35 and 21°C groups, respectively, they were 11.66±10.22% and 83.06±0.90% in the 35 and 26°C groups, respectively, at the 50th day of experiment (Table 1). Average survival rates of groups reared at different temperatures were generally decreased with increasing and decreasing temperature. Generally, survival rates of higher heat groups were much lower than that of lower heat groups at 11th day of experiment since, there was a significant differences ($p<0.05$) between the higher heat groups (29, 32 and 35°C) and lower heat groups (26, 22.5 and 21°C) whil,e no significant differences ($p>0.05$) were find with the temperature group of 19°C at 11th day of the experiment. The highest survival rate of 83.06±0.90% in temperature group of 26°C was not significantly differed ($p<0.05$) from the temperature groups of 19, 21 and 22.5°C but significantly differed ($p>0.05$) from the temperature groups of 29, 32 and 35°C at the 50th day of the experiment.

Growth parameters: The highest initial mean length of 7.59±0.05 mm at 26°C was not significantly differed ($p>0.05$) from the other temperature groups except the group reared at 29°C, while, the mean final length of group of 19, 21, 22.5 and 26°C were significantly differed ($p<0.05$) from the groups of 29, 32 and 35°C (Table 1). The highest final mean length of temperature group of 21°C (16.48±0.12 mm) did not significantly differed ($p>0.05$) only from the temperature group of 26°C. Generally, the final mean length was high in the temperature group of 26°C and lower. While, there was no significant differences ($p>0.05$) between all temperature groups in terms of initial mean weight, the highest mean final weight of 42.63±0.34 mg at 26°C were significantly ($p<0.05$) higher from the all temperature groups. Same trend was observed in terms of weight that 26°C and lower reared groups were heavier than the temperature groups of 29, 32 and 35°C.

The highest SGR%, RGR% and WG of 3.73±0.10, 559.92±34.55 and 37.28±1.44, respectively, obtained in the temperature group of 26°C were significantly differed ($p<0.05$) from the all temperature groups except the group reared at 19 and 22.5°C. The other temperature groups

Table 1: Survival rates (%±SE) of newly hatched guppy fry at 11 and 50th day of the experiment and growth and some reproductive parameters of newly hatched fry and gravid females of guppy, respectively, treated with different temperatures

Temperature (°C)	Survival rate at 11th day (%)	Survival rate at 50th day	Initial mean length (mm±SE)	Final mean length (mm±SE)	Initial mean weight (mg±SE)
19	61.00±2.08^{ab}	59.00±2.33^{bc}	7.90±0.12^{bc}	16.14±0.18^{c}	6.33±0.27^{a}
21	93.46±1.85^{c}	82.24±3.39^{c}	7.60±0.10^{ab}	16.48±0.12	7.43±0.29^{a}
22.5	71.84±4.98^{bc}	68.93±4.42^{bc}	7.73±0.11^{ab}	16.08±0.27^{c}	7.70±0.25^{a}
26	91.66±0.70^{c}	83.06±0.90	7.59±0.05^{a}	16.21±0.05^{c}	6.83±0.21^{a}
29	68.75±3.61^{ab}	57.64±2.41^{abc}	8.23±0.18^{c}	12.46±0.14^{a}	7.07±0.23^{a}
32	47.83±7.81^{ab}	34.06±8.52 ^{ab}	7.60±0.11^{ab}	14.88±0.31^{b}	7.36±0.12^{a}
35	22.70±8.55^{a}	11.66±10.22^{a}	7.43±0.10^{a}	12.95±0.34^{a}	7.63±0.58^{a}

Temperature (°C)	Final mean weight (mg±SE)	SGR	RGR	WG	Mean brood interval	Mean brood size
19	29.37±1.75^{b}	3.05±0.28^{abc}	369.41±61.77^{abc}	23.33±3.48^{abc}	40.33±0.88^{b}	44.33±4.27^{a}
21	27.13±1.14^{b}	2.65±0.04^{ab}	276.30±7.87^{ab}	20.57±1.26^{ab}	41.50±0.41^{b}	66.50±6.49^{ab}
22.5	30.59±1.36^{b}	2.92±0.19^{abc}	334.93±39.00^{abc}	25.97±3.69^{abc}	38.50±0.41^{b}	63.50±17.5^{ab}
26	42.63±0.34^{c}	3.73±0.10^{c}	559.92±34.55^{c}	37.28±1.44^{c}	29.06±0.46^{a}	76.10±11.89^{b}
29	18.39±0.9^{a}	1.90±0.16^{a}	160.83±21.47^{a}	11.27±1.11^{ab}	×	×
32	28.20±2.13^{b}	2.70±0.58^{ab}	319.71±126.33^{ab}	23.30±8.94^{ab}	×	×
35	21.05±2.28^{a}	2.26±0.45^{ab}	224.19±65.15^{ab}	17.70±6.12^{ab}	×	×

were not differed significantly ($p>0.05$) between each other. Generally, the fish reared at 26°C and lower temperature performed better than that of reared at 29°C and higher temperature.

Reproductive parameters: The mean brood interval at 19, 21 and 22.5°C were 40.33±0.88, 41.50±0.41 and 38.50±0.41days, respectively. These were significantly ($p<0.05$) longer than at 26°C, being 29.06±0.46 days (Table 1). The highest mean brood size of 76.10±11.89 at 26°C was significantly differed from the temperature group of 19°C ($p<0.05$) but was not significantly differed from the temperature group of 21 and 22.5°C ($p>0.05$).

DISCUSSION

Growth rate of guppy larvae was significantly influenced by temperature. Final mean weight was highest at 26°C. However, at 19 and 21°C growth rate in terms of SGR, RGR and WG was not significantly differed ($p<0.05$) from the temperature group of 26°C. Similar growth performance of the temperature group of 19°C might be resulted from the low survival rate of this group amongst the other low temperature groups enabling to the fry to be fed better because of no competion. Overall significantly low growth rate was obtained in fish reared at 29°C and over. As a summary, growth parameters increased with increased water temperature, reached a maximum at 26°C then dropped sharply at higher temperatures and it it was skewed towards to low temperature. The temperature range for guppy was reported as 18-28°C (www.fishbase.org). The optimal temperature for optimal larval growth, identified at 22.5-26°C in this study is similar with the study of Gibson and Hurst (1955) cited in Dzikowski *et al.* (2001) to be 23-25°C but lower than the study of Dzikowski *et al.* (2001), who reported the optimal temperature for reproduction as 26-27°C. On the other hand, Dzikowski *et al.* (2001) suggested that the significant effect of temperature on fry production of female guppy mediated through its effect on the duration of the brood interval (gestation time), since, the mean number of fry per brood was not affected by temperature. In this study similar result was obtained that temperature was only affected the mean brood interval but the mean brood size was similar in the temperature group of 21, 22.5 and 26°C at which the minumum brood interval and maximum brood size were obtained. Thus, guppy fry grown better at temperature between 22.5-26°C and the gravid female guppy produced high number of fry at temperature between 21-26°C showing lower temperature preference than fry may support the hypothesis that an ontogenetic reduction in temperature optima with increasing size for growth and the optimum tempereature for growth broadness as body increases, although, there were insufficient treatments to accurately estimate the values since, the experiment was not design for the optimum temperature for all size or age classed guppy from larvae to adult. A downward shift and broadness in the optimum temperature for growth with increasing body size has been reported in mostly cultured farmed fish such as in striped bass, Morone saxatilis (Duston *et al.*, 2004), cod, Gadus morhua (Björnsson and Steinarsson, 2002), blue fish, Pomatomus saltatrix (Buckel *et al.*, 1995), plaice, Pleuronectes platessa (Fonds *et al.*, 1992), turbot, Scophthalmus maximus (Imsland *et al.*, 1996) and halibut, Hippoglossus hippoglossus (Aune *et al.*, 1997). To our knowledge, there no reports about this hypothesis on any aquarium fish species and this study is the first report in this respect.

In the present study, generally a significantly lower survival rates observed in the temperature groups of 29°C and over (overall average 0.47±0.07% at 11 and 0.35±0.07% at 50 day of the experiment) comparing with the temperature groups of 22.5°C and lower (overall average 0.75±0.07% at 11 and 0.70±0.06% at 50 day of the experiment) may indicate sensitivity of guppy fry to high temperature regimes. Similar observation on the dead of all gravid female guppies in the temperature groups of 29°C and over showed that also the gravid female were much more susceptible to high temperature in this study. This result is not surprising because of the need for more oxygen related to cellular energy requirements of fry in mother's ovary causing insufficient oxygen level in the body fluids at high temperatures. In addition to this, any increases in temperature increase the feed intake to a maximum then decrease rapidly before the upper limit for thermal tolerance and finally prevented (Pörtner, 2002). The dead of gravid guppy fry at high temperature (32°C) were also reported by Dzikowski *et al.* (2001). The dead of gravid females in one of the replicate of 21 (B3) and 22.5°C (C3) treatment could be resulted from poor management. Fish generally have temperature ranges at which growth and survival are optimum (Gadomski and Caddell, 1991). Thus, the preference of fry and gravid female guppy to lower temperature than 29°C coincided with the optimal temperature for growth in this study. However, the preferred temperatures reported by Johansen and Cross (1980) as 24.5°C for male and 28.2°C for female and young were higher than the present study for gravid female and fry guppy. On the other hand no sex dependent differences was detected in terms of CTM (Critical thermal maxima) and DP (death point) to be ranged from 38.95-40.33 and 39.48-40.61 for male and female guppy, respectively while, the CTM and DP were

statistically different among acclimation temperatures, with positive relationships between temperature tolerances (CTM and DP) and acclimation temperatures (Chung, 2001). Laudien and Schlieker (1981) reported that male sexual behaviour was not differed after acclimization to different remperature range of 20-30°C. It can be concluded from these study that growth or behaviour at a given temperature might depend upon the rearing history of the fish. The low preferred temperature of gravid female was also coincided with the study of Karayücel *et al.* (2005), who reported that female number increased gradually with decreasing temperature.

The guppy *Poecilia reticulate* is one of the most important tropical ornamental fish in the world, however, low fecundity (small brood size) affecting production costs and surplus of females in offspring affecting marketing efficiency are 2 major constraints in guppy farming. The thermoregulator knowledge of fish is useful for improving the culturing practises of interested fish. Although, the guppy has been under culture for a long time, there have been few studies on the effect of temperature on growth and efficient reproduction. In this sense, the optimum temperature for optimum growth of fry treated for 11 days after parturition and brood size of gravid female treated with different duration starting with 16th day of first parturition were determined as 22.5-26°C and 21-26°C, respectively. On the other hand the present study and previous works indicate that there may be size and sex dependent optimum temperature range for growth and reproduction of guppy. Thus, much more attention should be paid for optimum temperature needs of guppy.

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