

THE EFFECT OF TEMPERATURE VARIATION ON THE SEX COMPOSITION AND GROWTH OF *PEOCILLA MELANOGASTER* OFFSPRING

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Key words : Temperature, sex composition, growth, offspring, *Peocilia*.

ABSTRACT

The sexual differentiation of *P. melanogaster* embryos was examined among fish kept in glass aquaria (80x40x35 cm) at 23, 26 and 29°C with a pH=6.5.

Analysis of variance of the data revealed a significant variation (at the 5 % level) of sex composition relative to increase of water temperature. Two mathematical representations were predicted for males and females. Accordingly, the degrees at which all males, or all females, or 1:1 sex ratio had been determined was 18.95, 32.01 and 25.4°C respectively.

The mechanism of this temperature effect on the sex ratio was discussed where growth of fish was suspected to have played a role in this process.

INTRODUCTION

Sex Ratio

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 by several authors (Shaw & Mohler 1953; Kolman 1960; MacArthur 1965; Verner 1965; Myers, 1978; Conover, 1984 and Olivier & Kaiser, 1997). However, environmental factors such as pH may affect sex ratio in

some Cichlids and Poeciliids (Rubin, 1985).

According to Romer and Beisenherz (1995) a correlation between sex ratio of offspring of 33 different species of *Apistogramma* and temperature at certain pH-level could be demonstrated with high significance. At low temperature (23 °C) the sex ratio of offspring of 32 *Apistogramma* species was skewed towards females, whereas at high temperature (29°C) towards males and at a temperature of 26 °C the number of males and females was approximately balanced. However, *Apistogramma cacti* showed a reversed response to temperature. The previous authors declared that temperature could influence sex ratio of offspring during development within the first weeks of life. On the other hand, in *Apistogramma*, sex ratio was influenced by pH-level, too.

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).

The live-bearing fishes of the family Poeciliidae are widely known to exhibit unusual sex ratios. Examples of some reports include *Poecilia reticulata* (Haskins *et al.* 1961), *Gambusia mani* (Krumholz, 1963), *Heterandria formosa* (Colson, 1969), *Poeciliopsis monacha* (Moore, 1971) and *Gambusia holbrooki* (Vargas & Sostoa, 1996). Three other examples of fishes with temperature sex determination have been recently studied in the laboratory: (1) the Atlantic silver-side, *Menidia menidia* (Conover & Kynard, 1981; Conover & Heins, 1987 a,b and Conover *et al.* 1992), in which some genotypes produced excess of males at high temperatures and excess of females at low temperatures, while others in the same population were strictly genetic sex determination (GSD); (2) the hermaphrodite, *Rivulus marmoratus* (Harrington, 1967, 1971), a fish which at its normally warm tropical or subtropical temperatures fertilizes itself and produces hermaphroditic offspring, but when reared at 20°C, produces 75% males; and (3) *Poeciliopsis lucida*, which is a polymorphic fish with genotypes that have either genetic sex determination (GSD) or temperature sex determination (TSD). As

documented by Sullivan and Schuiz (1986) and Schuiz (1993).

In this paper, the effect of temperature on the sex ratio of *P. melanogaster* is assessed.

MATERIAL AND METHODS

Adult males and females of *Poecilia melanogaster* were collected from large fish tanks containing various fish species at the Department of Zoology II, University of Dtisseldorf (Prof. Dr. Greven, H., Germany). Fishes were separated and kept in glass aquaria (80 x 40 x 35cm) with constantly aerated water. Fresh water plants e.g. *Hydrilla* and *Pistia*, were provided to match the natural habitat of the fish. Fishes were maintained under 12 hrs. light/ 12 hrs. dark schedule. They were fed daily with commercially available fish food (Tetramin¹¹, Tetra-Werke, Melle, Germany). The experimental aquaria were kept at $23 \pm 1^\circ\text{C}$, $26 \pm 1^\circ\text{C}$, or $29 \pm 1^\circ\text{C}$ and pH = 6.5. The temperature was checked four times daily, and heating was carried out by heaters, while cooling was reached by use of ice, if such need arised during the experiment.

Statistics:

The influence of the applied temperatures (23 °C, 26°C and 29°C) on the sex ratios of the offspring were examined statistically, by a two-way ANOVA analysis (Neter and Wasserman, 1974). In order to describe the interaction between gender and temperature, a correlation analysis is applied (Pearson Product Moment Correlation).

RESULTS

Effect of temperature variation on the sex composition and growth of offspring:

A two-way analysis of variance (ANOVA) revealed a significant variation ($P < 5\%$) of sex composition relative to the increase of water temperature in favour of males over females among offspring of that fish. The overall average for males was 54% and 46% for female with a standard error for either sex of (± 0.6). It is worth to mention that the total number of offspring did not show any significant variation with temperature difference (Tables 1, 2 and 3).

The change in sex ratio due to temperature variation was statistically examined as follows:

The relationship between average percentage of male offspring and average temperature used in the experiment was found to follow a straight line (Fig.1) and predicted as follows: $P_m = -145.1653 + 7.6592 T_r = 0.997$, where P_m = percentage of number of males among total number of offspring, and T_r = average water temperature in centigrades. From this equation, the quantity $-a/b$ (Y-intercept value divided by the slope value as defined in this equation) of this relationship will define the temperature at which all offspring would be females. Which was found to be 18.95°C .

While the relationship between percentage of females and variation of water temperature was found to behave similarly (Fig. 2), and followed the mathematical equation:-

$$P_f = 245.161 - 7.6592$$

$$T_r = 0.997$$

Where P_f = percentage of number of females among total number of offspring, T_r = average water temperature in centigrades.

Again, the quantity $-a/b$ here, in this relationship, will define the average temperature at which all offspring would be all males; this was found to be 32.01°C . In addition, by extrapolation at the 50% of either sex it appears that the temperature at which the sex ratio 1:1 would be reached is 25.4°C . However, the average water temperature that would be effective in producing 1:1 males to females, could be calculated by summation of both temperature extremes and dividing by two (2). This was found to be 25.45°C which is not different from the extrapolated prediction.

The growth in length with age of the *P. melanogaster* offsprings with variation of temperature is shown in Table 4. From this table, it appears that there is a significant correlation between age and length of the offsprings at either the lower on higher temperature tanks, for either sex. However, the difference in growth rate of the offsprings in either tanks is more apparent during the first 10 days of life, than in the next 10 to 50 days. Thus, either males or females of the 23°C tank have higher growth rate than those of the 29°C tank. The difference is in favour of growth of the 23°C tank fish in the first 10 days of life, as well as during the next 40 days (10-50). However, this difference in growth among males in the first period is more than double (0.04) that of those fish of the second period (0.0198). On the other hand, the difference in growth of females in the first period is more than four times that of the second period.

DISCUSSION

Poecilia melanogaster is a viviparous top-minnow belonging to the family *Poeciliidae*. This family is native to the New World (Southern United States and West Indies) where their distribution is principally tropical, [Gunther, 1866; Rosen and Bailey, 1963 Innes, 1979; Nelson, 1984 and Meyer *et al.* 1985]. The present preliminary investigation of sex ratio, in the viviparous Poeciliid teleost *Poecilia melanogaster* have clearly demonstrated that; sex ratio and thus very probably sex differentiation is temperature dependent in this species.

The statistical analyses of the mathematical representation of the relationship for the species under study has proved significantly the effect of temperature on determination of sex ratio. Such a finding was previously reported in a different species (*Apistogramma*) by Romer and Beisenherz (1995). However, it is only in this study, at temperature 18.95°C the offspring would be all females, and that at 32.01°C they would be all males. The equally estimated sex ratio was at 25.4°C. Although such quantification is not so far present in the literature, genotypic sex determination has been shown in many fish species. Environmental sex determination (BSD) in fish has been shown so far in a few species (Conover and Kynard, 1981; Rubin, 1985; Sullivan and Schultz, 1986; Schultz, 1993; and Romer and Beisenherz, 1995).

In the Atlantic silverside fish *Menidia 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 (11-19°C), while at warm fluctuating temperature (17-25°C) the ratio is 1:1 or favoured males (Conover and Kynard, 1981; Conover, 1984 and Middaugh and Hemmer, 1987).

Moreover, in the poeciliid, *Xiphophorus helleri* the sex ratio was affected by (pH) and water temperature (Rubin, 1985); and the poeciliid fish *Poeciliopsis lucida* (Sullivan and Schuiz 1986 and Schuiz, 1993). In these species, sex appears to be determined by genetic factors, temperature level and genotype-temperature interaction (Price, 1984) 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 (Sullivan and Schuiz, 1986 & Mair *et al.* 1997).

The hypothesis of environmental sex determination (ESD) suggests environmental determination of sex whenever the environment affects the relative fitness of males and females differentially (Charnov and bull, 1977 and Charnov *et al.*, 1981). 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 (Trivers and Willard, 1973). Adaptive variation in environmental and genetic sex determination in *Menidia menidia* according to this hypothesis could be demonstrated by Conover and Heins (1987a,b). Such mechanism is now suspected throughout the fish growth as emphasized in this study. Thus, on considering the growth in length of the offspring, statistical analysis showed differences which may throw some light on the effect of temperature on this fish. The first result that comes out of these analyses is that, fish kept in 29°C tanks have low growth rate than those kept in 23°C tanks. The second result is that, there is a conspicuous decrease in growth in both sexes after the day 10 to 50 after birth.

However, a third outcome of these results is that males have though slight, but lower growth rate than females. Could it be that lowering growth rate of offspring, due to raised temperature, activates, in some unknown way, the biological conversion of sex from females into males. No other evidence, so far, could be found in the previous literature confirming such finding.

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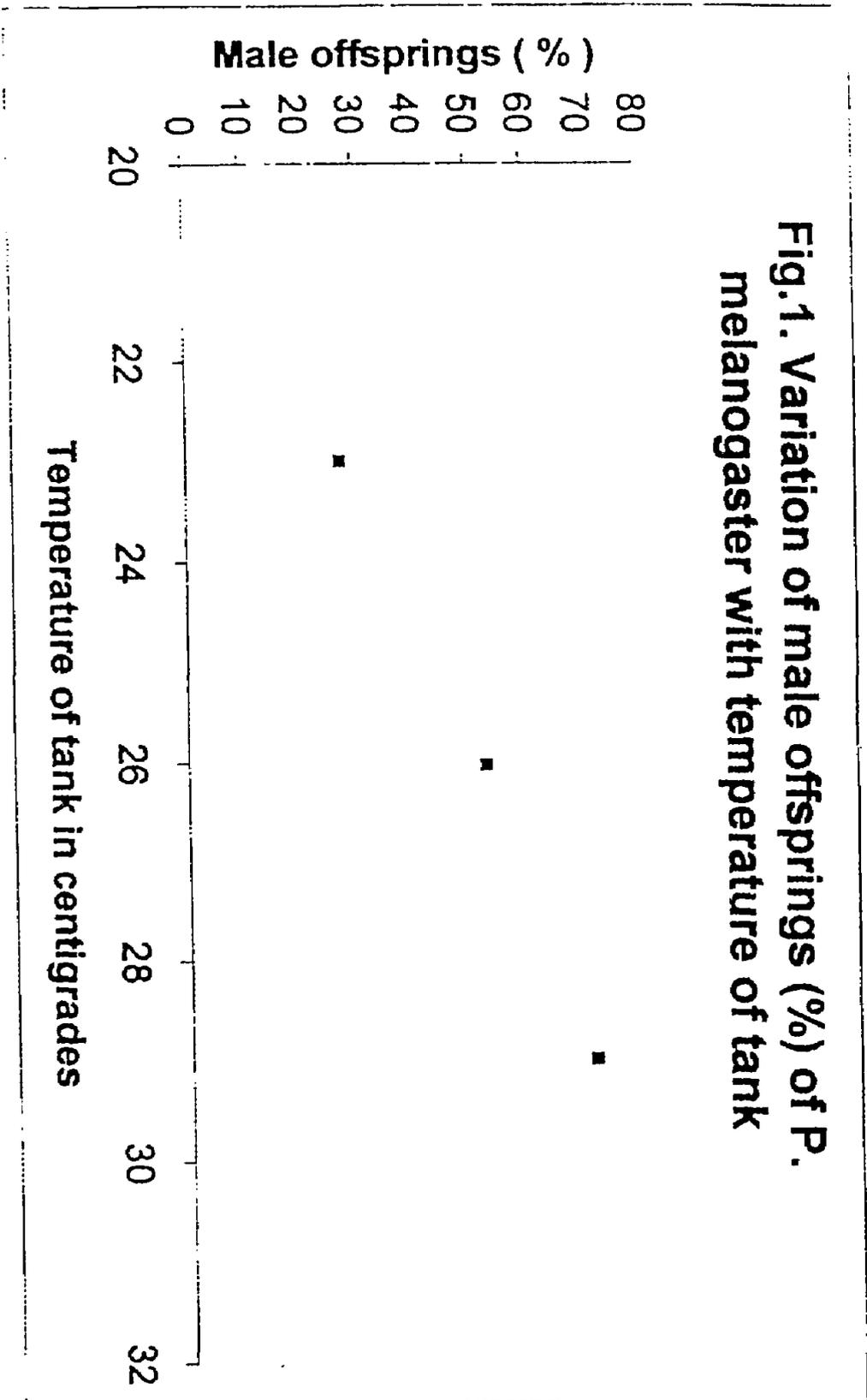
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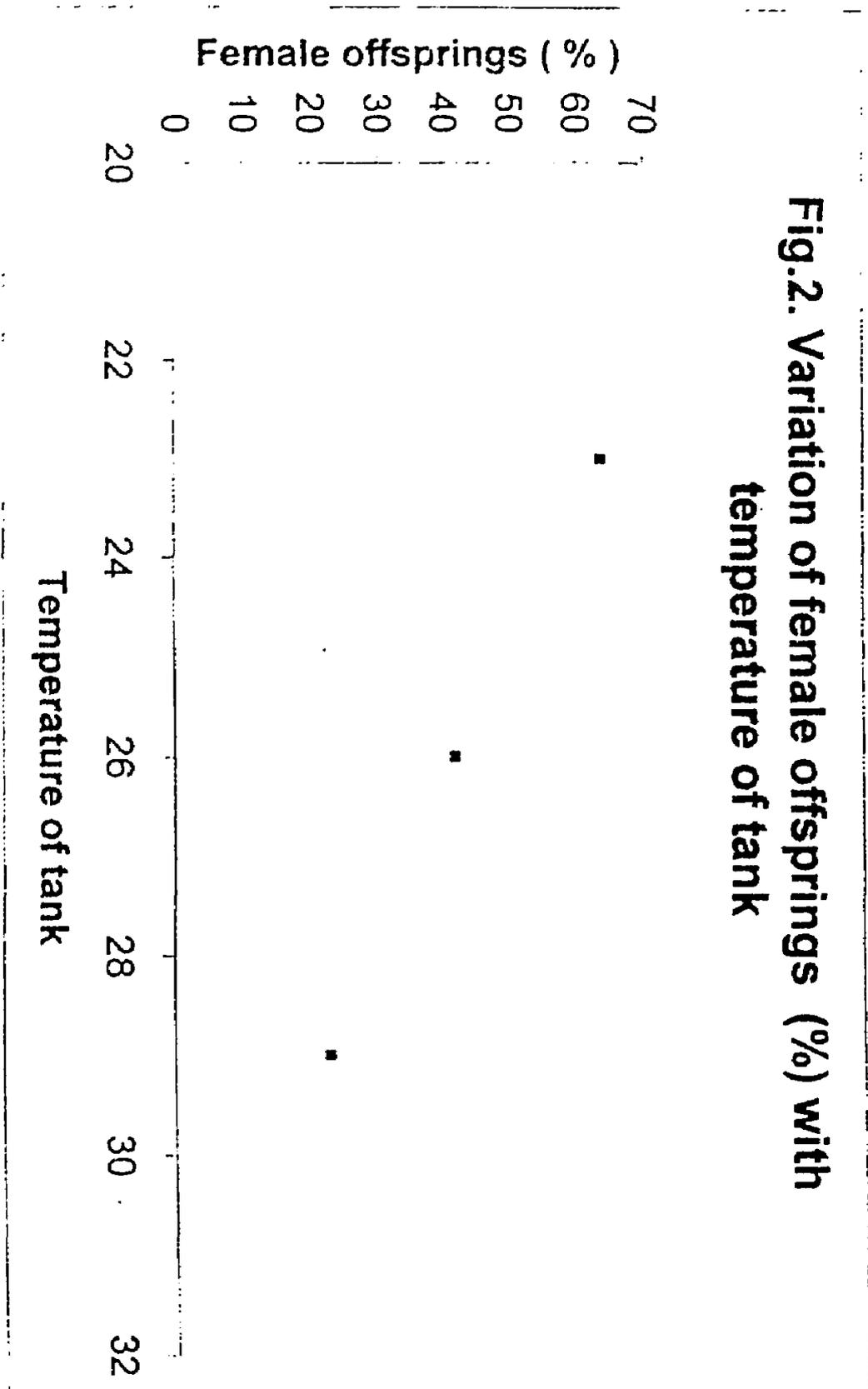
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Experiment (1)					
Table (1) Data collected on <i>P. melanogaster</i> kept at 23°C water tanks					
Number of generation	Number of collected embryos	F	M	Mortality	Undifferentiated
1st gen.	20	11	7	2	0
2nd gen.	22	15	4	3	0
3rd gen.	23	14	7	2	0
4th gen.	23	14	8	1	0
5th gen.	22	16	4	0	2
Total	110	70	30	8	2
%		63.6	27.3	7.3	1.8
F: Female					
M: Male					
Experiment (2)					
Table (2) Data collected on <i>P. melanogaster</i> kept at 26°C water tanks					
Number of generation	Number of collected embryos	F	M	Mortality	Undifferentiated
1st gen.	20	8	10	2	0
2nd gen.	24	9	15	0	0
3rd gen.	25	11	13	1	0
4th gen.	25	10	14	0	1
5th gen.	26	12	12	0	2
Total	120	50	64	3	3
%		41.7	53.3	2.5	2.5
F: Female					
M: Male					

Experiment (3)						
Table (3): Data collected on <i>P. melanogaster</i> kept at 29°C water tanks.						
Number of generation	Number of collected embryos	F	M	Mortality	Undifferentiated	
1 st gen.	23	5	16	2	0	
2 nd gen.	23	4	17	1	0	
3 rd gen.	22	6	14	2	0	
4 th gen.	20	5	15	0	0	
5 th gen.	23	5	17	0	1	
Total	110	25	79	5	1	
\bar{x}_n		71.1	71.8	4.6	0.9	
F : Female						
M : Male						
Table (4): Analysis of offspring growth in length with variation in temperature:						
Age in days	Male			Female		
	r	b	d	r	b	d
0-10 days						
23°C	0.9887	0.335		0.9962	0.375	
29°C	0.9961	0.295	-0.04	0.998	0.345	-0.03
10-50days						
23°C	1	0.1		0.9928	0.1243	
29°C	0.9914	0.0802	-0.0198	0.9959	0.1078	-0.0065
r = Correlation coefficient between age length of offspring in days.						
b = Slope of the line of such correlation equivalent to growth rate constant.						
d = Difference in growth rates due to temperature variation.						