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Differential Courtship Activity and Alterations of Reproductive Success of Competing Guppy Males (*Poecilia reticulata* Peters; Pisces: Poeciliidae) as an Indicator for Low Concentrations of Aquatic Pollutants

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Differential courtship activity of guppy males competing for the same females was used as a bioindicator for low concentrations of water-borne pollutants in a previous study (Schröder and Peters 1988). Water contamination of only 1 μgL^{-1} lindane as well as a 10% addition of wastewater drawn from the last clearing basin of a Munich water purification plant caused a decrease of the mean differential sigmoid courtship activities and an increase in gonopodial thrusting as compared to the control values. Patterns of male sexual activity were chosen because they determine reproductive success; more active males have a greater chance of encountering receptive females and are therefore preferred by females (Farr 1980a). When males compete for the insemination of the same females, their reproductive success roughly corresponds to the relative amount of courtship activity previously exhibited (Farr 1980a). Both quantitative male sexual behavior patterns and intermale aggressiveness are patriclinously inherited, autosomal factors modifying the primary influence of Y-chromosomal genes (Farr 1983; Pohla 1986). As known from previous studies (Farr 1980a), the mean difference between courtship activities of two male competitors determines the relative fitness of the male in question. Accordingly, the decrease in mean differential courtship after exposure to aquatic contaminants was predicted to cause a corresponding change in the relative reproductive success. However, this prediction could not be tested in the previous study (Schröder and Peters 1988), because only gravid females were used. The present study completed the previous one by repeating the experiment with a 10% addition of wastewater drawn from the last clearing basin of a Munich purification plant this time using virgin (non-inseminated) females which were receptive to male courtship (Farr 1980b). The females subsequently were allowed to produce as many offspring as possible. The number of young guppies sired by individual male competitors could easily be traced by the use of sex-linked phenotypic color patterns as markers. The purpose of these two studies was to show that the quantification of sexual activities of male guppies is useful for monitoring environmental alterations which affect fitness characters.

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MATERIAL AND METHODS

The sigmoid courtship display of the male guppy (Poecilia reticulata Peters) was described in the previous paper (Schröder and Peters 1988). It consists of an arching of the body with the unpaired fins either fully spread ("open display", O) or closed ("closed display", C; Baerends et al. 1985; Liley 1966; cf. Fig. 1 in Schröder and Peters 1987). Rates of C and O vary with changes in female fertility and receptivity (Liley 1966; Farr 1980a). During a copulation attempt or "gonopodial thrusting" (T) the male brings his gonopodium (anal fin modified into a copulatory organ) into a forward position and attempts to insert the gonopodial tip into the female's genital pore in order to inseminate the female. T occurs more often when females are not in the receptive portion of their brood cycle (Liley 1966; Farr 1980a,b). Virgin females as used in the present study normally respond to the activities of courting males and therefore are considered receptive (Farr 1980b).

Each pair of competing males was tested three times per day for the first two days and twice for the third day thus producing 8 replicates for each pair. Because one observation period lasted 20 min, each pair of competing males was tested for 160 min. To score both frequency and duration of the three male sexual activities (O, C, and T), an event recorder ('Peiseler Stopprechner') was used. Each pair of male competitors consisted of one male belonging to the Wild Maculatus (Ma) stock while the other was a Blue Iridescence (Ir) male. Four pairs of competing males (Ma versus Ir) were used for the present experiment, two pairs being exposed to 10%-Gross-Lappen (GL) wastewater and the remaining two served as controls (Munich tap water only).

To prevent habituation of courting males to the virgin (wildtype) females, males were only exposed to two females immediately before the onset of a observation period. After each 20-min observation both females of a given pair of male competitors were removed and males and females remained separated from each other until the beginning of the next observation. Because no true copulation was observed, the females were virgins up to the end of the experiment. After the last observation, male pairs were continuously kept together with the females for an additional period of 3 wk to allow the males to inseminate the females. Afterwards the females were isolated and routinely checked for newborn fry by daily examinations. Because the broods were sometimes born over a period of several hours and because some females might have been cannibalistic, all fry were immediately removed to small glass vessels and later transferred to rearing aquaria. Aquatic plants, Fontinalis, Riccia, and/or Myriophyllum, were grown in aquaria in which broods were anticipated to enable newborn fish to hide in the vegetation. All broods were brought up in separate tanks. After maturation, the fish were sexed and checked for the presence of the two Y-chromosomal marker genes, Ma or Ir, respectively. According to the presence or absence of the more conspicuous marker, Ma, all offspring were subdivided into two groups, viz. Ma and non-Ma (Table 2).

With the exception of pure inbred strains, all our guppy stocks are routinely maintained in collective breeding aquaria where fish mate at random. It can be assumed that maintenance of the fish in small population sizes and occasional "bottle-necking" of the populations over the years have resulted in a reduction in genetic variability in the stocks and a subsequent high degree of homozygosity among the individuals of a given stock. A detailed description of the Neuherberg guppy stocks is given by Farr (1980a) and Schröder (1983).

As in the previous study (Schröder and Peters 1988), the present experiments were conducted in 37.5-L aquaria filled with Munich tap water (Control; ca. 170 mg/L $CaCO_3$; 537 µs/cm conductance; 10. mg O_2/L oxygen content; pH 7.3; 22.4°C) or a 10%-dilution of Gross-Lappen (GL) wastewater, respectively. The GL- wastewater stemmed from the last sewerage plant at Gross-Lappen near Munich, West Germany. This water is normally collected in a collection tank before being released directly into the Isar river.

RESULTS AND DISCUSSION

The mean values (± standard error) of the three male sexual activities (O, C, and T) are given in Table 1. Figure 1 presents the

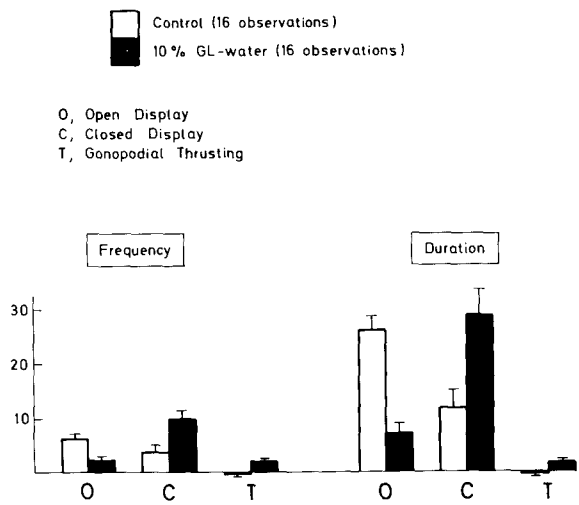

Figure 1. Change of mean differential courtship activity (mean ± standard error of 2 pairs of male competitors) as determined by the difference between ♂ Wild Ma - ♂ Blue Ir after addition of 10% wastewater drawn from the last clearing basin of a Munich water purification plant (Gross-Lappen, GL). All differences between control and 10% GL-wastewater were found to be significant (t test; $P < 0.05$).

change of mean differential sexual activities for the competing males, Ma versus Ir. The reproductive success as expressed by the offspring sired by the competing males is given in Table 2, discriminating between Ma and non-Ma progeny. Finally, Table 3 summarizes O and C courtship activities of Ma and Ir competitors.

Apart from both frequency and duration of the courtship activities (C and O) of Ir males, all differences between controls and 10%-GL treatment were found to be significant (Table 1). As far as Ma males are concerned, all activities were significantly changed. Both frequency and duration of C decreased in 10%-GL wastewater, but C and T increased. Ir males only exhibited a significant increase of T after GL exposure. These differences between Ma and Ir competitors in the response to wastewater constitute the change of mean differential sexual activities presented by Figure 1. While both frequency and duration of O decreased after GL treatment, mean differences of C and T increased significantly. Corresponding to higher rates of C and T of Ma males in GL wastewater, more Ma offspring were sired (Table 2). The higher incidence of O in the control apparently did not affect the reproductive success which is opposite to expectation (Farr 1980a). However, if one summarizes C and O activities as done in Table 3, no significant difference between control and and wastewater treatment was found. Consequently, the higher reproductive success of Ma males in wastewater may be determined by higher rates of both C and T. Because there was no significant alteration of non-Ma offspring in the GL-water as compared to the control group ($P = 0.40$), the excess of offspring in the GL treatment group is exclusively on excess of Ma offspring (27 versus 1; $P < 0.00004$; all two-tailed probabilities were determined by the use of the approximation of the binomial distribution by the normal distribution according to Sachs 1973). This result

Table 1. Courtship activities of competing guppy males
(mean ± standard error)

	Frequency/20 min						Duration (secs)					
	O		C		T		O		C		T	
Treatment	Ma	Ir	Ma	Ir	Ma	Ir	Ma	Ir	Ma	Ir	Ma	Ir
Tapwater (Control)	6.94 ± 0.97	0.63 ± 0.22	5.19 ± 1.20	1.44 ± 0.52	0.19 ± 0.14	0.44 ± 0.22	28.44 ± 2.40	2.13 ± 1.03	13.50 ± 3.21	1.56 ± 0.51	0.19 ± 0.14	0.38 ± 0.18
10%-GL wastewater	3.69 ± 0.86	1.25 ± 0.60	10.94 ± 1.99	1.19 ± 0.62	3.88 ± 0.60	1.94 ± 0.53	12.69 ± 2.98	5.31 ± 2.76	31.56 ± 5.87	2.19 ± 1.42	4.44 ± 0.66	2.75 ± 0.72
GL/Control	0.5 *	2.0	2.11 *	0.8	20.4 *	4.4 *	0.5 *	2.5	2.3 *	1.4	23.4 *	7.2 *

O, open display; C, closed display; T, gonopodial thrusting; Ma, Maculatus male; Ir, Iridescence male.
*) Significant differences between treatment groups (t test; $P < 0.05$)

completely agrees with the finding that all Ma values as presented by Tables 1 and 3 differ significantly from those from Ir males, i.e., the higher reproductive success of Ma males exposed to wastewater is due to the higher courtship activities of those males relative to the Ir competitors. In particular frequency and duration of thrusting was concerned which was about 20 to 23-fold greater in GL water in Ma males while only 4 to 7-fold greater in Ir males in GL water (Table 1).

Table 2. Reproductive success (fitness) of competing guppy males.

Treatment	Offspring Ma[a]	Offspring non-Ma[a]	%Ma
Tap water (Control)	1	28	3.45*
10%-GL wastewater	27	22	44.90*

[a]) Ma, dark spot in the dorsal fin; non-Ma, no dark spot in the dorsal fin
*) Short cut calculations of the Fisher-Yates "exact test" (Feldman and Klinger 1963); $P = 2.42 \times 10^{-6}$ (two-tailed)

The question arises of how to explain the higher reproductive sucess of Ma males in wastewater as compared to normal tap water (control). There are many examples of beneficial effects after exposure to either ionizing radiation or harmful chemicals. Thus, the weights of young platyfish, Xiphophorus maculatus, increased significantly by feeding them 3,4-benzpyrene and methylcholanthrene, a result which was not confirmed by the same treatment of the closely related species, Xiphophorus helleri. In addition, it could be shown that the intake of 3,4-benzpyrene and methylcholanthrene enhanced the respiration rate by 15 - 30% over that of controls (Schwanitz 1966).

Table 3. Pooled data of C and O courtship activities (mean ± standard error)

Treatment	Frequency Ma	Frequency Ir	Duration Ma	Duration Ir
Control	6.07 ± 0.77	1.04 ± 0.46	20.97 ± 2.39	1.85 ± 0.57
10%-GL wastewater	7.32 ± 1.25	1.22 ± 0.43	22.13 ± 3.65	3.75 ± 1.55

There is still another discrepancy between the present findings and those of the previous paper (Schröder and Peters 1988) which requires explanation. While all differential courtship activities (O and C) for controls were higher in the previous study (Figure 3), only differential O was higher in the present study (Figure 1) but differential C was lower for control than for GL-treated guppy males.

Opposite to expectation (Farr 1980b), the amount of C directed to virgin females was higher than that toward pregnant ones. Whether or not the influence of changing concentrations of pollutants in the wastewater drawn from the last clearing basin of the sewerage plant may play any role in causing the observed differences between both experiments cannot be decided from the present stage of our knowledge.

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