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Guppy Behavior During Exposure to a Sub-Lethal Concentration of Phenol

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Natural populations can be seriously harmed by pollutants in concentrations too low for outright death if these pollutants have indirect but deleterious effects as interfering with feeding, predation avoidance, and/or reproduction. The present study centers around a prevalent aquatic pollutant, phenol, which has received some attention with regard to fish behaviour. Experiments reported to date include avoidance experiments with sunfish (Lepomis spp) (SHELFORD 1917), salmonids (SPRAGUE & DRURY 1969), and minnows (Phoxinus phoxinus) (JONES 1951; STOTT & BUCKLEY 1979), conditioning in guppies (Poecilia reticulata) (MATEY 1970), sensory discrimination in various minnows (HASLER & WISBY 1949; ISHIO 1965), and temperature selection in brook trout (Salvelinus fontinalis) (MILLER & OGILVIE 1975). KOBAYASHI (1978) and KOBAYASHI et al. (1979) have reviewed the physiology of phenolic compounds in fish and SHMAL'GAUZEN (1973) has considered the developmental effects.

The present work extends earlier research in our laboratory on the disruptive effects of sub-lethal concentrations of phenol on courtship behavior and runway learning in guppies, and views the guppy test system as a model for natural systems affected by phenol. The objective of this study is to characterize the effects of such treatment on male courting acts and on temperature selection in both sexes in a controlled manner.

METHODS

To determine an appropriate sub-lethal concentration of phenol, 96 h LC50 experiments were conducted using static aerated aged tap water of pH 6.5 and hardness of 80 ppm as carbonate at 22-25C. The water of the control fish and the phenol solutions of the phenol-exposed fish was changed every 48 h. Seven different experiments were performed, and the resulting data were pooled to determine the 96- h LC50. The nominal phenol concentration was checked with UV spectrophotometry at 270 nm; the nominal and measured values were found to agree within $\pm$ 10%.

Up to 10 fish, from a polymorphic laboratory population, and totaling approximately 2 g in weight, were placed in 3 L of water or phenol test medium. Fish were fed once daily on Tetramin guppy diet. Dead fish were removed at regular frequent checks of the tanks. Fish ranged in size from 25 - 39 mm for females and 20-27

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mm for males. At 96 h the experiments were terminated and the data for males and females from the several experiments were analyzed using the logit model of the Generalized Linear Interactive Modeling package at Queen's University.

To study male courting acts, two males and three females were randomly placed in each of six visually isolated aquaria measuring 30 x 16 x 20 cm deep and containing 2 cm of fine gravel. Fish were fed daily (at least one h before any observations were made) and allowed to habituate for two weeks to the surroundings of the room which was kept on a 14:10 light:dark cycle at 24 $\pm$ 1C. During the three stages of the experiment (baseline 1 (B1), test (T), and baseline 2 (B2)) the water in the aquaria was replaced every 48 h with aged tap water, except for the three randomly chosen experimental aquaria during T, for which replacement was with a 10 mg/L phenol solution. The phenol levels were monitored daily spectrophotometrically and degradation was very slight between replacements.

In each experimental stage, which lasted about 10 days, each tank was observed for 20 min at a fixed time of day on 5 days, according to a split plot experimental design. Each male was observed for 10 min (focal-animal sampling of ALTMANN (1974)). The observer sat quietly in a darkened portion of the room and recorded acts in the following categories (after BAERENDS et al. 1955): category 1(C1, low intensity courtship acts: Approach, Retreating, Swimming with Fins Folded), category 2 (C2, medium intensity courtship acts: Biting, Following, Luring, Posturing), category 3 (C3, high intensity courtship acts: Sigmoid and Copulation Attempt), and category 4 (C4, general non-courting acts: general locomotion, feeding, chafing, snapping, and inter-male aggression).

The data were broken into dyadic transitions (such as Approach Biting) and, for each group of fish and experimental stage, collapsed across fish. Tables in which the number of times, n_{ij}, that category i(in row i) was followed by category j(in column j), were then drawn up.

To study temperature selection, guppies were exposed for seven days to 10 mg/L phenol in a continuous flow tank of 15 L at 25C with a flow rate of 300 mL/min. The water was continuously aerated and the fish fed twice daily on Tetramin guppy food. The phenol dosing solution was made fresh daily and was metered into a mixing funnel from a Mariotte flask along with 25C water from a constant head tank. The actual concentration present in the fish tank was checked daily and ranged between 8 and 12 mg/L due probably to the imperfect control over the flow rate of water and phenol concentration.

Temperature selection was determined in a horizontal temperature gradient measuring 180 cm x 15 cm wide with a water depth of 20 mm over the epoxy coated heated copper bottom. The gradient was divided into 18 zones for observation convenience and the water was progressively warmed as it flowed along the gradient by approximately 1C per zone. The temperature limits for the gradient were 18C at the cool end and 36C at the warm end. The established gradient was stable over time and readily reestablished from day to day. The temperature of each zone was recorded at mid-point and mid-depth with a YS1 telethermometer and thermistor probes. (For more details see ROY & JOHANSEN 1970; JOHANSEN & CROSS 1980.)

For each treatment a group of 3 fish of the same sex was placed into the gradient and left undisturbed for one hour following which the position of the fish in the gradient was recorded every 30 sec for 25 min. In total 7 replicates of each sex from each treatment were tested with no fish being tested more than once. The most frequent temperature zone (mode) for the group was the measure of selected temperature and the 7 values so obtained for each sex-treatment combination was used to calculate a mean selected temperature and t-tests performed on these data.

RESULTS

Table 1 presents the results of the 96-h LC50 tests. The estimated LC50's values for females and males are 44 $\pm$ 14 and 40 $\pm$ mg/L respectively. The difference of 4 mg/L is significant ($P < 0.05$).

In phenol concentrations of 20 mg/L and higher, but not 10 mg/L, the guppies very quickly appeared ill. They swam about in an agitated fashion with folded fins and became more melanistic in colour. In the higher concentrations (25 to 55 mg/L) that produced some mortality the fish displayed a loss of balance and slow swimming movements that lasted for some time prior to death. At these concentrations even the fish that maintained normal posture and did not die showed no interest in food while those at 10 and 20 mg/L concentration were seen to feed.

Though static assays are not as desirable as flow through assays we found no decline in phenol concentration over 24 h and after 48 h a variable decline of 0 to 20% with most showing only a modest decline of 10% or less. Nonetheless these tests serve to confirm that the 10 mg/L concentration selected for dosing the fish in the temperature selection and courtship studies was a non-lethal concentration over several days.

For the courting study, the results from one control male were omitted because he died at the end of baseline 1 and was not replaced since any replacment would lack such baseline data. No young were born during the course of the experiment. Table 2 presents the transition dyad frequencies per session per fish by treatment and experimental stage. As is usual with such behavioral transition tables, the data show a very skewed distribution, with some transitions (such as between low and

TABLE 1. Percent dead (actual number dead in brackets) at end of 96 h exposure to various concentrations of phenol. Data pooled from 7 experiments.

Concentrations	pooled	Females alone	Males alone
10	n = 10 0	n = 5 0	n = 5 0
20	n = 16 0	n = 5 0	n = 11 0
25	n = 8 12(1)	n = 2 0	n = 6 17(1)
30	n = 27 18(5)	n = 13 8(1)	n = 17 24(4)
35	n = 32 12(4)	n = 20 0	n = 12 33(4)
40	n = 42 62(26)	n = 25 44(11)	n = 17 88(15)
45	n = 30 83(25)	n = 18 72(13)	n = 12 100(12)
50	n = 30 80(24)	n = 18 (67(12)	n = 12 100(12)
55	n = 12 75(9)	n = 12 75(9)	

TABLE 2. Transition Dyad Frequencies Per Session Per Fish by Treatment and Experimental Stage.

		CONTROL				EXPERIMENTAL			
		C1	C2	C3	C4	C1	C2	C3	C4
B1	C1	6.7	12.1	4.8	14.9	8.6	12.5	9.9	17.6
	C2	14.3	15.5	3.1	1.6	16.2	23.2	7.3	1.2
	C3	2.8	5.2	0.3	0.0	6.3	11.0	1.0	0.1
	C4	14.6	1.7	0.2	6.7	17.4	1.2	0.2	3.3
T	C1	7.2	11.4	4.3	12.8	6.7	10.8	3.1	12.9
	C2	13.4	22.4	5.8	0.8	11.9	10.9	3.8	1.3
	C3	2.0	7.6	0.5	0.1	2.4	4.7	0.1	0.0
	C4	12.8	1.0	0.1	7.9	12.9	1.6	0.1	4.6
B2	C1	5.8	7.5	4.4	10.8	7.2	8.8	7.1	14.6
	C2	10.4	15.4	4.4	0.8	12.7	13.0	3.9	1.0
	C3	1.9	6.7	0.4	0.0	4.0	7.4	0.4	0.0
	C4	10.7	1.1	0.2	4.5	14.1	1.5	0.2	3.3

medium intensity courtship acts) very common and others (such as between high intensity courtship and general non-courting acts) very rare. Examining for possible correlations of total daily act numbers between pairs of males sharing aquaria (resulting in Spearman rank correlation coefficients of -0.43, 0.26, 0.28, 0.29, and 0.44, n = 18 overall $P > 0.05$) indicates that males may be treated as independent.

Fig. 1 shows the changes from B1 in occurrence of each act category for each group over the experimental stages. Between the baseline 1 and test stages, the rates of occurrences of all three courtship act categories decreased in the experimental group (paired t-test, $P < 0.05$ in each case) but not the control group. The control and experimental groups did not differ during baseline 2 (B2) for any act category. The rates of occurrence of general non-courting acts changed little over the course of the experiment for either group of fish.

Two further features of the data were investigated. First, the diversity information H (LOSEY 1978) was calculated to examine possible changes in information content over the three experimental stages. However, both the control group (for which H = 3.40, 3.38, and 3.43 in B1, T, and B2 respectively) and the experimental group (H = 3.43, 3.41, and 3.45) showed very little variation. Second, it was of interest to examine the persistence of the behavior (i.e. the extent to which acts are repeated). A ready measure of persistence is the proportion of the transitions falling on the diagonals of the transition tables (Table 2). When the control and experimental groups are compared for the difference in these measures between the baseline 1 and test stages, there is weak evidence for decreased persistence in the experimental fish (mean difference = 9.5%; U = 6, P = 0.063). The equivalent test for the difference in these measures between the test and baseline 2 stages is not significant (mean difference = 2.3%; U = 12, P = 0.331).

Temperature selection behaviour was not influenced by the phenol treatment in either sex. The mean selected temperature for treated and control females was 26.6 ± 3.0C and 26.6 ± 2.4C respectively while treated and control males averaged 24.4 ± 0.7C and 24.0 ± 0.9C respectively.

DISCUSSION

Our 96-h LC50 values of 40 mg/L for males, and 44 mg/L for females are comparable to those of RUESINCK & SMITH (1975) who obtained 96-h LC50 values of 36 mg/L at 15C and 24 mg/L at 25C for the minnow *Pimephales promelas* in a continuous flow assay. JONES (1951) found that concentrations below 0.002-0.003% (20-30 mg/L) were note rapidly lethal to the minnow, *Phoxinus phoxinus*, while OKSAMA & KRISTOFFERSON (1979) report a 96-h LC50 value of 10 ppm for this species resident and tested in brackish water. Overall, our choice of 10 mg/L as a sub-lethal dose seems reasonable in the light of our LC50 values and many of those published for other fish in fresh water.

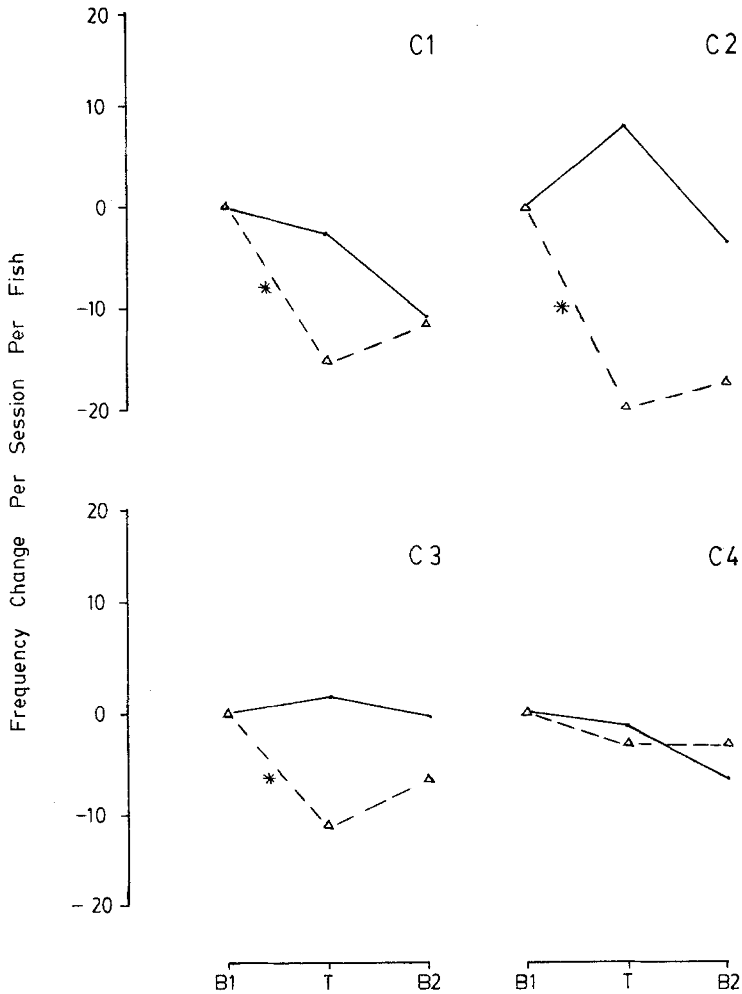

Fig. 1. Changes from B1 in occurrence of each act category for each group over the experimental stages. o: controls, :Δ experimentals. Act categories: C1: low intensity courtship, C2: medium intensity courtship, C3: high intensity courtship, C4: non-courting acts. Experimental stages: B1: baseline 1, T: test, B2: baseline 2. Bars indicate s.e. Significant differences indicated with an asterisk.

The results clearly demonstrate the depressing effect on male courtship behavior of phenol at a sub-lethal concentration. The rates of occurrence of courting acts at all three levels of intensity decreased markedly during exposure, returning to control levels subsequently. The decrease in persistence, if real, may reflect another aspect of the disorganizing effect of phenol on fish behavior as discussed by MATEY (1970). Such a decrease may be especially disruptive for the reproductive behavior of species such as guppies in which sexual selection and elaborate courtship play dominant roles. In both the present study and in other work in our laboratory in which female guppies have been exposed to 10 mg/L phenol for several weeks, we have noted the absence of the birth of young. This finding suggests a long-term detrimental effect of such treatment on reproductive output in this species.

A seven-day exposure to 10 mg/L phenol had no effect on the selected temperature of the guppy which is in contrast to the findings of MILLER & OGILVIE (1975) for brook trout. They found that exposure to phenol concentrations of 7.5 and 10.0 ppm (mg/L) for only 24 h was followed by a significant lowering of the selected temperature. This difference between our results for the guppy and those for trout probably reflects the more deleterious effects of these phenol concentrations on trout in contrast to that of the guppy. In brook trout 10 ppm phenol for 24 h was lethal to 30% of the sample whereas this concentration is not lethal to the guppy even after seven days exposure. In all probability the surviving trout were suffering from greater physiological damage which is reflected in altered selected temperature than the guppy.

As reported by JOHANSEN & CROSS (1980) we found that males selected a significantly lower temperature than females, and this sex-related difference is unaffected by phenol exposure.

The overall implication of the results of our study, in conjunction with those from the earlier studies cited, is that effective pollution control criteria must be based on more sensitive measures than simple ones such as LC50 determinations and that not all physiological-behavioral features are affected by relatively low concentrations.

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