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Oxygen Consumption and Filtering Rate of *Daphnia pulex* After Exposure to Water-soluble Fractions of Naphthalene, Phenanthrene, No. 2 Fuel Oil, and Coal-Tar Creosote

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Respiration and filtering rates of freshwater zooplankton are influenced by body size, metabolic state, and life history stage, as well as by numerous environmental factors such as food concentration, oxygen concentration, light, pH and temperature. As such, respiration and filtering rates in zooplankton are closely linked, not only with each other, but also with nutrition and growth rates (BUIKEMA 1973b, 1975).

Previous studies (GEIGER 1979, GEIGER et al. 1980) indicated that lifetime exposure of Daphnia pulex to sublethal levels of water-soluble fractions (WSFs) of hydrocarbons greatly depresses growth (measured as change in body length) and reproduction (measured by number of live young) rates. Because filtering rates and oxygen consumption indicate not only the amount of food taken in by daphnids but also provide a physiological "index" of metabolism, such data could help explain the reduced growth and reproduction rates previously noted. In addition, changes in filtering rate and oxygen consumption could provide a rapid biological monitor of the effects of environmental perturbation upon key physiological processes in zooplankton.

The following study was designed to examine the effects of short-term exposure to WSFs of naphthalene, phenanthrene, No. 2 fuel oil, and coal-tar creosote upon oxygen consumption and filtering rates of D. pulex.

MATERIALS AND METHODS

Animals. Daphnia pulex were obtained from stock cultures maintained continuously in our laboratory for several years. Stock cultures of daphnids were acclimated to a 20±1C test temperature and 16L:8D photoperiod. Cultures were fed the unicellular green alga Chlamydomonas reinhardi (wild type, negative strain) ad libitum, and their diet was supplemented once weekly with a "trout chow" plus cerophyll extract. All water was Blacksburg carbon dechlorinated tap water, millipore filtered (0.45 µm) before use.

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Treatment and Exposure. Preparation of test solutions is described in detail by GEIGER et al. (1980) and is described here briefly. Test solutions of 10% WSF were prepared from No. 2 fuel oil and coal-tar creosote with the method described by ANDERSON et al. (1974a). Stock solutions prepared from naphthalene and phenanthrene contained 0.034 and 1.0 gm/L, chemical respectively. All WSF stock solutions were millipore filtered (0.45 μm) before use. Acute toxicity tests were first performed and 48-hr LC50s were calculated (Table 1).

TABLE 1. Acute 48-hr LC50 values and estimated LC20 and LC30 concentrations for water-soluble fractions utilized in oxygen and filtration rate experiments.

	48-hr LC50		Estimated Values (mg/L or % WSF)	
	% WSF	mg/L[a]	LC20	LC30
Naphthalene	57.52 (46.02-76.79)	3.40	0.28-0.38	0.51-0.68 (mg/L)
Phenanthrene	>>100 (nc)[b]	1.14	0.096-0.13	0.31-0.41 (mg/L)
No. 2 Fuel Oil	34.10 (24.94-41.78)	--	5.6	10.0 (% WSF)
Creosote	2.91 (2.52-3.36)	--	1.0	1.8 (% WSF)

[a] determined by gas chromatography/mass spectroscopy of stock solution.

[b] nc - fiducial limits could not be calculated by probit analysis.

The LC20 and LC30 concentrations (corresponding to concentrations calculated to kill 20% and 30%, respectively, of the test organisms in 48 hr) were used during daphnid exposure. Test stock solutions were freshly prepared every three days. Glass aquaria containing 3 L of appropriate test solution were placed in an environmental growth chamber under the same temperature and photoperiod as previously described and aerated gently. Approximately 60 young Daphnia were added to each aquaria and exposed to test WSF's for at least three molt cycles (≈ 9 days). Test solutions were changed every three days and daphnids fed C. reinhardi ad libitum. Previous work indicated a three-molt exposure period appears sufficient to allow any potential effect of WSF's to Daphnia (GREEN 1957, BUIKEMA 1973b, GEIGER 1979).

Oxygen Consumption. D. pulex in test and control aquaria were not fed for 12 hr preceeding each test. Daphnids were collected, sized (1.2-1.4 mm using an ocular micrometer measuring from the

top of the head to the base of the caudal spine), and five Daphnia each placed in glass stoppered 60 mL pyrex bottles (previously calibrated by weight). Respirometers were stoppered, checked for air bubbles, then placed horizontally in the environmental chamber. After 24 hr, the amount of dissolved oxygen was determined by the azide modification of the Winkler Method (AMERICAN PUBLIC HEALTH ASSOCIATION et al. 1975). Oxygen consumption values were corrected for respirometer volume, Winkler reagent dilution, and differences between control and experimentals. Each test consisted of one control respirometer (5 similar-sized unexposed daphnids) and three replicates per WSF concentration. Each test was conducted three times. Data were expressed as µL O_2/Daphnia/day.

Filtering Rate. D. pulex were exposed and isolated as previously described for the oxygen consumption experiments. Log-phase C. reinhardi were added to each experimental and control bottle at a concentration of 30,000 cells per mL. Bottles were placed horizontally in the environmental chamber, and gently rotated at least twice during each test. These experiments were conducted in the dark to minimize algal growth. After 24 hr two drops of 40% formalin were added to kill both algal cells and Daphnia. Algal counts were made using an Electrozone electronic particle counter (Model 112, Particle Data, Inc., Elmhurst, Illinois) interfaced with a PDP-11 minicomputer using a 95 µ oriface, 500 µL volumetric section, with current set at 1/4 and gain at 68. At least three counts were performed on each bottle. Filtering rate was computed after BUIKEMA (1973a). Each test consisted of two control bottles (one with algae; one with unexposed control animals plus algae) and three replicates per WSF concentration. The concentration of cells filtered by Daphnia was considered the difference between experimental and control bottles. Each test was conducted three times. Data were expressed as mL/Daphnia/day.

Water chemistry analysis (APHA et al. 1975) was performed once during each test. Dissolved oxygen ranged from 7.0 to 8.5 mg/L, alkalinity from 42 to 46 mg/L ($CaCO_3$), hardness from 43 to 48 mg/L ($CaCO_3$) and pH from 6.8 to 7.5 units. Data were analyzed by GLM and Duncans test procedures (BARR et al. 1976). Statements of significance refer to $P<0.05$.

RESULTS

The results of oxygen consumption test are summarized in Table 2. Test concentrations are denoted as the abbreviation of the parent compound with a 2 or 3 to designate the respective LC20 or LC30 concentration (that is, "creo-2" refers to the creosote LC20 concentration). There were no significant differences between control oxygen consumption rates and any

of the experimental concentrations. The Duncan procedure identified only two real differences in the pooled data. The creo-3 animals had the highest oxygen consumption of all test groups, and was significantly different from the nap-3 exposed organisms, which had the lowest oxygen consumption rate.

TABLE 2. Combined oxygen consumption data for Daphnia pulex after exposure to water-soluble fractions of petroleum hydrocarbons. Means with the same letter are not significantly different (α = 0.05; GLM P>F, 0.34).

Compound	N	µL O_2/Daphnia/day mean(± S.D.)	Duncan Ranking	
Creo-3	9	2.87 (1.14)	A	
Control	6	2.42 (1.27)	A	B
Oil-3	9	2.27 (1.22)	A	B
Oil-2	9	2.06 (1.33)	A	B
Phe-2	9	1.99 (0.97)	A	B
Creo-2	9	1.92 (1.49)	A	B
Phe-3	9	1.89 (0.92)	A	B
Nap-2	9	1.69 (0.66)	A	B
Nap-3	9	1.60 (0.41)		B

The results of the filtering rate experiments are summarized in Table 3. Creo-2 organisms had the highest filtering rate of all compounds tested, and was significantly different from all other test means. Oil-2, creo-3, and oil-3 test groups had the next highest mean filtering rates. Nap-2, nap-3, controls, and phe-3 test means were not statistically different from each other. The phe-2 animals had the lowest filtering rates measured. The two concentrations which exhibited the highest (creo-2) and lowest (phe-2) filtering rates were not those which most affected growth and reproduction GEIGER et al. 1980). The filtering rates of nap-2, creo-2, and oil-2 exposed animals were always higher than those exposed to the representative LC30 respective test concentrations. The only exception was phenanthrene where the phe-3 test group had a significantly higher filtering rate than did the phe-2 group. These data indicate that there may be a threshold concentration for these compounds.

TABLE 3. Combined filtering rate data for Daphnia pulex after exposure to water-soluble fractions of petroleum hydrocarbons. Means with the same letter are not significantly different (α = 0.05; GLM P>F, 0.0001).

Compound	N	mL/Daphnia/day mean (± S.D.)	Duncan Ranking
Creo-2	27	13.68 (1.98)	A
Oil-2	27	10.52 (3.10)	B
Creo-3	27	9.99 (3.62)	B
Oil-3	27	9.58 (2.78)	B
Control	18	5.48 (4.45)	C
Phe-3	27	5.32 (3.62)	C
Nap-2	27	4.68 (4.54)	C
Nap-3	27	4.14 (3.98)	C
Phe-2	27	1.47 (2.65)	D

DISCUSSION

The metabolic rate of control Daphnia was 0.10 µL O_2/Daphnia/day, a rate similar to that obtained by RICHMAN (1958) for similar-sized D. pulex. In comparing our test results to those for marine and estuarine organisms exposed to various hydrocarbon stresses no consistent effects upon respiration were noted. Mysid shrimp exposed to No. 2 fuel oil WSF's exhibited an increase in oxygen consumption (ANDERSON et al. 1974b), while grass shrimp exposed to naphthalene exhibited a reduction in respiration rate (TATEM 1976). Sub-lethal exposure to crude oil WSF's had little effect on Alaska King Crab respiration; however, acute WSF's quickly depressed oxygen consumption (RICE et al. 1976). Respiration rates and heart rates of mussels exposed to sublethal levels of aromatic hydrocarbon WSF's were variable and inconsistent (SABOURIN & TULLIS 1981). Phenanthrene and creosote WSFs produced extreme reduction in growth and number of live daphnids (GEIGER et al. 1980), yet only the creosote LC30 WSF exhibited significant differences from controls and other test groups. Data on the effects of oil on fish metabolism are just as variable as those for the invertebrates (see STRUHSAKER et al. 1974, ANDERSON et al. 1974b, c; THOMAS & RICE (1975). Monitoring respiration does not appear to be a consistent indicator of hydrocarbon-induced stress.

Mean control filtering rates in this study was 5.48 mL/Daphnia/day. BUIKEMA (1973a) obtained a value of 5.96 mL/Daphnia/day using C. reinhardi and D. pulex approximately the same size (1.36 mm versus 1.2-1.4 mm) and the same density (1 animal/10 mL versus 1 animal/12 mL). The agreement between these independently-obtained values indicate the filtering rates obtained are reasonable.

Literature data on filtering rates for organisms exposed to environmental contaminants are limited. Oil-water emulsions of bunker fuel increased filtering rates of soft-shelled clams; however, exposure to WSF's of the same fuel did not affect filtering rates (ANDERSON 1972). COOLEY (1977) noticed effects on filtering rates in Daphnia exposed to pulp mill effluent. Phenanthrene and creosote WSF's, which greatly reduced growth and reproduction of daphnids during a lifetime chronic test (GEIGER 1979, GEIGER et al. 1980) produced significantly different filtering rates compared to controls and to each other (Table 3). Our data and that of COOLEY (1977) indicate that changes in filtering rates may be a sensitive indicator of sublethal stress and that such data are useful in interpreting chronic effects. For example, creo-3 exposed animals had a significantly higher filtering rate and no significant change in oxygen consumption yet chronic data (GEIGER et al. 1980) showed significant reductions in growth and reproduction. This seems to indicate available food was not being utilized for growth and reproduction. Similar differences were also observed for D. pulex exposed to various light conditions (BUIKEMA 1975).

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