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Oxygen Consumption in *Daphnia pulex* Exposed to 2,4-D or 2,4,5-T

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Although herbicides are extensively used and reach waterways through direct application and runoff (BOVEY et al. 1974), sublethal response of aquatic animals to herbicides is inadequately known (SCHOBER and LAMPERT 1977). KLEKOWSKI and ZVIRGZDS (1971) have examined variability in oxygen consumption resulting from exposure to 2,4-D. SCHOBER and LAMPERT (1977) have determined the effect of atrazine on growth and reproduction in Daphnia pulex.

The objective of this study was to determine the effect of 2,4-D (2,4-dichlorophenoxyacetic acid, butoxyethanol ester) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid, butoxyethanol ester) on oxygen consumption in D. pulex. Daphnia is an important freshwater animal because of its role as a food chain intermediate in temperate-zone lakes and ponds receiving agricultural runoff (CROSLEY and TUCKER 1966). Oxygen consumption is a useful measure of sublethal effects because energy processes serve as an indicator of overall physiological state (KLEKOWSKI and ZVIRGZDS 1971).

MATERIALS AND METHODS

Daphnia were cultured in the laboratory in a 19-L plastic container using pond water and were fed yeast daily. The animals in culture water were transferred to liter containers for acclimation at 20, 25, or 30°C with a 12-h light, 12-h dark photoperiod. Acclimation time varied with the difference in temperature between the stock culture and the acclimation temperature. Daphnia were held in the incubators one day prior to respiration measurements at 20°C (the ambient room temperature) but were maintained in incubators several days prior to measurement of respiration at 25° and 30°C.

Daphnia were exposed to herbicide shortly before measuring oxygen consumption. Herbicides were supplied by Amchem Products, Ambler, PA. Solutions of 1 and 3 ppm (expressed as acid equivalents) of the butoxyethanol esters of 2,4-D and 2,4,5-T were prepared from the pond

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water and animals maintained at each temperature. Five mature Daphnia were transferred to each Gilson respirometer flask (14 mL capacity) with approximately 6 mL of herbicide solution. Oxygen consumption was measured using a Gilson differential respirometer with readings taken at two-h intervals over a six to nine h period. Measurements were taken over approximately the same time of day for different runs.

Wet weights were determined using a Cahn Electrobalance. Excess water was blotted from the animals prior to weighing. Readings were expressed as µL oxygen consumed/mg wet weight x hour^{-1}.

RESULTS

Data were analyzed using Analysis of Variance, fixed effects models, with temperature and exposure concentration as main effects (SOKAL and ROHLF 1969). Sample size (N) is the number of flasks from which respiration was determined. Neither temperature nor dose had a statistically significant effect on oxygen consumption for Daphnia exposed to 2,4-D (N=229). Mean respiration did increase with increasing temperature (Table 1). Variation in mean respiration with dose appeared less predictable although the highest respiration was achieved in the 3 ppm treatment at 30°C (Table 1).

TABLE 1

Mean Oxygen Consumed (µL/mg wet weight x hour^{-1}) at Various Temperatures and 2,4-D Concentration ± Standard Deviation. Sample size for each is given in parentheses.

	CONCENTRATION (ppm)		
TEMPERATURE (°C)	0	1	3
20	1.12±0.80 (30)	1.51±1.13 (28)	1.65±1.37 (27)
25	1.57±1.07 (22)	0.70±0.71 (13)	1.36±1.56 (17)
30	1.66±1.38 (32)	1.38±0.93 (33)	2.04±1.26 (28)

Both temperature ($P<0.001$) and herbicide concentration ($P<0.025$) contributed significantly to variation in respiration for animals exposed to 2,4,5-T (N=227). Respiration was greatest at 30°C and increased with the 3 ppm treatment at 30°C (Table 2).

TABLE 2

Mean Oxygen Consumed (μL/mg wet weight x hour^{-1}) at Various Temperatures and 2,4,5-T Concentrations ± Standard Deviation. Sample size for each mean is given in parentheses.

	CONCENTRATION (ppm)		
TEMPERATURE (°C)	0	1	3
20	0.64±0.45 (14)	0.58±0.37 (30)	0.77±0.40 (22)
25	0.79±0.67 (21)	1.00±0.73 (29)	0.90±0.55 (32)
30	1.12±0.62 (21)	1.16±1.11 (27)	1.90±1.86 (32)

DISCUSSION

The general trend of increased respiration at 30°C was apparent in both the 2,4-D and 2,4,5-T experiments although the differences achieved statistical significance only in the 2,4,5-T data. The lower oxygen consumption at 25°C, 1 ppm 2,4-D (Table 1) was not significantly different from values obtained at 20 and 30°C. This shows that these data are quite variable.

Accelerated rate of oxygen uptake at elevated temperatures is expected due to the increased rate of reaction of enzyme systems involved in energy use (KEISTER and BUCK 1974). Oxygen uptake did not increase with temperature between the 20 and 25°C groups. This phenomenon has been observed frequently in other animals, but the explanation for it is not clear. KEISTER and BUCK (1974) suggested that several systems are affecting the overall oxygen consumption rate and that these systems are differentially affected by increasing temperature. Plateaus and sharp increases in the oxygen consumption-temperature curve result from the net effect of temperature on these different systems.

The physiological basis for the effect of herbicides on respiration is not known. A variety of insecticides cause both stimulation and depression of respiration depending on their concentration. Neurotoxins which increase spastic muscle activity cause an increase in oxygen consumption but when sufficient levels are present to cause some cell death, depression of oxygen consumption results (KEISTER and BUCK 1974).

The 3 ppm concentration used in this experiment resulted in increased respiration at 30°C. KLEKOWSKI

and ZVIRGZDS observed depression in repiration in another crustacean at higher concentrations of herbicide than those used in this study. The effects of these herbicides may be similar to the overall effect of the insecticides; however, the physiological systems important in the response have not been identified. Sharp increases in oxygen consumption with increased temperature and herbicide concentration could be due to the net effect of herbicide and temperature on interacting physiological systems just as KEISTER and BUCK (1974) proposed for sharp increases in the oxygen consumption-temperature curve.

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