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Enhanced Oxygen Uptake Rates in Dragonfly Nymphs (*Somatochlora cingulata*) as an Indication of Stress from Naphthalene

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The problem of pollutant bioaccumulation and subsequent physiological trauma is often reflected in the oxygen-uptake rate either through disrupted metabolism or in the mobilization of a compensatory homeostatic mechanism. Consequently, the respiration rate provides a critical index of environmental suitability and the cost for survival. While the resort to diversity indices to assess areas of stress is completely valid, such a strategy is costly and time-consuming. To identify biologically critical sampling stations, and evaluate suspect waters at chronic levels of toxicity, a rapid field method is desirable. Accordingly, the objective of this research was to determine the feasibility of interpreting variations in the respiration rates of the dragonfly *Somatochlora cingulata* as short-term indicators of stress incurred by exposure to aromatic hydrocarbons. Polycyclic aromatic hydrocarbons (PAH) are a widely distributed complex mixture of compounds, of which naphthalene and its derivates are the most toxic fraction absorbed by aquatic organisms (ANDERSON et al. 1974b; NEFF et al. 1976). Recourse to Odonata for toxicity testing has become increasingly more frequent (BELL&NEBERKER 1969; BELL 1971; JULIN&SANDERS 1977; MUIRHEAD-THOMSON 1978a,b). Observations have been confined, however, to discrete quantum type data rather than to the continuum of responses of nonlethal stresses.

MATERIALS AND METHODS

Prior to the initiation of the experiment, pilot tests were performed to determine the level at which naphthalene ceased to be acutely toxic, and the range that was both most stable and most prevalent in the aquatic environment. Our data indicated that the static 96 h LC50 value for naphthalene with *Somatochlora cingulata* was between 1 and 2.5 ppm, and that no deaths occurred before 1.5 ppm. Within this time frame, at 0.1 and below, naphthalene concentrations did not measureably decrease, though at 1 ppm and above the compound degraded rapidly. On the basis of the aforegoing considerations, the test concentrations chosen were 0.01 and 0.1 ppm.

Dragonfly nymphs collected in the fall from Cranberry pond in Leverett, Massachusetts, were acclimated in an aquarium at 21 ± 1°C in pond water for a minimum of seven days on a diet of mayfly nymphs. Feeding was stopped two days prior to the initiation of the experiment. The live weight of the 168 specimens used varied between 0.01–0.55 g.

Each of thirty sets of 4 nymphs was placed in 3 L of filtered water from Cranberry pond containing either 0,0.01 or 0.1 ppm of naphthalene. After 0,2,4,8,12,24 and 48 h, the accumulation of naphthalene in whole insects was measured. The remaining 9 sets were then transferred to control water to measure the depuration process at intervals of 24, 72 and 120 h.

To determine the concentrations of naphthalene in tissues, the methods described by NEFF&ANDERSON (1975) and modified by CORREA (1980) were adopted. The whole body was blotted, weighed and then homogenized for 1-2 min in a mixer with 10 mL of reagent grade hexane. The samples were maintained in a shaker bath at a 21°C for 24 h. The hexane supernatant was poured into a glass tube with a 0.5g of Florisil and activated by heating to 200°C for 24 h. The tube was shaken several times over 12-24 h. The extracts were then filtered through glass wool and their UV spectra determined at 221 nm. The concentrations were computed by means of simultaneous equations from the absorbance of standards in hexane. To determine the uptake of naphthalene, the following equations was used:

$$\text{Concentration in body: } \frac{\text{Vol in mL used}}{\text{Weight in g}} \times \text{concentration in the cuvette } (\mu g/mL)$$

Respiration was estimated by the Azide modification of the Winkler Method (STANDARD METHODS FOR EXAMINATION OF WATER AND WASTEWATER 1980). DO bottles were filled with filtered pond water saturated with oxygen containing 0,0.01, or 0.1 ppm or naphthalene. Forty eight nymphs were blotted, weighed, and placed into DO bottles (one each). DO bottles from each group were analyzed (4 per period of time) at 2,6,12 and 24 h after initial exposures.

Oxygen consumption rates at the designated naphthalene concentrations were evaluated by regression analysis and performed on both dependent (02 consumption) and independent (weight) variables to normalize the variances. Statistical differences between means were evaluated using analysis of variance.

RESULTS AND DISCUSSION

Oxygen consumption in *Somatochlora cingulata* increased with the concentration of naphthalene in the water (Table 1). The respiration rate at 0.1 mg/L was twice that of the controls (Fig. 1). While not as pronounced as the 0.1 treatment, the 0.01 ppm treatment elicited a clearly discernible increase in respiration. The metabolic rate was initially high in all treatment probably as a consequence of stress through handling or through their introduction to a new environment. It is interesting that the extent of this heightened uptake was magnified by the naphthalene concentrations. Oxygen consumption, per unit body weight, was constant (Fig. 2), so it would seem that the differences observed had not been compounded by sampling errors in size and age.

It is noteworthy that the greatest r^2 values were obtained from the experimentals. Perhaps this is a relfection of the over-

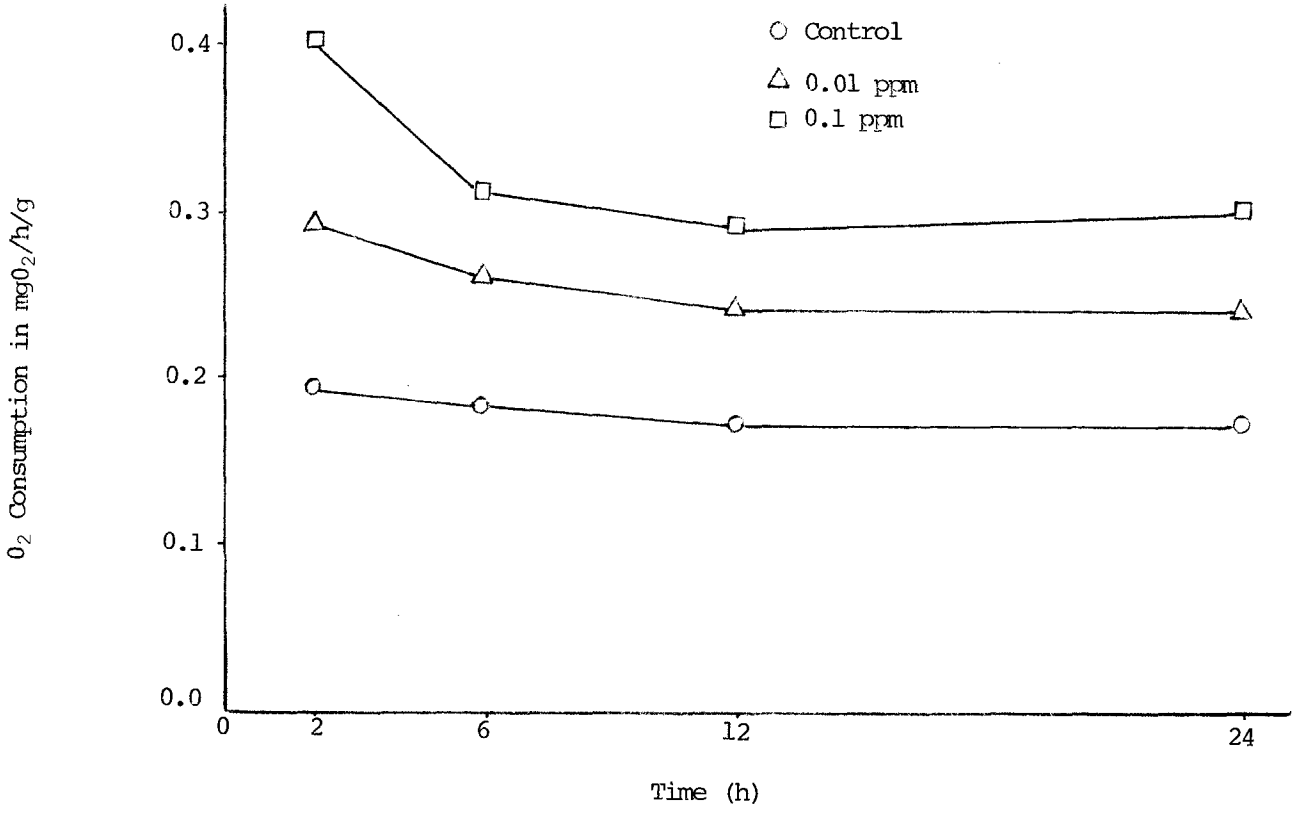

Fig. 1 Oxygen consumption $mgO_2/h/g$ by the dragonfly *Somatochlora cingulata* exposed to 0,0.01 and 0.1 mg/L of naphthalene during 24 hours.

riding response to stress. It could be that the narrower range of responses in stressed organisms may be as significant as magnitude, particularly if respiration is measured fairly soon before the organism has achieved an equilibrium. Our data support the observations of ISTENRI (1963), PETITPREN&KNIGHT (1970), and KAPOOR&

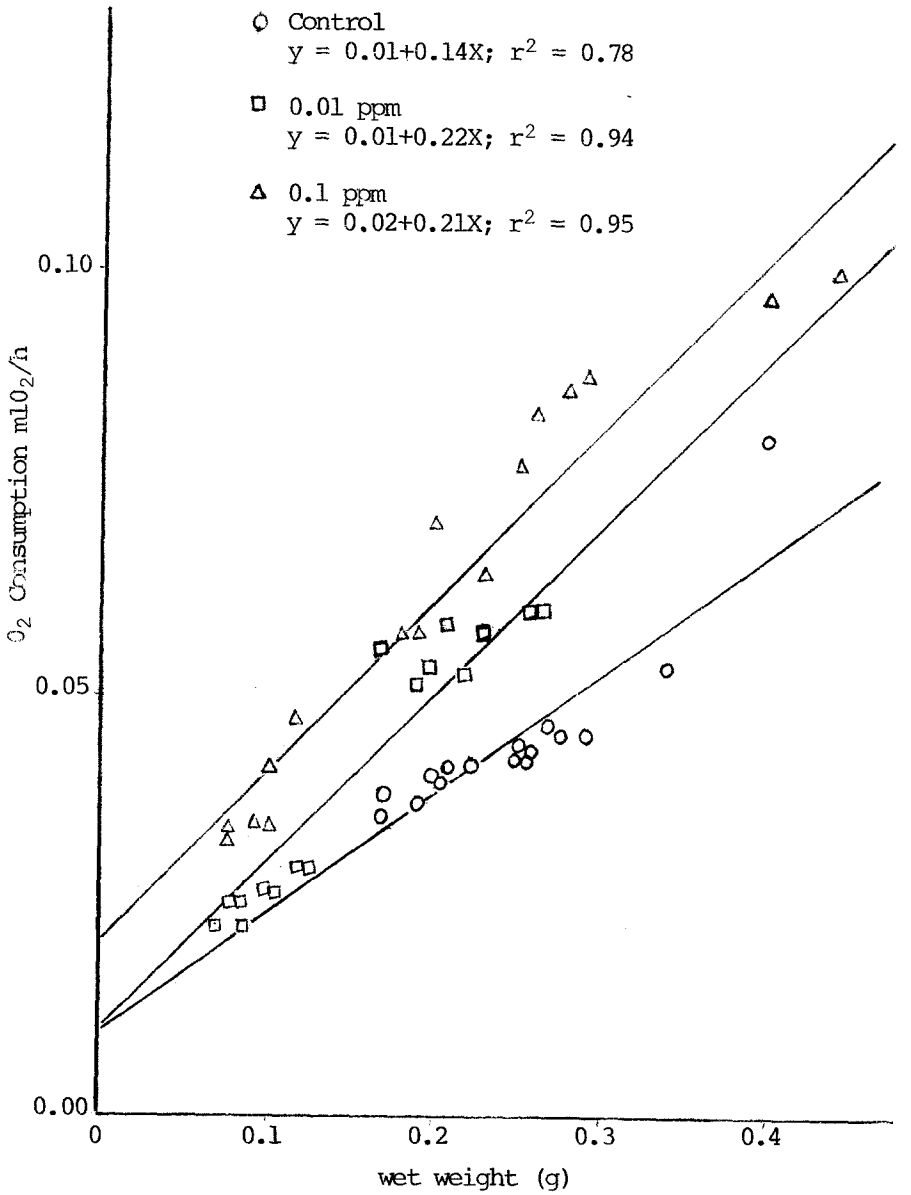

Fig. 2 Body wet weight - respiration relationship of dragonfly *Somatochlora cingulata* exposed to different concentrations of naphthalene during 24 h.

GRIFFITHS (1975) who found that there was no significant correlation in some aquatic insects between respiratory rate per unit weight and size. An analysis of variance to relate the effect of the concentration of naphthalene in the water to oxygen consumption of *S. cingulata* during 24 h exposures showed that very significant differences ($P < 0.001$) existed.

Table 1. Wet weight - respiration relationships of the dragonfly (*Somatochlora cingulata*) to indicated concentrations of naphthalene during 24 h at 21°C.

Treatment (µg/L)	No. of Samples	Time	Mean O_2 Consumption ($mL/O_2/h/g$) ± SD	CV
0	16	2	0.1917 ± 0.027	14.08
		6	0.1815 ± 0.023	12.67
		12	0.1782 ± 0.026	14.59
		24	0.1770 ± 0.013	7.34
0.01	16	2	0.2882 ± 0.011	3.81
		6	0.2625 ± 0.043	16.38
		12	0.2490 ± 0.014	5.62
		24	0.2492 ± 0.009	3.61
0.1	16	2	0.4082 ± 0.016	3.91
		6	0.3165 ± 0.044	13.90
		12	0.2857 ± 0.029	10.15
		24	0.3007 ± 0.044	14.63

The test organisms were analyzed to determine the availability of the naphthalene concentrations. Figure 3 shows the amount of naphthalene (µg/g) in intact *S. cingulata* specimens during a 48 h exposure to the two different concentrations, and a control followed by 120 h of depuration. Naphthalene was rapidly taken up with the concentration factors varying, respectively, after 48 h from 200-1550 and 20 to 180 times the initial water levels of naphthalene (0.01 and 0.1 mg/L) (Table 2).

The results of both exposure levels demonstrated that the rate of accumulation indicated by the concentration factor was highest in the first 8 hr of exposure (600 and 90 times, respectively). Apparently, after an 8 h period, *S. cingulata* increased naphthalene detoxification. Though the 0.1 ppm levels of naphthalene elicited a higher uptake rate, it was not proportionately greater than that of the 0.01 ppm experimental.

The elimination rate was higher during the first 24 h for both concentrations (32 and 42%, respectively) and lower during the remainder of the test period (4 days) (Fig. 3). Similar relationships during the accumulation and elimination process were found by MELANCON&LECH (1978) with rainbow trout for short and long-term exposures. An analysis of variance to relate concentrations of

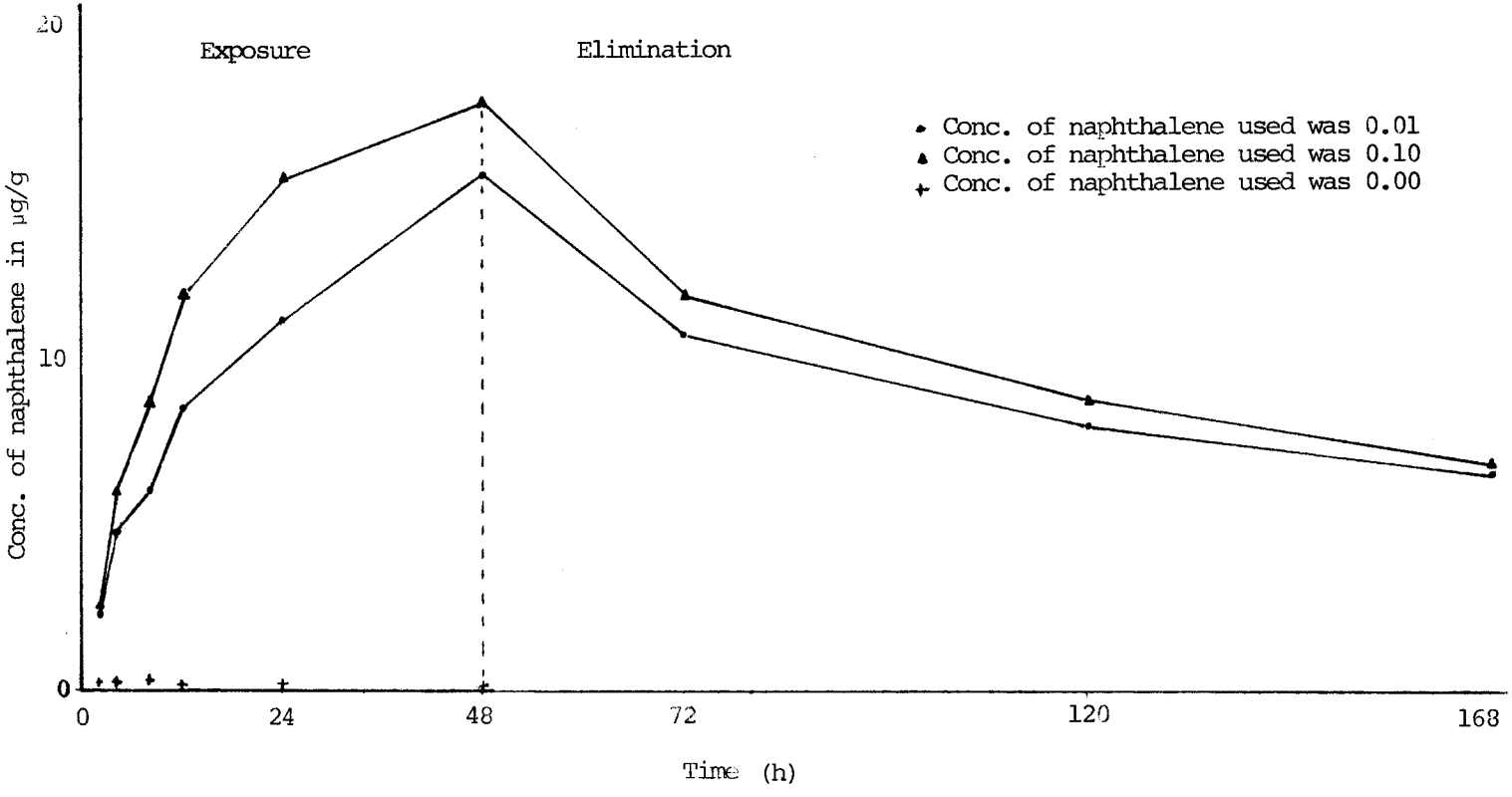

Fig. 3 Concentration levels of naphthalene in whole dragonfly nymphs during 48 hr exposure to naphthalene (0.01 and 0.1 mg/L) and subsequent elimination. Data are the average of value from 4 nymphs per period of time.

naphthalene in the water and in the whole insect for each specific period of time, showed that the respective rates were significantly different ($P < 0.05$) during the accumulation and elimination process.

Table 2. Naphthalene uptake by *S. cingulata* and concentration factor (CF) as $\frac{\mu g \text{ naphthalene/g wet weight of tissue}}{\mu g \text{ naphthalene/g water}}$ during 48 h exposure. Each value is an average of four dragonfly nymphs ± SD and CV (coefficient of variations).

	Naphthalene in test water			
	0.01 mg/L		0.1 mg/L	
Time (h)	Naphthalene uptake (µg/g ± SD)	CV	Naphthalene uptake (µg/g ± SD)	CV
0	*		*	
2	2.4 ± 0.4	16.6	2.5 ± 0.1	3.2
CF	241		(24.9)	
4	4.9 ± 0.2	13.9	6.0 ± 0.6	10.5
CF	488.2		(60.2)	
8	6.0 ± 0.1	1.8	8.8 ± 0.4	4.3
CF	599.7		(88.3)	
12	8.6 ± 0.8	8.9	12.0 ± 0.2	1.6
CF	865.3		(120.30)	
24	11.3 ± 0.6	5.5	15.4 ± 1.3	8.3
CF	1128		(154.2)	
48	15.5 ± 0.4	2.5	17.7 ± 1.1	6.1
CF	1548		(177.5)	

The fact that increased naphthalene uptake elicited a heightened, but constant metabolism suggests that the organism has acclimated to the stress, but at an increased energy expenditure. Were naphthalene solely exerting an excitatory effect, we could look for a positive correlation with tissue burden. It appears that recourse to *S. cingulata* as a preliminary screening tool to identify chronic levels of toxicity, offers promise, particularly when exposure periods are of short duration (± 2 h).

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REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTE WATER. 15th ed. New York, American Public Health Association, Inc. (1980).

ANDERSON, J.W., J.M. NEFF, B.S. COX, H.E. TATUM, G.M. HIGHTOWER: Mar. Biol. 27:75 (1974).

BELL, H.L. and A.V. NEBEKER: J. Kansas Entomol. Soc. 42:230 (1969).
BELL, H.L.: Water Res. 5:313 (1971).
CORREA, M.: Estudio sobre la acumulacion y elimacion de naftaleno en *Mugil curema*. Masters thesis. Institute Oceanographic. Universidad de Oriente Cumaná, Venezuela. (1980).
ISTERNIC, L.; Raspr. Slov. Akad. Znon. Umet. 4:201 (1963).
JULIN, A.M. and H.O. SANDERS: Trans. Am. Fish. Soc. 106:386 (1977).
KAPOOR, N.N. and W. GRIFFITHS: Can. J. Zool. 53:1089 (1975).
MELANCON, M.Y. and J.J. LECH: Arch. Environ. Contam. Toxicol. 7:207 (1978).
MUIRHEAD-THOMSON, R.C.: Arch. Environ. Contam. Toxicol. 7:129 (1978a).
MUIRHEAD-THOMSON, R.C.: Arch. Environ. Contam. Toxicol. 7:138 (1978b).
NEFF, J.H. and J.W. ANDERSON: Bull. Environm. Contam. Toxicol. 14: 122 (1975).
NEFF, J.M., B.A. COX, D. DIXIT, J.W. ANDERSON: Mar. Biol. 38:279 (1976).
PETITPREN, M.F. and A.W. KNIGHT: J. Insect. Physiol. 16:449 (1970).

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