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Assessment of the Impact of Naphthalene Contamination on Mangrove Fauna Using Behavioral Bioassays

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Pollution of the marine and estuarine environments by petroleum hydrocarbons is a world wide phenomenon (Connell and Miller 1980) and whilst large scale crude oil spills are the most obvious source of pollution, since the 1970's the impact of chronic, low level hydrocarbon input from sources such as oil refineries has been recognised as having long term ecological consequences, even when there may be no visible evidence of acute effects (Connell and Miller 1980). Mangroves are perhaps the dominant and most important intertidal habitat along subtropical and tropical coastlines and estuaries and as such are located in areas of high risk of acute or chronic petroleum hydrocarbon pollution. Further, once contamination occurs, high levels of hydrocarbons may be expected to remain in mangrove sediments as conditions are not favorable for hydrocarbon depletion by sediment transport or degradation by aerobic bacteria.

Much research has focused on determining the acute toxicity of the water soluble fraction of crude or fuel oil to aquatic fauna but relatively little attention has been given to individual hydrocarbons. The medium to low boiling point aromatics such as naphthalene and its alkyl derivatives are the most toxic petroleum fraction to marine organisms (Anderson *et al.*1974; Moore and Dwyer 1974) and they are known to provoke behavioral responses in marine animals at sublethal concentrations (Hargrave and Newcomb 1973; Linden 1977; Nagarajah *et al.* 1985). The goal of this investigation was to investigate the effects of a single aromatic petroleum hydrocarbon, naphthalene, in a subtropical mangrove environment, through the use of behavioral bioassays. The test organism chosen was the intertidal gastropod *Ophicardelus quoyi,* which is abundant in mangroves throughout eastern Australia.

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MATERIAL AND METHODS

Ophicardelus quoyi (approximately 10 mm long) were collected from mangroves on Bulwer Island in the Brisbane River (27°24'3"S, 153°8'11"E). The site is close to two oil refineries, a large sewage effluent and a busy port. In the laboratory, snails were placed in containers of aerated, commercial aquarium grade seawater (34 ppt) and acclimated at 25°C for 7 d during which time their normal laboratory behavior was documented. At the place of collection, sediment samples were taken from the top 1 cm of mud across an area of 1 m^{-2}, at the high, mean, and low water marks. Reference sediment samples were also taken from mangroves 25 km upstream from this site. Sediments were preserved with 5 mL concentrated hydrochloric acid and frozen at -20°C for chemical analysis the following day.

A solids analysis was carried out on each sediment sample. Naphthalene was extracted from the sediments by weighing circa 20 g of sediment onto glass fibre filter papers with a backing of aluminium foil. The filter papers were rolled into cylinders and placed into glass stoppered 250-mL conical flasks. Distilled freon (50 mL), several grams of anhydrous Na_2SO_4 and 3 g of activated Florisil was added to each flask. Flasks were placed on a shaker at 60 cycles min^{-1} for 24 hr and then subjected to sonification for 10 min. The flasks' contents were then filtered through glass wool and the filtrate collected in round bottom flasks. The filter residue was re-extracted with a further 50 mL of freon, sonicated for 10 min, filtered in the same way and the filtrate combined with the first extract. The combined extract was then washed into 50 ml-volumetric flasks and made up to volume. Finally a further 2 g of activated Florisil was added and the extract was mixed for 10 min to eliminate the polar biogenic hydrocarbons. The naphthalene content of the extract was determined by UV spectroscopy. Samples were scanned from 320 to 270 nm using 1 or 4-cm quartz cells on a Varian DMS-100 spectrometer and the absorbance read at 285 nm (ASTM 1986). Often the base lines were above zero and so the absorbance at 310 nm was subtracted from each reading (Anderson *et al.* 1977). The accuracy of this method suffers from several sources of interference; however, recovery of naphthalene added to uncontaminated garden soil was an acceptable 109%. Precision was evaluated as 4.3 % RSD by repeat (n=3) analyses on a sample. Extract concentrations were calculated according to Beers' Law using an extinction coefficient of 33.7 cm.L g^{-1} and converted to sediment mg/kg dry weight concentrations using the results of the solids analysis.

Behavioral bioassays, using an index of activity (crawling rate) were carried out to ascertain initial and longer term effects of naphthalene on the snails. The bioassays were performed mostly in the early morning and early evening, the periods in which snails exhibited greatest diurnal activity in the laboratory. Three similarly sized snails were placed in a 500-mL glass measuring cylinder holding oxygenated seawater containing the toxicant. When snails were active, placement in sea water generally induced them to move vertically up the sides of the container. As snails were exposed to the naphthalene in the water only for the short time before they crawled out of the water, this was classed as short term exposure. Activity was determined as the vertical distance moved over a period of time. Measurements were taken at approximately 5 min intervals over 30 min. The mean distance moved by the three snails was plotted against time and the slope of the line (crawling rate, mm min^{-1}) used as the index of activity. Since the habitat of the snails is only inundated for a few hours each day, long term exposure was defined as exposure to the toxicant for 60 min. To assess long term exposure, animals were kept immersed in the test solutions for 60 min; they were then rinsed in seawater several times and assayed, as above. Naphthalene stock solutions were prepared fresh for each experiment by adding approximately 5 mg solid, A.R. grade naphthalene to 1 L seawater and mixing the solution for 1 hr. The solution was then filtered through a Whatman No. 1 filter paper into a capped bottle. The solubility of naphthalene in seawater was analysed by UV spectroscopy and found to be 4.3 mg/L at 22°C, slightly higher than the previously recorded value of 2 mg/L at 20°C (Neff and Anderson 1981).

Regression models, standard errors of regression coefficients and means and their standard deviations were calculated using STATGRAPHICS software. Regression coefficients were compared by t-test.

RESULTS AND DISCUSSION

The results of short term exposure of *O. quoyi* to various naphthalene concentrations are shown in Table 1. There are significant differences ($P<0.05$) in snail activity between control experiments undertaken at different times probably reflecting differences in diurnal activity. This observation is similar to that of Hargrave and Newcomb (1973) who found an almost three-fold difference in activity in control animals. Although observations cannot be pooled because of this factor, it is clear from Table 1 that naphthalene concentrations of around 1 mg/L increase snail activity significantly and activity may be increased at

concentrations as low as 0.09 mg/L.

Table 1. The effects of short term exposure to various naphthalene concentrations on the activity (crawling rate) of *Ophicardelus quoyi* (* significantly different from control, P <0.05; activity is mean l±s.e.).

Exp No	Time hr	Naphthalene mg/L	Activity mm min^{-1}	R^2	n	P
1	09.00	0	12.1±1.0	0.99	6	<0.001
2	10.00	0	14.4±0.4	0.99	4	<0.001
3	21.00	0	9.7±1.4	0.94	5	0.006
		0.01	13.6±1.8	0.97	4	0.016
		0.05	9.0±1.1	0.95	5	0.004
4	12.00	0	8.7±0.1	0.99	9	<0.001
		0.09	*11.4±0.6	0.98	9	<0.001
		0.93	*10.5±0.7	0.96	9	<0.001
5	20.00	0	9.7±1.0	0.97	5	0.002
		0.2	11.7±1.2	0.97	5	0.002
		1.0	*15.0±0.2	0.99	4	<0.001

The effect of long term (60 min) exposure to naphthalene on the activity of *O. quoyi* is summarised in Table 2. Exposure for 1 hr caused a significant decrease in activity at all concentrations tested. These effects were observed at naphthalene concentrations well below the average naphthalene LD_{50} for invertebrates of 1.9 mg/L (Neff 1979). Snails exposed to the highest concentration appeared narcotised and were immobile but recovered on being placed in seawater.

This experimentation clearly demonstrated the utility of behavioral bioassays in assessing the possible chronic effects naphthalene may have on intertidal fauna. Two responses were elicited. Increased activity, provoked by a short term exposure to the toxicant, can be interpreted as an avoidance response to the toxicant (Linden 1977; Neff and Anderson 1981). Decreased activity, induced by long term exposure, suggests a direct physiological consequence of the toxicant on the organism.

Behavioral bioassays have been used by a number of authors to assess chronic hydrocarbon toxicity, sometimes with contradictory results (Hargrave and Newcomb 1973 ; Atema 1976; Linden 1977 ; Taylor and Karinen 1977; Chapman et al. 1988). This may be due to the fact that the toxicant tested often has been a mixture of hydrocarbons (the water soluble fraction of crude oil or diesel) rather than a specific hydrocarbon. The experiments reported here show that behavioral bioassays can provide fast and highly sensitive screening of specific hydrocarbons for chronic toxicity and operate at concentrations several orders of magnitude less than the LD_{50}.

Table 2. The effects of long term (60 min) exposure to various naphthalene concentrations on the activity of *Ophicardelus quoyi* (* significantly different from control, P <0.05; activity is mean 1±s.e.).

Exp. Number	Naphthalene mg/L	Activity mm min^{-1}	R^2	n	P
1	0	6.4±0.3	0.99	4	0.002
	0.007	*2.6±0.3	0.96	6	<0.001
	0.69	*1.9±0.3	0.92	5	0.01
2	0	5.6±0.3	0.99	7	<0.001
	0.043	*4.4±0.5	0.94	7	<0.001
	0.43	*0.8±0.1	0.90	6	0.004
	4.3	*0.0±0.0	0.99	6	<0.001

A response to the toxicant was elicited at a naphthalene concentration as low as 0.04 mg/L. It might be expected that a snail exposed to these levels in the natural environment would have their normal behavior patterns, such as feeding responses, disrupted to a significant extent.

The mangrove sediments where the test animals were collected are clearly contaminated with naphthalene (Table 3) and the highest concentrations are found at mid-shore, where the population density of *O. quoyi* tends to be highest, yet the bioassays suggest these concentrations should evoke a significant response by the snails to the toxicant.

Table 3. Naphthalene concentrations in mangrove sediments (mg/kg sediment dry weight 1±s.d.).

	Naphthalene, mg/kg		
Sample Position	Reference Site	Study Site	n
High Water	<2.0	5.1±2.1	7
Mean Water	2.0	24.3±6.5	4
Low Water	<2.0	14.0±7.0	3

Exposure to naphthalene in the sediment would come from two sources: that in the interstitial water and that bound in the sediment. The concentration of naphthalene in the interstitial water may be calculated from its partition coefficient between the aqueous and solid phases, the proportion of organic carbon in the sediments (0.15 from the solids analysis) and the relationship between $\log K_{ow}$ and $\log K_{ow}$ for naphthalene (Kayal and Connell 1990). This gives a value of 1 µg/kg for naphthalene in the interstitial water. The naphthalene concentration in the water in the Brisbane River estuary is 25 ng L^{-1} (Kayal and Connell 1989). Both this and the concentration of naphthalene in the interstitial water are well below the lowest concentration tested that provoked a response. It seems unlikely that such low concentrations would effect snail behavior but further testing is required to confirm this. Sediment bound naphthalene concentrations are much higher. Although naphthalenes may be readily absorbed by fauna from the aqueous phase, they may not be taken up or retained to any significant extent from contaminated sediment (Anderson 1977). Data from Shaw *et al.* (1976) and Taylor and Karinen (1977) indicate that toxic effects from sediment bound naphthalene do not occur until concentrations are much higher than those recorded in this study.

The presence of elevated levels of naphthalene in the sediment might suggest that elevated levels of other petroleum hydrocarbons toxic to the fauna are also present. Although such contaminants might act additively, their effects are likely to be small. The partition coefficients of polycyclic aromatics are such that their water solubilities and hence bioavailability are extremely low. Simple single ring aromatics have

partial pressures high enough to ensure their rapid disappearance from water and sediments through evaporation. Naphthalene derivatives would be the most abundant and toxic petroleum hydrocarbon contaminants to which the fauna would be exposed.

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