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INFLUENCE OF CONCURRENT EXPOSURE TO CRUDE OIL AND DIGENEAN INFECTION ON THE SURVIVAL OF THE MUD SNAIL, *CERITHIDEA CINGULATA*

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ABSTRACT: The influence of concurrent exposure to crude oil and digenean infection on the survival of the mud snail, *Cerithidea cingulata* (Gmelin, 1790) (Gastropoda: Prosobranchiata) was studied. Mud snails from Kuwait Bay, naturally infected with a cyathocotylid digenean and uninfected, were exposed for 8 weeks to sediment contaminated with various concentrations (0.1-200 mg/g) of crude oil. Mortality in the exposed snails increased with increase in concentration of the oil. However, no significant difference was observed in the mean mortality values obtained for infected and uninfected snails. Snails exposed to 200 mg/g of oil showed symptoms of acute toxicity and died within two weeks, while snails exposed to lower concentrations displayed avoidance responses.

KEY WORDS: Crude oil, Digenea, Cyathocotylidae, biological effects, Gastropoda, *Cerithidea cingulata*, Kuwait Bay.

INTRODUCTION

Numerous studies have shown that chronic exposure of marine organisms to oil-contaminated sediments can induce a wide range of patho-physiological effects. However, variables other than the concentration of the aromatic hydrocarbons have seldom been considered (HAENSLY *et al.*, 1982; FLETCHER *et al.*, 1982; KHAN & KICENIUK 1983; KICENIUK & KHAN, 1983). Studies by KHAN (1987, 1991) on the combined effects of crude oil and parasites on fish have indicated differential mortality: infected fish died at a higher rate than uninfected. Although there have been a number of studies concerning gastropods' tolerance to oil (MIRONOV, 1967; GRIFFITH, 1970; CRAPP, 1971; DAMBO, 1993), the influence of parasitism on this response has been neglected.

Members of the genus *Cerithidea* Swainson, 1840 are common gastropods in subtropical and tropical regions of all continents and typically inhabit intertidal mud flats of bays and estuaries (HOUBRICK, 1984), areas where high concentrations of pollutants are fairly often found. Studies from various geographical regions have indicated high susceptibility of *Cerithidea* snails to a variety of digenean infections (CABLE, 1956; HOLLIMAN, 1961; YOSHINO, 1975; ABDUL-SALAM & AL-KHEDERY, 1992). *Cerithidea cingulata* (Gmelin, 1790) is the most abundant gastropod on mud flats of Kuwait Bay and serves as intermediate host for at least six digenean species (ABDUL-SALAM &. SREELATHA, 1991). The present study was undertaken to ascertain experimentally the combined effects of natural digenean infection and crude oil exposure on mortality in *C. cingulata*.

MATERIAL AND METHODS

Mud snails, *C. cingulata* (Fig. 1), naturally infected with digeneans and uninfected, were collected from mud flats south of Kuwait Bay, about 10 km west of Kuwait City. In the laboratory the snails were kept in aquaria filled with sea water and continuously aerated. Snails used measured between 25 and 30 mm in length and all had been ascertained for infection by cercaria shedding. Liberated cercariae were vitally stained with 0.5% neutral red and identified by gross morphology (SCHELL, 1985). Snails shedding cyathocotylid cercariae (Fig. 2) and uninfected were used within 48 h of collection.

Oil-contaminated sediment was prepared by mixing Kuwait crude oil (density at 15° C = 0.8682 g/ml; gravity = 31.4° API; sul-

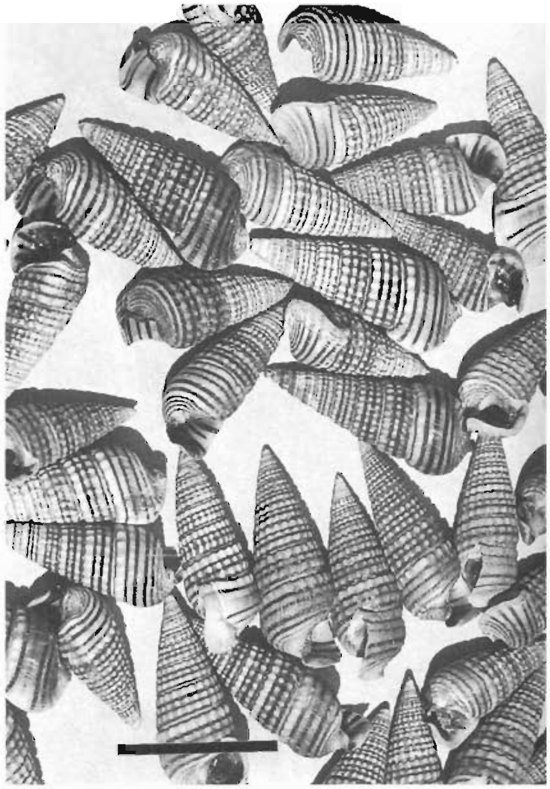

Fig. 1.– Shells of *Cerithidea cingulata*. Scale bar = 2.0 cm.

fur 2.56 Wt.%) with washed and thoroughly dried beach sand in varying concentrations (0.1, 1, 10, 100 and 200 µg/g). The oil-contaminated sand was placed in 2-liter aquaria, about 0.6 cm deep, and covered with 100 ml of sea water, about 10 cm deep. Two trials in duplicates, involving both infected and uninfected snails, were performed with groups of 10 snails, held in control or oil-contaminated aquaria. Two-way analysis of variance (ANOVA) was used in comparing the data. The lengths of uninfected snails used in each trial were matched as closely as possible to those of the infected individuals. Sex of snails was not considered in this study. Snails were fed twice a week with a speckle of powdered small animal diets (Pillsbury's Ltd.). Mortality was recorded every 24 h and was defined as immobilization and no response to touch. All dead snails were crushed and examined for digenean infections. Burrowing, crawling and retraction activities of the snails were observed and recorded daily.

RESULTS

Survival of snails

Results obtained on mortality of uninfected and infected snails exposed to sediment contaminated with various concentrations of crude oil and controls are shown in Tables 1 and 2. After 6 weeks, mortality in infected and uninfected controls was 10 and 22% respectively, while in exposed groups it increased significantly (p = 0.0014) with increase in concentration of the oil. The differences observed, however, were not significant (p = 0.8266) for infected and uninfected groups.

Exposure (weeks)	Mean % mortality at each concentration (µg/g) 0	0.1	1	10	100	200
1	0	0	0	0	0	3
2	3	0	0	0	33	100
3	3	0	0	10	83	
4	3	0	0	33	97	
5	7	47	27	73	100	
6	10	90	87	100		
7	67	100	100			
8	70					

Table 1.– Mean percentage mortality values of uninfected *Cerithidea cingulata* exposed to sediment contaminated with various concentrations of crude oil.

Exposure (weeks)	Mean % mortality at each concentration (µg/g) 0	0.1	1	10	100	200
1	0	0	0	0	3	27
2	0	0	0	0	87	100
3	0	0	0	7	100	
4	0	10	0	13		
5	10	50	43	60		
6	22	100	100	100		
7	52					
8	60					

Table 2.– Mean percentage mortality values of *Cerithidea cingulata* infected with digeneans and exposed to sediment contaminated with various concentrations of crude oil.

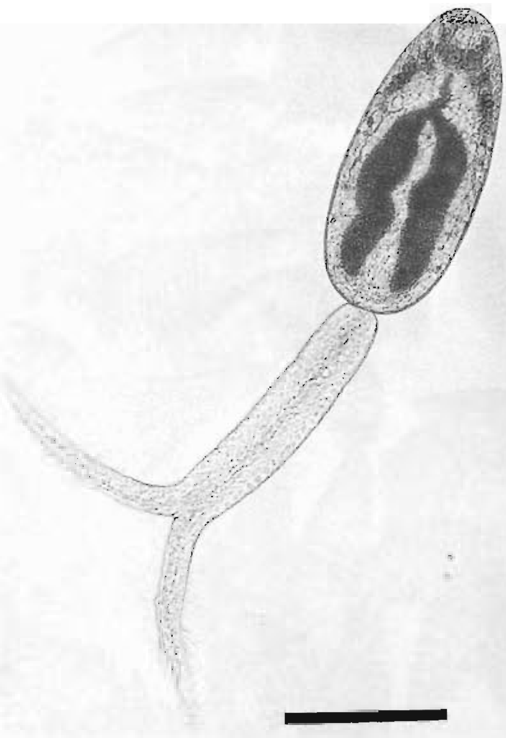

Fig. 2.– Cyathocotylid cercaria from *Cerithidea cingulata*, vitally stained with neutral red. Scale bar = 0.2 mm.

Behavioural responses

Snails exposed to 200 µg/g of oil showed acute total immobilization and died within two weeks, with the cephalopedal mass fully extended from the shell aperture. Snails exposed to lower concentrations demonstrated normal avoidance behaviour, burrowing beneath the sediment and quick retraction and aperture closing response, for the first two weeks. This was followed by a gradual display of intoxication symptoms, impaired locomotor and response activities. Gradual impairment of retraction of the cephalopedal mass inside the shell indicated paralysis of the columellar muscle, which is responsible for body retraction and aperture-closing movement of the snail.

DISCUSSION

Experimental evidence suggests that more than half of spilled oil drifts to shores and becomes adsorbed to bottom sediment (KNAP & WILLIAMS, 1982), posing significant threats to estuarine benthic species, particularly crustaceans and molluscs. Results of the present study demonstrated short and long-term toxic effects of crude oil to *C. cingulata*, a typically estuarine snail prevalent on mud flats of the eastern shores of the Arabian Gulf. Mortalities in infected and uninfected snails exposed to crude oil have been shown to be a concentration-time response; snails exposed to high concentration (200 mg/g) displayed acute toxicity symptoms and died within two weeks, while snails exposed to lower concentrations showed gradual toxicity symptoms and survived for up to six weeks. The observed mortality trends probably simulate consequences of acute and chronic exposures to oil spillage on marine organisms. Although chronic exposure to low doses of oil appears to be less dramatic than acute consequences of massive oil spillage, its long-term influences is more detrimental to marine ecosystems because it leads to accumulation of toxic oil derivatives in organisms of different ecological trophics (ANDERSON, 1982). BOROWSKY, AITKEN-ANDER & TANACREDI (1993) have shown that low doses of crankcase oil induced measurable sublethal morphological changes in the estaurine amphipod *Melita nitida* Smith, although survival of adults was not reduced by exposure to same doses.

Field studies have shown that *C. californica*, on coastal mud flats of San Francisco Bay display a wide range of physiological tolerance to high water temperatures and desiccation (RACE, 1981). Laboratory studies by SOUSA & GLEASON (1989) have shown that uninfected and naturally infected *C. californica* with digeneans exhibit a remarkable physiological tolerance to extremes of desiccation, salinity and water temperature. SOUSA & GLEASON concluded that mortality of both uninfected and infected snails due to these particular environmental conditions is likely to be an important factor in snail population density changes. Findings of the present study concur with results of the studies on *C. californica* by demonstrating nonselective mortality in *C. cingulata* due to infection alone, while exposure to a catastrophic event such as oil pollution had a drastic indiscriminate effect on the survival of both infected and uninfected snails. This is in contrast to the results of studies on infected fresh water snails exposed to molluscicides, which have demonstrated a synergistic increase in mortality (STURROCK, 1966; MASSOUD & WEBBE, 1969; HIRA & WEBBE, 1972; JANTATAEME, 1992). The use of experimentally rather than naturally infected snails in the molluscicide testing studies could be one of the reasons for the apparent discrepancy. Discrepancies in the results of studies on gastropods exposed to pollutants may also originate from differences in species of snail and parasite tested, chemical composition of the pollutant and laboratory procedures.

Variations in the tolerance of aquatic organisms to different types of pollutants have been broadly attributed to physiological and behavioural characteristics, and the degree of damage to the organism has been correlated with the penetration rate and biochemical action of the toxic element of the pollutant. Although the mechanism of oil toxicity in molluscs is not known, exposure to crude oil undoubtedly affects branchial function and, therefore, influences the respiration rate. SOUSA & GLEASON (1989) have found that anoxia was the only environmental stress that has a profound effect on survival of *C. californica*. Studies of the effects of pollutants on different systematic groups of marine organisms have indicated modification in behavioural patterns, particularly in swimming and burrowing activities. In the present study, snails exposed to sublethal concentrations of oil responded by isolating themselves from the polluted medium by retracting into their shells and burrowing beneath the sediment. Similar avoidance behaviours have been reported in gastropods and bivalves exposed to irritants or stress (HARRY & ALDRICH, 1963; BRYAN, 1969; CRAPP, 1971; DICK, 1976; RACE, 1981) and the degree and duration of the response have been accounted for by differences in the tolerance among different species (DAMBO, 1993).

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