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Metamorphosis of *Heteroxenia fuscescens* Planulae (Cnidaria: Octocorallia) is Inhibited by Crude Oil: a Novel Short Term Toxicity Bioassay

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ABSTRACT

By using TPA (12-tetra-decanoyl-phorbol-13-acetate) an artificial inducer for metamorphosis, it was possible to determine the effect of crude oil on settlement and metamorphosis of planulae of the soft coral Heteroxenia fuscescens. *In the absence of crude oil, TPA induced metamorphosis in 97% of these planulae. The effect of crude oil on metamorphosis and appearance of deformed primary polyps was concentration dependent. Only 50% of the planulae grown in experimental vessels with crude oil at a concentration of 0.1 ppm covering the bottom and walls of the vessels underwent metamorphosis when triggered by TPA. Of those planulae exposed to 100 ppm of the pollutant only 3% metamorphosed after being induced by TPA. Furthermore, oil film on the water surface was less toxic to the larvae than the crude oil covering the bottom and walls of the experimental vessels. Some of the oil treated planulae died, while others remained viable, looked normal, but did not metamorphose after being presented with TPA. These findings suggest that even at very low concentrations crude oil affects larvae of* H. fuscescens *preventing their settlement and metamorphosis. Therefore it is possible that oil spills affect coral recruitment by decreasing the viability and the settlement of coral planulae. This assay represents a new sensitive bioindicator to detect the impact of oil pollution on tropical and subtropical marine environments. Copyright © 1996 Elsevier Science Ltd*

INTRODUCTION

The catastrophic oil spills in the Persian Gulf during 1991 refocused public attention on the problem of oil pollution in the marine environment, confronting coastal nations with

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the issue of massive oil pollution. Oil pollution can be caused by shipping accidents, chronic contamination by offshore oil production and coastal refineries, discharge of contaminated ballast, wash waters and input from rivers. Although oil pollution in the marine environment has been regarded as a major environmental hazard for several decades, little is known about the mechanisms that crude oil affects in natural marine populations and communities.

It has been previously suggested that crude oil may not cause immediate mortality or visible damage to corals, although certain physiological responses may affect the corals' normal behaviour (Reimer, 1975; Knapp, 1987; Cohen *et al.*, 1977; Dodge, 1985). Studies on the stony coral *Stylophora pistillata* in a chronically oil polluted reef at Eilat (Red Sea) showed detrimental effects of crude oil to these corals. A high mortality rate, a decrease in the number of breeding colonies and gonad volume, as well as fewer planulae, were noted (Loya & Rinkevich, 1979, 1980). In addition, there was a decrease in viability and successful settlement of planulae released during an oil spill (Rinkevich & Loya, 1977, 1979; Reimer, 1975; Seng *et al.*, 1987).

To examine harmful effects of marine pollution, previous studies used eggs of marine invertebrates or their larvae as bioindicators (Spangenberg, 1984; Goh, 1991; Kobayashi, 1991; Te, 1991). Invertebrates have been proposed as potentially useful in ecotoxicological studies as well as for monitoring pollution stresses on coral reefs. Whereas many bioassays use lethal doses of pollutants, there is increasing interest in examining how sub-lethal doses affect marine organisms and their larvae (Brown, 1988; Martin & Richardson, 1995).

Many chemical and physical factors can influence patterns of initial settlement of marine invertebrate larvae (Chia & Bickell, 1978; Morse *et al.*, 1979; Pawlik & Hadfield, 1990; Pechenik, 1990; Morse & Morse, 1991). Potent artificial inducers of metamorphosis have also been described (Hofmann & Brand, 1987; Eyster & Pechenik, 1988; Freeman & Ridgway, 1990; Coon *et al.*, 1990; Henning *et al.*, 1991). The soft coral *Heteroxenia fuscescens* is common on the Red Sea reefs and reproduces by releasing planulae all year round (Benayahu, 1991). TPA (12-tetra-decanoyl-phorbol-13-acetate) at a concentration of 8.1×10^{-8} M was found to induce metamorphosis in 90% to 100% of *H. fuscescens* planulae (Henning et al., submitted). Therefore the use of this inducing agent may aid in the testing of the effects of various pollutants on metamorphosis in *H. fuscescens*.

The current study examines the deleterious effects of crude oil on planulae of *H. fuscescens*. We propose for further development a bioassay to test the capability of planulae to settle and undergo metamorphosis following exposure to various crude oil concentrations in the presence of a defined chemical inducer of metamorphosis.

MATERIALS AND METHODS

Collection and maintenance of planulae

During summer 1993 mature colonies of *H. fuscescens* were collected from the reef in front of the Marine Biological Laboratory (MBL) at Eilat (Red Sea) at a depth of 5–12 m. In the laboratory the colonies were maintained separately in an aerated aquaria and freshly released planulae were collected with Pasteur pipettes. All planulae were rinsed 3–4 times in natural Millipore-filtered seawater (0.2 μm). To prepare antibiotics containing seawater (ACS), 100 mg/ml of each of the following components were added to filtered

seawater: penicillin-G potassium salt; neomycin sulphate; and streptomycin sulphate (Sigma).

Preparation of TPA

TPA (12-tetra-decanoyl-phorbol-13-acetate), obtained from Sigma, was predissolved in Methanol and further diluted by ACS to a final concentration of 8.1×10^{-8} M. All TPA solutions were freshly prepared prior to each of the experiments (Henning *et al.*, in press).

Bioassay with crude oil

By using TPA as an artificial inducer in the presence of crude oil, it was possible to synchronise the metamorphosis of the planulae and to determine the impact of various concentrations. The sublethal effects of crude oil on this process are determined and its development as a bioindicator for oil pollution is proposed. All bioassays were carried out in sterile 24-well cell culture plates (thereafter referred to as vessels) containing ACS. In order to test the effects on the planulae of crude oil found on the water surface, different concentrations of crude oil were used (obtained from Haifa refineries, Israel; density: 0.8497 g/ml) diluted in *N*-pentane to a final concentration ranging from 1 to 5000 ppm. The different concentrations were applied onto the water surface in vessels containing ACS. The vessels were left uncovered for 24 h to evaporate the *N*-pentane. In experiments testing the effects on the larvae of crude oil covering the vessels' surface, the vessels were coated with crude oil diluted in *N*-pentane at concentrations ranging from 0.1 to 5000 ppm. After evaporation of the pentane, ACS and planulae were added. All bioassays were run at 25 °C in a controlled temperature room. After 3 d of incubation with the crude oil, TPA solution was added to each of the vessels. TPA controls for each experiment were conducted in vessels containing evaporated *N*-pentane without crude oil. In each experimental well ten planulae of *H. fuscescens* were introduced and three replicates were set for a given treatment. The experiments were run for a maximum of 8 d after adding the artificial inducer TPA. The vessels were monitored daily using a stereomicroscope, and the number of normal live, metamorphosed, and dead planulae was recorded.

RESULTS

In the all TPA control, a mean percentage of 97% metamorphosis was observed (Figs 1 and 2). The developing primary polyps showed no deformations.

When exposed to different crude oil concentrations on the water surface the planulae progressively lost their ability to undergo metamorphosis with the increase in crude oil concentration (one factor ANOVA, $p < 0.01$; Fig. 1). Only 50% metamorphosis occurred at 10 ppm crude oil. The remaining, normal-looking planulae, survived, but did not metamorphose. This figure also showed a variable percentage of dead planulae (one factor ANOVA, $p > 0.05$). For example, at 1000 ppm, 33% of the planulae died, whereas at 500 ppm, 67% died.

A thin layer of crude oil coating the surface of the experimental vessels caused inhibitied metamorphosis at concentrations as low as 0.1 ppm (one factor ANOVA, $p < 0.001$; Fig. 2). Planulae maintained in 0.1 ppm oil and TPA underwent 50% metamorphosis and

only 3.3% metamorphosis occurred at 100 ppm oil. The inhibition of metamorphosis by the crude oil as well as the percentage of dead planulae were dependent on the crude oil concentration (one factor ANOVA, $p < 0.001$). In addition, after metamorphosis there was an increase in the number of deformed primary polyps compared to the control (Fig. 3(a and b)). The deformed polyps were elongated and had short non pinnate tentacles (Fig. 3(b)).

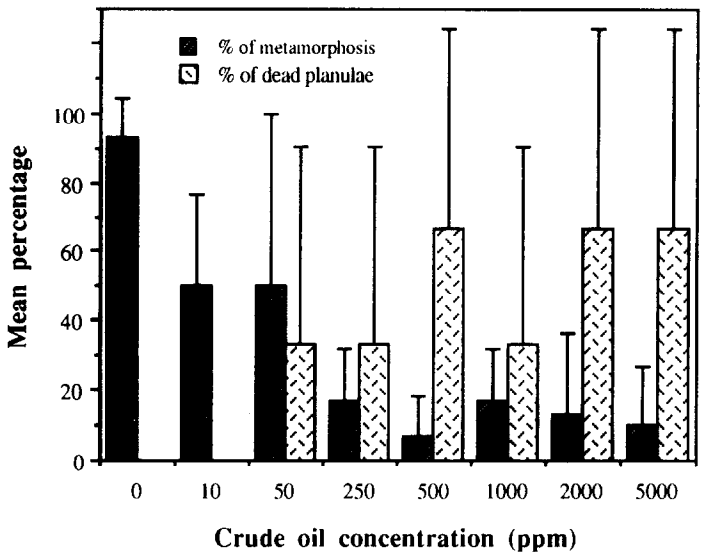

Fig. 1. Effects of different crude oil concentrations found on the water surface in presence of inducer (TPA) on metamorphosis and mortality percentages of *Heteroxenia fuscescens* planulae at day 8 of the experiment.

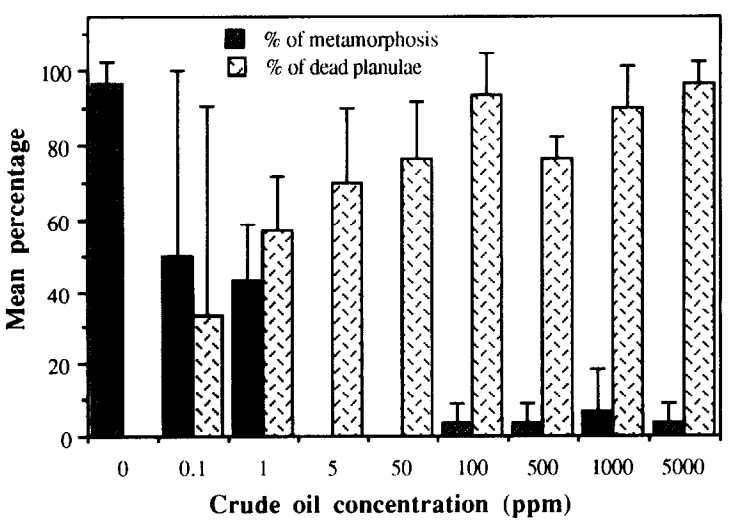

Fig. 2. The effects of different crude oil concentrations covering the vessel surface in the presence of the inducer (TPA) on metamorphosis and mortality percentages of *Heteroxenia fuscescens* planulae at day 8 of the experiment.

Of the 240 tested planulae 43 underwent metamorphosis in the vessels with crude oil coating their surface in the presence of the inducer (TPA). Among the metamorphosed planulae 49% settled on the surface and 51% did not settle at all and floated in the media. In contrast, when oil was applied to the water surface, planulae settled and metamorphosed on the bottom and walls of the vessels, with a greater number of settlements on the vessels' bottom. In the control, 62% of the metamorphosed planulae settled on the surface and 38% floated freely. One factor ANOVA showed significantly higher rates of free floating polyps when oil was coating the vessels surface compared to the control at oil concentrations of 500, 1000 and 5000 ppm.

DISCUSSION

Previous studies on pollution in the marine environment emphasised the need for base line studies on toxic effects of sublethal concentrations, which might lead to better evaluation and quantification of their effects on organisms. Lower reproduction rates after an oil spill cause damage to a coral reef by decreasing the number of planulae available for settlement (Rinkevich & Loya, 1977, 1979; Reimer, 1975; Loya & Rinkevich, 1980; Spangenberg, 1984; Seng *et al.*, 1987; Goh, 1991; Suchanek, 1993). The present study shows that fewer *H. fuscescens* planulae underwent metamorphosis when exposed to sublethal concentrations of crude oil. Planulae also settled less frequently on the oil-covered surfaces. Thus, on the reef, even in the presence of low concentrations of crude oil a decrease in both viability and successful settlement of coral planulae following an oil spill event is possible. The decline in settlement would mean that surviving planulae of a given species spend longer periods in the plankton (Moran & Grant, 1993), and consequently may be affected by: (a) a reduced capability of locating suitable settling sites; (b) a limited ability to attach to the substratum caused either by smothering it or the toxic effects of the crude oil

Fig. 3. Normal (a) and deformed primary polyps (b) of *Heteroxenia fuscescens* due to crude oil treatment. The deformed polyps were elongated and had short non pinnate tentacles (b).

(Knapp *et al.*, 1983); or (c) the lack of ability to grow or mature (Suchanek, 1993). The proposed use of dispersants after an oil spill could increase the mixing of the pollutant and its penetration into the sediments (position paper on dispersants, in: Bonn agreement 1988). This in turn would increase the toxic effects and longevity of the pollutant in the environment.

Almost all test organisms conventionally used for pollution bioassays were derived from temperate areas. These standards may be invalid when adopted for the tropics (Brown, 1988; Thorhaug, 1989). The usual tests for toxicity of substances discharged into the environment use mortality rates as an indicator for measuring harmful effects. However, toxins may affect the reproduction or physiology of the reef organisms without killing them. Coral planulae have been proposed as potentially useful in ecotoxicological work as well as for monitoring stress on coral reefs (Brown, 1988; Goh, 1991). Data in the literature indicate that lethal effects on a variety of marine invertebrates resulted from soluble fractions of oil, in the 1 to 100 ppm range (Suchanek, 1993). Fisher & Foss (1993) reported LD_{50} values for larval stages of 1.2–3.5 ppm total hydrocarbons in water soluble fractions of fuel oil. For more sensitive larval stages, mortality may even occur at lower concentrations of 0.1–1 ppm (Suchanek, 1993).

The present study found that metamorphosis and settlement of planulae were significantly affected by concentrations as low as 0.1 ppm of crude oil. In addition, mortality of planulae was variable in crude oil concentrations >0.5 ppm on vessel surfaces and >10 ppm on water surface (Figs 1 and 2). It is concluded that mortality rate is not a sensitive parameter for toxicity tests. Crude oil should be considered not only with regard to lethal effects but also to the sublethal effects in the range of 0.1–1 ppm.

Inhibition of metamorphosis could be useful for monitoring harmful effects of crude oil pollution, provided that it is feasible to synchronise the induction of metamorphosis under controlled laboratory conditions. TPA induces such synchrony. The rate of metamorphosis of settled planulae in the control experiment was 97% after TPA induction, compared with 55% settlement of *Stylophora pistillata* planulae when no artificial inducer was used (Rinkevich & Loya, 1977). In other studies, even lower percentages of settlement were found in the absence of artificial inducer (Te, 1991). In the present study planulae exposed to oil for 3 d were not altered morphologically. However, adding TPA showed that their competence to metamorphose decreased in the presence of the pollutant. These findings lead us to suggest that toxic effects of low crude oil concentrations cannot be detected without initiation of metamorphosis. Even after induction, planulae that did not undergo metamorphosis appeared normal, although they had in fact already been harmed by the toxic components of the crude oil.

In the Red Sea *H. fuscescens* planulae are obtainable throughout the year (Benayahu, 1991). The described bioassay is relatively easy to perform, since the planulae are large, competent to metamorphose within hours after release, do not require feeding and can be easily manipulated in the laboratory. We propose that this novel bioassay could be further developed as a model for evaluating a variety of other toxic substances at sublethal concentrations. However, more work is necessary to standardise the bioassay.

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