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The Effect of Naphthalene on Survival and Activity of the Amphipod *Parhyale*

W. Y. Lee, and J. A. C. Nicol

University of Texas, Marine Science Institute, Port Aransas Marine Laboratory, Port Aransas, Tex. 78373

The majority of experiments dealing with the biological effects of petroleum oils and constituents of oils have been carried out in open systems in which the volatile compounds are able to evaporate. Consequently, the animals are exposed to a medium in which some of the toxic components progressively decrease in concentration. In our experiments with amphipods, we have exposed amphipods to oils and aromatic compounds in open bowls; the solution was renewed daily for seven days (LEE et al., 1977a). One of the major toxic components of petroleum oils is believed to be naphthalene, which largely evaporates from solution within 3h at 25°C (LEE et al., 1977b). In order to determine the effect of sustained exposure to naphthalene, some experiments were carried out in a closed system, using the amphipod Parhyale hawaiensis. To obtain data for comparison, a parallel experiment was carried out in an open system.

Materials and Methods

Specimens of Parhyale hawaiensis (Dana) were collected from red weed occurring on the stone jetties at Port Aransas. They were placed in culture dishes (diameter 19 cm) containing about 1 l of sea water, which was changed every 2 days. From these stock cultures, adult animals were withdrawn as required. The dishes were aerated and the animals were fed dried sea lettuce and tropical fish food.

All sea water used in this research was collected offshore from Port Aransas. It was filtered through glass wool and a millipore filter (pore size, 0.45 μm), and the salinity was adjusted to 30 o/oo. Antibiotics were added, penicillin G at 25 mg and streptomycin sulphate at 50 mg l^{-1}.

Data are available for survival of the amphipod Elasmopus in solutions of naphthalene in open containers (LEE, unpublished information), but not for Parhyale. Therefore, an experiment was carried out

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in which Parhyale was exposed to naphthalene at concentrations of 3 to 20 ppm in open culture dishes (diameter 19 cm). Ten animals were placed in a dish containing 1 l of sea water or naphthalene solution. Controls and experimentals were duplicated (total of 20 animals each). They were not fed, the medium was aerated and the dishes were covered with thin polythene film. After 24h the animals were transferred to clean sea water and fed; the water was aerated and changed daily or every second day for a week.

To study biological effects in a closed system, BOD bottles (capacity 300 ml) were used. Sea water and bottles were sterilized. A preliminary experiment with two amphipods was done to ascertain that confinement in a closed bottle, without food, had no detrimental effect. For the definitive experiments, two animals were placed in each bottle, filled with solution, and the bottles were stoppered. A total of 10 bottles (20 animals) was used for the control and for each concentration of naphthalene tested. After 24h the animals were examined and these particulars were noted: whether the animals were alive, swimming, moving appendages, or dead. A rating system was employed: 0, flexed body, no movement; 1, slightly twitching appendages at infrequent intervals; 2, twitching appendages often and flexing body; 3, moving appendages actively and progressing short distances on the bottom; 4, swimming short distances; 5, swimming actively. Survivors were transferred to clean sea water in open culture bowls, fed, aerated and the sea water was changed daily. The condition of the animals was noted each day. For those behaving normally, observations were continued for one week, for those whose movement was impaired observations continued until they recovered or died.

All experiments were carried out at room temperature, 22°C.

Solutions of naphthalene were prepared by adding 10 or 20 µg to 1 l of sea water, the flask was sealed and the solution was stirred for 3 days with a magnetic stirrer. It was filtered through glass wool, and dilutions were made as required.

Results

The majority (85 to 95%) of Parhyale in the open bowls tolerated naphthalene at 6 to 10 ppm (Table 1). Chi-square tests showed that survival of amphipods in various test solutions is significantly different from

each other ($\chi^2 > 6.63$ at $p = 0.01$) except for control — 3 ppm, control — 4 ppm, 3 ppm — 4 ppm, and 6 ppm — 10 ppm.

TABLE 1

Survival of Parhyale in naphthalene: 24h in open bowls

Concentration of naphthalene	Number of animals	Number of survivors	Percentage of survivors
3 ppm	20	20	100
4	20	20	100
6	20	19	95
8	20	17	85
10	20	19	95
15	20	11	55
20	20	1	5
Control	20	20	100

The behavior was altered for a few hours, the animals lay about, rapidly moving their appendages; after 24h most of them began swimming actively. Mortalities were high at concentrations of 15 and 20 ppm (Fig. 1).

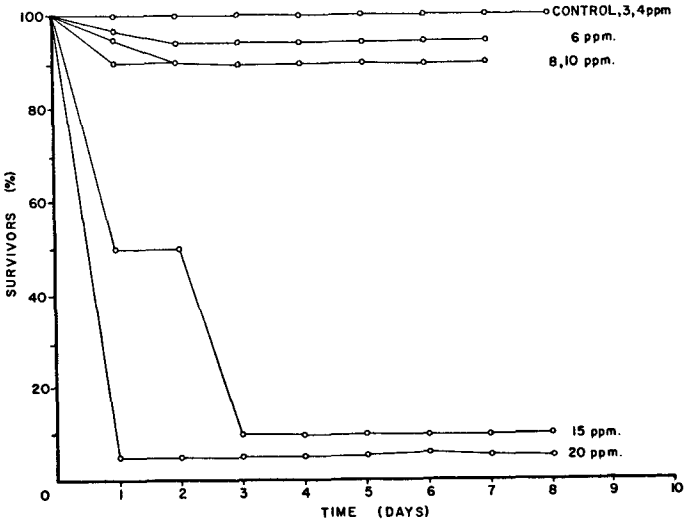

Fig. 1. Survival of Parhyale in open bowls after exposure to naphthalene for 24h.

The mean activity rating of the survivors at naphthalene concentrations of 4 to 8 ppm was 5, equal to the controls. At higher concentrations (10 ppm) there was a persistent toxic effect on some of the survivors; after a week half the surviving animals had not fully returned to normal (Figs. 2 and 3). All survivors had fully recovered in two weeks.

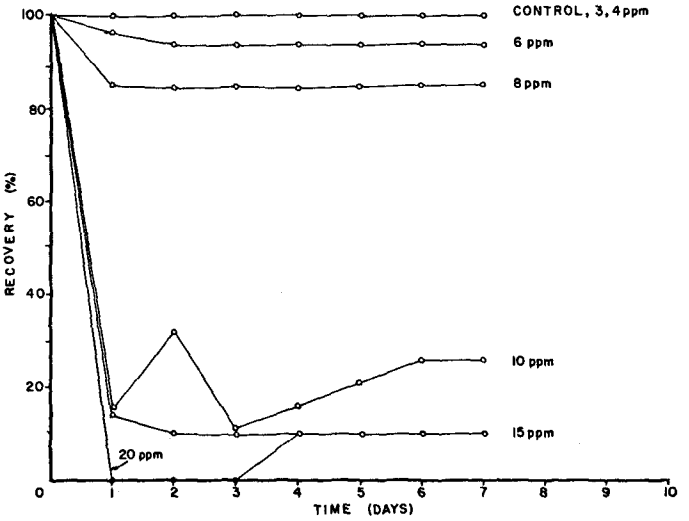

Fig. 2. Numbers of survivors (percentages) that showed a rating of 4 or 5 (swimming activity) following exposure to naphthalene in open bowls.

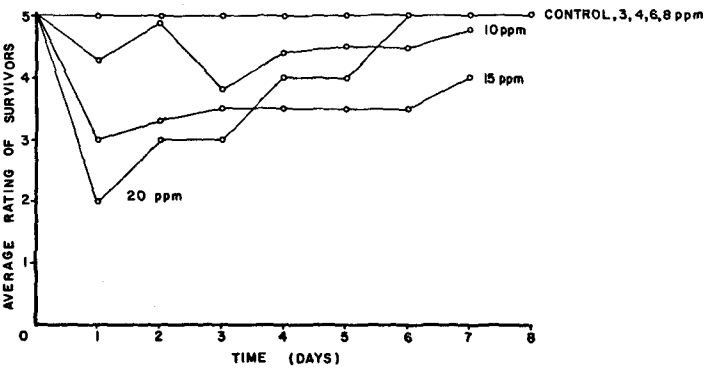

Fig. 3. Average rating of survivors following treatment with naphthalene in open bowls. The points for 15 and 20 ppm refer to only 2 and 1 animals after the third and first day.

In closed bottles heavy mortality (>50%) was caused by naphthalene > 5 ppm (Fig. 4, Table 2).

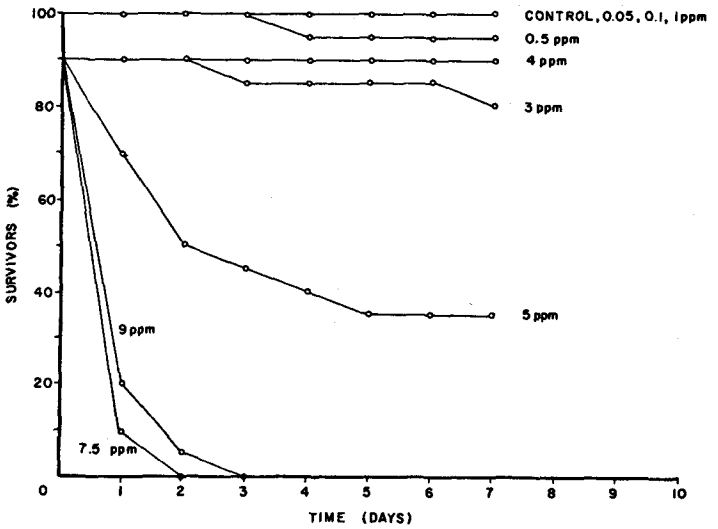

Fig. 4. Survival of amphipods during 7 days after exposure to naphthalene in closed BOD bottles (percentages).

TABLE 2

Survival of Parhyale in naphthalene: 24h in closed BOD bottles.

Concentration of naphthalene	Number of animals	Number of survivors	Percentage of survivors
9 ppm	20	4	20
7.5	20	4	20
5	20	14	70
4	20	18	90
3	20	18	90
1	20	20	100
0.5	20	20	100
0.1	20	20	100
0.05	20	20	100
Control	20	20	100

A Chi-square test showed that survival of amphipods in the control and the experimental groups was significantly different (χ^2 > 6.63 at p = 0.01) except among those of control, 0.05 ppm and 0.1 ppm, and the following:

0.5 ppm — 1 ppm, 3 ppm — 4 ppm and 7.5 ppm — 9 ppm. All animals in 7.5 and 9 ppm naphthalene were dead after 3 days (Fig. 4). The deleterious effects of naphthalene were also shown in other ways. At all concentrations above 3 ppm, swimming ability initially was depressed (Figs. 5 and 6). All survivors in naphthalene at 3 and 4 ppm were swimming by day 3.

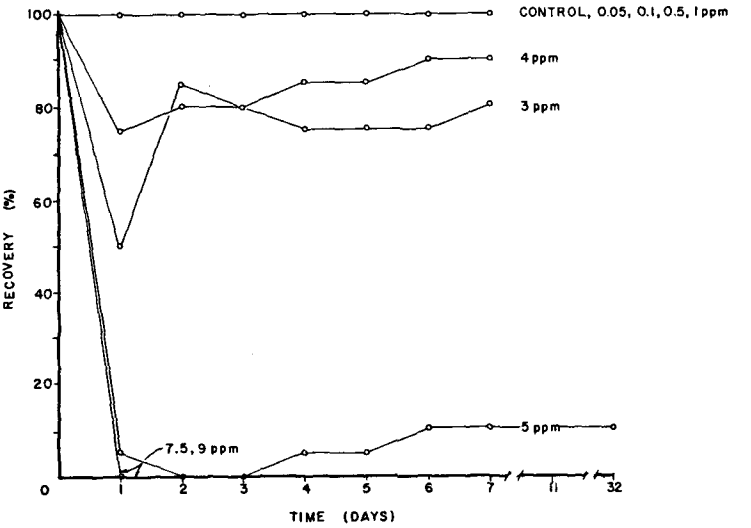

Fig. 5. Numbers of survivors (as percentages) that showed a rating of 4 or 5 (swimming activity) on 7 consecutive days after exposure to naphthalene in closed BOD bottles.

During the first two days some of the animals were nonmotile, only moving their appendages. In naphthalene 5 ppm, 5 animals that could not swim lived for 9 to 30 days.

Discussion

The experiments with a closed system show that naphthalene has a deleterious effect on Parhyale at a concentration beginning at 3 ppm, at which most of the animals recovered, the critical level was 5 ppm. In a parallel experiment with Elasmopus sp., in which animals were exposed to naphthalene solutions renewed daily in covered bowls, the critical level again was

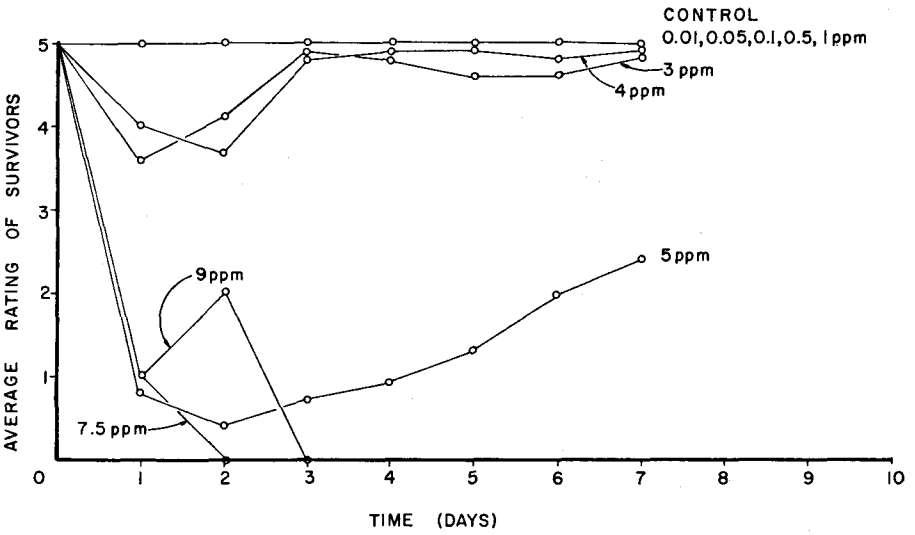

Fig. 6. Average rating of survivors, after treatment with naphthalene in closed BOD bottles (maximum score, 5). The points on the curves for 7.5 and 9 ppm are for 1 animal only.

5 ppm (W. Y. LEE, unpublished information). Much higher levels of naphthalene (2 or 3x) were required to kill Parhyale in open vessels compared with closed containers for a 24h exposure. A persistent damaging effect was found in some of the survivors from both open and closed systems, this effect appeared at much lower concentrations among survivors from the closed vessels. Under natural conditions these debilitated animals would be vulnerable because they could not move about or feed.

Naphthalene is regarded as one of the major toxic components of petroleum oils. For shrimp (Palaemonetes, Penaeus), the critical levels (LC_{50} 24h) of naphthalene were about 2.5 ppm (ANDERSON et al., 1974b). A fuel oil (No. 2, Exxon Baytown) was found to be lethal to amphipods Gammarus mucronatus and Amphithoe valida at concentrations $\geq$ 0.8 ppm (LEE et al., 1977a); this is a very toxic oil to marine animals. The concentration of total benzene extractable organics in sea water extracts of this oil was 20 to 21 mg l^{-1} (WINTERS et al., 1977; LEE et al., 1977b) and the concentration of naphthalene was 0.75 mg l^{-1}, and of naphthalene plus

alkyl naphthalenes, 1.93 mg l^{-1} (ANDERSON et al., 1974a; LEE et al., 1977b). It is obvious that the toxicity of No. 2 fuel oil cannot be ascribed to naphthalene alone. However, some of the substituted naphthalenes, for example dimethyl naphthalene, are much more toxic than naphthalene itself, and the same holds true for the substituted benzenes found in fuel oil (DONAHUE et al., 1977). Moreover, there is the possibility of synergistic effects between the aromatic components, and LEE (unpublished information) has found that mixes of naphthalene and 1,2,4-trimethyl benzene are more toxic than either component alone at the same concentration.

Acknowledgement

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