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Altered Grazing Patterns in an Experimental Copepod-Alga Ecosystem Exposed to Naphthalene and Kuwait Crude Oil

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Dissolved petroleum hydrocarbons can effect and alter a wide range of biological processes, including changes in behavior and feeding patterns. Such changes have been shown to occur at remarkably low concentrations (20-200 ppb, Hall et al. 1978, Elmgren et al. 1980). One potential consequence of this is that the disruption of, for example, the behavior of one organism may in turn alter the predator-prey balance with another. Such hydrocarbon-induced changes in predation pressure by certain major predators is thought to have led to complex community changes in large mesocosm studies (Grassle et al. 1981; Elmgren et al. 1980). In those studies the community changes came about after chronic low-level exposures to petroleum hydrocarbons. However, as yet no direct cause-and-effect relationship has been documented between petroleum hydrocarbon exposure and behavioral changes that subsequently could lead to such changes in food-web interactions.

We became interested in the potential disruption of predator-prey relationships after we observed that naphthalene, as well as a number of oils, changed the swimming behavior of the unicellular flagellate alga Pavlova lutheri (formerly Monochrysis lutheri) (Vandermeulen et al. 1983). With increasing concentrations of the hydrocarbons the swimming behavior of this phytoplankton changed, in a predictable way, from a normally motile form through sequential stages of immobilization, leading eventually to death of the cells. Reasoning that alterations in the motility of a prey species would render it more susceptible to predation, we examined the hydrocarbon-induced changes in predation success in a simple two-member prey-predator system consisting only of P. lutheri and the marine copepod Calanus finmarchicus. The organisms were exposed, together, to low concentrations of either naphthalene or Kuwait crude oil dissolved in seawater, and the feeding efficiency of the copepods under these conditions was measured by counting the survival of algal cells (Sheldon and Parsons 1967). Naphthalene was chosen because it is a relatively simple toxic aromatic hydrocarbon, common to all crude oils and most refined products and their aqueous extracts (water-soluble fractions) (for example 0.15 ppm in seawater extract of kerosene, Boylan and Tripp 1971). Kuwait crude oil was used as a representative oil mixture more commonly encountered under spillage conditions.

MATERIALS AND METHODS

Algal cultures were newly established from standard laboratory stocks, routinely maintained in f/2 medium (Guillard and Ryther 1962), and were taken through several transfers before experimentation. Only log-phase cultures were used experimentally. Copepods were collected at nearby St. Margaret's Bay, by plankton tow (#0 net) towed at 30 meters, and were isolated by hand. No distinction was made between males and females, and as much as possible only adult specimens were taken. They were then transported to the Bedford Institute of Oceanography laboratories and there were maintained at 3 oC in filtered seawater until use, usually within 48-72h. They were acclimated to 10 oC for two hours before each experiment.

All grazing studies were done in triplicate, and carried out in 500 mL Erlenmeyer flasks, containing 100 mL of a known concentration of algal culture prepared in filtered sea water (0.45 microns; 10 mL algal culture + 80 ml filtered sea water). For predation studies, ten 72-h starved mature copepods were added to the flasks, and allowed to graze on the algal cells for 24 h and 48 h periods.

Known aliquots of naphthalene or Kuwait crude oil, previously dissolved in acetone to known solutions and further dissolved in seawater, were then added to the test flasks at the start of each experiment to yield nominal concentrations of 0.1, 0.5 and 1.0 ug/L naphthalene in seawater . The total amount of acetone added to the experimental flasks in this manner never exceeded 0.05 mL. Cultures containing only seawater or only acetone and seawater served as controls.

Grazing pressure was determined by measuring particle volumes of algae, with and without copepods, by Coulter counting. This method allows less than 3% error per particle diameter, and less than 10% error for area under the particle-size spectrum curve (Sheldon and Parsons 1967, Sheldon unpublished results). All experiments were carried out in the light, in tin-foil capped Erlenmeyer flasks at 10 oC. Data were examined statistically but difficulty was experienced, possibly because of the high variability that was observed.

RESULTS AND DISCUSSION

Cultures of P. lutheri normally show a characteristic size spectrum, from three to eight microns in diameter, with over 55% of the cells averaging five microns in diameter. Without any contaminant present, the predation by C. finmarchicus on P. lutheri accounted for circa 25% of the algal volume over a 48-h period. Feeding apparently was non-selective, with predation approximately equal over the whole size range of the phytoplankton.

Figure 1 shows representative results of grazing experiments under the different experimental conditions, and indicates the general influence of naphthalene on grazing effectiveness. The

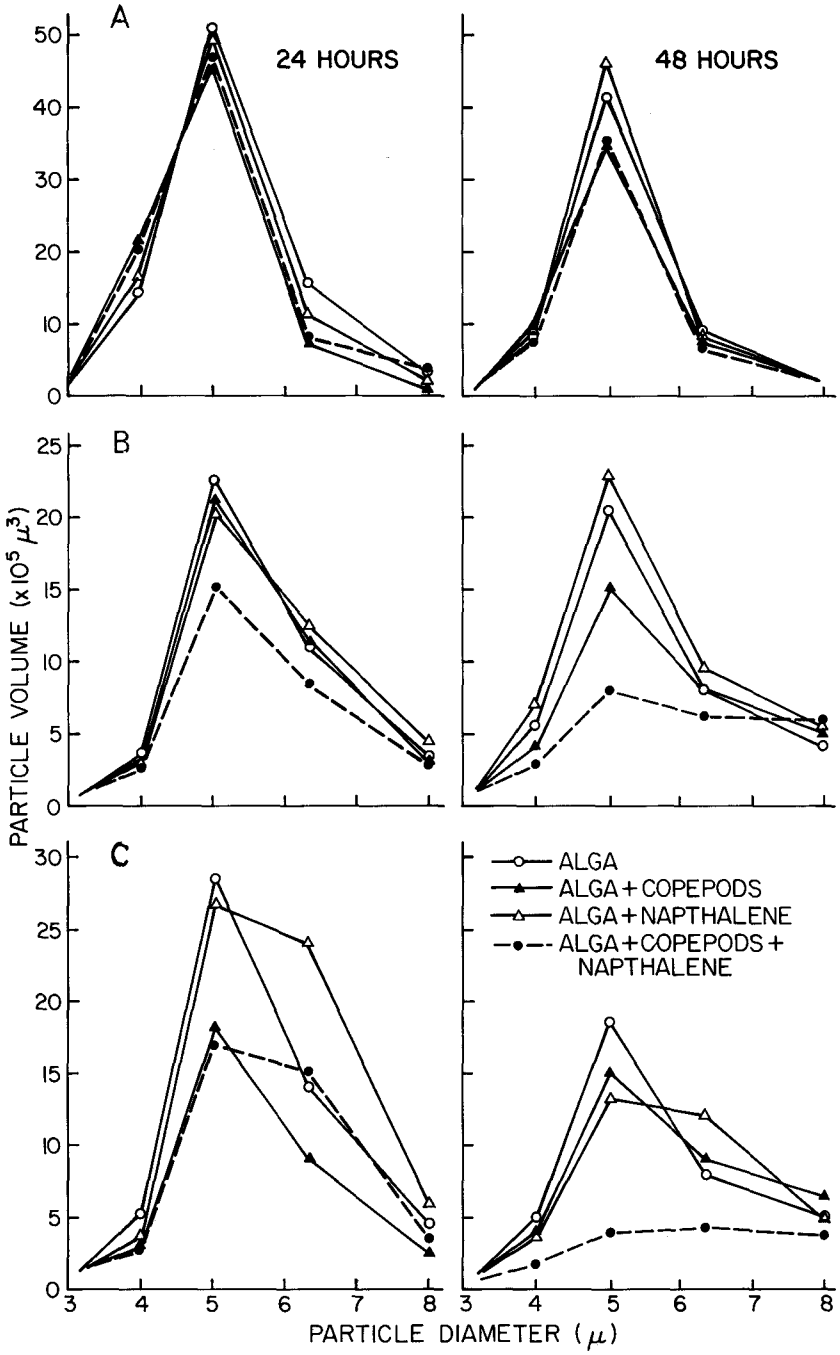

Figure 1. Effect of naphthalene on feeding effectiveness of the copepod Calanus finmarchicus on the unicellular phytoplankton Pavlova lutheri as measured by Coulter counting of algal cell volume after 24 and 48h. 1A - 0.1 ug/L, 1B - 0.5 ug/L, 1C - 1.0 ug/L naphthalene.

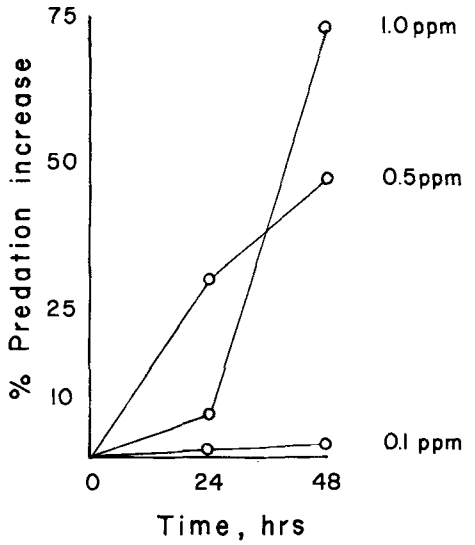

Figure 2. C. finmarchicus predation pressure on P. lutheri as a function of naphthalene concentration and of exposure time.

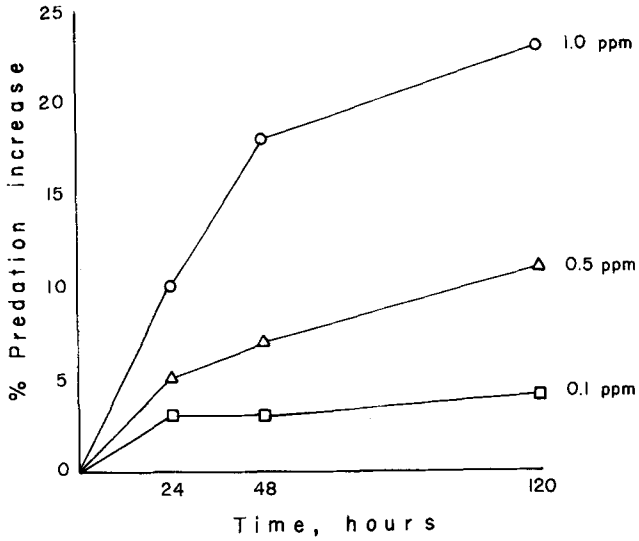

Figure 3. Predation pressure by C. finmarchicus on P. lutheri, as a function of concentration of Kuwait crude oil and exposure time.

survival of algae alone, at 0.1 and 0.5 ug/L naphthalene and as measured by Coulter counting, in the absence of any copepods, did not differ appreciably from survival in control cultures, both after 24 and 48 h (Figures 1A, 1B). Size spectra curves for both experiments overlapped after 24 h, and were only slightly different after 48 h. At 1.0 ug/L naphthalene also, there was no significant difference (within 1% error, Sheldon and Parsons 1967) difference after 24 h in survival from control cultures (Figure 1C). A discernible difference in algal survival was observed at the longer exposure period, 48 h.

Feeding pressure by the copepods, at the lowest concentration of naphthalene (0.1 ug/L), did not differ measurably from control cultures, both over 24 and 48 h (Figure 1A). This, however, changed markedly at the increased concentration of 0.5 ug/L naphthalene, with a decrease in survival, both as a function of time and of naphthalene concentration (Figure 1B). For example, after 24h at 0.5 ug/L naphthalene, the copepods had removed ca 30% more algae than in control flasks. Feeding success increased to 47% fewer algae surviving predation after 48-h in 0.5 ug/L naphthalene.

Grazing pressure, at the highest concentration of naphthalene tested (1.0 ug/L, Figure 1C) after 24 h was slightly increased over that with 0.5 ug/L. But after 48-h continuous exposure to 1.0 ug/L naphthalene algal survival was down 73% as compared to control flasks. At no time did control cultures, containing only acetone in seawater, differ significantly from control cultures in seawater alone. Nor was there evidence of dead algal cells on the bottom of the flasks.

These results are summarized in Figure 2, showing the relative changes in predation efficiency in the presence of increasing concentrations of naphthalene. Similar results were obtained with Kuwait crude added to the culture medium (Figure 3). Both 24-h and 48-h exposures of copepod and algae together to 0.5 and 1.0 ppm Kuwait crude in seawater preparations resulted in marked decreases in the algal biomass. The ca. 3% decrease in algal survival after 24 h exposure to 0.1 ppm Kuwait crude oil is not significant, falling within the experimental variability.

These results very suggestively lead to the conclusion that predation success of C. finmarchicus, feeding on M. lutheri, is enhanced in some manner by exposure to hydrocarbons, i.e. the presence of the hydrocarbons elicited an increased feeding efficiency. Furthermore, the effect appeared to be a direct function of both time and of hydrocarbon concentration. This was shown schematically in the summary Figures 2 and 3. The corollary of these observations was that, under our experimental conditions, the same incremental change in predation efficiency could be obtained equally by either lower concentrations of the contaminants at the longer exposure times, or by higher concentrations at the shorter exposure times.

At first glance these results seem very clear-cut and straight

forward, pointing to a very simple enhancement of predation pressure, and presumably with little or no effect on the copepods. However, they are unexpected because they run completely counter to the more established expectations that oil-stressed zooplankton are sensitive to hydrocarbons and that their feeding is suppressed by hydrocarbons (e.g. Berdugo et al. 1977; Berman and Heinle 1980). In fact, inhibition of feeding by zooplankton was thought to be one underlying factor in the observed changes in phytoplankton composition in the MERL mesocosm studies (Elmgren and Frithsen 1982; Elmgren et al. 1980), and was also the explanation put forward as a likely cause for the increases in phytoplankton biomass that were recorded after the TSESIS oilspill (Johansson et al. 1980). We can not, from these experimental results, resolve this quandary. No doubt the problem arises in part from the fact it is rarely a simple matter to isolate two components from a complex foodweb/communtiy and expect their relationship to continue unchanged from what it was in the context of the larger system where many factors, other than a simple feeding unchallenged effectiveness, determine the final balance between prey and predator.

One possible explanation may be that the change in feeding success measured in these experiments is in some way linked to a greater sensitivity of the algae to the hydrocarbons, and to its consequent decreasing motility. Such a difference in sensitivity to toxicity would not be unique, and is well known among invertebrates and phytoplankton. Also P. lutheri has been shown to be extremely sensitive to naphthalene (Vandermeulen et al. 1983) at low concentrations, and is known, at the concentrations of hydrocarbons used here, to switch from a motile pattern of swimming to a more stationary spinning behavior (Vandermeulen et al. 1983). Presumably in that state it would be rendered an easier prey for the copepods, resulting in increasing feeding effectiveness. The problem with this explanation is that the suite of behavior changes that occur in this alga, at the concentrations of hydrocarbons used here, usually occur rapidly, certainly within the first hour of exposure. However, the grazing changes that we have recorded are not really measurable until after 24 h, leading to a major difference in response times between the two indices of toxicity, changes in algal motility and increases in copepod predation. At present we have no explanations for this question. On the other hand, without further information on any sublethal effects of hydrocarbons on C. finmarchicus we can not rule out the possibility of even a slightly elevated feeding/filtration activity by the copepod occurring at these concentrations.

One point worth noting here is that the first indication of a change in predation pressure (here seen after 24h with 0.5 ug/L naphthalene, Figure 1B) occurred well before there was any noticeable decrease in algal survival due to naphthalene alone (seen after 48h at 1.0 ug/L naphthalene, Figure 1C), and that this occurred consistently through repeated experiments. This would suggest that the increased predation success by the copepods may be a function of a more subtle change in algal behavior than simply that of immobilization and/or death.

These results must be interpreted very cautiously in any attempt to relate them to any impact of hydrocarbons in the field, such as for pelagic plankton communities. As pointed out above, a laboratory experiment such as this represents an artificial simulation of the actual event in the field, and without knowing all the parameters the results obtained may not be those that one would expect from a field situation. Nonetheless, experimental results such as these can point to potential problem areas or to interactions that require further experimentation and understanding. For example, it does seem clear that apparently innocuous concentrations of naphthalene (as low as 0.5 ug/L over 24-h exposure) can elicit an unexpected change in survival fitness, that would more normally be expected to occur at much higher concentrations.

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REFERENCES CITED

Berdugo V, Harris RP, O'Hara SC (1977) The effect of petroleum hydrocarbons on reproduction of an estuarine planktonic copepod in laboratory cultures. Marine Pollution Bulletin 8:138-143

Berman MS, Heinle DR (1980) Modification of the feeding behavior of marine copepods by sub-lethal concentrations of water-accommodated fuel oil. Marine Biology 56:59-64

Boylan DB, Tripp BW (1971) Determination of hydrocarbons in seawater extracts of crude oil and crude oil fractions. Nature 230: 4447-4449

Elmgren R, Vargo GA, Grassle JF, Grassle JP, Heinle DR, Langlois G, Vargo SL (1980) Trophic interactions in experimental marine ecosystems perturbed by oil. In: Giesy JP Jr (ed) Microcosms In Ecological Research. US Tech Inform Ctr, US Dept of Energy Symp Ser 52 (CONF-781101) p 779

Elmgren R, Frithsen JB (1982) The use of experimental ecosystems for evaluating the environmental impact of pollutants: a comparison of an oil spill in the Baltic Sea and two long-term, low-level oil addition experiments in mesocosms. In: Grice GD, Reeve MR (ed) Marine Mesocosms. Springer-Verlag, New York, US, p 153

Grassle JF, Elmgren R, Grassle JP (1981) Response of benthic communities in MERL experimental ecosystems to low level, chronic additions of No. 2 fuel oil. Mar Envir Res 4:279

Hall CAS, Howarth R, Moore B, Vorosmarty CJ (1978) Environmental impacts of industrial energy systems in the coastal zone. Ann Rev. Energy 3:395

Johansson S, Larsson U, Boehm P (1980) The TSESIS oil spill. I. Impact of the pelagic ecosystem. Mar Poll Bull 11:284

Sheldon RW, Parsons TR (1967) A Practical Manual on the Use of the Coulter Counter in Marine Science. Coulter Electronics Sales Co Canada, 66 pp

Vandermeulen JH, Silvert WM, Foda A (1983) Sublethal hydrocarbon phytotoxicity in the marine unicellular alga Pavlova lutheri Droop. Aquatic Toxicol 4:31-49

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