Marine Biology 78, 45–51 (1983)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Effects of exposure to sublethal concentrations of crude oil on the copepod *Centropages hamatus*

I. Feeding and egg production *

T. J. Cowles and J. F. Remillard

Woods Hole Oceanographic Institution; Woods Hole, Massachusetts 02543, USA

Abstract

Female copepods of the species *Centropages hamatus* show decreased ingestion rates and decreased egg viability when exposed to crude oil/seawater dispersions having crude oil concentrations of 10–80 ppb. However, rates of egg production were not significantly affected by these exposure levels. In addition, we found no evidence for accumulation of petroleum hydrocarbons by copepods exposed to 200 ppb of South Louisiana crude oil. The results imply that biosynthetic pathways involved in oogenesis may be influenced by sublethal concentrations of crude oil or that petroleum hydrocarbons directly affect the viability of eggs. Recruitment into field populations of copepods could be severely reduced as a consequence of exposure to low levels of physically dispersed crude oil.

Introduction

Marine organisms are frequently exposed to a range of hydrocarbon concentrations as a result of oil spills, oil seepage, chronic coastal oil pollution from harbors, and, to a small extent, biogenic hydrocarbon production. Marine zooplankton, particularly copepods, are an important connection between planktonic primary production and upper trophic level harvestable yield and, as such, are critically important in determining the level of living resources in the pelagic environment. Given the importance of this link in the marine food chain, it is necessary to understand how petroleum hydrocarbons influence and modify the transformation of energy within the planktonic system.

Copepods show decreased rates of fecal pellet production (Spooner and Corkett, 1979) when exposed to crude oil which has been chemically dispersed, and ingestion rates decline following exposure to the water soluble fraction of aromatic heating oils (Berdugo *et al.*, 1977; Berman and Heinle, 1980). It has also been demonstrated that exposure to 0.5 to 1 ppm of petroleum hydrocarbons can have toxic effects (Mironov, 1969; Berdugo *et al.*, 1977; Ott *et al.*, 1978). Copepods can ingest oil droplets (Conover, 1971; Hebert and Poulet, 1980) and copepods in the vicinity of the "Argo Merchant" spill were observed to have oil droplets in their guts (Polak *et al.*, 1978). The rates of egg production by copepods have also been shown to decline under exposure to aromatic hydrocarbons under static bioassay conditions (Berdugo *et al.*, 1977; Ott *et al.*, 1978). As Ott *et al.* (1978) suggested, it is not clear from the results that hydrocarbon exposure had a direct effect on fecundity, since inhibition of feeding rates could have the same effect on egg production.

One of the difficulties faced in interpreting the results of earlier studies is that all used static exposures to hydrocarbons rather than continuous flow exposure systems. In static systems various hydrocarbons can undergo substantial modification during the experiment as volatile constituents leave solution and biodegradation takes place. We have adopted the continuous flow system of Capuzzo *et al.* (1976), which provides uniform exposure conditions throughout the experiment. This paper reports the results of a study designed to examine the interactions between continuous exposure to sublethal concentrations (10–80 ppb) of crude oil–seawater solutions, feeding behavior, and fecundity in the copepod *Centropages hamatus*. Specifically, we examined the effects of exposure to crude oil–seawater dispersions on

(1) the ingestion rate of *C. hamatus*,
(2) the rate of egg production per female copepod,
(3) the viability of eggs produced by exposed and control copepods,
(4) the accumulation of petroleum hydrocarbons in the copepods, and
(5) the activity patterns of *C. hamatus* as they relate to swimming and feeding.

* Contribution No. 5361 of the Woods Hole Oceanographic Institution

This paper will focus on the results of the experiments which addressed the first four points listed above. The results of the activity pattern studies are reported in a companion paper (Cowles, 1983).

Materials and methods

Adult females of the species *Centropages hamatus* were isolated from plankton tows made in Vineyard Sound and Great Harbor, Woods Hole, Massachusetts, USA. The copepods were maintained at 20 °C and were fed a mixture of the dinoflagellates *Prorocentrum micans, Scrippsiella trocoidea,* and *Gymnodinium nelsoni.* The algae were maintained at 15 °C, in log phase growth on f/2 medium (Guillard and Ryther, 1962) on a 14 hL:10 HD cycle. The parameters describing the experimental design are shown in Table 1.

An oil–seawater stock mixture was made each day for use on the following day (Capuzzo and Lancaster, 1981) by mixing South Louisiana crude oil and 1-μm filtered seawater for 24 h at 5 °C according to the method of Anderson *et al.* (1974). Aliquots were drawn from beneath the slick of the stock mixture, diluted with filtered seawater to the appropriate concentration (based on analysis of the stock mixture), and added to the reservoirs of the continuous flow system. Hydrocarbons in the stock solution and in the dilutions were extracted three times with methylene chloride, dried overnight over sodium sulfate, rotary evaporated, dried just to the point of dryness under a gentle stream of nitrogen, then brought to final volume in hexane. Concentrations of total hydrocarbons in the stock mixtures and in the experimental dilutions (see Table 1) were determined daily, relative to standards, with glass capillary gas chromatography and gravimetric analysis (Boylan and Tripp, 1971). Fresh oil-suspension reservoirs containing the experimental concentrations of dispersed crude oil were set up every 8 h from the initial stock mixture for that day in order to minimize degradation of the oil mixture in the reservoirs.

Table 1. Bioassay system parameters

Variable	Value
Hydrocarbon concentration (South Louisiana crude)	0, 10±5, 20±5, 80±20 ppb* (Feeding and egg prod) 0, 200±20 ppb* (Accumulation expts)
Algal food:	
Prorocentrum micans	50–100 cells ml^{-1}
Scrippsiella trocoidea	50–100 cells ml^{-1}
Gymnodinium nelsoni	25– 50 cells ml^{-1}
No. of exposure experiments (with 4 treatments × 3 replicates)	7 runs
Duration of exposures	48–64 h
Size of bioassay chamber ($n = 12$)	500 ml
No. of copepods $chamber^{-1}$	10–20
Flow rate through chamber (food sol'n + oil sol'n)	800 ml h^{-1}

* Mean + 2 SE

The three dinoflagellate cultures were counted daily, with aliquots drawn from each every 8 h for dilution with 1-μm filtered seawater to the appropriate concentration for the algal reservoirs. The diluted oil suspension and the algal mixture thus entered the continuous flow system separately, and the algal mixture was in contact with the oil dispersion only in the exposure chambers (approximately 40-min residence time).

Copepods were added to each of the twelve 500-ml assay vessels, with three replicate vessels for each oil treatment plus three control vessels receiving uncontaminated seawater (Table 1). The food and oil were pumped into the assay vessels from separate 12-l reservoirs by a peristaltic pump at 800 ml h^{-1}. The quantity of food in the inflow and outflow of the bioassay system was monitored with a Coulter Counter Model TA II. The bioassay system was maintained at 20 °C during the experimental series.

The possibility of accumulation of petroleum hydrocarbon into copepod body tissue was examined through application of thermal distillation pyrolysis techniques (Whelan *et al.*, 1980). The results from an initial run at 80 ppb indicated that body hydrocarbon levels were barely above background for both control and oil-exposed copepods. In subsequent runs for thermal distillation pyrolysis analyses, the concentration of crude oil in the reservoirs was increased to 200 ppb. The copepods were exposed to 200 ppb for 48 h, with the food conditions as noted in Table 1. At the end of the exposure period, control and treated copepods were removed from the bioassay system, rinsed with filtered seawater, placed in small quartz tubes, and frozen until analysis. In this application of the technique, a small sample, consisting of 100–150 copepods (all samples > 10 mg dry weight), was heated to 800 °C (40C° min^{-1}) while in a stream of helium. The quantity of hydrocarbons (C1–C30) released was measured as a function of temperature. The first peak (P1) consists of volatile, absorbed hydrocarbons, whereas the second peak (P2) consists primarily of products of cracking due to pyrolysis. Glass capillary gas chromatography was used to examine the composition of the two peaks obtained through pyrolysis. The technique is described in detail in Whelan *et al.* (1980).

Feeding rates of treated and untreated female *Centropages hamatus* were measured immediately following the 48- to 60-h exposure. Replicate groups of four copepods were separately added to 500-ml bottles containing the same food mixture and concentration seen by them in the bioassay system. Bottles were placed on a slowly rotating wheel for 6 h, after which the copepods were removed and particle counts were made using the Coulter Counter. Final counts were compared to initial and final control values for calculation of ingestion rates (Frost, 1972).

Egg production rates were determined for individual female copepods from each oil treatment following the exposure period. At least eight copepods were removed from each treatment and placed in individual beakers containing uncontaminated seawater with the same food mixture and concentration used during the period of treatment. Each beaker was examined daily for freshly released eggs, and the copepod transferred to another beaker containing uncontaminated seawater and food. Cannibalism of the eggs was prevented by a 333-μm mesh screen through which all eggs fell, out of the reach of the feeding copepod. Egg production was monitored for at least 4 d for each copepod following exposure to the various oil treatments in the bioassay system. Eggs were collected each day and monitored for viability.

Results

Composition of oil–seawater dispersions

Figure 1 shows a gas chromatogram (glass capillary) of the South Louisiana crude oil used in these experiments. Comparison of this chromatogram with one extracted from the crude oil–seawater dispersion used in the bioassay system (Fig. 2) indicates that the experimental dilutions contained the whole oil fraction, not just the water soluble fraction, with some enhancement of the > C15 hydrocarbons. The chromatograms show "total" hydrocarbons, including aromatics, as our analytical techniques detected the concentrations in both the dispersed droplets of crude oil and in the water soluble fraction. As discussed by Capuzzo and Lancaster (1981), a whole oil dispersion in seawater is more realistic than the water soluble fraction alone, as planktonic organisms are likely to ingest microdroplets of dispersed crude oil (Conover, 1971). The use of the continuous flow exposure system, with frequent changes of oil dispersion reservoirs and daily monitoring of hydrocarbon concentrations, assured us that the copepods were exposed to uniform conditions during the experiments.

Accumulation of petroleum hydrocarbons

There is no evidence for bioaccumulation of hydrocarbons by *Centropages hamatus* during exposures up to 200 ppb South Louisiana crude oil, based on analysis of copepod tissue through thermal distillation pyrolysis and subsequent gas chromatography. [As mentioned above, 200-ppb exposures were carried out to verify that the lack of bioaccumulation signal obtained at 80 ppb was not an analytical problem. Only the experiments destined for thermal distillation pyrolysis were run at 200 ppb (Table 1)]. Figures 3a and 3b show typical gas chromatograms obtained from the P1 peak (absorbed total hydrocarbons). Both the treated and untreated copepods have essentially identical patterns of hydrocarbon peaks, with no evidence of the characteristic n-alkane peaks found in the crude oil chromatograms (Figs. 1 and 2). The chromatograms from the P2 peak also showed no difference between control and treated copepods (not shown). The

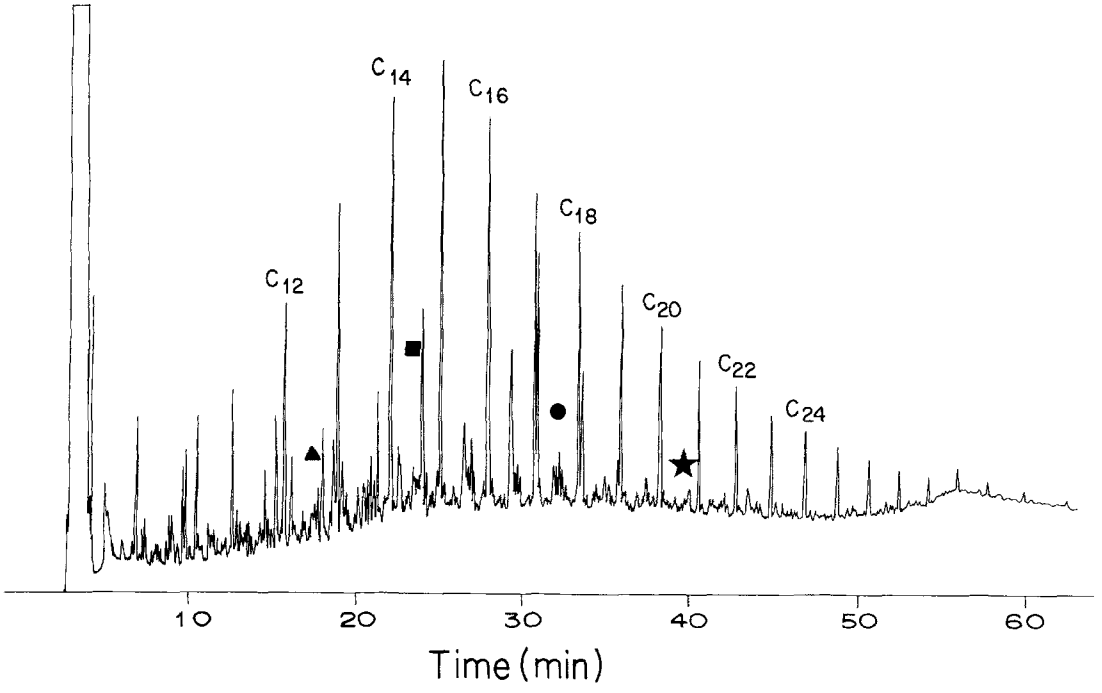

Fig. 1. Gas chromatogram of South Louisiana crude oil. GC conditions: HP 5840A FID; glass capillary column with SE-54, 20 m × 0.32 mm ID. Temperature program: 40°–280 °C at 4 C° min^{-1}. Alkane peaks determined from reference standards. Reference aromatic compounds indicated by the following symbols: (▲) naphthalene, (■) 2,6 dimethylnaphthalene, (●) phenanthrene, (★) pyrene

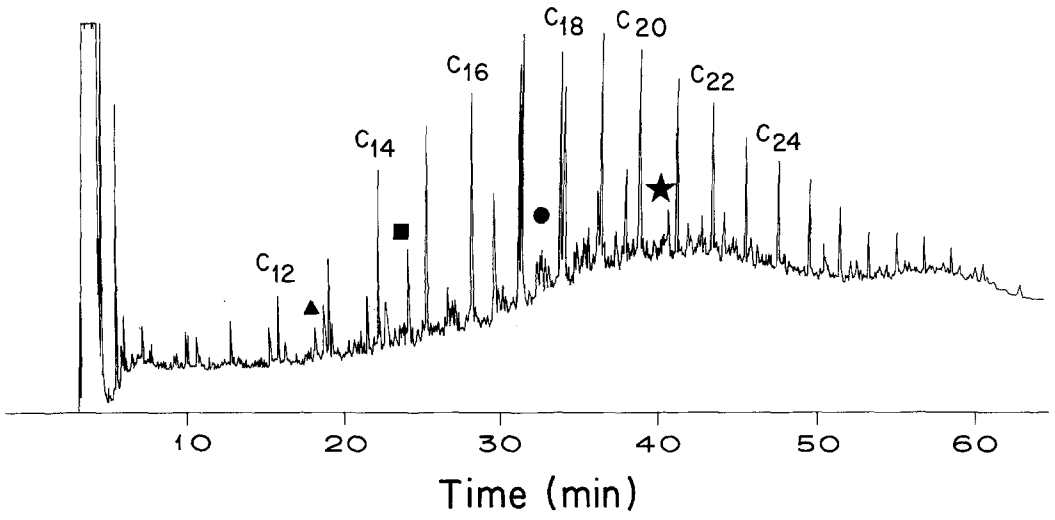

Fig. 2. Gas chromatogram of extract of mixture of South Louisiana crude oil and filtered sea water (see text for details). GC conditions as in Fig. 1. Symbols as in Fig. 1

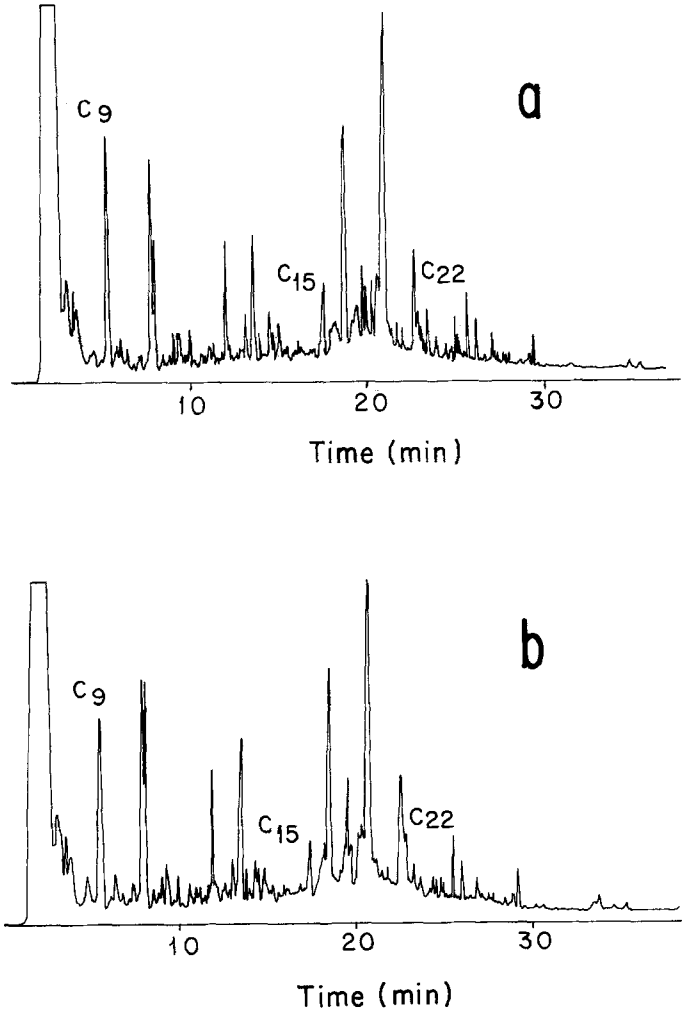

Fig. 3. *Centropages hamatus.* Gas chromatogram of P1 peak from thermal distillation pyrolysis of (a) control, and (b) oil-exposed *C. hamatus.* GC conditions: Quadrex 007 methylphenyl (5%) silicone fused silica capillary column; 25 m × 0.25 mm ID. Temperature program: 60°–270 °C at 10 C° min^{-1} after initial hold of 5 min at 60 °C

total quantity of hydrocarbons in the copepod tissue, based on integrations of the capillary GC data, are given in Table 2, and reveal no significant differences between oil-exposed and control treatments.

Rates of ingestion

Centropages hamatus showed a marked decline in ingestion rate in response to exposure to crude oil concentrations of 10 ppb and higher. The results from a typical experiment (Fig. 4) illustrate the feeding response of *C. hamatus* across the size distribution of available food. The mean hourly ingestion rate of females exposed to 80 ppb was less than 50% of that for unexposed females (Fig. 5), while exposure to 20 ppb resulted in a 20% decline in ingestion rate from control levels. The field estimates of ingestion rates of *C. typicus* found by Dagg and Grill (1980) indicate that maximal ingestion rates probably occur at food levels greater than 3-ppm particulate matter, levels equivalent to food levels used in this study. Labora-

Table 2. *Centropages hamatus.* Hydrocarbons in copepods exposed to South Louisiana crude oil. Details of analysis given in legend for Fig. 3. Values reported as means ± 1 SD (Units = ng hydrocarbon mg^{-1} wet weight)

	Total		nC7–nC26 (alkanes)	
	P1	P2	P1	P2
Exposed (200 ppb) $n=4$	3 167 ± 620	2 676 ± 1 500	963 ± 102	989 ± 653
Control (0 ppb) $n=3$	2 803 ± 838	2 907 ± 981	1 181 ± 372	954 ± 156

tory experiments indicate that *C. hamatus* and *C. typicus* have similar functional responses (Cowles, unpublished data), although the absolute level of ingestion rate saturation is frequently a function of prior feeding history (Mayzaud and Poulet, 1978). The dotted line in Fig. 5 represents an estimate of the maximal ingestion rates of freshly captured *C. hamatus* which had not been acclimated to a laboratory food source, based on our laboratory data (Cowles, unpublished data) and the data for *C. typicus* from Dagg and Grill (1980). All our ingestion rate measurements lie above the dashed line in Fig. 5, indicating that the copepods in the bioassay chambers were acclimated to the food source and were not likely to be food limited.

Rates of egg production

Freshly caught females of *Centropages hamatus* typically had egg production rates of 20–40 eggs per female per day over the first four days following capture, under the saturating food conditions used in our experiments (Table 1). *C. hamatus* females maintained in the control treatments of the bioassay system had rates equivalent to this level. Exposure to 10 to 80-ppb crude oil did not significantly decrease the rate of egg production in *C. hamatus* (Fig. 6a). In 40% of the experiments, copepods exposed to 80-ppb crude oil had higher egg production rates than the controls. In 20% of the experiments, copepods exposed to 80-ppb crude oil had lower rates than the controls, but in none of the experiments did a statistically significant difference in egg production rate emerge as a function of exposure to these concentrations of crude oil.

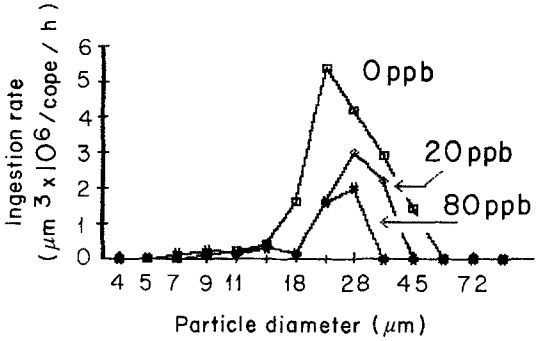

Fig. 4. *Centropages hamatus.* Ingestion rate of control and oil-exposed females across the size distribution of available food (from Expt 6, November 1981)

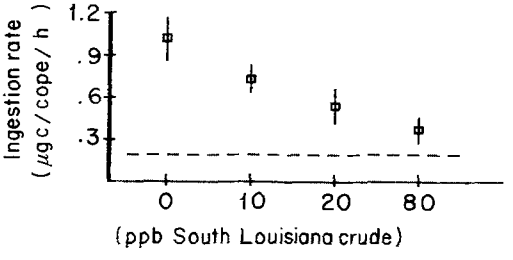

Fig. 5. Summary of ingestion rate response to oil exposure (means of all experiments ± SD). (0 ppb, $n=18$; 10 ppb, $n=12$; 20 ppb, $n=12$; 80 ppb, $n=18$). Dashed line represents level of ingestion below which egg production rates could decline (see text for details)

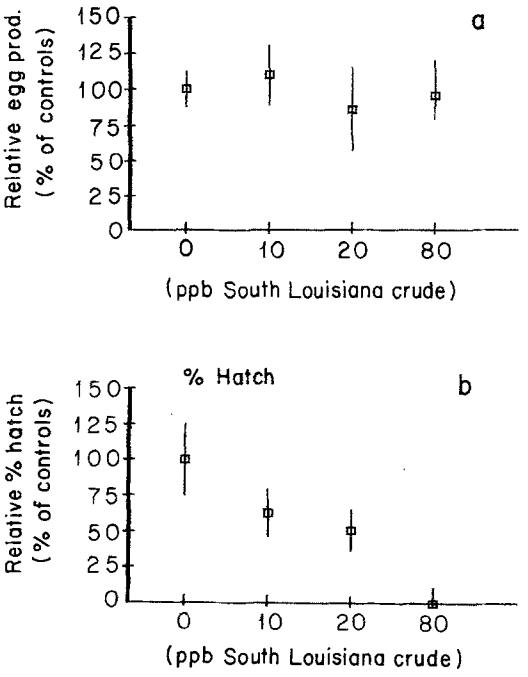

Fig. 6. (a) Response of egg production rate to oil exposure relative to controls (means of all experiments ± SD; 0 ppb, $n=48$; 10 ppb, $n=39$; 20 ppb, $n=36$; 80 ppb, $n=48$). (b) Percentage hatch of eggs laid under oil exposure relative to controls

Viability of eggs produced by oil exposed copepods

Exposure to concentrations of crude oil (> 10 ppb) dispersed in seawater had deleterious effects on the hatching success of eggs laid over a period of four days by *Centropages hamatus* after 48- to 64-h of exposure. Figure 6b shows the mean hatching success of eggs laid following exposure, in terms of % hatching relative to controls. Control copepods had hatching success greater than 85% in all experiments. No nauplii survived beyond stage 3 from eggs laid by females exposed to concentrations of dispersed crude oil > 10 ppb.

Discussion

The basic hypothesis for this work, at the outset, was that sublethal concentrations of crude oil–seawater mixtures would have deleterious effects on most, if not all, physiological and behavioral processes in marine copepods. While the data reported here support the initial hypothesis for ingestion rates, some unexpected results have been obtained with the egg production experiments for oil-exposed copepods. We interpret our results as being consistent with the findings of Lu *et al.* (1977) that some aquatic organisms may metabolize sublethal concentra-

tions of petroleum hydrocarbons so that no accumulation of untransfomed material can be found.

Egg production in copepods is known to vary in response to levels of available food (Marshall and Orr, 1952; Heinle, 1966; Mullin and Brooks, 1967; Paffenhöfer, 1970; Gaudy, 1971; Dagg, 1977; Corkett and McLaren, 1978; Checkley, 1980), with decreased rates of egg production at low ingestion rates or with intermittent exposure to food. The sharp decline in daily ingestion of exposed *Centropages hamatus* would suggest that egg production rates should show a similar decline. Since, however, total ingestion, even at 80 ppb (Fig. 5), was above that typically found for freshly caught copepods exposed to "saturating" food levels, it can be concluded that food limitation did not occur in any treatment. The data of Checkley (1980) for the copepod *Paracalanus parvus* further support that conclusion. Checkley found that egg production in that species reached a plateau at ingestion rates greater than 0.2-μg C copepod^{-1} h^{-1} (calculated from Fig. 10 in Checkley (1980) and using a C:N ratio of 7). Under the food conditions of the oil-exposure experiments reported here, the ingestion rates of *C. hamatus* were always above 0.3-μg C copepod^{-1} h^{-1}, even after exposure to 80 ppb of crude oil. The dashed line in Fig. 5 represents the level of ingestion below which food limitation might cause a decline in egg production rates. Therefore, it is not surprising that *C. hamatus* egg production rates were not affected by the decline in total ingestion we observed in the oil-treated copepods.

Unlike the results of Berdugo *et al.* (1977) and Ott *et al.* (1978) with the copepod *Eurytemora affinis,* our results with *Centropages hamatus* suggest that egg production per se is not necessarily inhibited by exposure to sublethal concentrations of crude oil less than 100 ppb. Egg viability is, however, strongly affected by such exposures. Berdugo *et al.* (1977) used short-term (less than 4 h) exposures of *E. affinis* to concentrations greater than 1 ppm of the water soluble fraction of aromatic heating oil, as well as to 1 ppm of naphthalene. Short-term exposures often resulted in depression of egg production over the remainder of the life of the copepod. In the same paper, Berdugo *et al.* (1977) report that 10-d exposures to 10- and 50-ppb naphthalene did not inhibit egg production, but they did not report any data for survival of nauplii. The data of Ott *et al.* (1978) indicate that up to 29 d of exposure of *E. affinis* to 10-ppb naphthalene led to decreased rates of egg production, brood size, and number of nauplii produced. The authors point out that an inhibition of feeding could have led to the decreased rates of egg production. Both of the above studies used static exposure conditions, making comparison with the present study difficult. In addition, the data of Anderson *et al.* (1974) indicate that naphthalene is only 0.5% of the total hydrocarbons in South Louisiana crude oil. Copepods would thus only encounter 10-ppb naphthalene in the sea if the concentration of dispersed South Louisiana crude oil was 2 ppm, levels probably in excess of sublethal concentrations.

There is evidence that feeding rates can recover from the effects of hydrocarbon exposure if the animals are returned to control conditions (Spooner and Corkett, 1979). In addition, Cowles (1983) showed that behavioral activity (related to feeding) in *Centropages hamatus* quickly returned to normal after transfer to uncontaminated conditions. The experiments of Dagg (1977) indicated that starved female *C. typicus* increased egg production within a few hours of transfer to abundant food. These results would suggest that ingested material is mobilized into oocyte lipid within a few hours of ingestion in this genus. Smith and Hall (1980) found that radiolabeled food was incorporated into oocytes within the ovary of the copepod *Temora longicornis* within 24 h of ingestion and that radiolabel assimilated by *C. typicus* and *C. hamatus* was retained at a constant level for at least seven days. Our observation of severely depressed egg viability in *C. hamatus,* even after 4–6 d of depuration, is consistent with the findings of Smith and Hall (1980). The lack of accumulation of petroleum hydrocarbons in *C. hamatus* (Table 2), coupled with the loss of egg viability, suggests (1) that toxic components of the hydrocarbon dispersion or their metabolic degradation products were incorporated into oocyte tissue, or (2) that biosynthetic pathways involved in oogenesis were affected by exposure to petroleum hydrocarbons. This latter suggestion is supported by the recent results of Capuzzo and Lancaster (1981), who found that lipid biosynthesis in larval lobsters was modified as a consequence of exposure to sublethal concentrations of crude oil. Crustaceans are known to have mixed function oxidase systems capable of hydrocarbon transformations (Lee, 1975; Lu *et al.,* 1977; Sanborn and Malins, 1977, 1980; Stegeman, 1981). Calanoid copepods metabolize petroleum hydrocarbons (Corner *et al.,* 1976) and there is evidence that the activity of the enzyme involved in hydrocarbon metabolism, aryl hydrocarbon hydroxylase (AHH), can be increased in *Calanus helgolandicus* by exposure to sublethal concentrations of aromatic hydrocarbons (Walters *et al.,* 1979). It is likely that the products of metabolic transformation of the petroleum hydrocarbons influence the physiological processes we have observed in this study, but we have no direct evidence that *C. hamatus* was affected in this way.

The lifetime of many adult copepods is sufficiently short (< 2 wk) that we conclude that a 4- to 6-d depuration period following exposure to crude oil will not permit a female copepod to return to normal levels of reproductive effort. Our data indicate that populations of marine copepods, as represented by *Centropages hamatus,* are likely to have severely reduced recruitment rates if exposed to sublethal concentrations of physically dispersed crude oil.

Acknowledgements. This work was supported by Contract no. NA80RAD00037 from the National Oceanic and Atmospheric Administration. We thank Drs. J. Farrington and J. Capuzzo for advice and assistance in the early stages of this research. We thank Dr. J. Whelan and M.

Tarafa for sample analysis using thermal distillation pyrolysis techniques and for the gas chromatograms which were obtained from those analyses. N. Copley provided valuable technical assistance with experiments and data analysis. We thank Drs. J. Stegeman, J. Capuzzo, and J. Whelan for constructive comments which have improved the manuscript.

Literature cited

Andersen, J. W., J. M. Neff, B. A. Cox, H. E. Tatum and G. M. Hightower: Characteristics of dispersions and water soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. *27,* 75–88 (1974)

Berdugo, V., R. P. Harris and S. C. M. O'Hara: The effect of petroleum hydrocarbons on reproduction of an estuarine planktonic copepod in laboratory cultures. Mar. Pollut. Bull. *8,* 138–143 (1977)

Berman, M. S. and D. R. Heinle: Modification of the feeding behavior of marine copepods by sub-lethal concentrations of water-accommodated fuel oil. Mar. Biol. *56,* 59–64 (1980)

Boylan, D. B. and B. W. Tripp: Determination of hydrocarbons in seawater extracts of crude oil and crude oil fractions. Nature, Lond. *230,* 44–47 (1971)

Capuzzo, J. M. and B. A. Lancaster: Physiological effects of South Louisiana crude oil on larvae of the American lobster (*Homarus americanus*). *In:* Biological monitoring of marine pollutants, pp 405–423. Ed. by F. J. Vernberg, A. Calabrese, F. P. Thurberg and W. B. Vernberg. New York: Academic Press 1981

Capuzzo, J. M., S. A. Lawrence and J. A. Davidson: Combined toxicity of free chlorine, chloramine and temperature to Stage I larvae of the American lobster *Homarus americanus.* Water Res. *10,* 1093–1099 (1976)

Checkley, D. M.: The egg production of a marine planktonic copepod in relation to its food supply: laboratory studies. Limnol. Oceanogr. *25,* 430–446 (1980)

Conover, R. J.: Some relations between zooplankton and Bunker C oil in Chedabucto Bay following the wreck of the tanker ARROW. J. Fish. Res. Bd Can. *28,* 1327–1330 (1971)

Corkett, C. J. and I. A. McLaren: The biology of *Pseudocalanus.* Adv. mar. Biol. *15,* 1–231 (1978)

Corner, E. D. S., R. P. Harris, C. C. Kilvington and S. C. M. O'Hara: Petroleum compounds in the marine food web: short-term experiments on the fate of naphthalene in *Calanus.* J. mar. biol. Ass. U.K. *56,* 121–133 (1976)

Cowles, T. J.: Effects of exposure to sublethal concentrations of crude oil on the copepod *Centropages harmatus* II. Activity patterns. Mar. Biol. *78,* 53–57 (1983)

Dagg, M.: Some effects of patchy food environments on copepods. Limnol. Oceanogr. *22,* 99–107 (1977)

Dagg, M. and D. W. Grill: Natural feeding rates of *Centropages typicus* females in the New York Bight. Limnol. Oceanogr. *25,* 597–609 (1980)

Frost, B. W.: Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus.* Limnol. Oceanogr. *17,* 805–815 (1972)

Gaudy, R.: Etude experimentale de la ponte chez trois especies de copepodes pelagiques *Centropages typicus, Acartia clausi* and *Temora stylifera.* Mar. Biol. *9,* 65–70 (1971)

Guillard, R. R. and J. H. Ryther: Studies on marine planktonic diatoms. 1. *Cyclotella nana* Hudstedt and *Detonula confervacae* (Cleve) Gran. Can. J. Microbiol. *8,* 229–239 (1962)

Hebert, R. and S. A. Poulet: Effect of modification of particle size of emulsions of Venezuelan crude oil on feeding, survival and growth of marine zooplankton. Mar. environ. Res. *4,* 121–134 (1980)

Heinle, D. R.: Production of a calanoid copepod, *Acartia tonsa,* in the Patuxent River Estuary. Chesapeake Sci. *7,* 59–74 (1966)

Lee, R. F.: Fate of petroleum hydrocarbons in marine zooplankton. *In:* Proc. 1975 Conf. Prevention and Control of Oil Pollution held at San Francisco, California, March 25–27, pp 549–553. American Petroleum Institute, Washington, D. C. 1975

Lu, P.-Y., R. L. Metcalf, N. Plummer and D. Mandel: The environmental fate of three carcinogens: benzo[a]pyrene, benzidine, and vinyl chloridae evaluated in laboratory model ecosystems. Arch. environ. Contam. Toxicol. *6,* 129–142 (1977)

Marshall, S. M. and A. P. Orr: On the biology of *Calanus finmarchicus.* VII. Factors affecting egg production. J. mar. biol. Ass. U.K. *30,* 527–547 (1952)

Mayzaud, P. and S. A. Poulet: The importance of the time factor in the response of zooplankton to varying concentrations of naturally occurring particulate matter. Limnol. Oceanogr. *23,* 1144–1154 (1978)

Mironov, O. G.: The effect of oil pollution upon some representatives of the Black Sea zooplankton. Zool. Zh. *48,* 980–984 (1969)

Mullin, M. M. and E. Brooks: Laboratory culture growth rate and feeding behavior of a planktonic marine copepod. Limnol. Oceanogr. *12,* 657–666 (1967)

Ott, F. S., R. P. Harris and S. C. M. O'Hara: Acute and sublethal toxicity of naphthalene and three methylated derivatives to the estuarine copepod *Eurytemora affinis.* Mar. environ. Res. *1,* 49–59 (1978)

Paffenhöfer, G.-A.: Cultivation of *Calanus helgolandicus* under controlled conditions. Helgol. wiss. Meeresunters. *10,* 346–359 (1970)

Polak, P., A. Filion, S. Fortier, J. Lanier and K. Cooper: Observations on ARGO MERCHANT oil in zooplankton of Nantucket Shoals. *In*: In the wake of the ARGO MERCHANT. Proceedings of a Symposium held 11–13 January 1978, University of Rhode Island. COMS 1978

Sanborn, H. R. and D. C. Malins: Toxicity and metabolism of naphthalene: a study with marine larval invertebrates. Proc. Soc. exp. Biol. Med. *154,* 151–155 (1977)

Sanborn, H. R. and D. C. Malins: The deposition of aromatic hydrocarbons on adult spot shrimp (*Fundulus platyceros*) and the formation of metabolites of naphthalene in adult and larval spot shrimp. Xenobiotica *10,* 193–200 (1980)

Smith, S. L. and B. K. Hall: Transfer of radioactive carbon within the copepod *Temora longicornis.* Mar. Biol. *55,* 277–286 (1980)

Spooner, M. F. and C. J. Corkett: Effects of Kuwait oils on feeding rates of copepods. Mar. Pollut. Bull. *10,* 197–202 (1979)

Stegeman, J. J.: Polynuclear aromatic hydrocarbons and their metabolism in the marine environment. *In*: Polycyclic hydrocarbons and cancer, Vol. 3, Ed. by Gelboin and Ts'o. New York: Academic Press 1981

Walters, J. M., R. B. Cain, I. J. Higgins and E. D. S. Corner: Cell-free benzo[a]pyrene hydroxylase activity in marine zooplankton. J. mar. biol. Ass. U.K. *59,* 553–563 (1979)

Whelan, J. K., J. M. Hunt and A. Y. Huc: Applications of thermal distillation-pyrolysis to petroleum source rock studies and marine pollution. J. anal. appl. Pyroly. *1,* 79–96 (1980)

Date of final manuscript acceptance: October 12, 1983.
Communicated by S. K. Pierce, College Park