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EFFECTS OF SUB-LETHAL OIL LEVELS ON THE REPRODUCTION OF A COPEPOD, NITOCRA AFFINIS

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ABSTRACT

Zooplankton are a major component of the diets of almost all species of fishes during some part of their life cycles. Any factor which reduces the numbers of zooplankton can indirectly affect fishes by reducing an important part of their food supply. In the ocean and estuaries the major component of the zooplankton is copepods. The findings of this study are that the soluble fraction from 200 ul crude oil per liter sea water significantly reduced egg production by the harpacticoid copepod, Nitocra affinis (Gurney) as did one-half and one-fourth dilutions of the oiled water with filtered sea water. The mean brood size of controls was 14.41 eggs per female while those of the undiluted, one-half, and one-fourth dilutions were 8.37, 8.44, and 8.35, respectively. There were no statistical differences among the mean lengths of life nor among the mean number of broods produced for any of the groups.

INTRODUCTION

It has been estimated that 10^9 metric tons of oil per year are spilled in the oceans (Zitko and Carson, 1970). Although much work has been done on oil spills (Smith, 1968; Nelson-Smith, 1970; Thomas, 1971) and on the toxicity of oils to organisms (LaRoche et al., 1970; Anderson et al., 1974; Moore and Dwyer, 1974), sub-lethal effects are just beginning to attract attention. Hargrave and Newcomb (1973) studied effects of crude oil on crawling rate and respiration of the periwinkle, Littorina littorea. Eisler (1973) looked at the predation rate of the gastropod, Drupo granulata, with both predator and prey contaminated with high, but sub-lethal oil concentrations. Atema and Stein (1974) observed changes in the feeding behavior of the lobster, Homarus americanus, after exposure to low levels of crude oil. Spooner and Corkett (1974) measured a decrease in fecal pellet production by copepods exposed to an oil droplet concentration of 10 ppm for 20 hours.

Spooner and Corkett's (1974) study is one of the first carried out on low level oil effects on zooplankton. However, they did not consider long term effects. Many investigators feel that long term effects may be more damaging to organisms than the acute toxicity of large amounts of oil (Blumer, 1969; Tarzwell, 1971). Sub-lethal effects are usually subtle and can result in physiological or behavioral changes, decreased growth, lessened motility, or reduced reproduction (Sprague, 1971; Morgan et al., 1973). This paper examines the long term effect of low oil levels on the reproduction of a copepod.

MATERIALS AND METHODS

I prepared the oiled water according to the procedure of Boylan and Tripp (1971). First a known volume of oil was added to each liter of filtered water (Whatman GF/C) from Bogue Sound, N.C. (approximate salinity 26 °/oo). Next, the mixture was stirred with a Teflon stirring bar and magnetic stirrer at a rate such that the funnel of oil produced reached no farther than one-fourth the distance to the bottom of the container. The stirring lasted 20 to 24 hours for optimal extraction of soluble materials with minimal bacterial contamination (Anderson et al., 1974). After the mixture stood for six hours to allow for separation of the oil and water, the water was drained into a second container and stored at 4C in a refrigerator. The container was shaken before 5, 10, and 20 ml samples were taken and allowed to equilibrate to room temperature before animals were put into them. Room temperature averaged 25.1C with a range of 20.9 - 29.0C. The final volume for all experiments was 20 ml.

In most experiments, the oiled water was used undiluted (FO), diluted by one-half (HO), and by one-fourth (QO) with filtered water. Offshore Louisiana crude oil, with a paraffin base, was used in all tests. Soluble hydrocarbons were extracted twice in hexane and the samples sent for analysis by gas chromatography according to the methods of Miller et al.,(1977).

Harpacticoid copepods were isolated from surface plankton samples in spring 1975. Longipedia helgolandica (Klie) was taken from the Newport River estuary near Beaufort, N.C., and Nitocra affinis

(Gurney), from the Cape Fear River estuary near Southport, N.C. Both species were cultured in two-liter flasks with filtered sound water and fed a mixture of Chlorella and Chlamydomonas spp. (Heinle, 1969).

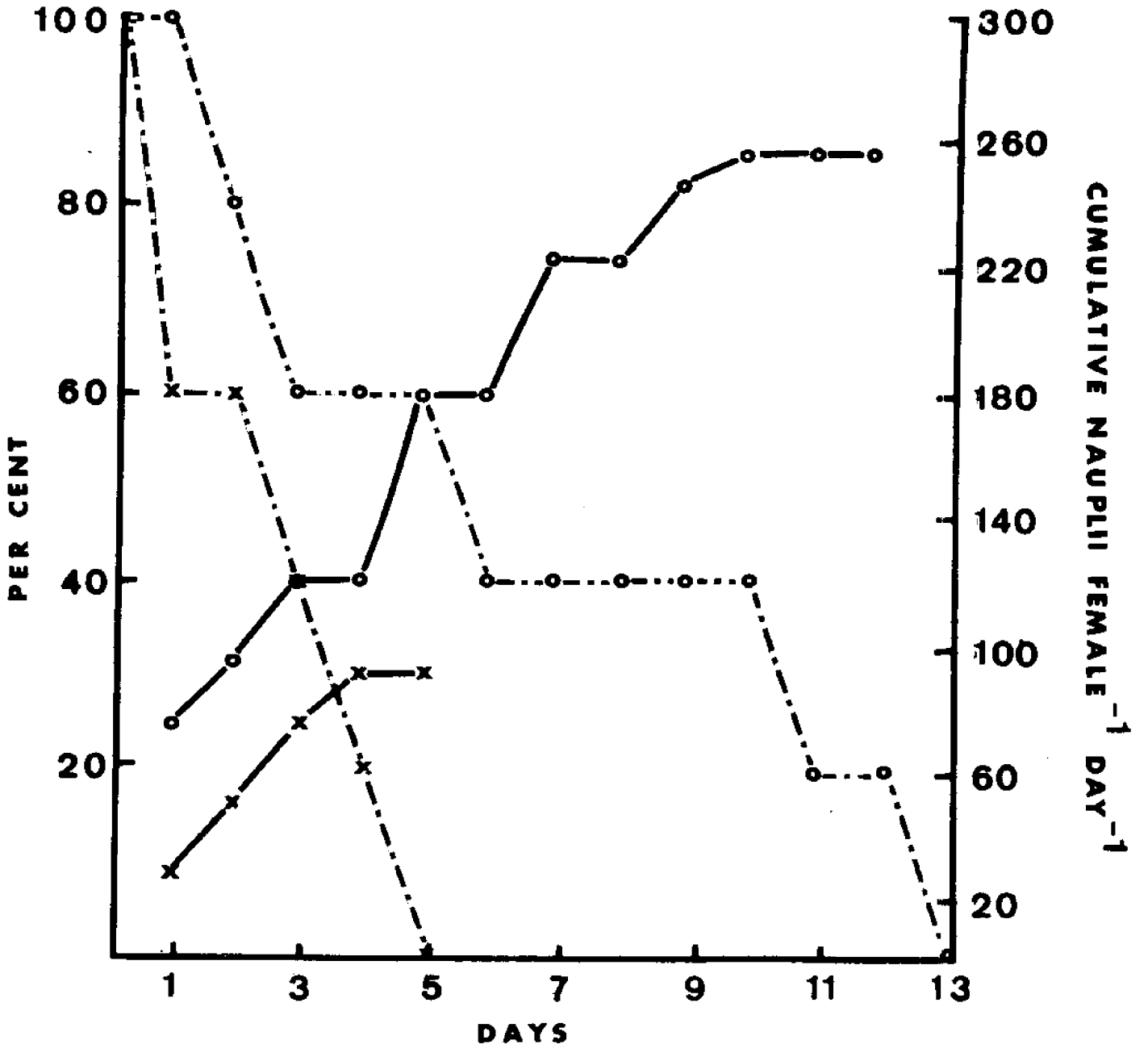
Sibling groups of copepods were grown in 125 ml flasks in 50 ml filtered water and fed a ration of Chlamydomonas sp. When females in these groups produced their first egg sacs (at 11 - 14 days) they were randomly assigned to treatments. All copepods were transferred into fresh medium and fed daily. All offspring and unhatched eggs produced were removed daily and preserved in 10% formaldehyde for counting. The food ration was adjusted so that the number of algal cells was between 7.0 and 8.0 x 10^5 cells ml^{-1} ($\bar{X}$ = 7.47 $\pm$ 1.27 x 10^5) in the experimental flasks. The algal numbers were obtained by a least squares regression of absorption at 440 nm on cell counts, determined by a hemocytometer. In preliminary experiments, adult animals were taken directly from stock cultures and were not raised from individual broods.

The results were analyzed by a one-way analysis of variance. If the calculated F was significant at $p = 0.05$, the treatment means were compared to the control using the least significant difference (LSD) test (Steele and Torrie, 1960).

RESULTS

A preliminary experiment indicated that the soluble fraction from 25 ml crude oil per liter of water would kill adult female L. helgolandica within 24h as would the one-half and one-fourth dilutions. The controls lived for one week and produced nine broods during that time.

Figure 1. Cumulative number of nauplii (solid line) and percent surviving female L. helgolandica (broken line) in oiled water (x's) and controls (open circles).



In a second preliminary test, I found that 100 ul oil liter^{-1} produced significant sub-lethal effects. Each treatment consisted of five gravid female L. helgolandica. The oiled group all died within five days while the controls died in thirteen (Fig.1). The cumulative number of nauplii produced was greater in the control than in the oiled group (Fig.1). The mean size of the initial broods was similar for both groups (Table 1) indicating similar egg production among females prior to the test. However, mean production per female of subsequent broods is significantly different; and while the other comparisons in Table 1 are not statistically significant, there is a trend indicating an adverse effect of the oil.

To examine this trend in more detail, I used the soluble fraction from water mixed with 200 ul oil liter^{-1} as the undiluted treatment. Other treatments were one-half and one-quater dilutions of the oiled water, and algal control, and a starved control. Ten adult female Nitocra affinis from 12 separate broods were randomly assigned to each treatment. Only N. affinis were used in this experiment since stock cultures of L. helgolandica suddenly decreased in numbers. The results are summerized in Table 2. There were no differences in sizes of the first broods suggesting similar developments for the individuals used in each treatment. Also, except for the starved group, there were no differences among the mean lengths of life of the copepods. There is a trend, however, towards a longer life with a greater dilution of the dissolved hydrocarbons. Nonetheless, this comparison indicates that any decrease in offspring production is not due to a significant

	Control	Oiled	F (df)
Mean length of life (days)	7.0	2.8	3.30 (1,8)
Mean size of first brood	23.75	22.75	0.07 (1,6)
Mean number of broods per female	2.4	0.8	2.84 (1,8)
Mean production per female (after first brood)	32.4	0.0	3.53 (1,8)**

Table 1. Length of life and brood production of L. helgolandicus in filtered seawater and in water with soluble fraction of 100 ul liter^{-1} oil. (** significant at p = 0.01)

shortening of the reproductive lives of the animals. This is supported by the mean number of broods produced; again, there are no statistical differences among the groups. However, the animals exposed to the oiled water produce significantly fewer nauplii than the controls. The average brood size and, consequently, the mean daily production and the mean total production are all statistically lower in the treated groups than in the algal control.

The dissolved substances do not appear to inhibit the copepods' filtering rates as estimated by fecal pellet counts (Table 2). These counts were done only for the first week of the experiment and were discontinued because of the time involved. Thus any later effects of the oil on feeding were not observed. However, the production of the animals during that period already shows a significant decrease for the treated organisms (Table 2). There also is no statistitical evidence for a decrease in life span for any of the animals, supporting the conclusion from fecal pellet production that oil did not affect the animals' feeding abilities.

The gas chromatographs of the crude oil and the oiled water extract are different (Figs.2,3). There are few low boiling compounds in the water extract while the crude oil has a large number of them. They were probably lost in the oiled water through volatization and microbial degredation (Dean, 1968). Atema and Stein (1974) observed a similar decrease in the low boiling fraction in an oil-sea water mixture over a 10-day period.

	AC	QO	HO	FO	S	F (df)	LSD
Mean length of life (days)	27.8 (9.5)	20.9 (8.8)	20.7 (9.3)	18.4 (11.6)	11.5 (4.1)"	3.79 (4,45)**	11.45
Mean size of first brood	17.7 (3.4)	15.5 (4.7)	12.4 (5.4)	16.3 (8.5)	17.4 (6.8)	1.00 (4,45)	____
Mean size of subsequent broods	14.41 (3.47)	8.35 (5.15)"	8.44 (3.94)"	8.37 (5.64)"	____	4.82 (3,36)**	5.28
Mean number of broods	5.6 (1.85)	4.1 (2.59)	3.9 (2.02)	3.4 (2.11)	____	1.73 (3,36)	____
Mean daily production (after first brood) nauplii/female/day	2.50 (1.05)	1.32 (1.22)"	1.29 (0.80)"	1.18 (0.91)"	____	4.41 (3,36)**	0.177
Mean total production (after first brood)	70.5 (34.4)	39.5 (37.4)'	30.5 (23.4)"	26.0 (23.4)"	____	4.02 (3,36)**	38.91
Mean production first seven days (less first brood)	22.3 (6.6)	6.3 (7.3)"	9.6 (10.9)"	7.3 (10.4)"	____	83.93 (3.36)**	5.09
Mean fecal pellet count	17.82 (8.65)	11.98 (6.23)	17.39 (7.04)	14.17 (6.76)	2.83 (2.97)"	6.27 (4,45)**	9.26
Mean number unhatched eggs	9.1 (8.3)	6.1 (6.5)	7.6 (6.6)	6.9 (6.9)	1.5 (1.4)	1.76 (4,45)	____

Table 2. Length of life, brood production, and fecal pellet production of N. affinis in filtered sea water and with dilutions of soluble fraction of crude oil (200 ul l^{-1}). AC = algal control; QO = one-fourth dilution; HO = one-half dilution; FO = undiluted oiled water; S = starved control. Standard errors in parentheses. LSD calculated at p = 0.01. (** = F significant at 0.01; " = LSD comparison significant at 0.01; ' = LSD comparison significant at 0.05).

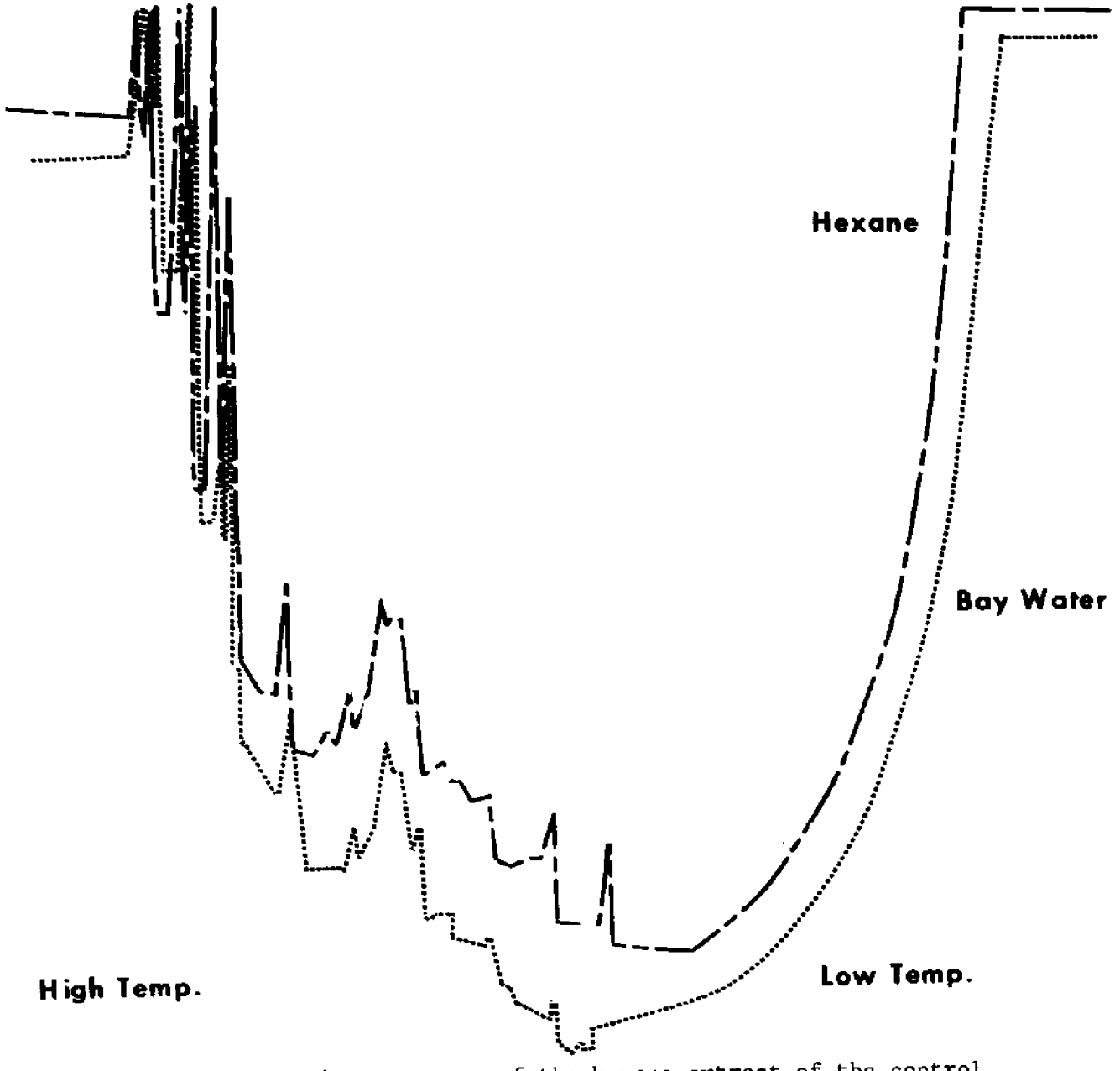


Figure 2. Gas chromatograms of the hexane extract of the control water and of the hexane. Lower weight compounds appear at the low temperature side and higher weight compounds at the high temperature side.

DISCUSSION

The soluble contaminants from the oil seem to affect egg production. All of the groups produced similar numbers of egg sacs and lived for a similar length of time, yet all of the treated groups produced fewer offspring. This result is similar to that of Linden (1976) who found that Gammarus oceanicus produced significantly fewer larvae than controls when exposed to oil concentrations of 0.3 - 0.4 ppm. In my study, the mean brood size of all the oiled groups differed by only 0.09, suggesting that there is a threshold level of contaminants affecting the animals and that all of the treated groups were above threshold.

The data indicate that the decrease in egg production was not the result of a decrease in N. affinis's filtering ability. Spooner and Corkett (1974) did get a reduction in fecal pellet production which they attributed to a reduced filtering rate. Heinle et al. (1974) have shown a reduction in brood size associated with a reduction in the amount of food available for the copepod Eurytemora affinis. However, they always observed a significant decrease in length of life associated with the diets that produced significant decreases in both brood size and total production. Because of the similarities in the first week's fecal pellet counts, in the life spans of the animals, and in the number of broods produced (Table 2), I do not believe that the reduced production was due to a decrease in food uptake.

Moore and Dwyer (1974) state that the toxic responses from oils are primarily caused by the aromatic hydrocarbons and that sub-lethal

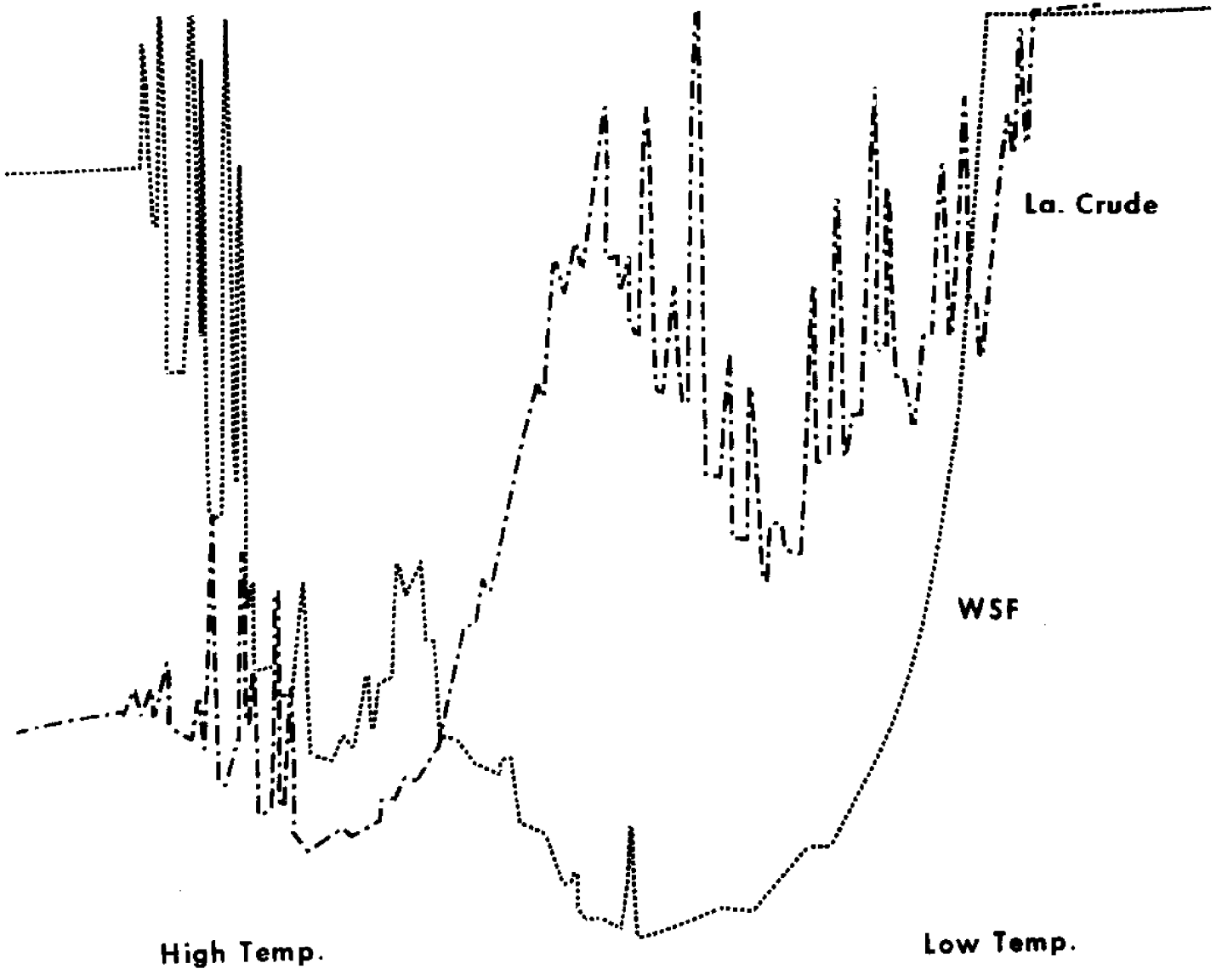


Figure 3. Gas chromatograms of Louisiana Crude oil dissolved in hexane and the hexane extract of the water soluble fraction (WSF) of Louisiana Crude oil.

effects may be caused by a "soluble aromatic derivative" concentration of 10 ppb. In this study, only those compounds larger than 17 carbons appear on the chromatograms (Figs. 2,3). There is a question whether some aromatics are appearing as contaminants in the chromatograms since the peaks appear in a different time sequence than the mixed alkane standard used (M. Miller, pers. comm.). Nonetheless, something was transferred to the water and affected the copepods.

The question of how these compounds act has yet to be answered. Do they act directly by interfering in some step(s) of egg production? Or do they act indirectly by increasing maintenance energy costs and, consequently, removing energy from egg production? It is clear that they do not affect egg hatching success (Table 2). Another question concerning these effects is how do they affect the larval stages: do they delay growth and development; at what stage are the animals most sensitive; are the concentrations used here lethal or sub-lethal for nauplii or copepodids? If concentrations of these magnitudes reduce larval survival then the population faces a double threat: a lowered production rate by adults and an increased death rate of larvae. These could seriously reduce the animal's chances of survival in its environment (Sprague, 1971).

Chronic, low levels of oil in shallow estuaries may have an impact on higher trophic levels by decreasing the zooplankton population. A small standing crop of zooplankton is typical of shallow estuaries (Williams et al., 1968). Nonetheless, Thayer et al., (1974) feel that the zooplankton control the survival of fishes during the

transition from larval to juvenile stages. In support of this, Kjelson et al., (1975) have shown that 76 - 99% of the gut contents of larval pinfish, spot, and menhaden in the Newport River estuary were copepods. If low levels of oil affect other copepods by reducing egg production, then they pose a threat to fish populations also. There is some evidence that this threat does not have to come from a direct oil source in the estuary itself. Levy and Waton (1973) have found the Atlantic waters outside the Gulf of St. Lawrence to be the dominant source of petroleum residues to the Gulf. Since estuaries can act as pollution traps as well as nutrient traps (Odum, 1970), low levels of off-shore petroleum may become concentrated in the estuaries and affect zooplankton production which can, in turn, affect other organisms.

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