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The Effects of Petroleum Hydrocarbons on Lipid Metabolism and Energetics of Larval Development and Metamorphosis in the American Lobster (*Homarus americanus* Milne Edwards)

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ABSTRACT

In studies with larval stages of the American lobster (Homarus americanus) *exposed to a sublethal concentration of South Louisiana crude oil, the normal patterns of lipid storage, utilization and synthesis during larval development and metamorphosis were altered with oil exposure. Increased rates of protein catabolism, delayed molting and reduced growth were also evident among oil-exposed lobster larvae and suggest either deficiency or immobilization of lipid reserves. From analyses of lipid class composition of control and oil-exposed lobster larvae, it is evident that oil-exposed larvae have consistently lower levels of triacylglycerols (the major energy store) and higher levels of sterols than control larvae. Decreased ratios of non-essential fatty acids (both saturated and unsaturated) and increased ratios of essential fatty acids were detected in the neutral lipid pools of oil-exposed larvae that suggest both decreased storage of fatty acids in energy reserves and decreased mobilization of essential fatty acids into phospholipid pools. The fatty acid distribution in the phospholipid pools of oil-exposed larvae, however, varied little from control values and reflects a tendency to preserve the integrity of these structural lipids. Increases in sterol levels may be related to decreased mobilization of cholesterol or other sterols and/or transformation of cholesterol into metabolic pathways including the biosynthesis of ecdysterone.*

Oil-exposed animals contained trace quantities of benzene, thiophene, toluene, alkylcyclohexane and alkylbenzenes, detected with GC–MS analyses. No naphthalene, dimethylnaphthalene, higher molecular weight aromatics or their phenolic derivatives were detected; their absence may

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be related to either the detection limits of the methodology used or extremely rapid metabolism and turnover of these components. Alterations in lipid metabolism may account for the developmental and energetic abnormalities observed in marine crustaceans. It cannot be ruled out, however, that decreased lipid utilization, synthesis and mobilization may be a defense mechanism against incorporating lipophilic components of petroleum hydrocarbons in metabolic pathways, and that disruption in energetics and development is a consequence of the reduction in energy available for growth and molting.

INTRODUCTION

The effects of crude oil and refined oils on the survival, metabolism and energetics of marine crustaceans have been the subject of many recent investigations (Wells & Sprague, 1976; Edwards, 1978; Laughlin *et al.*, 1978; Capuzzo & Lancaster, 1981, 1982). Disruption in energetics and development of larval crustaceans as a result of exposure to petroleum hydrocarbons has been reported by several investigators. Delayed development (Wells, 1972; Katz, 1973; Wells & Sprague, 1976; Caldwell *et al.*, 1977; Winters *et al.*, 1977; Laughlin *et al.*, 1978; Laughlin & Neff, 1979; Cucci & Epifanio, 1979), reduced growth (Tatem, 1977; Laughlin & Neff, 1980; Johns & Pechenik, 1980), inhibition of molting (Winters *et al.*, 1977; Mecklenburg *et al.*, 1977; Laughlin & Neff, 1979; Cucci & Epifanio, 1979), and the presence of abnormal intermediate larval stages (Wells & Sprague, 1976; Laughlin & Neff, 1979; Cucci & Epifanio, 1979) have been reported as developmental abnormalities associated with oil exposure.

The mechanisms responsible for developmental and energetic abnormalities are not well understood. It is evident that successful development and metamorphosis of the larval stages of marine crustaceans are dependent on the balance and efficient utilization of energy reserves (Holland, 1978), with lipid reserves being either of primary or secondary importance in the energetics of crustacean development. Several recent studies (Anger & Dawirs, 1981; Anger *et al.*, 1981) have demonstrated the critical importance of accumulation and efficient utilization of energy reserves in survival and development of planktotrophic decapod larvae. Capuzzo & Lancaster (1981, 1982) have recently demonstrated the impairment of lipid utilization, reduced respiratory rates and delayed molting in larval lobsters with oil exposure and suggested that the disruptions in energetics were caused by alterations in lipid metabolism.

The effects of oil exposure on marine crustaceans are modified by the bioavailability of crude and refined oils, the ability of the organism to accumulate and metabolize various hydrocarbon components and the interference with normal metabolic pathways. In order to understand the effects of oil exposure on the energetics of larval development and metamorphosis of the American lobster, it is important to ascertain the relationship of hydrocarbon accumulation and/or toxicity with the interference of lipid metabolism. The specific goals of our research have been:

(1) to relate body burden of hydrocarbon accumulation with observed changes in respiration and lipid utilization and storage in larval and postlarval lobsters;
(2) to compare the lipid classes and component fatty acids of control and oil-exposed larval and postlarval lobsters; and
(3) to relate the release of accumulated hydrocarbons (or by-products) with the restoration of normal lipid utilization and storage patterns.

MATERIALS AND METHODS

Culture of larval and juvenile lobsters

Female egg-bearing lobsters were obtained from local fishermen and maintained in flowing seawater (30–31‰ salinity) at ambient temperatures on a diet of mussels and squid. The eggs developed normally and began to hatch when seawater temperatures reached 18–20 °C. After hatching, stage I larvae were transferred to fiberglass plankton-kreisels, described by Hughes *et al.* (1974), and maintained in flowing seawater at 20–22 °C on a diet of live or frozen brine shrimp (*Artemia* sp.). Stage IV lobsters were transferred to compartmented polypropylene trays and maintained in flowing seawater under the same temperature and feeding regime.

Bioassay system

The bioassay system was a continuous flow system consisting of twenty-four 500 ml Fleakers® (Corning Glass) as the assay chambers. Each assay chamber was modified by the addition of an intake tube and an outflow port at the 400 ml mark covered with Nitex® screening to allow

continuous flow (Capuzzo *et al.*, 1976). Seawater–crude oil dispersions were supplied to the assay chambers by peristaltic pumping at a flow rate of 3·35 ml/min and temperature and flow rates were maintained at constant levels. Seawater or seawater–crude oil dispersions (0·25 ppm) were delivered from 13 liter glass carboys. Seawater–crude oil stock dispersions of 250 ppm SLCO (300 μl/liter, w/v) were prepared in 500 ml Erlenmeyer flasks using 1 μm filtered seawater. The flasks were tightly covered to minimize loss of volatiles and stock solutions were mixed for 24 h at 10 °C using a magnetic stirrer with the vortex extending approximately 25 % of the distance to the bottom of the flask. Aliquots of the 250 ppm oil–seawater dispersion were withdrawn by pipet from below the surface of the stock solution and added to the carboys directly and diluted at the designated concentration. Carboys were replaced every 6 h and the turnover time in each assay chamber was 1 h. Hydrocarbon concentrations of oil–seawater dispersions supplying the assay chambers were assayed by gas chromatography.

For each larval stage, 5 larvae were added to an assay unit and maintained on a diet of live *Artemia* nauplii to reduce cannibalism. Larvae were monitored for survival, molt stage, respiration rates, ammonia excretion rates and O:N ratios at selected times (0, 24, 72 and 96 h) during a 96 h exposure period at 20 °C and compared with control animals maintained under identical conditions. At the end of the 96 h exposure period animals were subsampled for hydrocarbon and lipid analyses.

To evaluate the effect of crude oil exposure on the molt to the postlarval form, stage IV larvae were exposed to 0·25 ppm South Louisiana crude oil–water dispersions for 96 h and molt stage, respiration rates, ammonia excretion rates and O:N ratios were monitored daily. After the 96 h exposure period (day 4), lobsters were transferred to clean seawater and maintained for 1 week to assess success of molting to the postlarval form, physiological changes associated with molting and degree of recovery from oil exposure. During the post-exposure period the various physiological parameters described above were measured at 96 h (day 8) and 168 h (day 11).

Physiological techniques

Respiration rates of individual larvae were measured using both microrespirometers and a Gilson differential respirometer according to

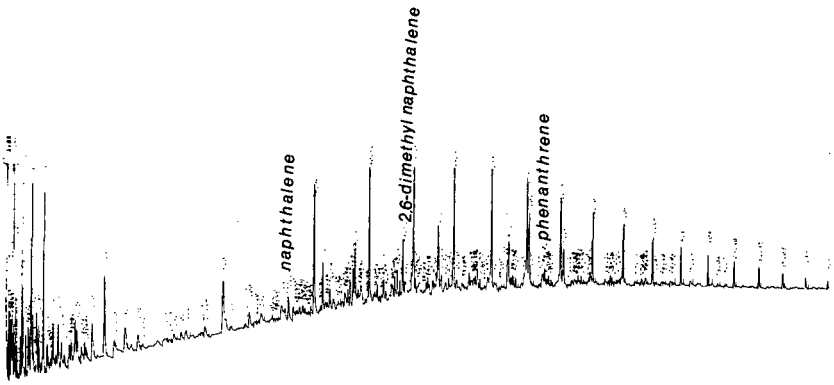


Fig. 1. Glass capillary gas chromatogram of South Louisiana crude oil–seawater mixture: column 20 m × 0·32 mm i.d., SE52WCOT on Silyl interlayer; carrier gas He at 1·6 ml/min; injection temperature 35 °C, splitless; program start at 25 min, 3 °/min for 90 min. Hydrocarbon standards injected simultaneously for comparison of retention times.

the techniques described by Capuzzo & Lancaster (1979). Equilibrium between the gas and liquid phase of O_2 diffusion in the respirometer flasks was enhanced by gently shaking the respirometer flasks. At the end of each set of oxygen uptake measurements, the seawater in the respirometer flasks was analyzed for NH_4^+–N by the method of Solorzano (1969) in order that an *in situ* estimate of ammonia excretion rates and the O:N ratio (atomic ratio of oxygen consumed to NH_4^+–N excreted) could be made; ammonia levels were compared with control blanks.

Hydrocarbon analyses

Seawater samples were extracted with hexanes and concentrated using a Bucchi rotary evaporator. Hydrocarbon content was determined by glass capillary GC using an HP 5830A gas chromatograph with an SE52WCOT on Silyl interlayer, column 20 m × 0·32 mm i.d. The gas chromatogram (Fig. 1) reveals that a complex mixture of hydrocarbons—including microdroplets and water-soluble fractions—was available in the bioassay experiments. Comparison with a gas chromatogram of South Louisiana crude oil was consistent with that presented in Fig. 1. The concentration of total hydrocarbons during the bioassay experiments was $0{\cdot}25 \pm 0{\cdot}05$ ppm (mean of 6 determinations $\pm$ 1 SD).

Because of the small size of individual animals, hydrocarbon content of

larval and postlarval lobsters was determined by direct analysis using gas chromatography–thermal distillation pyrolysis (Whelan *et al.*, 1980). Tissues were dissected by microtechnique under a dissecting microscope (American Optical Stereo-Zoom). The GC–TDP procedure combines the techniques of GC–thermal distillation (designed to recover volatile, absorbed organic molecules without significant alteration) and GC–pyrolysis (designed to crack the molecules and use the molecular patterns of cracked products for identification). Such techniques require a small sample size (0·1–300 mg) and are more rapid and less expensive than extraction procedures. A sample is heated from 100 to 800 °C at a rate of 40 °C/min in a helium stream and total hydrocarbons evolved are measured as a function of temperature. Two peaks are measured; the first (P_1) represents free absorbed hydrocarbons and the second (P_2) cracking products obtained by pyrolysis. The components of each peak were analyzed in more detail by capillary GC and GC–mass spectrometry (GC–MS). The technique is limited to hydrocarbon determinations within the range of C_1–C_{30} (Whelan *et al.*, 1980).

Biochemical analyses

At the end of the exposure periods, animals were weighed, dried at 70 °C for 24 h and reweighed, and assayed for biochemical composition and caloric content. The relative percentages of protein, lipid, carbohydrate, chitin and ash were determined for each stage of oil-exposed and control lobsters. Protein, carbohydrate, chitin and ash were determined by the methods described by Raymont *et al.* (1964) using dry tissues; lipid content was measured as described below. Caloric content was determined using a Phillipson Microbomb calorimeter.

Lipid class and fatty acid analyses

Lipids were extracted with chloroform–methanol by a method adapted from Bligh & Dyer (1959) and Folch *et al.* (1957); the extracted sample was split into three aliquots, one assayed for total lipids by gravimetric analysis, the second for lipid class composition and the third for fatty acid composition. The class structure was determined using an Iatroscan TH-10. This instrument employs a quartz rod coated with silica which is treated in a manner similar to thin-layer chromatography (TLC) plates for sample application and separation. The polar lipids were separated

using the chloroform–methanol–water (80:35:3) system of Innis & Clandinin (1981); the neutral lipids were separated by an adapted version of the solvent system of Christie & Hunter (1979) which consisted of dichloroethane–chloroform–glacial acetic acid–isopropanol (92:8:0·1:0·03) (Sasaki, in preparation). Together these solvent systems differentiate the following lipid classes: sterols, steryl esters, mono-, 1,2-di- and triacylglycerols, free fatty acids, phosphatidyl (P.) choline, P. ethanolamine, P. serine, P. inositol and sphingomyelin.

Fatty acids were analyzed as fatty acid methyl esters (FAME) by packed column gas chromatography with a flame ionization detector. A 6 ft, 2·1 mm i.d. stainless steel column packed with 10% SP-2330 on Chromosorb 100/120 W AW (Supelco, Inc.) was used at 200 °C with a nitrogen flow of 20 ml/min. The major lipid classes were separated for fatty acid analysis using thin-layer chromatography. FAME were formed from these classes by minor modifications of the boron trifluoride–methanol method of Metcalfe *et al.* (1966) after saponification with 0·5 N NaOH in methanol.

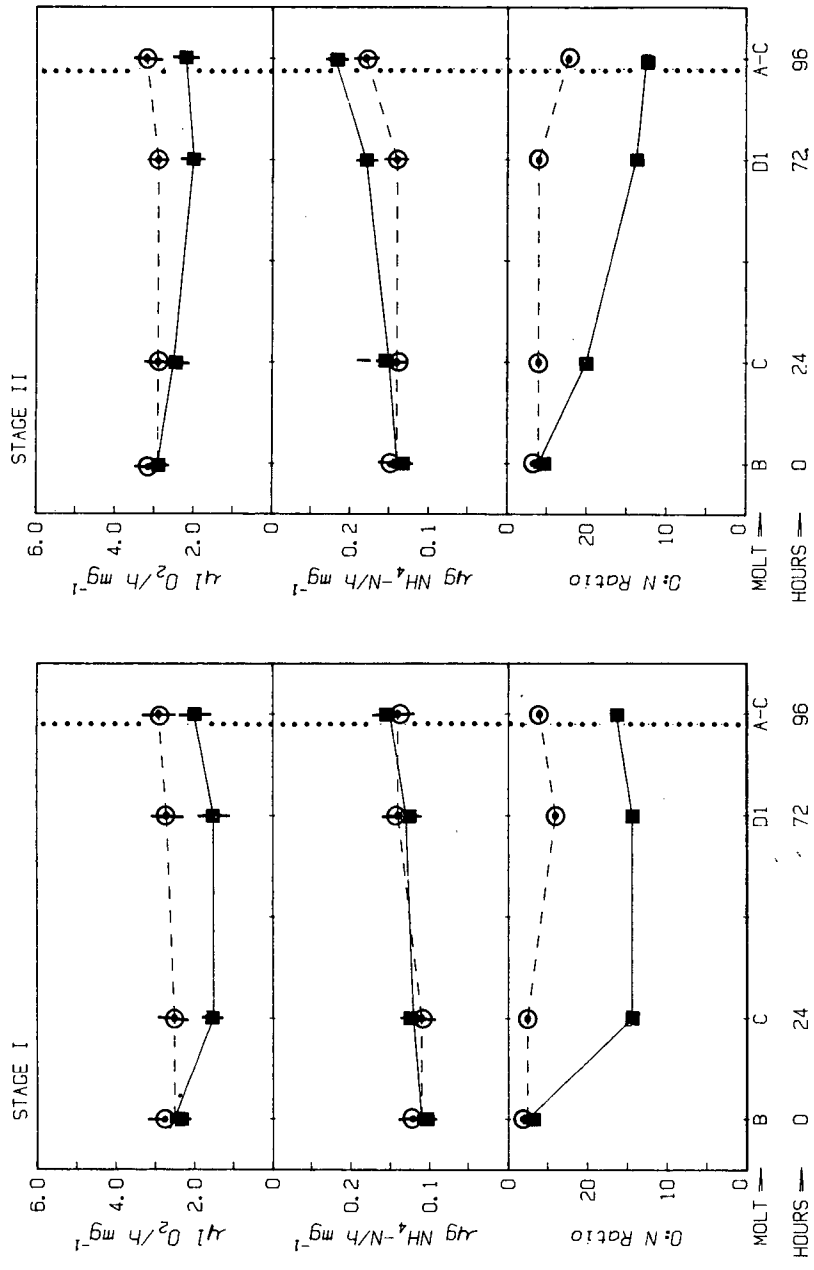
Statistical analyses

Differences among physiological and biochemical parameters measured from the oil-exposed and control lobsters were assessed by analysis of variance (Sokal & Rohlf, 1969).

RESULTS

Survival of larval lobsters exposed to 0·25 ppm South Louisiana crude oil was not significantly different from the survival of control animals and ranged from 41·3–51·1% (±1·5% SE) for stages I–III and 92·5–96·0% (±1·5% SE) for stage IV lobsters; the higher mortality observed among the early larval stages was attributed to cannibalism.

As illustrated in Fig. 2, respiration rates of control lobsters increased slightly during the molt cycle of each larval stage (molt stage B–D_1) and with the molt to the subsequent stage with the exception of the molt to the first postlarval form (stage V). Weight-specific respiration rates increased with each molt stage and were highest among stage IV larvae, then decreased just prior to the molt to stage V (D_3, 80 h, Fig. 3). Ammonia excretion rates of control lobsters followed similar patterns as respiration


STAGE II
STAGE I
μl O2/h mg-1
μg NH4-N/h mg-1
O:N Ratio
MOLT
HOURS
B
C
D1
A-C
0
24
72
96

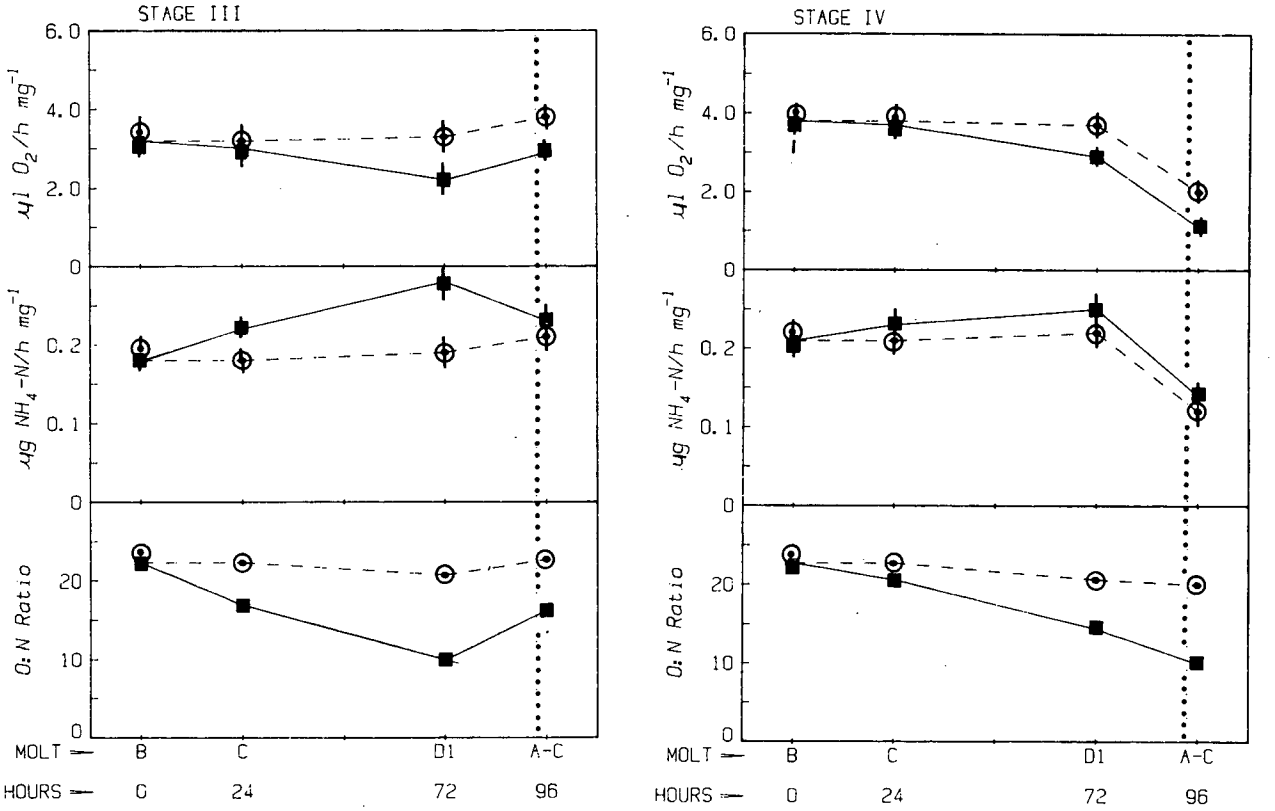


Fig. 2. Respiration rates, ammonia excretion rates and O:N ratios of larval lobsters exposed to 0·25 ppm South Louisiana crude oil for 96 h at 20 °C. ○, control values, ■, oil-exposed values. Each point is the mean of 24 replicates, bars represent 1 standard error; dotted line indicates molt to next stage.

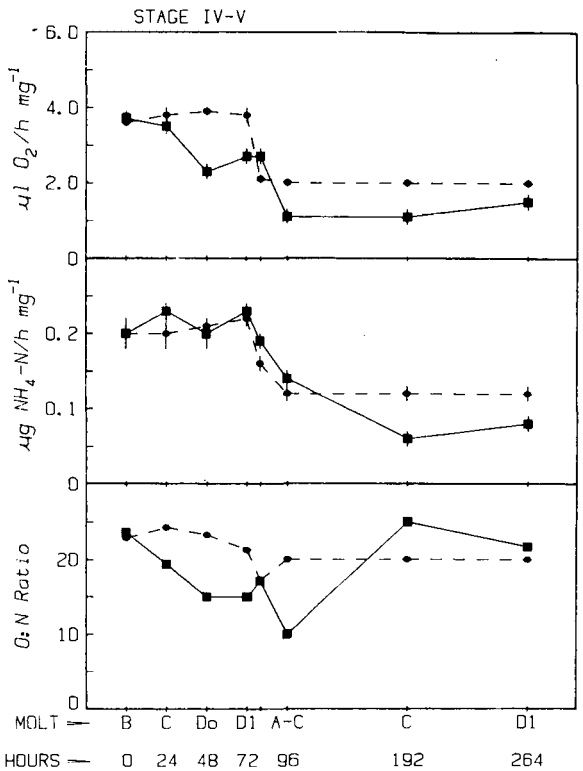


Fig. 3. Respiration rates, ammonia excretion rates and O:N ratios of stage IV and V lobsters exposed to 0·25 ppm South Louisiana crude oil for 96 h at 20 °C and maintained for 1 week following exposure to clean seawater. ●, control values; ■, oil-exposed values. Mean of 8 replicates, bars represent 1 standard error. Mark between 72 and 96 h represents 80 h sampling time.

rates among all larval stages and the first postlarval stage, with slight increases occurring immediately after molting in each larval stage (Fig. 2) and decreases just prior to molting to stage V (Figs 2 and 3). The calculated O:N ratios decreased slightly with each larval stage and were at the lowest value just prior to molting to stage V (Figs 2 and 3). These results indicate that the larval stages are dependent on the catabolism of mixed substrates for energy needs with an increased dependence on protein catabolism during the later stages of development.

With oil exposure respiration rates were significantly reduced after only 24 h of exposure for stage I ($P < 0{\cdot}01$) and stage II ($P < 0{\cdot}05$) larvae. After 72 h, respiration rates of all larval stages were significantly reduced ($P < 0{\cdot}01$), and although they increased at 96 h with the molt to subsequent

larval stages, they remained significantly lower ($P < 0{\cdot}01$) than control values (Fig. 2). Although respiration rates were significantly reduced among oil-exposed stage IV larvae, the pattern of respiratory changes was similar to the pattern observed for control larvae with the exception of a delay in the decline in respiration rate associated with the pre-molt condition (Fig. 3); at the 80 h sampling time, oil-exposed larvae had not yet proceeded to molt stage D_3. Following transfer of oil-exposed lobsters to clean seawater, respiration rates were maintained at reduced levels up to 7 days following transfer (Fig. 3).

Ammonia excretion rates were generally not significantly different between control and oil-exposed lobsters. Exceptions were observed for stage II larvae at 72 h and with the molt to stage III larvae at 96 h ($P < 0{\cdot}01$), for stage III larvae at both the 24 h and 72 h sampling times ($P < 0{\cdot}01$) (Fig. 2), and for stage IV larvae at 80 h with the delay to molt stage D_3 (Fig. 3). Following transfer to clean seawater, ammonia excretion rates were significantly reduced ($P < 0{\cdot}01$) among stage V lobsters (Fig. 3).

Calculated O:N ratios were reduced among all larval stages exposed to oil (Fig. 2), indicating an increased dependence on protein catabolism and a possible impairment of lipid metabolism in comparison with control larvae. Following transfer to clean seawater, O:N ratios were restored to control values although the magnitude of both respiration rate and ammonia excretion rates was reduced (Fig. 3).

Growth data of control and oil-exposed larval and postlarval lobsters are presented in Table 1. All oil-exposed lobster larvae showed significant delays ($P < 0{\cdot}01$) in molting to subsequent larval stages. At the end of the 96 h exposure period, control lobsters had developed to intermolt stage C (Aiken, 1973) of the subsequent larval or postlarval stage, whereas oil-exposed larvae had only developed to stage A or B, the early post-molt stages. The mean time to molt was 84 h ($\pm 1{\cdot}0$ h) for control larvae and 96 h ($\pm 2{\cdot}0$ h) for oil-exposed larvae. In addition to delays in molt, reduced growth rates were also observed among oil-exposed lobsters, as they were significantly smaller after the molt than control animals (Table 1). Differences in caloric content of lobsters sampled at the beginning and end of the exposure period were used to calculate the energy channeled to growth during a specific molt (Q_G) and on an hourly basis during the molt ($\dot{Q}_G$). In all cases the energy available for growth was reduced among oil-exposed lobsters (Table 1), presumably as a result of the failure to catabolize energy-rich lipid substrates. With the exception of slight

TABLE 1
Growth of Control and Oil-Exposed Larval and Postlarval Lobsters

Stage	*Initial wt (mg)*[a]	*Final wt (mg)*[a]	Q_G *(cal)*[c]	$\dot{Q}_G$ *(cal/h)*[c]
I–II–B[b] control	0·90 ± 0·05	1·70 ± 0·05	3·24 ± 0·05	0·039
oil-exposed	0·90 ± 0·05[f]	1·55 ± 0·05[d]	2·63 ± 0·04[d]	0·027
II–III–B[b] control	1·70 ± 0·05	3·70 ± 0·05	7·00 ± 0·10	0·083
oil-exposed	1·70 ± 0·05[f]	3·50 ± 0·05[e]	6·30 ± 0·10[d]	0·065
IV–V–B[b] control	7·5 ± 0·3	15·1 ± 0·1	23·8 ± 0·2	0·28
oil-exposed	7·5 ± 0·3[f]	14·3 ± 0·1[d]	21·3 ± 0·2[d]	0·22

[a] Mean of 6 replicates ± 1 SE.
[b] Exposed as stages I, II and IV, respectively, and molted to subsequent stage during exposure.
[c] Calories channeled to growth before molt to subsequent stage; based on caloric content at beginning and end of exposure and molting time of 84 h for control larvae and 96 h for oil-exposed larvae.
[d] $P < 0·01$. [e] $P < 0·05$. [f] Not significant.

increases in the lipid content of oil-exposed lobsters, there were no significant differences in biochemical composition between control and oil-exposed lobsters (Table 2).

Analysis of total lipids of control and oil-exposed larval and postlarval lobsters is presented in Table 3. For stage II-B lobsters the greater percentage of lipids is found in the polar lipid pool. In stages III-B and V-B there are consistent decreases in the polar lipid pool and increases in the neutral lipid pool. The ratio of neutral lipids to polar lipids is 3–5 times higher in postmetamorphic animals, possibly reflecting the importance of long-term energy stores in postlarval animals. Analyses of neutral lipid and polar lipid content of control and oil-exposed lobsters indicate significant ($P < 0·01$) reductions in neutral lipid pools of stage III-B and stage V-B lobsters and significant ($P < 0·05$) increases in the polar lipid pools of stage V-B lobsters; for stage II-B lobsters, no significant differences in either lipid pool were observed.

Analysis of lipid class composition of control and oil-exposed larval and postlarval lobsters is presented in Table 4. Oil-exposed lobsters had consistently lower levels of triacylglycerols (the major lipid energy store) and higher levels of sterols than control lobsters. There were no consistent differences in polar lipid classes between control and oil-exposed lobsters, although there were slight increases in phosphatidylcholine among stage

TABLE 2
Biochemical Composition of Control and Oil-Exposed Larval and Postlarval Lobsters[a]

Stage[b]	% *Protein*	% *Lipid*	% *Carbohydrate*	% *Ash*	% *Chitin*
II–B control	61·2 ± 0·5	9·0 ± 0·1	1·9 ± 0·1	19·0 ± 0·4	8·9 ± 0·4
oil-exposed	61·2 ± 0·5[e]	9·3 ± 0·1[c]	1·9 ± 0·1[e]	18·9 ± 0·3[e]	8·7 ± 0·3[e]
III–B control	59·6 ± 0·6	9·6 ± 0·0	1·5 ± 0·1	21·2 ± 0·5	8·1 ± 0·3
oil-exposed	60·1 ± 0·5[e]	9·7 ± 0·0[d]	1·5 ± 0·1[e]	20·5 ± 0·5[e]	8·2 ± 0·4[e]
V–B control	55·0 ± 0·5	8·3 ± 0·0	1·4 ± 0·1	23·0 ± 0·5	12·3 ± 0·3
oil-exposed	55·4 ± 0·6[e]	8·8 ± 0·3[d]	1·4 ± 0·1[e]	22·5 ± 0·5[e]	11·9 ± 0·5[e]

[a] Mean of 6 replicates ± 1 SE; % dry weight basis.
[b] Exposed as stages I, II and IV, respectively, and molted to subsequent stage during exposure.
[c] $P < 0.01$. [d] $P < 0.05$. [e] Not significant.

II-B and stage III-B larvae and slight decreases in phosphatidylethanolamine among stage III-B larvae. The ratios of the various lipid classes in control and oil-exposed lobsters are presented in Fig. 4. The differences in triacylglycerols and sterol composition reflect both a decreased synthesis of energy reserves and a decreased mobilization of sterols among oil-exposed lobsters.

Differences in the profiles of non-essential fatty acids (16:0, 16:1 ω7, 18:0, 18:1 ω9) were not consistent in the neutral lipid pools of control and

TABLE 3
Lipid Analyses of Control and Oil-Exposed Larval and Postlarval Lobsters[a]

Stage[b]	% *Neutral lipids*	% *Polar lipids*	*NL/PL*
II–B control	19·4 ± 0·8	80·6 ± 1·0	0·24
oil-exposed	19·3 ± 0·5[e]	80·7 ± 0·9[e]	0·24
III–B control	29·2 ± 0·5	70·8 ± 2·0	0·41
oil-exposed	27·1 ± 0·4[c]	72·9 ± 1·0[e]	0·37
V–B control	59·8 ± 0·6	40·2 ± 0·7	1·49
oil-exposed	57·5 ± 1·0[d]	42·5 ± 1·2[d]	1·35

[a] Mean of 3 replicates ± 1 SE.
[b] Exposed as stages I, II and IV, respectively, and molted to subsequent stage during exposure.
[c] $P < 0.01$. [d] $P < 0.05$. [e] Not significant.

TABLE 4
Lipid Class Composition of Control and Oil-Exposed Larval and Postlarval Lobsters[a]

Stage	*Neutral lipids*			*Polar lipids*			
	Tri	*Sterols*	*1,2-Di*	*PE*	*PC*	*SM*	*Other*
II–B control:							
%	6·2 ± 0·3	11·2 ± 0·4	1·9 ± 0·2	25·8 ± 1·1	43·1 ± 0·3	3·1 ± 0·2	8·7 ± 0·3
μg	9·4 ± 0·4	17·0 ± 0·6	2·9 ± 0·3	39·2 ± 1·7	65·5 ± 0·5	4·7 ± 0·3	13·2 ± 0·5
μg/mg	5·5 ± 0·2	10·0 ± 0·4	1·7 ± 0·2	23·1 ± 1·0	38·5 ± 0·3	2·8 ± 0·2	7·8 ± 0·3
oil-exposed:							
%	5·1 ± 0·5[d]	12·7 ± 0·2[c]	1·5 ± 0·2[e]	25·5 ± 1·3[e]	45·1 ± 0·7[c]	3·4 ± 0·2[e]	6·7 ± 0·3[c]
μg	7·4 ± 0·6[c]	18·4 ± 0·3[d]	2·2 ± 0·3[d]	37·0 ± 1·9[e]	65·4 ± 1·0[e]	4·9 ± 0·3[e]	9·7 ± 0·4[c]
μg/mg	4·8 ± 0·4[d]	11·9 ± 0·2[c]	1·4 ± 0·2[e]	23·9 ± 1·2[e]	42·2 ± 0·6[c]	3·2 ± 0·2[e]	6·3 ± 0·3[c]
III–B control:							
%	18·8 ± 0·5	8·3 ± 0·3	2·1 ± 0·1	24·0 ± 0·2	37·8 ± 0·6	2·5 ± 0·2	6·5 ± 0·6
μg	67·9 ± 1·8	30·0 ± 1·1	7·6 ± 0·4	86·6 ± 0·7	136·5 ± 2·2	9·0 ± 0·7	23·5 ± 2·2
μg/mg	18·4 ± 0·5	8·1 ± 0·3	2·1 ± 0·1	23·4 ± 0·2	36·9 ± 0·6	2·4 ± 0·2	6·4 ± 0·6
oil-exposed:							
%	15·3 ± 1·0[c]	9·7 ± 0·4[c]	2·2 ± 0·4[e]	21·2 ± 0·8[c]	40·1 ± 0·9[d]	2·7 ± 0·2[e]	8·8 ± 0·2[c]
μg	51·3 ± 3·4[c]	32·5 ± 1·3[e]	7·4 ± 1·3[e]	71·0 ± 2·7[c]	134·3 ± 3·0[e]	9·0 ± 0·7[e]	29·5 ± 0·7[c]
μg/mg	14·7 ± 1·0[c]	9·3 ± 0·4[d]	2·1 ± 0·4[e]	20·3 ± 0·8[c]	38·4 ± 0·9[e]	2·6 ± 0·2[e]	8·4 ± 0·2[c]
V–B control:							
%	51·0 ± 0·6	6·7 ± 0·3	2·1 ± 0·1	10·7 ± 0·3	21·5 ± 0·4	2·3 ± 0·4	5·7 ± 0·3
μg	704·3 ± 8·3	92·5 ± 4·1	29·0 ± 0·1	147·8 ± 4·1	296·9 ± 5·5	31·8 ± 5·5	78·7 ± 4·1
μg/mg	46·6 ± 0·5	6·1 ± 0·3	1·9 ± 0·1	9·8 ± 0·3	19·7 ± 0·4	2·1 ± 0·4	5·2 ± 0·3
oil-exposed:							
%	47·2 ± 1·4[d]	8·2 ± 0·2[c]	2·2 ± 0·1[e]	11·5 ± 0·8[e]	22·6 ± 1·1[e]	2·5 ± 0·6[e]	5·8 ± 0·3[e]
μg	621·1 ± 18·4[c]	107·9 ± 2·6[c]	29·0 ± 0·1[e]	151·3 ± 10·5[e]	297·4 ± 14·5[e]	32·9 ± 7·9[e]	76·3 ± 3·9[e]
μg/mg	43·4 ± 1·3[d]	7·5 ± 0·2[c]	2·0 ± 0·1[e]	10·6 ± 0·7[e]	20·8 ± 1·0[e]	2·3 ± 0·6[e]	5·3 ± 0·3[e]

[a] Tri, triacylglycerols; 1,2-Di, 1,2-diacylglycerols; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin. Mean of 3 replicates ± 1 SE.
[b] Exposed as stages I, II and IV, respectively, and molted to subsequent stage during exposure.
[c] $P < 0.01$. [d] $P < 0.05$. [e] Not significant.

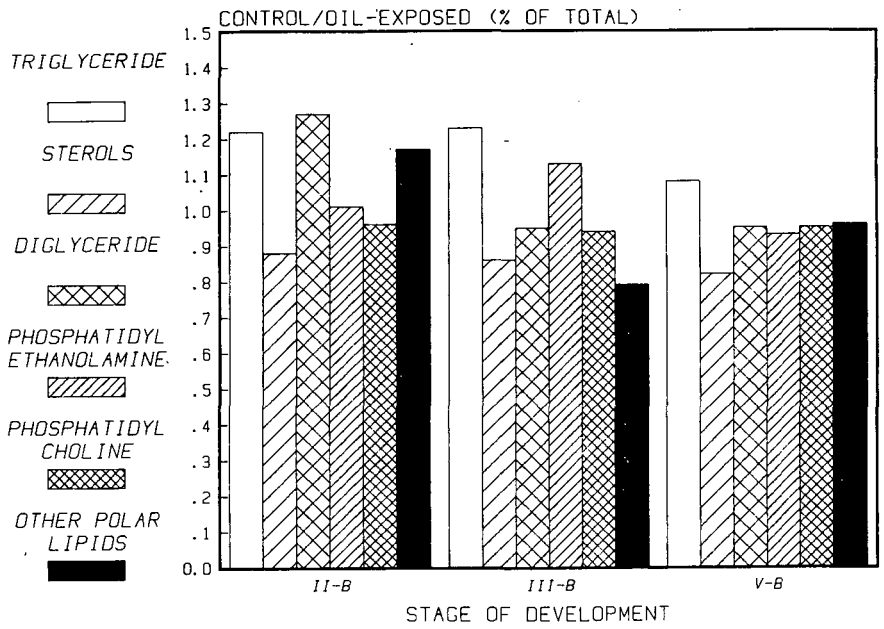


Fig. 4. Lipid class composition of larval and postlarval lobsters: ratio of control to oil-exposed values.

oil-exposed lobsters, but varied little in the polar lipid pools (Table 5). The greatest difference in fatty acid composition was the increased content of essential fatty acids (20:5 ω3 and 22:6 ω3) in the neutral lipid pools of oil-exposed lobsters, suggesting a decreased mobilization of essential fatty acids to polar lipid pools. The total content (μg) and concentration (μg/mg) of both saturated and unsaturated non-essential fatty acids were reduced in the neutral lipid pools of oil-exposed lobsters; the fatty acid content of polar lipid pools among oil-exposed lobsters, however, showed little difference from control values, or in some instances was increased. These results suggest that although decreased storage of non-essential fatty acids in energy reserves and decreased mobilization of essential fatty acids from neutral lipid pools may be occurring among oil-exposed animals, the non-essential and essential fatty acids are being conserved in polar lipid pools. The ratios of fatty acids in netural lipid and polar lipid pools of control and oil-exposed lobsters are presented in Fig. 5.

Hydrocarbon content of control and oil-exposed lobsters is presented in Table 6. There were few differences in the chromatograms of control

TABLE 5
Fatty Acid Composition of Control and Oil-Exposed Larval and Postlarval Lobsters

Stage[a]	NL							PL						
	16:0	*16:1*	*18:0*	*18:1*	*20:5*	*22:6*	*Other*	*16:0*	*16:1*	*18:0*	*18:1*	*20:5*	*22:6*	*Other*
II–B control:														
%	11·95	14·21	4·74	35·74	6·51	<0·1	26·85	11·54	6·20	7·57	22·67	18·11	6·49	27·42
μg	1·47	1·75	0·58	4·40	0·80	—	3·30	9·36	5·03	6·14	18·39	14·69	5·26	22·24
μg/mg	0·86	1·03	0·34	2·59	0·47	—	1·94	5·51	2·96	3·61	10·82	8·64	3·09	13·08
oil-exposed:														
%	12·79	14·74	3·94	36·20	9·86	4·59	17·88	11·85	6·20	7·57	22·89	17·72	6·66	27·11
μg	1·23	1·41	0·38	3·48	0·95	0·44	1·72	9·40	4·92	6·01	18·17	14·07	5·29	21·53
μg/mg	0·79	0·91	0·25	2·25	0·61	0·28	1·11	6·07	3·17	3·89	11·72	9·07	3·41	13·89
%C/%O	0·93	0·96	1·20	0·99	0·66	0·02	1·50	0·97	1·00	1·00	0·99	1·02	0·97	1·01
III–B control:														
%	12·17	14·24	4·23	35·38	5·88	<0·1	28·10	11·78	6·25	7·26	22·24	17·29	6·19	28·99
μg	9·19	10·75	3·19	26·71	4·44	—	21·22	20·36	10·80	12·56	38·46	29·90	10·70	50·13
μg/mg	2·48	2·91	0·86	7·22	1·20	—	5·73	5·50	2·92	3·39	10·39	8·08	2·89	13·55
oil-exposed:														
%	10·69	11·66	4·90	31·79	9·95	3·76	27·25	12·88	6·36	7·25	22·80	16·15	5·52	29·04
μg	6·28	6·84	2·88	18·66	5·84	2·21	15·99	20·49	10·11	11·53	36·27	25·70	8·78	46·21
μg/mg	1·79	1·96	0·82	5·33	1·67	0·63	4·57	5·86	2·89	3·29	10·36	7·34	2·51	13·20
%C/%O	1·14	1·22	0·86	1·11	0·59	0·03	1·03	0·91	0·98	1·00	0·98	1·07	1·12	1·00
V–B control:														
%	13·42	7·61	3·65	35·34	1·67	1·17	37·14	12·89	4·92	6·87	23·24	10·26	7·37	34·45
μg/mg	6·52	3·70	1·77	17·16	0·81	0·57	18·04	2·94	1·12	1·57	5·30	2·34	1·68	7·86
oil-exposed:														
%	14·24	7·46	4·33	36·11	1·68	1·54	34·64	13·16	4·60	7·08	21·67	11·17	7·59	34·73
μg	92·57	48·50	28·15	234·75	10·92	10·01	225·19	45·76	16·00	24·62	75·36	38·84	26·39	120·77
μg/mg	6·47	3·39	1·97	16·42	0·76	0·70	15·75	3·20	1·12	1·72	5·27	2·72	1·85	8·45
%C/%O	0·94	1·02	0·84	0·98	0·99	0·76	1·07	0·98	1·07	0·97	1·07	0·92	0·97	0·99

[a] Exposed as stages I, II and IV, respectively, and molted to subsequent stage during exposure.

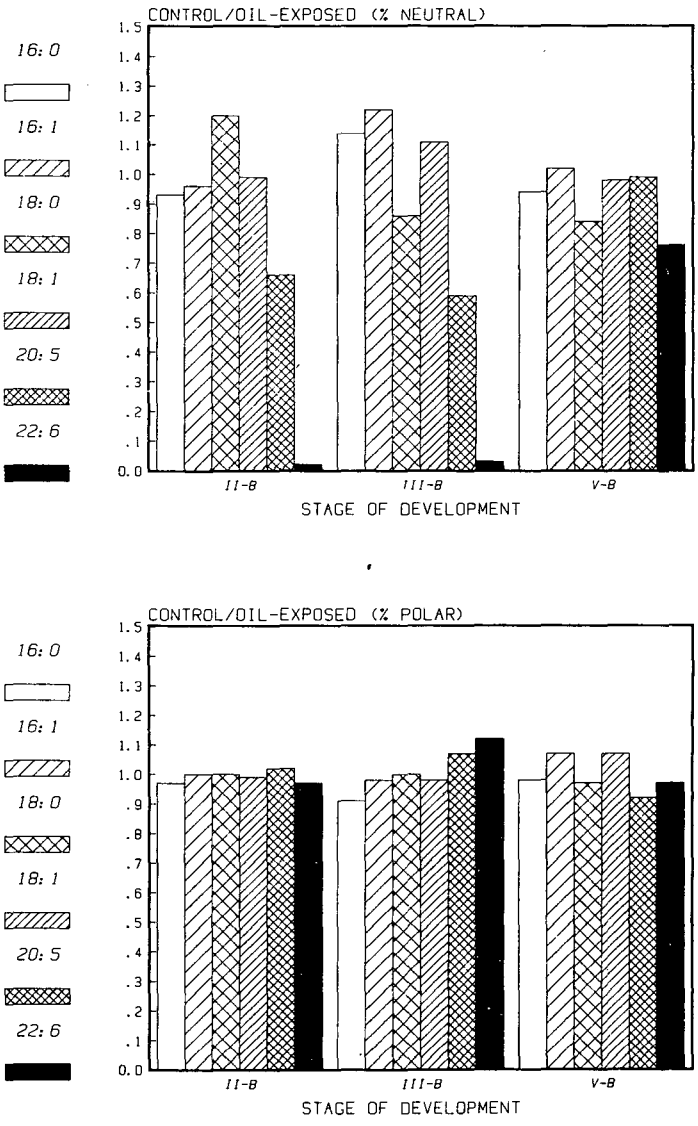


Fig. 5. Fatty acid composition of neutral lipid and polar lipid pools of larval and postlarval lobsters: ratio of control to oil-exposed values.

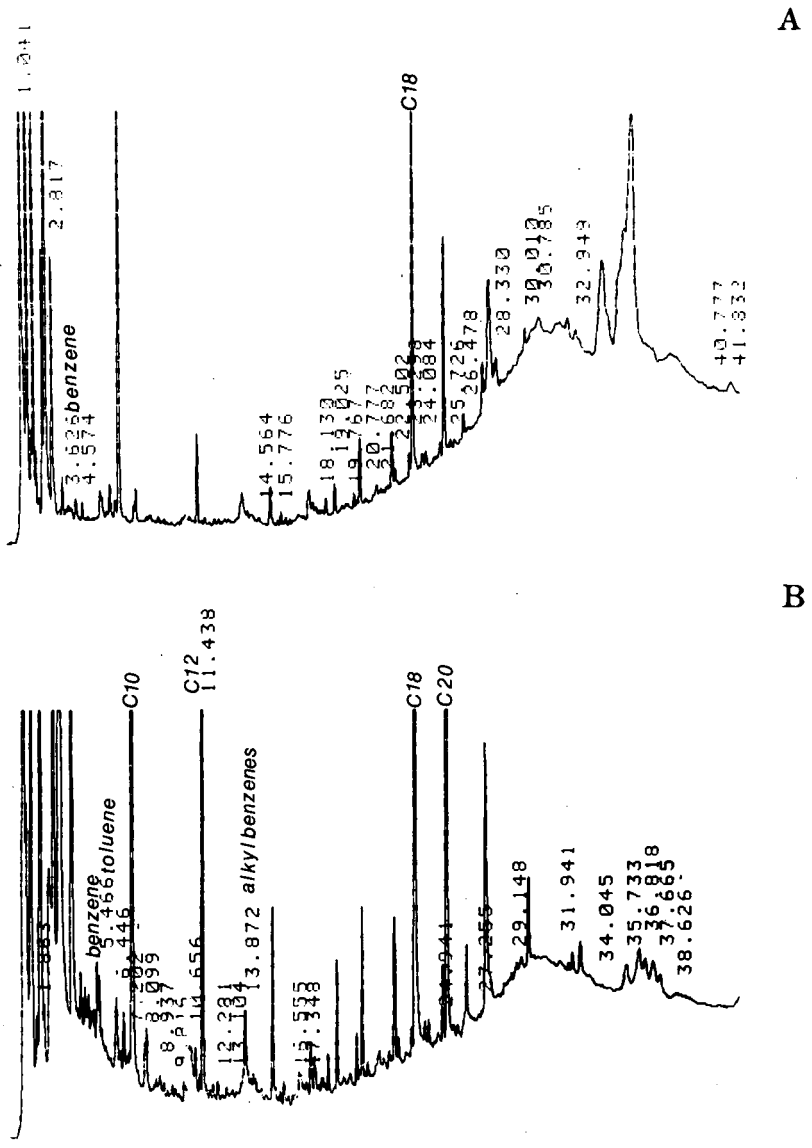


Fig. 6. Gas chromatogram of hepatopancreas of stage I lobster larvae exposed to 0·25 ppm South Louisiana crude oil for 96 h at 20 °C. A, control; B, oil-exposed.

TABLE 6
Hydrocarbon Content of Control and Oil-Exposed Larvae and Postlarval Lobsters[a]

Stage[b]	*Hydrocarbon content (μg/mg wet wt)*		
	Whole animal	*Tail muscle*	*Hepatopancreas*
II–B control	1·00 ± 0·10	0·75 ± 0·05	2·4 ± 0·1
oil-exposed	1·10 ± 0·10	0·80 ± 0·10	3·9 ± 0·1
III–B control	0·75 ± 0·10	0·60 ± 0·05	1·2 ± 0·1
oil-exposed	1·00 ± 0·10	0·80 ± 0·10	2·6 ± 0·2
V–B control	0·95 ± 0·05	0·80 ± 0·05	1·5 ± 0·1
oil-exposed	1·10 ± 0·10	0·80 ± 0·10	2·4 ± 0·1

[a] Mean of 3 replicates ± 1 SE.
[b] Exposed as stages I, II and IV, respectively, and molted to subsequent stage during exposure.

and oil-exposed lobsters and no obvious 'oil' signal was evident in exposed animals (Fig. 6), suggesting either limited bioaccumulation or rapid turnover and metabolism of hydrocarbon components by oil-exposed lobsters. Some enhancement of the C_{14}–C_{16} components (retention time = 12–18 min) was evident in oil-exposed lobsters, but there was no accumulation of petroleum-derived straight-chained alkanes and there appeared to be no petroleum-concentrating effect, at least within the limit of detection (10 ng hydrocarbon absolute, 2 ng/mg lobster = 2 ppm). In control animals greater peak heights in the chromatogram peaks above the 20 min retention time were observed that represent the lipid fraction chromatographed by the GC–TDP technique (C_{18} and higher), and are consistent with the packed column GC analyses of fatty acid composition. Oil-exposed animals contained trace quantities (10–200 ppm) of benzene, thiophene, toluene, alkylcyclohexane and alkylbenzenes detected by GC–MS analyses; these compounds (with the exception of trace concentrations of benzene < 10 ppm) were not detected in control animals. No naphthalene, dimethylnaphthalene, higher molecular weight aromatics or their phenolic derivatives were detected in either control or oil-exposed lobsters; their absence in oil-exposed animals may be related to either the detection limits of the methodology used or extremely rapid metabolism and turnover of these components. Stage V lobsters sampled 1 week after transfer from oil exposures to clean seawater also contained trace amounts of benzene and alkylbenzenes and

these components did not appear to be depurated to any appreciable extent during the post-exposure period.

DISCUSSION

Offshore oil spills such as those caused by the stranding of the *Argo Merchant* and *Amoco Cadiz* resulted in high surface water concentrations of petroleum hydrocarbons persisting for several days after the initial spill (Grose & Mattson, 1977; Law, 1978; Mackie *et al.*, 1978), thus impacting the pelagic ecosystem. The abundance of the larval stages of many commercially important species of fish and shellfish in surface waters of the world's oceans dictates our need to understand the effects of oil on the early development of these species. Exposure of planktonic larval stages to oil dispersed in surface waters could result in reduced survival, increased susceptibility to other environmental stresses, changes in the rates of growth and development, and reduced recruitment. Evaluations of the effects of petroleum hydrocarbons on metabolic processes that affect development and recruitment success are critical to understanding and predicting such impacts.

Alterations in energetics and lipid storage metabolism were observed among larval lobsters with exposure for 96 h to sublethal concentrations of South Louisiana crude oil. The early larval stages were the most sensitive to exposure as evidenced by reductions in respiration rate within 24 h of exposure. All larval stages, however, showed similar reductions in respiration rate during the 96 h exposure period, in addition to reductions in the O:N ratio, delays in molting and reductions in growth.

Delayed molting and reduced growth are not unique to larval lobsters but have been observed by other investigators with exposure of crustaceans to crude and refined oils (Table 7); the effects, however, are very greatly reduced among postmetamorphic animals. With chronic exposure of the mud crab *Rhithropanopeus harrisii* to water-soluble fractions of No. 2 fuel oil, Laughlin *et al.* (1978) found the early zoeal stages to be the most sensitive and larval development was significantly delayed as a result of oil exposure. The growth rates of crab stages and the size distribution and sex ratio of animals at the end of the 6-month exposure period, however, were not significantly different between control and oil-exposed groups. Similarly, postlarval lobsters (*Homarus americanus*) were unaffected by exposure to South Louisiana crude oil for

TABLE 7
Larval Crustaceans in Which Delayed Development and Inhibition of Growth and Molting Have Been Attributed to Exposure to Petroleum Hydrocarbons

Species	*Oil or hydrocarbon component*	*Reference*
Balanus sp.	'Oil products'	Mironov (1972)
Cancer irroratus	No. 2 fuel oil	Johns & Pechenik (1980)
Cancer magister	Naphthalene, Benzene	Caldwell *et al.* (1977)
Clibinarius vittatus	No. 2 fuel oil	Winters *et al.* (1977)
Eurypanopeus depressus	Kuwait crude oil	Cucci & Epifanio (1979)
Homarus americanus	Venezuelan crude oil	Wells (1972); Wells & Sprague (1976)
	South Louisiana crude oil	Capuzzo & Lancaster (1982)
Hyas araneus	Ekofisk crude oil	Christiansen & Stormer (1978)
Libinia dubia	No. 2 fuel oil	Winters *et al.* (1977)
Neopanaope texana	Venezuelan crude oil	Katz (1973)
Palaemonetes pugio	No. 2 fuel oil	Tatem (1977)
Pandalus hypsinotus	Cook Inlet crude oil	Mecklenburg *et al.* (1977)
Paralithodes camtschatica	Cook Inlet crude oil	Mecklenburg *et al.* (1977)
Rhithropanopeus harrisii	No. 2 fuel oil	Laughlin & Neff (1978)
	Phenanthrene	Laughlin & Neff (1979, 1980)

78 days (Tomei, 1979; Capuzzo & Lancaster, 1981) or Venezuelan crude oil for 30 days (Wells & Sprague, 1976). Cucci & Epifanio (1979), however, found significant delays in molting throughout development (zoea I to crab V) of the mud crab *Eurypanopeus depressus*, exposed to the water-soluble fraction of Kuwait crude oil beginning with the first zoeal stage. When exposure commenced later in development (zoea II, III or IV) no significant difference in molting rate or growth between exposed and control animals was detected. No increase in mortality above control levels was observed among organisms first exposed to oil as zoea III or IV, and the authors suggested that the mortality of these larval stages in other exposure groups was due primarily to an accumulation of hydrocarbons by earlier larval stages.

Impairment of growth and molting as a result of oil exposure appears to be related to the interference of hydrocarbon components with normal energetic pathways. Edwards (1978) observed changes in respiration rate, growth rate and net carbon turnover in the sand shrimp, *Crangon crangon*, with chronic exposure to the water-soluble components of North Sea Brent Field crude oil. Acute exposure (24–48 h) of shrimp to

low concentrations (5% WSF = 1 ppm) resulted in a reduction in respiration rate, but increases and subsequent decreases in respiration rate were observed at higher concentrations (10% WSF = 2 ppm). Similar changes in respiratory activity were observed by Lee *et al.* (1978) in the shrimp *Lucifer faxoni*, by Anderson *et al.* (1974) in the shrimps *Penaeus aztecus* and *Palaemonetes pugio*, and by Percy (1977) in the amphipod *Onisimus* (*Boekisimus*) *affinis*. Anderson (1977) suggested that these changes in respiratory activity might be related to a metabolic response to the naphthalene component of water-soluble fractions, although Cantelmo *et al.* (1982) found similar changes in respiration rate with exposure of the blue crab *Callinectes sapidus* to sublethal concentrations of benzene and dimethylnaphthalene.

A change in energetics as a result of oil exposure may be most crucial as a larval organism approaches metamorphosis and begins to shift its habitat preference from pelagic to benthic or adapts new behavioral strategies; such an impact may affect both larval recruitment and postlarval survival as energy reserves may not be sufficient during this transition period. Stage IV lobsters exposed to 0·25 ppm crude oil for 96 h showed reductions in the O:N ratio and delays in molting but successfully molted to the postlarval form. Recovery of lobsters was not immediate upon transfer to clean seawater; reduced metabolic rates but normal O:N ratios were observed during the post-exposure period of 1 week. These findings may be an indication that although respiration rates and ammonia excretion rates are reduced relative to control values during the post-exposure period, the normal pattern of energy metabolism and utilization is slowly being restored.

The mechanisms responsible for developmental and energetic disruptions in larval lobsters in this study appear to be related to alterations in the normal patterns of lipid storage, utilization and mobilization. Both decreased storage and utilization of lipid reserves and increased accumulation of sterols were detected among oil-exposed lobsters. These alterations in lipid metabolism possibly result from: (1) decreased storage of non-essential fatty acids into triacylglycerol reserves; and (2) decreased mobilization and/or transformation of cholesterol into metabolic pathways including the biosynthesis of β-ecdysone. The fatty acid distribution of phospholipid pools of oil-exposed larvae, however, varied little from control values and reflects a tendency to preserve the integrity of these structural lipids.

Such alterations in biochemical composition and utilization of reserves

are critical to the development of larval crustaceans and may result in the developmental abnormalities experienced with oil exposure. The reduction in lipid utilization of larval lobsters evident in the present study with oil exposure could account for the sublethal effects on growth and development observed among the larval stages of other crustacean species. It cannot be ruled out, however, that decreased lipid utilization, storage and mobilization may be a defense mechanism against incorporating lipophilic petroleum hydrocarbons in metabolic pathways and that disruption in energetics is a consequence of the reduction in energy available for growth and molting.

It is apparent that the physiological effects of oil exposure are modified by the ability of the organism to accumulate and metabolize various components of crude and refined oils. Anderson *et al.* (1980) investigated the relationship of mortality and hydrocarbon accumulation of mysids (*Neomysis awatschensis*) and two species of shrimp (*Hippolyte clarkii* and *Pandalus davae*) exposed to Prudhoe Bay crude oil. Accumulation of di- and triaromatic compounds in the tissues of these crustaceans was not cumulative and could not be used to explain toxic effects. The authors suggested that further analysis of monoaromatic hydrocarbons and metabolites might provide a better clue to the relationship between body burden and toxic effects. Lee (1977) discussed the accumulation and turnover of petroleum hydrocarbons in marine crustaceans, and Malins (1977) described several mechanisms of bioconversion of hydrocarbons that have been identified in marine organisms. Some marine crustaceans possess a mechanism for hydroxylating certain aromatic hydrocarbons (Lee, 1975; Sanborn & Malins, 1977; Corner, 1978), and Sanborn & Malins (1980) reported that larval spot shrimp (*Pandalus platyceros*) have the capacity to convert naphthalene to conjugated and non-conjugated structures, such as glucuronide, sulfate, dihydrodiol and phenolic derivatives.

Trace quantities of benzene, thiophene, toluene and alkylbenzenes were detected in oil-exposed animals, although no naphthalene, dimethylnaphthalene, higher molecular weight aromatics or their phenolic derivatives were detected. The absence of the latter compounds may be related to either the detection limits of the GC–TDP technique or extremely rapid metabolism and turnover of these components. Using a bioconcentration factor of 100 for naphthalene (Sanborn & Malins, 1977, 1980) and a naphthalene concentration in the seawater–crude oil mixtures of 10 ppb (derived from glass capillary GC, Fig. 1), the

calculated concentration of naphthalene in the oil-exposed lobsters would be equivalent to 1·0 ng/mg of animal tissue; for a 5 mg sample, this value would be below the detection limit of 10 ng. Higher accumulation, however, would be expected in the more lipid-rich hepatopancreas and using a concentration factor of 10^3 for naphthalene would yield a concentration of 10 ng/mg of hepatopancreas, within the detection limits of the technique; failure to detect naphthalene and the alkyl-substituted naphthalenes in the hepatopancreas suggests that they are rapidly metabolized and eliminated. The alkyl-substituted benzenes have concentration factors as high as ~400 in the whole animal tissues (Sanborn & Malins, 1980). The concentration of benzenes plus alkyl benzenes in crude oil–seawater mixtures was 25 ppb and the expected concentrations in oil-exposed lobsters would approximate 10 ng/mg in the tail muscle and perhaps as high as 100 ng/mg in the hepatopancreas. These components were not rapidly depurated from lobster tissue, as evidenced by their persistence in lobster tissues after a 7-day post-exposure period. Postlarval lobsters at the end of the post-exposure period also showed restoration of normal lipid utilization patterns, although they continued to show reductions in metabolic rates (respiration rates and ammonia excretion rates).

The results of this study suggest that the alterations in lipid metabolism and reductions in respiration rate observed among larval lobsters are responses to exposure, uptake and metabolism of specific hydrocarbon components. Specific changes in steroid metabolism may be related to detoxification reactions of aromatic hydrocarbons mediated by cytochrome P-450 mixed-function oxygenases (Lee *et al.*, 1982). The relationship between hydrocarbon metabolism, steroid metabolism and disruption in energetics of marine crustaceans warrants further exploration.

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