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LIPIDS IN THE MARINE ENVIRONMENT

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INTRODUCTION

Lipid utilization in the marine environment has been the subject of investigation in our laboratory. We have studied the composition and metabolism of lipids in members of a planktonic marine food chain: diatoms, copepods, and anchovies and sardines. We have discovered polyunsaturated hydrocarbons in diatoms and other marine algae and large amounts of wax esters, a reserve fuel for many copepods, were recognized during the course of our work (Lee *et al.*, 1970 a, 1970 b). A wax ester is a fatty acid esterified to a long chain alcohol, while a triglyceride is composed of fatty acids esterified to glycerol. Triglycerides, which are the normal reserve lipid in most animals, are used only for short term fuel needs in the copepods. The wax esters are used for long term fuel needs, and a special wax ester lipase is used for the mobilization of wax esters. Starvation experiments with bathypelagic copepods and seasonal studies of the lipid composition of Arctic copepods have demonstrated the important role of wax esters as reserve fuels during starvation and nutritional stress. The copepod, *Calanus helgolandicus*, was cultured on a known concentration of phytoplankton (expressed as μg carbon per liter) in the laboratory (Paffenhöfer, 1970), and the results of lipid determinations were compared with values from field-collected copepods. We have also measured the lipid changes during the life cycle of *Calanus helgolandicus* and *Euchaeta japonica*. Studies on the food of sardines and anchovies have shown that the major food was copepods (Hand and Berner, 1959; DeCiechomski, 1967; Haushi, 1967; Baxter, 1967; Nakamura and Wilson, 1970). We were concerned with the metabolism of wax esters and the transport of lipids in the blood of these fish.

Besides our findings on "natural" marine lipids, we have also included in this report our preliminary results on the uptake and discharge of petroleum hydrocarbons by marine invertebrates.

METHODS

Field Collections

Copepods from several depth intervals were collected using bongo nets of .333 mm mesh and a 3-m Isaacs-Kidd midwater trawl, on two cruises of the R/V MELVILLE about 450 km off the coast of southern California (31°N, 119°W). The live copepods were sorted as to stage or sex and taxonomic group aboard the ship, and lipid extraction was carried out as described below. Specimens of *Euchaeta japonica* were collected in Indian Arm Inlet near Vancouver, British Columbia. Copepods from the Arctic station T-3, were frozen and kindly sent to us by the Arctic Research Program of the University of Southern California. The sardines and anchovies were obtained from bait barges in San Diego, California.

Copepod Rearing

The method used for rearing *Calanus helgolandicus* has been described by Paffenhöfer (1970), while the rearing of *Euchaeta japonica* was described by Lewis and Ramnarine (1969).

Lipid Analysis

The lipids from algae, copepods, and fish were extracted in chloroform-methanol (2:1 v/v) and dried under nitrogen. The lipid was weighed, and for those experimental groups from which more than 6 mg were available, the lipid was fractionated on a silicic acid column, eluting the different lipid classes with solvents of increasing polarity as described by Nevenzel *et al.* (1965), and then each fraction was weighed. The procedure for analyzing the different lipid fractions (hydrocarbons, wax esters, triglycerides, free fatty acids, sterols, and phospholipids) by gas-chromatography is given in the paper by Lee *et al.* (1971b). When less than 6 mg of total lipid was available, the lipids were separated by thin-layer chromatography, and the amount of different types of lipid was determined spectrophotometrically by the procedure of Armenta (1964) using acid dichromate digestion and measurement at 250 mμ.

Petroleum Uptake

Mineral oil, 1, 2, 3, 4, -tetrahydronaphthalene and ^{14}C-heptadecene were used as hydrocarbon substrates. Approximately 2 gm of diatomaceous earth (Celite) were added to a 100 ml beaker containing 75 ml of sea water. The mixture was sonicated for several minutes, and the sonicated oil and water mixture was added to a 10 liter aquarium containing the mussel, *Mytilus californianus*. Some experiments were performed without sonication so that an oil slick was formed on the surface of the water. Mussels were taken out at various times, extracted with chloroform: methanol (2:1 v/v), and the lipid was fractionated using a silicic acid column in order to obtain the hydrocarbon fraction. The hydrocarbon fraction was weighed and analyzed by gas-liquid chromatography. For the ^{14}C-heptadecene experiments the mussel extraction mixture was assayed by counting in a liquid scintillation counter.

RESULTS

1. Algae

Since diatoms and dinoflagellates were used as food for rearing *C. helgolandicus,* we carried out extensive analysis of diatom and dinoflagellate lipids (Lee *et al.,* 1970c, 1971a). No wax esters were detected in these marine algae, but a unique lipid of most marine algae was identified as a 21:6 hydrocarbon (21 carbon atoms and 6 double bonds). This hydrocarbon occurs in diatoms, dinoflagellates, brown algae, euglenids, a few marine green algae, and cryptophytes (Table 1). *C. helgolandicus* adults showed no trace of this 21:6 hydrocarbon, so that excretion or metabolism of this hydrocarbon must occur. On the other hand, *Euchaeta japonica* and *Rhincalanus nasutus* did contain the hydrocarbon, probably by storing it from their algal or small zooplankton food. Blumer *et al.* (1970a) recently reported the occurrence of the 21:6 hydrocarbon in *Rhincalanus* and described its distribution in marine phytoplankton. The extent to which the 21:6 hydrocarbon occurs in higher members of the food chain needs further investigation.

TABLE 1

The Percent Lipid and Hydrocarbon in Algae

Organism	Lipid (Percent of dry weight)	Hydrocarbon (Percent of lipid)	21:6 Hydrocarbon (percent of total hydrocarbon)
Green Algae—Chlorophyta			
Platymonas sp.	6.6	7.8	82
Dunaliella tertiolecta	----	--	absent
Euglenids—Euglenophyta			
Euglena gracilis	10.1	5.2	1
Eutreptia viridis	18.8	4.2	12
Dinoflagellates—Pyrrhophyta			
Gymnodinium splendens	16.2	0.4	80
Exuviaella cassubica	5.2	3.7	20
Peridinium sociale	16.2	0.9	80
Cryptomonads—Cryptophyta			
Crytomonas ovata	18.3	12.3	20
Rhodomonas lens	16.8	11.7	45
Diatoms—Bacillariophyta			
Chaetoceros curvisetus	9.1	13.2	90
Cylindrotheca fusiformis	11.8	3.7	90
Skeletonema costatum	9.2	15.0	90
Golden Algae—Chrysophyta			
Isochrysis galbana	26.0	4.6	90
Prymnesium parvum	4.2	7.6	80
Brown Algae—Phaeophyta			
Ectocarpus sp.	8.7	7.6	85
Laminaria sp.	--	5.8	30

2. Copepods

There are some major differences between the nature of lipids in terrestrial and marine organisms. In most terrestrial animals, triglycerides serve as the only type of storage lipid. In starvation experiments with marine calanoid copepods, we found that triglycerides are only used for short-term fuel needs (Table 2 and 3), and that wax esters are the major metabolic reserve fuel. Also, in contrast to the saturated wax esters of terrestrial plants and animals, the copepod wax esters have appreciable amounts of polyunsaturated acids, and copepods living near the sea surface contain polyunsaturated alcohols (Tables 4 and 5). Wax esters of terrestrial organisms may provide structural or protective properties, whereas the waxes of marine copepods are utilized for metabolism and buoyancy regulation.

TABLE 2

Starvation Experiment with Females of the Bathypelagic Copepod *Gaussia Princeps*

The copepods were placed in the dark in plastic containers with about 10 liters of sea water previously cooled to 5°C and passed through 35 μ mesh nylon netting. Values are expressed as weight percent of total lipid.

Lipid Type	Hours of Starvation		
	0	80	120
Hydrocarbons	1	1	2
Wax esters*	49	64	61
Triglycerides	18	9	2
Polar lipids†	15	10	23
Phospholipids	17	16	22
Total lipid (percent of body dry weight)	26	22	23

* Includes a trace of sterol esters.
† Includes sterols, free fatty acids, free alcohols, and pigments.

TABLE 3

Starvation Experiment with Females of the Copepod *Euchaeta Japonica* Collected at Indian Arm Inlet, British Columbia in Net Hauls from 200m

The copepods were kept in seawater at 10°C. Values are expressed as weight percent of total lipid.

Lipid Type	Hours of Starvation	
	0	168
Hydrocarbons	2	2
Wax esters*	54	68
Triglycerides	18	2
Polar lipids†	12	4
Phospholipids	14	20
Total Lipid (percent of body dry weight)	41	39

* Includes a trace of sterol esters.
† Includes sterols, free fatty acids, free alcohols, and pigments.

A study of the changes of lipid composition during the life cycle of *Calanus helgolandicus* and *Euchaeta japonica* has indicated some important differences between these two copepods. In *C. helgolandicus* the eggs, the nauplii, and copepodite-I do not contain wax esters, but an increasing amount of lipid and proportion of wax ester occurred from copepodite-III to copepodite-V (Table 6). *E. japonica* showed sizeable amounts of wax esters in the eggs and also in the first naupliar stage, but a decreasing amount of lipid and wax esters was found in the remaining naupliar stages and in the first copepodite stage. An increase in lipid and proportion of wax was found from copepodite-III to V (Table 7). The largest quantity of lipid and wax esters was obtained in the copepodite-V of all species of copepods examined (Table 6 and 7, and unpublished work.)

Two cruises aboard the R/V MELVILLE enabled us to collect copepods from various depths down to 2500 m. Preliminary results are shown in Table 8. The deep-water copepods (below 750 m) appeared to have a higher proportion of wax ester content (expressed as a per cent of the total lipid) than did the near-surface copepods. The copepods collected at 2000 m showed no significant differences in total lipid or wax ester content from copepods collected at 1000 m. Why the bathypelagic copepods should have a higher lipid and wax ester content is an intriguing question. The high lipid content appears to function as a reserve fuel during long periods of nutritional stress, but the reason for the replacement of triglyceride by wax esters in copepods as the depth of the habitat increases or the temperature decreases is not easily answered. We have evidence that there is a slower rate of utilization of wax esters relative to triglycerides. Analysis of zooplankters other than copepods and micronekton captured in mid-water trawls taken to 2500 m also showed the presence of appreciable amounts of wax esters (Table 9).

At higher latitudes, such as the Arctic or the North Pacific off British Columbia, we have noted that the near-surface copepods (upper 200 m) have both high lipid and wax ester content. In Table 10 we report the seasonal change in lipid composition of two Arctic copepods, *Calanus hyperboreus* and *Metridia longa*, which were obtained at monthly intervals (November, 1969 to October, 1970) from the Arctic Island T-3. The storage of lipid occurred during the months of July, August, September, and October, which is correlated with the phytoplankton blooms in this area (English, 1961: Grainger, 1959: Marshall, 1958). During the dark winter months, these lipid reserves serve as fuels as indicated by the gradual decrease in lipid content (Table 10). *Calanus hyperboreus* has been shown by Mullin (1963) to be mainly herbivorous; the lipid reserve allows this animal to survive during the remaining 7 months of phytoplankton paucity. The females in June, which contain 30% lipid may be from a different generation than the females in July which contain 73% lipid. The females produce eggs in January and February (Conover, 1965) and would presumably not live much longer.

TABLE 4

Fatty Acid Composition of Wax Esters from Several Species of Calanoid Copepods

Values are weight % as methyl esters.

Fatty Acid*	Species of Copepod				
	Rhincalanus nasutus	*Gaussia princeps*	*Calanus hyperboreus*	*Calanus helgolandicus*	*Gaetanus brevicornis*
12:0	t	--	t	--	t
12:1	t	--	--	--	t
13:0	--	--	--	--	t
14:0	1.4	0.3	4.3	11.8	0.4
14:1	0.4	--	0.5	--	0.7
15:0	--	0.2	--	--	--
15:1	--	--	--	--	t
16:0	2.9	0.8	2.6	29.0	0.5
16:1	38.9	8.8	27.9	13.2	12.7
16:2	t	t	--	--	0.5
16:3	2.7	--	1.3	t	--
17:0	--	t	t	--	--
17:1	t	2.6	--	0.6	--
18:0	t	0.3	--	4.3	2.5
18:1	32.4	70.5	5.4	12.1	63.7
18:2	5.9	1.7	2.2	3.6	0.5
18:3	3.2	0.5	--	--	--
20:0	t	1.8	--	--	t
20:1	--	5.6	14.4	7.3	4.7
20:2	0.3	--	t	5.1	--
20:3	--	--	--	0.6	--
20:4	2.3	0.2	13.7	7.6	--
20:5	9.2	5.8	23.1	2.0	11.3
22:0	--	--	--	--	--
22:1	--	--	1.1	--	--
22:2	t	0.8	--	--	0.5
22:6	--	--	3.2	2.1	1.9

* Chain length: number of double bonds.

TABLE 5

Long Chain Alcohol Composition of Wax Esters from Several Species of Copepods

Values are weight % as trifluoroacetate esters.

Alcohol*	Species of Copepod				
	Rhincalanus nasutus	*Gaussia princeps*	*Calanus hyperboreus*	*Calanus helgolandicus*	*Gaetanus brevicornis*
12:0	t	t	t	--	t
12:1	t	--	--	--	--
14:0	18.8	7.6	5.5	1.5	6.7
14:1	t	t	--	--	4.6
15:0	--	1.5	t	--	t
15:1	--	t	t	--	0.1
16:0	52.1	54.9	61.7	13.0	44.5
16:1	0.7	t	9.6	1.0	1.0
16:2	--	--	--	--	--
16:3	--	--	--	--	--
17:0	--	--	--	--	--
17:1	--	3.0	0.8	1.2	5.2
18:0	15.7	1.2	0.1	0.8	1.7
18:1	--	11.6	2.2	3.8	11.3
18:2	--	0.5	t	0.2	4.3
18:3	3.0	--	--	--	--
20:0	0.9	--	--	--	t
20:1	t	4.9	14.3	18.4	5.1
20:2	5.4	--	--	--	--
20:3	--	--	--	--	--
20:4	0.3	--	--	40.9	--
20:5	1.4	--	--	--	--
21:0	--	--	0.3	--	--
22:0	0.2	--	--	--	--
22:1	0.3	11.5	5.1	--	5.2
22:2	0.5	--	--	--	--
22:3	--	--	--	8.4	--
22:4	0.4	--	--	--	--
22:6	--	--	--	8.1	--
23:1	--	--	--	--	2.2
24:1	0.4	3.3	0.1	--	7.9
24:5	--	--	--	1.7	--

* Chain length: number of double bonds.

3. *Fish*

The manner in which the copepod wax esters are digested by sardines and anchovies was the subject of our investigation into the alteration of the lipid composition of prey by predators in a marine food chain. The first operation involves hydrolytic cleavage of the wax ester catalyzed by a wax ester lipase. The wax ester lipase activity was highest in the pyloric caecum of the anchovy. Liver, red muscle, and white muscle wax ester lipase was much less active. Wax ester lipase releases long chain alcohols, but an analysis of both sardine and anchovy blood showed an absence

of long chain alcohols. The long chain alcohols may be oxidized to the corresponding fatty acids before being released into the blood. Analysis of serum lipoproteins of sardine blood (Table 11) showed large amounts of cholesterol esters.

4. Uptake of Petroleum Hydrocarbons by Marine Invertebrates

Blumer's *et al.* (1970b) recent study of oysters after an oil spill in Buzzards Bay, Massachusetts, indicated that oysters were able to take up petroleum hydrocarbons. We were interested in doing laboratory studies concerned with the uptake and discharge of petroleum hydrocarbons by various marine invertebrates. We used the mussel, *Mytilus californianus,* for our work, since it is a filter feeder easily obtained in our area. Purified paraffinic mineral oil was used in our experiments. It contained no aromatic compounds, and consisted only of straight chain, branched chain, and cycloparaffins. Uptake of a suspension of mineral oil in sea water was rapid during the first 24 hours, but no further increase was observed after 10 days of exposure to mineral oil (Table 12). Sonication of the mineral oil with Celite (diatomaceous earth) in the sea water did not increase uptake rate, nor did it increase the total amount of uptake in 48 hr. Discharge of the mineral oil was rapid and 99% of the mineral oil was discharged in 4 days after exposure to 7 liters of oil-free sea water. After exposure to oil-free sea water for 120 hr, the residual mineral oil could still be detected by gas chromatography of the hydrocarbon fraction from the mussel. Approximately 1% of the lipid of *M. californianus* is hydrocarbon. The identification of these natural hydrocarbons has not been completed, but a branched C_{19}, C_{20}, and C_{21} were important natural hydrocarbons. The mineral oil hydrocarbons were quite different from natural mussel hydrocarbons, and higher boiling point components

TABLE 6

The Changes in Wax Esters and Other Lipids During the Life Cycle of *Calanus Helgolandicus*

Fertile female *Calanus helgolandicus* were collected offshore and fed a large concentration (600μg C/liter) of *Thalassiosira fluviatilis* which caused the females to deposit their eggs. Approximately 1000 eggs were collected and placed in filtered sea water to remove diatoms and 200 eggs were allowed to become naupliar 1. Thus 200 naupliar 1 and 800 eggs were extracted for lipid analysis. Another batch of eggs was used to obtain the different copepodid stages which were fed on a mixture of *Lauderia borealis* and *Gymnodinium.*

Stage	Lipid (percent of dry weight)	Wax Ester (percent of total lipid)	Triglyceride (percent of total lipid)
Eggs	--	absent	60
Naupliar I	--	absent	15
Copepodid I	--	absent	12
Copepodid III (early)	--	1	5
Copepodid III (late)	--	10	2
Copepodid IV (early)	--	16	6
Copepodid IV (late)	--	22	5
Copepodid V	--	50	3
Adult	28	41	12

TABLE 7

The Changes in Wax Esters and Other Lipids During the Life Cycle of *Euchaeta Japonica*

Females with egg-sacs were collected in vertical net hauls (0–200m) at Indian Arm Inlet near Vancouver in September, 1971. The egg-sacs were removed from the females and placed in filtered sea water. Naupliar stages 2–4 were reared in the filtered sea water. Naupliar stages 5 to copepodid II were raised in the laboratory by feeding a mixture of *Dunaliella tertolecta* and *Phacodactylum tricornutum* (Lewis and Ramnarine, 1968). Lipid extraction and analysis were performed as described in *Methods.*

Stage	Lipid (percent of dry weight)	Wax Ester (percent of total lipid)	Triclyceride (percent of total lipid)
eggs	64.4	58	19
eggs (late stage)	59.1	50	17
Naupliar 2	43.8	61	17
Naupliar 3	30.8	56	5
Naupliar 4	25.0	20	3
Naupliar 5	21.2	15	1
Naupliar 6	17.1	12	0
Copepodid I	14.2	9	0
Copepodid I (field-collected)	23.6	29	0
Copepodid II	11.6	12	0
Copepodid IV (field-collected)	31.2	40	3
Copepodid V (field-collected)	50.1	81	2
Adult (field-collected)	41.3	54	18

TABLE 8

Lipid Composition of Calanoid Copepods from Various Depth Intervals Collected During Two Cruises of the R/V *Melville*

Species	Lipid per individual (mg)	Percent lipid of dry weight	Percent triglyceride of total lipid	Percent wax ester of total lipid
Sample depth interval: 1–10 m				
Candacia sp.	0.02	9	11	1
Euchirella sp.	0.14	19	37	absent
Undeuchaeta sp.	0.16	21	30	1
Calanus gracilis	0.08	26	17	21
Rhincalanus nasutus	0.12	48	9	69
Sample depth interval: 0–250 m				
Scottocalanus persecans	0.11	8	22	absent
Gaidius sp.	0.05	12	18	2
Pleuromamma xiphias	0.03	16	31	3
Gaetanus sp.	----	29	2	36
Eucalanus bungii	0.08	31	42	1
Sample depth interval: 325–625 m				
Metridia princeps	0.10	12	4	41
Gaetanus unicornis	0.20	16	26	5
Undeuchaeta sp.	0.11	18	38	14
Pleuromamma sp.	0.08	19	32	2
Disseta sp.	0.44	29	4	51
Euchirella galeata	0.06	43	20	absent
Disseta maxima	1.30	45	10	67
Paraeuchaeta sp.	0.65	46	2	68
Euaugaptilus sp.	0.30	50	2	59
Sample depth interval: 625–750 m				
Metridia sp.	0.30	24	5	72
Disseta sp.	0.20	27	4	69
Lucicutia bicornuta	0.14	31	12	63
Sample depth interval: 750–1600 m				
Bathycalanus sp.	31.1	59	11	77
Heterorhabdus sp.	0.11	62	1	82
Sample depth interval: 1300–2500 m				
Lucicutia sp.	0.07	15	1	50
Gaetanus sp.	----	47	4	62

of mineral oil make it easy to identify in the mussel.

Uptake and discharge rates of ^{14}C-heptadecene by the mussel were similar to that of mineral oil (Table 13). The aromatic hydrocarbon, 1, 2, 3, 4-tetrahydronaphthalene, killed the mussels at very low concentrations, but uptake at one hundred parts per million was shown by gas chromatography of the aromatic fraction.

TABLE 9

Various Zooplankton and Micronekton Taken in a 3-m Isaacs-Kidd Midwater Trawl from 0–2500 m

Lipid extraction and analysis was performed as described above.

Taxonomic group	Lipid (Percent of dry weight)	Triglycerides (Percent of total lipid)	Wax ester (Percent of total lipid)
Alciopidae sp. (Polychaeta)	55	11	76
Gammaridae (Amphipod)	57	13	73
Chaetognatha sp. A	13	22	18
Chaetognatha sp. B	40	11	71
Oegopsidae sp. (Cephalopoda)	23	6	27
Gennadas sp. (Mysidacea)	21	35	10
Gnathophausia sp. (Mysidacea)	42	12	69
Argyropelecus sp. (Hatchet fish)	--	7	22
Stomias atriventer	--	32	9
Cyclothone sp.	22	16	53

TABLE 10

Arctic Copepods

Date of Collection	Depth (meters)	Lipid (percent of dry weight)	Triglyceride (percent of total lipid)	Wax ester (percent of total lipid)
Species—*Calanus hyperboreus* (female)				
Nov 18	0–500m	62.0	2	92
Feb 27	0–100m	46.5	1	67
March 17	0–100	46.7	2	70
April 18	0–100	41.6	1	61
June 6	300–400	37.8	absent	52
June 6	50–150	30.4	absent	46
July 8	300–400	63.2	2	79
July 8	50–150	76.6	2	93
Aug 14	300–400	72.3	6	88
Aug 14	50–150	66.2	4	88
Sept 9	50–150	62.1	2	93
Oct 8	50–150	63.7	3	91
Species—*Metridia longa* (female)				
Nov 18	0–500	56.9	2	81
Feb 27	0–100	52.9	9	76
March 27	410–500	59.1	2	75
April 18	0–100	31.2	absent	36
June 6	300–400	37.2	1	29
June 6	50–150	30.4	absent	24
July 8	300–400	63.2	3	59
July 8	50–150	76.6	4	92
Aug 14	300–400	72.3	6	90
Aug 14	50–150	56.2	7	82
Sept 9	50–150	52.1	9	72
Oct 8	50–150	53.7	6	70
Oct 8	300–400	57.3	10	76
Species—*Calanus finmarchicus* (copepodite V)				
Nov 7	0–500	52.4	1	62
Species—*Calanus finmarchicus* (female)				
Nov 7	0–500	50.4	9	51
Species—*Euchaeta* sp. (copepodite V)				
Nov 7	0–500	30.8	5	46

DISCUSSION

1. Copepods

The studies of many workers, including our own, have emphasized the importance of lipid to marine zooplankton. Our early work was done on *Calanus helgolandicus* cultured at various concentrations of food (Lee *et al.*, 1970a, 1971b). A linear relationship was noted between the amount of food and the per

TABLE 11

Lipoproteins of Sardine Blood

Blood was taken from sardines and serum lipoproteins were fractionated into three density classes by the method of preparative ultracentrifugation. These density classes are very low density lipoproteins, VLDL (less than 1.006gm/cc); low density lipoproteins, LDL (1.006–1.063 gm/cc); and high density lipoproteins, HDL (1.063–121 gm/cc). The lipid of each of these density classes was fractionated into the different lipid classes (hydrocarbons, sterol esters, triglycerides, polar lipids, and phospholipids) by silicic acid column chromatography.

Lipid Type	Lipoprotein Class		
	VLDL	LDL	HDL
Hydrocarbons	trace	trace	trace
Sterol Esters	16	16	20
Triglycerides	31	31	10
Polar lipids*	15	15	15
Phospholipids	35	35	55

* Includes sterols, free fatty acids, and pigments.

TABLE 12

Uptake of Mineral Oil by the Mussel, *Mytilus Californianus*

The mussels were placed in a 7 liter aquarium and a mineral oil "slick" was formed by adding approximately 1 gram of oil. Mussels were taken out after various times of exposure and the procedure for analysis is given in *Methods*.

Time of Exposure (days)	mg of hydrocarbon/gm of mussel	percent of hydrocarbon (percent of lipid)
0	0.5	3
2	5.9	30
4	6.1	36
10	4.8	--

TABLE 13

Uptake and Discharge of ^{14}C-Heptadecene by the Mussel, *Mytilus Californianus*

Mussels were placed in a 500 ml beaker filled with sea-water containing ^{14}C-heptadecene (approximately 2×10^6 cpm), which was dispersed by sonication. Mussels were removed at various times and analysis performed as described in *Methods*.

Hours	Cpm/gm of wet tissue
3	45×10^2
5	180
24	1135
Transferred to radioactive-free sea water after 24 hours exposure to ^{14}C-heptadecene.	
20	20
48	80

cent lipid of the body dry weight. The number of carbon atoms in the wax esters of *C. helgolandicus* decreased with a decrease in the concentration of food, which indicated that the proportion of saturated wax esters probably increased at low food concentrations. Much of our recent work has been concerned with calanoid copepods, many of which contained large quantities of wax esters as reserve lipids. Wax esters were shown to be metabolized after triglyceride utilization was completed; wax esters are therefore probably the more lasting sources of reserve energy.

Littlepage (1964), Raymont *et al.*, (1969), and Orr (1934) have reported seasonal changes in the biochemical composition of zooplankters including euphausiids, copepods, and other crustaceans. Our seasonal study of lipid from Arctic copepods has indicated that the wax esters are slowly consumed during the long winter months. The copepodite stages (C-I to C-V) of wax ester-containing adult copepods have wax esters, but the eggs and naupliar stages of these same copepods may or may not have wax esters. The C-V contains the largest amount of wax esters of any stage in the life history (Tables 6 and 7), and the C-V is the stage which allows the copepod to "over-winter" or to withstand other times of food scarcity.

A study of the lipids of copepods from various depths (0 to 2500 m) has indicated that copepods from the depths below about 750 m invariably contain large amounts of wax esters and lipid, while copepods nearer the surface (0 to 250 m) contain less lipid and often do not contain appreciable amounts of wax esters.

2. *Food Chains*

An examination of food chains at shallow and great depths allows some interesting observations. A study of a near surface food chain consisting of phytoplankton—*C. helgolandicus*—anchovy and sardine, has shown the presence and often predominance of wax esters in the copepod, *C. helgolandicus,* but complete absence of wax esters in the phytoplankton and anchovies and sardines. Thus, wax ester lipases are probably essential enzymes in copepod-eating fish. The fatty acid composition of the wax esters of *C. helgolandicus* has been shown to closely resemble the fatty acid composition of the species of phytoplankton used as food (Lee *et al.*, 1971b) and therefore synthesis of wax ester is supplied by acids in the diet. A study of sub-tropical copepods indicated that many of the copepods in the upper 200 m have very low levels of both lipid and wax esters. On the other hand, a study of bathypelagic food chains indicates that perhaps all members of these food chains contain sizeable amounts of wax esters, since an examination of all specimens taken below 1000 m showed appreciable amounts of wax esters (Tables 8 and 9). The work of Nevenzel (1969, 1970) and our present studies reveal the importance of wax esters in the deep-water fish belonging to the families Myctophidae, Latimeridae, Gempylidae, Gonostomatidae, and Stomatidae. Copepods were observed in the stomachs of deep-water myctophids, gonostomatids, and stomatids by Gordin *et al* (unpublished data), so that wax esters in the copepods may go into the fish without modification to serve as fuel reserves.

Many of the copepods and fish obtained in deep midwater trawls are large relative to forms living in shallower water. Abyssal copepods have well-developed raptorial feeding apparatus, and the fish have developed a carnivorous mode of feeding adapted to infrequent encounters with other large individuals (Hardy, 1958). If most deep-living organisms do in fact feed infrequently at very low concentrations of prey, it would be of survival value for the predator to have: 1) an efficient digestive system, 2) a low metabolic rate, and 3) a large reserve of energy to sustain metabolic needs related to their environment. It therefore appears intuitively reasonable that near-surface omnivorous copepods living in subtropical or tropical regions do not need large energy reserves in the form of lipids, and that deep-living groups must store large energy reserves.

In the case of near-surface copepods from boreal and polar regions, much of the annual primary production occurs in short blooms during a few spring or summer months (McAllister *et al.*, 1960). Herbivores must feed and store this energy in preparation for winter months of relatively lower production and food abundance. When the supply of food is inhomogeneously distributed in time and is relatively abundant for only short periods, it should be an advantage for an organism to have a metabolism similar to that of the deep-living carnivores described above. One would expect that the deep-living organisms of high latitudes also follow the metabolic pattern of carnivores in deep water at lower latitudes.

3. *Hydrocarbons*

An important natural hydrocarbon of marine phytoplankton is the polyunsaturated 21:6 hydrocarbon. This hydrocarbon is not generally found at higher levels of the food chain although a few copepods, such as *Rhincalanus nasutus* and *Euchaeta japonica,* can store the 21:6 hydrocarbon (Blumer *et al.*, 1970a and the present work). Pristane is generally found in members of a food chain along with a series of straight chain compounds from C_{15} to C_{36} (Clark and Blumer, 1967; Lee *et al.*, 1971a).

A study of the distribution of "natural" hydrocarbon is important for any study of petroleum hydrocarbon uptake. Since marine life contains significant amounts of "natural" hydrocarbon, biosynthesis and metabolism of hydrocarbons occurs in these systems. Studies with *Mytilus californianus* demonstrated that filter feeding mussels can rapidly take up petroleum hydrocarbons (Tables 12 and 13). Discharge of the petroleum hydrocarbons is also rapid, but a low level of the petroleum hydrocarbons will remain for an unknown length of time. We have recently begun studies on the uptake of petroleum hydrocarbon by zooplankton, so that questions concerning the effects of oil spillage on a marine food chain can perhaps be answered.

SUMMARY

1. Wax esters are a major lipid of bathypelagic copepods and copepods from all depths in polar and temperate waters. Starvation experiments with copepods demonstrated that triglycerides serve for short-term fuel needs, while wax esters have a secondary reserve function.

2. In members of the shallow-water marine food chain, phytoplankton—copepods—anchovies and sardines, only the copepods contained wax esters, so that active synthesis of wax esters from phytoplankton lipid occurred. Sardines and anchovies contained an active wax ester lipase in their pyloric caecum, for the hydrolysis of wax esters from their copepod food.

3. All members of bathypelagic food chains contained high amounts of lipid and wax esters. Deep-living copepods in tropical and subtropical regions were found to contain a higher amount of lipid (as per cent of dry weight) and a higher content of wax esters (as per cent of lipid) than did copepods from more shallow waters. Upper water (upper 250 m) copepods contained polyunsaturated alcohols in their wax esters, while saturated and monounsaturated alcohols were found in the wax esters of bathypelagic species.

4. Seasonal studies of Arctic copepods (*Calanus hyperboreus* and *Metridia longa*) demonstrated that a large buildup of lipid in the form of wax esters occurred in the phytoplankton-rich summer months (July through October) followed by slow utilization of the wax esters during the dark winter months.

5. A study of "natural" hydrocarbons in marine algae showed the presence of large amounts (1/10% of the lipid) of a polyunsaturated hydrocarbon, 21:6 (21 carbons and 6 double bonds). This and similar hydrocarbons are good markers for "natural" hydrocarbons because they are absent from petroleum hydrocarbon. Preliminary results with the mussel, *Mytilus californianus*, indicated that petroleum hydrocarbons (mineral oil) are rapidly taken up by these filter feeders without apparent toxic effects. Discharge of the petroleum hydrocarbons was rapid when the mussels were placed in oil-free sea water. The aromatic hydrocarbon, 1, 2, 3, 4-tetrahydronaphthalene, shows toxic effects on mussels at low concentrations (10 parts per million) and like compounds may have future detrimental effects on the marine environment.

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