FATE OF PETROLEUM HYDROCARBONS IN MARINE ZOOPLANKTON

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ABSTRACT

Several groups of zooplankton from the coasts of California, British Columbia, and in the Arctic, including copepods, euphausiids, amphipods, crab zoea, ctenophores, and jellyfish rapidly took up ^{3}H-benzpyrene, ^{14}C-benzpyrene, ^{3}H-methylcholanthrene, and ^{14}C-naphthalene from seawater solution. These hydrocarbons were metabolized to various hydroxylated and more polar metabolites by crustaceans but not by ctenophores or jellyfish. Up to 22×10^{-4} µg of benzpyrene was ingested by the temperate water copepod ***Calanus plumchrus****, and transfer of this copepod to fresh seawater resulted in the discharge of most benzpyrene with less than 1×10^{-5} µg remaining after 17 days. When depuration was continued beyond 17 days, no further hydrocarbon loss was observed.* ***Calanus hyperboreus*** *from the Arctic took up to 11×10^{-4} µg of ^{3}H-benzpyrene and a 28-day depuration experiment still showed the presence of benzpyrene in the copepod although again less than 1×10^{-5} µg.*

INTRODUCTION

The results of analyses of marine organisms exposed to oil spills has demonstrated their ability to take up and store hydrocarbons [1-5]. Under laboratory conditions several species of fish and benthic invertebrates also take up and metabolize petroleum hydrocarbons [6-8]. Benthic molluscs, however, apparently lack the ability to metabolize hydrocarbons [8]. The work described here is concerned with the uptake, metabolism, storage, and discharge of petroleum hydrocarbons by marine zooplankton, an important link in the marine food web. Both paraffinic and polycyclic aromatic hydrocarbons were added to seawater containing various species of zooplankton collected off California, British Columbia, and in the Arctic.

Methods

1. Collecting and maintenance of zooplankton

Zooplankton were collected on cruises off California and British Columbia, Canada, using nets of 0.333- or 0.505-mm mesh. In addition copepods were collection with plankton nets through holes in the ice on Fletcher's Ice Island (T-3) in the Arctic. Zooplankton collected for the experiments were the following species: (1) copepods (*Calanus plumchrus, Calanus pacificus, Eucalanus bungii, Gaetanus intermedius*); ctenophore (*Pleurobrachia pileus*); amphipods (*Parathemisto pacifica, Euprimno abyssalis, Cyphocaris challengeri*); euphausiids (*Euphausia pacifica, Thysanoessa raschii*);and unidentified species of jellyfish and crab zoea from British Columbia; (2) copepods (*Calanus hyperboreus*) from the Arctic; (3) copepods (*Calanus helgolandicus, Euchaeta media, Rhincalanus nasutus, Labidocera trispinosa*, and *Acartia tonsa*) from California. The animals were sorted from the samples into homogenous groups as to species and stage or sex as soon as possible after capture. Animals were maintained in the laboratory in seawater passed through a 0.45 µfilter (Millipore Co.). For feeding experiments, copepods were fed 6-day cultures of the diatom *Skeletonema costatum* at a concentration equivalent to 500 of carbon per liter.

2. Radioactive hydrocarbon experiments

Animals were placed in one liter beakers containing 800 ml of filtered seawater which contained one of the following isotopes: ^{14}C-1-naphthalene (15.6 µci/mg), ^{14}C(3,6)-3, 4-benzpyrene (76 µci/mg), ^{14}C-1-octadecane (98 µci/mg), ^{3}H(G)-3, 4-benzpyrene (52 mci/mg) and ^{3}H(G)-20-methylcholanthrene (56 mci/mg). All isotopes were purified on silicic acid thin-layer plates before use and were dissolved in either benzene or ethanol before addition to the seawater. To follow losses from the water, a 1-ml sample was counted each time zooplankton were removed for extraction and counting. In experiments with ^{3}H-benzpyrene the water contained 50 ppb of petroleum hydrocarbons made by adding an aliquot of a stock solution of fuel oil #2 containing 15 ppm petroleum hydrocarbons. For depuration experiments, zooplankton were transferred to filtered seawater, free of radioactive hydrocarbons after the zooplankton were exposed for various periods to radioactive hydrocarbons. The water was changed every four days. At specified times, zooplankton were removed, rinsed with seawater, and their lipids extracted ($CHCl_3$:MeOH, 2:1 v/v). The lipid extract was dried under nitrogen and taken up in benzene: methanol (10:1 v/v). The radioactivity of the extract was determined by liquid scintillation counting (Beckman DPM-100). The carcass left after lipid extraction was also minced and counted to determine radioactivity not extracted. A portion of the lipid extract was applied to silicic acid thinlayer plates (Merck and Co.). After the application of authentic standards to help locate the position of the hydrocarbon and hydroxylated metabolites, plates were run in benzene:ethanol (10:1 or 3:1 v/v). The hydrocarbon and the different metabolites were scraped from the thin-layer plates, placed in scintillation fluid, and counted. As a check, the mobility of the radioactive hydrocarbon and metabolites were determined by radioautography using Single Coated Blue Sensitive X-ray film (Eastmak Kodak Co.).

3. Adsorption, loss by volatilization, photooxidation and bacterial decomposition of hydrocarbons

Between 10% and 15% of the radioactive hydrocarbon was adsorbed to the sides of the beakers and thus was lost from the water phase. Most of the work was with high molecular weight hydrocarbons, and little loss to the vapor phase was expected. However, using gas-tight containers it was found that up to 10% of the benzpyrene, octadecane and methylcholanthrene occurred in the vapor phase. The naphthalene was readily lost from the containers, and there was a gradual decrease in the seawater in all containers, so that after 4 days approximately 50% of the naphthalene had entered the vapor phase. In ten days approximately 10% of both benzpyrene and methylcholanthrene was oxidized in 800 ml of filtered seawater in the dark. A further loss was due to bacterial decomposition of the hydrocarbons. Using gas-tight

bottles and measuring $^{14}CO_2$ released, the bacterial decomposition of ^{14}C-hydrocarbons in filtered seawater was 1% in 10 days.

Results

Most of the experiments were with copepods because of ease of maintenance and low mortality. However, short-term experiments (generally less than 5 days) were carried out with other zooplankton groups; and the results with these species will be discussed. The problem of the form of the hydrocarbon, whether dissolved or dispersed in seawater, was of some concern. A series of samples (0.1 ml) from different parts of the containers indicated that the radioactivity was evenly distributed throughout the containers. According to available data, the hydrocarbons, at the concentrations used, should be in the dissolved state [9-11].

1. Uptake

During the first 24 hours there was a linear increase in the amount of hydrocarbon taken up by zooplankton and a more gradual increase over the next 24 hours. No increase in the amount of hydrocarbon per animal was noted after 3 days (figure 1; tables 1, 3, 5, 6, 8). The size of the animal appeared to be a factor in the amount of hydrocarbon taken up since the large copepods, *Calanus plumchrus* (1.0 mg, dry wt.) and *Calanus hyperboreus* (2.2 mg dry wt.), took up 3 times as much ^3H-benzpyrene as the smaller copepod, *Calanus helgolandicus* (0.3 mg, dry wt.). Since *C. plumchrus* and *C. hyperboreus* were maintained at a lower temperature than *C. helgolandicus*, metabolic rate differences may also have influenced uptake. *C. plumchrus* from temperate water had up to 22 $\times 10^{-4}$ μg of ^3H-benzpyrene, whereas *C. hyperboreus* from Arctic waters took up only 11 $\times 10^{-4}$ μg. Using data from table 1, approximately 500 *C. plumchrus* should clear 800 ml of water of 1 μg of benzpyrene in two days. Since this water actually contained 50 ppb of petroleum hydrocarbons and assuming the uptake rate of penzpyrene is representative of other hydrocarbons, then actually 50 ppb of petroleum could be taken from the water by the copepods.

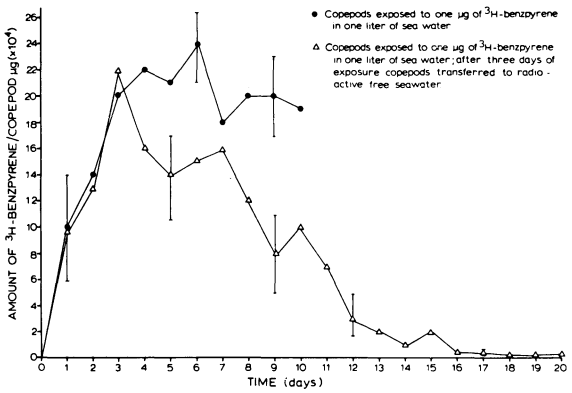

Figure 1. Uptake and discharge of ^3H-benzpyrene by the copepod, *Calanus plumchrus*

The amount of naphthalene taken up (1 $\times 10^{-2}$ μg) was the highest of any hydrocarbon but naphthalene was also present in the highest concentration in the water (table 8). Benzpyrene, methylcholanthrene, and octadecane reached levels of 22 $\times 10^{-4}$, 5 $\times$

Table 1. Uptake and discharge of ^3H-benzpyrene by the copepod, *Calanus plumchrus*

Each one-liter beaker contained 6 copepods, 800 ml of filtered seawater at 14°C, 50 ppb of a water extract of fuel oil #2 and 1 μg of ^3H(G)-3, 4-benzpyrene (50 $\times 10^6$ cpm). For the depuration experiments copepods were transferred to one liter of filtered seawater. The radioactivity listed for each time interval is the mean for 6 copepods extracted and counted separately. Benzpyrene is abbreviated by BP.

Time with ^3H-BP (days)	Depuration time (days)	Radioactivity per copepod (cpm)	Amount of ^3H-BP per copepod [μg(x10^4)]	Amount of ^3H-hydroxyBP per copepod [μg(x10^4)]	Amount of ^3H-polar metabolites of BP per copepod [μg(x10^4)]
1	-	55,000	10	0	1
2	-	80,000	14	1	2
3	-	130,000	22	1	3
3	1	120,000	16	2	6
3	2	90,000	14	1	3
3	3	80,000	15	0	1
3	4	90,000	16	0	1
3	5	70,000	12	1	1
3	6	70,000	8	1	5
3	7	60,000	10	1	1
3	8	60,000	7	1	4
3	9	40,000	3	1	4
3	10	25,000	2	0	3
3	17	400	<0.1	<0.1	<0.1

Table 2. Uptake and discharge of ^{3}H-methylcholanthrene by the copepod *Calanus plumchrus*

Each one-liter beaker contained 6 copepods, 800 ml of filtered seawater at 14°C and 0.2 μg of ^{3}H(G)-20-methylcholanthrene (10 x 10^6 cpm). For the depuration experiments copepods were transferred to one liter of filtered seawater. The radioactivity listed for each time interval is the mean of 6 copepods extracted and counted separately.

Time with ^{3}H-methylcholanthrene (days)	Depuration time (days)	Radioactivity per copepod (cpm)	^{3}H-methylcholanthrene per copepod [μg(x10^4)]
0.5	-	13,000	2.4
0.5	1	10,000	1.6
0.5	2	3,000	0.4
0.5	3	2,500	0.3
0.5	4	900	0.1
1	-	28,000	5.2
1	1	26,000	4.4
1	2	18,000	2.8
1	3	10,000	1.6
1	8	2,200	0.3

Table 3. Uptake and discharge of ^{14}C-1-oxtadecane by the copepod *Calanus plumchrus*

Each one-liter beaker contained 6 copepods, 800 ml of filtered seawater at 14°C and 5 μg of ^{14}C-1-octadecane (1 x 10^6 cpm). The activity listed for each time interval is the mean of 3 groups of copepods with each group consisting of 6 copepods. Copepods were transferred to one liter of filtered seawater for depuration experiments.

Time with ^{14}C-octadecane (days)	Depuration time (days)	Radioactivity per copepod (cpm)	^{14}C-octadecane per copepod [μg(x10^4)]
1	-	75	3.1
2	-	120	4.6
3	-	170	5.2
4	-	100	2.2
2	3	40	0.1

Table 4. Uptake and discharge of ^{3}H-benzpyrene by the copepod *Calanus helgolandicus*

Each one-liter beaker contained 6 copepods, 800 ml of filtered seawater at 16°C, 50 ppb of fuel oil #2 water extract and 1 μg of ^{3}H(G)-3,4-benzpyrene (50 x 10^6 cpm). For the depuration experiments copepods were transferred to one liter of filtered seawater. The radioactivity listed for each time interval is the mean for 6 copepods extracted and counted separately.

Time with ^{3}H-benzpyrene (days)	Depuration time (days)	Radioactivity per copepod (cpm)	^{3}H-benzpyrene per copepod [μg(x10^4)]
1	-	28,000	5.0
2	-	30,000	5.6
2	1	25,000	4.4
2	2	21,000	3.6
2	3	15,000	2.2
2	5	5,000	0.6
2	7	2,000	0.2
2	9	500	0.1

10^{-4} and 5 and 10^{-4} μg respectively in *Calanus plumchrus* (tables 1, 2, 3).

To study the adsorption of hydrocarbons, copepods (*Calanus helgolandicus*) were removed at various times from beakers containing ^{3}H-benzpyrene. The copepods were successively rinsed with hexane and each rinse was counted separately. There was a progressive decrease in the radioactivity so that the fourth rinse had no radioactivity. Radioactivity extracted by chloroform:methanol (2:1 v/v) after four rinses with hexane was assumed to be hydrocarbon or its metabolites contained within the copepod. The adsorption of hydrocarbon showed a linear increase for 12 hours but no further adsorption after that time. After 24 hours each copepod had 4.4 x 10^{-4} μg of ^{3}H-benzpyrene of which 1.1 x 10^{-4} μg was adsorbed (table 1). The adsorbed hydrocarbon was completely lost after 8 days of depuration. Uptake experiments with dead copepods (heat killed) showed that adsorption of ^{3}H-benzpyrene occurred.

Table 5. Uptake and discharge of ^{3}H-benzpyrene by the copepod *Calanus helgolandicus* during feeding

Each one-liter beaker contained 6 copepods, 800 ml of filtered seawater at 16°C, 50 ppb of fuel oil #2 water extract and 1 μg of ^{3}H(G)-3,4-benzpyrene (50 x 10^6 cpm). For the depuration experiments copepods were transferred to one liter of seawater containing diatoms (Skeletonema costatum) at a concentration equivalent to 500 μg of carbon per liter. The radioactivity listed for each time interval is the mean for 6 copepods extracted and counted separately.

Time with ^{3}H-benzpyrene (days)	Depuration time (days)	Radioactivity per copepod (cpm)	^{3}H-benzpyrene per copepod [μg(x10^4)]
1	-	26,000	4.4
2	-	29,000	5.2
3	-	32,000	5.8
4	-	28,000	5.2
2	1	20,000	3.6
2	2	18,000	3.0
2	3	7,000	0.8
2	5	1,500	0.1
2	7	140	<0.1

Table 6. Uptake and discharge of ^{3}H-benzpyrene by the Arctic copepod *Calanus hyperboreus*

Each one-liter beaker contained 6 copepods, 800 ml of filtered seawater at 3°C, 50 ppb of fuel oil #2 water extract and 1 μg of ^{3}H(G)-3,4-benzpyrene (50 x 10^6 cpm). The radioactivity listed for each time interval is the mean of 6 copepods extracted and counted separately. For the depuration experiments copepods were transferred to one liter of filtered seawater.

Time with ^{3}H-benzpyrene (days)	Depuration time (days)	Radioactivity per copepod (cpm)	^{3}H-benzpyrene per copepod [μg(x10^4)]
1	-	40,000	7.7
2	-	62,000	8.1
3	-	110,000	11.2
4	-	87,000	7.0
2	1	92,000	7.4
2	2	42,000	5.0
2	3	29,000	3.1
2	8	12,000	1.0
2	17	3,000	0.2
2	28	200	<0.1

Table 7. Uptake and discharge of ^{3}H-methylcholanthrene by the copepod *Euchaeta japonica*

Each one-liter beaker contained 6 copepods, 800 ml of filtered seawater at 10°C and 0.2 μg of ^{3}H(G)-20-methylcholanthrene (10 x 10^6 cpm). For the depuration experiments, copepods were transferred to one liter of filtered seawater. The radioactivity listed for each time interval is the mean of six copepods extracted and counted separately.

Time with ^{3}H-methylcholanthrene (days)	Depuration time (days)	Radioactivity per copepod (cpm)	^{3}H-methylcholanthrene per copepod [μg(x10^4)]
1	-	26,000	4.8
1	1	15,000	2.6
1	2	8,000	1.2
1	3	6,000	1.0
1	8	2,000	0.2
2	-	29,000	5.6
2	1	20,000	3.8
2	3	11,000	1.8
2	8	2,000	0.2

2. Metabolism of hydrocarbons by zooplankton

All of the crustaceans examined, which included copepods, amphipods, crab zoea, and euphausiids, had the ability to metabolize naphthalene, benzpyrene, methylcholanthrene, and octadecane. Octadecane was rapidly metabolized and excreted in contrast

Table 8. Uptake and discharge of ^{14}C-1-naphthalene by the copepod *Euchaeta japonica*

Each one-liter beaker contained 6 copepods, 800 ml of filtered seawater at 10°C and 80 μg of ^{14}C-1-naphthalene (2 x 10^6 cpm). The radioactivity listed for each time interval is the mean of 3 groups of copepods with each group consisting of 6 copepods. For the depuration experiments copepods were transferred to one liter of filtered seawater.

Time with ^{14}C-naphthalene (days)	Depuration time (days)	Radioactivity per copepod (cpm)	^{14}C-naphthalene per copepod [μg(x10^3)]
1	-	300	10
2	-	350	5
3	-	600	7
4	-	400	5
2	1	400	2
2	2	200	1
2	8	10	<0.1

Table 9. Uptake and discharge of ^{14}C-benzpyrene by amphipods

Each one liter beaker contained 800 ml of filtered seawater at 14°C, 3 amphipods and 15 μg of ^{14}C(3,6)-3,4-benzpyrene (4 x 10^6 cpm). The activity listed for each time interval is the mean for 3 amphipods extracted and counted separately. Amphipods were transferred to one liter of filtered seawater for depuration experiments.

Amphipod species	Time with ^{14}C-benzpyrene (days)	Depuration time (days)	Radioactivity per amphipod (cpm)	^{14}C-benzpyrene per amphipod [μg(x10^4)]
Parathemisto pacifica	1	-	32,000	11
	2	-	36,000	10
	2	1	29,000	5
	2	3	20,000	2
	2	14	100	<0.1
Cyphocaris challengeri	1	-	38,000	13
	2	-	45,000	15
	2	3	25,000	7
	2	6	3,000	<0.1

to slower metabolic losses of benzpyrene and methylcholanthrene (compare tables 1, 2, 3). Of the different zooplankton species, the amphipod *Parathemisto pacifica* showed the most rapid degradation of ingested hydrocarbons (table 9). After 24 hours, over 50% of ingested naphthalene, octadecane, benzpyrene, or methylcholanthrene was metabolized by this amphipod.

Such metabolites as naphthol, hydroxybenzpyrene, hydroxymethylcholanthrene and hydroxyoctadecane were identified by identical mobility on silicic thin-layer plates to authentic standards. Using different solvent systems more polar metabolites were tentatively identified, e.g., octadecanoic acid. There appeared to be rapid excretion of the metabolites since little storage was observed (tables 1-8). In table 1 the amounts of hydroxybenzpyrene and more polar metabolites of benzpyrene produced by *Calanus plumchrus* are presented. In the other tables only the amount of hydrocarbon is given but the quantity of the metabolites produced can be calculated by converting the total radioactivity to micrograms.

Experiments with the ctenophore *Pleurobrachia pileus* and an unidentified species of jellyfish showed no metabolism of benzpyrene. Two ctenophores were fed 10 copepods labeled with ^{3}H-benzpyrene (11×10^{-3} μg in the ten copepods), and after six days the ctenophores contained 2×10^{-3} μg of benzpyrene.

3. Storage

All zooplankton had detectable levels of hydrocarbon even after 28 days of depuration (tables 1-9). After 8 days of depuration the amount of hydrocarbon present was less than 1% of the total originally taken up. It may be that hydrocarbon remaining after a long depuration period is present in storage tissue and will not be discharged during the life of the animal.

4. Discharge

The discharge of hydrocarbons was studied by depuration experiments where animals, after various lenghts of exposure to radioactive hydrocarbons, were transferred to radioactive-free seawater. In all species discharge took place during these experiments (figure 1; tables 1-9) with a gradual decrease during the first few days and then a more dramatic decrease after 3 days. A residual amount of hydrocarbon remained in all animals even after a 28-day depuration experiment (table 6). Active feeding during depuration appeared to allow hydrocarbon to be discharged at a faster rate (compare tables 4 and 5). For *Calanus plumchrus*, no further decrease in stored hydrocarbon was seen after 17 days of depuration (figure 1). A comparison of discharge rates for different hydrocarbons indicated that methylcholanthrene was both metabolized and discharged faster than benzpyrene by *Calanus plumchrus* (compare tables 1 and 2).

5. Effects of petroleum extracts on zooplankton

Although the primary goal of the work was to study the transfer of hydrocarbons through zooplankton, a few effect studies were carried out. At the various concentrations of hydrocarbons used for uptake experiments no mortality of zooplankton was noted. Using a water extract of fuel oil #2 some mortality occurred after 24 hours when the concentration in the water was above 500 ppb. Below this concentration (between 500 and 200 ppb) a partial paralysis was noted, identified by the loss of ability to avoid a solid glass rod. The recovery of this response occurred after 24 hours. Also paralysis effects were not noted when the water was allowed to stand in an open beaker for 12 hours before copepods were added. We hypothesize that volatile components of petroleum produce this paralysis. Experiments with the copepod, *Calanus helgolandicus* and *Calanus plumchrus*, indicated that animals starved for longer than 7 days were more susceptible to petroleum at 500 ppb than feeding copepods.

Preliminary results (Lewis and Lee, unpublished data) have indicated that concentrations of petroleum above 100 ppb decrease the number of eggs of the copepod *Euchaeta japonica* which reached the second naupliar stage. Because of the importance of alkalated naphthalenes in petroleum, their relatively high solubility in seawater, and their toxicity [12], we tested the effect of 1-methylnaphthalene and 2-methylnaphthalene on the percentage of survival of eggs to naupliar 2. At 80 ppb of either methylnaphthalene there was a decrease from 70% survival for controls to 40% for treated eggs. Mineral oil and naphthalene at these same concentrations showed no effect.

Discussion

The feeding of copepods on oil droplets present in the water after an oil spill has been reported [13]. The present paper presents data on the uptake of dissolved hydrocarbons by various zooplankton species, including copepods. Uptake was linear for 24 hours with no further increase in stored hydrocarbon after that time. Most ingested hydrocarbon was metabolized and discharged by the various crustacean species although a small percentage (less than 1% of the ingested hydrocarbon) was stored by all species even after a long depuration time. Blumer et al. [14] has shown that the biogenic hydrocarbon, pristane, is not lost during starvation.

The metabolism of hydrocarbons by zooplankton appears to follow pathways similar to those found in mammals [15-17]. Corner et al. [6] demonstrated that naphthalene is metabolized by similar pathways in the benthic crustacean *Maia squinado*. Both jellyfish and ctenophores appear to lack the ability to metabolize hydrocarbons although discharge of ingested hydrocarbon occurred.

The results presented here suggest additional experiments that could be carried out with zooplankton. Most of the work reported here dealt with the uptake of hydrocarbons by copepods from filtered seawater. The fate of hydrocarbons adsorbed to phytoplankton and ingested by zooplankton remains to be explored. Preliminary work by us has suggested that hydrocarbons are metabolized and discharged by copepods at a greater rate when adsorbed to ingested algae. No metabolism of hydrocarbons by the algae cells was noted. Further work needs to be done to clarify differing rates of metabolism for different hydrocarbons since our results suggested that paraffinic hydrocarbons are metabolized at a faster rate than aromatic hydrocarbons. Also, alkylated aromatic hydrocarbons

appeared to be metabolized at a higher rate than the nonalkylated aromatic hydrocarbons. Similar results have been seen in studies on the bacterial decomposition of hydrocarbons. The differences in rate of hydrocarbon metabolism or lack of this ability in different zooplankton species needs further study.

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