

BIODEGRADATION OF AROMATIC COMPOUNDS BY
HIGH LATITUDE PHYTOPLANKTON

by

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SUMMARY

It was the purpose of the work undertaken to bring into pure culture representative diatoms from the Cook Inlet and the ice-edge in the Bering Sea and to examine their capacity for the oxidation of aromatic compounds using naphthalene as a model substrate. Three diatoms from the Cook Inlet (Kasitsna Bay) were shown to metabolize naphthalene at 6 or 12°C to 1-naphthol and other unidentified ethyl acetate and water-soluble products. Likewise, three diatoms isolated from samples collected at the ice-edge in the Bering Sea also formed small amounts of 1-naphthol from naphthalene when incubated in the light at 0 or 10°C.

We have not been able to rigorously prove that any algal cell, be it a blue-green alga, a green alga, or a diatom can metabolize (1-¹⁴C) naphthalene far enough to produce ¹⁴CO₂. However, if we assume a stoichiometry of one 1-naphthol in the algae equivalent to one CO₂ in bacteria, then for mesophilic algae, the rate of 1-naphthol production is roughly estimated as 10% of the in situ marine potential, and perhaps higher if only the photic zone is considered. We have as yet, no corresponding values for rate of 1-naphthol formation from naphthalene by cold-adapted or psychrophilic diatom cultures, however, it seems reasonable to suggest that algal aromatic transformations may also be a significant fraction of bacterial activity in cold environments. In addition to studies on the oxidation of naphthalene we have also examined the sensitivity of the Bering Sea psychrophilic diatoms to crude oil samples from Cook Inlet and Prudhoe Bay. The results with pure cultures indicate that the toxicity of crude oil was enhanced in psychrophilic diatoms growing at 0°C or 10°C as compared to previous studies with mesophilic forms.

There are several important consequences of the results for Alaskan OCS oil and gas development. It is now clear that pure cultures of diatoms isolated from either the Lower Cook Inlet or from the ice-edge in the Bering Sea can oxidize aromatic compounds such as naphthalene. Whether the metabolites persist through the food chain and will be more or less toxic than naphthalene itself is not known. The results with naphthalene also imply that the photic zone can be an important sink for aromatic hydrocarbon transformations. There are certainly differences among microalgae in the capacity to oxidize naphthalene. It seems prudent, therefore, to insure, via monitoring, that accidental introduction of aromatic compounds in Alaskan waters does not cause a selective or enrichment effect on existing phytoplankton populations.

A second area of environmental concern is the suggestion of an enhanced crude oil toxicity in slower growing psychrophilic diatoms as compared to their mesophilic cousins. Crude oil spills near or under the sea ice may severely impact primary productivity, and thereby higher trophic level.

INTRODUCTION

The results are presented in two sections, one dealing with the Cook Inlet isolates and the other with the isolates from the ice-edge in the Bering Sea. Each section has an introduction, description of materials and methods, results and discussion section, and references.

THE OXIDATION OF NAPHTHALENE BY DIATOMS ISOLATED FROM THE KACHEMAK BAY REGION OF ALASKA

Aromatic hydrocarbons have been found to be widely distributed in open ocean waters (Brown and Huffman, 1976). Many of these compounds and/or their metabolites have toxic properties which include initiation of tumor formation and cancer (Miller and Miller, 1976). In studies of the fate of hydrocarbons in aquatic ecosystems, a considerable amount of information is available on the bacterial and fungal degradation of these compounds and their derivatives (Atlas, 1981; Cerniglia, 1981). In view of the fact that cyanobacteria and microalgae are widely distributed in many aquatic environments and may be important in the catabolism of hydrocarbons, we initiated a research program on the algal oxidation of aromatic hydrocarbons (Cerniglia et al. 1979, 1980 a,b,c).

Most of the studies on the microbial oxidation of hydrocarbons have been conducted at temperatures between 20°C to 30°C. Since there has been increased activities of oil exploration and transport of petroleum in Alaskan waters, there has been recent interest in the **microbia** degradation of crude oil at low water temperatures (Atlas, 1981).

In this investigation, we report on three diatoms isolated from the Kachemak Bay region of Alaska which have the ability to metabolize the aromatic hydrocarbon, **naphthalene** at low temperatures.

MATERIALS AND METHODS

Organisms and Growth Conditions. The diatoms KIA (Navicula sp.), K8A (Nitzschia sp.) and 40 (Synedra sp.) were isolated via enrichment culture at 6 to 10°C from oblique net (20 µm Nitex nylon) tows made during August 1979 and April, 1980 in the Kachemak Bay region, south of Homer, Alaska. The enrichment medium was local sea water plus 5, 20, or 50% ASP-2 medium (Van Baalen, 1962). Pure cultures were obtained by repeated streaking or by several minutes treatment with ultraviolet radiation (254 nm, 15 W germicidal lamp) and subsequent pour plates. Organism N-1 (Cylindrotheca sp.) was isolated from a water sample taken from the Pass adjacent to the Port Aransas Marine Laboratory (Estep et al., 1978). The organisms were grown on ASP-2 medium containing 125 mg l⁻¹ Na₂ SiO₃·9H₂O, 4 µg l⁻¹ vitamin B₁₂ and 250 µg l⁻¹ thiamine in 22 x 175 mm Pyrex test tubes at 12°C. The growth tubes were illuminated with fluorescent lamps F20T12-WWX two on each side of the water bath, 8 cm from the front edge of the lamp to the tube center. The cultures were continuously aerated with 1 ± 0.1% CO₂ enriched air. The generation times under these conditions for the four organisms were about 24 hours.

Naphthalene Biotransformation Experiments. [1-¹⁴C]-Naphthalene experiments were conducted in order to determine the amount of naphthalene oxidized by each organism. Cells (0.5 to 0.8 mg) were pooled from several growth tubes and placed in 22 x 175 mm screw cap tubes, final volume 10 ml. [1⁴C]-Naphthalene (1 µCi in 20 µl ethanol, 6.9 mg/liter) was added just before closing the tube with a plastic top lined with a chromatography septum, aluminum foil and 1 ml Teflon film. Carbon dioxide was added

through a small hole in the plastic top with a gas tight syringe to an initial concentration of 1%. The screw cap tubes were clamped to a glass rod and rotated slowly in the same illuminated water bath used for growing the cultures. The tubes were incubated at either 6°C or 12°C.

After 22 hr incubation, cells were removed by **centrifugation** and each **supernatant** extracted with five thirty ml volumes of ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate and the solvent was removed in vacuo at 42°C. Each residue was redissolved in methanol and analyzed by high pressure liquid chromatography. The ratio of ethyl acetate soluble metabolizes to water soluble metabolizes was determined by taking each organic soluble extract and redissolving in 50 μ l of acetone and 10 μ l **aliquots** was added to vials containing 10.0 ml of scintillation fluid. The radioactivity present was determined in a liquid scintillation counter. Corrections were made for machine efficiency and quenching.

An experiment with unlabeled **naphthalene** was conducted with organism K8A in order to obtain sufficient material for the isolation and structure elucidation of the **naphthalene** biotransformation products. Four 10 ml samples of organism K8A were incubated in screw cap tubes as described above with 6.9 mg/liter **naphthalene** at 12°C. After 22 hr the cells were centrifuged and the **supernatant** was extracted and concentrated as described above. The residue was redissolved in methanol and analyzed by gas-chromatography and mass spectrometry.

Analysis of Metabolic Products. High pressure liquid chromatography (**hplc**) was used for the separation of metabolizes. All hplc analysis were

performed on a Beckman Model 332 hplc and Model 155-10 variable wavelength absorbance detector (Beckman Instruments, Inc., Berkeley, CA, USA) operated at 254 nm. An Altex Ultrasphere-ODS Column (25 cm x 4.6 mm id) [Altex Scientific, Inc., Berkeley, CA, USA] was used for the separation of **naphthalene** metabolizes, which was achieved with a programmed methanol/water gradient (50 to 95%, v/v, 30 min.) with a flow rate of 1 ml/min. In experiments with [¹⁴C]-**naphthalene**, 0.5 ml fractions were collected at 0.5 min. intervals in scintillation vials and 5.0 ml of **Aquasol-2** (New England Nuclear Corp., Boston, MA, USA) was added to each vial. The radioactivity present in each fraction was determined in a Beckman **LS-250** liquid scintillation counter.

Gas **chromatographic** and mass spectral analysis (**GC-MS**) of **naphthalene** metabolizes was performed on a **Finnigan** Model 3100 mass spectrometer coupled to a gas chromatography equipped with a glass column (2 m x 1.5 mm id) packed with 3% OV-1 on **Chromosorb Q**. The injection temperature was 50°C with a temperature program of 100-250°C at 8°C/min. The carrier gas was helium, with a flow rate of 30 ml/min. The following conditions were used for mass spectrometry: molecular separator temperature 350°C; ion source temperature 100°C ionization beam 70 eV; and ionization current 200 uA.

Chemicals: **Naphthalene** (99.9%) was from Aldrich Chemical Co., Milwaukee, Wis., USA. [**1(4,5,8)-¹⁴C**]-**Naphthalene** [5 mCi/mmol] was from **Amersham Searle**, Arlington Heights, Ill., USA. All **naphthalene** derivatives were purified as described previously (**Cerniglia** and Gibson, 1977). Solvents for hplc were purchased from Burdick and Jackson Laboratories, Muskegon, Mich., USA.

RESULTS AND DISCUSSION

Three pure cultures of diatoms isolated from Alaskan waters (strains K8A, 4D and KIA) were incubated with [¹⁴C]-naphthalene at either 6°C or 12°C. The hplc elution profile of the ethyl-acetate soluble naphthalene metabolites formed by each diatom is shown in Fig. 1. For comparative purposes the chromatographic properties of synthetic naphthalene derivatives is shown in Fig. 1A. All of the organisms oxidized naphthalene to a compound which co-chromatographed with 1-naphthol. These results are similar to our earlier studies on the oxidation of naphthalene by cyanobacteria and microalgae (Cerniglia *et al.*, 1980b).

In order to confirm that 1-naphthol was the major metabolite in the oxidation of naphthalene, cells of Nitzschia sp. strain K8A were incubated for 22 hr. in the presence of naphthalene and the ethyl acetate soluble extract analyzed by GC-MS. The GC-MS analysis of the ethyl acetate extract of the metabolism of naphthalene by Nitzschia sp. strain K8A showed a compound that had a similar retention time (9.5 min.) and mass spectrum (m/e 144) to that of authentic 1-naphthol.

Table 1 shows that these diatoms oxidized naphthalene to both organic soluble and water soluble derivatives. The amount of naphthalene oxidized ranged from 0.7 to 1.2%. It is also interesting to note that Cylindrotheca sp. strain N-1 when grown at 12°C, wherein it had a similar rate as organism 40 gave less total naphthalene oxidation (Table 1). This data suggests that cold-water adapted microalgae may prove to be more metabolically active than is implied by their slow growth rates.

In an earlier study we showed that the cyanobacterium Oscillatoria sp. strain JCM oxidized 4.8% of the added naphthalene. The

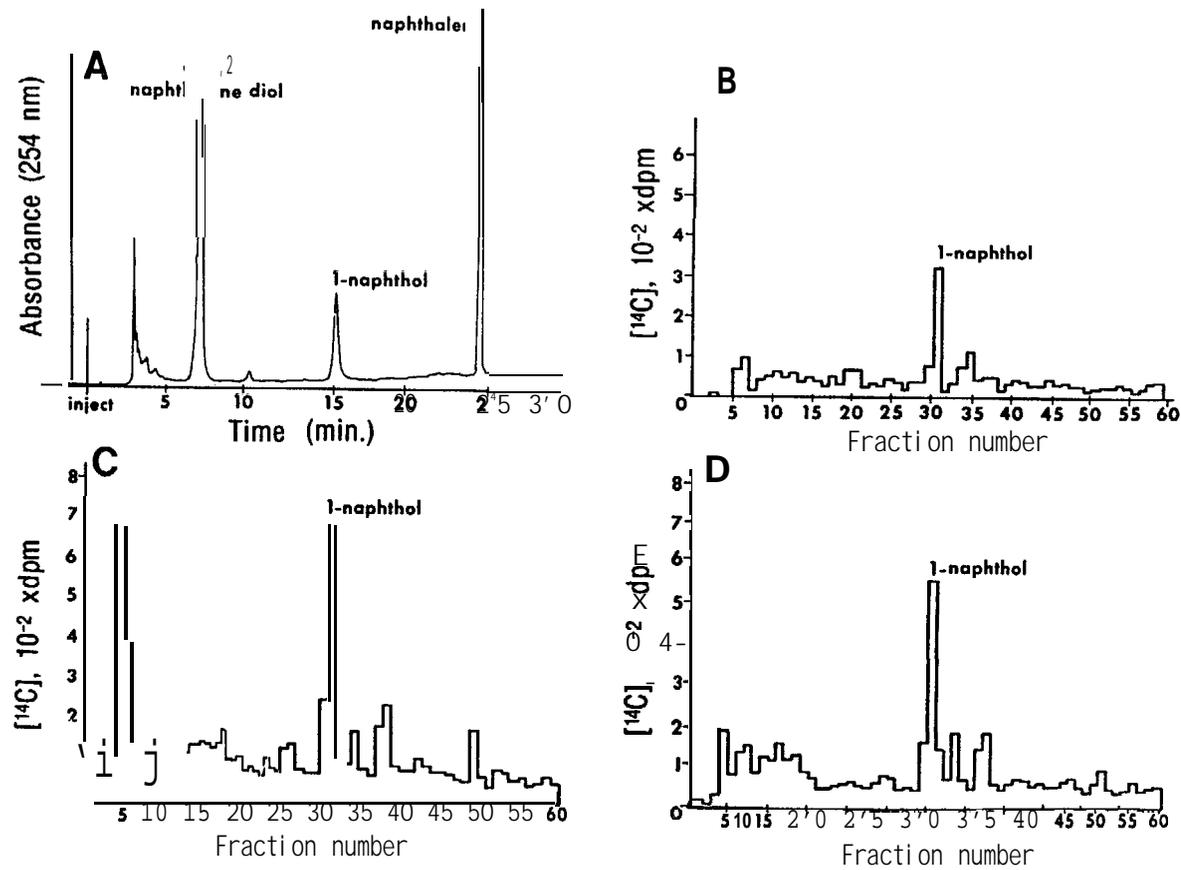


Figure 1. Hplc elution profile of metabolites formed from $[^{14}\text{C}]$ -naphthalene by different diatoms. A, resolution of a mixture of synthetic naphthalene derivatives. B, *Nitzschia sp.* strain K8A; C, *Synechococcus sp.* strain 4D; D, *Navicula sp.* strain KIA.
Hplc conditions were as described in Methods.

Table 1. Distribution of Radioactivity in the Ethyl Acetate Soluble and Water-Soluble Metabolizes Formed from [^{14}C]-Naphthalene by Diatoms.

Organism	d.p.m. mg dry wt ⁻¹		Total Radioactivity	Percentage Metabolism of Naphthalene
	Organic-Soluble	Water-Soluble		
Nitzschia sp. strain K8A	8,965 (49)*	9,311 (51)	18,276	0.7
Synedra sp. strain 4D	18,044 (58)	13,332 (42)	31,376	1.2
Navicula sp. strain KIA	9,658 (55)	7,987 (45)	17,645	0.7
Cylindrotheca sp. strain N-1	6,550 (43)	8,784 (57)	15,334	0.6

* percent of total metabolizes

ratio of ethyl acetate soluble metabolizes to water-soluble metabolizes was **41:59**. Table 1 shows that all of the diatoms formed water-soluble products. The identification of these products remains to be determined but these results suggest that diatoms may have the ability to oxidize **naphthalene** to ring cleavage or conjugated products.

The results herein extend the original observations on the oxidation of **naphthalene** by temperate forms (**Cerniglia et al., 1980b**) to cold-adapted diatoms and reinforce the view that the capacity for oxidation of aromatic compounds is a general metabolic feature in the **microalgae**. Algal rates of aromatic oxidation versus rates for the aerobic **heterotrophic** microbial populations in the **photic** zone are unknown. However, the **photic** zone in the sea may prove to be a **major** sink for transformations of aromatic compounds in nature. Whether this will increase or decrease their toxicity for zooplankton and higher **trophic** levels is unknown.

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BERING SEA DIATOMS: GROWTH CHARACTERISTICS, OXIDATION OF NAPHTHALENE,
AND SENSITIVITY TO CRUDE OIL

It has been known for many years that a rich assemblage of **microalgae**, primarily diatoms and small flagellates, is associated with the underside of the sea ice, the so-called ice algae (1). These ice forms are believed to contribute importantly to the primary production of the polar regions (2,3). In the Bering Sea the ice algae comprised the first spring bloom well preceding blooms that occurred in the open water further south (3). We herein describe the **photoautotrophic** growth characteristics of pure cultures of psychophilic diatoms isolated from water and ice samples taken at the ice edge in the Bering Sea, February-March, 1981 (4). In addition, because of the very real impact that petroleum exploration and production in the Arctic may have on the ice **microflora** (5) we have measured growth rates in the presence of two representative crude oils from the Cook Inlet and **Prudhoe** Bay.

Samples were collected from water pumped from the bow intake system of the R/V Surveyor, from melted ice cores, or from small pieces of floating, brown colored ice (Table 2). The samples were submitted to enrichment culture within one hour of collection. The medium for the enrichment cultures was composed of filtered (**0.4 μ m**) $\frac{1}{2}$ local sea water plus $\frac{1}{2}$ of a synthetic algal medium, **KASP-2** (6). Medium **KASP-2** contained per liter of glass distilled water: 18 g **NaCl**; 5 g **MgSO₄·7H₂O**; 0.60 g **KCl**; 0.37 g **CaCl₂·2H₂O**; 1 g **NaNO₃**; 0.05 g **KH₂PO₄**; 1 g tris(hydroxymethyl)-**aminomethane**; 0.03 g ethyl **enediaminetetraacetic** acid, **disodium** salt,

Table 2. Water and ice samples; Leg II Surveyor S132, 22 Feb. to 14 Mar., 1981

GMT	Date	Latitude	Longitude	Type
1800	Feb. 23	55°44.4'	157°24.1'	Shipboard pumped sea water filtered through 10 μm Nitex nylon net
1900	Feb. 24	55°4.3'	162°11.1'	Pumped sea water and 10 μm net
1830	Feb. 25	55°57.5'	168°45.3'	Pumped sea water and 10 μm net
1730	Feb. 26	58°7.5'	173°17.4'	Two ice cores, courtesy of Seelye Martin, bottom 3-4 cm thawed and filtered through 0.2 μm sterile filter
1830	Feb. 26	58°13.4'	173°8.6'	Pumped sea water and 10 μm net, sea water temp. 1.0°C ^a
1745	Feb. 27	58°26.6'	172°51.2'	Piece of "brown ice" thawed in 10 μm net
1930	Feb. 27	58°29.0'	172°37.1'	Pumped sea water and 10 μm net, sea water temp. 0°C
1900	Feb. 28	58°07.6'	173°17.8'	Pumped sea water and 10 μm net
1830	Mar. 1	58°39.9'	172°19.6'	Pumped sea water and 10 μm net
1800	Mar. 3	59°15.5'	171°12.0'	"Brown ice" thawed in 10 μm net, sea water temp. -1.5°C
1800	Mar. 4	59°18.2'	171°29.2'	Pumped sea water and 10 μm net
0400	Mar. 5	59°17.0'	171°41.8'	Surface tow, 10 μm net in clearings around ice
1900	Mar. 5	59°08.8'	171°55.0'	Pumped sea water and 10 μm net, sea water temp. -1.2°C
2300	Mar. 5	59°07.7'	171°50.4'	Pumped sea water 10 μm net
1830	Mar. 7	58°43.0'	172°15.4'	Pumped sea water 10 μm net
1930	Mar. 8	58°46.3'	172°51.7'	Pumped sea water 10 μm net, sea water temp. 0.6°C
1900	Mar. 9	58°32.9'	173°29.4'	Pumped sea water 10 μm net

^a Not precise temperatures, listed only to give some idea of the prevailing temperatures at the time the algal samples were collected.

dihydrate; 0.004 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; 0.034 g H_3BO_3 ; 0.004 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 670 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 38 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; 12 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; 0.3 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and supplemented with 0.125 g $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, 300 g thiamine; 8 g vitamin B_{12} ; 30 g **biotin**. The enrichment cultures were incubated in continuous light at -1 to 0°C in the shipboard flowing water system (one overhead fluorescent fixture) or at 5 to 7°C in a refrigerator (one 40W tungsten lamp). The cultures were frequently examined microscopically and transferred to fresh medium as appropriate. **Unialgal** cultures were purified by repeated streaking on petri dishes containing **agarized** (1% low gelling temperature **agar**, No. **A4018**, Sigma Chemical Co., St. Louis, MO) medium composed of $\frac{1}{2}$ offshore Gulf of Mexico sea water plus $\frac{1}{2}$ medium **KASP-2**. The dishes were incubated in continuous fluorescent or tungsten light in sealed plastic containers in an atmosphere of 0.5 to 1% **CO₂-in-air** at 5 or 10°C. After 5 to 15 days suitable micro-colonies were excised, transferred to agar slants, and examined for purity microscopically and in the basal medium supplemented with complex organic materials; 0.1% each of yeast extract, **trypticase**, and soytone (all products of **Difco** Laboratories, Detroit, MI). Stock cultures were routinely maintained as slants in a refrigerator at 5-10° with illumination provided by one 40W tungsten lamp 25 to 40 cm from the cultures.

The cultures were kindly identified by Professor **Qi Yu-zao** of the Department of Biology, **Jinan** University, **Guangzhou, P.R.C.** as: **Thalassiosira** sp. (our notation **D1-2**), **Navicula** sp. (J-4), **Nitzschia** sp. (**K3-3**), **Chaetoceros** sp. (**K3-10**) and **KD-50**). Organisms **K3-10** and **KD-50** isolated from two different samples may be the same species, tentatively

C. lacinosus Schutt, but they were sufficiently different in physiology to warrant experimentally being considered two different organisms. It should be noted that these diatoms isolated from the enrichment cultures, while certainly not all the organisms present, were common in numerous fresh samples examined on shipboard.

The light-temperature gradient plate (7) was used to survey the general growth characteristics of the isolates from 6 to 22°C (Fig. 2). All the cultures were clearly cold-adapted. Only one strain, the Chaetoceros sp. (K3-10), grew well at 18°C. The optimum temperatures were from 10 to 14°C. It was not practical to operate the light-temperature gradient plate below 6°C nor was the plate useful for measuring growth rates. Growth rates were therefore measured in liquid cultures at 0 or 10°C (Table 3). Four of the isolates, KD-50, K3-10, K3-3, and J-4 maintained reproducible generation times at 0°C of from 5 to 7 days. At 10°C the growth rates were 1 to 2½ days. The Thalassiosira sp. (111-2) grew at such a slow rate even at 10°C as to preclude useful experimental work. Organism K3-3 was found to require vitamin B₁₂, organism J-4 was stimulated by vitamin B₁₂. The other cultures grew without added vitamins. Of particular interest were the exceedingly slow growth rates, especially at 0°C. We have looked for chemical or physical factors having significant effect on the growth rate. Light and dark cycles (18L:6D) or addition of reduced nitrogen, NH₄Cl or organic nitrogen in the form of casamino acids, had little effect. The choice of lamps, deluxe warm-white phosphor fluorescent lamps shielded by one screen to cut intensity, was made on the basis of extensive early screening of different combinations of phosphors

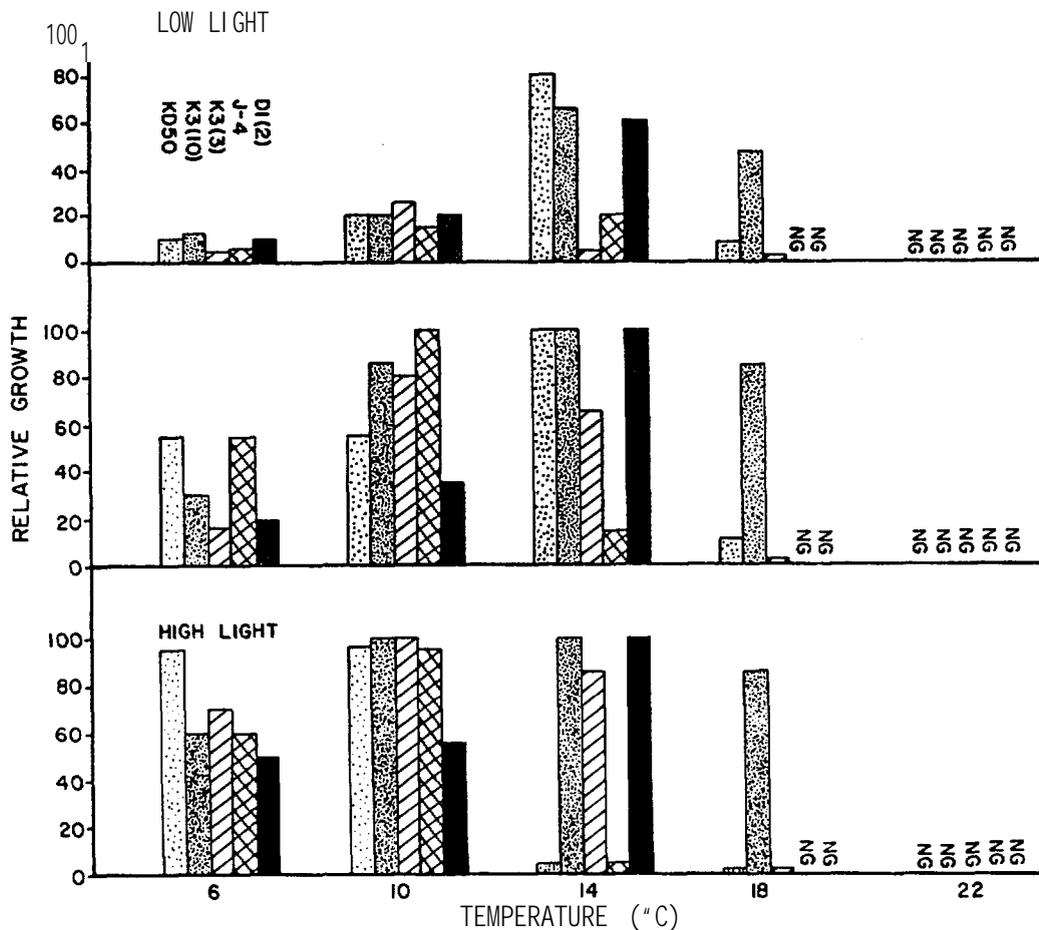


Fig. 2. Relative growth of ice edge diatoms as a function of temperature and light intensity. The aluminum light-temperature gradient plate was 46x63.5x1.27 cm. It was illuminated by two rows (2 lamps per row placed end to end) of **F20T12-WWX** lamps placed 34 cm above the front edge. The light intensity over the front edge of the plate was $420\mu\text{w}/\text{cm}^2$ (Model 65 Radiometer, YSI Co., Yellow Springs, OH). Pyrex petri dishes, 60x15 mm, containing 10 ml of medium (1/2 **KASP-2** plus 1/2 sea water) **plus inoculum**, were placed at desired locations on the plate. Growth was judged visually or optically, if dense enough. For each organism the data were recorded relative to the position on the plate which gave the best growth. The experiments were purposely terminated after 9-12 days at relatively low cell densities to avoid severe CO₂ or light limitations on growth. The notation NG means no growth.

Table 3. Growth rates, as generation times in hours, of diatoms isolated from the ice edge in the Bering Sea and inhibition of their growth by two crude oils.

Strain No.	Temperature (°C)									
	0			10			0		10	
	Cook Inlet Crude (ppm)			Prudhoe Bay Crude (ppm)						
	0	50	500	0	50	500	50	500	50	500
KD-50	144	144	0	30	33	36	0	0	42	72
K3-10	120	120	0	30	30	39	120	0	36	55
K3-3	144	0	0	48	48	NG*	0	0	45	NG
J-4	170	ND†	ND	60	60	60	ND	ND	72	103

*NG means no growth. †ND means not determined. Continuous illumination was provided by two F20T12/WWX fluorescent lamps 10 cm from the lamp center to the growth tube center. Lamp output was cut to approximately 60% by one copper screen inserted between the lamps and the growth bath. Temperatures were held to ± 0.2 at 1°C and ± 0.5 at 10°C. The growth tubes were continuously bubbled with $1 \pm 0.1\%$ CO₂ in air, cell concentration was measured turbidimetrically or by collecting cells on a 0.4 µm filter and drying at 45°C in a vacuum oven over P₂O₅. The crude oils were sterilized by filtration with pressure (N₂) through 0.45 µm silver membranes (Selas Corp., Dresher, PA). The crude oil was absorbed onto washed 12.7 mm filter paper discs and the discs placed directly into the culture tubes. Crude oils presented in this manner remain absorbed on the discs and in contact with the algae (15). The generation times shown are conservatively good to + 15%.

and intensities. Moreover short-time photosynthesis measurements ($^{14}\text{CO}_2$ fixation) carried out under these same lighting conditions gave linear and saturated rates of CO_2 uptake over several hours. By several fundamental criteria of algal culture, cell density and elementary analysis, these cultures are behaving as expected. Cell yields of **0.5mg dry weight ml^{-1}** were routinely achieved. The elemental analysis of organism **KD-50** grown at 0° or 10°C was: **%C**, 32.29 and 32.91; **%H**, 4.99 and 4.98; **%N**, 5.16 and 5.42, **%residue**, 34.9 and 30.9. On an ash-free basis these values compare very favorably with a variety of algal cells (8).

There are, then, two very interesting features which emerge from the characterization of growth in these ice edge diatoms. First, these organisms fit the textbook definition of obligate **psychrophiles**, micro-organisms that can grow well at **0°C** and that do so optimally below 20°C (9). In other words these are not just **mesophilic** forms capable of growth at **0°C** but with optimum temperatures above **20°C** , but rather strains restricted to temperatures below 18°C (Fig. 2). Their second significant characteristic was their exceedingly slow measured generation times, 5 to 7 days at **0°C** . Such very slow generation times are not anticipated from the existing large body of information primarily on **mesophilic microalgae** (10). Indeed a theoretical treatment of algal growth rates versus temperature predicted generation times approaching 1 day at **0°C** (11). In work with **unialgal (bacterized)** cultures of four Arctic ice diatoms at 5°C generation times of 1 to 2 days were found (12). A **unialgal** strain of **Skeletonema costatum**, a typical **mesophilic** form, had an estimated generation time of approximately 2 days at **0°C** (13).

The generation times measured herein at 0°C with pure cultures of cold-adapted diatoms appear to be the first of their kind. The very slow growth rates at 0°C may perhaps be a reflection of one or several enzymes with unavoidably low turnover times at 0°C. However, the very marked increase in the solubility of oxygen at low temperatures may cause special problems for a photosynthetic cell, for example, with the oxygenase reaction catalyzed by **ribulose 1,5-bisphosphate carboxylase** (14). If generation times approaching one week are typical under supposedly optimum conditions in the lab for ice edge algae then their turnover times in situ may be much lower. These unique Arctic (probably Antarctic as well) ice phytoplankton and hence these ecosystems may truly **merit** the appellation of fragile.

Notwithstanding the slow growth rates of these psychrophilic diatoms, we have been able to grow enough cells to examine their capacity for oxidation of aromatic hydrocarbons using **naphthalene** as a model substrate. Figures 3 and 4 demonstrate that **1-naphthol** was formed from **(1-¹⁴C) naphthalene** at 0 or 10°C. The amounts were very small but are real and suggest that cold-adapted **microalgae** can oxidize aromatic hydrocarbons as is now well-described in **mesophilic** forms (see page 1).

The observations on the toxicity of crude oils (Table 3) also suggest that cold-adapted diatoms will generally prove more sensitive to any accidental crude oil spills in or around the ice edge in the Bering Sea. Lethality was evident in two of the diatoms, **KD-50** and **K3-3** at 50 ppm at 0°C, while 500 ppm was lethal to all four organisms. At 10°C toxicity was lessened. For comparison the same Prudhoe Bay crude had no effect at

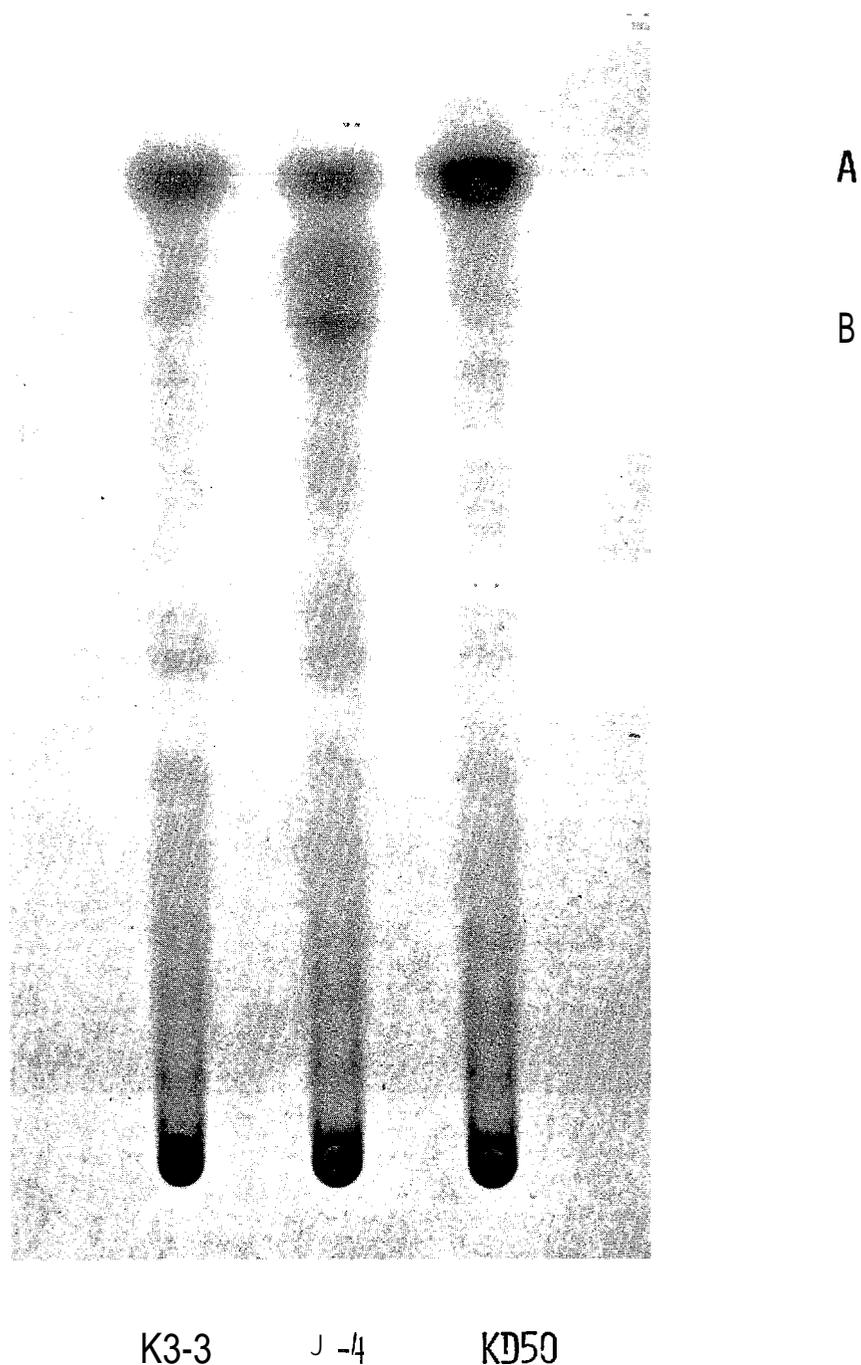


Fig. 3. Radioautogram of products formed from (1-¹⁴C) naphthalene by psychrophilic diatoms grown and incubated with naphthalene at 0 ± 0.1°C. The organisms are identified in the text. Naphthalene, 1μCi (specific activity 1μCi/μmol) was added to 10ml of diatom culture (approx. 0.5mg dry weight/ml) in a screw cap tube. After incubation for 24 hours in the same bath as used for growing the cells, the cells were removed by centrifugation and the supernatants from 3 tubes (30ml total) were extracted with ethyl acetate. The ethyl acetate extract was dried over Na₂SO₄, evaporated, and the whole sample chromatographed on silica gel plates using chloroform-acetone (4:1). The region marked A on the radioautogram is naphthalene, region B is 1-naphthol.

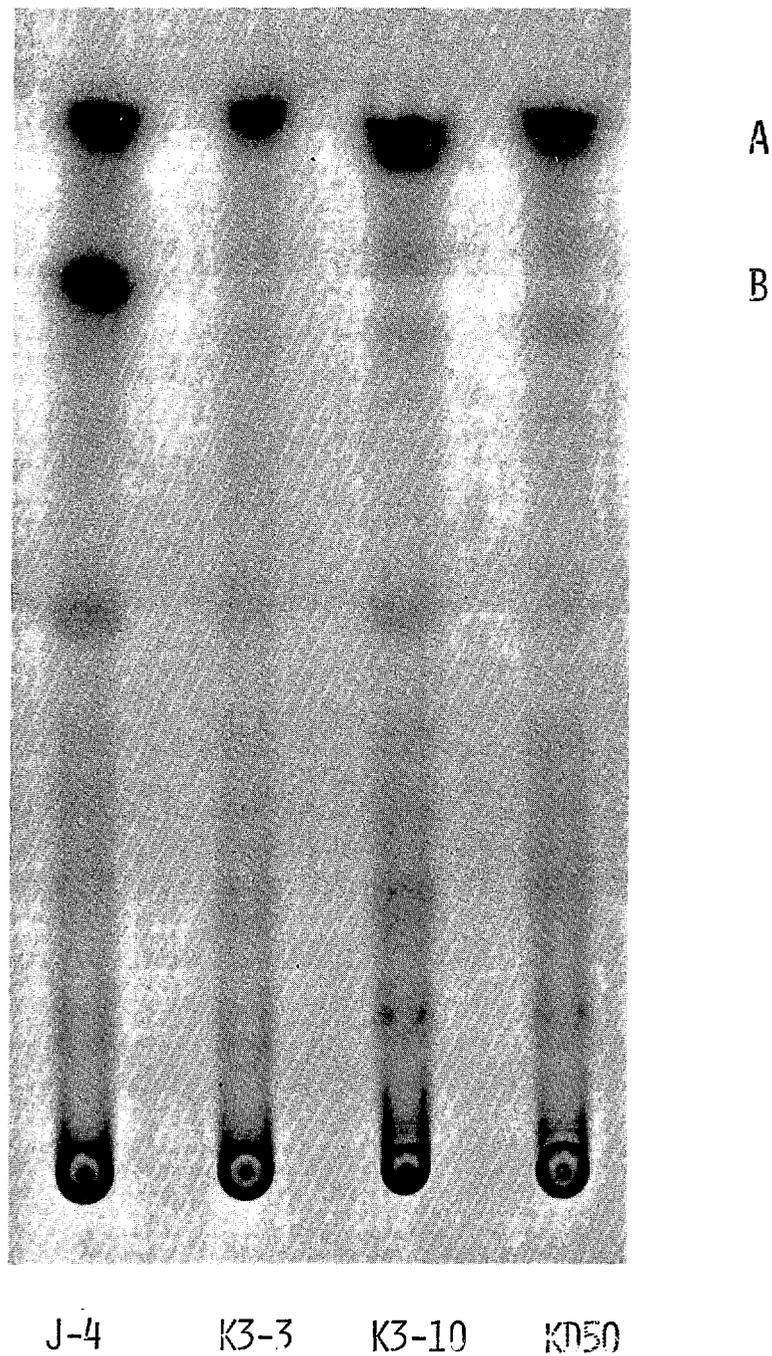


Figure 4. Radioautogram of products formed from (1-¹⁴C) naphthalene by psychrophilic diatoms grown and incubated with naphthalene at 10 ± 0.5°C. Experimental details were the same as in Figure 3.

500 ppm and caused only slight lags in growth at 1500 ppm when tested at 30°C against three **mesophilic** algae, a blue-green alga, a green alga, or a diatom (15). Work with four **unialgal** cultures isolated from the southern Beaufort Sea growth of diatoms and a green flagellate was markedly inhibited by crude oil concentrations higher than 100 ppm but diatoms seemed more sensitive than the green flagellate (16). Curiously, in this work greater inhibition was observed with longer exposure at temperatures between 5 to 10°C than at 0°C.

The capacity for oxidation of aromatic hydrocarbons and enhanced toxicity of crude oil in psychophilic diatoms may, in the case of an oil spill, be important to maintenance of primary production levels and therefore to higher **trophic** levels in the Bering Sea. These observations need broader confirmation both in laboratory and field studies.

With the enrichment and isolation in pure culture of these psychophilic Arctic diatoms, especially with the easily cultivated **Nitzschia** sp. (K3-3) and the **Chaetoceros** sp. (K3-10, KD-50) as experimental tools, we should now gain further understanding of regulation of photosynthetic and biosynthetic pathways in cold-adapted **microalgae**.

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RATE STUDIES OF 1-NAPHTHOL FORMATION IN MESOPHILIC ALGAE

To determine if **microalgae** can degrade **naphthalene** to **CO₂** we have incubated a blue-green alga, a green alga, and a diatom in closed flasks at 30°C with **(1-¹⁴C) naphthalene** and recovered **CO₂** from the gas phase by precipitation as **BaCO₃**. The **BaCO₃** was carefully washed with water, ethanol and again with water, then acidified and any radioactivity trapped in 5 ml of **0.1N NaOH**. Part of the NaOH solution was added to scintillation cocktail and counted. The above procedure completely eliminated any carry over of **naphthalene**. Recoveries using **NaH¹⁴CO₃** carried through the precipitation, washing, acidification and trapping in **NaOH** steps were 90% or better. We have not found any evidence that the above cultures can metabolize **naphthalene** to **¹⁴CO₂**. We have examined the time course of **1-naphthol** formation in the blue-green alga, **Oscillatoria** sp. our strain JCM (Fig. 5). We estimate from such data that strain JCM can form 20 nmol of **1-naphthol** per mg dry weight of cells in 24 hours. If we assume that the experimentally measured algal rate formation of **1-naphthol** can be equated with bacterial hydrocarbon biodegradation rates (**Bartha** and Atlas, 1977) then at the reasonable level of 1 **µg chlorophyll a/liter** in a **natural** system algal hydrocarbon oxidation can amount to 10% of the "in situ" marine potential.

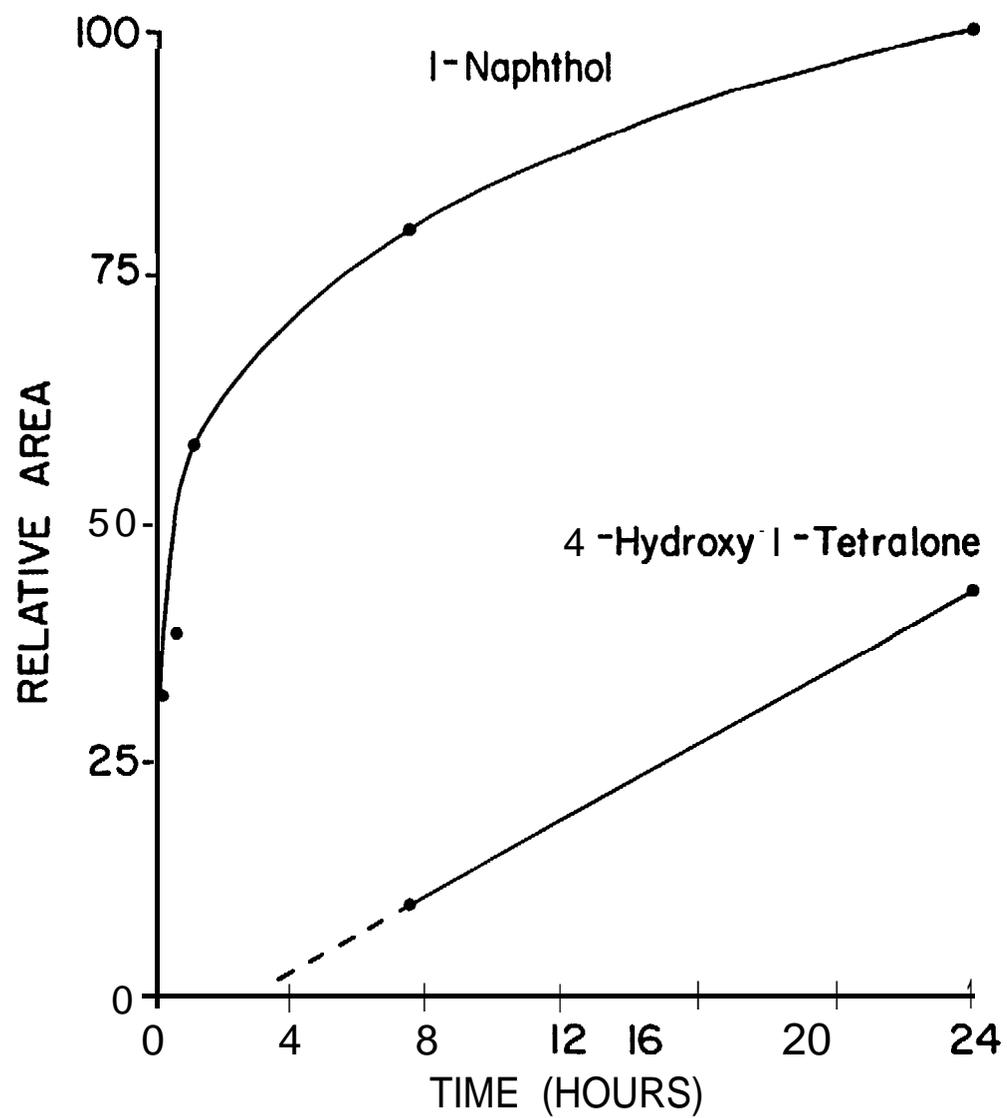


Figure 5. Time course of formation of 1-naphthol and 4-hydroxy-1-tetralone by the blue-green alga, Oscillatoria sp. strain JCM.

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