MOLECULAR AND ISOTOPIC FINGERPRINTING
OF ALIPHATIC HYDROCARBONS IN
CONCEPTION BAY, NEWFOUNDLAND

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Molecular and Isotopic Fingerprinting
of
Aliphatic Hydrocarbons
in
Conception Bay, Newfoundland

by

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ABSTRACT

The distributions and isotopic compositions of the aliphatic hydrocarbons of plankton, sediments and biota of a typical northern fjord (Conception Bay, Newfoundland) were studied by standard gas chromatography-mass spectrometry (GC-MS), and by the novel technique of compound-specific isotope ratio mass spectrometry (IRMS). Sediments contained compounds of terrestrial, marine biogenic, and bacterial origins, with petroleum appearing to contribute primarily an unresolved complex mixture (UCM), occasional pristane and phytane, and typical trace cyclic terpenoids. A suite of eight C_{25} highly branched isoprenoid alkenes containing 3 to 5 degrees of unsaturation was the dominant feature in plankton, sediments, and some benthic biota in Conception Bay, as well as in samples from other coastal and offshore areas around Newfoundland. Although attempts to isolate the source of these compounds by culturing common local diatom species were unsuccessful, the molecular distribution and isotopic signature of these alkenes suggest that these hydrocarbons are synthesized by one particular organism during the spring bloom. Measurement of the $\delta^{13}C$ (or carbon isotopic composition) of individual compounds proved to be a useful tool in recognizing the input of different sources of compounds to sedimentary organic matter pools. For example, variations in the isotopic compositions of even chain-length n-alkanes in sediments suggest the input of at least three main sources of n-alkanes (terrestrial leaf debris, marine algae, and a third source, proposed to be marine bacteria). The $\delta^{13}C$ of biogenic hydrocarbons

appeared to be relatively unchanged during early degradation in the water column and surface sediments. Although the existence of kinetic isotope fractionation during biosynthesis was indicated by co-variations in the distribution and isotopic signature of algal products in cultures, biochemical isotope effects generally appeared to be masked in the complex environmental samples analyzed. Compounds from distinct sources were generally found to all have distinct isotopic signatures. For example, known phytoplankton products featured $\delta^{13}C$ values above -28‰, whereas terrestrial n-alkanes (nC_{23-33}) generally featured $\delta^{13}C$ values below -30‰. The isotopic effects of petroleum input in Conception Bay appeared to be minor, but could not be quantitatively determined due to difficulties in separating compounds of interest from the large UCM typically found in petroleum sources. Petroleum input, however, could be effectively recognized using accepted molecular markers (hopanes, steranes, tricyclic terpanes). More detailed study of the isotopic signatures of individual compounds in sources such as terrestrial leaf wax alkanes and lubricating oils is needed in order to explain all of of the complex isotopic patterns seen in sediments.

ACKNOWLEDGEMENTS

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I. INTRODUCTION

a. GENERAL

The hydrocarbons found in environmental samples belong to three broad groups: those of recent biogenic origin, those of ancient origin (petroleum and recycled kerogen), and those formed during the combustion of organic material. Biogenic compounds, which dominate unperturbed environments, can be further grouped into those of terrestrial and marine origins. Despite many advances in the capability to detect and identify hydrocarbons since the development of gas chromatography-mass spectrometry (GC-MS), it is still often difficult to determine the sources or study the mixing of these compounds in complex environmental systems. Not only can many different sources contribute the same compounds to such systems, but these compounds may undergo extensive degradation and mixing as they are cycled. A promising advancement in this field is the development of a new analytical technique, compound-specific isotopic analysis (CSIA), which allows the routine measurement of the carbon isotopic composition of individual compounds. This isotopic signature may be used as a marker or tracer to distinguish hydrocarbons of different origins, and to estimate the contribution of various inputs to common pools. Although some studies have applied CSIA to the study of environmental compounds of recent origin such as leaf-wax alkanes (Rieley *et al.*, 1993), bivalve fatty acids (Murphy and Abrajano, 1994), and polycyclic aromatic hydrocarbons (O'Malley *et al.*, 1994), most CSIA research to date has focussed on ancient sedimentary hydrocarbons. It would appear important, even critical, that more study of the compound-specific

study of the compound-specific isotopic composition of modern environments take place, so that fossil isotopic signatures can be correctly interpreted. The following investigation is, hopefully, a step in that direction.

b. AIM

The objective of this study was to examine the molecular distribution and stable carbon isotopic composition of aliphatic hydrocarbons in a typical northern fjord. Sources and sinks of hydrocarbons in Conception Bay, on the northeast coast of the island of Newfoundland, were analysed to investigate the potential of the isotopic signature of individual hydrocarbons as a tool in tracing the origin and mixing of these compounds in modern environments. In particular, it was hoped that an investigation using this novel analytical technique would shed some light on the origin of an enigmatic group of highly branched isoprenoid alkenes found in Conception Bay and other sediments world-wide. This study was also intended to contribute to a better understanding of the fractionation of carbon isotopes among hydrocarbons in contemporary depositional environments in general.

c. BACKGROUND

1. HYDROCARBONS IN THE ENVIRONMENT

Aliphatic hydrocarbons are organic compounds consisting of only carbon and hydrogen atoms without aromatic rings. They may be saturated or unsaturated, and may

have linear, branched or cyclic structures (Figure 1). These compounds are commonly represented in shorthand form by the number of carbon atoms and double bonds they contain, with the prefixes *n*, *br*, and *c* representing linear, branched and cyclic structures, respectively. For example, nC_8 denotes n-octane, and $brC_{25:3}$ denotes a branched compound with 25 carbon atoms and three double bonds.

i. Recent biogenic hydrocarbons
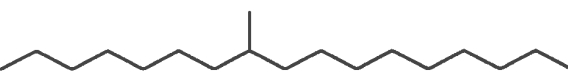
Synthesis and functions: Hydrocarbon synthesis in organisms is not fully understood at present, but is known to involve at least two primary pathways, summarized in Figure 2. Straight chain alkanes (and presumably alkenes) are formed in many organisms by decarboxylation of fatty acids, which themselves have been built up from two-carbon acetyl groups by the fatty acid synthetase enzyme system (Han *et al.*, 1969; McInnes *et al.*, 1980). Since the majority of the fatty acids produced this way contain even numbers of carbon atoms, and decarboxylation involves the removal of one carbon, this mode of formation accounts for the odd carbon chain length predominance seen in most biogenic alkane assemblages. Irregular branched alkanes, such as the algal 7- and 8-methylheptadecanes, are believed to be formed by the addition of the *S*-methyl group of the amino acid methionine to previously formed straight carbon chains (Han *et al.*, 1969, Fehler and Light, 1972). Isoprenoid hydrocarbons are formed essentially by the linking of two to ten or more branched five-carbon isoprene units, which are themselves also formed from acetate units via the mevalonate pathway. Cyclization of isoprenoids of various lengths (via different pathways in different organisms) yields a

4
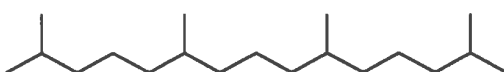
Figure 1. Representative hydrocarbon structures:

      a. linear (n-) alkane
      b. linear (n-) alkene
      c. methyl branched alkane
      d. isoprenoid alkane
      e-g. C_{20}, C_{25}, and C_{30} highly branched isoprenoid alkene structures
          (exact location and configuration of double bonds uncertain)
      h. isoprenoid alkene
      i. pentacyclic terpane (common petroleum marker)
      j. pentacyclic terpene (common bacterial marker)
      k. tricyclic terpane

a. n-hexadecane

b. n-heneicosahexaene

c. 8-methylheptadecane

d. pristane

e. . 2,6,10-trimethyl-7-(3-methyl)-dodecane
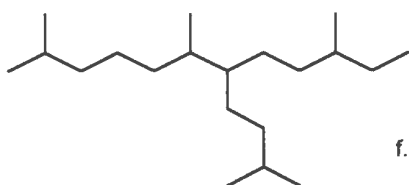
g. 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane
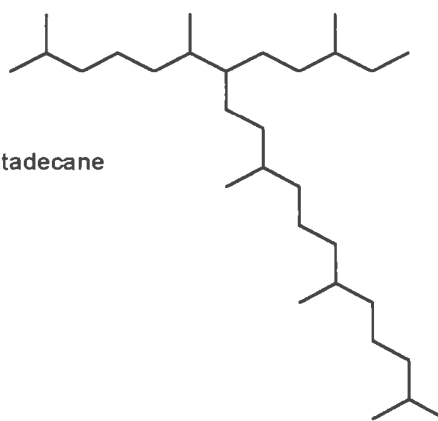
f. 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane

h. squalene

$R=H - C_{10}H_{21}$
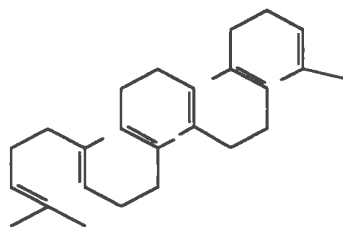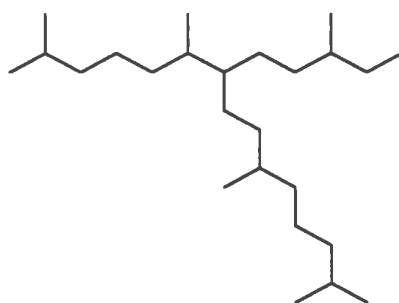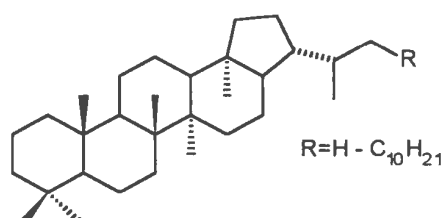
i. hopane skeleton

j. diploptene

$R= H - C_{12}H_{35}$
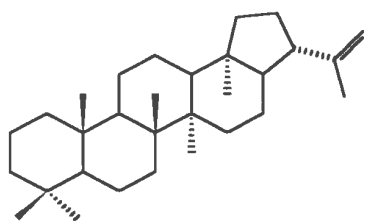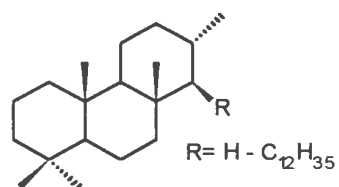
k. tricyclic terpane skeleton

Figure 2. General scheme of hydrocarbon biosynthesis

pyruvate

↓

acetate

fatty acid synthesis via
condensation, reduction, dehydration
elongation and desaturation

condensation followed
by decarboxylation
(mevalonate pathway)

fatty acids

isopentenyl pyrophosphate

decarboxylation

polymerization

linear hydrocarbons

isoprenoid compounds

addition of -CH_3 from methionine

cyclization

**irregular branched
hydrocarbons**

cyclic terpenoids

myriad of compounds containing one to five saturated rings known as the cyclic terpenoids (Nevenzel, 1989).

The exact function or purpose of relatively few of the vast array of hydrocarbons synthesized by organisms is known. Some have highly specific functions, such as the C_8 and C_{11} sexual pheromones released by brown algae (Jaenicke, 1977). The various triterpenoid hopanes found in bacteria are believed to serve a purpose similar to that of the sterols in higher organisms in maintaining cell membrane rigidity and fluidity. Long-chain alkanes produced by terrestrial plants form part of the waxy leaf cuticle that guards against excessive water loss. On the other hand, many hydrocarbons are intermediates in the synthesis of other biomolecules. For example, the C_{30} isoprenoid hydrocarbon squalene is the precursor to sterols in most organisms. Unsaturated C_{20} and C_{40} isoprenoid sub-units are, respectively, integral parts of the ubiquitous chlorophyll and carotenoid photosynthetic pigments. Finally, some of the hydrocarbons encountered in organisms are simply digestive transformations of dietary compounds. For instance, the pristane commonly found in marine copepods is believed to be derived primarily from the breakdown of the phytol side chain of chlorophyll from dietary phytoplankton (Avigan and Blumer, 1968). These types of transformations are likely especially common (and complex) among the bacteria and fungi, many of which utilize hydrocarbons directly as energy sources.

Sources and fates: The most prominent sources of biogenic hydrocarbons in most estuarine environments are marine phytoplankton and zooplankton, terrestrial plants, and

bacteria. The hydrocarbon assemblages produced by marine algae tend to consist of small numbers of specific, often unsaturated and branched compounds, and commonly include n-heneicosahexaene (HEH, $nC_{20:6}$), squalene ($brC_{30:6}$), pristane, and C_{15} and C_{17} n-alkanes (Nevenzel, 1989). Terrestrial plants contribute predominantly n-alkanes in the range of C_{23} to C_{35},, with a strong predominance of odd as compared to even homologues. The hydrocarbon contribution of bacteria is less well understood, but is known to include a variety of hopanoid (pentacyclic triterpenoid) compounds. Bacteria have been proposed as the source for some unusual C_{18} to C_{28} n-alkane distributions dominated by even chain length homologues (e.g., Kennicutt and Brooks, 1990). The biomarkers of sedimentary bacteria are often the most distinct feature of sedimentary hydrocarbon assemblages, as these organisms are usually the last to impart a fingerprint on organic matter before it is preserved.

Degradation rates of biogenic hydrocarbons in the water column and sediment are highly variable, and are partly determined by factors such as volatility, solubility, and bioreactivity. Straight chain and unsaturated compounds are especially susceptible to microbial degradation, whereas branched and cyclic hydrocarbons, and those associated with a matrix originating from outside the marine environment (such as the long chain waxes from leaf debris) tend to be more durable and persistent (Lee and Wakeham, 1988). Some compounds, such as the highly branched isoprenoids, may be preserved after burial as sulfur derivatives (thiophenes) (*e.g.*, Sinninghe-Damsté *et al.*, 1989, 1993).

ii. Ancient or petroleum hydrocarbons

Synthesis: The formation of petroleum hydrocarbons over geologic time is an entire science unto itself, and any introductory discussion such as the following one is unfortunately but inevitably a vast oversimplification. Petroleum consists of an enormously complex mixture of hydrocarbons formed by the combined action of bacteria, temperature, pressure, and sediment on buried organic matter over the course of millions of years. In essence, buried proteins, carbohydrates, and lipids (including the hydrocarbons discussed above) are microbially degraded and recombined into an intermediate phase (kerogen), from which, given time and the right conditions, organic molecular fragments are released. These fragments then are further differentially degraded and transported under pressure through sedimentary layers and accumulate where their migration is impeded. This crude oil contains both hydrocarbons inherited from the original organic matter, whose structure has remained to varying degrees intact, and compounds formed anew from the kerogen. Considerable debate exists as to the extent of preservation of original hydrocarbons during petroleum formation, but compounds such as pristane, and the hopanes and porphyrins are widely accepted to be molecular fossils or biomarkers whose structure has changed little since their burial. Petroleum n-alkanes, especially those shorter than C_{20}, on the other hand, are believed to be largely formed *de novo* during oil generation (Tissot and Welte, 1978). This origin presumably accounts for the equal ratio of odd and even n-alkane homologues found in most mature petroleums as compared with the odd-preference seen in most recent

biogenic assemblages. Crude oil hydrocarbons consist, on average of roughly 75 to 80% straight, branched and cyclic alkanes, and 20 to 25% aromatic compounds, as well as varying proportions of N, S and O derivatives (Clark and Brown, 1977). Notably absent from crude oils are the more reactive or unstable unsaturated aliphatics (olefins) that commonly characterize recent biogenic hydrocarbon assemblages.

The composition of crude petroleum is radically altered during refining, in which components are separated, purified, and chemically altered by a variety of processes including distillation, solvent washing, catalytic and thermal cracking, and polymerization (Hatch and Matar, 1981; Gary and Handwerk, 1975). As refining technologies and markets differ worldwide, the compositions of refined products vary widely. Products may contain mostly aromatic compounds (*e.g.*, solvents, tars), or consist entirely of aliphatics (*e.g.*, mud based drilling fluids). In general, though, gasolines and other light fuel cuts contain primarily low molecular weight (C_6 to C_{12}) straight, branched and cyclic alkanes and alkylbenzenes. Higher-boiling point products, such as kerosenes, heating oils and diesels contain progressively longer chain alkanes, up to n-C_{30} and greater. Lubricating oils, derived essentially from the less volatile material left after gasoline production, are generally composed of a complex mixture of higher boiling, highly branched, cyclic, and cross-linked compounds including relatively few n-alkanes (Crompton, 1985).

Sources and fates: Petroleum hydrocarbons may enter marine and estuarine systems by natural seepage, direct spillage, or as runoff and fallout from urban areas.

Although localised spills of oil tend to attract the most attention, it is generally accepted that by far the greatest proportion of anthropogenic petroleum hydrocarbons in estuarine systems come from non-point or diffuse sources such as atmospheric fallout and road runoff (*e.g.*, Laflamme and Hites, 1978, Green and Trett, 1989). Crankcase oil that has leaked past worn engine seals into automobile exhaust or directly onto roads has been shown by numerous workers to be a major source of this type of contamination (*e.g.*, Eganhouse and Kaplan, 1982). Vessels, especially those powered by outboard motors, may also contribute significant quantities of both lubricating oil and gasoline hydrocarbons to some coastal environments (Brammer and Puyear, 1982).

Many variables affect the distribution and fate of these compounds in the environment, including most importantly their relative volatility, solubility, adsorption behaviour, and resistance to microbial degradation. Components lighter than C_{15} (basically all of the light fuel cuts) generally evaporate rapidly. The remaining compounds are subjected to physical, chemical, and biological weathering, with the n-alkanes being particularly susceptible to microbial degradation. After only a relatively short period of exposure to the environment, most petroleum hydrocarbon assemblages are reduced to an unresolved complex mixture and a small number of more resistant compounds such as the cyclic terpenoids and the isoprenoids pristane and phytane (National Research Council, 1985).

iii. Other hydrocarbon types

Another major group of hydrocarbons commonly encountered in the environment

consists of those compounds that are produced as a result of the incomplete combustion of organic matter. Most of the hydrocarbons formed during burning, though, are aromatic hydrocarbons and soots, which are not examined in this study (c.f., O'Malley, 1994).

Synthetic or man-made compounds are also often found when analysing for hydrocarbons. Most of these compounds, however, are not true hydrocarbons, as they generally contain elements other than carbon and hydrogen (particularly oxygen and chlorine). Examples of these include the polychlorinated biphenyls (PCBs), the various esters made for use in synthetic motor oil, and the phthalate ester plasticizers. These last compounds, present in most plastics, are notorious sample (laboratory) contaminants.

2. CARBON ISOTOPIC COMPOSITION OF ALIPHATIC HYDROCARBONS

i. Stable Isotope Analysis

The two stable isotopes of the element carbon, ^{12}C and ^{13}C, make up 98.89% and 1.11% of the global carbon pool, respectively. Fractionation of isotopes during chemical, physical, and biological processes, due to differences in the masses and interatomic bond strengths of the two isotopes, leads to the formation of pools of carbon with isotopic compositions that vary from the global average. For example, the preferential incorporation of ^{12}C compounds during photosynthesis is responsible for the general depletion of all organic matter in ^{13}C relative to the inorganic carbon pool. Because of the lower vapour pressure of $^{13}CO_2$ as compared to $^{12}CO_2$, dissolved (aqueous) inorganic

carbon tends to be enriched in ^{13}C compared to the atmospheric CO_2. This difference is preserved in the organic matter synthesized from these inorganic carbon pools by terrestrial and aquatic primary producers. Stable isotope analysis, which is based on the measurement of such differences, has proven very useful in tracing the movement of carbon between various organic and inorganic reservoirs. Whereas past research was generally limited to measuring the carbon isotopic composition of bulk organic matter, new analytical instruments combining gas chromatography (GC) with isotope ratio mass spectrometry (IRMS) now allow the rapid and routine measurement of the isotopic composition of individual compounds, such as the hydrocarbons.

In general, the three primary determinants of the isotopic composition of a particular compound are:

1. the isotopic composition of precursor material,
2. the reactions involved in synthesis, and,
3. the physical conditions (*e.g.*, temperature) of formation.

Although Galimov (1981) argues that the isotopic composition of a biosynthetic compound depends less on these factors than on the intramolecular thermodynamics of the bonding and structure of that compound, it is still generally accepted that compounds produced from different ultimate sources, by different synthetic pathways, or under different conditions may all have different isotopic compositions (as reviewed by Hayes *et al.*, 1989). The stable isotope composition of a compound can thus potentially provide information about each of the above three aspects of the origin of a compound.

Additionally, this signature can serve as a label for tracing the movement and mixing of that compound in a complex system such as the environment. As previously outlined, the hydrocarbons encountered in environmental samples can be formed by a variety of pathways, under widely differing environmental conditions, and from various isotopically distinct carbon pools and precursors. Significant variations in isotopic composition can thus be expected, and have, in fact, already been documented, between different hydrocarbons.

ii. Isotopic composition of hydrocarbons

Recent biogenic hydrocarbons: In general, the entire lipid fraction of organisms and sediments is depleted in ^{13}C (by from approximately 2 to over 10‰) compared with non-lipid components, as documented by Park and Epstein (1961), Degens *et al.* (1968), Kennicutt *et al.* (1992) and many others. This depletion has been shown by Monson and Hayes (1982) to be at least partly due to kinetic fractionation during the formation of acetate by decarboxylation from pyruvate. Pyruvate itself appears generally to have the same isotopic composition as glucose, the universal metabolic fuel and primary photosynthetic product from which it is derived (Abelson and Hoering, 1961, De Niro and Epstein, 1977). The decarboxylation of pyruvate, mediated by the the pyruvate dehydrogenase enzyme complex, however, preferentially removes $^{13}CO_2$, so that carbon in the carboxyl position of the acetate thus formed becomes relatively depleted in ^{13}C. Carbon in the methyl position appears not to be involved in the decarboxylation, and thus

retains the same isotopic composition as the precursor pyruvate. As a result, then, all of the acetyl-CoA units used in alkyl chain synthesis, and thus fatty acids and hydrocarbons, tend to be depleted in ^{13}C by at least 3 per mil relative to pyruvate. Longer fatty acids and complex lipids are even further depleted in ^{13}C as a result of the preferential reaction of fatty acid fragments with "lighter" terminal carboxyl groups later during lipid synthesis (Monson and Hayes, 1980, Fang *et al.*, 1993).

Hayes *et al.* (1989) have further proposed that the difference in the isotopic composition of the carboxyl and methyl carbons of acetate may lead to a disparity in the isotopic compositions of n-alkyl and isoprenoid compounds. Although both groups of compounds are formed ultimately from the same acetate units, n-alkyl chains contain an equal ratio of methyl-derived and carboxyl-derived carbons, whereas the five-carbon building block of the isoprenoids is composed of three methyl- and two carboxyl-derived carbons. Linear chain compounds such as the fatty acids and n-alkanes, containing a greater proportion of "lighter", carboxyl-derived carbon, are thus suggested to be biochemically more depleted in ^{13}C than their isoprenoid counterparts. Although such a disparity has been documented for some crude oils (Ivlev *et al*, 1978; Bogacheva *et al.*, 1980), it has not yet been investigated or proven in recent biogenic compounds. On the other hand, Meinschein *et al.* (1974) and Galimov (1981) found the opposite relationship between the isotopic composition of carboxyl and methyl groups, and dispute the importance of kinetically derived isotopic differences in lipid biosynthesis. All of these studies, however have examined only a very limited number of organisms, and thus this

issue is currently still not resolved.

A progressive depletion in ^{13}C has been shown to accompany fatty acid chain elongation and desaturation in some organisms (Fang *et al.*, 1993), so that related longer-chain n-alkanes might also be expected to be progressively more depleted in ^{13}C. The final decarboxylation converting a fatty acid into an n-alkane may also involve an isotopic fractionation, although the direction and magnitude of this effect has not been investigated in detail. Rieley *et al.* (1993) reported leaf wax alkanes to be consistently more depleted in ^{13}C (by 1.9 to 3.7‰ on average) than the total leaf wax lipid extract, suggesting a preferential loss of $^{13}CO_2$ during the formation of these compounds. Similarly, Hayes *et al.* (1982) found the neutral lipid fraction of bacterial cultures to be depleted by almost 13‰ relative to the acidic fraction. The carbon isotope fractionations possibly associated with the addition of methyl groups from methionine to existing carbon skeletons have not yet been investigated.

Based on these observations, then, biogenic hydrocarbons (at least those synthesized by primary producers) would be expected to have an isotopic composition consistently slightly lighter than, but related to, that of the bulk of the organism producing them (-22 to -27‰ for aquatic, and -27 to -33‰ for terrestrial organisms). Very few data exist on the isotopic composition of recent biogenic hydrocarbons, but these predictions have been partially borne out by several recent studies. Rieley *et al.* (1993) found plant leaf wax n-alkanes to be consistently depleted in ^{13}C compared to bulk leaf tissue, but observed significant differences in the intensity of these depletions, as

well as in the signatures of odd and even homologues among plants using different photosynthetic pathways and between different species. Rieley *et al.* (1991) found the isotopic composition of leaf wax n-alkanes from six common temperate deciduous trees to range from -30.1 to -38.4‰, again with considerable variation both between species and among different alkanes of a single species. Kennicutt and Brooks (1990) found terrestrial biowax n-alkanes (CPI[1] > 5) in offshore New Zealand sediments to be significantly lighter in ^{13}C than alkanes from unusual even-dominant assemblages (suggested to be bacterial in origin). In both even and odd dominated assemblages in New Zealand sediments, the odd carbon number alkanes were consistently lighter in ^{13}C than even carbon number homologues, suggesting either a separate source or synthetic pathway for even and odd homologues. The hydrocarbon and fatty acid isotopic signature of heterotrophic organisms, especially those from higher positions in more complex food webs, have been shown to be complicated by dietary and metabolic effects (*e.g.*, Murphy and Abrajano, 1994).

As mentioned earlier, many of these explanations and generalisations are based on analyses of single species in pure cultures, or of sedimentary mixtures, and thus may be somewhat premature given our relatively poor understanding of hydrocarbon biosynthesis. It must be remembered that, as of yet, only one fractionation factor has been formally measured for part of one of the lipid synthesis pathways described earlier,

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[1]CPI (Carbon Preference Index), a measure of the odd or even dominance of an alkane assemblage, is generally defined as the ratio of the abundance of odd-chain length n-alkanes to the abundance of even chain length n-alkanes (over a specifically defined range of n-alkanes).

and that some debate still exists as to the fundamental factors controlling the isotopic composition of biosynthetic compounds. Despite the uncertainty in these areas, however, it is still likely that in addition to differences expected between marine and terrestrial biogenic hydrocarbons, there will be significant inherent isotopic disparities between hydrocarbons of different species, between various classes of compounds within organisms, and possibly even among homologues of a series such as the alkanes.

Petroleum hydrocarbons: Isotopically, petroleum reflects its origin in organic matter in that it is generally depleted in ^{13}C (bulk oil values range from -25 to -35‰). Most oils are marine in origin, and are slightly depleted in ^{13}C compared to the bulk of marine sedimentary organic matter, which averages approximately -20 to -23‰. The isotopic composition of petroleum thus closely resembles that of the biochemically ^{13}C-depleted lipid fraction of these sediments, supporting the premise that lipids are the primary precursors of petroleum. This correlation is, however, not evident for all oils and source rocks, probably due to the variety of processes and conditions that can alter the original isotopic signature of organic matter during oil generation. In general, ^{12}C is preferentially lost from buried organic matter during methane generation and maturation, resulting in an enrichment of ^{13}C in residual kerogen or oil. Oils thus generally become isotopically heavier with increasing maturity (Sofer, 1984), although the opposite trend has also been documented in some fields (*e.g.*, Hughes *et al.*, 1985). The preferential loss of ^{12}C may account for the observation that the saturated fraction, formed first during maturation, is commonly more depleted in ^{13}C than the bulk oil, with

aromatics, hetero-compounds, and finally the asphaltenes being progressively more enriched in ^{13}C (Chung *et al.*, 1992, Peters and Moldowan, 1993). The n-alkane fraction has, in some crude oils, been reported to be depleted in ^{13}C relative to both the iso-paraffins (Stahl *et al.*, 1977), and to the branched isoprenoids (Schoell *et al.*, 1992). Biomarkers in petroleum, such as pristane and the porphyrins have been shown to retain their biochemical isotopic signature (Hayes *et al.*, 1989). This has been used to speculate on the origin of the cyclic triterpenoids, some of which are consistently the most ^{13}C-depleted compounds in crude oils (Moldowan *et al.*, 1991; Schoell *et al.*, 1992 and 1994). While the isotopic composition of the saturated fraction of some crankcase oils has also been reported to be depleted in ^{13}C relative to the aromatic fraction (O'Malley, 1994), the signature of some crude oil hydrocarbons could potentially be scrambled by the mixing and chemical reactions involved in refining. No compound specific isotope analyses of refined petroleum products have yet been published. In summary, then, the isotopic compositions of petroleum hydrocarbons generally lie between -20 and -35‰, and are a composite of the original signature inherited from the source organic matter and the combined effects of maturation and refining.

3. STUDY AREA

Conception Bay is a typical northern fjord located on the north east coast of the island of Newfoundland (see Figure 3). The bay has a maximum depth of 300 m, and is separated from the open ocean by a sill of 170 m depth (Slatt, 1974b). Winds, tides,

and the general counterclockwise circulation of water created by the input of the cold Labrador Current from the northwest keep the upper layers of the bay fairly well mixed, while the deeper waters (below 100 m) are less regularly exchanged (Pomeroy *et al.*, 1991). Whereas surface water temperatures vary seasonally, water temperature below 100 m depth remains below 0°C. The salinity ranges from 31.5 ppt at the surface to 33.3 ppt in deeper water (Scheibe, 1991). The depth and intensity of the thermocline present during the summer months varies with weather conditions (Ostrom, 1992). The bay is fed by numerous small rivers draining areas covered with a variety of vegetation types, including primarily coniferous and mixed forests and peat bogs. Approximately 40,000 people inhabit the shores of the bay in over 70 communities, concentrated primarily along the southern coastline. Little industry or manufacturing takes place along these shores, and vessel traffic in Conception Bay is relatively light, consisting largely of several ferries, commercial fishing vessels, and small recreational boats.

The waters of Conception Bay support a typical major spring phytoplankton bloom dominated by common cold-ocean diatoms of the genera *Chaetoceros*, *Thalassiosira*, *Fragilariopsis*, *Skeletonema* and *Rhizosolenia*. A less intense bloom, also featuring coccolithophorids and dinoflagellates, occurs in the fall. A wide variety of typical cold water zooplankton and bacteria also flourish at different times, depending on weather conditions and the influence of the Labrador Current (C. Mackenzie, pers. comm.). Microbial utilization of sinking organic matter appears to be strongly limited by the constant low temperatures in deeper waters of the bay (Pomeroy and Deibel,

Figure 3. Map of study area (point labelled sampling site marks core location and approximate location of plankton tows)

ISLAND
of
NEWFOUNDLAND

Bonavista Bay

Trinity Bay

Conception Bay

Fortune Bay

GRAND BANKS

Job's Cove

N

200 m

100 m

200 m

47°50

Conception Bay

200 m

47°40

Spaniard's
Bay

SAMPLING
SITE

Bell Isl.

St. Phillip's

St John's

South
River

Topsail

Mundy
Pond

47°30

Avondale

Holyrood

48°00

53°20   53°00   52°40
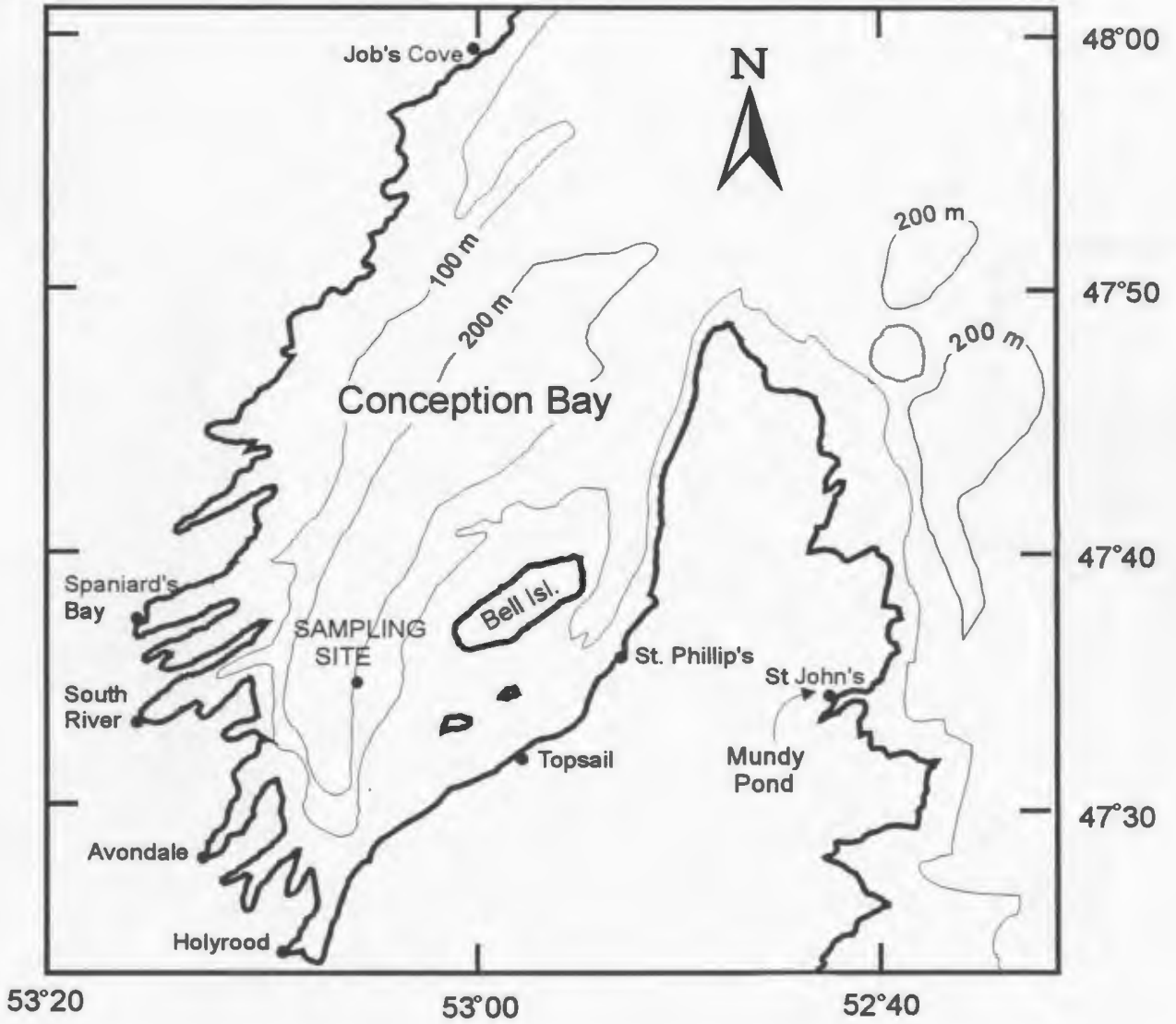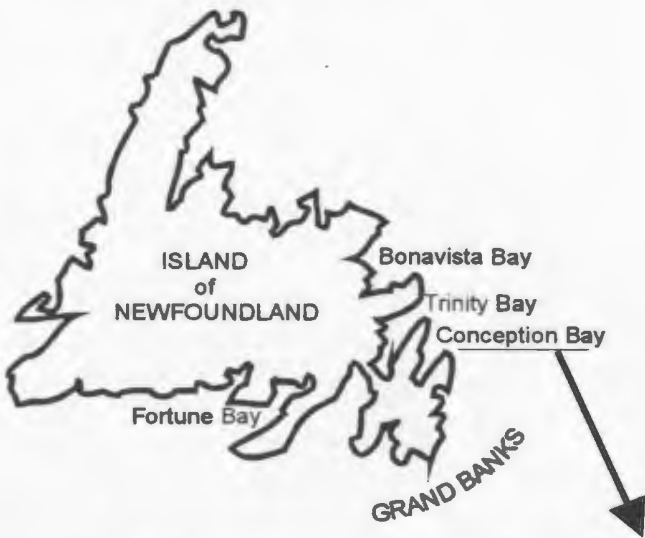
1986), so that these phytoplankton blooms support a diverse food chain.

The sediment cover in Conception Bay varies, but in the deepest portions consists of mud and sandy mud (Slatt, 1974a), and is accumulating at a rate of approximately 0.5 cm/yr (Ostrom, 1992). The upper 5 to 10 cm are well oxygenated, whereas sediment deeper than 10 cm appears to be generally anoxic (O'Malley, 1994). A relatively high density of burrowing sediment-feeders, including polychaetes, mud stars, and bivalves, keep the sediment highly bioturbated (Scheibe, 1991). The average organic carbon content of sediments of Conception Bay ranges from 0.8% in muddy sands to 3.5% in muds (Slatt, 1974a). Ostrom and Macko (1992) propose, on the basis of bulk sediment isotopic signatures (average $\delta^{13}C = -21.3‰$), that the input of terrestrial organic matter into this system is relatively minor, especially in deeper portions of the bay. Hellou *et al.* (1993) found the unsaturated hydrocarbon assemblage of sediments and some organisms of Conception Bay to be dominated by a suite of at least three highly branched C_{25} alkenes. These compounds (and other similar compounds based on structures d - f in Figure 1) have been identified in aquatic (mostly marine) environments worldwide in many studies (Rowland and Robson, 1990). Although Volkman *et al.* (1994) recently showed several of these compounds to be synthesized by diatom species, the exact source of these hydrocarbons in most environments is still not known for certain. Not all of the diatom species commonly found in Conception Bay have been analyzed for hydrocarbons, and thus the possibility exists that diatoms are responsible for the presence of these compounds in this locality as well.

II. EXPERIMENTAL

a. SAMPLE COLLECTION, EXTRACTION, AND PURIFICATION

1. SAMPLING AND SAMPLE PREPARATION

Plankton: Plankton samples were collected at several times during the year from surface waters of Conception Bay by towing a 20 μm mesh hoop net for various lengths of time at a depth of 5 - 10 m. Samples were kept chilled with ice during the brief period of time between collection and processing. Plankton was concentrated by filtration through glass fibre filtres (Whatman GF/F) which had been cleaned by rinsing with hexane. After weighing, samples were dried overnight in a darkened fume hood, re-weighed, and extracted immediately. For comparison, several plankton samples were also collected by a similar method from the surface waters of the Grand Banks, at a position of approximately 47°30' N, 52°30'W.

Diatom cultures: Based on preliminary results, it was decided to investigate individually the most abundant species of diatoms present in the spring plankton bloom which had not been previously analysed. Log-phase diatom cultures (approximately 1 litre each of the species *Thalassiosira nordenskioldii, Fragilaria striatula, Nitzschia seriata,* and *Fragilariopsis cylindrus*) were purchased from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton (Boothbay Hr., ME, U.S.A). Culturing conditions are described in Appendix 4. The cultures were filtered, dried and weighed in the same fashion as described for the natural phytoplankton samples.

Sediments: Sediments were obtained from Conception Bay (47°34.6'N, 53°8.3'W)

and, for comparison, from the Grand Banks (47°32.7N, 52°35.2W) using a 30 cm piston corer. Sediments were also collected from different points along various rivers flowing into Conception Bay, as well as from several freshwater ponds in the same area using a simple piston corer. Sample sites included unpolluted upstream locations (Job's Cove Pond, Holyrood River), polluted and unpolluted freshwater river mouths (St. Phillip's harbour and Topsail Beach Pond), and brackish water river mouths (Spaniard's Bay and South River). All sediment samples were kept frozen at -20°C until further processing. Sediments were dried overnight in a darkened fumehood, and approximately 30 g were weighed out for each extraction.

Biota: Previously extracted and purified unsaturated hydrocarbons of the hepatopancreas of snow crabs (*Chionectes opilio*) and spider crabs (*Hyas coarctatus*) collected from Conception Bay (Hellou *et al.*, 1994) and of the visceral mass of scallops (*Placopecten magellanicus*) collected from the St. Pierre Bank south of Newfoundland (Hellou *et al.*, 1993) were also analysed.

Petroleum: Samples of unused, common automotive lubricating and outboard motor oils were purchased from local commercial sources. Three different brands of each oil type (see Appendix 5) were analyzed, both individually, and pooled together with others of the same type. Samples of used crankcase motor oil were obtained from the oil storage tank of a service station. The aliphatic hydrocarbon fractions of samples of a No. 2 fuel oil, automotive muffler soots and St. John's roadsweeps (collected and extracted as described by O'Malley, 1994) were also acquired for analysis. Samples of

used outboard motor oil were obtained by sampling the water (surface slick and subsurface) of a tank used to test outboard motors at a commercial marine equipment dealership.

2. HYDROCARBON EXTRACTION AND PURIFICATION

Extraction: All sediment and plankton samples were Soxhlet extracted with dichloromethane (CH_2Cl_2) for 24 hrs. Anhydrous sodium sulfate (Na_2SO_4) was added to all samples (roughly 10% w/w) during extraction in order to remove any residual water. Extracts were reduced in volume to approximately 1 mL using a rotary evaporator (at 30°C), blown down to dryness under a stream of N_2, weighed, and then taken up in approximately 0.5 mL hexane for column chromatography.

To test the efficiency of Soxhlet extraction, and to estimate the concentration of bound hydrocarbons in sediments, some sediment samples were hydrolyzed and re-extracted. The Soxhlet extracted sediment was refluxed for 6 h in 100 mL of a 10% (w/w) solution of KOH in methanol, neutralised with HCl, and extracted three times with 80 mL of hexane. The combined hexane extracts were then reduced in volume to 0.5 mL using a rotary evaporator. Hydrocarbons in the outboard motor test tank water were recovered by extracting 30 mL of the water sample with 60 mL of hexane three times. The combined extracts were then reduced in volume to 0.5 mL. The unused automotive and outboard motor oils, as well as the used crankcase oil samples, required no other processing prior to column chromatography.

Column chromatography: The aliphatic hydrocarbons of extracts and oils were chromatographically isolated and separated using columns of alumina and silica gel eluted with combinations of hexane and dichloromethane. The plankton samples were processed using the same procedure as used by Hellou *et al.* (1993, 1994) for the crab and scallop samples. For each sample, two columns (1 cm diameter by 30 cm long) one loaded with 7.5 g of silica gel and and the other with 5% deactivated alumina (both of mesh size 100-200) were prepared and cleaned by eluting with 30 mL of dichloromethane followed by 50 mL of hexane. The hexane soluble fractions of the extracts were first purified on the alumina column, evaporated down to 0.5 mL, and then chromatographed on the silica column. The saturated and aromatic/unsaturated fractions were eluted from this column with 30 mL of hexane followed by hexane dichloromethane (1:1), respectively. These fractions were reduced in volume and blown down under a stream of N_2 to a constant weight. After weighing, they were redissolved in hexane in for GC-MS analysis (at a concentration of approximately 50 ng/μL).

In order provide better separation between some unsaturated compounds found to coelute during early GC analysis, a second chromatographic scheme, based on that of Llorente *et al.* (1987), in which alkenes with less than 3 double bonds are collected with the alkane in the hexane fraction, was used for the most of the samples. A single column, loaded with 8 g of alumina on top of 8 g of silica (both 7.5% deactivated) was prepared and cleaned as described above. Approximately 0.5 g of activated granular copper was added to the top of columns used for sediment samples to remove elemental sulfur. After adding the hexane soluble portion of the extracts to these columns, alkanes and most alkenes were eluted with 30 mL of hexane, while more unsaturated aliphatics

Figure 4. Flow chart of sample work up and analysis
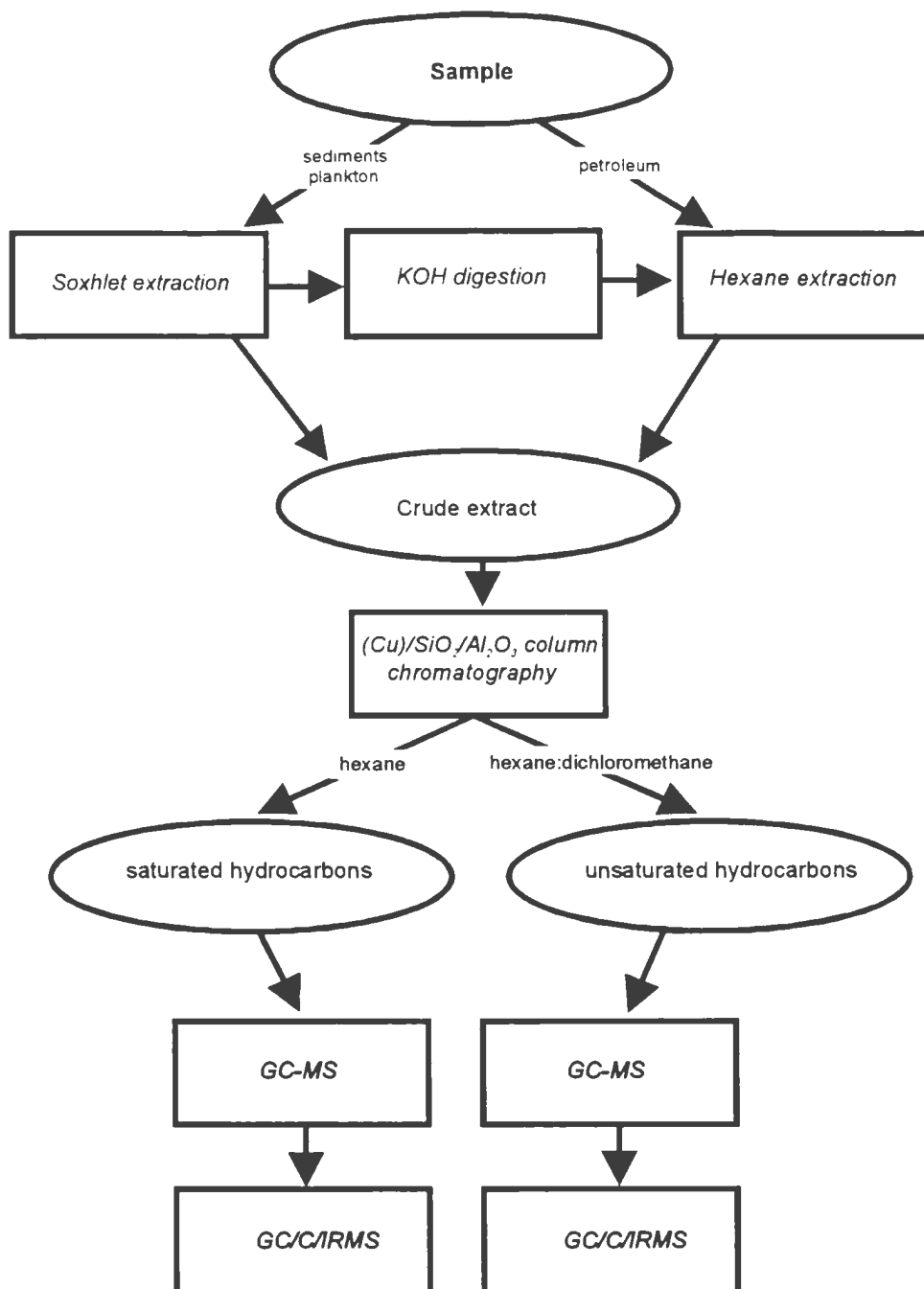
(and aromatics) were eluted with 30 mL of 9:1 hexane:dichloromethane.

b. SAMPLE ANALYSIS

1. GC-MS ANALYSIS

Hydrocarbons were identified and quantified by gas chromatography-mass spectrometry using a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett Packard Series 5970 mass selective detector, and a Hewlett Packard Series 300 data system. The gas chromatograph was equipped with a CP-Sil 5 column (25m $\times$ 0.25 mm i.d., film thickness 0.12 μm), with helium as the carrier gas (flow: 1 mL/min). All injections (1 μL) were splitless, and injector and detector temperatures were maintained at 275°C and 300°C, respectively. The oven temperature program started at 35°C for 1.5 min, and increased to 280°C at 2°C/min, where it remained for 10 min. Mass spectral data was acquired in the electron impact mode (70 eV), scanning the ion range of m/z = 50 to 450 atomic mass units (a.m.u.) once every 1.2 sec. Hydrocarbons were identified by comparison of retention indices and fragmentation patterns with literature data. Detector response was calibrated using standard solutions of n-C_{19} and n-C_{30}. Sample hydrocarbons were assumed to have response factors similar to the average of these two standards, and concentrations were determined from total ion current trace peak heights. Blanks of the filters, extraction thimbles, columns, and solvents used were analysed to determine background hydrocarbon levels.

2. GC/C/IRMS ANALYSIS

The isotopic compositions of the individual hydrocarbons were determined using

a VG Isochrom system consisting of a Hewlett Packard 5890 gas chromatograph coupled via a combustion interface and cold trap to a VG Optima isotope ratio mass spectrometer. The overall design, specifications, and performance of this machine are discussed by Freedman *et al.* (1988), and the MUN facility is specifically described by O'Malley *et al.* (1994), and O'Malley (1994). Gas chromatographic conditions were identical to those used for GC-MS analyses. All carbon isotopic measurements are reported in conventional delta (δ) notation:

$$\delta^{13}C \ (\text{in } \textperthousand) = 1000(R_S/R_{PDB} - 1),$$

where R represents the ratio $^{13}C/^{12}C$, and the subscripts s and PDB refer to the sample and standard Pee Dee Belemnite, respectively.

As reported by O'Malley *et al.* (1994), and O'Malley (1994), measurements generated by the machine used generally have a precision of less than $0.3\textperthousand$, and are accurate to within approximately $0.6\textperthousand$, depending on the nature of the sample. Inaccuracies in GC/C/IRMS can arise due to the coelution of compounds and as a result of errors associated with the removal of the isotopic contribution of the background (Matthews and Hayes, 1978; Hayes *et al.*, 1991). Coelutions, however, can generally be recognised by careful examination of the trace of the ratio of ion m/z = 45 to ion m/z = 44. Isotopic values of questionable integrity were generally not included in comparisons of the data. Samples with a significant background UCM (unresolved complex mixture) were spiked with pyrene and benzo(b)fluoranthene of known isotopic compositions as internal standards to monitor the effectiveness of the background correction. No problems due to background interference were encountered in the samples studied.

III. RESULTS

a. PLANKTON SAMPLES[2]

1. NATURAL PLANKTON TOWS[3]

Molecular signature: The hydrocarbon assemblage found in the spring bloom plankton tows was dominated by the presence of n-heneicosahexaene ($nC_{20:6}$), squalene ($bC_{30:6}$), isomers of a series of C_{25} highly branched isoprenoid (HBI) alkenes ($bC_{25:3,4,5}$), pristane ($bC_{17:0}$), and three isomers of phytadiene ($bC_{20:2}$).   Also present in lower concentrations in these samples was a branched C_{17} mono-olefin, two isomers of a branched C_{21} olefin, and a series of n-alkanes ranging from nC_{15} to nC_{31} maximizing at approximately C_{25-27} and displaying little or no odd or even predominance. Plankton tows taken later during the summer contained only n-heneicosahexaene, squalene, pristane, and n-alkanes from nC_{15} to nC_{35}. The n-alkanes of these later samples display a marked odd over even predominance over the range of nC_{25-33} (see Figure 5). The amount of material collected in the plankton tows from the Grand Banks was unfortunately too small to analyse, although traces of some HBI alkenes were detected in one of these samples. The C_{25} HBI alkenes in the spring bloom plankton are the same compounds as those identified in scallops and Conception Bay sediments and crabs by Hellou *et al.* (1993, 1994). Additional isomers of each compound are also present, so that a total of eight

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[2]Concentrations and isotopic compositions of individual compounds are tabulated in Appendices 1 (plankton tows) and 2 (sediments). Retention indices of all compounds are tabulated in Appendix 6.

[3]The species compositions of plankton tows are shown in Appendix 3.

separate peaks, consisting of two isomers of each of a triene, two tetraenes, and a pentaene, are actually distinguishable on GC traces (see Figure 6). Based on their relative retention time and fragmentation pattern (shown in Figure 7), these compounds all appear to have similar structures, differing only in the number of double bonds and the stereochemistry around these bonds.

Both spring and fall samples also feature a small UCM in the unsaturated hydrocarbon fraction, responsible for a background total ion current (TIC) of roughly the same size as that of the n-alkanes. Plotting the intensity of ions m/z = 191 and 217 a.m.u. revealed the presence of distinct suites of hopanoid and steroid hydrocarbons indicative of geologically mature (petroleum) hydrocarbon input as part of this UCM (see Figure 8). The phytoplankton tow samples were in general marked by a low ratio of tricyclic to pentacyclic (hopanoid) terpanes.

Isotopic signature: The isotopic compositions of the plankton tow hydrocarbons ranged from -21.4 to -36.4 per mil. Individual spring and fall samples are shown in Figure 9, and are summarized in Figure 10. Meaningful values could not be obtained for some compounds in some samples due either to low abundance, or to peak overlap with other compounds. Isotopic values in these cases, such as for n-C_{21} in phytoplankton tow samples (which coelutes with brC_{25}4a'), are not included in data analyses. Among the compounds other than the alkanes, there appear to be two broad groups, one with isotopic compositions between -22 and -28‰, and the other with isotopic compositions less than -28‰ (see Figure 10). The former includes heneicosahexaene, squalene,

Figure 5. Sample extracted ion chromatograms of m/z = 57 a.m.u. of
        Conception Bay samples (hexane or saturated fraction):

     a. spring bloom plankton tow (CPI $\approx$ 1)
     b. fall plankton tow (CPI > 1)
     c. surface sediment
     d. 30 cm deep sediment

     (note similarity between c and d)
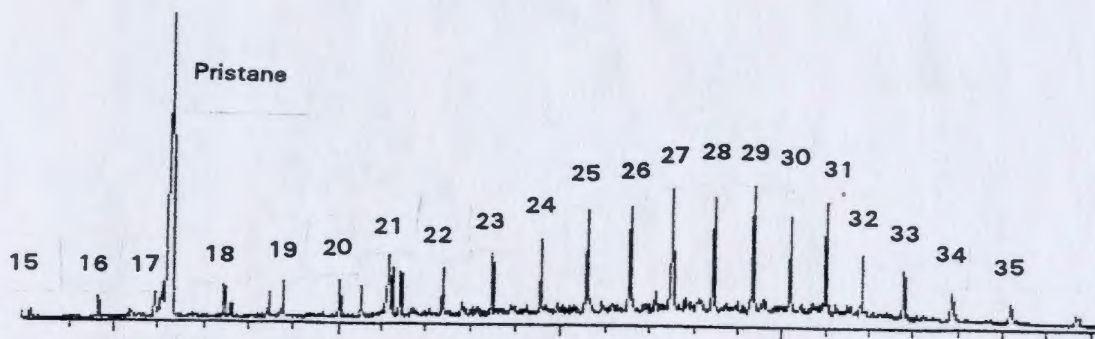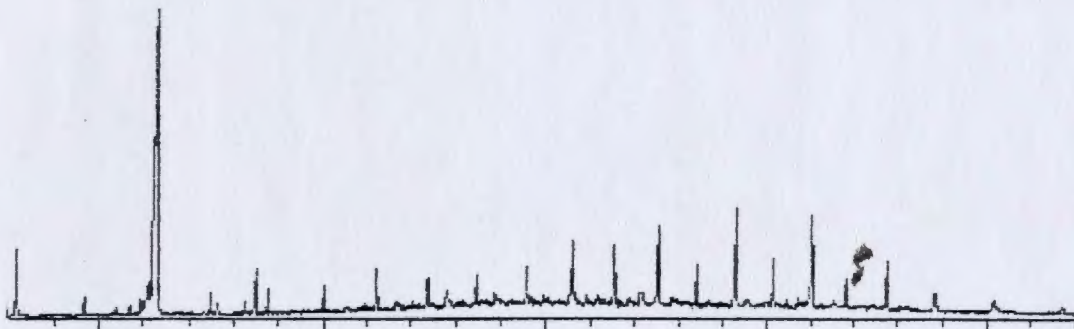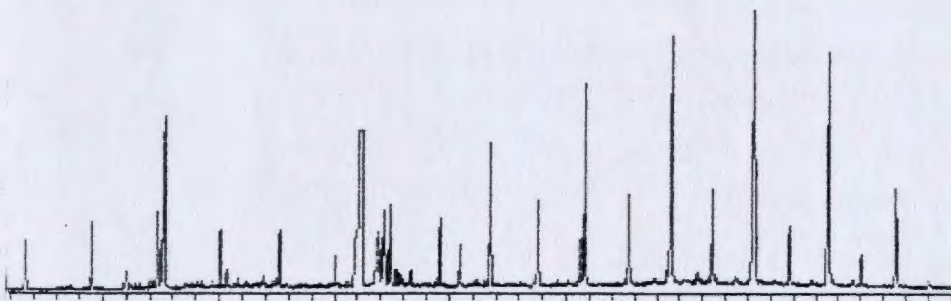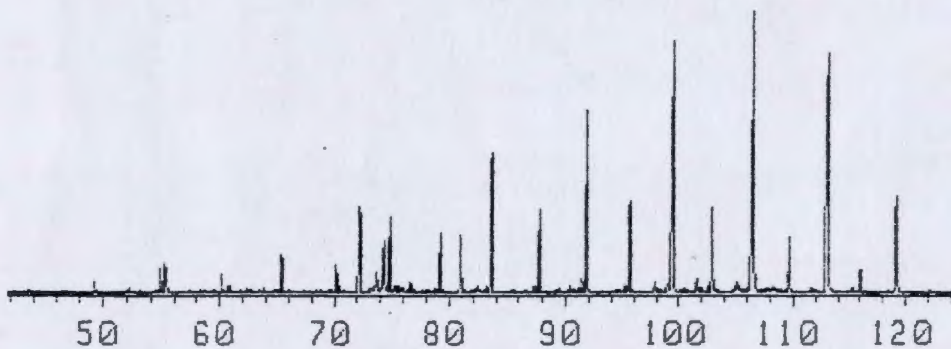
TIME (min.)

Figure 6. Total ion traces showing C_{25} HBI alkene distributions in marine samples:

    a. Conception Bay spring bloom plankton tow
    b. Conception Bay surface sediment
    c. Grand Banks surface sediment
    d. scallop visceral mass
    e. crab hemolymph
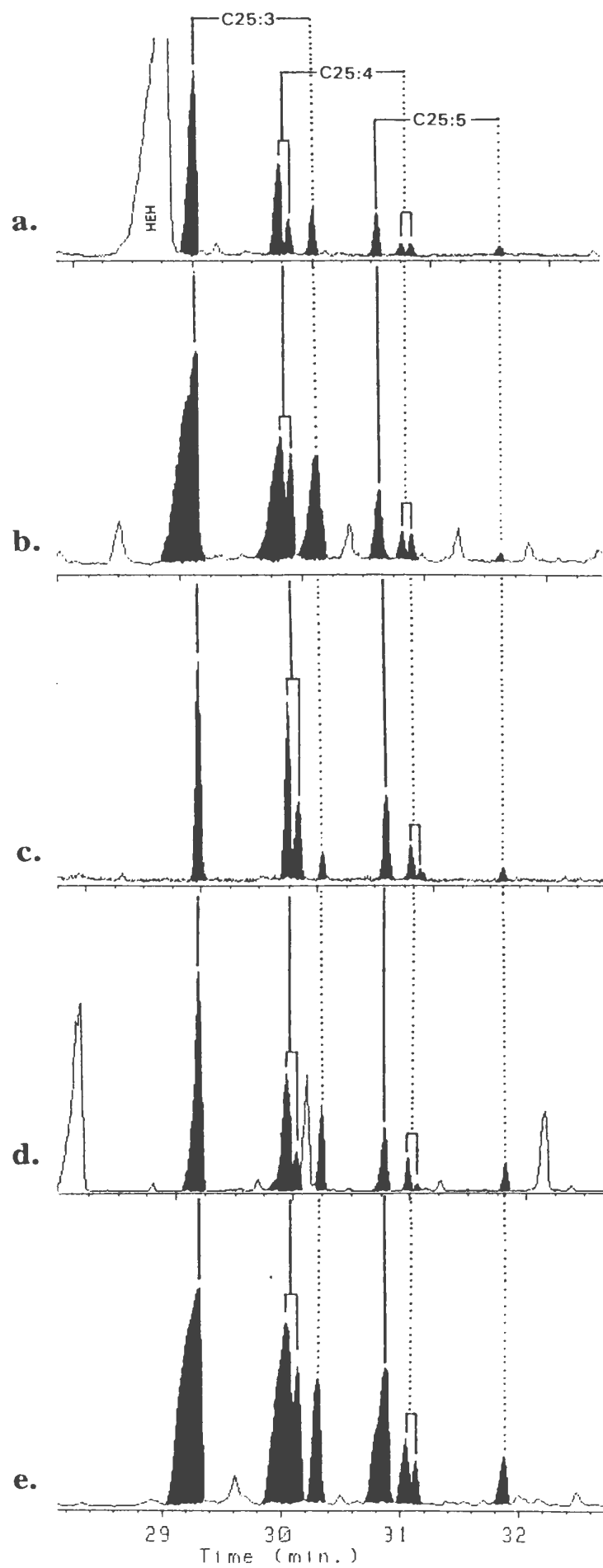
Figure 7. Electron impact spectral patterns of C_{25} HBI alkenes
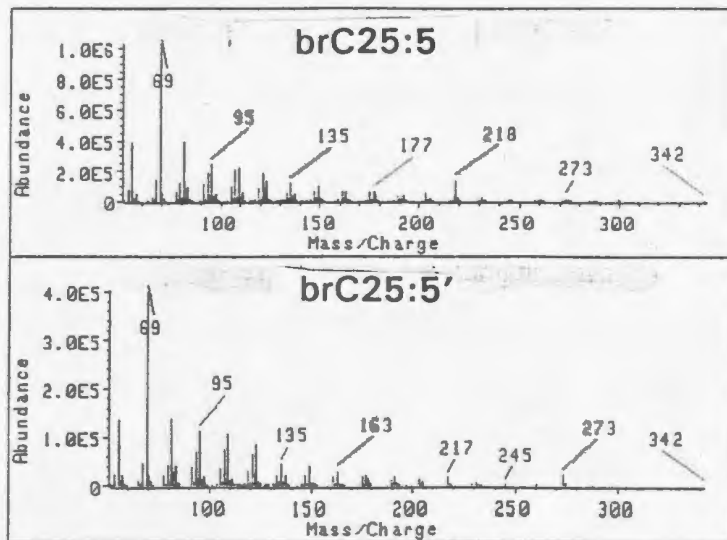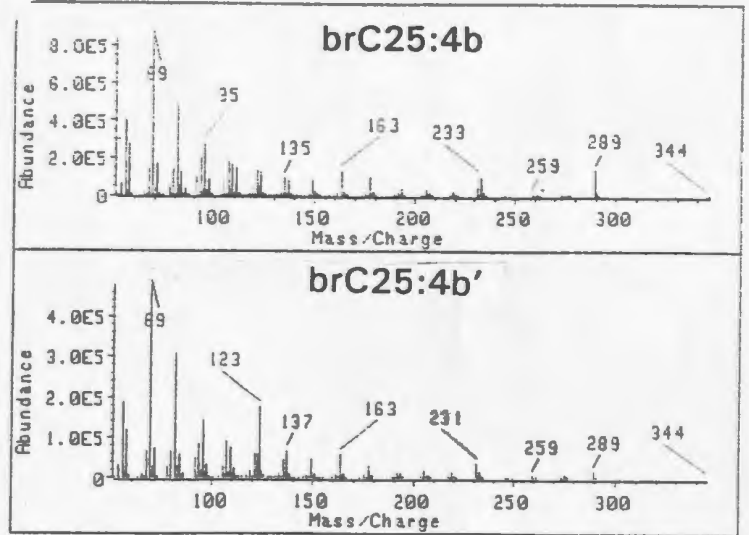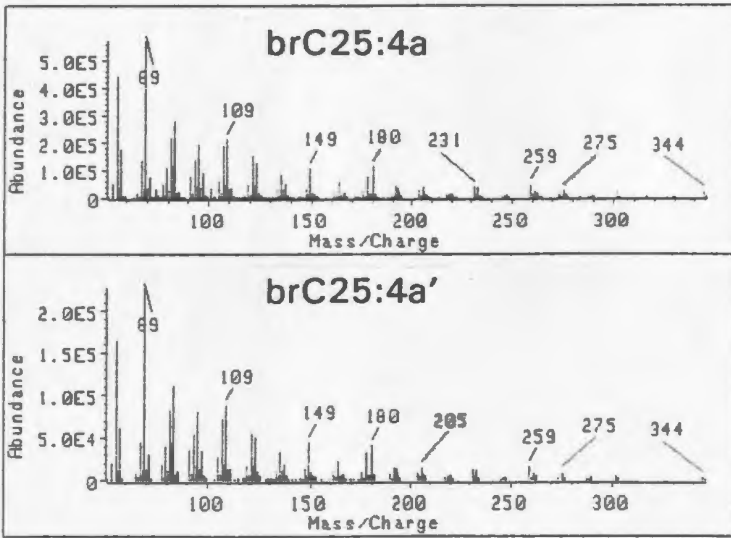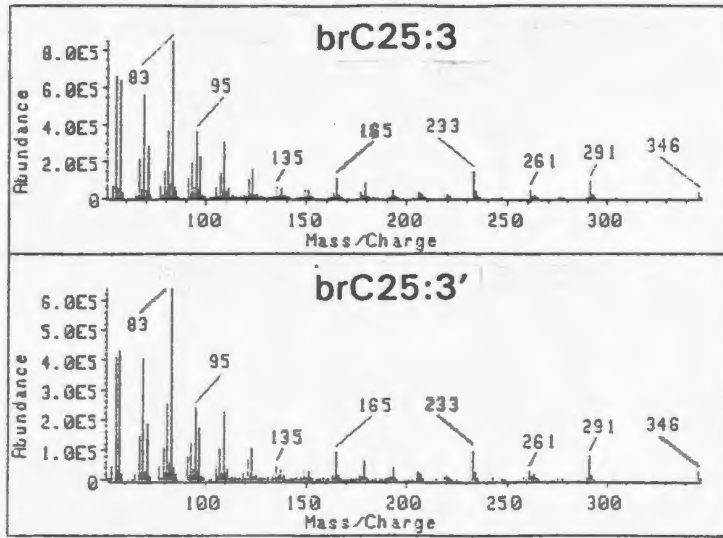
Figure 8. Extracted ion chromatograms of m/z = 191 a.m.u. of
hexane fractions of:

a. outboard motor oil (composite)
b. phytoplankton tow (hexane fraction)

(note predominance of hopanes over terpanes)

a.

b.

TRICYCLIC TERPANES

HOPANES

C_{29}

C_{30}

C_{31}

C_{32}

C_{33}

C_{34}

C_{35}

40 50 60 70

TIME (min.)
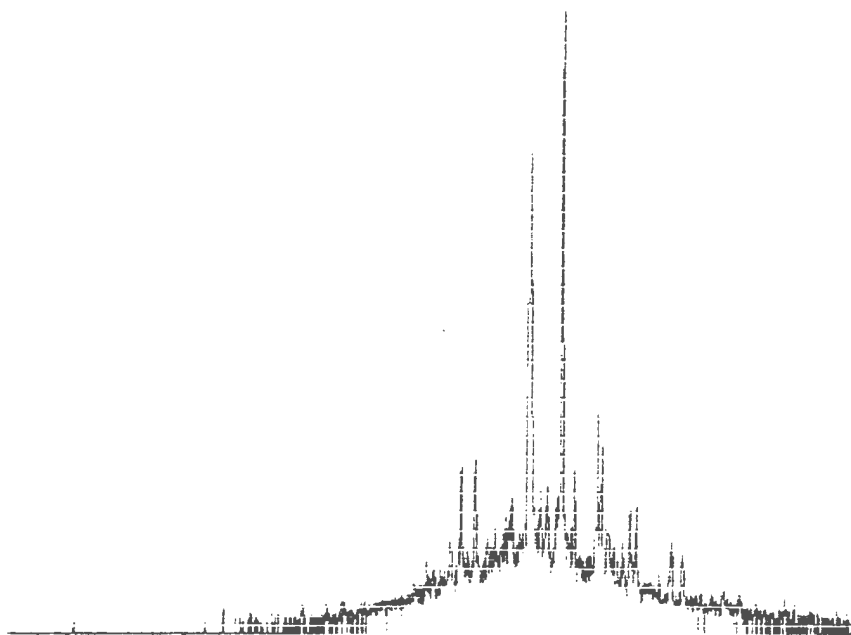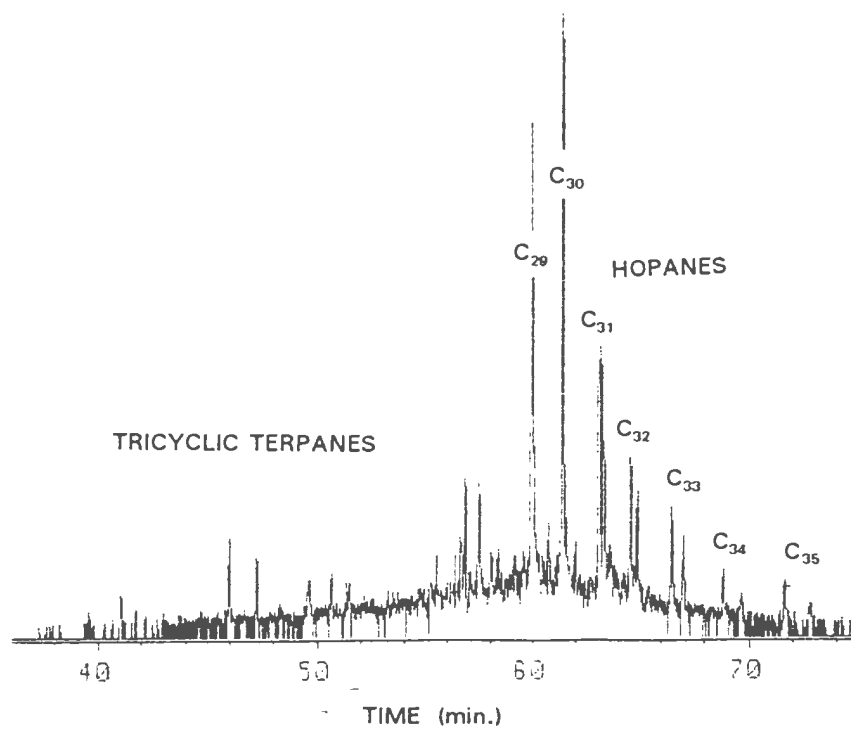
Figure 9. N-alkane abundances and isotopic compositions of mid-spring bloom, late spring bloom, end of spring bloom, and fall bloom plankton in Conception Bay

(Bars represent concentrations; points denote isotopic values)

Mid-bloom
n-alkanes
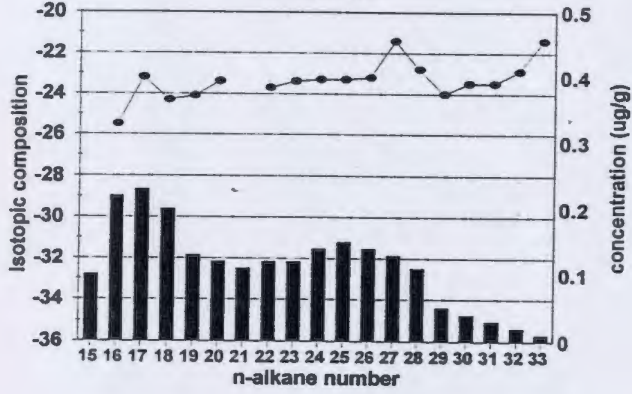
Late bloom
n-alkanes
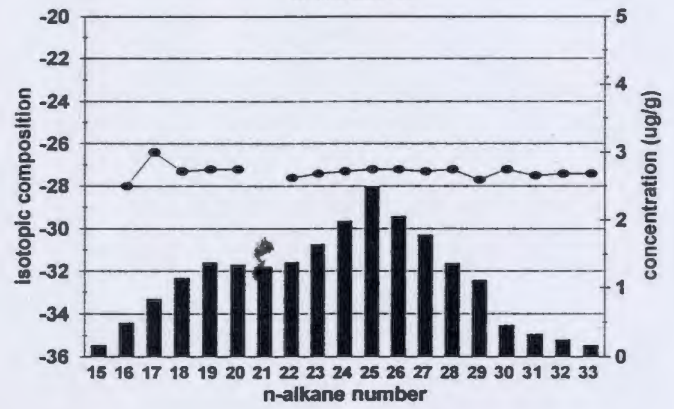
End of Bloom
n-alkanes
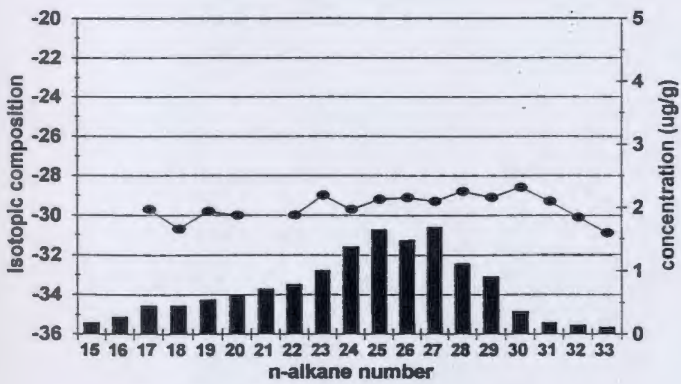
Fall plankton
n-alkanes
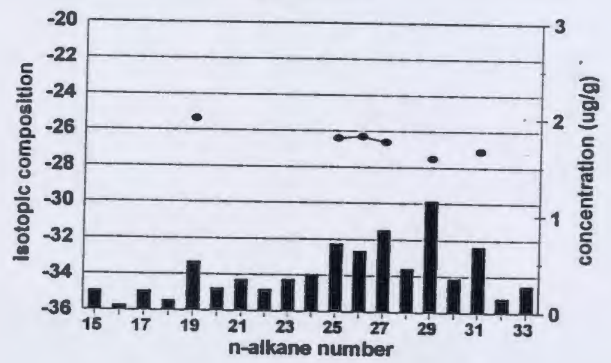
Figure 10. Summary of isotopic compositions of plankton samples

Phytoplankton tow aliphatics
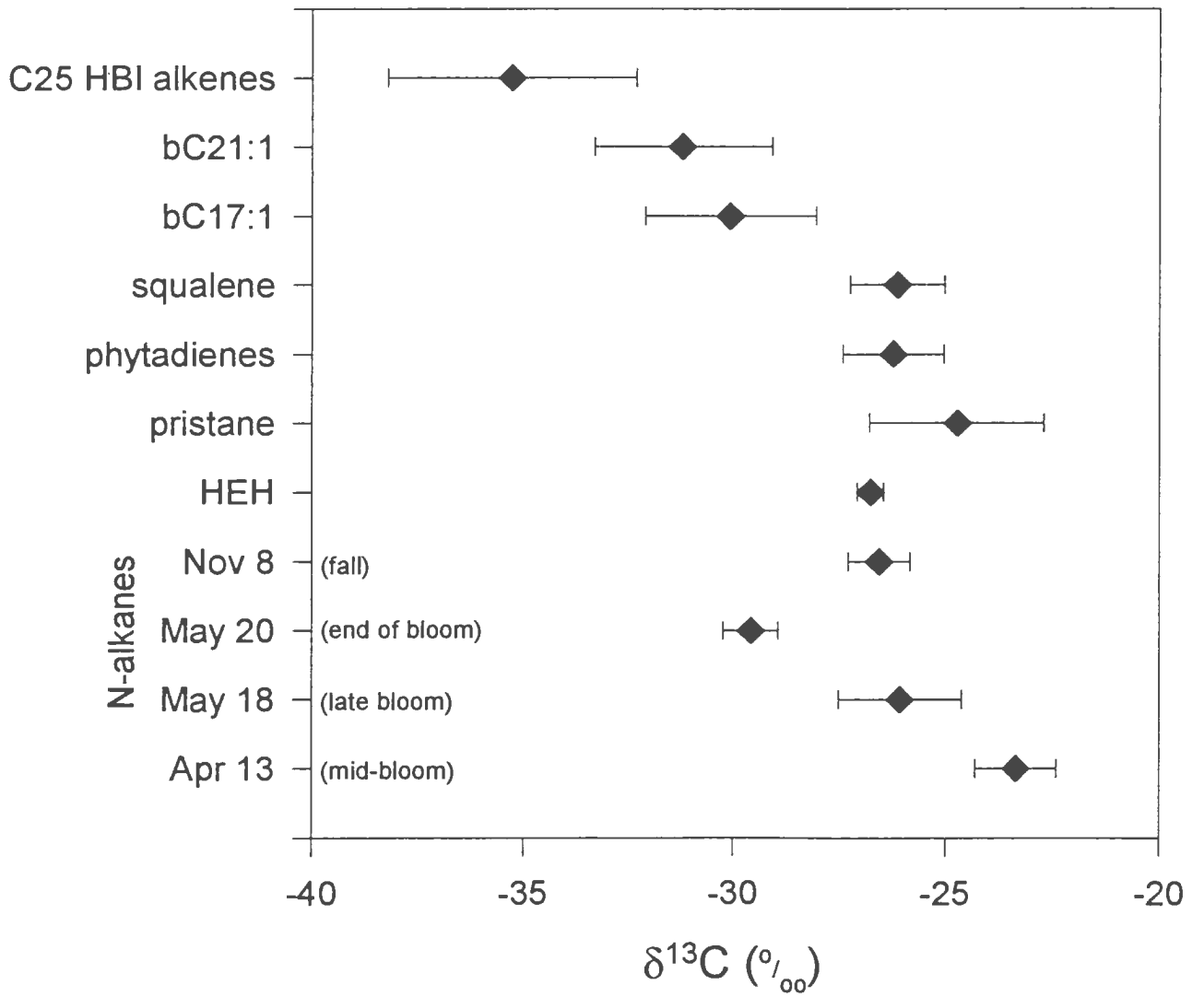
pristane, and the phytadienes (all known phytoplankton products), while the latter consists of the C_{25} HBI alkenes, $bC_{17:1}$, and the two isomers of $bC_{21:1}$. The n-alkane isotopic compositions change from an average of -23.7‰ at the height of the spring bloom to an average of -29.6‰ at the end of the bloom. Hopanes and steranes were not abundant enough to be resolved from the UCM in these samples, and thus their isotopic compositions could not be determined.

2. DIATOM CULTURES

Molecular signature: The hydrocarbon assemblage of all of the diatom cultures analysed consisted almost entirely of n-heneicosahexaene. Minor squalene was also detected in all cultures except that of *T. nordenskioldii*. Small amounts of pristane, phytane, and a series of n-alkanes from nC_{15} to nC_{32}, as well as several phthalates were also detected, but are all likely to be contaminants from the culture facility as they were also present in the culture blanks. Production of n-heneicosahexaene ranged from 52 to 254 μg/g dry weight of phytoplankton as shown in Figure 11. The HEH produced by the diatoms *T. nordenskioldii*, *F. cylindrus*, and *N. seriata* consisted entirely of one isomer, whereas *F. striatula* produced also an equal quantity of another (earlier eluting) isomer. No C_{25} HBI alkenes were detected in these cultures.

Isotopic signature: The isotopic composition of the HEH produced by the diatom cultures is also depicted in Figure 11. The isotopic composition of the single isomer of HEH produced by *T. nordenskioldii*, *F. cylindrus*, and *N. seriata* (-26.3 $\pm$ 0.6‰) is

Figure 11. Abundance and isotopic composition of HEH produced by diatom cultures
(bars represent concentrations; points denote isotopic values)

-Note distinct isotopic composition produced by *F. striatula*

Diatom HEH production
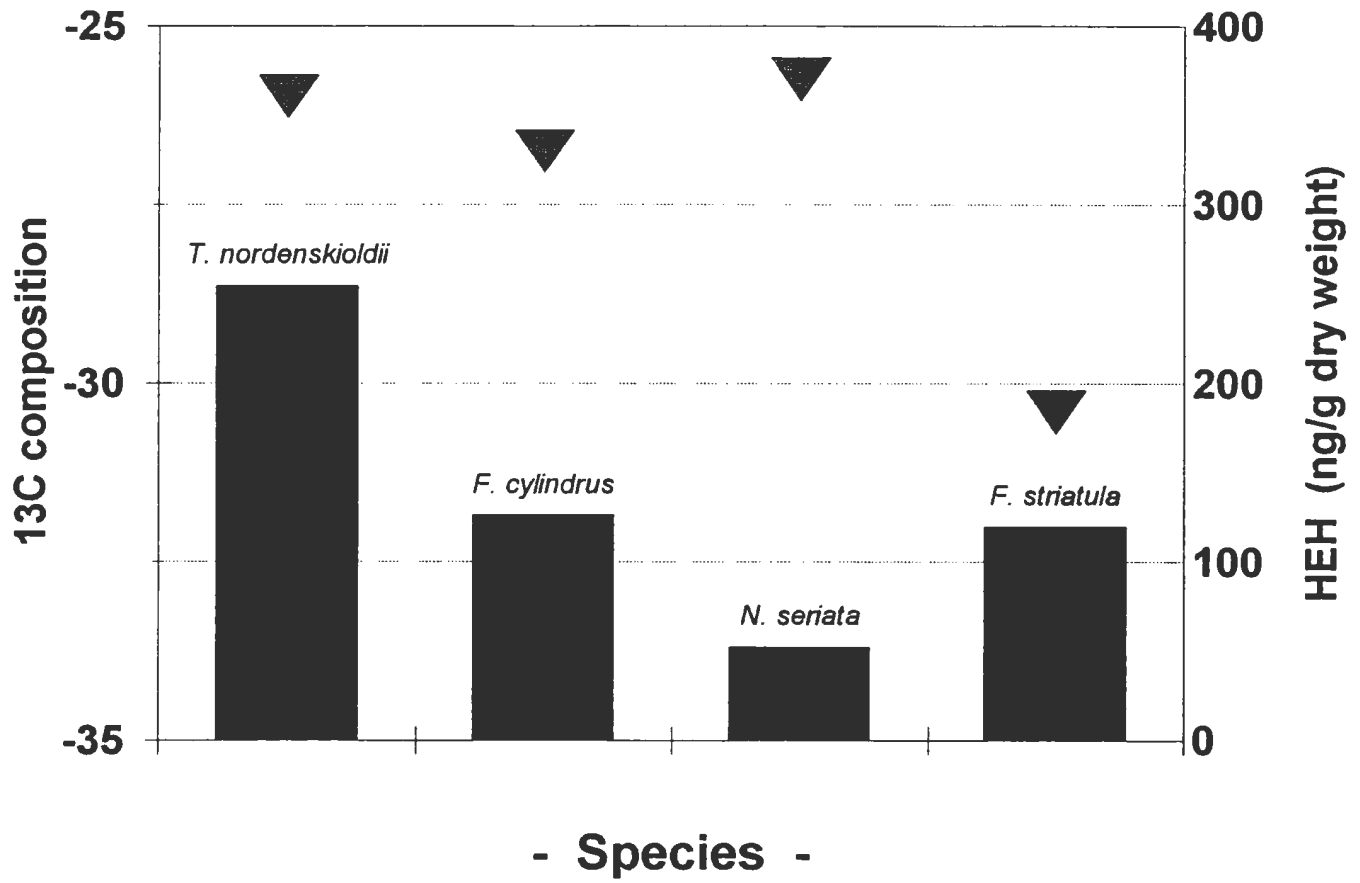
approximately the same as that of the HEH extracted from natural phytoplankton in Conception Bay. Both of the isomers of HEH produced by *F. striatula* are depleted in ^{13}C by almost 4‰ relative to that synthesized by the other species. The isotopic composition of squalene could not be determined due to the low abundance of this compound in these samples.

b. PETROLEUM SAMPLES

1. AUTOMOTIVE OILS, MUFFLER SOOTS AND ROADSWEEPS

Molecular signature: The unused automotive lubricating oils analysed all had similar chromatographic features, consisting almost entirely of a unimodal, symmetrical UCM spanning Kovats retention indices (RI)[4] 1700 to 3300, and maximizing consistently at an RI of approximately 2500 (see Figure 12a). The only resolved components of this mixture were typically pristane, phytane, and a series of n-alkanes from nC_{16} to nC_{22} showing no odd-even predominance. The chromatograms of all of the individual unused oils analysed were virtually identical to the composite profile shown in Figure 12a. The extracted ion chromatograph of m/z = 191 a.m.u. from these oils (Fig. 13a) typically revealed an relatively high ratio of tricyclic to pentacyclic terpanes, with the C_{23} homologue of the extended tricyclic terpane series dominating over norhopane and

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[4]The first two digits of a Kovats Retention Index of a compound denote the carbon chain length of the n-alkane eluting directly before the compound; the second two represent the time *after* this reference n-alkane that the compound elutes expressed as a percentage of the total time between elution of the reference n-alkane and the following n-alkane.

Figure 12. Total ion chromatograms of the hexane fraction of:

        a. unused automotive oil
        b. used crankcase oil
        c. muffler soot

a. UNUSED AUTOMOTIVE OIL

b. USED CRANKCASE OIL

C_{12} C_{13} C_{14} C_{15} C_{16} C_{17} C_{18} C_{19} C_{20} C_{21}

c. MUFFLER SOOT

TIME (min.)
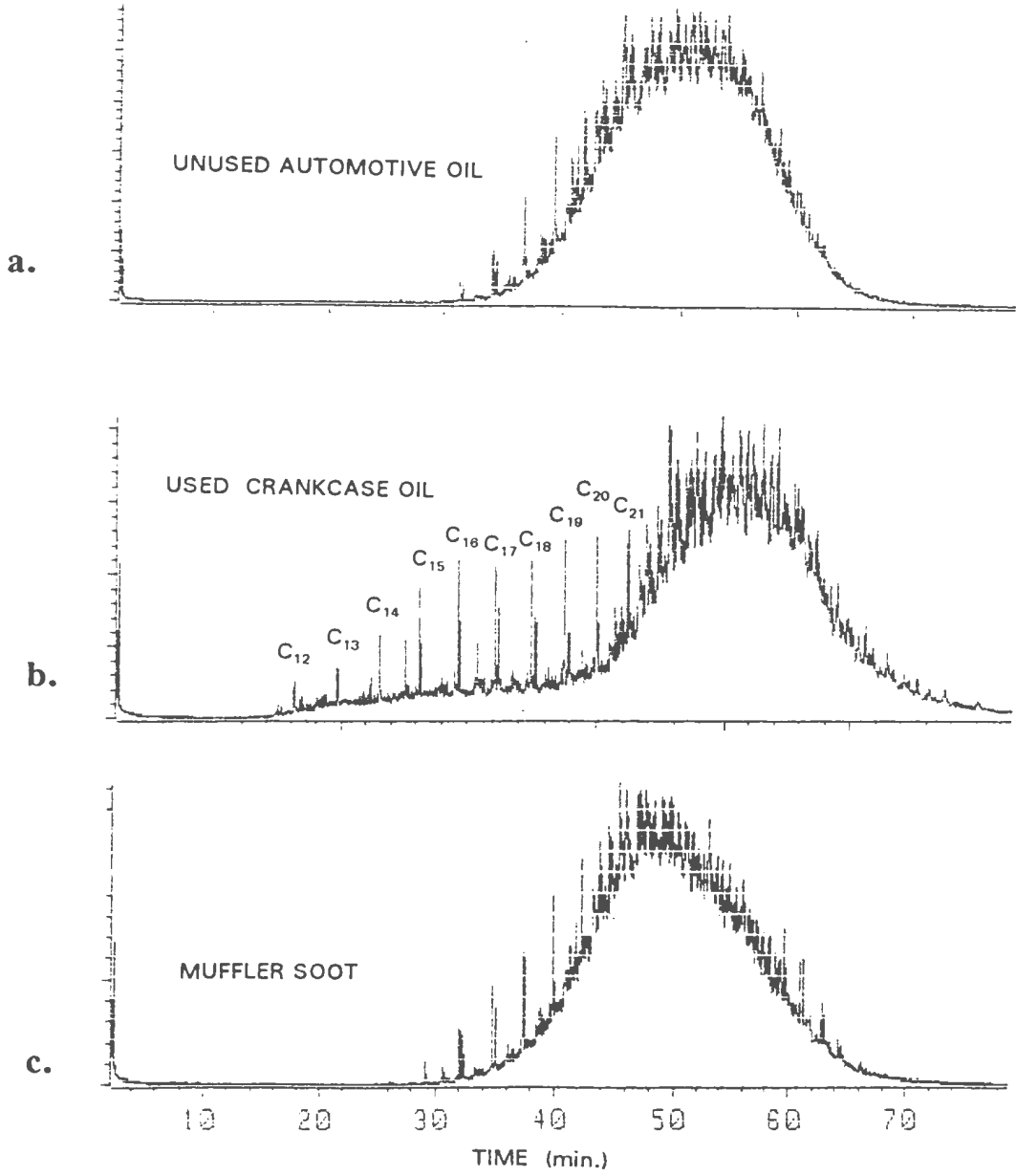
Figure 13. Extracted ion chromatogram of m/z = 191 a.m.u. of
hexane fractions of:

a. fresh, unused automotive oil
b. Mundy Pond surface sediment
c. Conception Bay surface sediment

(note greater abundance of terpanes)

a.

b.

HOPANES

diploptene

C_{30}

C_{29}

C_{31}

C_{32}

TRICYCLIC TERPANES

C_{20} C_{21} C_{22} C_{23} C_{24} C_{25} C_{26} C_{27}

C_{33}

C_{34} C_{35}

c.

C_{30}

diploptene

C_{29}

70   80   90   100   110   120

TIME (min.)
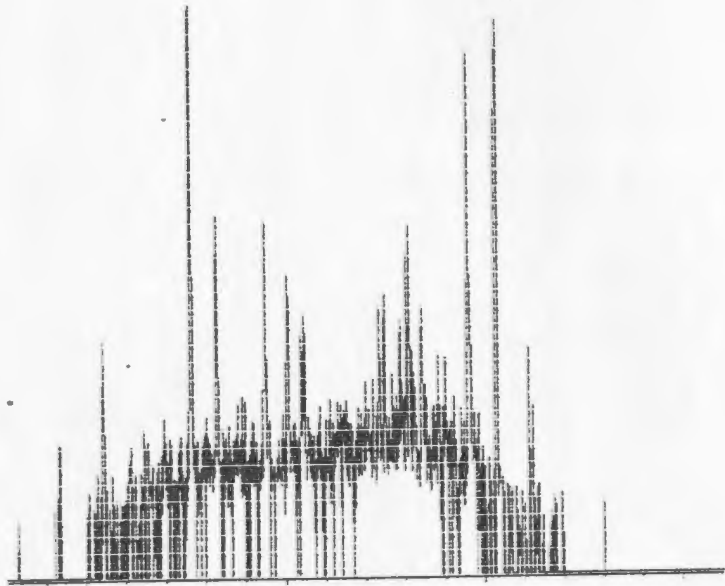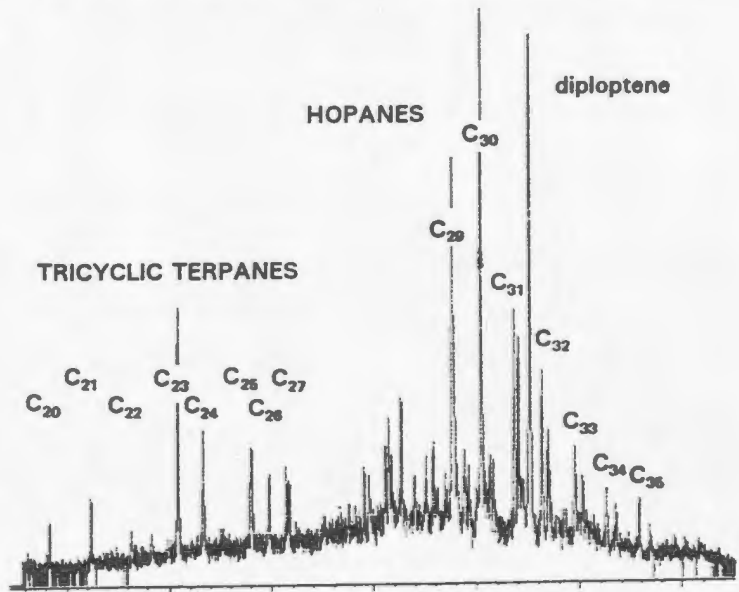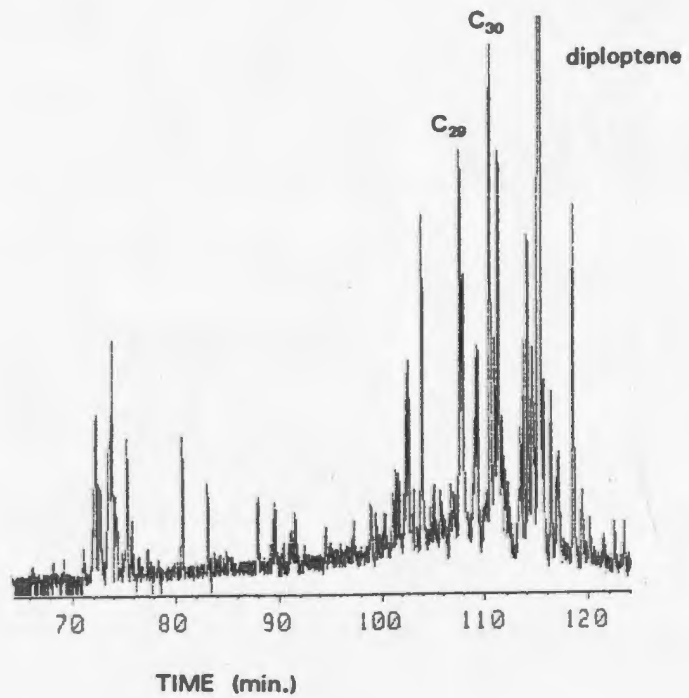
hopane. Extended hopanes (C_{31} to C_{33} homologues) were present in relatively low concentrations in unused automotive oils. The shape of the chromatogram of the used crankcase oil was overall very similar to that of the unused oils (Fig. 12b). Tricyclic terpanes were, however, greatly reduced in abundance relative to the hopanes, whereas the extended hopanes (from C_{31} to C_{35}) were more pronounced than in unused oil. The muffler soot and roadsweep samples contained hydrocarbon distributions almost identical to those of the fresh crankcase oil, with the exception that n-alkanes from nC_{12-22} were more pronounced relative to the UCM in the muffler soot samples.

Isotopic signature: Because of the large background associated with the UCM in these oils, and the small size of the alkane peaks, it was not possible to generate any meaningful or reliable measurements of the isotopic composition of any individual components of these oils.

2. OUTBOARD OILS AND EXHAUST WATERS

Molecular signature: The aliphatic hydrocarbons of outboard motor oils consisted predominantly of a bimodal UCM, with maxima at approximately RI 1150 and 2450 (see Fig. 14). The lower boiling range of this fraction included an assortment of branched and cyclic alkanes and alkenes (C_{10} to C_{13}). The regular alkanes (nC_{10} to nC_{23}), and pristane and phytane were present, but were less abundant relative to the UCM than in automotive oils. The unused outboard motor oils were found to contain almost no tricyclic terpanes, but relatively high concentrations of C_{31-35} extended hopanes (see Figure 10). Overall, norhopane and hopane were the dominant cyclic terpenoids present.

Figure 14. Total ion chromatograms of hexane fractions of:

      a. fresh unused outboard motor oil
      b. surface slick of outboard test tank
      c. subsurface water of outboard test tank

a. FRESH OIL

b. SURFACE SLICK

C_{11} C_{12} C_{13} C_{14} C_{15} C_{16} C_{17} C_{18} C_{19} C_{20}

c. WASTE WATER

TIME (min.)
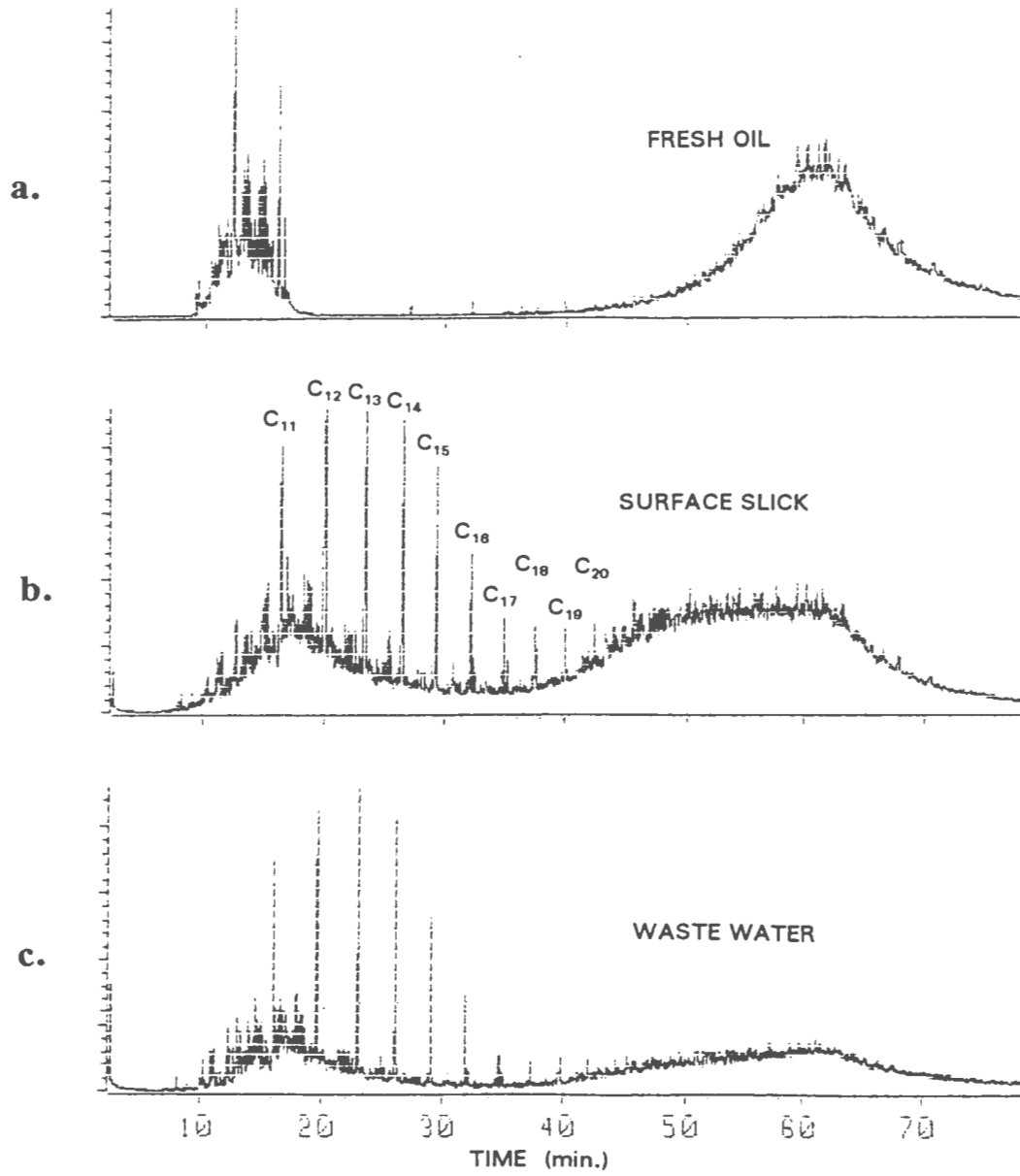
The GC profiles of both the surface slick and subsurface waters of the outboard motor test tank were significantly different from that of the fresh oil (Figure 14). In both of these samples, the n-alkanes from C_{12} to C_{20} were more pronounced, and the maxima of the UCM had widened and moved closer to one another in terms of retention time. This was most evident in the subsurface water sample, in which the UCM beyond RI 1700 closely resembled that of the phytoplankton tows (Figure 5a). Except for an increase in the abundance of tricyclic terpanes, the m/z = 191 a.m.u. profile of the test tank water samples generally coincided with that of the unused oil.

Isotopic signature: As with the crankcase oils, no reliable isotopic values were obtained for these oil samples.

c. SEDIMENT SAMPLES

1. CONCEPTION BAY

Molecular signature: The dominant hydrocarbons identified in Conception Bay surface sediments included the identical series of HBI alkenes discovered in the plankton samples (Fig. 6), pristane, HEH, squalene, and n-alkanes ranging from C_{15} to C_{35}. The branched C_{17} monoene and the phytadienes were also identified in the sediments, although the two C_{21} monoenes were not detected. Two isomers of a C_{20} HBI monoene homologous to the C_{25} HBI compounds (Barrick _et al._, 1980) were also identified. Many of the compounds collected in the hexane:dichloromethane (9:1) fraction of these sediment extracts could not be identified. This assemblage was underlain by a broad (RI

1700-3350), low UCM maximizing around RI 2700-2900. The extracted ion chromatogram of m/z = 191 a.m.u. of these samples (Figure 13c) revealed the presence of cyclic terpenoids of both biogenic and petroleum origins. The dominant biogenic hopanoids present were trisnorhop-17(21)-ene, 22,29,30-trisnorneohopane, diploptene, and 17β(H)-homohopane, all considered to be derived directly or indirectly from bacteria (Simoneit, 1986, Volkman *et al.*, 1992). The suite of petroleum hopanoids present was very similar to those of the petroleum sources discussed above (dominated by hopane, norhopane, and the extended hopanes). The ratio of tricyclic to pentacyclic terpanes among these compounds was intermediate between that of the automotive and outboard motor oils.

Sediment from deeper intervals in the core featured the same basic hydrocarbon profile as that from the surface, dominated by the HBI alkene isomers and odd-numbered alkanes (see Figures 5c and d). However, n-alkanes lighter than nC_{21} and pristane were found to be much less abundant at depth than at the surface, and HEH and squalene were not detected at all. In addition, almost no UCM was found in deeper sediments. The same biogenic hopanoids identified at the surface were also abundant at depth, but the complete suite of petroleum hopanes and tricyclic terpanes was generally absent. Petroleum steranes were also not detected in deeper sediments. In general, then, the deeper sediments feature lower relative levels of some of the lighter, more labile compounds still preserved at the surface, and do not appear to contain any petroleum hydrocarbons.

Figure 15. N-alkane abundances and isotopic compositions of surface and 30 cm deep sediments in Conception Bay, and of Grand Banks surface sediment.

(Bars represent concentrations; points denote isotopic values)

Conception Bay surface sediment
n-alkanes
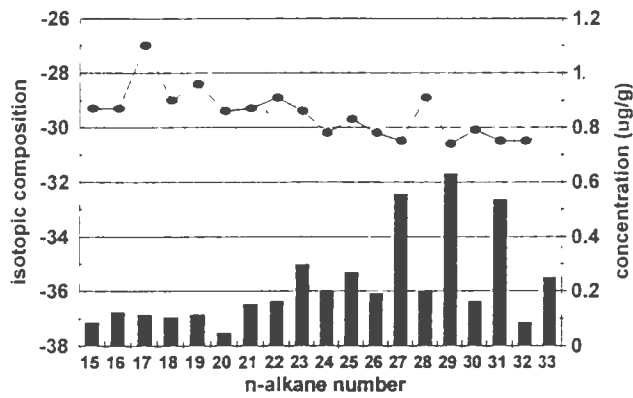
Conception Bay 30 cm sediment
n-alkanes
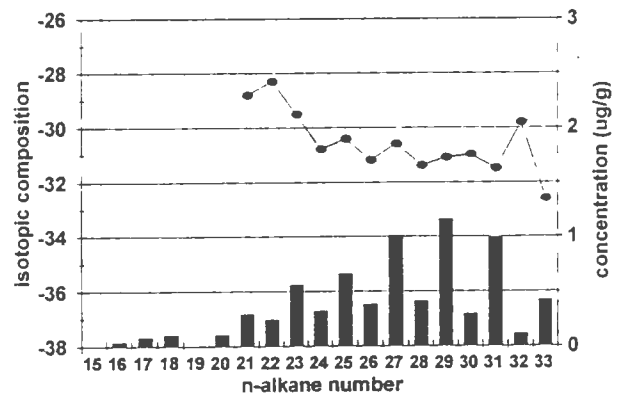
Grand Banks surface sediment
n-alkanes
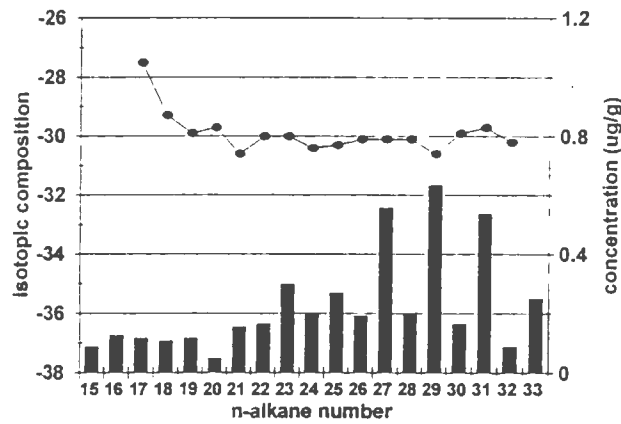
Figure 16. Summary of isotopic compositions of aliphatic hydrocarbons of Conception
         Bay sediments. Note similarity of surface and subsurface n-alkanes,
         and similarity of known algal products HEH, pristane, phytadienes,
         and squalene.

Conception Bay sediment aliphatics
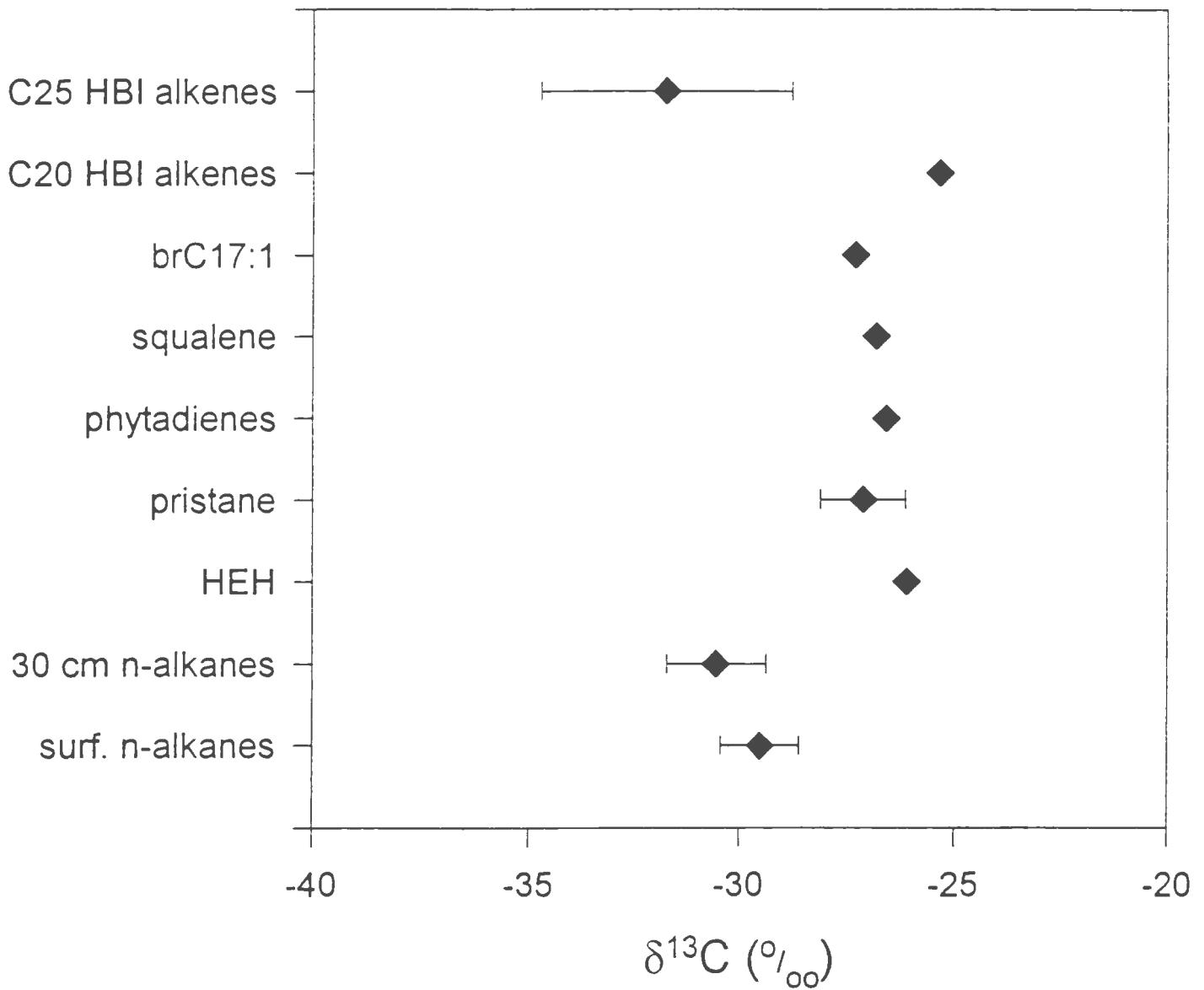
Figure 17. Summary of isotopic compositions of Grand Banks sediment aliphatic hydrocarbons

Grand Banks sediment aliphatics

$\delta^{13}C$ (°/$_{oo}$)
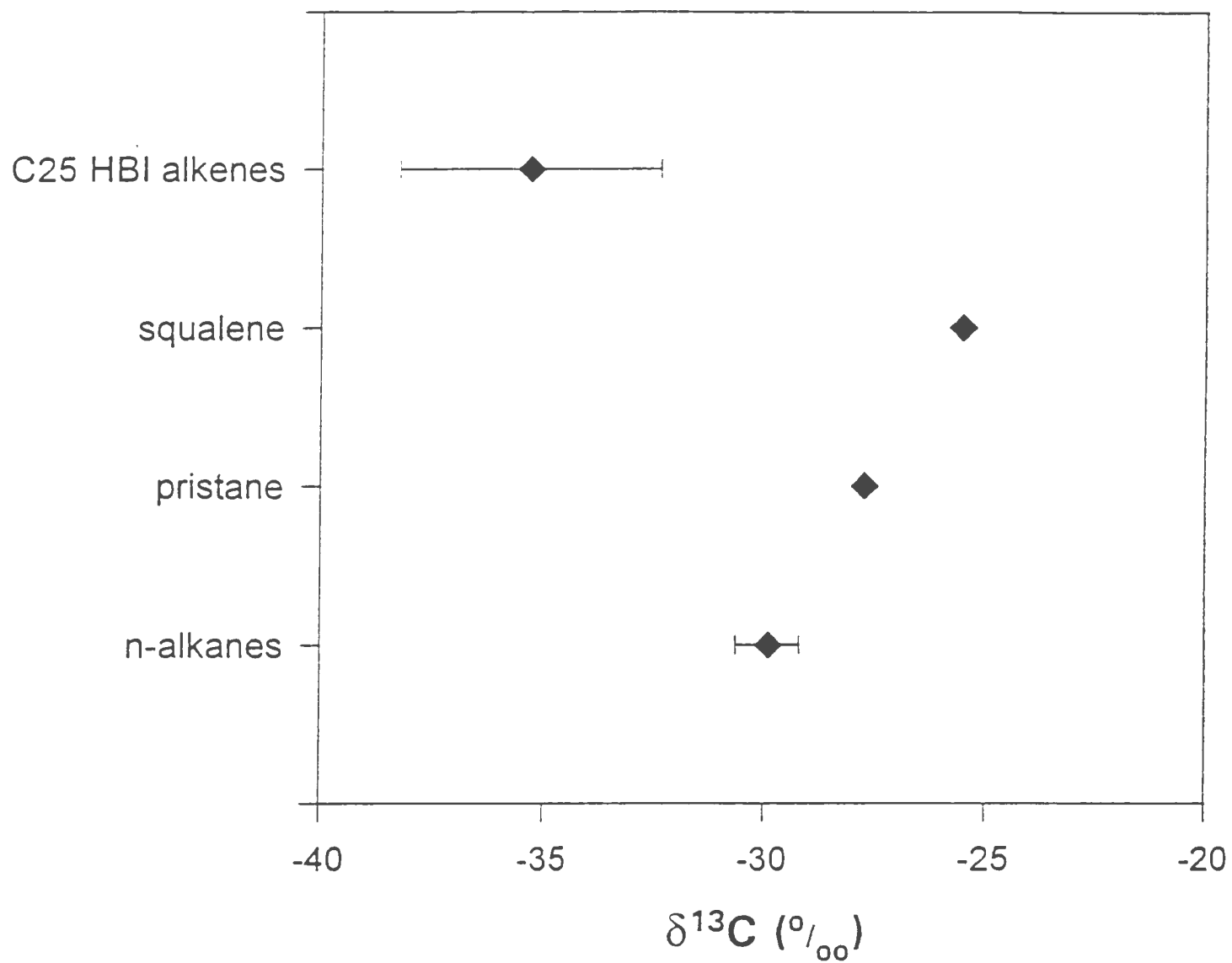
Hydrolysis of the Soxhlet-extracted sediment revealed that a significant proportion of some hydrocarbons was more tightly bound to the sediment matrix, and had not been recovered by the first extraction. This was most pronounced for the phytadienes in the surface sediment, which were actually present predominantly in the bound form. An additional 20 to 30% of the HBI alkenes was also recovered after hydrolysis. Small amounts of pristane, n-alkanes from C_{17} to C_{32}, and a UCM were also released by this process. The bound n-alkanes showed no odd or even predominance.

Isotopic signature: The isotopic compositions of individual n-alkanes extracted from Conception Bay ranged from -25.3 to -34.8‰ (Fig. 15), and are summarized in Figure 16. In general, the isotopic compositions of the marine biogenic compounds are similar to those observed in the plankton tows, with pristane, the phytadienes, squalene, and HEH ranging from -25 to -28‰ (Figures 10 and 16). Again, the C_{25} HBI alkenes are the most depleted compounds present. The n-alkanes all fall within a relatively narrow range of -28.4 to -30.5‰. Among the lighter n-alkanes ($<C_{21}$), most even chain length homologues tend to be depleted in ^{13}C relative to odd numbered variants, whereas the opposite relationship is displayed among most heavier n-alkanes ($>C_{27}$) (Figure 15). The only hopanoid present in a concentration great enough to be isotopically analyzed was diploptene, which in the surface sediment had an isotopic composition of -27.2‰. No significant or consistent difference was found between the isotopic compositions of any of the free (Soxhlet extractable) *vs.* bound hydrocarbons.

2. GRAND BANKS SEDIMENT

Molecular signature: The surface sediment sample from the Grand Banks (Figure 15) contained primarily n-alkanes from C_{17} to C_{32} (with a moderate odd predominance over the range of C_{22} to C_{32}), squalene, and the same suite of eight C_{25} HBI alkene isomers found in Conception Bay (see Figure 6). The only other hydrocarbons identified in these samples were the bacterial hopanoids diploptene and trisnorhop-17(21)-ene, as well as traces of hopane and norhopane.

Isotopic signature: Isotopically, the Grand Banks hydrocarbons (summarized in Figures 15 and 17) resembled those from Conception Bay. The n-alkanes ranged in composition from -29.3 to -30.6‰, but did not appear to show any consistent odd *vs.* even preference or pattern. The C_{25} HBI alkenes were again the most ^{13}C-depleted compounds present, with an overall mean isotopic composition of -34.8‰ .

3. RIVER AND POND SEDIMENTS

Molecular signature: The sedimentary hydrocarbon profiles of rivers and ponds around Conception Bay generally consisted of combinations of the following elements:

1. n-alkanes from C_{15} to C_{33} with a strong odd-chain predominance and maximising at C_{25} to C_{29},
2. pristane and phytane in varying ratios,
3. several branched or cyclic C_{20-25} alkenes
4. a suite of biogenic and petroleum derived cyclic terpenoids, and
4. a broad UCM, generally maximizing at approximately RI 2700.

Not all of these components were found in all samples. For example, the Holyrood River sediment sample, consisting almost entirely of leaf debris, was found to contain only C_{23} to C_{31} n-alkanes. The profile of sediment from a pond within the city of St. John's (Mundy Pond), on the other hand, consisted almost entirely of a UCM very similar to that of crankcase oil. The presence of phytane and petroleum derived cyclic terpenoids was generally correlated with the presence of a UCM. In general, the abundance of these petroleum markers relative to the size of the UCM was much higher in all of these samples than in any of the petroleum products analyzed. This is to be expected, as these highly cyclic and branched hydrocarbons are much more resistant to biodegradation than most of the other compounds that make up the UCM (Prince *et al.*, 1994). The dominant biogenic terpenoids were, as in Conception Bay sediments, diploptene, several trisnorhopanoids, and 17β(H)-homohopane. All of the samples taken from sites close to harbours or communities also contained petroleum hopane and sterane assemblages very similar to those of the petroleum sources analyzed. Some of the alkenes in several of these sediments, such as a C_{20} HBI monoene, and a C_{25} HBI triene and tetraene, appeared, on the basis of retention time and fragmentation pattern, to be the same compounds as those identified in plankton and sediments of Conception Bay. However, others of this group were distinct, previously unreported compounds, and could not be identified by comparison with literature data (see Figures 18-19 as compared to Figures 6-7). It is possible that some of these compounds are cyclic.

Isotopic signature: The $\delta^{13}C$ of the n-alkanes of these samples displayed a total

Figure 18. Comparison of marine and brackish water C_{23-25} alkene distributions revealed in TIC traces of:

    a. Conception Bay spring bloom plankton tow
    b. Spaniard's Bay surface sediment
    c. South River surface sediment
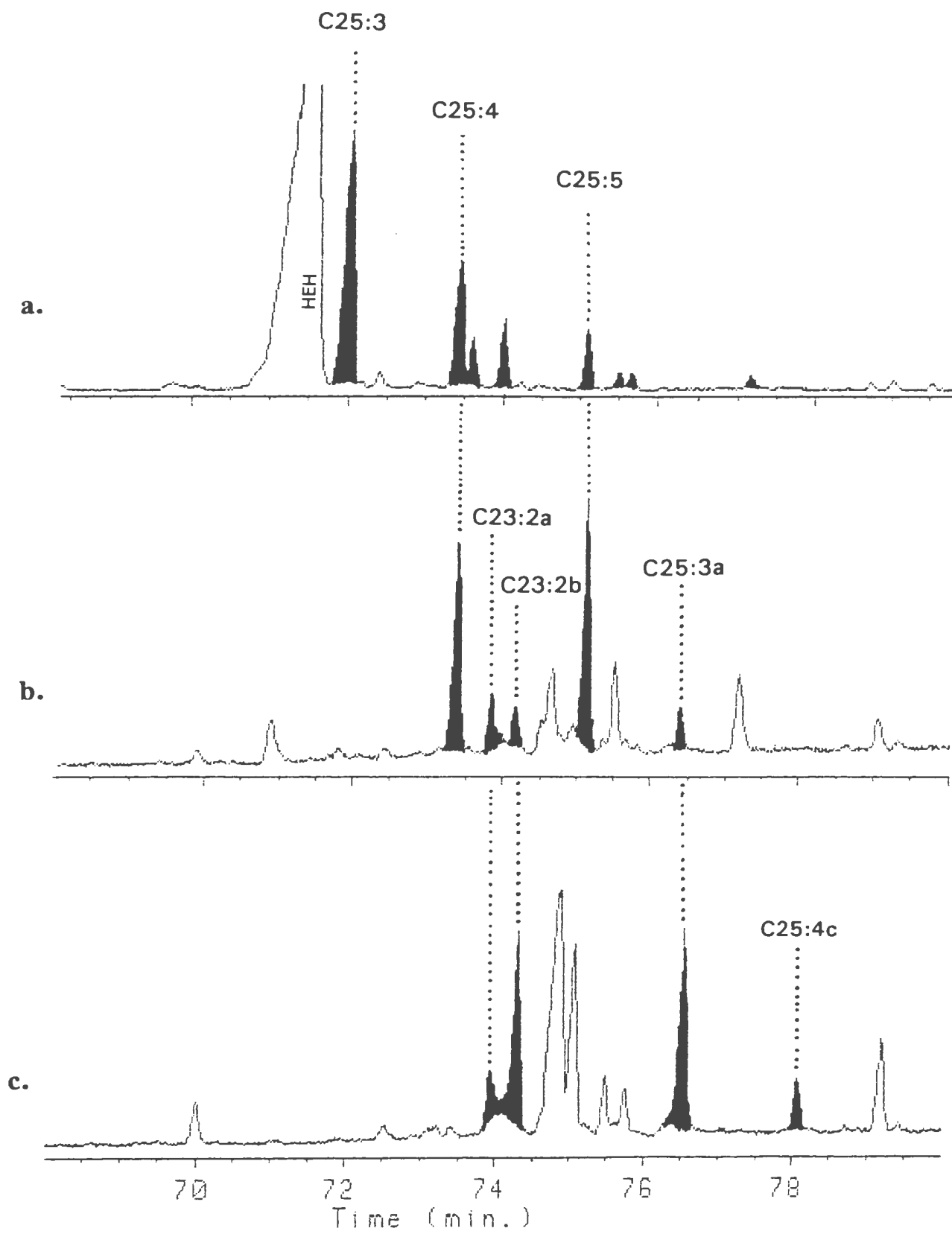
Figure 19. Electron impact spectral patterns of C_{23-25} alkenes extracted from Spaniard's Bay and South River surface sediment

brC23:2a

brC23:2b

brC25:3a

brC25:4c
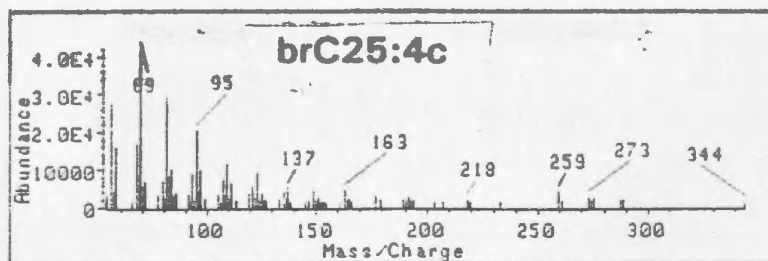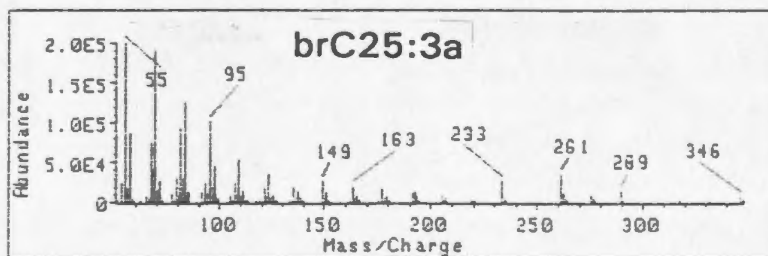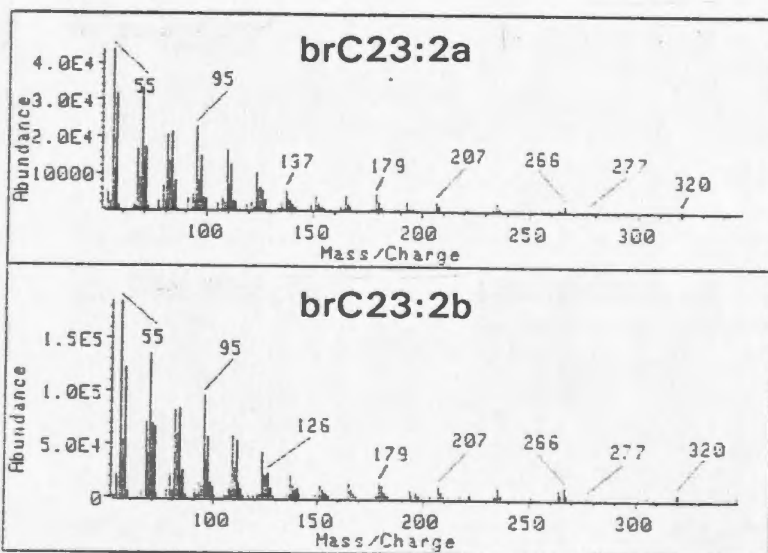
Figure 20a. Abundances and isotopic compositions of n-alkanes extracted from sediments in river inlets and ponds around Conception Bay

(Bars represent concentrations; points denote isotopic values)

Holyrood River
n-alkanes
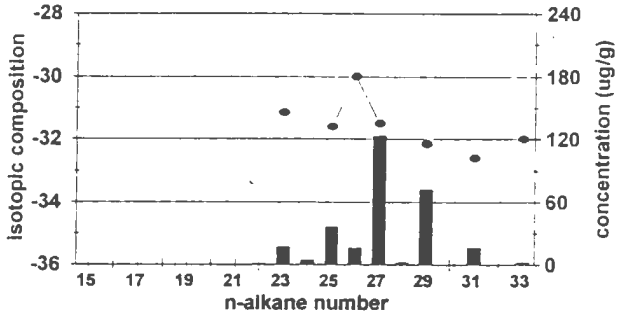
Job's Cove
n-alkanes
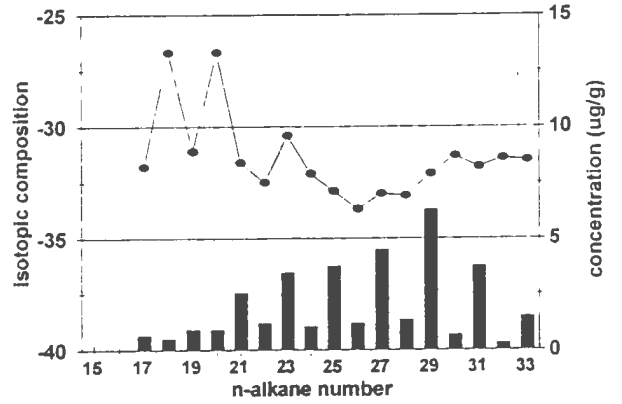
Topsail Beach
n-alkanes
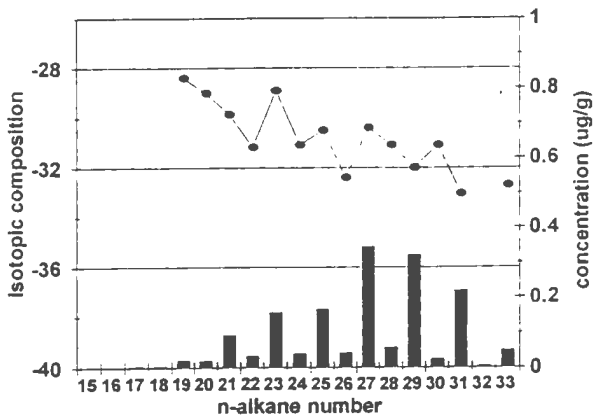
South River
n-alkanes
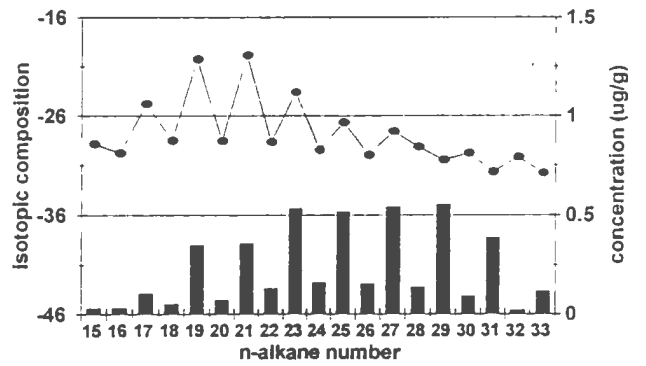
Figure 20b. Abundances and isotopic compositions of n-alkanes extracted from sediments in river inlets and ponds around Conception Bay (continued)

(Bars represent concentrations; points denote isotopic values)

Spaniard's Bay
n-alkanes
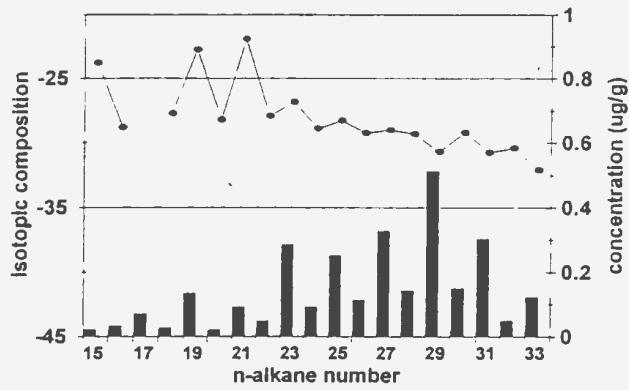
St. Phillip's
n-alkanes
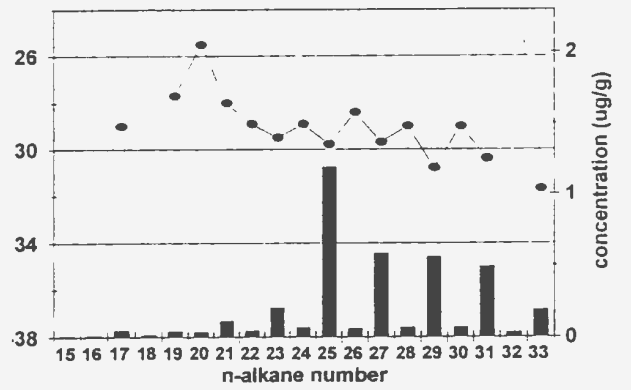
Avondale
n-alkanes
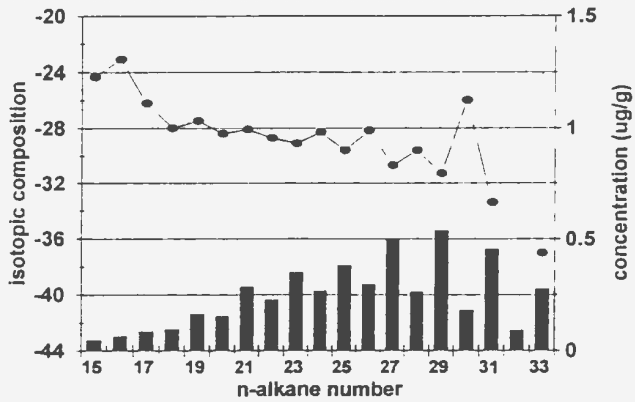
Mundy Pond
n-alkanes
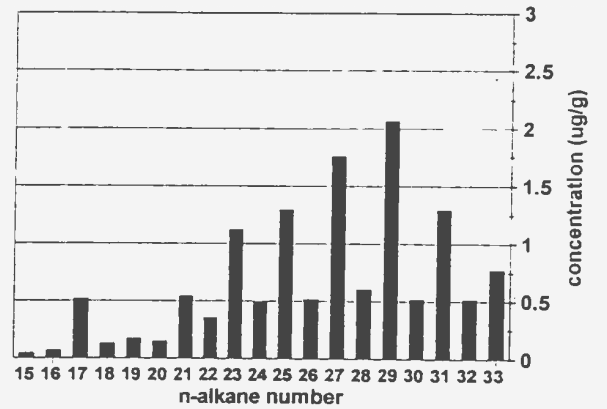
Figure 21. Summary of isotopic compositions of sedimentary n-alkanes.

Note: this plot is a summary comparing sediments as a *whole*. The magnitude of the error bars in this plot is due to the inclusion of the isotopic values of *all* of the alkanes in each sample. The high intra-sample variance of n-alkane compositions generally masks trends that are even more distinct when comparing individual compounds.

Surface sediment n-alkanes
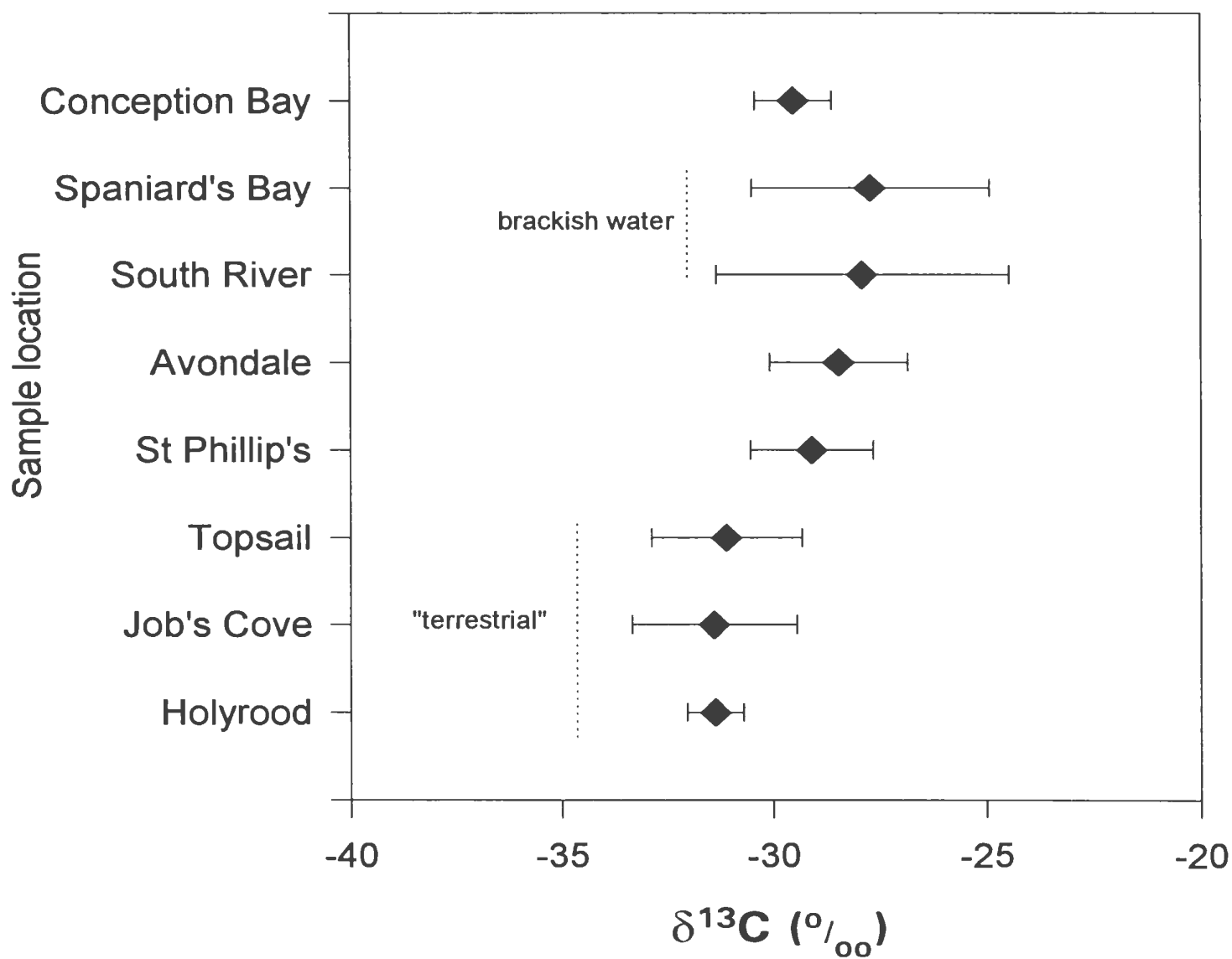
Figure 22. Overall average of sediment n-alkane isotopic compositions

Average alkane 13C composition
of all sediments
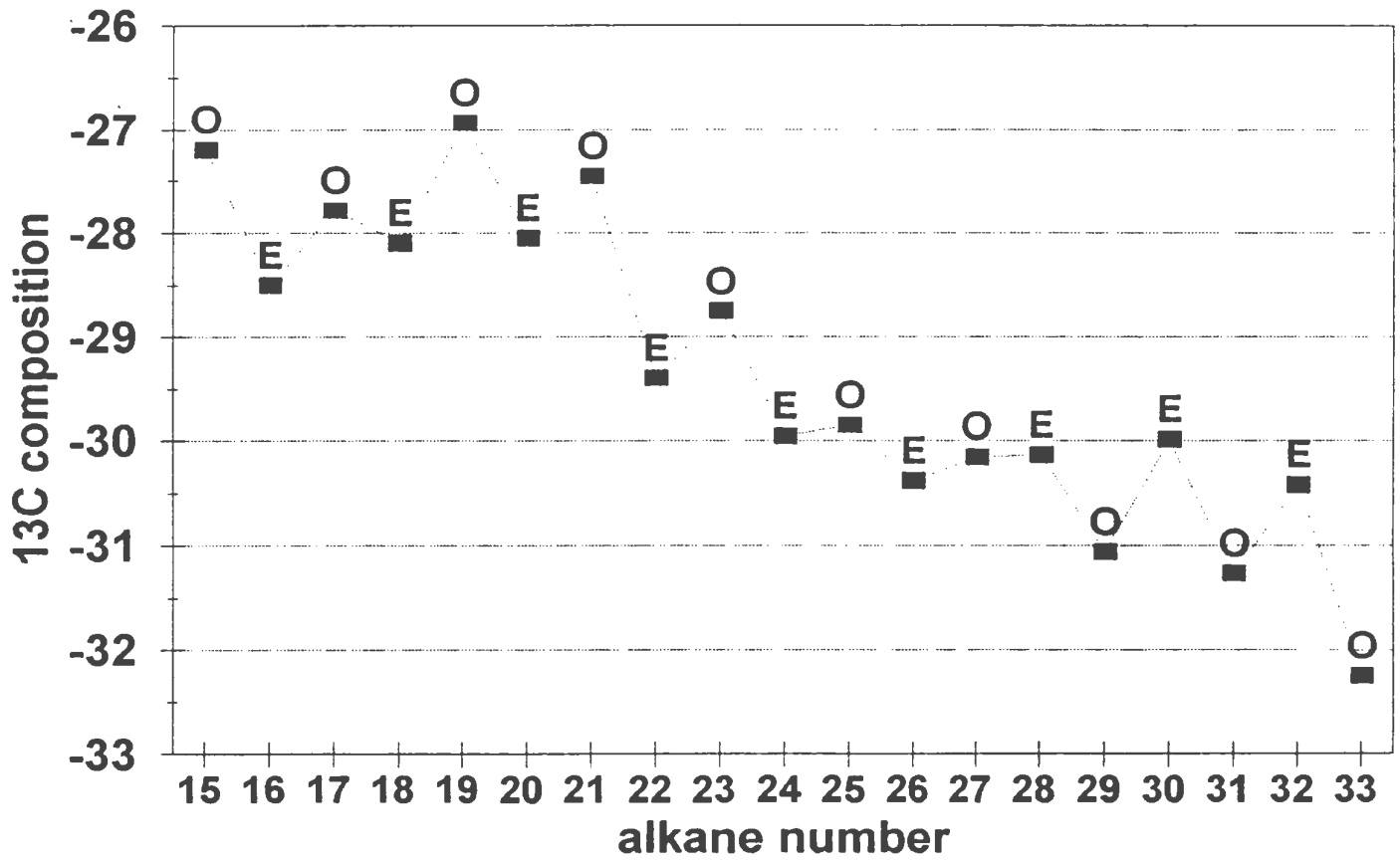
range of -21.5 to -33.7‰. Abundances and isotopic compositions of river and pond hydrocarbons are tabulated in Appendix 2, and are shown in Figures 20a and b. As summarized in Figure 21, the most ^{13}C depleted alkanes are found in the samples from upper reaches of rivers and ponds (Holyrood and Job's Cove), whereas the most ^{13}C-enriched n-alkanes are found in brackish water rivermouths (Spaniard's Bay and South River). Higher molecular weight alkanes ($>C_{25}$) are generally slightly more depleted in ^{13}C than their lighter counterparts. The same trend among odd and even homologues of the n-alkane series was observed in most of these riverine and lacustrine samples as discussed for the Conception Bay sediments: low molecular weight odd homologues, and high molecular weight even homologues were generally enriched in ^{13}C. This reversing "sawtooth" trend is clearly summarized in a plot of the mean values of all sediment n-alkanes (Fig. 22). Pristane and phytane (mean $\delta^{13}C$ = -29.3 and -27.5‰ respectively) were significantly more depleted than the pristane in the phytoplankton tows. All of the C_{20-25} alkenes found in these sediments were significantly enriched in ^{13}C relative to co-occurring n-alkanes, and to the C_{25} HBI alkenes in Conception Bay samples.

d. BIOTA

1. CRABS AND SCALLOPS

Molecular signature: As originally described by Hellou *et al.* (1993, 1994), the unsaturated hydrocarbons of the hepatopancreas of both species of crab, as well as of the scallop visceral mass, consist almost entirely of branched C_{25} HBI alkenes and squalene.

Closer examination of these fractions revealed that in addition to the three compounds identified in earlier studies, the additional isomers found in the plankton samples were also present in these extracts. The suite of C_{25} HBI alkenes in the scallop and crabs samples is thus identical to that found in the spring bloom plankton and sediment of Conception Bay (see Figure 6). Squalene was also abundant in the crab hepatopancreas samples.

Isotopic signature: The alkenes in the crab and scallop samples are isotopically similar to the same compounds in plankton tows and sediments. Squalene is relatively enriched in ^{13}C (mean δ^{13}C = -24‰), whereas the C_{25} HBI alkenes are all strongly depleted (from -30.6 to -40.5‰).

IV. DISCUSSION

A. N-ALKANES

The molecular characteristics of the spring bloom plankton tows would initially appear to indicate a petroleum origin for the n-alkanes in these samples. However, while the n-alkanes display a CPI close to 1, and there is a small UCM and a suite of hopanes resembling that of outboard motor oil present, several other observations suggest that petroleum is unlikely to be the primary source for these n-alkanes. The common petroleum sources analysed contained a very low abundance of n-alkanes relative to the size of the UCM. This ratio would be expected to be even lower in environmental samples as a result of the preferential degradation of n-alkanes during petroleum weathering. The plankton samples, however, feature an *enhanced* ratio of n-alkanes to unresolved compounds relative to the petroleum sources. Secondly, a smooth nC_{15-31} alkane distribution is seen only in the spring bloom samples, and is replaced with a series of n-alkanes displaying a pronounced odd:even preference in the summer and fall. As boat traffic increases during the summer and fall, this is also the opposite of what would expected if petroleum were the primary source for these compounds. While a small UCM is present in samples during the whole year, a low-CPI n-alkane assemblage is present only during the spring bloom. Hence the two signals are unlikely to have a common origin. Thirdly, the entire n-alkane assemblage becomes significantly isotopically lighter over the course of the spring bloom. While the effect of weathering on the isotopic composition of petroleum n-alkanes has not been studied, it would appear

unlikely that it would involve a depletion in ^{13}C, especially such a rapid one (see introductory chapter).

It is also possible that the spring bloom n-alkanes are derived from atmospheric fallout. Several studies have documented smooth n-alkane distributions over the range of C_{20-30} in urban air (Gelpi *et al.*, 1970; Hauser and Pattison, 1972). The appearance of a smooth distribution of n-alkanes in the spring samples could be due to the sudden input, through spring runoff, of atmospheric fallout accumulated on the land over the winter. Normally, however, the input of atmospherically derived semi-volatile organic compounds such as n-alkanes reaches a maximum in late summer or fall (*e.g.*, Simonich and Hites, 1994). Most importantly, though, this origin cannot readily account for the observed shift in $\delta^{13}C$.

A biological origin for these compounds, on the other hand, could account for such a change. It has been well documented, both generally (Calder and Parker, 1973), and in Conception Bay itself (Ostrom, 1992), that during periods of high productivity, when seawater dissolved inorganic carbon (DIC) is being more efficiently and completely used, phytoplankton become, in bulk, isotopically heavier. This could explain the observation that n-alkanes synthesized during the peak of a bloom (April) are isotopically heavier than compounds synthesized during less productive periods (late May). Similar long-chain n-alkane distributions have in fact been documented in unpolluted regions by many researchers (for example, Osterroht *et al.* 1983; Serrazanetti *et al.* 1991; Nichols *et al.*, 1988). While it does appear that biological sources do exist for these

assemblages, debate exists as to whether these compounds are synthesized by bacteria or algae (Volkman *et al*, 1992). The isotopic shift observed in the Conception Bay samples could be associated with either bacterial or algal synthesis. However, given the relatively constant isotopic composition of algal hydrocarbons such as heneicosahexaene and pristane over the course of the bloom, it would seem more likely that these n-alkanes are microbial in origin.

The distribution of the n-alkanes in the summer and fall plankton samples, as well as those of all of the sediments, indicates a dominant input from terrestrial plant (leaf) debris. Although there is considerable petroleum contamination in the more recent surface sediments of Conception Bay, the n-alkane distribution at the surface differs little from that of uncontaminated sediments at 30 cm depth (Figure 15). This observation further supports the conclusion arrived at above that the petroleum contribution to n-alkanes is virtually absent in Conception Bay. Only n-alkanes smaller than nC_{20} are more abundant in surface sediments, and while this could be due to petroleum contamination, the relative abundance of these more labile, shorter-chain homologues in more recent sediments may simply reflect the shorter length of time for which they have been exposed to degradation.

The sawtooth trend in the average isotopic composition of n-alkane homologues from all sediment samples (Fig. 22) could be due either to the mixing of separate assemblages of n-alkanes with different distributions and isotopic compositions, or could be a biosynthetic pattern inherited intact from primary sources. The general decrease in

$\delta^{13}C$ with increasing chain length, for example, could be either a reflection of the predominantly terrestrial source of longer n-alkanes, or could be the result of fractionation of carbon isotopes during the progressive elongation of precursor fatty acids as suggested by Fang *et al.* (1993). Rather than a smooth, gradual decline in $\delta^{13}C$ from n-C_{15} to n-C_{33}, the trend in Figure 22 actually appears to flatten out over the ranges of C_{15-21} and C_{24-33}, suggesting that mixing of two isotopically distinct long- and short-chain assemblages may be the most likely explanation.

Similar alternatives could account for the relatively depleted nature of even chain length homologues in the lower boiling range, and of odd-chain homologues in the higher boiling range. Dissimilarities in the isotopic composition of odd and even n-alkane homologues in sources themselves could arise due to the slight differences in the early stages of the synthesis of odd and even fatty acids. The sediment sample from Holyrood, which consisted almost entirely of leaf debris, unfortunately did not contain enough even chain length n-alkanes to show the complete, unaltered isotopic signature of local leaf wax alkanes. Previous studies of leaf wax alkanes (*e.g.*, Rieley *et al.*, 1991; 1993) have not found any consistent differences between odd and even n-alkane homologues. The presumably biogenic n-alkanes in the spring plankton tows also show no consistent odd *vs.* even isotopic pattern. The pattern in Figure 22 is thus more likely the result of the mixing of suites of alkanes of distinct isotopic compositions from different sources. This sawtooth isotopic pattern may derive from the odd-chain length n-alkane dominance seen in most organisms. Given the isotopic composition of other compounds of algal

origin such as HEH, Conception Bay phytoplankton would be expected to produce primarily isotopically relatively heavy ($\delta^{13}C \approx$ -24 to -27‰) odd-length short-chain (C_{15-19}) n-alkanes. Terrestrial vegetation contributes almost exclusively isotopically lighter ($\delta^{13}C \approx$ -30 to -33‰), odd-length long-chain homologues. The isotopic compositions of these more abundant odd-chain length compounds would tend to be unchanged by minor inputs of isotopically distinct sources. The smaller pools of even-chain length homologues, on the other hand, would be more strongly isotopically altered by such mixing. As seen in Figure 22, the $\delta^{13}C$ values of even-numbered alkanes appear to be consistently deflected towards the center of the plot, suggesting the addition of a source of n-alkanes of an approximate isotopic composition of -28 to -29‰.

Uzaki *et al.* (1993) found a similar pattern among C_{27-33} n-alkanes in Tokyo Bay surface sediments, and concluded that the enrichment in ^{13}C in even chain-length n-alkanes was due to the input of isotopically relatively heavy ($\sim$ -28‰) petroleum n-alkanes. The sawtooth n-alkane isotopic pattern in Conception Bay, however, is also present in some samples that, on the basis of hopane and sterane signatures, contain no petroleum contamination. Furthermore, the most common petroleum sources in the study area contain very low concentrations of n-alkanes beyond C_{25}. Thus, petroleum would appear unlikely to be the source of the n-alkanes in question. Alternatively, the smooth distribution of n-alkanes produced during the spring bloom could be responsible for these deflections. The mean isotopic composition of n-alkanes in spring plankton tows, however, is approximately -27‰, which is too high to account for the deflections seen

Figure 23. Model of sources of n-alkanes in and around Conception Bay

Sediment n-alkanes
Source model
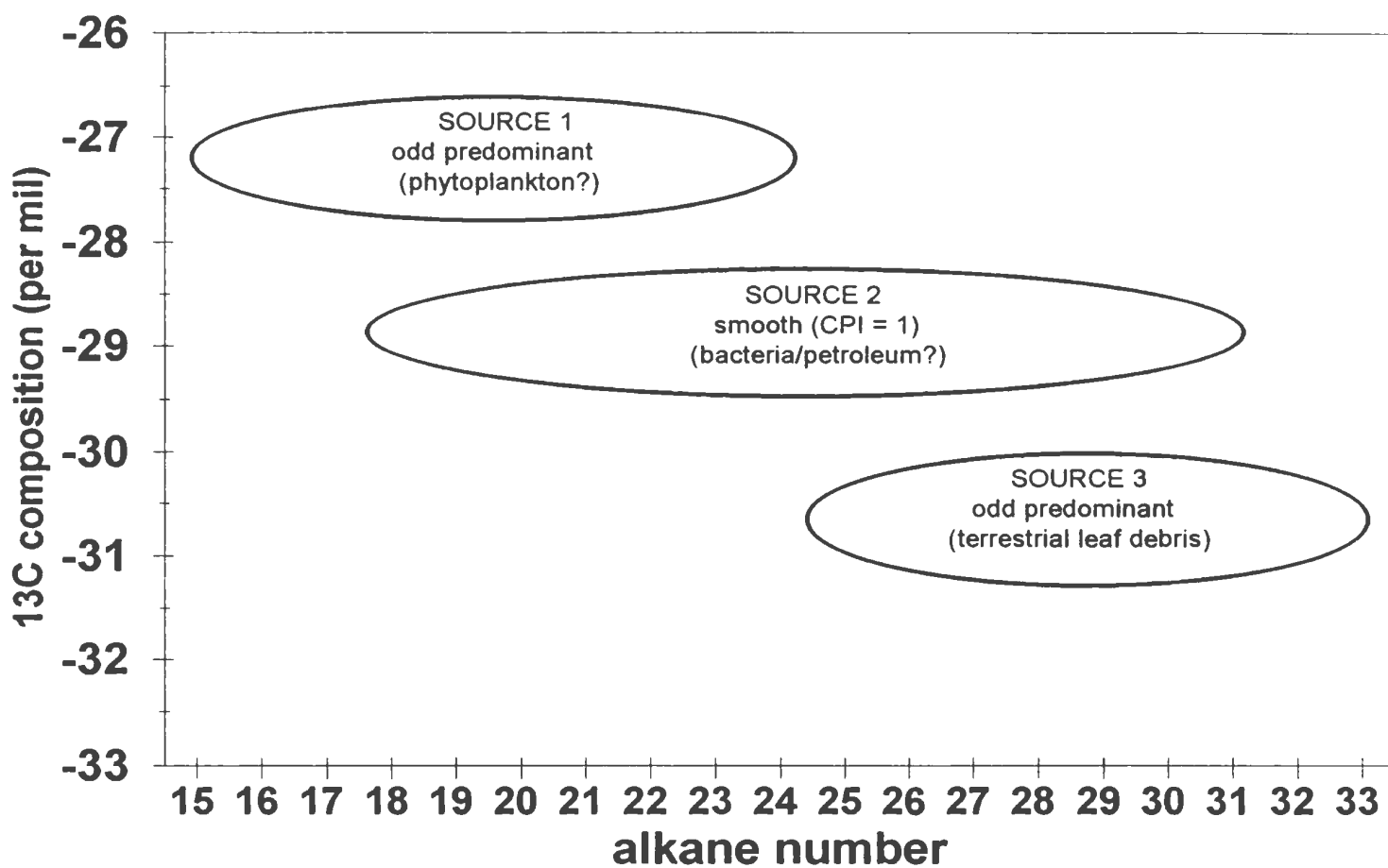
in short-chain length sediment n-alkanes. This mean sample value, though, may not represent the total average isotopic composition of the n-alkanes synthesized over the entire spring bloom, so that this source cannot be entirely ruled out.

In summary then, both the molecular and isotopic compositions of the sediment n-alkanes are consistent with the mixing of three end members with the following general attributes (depicted in Figure 23):

Source 1: predominantly odd, short-chain, isotopically heavy (-24 to -27‰) n-alkanes
Source 2: a series of evenly distributed n-alkanes of intermediate $\delta^{13}C$ (-28 to -29‰)
Source 3: predominantly odd, long-chain, isotopically light (-30 to -33‰) n-alkanes.

The first and third sources are most likely algal production and terrestrial leaf debris, respectively, while the second source may be petroleum or bacteria. This second source appears to dominate the spring bloom, whereas sources 1 and 3 likely dominate alkane inputs in the summer and fall.

b. HENEICOSAHEXAENE

Heneicosahexaene is a very labile compound (Schultz and Quinn, 1977) produced primarily by diatoms (Blumer *et al.*, 1970). The susceptibility of this compound to microbial degradation likely accounts for the fact that while it is by far the most abundant hydrocarbon in plankton tows, it is much less abundant than the C_{25} HBI alkenes in surface sediments, and completely absent at 30 cm sediment depth. Since the heneicosahexaene in the upper sediment layer (-26.1‰) is isotopically similar to that produced in the surface waters of the bay (-26.7 $\pm$ 0.4‰), the isotopic composition of

this compound does not appear to be greatly affected by the extensive degradation it undergoes as it sinks and is buried.

HEH is believed to be formed by decarboxylation of a n-$C_{22:6}$ fatty acid (Blumer *et al.*, 1970). If, as suggested by Fang *et al.* (1993), desaturation of fatty acids involves a kinetic depletion in ^{13}C, one might expect HEH, with six degrees of unsaturation, to be depleted in ^{13}C compared with other linear hydrocarbons formed from fatty acids. In fact, the mean isotopic composition of HEH in phytoplankton samples (-26.8‰) is very close to the average $\delta^{13}C$ of the n-alkanes smaller than n-C_{22} in plankton samples. The HEH in Conception Bay, also does not appear to be significantly more depleted in ^{13}C than any of the other algal hydrocarbons (e.g., pristane, squalene) in the plankton tows (Figures 12 and 20). While these compounds all may have separate origins (as reflected in the more constant and time-invariant isotopic composition of HEH as compared with the plankton tow n-alkanes) the formation of six double bonds during the synthesis of HEH in general does not appear to have led to any significant depletion in ^{13}C in this compound. Together with a $nC_{20:5}$ fatty acid, a n-$C_{22:6}$ fatty acid has been shown to be the most abundant polyunsaturated fatty acid in the Newfoundland coastal environment (Murphy and Abrajano, 1994). These authors found the n-C22:6n3 fatty acid in Conception Bay mussels to have an isotopic composition of approximately -29‰.

As seen in the diatom cultures, which were grown under identical conditions in this study, the isotopic composition of heneicosahexaene produced by different organisms or via different biosynthetic pathways may vary widely. Interspecific variation in the

isotopic composition of biogenic hydrocarbons has been demonstrated before (in leaf wax alkanes) by Rieley *et al.* (1991). In the case of the heneicosahexaene in this study, both the molecular and isotopic signature of this compound differed between *Fragilaria* and the other species, suggesting that different fractionation factors are associated with the two separate pathways operating in these organisms. This observation again seems to contradict the contention of Galimov (1981) that the isotopic compositions of biological molecules are determined primarily by intramolecular thermodynamics and not by kinetic fractionations during synthesis. Only the late-eluting, isotopically relatively heavy isomer of HEH is found in Conception Bay. Thus, both on the basis of molecular and isotopic evidence, it would appear that none of the dominant diatoms in Conception Bay synthesize heneicosahexaene via the pathway used by *Fragilaria striatula*. If all of the diatom species in Conception Bay synthesize heneicosahexaene of similar isotopic composition, the usefulness of isotopic measurements in determining the relative contributions of individual species is somewhat limited. A more detailed and comprehensive study than the present one, including all of the species in this environment would be required, however, to justify drawing such a conclusion.

In summary, compounds such as HEH may have varying isotopic signatures depending on the organism or synthetic sheme by which they are synthesized. The isomer of HEH found in Conception Bay was of the same isotopic composition as the that isomer in cultures of common diatoms, and this composition appears to be both relatively invariant with time (unlike the n-alkanes) and largely preserved during the early sedimentary degradation.

C. IRREGULAR BRANCHED HYDROCARBONS

The synthesis of irregular branched hydrocarbons has been investigated the least among the suite of biogenic hydrocarbons studied in the present work. Consequently, the isotopic signature of these compounds is most difficult to interpret. Interpretation is further complicated by the coelution of some of these compounds with other hydrocarbons, and the fact that the exact structures of the branched compounds detected in the present work could not be determined. Overall, one might expect irregular branched hydrocarbons to have isotopic compositions similar to the n-alkanes they are presumably derived from. In fact, these compounds are generally among the more ^{13}C-depleted hydrocarbons present in samples. In the plankton tows, for example, the branched C_{17} monoene and the two branched C_{21} monoenes have average isotopic compositions of -30.1‰ and -31.1‰ respectively, which is roughly 3 per mil depleted relative to the regular isoprenoids, HEH, and squalene. This depletion may be a reflection of the source of these compounds in bloom organisms other than algae (the source of neither of these compounds is known). Conversely, if these compounds are algal products, this depletion may result from the preferential incorporation of ^{12}C methyl groups during the formation of branches, or may be due to an intense depletion in the methyl groups being donated by methionine.

D. REGULAR ISOPRENOIDS AND SQUALENE

Pristane and phytane: Pristane and phytane are products of the degradation of the phytol side chain of chlorophyll, and are also common components of petroleum.

All of the petroleum sources analysed in this study contained roughly equal amounts of pristane and phytane, and thus, the amount of petroleum-derived pristane in any sample would appear to be limited to an amount equal to the amount of phytane present. Pristane present in excess of this amount can be considered to be of recent biogenic origin. In most samples with abundant pristane, then, as in all of the plankton tows and in Conception Bay sediments, pristane would appear on the basis of these molecular ratios to be predominantly of recent biogenic origin.

Chlorophyll is abundant both in terrestrial and aquatic photosynthetic organisms, and thus both terrestrial leaf debris and phytoplankton are sources of recent biogenic pristane and minor phytane. The distinct isotopic difference between pristane in lacustrine/nearshore samples (mean $\delta^{13}C$ = -29.9‰) and that in phytoplankton tows (mean $\delta^{\delta 13}C$ = -25.0‰) is likely simply a reflection of the general depletion in ^{13}C of terrestrial organic matter. The $\delta^{13}C$ of pristane in Conception Bay surface sediments is, at -27.8‰, intermediate between that of pristane in phytoplankton and terrestrial sources. Assuming $\delta^{13}C$ values of -29.9 and -25.0‰ respectively for pristane from terrestrial and marine (phytoplankton) sources (and disregarding the minor input of pristane from petroleum), one can use a simple mixing equation to calculate that roughly 45% of the pristane in Conception Bay sediments appears to be derived from phytoplankton, with the remaining 55% being derived from terrestrial vegetation. Given the conclusion drawn from bulk organic isotopic measurements (Ostrom and Macko, 1992) that most of the organic matter in Conception Bay is of marine biogenic origin, this finding suggests that

the behaviour of individual hydrocarbons may differ from that of the bulk organic matter with which they enter an estuarine system such as Conception Bay. This must be taken into consideration when using hydrocarbons and their isotopic compositions as indicators of the origins of organic matter.

While the long-chain n-alkanes of the relatively undegraded Job's Cove and Holyrood River samples are significantly depleted relative to the average terrestrial pristane, a distinct difference is not observed (on average) between terrestrial pristane and C_{23-33} n-alkanes in terrestrial samples as a whole. Thus, the inherent "biochemical" isotope difference proposed by Hayes *et al.* (1989) to exist between linear and isoprenoid compounds as a result of the varying proportions of carboxyl- and methyl-derived carbon atoms in these compounds is not evident in this study. Since the compounds analysed in this study are relatively fresh, or recent, while the model of Hayes *et al.* (1989) is based primarily on analyses of crude oils and ancient sedimentary organic matter, it is possible that the previously reported differences between linear and isoprenoid compounds are diagenetic rather than synthetic in origin. Due to the low abundance of pristane in many of these samples, some of these isotopic measurements are somewhat imprecise, and thus further study will be required to resolve this issue.

Phytadienes: Phytadienes are formed directly from chlorophyll by dehydration of the phytol side chain (Blumer and Thomas, 1965). The origin of these compounds in marine phytoplankton is reflected in the average isotopic composition of the three isomers identified in Conception Bay plankton (-26.2‰), which is fairly close to the $\delta^{13}C$ of

pristane in the same samples. The average isotopic composition of the three phytadienes in Conception Bay sediments (-26.6‰) is the very close to the average in surface plankton tows, indicating that water column and sedimentary phytadienes in Conception Bay are derived entirely from marine chlorophyll, with no terrestrial contribution. The slight depletion in ^{13}C relative to marine derived pristane is perhaps to be expected for these compounds as dehydration may take place less easily if ^{13}C atoms occupy the sites in the phytol side chain from which hydrogen atoms are to be lost to form double bonds. In general, though, the phytadienes have the same isotopic composition as the other products of the breakdown of chlorophyll, indicating that the isotopic signature of these biomarkers during the early degradation of organic matter is relatively resistant or stable (*cf.*, Hayes *et al.*, 1989).

Squalene: Squalene on average has an isotopic composition very close to that of the regular isoprenoids and HEH (see Figures 10 and 16), suggesting that this compound in Conception Bay is also derived primarily from marine organisms. The six double bonds in squalene are not created after the C_{30} backbone has been assembled, but are derived from the six isoprene units that polymerize to form each molecule of this compound. The isotopic similarity between squalene and other saturated (*e.g.*, pristane) and highly unsaturated (*e.g.*, HEH) algal hydrocarbons again suggests that double bond formation does not greatly affect the isotopic composition of biogenic hydrocarbons of this size.

In conclusion, squalene and the regular isoprenoid compounds derived from

chlorophyll were found to generally have similar isotopic compositions. The mixing of terrestrial and marine derived pristane could be recognized in Conception Bay sediments on the basis of the observed $\delta^{13}C$ values. However, "biochemical" isotope effects, predicted by previous studies to be related to the degree of unsaturation of compounds, and to the proportion of carboxyl or methyl derived carbon, were not recognized among these compounds.

E. HIGHLY BRANCHED ISOPRENOIDS

C_{25} HBI alkenes: The resemblance of the isotopic composition and molecular distribution of C_{25} HBI alkenes in sediments and biota of Conception Bay to those of spring bloom plankton samples shows clearly that some organism(s) present in the spring bloom must be the source of these compounds. In fact, both the molecular and isotopic signatures of the C_{25} HBI alkenes in all plankton, sediments and benthic organisms from marine environments around Newfoundland are virtually identical (see Figure 6). The presence of the identical assemblage of isomers over a wide geographic region (on the northeast and south coasts, and far out on the Grand Banks) featuring varying distributions of planktonic organisms suggests that a single species is responsible for the occurrence of these alkenes. Hellou et al. (1994) proposed dinoflagellates as a source, as the only molluscs found by these authors to contain C_{25} alkenes (scallops) were also the only ones containing abundant dinoflagellates. In light of the present study, however, this seems unlikely, as virtually no dinoflagellates are found in the spring bloom, and C_{25} HBI alkenes are not found in the fall plankton samples, which are dominated by

dinoflagellates. Although no C_{25} HBI alkenes were found in the diatoms cultured, diatoms would still appear to be the most likely source for these compounds. Volkman *et al.* (1994) recently found a series of C_{25} HBI alkenes with 3, 4, and 5 degrees of unsaturation in pure cultures of the diatom *Haslea ostrearia*. It is unlikely that this particular species contributes these compounds to Conception Bay, as this diatom is a warm water species not known to occur in waters around Newfoundland, and the series of isomers reported by these authors is different from that found in Conception Bay. Many of the major diatom species present in the spring bloom in Conception Bay have been cultured and analyzed as part of various studies of the production of hydrocarbons by marine algae (e.g., Blumer *et al.*, 1971; Youngblood *et al.*, 1971), but C_{25} HBI alkenes have never been reported in any of these species. HBI alkene production, however, may not be exhibited at all times by all species capable of synthesizing these compounds. Volkman *et al.* (1994) found isomers of C_{30} HBI alkenes in cultures of *Rhizosolenia setigra*, a species of diatom common in Conception Bay, and present in the late spring bloom plankton tows. No C_{30} HBI alkenes, however, were found in Conception Bay plankton tows or sediments. Some specific environmental factors or stimuli thus may control or induce the synthesis of these compounds. The conditions under which diatoms were cultured in this and in other studies may simply not have been conducive to HBI alkene synthesis, and thus, none of the diatoms analysed to date can be completely ruled out as potential sources of HBI alkenes. If diatoms are indeed the source . these compounds in Conception Bay, then the relatively high concentrations of HBI alkenes in the plankton tow samples suggests that these compounds are being

synthesized by one of the more abundant species in the Conception Bay spring bloom (one of the genera *Thalassiosira sp.*, *Fragilariopsis sp.*, or *Chaetoceros sp.*). The species found by Volkman (1994) to produce C_{25} HBI alkenes, *Haslea ostrearia*, is a pennate diatom, and thus diatoms of the genus *Fragilariopsis*, the most abundant pennate genus in Conception Bay, may merit closer examination.

The difference in the isotopic compositions of the plankton tow C_{25} HBI alkenes from those of all other co-occurring hydrocarbons of known algal origin (HEH, pristane, phytadienes, squalene), however, may indicate that the HBI alkenes have a source other than diatoms. Although it has never been clearly demonstrated, marine heterotrophic bacteria could potentially synthesize highly ^{13}C-depleted compounds. Although no bacterial markers were identified in the plankton tow samples to test this suggestion, the bacterially derived compound diploptene (-27.2‰) in Conception Bay sediments is only slightly depleted relative to the algal products in general. If this isotopic value is representative of bacterially derived compounds, then a bacterial origin for the C_{25} HBI alkenes is not indicated on the basis of isotopic composition. The absence of C_{25} HBI alkenes in the fall plankton tows, taken at a time during which one would expect bacterial abundances similar to spring bloom levels, also does not support this suggestion. The depletion seen in C_{25} HBI alkenes, of course, could also arise in diatoms if a particularly strong kinetic isotope effect existed in the formation of these compounds. Such an effect would be fully expressed especially if the synthesis of these compounds utilised only a small fraction of the carbon flowing along a particular metabolic pathway. This may actually be the case, if, as suggested earlier, HBI alkenes are non-essential compounds synthesized only under certain conditions.

The relationship between the isotopic compositions of the HBI alkene isomers is shown in Figure 24. While there does not appear to be a consistent relationship between isotopic composition and the degree of unsaturation among these compounds, the later-eluting isomer is in each case and in all samples enriched in ^{13}C relative to the corresponding (more abundant) earlier eluting isomer. The consistency of this relationship in all samples further supports the suggestion that only one organism, imparting a distinct isotopic fingerprint on these compounds, is responsible for the occurrence of C_{25} HBI alkenes in Newfoundland waters.

The C_{25} alkenes in Spaniard's Bay and South River mouth sediments feature both (1) different molecular distributions and (2) different isotopic compositions than those in the adjacent marine environment. As seen in the diatom cultures, organisms synthesizing different assemblages of similar compounds, perhaps by separate pathways, can produce compounds of widely differing isotopic composition. However, it is difficult to explain the consistent enrichment in all of the C_{25} alkenes from these brackish water environments, especially in those also found in the bay. Depending on the extent of ^{13}C-depleted groundwater input, fresh water DIC tends to be even more depleted in ^{13}C than marine bicarbonate (Galimov, 1981; Vogel, 1969), and one would expect this to be reflected in the isotopic composition of compounds (especially of hydrocarbons) produced in brackish or fresh water. An increased uptake of ^{13}C due to higher water temperatures and resultant more intense competition for dissolved inorganic carbon in brackish water could possibly partially explain the relative enrichment observed. On the other hand these brackish water compounds could simply be derived from isotopically distinct

Figure 24. Summary of C_{25} HBI alkene isotopic compositions (note consistent ^{13}C enrichment of later eluting isomers)

C25 HBI alkene summary
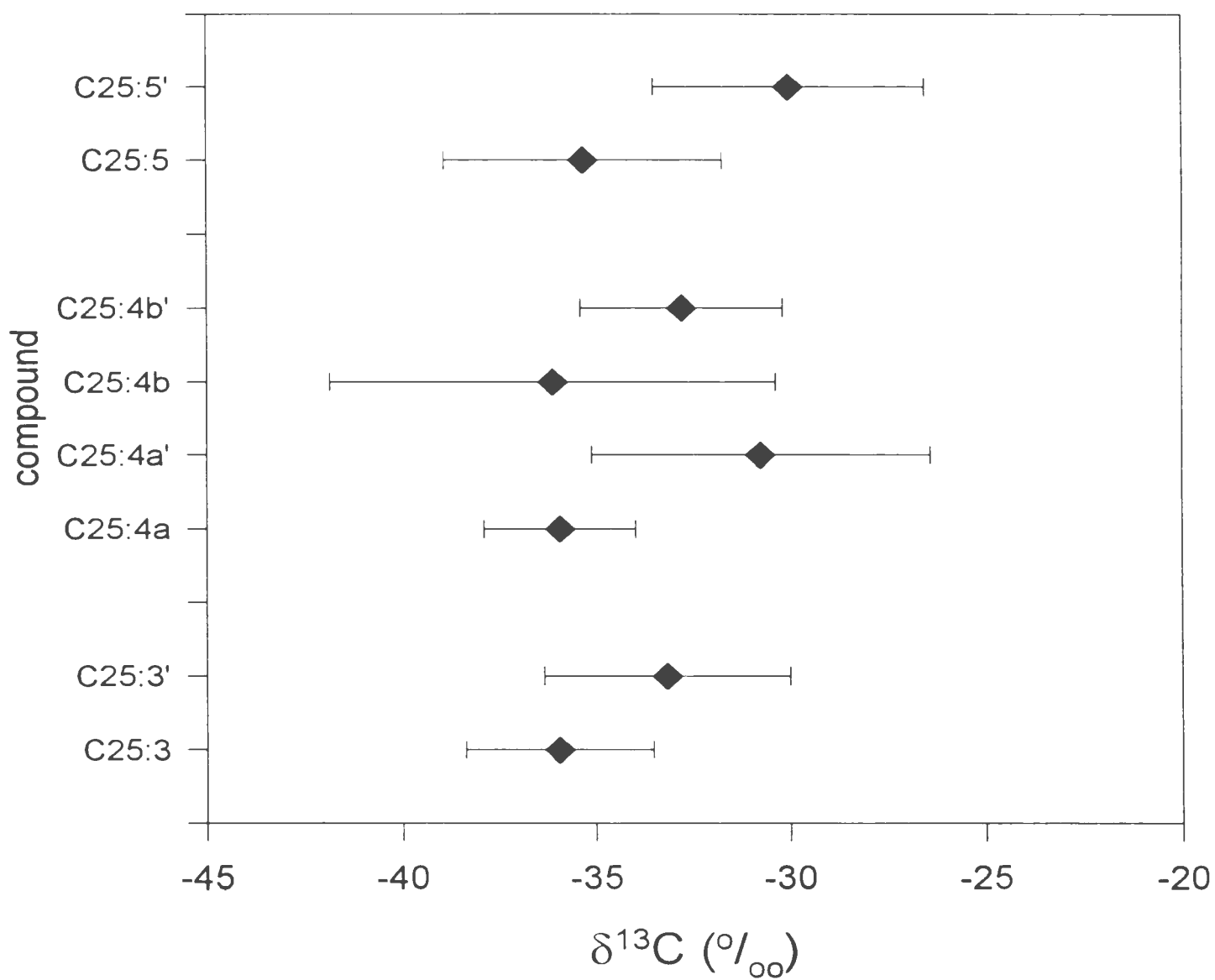
Page 103 missing from thesis bound book

precursors, although their high $\delta^{13}C$ values would appear to rule out terrestrial hydrocarbons as precursors. Nevertheless, based on their isotopic signature, these riverine C_{25} HBI alkenes do not appear to contribute significantly to the assemblage of compounds in Conception Bay sediments.

C_{20} HBI alkenes: The two C_{20} HBI isomers identified in Conception Bay, Spaniard's Bay, and South River sediments (*i.e.*, in all of the marine and brackish water sediments) were not found in plankton tows. It is possible that these compounds are also synthesized by a marine diatom during the spring bloom, but were not being produced at the time of sampling. On the other hand, these compounds may be produced only in fresh or brackish water such as South River and Spaniard's Bay, and might be distributed throughout Conception Bay from these sources. The absence of these compounds from phytoplankton tows, and the relatively high $\delta^{13}C$ values (mean $\delta^{13}C$ = -24.1‰) of the first C_{20} HBI alkene isomer in Conception Bay sediments, together suggest that the second origin may be the most likely one. C_{20} HBI hydrocarbons have, in fact, been most commonly detected in freshwater and intertidal or brackish environments (Robson and Rowland, 1986).

In summary, while the highly depleted isotopic signature of C_{25} HBI alkenes indicates that these compounds are formed either by some organism other than algae or common marine bacteria, or via some unique biosynthetic route, a single species of marine diatom still appears to be the most likely source for these compounds in Newfoundland. The C_{20} HBI alkenes in Conception Bay, on the other hand, are more likely produced by brackish water organisms in the lower sections of rivers flowing into the bay.

F. CYCLIC TERPENOIDS

Petroleum terpenoids: The cyclic terpenoids are among the most durable and stable hydrocarbons (Prince et al., 1994), and have long been used to fingerprint and trace petroleum (e.g., Broman et al., 1987, Peters and Moldowan, 1993). The resistance of these compounds to degradation is demonstrated in Figure 25, which shows that as samples in this study become more weathered, the two most abundant petroleum hopanes (17α(H),21β(H)-29-norhopane and 17α(H),21β(H)-hopane) become progressively more abundant or pronounced relative to the UCM. Assuming a sedimentation rate of approximately 0.5 cm/yr, sediment from a 30 cm depth in Conception Bay was deposited roughly 60 years ago. At that time, there would have been very little automotive or motorized vessel traffic around Conception Bay. This is reflected in the absence of petroleum hopanes from sediments at this depth. It is unlikely that the absence of these compounds at this depth is due to degradation, as recent biogenic bacterial hopanes (discussed below) are equally abundant at the surface as at depth.

As shown in Figures 10 and 13, automotive and outboard oils consistently display distinct ion fragment $m/z = 191$ a.m.u. traces. While both oil types contain hopanes, automotive oils also contain significant amounts of tricyclic terpanes. This difference can be used to estimate the relative contribution of these two petroleum sources to some samples. The surface sediments samples from St. John's Harbour and Mundy Pond, for example, both contain abundant tricyclic terpanes, as would be expected in a modern urban environment with substantial automobile oil loading. The phytoplankton samples

Figure 25. Ratio of petroleum terpenoids to UCM in petroleum, plankton, and sediment samples. Higher relative ratios of the more resistant terpenoids indicate increased exposure to weathering (petroleum terpenoid values estimated by sum of peak heights of C_{23} tricyclic terpenoid and hopane in the extracted ion trace of m/z = 191 a.m.u.; UCM size measured as height of total ion current trace above background at RI 2700-2800).

FRESH OIL ┊ WEATHERED OIL

Avondale
South River
St. Phillip's
Spaniard's Bay
Conc. Bay sediment
roadsweep
Mundy Pd.
St. John's Hr.
plankton tow (mean)
outboard slick
outboard water
used auto oil
muffler soot
auto oil
outboard oil

-5   5   15   25   35

Ratio of petroleum terpenoids to UCM
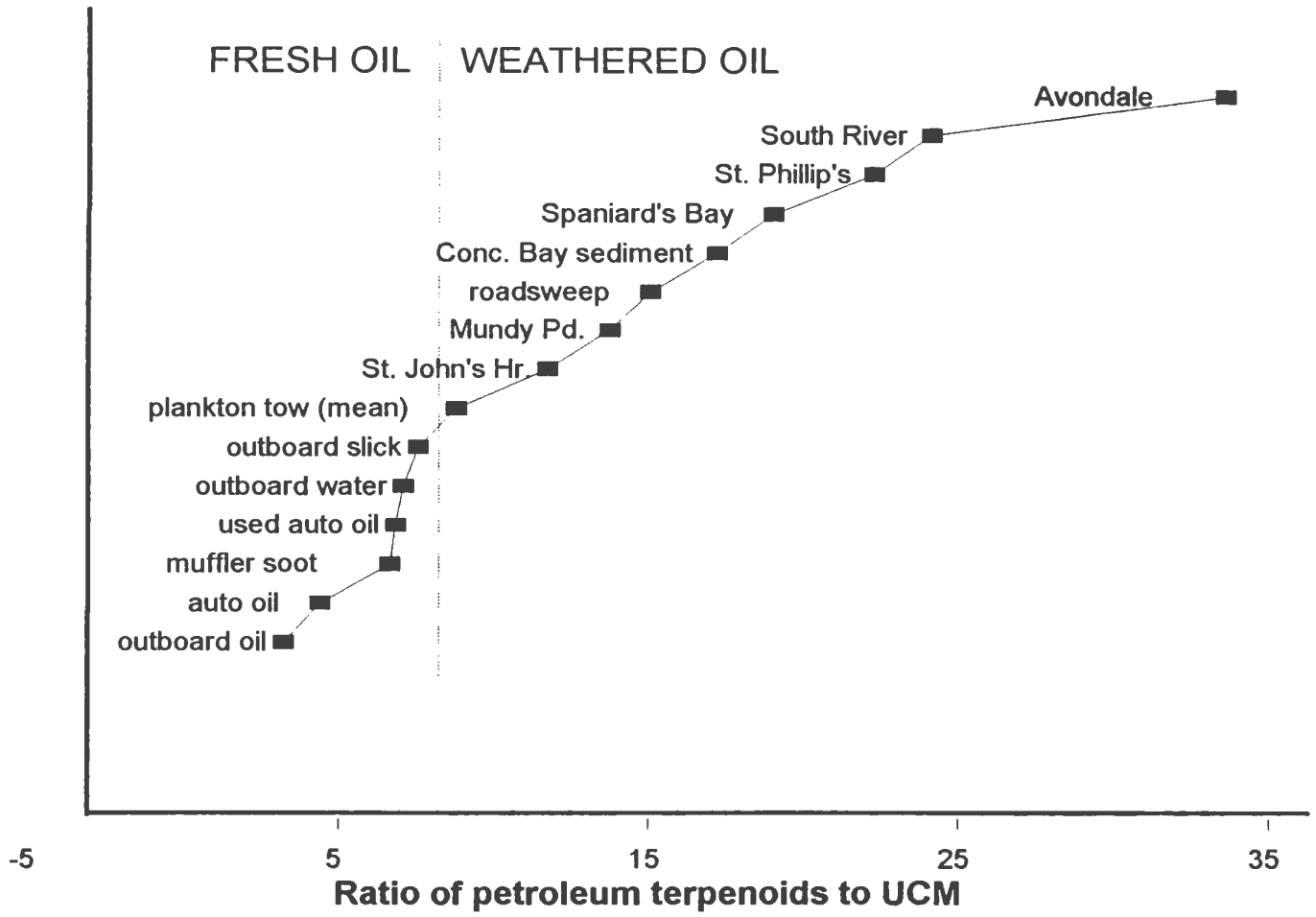
Figure 26. Plot of hopane:UCM ratio *vs.* C_{23} tricyclic terpane:hopane ratio. Note that as hopane/UCM increases (increasing weathering), the abundance of C_{23} terpane relative to hopane decreases (as shown by arrow), indicating that the C_{23} tricyclic terpane is degrading more quickly than hopane.
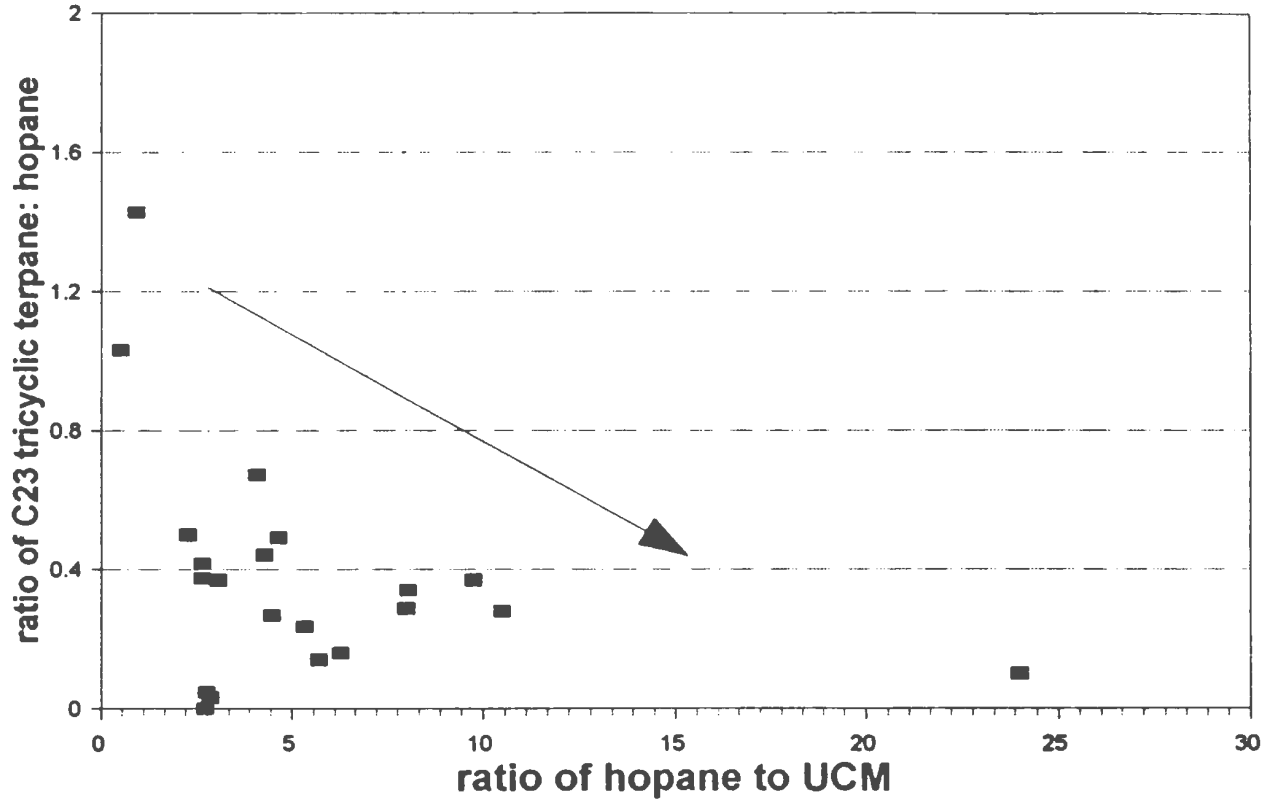
from Conception Bay, on the other hand, show a very low ratio of tricyclic to pentacyclic terpanes, suggesting that they are contaminated primarily with oil from outboard motors. Surface sediments in Conception Bay contain tricyclic and pentacyclic terpanes in a relative ratio of approximately half that of urban sediments. This lower relative abundance of tricyclic terpanes in these sediments may be due to the added input of outboard motor oil. Since the terpanes in Conception Bay sediments are roughly half as abundant as in the urban sediment samples, one might estimate that roughly half of the petroleum contamination in Conception Bay is derived from the operation of outboard motors. As shown in Figure 26, however, the abundance of tricyclic terpanes (relative to the size of the UCM) tends to decrease as the relative abundance of the hopanes increases, suggesting that the tricyclic terpanes weather faster than the hopanes. As a result, a large part of the reduction in tricyclic terpanes in Conception Bay may be the result of greater or longer exposure to degradation in these sediments. Thus, while the ratio of tricyclic to pentacyclic terpenoids may effectively distinguish fresh automotive and fresh outboard oil sources, differences in the degradation rates of these compounds may make this ratio unreliable for source apportionment in more weathered or older sediments.

There is no clear relationship evident between the concentrations of petroleum terpenoids present in samples and the isotopic compositions of either the n-alkanes or pristane. This finding further supports the conclusion arrived at earlier that petroleum sources contribute no significant quantities of n-alkanes or pristane to Conception Bay.

Recent biogenic hopanoids: As none of these compounds were detected in plankton tow samples, the recent biogenic terpenoids found in Conception Bay sediments would appear to be primarily derived from sedimentary bacteria. It is possible, however, that these compounds were not found in plankton tows as the net used to collect these samples may not have filtered out many particles as small as bacteria. Diploptene, the most common biogenic hopanoid detected, was present in all sediment samples except the recent leaf litter from Holyrood River. Except for this compound, all of the other biogenic hopanoids were present in concentrations too low to be isotopically analyzed. The isotopic composition of the diploptene in Conception Bay (-27.2‰) is similar to that reported by Schoell *et al.*, (1992) for other biogenic hopanoids and steranes derived from marine photosynthetic and heterotrophic bacteria. The similarity of the isotopic composition of diploptene with that of squalene suggests that the fractionation of carbon isotopes during the cyclization of squalene and final synthesis of larger terpenoids is relatively minor.

V. SUMMARY AND CONCLUSIONS

The aliphatic hydrocarbons of Conception Bay include mainly compounds derived from terrestrial vegetation, marine phytoplankton, bacteria, and petroleum. Terrestrially derived biogenic hydrocarbons present include primarily odd chain length C_{25-33} n-alkanes, as well as pristane. Phytoplankton, both in the rivers flowing into Conception Bay, and in the waters of the bay itself, contribute a complex, variable mixture of mostly unsaturated and branched compounds, including a series of C_{25} highly branched isoprenoid alkenes. The consistency of the molecular and isotopic signature of these C_{25} alkenes in plankton, sediments, and biota in Conception Bay and other areas around the coast of Newfoundland suggests that these compounds are produced by one particular species of marine diatom during the spring bloom. A series of n-alkanes from C_{15-31} showing low CPI are also produced during the spring bloom. Lubricating oils, from both automobiles and outboard motor oils, contribute an unresolved complex mixture to sediments and waters, as well as an assemblage of tricyclic and pentacyclic triterpanes and steranes. Numerous cyclic terpenoids, most likely derived from sedimentary bacteria, are also widely distributed in sediments in and around the bay.

In general, most of the aliphatic hydrocarbons in Conception Bay are significantly depleted in ^{13}C relative to bulk sedimentary and particulate organic matter. Although pronounced isotopic differences exist between hydrocarbons of different origins (*e.g.*, marine *vs.* terrestrial compounds), none of the "inherent" or biochemically derived isotopic differences predicted by or observed in previous studies between various

chemically distinct hydrocarbon groups were unequivocally or clearly affirmed in this investigation. The $\delta^{13}C$ of known phytoplankton hydrocarbons, including diverse structural types, ranged only from -22.9 to -27.9‰, with no consistent differences evident between, for example, linear and isoprenoid, or saturated and unsaturated compounds. Thus, kinetic isotope fractionations during biosynthetic reactions, if present, appear to be largely masked by effects related to the carbon fixing pathways or carbon pools utilised by organisms synthesizing the compounds, or to mixing of sources. The only biochemical isotope trend possibly in evidence among the environmental hydrocarbons in this study is a depletion in ^{13}C with increasing n-alkane chain length. However, this is more likely the result of the mixing of isotopically "heavy" short-chain alkanes of marine origin with isotopically "light" long-chain terrestrial n-alkanes. The potential significance of kinetic fractionations (and the complexity of the isotopic signatures of biomolecules in general), however, was demonstrated by the observation of variations in the isotopic composition of the heneicosahexaene produced by different species of diatoms grown in culture, depending on the the distribution of isomers synthesized.

No consistent, significant difference was found between the hydrocarbons in sources and sediments. For example, squalene, HEH, and the various isoprenoid breakdown products of the phytol side chain of chlorophyll displayed the same isotopic composition in plankton tows as in sediments. Thus, the isotopic composition of most hydrocarbons appears to be generally relatively unaffected by early degradation in the

water column and upper layers of the sediment, and primarily reflects mixing of sources.

The measurement of the isotopic composition of petroleum alkanes themselves was precluded by interference by the large UCM associated with weathered petroleum and lubricating oils, which would require more extensive sample treatment (adsorption on a molecular sieve) to remove. Petroleum contamination in some samples was, however, revealed by the presence of a distinct assemblage of tricyclic terpanes, hopanoids, and steranes. Although relatively fresh sources of automotive and outboard motor oil could be distinguished on the basis of these molecular signatures, differential weathering of these compounds appears to complicate the use of these markers for source apportionment in more weathered sediments. The lack of a relationship between the abundance of petroleum cyclic terpenoids and the isotopic composition of sedimentary pristane or n-alkanes suggests that while detectable quantities of lubricating oils and other petroleum products enter Conception Bay, these sources do not contribute significant amounts of n-alkanes to Conception Bay. This conclusion is also supported by the similarity between the isotopic and molecular signatures of these compounds in surface and deeper (uncontaminated) sediments.

In general, the combined analysis of the molecular distribution and the isotopic signature of source and sedimentary hydrocarbons allowed a more precise determination of the sources of hydrocarbons in Conception Bay to be performed. While the use of petroleum markers such as the cyclic terpenoids proved to be the most effective way to recognize petroleum contamination, variations in the isotopic signatures of some

compounds, especially of the n-alkanes, allowed the input of multiple sources to be recognized. More detailed analysis of the isotopic signature of sources, such as local terrestrial leaf debris and lubricating oil, is required before the complex isotopic patterns seen among some sedimentary compounds can be fully explained. Once source signatures are more clearly defined, compound-specific isotope analysis promises to become an even more effective tool for apportioning the input of multiple sources of compounds into estuarine environments such as Conception Bay.

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Phytoplankton tow data

(all concentrations expressed in terms of dry weight)

	Sample date:											
	April 15		May 18		May 18		May 20		Sept. 2		Nov. 8	
Compound	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C
n-alkanes												
nC15	0.3	-	0.2	-	0.1	-	0.2	-	0.2	-	1.1	-
nC16	0.7	-25.5	0.5	-28.0	0.3	-	0.3	-	0.3	-	0.3	-
nC17	0.7	-23.2	0.8	-26.4	0.5	-23.5	0.5	-29.7	0.4	-	0.6	-
nC18	0.6	-24.3	1.1	-27.3	0.5	-26.4	0.5	-30.7	0.2	-	0.4	-
nC19	0.4	-24.1	1.4	-27.2	0.6	-25.8	0.5	-29.8	0.2	-	2.5	-25.4
nC20	0.4	-23.4	1.3	-27.2	0.6	-25.8	0.6	-30.0	0.3	-	1.1	-
nC21	0.3	-	1.3	-29.1	0.8	-28.6	0.7	-30.6	1.6	-	1.4	-
nC22	0.4	-23.7	1.4	-27.6	0.8	-25.0	0.8	-30.0	0.5	-	1.1	-
nC23	0.4	-23.4	1.6	-27.4	1.2	-24.6	1.0	-29.0	0.6	-	1.7	-
nC24	0.4	-23.3	2.0	-27.3	1.2	-24.4	1.4	-29.7	1.1	-	2.3	-
nC25	0.5	-23.3	2.5	-27.2	1.6	-24.4	1.6	-29.2	1.1	-	3.5	-26.4
nC26	0.4	-23.2	2.1	-27.2	1.7	-24.4	1.5	-29.1	0.8	-	3.4	-26.3
nC27	0.4	-21.4	1.8	-27.3	2.0	-24.6	1.7	-29.3	1.5	-	5.0	-26.6
nC28	0.3	-22.8	1.4	-27.2	1.5	-24.4	1.1	-28.8	0.9	-	2.5	-
nC29	0.2	-24.0	1.1	-27.7	1.3	-24.9	0.9	-29.1	1.3	-	6.5	-27.5
nC30	0.1	-23.5	0.5	-27.2	0.8	-24.4	0.4	-28.6	0.4	-	3.2	-
nC31	0.1	-23.5	0.3	-27.5	0.4	-24.6	0.2	-29.3	1.2	-	3.4	-27.1
nC32	0.1	-22.9	0.3	-27.4	0.3	-24.2	0.1	-30.1	0.4	-	0.6	-
nC33	0.0	-21.4	0.2	-27.4	0.1	-24.1	0.1	-30.9	0.8	-	1.2	-
isoprenoids												
pristane	5.8	-23.5	7.7	-25.0	6.2	-22.9	1.9	-27.5	8.8	-	22.0	-26.3
phytane	0.3	-25.4	0.5	-27.6	0.3	-	0.2	-30.8	-	-	0.4	-
neo-phytadiene	2.1	-26.8	1.5	-26.7	1.8	-24.5	1.1	-24.9	-	-	-	-
1,3-phytadiene	2.2	-27.9	1.6	-27.5	1.6	-25.2	1.2	-25.5	-	-	-	-
2,4-phytadiene	2.1	-27.4	1.3	-27.3	2.2	-25.1	1.1	-25.8	-	-	-	-
brC25:3	15.4	-34.2	9.9	-38.7	11.5	-	9.9	-38.5	-	-	-	-
brC25:3'	5.8	-34.8	2.4	-35.7	2.6	-35.5	3.1	-35.6	-	-	-	-
brC25:4a	9.2	-35.2	5.2	-37.2	7.4	-36.5	4.9	-36.0	-	-	-	-
brC25:4b	8.0	-	2.4	-37.4	4.1	-42.5	2.4	-39.7	-	-	-	-
brC25:4a'	0.8	-	1.2	-34.3	1.3	-33.1	0.9	-30.7	-	-	-	-
brC25:4b'	0.6	-	1.2	-35.4	1.2	-31.5	0.9	-31.3	-	-	-	-
brC25:5	2.9	-	2.2	-36.6	4.0	-36.5	2.6	-34.4	-	-	-	-
brC25:5'	0.5	-	1.0	-34.5	1.0	-32.8	1.3	-28.8	-	-	-	-
Squalene	12.3	-26.3	10.3	-25.1	6.9	-27.6	8.2	-25.5	4.5	-	5.1	-
Other										-		-
HEH	24.6	-26.9	15.0	-26.6	1.7	-27.1	15.6	-26.4	9.7	-	22.5	-
brC17:1	0.9	-27.9	2.0	-31.9	1.2	-30.4	0.2	-	-	-	-	-
brC21:1	2.0	-29.3	3.0	-32.3	3.6	-32.3	1.7	-35.0	-	-	-	-
brC21:1'	1.2	-28.3	2.0	-30.1	2.4	-30.3	0.8	-32.1	-	-	-	-

Sediment data

(all concentrations expressed in terms of dry weight)

Compound	C. Bay (surf.)		C. Bay (surf.) bound		C. Bay (30 cm)		G. Banks (surf.)		Holyrood		Job's Cove	
	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C
n-alkanes												
nC15	0.1	-29.3	-	-	-	-	-	-	-	-	-	-
nC16	0.1	-29.3	-	-	-	-	-	-	-	-	-	-
nC17	0.1	-27.0	0.1	-	0.1	-	0.1	-27.5	-	-	0.6	-31.8
nC18	0.1	-29.0	0.1	-	0.1	-	0.1	-29.3	-	-	0.5	-26.7
nC19	0.1	-28.4	0.1	-	0.0	-	0.1	-29.9	-	-	0.9	-31.1
nC20	0.0	-29.4	0.1	-	0.1	-	0.1	-29.7	-	-	0.9	-26.7
nC21	0.2	-29.3	0.2	-28.4	0.3	-28.8	0.1	-30.6	-	-	2.5	-31.6
nC22	0.2	-28.9	0.3	-28.7	0.2	-28.3	0.1	-30.0	0.8	-	1.2	-32.5
nC23	0.3	-29.4	0.2	-29.2	0.6	-29.5	0.1	-30.0	17.0	-31.5	3.4	-30.4
nC24	0.2	-30.2	0.2	-29.2	0.3	-30.8	0.2	-30.4	4.2	-	1.0	-32.1
nC25	0.3	-29.7	0.2	-29.2	0.7	-30.4	0.3	-30.3	36.1	-31.5	3.7	-32.9
nC26	0.2	-30.2	0.1	-29.4	0.4	-31.2	0.2	-30.1	16.1	-30.0	1.2	-33.7
nC27	0.6	-30.5	0.2	-29.7	1.0	-30.6	0.2	-30.1	123.1	-31.1	4.5	-33.0
nC28	0.2	-28.9	0.2	-	0.4	-31.4	0.2	-30.1	2.1	-	1.3	-33.1
nC29	0.6	-30.6	0.2	-29.4	1.2	-31.1	0.2	-30.6	72.2	-31.9	6.3	-32.1
nC30	0.2	-30.1	0.1	-	0.3	-31.0	0.2	-29.9	-	-	0.7	-31.3
nC31	0.5	-30.5	0.1	-	1.0	-31.5	0.1	-29.7	16.6	-32.0	3.7	-31.8
nC32	0.1	-30.5	0.0	-	0.1	-29.8	0.1	-30.2	-	-	0.3	-31.4
nC33	0.2	-	0.0	-	0.4	-32.6	-	-	2.1	-31.6	1.5	-31.5
Isoprenoids												
pristane	0.4	-27.8	0.2	-	0.2	-	-	-	-	-	-	-
phytane	-	-	-	-	-	-	-	-	-	-	-	-
neo-phytadiene	-	-	0.2	-26.6	tr.	-	-	-	-	-	-	-
1,3-phytadiene	-	-	0.2	-26.5	tr.	-	-	-	-	-	-	-
2,4-phytadiene	-	-	0.3	-26.6	tr.	-	-	-	-	-	-	-
brC20:1	-	-11.5	-	-	-	-	-	-	-	-	-	-
brC20:1'	-	-	1.3	-	-	-	-	-	-	-	-	-
brC25:3	1.1	-34.8	0.4	-36.4	1.2	-32.3	0.2	-36.5	-	-	-	-
brC25:3'	0.6	-30.2	0.6	-30.0	0.6	-27.7	0.0	-35.2	-	-	-	-
brC25:4a	0.7	-34.4	0.1	-35.6	0.4	32.9	0.1	-35.4	-	-	-	-
brC25:4b	0.6	-31.7	0.3	-34.3	0.3	-26.9	0.1	-38.5	-	-	-	-
brC25:4a'	0.2	-35.8	0.1	-29.7	0.2	-29.7	0.1	-29.1	-	-	-	-
brC25:4b'	0.2	-30.2	0.2	-30.4	0.2	-31.6	0.0	-36.7	-	-	-	-
brC25:5	0.4	-33.2	0.1	-30.6	0.3	-28.3	0.1	-35.9	-	-	-	-
brC25:5'	-	-26.3	0.1	-26.6	0.2	-27.7	-	-	-	-	-	-
Squalene	0.4	-	0.0	-	0.5	-	0.1	-25.5	-	-	-	-
Other									-	-	-	-
HEH	0.5	-26.1	-	-	-	-	-	-	-	-	-	-
brC17:1	0.1	-26.8	-	-	0.1	-	-	-	-	-	-	-
brC21:1	-	-	-	-	-	-	-	-	-	-	-	-
brC21:1'	-	-	-	-	-	-	-	-	-	-	-	-
brC23:2a	-	-	-	-	-	-	-	-	-	-	-	-
brC23:2b	-	-	-	-	-	-	-	-	-	-	-	-
brC25:4c	-	-	-	-	-	-	-	-	-	-	-	-
brC25:3b	-	-	-	-	-	-	-	-	-	-	-	-

| Sample site: | Topsail | | South River | | Spaniard's Bay | | St. Phillip's | | Avondale | | Mundy Pond | |
Compound	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C	conc. (ug/g)	del 13C
n-alkanes												
nC15	-	-	0.1	-28.9	0.1	-23.8	-	-	0.1	-26.8	-	-
nC16	-	-	0.1	-29.8	0.1	-28.8	-	-	0.1	-26.1	0.1	-
nC17	-	-	0.1	-24.8	0.1	-	0.1	-29.0	0.1	-26.6	0.5	-
nC18	-	-	0.1	-28.6	0.0	-27.7	-	-	0.1	-27.3	0.1	-
nC19	0.1	-28.4	0.3	-20.3	0.1	-22.6	0.1	-27.7	0.2	-27.1	0.2	-
nC20	0.1	-29.0	0.1	-28.6	-	-28.1	0.1	-25.5	0.2	-27.4	0.1	-
nC21	0.1	-29.8	0.4	-19.9	0.1	-21.5	0.1	-28.0	0.3	-27.6	0.5	-
nC22	0.0	-31.5	0.1	-28.7	-	-27.9	0.1	-28.9	0.2	-27.9	0.4	-
nC23	0.2	-28.9	0.5	-23.6	0.3	-26.2	0.2	-29.5	0.3	-28.5	1.1	-
nC24	0.0	-31.1	0.2	-29.5	0.1	-28.5	0.1	-28.9	0.3	-28.1	0.5	-
nC25	0.2	-30.3	0.5	-26.7	0.3	-27.9	1.2	-29.8	0.4	-29.0	1.3	-
nC26	0.0	-32.9	0.2	-30.0	0.1	-28.9	0.1	-28.4	0.3	-28.4	0.5	-
nC27	0.3	-30.6	0.5	-27.6	0.3	-28.6	0.6	-29.7	0.5	-29.7	1.8	-
nC28	0.1	-31.2	0.1	-29.2	0.1	-28.9	0.1	-29.0	0.3	-29.4	0.6	-
nC29	0.3	-32.2	0.5	-30.5	0.5	-30.1	0.6	-30.8	0.5	-30.7	2.1	-
nC30	-	-31.5	0.1	-29.8	0.1	-28.8	0.1	-29.0	0.2	-28.5	0.5	-
nC31	0.2	-33.5	0.4	-31.7	0.3	-30.4	0.5	-30.4	0.5	-31.2	1.3	-
nC32	-	-	-	-30.2	-	-	-	-	0.1	-	0.5	-
nC33	0.1	-34.5	0.1	-31.8	0.1	-32.4	0.2	-31.7	0.3	-31.9	0.8	-
Isoprenoids												
pristane	0.1	-30.5	0.1	-29.5	tr	-	0.1	-29.8	0.1	-27.5	-	-
phytane	-	-	0.1	-26.5	tr	-	0.1	-29.1	0.1	-27.0	-	-
neo-phytadiene	-	-	-	-	-	-	-	-	-	-	-	-
1,3-phytadiene	-	-	-	-	-	-	-	-	-	-	-	-
2,4-phytadiene	-	-	-	-	-	-	-	-	-	-	-	-
brC20:1	-	-	0.1	-16.4	0.1	-22.4	-	-	-	-	-	-
brC20:1'	-	-	0.1	-	0.1	-	-	-	-	-	-	-
brC25:3	-	-	-	-	-	-	-	-	-	-	-	-
brC25:3'	-	-	-	-	-	-	-	-	-	-	-	-
brC25:4a	-	-	-	-	0.5	-20.1	-	-	-	-	-	-
brC25:4b	-	-	-	-	-	-	-	-	-	-	-	-
brC25:4a'	-	-	-	-	0.2	-21.1	-	-	-	-	-	-
brC25:4b'	-	-	-	-	-	-	-	-	-	-	-	-
brC25:5	-	-	-	-	0.5	-17.6	-	-	-	-	-	-
brC25:5'	-	-	-	-	0.2	-23.6	-	-	-	-	-	-
Squalene	-	-	-	-	-	-	-	-	-	-	-	-
Other							-	-	-	-	-	-
HEH	-	-	-	-	-	-	-	-	-	-	-	-
brC17:1	-	-	-	-	tr	-	-	-	-	-	-	-
brC21:1	-	-	0.1	-	-	-	-	-	-	-	-	-
brC21:1'	-	-	-	-	-	-	-	-	-	-	-	-
brC23:2a	-	-	0.1	-18.8	0.1	-19.5	-	-	-	-	-	-
brC23:2b	-	-	0.3	-	0.1	-21.7	-	-	-	-	-	-
brC25:4c	-	-	0.3	-18.0	0.1	-24.2	-	-	-	-	-	-
brC25:3b	-	-	0.3	-20.6	-	-	-	-	-	-	-	-

Appendix 3

Estimated plankton tow species compositions

(values are estimated weight percentages, pr denotes present)

Grand Banks, Spring bloom, 1993	April 17		
Thalassiosira nordenskioldii and *hyalina*	65		
Fragilariopsis sp.	20		
Chaetoceros concavicornis	10		
Navicula sp.	5		

Conception Bay, Spring bloom 1993	April 19	May 18	May 20
Thalassiosira nordenskioldii and other sp.	45	50	45
Fragilariopsis sp.	25	35	35
Chaetoceros conca\ .ornis	10	5	5
Rhizosolenia setigra	2	5	7
Unspecified pennate diatoms	2	2	2
Unspecified dinoflagellates	2	2	2
Eucampia zoodiaca	pr	pr	pr
Coscinodiscus sp.	pr	pr	pr
Leptocylindricus danicus		pr	pr

Conception Bay, Fall bloom, 1993	Sept. 2	Nov. 8	
Serratum arcticum and *Serratum fusus*	75	65	
Dinophysis sp.	25	35	

Appendix 4

Diatom culturing conditions

All cultures were obtained from:
Center for Culture of Marine Phytoplankton (CCMP)
Bigelow Laboratory for Ocean Sciences
West Boothbay Harbor, Maine, 04575 U.S.A.

Thalassiosira nordenskioldii (axenic)
CCMP clone number: 992
Origin: Gulf of Maine, NW Atlantic
Medium: f/2
Temperature: 2-15 °C
Culture volume: 1575 ml
Final dry weight: 0.1739 g

Fragilariopsis cylindrus
CCMP clone number: 557
Origin: SW Atlantic
Medium: f/2
Temperature: -2-4 °C
Culture volume: 1580 ml
Final dry weight: 0.1346 g

Nitzschia seriata
CCMP clone number: 1309
Origin: Resolute Passage, Barrow Strt, NWT
Medium: f/2
Temperature: -2-2 °C
Culture volume: 1450 ml
Final dry weight: 0.1142 g

Fragilaria striatula (axenic)
CCMP clone number: 1094
Origin: Kachemak Bay, Alaska
Medium: f/2
Temperature: 0-6 °C
Culture volume: 1400 ml
Final dry weight: 0.1013 g

Appendix 5

Manufacturers of unused lubricating oils analysed

Automotive (Crankcase) Oils

Irving 10W30 Engine Oil
Motomaster 5W30 Engine Oil, blended by Imperial Oil Products, Canada
Ultramar 5W30 Supreme Ultralube Engine Oil

Outboard Motor (Two Cycle) Oils

Irving Outboard Motor Oil
Ultramar Outboard Motor Oil (Injection, 20-100:1 Mix), blended by Ultramar Canada
Motomaster Superior Protection Outboard Motor Oil (16-100:1 Mix), blended by Shell
Canada Products.

Appendix 6

Retention (Kovat's) Indices of hydrocarbons analysed

(all values reported for 25 m × 0.25 mm i.d. CP-Sil 5 column, film thickness 0.12 μm as described in Experimental section)

Compound	Retention Index
pristane	1710
phytane	1810
br $C_{17:1}$	1680
br $C_{20:1}$	1688
br $C_{20:1}$'	1702
br $C_{21:1}$	2092
br $C_{21:1}$'	2110
neo-phytadiene	1850
1,3-phytadiene	1880
2,4-phytadiene	1910
HEH	2040
br $C_{25:3}$	2050
br $C_{25:3}$'	2090
br $C_{25:3}$b	2170
br $C_{25:4}$a	2075
br $C_{25:4}$a'	2080
br $C_{25:4}$b	2120
br $C_{25:4}$b'	2125
br $C_{25:4}$c	2130
br $C_{25:5}$	2110
br $C_{25:5}$'	2160
br $C_{23:2}$a	2090
br $C_{23:2}$b'	2105
squalene	2820
norhopane	2995
hopane	3005
diploptene	3120