TERRESTRIAL INPUT TO ESTUARINE BIVALVES AS MEASURED BY MULTIPLE STABLE ISOTOPES TRACERS

BY

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STABLE ISOTOPES STUDY OF ESTUARINE BIVALVES

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ABSTRACT

Stable isotope ratios of carbon, nitrogen, hydrogen and oxygen were used here, to trace the extent of terrestrial input to estuarine bivalves (*Mytilus edulis*) during the summer of 1984 and 1985. Salinity records indicate a stronger river influence in 1985 compared to 1984.

Fatty acids ratios (C24/C16, C24/C14) are intercalibrated with stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to characterize the sediments of a Negro Harbour, a small estuary which receives mixed terrestrial input. The relative contribution of terrestrial and organic matter calculated through fatty acid and stable isotope ratios were generally in agreement for sample sites in the upper part of the estuary. However, changes in the relative contribution of peat compared to higher terrestrial plants are more readily noticed using fatty acid ratios than with isotope ratios.

$\delta^{13}C$ and $\delta^{15}N$ were both compared in the organic matrix (lifetime average diet) and in the flesh (short-time variation) as well as in the stomach content (which were assumed to reflect the diet), the sediments, POC and marine plants. Based on $\delta^{13}C$ of the flesh more so than for $\delta^{15}N$ values, it seems that *Mytilus edulis* take up

some of the terrestrial material coming from Clyde river. The uptake was higher in 1985 which agrees with the river influence being stronger during that period of time.

The isotopic fractionation between the organic matrix of M. *edulis* and the stomach content (assumed to be the immediate diet) average about 4‰ for carbon and 2.0‰ for nitrogen. There seems to be less fractionation between the flesh and the organic matrix using nitrogen isotope compared to carbon isotope ratio. $\delta^{13}C$ and $\delta^{15}N$ of organic matrices do not change over a period of one year and are therefore useful in evaluating long-term changes in diet of bivalves and regional differences. The organic matrix resemble in certain characteristics, the collagen of bone, which is widely used in paleodiet studies. Thus, the organic matrix could be used in well preserved fossils to learn about the past diet of molluscs.

Stable isotopes of carbon and oxygen of the calcitic shell of M. *edulis* were also investigated since carbonates are often used to indicates freshwater input in paleoenvironments. In general, shells were close to equilibrium with the surrounding water. But, the results obtained here showed that the use of carbonates to detect past estuarine environment could lead to misinterpretation due to possible biological effects that inhibits calcification (as noted near the head of the estuary).

A Ati,

pour l'amour, le perpétuel encouragement et les choses.

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CHAPTER 1. INTRODUCTION

Knowledge of the relative contribution of organic material of marine and terrestrial origin to estuarine and coastal food webs is important to understanding and managing these ecosystems. It is also an aid in determining the source of organic matter in sediments, the origin of geolipids, and the fate of organic pollutants.

Numerous studies have examined the flow of organic carbon of different plant sources through estuarine food webs using ratios of the stable isotopes of carbon as natural labels (Haines, 1976; Haines and Montague, 1979; McConnaughey and McRoy, 1979b).

The various isotopic ratios of plants are apparently related to differences in photosynthetic pathways of carbon fixation (Wong and Sackett, 1978) and these ratios, expressed as $\delta^{13}C$ values, appear to be only slightly altered by the metabolism of consumers (Deniro and Epstein, 1978; McConnaughey and McRoy, 1979a) by about 1-2‰. The range of $\delta^{13}C$ values for primary producers of interest may be narrow, and the trophic relationships of consumer populations complex. Consequently the $\delta^{13}C$ values of many estuarine consumers fall into a range where pathways of carbon flow are

difficult to demonstrate with any confidence (Schwinghammer *et al.*, 1984; Fry and Parker, 1979).

There are, however, a number of promising approaches which involve a combination of δD, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ for sediment and food web analysis (Peterson *et al.*, 1985; Sweeney and Kaplan, 1980; Sweeney *et al.*, 1980; Macko, 1983; Rau *et al.*, 1980).

In most estuarine systems, organic matter in sediments and particulate organic matter (POM) in the water are expected to be dominated by two major sources of fixed carbon: terrestrial vascular plants and marine phytoplankton. The proportion of each varies depending on proximity to each carbon source (Fry and Sherr,1984). Isotopic or chemical labelling of these sources allows us to test the relative importance of each source, when sampling along a gradient between these sources (Schultz and Calder, 1976). Stable carbon isotopes have been used as such a tracer (Gearing *et al.*, 1984; Peters *et al.*, 1978) but if more than two sources are suspected of being present, carbon isotope studies alone cannot detect the contribution of other sources (Schwingammer *et al.*, 1983). Analyses of lignin (Hedges and Parker, 1976) and lipid components (Volkman *et al.*, 1980, 1981) in sediments provide complementary information which helps to "fingerprint" the individual sources of fixed carbon in multiple source environments.

Input of a particular source (marsh plants, peat, sewage, etc.) in a nearshore area may influence the adjacent fauna. Rau *et al.* (1980) used multiple isotope ratios ($\delta^{13}C$, $\delta^{15}N$ and δD) to trace the fate of sewage in the gulf of California. This study showed an uptake of pollutants in commercial species such as the ridgeback prawn (*Sicyonia digentis*) and the Dover sole (*Microstomus pacificus*).

The importance of peatland contribution to nearshore food webs has been discussed by Schell (1983). Inputs of terrestrial peat to the nearshore Alaskan Beaufort Sea from erosion and fluvial transport are of the same magnitude as *in situ* primary production within 10 km of the coastline. Nevertheless, $\delta^{13}C$ and ^{14}C abundances in marine organisms show that only small amounts of terrestrial carbon are transferred beyond the microbial level. Freshwater organisms, however, are heavily dependent on peat, as shown by pronounced seasonal radiocarbon depression in resident fish and ducks. On the other hand, Incze et al. (1982), Stephenson and Lyon (1982), and Lyon and Stanley (1985) found that corresponding trends in $\delta^{13}C$ values for particulate organic carbon, (POC), and the tissues of several filter-feeding bivalve mollusks in estuaries, indicate assimilation of terrestrial organic matter by these estuarine organisms.

Tieszen *et al.* (1983) suggested that information about a herbivorous vertebrate's food intake would best be maximized by an analysis of bone collagen, soft tissue and feces or stomach contents. These multiple analyses would reveal the animal's average long term diet, its immediate diet, and whether or not any shifts in food habits had occurred recently.

My purpose was to study an estuarine environment and determine the flow of nutrients using multiple isotopes and organic chemistry. Two aspects of this problem were investigated: (1) simultaneous use of geochemical, ($\delta^{13}C$, $\delta^{15}N$, δD), and geolipid tracers, (fatty acids), to characterise possible terrestrial (peatland) and marine food sources available to estuarine bivalves; and (2) evaluating the use of the organic matrix contained in molluscan shell as a diet (paleodiet?) indicator.

Stable isotope ($\delta^{13}C$, $\delta^{15}N$, δD) analysis of soft tissues is a well known technique in determination of the diet, but this implies preservation of the tissues prior to analysis. Use of the shell organic matrix instead of soft tissues to record the lifetime diet could be a useful alternative. There are several advantages in using this organic matrix:

- the organic matrix corresponds to the integrated overall diet of the animal since it is

produced and preserved within the shell as the organism grows,

- original shell matrix can be preserved in the geological record, so comparative studies could be done of modern and fossil populations,

- shells are more easily collected and preserved than tissues.

CHAPTER 2. STUDY AREA

Peatlands are prominent elements in the terrestrial environment of Southwest Nova Scotia (figure 2.1). Peatland discharge in Nova Scotia can vary considerably over a year, depending on the availability of water at or near the peat surface.

Proposals have been made to develop some of the Nova Scotian deposits as local energy sources, particularly Barrington Bog, which is located near the mouth of the Clyde River. Wetlands are dominated by treed swamps and bogs (MacDoughall and Cann, 1961). The dominant peat-forming material is *Sphagnum* moss.

The Clyde River is characterized, like other rivers of Southwest Nova Scotia, by naturally acidic, highly coloured waters. It flows south, sixty-five km from the central highlands of Shelburne and Yarmouth Counties to the Atlantic Ocean, draining an area of 361 square km. The watershed is characterized by many scattered wetlands in depressions (MacDoughall and Cann, 1961).

The Clyde River flows into Negro Harbour, a 15 km long estuary with a shoreline covered with sandy gravel, boulders and scattered marsh. The bottom of the estuary is largely covered by mud (98% <63 μm).

A mild maritime climate dominates the watershed. Mean daily temperature ranges from 18°C in July and August to -3°C in Febuary. The mean annual precipitation is 1337 mm, of which 156 mm falls as snow (Atmospheric Environment Service, 1982).

The two sampling seasons (summer 1984 and 1985) were very different in terms of precipitation. The summer of 1984 in Southwestern Nova Scotia was very dry with some river levels below average, and the summer of 1985 was comparatively wet, with twice normal rainfall (Climatic Perspective 1984, 1985). The gradient in salinity was steeper in 1985 compared to 1984 (Figure 2.2).

Figure 2.1. Study area and its peat deposits.

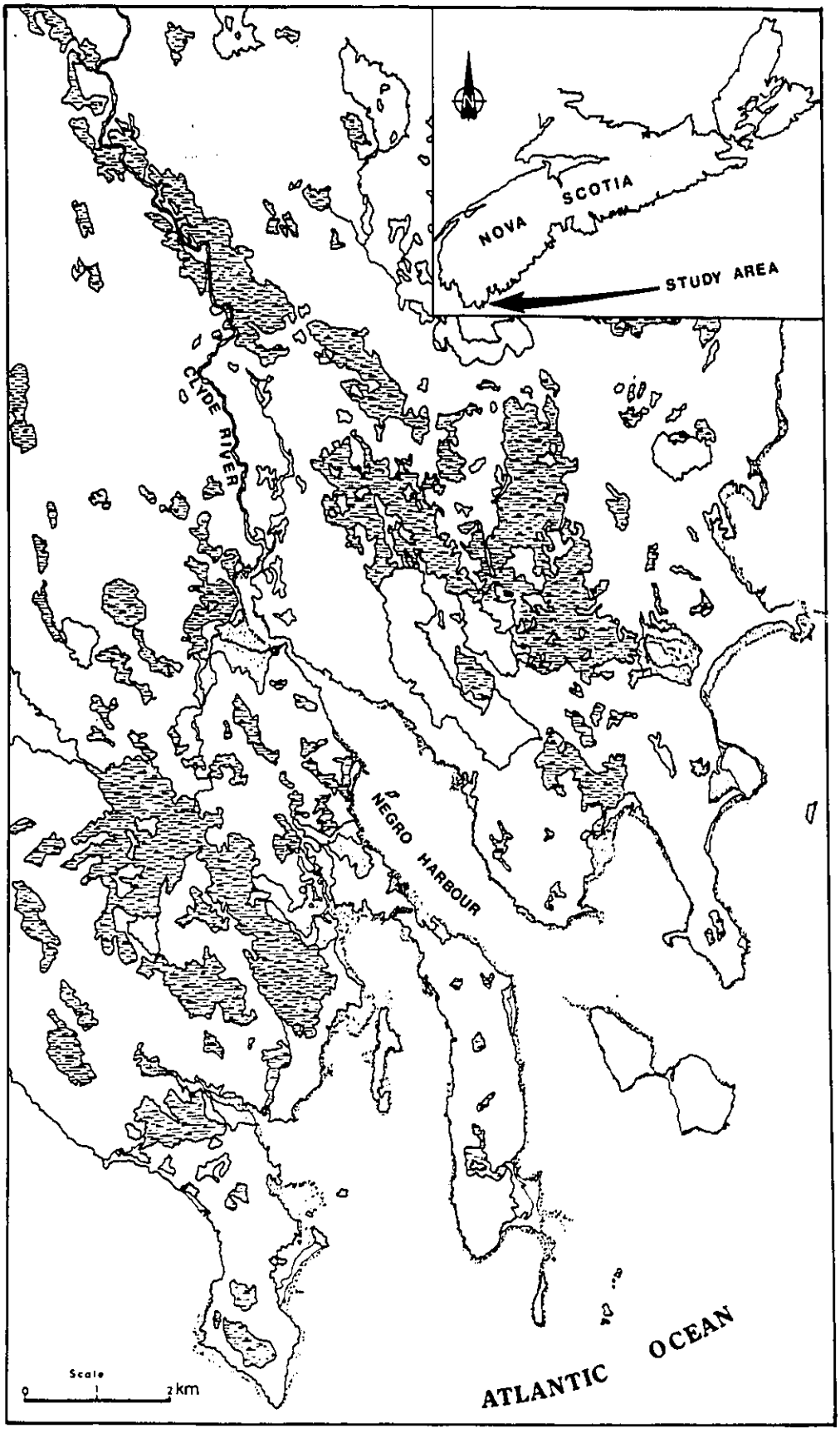
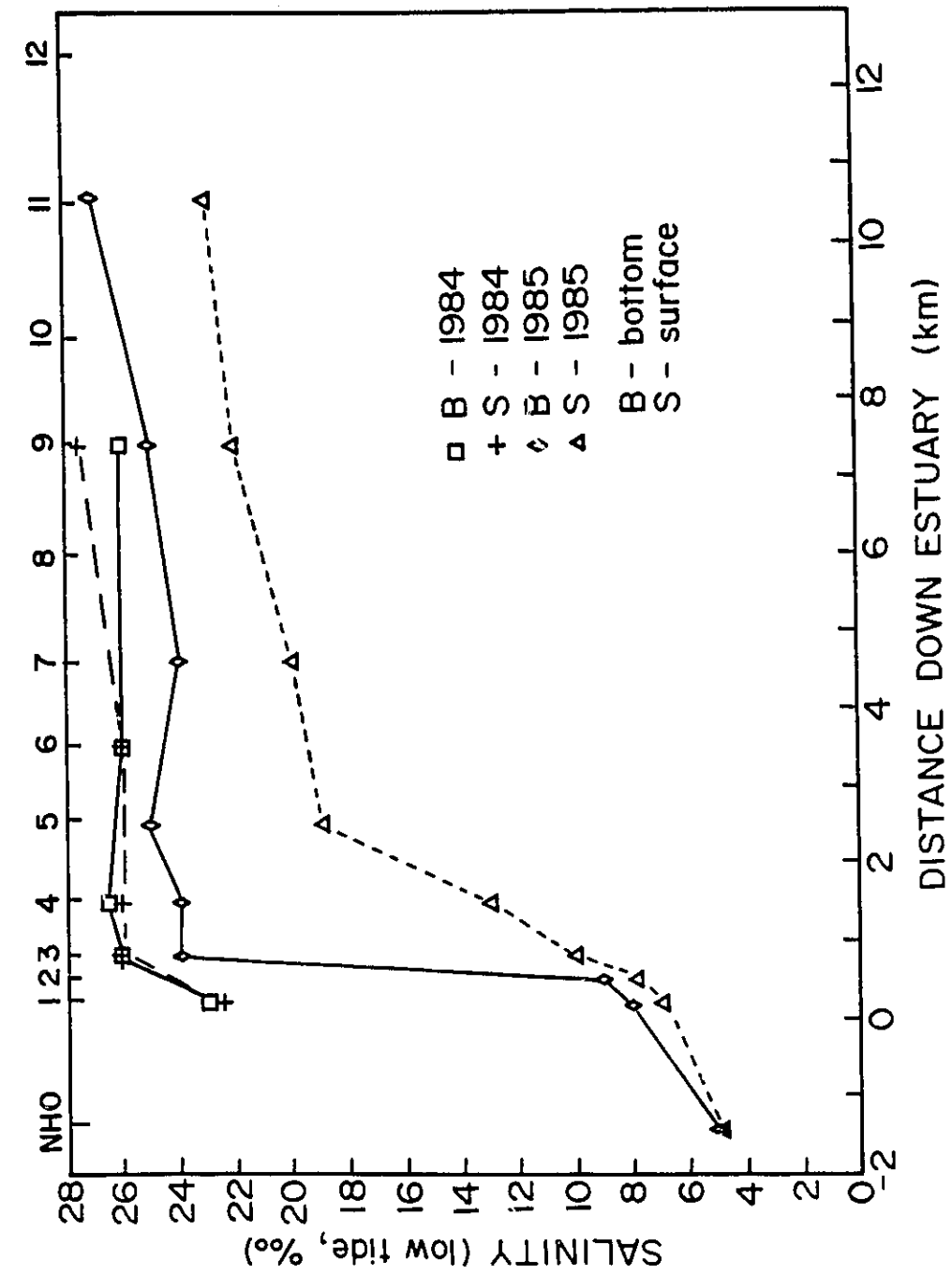

NOVA SCOTIA
STUDY AREA
CLYDE RIVER
NEGRO HARBOUR
ATLANTIC OCEAN
Scale
0 1 2 km

Figure 2.2. Salinity profiles at low and high tide for 1984 and 1985. Numbers at top refer to sampling stations on downstream traverse, to be discussed later.


SALINITY (low tide, ‰)
DISTANCE DOWN ESTUARY (km)
B - bottom
S - surface
□ B - 1984
+ S - 1984
◇ B - 1985
△ S - 1985
NHO
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
12
11
10
9
8
7
6
5
4
3
2
1
0
2
4
6
8
10
12

CHAPTER 3. EXPERIMENTAL METHODS

3.1. FIELD TECHNIQUES

One method for evaluating the significance of relatively small differences in $\delta^{13}C$ of a consumer species is to look for trends in specimens along a gradient between differing nutrient sources. A gradient of progressive mixing of terrestrial and marine inputs to an estuarine food web represents such a situation (Incze et al., 1982).

Samples of sediments were collected by SCUBA at thirteen sites (Figure 3.1) along a traverse along the axis of Negro Harbour during both sample years (summer 1984,1985). The sediment samples were obtained by scraping approximately the top 1-2 cm of sediment into sampling jars.

Samples of plants and peat from different depths (0-50 cm, 100-150 cm) were collected from Barrington bog in August 1984. Plankton samples were collected outside the mouth of the estuary (Figure 3.1, P-1) in August 1985, using a conical plankton net sampler of 63 µm mesh size. Water samples from different points in the river (figure 3.2) in August 1984 and 1985, were filtered in the field through Whatman 934-AH glass fiber filters

Figure 3.1. Location of sediment sample sites.

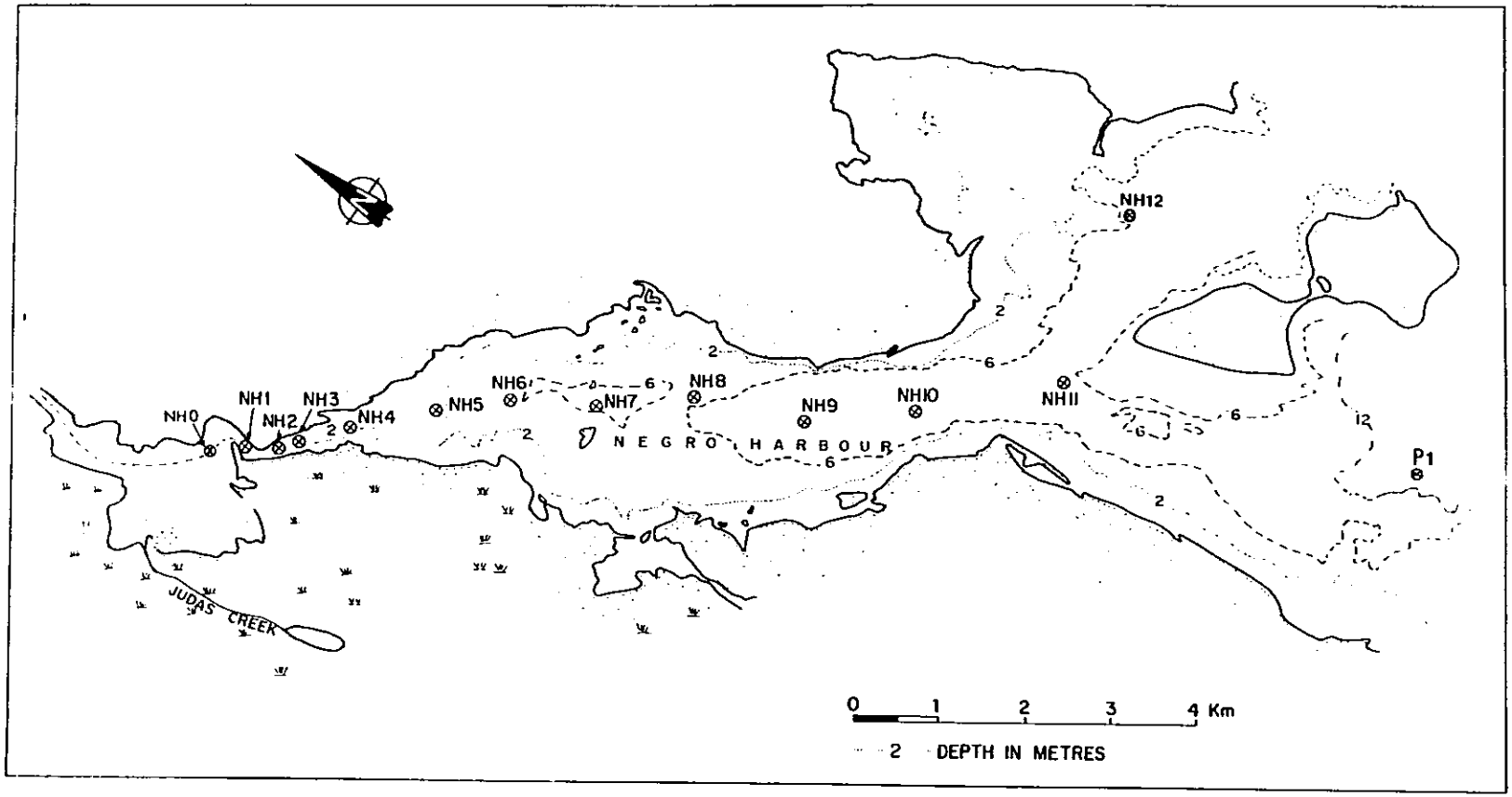
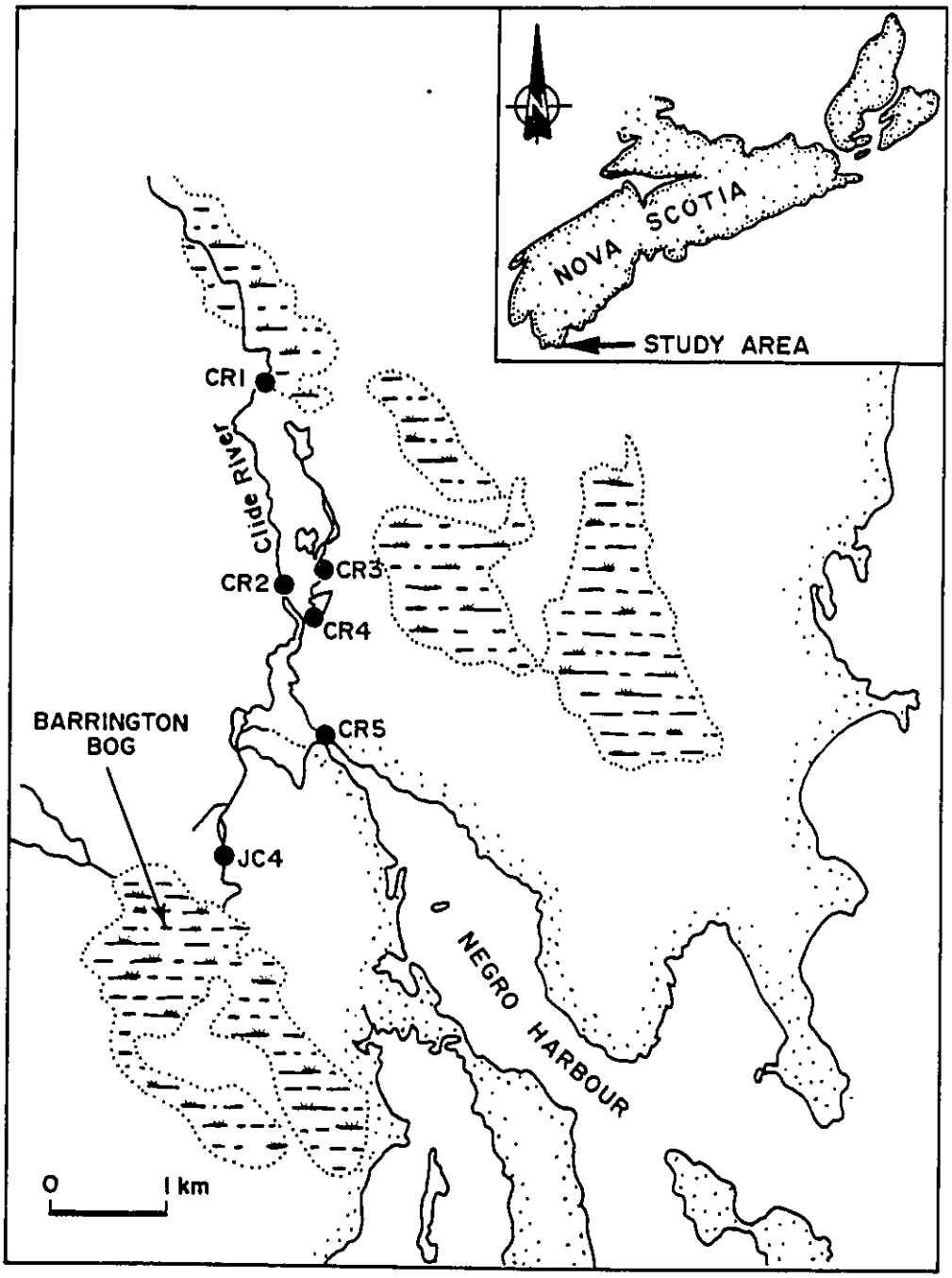

NH0
NH1
NH2
NH3
NH4
NH5
NH6
NH7
NH8
NH9
NH10
NH11
NH12
P1
NEGRO HARBOUR
JUDAS CREEK
2
6
12
0 1 2 3 4 Km
2 DEPTH IN METRES

Figure 3.2. Location of river water sample collection sites.


NOVA SCOTIA
STUDY AREA
CR1
Clyde River
CR2
CR3
CR4
CR5
BARRINGTON BOG
JC4
NEGRO HARBOUR
0
1 km

nominal pore size, ~1.5µm), to extract particulate organic matter (POM) for determination of its isotopic composition.

3.2. SAMPLE PREPARATION

3.2.1. SEDIMENT AND POM

The sediment and filters containing POM were kept frozen from the time of collection until they were freeze-dried in the laboratory and then stored until analysis.

3.2.2. MOLLUSCS

3.2.2.1. Soft tissues and stomach contents

The entire mollusks were kept frozen from the time of collection. In the laboratory the flesh and stomach contents (guts) of each mollusk to be analysed, were isolated from the shell (which was kept for carbonate or organic matrix analyses), numbered and freeze-dried separately. All samples were ground to powder and stored until analysis.

3.2.2.2. Extraction of insoluble component of the Organic Matrix from molluscan shells:

Three methods have been tested for the dissolution of calcium carbonate and recovery of only the insoluble component of the organic matrix on both *Mytilus edulis* and *Arctica islandica* (all at 20°C).

Organic matrix was first extracted according to

the method of Weiner (1979):

The periostracum of the shell was first removed mechanically and the shell were crushed. Fragments larger than about 1 mm are decalcified by dialysis (Spectropore 6 tubing; 1KD (kilodalton) MW cut-off) against 10% (wt/vol) ethylenediaminotetraacetic acid (EDTA) buffered with 0.05M sodium phosphate to pH 7.0. After complete decalcification, the contents of the dialysis bag are dialyzed against cold running tap water. The insoluble and soluble fractions are then separated by low speed centrifugation (2000g for about 3 minutes). The supernatant contains the soluble fraction. The final pellet is the insoluble fraction of the organic shell matrix. This pellet is then freeze-dried and kept until analysis.

With dissolution in EDTA, fragmented shells of *Mytilus edulis* took about a month to dissolve compared to less than 1 week for *Arctica islandica*. The $\delta^{13}C$ and $\delta^{15}N$ of *Mytilus edulis* organic matrices were similar for 3 replicate samples, as were those of *Arctica islandica* samples (table 3.1).

In order to accelerate the dissolution process, I attempted to dissolve the shells in dilute hydrochloric acid, using 2 different concentrations of HCl, 1N and 3N. Shell fragments of about 3 to 5mm were placed in a beaker and covered with ~20ml of acid. More acid was added each day after having carefully removed with a pipette a

Table 3.1. Dissolution test on shells ($\delta^{13}C$, $\delta^{15}N$)
Average in brackets. Replicates of a batch sample (~ 50g of homogenized crushed shells).

Mytilus edulis	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
EDTA	-16.2, -16.1, -16.2 (-16.2)	8.7, 9.0, 9.0 (8.9)
HCl 1N	-16.9, -16.2, -16.4 (-16.5)	9.0, 8.9, 9.5 (9.1)
HCl 3N	-17.4, -17.4, -17.2, -17.0, (-17.2)	9.9, 10.0, 9.7 (9.9)

Arctica islandica	$\delta^{13}C$ ‰)
ETDA	-17.3, -17.0, (-17.2)
HCl 1N	-17.4, -17.4
HCl 3N	-13.5, -12.0, -11.3 (-12.3)

corresponding volume of supernatant solution, without disturbing the insoluble portion. This procedure was repeated until complete decalcification (about 1 week for both species). The insoluble residues were then soaked in distilled water, which was changed regularly until neutral pH was reached. The residues were then lyophylized and stored until further analysis. This process probably removes some small protein chains and amino acids but the $\delta^{13}C$ and $\delta^{15}N$ results are statistically equivalent (p=0.95) to those obtained through EDTA dissolution (table 3.1). Dissolution in 3N HCl differs from that using 1N HCl in that no solution was discarded until the centrifugation process. NaOH was then added to neutralize the acid; phenolphtalein was also added to indicate the neutral point. This mixture was then transfered to a dialysis bag (1KD) and dialysed against running water to eliminate Na, Cl, Ca ions and phenolphtalein. The solution was then centrifuged. the supernatant was discarded and the residue collected and lyophylized. Stable carbon and nitrogen isotopes of organic matrix obtained through 3N dissolution were statistically (p=0.95) different in both *M. edulis* and *A. islandica* from the EDTA and 1N HCl values.

After these preliminary experiments on the extraction process, dissolution in 1N HCl was the method preferentially used because it was more efficient.

3.2.2.3. Preparation of shell for carbonate analysis:

The periostracum of the shell was removed mechanically. Only the outer calcitic layer of the shell was used for the analyses. Two valves of each sample were treated as replicates. A drill was used to obtain an equivalent of about 5 mg of calcite.

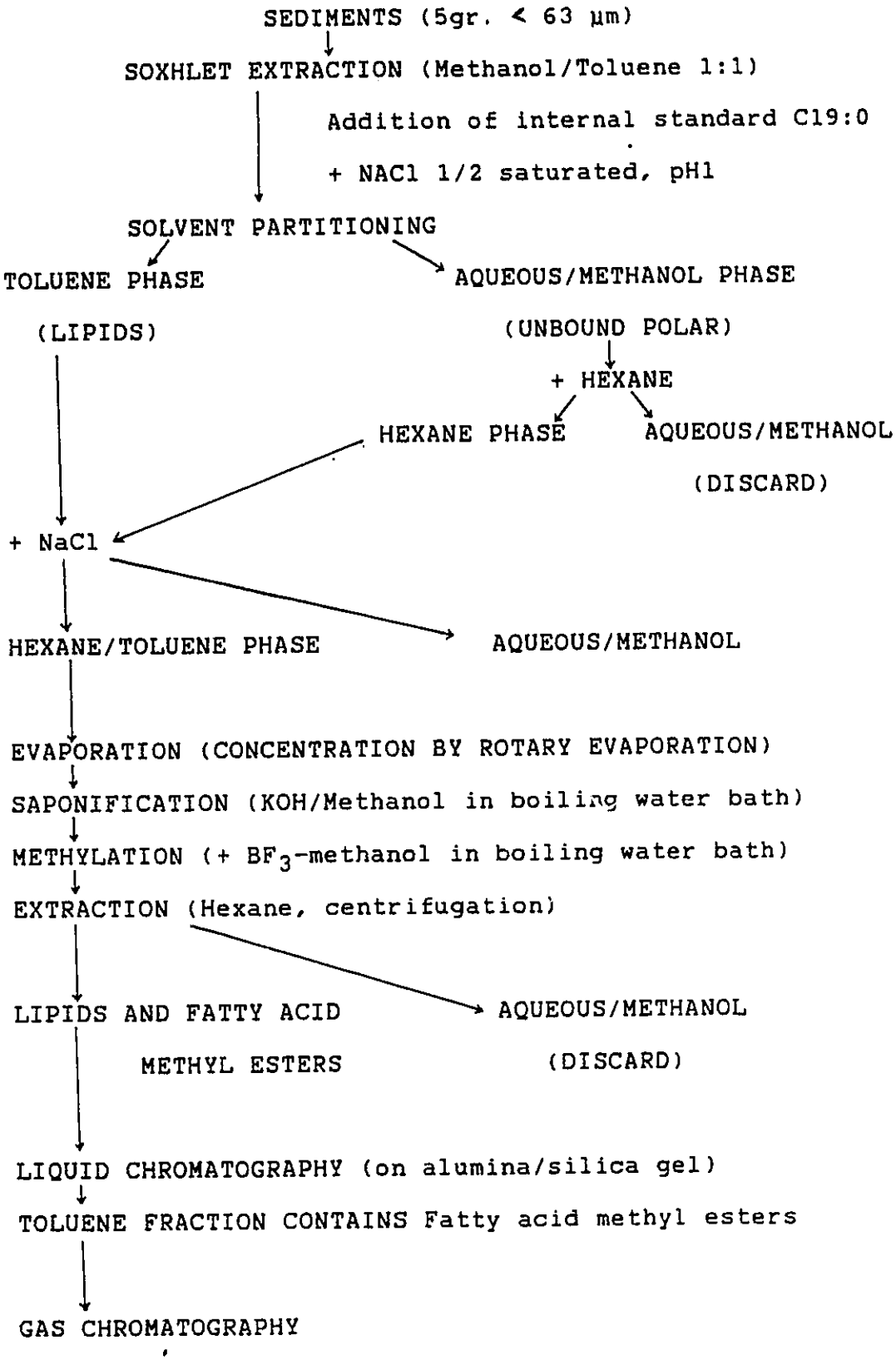
3.3. ANALYTICAL METHODS

3.3.1. FATTY ACID ANALYSIS:

Fatty acids were extracted from sediment and plant samples according to the method of Leenheer *et al.*, (1984), with slight modifications (figure 3.3). First, the unbound lipids in the sample (sediment, peat or plant) were extracted in a Soxhlet apparatus with 1:1 toluene:methanol for a total of 48 hours, changing solvent after 24 hours. Nonadecanoic acid (C19:0) was added as an internal standard to the extract. Following this, the extract was partitioned with the aid of half-saturated NaCl @ pH=1 to separate the unbound non-polar from the unbound polar fraction. Non-polar lipids remained in the organic (toluene) phase and the aqueous/methanol phase contained polar lipids. The aqueous/methanol phase was reextracted two times with hexane. These extracts were combined with the initial toluene phase and evaporated to near dryness by rotary evaporation.

The unbound non-polar fraction was dissolved with methanol:toluene and then saponified with 0.5 N KOH in

Figure 3.3. Flow-diagram representing the extraction of fatty acid methyl esters.


SEDIMENTS (5gr. < 63 µm)
SOXHLET EXTRACTION (Methanol/Toluene 1:1)
Addition of internal standard C19:0
+ NACl 1/2 saturated, pH1
SOLVENT PARTITIONING
TOLUENE PHASE
(LIPIDS)
AQUEOUS/METHANOL PHASE
(UNBOUND POLAR)
+ HEXANE
HEXANE PHASE
AQUEOUS/METHANOL
(DISCARD)
+ NaCl
HEXANE/TOLUENE PHASE
AQUEOUS/METHANOL
EVAPORATION (CONCENTRATION BY ROTARY EVAPORATION)
SAPONIFICATION (KOH/Methanol in boiling water bath)
METHYLATION (+ BF3-methanol in boiling water bath)
EXTRACTION (Hexane, centrifugation)
LIPIDS AND FATTY ACID
METHYL ESTERS
AQUEOUS/METHANOL
(DISCARD)
LIQUID CHROMATOGRAPHY (on alumina/silica gel)
TOLUENE FRACTION CONTAINS Fatty acid methyl esters
GAS CHROMATOGRAPHY

95% methanol and heating for 20 min in a boiling water bath. The saponification is followed by methylation of the fatty acids with BF_3-methanol (Metcalfe and Schmitz, 1961) and heating for 5 min to produce fatty acid methyl esters (FAME).

The non-polar lipids were partitioned into three fractions by chromatography in a column 9 mm in diameter, packed with 2 grams of 5% deactivated aluminium oxide (Al_2O_3) over 2 grams of 5% deactivated silica gel. The column was eluted with three solvents; first, a mixture of hexane/toluene (85/15) was used to elute hydrocarbons, next toluene (100%) eluted FAME and finally ethyl-acetate/toluene (1/1) eluted hydroxy-lipids.

Analyses of FAME were carried on a Hewlett-Packard 5890A gas-liquid chromatograph equipped with an on-column capillary inlet system, 30m x .32mm i.d. fused silica column (DB-1) and standard flame-ionization detector. Procedural blanks show minor amounts of contaminants, but nevertheless results are corrected for these blank values.

Fatty acid concentrations are corrected for blanks run at the same time as the samples. Using the areas from the GC output, if any amount of the fatty acids retained for this study (myristic, C14:0; palmitic, C16:0 and lignoceric, C24:0) is found in the blanks, it

is subtracted from the GC output of the samples previously measured, and normalized to the fatty acid standard (C19:0) peak area.

3.3.2. STABLE ISOTOPES ANALYSES:

3.3.2.1. Stable carbon and nitrogen isotope ratios of organic material.

Stable carbon and nitrogen isotope ratios of organic material are determined by converting the material into CO_2 and N_2 gas after the method of Macko (1981). Freeze-dried samples of organic material (POM, plankton, tissues, organic matrix and stomach contents of *Mytilus edulis)* and sediment (which were previously acidified with 1N HCl to remove any carbonates) together with cupric oxide (CuO) are sealed in precombusted Pyrex tubing (6mm and 9mm diameter) and evacuated. Three to five mg of organic material and 300 mg of sediments are needed for carbon isotope analyses and 10 to 15 mg of organic material and 400 to 800 mg of sediments are needed for nitrogen isotope ratios determination. The Pyrex tubes are heated to a temperature of 550°C for two hours. The resulting CO_2 and N_2 gas are purified (using a dry ice/ isopropyl alcohol bath and liquid nitrogen, respectively) before injection into the isotope ratio mass spectrometer (VG micromass 602D). The molecular sieve used by Macko (1981) for concentrating the N_2 gas

before mass spectrometry was omitted. Vycor tubing (96% quartz) had to be used for nitrogen isotope ratio analyses of sediments samples due to the weakness of Pyrex tubing (9mm) when submerged in liquid nitrogen. This change in tubing should not affect the results since Sofer (1980) found no difference using either Pyrex tubing or quartz for 2 different temperatures (550° and 900°C).

3.3.2.2. Stable hydrogen ratios

Samples for the determination of the stable hydrogen isotope ratio are prepared as follows. Samples of freezed-dried organic material are put into Pyrex tubing with CuO and evacuated while heated at a temperature of 100°C for at least 8 hours to remove any non-organically bound water. The tubes are then sealed and heated at 550°C. The cooked samples (which contain H_2O, CO_2 and N_2 gases) are then placed onto another vacuum line to isolate the organically bound water from the total sample. The tubing is cracked and the water isolated in a Pyrex tube placed into a dry-ice/isopropyl alchohol bath, which will freeze the water but neither CO_2 nor N_2. The tube containing the water is then sealed. The extracted water samples were then sent to Dr. C. Yonge (Physics department, Univ. of Calgary) for δD determination.

3.3.2.3. Oxygen isotope ratios of water

The water samples used for the $\delta^{18}O$ analyses (CR1, CR5) were provided by Dr. R. Bourbonniere (Canada Center for Inland Waters, Burlington) and were analysed by Martin Knyf. The $\delta^{18}O$ values were determined on CO_2 equilibrated with 2 ml of water sample at 25°C. The samples were prepared according to the following method. The sample of water is put into a vial and acidified with concentrated H_2SO_4, which will accelerate the equilibration process. The sample is then sealed under vacuum. Next, the vial is put into a dry ice/ isopropyl alchohol bath to freeze the water and then evacuated of any CO_2 from the water. The sample is thawed and the previous step repeated. Next, a known volume of CO_2 is added to the water sample and the sytem is equilibrated under vacuum in a water bath for 48 h at 25°C. The sample is analysed by freezing the water and expanding the CO_2 gas into the mass spectrometer. The values are reported relative to the SMOW standard.

3.3.2.4. Carbonates

The CO_2 samples were prepared following a modified version of McCrea's 1950 method.. The sample of calcite or aragonite was reacted with 100% phosphoric acid (H_3PO_4) at 47.5°C under vacuum for about 20 minutes or until all calcite had reacted with the acid. The evolved CO_2 is then cleaned of any contaminant gases,

using fractional freezing, and collected in a cold finger. After collection, the CO_2 is introduced into the mass spectrometer for comparison with CO_2 from a standard calcite (GCS) by reacting it with H_3PO_4 at the same temperature at which the gas sample was evolved. The isotopic values wer converted to $\delta^{13}C$and $\delta^{18}O$ values relative PDB for both carbon and oxygen.

3.3.2.5. Stable isotope ratio expression; precision of analyses.

Stable isotope ratios are expressed as:

$$\delta^*X = \frac{R(sample) - R(standard)}{R(standard)} \times 1000,$$

in ‰, where R=*X/X and *X is the heavy isotope (^{13}C, ^{15}N, ^{18}O and D) with respect to the standards PDB, atmospheric air and SMOW respectively.

Differences between duplicate $\delta^{13}C$(organic) analyses range from 0.05 to 0.53‰, giving an average replication error of 0.22‰. Errors between replicates for δD , $\delta^{15}N$ (organic), $\delta^{13}C$ (calcite) and $\delta^{18}O$ (calcite) determination were respectively 3‰, 0.45‰, 0.09‰ and 0.16‰.

3.3.3. TOTAL ORGANIC CARBON

Percentages of total organic carbon (TOC) in the sediments were determined using a LECO Carbon Determinator. Preweighed samples of the sediments were

acidified with concentrated sulfurous acid, dried, and combusted. The CO_2 evolved was determined by an infrared detector.

3.3.4. SALINITY AND TEMPERATURE

The salinity and temperature profile at low and high tide were taken along a down estuary trend at different sites (figure 2.2) using a Salinity-Conductivity-Temperature-meter (YSI brand instruments). The SCT-meter was calibrated with freshwater, in the laboratory before leaving for the field. The highest salinity recorded was 28‰, which still indicates a freshwater influence at the end of the estuary (NH11).

CHAPTER 4. NEGRO HARBOUR SEDIMENT ANALYSIS.

Particulate organic matter (POM) in the estuary is assumed to be derived from a mixture of two end-members: a terrestrial source composed mainly of peat and higher plant detritus and a marine source derived from plankton and higher marine plant detritus.

4.1. THEORETICAL REVIEW

4.1.1. CARBON ISOTOPE RATIOS IN SEDIMENTS

In spite of some isotopic intra-group variation, organic matter in marine plants, autotrophic bacteria, and peats can often be separated by $\delta^{13}C$ values into fairly distinct groups (Table 4.1). These divisions into groups are important for constructing mixing models and for testing the importance of a specific source, by sampling along a gradient towards that source (Schultz and Calder, 1976).

One of the most important processes affecting changes in carbon isotopic composition in the geochemical cycle is the absorption of carbon from the carbon dioxide reservoir of the atmosphere and surface waters by photosynthetic fixation in the form of complex organic molecules. In general, such carbon shows a high degree of enrichment in the light isotope compared to its source.

Table 4.1. General range of $\delta^{13}C$ values of carbon sources in coastal ecosystems.

SOURCE	Usual $\delta^{13}C$ range (‰)
Terrestrial C_3 plants	-23 to -30
Terrestrial C_4 plants	-10 to -14
River seston (POC)	-25 to -27
Peat deposits	-12 to -28
C_3 marsh plants	-23 to -26
C_4 marsh grasses	-12 to -14
Seagrasses	-3 to -15
Macroalgae	-8 to -27
Benthic unicellular algae	-10 to -20
Temperate marine phytoplankton	-18 to -24
River-estuarine phytoplankton	-24 to -30
Autotrophic sulfur bacteria	-20 to -38
Methane-oxidizing bacteria	-62

From Fry and Sherr (1984)

Carbon fixation in photosynthesis may proceed by two main pathways which differ with respect to the number of carbon atoms in the first formed intermediate compounds. The two pathways are known as the Calvin-Benson (C_3) and the Hatch-Slack (C_4) cycle respectively. $\delta^{13}C$ values for C_3 plants fall around -26‰ and for C_4 plants around -13‰ (Smith and Epstein, 1971). The isotopic composition of soil CO_2 will generally be determined by that produced by decay and respiration. As decomposed wood, peat, and lignite have approximately the same $\delta^{13}C$ values as fresh wood (Craig, 1953), and respired CO_2 is isotopically similar to the plant carbon in C_3 and C_4 plants (Park and Epstein, 1960, 1961; Smith, 1971), one can expect that soil CO_2 from these sources has carbon isotopic compositions close to that of the local plant cover.

While the difference in stable carbon composition between C_3-dominated terrestrial plant material ($\delta^{13}C$ of ~ -26‰) and organic matter of marine origin (i.e. phytoplankton photosynthesis, $\delta^{13}C$ of -21‰) is fairly small (only 5‰), analysis of samples collected along riverine-offshore transects reveals very consistent and similar patterns of isotopic change from terrestrial to marine values (Hedges and Parker, 1976; Shultz and Calder, 1976; Tan and Strain, 1979). In these studies the transition to marine values of -21‰ typically occurs

fairly sharply near river mouths. Transect studies of sediments and POC often show clear-cut patterns of isotopic change that can be directly related to the proximity of various carbon sources (Fry and Sherr, 1984), isotopic changes during plant decomposition being small ($\leq$ 2‰).

Material produced in surficial water sinks upon death and undergoes decay. Diagenetic processes can affect the isotopic composition of the residual matter. These processes include loss of biochemical fractions, hydrolysis of terminal groups from the chemical structure, or other bacterial alterations of the structure (Macko and Estep, 1983).

Relatively little is known about the isotope fractionations associated with microorganims that require organic substances for their growth. However it seems that heterotrophic bacteria can change to a considerable extent the isotopic composition of organic matter. The $\delta^{13}C$ and the $\delta^{15}N$ of microorganisms are regulated by incorporation by the cell of the substrate, into biosynthetic pathways, the C/N of the substrate, and nitrogen loss of ammonia during growth and respiration. Most studies on $\delta^{13}C$ determination of bacterial growth have been made in culture on pure compounds. In natural environments with species substrate diversity, these large isotope fractionations could cancel each other and

not be observed (Macko and Estep, 1983).

4.1.2. NITROGEN ISOTOPE RATIOS IN SEDIMENTS

More than 99% of the known nitrogen on or near the Earth's surface is present as N_2, either in the atmosphere or dissolved in sea water. Only a minor amount is combined with other elements, mainly C, H, and O.

Fixation into a utilizable form is performed by microorganisms in the soil and blue-green algae (cyanobacteria) in water. Considering the isotopic fractionation for nitrogen fixation (α = 1.000, Hoering and Ford, 1960), newly formed compounds in the soil should have a $\delta^{15}N$ close to the atmospheric $\delta^{15}N$ (0.0‰). In soils, ammonium ion, the dominant form of inorganic nitrogen, is mobilized as nitrate, and can be utilized by vascular plants. The isotopic ratio of non-hydrolyzable nitrogen (-3‰ to +1‰) is similar to the air input, whereas the hydrolyzable forms of nitrogen may be quite different (Cheng *et al.*, 1964). So, although soil nitrogen may have a large range in $\delta^{15}N$ values, the residual organic matter of vascular plant origin has an isotopic composition corresponding nearly to that of atmospheric nitrogen (i.e. 0.0‰). This more refractory non-hydrolyzable nitrogen would be expected to be preferentially retained during transport by rivers to

lakes or the ocean. Study suggests that organic nitrogen compounds contributed to coastal waters by rivers are stable during the period (2-3 months) of their transfer over the continental shelf and that nitrogen is associated with refractory organic compounds (Gardner and Stevens, 1978).

Under normal oceanic conditions nitrate is the most stable, and thus the most common form of combined nitrogen in the water. In sediments and interstitial waters, N is primarily in the form of organic N, although significant levels of ammonia are present (Macko, 1981).

The major biological processes affecting the chemical nature of nitrogen in the marine environment include fixation, synthesis, decomposition, nitrification, assimilation and dissimilation.

In boreal, temperate waters, where nitrate is the main nitrogen source, the $\delta^{15}N$ value of plankton is regulated by the kinetic isotope effect in the process of nitrate assimilation. In temperate regions (e.g. Nova Scotia) nitrate assimilation ought to be the dominant source of nitrogen utilized by organisms. Nitrate is transported from deeper layers to the euphotic layer by vertical water mixing, which occurs most prominently at higher latitudes and in upwelling regions. It appears that little isotope fractionation occurs during nitrate assimilation (Wada and Hattori, 1976). In this

assimilation process nitrate (NO_3^-) is first reduced to nitrite ($NO_2^=$), then to ammonia (NH_4^+), and is finally converted into amino acids. Miyake and Wada (1967) have shown, for the Northwest Pacific Ocean that the average $\delta^{15}N$ value of nitrate, phytoplankton, and seaweeds is +7‰ on the average, and those in zooplankton and fishes is +10‰ and +15‰ respectively .

4.1.3. FATTY ACIDS

Lipids are generally more stable than carbohydrates and proteins and are sometimes found associated with humic materials and clay minerals. Lipid components have been studied in many recent sedimentary environments and can be related to their sources (Brooks *et al.*,1976; Volkman *et al.*, 1980,1981) . Sterols have proven to be particularly useful source indicators and have been studied in sediments from estuarine environments using specific terrestrial and marine biomarkers (Huang and Meinschein, 1976). In a study of lipid distribution in recent sediments , Brooks *et al.*, (1976) found that carbon chain length distributions of both hydrocarbons and fatty acids were, in most cases, similar to those typically found in organisms. Also, bimodal distributions of hydrocarbons and fatty acids were observed in almost all of their samples. The presence of higher carbon chain components

(>C20) was taken to indicate higher plant contribution, while lower carbon chains (<C20) indicate autochthonous contribution (algae and/or bacteria). The variation in proportions of the two groups was related to the difference in sedimentary environments. Although variations in types and amounts of fatty acids can occur at different locations in an estuary, there appear to be no temporal variations at one location over a one-year period (Farrington and Quinn, 1973). Cranwell (1974) has shown that autochthonous material is the main source of the C12-C18 n-alkanoic acids while allochthonous terrestrial sources provide the C22-C28 n-alkanoic acids. Aquatic plant sources (plankton and macroalgae) are not so easily distinguished because many compounds present in aquatic organisms also occur in land plants.

In many studies, identification of specific polyunsaturated acids was shown to be particularly useful for source assignment (Volkman *et al.* , 1980). However quantitative assessment of relative source contributions is complicated by the rapid degradation of such biomarkers (Johns *et al.*, 1978). Leenheer *et al.*, (1984) used the ratio of saturated C16 over C24 fatty acid to determine the relative contribution of allochthonous (terrestrial) vs autochthonous (algae/bacteria) sources. They found an increase in the

C16/C24 saturated fatty acids ratio in surficial sediments with increasing distance from the the source of terrestrial material i.e. from lakes to river to coastal waters.

Although lipids represent only a small portion of the biologically produced organic matter in seawater and sediments, lipids (e.g. hydrocarbons, fatty acids, fatty alcohols, steroids, wax esters, etc.) are key biochemicals in marine organisms; from the point of view of energy storage and regulation, membrane structural components, and hormonal regulation of stimulation, reproduction, and metabolic processes. It is the labile organic compounds like lipids, rather than the more resistant organic matter, which take part in the nutritional and chemical communication processes of marine organisms. Since these compounds play such a critical role in the life cycle of marine organisms, and many of them are stable for long periods of time, they serve as excellent markers of the biota living on and above the sea floor.

Geolipids are subject to alterations over geologically long time spans. In addition, some types of lipids are altered more than others (Farrington and Meyers, 1976; Barnes and Barnes, 1978). Fatty acids in particular seem to be relatively reactive in modern sedimentary environments (Rodier and Khalil, 1982).

Total fatty acid levels in sediments decrease with depth, and rate of decrease is greater for unsaturated than for saturated acids (Farrington and Quinn, 1971). It is also possible that fatty acids and hydrocarbons, which comprise only a few percent of the total organic carbon, may not always be dependable indicators of the sources of all the organic constituents in subaqueous sediments since the rate of alteration seems to be slower for terrestrial material (Meyers *et al.*, 1980). Although variations in types and amounts of fatty acid can occur at different locations in an estuary, there appear to be no temporal variations at one location over a one-year period (Farrington and Quinn, 1973).

In this study, saturated C24, C16 and C14 fatty acids are discussed. C24 (lignoceric acid) is commonly considered to originate in higher plant matter (Volkman *et al.*, 1980). It is totally absent from marine phytoplankton. Palmitic acid (C16), also found in higher plants, is the most common saturated fatty acid in marine organisms . Phytoplankton often accumulate appreciable amounts of myristic acid (C14) together with polyunsaturated acids of the C16, C18, and C20 series (Chuecas and Riley, 1969; Lewis, 1969). It should therefore be possible to recognize the terrestrial component in the sediment from the ratio of C24 to other fatty acids (C16 and C14).

4.2. SOURCES OF ORGANIC MATTER IN NEGRO HARBOUR

$\delta^{13}C$ and fatty acid analyses of the sources of organic matter and their relative percentages in the sediments of Negro Harbour were the subject of a recent paper (LeBlanc *et al.*, 1989). The major points of this paper are reviewed here.

4.2.1. CARBON ISOTOPE RATIOS

Terrestrial and marine sources in Negro Harbour have more than one contributor. Peat and higher plants both contribute to a terrestrial signal. Plankton, marine seagrasses (*Zostera* and *Spartina*) and macrophytes (*Fucus, Laminaria*) are possible sources of marine origin (table 4.2). Peat is slightly enriched (~3‰) in ^{13}C compared with POM of terrestrial origin from the river (table 4.3). POM in the river is assumed to result from equal mixtures of peat and higher plant material with an average $\delta^{13}C$ of -29.0 ± 0.4‰. This value will be used to represent the terrestrial end-member. Some maple leaves (in a state of degradation) found at the bottom of station NH0 had a $\delta^{13}C$ of -26.8‰.

Marine organic matter is assumed to be derived mainly from plankton with an average value of -21.2‰ ± 0.4‰. This value compares very well with other studies (table 4.4). Other marine plants are present but rare in the estuary. $\delta^{13}C$ values of POM indicate a stronger

Table 4.2. $\delta^{13}C$ (‰) of terrestrial and marine sources of organic matter.

Terrestrial	
Peat (0-50cm):	-25.9
Peat (100-150):	-26.0
Maple leaves (NHO):	-26.9, -26.7
Marine	
Plankton:	-20.8, -21.0, -21.7
Spartina alterniflora	-12.3, -12.2, -13.6
Zostera marina	-14.9
Fucus vesiculosus	-17.1
Laminaria	-14.9

Table 4.3. $\delta^{13}C$ (‰) of POM in the Clyde River for 1984 and 1985.

Station	1984	1985
JC4	_	-27.3
CR1	-28.5	-28.9
CR2	_	-29.5
CR3	_	-29.5, -29.3
CR4	_	-28.5
CR5 low tide	-24.2	-26.8
high tide	_	-25.2

Table 4.4. $\delta^{13}C$ values of plankton and other material of temperate areas. Number of replicates in brackets

$\delta^{13}C$ (‰)	Reference	Area
-21.3 ± 1.1 (56)	Peterson *et al.* (1985)	Massachussets
-21.3 ± 1.1 (57)	Gearing *et al.* (1984)	Narraganset Bay
-21.2*	Incze *et al.* (1982)	Maine
-19.2 ± 0.5	Schwinghammer *et al.* (1983)	Nova Scotia
-23.8 ± 1.8	Tan and Strain (1983)	Gulf of St-Lawrence
-21.2**	Macko (1981)	Chesapeake

* POC of marine sediments

** Offshore sediments

influence of the river (organic matter of terrestrial origin) in 1985 than in 1984. At site CR5 (figure 4.1), at the head of Negro Harbour, POM values varied with the tide, varying from a value at low tide of -26.8‰ to a high tide value of -25.2‰ in 1985. In 1984, $\delta^{13}C$ at CR5 at low tide was -24.2‰ an enrichment of 2.6‰ compared to 1985. This agrees with other evidence for a stronger influence of the river in the summer of 1985 as inferred from salinity records.

$\delta^{13}C$ in the sediments of Negro Harbour increased from -25.2 to -20.6‰ down estuary in 1984 and from -24.4 to -21.0‰ in 1985 (table 4.5). The two curves follow the same trend (figure 4.1) but for the first few km into the estuary the $\delta^{13}C$ indicates lesser terrestrial input to surficial sediments in 1985, which is in contradiction with the conclusion from salinity profiles that terrestrial input was more important in 1985 (figure 2.2).

4.2.2. FATTY ACID RATIOS

Fatty acid ratio analyses provide a different source of information on the nature of organic matter sources delivered into the estuary.

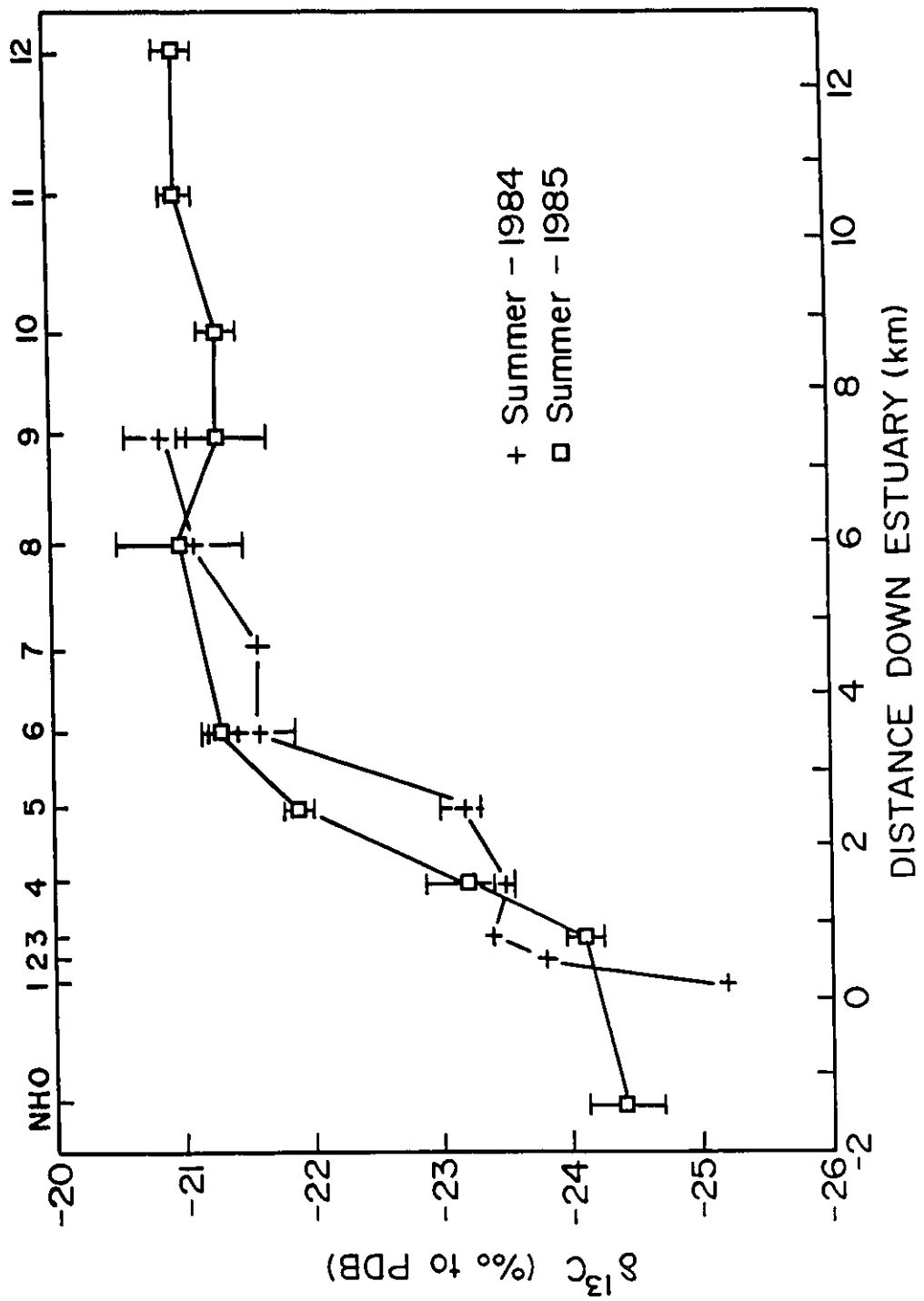
The concentration of C14, C16 and C24 in various plants sampled in Barrington Bog varies significantly, depending on the type of plant analysed (table 4.6).

Table 4.5. $\delta^{13}C$ (‰) of organic matter in sediments of Negro Harbour for 1984 and 1985.

Distance*	Station	1984	1985
-1.4	NH0		-24.1,-24.4,-24.7
0.2	NH1	-25.2	
0.5	NH2	-23.8	
0.8	NH3	-23.4	-24.0,-24.0,-24.3
1.5	NH4	-23.5,-23.6	-23.2,-22.7,-23.6
2.5	NH5	-23.0,-23.3	-21.8,-21.9
3.5	NH6	-21.9,-21.2	-21.1,-21.2,-21.5
4.6	NH7	-21.6	
6.0	NH8	-21.1	-20.8,-20.4,-21.7
7.4	NH9	-20.6,-21.1	-20.8,-21.5,-21.5
8.8	NH10		-21.5,-21.2
10.6	NH11		-20.9,-21.1
12.5	NH12		-21.2,-20.9

* Distance down estuary in km, measured from station CR5.

Figure 4.1. $\delta^{13}C$ of the sediments of Negro Harbour for 1984 and 1985.


+ Summer – 1984
□ Summer – 1985
DISTANCE DOWN ESTUARY (km)
δ13C (‰ to PDB)
NHO
-20
-21
-22
-23
-24
-25
-26
-2
0
2
4
6
8
10
12

Overall, C14 and C16 were respectively the least and most abundant in a particular plant. Within a single genus (*e.g. Sphagnum*), the abundances are very regular as are the ratios. Higher plants like maple have a relatively high concentration of C16 and to a certain extent, C14 but not C24, giving them lower C24/C14 and C24/C16 ratios.

Higher marine plants showed C24/C14 ratios quite similar to higher terrestrial material (~2.00) but their C24/C16 ratio is lower (~0.10). The plankton sample shows a very high concentration of myristic acid (C14) and C16. There was no C24 in the sample so therefore we assumed that this plankton sample was free of terrestrial material. In other words, an entirely marine sample will have both C24/C14 and C24/C16 ratios of 0. Leenheer et al. (1986) used the C16/C24 ratio in their study, but since C24 is absent in plankton, it made more sense to have a ratio of a marine signal tending toward zero than infinity.

The sediments were sampled in May of 1984 to test the fatty acid ratios as tracers of these various sources. Triplicate samples were obtained from 5 sites , NH3, NH4, NH7 NH8 and NH10 to verify the variations between samples . Unfortunately the samples were analysed without blanks so a correction cannot be applied to these samples. However, since the corrected values calculated

Table 4.6. Fatty acid concentration (ug/g) and ratios in various plants from Negro Harbour and Barrington bog area .

PLANTS	C14	C16	C24	C24/C16	C24/C14
Terrestrial:					
Sphagnum rubellum	35	506	345	0.68	9.84
Sphagnum fuscum	21	426	267	0.63	12.91
Sphagnum magellanicum	48	676	281	0.41	5.86
Sphagnum sp1	73	767	386	0.50	5.30
Sphagnum sp2	65	438	319	0.73	4.91
Peat (0-10 cm)	44	211	411	1.94	9.40
Peat (10-20 cm)	40	155	559	3.59	14.13
Peat (90-100 cm)	4	185	769	4.15	16.29
Maple leaves (fresh)	150	1737	298	0.17	1.98
Maple leaves (@ NH0)	210	1041	504	0.48	2.40
Marine:					
Zostera marina	52	905	133	0.15	2.56
Spartina alterniflora	74	2596	118	0.05	1.61
Plankton	558	1149	0	0	0

from the summer of 1984 and 1985 are but slightly different (on the average ~ 10%) from the uncorrected values (table 4.7). The magnitude of the blank correction of the FAME ratio varies between 0-29.3 %, 0.6-15.5%, 4.1-21.9% and 0-10.4% for the analyses of 1984 values of C24/C16, and C24/C14, and 1985 values of C24/C16 and C24/C14 respectively. The blank corrections are smaller in general with C24/C14 ratios, which is to be expected since the ratio values are usually higher.

The values of the preliminary study, for samples from the same site are very similar to each other, which indicates that one analysis can be taken as being representative of the environment. These Spring, 1984 values do not differ from the samples obtained 3 months later (August 1984) (table 4.8). The ending of Spring run-off can explain this resemblance in these values. The only difference is at NH3 where the values are higher in the summer.

The content of lipids in oceanic sediment is generally correlated with total organic matter (Romankevich, 1984). This was confirmed here by the data from 1985, showing that the concentration of C16 and C24 fatty acids in Negro Harbour sediment is correlated with total organic carbon (TOC) (Table 4.9 and Figure 4.2). In the sediment, the concentration of C14 was found to be very low relative to C16 and C24. Data collected

Table 4.7a. Fatty acid ratio (corrected, uncorrected, % difference) for the 1984 sediment samples.

Site	C24/C16			C24/C14		
	Cor	Uncor	% diff	Cor	Uncor	% diff
NH1	0.37	0.36	2.7	1.71	1.70	0.6
NH2	0.51	0.39	23.5	2.19	1.85	15.5
	0.69	0.52	24.6	2.54	2.16	15.0
NH3	1.23	1.16	5.7	5.62	5.41	3.7
NH4	0.63	0.57	7.9	2.73	2.60	4.8
NH5	0.49	0.46	6.1	2.01	1.97	2.0
NH6	0.52	0.47	9.6	2.06	1.98	3.9
NH7	0.72	0.56	22.2	1.99	1.80	9.5
	0.75	0.53	29.3	2.27	1.92	15.4
NH8	0.46	0.43	6.5	1.55	1.53	1.3
NH9	0.25	0.25	0.0	0.75	0.76	1.3
			$\bar{X}$= 12.6 %			$\bar{X}$= 6.6 %

Table 4.7b. Fatty acid ratio (corrected, uncorrected, % difference) for the 1985 sediment samples.

Site	C24/C16			C24/C14		
	Cor	Uncor	% Diff	Cor	Uncor	% Diff
NH0	0.41	0.37	9.8	1.74	1.74	0.0
	0.60	0.54	10.0	3.47	3.20	7.8
	0.54	0.48	11.1	2.92	2.66	7.5
NH3	0.79	0.70	11.4	3.02	3.00	0.7
	0.82	0.71	13.4	2.09	1.98	5.3
	0.86	0.76	11.6	3.66	3.38	7.7
NH4	0.58	0.53	8.6	2.12	2.16	1.9
	0.62	0.56	9.7	2.66	2.51	5.6
NH5	0.49	0.47	4.1	1.95	1.90	2.6
NH6	0.84	0.77	8.3	3.22	3.22	0.0
NH8	0.60	0.52	11.9	2.34	2.34	0.0
NH9	0.41	0.32	21.9	1.65	1.65	0.0
	0.34	0.29	14.7	1.63	1.46	10.4
NH10	0.15	0.10	33.3	0.79	0.79	0.0
NH11	0.06	0.05	16.7	0.39	0 36	7.7
NH12	0.06	05	16.7	0.34	0.34	0.0
			$\bar{X}$= 13.3 %			$\bar{X}$= 3.6 %

Table 4.8. Spring (1984) C24/C16 uncorrected values.

Site	C24/C16 (µg/g)
NH3	0.58, 0.52, 0.53
NH4	0.61, 0.58, 0.65
NH7	0.66, 0.58, 0.57
NH8	0.41, 0.45, 0.48
NH10	0.08, 0.06, 0.07

Table 4.9. Fatty acid concentrations (ug/g) and % TOC for 1984 and 1985.Average in brackets.

Station	C24(ug/g)		C16(ug/g)		C14(ug/g)		% TOC	
	1984	1985	1984	1985	1984	1985	1984	1985
NH0	-	10.7	-	25.7	-	6.1	-	1.59
		10.5		19.6		3.8		
		13.1		21.7		3.6		
		(11.4)		(22.5)		(4.5)		
NH1	12.2	-	33.1	-	7.1	-	0.25	-
							0.31	
							(0.28)	
NH2	10.3	-	19.0	-	4.7	-	1.45	-
	13.1		20.1		5.1		1.63	
	(11.7)		(20.0)		(4.9)		1.51	
							(1.53)	
NH3	20.0	19.8	16.3	24.9	3.6	6.6	2.39	1.91
		15.5		18.9		4.7		
		17.2		20.0		7.4		
		(17.5)		(21.3)		(6.2)		
NH4	6.7	20.9	10.5	36.2	2.4	9.9	1.91	2.56
		18.7		30.4		7.0	2.12	
		(19.8)		(33.3)		(8.4)	(2.01)	

Table 4.9. Continued

NH5	9.0	32.6	18.5	66.7	4.5	16.8	4.87 3.50 (4.18)	4.58
NH6	5.1	32.0	9.9	38.3	2.5	9.9	2.63 2.59 (2.61)	4.65
NH7	10.9	-	14.4	-	4.8	-	-	-
NH8	7.2	15.8	15.7	26.9	4.7	6.8	2.96	2.79
NH9	6.0	5.4 5.5	23.9	13.3 16.0 (14.6)	8.0	3.3 4.0 (3.6)	1.57	1.28
NH10	-	1.1	-	7.5	-	1.4	-	0.26
NH11	-	1.2	-	21.7	-	3.0	-	0.45
NH12	-	0.9	-	-	-	2.6	-	0.17

Table 4.10. Fatty acid ratios in the sediments of Negro Harbour. Average in brackets.

Station	C24/C16		C24/C14	
	1984	1985	1984	1985
NH0	-	0.60	-	3.47
	-	0.53	-	2.92
	-	(0.57)	-	(3.19)
NH1	0.37	-	1.71	-
NH2	0.51	-	2.19	-
	0.69	-	2.54	-
	(0.60)	-	(2.36)	-
NH3	1.23	0.79	5.62	3.02
		0.86		3.66
		(0.83)		(3.34)
NH4	0.63	0.58	2.73	2.12
		0.61		2.66
		(0.60)		(2.39)
NH5	0.48	0.49	2.01	1.95
NH6	0.52	0.83	2.06	3.22
NH7	0.72	-	-	-
NH8	0.46	0.50	1.55	2.34

Table 4.10. Continued

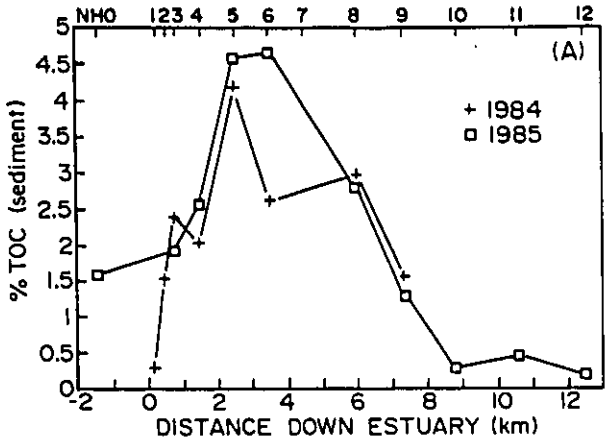
NH9	0.25	0.41	0.75	1.65
		0.34		1.63
		(0.37)		(1.64)
NH10	-	0.15	-	0.79
NH11	-	0.06	-	0.39
NH12	-	0.06	-	0.34

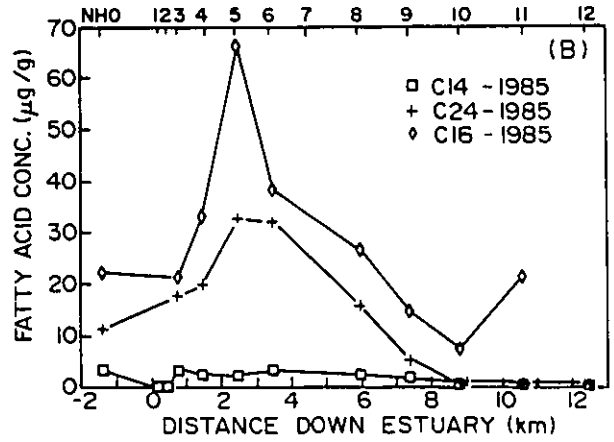
Figure 4.2. Fatty acid concentration and % TOC in the sediments of Negro Harbour for 1984 and 1985. Curves plotted with averaged values.

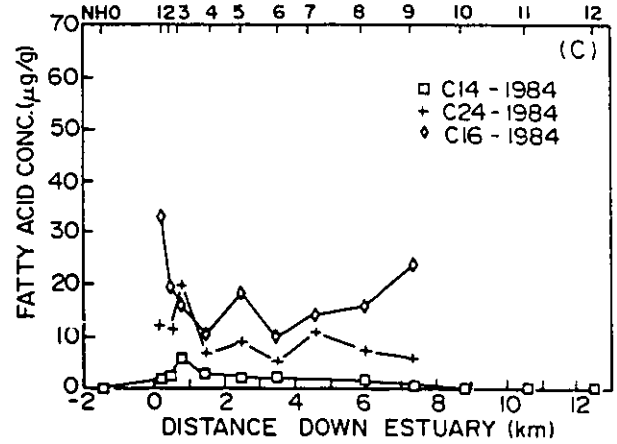
a) % TOC down estuary for 1984 and 1985

b) Fatty acid concentration down estuary for 1985

c) Fatty acid concentration down estuary for 1984


NHO 123 4 5 6 7 8 9 10 11 12
(A)
+ 1984
□ 1985
% TOC (sediment)
DISTANCE DOWN ESTUARY (km)


NHO 123 4 5 6 7 8 9 10 11 12
(B)
□ C14 - 1985
+ C24 - 1985
◊ C16 - 1985
FATTY ACID CONC. (µg/g)
DISTANCE DOWN ESTUARY (km)


NHO 123 4 5 6 7 8 9 10 11 12
(C)
□ C14 - 1984
+ C24 - 1984
◊ C16 - 1984
FATTY ACID CONC.(µg/g)
DISTANCE DOWN ESTUARY (km)

in 1985 (Figure 4.2b), show that the peak values for fatty acid concentrations corresponded to those for the TOC. This fatty acid-TOC correlation was not found when studying the 1984 data (figure 4.3c). At all sites, fatty acid concentrations were higher in 1985, whereas TOC was not found to be uniformly higher.

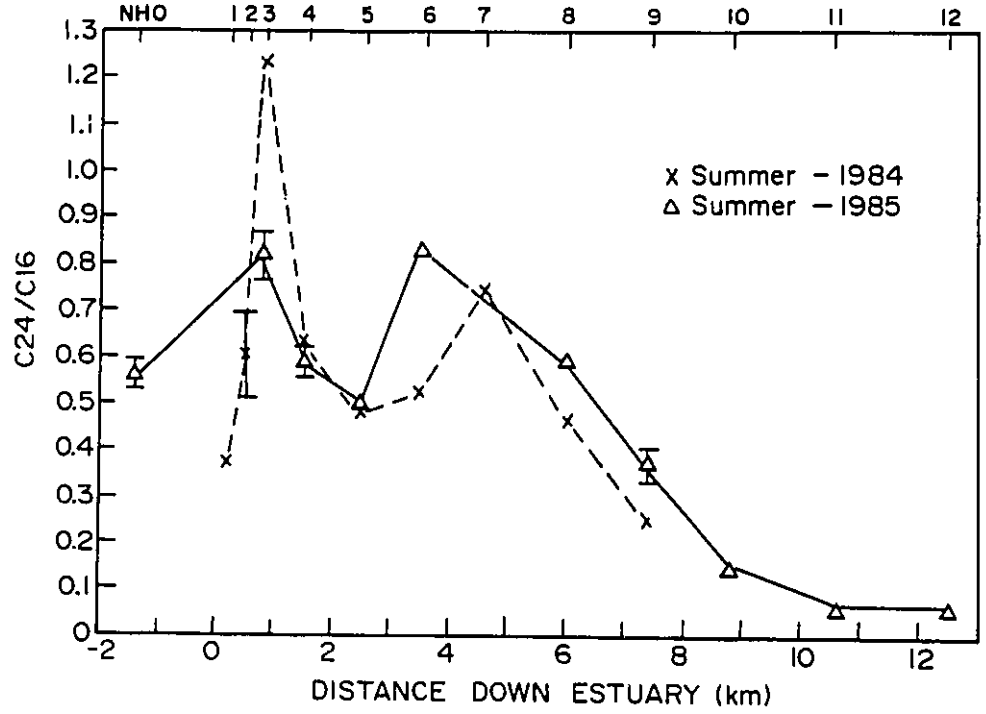
Graphs of fatty acid ratios give a rather different pattern than do the isotope curves of the relative contributions of marine and terrestrial organic matter. At all sites fatty acid concentration was higher in 1985 and fatty acid ratio in the sediments showed a complexity that was not present with $\delta^{13}C$ (figure 4.3, figure 4.1, table 4.10). Fatty acid ratio decreases toward zero down estuary as expected. Theses ratios agree with the fact that 1985 was more terrestrially influenced than 1984. There seems to be a larger influence of peat material in 1985, which could explain the $\delta^{13}C$ results in sediments, since peat is enriched in ^{13}C compared to higher terrestrial plants.

4.2.3. NITROGEN ISOTOPES

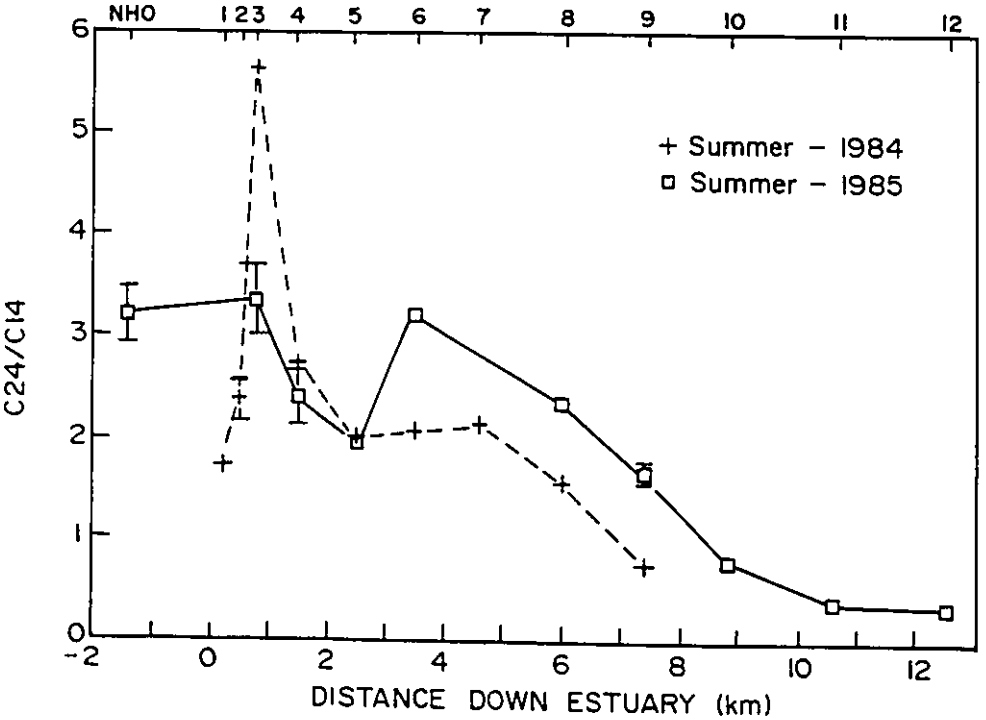
4.2.3.1. Organic inputs

First, the sources of organic matter to the estuary measured through nitrogen isotopes are discussed. Plankton $\delta^{15}N$ values are similar for a plankton sample from 1985 (+5.6‰) and 1987 (+5.9‰). Table 4.11 compare

Figure 4.3 Fatty acid ratio in the sediments of Negro Harbour for a) C24/C16 and b) C24/C14.


NHO 1 2 3 4 5 6 7 8 9 10 11 12
C24/C16
x Summer – 1984
Δ Summer – 1985
DISTANCE DOWN ESTUARY (km)

A


NHO 1 2 3 4 5 6 7 8 9 10 11 12
C24/C14
+ Summer – 1984
□ Summer – 1985
DISTANCE DOWN ESTUARY (km)

B

Table 4.11. $\delta^{15}N$ values of plankton.

$\delta^{15}N$ (‰)	Reference
6.2	Sealy *et al.* (1987)
5.6 ± 1.7	Minagawa and Wada (1984)
6.2*	Macko (1981)
4.2	Wada and Hattori (1976)
8.6 ± 1.0	Peterson *et al.* (1985)
8.0 ± 1.8	Mariotti *et al.* (1984)
8.9	Sweeney and Kaplan (1984)

* Offshore sediments

these two values with $\delta^{15}N$ values of plankton from other studies. The $\delta^{15}N$ values of the present study are well within the expected values for plankton. Marine seagrasses and macrophytes also have $\delta^{15}N$ values which compare well with other studies (table 4.12).

No $\delta^{15}N$ values for POM could be obtained from Clyde River due probably to the very low concentration of nitrogen in these plants. For example Lévesque *et al.* (1980) found that the C/N ratio in peat samples from Quebec can be as high as 99.2 (low of 15.4) so that terrestrial nitrogen is not expected to be of importance in the Negro Harbour ecosystem. $\delta^{15}N$ values for terrestrial component is assumed to be close to the atmospheric value of 0‰, since not enough N_2 gas was obtained through the laboratory procedure to procede with the mass spectrometric analysis.

4.2.3.2 $\delta^{15}N$ results from sediments

The $\delta^{15}N$ values of sediments are about the same for both years (figure 4.4). $\delta^{15}N$ values range between 2.8‰ and 6.0‰ (table 4.13). NH9 is the most enriched station (6.0‰) and corresponds to a planktonic origin of organic matter. The only offset occurs between NH5 and NH6 where there is a peak in 1985 and 1984, respectively. The curves of $\delta^{15}N$ in the sediments down the estuary do

Table 4.12. $\delta^{15}N$ in marine seagrasses

PLANT	$\delta^{15}N$ (‰)	REFERENCE
Zostera marina	4.2	This study
Laminaria sp.	5.7	This study
Spartina alterniflora	6.0 ± 2.1	Peterson and Howarth (1987)
Laminaria sp.	3.2	Sealy *et al.* (1987)
Marine algae	5.2-9.7 (x = 7.5)	Miyake and Wada (1967)

Figure 4.4. $\delta^{15}N$ in the sediments of Negro Harbour for 1984 and 1985.

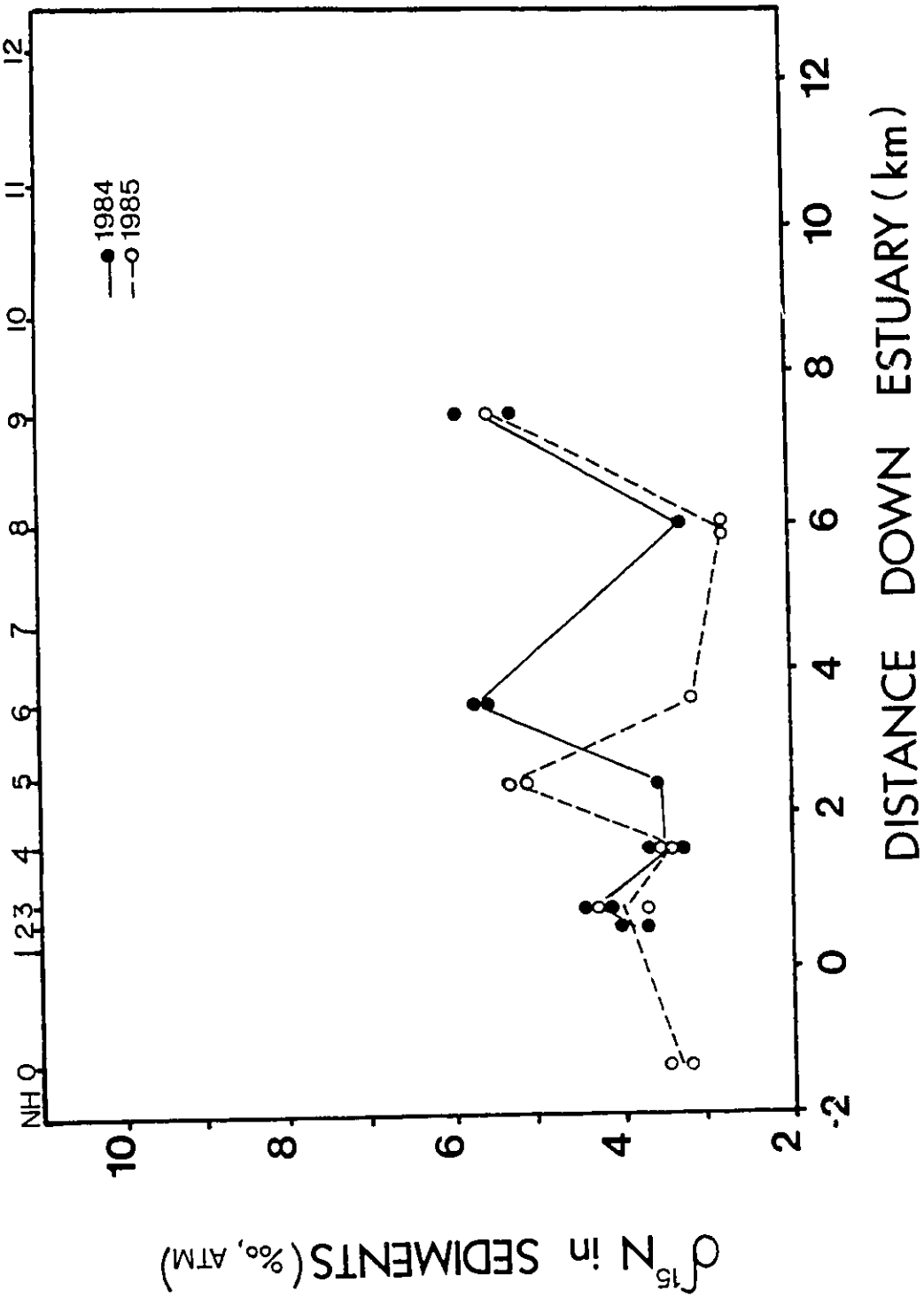
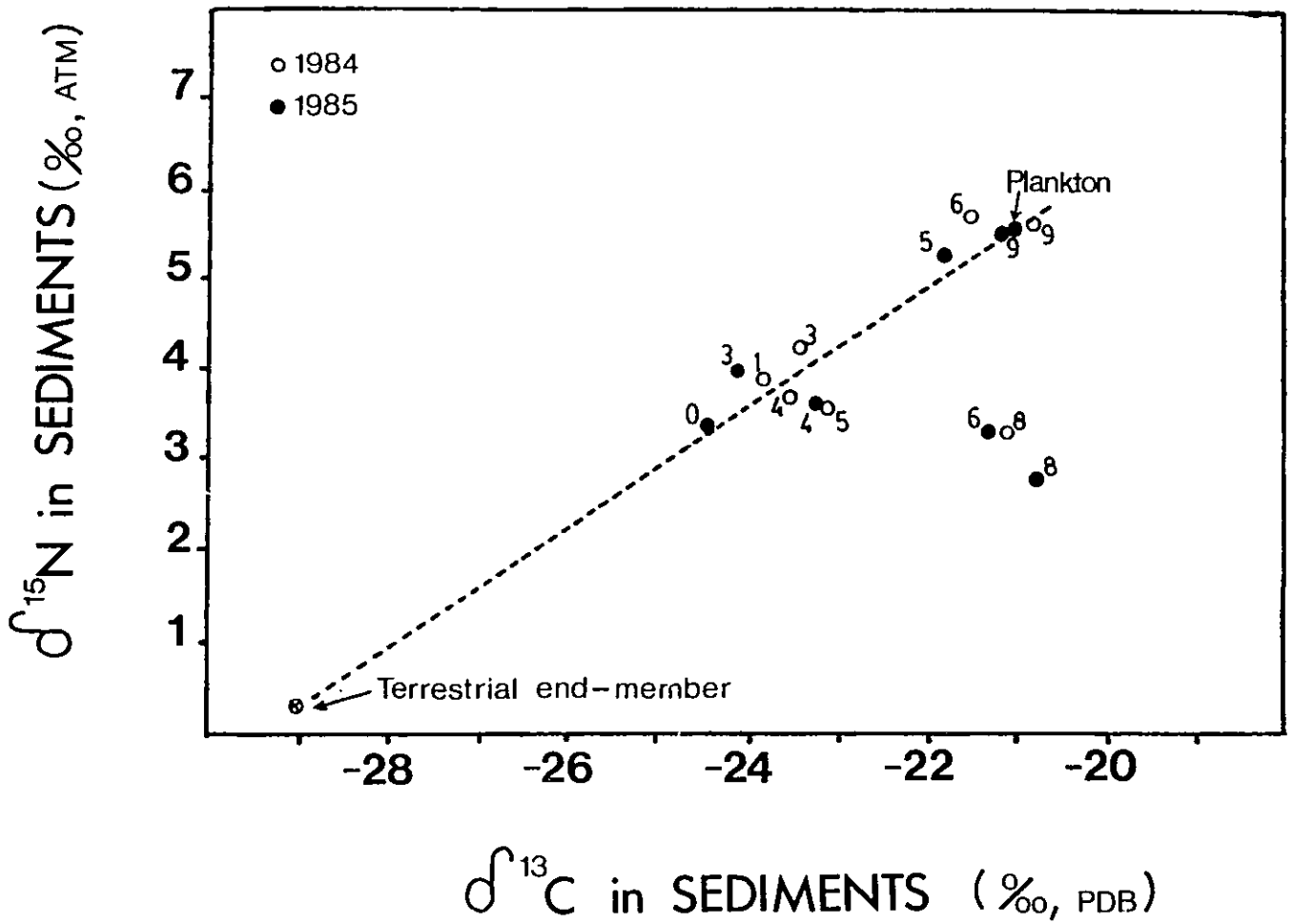

δ15N in SEDIMENTS (‰, ATM)
DISTANCE DOWN ESTUARY (km)
1984
1985
NH 0
1 2 3 4 5 6 7 8 9 10 11 12
-2 0 2 4 6 8 10 12
2 4 6 8 10

Table 4.13. $\delta^{15}N$ values in Negro Harbour sediments

Sites	1984	1985
NH0	-	3.5, 3.3
NH2	4.1, 3.8	-
NH3	4.5, 4.2	3.8, 4.1, 4.4
NH4	3.4, 3.7	3.6, 3.5
NH5	3.5	5.2, 5.4
NH6	5.8, 5.7	3.2
NH8	3.3, 2.8	2.8, 2.8
NH9	6.0, 5.4	5.6

Figure 4.5. Plot of $\delta^{13}C$ against $\delta^{15}N$ sediment values of Negro Harbour.


o 1984
● 1985
Plankton
Terrestrial end-member
7
6
5
4
3
2
1
-28
-26
-24
-22
-20
δ15N in SEDIMENTS (‰, ATM)
δ13C in SEDIMENTS (‰, PDB)

not follow as a clear trend as do $\delta^{13}C$ values, which have sigmoidal curves, indicating a rapid change of sources of organic matter in the sediments. The $\delta^{15}N$ signals form a series of peaks as was found using fatty acid ratios.

Figure 4.5 represents the plot of $\delta^{13}C$ values against $\delta^{15}N$ values in the sediments. Surficial sediments at station NH9 definitly receive most of their organic matter from a planktonic source, as do stations NH5 (1985) and NH6 (1984). Stations NH0, NH2, NH3 and NH4 receive a mixture of terrestrial and marine organic matter. All stations fall along a line with the exception of stations NH8 (both 1984 and 1985) and NH6 (1985). These stations have $\delta^{13}C$ corresponding to a marine component (-21.2‰) but their lower $\delta^{15}N$ values suggest a more terrigenous source. The remainder of the stations show a statistically significant ($p= 0.01$) linear correlation with $r= 0.92$. The regression equation for the analyses is $\delta^{15}N= 20 + 0.68\delta^{13}C$. The $\delta^{13}C$ value for the terrestrial member is obtained by averaging the carbon isotope ratios of the POM samples obtained from the river filters (CR1, 2, 3 and 4) which is -29.0‰ $\pm$ 0.4‰. The corresponding $\delta^{15}N$ terrestrial value using the regression equation is 0.3‰. This value is close to the estimated value for a terrestrial input given earlier and is very similar to the $\delta^{15}N$ of the terrestrial end-member found by Macko (1983) in Chesapeake Bay and other

adjacent estuarine systems. Macko (1983), who found a similar correlation, suggested that this linear relationship indicates that similar processes are affecting both nitrogen and carbon in the resultant diagenesis of the original organic matter.

4.2.4. MIXING EQUATION

In order to characterize the sources of organic matter contributing to the sediments, we have calculated the percentage of terrestrial organic matter through mixing equations.

4.2.4.1. Carbon isotopes

We attempted to calculate the percentage of terrestrial material through carbon isotope ratios. We can express carbon isotope data in terms of percentage of terrestrial organic carbon using an isotopic mixing equation (Schultz and Calder, 1976) :

$$T_{ic} = \frac{\delta^{13}C - \delta^{13}C_m}{\delta^{13}C_t - \delta^{13}C_m} \times 100 \quad \text{where,}$$

T_{ic} is the percent fraction of terrestrial material, $\delta^{13}C$ is the value of the sediment sample, $\delta^{13}C_t$ is the value of terrestrial organic carbon and $\delta^{13}C_m$ is the value of marine organic carbon. $\delta^{13}Ct$ obtained from the river filters is -29.0‰ ± 0.4‰.

$\delta^{13}Cm$ is taken to be the $\delta^{13}C$ of phytoplankton (-21.2‰). *Zostera marina* and *Spartina alterniflora* are

here neglected as marine components since they are not abundant in the estuary.

The percentages of terrestrial material in Negro Harbour sediments (table 4.14 and figure 4.6a) decrease seaward as expected. However percentages for the same site are significantly different in the two years of sampling. In the summer of 1985, the Ti values between NH0 to NH6 are on the average about 15% less at each site compared to 1984. Since the discharge of the Clyde River was larger in 1985, a decrease in the amount of organic matter delivered to the estuary is unlikely to be the cause of the lesser terrestrial component. We shall return to this problem after presenting comparative data from fatty acid analyses.

4.2.4.2. FATTY ACID RATIOS

Using the same principle as for isotopic mixing equation, we can define a fatty acid mixing equation as :

$$T_{fa} = \frac{FA - FA_m}{FA_t - FA_m} \times 100 \text{ , where}$$

T_{fa}, FA, FA_m and FA_t are respectively the percentage of terrestrial component in sediment, fatty acid ratio (C24/C16 or C24/C14) of the sample, fatty acid ratio of marine component which here is equal to 0 (plankton) and the fatty acid ratio of the terrestrial component. The terrestrial component is assumed to be a combination of

Table 4.14. Percentage of terrestrial organic matter deduced from $\delta^{13}C$, $\delta^{15}N$ and fatty acid ratios for 1984 and 1985.

Station	$\delta^{13}C$	$\delta^{15}N$	C24/C16	C24/C14
1984				
NH1	51	-	21	22
NH2	33	31	34	31
NH3	28	24	70	73
NH4	30	39	36	36
NH5	25	38	27	26
NH6	4	-2	29	27
NH7	5	-	-	-
NH8	0	-	26	20
NH9	0	-2	14	10
1985				
NH0	41	41	32	41
NH3	37	28	47	43
NH4	27	38	34	31
NH5	8	6	28	25
NH6	1	-	47	42
NH8	0	-	33	30

Table 4.14. continued.

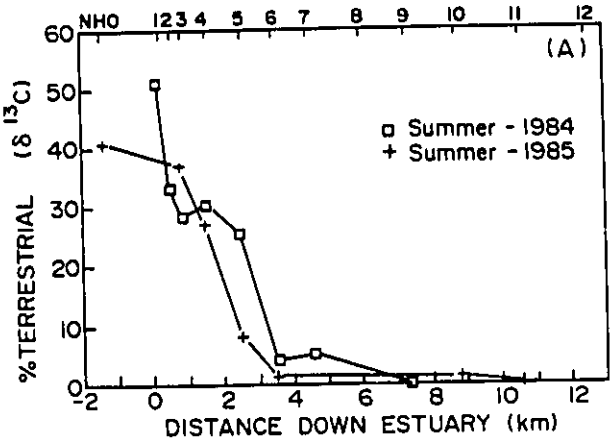
NH9	1	0	21	21
NH10	1	–	8	10
NH11	0	–	3	5
NH12	0	–	3	4

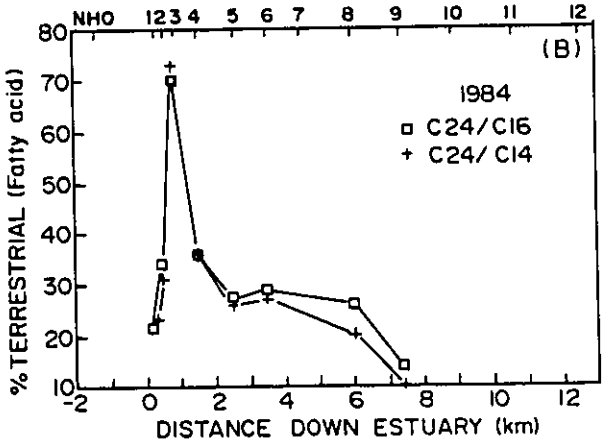
Figure 4.6. Percentage of terrestrial organic matter down estuary as measured by :

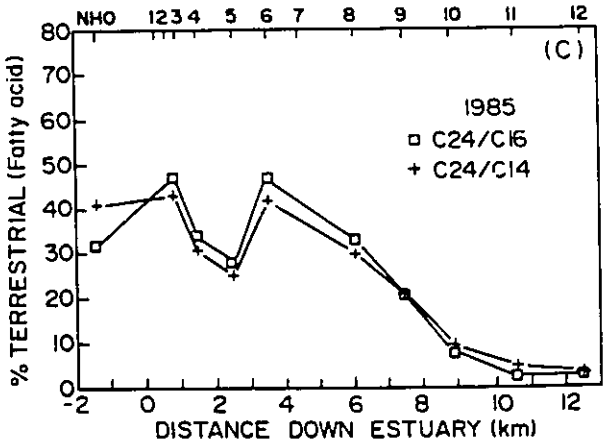
a) stable carbon isotope ratios

b) Fatty acid ratios - 1984

c) Fatty acid ratios - 1985


NHO 1 2 3 4 5 6 7 8 9 10 11 12
(A)
% TERRESTRIAL (δ 13C)
□ Summer - 1984
+ Summer - 1985
DISTANCE DOWN ESTUARY (km)


NHO 1 2 3 4 5 6 7 8 9 10 11 12
(B)
% TERRESTRIAL (Fatty acid)
1984
□ C24/C16
+ C24/C14
DISTANCE DOWN ESTUARY (km)


NHO 1 2 3 4 5 6 7 8 9 10 11 12
(C)
% TERRESTRIAL (Fatty acid)
1985
□ C24/C16
+ C24/C14
DISTANCE DOWN ESTUARY (km)

higher vascular plant material and peat material (as for the isotopes) Using table 4.6 we assume an equal input from each source. The average value for C24/C16 of peat is 3.22 and 13.3 for C24/C14. C24/C16 and C24/C14 ratios of maple leaves representing higher plant components are 0.36 and 2.19 respectively. Assuming equal contributions from the two terrestrial groups, the average value for the terrestrial component is 1.77 using the C24/C16 ratio and 7.7 for C24/C14 . Since FA_m is equal to 0, the T_{fa} values are given directly by FA/7.70 and FA/1.77, for C24/C14 and C24/C16 respectively. We also assume that bacterial degradation occurred after deposition and that their fatty acid contribution to the sediments is minimal. However as peat has already been degraded by soil microorganisms prior to transport into the aquatic environment, further degradation should be a rather slow process.

The percentage of terrestrial material calculated (Table 4.14) ranges from maximum values of about 70% (at station NH3) to minima of a few per cent. There are no noticeable differences between stations using either ratio for the same year of sampling. There is also little variation between the two years of sampling, but the T_{fa} values for 1985 are more regular, varying only between 28 to 49% (C24/C16) and 25 to 48% (C24/C14) for station NH0 to NH6 with no particular trend (Figure 4.6b,

c). The T_{fa} data for 1984 show that about 70% of the organic material is of terrestrial origin. The $\%T_{fa}$ decreases dowstream from NH6 in all cases.

These calculated percentages are of the same order of magnitude as the T_{ic} calculated with the isotopic mixing equation . The first major difference between the two methods occurs downstream from site NH5, where the fraction of terrestrial material calculated through fatty acid ratios makes up about 30% of the organic material and gradually decreases seaward, whereas T_{ic} is equal to zero along this part of the traverse. Secondly the T_{ic} values indicate that the amount of terrestrial material contributed in 1985 is less than in 1984; the $\%T_{fa}$ values show the opposite.

4.2.4.3 Nitrogen isotopes

Knowing the $\delta^{15}N$ of the terrestrial end-member value we can calculate the percentage of the terrestrial component in the sediments. The mixing equation using nitrogen isotope ratios is:

$$\% T_{in} = \frac{\delta^{15}N - \delta^{15}N_m}{\delta^{15}N_t - \delta^{15}N_m} X 100$$

where $\delta^{15}N$, $\delta^{15}N_m$ and $\delta^{15}N_t$ are the nitrogen isotope ratios of the sediments, the marine end-member (plankton= +5.6‰) and of the terrestrial end-member (0.3‰). The values are similar to the percentage obtained through $\delta^{13}C$ (table 4.14).

4.3. COMPARISON OF MIXING EQUATION DATA ($\delta^{13}C$, $\delta^{15}N$, FATTY ACIDS)

There are striking differences between the down estuary gradients in % terrestrial as estimated by $\delta^{13}C$, $\delta^{15}N$, and fatty acid ratios in the sediments. The curves of $\delta^{15}N$ and of fatty acid ratios (figures 4.3 and 4.4) give similar signals in 1985 but not in 1984. Fatty acid ratios indicate a major peak in sediments collected in the summer of 1985 at NH4 and NH5 suggesting material of marine origin. This peak is also found at NH5 using nitrogen isotope ratios. The curve for 1984 does not follow the same trend. Fatty acid curves show an important terrestrial input to the sediments at NH3. This input is not seen with the use of nitrogen isotope ratios. The $\delta^{15}N$ values show an important terrestrial input at NH8 and a marine input at NH6.

Most of the carbon atoms in sediments are in non lipid species (*e.g.* lignin, cellulose) so that fatty acid and carbon isotopic signatures can be (and are obviously) decoupled. Lipids make up only a few percent of TOC, thus a change in the fatty acid composition may not necessarily correlate with $\delta^{13}C$ and $\delta^{15}N$ values. It is possible however, that the organic material in 1985 was rich in peat-like fatty acids. The enrichment in $\delta^{13}C$ in 1985 could be due to the presence of a larger fraction of peat derivatives in which $\delta^{13}C$ is heavier

by ~3‰ compared to higher vascular plants rather than a lower concentration of terrestrial material as a whole. A dilution effect could also explain this situation in 1985, since the rate of flocculation depends on the extent of particle collisions and thus on particulate matter concentration (Postma, 1967). Diagenesis may also have been slower in 1985 due to lower sedimentation rate. But the %TOC values in the sediments were definitely higher than in 1984 so that an increase in peat detritus to the sediments is more probable than a low sedimentation rate effect. Fatty acid and carbon isotopic ratios gave quite similar patterns concerning the amount of terrestrial material in the Negro Harbour sediments. However a rather interesting fact is that T_{ic} and T_{in} (although less obvious) have their maximum in the Clyde river (where it should be), whereas Tfa peaks at NH3. River-borne particulate matter, of which a considerable fraction may be colloidal (e.g. some clay-minerals) is subject to flocculation in the early stages of estuarine mixing. This mixing, according to salinity profiles, occurs basically at the head of the estuary, between NH1 and NH3, so the peak at NH3 could be explained by peat-derived flocculants.

4.4. CONCLUSIONS

$\delta^{13}C$, $\delta^{15}N$ and fatty acid analyses of sediments

can give rather different signals of terrestrial inputs but the comparison of the three indices is very useful for characterization and quantification of the sources of organic material in the sediments.

Discrepancies between the indices can arise due to local concentration of C-sources that have variable fatty acid ratios but normal $\delta^{13}C$ signatures. Local high concentrations of peat-like fatty acid content (as at NH3) can be due to microsedimentary effects (flocculation, turbulence, resuspension of sediment by tidal currents, seasonality, etc.) which do not affect the macro-detritus which determines $\delta^{13}C$.

Despite all these assumptions, the relative contribution of terrestrial and marine organic matter calculated from Tfa values and carbon (and nitrogen) isotope analyses are generally in agreement, for sample sites in the upper part of the estuary (before NH5). Also the Tfa, calculated using C24/C16 or C24/C14 are similar, implying a consistent relation between the individual fatty acid concentration and a particular source of organic material. Thus, fatty acid ratios analysed in the sediment should be considered as a very useful tool in the quantification of different sources of organic material in modern sediments particularly to follow the fate of organic pollutants in estuaries and coastal waters.

CHAPTER 5. *Mytilus edulis* AND FEEDING BEHAVIOUR

Mytilus edulis, commonly called the blue mussel, was selected for this study. Mussels are not usually found in upper estuary salt marshes, but are abundant on intertidal flats, where they occur crowded together in immense numbers on rock ledges on the lower, level part of the shore, and in deeper channels in a more seaward direction. Its broad environmental tolerances with respect to temperature, salinity, depth, and geography make it an ideal species to work with.

As a non-selective filter-feeder *Mytilus edulis* can utilize plankton and detritus, food resources that are brought to it by tidal currents. *Arctica islandica*, found at NH11 is also a suspension-feeder. Literature on the chemical composition of suspended particulates in the sea contains little comprehensive information on seasonal changes in the food available to estuarine suspension feeders. There is also a paucity of data on the feeding behaviour of bivalve mollusks under natural conditions.

Vernal phytoplankton blooms are an important phenomenon in northern waters. Diatoms generally dominate these blooms (Parsons *et al.*, 1977). Phytoplankton production is much more closely coupled with the benthos in estuaries than is the case in the ocean. Algal blooms,

which are not immediately consumed by zooplankton because of lack of synchrony between the plant and animal population, can quickly sink to the bottom before the cells have time to decompose and recycle their nutrient contents (Officer *et al.*, 1981). During the colder months (October to Febuary) phytoplankton are practically absent (Widdows *et al.*, 1979). Widdows *et al.* (1979) found that the low amounts of non-phytoplankton particles available to mussels in late August suggest that the importance of this particulate material to *M. edulis* was comparatively small, whereas the phytoplankton constitute a critical element in the diet of the mussels. On the other hand, Incze *et al.*. (1980) concluded that phytoplankton alone could not meet the food requirements of *M. edulis*, and that the additional particulate food material is probably detrital or bacterial in origin, especially in the autumn and winter.

Previous studies (e.g. Widdows *et al.*, 1979) have shown that a large proportion of the particulate organic matter was found to be refractory and not utilized as an energy substrate by heterotrophic organisms, the "food" being less well digested or of poor nutritive quality.

Recent studies have shown that the planktonic bacteria are important components of estuaries and coastal waters, reaching high population densities and accounting for a large fraction of the production of

particulate matter in these systems (Fuhrman and Azam, 1980). Zobell and Feltham (1938), by laboratory experiments, demonstrated that the California mussel, *Mytilus californianus*, ingests and digests bacteria. However, these authors concluded that it is doubtful that bacteria are sufficiently abundant in sea water to constitute an appreciable item in the diet of marine animals. In marine bottom deposits and as a constituent of the slime on solid surfaces, bacteria may be sufficiently abundant to nourish certain animals (detritus-feeders). Wright *et al.*. (1982) showed that blue mussels removed a substantial proportion of phytoplankton from water passing over the mussel bed, but had no measurable effect on the bacterial plankton. Bayne *et al.* (1977) studied particle selection by *M. edulis* in the estuarine environment and showed that all particles greater than 2 to 5 µm are filtered with 100% efficiency.

Seaweeds, fleshy attached macroalgae, are found predominantly in the rocky subtidal habitat on the South Coast of Nova Scotia. Most of the unsuitable shoreline for the occurence of macroalgae occured in estuarine areas. Negro Harbour is one such area (Moore and Miller, 1983). Some kelp beds are, however, observed at the outer edge of Cape Negro Island. Kelp beds are highly productive. In general, very little of the production is removed by grazers; most is released as particulate and

dissolved matter from eroding blades (Robinson *et al.*, 1982), which later becomes detritus. Also, the importance of kelp detritus rapidly drops within a short distance from the kelp bed. The major changes in carbon isotope ratios of sediments occur inside Negro Harbour (figure 5), thus it is doubtful that macroalgae are an important source of detritus.

Seasonal changes in flesh weight and composition in *Mytilus edulis* result from the storage and utilisation of food reserves in relation to the complex interactions of food availability and temperature with growth and reproductive processes.

In the winter *Mytilus edulis* enters a period either of zero growth, when the available food is equivalent to the maintenance ration, or of negative growth and utilization of body reserve, when the available food is less than the maintenance ration (Widdows *et al.*, 1979; Tieszen *et al., 1983*). The ration level required to maintain a mussel increases with body size (Widdows *et al.*, 1979). There is winter loss of protein and lipid since winter is the time when metabolic demands are maximal due to gametogenesis (Tieszen *et al.*, 1983). Carbohydrates on the other hand are the main energy reserves in winter. There may be enough food in winter to support small individuals, but insufficient to prevent the utilization of body reserve in larger

mussels. At times when carbon-rich reserves are limiting, however, as is increasingly the case with decreasing net growth, *Mytilus edulis* may catabolize protein to satisfy at least 67% of the mass equivalent of maintenance requirements (Hawkins, 1985). The storage of protein within adipogranular cells, as well as within muscle, may constitute one means by which protein degradation is reduced with growth in *M. edulis* (Hawkins, 1985). Whatever the mechanism(s) enabling such conservation of protein, it is clear that this effect may be advantageous to mussels, being sessile organisms which are virtually ubiquitous in coastal ecosystems where pronounced seasonal variations of nitrogen abundance commonly limit organic production (Mann, 1982). Hawkins (1985) also found lower elemental turnover of nitrogen, relative to carbon, to be in agreement with previous observations that bivalves utilize carbohydrate as the preferred respiratory substrate.

This suggests that no seasonal trends could be expected to be found in the $\delta^{15}N$ and $\delta^{13}C$ signatures of mussel flesh. Thus, for example, Lyon and Stanley (1985) used $\delta^{13}C$ analysis in an attempt to help identify the mussel's food. The values they found suggested a continuous change from more "terrestrial" food to more "marine" food, with no apparent seasonal trend.

CHAPTER 6. STABLE ISOTOPES IN THE SOFT TISSUES OF *Mytilus edulis*.

As suggested by Tieszen *et al.* (1983), information about an herbivore's diet can be maximized by analyzing as many components as possible. We carried out analysis of the soft tissues, stomach content and the organic matrix extracted from the molluscan shell in order to determine the immediate diet (more closely represented by the stomach content), short term variation or shifts in food intake (flesh) and the average lifetime diet (organic matrix). In this way, one can tell if any shifts in food habits have occured recently. The fact that there were some variations in river input between the 2 summers of sampling mean that a diet variation in *Mytilus edulis* could be observed. In this chapter we shall discuss soft tissues and gut contents. In the following chapter we will discuss the organic matrix of the shell.

6.1. STABLE CARBON ISOTOPES

6.1.1 REVIEW OF $\delta^{13}C$ IN CONSUMERS

Organic carbon enters the food chain entirely at the primary producer level and undergoes similar metabolism by all animals.

DeNiro and Epstein (1978) showed that the isotopic composition of the whole body of an animal varies with the isotopic composition of its diet, but the animal is on average enriched in ^{13}C by about 1.5‰ relative to the diet. In almost every case examined, the ^{13}C enrichment of the whole body relative to its diet is balanced by a ^{13}C depletion of the respired CO_2. Thus enrichment of ^{13}C occurs because animals selectively respire ^{12}C and retain ^{13}C, consequently raising the $^{13}C:^{12}C$ ratio as carbon passes through the food web (DeNiro and Epstein, 1978; McConnaughey and McRoy, 1979a). DeNiro and Epstein (1978) also found that the isotopic relationships between the whole bodies of animals and their diets are similar for different species raised on the same diet and for the same species raised on different diets. Thus for the purpose of dietary analysis, the determination of $\delta^{13}C$ values of several tissues of an animal will allow for a better estimate o the $\delta^{13}C$ value of its diet than would the analysis of a single tissue (De Niro and Epstein, 1978). Moreover, the major biochemical fractions (namely lipids, carbohydrates, and protein) have characteristically different $\delta^{13}C$ values. The lipids of an organism normally possess lower $^{13}C/^{12}C$ ratios than proteins and carbohydrates. The latter two are closer to the diet.

Because isotopes are conserved in metabolism, the

extent of ^{13}C enrichment in a consumer (compared to its food) must reflect the carbon budget of the consumer and its hierarchy in the food web.

6.1.2. RESULTS FROM NEGRO HARBOUR

In both 1984 and 1985 the $\delta^{13}C$ values of the stomach contents of the mollusks were uniform throughout the estuary for both years (figures 6.1, 6.2, table 6.1 and 6.2), but the values for 1984 (-20.5‰) were enriched in ^{13}C by about 1 per mil compared to 1985 (-21.3‰). This comfirms that *Mytilus edulis* uptake mainly material of marine origin but also that river discharge was more important in 1985 and thus that more terrestrial material was available to *Mytilus edulis*. Stomach contents are here assumed to be representative of the food filtered out from the water and not yet chemically taken up (processed) by the bivalve. There are some similarities and differences between the stomach content and soft tissue data from 1984 and 1985. The difference between stomach contents and the flesh of the bivalve is 1.1‰ in 1984 and 1.2 to 1.5‰ (with the exception of NH7) in 1985. These data conform well with the literature (DeNiro and Epstein, 1976; Incze *et al.*, 1982). In fact, other authors (table 6.3) found similar fractionation between the flesh and the available source in *Mytilus edulis* or other bivalves having the same habitat (Incze *et al.*, 1982; Peterson *et al.*, 1985; Stephenson and Lyon, 1982).

Figure 6.1 $\delta^{13}C$ values of different tissues of *Mytilus edulis* down estuary (1984).

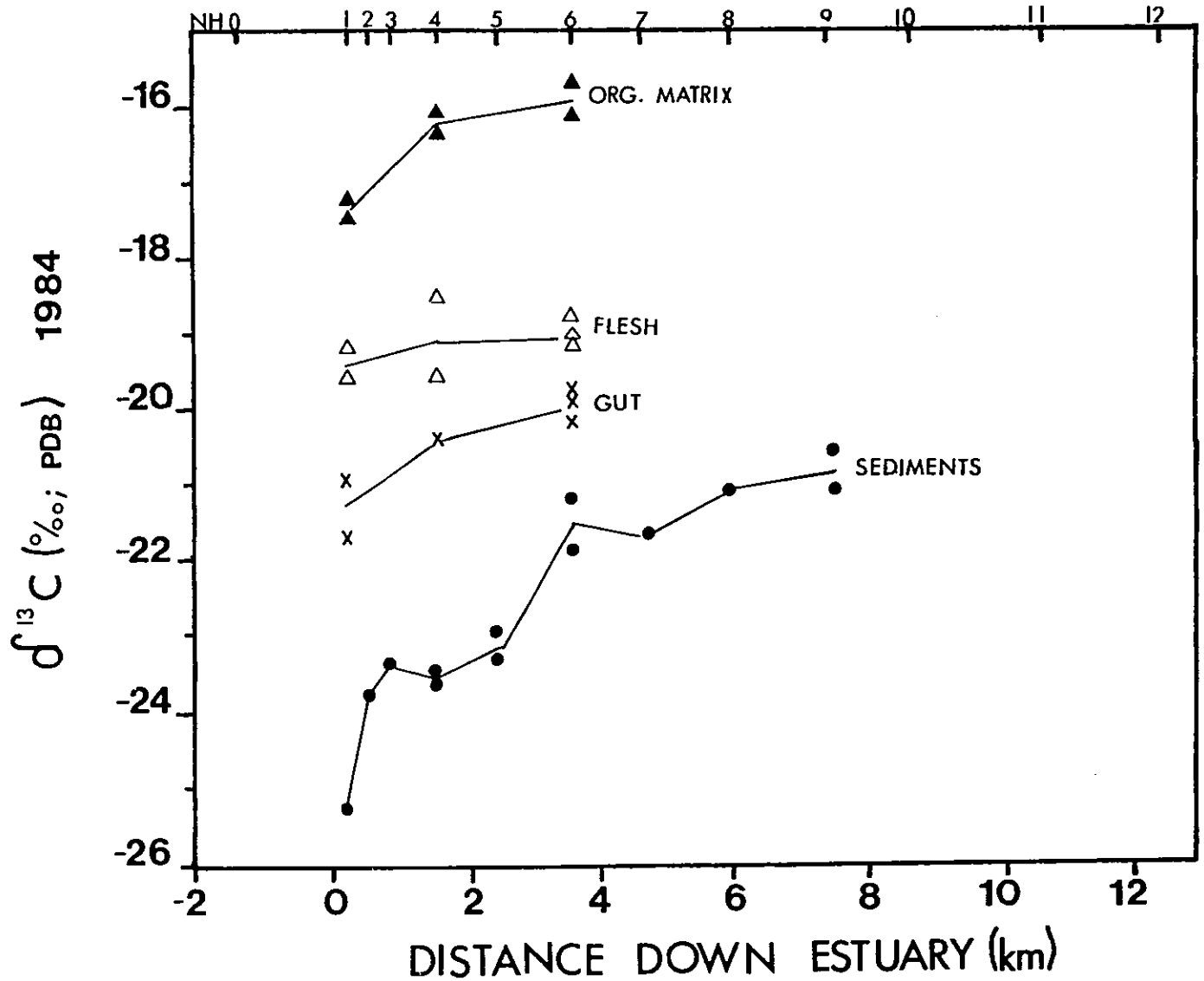

NH 0
1
2
3
4
5
6
7
8
9
10
11
12
-16
-18
-20
-22
-24
-26
ORG. MATRIX
FLESH
GUT
SEDIMENTS
δ13C (‰; PDB) 1984
-2
0
2
4
6
8
10
12
DISTANCE DOWN ESTUARY (km)

Figure 6.2 $\delta^{13}C$ values of different tissues of *Mytilus edulis* down estuary (1985).

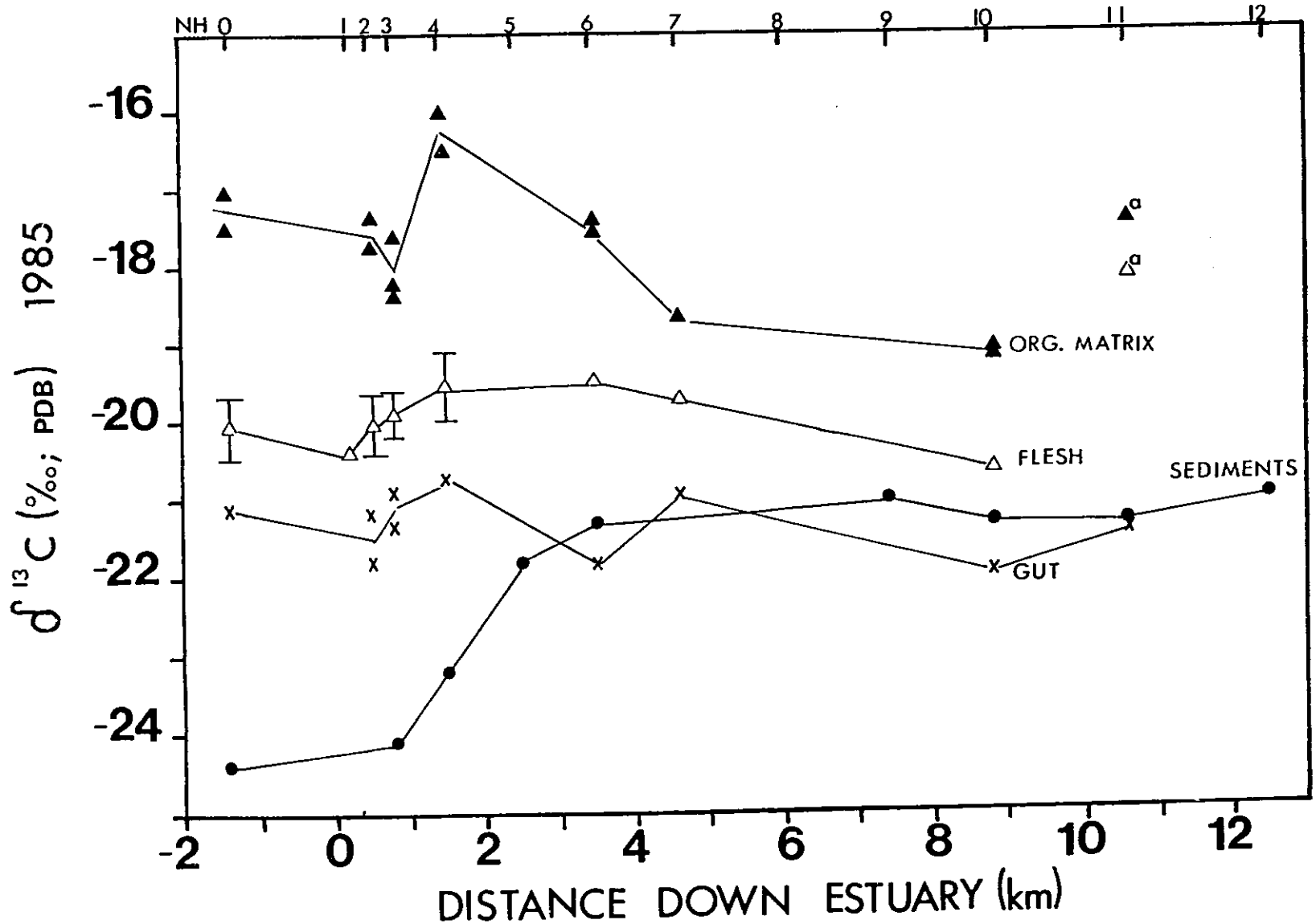

NH 0
1 2 3
4
5
6
7
8
9
10
11
12
-16
-18
-20
-22
-24
$\delta^{13}C$ (‰; PDB) 1985
ORG. MATRIX
FLESH
SEDIMENTS
GUT
-2
0
2
4
6
8
10
12
DISTANCE DOWN ESTUARY (km)

Table 6.1. Carbon isotope ratios (‰) from the flesh, organic matrix and stomach contents of *Mytilus edulis* from the summer of 1984. Average value in brackets. Each value represent the analysis of a single individual.

Site	Flesh	Stomach contents	Organic matrix
NH1	-19.2	-21.7	-17.2
	-19.6	-20.9	-17.5
	(-19.4)	(-21.3)	(-17.3)
NH4	-19.6	-20.4	-16.1
	-18.5		-16.4
	(-19.0)		(-16.2)
NH6	-19.2	-19.9	-15.7
	-19.1	-20.2	-16.2
	-18.8	-19.8	(-15.9)
NH11*			-17.4
			± 0.3 (10)

* *Arctica islandica*

Table 6.2. Carbon isotopes ratios (‰) for the flesh stomach contents and organic matrix from *Mytilus edulis* from the summer of 1985. Average value in brackets. Each value represent the analysis of a single individual.

Site	Flesh	Stomach contents	Organic matrix
NH0	-20.5, -19.4	-21.1	-17.0
	-20.0, -20.4		-17.5
	(-20.1)		(-17.3)
NH1	-20.4*	-24.2*	
NH2	-20.2, -20.1	-21.8	-17.7
	-19.7, -19.4	-21.2	-17.4
	-20.5, (-20.0)	(-21.5)	(-17.6)
NH3	-19.4, -20.2	-21.3	-18.2, -17.6
	-19.9, -20.1	-20.9	-18.3, (-18.0)
	-19.8, (-19.9)	(-21.1)	
NH4	-19.7, -20.1	-20.7	-16.1
	-19.0, -18.8		-16.5
	-19.7, (-19.5)		(-16.3)

Table 6.2. continued.

NH6	-19.9, -19.3	-21.8	-17.6 Ar
	-18.8, (-19.3)		-17.5 Bk
NH7	-19.8	-21.8	-18.6
	-19.7	-19.9	
		(-20.9)	
NH10	-20.5, -20.7	-21.7	-19.0
	-20.7	-22.1	-19.1
	(-20.6)	(-21.9)	
NH11**	-17.8, -18.4	-21.4	-17.4
	-18.1, -18.3		
	-17.8, -18.1		
	(-18.1)		

* This sample was collected in May of 1985.

** NH11 is the only station with *Arctica islandica* and without *Mytilus edulis*.

Table 6.3 $\delta^{13}C$ values from the flesh of bivalves (filter-feeders).

Bivalve	$\delta^{13}C$ (‰)	Reference
Mytilus edulis	-18.7 to -22.5	Incze *et al.* (1982)
Mytilus edulis	-19.4	Peterson *et al.* (1985)
Chione sp	-16.7 to -23.5	Stephenson and Lyon, (1982)

The $\delta^{13}C$ of stomach contents are consistently heavier than the $\delta^{13}C$ in the sediment between NH0 and NH4. Let us consider why there should be such a difference. *Mytilus edulis* could preferentially select phytoplankton over bulk seston. This could be simply because the terrestrial material is too fine or otherwise too indigestible to be taken up by *Mytilus edulis*. Wright *et al.* (1982) and Peterson *et al.* (1985) noticed that the bivalve *Geukensia demissa* could efficiently remove bacterioplankton and fine detritus from the surrounding water while *Mytilus edulis* found in the same area failed to do so and was only efficient in removing plankton of 2 µm and up. These authors attributed the efficiency of removal of fine particles by the filter-feeding *Geukensia demissa* to the closer spacing and more lateral overlapping of filtering cilia of the gills. Incze *et al.* (1982) studied the effect of river input to an estuarine system and observed a discrepancy between the slopes of the POC regression and the bivalves, and sediments, $\delta^{13}C$. They explained this dicrepancy by the fact that that the sedimentary organic matter derives part of its carbon isotope composition from the more refractory component of organic material delivered to it, while bivalve tissues tend to be made from a more labile component. This labile material must be primarily of marine origin (i.e. phytoplankton). Only the more refractory components of

terrestrial organic matter remain by the time this material is delivered to the lower estuary. Incze *et al.*(1982) also made a transfer experiment on *Mytilus edulis*. Mussels were taken from a dominantly marine estuary (Damariscotta) to an estuary with important terrestrial input (Sheepscot). $\delta^{13}C$ values of mussels transplanted changed from -18.3‰ to -21.2‰ over 6 months. The authors suggest that a measurable terrestrial influence on the organic carbon composition of bivalves exists as well as for POC $\delta^{13}C$. Similarly, the depletion by about 1.0‰ in the flesh of *Mytilus edulis* observed here in 1985 compared to 1984 can be accounted for as an incorporation of a larger amount of terrestrial material in its tissues, during this year of higher stream flow.

6.2. STABLE NITROGEN ISOTOPES

6.2.1. REVIEW OF STABLE NITROGEN RATIOS IN CONSUMERS

The general scheme of nitrogen metabolism in animals is as follows. Protein introduced into the digestive tract is digested into amino acids which largely undergo transamination in the metabolic recycling system. Waste nitrogen generated from catabolism of proteins is excreted as ammonium ion (in bivalves), the final biological decomposition state.

Animals have been observed to contain an excess of ^{15}N relative to their food (DeNiro and Epstein, 1981;

Macko *et al.*, 1982b). DeNiro and Epstein (1981) in a study of laboratory-raised animals, established that the fractionation can vary from -0.5 to +9.2‰, averaging +3.0 ± 2.6‰. Furthermore, they found that while a species raised on different food sources had similar isotopic fractionation with respect to its diet, two species can show quite different fractionations for the same food. The mechanism by which this fractionation occurs is still not well known. However, Gaebler *et al.*. (1966) suggested that it was due to the accumulation of ^{15}N during the transamination of α-keto acids. Transamination is the major process occuring in a cell whereby NH_2 is transferred from one amino acid to an α-keto acid, thus forming a new amino acid. Macko et al. (1982a) have studied the stable nitrogen isotope effects in the transamination of amino acids. They concluded that enrichment in higher trophic levels of food webs can not simply be due to the process of transamination and that a more complex pathway is likely in the assimilation and metabolism of organic nitrogen. The enrichment of ^{15}N is widespread among most animals collected from several different ecosystems, even if they belong to different trophic levels. Isotopic enrichment occurs independently of habitat, form of nitrogen excreted, and growth rate (Minagawa and Wada, 1984). The nitrogen compositions of these are, on the other hand, influenced

by the ultimate source of the fixed nitrogen. Furthermore, it has been shown that the $\delta^{15}N$ of soft animal tissues is almost constant during the life span of two marine mussels (Minagawa and Wada, 1984). Consequently it appears that nitrogen isotopes can be used as a tracer, not only for dietary analysis, but also for determining the trophic level of given animals. This was shown more generally by Schoeninger and DeNiro (1984).

6.2.2. RESULTS FROM NEGRO HARBOUR

The $\delta^{15}N$ values of the stomach content from the mussels collected in 1984 follow the same trend as the sediments but there is an enrichment of ~ 3-4‰ in the stomach contents with values between 5.4 to 7.8‰ (table 6.4, figure 6.3). The lack of data from some stations in 1984 does not permit a definite comparison with 1985 (table 6.5, fig. 6.4) but the stomach contents seem to be very similar between the 2 years. We conclude that there was no major change in the origin of the nitrogen source.

$\delta^{15}N$ values of the flesh from samples collected in 1985 follow mostly the same geographical trend as the sediments. There are a few stations at which the flesh and stomach content have the same $\delta^{15}N$ values (NH0, NH3 and NH4). $\delta^{15}N$ of the flesh in 1984 are more variable (table 6.4, figure 6.3). The values do not follow the curve of the sediments or of the stomach contents. There is also an enrichment of 1.5 to 4.5‰ between the stomach

Table 6.4 $\delta^{15}N$ values in different tissues of *Mytilus edulis* from 1984. Average value in brackets. The replicates are from separate individuals.

Site	Flesh	Stomach contents	Organic matrix
NH1	9.1, 8.7, 8.9 (8.7)	7.3	8.4, 8.4
NH4	9.8, 9.0 (9.4)	5.4	8.9, 8.9, 8.4 (8.7)
NH6	8.9, 8.1 (8.5)	6.8	8.3, 8.6 (8.4)
NH11*			6.7 ± 0.7 (10)

* *Arctica islandica*

Table 6.5. $\delta^{15}N$ values in different tissues of *Mytilus edulis* from 1985. Each value represent the analysis of a single individual. Average value in brackets.

Site	Flesh	Stomach contents	Organic matrix
NH0	7.6, 7.5	7.8	8.7, 8.2 (8.4)
NH1*	9.2	7.1	8.9
NH2	9.0, 9.0	7.3, 7.2	9.0
NH3	7.7, 7.6, 7.8 (7.6)	7.5, 7.1 (7.3)	8.9, 8.7 (8.8)
NH4	8.2, 7.8 (8.0)	7.7	8.7, 9.1 (8.9)
NH6	9.1, 9.1		8.9 Ar 8.7, 8.8 Bk
NH7	7.8	6.0	8.4, 8.8, 8.8
NH10	9.4	6.3, 6.8 (6.6)	8.3
NH11**	9.0, 9.8 (9.4)	7.0	

* Sample collected in May of 1985

** Values from *Arctica islandica*

Ar= aragonite; Bk= bulk of shell (aragonite and calcite)

Figure 6.3 $\delta^{15}N$ values for different tissues of *Mytilus edulis* down estuary (1984).

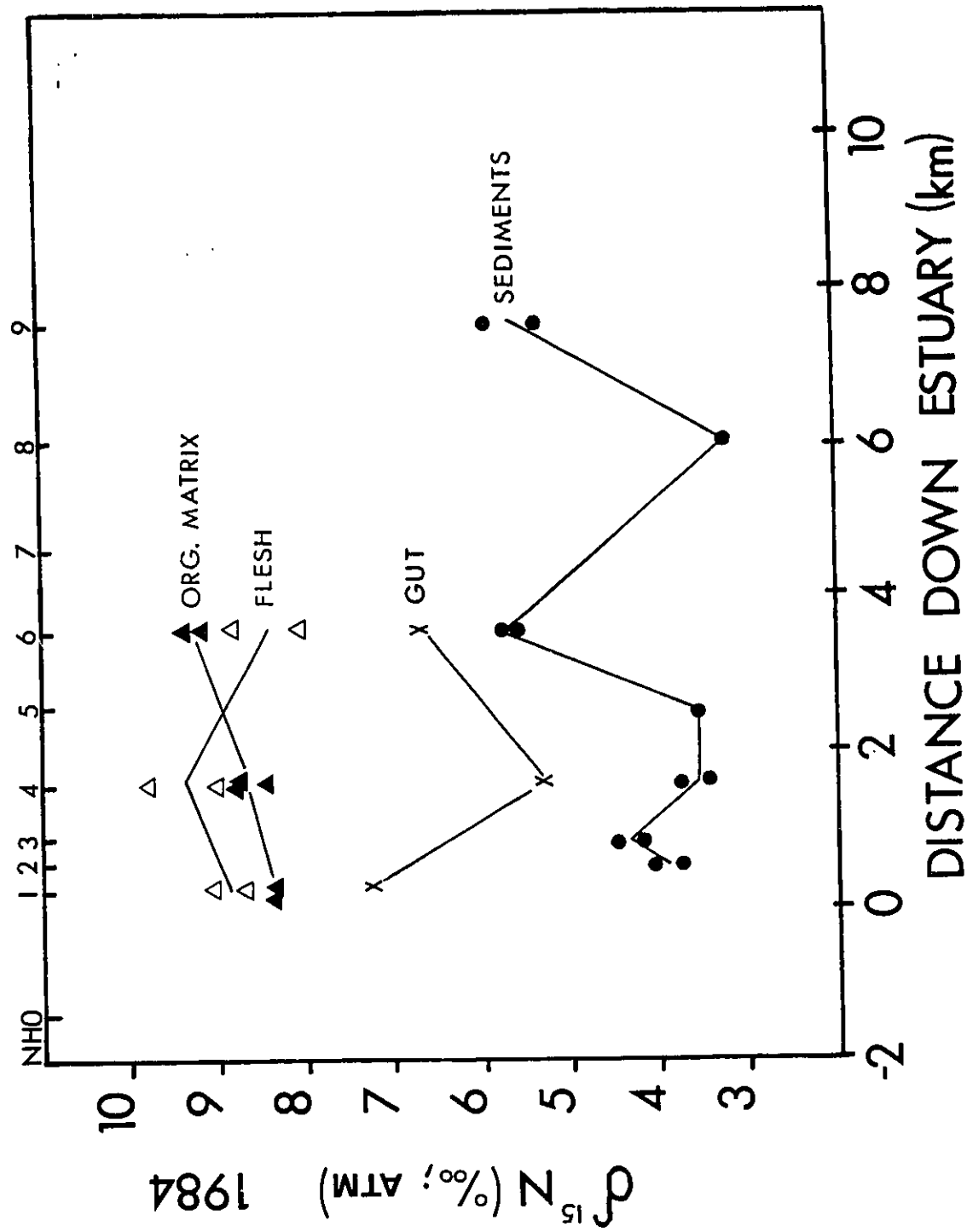
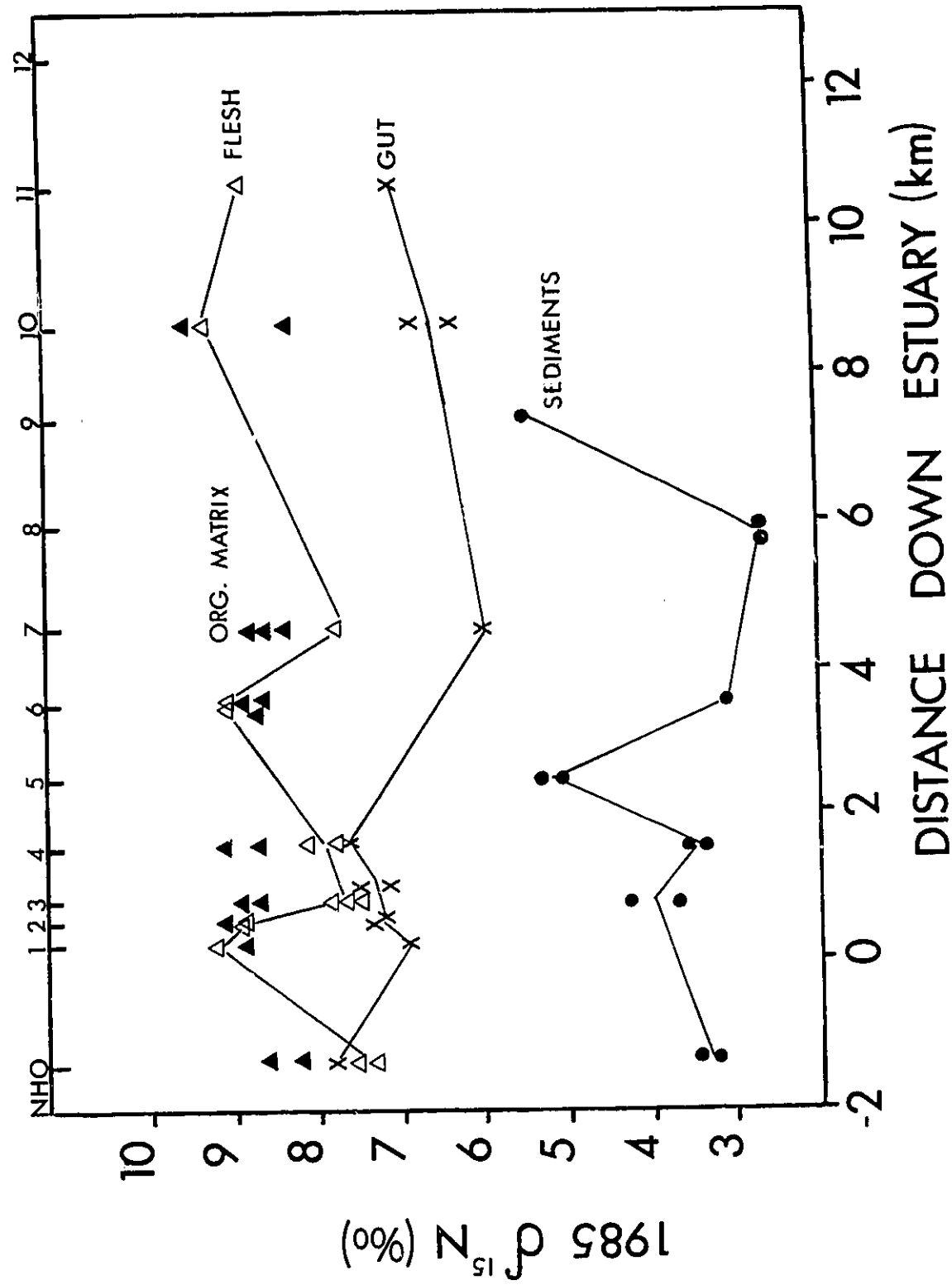

1984
$\delta^{15}N$ (‰; ATM)
10
9
8
7
6
5
4
3
NHO
1 2 3 4 5 6 7 8 9
ORG. MATRIX
FLESH
GUT
SEDIMENTS
-2 0 2 4 6 8 10
DISTANCE DOWN ESTUARY (km)

Figure 6.4 $\delta^{15}N$ values in different tissues of *Mytilus edulis* down estuary (1985).


1985 δ15N (‰)
DISTANCE DOWN ESTUARY (km)
FLESH
GUT
ORG. MATRIX
SEDIMENTS
NHO

contents and the flesh. $\delta^{15}N$ values of the flesh samples collected in 1984 are slightly enriched ("more marine") compared to 1985, an effect which was also seen in the $\delta^{13}C$ values.

Table 6.6 represents the $\delta^{15}N$ values other authors found in the flesh of bivalves which have the same habitat as *Mytilus edulis*. The $\delta^{15}N$ of these bivalves (Minagawa and Wada, 1984; Sealy *et al.*, 1987) are similar with respect to the present study. These studies also had a marine component (table 4.11) which should also have had $\delta^{15}N$ values of 5.6 to 5.9‰ for the plankton samples. Thus *Mytilus edulis* fractionated its food in a similar way as for the other bivalves.

6.3 STABLE HYDROGEN ISOTOPES

6.3.1. THEORETICAL REVIEW

Although many organic hydrogen atoms are exchangeable with environmental water, once a carbon-hydrogen bond is formed in an organism, the hydrogen is no longer readily exchanged (Estep and Hoering, 1980). Heavy isotopes of hydrogen (D) tend to be excluded from plant matter.

Transpiration is the major process fractionating hydrogen isotopes in plants with a further offset by photosynthesis. Additional fractionation of hydrogen isotopes occurs during biosynthesis. δD in wood of

Table 6.6 $\delta^{15}N$ values (‰) in flesh of bivalves (filter-feeders).

Bivalve	$\delta^{15}N$	Reference
Mytilus edulis	8.7 ± 0.3	Minagawa and Wada (1984)
Septifer virgatus	9.0 ± 0.6	Minagawa and Wada (1984)
Choromytilus meridionalis	8.5 ± 0.3	Sealy *et al.* (1987)

growing trees varied as a function of species and location and on average, whole wood is about 30‰ depleted in D relative to the average D/H of local precipitation. Edwards *et al.* (1985) pointed out that a good first-order approximation of the average daytime relative humidity during the growth season at a site can be based on the linear correlation that exists between humidity and cellulose enrichment, independent of temperature. However δD in plants is more related to δD of precipitation than to humidity.

6.3.2. RESULTS FROM NEGRO HARBOUR

The mean annual δD of the meteoric waters in the area studied is calculated using an averaged $\delta^{18}O$ of the Clyde river water as it is well known that the $\delta^{18}O$ and the δD in the meteoric waters are related by the equation of Craig (1961): $\delta D = 8\delta^{18}O + 10$. A value of 6.0‰ was found in Clyde river (table 6.7) and gave a δD value of -38‰. This δD value lies between the -50 and -30‰ boundaries given by Sheppard *et al.* (1969). Adding a fractionation factor of 1.033 (-33‰ average depletion in wood compared to environmental water; Edwards *et al.* (1985) are a value of about -73‰. This approximated value agrees with the value measured from a peat sample (0-50cm) of -78‰ (table 23). On the other hand plankton values are similar (-77‰). Estep and Hoering (1980)

Table 6.7 $\delta^{18}O$ values of the water from Clyde river collected at two sites, CR1 (freshwater) and CR5 (mixture of fresh and sea waters) in the summer of 1984 and 1985 and the winter of 1985.

Date	Site	$\delta^{18}O$(‰, SMOW)
07/84	CR1	-5.4
	CR5	-2.6
02/85	CR1	-6.5
	CR5	-3.7
08/85	CR1	-6.1
	CR5	-4.7
Average	CR1	-6.0 ± 0.5
Average	CR5	-3.7 ± 0.8

found that on average phytoplankton and mixed natural population of phytoplankton and zooplankton samples from waters with a wide δD range have δD (of organic H) values of -169 to -98‰ with an average of -120‰. We have no explanation for why our plankton sample so enriched compared to this average value.

Preliminary results for δD in Mytilus samples from Negro Harbour give identical values to those of terrestrial (and marine) material, with the exception of the mussels at stations NH3 and NH10 of 1985 which show a depletion in D (-96, -97‰) compared to the other sites. The estimated trophic level effect is 0‰. NH3 also shows a discrepancy in fatty acid ratio. Should the depletion be an indication of a marine influence, these data would agree with FAME and carbon isotope data.

6.3.3. δD in biogenic silica

If the samples of plankton contained many diatoms, it is possible that water contained in the silica frustule contributes to the final δD of plankton, because the analysis procedure involves heating the sample to 550°C which would liberate water. In diatoms, the opaline silica frustule (covered by an organic skin) may represent up to 40% of the dry weight of the cell but this amount can vary by a factor of at least 5 depending on the availability of silicon in the surrounding

Table 6.8. δD values (SMOW) from a plankton sample, peat sample, the flesh and organic matrix of *Mytilus edulis*.

SAMPLE	δD(‰, SMOW)
Plankton	-77
Peat (0-50 cm)	-78
Mytilus edulis	
Flesh at NH0-1985	-79
Flesh at NH2-1985	-80
Flesh at NH3-1985	-96
Flesh at NH4-1985	-72
Flesh at NH10-1985	-97
Organic matrix at NH0-1985	-60
Organic matrix at NH2-1985	-67
Organic matrix at NH3-1985	-75
Organic matrix at NH4-1985	-70
Organic matrix at NH10-1985	-59

water (Parsons *et al.*, 1976). Table 6.9 shows the percentage of material left after heating two plankton samples in air at different temperatures. At 900°C what is left of the samples should be silica. The silica sample gave weight percentages of 46 and 57% of silica since organic carbon burns off at less than 650°C and calcium carbonate at about 800°C. Water is an essential constituent of naturally occuring opaline silicas. Most opals contain between 3 and 10% H_2O (Segnit *et al.*, 1965). This opaline silica ($SiO_2.nH_2O$) is dehydrated at high temperature. Knauth and Epstein (1975) reported that water extracted from marine opal by heating was depleted in D relative to seawater by -56 to -87‰. Some of the hydrogen in opal is readily exchangeable at 25°C, while other hydrogen was more resistant to isotopic exchange on a laboratory time scale. Starting from less than 2% of water in opal at room temperature, there is less than 1.5% left after dehydration at 100°C. δD of the extracted water varies between -17.6 and -79.6‰ for extraction temperatures between 25 and 1000°C. The combined δD value of these increments is -57‰. It has long been recognized that most of the water in opal is physically adsorbed, as most of it is driven off at 100°C (Frondel, 1962) and shown through differential thermal analysis (DTA) (Segnit *et al.*, 1965). All opals of the "amorphous" type examined by Segnit *et al.* (1965) gave no

Table 6.9 Weight and percentage of material left (opaline) of two plankton samples after heating experiment at 550° and 900° C.

TEMPERATURE	SAMPLE 1		SAMPLE 2	
	Weight (mg)	%	Weight (mg)	%
22°C (start)	157.8	100	330.6	100
550°C (2 hrs)	103.2	65	235.2	71
900°C (1 hr)	73.1	46	188.1	57

more than a broad shallow endotherm in their DTA curves and with a residual (non-exchangeable) water content of less than 3% (initially 10%) in a diatomite sample after heating. In our plankton sample we probably released most of the exchangeable water when preparing the sample since all samples were heated under vacuum at 100°C for 24 hours before extraction of the organic water. There is probably some high temperature water coming from the opaline in the plankton sample, but this can only be but a small contamination.

CHAPTER 7. STABLE ISOTOPE IN THE ORGANIC MATRIX OF SHELL

7.1. THEORETICAL REVIEW

Schimmelman *et al.* (1986) have analyzed the chitin of several crustaceans in order to determined its usefulness in reconstructing paleodiet (paleoenvironment) of these organisms. Schoeninger and DeNiro (1984), and Chisholm *et al.* (1982, 1983) have analyzed bone collagen for similar reasons. Its isotopic composition ($\delta^{13}C$, $\delta^{15}N$) reflects that of the animal's diet (DeNiro and Epstein, 1978, 1981), its chemistry is well defined, and its amino acid composition varies only slightly between species. The differences in the $\delta^{15}N$ and $\delta^{13}C$ values of collagen from different animals should reflect differences in the isotopic composition of their diet and not differences in chemical composition. Additionally, the choice of bone collagen allows analysis of the remains of animals from prehistoric and earlier periods if the bones still contain collagen. The collagen content of buried bones decreases by half in about 5,000 years.

Although the amino acid sequences of the organic matrix contained in mollusk shells is not perfectly known, the approach is the same.

Mineralized tissues are usually formed by the initial elaboration of a structural organic framework

composed of proteins and polysaccharides into which ions of the mineral phase permeate and crystallize (Lowenstam and Weiner, 1983; Weiner and Traub, 1984). Mollusks have adopted this so-called "organic-matrix-mediated" mineralization process (Lowenstam and Weiner, 1983). The mineral crystals are characteristically aligned in a preferred direction and often adopt habits distinctly different from their inorganically formed counterparts (Lowenstam, 1981).

While the organic matrix undoubtedly plays an important role in processes leading to the formation of mollusk shells, not all components of the matrix necessarily have a function in the mineralization process nor is mineralization the only function of the matrix. For example, the organic matrix appears to have a role in determining the mechanical properties of the shell (Currey and Taylor, 1974; Palmer, 1983).

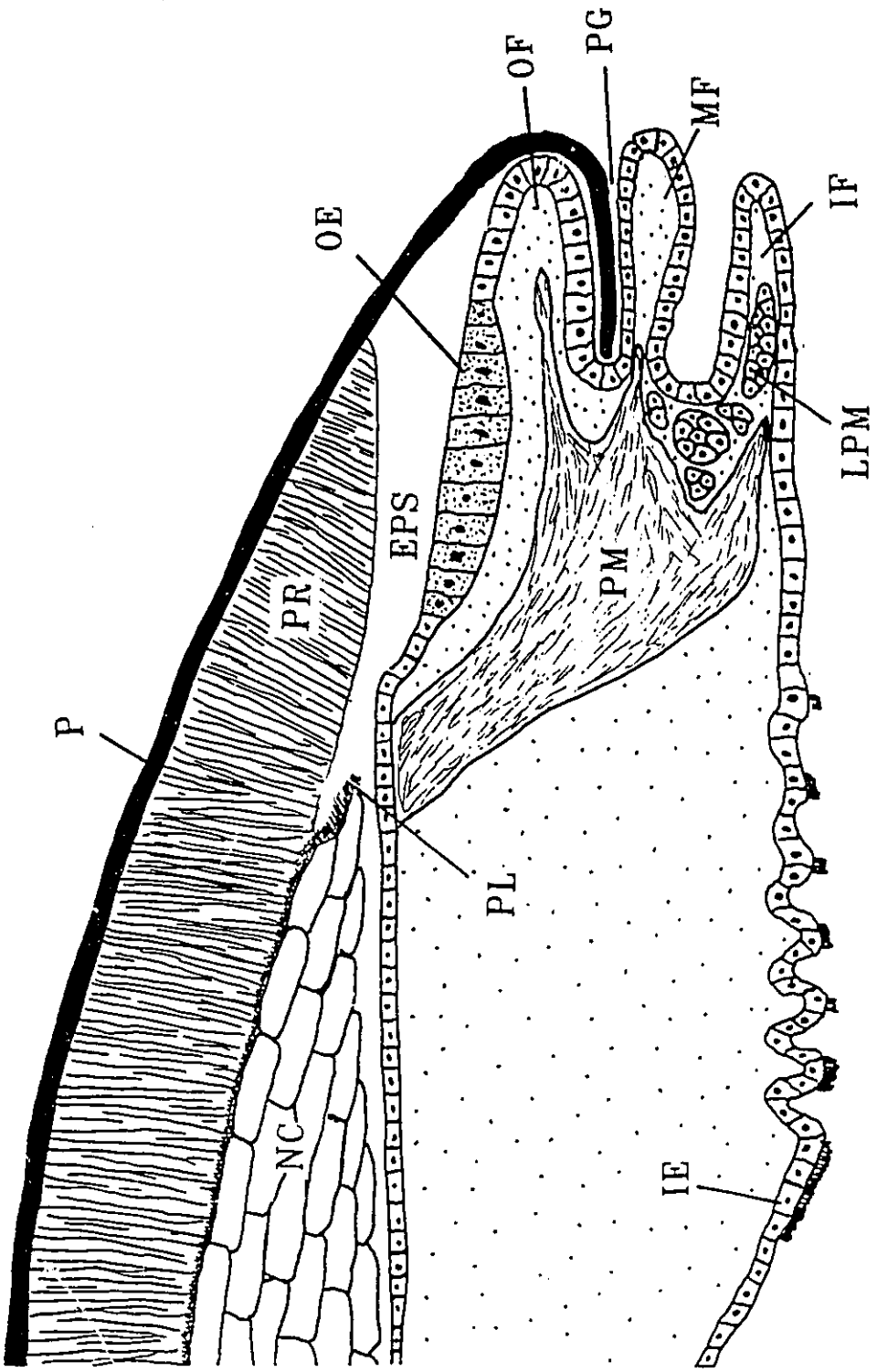
Biochemical analyses of mollusk shell organic matrices were initiated more than a century ago. These studies in essence report that the organic matrix generally comprises between 0.01 to almost 10% by weight of the shell, (Hare and Abelson, 1965) and is composed primarily of proteins and polysaccharides. In the analysis of shells of 14 species of estuarine bivalves a range of 1.4 to 21.4% was found (Price *et al.*, 1975). Reported values for a particular species vary

considerably. In addition Hare and Abelson (1965), studying the amino acid content of about one hundred different shells, found that the protein of related species are of similar composition, and noted large differences in species not closely related. They also found correlations between type of shell structures and the amount of organic matter contained in the shells. The nacreous and prismatic structures are apparently restricted to shells of high organic content, whereas the crossed lamellar structure covers a wider range. Moreover, protein amino acid composition differs between shell layers of the same species (Hare, 1963).

Shell formation can be described in terms of two major phases; (1) cellular processes of ion transport, protein synthesis, and secretion, and (2) a series of physicochemical processes in which crystals of $CaCO_3$ are nucleated, oriented, and grown in intimate association with the secreted organic matrix (Lutz, 1980).

The extrapallial fluid, found between the mantle and the shell (figure 7.1), is the microenvironment of shell deposition, with its inorganic substances contributed by the mantle. Whether an increase in shell growth will occur will depend ultimately on the condition within the extrapallial fluid and at the site of $CaCO_3$ deposition on the inner shell surface (Wilbur and Saleuddin, 1983). The inorganic ions of the extrapallial

Figure 7.1 Radial section of the mantle edge of a shell to show the relationship between the shell and mantle (not to scale). EPS, extrapallial space; IE, inner epithelium; IF, inner fold; LPM, longitudinal pallial muscle; MF, middle fold; NC, nacre; OE, outer epithelium; OF, outer fold; P, periostracum; PG, periostracum groove, PL, pallial line; PM, pallial muscle; PR, prismatic shell layer (after Lutz and Rhoads, 1980).


OF
PG
MF
IF
OE
LPM
EPS
PM
PR
P
PL
NC
IE

fluid are derived primarily from the hemolymph in the mantle after diffusion or active transport across the outer mantle epithelium. The organic compounds of extrapallial fluids include amino acids, proteins, mucopolysaccharides, organic acids, and probably lipids since lipids are found in shells (Wilbur and Simkiss, 1968). Peptides and free amino acids of extrapallial fluids have been given relatively little detailed attention.

The organic substances of mollusk shells can be obtained by dissolution of the mineralized layers of the shell. This procedure (Weiner, 1979) yields two fractions, one that is soluble and the other insoluble in aqueous solutions.

The soluble matrix has been regarded to be intracrystalline (Crenshaw, 1972; Meenakshi *et al.*, 1971), while the insoluble matrix is thought to be intercrystalline (Gregoire, 1972). The concentration of soluble matrix components varies widely amoung species, ranging from 14 to 64% (Wilbur, 1976). The soluble matrices from bivalves and cephalopods are 40 to 80% protein. The protein core may be a polymer of (Asp-Y)n, where Y is predominantly glycine or serine (Weiner and Hood, 1975). This regularly negatively charged aspartic acid molecule may function as a template upon which mineralization occurs (Weiner and Hood, 1975). Soluble

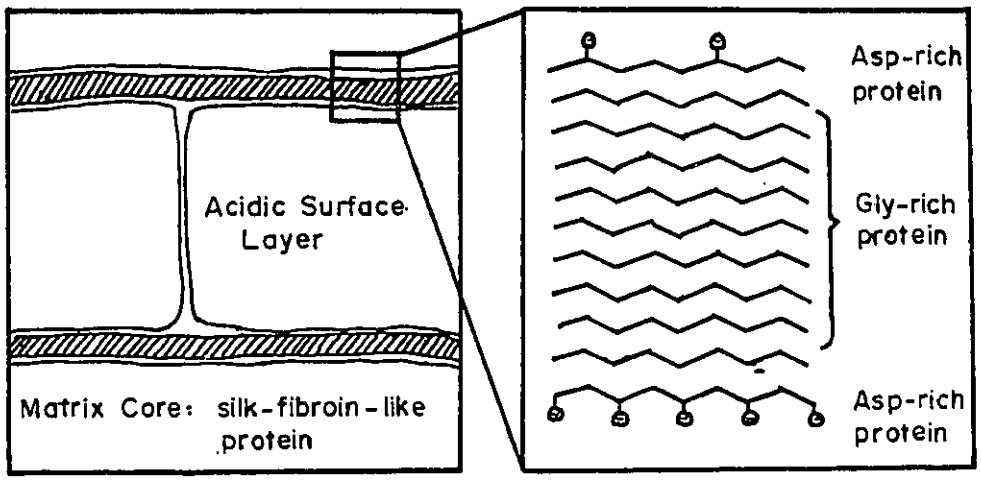
matrices from *Mercenaria mercenaria* and *Crassostrea virginica* have been reported to bind calcium (Crenshaw, 1972). However, the soluble matrix of *C. virginica* also prevents or reduces the growth of calcium carbonate crystals (Wheeler *et al.*, 1981).

The insoluble fraction is primarily composed of proteins rich in the amino acids glycine, alanine, phenylalanine, and tyrosine (Meenakshi *et al.*, 1971). The insolubility of this fraction is probably due, in part, to cross-linking of proteins by phenoloxidase, an enzyme which has been isolated from mollusk shells (Gordon and Carriker, 1980). Cross-linked polymerization of protein chains (commonly referred to as tanning or sclerotization) is a distinctive feature of biological materials serving a structural function (e.g. silks, collagen, hair, cuticules, etc.).

Weiner *et al.* (1982), and Weiner and Traub (1984) envisage an individual matrix layer to be generally composed of a core of silk-fibroin-like protein, covered on both surfaces by layers of soluble matrix constituents (figure 7.2).

The functions performed by different matrix constituents are still poorly understood. As a working hypothesis, Weiner and Traub (1981) have proposed that the silk-fibroin-like protein core, where present, acts primarily as a non-mineralized structural framework.

Figure 7.2 Layers of organic matrix (after Weiner *et al.*, 1982).


Acidic Surface Layer
Matrix Core: silk-fibroin-like protein
Asp-rich protein
Gly-rich protein
Asp-rich protein

Weiner *et al.* (1976) identified fossil shell proteins which still contain recognizable mollusk shell organic matrices.

The organic matrices of fossil shells are almost entirely soluble (Weiner and Lowenstam, 1979; Weiner *et al.*, 1979), despite the fact that the soluble fractions of living mollusk shell matrices generally only comprise the smaller fraction of the total organic matrix (Wilbur and Simkiss, 1968). The similarity in chromatographic behaviour of the soluble extant and fossil cephalopod matrices suggests that the original soluble fraction has been preferentially preserved (Weiner *et al.*, 1979). The preservation of the soluble matrix components could be due to the close interaction between these molecules and the bioinorganic phase which would provide a stabilizing influence on the organic material (Weiner and Lowenstam, 1979). Diagenesis, even in unusually well preserved fossil shells, does alter the properties of the organic matrix components (Weiner and Lowenstam, 1979). On the other hand, Ca-binding proteins containing functional polypeptide chains have also been detected in shells of 3 different fossil oysters from the Cretaceous and Jurassic period (Samata and Krampitz, 1982). Their chemical compositions and their physiochemical behavior were very much like those of recent oysters (*Crassostrea gigas*).

7.2. SHELL AND ORGANIC MATRICES OF *Mytilus edulis* AND *Arctica islandica*.

The organic matrices of two bivalves (*Mytilus edulis, Arctica islandica*) are used in this study, *Mytilus edulis* is the principal subject of the study being present at 8 sites in the estuary (NH0, NH1, NH2, NH3, NH4, NH6, NH7, NH10). *Arctica* was only found at site NH11.

In *Mytilus edulis* is found a 2-layered aragonitic and calcitic shell. The calcite occurs in an outer, finely prismatic layer and the aragonite occurs as an inner nacreous layer (figure 7.3). The shell of *Arctica islandica* is made of an aragonitic, homogeneous structure. The shell is dense and porcellaneous and is built up of carbonate granules set in an organic matrix (Taylor *et al.*, 1974).

Prismatic and nacreous structures have a larger amount of organic matrix than the other structures (Taylor *et al.*, 1974). A number of authors have worked on the organic matrix of *Mytilus edulis* or its relatives (table 7.1) .

7.3. EDTA-HCl comparison

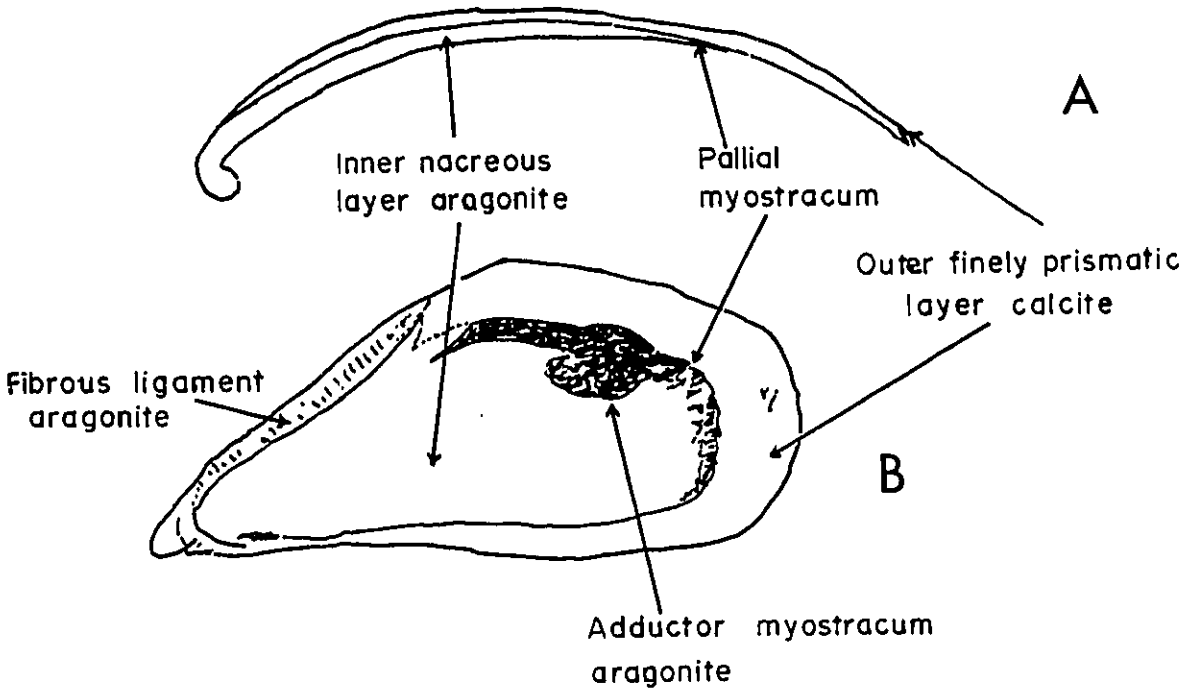
The percentage of total organic matrix (weight) recovered from EDTA dissolution averaged 1.08% for *Mytilus edulis* and 0.27% for *Arctica islandica*. The

Figure 7.3. Cross section of a *Mytilus edulis* shell displaying the outer calcitic layer and the inner aragonitic layer.

a) radial section showing the distribution of the shell layers.

b) General view of the shell interior.

From Taylor *et al.* (1973)



MYTILACEA: Mytilus edulis

Table 7.1 Studies on the shell and organic matrix of *Mytilus edulis* and relatives.

Grégoire, C. 1967	Prismatic structure
Grégoire, C. 1972a	Prismatic structure
Grégoire, C. 1972b	Structure of molluscan shell
Hare, P.E. 1963	Amino acid composition
Hudson, J.D. 1967	Elemental composition of organic matrices of different species
Price *et al.*, 1976	Percentage of organic matrix
Taylor, Kennedy and Hall. 1973	Shell structure.
Weiner, S., Lowenstam and Hood 1977	Soluble fraction
Weiner, S. and Traub, 1980. (protein conformation).	X-ray diffraction on the insoluble matrix
Weiner, S. 1983.	Soluble protein association with calcite deposition.

TABLE 7.2. Yields of organic matrix.

a) Test samples: replicates of single homogenized batch of *Mytilus edulis* and *Arctica islandica* shell.

SAMPLE MATRIX	SHELL weight (g)	ORGANIC MATRIX weight (g)	% ORGANIC
M-EDTA	5.04980	0.05775	1.14
M-EDTA	3.99595	0.04115	1.03
M-1N HCl	4.98130	0.03975	0.8
M-1N HCl	4.98600	0.03395	0.68
M-1N HCl	5.00370	0.05085	1.01
M-3N HCl	4.99180	0.04095	0.82
M-3N HCl	4.97685	0.02415	0.49
M-3N HCl	5.02515	0.02295	0.46
A-EDTA	5.06565	0.01355	0.27
A-1N HCl	6.57520	0.01790	0.27
A-3N HCl	5.03150	0.05450	1.08

M= *Mytilus edulis*; A= *Arctica islandica*

Table 7.2. Yields of organic matrix.

b) Samples of *Mytilus edulis* from sites in Negro Harbour estuary.

SAMPLE MATRIX	SHELL Weight (g)	ORGANIC MATRIX weight (g)	% ORGANIC
NH1.1-84	7.66100	0.08070	1.05
NH1.2-84	8.31400	0.06410	0.77
NH4.1-84	6.09000	0.04910	0.81
NH4.2-84	6.02400	0.04105	0.68
NH4.3-84	6.10600	0.03710	0.61
NH6.1-84	6.05200	0.04750	0.79
NH6.2-84	6.10600	0.03405	0.56
NH6.3-84	6.00500	0.04830	0.80
NH0.1-85	3.90045	0.02985	0.77
NH0.2-85	4.00440	0.01915	0.48
NH2.1-85	4.22675	0.03775	0.89
NH2.2-85	5.81665	0.05920	1.02
NH3.1-85	3.93105	0.02070	0.53
NH3.2-85	3.71170	0.02915	0.79
NH4.1-85	4.97770	0.02080	0.42
NH4.2-85	3.90695	0.03605	0.92
NH6.1-85A*	5.23845	0.06865	1.31
NH6.1-85B*	7.54630	0.03230	0.43
NH7.1-85	4.57830	0.01985	0.43
NH7.2-85	4.19205	0.03040	0.72
NH10.1-85	4.01145	0.01615	0.40
NH10.2-85	3.69770	0.02505	0.68
NH11.1-85*	6.57520	0.01790	0.27

A* = Aragonite

B* = Bulk

NH11* = A. islandica

percentage obtained through mild acidic (1N HCl) dissolution are comparable, with an average of 0.83% and 0.27% for *Mytilus edulis* and *Arctica islandica* respectively (table 7.2). Average yields of organic matrix from *M. edulis* shell are lower (0.59%) when using a stronger concentration of the acid (3N HCl),and on the contrary are much higher in *A. islandica* (1.08%).

There were no differences between the $\delta^{13}C$ and $\delta^{15}N$ (see table 3.1. p19) of organic matrices after extraction with either EDTA or a mild solution of HCl (1N) as shown also in collagen extracted by the same two techniques (Tuross *et al.* 1988). Both EDTA and mild acid dissolution are comparable. The fact that both methods gave similar isotopic signature exclude a possible contamination by EDTA residue in the extract and/or a differential degradation of the protein's amino acids by the acid. But when using a stronger acid concentration (3N HCl), there is a marked enrichment in the organic matrix in ^{15}N and a slight loss in ^{13}C in *Mytilus edulis* and an enrichment in ^{13}C from the organic matrix of *Arctica islandica*. The variations observed in the present study could be due to selective hydrolysis or degradation of specific amino acids. Akiyama (1978) studied the effect of diagenesis on polypeptide chains and found that serine residues in peptide linkages may be preferentially altered to glycine and alanine as observed

in degradation of free molecules of serine by heating (with 6N at 150°C). The most abundant amino acids in *Mytilus edulis* are glycine, alanine, serine and aspartic acid (Hare and Abelson, 1965). Weiner and Lowenstam (1978) found that the extent to which the total organic matrix has been degraded is illustrated by the fact that the amino acid composition of the undialyzable fraction is greatly enriched in stable amino acid like glycine, alanine and glutamic acid and contains small amounts of serine, tyrosine and aspartic acid. Serine is the non-essential amino acid most depleted in ^{15}N, and it can be derived from glycine or alanine by hydroxypyruvate-amino transferase reactions, which cause a further depletion in ^{15}N (Macko *et al.*, 1982a). Because of the large number of distinct atoms, carbon isotope fractionations are more complex . Glycine, tyrosine and serine were the most enriched in ^{13}C and leucine, aspartic acid and alanine were the most depleted (Hare and Esters, 1983). This differential loss or degradation of amino acids could explain the enrichment, especially in ^{15}N, in the organic matrices extracted with strong acid, since serine is lost through acid extraction and being the most depleted amino acid in ^{15}N, could cause an enrichment in ^{15}N in the residual organic matrix. The difference in $\delta^{13}C$ is not as large as for $\delta^{15}N$.

7.4. RESULTS FROM NEGRO HARBOUR.

7.4.1 CARBON ISOTOPE RATIOS

$\delta^{13}C$ of the organic matrix is similar at NH4 but not at NH6 over the two year period. Although there is insufficient data to confirm that the organic matrix corresponds to a life-time average of diet, I continue to support this assumption. Unusual variations in the river discharge should not affect the $\delta^{13}C$ of the organic matrix (table 6.1 and 6.2). The curve for the organic matrix does not follow the same trend as for the soft tissues (flesh) of the bivalves and the stomach contents (figures 6.1 and 6.2). The difference between the organic matrix of *M. edulis* and the flesh varies between 1.1 and 2.9‰ in 1985 and between 1.9 and 3.8‰ in 1984. There is however a major peak (enrichment) at site NH4-1985 which indicates uptake of a significant amount of marine material. The fractionation between the flesh and the organic matrix in *Arctica islandica* is only 0.7‰.

We wish to determine whether isotopic composition of the matrix of the aragonitic part of bivalve shell (if two types of polymorphs are present) was the same as the calcitic fraction. The outer calcitic layer was mechanically removed from the shell of *Mytilus edulis* to leave only the aragonitic layer. The matrix was extracted from this aragonite to compare with the organic matrix extracted from a bulk sample (calcite and aragonite).

There was no isotopic difference between the organic matrix extracted from the aragonite and the matrix extracted from a bulk sample (table 6.2). So change in the ratio aragonite/calcite due to environmental fluctuations like salinity and temperature (Dodd, 1963) should not interfere in the determination of $\delta^{13}C$ of the organic matrix of bivalve shells which are made of two different polymorphs of Ca-carbonates.

7.4.2 NITROGEN ISOTOPE RATIOS

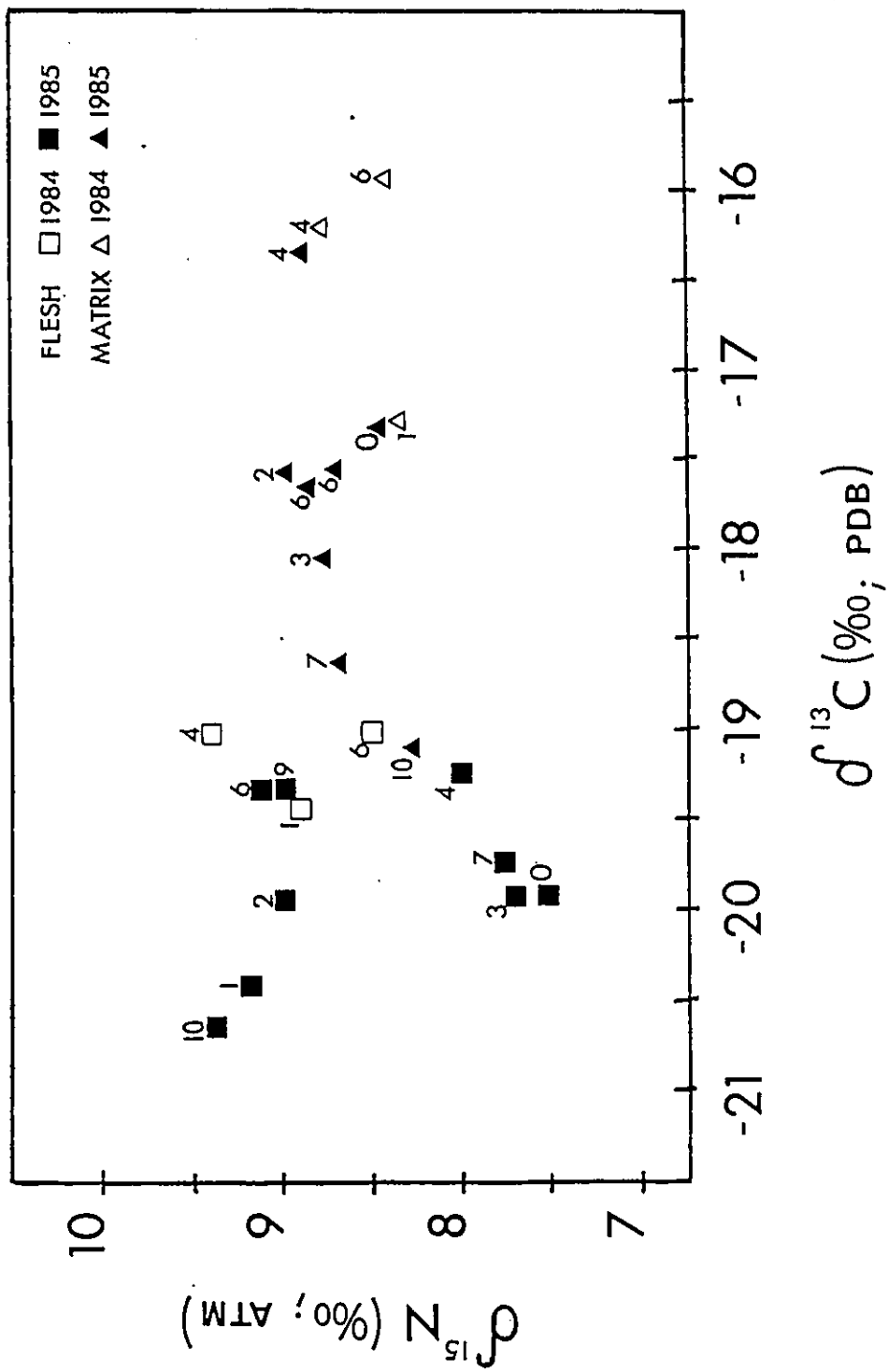
The organic matrices extracted from *Mytilus edulis* shells have $\delta^{15}N$ values that are similar from one year to the next as for the $\delta^{13}C$ data (table 6.4 and 6.5). There is almost no change in the organic matrices throughout the estuary (figures 6.4, 6.5). The organic matrices have $\delta^{15}N$ values comparable to those of the corresponding flesh sample. The organic matrix was on average enriched by about 1.6‰ compared with the guts, with a few exceptions like NH4 (1984). At this station there is an enrichment of about 4‰. This enrichement corresponds to a depletion in ^{15}N of the stomach contents which also followed the sediment $\delta^{15}N$ pattern. Otherwise, the $\delta^{15}N$ value at NH4 agrees with the other stations, suggesting the presence of a non-digestable material with low $\delta^{15}N$ (terrestrial) value like peat, for example. The same exclusion of low ^{15}N material is observed in the

flesh of *Mytilus edulis*. Overall the $\delta^{15}N$ in the flesh are slightly depleted (-0.6‰) relative to the organic matrices in 1985 and are slightly enriched in 1984 (+0.3‰). The change is not large, but the lighter values in the flesh of the mussels in 1985 could indicate a change in diet to one which contains a more significant amount of terrestrial material than in 1984. This statement agrees with the $\delta^{13}C$ records in the flesh and sediment analyses.

7.5. COMPARISON BETWEEN $\delta^{13}C$ AND $\delta^{15}N$ RESULTS

When comparing $\delta^{15}N$ and $\delta^{13}C$ data for both organic matrix and flesh samples (figure 7.4), one can see that the flesh values vary between stations with respect to both nitrogen and carbon in an irregular fashion. But the organic matrix isotope ratios vary predominantly with respect to $\delta^{13}C$. With respect to the diet, the long term significance of the organic matrix indicates that the mussels get their nitrogen supply mainly from the marine source (plankton). Although there are short-term variations in $\delta^{15}N$ of the flesh (~2‰), the values get averaged out in the matrix. Terrestrial detritus is a negligible source of nitrogen to the mussels. Terrestrial carbon, is on the other hand a significant source to the mussels at least in the upper estuary where the terrestrial input is most important.

Figure 7.4. Plot of $\delta^{13}C$ values in the flesh and organic matrix at all sites against its corresponding $\delta^{15}N$ value.


FLESH □1984 ■1985
MATRIX △1984 ▲1985
$\delta^{15}N$ (‰; ATM)
$\delta^{13}C$ (‰; PDB)
10
9
8
7
-21
-20
-19
-18
-17
-16

7.6. DISCUSSION

The organic matrices of fossil mollusc shells have been extensively studied, following the suggestion by Abelson (1954) that this material could provide valuable information to further our understanding of molecular evolution. Indication that the organic matrix could be use as an indicator of diet has been only scarcely noted (DeNiro and Epstein, 1978; 1981; Croker *et al.*, 1986). One of the few studies on the importance of the organic matrix as diet indicator was that of Croker *et al.* (1986). These authors measured $\delta^{13}C$ values of the insoluble organic matter in modern *Nautilus* siphuncules in order to study changes in the $\delta^{13}C$ values of the food sources of *Nautilus* during its lifetime. They found that the animal's diet did not change before the spawning season. Another study by Weiner and Lowenstam (1978), compared the carbon isotopic composition of the total shell insoluble organic matrix of well-preserved specimens of two bivalves to the $\delta^{13}C$ of the organic matter in which they were found. At the two sites of study, shell organic matrix was slightly enriched in ^{13}C compared to the sediment's organic matter. The authors suggested that since the carbon isotopic composition of the fossil's organic matrices are much lighter than those reported for the extant shell matrices (Hare and Hoering, 1977) the fossil organic matrices themselves have likely

undergone diagenetic alteration. Our data for matrix obtained by 3N acid dissolution of shell, suggest that such an enrichment is possible, although we observed this difference in the case of *Arctica islandica* but not *Mytilus edulis*. This difference could also be due to the fractionation between the organic matter found in the sediment which approximates the food intake and the organic matrix.

Magwood (1985) did a study on mollusks from different habitats and their food intake, comparing both the $\delta^{13}C$ and $\delta^{15}N$ in the flesh and organic matrices. He concluded from his results, that the organic matrix could not be useful as a diet indicator because of the large and variable differences between it and the flesh. Data on the actual food intake (stomach content) is missing from this research. It is true, from the results of the present research, that a comparison between flesh (short term variations) and the organic matrices (average lifetime diet) is bound to encounter some differences, more so if there were changes in the food supply. It is also probable from Magwood's work that the fractionation (either carbon or nitrogen isotope ratios) between the diet and the organic matrix won't be the same for all bivalves since there are actual variations (Weiner and Lowenstam, 1978) in the biochemical composition of the organic matrix. But, one could always observe this

fractionation by studying the organisms in nature in a similar way as the present work on *Mytilus edulis*, as long as the species is extant.

$\delta^{13}C$ values of the organic matrix are enriched compared to $\delta^{13}C$ of flesh. This is not the case for nitrogen isotopic ratios. Both the flesh and the organic matrix are composed of proteins and any important $\delta^{15}N$ fractionation occurs during amino acid production prior to the formation of proteins. As long as the average amino acid composition of matrix is not significantly different from the flesh, then the fractionation should be small. The same argument should apply to $\delta^{13}C$ values, but it does not. The point is that, due to transamination, there is little $\delta^{15}N$ variation between most amino acids whereas the $\delta^{13}C$ values tend to differ between amino acids and especially between essential and non essential amino acids (Hare and Estep, 1983). The results on the amino acid content of a mantle sample of *Mytilus edulis* obtained through Dr. J.F. Wehmiller (Univ. of Delaware) show that the flesh of mussels is rich in glutamic acid, glycine and aspartic acid (table 7.3). These results compared very well with others studies (Hare, 1963, 1965). The organic matrix is composed mainly of glycine but also of alanine, serine and aspartic acid (Hare, 1965). Glycine is also abundant in collagen, and is conspiciously enriched in ^{13}C relative to the other

Table 7.3 Amino acid fractions (mole) in a flesh sample of *Mytilus edulis*. (Wehmiller, personal communication)

ACID	Aspartic	Threonine	Serine	Glutamic	
	0.111	0.059	0.065	0.144	0.38
NEUTRAL	Glycine	Alanine	Valine	Isoleucine	
	0.139	0.073	0.054	0.047	0.31
SULFUR/ AROMATIC	Leucine	Tyrosine	Phenyl-alanine	Methionine	
	0.076	0.025	0.033	0.024	0.16
BASIC	Histidine	Lysine	Arginine		
	0.020	0.080	0.05		0.15
					1.00

major amino acids (Hare and Estep, 1983). This could explain the enrichment in the organic matrix since glycine is not as predominant in the flesh tissues. This enrichment is also observed in collagen to the same degree (DeNiro and Epstein, 1978; Schwarcz *et al.* 1984). For $\delta^{15}N$ values, glutamic acid, aspartic acid and alanine are most enriched in ^{15}N and glycine, serine and arginine are the most depleted amino acids (Hare and Estep, 1983). The relative patterns of carbon and nitrogen isotope fractionations in amino acids are similar both for a specific organism cultured by vastly different methods and between algae and bacteria (Macko and Ester 1983). Also, nitrogen in the flesh and organic matrix is mainly bound into amino acid and protein, while the carbon in the flesh is bound into carbohydrate, lipids and proteins. This can lead to a difference in $\delta^{13}C$ between these two tissue (flesh and organic matrix) but may not necessarily cause a difference in $\delta^{15}N$. The same phenomenon is observed in collagen (DeNiro and Epstein, 1978; 1981) as the nitrogen isotopic fractionation between the collagen and the flesh is close to 0‰ whereas the carbon isotopic fractionation is about 4‰.

It is possible that the organic matrix of shell can be used as a paleodiet indicator. Its conformation and composition are very similar to collagen which is widely used in this way. Amino acids are by far the most

abundant organic components found in fossils, although the remains of lipids have also been found (Wycoff, 1970). However, there are changes in skeletal amino acids even in the best preserved fossils. The most obvious change is in absolute abundance. The greatest absolute loss of skeletal amino acids occurs very quickly, with about 80% disappearing in less than 2 million year, and possibly within the first few thousands or ten of thousands of years (Curry, 1988). There is often a clear trend of relatively rapid initial decay, followed by a period of much lower degeneration, itself followed by a further levelling off of the rate of decay (Akiyama, 1971). Such rapid initial decay can probably be attributed to the breakdown and leaching away of the more exposed intercrystaline molecules of the organic shell matrix. Miller and Hare (1980) studied the integrity of the carbonate matrix in molluscan fossils by amino acid geochronology. Their data indicate that there is a rapid decrease in total amino acid content of molluscan fossils during the earliest stages of diagenesis but the relative abundances of amino acids are not altered. They also found that some molluscan genera yield remarkably unchanged compositional patterns in sedimentary environments of exceptional preservation for 10^7 to 10^8 years.

Macko and Estep (1983) found that the isotopic

signatures from modern collagen are still present in the amino acids of fossil collagen. They also found that highly degraded collagen was more negative in $\delta^{13}C$ than that of an intact collagen from a similar fossil. Mollusc shells are an important constituent in the fossil record and their dense calcite or aragonite matrix provides a tight, inert medium for preserving organic matter. However, since there is a loss of material with time, the amount of shell needed to extract enough of organic material for isotopic analysis has to be increased by an order of magnitude. In the present study, the shell of *Mytilus edulis* yielded on average, 1% of insoluble organic matrix. The total amount of organic matter necessary for both carbon and nitrogen isotopic analyses, is about 15mg for one replicate. Thus, 1 gram of shell has to be dissolved. With a loss of 80% of organic material, 10 grams of shell would be necessary to obtained 20 mg (or so). It is also suggested to dissolve the fossil carbonate material in EDTA rather than acid. Although $\delta^{13}C$ and $\delta^{15}N$ values for collagen replicas obtained after HCl or EDTA demineralization were similar, Tuross *et al.* (1988) shown that the yield of collagen obtained with EDTA was consistently higher than extraction procedures that used HCl (1M). The same conclusion applied to molluscan organic matrix from the results of this study. Acid dissolution was retained in

the present study, because of a faster rate of decalcification. The use of EDTA dissolution with fossil material would also permit the recovery of soluble material since most organic matrix is almost entirely soluble in fossil shells. The fact that the organic matrices are almost entirely soluble after decalcification may be due to some diagenetic solubilization process of what was originally the insoluble fraction. The latter is in fact the most abundant component of the organic matrix. The soluble component of the matrix may also be more stable since it occurs in close association with the inorganic mineral phase.

CHAPTER 8. STABLE ISOTOPES IN THE CARBONATES OF MOLLUSC SHELLS

Carbon and oxygen isotope ratios of the carbonate of shells have been investigated since systematic differences between marine and freshwater shells can be observed (Clayton and Degens, 1959; Keith and Parker, 1965; Keith *et al.* 1964; Mook, 1971). Mollusks are believed to exert only a minimal vital effect on oxygen isotopic fractionation.

Oxygen isotopic composition of shells is determined by the temperature of the surrounding water (Epstein *et al.*, 1951, 1953; Craig, 1965), and by $\delta^{18}O$ of that water. The latter leads to a salinity effect which may be used to determine the average salinity of the water in which a shell grew (Mook, 1971; Eisma *et al.*, 1976). Changes in the environment (temperature and salinity) can thus be shown by analysing the shell carbonate which permits us to evaluate the influence of marine versus freswater input on a down-estuary trend. Correlation should be possible with the mussel's diet as inferred from other isotopic analyses as presented earlier.

8.1 THEORETICAL REVIEW

8.1.1 Oxygen isotopes

When a mollusk deposits its shell carbonate in isotopic equilibrium with the surrounding water, the oxygen isotopic composition depends on two factors: the ^{18}O-content of the water and the temperature at which the deposition takes place.

McCrea (1950) first demonstrated that there is a temperature-dependent isotope fractionation of the $^{18}O/^{16}O$ ratio between $CaCO_3$ and water. Epstein *et al.* (1953) showed that some molluscs also precipitated calcite in isotopic equilibrium and also that aragonite-calcite fractionation was small. A carbonate-water isotopic scale was determined by Epstein *et al.* (1951, 1953) and later modified by Craig (1965) to give the following paleotemperature equation:

$$T(^{o}C) = 16.9 - 4.2\ (\delta^{18}O_c - \delta^{18}O_w) + 0.13\ (\delta^{18}O_c - \delta^{18}O_w)^2$$

where $\delta^{18}O_c$ is the $\delta^{18}O$ of the sample CO_2 and $\delta^{18}O_w$ is the isotopic composition of the water in which the animal grew.

Epstein and Mayeda (1953) showed that there could be large variations in the ^{18}O content of natural waters. Variations of the $^{18}O/^{16}O$ ratio in the hydrosphere depends mainly on the fact that the vapor pressure of $H_2{}^{16}O$ is greater than that of $H_2{}^{18}O$. The amount of

isotopic fractionation changes with water temperature. Seawater is isotopically heavier than freshwater (Craig, 1965) and therefore the $^{18}O/^{16}O$ ratio of brackish water is linearly related to salinity. At constant salinity, therefore, and $^{18}O/^{16}O$ ratio of seawater, the incorporation of oxygen isotopes into the shell is influenced mainly by the temperature. Rye and Sommer (1980) reviewed the use of oxygen isotopes in paleothermometry and paleosalinity reconstruction.

8.1.2. Carbon isotopes

The principal inorganic reservoirs or sources of carbon, in order of decreasing carbon-13 are: (1) ocean water bicarbonate, with a $\delta^{13}C$ of about -2‰ (2) atmospheric carbon dioxide (CO_2) with a $\delta^{13}C$ of about -7‰ and (3) freshwater bicarbonate with a widely variable $\delta^{13}C$, generally less than -8‰. The land plant and humus reservoirs of carbon affect the bicarbonate, the food web and the biologic communities of continental waters with consequent ^{13}C-deficiency in those of continental waters relative to those of the ocean (Keith *et al.*, 1964).

Mook and Vogel (1968) analyzed a series of shells of several different bivalve species collected alive from 2 estuaries and found that $\delta^{18}O$ and $\delta^{13}C$ of the shell carbonates showed a linear relation. There also appeared

to be correlation between the isotopic contents and the freshwater/saltwater mixing ratio, the suggestion being that not only $\delta^{18}O$ (Epstein *et al.*, 1953) but also $\delta^{13}C$ of the shell, is determined by the chemical environment in which the mollusks live.

The temperature dependance of carbon isotope fractionation is not accurately known but Mook and Vogel (1968) report a $d\alpha(^{13}C)_{C-W}/dt = +0.07‰/^{o}C$.

7.1.3. $\delta^{13}C$ and $\delta^{18}O$ in bivalve shells.

Keith *et al.*(1964) found $\delta^{13}C$ values ranging from +4.2 to -1.7‰ in the shells of fossil marine mollusks and -0.6 to -15.2‰ in freshwater species. The freshwater shell samples were also ^{18}O-deficient, relative to the marine samples, an expected consequence of the low $\delta^{18}O$'s typical of freshwaters. Although the variation is small, both carbon and oxygen isotope ratios decrease toward the less saline environment (Seward, 1978).

Coastal waters cannot be expected to be purely marine. Since in brackish waters the $\delta^{13}C$ and $\delta^{18}O$ content are largely determined by the mixing ratio of river and sea water (Mook, 1970), the isotopic composition of the carbonate also depends on the salinity (Eisma *et al.*, 1976). $\delta^{18}O$ values of carbonates from marginal environments are difficult to interpret because the ^{18}O-content depends on both the temperature and the

isotopic composition of the water. Keith and Parker (1965) concluded that $\delta^{18}O$ could not be used as an indicator of the temperature of marginal environment and that it was more generally meaningful to relate the $\delta^{13}C$ and $\delta^{18}O$ of fossils to proximity to sources of continental carbon, rather than salinity. Along the same lines, Lloyd (1964) studied the isotopic composition of mollusk shells from the shallow waters of Florida Bay and attributed an observed landward decrease in $\delta^{13}C$ to the relative effect of CO_2, derived locally by oxidation of organic detritus and to an increase in the relative contribution of mangrove detritus over marine grass since there is a ^{13}C-deficiency in land plants compared to marine bicarbonate.

A problem when using shell carbonates is determining what polymorph was deposited; calcite or aragonite. At 25°C aragonite is 0.6‰enriched in ^{18}O relative to calcite (Tarutani *et al.* 1969), whereas the carbon isotope fractionation (@ 25°C) of calcite and aragonite with respect to dissolved bicarbonate were reported by Rubinson and Clayton (1969) as 0.9‰ and 2.7‰ respectively. The *Mytilus edulis* shell is made of an outer calcitic layer and inner aragonitic layer. Mussels form a thin inner layer of aragonite during each growing period and a rim of calcite along the edges. Only the calcitic edge was analyzed.

Recent stable isotope studies on carbonates have emphasised the role of temperature of deposition on $\delta^{18}O$ of bivalves (Brand *et al.*, 1987) which can be used to determine age at death for shell grown in temperate areas where the temperature of the water in the colder months is significantly different than in the warmer months (Margosian *et al.*, 1987). Also stable isotopes in shells can help to detect upwelling events (Killingley and Berger, 1979; Brand *et al.*, 1987).

8.2. RESULTS FROM NEGRO HARBOUR

The $\delta^{18}O$ values observed in *Mytilus edulis* shell calcite vary between -1.35 and 0.42‰ while the $\delta^{13}C$ varies between -0.23 and 0.29‰ (table 8.1). As expected, both $\delta^{13}C$ and $\delta^{18}O$ values became heavier down estuary with increasing salinity (and decreasing temperature). When these values are plotted against position down the estuary (figure 8.1) the $\delta^{18}O$ data parallel the low-tide salinity and are inverse to the low-tide temperature (figure 8.2). This is true mainly for the 1985 data. The 1984 data are too sparse to be conclusive. Apparently, both salinity and temperature seem to affect the δ-value of bivalve carbonate shells. There were more changes in 1985 than in 1984 in the salinity and temperature of the water at high and low tide.

If we take into account that $\delta^{13}C$ of carbonates

Table 8.1 $\delta^{13}C$ and $\delta^{18}O$ observed in *Mytilus edulis* shell calcite (replicates from opposite valves).

SAMPLE	$\delta^{13}C$ (‰, PDB)	$\delta^{18}O$ (‰, PDB)
NH4.1-84	0.10	0.38
NH6.1-84	0.17	-0.08
NH6.2-84	0.03	0.04
NH0.1-85	-0.14	-1.35
NH0.2-85	-0.23	-1.13
NH2.1-85	-0.02	-1.24
NH2.2-85	0.01	-0.93
NH3.1-85	0.05	-0.86
NH3.2-85	0.11	-0.87
NH4.1-85	0.28	-0.03
NH4.2-85	0.48	0.22
NH7.1-85	0.21	-1.04
NH7.2-85	0.29	-1.06
NH10.1-85	0.16	0.25
NH10.2-85	0.09	0.42

Figure 8.1. $\delta^{13}C$ and $\delta^{18}O$ values in the shell calcite of *Mytilus edulis* down estuary for 1984 and 1985.

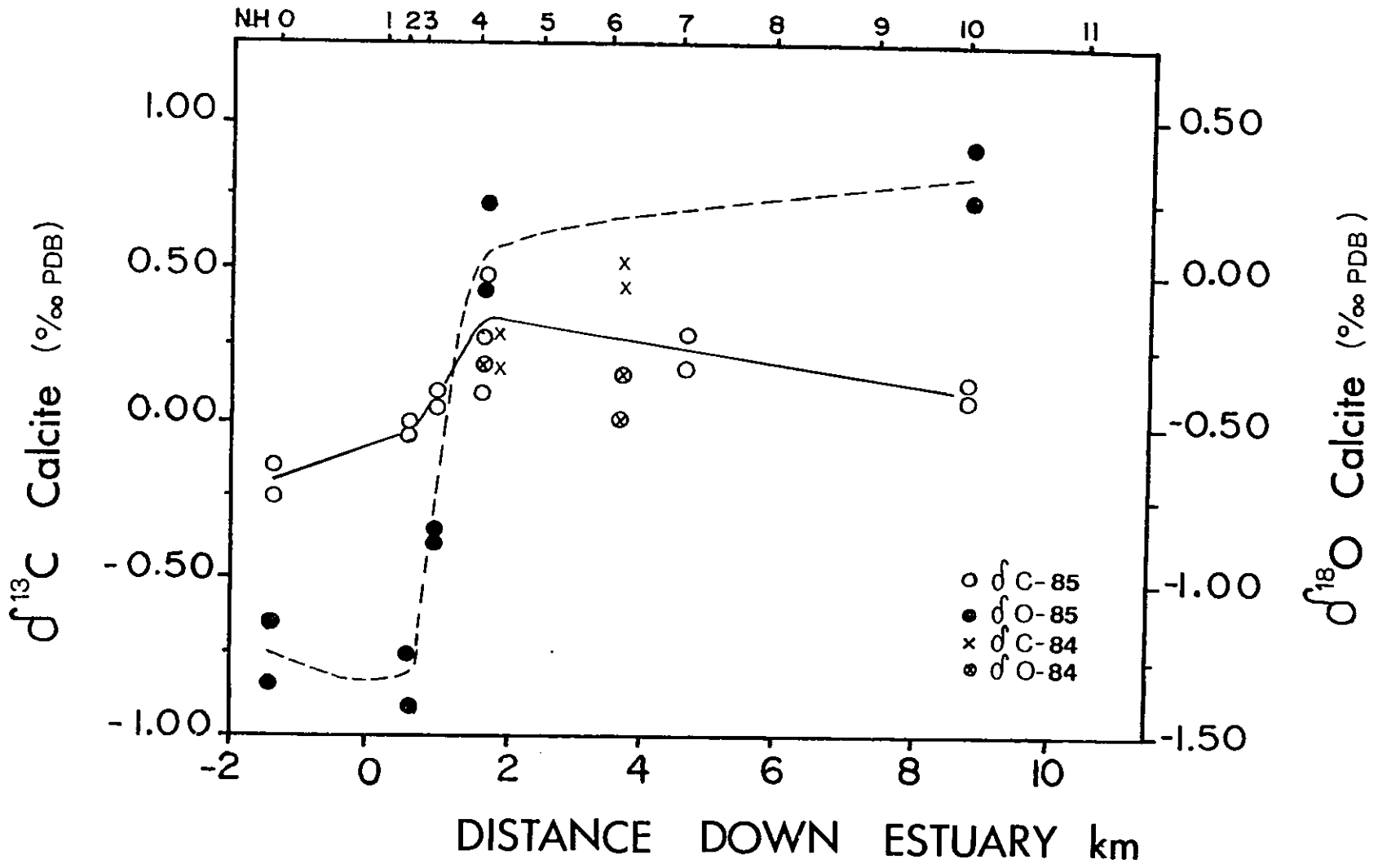
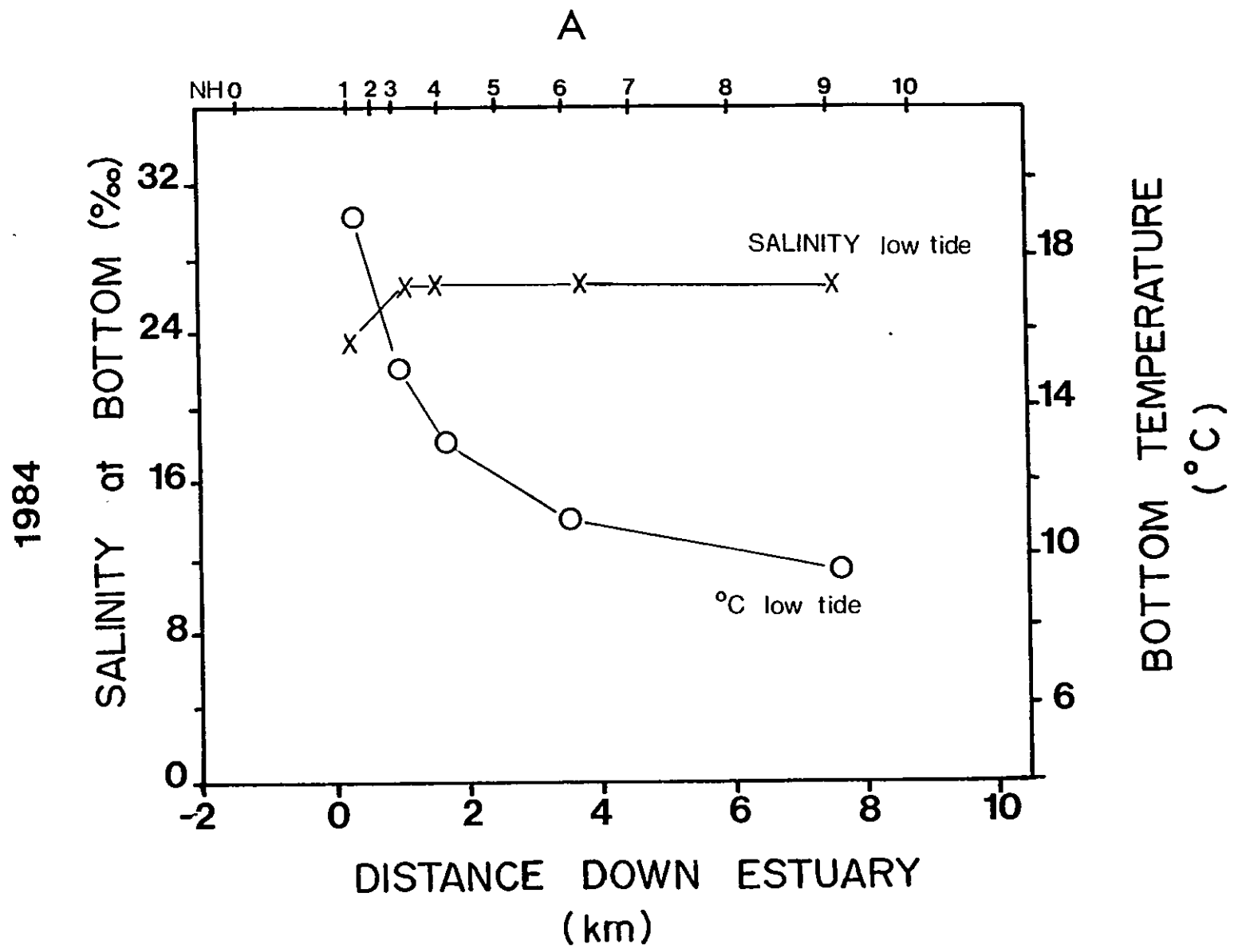
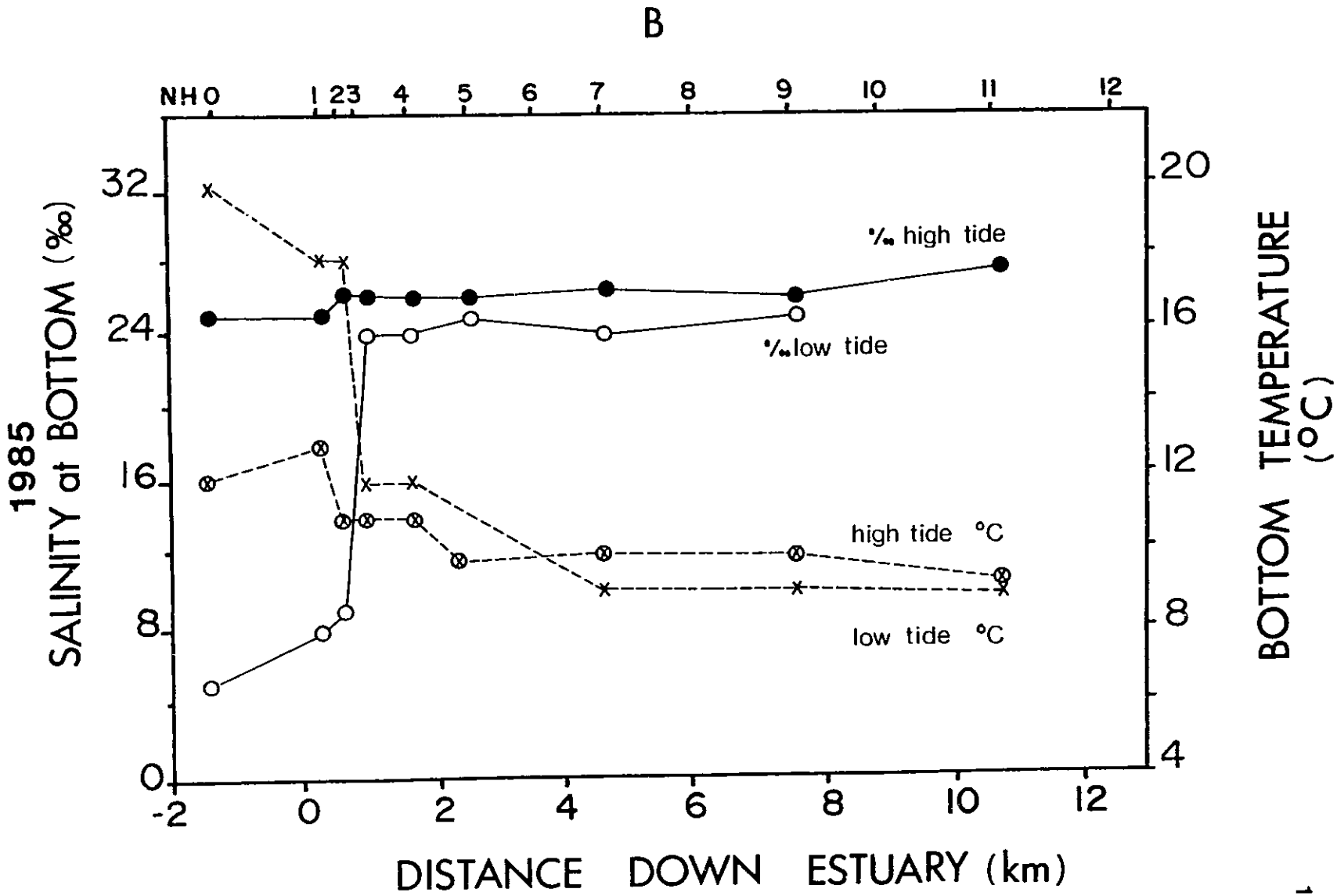

NH 0
1 2 3
4
5
6
7
8
9
10
11
1.00
0.50
0.00
-0.50
-1.00
0.50
0.00
-0.50
-1.00
-1.50
-2
0
2
4
6
8
10
δ13C Calcite (‰ PDB)
δ18O Calcite (‰ PDB)
DISTANCE DOWN ESTUARY km
δ C-85
δ O-85
δ C-84
δ O-84

Figure 8.2. Temperature and salinity gradient at low tide for the summer of 1984 and 1985.


A
NH 0
1 2 3 4 5 6 7 8 9 10
SALINITY low tide
°C low tide
SALINITY at BOTTOM (‰)
BOTTOM TEMPERATURE (°C)
1984
32
24
16
8
0
18
14
10
6
-2 0 2 4 6 8 10
DISTANCE DOWN ESTUARY
(km)


B
NH 0 1 2 3 4 5 6 7 8 9 10 11 12
32
24
16
8
0
20
16
12
8
4
-2 0 2 4 6 8 10 12
‰ high tide
‰ low tide
high tide °C
low tide °C
1985
SALINITY at BOTTOM (‰)
BOTTOM TEMPERATURE (°C)
DISTANCE DOWN ESTUARY (km)

is determined mainly by the source of dissolved inorganic carbon, the $\delta^{13}C$ values in the shells suggest that there is little variation in this carbon source downstream. As noted in section 8.1.3 it is to be expected that marine bicarbonate is the major source of carbon for shell deposition since the higher concentration of HCO_3^- in seawater masks the freshwater carbon contribution.

No seawater samples were collected during the surveys. However, if we assume that the shell grew in oxygen isotope equilibrium with the water, then we can estimate the $\delta^{18}O$ of the marine water component in the estuary ($\delta^{18}O_w$), knowing that the temperature of the estuary remains at 9°C (for a salinity of 28‰) near the mouth of the harbour during the full tidal cycle and for both consecutive years. The $\delta^{18}O_c$ estimate is taken to be the two values obtained at NH10, which are 0.25 and 0.42‰. The corresponding $\delta^{18}O_w$ values found through the paleotemperature equation are -1.58 and -1.44‰ respectively, the average value being -1.51‰. This value falls between the values of -2.56‰ by Margosian *et al.* (1987), taken outside an harbour also on the southwest coast of N. Scotia and of -1.04‰ by Epstein and Mayeda (1953) from the open ocean off the Maine coast.

To verify if *Mytilus edulis* deposits its shell in equilibrium with the seawater , we can calculate what the $\delta^{18}O_c$ should be at a known temperature and salinity. A

$\delta^{18}O_w$ *versus* salinity profile can be used to calculate this $\delta^{18}O_c$ at the different sites. The $\delta^{18}O$ of the freshwater component was obtained through the water samples collected in summer and winter at CR1 by Dr R.A. Bourbonniere of the Canada Centre for Inland Waters (table 6.7). CR5 is situated at the head of the estuary and correspond to a mixture of fresh and seawater. Figure 8.3 shows the expected variation in $\delta^{18}O$ downstream against the salinity and assuming a simple mixing of this freshwater ($\delta^{18}O$= -6.0‰ at a salinity of 0‰ with seawater with a $\delta^{18}O$ of -1.5‰ and salinity of 28‰. $\delta^{18}O$ of the water at CR5 (3.7‰) and its average salinity (16 parts per thousand) were also plotted and fell on the line. At each point in the estuary with a known average salinity, we can estimate the $\delta^{18}O$ of the ambient water at the time of deposition (table 8.2). This $\delta^{18}O_w$ is then used in the paleotemperature equation to give the $\delta^{18}O$ of the carbonates with a known temperature at the time of collection. These values are shown compared to the observed $\delta^{18}O_c$ given in table 8.1 and figure 8.4. Except for NH0 and NH2, where there are differences of about +1.8‰ between the observed value and the calculated one, all the observed ratios are close to the calculated values, showing that there is approximate isotopic equilibrium between the calcite and the ambient water. The value (-0.46‰) calculated for both NH3 and NH4

Table 8.2 Calculated and observed averaged $\delta^{18}O$ in *Mytilus edulis* shell calcite ($\delta^{18}O_{cal}$, $\delta^{18}O_{ob}$), $\delta^{18}O_w$ (extrapolated), the isotopic difference between the observed and calculated $\delta^{18}O$ and averaged temperature and salinity at time of collection.

SAMPLE	$\delta^{18}O_{ob}$ (‰)	$\delta^{18}O_{cal}$ (‰)	$\delta^{18}O_w$ (‰)	Δ_{ob-cal} (‰)	°C	Salinity
NH4-84	-0.64	+0.38	-1.60	+1.02	13.0	26.5
NH6-84	-0.13	-0.04	-1.75	+0.09	9.5	25.5
NH0-85	-3.28	-1.29	-3.50	+2.00	16.0	15.0
NH2-85	-2.66	-1.08	-3.25	+1.58	14.5	16.5
NH3-85	-0.46	-0.86	-1.80	-0.40	11.5	25.0
NH4-85	-0.46	+0.09	-1.80	-0.55	11.5	25.0
NH7-85	+0.12	-1.05	-1.60	-0.97	11.0*	26.5*

* Low tide only.

Figure 8.3 Expected variations in $\delta^{18}O$ values of the water relative to the salinity.

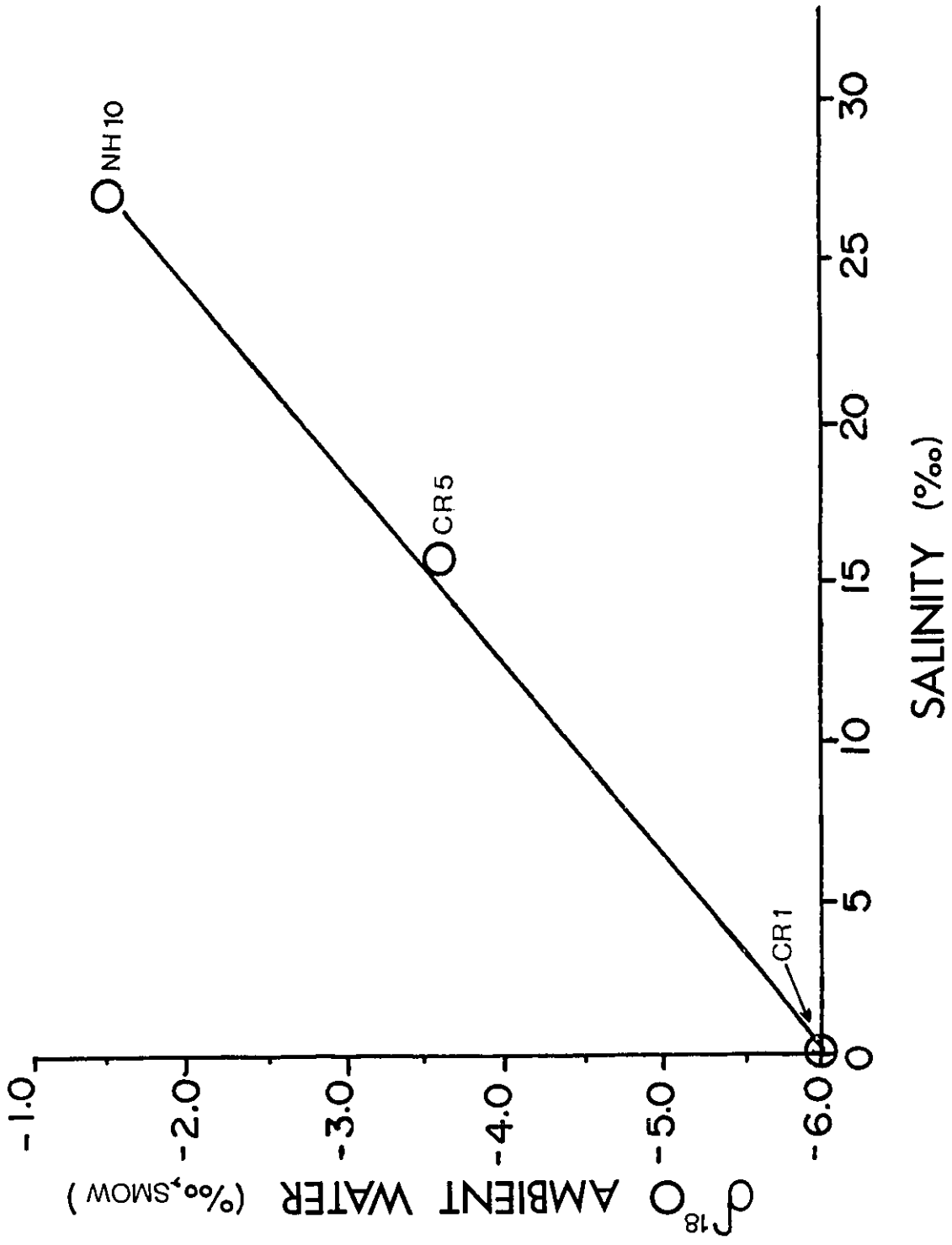
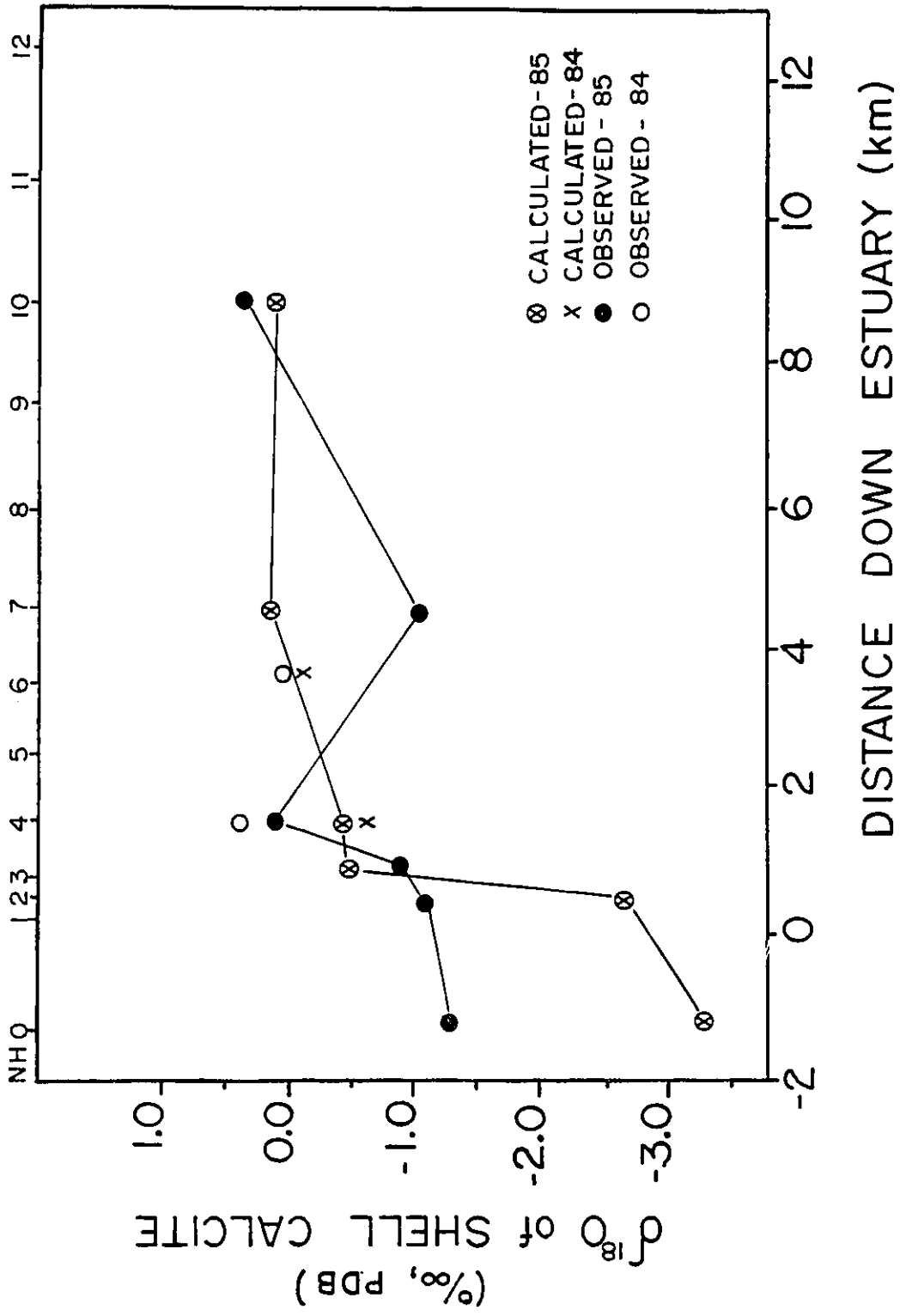

NH10
CR5
CR1
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
0
5
10
15
20
25
30
SALINITY (‰)
δ18O AMBIENT WATER (‰, SMOW)

Figure 8.4 Caculated and observed $\delta^{18}O$ in *Mytilus edulis* shell calcite, down estuary.


⊗ CALCULATED-85
x CALCULATED-84
● OBSERVED-85
○ OBSERVED-84
DISTANCE DOWN ESTUARY (km)
δ18O of SHELL CALCITE
(‰, PDB)
NHO
1.0
0.0
-1.0
-2.0
-3.0
-2
0
2
4
6
8
10
12

(1985) falls between the two observed ratios for these sites, so that there might be more variation in the temperature and salinity than what has been observed. The overall agreement is satisfactory, considering that $\delta^{18}O_{c-obs}$ averages over several years of growth whereas the calculated values are based on temperature and salinity values observed on a single day.

There seems to be some vital effect or environmental stress affecting the calcification process at NH0 and NH2, stations where there were large variations in temperature and salinity. One explanation could be that low salinity (down to 5 parts per thousand) could inhibit calcite or aragonite deposition. Malone and Dodd (1965) found that maximum calcification rates for *Mytilus edulis* increased with salinity. A lower salinity threshold for calcification between 14 and 20 parts per thousand is suggested by the results of their experiments. Their results do suggest that, in an environment with variable salinity, calcification is likely to be more rapid during times of high salinity. As for the effect of temperature, they found that at 5°C and lower the specimens appeared to be active with the valves open and the animal pumping, but calcification slow or nonexistent.

The bivalves *Mytilus edulis*, *Calliostoma* and *Clinocardium* from Lucy Island (B.C) precipitate shells in carbon isotopic equilibrium, but not oxygen, with ambient

seawater (Brand *et al.*, 1987). Brand *et al.* (1987) postulated that growth/ precipitation rate, is possibly triggered by temperature and/or nutrient supply causing a negative shift in $\delta^{18}O$ values. It is possible that *Mytilus edulis* requires certain temperature and/or nutrition level to calcify year round. Below these optimum water conditions, calcification is irregular and/or intermittent, or the rate is changed, which could account for the anomalous isotopic values observed in the shells of the bivalves from Lucy Island, B.C. as well as Negro Harbour. Brand *et al.* (1987) concluded that further studies are needed to determine why at one locality a specific organism shows no isotopic vital effects, whereas at others it does. This fact is of serious consequence to the use and interpretation of stable isotopes of fossils. The values reported here may also demonstrate the effect of an environmental stress on the calcification process.

CHAPTER 9. GENERAL CONCLUSIONS

Estuaries are very productive but also very vulnerable areas. A large proportion of the world's population is concentrated along the coastline and along the banks of rivers which drain into coastal waters. The double impact by man of adding pollutants while harvesting plants and animals places a great strain on coastal aquatic ecosystems (Mann, 1982).

Mytilus edulis is a more or less accepted index species for certain substances, and a popular species for pollution monitoring (Goldberg, 1976). Here different tissues of *Mytilus edulis* were analyzed to determine the importance of input of terrestrial matter in a small estuary.

First the terrestrial input in the estuary was followed through stable isotopes and fatty acid ratios in the sediments. The sediment $\delta^{13}C$, $\delta^{15}N$ and fatty acid ratios showed that accumulation of terrestrial organic matter is occuring between stations NH0 and NH4 (e.g. within 2km from the mouth of the Clyde river). The terrestrial influence was not as pronounced in the summer of 1984 as in 1985. The combination of stable isotopes and fatty acid ratios was very informative. The isotopic ratios shed light on the macrodetritus signal, while the

fatty acid ratios give information on the microdetritus.

Based on $\delta^{13}C$ values, it seems that *Mytilus edulis* can take up terrestrial material coming from the Clyde river. This is mainly shown by the gradient in $\delta^{13}C$. Also, the quantity of terrestrial material ingested was higher in 1985 since $\delta^{13}C$ values from the flesh and stomach content in 1985 are depleted by 1‰ compared to 1984. There is, however, a discrepancy between the $\delta^{13}C$ of sediments and the $\delta^{13}C$ of flesh and stomach contents which could be due to the fact that the terrestrial material does not pass through the mussel filtering system (or that it is too refractory to be ingested.)

The carbon isotope fractionation between *Mytilus edulis* and its food (assuming that the stomach contents represent the dietary input) is very regular (1-1.5‰ enrichment) and agrees with the literature. The carbon isotope fractionation between the organic matrix and the stomach content is more constant at about 4‰. Also there is no significant depletion in 1985 in the organic matrix as seen in the flesh, as expected, since this matrix represents a life-time average of the dietary input. The very dry season (1984) and very wet season (1985) gave us an idea of extreme conditions for *Mytilus edulis*. The organic matrix should represent the norm.

The fractionation between the flesh and the organic matrix seems to be dependent on species. The

organic matrix in *Arctica islandica* was enriched by only 0.7‰ compared to an average of 2‰ for *Mytilus edulis*. Could there be a relation between the type of shell structure (i.e. homogeneous, cross-lamellar,...) and the fractionation factor or is the fractionation dependent only on species? Further research on this is needed.

There seems to be less fractionation between the flesh and the organic matrix using $\delta^{15}N$. This is normal since the nitrogen both in the flesh and the organic matrix is bound into amino acids and protein. Also the main amino acids composing protein are isotopically uniform in both flesh and organic matrix. A change in the food source will be readily noticed.

Altogether the isotopic composition of *Mytilus edulis* was up by 1‰ for both carbon and nitrogen in 1985, relative to the dry year of 1984. This corresponds to an increase of about 20% in the terrestrial component of the diet.

In the fossil record, estuaries have been mainly studied using stable isotope ratios in carbonates. The results obtained in the present study showed that the use of carbonate to detect past estuarine environment can lead to misinterpretations due to biological effects in the upper part of the estuary. When freshwater input is too high the brackish species do not deposit their shells in equilibrium with the surrounding water, possibly

because the calcification process is inhibited. Therefore the carbonate data suggests a more saline environment (more positive $\delta^{18}O_c$ values) than was actually present.

Study of the organic matrix (present and fossil) or flesh (present) of estuarine bivalves might also lead one to conclude less terrestrial input than actually exists since bivalves can be selective as to the particle size and quality (refractory or labile) of the particulate organic material available for consumption. A thorough study of the sediments, tissues of bivalves and the carbonates of shells is required to demonstrate the input of terrestrial matter into an estuarine or any other coastal environment.

Mollusc shells are a common component of the fossil record and their dense calcite and/or aragonite matrix provides a tight, inert medium for preserving organic matter. The most abundant amino acids present in the organic matrix are very similar in composition and proportion to those found in collagen, i.e., glycine, aspartic acid, glutamic acid and serine. Thus, the organic matrix could be used in well preserved fossils to learn about the diet of an animal, as is the case for collagen in bone.All but serine are very stable during diagenesis (Macko and Estep, 1983). It is recommended that a deeper study on the use of the organic matrix as a paleodiet indicator should be done.

How do $\delta^{18}O_{calcite}$, $\delta^{13}C_{calcite}$, $\delta^{13}C_{org}$, $\delta^{15}N_{org}$ and δD compare as (palaeo)environmental indicators in estuaries? To best answer this question, it is important to understand which signals are best preserved with time and also least dependent on assumptions.

The isotopic signature of the organic material present in estuaries sediments is usually the result of a mixture of terrestrial and marine (planktonic) sources. The difference in the carbon and nitrogen stable isotopic composition between the two sources is sufficient to differentiate the two sources. The difference is ~ 6‰ for both $\delta^{13}C$ and $\delta^{15}N$. The stable isotope ratio of carbon is however more reliable than nitrogen because of its stablity with time and also because mollusc appears to be more selective as to their nitrogen source (e.g. plankton) than to their carbon source. The δD signature of terrestrial matter depends on geographical location. In the present study, the δD value of plankton and terrestrial matter were similar to one another, but the use of hydrogen in other areas is strongly recommended if only because of its apparent lack of fractionation between trophic levels which makes it easier to relate directly to original food source. The assumption of mixing of sources is usually easily defined. In cases where more than two sources could be present, the signal is harder to interpret.

Faunal remains are appropriate to use as monitors of trophic input. However, the real terrestrial input (here, to bivalves) can be misinterpreted if the studied species is a selective feeder either because the detritus are not filtered due to their size or are too refractory for assimilation. Comparison between the surrounding sediments and the bivalve tissues will give information on the selectivity of the animal.

Carbon and nitrogen isotopes have been used for studying palaeodiets using collagen in bone (Chisholm *et al.* 1982, 1983; Schwarcz *et al.* 1985; Tuross *et al.* 1988). Tuross *et al.* (1988) showed a lack of alteration in the chemical and isotopic compositions of the collagen from well preserved bones. Since the structure and composition of the organic matrix compares with that of collagen, the possibility of using the stable isotopes in the organic matrix of molluscan shell as paleoenvironmental tracers appears promising.

The use of stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) to study the environment through analysis of the carbonate of the shell is also an important field of research. However, there seems to be a wrong conception about the carbonate of the bivalve shell being in equilibrium with surrounding waters which can lead to misinterpretation of the paleoenvironment (salinity, temperature). Brand *et al.* (1987) noticed it in their study on bivalve

populations off the coast of British Colombia and the present study seems to confirm that there is a biological (stress-related) effect that inhibits calcification. In the present study this is due most probably to a drastic change of temperature and salinity during a tidal cycle at least in the upper part of the estuary. Thus, calculations through the paleotemperature equation of temperature would give a temperature at which bivalves can deposit its shell but not necessarily the actual averaged temperature of the surrounding waters. In any case, only well preserved samples, unaffected by diagenesis can be used for the carbonate analysis. Carbonates are easily exchangeable with ground water which can modify tremendously the isotope signature (Curry, 1988). The organic matrix in the shell would not necessarily be affected by these changes; this improves its chance of being a better (palaeo)environmental indicator. Also, the shell material is less subsceptible than bone (which is more porous) for contamination because of its closed system.

The use of stable isotopes to identify ancient environment through mollusks could only be done so far through carbonate analysis, but the potential use of the organic matrix as a paleodiet indicator opens a new and promising field of research.

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APPENDIX 1

GLOSSARY

DTA: Differential Thermal Analyses

EDTA: Ethylenediaminotetraacetic acid

FAME: Fatty Acid Methyl Esters

GCS: Grenville Calcite Standard

PDB: Pee Dee Belemnite Standard

POC: Particulate Organic Carbon

POM: Particulate Organic Matter

SMOW: Standard Mean Ocean Water

T_{fa}: Percentage Terrestrial Material Calculated With Fatty Acid Ratios

T_{ic}: Percentage Terrestrial Material Calculated With Stable Carbon Isotope Ratios

T_{in}: Percentage Terrestrial Material Calculated With Stable Nitrogen Isotope Ratios

TOC: Total Organic Carbon