AN ASSESSMENT OF THE IMPORTANCE OF TERRESTRIAL PRIMARY PRODUCTIVITY TO AN ARCTIC AND A TEMPERATE ESTUARINE TIDAL FLAT USING STABLE ISOTOPSE RATIOS OF CARBON AND NITROGEN

AN ASSESSMENT OF THE IMPORTANCE OF TERRESTRIAL PRIMARY PRODUCTIVITY TO AN ARCTIC AND A TEMPERATE ESTUARINE TIDAL FLAT USING STABLE ISOTOPE RATIOS OF CARBON AND NITROGEN

by

James P.A. Magwood

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ABSTRACT

The isotopic composition of the organic components of an animal's body, with respect to carbon and nitrogen, reflect the weighted average of the isotopic compositions of the animal's food sources, with a certain degree of enrichment in the heavier isotopes. Thus, by comparing the isotopic compositions of the animal and all the potential food sources, it is possible to ascertain the relative proportions of each availible food source in its diet, if the various food sources are sufficiently isotopically distinct.

This approach is particularly usefull in estuarine communities where food-webs tend to be complex and where there are several sources of primary productivity. In this study it was used on two types of clam in an arctic and a temperate estuarine tidal flat in order to assess the importance of terrestrially fixed organic matter to each community. The results indicated that while marine and terrestrial organics were important food sources in the arctic tidal flat, the clams in the temperate site depended mostly on marine organics.

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1. INTRODUCTION

Estuarine environments typically support complex food-webs. The organisms exibit a wide variation in habitat and life habit between and within species, especially with respect to feeding strategies. There are also many potential food sources including marine and terrestrial organic matter and primary productivity within estuaries themselves. (Gearing et.al, 1984; Haines and Montague, 1979, Incze et.al., 1982; and others)

The understarding of these food-webs is important for the management of these environments (Stephenson and Lyon, 1982). The complexity of the food-webs, together with the fact that detritus is a major food source in estuaries, makes it very difficult to assess the relative importance of any one source of primary productivity to a particular consumer organism in the system (Haines and Montague, 1979; McConnaughey and McRoy, 1979b; Peterson et.al., 1985).

Many attempts have been made to assess the proportion of the availible food sources making up a consumer's diet by using chemical tracers such as comparing fatty acids in consumers and plants , and by following radio-isotopes through food chains (Haines and Montague, 1979).

One of the newest and most promising methods is the comparison of the stable isotope ratios of common, important elements (C,N,S and H) in the organic components of consumers and their potential food sources. The basis of this method is that different souces of primary productivity carry different isotopic compositions and that these isotopic signatures are passed on to the consumer (DeNiro and Epstein, 1978 and 1981). The

average isotopic composition of the consumer is related to the average of all its food sources: thus, if the source isotopic signatures are sufficiently distinct and enough isotope sets are used, it is a simple mathematical problem to calculate the relative proportions of each source in the diet from the average isotopic compositions of the consumer (DeNiro and Epstein, 1978 and 1981).

The aim of this report is to use carbon and nitrogen stable isotope ratios as tracers of organic matter in food chains to compare the importance of terrestrially fixed organic matter to an arctic and a temperate estuar ne intertidal community. A filter-feeding and deposit feeding bivalve species was used at each site as test subjects. The carbon and nitrogen isotopic composition of the organic components of the clams, all the possible sources of primary productivity (terrestrial and marine) and sediment will be compared to this end.

This approach has been used extensively with respect to carbon isotopes, and more recently using nitrogen and sulphur isotopes as well, to assess the importance of various food sources to coastal and estuarine ecosystems where there are more than one isotopically distinct food sources availible (Haines, 1976; Haines and Montague, 1979; Incze et.al., 1982; McConnaughey and McRoy, 1979b; Peterson et.al., 1985; Stephenson and Lyon, 1982).

1.1 THEORY

There are three assumptions inherent in the use of this method (Gearing et.al., 1984).

1) Each source of primary productivity has a constant isotopic composition .

2) There is no fractionation of isotopes with the breakdown and decomposition of organic matter to form detritus.

3) There is little or no fractionation between an organism's body and its food.

None of these assumption is perfectly valid; however, the departures from the ideal condition can be accounted for so that a reliable picture may be ascertained.

Differences in the isotopic composition of organic matter can be caused by two factors. The inorganic sources may have different isotopic compositions, or there may be fractionation of the isotopes during uptake and fixing of organic compounds (Anderson and Arthur, 1983; Kaplan, 1983). Fractionation is caused by two effects. The kinetic effect results from the fact that in a given reaction different isotopes have different rates of reaction (Anderson and Arthur, 1983; Kaplan, 1983). Thus the products will become enriched in the isotope with the faster reaction rate (Anderson and Arthur, 1983; Kaplan, 1983). The magnitude of this effect decreases as the percentage of the pool of reactants used up increases. The equilibrium effect results from the fact that the thermodynamic stability of a compound depends on the isotopic composition

of its elements. If a system is undergoing a reversible reaction that involves isotopic exchange, the isotopes will redistribute themselves so as to maximize the stability of the system (Anderson and Arthur, 1983; Kaplan, 1983).

The differences in the carbon isotope composition of different sources of photosynthetically fixed carbon are primarily due to kinetic fractionation during the uptake of CO_2 (Anderson and Arthur, 1983). There is a preferential uptake of $^{12}CO_2$ at the leaf surface. There are further enrichments in ^{12}C during the fixing of CO_2 into organic acids. The magnitude of this depends on the the particular biochemical pathway used. C_4 plants exibit a much smaller fractionation than C_3 plants (Anderson and Arthur, 1983;Deines, 1980; Peterson et.al.,1985), possibly because C_4 plants utilize a greater proportion of captured carbon dioxide. There are also source differences as marime macrophytes take up dissolved bicarbonate ion which is enriched in ^{13}C relative to atmospheric CO_2 due to an equilibrium effect (Anderson and Arthur, 1983). Phytoplankton takes up dissolved CO_2 and is depleted in ^{13}C (Anderson and Arthur, 1983; Deines, 1980). This fractionation is temperature dependent, as the solubility of CO_2 decreases with increasing temperature and the phytoplankton use proportionately more of the availible carbon dioxide (Anderson and Arthur, 1983; Deines, 1980).

Nitrogen isotopes also show fractionation effects. N_2 fixation preferentially fixes the lighter isotope, ^{14}N. However, dissolved N_2 and NH_4^+ in soil waters in, equilibrium with the atmosphere, become enriched in ^{15}N (Kaplan, 1983; Letolle, 1980). Most plants take up prefixed

nitrogen in solution. Biological uptake results in enrichment in the lighter isotope (Letolle, 1980).

Most of the isotopic fractionation in the fixing of carbon and nitrogen in organic compounds are physical effects. Thus, concerning the first of the assumptions listed on page 2, different sources of primary productivity will have constant isotopic compositions if the physical conditions of the environment are constant (Gearing et.al., 1984). This must be taken into account when plant sources are not sampled directly.

Concerning the second assumption, processes occuring in the soil, mediated primarily by bacteria, are extremely complex (Letolle, 1980; Macko and Estep, 1983). The many biochemical processes exibit a wide variety of degrees of fractionation when isolated (Macko and Estep, 1983). It appears that, on average, the fractionation of carbon isotopes is negligible (Gearing et.al., 1984). It is not known yet what the overall effect is for nitrogen isotopes (Macko and Estep, 1983).

The third assumption was studied extensively by DeNiro and Epstein (1978 and 1980) resulting in the finding that there is a definite fractionation with the uptake of organic carbon and nitrogen. This fractionation leads to an enrichment in the animal of the heavier isotope in both cases (DeNiro and Epstein, 1978 and 1980). The fractionation in carbon is due to the faster rate of respiratory $^{12}CO_2$ loss compared to heavy CO_2 (DeNiro and Epstein, 1978). It appears to be independent of species or diet but there is significant variation among individuals (DeNiro and Epstein, 1978).

The magnitude of the fractionation was more variable for nitrogen, with significant variation between individuals, species and diets (DeNiro and Epstein, 1981). Variation with diet was the smallest of the three (DeNiro and Epstein, 1981).

The conclusions were that it was possible to get an estimate of the average isotopic composition of the diet from the isotopic composition of the whole body of the animal, especially if the assimilation fractionation factors are known (DeNiro and Epstein, 1978 and 1980). Thus the use of carbon and nitrogen stable isotope ratios to estimate the proportion of isotopically distinct food sources in the diet of an animal is a valid technique (DeNiro and Epstein, 1978 and 1980).

Some studies have been made on the biomagnification of ^{13}C and ^{15}N in natural, well understood food-webs (McConnaughey and McRoy, 1979b; Minagawa and Wada, 1984). These have shown that on average an enrichment in ^{13}C and ^{15}N, of about 1.5 and 3.5 permil respectively, occurs at each trophic exchange.

2. MATERIALS AND METHODS

2.1 SITE LOCATION

The two sample sites were Pangnirtung Fjord, on the Cumberland Penninsula, Baffin Island, and Lyle's Bay, in the Clyde River-Negro Harbour estuary, Nova Scotia (fig. 1). At each site the sampling stations were located on the tidal flats at elevations that were well covered at high tide and exposed or in shallow water at low tide.

At Pangnirtung, all the samples were taken at 6 sampling stations along a transect run perpendicular to shore on a section of the flat just south of the town site (fig. 2). This was the location judged to be the least influenced by fuel and waste pollution from the town site. At Lyle's Bay the samples were taken from four stations around the perimeter of the bay (fig. 3)

2.2 SAMPLE COLLECTION

At each of the two sites, representative filter-feeding and deposit-feeding clam species were chosen. At both sites the deposit-feeder choosen was *Macoma balthica*. The filter-feeder at Pangnirtung was *Hiatella arctica*, and at Lyle's Bay *Mya arenaria*. The same genus could not be sampled at both sites as *Hiatella* is a deep water form at temperate latitudes and *Mya* could not taken in quantity at Pangnirtung due to competition with the town's people.

FIG. 1 LOCATION MAP FOR COLLECTION SITES AND SITES FOR STUDIES FROM WHICH DATA WERE BORROWED

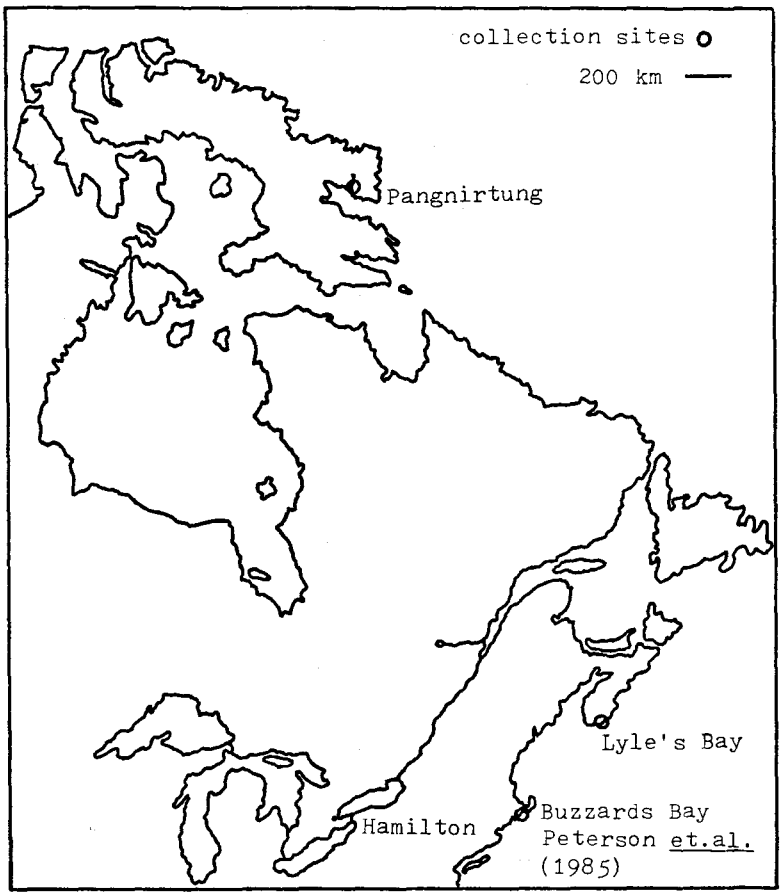


FIG. 2 SAMPLING STATIONS ON THE PANGNIRTUNG TIDAL FLAT

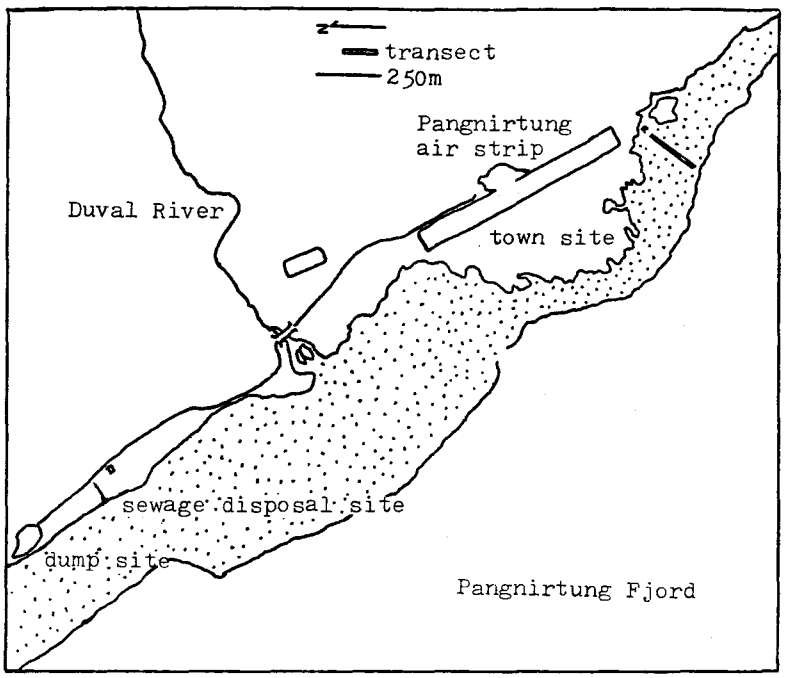
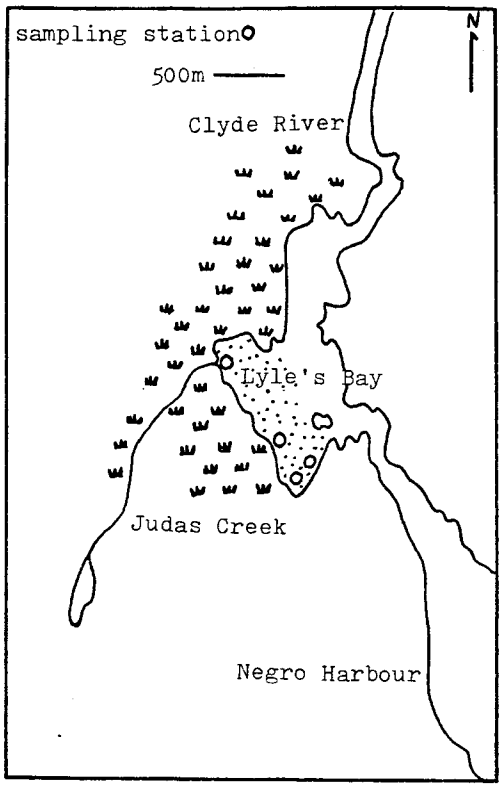


FIG. 3 SAMPLING STATIONS ON THE LYLE'S BAY TIDAL FLAT



At both, sites live clams of both species were taken at each station where found. A sample of the top five centimetres of sediment was also taken at each station. The clams at Pangnirtung were kept in fresh fjord water for 48 hours after sampling to allow them to evacuate their guts. Only the Macoma were able to survive this treatment, however, so that the Hiatella probably did not completely clear their guts. The clams from Lyle's Bay were not allowed to clear their guts. At both sites the clams and sediment were frozen within hours of sampling or the 48 hour cleaning period.

At Pangnirtung, samples of the potential food sources were also taken. These included samples of terrestrial vegetation, Fucus and seston samples. The terrestrial vegetation was taken just in from the landward end of the transect. Sampling was subjective, the majority of the samples being peat moss along with some grasses and woody shrubs. Fucus was cut from live specimens along the transect. Seston samples were taken by filtering 2 litre water samples through glass fibre filters. The water was collected off the seaward end of the transect at high tide. The plant samples and filters were frozen immediately after sampling. These samples were not available from Lyle's Bay.

Samples were taken on July 18 and August 4, 1984 at Pangnirtung and once in early August 1984 at Lyle's Bay. All samples were transported, frozen, to McMaster University for pretreatment and analysis with a mass spectrometer.

2.3 SAMPLE PREPARATION

To ensure that only organic carbon and nitogen were analysed each, type of sample was given a specific pretreatment prior to analysis on the mass spectrometer, as follows:

2.3.1 PLANTS (inluding *Fucus*)

The samples were thawed to room temperature then washed in distilled water. Samples were then freeze-dried under vacuum with liquid nitrogen. Dried samples were crushed to a powder.

2.3.2 CLAMS

Two components of the clams were analysed seperately, the body tissues (whole bodies) and the organic matrix of the shell. The samples were thawed to room temperature and the bodies were removed from the shell with a blunt knife blade.

2.3.2.1 BODY TISSUES

All pieces of periostracum and tissue with algal growths were removed. the bodies were then rinsed thoroughly in distilled water. Samples were then freeze dried under a vacuum with liquid nitrogen. No N_2 remains in the tissue as a result of this process (M.Knyf, personal comm.). The dried samples were then crushed. Samples were made up of a number of individuals from all stations at each site.

2.3.2.1 SHELL MATRIX (insoluble fraction)

The shells were all rasped to clean off any periostracum, remaining tissue, ligament or algal growths. The shells were then rinsed in distilled water, dried, and crushed into approximately 2 mm fragments. Five- to six-gram samples of shell fragments were placed in cellulose dialysis bags (20,000 MW) with distilled water. The bags were dialyzed against a saturated EDTA solution, buffered to pH=7 with a phosphate buffer, until all the $CaCO_3$ had been dissolved. This took about one month. The bags were then soaked in running water, kept cold to inhibit bacteria, for three days to dialyse away the EDTA. The bag contents were then centrifuged 3 times in distilled water, discarding the supernatant. The final pellet was the insoluble fraction of the organic shell matrix. This was oven dried at 90°C for 24 hours and then crushed to a powder. Samples were made up of all the individuals from a station.

2.3.3 SESTON

The glass fibre filters were dried at 90°C for 1 hour and shredded.

2.3.4 SEDIMENT

The sediment samples were thawed to room temperature. Samples were then washed in 3N HCl solution for 30 minutes to dissolve any carbonates, and left to settle. the washing proceedure was repeated twice more with distilled water. The samples were then oven dried for 24 hours at 90°C and crushed. All clams were removed from the sediment prior to pretreatment.

2.4 ISOTOPIC ANALYSIS

For analysis, the organic material was converted to CO_2 and N_2 gas which retains the same carbon or nitrogen isotopic composition as the carbon and nitrogen in the original organic materiel. The crushed samples were placed in glass tubes with purified cuprous oxide fragments (CuO) and the tubes vacuum sealed. The quantity of sample placed in the tube depended on the estimate of its organic content and the element to be analysed. For carbon isotope ratio analyses 1 to 5 micrograms of organic matter was needed, and for nitrogen 5 to 15 micrograms. The sealed tubes were baked at 550°C for 2 hours to react the organics with the CuO. The resulting CO_2 or N_2 gas was analysed on a VG MICROMASS 602D mass spectometer against standard CO_2 and N_2 gases. The raw data were corrected for ^{18}O, for the carbon analyses, and both were corrected to the appropriate international standard gases.

Isotopic ratios are expressed relative to a standard gas in the del notation as follows (Anderson and Arthur, 1983; Kaplan, 1983):

$$dX=((R_{sample}/R_{standard})-1)*1000 \text{ permil}$$

where X= the heavier isotope ^{13}C or ^{15}N

R= the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$

standards: carbonate from PEEDEE Belemnite (PDB) for carbon
atmospheric N_2 (ATM) for nitrogen

$$DdX_{b-a}=dX_b-dX_a$$

By this notation heavy isotope enrichment increases with del value.

The data were analysed for differences between classes using the Wilcoxon rank sum test (Mann-Whitney test) (Snedecor and Cochran, 1967).

3. RESULTS

3.1 COLLECTION SITES

Both sites were estuarine tidal flats.

The arctic site was located in a glacial U-shaped valley. The major river input was from the Weasel River which drains the Penny Ice Cap. The river bed consisted of gravel with some sparse vegetation. There were several smaller rivers emptying into the fjord closer to the sample site. These areas were covered in peat and small shrubs. The river water was clear and colourless. In July the fjord was covered with a lid of fresh water, salinity 7.3 permil, about 1 metre thick. The underlying water mass had a salinity of about 21 permil. In August the whole water column had a salinity of about 21 permil. The water temperature was 4.8°C for July and August.

The transect was located on a tidal flat consisting of mud and sand with a surface layer of fecal mud. The substrate was seen to contain woody and leafy detritus as well as Fucus detritus. The substrate was oxidized (brown) to a depth of about 5 cm, where it made a sharp transition to a reduced (black) condition. The dominant vegetation on the flats was marrophytic algae, mostly Fucus.

The temperate site was located in a brackish estuary (salinity>20,temp 10-15°C). The area around Lyle's Bay was a salt marsh where the dominant vegitation was the sea grass Spartina, a C_4 plant. The rivers had dark brown water indicating a heavy concentration of organic

acids. The rivers drained areas of forest and scrub, all of which were assumed to be C_3 plants. There was no major agriculture in the area. The tidal flat consisted of a sandy substrate with abundant Spartina detritus and some woody fragments. There was no obvious reduced layer in the upper 10 cm.

3.2 ISOTOPIC ANALYSES

The isotopic analyses reported here were made over a period of several days The precision of the mass spctrometer varied from day to day but the error in individual results was always less than .5 permil and usually less than .1 permil.

3.2.1 PLANT TISSUE

Plant and seston samples were only available from the arctic site. The results of the analyses are given in table 4. Owing to the low nitrogen content of these samples, only carbon analyses could be performed with the available equipment.

For the terrestrial plants the mean $d^{13}C_{PDB}$ value was -27.8 permil (SD=0.5,N=2). The mean $d^{13}C_{PDB}$ value for Fucus was -12.3 permil (SD=0.97,N=4). These values are within the ranges reported in the literature for these types of plant tissue (Anderson and Arthur, 1983; Haines, 1976; Haines and Montague, 1979; Peterson et.al., 1985).

Fragments of the seston filters were examined under binocular and compound microscopes. They contained abundant terrestrial plant debris and mineral grains, but no phytoplankton or zooplankton were seen on the filter fragments observed. Thus, at least in July and early August the fjord water contains insignificant quantities of plankton relative to the quantity of terrestrial debris. The mean $d^{13}C_{PDB}$ for the particulate organic matter on the filters was -25.7 permil (SD=1.4,N=4). This value is similar to the values for suspended particulate organic matter reported by Incze et. al. (1982) for the Sheepscot River estuary (Maine, USA) which drains a large area of terrestrial vegitation.

The mean $d^{13}C$ values of Fucus and seston were significantly different at the 5% level. The Fucus and terrestrial plant values were not significantly different at the 5% level, however, even the though the spread between the two ranges was greater (fig. 4). This indicates that the Mann-Whitney test may be inaccurate when small sample sizes (N=2,3) are used.

To supplement the lack of data for the Lyle's Bay site, data from a stable isotope study of the Sippewissett Marsh, part of Buzzards Bay Massachusetts, (Peterson et. al., 1985) were used. As this site is at a similar latitude and longitude as Lyle's Bay, the physical conditions at the two sites are likely to be similar. The $d^{13}C$ values are within the ranges qouted in other studies for similar samples at temperate latitudes (Anderson and Arthur, 1983; Gearing et.al., 1984).

3.2.2 CLAM TISSUES

The data from the clam soft tissue and shell matrix are listed in tables 1 and 2 respectively. It should be noted that both soft tissue and shell matrix samples are of pooled individuals. Thus the variance in data underestimates the variation among individuals. The soft body data furnished by C. Leblanc (fig.1) was from indidual clams. The standard deviations from these samples are an order of magnitude greater than the standard deviations for the same data classes of similar size from this report. This should be noted when interpreting the results of statistical analysis on clam tissue.

The following trends were observed in the isotopic composition of the clam tissues.

There was a seasonal shift in isotopic composition. From July 18 to August 4 there was depletion in ^{13}C of about 1 permil in the body tissues of both species. There were smaller seasonal shifts in the abundance of ^{15}N. However, none of the seasonal shifts were significantly different from 0 ($p>=0.1$ in all cases), although this may be due to the small sample sizes (N=3). The data for the two sampling dates were pooled for subsequent discusions.

In the arctic site the filter-feeding species was depleted in ^{13}C compared to the deposit-feeder by 1.8 permil in the body tissue and by 3 permil in the shell matrix. Both these differences were significant at probability level of 5% . The filter-feeder was also depleted in ^{15}N compared to the deposit-feeder by 2.6 permil in the shell matrix. This was significant at the 5% level. There was no significant difference in

In the temperate site there was no significant difference $d^{13}C$ between species in either the body tissues or shell matrix at the 5% level. However the filter-feeder had a significantly greater $d^{15}N$ in the body tissue (p=0.012) although this was not reflected in the shell matrix.

There was a consistant trend, at both sites, in the relationship between the isotopic composition of the whole body and the insoluble organic shell matrix (table 3). The matrix was always enriched in ^{13}C compared to the whole body and depleted in ^{15}N. There was a large range, however, in the degree of enrichment or depletion between species and sites. All but two of the $DdX_{shell\ matrix-body}$ were significantly different from 0 at the 5% level. The two exeptions were the Lyle's Bay Macoma ^{15}N and Mya ^{13}C.

3.2.3 SEDIMENT

The sediment $d^{13}C$ values are reported in table 4. As was the case in the plant samples, the low nitrogen content in the sediment prevented a nitrogen stable isotope analysis. No estimate could be made of the sediment $d^{15}N$ as the values for sediment are quite variable (Kaplan, 1983; Macko, 1983). The sediment ^{13}C abundance was similar for both sites and similar to values reported in the literature (Grearing et.al., 1984; Haines, 1976; Macko, 1983).

TABLE 1 CLAM SOFT TISSUE ANALYSES

PANGNIRTUNG				LYLE'S BAY	
MACOMA		HIATELLA		MACOMA	MYA
JULY	AUGUST	JULY	AUGUST	AUGUST	AUGUST
$d^{13}C_{PDB}$ (PERMIL)					
-19.637	-21.057	-21.807	-22.646	-16.776	-19.098
-19.961	-20.312	-21.617	-22.706	-16.759	-17.566
-19.451	-20.802	-21.498	-22.035	-16.646	-16.748
				-16.866	-16.702
			$\bar{X}$	-16.762	-17.529
				-15.910	-15.207
				-18.160	-16.634
				-16.376	-16.859
				-18.180	-19.450
				-17.440	-20.020
					-17.280
					-17.650
			$\bar{X}$*	-17.213	-17.400
$\bar{X}$	-19.683	-20.724	-21.641	-22.462	-17.013	-17.565
S_X	0.258	0.379	0.156	0.371	0.771	1.427
N	3	3	3	3	9	11
$d^{15}N_{ATM}$ (PERMIL)						
10.357	11.583	10.119	8.463	8.339	7.902
11.904	9.959	8.281	9.972	7.737	8.718
9.878	9.967	8.081			
			$\bar{X}$	8.038	8.310
				8.036	8.102
				8.526	8.676
				8.376	8.916
				7.427	8.916
				7.371	8.732
					11.045
					8.548
			$\bar{X}$*	7.954	8.991
$\bar{X}$	10.713	10.503	8.827	9.218	7.978	8.839
S_X	1.059	0.935	1.123	1.067	0.477	0.897
N	3	3	3	2	7	9

* data courtesy of C. LeBlanc

TABLE 2 CLAM SHELL INSOLUBLE ORGANIC MATRIX ANALYSES

	PANGNIRTUNG MACOMA	PANGNIRTUNG HIATELLA	LYLE'S BAY MACOMA	LYLE'S BAY MYA
	$d^{13}C_{PDB}$ (PERMIL)			
	-16.288	-19.248	-15.196	-16.466
	-16.068	-19.116	-15.252	-16.282
	-16.135	-18.979		-16.789
	-15.527	-18.890		
	-15.617			
	-16.734			
	-15.030			
	-15.935			
	-15.647			
$\bar{X}$	-16.006	-19.058	-15.224	-16.512
S_X	0.368	0.157	0.040	0.257
N	9	4	2	3
	$d^{15}N_{ATM}$ (PERMIL)			
	8.697	6.215	4.878	5.583
	8.904	6.468	6.251	5.794
	9.904	6.804		5.762
	8.951	6.877		
	9.229			
	9.701			
$\bar{X}$	9.192	6.591	5.565	5.713
S_X	0.418	0.308	0.971	0.114
N	6	4	2	3

TABLE 3 RELATIONSHIP BETWEEN THE MEAN ISOTOPIC COMPOSITION OF WHOLE CLAM BODIES AND THE INSOLUBLE ORGANIC SHELL MATRIX

	PANGNIRTUNG MACOMA	PANGNIRTUNG HIATELLA	LYLE'S BAY MACOMA	LYLE'S BAY MYA
(permil)				
$Dd^{13}C_{PDB}$ shell-body	4.198	2.994	1.789	1.053
$Dd^{15}N_{ATM}$ shell-body	-1.416	-2.432	-2.413	-3.126

TABLE 4 PLANT AND SEDIMENT ANALYSES

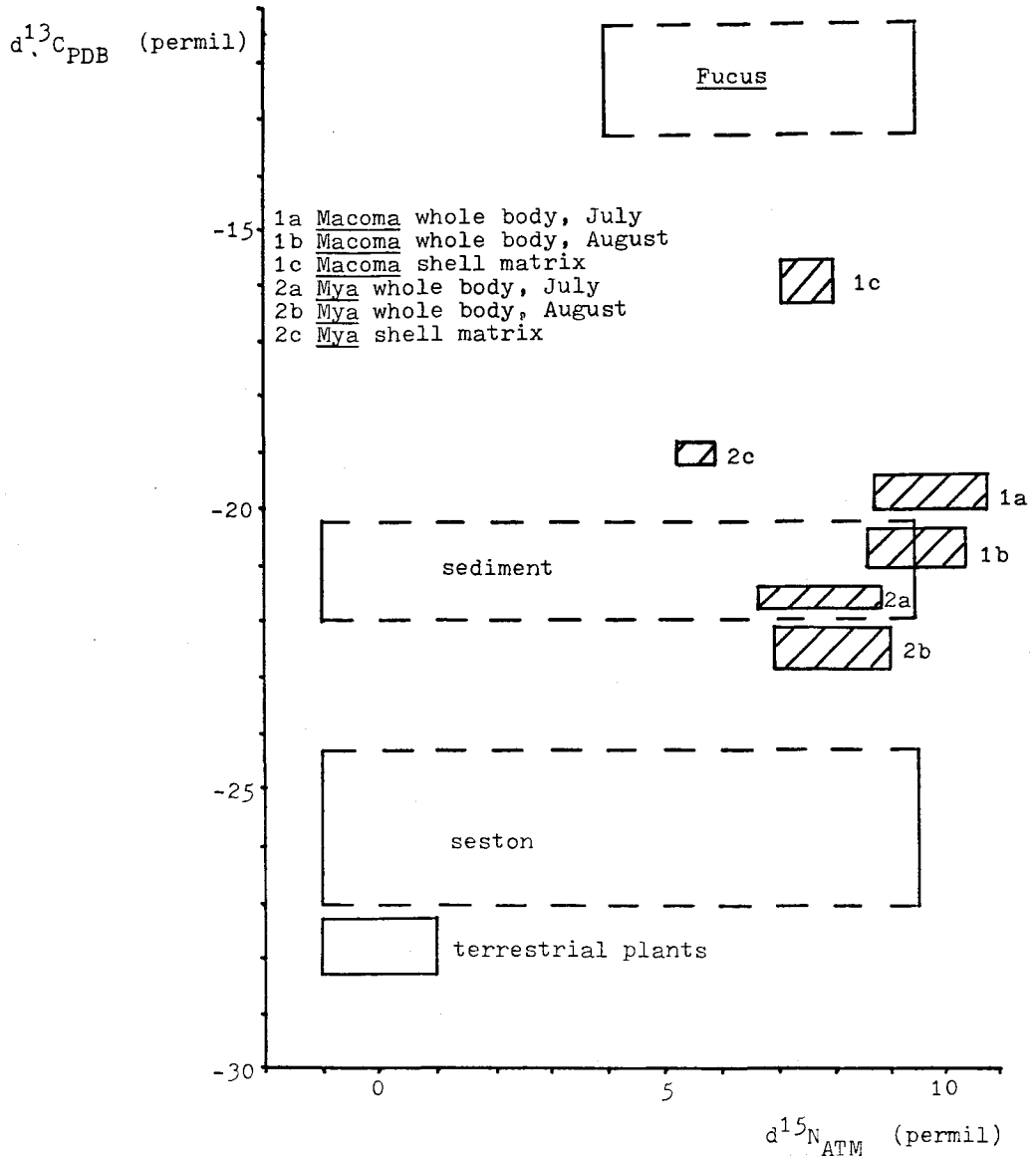
	PANGNIRTUNG FUCUS	LAND PLANTS	SESTON	SEDIMENT	LYLE'S BAY SEDIMENT
	$d^{13}C_{PDB}$ (PERMIL)				
	-13.371	-28.135	-26.983	-20.889	-22.571
	-12.860	-27.505	-26.838	-21.078	-21.600
	-11.519		-24.682	-22.273	-22.260
	-11.450		-24.387	-20.154	-25.150
$\bar{X}$	-12.300	-27.820	-25.723	-21.099	-22.895
S_X	0.965	0.445	1.378	0.879	1.557
N	4	2	4	4	4

TABLE 5 PLANT ANALYSES FROM THE NEW ENGLAND SALT MARSH

	$d^{13}C_{PDB}$ (PERMIL) MEAN	S_X	$d^{15}N_{ATM}$ (PERMIL) MEAN	S_X
UPLAND C_3 PLANTS	-28.6	1.3	-0.6	1.2
MARSH C_4 PLANTS	-13.1	0.8	3.8	2.6
PLANKTON	-21.3	1.1	8.6	1.0

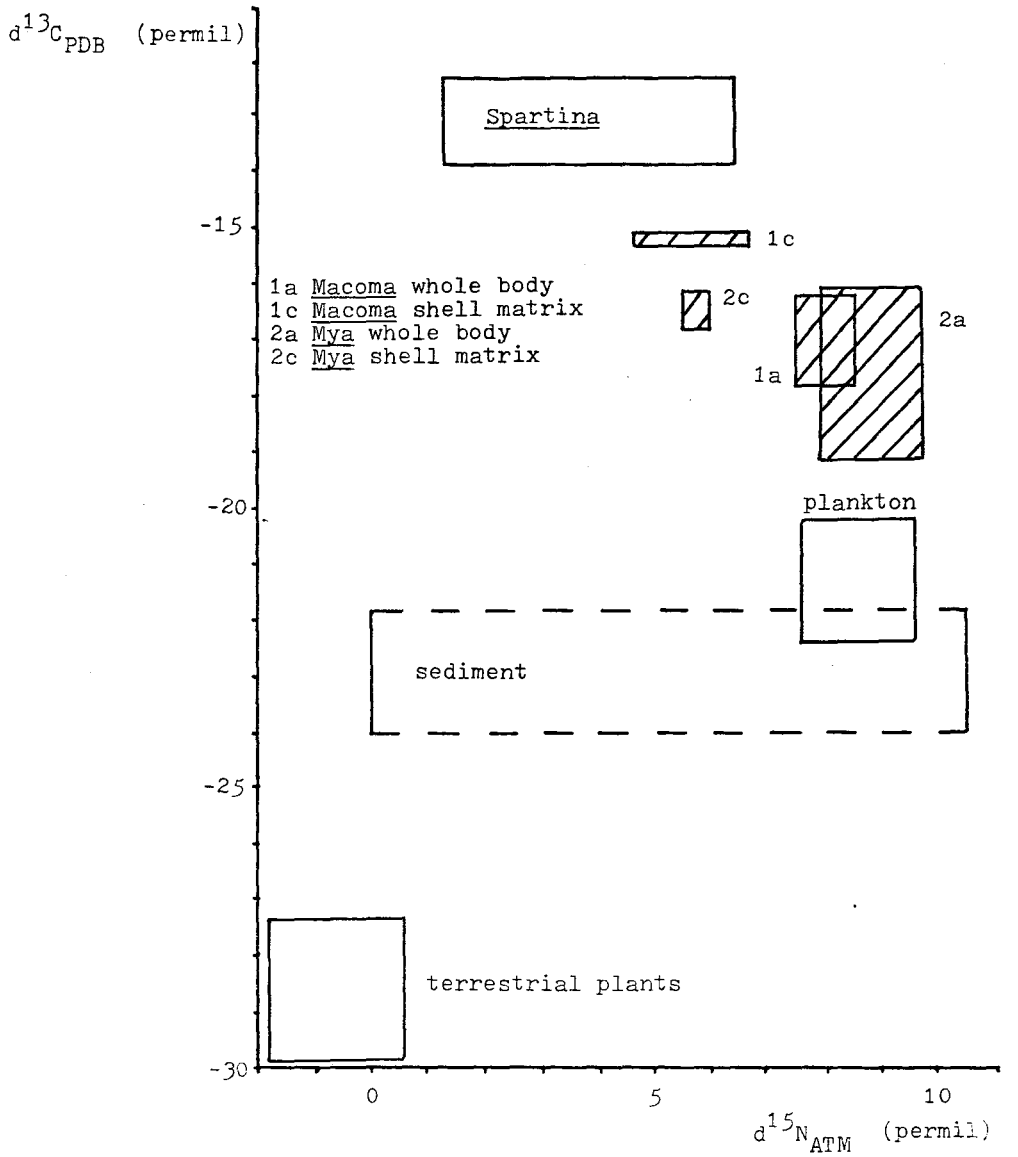
from Peterson et. al., 1985

FIG. 4 RELATIONSHIP BETWEEN THE ISOTOPIC COMPOSITION OF THE PLANTS, SEDIMENT, AND CLAMS ON THE PANGNIRTUNG TIDAL FLAT



areas enclose 1 SD on either side of the mean
dashed lines indicate unknown values
terrestrial plant data from: Peterson et.al. (1985) and others

FIG. 5 RELATIONSHIP BETWEEN THE ISOTOPIC COMPOSITION OF THE PLANTS, SEDIMENT, AND CLAMS ON THE LYLE'S BAY TIDAL FLAT



areas enclose 1 SD on either side of the mean
dashed lines indicate unknown values
plant and plankton values from: Peterson et.al. (1985)

4. DISCUSION

4.1 METHOD

As stated earlier, stable isotope ratios in organic matter are used to assess the proportion of an animal's diet made up by a given food source. This is done by comparing the isotopic composition of the animal's diet, calculated from the whole body isotopic composition, with the isotopic composition of all the possible food sources (DeNiro and Epstein, 1978 and 1980). This can be done graphically, as in figs. 4 and 5, by comparing the spatial relationships of the isotopic distributions of each group, to yield a qualitative assessment. A more quantitative assessment can be made by solving a set of mutually dependent linear equations, one equation per isotope set used, where the variables are the proportions of the animal's diet made up by each food source (x_j) and the co-efficients are the del values for the plant sources (d_j). For each isotope set, the sum of $d_j * x_j$ for all the plant sources must equal the del value for the animal's diet. This method requires the use of at least as many isotope sets as number of potential food sources. Thus the graphical approach will be used in this report.

For both methods the isotopic compositions of each plant source must be well separated. Thus the method is only useful for broad classes of primary producers such as marine versus terrestrial or C_3 versus C_4 (DeNiro and Epstein, 1978 and 1981). The resolution of the different

plant sources is improved by using many isotopes at the same time (Peterson et.al., 1985).

4.2 RESULTS

4.2.1 PLANT ANALYSES

At the arctic site the spread in $d^{13}C$ between the two plant sources (15.5 permil) is sufficient to distinguish them.

Phytoplankton was not identified in either the seston or the sediment. Marine phytoplankton $d^{13}C$ decrease with temperature (Anderson and Arthur, 1983; Deines, 1980) as explained previously. Given the water temperature in Pangnirtung Fjord the expected $d^{13}C$ values would be between -28 and -23 permil (Anderson and Arthur, 1983; Deines, 1980). This would make planktonic and terrestrially fixed organic matter indistinguishable on the basis of $d^{13}C$ alone. The distinction could possibly be made if $d^{15}N$ values were availible as terrestrial C_3 plants have $d^{15}N$ values close to zero (Kaplan, 1983; Letolle, 1980; Peterson et.al., 1985) while marine values are usually higher (Kaplan, 1983; Peterson et.al., 1985) (a d15N value of 0 was assumed for the terrestrial plants in figure 4).

It is possible that there are phytoplankton blooms in Pangnirtung Fjord later in the summer as the salinity rises. Considering that the day lengths decrease rapidly after August, it is unlikely that phytoplankton would contribute a significant amount of primary productivity at this site.

At the temperate site the sources of primary productivity: Spartina, terrestrial plants, and plankton, are all distinguishable with $d^{15}N$ and $d^{13}C$ data (Peterson et. al., 1985).

4.2.2 SEDIMENT ANALYSES

Only carbon analyses were made on sediment samples. As microbial degredation and detritus formation have little effect on the $d^{13}C$ of organic matter (Gearing et.al., 1984) the $d^{13}C$ of the sediment organic matter should reflect the weighted average of all the detritus sources. It has been suggested (McConnaughey and McRoy, 1979a) that the action of detritivore meiofuana could enrich the substrate in organic ^{13}C through respiratory fractionation. There estimate for the Bering Sea was a 1.4 permil enrichment in ^{13}C in the sediment over the detritus source, plankton. Thus the sediment organics have a $d^{13}C$ value of between 0 and 2 permil greater than the weighted average for all the detritus sourses.

For the Pangnirtung sediments, the mean $d^{13}C$ (see fig. 4) is 2.1 permil closer to the value for terrestrial plants than Fucus, suggestng that terrestrial detritus is more abundant on the tide flat. This is in agreement with the visual impression of the sediment detritus.

For the Lyle's Bay sediments the, mean $d^{13}C$ (see fig. 5) is 5.7 permil greater than the terrestrial C_3 value, 1.6 permil less than the plankton value and 9.8 permil less than the Spartina value. Since there are 3 potential sources of detritus and only one set of isotopes values to work with, it is theoretically impossible to work out the proportions of each type of detritus in the sediment. One must also consider the

value for the whole body. A better alternative would be to experimentally determine the $Dd^{13}C_{a-d}$. (DeNiro and Epstein, 1978)

The trends for nitrogen isotopes were less straitforward (DeNiro and Epstein, 1981). Although the $Dd^{15}N_{a-d}$ was usually greater than 0, there was a large variation among individuals. The mean value was 3.0 permil (SD=2.6). There was also a large range in mean $Dd^{15}N_{a-d}$ values between the same species on different diets and different species on the same diet, as high as 3 or more permil in each case. (DeNiro and Epstein, 1981)

This suggests that, unlike $Dd^{13}C_{a-d}$, $Dd^{15}N_{a-d}$ has a significant relationship to the particular species and diet. Thus $Dd^{15}N_{a-d}$ values should be experimentally determined for use in food-web tracing.

Using $d^{13}C$ values alone , fig. 4 it shows that the $d^{13}C$ of the clam diets, estimated as the mean whole body values less 1 permil, are 4 permil closer to the terrestrial values for Mytella (-23 permil) and are half way between the terrestrial and Fucus values for Macoma (-21 permil). Thus the data suggest that terrestrial and marine primary productivity are about equally important to the arctic tidal flat. The slightly more terrestrial signature for the filter-feeder may be due to less assimilation of the sediment organics, which are enriched in ^{13}C by the respiratory fractionation of benthic detritivores.

The $d^{15}N$ values for whole clam bodies are typical of marine animals or extremely enriched land animals (Kaplan, 1983). If the conclusion regarding the importance of terrestrial organic matter is correct these values could indicate one of several situations. Since ^{15}N enrichment

presence of benthic algae, probably mostly diatoms, which have $d^{13}C$ values 2 to 4 permil lower than that of the phytoplankton (Haines and Montague, 1979).

By observation, however, Spartina detritus makes up a large proportion of the total detritus. Thus, from the relationships in fig. 5, terrestrial C_3 organic matter must make up a similar proportion to account for the low sediment $d^{13}C$. This could come in the form of particulate matter and in the large concentrations of organics acids brought in by the rivers. These acids enter the food-web through assimilation by bacteria (M. Risk, personal comm.). It is not possible to assess the proportion of algal detritus from these data.

4.2.3 CLAM ANALYSES

4.2.3.1 SOFT TISSUES

DeNiro and Epstein (1978 and 1981), in a series of laboratory feeding studies, noted the following isotopic relationships between animals and their food. For carbon the $d^{13}C$ of the whole organism was almost always larger than that of its food. The mean difference between animal and diet, $Dd^{13}C_{a-d}$, was 0.8 permil (SD=1.1). This was primarily due preferential respiratory loss of $^{12}CO_2$. There was a variation among individuals of between 0.2 and 1.8 permil. The range of mean values for different species on the same diet and the same species on different diets was 1 permil or less. Thus, a reasonable estimate the $d^{13}C$ of an animal's diet can be can be made by subtracting about 1 permil from the

generally occurs at each trophic level (DeNiro and Epstein,1981; Minagawa and Wada, 1984), the data could indicate that the organic nitrogen has passed through several consumer organisms before reaching the clams. This is a possibility in a detritus dominated food-web. The data could also indicate that the clams get most of their nitrogen from marine organic matter, or that there is some microbial activity in the sediment that is causing a large fractionation in the organic nitrogen isotopes (Macko and Estep, 1983). It is not possible to discriminate between these or other possibilities with the data at hand.

The data in table 1 show a trend toward lower $d^{13}C$ values from July to August in both clam species. There are two explanations for this. One is that since the river flow drops off at the end of the summer the clams depend more on marine carbon at the end of the summer. This would be expressed as heavier carbon isotope signatures at the start of the next summer. As they take up the terrestrial organics brought in by the rivers in early summer their body $d^{13}C$ would drop. The other explanation is that the clams are building up lipid reserves over the summer. Since lipids are ^{13}C depleted (Deines, 1980; DeNiro and Epstein, 1978; McConnaughey and McRoy, 1979a) this would also lower the body $d^{13}C$ value.

Unlike the clams in the arctic site, there was no significant difference in the $d^{13}C$ of body tissues between the filter and deposit feeding clams. This could be due to the fact that although Macoma is preferentially a surface deposit-feeder of muddy substrates,it will filter-feed from the water column to suplement this (Tunnicliffe and Risk, 1977). Since the sediment in Lyle's Bay is very sandy, the Macoma

may spend much of its time filter-feeding. Thus the similar isotopic signature for the two species may be the result of a similar feeding pattern.

For the Lyle's Bay clams fig. 5 shows that the estimate of the $d^{13}C$ of the diets of both clams is about -18 permil, given a $Dd^{13}C_{a-d}$ of 1 permil. This value is 2 permil closer to the value for plankton than for Spartina grass. Given a $Dd^{15}N_{a-d}$ >0, the $d^{15}N$ of the diet would be intermediate to plankton and Spartina. The data combine to suggest that plankton and Spartina are of about equal importance as food sources for the two clam species, with plankton perhaps a bit more so. Thus, unlike the arctic site, the clams from the temperate site are not utilizing terrestrial organic matter to a significant extent. This is inspite of the fact that the sediment shows good isotopic evidence of containing terrestrial detritus. The presumptive conclusion is that the terrestrial organics are in a form that is not taken up by the clams. Perhaps the bacteria that are absorbing the terrestrial organic acids are not resuspended into the water column where where they would be availible to suspension-feeders.

4.2.3.2 CLAM SHELL INSOLUBLE ORGANIC MATRIX ANALYSES

DeNiro and Epstein (1978, 1981) investigated the relationship between the isotopic composition of animal's diet and various different tissues and biochemical components in its body. Although there were obvious relationships, they were often species and diet dependent. Since the differences between DdX (biochemical fraction-diet) values for

individuals on different and, in some cases, similar diets were quite large (up to 3 permil for $d^{13}C$ and 9 permil for $d^{15}N$) , it is not possible to generalize (DeNiro and Epstein, 1978 and 1981). This is due, in part, to the fact that some biochemical components can not be synthesized by some animals (DeNiro and Epstein, 1978). There is also fractionation of isotopes associated with some biochemical reactions (Macko and Estep, 1983). Thus the isotopic composition of some biochemical components will approximate that of the dietary average, while others may reflect the isotope ratio of a specific component of the diet, or the fractionation from a certain reaction.

The data from this report (table 3) show that there is a range of several permil in the difference between the isotopic composition of the shell matrix and the whole body. Since the whole body del values reflect the average values of the diet, there is must also a range in values for the difference between the shell matrix isotope composition and that of the diet for the clams in this study.

It would be advantageous if further research could account for the differences in the $Dd^{13}C$ and $Dd^{15}N$ for shell matrix-diet since there are several ways in which the shell matrix is better for use in diet studies than the whole body.

1) The shell matrix provides del values that are integrated over the life of the animal, where as that of the body tissues will change with changing isotope ratios in the diet. The change in body isotope composition will also lag behind that of the diet, depending on the rate of overturn of organic components in the organism. The shell will only

provide an integrated value if there is no loss of shell material over time. Loss of shell material could occur by breakage or abrasion as was observed in the Macoma at Pangnirtung. It could also occur by selective dissolution when the clam uses the shell carbonate to buffer its body pH during anaerobisis, a common phenomenon in intertidal clams that must spend time with their valves tightly shut.

2) The shell matrix data have smaller errors. This is because the shell matrix samples are made up of many individuals. Large variation between individuals could be due to individuals having different contents of certain biochemical components, such as lipids,which have distinctive isotopic compositions. Also the problem of homogenizing whole bodies when taking microgram samples is avoided when using the shell matrix.

3) The clams need not clear their guts when shell matrix samples are used.

4) The insoluble organic shell matrix can be isolated from fossil shells for analysis and compared to analyses of modern shell matrices. The shell matrix is often well preserved in fossils and in such cases the isotopic composition is unchanged as atoms are held against isotopic exchange by covalent bonds (DeNiro and Epstein, 1978 and 1981).

5. CONCLUSIONS

1) Organic matter from terrestrial primary producers was an important food source for the two test species in the Pangnirtung tidal flat. In Lyle's Bay the test clams utilized plankton and sea grass but did not take up the terrestrial organic matter present in the sediment in any significant amount.

2) There was a consistent trend in the relationship between the isotopic composition of the clam whole bodies and the insoluble organic shell matrix. The shell matrix was depleted in ^{15}N and enriched in ^{13}C as compared to the whole bodies. There was a wide range in the magnitude of these depletions and enrichments between sites and species.

The shell matrix is not a suitable tissue to use in isotopic diet studies at this time, however, as the isotopic relationship between the diet and the shell matrix is variable.

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