30 pages

93/41

The Stable Carbon Isotope Composition of Green-Lipped Mussels *Perna canaliculus*, Marlborough Sounds

Graeme L Lyon
Robert W Hickman

1993

Institute of
GEOLOGICAL
& NUCLEAR
SCIENCES
Limited

The Stable Carbon Isotope Composition of Green-Lipped Mussels *Perna canaliculus*, Marlborough Sounds

Graeme L Lyon and Robert W Hickman

Institute of Geological & Nuclear Sciences Science Report 93/41

Institute of Geological & Nuclear Sciences Limited
Lower Hutt, New Zealand
May 1993

RECOMMENDED BIBLIOGRAPHIC REFERENCE

Lyon, Graeme L; Hickman, Robert W: The stable carbon isotope composition of green-lipped mussels *Perna canaliculus*, Marlborough Sounds. Institute of Geological & Nuclear Sciences Science Report 93/41. 26 pp.

Graeme L Lyon, Nuclear Sciences Group, Institute of Geological & Nuclear Sciences, PO Box 31 312, Lower Hutt
Robert W Hickman, Aquaculture Section, MAFFisheries, PO Box 297, Wellington

ISSN 1171-9184
ISBN 0-478-07076-4

CONTENTS

Contents (Ctd)

Page

Tables

Figures

ABSTRACT

Variations are shown for the $\delta^{13}C$ values of green-lipped mussels, *Perna canaliculus*, collected on several occasions between October 1983 and April 1985 from six sites in the Marlborough Sounds, South Island, New Zealand. Additional limited data is presented for mussels from two other sites and on other fish and particulate matter.

The $\delta^{13}C$ in *P canaliculus* was found to vary seasonally, with the least negative values in winter 1984. The $\delta^{13}C$ values ranged from -16.7 to -21.3 ‰, with males on average 0.7 ‰ less negative than females. Phytoplankton composition would be expected to also vary in $\delta^{13}C$ with least negative values when at their greatest production rate, i.e., in spring or summer, and thus mussel compositions appear to show a lag of several months. There was no evidence that terrestrial food was important.

Keywords

Carbon-13; Mussel; Perna; New Zealand; Shellfish; Aquaculture.

INTRODUCTION

Estuarine ecosystems are characterised by complex food webs which span terrestrial and aquatic environments and include fauna with a variety of feeding strategies. The detritus pathway of carbon flow may dominate in estuaries and can obscure the origins of the organic matter which forms the base of individual food chains. The carbon isotopic composition of flora and fauna has been used to elucidate specific food sources for estuarine animals (Haines & Montague, 1979; Stephenson & Lyon, 1982). However, in some marine situations, this method has not been successful (Lyon & Richardson 1983, Stephenson *et al.* 1984).

The Marlborough Sounds of New Zealand are not strictly an estuarine system, but a series of inlets, extending up to 70 km from the open ocean, and therefore with limited circulation of seawater. A large number of marine farms have been established in Pelorus Sound, for the production of the green-lipped mussel *Perna canaliculus* Gmelin (Hickman, 1980). The food and habitat conditions for optimal growth of farmed mussels were investigated during 1983-85 by a team co-ordinated from MAFFisheries, Greta Point, Wellington (Hickman *et al.*, 1991; Gibbs *et al.*, 1991; Gibbs *et al.* 1992). Water quality parameters measured during this study included temperature, salinity, chlorophyll *a*, nitrogen, carbon, phosphorus, particulates and dissolved oxygen, as well as current velocity. The condition index of the mussels was also measured. Growth rate and condition cycle measurements of green-lipped mussels in the Marlborough Sounds had also formed part of an earlier study (Hickman, 1979; Hickman & Illingworth, 1980). As part of the 1983-85 study, mussels were collected from a number of sites, several times over 18 months, and analysed for their stable carbon isotope ratio.

The green-lipped mussel is a filter-feeder and, at commercial farms, is grown on ropes suspended from surface buoys to depths of 10 m. Each licensed farm occupies an area of about 3 hectares, adjacent to the shore, with up to 400 ropes suspended vertically from buoyed long lines that make up the mussel farm. The water conditions at farms vary considerably from shallow, sheltered embayments to deep water with high tidal velocities. Temperature and salinity vary depending on location, season and recent rainfall.

The carbon isotope composition of the mussel's food supply (the suspended particulates) was expected to show some variation, and this would be reflected in the mussel composition (Haines & Montague, 1979; DeNiro & Epstein, 1978). This has the advantage over stomach (ingestion) analysis, or bulk particulate collection, in that the mussel composition results only from assimilated carbon, and some of the total particulates may not be available or may be rejected or excreted by the animal. Terrestrial detrital material from the surrounding farm and forest lands of the Marlborough Sounds would have $\delta^{13}C$ values of about -25‰ to -30‰ (Rounick *et al.*, 1982) and be considerably different from marine plankton (about -19‰) as found in the Avon-Heathcote estuary (Stephenson & Lyon, 1982). This study was to determine if terrestrial detritus was significant in the nutrition of mussels. The amount of terrestrial detritus in the diet should vary depending on the proximity of the mussel farm site to major rivers, which enter the Sound at Havelock or the open sea (Fig. 1).

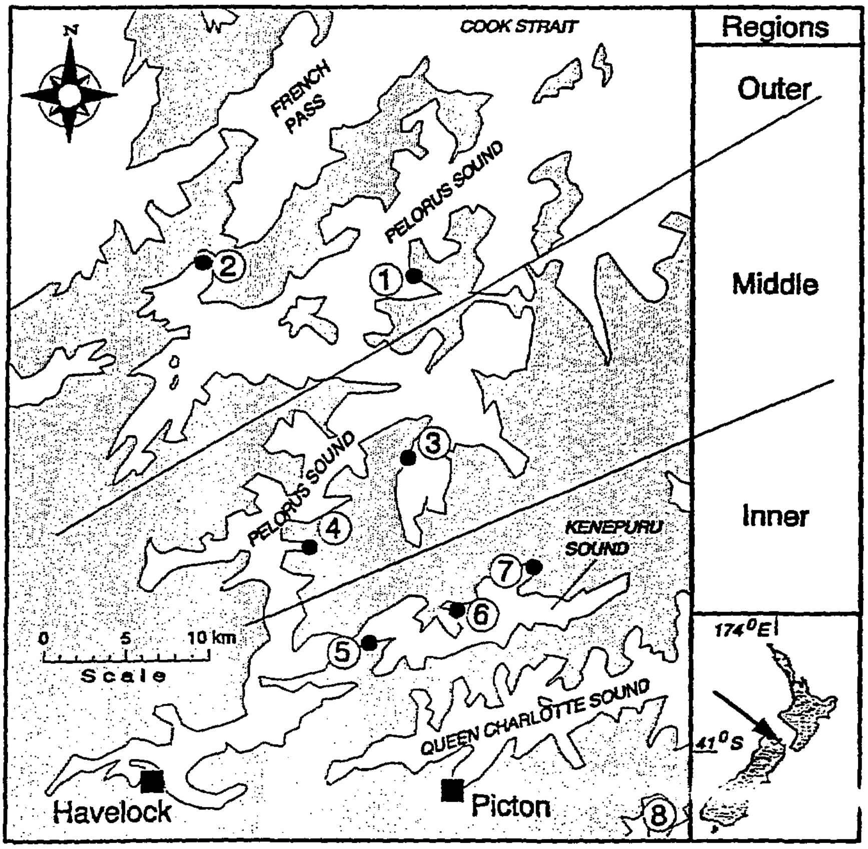

Fig. 1 Map of mussel farm sampling sites, Pelorus Sound, South Island, New Zealand (from Hickman *et al.* 1991). 1. Richmond Bay
2. Hallam Cove
3. Crail Bay
4. Four Fathom Bay
5. Schnapper Point
6. Mills Bay
7. Waitaria Bay
8. Port Underwood

Preliminary analyses of various fish were made (Table 1) showing that a range of values occurs and thus there could be useful variations in mussels. Table 1 also shows that variations occur in phytoplankton $\delta^{13}C$. The site locations for the samples in Table 1 are listed in Table 2A, and shown in Fig. 2.

METHODS

Mussels were collected from the sites shown in Fig. 1 and detailed in Table 2B (water depth from Gibbs *et al.*, 1991), on up to eight occasions from October 1983 to April 1985 during cruises of the MAFFisheries research vessel "Kaharoa". No samples were collected on the research cruises of April 1983, August 1983, and February 1984. In general, at each of sites 1 to 6 on each cruise, the vessel would be stationed for 12 to 24 hours, making water quality measurements close to a mussel farm. From the nearest adjacent farm, usually two or more mussels of 50-150 mm length, depending on availability, were harvested from 0.5-3 m depth. Two other sites were also visited and sampled but have limited sample numbers and hydrodynamic data. In some cases juveniles were also sampled, and some blue mussels *(Mytilus edulis)* were also collected. The animals were frozen on board, and returned to the laboratory. After defrosting, the mussels were opened and sexed by the colour of the mantle, although this was difficult for juveniles. The mantle (or the whole flesh for small animal) was freeze-dried, and then pulverised for subsequent analysis of about 5 mg of dried matter. Particulate matter was sampled, as described in Gibbs *et al.* (1992), by pumping water through a 100 µm filter on to glass-fibre 0.45 µm filter papers (pre-combusted), frozen on board and freeze-dried in the laboratory. The glass-fibre filters were then treated with 1M HCl solution to remove carbonate, and re-dried before stable isotope analysis.

For analysis the sample was combusted to CO_2 in a closed tube (Stephenson & Lyon 1982) and the CO_2 analysed for its $^{13}C/^{12}C$ by mass spectrometry. The precision of the method is estimated from repeat analyses of NBS21 graphite for which $\delta^{13}C$ = -27.99‰ ± 0.12‰ one standard deviation (n=13) where the $\delta^{13}C$ value is the deviation of $^{13}C/^{12}C$ from that of the international standard PDB, i.e., $\delta^{13}C_{PDB}(\text{sample}) = 1000[^{13}C/^{12}C(\text{sample})/^{13}C/^{12}C(\text{PDB}) -1]$. More than 150 analyses were made.

The data are listed in Tables 3A (males), 3B (females), and 3C (all samples).

RESULTS AND DISCUSSION

The range of $\delta^{13}C$ for this one species of mussel is from -16.7‰ to -21.3‰. This presumably must reflect some differences of food supply. However, metabolic variations could account for some of the variation. For example, fats are always more depleted in ^{13}C (more negative $\delta^{13}C$ values) than are proteins or carbohydrates, therefore the relative proportions of fats to protein and carbohydrate will affect $\delta^{13}C$. This is likely to be minor as fat is only a small proportion of the mussel (e.g., in *Mytilis platenisis*, 0.9-1.8% wet weight, Gosling, 1991, p 267).

Fig. 3 shows the data from Tables 3A and 3B with Fig. 4 summarising the data. The figures show that there are clear temporal patterns which are consistent for both males and females

Table 1: Fish, shellfish, seaweed, and phytoplankton, collected by C Hay, NZOI Cruise #1143, February 1983, Marlborough Sounds Analyst - J S Rounick

Fish	Site	$\delta^{13}C_{PDB}$
Parika scaber - leather jacket	S461	-17.4
Pseudophycis bachus - red cod	Dryden Bay	-14.9
Unknown fish	S442	-15.2
Helicolenus percoides - Jock Stewart	S427	-14.4
Galeorhinus australis - school shark	Dryden Bay	-15.2
Arripis trutta - kahawai	S465	-15.9
Pseudolabrus celidotus - spotty	S461	-15.4
Thyrsites atun - baracouta	Dryden Bay	-16.0
Invertebrates		
Perna canaliculus - green mussel (gonads)	S421	-16.2
Perna canaliculus - green mussel	S421	-18.2
Atrina zelandica - horse mussel	S424	-19.0
Haliotis iris- paua	S442	-13.8
Glycymeris laticostata - dog cockle	S429	-16.0
Colonial tunicate	S420	-15.9
Squid tissue	Dryden Bay	-17.5
Munida gregaria - krill	Dryden Bay	-21.6
Seaweed		
Cystophora scalaris - brown seaweed	S421	-16.4
Ecklonia radiata - kelp	S442	-15.8
Caulerpa sedoides - green seaweed	S421	-21.2
Carpophyllum sp - brown seaweed	S461	-15.0
Ulva sp - sea lettuce	S461	-16.2
Macrocystis pyrifera - kelp	S424	-18.3
Phytoplankton		
Phytoplankton 200 µm pre-filter	S405	-18.5
" " "	S422	-23.1
" 100 "	S417	-18.6
Sediment		
63-210 µm	Kenepuru Sd	-23.2
210-420 "	"	-23.5
420-850 "	"	-23.4
63-210 "	S452	-21.8
210-420 "	"	-24.3

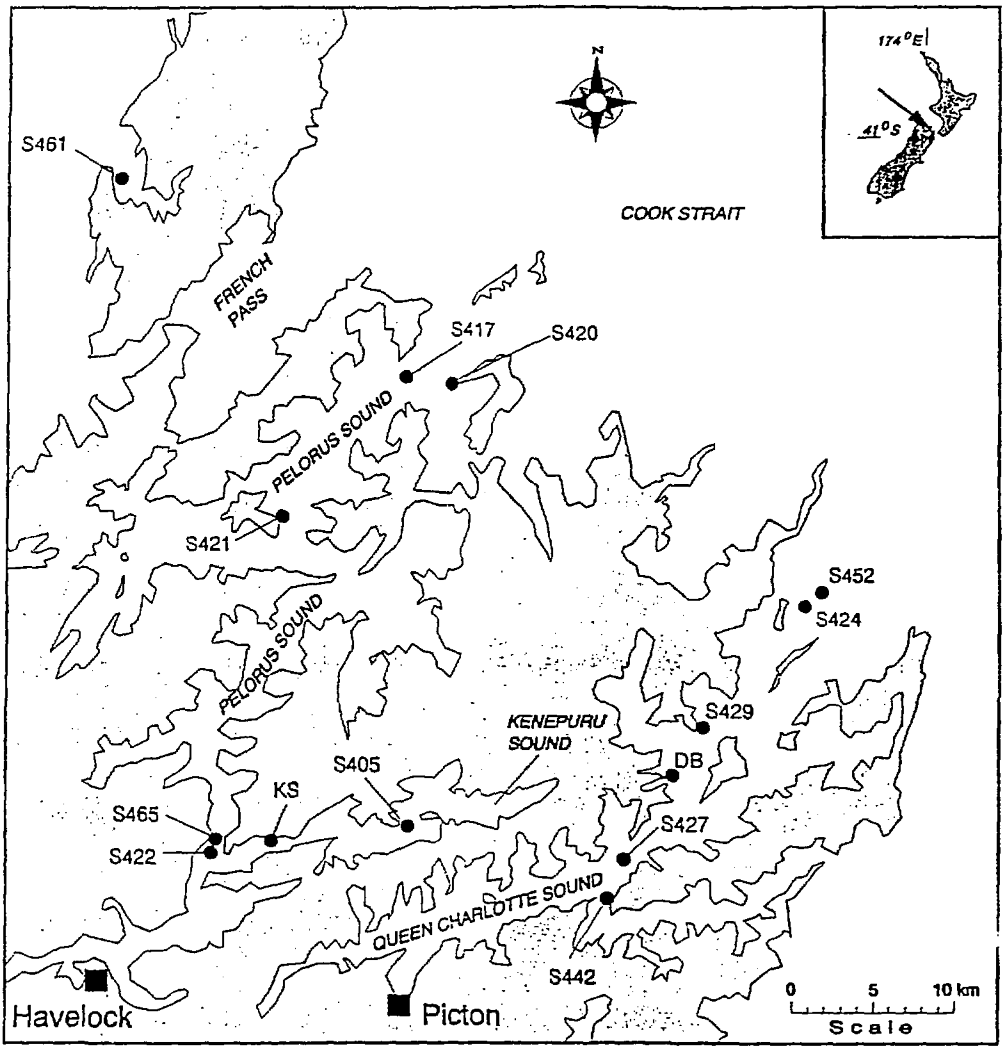

Fig. 2 Sample locations for the collections listed in Table 1.

Table 2: Marlborough Sounds sample locations (±200-500 m)

A. Locations for samples in Table 1 (see Fig. 2)

		Grid ref NZMS260
S405	Near Portage, Pelorus	P27/945010
S417	Near entrance to Pelorus	P26/945275
S420	Duffer's Reef, Forsyth Bay	P26/975275
S421	Maud Island, Pelorus	P26/864192
S422	Hikapu Reach, Pelorus (nr S465)	P27/825995
S424	N entrance Queen Charlotte	Q26/185137
S427	Snake Pt, Queen Charlotte (SCUBA)	P27/085993
S429	Scott Pt, Queen Charlotte, SCUBA	Q27/130065
S442	W entrance Tory Channel	P27/070960
S452	Close to S424, Queen Charlotte	Q26/195140
S461	Greville Harbour, Durville Island	P25/770410
S465	Hikapu Reach	P27/828000
DB	Dryden Bay 22/2/83 (nr stn S428)	P27/095030
KS	Kenepuru Sound (from nr S403)	P27/860000

B. Collection sites for mussels (see Fig. 1)

	Long E	Lat S	Grid Ref NZMS260	Approx dist from Cook Strait (km)	Approx water depth (m)
1 Richmond Bay	173°58'	41°00'	P26/918220	15	24
2 Hallam Cove	173°49'	41°00'	P26/794220	28	25
3 Crail Bay	173°58'	41°05'	P26/914120	30	[illegible]
4 Four Fathom Bay	173°52'	41°09'	P27/840060	33	9
5 Schnapper Point	173°56'	41°12'	P27/890001	52	13
6 Mills Bay	174°00'	41°11'	P27/935024	58	8
7 Waitaria Bay	174°02'	41°10'	P26/970043	62	6
8 Port Underwood	174°09'	41°16'	P27/060900	5	18

Table 3A
Green Mussels, (*Perna canaliculus*) Marlborough Sounds
Males delta 13C (o/oo)

Site		Oct-83	Apr-84	Jun-84	Aug-84	Sep-84	Dec-84	Feb-85	Apr-85
1	Richmond Bay	-19.9	-19.1		-19.3	-18.1	-20.7	-19.9	-18.1
		-19.5			-19.7		-19.7	-19.1	-18.5
					-18.1		-20.4		-19.8
					-17.9				
2	Hallam Cove	-19.5			-18.6	-19.6	-19.7	-19.4	-19.0
		-19.4			-19.0	-19.9	-19.0	-19.2	-19.6
					-18.9	-19.2		-19.6	-19.1
					-18.7				
3	Crail Bay	-19.2	-18.1	-17.1	-17.4	-17.5	-18.8	-19.0	-19.2
		-18.9	-18.0	-16.9	-17.7	-18.0	-18.9	-18.2	-18.9
						-18.2	-19.0	-18.3	-19.3
4	Four Fathom Bay	-20.5		-19.1	-19.0	-18.2	-20.6	-19.3	-18.7
				-19.4	-17.9	-18.3	-20.2	-19.5	-18.6
					-17.9	-17.6		-19.5	
					-17.7	-17.7			
						-17.6			
5	Schnapper Point	-20.0	-20.0	-18.8	-18.1	-16.9	-19.9	-19.6	-19.0
			-19.8	-19.8	-18.2	-17.1	-19.9	-19.4	-19.2
						-16.7	-20.1	-19.2	
6	Mills Bay	-20.4		-18.8	-18.1	-18.0	-20.9	-19.3	-19.6
					-19.4	-18.6	-19.9	-19.4	-19.8
					-18.2	-19.4	-20.3		
					-18.4				
7	Waitaria Bay	-21.2	-19.6					-19.4	-19.7
		-20.6						-20.2	-19.7
									-19.4
8	Port Underwood				-19.4				
					-19.8				

									All
Means: sites 1-6	-19.70	-19.00	-18.56	-18.41	-18.14	-19.88	-19.24	-19.09		-18.96
s.d.	0.54	0.93	1.12	0.64	0.91	0.66	0.44	0.49		0.91
No.	9	5	7	20	18	16	16	15		106
Means : all	-19.92	-19.10	-18.56	-18.52	-18.14	-19.88	-19.31	-19.18		-19.04
s.d.	0.70	0.87	1.12	0.70	0.91	0.66	0.47	0.49		0.92
No.	11	6	7	22	18	16	18	18		116

Table 3B
Green Mussels, (Perna canaliculus) Marlborough Sounds
Females delta 13C (o/oo)

Site		Oct-83	Apr-84	Jun-84	Aug-84	Sep-84	Dec-84	Feb-85	Apr-85		All
1	Richmond Bay	-20.8 -19.8	-19.6	-19.8 -20.0		-18.8		-19.6 -19.4	-19.1		
2	Hallam Cove		-19.8	-19.8 -18.6			-20.8		-19.8		
3	Crail Bay					-18.4					
4	Four Fathom Bay						-21.3		-19.0 -19.2		
5	Schnapper Point					-18.1 -18.0 -18.0			-20.2 -20.5		
6	Mills Bay			-20.5				-20.1 -19.7	-19.8 -19.6		
7	Waitaria Bay		-21.0					-20.3			
8	Port Underwood										
	Means: sites 1-6	-20.30	-19.70	-19.74		-18.26	-21.05	-19.70	-19.65		-19
	s.d.	0.71	0.14	0.70		0.34	0.35	0.29	0.53		0
	No.	2	2	5	0	5	2	4	8		
	Means : all	-20.30	-20.13	-19.74		-18.26	-21.05	-19.82	-19.65		-19
	s.d.	0.71	0.76	0.70		0.34	0.35	0.37	0.53		0
	No.	2	3	5	0	5	2	5	8		

Table 3C
Green Mussels, (Perna canaliculus) Marlborough Sounds
All mussels delta 13C (o/oo)

	Oct-83	Apr-84	Jun-84	Aug-84	Sep-84	Dec-84	Feb-85	Apr-85		All
Means: sites 1-6	-19.81	-19.20	-19.05	-18.41	-18.17	-20.01	-19.34	-19.29		-1
s.d.	0.58	0.83	1.11	0.64	0.82	0.73	0.45	0.56		
No.	11	7	12	20	23	18	20	23		
Means : all	-19.98	-19.44	-19.05	-18.52	-18.17	-20.01	-19.42	-19.32		-1
s.d.	0.68	0.94	1.11	0.70	0.82	0.73	0.50	0.54		
No.	13	9	12	22	23	18	23	26		

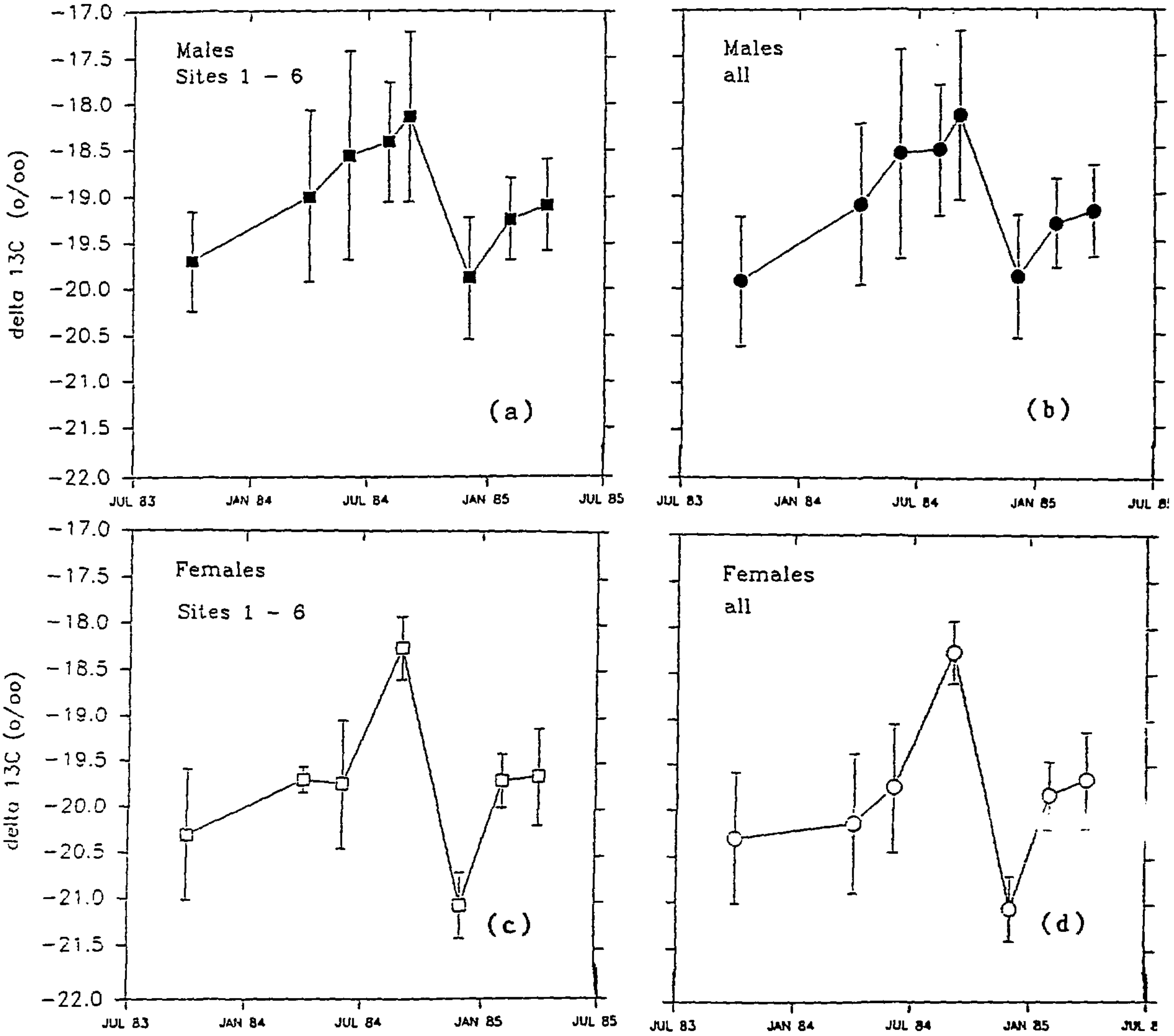

Fig. 3 $\delta^{13}C$ data. Mean and standard deviation values from Tables 3A, 3B at each sampling date: (a) Males from sites 1-6, (b) all males, (c) females from sites 1-6, (d) all females.

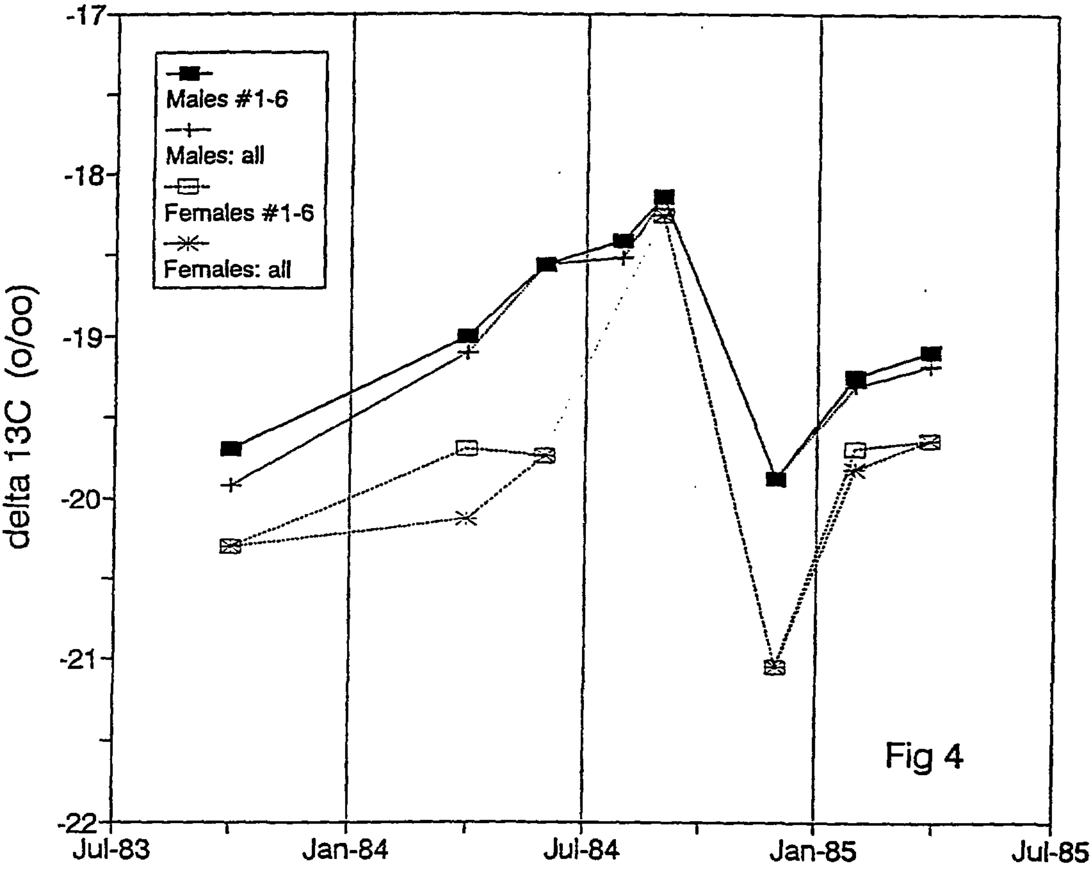

Fig. 4 Comparison of mean $\delta^{13}C$ values.

through the 18 months period of collection, and that there are differences between the male and female sample sets. For completeness, Figs 3 and 4 show mean values for the whole set as well as for only sites 1 to 6. Sites 7 and 8 have few or no samples on some dates, and no females were collected on the August 1984 cruise. The samples from sites 7 and 8 can be seen to shift the means for all sites combined to more negative values.

Intra-site variations

At one site, Schnapper Point (site 5) in September 1984, three individuals were analysed from each of 0.5 m and 3.0 m depth. There was no difference in $\delta^{13}C$ between depths. Some comparisons were also made between small and large males in one collection. There were no clear trends for animal size.

Analyses of mussels of both male and female sex from the same site at the same time (Table 4, Figs 4, 5, 6) show that males (pale coloured mantle) were always with less negative $\delta^{13}C$ values compared to females (with bright orange mantle). The mean difference was +0.7 ± 0.5‰. Fig. 6 shows the site data for females as variations with time, and shows that there are large data gaps, although as also shown in Fig. 3, temporal trends are similar to those for males.

For most further discussions only male animals (which were the more common) are considered, and therefore some collections of only females, have been eliminated from those discussions.

The main group of analyses of male mussels is given in Table 3A. Some collections showed considerable variation, as much as 1.8‰ (e.g., Richmond Bay 8/84: -17.9‰ to -19.7‰, Richmond Bay 4/85: -18.1‰ to -19.8‰), hence there can be considerable natural variability of $\delta^{13}C$ in any one population. The results of the analyses from the six main sites have been averaged for each collection trip, and these data are plotted in Fig. 3(a) and 4.

Inter-site variations

Samples of mussels from Crail Bay (site 3) were usually less negative $\delta^{13}C$ relative to most other sampling sites (Table 3, Fig. 5). The location in Crail Bay is open to well mixed sea water inflow, but no more so than Richmond Bay which is closer to Cook Strait, so it is difficult to explain the ^{13}C enrichment as being due to a greater degree of marine influence. The cruise report for April 1985 records low salinity water at Crail Bay after a flood.

As perhaps the location of a site relative to Cook Strait may be a significant factor, the sites were divided into Outer Sounds, Inner Sounds (Kenepuru Sound) and a middle region as in Fig. 1, and the male data from Table 3A recalculated (Table 5) and plotted (Fig. 7) with averages for two sites in each region. Fig. 7 shows more clearly than Fig. 5 that except at the September 1984 collection the middle region has the most enriched $\delta^{13}C$ values.

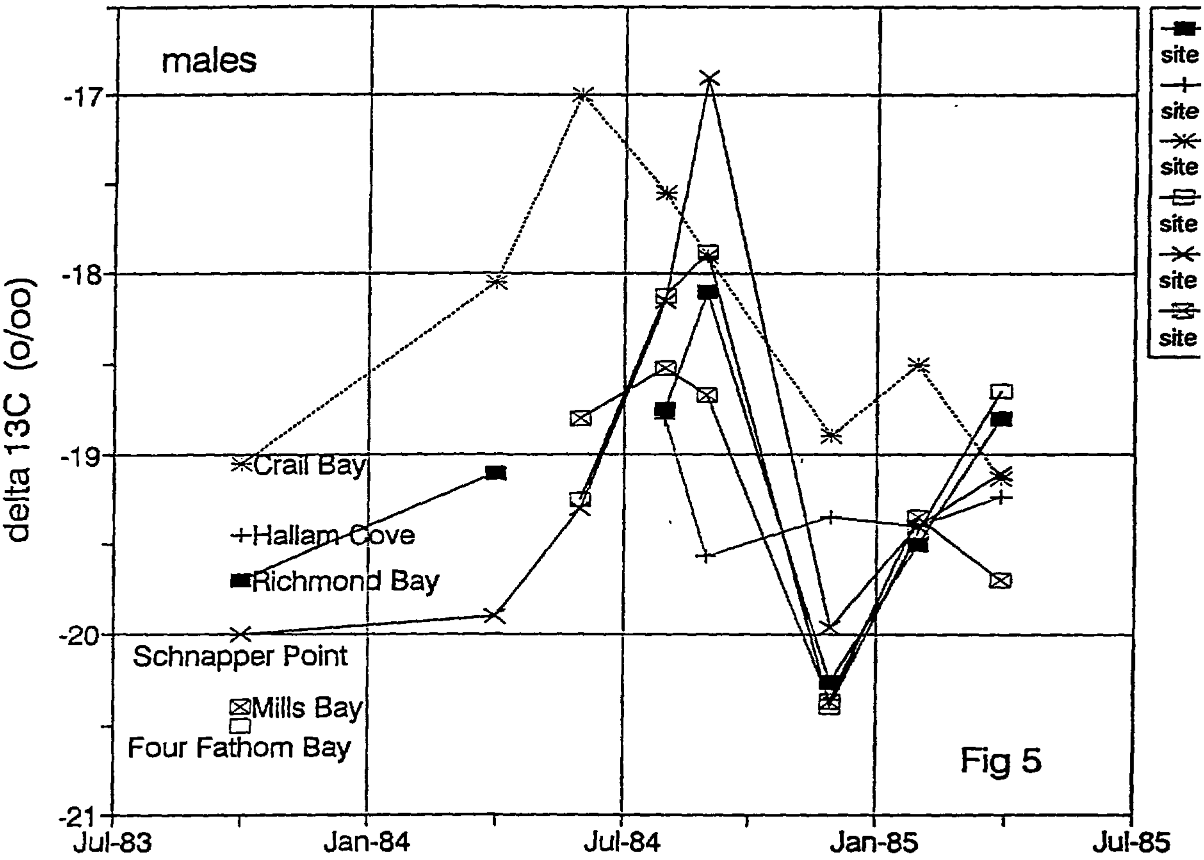

Fig. 5 $\delta^{13}C$ data for males: variation of mean values with time for sites 1 to 6.

Table 4. Differences in delta 13C : male - female

Site		Oct-83	Apr-84	Jun-84	Aug-84	Sep-84	Dec-84	Feb-85	Apr-85		
1	mean diff M-F	0.6	0.5			0.7		0.0	0.3		
2	mean diff M-F						1.4		0.6		
3	mean diff M-F					0.5					
4	mean diff M-F						0.9		0.5		
5	mean diff M-F					1.1			1.3	all differences	
6	mean diff M-F			1.7				0.5	0.0	av	0.7
7	mean diff M-F		1.4					0.5		s.d.	0.5
8	mean diff M-F									no.	17

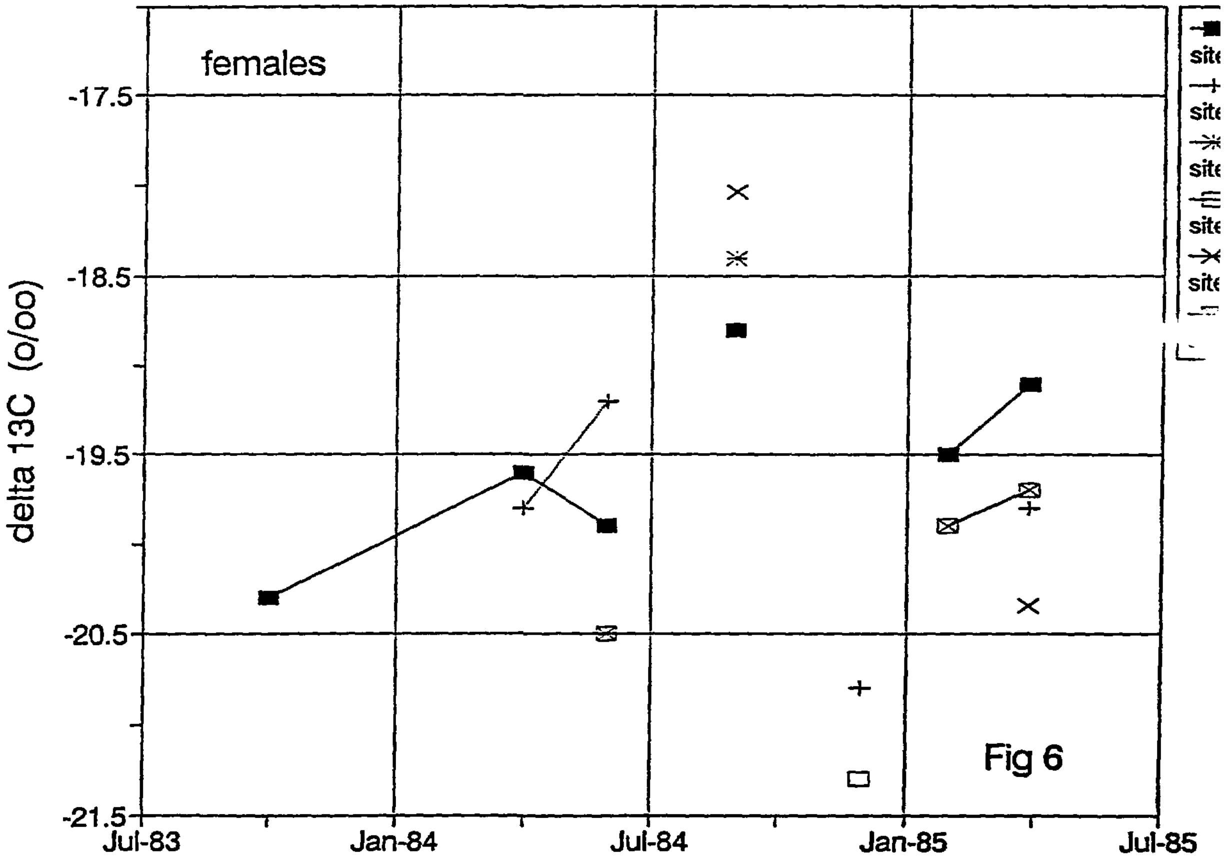

Fig. 6 $\delta^{13}C$ data for females: variation of mean values with time for sites 1 to 6.

Table 5. Zone averages for males

Site	Zone	Oct-83	Apr-84	Jun-84	Aug-84	Sep-84	Dec-84	Feb-85	Apr-85
1+2	Outer : Mean	-19.6	-19.1		-18.8	-19.2	-19.9	-19.4	-19.0
	s.d.	0.2			0.6	0.8	0.7	0.3	0.6
3+4	Middle: Mean	-19.5	-18.1	-18.1	-17.9	-17.9	-19.5	-19.0	-18.9
	s.d	0.9	0.1	1.3	0.6	0.3	0.8	0.6	0.3
5+6	Inner : Mean	-20.2	-19.9	-19.1	-18.4	-17.8	-20.2	-19.4	-19.4
	s.d.	0.3	0.1	0.6	0.5	1.1	0.4	0.1	0.4

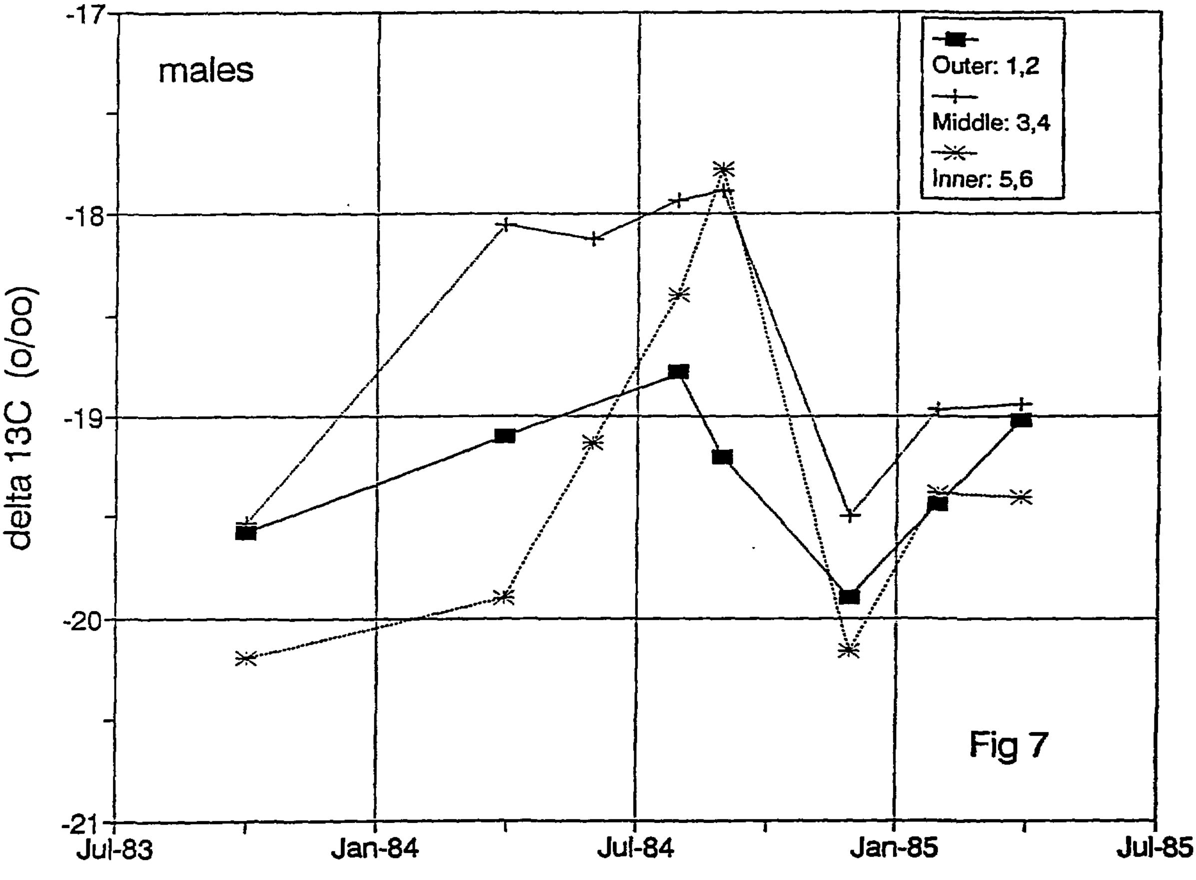

Fig. 7 δ^{13}C data for males sorted by zones (after Hickman *et al.* 1991), showing mean values for the sites listed in the legend.

Temporal variations

The results in Figs 3-7 show a general pattern of carbon isotope variation, with enrichment in the winter of 1984 and depletions in early spring of 1984 and early summer 1984-85. It is not clear, however, if this pattern would be a truly annual one, with variability of a few months for maxima and minima, or if it is a less regular pattern. The mussel will, though, not respond to day-to-day variations but a longer-term average of whatever variable it is, that can only change as the animal carbon is replaced. This general trend of more enriched carbon (less negative $\delta^{13}C$) in winter, and more depleted in summer, is opposite to that shown for the temperature dependence of phytoplankton $\delta^{13}C$ (Sackett *et al.*, 1965). However, Sackett's data was very scattered, and based on averages from 0°C (Antarctic) and 25°C; at each temperature there was a range of about 5‰. The total range of mussel values here is only 5‰. More recent work such as Rau *et al.* (1992) relates plankton $\delta^{13}C$ to the concentration of dissolved CO_2 [$CO_2(aq)$] which in turn is temperature dependent, with plankton having less negative $\delta^{13}C$ at warmer temperatures.

An attempt was made to measure the $\delta^{13}C$ values of particulate organic carbon (POC). The POC composition was routinely monitored by DSIR Marine and Freshwater Research (Hickman *et al.*, 1991) and comparison with total particulate matter gives a measure of the nutrient status of this material, which is mostly phytoplankton, but also includes silt and detrital material. Table 6 lists the isotope and carbon content of collections of POC. The carbon content values are ±10%. Most of the $\delta^{13}C$ values are close to -21‰, but there is some variability. The two extreme $\delta^{13}C$ values (-18.0‰, -24.1‰) occur with the highest POC concentrations. Crail Bay had, in February 1985, but not April, a more positive $\delta^{13}C$ for the particulates. This is similar to the more positive values for most mussels from Crail Bay. Perhaps this implies an unusually high production rate in this part of the Sound, which would tend to isotopically enrich the dissolved carbon dioxide if the CO_2 reservoir becomes significantly reduced in concentration. Alternatively, different species of plankton may dominate in different areas. Diatoms are known to dominate in winter after floods (Bradford *et al.*, 1987).

In contrast, the POC $\delta^{13}C$ from shallow Four Fathom Bay in April 1985 was depleted, and there was high silt load, but also high POC concentration probably including much terrestrial detritus. Terrestrial detritus of fine particular organic matter in New Zealand streams has $\delta^{13}C$ values ranging from -24.1‰ to -29.1‰ (Rounick *et al.*, 1982; Rounick & Hicks, 1985), and this was probably present in the flood-water silt near the surface at that collection.

During high rainfall periods, which may occur at any season and are variable from year-to-year (Heath, 1982), terrestrial detritus may be added to the mussel food and incorporated in the animal. This would then have an effect, though delayed according to the rate of carbon turnover in the animal, on the $\delta^{13}C$ composition of filter-feeding animals. Such an effect would be expected to be more marked the greater the distance from the open sea. This agrees with the more negative $\delta^{13}C$ values found for most Waitaria Bay samples, since that bay is shallow and the sediments, including terrestrial detritus, are often resuspended by wind. The contrasting situation is at Crail Bay, where although not as close to Cook Strait as Richmond Bay, the sampling site is not near any significant streams, and hydrologically sheltered from

Table 6: Particulate $\delta^{13}C$

Collection		$\delta^{13}C_{PDB}$ (‰)	Carbon content µg/litre
Richmond Bay April'85	1 m	-21.1, -20.9	86
Hallam Cove April'85	1 m	-21.3, -21.2	181
Crail Bay February'85	1 m	-18.0, -19.1	310
Crail Bay April'85	1 m	-22.3, -22.2	151
Four Fathom Bay April'85	1 m	-24.1, -23.7	376
Four Fathom Bay April'85	7 m	-21.2, -21.0	153
Mills Bay February'85	1 m	-21.5	not determined
Mills Bay April'85	1 m	-20.7, -20.3	206
Schnapper Pt April'85	1 m	-20.3, -20.5	156
Waitaria Bay April'85	0.5 m	-22.5	220

Table 7: Kaituna marsh plants

Plant	Location	$\delta^{13}C_{PDB}$ (‰)
Juncus sp	Upper Marsh	-27.3
Zostera	Creek site	-10.8
Enteromorpha	Creek site	-15.3
Leptocarpus	Upper Marsh	-24.5
Unknown (*Ruppia*-like)	Creek site	-11.2
Ruppia	Creek site	-26.1
Spartina	Creek site	-13.1

major floodwaters in the Sound (Gibbs *et al.*, 1991, 1992). Thus Crail Bay mussels attain the least negative $\delta^{13}C$ values.

Alternatively, floodwater can promote growth of mussels, not by direct addition of organic detritus as food, but by the addition of nutrients such as N and P to the sea water, promoting the growth of phytoplankton and therefore increasing mussel food. This is shown by other concurrent studies (Gibbs *et al.*, 1992).

The very large "jump" to more negative $\delta^{13}C$ values between September and December 1984 appears to relate to the regular spring phytoplankton bloom (Hickman, cruise reports) which had not occurred before the September 1984 sampling, but had before the October 1983 collection.

The analyses in this study reflect the average $\delta^{13}C$ of the food eaten by the mussel over some period, along with any enrichment by metabolism. Fry & Arnold (1982) showed that for shrimps, a four-fold weight increase sufficed to achieve a close isotopic resemblance of 1‰ or less to a new diet. Growth will effectively dilute carbon from earlier diet, but there will also be carbon turnover of the whole-body which may be a slower effect than growth for most organisms in immature stages. The mussels in this study were, in general, smaller than the asymtotic size for *P canaliculus* of about 140 mm shell length and the mussels can increase in length by 0.1-0.2 mm/day (Hickman, 1979). Thus the $\delta^{13}C$ value may reflect food over the previous 3-6 months. The surprisingly rapid change observed between September and December 1984 may have been influenced by the fact that on those occasions, at all except Richmond Bay, smaller individuals were sampled on the later date than on the earlier one.

The smaller individuals, because they are growing more rapidly, may reflect recent changes (as a fraction of body weight) more readily than larger individuals. Perhaps the September samples were dominated by the previous summer's food, whereas the December samples reflect the food available in winter, which would be expected to be more negative $\delta^{13}C$.

Condition variations

The detailed report of Hickman *et al.* (1991) uses condition index [CI = (meat weight × 100)/(total weight - shell weight)] as a measure of economic efficiency of the mussel farms. CI is related at the sites studied here for $\delta^{13}C$, and to some environmental factors. The condition may affect the $\delta^{13}C$ which depends on the biochemistry of the sample. Fats are consistently lighter isotopically than protein and carbohydrate (Fry & Parker, 1979), thus fat animals or flesh may have more negative $\delta^{13}C$ than lean animals. In a study in the Bering Sea, McConnaughey & McRoy (1979) measured C/N ratios on their samples and applied a correction factor for fat content. This was not done in this study but inspection of the Bering Sea data shows that for the six mollusc species, corrections (normalising to C:N = 4.0, presumably atomic ratio) were from -0.1 to +1.4‰. However, in that study, whole animal flesh was used, whereas in the present study only mantle material, in general, was analysed which although probably having a higher fat content than whole mussel flesh has weak seasonal variations (Gosling, 1992, p 267). The observed lipid concentrations (Gosling, 1992) would be expected to alter $\delta^{13}C$ values by less than 0.3‰, from the value for 100% protein or carbohydrate.

Hickman *et al.* (1991) used the data on condition index to divide the Pelorus Sound sites into three groups, and these are compared with male mussel $\delta^{13}C$ variations in Fig. 8. The winter high condition of 1984 correlates with less negative $\delta^{13}C$ values, and the sharp change in $\delta^{13}C$ follows a lowering of condition, but lack of samples prevents defining whether a similar effect was observed in spring 1983.

The data for sites 1-7 are regrouped in Table 8 and Fig. 9 according to the condition groups shown in Fig. 8. The high condition mussels are almost always, on average, less negative than the low and medium condition animals. This suggests that for identical food sources, the high condition mussels have less fat than the other mussels, although Table 8 shows that the spread of data for each group are over-lapping, and the low fat contents (0.9-1.8% wet weight) reported in Gosling (1992) show that fat content is not the cause of $\delta^{13}C$ variations.

Carbon source

The particulate matter $\delta^{13}C$ values may vary for several reasons:

a) Variations in plankton $\delta^{13}C$

Recent studies by Rau *et al.* (1992) confirm for the marine situation that the $\delta^{13}C$ of organic matter is inversely related to the concentration of dissolved carbon dioxide [CO_2(aq)]. Early studies by Calder & Parker (1973) on freshwater algae and the model of Farquhar *et al.* (1982) for terrestrial plants suggested that phytoplankton should vary in this way. For the North Atlantic data of Rau *et al.* (1992) the suspended particulate organic matter (POM) $\delta^{13}C$ was shown to respond to [CO_2(aq)] in an inverse, linear relationship due to biological demand. An inverse, non-linear relationship is shown to be expected for constant biological demand. As demand increases then the biological production removes depleted CO_2 and the remaining CO_2(aq) becomes more enriched as production increases. The maximum rate of photosynthesis production might be expected to be in summer, leading to an enrichment in $\delta^{13}C$ in mussels as they replace or add to their carbon store with new carbon. Because of stored carbon, any changes to the mussel composition, and the rate of change, would depend on relative growth rate, i.e., amount of new carbon added, as well as the turnover time of old carbon.

Changes to less negative values are observed (Figs 3, 4, 5) from October 1983 to September 1984 and again from December 1984 to April 1985. These agree with the above hypothesis except that depletion toward more negative "winter" values would be expected to occur perhaps from May onward, but in the only winter sampled, June-August 1984, values continued to become more positive. The time of year with significant $\delta^{13}C$ change in mussels would depend to a large extent on the effective lag time that is relevant for these animals.

The degree of change and timing of $\delta^{13}C$ of mussel food depend on not only the relationship between plankton and $\delta^{13}C$[CO_2(aq)] but could also vary with any phytoplankton species change. Gibbs *et al.* (1992) record increases in silica which could favour diatom growth over other species, but Rau *et al.* (1992) show that $\delta^{13}C$ variations were independent of species

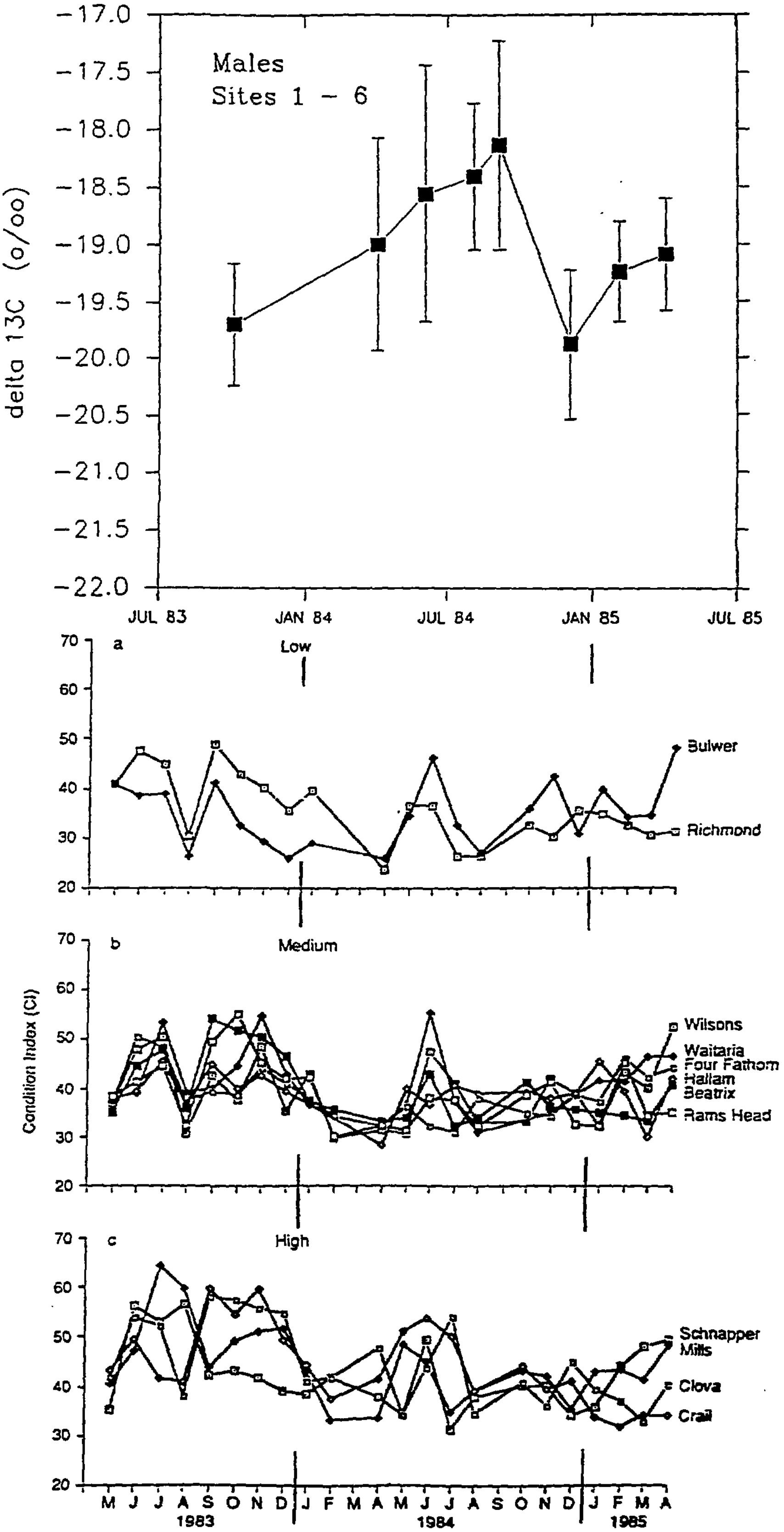

Fig. 8 Condition summaries (from Hickman *et al.* 1991, Fig. 2) compared with $\delta^{13}C$ data for male mussels.

Table 8. Condition mean delta 13C values for males

Condition		Oct-83	Apr-84	Jun-84	Aug-84	Sep-84	Dec-84	Feb-85	Apr-85
	Sites								
High	3,5,6 Mean	-19.6	-19.0	-18.3	-18.2	-17.8	-19.7	-19.1	-19.3
	s.d.	0.7	1.1	1.2	0.6	0.9	0.7	.0.5	0.3
	No.	4	4	5	8	9	9	8	7
Med	2,4,7 Mean	-20.2	-19.6	-19.3	-18.5	-18.5	-19.9	-19.5	-19.2
	s.d.	0.8		0.2	0.5	0.9	0.7	0.3	0.4
	No.	5	1	2	8	8	4	8	8
Low	1 Mean	-19.7	-19.1		-18.8	-18.1	-20.3	-19.5	-18.8
	s.d.	0.3			0.9		0.5	0.6	0.9
	No.	2	1	0	4	1	3	2	3

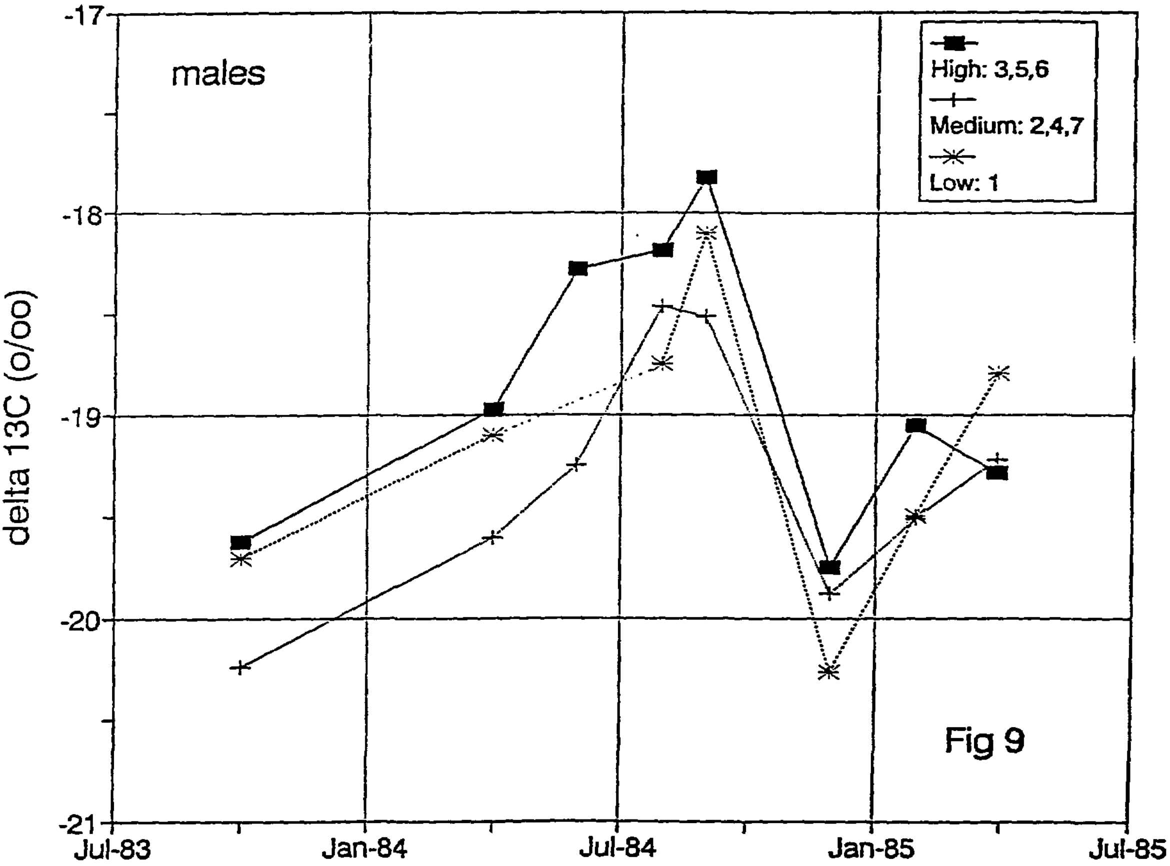

Fig. 9 $\delta^{13}C$ data for males sorted by low, medium and high condition (after Hickman *et al.* 1991), showing mean values for the sites listed in the legend.

composition.

b) Changes of POC $\delta^{13}C$ values due to freshwater $\delta^{13}C$

Tan and Strain (1983) show that estuarine dissolved inorganic carbon (DIC) is depleted in ^{13}C relative to marine DIC, with values near -10‰, and thus phytoplankton in such an environment is more negative, near -30‰. However, Table 6 shows that the plankton $\delta^{13}C$ values are marine-like values, and the salinity data (Gibbs *et al.*, 1991) show little variation from marine values of 34.0 ppt at Richmond Bay to 31.8 ppt at Mills Bay, except for occasional freshwater influx from storm events (Fig. 10). This data is insufficient to determine if plankton $\delta^{13}C$ relate to the mussel $\delta^{13}C$ data. In any case, chlorophyll *a* concentrations showed poor correlation with condition index.

c) Addition of terrestrial detritus

Detrital material brought in to the Sound will be mostly of terrestrial material such as found by Rounick *et al.* (1982), but some may be flushed from the estuaries of the only two rivers, the Kaituna and Pelorus near Havelock. These form extensive delta areas and marshes which are colonised by several species of native and exotic plants. Some $\delta^{13}C$ analyses have been made for Professor G Knox (Table 7). They show that several species: *Zostera, Entromorpha, Spartina,* and an unknown plant, have less negative $\delta^{13}C$ values than most terrestrial plants which have values between -23‰ and -30‰. Although unlikely to be of dominance in flood detritus, the compositions of these plants would reduce any $\delta^{13}C$ difference between terrestrial detritus and marine phytoplankton.

Both effects (b) and (c) would add ^{13}C-depleted components to the food. And both would occur more readily during periods of increased runoff. However, such events, especially major storms enough to affect most of Pelorus-Kenepuru Sound, would be occasional and ephemeral and unlikely to have long term effects. More significant may be the general trends recorded by Gibbs *et al.* (1991) and Hickman *et al.* (1991) of a small average decrease in salinity toward the head of the Sound. This is inconsistent with the trends shown in Fig. 7, in which the samples are grouped into three different zones (Fig. 1) as the middle zone samples are the most enriched mussels. However, in the late winter July-August of 1984, when the wettest time of year might be expected, the Inner Sound mussels became enriched in ^{13}C. A possible confused picture may arise if mussels at Mills Bay (site 6) are able to feed on particulate matter (either food or fecal material) from the nearby salmon farm, if the salmon feed is composed of material with rather different $\delta^{13}C$, especially if it varied during the period of study. However, Fig. 5 shows that Mills Bay samples are rarely outside the range of values for other sites.

Environmental variability does not readily account for the isotope variations, or the variations in condition index. Hickman *et al.* (1991) report that parallel gradients, and therefore cross correlations, of increasing salinity, decreasing (summer) and increasing (winter) water temperature and decreasing food concentration (chlorophyll *a*, total particulate matter, and particulate carbon) which occurred from the inner Kenepuru to the outer Pelorus Sound prevent clear identification of a single environmental factor determining condition index. Since environmental conditions provide important control on energy storage and reproductive

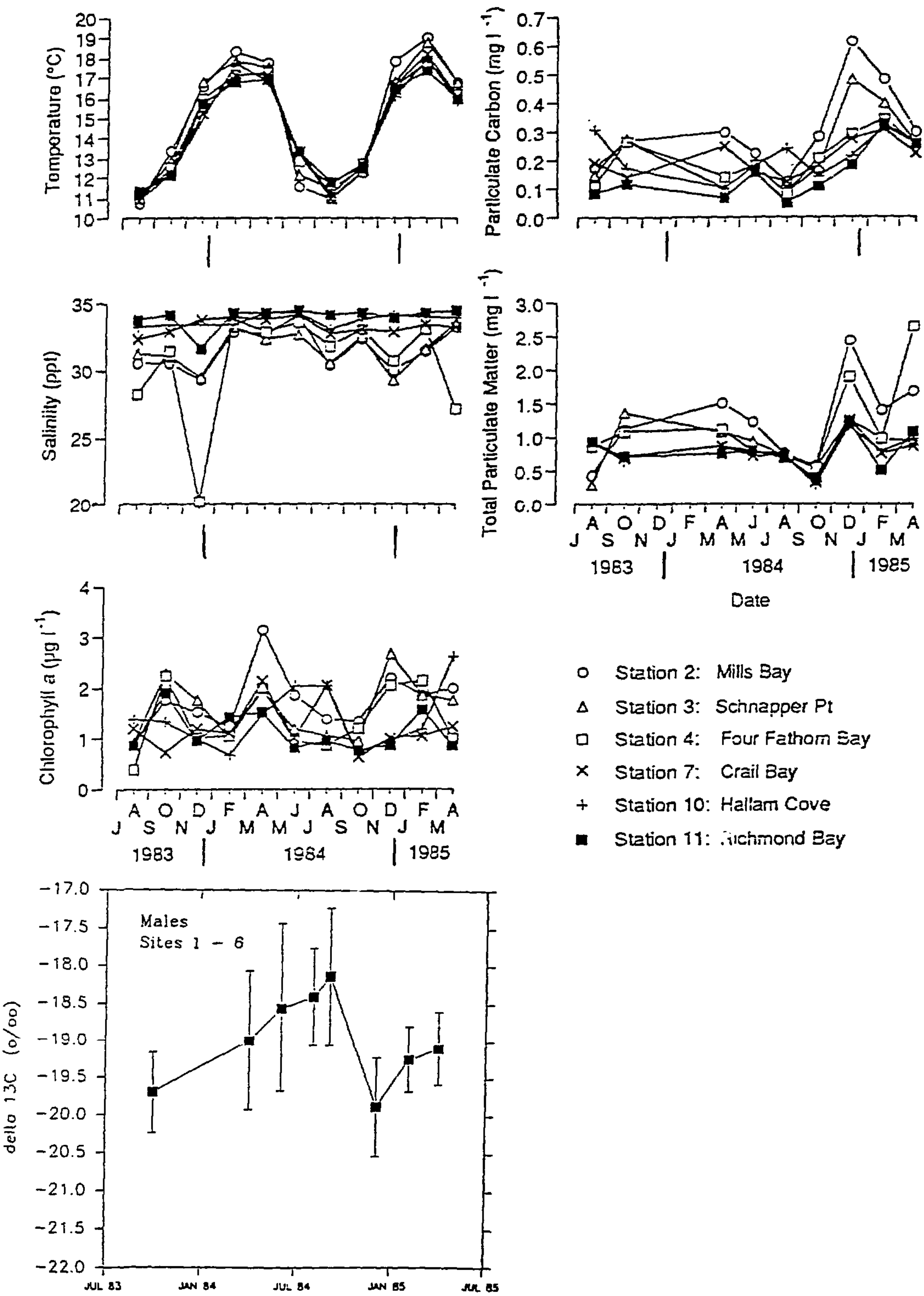

Fig. 10 **Environmental data (from Hickman *et al.* 1991, Fig. 3) compared with $\delta^{13}C$ data for male mussels.**

activity, a detailed set of data for those factors, over the preceding several months prior to sampling, may elucidate explanations for the isotope variability.

General discussion

Some other studies such as Peterson *et al.* (1985) have shown advantages in multiple isotope studies in estuarine systems. Although $^{15}N/^{14}N$ has possibilities, some marsh plants have much variability and little difference from plankton, and the method is not available at the Institute of Geological & Nuclear Sciences. Perhaps a more useful indicator would be sulphur isotopes $^{34}S/^{32}S$, where any negative excursion from plankton values of $\delta^{34}S_{CDT}$ from +20 ‰ (similar to sea water) would imply terrestrial input (Peterson *et al.*, 1985).

CONCLUSION

These data provide no evidence for terrestrial food being significant for green-lipped mussels *P canaliculus*. There are, however, significant variations in mussel $\delta^{13}C$ values, with a seasonal component. This can be explained by an apparent lag of about six months, in the $\delta^{13}C$ composition variation relative to expected variations of phytoplankton composition. This is despite data showing steady growth, and observed rapid changes in condition index.

Acknowledgements

We thank crew of the Kaharoa for assistance with sampling, Mark James for helpful comments on this report, and K Stanley, M Oelsner, P Chapman and R Ramaratnam for analytical assistance.

References

Bradford, J M, Chang, F H, Baldwin, R, Chapman, B, Downes, M and Woods, P. 1987. Hydrology, plankton, and nutrients in Pelorus Sound, New Zealand, July 1981 and May 1982. *N Z Journal of Marine and Freshwater Research 21*, 223-233.

Fry, B and Arnold, C, 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia 54*, 200-204.

Fry, B and Parker, P L, 1979. Animal diet in Texas seagrass meadows: $\delta^{13}C$ evidence for the importance of benthic plants. *Estuarine and Coastal Marine Science 8*, 499-509.

Gibbs, M M, James, M R, Pickmere, S E, Woods, P H, Shakespeare, B S, Hickman, R W and Illingworth, J, 1991. Hydrodynamic and water column properties at six stations associated with mussel farming in Pelorus Sound, 1984-85. *N Z Journal of Marine and Freshwater Research 25*, 239-254.

Gibbs, M M, Pickmere, S E, Woods, P H, Payne, G W, James M R, Hickman, R W and Illingworth, J, 1992. Nutrient and chlorophyll *a* variability at six stations associated with mussel farming in Pelorus Sound 1984-85. *N Z Journal of Marine and Freshwater Research 26*, 197-211.

Gosling, E (editor) 1992. The mussel *Mytilus*: ecology, physiology, genetics and culture. Developments in Aquiculture and Fisheries Science vol 25, Elsevier, Amsterdam. 589 pp.

Haines, E B and Montague, C L, 1979. Food sources of estuarine invertebrates analysed using ^{13}C/^{12}C ratios. *Ecology 60*, 48-56.

Heath, R A, 1982. Temporal variability of the waters of Pelorus Sound, South Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research 16*, 95-110.

Hickman, R W, 1979. Allometry and growth of the green-lipped mussel *Perna canaliculus* in New Zealand. *Marine Biology 51*, 311-327.

Hickman, R W, 1980. Management problems in mussel farming. *N Z Veterinary Journal 28*, 226-230.

Hickman, R W, 1985. What makes a mussel? (Abstract only) *N Z Marine Sciences Society Newsletter (28)*, 11-12.

Hickman, R W and Illingworth, J, 1980. Condition cycle of the green-lipped mussel *Perna canaliculus* in New Zealand. *Marine Biology 60*, 27-38.

Hickman, R W, Waite, R P, Illingworth, J, Meredyth-Young, J L and Payne, G, 1991. The relationship between farmed mussels, *Perna canaliculus*, and available food in Pelorus-Kenepuru Sound, New Zealand, 1983-1985. *Aquaculture 99*, 49-68.

Lyon, G L and Richardson, J, 1983. Non-terrestrial food source for Fiordland brachiopods: carbon isotope evidence. Institute of Nuclear Sciences, Lower Hutt. INS-R--317.

McConnaughey, T and McRoy, C P, 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology 53*, 257-263.

Peterson, B J, Howarth, R W and Garritt, R H, 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science 227*, 1361-1363.

Rau, G H, Takahashi, T, DesMarais, D J, Repeta, D J and Martin, J H, 1992. The relationship between δ^{13}C of organic matter and [CO$_2$(aq)] in ocean surface water: data from a JGOFS site in the northeast Atlantic Ocean and a model. *Geochimica et Cosmochimica Acta 56*, 1413-1419.

Rounick, J S and Hicks, B J, 1985. The stable isotope ratios of fish and their invertebrate prey in four New Zealand rivers. *Freshwater Biology 15*, 207-214.

Rounick, J S, Winterbourn, M J and Lyon, G L, 1982. Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. *Oikos 39*, 191-198.

Sackett, W M, Eckelmann, W R, Bender, M L and Be, A W H, 1965. Temperature dependence of carbon isotope composition in marine plankton and sediment. *Science 148*, 235-237.

Stephenson, R L and Lyon, G L, 1982. Carbon-13 depletion in an estuarine bivalve: detection of marine and terrestrial food sources. *Oecologia 55*, 110-113.

Stephenson, R L, Tan F C and Mann, K H, 1984. Stable carbon isotope variability in marine macrophytes and its implications for food web studies. *Marine Biology 81*, 223-230.

Tan, F G and Strain, P M, 1983. Sources, sinks and distribution of organic carbon in the St Lawrence Estuary, Canada. *Geochimica et Cosmochimica Acta 47*, 125-132.

Waite, R P, 1985. The nutritional biology of *Perna canaliculus* within mariculture systems. (Abstract only) *N Z Marine Sciences Society Newsletter (28)*, 21-22.

Waite, R P, 1986. Effects of food concentration, temperature and mussel size on the nutrition and growth of *Perna canaliculus*. (Abstract only) *N Z Marine Sciences Society Newsletter (29)*, 27-28.