Stable isotopes of carbon and oxygen in the shells of terrestrial molluscs

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ABSTRACT

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The oxygen and carbon isotopic composition of terrestrial mollusc shell aragonite has been investigated using a series of controlled laboratory experiments, and two field case studies of modern and fossil snail shells. A standardised method of treating mollusc shell carbonate prior to mass spectrometric analysis, to remove external and intercrystalline organic material, involving two stages of bleaching, after which samples were vacuum roasted (at 380°C) and then placed in an oxygen plasma furnace, has been developed.

Base populations of five species - *Helix aspersa, Cepaea nemoralis, Cernuella virgata , Rumina decollata* and *Ferussacia folliculus* - were maintained to supply snails for three laboratory experiments. The first experiment investigated the effect of environmental temperature (from 10-24°C) on the growth of the snails and isotopic composition of the shells. The second investigated the effect of introducing diurnal cycles of temperature and light, and the third the effect of changing the experimental design with some snails maintained (as controls) in the same way as in the first two experiments. The oxygen isotopic composition of the water and the carbon isotopic composition of the organic and inorganic fractions of the snails' diet were measured.

The temperature effect upon the $\delta^{18}O$ composition of the shells was close to that expected (around 0.2‰ per °C), although above approx. 15°C, true temperature effects were masked by variable evaporation and metabolic effects. These effects may explain why the oxygen isotopic compositions of the shells were enriched by up to 13‰ over isotopic equilibrium with the snails' environmental water The evaporation effect was quantified from the results of the third experiment, and from this metabolic effects were seen to cause 2-3‰ enrichment in ^{18}O over equilibrium values expected from the snails' environmental water. In natural systems snails are active under conditions of high humidity. In the experiments, relative humidities in the microenvironment were constantly near saturation, but the containers in which the snails were maintained were not sealed (except in the third experiment) resulting in a strong evaporation effect. The metabolic (vital) effect may be a two stage fractionation, firstly between the extrapallial fluid and the shell, but also between the external fluid (environmental water) and the blood fluid (haemolymph) which could vary according to the activity levels and metabolic rate of the snails.

The Jamaican case study concentrated on four endemic species collected from 96 sites across the island. Shell isotopes in relation to location, elevation and wetness of environment were investigated. A large amount of isotopic variation was found within and between species of snail, locally and regionally. An expected trend towards more depleted $\delta^{18}O$ values with increasing elevation along coast to inland transects was not found. The limited data on Jamaican environmental waters, and uncertainties as to the temperatures in the snails' microenvironments, suggested that shell secretion was close to isotopic equilibrium with environmental waters (predominantly rainfall), although a vital effect of up to 2‰ could still be inferred. Carbon isotopes in the Jamaican shells have been linked with the type and relative proportions, of inorganic : organic carbon available to, and used by, each species of snail in relation to their ecologies. It may be possible to derive information on the habits of other Jamaican species once a typical $\delta^{13}C$ for a species has been assessed.

Fossil shells of the species *Arianta arbustorum* from two Late-glacial to Post-glacial sections at Holywell Coombe, Folkestone, Kent were investigated as part of a multi-disciplinary project. A high degree of isotopic variation was found within and between sample horizons, making it difficult to interpret palaeo-environmental or climatic changes. Comparison of shell and some tufa oxygen isotope ratios suggested that an evaporation effect of around 2‰ resulted in isotopic enrichment in shell isotope values over the environmental water (spring water from which the tufas precipitated).

In both field studies it was difficult to interpret the results because of the complexity of natural systems.The lab experiments suggest that temperature changes may be masked by compositional effects acting upon the environmental waters used by the snails. However, if the fractionations between water and shell are taken into account, then the oxygen isotopic composition of land snail shell material should give a good estimation of the isotopic composition of local environmental waters (generally precipitation).

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CONTENTS

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTORY REMARKS AND METHOD OF APPROACH

Shells of terrestrial molluscs are often well preserved in the geologically recent (Pleistocene and Holocene) fossil record. Provided that the shells have not been transported after the animal's death, then the shell provides a datable and local source of palaeoenvironmental information.

Many studies of land snails concentrate on faunal analyses, where assemblages, relative abundances of species or shell morphologies are compared with modern populations and specific distributions in relation to environmental variables. However, provided that the shell material is well preserved, mollusc carbonate shells also contain an oxygen and carbon stable isotope record from the time of their secretion. From studies of field populations of modern snails, stable isotope signatures, in the form of the ratios of ^{18}O to ^{16}O and ^{13}C to ^{12}C present in the shells, have been shown to reflect environmental variables.

Recent work has linked carbon isotope ratios in terrestrial mollusc shells with the nature of the plant community upon which the snails feed and to the amount of rainfall in their habitat (Goodfriend and Magaritz, 1987). Oxygen isotope ratios in shells have been associated with the isotopic composition of local precipitation or water vapour, with modifications thought to result from a number of factors, including some or all of the following: evaporation effects linked with local relative humidities (Yapp 1979); seasonal or geographic effects (Magaritz and Heller 1980, 1983 and Magaritz *et al.* 1981); a relatively constant biological fractionation or vital effect, relating to the snails' metabolism (Lecolle 1985) and from variable metabolic effects relating to activity levels of the snails , with some influence from evaporation (Goodfriend and Magaritz 1987, Goodfriend *et al.* 1989). The oxygen isotopic composition of shell aragonite should also reflect the environmental temperature at the time the shell was secreted, and although the magnitude of the effect is not very great (around -0.2‰ per °C, measured in marine gastropod shells, Grossman and Ku, 1986, Hudson and Anderson, 1989), a large shift in environmental temperatures (such as that experienced at the Devensian - Flandrian transition at the demise of the last glaciation) should be recorded in the oxygen isotope record of contemporaneous terrestrial mollusc shells.

The studies noted briefly above have concentrated upon field surveys of natural mollusc populations. No work has been carried out using laboratory populations. Laboratory experiments have an advantage over field surveys in that they allow the control of particular environmental factors independently of others. Thus, it should be possible to isolate specific isotopic effects resulting from a particular environmental variable. Such laboratory experiments should allow a better appreciation of how the stable isotopic composition of shells is affected by specific environmental variables. From this, a greater understanding of the types of palaeoenvironmental information indicated by the stable isotope signatures of terrestrial mollusc shells should be realised.

The approach of this study has, therefore, been to investigate the isotopic composition of terrestrial mollusc shells secreted during controlled laboratory experiments, with a view to assessing the trends likely with changing environmental temperatures and other conditions. It is also hoped that the experiments will show how the isotopic composition of the primary sources of carbon (organic

and inorganic dietary components and atmospheric carbon, Goodfriend and Hood, 1983); and oxygen (environmental water, atmospheric sources and dietary components) may be reflected in the resultant shell isotopic compositon. For carbon isotopes, this should allow a better understanding of how carbon isotopes in mollusc shells relate to diet and environment. For oxygen isotopes it should show whether the isotopic composition of the water available to the snails (which in a natural environment would be rain, water vapour, or a mixture of the two), is reflected in shell isotope ratios, *i.e.* whether the shell is secreted at, or near, isotopic equilibrium with the original water. Any deviations from equilibrium values might then be quantified and accounted for.

In addition to the laboratory experiments two case studies will be presented, investigating stable isotopes in the shells of modern and fossil terrestrial molluscs from natural systems, with a view to interpreting the stable isotope records in the light of the findings of the experimental work.

1.2 STABLE ISOTOPE RATIOS

Stable isotopes are measured as relative abundances with respect to a standard value. The relative isotope concentrations are measured much more easily than absolute abundances, and with great accuracy using mass spectrometric techniques. The relative difference is known as the delta value, and is defined as

$$\delta x = \frac{R_x - R_{std}}{R_{std}} \qquad (1.1)$$

where R_x represents isotope ratio of a sample (for example, $^{13}C/^{12}C$ or $^{18}O/^{16}O$) and R_{std} is the corresponding ratio in a standard. The δ value is expressed as parts per thousand (per mil, ‰) and written as

$$\delta x = \left(\frac{R_x}{R_{std}}\right) - 1 \quad \times 1000 \qquad (1.2)$$

Thus, a sample with a $\delta^{13}C = -8‰$ has 8‰ less ^{13}C than the standard, and a sample with a $\delta^{13}C$ of +6‰, is enriched in ^{13}C by 6‰ relative to the standard. Generally, a convenient 'working standard' is used by each stable isotope laboratory, and these are calibrated with internationally recognised standards. For carbonates, oxygen and carbon isotope ratios are measured relative to a belemnite rostrum (*Belemnitella americana*) from the Cretaceous, Pee Dee formation, South Carolina, abbreviated and known as the PDB standard. For oxygen isotopes in waters, the standard is a sample close to that of an average sea water sample, known as Standard Mean Ocean Water, or SMOW (Fritz and Fontes, 1980; Hoefs, 1987).

Because of their different atomic masses, the isotopes of a particular element may be found in different amounts between substances or phases. The partitioning between two substances or phases with different isotopic ratios is called isotopic fractionation, and the fractionation factor (α) is defined as the ratio of the numbers of any two isotopes in one chemical compound, A, divided by the corresponding ratio for another compound, B.

The methods of analysing isotope ratios in carbonates, such as mollusc shell aragonite, and waters are presented in section 2.6

1.3 AIMS AND STRUCTURE OF THE THESIS

The primary aim of this research was to investigate, and to improve the understanding of, the factors that influence the stable isotopic composition of terrestrial mollusc shells. From this it is hoped to be better able to interpret the stable isotope records of fossil mollusc material in relation to environmental variables, and thus to test the potential of stable isotopes in fossil mollusc shells as a tool in the reconstruction of continental palaeoenvironments and palaeoclimates.

The chosen method of study was primarily through the use of controlled laboratory experiments, followed by two case studies of modern and fossil snail shells.

The structure of this thesis is as follows: Firstly, the experimental methods for the controlled growth experiments are outlined, along with the development of standard sample preparation techniques. Following this, the results of the various growth experiments (three in all) are presented and discussed, and the findings of each experiment are summarised. The findings of the first case study - investigations of stable isotopes in the shells of modern snail populations from Jamaica, West Indies, in relation to local environmental variables - are then presented. The second case study follows on. This study involved analysis of fossil mollusc shells located in two sections through ?Late Devensian to Flandrian deposits in Kent, Southeast England. Finally the overall findings of the various aspects of the research are summarised and discussed.

CHAPTER 2

EXPERIMENTAL METHODS & DEVELOPMENT OF TECHNIQUES

2.1 INTRODUCTION

The primary aims of growing land snails under constant controlled conditions were two-fold

(a) To ascertain whether shell deposition for terrestrial snails is at or near isotopic equilibrium with a snail's environmental water.

(b) To see how the isotopic composition of laboratory reared snails would relate to their growth temperature and possibly other variables, and thus whether fossil terrestrial snail shells (where original shell material is preserved) might be useful as a continental palaeoclimatic indicator.

In all, three experiments were carried out to produce snails whose shells could be analysed for the stable isotopes of carbon and oxygen. Snails were grown under controlled constant temperatures, maintaining and monitoring as many of the other environmental variables as possible. The three experiments were:

(1) Growth of at least four species of snails under a series of constant temperatures, from approximately 10° to 24°C. Each period of growth lasted for six weeks, and snails were grown at six different temperatures. Thus, Experiment 1 comprised six phases.

(2) Comparison of shells of three species grown under three temperature regimes. One population was grown at a higher temperature in illuminated conditions, one at a lower temperature, in darkness, and the third moved between the two temperature extremes on a daily basis.

(3) A similar scenario to (2), although with further investigation of the effects of dark and light in the daily cycles, with the snails being maintained under different environmental conditions from those imposed in (1) and (2).

The growth experiments were carried out in thermostatically controlled rooms in the Zoology Dept. (now the Dept. of Ecology and Evolutionary Biology) at Liverpool University. Two rooms were available. The first, the 'warm room' was used for temperatures of above 19°C. The other the 'cool room', was used during the first experiment for growth over a range of temperatures between 10 - 16°C. The 'cool-room' was not freely available at the times of experiment two and three. However, it was found that the open corridor in the basement area adjacent to the thermostatically controlled rooms was suitable for use as a cool-room base at a fixed temperature, providing that a permanent record of the air temperature was taken. Thus the corridor was used along with the warm-room during the second and third growth experiments.

2.2 BASE POPULATIONS

2.2.1 Initiation, care and maintenance

To obtain snails for experimental work, it was necessary to set up and maintain breeding populations of the chosen snail species. Several populations of five terrestrial species, all pulmonates, were set up during late October 1986. The species chosen were *Helix aspersa*, *Cepaea nemoralis*, *Rumina decollata*, *Cernuella virgata*, and *Ferussacia folliculus*.

Throughout the experimentation period, up to the end of 1988, populations of these snails were maintained in the Dept. of Zoology here at Liverpool. The base populations were grown in transparent plastic lunch boxes 28 x 16 x 9cm or 17 x 11.5 x 5cm in dimensions, with lift-off lids that were partially constucted of fine netting. The netting was stretched across most of the surface up to within 2cm of the edges of the lids of the larger boxes, and across one half of the smaller boxes (Fig 2.1, and also Cowie, 1982). Feeding was carried out, on average, every five days, by placing food onto solid areas of the lunch-box lids that had been cleaned and wetted. The food was a 1:1:1 mixture of three ingredients - 'Marvel' milk powder, 'Ready-brek' oat cereal and General Purpose Reagent (GPR) calcium carbonate. These named brands were used throughout, and the ingredients were weighed out in equal proportions before the mixture was ground to a fine powder using a pestle and mortar. The diet supplied protein, carbohydrate, vitamins and minerals and a constant supply of calcium carbonate. Any remaining uneaten food was removed by scrubbing the lids prior to the next feeding. The boxes were approximately half filled with a base of potting compost, usually 'John Innes Seed Compost'. The soil was dampened at each feeding with water, and a plant spray-mister used to coat the remainder of the box interior and snails with moisture. Almost immediately after feeding, (generally within five to fifteen minutes) the snails could be seen crawling up the sides of the boxes to obtain their food from the inside of the box lids.

The constant feeding and dampening of the soil induced all of the species to breed, although it was found that a high density of snails would often inhibit further reproduction. Extra inducement to reproduce was found to occur under several conditions:

(1) Rehousing snails in new soil and/or boxes.

(2) Thinning existing populations within boxes.

(3) Allowing the period between feeding, and especially between watering, to increase, and then stepping up the amount and regularity of feeding and watering.

The soil compost was turned every few weeks or so using a flat knife, although care was taken not to disturb eggs so this overturning was not carried out on a strict regular basis. The overturning allowed aeration of the soil, and helped stop the substrate becoming packed down as a result of snails travelling across the surface and with the addition of water at the soil surface.

It gradually became possible to make reasonably sure that baby snails would be available for the start of experimental periods, for transfer to the controlled temperature rooms. It was most important to maintain base populations and to achieve a steady rate of reproduction, as young snails were to be used in all the proposed growth experiments.

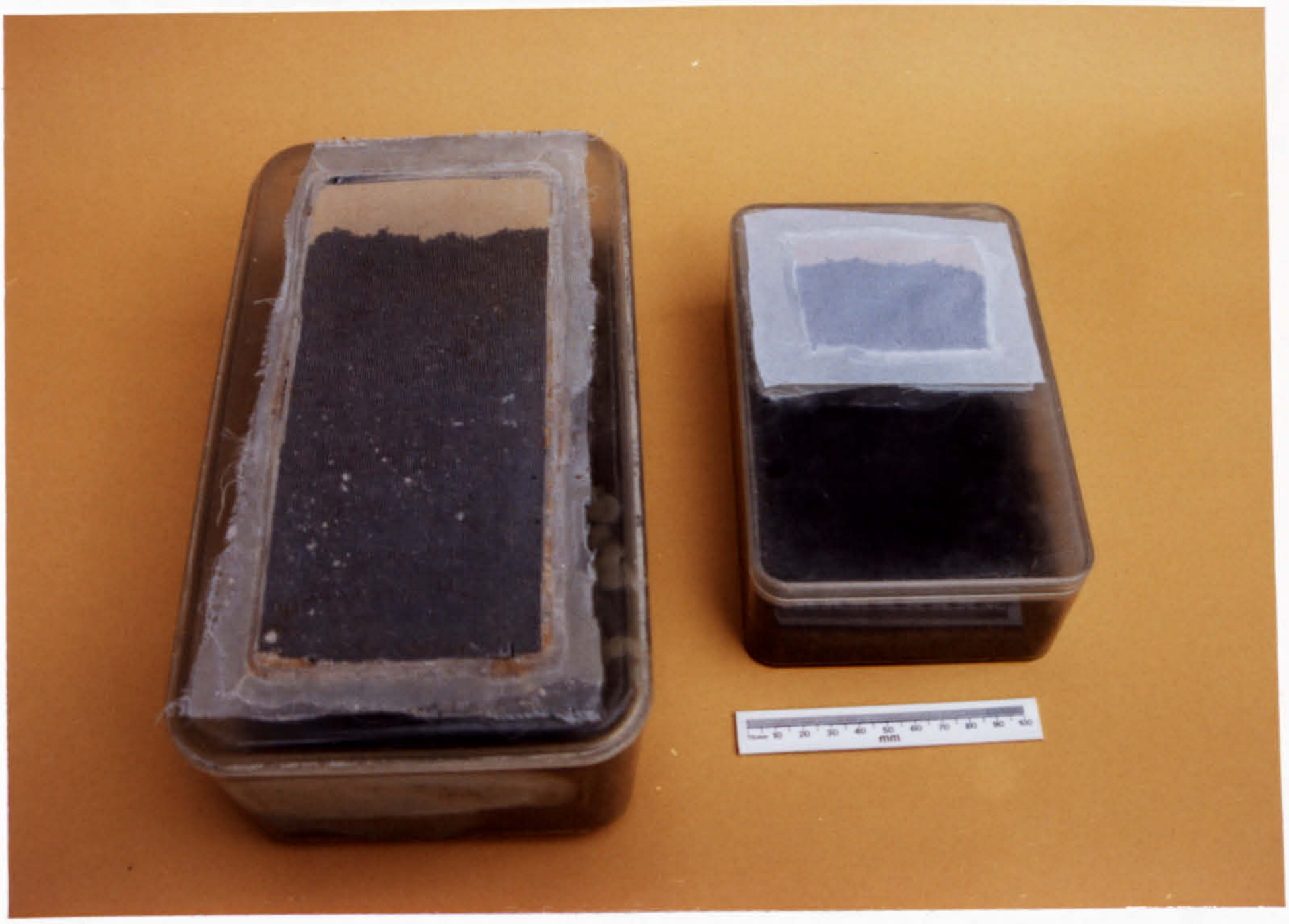


Figure 2.1 Containers used for housing the base populations of snails, 28 x 16 x 9cm and 17 x 11.5 x 5cm. (These containers were also used throughout the growth experiments).

2.2.2 Choice of snail species

Snail species were chosen for the availability, ease and maintenance of breeding populations and relatively large shell size.

It was decided to work with northern temperate and Mediterranean species to see how the different ecologies of the snails might influence the results. It was also hoped to be able to compare isotope data from the shells of egg layers to those from viviparous snails.

The pertinent factors and the suitability of each of the five species are outlined in Table 2.1.

	DIMENSIONS Ht x Bdth mm suitable size ?	ECOLOGY well known + understood ?	LAB REARING rel easy ?	COMMONLY used in lab expts?	DISTRIBUTION		REPRODUCTION		AVAILABILITY		
					N. Temp. species	Med. species	Egg layer	Live bearer	Current lab. pops. ?	Domestic pops ?	Field coll. ?
Helix aspersa	globular 25-35 x 25-40 *yes*	herbivore introduced synanthropic gregarious widespread *yes*	*yes*	*yes*	*yes*	--	*yes*	--	---	*yes*	---
Cepaea nemoralis	globular 12-22 x 18-25 *yes*	herbivore post-gl migrant variable habitat cold susceptible f. widespread *yes*	*yes*	*yes*	*yes*	--	*yes*	--	--	*yes*	*yes*
Cernuella virgata	globular 6-19 x 8-25 *lgr. ones preferred*	herbivore ? introduced xerophile dry open coastal habitats f. widespread *somewhat*	?	?	*yes*	--	*yes*	--	--	--	*yes*
Rumina decollata	tapering cylindrical 22-35 (ht) *yes*	herbivore not in Britain dry open habitats throughout Med *somewhat*	*yes*	--	--	*yes*	*yes*	--	*yes*	--	--
Ferussacia folliculus	slender conical oval 5-6 x 2-4 *rather small*	herbivore not in Britain dry open habitats throughout Med *somewhat*	*yes*	--	--	*yes*	--	*yes*	*yes*	--	--

Table 2.1 Suitability of chosen snail species for experimental work

2.3 ECOLOGY AND LABORATORY REARING OF SPECIES

The five chosen snail species are illustrated in Figures 2.2a-e.

2.3.1 *Helix aspersa* Müller

This snail, of the family Helicidae, is also known as the common garden snail. It has a globular shell with 4.5 - 5 rapidly expanding whorls. The shell colour is variable but generally pale brown to yellow, with 0 - 5 dark spiral bands, and the shell has a characteristic wrinkle-like sculpture. It reaches up to 25-35 x 25-40mm in size (Kerney and Cameron, 1979), and the natural life cycle is thought to be completed within nine to fifteen months, although some helicids may live for several years (Boycott, 1934). The snail is gregarious in habit, and large groups of the snails often hibernate together. The reproduction of this helicid, (and also that of *Cepaea*), is stimulated by the firing of calcareous "love-darts" in to the flesh of the partner as a sexual stimulant. These hermaphrodite snails cross-fertilise, and clutches of eggs are produced.

Helix aspersa is synanthropic, and is widely distributed throughout the British Isles, north to the Outer Hebrides and Orkney, although it is rare in eastern Scotland due to its susceptibility to winter frosts. It is also found in much of Western Europe. It occurs in gardens, hedgerows and waste ground (Kerney and Cameron, 1979).

It is thought that this snail was introduced into this country by the Romans in the first century AD, presumably as a source of food (Evans, 1972).

For laboratory rearing, *Helix* was always kept in the larger of the two standard sized boxes. Sub-adult snails were used to start the laboratory cultures, and these were taken from two garden populations, one in Liverpool and one from the Wirral. Initially, the two populations and their succeeding generations were kept separately, although individuals from each parent population were later mixed to begin new cultures. The first sub-adults matured, bred, and laid clutches of eggs within four months. The first eggs were noted on 17-2-87, and hatchlings were seen sub-surface by 9-3-87. The adults were sometimes seen in the process of copulation, although it was difficult to relate the mating times to specific clutches of eggs laid. The snails burrowed into the soil, to the base of the plastic boxes, to lay their off-white eggs. The eggs, each approximately 4mm in diameter, were laid in clutches of about thirty. The clutches were visible through the base of the transparent plastic boxes.

The reproduction of *Helix* was probably the most prolific of the five base populations, although its reproductive capacity was susceptible to overcrowding. Four sub-adults or adults, within one of the larger plastic boxes, is probably near maximum density for successful breeding (A. J. Cain pers. comm.,1989). It was found that rehousing, along with thinning of the *Helix* cultures would generally induce breeding. The life expectancy of the cultured *Helix* was generally about one year, although some of the F1 generation survived for well over two years throughout the experimentation period. With periodic thinning of the five to ten cultures, and with some forward planning, baby *Helix* from the base populations were always available at the onset of each growth experiment.

Fig. 2.2a *Helix aspersa*

20mm

Fig. 2.2b *Cepaea nemoralis*

20mm

Figure 2.2a-e Illustrations of the five species used in the growth experiments. (a) *Helix aspersa* (b) *Cepaea nemoralis*, (c) *Cernuella virgata*, (d) *Rumina decollata* and (e) *Ferussacia folliculus*

2.3.2 *Cepaea nemoralis* (Linné)

This helicid snail has a globose shell with 5.5 convex whorls. It is generally 12-22mm x 18-25mm in size, and has been known by the synonym *Helix nemoralis*. The shell is brightly coloured (yellow-pink-golden brown), with zero to five dark spiral bands. The umbilicus is sealed by a thickened lip which is usually dark brown but occasionally white. *Cepaea nemoralis* and the similar species *Cepaea hortensis* have been the subjects of much research relating to their genetic characteristics, phenotypically displayed by their polymorphic shell colour and banding (*e.g.* Cain, 1977, and Cain *et al.*, 1968). *Cepaea nemoralis* and C. hortensis are the most variable in colour and banding pattern of all N.W. European land snails (Kerney and Cameron, 1979).

Cepaea nemoralis is widespread in England and Wales, and is found north to Central Scotland and coastal areas of S. Norway, Finland and Sweden. It is believed to have been spread by man at its northern limits. It is a cold-susceptible species, and is not found in certain valley areas of chalk in southern England that are thought to be frost hollows. The species arrived in Britain in the early Post-Glacial in the first wave of thermophile migration, and it was also present during Hoxnian and earlier interglacials in Britain. In the Post-Glacial it has been found in Mesolithic, Neolithic and Bronze Age sites from which today it is absent. The contraction of its range (between 2000 and 0 BC) may relate to clearance of forests and the resultant induction of local climatic gradients and frost hollows in the newly open downland (Evans, 1972).

The first lab culture of *Cepaea* was set up at the beginning of October 1986, and consisted of four mature individuals, three of which had been collected by me in a Norfolk chalk pit. The fourth snail came from the Wirral. Eggs were seen in the culture by January 1987, and crawling baby snails were visible by early February. Eggs were laid in in clutches, at the base of the plastic boxes, in groups of 20-30, each egg being approximately 1.5mm in diameter. *Cepaea* proved to be relatively easy to breed although it appeared to be even less tolerant of overcrowding than *Helix*. Sub-colonies were begun early in 1987, and produced plenty of young throughout 1987 and into 1988. The populations were thinned twice. After mid-1988, the colonies of *Cepaea* appeared to be declining, although rehousing of all individuals, towards the end of the summer of 1988, did provide a few infant snails for the third growth experiment. It was noticed that by the Spring-Summer of 1988, the individual *Cepaea* were growing at a slower rate than the earlier generations. The reasons for the general decline in the laboratory populations are not known.

2.3.3 *Cernuella virgata* (da Costa)

The shell of this helicid snail is globular, with a high, convex spire. It has several synonyms, *Helicella virgata* (da Costa), *H. maritima* (Draparnaud) and *H. variabilis* (Draparnaud). The shell has 5-7 convex whorls with moderate sutures, and a narrow umbilicus partly obscured by a reflected columellar lip. It is very variable in size, shape and colour, although it is usually between 6 - 19mm in height and 8 - 25mm in diameter, and the shell is white or ginger, usually with dark brown spiral bands which may be interrupted, blotched or fused. This variability was noted in the laboratory cultures.

Fig. 2.2c *Cernuella virgata*

Fig. 2.2d *Rumina decollata*

This species has a more southerly distribution than the previous two helicids, and is considered a xerophile species by Boycott (1921). It occurs in southeast England mainly on chalk, with lesser frequencies in central and northern England. It is also found in coastal areas of England and Eire, and in addition occurs in western France, along the coasts of Belgium and the Netherlands, and in Mediterranean regions. It inhabits moderately dry and open sites including dunes, grassland and hedgerows.

Evans (1972) states that *Cernuella* is probably an introduced species, as it has been found in allegedly Iron Age sediments. In southeast England it is present in a possible thirteenth century deposit (Kerney, 1966) and it is also noted by Sparks (1964) as present in Britain during the Ipswichian interglacial.

The laboratory population was established from a large number (at least thirty) of adolescent to mature individuals collected by me from the Norfolk coast, in October 1986. The larger plastic containers were used for this species. In the laboratory, the soil in the base populations of *Cernuella* was kept shallower than for the other species, and also some dried leaf litter was added to the cultures. Both these factors facilitate the laboratory rearing of this species (A.J. Cain, pers. comm., 1986). The snails laid clutches of eggs at, or just below, the soil surface. The clutches consisted of 20-30 eggs, each translucent grey-white egg being approximately 1mm in diameter. Large numbers of hatchlings were noted by the beginning of February 1987.

The base populations of *Cernuella* seemed to progress through good and bad cycles. High mortality sometimes occurred, and even with rehousing and thinning, reproduction could not always be assured. During 1987, large numbers of offspring were still being produced, although by mid 1988 the population appeared to be dwindling severely. Consequently, immature snails were not available for the second or third growth experiments.

2.3.4 *Rumina decollata* (Linné)

This snail has a long narrow tapering shell, characteristic of the Subulinidae, generally 22 - 35mm, but occasionally up to 40mm in height. The shell is a pale brown to cream colour and is rather glossy. Juveniles are slender and tapering, with a blunt apex (which is a reflection of the large egg size, 2-2.5mm). Adults are sub-cylindircal rather than tapering, as a result of deliberate loss of the earlier whorls (decollation), with the apical opening being sealed by a shelly plate (Kerney and Cameron, 1979). Some of the laboratory-cultured specimens did not entirely decollate, or just lost the very early juvenile whorls. This produced mature snails still with an elongated profile, and with more than the 3-6 whorls usually seen in wild specimens.

Rumina is a common Mediterranean species, just extending into southwest France. Elsewhere it is only an occasional adventive (Kerney and Cameron, 1979). It inhabits dry open places, waste ground, scrub and grassy screes, and is found mostly on calcareous soils. It is also said to be subterranean.

The laboratory cultures of *Rumina* were begun with six mature individuals from a colony being kept by Prof. A.J. Cain. The snails originally came from the Isthmus of Corrinth, Greece, where they were found under rocks and stones in a rubbish tip adjacent to the Corrinth Canal (A.J. Cain pers. comm., 1989). The six mature individuals were placed singly in the smaller sized lunch boxes,

Fig. 2.2e *Ferussacia folliculus*

2 mm

with fairly deep soil. The first eggs, opaque, white and slightly calcareous looking, were noted in one box in less than one week. Several of the other parent *Rumina* produced eggs with almost equal rapidity, with up to three clutches (generally 10-20 eggs in each) visible in three of the six sub-colonies within a further three weeks. However, the *Rumina* eggs were very slow to hatch, possibly as a result of the fairly low ambient temperatures in the rearing laboratory at this time of year. Some hatchlings were noted in one sub-culture by the very end of January, eight weeks after being laid. Eggs in four of the other sub-colonies hatched out in mid February to early March, and so after a slow start, a healthy base population of *Rumina* developed. Thinning of adolescent and mature snails was necessary on two occasions, and colonies were transferred to the larger plastic boxes to ease overcrowding and to allow a greater supply of food to the snails. Healthy populations of *Rumina* generally ate all the food available to them and, unlike *Helix* , *Cepaea* and *Cernuella*, did not deposit their faecal material on the box lids.

2.3.5 *Ferussacia folliculus* Gmelin

This species is a fairly small snail, up to 5-6mm in height. The vitreous, pale brown shell is narrow and slightly elongate in shape, with a relatively large body whorl. In contrast to the pale brown transluscent shell, the foot is a striking yellow - green colour. This species is viviparous, *i.e.* it bears live young.

Like *Rumina* , *Ferussacia* is a Mediterranean species. It occurs around the whole of the Mediterranean, including Africa, and is found under stones, dead leaves and rotting bark, but generally in damp situations (Germain, 1930).

The cultures of *Ferussacia* were begun using adult specimens from laboratory populations already established by Prof. A.J. Cain. The wild population originated from the Isthmus of Corrinth in Greece, where the *Ferussacia* were found coexisting with *Rumina decollata* (section 2.3.4). Specimens of both were brought to Liverpool by Prof. A.J. Cain in 1985.

Six *Ferussacia* sub-colonies were set up at the end of October 1986, with adult specimens, and using the smaller plastic boxes. At first reproduction was slow, and more adults were added to each of the sub-colonies in January 1987, and then again in May 1987. Gradually the populations began to expand in numbers. It was found that rehousing the populations in the larger boxes induced greater activity and reproduction, and that the species seemed to thrive after a short period of desiccation, as might be expected of a Mediterranean species. The initial sluggishness of the sub-colonies to produce offspring is thought to be partially the result of low ambient temperatures in the rearing laboratory over the winter period, which also appears to have affected *Rumina* .

2.4 GROWTH EXPERIMENTS : METHODS

2.4.1 Introduction

There were three main growth experiments, each using up to five species of snails and with replicate populations. The first experiment was designed to investigate how the oxygen and carbon stable isotopes, present in the shells of the snails under investigation (hereafter referred to as experimental snails), would be affected by increasing ambient temperatures (10.2 to 24°C), and whether or not the oxygen isotopes measured in the shells would be at isotopic equilibrium with the environmental water from which they were secreted. The second experiment was designed to test how a diurnal cycle of light and dark might affect the resultant isotopic compositions of the shells. The third experiment was originally designed to be a more refined version of the second, but also allowed an estimation of the oxygen isotope effect caused by evaporation from the environmental waters.

The techniques used in the three growth experiments are described below. The methods used in experiment 1 are presented in full. For experiments 2 and 3, only those features of the experiments that differed from previous are detailed.

2.4.2 Experiment 1

(a) Outline

Experiment 1 comprised six stages, whereby snails taken from the base populations were grown under standardised, controlled conditions at six different temperatures, ranging from 10.2°C to 24°C. Two thermostatically controlled rooms - the 'warm room' and the 'cool room' - were used to house the growing snails. Where possible, snails of all five species from the base populations were used in each of the phases of experiment 1, but *Ferussacia folliculus* was not always available.

(b) Conditions of growth and maintenance

Recently hatched individuals were selected for the all the growth experiments. These snails had hopefully survived the risk of post-natal mortality - the stage when most offspring die - and therefore were reasonably likely to survive. However, these same snails were, conversely, small enough so that the majority of the shell laid down over the experimental period would be new shell and not interior thickening. Using baby snails, of a similar size, allowed comparison of the relative growth within and between species kept under different thermal regimes, and minimised potential overcrowding problems.

The numbers of baby snails comprising each experimental culture would have to be:

a) Sufficient to allow for some mortality

b) Sufficient to make statistical comparisons possible

c) Insufficient to cause any immediate overcrowding

d) Insufficient to cause any competition for resources

e) Sufficient to allow for further isotopic analyses should primary samples be lost

The absolute numbers of snails used for each experimental phase (Table 2.2a-c) depended on the availability of baby snails at that time. Three replicate populations (*i.e.* boxes) of each species of snail were maintained at each temperature. Infant snails were removed from the base populations in the rearing laboratory, and placed in identical transparent plastic boxes which had been previously half filled with 'John Innes Seed Compost'.

The boxes were closed with tightly fitting lids, partially composed of fine netting, as described in section 2.2.1. The same basic diet was given to the experimental snails as to the base populations. For experiment 1, the source of calcium carbonate in the food mixture (Food 1), was a first batch of GPR calcium carbonate ((GPR 1) ($\delta^{13}C$ = -13.09 ‰, $\delta^{18}O$ = -5.02 ‰)) The food was administered on the lids of the plastic containers in a similar way to that described for the base populations, except that at each feeding a clean, dry lid was used, so that the only water available to the snails was that administered by a plant spray-mister, either on the dry lid to hold the food in place, or directly on the insides of the box and the soil surface. This water came from on-site storage aspirators (see below). Feeding (and watering) took place on Monday, Wednesday and Friday mornings, right through the experimental periods. Samples of Food 1, and each separate component were taken for stable isotopic analysis.

The only water available to the snails under the experimental conditions, was that kept in 60 litre aspirators in each of the controlled temperature rooms, so as to be at ambient temperatures. Distilled water was used throughout. The aspirators were filled prior to the beginning of experiment 1. The translucent aspirators were stood off the floor, and covered with black opaque plastic bin-liners so as to prevent any surviving micro-organisms respiring etc. and so effecting the stable isotopic composition of the water. At each feeding, freshly decanted water was sprayed onto the soil surface, and onto the box lids to receive the food. Samples of the administered water were transferred to 30 ml Nalgene (high density polyethylene) bottles, to which a few milligrams of sodium azide had been added. The sodium azide poisons any micro-organisms or algae, and effectively 'sets' the isotopic composition of the water. The Nalgene bottles were filled to the brim and tightly capped. The screw cap was further protected using "parafilm" to seal the bottles effectively. The water samples were stored in a cool drawer, inside a black polythene bag, to await isotopic analysis.

As compared to the base populations, the experimental snails were kept much more moist, in an attempt to offset any potential evaporation. Such evaporation from the environmental water, or from the body tissues, of the snails might influence to some degree the isotopic composition of the resultant secreted shell material. The snails and soil were, therefore, well sprayed with water at each feeding, and generally by the fourth to fifth weeks of each experimental period, the soil in the boxes was well saturated.

The growth experiments were carried out over a standard six week period. It was thought that six weeks would be sufficient time for the young snails to adjust to their experimental habitat (acclimatize), and to secrete enough shell material to make stable isotopic analyses possible. However, at the coolest experimental temperature 10.2°C (see below), the experimental period was extended for a further two weeks for some of the snails, to ensure that sufficient growth had taken place.

Each phase of experiment 1 (and subsequent experiments) was begun with baby snails on the Monday of week 0, and ended with the killing of the experimental snails on the Monday of week 6, (with the exception noted above for the coolest temperature).

EXPERIMENT 1 W1-3, C1-3	*Helix*	*Cepaea*	*Cernuella*	*Rumina*	*Ferussacia*
No. of BOXES	3	3	3	3	3
No. of SNAILS in each box	6-10	8-10	>15 gen. 20-30	4-8	10-12

Table 2.2a

EXPERIMENT 2	*Helix*	*Cepaea*	*Rumina*
No. of BOXES	3	3	3
No. of SNAILS in each box	10-12	10-12	6-8

Table 2.2b

EXPERIMENT 3	*Helix*	*Cepaea*	*Rumina*	*Ferussacia*
No. of BOXES	9	7	9	7
No. of SNAILS in each box	8-10	3-5	8-10	10-12

Table 2.2c

Table 2.2a - c Numbers and species of snail used in growth experiments (a) Experiment 1, (b) Experiment 2 and (c) Experiment 3.

During weeks 4 and 5 of each phase of experiment 1, faecal samples were taken again, for possible isotopic analysis. The faecal matter, mostly white in colour, was picked from the box lids prior to the next feeding, and stored in clean glass vials.

Within the controlled temperature rooms the boxes of experimental snails were always sited in the same place, and at the same level, upon the fixed shelving.

For the entire period of experiment 1, the room lights (fluorescent strips) were left permanently on in the controlled temperature chambers. Even so, the rooms appeared dull rather than brightly lit, as pillars and shelving partially blocked light directly reaching the boxes of snails.

(c) Temperature and humidity

A number of methods were adopted to estimate the actual temperatures being experienced by the snails in their boxes. The room temperature was set with a thermostat, but further measurements were made to assign a 'set' temperature to each of the regimes. A standard graduated thermometer was used along with a 'Vaisala' digital temperature/humidity probe (which measured to 0.1°C). In addition to background room temperatures, measurements were taken using the probe inserted in boxes similar to those containing the snails. Three holes were drilled in the ends of the plastic boxes into which the probe could be inserted. Rubber bungs were used to plug the holes in between readings (Figure 2.3). Daily measurements were made generally for 15-20 consecutive days during each experimental phase.

The first of the control boxes contained no soil, to indicate the effect of the box alone on ambient room temperature (and humidity). The second box was half filled with soil, and the three holes were positioned so that the lowest was below the soil surface (BS), the next was at the soil surface (SS), and the third above the soil (AS), (Figure 2.3). Measurements were made through each of the holes. For three of the six temperature regimes in experiment 1, a third larger box, with similarly located holes and containing soil, was used. For all probe measurements the same fine mesh brass covering on the probe head was used.

As the populations of snails were deliberately kept very moist, it was decided to use the graduated thermometer to take measurements of an open tank of water kept in the two controlled temperature rooms. Although not carried out initially, measurements of water temperature were made for four out of the six temperature regimes, to assist in the assessment of overall temperature.

Table 2.3, shows the mean values for the different temperatures measured over each of the six temperature regimes. Also indicated is the number of days over which the measurements were made. "W" temperatures are from the 'warm room', and "C" temperatures are from the 'cool room'. The final representative 'set' temperatures are averages of the other measurements, for each of the six growth phases of experiment 1.

In an attempt to minimise potential evaporation from the snail's environmental water, or from their body fluids, it was necessary to keep the ambient humidity as high as possible. This was achieved by keeping the soil and the box walls and lids, as moist as possible. The soil surface, box and fresh lids were well sprayed at each feeding, so that within the boxes the air would constantly be close to saturation. However, some loss of moisture must have occurred across the netting lids, although the box lids sometimes remained damp in-between feeds (though not at the higher experimental temperatures during experiment 1). Generally snails remained active throughout the experimental period, rather than sealing themselves down onto the plastic surface of the boxes, as a protective measure to prevent moisture loss.

Figure 2.3 Control container for temperature and humidity measurements showing position of rubber bungs where temperature/humidity probe was inserted.

CODE	C1	C2	C3	W1	W2	W3
No. OF READINGS	15	16	10	15	20 + 20	15
$\bar{X}$ PROBE T°C ROOM	10.3	12.8	15.5	24.2	23.3	18.9
$\bar{X}$ PROBE T°C CONTROL BOX *	10.1	12.9	15.5	23.9	22.9	19.0
$\bar{X}$ PROBE T°C AS Above Soil surface	10.2**	12.9	15.4	24.0**	22.6**	19.4
$\bar{X}$ PROBE T°C SS at Soil Surface	10.2**	12.9	15.3	23.5**	21.8**	19.3
$\bar{X}$ PROBE T°C BS Below Soil surface	10.0**	12.7	15.1	22.9**	21.0**	19.2
$\bar{X}$ THERMOMETER T°C DRY AIR TEMP.	--	13.9	17.0	25.4	--	20.7
$\bar{X}$ THERMOMETER T°C STANDING WATER	--	13.6	16.1	24.2	--	18.9
SET TEMP. °C	10.2	13.1	15.7	24.0	22.3	19.3

Table 2.3 Measured and 'set' growth temperatures for each of the six phases of Experiment 1. 'Set' temperatures calculated as a mean of all measured values from each thermal regime.

AS = Above soil surface
SS = At soil surface
BS = Below soil surface

* = An average value of AS SS and BS readings taken in a control box, containing no soil.
** = Mean values from two boxes, one standard size and one slightly larger.

To test the effectiveness of the boxes at maintaining a localised high relative humidity (above 95%, *i.e.* near saturation), a series of test measurements was made. This involved using the same control boxes as described above. After adding fresh seed compost, the relative humidity within the control boxes was generally over 95%, so no additional water was added. Relative humidity was measured, in the same way as temperature, through the holes in the sides of the plastic boxes into which the probe-head could be inserted. Between ten and twenty humidity readings were taken, generally on consecutive days, to see how the relative humidity within the boxes declined with no further addition of water. Measurements were also made of the ambient room humidity, and the humidity within a box containing no soil. Relative humidity data from three of the temperature regimes in experiment 1 (W1, W2, and C1), are shown graphically in Figure 2.4a-c.

The plots of W1 (Figure 2.4a) and C1 (Figure 2.4c) consist of fifteen, and W2 (Figure 2.4b) of twenty, consecutive measurements. Also plotted on each figure is the background room humidity, and the relative humidity within the box containing no soil. From Figures 2.4a-c, the following points may be noted.

1) The relative humidities in the boxes with soil were always higher or much higher than the background room humidities.

2) The SS and BS readings were generally consistently between 90-95% or even higher, and the AS data above 85% throughout, *i.e.* the highest humidities were recorded below the soil surface and lower humidities were recorded above the soil nearer to the lid/air interface.

3) The humidity data from the control box containing no soil closely followed the trace of the background room humidity.

4) For trace W1, over the fifteen daily readings, the background and the AS humidity both slightly declined. The SS and BS humidities remain fairly constant.

5) For trace W2, over 20 daily readings, the background humidity slightly increased. The AS humidity appeared to decline from day 6-7 onwards, and the SS reading from day 9 onwards. The BS humidity remained consistently high, around 95%.

6) For trace C1, over 15 consecutive readings, the background humidity was high though somewhat variable, There was a slight increase in the AS, and perhaps the SS, relative humidities, but all three within-box humidities remained consistently high throughout the period over which measurements were recorded.

From these points it may be seen that the moisture held within the soil is an extremely important factor in influencing the relative humidity within the boxes. In the W2 regime, which had the lowest background humidities, some decline in the AS and SS humidities was seen; however, there was still moisture held within the soil. During these test measurements, no water was added to the soil, and little or no decline in the relative humidity within the boxes was seen. In the actual growth experiments, water was added to the boxes three times each week by spraying the soil, box sides and lid (see above). With this continual addition of moisture, it seems likely that relative humidities in excess of those measured purely with the fresh seed compost were maintained within the boxes over the experimental periods. Saturation of the soil was generally reached towards the end of each experimental period. However, there must have been some loss of moisture to the surroundings, but it was hoped that experiment 1 was carried out under conditions near 100% relative humidity.

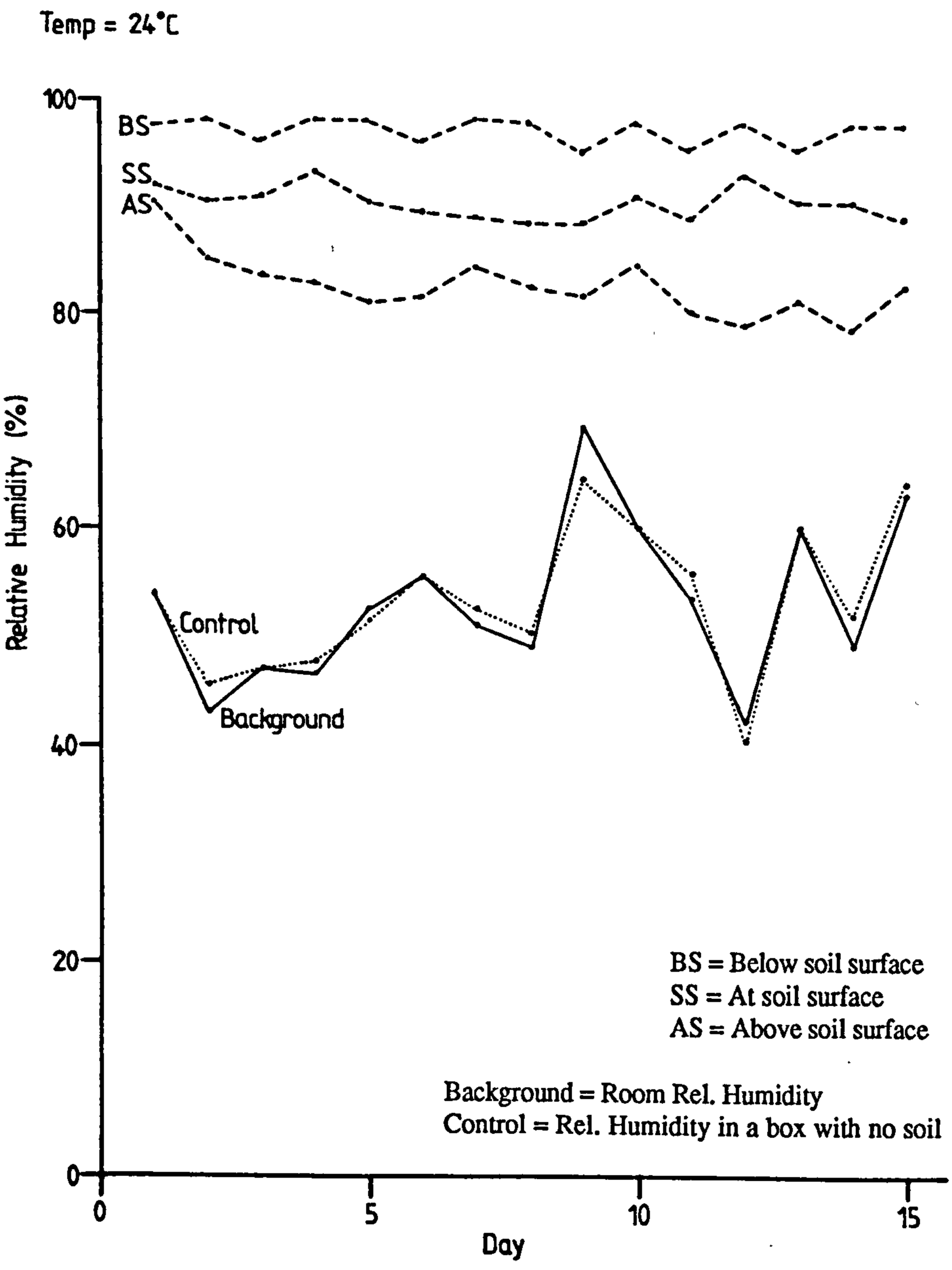


Fig. 2.4a Relative humidities over 15 daily readings from the W1 regime (Experiment 1) at 24°C

Figure 2.4a-c Relative humidity data from three of the temperature regimes of Experiment 1, over 15 or 20 consecutive daily readings and with no addition of water. Measurements of the background room humidity, the humidity in a control box (containing no soil), and humidities inside a box with soil (at three levels - see key)) were made using a Vaisala, digital temperature / humidity probe. (a) W1 at 24°C, (b) W2 at 22.3°C and (c) C1 at 10.2°C

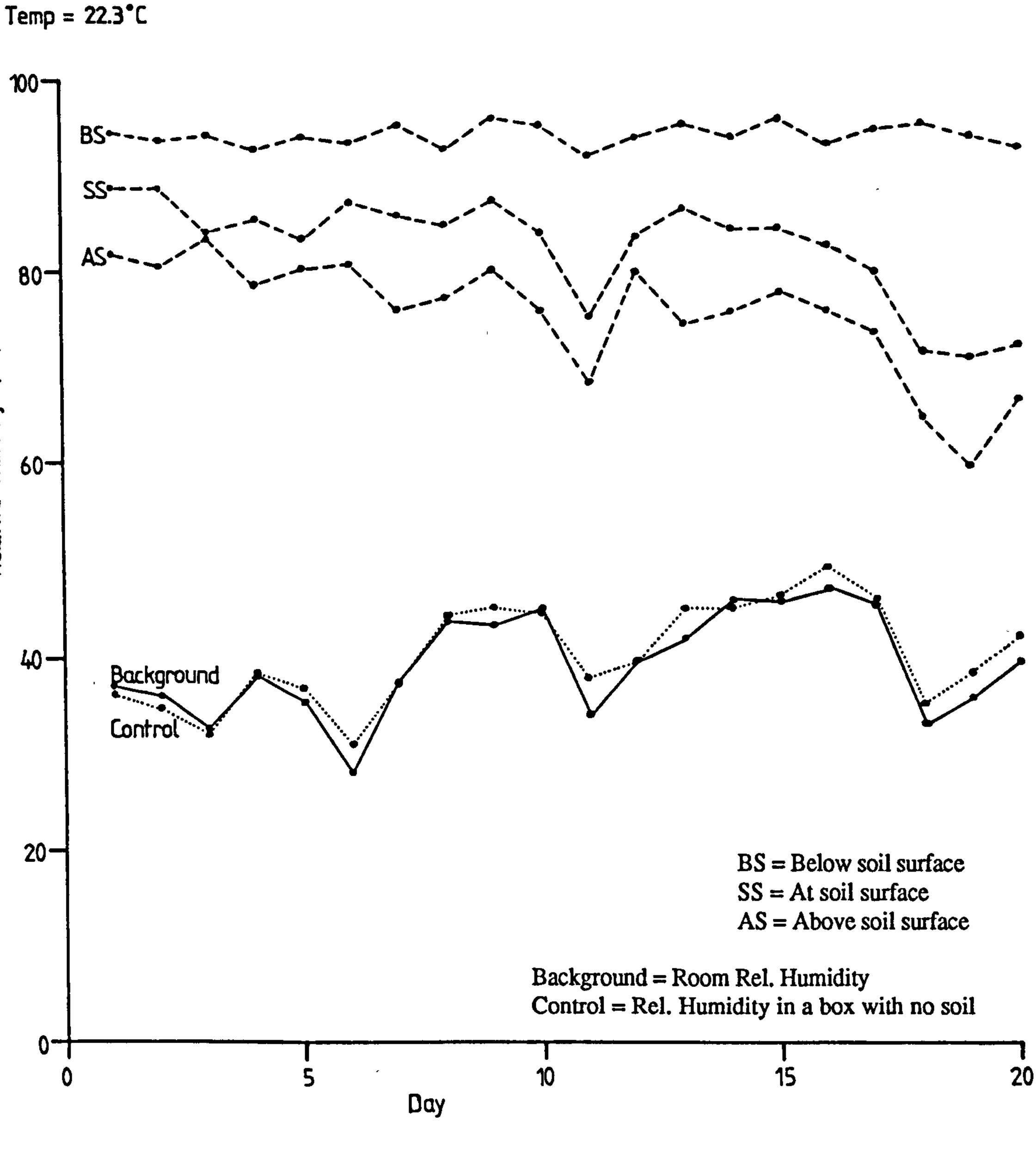


Fig. 2.4b Relative humidities over 20 daily readings from the W2 regime (Experiment 1) at 22.3°C

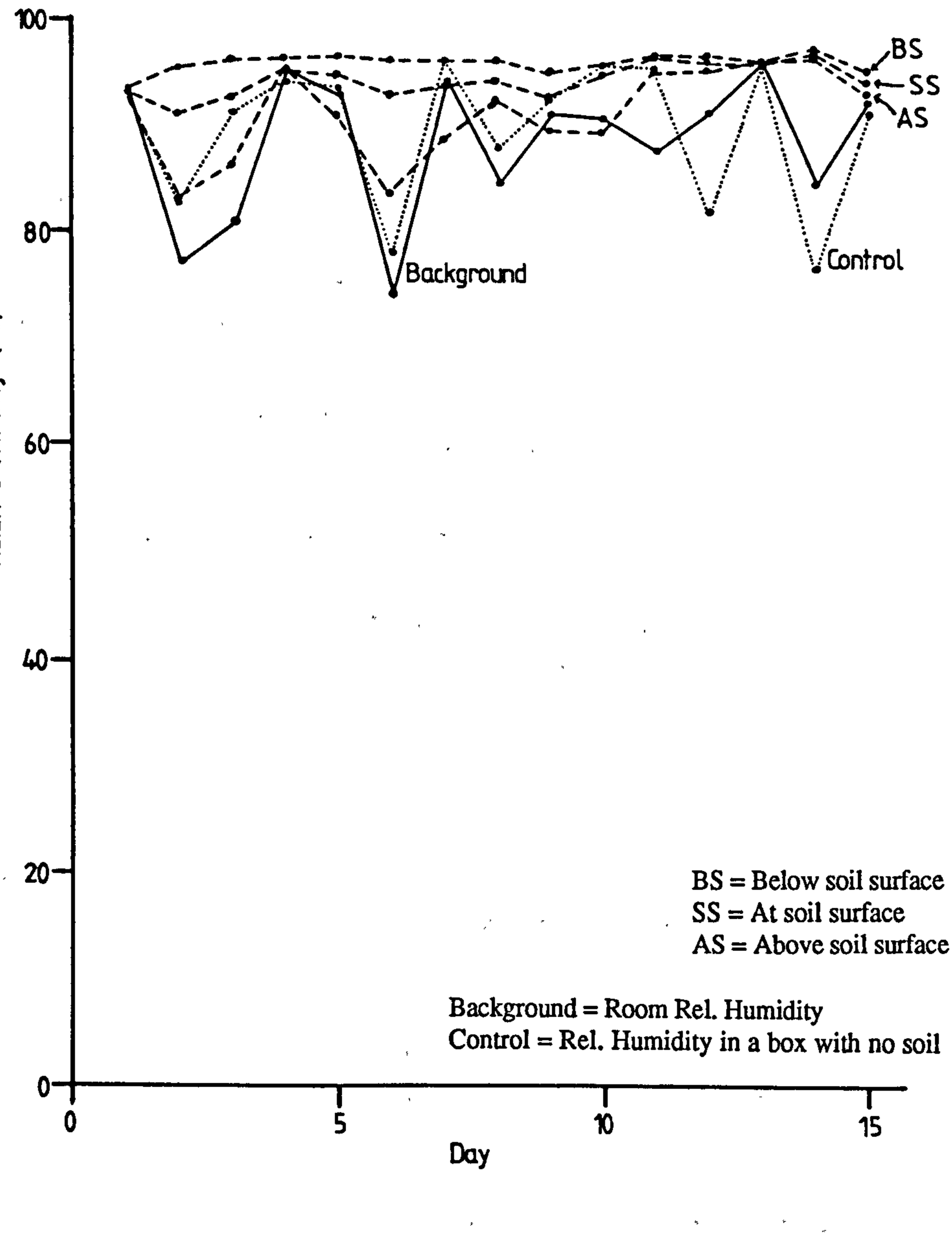


Fig. 2.4c Relative humidities over 15 daily readings from the C1 regime (Experiment 1) at 10.2°C

d) Snail killing

At the six week mark, the snails were removed from their experimental colonies and all, or those selected (if there were sufficient snails available), were killed and the bodies extracted from the shell.

Initially, as a method of instantaneous death, the snails were immersed in liquid nitrogen. This method had the desired effect, although the bodies and visceral mass had to be thawed by immersion in hot water before they could be removed. Unfortunately this resulted in tissue breakdown, and the snail bodies proved immensely difficult and messy to remove from their shells. A second method of dispatch proved to be more successful. Snails were immersed in boiling water for approximately thirty seconds. For the larger snails *Helix* and *Cepaea*, it was found that the bodies and visceral mass could then be cleanly skewered out of the shells. The larger *Cernuella* shells could also be dealt with in the same way, although the smaller individuals proved to be rather fiddly. It was found that only rarely could a body be completely cleanly removed from the high spired *Rumina* shells, especially as the foot was often withdrawn well into the shell at death, and the aperture area of the shell was often very thin and brittle. For the shells of *Rumina* a method was adopted of slicing off the last two whorls of the shell, and skewering the body and any visceral mass out of this portion of shell only. The rest of the shell was disgarded. *Ferussacia* shells were also treated in a similar way, with the last 1 - 1.5 whorls being sliced off.

After removal of the body tissues, the empty shells were soaked and washed in hot water, to help remove any exterior slime or remaining tissue. The shells were then oven dried and, if necessary, were further cleaned in an ultrasonic bath before being labelled to await preparation for stable isotope analysis. This method was used throughout the experimentation period.

2.4.3 Experiment 2

(a) Outline

This experiment involved populations of snails being grown under two temperature regimes, one warm and one cool, whilst a third set of snails was moved between the two temperature extremes on a daily basis. Three boxes of each of the three populations of snails were initiated.

Helix aspersa, *Cepaea nemoralis*, and Rumina decollata were the species available at the onset of the experiment. The numbers of these snails used are indicated in Table 2.2b.

(b) Conditions of growth and maintenance

The snails were grown under the same standardised conditions as experiment 1, for the same time period of six weeks, being maintained as described in section 2.4.2 with the following exceptions.

The mixed diet (Food 2) included a second batch of GPR $CaCO_3$ (GPR 2), as a source of inorganic carbon for the snails ($\delta^{13}C = -43.35‰$, $\delta^{18}O = -28.40‰$).

Water from the 'warm room' aspirator was used for all the snail populations in experiment 2. The aspirator was refilled prior to the beginning of the experiment, and samples were taken for isotopic analysis (section 2.4.2).

Of the three populations of snails the first set (Pop 1) was maintained permanently in the 'warm room' whilst another (Pop 3) was always located in the representative 'cool room' (the corridor).

The remaining snails (Pop 2), were moved between the warm and cool localities with an approximate 12 hour change-over period, being moved to the 'cool room' between 8-10pm, and being returned to the 'warm room' the following morning between 8-10am. To stabilise the immature snails before subjecting them to the daily cycle, all three populations were acclimatized to the 'warm room' temperature for the first two weeks of the standard six week growth period. After two weeks, Pop 2 boxes were then moved on a twice daily basis, whilst Pop 3 boxes were moved permanently to the cooler environment.

During experiment 2, lights in the 'warm room' were left on whilst those in the corridor ('cool room') were permanently switched off. Thus, Pop 1 snails were subject to permanent light, Pop 3 to continual darkness and Pop 2 snails were subject to a daily cycle of light to dark in addition to a daily cycle of changing temperature.

All the snails were killed, after a six week total growth period, as described in section 2.4.4. Individual snails were measured and weighed before being prepared for stable isotopic analysis.

(c) Temperature and humidity

For this experiment, two fixed temperatures were required. These were achieved by using the 'warm room' and the adjacent corridor (section 2.1). The thermostat in the 'warm room' was set at the same temperature as that of W2 in experiment 1. The background temperature of the 'warm room' and the corridor was monitored with a thermograph chart-recorder. The 'warm room' thermograph followed a saw-toothed pattern, indicating the immediate impact of the thermostatically controlled heating, and then the gradual decline in temperature until heating was resumed. Thus, for the 'warm room' the same environmental temperature as that of W2 in experiment 1, was taken as the 'set' temperature, being 22.3°C.

The corridor background temperature increased slightly over the experimental period, mainly because of slightly elevated external temperatures during the experiment (early June). At the initiation of experiment 2, the corridor temperature was 16°C. By the end of the third, and into the fourth week of the six week experimental period, the temperature as recorded on the thermograph had reached 17°C. For the final week, the temperature was as high as 18°C. Taking into account the likely cooling effect of the soil in the boxes, as noted in experiment 1, (Table 2.3), a 'set' temperature of 16.5°C was chosen as a likely mean temperature over the six week experimental period. This was 1°C higher than the previous warmest 'cool room' temperature of experiment 1. The assigned temperatures for the 'warm room' and the 'cool room' are shown in Table 2.4.

Experiment 2 was carried out under conditions similar to those of the first experiment, with slight modifications. It is therefore assumed that the relative humidity within the boxes was maintained near to 100% through the experimental period, as noted for experiment 1 (section 2.4.2c)

	SET GROWTH TEMPERATURES °C 'warm room'	'cool room'
EXPERIMENT 2	22.3	16.5
EXPERIMENT 3	22.3	14.5

Table 2.4 'Set' growth temperatures for Experiments 2 and 3, see sections 2.4.3 and 2.4.4 for method.

POPULATION NUMBER	ROOM	BAG
1	warm	black
2	warm	clear
3	warm	black - clear *
4	warm - cool **	black
5	warm - cool **	clear
6	cool	black
7	cool	clear
8	warm	none
9	cool	none

Table 2.5 Experimental conditions and status of the nine populations in Experiment 3.

* = Exchange every 12 hours from black to clear plastic bag, and vice versa.
** = Exchange every 12 hours from 'warm' to 'cool' room and vice versa.

'warm' room at 22.3°C
'cool' room at 14.5°C
P4 and P5 subject to a mean temperature of 18.4°C.

2.4.4 Experiment 3

(a) Outline

In this experiment it was hoped to investigate further the isotopic variation found between shells subject to diurnal changes in illumination as well as temperature.

Helix aspersa, *Cepaea nemoralis*, *Rumina decollata* and *Ferussacia folliculus* were the species used in this experiment. The numbers of snails used are indicated in Table 2.2c.

(b) Conditions of growth and maintenance

The snails were grown under the same standardised conditions as experiment 1, with the following exceptions.

The mixed food (Food 3) included a different source of inorganic carbonate. This was a sample of Mid-Cenomanian chalk (MCC) which originated from Southerham chalk-pit in Sussex. The chalk was almost pure, being composed mostly of coccoliths with about 10% clay content (P. W. Ditchfield, pers. comm., 1988). The chalk ($\delta^{13}C = 2.24‰$, $\delta^{18}O = -3.33‰$) was ground to less than 100μm, a similar grain size to the GPR calcium carbonate, before being used a component in Food 3.

The water source for this experiment was distilled water from the aspirator housed in the 'warm room'. The aspirator was refilled prior to the onset of experiment 3 and samples were taken, as in section 2.4.2, for isotopic analysis.

The room lights in both the 'warm room (22.3°C), and the corridor (the 'cool room', 14.5°C) were left on over the whole of the experimental period. To control the light reaching the snails, some of the populations were kept in black plastic, garden refuse bags, through which virtually no light could penetrate. To test for any resultant environmental or vital effect of the black plastic bags, other populations were kept in clear polythene bags. It was found that a pair of boxes fitted conveniently into each of the clear or black polythene bags. Therefore, *Helix* were housed with *Cepaea* (larger boxes) under the same conditions, and *Rumina* with *Ferussacia*, giving two bags, each containing a pair of boxes, for each population. Seven populations were set up in this way. The bags were held closed with twisted plastic-coated wire. To assess the effect of keeping the snail populations in the polythene bags, two control populations each of *Helix* and *Rumina* were maintained without bags, in the same way as experiments 1 and 2. Thus, nine populations were set up in total.

The situation of each of the nine populations (P1 to P9) is shown in Figure 2.4, and is summarised in Table 2.5.

P1, P2, P3 and P8, were maintained constantly in the 'warm room'. P4 and P5, were acclimatized in the 'warm room' for the first two weeks, before being subjected to the twelve hour movements between the two temperature extremes. P3 was acclimatized in a clear polythene bag in the 'warm room' for two weeks, before being switched from a black to a clear bag every twelve hours, for the next four weeks. All other bagged populations were maintained in the same bags for the whole of the six week period, except at feedings. P6 and P7, were kept for the entire six week period in the 'cool room' to maximise potential shell growth. The exchanges between rooms, and from bag to bag, took place as follows. Between 8-10am. each day, from week three onwards, P4 and P5 (two pairs of boxes in each population) were moved from the 'cool room' to the 'warm room', and P3 snails (two pairs of boxes) were transferred from black to clear polythene bags. The reverse movements were made each day between 8-10pm.

(c) Temperature and humidity

Two set temperatures were required for experiment 3, which took place in November as opposed to May/June for experiment 2. The thermostat in the 'warm room' was maintained at the same temperature as for experiment 2, and W2 of experiment 1. Thus, the 'set' temperature for the 'warm room' was taken as 22.3°C. The corridor was again used to achieve a fixed temperature 'cool room'. The temperature was slightly lower than that during experiment 2, as the exterior temperature was very much cooler in November than in May/June. Over the six week period, a temperature range of 13.5 to 16°C, was recorded on the thermograph, but the temperature was generally between 14.5 and 15°C. On consecutive days, three sets of additional measurements were made. These were background room temperature and humidity measurements using a probe, and temperature readings using a graduated thermometer. Also measured over the two day period, were temperatures inside the polythene bags in which the boxes of snails were kept for this experiment. The 'inside bag' temperatures were measured with the probe and a graduated thermometer. Unfortunately, the digital probe used during experiment 1, was not functioning, however a slightly less precise Vaisala instrument was available. This was a non-digital instrument, which allowed measurement of temperature to approximately 0.5°C, and humidity to within approximately 0.5%. Background and 'bag' temperatures for the corridor 'cool room', during experiment 3, are shown in Table 2.6.

Using the thermograph temperatures, and the background and 'bag' thermometer and probe readings, an overall 'set' temperature of 14.5°C was attributed to the 'cool room' snails of experiment 3. This 'set' temperature was 2°C lower than that during experiment 2. This would be the result of seasonal differences in outside temperatures somewhat buffered by the heat emanating from the building.

The assigned growth temperatures for the 'warm room' and the corridor 'cool room' from experiments 2 and 3, are shown in Table 2.4.

Housing the snails in boxes inside polythene bags also introduced a further barrier to evaporative loss from the snails' environmental water. The populations were only removed from the sealed bags to allow feeding and watering. The potential for evaporation would therefore be less with the limited amount of surrounding air. Relative humidities within the polythene bags, but outside the boxes, were measured three times over two consecutive days. The bags of boxes kept permanently in each temperature regime were used for these measurements, which gave relative humidity data for four bags from either room. Background room humidities were also measured (Table 2.7). As mentioned above, a different Vaisala temperature/humidity probe to that available during experiment 1, was used in this case, although the measurements from the two experiments should be comparable.

A slight decline in humidities over the measurement period probably relates to the time elapsed since the previous feeding and watering. The measurements were made on a Wednesday afternoon, and then twice during the following day. Feeding and watering had taken place, as standard, on the Wednesday morning. Thus, it appears that there is some gradual loss of moisture from within the plastic bags. However, the humidities within the bags were generally between 85 to 90%. The humidities inside the boxes, in the snails' immediate environment, would be likely to be even higher. Therefore, it seems reasonable to assume that the relative humidity within the boxes would be constantly close to saturation.

	COOL ROOM	BLACK BAG LARGE	BLACK BAG SMALL	CLEAR BAG LARGE	CLEAR BAG SMALL
1st PROBE READING	16.0	15.0	16.0	15.0	15.5
1st THERMOMETER READING	15.0	13.25	14.0	13.75	14.0
2nd PROBE READING	16.5	16.0	16.5	16.5	17.0
2nd THERMOMETER READING	14.0	14.0	13.75	14.0	14.25
3rd PROBE READING	16.5	16.0	16.25	16.0	16.75
3rd THERMOMETER READING	14.75	13.75	14.0	14.0	14.5

Table 2.6 Background room and 'in bag' temperatures (°C) on consecutive days, in the 'cool room', during Experiment 3, measured with Vaisala temperature / humidity probe.

All readings °C
1st reading - Wednesday p.m.
2nd reading - Thursday a.m.
3rd reading - Thursday p.m.

Over the 6 week growth period, room thermograph = 13.5°C (min) to 16°C (max), but generally 14.5 to 15°C.

READING	WARM ROOM	COOL ROOM	Lge. Box BAG 1 WARM	Sml. Box BAG 1 WARM	Lge. Box BAG 2 WARM	Sml. Box BAG 2 WARM	Lge. Box BAG 6 COOL	Sml. Box BAG 6 COOL	Lge. Box BAG 7 COOL	Sml. Box BAG 7 COOL
(1) WED. pm	54.0	40.0	90.5	90.0	92.0	89.0	88.0	80.0	89.0	89.0
(2) THURS. am	57.0	39.5	88.0	85.0	89.0	86.0	87.0	78.0	88.0	81.0
(3) THURS. pm	40.0	44.0	84.0	80.0	86.0	82.0	84.0	81.0	84.0	80.0

Table 2.7 Background room and 'in bag' relative humidities (%) on consecutive days during Experiment 3, measured with Vaisala temperature / humidity probe. 'In bag' humidities measured outside snail boxes.

'Warm room' at 22.3°C
'Cool room' at 14.5°C

2.5 LABORATORY PREPARATION FOR STABLE ISOTOPIC ANALYSIS

2.5.1 Structure and organic components of the molluscan shell

A terrestrial gastropod shell is a complex structure of crystals of calcium carbonate, usually aragonite, bound together in a proteinaceous matrix. The organic matrix serves to separate individual crystals, and to bind the crystals and crystal layers into a unified structure. The matrix is composed primarily of proteins, amino acids and carbohydrates (Wilbur and Saleuddin, 1983). The organic content of the shell also includes the external covering of the shell, the periostracum, which is composed of tanned scleroprotein (Saleuddin and Petit, 1983; Waite, 1983). The organic component of molluscan shells is generally less than 10% of the total shell weight (Price *et al* ., 1976), although Wilbur and Saleuddin (1983) report that some published values of shell organic content may be too high because of an error, due to loss of carbonate during combustion - the most commonly used method of determination. Details of the organic components of the periostracum and calcified layers in a molluscan shell, are given in Meenakshi *et al* ., (1975).

The basic requirement of the processing was to produce clean, pure calcium carbonate without any organic contaminants. Organic components are thought to affect adversely the stable isotope analyses, and worse still to contaminate the mass spectromenter. The pretreatment of the snail shells was thus to remove both the internal and external organic material from the freshly killed snails used in the the growth experiments.

2.5.2 Preliminary cleaning

After removal of the body and visceral mass, the shells of the freshly killed snails were cleaned manually and ultrasonically in hot water, to remove any remaining tissue, slime, soil or excreta adhering to the shell surface. The shells were then oven dried, and in some cases were weighed (mg) and measured for their height and diameter (mm), before any further treatment was carried out.

2.5.3 Bleaching : method and testing

To prepare fossil bryozoa for stable isotope analysis. Forester *et al*., (1973) immersed the bryozoa in bleach for thirty or sixty minutes. They used the commercial bleach 'Chlorox' (NaOCl), which was mixed on a 1:4 proportion with distilled water to give an approximate 1% solution. Forester *et al*., reported that unlike the roasting of samples in helium, the 'Chlorox' method did not appear to effect the isotopic composition of the bryozoa. The authors also noted that although the bleaching process did not remove all the organics, it was thought to stabilise any remaining organic material so that no further isotopic exchange with the skeletal carbonate would occur. Thus, this process was deemed the most suitable for the removal of organic contaminants by those authors.

It was decided to run test samples of *Helix aspersa* in 'Chlorox' (sodium hypochlorite) solution of a similar strength to that used by Forester *et al*., (1973). It was hoped that the 'Chlorox'

solution would act to remove the periostracum whilst the shell was still intact, rather than working with a powdered sample in a solution.

Shells of *Helix aspersa* were immersed in 1% 'Chlorox', which was warmed to 60-80°C for up to 48 hours. Little immediate difference was seen in the external appearance of the shells, although after 48 hours, some difference in the coloration of the shell could be seen, and in places the periostracum appeared to be beginning to flake off or dissolve. However, after rinsing and drying, it could be seen that some external organic matter was still present, and the shells were not efficiently bleached.

A stronger alternative bleaching agent was subsequently tested. This was General Purpose Reagent (GPR) 30% hydrogen peroxide (H_2O_2). *Helix* shells were immersed in the peroxide, at room temperature, for up to 48 hours. In addition, shells that had previously been bleached in the 'Chlorox' for 48 hours, were transferred to the peroxide solution after they had first been well rinsed.

After 48 hours in the peroxide, and having been thoroughly rinsed and dried, it could be seen that the hydrogen peroxide had a fairly strong effect upon the external appearance of the shell, with some loss of the glossy outer covering. However, it was noted that the shells most effectively bleached, were those that had been immersed in 'Chlorox' and then subsequently transferred to the peroxide solution.

Provided that such a bleaching process would not effect the stable isotopic composition of the snail shells (see section 2.5.6), this treatment, of 48 hours in warm 'Chlorox' followed by 48 hours in hydrogen peroxide at room temperature, appeared to be a suitable method for removing external organic matter from the snail shells.

2.5.4 Rinsing, drying and grinding

After removal of shell samples from the peroxide solution, the shells were thoroughly rinsed and oven dried (at approximately 80°C) for at least 24 hours. The portion of the shell that was to be analysed for oxygen and carbon stable isotopes was then removed for further processing. For shells of *Helix* , *Cepaea* and *Cernuella*, this involved slicing, or breaking off, the last whorl of shell laid down under the experimental conditions. Shells of *Rumina* and *Ferussacia* had been sliced at the time of killing, leaving the last two whorls for further processing. In one or two cases when more specific investigations of the intra-shell stable isotope composition were to be made, a micro-drill with an arrow-head bit was used to drill out minute portions of shell.

After selection of the critical portion of shell, it was necessary to process further these sub-samples to remove the inter-crystalline organic material, plus any remaining external organics. It was therefore necessary to grind the samples to a suitable grain size so that the inter-crystalline organics could be removed. Grinding the shells to less than 100μm, using a small agate pestle and portar, was relatively straightforward, and it was hoped that at this grain size most of the internal sheets and prisms of calcium carbonate would be broken, and the organic material between and around them exposed. SEM micrographs of nacreous and prismatic crystal layers in gastropod shells (Wise and Hay, 1968), indicate that individual carbonate crystals are approximately 10μm in width. However, the organic matter in nacreous layers forms sheets between the contiguous crystal stacks, and in the prismatic layers organic material surrounds and is irregularly placed within the prisms of crystals (Wilbur and

Saleuddin, 1983). Thus, it was felt that grinding down to less than 100μm would effectively break into the layers of organic matrix, and leave them open for attack and removal. Therefore, this size fraction, less than 100μm, was used throughout.

2.5.5 Roasting : method and testing

The method selected for the breakdown of the inter-crystalline organics (proteins, amino acids and carbohydrates),was to roast the powdered samples, under vacuum, at a high temperature. This method was thought to be preferable to further bleaching of the powdered samples in solution, especially if dealing with small samples that might easily be lost during transfer from soaking to rinsing to drying vessels. The roasting was carried out on 15-20mg sub-samples, or total samples if less than this weight was available, of the ground powders (≤100μm). The powdered samples were placed in glass thimbles held in finger shaped vessels with a vacuum seal. The samples and vessels were evacuated to a high vacuum, using rotary and diffusion pumps, before being sealed. The evacuated vessels were then placed in a 'Techni Dri-Block', with the lower third of the vessel containing the thimble being immersed in the heated block. The roasting was carried out in a vacuum to prevent isotopic exchange between atmospheric oxygen and the carbonate.

The temperature of the roasting process needed to be high enough to effect the breakdown of the organics, but not so high as to allow any combustion or isotopic exchange which might influence the stable isotope signature of the sample. A maximum temperature for roasting would be somewhere around 400°C. At such temperatures, roasting would break apart the complex organic molecules forming the inter-crystalline matrix. The products of the breakdown would then be removed by the final processing procedure, in the oxygen plasma furnace (section 2.5.7), to provide pure carbonate for stable isotope analysis.

To monitor the effects of the roasting process, and to assess the most suitable operating temperature and time, a number of test samples was prepared. These were samples of the Liverpool Stable Isotope Laboratory lab' standard, Lincolnshire Limestone of the 105-180μm size fraction. The samples (3-5mg) were oven dried to remove any excess moisture from the powder, weighed prior to evacuation, and were roasted for 6, 12, 24 and 36 hours before being cooled and reweighed (Table 2.8). All but one sample (number 9) lost weight, and this one anomaly may be accounted for by inaccuracy in weighing. Taking into account this level of possible inaccuracy (±0.02mg), most of the weight losses were low, and would probably be the result of water, or other constituents of the limestone, vaporising. The percentage weight losses (up to 3.64%, mean value for samples 4, 5 and 6), may seem relatively high, but as the samples are small, a weighing inaccuracy of only ±0.02mg would, in some cases, be equivalent to a 1% loss in weight.

Some slightly larger samples (10 -15mg) of two *Helix* and two *Cepaea* shells were also put through the roasting process. These shells had previously been bleached for 48 hours in both sodium hypochlorite ('Chlorox'), and hydrogen peroxide, before being ground to less than 100μm. The mean percentage loss in weight for these four samples was 2.3%, which could be accounted for through vapourisation of water or other shell constituents.

SAMPLE No.	SAMPLE Wt. mg	ROASTING TEMP °C	ROASTING TIME Hrs	Wt. LOSS mg	% Wt. LOSS	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
(1) 11	5.60	380	6	0.07	1.25	3.38	-0.11
(2) 12	3.82	380	6	0.07	1.83	3.34	-0.23
(3) 13	3.60	380	6	0.17	4.72	--	--
			MEANS	0.10	2.60	3.36	-0.17
(4) 14	4.55	380	12	0.09	1.98	3.38	-0.48
(5) 15	4.44	380	12	0.14	3.15	3.42	-0.07
(6) 16	3.45	380	12	0.20	5.79	3.30	-0.27
			MEANS	0.14	3.64	3.37	-0.27
(7) 17	4.27	380	24	0.11	2.57	3.38	-0.16
(8) 18	2.82	380	24	0.03	1.06	3.35	-0.17
(9) 19	4.04	380	24	+0.02	+0.50	3.30	-0.24
			MEANS	0.04	1.04	3.34	-0.19
(10) 20	2.77	380	36	0.09	3.25	3.37	-0.20
(11) 21	2.92	380	36	0.03	1.03	3.39	-0.27
(12) 22	3.76	380	36	0.03	0.80	3.40	-0.17
			MEANS	0.04	1.69	3.39	-0.21
(13) 23	3.16	425	24	0.09	2.85	2.99	-0.25
(14) 24	3.95	425	24	0.09	2.28	3.06	-0.08
(15) 25	4.79	425	24	0.10	2.08	3.06	-0.19
			MEANS	0.09	2.40	3.04	-0.17
(16) 26	4.81	425 then 450	24 then 8	0.00	0.00	2.95	-0.29
(17) 27	4.99	a/a	a/a	0.07	1.40	3.02	-0.20
(18) 28	4.54	a/a	a/a	0.02	0.44	3.00	-0.16
			MEANS	0.03	0.61	2.99	-0.22
Cepaea 1	12.43	380	24	0.25	2.01	–	–
Cepaea 2	13.55	380	24	0.39	2.88	–	–
Helix 1	11.56	380	24	0.24	2.08	–	–
Helix 2	13.48	380	24	0.33	2.39	–	–
			MEANS	0.30	2.34	–	–

Table 2.8 Roasting : Results of test samples. Samples (1) to (16) Lincolnshire Limestone lab' standard, 105-180μm size fraction. All samples oven dried for at least 24 hours prior to roasting

Weight loss - see section 2.5.5
Stable isotope stability - see section 2.5.6 and Table 2.9 for comparison with stable isotope ratios

To find the optimum roasting temperature and period, the limestone test samples were further analysed for their stable isotope signatures, to assess how the roasting process might affect the carbon and oxygen stable isotopes. (see below).

2.5.6 Stability of stable isotopes during pretreatment

Eighteen samples of the laboratory standard (Lincolnshire limestone), size fraction 105-180μm, were roasted for time periods of 6-36 hours, at three temperatures. After roasting, carbon dioxide produced from the limestone samples, by acid digestion, was analysed in a triple collecting mass spectrometer, to measure the ratios of carbon and oxygen stable isotopes (section 2.6). The results are expressed as the per mil difference (δ‰) between the isotope ratios in the sample and those in a standard (PDB). The delta values are shown in Table 2.8, for the various samples. For comparison, eight isotopic analyses were carried out on untreated samples of the same limestone (Table 2.9).

The variability of $\delta^{18}O$ in the untreated samples (Table 2.9), suggests some isotopic inhomogeneity in the limestone, although the carbon isotope data are very consistent. With the roasted samples, there appears to be little or no difference between the resultant isotope ratios of samples roasted over the time period 6-36 hours at 380°C. However, at a more elevated temperature (425°C and then 425 to 450°C), the carbon isotope ratios, normally consistently between 3.3 and 3.4‰ , drop by 0.4‰ to around 3.0‰ (Table 2.8). This may indicate some isotopic fractionation resulting from combustion of carbon in the carbonate, during roasting. Thus it would seem that 425°C is too high a temperature for roasting, without also influencing the isotopic composition of the carbonate. At 380°C, no such effect is seen, therefore this temperature was selected as the standard for all sample preparation.

In selecting a roasting period for a shell sample, it was necessary to ensure that breakdown of organic consituents was complete, therefore a time period longer than six hourswas thought to be preferable. With no apparent influence upon the isotopic composition of the test samples, and for relative ease of maintenance, a roasting period of 24 hours was selected as the standard for all samples.

To test further isotopic stability of carbonate during bleaching followed by roasting, stable isotope analyses were carried out on six Lincolnshire Limestone samples (0.5-1.5mm), that had been bleached under various regimes, ground to ≤100μm, and then roasted at 380°C for 24 hours (Table 2.10). The coarser grain size was used to minimise sample loss during processing, especially critical during the rinsing stages which were carried out in a centrifuge. The weight losses on roasting were roughly equivalent to those measured in the shell and limestone samples (Table 2.8 and section 2.5.5), and again these weight losses could be accounted for by vapourisation of constituents, possibly including water.

The stable isotope analyses were carried out on approximately 3mg sub-samples of the ground, roasted limestone powders. As might be expected, the isotope ratios of this size fraction of limestone (0.5-1.5mm) were slightly different from the untreated samples of the finer grain size (Table 2.9). However, the isotope data from the bleached, ground and roasted samples (Table 2.10) are, with one exception, fairly consistent. The exception was sample 3, which had been in 'Chlorox' for 48 hours before being ground and roasted. This sample also lost 5% of its weight during roasting. The

	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰
1	3.43	0.26
2	3.40	-0.13
3	3.39	-0.04
4	3.39	-0.03
5	3.40	0.08
6	3.40	-0.07
7	3.42	-0.32
8	3.39	0.35
MEAN	3.40	-0.01

Table 2.9 Stable isotopic analysis of untreated Lincolnshire Limestone lab standard, 105-180μm size fraction.

See Table 2.10 for comparison with isotope data from an unbleached, but roasted sample (No. 4) of Lincolnshire Limestone, >500μm size fraction.

SAMPLE No.	CHLOROX TIME Hrs.	PEROXIDE TIME Hrs.	SAMPLE Wt. mg	ROASTING TEMP. °C	ROASTING TIME °C	Wt. LOSS mg	% Wt. LOSS	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
1	48	48	9.72	380	24	0.15	1.54	3.34 3.02	-0.21 -0.30
2	48	24	9.86	380	24	0.15	1.52	3.30	-0.55
3	48	x	10.87	380	24	0.55	5.06	3.07 3.06	-1.20 -1.17
4	x	x	14.61	380	24	0.27	1.85	3.28 3.25	-0.53 -0.67
5	x	48	9.52	380	24	0.42	4.41	3.34 3.30	-0.52 -0.60
6	x	24	10.95	380	24	0.19	1.73	3.26	-0.70

Table 2.10 Bleaching and roasting : results of test samples. All samples are Lincolnshire Limestone lab' standard >500µm size fraction.

See Table 2.9 for comparison with isotope data from roasted samples of Lincolnshire Limestone, 105-180µm size fraction.

anomalous carbon and oxygen isotope ratios of this sample, may be the result of residual 'Chlorox' being incorporated into the ground powder, if the sample was insufficiently rinsed. This indicates the necessity of washing thoroughly all samples after each stage of the bleaching process.

From these data, it would appear that the bleaching process did not influence to any high degree the stable isotopic composition of the test limestone samples, providing that samples were rinsed sufficiently. Thus the standard bleaching treatment of 48 hours in warm 'Chlorox', followed by 48 hours in hydrogen peroxide was adopted for all subsequent shell samples.

2.5.7 Oxygen plasma furnace

After shell samples had been roasted, the powdered samples often appeared greyish in colour, whereas previously they had been white to creamy-beige. The roasted samples also sometimes gave off a sometimes strong hydrocarbon / tar-like aroma. Thus, it appeared that the roasting method was effective at breaking down the organic components of the snail shells.

To remove the organic residues left after roasting, samples were placed in a low-temperature oxygen plasma furnace for four hours. On removal from the furnace, the samples had lost all hint of hydrocarbon smell, and again appeared cream to pure white in colour. Previous laboratory tests, using the Lincolnshire Limestone standard, have shown that plasma roasting does not appear to affect the stable isotopic composition of that carbonate (H. P. Attenborough., pers. comm., 1989). All shell samples were therefore subjected to four hours in the plasma furnace, before finally, being deemed clear of organic constituents, and therefore suitable for stable isotope analysis on the mass spectrometer.

2.5.8 Summary of sample preparation of gastropod shells

The preparation of all shell samples is summarised in flow-chart form (Figure 2.5).

The same basic procedure was applied to all other modern and fossil shells discussed in Chapters 4 and 5.

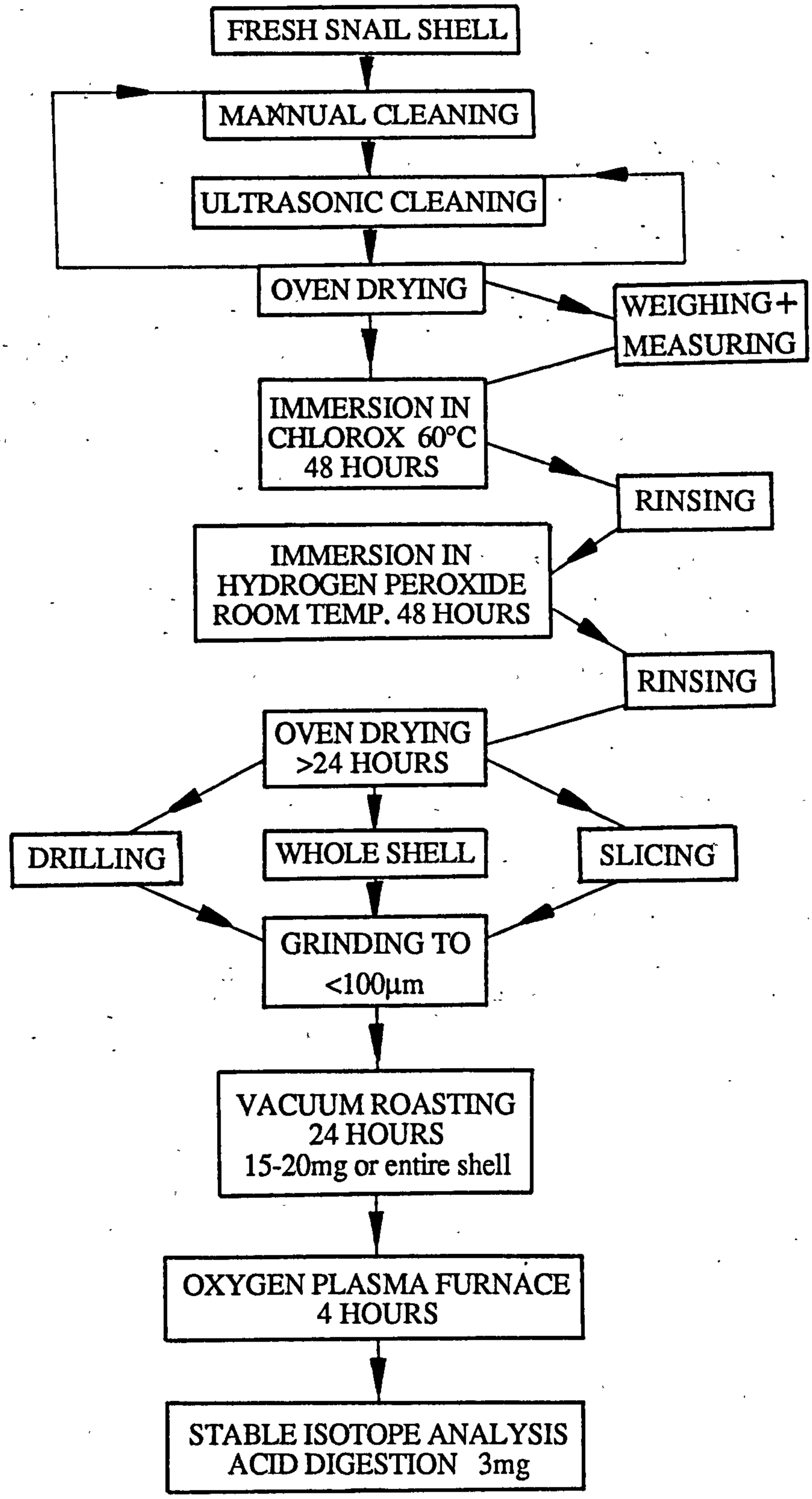


Figure 2.5 Flow chart of standard sample preparation. This procedure was followed for all samples of mollusc shell carbonate.

2.6 ANALYTICAL TECHNIQUES

2.6.1 Stable isotopic analysis of shell carbonates

All isotopic analyses of carbonate were carried out in the Stable Isotope Laboratory at Liverpool University by this author.

Analyses were carried out on approximately 3mg sub-samples of the purified carbonate powders (snail shell, dietary carbonate or tufa samples - see below). Sample powders were reacted with an excess of 100% orthophosphoric acid (generally approximately 2ml of H_3PO_4), under vacuum, at 25°C. The carbon dioxide produced was then cleaned of water, acid vapour and non-condensibles (O_2 and N_2) by isolation of the gas in a cryogenic acetone trap and subsequently in liquid nitrogen. The purified carbon dioxide evolved from each carbonate powder was then transferred to an automated VG Isogas Sira 12 triple collecting mass spectrometer, to measure the ratios of the masses of the various isotopes present (*i.e.* masses 44, 45 and 46) relative to a reference gas.

Samples and internal standards (Lincolnshire limestone) were analysed, in batches of ten or twenty, relative to an internal reference carbon dioxide. The reference gas was calibrated to the PDB international standard assuming values of $\delta^{13}C = 3.401$ and $\delta^{18}O = 0.014$ for the calcite Linclonshire limestone standard. Generally three or four internal standards were included in each batch of twenty gas samples. Raw sample and internal standard δ45 and δ46 values were corrected following the procedure of Craig (1957), and using a fractionation factor (α) of 1.01034 for carbon dioxide liberated from aragonite by reaction with 100% orthophosphoric acid, or an α of 1.01025 for the reaction from calcite (Friedman and O'Neil, 1977). Thus values for $\delta^{13}C$ and $\delta^{18}O$ relative to the international PDB standard were produced.

X-ray diffraction of samples of finely powdered snail shells of base population species revealed all to be aragonite. Most terrestrial mollusc shells are aragonitic in nature, and thus the isotopic ratios of all snail shell specimens reported in this thesis (growth experiments and case studies) have been considered as aragonite. Samples of the dietary carbonate (GPR calcium carbonate or Cretaceous chalk) given to the snails in the growth experiments have been treated as calcites, as have the tufas analysed as part of the case study presented in Chapter 5.

Analytical precision (reproducibility of the isotope data) was estimated by analysing four samples (a shell sample from the growth experiments; a shell sample from each of the two case studies presented; and a sample of the GPR calcium carbonate fed to the growth experiment snails) five times each. The repeat samples were analysed within the same batch of samples. Analytical precision, given as the mean of the standard deviation from each set of analyses, was determined as 0.02‰ for $\delta^{13}C$ and 0.03‰ for $\delta^{18}O$ (Table 2.11). Other workers at the Liverpool laboratory report analytical reproducibilities of duplicate analyses not run in the same batch which, therefore, are greater than those in Table 2.11, being 0.06‰ for $\delta^{13}C$ and 0.08‰ for $\delta^{18}O$ (Rowse, 1988) and 0.04‰ for $\delta^{13}C$ and 0.06‰ for $\delta^{18}O$ (Hendry, 1990). The overall reproducibility of the data may be considered as better than 0.1‰ for both $\delta^{13}C$, for $\delta^{18}O$.

The data presented in this thesis represent the analysis of over 700 shell samples, and over 750 carbonate samples in all, excluding internal standards and duplicate samples. The full data set represents over a thousand analyses.

	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
SAMPLE 1	-10.781	-5.963
Helix aspersa	-10.723	-5.919
snail from Expt.1 P1	-10.758	-5.905
	-10.745	-5.857
	-10.735	-5.904
Mean	-10.748	-5.910
Std. Dev.	0.022	0.038
SAMPLE 2	-8.892	-3.044
Arianta arbustorum	-8.910	-3.084
snail from Holywell	-8.915	-3.136
Coombe T5 section	-8.928	-3.127
	-8.898	-3.094
Mean	-8.909	-3.097
Std. Dev.	0.014	0.037
SAMPLE 3	-13.094	-5.004
GPR 1	-13.103	-5.023
inorganic carbonate snail	-13.089	-5.037
food from Expt. 1	-13.069	-4.978
	-13.092	-5.066
Mean	-13.089	-5.022
Std. Dev.	0.013	0.033
SAMPLE 4	-8.322	-3.764
Lucidela aureola	-8.353	-3.789
snail from Jamaican	-8.393	-3.767
case study	-8.340	-3.757
	-8.366	-3.781
Mean	-8.349	-3.772
Std. Dev.	0.027	0.013
Overall Mean Std. Dev. (anal. reprod. within the same batch of samples)	0.019	0.030

Table 2.11 Analytical reproducibility of carbon and oxygen isotope data from samples analysed within the same batch.

2.6.2 Stable isotopic analysis of waters

All stable isotopic analyses of water samples, for $\delta^{18}O$ (and additionally for $\delta^{2}H$), were carried out by staff members at the Institute of Hydrology at Wallingford, Oxfordshire - part of the British Geological Survey.

Oxygen isotope analyses were performed on approximately 5ml sub-samples of the collected specimens (waters fed to experimental snails during the growth experiments, or, from the Jamaican case study). The sub-samples were evacuated and subsequently equlibriated with a carbon dioxide gas (of known isotopic composition relative to an internal standard water - Wallingford Tap Water, WTW), overnight at 25°C. The equilibriated carbon dioxide was then extracted and purified, before being analysed in a VG 602E double collector mass spectrometer relative to carbon dioxide (produced from WTW) of known isotopic composition, relative to the SMOW and SLAP international standards.

Raw isotope data were corrected using standard procedures outlined in the IAEA Technical Reports Series 210, (Gat, 1981) and the precision of the results is within ± 0.2‰ (G. W. Darling, pers. comm., 1990).

Deuterium analyses were performed on 20 microlitre sub-samples of the water specimens following the "zinc method" (Coleman *et al.*, 1982). Isotopic analyses were performed on the same mass spectrometer as the carbonate analyses but on a separate head.

CHAPTER 3

GROWTH EXPERIMENTS - RESULTS

3.1 INTRODUCTION

In this chapter, the results of stable isotopic analyses of the shells produced in the growth experiments described in the previous chapter are presented.

Firstly, results of some preliminary analytical work to establish the degree of intra-specific and intra-shell variation in isotopes will be presented. Secondly, the mechanics of terrestrial mollusc shell secretion will be outlined and the potential sources of oxygen and carbon available to the snails for shell secretion, and their possible pathways into the shell, will be outlined. Following these, results from the three growth experiments, along with other pertinent data, will be considered sequentially.

3.2 ISOTOPIC VARIATION

3.2.1 Variation within a population

In order to assess isotopic variation between individuals of the same species of snails grown under 'base population', and 'experimental' conditions, two sets of isotopic analyses were carried out.

The first involved whole shells of recently deceased *Ferussacia folliculus*, taken from the base population of this species. As the shells were already empty, it was possible to prepare and analyse whole shells. Ten shells were analysed for their oxygen and carbon stable isotopic composition. The results are shown in Table 3.1a. Mean $\delta^{13}C$ = -11.94‰, standard deviation (sd) ($\sigma n-1$) = 0.19‰; mean $\delta^{18}O$ = 3.78‰, sd = 0.39‰.

Following this, shells of *Cernuella virgata* grown under the W2 regime of experiment 1 (see section 2.4.2) were examined. The last whorls from six shells grown for the standard six week experimental period were analysed. Subsequently, eight more analyses were carried out on the last whorls of *Cernuella* maintained under the same conditions for an additional six weeks. For these data, overall mean $\delta^{13}C$ = -13.27‰, sd = 0.13‰; and overall mean $\delta^{18}O$ = 3.12‰, sd = 0.37‰. The data from both *Ferussacia* and *Cernuella* are plotted on Figure 3.1, which allows a further assessment of the degree of similarity between the snails.

Figure 3.1 shows that the isotopic signatures of the two species grown under different conditions are dissimilar. It is apparent that a greater variation may be expected in the oxygen isotopic composition of these laboratory grown shells, than the carbon, *i.e.* for *Cernuella* , sd $\delta^{13}C$ = 0.13‰ (overall) whilst sd $\delta^{18}O$ = 0.37‰. The smaller variation in the carbon isotope signature may well reflect the constancy of the carbon available to the snails from their diet and the atmosphere, whereas the sources of oxygen were more likely to vary in their stable isotope compositions.

For both sets of results shown on Figure 3.1 and in Table 3.1a and 3.1b, there appear to be one or two data points that depart from the majority *e.g.* for *Ferussacia* , two samples (numbers 6 and

	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰
1	-11.98	3.84
2	-12.11	3.57
3	-11.56	3.52
4	-12.15	3.87
5	-11.68	3.47
6	-12.02	4.57
7	-11.91	3.65
8	-12.09	3.37
9	-12.06	3.63
10	-11.88	4.33
Max.	-11.56	4.57
Min.	-12.15	3.37
Mean	-11.94	3.78
Std. Dev.	0.19	0.39

Table 3.1a Intraspecific variation in $\delta^{18}O$ and $\delta^{13}C$ in the base population of *Ferussacia folliculus* (samples represent analysis of single whole shells)

	GROWTH WEEKS	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰
1	6	-13.47	3.07
2	6	-13.34	3.34
3	6	-13.51	3.54
4	6	-13.36	3.11
5	6	-13.25	2.92
6	6	-13.30	2.74
7	12	-13.04	3.13
8	12	-13.21	2.84
9	12	-13.17	2.52
10	12	-13.12	3.78
11	12	-13.11	3.37
12	12	-13.30	2.59
13	12	-13.30	3.28
14	12	-13.29	3.41
	Max.	-13.04	3.78
	Min.	-13.51	2.52
	Mean	-13.27	3.12
	Std. Dev.	0.13	0.37

Table 3.1b Intraspecific variation in $\delta^{18}O$ and $\delta^{13}C$ in shells of *Cernuella virgata* (samples represent last whorls of shell) from Experiment 1. (W2 regime at 22.3°C).

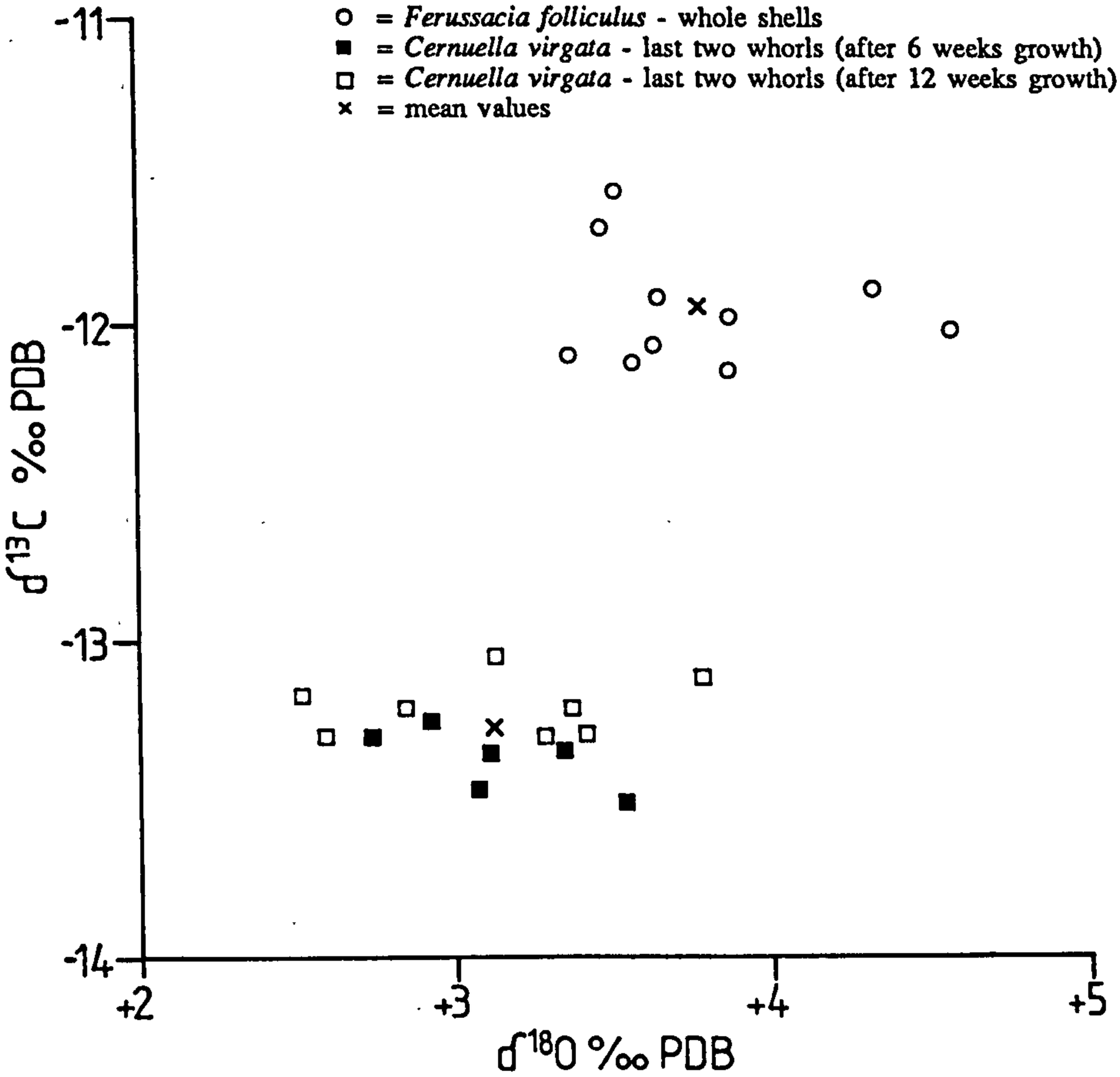


Figure 3.1 Isotopic variation within two populations of snail - *Cernuella virgata* and *Ferussacia folliculus*.. Shells of *Ferussacia* were taken from the base populations and shells of *Cernuella* were taken from the W2 (22.3°C) regime of experiment 1.

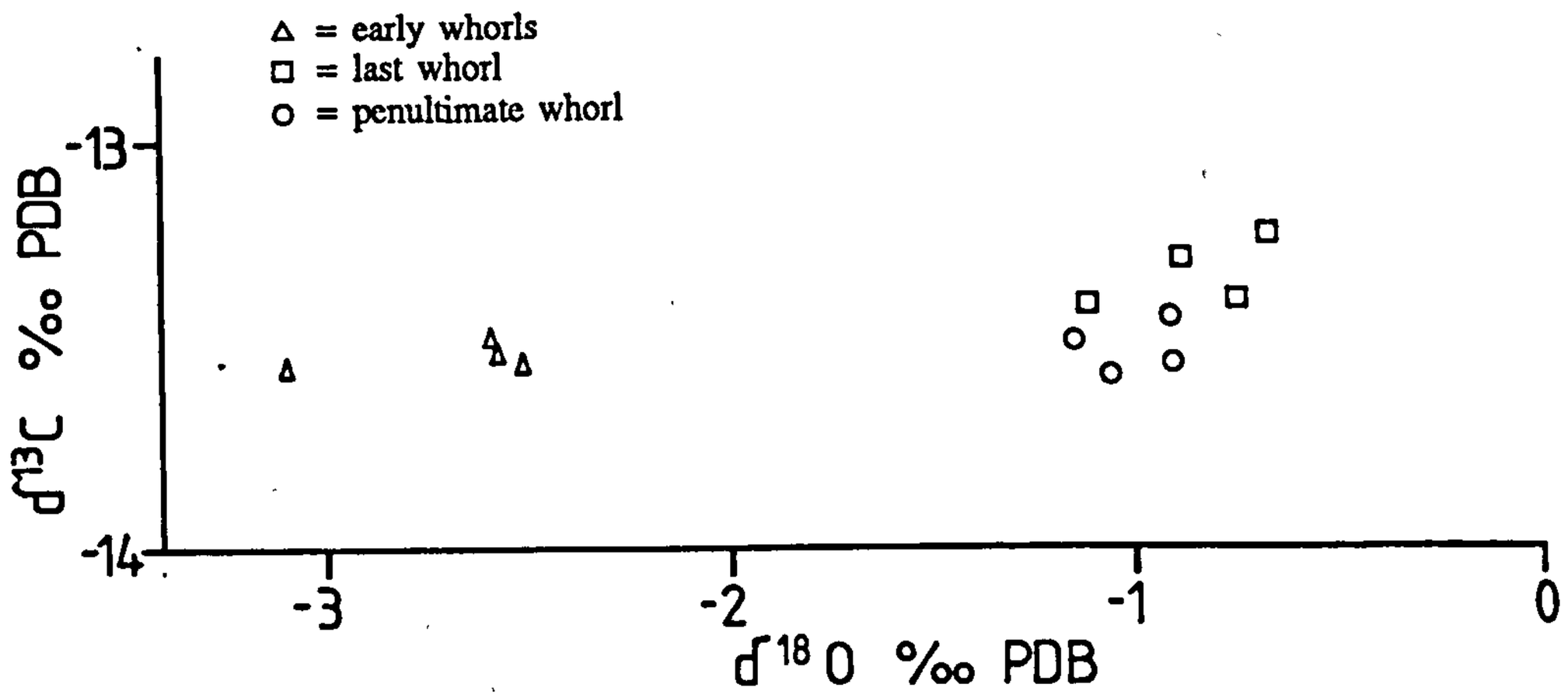


Figure 3.2 Isotopic variation within four shells of *Rumina decollata*, taken from the W1 (24°C) regime of Experiment 1.

10) have oxygen isotope values considerably above the mean value of 3.78‰, being 4.57‰ and 4.33‰, respectively. A similar observation may be seen in the data from the second batch of *Cernuella* shells (grown for twelve weeks) where sample 10 ($\delta^{18}O = 3.78‰$) is enriched in ^{18}O as compared to other shells grown under the same conditions, mean $\delta^{18}O = 3.12‰$. The *Cernuella* isotope data are slightly less variable than those from the *Ferussacia* shells, which partially reflects the effectiveness of regulating the environmental variables of temperature, water and feeding, on the *Cernuella* population as compared to the base populations of *Ferussacia* . The smaller variation in the data from *Cernuella* , as compared to *Ferussacia* , may also reflect the fact that the data from *Ferussacia* represent whole shell values not necessarily produced over the same time period, whereas the data from *Cernuella* represent only the last whorls of shell which were produced over the same time period. However, the difference in variability between the two populations is small, and may well reflect the fact that the data are from different species of snail.

In summary, it appears the expected intra-specific isotopic variation for snails grown under laboratory conditions is near ± 0.15 for $\delta^{13}C$, and ± 0.35 for $\delta^{18}O$. The variation may be accounted for through very localised environmental effects, and as a result of vital effects within the bodies of the various animals.

3.2.2 Variation within shells

The degree of intra-shell isotopic variation was tested on shells of *Rumina decollata.* This species was selected, as it is relatively easy to slice off individual whorls from the cylindrical shells. Three portions of each of four individual shells were analysed. The snails had been grown under the W1 regime of experiment 1 at 24°C. The *Rumina* snails appeared to grow well at this temperature, and laid down sufficient shell to allow several isotopic analyses, if necessary. The three portions of shell analysed for their isotopic composition were:

1) The early whorls
2) The penultimate whorl
3) The last whorl

The early whorls have $\delta^{18}O$ values from -2.52 to -3.09‰ and $\delta^{13}C$ values from -13.49 to -13.57‰. The penultimate whorls have $\delta^{18}O$ values from -0.91 to -1.15‰ and $\delta^{13}C$ values from -13.44 to -13.58‰. The last whorl $\delta^{18}O$ values lie between -0.67 and -1.11‰, and $\delta^{13}C$ values from -13.23 to -13.41‰. The results are shown in Table 3.2 and are plotted on Figure 3.2.

It is apparent that within these four shells, there is an increase in $\delta^{18}O$ between the early whorls (from -2.5 to -3‰) and the last whorls (generally around -0.75‰). The 'early whorls' data include juvenile whorls that may have been secreted before the snail was placed under the experimental conditions, or, before the the snail had fully acclimatized to the W1 high temperature regime. This probably accounts for the change in $\delta^{18}O$ through the shells. This positive shift in $\delta^{18}O$ was consistent with that seen between shells of *Ferussacia folliculus*, from the base population (mean $\delta^{18}O = +3.78‰$) and those grown under the W1 regime of experiment 1 (mean $\delta^{18}O = +0.56‰$).

The carbon isotope data are also slightly more enriched in $\delta^{13}C$ in the last whorls, as compared to the data from the early whorls, with enrichments between 0.08 and 0.32‰ being recorded. This may also reflect acclimatization of the snails. However, there is no major shift in $\delta^{13}C$ (mean

		EARLY WHORLS	PENULTIMATE WHORL	LAST WHORL
1	$\delta^{13}C$ ‰	-13.57	-13.50	-13.30
	$\delta^{18}O$ ‰	-3.09	-1.15	-0.89
2	$\delta^{13}C$ ‰	-13.49	-13.58	-13.41
	$\delta^{18}O$ ‰	-2.59	-1.06	-1.11
3	$\delta^{13}C$ ‰	-13.55	-13.44	-13.23
	$\delta^{18}O$ ‰	-2.52	-0.91	-0.67
4	$\delta^{13}C$ ‰	-13.52	-13.56	-13.40
	$\delta^{18}O$ ‰	-2.58	-0.91	-0.75

Table 3.2 Intrashell variation in $\delta^{18}O$ and $\delta^{13}C$ for four shells of *Rumina decollata* from Experiment 1 (W1 regime at 24°C).

enrichment = +0.19‰), as was seen with the oxygen isotopes (mean enrichment = +1.85‰). The food source was the same for the base population as for these experimental snails, which may explain the consistency of the data, and also suggests that the food is a significant source of carbon for the snails' shells, with only a limited isotope effect resulting from the prevailing environmental conditions.

In the light of the intra-shell isotope variation recorded in *Rumina*, it is necessary, for this species and for other snails used in the experimental work, to analyse only the latest shell material deposited, which would have been secreted, hopefully, after full thermal acclimatization. Earlier shell whorls may represent a mixture of pre- and post-acclimatization shell, with previously secreted shell being internally thickened at a later date. It was for this reason that in the growth experiments, only the latest secreted shell material was processed and analysed for its stable isotope signature. The visual increase in shell size, over the experimental period, made it possible to be virtually certain that the shell material in question had been deposited under the experimental conditions, and indeed usually in the latter part of the growth period. Marking of individual shells at the onset of each experiment, with a view to recording a 'starting point' was considered. The marking material would have to adhere strongly to the shell, and be non-edible and non-toxic to the tiny snails. Nail varnish was considered a possibility (A. J. Cain, pers. comm., 1987), and was tested upon base population sub-adult to adult *Helix aspersa*. However, application of the varnish appeared to cause immediate stress to the snails as they expelled large amounts of watery mucus in a frothy mass. This method was therefore deemed unsuitable for the juvenile snails, as mass mortality may have resulted. The marking of the baby snails that were used in the experiments, would also have been impractical due to the tiny amount of the shell material present initially.

3.3 THE DISTRIBUTION AND MOVEMENT OF OXYGEN AND CARBON ISOTOPES THROUGH A SNAIL, AND SECRETION OF SHELL MATERIAL

3.3.1 Introduction

A snail's body may be considered in terms of four individual compartments (Wilbur and Saleuddin, 1983),

(1) the external medium

(2) the blood fluid (haemolymph) and body tissues

(3) the extrapallial fluid and space (between the mantle and the inner shell surface); compartments (2) and (3) form the body fluid

(4) the shell

The extrapallial fluid (from which the shell is secreted) is composed of organic compounds such as proteins and amino acids, but is composed primarily of inorganic ions such as calcium and bicarbonate from which the shell is constructed, plus water. Material is transferred through the various compartments by passive movement down electrochemical gradients; by active transport by the snail; by movement of ions coupled with other ions; and possibly by intercellular transport (Wilbur and Saleuddin, 1983).

In this section the possible sources of oxygen and carbon available to the experimental snails will be examined and the isotopic fractionations likely to occur during uptake, transport and secretion of the materials will be presented. The effect of temperature upon the various fractionation processes will be considered with a view to outlining the nature of the results that might be expected over the range of environmental temperatures used in the growth experiments. Additional factors that could influence the isotopic composition of the secreted shells will also considered.

3.3.2 Oxygen and oxygen isotopes

(a) Sources and pathways

The ultimate sources of oxygen available to a snail for use in shell secretion are from its environmental water, from the atmosphere and from the organic and inorganic fractions of its diet. The oxygen held in the carbonate shell is taken from the bicarbonate ion pool in the extrapallial fluid. The possible pathways for oxygen to enter this fluid are as follows:

1) Oxygen dissolved in environmental water. This could be absorbed through the body wall along osmotic gradients, or ingested with the food. Water entering the the body fluid reservoir from this source will exchange its oxygen isotopes with the water already present with no isotopic fractionation - a simple mixing process.
2) Atmospheric oxygen taken into the snail by external respiration. This oxygen will dissolve into the body water, where, once dissolved its isotopes would freely mix with the isotopes already present.

3) Oxygen held in metabolic water, produced by the breakdown of organic foodstuffs. The oxygen in metabolic water has been shown to be derived from atmospheric oxygen (Lifson *et al* ., 1949).

4) Oxygen held in metabolic carbon dioxide. This will exchange its isotopes with the water produced by metabolism, and with atmospheric oxygen.

The oxygen of bicarbonate rapidly equilibrates isotopically with the water it is dissolved in (Goodfriend and Magaritz, 1987). Therefore, shell carbonate (aragonite), which is secreted from body fluid bicarbonate, should have an oxygen isotope composition reflecting that of the snail body water once the known fractionation between water and aragonite and the temperature of precipitation are taken into consideration (see below).

Thus, the resultant oxygen isotopic composition of the snail body fluid will depend on the relative importance of the sources outlined above and their initial oxygen isotope composition, but perhaps more significantly, on whether the body water composition may be further modified by processes prior to shell secretion (see section (c) below).

(b) Temperature effects

Temperature and oxygen isotopes in mollusc shell aragonite (the carbonate forming the shells of the land snails investigated here, as shown by X-ray diffraction - see section 2.6.1) have been shown to be related such that:

$$T°C = 21.8 - 4.69\ (\delta^{18}Oar - \delta^{18}Ow) \qquad (3.1)$$

where, $\delta^{18}Oar = \delta^{18}O$ of CO_2 generated from the aragonite shell at 25°C (PDB) and
where, $\delta^{18}Ow = \delta^{18}O$ of CO_2 generated in equilibrium with water at 25°C (PDB).

This relationship was established for marine gastropods collected from the continental margins of southern California and Texas, USA, and Mexico (Grossman and Ku, 1986).

Friedman and O'Neil (1979), (after Craig, 1961), state that the $\delta^{18}O$ value of CO_2 produced by the reaction of 100% phosphoric acid with PDB calcite at 25°C, is +0.22‰ relative to CO_2 equilibrated at 25°C with SMOW. Thus the value for $\delta^{18}Ow$ in (3.1) may be calculated as:

$$\delta^{18}Ow = \delta^{18}O_{water\ (SMOW)} - 0.22‰ \qquad (3.2)$$

In the experimental work, the value of $\delta^{18}O$water (SMOW) is taken as the $\delta^{18}O$ value of the water given to the snails. Equation (3.1) indicates that with increasing temperature, and with a known constant value of $\delta^{18}Ow$, the resultant aragonite shell will become depleted in ^{18}O, by 0.21‰per°C. This is indicated on Figure 3.3, where for a series of values of δw, calculated equilibrium $\delta^{18}O$ aragonite values are plotted against temperature. Therefore as environmental temperature increases, the oxygen isotope value of mollusc shell aragonite should become more negative, providing that other factors (such as any modification of the the body fluid oxygen isotope composition by other processes) remain constant.

n. b. In a recent publication, Hudson and Anderson (1989), have re-defined the equation of Grossman and Ku (1986), taking into account the difference between CO_2 equilibrated at 25°C

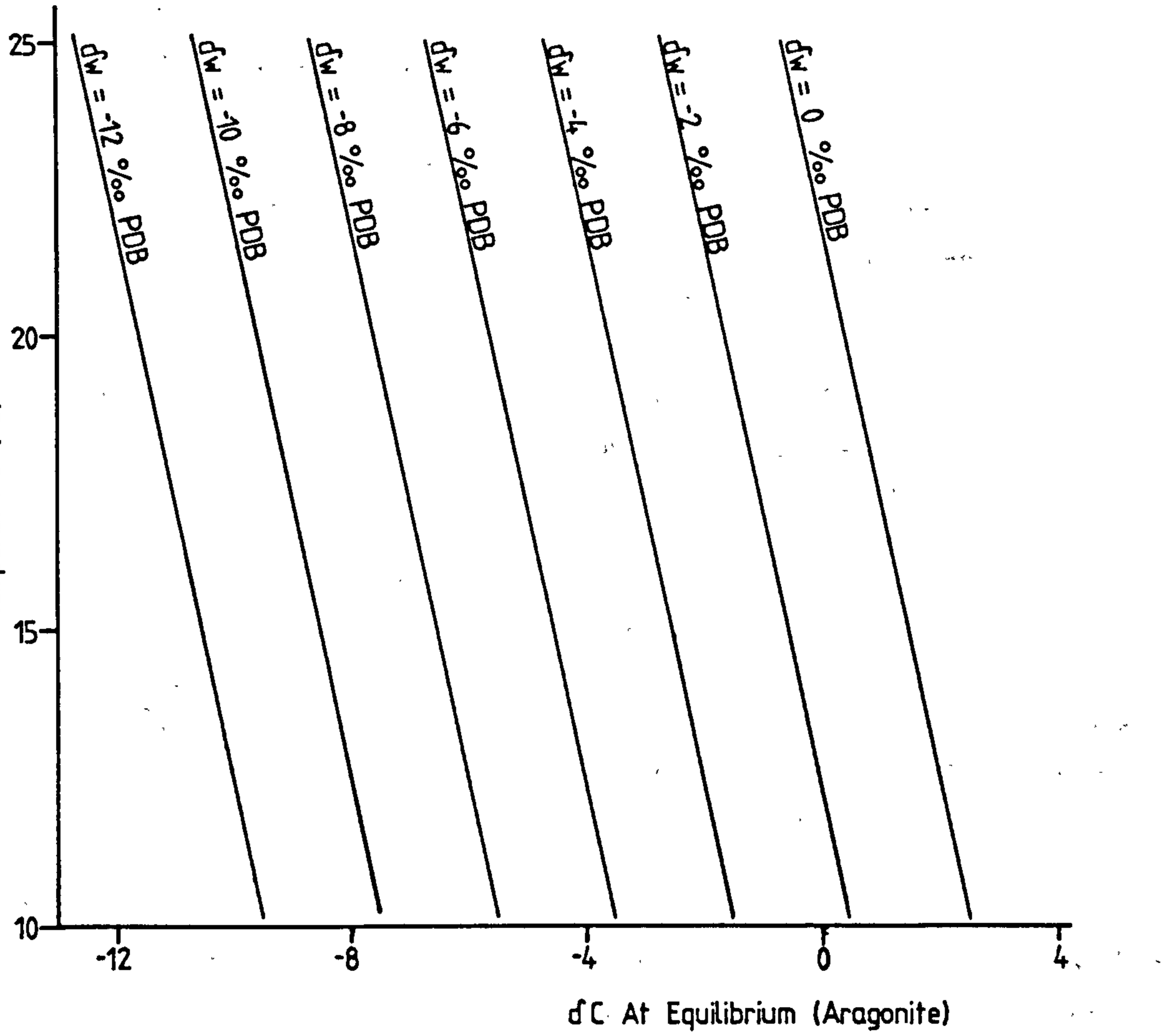


Figure 3.3 Relationship of temperature and $\delta^{18}O$ in aragonite at equilibrium with δw, over a range of temperatures. Based on equation 3.1 (after Grossman and Ku, 1986), which relates $\delta^{18}O$ PDB aragonite and δw with temperature. δw is calculated from $\delta^{18}O$ SMOW environmental water (see equation 3.2).

(SMOW) and CO_2 produced from acid digestion at 25°C (PDB). Thus, Hudson and Anderson (1989) have a single temperature equation where $\delta^{18}O$ PDB Arag. values and $\delta^{18}O$ SMOW Water values are directly comparable, such that:

$$T°C = 19.7 - 4.34 (\delta A - \delta W) \qquad (3.2a)$$

where $\delta A = \delta^{18}O$ of aragonite on the PDB scale, and
where $\delta W = \delta^{18}O$ of water on the PDB scale

The application of this single equation provides the same result, in terms of the temperature obtained, as that achieved by using equations 3.1 and 3.2. Throughout subsequent sections of this thesis, the Grossman and Ku equation has been cited, although the Hudson and Anderson equation is equally viable.

(c) Other mechanisms affecting the oxygen isotope composition of snail body fluid

If shell precipitation is at isotopic equilibrium, then the oxygen isotope composition of the secreted shell is set by the isotopic composition of the body fluid and the environmental temperature. Thus, the isotopic composition of the shell will reflect that of the environmental water available to the snail, provided that the oxygen isotopes in the fluid are not modified by other processes. Two mechanisms have been proposed that may affect the isotopic composition of the body water - evaporation and snail metabolism. Both these processes lead to oxygen isotope enrichment of the body fluid and have been used to explain observed differences between shell $\delta^{18}O$ values relative to isotopic equilibrium with environmental waters (such as rain, dew or water vapour) and actual measured shell $\delta^{18}O$ values (Lecolle, 1985; Goodfriend and Magaritz, 1987; Goodfriend *et al* ., 1989).

1) Evaporation

Evaporation results in ^{18}O enrichment in the remaining reservoir, as the lighter isotope (^{16}O) is preferentially lost from the system. Evaporation would effect the external water reservoir prior to uptake by the snails, or could effect the internal body water by evaporative loss through the integument from either an active or an inactive snail. However, recent work by Goodfriend *et al* . (1989), suggests that water loss from a snail's body may be non-fractionating. This will be discussed further in ensuing sections

2) Metabolic effects

Metabolic effects may also result in oxygen isotope enrichment of the snail body fluid reservoir.

Metabolism, or internal, tissue or cell respiration, produces water and carbon dioxide (as well as energy) through the aerobic breakdown of organic molecules such as glycogen and glucose, as follows:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

The oxygen is supplied by external respiration. The water produced through metabolism mixes with body water already present, and the carbon dioxide exchanges its oxygen with body water

during the processes of transport through the body (*e.g.* traversal of intracellular and interstitial fluid, carriage by the blood) until removed from the body by external respiration (Lifson *et al* ., 1949).

The oxygen in metabolic water is derived from atmospheric oxygen (Lifson *et al* ., 1949) through external respiration. Atmospheric oxygen has a $\delta^{18}O$ of around +23‰ SMOW (Hoefs, 1987) which is greatly enriched in ^{18}O over meteoric (*e.g.* the average value for worldwide precipitation ≈ -4‰ Craig and Gordon, 1965) or environmental waters (*e.g.* the water given to snails during this experimental work = -9.4‰ SMOW (see Table 3.5)). Thus, increased throughput of atmospheric oxygen could be one source of oxygen isotope enrichment over "expected" equilibrium values.

The oxygen in the metabolic carbon dioxide is in part derived from dietary material which may also be enriched in ^{18}O relative to water taken into the snail's body (Goodfriend and Magaritz, 1987). Metabolic alteration of snail body water has been attributed to some of the oxygen isotope enrichment found in calculated (Goodfriend and Magaritz, 1987) and measured (Goodfriend *et al* ., 1989) snail body water compositions, when compared to meteoric or environmental waters.

Goodfriend and Magaritz (1987), have related the degree of metabolic enrichment to the activity level of the snails they studied. During periods of inactivity, direct input of environmental water would cease and thus body water would become relatively enriched in ^{18}O as a result of metabolic activity, as outlined above. In active snails, water fluxes are higher, and thus the relative effects of metabolic enrichment would be lessened.

3.3.3 Carbon and carbon isotopes

(a) Sources and pathways

The ultimate sources of carbon for mollusc shell carbonate are

1) organic foodstuffs (usually plant carbon)

2) atmospheric carbon dioxide

3) inorganic carbon sources such as limestone

(Goodfriend and Hood, 1983; Wilbur and Saleuddin, 1983; Goodfriend and Magaritz, 1987).

Bicarbonate in the extrapallial fluid is derived mainly from the bicarbonate of the haemolymph (blood fluid), although some may be derived from direct diffusion of carbon dioxide at the mantle edge, and possibly by the breakdown of urea by the enzyme urease (at least in those molluscs that possess urease) (Goodfriend and Hood, 1983). The processes and pathways leading to carbonate shell secretion are shown in Figure 3.4 (after Goodfriend and Hood, 1983). In this scheme, the pathways that involve isotopic fractionation are indicated. The main fractionations involved within the snail carbon cycle and how they are influenced by temperature are as follows:

1) The dissolution and precipitation of solid carbonate, including the breakdown of inorganic carbonates ingested by the snail (Goodfriend and Hood, 1983; Goodfriend and Magaritz, 1987).

i.e. $HCO_3\text{-(aq)} \rightleftharpoons CaCO_3\text{(s)}$

This fractionation may be important in the secretion of the shell from the extrapallial bicarbonate pool. Following a series of inorganic laboratory experiments, Emrich *et al.* (1970), found that at 20°C, ^{13}C will be enriched in the solid carbonate phase (presumably calcite) by 1.85‰, with respect to the

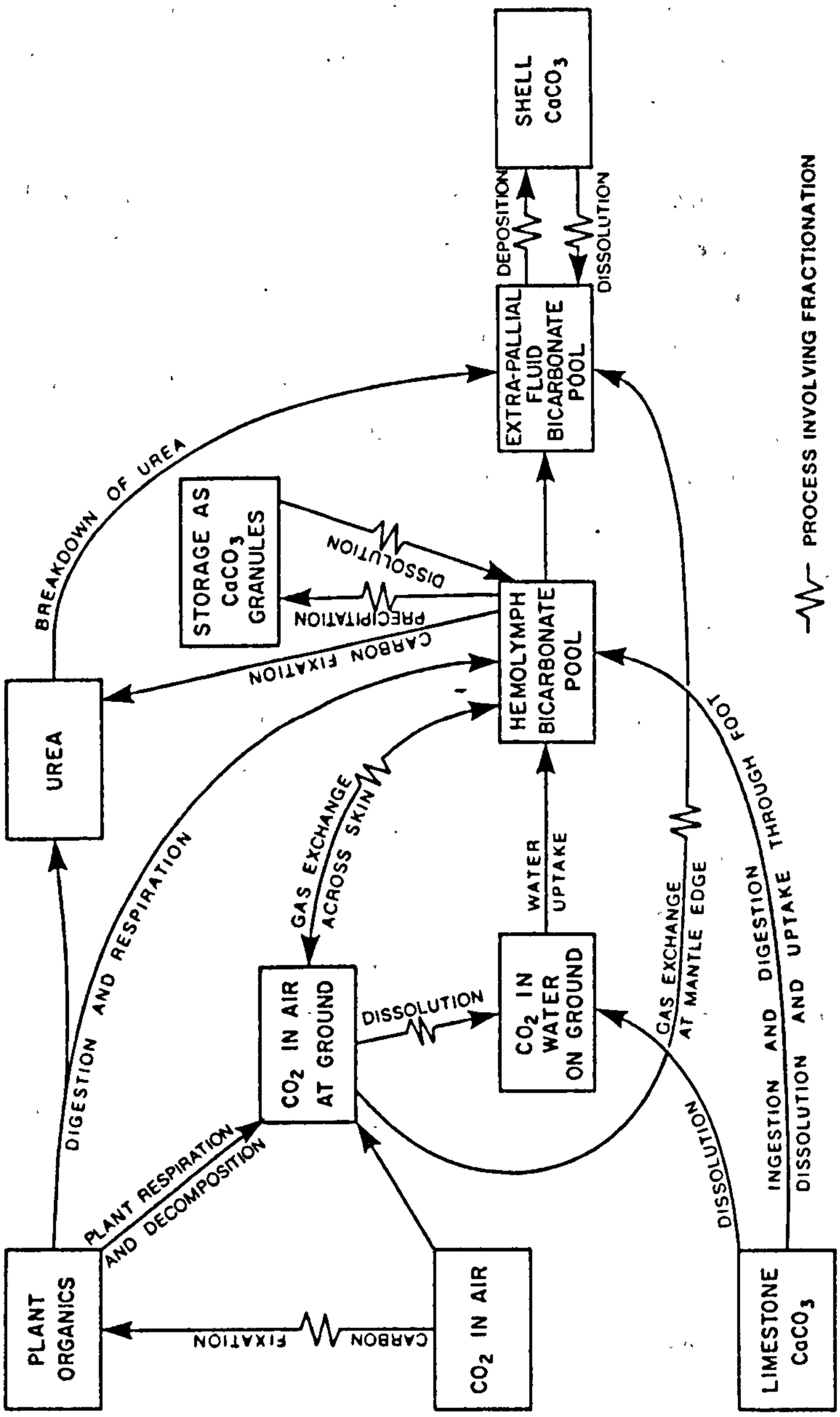


Figure 3.4 Processes and pathways of carbon leading to carbonate shell secretion (after Goodfriend and Hood, 1983).

aqueous bicarbonate. Rubinson and Clayton (1969), investigated inorganic precipitation of aragonite (in addition to calcite) from bicarbonate solution. They reported that at 25°C aragonite was enriched in ^{13}C by +2.7‰ relative to the bicarbonate solution, assuming isotopic equilibrium precipitation had occurred. This fractionation is weakly temperature dependent (0.035‰per°C - Emrich *et al* ., 1970). Thus with increasing temperature, the isotopic value the solid carbonate would increase very slightly, and the bicarbonate solution would become relatively more depleted in ^{13}C.

From the work on inorganic systems outlined above, at equilibrium the shell carbonate should be enriched by 1.8‰ at 20°C relative to the bicarbonate solution (Emrich *et al* .,1970), or by 2.7‰ at 25°C (Rubinson and Clayton, 1969)

Goodfriend and Hood (1983), have investigated carbon isotope fractionation effects between extrapallial fluid bicarbonate and aragonite shell carbonate *i.e.* in a biological system. They found that the aragonite shells were depleted by approximately 2‰ relative to the bicarbonate. This finding contrasts with the situation outlined above for the fractionation between aqueous bicarbonate and solid calcium carbonate. However, Goodfriend and Hood suggested that this fractionation could only be approximate, as they had compared whole shell $\delta^{13}C$ values to $\delta^{13}C$ of haemolymph collected at one time from mature shells, and that the enriched shell ^{13}C could be accounted for through the breakdown of urea (see Figure 3.4).

Evidence that aquatic molluscs secrete their shell at equilibrium with the aqueous carbonate species of their habitats has been presented by Mook and Vogel (1968), Mook (1971), and Fritz and Poplawski (1974). Although Goodfriend *et al*.. (1989), have shown a fractionation between $\delta^{18}O$ in body water and that in shell aragonite, there is limited evidence to suggest that carbon isotopes, in the aragonite of landsnail shells, are likely to be deposited out of equilibrium with extrapallial bicarbonate.

In a snail's body, the bicarbonate reservoir is small and limited (unlike the inorganic experiments carried out Emrich *et al* ., and Rubinson and Clayton, noted above). Under such conditions a positive fractionation of initially deposited shell would produce a negative fractionation of the bicarbonate. This would counteract further fractionation effects in the shell (Goodfriend and Hood, 1983). In addition, Goodfriend and Hood (1983) state that secretion of shell carbonate may occur too rapidly for any equilibrium to be reached and that there is no evidence for carbon isotope fractionation during the precipitation of land snail shell aragonite from the bicarbonate of the extrapallial fluid. Such a 'rate effect' was also demonstrated in inorganic systems by Turner (1982), who carried out a series of experiments measuring the extent of the fractionation between $CaCO_3$ and HCO_3^-. At rapid rates of precipitation, the $\delta^{13}C$ of the carbonate approaches that of the dissolved bicarbonate and only at very slow rates is true equilibrium fractionation reached. Thus, rapid shell secretion could produce a precipitated carbonate with an isotopic composition identical to the aqueous carbon reservoir.

2) The exchange of gaseous carbon dioxide (atmospheric CO_2) with dissolved carbon dioxide and ultimately dissolved bicarbonate, both across the epithelium and at the mantle edge (Goodfriend and Hood, 1983; Goodfriend and Magaritz, 1987).

i.e. $$CO_2(g) \rightleftharpoons CO_2(aq) \rightleftharpoons HCO_3^-(aq)$$

Emrich *et al.* (1970), found that at 20°C, ^{13}C will be enriched in the dissolved bicarbonate by +8.38‰ relative to gaseous CO_2. A enrichment of +8.46‰ is reported by Mook *et al* ., (1974). For

any snail, the gaseous carbon dioxide under consideration is predominantly that in the atmosphere. Atmospheric carbon dioxide has a $\delta^{13}C$ of around -8‰ PDB (Goodfriend and Magaritz, 1987), which would give a value of dissolved carbon dioxide, at isotopic equilibrium, of around +0.4‰. This fractionation is only slightly temperature dependent (-0.1‰per°C), and with increasing temperature the amount of enrichment would decrease, leaving the bicarbonate relatively depleted in ^{13}C (Emrich *et al* ., 1970).

3) The digestion of organic material, whereby carbon dioxide produced by metabolism is converted to bicarbonate, facilitated by the presence of the enzyme carbonic anhydrase (Wilbur and Saleuddin, 1983).

A snail's body fluid is around pH 7.5, and at this pH around 85 to 90% of the carbon dioxide produced by metabolism is converted to bicarbonate (G. A. Goodfriend, pers. comm., 1990).

Investigation of an inorganic system by Mook *et al.* (1974), showed that at 20°C dissolved bicarbonate would be enriched in ^{13}C by 9.54‰, with respect to dissolved carbon dioxide. (From gaseous carbon dioxide to dissolved bicarbonate, the fractionation is that noted above, *i.e.* an 8.4‰ enrichment in the bicarbonate phase). The fractionation is only slightly temperature dependent (-0.1‰per°C). Thus, with increasing temperature the amount of enrichment would decline, leading to less enrichment in $\delta^{13}C$ in the dissolved bicarbonate at higher temperatures.

Goodfriend and Hood (1983), considered that in the natural system of a snail processing its food, carbon isotopes would not be fractionated (see Figure 3.4). The experiments carried out by Mook *et al* ., (1974) and Emrich *et al* ., (1970) were based on inorganic systems where a very large pool of carbon dioxide was available - large enough so that the original isotopic composition of the carbon dioxide is not altered although producing an equilibrium fractionation in the bicarbonate (around +8 to +9‰) with respect to the carbon dioxide. As discussed above, the snails bicarbonate reservoir is limited, as is the supply of carbon dioxide from its food. If all the dietary material is converted to carbon dioxide and this carbon dioxide is in no way supplemented by atmospheric carbon dioxide *i.e.* assuming a closed system, then mass balance considerations dictate that the $\delta^{13}C$ of the bicarbonate produced from the dietary carbon dioxide will be only very slightly different from the $\delta^{13}C$ value of the organic foodstuffs (Goodfriend, pers. comm., 1990). Because the system is closed, the carbon isotope ratio of the carbon dioxide produced from the breakdown of the food (respiratory, or metabolic carbon dioxide) must be balanced with isotope ratios of the bicarbonate pool plus the metabolic carbon dioxide.

In reality, the snail's body is not a closed system. Some atmospheric carbon dioxide will enter the bicarbonate pool where it will undergo fractionation up to the equilibrium value noted in the preceding sub-section. However, if the internal respiration of the food is entire, then the carbon dioxide produced from this source will have a $\delta^{13}C$ value similar to the original food material (*i.e.* around -27‰).

(b) Temperature effects

The carbon isotope effects linked directly with environmental temperatures are small, although from the calculated theoretical fractionation factors and the temperature dependence of the fractionations, increasing temperature would lead to slight overall ^{13}C depletion in the extrapallial bicarbonate.

(c) Variation in carbon sources and use of resources

As noted above, the temperature dependence of the reactions involving carbon isotopes is small. Therefore to assess the $\delta^{13}C$ shell isotope signatures the initial isotopic compositions of material entering the snail body from the three main sources (inorganic carbon, atmospheric carbon dioxide and organic foods) must be considered. The relative importance of these three sources under various environmental conditions (including temperature) must be taken into account. This same principle has been applied by Goodfriend and Hood (1983), and Goodfriend and Magaritz (1987). For the experimental snails, the carbon sources available to the snails are as follows.

1) Inorganic carbon

The only source of inorganic carbon available to the snails was that supplied in the snail's diet. In each of the experiments the $\delta^{13}C$ value of the inorganic carbon (as carbonate) in the diet was known and, in each experiment, was constant. The amount of carbon available to the snails from this source did not vary, and was available in excess.

2) Atmospheric carbon dioxide

The atmospheric carbon dioxide used by the snails would have a $\delta^{13}C$ value of approximately -8‰ (Goodfriend and Magaritz, 1987). As indicated above, ^{13}C from gaseous carbon dioxide will be enriched in the dissolved bicarbonate of the snail body fluids by up to 8.42‰ (Emrich *et al.*. 1970, Mook *et al.* 1974). This would give a resultant bicarbonate solution (and ultimately the shell) with a $\delta^{13}C$ of +0.42‰ at isotopic equilibrium, if all the carbon in the snail's system was from this source and complete exchange had occurred.

Some carbon dioxide is also held within the soil, and generally has ^{13}C compositions close to those produced by a mixture of decay and respiration of local plants - generally between -15 and -30‰ (Deines, 1980). Some soil carbon dioxide might have been available to the snails, but the relatively much larger atmospheric carbon dioxide reservoir would buffer any isotopic effect from the use of carbon dioxide from this source. Also, the soil used in the experiments had an open structure and therefore carbon dioxide from the soil would equilibrate quickly with that from the atmosphere. Finally the soil surface only accounted for approximately 25% of the crawling surface area for the snails. Therefore, the influence from soil carbon dioxide is assumed to be negligible.

3) Organic carbon in the diet.

The organic constituents of the diet, milk powder and oat cereal, have $\delta^{13}C$ values of -26.9 and -26.7‰ respectively. As the food sources were given in equal amounts, this gives a mean $\delta^{13}C$ organic carbon value of -26.8‰. This value indicates that the milk and cereal originated from plants following the C3 photosynthetic cycle (Calvin Cycle; Deines, 1980). The breakdown of this organic material transfers the carbon to dissolved carbon dioxide which mostly becomes bicarbonate in the snail body fluid. If the oxidation (internal respiration) of the food is complete then the carbon dioxide produced will have the same $\delta^{13}C$ value as the organic food stuff. Thus, if all the carbon in the shell originated from metabolic carbon dioxide, then the shell would have an isotopic composition close to that of the organic component of the diet - in this case around -26.8‰.

Other organic carbon might be available to the snails from the soil base. However, the faeces of the snails were predominantly off-white in colour, containing limited soil debris, therefore it is assumed that dietary organic carbon was processed from the milk powder and oat cereal.

Therefore for the experimental snails, as the carbon isotope composition of the inorganic carbon in the diet was fixed, variation in ^{13}C will reflect the predominance of the utilisation of metabolic versus atmospheric carbon dioxide.

More enriched $\delta^{13}C$ shell values will occur when atmospheric carbon dioxide is the more important component reaching the extrapallial bicarbonate fluid, up to values greater than 0‰ where 100% of the carbon in the bicarbonate is from the atmosphere. Conversely, more depleted $\delta^{13}C$ values will be present in the shell carbonate if the input of metabolic carbon dioxide predominates, producing $\delta^{13}C$ values similar to the organic food where 100% of the carbon in the bicarbonate is from this source. This relationship was established ~~by~~ by Goodfriend and Magaritz (1987), in their study of Israeli landsnails from different geographic localities. In natural systems, both the $\delta^{13}C$ of the inorganic carbon and the amount of such carbon available to the bicarbonate pool would be variable, and neither atmospheric nor metabolic carbon dioxide would be the sole contributor to the bicarbonate pool.

3.4 EXPERIMENT 1 : ISOTOPIC COMPOSITION OF SHELLS OF SNAILS GROWN OVER A RANGE OF TEMPERATURES

3.4.1 Introduction and aims

Experiment 1 was designed to reveal how the oxygen and carbon isotopic compositions of the shells of the test snail species might be affected by increasing environmental temperatures, if all other factors were maintained as constant. It was also hoped to relate the oxygen isotopic composition of the shells to the environmental water available to the snails to assess whether shell deposition was at isotopic equilibrium with that environmental water. Details of the experimental design were presented in section 2.4.2. Shells produced from the six phases of experiment 1 were analysed for their carbon and oxygen stable isotope signatures (section 2.6). Isotopic analyses were carried out on at least four individual shells (where available) from each species, in each of the six temperature regimes. The results of all the isotopic analyses are presented in Appendix 1.

3.4.2 Results : Experiment 1

(a) Outline

The complete data from Appendix 1. are summarised in Table 3.3. Also included are standard deviations ($\sigma n-1$) for each mean isotope value, and the number (n) of data analyses on which the means and standard deviations are based.

From Table 3.3, it may be seen that, as was noted for the intra-specific variation (section 3.2.1), the $\delta^{13}C$ data are less variable (mean standard deviation = 0.15‰) than the $\delta^{18}O$ data (mean standard deviation = 0.39‰). The oxygen isotope data have been plotted on a series of graphs, for each of the snail species (Figures 3.5a-e), and then similarly for the carbon isotope data (Figures 3.6a-e). These figures are based upon all the data included in the statistical summary (Table 3.3) and show range bars and mean values for each temperature regime. Lines have been drawn through the mean values to show overall trends with temperature for each snail species, for both oxygen and carbon isotopes.

(b) Oxygen isotopes

Oxygen isotope values range from -4.01‰ to +4.40‰. Generally, more depleted oxygen isotope values occur over the lower temperatures (13.1 to 15.7 and possibly 19.3°C), with more positive *i.e.* more enriched values present in shells from the two warmest regimes (22.3 and 24°C. However the pattern is not a linear relationship, as predicted by the equation of Grossman and Ku (1986) and Hudson and Anderson (1989), section 3.3.2, as may be seen from Figures 3.5a-d. Instead, with increasing temperature, each oxygen isotope curve follows a broad 'S' shape. This same trend is apparent for *Helix*, *Cernuella*, *Cepaea* and *Rumina*, with only slight differences between the species. The plots for *Helix aspersa* and *Cernuella virgata* are the most comparable, but those for *Cepaea nemoralis* and *Rumina decollata* also fairly closely follow a similar path.

At the lowest temperature (C1 10.2°C), the oxygen isotope data are the most enriched of the three 'cool' temperatures (-2.76 to -0.70‰), and with increasing temperature the $\delta^{18}O$ values become

TEMP. °C	$\delta^{13}C$ ‰	Std. Dev.	$\delta^{18}O$ ‰	Std. Dev.	n
Helix					
10.2	-12.26	0.17	-2.10	0.53	9
13.1	-11.81	0.07	-2.75	0.23	4
15.7	-12.07	0.09	-2.72	0.20	4
19.3	-12.64	0.07	-0.98	0.19	4
22.3	-13.42	0.11	3.88	0.36	4
24.0	-13.42	0.06	0.84	0.16	4
Cepaea					
10.2	-12.61	0.13	-1.46	0.89	7
13.1	-12.20	0.12	-3.06	0.33	4
15.7	-12.51	0.14	-1.97	0.15	4
19.3	-12.31	0.03	-1.23	0.14	4
22.3	-13.11	0.25	4.40	0.54	6
24.0	-13.16	0.09	2.50	0.18	6
Cernuella					
10.2	-10.90	0.17	-2.76	0.47	6
13.1	-11.17	0.28	-3.49	0.29	4
15.7	-11.55	0.58	-3.77	0.77	4
19.3	-12.39	0.24	-2.68	0.05	3
22.3	-13.27	0.13	3.12	0.37	14
24.0	-12.91	0.18	-1.46	0.41	6
Rumina					
10.2	-12.38	0.40	-0.70	0.30	2
13.1	-12.30	0.07	-3.63	0.20	6
15.7	-12.53	0.12	-3.71	0.54	4
19.3	-12.93	0.17	-3.79	0.64	4
22.3	-13.63	0.10	0.78	0.56	4
24.0	-13.34	0.09	-0.86	0.19	4
Ferussacia					
15.7	-11.39	0.01	-4.01	0.11	2
22.3	-12.56	0.10	-0.44	0.23	2
24.0	-12.68	0.20	0.56	1.19	4

Table 3.3 Mean carbon and oxygen isotope values of shells grown in the six temperature regimes of Experiment 1. (data from all species)

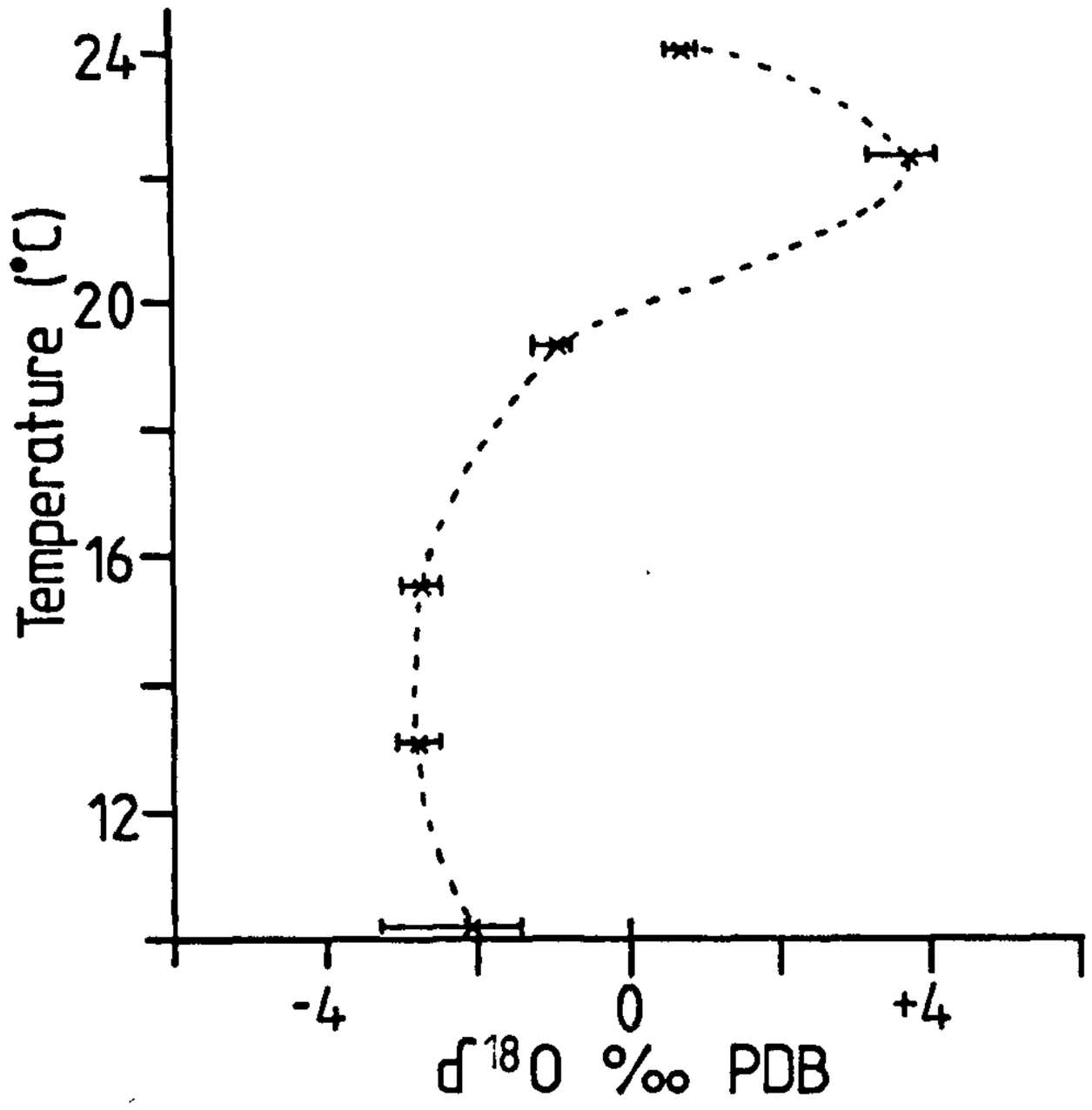


Figure 3.5a *Helix aspersa*

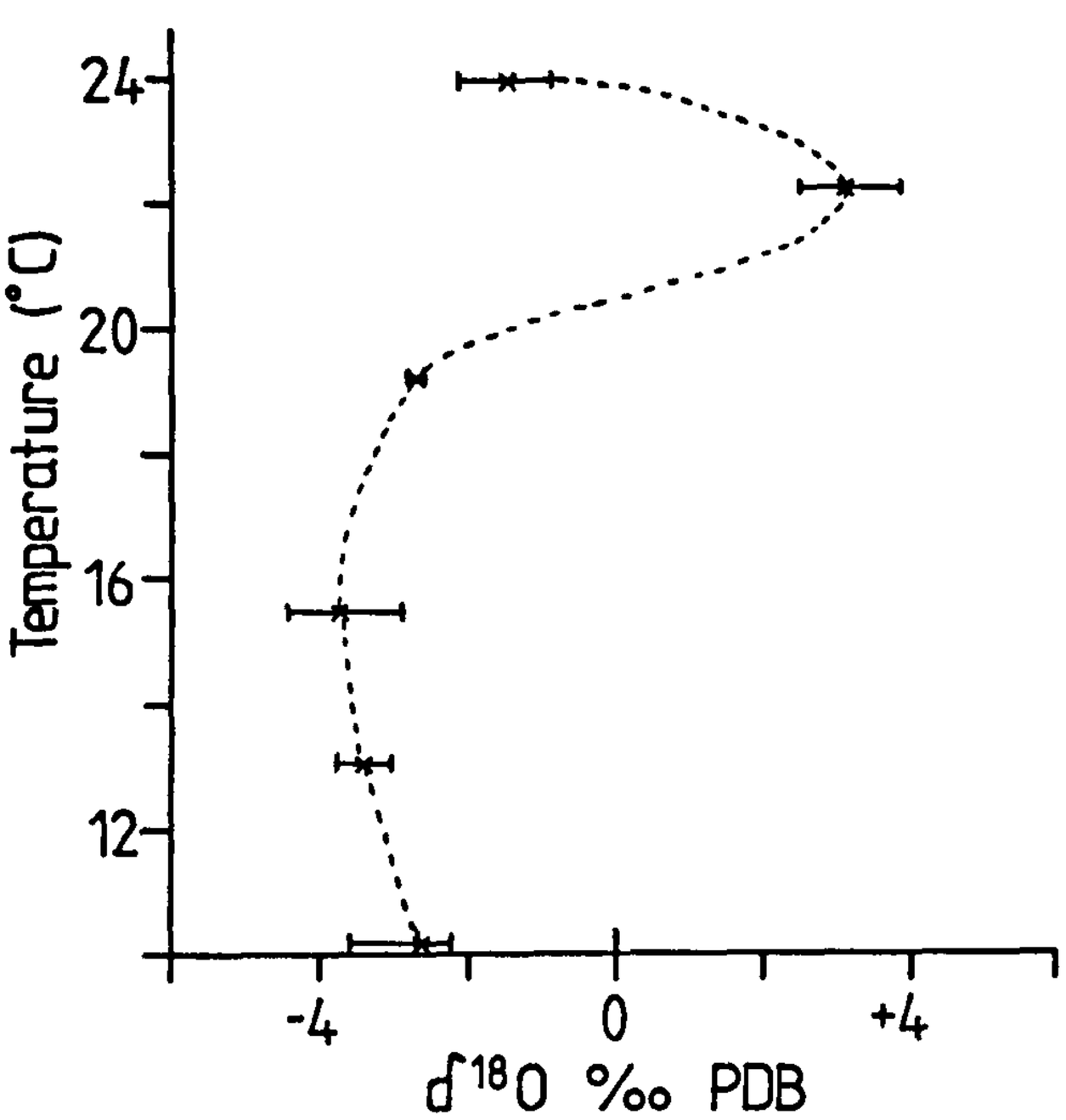


Figure 3.5b *Cernuella virgata*

Figure 3.5a-e Oxygen isotope trends with temperature : Experiment 1. The range of values at each temperature is shown, and lines are drawn through mean values, for (a) *Helix aspersa*, (b) *Cernuella virgata*, (c) *Cepaea nemoralis*, (d) *Rumina decollata* and (e) *Ferussacia folliculus*

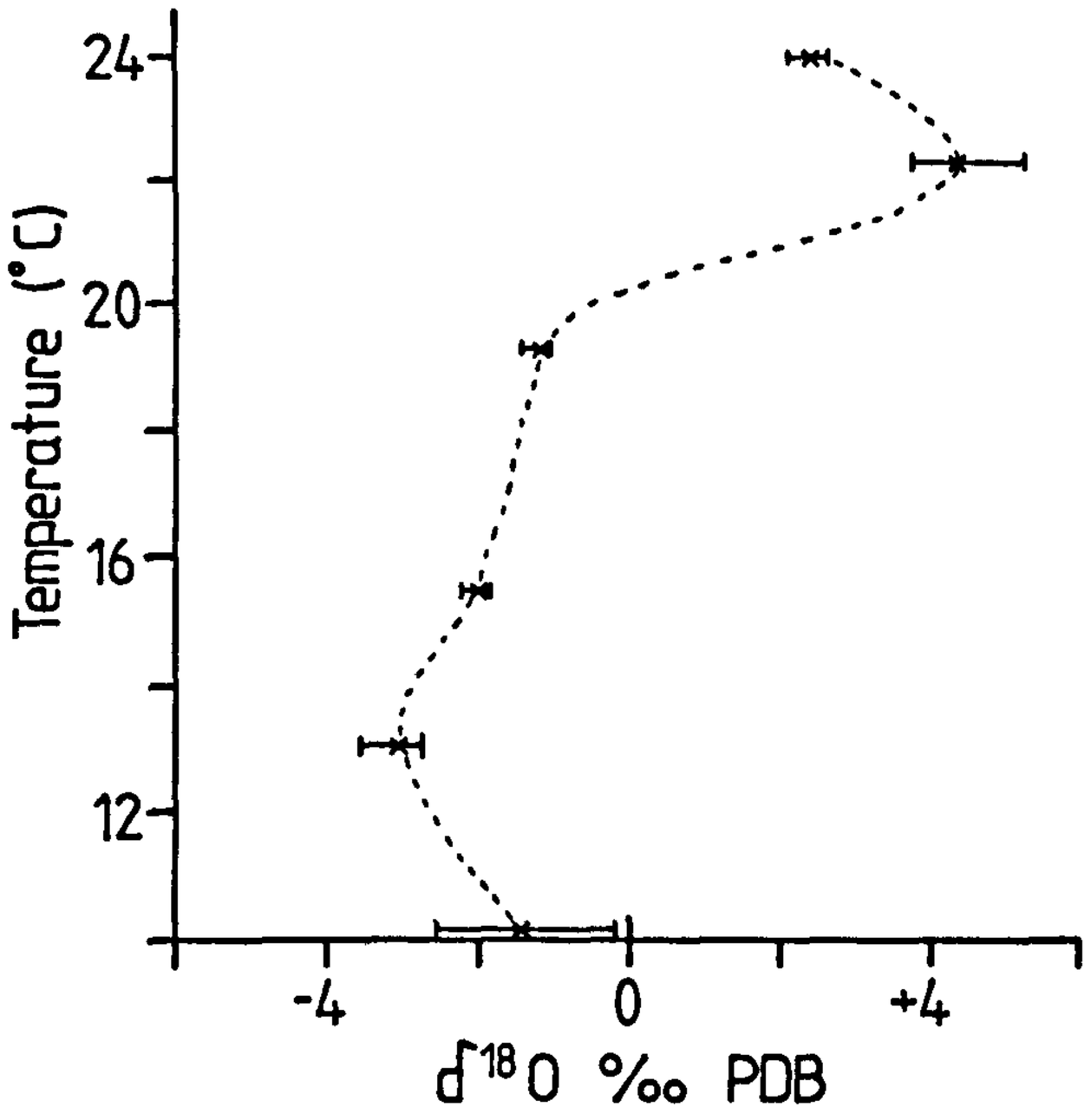


Figure 3.5c Oxygen isotope trends with temperature for *Cepaea nemoralis* from Experiment 1.

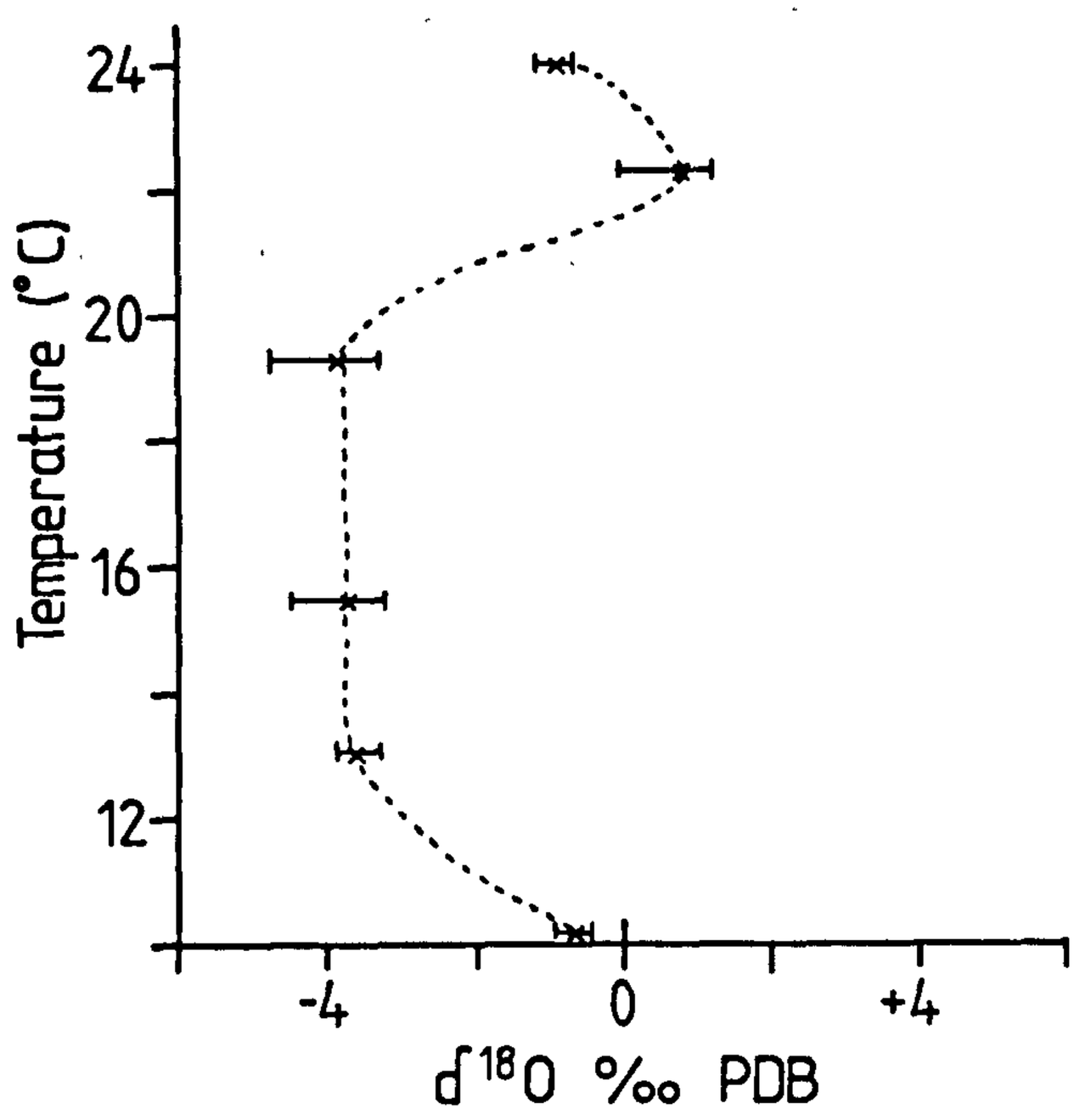


Figure 3.5d Oxygen isotope trends with temperature for *Rumina decollata* from Experiment 1.

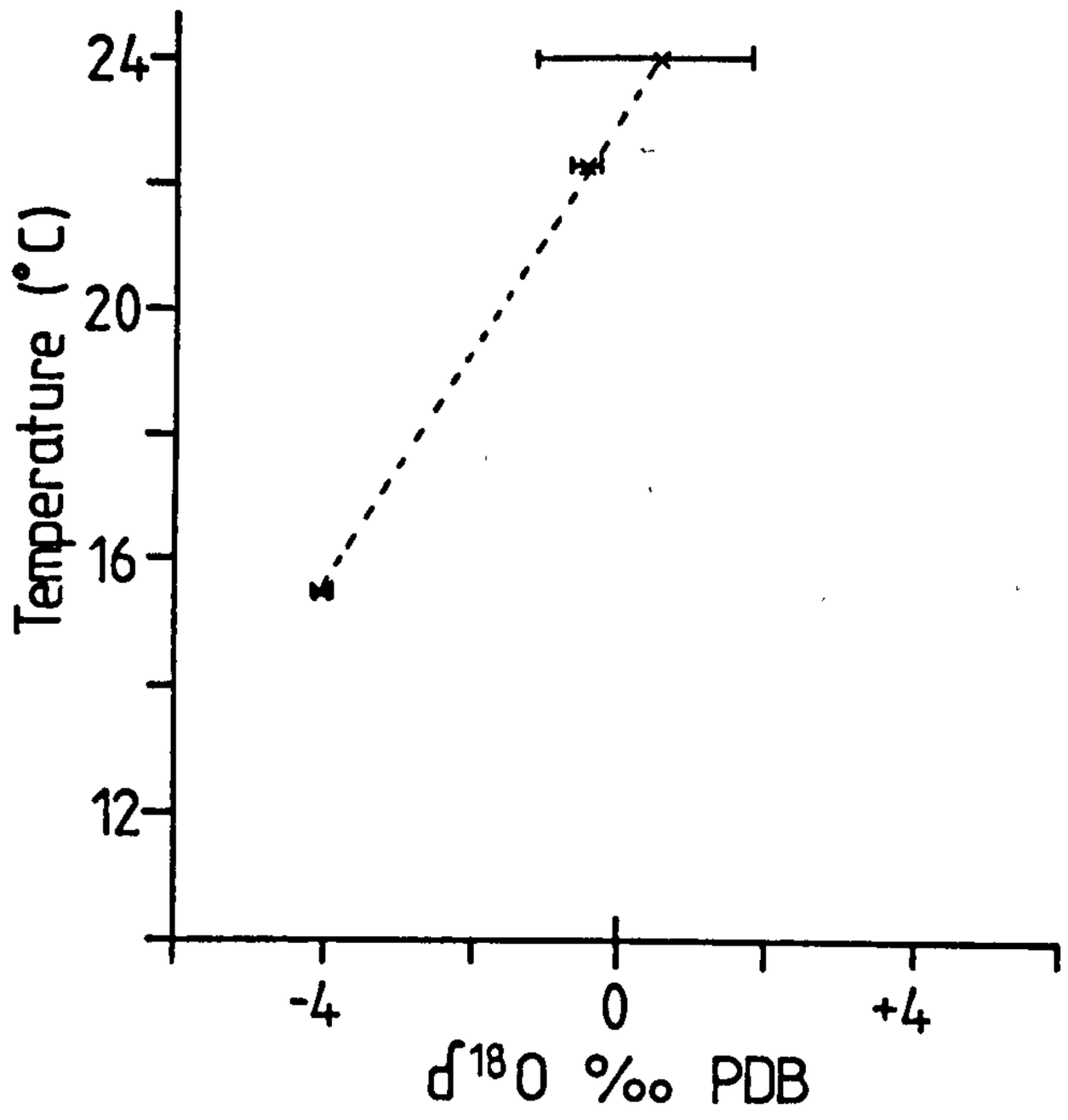


Figure 3.5e Oxygen isotope trends with temperature for *Ferussacia folliculus* from Experiment 1.

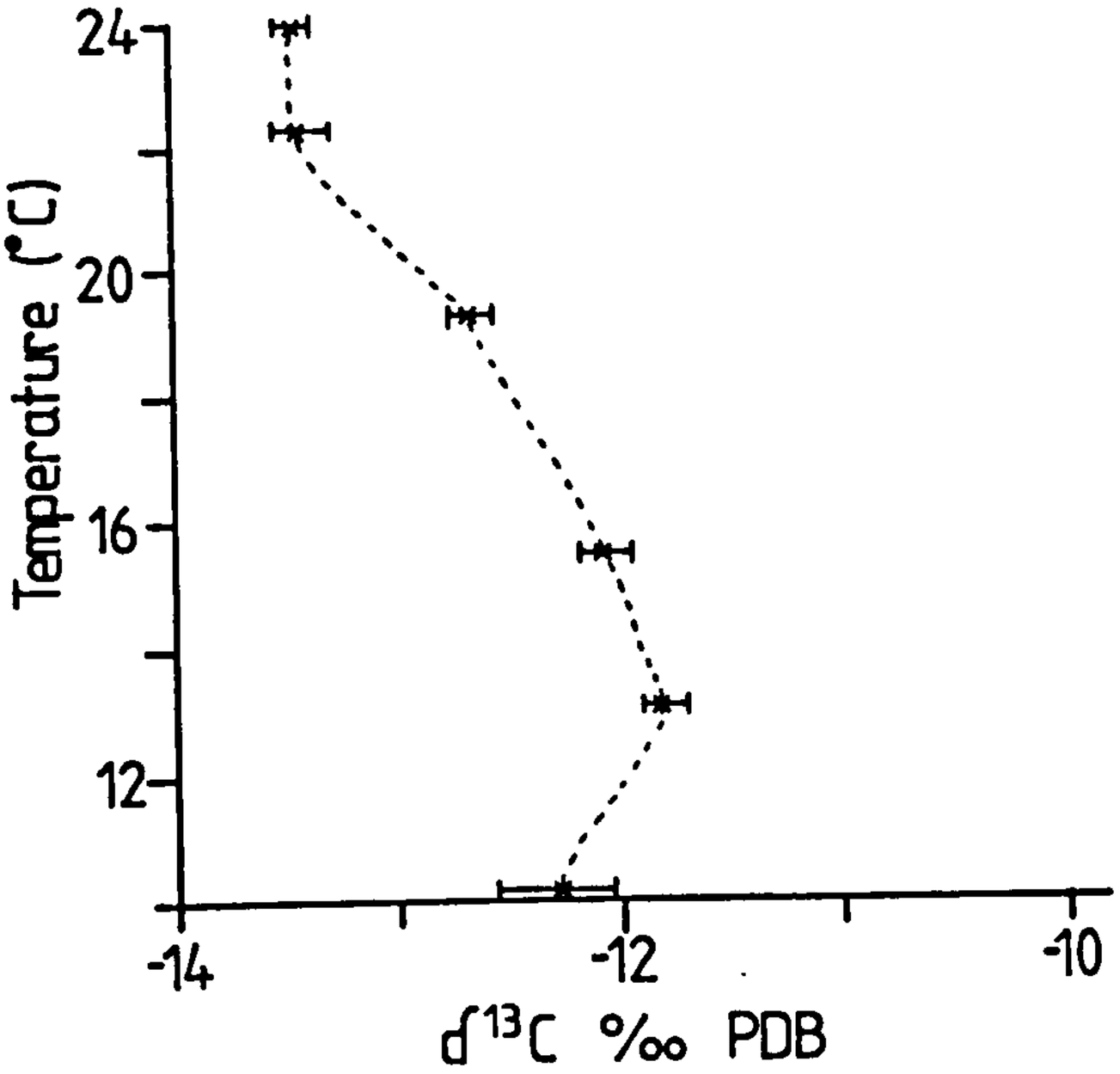


Figure 3.6a *Helix aspersa*

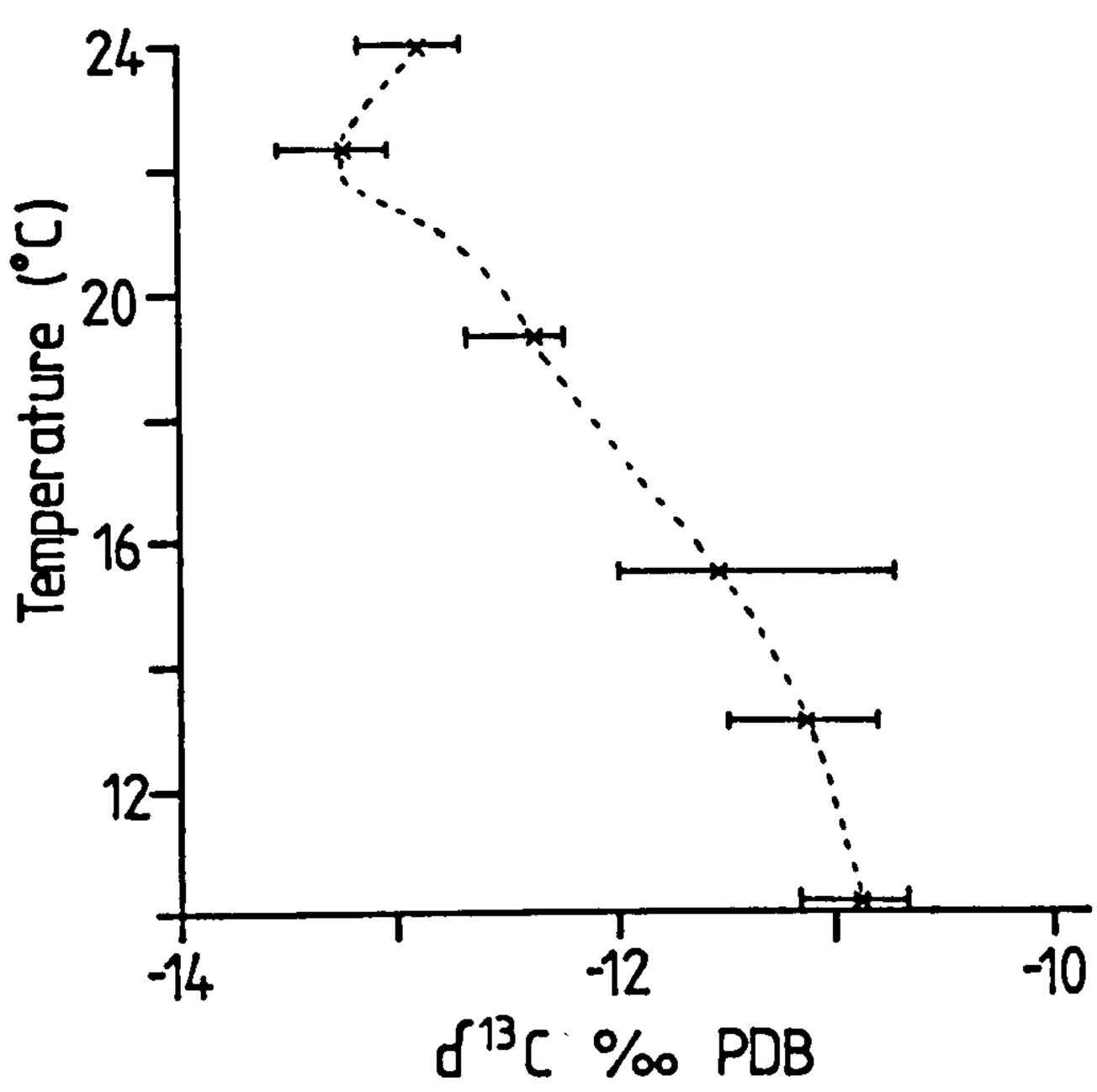


Figure 3.6b *Cernuella virgata*

Figure 3.6a-e Carbon isotope trends with temperature : Experiment 1. The range of values at each temperature is shown and lines are drawn through mean values, for (a)*Helix aspersa*, (b)*Cernuella virgata*, (c)*Cepaea nemoralis*, (d)*Rumina decollata* and (e)*Ferussacia folliculus*.

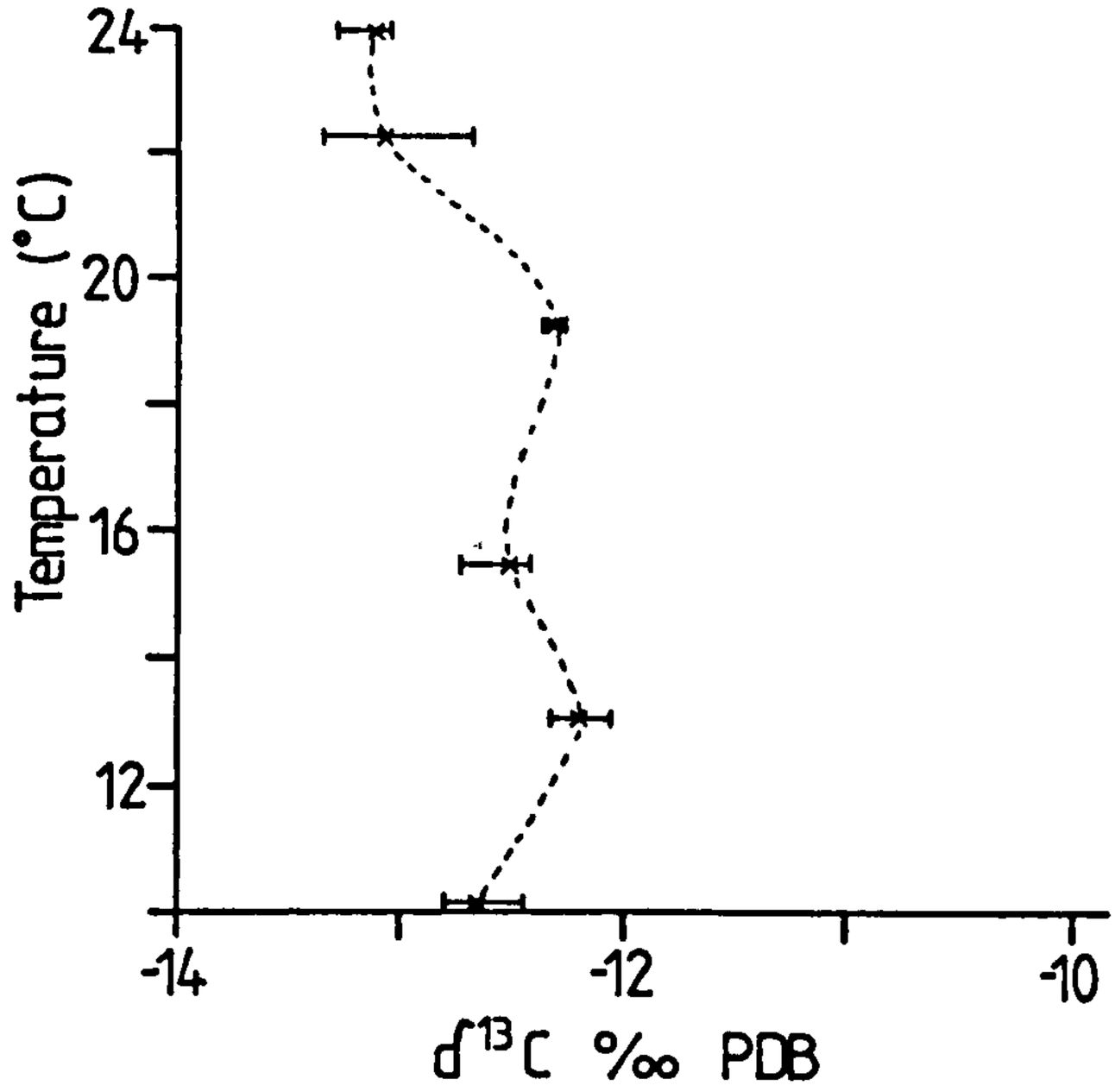


Figure 3.6c Carbon isotope trends with temperature for *Cepaea nemoralis* from Experiment 1.

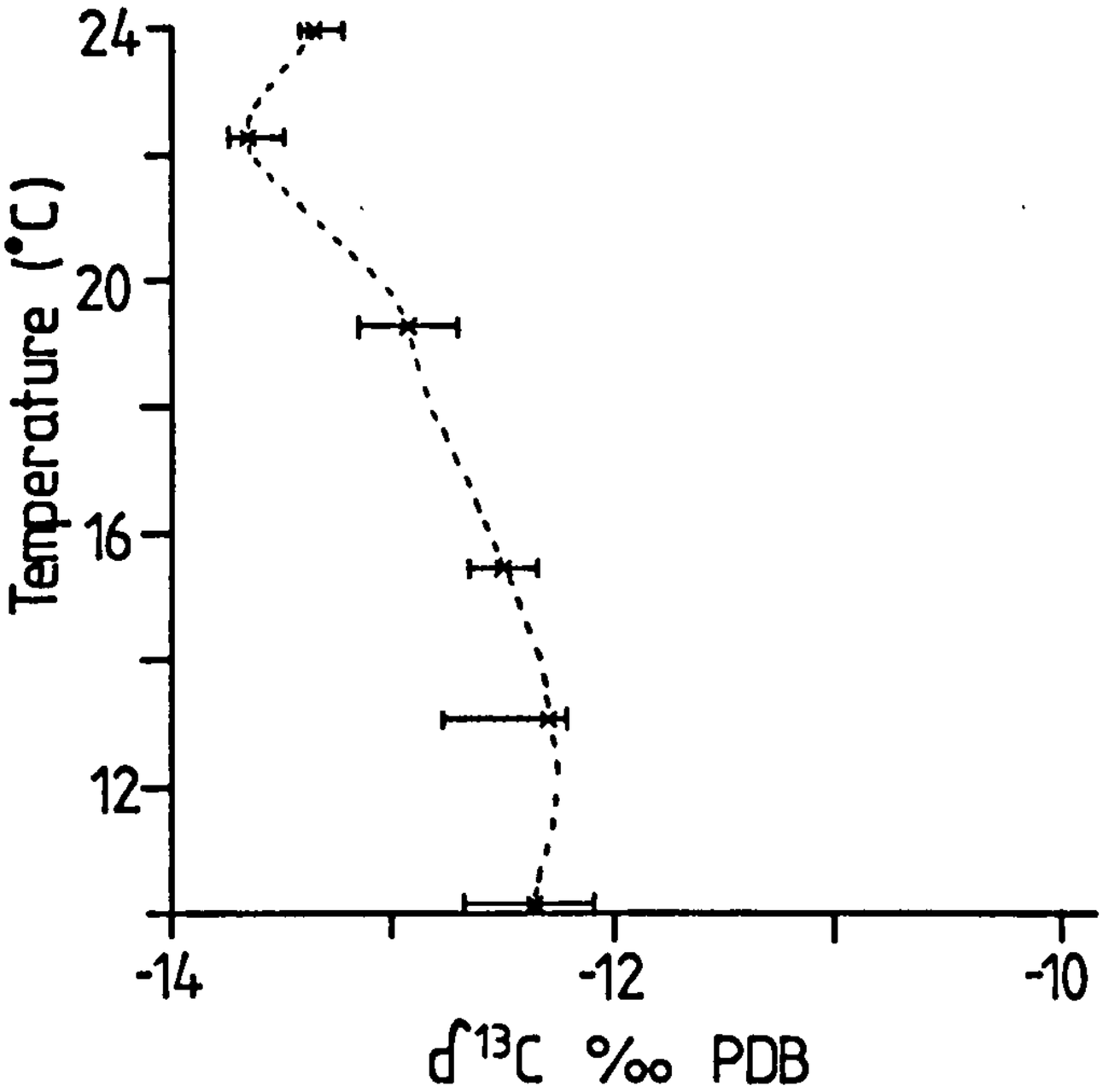


Figure 3.6d Carbon isotope trends with temperature for *Rumina decollata* from Experiment 1.

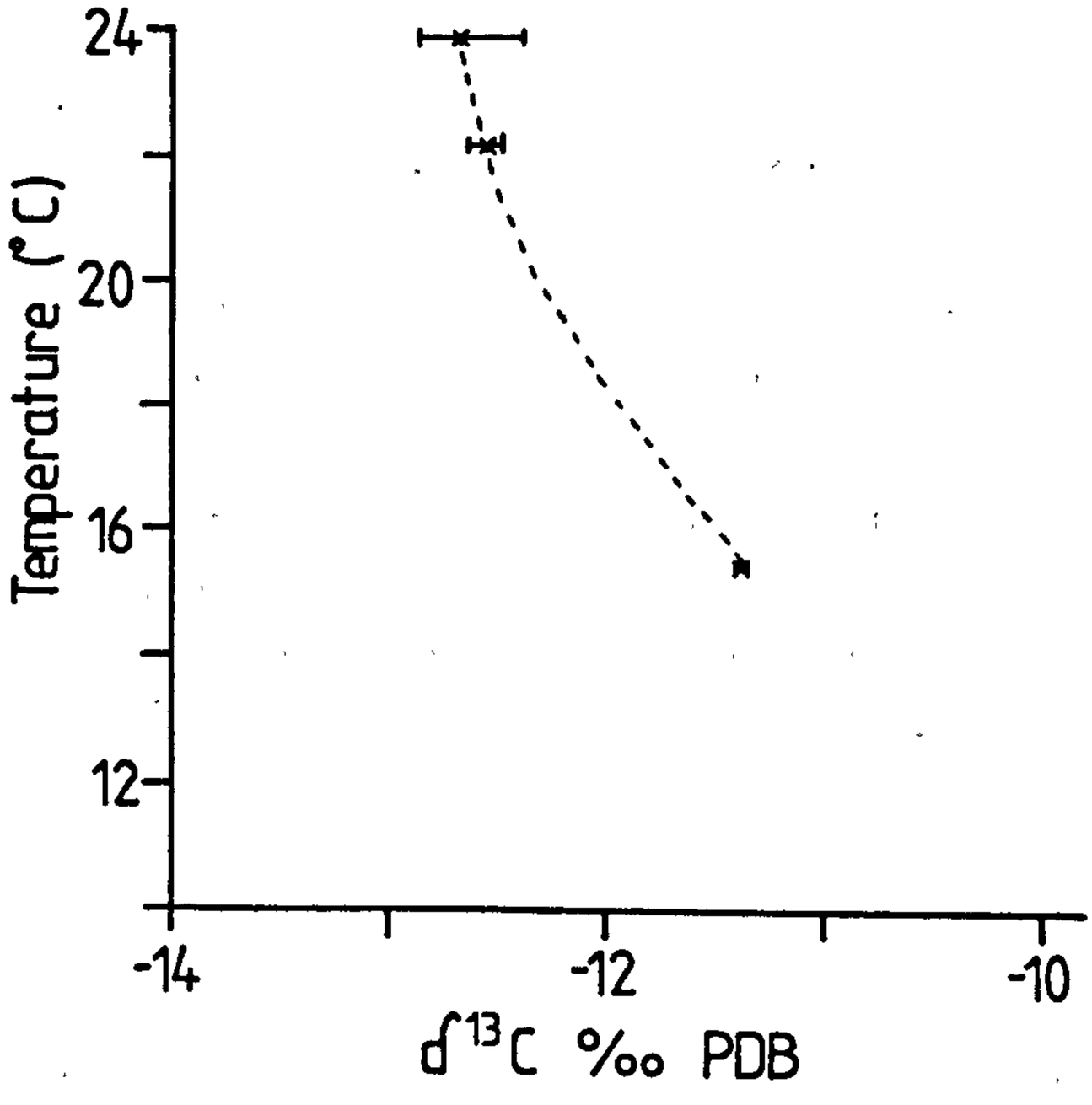


Figure 3.6e Carbon isotope trends with temperature for *Ferussacia folliculus* from Experiment 1.

slightly more depleted up to the C3 (15.7°C) regime (-3.77 to -1.97‰). Above this temperature, the oxygen isotopes move towards more enriched, and generally positive, values. For *Cepaea* this reversal in trend occurs after the C2 (13.1°C) regime, and for *Rumina* does not occur until after the W3 (19.3°C) regime. For all four species the most enriched oxygen isotope data are seen in the W2 (22.3°C) regime, and after this temperature the trend is again reversed with more depleted $\delta^{18}O$ values at W1 (24°C) as compared to those from W2.

Shells of *Ferussacia* were only available from three temperatures C3, W2 and W1 (Figure 3.5e), although the limited data from this snail suggest a trend towards more enriched $\delta^{18}O$ values over these temperatures, which is consistent with the data from the other four species.

(c) Carbon isotopes

Shell carbon isotope values lie between -10.9‰ to -13.63‰. The overall trend is towards more depleted $\delta^{13}C$ values with increasing temperature, with only slight differences between the species, generally at the temperature extremes. As with the oxygen isotopes, plots of the carbon isotope data for the four species *Helix, Cernuella, Cepaea* and *Rumina* (Figures 3.6a-d) all show a similar trend.

Helix and *Cepaea* show more depleted $\delta^{13}C$ values at C1 (10.2°C) than at C2 (13.1°C). However this was not the case for *Cernuella* and *Rumina*. At the other temperature extreme, *Helix* and *Cepaea* show fairly consistent values between W2 and W1, whereas for *Cernuella* and *Rumina* there is a return to more enriched $\delta^{13}C$ values. In other words, for *Helix* and *Cepaea* the overall trend towards ^{13}C depletion declines, but for *Cernuella* and *Rumina* it reverses.

The most depleted $\delta^{13}C$ values are, for all species, recorded in shells from the 22.3°C, W2 regime (-12.56 to -13.63‰; and generally the least depleted data are at C2 (13.1°C), from -11.17 to -12.30‰.

The limited data for *Ferussacia* (Figure 3.6e) also suggest a trend towards more depleted ^{13}C values with increasing temperature, and a reduction in the rate of change as higher temperatures are reached.

The carbon and oxygen isotope data plots appear, at least to some degree to mirror each other although the per mil shifts are not always equivalent. This may be seen particularly if the carbon and oxygen plots of *Helix* and *Cernuella* are compared.

(d) Shell sizes and weights

To indicate the shell size and weights achieved by the snails under the various temperature regimes, a series of test measurements were made.

The snails measured were those that were still available, untreated, after all isotopic analyses had been carried out. Using measurements from these shells, it is not possible to compare directly individual measurements with shell isotopic composition. However, it is still possible to assess relative rates of growth under the six regimes, and to see how this may relate to the observed isotopic data from other shells of the same populations.

Shell diameter and height were measured to 0.1mm using Vernier callipers. The breadth (*i.e* the largest diameter) was measured between the outer edge of the shell aperture and the opposite side of the outer whorl. The maximum height was measures from the shell apex to the base of the aperture. (see Oosterhoff, 1977; and Kerney and Cameron, 1979). *Helix*,*Cepaea* and *Cernuella* were measured

in this way. For the remaining portions of the cylindrical *Rumina* shells, the diameter was measured as the maximum breadth of the last whorl. Measurements were not made of *Ferussacia* . Whole shell weights were measured to 0.1mg on a Sartorius balance. Data are shown in full in Appendix 1.

Mean values of shell breadth, height and weight, and the numbers of shells measured are indicated in Table 3.4. Figure 3.7a is a plot of maximum diameter versus temperature for the four snail species. To assess further the pattern of growth with increasing temperature, Figure 3.7b shows mean weight achieved (plotted on a log scale) against temperature.

It may be seen that Figures 3.7a and 3.7b are similar, despite Figure 3.7b having a logarithmic x-axis. This indicates that generally size and weight increases were proportional, and that even under the artificial laboratory conditions, the snails did not appear to produce thin (and thus light-weight) large shells, particularly over the higher temperatures.

The data suggest an overall trend of increasing size and weight with increasing environmental temperature up to a maximum generally reached at W2 (22.3°C), followed by a slight decline in size. The individual species show some variation from this pattern.

a) *Helix*

This snail achieved a maximum size and weight under the W2 regime, but also appeared to grow well at C2 (13.1°C), where the size and weight reached at this temperature was greater that the next two warmer regimes.

b) *Cepaea*

For this snail, maximum growth occured at W3 19.3°C, although snails from the W1 (24°C) regime also reached a similarly large size. This species did not appear to fair too well at the lower temperatures, but growth was much increased between the C3 (15.7°C) and W3 regimes.

c) *Cernuella*

The trend for this species is very similar to that for *Helix*, with two 'peaks' in growth firstly at C2, and then again at W2 where maximum growth was recorded.

d) *Rumina*

The increase in size with temperature is negligible at the lower experimental temperatures. However, diameter then steadily increases above C2, to reach a maximum at W2.

Although young, recently hatched snails were utilised for all the experimental populations, it was not always possible to begin each phase of the experiment with baby snails of an identical size and age. Discrepancies in the sizes of snails, and the general state of health of individuals, at the onset of each phase may account for some of the tends noted above for the individual species and for the zig-zag nature of the growth curves. For example, as part of the subsequent experiment 2, fifteen juvenile *Helix aspersa*, similar in size to those used in that experiment, were weighed. The fifteen shells ranged in weight from 4.8 to 11.0mg; the mean weight was 8.55mg and the standard deviation was 1.92mg.

(e) Environmental water

Results of oxygen isotope analyses of the water given to the snails during the six phases of experiment 1 , are shown in Table 3.5. Three or four samples were collected and analysed from each experimental phase. The samples were taken at evenly spaced intervals during each experimental period. The water samples originated from the same distilled water source, and the data all lie between -8.3 to -10.1‰ relative to the SMOW standard, with an overall mean value of -9.36‰. However, there

		C1 10.2°C	C2 13.1°C	C3 15.7°C	W3 19.3°C	W2 22.3°C	W1 24°C
Helix	n	9**	5	10	6	2	6
	Ht. mm	8.2	16.0	11.1	13.2	20.1	17.7
	Diam. mm	9.1	16.5	12.5	14.4	23.2	18.7
	Wt. mg	44.7	247.6	80.5	137.8	470.1	356.0
Cepaea	n	9**	4	5	5	6	6
	Ht. mm	6.0	6.7	6.1	14.4	11.3	13.8
	Diam. mm	7.9	8.9	8.0	17.8	14.7	17.4
	Wt. mg	50.5	63.5	42.1	373.6	184.3	300.8
Cernuella	n	6*	5	3	4	3	6
	Ht. mm	3.3	5.2	3.2	3.9	6.7	6.3
	Diam. mm	4.6	7.1	4.6	5.4	8.6	8.4
	Wt. mg	10.1	30.1	10.0	13.0	61.0	43.9
Rumina	n	2*	5	6	6	1	6
	Diam. mm	4.0	4.1	6.3	7.9	9.0	8.2

Table 3.4 Mean shell size and weights achieved during each phase of Experiment 1.

* = mean value after eight weeks growth
** = mean value of six and eight weeks growth.

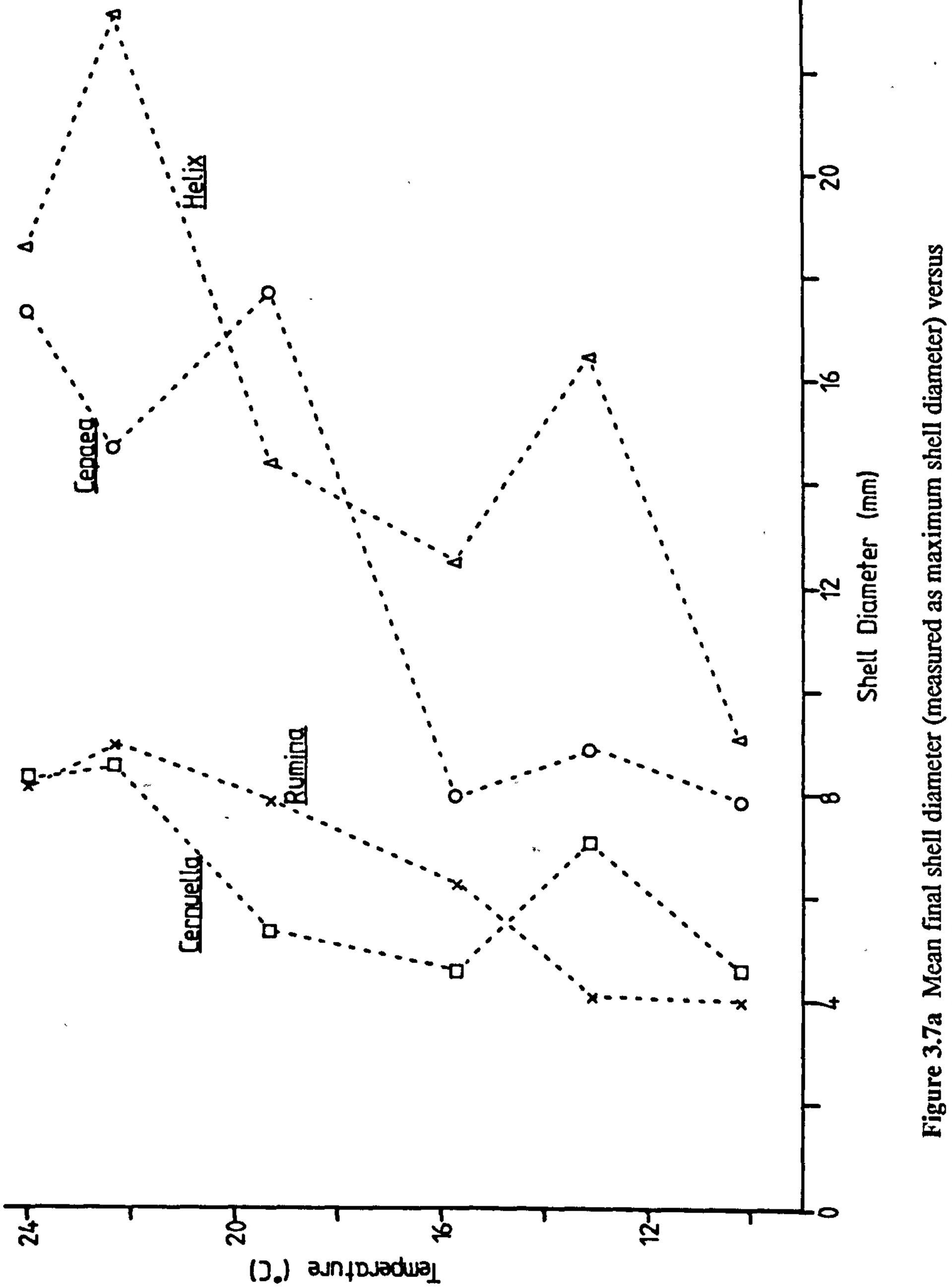


Figure 3.7a Mean final shell diameter (measured as maximum shell diameter) versus temperature for the four main species of snail in Experiment 1 - *Helix aspersa*, *Cepaea nemoralis*, *Cernuella virgata* and *Rumina decollata*.

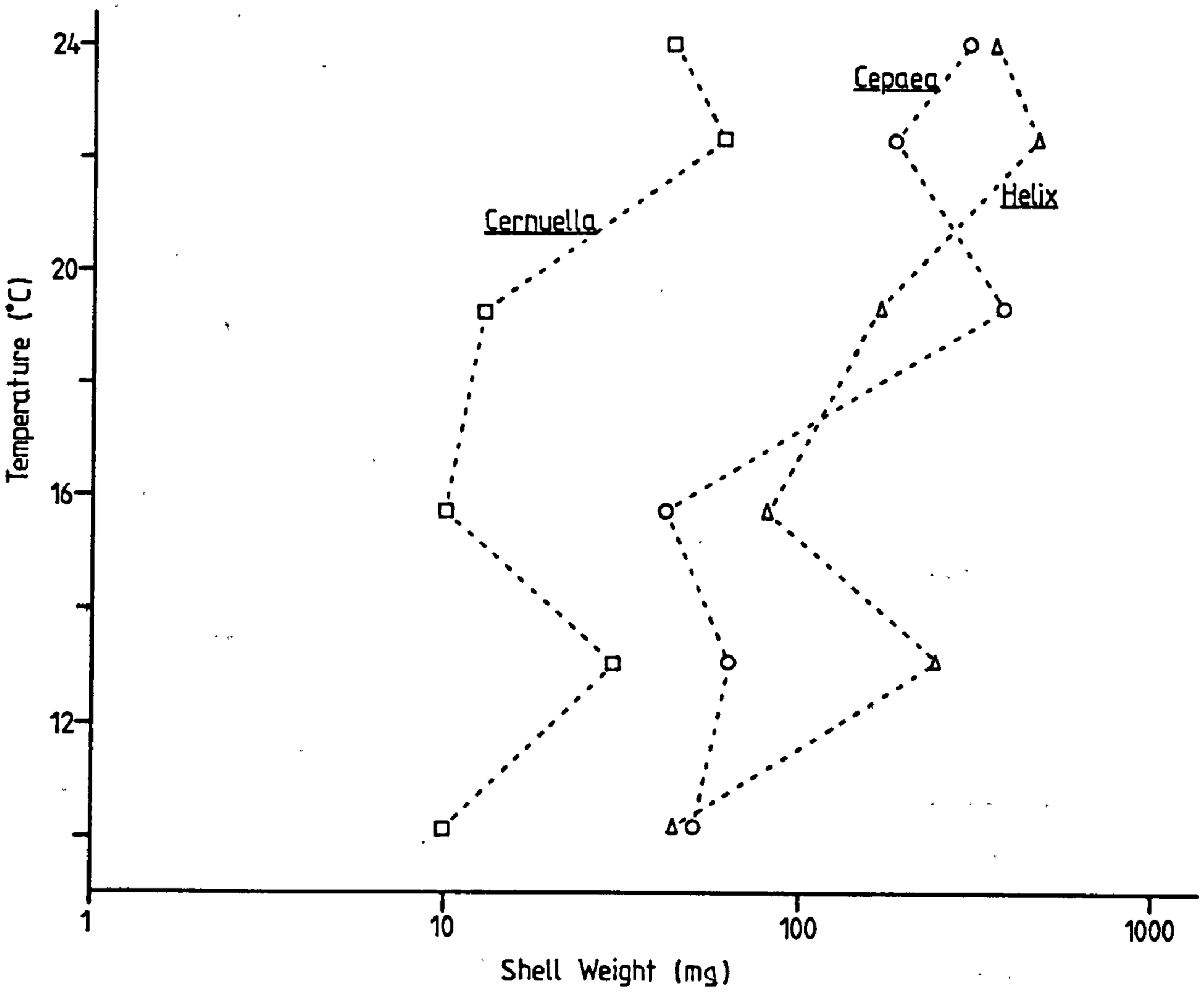


Figure 3.7b Mean final shell weight (logarithmic scale) versus temperature for the four main species of snail in Experiment 1 - *Helix aspersa*, *Cepaea nemoralis*, *Cernuella virgata* and *Rumina decollata*.

Aspirator Location	Temperature °C	$\delta^{18}O$ ‰ SMOW Water	Mean $\delta^{18}O$‰ SMOW Water
'Cool' room	10.2	-9.9 * -9.4 -9.7 -9.9	-9.70
'Cool' room	13.1	-9.3 -10.1 -9.9	-9.77
'Cool' room	15.7	-9.7 -10.1 -9.9 -9.8	-9.88
'Warm' room	19.3	-9.4 -8.8 -8.6 -8.3	-8.78
'Warm' room	22.3	-9.0 * -9.5 -8.9	-9.13
'Warm' room	24.0	-9.6 -8.5 -9.1 -8.5	-8.92

Table 3.5 Oxygen isotopic composition of environmental water during each phase of Experiment 1.

'Cool' room mean $\delta^{18}O$ = -9.78‰ SMOW 'Warm' room mean $\delta^{18}O$ = -8.94‰ SMOW

* = Initial reading - samples from full aspirator one week after filling, during which time aspirator was sealed and covered

SPECIES	TEMPERATURE RANGE	DEPLETION ‰ PER °C
Helix aspersa	10.2 to 15.7	0.11
Cepaea nemoralis	10.2 to 13.1	0.55
Cernuella virgata	10.2 to 15.7	0.18
Rumina decollata	10.2 to 19.3	0.34
	Mean	0.295

Table 3.6 The rate of oxygen isotope depletion with increasing temperature, for the four main species of snail.

is a broad separation between the oxygen isotope data from water stored in the 'warm room' aspirator (mean $\delta^{18}O$ = -8.94‰) and those from the 'cool room' aspirator (mean $\delta^{18}O$ = -9.78‰).

The initial water samples, which were taken form each completely full, sealed aspirator one week after filling also show this difference (W2 sample 1, $\delta^{18}O$ = -9.0‰, and C1 sample 1, $\delta^{18}O$ = -9.9‰). This indicates that the isotopic difference between the two reservoirs is primary, and probably relates to the still from which the water was taken. For example, if the water decanted from the still to fill the first aspirator (that in the 'warm room') had been distilled sometime previously, it may have become slightly enriched in $\delta^{18}O$ through evaporation or isotopic exchange with the atmosphere. However, the water for the 'cool room' aspirator was freshly distilled, immediately decanted from the distilling reservoir and removed to the sealed covered 'cool room' aspirator.

3.4.3 Discussion of results : experiment 1.

(a) Oxygen isotopes of shell aragonite

1) 'Cool' temperatures - 10.2 to 15.7°C

As outlined in section 3.3.2, following the temperature equation proposed by Grossman and Ku (1986) and modified by Hudson and Anderson (1989), with increasing environmental temperatures, the oxygen isotope composition of mollusc shell aragonite should become increasingly depleted with respect to ^{18}O, *i.e* delta values will become more negative by 0.213‰ per°C. Over the lower experimental temperatures, the $\delta^{18}O$ of the snail shells (Figures 3.3a-d) do indeed follow this expected trend.

For the four main species *Helix aspersa*, *Cepaea nemoralis*, *Cernuella virgata* and *Rumina decollata* , the rate of depletion for each °C is shown in Table 3.6. Also indicated in this table is the temperature range over which each depletion rate was calculated. The mean rate of depletion is 0.295‰.°C, which is slightly higher than that predicted by the Grossman and Ku (1986) equation. However, the rates of depletion shown in Table 3.6 range from only 0.11 (*Helix*) up to 0.55 ‰ per°C (*Cepaea*). The very high depletion rate for *Cepaea* reflects the fact that the main isotopic difference was measured between the C1 and C2 regimes - with a temperature difference of only 2.9°C. Rates of depletion measured over a wider temperature range, and between more than two of the set growth temperatures may be more realistic, and using these data would reduce the mean rate of oxygen isotope depletion to a value close to that in Grossman and Ku (1986).

However, over the temperature range of approximately 10-15°C, whilst other factors remained constant, external temperature appears to be reflected in the oxygen isotope signature of the resultant aragonite shell.

2) 15.7 to 22.3°C

Above approximately 15°C, there is a noticeable shift towards enriched shell $\delta^{18}O$ values. This suggests that other factors apart from, or in addition to, temperature must be affecting the isotopic composition of the body fluid from which the snail shell is secreted. Possible sources of oxygen isotope enrichment are from evaporation and metabolic effects (section 3.3.2) - these are discussed below.

Molluscs have a high and very variable water content, with highest values found during the summer in active animals (Machin, 1975). *Theba pisana* from the coastal plain of Israel, investigated by Goodfriend et *al* ., (1989) were about 70% water, measured as percent water content of shells (body plus shell). Total body water content (percent wet weight less shell) of *Cepaea nemoralis* and *Helix pomatia*lie between approximately 80 and 90% (Machin, 1975).

Water is mainly absorbed through the snail's epithelium, although small amounts are thought to be ingested with the food. Water is lost from exposed skin surfaces once the ambient relative humidity falls below the blood equilibrium humidity (about 99.5%) (Machin, 1975). Most molluscan tissue is highly permeable, and water flows by osmosis from one compartment to another when concentration differences exist. The vapour loss from the tissue surface depends upon the vapour pressure gradients adjacent to the exposed surface. The pressure gradients are influenced by external humidity, temperature and air movements. Mucus production, to aid locomotion and to prevent external surfaces drying out, is also thought to play an important role in the transport of water to the skin surface (Machin, 1964, 1966, 1975).

Measurements of snail body fluid $\delta^{18}O$ and δD (deuterium) of *Theba pisana* over a period of several weeks and in relation to rainfall events in the locality (the coastal plain of Israel) were made by Goodfriend *et al*., (1989). Enrichment in $\delta^{18}O$ was recorded in between rain events, but a similar enrichment was not consistently seen in the deuterium isotopes, suggesting that the effect on the oxygen isotopes was not the result of evaporation. However, water loss through locomotion *and* evaporation are documented by Machin (1975), although pedal losses during activity were variable and much greater than losses by evaporation. In active snails then, the majority of water loss is thought to be due to the loss of mucus used in locomotion, rather than by evaporative loss, and the loss of water through mucus is thought to be non- fractionating (G. A. Goodfriend, pers. comm., 1990). ~~As snails in natural environments are only active under very~~ In the case of the experimental snails, the ambient humidity within the boxes was maintained at constantly elevated levels, near saturation. Thus, it is considered that the active snails would have been subject to negligible evaporation, and the water uptake and loss would have been non-fractionating with respect to oxygen isotopes.

Most pulmonate snails are efficient at maintaining internal moisture during inactivity (Machin, 1966, 1972). The experimental snails were not subject to shifts in conditions that might have stimulated inactivity, and although no actual observations were recorded, the regular feeding and watering meant that the experimental snails were not inactive for sustained periods. Therefore this potential source of evaporative enrichment of oxygen isotopes would, in the case of the experimental snails, have been insignificant .

Another source of evaporation would have been from the water given to the snails before uptake. The water was administered by a plant spray-mister, and the action of spraying may have encouraged some evaporation from the fine mist prior to settling, or from the box and soil surfaces. The design of the experiment meant that the boxes in which the snails were housed were not completely sealed, as the lids were partially constructed of fine netting (see section 2.4.2). Such boxes were chosen as they were identical to those housing the base populations and seemed to suit the laboratory populations of the species involved. It was hoped that water loss from the boxes would have been limited, but as extra water was added throughout most of the six week growth periods, some evaporative loss may have occurred. Thus, evaporation of the water would have been possible before the snails were able to absorb the water given to them at each feeding. It is felt that such evaporation

effects may have been concentrated upon the test populations maintained within the 'warm room' (*i.e* those with the "w" prefix). This is because in the 'warm room' temperatures were maintained by a thermostatically controlled fan heater, which when active would have facilitated the movement of warm air currents within the room and so would have encouraged greater amounts of evaporation from the snail boxes. It is also possible that evaporation effects influenced the shell oxygen isotope compositions at the lower experimental temperatures, although in the 'cool room' the air temperatures were maintained by a cooling system, so the effects of evaporation may have been considerably less than those in the 'warm room'. Hence, the 'cool room' regimes do still show the expected oxygen isotope trend with temperature although the depletion rates seen could have been partially offset by a limited evaporation effect.

It has been shown that evaporation effects would primarily be those acting upon the environmental water prior to uptake by the snails and these would be more prevalent within the 'warm room' test populations. This may at least partially explain the shift towards more enriched shell $\delta^{18}O$ values in the snails from the warmer temperature regimes..

Evaporation would be expected to increase with increasing temperature and up to the W2 (22.3°C) regime, further ^{18}O enrichment in the shells is recorded. However, isotope fractionation or the rate of enrichment decreases with increasing temperature, as the heavier isotope becomes less resistant to being expelled from the system (relative to the light isotope) as more energy in the form of heat is added.

The oxygen isotope composition of snail body fluid may also be influenced by metabolic effects.

For the experimental snails' diet, the $\delta^{18}O$ of the milk powder and the oat cereal are not known. However, $\delta^{18}O$ GPR1 = -5.02‰ PDB = +25.69‰ SMOW. Longinelli (1984), and Luz *et al.* (1984), have shown that for mammals, with a constant body temperature, metabolic activity enriches body water in ^{18}O relative to environmental (drinking) water, by several per mil. For terrestrial molluscs, metabolic activity has been used to explain oxygen isotope enrichment in snail body waters as compared to meteoric of environmental waters (Goodfriend and Magaritz, 1987; Goodfriend *et al* ., 1989). The degree of metabolic enrichment has been related to the activity level of the snails. The body waters of inactive snails, with no direct input of environmental water, would become isotopically enriched as a result of metabolic activity. In active snails water uptake resumes and metabolic enrichment would decline (Goodfriend and Magaritz, 1987).

Following this line of reasoning, continuing isotopic enrichment (from metabolic effects) with increasing environmental temperature, should be linked to periods of cessation in activity of the experimental snails at the higher temperatures. Unfortunately, no actual observations of activity patterns were recorded. However, Cameron (1970), has shown that over daylight hours, for three species of helicid snail (*Cepaea nemoralis*, *Cepaea hortensis* and *Arianta arbustorum*) , maximum activity levels were recorded at 8°C (over a temperature range from 0-25°C). No readings were taken over the temperature range 8-17°C, although after 8°C there appeared to be a slight decline in the daylight activity pattern of the snails. The decline was most noticeable in *Arianta arbustorum.* Although the night-time activity patterns he recorded were slightly different, the experimental snails studied here were kept in continually illuminated conditions, so it is probably more realistic to refer to daylight activity patterns. Even considering the total activity patterns (day + night) of the three species, the levels of activity decline above 17°C under conditions of virtually 100% humidity. The

findings of Cameron suggest that above the 'C' temperatures, the experimental snails were perhaps active only periodically, allowing greater metabolic enrichment of their body fluids.

However, from Table 3.4 and Figures 3.7a and 3.7b, greater growth was achieved over the higher experimental temperatures. Could larger snails be produced by periodic as compared to constant activity?, and how is the metabolism of snails affected by increasing temperature?

To test the effects of population density on activity and growth in three species of helicid snail *Cepaea nemoralis*, *Cepaea hortensis* and *Helix aspersa*; Cameron and Carter (1979) set up a series of experiments. They showed that growth rate and activity of juveniles were inversely related to density in their test boxes. Their results also indicate that increasing activity leads to an increase in amount of shell secreted over the set experimental growth period. The most active snails put down more shell per active period than snails that were not growing so freely due to density stress. In other words, the snails under stress needed more active occasions to put down the same amount of shell as the snails growing more favourably. This suggests that snails living under favourable conditions might not require to be active for such long periods to be able to assimilate sufficient metabolites (to produce a large strong shell) as smaller snails not living under such favourable conditions, and therefore may become more isotopically enriched.

Metabolism has to occur to provide the building blocks for shell deposition. The products of metabolism include water and carbon dioxide (these may also enter the snail by diffusion and/or direct uptake), and with the aid of the enzyme carbonic anhydrase they produce the bicarbonate necessary for shell secretion (Wilbur and Saleuddin, 1983). Therefore, a snail placed in favourable conditions, with plentiful food and water sources might ingest a capacity load of metabolites fairly rapidly and then become inactive so as to minimise water loss. Metabolism would then occur in the inactive snail and the body water would become isotopically enriched in ^{18}O because throughput of water declined, as outlined by Goodfriend and Magaritz, (1987). Increasing environmental temperatures would encourage metabolism and internal respiration, as well as external respiration through the pneumostome into the snail's body cavity, as shown by Richardson, (1975); Riddle (1977); Barnhardt and Armitage, (1979) and Armitage and Stinson, (1980).

These authors measure respiration as the volume of oxygen consumed, generally as µl per unit tissue weight per unit time, expressed as VO_2 or QO_2.

Richardson (1975), reported that for juvenile *Cepaea nemoralis* respiration increases with temperature, but the rate declines above 30°C (although from the figure of the data the rate of increase appears to decline below this temperature, perhaps nearer 20-25°C). Investigating *Helix aspersa*, Riddle (1977) found that strong regulation in oxygen consumption was apparent above 25°C, above which temperature oxygen consumption declined. Barnhardt and Armitage (1979), investigated the oxygen consumption of the polygyrid *Stenotrema leai* as a function of temperature and season. For this snail, increasing oxygen consumption was seen up to 35°C, although the rate of increase declined at higher temperatures. The rate of increase of a process with temperature is known as the temperature coefficient Q10, which is the increase in the rate of a process, expressed as a multiple of the initial rate, by increasing the temperature by 10°C. Armitage and Stinson (1980), working with *Stenotrema leai*, found that Q10 values declined from approximately 2.5 - 4 down to approximately 1.5 with increasing environmental temperature above 15-20°C, although the exact patterns presented varied with the acclimatisation temperature (the temperature at which the snails had been kept for 21 days before being placed in the respirometers to assess oxygen uptake).

From these findings it would appear that the increasing environmental temperatures of experiment 1. should be encouraging the snails to respire and thus to metabolise their dietary intake, even if activity becomes more periodic.

Therefore, the movement towards more enriched $\delta^{18}O$ values from C3 to the W2 regimes (15.7 to 22.3°C) reflects an increase in evaporation of the environmental water prior to uptake by the snails, and also probably a rise in the metabolic effect as a result of the increasing temperatures.

3) Above 22.3°C

Above the W2 (22.3°C) regime, the four main species all show a shift towards more depleted $\delta^{18}O$ values at the W1 (24°C) regime. This might indicate that the environmental temperature was then high enough to have a limiting effect upon the snails' respiration and metabolism whatever the activity patterns of the experimental snails. The high environmental temperature may also have caused an increase in fluid processing by the snail with little chance for metabolic enrichment to influence the isotopic composition of the body waters. With a constant ambient temperature of 24°C (much above that experienced by snails in their natural environment) it seems likely that the snails would be subject to some degree of biological stress. The return to more depleted values might also indicate a decline in the relative importance of combined evaporation and metabolic isotope effects and a return to the temperature dominated isotope effects thought to be occuring at the lower experimental temperatures. Additionally, the shift could be a combination of one or more of the three scenarios noted above, and the extreme temperatures appear to have influenced all the test species.

4) Variation between species

The most enriched ^{18}O data, for all species, occur at the W2 (22.3°C) regime. *Helix aspersa*, *Cepaea nemoralis* and *Cernuella virgata* (the northern temperate species) all show mean W2 $\delta^{18}O$ values close to +4‰; whereas *Rumina decollata* and *Ferussacia folliculus* (the two Mediterranean species) have W2 $\delta^{18}O$ values of +0.78 and -0.44‰ respectively.

This separation between the temperate and Mediterranean species may indicate that the evaporative or metabolic effects influence the temperate N.W. European species to a greater degree than the Mediterranean species, which may be physiologically and genetically better suited to continuous high temperatures.

An average N.W. European snail is probably only subjected to temperatures as high as 22-24°C on relatively few days each year, and under such conditions, unless it was also very damp, the snails probably remain inactive. For 1970 and 1971, Richardson (1975), recorded mean monthly temperatures (at grass surface) for a sand dune system in N. Devon. The warmest months were June, July and August, which saw mean monthly temperatures from 15.8 to 20.9°C. In a natural system, the temperature would be subject to diurnal rhythms, whereas for experiment 1., temperatures were continuous. Thus, the snails' diurnal rhythms might have been disturbed, which could have influenced the degree of isotopic enrichment seen.

(b) Oxygen isotopic composition of environmental water

1) The question of equilibrium aragonite precipitation

The oxygen isotopic compositions of the shells of the experimental snails have been shown to reflect the environmental temperature, but also to be influenced by evaporation and metabolic effects. However, the isotopic composition of the water given to the snails must also be considered, as this is another potential source of variation. The $\delta^{18}O$ values of the waters given to the snails were fairly consistent (overall mean = -9.36‰ see section 3.4.2). Therefore, it would appear that the trends in shell oxygen isotopes discussed above can not be explained simply in terms of a primary difference in the environmental water.

Instead, these water isotope data may be used to indicate, for each experimental temperature, the values of shell aragonite that would be produced if shell deposition took place at or near isotopic equilibrium. Equilibrium shell deposition has been suggested for freshwater molluscs (Stuiver 1970, Fritz and Poplawski, 1974), and has been assumed by Abel (1985).

Using the temperature equations presented in section 3.3.2, and the mean water $\delta^{18}O$ SMOW values shown in Table 3.5; values have been calculated for aragonite precipitated at isotopic equilibrium for each of the six temperature regimes. These results are shown in Table 3.7. Also indicated, for each species at each temperature, is the difference (Δ) between the 'expected' equilibrium values and the mean oxygen isotope values actually measured in the shells. Figure 3.8 is a plot of the 'expected' and measured $\delta^{18}O$ shell aragonite values, with temperature.

From Figure 3.8 and Table 3.7, it can be seen that the measured $\delta^{18}O$ values are always more enriched in ^{18}O than equilibrium values by at least 4.5‰, up to a maximum of 13.76‰. The largest differences (Δ) occur in the data from the W2 (22.3°C) regime, which also showed the most enriched $\delta^{18}O$ aragonite values as outlined in sections 3.4.2 and above. There is a slight increase in the $\overset{\Delta}{\lambda}$ values with increasing temperature, which reaches a peak in the data from the W2 regime and is then followed by a slight decline to the data from W1 (24°C). These $\overset{\Delta}{\lambda}$ values indicate that the aragonite shell is far from isotopic equilibrium with the environmental water given to the snails and this suggests that the oxygen isotopes have been fractionated quite significantly in the process of becoming incorporated into the secreted shells.

As described for the ^{18}O enrichment trend in the shells themselves (section above), the $\overset{\Delta}{\lambda}$ value must be a compound factor relating to evaporation processes outside the snails' bodies, and vital effects linked to gaseous exchange, metabolism, activity levels and possibly the mechanism of shell construction.

2) Comparison with previous findings

Biological fractionation, leading to ^{18}O enrichment relative to equilibrium aragonite precipitation, has also been reported by other researchers investigating isotopes in land snail shells (a limited field).

Lecolle (1983), investigated a variety of land snails from N.W. Europe from oceanic, Alpine and Mediterranean climates. He reported enrichment in ^{18}O in the snail shells (averaging 5.2‰) relative to that calculated from isotopic equilibrium of aragonite with rainwater, at the mean annual temperature at each locality studied. As this difference was constant with changing temperature,

TEMP. ℃	SNAIL SPECIES	MEAN $\delta^{18}O$‰ SMOW WATER MEAS.	MEAN $\delta^{18}O$‰ PDB ARAG. MEAS.	EQM. $\delta^{18}O$‰ PDB ARAG. CALC.	Δ EQM - $\delta^{18}O$‰ PDB ARAG. MEAS. - CALC.
10.2	*Helix*	-9.70	-2.10	-7.45	5.35
	Cepaea	"	-1.46	"	5.99
	Cernuella	"	-2.76	"	4.69
	Rumina	"	-0.70	"	6.75
		Mean	-1.76		5.69
13.1	*Helix*	-9.77	-2.75	-8.13	5.38
	Cepaea	"	-3.06	"	5.07
	Cernuella	"	-3.49	"	4.64
	Rumina	"	-3.63	"	4.50
		Mean	-3.23		4.90
15.7	*Helix*	-9.88	-2.72	-8.80	6.08
	Cepaea	"	-1.97	"	6.83
	Cernuella	"	-3.99	"	5.03
	Rumina	"	-3.71	"	5.09
	Ferussacia	"	-4.01	"	4.79
		Mean	-3.24		5.56
19.3	*Helix*	-8.78	-0.98	-8.47	7.49
	Cepaea	"	-1.23	"	7.24
	Cernuella	"	-2.68	"	5.79
	Rumina	"	-3.79	"	4.68
		Mean	-2.17		6.30
22.3	*Helix*	-9.13	3.88	-9.46	13.34
	Cepaea	"	4.40	"	13.86
	Cernualla	"	3.12	"	12.58
	Rumina	"	0.78	"	10.24
	Ferussacia	"	-0.44	"	9.02
		Mean	2.35		11.81
24.0	*Helix*	-8.92	0.84	-9.61	10.45
	Cepaea	"	2.50	"	12.11
	Cernuella	"	-1.46	"	8.15
	Rumina	"	-0.86	"	8.75
	Ferussacia	"	0.56	"	10.17
		Mean	0.32		9.93

Table 3.7 Calculated oxygen isotope equilibrium values for oxygen isotope values : Experiment 1. Equilibrium values calculated using equation 3.1 (section 3.3.2) (Grossman and Ku, 1986). Differences between actual measured $\delta^{18}O$ shell values and calculated equilibrium values are shown as Δvalues.

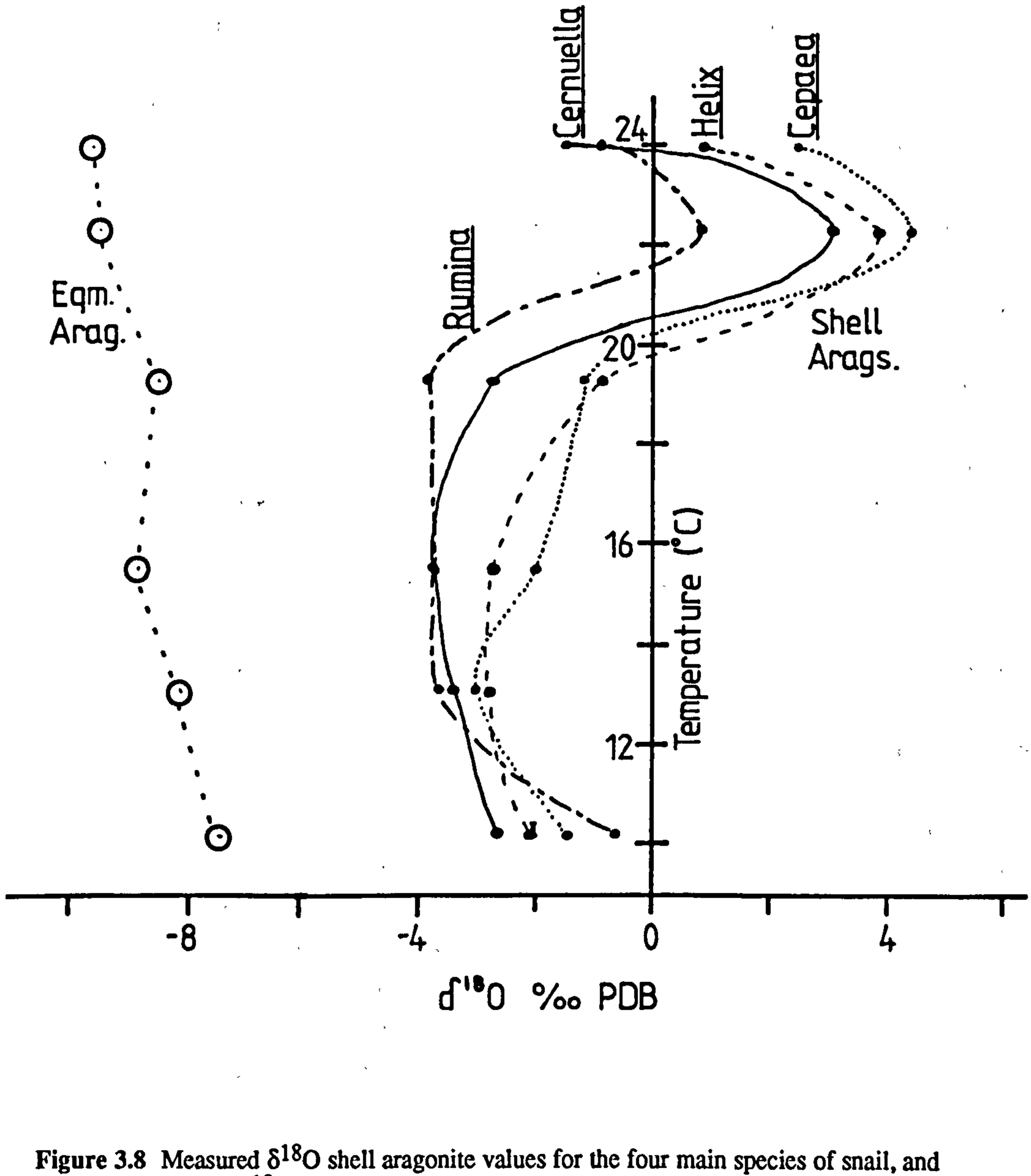


Figure 3.8 Measured $\delta^{18}O$ shell aragonite values for the four main species of snail, and 'expected' aragonite $\delta^{18}O$ values at isotopic equilibrium with the water given to the snails, under the various thermal regimes of Experiment 1.

Lecolle considered that the enrichment was related to the metabolism of the snails, and that for the examples studied the maximal enrichment caused by evaporation was 1‰.

In land snails sampled from the southern Levant, Israel (*Trochoidea seetzeni*, *Xeropicta* spp.and Sphincterochila spp.), Goodfriend and Magaritz (1987) found that shell $\delta^{18}O$ values were enriched by 4 to 8‰ relative to isotopic equilibrium with environmental waters (local precipitation). The authors also reported that the snails were enriched in ^{18}O by 2 - 4‰ relative to equilibrium with water vapour, if their source of water was predominantly from condensation (*i.e* dew). In the case of the experimental snails investigated here, it is felt that most of the water required by the snails would be obtained by direct uptake through the integument, or ingested with the food. Condensation was not seen upon the insides of the boxes.

Goodfriend *et al* .(1989), actually measured the isotopic composition of snail body fluids, in addition to shell material secreted over a corresponding known period. The snail investigated was *Theba pisana* from Rehovot in the southern coastal plain of Israel. The authors reported that snail body water showed a minor enrichment (up to 2‰) in $\delta^{18}O$ relative to equilibrium with water vapour (or dew), and that the enrichment became more pronounced after periods of inactivity. On average, the body water $\delta^{18}O$ values were also enriched by about 2‰ relative to rain water $\delta^{18}O$ values, as water vapour in the Mediterranean coastal plain of Israel is only slightly out of equilibrium with rainfall. The authors also reported that shell carbonate was enriched in $\delta^{18}O$ by 1 to 2‰ relative to equilibrium with body fluid. This suggests that there may be another biological fractionation factor involved in shell secretion affecting the shell $\delta^{18}O$ values, which is independent of the evaporation or metabolic effects.

The amount of enrichment (for the experimental snails as compared to equilibrium values) at the lower 'C' temperatures (10.3 to 15.7°C), is approximately 5‰. This is in agreement with, or slightly higher than the findings of the workers noted above. Above 15.7°C, the isotopic effects related to evaporation and vital effects, become much more pronounced, and much greater than reported elsewhere being from around 7 to 13‰. However, at these temperatures (19-24°C), the snails continued to grow well and were therefore consuming food and active for at least some of the time. Therefore, any stress felt by the snails due to the continual high ambient temperatures, might have been sufficient to disturb their metabolism somewhat, but was insufficient to disrupt snail growth. The continual high temperatures may also have acted to increase evaporation of water, particularly prior to uptake by the snail. The amount of enrichment may reflect (particularly for those shells enriched by more that 5‰ over equilibrium values) an increased evaporation effect, and the degree of 'metabolic stress' endured by the snails.

3) Variation between species

Helix and *Cepaea* generally show the most ^{18}O enrichment, relative to equilibrium with their environmental water, followed by *Cernuella* and lastly *Rumina*. Data for *Ferussacia* , where available, were generally enriched in ^{18}O even less than *Rumina*. This may indicate that the Mediterranean species *Rumina* and *Ferussacia* are less susceptible to 'metabolic stress' especially at the higher experimental temperatures, than the helicids that originate from a more temperate climate.

At the lowest temperature (10.2°C), *Rumina* is the species most enriched relative to equilibrium. This may reflect the fact that at this temperature the *Rumina* snails grew very little, and

may have been subject to metabolic enrichment related to inactivity (Goodfriend and Magaritz, 1987), as compared to the other snail species.

(c) Carbon isotopes of shell aragonite

1) Trends with temperature

For all five species the overall trend is towards more depleted $\delta^{13}C$ values at higher temperatures, with the most depleted values recorded in the shells from the W2 regime, 22.3°C (-12.56 to -13.63‰) and the least depleted values in the C2 13.1°C regime (-11.17 to -12.30‰).

This trend is that expected from the temperature dependence of the carbon isotope fractionations probably involved in the processing and shell secretion (section 3.3.3). However, the limited temperature dependencies of these reactions would not by themselves explain the degree of ^{13}C depletion with temperature seen in the data from the experimental snails. Therefore the isotopic shifts recorded in the data are likely to signify fluctuations in the relative importance of each source of carbon to the extrapallial fluid, with those causing greater depletion in ^{13}C taking prominence with increasing temperature. As the carbon isotope composition of the inorganic carbon available to the snails (GPR1) was constant (-13.09‰ PDB) and this carbon was available in excess, the isotopic shifts must represent changes in the amounts of metabolic or atmospheric carbon dioxide being utilised by the snails under the different temperature regimes (section 3.3.3).

2) Metabolic versus atmospheric carbon dioxide

The trend towards more depleted $\delta^{13}C$ shell values with increasing temperature, indicates that metabolic carbon dioxide production was becoming increasingly important as compared to atmospheric carbon dioxide uptake.

Goodfriend and Magaritz (1987), have linked a ^{13}C depletion in body fluid bicarbonate to an increase in rate of activity of the snails when, they suggest, metabolism of the snails will be increased. During periods of inactivity, atmospheric carbon dioxide exchange will become relatively more important and aqueous bicarbonate $\delta^{13}C$ values will become more enriched. This is in turn reflected in the shell $\delta^{13}C$ values. Herreid (1977), has shown that metabolism of inactive snails is greatly reduced as compared to active snails. From this point, Goodfriend and Magaritz (1987), were able to link the periods of greater ^{13}C shell depletion from greater activity to increased availability of moisture in the snails' local environment.

Therefore, in the experimental snails, the trend towards more depleted ^{13}C values with increasing temperature may be the product of greater activity, production of metabolic carbon dioxide and overall shell growth. As the largest snails were obtained from the W2 regime, this argument might also account for the shift towards slightly more enriched ^{13}C values in the W1 snails. However, the depleted ^{13}C values recorded in the snails from the coldest environmental temperature for *Helix* and *Cepaea* do not fit in with this line of reasoning.

Referring back to the discussions of oxygen isotopes with temperature (section 3.4.3a), ^{18}O enrichment with increasing temperature was explained partially in terms of increasingly periodic activity leading to metabolic enrichment of body waters. However, the carbon isotope data appear to contradict this and suggest increasing or perhaps continual activity with increasing temperature.

Herreid (1977), carried out a series of experiments measuring the relative metabolism of dormant, aroused, and aroused and active *Otala lactea*. Metabolism was measured as volume of oxygen used per gram weight per hour, and the active snails consumed the most oxygen. However, none of the experiments allowed for the snails to consume any food, so presumably the digestive tracts of the freshly aroused snails were empty. In the experimental snails studied here, food was always in plentiful supply, and during activity, the snails would probably consume sufficient food to induce continuous tissue metabolism for sometime after locomotor activity had ceased. Thus, a resting snail with a full digestive tract should require more oxygen for tissue metabolism than an inactive snail that had not recently eaten food. Therefore, although the experimental temperatures may have been sufficiently high to reduce snail activity (Cameron, 1970) so as to prevent moisture loss, sufficient food would have been consumed during the active phase and ambient temperatures would have been warm enough to allow high rates of tissue metabolism. This would allow metabolic carbon dioxide to enter the snail bicarbonate pool, leading to ^{13}C depletion on the shells. This argument implies that increasing tissue metabolism may not always be a consequence of an increase in locomotor activity, and the two factors may be independent of each other.

Alternatively, if the ^{13}C depletion with increasing temperature does truly reflect increased activity, then the trend seen in the oxygen isotope data would primarily be the result of evaporative isotope effects, with limited influence from metabolic effects.

3) Variation between species.

The most ^{13}C depleted data, for all snail species, occur close to the W2 regime temperature (22.3°C), with $\delta^{13}C$ values around -13‰, indicating greatest tissue metabolism at this temperature.

^{13}C is more enriched in *Cernuella* and *Ferussacia* as compared to the other species. For example, over the 'C' temperatures *Cernuella* ^{13}C is -11‰ whereas *Helix, Cepaea* and *Rumina* have $\delta^{13}C$ values close to -12.5‰. With increasing temperatures, *Cernuella* ^{13}C are still approximately 0.5‰ more enriched in ^{13}C than *Rumina* shells, but are similar to *Helix* and *Cepaea* .

This consistent difference between *Cernuella* and the other species, over the lower experimental temperatures, may indicate that *Cernuella* were producing less metabolic carbon dioxide and were exchanging more carbon dioxide with the atmosphere than the other species. Perhaps this species was more sensitive to the artificial conditions it was being grown under and as a result underwent physiological effects apparent only in this species. However, any species specific vital effect was not substantiated by the oxygen isotope data for this species.

3.4.4 Summary of findings and development of Experiments 2 and 3

1) The predicted trend of ^{18}O depletion with increasing temperature was only evident at the lower experimental temperatures (below 15°C). Above this temperature other factors counteracted any temperature effect and oxygen isotope ratios became more enriched in ^{18}O. The factors responsible for the shift towards more enriched values are thought to be a combination of evaporation and metabolic effects, possibly relating to the activity patterns (and shell growth rates) of the snails.

2) The oxygen isotopic compositions of the shells secreted by the snails were not at, or even close to, isotopic equilibrium with the waters given to the snails. The shells were consistently more enriched than "expected" equilibrium values, by at least 4.5‰up to a maximum of almost 13.8‰. The differences between actual and equilibrium values were greatest in the 'warm room' regimes (above 15.5°C) and especially in the W2 (22.3°C) regime. These differences would be caused by an evaporation effect on the external waters prior to uptake by the snails and/or a metabolic effect.

3) For the carbon isotopes, a trend towards more depleted $\delta^{13}C$ values with increasing temperatures was found, which is thought to reflect an increasing contribution of metabolic carbon dioxide to the snails' body fluids, with a decline in the proportion of atmospheric carbon dioxide being utilised. This effect could be linked to increased activity at the higher experimental temperatures.

4) The trends in isotopic signatures were fairly consistent between the five species used in the experiments. However, the Mediterranean species *Rumina decollata* and *Ferussacia folliculus* have somewhat different $\delta^{18}O$ values than the temperate species. Also, the carbon isotope values of *Cernuella virgata* are somewhat different from the other species.

The general consistency of the isotopic trends obtained for each species may reflect the artificial experimental conditions. In carrying out further investigations, it was decided to attempt to modify the experimental design whilst still maintaining controlled conditions. This was achieved in experiments 2 and 3 by introducing a daily cycle of temperature and/or lighting.

The temperature regimes of experiment 1 were also unreal, being constant and, in the case of the 'warm room' regimes, much higher than in a natural system. Under such conditions, the snails might have been put under physiological stress, which may have further interrupted their natural patterns of activity and growth. Therefore, it was decided to grow populations of snails at one temperature for a time and then change that temperature to assess the affect on the isotopic composition of the shells. This investigation (experiment 1B) was carried out in conjunction with experiment 1. Isotopic analyses were performed on several samples from within individual shells.

The oxygen isotopic compositions of the snails' shells have been influenced by evaporation and/or metabolic effects. To test the relative importance of the two effects, it was decided to try and create experimental conditions where evaporation effects could be further minimised, whilst control populations would be maintained in the same way as for experiment 1. This was carried out in experiment 3.

It has been suggested that there may be some link between shell isotopic composition and shell size, related to metabolic activity and rate of shell growth. Therefore, in experiments 2 and 3, it was decided to weigh and measure individual shells prior to preparation for isotopic analysis to test for a relationship.

3.5 EXPERIMENT 1B : COMPARISON OF THE ISOTOPIC COMPOSITION OF SHELLS GROWN OVER TWO SEQUENTIAL TEMPERATURES

3.5.1 Introduction and aims

The aim of this investigation was to trace the changes in the isotopic composition of individual shells that resulted from altering the environmental temperature, whilst other environmental factors were maintained constant.

3.5.2 Methods

This investigation coincided with experiment 1, and thus has been termed experiment 1B. Snails were grown initially at W1 (24°C) and then maintained for a further period at W3 (19.3°C).

After the standard six week growth period at W1, one box of each of the species *Helix aspersa, Cepaea nemoralis, Cernuella virgata* and *Rumina decollata* was maintained at that temperature for a further three week period, until base population snails became available to initiate the W3 regime. Therefore, the snails had been kept at 24°C for a total of nine weeks before the room temperature was lowered to 19.3°C. To balance out this time period, the snails were continued at 19.3°C for a further eight weeks before being killed, and chosen individuals prepared for stable isotopic analysis. By this time the experimental snails had reached an almost mature or fully mature state, with interior thickening of the lip in the large helicids (white in colour for *Helix*, brown in *Cepaea*). *Rumina* shells also showed the beginnings of modified apertures and *Cernuella* reached a size equivalent to the largest individuals in the base populations (up to 10mm x 15mm, although this species does not modify its aperture, and the size of mature individuals is very variable).

It was expected that the change in conditions, moving from W1 to W3, would result in a clear break in shell deposition. However, in practice, it was difficult to discern where the actual change came, and so, to compare isotopic data. In retrospect, marking of the leading shell edge after the time at W1 would have been a helpful addition in considering the results of the stable isotopic analyses of these particular shells.

Two individual shells each of *Helix, Cepaea* and *Rumina*, and one *Cernuella* shell were selected for isotopic analysis. A number of sub-samples were taken from each shell. For *Helix* and *Cepaea*, a micro-drill was used to make either single, or a series of, holes along growth lines parallel with the aperture, at approximately 10mm intervals through the body whorl. The earlier whorls were drilled between the sutures. This gave five or six sub-samples per shell. For *Rumina*, complete or half whorls were broken off in succession, to give five to seven sub-samples per shell. For *Cernuella*, slices of whorls were taken giving five sub-samples of the shell.

3.5.3 Results : Experiment 1B

Brief notes describing each shell; its dimensions (height x breadth) and measured or estimated distances behind the aperture for each sub-sample, are shown in Table 3.8a. This table also includes the carbon and oxygen isotope values for each of the samples.

The oxygen isotope data for *Helix* and *Cepaea* are all positive, ranging from +0.06 to +3.81‰. However the pattern within each shell is not the same. For the two shells of *Rumina*, the oxygen isotope values are negative and range from -2.15 to -0.56‰. In both shells there is a trend towards more enriched $\delta^{18}O$ values through time. The single shell of *Cernuella* investigated in this way has $\delta^{18}O$ values from -4.48 to +0.10‰ with more enriched $\delta^{18}O$ values towards the aperture as compared to the earliest whorls.

The carbon isotope data are similar for all species (ranging from -13.67 to -12.4‰) and there are no consistent trends in individual shells.

Table 3.8b compares the isotope data from this experiment with those from experiment 1. The table shows mean carbon and oxygen isotope data, for each species, from the 24°C (W1) and 19°C (W3) regimes of experiment 1, compared with mean whole-shell data from experiment 1B. Also included in Table 3.8b mean body-whorl data from experiment 1B, as it was felt that the values for this part of the experiment 1B shells might be closer to those from the W3 regime of experiment 1.

Considering the mean shell data from experiment 1B, ll the snails in experiment 1B (with one exception - see below) show oxygen isotope values close to those for the W1 snails from experiment 1. For example, W1 *Helix* = +0.84‰ and expt.1B *Helix* = +1.41‰ whereas W3 *Helix* = -0.98‰; W1 *Cernuella* = -1.46‰ and expt.1B *Cernuella* = -1.40‰ whereas W3 *Cernuella* = -2.68; W1 = -0.86‰ and expt.1B *Rumina* = -1.17 and -1.10‰ whereas W3 *Rumina* = -3.79‰ (Table 3.8b). This indicates that the majority of growth appears to have taken place during, or, has been positively influenced by the first and higher of the two test temperatures (W1 24°C). The exception is the oxygen isotope data from within the shell of *Helix* 2, which are all close to +3.5‰ (Table 3.8a). This value is similar to the $\delta^{18}O$ value to that found in shells of *Helix aspersa* from the W2 (22.3°C) regime of experiment 1 (+3.88‰, see Table 3.3). Therefore, for this snail only, the isotope data appear to be consistent with those from snails grown at a mean of the two environmental temperatures, as found in experiment 1.

The mean last whorl isotope values from experiment 1B are not similar to those from the W3 (19°C) regime even though these latter portions of shell may have been secreted during the time the snails were under the W3 regime. For example, W3 *Cepaea* = -1.23‰ whereas expt.1B mean last whorl *Cepaea* = +1.40 and +1.86‰ (Table 3.8b).

The carbon isotope data (mean shell data) of all the snails apart from *Rumina*, lie between the $\delta^{13}C$ values seen in the separate W1 and W3 populations from experiment 1. For example, for *Helix*, W1 $\delta^{13}C$ = -13.42 and W3 $\delta^{13}C$ = -12.64‰, whereas expt.1B $\delta^{13}C$ = -12.97 and -13.37‰, (Table 3.8b). *Rumina* ^{13}C data (-13.50 and -13.44‰) are closer to those data from shells of the W1 regime (-13.34‰) than to shells from the W3 regime (-12.93‰). However, the spread in all the carbon isotope data is small.

If there had been continuous growth from W1 to W3, then from the results of experiment 1, the trends that might be expected through time may be plotted. For oxygen, the expected trend would be towards more depleted $\delta^{18}O$ values though the course of the experiment (Figure 3.9a), with an expected shift of several per mil. This shift would be the result of evaporation and metabolic

SPECIES + DESCRIPTION	SAMPLE	Dist. from Aperture mm	Last Whorl?	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
Helix 1	1A	11	✓	-12.73	0.06
mature,	1B	28	✓	-12.73	0.64
thickened lip.	1C	41	✓	-12.81	2.23
27.4 x 29.3mm	1D	51	x	-12.90	1.46
	1E	58	x	-13.30	1.04
	1F	70	x	-13.34	3.05
Helix 2	2A	10	✓	-13.54	3.47
almost mature	2B	25	✓	-13.31	3.81
slight thickening	2C	31	✓	--	--
26 x 28.7mm	2D	46	x	-13.41	3.55
	2E	63	x	-13.21	3.37
Cepaea 1	1A	5	✓	-12.84	0.98
mature, brown	1B	17	✓	-12.70	2.12
lip, 3 banded	1C	29	✓	-12.82	1.35
yellow	1D	41	✓	-12.91	1.15
18 x 24.2mm	1E	57	x	-13.02	1.02
Cepaea 2	2A	5	✓	-12.73	0.85
mature, brown	2B	18	✓	-13.28	2.32
lip, 1 band	2C	33	✓	-12.51	2.41
pink	2D	45	✓	-12.71	1.85
17.8 x 22.9mm	2E	56	x	-12.71	1.22
Rumina 1	1A	20	✓	-13.28	-0.24
mature, slight	1B	40	✓	-13.50	-0.77
thickening	1C	56	x	-13.55	-0.99
	1D	70	x	-13.67	-1.76
	1E	RoS	x	-13.52	-2.07
Rumina 2	2A	10	✓	-13.19	-0.67
(as 1)	2B	20	✓	-13.38	-0.86
	2C	30	✓	-13.51	-0.56
	2D	40	✓	-13.50	-0.57
	2E	48	x	-13.40	-1.23
	2F	56	x	-13.55	-1.66
	2G	64	x	-13.52	-2.15
Cernuella 1	1A	0-20	✓	-12.47	-0.35
large shell	1B	21-30	✓	-12.59	0.07
	1C	31-43	x	-12.70	0.10
	1D	44-51	x	-12.76	-2.33
	1E	52-RoS	x	-12.40	-4.48

Table 3.8a Descriptions and dimensions of shells investigated and results of carbon and oxygen isotope analyses : Experiment 1B.

RoS = Rest of Shell

	W1 24°C		W3 19.3°C		WHOLE SHELL Expt. 1B		LAST WHORL Expt. 1B	
	$\delta^{13}C‰$	$\delta^{18}O‰$	$\delta^{13}C‰$	$\delta^{18}O‰$	$\delta^{13}C‰$	$\delta^{18}O‰$	$\delta^{13}C‰$	$\delta^{18}O‰$
Helix	-13.42	0.84	-12.64	-0.98	-12.97	1.41	-12.77	0.98
					-13.37	3.55	-13.43	3.64
Cepaea	-13.16	2.50	-12.03	-1.23	-12.86	1.32	-12.82	1.40
					-12.79	1.73	-12.56	1.86
Rumina	-13.34	-0.86	-12.93	-3.79	-13.50	-1.17	-13.39*	-0.51*
					-13.44	-1.10	-13.32*	-0.67*
Cernuella	-12.91	-1.46	-12.39	-2.68	-12.58	-1.40	-12.53	-0.14

Table 3.8b Comparison of shell isotope data from Experiment 1B with that from individual temperatures from Experiment 1.

* = mean last two whorls

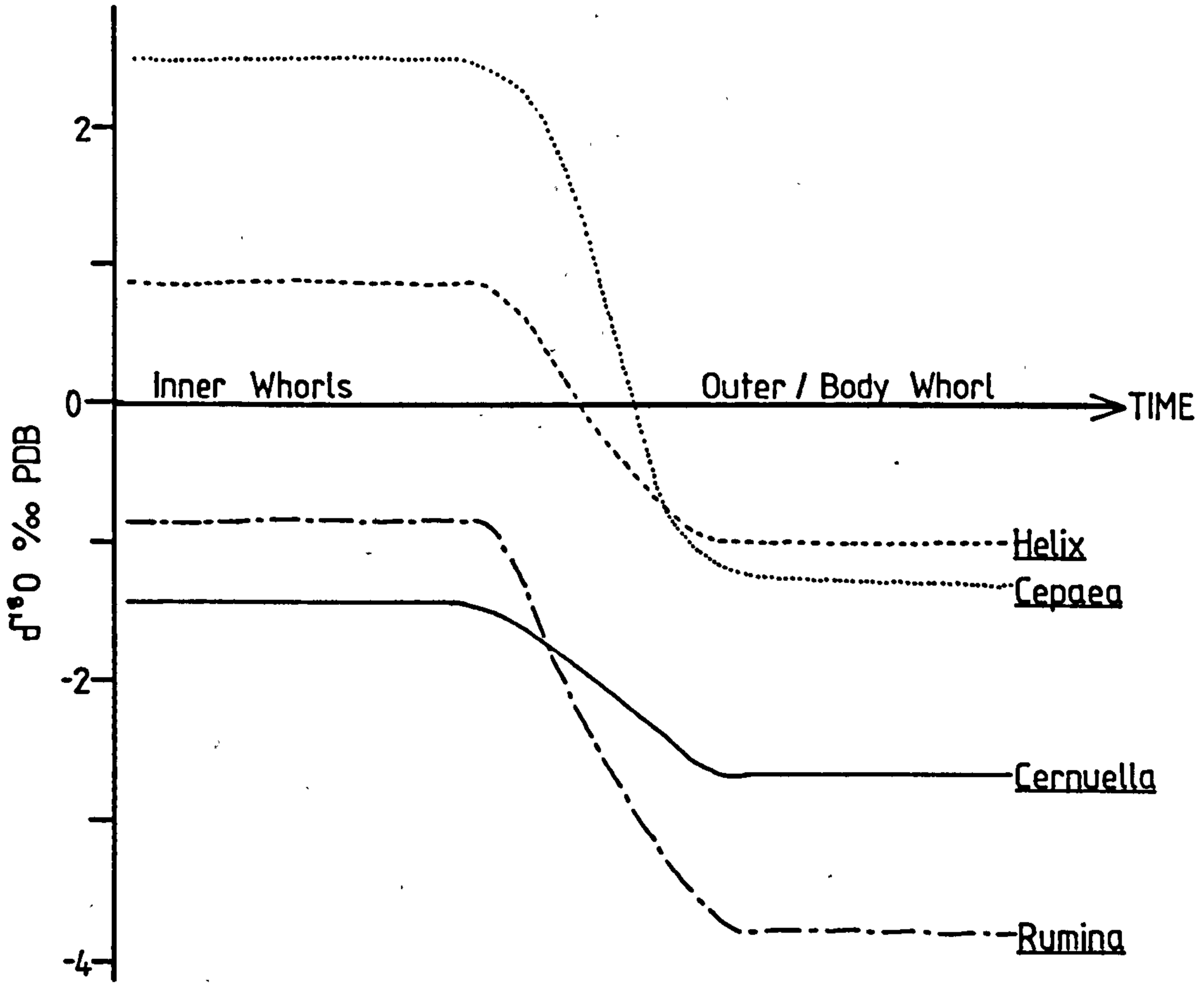


Figure 3.9a 'Expected' trend of oxygen isotopes through time in Experiment 1B, with removal of snails from 24°C to 19.3°C habitat, in light of the results of Experiment 1.

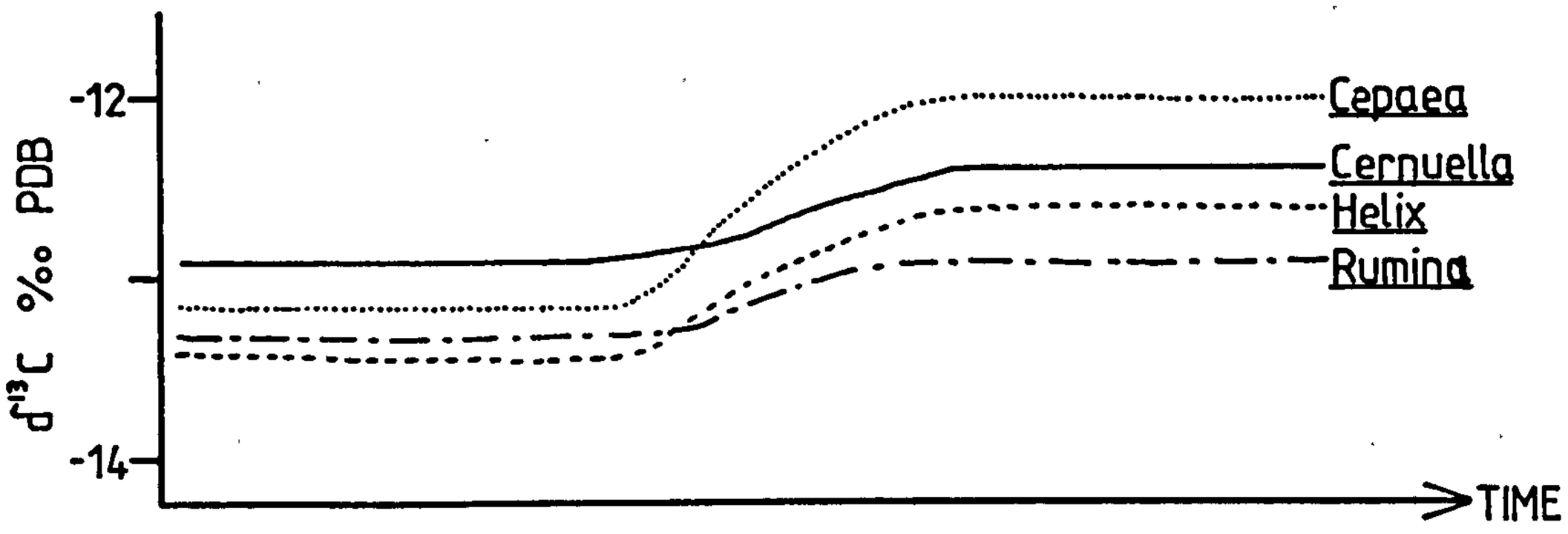


Figure 3.9b 'Expected' trend of carbon isotopes through time in Experiment 1B, with removal from 24°C to 19.3°C habitat, in light of the results of Experiment 1.

enrichment effects becoming less important as environmental temperature fell. Temperature effects alone would produce a less pronounced shift in the opposite direction. For carbon a shift of between 0.5 and 0.8‰ might be expected (Figure 3.9b) moving towards less depleted $\delta^{13}C$ values over the course of the experiment. These expected trends (Figures 3.9a and 3.9b) may then be compared to the patterns actually observed within the shells, which for oxygen isotopes are shown on Figures 3.10a-d for *Helix, Cepaea, Rumina* and *Cernuella*, respectively. Carbon isotope data within each shell are shown in a similar fashion in Figures 3.11a-d.

The following section outlines the differences between the expected and observed trends. A greater amount of isotopic variation was seen between and within shells for the oxygen isotopes as compared with the carbon isotopes. Therefore, data from individual species will be considered in terms of their oxygen isotopic composition, whereas the carbon isotope results will be considered more generally.

3.5.4 Discussion of oxygen isotope results

(a) *Helix aspersa*

The oxygen isotope data from the two shells of *Helix aspersa* are plotted on Figure 3.10a.

Helix 1 shows an overall trend towards more depleted $\delta^{18}O$ values through the course of the experiment as might be expected following the results of experiment 1. However this trend, as shown on Figure 3.10a, does not follow accurately the hypothetical trend described in Figure 3.9a. In addition, the mean oxygen isotope value of the three samples from the last whorl, is almost 2‰ more enriched than the shells of *Helix* from the W3 (19.3°C) regime in experiment 1. (*i.e.* $\delta^{18}O = +0.98‰$ in experiment 1B, compared with -0.98‰ in experiment 1 - see Table 3.8b) This difference might be explained if the body whorl samples from the experiment 1B snails were influenced by a 'memory effect' from the W1 (24°C) temperature. It is also possible that the sub-samples taken from closest to the apex contain some 'memory' of the base population conditions, hence the first drop in $\delta^{18}O$ from 70 to 60mm behind the aperture, before a further increase in $\delta^{18}O$ up to 40mm (Figure 3.10a). The 'memory effect' (of base population conditions in the earlier whorls) might also explain why the body whorl $\delta^{18}O$ mean value for this shell (+0.98‰) is closer than the whole shell mean $\delta^{18}O$ value (+1.41‰) to $\delta^{18}O$ of *Helix* during experiment 1 (+0.84‰ in W1, and -0.98‰ in W3).
Alternatively, the first drop in $\delta^{18}O$ values (70-60mm behind the aperture) may reflect later internal shell thickening at the lower temperature onto shell previously secreted under the warmer conditions. Although no actual measurements were taken, the snails did continue to grow and secrete new shell material during the second phase of the experiment. However, it is not certain whether internal thickening of part, or of the entire shell took place as the snails approached their sub-adult size. Therefore, it is not possible to say whether the difference between the actual and "expected" trend is the result of slow thermal acclimatisation (with a time lag until the snail's metabolism adjusted to the new thermal regime), or whether the disparity is the result of differential internal thickening.

The other *Helix* shell (*Helix* 2), has $\delta^{18}O$ values of around +3.5‰ consistently throughout the shell. This means that *Helix* 1 and 2, grown together under identical conditions have oxygen isotopic compositions up to 3.5‰ apart, which is somewhat unexpected. *Helix* 2 also has oxygen isotope values more enriched than either W1 or W3 *Helix* from experiment 1. It is felt that the

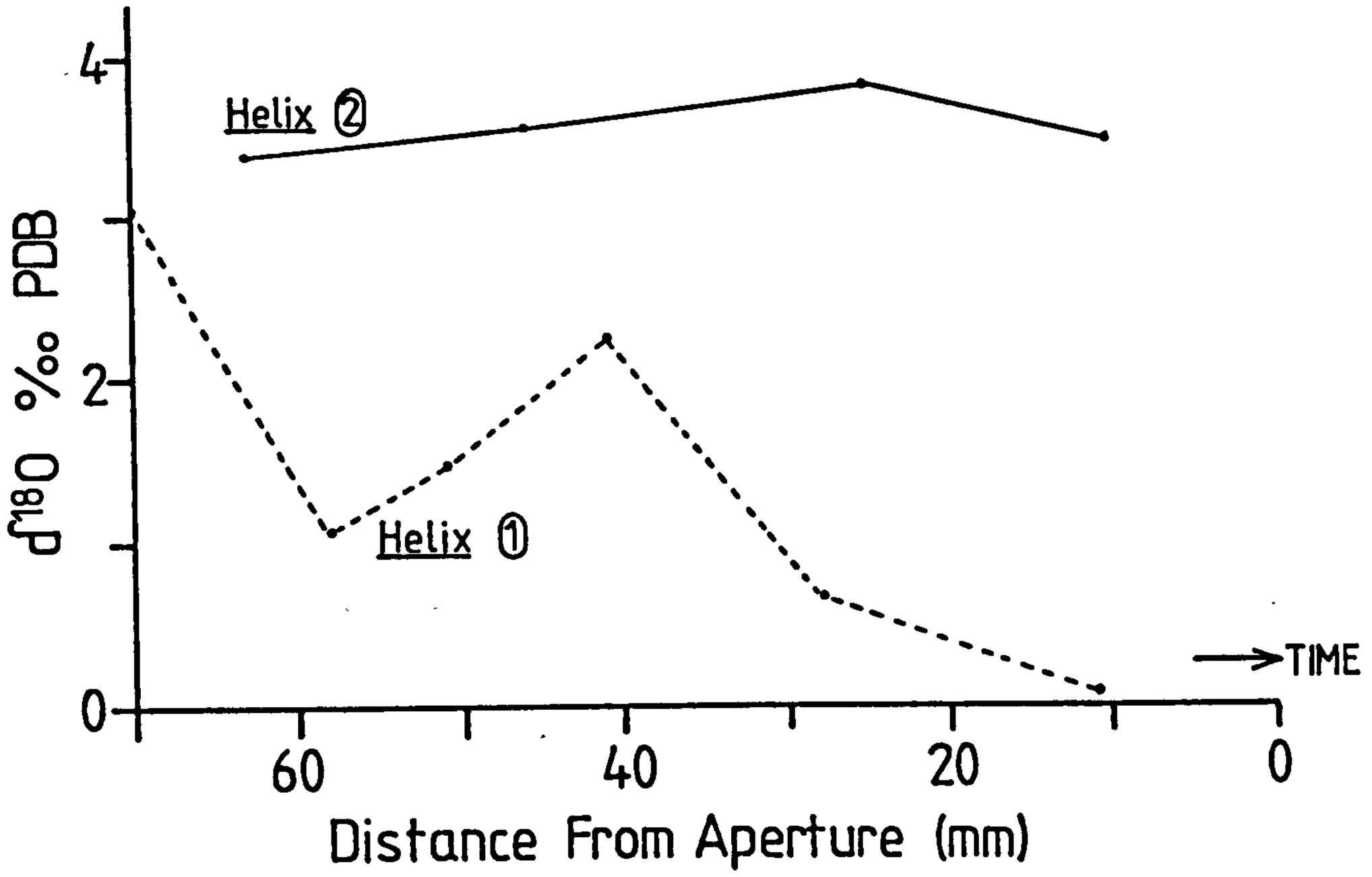


Figure 3.10a Oxygen isotope profile through time - measured as approximate distance (mm) of sample from the aperture - for two individual shells of *Helix aspersa* from Experiment 1B.

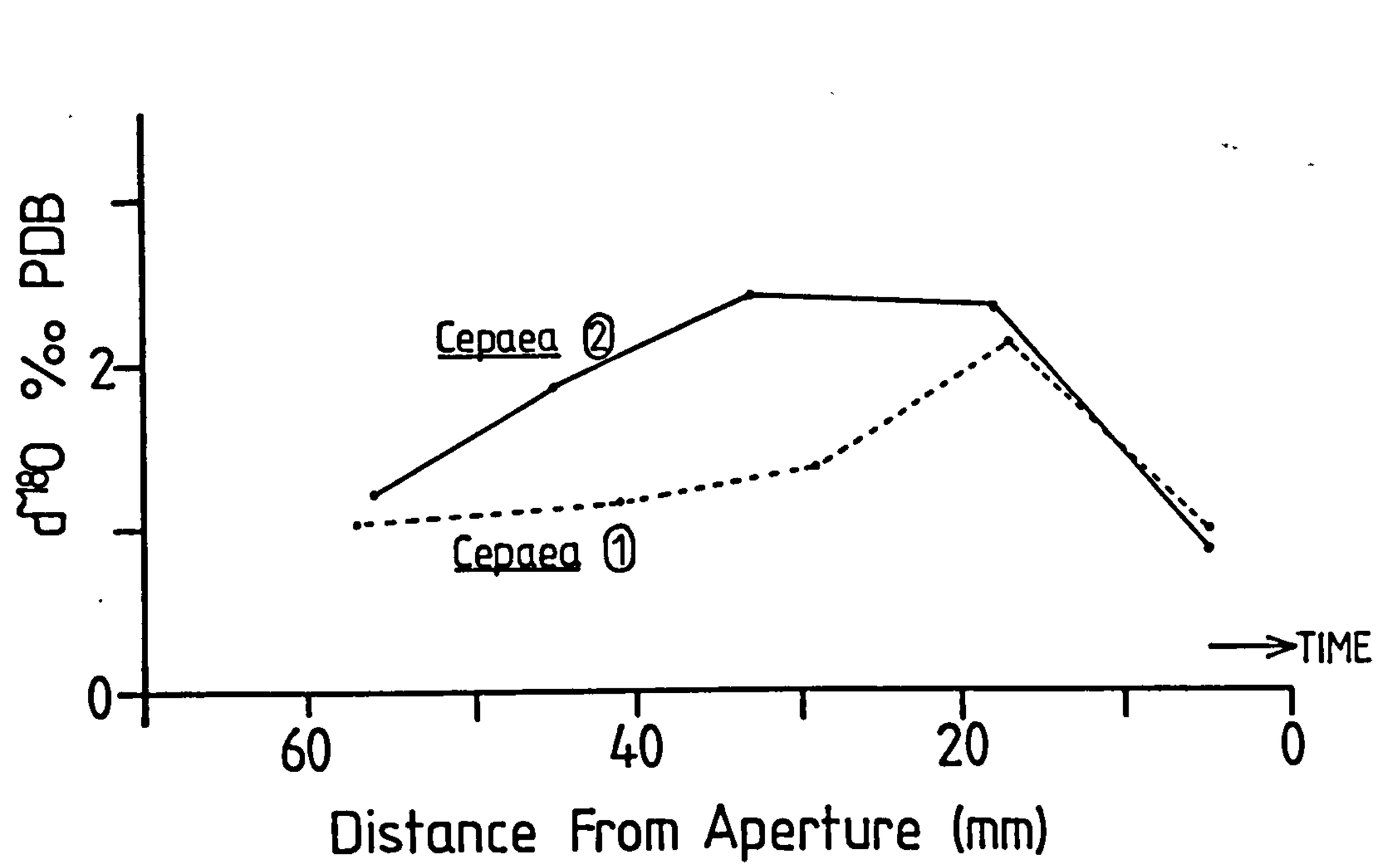


Figure 3.10b Oxygen isotope profile through time for two individual shells of *Cepaea nemoralis* from Experiment 1B.

Figure 3.10a-d Oxygen isotope data versus approximate distance from shell aperture (mm), for individual shells from Experiment 1B.

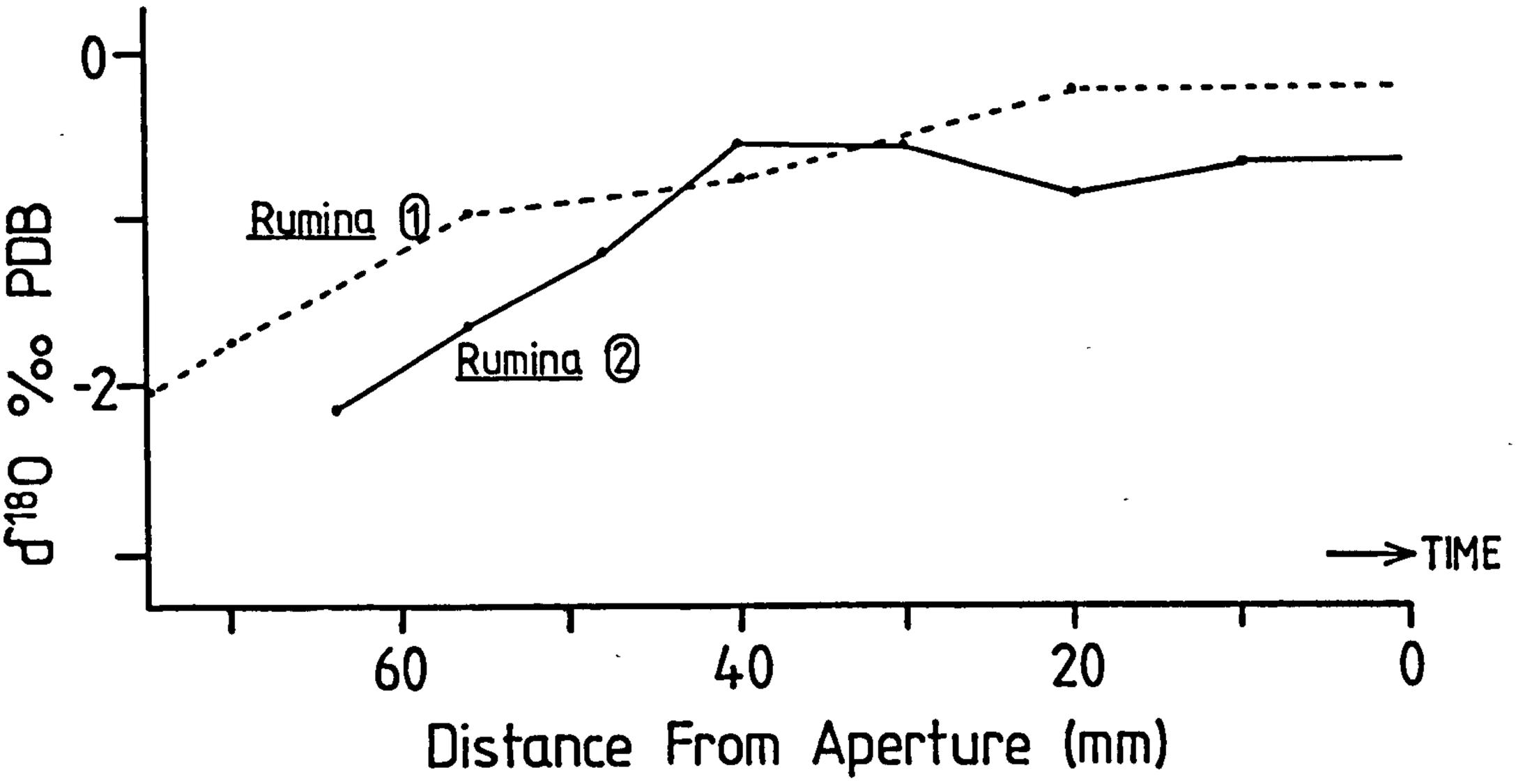


Figure 3.10c Oxygen isotope profile through time - measured as approximate distance (mm) of sample from the aperture - for two individual shells of *Rumina decollata* from Experiment 1B.

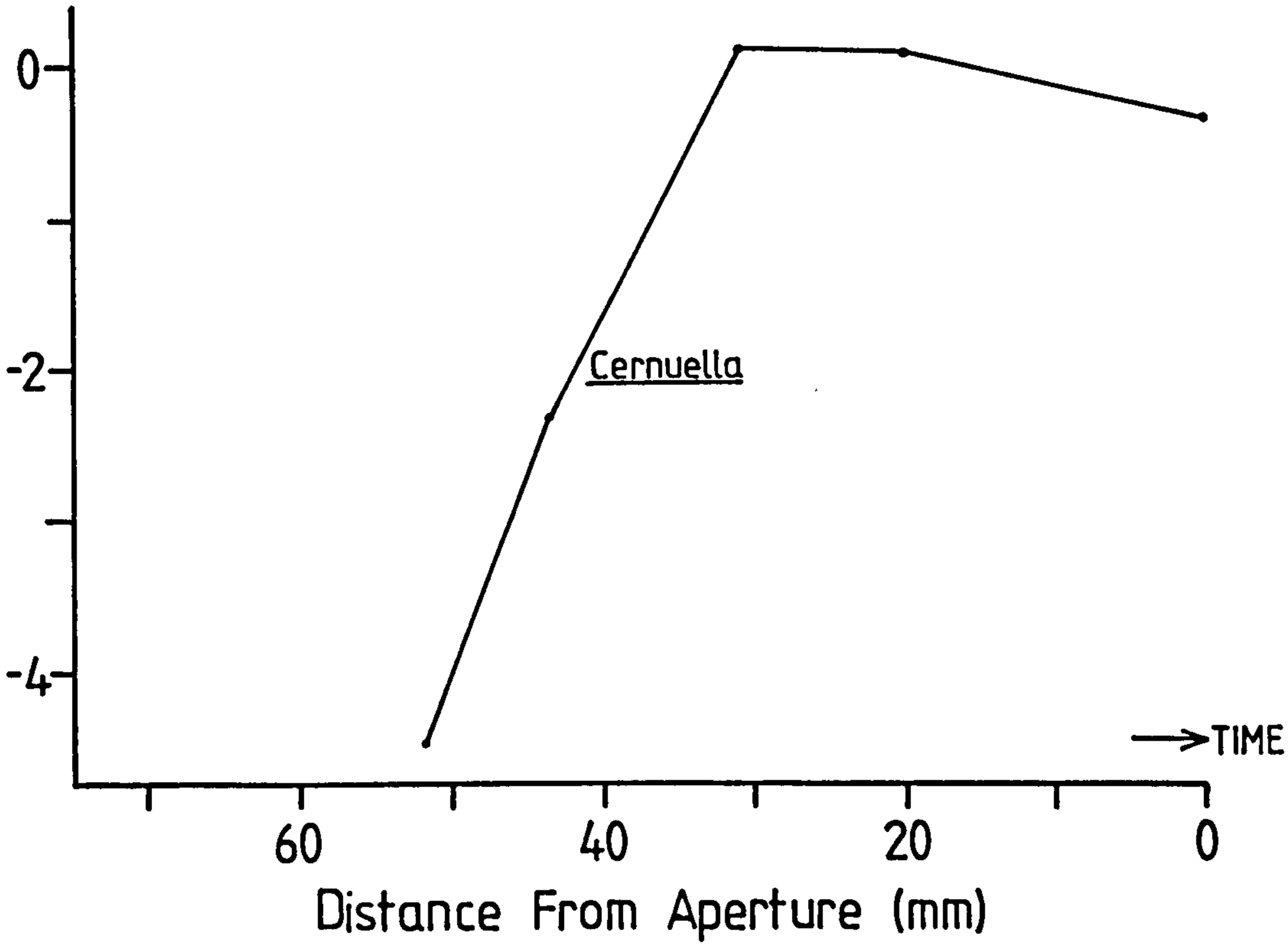


Figure 3.10d Oxygen isotope profile through time for one shell of *Cernuella virgata* from Experiment 1B.

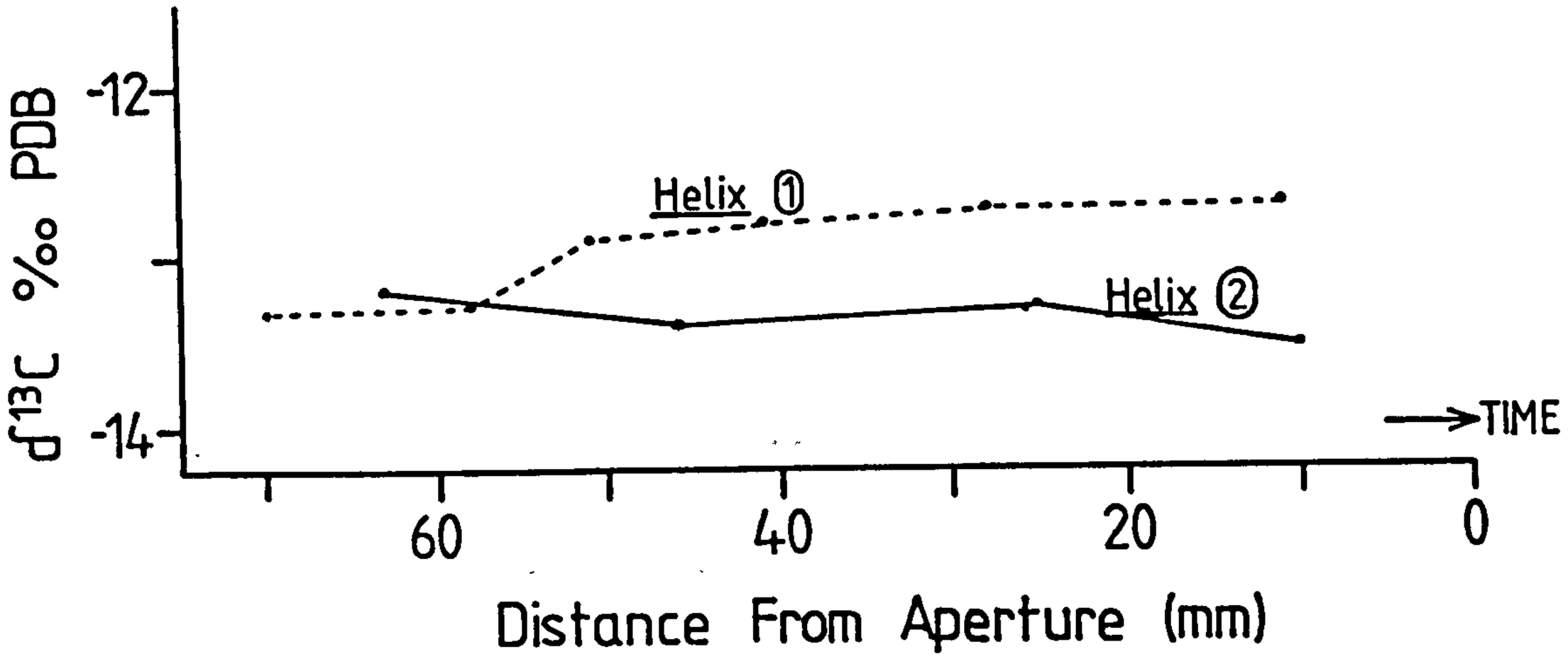


Figure 3.11a Carbon isotope profile through time - measured as approximate distance (mm) of sample from the aperture - for two individual shells of *Helix aspersa* from Experiment 1B.

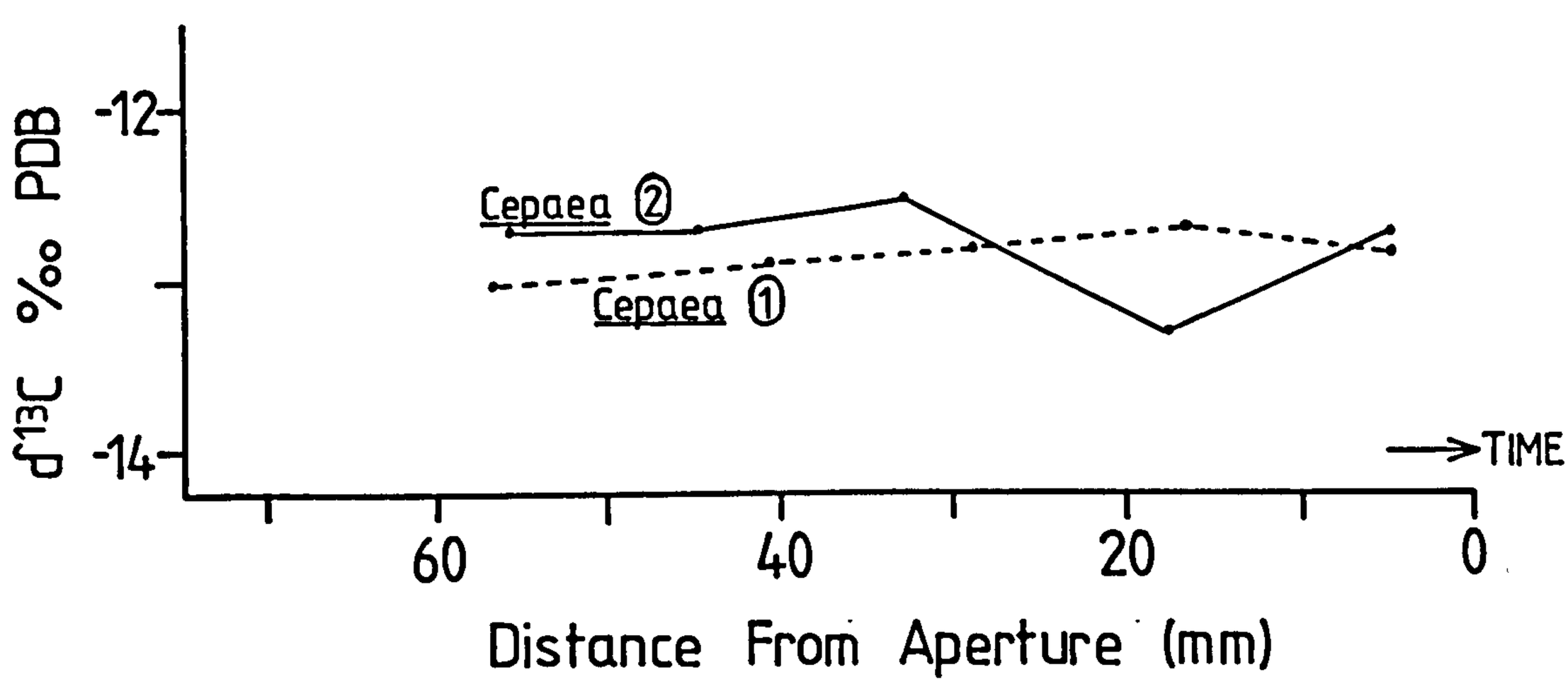


Figure 3.11b Carbon isotope profile through time for two individual shells of *Cepaea nemoralis* from Experiment 1B.

Figure 3.11a-d Carbon isotope data versus approximate distance from shell apex (mm), for individual shells from Experiment 1B.

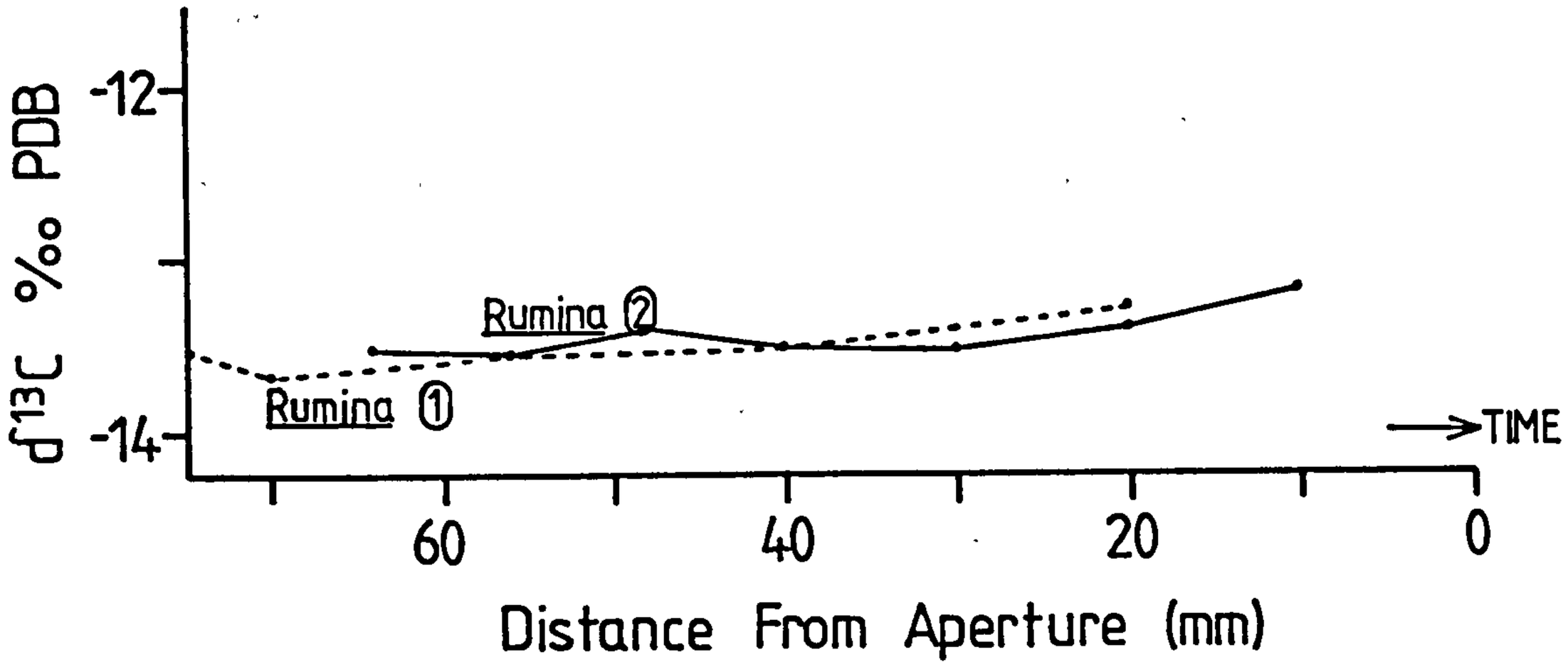


Figure 3.11c Carbon isotope profile through time - measured as approximate distance (mm) of sample from the aperture - for two individual shells of *Rumina decollata* from Experiment 1B.

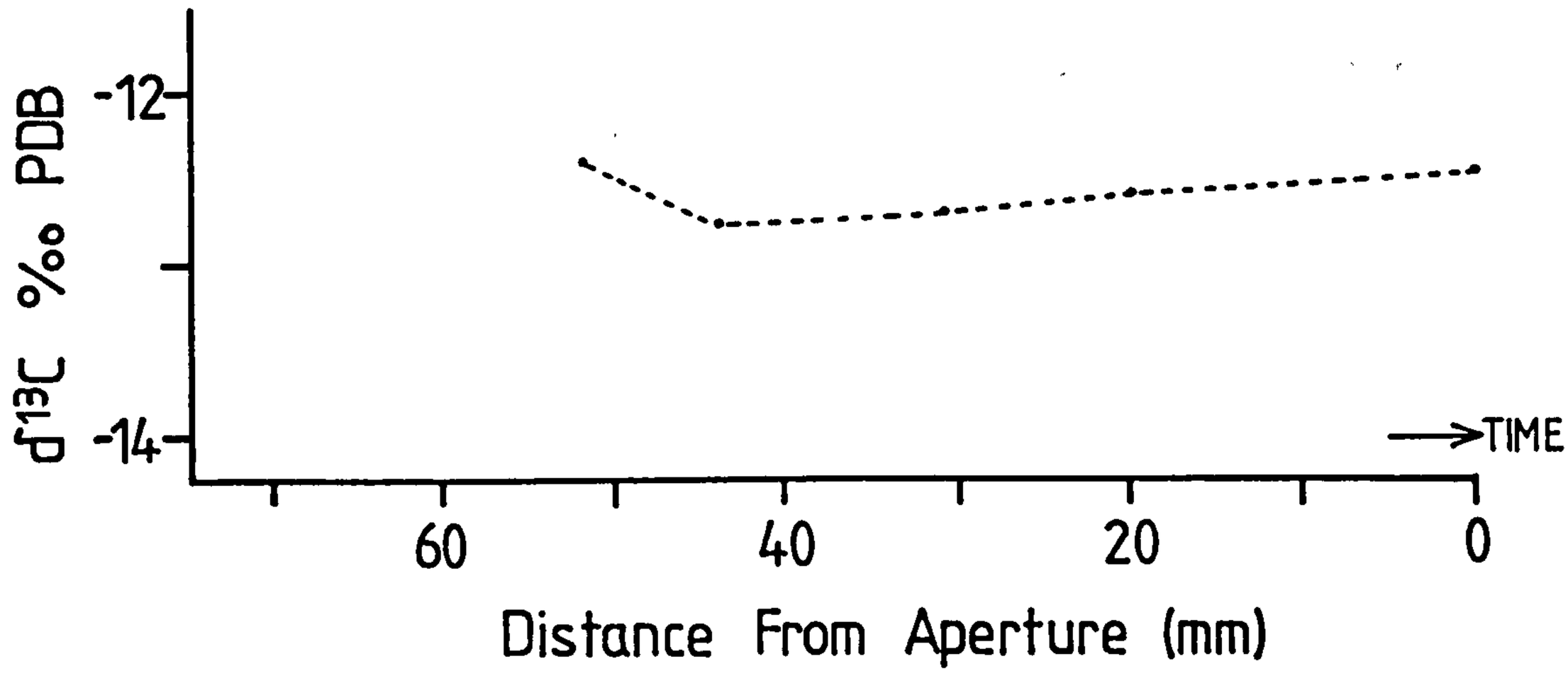


Figure 3.11d Carbon isotope profile through time for one shell of *Cernuella virgata* from Experiment 1B.

activity of this individual may have been inhibited by the density of sub-adults to adults also housed with it, hence the significantly more enriched $\delta^{18}O$ value. Six *Helix aspersa* individuals were housed in the standard boxes for experiment 1B. Overcrowding, or the influence of a greater amount of secreted mucus and slime within the box, may have had an inhibitory effect upon the growth of this snail, which unlike *Helix* 1 and the other *Helix* individuals, had not reached full maturity. Cameron and Carter (1979), reported that for the helicids they studied, density of snails was inversely proportional to growth rates and activity in juvenile snails, and that the activity of *Cepaea nemoralis*, *C. hortensis* and possibly *Helix aspersa* was reduced when they were housed in boxes that had been pretreated with mucus trails of the same species of snail. Four, or fewer, large helicid adults (for example, *Helix* or *Cepaea*) housed in the boxes used in this experiment , may be a maximum density before space becomes a limiting factor (A. J. Cain, pers. comm., 1987). Therefore, the activity of *Helix* 2 may have been inhibited by density stress or by the presence of mucus, leading to increased metabolic enrichment during periods of inactivity, and hence the high $\delta^{18}O$ values throughout this shell.

(b) *Cepaea nemoralis*

The oxygen isotope data for the two shells of this species follow a similar pattern (Figure 3.10b), with a slight trend to ^{18}O enrichment through the interior shell sub-samples and a drop in $\delta^{18}O$ values to the ultimate sub-sample. This decline in ^{18}O towards the aperture over the last two to three sub-samples follows the 'expected' trend as seen in experiment 1. However, as with *Helix aspersa*, the last whorl sub-samples from both *Cepaea* shells were over 2‰ more enriched than *Cepaea* from the W3 regime of experiment 1 (*i.e.* experiment 1B mean last whorl, *Cepaea* 1 = +1.40‰ and *Cepaea* 2 = +1.86‰; experiment 1, W3 19.3°C, mean *Cepaea* = -1.23‰).

The samples from the last whorl of the shells may therefore denote a 'memory effect' from the 24°C regime upon later growth, or that the last whorls of the shells were a mixture of shell material secreted over the two temperatures. This would occur if the the initial shell was secreted at 24°C, and then thickened on the interior during the following period at 19.3°C.

Interior thickening may also explain some of the less enriched samples from earlier in the shells, if this early part of the shells was thickened after the snails were switched to the lower temperature.

(c) *Rumina decollata*

The oxygen isotope compositions of the two *Rumina* shells follow the same trend, although the two shells were sampled over different intervals. *Rumina* 1 represents four complete whorls (samples A-D) and then the initial two-and-a-half whorls from the decollation point (sample E). *Rumina* 2 includes the last three-and-a-half whorls at half whorl intervals (samples A-G), and the initial whorls from the decollation point were not sampled for analysis (Table 3.8a). Even though the sample interval was different, the oxygen isotopes of both shells (when considered in terms of the distance from the aperture) show a trend towards more enriched $\delta^{18}O$ values through time, which levels off around 30-40mm behind the aperture (approximately two whorls from the aperture), Figure 3.10c. This overall trend does not fit that 'expected' from the data of experiment 1, although the mean $\delta^{18}O$ value for the last whorl (approximately -0.6‰) is close to that from the W1 (24°C) regime (-0.86‰), but is still 3‰ more enriched than a mean $\delta^{18}O$ value for *Rumina* from the W3 (19.3°C regime in experiment 1 (-3.79‰).

The trend seen could be the result of one or more effects. Firstly, the interior whorls may show a 'memory effect' of conditions of the base populations, and the latter whorls a 'memory effect' of the 24°C conditions. A similar idea has been proposed to explain some of the phenomena described above for the helicids. If this effect does influence the oxygen isotopic composition of the snail shells, then it would be vital to allow sufficient time for snails to acclimatize fully to environmental conditions before sampling their shells for stable isotopes. A similar 'memory effect' was seen in the initial analyses of *Rumina* shells (section 3.2), where the isotopic variation present in single shells was investigated, and where the early whorls showed significantly more depleted $\delta^{18}O$ values than the later whorls.

An alternative explanation for the pattern within the *Rumina* shells, as suggested for the previous two species, is that it reflects interior thickening of the shells. If most of the forward growth of the shells was achieved during the period at 24°C, then the lower $\delta^{18}O$ values of the earlier whorls may indicate interior thickening of these whorls at 19.3°C, leading to a mixture of shell secreted over the two temperatures. However, total interior thickening was not complete when the snails were killed, hence the $\delta^{18}O$ values from later whorls are closer to those 'expected' after experiment 1. This idea is supported by the fact that overall, the shells from experiment 1B had much greater strength (*i.e.* it was not easy to crush the shells between the fingers) than the shells from experiment 1, and also that the *Rumina* shells investigated in experiment 1B may still not have reached a completely mature state when they were killed.

The relatively depleted interior whorls might also be the result of a juvenile snail metabolic effect, with less metabolic enrichment occurring in immature snails than in adults. This idea would also be supported by the findings reported earlier in section 3.2.

A fourth explanation is that the oxygen isotope data from these shells primarily reflect the influence of changing temperature, rather than evaporative or metabolic isotope effects. In the light of the discussions of the results of experiment 1, where at temperatures above 15°C there appeared to be little evidence for a direct link between environmental temperature and shell $\delta^{18}O$ values, this is thought to be unlikely.

(d) *Cernuella virgata*

The single *Cernuella* shell shows a strong 2-4‰ shift in $\delta^{18}O$ after the early whorls. After this strong shift, the oxygen isotope data are fairly consistent with a slight depletion to the ultimate sample (two-thirds of the last whorl) Figure 3.10d. The depleted ^{18}O values from samples D and E (the earliest samples) may reflect shell material deposited prior to the snail being placed under the experimental conditions, *i.e.* it was secreted in the base population. Sample D ($\delta^{18}O$ = -2.33‰) may include some shell secreted in the W1 24°C regime.

3.5.5 Discussion of carbon isotope results

Only a small shift towards more enriched $\delta^{13}C$ values through the course of this experiment, might be expected in view of the results of experiment 1, where mean $\delta^{13}C$ values of shells from the W1 (24°C) regime were -12.91 to -13.42‰, and mean $\delta^{13}C$ values of shells from the W3 (19.3°C) regimes were from -12.03 to -12.93‰ (Figure 3.9b).

In experiment 1B, most of the ^{13}C data are fairly consistent within each shell with a slight shift towards more enriched $\delta^{13}C$ values being present in *Helix* 1 (Figure 3.11a); *Cepaea* 1 (Figure 3.11b); both *Rumina* shells (Figure 3.11c); and also in *Cernuella* after initially being slightly more enriched (Figure 3.11d). *Helix* 2 is the only shell which has somewhat more depleted $\delta^{13}C$ values towards the aperture. This shell also gave unexpected oxygen isotope data, which were thought to be the result of density stress and inhibition of activity due to mucus secretion by the other snails, both leading to an increased metabolic effect. This same phenomenon might also account for the $\delta^{13}C$ values being about 0.5‰ more depleted than *Helix* 1.

With only slight variations in the carbon isotopes, it is difficult to interpret the data in terms of the memory effects or, interior thickening effects, (as suggested above for the oxygen isotopes) related to the change in the temperature regime experienced by the snails.

3.5.6 Summary

The limited results of experiment 1B suggest several points.

1) A 'memory effect', particularly evident in the oxygen isotope ratios of the shells, may last for some time after the snails are moved from one environment to another. Snails should be allowed sufficient time to acclimatize before shell material sampled for stable isotopic analysis will be certain to reflect the prevailing environmental conditions. This could be because metabolites (water, oxygen, carbon dioxide and bicarbonate) produced from earlier environmental conditions may take some time to be completely replaced by those relating to prevailing conditions.
2) Interior thickening of the shell may also be an important factor, particularly in the later stages of growth of the shell. More accurate experiments would be required to trace the relationship between forward growth and interior thickening for different species under various conditions. Because of the effects of interior thickening, shell isotope ratios will reflect a mixture of environmental temperatures and conditions, if these are not maintained constant. Points (1) and (2) suggest that in natural systems, shell isotope values may reflect average prevailing conditions over the whole period of growth of a shell rather than representing day to day or week by week fluctuations.
3) Overcrowding of sub-adult or adult snails may result in anomalous oxygen, and perhaps carbon, isotope data, as activity and metabolism are disrupted leading to ^{18}O enrichment and ^{13}C depletion.
4) To interpret results better from similar experiments it would be useful to:
   i) Mark the extent of growth before the start of the experiment and after the first temperature regime.
   ii) Analyse stable isotope ratios in a series of sections across the thickness of a shell. This might reveal the pattern of interior thickening relative to forward shell growth, if a method for extracting the tiny portions of shell necessary for this type of analysis were available. The thin nature of the shells investigated here would make this proposition very difficult.
   iii) Maintain some of the snails at the first temperature from juvenile to mature state, for comparison with other snails that had been moved. Thus shell isotope ratios characteristic of juvenile stages could be isolated from those of mature shells.

3.6 EXPERIMENT 2 : ISOTOPIC COMPOSITION OF SHELLS OF SNAILS SUBJECT TO A DIURNAL CYCLE OF TEMPERATURE AND LIGHT

3.6.1 Introduction and aims

The aims of this experiment were twofold. Firstly, to introduce diurnal variation in the conditions experienced by the snails in an attempt to minimise the artificiality of the experimental environments. Secondly, to compare the isotopic compositions of snail shells secreted under variable conditions with those from snails maintained under continuous standard conditions.

The method of this experiment has been described in detail in section 2.4.3. Population 1 snails (Pop1) were maintained at 22.3°C in permanently lit conditions. Population 3 snails (Pop3) were kept at 16.5°C, permanently in the dark. Population 2 snails (Pop2) were moved between the two extremes, therefore undergoing a daily cycle of approximately 12 hours of darkness at 16.5°C, followed by 12 hours in fluorescent light at 22.3°C.

3.6.2 Results

(a) Outline

Isotopic analyses were carried out on between four to eight shells from each population of the three species investigated (*Helix aspersa*, *Cepaea nemoralis* and *Rumina decollata*). Dimensions (height x breadth in mm) and weight (mg) of all shells of snails killed at the close of the six week growth period were measured. This was to investigate a possible correlation between growth rate and shell isotopic composition and to assess the relative growth of the three test populations. The dimensions of fifteen additional shells of juvenile *Helix aspersa* (removed from the base populations in conjunction with those snails actually used in the experiment but killed immediately) were measured to give an estimate of initial size and weight.

Table 3.9a shows height, breadth, final weight measurements and oxygen and carbon isotope data (where measured) for Pop1 *Helix aspersa*. Tables 3.9b and 3.9c comprise the same data, but for Pop2 and Pop3 *Helix aspersa*, respectively. In a similar way, Tables 3.10a-c show data from the three populations of *Cepaea nemoralis* , and Tables 3.11a-c for *Rumina decollata* (although for this snail, the diameter of the last whorl was the only dimension measured).

Two samples of the water given to the snails during the experiment were analysed for their oxygen isotope composition. The first sample was taken at the onset of the experiment ($\delta^{18}O$ = -8.3‰ SMOW) and the other at the end ($\delta^{18}O$ = -8.8‰SMOW), giving a mean $\delta^{18}O$ water value of -8.55‰ SMOW.

The stable isotopic composition of the inorganic carbon (GPR 2)given to the snails in their mixed diet (Food 2) was measured. $\delta^{18}O$ of GPR2 = -28.40‰ PDB, and $\delta^{13}C$ = -43.35‰ PDB.

Helix POP 1	HEIGHT mm	BREADTH mm	WEIGHT mg	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
1	18.8	18.6	379.9	-19.85	1.33
2	18.3	19.5	226.0	-18.14	1.23
3	19.3	20.4	324.7	-19.57	1.35
4	18.0	20.0	354.1	-19.85	1.58
5	21.3	21.8	477.6	-18.90	1.37
6	23.7	23.2	700.7	-21.16	1.76
7	16.9	18.2	263.0	-19.74	1.96
8	15.8	16.8	198.3	-20.02	1.90
9	16.5	19.4	283.8	-	-
10	16.5	17.0	217.5	-	-
11	16.0	16.8	230.0	-	-
Means	18.28	19.25	332.30	-19.66	1.56

Table 3.9a

POP 2					
1	16.1	18.7	272.2	-20.59	1.77
2	14.9	16.7	277.6	-20.20	1.25
3	14.7	16.7	243.0	-20.47	1.62
4	15.3	17.3	230.3	-19.77	1.07
5	17.9	20.1	392.4	-20.44	1.61
6	17.8	19.0	342.6	-20.22	1.26
7	9.8	12.5	102.4	-19.89	1.24
8	12.7	14.1	194.3	-20.68	1.00
9	14.8	16.2	272.2	-	-
10	12.1	13.9	192.8	-	-
11	14.4	16.2	217.6	-	-
Means	14.59	16.49	248.90	-20.28	1.35

Table 3.9b

POP 3					
1	14.9	15.9	207.6	-19.35	1.11
2	13.1	15.6	125.3	-19.18	1.32
3	13.6	15.5	196.3	-20.16	1.47
4	12.2	13.3	126.9	-19.59	1.30
5	15.0	16.5	185.1	-19.69	1.87
6	13.5	14.5	172.0	-19.49	1.42
7	14.1	16.1	213.3	-19.89	1.48
8	10.6	12.2	81.7	-19.14	1.72
9	13.3	15.1	185.1	-	-
10	11.6	12.9	119.3	-	-
11	13.7	15.5	150.3	-	-
Means	13.24	14.83	160.3	-19.56	1.46

Table 3.9c

Table 3.9a-c Dimensions, final weight, oxygen and carbon isotope data for shells of *Helix aspersa* from Experiment 2.

Cepaea POP 1	HEIGHT mm	BREADTH mm	WEIGHT mg	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
1	9.5	12.3	105.4	-22.30	1.82
2	8.6	12.2	97.7	-21.90	2.01
3	9.8	13.4	148.0	-22.50	1.95
4	6.4	8.7	44.8	-22.51	1.07
5	8.3	11.3	93.9	-22.21	1.79
6	9.7	12.8	136.5	-22.17	2.21
7	8.2	10.9	81.8	-22.44	1.54
8	7.2	9.8	70.7	-	-
9	5.7	7.9	33.3	-	-
10	6.9	9.5	58.4	-	-
11	9.3	12.4	113.8	-	-
Means	8.15	11.02	80.86	-22.29	1.77

Table 3.10a

POP 2					
1	8.4	11.1	99.8	-22.55	2.30
2	8.9	11.9	106.4	-22.41	2.69
3	8.2	11.1	94.3	-22.95	2.86
4	8.1	10.9	88.69	-21.79	2.55
5	8.2	11.0	84.9	-23.25	1.58
6	7.0	9.6	67.8	-22.10	2.43
7	6.4	8.5	44.1	-22.53	3.05
8	5.6	8.0	36.4	-	-
9	7.7	9.8	74.3	-	-
10	7.0	8.8	54.6	-	-
11	4.2	5.6	15.5	-	-
Means	7.25	9.65	69.73	-22.51	2.51

Table 3.10b

POP 3					
1	7.6	9.6	55.6	-20.92	1.62
2	7.1	9.5	50.4	-21.61	1.98
3	7.0	9.3	48.0	-20.82	1.99
4	7.3	9.6	60.5	-22.16	0.85
5	5.9	7.8	39.0	-22.36	1.00
6	6.2	7.8	38.2	-21.88	1.83
7	4.5	6.4	18.8	-22.96	2.87
8	6.3	8.5	41.8	-	-
9	5.9	7.8	31.3	-	-
10	5.1	7.0	24.8	-	-
11	4.2	5.8	17.9	-	-
12	3.7	5.2	13.0	-	-
Means	5.90	7.86	36.61	-21.82	1.73

Table 3.10c

Table 3.10a-c Dimensions, final weight, oxygen and carbon isotope data for shells of *Cepaea nemoralis* from Experiment 2.

Rumina POP 1	DIAM. LAST WHORL mm	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
1	7.8	-20.29	-3.33
2	7.6	-20.65	-2.67
3	7.6	-21.33	-1.30
4	7.2	-21.14	-1.63
5	6.6	-22.03	-2.11
6	6.8	-	-
7	6.5	-	-
8	6.3	-	-
9	4.4	-	-
Means	6.76	-21.09	-2.21

Table 3.11a

POP 2			
1	7.0	-21.62	-1.37
2	5.8	-21.94	-1.25
3	6.7	-21.38	-1.16
4	7.1	-20.67	-0.67
5	6.9	-	-
6	5.9	-	-
7	5.6	-	-
8	5.6	-	-
9	5.9	-	-
10	5.1	-	-
Means	6.16	-21.40	-1.11

Table 3.11b

POP 3			
1	5.9	19.19	-0.18
2	5.2	-19.39	-1.22
3	4.8	-20.25	-1.99
4	4.9	-19.40	-0.09
5	4.7	-19.64	-0.06
6	4.7	-	-
Means	5.03	-19.72	-0.71

Table 3.11c

Table 3.11a-c Dimensions, final weight, oxygen and carbon isotope data for shells of *Rumina decollata* from Experiment 2.

(b) Shell growth

Figures 3.12a and 3.12b are plots of the height and breadth of *Helix* and *Cepaea*, respectively. (For *Rumina*, mean values of the diameter of the last whorls are shown in Tables 3.11a-c).

Figures 3.12a and 3.12b and the data from *Rumina* in Tables 3.11a-c, show that for all three species, Pop1 snails put down the most shell under the warmer, permanently lit conditions (*e.g.*, for *Helix* , Pop1 mean height 18.28 x 19.25mm, Pop2 = 14.59 x 16.49mm and Pop3 = 13.24 x 14.83mm; and Pop1 mean weight = 332.3mg, Pop2 = 248.9mg and Pop3 = 160.3mg). The least shell was secreted by the snails kept in the cooler, permanently dark conditions (Pop3). In terms of weight, shells of *Helix* and *Cepaea* from Pop1 secreted more than double the weight of shell as the Pop3 snails. the increase in shell weight was greater between the Pop3 and Pop2 shells than between the Pop2 and Pop1 shells (*e.g.*, percentage increase in shell weight from Pop3 *Helix* as compared with Pop2 *Helix* = 55% whereas the percentage increase in weight from Pop2 as compared with Pop1 = 33%). Data from the Pop2 snails lie between the other two data sets, but with some considerable overlap. Some Pop2 snails were larger than individuals from Pop1, and some smaller than those from Pop3.

(c) Oxygen isotopes

The mean oxygen isotope data from the three populations of *Helix aspersa* are all fairly similar, ranging from +1.35 to +1.56‰ (Tables 3.9a-c).

For *Cepaea nemoralis* (Tables 3.10a-c), the oxygen isotope data from Pop1 and Pop3 snails are similar (+1.77 and +1.73‰, respectively), but the mean value from Pop2 snails is more enriched by 0.8‰ as compared to the other snails ($\delta^{18}O$ = +2.51‰).

Rumina decollata is the only one of the three species to show a trend from more depleted $\delta^{18}O$ values in Pop1 (mean $\delta^{18}O$ = -2.21‰), through Pop2 (-1.11‰) to Pop3 (-0.71‰) (Tables 3.11a-c). Thus Pop2 *Rumina* shells have $\delta^{18}O$ values which lie between those from the other populations, but slightly nearer to those from Pop3 than Pop1. *Rumina* shells also show negative oxygen isotope values, whereas the data for *Helix aspersa* and *Cepaea nemoralis* are always positive and enriched by more than 2‰, compared to $\delta^{18}O$ values from shells of *Rumina*..

(d) Carbon isotopes

The mean carbon isotope data for the three populations of *Helix aspersa* are all fairly similar (Tables 3.9a-c), although the Pop2 mean $\delta^{13}C$ value (-20.16‰) is slightly more depleted than the mean values from Pop1 and Pop3 (-19.66 and -19.56‰, respectively).

Carbon isotope data from the three populations of *Cepaea nemoralis* are also fairly similar, ranging from -22.51 to -21.82‰ (Tables 3.10a-c). As was seen with *Helix aspersa*, the Pop2 mean $\delta^{13}C$ value (-22.51‰) is slightly more depleted than the mean value for Pop1 (-22.29‰), and Pop3 $\delta^{13}C$ data are the least depleted (mean value = -21.82‰). The carbon isotope data from *Cepaea* are consistently the most depleted of the three species investigated, by up to 2‰.

Pop2 *Rumina decollata* shells also show the most depleted mean $\delta^{13}C$ value of the three populations of this snail (-21.40‰). Pop1 snails have a mean $\delta^{13}C$ value of -21.09‰ (Tables 3.11a-c). However, and for this species only; Pop3 snails are considerably less depleted in $\delta^{13}C$, the mean value being -19.72‰, approximately 1.5‰ less depleted than *Rumina* from Pop1 and Pop2.

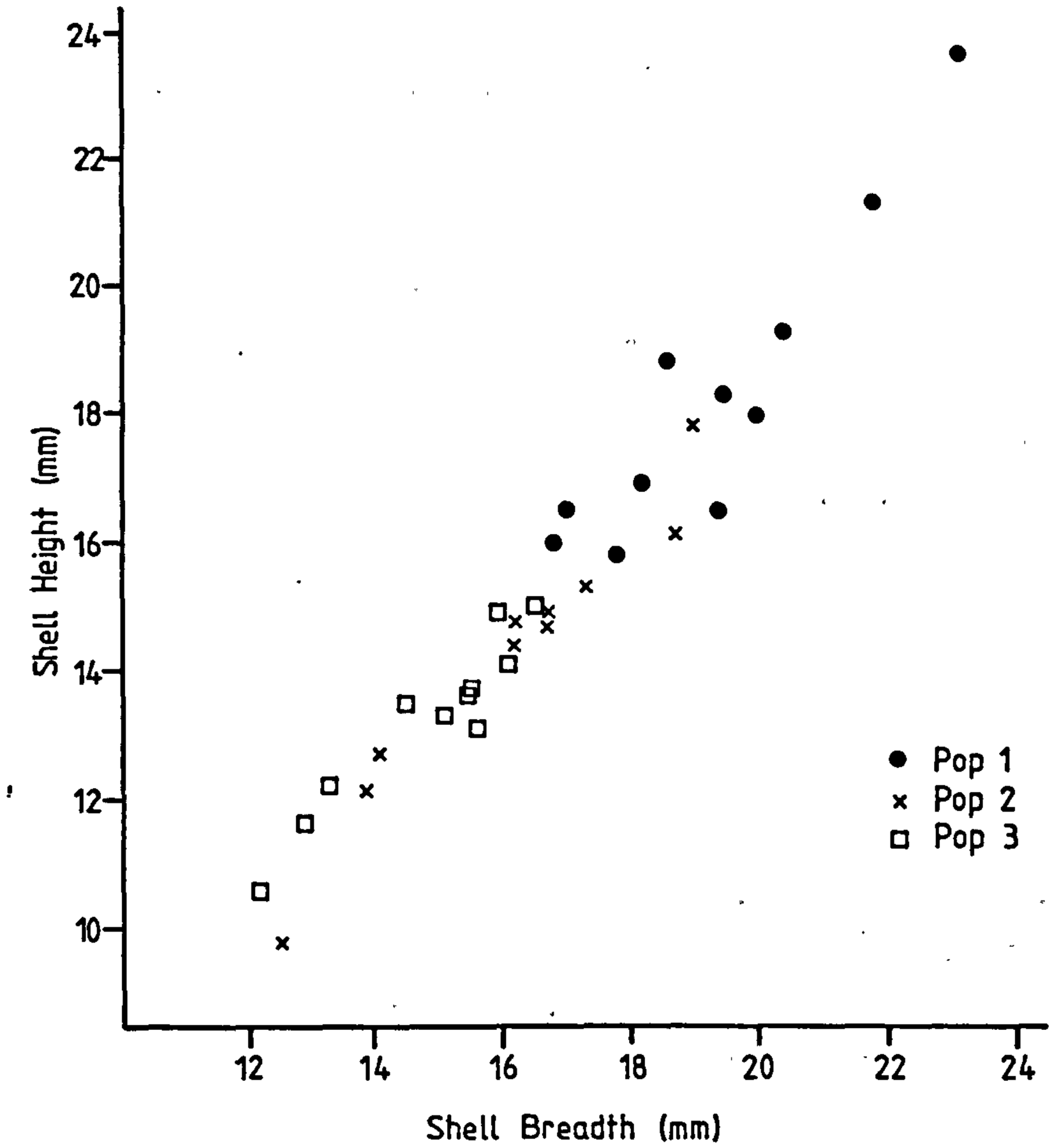


Figure 3.12a Height versus breadth (mm) for shells of *Helix aspersa* at the end of Experiment 2.

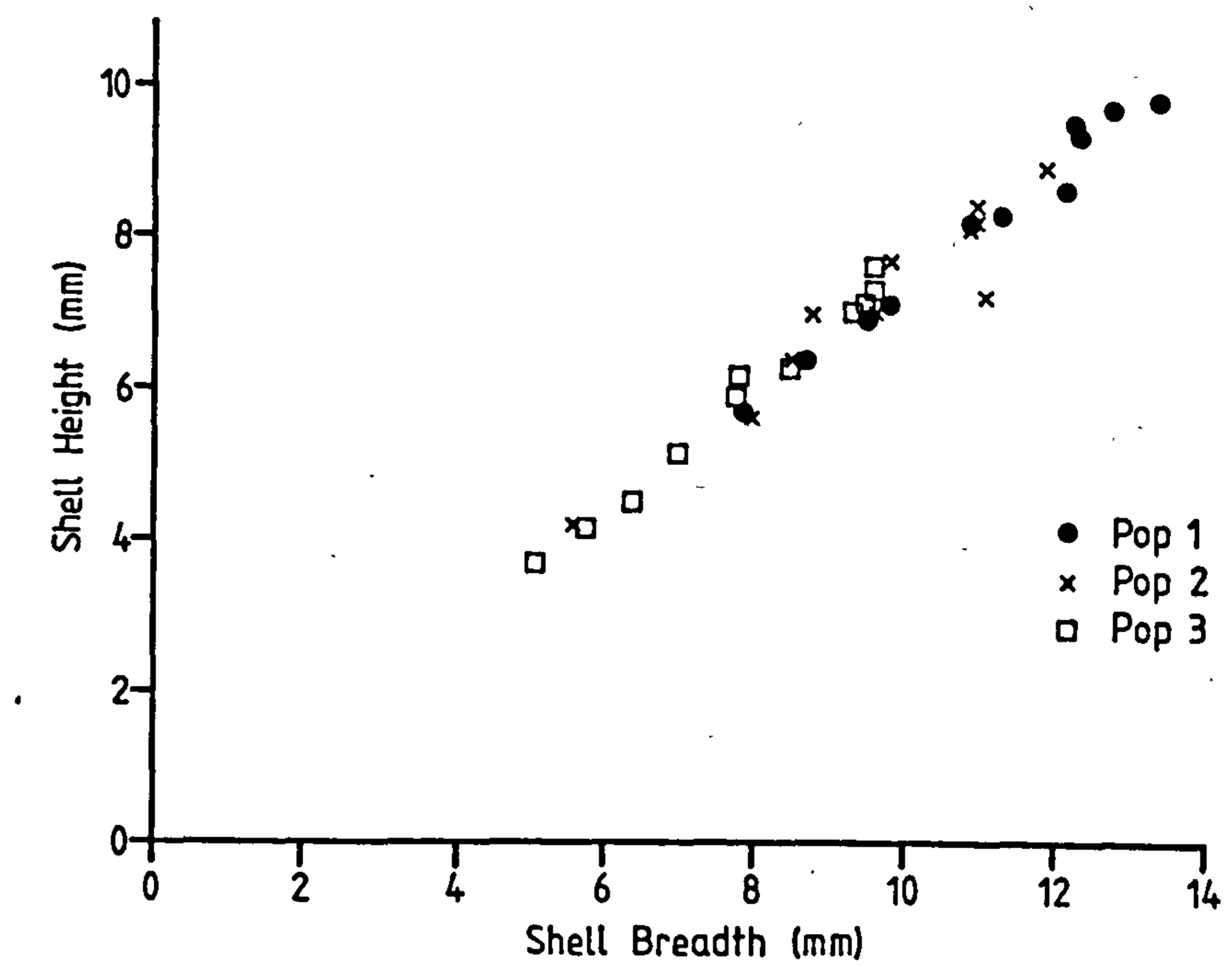


Figure 3.12b Height versus breadth (mm) for shells of *Cepaea nemoralis* at the end of Experiment 2.

3.6.3 Discussion of results : Experiment 2

(a) The effect of the diurnal cycle on the comparative growth of the snails.

To reiterate, Pop1 snails secreted the most shell, Pop3 snails consistently grew the least and Pop2 snails showed intermediate growth (Figures 3.12a and 3.12b).

The results suggest that under these experimental conditions, growth of shell material is positively influenced by increased temperature and/or by the presence of permanent fluorescent light.

No actual patterns of activity were recorded, however, note was made of the condition of the boxes prior to the next feeding during weeks four and five of the experimental period. At this time, Pop1 snails appeared to have consumed most, if not all, their food since the previous feeding, and for Pop1 *Helix* little, if any, faecal debris was visible, leading one to conclude that the snails may have also consumed the excreta. Comparatively more food remained in the Pop2 boxes, and Pop3 boxes contained more untouched food than Pop2 boxes. Therefore, Pop3 snails appeared to the least efficient at gathering food, which is reflected in their relatively smaller sized shells.

Although, for the Pop1 snails, temperature and light do not appear to have been limiting factors, by the latter stages of the experiment, competition for resources, availability of food and the density of the snails may have become limiting factors. This may explain why some of the snails grew less well than their counterparts *e.g.* Pop1 *Helix* specimen 6, reached 23.7 x 23.2mm and 700.7mg, whereas Pop1 *Helix* 8 reached 15.8 x 16.8mm and 198.3mg.

Bailey (1975), studied the locomotor activity of mature *Helix aspersa* by automatic recording in a soil-filled outdoor arena. Although activity was predominantly controlled by moisture, the nocturnal habitat of the snails was strongly evident with most activity occurring at night. Laboratory specimens also showed a peak in activity after an artificial dusk.

Cameron (1970), measured the activity of *Cepaea nemoralis, C. hortensis* and *Arianta arbustorum* with increasing temperature (from 0-26°C), at 100% relative humidity and with a 16 hours light and 8 hours dark daily cycle. With increasing temperature, all three species became increasingly nocturnal. Cameron stated, however, that the experimental conditions under which his snails were maintained were very artificial, but that the tendency for snails to be more active in the dark than in the light is also true for natural wild populations. Cameron also stated that his method of scoring activity (positive scoring if the snail was crawling or, if immobile its body was extended and the tentacles fully everted) took no account of the rate of activity or of the metabolic rate.

The reduction in activity rate over daylight hours noted by Bailey (1975) and Cameron (1970), is the result of the snails attempting to minimise potential water loss, since high daytime temperatures are often associated with saturation deficits. Therefore, under natural conditions snails would, necessarily, be more active in the dark. By contrast, in experiment 2, the environment was constantly very moist, and under these conditions, warmer temperatures and/or the presence of constant light have encouraged greater food gathering and shell growth. As the experimental snails were not kept under constantly light and constantly dark conditions over a range of temperatures, it is difficult to assess whether the increase in temperature, or, the presence of light was the predominant factor inducing greater growth. However, under natural conditions, snails are more active in the dark, suggesting that for the experimental snails the environmental temperature and moisture availability may have been more important than the lighting.

(b) The effect of the diurnal cycle on the stable isotope ratios of shells.

1) Oxygen isotopes

No consistent patterns were found between the oxygen isotopic compositions of the three snail populations. However, the isotopic effect resulting from the influence of the light/dark cycle, rather than from the combined effects of lighting and temperature, may be assessed by comparing the isotope data from this experiment with those collected from experiment 1 over similar temperatures. Pop1 data should be comparable to data from the W2 regime in experiment 1, which was carried out at the same temperature (22.3°C). The nearest temperature from experiment 1 to that of Pop3 is that from the C3 regime (15.7°C). Pop2 snails would have been exposed to an approximate mean temperature of 19.4°C. This is closest to the W3 temperature of 19.3°C from experiment 1. Table 3.12a-c compares the oxygen (and carbon, for later discussion) isotope data from experiments 1 and 2. The comparative oxygen isotope data have been plotted on Figures 3.13a-c for *Helix*, *Cepaea* and *Rumina* , respectively.

From Table 3.12a, Pop1 snails (at 22.3°C) were considerably less enriched in $\delta^{18}O$ than snails from the W2 regime in experiment 1 (also at 22.3°C). This was a surprising result since the snails from the two experiments were grown under the same temperature and lighting conditions. Pop1 *Helix aspersa* were 2.3‰ less enriched, *Cepaea nemoralis* by 2.6‰ and *Rumina decollata* by 3.0‰ than their counterparts from experiment 1. The W2 $\delta^{18}O$ values from experiment 1 were considerably more enriched in ^{18}O as compared to snails from the other 'warm-room' temperature regimes. Therefore, the results from experiment 2 may indicate that for some reason, anomalously enriched oxygen isotope ratios were obtained from all the W2 snails in experiment 1. However, some of the differences between the snails from the two experiments might be accounted for through primary differences in the environmental water or diet given to the snails.

The water given to the snails during this experiment had a mean $\delta^{18}O$ value of -8.55‰ SMOW. This compares with -9.13‰ for W2; -8.78‰ for W3; and -9.88‰ for C3 during experiment 1. This difference would give rise to slightly lower $\delta^{18}O$ equilibrium values in the snail shells from experiment 2 as compared to experiment 1, but the difference is not sufficient to account for the 2-3‰ discrepancy in $\delta^{18}O$ values actually recorded.

Considering the food given to the snails, the organic component of the diet (milk powder and oat cereal) was identical to that given in experiment 1, and the proportion of organic : inorganic components was the same (2:1). However, the oxygen isotope composition of the inorganic carbonate used in the diet was not the same in the two diets. In experiment 1, GPR1 was used ($\delta^{18}O$ = -5.02‰) whereas in experiment 2, GPR2 was used ($\delta^{18}O$ = -28.40‰) therefore being much more depleted in ^{18}O. The Pop1 snail shells from experiment 2 also contained less ^{18}O than experiment 1 snails. This suggests that the oxygen isotopic composition of inorganic carbon in a snail's diet may exert some influence upon the resultant isotopic composition of the snail shell. This effect might be more discernible where the inorganic carbon had an anomalously high, or, as in this case, low oxygen isotope composition.

A similar depletion in Pop2 and Pop3 snails as compared to their counterparts in experiment 1 is not evident. However, these snails were not grown under identical environmental conditions as their correlatives in experiment 1, and the differences in oxygen isotope ratios between the two data sets may

	$\delta^{13}C$ ‰ Expt. 1 W2	$\delta^{13}C$ ‰ Expt. 2 Pop 1	$\delta^{18}O$ ‰ Expt. 1 W2	$\delta^{18}O$ ‰ Expt. 2 Pop 1
Helix	-13.42	-19.66	3.88	1.56
Cepaea	-13.11	-22.29	4.40	1.77
Rumina	-13.63	-21.09	0.78	-2.21

Table 3.12a Comparative isotope data from Experiment 1 W2 (22.3°C) and Experiment 2 Pop 1 (22.3°C).

	$\delta^{13}C$ ‰ Expt. 1 W3	$\delta^{13}C$ ‰ Expt. 2 Pop 2	$\delta^{18}O$ ‰ Expt. 1 W3	$\delta^{18}O$ ‰ Expt. 2 Pop 2
Helix	-12.64	-20.16	-0.98	1.35
Cepaea	-12.31	-22.51	-1.23	2.51
Rumina	-12.93	-21.40	-3.79	-1.11

Table 3.12b Comparative isotope data from Experiment 1 W3 (19.3°C) and Experiment 2 Pop 2 (19.4°C).

	$\delta^{13}C$ ‰ Expt. 1 C3	$\delta^{13}C$ ‰ Expt. 2 Pop 3	$\delta^{18}O$ ‰ Expt. 1 C3	$\delta^{18}O$ ‰ Expt. 2 Pop 3
Helix	-12.07	-19.56	-2.72	1.46
Cepaea	-12.51	-21.82	-1.97	1.73
Rumina	-12.53	-19.72	-3.71	-0.71

Table 3.12c Comparative isotope data from Experiment 1 C3 (15.7°C) and Experiment 2 Pop 3 (16.5°C).

Table 3.12a-c Comparative isotope data from Experiments 1 and 2

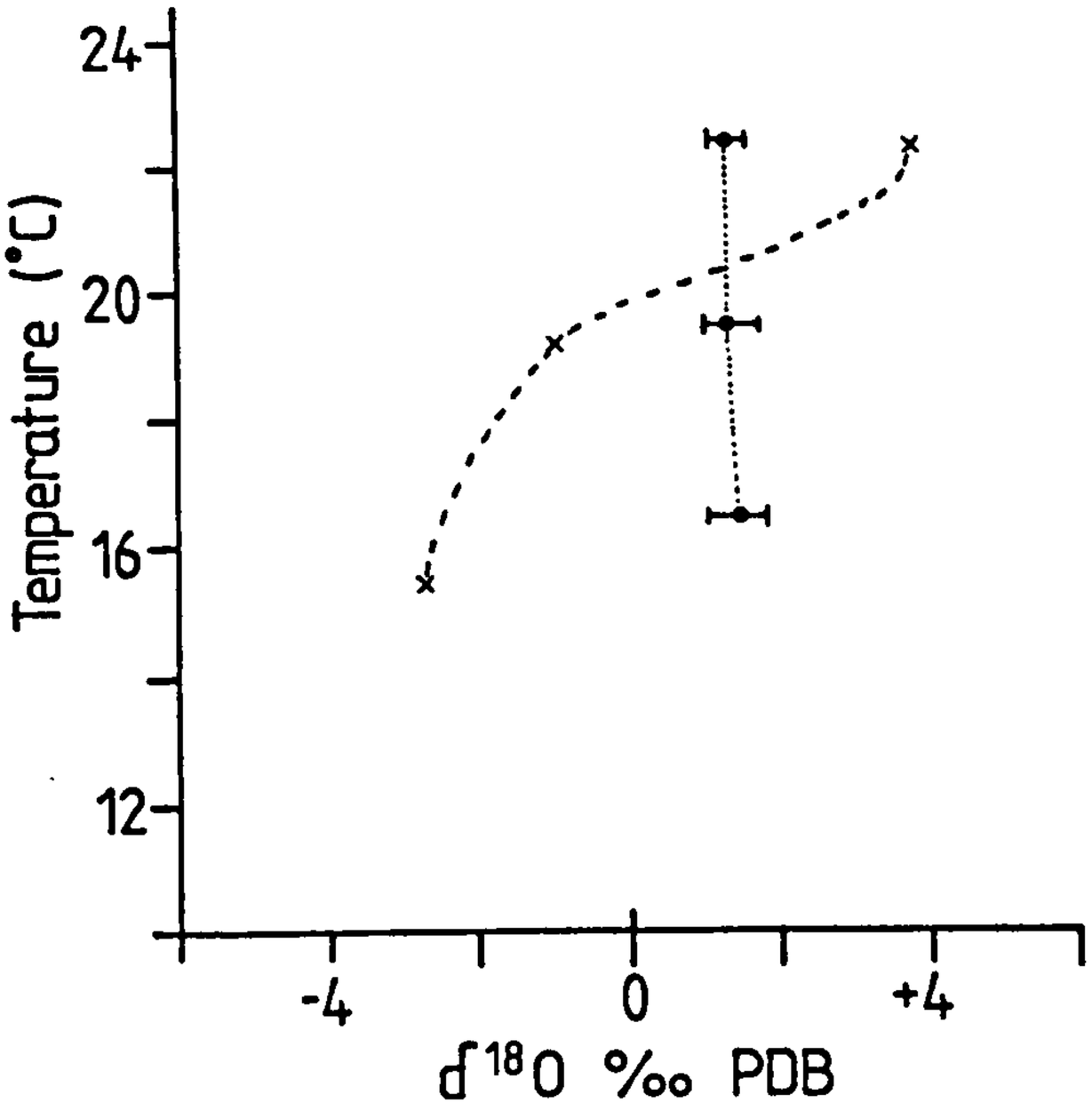


Figure 3.13a *Helix aspersa*

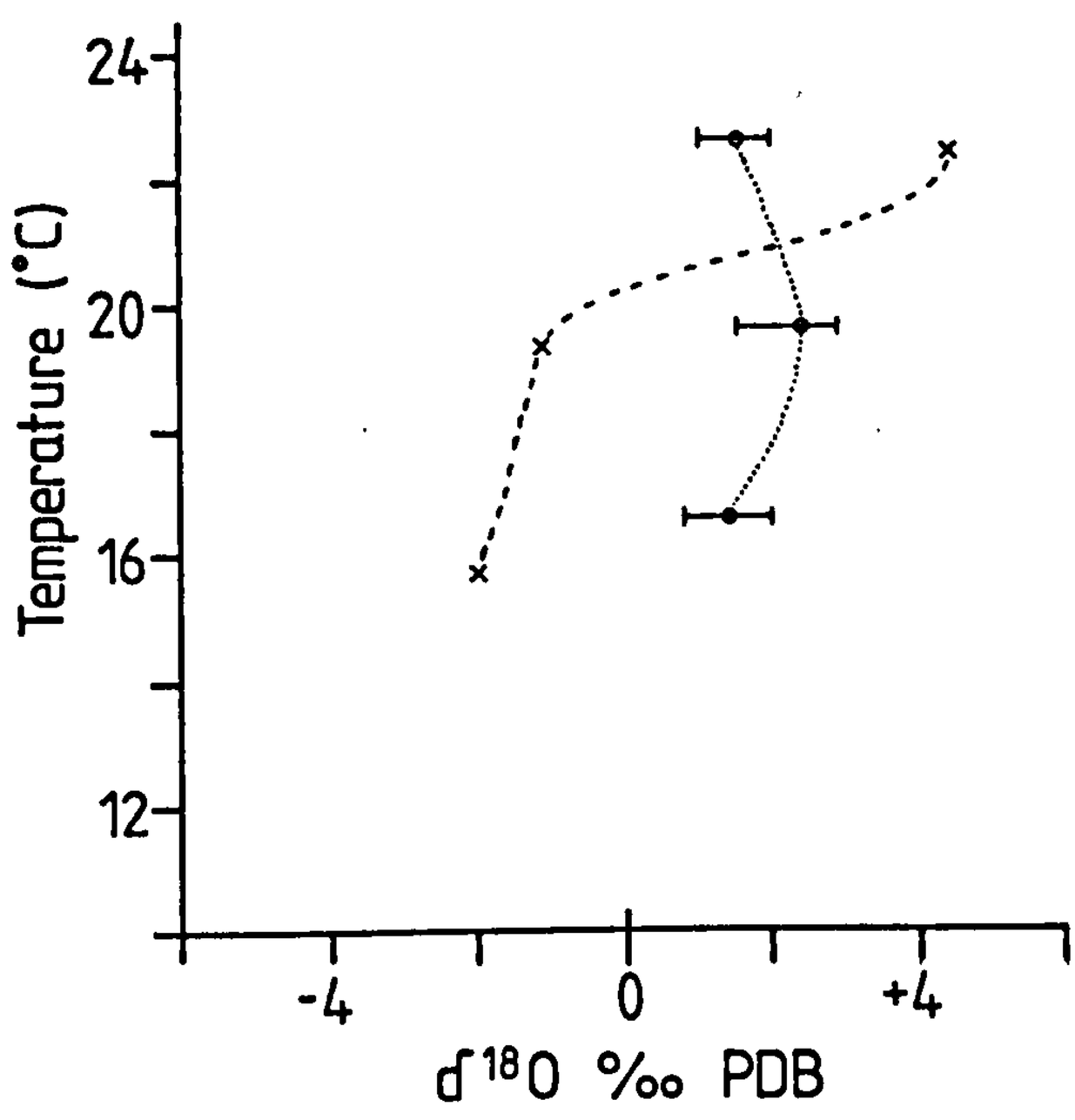


Figure 3.13b *Cepaea nemoralis*

Figure 3.13a-c Oxygen isotope trends with temperature : Experiment 2. The range of values at each temperature is shown and lines are drawn through mean values; also shown are mean values of shells grown under comparable temperatures in Experiment 1, for (a) *Helix aspersa*, (b) *Cepaea nemoralis* and (c)*Rumina decollata*.

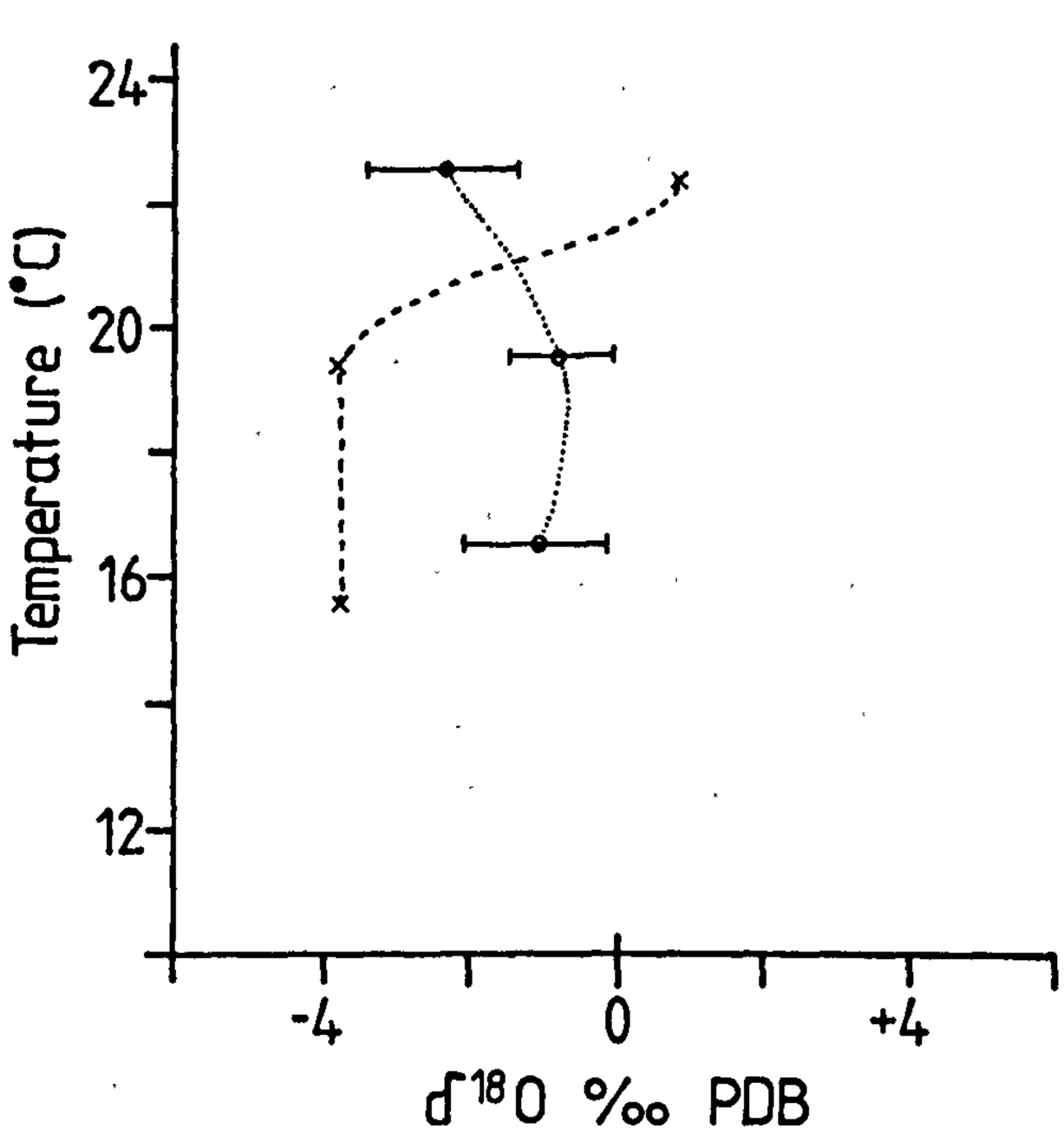


Figure 3.13c Oxygen isotope trends with temperature for shells of *Rumina decollata* from Experiment 2, with data from comparable temperatures in Experiment 1.

reflect the effects of a warm - cool and/or a light - dark cycle, or permanent darkness. Any isotopic depletion related to the isotopic composition of the GPR2 is masked by the apparent enrichment caused by the introduction of darkness, or by diurnal variation in light.

Pop2 snail shells were, in contrast to Pop1, considerably more enriched in ^{18}O than those from W3 in experiment 1 which were grown at a temperature similar to the mean temperature experienced by snails of Pop2 (19.4°C mean temperature for Pop2 snails and 19.3°C for W3 snails). Pop2 shells of *Helix aspersa* were enriched by 2.3‰; *Cepaea nemoralis* by 3.8‰; and *Rumina* by 2.7‰ compared with W3 snails from experiment 1 (Table 3.12b).

If the Pop2 snails manufactured the majority of their shell during the warmer part of their daily cycle (under the same conditions as Pop1), this may explain why their oxygen isotope ratios are not close to W3 snails in experiment 1, but are nearer to the ^{18}O enriched values seen for W2 in experiment 1 and measured for Pop1 snails in this experiment.

The oxygen isotope enrichment of Pop2 snails as compared to W3 snails from experiment 1 may also be the result of the introduction of the lower temperature in darkness. If the snails were less active during the cooler dark part of the daily cycle this might lead to increased metabolic enrichment of oxygen isotopes within the snails' bodies during the dark phase.

Thus, the enriched oxygen isotope values of Pop2 snails as compared to W3 snails form experiment 1, may be the result of increased evaporation during the warm and lighted part of the daily cycle, coupled with increased metabolic enrichment during the dark cooler phase.

Pop3 snails, kept in constant darkness, also have shells considerably more enriched in ^{18}O than C3 snails from experiment 1. *Helix aspersa* being 4.2‰ more enriched: *Cepaea nemoralis* 3.7‰ and *Rumina decollata* being 3.0‰ more enriched than their counterparts from experiment 1 (Table 3.12c).

The slight difference in temperature between Pop3 (16.5°C) and C3 (15.7°C) would not account for the large isotopic difference between the two groups of snails. The enriched oxygen isotope ratios of the Pop3 snails are likely to be the result of an increase in metabolic enrichment during periods of inactivity in this cool and permanently dark environment. The idea of decreased locomotor activity for food gathering, and longer periods of cessation in activity when snail body waters would become metabolically enriched, is supported by the fact that the Pop3 snails grew least of the three populations of snails in this experiment (see previous sub-section). However, the isotopic compositions of Pop1 snails, which were less enriched than those from experiment 1, were thought to be at least partially the result of ^{18}O depleted inorganic carbonate becoming incorporated in the snails' shells. If this inorganic carbonate also affected the Pop2 and Pop3 snails in a similar way, it would have had the effect of reducing the amount of enrichment as compared to the experiment 1 counterparts. This suggests that the isotopic effect of cyclic light and dark or, permanent darkness, upon the resultant isotopic composition of the snails' shells could have been greater even than the 2-4‰ effect noted for the Pop2 and Pop3 snails. Figures 3.13a-c show oxygen isotope data *versus* temperature, from this experiment and from experiment 1, for the species *Helix aspersa*, *Cepaea nemoralis* and *Rumina decollata*. The large differences between the Pop2 and Pop3 shells compared with the data from shells grown over comparable temperatures in experiment 1, is clearly evident on these figures.

2) Oxygen isotope equilibrium values

It has been suggested that the presence of continual darkness, and possibly a daily cycle of light and darkness, may have induced a pronounced oxygen isotope effect upon the snails. This 'environmental' effect, related to the artificiality of the experimental conditions, may be assessed if oxygen isotope equilibrium values for the aragonite shells are calculated at each temperature. The greater the enrichment in shell ^{18}O, over equilibrium values, the greater the degree of evaporative or metabolic isotope enrichment resulting from the experimental conditions. The deviation from equilibrium values may again be compared to data from the snails grown in experiment 1. Equilibrium values have been calculated using the equations in section 3.3.2, and a mean $\delta^{18}O$ water value of -8.55‰ SMOW. The calculated values for the three populations, and the data from experiment 1 (extracted from Table 3.7) are shown in Tables 3.13a-c.

From these tables, it can be seen that only Pop1 data from experiment 2 are nearer to equilibrium values (*i.e.* δ values are smaller) than comparable data from experiment 1. Both Pop2 and Pop3 data (Tables 3.13b and 3.13c) have greater δ values than their counterparts from experiment 1, indicating that the nature of the experimental conditions (*i.e.* the introduction of darkness) has led to a greater isotope effect. Therefore, maintaining the snails in continual darkness in cool conditions, or in a daily cycle of warmer conditions in light and darkness, does not appear to have aided in the reduction of the artificiality of the environments, in terms of the resultant oxygen isotope ratios of the shells produced under such conditions. An alternative explanation could be that all the experimental snails are subject to a marked biogenic fractionation (vital effect) of oxygen isotopes, and the more 'natural' conditions during experiment 2, have allowed the snails to achieve this fractionation more completely. The lower δ values for Pop1 as compared to W2 snails from experiment 1, are thought to be the result of the influence of the isotopic composition of the carbonate in the snails' diet, as outlined above. Because of this effect, it is difficult to assess quantitatively the influence of the changing diurnal cycle of light and temperature, and the effect of continual darkness upon the oxygen isotopic compositions of the shells.

In summary, Pop1 snails appear to have less positive $\delta^{18}O$ values than might be expected, from the results of experiment 1, due to the effect of ^{18}O depleted inorganic carbon in the snails' diet.

Pop2 snails are more enriched in ^{18}O than might be expected, due to an increased environmental effect with greater evaporation effects during the artificial daylight hours at warmer temperatures; and/or increased metabolic enrichment due to inactivity during the hours in darkness.

Pop3 snails are also considerably more enriched than might be expected due to a greater amount of metabolic enrichment, as the permanent darkness appears to have had a negative effect upon the growth potential of this group of snails.

3) Carbon isotopes

The very depleted $\delta^{13}C$ values (generally around -21‰) as compared to experiment 1 values (generally around -12.5‰), Tables 3.12a-c, are a reflection of the ^{13}C depleted inorganic carbon (GPR2 $\delta^{13}C = -43.35$‰) included in the snails' diet for experiment 2. The other sources of carbon available to the snails were the same as for experiment 1. This indicates that inorganic carbon in a snail's diet plays an important role in shell secretion.

	Expt. 1 W2 22.3°C			Expt. 2 POP 1 22.3°C		
	$\delta^{18}O$ Arag. Meas.	Eqm. $\delta^{18}O$ Arag. Calc.	Meas. - Calc. (Δ)	$\delta^{18}O$ Arag. Meas.	Eqm. $\delta^{18}O$ Arag. Calc.	Meas. - Calc. (Δ)
Helix	3.88	-9.46	13.34	1.56	-8.88	10.44
Cepaea	4.40	-9.46	13.86	1.77	-8.88	10.65
Rumina	0.78	-9.46	10.24	-2.21	-8.88	6.67

Table 3.13a Experiment 1 W2 (22.3°C), and Experiment 2 Pop1 (22.3°C).

	Expt. 1 W3 19.3°C			Extp. 2 POP 2 19.4°C		
	$\delta^{18}O$ Arag. Meas.	Eqm. $\delta^{18}O$ Arag. Calc.	Meas. - Calc. (Δ)	$\delta^{18}O$ Arag. Meas	Eqm. $\delta^{18}O$ Arag. Calc.	Meas. - Calc. (Δ)
Helix	-0.98	-8.47	7.72	1.35	-8.26	9.61
Cepaea	-1.23	-8.47	7.24	2.51	-8.26	10.77
Rumina	-3.79	-8.47	4.68	-1.11	-8.26	7.15

Table 3.13b Experiment 1 W3 (19.3°C), and Experiment 2 Pop2 (19.4°C).

	Expt. 1 C3 15.7°C			Extp. 2 POP 3 16.5°C		
	$\delta^{18}O$ Arag. Meas.	Eqm. $\delta^{18}O$ Arag. Calc.	Meas. - Calc. (Δ)	$\delta^{18}O$ Arag. Meas	Eqm. $\delta^{18}O$ Arag. Calc.	Meas. - Calc. (Δ)
Helix	-2.72	-8.80	6.08	1.46	-7.64	9.10
Cepaea	-1.97	-8.80	6.83	1.73	-7.64	9.37
Rumina	-3.71	-8.80	5.09	-0.71	-7.64	6.93

Table 3.13c Experiment 1 C3 (15.7°C), and Experiment 2 Pop3 (16.5°C).

Table 3.13a-c Comparative mean oxygen isotope values (measured) and calculated equilibrium values (see equation 3.1 and section 3.3.2 for method) from Experiments 1 and 2.

The input of both atmospheric, and metabolic carbon dioxide will also have provided carbon for shell secretion, but from these results alone, it is difficult to evaluate whether metabolic or atmospheric carbon dioxide was the more important source of carbon for shell secretion. More depleted $\delta^{13}C$ values could indicate a greater input of inorganic carbon (the GPR2) although, as the proportion of this inorganic carbon in the diet was always constant, this is probably unlikely. Therefore, a trend towards depleted $\delta^{13}C$ values would imply that metabolic carbon dioxide was the more important source of carbon for shell secretion, as opposed to atmospheric carbon dioxide.

$\delta^{13}C$ values from Pop3 snails were not as depleted as those from the other populations - for all species but especially for *Rumina decollata*. This may indicate that for Pop3 snails, atmospheric carbon dioxide (equilibrium value in aqueous bicarbonate = approximately +0.5‰ see section 3.3.3) was a more important constituent than metabolic carbon dioxide (close to -27‰ in aqueous bicarbonate). This would occur if the snails were receiving a larger proportion of carbon dioxide by direct uptake from, or exchange with, the atmosphere, rather than from metabolism of organic foodstuffs. Pop3 snails grew least and larger amounts of unconsumed food remained between feedings, as compared to the other populations, supporting this idea.

(c) The isotopic composition of individual shells and rate of shell growth

From the preceding sub-section it appears that, to some extent, the relative growth of the three populations (in terms of final size and weight of shell secreted) may have influenced the resultant isotopic compositions of the shells. If rates of growth were enhanced or diminished by the prevailing environmental conditions, this may have increased the potential for metabolic effects to influence the shell isotopic composition.

Berger *et al.* (1978), have investigated oxygen and carbon isotopes in several genera of planktonic foraminifera, located in cores from deep-sea carbonates in the west equatorial Pacific. These authors have compared the size of tests to both $\delta^{18}O$ and $\delta^{13}C$ data. They were able to show that, in general, the larger shells tended to be enriched in ^{18}O. This trend was explained in terms of several factors related to the depth and temperature of the growth habitat affecting the size of adult tests, and that the degree of isotopic disequilibrium might alter during the growth cycle. A relationship was also found between carbon isotopes and test size whereby, the amount of ^{13}C increased with size. This was not thought to be related to the inferred habitat depth, but marked a decrease in the 'vital effect' disequilibrium with size, and hence, rate of growth. During active growth (small test size), the test carbon isotopic composition would be primarily influenced by metabolic processes. As tests reached their slower, or terminal growth phase, metabolism would be greatly reduced, and the shell carbon isotope ratios would move towards equilibrium values.

From the results of experiment 1 and those discussed so far for experiment 2, for a population of land snails grown under identical conditions, the larger individuals might be expected to be more enriched in ^{18}O as a result of metabolic effects during and in between bouts of activity. Tiny snails might also be expected to be relatively enriched in ^{18}O, if they only periodically ingested metabolites, and thus underwent a greater proportion of metabolic enrichment. Conversely, for carbon isotopes, larger snails within a population might be expected to be more depleted in ^{13}C where metabolic carbon dioxide was a relatively more important source of carbon than atmospheric carbon dioxide.

Atmospheric carbon dioxide would become more important where locomotor and metabolic activity were restricted, producing smaller snails with less depleted $\delta^{13}C$ values.

Within each population of the experimental snails, a range of sizes and weights of shells was achieved. Therefore, it was decided to investigate possible relationships between the rates of growth of individual shells of *Helix aspersa* and *Cepaea nemoralis* from each population, and their stable isotopic compositions. Shells of *Rumina decollata* could not readily be investigated in this manner as, after killing, only the latter portions of the tapering cylindrical shells remained.

For *Helix aspersa* rate of growth was calculated as:

$$\text{Rate of Growth (mg per day) (}\textit{Helix}\text{)} = \frac{\text{Final Wt. - Initial Wt.}}{\text{Growth Period (days)}} \qquad (3.3)$$

The initial shell weight of *Helix aspersa* was calculated as the mean shell weight (range 4.8 to 11.0mg, standard deviation = 1.92mg) of fifteen additional juvenile *Helix aspersa* removed from the base populations at the onset of the experiment. The additional *Helix* were immediately killed, and after air drying, the shells were individually weighed. The mean initial shell weight was 8.55mg. The total growth period was six weeks, *i.e.* 42 days.

Insufficient juveniles of *Cepaea nemoralis* were available to carry out the experiment and to kill certain individuals so as to assess a mean initial weight. Therefore the rate of growth was estimated from the data on *Helix aspersa* in the following way. The mean final weight of the three populations of *Helix aspersa* was 248.9mg. Thus the mean growth increment of *Helix* would be 248.9 - 8.55mg which is 240.35mg. 8.55mg is 3.43% of this growth increment. Therefore, as an approximation, the initial shell weight of *Cepaea nemoralis* was taken as 3.43% of the final weight of each shell.

Tables 3.14a-c show calculated rates of growth and oxygen and carbon isotope data for Pop1, Pop2 and Pop3 *Helix aspersa*, respectively. For this species, the mean rate of growth for Pop1 snails was 8.5mg per day, for Pop2 5.91mg per day and for Pop3 3.67mg per day. Tables 3.15a-c show similar data but for the three populations of *Cepaea nemoralis*. In this case the mean rate of growth was for Pop1 snails 2.33mg per day, for Pop2 1.94mg per day and for Pop3 1.02mg per day.

To investigate the relationship between rate of growth and shell isotopic composition for each population of *Helix* and *Cepaea*, regression analyses were performed, where rate of growth was the independent variable (X), and the isotope in question was the dependent variable (Y).

thus, $$Y = a + bX \qquad (3.4)$$

and $$a = Y - bX \qquad (3.5)$$

and $$b = \frac{\Sigma XY - \bar{X}.\Sigma Y}{\Sigma X^2 - \bar{X}.\Sigma X} \qquad (3.6)$$

Helix POP 1	Rate of Growth mg per day	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
1	8.84	-19.85	1.33
2	5.18	-18.14	1.23
3	7.53	-19.57	1.35
4	8.23	-19.85	1.58
5	11.17	-18.90	1.37
6	16.48	-21.16	1.76
7	6.06	-19.74	1.96
8	4.52	-20.08	1.90
Means	5.91	-19.66	1.56

Table 3.14a

POP 2	Rate of Growth mg per day	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
1	6.28	-20.59	1.77
2	6.41	-20.20	1.25
3	5.58	-20.47	1.62
4	5.28	-19.77	1.07
5	9.14	-20.44	1.61
6	7.95	-20.22	1.26
7	2.23	-19.89	1.24
8	4.42	-20.68	1.00
Means	5.91	-20.16	1.35

Table 3.14b

POP 3	Rate of Growth mg per day	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
1	4.74	-19.35	1.11
2	2.78	-19.18	1.32
3	4.47	-20.16	1.47
4	2.82	-19.59	1.30
5	4.20	-19.69	1.87
6	3.89	-19.49	1.42
7	4.88	-19.89	1.48
8	1.74	-19.14	1.72
Means	3.67	-19.56	1.46

Table 3.14c

Table 3.14a-c Calculated growth rates, carbon and oxygen isotope data for *Helix aspersa* , Experiment 2. (For calculation of growth rates see equation 3.3, section 3.6.3c).

Cepaea POP 1	Rate of Growth mg per day	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
1	2.42	-22.30	1.82
2	2.25	-21.90	2.01
3	3.40	-22.50	1.95
4	1.03	-22.51	1.07
5	2.16	-22.21	1.79
6	3.14	-22.17	2.21
7	1.88	-22.44	1.54
Means	2.33	-22.29	1.77

Table 3.15a

POP 2	Rate of Growth mg per day	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
1	2.30	-22.55	2.39
2	2.45	-22.41	2.69
3	2.15	-22.95	2.86
4	2.04	-21.79	2.55
5	1.95	-23.25	1.58
6	1.56	-22.10	2.43
7	1.01	-22.53	3.05
Means	1.94	-22.51	2.51

Table 3.15b

POP 3	Rate of Growth mg per day	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
1	1.28	-20.92	1.62
2	1.16	-21.16	1.98
3	1.10	-20.82	1.99
4	1.39	-22.16	0.85
5	0.90	-22.36	1.00
6	0.88	-21.88	1.83
7	0.43	-22.96	2.87
Means	1.02	-21.82	1.73

Table 3.15c

Table 3.15a-c Calculated growth rates, carbon and oxygen isotope data for Cepaea nemoralis, Experiment 2.

Correlation coefficients (r values) have been calculated where

$$r = \frac{\Sigma XY - \bar{X}.\Sigma Y}{\sqrt{(\Sigma X^2 - \bar{X}.\Sigma X)\ (\Sigma Y^2 - \bar{Y}.\Sigma Y)}} \qquad (3.7)$$

Values for the intercept (a); the gradient (b); and the correlation coefficient (r); for each population of *Helix* and *Cepaea* are shown in Tables 3.16a and 3.16b.

The regression analyses performed on the data are not consistently in agreement with the 'expected' trends and generally the correlation coefficients for both carbon and oxygen are too low to show any significant correlation between rate of growth and shell isotopic composition. Student 't" tests of the correlation coefficients ('r' values shown in Tables 3.16a and 3.16b) showed that at the 0.05 level (95% confidence limits) only one correlation (that for *Cepaea*, between rate of growth and shell oxygen isotopic composition) was statistically significant. In all other cases the correlations were not significantly different from zero and could have occurred by chance.

The poor correlation found between isotopic composition and rate of shell growth may be the result of several factors, including:

i) The data sets may contain insufficient points, bearing in mind the variation in both isotopic composition and rate of growth. Therefore, this statistical method of analysis may be inappropriate.

ii) One or two extreme isotope values may grossly effect the overall trend, especially if the data set is limited. As a result, the isotopic variation between individual snails could be as great as that between populations subject to different environmental conditions.

iii) The oxygen and carbon isotope compositions of the shells are not directly linked to the rate of growth of individual snails within a population, resulting from differential metabolic effects through the life history of the snail.

3.6.4 Summary

1) Under the conditions in which the experimental snails were maintained, the growth rate of shell is positively influenced by the presence of permanent light and higher ambient temperatures.
2) The oxygen isotopic composition of the three populations were similar, with no consistent pattern between them. This suggests that the individual variation between these snails may be greater than any variation caused by environmental factors. However, comparison of the oxygen isotope data from this experiment with those produced at similar temperatures in experiment 1, allowed the isotopic effects as a result of the change in lighting conditions to be separated from those relating to changes in temperature and light. Pop2 (mean temperature 19.4°C) and Pop3 (16.5°C) snails were enriched by 2-4‰ compared with snails from equivalent temperatures in experiment 1, as a result of a greater metabolic effect with daily changes in lighting and temperature, or, with conditions of constant darkness. Therefore the oxygen isotope effect was not reduced by attempting to make the environmental conditions experienced by the snails less artificial. The Pop2 and Pop3 snails also showed a greater difference between shell $\delta^{18}O$ values and equilibrium values based on the isotopic composition of their environmental waters, as compared with their

Helix	$\delta^{18}O$ ‰			$\delta^{13}C$ ‰		
	a	b	r	a	b	r
POP 1	1.56	0.00	0.01	-18.59	-0.13	0.56
POP 2	1.02	0.057	0.43	-19.99	-0.05	0.33
POP 3	1.62	-0.04	0.20	-18.80	-0.21	0.68

Table 3.16a

Cepaea	$\delta^{18}O$ ‰			$\delta^{13}C$ ‰		
	a	b	r	a	b	r
POP 1	0.81	0.413	0.88	-22.40	0.048	0.17
POP 2	2.98	-0.25	0.26	-22.34	-0.09	0.09
POP 3	3.23	-1.47	0.69	-23.42	1.573	0.65

Table 3.16b

Table 3.16a-b Regression analysis and correlation coefficients between growth rates and shell isotopes for (a) *Helix aspersa* and (b) Cepaea nemoralis. a = intercept, b = gradient, r = correlation coefficient (see equations 3.4 to 3.7 section 3.6.3c).

counterparts in experiment 1. Pop1 snails (22.3°C) were, on the other hand, more depleted in ^{18}O than snails from experiment 1, even though both sets of snails were grown under the same conditions of temperature and lighting. It is thought that the resultant shell composition may have been influenced by the oxygen isotopic composition of the carbonate in the snail's diet, which during experiment 2 had a very depleted oxygen isotopic composition (around -28.4‰).

3) No consistent patterns in shell carbon isotope values were seen between shells from the three test populations. However, the depleted $\delta^{13}C$ values in this experiment as compared to those from experiment 1, are thought to reflect the ^{13}C depleted carbonate in the snail's diet (for experiment 2, $\delta^{13}C$ = -43.35‰ whereas for experiment 1 $\delta^{13}C$ = -13.09‰).

4) No statistically significant relationship was found between the isotopic composition of individual shells (carbon and oxygen) and their rates of growth.

3.7 EXPERIMENT 3 : FURTHER INVESTIGATION OF THE ISOTOPIC COMPOSITION OF SNAILS SUBJECT TO DIURNAL CYCLES OF LIGHTING AND TEMPERATURE, MAINTAINED UNDER ALTERNATIVE ENVIRONMENTAL CONDITIONS

3.7.1 Introduction and aims

Experiment 2 was designed primarily to reveal isotopic differences between snails kept at two extreme temperatures, and snails moved between the two on a daily basis. The comparison of the results with those of experiment 1 has shown that the stable isotopic compositions of the snail shells were influenced strongly by the presence, or absence of light, along with the change in temperature. It was decided, therefore, to investigate further the effects of the presence or absence of light upon the patterns of growth and shell isotopic compositions.

The details of this third experiment are presented in section 2.4.4; the following outlines the procedure. Seven populations, P1 to P7, (no replicates) of four species (*Helix aspersa, Cepaea nemoralis, Rumina decollata* and *Ferussacia folliculus*) were set up in such a way that the light reaching the boxes of snails was controlled by enclosing the boxes of snails within black opaque or, transparent polythene bags. Populations were maintained in the 'warm' and 'cool room' (22.3 and 14.5°C, respectively); some were moved between the two extremes on a daily basis; and one population in the 'warm room' was transferred between a clear and an opaque polythene bag every twelve hours. See Table 2.5 for a summary of the conditions of the experimental populations.

Two control populations (P8 and P9) of *Helix aspersa* and *Rumina decollata* were maintained as in the previous experiments, outside the polythene bags; P8 in the 'warm room' at 22.3°C and P9 in the 'cool room' at 14.5°C. Both control populations received permanent fluorescent light as the room lights were left on continually. Insufficient juvenile *Cepaea nemoralis* were available to stock both test and control populations of this species.

Differences between measured oxygen isotope ratios in the experimental snails, and calculated equilibrium values were, in Experiments 1 and 2, interpreted as a consequence of evaporation and metabolic effects. By housing the boxes of snails within polythene bags, sealed with 'twist ties' (from which the boxes were only removed to allow feeding and watering), it was found that relative humidities within the bags, and even more so within the boxes inside the bags, were kept at near saturation levels (section 2.4.4c). Thus the potential for evaporation was minimised. An additional aim of the experiment was, therefore, to evaluate the differences between the measured shell oxygen isotope ratios and calculated equilibrium values under the conditions of minimal evaporation. This would give, in effect, a quantitative assessment of the metabolic effect characteristic of these species of landsnail.

3.7.2 Results : Experiment 3

(a) Outline

Stable isotopic analyses were carried out on shells of five *Helix aspersa*, three *Cepaea nemoralis* and three *Rumina decollata* from each of P1 to P7. The last whorls of between eight and twelve individuals from each population of *Ferussacia folliculus* (those surviving the six week experimental period) were amalgamated to give a single isotopic sample for each *Ferussacia* population (P1 to P7). Five additional *Helix aspersa* shells and three *Rumina decollata* shells from each of the control populations (P8 and P9) were also analysed for their oxygen and carbon isotope ratios.

As in experiment 2, dimensions (height x breadth, mm, and weight, mg (where possible)) of all snails killed at the end of the six week growth period were measured. For *Rumina*, the diameter of the last whorl was measured, but the fragments of shell were not weighed. *Ferussacia* shells from all seven populations were of comparable size. Initial weights and dimensions of comparable shells were not measured in this experiment.

The data are shown in full in Appendix 1. Table 3.17a comprises mean data for each population (mean temperatures, isotopic analyses and dimensions) of *Helix aspersa*. Tables 3.17b-d include similar data for *Cepaea nemoralis*, *Rumina decollata* and *Ferussacia folliculus* .

Samples of the water given to the snails were taken, at the beginning of each of the first four weeks of the six week experimental period, for oxygen isotope analysis. On each occasion a pair of samples was taken. The results of these eight analyses are shown in Table 3.18, and gave an overall mean oxygen isotope value for the water given to the snails during experiment 3, of -9.0‰ SMOW.

The stable isotopic composition of the inorganic carbon (CHALK) given to the snails in their mixed diet (Food 3) was measured, such that $\delta^{18}O$ CHALK = -3.33‰ PDB, and $\delta^{13}C$ CHALK = +2.24‰ PDB.

(b) Shell growth

Figure 3.14a is a plot of the final size (in this case, mean breadth) against mean environmental temperature for each population of *Helix aspersa*, *Cepaea nemoralis* and *Rumina decollata*. It also includes data from the two control populations of *Helix* and *Rumina*. For referral, the key for this figure, and succeeding figures, shows the status (temperature and type of bag) of the seven test and and two control populations. Figure 3.14b plots the mean final weight of *Helix* and *Cepaea* against the mean temperature experienced by the each population of snails. The data shown in these figures may be found in Tables 3.17a-c.

For all three species shown in Figure 3.14a, there is an overall increase in size with increasing temperature. Within each temperature regime there are also differences between species. P1 *Helix* (opaque bag) grew more than P2 *Helix* (clear bag), whereas for *Cepaea* , P2 snails in the clear bag grew more than P1. P1 and P2 were housed in the 'warm room'. For the snails housed in the 'cool room', (P6 and P7), the relationship between *Helix* and *Cepaea* is reversed, with P6 *Helix* (opaque bag) being smaller than P7, and for *Cepaea* , P6 snails grew more than P7. Therefore, for *Cepaea* in warm conditions (22.3°C), higher rates of growth were achieved in lit conditions but for *Helix* , better growth was achieved in constant darkness. At cooler temperatures (14.5°C), *Helix* grew better when in constant light and *Cepaea* better when in permanent darkness. There is no obvious

Helix	Mean Temp. °C	Mean Final Height mm	Mean Final Breadth mm	Mean Final Weight mg	n1	$\delta^{13}C$ ‰ PDB	Std. Dev.	$\delta^{18}O$ ‰ PDB	Std. Dev.	n2
P1	22.3	12.05	12.68	64.76	8	-11.52	0.52	-6.03	0.09	5
P2	22.3	9.24	10.49	36.66	7	-12.06	0.49	-6.47	0.57	5
P3	22.3	10.50	11.37	54.73	7	-12.19	0.24	-6.55	0.08	5
P4	18.4	10.34	11.03	51.99	8	-11.19	0.48	-6.08	0.46	5
P5	18.4	9.63	10.54	45.36	7	-11.15	0.34	-5.95	0.17	5
P6	14.5	6.50	7.60	24.49	10	-11.40	0.79	-4.39	0.35	5
P7	14.5	7.12	8.42	29.12	9	-11.12	0.80	-4.78	0.74	5
P8	22.3	12.98	13.63	97.86	8	-9.93	0.30	2.19	0.26	5
P9	14.5	6.90	8.36	29.54	8	-10.15	0.84	0.83	0.85	5

Table 3.17a *Helix aspersa*

Cepaea	Mean Temp. °C	Mean Final Height mm	Mean Final Breadth mm	Mean Final Weight mg	n1	$\delta^{13}C$ ‰ PDB	Std. Dev.	$\delta^{18}O$ ‰ PDB	Std. Dev.	n2
P1	22.3	5.50	7.58	22.78	5	-11.54	0.64	-5.62	0.48	3
P2	22.3	7.07	9.57	58.63	3	-10.93	0.89	-6.15	0.13	3
P3	22.3	5.93	8.07	41.50	3	-11.54	0.21	-5.99	0.11	3
P4	18.4	6.47	8.87	50.53	3	-10.25	0.22	-5.73	0.11	3
P5	18.4	6.10	8.40	45.57	3	-10.04	0.05	-5.54	0.10	3
P6	14.5	4.20	5.70	19.73	3	-10.27	0.28	-4.81	0.27	3
P7	14.5	3.77	4.93	14.70	3	-10.63	0.63	-4.89	0.40	3

Table 3.17b *Cepaea nemoralis*

n1 = number of snails
n2 = number of analyses

Table 3.17a-d Mean shell dimensions and stable isotope data from Experiment 3, P1 - P9. Shells measured after six week growth period at time of killing. P1 to P7 = 'test' populations, P8 and P9 = 'control' populations. (a) *Helix aspersa* , (b) Cepaea nemoralis, (c) *Rumina decollata* and (d) *Ferussacia folliculus.*

Rumina	Mean Temp °C	Mean Final Diam. mm	n1	$\delta^{13}C$ ‰ PDB	Std. Dev.	$\delta^{18}O$ ‰ PDB	Std. Dev.	n2
P1	22.3	7.33	9	-9.92	0.06	-6.06	0.04	3
P2	22.3	7.03	8	-10.21	0.05	-6.36	0.06	3
P3	22.3	7.00	7	-9.97	0.21	-6.20	0.20	3
P4	18.4	5.54	8	-9.82	0.10	-5.99	0.06	3
P5	18.4	5.88	8	-9.77	0.08	-5.94	0.08	3
P6	14.5	3.80	7	-9.19	0.16	-5.14	0.08	3
P7	14.5	4.10	5	-9.07	0.25	-5.68	0.12	3
P8	22.3	7.31	7	-9.42	0.16	-0.70	0.19	3
P9	14.5	3.89	7	-8.66	0.14	-0.89	0.39	3

Table 3.17c *Rumina decollata* (full caption on prev. page)

n1 = number of snails
n2 = number of analyses

Ferussacia	Mean Temp. °C	n1	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
P1	22.3	11	-9.30	-5.78
P2	22.3	12	-9.38	-6.34
P3	22.3	9	-9.49	-6.20
P4	18.4	11	-9.13	-5.82
P5	18.4	10	-9.14	-5.95
P6	14.5	8	-8.61	-5.01
P7	14.5	9	-8.47	-5.32

Table 3.17d *Ferussacia folliculus* (full caption on prev. page)

Sample No.	BOTTLE 1 $\delta^{18}O‰$ SMOW	BOTTLE 2 $\delta^{18}O‰$ SMOW
1	-9.2	-9.0
2	-8.9	-9.2
3	-9.0	-8.8
4	-8.7	-9.1

overall mean = -9.00‰ SMOW

Table 3.18 $\delta^{18}O‰$ SMOW values of environmental waters in Experiment 3.

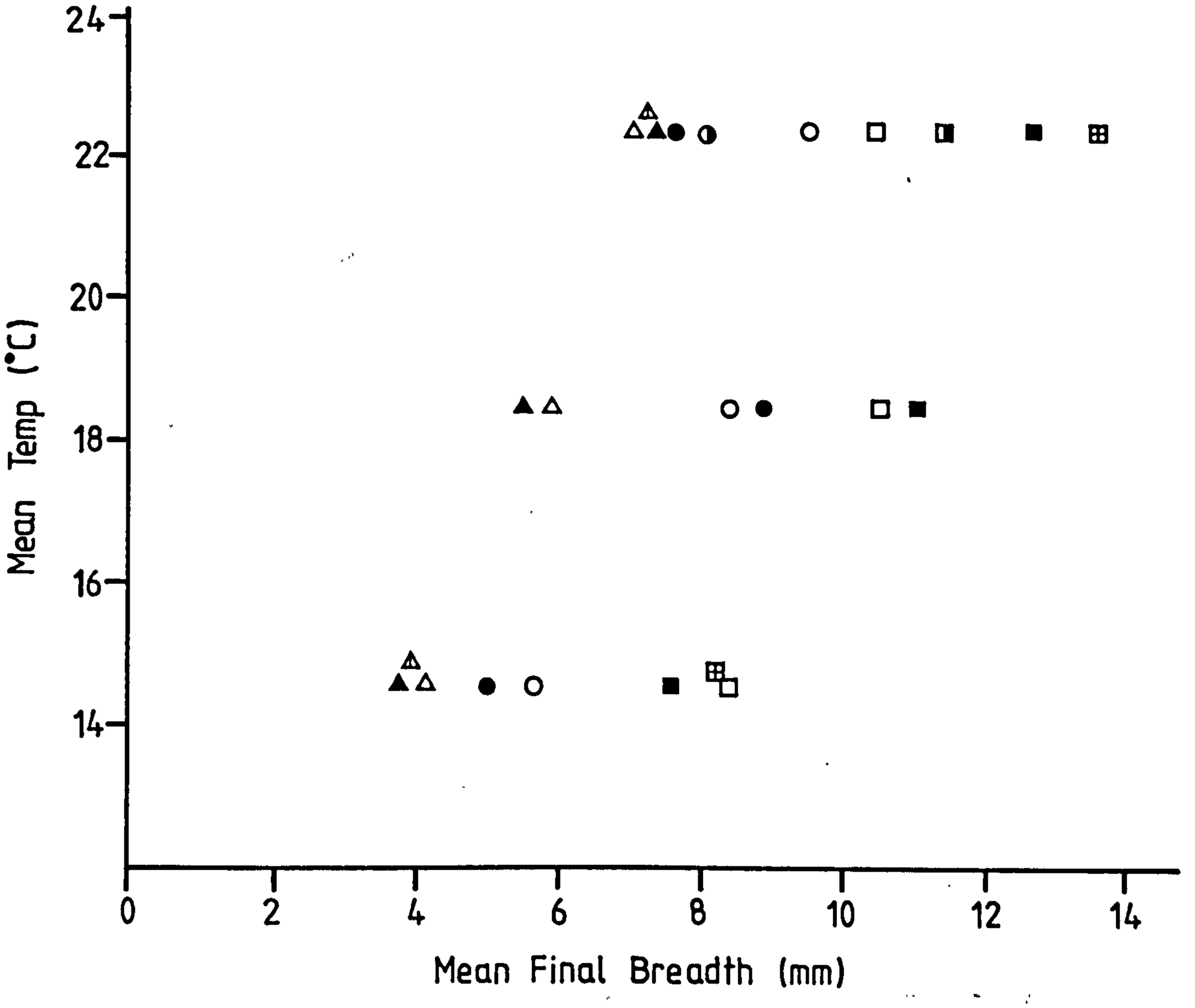


Figure 3.14a Mean final shell breadth of *Helix aspersa*, *Cepaea nemoralis* and *Rumina decollata* versus temperature for P1 - P9 : Experiment 3.

Pop^{n} No.	Mean T°C	Bag	Species			
			Helix	*Cepaea*	*Rumina*	*Ferussacia*
P1	22.3	opaque	■	●	▲	◆
P2	22.3	clear	□	○	△	◇
P3	22.3	opaque/clear exch.	◨	◑	▲	◆
P4	~18.4	opaque	■	○	▲	◆
P5	~18.4	clear	□	○	△	◇
P6	14.5	opaque	■	●	▲	◆
P7	14.5	clear	□	○	△	◇
P8	22.3	none-control	⊞		◬	
P9	14.5	none-control	⊞		◬	

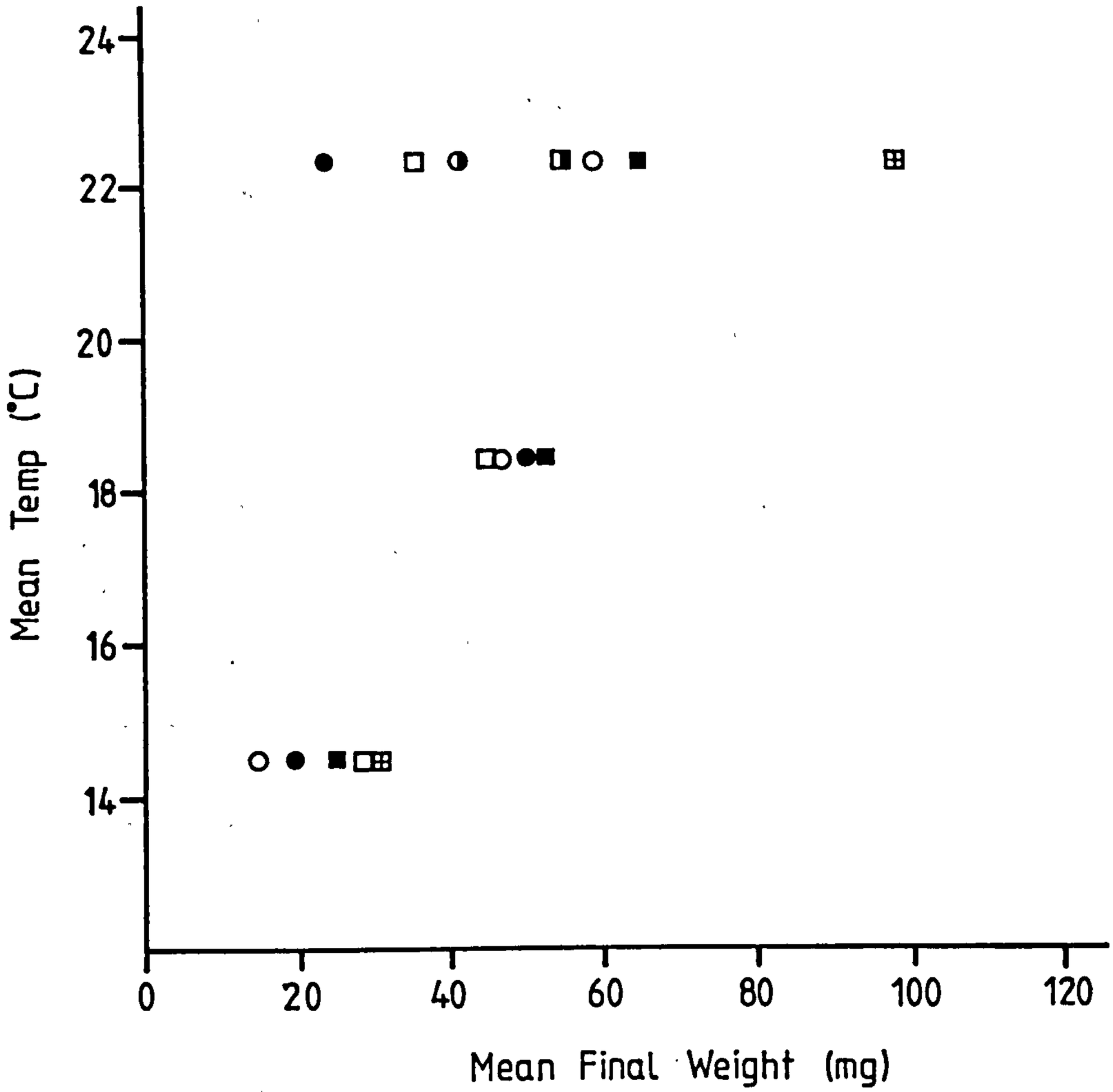


Figure 3.14b Mean final shell weight of *Helix aspersa* and *Cepaea nemoralis* versus temperature for P1 - P9 : Experiment 3. (see Fig. 3.14 a for key)

difference between P1 and P2 or P6 and P7 *Rumina*, and the snails in each temperature regime in clear and opaque bags grew similar amounts.

P3 snails lie mid-way in size between the P1 and P2 snails for *Helix* and *Cepaea* . All the *Rumina* snails from the 'warm room' (22.3°C, P1-P3) reached a similar size.

P4 and P5 snails were moved between the 'warm room' and the 'cool room' on a daily basis; P4 in an opaque bag, and P5 in a clear bag and were subject to a mean temperature of approximately 18.4°C. For *Helix* and *Cepaea* , P4 snails grew slightly more than P5 snails. However, P4 and P5 were larger than P2 (22.3°C, clear bag) for *Helix* , and larger than P1 and P3 for *Cepaea* . On the other hand, P4 and P5 *Rumina* were noticeably smaller than *Rumina* from P1 to P3, but still larger than P6 and P7 *Rumina*. For all three species, P6 and P7 snails (14.5°C) are appreciably smaller than P1 to P5.

Figure 3.14b shows that, as with the shell size, there is an overall increase in weight of shell secreted with increasing mean environmental temperature.

The control population P8 grew to a similar magnitude to P1-3, also permanently housed at 22.3°C, although P8 *Helix* were slightly larger than their counterparts within the bags. Correspondingly, *Helix* and *Rumina* from P9 (at 14.5°C) reached approximately the same size as P6 and P7 snails of these species. However, P8 *Helix* were much heavier than the P1 to P3 of this species (*i.e.* P8 = approximately 98mg, whereas P1 to P3 have a mean weight of approximately 52mg (see Figure 3.14b). Thus, P8 snails appear to be of a similar size to the other 'warm room' snails, but are heavier, therefore thicker shelled.

The sizes and weights of the helicids, and the diameter of the last whorl of *Rumina*, achieved during this experiment in both test and control populations (Tables 3.17a-c), are all less than the dimensions recorded in experiment 2 (Tables 3.9 to 3.11).

(c) Oxygen isotopes

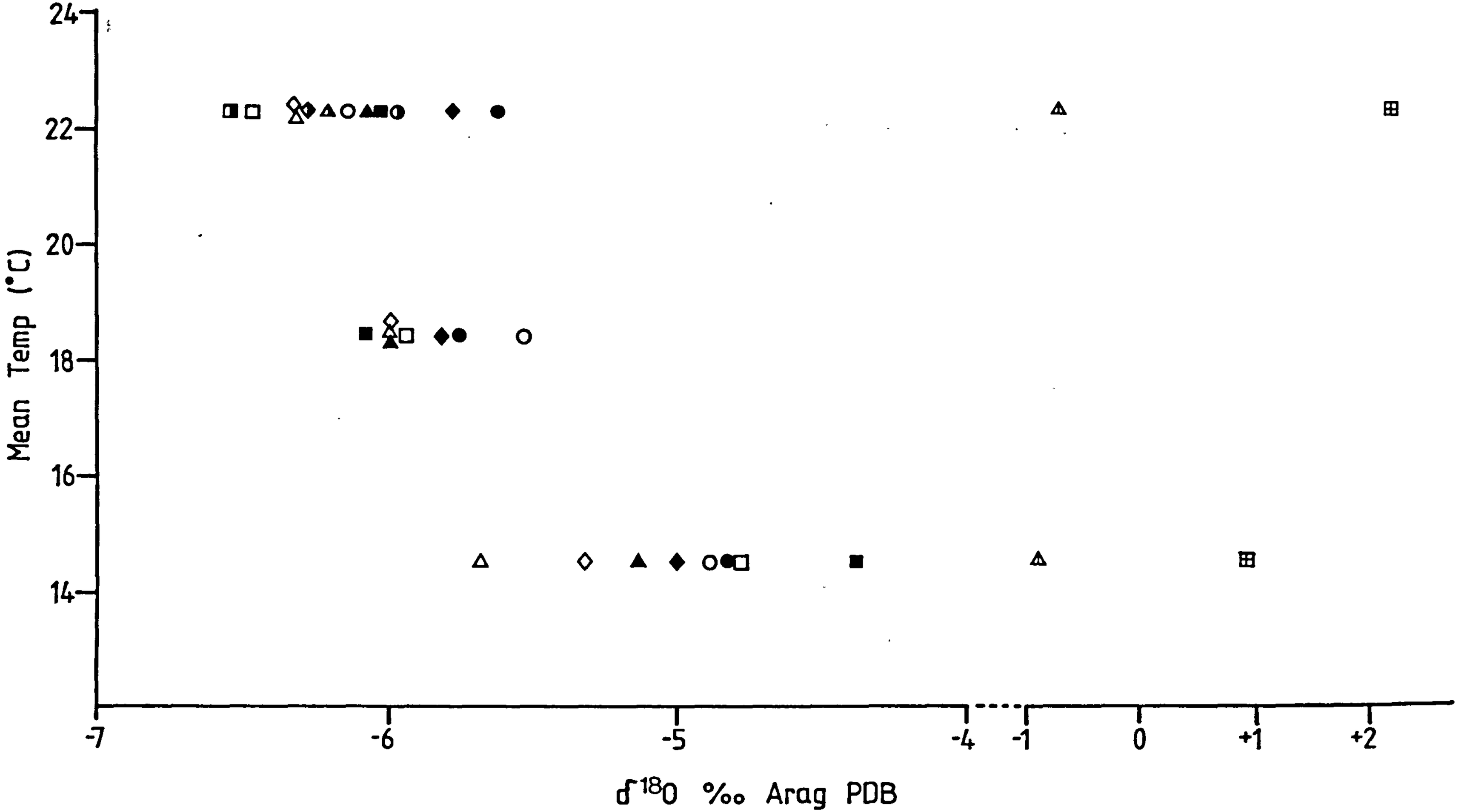
The oxygen isotope data shown in Tables 3.17a-d have been plotted against mean environmental temperature (Figure 3.15a).

The data from P1 to P7 all have negative $\delta^{18}O$ values, and lie between -4.5 to -6.5‰. The isotope values from all four species within each population are similar, although there is a larger spread in the data from P6 and P7 than in the other numbered populations. The isotope ratios of the Mediterranean species *Rumina decollata* and *Ferussacia folliculus* are not distinctly different to those from the temperate helicids. With increasing temperature, there is an overall drop in ^{18}O from values near -5‰ to values around to -6‰ for P1 - P7, and then a large shift (four to six per mil) to $\delta^{18}O$ values of the control populations of *Helix* and *Rumina* whose oxygen isotope ratios are close to zero, or slightly positive.

Of the populations housed in the 'warm room', for all four species, P1 (opaque bag) snails are approximately 0.5‰ more enriched in ^{18}O than P2 snails maintained in the clear bags. Except for *Helix* , where P3 snails are slightly more depleted in ^{18}O than P1 and P2, P3 snails have oxygen isotope ratios intermediate between P1 and P2.

In the 'cool room' populations (P6 and P7), as for the 'warm room' (P1 to P3) and for all species, P6 snails (black bag) are more enriched (0.2 to 0.5‰) than snails from P7. The snails from the 'cool room', at 14.5°C, are 0.5 to 1.5‰ more enriched in ^{18}O than their counterparts at 22.3°C in

Figure 3.15a Mean shell $d^{18}O$ versus temperature, Experiment 3. (see Fig. 3.14a for key)



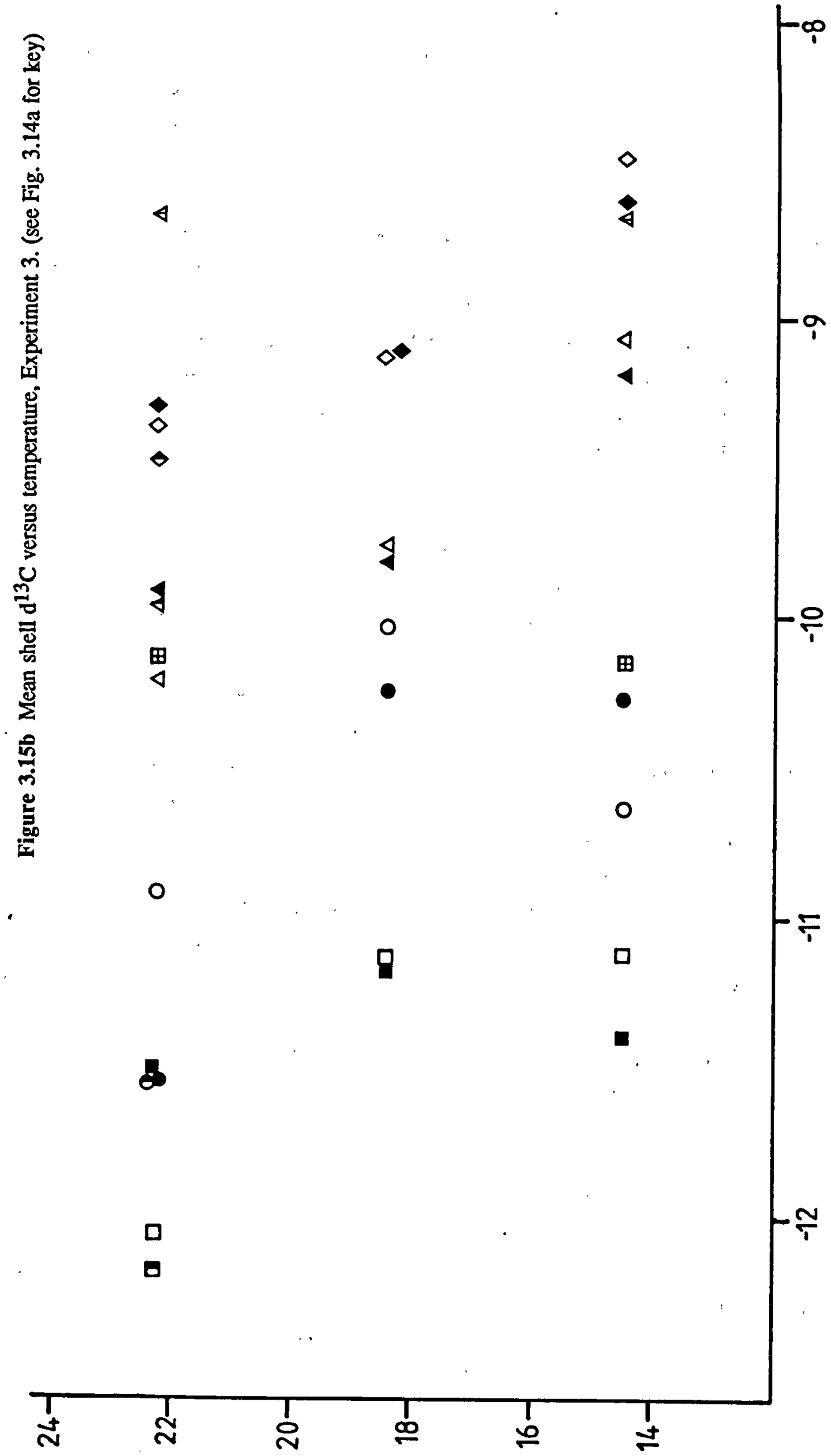


Figure 3.15b Mean shell $d^{13}C$ versus temperature, Experiment 3. (see Fig. 3.14a for key)

the 'warm room', as would be expected from the temperature equation for the precipitation of aragonite (section 3.3.2).

The $\delta^{18}O$ values of P4 (black bag) and P5 (clear bag) snails which were moved between the 'warm' and 'cool' rooms and were subject to a mean temperature of 18.4°C, lie between the values from shells of snails kept permanently in the 'warm' or 'cool' rooms. The isotopic differences between P4 and P5 snails are very slight; P5 snails are generally more enriched in ^{18}O than P4 snails, although by not more than 0.2‰.

The control populations of *Helix* and *Rumina* have $\delta^{18}O$ values that are substantially different from the seven test populations. P8 *Helix* (22.3°C) has a $\delta^{18}O$ value of +2.0‰, and P9 (14.5°C) of +0.8‰. For *Rumina* the P8 value is -0.6‰ and for P9 is -0.9‰. These positive $\delta^{18}O$ values are of a similar magnitude to the values for *Helix* and *Rumina*, grown over equivalent temperatures, from Experiments 1 and 2. P8 $\delta^{18}O$ values are more positive than those from P9 snails, and there is a difference of over 2‰ between the data from the two species. However, P8 and P9 snails are four to six per mil more enriched in ^{18}O than snails from the seven test populations.

(d) Carbon isotopes

Carbon isotope data against mean environmental temperature are shown in Figure 3.17b. There is a trend towards more depleted $\delta^{13}C$ values with increasing temperature for all four species, however, unlike the oxygen isotope data, the carbon isotope data from each species are distinct. The pair of helicid species *Helix aspersa* and *Cepaea nemoralis* closely follow the same trend, and the two Mediterranean species *Rumina decollata* and *Ferussacia folliculus* also closely follow each other 'as a pair'.

The carbon isotope data lie between -8.5 and -12.2‰, with *Helix* and *Cepaea* from -9.9 to -12.2‰ and *Rumina* and *Ferussacia* from -8.5 to -10.2‰.

For all four species, the most depleted $\delta^{13}C$ values occur in the shells of the snails kept in the warm room, P1-P3, at 22.3°C. For all but *Cepaea* , $\delta^{13}C$ values are slightly lower in P2 snails (clear bags) as compared to those housed in the opaque bags from P1. For *Cepaea* the opposite is true, as P2 *Cepaea* (-11‰) are 0.5‰ more enriched than P1 (-11.5‰). P3 $\delta^{13}C$ values are generally as depleted, if not slightly more so, than P1 data; except for *Rumina* which has a $\delta^{13}C$ value intermediate between P1 and P2.

P6 and P7 snails, kept in the cooler environment (14.5°C), have $\delta^{13}C$ values up to 1‰ more enriched than the populations maintained at 22.3°C. For the 'cool room' populations, P6 snails (opaque bag) have more depleted $\delta^{13}C$ values than P7 snails (clear bag), the opposite of what was seen in the 'warm room', but again *Cepaea* is the 'odd man out'. Therefore, as with the pattern of relative growth and the oxygen isotopes, the pattern of carbon isotope ratios for *Cepaea* is somewhat different from the other snail species, in terms of the differences between snails kept in the black and clear bags under the same conditions.

P4 and P5 snails were moved between the two temperature extremes twice daily and were subject to a mean environmental temperature of 18.4°C. For the helicids, P4 and P5 $\delta^{13}C$ values are approximately 1‰ more enriched than the shells from P1 to P3, and are also slightly more enriched than the shells in P6 and P7. However, the pattern is different in the Mediterranean species *Rumina* and *Ferussacia* , where the $\delta^{13}C$ of P4 and P5 shells lie between those from snails kept permanently in the warm and cool environments.

The control populations of *Helix* (P8 and P9) have $\delta^{13}C$ values more enriched than their counterparts kept under the same conditions but within the plastic bags. P8 *Helix* (22.3°C, no bag) are 2‰ more enriched than P2 *Helix* (22.3°C, clear bag); and P9 *Helix* (14.5°C, no bag) are 1.5‰ more enriched than P7 *Helix* (14.5°C, clear bag). The control populations of *Rumina* are also more enriched than their test population counterparts, with P8 being 0.8‰ more enriched than P2; and P9 *Rumina* being 0.4‰ more enriched than P7 shells.

3.7.3 Discussion : Experiment 3

(a) The effect of environmental conditions and diurnal cycles upon shell growth

As was indicated in Experiments 1 and 2, environmental temperature is positively correlated with the amount of shell secreted over the experimental period. Housing the snails within the bags has not reduced the size of shell produced, although the weight of shell secreted appears to have been negatively influenced by keeping the snails in bags in the warmer regime. This suggests that under warm environmental temperatures, the most favourable conditions for shell secretion would be those where some movement of air and loss of water from the microenvironment would be possible.

For the helicids *Helix aspersa* and *Cepaea nemoralis*, moving the populations from the 22.3°C to the 14.5°C regime, whilst still housed within the bags, also appears to have been beneficial to growth. P4 and P5 *Helix* achieved a larger size than P2 (mean final shell weight = 51.99 and 45.36mg for P4 and P5, respectively and 36.66mg for P2), and for *Cepaea* P4 and P5 snails (mean final shell weight = 50.53 and 45.57mg, respectively) were larger in size than P1 and P3 snails (mean final shell weight = 22.78 and 41.50mg, respectively). However, this pattern was not repeated for the Mediterranean species *Rumina*, where P4 and P5 snails were considerably smaller than individuals from P1 to P3 (mean final shell breadth = 5.54 and 5.88mm for P4 and P5, respectively and 7 to 7.33mm for P1, P2 and P3), suggesting that for this snail constantly warm temperatures allow greater snail growth. The helicids either prefer a diurnal cycle in temperature, or, have benefitted from being placed in conditions where the mean temperature was lower than 22.3°C. A constant temperature as high as 22.3°C ('warm room' temperature) may have reduced potential growth even where space and the availability of food were not limiting factors.

The two helicid species appear to have reacted differently (in terms of the relative amounts of shell they secreted) to being placed permanently in the opaque or clear polythene bags. Under warmer conditions, *Helix* faired better in the opaque bags and *Cepaea* in the clear. Under the cooler conditions the opposite was found, with *Cepaea* growing better in the dark conditions. This implies that under the same experimental conditions, the two species were reacting differently. The reduction in size when comparing the final weights of the helicid snails produced in experiment 2 (for *Helix* from 160.3 to 332.3mg and for *Cepaea* from 36.6 to 80.9mg, Tables 3.9 and 3.10) to those from experiment 3 (for *Helix* from 29.5 to 97.9mg and for *Cepaea* from 14.7 to 58.6mg, Table 3.17), may reflect several factors. In experiment 3, the juvenile *Helix* snails came from overcrowded base populations (many babies, from several clutches, had hatched almost simultaneously), and thus, it may have taken a larger proportion of the six week growth period for the snails to acclimatise to the environmental conditions, as compared to the snails used in experiment 2. For *Cepaea* , the juvenile snails used in the test populations were very tiny when they were removed from the base populations. Thus, their initial

sizes were smaller than the snails of this species used in experiment 2, and they might also have required a longer adjustment period.

(b) The effect of environmental conditions and diurnal cycles upon oxygen isotope ratios of shells

1) Trends with temperature

The snails maintained in bags under constant conditions at 22.3°C (P1 and P2) have lower $\delta^{18}O$ values than those snails kept under identical conditions in the at 14.5°C. The differences between $\delta^{18}O$ from P1 and P6 (opaque bags), and P2 and P7 (clear bags), are shown in Table 3.19a as Δvalues. The measured differences range from 0.77 to 1.69‰ and in all cases the oxygen isotope ratios of the 'warm room' snails are more depleted in ^{18}O than the shells of snails from the 'cool room'. Based on the depletion in ^{18}O of 0.213‰ per °C for the precipitation of marine gastropod aragonite shell (Grossman and Ku, 1986), see section 3.3.2, the Δ values are also indicated in Table 3.19a as differences in temperature (ΔT) between the two groups of populations. The actual temperature difference between the 'warm' (22.3°C) and 'cool' (14.5°C) rooms , was 7.8°C. This temperature difference is comparable to the ΔT values for *Helix* (7.70 and 7.93°C), but is 1.9°C higher than the ΔT for *Cepaea* kept in the clear bags (5.92°C), and is 4°C higher than the ΔT values for *Cepaea* maintained in the opaque bags (3.80°C).

In the light of the results from experiment 1, where evaporation and metabolic effects appeared to play such an important role in the trend of $\delta^{18}O$ with temperature, it was somewhat surprising to find that the helicids (especially *Helix*) kept in the clear bags, *i.e.* subject to constant light, followed quite closely the depletion rate expected with temperature. This implies that evaporation, and perhaps metabolic effects have a lesser influence upon the helicids kept within the plastic bags, than those maintained without, as in Experiments 1 and 2.

Smaller temperature differences (ΔT = 3.19 to 4.79°C) were calculated for the Mediterranean species *Rumina* and *Ferussacia* , suggesting that for these species (and perhaps for *Cepaea* maintained in permanent darkness) additional factors, either causing less enrichment in the snails from the cooler environment or greater depletion in the snails from the warmer regime, may be influencing the isotopic compositions of the shells. The growth of *Rumina* was restricted in the cooler regime (P6 and P7, see previous sub-section), and under such conditions the shells might be expected to become metabolically enriched in ^{18}O, as activity became restricted. If this were the case, then the $\delta^{18}O$ values of the shells from P6 and P7 might be expected to be more enriched in ^{18}O, relative to those shells from the warmer regime (P1 and P2), than they actually are.

2) The effect of opaque and transparent bags and the exchange between the two

All the snails, from both warm and cool regimes, show more depleted $\delta^{18}O$ values (*i.e.* more negative values) in the shells of snails maintained in the transparent bags rather than the opaque bags. This may be seen in Table 3.19a, if P1 data are compared to P2 data, and P6 to P7. The difference is generally between 0.3 and 0.5‰. This fairly small isotopic difference must reflect a vital effect related to the light reaching the snails. It does not appear to be linked directly to the rates of growth of the snails, as the growth patterns of *Helix, Cepaea* and *Rumina* from the populations in question are dissimilar, especially when comparing P1 with P2 (Figure 3.14a).

	P1 $\delta^{18}O$ OPAQUE	P6 $\delta^{18}O$ OPAQUE	P1 - P6 Δ ‰	Δ T	P2 $\delta^{18}O$ CLEAR	P7 $\delta^{18}O$ CLEAR	P2 - P7 Δ ‰	Δ T
Helix	-6.03	-4.39	1.64	7.70	-6.47	-4.78	1.69	7.93
Cepaea	-5.62	-4.81	0.81	3.80	-6.15	-4.89	1.26	5.92
Rumina	-6.06	-5.14	0.92	4.32	-6.36	-5.68	0.68	3.19
Ferussacia	-5.78	-5.01	0.77	3.62	-6.34	-5.32	1.02	4.79

Table 3.19a Comparison of oxygen isotope data from P1 with P6 (populations maintained in opaque bags at 22.3 and 14.5°C, respectively), and P2 with P7 (populations maintained in clear bags at 22.3 and 14.5°C, respectively) from Experiment 3. The per mil difference between the two temperature regimes (δ) is in each case assessed as a calculated difference in temperature (ΔT), based on a rate of ^{18}O depletion of 0.213‰ per °C (see section 3.7.3b). These calculated temperature differences may be compared with the actual temperature difference, AΔT, of 7.8°C.

	P2 $\delta^{18}O$ 22.3°C CLEAR	P8 $\delta^{18}O$ 22.3°C NO BAG	P2 - P8 Δ ‰	P7 $\delta^{18}O$ 14.5°C CLEAR	P9 $\delta^{18}O$ 14.5°C NO BAG	P7 - P9 Δ ‰
Helix	-6.47	2.19	8.66	-4.78	0.83	5.61
Rumina	-6.36	-0.70	5.66	-5.68	-0.89	4.79

Table 3.19b Comparison of oxygen isotope data from test and control populations grown under similar conditions (temperature and lighting) in Experiment 3. Test populations (P2 and P7) were maintained in clear polythene bags, whereas control populations (P8 and P9) were maintained without plastic bags, in the same way as in Experiments 1 and 2.

For all species except *Helix* , the $\delta^{18}O$ values of P3 snails (moved between the opaque and clear bags in the 'warm room') lie between those from P1 and P2, but slightly nearer those of P2 (Tables 3.17a-d). The P3 data for *Helix* are only very slightly more depleted (0.08‰), than those from P2. Thus, subjecting the snails to a diurnal cycle of light and dark, under the experimental conditions has had a limited effect upon the oxygen isotope ratios.

3) The effect of the exchange between the 'warm' and 'cool' room

P4 snails (opaque bag), have $\delta^{18}O$ values almost identical to those from P1, for all four species (Tables 3.17a-d), suggesting that in continual darkness the shells reflect the environmental conditions of the warmer half of the daily cycle. P5 snails (clear bag) are approximately 0.5‰ more enriched in ^{18}O than comparable snail shells from P2, indicating that when kept in permanent light, some of the shell processing and secretion reflects conditions in the cooler part of the daily cycle.

4) The effect of keeping the snails boxes inside polythene bags

The pronounced difference between the $\delta^{18}O$ values of *Helix* and *Rumina* from P8 as compared to P2 (both in permanent light at 22.3°C in the 'warm room'; and P9 as compared to P7 (both in permanent light at 14.5°C in the 'cool room') may be clearly seen on Figure 3.15a and in Table 3.19b, where Δ values have been calculated. The largest difference caused by the presence of the bags, is 8.66‰ between P8 and P2 *Helix* . The difference between 'bagged' and 'non-bagged' *Helix* in the 'cool room' was 5.61‰. For *Rumina*, the difference in the 'cool room' (4.79‰) was also slightly lower than that from the 'warm room' where the Δ value was 5.66‰.

These considerable differences in shell oxygen isotope ratios, between the control and test populations, must reflect a dramatic decline in evaporative enrichment of body and environmental waters in the snails kept within the bags compared to those kept just in boxes with fine netting lids.

It had been hoped, in experiment 1, that by keeping the boxes and soil continually moist and with constant high relative humidities, that evaporation from the microenvironment would have been minimal. However, by comparison of the test and control populations here, a large isotopic effect related to evaporation has become apparent, and it is likely that an effect of similar magnitude (5 to 8‰) occurred during Experiments 1 and 2.

The results also show that the considerable evaporation effect appears to be more pronounced at higher environmental temperatures, and that the effect may be less extreme in the Mediterranean, as opposed to the northern temperate, species. More data would be required to assess better the differences in the evaporation effect between snail species, and over a range of environmental temperatures. However, under normal temperatures prevailing during the growth season (around 15°C), evaporation effects may lead to enrichment of body waters and the resultant shell $\delta^{18}O$ values by around 5‰. This will be considered further in section 3.7.3(f).

Keeping the experimental snails within polythene bags could have upset the natural functioning of the snails metabolic systems, and the low $\delta^{18}O$ value seen in the snails shells from the bagged populations could be the result of a strong vital effect. However, vital effects linked to snails' metabolism are thought to produce oxygen isotope enrichment as a consequence of a reduction in activity and water fluxes through the snails' bodies (Goodfriend and Magaritz, 1987), and not depletion as seen here. Also, the snails kept within the bags produced shells of comparative size to those of the

control populations, suggesting that the 'bagged' snails were not suffering undue environmental stress. Thus, their metabolism and, ultimately, their shell secretion were not likely to be strongly affected.

(c) Comparison with oxygen isotope data from snails in Experiments 1 and 2

Oxygen isotope data from groups of snails, in each experiment, that have been grown under comparable conditions, should be comparable. In experiment 1, W2 snails were kept at 22.3°C in lighted conditions, as were Pop 1 snails in experiment 2 and P8 (no bag) in experiment 3. Mean oxygen isotope data from these test populations of *Helix aspersa* and *Rumina decollata* (the two species for which all data are available)) from each experiment are shown in Table 3.20a. For both species the data are not consistent across the three experiments *i.e.* for *Helix* expt. 1 $\delta^{18}O$ = +3.88, expt. 2 = +1.56 and expt. 3 = +2.19‰; and for *Rumina* expt. 1 $\delta^{18}O$ = +0.78, expt. 2 = +1.46 and expt. 3 = -0.70‰.

The differences between the mean values shown in Table 3.20a and noted above, may be partially explained in terms of primary effects, whereby, the oxygen isotopic composition of the food and water source may have exerted some influence upon the resultant isotopic composition of the shell. In such a way, it was thought that the depleted $\delta^{18}O$ value of the inorganic carbonate in the snails' diet in experiment 2, has affected the isotopic composition of, especially, *Helix aspersa*, which were 2‰ lower in ^{18}O than the shells of this species from experiment 1. The differences may also have occurred if, under certain environmental conditions, resources became limited, and overcrowding became a problem.

At a lower experimental temperature (experiment 1, C3 (15.7°C); experiment 2, Pop 3 (16.5°C); experiment 3, P9 (14.5°C)), comparable oxygen isotope data have also been collated (Table 3.20b). In this case, the experiment 2 snails were kept in darkness whilst the rest of the data are from snails kept in conditions of permanent light. Again the data from the three experiments are dissimilar *i.e.* for *Helix* expt. 1 $\delta^{18}O$ = -2.73, expt. 2 = +1.46 and expt. 3 = +0.83‰; and for *Rumina* expt. 1 $\delta^{18}O$ = -3.71, expt. 2 = -0.71 and expt. 3 = -0.89‰.

For the data presented in Table 3.20b, the experimental conditions were not identical, with some variation in temperature and, additionally, experiment 2, Pop 3 snails were kept in permanently dark conditions whereas the other data are from shells of snails maintained in constant light. Further to this, experiment 1 'cool' populations were housed in a separate sealed, controlled temperature chamber. During Experiments 2 and 3, the outer corridor was used. This difference in locations may explain the lower $\delta^{18}O$ values from experiment 1 as compared to Experiments 2 and 3, if evaporation effects were enhanced in the outer corridor as compared to the sealed room.

It has already been noted that evaporation effects must have influenced the resultant shell isotopic compositions to some considerable extent (probably causing enrichment in the range of 5‰). The amount of water sprayed into the boxes at each feeding was not measured, therefore, the amount of water available to be lost from a snail, or to be evaporated prior to uptake, would have been variable. Also it is known that the relative humidities within the rooms were not constant, but varied with external weather conditions. These factors could have introduced variation in the amount of evaporation occurring in each experiment.

Therefore, the differences between the shell oxygen isotope data from the three experiments may be explained in terms of primary variation in the isotopic composition of food and water given to the snails, and the exact environmental conditions with regards to temperature, light and location.

	EXPERIMENT 1 W2 $\delta^{18}O$ 22.3°C	EXPERIMENT 2 Pop 1 $\delta^{18}O$ 22.3°C	EXPERIMENT 3 P8 $\delta^{18}O$ 22.3°C
Helix	3.88	1.56	2.19
Rumina	0.78	1.46	-0.70

Table 3.20a At 22.3°C

	EXPERIMENT 1 C3 $\delta^{18}O$ 15.7°C	EXPERIMENT 2 Pop 3 $\delta^{18}O$ 16.5°C *	EXPERIMENT 3 P9 $\delta^{18}O$ 14.5°C
Helix	-2.72	1.46	0.83
Rumina	-3.71	-0.71	-0.89

Table 3.20b From 14.5 - 16.5°C

Table 3.20a-b Mean oxygen isotope data from shells of *Helix aspersa* and *Rumina decollata* grown under comparable temperatures and conditions from Experiments 1, 2 and 3. (a) at 22.3°C - 'warm' room temperature and (b) from 14.5 to 16.5°C - 'cool' room temperatures

* = permanent darkness, all others in permanent light

Secondary effects related to the degree of evaporation suffered by individual populations, may also explain some of the variation.

(d) The effect of environmental conditions and diurnal cycles upon carbon isotope ratios of shells.

1) Trends with temperature

The $\delta^{13}C$ values from P1 and P2 (maintained under constant conditions at 22.3°C in the 'warm room') are compared to the carbon isotope ratios of P6 and P7 shells (same conditions but 7.8°C cooler) in Table 3.21a. All four species show more depleted $\delta^{13}C$ values in the snails from the warmer environment, with a 0.12 to 1.27‰ depletion (shown in Table 3.21a as Δ values) recorded with increasing temperature. The main carbon isotope fractionations involved in the production of snail shell aragonite are the uptake and transfer of atmospheric carbon dioxide, balanced against the evolution of metabolic carbon dioxide. Such processing involves fractionations that are weakly temperature dependent (-0.1‰ per °C) and lead to lower $\delta^{13}C$ values with increasing temperatures (see section 3.3.3). The temperature effect may, therefore, account for a small portion of the Δ values.

The more depleted shells in the P1 and P2, as compared to the P6 and P7 shells may also signify that the P1 and P2 shells were influenced by a larger proportion of metabolic, relative to atmospheric, carbon dioxide. This idea is supported by the resultant sizes of the two groups of snails, as P1 and P2 were larger than P6 and P7 snails, suggesting a greater throughput of metabolites and consequently a reduced contribution of atmospheric carbon dioxide in the snails which grew more, resulting in more depleted $\delta^{13}C$ values.

The separation between the data from the four species (Table 3.21a and Figure 3.15b) indicates that each species utilises a different ratio of organic to inorganic sources of carbon, for shell secretion. The Mediterranean species *Rumina decollata* and *Ferussacia folliculus* have less depleted $\delta^{13}C$ values than the temperate helicids *Helix aspersa* and *Cepaea nemoralis*, Thus, the Mediterranean species may employ a greater proportion of atmospheric, as opposed to metabolic, carbon dioxide, or else they incorporate a greater proportion of inorganic carbon into their shells. For this experiment, the $\delta^{13}C$ value of the inorganic carbon in the diet was +2.24‰.

2) The effect of opaque and transparent bags and the exchange between the two.

It has been suggested above (after Goodfriend and Magaritz, 1987), that under cooler conditions, when snails secrete less shell, that $\delta^{13}C$ values would move towards equilibrium with atmospheric carbon dioxide (up to around +1‰). With increased growth, more metabolic carbon dioxide would be produced and shell isotope values would move towards equilibrium with metabolic carbon dioxide.

However, for the helicids under the 'warm room' conditions, P2 *Helix* (clear bag) grew less than P1 *Helix* (opaque bag), but had more depleted $\delta^{13}C$ values (P2 = -12.06 and P1 = -11.52‰); whereas, P2 *Cepaea* grew more than P1 *Cepaea* , but had less depleted $\delta^{13}C$ values (P2 = -10.93 and P1 = -11.54‰). Conversely, in the cooler environment, P7 *Helix* (clear bag) grew more than P6 *Helix* (opaque bag), but had less depleted $\delta^{13}C$ values (P7 = -11.12 and P6 = -11.40‰); whilst P7 *Cepaea* grew less than P6 *Cepaea* and had more depleted $\delta^{13}C$ values (P7 = -10.63 and P6 = -10.27‰) (see Figures 3.14a and 3.14b and Tables 3.17a and 3.17b for mean sizes of shell secreted by each species). Therefore, for these two species of helicids under the conditions in the plastic bags, the

	P1 $\delta^{13}C$ OPAQUE	P6 $\delta^{13}C$ OPAQUE	P1 - P6 Δ ‰	P2 $\delta^{13}C$ CLEAR	P7 $\delta^{13}C$ CLEAR	P2 - P7 Δ ‰
Helix	-11.52	-11.40	0.12	-12.06	-11.12	0.94
Cepaea	-11.54	-10.27	1.27	-10.93	-10.63	0.30
Rumina	-9.92	-9.19	0.73	-10.21	-9.07	1.14
Ferussacia	-9.30	-8.61	0.69	-9.38	-8.47	0.91

Table 3.21a Comparison of carbon isotope data from P1 with P6 (populations maintained in opaque bags at 22.3 and 14.5°C, respectively) and P2 with P7 (populations maintained in clear bags at 22.3 and 14.5°C, respectively) from Experiment 3. The differences between the regimes are shown as Δ‰ values.

	P2 $\delta^{13}C$ 22.3°C CLEAR	P8 $\delta^{13}C$ 22.3°C NO BAG	P2 - P8 Δ ‰	P7 $\delta^{13}C$ 14.5°C CLEAR	P9 $\delta^{13}C$ 14.5°C NO BAG	P7 - P9 Δ ‰
Helix	-12.06	-9.93	2.13	-11.12	-10.15	0.97
Rumina	-10.21	-9.42	0.79	-9.07	-8.66	0.41

Table 3.21b Comparison of carbon isotope data from test and control populations grown under similar conditions (temperature and light) in Experiment 3. Test populations (P2 and P7) were maintained in clear polythene bags, whereas control populations (P8 and P9) were maintained without polythene bags, in the same way as in Experiments 2 and 3.

trends predicted by the balance between metabolic versus atmospheric carbon dioxide (in relation to relative shell growth) are contradicted. Under the conditions within the polythene bags, the maximisation of potential growth might require larger amounts of atmospheric oxygen and thus carbon dioxide. This might explain some of the anomalous $\delta^{13}C$ data from the 'warm' and 'cool' populations of *Helix* and *Cepaea* . However, the data may also reflect some disruption of the natural functioning of the snails because of the presence of permanent darkness (P1 and P6) or permanent light (P2 and P7) which affected the processing of the carbon isotopes.

For *Rumina* and *Ferussacia* , there were small differences between the $\delta^{13}C$ values of snails kept in the clear and opaque bags. At 22.3°C, P2 snails (clear bag) were 0.29‰ more depleted in ^{13}C than P1 (opaque bag) and P2 snails were slightly the smaller; but at 14.5°C, in the 'cool room', P7 shell $\delta^{13}C$ values (clear bag) were 0.12‰ higher than those from P6 (opaque bag) (Table 3.21a), and in this case the P7 snails attained a larger final size.

The P3 snails (moved every twelve hours from the opaque to clear bag) have $\delta^{13}C$ values the same as, or slightly lower than P1 snails, and, apart from *Rumina*, also lower than P2 snails (Tables 3.17a-e and Figure 3.15b). This would indicate that the P3 snails were utilising more metabolic carbon dioxide relative to atmospheric carbon dioxide, than P1 and P2 snails. However, this is not reflected in the final sizes of the snails, as P3 snails were not consistently larger than P1 and P2 snails.

Thus, housing the snails in the opaque, or clear, bags does not give rise to systematic differences in the shell carbon isotope ratios, although being moved between the two environments appears to lead to ^{13}C depletion. Further, the isotope effects revealed are not consistently linked to the size of shell produced, and therefore do not provide evidence of a relationship between the amount of metabolic versus atmospheric carbon dioxide and the rate of shell growth.

3) The effect of exchange between the 'warm' and 'cool' room

For all four species, P4 and P5 snails (moved between the two rooms and therefore subject to a mean temperature of 18.4°C) have carbon isotope ratios that are up to 1.25‰ more enriched in ^{13}C than their counterparts grown at 22.3°C, showing the effect upon this isotope of the diurnal change in temperature.

For the helicids (*Helix* and *Cepaea*), P4 and P5 snails have $\delta^{13}C$ values considerably more enriched (from 0.5 to 1.25‰ more enriched) than the populations from the 'warm room' at 22.3°C (P1 and P2), but have $\delta^{13}C$ values close to those from snails maintained at 14.5°C in the 'cool room' (P6 and P7), (Tables 3.17a-d and Figure 3.15b). This suggests that the 'cool room' conditions play a more dominant role in the processing and shell secretion of the carbon isotopes of the helicids moved between the two rooms.. However, the predominance of the 'cool room' conditions was not so evident in the oxygen isotope ratios for these species (see previous sub-section).

The Mediterranean species *Rumina* and *Ferussacia* , have $\delta^{13}C$ values for P4 and P5 snails which lie between the values for these snails kept permanently in the 'warm' and 'cool' rooms, but somewhat closer to the carbon isotope ratios of the snails in the 'warm room'; *i.e.* the P4 and P5 snails are 0.1 to 0.4‰ more enriched in ^{13}C than snails grown at 22.3°C (P1 to P3), but are 0.5 to 0.7‰ more depleted in ^{13}C than the snails grown at 14.5°C (P6 and P7). Thus, these species again show a pattern distinct from the temperate helicids.

4) The effect of keeping the boxes of snails inside polythene bags

As with the oxygen isotopes, this may be best assessed by comparing the isotopic compositions of the control and test populations kept under similar conditions, *i.e.* P8 ('warm room', no bag) with P2; and P9 ('cool room', no bag) with P7. These data are shown in Table 3.21b, which includes Δ values.

The snails kept in the polythene bags were, in all cases, more depleted in ^{13}C than the control populations. The Δ values shown in Table 3.21b are of a smaller magnitude to those noted for the oxygen isotope data. The largest Δ value is between *Helix* from P2 and P8, being 2.13‰. However, for carbon isotopes in the experimental snails, this is still a considerable shift. For the 'cooler' populations of *Helix* , Δ = 0.97‰, again a marked difference. *Rumina* Δ values are just under half those of *Helix* , implying that *Rumina* is less influenced by the presence or absence of bags. This was also noted in the oxygen isotope data where the Δ values for *Rumina* were lower than those of *Helix* (see Table 3.20b).

The lower $\delta^{13}C$ values in the snails kept in the bags would be indicative of a greater proportion of metabolic, relative to atmospheric, carbon dioxide being processed through the snail body and into the shell. This may reflect the fact that being in boxes enclosed within loosely sealed bags, the snails did not have access to a complete atmospheric carbon dioxide reservoir, as compared to those snails housed just in the boxes.

(e) Comparison with carbon isotope data from Experiments 1 and 2

Mean carbon isotope ratios for *Helix* and *Rumina* grown under comparable conditions from each experiment are shown in Tables 3.22a and 3.22b for the 'warm room' at 22.3°C and 'cool room' (14.5 to 16.5°C) temperatures, respectively.

The differences between the data are best explained in terms of the variable isotopic composition of the inorganic carbon included the diet of the snails in each experiment as the rest of the diet was of a constant isotopic composition. For experiment 1, the $\delta^{13}C$ value of the inorganic carbon (GPR 1) = -13.09‰; in experiment 2, inorganic carbon (GPR 2) = -43.35‰; and in experiment 3, inorganic carbon (CHALK) = +2.24‰. Knowing the carbon isotopic composition of the inorganic carbon given to the snails, and using the carbon isotope data from shells grown under identical conditions, it is possible to assess the percentage of carbon in the shells which came from the inorganic carbon fed to the snails in their diet.

The three sources of carbon for shell secretion are from organic carbon, atmospheric carbon dioxide and inorganic carbon. The ultimate carbon isotopic composition of the shell will be a function of the proportion of these three components - organic carbon (pOC), atmospheric carbon (pAC) and inorganic carbon (pIC), therefore

$$pOC + pAC + pIC = 1 \qquad (3.8)$$

and

$$\delta^{13}C \text{ shell} = f(pOC + pAC + pIC) \qquad (3.9)$$

In all three growth experiments, the isotopic compositions of the first two components were fixed (although the proportions of each component used by the snails have been shown to be variable). Thus, providing that neither OC nor AC were limited, then the total input from these two sources may

	EXPERIMENT 1 W2 $\delta^{13}C$ 22.3°C	EXPERIMENT 2 Pop 1 $\delta^{13}C$ 22.3°C	EXPERIMENT 3 P8 $\delta^{13}C$ 22.3°C
Helix	-13.42	-19.66	-9.93
Rumina	-13.63	-21.09	-9.42

Table 3.22a At 22.3°C

	EXPERIMENT 1 C3 $\delta^{13}C$ 15.7°C	EXPERIMENT 2 Pop 3 $\delta^{13}C$ 16.5°C *	EXPERIMENT 3 P9 $\delta^{13}C$ 14.5°C
Helix	-12.07	-19.56	-10.15
Rumina	-12.53	-19.72	-8.66

Table 3.22b From 14.5 - 16.5°C

Table 3.22a-b Mean carbon isotope data from shells of *Helix aspersa* and *Rumina decollata* grown under comparable temperatures and conditions from Experiments 1, 2 and 3. (a) at 22.3°C - 'warm' room temperature and (b) from 14.5 to 16.5°C - 'cool' room temperatures.

* = permanent darkness, all others in permanent light

be assumed to be a constant. Therefore, using the carbon isotope values of shells of *Helix aspersa* maintained at 22.3°C from the three experiments (Table 3.22a):

In experiment 1

$$-13.42 = pOC + pAC + pIC\ (-13.09) \qquad (3.10)$$

In experiment 2

$$-19.66 = pOC + pAC + pIC\ (-43.35) \qquad (3.11)$$

In experiment 3

$$-9.93 = pOC + pAC + pIC\ (+2.24) \qquad (3.12)$$

Values for pIC may be obtained by subtracting equation 3.11 from 3.10; 3.12 from 3.10; or 3.12 from 3.11. In each case, pOC and pAC will cancel. For *Helix aspersa*, at 22.3°C values for pIC are 0.21, 0.23 and 0.21 - the slight differences between the values being the result of experimental errors in the measurement of the isotopic composition of shell and food materials. For *Rumina decollata* at 22.3°C, values for pIC are 0.25, 0.27 and 0.26. Thus, under the prevailing experimental conditions, shells of *Helix* are composed of approximately 22% inorganic carbon with 78% of the carbon coming from organic carbon through metabolism, or from atmospheric carbon dioxide. For *Rumina* around 26% of shell carbon appears to be from inorganic carbon sources.

Similar calculations have been carried out using the carbon isotope data from shells of *Helix* and *Rumina* from 'cool room' populations (Table 3.22b). However, in this case the conditions of temperature and lighting were not the same during all three experiments, hence the data are not directly comparable. Values for pIC from shells of *Helix* are 0.25, 0.13 and 0.20, and from shells of *Rumina* are 0.24, 0.25 and 0.24.

Such calculations take no account of possible fractionations between the calcitic foodstuffs and the aqueous bicarbonate from which the shell is secreted, and between that bicarbonate and the aragonitic shell. However the inclusion of such fractionations would not affect the calculated proportions, as the differences between the phases would remain the same.

(f) Assessing the relative importance of metabolic and evaporation effects on shell isotope composition using calculated oxygen isotope equilibrium values

The comparison of the oxygen isotope data from the control and test populations (sub-section 3.7.3b) revealed a large isotopic difference between the two, which was interpreted as being the result of the presence or absence of evaporation effects. Therefore, considering the test populations maintained within the bags and assuming that these populations were subject to negligible evaporation effects,, the difference between the measured shell oxygen isotope ratios, and aragonite precipitated at isotopic equilibrium at that temperature from the environmental water given to the snails (of known isotopic composition), should be primarily attributable to metabolic, or vital, effects. The control populations should, in a similar way, reveal metabolic plus evaporation effects.

Equilibrium oxygen isotope values have been calculated for *Helix* and *Rumina* f rom P2 and P8 (both at 22.3°C, and under permanently lit conditions), and from P7 and P9 (both at 14.5°C, and under constant light) using the temperature equation of Grossman and Ku (1986) and Hudson and

Anderson (1989), (see section 3.3.2) and the mean oxygen isotopic composition of the water given to the snails of -9.0‰ SMOW (see section 3.7.2a and Table 3.18).

These calculated equilibrium values, and the differences between them (Δ1 and Δ2) are shown in Tables 3.23a and 3.23b. Δ1 values represent, primarily, a metabolic effect, whereas, Δ2 values encompass evaporation plus metabolic effects. A value for the oxygen isotope enrichment in the control populations, caused primarily by evaporation effects, is given by subtracting Δ1 from Δ2.

Metabolic, or vital, effects account for between 1.98 and 3.00‰ of enrichment in $\delta^{18}O$ over calculated equilibrium values. The lowest Δ1 value (1.98‰) is from *Rumina* shells grown at 14.5°C. At 22.3°C, the Δ1 value for *Rumina* is 3.00‰, suggesting, for this species, that vital effects are more important at higher temperatures, and at temperatures closer to those of its natural environment (Mediterranean climate). For *Helix* , the two Δ1 values shown in Tables 3.23a and 3.23b, are almost identical (2.89 and 2.88‰). This suggests that for temperate and active Mediterranean species, a vital effect resulting in a 3‰ enrichment of shell oxygen isotopes over equilibrium values, should be expected. It is possible that some of the Δ1 effect could also be the result of evaporation, as the polythene bags were not completely sealed. However, it is felt that loss of water vapour from the bags would have been limited, and that a vital effect of 2.5 to 3‰ could still be inferred from these data.

The ^{18}O enrichments seen as a result of evaporation (Δ2 - Δ1) range from 4.79 to 8.66‰. Larger evaporation effects were recorded over the warmer temperature, and also for *Helix* rather than *Rumina*. The difference between the two species was greater at the higher temperature, suggesting that at elevated temperatures, the water used by the Mediterranean species, *Rumina*, has had less time to become isotopically enriched by evaporation than that used by the temperate species *Helix* . This in turn suggests that *Rumina* may take in sufficient water for its needs as soon as that water becomes available, whereas *Helix* may continually absorb water from its environment, which under the experimental conditions would become progressively more enriched by evaporation. This implied difference in water collection strategies, between the two species, may be related to the natural habitats and environmental conditions experienced by the snails. *Rumina decollata*, lives in a natural environment with arid seasons and only periodic precipitation. Therefore it would be necessary for this species to be able to become active immediately water becomes available and to take in rapidly, and possibly to store, sufficient moisture for continued existence. *Helix aspersa* is found generally in temperate climates and moist habitats, and with a more continuous supply of moisture this species may only take in that water for its immediate requirements, rather than storing any excess. However, as the range of this species does extend into the Mediterranean region, then by comparison of isotope data from Mediterranean *Helix* with north European *Helix*, it may be possible to test for habitat-specific differences.

(g) Comparison with experiments 1 and 2, and assessment of overall evaporation and metabolic effects on oxygen isotope ratios of shells

Δ2 (*i.e.* the difference between measured and equilibrium $\delta^{18}O$ shell aragonite values) for all species (where available) over the three experiments are shown in Table 3.24a. This table includes data from C3, 15.7°C (expt. 1), Pop 3, 16.5°C (expt. 2), and P9 14.5°C (expt. 3) to represent the 'cool' temperatures; and W2 (expt 1.), Pop 1 (expt. 2) and P8 (expt. 3) all at 22.3°C, to represent the 'warm' temperatures.

	BAG P2 $\delta^{18}O$ Meas.	22.3°C Eqm. $\delta^{18}O$ Calc.	VITAL EFFECT Δ 1 Meas. - Calc.	NO BAG P8 $\delta^{18}O$ Meas.	Δ 2 Meas. - Calc.	EVAP. EFFECT Δ 2 - Δ 1
Helix	-6.47	-9.36	2.89	2.19	11.55	8.66
Rumina	-6.36	-9.36	3.00	-0.70	8.69	5.69

Table 3.23a Comparison of oxygen isotope data from 'warm' room (22.3°C) test and control populations with equilibrium oxygen isotope values, in order to estimate the per mil oxygen isotope enrichment resulting from metabolic (vital) effects and evaporation effects. Equilibrium oxygen isotope values calculated using equation 3.1 after Grossman and Ku, 1986 (see section 3.3.2.

Δ1 = ^{18}O enrichment due to metabolic (vital) effects, calculated from measured $\delta^{18}O$ shell aragonite (test populations, in clear bags) and calculated equilibrium aragonite.
Δ2 = Measured $\delta^{18}O$ shell aragonite (control populations, no bags) minus calculated equilibrium aragonite, indicating ^{18}O enrichment due metabolic plus evaporation effects.
Δ2 - Δ1 = ^{18}O enrichment resulting from evaporation effects.

	BAG P7 $\delta^{18}O$ Meas.	14.5C Eqm. $\delta^{18}O$ Calc.	VITAL EFFECT Δ 1 Meas. - Calc.	NO BAG P9 $\delta^{18}O$ Meas.	Δ 2 Meas. - Calc.	EVAP. EFFECT Δ 2 - Δ 1
Helix	-4.78	-7.66	2.88	0.83	8.49	5.61
Rumina	-5.68	-7.66	1.98	-0.89	6.77	4.79

Table 3.23b Comparison of oxygen isotope data from 'cool' room (14.5°C) test and control populations with equilibrium oxygen isotope values (see caption above).

Δ1 = ^{18}O enrichment from metabolic effects.
Δ2 = ^{18}O enrichment from to metabolic plus evaporation effects.
Δ2 - Δ1 = ^{18}O enrichment from evaporation effects.

	Expt. 1 C3 15.7°C Δ 2	Expt. 2 Pop 3 16.5°C Δ 2	Expt. 3 P9 14.5°C Δ 2	Expt. 1 W2 22.3°C Δ 2	Expt. 2 Pop 1 22.3°C Δ 2	Expt. 3 P8 22.3°C Δ 2
Helix	6.08	9.10	8.49	13.34	10.44	11.55
Cepaea	6.83	9.37	–	13.86	10.65	–
Cernuella	5.03	–	–	12.58	–	–
Rumina	5.09	6.93	6.77	10.24	6.67	8.69
Ferussacia	4.79	–	–	9.02	–	–

Table 3.24a Δ2 values, calculated from mean $\delta^{18}O$ shell aragonite (meas.) minus equilibrium aragonite values (calc.) for all species where available, from Experiments 1, 2 and 3; at 'cool' room temperatures of 14.5 to 16.5°C and at a 'warm' room temperature of 22.3°C.

	Δ 2 'Corrected' Cool T°C	Δ 1 Assumed Cool T°C	Δ 2 - Δ 1 Cool 15.6°C	Δ 2 'Corrected' Warm T°C	Δ 1 Assumed Warm T°C	Δ 2 - Δ 1 Warm 22.3°C
Helix	7.58	2.75	4.83	11.74	2.75	8.99
Cepaea	8.33	2.75	5.58	12.26	2.75	9.51
Cernuella	6.53	2.75	3.78	10.98	2.75	8.23
Rumina	6.59	2.00	4.59	8.64	2.75	5.89
Ferussacia	6.29	2.00	4.29	7.42	2.75	4.67

Table 3.24b Corrected Δ2 values for all five species at a mean 'cool' temperature of 15.6°C and a 'warm' temperature of 22.3°C (see section 3.7.3f for method and assumed values of Δ1 (metabolic effect). From the corrected Δ2 values and the assumed Δ1 values, the oxygen isotope enrichment likely to have resulted from evaporation effects (Δ2 - Δ1) for all five species, at two mean temperatures (15.6 and 22.3°C, have been calculated.

Over the 'cool' temperatures, the Δ2 values from experiment 1 (4.79 to 6.83) are lower than those from the same species in experiments 2 and 3 (6.77 to 9.37). These variations reflect the somewhat different temperatures and conditions between the three experiments, and also, possibly, the the influence of the oxygen isotope composition of the inorganic carbonate in the diet given to the snails (see section 3.6.3b). The lowest Δ2 values occur in the data from experiment 1. For this first experiment, the snails were housed in a closed, controlled temperature chamber; thus, evaporation effects may have been reduced as compared to experiments 2 and 3, where the outer corridor was used as a 'cool room' base.

For the 'warm room', Δ2 values from experiment 1 are consistently higher than those from experiments 2 and 3, suggesting that either evaporation or vital effects were greatest in the first experiment. As the W2 regime (22.3°C) was the first phase of experiment 1 to be carried out, it is possible that less water was added at each feeding, and consequently evaporation was higher than in experiments 2 and 3, and in the later phases of experiment 1. Volumes of water added at each feeding were not measured, but water was sprayed on to the soil and boxes until a good coating was visible on the boxes, and the soil surface appeared well dampened.

To assess the overall effects of evaporation and metabolism for all five species over the course of all three experiments, it was necessary to calculate a 'corrected' Δ2 value for each species, using the Δ2 data from all three experiments for *Helix* and *Rumina* relative to that from experiment 1 (where Δ2 values are available for all species). This was carried out in the following way.

Over the 'cool' temperatures (mean temperature = 15.6°C), the mean Δ2 values for *Helix* and *Rumina* over the three experiments were 7.89 and 6.26‰, respectively. These mean values are 1.8 and 1.2‰ more enriched, respectively, than the Δ2 for these species from experiment 1. Therefore, to give a 'corrected mean Δ2 value for each species over the three experiments (at a mean temperature of 15.6°C), 1.5‰ (the mean of 1.8 and 1.2‰) was added to the Δ2 value calculated from the data for each species individually in experiment 1.

For the 'warm room' data, mean values of Δ2 for *Helix* and *Rumina* over the three experiments were 11.78 and 8.53‰, respectively. These mean values are 1.56 and 1.71‰ lower than the Δ2 values for these species from experiment 1, giving a mean difference from experiment 1 data of 1.6‰. To gain a 'corrected' Δ2 value for each species over the three experiments at a temperature of 22.3°C, 1.6‰ was subtracted from the Δ2 value for each species in experiment 1.

In the previous sub-section, Δ1 values (metabolic or vital effects) were assessed as between 2.5 and 3.0‰, although a slightly lower value was suggested for the Mediterranean species at lower temperatures. Therefore, Δ1 has been assumed as 2.75‰ for all the helicids and for the 'warm room' Mediterranean species, and as 2.0‰ for the Mediterranean species in the 'cool room'.

The corrected mean Δ2 values, and set Δ1 values for all five species over a mean 'cool room' temperature of 15.6°C, and a 'warm room' temperature of 22.3°C, are shown in Table 3.24b. From these data, values for Δ2 - Δ1 have been calculated, and are also shown in Table 3.24b. These Δ2 - Δ1 values are an estimate of the per mil enrichment caused by evaporation effects alone.

At 15.6°C, evaporation effects appear to cause enrichments of between 3.78‰ (*Cernuella virgata*), and 5.58‰ (*Cepaea nemoralis*). At this temperature, and with the reduced metabolic effect, the Mediterranean species appear to be subject to an enrichment of approximately 4.5‰, not significantly different from the temperate helicids. The overall mean oxygen isotope enrichment caused by evaporation, at 15.6°C, is 4.6‰.

For all species, the evaporation effect is greater at the higher temperature, although the difference between 'warm' and 'cool' is much larger for the temperate helicids than for the Mediterranean species. For the helicids, the enrichment caused by evaporation, at 22.3°C, is between 8.23 and 9.51‰, with a mean enrichment of 8.9‰. For the Mediterranean species, the mean evaporative enrichment is 5.28‰. However, both groups of snails should be equally susceptible to evaporation of environmental water prior to uptake by the snails. Therefore, the difference between the groups may indicate that the helicids continued to take in environmental water which was progressively enriched in ^{18}O due to evaporation whereas the Mediterranean species only took in water close to the time of feeding. The differences between the two groups may therefore relate to their ecologies and natural distributions, as discussed in the previous sub-section. Water loss from a snail's body fluid is thought to be a simple transfer process which does not result in isotopic enrichment of body fluids (Goodfriend *et al.*, 1989). However if the loss of water from a snail does result in some oxygen isotopic enrichment of the body fluid, then at higher temperatures, the helicids appear to be susceptible to a much greater water loss and degree of isotopic enrichment as compared to the Mediterranean species.

In summary, metabolic, or vital, effects appear to cause oxygen isotope enrichment in shell aragonite of about 2.75‰. This enrichment may be less for Mediterranean species as compared to temperate helicid species at temperatures close to 15.6°C. From the limited data available, it does not appear that the amount of metabolic enrichment increases with temperature.

Evaporation causes at least twice as much oxygen isotope enrichment as metabolic effects. At temperatures close to 15.6°C, evaporation effects appear to result in oxygen isotope enrichment of approximately 4.6‰. At higher temperatures, Mediterranean species appear to be less vulnerable to evaporation effects (5.3‰ enrichment), than the temperate helicids (8.9‰ enrichment).

3.7.4 Summary

1) Shell growth was favoured at higher environmental temperatures, as was noted in experiments 1 and 2. Shell size was comparable between populations maintained in and outside of the bags although final shell weight was less in the bagged populations, suggesting that such an environment was not ideal for the snails. The effect of keeping snails in the opaque and transparent bags, in relation to the growth patterns of the snails, was different for the various species.

2) The oxygen isotopic compositions of the shells were lower (more depleted) at higher environmental temperatures, and the rate of depletion with increasing temperature was in fairly good agreement with that predicted for the secretion of aragonite shell (around -0.213‰ per °C, Grossman and Ku, 1986). The range of oxygen isotope values of shells at the three mean environmental temperatures (14.5, 18.4 and 22.3°C) were distinct, although there was overlap in the isotope data from individual species within each temperature level.

Slightly more depleted $\delta^{18}O$ values were recorded in the shells of snails kept in transparent bags as opposed to opaque bags - the difference was generally 0.3 to 0.5‰. For those populations moved between the warmer and cooler environment every twelve hours, under conditions of continual darkness (*i.e.* in the opaque bags) the oxygen isotopic composition of the shells appeared to reflect conditions in the warmer part of the daily cycle, although shells in the transparent bags reflected growth

over both temperature regimes. These findings suggest that the snails found conditions in the opaque bags less favourable than those in the clear bags, especially at lower environmental temperatures.

A large difference was found between the $\delta^{18}O$ values of the bagged and control populations of snails, with shells from the control populations being 5 to 8‰ more enriched than the shells from the bagged populations at similar temperatures. This marked difference is thought to reflect a much reduced evaporation effect in the bags as opposed to the control populations. The evaporation effect upon the control populations, and thus the difference between the bagged and control populations, was greater at a higher environmental temperature.

Differences between the oxygen isotope compositions of shells grown over comparable conditions from the three experiments are thought to reflect primary differences in the oxygen isotopic composition of the environmental water, and possibly the food, given to the snails; and also to relate to the exact conditions of temperature, light and location of the three experiments.

3) The carbon isotopic compositions of the snail shells were slightly lower (more depleted in ^{13}C) at higher environmental temperatures, as noted in experiment 1. This is thought to reflect the use of a greater proportion of metabolic carbon dioxide, as opposed to atmospheric carbon dioxide, with increasing temperature, which in turn is linked with increased shell growth at the higher temperatures.

Each snail species was found to have characteristic carbon isotope ratios, with no overlap between the $\delta^{13}C$ values of each species within each of the three temperatures, suggesting that each species uses a different balance of organic to inorganic constituents in the diet, or else processes the material differently. The range of values from each temperature regime was greater than that for the oxygen isotopes.

Maintaining the snail populations in clear or opaque plastic bags did not give rise to any systematic isotope effects, as had been the case for oxygen isotopes.

The carbon isotope ratios of snail shells from the bagged populations were between 0.4 and 2.0‰ more depleted than the control populations (no bags) of the same species grown at the same temperatures. This suggests that the snails in the bags were using more metabolic, rather than atmospheric, carbon dioxide as compared to the control populations.

The differences between carbon isotope compositions of snail shells produced under similar conditions in all three experiments reflects the changes in the carbon isotopic composition of the inorganic carbon fraction of the snails' diet. Comparison of the carbon isotope data from the three experiments allowed the proportion of shell carbon formed from the inorganic carbon supplied in the diet, to be estimated. At 22.3°C, around 22% of the carbon isotopic composition of shells of *Helix aspersa* was attributed to the inorganic carbon in the snails' diet, and for *Rumina decollata* the value was around 26%.

4) Oxygen isotope values of shell material secreted at isotopic equilibrium with the environmental water given to the snails were calculated. The differences between these calculated equilibrium values and the measured shell oxygen isotope values ($\Delta 1$), for the test populations, were thought to represent a vital isotope effect relating to the metabolism of the snails with little or no evaporation effect. From the results of experiment 3, a vital effect, causing oxygen isotope enrichment, of between +2 to +3‰ was indicated. The differences between calculated equilibrium values and measured shell values for the control populations ($\Delta 2$) were much larger and were thought to reflect metabolic plus evaporation

effects. Subtraction of Δ1 from Δ2 indicated the oxygen isotopic enrichment resulting from evaporation effects which affected the control populations. The evaporation effect was between +4.8 to +8.7‰.

In the light of these findings the results of experiments 1 and 2 were reconsidered and values of Δ2 - Δ1 were assessed over two mean environmental temperatures (15.6 and 22.3°C) for all five of the species used in the experimental work. At temperatures around 15.6°C, the oxygen isotope enrichment in shells resulting from evaporation effects was between +3.8 to +5.6‰, with a mean enrichment of +4.6‰ for the five species. At temperatures around 22.3°C, the evaporation effect was as much as +8.9‰ for the temperate helicid species, but a lower +5.3‰ for the two Mediterranean species. Metabolic effects were seen to cause a constant oxygen isotope enrichment of around +2.75‰, although this value was probably nearer +2‰ for the two Mediterranean species at 15.6°C.

CHAPTER 4

INVESTIGATION OF THE STABLE ISOTOPIC COMPOSITION OF SHELLS OF SELECTED TERRESTRIAL MOLLUSCS FROM JAMAICA, WEST INDIES : A CASE STUDY

4.1 INTRODUCTION AND AIMS

In conjunction with the extensive laboratory experiments presented in the preceeding chapter, it was hoped to investigate the stable isotopic compositions of shells of terrestrial molluscs from natural environments in order to assess relationships between shell isotopes and local environmental variables and between the environmental waters utilised by the snails and the shell material ultimately secreted.

Jamaica, part of the Greater Antilles of the Caribbean, was selected as a site for such a case study for a wide variety of reasons

1) Terrestrial snails are widely distributed across the island as much of the bedrock and soils are calcareous in nature.
2) The land snail fauna of this small island shows a remarkable diversity with some 400-450 species being recognised (Goodfriend and Mitterer, 1988) as compared with the whole of north west Europe where 279 species of snail and slugs are known (Kerney and Cameron, 1979). Also unusual is the very high proportion of endemism among the land snail fauna with about 95% of the species occurring only in Jamaica (Goodfriend and Mitterer, 1988).
3) The island land snail fauna is strongly regional in character responding to different climatic regions (Paul 1982, Goodfriend, 1986a and 1986b, Goodfriend and Mitterer, 1988). Recent research has shown that some species only occupy limited geographic areas, and one species may be completely replaced by another of the same genus over a very short distance (C. R. C. Paul pers. comm., 1987). Such a degree of specialisation is rare.
4) Both topographic and climatological gradients are sharp over a small geographic distances, although precise meteorological data are limited.
5) Co-operation exists between the Departments of Geology, University of West Indies, Jamaica, and Earth Sciences here at Liverpool.
6) Previous trips to Jamaica were undertaken by C. R. C. Paul in 1979 and 1986, and thus background information upon aspects of terrestrial mollusc taxonomy and distributions was available and specific areas of investigation could be targeted.

With these points in mind, the main aims of the Jamaican case study were,

1) To look for patterns in the stable isotopic compositions of land snail shells, in relation to environmental variables, along transects from the coastal plains into the hilly interiors.
2) To collect samples of the environmental waters available to the snails, and in a similar way to the experimental work, to assess whether the shell would be secreted at or near isotopic equilibrium with the collected waters.

4.2 JAMAICA : GEOGRAPHIC SETTING AND BACKGROUND INFORMATION

4.2.1 Location and setting

Jamaica lies in the north western sector of the Caribbean archipelago between latitudes 17° 43' and 18° 32' North and longitudes 76° 11' and 78° 21' West. The island, covers an area of 11,396 km^2 (4,400 square miles), which is similar in size to the county of Yorkshire (Clarke, 1974) measuring approximately 150 miles from east to west coasts and between 25 to 50 miles from north to south coasts. Jamaica forms part of the group of islands in the Caribbean sea known as the Greater Antilles. The closest neighbours to Jamaica are Cuba and the Island of Hispaneola (Figure 4.1).

Jamaica (population close to two million) is subdivided into fourteen parishes which run inland from the coasts and are divided into northern and southern groups by a line that runs across the island. The parishes constitute the major geographic divisions of Jamaica. The only large population centre is that of Kingston with the rest of the population living in small towns, villages and dispersed rural dwellings. The parishes and major towns are shown in Figure 4.2.

4.2.2 Topography and geology

Jamaica has three main topographic types: the interior mountain ranges; the dissected limestone plateaux and hills; and the coastal plain and interior valleys (Bent and Bent-Golding, 1966).

The mountain ranges form the core of the island and rise to nearly 2,500 metres (over 7,000 feet) in the east of the island (the Blue Mountain range). The central and western ranges are much lower but reach slightly over 1,000 metres (3,000 feet) above sea level.

Dissected limestone plateaux and hills surround the interior mountain ranges and are usually below 3,000 feet. The plateaux often show well developed karst topography with rounded, conical or elongated hummocks with depressions between them. This type of karst landform covers much of the limestone plateaux and is most prominent in the Cockpit Country of Trelawny.

The coastal plains are broadest to the south of the island, being composed of alluvial sands, gravels and loams. On the north coast the plains are narrow, but in places extend inland to the limestone foothills, and flat bottomed valleys, or poljes, may be found in the karst regions of the limestone hills.

The broad geological divisions are evident from the topography and the geology also determines the pedology and hydrology. The overall structure is anticlinal, with an east to west axis.

The eastern portion of Jamaica is dominated by a basal complex of igneous and metamorphic rocks, whilst much of the remainder of the island is formed by white and yellow limestone, which lie unconformably upon the older basement, which is composed primarily of...

... shales, limestones conglomerates, tuffs, some intrusive and extrusive igneous rocks and metamorphic marbles. Towards the end of the Cretaceous, uplift and folding formed the Blue Mountain Range. The Tertiary period was characterised by submergence and the deposition of the Yellow Limestone followed by the more extensive White Limestone formations right across the island, with probably only the peaks of the Blue Mountain range remaining uncapped. Elevation and block faulting

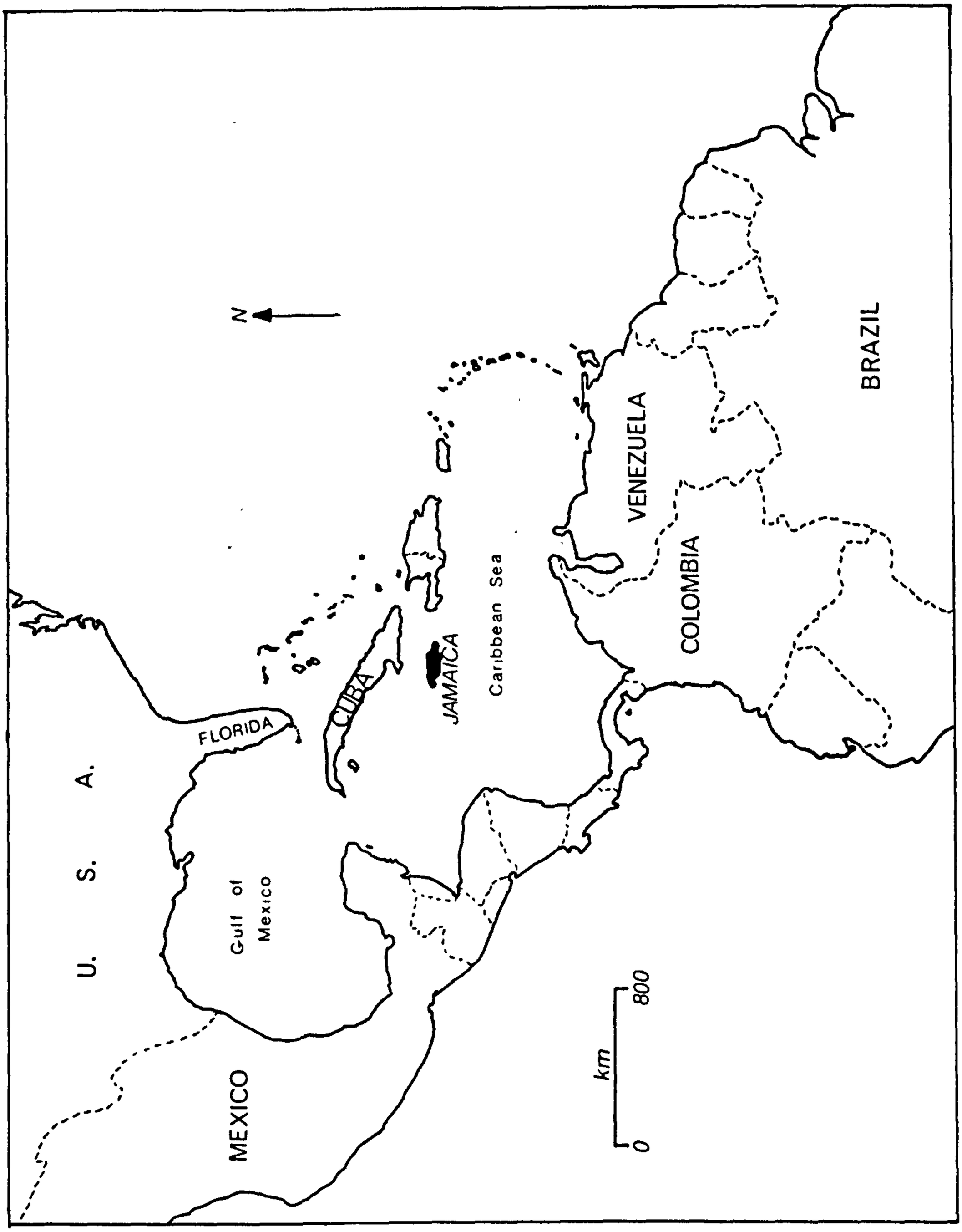


Figure 4.1 Location of Jamaica in the Caribbean Sea

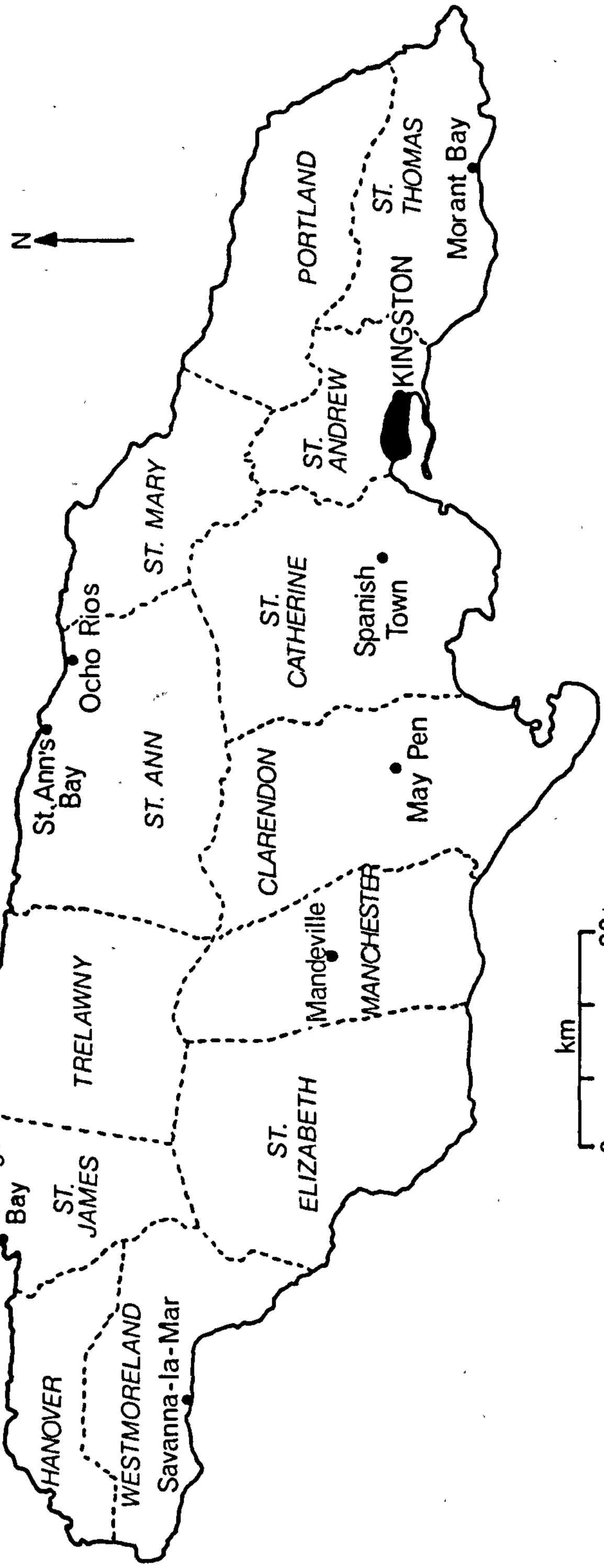


Figure 4.2 Parishes and major towns of Jamaica

with folding occurred in the late Tertiary, with faults running north-south and east-west. This period of uplift brought the Blue Mountains up to around 2,500m (7,000 ft) whereas the central and western parts of the island reached only 700 to 1,000m (2,000 - 3,000 ft). At this time the Blue Mountain rocks were also subjected to metamorphism.

The Quaternary has seen the karstification and erosion of the White and Yellow Limestones. Terra rossa soils rich in bauxites have been produced in White Limestone areas. In central and western districts, the capping of the limestones has been breached revealing inliers of the Cretaceous shales, conglomerates, limestones and tuffs. Pronounced fault scarps have developed, limiting interior basins, coastal plains and drainage. The Blue Mountain Range has also been subject to intense erosion and this coupled with uplift has produced river valleys cut to depths of several hundred metres. The most recent deposits have been in the coastal and interior valleys, where levels of sediment are being progressively raised. Off the south coast, a broad shallow shelf extends for several miles with coral and algal reefs and cays of sand and coral detritus. The north coast is dominated by fault scarps and fringing reefs and barriers are restricted. Quaternary uplift has led to a series of raised reefs and shorelines above the present sea level (Digerfelt and Hendry, 1987). Therefore the northern margins are being uplifted with the southern margin tilting down. The topography and geology of Jamaica are summarised on Figures 4.3a and 4.3b respectively.

4.2.3 Climate and Vegetation

Jamaica lies in the path of the north east trade winds and experiences a tropical maritime climate. The climate is characterised by little change in seasonal temperature, although the mountainous nature of the island produces regional and altitudinal variations. Sea level stations record averages of about 27°C and ranges between January and July of less than 5°C (Clarke, 1974).

The rainfall is, on the other hand, distinctly seasonal with maxima occurring in May and October. The heaviest rainfall is orographic and thus is concentrated over the Blue Mountains on the eastern portion of the island. A marked rainshadow effect occurs to the leeward side of the mountains. For example, Kingston records less than 76 cm (30 inches) of rain annually whereas over 500 cm (200 inches) falls on the Blue Mountains. In parts of the Central plateau average annual precipitation exceeds 190 cm (75 inches), but falls below 125 cm (50 inches) on the sheltered south coast and near Montego Bay. However, the high Cockpit country experiences more than 250 cm (100 inches) (Clarke, 1974).. The annual rainfall may be augmented by hurricanes generated in the Atlantic which generally track westerly across the Caribbean. Hurricane season extends from June to November. The climate and rainfall experienced by Jamaica are shown in Figure 4.4 (after Clarke, 1974).

Relief and climate influence the pattern of natural vegetation in Jamaica. However the impact of human settlement and cultivation is strongly evident and natural vegetation only survives in the most isolated and inhospitable regions. Much of the island is cultivated to produce crops such as sugar cane, banana, citrus, coffee, cocoa, coconuts, ginger and pimento. Asprey and Robbins (1953), have produced a checklist of plants occurring on Jamaica and they suggest that the total flora is around 4,000 plants, excluding fungi and lichens, around 20% of which (a minimum value) are endemic. The vegetation types may be categorised as: coastal communities, including marine, shoreline and cay communities; lowland communities, comprising dry limestone scrub forest, wet limestone forest and

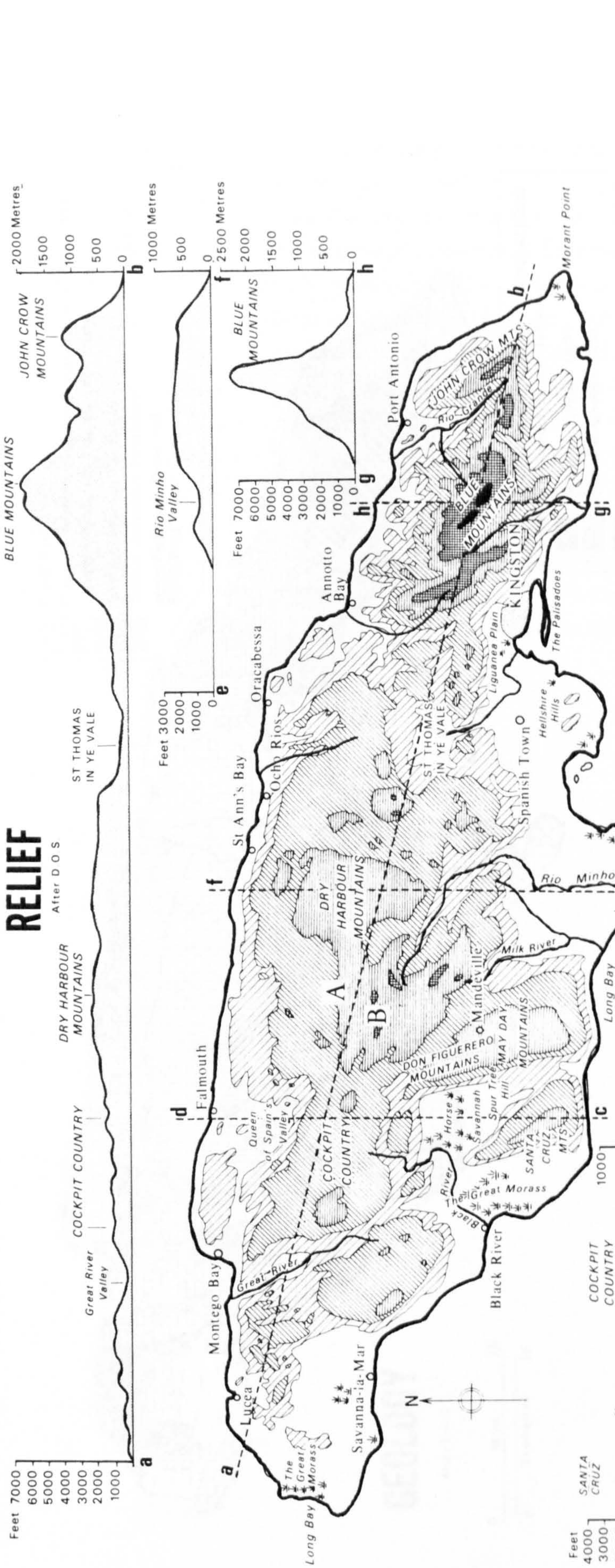


Figure 4.3a Topography of Jamaica (after Clarke, 1974)

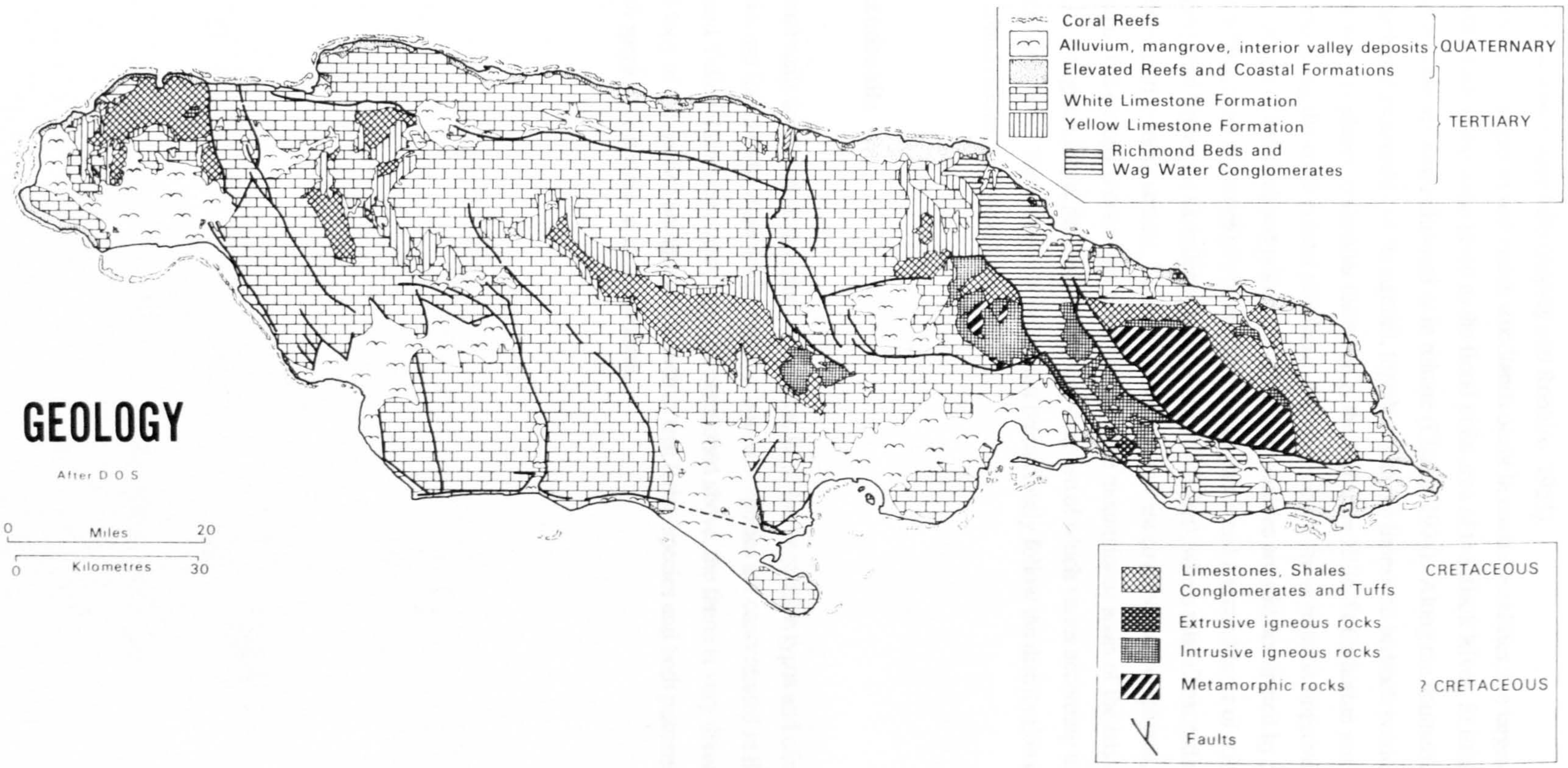


Figure 4.3b. Geology of Jamaica (after Clarke, 1974)

bauxite plateaux; alluvium communities; swamp and marsh communities; montane communities; and vegetation on the lower shale hills (Asprey and Robbins, 1953).

Swamps, mangroves and marsh woodlands occur in coastal localities, the largest being the Great Morass which is the name given to the flood plain area of the Black River, St Elizabeth. Much of the lowland areas are now cultivated or in ruinate (Clarke, 1974). Along the southern margins of the island, the lack and seasonality of the rainfall, together with the limestone bedrock produces a low scrub forest with, in places, col·umnar cactus (*e.g.* the Hellshire Hills, St Catherine just to the west of Kingston, and along the north coastal parishes). Moving inland, the vegetation improves with a more continuous canopy of red birch and cotton trees, but communities are still dominated by plants adapted to seasonal aridity, such as xerophytic bromeliads, small palms and a ground layer of a few ferns, cacti or woody perennial herbs. At elevations above 300m (1000 feet), where rainfall exceeds 190 cm (75 inches), wet limestone forest occurs. Trees are evergreen and are covered with epiphytes, bromeliads and tree ferns. The canopy is dense but never closed. The mountainous areas of the island are dominated by evergreen forest, the components and structure of which varies according to the altitude and degree of exposure. The changes in vegetation types closely follow the distribution of rainfall across the island (shown in Figure 4.4).

4.2.4 Landsnails

Landsnails are found across most of Jamaica, in all the vegetation types and climatic zones. Some species are found away from the limestones, but most species are concentrated in the areas where the White and Yellow Limestones crop out. As mentioned above, the fauna is very diverse and highly endemic. Many of the species are specialists such as tree living species and both pulmonate and prosobranch species are present in large numbers.

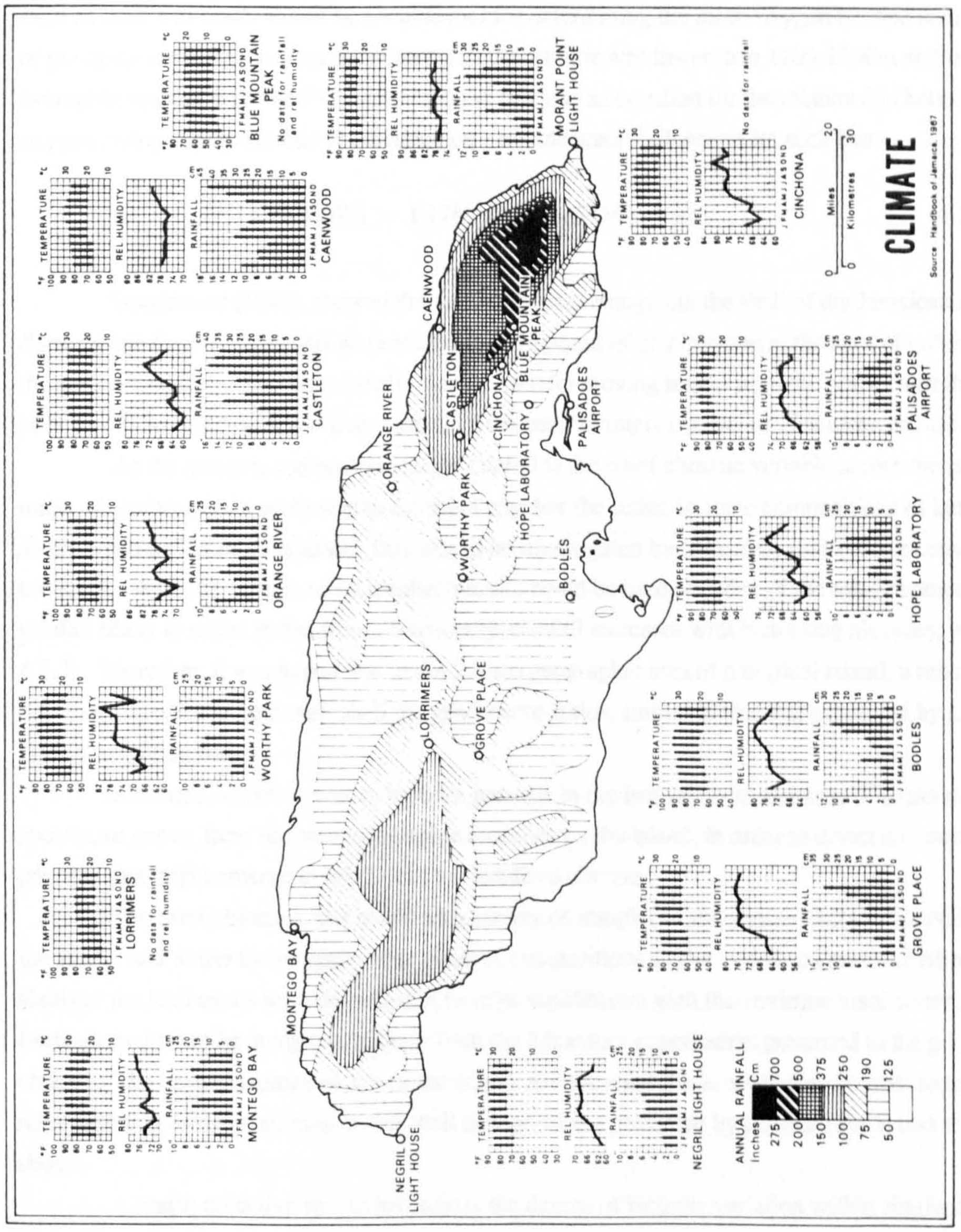


Figure 4.4 Jamaican climate and rainfall (after Clarke, 1974)

4.3 OBJECTIVES

Lecolle (1985), found that for snails in western Europe the oxygen isotope ratios of their shells correlated with altitude, with shell isotope values becoming more depleted in ^{18}O at higher altitudes. The slope of the function varied depending upon the range in altitude, but for the four species shown was between -3 and -8‰ per 1000m. Furthermore, as the oxygen isotope composition of precipitation would be related to temperature and altitude, Lecolle maintained that the oxygen isotope ratio of landsnail shells would be a viable tool for determining the mean oxygen isotope composition of precipitation at that locality, providing that the altitude was lower than 1200-1300m and/or with an average temperature above 6°C. He went on to produce an equation for the relationship between the oxygen isotope compositions of precipitation and land snail shell aragonite such that

$$\delta^{18}O_P(SMOW) = 1.17\delta^{18}O_{S\ arag}(PDB) - 5.91 \qquad (4.1)$$

Goodfriend (1987), showed that morphological changes in the shell of the Jamaican landsnail *Pleurodonte lucerna* (Müller) were related to the amount of precipitation at the sites of collection of the shells. Goodfriend collected shells along a transect moving inland from the north coast through the parish of St Ann and he found that mean shell diameter strongly correlated with mean annual rainfall.

As the amount, and seasonality, of rainfall is the chief climatic variable across the island, the major objective of this study was to ascertain whether the stable isotopic compositions of land snail shells could be linked to rainfall. This was to be investigated by collecting snails along coast to inland transects, where the altitude of each collection site could be used an index of the relative amount of rainfall likely to occur at that place. (Generally, rainfall increases with increasing altitudes, see section 4.2.3). Therefore, it was hoped that in the limited geographic area of a tropical island, a relationship between altitude and land snail shell oxygen isotope ratios, similar to that that presented by Lecolle (1985), might be found.

A second objective was to look for patterns in the isotopic compositions of shells of the same species, or genus, from different geographic locations on the island, in order to detect any trends or patterns that might emerge in relation to the localised climate.

The third objective was to collect a variety of samples of the waters likely to be available to the snails, and to use the oxygen stable isotopic compositions of the waters to assess whether the shells of the land snails were deposited at isotopic equilibrium with the environmental waters. These findings could then be compared to those from the laboratory experiments presented in the preceeding chapter. The oxygen isotope values of the waters and the snail shells could also be used to test the relationship between precipitation and shell isotope values presented by Lecolle (1985) and shown above.

A fourth objective was to investigate the degree of isotopic variation within single shells and to look for any patterns of seasonal or annual growth.

4.4 METHODS

4.4.1 Field sampling

(a) Selection of, and constraints upon, field sampling areas

As Jamaica is a very mountainous island, and is essentially a developing country, much of the island interior is largely inaccessible by road. Time constraints and practical considerations meant that the majority of the field collections were made close to Kingston. Consequently, much of the analytical work was carried out on shells from sites in the Red Hills to the north west of Kingston in the parishes of St. Andrew and St. Catherine, where the bedrock is the White Limestone. Other minor collections were made in the Santa Cruz Mountains of St Elizabeth, and a few sites on the edge of the Cockpit Country were sampled on a brief sortie into Trelawny and St James.

Much of the scrub and forest is difficult and hazardous to penetrate, and consequently coast to inland transects largely followed the existing paved roads. Collections were made at the road side, although where possible the ground cover and any exposed bedrock adjacent to the roads was investigated.

(b) Collection methods - snails

Route plans for each collection day were drawn up, but it was not always possible to negotiate, or even locate, some of the roads marked on the topographic base maps. However, with the large scale maps available (1 : 50,000) it was possible to locate collection sites and to collect snail shells at fairly regular altitudinal or geographic intervals.

Each collection site was marked on the base map and a six figure grid reference and an approximate altitude (generally to within 100ft) assigned. At the time of the field collections, new metric topographic maps of Jamaica were not yet available for the whole of the island. Therefore, the old non-metric maps were used throughout and all altitudes are quoted in feet above sea level.

Foraging for shells in the roadside vegetation and/or outcrops generally produced a good collection of specimens within ten to twenty minutes, especially if locals arrived upon the scene to lend helping hands. It was evident, by the drop in numbers of shells and diversity of species, when the geological boundaries between calcareous and non-calcareous bedrock, were crossed. Only rarely were live specimens noted and these were generally inactive. The majority of shells collected were empty. Weathered or bleached shells were not taken if more recently vacated shells could be located. Where plant litter was present sieves were sometimes used to look for smaller individuals (approximately 0.5 to 2mm in diameter). However, the collections concentrated on the larger and more readily visible species. Shells were sorted and stored in small polythene bags, labelled with the location name and assigned site number. Collections were made at a total of 96 sites, and the distribution of these sites across the island is shown on Figure 4.5.

The collections, made over two short trips to Jamaica, may not represent complete faunas at each site, especially where only the larger shells, easily visible to the eye, were sampled. Additionally, species and even generic identifications of some taxa could only be tentative, as no index of the complete Jamaican fauna has been published. However, of the shells collected, over fifty taxa (genera and species) have been separated. The field collections await complete identification and tabulation.

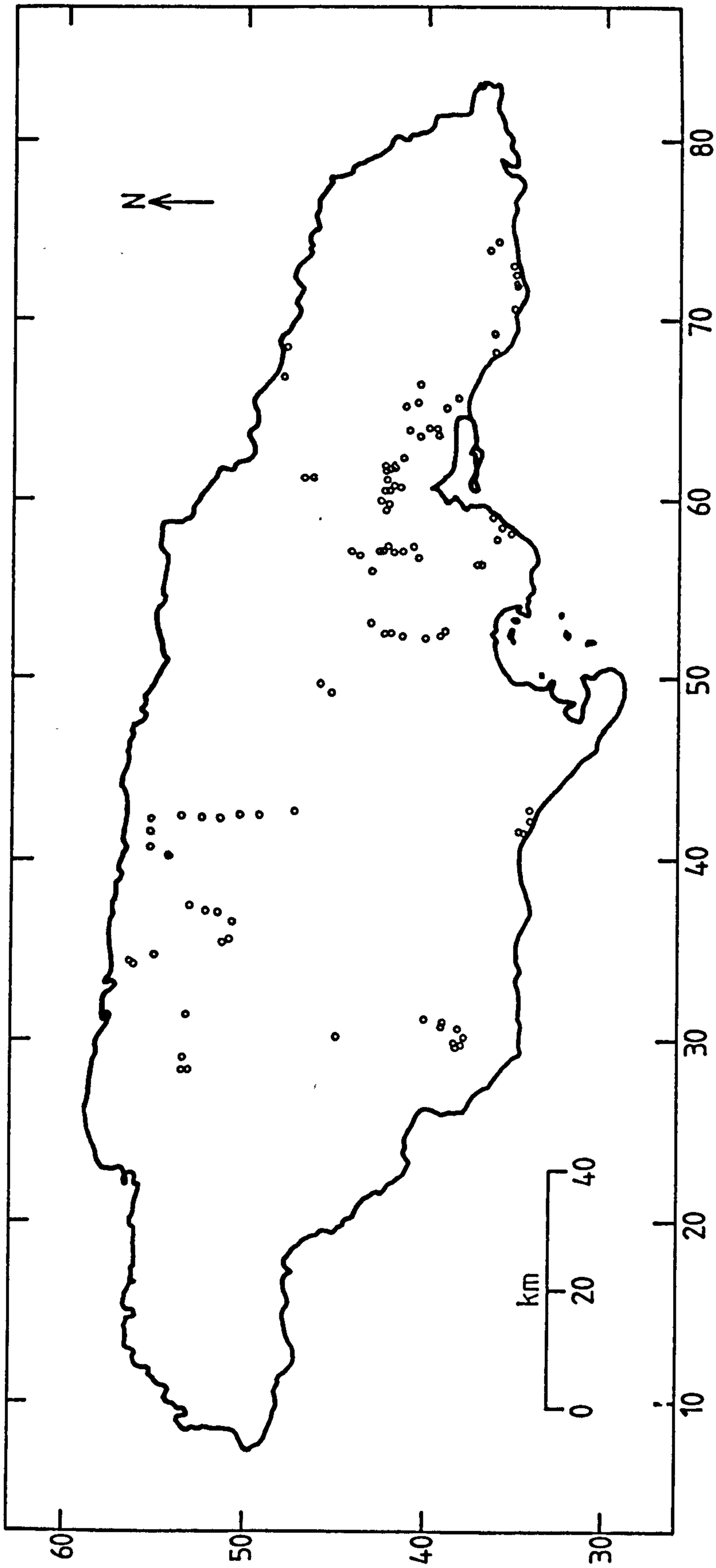


Figure 4.5 The distribution of the 96 collection sites across Jamaica grid = 1 : 50,000 topographic map grid

However, it is possible to recognise with certainty the most common taxa found in Jamaica, and to separate different species. Of these taxa, certain species were targeted for collection for later stable isotopic analysis.

(c) Selection of target species and notes on their ecologies

In selecting potential snail species for isotopic analyses, several factors were taken into consideration. It was decided to choose taxa that were relatively large in shell size so they would be more visible to the collector's eye. It was also necessary to choose species that were likely to be found over a relatively wide geographic area and over a range of altitudes. Ideally, information on the ecology and island wide distribution of the species would be available, and the species would be present in sufficient numbers to facilitate location of specimens.

The species selected as the principal target of collection, and subsequent stable isotopic analysis, was the endemic pulmonate *Pleurodonte sublucerna* (Pils.) of the family Camaenidae. This species forms a semispecies complex with *Pleurodonte lucerna* (Müller), and the two semispecies are found over much of the island (Goodfriend, 1983). Although the two species may be separated by examination of the soft tissues and especially the reproductive organs, the shells of the both species are very variable in size and shape and the two species are not necessarily distinct. However, Goodfriend (1983), has collected specimens of the snails from much of Jamaica, and showed that *P. lucerna* is present to the north and west of the island, and *P. sublucerna* to the south and east, including the area of the Red Hills and encompassing the parishes of Clarendon, St. Catherine, St. Andrew and Kingston and St. Thomas. The two species have a narrow hybrid zone in the south of St. Ann. As the majority of collection sites sampled in this study are in the Red Hills and surrounding districts, it may be assumed that the species located was *Pleurodonte sublucerna* .

The shell of *Pleurodonte sublucerna* is rather large in size but very variable in both shape and thickness of shell (collected specimens ranged in size from 15 x 29 mm to 30 x 52mm, with four to six depressed whorls. Mature individuals have thickened reflected lips, a closed umbilicus and may develop apertural denticles. The periphery of the shell may be rounded or somewhat keeled, and the shells are yellow-brown to brown in colour, sometimes with a purplish tint in the under side. The snail is a ground dwelling species, and mature individuals are nocturnal (Goodfriend, 1983). This species is shown in Figure 4.6a. The morphology of the other species of the complex (*P. lucerna*), has been shown to be linked to rainfall (Goodfriend 1983 and 1987), and also the shells of this species are of sufficient size to allow investigation of the intra- shell pattern of oxygen and carbon stable isotopes, as several sequential sub-samples could be taken. Thus, *P. sublucerna* was selected for investigation.

Another group of land snails widely distributed across island are species of the pulmonate family Urocoptidae, including the genus *Urocoptis* . Shells of *Urocoptis* are cylindrical in shape, generally with 8 to 10 whorls, and between 15 to 30mm in height depending upon the species. The shells may be pink to yellow-brown, pale brown or white in colour. The snails of this genus are ground dwelling and a high degree of local endemism is apparent with species occupying only small areas of the island (Pilsbry, 1903). Various species of *Urocoptis* were collected, but stable isotopic analysis was limited (due to time constraints) to the xerophile *Urocoptis brevis* (Pfr.) collected from various habitats along the southern coastal margins of Jamaica, and to a few specimens of the much

Figure 4.6a *Pleurodonte sublucerna*

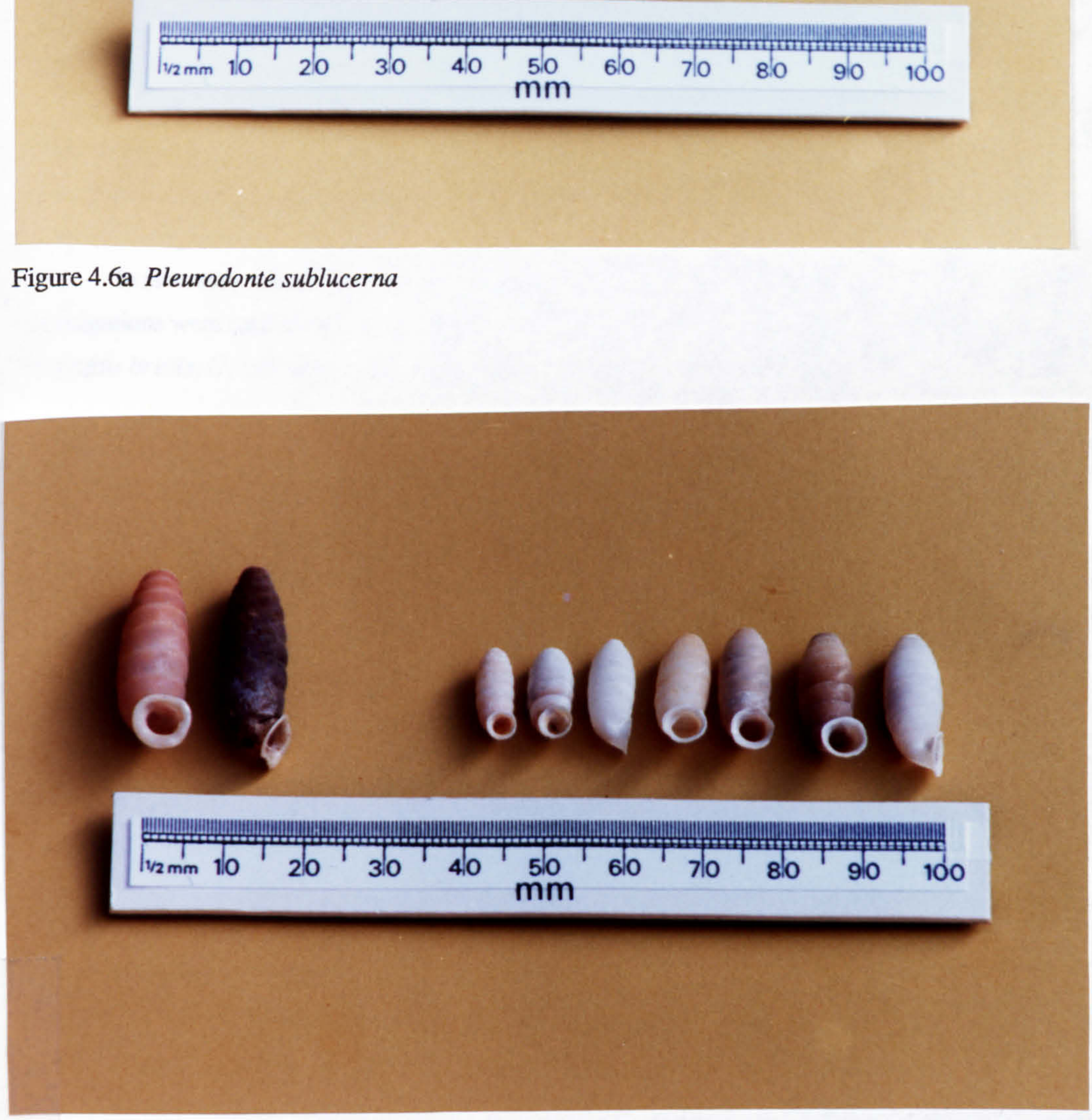


Figure 4.6b *Urocoptis brevis* (right) and *Urocoptis cylindrus* (left). The specimens of *U. brevis* indicate the wide variety of shell morphologies within this urocoptid species

Figure 4.6a-c The four species of Jamaican land snail investigated in this study

larger *Urocoptis cylindrus* (Chemnitz) found in the wet limestone forest of the Cockpit Country. These species are shown in Figure 4.6b.

To contrast with the isotopic analysis of the two pulmonate genera (*Pleurodonte* and *Urocoptis*) it was decided to investigate the stable isotopic composition of shells of an operculate snail. As with the urocoptids, many of the prosobranch snails have very localised distributions, *e.g.* species of the genera *Adamsiella; Alcadia; Annularia; Helicina; Parachondria* and *Tudora* although no data on the distributions of these taxa have been published (C. R. C. Paul, pers. comm., 1989). However a few species are more widely distributed, and one of these, *Lucidella aureola* (Fér.), of the family Helicinidae, was collected from many of the sites investigated, and from most of those in the Red Hills. It was therefore decided to carry out an investigation of how the isotopes of this operculate snail might vary with rainfall/altitude and to compare the findings with those from the pulmonate *Pleurodonte sublucerna* .

The shell of *Lucidella aureola* is relatively small, (around 6 x 8mm), triangular in shape and is coloured yellow to pink to reddish brown. Shells of dead individuals were located on the ground, often in large numbers, but this species is arboreal (C. R. C. Paul, pers. comm. 1990). This species is shown in Figure 4.6c.

In summary, of the many taxa collected from the field expeditions, the stable isotopic investigations were carried out on a limited range of species. These were *Pleurodonte sublucerna, Urocoptis brevis, U. cylindrus* and *Lucidella aureola.*

(d) Water samples

Samples of water were taken from streams, rivers, from a spring, as freshly fallen rainwater or storm runoff and by sweeping over vegetation dripping with early morning dew. Water samples were stored in 30ml Nalgene (dense plastic) screw cap bottles to which a few milligrams of sodium azide had previously been added. The azide would act as a poison to kill any algae or microbes that might alter subsequently the isotopic composition of the water. The bottles were filled to the brim and the closures of the bottles were secured with 'parafilm'. The bottles were stored in a black polythene bag to which little or no light could penetrate, and the bag was kept as cool as possible.

Surface water was close at hand at only a very few of the collection sites. The chosen sampling points were allocated six figure grid references.

4.4.2 Laboratory analysis

(a) Measurement of shell samples

All *Pleurodonte* shells were measured (height and diameter (see section 3.4.2) prior to preparation for isotopic analysis. The measurements were to within 0.5mm. Dimensions of shells of *Urocoptis* and *Lucidella* were not measured as they were much less variable than the *Pleurodonte*

(b) Preparation for isotopic analysis

All shell samples were prepared following the scheme presented in section 2.5 and in Figure 2.5. However, owing to the varying sizes of the shells investigated, different portions of shell from the different species were utilised.

Figure 4.6c *Lucidella aureola* The specimens have basal diameters of 6mm

1) *Pleurodonte sublucerna*

The large size and strong nature of the shell of this species, meant that preparing and grinding up entire shells would have been time consuming and impractical. Therefore, sub-samples were taken from each shell by drilling out a slice of shell from just behind the aperture. A small micro drill with a circular, flat-ended bit was used, and the shells were held in a vice padded with tissue to allow stability and prevent crushing during the drilling process. Slices of shell were cut from just below the suture to an equal distance below the keel, and were generally around 0.5 to 1cm in width and approximately 1.5 to 2cm in height (depending upon the original size of the shell). Manual cleaning took place prior to and after drilling, and the drilled slices were then processed following the standard procedure of bleaching, grinding, roasting etc, before the powdered samples were finally ready for isotopic analysis.

Where a number of sub-samples was required from an individual shell, the complete shell was cleaned and bleached, and then an arrow-head drill bit was used to drill out small portions of shell at regular intervals behind the aperture. The drill holes were made on the upper surface of the shell just below the sutures. The powdered samples produced were then further processed following the standard technique.

2) *Urocoptis brevis* and *U. cylindrus*

The cylindrical nature of the urocoptids meant that it was relatively easy to take slices of shell, cutting along the sutures. This same method had been adopted for the cylindrical species *Rumina decollata* in the laboratory growth experiments. Therefore, for both species of *Urocoptis*, the last two whorls were sliced off from the remainder of the shell using a scalpel. The whole of the last two whorls from each shell were processed and analysed for their stable isotopic composition.

3) *Lucidella aureola*

For this species entire shells were processed as shell size and weights were relatively small, and as the inner whorls are resorbed, manual cleaning was relatively straightforward.

(c) Stable isotopic analysis

The method of analysis of the powdered shell samples has been described in section 2.6. All carbon and oxygen isotope values are relative to the PDB standard and are presented as ‰ values.

Oxygen and deuterium isotopic analyses of five water samples were carried out at the B.G.S. Stable Isotope Laboratory at Wallingford (see section 2.6). The results are relative to the SMOW standard.

Firstly, the results of the investigation of isotopic variation within individual Jamaican shells are presented below, in order to establish the range of values likely within shells. Following this, the results of investigations of shell isotopic compositions along coast to inland transects, and local and regional variations in shell isotope ratios, will be presented. The data from all shell samples *i.e.* isotopic analyses, site numbers, grid references, altitudes, and where applicable, shell heights and diameters for each sample are shown in full in Appendix 2.

4.5 ISOTOPIC VARIATION WITHIN INDIVIDUAL SHELLS

4.5.1 Introduction

To investigate the degree of isotopic variation likely within individual shells and to look for any patterns of seasonal or annual growth, it was decided to use shells of *Pleurodonte sublucerna*. Shells of this species are large enough to generate several sub-samples (up to eleven were taken) from within each specimen by using a micro-drill (see section 4.4.2 for method). The six chosen specimens came from four sites: from Cave Valley and nearby Culloden in St. Ann; from Mandeville in Manchester and from the Hellshire Hills of St. Catherine. These particular shells were collected by C. R. C. Paul in 1987.

One shell of *Urocoptis cylindrus* collected from Glasgow in the Cockpit Country of Trelawny was also analysed in a series of sub-samples to assess isotopic trends with growth. The single shell of this species that was analysed was large enough to allow eight sub-samples to be taken, each one being composed of a complete whorl slice. Time constraints prohibited analyses on further shells of this species. Shells of *Lucidella aureola* were not suitable for work of this kind, being too small for sequential sampling.

4.5.2 Results

The results of the isotopic analyses are shown in full in Appendix 2.

(a) *Pleurodonte sublucerna*

For the shells of *Pleurodonte*, mean oxygen isotope values for each shell range from -2.04 to -3.93‰ with standard deviations from 0.27 to 0.78‰ (n = 6). Mean carbon isotope values range from -9.12 to -11.20‰ with standard deviations from 0.16 to 0.54‰ (n = 6). The intra-shell data are plotted on Figure 4.7a and 4.7b, which show the oxygen and carbon isotope data of each sub-sample plotted against the approximate distance of that sub-sample from the shell apex (measured in cm).

More variation is seen in the oxygen isotope data within single shells than for the carbon isotope data. In two shells (J20-1A and J29-4) there are marked shifts in the oxygen isotope values half way through the shell growth. In both cases the shifts are towards more enriched values and are close to +2‰. However, this feature is not present in the other shells. There appears to be no consistent pattern of isotope values through the period of shell growth.

(b) *Urocoptis cylindrus*

For the seven intra-shell samples from the single *U. cylindrus* analysed the mean oxygen isotope value is -1.47‰ and the standard deviation is 0.2‰. The mean carbon isotope value is -6.86‰ with a standard deviation of 0.42‰ (n = 7).

The oxygen isotope values are fairly consistent throughout the shell, lying between -1.10 and -1.77‰. For the carbon isotopes, apart from the apical and preliminary whorls, there is a trend towards

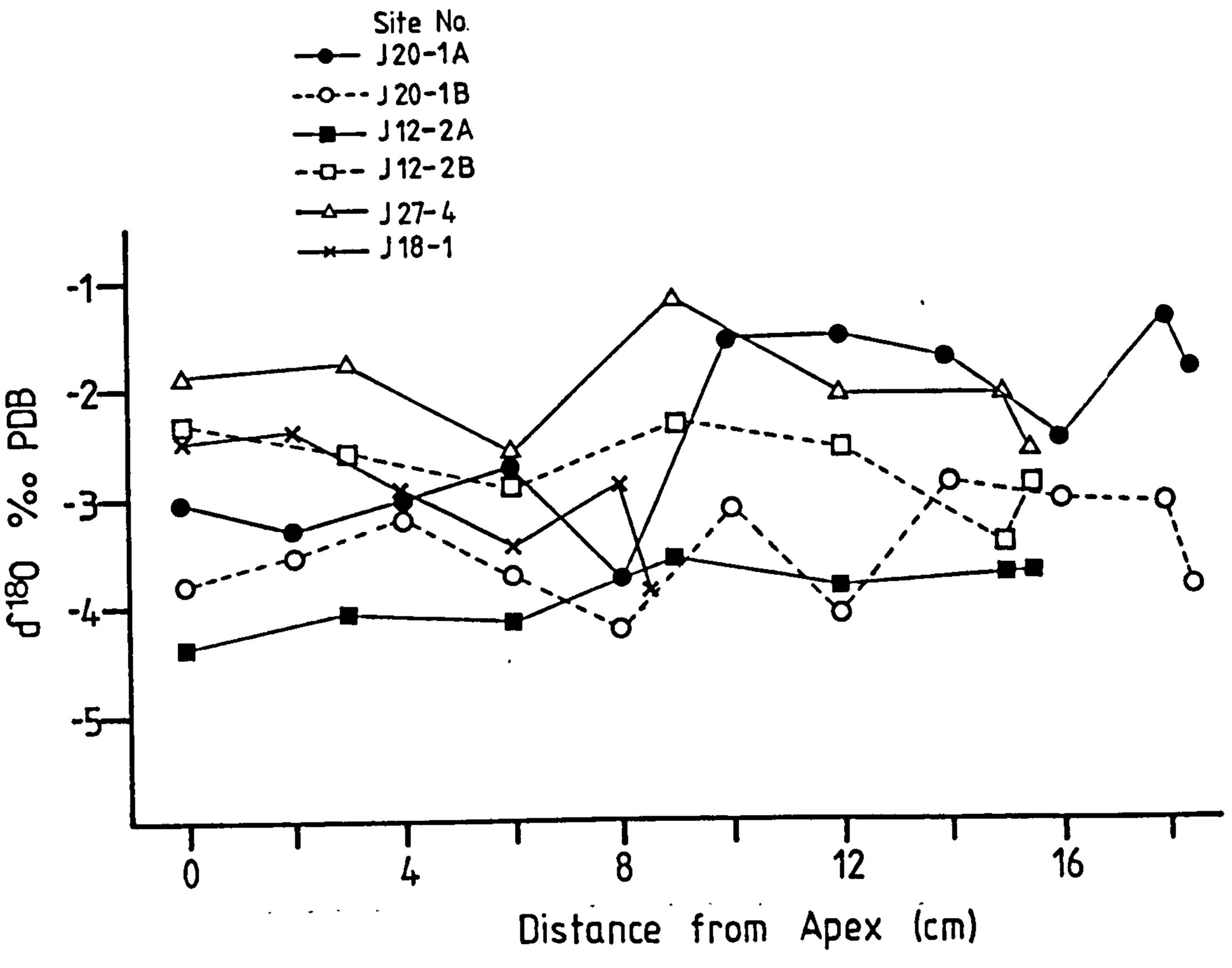


Figure 4.7a Oxygen isotope data within six individual shells (from four sites) of *Pleurodonte sublucerna*; plotted as distance from shell apex (mm)

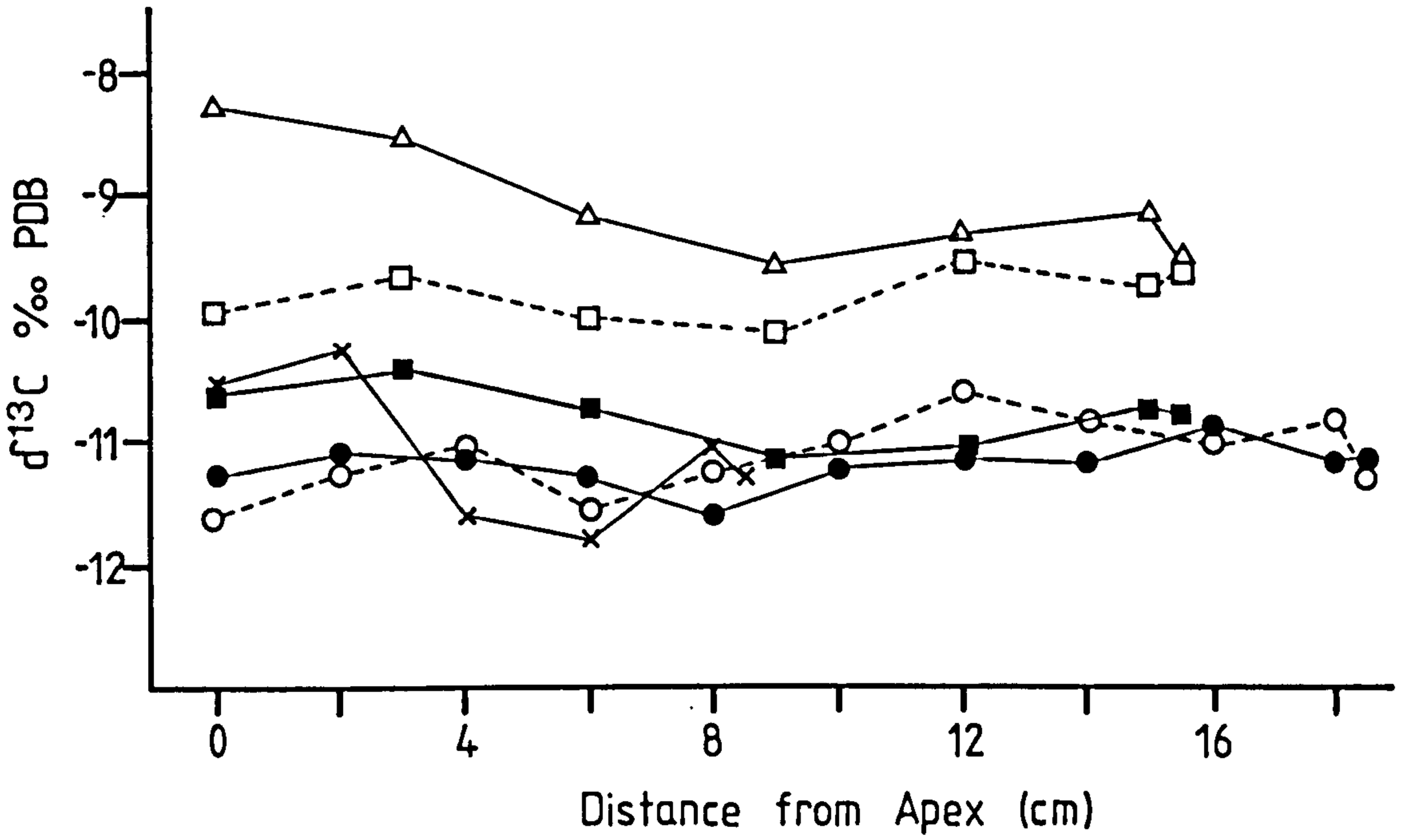


Figure 4 7b Carbon isotope data within six individual shells (from four sites) of *Pleurodonte sublucerna*; plotted as distance from shell apex (mm)

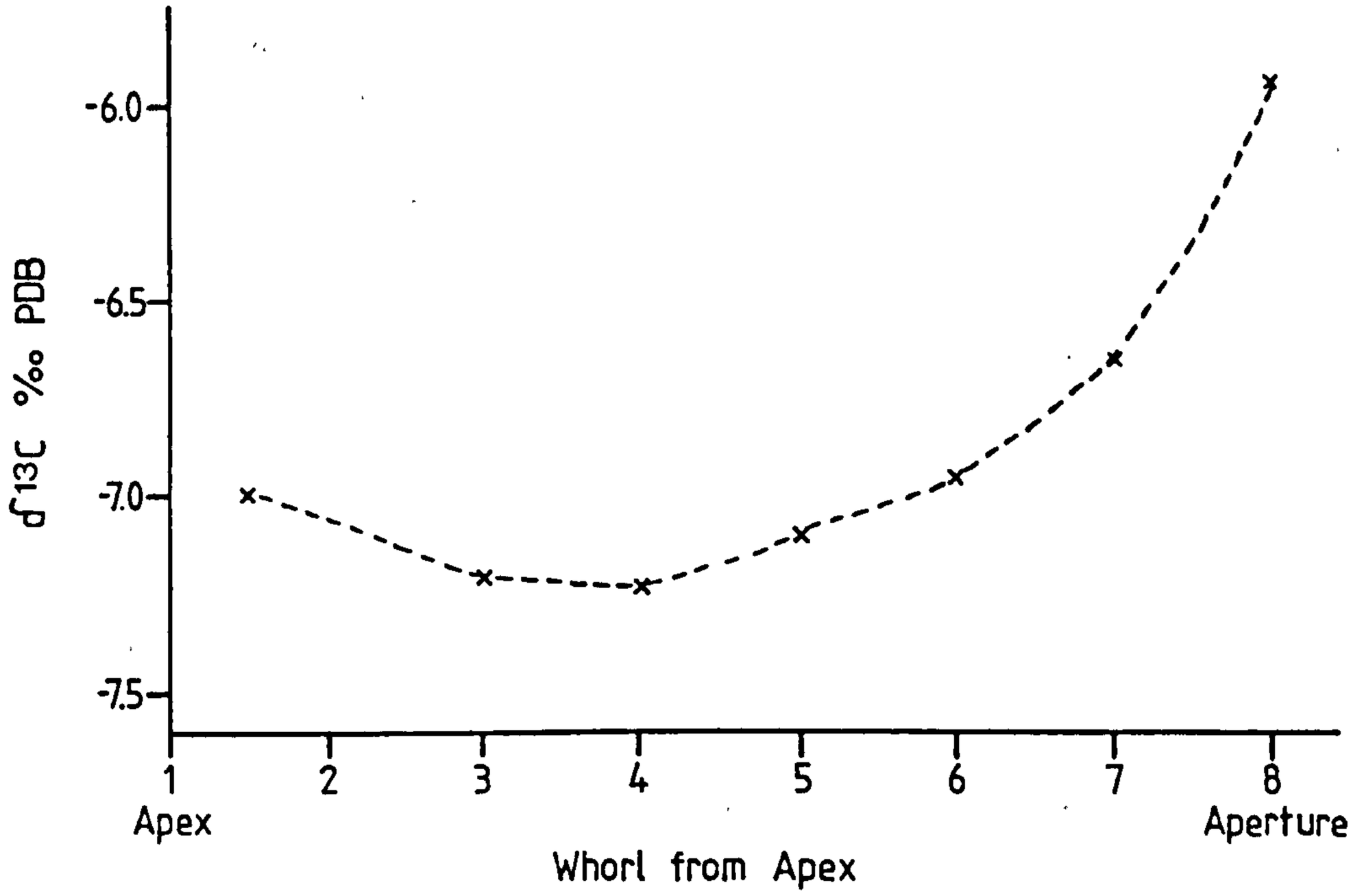


Figure 4.8 Carbon isotopes ($\delta^{13}C$) within single whorl slices of a single shell of *Urocoptis cylindrus*; plotted as whorl number from shell apex

more enriched $\delta^{13}C$ values from -7.2‰ at the third whorl to -5.93‰ at the body whorl and aperture. This may be seen in Figure 4.8.

4.5.3 Discussion

The shells that have fairly consistent isotope values throughout their growth are likely to have been secreted continually and under stable environmental conditions. The abrupt two per mil shift in the two shells of *Pleurodonte* may indicate that these snails underwent an interruption in shell deposition (leading to metabolic enrichment of oxygen isotopes in the body fluid), or else used a source of water that had undergone evaporative enrichment before being used by the snails. However, as no equivalent shift is seen in the carbon isotope ratios the evaporation effect may be the more likely of the two scenarios.

The pattern of carbon isotope ratios in the shell of *U. cylindrus* may be related to the rate of growth of the shell and metabolic effects upon the bicarbonate in the snail body fluid. Initial growth, over the preliminary whorls would be continuous, with metabolic carbon dioxide depleted with respect to ^{13}C in the snail's system. After the initial period of rapid growth, shell secretion would become more periodic, and with less metabolic carbon dioxide in the system, the body fluid bicarbonate would move closer to equilibrium with atmospheric carbon dioxide and thus would become more enriched in ^{13}C. However, as this species loses its early whorls through decollation, for the remaining whorls there may be no change in the rate of shell growth with time. Instead the trend may represent later internal thickening of the shell, with a resultant mixing of the isotope signature.

The exact mode of shell growth of these two species is not known. Observations by this author and C. R. C. Paul (pers. comm., 1990) suggest that *Pleurodonte* rapidly secrete thin shells to reach a mature size and then substantially thicken the aperture and existing shell by secreting further internal layers. Therefore any isotopic trends might be masked by the process of later internal thickening. For *Urocoptis cylindrus* , immature specimens of this species have been observed to have much thinner shells than adults, supporting the idea of internal thickening.

In summary then, it is thought that the isotope ratios in the shells of *Pleurodonte* are indicative of continual forward growth and later internal thickening under stable conditions. Shifts in oxygen isotopes in two shells probably represent a change of, or variation in, the environmental water used by the snails. The isotopic composition of the *U. cylindrus* shell indicates that this shell may have been secreted only periodically, or that later internal thickening is significant. Analysis of further shells of both species would be required to confirm these speculative conclusions.

These limited results do not indicate periodic or seasonal trends in isotopic composition and suggest that providing the same portion of shell is sampled (to allow comparative studies within and between species), then a single isotope sample from a shell should give a reasonable indication of overall shell isotope values for that shell.

4.6 ALTITUDINAL VARIATIONS IN STABLE ISOTOPES AND SHELL MORPHOLOGY IN THE RED HILLS, JAMAICA

4.6.1 Introduction

The investigation of the relationship between altitude (as an index of amount of rainfall) and isotopic composition of land snail shell was carried out primarily on shells of the species *Pleurodonte sublucerna*, but also on specimens of *Lucidella aureola* . Previous work by Lecolle (1985), has suggested that with increasing altitude, $\delta^{18}O$ values of land snail shells should become more depleted (by over -3‰ per 1,000m). It was also expected, from the work of Goodfriend (1983, and 1987) that the size of shells of *Pleurodonte sublucerna* would increase with elevation.

4.6.2 Results : *Pleurodonte sublucerna*

Isotopic analyses were carried out on a total number of sixty one specimens of this species. Of these, fifty were collected from the area of the Red Hills, and of these fifty, forty four represent mature and recently deceased individuals (*i.e.* the shells were complete and were unbleached), collected from twenty five sites. Up to three individuals from single sites were analysed.

The data from the forty four Red Hills *Pleurodonte sublucerna* samples are shown in Table 4.1. The altitudes of the sites range from 100 to 2250ft, and the shells range in diameter from 30 to 51mm. The oxygen isotope values of the shells range from -0.1 to -3.86‰, with a mean value of -1.79‰ (standard deviation = 0.85‰). The carbon isotopes of the shells lie between -8.30 and -15.71‰ with a mean value of -11.17‰ (standard deviation = 1.26‰). The correlations between shell size, oxygen and carbon isotope ratios and altitude are as follows.

(a) Shell diameter and altitude

The size of shell (diameter) increases with increasing altitude. The correlation coefficient of the relationship (r) is 0.76 and the correlation is statistically significant at the 0.05 level. This is shown on Figure 4.9 where the shell diameters of the forty four Red Hills specimens are plotted against the altitude of the collection site. The range of shell size is quite large at certain altitudes, *e.g.* at 500ft the diameters range from 32 to 47mm. However, regression analysis has been carried out on the data, and the regression line has been plotted on Figure 4.9, along with a dotted line through the mean value at each of the altitudes at which shells were collected. The gradient of the regression line indicates that shell diameter increases by 6.14 mm per 1000ft increase in altitude. Therefore, even with the wide spread in shell diameter at some elevations, the size of shell is strongly related to the altitude and it therefore follows that the shell diameter is also likely to be correlated with mean annual rainfall, as was demonstrated for *Pleurodonte lucerna* in north-central Jamaica by Goodfriend (1987).

(b) Oxygen isotopes and altitude

These data are plotted against each other on Figure 4.10a. The correlation coefficient of the relationship is only 0.33, which is indicative of the large spread in the data. A large spread in the data

ELEVATION ft.	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	
100	-11.92	-0.79	
100	-12.37	-0.55	
100	-12.49	-0.32	
250	-10.94	-2.52	
250	-10.76	-2.19	
250	-11.25	-1.96	
250	-10.74	-2.04	
250	-12.96	-0.95	
250	-9.03	-0.44	
250	-10.48	-2.86	
250	-10.43	-0.90	
500	-11.85	-1.71	
500	-11.00	-1.69	
500	-10.67	-2.69	
500	-10.99	-2.24	
500	-12.21	-1.34	
500	-11.04	-2.09	
600	-12.56	-1.13	
750	-13.04	-1.69	
1000	-12.34	-1.92	
1000	-10.64	-0.64	
1000	-12.12	-0.56	
1000	-10.96	-1.46	
1000	-15.71	-0.10	
1000	-11.31	-2.39	
1000	-12.20	-2.29	
1000	-10.71	-1.83	
1250	-11.04	-2.89	
1250	-10.03	-1.35	
1500	-11.26	-2.68	
1500	-8.30	-1.43	
1500	-11.52	-2.78	
1500	-11.10	-3.86	
1500	-10.68	-1.48	
1750	-8.85	-3.23	
1750	-9.63	-1.43	
1750	-11.26	-1.54	
2000	-10.90	-1.87	
2000	-10.96	-2.04	
2000	-10.10	-3.02	
2000	-11.02	-1.78	
2250	-12.15	-2.65	
2250	-10.59	-2.23	
2250	-9.25	-1.14	
	-11.17	-1.79	Mean
	1.26	0.85	Std. Dev.

Table 4.1 Stable isotope data and elevations (ft) for specimens of *Pleurodonte sublucerna* - slices from whole, mature and unbleached specimens - from the Red Hills

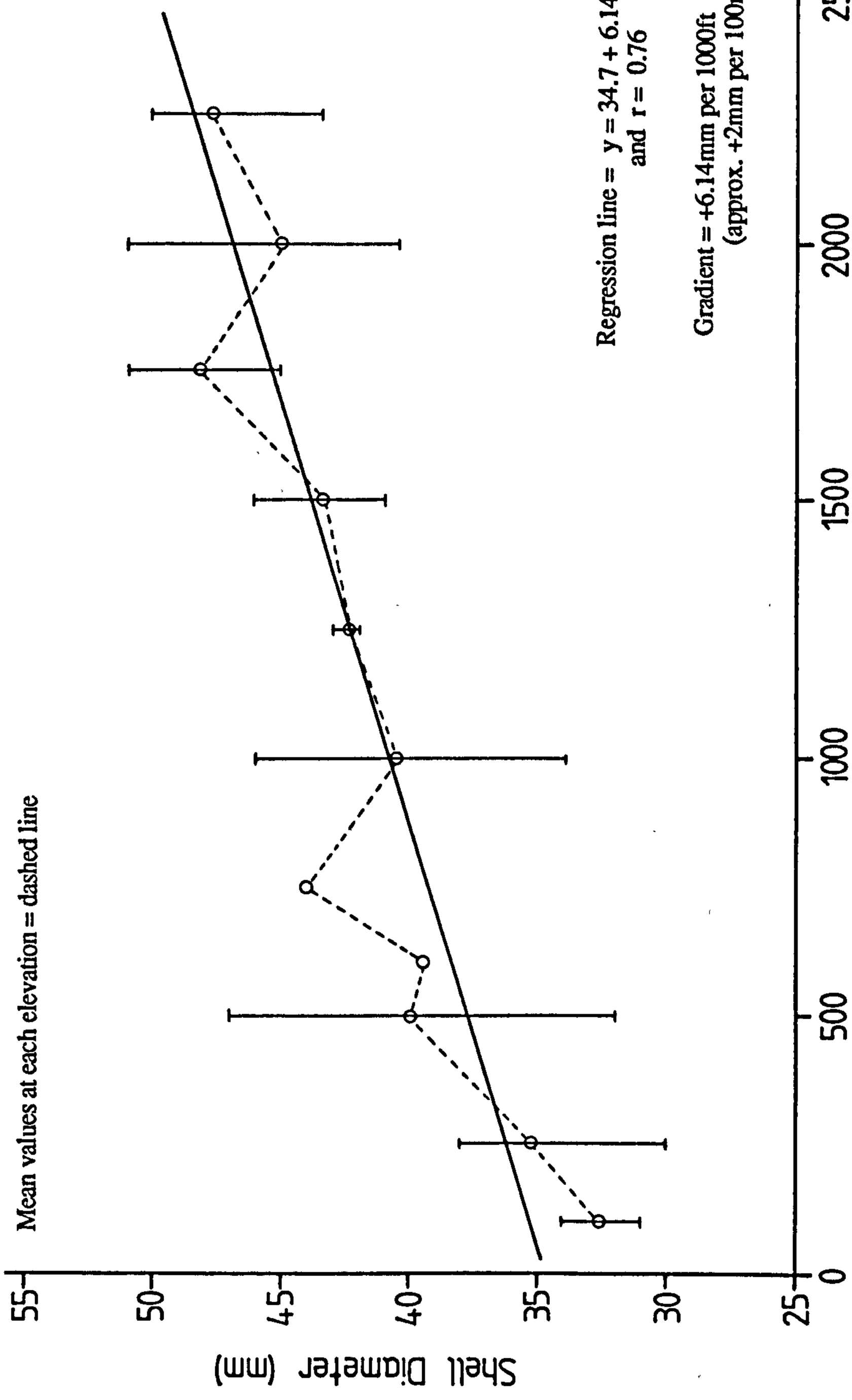


Figure 4.9 Shell diameter (mm) versus elevation (ft) for shells of *Pleurodonte sublucerna* (n = 44 from 25 sites) from the Red Hills, Jamaica

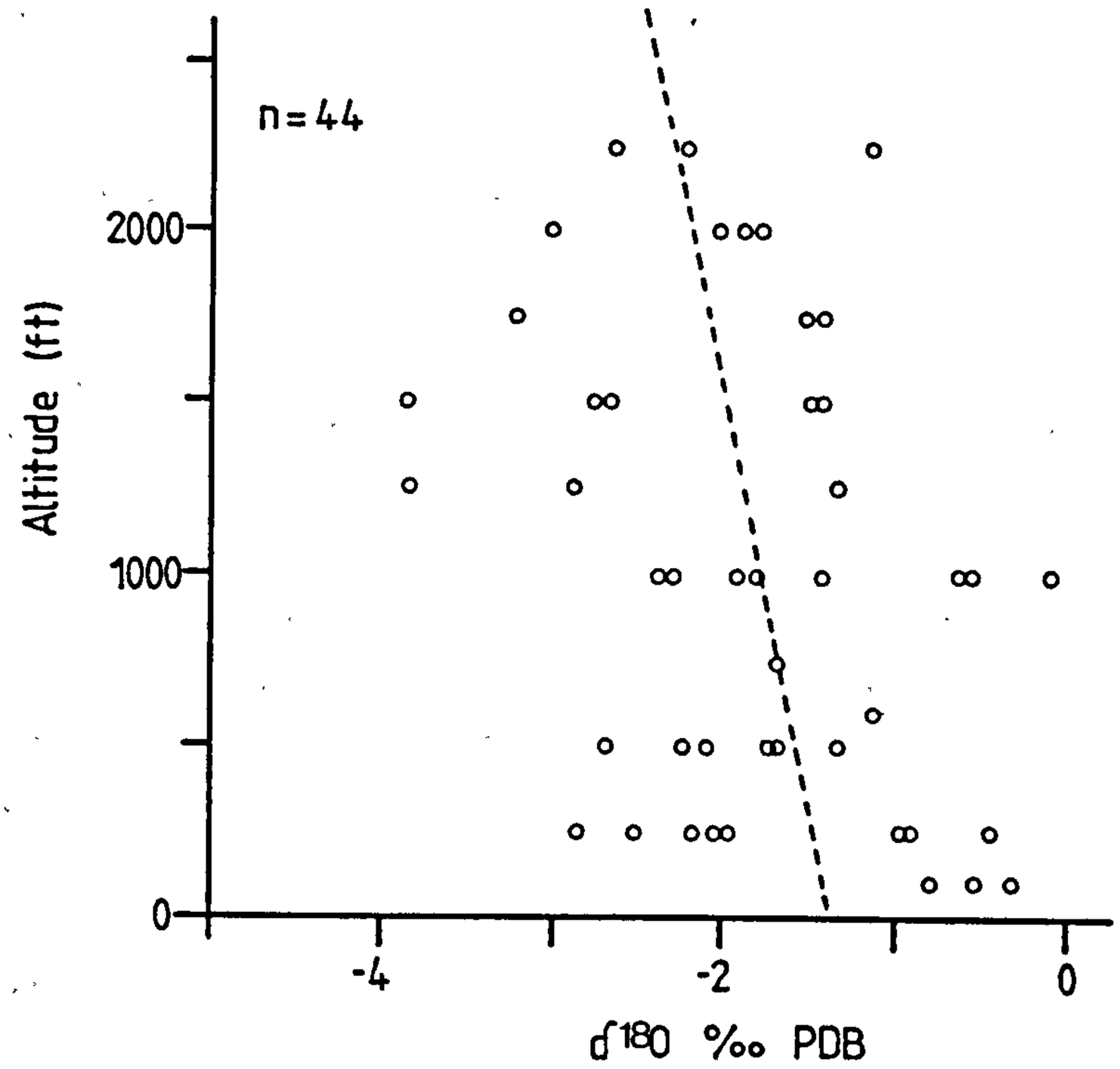


Figure 4.10a $\delta^{18}O$ versus elevation (ft) for shells of *Pleurodonte sublucerna* from the Red Hills (n = 44), with regression line. r = -0.33, not significant at 0.05 level

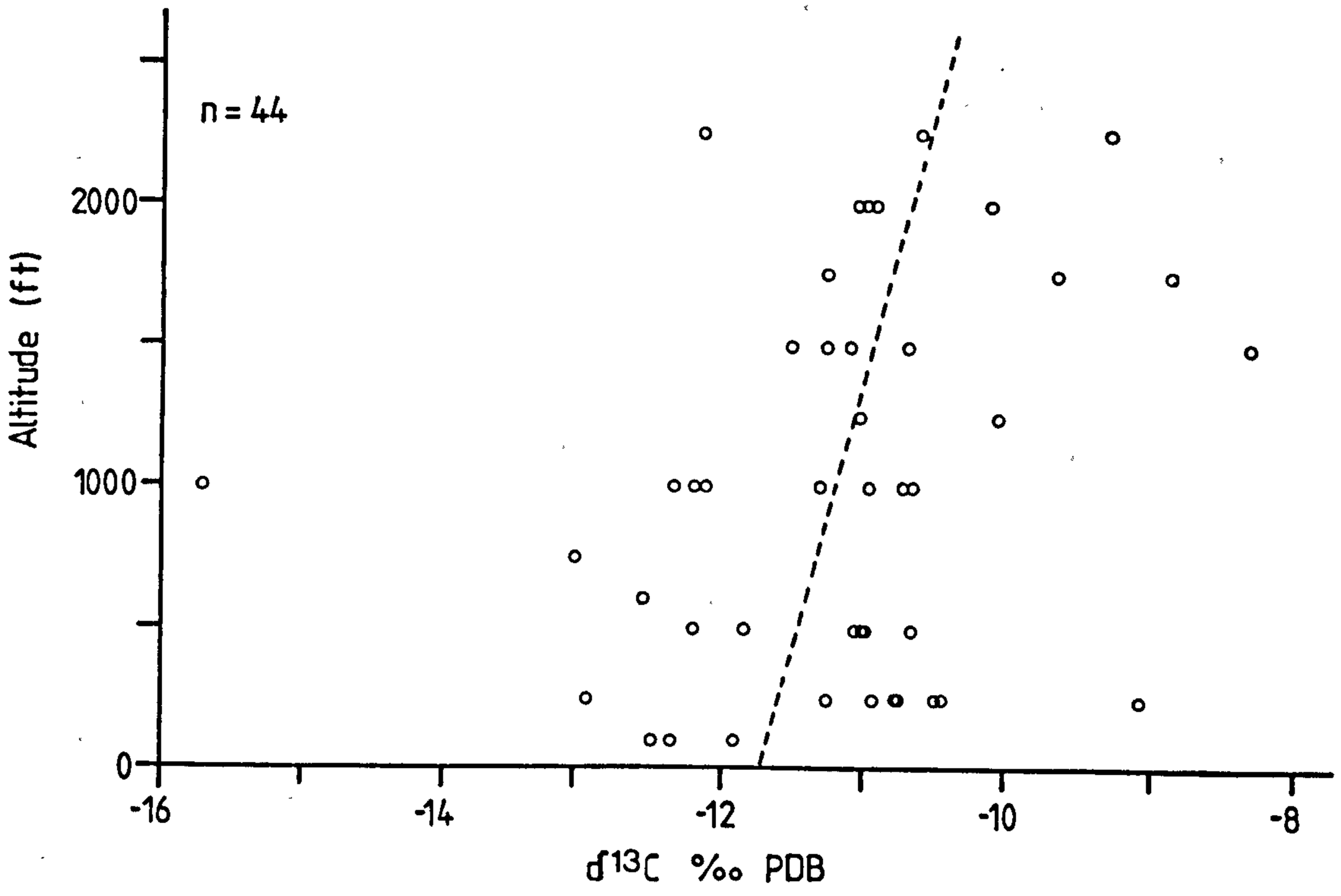


Figure 4.10b $\delta^{13}C$ versus elevation (ft) for shells of *Pleurodonte sublucerna* from the Red Hills (n = 44), with regression line. r = 0.26, not significant at 0.05 level

at individual elevations is evident (*e.g.* at 250ft $\delta^{18}O$ values range from -0.44 to -2.86‰). The regression line of the data has been plotted, and the gradient of the line is -0.4‰ per 1000ft. However, the relationship between oxygen isotopes and altitude is not statistically significant at the 0.05 level.

(c) Carbon isotopes and altitude

Figure 4.10b represents these data. The spread in the carbon isotope values is quite marked, and is greater than that from the oxygen isotopes, but is exaggerated by one or two extreme values such as that from a shell at 1000ft with a $\delta^{13}C$ of -15.71‰, more than two per mil more depleted in ^{13}C than any other shell. The spread in the data is shown by the standard deviation being as high as 1.26‰.

Regression analysis shows that the $\delta^{13}C$ values become more enriched with increasing altitude, by 0.52‰ per 1000ft (see regression line of Figure 4.8b). However the correlation coefficient for these data is only 0.26, again reflecting the variation in the data, and this correlation is not statistically significant at the 0.05 level. Therefore, for both carbon and oxygen isotopes, the weak trends are present but these trends are statistically insignificant.

4.6.3 Results : *Lucidella aureola*

Stable isotopic analyses were carried out on thirty individual shells of *Lucidella aureola* collected from eighteen sites in the Red Hills over an altitudinal range of 100 to 2250ft. Analyses were carried out on complete, mature individuals that were unbleached. The data from these analyses are summarised in Table 4.2.

The shells of *Lucidella* were all similar in size, so no measurements of shell dimensions were taken.

The oxygen isotope values range from -0.72 to -4.34‰ with a mean value of -2.36‰ and a standard deviation of 0.94‰. The carbon isotope data range from -5.63 to -11.96 ‰ with a standard deviation of 1.41‰, indicating the degree of spread in the carbon isotope data from the shells of this species.

The correlations between shell isotopes and altitude are shown below.

(a) Oxygen isotopes with altitude

As with the data from *Pleurodonte sublucerna*, the oxygen isotope values are plotted against elevation (Figure 4.11a). This figure shows that no shells were collected from sites between 1000 and 1750ft, and a wide degree of variation in the oxygen isotopes is apparent in the shells originating from other elevations and from individual sites *e.g.* three shells at 1750ft which were collected from the same site have $\delta^{13}C$ values of -0.72 -2.58 and -3.94‰, a range of over 3‰.

The visual impression of Figure 4.11a, is that there is no obvious correlation between altitude and the oxygen isotope composition of shells of *Lucidella aureola* . This view is substantiated by regression analysis of the data. The correlation coefficient between altitude and $\delta^{18}O$ is only 0.18, and the correlation is not significant at the 0.05 level. The regression line for these data shows an enrichment of 0.224‰ per 1000ft, although this relationship may have occurred by chance.

ELEVATION ft.	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	
100	-9.73	-1.19	
250	-9.72	-3.12	
250	-10.32	-2.41	
250	-9.96	-2.70	
250	-9.81	-1.39	
500	-10.23	-1.91	
500	-10.12	-1.75	
500	-8.64	-1.95	
600	-5.63	-3.77	
600	-5.81	-2.96	
600	-8.39	-3.84	
750	-9.58	-1.66	
750	-9.41	-1.73	
750	-11.96	-2.44	
850	-6.89	-4.23	
1000	-11.54	-1.67	
1000	-9.82	-2.03	
1750	-8.47	-0.72	
1750	-10.29	-2.58	
1750	-9.41	-3.94	
1750	-10.98	-1.45	
1750	-9.77	-2.45	
1750	-9.08	-2.02	
2000	-9.45	-1.80	
2000	-9.26	-2.23	
2000	-9.21	-1.57	
2250	-7.43	-1.49	
2250	-8.85	-3.21	
2250	-9.36	-2.28	
2250	-9.98	-4.34	
	-9.30	-2.36	Mean
	1.41	0.94	Std. Dev.

Table 4.2 Stable isotope data and elevations (ft) for specimens of *Lucidella aureola* - entire shells - from the Red Hills

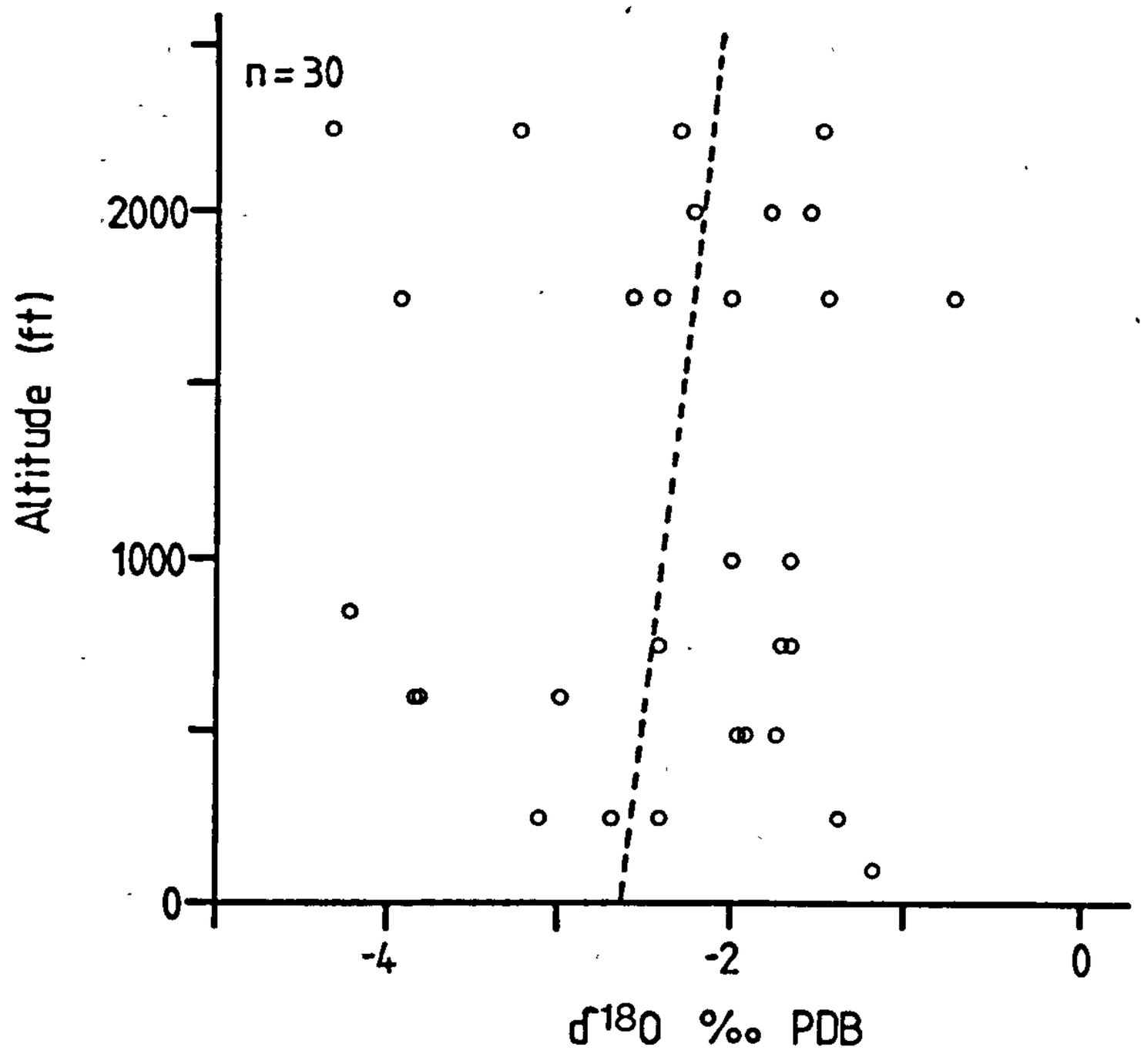


Figure 4.11a $\delta^{18}O$ versus elevation (ft) for shells of *Lucidella aureola* from the Red Hills (n = 30), with regression line, r = 0.18, not significant at 0.05 level

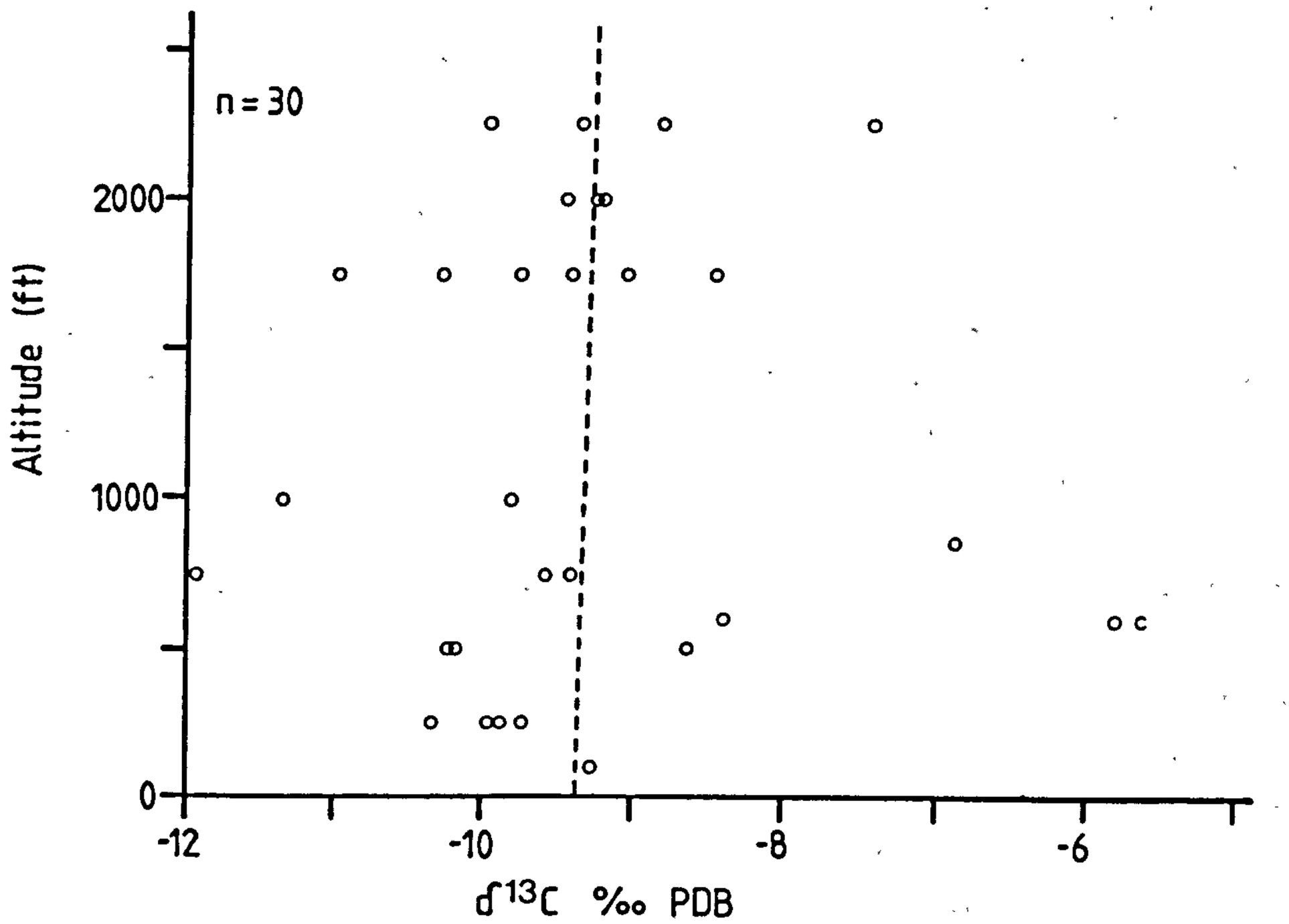


Figure 4.11b $\delta^{13}C$ versus elevation (ft) for shells of *Lucidella aureola* from the Red Hills (n=30), with regression line, r = 0.01, not significant at 0.05 level

(b) Carbon isotopes and altitude

These data are shown on Figure 4.11b. Again, as was seen in the data from *Pleurodonte sublucerna*, there appear to be one or two extreme values, notably in this case those more enriched in ^{13}C than -7‰. Overall, the data are well spread with a standard deviation as high as 1.41‰, and no trend between altitude and $\delta^{13}C$ is apparent. Regression analysis gives an almost vertical regression line (gradient = 0.024‰ per 1000ft), but the correlation coefficient of this relationship is a statistically insignificant 0.012.

4.6.4 Discussion

(a) Oxygen isotopes

In view of the positive correlation found between shell diameter of *Pleurodonte sublucerna* and altitude, and in the light of the findings presented by Lecolle (1985), the lack of any statistically significant correlation between shell $\delta^{18}O$ and altitude was somewhat unexpected.

The amount of isotopic variation within and between sites was also somewhat unexpected, and the degree of spread in the data sets for the two species investigated was augmented by the presence of a small number of values that deviated strongly from the mean.

The variation in the data sets points to the fact that the shells (even those collected from the same locality) were not necessarily secreted over the same time and under similar conditions. To get over this it would be necessary to collect shells of live, and probably mature, specimens from a number of sites at a series of altitudes so that the degree of isotopic variation at and between sites could be established. Some, but not many, live specimens of *Pleurodonte* were discovered at the chosen collection sites, but live *Lucidella aureola* were not seen. Location of significant numbers of live specimens would have involved penetration into areas of scrub and forest. This was not feasible within the time constraints of this study and in light of the logistics involved in such a proposal. It was a necessary drawback of the field collections that the collections were made adjacent to existing roads or tracks, which would not necessarily be suitable habitats for *Pleurodonte* and *Lucidella* .

With the passage of a rainstorm up and over a mountain range, the oxygen isotopic composition of rainwater (the ultimate water source for land snails), should become more depleted in ^{18}O as more of the heavier isotope is lost progressively. Therefore with increasing distance from the initial condensation point (generally the sea) and/or increasing altitude, the oxygen isotopic composition of the rain will become more depleted. A depletion of between -0.15 to -0.5‰ per 100 metres (altitude) was shown by Gat (1980), and Lecolle (1985) used a depletion rate of -0.3‰ per 100m. At an elevation of 2250ft (approximately 680m) rainwater should be around 2‰ more depleted in ^{18}O than at sea level.

In the Red Hills of Jamaica then, at higher elevations, it might be expected that the rain would have more depleted $\delta^{18}O$ values, as a result of this 'rainout' effect, which should be reflected in the isotopic compositions of the land snail shells (providing that the oxygen isotopic composition of the water is not significantly altered before or after uptake by the snail). However, this argument holds true only if the source of rain is offshore to the south of the Red Hills. Indeed this is a source of rainfall in the hills. However, an additional source of rain is from the north east trade winds which reach the Red Hills after traversing the Blue Mountain range and subsequently will already be depleted

in ^{18}O by the time they reach the summits of the Red Hills. Thus, any rainfall at lower elevations to the south and south west of the Red Hills peaks may be more depleted with respect to ^{18}O if its source is from the north east rather than the south shore of Jamaica. Therefore, the overall pattern of oxygen isotopes in localised environmental waters, and in land snail shells, with respect to elevation, may be a compound factor relating to the the actual source of the precipitation and its progress inland and not just simply upon the altitude of the site. This may help explain the lack of correlation between altitude and shell oxygen isotopes actually observed in the snails collected from the Red Hills.

The actual $\delta^{18}O$ values of the snails' shells may depend on other factors in addition to the source and track of the precipitation. What happens to the water after falling as precipitation, prior to and after uptake by a snail may also be important, and the actual temperature experienced by the snail will affect the resultant isotopic composition of the shell. These factors, and how they may relate to location and altitude in the Red Hills, will now be considered.

At higher altitudes in the Red Hills the amount of rainfall is much greater than at elevations close to sea level *e.g.* above 1500ft precipitation may reach 190 to 250cm (75 to 100 inches) per annum, whereas close to sea level it may fall below 125cm (50 inches). This variation in rainfall is evidenced by the amount and type of vegetation which at lower elevations is dry scrub forest and which at altitudes above 1000ft grades into wet limestone forest. Thus the environments at higher altitudes not only receive more rainfall but will be more efficient at retaining moisture than environments at lower elevations, particularly on the forest floor and beneath a more complete canopy. In this way, snails in permanently moist environments at higher altitudes, and subject to favourable tropical temperatures, can be active for most of the time and shell material may be deposited continually. Under such conditions the snails will have a high water flux with little chance for metabolic enrichment of the oxygen isotopes in their body fluids (see section 3.3.2). In addition to this, and as a result of the nature of the habitat, humidities will be consistently high with little chance for evaporative enrichment of the environmental waters prior to uptake by the snails. In contrast to this situation, at lower elevations, waters may be subject to more enrichment in ^{18}O as a result of evaporation before being used by the snails, and under the drier conditions the snails may only be periodically active and in between active periods the body waters may become metabolically enriched in ^{18}O.

The net result of this reasoning is that with increasing altitude the oxygen isotopes in the snail shells will become more depleted in ^{18}O *i.e.* the same trend as that presented above as the 'rainout' effect from storms tracking inland from the south coast of Jamaica.

In addition to the effect of altitude upon the oxygen isotopic composition of rainfall, and the possible indirect link with evaporation and metabolic effects, relative environmental temperatures may exert some influence upon the snails.

Although Jamaican temperatures do not vary greatly on a seasonal basis, air temperatures decrease with increasing altitude. Working in Jamaica, Goodfriend (1983) has shown that air temperature decreases by 5.2°C per 1000m increase in altitude, and such a rate of cooling is close to the wet adiabatic lapse rate (the rate of cooling of rising saturated air). Temperature differences may be exaggerated by the presence of forest canopy at higher elevations with more exposed habitats nearer the coast. Goodfriend (1983) noted that the actual local temperatures experienced by the snails will be affected by the direction of exposure of slope; the degree of horizon obstruction (presumably the degree of shadow cast by surrounding hills); the amount of ground moisture present; and the amount of

shading by vegetation. The localised effects upon temperature are therefore likely to be very strong. However, higher temperatures at lower elevations will result in more depleted $\delta^{18}O$ values in snail shells. A depletion rate of approximately 0.2‰ per °C for aragonite precipitation was shown by Grossman and Ku (1986) (see section 3.3.2). Therefore temperature effects may result in oxygen isotope enrichment with increasing altitude.

In summary, a combination of factors may influence the pattern of oxygen isotopes in the land snail shells of the Red Hills in relation to the altitude of the habitat. Of the factors considered here, 'rainout' of moisture from the south, metabolic and evaporation effects will result in relative ^{18}O depletion at higher elevations. However, these effects may be partially offset by enrichment in shell ^{18}O resulting from cooler temperatures at higher altitudes and from 'rainout ' of moisture from the north east. The likely variations in these factors depending on the specific characteristics of each collecting locality may explain the variation in the data sets and the absence of significant correlations between shell oxygen isotopes and altitude for the two species of snail investigated here.

(b) Carbon isotopes

No significant relationship was measured between shell carbon isotopes and altitude and the amount of variation in the isotope values was unexpected. For both species, the spread in values was as much as 7‰, although only a small number of points deviate widely from the regression lines.

Increasing wetness of the habitats with increasing altitude should promote continuous snail activity and shell production with lots of metabolic carbon dioxide entering the body fluid bicarbonate pool, which would be depleted with respect to ^{13}C. In drier environments (lower elevations) activity might be more periodic and with less metabolic carbon dioxide in the system, the body waters would approach equilibrium with atmospheric carbon dioxide. These effects would result in depletion in shell ^{13}C with increasing altitude. However, as discussed above for oxygen isotopes, temperature effects would work in the opposite direction to metabolic effects, with more enriched $\delta^{13}C$ values expected at cooler sites in the Red Hills (higher altitudes). Even so, for carbon isotopes the temperature dependence of the fractionations is fairly weak, and consequently temperature effects may not fully offset metabolic effects.

The variation in the carbon isotope data may reflect a variation in the sources of organic carbon utilised by the snails. The arboreal *Lucidella* feed on unicellular epiphytic algae and lichens (C. R. C. Paul, pers. comm., 1990) and would get their carbon from these sources, with little or no input from inorganic carbon. Therefore the range in the shell $\delta^{13}C$ values is hard to explain unless the activity patterns of the snails (when they were living) were very variable. The ground dwelling *Pleurodonte* might ingest some carbonate from the white limestone bedrock, and might graze a variety of organic materials. Goodfriend (1983), reports that *Pleurodonte lucerna* feed on freshly fallen plant debris, primarily leaf litter but also fruits and flowers, and that they are fairly selective feeders only using around 10% of available species. The plants ingested by *Pleurodonte* could have a variety of carbon isotope signatures, particularly if *Pleurodonte* feed on plants with different photosynthetic pathways which have characteristic carbon isotope values (Deines, 1980).

For both oxygen and carbon isotopes, relationships between shell isotope compositions and altitude would be best investigated where all the influencing factors would operate in the same direction, so that any isotope effects might not cancel each other out. An ideal locality would be one where precipitation came from a known single source and where lower elevations were cooler and drier, or warmer and wetter, than higher altitudes. In reality this ideal locality probably does not exist.

4.7 LOCAL AND REGIONAL VARIATIONS IN STABLE ISOTOPES OF JAMAICAN SNAIL SHELLS

4.7.1 Introduction

To investigate possible isotopic differences between shells of the same species from different regions (*i.e.* parishes) of Jamaica, the species selected was *Urocoptis brevis* . This small urocoptid occupies the hot and arid southern coastal fringes of Jamaica. It was located at 18 sites in all; four sites were in the region of St Andrew to the north east of Kingston, two were in the Yallahs River area and coastal lowland of St Thomas and six sites in the Hellshire Hills of St Catherine. Three further sites were in the environs of Milk River Bath on the coast of Clarendon and the final three sites were located close to the foothills of the Santa Cruz Mountains in St Elizabeth.

Shells of *Urocoptis brevis* were commonly found in rock crevices or at the foot of low outcrops. The sites were generally very arid, with open scrub and cactus being present at most, providing only limited shading. Some live specimens were discovered, although it was often difficult to tell whether of not a specimen was actually alive as the soft tissues were retracted well back into the shells. No active specimens were seen. Some of the live specimens were brought back to Liverpool, but even with Jamaican soil and plant litter, and with the snails being maintained in warm moist conditions, the specimens did not survive for more than a couple of months.

Smaller scale variations in shell isotopic compositions have been investigated using the isotope data from shells of *Pleurodonte sublucerna* and *Lucidella aureola* presented in section 4.6. The data from these species, collected from different areas in the Red Hills, are used to investigate potential local, as opposed to regional, isotopic variations.

Shells of *Pleurodonte sublucerna* were collected from 27 sites in the Red Hills and *Lucidella aureola* was found at 18 sites. All the collecting sites were located on, or adjacent to, the three negotiable paved roads from the coastal lowlands up and into the Red Hills. Therefore, in order to assess variations in the shell isotope data from within the Red Hills, the data sets have been subdivided into three areas that correspond to the three routes. The three areas are:

1) The East Red Hills route (ERH) - on the road from Constant Spring in the Liguanea Plain of Kingston to Rock Hall and Coopers Hill Peak.

2) The Spanish Town route (ST) - on the road from Spanish Town to Bog Walk via Stanberry Grove, Sligoville and Montpelier Peak.

3) The St John's Red Hills route (SJ) - on the road from Bushy Park to Bog Walk via Guanaboa Vale in the St Johns Red Hills.

Of these three collection routes, ERH is in the eastern part of the Red Hills, ST lies centrally in the hills and SJ follows the western margins of the hills.

4.7.2 Regional variations in shells of *Urocoptis brevis*

(a) Results

Isotopic analyses were carried out on thirty six individual shells collected from the eighteen localities in the five areas; Hellshire Hills (HH), St Andrew (StA), St Thomas (StT), Milk River Bath (MRB) and Santa Cruz Mnts (SCM). Generally, one or two specimens from each site were analysed although at one of the sites from the Hellshire Hills five individual shells were analysed.

The results of the isotopic analyses are shown in full in Appendix 2, with location abbreviations. Overall, the oxygen isotope values range from -0.06 to -6.26‰, and the carbon isotope values from -3.88 down to -11.81‰. For each of the areas, the mean oxygen and carbon isotope values and standard deviations have been calculated and are shown in Table 4.3. This table shows that mean oxygen isotope values for each area lie between -1.44 and -3.34‰ but that the standard deviation of the results is as high as 1.5‰. For carbon isotopes mean values for the five areas are much more consistent, ranging from -5.67 to -6.52‰; however, the standard deviations show the data to be even more variable than those of the oxygen isotopes with deviations from 0.95 to 2.17‰. It is evident from Table 4.3 that the standard deviation in the carbon isotope data is correlated with the number of determinations upon which the standard deviation is based *e.g.* for HH n = 13 and sd = 2.17, for MRB n = 9 and sd = 1.23, and for StT n = 3 and sd = 0.95. This suggests that the variation might be even greater if more determinations had been carried out.

The standard deviations appear to be similarly high within single sites. Five shells from one site in the Hellshire Hills were analysed and produced standard deviations of 1.48 and 1.2‰ for oxygen and carbon isotopes, respectively, and a range of oxygen isotope values from -2.55 to -5.64‰ and carbon isotope values from -3.79 to -6.75‰.

(b) Discussion

No consistent pattern is evident between the isotope values from the shells of *Urocoptis brevis* from the five areas. Furthermore the degree of isotopic variation within all the data sets, as indicated by the standard deviations, makes statistical comparisons between the areas impossible. There appears to be as much isotopic variation within areas and within single sites as between the five areas considered. Thus the data appear to signify that regional variations in stable isotope values in the shells of this species are not significant or else are masked by very localised effects which result in variable isotopic signatures for both oxygen and carbon isotopes.

Overall, the carbon isotope values are more variable than those for oxygen. This may relate to the fact that the snails obtained their carbon from sources which had a wider range of isotope signatures than their sources of oxygen.

4.7.3 Isotopic variation in shells of *Pleurodonte* and *Lucidella* from within the Red Hills

(a) Results

The collecting sites in the Red Hills have been subdivided into three areas (see section 4.7.1). Of the 27 localities where *Pleurodonte* were found, eleven were in the eastern area of the Red Hills

REGION	$\delta^{13}C‰$	Std. Dev.	$\delta^{18}O‰$	Std. Dev.	n of shells
HH	-6.00	2.17	-3.34	1.54	13
St. A	-6.34	1.00	-2.42	0.67	6
St. T	-6.24	0.95	-1.44	1.53	3
MRB	-5.67	1.23	-1.65	0.99	9
SCM	-6.52	1.11	-2.43	1.49	5

HH = Hellshire Hills
St. A = St. Andrew
St. T = St Thomas
MRB = Milk River Bath
SCM = Santa Cruz Mnts

Table 4.3 Mean isotope values and standard deviations for shells of *Urocoptis brevis* from five areas along the southern coastal margin of Jamaica

(ERH), nine in the central area above Spanish Town (ST) and seven in the western area in the St. John's Red Hills (SJ). Of the 18 sites where *Lucidella* were located, nine were in ERH, five in ST and four in SJ.

From these sites, stable isotopic analyses were carried out upon 30 individual shells of *Lucidella* and on 46 specimens of *Pleurodonte*. (To allow two additional sites to be considered, the number of analyses on *Pleurodonte* presented in this case includes analyses on two additional shells - one slightly bleached and one almost mature shell).

Mean isotope values (carbon and oxygen) from shells of *Pleurodonte* from each site have been calculated and are shown in Tables 4.4a-c for ERH, ST and SJ respectively. Similarly, isotope data from *Lucidella* are shown in Tables 4.5a-c.

For shells of *Pleurodonte*, mean oxygen isotope values from the three routes into the Red Hills are fairly similar, being -1.96, -1.90 and -1.40‰ for ERH, ST and SJ, respectively (Tables 4.4a-c). The standard deviations for these data sets are 0.71, 0.44 and 0.93‰. Mean carbon isotope values from the three areas are -10.76, -11.28 and -12.27‰ in the order of ERH, ST and SJ, thus showing an apparent trend towards more depleted values moving westwards through the Red Hills. However, the standard deviations for the carbon isotope data are 1.04, 0.86 and 1.84‰ and thus the variation within the sub-areas is as great, or greater, than that between them.

The mean oxygen isotope values from the shells of *Lucidella* (Tables 4.5a-c) are -2.55 (ERH), -2.14 (ST) and -2.30‰ (SJ); and the standard deviations are 1.00, 0.63 and 0.97‰ respectively. As with the data from *Pleurodonte*, the mean values from the three areas are similar, although the standard deviations within each data set are high. The situation is repeated in the carbon isotope data with mean $\delta^{13}C$ values of -9.18 (ERH), -10.11 (ST) and -9.55‰ (SJ) and standard deviations of 1.04, 0.87 and 2.21‰, respectively.

For both species of snail, the shell carbon isotope data are more variable (*i.e.* have higher standard deviations) than the oxygen isotope data.

(b) Discussion

Analysis of the stable isotopic data from shells of both species *Pleurodonte* and *Lucidellaa*, has shown that isotopic variation is as great within each sub-area of the Red Hills as between them

For carbon isotope ratios in shells of *Pleurodonte*, a trend towards more depleted $\delta^{13}C$ values moving westward was noted. However this 'trend' was the result of a very depleted $\delta^{13}C$ values at one SJ site (-15.7‰), and the standard deviations from within each of the three areas were as large or larger than the differences between them.

Although no local or regional patterns in shell isotopic compositions have been isolated, the data suggest that large variations in shell isotopic compositions may be expected island wide. This implies that isotopic analysis of single shells from individual localities may not give an accurate measure of mean shell isotopes in that area.

SITE	n of Shells	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	
3/1	1	-12.21	-1.34	
3/2	1	-11.04	-2.09	
3/3	2	-11.76	-2.34	
3/4	1	-11.26	-2.68	
5/1	2	-9.91	-2.11	
17/2	3	-11.24	-0.89	
17/3	1	-8.85	-3.23	
17/4	2	-11.37	-2.44	
5/2	2	-10.45	-1.49	
5/3	1	-11.02	-1.78	
5/4	1	-9.25	-1.14	
Total n	17	-10.76	-1.96	Mean
		1.04	0.71	Std. Dev.

Table 4.4a East Red Hills (ERH) route

SITE	n of Shells	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	
11/3	3	-10.98	-2.22	
11/4	3	-10.91	-1.14	
11/5	1	-13.04	-1.69	
5/6	1	-10.71	-1.83	
11/6	2	-10.53	-2.12	
5/5	2	-10.89	-2.67	
11/7	3	-10.65	-2.31	
11/8	1	-12.34	-1.92	
17/5	2	-11.43	-1.70	
Total n	18	-11.28	-1.90	Mean
		0.86	0.44	Std. Dev.

Table 4.4b Spanish Town (ST) route

SITE	n of Shells	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	
18/1	3	-12.26	-0.55	
18/2	2	-10.46	-1.88	
18/3	1	-10.67	-2.69	
18/4	1	-12.56	-1.13	
18/5	1	-13.21	-1.19	
18/6	1	-15.71	-0.10	
18/7	1	-10.99	-2.27	
Total n	10	-12.27	-1.40	
		1.84	0.93	Mean
				Std. Dev.

Table 4.4c Red Hills, St. John (SJ) route

Table 4.4a-c Mean isotope values for shells of *Pleurodonte sublucerna* from sites along three routes into the Red Hills

SITE	n of Shells	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	
3/1	2	-10.18	-1.83	
3/2	1	-8.64	-1.95	
17/1	1	-6.89	-4.23	
17/2	1	-9.82	-2.03	
17/3	3	-9.39	-2.41	
17/4	3	-8.55	-2.33	
5/2	3	-9.94	-1.96	
5/3	2	-9.24	-1.90	
5/4	1	-9.98	-4.34	
Total n	17	-9.18	-2.55	Mean
		1.04	1.00	Std. Dev.

Table 4.5a East Red Hills (ERH) route

SITE	n of Shells	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	
11/3	1	-9.72	-3.12	
11/4	1	-10.32	-2.41	
11/5	2	-9.50	-1.70	
11/7	1	-9.45	-1.80	
11/8	1	-11.54	-1.67	
Total n	6	-10.11	-2.14	Mean
		0.87	0.63	Std. Dev.

Table 4.5b Spanish Town (ST) route

SITE	n of Shells	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	
18/1	1	-9.73	-1.19	
18/2	2	-9.89	-2.05	
18/4	3	-6.61	-3.52	
18/5	1	-11.96	-2.44	
Total n	7	-9.55	-2.30	Mean
		2.21	0.97	Std. Dev.

Table 4.5c Red Hills, St. John (SJ) route

Table 4.5a-c Mean isotope values for shells of *Lucidella aureola* from sites along three routes into the Red Hills

4.8 OXYGEN ISOTOPIC COMPOSITION OF JAMAICAN ENVIRONMENTAL WATERS AND SHELL SECRETION

4.8.1 Introduction

Surface water was present at only a few of the collection sites. Water samples were therefore taken from a variety of sources, whenever the opportunity arose. Thirteen sites were sampled in total and the samples included specimens of rainwater; dew; fast flowing streams or falls; roadside stand pipes (presumably sourced from local wells) and from one spring. The analysis of these samples would indicate an overall oxygen isotope value for local surface and ground waters. Of the waters collected, isotopic analyses for oxygen and deuterium isotopes could only be carried out on five samples, due to practical difficulties and time constraints. The analyses were completed by the BGS Laboratory at Wallingford. The five chosen specimens were a sample of early morning dew collected by skimming a pan over dew-laden grass; two samples of rainwater; a sample of water from a cave resurgence; and one from a spring.

The results of these analyses will be used, in conjunction with information on local temperatures, to ascertain whether the shells of the Jamaican snails investigated in this study (specifically those from the Red Hills) have been secreted at or near isotopic equilibrium with their environmental water.

4.8.2 The question of shell secretion at isotopic equilibrium with environmental waters.

(a) Analytical results - waters

The results of the stable isotopic analyses are shown in Table 4.6. This table includes notes on the source of each sample, a grid reference of the location and the isotope values for oxygen ($\delta^{18}O$) and deuterium ($\delta^{2}H$) relative to the SMOW standard. The $\delta^{18}O$ values of the Jamaican water samples lie between -3.8 and +0.4‰.

Deuterium and oxygen isotopes in naturally occurring waters are related and values lie on or close to a line - the Mean Water Line (MWL) - represented by the equation

$$\delta D = 8\delta^{18}O + 10 \qquad (4.2)$$

(Anderson and Arthur, 1983). Deviations to the right of the MWL indicate that the water has been subject to evaporation effects, and thus the oxygen and deuterium values from the Jamaican waters can be used to assess whether the samples had been subject to evaporation effects prior to being collected and/or analysed. Of the five samples only one (JW13), collected in a Kingston garden during a rainstorm, deviates from the MWL. This particular sample may have been subject to evaporation during collection and prior to bottling. Excluding this particular sample, the mean $\delta^{18}O$ value of the other four water samples is -2.05‰ SMOW.

SAMPLE No.	COLLECTION Date	SOURCE	GRID. REF.	δd ‰ SMOW	$\delta^{18}O$ ‰ SMOW
JW 5	12-08-88	DEW - by skimmimg over vegetation	L636398	-21	-3.8
JW 8	03-08-88	STORM RAIN - in overflow channel	L637401	11	0.4
JW 11	09-08-88	CAVE RESURGENCE - flowing stream	C283535	-9	-2.4
JW 12	12-08-88	SPRING - entering Wallywash Pond	E268387	-12	-2.4
JW 13	13-08-88	RAINWATER - in Kingston garden	L634414	-19	-2.5

Table 4.6 $\delta^{18}O$ and $\delta^{2}H$ (δd) from five samples of Jamaican meteoric waters

From a worldwide survey of stable isotopes in precipitation, the value for the Caribbean is around -3.0‰ (Anderson and Arthur, 1983 after Yurtsever, 1975). This value is in fairly close agreement with the few analyses carried out in this study.

Due to the small number of water analyses carried out and because data are not available from snails' shells and environmental waters at identical localities, it is necessary to infer an oxygen isotope value typical of water available to the snails. Although local and regional effects relating to altitude, aspect, temperature will affect the oxygen isotope value of environmental water available to any Jamaican landsnail, an overall mean value of -2.24‰ (to include the value from Yurtsever, 1975, and excluding sample JW13) has been assigned. Actual values might deviate from this assumed mean by up to 2‰ either way. These values may be used with information on environmental temperatures to investigate whether the landsnails secrete their shells close to isotopic equilibrium with the oxygen isotopes in their environmental waters.

(b) Environmental temperatures

Seasonal variation in Jamaican temperature is small (Clarke, 1974). However, the environmental temperatures experienced by snails at any locality will depend upon the altitude, the direction of exposure of slope, the degree of horizon obstruction, the amount of ground moisture present and the amount of shading by other vegetation (Goodfriend, 1983).

Within the Red Hills (the area where most of the shell collections were concentrated) meteorological information is limited. Clarke (1974), shows information on mean monthly ranges in temperature for three stations in the vicinity of the Red Hills; at the Hope Laboratory in Kingston, at Bodles, located to the south west of Spanish Town in St. Catherine, and at Worthy Park, located on the elevated limestone plateau to the north west of the St. John Red Hills. For these stations, the January mean temperatures lie between 20 and 24°C and the July/August temperatures between 25 and 28°C. From the data presented by Clarke (1974), the mean annual air temperatures from the coastal foothills to the peaks of the Red Hills might be expected to range from approximately 23 to 26°C. Temperatures at or near ground level (the habitat of many Jamaican landsnails) might be differ from these approximate air temperatures, and night time temperatures would be lower. *Pleurodonte* is described as nocturnal (Goodfriend, 1983) and activity and possible shell secretion during the night and at ground level might be at temperatures, perhaps 5°C lower (*i.e.* between 18 to 21°C).

(c) Testing for shell secretion at isotopic equilibrium

In order to assess whether shell secretion occurs at or near isotopic equilibrium with the environmental water it is necessary to refer to the equation presented by Grossman and Ku (1986) and shown in Section 3.3.2. This equation relates the oxygen isotope ratio of shell aragonite and environmental water at equilibrium, to temperature. The relationship was represented in Figure 3.3.

For the Red Hills it is assumed that the mean annual air temperature lies between 23 and 26°C (see above), and that the oxygen isotope value of the environmental water will be approximately -2.2‰, possibly ranging from 0 to -4‰. Using these values and equations 3.1 and 3.2 (see section 3.3.2) the expected range values of oxygen isotopes from aragonite shells when secretion is at isotopic equilibrium, is shown in Figure 4.12. On this figure the area of equilibrium values is defined by the maxima and minima values of δw (given by equation 3.2) and the range of temperatures. Under such conditions, equilibrium shell secretion would produce aragonite with $\delta^{18}O$ values between -0.3 and -

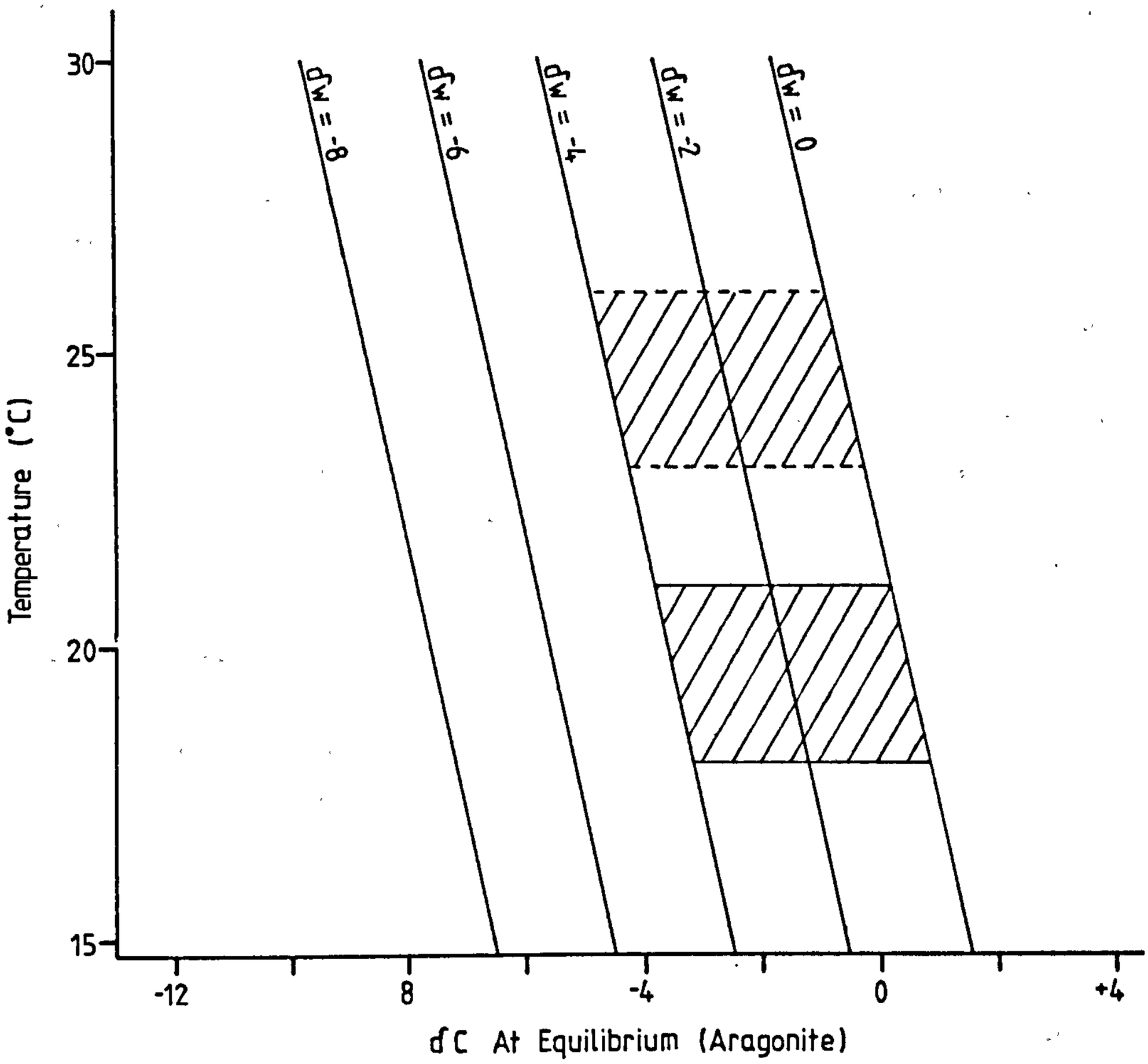


Figure 4.12 Relationship between temperature (°C) and aragonite ($\delta^{18}O$) at isotopic equilibrium with δw (water), following the equation of Grossman and Ku (1986), and showing shaded areas of equilibrium shell values for Jamaican shells defined by maximum and minimum values of δw over two ranges of temperature

4.9‰. However using a range of ground level temperatures (18 to 21°C), equilibrium shell secretion would produce aragonite with $\delta^{18}O$ values between +0.8 and -3.2‰.

These "expected" equilibrium values are close to those actually measured in shells of both *Pleurodonte sublucerna* ($\delta^{18}O$ from -0.10 to -3.86‰) and *Lucidella aureola* ($\delta^{18}O$ from 0.72 to -4.34‰) collected from the Red Hills. Although there are uncertainties in the estimation of both ambient temperatures and the isotopic composition of the water available to the snails, the findings suggest that these two species of snail are indeed secreting their shells at near isotopic equilibrium with their environmental waters.

(d) Discussion

Previous findings, presented in the preceeding chapter and by authors such as Lecolle (1985) and Goodfriend *et al.* (1989), have suggested that for land snails a vital effect exists and that shell secretion may not occur at isotopic equilibrium with environmental water. However, in the case of the Jamaican land snails, the limited available evidence indicates shell secretion at least close to isotopic equilibrium, with respect to oxygen isotopes.

In view of the lack of information on ambient ground level temperatures, it is difficult to know whether the temperature estimates are accurate. In addition, the isotopic data on the waters available to the snails are sparse. To assess truly whether the shells of these Jamaican snails are deposited under equilibrium conditions, or whether a consistent vital effect exists, would require samples of shells secreted over a known time period with samples of the environmental water available to the snails at that same time from the same locality. A detailed investigation of this type was carried out on land snails from the southern coastal plain of Israel by Goodfriend *et al.* (1989), where rainwater, body water and shell carbonate were measured. Goodfriend *et al.* (1989), found that shell carbonate was enriched by around 1-2‰ relative to equilibrium with body water. An enrichment of this magnitude could also be present in the Jamaican snails but would go unnoticed due to the range of calculated values.

The results of the experimental work presented in chapter three indicate that snail shell is enriched by around 2.75‰ over equilibrium values as a result of a metabolic or vital effect. Such an enrichment could also be present in the Jamaican snails and be masked by the inherent uncertainties. However, with a vital effect of this magnitude, it is somewhat unexpected that the shell carbonate values are not more enriched, as compared to equilibrium values, than they actually are. In the relatively moist and humid conditions of much of Jamaica, throughput of water may be large and continuous, and therefore, the snails may have little time to alter their body waters, and so shell $\delta^{18}O$ values are not widely different equilibrium values. In drier or more seasonal climates throughput of water would be slower, and greater metabolic enrichment would be possible.

As mentioned above, Lecolle (1985) produced an equation to relate the oxygen isotope composition of precipitation, relative to SMOW and land snail shell aragonite relative to PDB (see equation 4.1). For the Jamaican snails of the Red Hills the mean oxygen isotope values of shell aragonite for *Pleurodonte* and *Lucidella* are -1.8 and -2.4‰ PDB, respectively. The isotopic composition of Jamaican rainwater was measured as approximately -2.5‰ SMOW. However, using the above shell values in equation 4.1 (after Lecolle, 1985) would give a value for precipitation of between -8.0 and -8.7‰ - which is between 5 and 6‰ more depleted than that actually measured. It would appear that with respect to a tropical maritime climate, the relationship proposed by Lecolle cannot be used, and should be used with caution in other climatic regimes.

4.9 OTHER FINDINGS - SHELL ISOTOPE SIGNATURES AND LAND SNAIL ECOLOGIES

4.9.1 Introduction

The investigations carried out on the Jamaican land snails, the results of which have been presented in sections 4.5 to 4.8, have been of limited success and the findings are necessarily tentative due to the limitations of field collections, laboratory analysis, and available environmental information. The importance of having information concerning the mode of life of the snails in order to reach the best interpretation the isotopic data from their shells has been stated. In carrying out the objectives of the research, data have been collated from three groups of species - *Pleurodonte*, *Urocoptis* and *Lucidella*, each with a characteristic ecology and mode of life. On further investigation, beyond the original objectives of the Jamaican case study, the isotopic signatures of these three groups of species, were found to be distinctive. The results of these findings are presented below.

4.9.2 Results

The stable isotope data from the three groups of species are shown graphically in Figures 4.13a-c where the carbon isotopic composition of individual shells is plotted against the oxygen isotope values. Figure 4.13a represents the data from 61 individual shells of *Pleurodonte sublucerna*, from either slices or drill holes sampled from just behind the aperture. Isotope data from thirty specimens of *Lucidella aureola* (complete shells) are shown in Figure 4.13b. Figure 4.13c represents 37 shells of *Urocoptis brevis* and six individuals of *Urocoptis cylindrus*, a total of 43 specimens, each sample being measured from the last two whorls sliced off from individual shells.

For each of the three groups there is a wide spread in isotopic values generally around a 4 to 6‰ range in oxygen isotopes and a larger range of 6 to 8‰ in carbon isotopes. The data are summarised in Table 4.7, which shows mean oxygen and carbon isotope values, total ranges and standard deviations for the three groups. Although the oxygen isotope signatures for the three groups are similarly spread, the carbon isotope data of each group are characteristic with *Pleurodonte* having the most depleted $\delta^{13}C$ values (mean $\delta^{13}C$ = -11.18‰), and *Urocoptis* the most enriched $\delta^{13}C$ values (mean $\delta^{13}C$ = -6.22‰ with the data from *Lucidella* lying between the two extremes (mean $\delta^{13}C$ = -9.3‰).

The distinction between the three groups, as shown in Figures 4.13a-c, is partially masked by the degree of isotopic variation within the data sets. The variation within each of the three groups may be reduced if data points more than 1.5 times the standard deviation away from the mean are precluded. (This value was chosen arbitrarily although 1.5 times the standard deviation excludes only a small portion of data points, but on the other hand does eliminate extreme values.) This has been carried out to produce Figure 4.14, where for each of the groups the "area" encompassing the data points less than 1.5 times the standard deviation away from the mean oxygen and carbon isotope value is shaded. From Figure 4.14 it may be seen that there is a some overlap between the *Pleurodonte* and *Lucidella* areas, but that they are still distinct, with $\delta^{13}C$ values of the shells of *Pleurodonte* being more depleted in

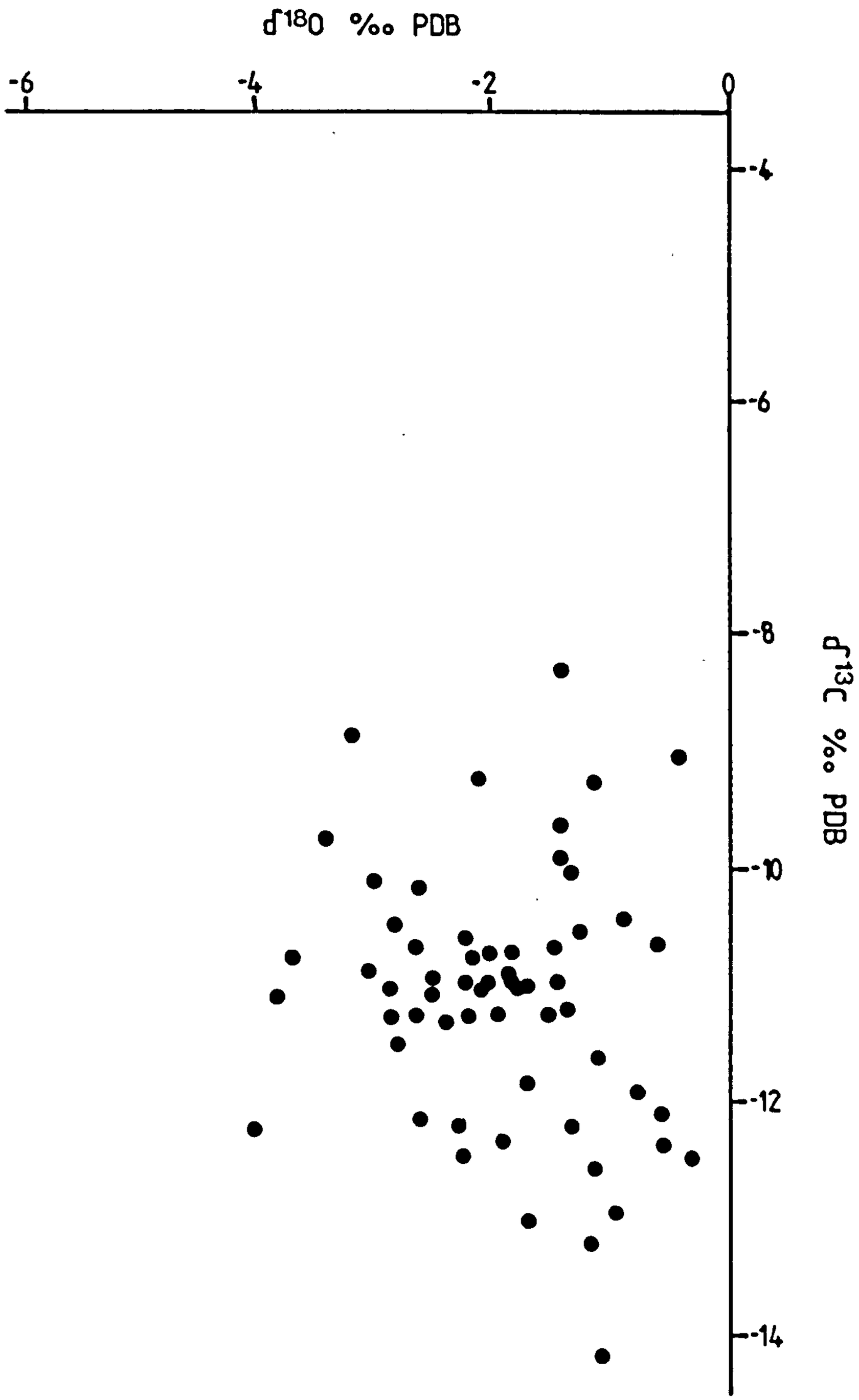


Figure 4.13a $\delta^{13}C$ versus $\delta^{18}O$ for shells of *Pleurodonte sublucerna* (n = 61)

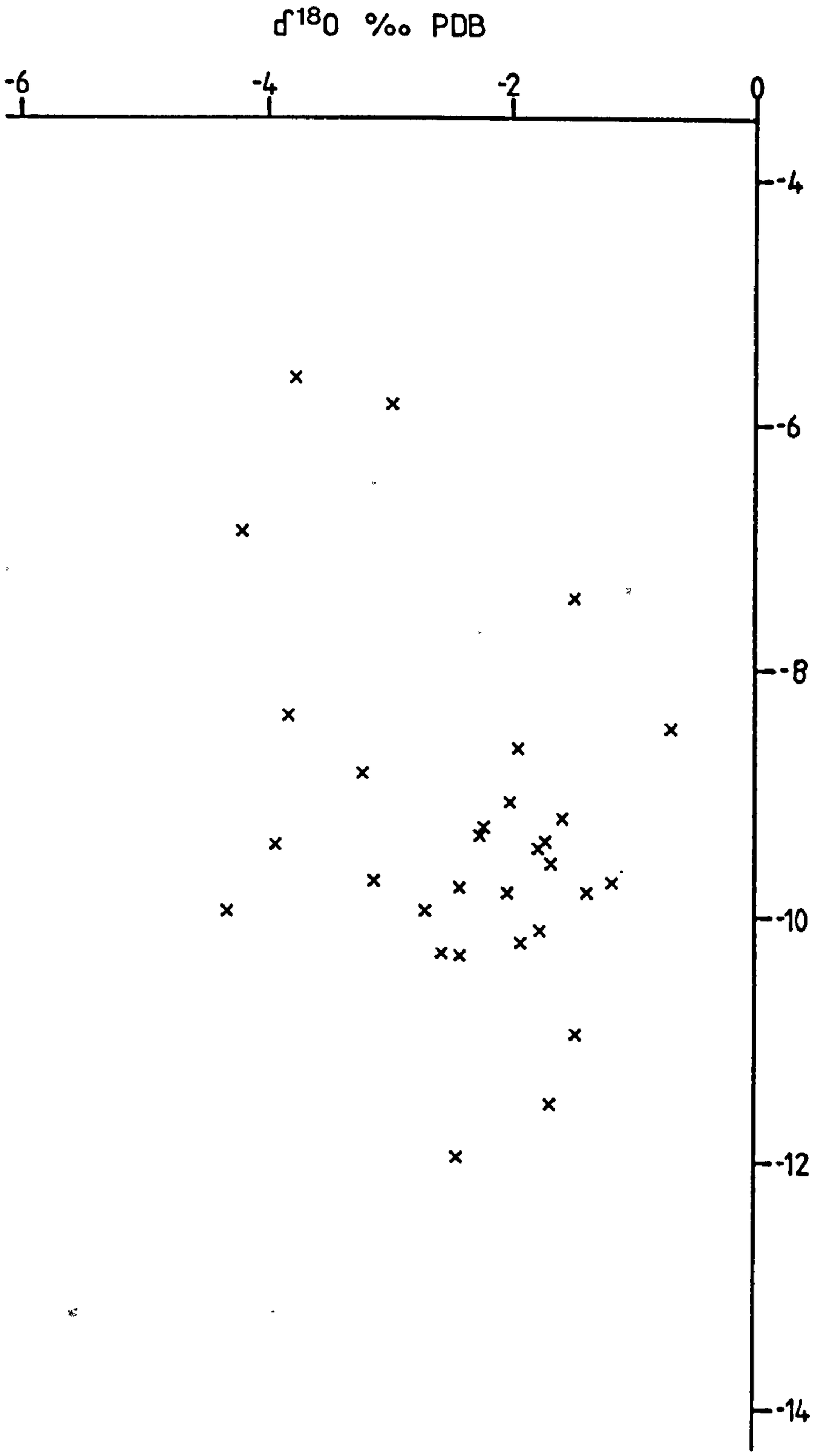


Figure 4.13b $\delta^{13}C$ versus $\delta^{18}O$ for shells of *Lucidella aureola* (n = 30)

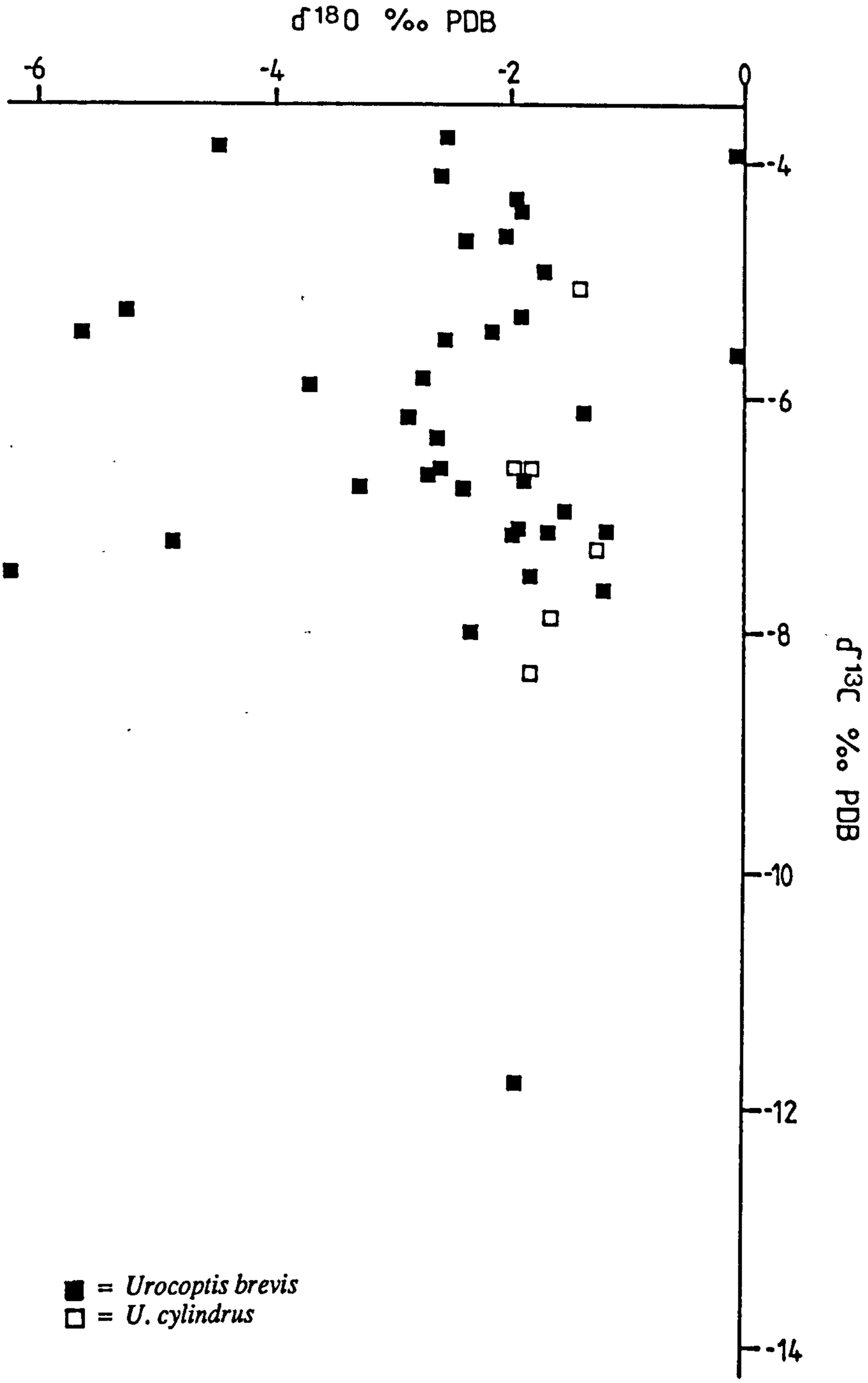


Figure 4.13c $\delta^{13}C$ versus $\delta^{18}O$ for shells of *Urocoptis brevis* (n = 37) and *Urocoptis cylindrus* (n = 6)

	Pleurodonte n = 61	*Lucidella* n = 30	*Urocoptis* n = 43
Mean $\delta^{13}C‰$	-11.18	-9.30	-6.22
Range $\delta^{13}C‰$	-8.30 to -15.71	-5.63 to -11.96	-3.88 to -11.81
Std. Dev.	1.25	1.41	1.50
Mean $\delta^{18}O‰$	-1.92	-2.36	-2.33
Range $\delta^{18}O‰$	-0.10 to -4.05	-0.72 to -4.34	0.28 to -6.26
Std. Dev.	0.89	0.94	1.35

Table 4.7 Mean oxygen and carbon isotope values, total ranges and standard deviations for shells of the three Jamaican taxa *Pleurodonte*, *Lucidella* and *Urocoptis*

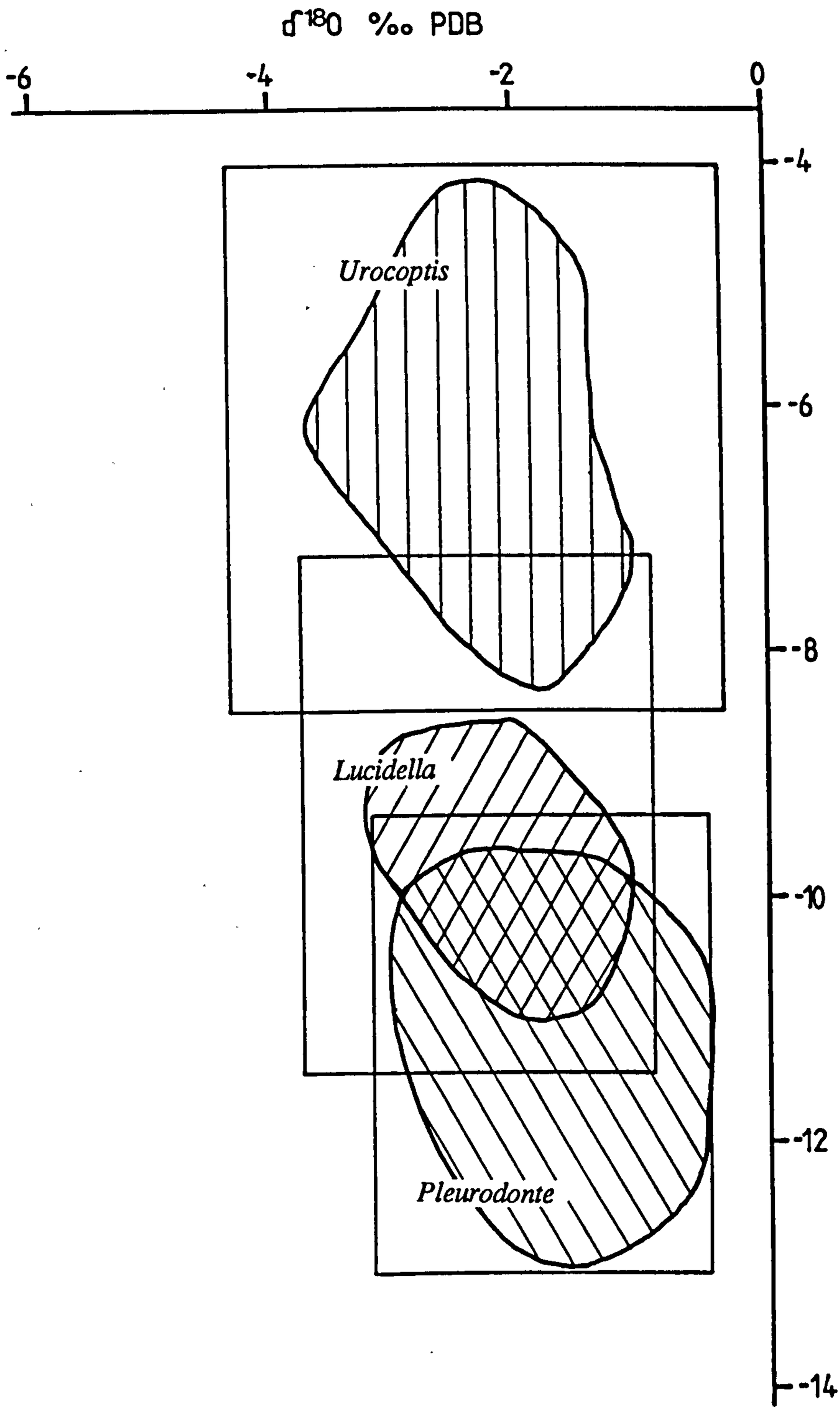


Figure 4.14 $\delta^{13}C$ versus $\delta^{18}O$ plot, showing the area encompassing data points from Figures 4.13a-c that are less than 1.5 times the standard deviation (defined by rectangles)away from the mean isotope values of each of the three main taxa *Pleurodonte*, *Lucidella* and *Urocoptis*

^{13}C than shells of *Lucidella*. The *Urocoptis* area is quite separate from those of the other two groups and shells from this group are evidently more enriched in ^{13}C.

4.9.3 Ecology of the three major groups of species

Pleurodonte sublucerna is a ground dwelling pulmonate. Adults are nocturnal and juveniles are diurnal (Goodfriend, 1983). Its natural habitat is the forest floor, although I found live specimens and empty shells on road side verges on the edges of forest, with relative ease. It is found mostly in hilly limestone areas but may spread beyond. Snails of this species have been observed to feed upon freshly fallen plant debris, including leaf litter, fruits and flowers (Goodfriend, 1983). The microenvironment occupied by the snails is damp, and so the snails would be active for much of the time without extended periods of hibernation. During the daily bouts of inactivity they are generally found among leaf litter on the forest floor, or on soil under rocks. Inorganic carbonate would only be available if the snails travelled across some rock or mossy outcrop, or if limey soil were ingested.

Lucidella aureola is an operculate snail. It is found island wide, even beyond limestone bedrock areas. It is thought to be arboreal (live specimens have only been seen active on tree trunks and leaves C. R. C. Paul, pers. comm., 1990). As a tree climbing species, its diet may consist of algae and lichens rasped off the tree limbs, or possibly leaves, although it is not known if this species would eat leaf material. If this species does spend its life cycle in trees it is likely that it gets all its carbon requirements from the organic material in its diet and from the atmosphere. Inorganic carbon from limestone would be unimportant.

Urocoptis brevis is a pulmonate snail that inhabits rocky outcrops in areas of the island subject to extremes of climate, with extended periods of drought in between rains. It is adapted to sustain life for long periods of time without water and retreats into holes and crevices in limestone outcrops before retracting well back into its shell. It probably feeds by rasping algae and lichens from the rock surfaces, hence consuming limestone along with the organic material, and snails of this species are presumably only active when their habitat is wet. I did not see any active specimens. It is likely that this species, the smallest *Urocoptis* on the island, is best adapted to the driest conditions by being able to reach a mature size quickly when the rains come. Thus there may be selective pressure for fast growth during immaturity to reach a size that will improve chances for survival during dry periods. In contrast, *Urocoptis cylindrus* has a much larger and robust shell. This species is restricted to the wet limestone forest. The few specimens analysed in this study all came from the periphery of the Cockpit Country (there is no road access into the heart of the Cockpit Country). This species lives on the ground and on rocky outcrops and like *U. brevis* probably feeds on algae and lichen rasped from the surfaces of rocks. Specimens were collected from mossy outcrops and under stones. Living in a continually moist environment, there may be less pressure to mature quickly, and hence the shells of this species are thicker walled, much larger and more robust than those from *U. brevis*.

4.9.4 Discussion

The chief differences between the three groups concern their respective habitats - of their microenvironments and their distribution across the island; their mode of life; and their diets, especially the amount of inorganic carbon likely to be ingested.

Firstly the two pulmonate genera *Pleurodonte* and *Urocoptis* will be considered (as the isotopic compositions of the two species of *Urocoptis* are similar, comparisons will be drawn at the generic level). The differences in carbon isotope ratios of the two genera are thought to be a result of the relative proportions of organic to inorganic carbon ingested by the snails. *Pleurodonte* probably gets much of its carbon from the plant material in its diet, which will have depleted carbon isotope ratios (a mean value for tropical vegetation is given as around -27‰, Goodfriend and Hood, 1983). In comparison, *Urocoptis* probably ingests a much larger proportion of limestone in its diet as it feeds by rasping at the surface of rock. Marine limestones such as the Yellow and White Limestones that cover much of Jamaica, will have carbon isotope ratios near to zero (*i.e.* the PDB belemnite standard reference point). Isotopic analyses of Pleistocene limestones and dolomites from the reefs of North Jamaica (Land and Epstein, 1970), have shown that the isotope ratios of the carbonates have been diagenetically altered by reaction with meteoric water. Even so, the carbon isotope ratios of the samples measured by Land and Epstein lie between +1.41 and -9.32‰. It is therefore likely that the limestones available to landsnails, whether or not the isotope ratios have been altered by diagenesis, will have carbon isotope ratios considerably more enriched in ^{13}C than the available carbon from organic sources. Therefore, for these two groups of snails, the carbon isotope ratios of their shells seem to reflect their life habit and diet. From this, it might be possible to infer details of the ecologies of other Jamaican species, knowing an average $\delta^{13}C$ value characteristic of a particular species or genus.

It was thought that the nature of the habitat, in terms of the moistness of the environment and its effect on the habits of the snails, might also be evident in the carbon isotope ratios of the snails. Thus, the periodically active *Urocoptis brevis* might have more enriched $\delta^{13}C$ values than the more continually active *U. cylindrus* and *Pleurodonte*, where more metabolic carbon dioxide - depleted in ^{13}C - would be produced. However, as the two species of *Urocoptis* have similar $\delta^{13}C$ values (and also similar oxygen isotope ratios), but very dissimilar habits and environments, this argument does not appear to hold true.

As the tree-dwelling *Lucidella* probably ingest little or no limestone, following the line of argument presented above, they might be expected to have $\delta^{13}C$ values even more depleted than those from *Pleurodonte*. However this is not the case as *Lucidella* are slightly more enriched in ^{13}C than shells of *Pleurodonte*. This is possibly because *Lucidella* uses atmospheric carbon dioxide to meet its bicarbonate requirements, as it does not have access to limestone. Atmospheric carbon dioxide has a $\delta^{13}C$ of around -7 to -8‰, but this is fractionated by around +8‰ upon dissolving in water in the snail or in water subsequently taken up by the snail (Goodfriend and Hood, 1983), giving as effective $\delta^{13}C$ of +1‰ (section 3.3.3). Even a slightly greater reliance upon this source of carbon, as compared to another species, might result in more enriched $\delta^{13}C$ shell values. However, as the $\delta^{13}C$ values of *Lucidella* are still close to those of *Pleurodonte* it would appear that the most important source of carbon to this prosobranch is still from the organic material in its diet. Some of the difference between the carbon isotope ratios of *Lucidella* and *Pleurodonte* might be explained if the carbon isotopic

composition of the epiphytes grazed by *Lucidella* was different from the leaf material consumed by *Pleurodonte*. The possibility that *Lucidella*, an operculate snail, processes its carbon in a different manner to the pulmonate snails could also be an explanation of the position of the $\delta^{13}C$ values of this species in relation to the other two groups.

In summary then, it would seem that the carbon isotope composition of these Jamaican snails is most closely influenced by the isotopic composition of carbon in the diet. Metabolic effects linked to activity levels, due to the relative aridity of the microenvironment are of less importance.

4.10 SUMMARY OF FINDINGS AND CONCLUSIONS

4.10.1 Isotopes within single shells

Within individual shells there was less variation in carbon isotopes than oxygen isotopes, the opposite of the situation seen in the investigations of shells from the Red Hills and other areas of Jamaica.

For shells of the species *Pleurodonte sublucerna*, intrashell isotope values were consistent, although two specimens showed a 2‰ shift in $\delta^{18}O$ which was attributed either to a possible evaporation effect acting on the environmental water used by the snails, or to an interruption in shell deposition.

The pattern of carbon isotopes within shells of *Pleurodonte* and within a single shell of *Urocoptis cylindrus* was thought to reflect changing metabolic effects over time, possibly linked to the rate of growth of shell, or to later internal thickening of the shell once adult size had been reached.

4.10.2 Altitude and shell isotopes

A good positive correlation was found between altitude (elevation ft) and shell diameter for shells of *Pleurodonte sublucerna* from the Red Hills. However, correlations between shell oxygen isotopes and altitude were poor and not statistically significant (a trend towards more depleted $\delta^{18}O$ values with increasing elevation had been expected). The poor relationship between shell oxygen isotopes and elevation is thought to reflect the fact that the isotopic composition of Jamaican land snail shells is not a simple function of increasing wetness of the environment with increasing elevation coupled with a rain-out effect. Factors such as the source of precipitation and its subsequent landward passage must be considered in addition to what may happen to the environmental water before it is utilised by a land snail, the environmental temperature as well as other aspects specific to each localised environment.

Both oxygen and carbon isotopes were very variable although carbon more so. For carbon, a spread of as much as 7‰ was noted for shells of each species collected from within the Red Hills. Subsequent analysis of these and other isotope data from the Jamaican case study (see section 4.10.5) suggests that the carbon isotope ratio of land snail shells is dominated by the initial carbon isotope ratios of the carbon sources in the diet, and the relative importance of each of those sources to that particular individual or species. Even so, the degree of isotopic variation within the data from a single species is hard to account for.

To assess accurately the relationships between altitude and shell isotopes an "ideal" locality where the variety of influencing factors would operate in the same rather than opposing directions. The initial objective of the study was, in hindsight, rather too simple for the complexity of the natural situation.

4.10.3 Local and regional isotope variations

On the regional level, no consistent patterns in the shell isotopes of *Urocoptis brevis* were found. The data appear to be equally variable across the whole of the south coastal margins of the island and there was as much isotopic variation within areas and within singe sites as between the five areas considered, although data from some areas were limited. Therefore, any regional isotope effects were masked by localised influences.

A similar conclusion was reached from the results of isotopic variation on a smaller scale, where there was as much isotopic variation in shells from within the three sub-areas of the Red Hills as between those areas.

The data suggest that large variations in shell isotopes might be expected right across the island, and therefore any future investigations of shell isotopes in Jamaican land snails would need to be based on more than a handful of isotopic analyses.

4.10.4 Isotopes in waters and shells

Uncertainties exist in the measurement of the isotopic composition of environmental waters (due to the paucity of analytical data) and in the assessment of ambient temperatures in the snails' microenvironments. In addition, data are not available for waters and shells from the same locality, or from where shell secretion was contemporaneous with a known isotopic composition of environmental water. However, the limited evidence suggests that shell secretion occurs at least close to isotopic equilibrium with respect to oxygen isotopes, although within the range of estimated values a small vital effect and/or an evaporation effect (within +1-2‰) acting upon the environmental water before being used by the snail, may be masked.

The data from the Jamaican landsnails were used to test the equation presented by Lecolle (1985) which related the oxygen isotopic composition of precipitation to land snail shell aragonite. However, the relationship was not in accord with the data from this tropical maritime climate.

4.10.5 Isotopes and snail ecologies

The carbon isotope composition of each of the three genera of snails under study (*Pleurodonte*, *Lucidella* and *Urocoptis*) was variable, but characteristic and is thought to be related to the mode of life and diet of each type of snail. It may be possible to use this finding to infer information on the ecologies of other Jamaican snails based upon their shell carbon isotope ratios. However further investigation would be required to confirm the idea.

The oxygen isotope ratios of the different genera were, in contrast, similar and equally variable.

4.10.6 Conclusions of the Jamaican case study

(1) Carbon isotopes in land snail shell aragonite are more variable that oxygen isotopes when comparing populations of Jamaican snails. However, the reverse was found within individual shells where oxygen isotopes were more variable. This suggests that although the isotopic composition of carbon sources is the more variable, that individual snails use the same basic source throughout their lifetime. Even so isotope ratios of both carbon and oxygen were very variable at individual sites, locally and regionally. Therefore, more than one shell needs to be analysed to obtain a 'typical' value for a site. However, from the isotope data from the Red Hills and that from *Urocoptis brevis*, once the range of values has been adequately assessed and a 'typical' isotope value obtained, it should apply to that species throughout the island.

(2) The oxygen isotopic composition of aragonite in Jamaican land snails is thought to reflect primarily the oxygen isotopic composition of the environmental water available to the snails, which is in itself likely to be very variable over space and time. Local temperatures and the wetness of the local environment along with metabolic effects acting upon the body water of the snails are seen as less important contributary factors. The limited data do not provide direct evidence to support a consistent vital effect of more than 1-2‰ in the snails.

(3) The predominant influence upon the carbon isotopic composition of the Jamaican land snails studied appears to relate to the type and relative proportions of organic and inorganic carbon available to, and utilised by, the snails. As a result it is suggested tentatively that shell $\delta^{13}C$ ratios may be used to infer information on the ecologies of other Jamaican species, but more work would be required to substantiate this. Metabolic effects, linked to the activity levels of the snails, are thought to have a lesser influence upon the resultant shell isotope compositions.

(4) The limited time available for field collections, and the logistical problems encountered, meant that it was not always feasible to collect sufficient information to investigate more successfully the objectives of the study. As a result, some of the investigations provided little in the way of definite results. However, the study did give light to several areas where further study might prove fruitful, particularly in relation to the issues of shell secretion at oxygen isotope equilibrium and to snail ecologies and carbon isotopes.

CHAPTER 5

STABLE ISOTOPES IN TERRESTRIAL FOSSIL SNAIL SHELLS FROM A LATE GLACIAL - EARLY POST GLACIAL SITE AT HOLYWELL COOMBE, NEAR FOLKESTONE, KENT : A CASE STUDY

5.1 INTRODUCTION AND AIMS

In addition to the laboratory experiments and Jamaican case study presented in chapters two to four, it was hoped to investigate the stable isotope record of some sub-fossil / fossil terrestrial mollusc shells to look for evidence of climatic change. The results of the various laboratory growth experiments have indicated that if other environmental factors remain reasonably constant then changes in ambient temperatures should be recorded in the shells secreted by contemporary land snails. However, it would be difficult to interpret changes in shell isotopes over time in terms of real climatic change, rather that just changes in the local environment over time, if other corrobative evidence were not available. Additional supporting evidence might come from studies on other fossil taxa present at a site, such as pollen, seeds and insects, as well as changes in a terrestrial mollusc assemblage itself. Changes in the lithology of a section would also be indicative of environmental or possibly climatic change.

It is necessary to assume that any fossil shell undergoing stable isotopic analysis records the original isotopic composition of that shell and that it has not undergone isotopic exchange with any meteoric fluids. Thus, well preserved shell material is essential.

During 1988, the opportunity arose to become involved with a multi-disciplinary project working on a series of late glacial to post glacial sections located in Holywell Coombe, near Folkestone in Kent. The sites, which were rich in land mollusc shells, other fossil taxa and archaeological remains, were adjacent to engineering works relating to the construction of the Channel Tunnel, and the research programme has been assisted financially by the construction consortium 'Eurotunnel'. Further details concerning the overall research project and information on the site and the sections sampled will be presented below.

Therefore the aim of this case study was to use isotopic analysis of fossil terrestrial mollusc shells from the sections at Holywell Coombe to look for evidence of climatic change, particularly in relation to the climatic amelioration marking the final demise of the Devensian glaciation (around 10,000 BP). The results of this investigation would then be considered in conjunction with the other aspects of the multi-disciplinary research project. However, at present the overall findings of the various aspects of research have not been assessed. Thus, the results of the stable isotopic analyses presented in this chapter will allow, at best, only a limited interpretation of past environments at the site.

5.2 HOLYWELL COOMBE - BACKGROUND INFORMATION ON THE SITE AND RESEARCH PROJECT

5.2.1 The development of the multidisciplinary project

In 1968 a trial pit was excavated at the mount of the escarpment coombe (Holywell Coombe) west of Sugarloaf Hill, Folkestone (Grid Reference TR 220379). The dig revealed Late Devensian and Flandrian slope wash and spring deposits containing fossil remains of molluscs, insects and plants (Kerney *et al* ., 1980). In 1969, to allow further examination of the site, three additional pits were excavated as close as possible to the original pit. Based on mollusc, beetle and pollen assemblages, and from the stratigraphy, a sequence of environments were inferred by Kerney *et al.* ,(1980) comprising three main stages.

The bases of the sections revealed chalky gravels and silts, thought to be the result of solifluction processes, which contained a flora (plant macrofossils) and molluscan assemblage representing an open environment of late-glacial character - the vegetation was discontinuous tundra with dwarf shrubs but no trees. Above these deposits were layers of organic silts and tufas representing a period of vigorous spring activity. The flora and fauna indicated an environment with some open woodland and marsh, although over time the forest became more dense and impinged on the marshy area of the valley floor and deciduous trees predominated. Above the tufas, there was a discontinuity, marking a phase of weathering, overlain by an accumulation of hillwash material, the latter thought to be associated with vegetational clearance and cultivation by prehistoric man. Radiocarbon analysis of organic silts and wood fragments gave dates between 9,960 ± 170 B.P. near the base of the sections and 7,500 ± 100 B.P. near the top of the tufa deposits (Kerney *et al.*, 1980).

In 1982 the area of Holywell Coombe was scheduled as a S.S.S.I (Site of Special Scientific Interest) by the Nature Conservancy Council. The proposed construction of the Channel Tunnel would have led to the destruction of the S.S.S.I. The building consortium (Eurotunnel) agreed to move the alignment of the proposed entrance tunnels a little to the south, to miss the head of the valley, and they also agreed to assist with funding a multidisciplinary research project to investigate the ecology of the coombe, both past and present. This initiative, presumably based on the need for good public relations, provided the impetus for further research in the valley.

The aims of the multidisciplinary research project were varied but covered five main areas. The first was to assess the changes in the sediments over the area of the coombe, so as to pin-point the best sources of various materials such as organics for radiocarbon dating, pollen or insect studies; or thick tufas for studies of freshwater organisms such as ostracodes. The second area of research was to look for deposits older than those already located (*i.e.* older than around 10,000 B.P.) to investigate further the transition from a late-glacial to a post-glacial climate and environment. The third area of study was expand, and attempt to date, the transition for boreal to deciduous forest during the accumulation of the tufa deposits. Leading on from this, the fourth line of research was to discover the time of forest clearance and the fifth to gain archaeological information about the prehistoric inhabitants of the coombe, from artifacts located in the hillwash sediments at the top of the sedimentary sequence.

To carry out these aims a line borehole survey was initiated across the valley to assess "key areas". Following this a large trench (40 metres x 8 metres) was excavated at the head of the axis of the valley (HVI trench). Such a large trench would provide sufficient surface material for archaeological research and also a reference point for other pit stratigraphies. The HVI trench was excavated, on one side, to a depth of almost five metres - a complex procedure with the water table sitting at around 1.5 metres below the ground surface. Other smaller pits were excavated at various points across the valley to look for older organic layers. These pits/small trenches included a fifth one in part of the coombe known as Horseshoe Springs (T5). A sketch map of the coombe with the locations of the excavations pertinent to this case study is shown in Figure 5.1.

Areas of study include: fossil mollusc shells (terrestrial and aquatic); pollen and plant macrofossils; fossil insects especially beetles; fossil ostracode valves (present in some of the tufas); a study of bones located at the site (both pre- and post- inhabitation by man); investigation of the magnetic susceptibility of magnetic minerals within the sediments; studies on the archaeological remains; and soil development and morphology. An integral part of the study involves a comprehensive survey of the flora and fauna present in and around the coombe at the time of the tunnel development.

5.2.2 How the stable isotope work fits in

With such a diverse project underway, and with a good potential supply of fossil shell material possibly available, I was invited by the project co-ordinator (Dr. Richard Preece, Zoology, Cambridge), to become involved. It was hoped that the isotope work might reveal chemical evidence to substantiate the changes in environment and climate inferred from the changes in biota. Of particular interest was the marked climatic amelioration thought to have occurred at the demise of the Devensian glaciation sometime around, or before, 10,000 to 10,500 B.P. The precise timing of the warming, whether a sudden marked climatic shift actually took place, or whether the amelioration was in fact more gradual is under constant debate between the various groups involved in Quaternary paleontology, palaeoentomology and palynology. If a sharp increase in temperature did occur then it might be recorded in the stable isotope ratios of the shells of the terrestrial molluscs living at that time, with the potential for a shift towards more depleted (*i.e.* more negative) oxygen isotope values as the climate warmed.

Of the various trenches and pits excavated in the vicinity of Holywell Coombe, it was decided to concentrate the stable isotopic study on the main trench at the head of the valley (HVI) and on the trench excavated in Horseshoe Valley (T5). Both pits contained large quantities of fossil shell material and some organic material suitable for radiocarbon analysis. It was considered that the results from the two pits might be comparable.

It was therefore hoped that any shifts in shell oxygen isotopes could be linked with climatic change, and that fluctuations in shell carbon isotope values over time could be identified with changes the nature of the local environment. These were the basic premises behind the investigations outlined below.

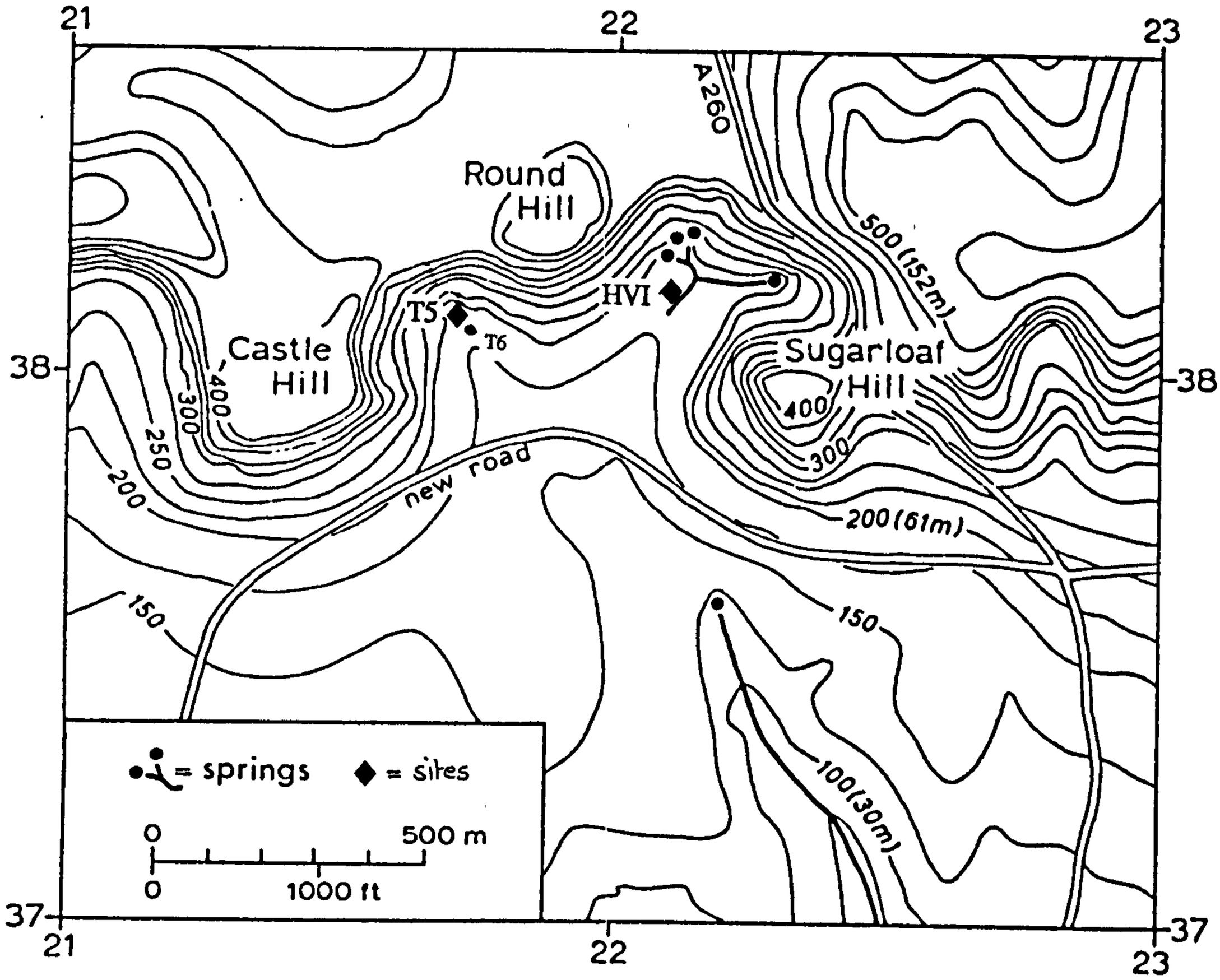


Figure 5.1 Location of excavations in Holywell Coombe, Folkestone. National Grid 1km co-ordinates are marked, contours in feet. (after Kerney *et al.*, 1980)

5.3 METHODS

5.3.1 Species selection

For the purposes of the stable isotope work it was necessary to select a species that was present in reasonable quantities throughout much of the sequence, and preferably into the lowermost part of the sequence thought to represent the Late glacial. Thus the species would, by inference, be one with a wide environmental tolerance. The species selected would have to be readily identifiable in fragmented form, as only fragments could be made available from the detailed catalogue of specimens previously collated from the various trenches. Further, the fragments would have to be of a sufficient size to survive the standard lab' preparation technique (see section 2.5), and to provide sufficient material for stable isotope analysis. Small, tightly coiled shells would have proved very difficult to clean of detrital calcareous matter, so a species with a larger more open shell structure was necessary.

The species selected was *Arianta arbustorum* (Linné), an helicid snail with a fairly large globular shell. Whole shell specimens of this species and a large number of fragments were located in the trench samples. Fragments of the shells of this species are readily distinguishable as they bear characteristic microsculpture. Shell fragments were separated from bulk samples by Dr. R. C. Preece, and sent to Liverpool for further cleaning and processing prior to isotopic analysis.

5.3.2 Section descriptions and radiocarbon dates and sampling procedure

The HVI trench had been excavated to a depth of five metres where the underlying Gault Clay was located. Above this were layers of calcareous silty clay with a few organic lenses and humic horizons. Tufas were found above 230cm and continued to around 80cm below the surface where they were replaced by hillwash deposits. The top 30 to 40cm at the trench were made up of modern plough-soil. A radiocarbon date of 12,280 ± 140 BP (OXa1752) was obtained from a scapula of an aurochs, *Bos primigenius* (an ancestor of modern cattle), found in the basal organic silt at around 430 - 440cm. A further date from the humic horizon at 320 to 330cm is pending. The organic lens at around 390cm is thought to represent approximately 11,500 BP and the top of the sequence of tufas is thought to represent between 7,000 to 7,500 BP (R. C. Preece, pers. comm., 1990). The sequence is shown in Figure 5.2 (see section 5.4).

In the HVI trench, terrestrial molluscs were found in small numbers in the basal organic silt (around 440cm) and thereafter throughout most of the section. As mentioned above, complete shells of *Arianta arbustorum* were not available for isotopic analysis. However shell fragments of this species were sampled and analysed from much of the HVI section from a depth of 50cm to close to 380cm. The sample interval was generally five or ten centimetres, depending upon the nature of the sediments being sampled, and whether any change in lithology was occurring. Overall, shell fragments of *Arianta* from 33 sample intervals were entered for stable isotopic analysis. Generally the analyses were carried out upon single fragments of shell. Duplicate analyses were possible where more than one fragment of a suitable size was available. To gauge the amount of isotopic variation likely within a single sample horizon, multiple analyses were carried out on several fragments (four to six) from within three different horizons.

The T5 trench was also excavated to around five metres below surface. Below 470cm blocky brecciated Gault Clay was present. Above this was fine glauconitic sand with chalk clasts and clay. This material became more like silty clay above 400cm and contained some wood fragments and shell material. Around 340-350cm a dark grey-brown organic lens was located which has been radiocarbon dated as 9,760 ± 100 BP (Q-2721). Above this a change in lithology occurred and the silty clays were replaced by tufas of varying textures. Within the tufas, a band of calcareous clay with charcoal was found around 250cm. The charcoal revealed a radiocarbon date of 9,460 ± 140 BP (OxA-2088). The tufas were capped by modern ploughsoil at a depth of 30-40cm although because of the discontinuity the tufas are though to represent time up until around 8,000 BP (R.C. Preece, pers. comm., 1990). The sequence is shown in Figure 5.3 (see results section below).

Shells and fragments of shells of terrestrial molluscs were located throughout the tufaceous sediments and silty clays, to a depth of around 400cm. In the T5 trench, the sample interval was five centimetres. Most of the sampled horizons contained fragments of *Arianta arbustorum*. Samples from 35 horizons were entered for stable isotopic analysis. As with the HVI samples, the analyses from each sample interval were carried out on individual or pairs of fragments. From two sample horizons four and five fragments were analysed so as to assess the amount of isotopic variation likely within horizons.

In the previous case study (Chapter 4) and in the laboratory growth experiments, it has been shown that the carbon isotope composition of the inorganic carbon available to a terrestrial snail is very important in determining the resultant carbon isotopic composition of the secreted shell. Therefore it was decided to investigate the carbon isotopic composition of the tufaceous material deposited contemporaneously with the snail shells - the likely source of inorganic carbon. Time constraints limited the extent of tufa analyses. However, tufas from five horizons in the HVI trench and from seven horizons in the T5 trench were entered for stable isotopic analysis. Tufas were chosen for analysis at fairly regular intervals (approximately 50cm) throughout each tufa sequence, and/or where a lithology change occurred.

5.3.3 Laboratory analysis

The shell fragments of *Arianta arbustorum* were prepared using the standard treatment method outlined in section 2.5, where each fragment was subjected to bleaching, high temperature vacuum roasting and to being placed in a low temperature oxygen plasma furnace. These procedures were designed to remove from the shell material any remaining external, plus all internal, organic substances. Analyses were carried out following the methods shown in section 2.6. All shell material has been considered as aragonite and the isotope data are relative to the PDB standard.

The tufa samples were ground to a fine powder and then treated in the low temperature oxygen plasma furnace for four hours prior to being analysed in the mass spectrometer. The tufas are assumed to be calcites and the isotope data are expressed relative to the PDB standard.

5.4 RESULTS

5.4.1 HVI - Head of the Valley Trench

A total of forty nine isotopic analyses were carried out on shell fragments of *Arianta arbustorum*. These fragments came from thirty one of the thirty three sample horizons from which material was available. Unfortunately, the fragments from two horizons proved to be too tiny to survive the pretreatment process and provide sufficient material for isotopic analysis. The results of the isotopic analyses are shown in full in Appendix 3.

The carbon isotope values of these fossil shell fragments lie between -7.85 and -10.78‰, with a mean value of -9.34‰ and a standard deviation of 0.84‰. The oxygen isotope values lie between -0.93 to -4.16‰ with a mean value of -2.57‰ and a standard deviation of 0.75‰.

The carbon isotope data are plotted against depth on Figure 5.2a and the oxygen isotope data on Figure 5.2b. These figures also include lithologies, radiocarbon dates and stable isotope data from the tufas. On each figure a line has been drawn through mean values, where more than one fragment was analysed, or else through the individual sample points. Where more than one fragment was analysed, the spread in the data is indicated by barred lines.

In the HVI section no shells of *Arianta* were available from 260 to 310cm. Further, only one analysis was carried out on a fragment from the oldest part of the section (at 375cm) as fragments from the deeper horizons were too small to process. If horizons did not provide *Arianta* fragments, this is indicated on Figures 5.2a and 5.2b by dotted lines. As can be seen from Figures 5.2a and 5.2b, most of the analyses were carried out on shell fragments preserved in the tufas, where *Arianta* were most abundant. The silty clays below the tufas provided only a few fragments for isotopic analysis.

The carbon isotope profile (Figure 5.2a) shows no major change through the sequence, although there are differences between adjacent levels of as much as 2‰. The fluctuations between levels continue right through the section. However, these fluctuations appear to be of a similar magnitude to the isotopic variation found within individual horizons where several shell fragments were analysed. The most enriched $\delta^{13}C$ value (-7.03‰) was found in the shell fragment from the lowest horizon (370-380cm).

The oxygen isotope profile (Figure 5.2b) also shows fluctuations throughout which are of a magnitude similar to, or slightly less than, those fluctuations seen between $\delta^{13}C$ values from adjacent horizons. In addition, and also in the same manner as for the carbon isotopes, there appears to be as much oxygen isotope variation within individual sample horizons as between them. The most depleted $\delta^{18}O$ value (-4.16‰) was found in the shell fragment from the lowest horizon.

The amount of variation found within three individual sample levels is summarised in Table 5.1. The standard deviations in both carbon and oxygen isotopes values from within each sample level are as high, or higher, than those from the complete section. However, the number of samples analysed from each horizon is small, and the standard deviations are least in the 120-125cm level where the most analyses were carried out (n=6, $\delta^{13}C$ standard deviation = 0.50‰, $\delta^{18}O$ standard deviation = 0.59‰). Even so, the spread in values for individual horizons lie between 1.49 and 2.08‰ for $\delta^{13}C$ and between 1.63 and 2.19‰ for $\delta^{18}O$.

The stable isotope values of the tufa samples from the HVI section are shown in Table 5.2a and are plotted on Figures 5.2a and 5.2b. It may be seen that the carbon isotope values of the two

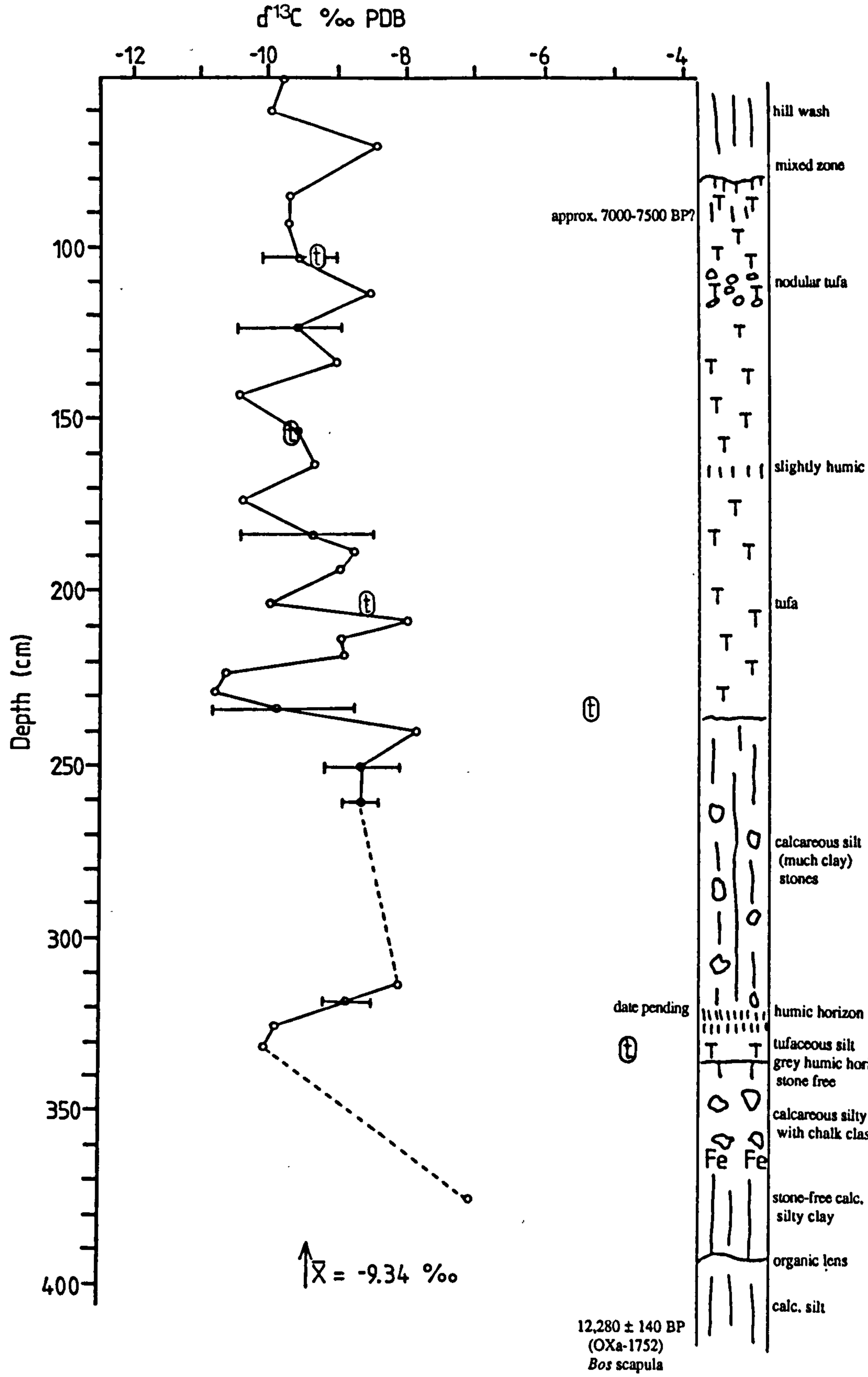


Figure 5.2a HVI trench Holywell Coombe, section and $\delta^{13}C$ profile, from shells of *Arianta arbustorum*. Range bars and mean values indicated. Solid line through mean values.
ⓣ= $\delta^{13}C$ tufa samples

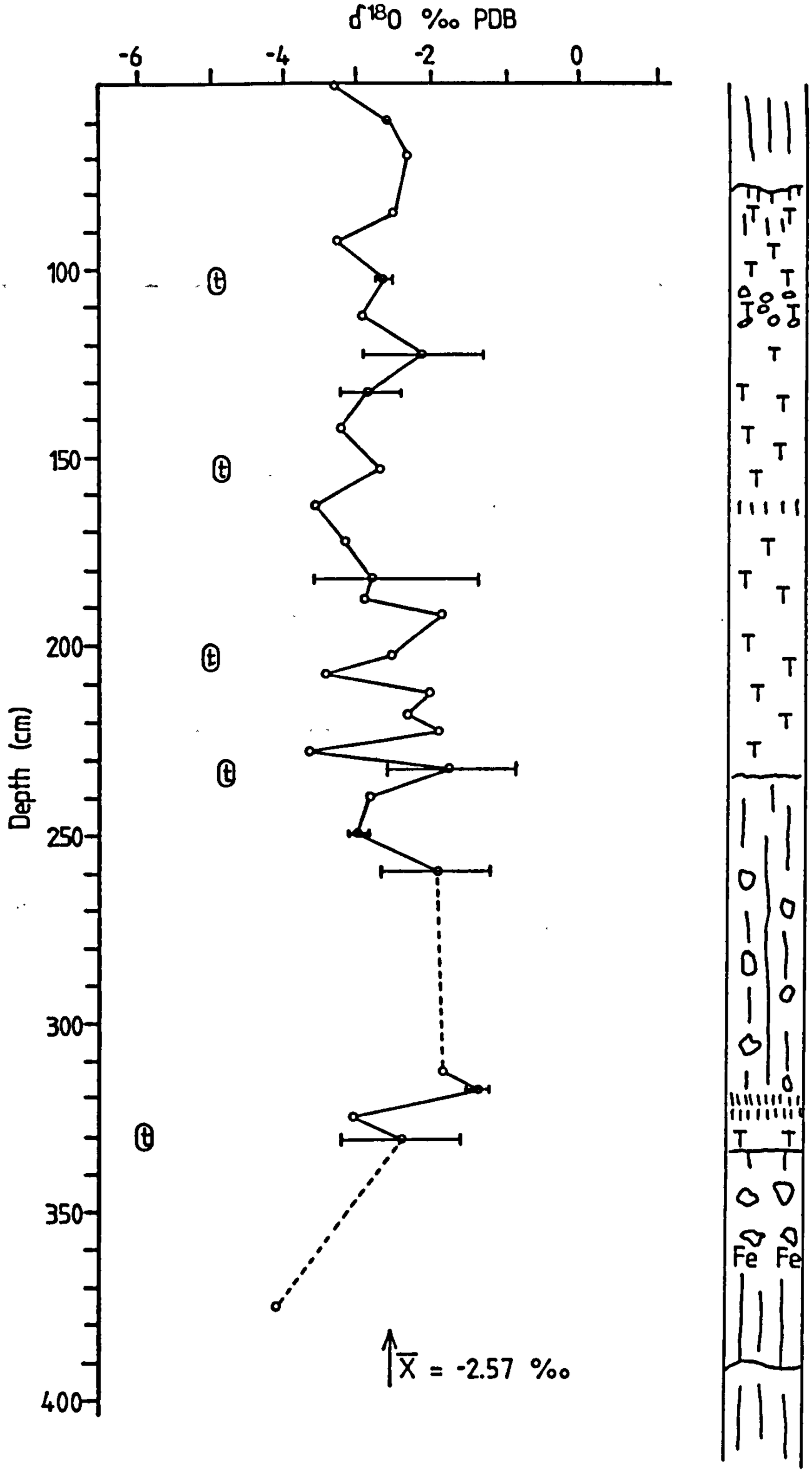


Figure 5.2b HVI trench Holywell Coombe, section and $\delta^{18}O$ profile, from shells of *Arianta arbustorum*. Range bars and mean values indicated. Solid line through mean values.
ⓣ= $\delta^{18}O$ tufa samples

	HVI Sample Level			Whole HVI Section
	120-125 cm	180-185 cm	230-235 cm	
No. of analyses	6.00	4.00	5.00	49.00
Mean $\delta^{13}C‰$	-9.58	-9.41	-9.88	-9.34
Range $\delta^{13}C‰$	-8.95 to -10.44	-8.49 to -10.37	-8.74 to -10.82	-7.85 to -10.78
Std. Dev.	0.50	0.77	0.87	0.84
Mean $\delta^{18}O‰$	-2.16	-2.87	-1.83	-2.87
Range $\delta^{18}O‰$	-1.35 to -2.98	-1.43 to -3.62	-0.93 to -2.66	-0.93 to -4.16
Std. Dev.	0.59	0.98	0.62	0.75

Table 5.1 Carbon and oxygen isotopic variation within three sample levels, HVI section, Holywell Coombe. Variation over entire section included, for comparison

DEPTH cm	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
100-105	-9.32	-4.94
150-155	-9.64	-4.88
200-205	-8.59	-5.05
230-235	-5.35	-4.82
327-335	-4.77	-5.90
Mean	-7.53	-5.12
Std. Dev.	2.30	0.45

Table 5.2a HVI section tufas

DEPTH cm	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
55-60	-9.01	-4.69
105-110	-9.81	-5.28
155-160	-9.11	-5.13
205-210	-8.44	-4.98
250-255	-9.06	-5.55
305-310	-8.78	-5.65
330-340	-8.32	-5.58
Mean	-8.93	-5.27
Std. Dev.	0.49	0.36

Table 5.2b T5 section tufas

Table 5.2a-b $\delta^{13}C$ and $\delta^{18}O$ data from selected tufa samples from (a) HVI section and (b)T5 section,from Holywell Coombe.

lowest tufa samples (-5.35 and -4.77‰) are considerably different from the rest which have $\delta^{13}C$ values from -8.59 to-9.64‰. This shift, of three to four per mil, occurs just above the base of the main sequence of tufas. The two samples enriched in ^{13}C were from the transition between the silty clays and the main tufas, and from a lower tufaceous silt horizon. The oxygen isotope values of the tufas are, on the other hand, similar throughout the sequence ($\delta^{18}O$ between -4.82 to -5.90‰), mean $\delta^{18}O$ = -5.12‰, standard deviation = 0.45‰), and are consistently more depleted in ^{18}O than the shells. The lowest tufa $\delta^{18}O$ value (-5.90‰), was that found in the lowest sample (327-335cm), however the tufa samples are generally 2-3‰ more depleted in ^{18}O than the *Arianta* shells.

5.4.2 T5 - Horseshoe Spring trench

Stable isotopic analyses were carried out on 53 shell fragments from the T5 section from a total of 35 horizons. The results of these analyses may be found in full in Appendix 3.

The carbon isotope values of the shell fragments lie between -6.51 and -11.43‰, with a mean value of -9.35‰ and a standard deviation of 1.19‰. The oxygen isotope values range from -0.36 to -4.00‰, with a mean value of -2.4‰ and a standard deviation of 0.73‰. These mean values, ranges and standard deviations are similar to those noted in the HVI section.

The isotopic profiles of the T5 section are shown in Figures 5.3a (carbon) and 5.3b (oxygen) in the same manner as the data from HVI. Shell fragments of *Arianta* were present in most of the samples horizons from the T5 section, and unlike the HVI section, analyses were possible on several fragments from the lower silty clays which are below the horizon radiocarbon dated as 9,760BP ± 100 (Q-2721).

The carbon isotope values of the shells in the basal silty-clay horizons appear to be the most enriched in ^{13}C, being from -6 to -8‰ approximately (Figure 5.3a). Between the base of the section (390cm) to around 180cm there appears to be a trend towards more negative $\delta^{13}C$ values. Between 180 and 170cm, there is a marked shift of 4‰ from -12.56 to -8.55‰, and above this the $\delta^{13}C$ values are fairly consistent, being between approximately -8.5 and -10‰.

The oxygen isotope profile (Figure 5.3b) is similar to that found in the HVI section, *i.e.* there are consistent small fluctuations between levels throughout the entire section but overall there are no obvious trends or major shifts.

As was noted for the HVI section, a spread in isotope values (both for carbon and oxygen isotopes) of as much as 2 to 3‰ was measured between fragments from the same horizon. These variations may be seen on the profile figures as the barred lines. The variations within samples from individual horizons appear to be as great, or greater than, the isotopic variations or shifts between horizons.

The stable isotope data from the tufa samples taken from the T5 section are shown in Table 5.2b. The carbon isotope values lie between -8.32 and -9.81‰, mean value = -8.93‰ and standard deviation = 0.49‰. In this section, the carbon isotope values of the tufas are all close to, or only slightly more enriched in ^{13}C than the shell values. In addition there is no shift in the $\delta^{13}C$ values of the tufas throughout the sequence analysed, as was seen in the HVI section and the mean value of the T5 tufas is close to that seen in the 'upper' tufa samples from HVI.

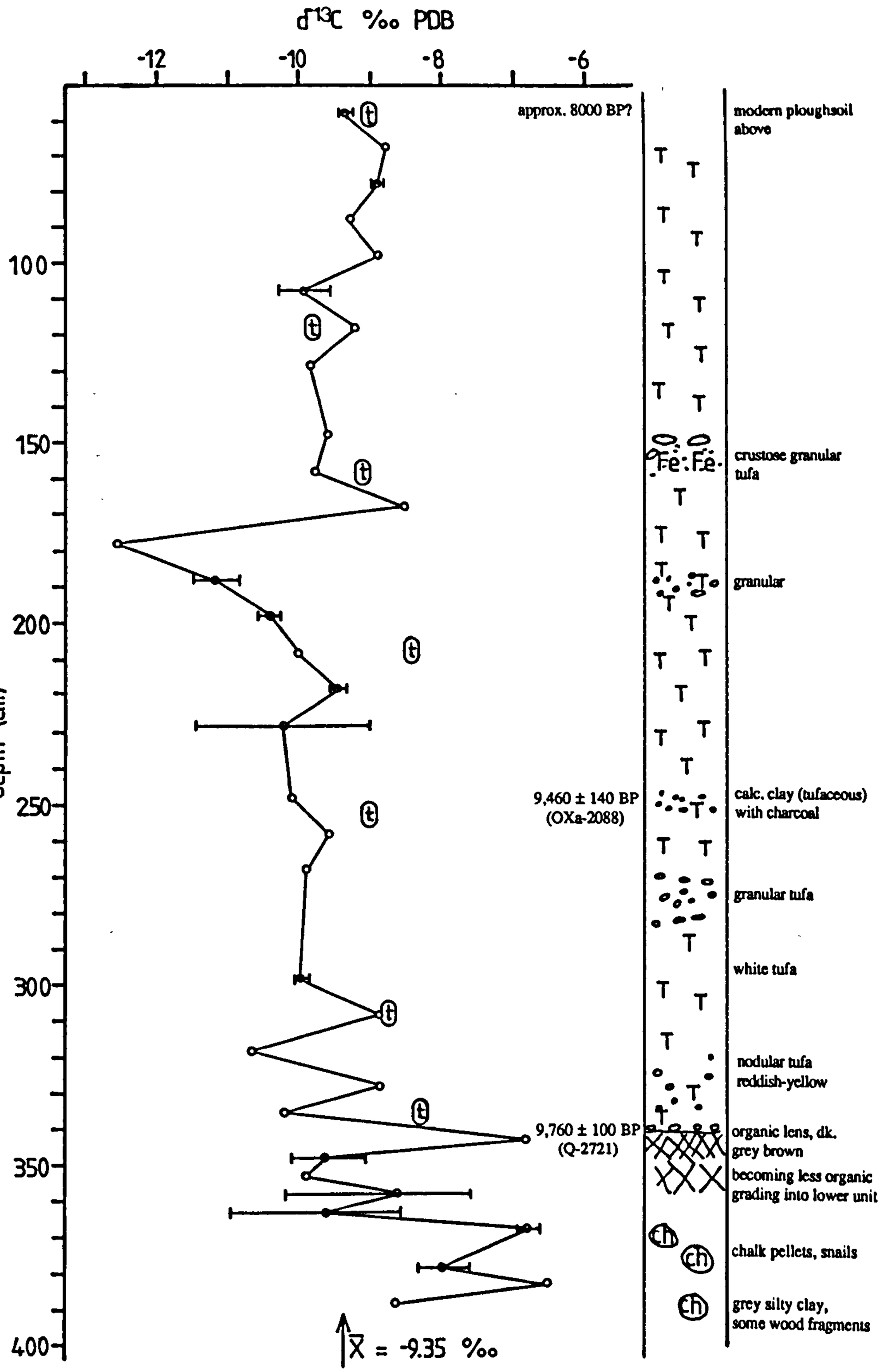


Figure 5.3a T5 trench Holywell Coombe, section and $\delta^{13}C$ profile, from shells of *Arianta arbustorum*. Range bars and mean values indicated. Solid line through mean values.
(t)= $\delta^{13}C$ tufa samples

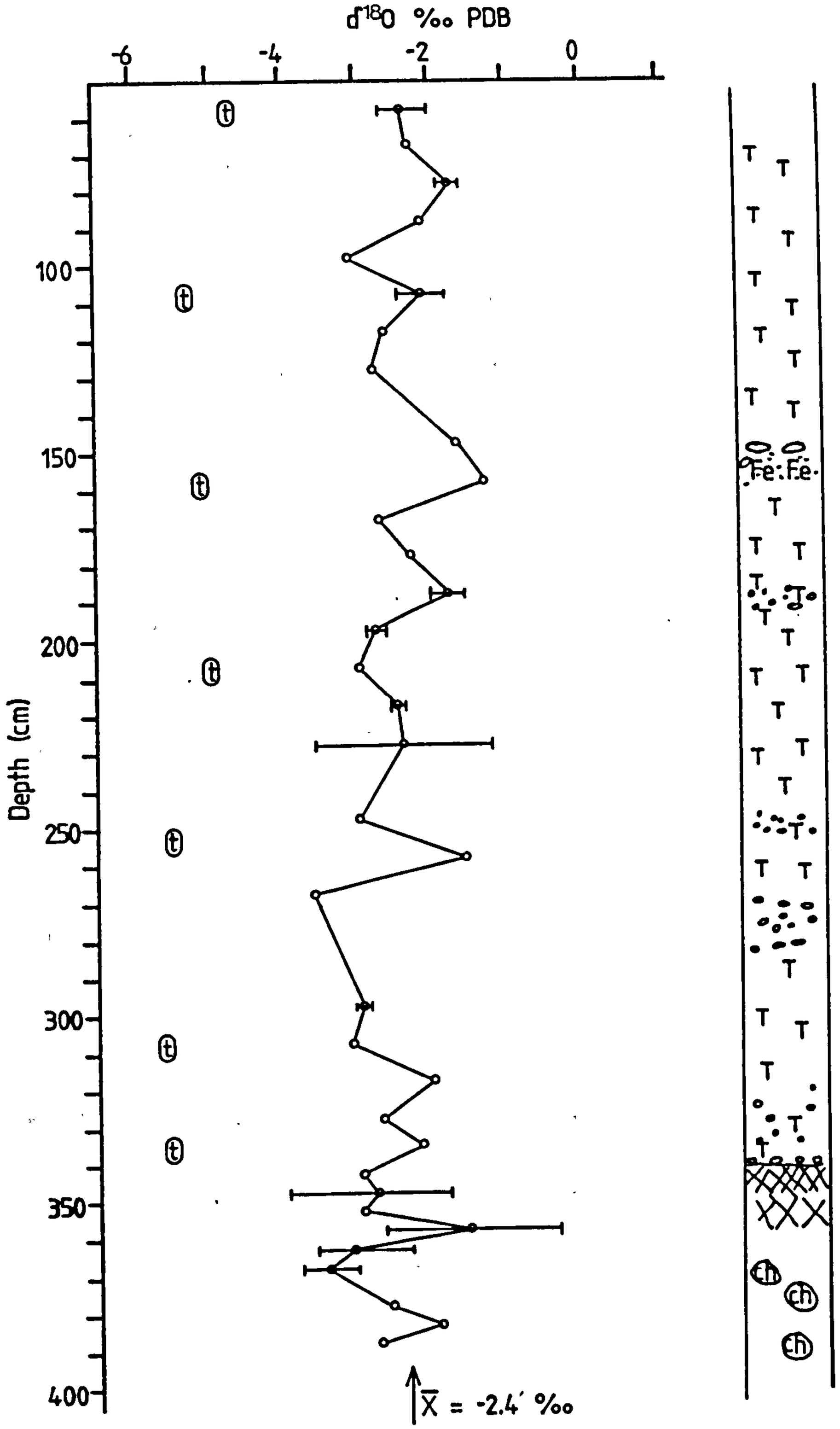


Figure 5.3a T5 trench Holywell Coombe, section and $\delta^{18}O$ profile, from shells of *Arianta arbustorum*. Range bars and mean values indicated. Solid line through mean values.
(t)= $\delta^{18}O$ tufa samples

The oxygen isotope profile of the T5 tufas is, on the other hand, very similar to that from HVI, with values from -4.69 to -5.65‰, mean = -5.27‰ and standard deviation = 0.36‰. The oxygen isotope values are similar throughout the section and as with the HVI samples, are more depleted in ^{18}O (generally by between 2 to 3‰) than shells from the same horizons.

5.5 DISCUSSION

It is apparent from the isotope profiles from the two sections studied that there is as much variation within individual levels as between them. This is the case for both carbon and oxygen isotopes and suggests that the fluctuations between levels are not necessarily significant, but may be explained in terms of a range of isotopic variation within sample levels. Such a degree of isotopic variation was somewhat unexpected. Even the most marked isotopic shift, that in the carbon isotope profile at the T5 trench around 180cm, might be insignificant as the carbon isotope ratios from both the 165-170 and 175-180cm levels are from single shell fragments. The amount of isotopic variation within sample levels (up to approximately 3‰) indicates that the shell fragments in any one layer were secreted over a range of environmental temperatures and conditions. The shells may have been secreted during different seasons, or in different years under a variety climatic and/or hydrological conditions. In addition, some degree of mixing between sedimentary horizons may have occurred to offset potential isotopic fluctuations. However, certain trends and isotopic values of both the shell fragments and the tufa samples may be used to infer information about the changing nature of the Late-glacial to Post-glacial environments in Holywell Coombe.

It had been hoped that a climatic shift marking the climatic amelioration at the end of the Devensian glaciation might be apparent in the oxygen isotope ratios of the shell fragments, with the increase in temperatures marked by a shift towards more negative $\delta^{18}O$ values. However this was not the case in the two sections investigated. This may be because a warming of several degrees Celsius would only result in approximately a one per mil shift in the shell oxygen isotope ratios. Such a shift could be present in the isotope data from these two sections but might be masked because of the amount of isotopic variation within samples levels. It may also be that the shell samples analysed represent time after the amelioration. In the HVI section the radiocarbon date on the humic horizon around 320cm is not yet available. Below this, very few shell samples were analysed although the radiocarbon date from the aurochs scapula (420-430 cm) does suggest that the basal sediments are indeed representative of Late-glacial times. Much of the T5 sequence is composed of tufas deposited in Post-glacial times. Beneath the tufas (older than 9,760 + 100 BP) several fragments of *Arianta* were analysed. However, these fragments did not have oxygen isotope ratios distinguishable from those from shell fragments found in the Post-glacial sediments above.

Difficulties in interpreting the oxygen isotope sequence arise because it is not always possible to find a species that is present right throughout a section, from a cool to a temperate climate and with a changing local environment. *Arianta arbustorum* was one of the most common specimens in the Holywell Coombe sections, but there are still gaps in the complete isotope profile through time. As is the case in many of the studies underway in the Holywell Coombe project, the full sequence of events during the Late-glacial to Post-glacial may only be obtained by combining the findings from various sections within the coombe. It is thought that trench T6, near Horseshoe Spring, has

sediments that represent the Late-glacial Younger Dryas (the last cold period that occurred from around 10,250 to 11,350 BP), and within the T6 sediments are fragments of *Arianta arbustorum* (R. C. Preece., pers. comm., 1990). This section may, therefore, provide a source of older shell material for oxygen isotopic analysis, from which climatic change could be inferred. Time constraints did not allow an investigation of the T6 section during this case study.

In the carbon isotope sequence from T5, there is some indication of a trend towards more depleted shell ^{13}C values from the base of the sequence (below 340-350cm) as compared to those from the tufas above. This trend continues from the base of the tufas (340cm) to around 180cm. Although there are uncertainties as to whether this trend is valid due to the amount of possible isotopic variation, the move towards more negative $\delta^{13}C$ values may be linked to the development of a forested landscape from a more open one. Snails process carbon for shell secretion from three sources, solid calcium carbonate; from carbon dioxide metabolised from their food; and from atmospheric carbon dioxide (Goodfriend and Hood, 1983 and see section 3.3.3). On the chalk downlands, increased proportions of either atmospheric CO_2 or solid $CaCO_3$ would result in shells with higher $\delta^{13}C$ values. Lower $\delta^{13}C$ values would result from the utilisation of a greater proportion of CO_2 metabolised from food stuffs as plant material commonly has $\delta^{13}C$ values around -26 to -27‰. Therefore, more depleted ^{13}C values could result from the snails having access to a richer supply of organic foodstuffs, which could in turn be linked to the increasing forestation of the area during the early Post-glacial period. Unfortunately a similar trend was not seen in the section from the HVI trench. However, although few samples from below the tufas in the HVI section could be analysed, the highest $\delta^{13}C$ value was seen in the shell fragment from the lowest sampled horizon (370cm). A study of modern and fossil shells of *Cepaea nemoralis* from a variety of sites in the United Kingdom was presented by Yates, (1987). Yates reported that shell $\delta^{13}C$ values appeared to reflect the degree of woodland cover at the sites investigated, whereby increasing woodland cover was accompanied by enrichment in ^{13}C. This opposes the results presented here where the trend towards more depleted ^{13}C values in the T5 sequence is thought to be linked to increasing forestation. Yates, however, did not present an explanation for his findings.

The limited number of isotopic analyses carried out on tufa samples may provide some additional information.

In both sections the oxygen isotope values of the tufas are 2-3‰ more depleted in ^{18}O than shell samples from the same horizon. Even accounting for the isotopic differences between the calcite tufas and the aragonite shells (around +0.6‰ for the difference between calcite and aragonite fractionation, Tarutani *et al.*, 1969), the difference between the tufa samples and contemporaneous shells is over 2‰. Tufas are deposited predominantly where groundwaters (such as springs) emerge, in intimate contact with vegetation, moss etc. which acts as a nucleus for precipitation. The oxygen isotope ratios of travertines, including the spongy textured and porous tufas, essentially reflect the isotopic composition of the water from which they precipitate and the relevant physiochemical conditions. Water is the main source of oxygen for travertines although there may be some input from dissolved CO_2 (Turi 1986). However, the deposition of travertines is rarely at equilibrium due to kinetic effects such as the evaporation of water molecules resulting in ^{18}O enrichment is the remaining liquid phase.

The spring water from which the tufa precipitated would have been agitated and thus, the water may have been subject to little evaporation prior to tufa precipitation. Conversely, the local land snail population may have been using water from spring overflow (and possibly additional water from dew

and/or from rainfall), which could have become enriched in ^{18}O by evaporation, prior to being used by any snail. Therefore the difference between the oxygen isotopic composition of the shell and the tufa samples may represent an evaporation effect, causing enrichment of up to 2‰ in the shells of local landsnails. Some, or all, of the 2‰ enrichment in shell $\delta^{18}O$ values over tufa values, might also be the result of a vital effect causing oxygen isotope enrichment in snail body fluids (as suggested in the growth experiments reported in Chapters 2 and 3). However there is no direct evidence to support this.

The carbon isotope values of the Holywell Coombe tufas are generally similar to contemporaneous shells, indicating that the tufas may have been an important source of inorganic carbon for local landsnails. However the two lowest tufa samples from HVI are both 3-4‰ more enriched in ^{13}C than the rest. As with terrestrial mollusc shells, the main sources of carbon going into tufa-depositing solutions are organic CO_2, atmospheric CO_2, and solid carbonates such as limestones (Turi, 1986). The chalk bedrock of the vicinity has a carbon isotope ratio of around +1‰ PBD (calcite) (P. W. Ditchfield., pers. comm., 1990). It may be that the two lowest tufa samples from HVI have incorporated a larger proportion of carbon from the chalk bedrock. Both these tufa samples were deposited before the main tufa sequence in the coombe - the upper one marking the boundary at the base of the main tufa.

5.6 SUMMARY

The interpretations of the stable isotope profiles of the two sections studied at Holywell Coombe (HVI and T5) have been limited. It is difficult to infer with much certainty climatic or environmental changes from the carbon and oxygen isotope records. This has been mainly due to the degree of isotopic variation found between shell samples taken from the same horizons. In many cases the variation within horizons was as great or even greater than the isotopic differences between adjacent horizons. A possible trend towards more depleted $\delta^{13}C$ values through time has been linked tentatively to the increasing forestation of the coombe during early Post-glacial times.

It is thought that the consistent difference between the oxygen isotope values of the tufas and shells of contemporary *Arianta arbustorum* may reflect an evaporation effect of around +2‰ acting upon the spring waters prior to it being utilised by the snails. The higher $\delta^{13}C$ values of two tufa samples from HVI are thought to have resulted from the incorporation of a greater proportion of carbon from the indigenous chalk bedrock in these samples, as compared to the other samples investigated.

Of the other sections and trenches excavated in Holywell Coombe, it is thought that a study of shells of *Arianta arbustorum* from trench T6 might provide additional and more precise information on the climatic changes thought to have occurred at the end of the Devensian glaciation. T6 contains fragments of *Arianta* that are thought to be older than those from either HVI or T5 and further work on shell fragments from T6 may help build up a better picture of climatic / environmental changes that have taken place in in the coombe.

CHAPTER 6

SUMMARY AND CONCLUSIONS

6.1 INTRODUCTION

This chapter aims to synthesise the findings of the various aspects of the thesis (experiments and case studies), and to assess the extent to which the initial aims of the project have been met.

Each of the laboratory growth experiments is summarised briefly, and then the overall findings of the experimental work will be discussed and conclusions presented. In a similar way the data from the two case studies (Jamaica and Holywell Coombe), will be summarised and discussed. Following this, the overall conclusions of the project will be presented, with recommendations for possible future research.

6.2 SUMMARY OF THE FINDINGS OF THE GROWTH EXPERIMENTS

6.2.1 Laboratory techniques

A technique for the cleaning and purification of mollusc shells has been developed, encompassing a two stage bleaching process, followed by the vacuum roasting, of individual samples. This technique allowed the removal of organic contaminants from exterior and intercrystalline portions of mollusc shells. Stable isotopic analysis of test samples of limestones showed that providing samples were rinsed thoroughly after the bleaching process (to remove all bleach residues), and that the samples were not vacuum roasted above approximately 380°C, then the isotopic composition of the carbonate would not be affected.

6.2.2 Experiment 1 and Experiment 1B

The aim of this experiment was to investigate the effect of environmental temperature upon the oxygen and carbon stable isotope composition of various populations of snails, whilst other environmental factors were maintained as constant as possible. The thermal regimes investigated were from 10.2 to 24°C. The second aim of this experiment was to see how the oxygen isotopic composition of the secreted shells related to the oxygen isotopic composition of the environmental water available to the snails.

The predicted trend of oxygen isotope depletion with increasing temperature was only evident at temperatures below approximately 15°C. Above this temperature, a shift towards more enriched values has been thought to indicate increasing evaporation effects and possibly metabolic effects. A similar trend was found for all species.

The oxygen isotopic compositions of the shells were not at isotopic equilibrium with the waters given to the snails, with the shells being more enriched than equilibrium values by at least 4.5‰, up to a maximum of almost 13.8‰. The difference was greatest at temperatures above 15°C, suggesting greater evaporation and/or metabolic effects at higher environmental temperatures.

For carbon isotopes, a trend towards more depleted $\delta^{13}C$ values with increasing temperatures was noted for all species, which has been linked with the increased use of metabolic, as opposed to atmospheric, carbon dioxide, coupled with increased snail activity and shell growth at higher environmental temperatures.

The isotopic trends were fairly consistent between the five species of snail investigated, although the two Mediterranean species *Rumina decollata* and *Ferussacia folliculus* had $\delta^{18}O$ values distinct from the temperate species (*Helix aspersa*, *Cepaea nemoralis* and *Cernuella virgata*); and the carbon isotope values of *Cernuella virgata* were somewhat distinct from the other species. Shell growth was greater at higher temperatures, although actual growth rates could not be quantified as initial shell weights were unknown and were not constant from one thermal regime to the next.

Experiment 1B aimed to trace the changes in shell isotope composition of individual shells that resulted from altering the environmental temperature, whilst other factors were maintained constant.

The isotopic trends within individual shells did not follow the patterns expected from the results of experiment 1 (which was run simultaneously). The differences have been interpreted as resulting from 'memory effects' of the previous thermal regime, and/or from the effects of internal thickening of shells once a mature shell size had been attained. This suggested that in natural systems, shell isotope values may reflect average prevailing conditions over their whole period of growth rather than daily or weekly fluctuations.

6.2.3 Experiment 2

The aim of this experiment was to examine the effect upon the isotopic composition of shells as a consequence of the introduction of diurnal variation in the temperatures and lighting experienced by the snails. This was in an attempt to minimise the artificiality of the experimental conditions. A further aim of this experiment was to compare the isotopic compositions of shells secreted under variable conditions with those from shells maintained in constant conditions. This was achieved by using three populations of snails. The first was kept under constant conditions (22.3°C and permanent light) as was the third (16.5°C and permanent darkness) whilst the second was moved between these two environments every twelve hours.

The oxygen isotopic compositions of shells from the three populations were similar, although there was a high degree of isotopic variation within each population. This result was unexpected, but suggested that variation between individual snails was greater than that caused by environmental factors. Comparison of the findings of this experiment with those of experiment 1, allowed separation of the isotopic effects resulting from the presence of light, from those resulting from light together with the environmental temperature. This analysis, and the comparison of actual shell $\delta^{18}O$ values with 'expected' equilibrium values, suggested that the isotope effects were greater in experiment 2 than in experiment 1, even though the environmental conditions were thought to be more 'natural'. The

results of this experiment also suggested that the oxygen isotopic composition of the shells may have been influenced by the oxygen isotopic composition of the inorganic carbonate in the snail's diet, which during this experiment was very depleted (-28.4‰).

No consistent differences in the carbon isotopic composition of shells from the three populations were found. Shell $\delta^{13}C$ values, from experiment 2 snails, were much more depleted than those from experiment 1, which reflected the different carbon isotopic composition of the inorganic carbonate in the snail's diets from the two experiments (for experiment 1 = -13.09‰, but for experiment 2 = -43.35‰.

Shell growth was influenced positively by the presence of permanent light and higher ambient temperatures. However, no statistically significant relationship was found between the isotopic composition of individual shells and their estimated rates of growth.

6.2.4 Experiment 3

This experiment was designed to investigate further the effects of the presence or absence of light upon the patterns of shell growth and shell isotopic composition, where the light reaching the snails was controlled by enclosing some of the boxes of snails within transparent or opaque polythene bags. Seven populations were set up; over three thermal regimes. Two other populations of snails were maintained without polythene bags, as in the previous experiments, acting as controls. Within the bags, relative humidities were high and constant, and evaporation effects were greatly reduced. Therefore an additional aim of this experiment was to attempt to separate and quantify the isotope effects resulting from evaporation and/or metabolic influences.

As noted in the previous experiments, shell growth was favoured at higher temperatures. Over the experimental period, snails from the 'bagged' and control populations secreted shells of similar size, although the final shell weights of the 'bagged' shells were comparatively less, which suggested that such experimental conditions were not ideal for the snails.

The $\delta^{18}O$ values of the shells secreted by the snails became, as expected, lower (more depleted in ^{18}O) with increasing environmental temperatures, and the range of values from the three mean environmental temperatures (14.5, 18.4 and 22.3°C) was distinct. The oxygen isotope data from the 'bagged' populations suggested that the snails found conditions in the opaque polythene bags less favourable than those in the clear bags, especially at lower ambient temperatures. A large difference was found between the oxygen isotope ratios of the 'bagged' and control populations, with the control populations being 5 to 8‰ more enriched in ^{18}O. This has been related to a greatly reduced evaporation effect within the bags, as compared with the control populations under similar conditions. The differences in shell oxygen isotope ratios of snails grown under comparable conditions from all three experiments have been thought to reflect primary isotopic differences in the water and possibly the food, and to the exact experimental conditions in operation, such as temperature, light and experiment location.

For carbon isotopes, the $\delta^{13}C$ values of shells were slightly lower at higher environmental temperatures (as had been the case in experiment 1), indicating a greater use of metabolic, as opposed to atmospheric carbon dioxide linked with increased shell growth at higher temperatures. Within each of the three temperature regimes, each species of snail showed characteristic carbon isotope ratios with no

overlap between species, suggesting that each species was using a different balance of organic : inorganic carbon sources in the process of shell secretion. No systematic carbon isotope effects, relating to the housing of the snails in the opaque or transparent bags, were found. However, the 'bagged' populations were seen to be 0.4 to 2‰ more depleted in ^{13}C than the control populations, indicating that the 'bagged' snails were using more metabolic carbon dioxide than the control population snails, although in this case the 'bagged' snails did not secrete larger or thicker shells as might have been expected. The variations in shell carbon isotope ratios of snails grown under comparable conditions from all three experiments have been related to primary differences in the carbon isotope ratios of the inorganic carbon component of the diet. Comparison of the carbon isotope data from the three experiments showed that for *Helix aspersa* grown at 22.3°C, around 22% of the isotopic signature of the shell could be attributed to inorganic carbon in the diet. For the Mediterranean species *Rumina decollata* the value was 26%.

Calculated values of $\delta^{18}O$ in aragonite at oxygen isotopic equilibrium with the environmental water given to the snails at a given temperature, were compared with the actual measured aragonite shell $\delta^{18}O$ values. For the 'bagged' populations, with effectively zero evaporation, the difference between calculated and measured values ($\Delta 1$) was thought to reflect a vital effect relating to the snail's metabolism. This vital effect has been estimated as causing a +2 to +3‰ enrichment in shell isotope values. For the control populations subject to much evaporation, the difference ($\Delta 2$) was much greater, encompassing evaporation plus vital effects. The oxygen isotope enrichment recorded in the oxygen isotope ratios of the control populations as a result of evaporation effects alone, calculated as $\Delta 2 - \Delta 1$, has been estimated as +4.8 to +8.7‰. In view of these findings, the results of experiments 1 and 2 were reconsidered. Under the prevailing experimental conditions, and at a temperature of around 15.6°C, the mean evaporation effect ($\Delta 2 - \Delta 1$) has been estimated as +4.6‰. At 22.3°C evaporation effects have been estimated as up to +8.9‰ for the helicid species, and around +5.6‰ for the Mediterranean species. Metabolic effects have been estimated as causing a fairly constant oxygen isotope enrichment of around +2.75‰, although a slightly lower figure (around +2‰) was estimated for the Mediterranean species at a mean temperature of 15.6°C

6.3 SYNTHESIS OF THE FINDINGS OF THE GROWTH EXPERIMENTS.

It would appear that if other factors - food, water, living conditions - remain constant, then the oxygen isotopic composition of the water (environmental water, and body water) from which the shells of terrestrial molluscs are secreted, should reflect the temperature of their environment. However the design of the growth experiments made it difficult to assess accurately the rate of oxygen isotope depletion with increasing temperature, as true temperature effects were masked by variable evaporation and possibly metabolic effects. Even so, from the results of experiment 1 at temperatures below around 15°C, and from the isotopic compositions of the 'bagged' snail populations of experiment 3, the magnitude of the depletion would seem to be close to that found in aragonite shells of marine molluscs by Grossman and Ku (1986), around -0.2‰ per °C. However, outside the laboratory, although a shift in environmental temperature of several degrees Celsius should be reflected in the oxygen isotope ratios of land snail shells, it is probable that such a shift in temperatures would be accompanied by other major alterations to the isotopic composition of the local environmental waters, which may mask temperature effects. These additional influences, may include changes in the oxygen isotopic composition of the local precipitation with the movement of new air masses and sources of water vapour; or the establishment of new microclimates with the expansion or contraction of vegetation zones. Therefore the waters will be influenced by a compositional effect in addition to the environmental temperature.

The experiments also showed that carbon isotopes in the shells of these snails may be related to the temperatures, if the isotopic composition of the various carbon sources remains constant. A depletion in $\delta^{13}C$ values with increasing environmental temperature, of a similar magnitude to that seen for oxygen isotopes, has been related to an increased use of metabolic carbon dioxide, as opposed to atmospheric carbon dioxide, at higher temperatures. This relationship was proposed by Goodfriend and Magaritz (1987), and is supported by the findings of the experimental work, as in all three experiments more shell material was secreted and rates of growth were greater at higher environmental temperatures. This finding suggests that the carbon isotope ratios of land snail shells may also be useful as an indicator of relative temperature changes, provided that the isotopic composition of the source materials does not alter.

The design of the experiments 1 and 2, was supposed to limit the evaporation effect upon the environmental waters used by the snails, so that the isotopic composition of the waters given to the snails could be related to the isotopic composition of the resultant shells secreted under the various temperature regimes, and any consistent differences could be quantified. In these first two experiments, surprisingly large differences were found between the measured oxygen isotopic compositions of the shells and those expected from the isotopic composition of the waters given to the snails at various temperatures. These differences, with shells being more enriched that expected (by up to 13.8‰), were thought to represent evaporation effects and possibly metabolic effects. However the results of experiment 3, showed that the design of the two previous experiments had been flawed in that evaporation effects were playing a major role in influencing the isotopic composition of the waters available to the snails. Although the conditions under which the snails were housed in experiment 3 may not have been ideal, from the snails' point of view (*i.e.*, boxes inside polythene bags), this final experiment quantified approximately the evaporation effect and showed that the shells had been secreted

within 2-3‰ of that expected from the initial isotopic composition of the waters the snails were given, *i.e.* the shells were only 2-3‰ more enriched than expected values.

This 2-3‰ difference has been explained in terms of a vital effect, relating to the processing of oxygen in a snail's body. Lecolle (1985), reported a consistent vital effect of around +5‰, between measured oxygen isotope ratios of western European land snails and some measured, and some inferred, oxygen isotopic compositions of local precipitations. Goodfriend and Magaritz (1987), investigating the isotopic composition of land snails from the Southern coastal plain of Israel, found that shell $\delta^{18}O$ values were enriched by 2 to 8‰ relative to isotopic equilibrium with environmental waters (local rainwater). The authors suggested that this enrichment resulted from metabolic effects enriching the oxygen isotopic composition of the snails' body waters. The degree of metabolic enrichment was related to the activity levels of the snails, with lower activity producing increased enrichment. The authors also suggested that some of the enrichment might be accounted for by evaporation effects. Although the experimental work reported in this thesis does not support such a large isotope effect as those suggested by Lecolle (1985) and Goodfriend and Magaritz (1987), it does confirm the findings of those authors, that the isotopic compositions of terrestrial mollusc shells are enriched relative to isotopic equilibrium with environmental waters. In this research the enrichment has been estimated as between 2-3‰.

The question remains as to where and how this fractionation takes place. Goodfriend *et al.*, (1989), analysed the isotopic composition of the body waters of some Israeli landsnails (oxygen and deuterium), in relation to the isotopic composition of local precipitations and the shell material secreted by the snails. These authors reported that, on average, body waters were enriched in ^{18}O by 2‰ relative to rainwater, but also that shell carbonate was enriched in ^{18}O by 1-2‰ relative to equilibrium with body water. Goodfriend *et al.* (1989), suggested that the fractionation between body fluid and shell carbonate could be explained if the isotopic composition of the body water is heterogeneous and that there may be local enrichment in the region of shell deposition, *i.e.*, in the extrapallial fluid. This suggests that the fractionations found between environmental water and shell carbonate in this study, and in those by Lecolle (1985) and Goodfriend and Magaritz (1987), may be a two stage phenomenon. The body fluid of a snail is composed of blood fluid (haemolymph) and extrapallial fluid. Metabolic effects may influence the isotopic composition of the haemolymph, and this could vary according to the activity levels and metabolic rate of the snails as suggested by Goodfriend and Magaritz (1987). A further fractionation may then take place in the extrapallial fluid at the site of shell secretion. This could occur if the extrapallial fluid is considered as an open system with material being replaced by, and exchanged with, material from the blood fluid and the external medium, through the process of respiration. Further experimentation and field studies would be required to assess fully the extent of the total vital effect (see section 6.7). However, once the isotope effects were accurately quantified, the oxygen isotopic composition of terrestrial mollusc shells should give a good estimation of the oxygen isotopic composition of precipitation.

In the experimental work, evaporation effects played a major role in determining the resultant oxygen isotopic composition of the snails' shells. In natural systems, shells become active only when moisture becomes available - after regular or seasonal rainfall, or with the formation of dew. In addition, land snails are, by necessity, very efficient at preserving internal moisture once any water source has been exhausted. Therefore, in field situations, evaporation effects should be limited to those acting upon environmental water prior to uptake by any snail, and these effects may not be very great,

particularly if ambient humidities remain high. This suggests that the oxygen isotope ratios of the shells of land snails could be used to infer relative humidities in the snails' microenvironment, as proposed by Yapp (1979). Once the magnitude of any vital effects had been quantified, then the difference between the 'expected' oxygen isotopic composition of the environmental water (if there was no evaporation), and the isotopic composition of environmental water inferred from the isotopic composition of the shells, should be a function of the local relative humidity

The design of the growth experiments allowed various factors influencing the stable isotopic composition of shells to be considered. It was thought that the artificial conditions under which the snails were maintained may have induced some of the isotope effects noted above. However, the introduction of daily cycles of temperature and light, in an attempt to reduce any environmental stress, did not appear to lessen the isotopic effects, even in experiment 3 where evaporation effects were minimised. This suggests that the isotope effects were not the result of environmental stress, but are inherent in the secretion of landsnail shells. However, where snails were placed in overcrowded situations, anomalous shell isotope values were recorded. Thus in any future experiments, overcrowding should be avoided. Some of the interpretations of the findings of the growth experiments could have been improved if a system of marking the leading shell edge (without causing abnormal reactions in the experimental snails) had been introduced

6.4 SUMMARY OF THE FINDINGS OF THE CASE STUDIES

6.4.1 Field studies in Jamaica

The aim of the Jamaican study was to examine the stable isotopic composition of shells of selected 'target species' of endemic Jamaican landsnails, in relation to their distribution and to local environmental variables, particularly along coast to inland transects. A secondary aim was to collect and analyse samples of local environmental waters to investigate whether the Jamaican snails were secreting their shells at or near isotopic equilibrium with such waters.

On a local and regional level, the carbon isotopes in landsnail shells were found to be much more variable than oxygen isotopes when comparing various populations of snails. The opposite was found in the investigation of individual shells where oxygen isotopes were much more variable than carbon. This indicated that although the sources of carbon are more variable isotopically, individual snails use the same basic source throughout their lives. Both the oxygen and carbon isotope ratios were very variable (within species) locally and regionally, and generally there was as much isotopic variation in shells from within each region or area, as that between regions. This suggested that several isotopic analyses would be required to obtain a value typical of that species, but that once the 'typical value' had been assessed, it should apply to that particular species throughout the island.

A good positive correlation was found between shell size of the species *Pleurodonte sublucerna* and altitude in the region of the Red Hills. However, for the same shells, an expected trend towards more depleted $\delta^{18}O$ values with increased altitude was not found.

The limited data on the oxygen isotopic composition of Jamaican environmental waters, and uncertainties as to the ambient temperatures in the snails' microenvironments, made it difficult to assess accurately whether shells had been secreted in isotopic equilibrium with the waters available to them. However, the data have suggested that secretion was close to equilibrium with the waters and thus the oxygen isotopic composition of the shells investigated reflects primarily the oxygen isotopic composition of the water available to the snails (predominantly rainfall), with local temperature effects, degree of wetness of the environment and additional metabolic effects being of limited influence. However, a small, and possibly consistent, vital effect of up to 2‰ might still be inferred. The data from the landsnail shells and the environmental waters were used to test the relationship presented by Lecolle (1985) which equated the oxygen isotopic composition of precipitation and landsnail shell aragonite. The relationship, which had been established for European snails, was not in accord with the data from the tropical maritime climate of Jamaica.

The carbon isotopic compositions of the Jamaican land snail shells have been linked to the type, and relative proportions, of organic to inorganic carbon available to, and utilised by, each snail species in relation to their ecologies and mode of life. From this it has been suggested that it may be possible to infer information on the habits of other Jamaican snails, knowing a 'typical' $\delta^{13}C$ values for a particular species.

6.4.2 ?Late-glacial and Post-glacial fossil shells from Holywell Coombe, Folkestone, Kent.

The aim of this study was to investigate the potential use of stable isotopes in the shells of fossil landsnail in revealing climatic and environmental changes, and to see if the isotopic evidence could be combined with that from other palaeoenvironmental investigations. Shells of *Arianta arbustorum* from two sections through Late-glacial to Post-glacial tufa and slope-wash deposits, found in Holywell Coombe near Folkestone in Kent, were investigated. Some samples of the tufas present in the sections were also analysed for their stable isotopic compositions.

Comparison of the findings of this research with other aspects of the multi-disciplinary study underway on the deposits from Holywell Coombe, has not yet taken place. Also, some radiocarbon dates from the sections are still outstanding. Therefore the interpretation of the isotope data from the fossil shells and the tufas from the two sections has been limited.

A high degree of isotopic variation within individual sample horizons was found. This variation was generally as great as any differences between sample horizons, and thus it has been difficult to infer any real trends, or environmental changes, from the stable isotope data. A possible trend towards more depleted $\delta^{13}C$ values through time has been linked tentatively, with increasing forestation of the area during early Post-glacial times.

From the limited number of tufa samples, a consistent difference between tufa and shell $\delta^{18}O$ values from both sections (with the shells being approximately 2‰ more enriched in ^{18}O) has been linked with an evaporation effect acting upon the spring waters prior to them being used by the snails. Carbon isotopic compositions of the tufas were found to be close to those in the snail shells, which has suggested that the tufas were an important source of inorganic carbon to the local landsnails. Two tufa samples with $\delta^{13}C$ values 4-5‰ higher than the shells from the same horizons, have been assumed to contain a greater proportion of carbon from the chalk bedrock than the rest of the tufa samples.

Of the other trenches and sections excavated at Holywell Coombe, it has been suggested that shells from trench T6, may provide additional and more precise information on climatic and environmental changes in the Coombe, particularly as it is thought that the sediments in this section go further back in time to the Late-glacial, from which time climatic changes may have been more extreme.

6.5 SYNTHESIS OF THE FINDINGS OF THE CASE STUDIES.

The variation in oxygen (and carbon) isotopes in shells of Jamaican landsnails was much greater than that reported by Lecolle (1985) in his study of landsnails from Western Europe, in which the variations in oxygen isotopes between different shells, or from one species to another in the same area, did not exceed 1‰. This suggests that over small geographic areas in Jamaica there is much greater variation in the snails' environmental water than over comparative areas in mainland Europe. Additional evidence for this came from the lack of a relationship between shell isotopes and elevation along coast to inland transects, which was thought to reflect the fact that the environmental waters used by the snails (considered to be mainly precipitation) came from more than one source area.

Jamaican shells appeared to be secreted close to isotopic equilibrium with their environmental waters, although a vital effect of up to 2‰ could be masked in the data because of uncertainties in the estimation of temperatures and the isotopic composition of environmental waters, at specific sites where landsnails were collected. More isotopic data, from specific sites where temperatures could be accurately measured, would be required to test fully the findings of the laboratory experiments.

Carbon isotopes in the shells of land snails have been linked to the nature of dietary constituents, of a particular species in a particular area, in relation to the isotopic composition of the dominant vegetation type (*i.e.* the photosynthetic pathway) by Goodfriend and Magaritz (1987). In the Jamaican study, this idea has been taken further, with the carbon isotope ratios of shells being linked tentatively with the mode of life and ecologies of different species. If this relationship can be tested further, and proved, it may provide useful information, given the lack of documented evidence on the distributions and ecologies of the diverse and highly endemic Jamaican landsnail fauna. Over time, fluctuations in the $\delta^{13}C$ values of a particular species might then be related to environmental change.

The interpretation of the stable isotope data from the Post-glacial sections at Holywell Coombe, has been restricted as isotope trends could not be distinguished due to the amount of isotopic variation within and between sample horizons. The analysis has been further limited because the findings have not yet been considered along with those of other aspects of the multi-disciplinary research project. However, comparison of the oxygen isotopic composition of shell and tufa samples has suggested an evaporative isotope effect of approximately 2‰ resulting in ^{18}O enrichment in the snail shells. It may be possible to infer palaeotemperatures, once the findings of the other aspects of the Holywell Coombe Project have been assessed. From this it may be possible to calculate equilibrium values of shell aragonite, which can than be compared to tufa and actual shell $\delta^{18}O$ values.

To obtain shell material from one species throughout the majority of the sections, the species would have to be one with wide environmental tolerences, such as *Arianta arbustorum*. Because of the environmental tolerance of this species, it is possible that the isotope signature may have not be greatly affected by environmental changes, *i.e.* any isotope effects may have been buffered.

6.6 OVERALL CONCLUSIONS

1) The oxygen isotopic composition of landsnail shell should reflect the isotopic composition of precipitation, although a ?two stage biological fractionation within the snail and external evaporation effects appear to modify the initial isotopic composition of the water. Temperature increases result in oxygen isotope depletion of around -0.2‰ per °C, but, in natural systems, this effect may be masked by compositional effects on the environmental water. Biological fractionation causes 2-3‰ enrichment in shell $\delta^{18}O$ over equilibrium values. That part of the fractionation that relates to the metabolism of the snails may not be constant, but may vary according to the effects of the local temperature and environment on the metabolism of the snails. External evaporation effects have not been quantified accurately, although they are not considered to cause large isotope effects since snails are only active once water is available, and they are efficient at retaining moisture. The Holywell Coombe case study suggested that evaporation effects may cause isotopic enrichment in the environmental water of approximately 2‰. Over time, fluctuations in temperature may be masked by changes in the actual isotopic composition of precipitation, although information on the past isotopic compositions of precipitation would be useful in palaeoenvironmental reconstruction.

2) Carbon isotopes in landsnail shells reflect the initial isotopic composition of the various source materials, and the relative proportions of each that are available to, and are used by, the snails. This in turn may be dictated by the ecologies and modes of life of individual species. Through time, changes in carbon isotope ratios should give information regarding environmental change, and may reflect relative changes in temperatures. Under optimum conditions, snails will produce larger proportions of metabolic carbon dioxide, as opposed to using atmospheric carbon dioxide. This should result in shells with depleted $\delta^{13}C$ values. Under less ideal conditions, the increased use of atmospheric carbon dioxide will result in shells with relatively more enriched $\delta^{13}C$ values.

3) The laboratory experiments indicated that landsnail aragonite shell material should be a good potential source of palaeoclimatic and palaeoenvironmental information. The aim of the laboratory experiments was to allow the control of particular environmental variables, in order to isolate and quantify specific isotopic effects. Unfortunately, it was not until the third growth experiment, that some of the isotopic effects could be properly identified, and time constraints did not allow further investigation of the proposed fractionation effects. Although laboratory investigations have certain advantages over field surveys, it is often difficult to transpose the results of experimental work to complex and interactive natural field systems. In the limited work carried out in Jamaica and on the fossil shell material from Holywell Coombe, this was often the case. An ideal situation might be one where palaeoenvironmental interpretations could be based on more that just isotopic evidence from mollusc shells, as had been the aim in the study of the deposits found at Holywell Coombe.

6.7 FUTURE RESEARCH

6.7.1 Laboratory experiments

1) The experimental conditions developed for use in experiment 3, could be repeated with several variants.

(a) A better estimate of the temperature dependence of oxygen isotope fractionation with increasing temperature could be obtained with control boxes of snails to monitor evaporation effects.

(b) Further experiments could be carried out where environmental conditions were constant, including temperature, but the oxygen isotopic composition of the environmental water given to the snails could be varied. The effect of this could then be measured by analysis of the resultant secreted shell material. This should give a good estimate of the total amount of biological fractionation from external water to shell. The results of this investigation could then be compared with those where the water composition was constant but the temperature was varied (as above).

(c) With careful monitoring of shell growth, it may be possible to relate particular phases of growth to the introduction of an environmental water and food of known isotopic composition. This could be carried out by choosing a species which secretes "frills", which are not then added to during subsequent growth.

(d) The experiments gave some indication that the processing of oxygen isotopes was not the same for the Mediterranean species as compared to the other species from temperate climates. Other species, from various climates, could be used in similar growth experiments, to look more closely for possible relationships, provided that viable laboratory base populations could be maintained to supply such experiments. As *Helix aspersa* extends into the Mediterranean, it may be useful to compare the isotopic compositions of shells of this species from various climatic regimes.

2) More work could be carried out using the techniques presented in Goodfriend *et al.*, (1989), where the body water and shell material of snails from field populations has been measured. Water extraction (using the garlic press method, or, for smaller snails with lower water contents and/or more squeamish workers, the distillation method from specimens frozen in liquid nitrogen) could be carried out on laboratory specimens being maintained under various environmental conditions, or on field populations of snails. If it is possible to separate haemolymph from fluids which have passed through the mantle, it would be useful to analyse the components separately. Snails can be induced to secrete frothy mucus if they are disturbed, as happened when attempts were made to mark the leading shell edges of base population specimens of *Helix aspersa*. Such secretions might be collectable and they represent non-blood fluid. Faecal droppings were collected during the growth experiments, and although time constraints did not allow close examination of their chemical and isotopic compositions, they may provide an additional source of information on the carbon and oxygen cycle within a snail.

6.7.2 Field investigations

1) With reference to field studies in Jamaica, more work will be required to test the proposed relationship between carbon isotopes in shells and the ecology and mode of life of individual species. Any additional work on distributions of species, in relation to the isotopic composition of their shells and environmental waters, may provide further insights into possible different mechanisms operating in various climatic zones (*i.e.* findings from Jamaica could be compared with those from Europe and the Middle East).

Some work has been carried out on fossil snail faunas discovered in cave deposits in the limestone areas of Jamaica (Goodfriend 1986b, Goodfriend and Mitterer, 1988), where amino-acid dating techniques have been used on the fossil shell material, and faunas have been compared to those currently inhabiting the area. This author discovered a similar cave-infill deposit in the Red Hills which showed an abundant, diverse and well preserved landsnail fauna, in addition to bone material of rodents, ?bats and ?lizards. It may be useful to investigate the stable isotopic compositions of some of these fossil shells, in comparison with modern specimens from the same localities, to provide additional evidence for climatic and/or environmental change.

2) With reference to the Holywell Coombe case study, it has already been suggested that further analysis of older sediments, and specimens of *Arianta arbustorum*, from the T6 section be carried out. If shell material becomes available it may be possible to undertake isotopic investigations on other land snail species.

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APPENDICES

APPENDIX 1 : Growth Experiments - Results

APPENDIX 2 : Jamaican Case Study - Results

APPENDIX 3 : Holywell Coombe Case Study - Results

APPENDIX 1

Growth experiments - Results

(A) Results of all isotopic analyses from the six phases of experiment 1. Also included are the results of the measurements of shell dimensions and weights, taken from replicate populations that were not used for isotopic analysis.

(B) Results of all isotopic analyses from the nine populations used in experiment 3. Also included are results of the measurement of shell dimensions and weights taken on the shells prior to the shells being processed for isotopic analysis.

n.b. Results of isotopic analyses forming experiment 1B are shown in full in Table 3.8 (section 3.5.2) Results of isotopic analyses, measurements of shell dimensions and weights for experiment 3 are shown in full in Tables 3.9, 3.10 and 3.11 (section 3.6.2).

C1 Regime - mean temperature = 10.2°C
- mean $\delta^{18}O$ SMOW water = -9.7‰

Species	$\delta^{13}C$‰ PDB	$\delta^{18}O$‰ PDB
Helix aspersa	-12.23	-2.28
	-12.12	-1.67
	-12.43	-2.40
	-12.33	-2.03
	-12.20	-2.06
	-12.04	-1.65
	-12.08	-2.18
	-12.33	-1.40
	-12.54	-3.24
Mean	-12.26	-2.10
Std. Dev.	0.17	0.54
Cepaea nemoralis	-12.44	-1.94
	-12.61	-0.80
	-12.78	-0.22
	-12.70	-0.78
	-12.44	-2.41
	-12.62	-1.55
	-12.68	-2.54
Mean	-12.61	-1.46
Std. Dev.	0.13	0.89
Cernuella virgata	-10.94	-2.41
	-10.68	-2.85
	-10.74	-3.61
	-11.16	-2.87
	-10.96	-2.54
	-10.90	-2.30
Mean	-10.90	-2.76
Std. Dev.	0.17	0.47
Rumina decollata	-12.66	-0.48
	-12.09	-0.91
Mean	-12.38	-0.70
Std. Dev.	0.40	0.30

C2 Regime - mean temperature = 13.1°C
- mean $\delta^{18}O$ SMOW water = -9.8‰

Species	$\delta^{13}C$‰ PDB	$\delta^{18}O$‰ PDB
Helix aspersa	-11.82	-2.98
	-11.82	-2.63
	-11.72	-2.48
	-11.80	-2.89
Mean	-11.81	-2.75
Std. Dev.	0.07	0.20
Cepaea nemoralis	-12.07	-2.92
	-12.13	-2.77
	-12.32	-3.54
	-12.26	-3.02
Mean	-12.20	-3.06
Std. Dev.	0.10	0.29
Cernuella virgata	-11.12	-3.56
	-11.48	-3.08
	-10.82	-3.77
	-11.26	-3.55
Mean	-11.17	-3.49
Std. Dev.	0.24	0.25
Rumina decollata	-12.26	-3.36
	-12.29	-3.76
	-12.22	-3.58
	-12.26	-3.46
	-12.42	-3.72
	-12.34	-3.90
Mean	-12.30	-3.63
Std. Dev.	0.07	0.18

C3 Regime - mean temperature = 15.7°C
- mean $\delta^{18}O$ SMOW water = -9.9‰

Species	$\delta^{13}C$‰ PDB	$\delta^{18}O$‰ PDB
Helix aspersa	-12.12	-2.45
	-12.05	-2.72
	-11.96	-2.94
	-12.16	-2.75
Mean	-12.07	-2.72
Std. Dev.	0.09	0.20
Cepaea nemoralis	-12.46	-1.95
	-12.42	-1.94
	-12.43	-1.82
	-12.72	-2.17
Mean	-12.51	-1.97
Std. Dev.	0.14	0.15
Cernuella virgata	-11.98	-4.40
	-11.51	-3.30
	-10.74	-2.93
	-11.97	-4.43
Mean	-11.55	-3.77
Std. Dev.	0.58	0.77
Rumina decollata	-12.63	-3.25
	-12.58	-3.64
	-12.57	-4.49
	-12.35	-3.46
Mean	-12.53	-3.71
Std. Dev.	0.12	0.54
Ferussacia folliculus	-11.38	-3.93
	-11.40	-4.08
Mean	-11.39	-4.01
Std. Dev.	0.01	0.11

Appendix 1A Stable isotope data - experiment 1

W3 Regime - mean temperature = 19.3°C
- mean $\delta^{18}O$ SMOW water = -8.8‰

Species	$\delta^{13}C$‰ PDB	$\delta^{18}O$‰ PDB
Helix aspersa	-12.58	-1.03
	-12.73	-0.71
	-12.59	-1.03
	-12.65	-1.16
Mean	-12.64	-0.98
Std. Dev.	0.07	0.19
Cepaea nemoralis	-12.28	-1.04
	-12.28	-1.28
	-12.32	-1.21
	-12.35	-1.38
Mean	-12.31	-1.23
Std. Dev.	0.03	0.14
Cernuella virgata	-12.24	-2.64
	-12.66	-2.66
	-12.26	-2.74
Mean	-12.39	-2.74
Std. Dev.	0.24	0.05
Rumina decollata	-12.94	-3.31
	-12.71	-4.71
	-13.13	-3.88
	-12.92	-3.75
Mean	-12.93	-3.79
Std. Dev.	0.17	0.65

Appendix 1A Stable isotope data - experiment 1 (cont.)

W2 Regime - mean temperature = 22.3°C
- mean $\delta^{18}O$ SMOW water = -9.1‰

Species	$\delta^{13}C$‰ PDB	$\delta^{18}O$‰ PDB
Helix aspersa	-13.49	4.06
	-13.27	3.95
	-13.41	4.15
	-13.51	3.35
Mean	-13.42	3.88
Std. Dev.	0.11	0.36
Cepaea nemoralis	-13.27	5.28
	-13.27	4.60
	-12.68	3.82
	-13.34	3.85
	-13.08	4.50
	-12.99	4.34
Mean	-13.11	4.40
Std. Dev.	0.25	0.54
Cernuella virgata	-13.47	3.07
	-13.34	3.34
	-13.51	3.54
	-13.36	3.11
	-13.25	2.92
	-13.30	2.74
	-13.04	3.13
	-13.21	2.84
	-13.17	2.52
	-13.12	3.78
	-13.11	3.37
	-13.30	2.59
	-13.30	3.28
	-13.29	3.41
Mean	-13.27	3.12
Std. Dev.	0.13	0.37
Rumina decollata	-13.72	1.10
	-13.64	0.85
	-13.49	1.20
	-13.66	-0.04
Mean	-13.63	0.78
Std. Dev.	0.10	0.57
Ferussacia folliculus	-12.63	-0.28
	-12.49	-0.60
Mean	-12.56	-0.44
Std. Dev.	0.10	0.23

W3 Regime - mean temperature = 24.0°C
- mean $\delta^{18}O$ SMOW water = -8.9‰

Species	$\delta^{13}C$‰ PDB	$\delta^{18}O$‰ PDB
Helix aspersa	-13.44	0.95
	-13.35	0.64
	-13.39	0.99
	-13.49	0.77
Mean	-13.42	0.84
Std. Dev.	0.06	0.16
Cepaea nemoralis	-13.10	2.35
	-13.27	2.21
	-13.17	2.50
	-13.09	2.68
	-13.26	2.63
	-13.04	2.50
Mean	-13.16	2.50
Std. Dev.	0.10	0.18
Cernuella virgata	-12.69	-1.56
	-13.16	-2.11
	-13.03	-1.51
	-12.86	-0.93
	-12.73	-1.52
	-12.96	-1.13
Mean	-12.91	-1.46
Std. Dev.	0.18	0.41
Rumina decollata	-13.30	-0.89
	-13.41	-1.11
	-13.23	-0.67
	-13.40	-0.75
Mean	-13.34	-0.86
Std. Dev.	0.09	0.19
Ferussacia folliculus	-12.71	1.61
	-12.78	1.77
	-12.39	-0.06
	-12.85	-1.08
Mean	-12.68	0.56
Std. Dev.	0.20	1.37

C1 Regime - Shell dimensions and weights

Species	Height mm	Breadth mm	Weight mg
Helix aspersa	9.9	11.2	72.2
	8.6	8.9	40.7
	8.1	8.9	46.1
	8.4	8.9	47.4
	7.4	8.3	40.6
	6.8	7.7	26.9
	6.1	7.0	22.0
	9.7	10.9	48.6
	6.8	7.9	33.1
Mean	8.2	9.1	44.7
Cepaea nemoralis	5.9	7.9	47.5
	5.7	7.6	46.1
	6.2	8.7	66.9
	7.4	9.7	74.8
	5.9	7.9	40.7
	6.0	7.9	50.1
	6.0	7.8	51.2
	5.5	7.4	41.6
	4.5	5.3	27.1
Mean	6.0	7.9	50.5
Cernuella virgata	3.5	5.3	13.2
	3.9	5.4	12.8
	3.2	4.8	11.2
	3.3	5.0	8.9
	3.0	3.7	7.2
	2.9	3.5	7.4
Mean	3.3	4.6	10.1

Rumina decollata	Diam. last Whorl mm
	4.5
	3.4
Mean	4.0

C2 Regime - Shell dimensions and weights

Species	Height mm	Breadth mm	Weight mg
Helix aspersa	17.7	19.7	284.9
	16.2	16.9	240.7
	15.1	15.9	315.9
	16.0	15.4	179.5
	15.0	16.6	216.8
Mean	16.0	16.5	147.6
Cepaea nemoralis	7.8	10.2	81.6
	7.1	9.4	81.5
	6.0	7.3	43.4
	6.0	8.5	47.5
Mean	6.7	8.9	63.5
Cernuella virgata	5.9	8.2	48.7
	4.6	5.3	18.3
	4.5	6.1	18.8
	5.5	7.7	38.0
	5.3	7.1	26.6
Mean	5.2	7.1	30.1

Rumina decollata	Diam. last Whorl mm
	4.5
	4.2
	3.9
	4.4
	3.7
Mean	4.1

C3 Regime - Shell dimensions and weights

Species	Height mm	Breadth mm	Weight mg
Helix aspersa	14.5	16.0	133.1
	12.8	13.9	84.5
	12.8	14.0	121.5
	10.7	12.3	83.1
	11.5	12.8	62.5
	11.6	12.3	86.8
	9.8	11.1	49.0
	9.4	11.5	56.1
	8.8	10.0	52.0
	9.4	11.3	76.2
Mean	11.1	12.5	80.5
Cepaea nemoralis	7.1	9.8	60.7
	6.5	8.1	45.2
	5.4	7.1	28.6
	5.8	7.9	42.3
	5.5	7.2	33.8
Mean	6.1	8.0	42.1
Cernuella virgata	3.8	5.4	14.7
	3.1	4.7	10.2
	2.6	3.8	5.0
Mean	3.2	4.6	10.0

Rumina decollata	Diam. last Whorl mm
	7.5
	5.5
	7.2
	5.7
	6.4
	5.2
Mean	6.3

Appendix 1A Shell dimensions and weights - experiment 1

W3 Regime - Shell dimensions and weights

Species	Height mm	Breadth mm	Weight mg
Helix aspersa	13.2	14.9	166.9
	13.3	14.8	172.4
	12.8	14.2	158.7
	14.3	14.9	202.2
	13.3	14.1	166.1
	12.4	13.6	140.3
Mean	13.2	14.4	167.8
Cepaea nemoralis	16.8	19.6	452.6
	14.8	18.9	468.1
	13.7	17.6	417.7
	13.6	17.1	280.6
	13.1	16.0	249.2
Mean	14.4	17.8	373.6
Cernuella virgata	4.6	6.8	18.2
	3.7	5.0	12.1
	3.5	4.9	8.3
	3.7	4.9	13.0
Mean	3.9	5.4	13.0

Species	Diam. last Whorl mm
Rumina decollata	7.7
	8.2
	7.8
	7.8
	8.4
	7.5
Mean	7.9

W2 Regime - Shell dimensions and weights

Species	Height mm	Breadth mm	Weight mg
Helix aspersa	20.9	25.4	621.9
	19.3	20.9	318.3
Mean	20.1	23.2	470.1
Cepaea nemoralis	12.8	15.1	173.8
	11.9	14.9	185.9
	9.8	13.8	138.8
	9.5	12.7	113.1
	11.9	15.7	234.7
	12.0	15.8	259.4
Mean	11.3	140.7	184.3
Cernuella virgata	9.8	10.2	115.2
	5.5	7.9	34.6
	4.9	7.6	33.1
Mean	6.7	8.6	61.0

Species	Diam. last Whorl mm
Rumina decollata	9.0

W1 Regime - Shell dimensions and weights

Species	Height mm	Breadth mm	Weight mg
Helix aspersa	21.7	22.9	540.2
	19.5	20.8	345.5
	18.2	18.5	295.9
	13.0	14.9	102.8
	22.3	23.1	625.8
	11.4	12.3	226.0
Mean	17.7	18.7	256.0
Cepaea nemoralis	15.8	19.2	333.9
	15.2	19.3	339.1
	15.0	19.2	352.4
	14.8	17.3	293.0
	11.9	15.5	218.7
	10.1	13.7	267.9
Mean	13.8	17.4	300.8
Cernuella virgata	6.4	8.6	49.7
	6.2	8.3	46.0
	6.1	8.1	39.7
	5.4	7.5	29.0
	5.6	7.8	35.9
	7.9	10.0	63.1
Mean	6.3	8.4	43.9

Species	Diam. last Whorl mm
Rumina decollata	8.7
	8.9
	7.8
	7.9
	7.9
	7.7
Mean	8.2

Appendix 1A Shell dimensions and weights - experiment 1 (cont)

POP. 1 - mean temperature = 22.3°C
- Pop 1 snails maintained inside black, opaque polythene bags

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	12.3	13.1	57.7	-11.61	-5.97
	13.8	14.8	142.1	-10.75	-5.91
	12.4	12.5	66.7	-11.74	-6.12
	14.5	14.9	89.2	-11.35	-6.02
	11.1	11.7	38.3	-12.14	-6.03
	12.3	12.8	56.4	-	-
	10.9	11.6	37.5	-	-
	9.1	10.0	30.2	-	-
Mean	12.1	12.7	64.8	-11.52	-6.03
Std. Dev.				0.52	0.09
Cepaea nemoralis	5.9	8.4	30.0	-11.10	-5.94
	6.1	8.2	46.5	-12.28	-5.07
	5.9	7.9	26.6	-11.26	-5.86
	5.5	7.4	26.8	-	-
	4.1	6.0	14.0	-	-
Mean	5.5	7.6	28.8	-11.54	-5.62
Std. Dev.				0.64	0.48

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	8.2	-9.86	-6.10
	7.7	-9.96	-6.05
	7.3	-9.42	-6.03
	8.0	-	-
	8.1	-	-
	7.2	-	-
	6.9	-	-
	4.8	-	-
Mean	7.3	-9.92	-6.06
Std. Dev.		0.06	0.04

Ferussacia folliculus	No. of Shells	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	11.0	-9.30	-5.78

POP. 2 - mean temperature = 22.3°C
- Pop 2 snails maintained inside clear polythene bags

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	12.1	12.9	67.8	-11.88	-6.59
	10.3	12.3	48.8	-11.93	-6.78
	7.9	9.1	23.1	-12.92	-5.45
	10.2	11.6	41.5	-11.88	-6.73
	8.0	9.3	32.5	-11.69	-6.79
	8.8	9.5	24.4	-	-
	7.4	8.7	18.5	-	-
Mean	9.2	10.5	36.7	-12.06	-6.47
Std. Dev.				0.49	0.57
Cepaea nemoralis	8.9	11.8	101.3	-10.73	-6.11
	6.1	8.3	32.5	-10.68	-6.04
	6.2	8.6	42.1	-11.38	-6.29
Mean	7.1	9.6	58.6	-10.93	-6.15
Std. Dev.				0.39	0.13

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	7.7	-10.23	-6.33
	7.3	-10.25	-6.33
	6.8	-10.15	-6.43
	7.3	-	-
	7.2	-	-
	7.1	-	-
	6.4	-	-
	6.4	-	-
Mean	7.0	-10.21	-6.36
Std. Dev.		0.05	0.06

Ferussacia folliculus	No. of Shells	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	12.0	-9.38	-6.34

Appendix 1B Stable isotope data, shell dimensions and weights - experiment 3

POP. 3 - mean temperature = 22.3°C
- Pop 3 snails moved from black, opaque to clear polythene bags, and vice versa, every 12 hrs

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	13.3	13.8	80.9	-12.18	-6.56
	12.6	12.8	73.4	-12.17	-6.61
	12.2	13.1	49.8	-12.30	-6.54
	10.7	12.3	81.0	-11.82	-6.43
	9.0	10.0	37.2	-12.47	-6.64
	9.7	10.3	46.6	-	-
	6.0	7.3	14.2	-	-
Mean	10.5	11.4	54.7	-12.19	-6.55
Std. Dev.				0.24	0.08
Cepaea nemoralis	7.1	9.6	68.5	-11.51	-5.86
	6.0	7.9	32.3	-11.35	-6.05
	4.7	6.7	23.7	-11.75	-6.06
Mean	5.9	8.1	41.5	-11.54	-5.99
Std. Dev.				0.21	0.11

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	7.7	-10.12	-6.39
	7.4	-9.73	-5.99
	7.1	-10.07	-6.21
	7.3	-	-
	7.2	-	-
	7.0	-	-
	5.3	-	-
Mean	7.0	-9.97	-6.20
Std. Dev.		0.21	0.20

Ferussacia folliculus	No. of Shells	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	9	-9.49	-6.20

POP. 4 - mean temperature = 18.4 °C
- Pop 4 snails maintained inside black opaque polythene bags, and moved between 22.3 and 14.5°C rooms, and vice versa, every 12 hrs

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	16.4	15.9	123.4	-10.71	-5.83
	14.9	15.1	71.0	-10.48	-5.67
	13.2	13.7	101.8	-10.63	-5.79
	10.0	10.9	45.8	-11.73	-6.73
	8.3	9.4	22.7	-11.69	-6.40
	6.7	7.8	20.6	-	-
	6.4	8.0	18.1	-	-
	6.8	7.4	12.3	-	-
Mean	10.3	11.0	52.0	-11.19	-6.08
Std. Dev.				0.48	0.46
Cepaea nemoralis	8.0	11.3	83.7	-10.24	-5.80
	5.9	8.2	39.8	-10.03	-5.60
	5.5	7.1	28.1	-10.47	-5.80
Mean	6.5	8.9	50.5	-10.25	-5.73
Std. Dev.				0.22	0.11

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	6.2	-9.72	-5.95
	5.3	-9.91	-6.06
	5.8	-9.83	-5.96
	6.2	-	-
	5.5	-	-
	5.5	-	-
	5.0	-	-
	4.8	-	-
Mean	5.5	-9.82	-5.99
Std. Dev.		0.10	0.06

Ferussacia folliculus	No. of Shells	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	11	-9.13	-5.82

Appendix 1B Stable isotope data, shell dimensions and weights - experiment 3 (cont)

POP. 5 - mean temperature = 18.4 °C
- Pop 5 snails maintained inside clear polythene bags, and moved between 22.3 and 14.5°C rooms, and vice versa, every 12 hrs

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	12.5	13.4	77.1	-11.01	-5.97
	12.1	12.5	73.0	-10.72	-5.94
	10.5	11.4	48.9	-10.10	-6.22
	8.4	9.4	22.8	-11.63	-5.90
	8.6	9.6	36.4	-11.28	-5.74
	7.3	8.8	26.9	-	-
	8.0	8.7	32.4	-	-
Mean	9.6	10.5	45.4	-11.15	-5.95
Std. Dev.				0.34	0.17
Cepaea nemoralis	7.2	10.7	79.1	-10.04	-5.50
	6.0	7.6	32.8	-10.09	-5.48
	5.1	6.9	24.8	-9.99	-5.62
Mean	6.1	8.4	45.6	-10.04	-5.54
Std. Dev.				0.05	0.10

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	6.9	-9.71	-6.03
	5.4	-9.87	-5.88
	6.4	-9.73	-5.90
	6.8	-	-
	5.9	-	-
	6.1	-	-
	5.2	-	-
	4.3	-	-
Mean	5.9	-9.77	-5.94
Std. Dev.		0.09	0.08

Ferussacia folliculus	No. of Shells	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	10	-9.14	-5.95

POP. 6 - mean temperature = 14.5 °C
- Pop 6 snails maintained inside black, opaque polythene bags

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	8.2	9.3	35.5	-10.91	-4.53
	8.7	9.2	40.7	-11.02	-4.62
	6.8	8.1	38.2	-11.02	-4.68
	6.2	7.1	18.7	-11.27	-4.56
	6.8	7.8	22.5	-12.79	-3.57
	5.9	7.3	20.5	-	-
	5.8	7.0	18.6	-	-
	6.0	7.1	21.4	-	-
	5.0	6.6	11.7	-	-
	5.6	6.5	17.4	-	-
Mean	6.5	7.6	24.5	-11.40	-4.39
Std. Dev.				0.79	0.46
Cepaea nemoralis	5.0	6.5	28.0	-9.97	-4.96
	4.3	5.7	19.5	-10.50	-4.50
	3.3	4.9	11.7	-10.34	-4.96
Mean	4.2	5.7	19.7	-10.27	-4.81
Std. Dev.				0.28	0.27

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	4.1	-9.33	-5.04
	3.8	-9.22	-5.20
	4.0	-9.02	-5.20
	3.7	-	-
	3.7	-	-
	3.9	-	-
	3.4	-	-
Mean	3.8	-9.19	-5.14
Std. Dev.		0.16	0.08

Ferussacia folliculus	No. of Shells	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	8	-8.61	-5.01

Appendix 1B Stable isotope data, shell dimensions and weights - experiment 3 (cont)

POP. 7 - mean temperature = 14.5 °C
- Pop 7 snails maintained inside clear polythene bags

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	9.5	10.9	48.7	-10.32	-5.34
	9.8	10.7	37.4	-10.55	-4.66
	8.3	9.7	30.7	-11.05	-4.90
	6.3	7.6	30.8	-11.30	-4.19
	5.7	6.9	23.5	-12.36	-3.40
	7.2	8.6	31.9	-	-
	6.4	7.8	21.5	-	-
	5.6	6.7	16.8	-	-
	5.3	6.9	20.8	-	-
Mean	7.1	8.4	29.1	-11.12	-4.50
Std. Dev.				0.80	0.74
Cepaea nemoralis	5.0	6.3	23.0	-10.07	-4.74
	3.4	4.5	12.8	-10.49	-4.59
	2.9	4.0	8.3	-11.32	-5.34
Mean	3.8	4.9	14.7	-10.63	-4.89
Std. Dev.				0.63	0.40

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	4.2	-9.02	-5.56
	4.7	-8.85	-5.79
	4.0	-9.34	-5.71
	3.8	-	-
	3.8	-	-
Mean	4.1	-9.07	-5.68
Std. Dev.		0.25	0.12

Ferussacia folliculus	No. of Shells	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	9	-8.47	-5.32

Appendix 1B Stable isotope data, shell dimensions and weights - experiment 3 (cont)

POP. 8 - mean temperature = 22.3°C
- Pop 8 snails maintained as in Expts. 1 and 2, *i.e.* without polythene bags

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	17.8	18.4	185.6	-9.60	2.16
	16.0	16.3	147.6	-9.74	2.40
	13.8	14.6	123.9	-9.60	2.32
	13.4	13.8	91.9	-9.86	2.42
	11.6	12.3	72.2	-9.96	1.72
	10.2	11.4	49.4	-10.48	2.11
	9.9	10.5	47.3	-	-
	11.1	11.7	65.0	-	-
Mean	13.0	13.6	97.9	-9.93	2.19
Std. Dev.				0.30	0.26

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	7.9	-9.59	-0.90
	7.5	-9.27	-0.68
	7.3	-9.41	-0.52
	7.7	-	-
	7.2	-	-
	7.2	-	-
	6.4	-	-
Mean	7.3	-9.42	-0.70
Std. Dev.		0.16	0.19

POP. 9 - mean temperature = 14.5°C
- Pop 9 snails maintained as in Expts. 1 and 2, *i.e.* without polythene bags

Species	Height mm	Breadth mm	Weight mg	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
Helix aspersa	8.8	10.1	42.4	-8.99	0.49
	8.1	10.5	43.0	-9.73	0.30
	7.1	8.4	29.1	-11.05	2.34
	6.7	8.2	33.7	-10.88	0.46
	5.9	7.3	24.5	-10.11	0.58
	6.4	7.5	23.0	-	-
	6.1	7.5	21.4	-	-
	6.1	7.4	19.2	-	-
Mean	6.9	8.4	29.5	-10.15	0.83
Std. Dev.				0.84	0.85

Rumina decollata	Diam. Last Whorl mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
	4.6	-8.52	-1.19
	4.3	-8.73	-1.03
	3.7	-8.45	-0.44
	3.7	-	-
	3.9	-	-
	3.5	-	-
	3.5	-	-
Mean	3.9	-8.66	-0.89
Std. Dev.		0.14	0.39

Appendix 1B Stable isotope data, shell dimensions and weights - experiment 3 (cont)

APPENDIX 2

Jamaican case study - Isotopic analyses and site information

(A) Stable isotope data from within individual shells of *Pleurodonte sublucerna* and *Urocoptis cylindrus*

(B) Stable isotope data from shells of *Pleurodonte sublucerna*, from the Red Hills predominantly

(C) Stable isotope data from shells of *Lucidella aureola*, all from the Red Hills area

(D) Stable isotpe data from shells of *Urocoptis brevis*, from the Southern coastal margins of Jamaica

(E) Stable isotope data from shells of *Urocoptis cylindrus*, from the Cockpit Country.

n.b. Grid references are quoted to six figures; the letter preceeding the grid reference represents the sheet upon which the site may be located. Altitudes are quoted in feet above sea level as, at the time of this study, the only maps available for the entire island were non-metric. Abbreviations are keyed in each section. For oxygen isotopic composition of Jamaican waters see Table 4.6 (section 4.8.2)

SHELL 1; loc. Cave Valley, GR G420428; Alt. 2000ft

Sample No.	Dist. from Apex (cm)	Whorl No.	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
J9.1J	0	1-2	-11.30	-3.05
J9.1I	2	3	-11.12	-3.30
J9.1H	4	3	-11.19	-3.00
J9.1G	6	4	-11.30	-2.72
J9.1F	8	4	-11.58	-3.73
J9.1E	10	4	-11.22	-1.55
J9.1D	12	5	-11.14	-1.52
J9.1C	14	5	-11.20	-1.76
J9.1B	16	5	-10.90	-2.51
J9.1A	18	5	-11.21	-1.37
J9.1K	18.5	umb	-11.09	-1.85
		Mean	-11.20	-2.40
		Std. Dev.	0.16	0.78

SHELL 2; loc. Cave Valley, GR G420428; Alt. 2000ft

Sample No.	Dist. from Apex (cm)	Whorl No.	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
J9.2J	0	1-2	-11.62	-3.79
J9.2I	2	3	-11.24	-3.55
J9.2H	4	3	-11.12	-3.17
J9.2G	6	4	-11.54	-3.70
J9.2F	8	4	-11.07	-4.22
J9.2E	10	4	-11.02	-3.12
J9.2D	12	5	-10.59	-4.07
J9.2C	14	5	-10.84	-2.88
J9.2B	16	5	-11.02	-3.04
J9.2A	18	5	-10.87	-3.06
J9.2K	18.5	umb	-11.17	-3.84
		Mean	-11.10	-3.49
		Std. Dev.	0.28	0.44

Shell 7; loc. Glasgow, GR D335482; Alt. 1500ft

Sample No.	Whorl No.	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
J8G	1-2 (apical)	-6.99	-1.45
J8F	3	-7.20	-1.35
J8E	4	-7.22	-1.10
J8D	5	-7.09	-1.47
J8C	6	-6.95	-1.77
J8B	7	-6.64	-1.46
J8A	8 + aperture	-5.93	-1.68
	Mean	-6.86	-1.47
	Std. Dev.	0.42	0.20

SHELL 3; loc. Nr. Culloden, GR G420519; Alt. 2000ft

Sample No.	Dist. from Apex (cm)	Whorl No.	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
J10.1F	0	1-2	-9.94	-2.35
J10.1E	3	3	-9.67	-2.55
J10.1D	6	4	-10.01	-2.91
J10.1C	9	5	-10.13	-2.30
J10.1B	12	5	-9.57	-2.54
J10.1A	15	5	-9.74	-3.44
J10.1G	15.5	umb	-9.63	-2.91
		Mean	-9.81	-2.71
		Std. Dev.	0.20	0.37

SHELL 4; loc. Nr. Culloden, GR G420519; Alt. 2000ft

Sample No.	Dist. from Apex (cm)	Whorl No.	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
J10.2F	0	1-2	-10.59	-4.39
J10.2E	3	3	-10.41	-4.07
J10.2D	6	4	-10.75	-4.14
J10.2C	9	5	-11.16	-3.56
J10.2B	12	5	-11.14	-3.87
J10.2A	15	5	-10.76	-3.73
J10.2G	15.5	umb	-10.79	-3.72
		Mean	-10.81	-3.93
		Std. Dev.	0.25	0.27

SHELL 5; loc. Mandeville, GR D375414; Alt. 2000ft

Sample No.	Dist. from Apex (cm)	Whorl No.	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
J11.1F	0	1-2	-8.36	-1.91
J11.1E	3	3	-8.56	-1.79
J11.1D	6	4	-9.22	-2.60
J11.1C	9	5	-9.59	-1.21
J11.1B	12	5	-9.36	-2.09
J11.1A	15	5	-9.22	-2.08
J11.1G	15.5	umb	-9.54	-2.59
		Mean	-9.12	-2.04
		Std. Dev.	0.44	0.45

SHELL 6; loc. Hellshire Hills, GR L563373; Alt 50ft

Sample No.	Dist. from Apex (cm)	Whorl No.	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
J11.2E	0	1-2	-10.58	-2.46
J11.2D	2	3-4	-10.26	-2.42
J11.2C	4	4	-11.58	-2.95
J11.2B	6	5	-11.78	-3.47
J11.2A	8	5	-11.27	-2.89
J11.2F	8.5	umb	-11.29	-3.54
		Mean	-11.13	-2.96
		Std. Dev.	0.54	0.44

Shells 1-6 = *Pleurodonte sublucerna*

Shell 7 = *Urocoptis cylindrus*

loc. = location
umb = umbilical area

Appendix 2A Stable isotope data from within individual Jamaican shells

Samples from slices of shell taken from behind the aperture (except for CP samples - see key)

Site No.	Grid Ref.	Alt. ft.	Sample No.	Site Code	Shell Ht. mm	Diam. mm	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
11/11 2	L562372	100	P11.2A	HH	17.0	29.0	-12.22	-4.05
"	"	"	P11.2B	HH	15.0	29.0	-11.64	-1.13
"	"	"	P11.2C	HH	16.0	30.0	-12.46	-2.26
11/11 3	L569407	250	P11.3A	ST	21.0	37.0	-10.94	-2.52
"	"	"	P11.3B	ST	17.0	30.0	-10.76	-2.19
"	"	"	P11.3C	ST	19.0	33.5	-11.25	-1.96
11/11 4	L572410	250	P11.4A	ST	22.0	38.5	-10.74	-2.04
"	"	"	P11.4B	ST	21.0	36.5	-12.96	-0.95
"	"	"	P11.4C	ST	21.0	33.0	-9.03	-0.44
11/11 5	L570418	750	P11.5B bl	ST	24.0	44.0	-13.04	-1.69
11/11 6	L573424	1250	P11.6A	ST	23.0	43.0	-11.04	-2.89
"	"	"	P11.6B	ST	23.5	42.0	-10.03	-1.35
11/11 7	L569429	2000	P11.7A	ST	29.0	44.0	-10.90	-1.87
"	"	"	P11.7B	ST	22.0	40.5	-10.96	-2.04
"	"	"	P11.7C	ST	24.0	45.0	-10.10	-3.02
11/11 8	L558433	1000	P11.8A	ST	23.0	39.0	-12.34	-1.92
"	"	"	P11.8B im	ST	20.0	37.5	-10.97	-1.84
17/11 2	L607419	1000	P17.2A	ERH	17.0	40.0	-10.64	-0.64
"	"	"	P17.2B	ERH	16.0	36.0	-12.12	-0.56
"	"	"	P17.2C	ERH	16.0	34.0	-10.96	-1.46
"	"	"	PS17.2A sf	ERH	30.0	52.0	-14.18	-1.06
"	"	"	PS17.2B sf	ERH	28.0	52.0	-11.09	-2.51
"	"	"	PS17.2C sf	ERH	18.0	30.0	-10.52	-1.27
17/11 3	L605422	1750	P17.3A	ERH	25.0	45.0	-8.85	-3.23
17/11 4	L603424	2250	P17.4A	ERH	25.0	43.5	-12.15	-2.65
"	"	"	P17.4B	ERH	26.0	50.0	-10.59	-2.23
17/11 5	L570440	500	P17.5A	ST	24.0	38.0	-11.85	-1.71
"		"	P17.5B	ST	20.0	39.0	-11.00	-1.69
17/11 7	G493453	750	P17.7A	LL	26.0	43.0	-10.17	-2.65
"		"	P17.7B	LL	19.0	35.0	-11.26	-2.20
17/11 8	G498462	750	P17.8A	LL	23.5	39.0	-9.91	-1.44
18/11 1	G562393	100	P18.1A	SJ	17.0	31.0	-11.92	-0.79
"	"	"	P18.1B	SJ	17.0	33.0	-12.37	-1.55
"	"	"	P18.1C	SJ	19.0	34.0	-12.49	-0.32
18/11 2	G525395	250	P18.2A	SJ	20.0	37.0	-10.48	-2.86
"		"	P18.2B	SJ	21.5	36.5	-10.43	-0.90
18/11 3	G523403	500	P18.3A	SJ	19.0	38.0	-10.67	-2.69
18/11 4	G524417	600	P18.4A	SJ	20.0	39.5	-12.56	-1.13
18/11 5	G526422	750	P18.5A im	SJ	21.0	40.0	-13.21	-1.19
18/11 6	G525424	1000	P18.6A	SJ	22.0	46.0	-15.71	-0.10
18/11 7	G530434	500	P18.7A	SJ	18.0	32.0	-10.99	-2.24
3/8 1	L617419	500	JP1	ERH	23.0	46.0	-12.21	-1.34
3/8 2	L617421	500	JP2	ERH	23.0	47.0	-11.04	-2.09
3/8 3	L617424	1000	JP3	ERH	20.0	41.0	-11.31	-2.39
"	"	"	JP4	ERH	25.0	46.0	-12.20	-2.29
3/8 4	L614424	1500	JP5	ERH	23.0	44.0	-11.26	-2.68
5/8 1	L610424	1500	JP7	ERH	23.0	42.0	-8.30	-1.43
"	"	"	JP8	ERH	22.0	41.0	-11.52	-2.78
5/8 2	L598422	1750	JP9	ERH	28.0	51.0	-9.63	-1.43
"	"	"	JP10	ERH	26.0	49.0	-11.26	-1.54
5/8 3	L596423	2000	JP11	ERH	25.0	51.0	-11.02	-1.78
5/8 4	L599424	2250	JP12	ERH	28.0	50.0	-9.25	-1.14
5/8 5	L572427	1500	JP13	ST	24.0	44.0	-11.10	-3.86
"	"	"	JP14	ST	24.0	46.0	-10.68	-1.48
5/8 6	L572420	1000	JP15	ST	21.0	42.0	-10.71	-1.83
CP 20 -1	G420480	2000	J9.1A CP	CC	23.0	51.0	-11.21	-1.37
"	"	"	J9.2A CP	CC	22.0	51.0	-10.87	-3.06
CP 12 -2	G420519	2000	J10.1A CP	CC	21.0	39.0	-9.74	-3.44
"	"	"	J10.2A CP	CC	24.0	39.0	-10.76	-3.73
CP 29 -4	D375414	2000	J11.1A CP	CC	22.0	38.0	-9.22	-2.08
CP 18 -1	L563373	50	J12.1A CP	HH	16.0	29.0	-11.27	-2.89

KEY : bl = bleached shell im = immature specimen sf = sub-fossil shell
CP = coll. by CRC Paul, prior to 1987. Samples taken as part of the investigation of within-shell isotopic variation (see Appendix 2A)
Sites 11/11, 17/11 and 18/11 = coll. during November, 1987
Sites 3/8 and 5/8 = coll. during August, 1988

SITE AREA CODES :
1) Non - Red Hills sites
HH = Hellshire Hills, LL = Lluidas Vale, CC = Cockpit Country
2) Red Hills sites
ERH = East Red Hills Route, ST = Spanish Town Route,
SJ = St. John's Red Hills Route

Appendix 2B Site information and stable isotope data from shells of *Pleurodonte sublucerna*

Appendix 2C Site information and stable isotope data from shells of *Lucidella aureola* from the Red Hills

Samples represent whole shells

Site No.	Grid Ref.	Alt. ft	Sample No.	Site Code	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
11/11 3	L569407	250	JL1	ST	-9.72	-3.12
11/11 4	L572410	250	JL2	ST	-10.32	-2.41
11/11 5	L570418	750	JL3	ST	-9.58	-1.66
"	"	"	JL4	ST	-9.41	-1.73
11/11 7	L569429	2000	JL5	ST	-9.45	-1.80
11/11 8	L558433	1000	JL6	ST	-11.54	-1.67
17/11 1	L608417	850	JL7	ERH	-6.89	-4.23
17/11 2	L607419	1000	JL8	ERH	-9.82	-2.03
17/11 3	L605422	1750	JL9	ERH	-8.47	-0.72
"	"	"	JL10	ERH	-10.29	-2.58
"	"	"	JL11	ERH	-9.41	-3.94
17/11 4	L603424	2250	JL12	ERH	-7.43	-1.49
"	"	"	JL13	ERH	-8.85	-3.21
"	"	"	JL14	ERH	-9.36	-2.28
18/11 1	G526393	100	JL15	SJ	-9.73	-1.19
18/11 2	G525395	250	JL16	SJ	-9.96	-2.70
"	"	"	JL17	SJ	-9.81	-1.39
18/11 4	G524417	600	JL18	SJ	-5.63	-3.77
"	"	"	JL19	SJ	-5.81	-2.96
"	"	"	JL20	SJ	-8.39	-3.84
18/11 5	G526422	750	JL21	SJ	-11.96	-2.44
3/8 1	L617419	500	JL22	ERH	-10.23	-1.91
"	"	"	JL23	ERH	-10.12	-1.75
3/8 2	L617421	500	JL24	ERH	-8.64	-1.95
5/8 2	L598422	1750	JL25	ERH	-10.98	-1.45
"	"	"	JL26	ERH	-9.77	-2.42
"	"	"	JL27	ERH	-9.08	-2.02
5/8 3	L596423	2000	JL28	ERH	-9.26	-2.23
"	"	"	JL29	ERH	-9.21	-1.57
5/8 4	L599424	2250	JL30	ERH	-9.98	-4.34

KEY : ERH = East Red Hills Route, ST = Spanish Town Route
SJ = St. Johns Red Hills Route
Sites 11/11, 17/11 and 18/11 = coll. during November, 1987
Sites 3/8 and 5/8 = coll. during August, 1988

Samples represent the last two whorls of individual shells

Site No.	Grid Ref.	Alt. ft	Sample No.	Site Code	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
10/11 1	L592368	50	JUB1	HH	-6.62	-2.60
"	"	"	JUB2	HH	-5.45	-5.64
"	"	"	JUB3	HH	-6.75	-3.29
"	"	"	JUB4	HH	-3.79	-2.55
"	"	"	JUB5	HH	-5.25	-5.28
10/11 2	L585361	50	JUB6	HH	-6.67	-2.71
"	"	"	JUB7	HH	-4.42	-1.94
10/11 3	L584357	50	JUB8	HH	-4.66	-2.40
"	"	"	JUB9	HH	-4.13	-2.60
10/// 4	L577364	250	JUB10	HH	-11.81	-1.94
"	"	"	JUB11	HH	-3.88	-4.49
11/11 2	L562372	100	JUB13	HH	-7.15	-1.69
13/11 1	L641400	750	JUB14	SA	-6.76	-2.44
"	"	"	JUB15	SA	-5.31	-1.93
13/11 2	L652394	850	JUB16	SA	-5.90	-3.72
"	"	"	JUB17	SA	-5.44	-2.19
13/11 3	L657387	500	JUB18	SA	-7.99	-2.32
13/11 6	N705356	750	JUB19	ST	-7.11	-1.96
6/8 2	H421344	50	JUB20	MRB	-4.63	-2.05
"	"	"	JUB21	MRB	-3.94	-0.06
"	"	"	JUB22	MRB	-7.51	-1.82
"	"	"	JUB23	MRB	-5.64	-0.08
"	"	"	JUB24	MRB	-6.18	-2.90
6/8 3	H427346	75	JUB25	MRB	-4.31	-1.98
"	"	"	JUB26	MRB	-5.53	-2.57
6/8 4	H414350	50	JUB27	MRB	-6.14	-1.38
"	"	"	JUB28	MRB	-7.16	-1.98
12/8 1	E297384	150	JUB29	SC	-5.84	-2.75
"	"	"	JUB30	SC	-7.24	-4.89
12/8 2	E296388	300	JUB31	SC	-4.94	-1.73
"	"	"	JUB32	SC	-7.64	-1.23
CP 27 -1	L675370	250	J1.1. CP	ST	-5.23	0.28
"	"	"	J1.2A CP	ST	-6.38	-2.64
CP 1 8-1	L563373	100	J2.2A CP	HH	-7.50	-6.26
CP 1 1-2	C292584	100	J3A CP		-7.12	-1.17
CP 1 -1	E344403	750	J4.2A CP	SC	-6.96	-1.54
CP 1 6-2	L658384	500	J5.2A CP	SA	-6.66	-1.91

Other *Urocoptis brevis* data (from sections of shells other than the last two whorls)

Site No.	Grid. Ref	Alt. ft.	Sample No.	Section of Shell(s)	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
CP 27-1	L675370	250	J1.2B CP	early whls, J1.1+ J1.2A	-7.09	-2.11
CP 18-1	L563373	100	J2.1 CP	whole shell	-7.39	-6.41
"	"	"	J2.2B CP	rest of J2.2A	-7.16	-5.64
CP 11-2	C292584	100	J3.B CP	rest of J3.A	-7.46	-1.06
CP 1-1	E344403	750	J4.1 CP	whole shell	-5.84	-2.85
"	"	"	J4.2B CP	rest of J4.2A	-7.53	-1.29
CP 16-2	L658384	500	J5.1 CP	whole shell	-6.85	-0.67
"	"	"	J5.2B CP	rest of J5.2A	-5.86	-1.10

KEY : Sites 10/11, 11/11 and 13/11 coll. during November 1987
Sites 6/8 and 12/8 coll, during August 1988
CP = coll. by CRC Paul prior to 1987

SITE AREA CODES : HH = Hellshire Hills, SA = St. Andrew, ST = St. Thomas
MRB = Milk River Bath, SC = Santa Cruz and Spur Tree

Appendix 2D Site information and stable isotope data from shells of *Urocoptis brevis* from the Southern coastal margins, Jamaica

Samples represent the last two whorls of individual shells
All samples from the Cockpit Country

Site No.	Grid Ref.	Alt. ft	Sample No.	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
8/8 3	C376532	850	JUC1	-7.87	-1.66
8/8 4	C372518	1500	JUC2	-5.07	-1.42
8/8 8	C372521	1250	JUC3	-6.62	-1.83
" "	"	"	JUC4	-6.61	-1.95
CP 2 0-1	G420480	2000	J6.A CP	-7.27	-1.27
CP 1 2-2	G420519	2000	J7.2A CP	-8.32	-1.84

Other Urocoptis cylindrus data (from sections of shell other than the last two whorls)

Site No.	Grid. Ref	Alt. ft.	Sample No.	Section of Shell(s)	$\delta^{13}C‰$ PDB	$\delta^{18}O‰$ PDB
CP 20-1	G420480	2000	J6.B	rest of J6.A	-8.08	-1.09
CP 12-2	G420519	2000	J7.1	J7.1	-9.03	-2.00
"	"	"	J7.2A	J7.2A	-9.64	-1.97

KEY : Sites 8/8 = coll. during August 1988
CP = coll. by CRC Paul, prior to 1987

Appendix 2E Site information and stable isotope data from shells of *Urocoptis cylindrus*

APPENDIX 3

Holywell coombe case study - Stable isotope data from shells of Holocene terrestrial molluscs (Arianta arbustorum) and tufas

(A) Isotope data from HVI (Head of Valley) trench

(B) Isotope data from T5 (Horseshoe Spring) trench

Sample Interval cm	Set Depth cm	Shell $\delta^{13}C‰$ PDB	Shell $\delta^{18}O‰$ PDB	Mean + Std. Dev. where applicable		Tufa $\delta^{13}C‰$ PDB	Tufa $\delta^{18}O‰$ PDB
45-55	50	-9.75	-3.55				
55-65	60	-9.96	-2.60				
67-75	70	-8.43	-2.36				
80-90	85	-9.68	-2.53				
90-95	93	-9.69	-3.29				
100-105	103	-9.00	-2.77	-9.54	-2.67	-9.32	-4.94
"	"	-10.07	-2.57	0.76	0.14		
110-115	113	-8.56	-2.96				
120-125	123	-9.34	-2.61				
"	"	-9.72	-2.98				
"	"	-8.95	-1.35				
"	"	-9.43	-2.20				
"	"	-10.44	-2.09	-9.58	-2.16		
"	"	-9.58	-1.72	0.50	0.59		
130-135	133	-8.97	-3.27	-9.03	-2.87		
"	"	-9.08	-2.47	0.08	0.57		
140-145	143	-10.41	-3.27				
150-155	153	-9.59	-2.76			-9.64	-4.88
160-165	163	-9.35	-3.64				
170-175	173	-10.37	-3.22				
180-185	183	-10.37	-3.07				
"	"	-9.30	-3.62				
"	"	-9.48	-3.35	-9.41	-2.87		
"	"	-8.49	-1.43	0.77	0.98		
185-190	188	-8.75	-2.96				
190-195	193	-8.98	-1.94				
200-205	203	-9.99	-2.60			-8.59	-5.05
205-210	208	-8.01	-3.49				
210-215	213	-8.96	-2.09				
215-220	218	-8.90	-2.40				
220-225	223	-10.59	-1.96				
225-230	228	-10.78	-3.71				
230-235	233	-9.48	-2.66			-5.35	-4.82
"	"	-10.68	-0.93				
"	"	-9.68	-1.87				
"	"	-8.74	-1.94	-9.88	-1.83		
"	"	-10.82	-1.77	0.87	0.62		
235-245	240	-7.85	-2.88				
245-255	250	-9.19	-2.93	-8.65	-3.04		
"	"	-8.11	-3.14	0.76	0.15		
255-265	260	-8.89	-2.72	-8.67	-1.99		
"	"	-8.44	-1.26	0.32	1.03		
310-315	313	-8.08	-1.91				
315-322	318	-8.47	-1.31	-8.84	-1.44		
"	"	-9.20	-1.57	0.52	0.18		
322-327	325	-9.88	-3.11				
327-335	331	-10.00	-3.25	-10.02	-2.47	-4.77	-5.90
"	"	-10.04	-1.68	0.03	1.11		
335-345	340	--	--				
370-380	375	-7.03	-4.16				
380-390	385	--	--				
	n = 49. Mean	-9.34	-2.57			-7.53	-5.12
	Std. Dev.	0.84	0.75			2.30	0.45

Appendix 3A Stable isotope data from HVI (Head of Valley) trench, Holywell Coombe

Sample Interval cm	Set Depth cm	Shell $\delta^{13}C$‰ PDB	Shell $\delta^{18}O$ ‰ PDB	Mean + where	Std. Dev. applicable	Tufa $\delta^{13}C$‰ PDB	Tufa $\delta^{18}O$ ‰ PDB
55-60	58	-9.44	-2.02	-9.36	-2.34	-9.01	-4.69
"	"	-9.27	-2.66	0.12	0.45		
65-70	68	-8.81	-2.28				
75-80	78	-8.97	-1.64	-8.93	-1.75		
"	"	-8.88	-1.85	0.06	0.15		
58-90	88	-9.27	-2.15				
95-100	98	-8.93	-3.10				
105-110	108	-9.58	-1.82	-9.93	-2.13	-9.81	-5.28
"	"	-10.27	-2.43	0.49	0.43		
115-120	118	-9.22	-2.63				
125-130	128	-9.84	-2.79				
145-150	148	-9.62	-1.67				
155-160	158	-9.79	-1.33			-9.11	-5.13
165-170	168	-8.55	-2.72				
175-180	178	-12.56	-2.31				
185-190	188	-10.84	-1.62	-11.17	-1.83		
"	"	-11.49	-2.04	0.46	0.30		
195-200	198	-10.54	-2.69	-10.41	-2.80		
"	"	-10.28	-2.91	0.18	0.16		
205-210	208	-10.03	-3.00			-8.44	-4.98
215-220	218	-9.36	-2.43	-9.47	-2.50		
"	"	-9.58	-2.57	0.16	0.10		
225-230	228	-9.05	-1.24	-10.24			
"	"	-11.05	-3.60	1.68	-2.42		
235-240	238	–	–		1.67		
245-250	248	-10.11	-3.00				
250-255	253	–	–			-9.06	-5.55
255-260	258	-9.61	-1.64				
265-270	268	-9.88	-3.63				
295-300	298	-10.05	-3.05	-9.88	-2.99		
"	"	-9.90	-2.93	0.11	0.08		
305-310	308	-8.91	-3.16			-8.78	-5.65
315-320	318	-10.66	-2.09				
325-330	328	-8.91	-2.74				
330-340	335	-10.20	-2.24			-8.32	-5.58
340-345	343	-6.85	-3.05				
345-350	348	-9.11	-4.00				
"	"	-10.13	-1.84	-9.61	-2.83		
"	"	-9.59	-2.65	0.51	1.09		
350-355	353	-9.91	-3.03				
355-360	358	-8.68	-1.69				
"	"	-8.57	-0.36				
"	"	-8.20	-1.92				
"	"	-7.60	-1.44	-8.65	-1.62		
"	"	-10.20	-2.70	0.96	0.85		
360-365	363	-8.59	-3.02				
"	"	-9.45	-3.56				
"	"	-9.42	-3.62	-9.60	-3.15		
"	"	-10.95	-2.38	0.98	0.58		
365-370	368	-6.93	-3.74	-6.80	-3.49		
"	"	-6.66	-3.13	0.19	0.50		
375-380	378	-7.63	-2.58	-7.99	-2.63		
"	"	-8.34	-2.68	0.50	0.07		
380-385	383	-6.51	-1.99				
385-390	388	-8.66	-2.77				
	n = 53. Mean	-9.35	-2.40			-8.93	-5.27
	Std. Dev.	1.19	0.73			0.49	0.36

Appendix 3B Stable isotope data from T5 (Horseshoe Spring) trench, Holywell Coombe