

A comparison of stable isotope ratios ($^{18}\text{O}/^{16}\text{O}$ and $^{13}\text{C}/^{12}\text{C}$) between two species of hydrothermal vent decapods (*Alvinocaris lusca* and *Munidopsis subsquamosa*)

Cindy Lee Van Dover*

Department of Biology, University of California, Los Angeles, California 90024, USA

ABSTRACT: Stable isotope ratios ($^{18}\text{O}/^{16}\text{O}$ and $^{13}\text{C}/^{12}\text{C}$) in calcium carbonate exoskeletons of shrimp *Alvinocaris lusca* and galatheid squat lobsters *Munidopsis subsquamosa* from hydrothermal vents may provide a record of post-molt water temperature and chemistry experienced by each individual. Shrimp and galatheids appear to occupy distinct environmental regimes which are well-defined in terms of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values, and which correspond to specific regions of vent habitats. Each species may occupy these areas in a complex manner, dependent on size, sex, and reproductive state. Several mechanisms that might maintain these differences in distribution are proposed.

INTRODUCTION

The environments of deep-sea hydrothermal springs in the Pacific Ocean are defined by gradients of temperature and modified water chemistry radiating from sources of venting water. Distributional relations of fauna to these gradients are of fundamental importance in view of the animals' unusual nutritional requirements, physiological tolerances, and adaptability to seemingly hostile chemical environments (Colliss et al. 1979, Arp & Childress 1981, Cavanaugh et al. 1981, Childress & Mickel 1985, Felbeck et al. 1985). Although a considerable effort has focused on determining distributions of sessile vestimentiferan worms and large bivalve molluscs found at vents (Desbruyères et al. 1982, Hessler & Smithey 1983, Hessler et al. 1985), little is known about distributions of decapod crustaceans. Hessler & Smithey (1983), relying on direct observations and photographs of Rose Garden (Galapagos Rift), do note that the shrimp *Alvinocaris lusca* Williams & Chace reached its highest densities among large vestimentiferan/mussel thickets. In contrast, the galatheid squat lobster *Munidopsis subsquamosa* Henderson occurred most abundantly

among serpulid worms and on rock surfaces adjacent to animal-occluded vent openings (Hessler & Smithey 1983).

Temperature and water chemistry records incorporated in $^{18}\text{O}/^{16}\text{O}$ and $^{13}\text{C}/^{12}\text{C}$ ratios, respectively, of decapod calcium carbonate exoskeletons may provide details of distributions of these animals with respect to venting water. I use stable isotope ratios to address the following questions: (1) Does this technique in fact detect differences in species' distributions described in Hessler & Smithey (1983)? (2) Within a given species, how do these ratios correlate with size, sex, reproductive state and exposure to vent water?

Temperature-related variations of $^{18}\text{O}/^{16}\text{O}$ ratios in calcium carbonate have been used extensively in the study of deep-sea stratigraphy and paleoceanography; application of $^{18}\text{O}/^{16}\text{O}$ isotopic analyses to problems of the ecology of living animals is a more recent development. Feasibility of the technique for study of an array of organisms, including bivalves, gastropods, corals, and barnacles, has been demonstrated (Killingley et al. 1980, Wefer & Killingley 1980, Mikkelsen et al. 1982, Jones et al. 1986). The present study is the first attempt to apply $^{18}\text{O}/^{16}\text{O}$ analyses to decapod crustaceans.

The use of carbonate $^{13}\text{C}/^{12}\text{C}$ ratios in ecological studies has been somewhat less successful than $^{18}\text{O}/^{16}\text{O}$ analyses, due in part to complications that result

* Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

from incorporation of metabolic carbon, in addition to dissolved inorganic carbon (ΣCO_2) from seawater, into calcareous exoskeletons. This phenomenon is known as the 'vital effect'. Furthermore, $\delta^{13}\text{C}$ composition of seawater varies only from $\sim 2.0\text{‰}$ in surface waters (due to ^{12}C depletion by its preferential uptake by phytoplankton during photosynthesis), through a minimum of -1.0‰ at an intermediate depth corresponding to the ΣCO_2 maximum, to 0.0‰ in deeper waters (Kroopnick 1985). Resolution of patterns of variation in $^{13}\text{C}/^{12}\text{C}$ within biological systems over this narrow range is poor. At Rose Garden hydrothermal vent site, however, $\delta^{13}\text{C}$ (ΣCO_2) of venting water was measured at -5.1‰ (Craig et al. 1980). This suggests the presence of relatively steep gradients in $^{13}\text{C}/^{12}\text{C}$ ratios toward less negative values as one moves away from sources of vent water.

MATERIALS AND METHODS

Two species of decapods, collected by the research submersible ALVIN from Rose Garden hydrothermal vent site ($00^\circ 48.247' \text{N}$; $86^\circ 13.478' \text{W}$) in spring of 1985 were used in this study. Hessler & Smithey (1983) provide a general description of the vent community at this location. The caridean shrimp *Alvinocaris lusca* were captured primarily in 'slurp gun' samples from within clumps of vestimentiferans, although some individuals were obtained from washings of worm tubes and mussels brought to the surface by ALVIN. Collections of galatheid squat lobsters *Munidopsis subsquamosa* were obtained when these animals, disturbed by ALVIN maneuvers, sought refuge within the submersibles' superstructure and were subsequently carried to the surface. The entire carapace of each of 11 shrimp and the 4th leg of each of 21 galatheids were air-dried. Samples were finely ground, soaked in warmed 5.25% sodium hypochlorite solution overnight to destroy organic material, then washed several times in distilled water and freeze-dried. Standard techniques for collection of CO_2 and mass spectrometry (McCrea 1950) were followed. The average difference between 2 replicates of samples was 0.44 ± 0.37 and $0.22 \pm 0.16\text{‰}$ for ^{18}O and ^{13}C , respectively ($n = 20$).

RESULTS AND DISCUSSION

The data presented here are interpreted with respect to a set of assumptions, stated below, regarding calcification and isotopic fractionations in decapod crustaceans. It is hoped that this study will serve as the impetus for research that will either prove or falsify these assumptions.

Because decapods molt intermittently and because calcium carbonate (CaCO_3) deposition is neither continuous nor uniform throughout the molt cycle, isotopic ratios measured in their exoskeletons represent values averaged over the time since the previous molt. These measurements are interpreted to be indicative of the environmental regime experienced by the individual over the post-molt period when CaCO_3 is being deposited. This post-molt period lasts 2 to 6 wk in shallow marine and fresh-water decapod crustacean analogues (Yamaoka & Scheer 1971, Welinder 1975, Vigh & Dendinger 1982).

The calcium carbonate exoskeleton of the 2 species studied here was determined by X-ray diffraction to be calcite.

Measurements of $\delta^{18}\text{O}$ of fluids emanating from Rose Garden vents have not been published. But the 350°C end member fluids of hydrothermal vents at 21°N on the East Pacific Rise are enriched in ^{18}O with respect to ambient seawater by $\sim 2\text{‰}$ (Craig, reported in Bowers & Taylor 1985). Fluid rising from Galapagos type vents at Rose Garden has been diluted by ambient seawater. The extent of this dilution is so great that $\delta^{18}\text{O}$ values equal to those of ambient seawater, i.e. -0.3‰ (Craig & Gordon 1965), may be assumed. This is the same value used by Killingley et al. (1980) in their discussion of stable isotope ratios in vent molluscs and barnacles.

The $\delta^{18}\text{O}$ vs $\delta^{13}\text{C}$ values for the shrimp and galatheid are plotted in Fig. 1. In each species there is a significant ($p < 0.001$) positive correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, and the slopes of the 2 regression lines describ-

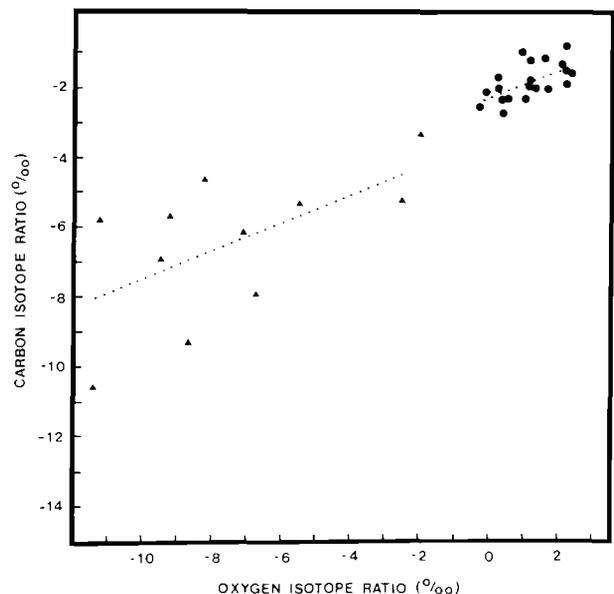


Fig. 1. $\delta^{18}\text{O}$ vs $\delta^{13}\text{C}$ expressed in ‰ (PDB) of exoskeleton calcium carbonate. (\blacktriangle) *Alvinocaris lusca*; (\bullet) *Munidopsis subsquamosa*. Where replicate samples were analyzed, points represent average values (similarly for Fig. 2 & 3)

ing these relations are nearly equal (0.36, shrimp and 0.39, galatheid). The ranges of the 2 species, in terms of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, are completely disjunct. This graph illustrates several important points:

(1) The positive correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ suggests that both of these signatures are reliable indices of degree of exposure to, but not necessarily temperature of, vent water at the Rose Garden site during the period of calcification of the exoskeleton. This correlation cannot simply be a consequence of a thermodynamic phenomenon since the proportion of ^{13}C precipitated in CaCO_3 has a small positive temperature dependence and hence would result in a slight negative correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. An alternative explanation for the positive correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ which cannot be ruled out is that incorporation of isotopically light, metabolic CO_2 increases with temperature. But if the first explanation is correct, then shrimp and galatheids occupy discrete environmental regimes, with shrimp in warmer, ^{13}C -depleted vent water and galatheids in cooler, peripheral water. In addition, shrimp occur over a broader range of temperature and exposure to vent water than galatheids. These interpretations are in agreement with photographic analyses of Hessler & Smithey (1983) and Hessler et al. (1985).

A barnacle from a peripheral location at the 21°N vent site on the EPR had average $\delta^{13}\text{C}$ values of $\sim -1.00\text{‰}$ and $\delta^{18}\text{O}$ values of $\sim 4.00\text{‰}$ (Killingley et al. 1980). Plotted on Fig. 1, this point would fall at the upper right-hand corner of the graph, in line with galatheid values. I analyzed a calcareous tube produced by a serpulid worm collected from the periphery of Rose Garden. The average of isotopic ratios for 3 replicates of this specimen ($\delta^{13}\text{C} = -1.64 \pm 0.26\text{‰}$, $\delta^{18}\text{O} = 2.67 \pm 0.13\text{‰}$) fall in the same region of Fig. 1. This is precisely where these values would be predicted to fall, based on the fact that galatheids, serpulids, and barnacles can all be found within the same region of vent communities (Killingley et al. 1980, Hessler & Smithey 1983, Hessler et al. 1985).

(2) Application of Epstein's paleotemperature equation (Epstein et al. 1953), assuming a value of -0.3‰ for the $\delta^{18}\text{O}$ of the surrounding seawater, to the limits of the $\delta^{18}\text{O}$ range observed (-11.40 to 2.36‰) leads to an unlikely result, namely, that temperatures to which the animals were exposed ranged from 6 to 81°C . *In situ* measurements at Rose Garden, with a probe placed within the throat of a vent, recorded temperatures no greater than 17°C (Hessler pers. comm.). Application of the barnacle temperature equation developed by Killingley & Newman (1982) gives numbers even further off-scale. Therefore, I interpret my $\delta^{18}\text{O}$ values to be indicative of relative rather than absolute temperatures. I assume that the relation

between temperature and $\delta^{18}\text{O}$ in decapod crustaceans will be nearly linear, as it is in other marine invertebrates (Epstein et al. 1953, Wefer & Killingley 1980, Killingley & Newman 1982, Mikkelsen et al. 1982), with some large amplification factor.

(3) The $\delta^{13}\text{C}$ values are more negative than might be expected on the basis of a -5.1‰ value measured in dissolved inorganic carbon of vent water. I attribute this either to a biogenic contribution of ^{13}C -depleted respiratory CO_2 from vent fauna to the $\text{CO}_2\text{-HCO}_3$ pool in the seawater, or to incorporation of metabolic CO_2 during calcification, or both. Cameron & Wood (1985) have demonstrated direct bicarbonate uptake from seawater during calcification in shallow-water *Callinectes sapidus* Rathbun and stated that metabolic and environmental CO_2 serve as sources for CO_2 in the carapace. The relative importance of these 2 sources is not known.

A significant ($p < 0.001$) positive correlation between size (carapace length) and $\delta^{18}\text{O}$ in *Alvinocaris lusca* is illustrated in Fig. 2. This correlation may

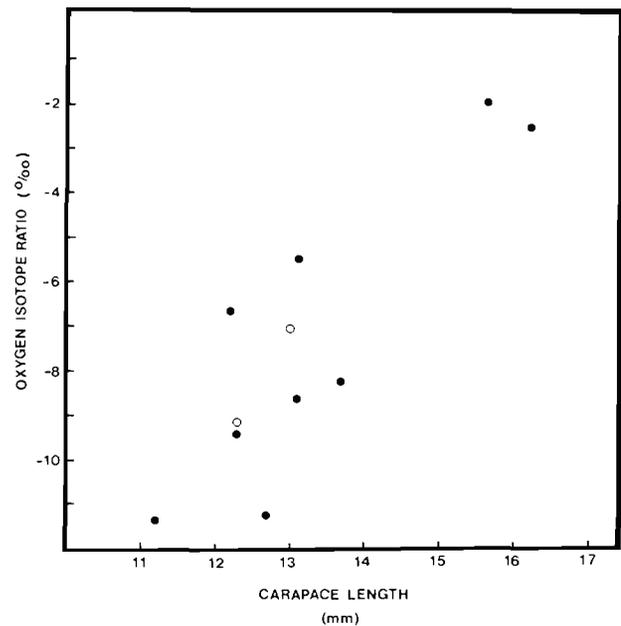


Fig. 2. *Alvinocaris lusca*. $\delta^{18}\text{O}$ vs carapace length. (○) Males; (●) non-gravid females

reflect a physiological change in the calcification process linked with size, resulting in a modified fractionation of oxygen isotopes. Alternatively, larger shrimp may spend more time in cooler water than smaller individuals. There is no correlation of sex with temperature, but the largest specimens in the coldest water were females.

The size- $\delta^{18}\text{O}$ relation in *Munidopsis subsquamosa* is considerably more complex (Fig. 3). Males show a significant ($p < 0.001$) negative correlation between

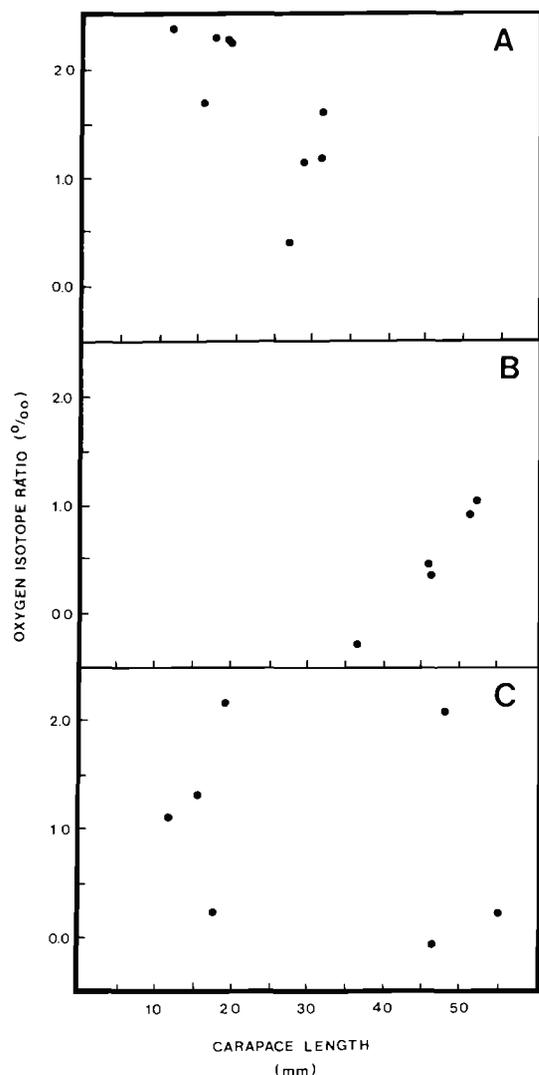


Fig. 3. *Munidopsis subsquamosa*. $\delta^{18}\text{O}$ vs carapace length. (A) males; (B) gravid females; (C) non-gravid females

size and $\delta^{18}\text{O}$ and have, with a single exception, more positive $\delta^{18}\text{O}$ values than gravid females (females with eggs attached to the pleopods). Gravid females show a significant ($p < 0.001$) positive correlation between size and $\delta^{18}\text{O}$. Non-gravid females show no correlation between $\delta^{18}\text{O}$ and size; the lack of correlation is expressed over a size range as great as that of males and gravid females combined. This last fact suggests that significant correlations between $\delta^{18}\text{O}$ and size observed among male and gravid female subpopulations of *M. subsquamosa* are not simply a reflection of a physiological phenomenon correlated with size or age of individuals. An alternative explanation is that male galatheids spend more time in colder water than gravid females, and that non-gravid females are less discriminating in their choice of a microhabitat.

To summarize, *Alvinocaris lusca* and *Munidopsis subsquamosa* are motile decapods with the freedom to

select and maintain themselves in a particular environmental milieu in the vicinity of hydrothermal vents. The importance of 'vital effects' in determining the fractionation of stable isotopes of carbon and oxygen in calcium carbonate is unknown in these animals. If these vital effects are minimal, then the exoskeleton of each species records the signature of a distinct environmental regime which is well-defined in terms of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values, and which corresponds to specific regions of vent habitats. Further, each species may occupy these regions in a complex manner, dependent on size, sex, and reproductive state. Several types of mechanisms that might, either independently or in combination, maintain these differences in distribution can be hypothesized:

- Diet*. Dietary requirements may be satisfied from different regions of vent habitats.
- Foraging time*. Diet may remain constant, but there may be differences in time required for collection or ingestion of food items from particular regions of vents.
- Tolerance to temperature or chemical environment*. It has been reported, for example, that large galatheids are much more tolerant of anoxic conditions than smaller individuals (Burd & Brinkhurst 1984).
- Predation effects*. Smaller individuals may be more susceptible to predation by other decapods, fish, etc. than larger ones; refuges may exist either at the periphery of vents where predator abundance might be low, or deep within the tangle of worm tubes and mussels where predators might be excluded.
- Reproductive behavior*. Mate selection and associated activities (e.g. territoriality) may take place in particular regions of vent sites. A preference by gravid females for warm environments may enhance their reproductive success; warmer water shortens the reproductive cycle in decapods (Amsler & George 1984), allowing production of additional broods.

Acknowledgements. Isotope analyses were carried out with the support and guidance of Dr. Ian Kaplan and Mr. David Winter, Institute of Geophysics and Planetary Physics, UCLA. The Master and crew of the R/V *Atlantis II*, the ALVIN group, Chief Scientist Dr. James J. Childress, and members of the scientific party of the Galapagos '85 Expedition all helped me obtain specimens. Dr. Robert Hessler facilitated my participation in the expedition. Drs Hessler, W. Berger, E. Druffel, and A. Williams reviewed the original manuscript. This study benefitted from discussions with and encouragement by many people, including Drs K. Dunn, G. Steen, J. Smith, R. Gore, and R. Hessler. Dr. James G. Morin served as my graduate advisor. To each person named, my thanks. Much of this work was completed during my tenure as an NSF graduate fellow at UCLA. The Galapagos '85 Expedition was funded by NSF.

LITERATURE CITED

- Amsler, M. O., George, R. Y. (1984). The effect of temperature on the oxygen consumption and developmental rate of the embryos of *Callinectes sapidus* Rathbun. *J. exp. mar. Biol. Ecol.* 82: 221–229
- Arp, A. A., Childress, J. J. (1981). Functional characteristics of the blood of the deep-sea hydrothermal vent brachyuran crab. *Science* 214: 559–561
- Bowers, T. S., Taylor, H. P. (1985). An integrated chemical and stable-isotope model of the origin of mid-ocean ridge hot spring systems. *J. geophys. Res.* 90 (B14): 12583–12606
- Burd, B. J., Brinkhurst, R. O. (1984). The distribution of the galatheid crab *Munida quadrispina* (Benedict 1902) in relation to oxygen concentrations in British Columbia fjords. *J. exp. mar. Biol. Ecol.* 81: 1–20
- Cameron, J. N., Wood, C. M. (1985). Apparent H^+ excretion and CO_2 dynamics accompanying carapace mineralization in the blue crab (*Callinectes sapidus*) following molting. *J. exp. Biol.* 114: 181–196
- Cavanaugh, C. C., Gardiner, S. L., Jones, M. L., Jannasch, H. W., Waterbury, J. B. (1981). Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. *Science* 213: 340–342
- Childress, J. J., Mickel, T. J. (1985). Metabolic rates of animals from the hydrothermal vents and other deep-sea habitats. In: Jones, M. L. (ed.) *Hydrothermal vents of the eastern Pacific: an overview*. *Bull. Biol. Soc. Wash.* 6: 249–260
- Corliss, J. B., Dymond, J., Gordon, L. I., Edmond, J. M., von Herzen, R. P., Ballard, R. D., Green, K., Williams, D., Bainbridge, A., Crane, K., van Andel, T. H. (1979). Submarine thermal springs on the Galapagos Rift. *Science* 203: 1073–1083
- Craig, H., Gordon, L. I. (1965). Deuterium and oxygen-18 variations in the oceans and marine atmosphere. In: Tongiorgi, E. (ed.) *2nd conference, stable isotopes in oceanographic studies and paleotemperature*. Consiglio Nazionale delle Ricerche, Roma, p. 9–130
- Craig, H., Welhan, J., Kim, K., Poreda, R., Lupton, J. (1980). Geochemical studies of the 21°N EPR hydrothermal fluids. *Trans. Am. geophys. Un. (EOS)* 61: 992
- Desbruyères, D., Crassous, P., Grassle, J., Khirpounoff, A., Reyss, D., Rio, M., van Praet, M. (1982). Données écologiques sur un nouveau site d'hydrothermalisme actif de la ride du Pacifique oriental. *C. r. hebdomadaire Séances Acad. Sci., Paris, Serie III* 295: 489–494
- Epstein, S., Buchsbaum, R., Lowenstam, H. A., Urey, H. C. (1953). Revised carbonate-water isotopic temperature scale. *Bull. geol. Soc. Am.* 64: 1315–1326
- Felbeck, H., Powell, M. A., Hand, S. C., Somero, G. N. (1985). Metabolic adaptations of hydrothermal vent animals. In: Jones, M. L. (ed.) *Hydrothermal vents of the eastern Pacific: an overview*. *Bull. Biol. Soc. Wash.* 6: 261–272
- Hessler, R. R., Smithey, W. M. (1983). The distribution and community structure of megafauna at the Galapagos hydrothermal vents. In: Rona, P. A., Bostrom, K., Laubier, L., Smith, K. (ed.) *Hydrothermal processes at sea-floor spreading centers*. Plenum Press, New York, p. 735–770
- Hessler, R. R., Smithey, W. M., Keller, C. H. (1985). Spatial and temporal variation of giant clams, tube worms and mussels at deep-sea hydrothermal vents. In: Jones, M. L. (ed.) *Hydrothermal vents of the eastern Pacific: an overview*. *Bull. Biol. Soc. Wash.* 6: 411–428
- Jones, D. S., Williams, D. F., Romanek, C. S. (1986). Life history of symbiont-bearing giant clams from stable isotope profiles. *Science* 231: 46–48
- Killingley, J. S., Newman, W. A. (1982). ^{18}O fractionation in barnacle calcite: a barnacle paleotemperature equation. *J. mar. Res.* 40: 893–902
- Killingley, J. S., Berger, W. H., MacDonald, K. C., Newman, W. A. (1980). $^{18}\text{O}/^{16}\text{O}$ variations in deep-sea carbonate shells from the Rise hydrothermal field. *Nature, Lond.* 288: 218–221
- Kroopnick, P. M. (1985). The distribution of ^{13}C of ΣCO_2 in the world oceans. *Deep Sea Res.* 32: 57–84
- McCrea, J. M. (1950). On the isotopic chemistry of carbonates and a paleotemperature scale. *J. chem. Phys.* 18: 849–857
- Mikkelsen, N., Erlenheuser, H., Killingley, J., Berger, W. (1982). Norwegian corals: radiocarbon and stable isotopes in *Lophelia pertusa*. *Boreas* 11: 163–171
- Vigh, D. A., Dendinger, J. E. (1982). Temporal relationships of post-molt deposition of calcium, magnesium, chitin, and protein in the cuticle of the Atlantic blue crab, *Callinectes sapidus* Rathbun. *Comp. Biochem. Physiol.* 72A: 365–369
- Wefer, G., Killingley, J. (1980). Growth histories of strombid snails from Bermuda recorded in their O-18 and C-13 profiles. *Mar. Biol.* 60: 129–135
- Welinder, B. S. (1975). The crustacean cuticle – II. Deposition of organic and inorganic material in the cuticle of *Astacus fluviatilis* in the period after molting. *Comp. Biochem. Physiol.* 51B: 409–416
- Yamaoka, L. H., Scheer, B. T. (1971). Chemistry of growth and development in crustaceans. In: Florkin, M., Scheer, B. T. (ed.) *Chemical zoology*, Vol. A. Academic Press, New York, p. 321–341