Vol. 219: 299–304, 2001 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published September 10

NOTE

Effects of storage and preservation on the $\delta^{13}C$ and $\delta^{15}N$ signatures of selected marine organisms

S. Kaehler[1,*], E. A. Pakhomov[2,]**

[1]Coastal Research Group, and [2]Southern Ocean Group, Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa

ABSTRACT: The effects of freezing, oven-drying, and formalin and ethanol preservation on stable isotope signatures of 3 unrelated marine species (fish, octopus and kelp) were studied over a period of 3 mo to investigate temporal changes in $\delta^{13}C$ and $\delta^{15}N$. The effects on stable isotope ratios during the short-term confinement of live littorinid snails were also examined. Freezing and oven-drying did not result in a significant change in the initial isotope ratio for both carbon and nitrogen over the period of investigation. Formalin preservation significantly decreased, while ethanol preservation significantly increased $\delta^{13}C$ values by ~0.6 to 1.5% after 1 to 12 wk in both the animals and the kelp. The $\delta^{15}N$ signatures tended to increase slightly, but not significantly, during both formalin and ethanol preservation. Data from this and previous studies suggest that preserved samples should not be used for $\delta^{13}C$ analysis as the effects of preservatives on carbon isotope signatures vary greatly between species and studies. In contrast, the effect of preservatives on $\delta^{15}N$ signatures was comparatively small, which may facilitate the use of preserved samples in studies of stable nitrogen isotopes. Animal confinement had an almost immediate effect on stable isotope signatures of littorinids. $\delta^{13}C$ exhibited a significant increase after 6 h and $\delta^{15}N$ became significantly depleted after 12 h of confinement. The results of this study suggest that the methods used for preserving samples for stable isotope analysis should be carefully chosen. Samples intended for isotope analysis should be frozen, freeze-dried or oven-dried, preservatives may be used only for $\delta^{15}N$ analysis and organisms should be sacrificed immediately after collection.

KEY WORDS: Stable isotopes · Preservation · Storage · Fish · Octopus · Kelp · Littorinid

Over the past 2 decades, an increasing number of biologists have started using stable isotopes of nitrogen and carbon in tropho-dynamic studies. A number of these studies have successfully identified sources of dietary matter and overall trophic pathways within a variety of marine and estuarine systems (e.g. Wada et al. 1986, Dunton et al. 1989, Thomas & Cahoon 1993, Hobson 1995, Bustamante & Branch 1996, Jennings et al. 1997, Kaehler et al. 2000, Lee 2000). Yet, while it is feasible to interpret a species isotope signature relative to another from the same study, inconsistencies in the use of techniques for preparing and storing samples prior to analysis preclude the comparison of results from different investigations. This is especially regrettable where previously collected and preserved museum specimens are available for comparison.

Due to the particular nature of isotope analysis, samples collected in the field cannot usually be analysed immediately. Instead, organisms are frequently frozen, dried or stored in a preservative until further preparation becomes possible (see references above). Recently, however, it has been suggested that the methods used for sample preparation may in themselves alter the signature ratios of carbon and nitrogen isotopes. For example, Bosley & Wainright (1999) illustrated that after several months of preservation, formalin, formalin/ethanol and mercuric chloride solution produced a significant increase in $\delta^{15}N$ values and a decrease in the $\delta^{13}C$ values of 2 marine organisms. They concluded that freezing was the only preservation method that did not significantly affect stable isotope ratios.

While the majority of recent studies have used either freezing or oven-drying as a means for preserving samples, certain studies attempt to keep animals alive until further preparation (e.g. Haines & Montague 1979, France 1995, Frazer 1996, Fantle et al. 1999). This may be done either because preservation is unavailable in the field or to facilitate the evacuation of gut contents from whole animal samples. We know of no studies that have attempted to investigate possible effects of

*E-mail: s.kaehler@ru.ac.za
**Present address: Department of Zoology, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa

short-term animal confinement in essentially closed systems.

The aim of this study is to quantify the effects of freezing, oven-drying, and ethanol and formalin preservation on the stable isotope signatures of 3 unrelated marine species (i.e. vertebrate: fish species, invertebrate: octopus species, plant: kelp species). The organisms were chosen because they are from different trophic levels (primary producer to top predator) and are, therefore, expected to have different isotopic compositions. Repeated analyses were carried out over a period of 3 mo to examine temporal changes in the effects of preservation and to determine whether preservation induced predictable shifts in $\delta^{13}C$ and $\delta^{15}N$ signatures. In addition, the effects on stable isotope ratios of short-term confinement of live littorinid snails *Nodilittorina africana* were quantified.

Materials and methods. ***Preservation:*** In order to guard against within species variability of isotope ratios, a single adult kob *Argyrosomus hololepidotus*, 1 subadult octopus *Octopus vulgaris* and 1 frond of kelp *Ecklonia radiata* were freshly caught/collected at Waterloo Bay, Eastern Cape, South Africa (27° 12′ E, 33° 30′ S). The animals were killed immediately and stored in the dark, on ice, for transportation to the laboratory. All organisms were prepared for preservation within 1 h of collection. One fillet of the cob (white muscle tissue only), 1 arm of the octopus, and the kelp frond were each subdivided into 39 approximately equal-sized sections. Three randomly chosen subsamples from each of the organisms were immediately oven-dried at 50°C for 24 h and prepared for isotope analysis (n = 3 samples × 3 species = 9). A further 9 subsamples from each organism were either oven-dried, frozen (–18°C), immersed in hexamine buffered 4% saline formalin or immersed in 70% ethanol (n = 9 samples × 3 species × 4 preservation treatments = 108). After 1, 4 and 12 wk, 3 randomly chosen subsamples from each organism and each preservation treatment were washed in distilled water and prepared for isotope analysis.

As we were interested primarily in relative changes of the isotope signatures over time, samples were neither decalcified nor defatted. At the allocated times (1, 4 and 12 wk) preserved samples were washed in distilled water, dried at 50°C for 24 h and crushed and homogenised with a mortar and pestle. Dried samples were stored in a desiccator and sent for analyses.

Live animals: 54 adult littorinid snails *Nodilittorina africana* were collected at Kowie Point, Eastern Cape, South Africa (26° 52′ E, 33° 40′ S) from within a 1 m^2 area on the high shore. Of these animals, 9 were immediately sacrificed in the field. The remaining live animals were placed into glass scintillation vials with 5 ml of water and kept in the dark at room temperature until further preparation (at ~20°C). As littorinids frequently spend extended periods of time out of the water and at very high temperatures (>40°C), we assumed that confinement conditions did not add to the desiccation and heat stress of the animals. After 6, 12, 24, 48 and 96 h, a further 9 animals were sacrificed and prepared for analysis.

After sacrificing the animals, their feet were dissected immediately, washed in distilled water and dried (see 'Preservation' above). In order to obtain sufficient sample mass for isotope analysis, the feet of 3 animals were pooled to provide 3 replicates (each containing 3 feet) for each sampling time period. Individual samples were then homogenised and stored in a desiccator until all samples had been processed. Samples were then sent for analysis.

Stable isotope and data analyses: Analyses were carried out at the UCT/FRD/Goldfields light stable isotope laboratory. $\delta^{13}C$ and $\delta^{15}N$ were determined on a Finnigan-MAT 252 stable light isotope mass spectrometer, after sample combustion in an on-line Carlo-Erba preparation unit. Merck gelatine, Sucrose and Valine were used as internal standards, calibrated against several IAEA reference materials. Results are expressed in the standard delta notation, as $X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X = element in question and R = ratio of the heavy over the light isotope. Repeated analyses of homogeneous material yielded a standard deviation of 0.04%.

The effects of preservation, length of preservation and time since confinement were investigated by performing ANOVAs on the stable isotope data (Statistica '99 software, StatSoft Inc., Tulsa, USA). No data transformations were attempted, as in the majority of cases, variances were homogeneous (Cochran's test $p > 0.05$). Student Neuman Keuls multiple comparison procedures were carried out to further investigate significant differences.

Results and discussion. Different preservation techniques resulted in different $\delta^{13}C$ signatures, which varied with length of preservation and also with the species analysed (Fig. 1, Table 1). Freezing and oven-drying were the only treatments that did not result in a significant change from the initial isotope ratio in any of the species and at any time of the experiment (Table 1). In contrast, formalin preservation resulted in depleted $\delta^{13}C$ values, with the fixative producing a significant effect over dried and frozen samples after 4 wk in animals and 1 wk in the kelp (Fig. 1). As suggested by Hobson et al. (1997), this depletion in $\delta^{13}C$ is most likely a result of the direct incorporation of isotopically light carbon from the formalin. Formalin binds to certain biochemical constituents of the tissues and contains its own source of carbon. It is likely, therefore, that changes in the isotope signatures of samples are at least partially dependent on the isotopic composition of the fixative and the amount of fixative bound to the tissues. The

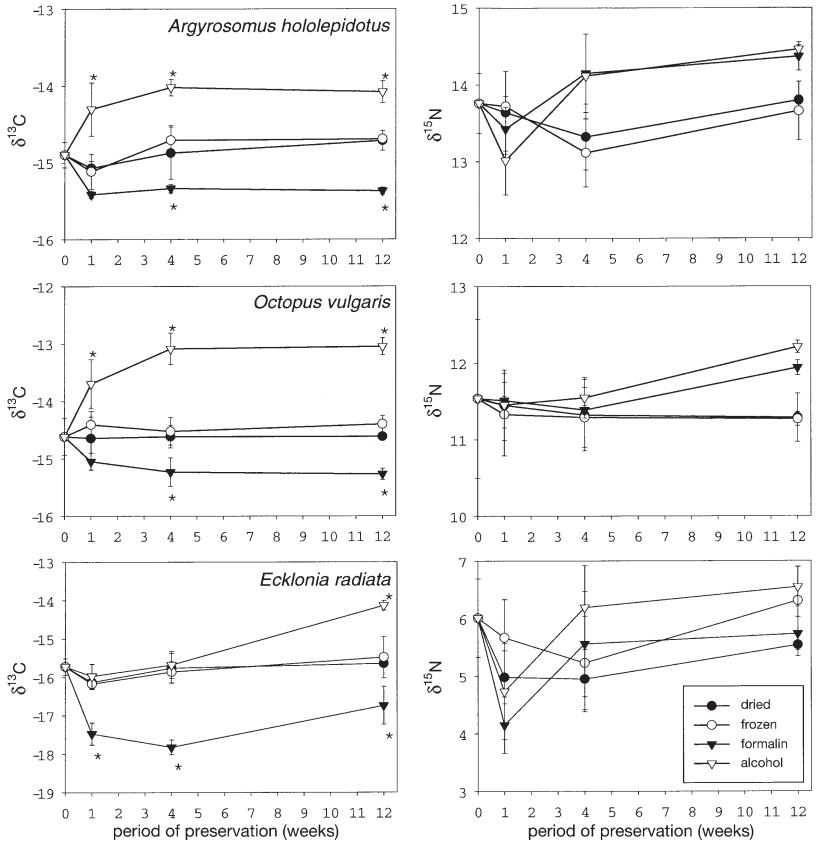

Fig. 1. Mean $\delta^{13}C$ and $\delta^{15}N$ signatures (±SD) of 3 marine organisms preserved for varying periods of time by oven-drying, freezing, formalin and ethanol immersion. At no time did the $\delta^{13}C$ and $\delta^{15}N$ signatures of dried and frozen samples deviate significantly from pre-treatment ratios. (*) Formalin and/or ethanol treated samples are significantly different ($p > 0.05$) in their isotopic composition from their dried and frozen counterparts (see Table 1)

potential effects of leaching of soluble organics into solution, however, may not be discounted. The mean decrease in $\delta^{13}C$ of approximately 0.6% in the animals and 1.5% in the kelp is close to that found for some (Hobson et al. 1997, Bosley & Wainright 1999), but not all previous investigations (Mullin et al. 1984, Gloutney & Hobson 1998). The extent of depletion varied greatly between species and it is therefore suggested that formalin should not be used as a preservative for storing samples intended for carbon isotope analysis.

Unlike previously suggested (Hobson et al. 1997, Gloutney & Hobson 1998), in the present study ethanol preservation significantly enriched the $\delta^{13}C$ of samples by 0.7 to 1.5%. The increase in the isotope signature relative to dried and frozen samples became significant after 1 wk in animals and after 4 wk in the kelp (Fig. 1, Table 1). While it is possible that the carbon contained within ethanol may have 'contaminated' the samples and thus affected their $\delta^{13}C$ ratios, it is more likely that ethanol acted as a solvent of isotopically lighter compounds present in the samples. For example, a variety of studies (e.g. DeNiro & Epstein 1976, 1978, McCounnaughey & McRoy 1979, Gloutney & Hobson 1998, Doucett et al. 1999, Bernard & Pakhomov unpubl. data) have shown that the extraction of isotopically lighter lipids from body tissues may enrich the whole body $\delta^{13}C$ of an organism (but see France & Peters 1997). As ethanol is known to act as a solvent as well as a preservative, the observed increase in the $\delta^{13}C$ may, therefore, be explained at least partially by lipid extraction (see also Leggett 1998). This solvent effect may also explain why ethanol has a more immediate effect on lipid-rich animal tissues when compared to lipid-poor plant samples. As the extent of ethanol-induced enrichment varies between species and over time, ethanol should not at this stage be used for storing isotopic samples. Should future studies show that the observed enrichment of $\delta^{13}C$ is indeed due primarily to lipid extraction, ethanol may turn out to be a useful preservative for defatted samples.

Similar to $\delta^{13}C$, neither drying nor freezing had any significant effect on the $\delta^{15}N$ signature of any of the samples at any time (Table 2). However, unlike the isotope ratios of carbon, $\delta^{15}N$ exhibited very high within-treatment variability (Fig. 1). Thus, while in the animal samples, formalin and alcohol preservation tended to result in an enrichment of $\delta^{15}N$ (Fig. 1), these differences were not statistically significant. It is likely, however, that larger replicate numbers would have resulted in a significant increase in $\delta^{15}N$, as has previously been shown for formalin (Bosley & Wainright 1999).

When comparing the results from this study to those from other investigations (Fig. 2), certain patterns become apparent. Formalin preservation always depletes the $\delta^{13}C$ signature of samples (marine and terrestrial), while ethanol preservation either enriches or slightly depletes the carbon signatures. While there was a very weak trend of increased effect with length of preservation, variation in the magnitude of the effect was highly variable (e.g. 0.28 to 3% depletion after formalin preservation, Fig. 2) and apparently unpredictable ($r^2 = 0.06$ and 0.19 for ethanol and formalin respectively). As $\delta^{13}C$ step-wise trophic enrichment is frequently <1% (reviewed by France & Peters 1997), formalin- and ethanol-preserved specimens are, therefore, of limited use for comparing carbon isotope signatures of different species from within the same or from different studies.

Table 1. ANOVAs and Student-Newman-Keuls (SNK) test of $\delta^{13}C$ data from 3 organisms on the length of preservation (time) and type of preservation (preservation)

ANOVA

Argyrosomus hololepidotus **($\delta^{13}C$)**

Source	df	MS	*F*	p
Time	3	0.193	6.141	0.002
Preservation	3	1.755	55.791	<0.001
Interaction	9	0.214	6.801	<0.001

Octopus vulgaris **($\delta^{13}C$)**

Source	df	MS	*F*	p
Time	3	0.193	2.767	0.057
Preservation	3	4.349	62.22	<0.001
Interaction	9	0.575	8.234	<0.001

Ecklonia radiata **($\delta^{13}C$)**

Source	df	MS	*F*	p
Time	3	2.372	28.23	<0.001
Preservation	3	5.355	63.70	<0.001
Interaction	9	0.900	10.71	<0.001

SNK tests

Dried:	Week 0 = Week 1 = Week 4 = Week 12
Frozen:	Week 0 = Week 1 = Week 4 = Week 12
Formalin:	Week 0 > Week 1 = Week 4 = Week 12
Ethanol:	Week 0 < Week 1 = Week 4 = Week 12
Week 1:	Formalin = dried = frozen < ethanol
Week 4:	Formalin < dried = frozen < ethanol
Week 12:	Formalin < dried = frozen < ethanol
Dried:	Week 0 = Week 1= Week 4 = Week 12
Frozen:	Week 0 = Week 1= Week 4 = Week 12
Formalin:	Week 0 = Week 1= Week 4 = Week 12
Ethanol:	Week 0 < Week 1< Week 4 = Week 12
Week 1:	Formalin = dried = frozen < ethanol
Week 4:	Formalin < dried = frozen < ethanol
Week 12:	Formalin < dried = frozen < ethanol
Dried:	Week 0 = Week 1= Week 4 = Week 12
Frozen:	Week 0 = Week 1= Week 4 = Week 12
Formalin:	Week 0 > Week 1= Week 4 < Week 12
Ethanol:	Week 0 = Week 1= Week 4 < Week 12
Week 1:	Formalin < dried = frozen = ethanol
Week 4:	Formalin < dried = frozen = ethanol
Week 12:	Formalin < dried = frozen < ethanol

Table 2. ANOVAs and Student-Newman-Keuls test of $\delta^{15}N$ data from 3 organisms on the length of preservation (time) and type of preservation (preservation)

Argyrosomus hololepidotus **($\delta^{15}N$)**

Source	df	MS	*F*	p
Time	3	0.796	6.268	0.002
Preservation	3	0.349	2.746	0.059
Interaction	9	0.430	3.386	0.005

SNK: ethanol at Week 1 < ethanol at Week 4, Week 12

Octopus vulgaris **($\delta^{15}N$)**

Source	df	MS	*F*	p
Time	3	0.156	0.469	0.705
Preservation	3	0.190	0.570	0.638
Interaction	9	0.094	0.283	0.974

SNK: no significant differences amongst the treatments

Ecklonia radiata **($\delta^{15}N$)**

Source	df	MS	*F*	p
Time	3	3.561	8.847	<0.001
Preservation	3	0.902	2.241	0.102
Interaction	9	0.607	1.508	0.187

SNK: formalin at Week 1 < Week 0, Week 4, Week 12

The effects of ethanol and formalin preservation on the $\delta^{15}N$ signatures of samples were less variable than those for $\delta^{13}C$ (r^2 = 0.54 and 0.23 respectively). More importantly, the overall change due to the preservatives was much smaller (with 2 exceptions <1%, Fig. 2) and in most cases not statistically significant (Mullin et al. 1984, Hobson et al. 1997, Gloutney & Hobson 1998, Bosley & Wainwright 1999). As $\delta^{15}N$ trophic enrichment is typically far greater (in the range of 3 to 4%; Owens 1987, Wada et al. 1991) than preservative-induced changes, it might therefore be possible to utilise preserved samples in tropho-dynamic studies that are concerned solely with the isotope signatures of nitrogen.

Animal confinement had an almost immediate effect on the $\delta^{13}C$ and $\delta^{15}N$ of littorinids (Fig. 3). $\delta^{13}C$ became significantly enriched within 6 h and $\delta^{15}N$ became significantly depleted within 12 h of collection. Ultimately, $\delta^{13}C$ and $\delta^{15}N$ were enriched by 2.3% and depleted by 1.2% respectively. While the present study cannot explain these rapid changes, it is likely that the $\delta^{13}C$ enrichment was partially due to the metabolism of lipids during fasting. We do not have any direct data on the lipid content of the littorinids. However, C:N ratios (as provided by the elemental analyser) steadily decreased over the period of the experi-

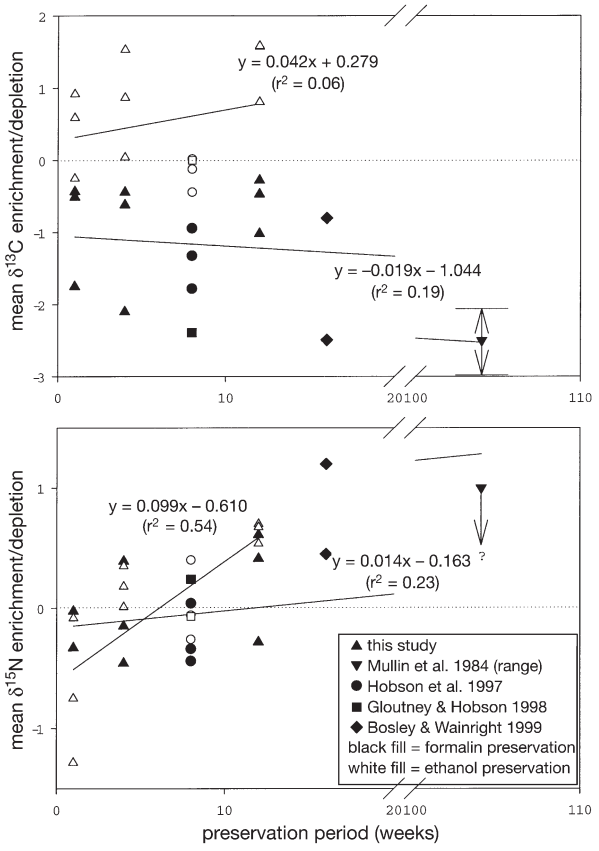

Fig. 2. Summarised effects (% enrichment/depletion) of the preservatives formalin (black) and ethanol (white) on the $\delta^{13}C$ or $\delta^{15}N$ signatures of samples. Data obtained from this and previous studies. Mullin et al. (1984): zooplankton; Hobson et al. (1997): quail and sheep; Gloutney & Hobson (1998): quail egg albumen; Bosley & Wainright (1999): flounder and shrimp. Arrows indicate ranges where mean effects were not available

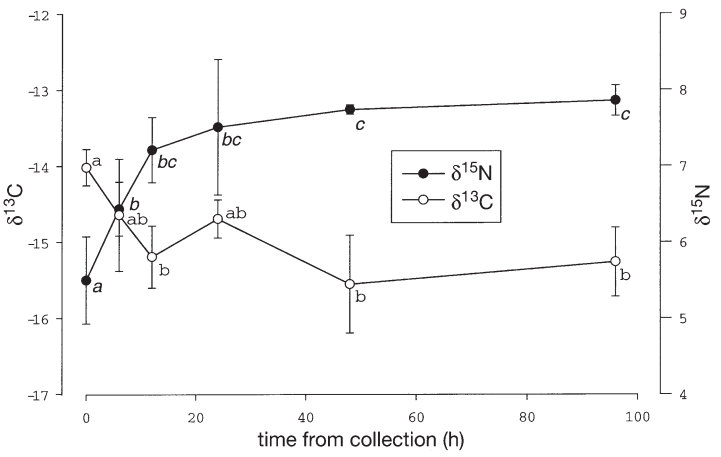

Fig. 3. Mean $\delta^{13}C$ (●) and $\delta^{15}N$ (○) signatures (±SD) of *Nodilittorina africana* after varying periods of confinement. Treatments with identical letters indicate no significant difference (Student Neumann Keuls-tests), while different letters indicate significant changes in $\delta^{13}C$ or $\delta^{15}N$ signatures. ANOVAs on $\delta^{13}C$ (df = 5, $F = 10.147$, $p < 0.001$) and $\delta^{15}N$ (df = 5, $F = 3.180$, $p = 0.026$)

ment, indicating a loss of lipids from the organisms. The rapid depletion of $\delta^{15}N$ may not be readily explained either. It is likely, however, that the re-ingestion of faeces or reduced voiding may have contributed to this process. While we do not fully understand the biochemical processes involved in the enrichment of $\delta^{13}C$ and the depletion of $\delta^{15}N$, it is likely that the small size and rapid turn-over rate of *Nodilittorina africana* facilitates fast changes in isotope ratios. Changes may be far slower in larger organisms with slower turn-over rates. Nonetheless, the data presented here illustrate the potential pitfalls of storing live animals, and it is suggested that organisms should be sacrificed as soon after collection as possible.

The results presented in this paper illustrate that different methods of storing and preserving of marine organisms have significantly different effects on isotope signatures. Combined data from this and other studies suggest that the effects of formalin and ethanol on carbon isotope signatures are highly variable between species and that the use of correction factors may, therefore, not be possible. In contrast, $\delta^{15}N$ signatures are affected to a far lesser degree, which may facilitate the use of preserved samples in trophodynamic studies that are concerned solely with stable isotopes of nitrogen. The current study suggests that freezing (see also Bosley & Wainright 1999) and also oven-drying are reliable methods for preserving samples intended for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis. Current methods of confining animals for gut-evacuation (e.g. DeNiro & Epstein 1978, Haines & Montague 1979, Dunton et al. 1989, France 1995, Frazer 1996, Kaehler et al. 2000) clearly require re-examination. At present, gut-evacuations are frequently carried out on small animals on which dissections are not feasible. As with the littorinids, their small size and fast turn-over rates are likely to facilitate rapid changes in their isotopic composition.

Acknowledgements. This study was partially funded by a National Research Foundation post-doctoral fellowship to S.K. and a Rhodes University Joint Research Comittee grant to E.A.P. We gratefully acknowledge John Lanham and Ian Newton (University of Cape Town, Archaeometry Lab) for helping with the preparation and analysis of the samples.

LITERATURE CITED

Bosley KL, Wainright SC (1999) Effects of preservation and acidification on the stable isotope ratios ($^{15}N:^{14}N$, $^{13}C:^{12}C$) of two species of marine animals. Can J Fish Aquat Sci 56: 2181–2185

Bustamante RH, Branch GM (1996) The dependence of intertidal consumers on kelp-derived organic matter on the west coast of South Africa. J Exp Mar Biol Ecol 196:1–28

DeNiro MJ, Epstein S (1976) You are what you eat (plus a few %); the carbon isotope cycle in food chains. Geol Soc Am Conf Abstract 8:834–835

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

Doucett RR, Booth RK, Power G, McKinley RS (1999) Effects of the spawning migration on the nutritional status of anadromous Atlantic salmon (*Salmo salar*): insights from stable-isotope analysis. Can J Fish Aquat Sci 56:2172–2180

Dunton KH, Saupe SM, Golikov AN, Schell DM, Schonberg SV (1989) Trophic relationships and isotopic gradients among arctic and subarctic marine fauna. Mar Ecol Prog Ser 56:89–97

Fantle MS, Dittel AI, Schwalm SM, Epifranio CE, Fogel ML (1999) A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120:416–426

France RL (1995) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol Oceanogr 40:1310–1313

France RL, Peters RH (1997) Ecosystem differences in the trophic enrichment of ^{13}C in aquatic food webs. Can J Fish Aquat Sci 54:1255–1258

Frazer TK (1996) Stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of larval krill, *Euphausia superba*, and two of its potential food sources in winter. J Plankton Res 18:1413–1426

Gloutney ML, Hobson KA (1998) Field preservation techniques for the analysis of stable-carbon and nitrogen isotope ratios in eggs. J Field Ornithol 69:223–227

Haines EB, Montague CL (1979) Food sources of estuarine invertebrates analysed using $^{13}C/^{12}C$ ratios. Ecology 60: 48–56

Hobson KA, Ambrose WG Jr, Renaud PE (1995) Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: insights from $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 128:1–10

Hobson KA, Gibbs HL, Gloutney ML (1997) Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Can J Zool 75:1720–1723

Jennings S, Reñones O, Moralis-Nin B, Polunin NVC, Moranta J, Coll J (1997) Spatial variation in the ^{15}N and ^{13}C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways. Mar Ecol Prog Ser 146:109–116

Kaehler S, Pakhomov EA, McQuaid CD (2000) Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 208:13–20

Lee SY (2000) Carbon dynamics of Deep Bay, eastern Pearl River estuary, China. II: Trophic relationships based on carbon- and nitrogen-stable isotopes. Mar Ecol Prog Ser 205:1–10

Leggett MF (1998) Food-web dynamics of Lake Ontario as determined by carbon and nitrogen stable isotope analysis. PhD thesis, University Waterloo, Ontario

McConnaughey T, McRoy CP (1979) Food-web structure and fractionation of carbon isotopes in the Bering Sea. Mar Biol 53:257–262

Mullin MM, Rau GH, Eppley RW (1984) Stable nitrogen isotopes in zooplankton: some geographic and temporal variation in the North Pacific. Limnol Oceanogr 29:1267–1273

Owens NJP (1987) Natural variation in ^{15}N in the marine environment. Adv Mar Biol 24:389–451

Thomas CJ, Cahoon LB (1993) Stable isotope analyses differentiate between different trophic pathways supporting rocky-reef fishes. Mar Ecol Prog Ser 95:19–24

Wada E, Terazaki M, Kabaya Y, Nemoto T (1986) ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep-Sea Res 34:829–841

Wada E, Mizutani H, Minagawa M (1991) The use of stable isotopes for food web analysis. Crit Rev Food Sci Nutr 30: 361–371

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: February 6, 2001; Accepted: May 10, 2001
Proofs received from author(s): August 3, 2001