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Measured and inferred gross energy content in diapausing *Calanus* spp. in a Scotian shelf basin

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Calanus finmarchicus stage-C5 and *C. hyperboreus* stage-C4 diapausing in Scotian Shelf basins are high-quality food sources because they are abundant and high in energy content. When combined, these two variables are informative for quantifying energy density, carrying capacity and energy transfer in marine ecosystems. However, measuring energy content directly can be difficult and expensive. Here, we present energy (caloric) content estimates for two co-located diapausing copepod species; *C. finmarchicus* C5 and *C. hyperboreus* C4 collected during late-summer when their energy content is at or near the annual maximum. We then develop several practical energy content calibrations, each designed to estimate energy content using simple measures of *Calanus* spp. body size or weight. Weight-specific energy content (energy quality, kJ g^{-1}) did not differ between *C. finmarchicus* and *C. hyperboreus*; i.e. body size explained the majority of inter-species variation in individual energy content (J $ind.^{-1}$). Energy content estimated directly using calorimetry did not differ from energy inferred from oil sac volume (OSV), demonstrating that energy content can be inferred when direct energy estimates are unavailable. We also assess the effect of formalin preservation on energy content to determine the utility of using archived samples when addressing energy-related questions. There was no difference between directly measured energy content in frozen- and formalin-preserved specimens. However, formalin preservation appears to alter oil sac shape in *C. hyperboreus*, thereby altering energy content estimates inferred from OSV for the species. Formalin-preserved samples can be used for direct energy content estimation if individuals with intact oil sacs are selected.

KEYWORDS: *Calanus*; Roseway Basin; Scotian Shelf; energy content; oil sac volume; preservation

INTRODUCTION

Ecological role of lipid energy stored in Scotian shelf copepods

Calanus spp. are one of the most ubiquitous and abundant members of the mesozooplankton in the North Atlantic and particularly on the Scotian Shelf where they are the primary food of many marine animals, ranging from euphausiids and juvenile silver hake to North Atlantic right whales (Båmstedt, 1986; Sameoto and Herman, 1990; Albers *et al.*, 1996; Baumgartner and Mate, 2003). The late-stage diapausing copepodites,

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typically C4 and C5, are a high-quality food source because they contain accumulated energy-rich storage lipids as an adaptation to the seasonal periods of food shortage (Lee, 1974; Hirche, 1996; Lee *et al*., 2006). The copepods accumulate lipids by feeding on phytoplankton near the surface during spring and summer. The lipid energy is stored primarily as wax esters in large oil sacs that generally comprise >50% of the body cavity (McLaren *et al*., 1989; Hirche, 1997; Pepin and Head, 2009). Throughout autumn, the C4s and C5s enter diapause and rest over the winter in Shelf basins at depths >100 m, surviving solely on their lipid reserves.

Estimates of copepod energy content are desirable for a variety of ecological applications that include models of ecosystem function, energy flow, carrying capacity and physiology; these are the applications of significance to the Scotian Shelf ecosystem and similar systems elsewhere. A key application of energy estimation in the field of trophic ecology is its use in calculating the energy density (kJ m^{-3}) of local habitat-scale copepod populations. Answers to energetic questions are typically inferred from simple abundance measurements (e.g. Beardsley *et al*., 1996; Baumgartner and Mate, 2003; Zhou *et al*., 2009), whereas others also estimate oil sac volume (OSV) as a proxy for energy content (Reiss *et al*., 1999; Pepin and Head, 2009). Less often, population level energy density is measured as the product of individual energy content and abundance (Michaud and Taggart, 2007, 2011). There is a clear advantage to incorporating direct estimates of energy content into such studies, but to do so reliably requires that various factors be considered. These include but are not limited to: (i) how do we approximate copepod energy in systems containing different species that fulfill similar ecological roles? (ii) how do we best infer energy content using the simplest of techniques? and (iii) can we derive reliable estimates from chemically preserved specimens? The answers to these questions may go a long way toward advancing the science by introducing the use of energy content into a broader spectrum of research programs and by providing estimates that can be drawn from the extant literature as well as archived material.

Environmental influences on *Calanus* spp. energy content

Diapausing copepods, dominated in late summer by *Calanus finmarchicus* stage-C5 copepodites (hereafter CF5) and *Calanus hyperboreus* stage-C4 copepodites (hereafter CH4), are co-located in deep basins on the western and central Scotian Shelf (Sameoto and Herman, 1990; Herman *et al*., 1991; Sameoto and Herman, 1992; Head *et al*., 1999). *Calanus hyperboreus* is a colder water species transported from the Labrador Sea through the Gulf of St Lawrence and along the Scotian Shelf via the Nova Scotia coastal current, whereas shelf populations of *C. finmarchicus* have multiple origins; from the Gulf of St. Lawrence, from Scotian Shelf basins and from the continental shelf slope waters. The co-location of the two species in central and western Shelf basins is due to water mass mixing on the continental shelf.

In response to the long periods of food shortage, especially in high-latitude arctic regions, *C. hyperboreus* tend to have a larger body size and accumulate a greater percentage of longer chain, higher energy, wax esters than do *C. finmarchicus* (Albers *et al*., 1996; Falk-Petersen *et al*., 2009). These two properties make the former species energetically more valuable to predators in Arctic food webs. This inter-species variation necessitates that the energy content and size distribution of each species be measured separately and employed separately in ecological applications. Both species, however, exhibit remarkable plasticity in body size, energy content and life span in response to their highly variable environments (Falk-Petersen *et al*., 2009) such that their relative energetic values at lower latitudes may vary.

Energy content of *Calanus* spp. inferred from OSV

OSV is a metric widely used to estimate relative changes in copepod energy content (Plourde and Runge, 1993; Reiss *et al*., 1999; Pasternak *et al*., 2001; Hassett, 2006; Pepin and Head, 2009) because it is easily estimated using morphometric dimensions obtained by microscopy and imaging. Direct energy content measurements require specialized personnel and equipment and a high degree of precision. In part, for these reasons, significant research effort has been expended to develop empirical relations between the OSV and energy content in marine zooplankton; particularly *C. finmarchicus* (e.g. Arts and Evans, 1991; Miller *et al*., 1998). The research addresses issues such as the geometrical shape equations used to approximate the OSV in relation to the orientation of the animal when measured, and the various techniques used to directly estimate energy content. OSV-inferred wax ester content is well correlated with the direct chromatographic measurements of wax ester, but there is evidence that the former overestimates the latter (Miller *et al*., 1998; Vogedes *et al*., 2010), and alternatives such as the oil sac area (OSA) being used as a proxy for energy content have been proposed (Vogedes *et al*., 2010).

The effect of preservation on energy content estimation

Sample freezing (typically flash-freezing in liquid nitrogen) is the standard preservation method used for energy content analysis of zooplankton (e.g. Omori, 1978; Miller *et al*., 1998, 2000; Michaud and Taggart, 2007). Most zooplankton samples collected for reasons other than energy content analysis are usually preserved in 4 to 10% formalin. Formalin collections represent a massive, readily available, historical catalog of biological material that is a potentially valuable source of information when energy content estimation becomes desirable or necessary at some later date. Formalin-preserved samples are not normally used for energetic studies because formalin causes some lipid loss by either oil sac leakage or exchange with the surrounding preservation solution through the anal pore after death (Morris, 1972; Steedman, 1976; Salonen and Sarvala, 1985). There is also some evidence that the hydrolysis of the lipid and the degradation of the polyunsaturated fatty acids may occur (Morris, 1972). However, researchers addressing energy content analysis use only those animals that retain intact oil sacs regardless of the preservation technique (e.g. Reiss *et al*., 1999). Thus, it is possible that some lipid loss may be tolerated as long as there are individuals that retain intact oil sacs. It should be noted that zooplankton samples may also be preserved in 95% alcohol, but these are not suitable for energetic analysis (Morris, 1972).

Objectives

Here, we address the above questions and associated issues by measuring the gross energy (J) content of diapausing CF5 and CH4 field samples collected in Roseway Basin (43°N, 65.2°W) on the western Scotian Shelf that were preserved by freezing or in 4% buffered formalin. We do so by examining empirical relations between and among direct energy content estimates for each species and the inferred energy content estimates. We also determine the value of formalin-preserved and formalin-archived samples as source material for energy content estimation.

METHOD

Samples

Zooplankton samples were collected at several stations in Roseway Basin aboard the R/V Dominion Victory in September 2007. September coincides with the timing of maximum CF5 concentration and energy content at depth (Michaud and Taggart, 2007, 2011). Our results and inferences extend only to copepodites that have recently entered diapause and have full oil sacs; that is, they are at or near their annual maximum in energy content as occurs elsewhere (Comita *et al*., 1966; Scott *et al*., 2000) and thus represent a high-quality food source. A Bedford Institute of Oceanography Net and Environmental Sampling System (BIONESS; Sameoto *et al*., 1980) towed at a nominal 1 m s^{-1} and equipped with seven 333-μm mesh nets was used to collect the samples. Only those nets ($n = 9$) that opened and closed at depths >108 m (range 108–150 m) were used in our analysis. Upon net retrieval, two 5-mL subsamples from each net were frozen in liquid nitrogen and later transferred to a −70°C freezer. The remainder of each sample was preserved in 4% buffered formalin for taxonomic and abundance analysis. Samples were preserved for 1–2 years before undergoing energy content analysis.

Energy content measured by calorimetry

All zooplankton were identified and counted following Michaud and Taggart (Michaud and Taggart, 2007). The average proportion of mesozooplankton within each net attributed to CF5s and CH4s was estimated, and energetic analyses were performed on each.

Gross energy content was estimated using a Parr® 1266 semi-micro oxygen-bomb calorimeter following Michaud and Taggart (Michaud and Taggart, 2007). Individuals were selected from all net collections as needed under the assumption that horizontal spatial variation among variables at scales <10 km within the Basin was negligible. Only animals with clearly intact oil sacs were selected for analysis. Copepodite prosome length (PrL, mm) and width (PrW, mm) were measured using a dissecting microscope and an ocular micrometer. PrL and PrW were converted to equivalent spherical diameter (ESD) using the prolate spheroid volume approximation. Copepods were then sorted among six geometric mean ESD size classes (Table I) selected to correspond with a range of digital size classes measured using an optical plankton counter (OPC) (Michaud and Taggart, 2011). We did this for the eventual estimation of size-specific energy terms using OPC abundance-at-size data where previously an average-energy term has been applied across all OPC size classes (e.g. Michaud and Taggart, 2011).

Three replicates from each of the frozen-preserved and two replicates from the formalin-preserved samples were analyzed in this manner. Calorimeter sensitivity did not allow for the analysis of individual copepods; hence each replicate consisted of 20 individuals within

Table I: Summary of the C. finmarchicus stage-C5, CF5, and C. hyperboreus stage-C4, CH4, sorting methods indicating preservation, size (ESD) ranges with the corresponding geometric mean ESD (μm) for each size class and the number of replicates for each measurement method (EC_{CAL} and EC_{OSV})

				Number of replicates	
THIS species	Preservation	Size range (μm)	Geometric mean ESD	EC_{CAL} [a]	EC_{OSV} [b]
Calanus finmarchicus (CF5)	Frozen	823–936	880	3	20
		943–1058	1001	3	20
		1064–1182	1123	3	20
		1187–1307	1247	3	20
	Formalin	823–936	880	2	20
		943–1058	1001	2	20
		1064–1182	1123	2	20
		1187–1307	1247	2	20
Calanus hyperboreus (CH4)	Frozen	1187–1307	1247	3	20
		1312–1434	1373	3	20
		1439–1562	1501	3	20
	Formalin	1187–1307	1247	2	20
		1312–1434	1373	2	20
		1439–1562	1501	2	20

[a] Each replicate contains a bulk sample of 20.
[b] Each replicate is an individual copepod.

each size class (35 bulk samples). Each bulk sample was wet-weighed using a Mettler® AJ 100 balance (±0.0001 g) and freeze-dried for 8 h. Samples were then dry-weighed, pressed into 3 mm diameter pellets and combusted in random order using one of the two calibrated Parr® 1107 oxygen micro-bombs. Details regarding calibration, corrections for nitric and sulfuric acid formation and conversion factors are provided in Michaud and Taggart (Michaud and Taggart, 2007). Energy content estimates are presented as dry-weight specific gross energy content (EC_{DW}; kJ g^{-1}) and gross energy content per individual copepod (EC_{CAL}, J) that is the product of EC_{DW} and the pellet dry weight divided by the number (20) of copepods in the bulk sample. Ten individuals from each species, size class and preservation technique were sorted from the original collections as above, freeze dried and individually weighed using a Sartorius® balance (±0.00001 g).

Inferred energy content

One replicate from each size class within each preservation technique destined for calorimetry was first photographed using a Nikon® Coolpix 995 digital camera for micrometer-calibrated image analysis. PrL, PrW, and oil sac length and width were measured using ImageJ (version 1.41) freeware. OSV (mm^3) was estimated using the cylindrical approximation (Miller *et al.*, 1998). Lipid content (g) was estimated from OSV using a lipid density of 0.9 g mL^{-1} (Miller *et al.*, 1998; Visser and Jonasdottir, 1999). Individual energy content (EC_{OSV}, J) was inferred from lipid content assuming a lipid-energy content of 39.5 kJ g^{-1} (Lamprecht, 1999). The prosome volume was calculated from PrL and PrW assuming a prolate spheroid and OSV was expressed as the proportion of the prosome volume. Comparisons of prosome and oil sac shapes were made among species and preservation techniques.

Preservation effects

We quantified the effects of preservation on individual dry weight, pellet dry weight, EC_{DW}, EC_{CAL} and EC_{OSV} by comparing all variables between the frozen- and formalin-preserved samples within and between species.

Statistical analyses

Size was treated as a covariate in our statistical analysis because the size classes were chosen for logistical reasons and not for any particular biological relevance. Statistical tests were performed separately for each species because their size ranges did not overlap across all size classes. Differences in EC_{DW} or DW (both individual copepod and bulk-sample pellet) among size classes and between preservation techniques were assessed using analysis of covariance (ANCOVA). Differences in EC_{DW} between species, ignoring size, were subsequently determined using Student's *t*-test. The effects of size, preservation and measurement methods (EC_{CAL} and EC_{OSV}) on individual energy content were determined using a generalized linear model that allowed us to (i) accommodate the unbalanced design (type III sum of squares) and (ii) use a mixture of covariates (size) and discrete explanatory variables (preservation and measurement methods). EC_{CAL} was expressed as a function of pellet dry weight and size, ignoring species, and analyzed using linear regression. Finally, the proportion of the prosome volume occupied by the OSV was compared between preservation techniques using a *t*-test on arcsine square-root transformed data. Averages for all relevant metrics are presented as ±1 SD.

RESULTS

Energy content measured by calorimetry

Calanus finmarchicus C5 and *C. hyperboreus* C4 copepodites comprised 64 ± 17 and 19 ± 9% of the mesozooplankton abundance, respectively, at collection depths >108 m in Roseway Basin. The remaining 17% was comprised of *C. finmarchicus* C4 and adult stages, *C. glacialis*, *Centropages* spp., *Metridia* spp. and *Pseudocalanus* spp.

Size explained the majority of variation in both dry weight and individual energy content. EC_{DW} did not vary with size within each species and there was no significant interaction between size and preservation (Table II; Fig. 1A and B). There was no difference in EC_{DW} between species ($t = 0.87$; $P = 0.390$; df = 27) and therefore the overall average was estimated at 27.9 ± 5.0 kJ g^{-1}. Pellet dry weight increased with size regardless of preservation and again there was no significant interaction between size and preservation (Table III, Fig. 1C and D). Similarly, the CF5 (Fig. 1E) and the CH4 (Fig. 1F) individual energy content estimates both increased as a function of size with no significant interaction between preservation techniques (Table IV). Estimates of EC_{CAL} among frozen CF5s increased with size from 4.4 ± 0.79 to 10.9 ± 2.2 J between the smallest and largest size class. The frozen CH4s contained greater energy content than frozen CF5s, varying between 9.4 ± 1.5 and 12.7 ± 2.5 J. Within the overlapping size class, there was no difference in EC_{CAL} estimated using frozen samples of the two species ($t = 0.56$; $P = 0.108$, df = 5). This means that, for all practical applications, EC_{CAL} varies only as a function of size and not with species or preservation ($r^2 = 0.75$, $P < 0.001$, Fig. 2A). EC_{CAL} can also be expressed as a function of dry weight; EC_{CAL} estimates increased with pellet DW across all size classes ($r^2 = 0.76$; $P < 0.001$, Fig. 2B).

Inferred energy content

Energy content estimates did not differ between the EC_{CAL} and EC_{OSV} measurement methods (EC_{METHOD}) for the two species and there were no significant interaction terms between EC_{METHOD} and size (Table IV). For the CH5s, there was also no EC_{METHOD} × preservation interaction term. There was, however, a significant EC_{METHOD} × preservation interaction term for CH4s. The difference between species in the EC_{METHOD} × preservation interaction term is apparent by comparing Fig. 1E–H. There was no effect of preservation on either species when energy content was measured using calorimetry (Fig 1E and F). When CH4 energy content was inferred from OSV, the EC_{OSV} estimate (Fig. 1H) was significantly smaller in the preserved animals than in the frozen animals at all sizes, whereas there was clearly no difference in EC_{CAL} between the frozen- and formalin-preserved CF5s (Fig. 1G).

Table II: Results of an ANCOVA test for the effects of size (μm), and preservation (Pres), on dry-weight specific energy content (EC_{DW}, kJ g^{-1}) of C. finmarchicus stage-C5, CF5, and C. hyperboreus stage-C4, CH4

Species	Source of variation	Sum of Squares	df	Mean Square	F	*P*-value
Calanus finmarchicus (CF5)	Size	112.03	1	91.77	2.52	0.132
	Pres[a]	4.92	1	13.45	0.37	0.552
	Pres × Size	15.64	1	15.64	0.43	0.522
	Error	582.79	16	36.42		
	Total	715.39	19			
Calanus hyperboreus (CH4)	Size	4.87	1	5.07	0.62	0.448
	Pres[a]	11.58	1	0.05	0.01	0.942
	Pres × Size	0.20	1	0.20	0.02	0.879
	Error	90.01	11	4.334		
	Total	106.66	14			

CF5: $r^2 = 0.19$; CH4: $r^2 = 0.16$.

[a] Preservation = frozen or formalin preserved.

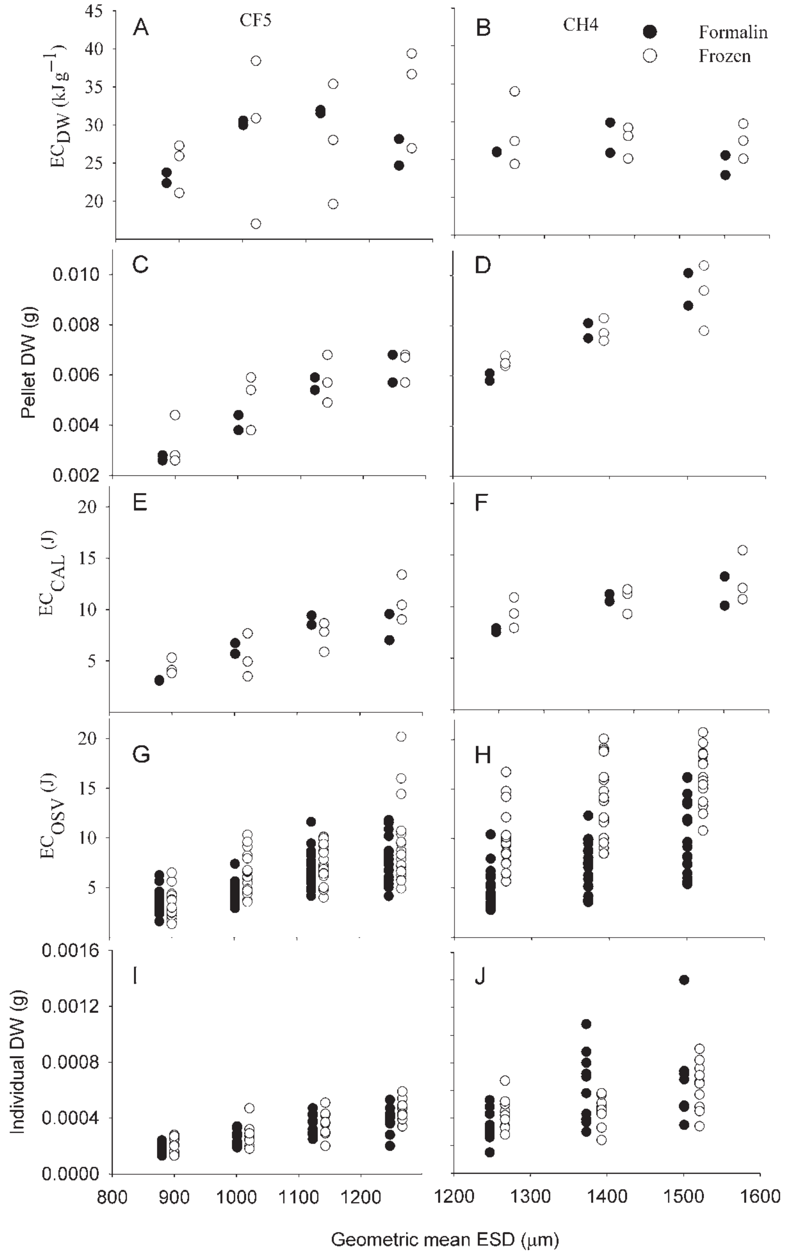

Fig. 1. Copepod energy content (EC) measured by the weight-specific calorific value (EC_{DW}, kJ g^{-1}; **A** and **B**), dry weight of the sample pellet used for calorimetry (**C** and **D**), individual energy content measured by calorimetry (EC_{CAL}; **E** and **F**) or inferred from volumetric oil sac approximations (EC_{OSV}; **G** and **H**), wherein each increases with size (geometric mean ESD, μm) in both frozen- (open symbols) and formalin-preserved (closed symbols) *C. finmarchicus* stage-C5, CF5 (A, C, E, G and **I**) and *C. hyperboreus* stage-C4, CH4 (B, D, F, H and **J**). Dry weight measured on individual copepods (I and J) also increased with size. For EC_{DW}, pellet DW and EC_{CAL}, each symbol represents a bulk sample of 20 copepods. Animals in the frozen-preserved ESD category are offset by 20 μm for clarity.

Preservation effects

There was no effect of preservation on EC_{DW} estimates among the CF5 (Table II) samples or on their individual energy content estimates (Table IV; frozen 6.04 ± 3.09 J; formalin 5.41 ± 2.49 J). There was also no effect of preservation on the CH4 estimates of EC_{DW} (Table II). However, the specimens of CH4 had different preservation effects on individual energy content depending on the measurement method (Table IV). There appeared to be no effect of preservation on EC_{CAL} (Fig. 1F), whereas formalin-preserved specimens had lower EC_{OSV} estimates than frozen-preserved specimens (Fig. 1H). Consistent with the lack of preservation effect on EC_{CAL} for either species, we

Table III: Results of an ANCOVA test for the effects of size (μm), and preservation (Pres), on pellet dry weight (g) of C. finmarchicus stage-C5, CF5 and C. hyperboreus stage-C4, CH4

Species	Source of variation	Sum of squares ($\times 10^{-5}$)	df	Mean square ($\times 10^{-5}$)	F	*P*-value
Calanus finmarchicus (CF5)	Size	3.00	1	2.99	87.62	<0.001
	Pres[a]	0.01	1	0.01	0.85	0.370
	Pres × Size	0.01	1	0.01	0.75	0.399
	Error	0.55	16	0.01		
	Total	3.57	19			
Calanus hyperboreus (CH4)	Size	2.22	1	2.26	49.22	<0.001
	Pres[a]	0.01	1	0.01	1.02	0.333
	Pres × Size	0.01	1	0.01	0.98	0.343
	Error	0.05	11	0.01		
	Total	2.29	14			

CF5: $r^2 = 0.82$; CH4: $r^2 = 0.77$.

[a] Preservation = frozen or formalin preserved.

Table IV: Results of a generalized linear model test for the effects of size (ESD, μm), preservation (Pres) and energy content measurement method (EC_{METHOD}) on individual energy content (J) of C. finmarchicus stage-C5, CF5, and C. hyperboreus stage-C4, CH4

Species	Source of variation	Type III sum of squares	df	Mean square	*F*-value	*P*-value
Calanus finmarchicus (CF5)	Size	649.87	1	294.02	70.44	<0.001
	EC_{METHOD}[a]	11.99	1	1.34	0.32	0.572
	Pres[b]	34.80	1	9.43	2.26	0.135
	EC_{METHOD} × Pres	1.22	1	1.22	0.29	0.589
	EC_{METHOD} × Size	3.19	1	2.41	0.58	0.449
	Size × Pres	13.36	1	13.36	3.20	0.075
	EC_{METHOD} × Pres × Size	0.77	1	0.12	0.10	0.750
	Error	722.12	172	4.17		
	Total	1436.55	179			
Calanus hyperboreus (CH4)	Size	747.13	1	198.36	26.41	<0.001
	EC_{METHOD}[a]	1.63	1	15.74	2.10	0.150
	Pres[b]	925.81	1	0.00	0.00	0.980
	EC_{METHOD} × Pres	76.43	1	76.43	10.18	0.002
	EC_{METHOD} × Size	14.69	1	15.31	2.04	0.156
	Size x Pres	1.91	1	1.91	0.25	0.615
	EC_{METHOD} × Pres × Size	0.10	1	0.10	0.02	0.681
	Error	961.28	127	7.51		
	Total	2729.65	134			

CF5: $r^2 = 0.48$; CH4: $r^2 = 0.63$.

[a] $EC_{METHOD} = EC_{CAL}$, EC_{OSV}.

[b] Preservation = frozen or formalin preserved.

did not find an effect of preservation on either pellet dry weight (Table III) or on individual dry weight (CF5 $P = 0.055$; CH4 $P = 0.295$) for either species (Fig. 1B, C, I and J).

The lower EC_{OSV} measured on the formalin-preserved specimens of CH4 compared with frozen-preserved specimens may be explained by variation in the oil sac shape. Formalin-preservation appeared to result in longer, thinner oil sacs in both species but the effect was more pronounced in CH4s (Fig. 3A and B). Variation in the oil sac width among CH4 individuals also decreased substantially in formalin. CH4 formalin-preserved oil sacs occupied 5–25% of the prosome volume, whereas frozen-preserved oil sacs occupied significantly more, ranging between 10 and 45% ($t = 6.03$; $P \leq 0.001$, df = 116, Fig. 3D and F). Differences were less substantial, though significant, among the CF5s, where frozen-preserved oil sacs occupied 10–60% of the body volume, whereas formalin-preserved sacs comprised between 10 and 40% ($t = 2.56$; $P = 0.011$, df = 154, Fig. 3C and E). Taken together, these results imply that when preserved in formalin, the larger CH4 oil sacs appear to change the shape compared with the smaller CF5 oil sacs.

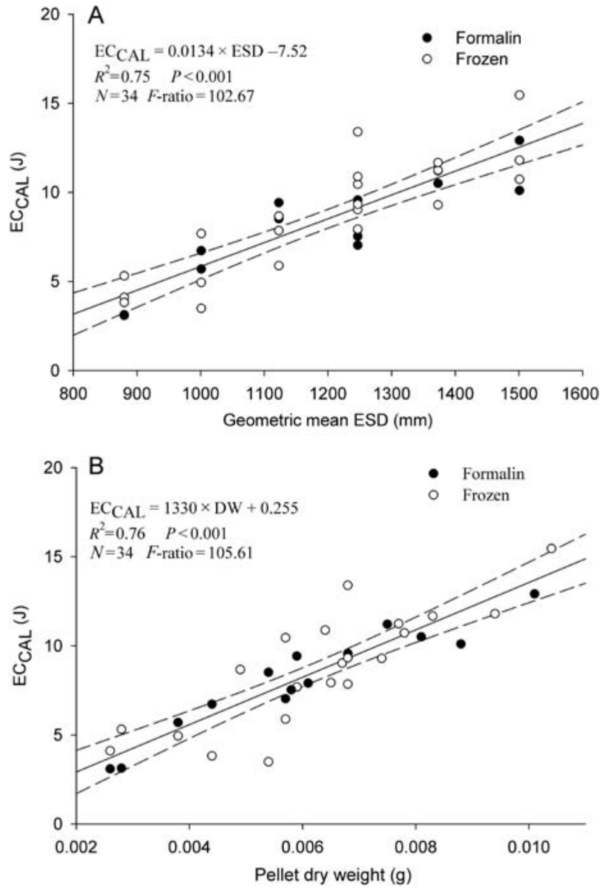

Fig. 2. Relationship between individual energy content (EC_{CAL}, J) and size (**A**) or pellet DW (**B**) for frozen-preserved (open symbols) and formalin-preserved (closed symbols) *C. finmarchicus* stage-C5, CF5, and *C. hyperboreus* stage-C4, CH4. Both species and preservation techniques were combined in a single regression equation because no statistical differences were found within each effect. Dashed lines are the 95% confidence intervals around the regression model and each symbol represents a bulk sample of 20 copepods.

DISCUSSION

Energy content

Using calorimetry, we directly measured gross energy content in diapausing *Calanus* spp. collected at depth on the Scotian Shelf during late summer and related the measures to morphometric quantities that are easily measured in the lab and (or) field. In making these measurements, we were able to determine a number of relations that provide greater insights into how energy content estimates can be determined when using frozen- or formalin-preserved material and using a range of metrics. First, we established that size (ESD) explains most of the variation in directly measured individual energy content (EC_{CAL}) and that the variation was not significantly influenced by the formalin-preservation technique. Second, we determined no difference in EC_{DW} (a measure of energy quality) between the species and the stages examined and that diapausing *C. finmarchicus* and *C. hyperboreus* on the Scotian Shelf have a dry weight-specific energy content of 27.9 ± 5.0 kJ g^{-1}. Third, we determined that among overlapping size classes, there is no difference in EC_{CAL} between the two species and the stages. Thus, we can conclude that ecologists can use these "calibrations" to estimate energy available to *Calanus* spp. predators and can do so by using relatively simple and readily available sampling devices and simple metrics (e.g. size).

The usefulness of our "calibrations" rest on the accuracy of the calorimetry measurements, something achieved through comparisons with energy measurements made on *Calanus* spp. elsewhere in the North Atlantic. Estimates of EC_{DW} among large copepods, including CF5s and CH4s, vary between 17 and 30 kJ g^{-1} at low-to-mid latitudes and increases at higher latitudes (Båmstedt, 1986), a range that brackets our average EC_{DW} estimate. Energy content in CF5s measured in nearby Grand Manan Basin was 32.1 ± 13.5 kJ g^{-1} (Michaud and Taggart, 2007), an estimate that also brackets our estimate, and more importantly, it is an estimate that did not vary across size (as in our results) or across the May through Oct season. From this, we can reasonably conclude that our results based on September samples would be constant over the same seasonal period, particularly because Comita *et al.* (Comita *et al.*, 1966) estimated 31.2 ± 0.8 kJ g^{-1} over the May though Oct period in the Clyde Sea. Thus, our results are consistent with the independent results above. The relatively small differences in EC_{DW} estimates among studies could be attributed to geographic variability, time of year, depth distribution or feeding conditions (Pepin and Head, 2009). We can also reasonably conclude that the effect of individual variability in energy content among copepods was minimized in our study relative to other studies (e.g. Båmstedt, 1988) by using diapausing copepods collected at depth during the time of year when oil sacs are near their maximum (Miller *et al.*, 2000) and have the lowest variability among individuals (Pepin and Head, 2009).

Environmental influences on *Calanus* spp. energy content

Higher trophic level predators in Roseway Basin feed on a mix of co-located CF5s and CH4s. We found that individual energy content in frozen CF5s and CH4s varied only because larger animals contained greater energy (EC_{CAL}) than smaller animals, and in contrast to Arctic food webs, this was not due to variation in energy quality (EC_{DW}) between species. This makes the

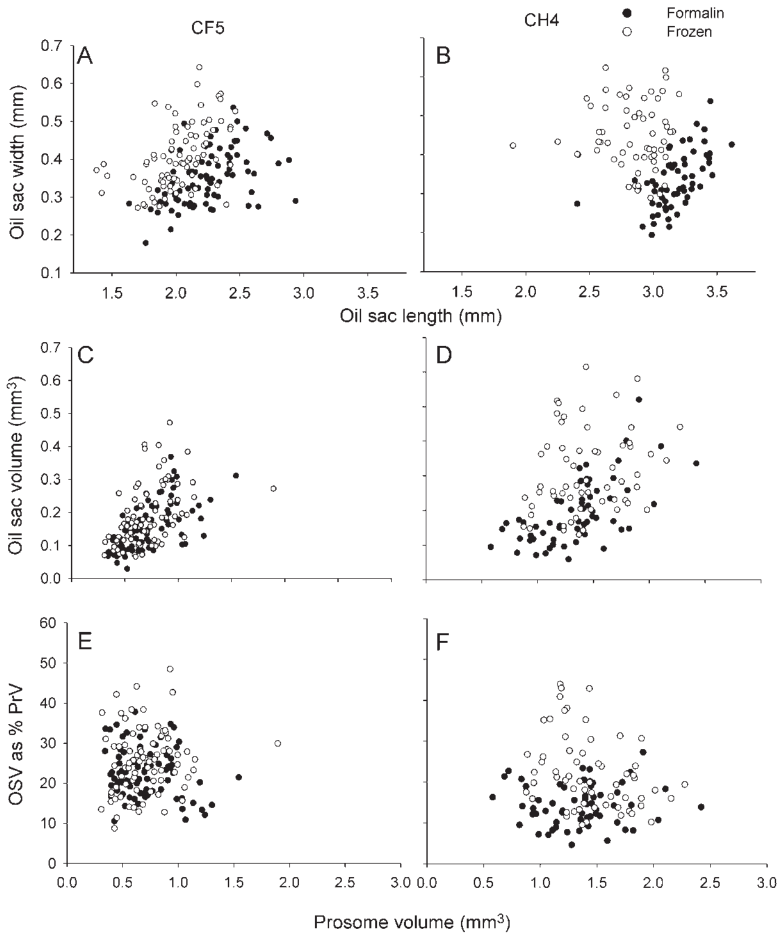

Fig. 3. Scattergrams of relations between oil sac width (mm) and oil sac length (mm) for frozen- (open symbols) and formalin-preserved (closed symbols) *C. finmarchicus* stage-C5, CF5 (**A**), and *C. hyperboreus* stage-C4, CH4 (**B**), illustrating the variation in the effect of preservation on oil sac shape between the two species. The differential preservation effect is further illustrated with CF5 (**C** and **E**) and CH4 (**D** and **F**) cylindrical OSV (mm^3) and cylindrical OSV as a percentage of the prosome volume, in relation to its prosome volume (mm^3).

application of energy terms to abundance-at-size measurements of the two co-located species straightforward in our study area, which is particularly useful when using remote-sensing techniques such as the optical plankton counter. Arctic *C. hyperboreus* have slightly greater calorific content than *C. finmarchicus* because wax esters in the former have a greater proportion of long-chain 22:1(*n*-11) fatty acids (Albers *et al.*, 1996), likely an adaptation to the extreme Arctic environment. Our data imply that the more temperate populations of *C. hyperboreus* need not accumulate such long-chain fatty acids because they do not experience the long periods of food shortage typically experienced by Arctic populations. This type of variation has been measured in Arctic (Fram Strait, 78°N) and north-temperate (North Sea, 58°N) populations of *C. finmarchicus* (Kattner, 1989).

The presence of the cold Nova Scotia coastal current on the Scotian Shelf means that individual copepods diapausing in the Shelf basins are more energetically valuable to their predators compared with those diapausing nearby in Grand Manan Basin, Bay of Fundy. We offer two explanations as to why this may be so. First, the coastal current brings *C. hyperboreus* to the Shelf basins but only periodically does it intrude further to the southwest into the warmer waters of the Bay of Fundy and Gulf of Maine (Aretxabaleta *et al.*, 2008, 2009), where this arctic species is typically found at low abundance ($8\,m^{-3}$; CF5:CH4 80:1; Michaud and Taggart, 2007). Second, lower temperatures promote larger copepod body weight (Kattner, 1989; McLaren *et al.*, 1989) and total energy scales positively with body weight as detailed above. It is not surprising, then, that we found individual CF5 energy content in Grand Manan Basin (Bay of Fundy) was on average less than half ($EC_I = EC_{CAL}$, 3.31 J, range 1–5 J; Michaud and Taggart, 2007) that reported here for Roseway Basin at 6.9 J (range 3–11 J). The range of CF5 prosome lengths was not different between the two basin habitats

(2–3 mm); however, individual CF5s in Roseway Basin weighed twice that per unit length ($\mathrm{DW} = -7.13 \times 10^{-4} + 4.40 \times 10^{-4}$ PrL) observed for CF5s in the Grand Manan Basin ($\mathrm{DW} = -3.8 \times 10^{-4} + 2.13 \times 10^{-4}$ PrL; Michaud, 2005). These are relevant ecological findings. For example, species such as endangered right whales rely on copepod populations in both habitats, and the energetic relationship between right whales and diapausing copepods is critical to several aspects of right whale recovery (Klanjscek *et al.*, 2007; Michaud and Taggart, 2011; Miller *et al.*, 2011).

Energy content of *Calanus* spp. inferred from OSV

Gross energy content inferred from OSV was not different from energy content measured using calorimetry on frozen samples ($\mathrm{EC_{CAL}}$). Thus, energy content inferred from OSV offers a simple and practical metric for addressing questions related to absolute energy content (i.e. converting relative measures into units of J using the same conversion factors we employed). Accordingly, studies that use copepod OSV in a relative manner (e.g. Plourde and Runge, 1993; Reiss *et al.*, 1999; Pasternak *et al.*, 2001; Hassett, 2006; Pepin and Head, 2009) may be interpretable in an absolute context if the analytical protocols we provide are employed. The OSV metric has an additional advantage over calorimetry because it can be estimated in the field using simple body size metrics.

Vogedes *et al.* (Vogedes *et al.*, 2010) argue that OSV-inferred energy content overestimates the chromatographic estimates of wax ester content by a factor of 2. The results of their study and ours are not directly comparable because we used fresh-frozen animals for photo-microscopy while they used live animals. However, we show no similar discrepancy between energy content estimated from calorimetry and that inferred from OSV. We were able to make a direct comparison between total energy ($\mathrm{EC_{CAL}}$) and oil sac energy ($\mathrm{EC_{OSV}}$) because our measurements used only copepods that had recently entered diapause and hence had full oil sacs; in this state, the relationship between CF5 calorific value and wax ester (oil sac) energy measured by chromatography centers around 1:1 (Michaud and Taggart, 2007). Vogedes *et al.* (Vogedes *et al.*, 2010) also developed an oil sac cross-sectional area calibration and concluded that the OSA approximation has greater predictive power for estimating wax ester content than does an OSV approximation. However, using OSA as a proxy for a volumetric quantity such as energy content assumes that the oil sac shape is constant. It is easily demonstrated that a spherical oil sac and a long, thin cylindrical oil sac of equal area have different volumes and hence estimates of wax ester content will be different. Variation in the oil sac shape among copepod species, and under different environmental conditions, may thus limit the predictive power of the Vogedes *et al.* (Vogedes *et al.*, 2010) OSA calibration. The area "proxy" may be unnecessary in any event because our results are consistent with the conventional use of OSV-inferred energy content.

The effect of preservation on energy content estimation

Archived samples of CF5s preserved in formalin can potentially be used to answer energetic questions. Direct and inferred estimates of energy content among lipid-replete CF5s preserved in formalin for up to 2 years did not differ from estimates derived from those preserved by freezing; the latter being the standard preservation method used in most energetic studies. We caution that oil sac integrity is not maintained by all individuals preserved in formalin. Oil (lipid) droplets were prevalent in the preservation solution in our sample collections and there were many individuals with obviously ruptured oil sacs. However, our analysis shows that a reliance on only those newly diapausing and preserved CF5 individuals with full and intact oil sacs can provide reliable, unbiased estimates of gross energy content. The degradation of the polyunsaturated fatty acids has been hypothesized to occur in formalin (Morris, 1972). Our results indicate that either degradation did not occur during the sample storage over 1–2 years or that degradation, presumably to shorter fatty acid chains, does not measurably decrease the energy quality, simply because $\mathrm{EC_{DW}}$ did not vary between the two preservation techniques.

In contrast to the CF5s, the CH4s appear to be more susceptible to oil sac shape change in formalin, and this may preclude the use of the simpler method of inferring energy from OSV. Both calorimetry and dry weight (direct measures) showed no effect of preservation on CH4 energy content, whereas inferred energy content did show a preservation effect. This preservation effect on inferred energy content could be due to the geometric shape approximation applied. To demonstrate this, we applied a correction factor that converted cylindrical OSV measured from the lateral aspect to the average between OSV measured from a lateral and a dorsal aspect, a metric provided by Miller *et al.* (Miller *et al.*, 2000; their Fig. 2) for use with CF5s because the oil sacs of larger organisms become oblate rather than circular in the transverse section, and we assumed that the larger CH4s underwent the same shape change. When applied, the correction decreased the difference in CH4 cylindrical EC_{OSV} between preservation techniques by 7%. We then assumed that a frozen CH4 oil sac reflects that of a fresh oblate spheroid oil sac and that formalin moulds it into a longer, more cylindrical phenotype, consistent with the morphometric changes we measured. Under this assumption, the difference between formalin-preserved and frozen animals declined by as much as 11%. It is possible, then, that the "effect" of formalin on EC_{OSV} was an artifact of the geometric approximation, and different approximations should be used in such a case.

There are two caveats when using formalin preserved CF5 specimens: (i) only those specimens with intact oil sacs should be chosen for analysis and (ii) time and handling likely affect oil sac integrity. Vigorous shaking may easily damage delicate oil sacs. The rate of passive lipid loss is probably highest in the first few weeks and declines thereafter. Dry weight loss was 37% (Giguere *et al.*, 1989) and 20% (Omori, 1978) in copepods preserved for 1 week, whereas after 1 year a similar 30–35% loss was measured (Bottger and Schnack, 1986).

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