

Storage lipids of the copepod *Calanus finmarchicus* from Georges Bank and the Gulf of Maine

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Abstract

Storage lipids of fifth copepodites (C5) of *Calanus finmarchicus* from Georges Bank and the Gulf of Maine were quantified from video images and by Iatroscan (thin-layer chromatography with flame-ionization detection, FID). Oil withdrawn from the oil sac by micropipette was pure wax ester (WE); triacylglycerols (TAG) were elsewhere in the body. Video images indicated more WE than could be demonstrated by Iatroscan. This was due to greater FID response to pure, commercially available waxes with low levels of unsaturation than to mixtures of mostly polyunsaturated wax in *C. finmarchicus*. A revised calibration scheme uses both WE and TAG purified in bulk by silica gel chromatography from *C. finmarchicus* itself. Use of quadratic calibration curves allowed analysis of single specimens, showing the individual variability of lipid class composition. C5 collected in April–July, while presumably storing lipid for the resting stage, showed a nearly constant level of TAG, $\sim 17 \mu\text{g ind.}^{-1}$, whereas WE amounts varied from nearly 0 to $>300 \mu\text{g ind.}^{-1}$. There appears to be a TAG quota that is filled before WE accumulates significantly and that is usually not exceeded. Many C5 collected in February and March had very high amounts and fractions of TAG. This likely results from transformation of stored WE to TAG in preparation for egg production.

Calanus finmarchicus (Gunnerus) is the dominant copepod of boreal North Atlantic waters from the Gulf of Maine east and north to the Atlantic entrance to the Arctic Basin. As part of the U.S. Global Ecosystem (GLOBEC) study of Georges Bank, we have been investigating the ecology and basic biology of *C. finmarchicus* in the southern Gulf of Maine and over Georges Bank. One of our specific tasks has been examination of the quantity and composition of storage lipids as they vary with location and through the seasons.

Species of the copepod family Calanidae store lipids in a membrane-bound organ, the oil sac, which extends from near the back of the prosome forward into the cephalosome. The greater the quantity of oil, the farther the sac extends toward the anterior end, eventually filling over half of the volume of the prosome. In *C. finmarchicus*, large oil sacs are most characteristic of the fifth copepodite (C5) stage, which is predominant in the resting population of summer and autumn months. We have been primarily concerned with the lipid types and quantities stored by C5s in preparation for diapause.

Lee et al. (1970) showed that calanid storage lipids include very large fractions of waxes, esters of long-chain alcohols with long-chain fatty acids (“wax esters,” WE). Most of the WE molecules have one or more *cis*-double bonds in both chains, producing strong curvature and making packing less efficient. This lowers the melting point such that these waxes remain liquid at deep ocean and winter temperatures and at substantial pressure (Yayanos et al. 1978). Polyunsaturated fatty acids (particularly 20:5 and 22:6 acids) are

also essential nutrients in many crustaceans (Kanazawa et al. 1979; Phillips 1984). Thus, their inclusion in *Calanus* oil sac wax ester may sequester them for use in later anabolic metabolism.

Comparison of oil sac lipid quantified by two techniques showed a large discrepancy. Quantification was by (1) video images of live specimens for approximate estimation of oil sac volume, and (2) automated thin-layer chromatography (Iatroscan; Shantha 1993). Approximate video estimates of oil sac volume, multiplied by a reasonable lipid density, implied about twice the WE shown by the Iatroscan. The source of this discrepancy is variation as large as a factor of 2.0 in the Iatroscan flame-ionization detection (FID) response among different wax molecules. An operational solution to this problem is to use the mixture of waxes actually present in *C. finmarchicus* as a calibration standard, a method we explain in detail.

Copepod storage lipids other than WE are mostly triacylglycerols (TAG; Lee et al. 1970). Seasonal sampling over and near Georges Bank produced a series of monthly comparisons of the relative amounts of these lipid classes in *C. finmarchicus*. The series reveals a seasonal change in storage lipid composition, which can be interpreted as reflecting a shift from preparation for reproductive activity in late winter to predominance of preparation for rest in spring.

Methods

Collections at sea—Sampling was conducted on a series of cruises to Georges Bank and the southern reaches of the Gulf of Maine (Table 1). Georges Bank is a shallow marine rise off New England centered at 41°30'N, 68°W. Differences between stations are not emphasized in this report, so stations are not shown. Station locations in 1995 were standard stations of the U.S. GLOBEC, Georges Bank Broad-scale Survey. Collections of copepods were made primarily with a 1-m² Moccness sampler (Wiebe et al. 1985) equipped

Acknowledgments

Our work was supported by the NSF (OCE 93-17506) as part of the U.S. GLOBEC Georges Bank Program. This is contribution 85 of the U.S. GLOBEC Program supported by NSF and NOAA. Thanks to Peter Wiebe for the invitation to join the program and to all our many shipmates for sharing the work at sea. Thanks to Mark Ohman for help with Iatroscan procedures.

Table 1. Cruises from which *Calanus finmarchicus* were analyzed in this study. All cruises were part of the U.S. GLOBEC Georges Bank Program.

Ship	Cruise No.	Dates
I. RV <i>Iselin</i>	CI-9407	25 May–16 Jun 94
II. RV <i>Albatross IV</i>	ALB-9410	8–18 Nov 94
III. RV <i>Endeavor</i>	EN-261	10–20 Feb 95
IV. RV <i>Endeavor</i>	EN-263	13–24 Mar 95
V. RV <i>Endeavor</i>	EN-265	11–22 Apr 95
VI. RV <i>Albatross IV</i>	ALB-9505	9–18 May 95
VII. RV <i>Albatross IV</i>	ALB-9506	5–15 Jun 95
VIII. RV <i>Albatross IV</i>	ALB-9508	10–20 Jul 95

with 150-mm mesh. Stratified hauls divided the water column into a varying number of strata depending on total depth. On all 1995 cruises, target depths were 0–15, 15–40, 40–100 m, and near bottom–100 m. Actual deepest samples on any given tow were from near bottom (3–6 m, usually) to the next target depth limit (e.g. 60–40 m over a 65-m bottom). Tow speeds were $\sim 75 \text{ cm s}^{-1}$. A few tows were taken in May–June 1994 with a 150-mm mesh net on a bridled 70-cm ring.

Individuals and groups of C5s for lipid analysis were sorted live from diluted portions of net samples. On 1994 cruises, groups of five were most commonly sorted, videorecorded, and frozen together in liquid nitrogen. At May–June 1994 sta. 35 (20 ind.) and 41 (6 ind.), we took video pictures of single specimens, then froze them singly in labelled vials. Lipids from these were extracted and chromatographed separately. In 1995 cruises all specimens were videorecorded, frozen, and analyzed separately. All 1995 analyses were based on single individuals. To limit analytical work, specimens for Iatrosan determinations from April to July 1995 cruises were selected by a stratified random procedure. Video estimates of oil sac volume were grouped into three sets with equal numbers of specimens: most oil, intermediate amount of oil, and least oil. Five specimens from each group were selected randomly from each month for analysis.

Video pictures of individuals (1994 and 1995) or groups (1994) were recorded in TIF computer format with a stereomicroscope-mounted video camera. Clear images of the outlines of the prosome and oil sac were thus captured. Most individuals were recorded in side view. Some were recorded in dorsal view as well for comparison of the performance of estimating functions for storage lipid volume. Images of stage-micrometer scales were made for calibration and checked for consistency at regular intervals. The microscope and videorecording system produced consistent scaling when in focus. After recording, we wicked water away from the copepods with tissue paper, transferred them to screw cap cryogenic vials, and promptly stored them in liquid nitrogen until analysis onshore.

Analysis of video pictures—Video pictures were analyzed using Sigma ScanImage software operating on a PC. Prosome lengths were measured by superimposing a line from the anterior dome of the head to the posteriormost extent of the fifth thoracic segment. The program calculates length

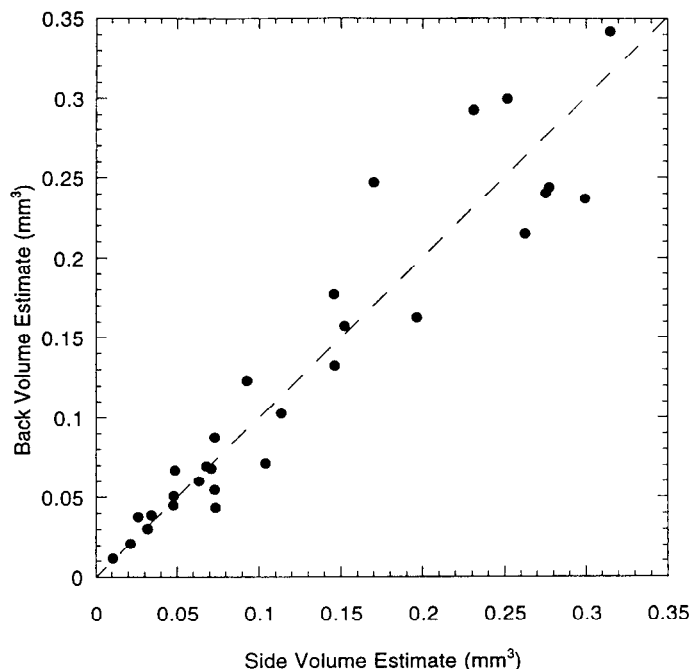


Fig. 1. *Calanus finmarchicus*, C5. Each point compares the lipid volume of a single individual estimated from video images taken in side view and dorsal view. The line represents equal estimates.

from a calibration derived from a stage-micrometer image. For estimation of oil sac volume, the operator traces the outline of the oil sac on the computer screen with a cursor. The program calculates (1) the length of the principal axis, L , and (2) the projected sac area, A , by comparing pixel counts in the outlined area to an area-per-pixel calibration. We got the most consistent estimates of sac volume, V , between dorsal and side views by using a cylindrical tube approximation, obtaining a diameter, D , of the cylinder as A/L . Substituting A/L for D in the obvious formula for rotated volume ($V = L\pi D^2/4$) gives the more direct formula

$$V = \frac{\pi A^2}{4L}.$$

Results from this formula are usually within 2% of estimates by a detailed numerical integration procedure (C. Miller in prep.). An oblate-spheroid approximation developed by Arts and Evans (1991) for oil inclusions in *Diaptomus sicilis* and used for *C. finmarchicus* by Plourde and Runge (1993) was less accurate. The oil sac of *C. finmarchicus* is too elongate to be treated as an oblate spheroid. The rough equivalence of side and dorsal view estimates (Fig. 1) indicates that the elongate oil sac is not preferentially flattened either dorso-ventrally or laterally.

Lipid class analysis—We produced Iatrosan estimates of major lipid class abundance following the general protocol for copepod lipids modified by Ohman (1988) from the techniques of Gardner et al. (1985) and Parrish and Ackman (1985). Copepods were ground to homogeneity in a glass tissue grinder with 100 ml of 2:1 (vol/vol) chloroform:methanol to extract lipids. A known amount of 1-nonadec-

canol was dissolved in the extraction solvent as an internal standard. Partitioning between the solvent and 0.9% NaCl solution removed protein contaminants. Chromatography by the Iatroscan technique uses elution of lipids along 1-mm (diam.) \times 15-cm silica rods, followed by FID on a Th-10, Mark IV Iatroscan machine. Extracted lipids were delivered to the origin by syringe, dried for 5 min, then rehumidified over CaCl₂ solution in a closed chamber for 5 min. Elution was by the double solvent scheme of Ohman (1988): 76:4 (vol/vol, 80 ml) hexane:ethyl ether for 20 min and 5 min drying, followed by 66:14:0.1 (vol/vol/vol, 80 ml) hexane:ethyl ether:formic acid for 20 min and 5 min drying. The rods were then scanned by the Iatroscan FID detector with data ported to a computer for storage and analysis by LabCalc software. Residual lipids developed by trial second scans (burns) were negligible, counter to the report of Ohman and Runge (1994). Peak integrals (arbitrary units) were compared to standard curves for determination of WE, TAG, phospholipid, and internal standard content. Standards during initial work were tripalmitin (TAG), stearyl palmitate (WE), phosphatidylcholine dipalmitoyl (phospholipid), and cholesterol (sterol). This selection followed Ohman (1988), except that he used palmityl palmitate to represent WE. Our internal standard, 1-nonadecanol (C₁₉-1-OH) chromatographed as a distinct peak between phospholipids remaining near the origin and TAGs.

Recalibration of Iatroscan using copepod wax—We eventually recalibrated storage lipid analyses using bulk copepod WE and TAG purified by column chromatography from three lots (from May–June 1994, February 1995, March 1995) of 124–125 ind. C5 *C. finmarchicus*. They were placed in a vial with 15 ml 2:1 (vol/vol) CHCl₃:MeOH sonicated for 10 min, centrifuged, and the supernatant was decanted. Sonication and centrifuging were repeated twice with 5 ml of new solvent. Combined supernatant was partitioned (3 \times) against water, adding hexane to invert the layers. The water layer was then partitioned twice more against 10 ml of hexane, which was combined with the original organic fraction. The hexane was partitioned against 50% saturated NaCl, then transferred to a flask and held 1 h over sodium sulfate to remove remaining water. After evaporation of solvent, samples were applied with hexane to columns containing 7 g of 5% deactivated silica gel, then eluted with 50 ml of hexane to remove hydrocarbons. Fraction-1 (F-1) was eluted with 40 ml of 1:1 toluene:hexane; F-2 was eluted with 15 ml of 2:98 ethyl acetate:hexane; and F-3 was eluted with 25 ml of 2:98 ethyl acetate:hexane. All elutions were rotary evaporated to dryness; elutions with toluene required a postcolumn addition of methanol (3 \times toluene volume) to promote toluene evaporation. Such elution protocols must be modified for specific batches of silica gel and for varying sample composition and quantity. Fractions were transferred in methylene chloride to vials, then dried under a flow of nitrogen. Iatroscan results showed that F-1 and F-2 were essentially pure WE; F-3 was TAG. Some TAG was probably left on the column.

Iatroscan standard curves were generated from each of these WE and TAG fractions for comparison with curves developed with pure commercial compounds. Gas chromatographic (GC) analyses were performed to determine the molecular composition of the WE fraction. Molecular identity was confirmed by GC–mass spectrometry. Detailed methods are available on request.

Iatroscan calibration curves and precision—For analyses of single *C. finmarchicus*, the Iatroscan method was used just above its sensitivity limit, near which the response of the FID is nonlinear. This was handled by using quadratic standard curves (Parrish and Ackman 1985) below 2.5 mg TAG and for all WE and internal standard peaks in analyses of single specimens. In final calculations of lipid quantities in the original samples, solvent losses during processing were accounted from the known initial mass of internal standard. All samples were chromatographed twice; reported data are means of the estimates. In all analyses we included a rod spotted with a sample of extraction solvent with internal standard and a known mass of WE, TAG, and phospholipid standards. This was a check for lipid contamination and provided a measure of solvent evaporation prior to extraction of copepods. Such evaporation was never significant.

We studied analytical variation of the Iatroscan in two ways. First, we ran three full developments and FID runs for separate applications of a solution of mixed standards on all 10 rods in the Iatroscan set. Separate one-way analyses of variance for each lipid showed significant differences among rods for WE, TAG, and phospholipid. We did not, however, remove this effect by separate calibrations for each rod, but allowed it to affect the data (and calibration curves) in return for simplified data handling. Confidence limits (95%) based on these data for single observations as estimates of the mean are ± 14 –15% of the mean for WE, TAG, and internal standard. They were $\pm 20.6\%$ of the mean for phospholipid. Coefficients of variation (overall standard deviation/overall mean) were 7–8%, except for PL (10.3%). Ohman (1988) reported coefficients of variation of 15.5% for applications of 1 mg lipid, declining to 6.2% for applications of 10 mg lipid. Our applications were typically in the range of 1–10 mg.

Second, we ran each of our samples (both copepod extracts and mixed standard run concurrently) in duplicate on adjacent rods. Wax ester data were the most consistent between pairs. Percentage coefficients of variation (C.V. = $100 \times \text{SD}/\text{mean}$) were the same on average at different estimated lipid concentrations; mean C.V. was 3.6%, with just more than half of the values less than that. Mean C.V. for TAG was 4.9%, with somewhat more high values than found for WE. Phospholipid analyses were more variable than those for storage lipids; mean C.V. was 10.9%. We do not use the phospholipid data extensively, so we did not strive to improve our technique for such analysis. These characterizations of variability only include differences resulting from rod spotting, FID performance, integration, and internal standard estimation. Variations in extraction efficiency are not accounted for.

Separation of oil sac contents—We achieved partial separation of oil sac contents from the remainder of the body by puncturing the oil sac with glass micropipettes produced

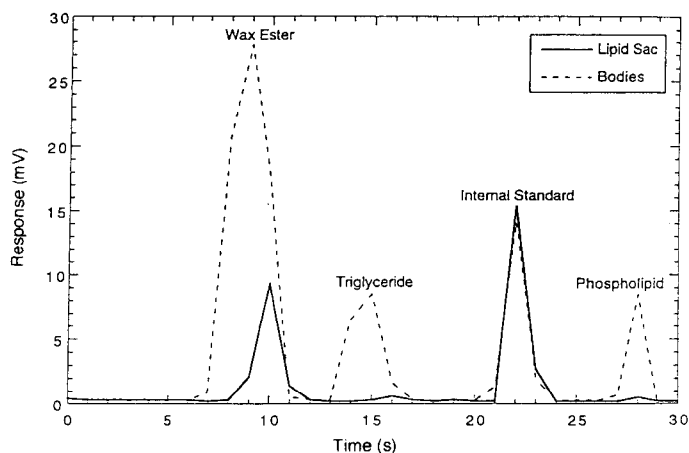


Fig. 2. *Calanus finmarchicus*, C5. Overlaid chromatograms for oil sac contents withdrawn by micropipette (---) and the remainder of the bodies (—). Note that relative to WE both TAGs and phospholipids are greatly reduced in the lipid sac content sample.

with a micropipette puller. Oil sac puncture was most effective with tubes of ~ 0.1 – 0.2 mm outside diameter. A group of frozen C5s were thawed (20 s) on a microscope slide previously cleaned with methylene chloride. We introduced the pipette through an anterior thoracic articulation into the oil sac. Mild suction was applied and as much oil as possible removed. The goal was not to get all of the oil but to minimize contamination by other body fluids. Oil was taken from all five specimens in the group with the same pipette, then blown from the capillary onto the wall of a 6×50 -mm test tube. Extraction solvent was added to this tube and the analysis carried out as for copepod extracts. The bodies of the copepods, with some remaining oil, were extracted and analyzed separately.

Results

Oil sac content is wax, triacylglycerols are elsewhere—Examination by video imaging and Iatroscan of lipids in single C5 of *C. finmarchicus* collected in 1994 (data not shown) demonstrated (1) a stronger correlation between video-estimated lipid weight (VELW) of the oil sac and Iatroscan estimates of WE content than between VELW and total storage lipids (WE + TAG), and (2) that TAG was usually a smaller fraction of total lipids when WE was abundant. These facts, illustrated for 1995 data below, led us to suppose that the oil sac must be filled entirely with WE, and that TAG must be elsewhere. Comparison (Fig. 2) of oil sac contents obtained by micropipette withdrawal with the remainder of the body (including sac contents not removed) showed dramatic relative reduction of TAG and phospholipid in the sac content, which was mostly WE. The slight amounts of TAG and phospholipid in the withdrawn sac content are due to contamination during puncture of the body.

Quantity of storage lipids by video analysis in single individuals—Total storage lipids (TL = WE + TAG) of single specimens from 1994 sta. 35, 41X, and 43X were mostly well correlated with estimates of lipid weight from video

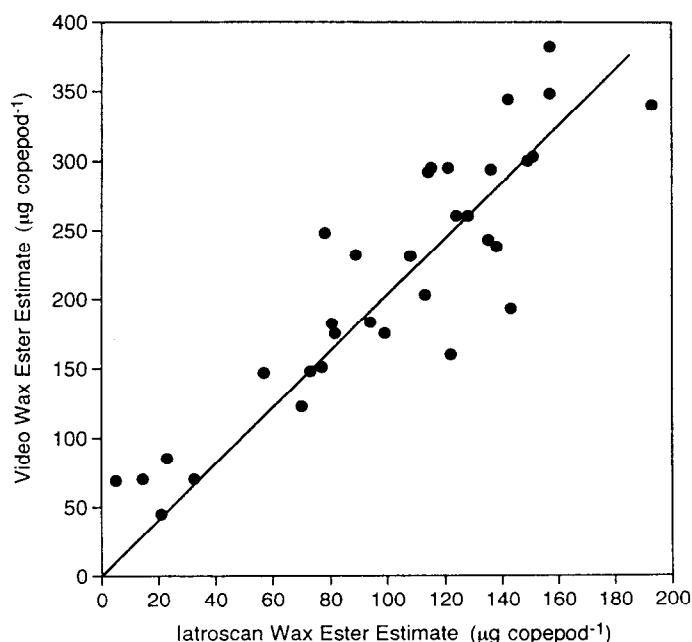


Fig. 3. *Calanus finmarchicus*, C5. Scatter diagram for oil sac lipid content of single copepods estimated from a video image vs. the Iatroscan chromatographic estimate of wax ester (mg per copepod) (1994 collections). Least-squares line forced through origin: Video estimate = $2.03(\text{Iatroscan estimate})$, $R^2 = 0.78$.

determinations of oil sac volume (VELW), assuming lipid density of 0.90 g ml^{-1} . However, correlation of VELW with just WE (Fig. 3) was considerably tighter than for TL, which partly suggested and agrees with our demonstration that sac oil is all or mostly WE. However, a fitted regression forced through the origin gave VELW of $2.0 \times \text{WE}$. The slope was the same for specimens from both Sta. 35 (20 ind.) and 41X (6 ind.).

Solution of the video vs. Iatroscan discrepancy—Confirmation that oil sac contents were entirely WE suggested VELW and chromatography should agree on average, (i.e. VELW = $1.0 \times \text{WE}$). In a first test, VELW gave reasonable approximation to lipid weight estimated from dry weight and lipid-free dry weight measures (method in Miller 1993) after approximate allowance for TAG (using an early version of Fig. 7). That result suggested that the problem lay in the calibration of the Iatroscan technique; that is, possibly different WE produced different FID response levels. As an initial test we produced calibration curves (Fig. 4) for a variety of commercially available waxes. These included (1) various chain length combinations and (2) C_{18} – C_{18} esters with all combinations of zero and one double bond per chain. All tested waxes required more mass of compound to produce a given peak area than our original choice of standard (stearyl palmitate). The overall result indicated that our choice of a WE standard was probably causing underestimates of copepod WE content.

Waxes with the large numbers of double bonds typically found in copepod oils (Sargent and Henderson 1986) are not commercially available. A calibration curve for chromato-

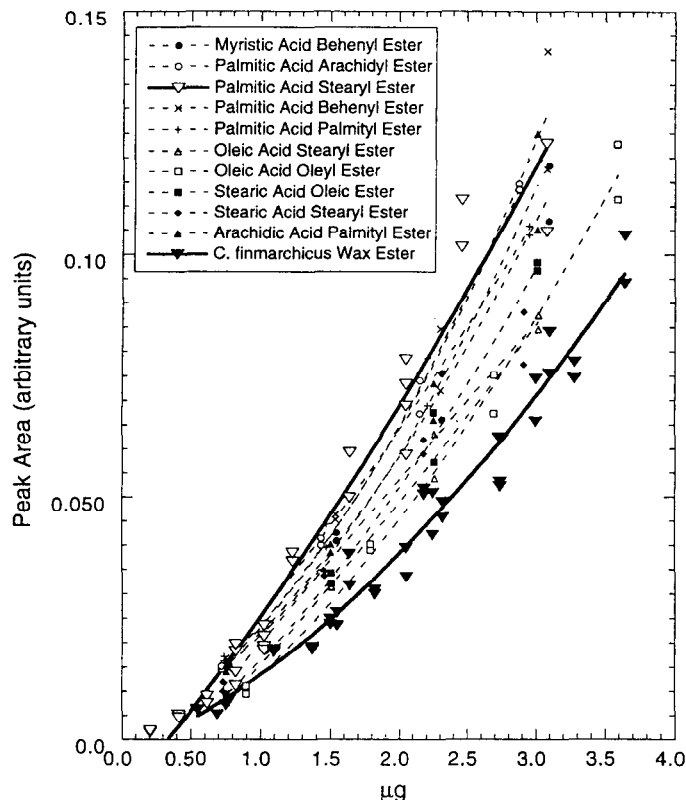


Fig. 4. Iatroscan calibration curves for a variety of commercially available waxes (dark line at left for our usual standard, stearyl palmitate) and for WE chromatographically purified from bulk *C. finmarchicus* (dark line at right).

graphically extracted WE from *C. finmarchicus* showed that highly unsaturated forms are required in greater mass to produce a given peak area than is needed of any specific wax available for test (Fig. 4). In the range from 1 to 2 μg lipid, the difference in peak area is close to the ratio of 2:1 required to explain the discrepancy we observed between video estimates and Iatroscan estimates based on stearyl palmitate calibration. The shift required in WE calibration of the Iatroscan then led us to test extracted copepod TAG as a standard for Iatroscan TAG estimates. The shift in calibration from the tripalmitin curve was close to the same as that for WE (Fig. 5). All of the 1995 Iatroscan data reported below are based on a calibration against purified *C. finmarchicus* WE and TAG.

Gas chromatography—Wax ester extracted from bulk *Calanus* and used for recalibration of the Iatroscan was analyzed by GC to determine the composition of fatty acids and alcohols. In terms of weight contributed to the mixture (Table 2), acids of 18 or more carbons and 20:1 alcohols predominated. The GC-mass spectrometry did not allow positive identification of acids with four or more double bonds, hence the notation $\text{C}_{20:4+}$.

Video-estimated lipid content vs. Iatroscan, 1995 data—Our new calibration scheme based on lipid classes extracted from *C. finmarchicus* gives improved agreement between

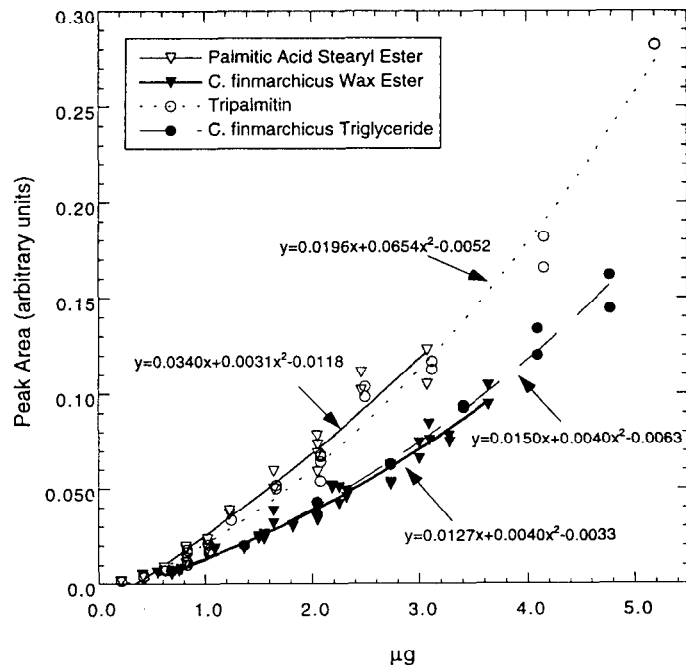


Fig. 5. Iatroscan calibration curves for both WE and TAG. Our original standards (open symbols) are compared to chromatographically purified WE and TAG from bulk *C. finmarchicus* C5s. Effects for the two lipid classes are comparable.

VELW and Iatroscan. Results by month (Fig. 6) show that discrepancies remain for WE; they are opposite in sign between February collections (Iatroscan > VELW) and the rest of the year (VELW > Iatroscan).

Content of TAG and WE—Our 1995 data reveal a strong pattern in the relative amounts of TAG and WE in C5 col-

Table 2. Relative abundance of specific fatty acids and fatty alcohols in wax ester extracted from bulk *Calanus finmarchicus*, mostly C5s. Analysis by gas chromatography (GC) with identification by GC-mass spectrometry (MS). Acids and alcohols are classed according to chain length and number of double bonds. The GC-MS did not resolve some acids with multiple double bonds, hence designations such as $\text{C}_{20:4+}$.

Fatty acid	Relative abund. (% C in fatty acid)	Fatty alcohol	Relative abund. (% C in fatty alcohol)
$\text{C}_{14:0}$	3.2	$\text{C}_{14:0}$	2.0
$\text{C}_{16:3+}$	1.2	$\text{C}_{16:1}$	3.3
$\text{C}_{16:1}$	3.2	$\text{C}_{16:0}$	21.2
$\text{C}_{16:2}$	0.6	$\text{C}_{18:1}$	10.5
$\text{C}_{16:0}$	8.8	$\text{C}_{18:0}$	2.7
$\text{C}_{18:4}$	22.9	$\text{C}_{20:1}$	60.5
$\text{C}_{18:2}$	4.7		
$\text{C}_{18:1}$	5.5		
$\text{C}_{18:0}$	1.1		
$\text{C}_{20:4+}$	14.6		
$\text{C}_{20:2+}$	3.9		
$\text{C}_{20:1}$	11.5		
$\text{C}_{22:2+}$	3.2		
$\text{C}_{22:1}$	15.8		

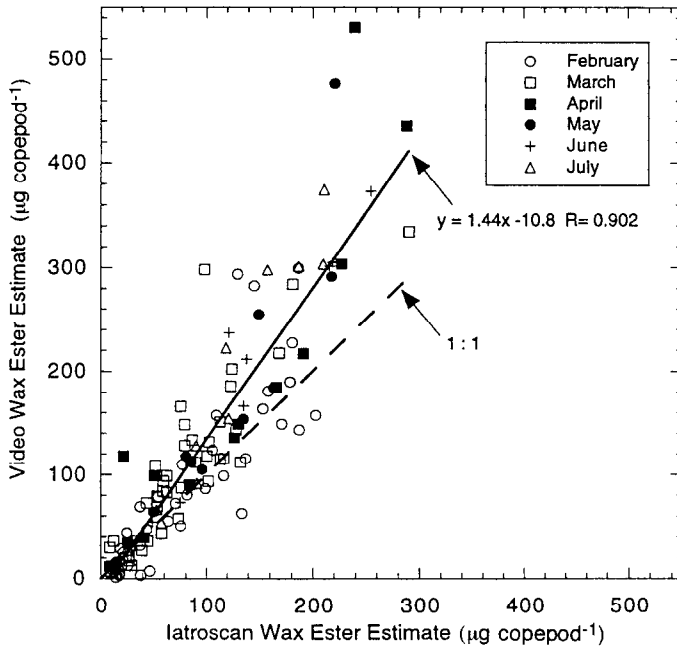


Fig. 6. *Calanus finmarchicus*, C5. Video-predicted lipid weight vs. Iatroscan estimate of WE for early months of 1995. Dashed line represents equal estimates.

lected from April–July on Broadscale cruises (Fig. 7). This pattern can be modelled from any one point. For example, fixing TAG at 17 mg estimated from the 34% TAG found at 50 mg of total storage lipid, all other points are well predicted by varying the content of WE. We interpret this as showing that *C. finmarchicus* storing lipid have a TAG quota, above which all additional storage is as WE. The same pattern was shown by most 1994 data for single individuals. Fifth copepodites collected on February and March cruises in 1995 did not follow this pattern (Fig. 8), although the model line for April–July roughly represents a lower limit to the fraction of TAG present. Many of the late winter specimens had much more TAG, as did specimens from two specific stations collected in May–June 1994.

Discussion

Iatroscan calibration — Various problems with the Iatroscan method have been demonstrated previously. Kaitaranta and Nicolaidis (1981) showed that its response factor depends on the amount of sample, but we think that is safely handled by keeping sample loading within the range of calibration. Crane et al. (1983) demonstrated strong variations of FID response with changes in burning conditions (gas pressure, flame composition, temperature). Consistency over time in our calibration curves gives us confidence that those conditions can be kept reasonably stable. Most importantly, Sipos and Ackman (1978) pointed out the strong differences between lipid classes in the amount of ionizable carbon produced by the flame in Iatroscan FIDs. It has been widely assumed that this problem can be managed by calibrating each lipid class separately, which has become standard practice.

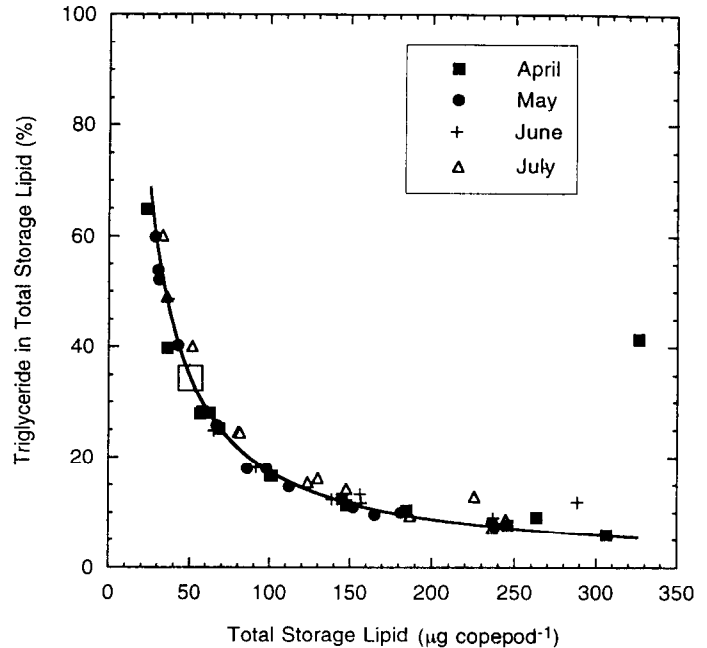


Fig. 7. *Calanus finmarchicus*, C5. %TAG, relative to TAG + WE, for individuals analyzed singly from April through July 1995. Curve is a single point model: TAG was held constant at the 17 mg calculated from 34% TAG at 50 mg total storage lipid, then WE was varied in the relation $\%TAG = 17/(17+WE)$ to generate the curve.

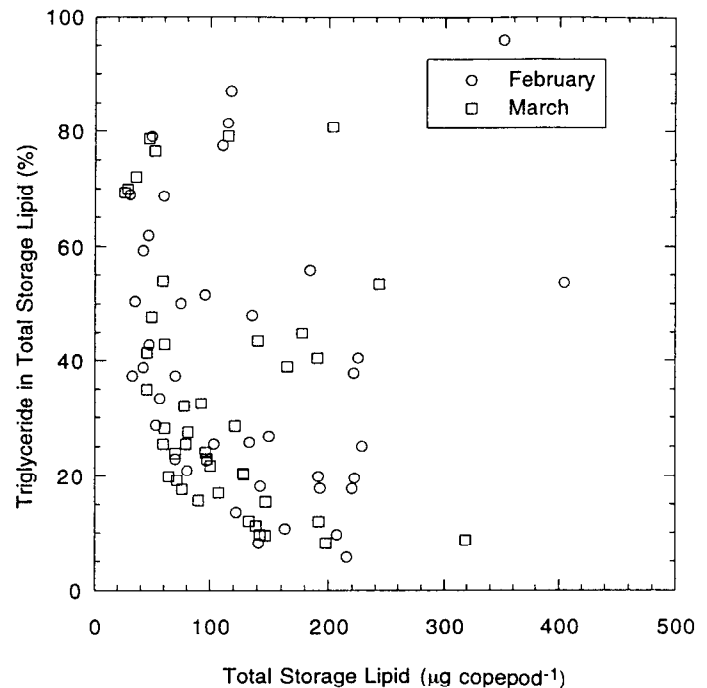


Fig. 8. *Calanus finmarchicus*, C5. %TAG, relative to TAG + WE, for individuals analyzed singly from February and March 1995.

Unfortunately, our results (Fig. 3) show up to twofold variation within the WE class dominant in *Calanus*. Similar variation was shown for TAG by Kramer et al. (1986) and by Fraser and Taggart (1988). Kramer et al. (1988) found similar FID responses relative to internal standard for several single TAG compounds, but lower response with mixtures. They recommended using mixtures close in composition to the lipid to be analyzed as standards. Fraser and Taggart compared Iatroscan calibrations with triolein and marinol, a polyunsaturated fish oil. Response to triolein was greater by a factor of 1.7. This discrepancy is discussed by Shantha (1993), who recommended several approaches to the problem. Our solution, to use bulk-extracted, chromatographically purified lipid from the organisms of interest as standard, may or may not suit all analytical circumstances. The shift in VELW vs. Iatroscan WE relationship between samples from February 1995 and later months suggests that highly specific calibration would be required for each temporal and spatial interval included in a study. Such calibration can only be done by very patient workers and for animal populations available in sufficient quantity to provide both calibrating lipid and individuals for sample estimates.

We have not carried the experimental analysis of differences among waxes far enough to determine which features of wax compounds determine the resulting FID response factor. Details of chromatographic performance are not a principal concern in our work. Among the waxes tested, no particular feature (not acid or alcohol chain length, total chain length, nor presence of one or two double bonds) was correlated with response factor. However, single wax compounds with large numbers of double bonds are not available for purchase, and possibly double bond number is significant beyond the available catalogue of 0 and 1 double bond per chain. Thus, multiple double bonds could cause the *C. finmarchicus* wax mixture to have the lowest response factor among waxes tested.

Most previous studies of copepod lipids using Iatroscan used single, nearly saturated compounds as WE standards: oleic acid oleyl ester (Håkanson 1984), palmitic acid palmityl ester (Parrish and Ackman 1985; Ohman 1988), palmitic acid oleyl ester (Ohman et al. 1989), palmitic acid arachidyl ester (Ohman and Runge 1994; this study). Fraser et al. (1989) stated that their method followed Fraser et al. (1985), which recommends palmitic acid oleyl ester as a WE standard.

Several papers (e.g. Fraser et al. 1989; Schnack-Schiel et al. 1991) cite Fraser et al. (1985) for basic methods. This earlier paper showed a comparison of a calibration curves for marinol, a predominantly TAG fish oil with >40% polyunsaturated acids, to one for hydrogenated marinol. The difference was slight, and confirmed to the authors' satisfaction that "markedly different degrees of unsaturation do not materially affect lipid quantitation by the Iatroscan." They did not show a similar comparison for waxes, but recommended replacing TAG with a wax such as palmitic acid oleyl ester when WE is an important constituent of the neutral lipid. The contradictory data of Fraser and Taggart (1988) were not mentioned by Fraser et al. (1989). Clearly, some published studies may suffer from calibration problems as large as a factor of 1.7. Mark Ohman (pers. comm.) has

performed Iatroscan calibration comparisons between common standards and lipids from *C. pacificus*. He found similar, but less dramatic, differences in calibration curves. Fig. 4 suggests that the calibration offsets between compounds becomes roughly constant with larger lipid loading. Thus, with very large applications (e.g. >10 μg), the offsets would represent a modest, sometimes acceptable fractional error. Benefits from analysis of single individuals, however, are only obtained by attention to the specificity of the response factor.

Anatomical partition of storage lipid classes—Benson et al. (1972) reported that the lipid carried in a discrete, membrane-limited oil sac by various species of the copepod family Calanidae is exclusively WE, while TAG is stored elsewhere. They were cited in the review by Sargent and Henderson (1986), where Corner and Gatten were also cited as having unpublished data establishing the fact. However, these workers did not publish explicit data. Benson et al. (1972) just stated that, "Isolation of oil from individual animals, 1 mm long, by M. Zalokar revealed that the oil sac of *C. helgolandicus* contained pure wax ester and that the animal's triglyceride component must be distributed elsewhere." We offer our chromatogram (Fig. 2) in evidence.

Gas chromatography results—Fatty acid composition of WE in *C. finmarchicus* from the Gulf of Maine (Table 2) differed somewhat from Kattner's (1989) results for the North Sea. Although our data are only for a single bulk sample, and thus should not be overemphasized, the differences bear discussion because they point to a need for further detailed study. We found proportionally less $\text{C}_{14:0}$ and $\text{C}_{22:2+}$ acids and proportionally more $\text{C}_{18:4}$ and $\text{C}_{20:1}$ acids. The fraction of $\text{C}_{18:4}$ acid was greater, even though Kattner (1989) selected for his North Sea vs. Arctic comparison only samples in which that acid was relatively high (>8%). Fractions of $\text{C}_{18:4}$ in Fladen Ground *C. finmarchicus* shown by Kattner and Krause (1987, 1989) are also lower (0.4–12%). The differences could be due to differences in dietary fatty acid composition. Kattner and Krause (1987) term $\text{C}_{18:4}$ fatty acids "markers" for diets predominantly of *Phaeocystis* or dinoflagellates, both of which are rich in this acid (Sargent et al. 1985). Kattner (1989) and Graeve and Kattner (1992) reported high proportions of $\text{C}_{20:1}$ and $\text{C}_{22:1}$ fatty alcohols (>30%) in *C. finmarchicus* from the Arctic and North Sea. We found an even higher proportion of $\text{C}_{20:1}$ alcohol, but $\text{C}_{22:1}$ was essentially absent. This agrees with low amounts of $\text{C}_{22:1}$ alcohol found by Falk-Petersen et al. (1987) in northern Norwegian fjords. Variation in relative amounts of fatty alcohols may be real, and if so needs explanation.

Comparison of total lipid estimates to prior data—Table 3 compares our 1995 Iatroscan data (calibrated against extracted copepod lipid) with all estimates in the literature for the lipid content of *C. finmarchicus*. On the whole, our video estimates were even higher, despite the new calibration scheme for Iatroscan. However, basing the comparison on the Iatroscan data allows inclusion of phospholipid, which has been usual in other studies. Our total lipid estimates are relatively high, and the extended range is confirmed by the

Table 3. Reported values of total lipid content ($\mu\text{g ind.}^{-1}$) in *Calanus finmarchicus*. Values are ± 1 SD.

Site	Season	Stage	Lipid content (μg)	Reference
Arctic Ocean	?	C6♀	220	Lee and Hirota 1973
Fram Strait	Summer	C6♀	67.6 \pm 16.5	Kattner 1989
		C5	71.5 \pm 32.9	
Fram Strait	24 Jun–14 Jul	C5	28–122	Kattner et al. 1989
		C6	19–93	
Fladen Ground	Feb	C5	5.4	Kattner and Krause 1989
	May	C5	37.4 \pm 19.2*	
Northern North Sea	Late Apr–early May	C6♀	26.9–37.7	Kattner and Krause 1987
		C6♂	21.7–85.3	
		C5	26.7–33.7	
Enclosure in Loch Thurnaig	May	C5	31.5	Fraser et al. 1989
Gulf of St. Lawrence	June	C6♀	104 \pm 17	Ohman and Runge 1994
St. Lawrence Estuary	March	C6♀	103 \pm 11	Ohman and Runge 1994
Gulf of Main and Georges Bank Groups of 5 only	May–Jun 1994	C5	35–237	This paper
	Nov 1994	C6♀	72–149	
Gulf of Main and Georges Bank Single specimens	Feb 1995	C5	31–141	
	Mar	C5	33–420	
	Apr	C5	39–341	
	May	C5	46–341	
	Jun	C5	35–334	
	Jul	C5	48–332	
		C5	37–258	

* $N = 2$.

approximate video estimates (Fig. 6). Ranges of May–June and November 1994 values (calibrated against palmitic acid stearyl ester) also extend beyond values previously reported in the literature. The lower range of our observations is about the same as available North Sea means. In part, this is because most prior studies have examined small numbers of samples of large numbers of individuals, while we have examined many samples of few (in 1995 one) individuals. The former strategy is suitable for determining a mean; the latter will produce a greater range of values. Different sampling strategy is a partial explanation of our higher estimates, but possibly Georges Bank has substantially fatter *C. finmarchicus* than does the rest of the North Atlantic. Not all of the older data were subject to severe calibration errors; some estimates were simply extracted lipid weight. Resolution of the discrepancy must await a wider comparative study.

Proportions of TAG and WE—The relationship demonstrated (Fig. 7) between %TAG and total storage lipid (TAG + WE) implies a TAG quota, above which additional storage lipid is converted to WE. Possibly TAG serves as a transfer form within the body. When it reaches a threshold concentration (a TAG quota), WE synthesis is enabled and conversion to WE holds TAG constant. Possibly a small, roughly fixed amount of TAG is involved in structure or function of specific body organs, requiring that it be stocked first before WE synthesis proceeds. The general result was anticipated by Sargent et al. (1977), who showed that “the small amounts of triacylglycerols are accumulated faster than the large amounts of wax esters, at least in the feeding Stage V” (quote from Sargent and Henderson 1986). Fractions of TAG found by Sargent et al. (1977) are low relative to those

shown in our Fig. 7 (calibrated against extracted copepod lipids), but are comparable to our results when calibrated against palmitic acid stearyl ester.

Many winter (February–March) and some spring (1994) specimens did not follow this pattern, but had much more TAG. Since TAG is the storage lipid deposited in eggs, the C5 of late winter (and C5 preparing for maturation in spring) are presumably converting WE back to TAG in preparation for egg production. Recent experimental evidence (Fessenden 1996) with *C. pacificus* suggests that exactly this transformation occurs. Fessenden found that C5s newly brought in from the field were high in WE and low in TAG. After several weeks of feeding, both newly molted females and the remaining C5s had much higher TAG, more than WE. This is reflected in the small oil sacs of females relative to those in C5s. Fessenden also found that newly matured males retain both large oil sacs and predominance of WE among storage lipid. Many C5s in the vicinity of Georges Bank do mature in April and May (T. Durbin unpubl. data). Had we analyzed individuals from more sites around Georges Bank during that period, we probably would have found some C5s with higher TAG fractions. The one point off the line in Fig. 7 would be joined by others.

This is the first report to our knowledge of large fractions of TAG recurrently found in the storage lipids of *C. finmarchicus* (Table 4). Our evaluations have been presented in terms of the fraction TAG/(WE + TAG), whereas most earlier reports are in terms of TAG/total lipid, with total lipid including phospholipids. For our purposes, therefore, we included phospholipids in the denominator in Table 4. Our range of values is high compared to previous studies. However, most earlier data were presented as single estimates for groups of specimens, i.e. as means. Because individuals with

Table 4. Previously reported fractionations of storage (neutral) lipid content in *Calanus finmarchicus*.

Site	Season	Stage	% triacylglycerol	Reference
Arctic Ocean	?	C6 ♀	11*	Lee and Hirota 1973
Balsfjorden	23 Oct	C5	1.3*	Falk-Petersen et al. 1987
	13 Jan	C5	0.8*	
	18 Mar	C5	Trace	
	09 Jun	C5	8.9*	
Fram Strait	Summer	C6 ♀	<20†	Kattner 1989
		C5	<15†	
Fram Strait	24 Jun–14 Jul	C5	<14.5‡	Kattner et al. 1989
		C6	<23§	
Fladen Ground Northern North Sea	May	C5	<12†	Kattner and Krause 1989
	Late Apr–early May	C6 ♀	<31†	
C6 ♂		<35†		
C5		<14†		
Enclosure in Loch Thurnaig	May	C5	3.1±0.4*	Fraser et al. 1989
Gulf of St. Lawrence	Jun	C6 ♀	2.1*	Ohman and Runge 1994
St. Lawrence Estuary	Mar	C6 ♀	0.34*	Ohman and Runge 1994
Gulf of Maine and Georges Bank Groups of 5	May–Jun 94	C5	2.2–41.4	This paper
		C6 ♀	1.8–25.7*	
Gulf of Maine and Georges Bank Single specimens	Nov 94	C5	0.8–11.0*	
	Feb 95	C5	8–92*	
	Mar 95	C5	8–78*	
	Apr 95	C5	5.5–4.2*	
	May 95	C5	7–50*	
	Jun 95	C5	7–33*	
	Jul 95	C5	6–53*	

* Based on total lipids.

† Difference from %WE in total lipid; TAG not directly analyzed.

‡ Average for 11 stations of difference from %WE in total lipids; TAG not directly analyzed.

§ Average for 10 stations of difference from %WE in total lipids; TAG not directly analyzed.

|| Percent of total lipid; one value of 81.8% TAG in 489 µg lipid not included; 37 of 70 values were >15% TAG.

large lipid stores contain a preponderance of WE, mean %TAG (and %TAG for animals analyzed in bulk) will not reveal the higher fractions in individuals with low total lipids. We think that the high frequency of high %TAG values in our data is well explained by our analysis of single specimens. The TAG quota model explains the relationship of %TAG to total lipid adequately. The composition of lipids we observed in November 1994 (0.8–11% TAG, tripalmitin calibration, consistently lower % TAG than in May–June) suggests, in agreement with Håkanson's (1984, 1987) conclusion for *C. pacificus*, that it is mostly WE that persists through the resting stage.

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Received: 26 July 1996

Accepted: 7 August 1997

Amended: 28 August 1997