

INITIAL; COLLATERAL MEASUREMENTS OF SOME PROPERTIES OF CALANUS FINMARCHICUS

by

Kenneth G. Foote, Tor Knutsen, Anna Ersland Bækkevold, Padmini Dalpadado,  
and Signe E. Johannessen

Institute of Marine Research, 5024 Bergen, Norway

ABSTRACT

In general, acoustic quantification of zooplankton such as Calanus finmarchicus requires the use of models, among other reasons, to aid in the interpretations of data collected on animals whose scattering properties change with development stage, season, and other environmentally linked factors. In conjunction with a project to determine acoustic scattering signatures of zooplankton and fish, a study is being performed to measure physical, morphometric, and biochemical properties of selected euphausiid species and Calanus finmarchicus. An important feature of this study is the performance of a suite of measurements on animals collected at the same time and place. The measurement methods being used to study Calanus are presented here together with results from the initial field experiment. The criticism of interested parties is solicited.

INTRODUCTION

Calanus finmarchicus is widely distributed in the North Atlantic Ocean. In the central gyre of the Norwegian Sea, for example, it is a dominant zooplankton species (Skjoldal et al. 1993). At present, it is sampled primarily by nets and pumps (Anon. 1968, 1977, Wiebe et al. 1976, 1985, Sameoto et al. 1980, Solemdal and Ellertsen 1984). On the scale size of a large ocean area, such sampling may be regarded as occurring essentially at point stations.

It may be imagined that the modality of acoustics could vastly increase both the magnitude and resolution of the sampling volume, effectively accomplishing continuous sampling in space and time. Basic distribution patterns of the copepod could be defined and associated with, for example, the high-productivity region of a front or the distribution and behavior of a predator, such as herring (Clupea harengus). Indeed, more may be expected through this basic acoustic imaging capacity, as in TransAtlantic Studies of Calanus (TASC) (Miller 1995).

Admittedly, the feasibility of acoustic registration depends critically on a number of factors. These include, among others, individual animal target strength, degree of concentration of the animal, background noise

level, and source level and receiver sensitivity of the observing acoustic system (Foote 1991). The first item on this list is given primacy, for modern echo sounders are so sensitive that the internal noise level in the receiver is comparable to that of the intrinsic thermal noise level, say to within 3-5 dB (Foote 1995). If, moreover, it were possible to detect a single animal, then the degree of concentration would be immaterial instead of critical, rendering application of the acoustic method more general too.

Determining the target strength of a single Calanus finmarchicus specimen is an evident challenge because of the smallness of the animal and its liquid-like qualities, rendering the contrast with sea water weak and the potential for producing an echo small (Wiebe et al. 1990, Stanton et al. 1993). Direct acoustic measurement offers one means to determine target strength; modelling offers another. Because of the large variability in biochemical composition of the animal (Tande 1982, Miller and Morgan 1995), which is shared by other fluid-like zooplankton species, such as Euchaeta norvegica (Båmstedt 1976a) and euphausiids (Båmstedt 1976b, Roschke 1977/78, Clarke 1980, Falk-Petersen 1981, 1985, Falk-Petersen et al. 1981, 1982, Saether et al. 1985), measurement under controlled conditions can sample at most a restricted range of variation. At the same time, measurement requires definition of the animal's properties. This provides one justification for the present work.

Certainly, properties of Calanus finmarchicus that are acoustically important have been determined before (Gross and Raymont 1942, Lowndes 1942, Køgeler et al. 1987), but apparently only incompletely apropos of the full range of requisite properties. Examination of representative models (Johnson 1977, Stanton 1989a,b), indicates that any one calculation requires specification of a number of properties for the same animal. For example, in the case of a fluid-like body, the properties might include density, i.e., mass density, sound speed, size, and shape. Insofar as lipid and protein content determine the density, the sum of biochemical properties is also important.

The major aim of this work then is to measure multiple properties of the same specimens, either on individuals or ensembles from the same catch, depending on feasibility. It is a further aim to begin to define the range in variability of the same properties. Because of the connection of this work with an EU-sponsored MAST-III project "Broadband acoustic scattering signatures of fish and zooplankton", which began in February 1996, not to mention related activities in TASC, it is especially important to document these early measurements to stimulate criticism, in case the present methods can be refined or supplanted by better ones.

## MATERIALS

### Venue

The laboratory measurements were conducted at the Espeland Marine Biology Field Station near Bergen. This is an onshore facility operated by the University of Bergen through the Department of Fisheries and Marine Biology. The facility is part of a Large Scale Facility for Marine Pelagic Food Chain Research, with sponsorship by the European Union.

### Catching operations

The *Calanus finmarchicus* specimens were caught in Raunefjorden (60°16'N, 5°09'E) (Fig. 1) near Bergen, Norway, on a number of occasions with R/V Aurelia, and once with a small boat with outboard engine, during the period late April-early June 1996. The 11-m-long research vessel is equipped with a Trimble Geophysical Pointing System (GPS), radar, echo sounder, and other instruments for navigation and fish finding, and a side winch for towing and hauling catching gear. The vessel-employed catching gear consisted initially of an Isaac-Kidd Midwater Trawl (IKMT), with 3' opening and 500- $\mu$ m mesh size, and later a small WP-like net with similar mesh size. This particular mesh size was used in order to avoid catching excessive quantities of phytoplankton and small gelatinous zooplankton. In lieu of MOCNESS bottles, a sturdy, transparent plastic bag of 30-l capacity, pre-filled with sea water, was used. This was attached to the codend of the net by means of a clamp.

Trawling was performed at a range of depths throughout the water column, but generally deeper than 50 m to avoid contamination with medusae and phytoplankton. On at least two occasions trawling was aimed at a scattering layer with vertical extension 10-20 m in the depth range 140-180 m in an attempt to secure euphausiids. The layer was detected by means of a Furuno Color Video Sounder FCV-292, with transducer operating at 28 and 200 kHz. Without a net sonde, however, it was difficult to know the precise depth of the trawl, which was generally regulated by boat speed, about two knots, and length of wire out, roughly 150-250 m, whatever the target. A typical towing time was 30 minutes. The catch of euphausiids was completely negligible, while the catch of *Calanus* was more or less constant at all depths, diminishing only with the advancing season, probably due to grazing by larger animals.

When the trawl was brought on board, the contents of the bag were emptied into a large plastic tub of 50-l capacity. Surface water was added as necessary. Relatively large animals, mainly ctenophores, but with some *Aurelia aurita* and a few euphausiids, were removed manually with a coarse sieve of 2-mm mesh size. Following this operation, the tub was covered with black plastic sheeting that was taped around the edge. The trawl was flushed with surface water either by manually wielded hose or by towing the net with open codend. Typically, three hauls were made, each netting quantities of *Calanus* of the order of 50-500 ml.

### Storage of live *Calanus*

Upon return to the quay at Espegrend, the tubs with the filtered *Calanus* specimens were transferred to a cold room maintained at about 10°C. Oxygenation was ensured by an air hose held on the bottom of the tub. Fresh sea water was added occasionally to enhance aeration by physical mixing as well as to lessen the effects of crowding by increasing the available water volume.

The animals were maintained in the cold room typically for a period of several days, during which subsamples were collected for laboratory measurements. These are now described.

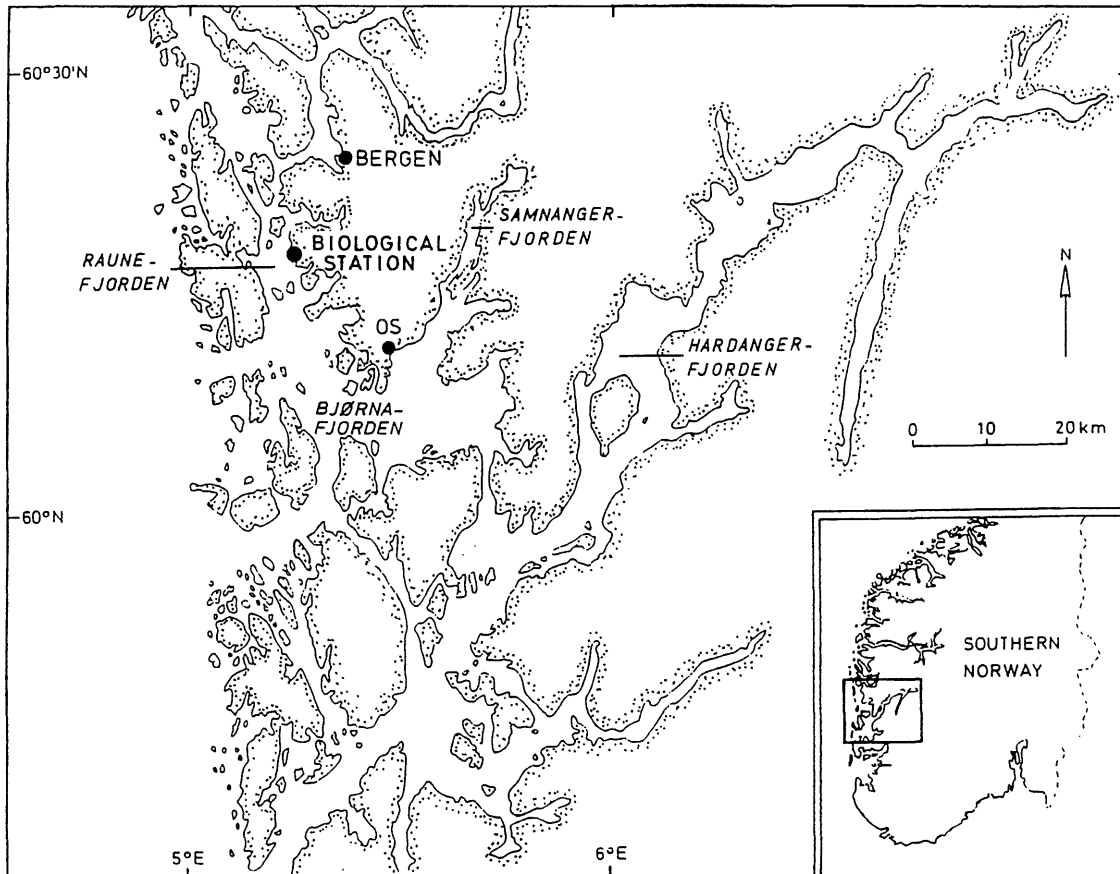


Fig. 1. Locality of sample collection in Raunefjorden.

## METHODS

### Salinity and temperature measurement

In order to characterize the Calanus habitat in Raunefjorden, two CTD stations were taken with a portable sonde, Gytre Mini-STD sonde model SD-200 (Gytre 1988), on 15 May and 3 June 1996. The profiles from the first station, which are most representative for the analyzed samples, are shown in Fig. 2.

Water samples were collected from the tubs where the Calanus specimens were maintained and from the apparatus used in the sound speed measurements reported below. The salinity was determined by standard procedures at the Institute of Marine Research. The temperature of the water samples during actual measurements in the velocimeter was monitored by a thermometer with 0.1°C gradation. Similar procedures were employed during sinking speed measurements, also reported below.

### Classification and sorting

A preliminary step in the preparation of Calanus samples for morphometry and biochemical analysis was classification and sorting. Initially, animals were collected from storage tubs in the cold room by dipping a Petri dish into the water to catch Calanus. The animals were thus transferred to the dish without air exposure. When outside of the cold room, the Petri dish was placed on a bed of crushed ice to avoid excessive heating and to keep the temperature close to the cold-room temperature of 10°C.

Species and copepod stages were identified by binocular microscope. Live animals were moved by pincette or pipette to separate Petri dishes maintained for each stage, namely the copepodites: C4 and C5, and adults: C6f and C6m. Among the identified copepods, Calanus finmarchicus dominated. Only a very few C3 copepodites were found, so these were not analyzed.

### Morphometry

Animals were analyzed individually in every case. A unique number was assigned to each segregated animal at the time of the initial measurement, which consisted either of video-filming, binocular-microscope measurement, or video-filming followed by binocular-microscope measurement.

Video-filming Some of the animals in large droplets of seawater were filmed on video through an attached microscope with 15X effective magnification for later analysis of shape and size. The animals were not necessarily horizontal when filmed. In addition, ensembles of animals taken from the surface layer of the cold-room-stored tubs were filmed on video. This material has not been analyzed; it constitutes a reserve for future analysis to supplement the measurements on the segregated individual Calanus specimens. The ensembles are identified solely by catch number.

Biocular-microscope measurement The cephalothorax length of a number of specimens was determined by binocular-microscope measurement. Excess water was removed by using a blotting paper. The usual position of the animal was horizontal, allowing the most precise measurement of length.

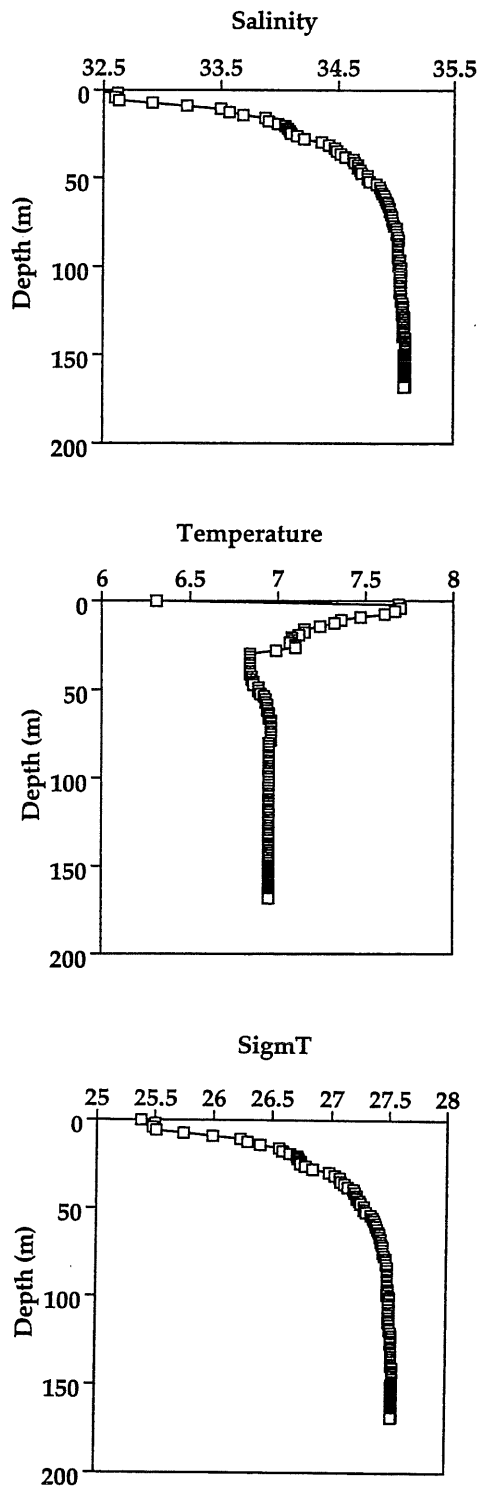


Fig. 2. Vertical profiles of salinity in parts per thousand, temperature in degrees Celsius, and  $\sigma_T$  as measured or determined from the CTD cast in Raunefjorden on 15 May 1996.

Some of the animals recorded on video film were also measured subsequently with the binocular microscope for control purposes. This was especially important because the magnification used in the video-filming was 15X, whereas that used in the binocular-microscope measurement was 25X.

Shape analysis Selected specimens on the video film were displayed on an electronic screen by means of the Zeus image-analysis system (Estep and MacIntyre 1989). A white line was usually drawn to mark the cephalothorax length, which was automatically registered. Individual images were printed in gray tones and transferred by manual tracing to graph paper with the white line coincident with a grid line. The outline was digitized and the data transferred to files on a digital computer.

#### Weighing

Following video-filming or binocular-microscope measurement, animals were stored individually in vials and frozen in liquid nitrogen. The same were later transferred to a freezer at  $-80^{\circ}\text{C}$ , freeze-dried in vacuo at  $-50^{\circ}\text{C}$ , weighed, and returned to the freezer at  $-80^{\circ}\text{C}$ . The freeze-dried samples were held in an exicator when outside of the freezer except during the weighing operation itself, which was done with due haste.

#### Biochemical analysis

The intention of this analysis was to determine both lipid and protein content, although in the event, due to a mishap, only the lipid analysis was performed.

The classified, video-filmed or binocular-microscope-measured, and weighed individual animals were combined by stage immediately prior to the lipid analysis. The combined sample was analyzed by means of an Iatroscan MK-5 TLC/FID, which is based on thin-layer chromatography. Lipid classes were determined with a flame-ionization detector. This measurement series was repeated a total of four times for each sample. The minimum recommended sample dry weight required for this method is 20 mg. The measurements were performed according to a protocol established at the Institute of Nutrition, Directorate of Fisheries, Bergen.

Determination of protein content would have been accomplished by means of the micro-Kjeldahl method. Nitrogen would have been measured by colorimetry as  $\text{NH}_4^+$ , and the protein content derived by multiplying the total nitrogen content by the numerical factor 6.25. The recommended minimum sample dry weight required for this method is approximately 40 mg. Again, the measurement would be made according to Institute of Nutrition protocol.

#### Density measurement

Determining the density of small particles can be surprisingly difficult. Determining the density of small aquatic animals is particularly thorny, because of the adherence of water to small parts of the body, for example, hairs and slender appendages, not to mention folds, as in scales or joints between carapace segments, and larger surfaces too, as by a thin film. The influence of surface tension is mighty on the microscopic scale; undoubtedly it is essential to the survival of animals that may enter the

turbulent, air-bubble-replete surface layer of the ocean, either naturally through vertical migration or inadvertently through forces of vertical convection. For an animal such as Calanus water is vital; to separate the animal from water, were it possible, would also be instantly destructive.

To avoid inevitably insurmountable problems in physically separating the animal and water, a method of density determination has been devised which is based on measurement of the animal in a seawater mixture. A quantity of the small animals in sea water is added to a volumetric flask of known mass. By weighing the combined system, the mass due to the mixture is determined, thence the density of the mixture,  $\rho$ . The density of sea water,  $\rho_0$ , is known a priori for the applicable conditions of temperature, salinity, and pressure by the equation of state (Millero 1982). The unknown density of the animal itself,  $\rho_1$ , is determined by the equation

$$\rho = \xi\rho_1 + (1 - \xi)\rho_0 \quad , \quad (1)$$

where  $\xi$  is the volume fraction of the animal. To determine  $\xi$ , the concentration and effective size composition of the animal in the volumetric flask sample is determined. Morphometry performed on individual animals enables single-animal volumes to be determined, thence the total volume of the animals by appropriate weighting of individual mean volumes by the distribution of sizes.

In the case where Calanus finmarchicus is staged and adults are distinguished by sex, with numerical concentration  $C_j$  for class  $j$ ,

$$\xi = \sum_j C_j v_j \quad , \quad (2)$$

where  $v_j$  is the mean volume of examined animals in class  $j$ , and the summation is performed over all classes. At present, an individual animal volume is determined by digitizing geometric cross sections of the animal, less appendages, in dorsal, ventral, or lateral aspects from printed video images, and computing the volume assuming cylindrical symmetry about the longitudinal axis of the animal. It is recognized that this is a fiction, but for purposes of volume determination, it is believed to represent an adequate first approximation (J. L. Watkins, pers. comm., J. Nejstgaard, pers. comm.).

#### Sound speed measurements

The basis of this measurement is the additivity of compressibility (Urick 1947), which follows from the definition

$$\kappa = -\frac{1}{V} \frac{\partial V}{\partial P} \quad (3)$$

where  $V$  represents the specific volume and  $\partial V/\partial P$  is the partial derivative of  $V$  with respect to pressure  $P$ . In the case of a binary mixture, say of some small particles with compressibility  $\kappa_1$  suspended in water with compressibility  $\kappa_0$ , the compressibility is

$$\kappa = \xi\kappa_1 + (1 - \xi)\kappa_0 \quad , \quad (4)$$



where  $\xi$  is the volume proportion of the small particles relative to the total volume, that is, the volume fraction. This equation holds a key to the measurement. If  $\kappa$  can be measured, then since  $\xi$  can be known by the way the mixture is prepared or analyzed, and since  $\kappa_0$  is known a priori given description of temperature, salinity, and pressure (Millero 1982),  $\kappa_1$  can be determined by simple solution.

In practice, for a liquid or fluid-like body,

$$\kappa = \frac{1}{\rho c^2} \quad , \quad (5)$$

where  $\rho$  is the density and  $c$  is the speed of sound. If the small particles are fluid-like, then equation (4) can be rewritten according to equation (5) as follows:

$$\frac{1}{\rho c^2} = \frac{\xi}{\rho_1 c_1^2} + \frac{1-\xi}{\rho_0 c_0^2} \quad , \quad (6)$$

where  $\rho_1$  and  $c_1$  are the respective density and sound speed of the small particles. If  $\rho_1$  is known, then

$$\frac{c_0}{c_1} = \left[ \frac{\rho_1 c_0^2}{\xi \rho c^2} - \frac{(1-\xi)\rho_1}{\xi \rho_0} \right]^{\frac{1}{2}} \quad (7)$$

This last equation suggests how the sound speed in the small particles can be determined by measuring the sound speed in the mixture. If a pulse of sound with characteristic wavelength that is much larger than typical dimensions of the small particles is transmitted over a fixed path length containing the mixture, then the time of flight can be measured. If the same measurement can be performed at the exact temperature for the liquid medium itself, without addition of particles, then the ratio of the travel times will equal the inverse ratio of the respective sound speeds. If the time of flight is measured by an electrical resistance  $R$ , for example, then

$$\frac{c_0}{c} = \frac{R}{R_0} \quad , \quad (8)$$

and, substituting in equation (7),

$$c_1 = c_0 \xi^{\frac{1}{2}} \left[ \frac{\rho_1 R^2}{\rho R_0^2} - (1-\xi) \frac{\rho_1}{\rho_0} \right]^{-\frac{1}{2}} \quad . \quad (8)$$

where  $\rho_0$  and  $c_0$  are known a priori (Mackenzie 1981, Millero 1982).

In the special case that  $\rho_1 = \rho_0$ ,

$$c_1 = c_0 \xi^{\frac{1}{2}} \left( \frac{R^2}{R_0^2} - 1 + \xi \right)^{-\frac{1}{2}} \quad . \quad (9)$$

If  $R = R_0 + \Delta R$ , where  $|\Delta R| \ll R_0$ , then

$$c_1 = c_0 \left( 1 - \frac{\Delta R}{\xi R_0} \right) \quad . \quad (10)$$

As in the determination of sound speed in Euphausia superba,  $R$  and  $R_0$  should be measured at or referred to the same temperature  $T$ . A method for making the referral is given in Foote (1990).

The small particles in this development could represent Calanus specimens, which are fluid-like in their properties, often conspicuously so through the presence of an oil sac and fluid-filled gut. For the square-wave-modulated sinusoid in the apparatus built by T. Gytre and used in Foote (1990), the carrier frequency is 500 kHz, hence the nominal wavelength is 3 mm. This is certainly larger than the largest dimension of most of the investigated Calanus specimens. While typical body lengths and widths are of the order of 2 and 0.6 mm, respectively, the requirement for use of equation (4) is at least partly met. It is observed that the time-of-flight equation applicable to particles that are large compared with the wavelength is not applicable here.

In the particular measurements, the animals were collected from the large holding tubs in the cold room by siphoning into a cup with sieve at the bottom. The animals were then introduced into the velocimeter in the form of an inverted T-shaped tube with a weak stream of sea water used to bear the animals. During the measurements, which otherwise followed the procedures described in Foote (1990), the animals were observed to be more or less uniformly distributed. The temperature was monitored, and a water sample was taken afterwards for later analysis of salinity.

#### Sinking speed measurements

In principle, measurement of the rate of sinking or rising of a body through a uniform fluid medium of different density than that of the body can be used to determine the density of the body. If the difference in densities is small, then the rate of sinking or rising is slow. If the body adopts a stable orientation, its speed will also be constant. However, the speed depends on the drag coefficient, which is generally unknown for finite bodies other than the sphere, for which Stoke's formula applies (Donnelly 1989). Thus translating a sinking speed measurement into a density value requires a separate determination, or calibration, for the very object that is to be measured.

For a body as complicated as the maturing Calanus finmarchicus is, the determination is daunting. In addition, the difference in medium densities may be accompanied by a difference in salinity or other chemical properties compared to that of the seawater medium natural to the animal. Diffusion is inevitable, thus changing the animal's composition during its density measurement.

Under certain conditions, the fact of sinking or rising may be associated unambiguously with a positive or negative difference in densities, thus putting a bound on the density.

In the present case, animals were prepared for the sinking experiment by anesthetization followed by removal of the antenna. Each animal was then immediately introduced into the constant-salinity column and its rate of sinking measured.

## RESULTS AND DISCUSSION

### Composition

The composition by stage of the Calanus finmarchicus specimens collected during the several catches in Raunefjorden is described in Table 1. In fact, this describes the number of animals in divided subsamples taken from the sound speed measurements. The possibility of non-representative sampling due to the period of time that elapsed between catching and use in the sound speed measurements is believed to be small because of the use of essentially all collected specimens to fill the velocimeter at a significant volume fraction. The volume of the apparatus covered by the acoustic transmission path is roughly 130 ml.

A substantial change in composition is seen from the catches in late April and early May to those in early June, which contain a much lower proportion of stage C4 and much higher proportion of adults, stage C6.

Other species were also observed in the catches. The larger animals were removed by filtering with a sieve, as described, but smaller animals remained. The numerical percentage of these was typically well under 1% and of the order of 1% in the worst case.

### Morphometry and sizing

A number of Calanus finmarchicus specimens were measured both by binocular microscope and by means of the Zeus video-image-analysis system. The largest number applies to stage C5. The results are presented through a scatter diagram in Fig. 3. While some video-measured cephalothorax lengths are greater than the respective binocular-microscope-measured lengths, most are less. This may be attributed to the near-horizontal orientation of the measurement with binocular microscope and generally skewed orientation of animals measured within a water droplet with the video system. The binocular-microscope measurement is believed to yield a better, more accurate measurement of length. However, for other measurements, the video system must be preferred.

Gross morphometric statistics of video-imaged and digitized Calanus finmarchicus specimens are presented in Table 2. Interestingly, very similar measures have been derived by D. Sameoto (pers. comm.) from some sampling stations on the Scotian Shelf in spring 1990, whereas at other stations the length and breadth measures are generally significantly larger. No interpretation of this variability is attempted here.

Exemplary video-taped and video-system-processed images of four specimens are shown in Fig. 4 together with the respective digitized images. The aspect is dorsal or near-dorsal.

### Dry weights

Dry weights have been measured and regressed on the cephalothorax length as measured both with binocular microscope and with video-image-analysis system. The results are expressed through the basic equation

$$m = a\ell^3 ,$$

Table 1. Composition of Calanus finmarchicus in the various catches as determined from samples taken from the subsequent sound speed measurements, together with the morphometrically determined volume fraction  $\xi$  in the same sound speed measurements. The bracketted number is highly uncertain.

Measurement series	subs.	Catch date	$V_s$ (ml)	$V_s/n$	Number of animals by stage				$\xi$
					C4	C5	C6f	C6m	
13	1	0429	25	64	103	33	5	4	0.365
18	1	0430	25	128	59	44	11	7	[0.600]
27	1	0506	50	256	95	55	20	12	0.917
29	1	0506	50	128	104	82	14	18	0.549
31	1	0506	50	128	169	111	24	16	0.808
33	1	0506	25	32	129	105	21	16	0.336
53	1a	0603	50	64	6	105	66	7	0.236
53	1b	0603	50	64	14	109	58	11	0.246
53	2a	0603	50	128	6	73	39	4	0.312
53	2b	0603	50	128	5	81	56	2	0.369

Table 2. Gross morphometric statistics of cephalothorax length  $\ell$  and maximum breadth  $b$  based on  $n$  video-system images for the respective stage. The average and standard deviations are shown together with the ratio  $b/\ell$  and average volume  $\bar{v}$  and corresponding standard deviation  $\Delta v$ .

Stage	n	--- Length measures (mm) ---				$\bar{b}/\bar{\ell}$	$\bar{v}$ (mm <sup>3</sup> )	$\Delta v$ (mm <sup>3</sup> )
		$\bar{\ell}$	$\Delta \ell$	$\bar{b}$	$\Delta b$			
C4	20	1.78	0.11	0.53	0.04	0.295	0.266	0.041
C5	14	2.30	0.06	0.66	0.06	0.287	0.565	0.138
C6f	17	2.49	0.19	0.74	0.05	0.298	0.731	0.101
C6m	6	2.29	0.28	0.68	0.06	0.299	0.626	0.186

Table 3. Regression coefficient  $a$  in equation  $m=a\ell^3$ , where  $m$  is the dry weight in milligrams and  $\ell$  is the cephalothorax length as determined by binocular-microscope or video-system measurement.

Stage	---- binocular microscope ----				----- video system -----			
	n	$a_1$	se( $a_1$ )	se	n	$a_2$	se( $a_2$ )	se
C4	19	0.00835	0.00051	0.0134	17	0.01330	0.00155	0.0392
C5	40	0.01303	0.00804	0.0614	22	0.01196	0.00079	0.0477
C6f	24	0.01018	0.00053	0.0436	15	0.01051	0.00078	0.0479
C6m	7	0.01306	0.01794	0.0286	8	0.01388	0.00078	0.0317

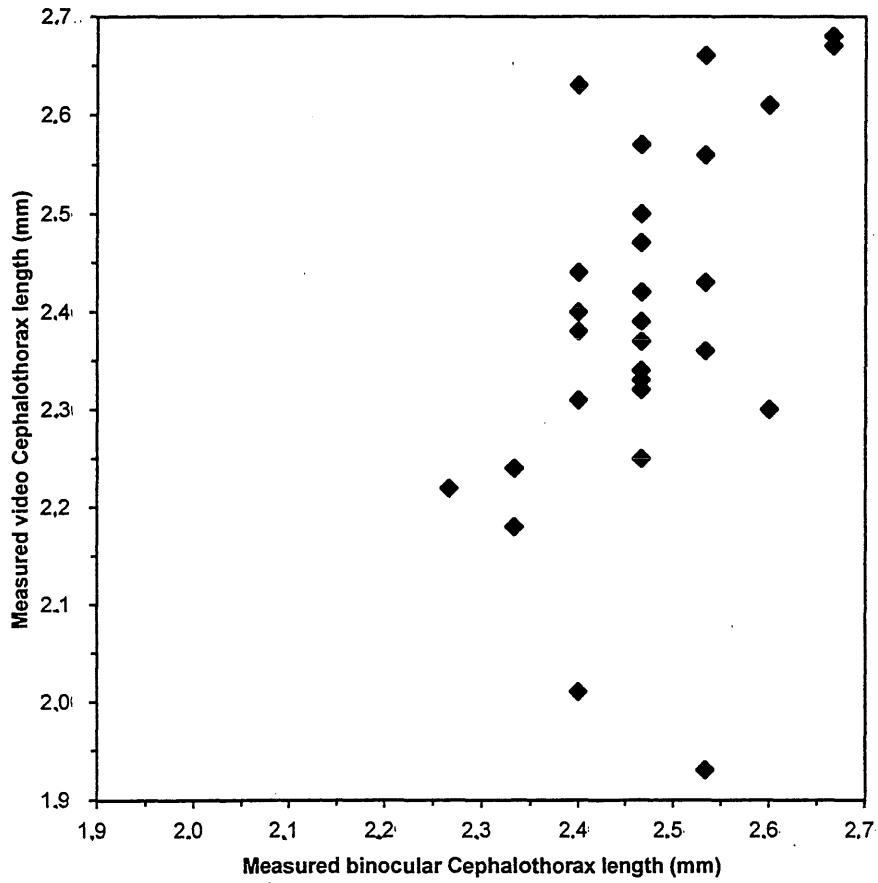


Fig. 3. Scatter diagram of cephalothorax lengths of the same 30 specimens representing both C5, C6f, and C6m, as measured with binocular microscope and video-image-analysis system.

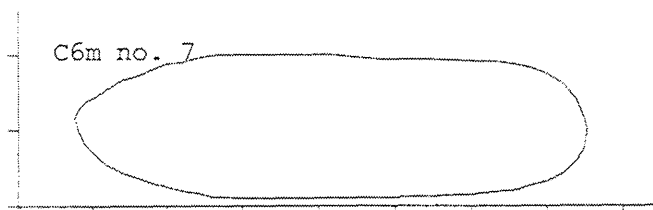
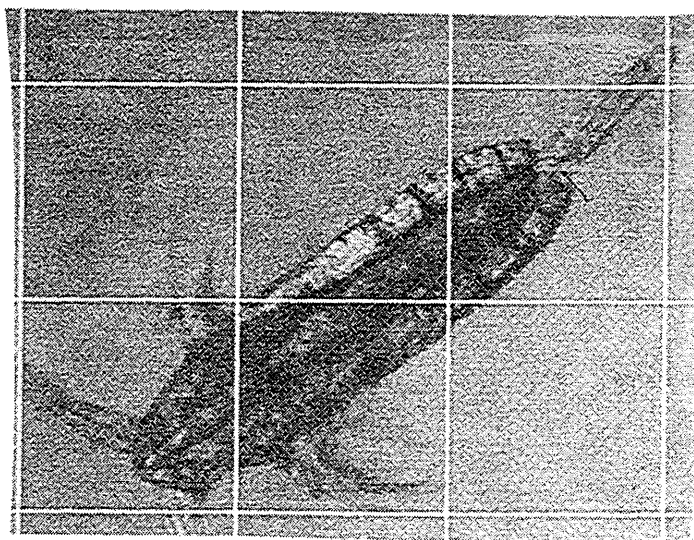
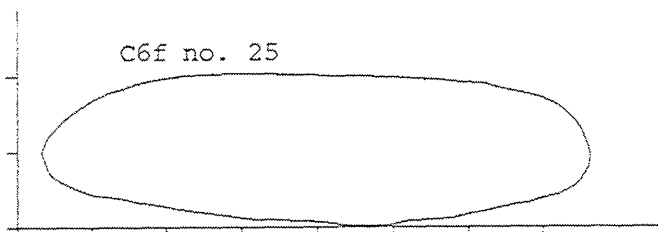
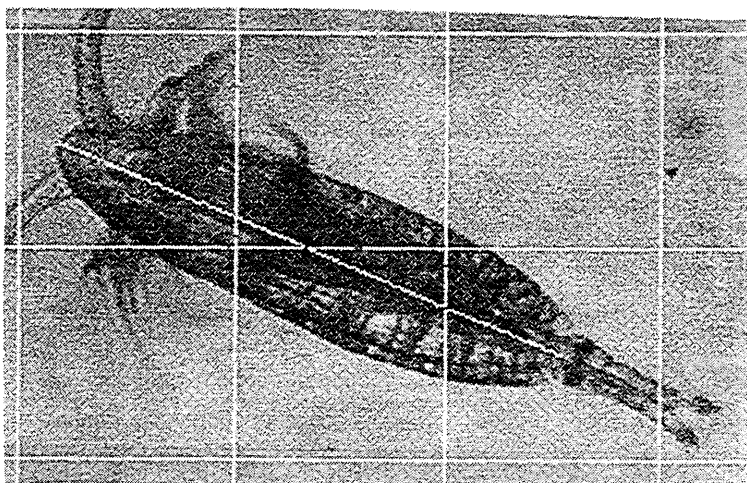
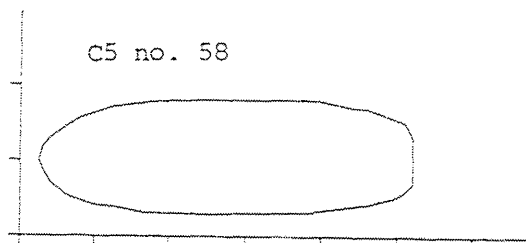
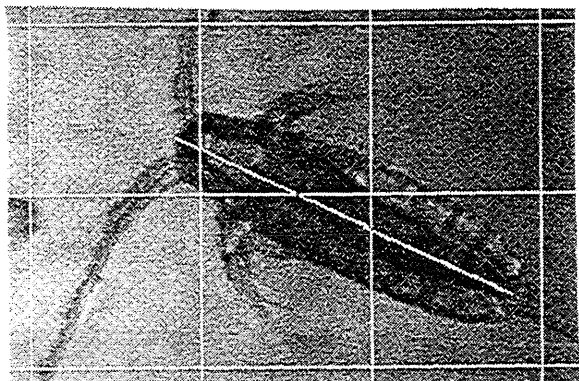
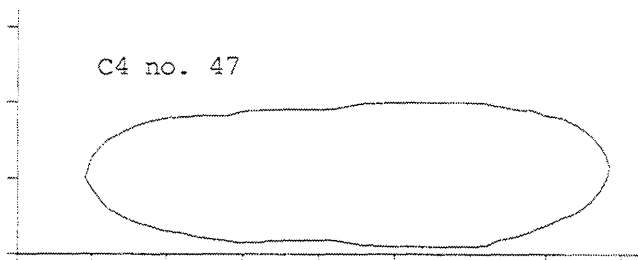
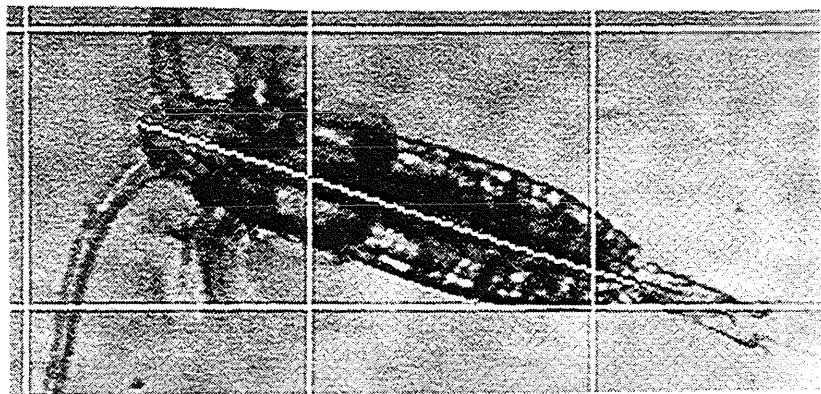


Fig. 4. Exemplary videotaped and processed images of four specimens of Calanus finmarchicus in dorsal aspect, with corresponding digitized geometric cross sections. The scale size of the video images is indicated by the superimposed squares of side length 1 mm in true size. The basic unit in the digitized images is 1 cm in terms of the actual printed video image.

where  $m$  is the mass in milligrams,  $l$  is the cephalothorax length in millimeters, and  $a$  is the regression coefficient. The standard error of the coefficient,  $se(a)$ , and standard error of the regression,  $se$ , are both presented with the coefficient  $a$  in Table 3. The underlying data for stage C5, as an example, are presented in Fig. 5.

#### Biochemical analysis

According to standard laboratory procedures, each analysis was performed a total of four times with the same sample. The sample characteristics are described in Table 4. The results for the lipid analysis are shown in Table 5. The categories of lipids are divided into two classes. The neutral lipids include waxy esters (WE), cholesterol (Chol), and other neutral lipids (NL rest), consisting mainly of triacylglycerides (TAG) and free fatty acids (FFA). The polar lipids include phosphatidylethanolamine (PE) with possible addition of phosphatidylserine (PS) and phosphatidylinositol (PI), phosphaticholine (PC), and other polar lipids (PL) mainly consisting of carotenoides, which are fat-soluble coloring agents, but also some lysophosphatedyl-compounds.

Very recent results on the lipid composition of Calanus finmarchicus are contained in a report by Miller and Morgan (1995). Because of the provisional nature of these results (C. B. Miller, pers. comm.), no comparison is attempted with the results in Table 5.

As mentioned above, the intended protein analysis was not performed, but solely for reasons of inadvertence.

#### Density and sound speed measurements

Measurements of mass density and relative sound speed are presented in Table 6. These apply to the described conditions of temperature and salinity in the velocimeter.

It is to be emphasized that both the animal mass density  $\rho_1$  and sound speed relative to that of the medium,  $c_1/c_0$ , both depend on the volume fraction  $\xi$ , which is presented in Table 1. This last quantity is determined by a new method which involves the following steps: filling a volumetric flask with a representative sample of animals, weighing this, determining the numerical composition of the contents by stage, and computation of the total animal volume by a morphometry performed on the basis of the video-analyzed images. The method, if simple in concept, was complicated by additional factors, namely, (1) addition of formalin droplets to the volumetric flask in all samples before those from June, requiring separate, admittedly imprecise compensation through a generalization of equation (1), (2) unanticipated delays in determination of the mass of the contents of the volumetric flask, and (3) application of morphometric data gathered on specimens from early catches for all of the density and sound speed measurements, notwithstanding major changes in composition observed over the time period of the catches, documented in Table 1.

For these reasons, the quality of results in Table 6 could in some cases be suspect at the outset. In fact, the values of density  $\rho_1$  relative to medium density  $\rho_0$  rather prove the point, especially in the light of the

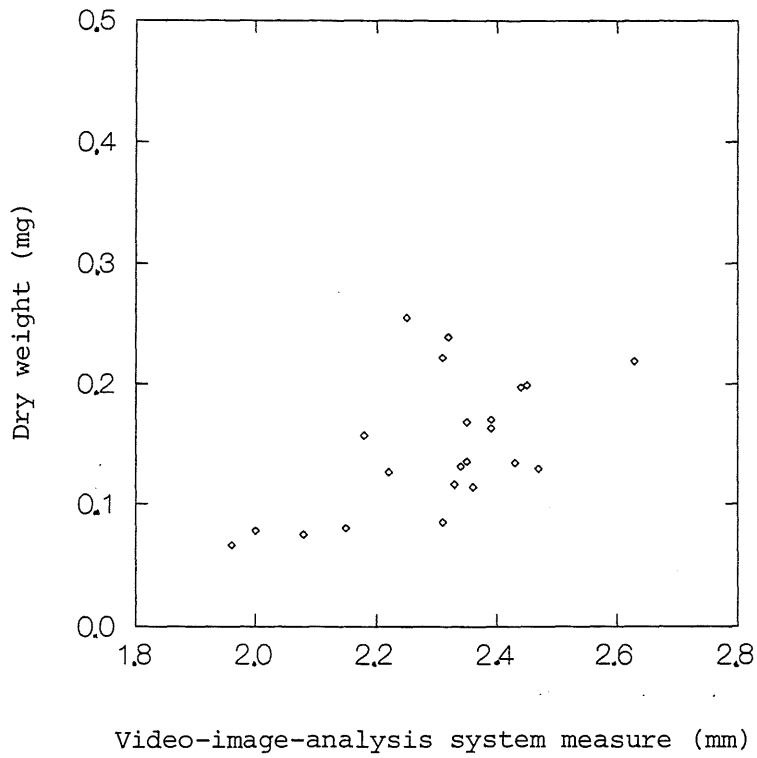
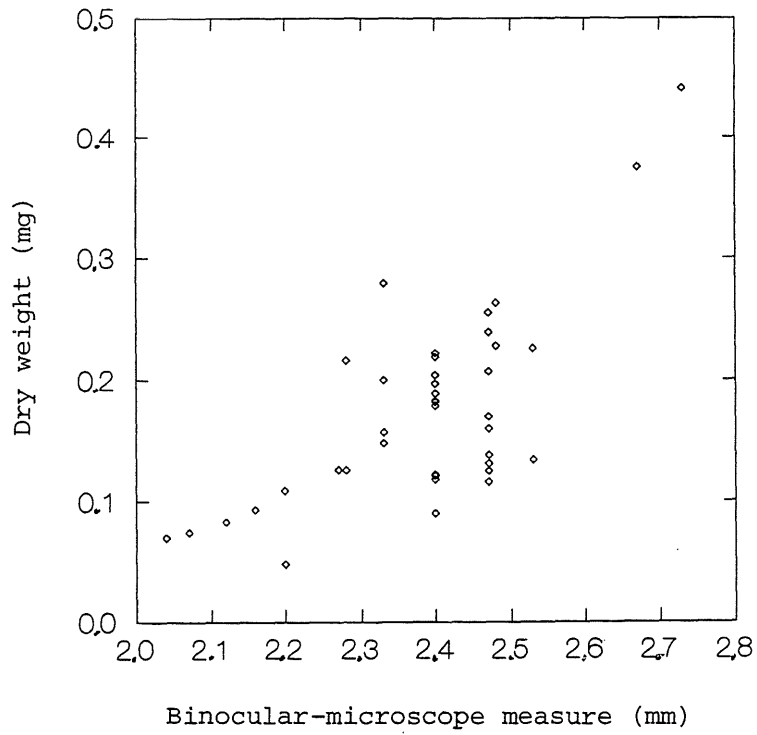


Fig. 5. Scatter diagrams of dry weight and cephalothorax length as measured with each of two systems.



Table 4. Characteristics of the samples analyzed for lipid content. The dry weight is denoted by m.

Stage	m (mg)	n	Dates of catch
C4	5.431	82	0430, 0503, 0506
C5	31.891	226	0430, 0503, 0506
C6f	3.798	26	0503, 0506
C6m	1.912	11	0506

Table 5. Lipid content by stage. See text for further explanation.

Stage	Quantity	NØYTRALE LIPIDER						POLARE LIPIDER						TOTAL LIPID		
		WE mg/ g DW	WE ug/ ind.	Chol mg/ g DW	Chol ug/ ind.	NL rest mg/ g DW	NL rest ug/ ind.	PE mg/ g DW	PE ug/ ind.	PC mg/ g DW	PC ug/ ind.	PL rest mg/ g DW	PL rest ug/ ind.	total mg/ g DW	total ug/ ind.	total % lipid
C4	Ave	<b>79,05</b>	5,24	<b>3,66</b>	0,24	<b>12,84</b>	0,85	<b>13,85</b>	0,92	<b>14,79</b>	0,98	<b>11,62</b>	0,77	<b>135,80</b>	8,99	<b>14</b>
C4	s.d.	<b>2,05</b>	0,14	<b>0,09</b>	0,01	<b>0,43</b>	0,03	<b>0,09</b>	0,01	<b>0,44</b>	0,03	<b>0,45</b>	0,03	<b>2,57</b>	0,17	<b>0,26</b>
C4	CV (%)	<b>2,59</b>	2,59	<b>2,40</b>	2,40	<b>3,35</b>	3,35	<b>0,64</b>	0,64	<b>2,99</b>	2,99	<b>3,88</b>	3,88	<b>1,89</b>	1,89	<b>1,89</b>
C5	Ave	<b>143,57</b>	20,26	<b>3,17</b>	0,45	<b>12,25</b>	1,73	<b>11,82</b>	1,67	<b>12,49</b>	1,76	<b>9,49</b>	1,34	<b>192,78</b>	27,20	<b>19</b>
C5	s.d.	<b>4,28</b>	0,60	<b>0,04</b>	0,01	<b>0,44</b>	0,06	<b>0,16</b>	0,02	<b>0,12</b>	0,02	<b>0,09</b>	0,01	<b>4,92</b>	0,69	<b>0,49</b>
C5	CV (%)	<b>2,98</b>	2,98	<b>1,14</b>	1,14	<b>3,61</b>	3,61	<b>1,37</b>	1,37	<b>0,92</b>	0,92	<b>0,95</b>	0,95	<b>2,55</b>	2,55	<b>2,55</b>
C6f	Ave	<b>62,99</b>	9,20	<b>3,82</b>	0,56	<b>13,15</b>	1,92	<b>13,61</b>	1,99	<b>15,32</b>	2,24	<b>11,69</b>	1,71	<b>120,58</b>	17,61	<b>12</b>
C6f	s.d.	<b>5,57</b>	0,81	<b>0,08</b>	0,01	<b>0,16</b>	0,02	<b>0,38</b>	0,05	<b>0,35</b>	0,05	<b>0,30</b>	0,04	<b>6,28</b>	0,92	<b>0,63</b>
C6f	CV (%)	<b>8,85</b>	8,85	<b>2,11</b>	2,11	<b>1,25</b>	1,25	<b>2,76</b>	2,76	<b>2,27</b>	2,27	<b>2,57</b>	2,57	<b>5,21</b>	5,21	<b>5,21</b>
C6m	Ave	<b>157,94</b>	27,45	<b>6,39</b>	1,11	<b>18,33</b>	3,19	<b>22,89</b>	3,98	<b>27,17</b>	4,72	<b>19,63</b>	3,41	<b>252,34</b>	43,86	<b>25</b>
C6m	s.d.	<b>5,98</b>	1,04	<b>0,08</b>	0,01	<b>0,11</b>	0,02	<b>0,56</b>	0,10	<b>0,11</b>	0,02	<b>0,34</b>	0,06	<b>6,22</b>	1,08	<b>0,62</b>
C6m	CV (%)	<b>3,78</b>	3,78	<b>1,18</b>	1,18	<b>0,58</b>	0,58	<b>2,45</b>	2,45	<b>0,39</b>	0,39	<b>1,71</b>	1,71	<b>2,46</b>	2,46	<b>2,46</b>

Table 6. Sound speed measurement conditions, densities, and relative sound speed. The medium density is denoted by  $\rho_0$ , measured density of the mixture by  $\rho$ , and derived density of Calanus finmarchicus by  $\rho_1$ . The relative sound speed in the animal is denoted by  $c_1/c_0$ .

Measurement series subs.	T(°C)	S(ppt)	---- Density (g/cm <sup>3</sup> ) ----			$c_1/c_0$
			$\rho_0$	$\rho$	$\rho_1$	
13 1	15.7	33.628	1.0248	1.0290	1.0344	1.0113
27 1	10.0	33.640	1.0259	1.0268	1.0089	1.0081
29 1	10.0	33.646	1.0259	1.0250	1.0624	1.0111
31 1	10.6	33.650	1.0258	1.0275	1.0272	1.0119
33 1	10.2	33.652	1.0259	1.0215	1.0082	1.0103
53 1	13.0	34.498	1.0260	1.0240	1.0176	1.0196
53 2	13.0	34.498	1.0260	1.0224	1.0153	1.0329

sinking speed measurements. These last-mentioned measurements were performed on stage C5 or females, following anesthetization and removal of the antennae, derived from the catch on 6 May only. In each of the 64 investigated cases, the animals sank. The density of sea water in the column was very near to  $1.0262 \text{ g/cm}^3$ . Admittedly, the antennae were absent, but the volume of these is rather small compared to that of the cephalothorax.

At present, a number of error sources have been identified. Making all measurements on the same ensemble of animals seems to be an obvious remedy, at least for proving the value of the new method for density determination.

In computing sound speed from the measurements with the velocimeter, the animal mass density was assumed to be identical with that of the seawater immersion medium. That is, the tabulated value for  $\rho_1$  was ignored, being replaced by the value for  $\rho_0$ . Thus equation (9) could be employed. Given the nearness of the value of sound speed to that of the pure seawater medium, the approximation in equation (10) would be equally useable for this determination. The numbers are credible, but with the important caveat of assumed neutrally buoyant state. Insofar as the animals sank, a higher density would translate into a slower sound speed, as is evident from equation (8).

The literature on density and sound speed of Calanus finmarchicus is thin, notwithstanding possible appearances. A careful reading of this indicates apparently only two independent measurements of density for the animal beyond the egg and nauplii stages, namely those performed by Gross and Raymont (1942) and Køgeler et al. (1987). In the first, the specific gravity, or mass density in grams per cubic centimeter, is described as follows: stage C5: 1.0255-1.0265, females: 1.043-1.045, and males: 1.043-1.047. These measurements, which were based on sinking rates, were criticized, however, by Salzen (1956), because of lack of treatment of possible effects of viscosity.

Køgeler et al. (1987) have determined the density by means of a density-gradient column. Measurements were performed on Calanus finmarchicus specimens with a prosome length in the range 2.2-3.0 mm. The measurements spanned a range of variation from 1.022 to 1.038  $\text{g/cm}^3$ , with demonstrated systematic seasonal pattern of variation. Interestingly, for the most comparable measurements, which were performed in late May, the density was 1.023-1.025  $\text{g/cm}^3$ .

The only sound speed measurements performed on Calanus finmarchicus that are known to these authors are those made by Køgeler et al. (1987). These indicate a contrast in sound speed of 1.021-1.036. However, the basic equation assumed by Køgeler et al. (1987) expresses the time of flight of the sound pulse as the sum of the times of propagation in the seawater medium and in the animals in the proportion of their respective total volumes. However, since the acoustic wavelength, nominally 3 mm, is not small compared to the animal size, it would seem that the compressibility equation given in equation (4) is the more appropriate. The particular values given in the cited work would consequently be questionable and of doubtful value for purposes of comparison.

## Error analysis

All of the reported measurements should be accompanied by an estimate of uncertainty or, alternatively, confidence limits. That such has generally been omitted merely reflects the preliminary nature of the work and desire for timely dissemination.

## SUMMARY

An attempt has been made to perform collateral measurements of diverse properties of Calanus finmarchicus on essentially the same ensemble of animals. At least this was the ambition at the outset. A number of practical difficulties have been encountered. Because of the limited time period available for the measurements, not to mention their inevitably heuristic nature for the authors themselves, a number of the results are apparently implausible and, in the case of density determination, contradictory. Nonetheless, it is hoped that later, more consistent application of the same methods may yield useful data.

The particular aim in hastening the presentation of this admittedly preliminary work is to solicit criticism of the methods. If these are sound, then the program of collateral measurements may be pursued, for, as already noted, calculation of acoustic scattering properties depends on knowledge of the properties of individuals. In some cases at present, as in density and sound speed measurements, for example, only ensemble values are available. Calling attention to this may stimulate a more fundamental consideration of the problems of individual-animal characterization.

## ACKNOWLEDGEMENTS

J. Egge is thanked for frequent, expert assistance with practical details at the field station, and K. O. Fjeldstad, for operating R/V Aurelia and assisting in the catching operations. H. Myran is thanked for performing the salinity analyses, and O. M. Gjervik, for assistance with CTD-sondes. The following are thanked for diverse communications or discussions: D. Aksnes, U. Båmstedt, A. Clarke, T. Gytte, H. P. Knudsen, C. B. Miller, J. Nejstgaard, D. Sameoto, and J. L. Watkins. The partial support of the European Union through contract no. MAS3-CT95-0031 is gratefully acknowledged.

REFERENCES

- Anon. 1968. Smaller mesozooplankton. Report of Working Party No. 2. In Zooplankton sampling, pp. 153-159. Ed. by D. J. Tranter. UNESCO, Paris. 174 pp.
- Anon. 1977. Report of the Working Group on North Sea Herring Larval Surveys. ICES Coop. Res. Rep., 68. 33 pp.
- Båmstedt, U. 1976a. Studies on the deep-water pelagic community of Korsfjorden, western Norway. Ecological aspects of individual variations in weight and protein and lipid content of Euchaeta norvegica (copepoda). *Sarsia*, 59: 31-46.
- Båmstedt, U. 1976b. Studies on the deep-water pelagic community of Korsfjorden, western Norway. Changes in the size and biochemical composition of Meganyctiphanes norvegica (Euphausiacea) in relation to its life cycle. *Sarsia*, 61: 15-30.
- Clarke, A. 1980. The biochemical composition of krill, Euphausia superba Dana, from South Georgia. *J. exp. mar. Biol. Ecol.*, 43: 221-236.
- Donnelly, R. J. 1989. Fluid dynamics. In A physicist's desk reference. The second edition of *Physics Vade Mecum*, Chap. 12, pp. 196-209. Ed. by H. L. Anderson. American Institute of Physics, New York. 356 pp.
- Estep, K. W., and MacIntyre, F. 1989. Counting, sizing, and identification of algae using image analysis. *Sarsia*, 74: 261-268.
- Falk-Petersen, S. 1981. Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: seasonal changes in body weight and the main biochemical composition of Thysanoessa inermis (Krøyer), T. raschii (M. Sars), and Meganyctiphanes norvegica (M. Sars) in relation to environmental factors. *J. exp. mar. Biol. Ecol.*, 49: 103-120.
- Falk-Petersen, S. 1985. Growth of the euphausiids Thysanoessa inermis, Thysanoessa raschii, and Meganyctiphanes norvegica in a subarctic fjord, north Norway. *Can. J. Fish. Aquat. Sci.*, 42: 14-22.
- Falk-Petersen, S., Gatten, R. R., Sargent, J. R., and Hopkins, C. C. E. 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: seasonal changes in the lipid class composition of Meganyctiphanes norvegica (M. Sars), Thysanoessa raschii (M. Sars), and T. inermis (Krøyer). *J. exp. mar. Biol. Ecol.*, 54: 209-224.
- Falk-Petersen, S., Sargent, J. R., Hopkins, C. C. E., and Vaja, B. 1982. Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: lipids in the euphausiids Thysanoessa raschii and T. inermis during spring. *Mar. Biol.*, 68: 97-102.
- Foote, K. G. 1990. Speed of sound in Euphausia superba. *J. acoust. Soc. Am.*, 87: 1405-1408.
- Foote, K. G. 1991. Acoustic sampling volume. *J. acoust. Soc. Am.*, 90: 959-964.

- Foote, K. G. 1995. Comparison of equal-area cylindrical and circular piston transducers. *ICES J. mar. Sci.*, 52: 67-75.
- Gross, F., and Raymont, J. E. G. 1942. The specific gravity of Calanus finmarchicus. *Proc. R. Soc. Edinburgh*, B61: 288-296.
- Gytte, T. 1988. Automatic calibration of the sensors used in a miniature STD instrument. *ICES C.M. 1988/C:13*. 6 pp. [mimeo.]
- Johnson, R. K. 1977. Sound scattering from a fluid sphere revisited. *J. acoust. Soc. Am.*, 61: 375-377.
- Køgelier, J. W., Falk-Petersen, S., Kristensen, Å., Pettersen, F., and Dalen, J. 1987. Density- and sound speed contrasts in sub-Arctic zooplankton. *Polar Biol.*, 7: 231-235.
- Lowndes, A. G. 1942. The displacement method of weighing living aquatic organisms. *J. Mar. Biol.*, 25: 555-574.
- Mackenzie, K. V. 1981. Nine-term equation for sound speed in the oceans. *J. acoust. Soc. Am.*, 70: 807-812.
- Miller, C. 1995. TransAtlantic Studies of Calanus (TASC) Working Group. *U.S. GLOBEC News*, (8): 9-11.
- Miller, C. B., and Morgan, C. A. 1995. Storage lipids of Calanus finmarchicus in the Gulf of Maine, a data report. Oregon State University, Corvallis, Oregon. 15 pp. + figs. + appendices.
- Millero, F. J. 1982. The thermodynamics of seawater. Part I. The PVT properties. *Ocean Sci. Eng.*, 7: 403-460.
- Roschke, V. N. 1977/78. Variabilität des Fett- und Proteingehalts beim Krill (Euphausia superba Dana). *Meeresforschung*, 26: 217-221.
- Saether, O., Ellingsen, T. E., and Mohr, V. 1985. The distribution of lipid in the tissues of Antarctic krill, Euphausia superba. *Comp. Biochem. Physiol.*, 81B: 609-614.
- Salzen, E. A. 1956. The density of the eggs of Calanus finmarchicus. *J. mar. biol. Ass. U.K.*, 35: 549-554.
- Sameoto, D. D., Jaroszynski, L. O., and Fraser, W. B. 1980. BIONESS, a new design in multiple net zooplankton samplers. *Can. J. Fish. Aquat. Sci.*, 37: 722-724.
- Skjoldal, H. R., Noji, T. T., Giske, J., Fosså, J. H., Blindheim, J., and Sundby, S., eds. 1993. *Mare Cognitum*. Science plan for research on marine ecology of the Nordic Seas (Greenland, Norwegian, Iceland Seas) 1993-2000. Institute of Marine Research, Bergen, Norway. 162 pp. + addendum 6 pp.
- Solemdal, P., and Ellertsen, B. 1984. Sampling fish larvae with large pumps; quantitative and qualitative comparisons with traditional gear. *Flødevigen rapportser.*, 1: 335-363.

- Stanton, T. K. 1989a. Sound scattering by cylinders of finite length. III. Deformed cylinders. *J. acoust. Soc. Am.*, 86: 691-705.
- Stanton, T. K. 1989b. Simple approximate formulas for backscattering of sound by spherical and elongated objects. *J. acoust. Soc. Am.*, 86: 1499-1510.
- Stanton, T. K., Clay, C. S., and Chu, D. 1993. Ray representation of sound scattering by weakly scattering deformed fluid cylinders: Simple physics and application to zooplankton. *J. acoust. Soc. Am.*, 94: 3454-3462.
- Tande, K. S. 1982. Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: generation cycles, and variations in body weight and body content of carbon and nitrogen related to overwintering and reproduction in the copepod Calanus finmarchicus (Gunnerus). *J. Exp. Mar. Biol. Ecol.*, 62: 129-142.
- Urick, R. J. 1947. A sound velocity method for determining the compressibility of finely divided substances. *J. Appl. Phys.*, 18: 983-987.
- Wiebe, P. H., Burt, K. H., Boyd, S. H., and Morton, A. W. 1976. A multiple opening/closing net and environmental sensing system for sampling zooplankton. *J. Mar. Res.*, 34: 313-326.
- Wiebe, P. H., Morton, A. W., Bradley, A. M., Backus, R. H., Craddock, J. E., Barber, V., Cowles, T. J., and Flierl, G. R. 1985. New developments in the MOCNESS, an apparatus for sampling zooplankton and mikronekton. *Mar. Biol.*, 87: 313-323.
- Wiebe, P. H., Greene, C. H., Stanton, T. K., and Burczynski, J. 1990. Sound scattering by live zooplankton and micronekton: Empirical studies with a dual-beam acoustical system. *J. acoust. Soc. Am.*, 88: 2346-2360.

