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SOUND SPEED MEASUREMENTS OF ANTARCTIC KRILL

by

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ABSTRACT

The longitudinal sound speed in Euphausia superba has been measured by time of flight of an acoustic pulse through a suspension of krill in a T-shaped tube with nominal path length of 20 cm. Results are presented.

RESUME: MESURES DE VITESSE DU SON DU KRILL DE L'ANTARCTIQUE

La vitesse longitudinale du son du krill de l'Antarctique, Euphausia superba, a été mesurée par le temps de déplacement d'une pulsation acoustique à travers une suspension de krill dans un tube en forme de T, de 20 cm de longueur nominale. On présente les résultats de cet essai.

INTRODUCTION

Zooplankton are being studied increasingly by means of acoustics. To support such studies, the density and speed of longitudinal sound waves in the animals are often measured. Knowledge of these two physical properties, together with data on size and shape, may enable the backscattering cross section or target strength of the animal to be computed. Anderson (1950), Johnson (1977), and Stanton (1988, in press), among others, describe theoretical models for this computation.

The alternative to computation of target strength is direct measurement. This is generally difficult and is limited to a particular condition or physical state of the animal, as defined by its density and sound speed. These quantities will vary with season (Køgelier et al. 1987), as biochemical composition changes throughout the year. There is, consequently, every reason to measure the density and sound speed of zooplankton whenever possible, even when target strength can be measured.

A significant gap in tabulations of the speed of longitudinal sound waves (Greenlaw and Johnson 1982, Køgelier et al. 1987), called merely sound speed hereafter, is that of Antarctic krill (Euphausia superba). This study reports on the first measurements of sound speed in this species, which were made

during the Krill Target Strength Experiment, conducted under the aegis of British Antarctic Survey, during the autumnal summer 1987-88. This study therefore supplements the preliminary report on target strength by Everson et al. (1988). Both the sound speed measurements reported here and final target strength data on krill have been documented by, respectively, Foote (1989 MS) and Foote et al. (1989 MS).

In this work two methods for measuring target strength in small objects are outlined. The application of one of these to Antarctic krill is then described. Results are given and discussed.

#### MEASURING SOUND SPEED IN SMALL OBJECTS

The object is regarded as a homogeneous fluid. It may, however, also be a homogeneous solid whose shear-wave sound speed is very small compared to the longitudinal-wave sound speed.

Two cases are considered, as the object size is less than or greater than the acoustic wavelength of measurement. In either case, the absolute size is considered to be too small for convenient measurement of the sound speed in an individual specimen.

The general method is that of time of flight of an acoustic pulse through an ensemble of the object suspended in a host fluid. By measuring the physical properties of the mixture, and knowing the properties of the host fluid, if by measurement, the sound speed in the object can be inferred.

The host fluid is characterized by its density  $\rho_o$ , sound speed  $c_o$ , and compressibility  $\kappa_o = (\rho_o c_o^2)^{-1}$ .

#### Small objects

The objects whose sound speed  $c_1$  is to be determined are assumed to be rather small compared to the acoustic wavelength of measurement. Mixing of the objects in the host fluid is assumed to result in a uniform random distribution.

The change in volume  $\Delta V$  of a volume  $V$  of fluid due to a uniform pressure increase  $\Delta P$  is the sum of the volume changes in host fluid and objects,  $\Delta V_o$  and  $\Delta V_1$ , respectively. Since, by definition, the compressibility  $\kappa$  is

$$\kappa = -\frac{1}{V} \lim_{\Delta P \rightarrow 0} \frac{\Delta V}{\Delta P} \quad , \quad (1)$$

$$\Delta V_o = -\kappa_o v_o V \Delta P \quad , \quad (2a)$$

and

$$\Delta V_1 = -\kappa_1 v_1 V \Delta P \quad , \quad (2b)$$

where  $v_0$  and  $v_1$  are the volume fractions of the host fluid and object in the mixture,  $v_0+v_1=1$ , and  $\kappa_1$  is the compressibility of the object. Thus, summing volumes, the compressibility of the mixture is

$$\kappa = \kappa_0 v_0 + \kappa_1 v_1 \quad . \quad (3)$$

However, for a fluid,

$$\kappa = (\rho c^2)^{-1} \quad , \quad (4)$$

where  $\rho$  is the density and  $c$  is the sound speed. For the particular fluid mixture,

$$\rho = \rho_0 v_0 + \rho_1 v_1 \quad . \quad (5)$$

Thus,

$$c = \left[ (\rho_0 v_0 + \rho_1 v_1) \left( \frac{v_0}{\rho_0 c_0^2} + \frac{v_1}{\rho_1 c_1^2} \right) \right]^{-1/2} \quad . \quad (6)$$

This result is given by Wood (1941) and is also found in the more accessible Urlick (1975).

Determination of  $c_1$  proceeds in the following way:  $\rho_0$  and  $c_0$  are known a priori or are measured,  $\rho_1$  is similarly known or measured,  $v_1$  is known by the manner of mixing object and host fluid,  $v_0+v_1=1$ , and  $c$  is measured by the time-of-flight method. Equation (6) is solved for the only remaining unspecified quantity,  $c_1$ .

#### Large objects

The objects are assumed to be rather large compared to the acoustic wavelength of measurement. It is sufficient for irregularly shaped objects that the mean dimension along the direction of flight of the acoustic pulse be rather large.

The time of flight  $t$  of an acoustic pulse through a mixture is the sum of times of flight through the host fluid,  $t_0$ , and object,  $t_1$ . These times are proportional to the corresponding volume fraction,  $v_0$  and  $v_1$ , and inversely proportional to the corresponding sound speed,  $c_0$  and  $c_1$ , thence

$$t = \ell \left( \frac{v_0}{c_0} + \frac{v_1}{c_1} \right) \quad , \quad (7)$$

where  $\ell$  is the path length over which  $t$  is measured, and  $v_0+v_1=1$ . Since

$t = \ell/c$ ,

$$\frac{1}{c} = \frac{v_0}{c_0} + \frac{v_1}{c_1} \quad (8)$$

The unknown sound speed  $c_1$  is found by solving this equation, given prior knowledge of  $c_0$  and measurement of  $c$  for the given volume fraction  $v_1$  of object in the mixture.

#### MEASUREMENT PRINCIPLE FOR ANTARCTIC KRILL

For typical animal sizes, characterized by total length of 30 mm, and approximate alignment along the propagation path, the measurement frequency of 500 kHz guarantees the applicability of the large-object condition. Equation (8) thus governs the measurement.

However, because the speed of sound in krill,  $c_1$ , is only slightly greater than that in sea water,  $c_0$ , it is difficult to make precise measurements directly from an oscilloscope. Turning a precision potentiometer to adjust the length of an independent square wave displayed through a second channel on the oscilloscope, operating in time-expanded mode, allows the travel time to be accurately gauged through a precision measurement of electrical resistance. If  $R$  denotes the result of this kind of measurement on the mixture of water and krill at volume fraction  $v$ ,  $R_0$  denotes that in water containing no krill, and  $R_1$  denotes that hypothetical result for krill alone, then

$$R = (1-v)R_0 + vR_1 \quad (9)$$

Since  $R$  and  $R_0$  can be measured and  $v$  is known,  $R_1$  can be determined. The sound speed in krill,  $c_1$ , relative to that in water,  $c_0$ , is given by the the simple relation

$$\frac{c_1}{c_0} = \frac{R_0}{R_1} \quad (10)$$

The problematical elements of the measurement are ensuring the uniformity of krill distribution in the sound path and accounting for variations in a laboratory without temperature control. These issues are addressed in the following sections.

#### MATERIALS AND EXPERIMENTAL METHODS

The physical apparatus for measuring sound speed closely resembles that used by K geler et al. (1987), as it was based on drawings provided by J. Dalen, Institute of Marine Research, Bergen. The velocimeter consists of a T-shaped tube and associated acoustic and electronic instrumentation. Ceramic

transducers resonant at 500 kHz, but damped for pulsed operation, are mounted at the ends of the horizontal part of the tube, about 20 cm distant from each other. Pulsing of one of the transducers, used for transmission, is controlled by circuitry built for the purpose by T. Gytre, Institute of Marine Research, Bergen. Additional circuitry, also designed and built by Gytre, generates single-shot square waves whose length can be adjusted to that of an early, easily identifiable feature of the received pulse as displayed on an oscilloscope. Measurement of the resistance of the potentiometer, whose fine tuning accomplishes the equalization process, allows precise determination of the travel time, thence sound speed. A standard Kikusui COS 5100 Oscilloscope at 100 MHz and Philips Manufacture PM 2519 Automatic Multimeter proved adequate for the measurements.

Krill were introduced into the tube at the open end. As these were alive and swimming, they had to be assisted into the flooded, horizontal, acoustically instrumented part of the tube. This was done using a slim piece of hollow glass boiler tubing, which could also double as a large pipette for removing excess water introduced into the vertical section of the tube with the krill.

A sound speed measurement series consisted of several distinct parts. Krill were transferred from their holding pen or cage in the bay at Stromness on South Georgia to a 100-l tub half-filled with sea water. This was immediately brought into the laboratory, 200 m distant from the holding site. The T-tube was flushed several times with this water and the first measurements made on water alone, for calibration purposes. Krill were typically added in net increments of 50 or 25 animals, and the travel time was measured for each of the new ensembles. The last valid krill measurement of a series was made when the horizontal portion of the tube was full of krill, introduced by prodding, but without compression or crushing. After the last krill measurement, the tube was emptied, flushed and refilled with tub water for additional water-only calibration measurements.

The ambient temperature in the laboratory was not controlled, and because the volume of the horizontal section of the T-tube was only 130.5 ml, the temperature of sea water-and-krill mixture generally varied in the course of the measurement series. The temperature was therefore measured immediately before or immediately after each travel-time measurement. The salinity of tub water was also measured once for each batch of krill.

Following the conclusion of each T-tube measurement series, the total volume of subject krill was measured by displacement in a graduated cylinder. The total length was then measured as the distance from the anterior edge of the eye to the tip of the telson, expressed to the nearest millimeter on or below by truncation. Density was measured occasionally by means of density bottles.

#### ANALYSIS

Several conditions must be fulfilled for equations (8)-(10) to be applicable. Firstly, the krill must be essentially uniformly distributed across the cross section of the sound path, hence the cross section of the horizontal section of T-tube. Because of the tendency of krill to sink at

the highest number densities, where swimming movements are limited by the proximity of other krill, only measurements made at the highest uncompressed densities were assumed to be valid for further analysis. At these densities, with estimated volume fraction between 29 and 40%, the krill appeared to fill the horizontal tube uniformly. Most of the animals were aligned with the axis of the tube, with thoracic legs usually in motion or showing signs of movement when touched, as by prodding.

A second condition for fulfillment of the equations is constancy of measurement conditions. For the aforementioned reasons of lack of temperature control in the laboratory and smallness of measurement volume, the temperature generally changed in the course of a measurement series. To account for this, therefore, and at the same time, make use of all available calibration measurements, done on water alone at the beginning and end of each measurement series, each  $R_o$ -measurement was referred to a constant temperature  $T$  and the resulting referred measurements averaged.

This referral process was effected by means of the approximate relation,

$$\frac{1}{R} = aT + b \quad , \quad (11)$$

where the coefficients  $a$  and  $b$  were determined in the following way. A succession of measurements of  $T$  and  $R$  were made on saline water samples while cooling from  $37^{\circ}\text{C}$  to the ambient laboratory temperature. Least mean squares regression of  $1/R$  on  $T$  determined the value  $a=4.5 \cdot 10^{-5} \text{ (k}\Omega \cdot \text{K)}^{-1}$ . For the particular velocimeter, this describes a temperature gradient in sound speed of  $2.9 \text{ m(s}\cdot\text{K)}^{-1}$ , which agrees closely with Wilson's finding as cited by Urick (1975). The resistance  $R_o(T)$  is determined by solving the equation pair:

$$\frac{1}{R_o(T_i)} = aT_i + b \quad (12a)$$

$$\frac{1}{R_o(T)} = aT + b \quad , \quad (12b)$$

where the measurement at temperature  $T_i$  is to be referred to that expected at temperature  $T$ . Solving the two equations,

$$R_o(T) = \frac{R_o(T_i)}{1 - aR_o(T_i)(T_i - T)} \quad . \quad (13)$$

For  $n$  measurements of  $R_o$ , the average is

$$\overline{R_o}(T) = \frac{1}{n} \sum_{i=1}^n \frac{R_o(T_i)}{1 - aR_o(T_i)(T_i - T)} \quad . \quad (14)$$

If the reference temperature is chosen to be that measured at the time of the applicable high-density krill measurement  $R(T)$ , the relative sound speed in krill is given by equation (10), viz.

$$\frac{c_1}{c_o} = \frac{\overline{R_o}(T)}{R_1(T)} \quad , \quad (15)$$

in this elaborated form. The quantity  $R_1(T)$  is determined by solving equation (9) where  $R_o$  is replaced by  $\overline{R_o}(T)$ . The volume fraction  $v$  in this cited equation was not determined from the direct volume measurement, which was judged to be too imprecise, but rather from the density bottle measurements. As determined by these data, gathered and analyzed by J. L. Watkins, British Antarctic Survey, Cambridge, the volume of a single krill,  $V$ , in cubic centimeters is

$$V = 0.9432 m - 0.0034 \quad , \quad (16)$$

where  $m$  is the wet weight. For a so-called standard krill, which is the applicable category for the present krill subjects,

$$m = 9.60 \cdot 10^{-6} \ell^{2.94} \quad , \quad (17)$$

where  $\ell$  is the total krill length in millimeters (Morris et al. 1988).

## RESULTS

A total of 51 measurement series were performed. This number includes series where the technique was being established and refined, and others in which the subject animals were dead, moribund or in such poor condition that the results could not be associated with healthy specimens. For the sake of consistency, only those measurement series were selected where the measurement was performed within 12 hours of the subject's removal from the sea and the subjects were in reasonably good condition, as evidenced by swimming activity and maintenance of pigmentation.

The results of the applicable 17 measurement series are shown in the table. Included are the number of krill specimens and associated length statistics, the volume fraction, and the time of measurement reckoned from the time of removal of animals from the sea.

The sound speed in krill exceeds that in sea water by 2.79% in the mean, with standard deviation of 0.24%. That is, the determined sound speed contrast is  $1.0279 \pm 0.0024$ . The associated mean length is 32.2 mm. If the data from measurement series 44 and 45 are excluded, because of their extreme lengths, the resulting relative sound speed increase is  $2.75 \pm 0.22\%$  for mean mean length  $31.4 \pm 1.0$  mm. The applicable density for krill of mean length 31 mm, as determined by J. L. Watkins, is  $1.0647 \pm 0.0069$  g/cm<sup>3</sup>.

Table. Relative increase in sound speed in krill over that of sea water at the same temperature,  $\Delta c_1$ , for 17 measurement series. Measures of length are expressed in millimeters. The elapsed time refers to the time from removal of the krill from the sea to their sound speed measurement. The measurement temperature is denoted by T, volume fraction by v, and sample size by  $n_s$ .

Series number	Date (1988)	$\Delta c_1$ (%)	$\bar{l}$	$\Delta l$	$\frac{l}{2}$	$l_{\min}$	$l_{\max}$	Elapsed time (min)	T (°C)	v (%)	$n_s$
19	22/1	2.57	33.8	4.8	34.2	25	45	623	7.5	33	150
20	23/1	2.63	29.4	2.1	29.5	23	34	454	7.0	31	225
24	26/1	2.65	30.4	2.0	30.5	26	35	200	6.2	34	225
25	26/1	2.67	30.9	2.6	31.0	24	37	255	6.2	40	250
27	28/1	2.85	30.1	2.2	30.2	24	35	535	7.2	36	225
30	1/2	2.76	31.8	2.5	31.9	26	39	167	5.6	35	200
31	1/2	2.47	31.7	3.0	31.8	25	41	202	5.9	35	200
32	1/2	2.96	32.1	3.2	32.2	24	42	241	6.4	36	200
34	2/2	2.46	31.7	2.8	31.8	25	39	194	5.3	27	150
41	8/2	3.16	31.0	2.2	31.1	24	36	347	8.6	32	200
42	8/2	2.86	31.9	3.0	32.1	25	42	375	8.1	35	200
44	12/2	3.06	38.9	3.8	39.1	28	50	89	5.5	32	101
45	12/2	3.13	37.9	3.7	38.0	29	49	201	6.3	29	99
46	15/2	2.80	30.8	3.6	31.0	22	41	349	12.1	32	200
47	15/2	3.08	32.2	3.6	32.4	27	41	396	11.4	34	186
48	17/2	2.94	31.1	3.1	31.2	25	41	371	11.8	34	200
49	17/2	2.46	31.6	3.5	31.8	24	44	421	11.6	34	200

## DISCUSSION

Some krill samples were measured more than 48 hours after their removal from the sea. Comparison of their sound speeds, not presented here, with those of related, earlier measured samples from the same batch, shows a marked decline in sound speed with increasing sample age. Clearly this is an effect of deteriorating tissue elasticity, in which the tissue becomes more fluidic. Association of sound speed with tissue elasticity has been proposed as a means of characterizing fish flesh, as for quality control (Gytre 1987).

In order to investigate the possible effect of aging on sound speed, as well as effects due to length, temperature of measurement, and density of krill in the T-tube, as expressed through the volume fraction, multiple linear regression analysis was employed. In most cases the residual sum of squares exceeded the regression sum of squares. That is, in these cases there was no statistically significant effect to be found.

When data spanning a long time period were selected, an effect was found. However, as in the other cases, the intrinsic variability of the particular data is simply too great to support a finer-grained analysis.

The potential accuracy of the method is probably limited by unavoidable variations in the uniformity of krill distribution in the sound path. The intrinsic measurement error due to uncertainties in measurement of the travel time and temperature is about 0.5% of the sound speed difference. Problems caused by a non-uniform distribution might be surmounted by sound speed measurement in individual specimens by means of Gytre's fork-probe (Gytre 1987).

The accomplishment of this study is measurement of the sound speed in living E. superba. Future sound speed measurements, to support collateral target strength modelling work, should attempt to disclose the precise causes and magnitude of variation in sound speed with biological state and physical condition of the specimens. This could be quite significant for acoustic estimation of krill abundance because of the sensitivity of target strength values to changes in density and sound speed contrasts, at least in the context of the fluid-sphere model (Anderson 1950, Johnson 1977, Greenlaw 1979). That differences are to be expected is a consequence of observed variations in krill biology between (Watkins et al. 1986) and even within (Watkins 1986) swarms and variations in biochemical composition (Clarke 1980), including especially fat and protein (Roschke 1977/78) and tissue lipids (Saether et al. 1985).

Predictions of target strength derived by exercising the fluid-sphere model, as expressed by Greenlaw (1979), with the new data are consistent with the measurements of target strength first reported by Everson et al. (1988). This work, together with final target strength values, has been documented by Foote et al. (1989 MS).

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