Speed of sound in *Euphausia superba*

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The speed of longitudinal sound waves in Antarctic krill has been measured by the time-of-flight method. The result of 17 separate measurement series on different assemblages of living krill is that the animal's sound speed exceeds that of seawater at the same temperature by 2.79 ± 0.24%. The mean lengths vary from 29.4 to 38.9 mm, with overall mean 32.2 and s.d. 2.5 mm. The corresponding density of krill of mean length 31 mm is 1.0647 ± 0.0069 g/cm^3. Measurement temperatures varied from 5.3 to 12.1 °C; corresponding salinities varied from 32.5 to 33.87 ppt, which also represent the ambient state. The ambient sea temperature was 2.0 ± 0.3 °C.

INTRODUCTION

Theoretical modeling of acoustic scattering by marine organisms requires knowledge of morphology and certain physical properties. In the case of small organisms without gas inclusions, the significant properties include contrasts in density and longitudinal-wave sound speed, compressibility, or bulk modulus with the surrounding medium.[1] Other elastic properties, such as the transverse-wave sound speed, modulus of rigidity of the organism, or stiffness of carapace might also be included.[2]

In much modeling work on zooplankton, the essential physical properties are the density and longitudinal-wave sound speed contrasts. This is the case with the well-known fluid sphere model,[3] its so-called high-pass version,[4] with liberation from typographical errors by Greenlaw,[5] and many successors, particularly those by Stanton.[6–8]

Two convenient sources of data on zooplankton density and longitudinal-wave sound speed, called simply sound speed hereafter, are the studies by Greenlaw and Johnson[9] and Køgeler et al.[10] It is assumed that the authors of the second study employed Greenlaw's procedure,[11] and did not in fact use their own equation, where all occurrences of the sound speed should be replaced by the corresponding reciprocal.

Clearly, from both the cited studies and examination of the larger literature, data on sound speed in *Euphausia superba* have been wanting. Thus, in order to estimate the backscattering cross section of the animal, Greenlaw et al.[12] had to assume a value, which was 1.02.

Perhaps as a consequence of this, the 1981 and 1984 FIBEX-connected Workshops on Krill[13] gave little credence to the work by Greenlaw et al. Estimates of target strength derived at the workshops were exceedingly large. Compared to recent measurements on *E. superba* of mean length 34 mm,[14] the 1984 FIBEX estimate for the target strength at 120 kHz was roughly 15 dB too high. Given such a discrepancy, application of acoustic methods in krill stock assessment could hardly decide between the widely varying estimates of abundance noted by Everson in his comprehensive review.[15]

The present aim is simple. It is to describe the first measurement of sound speed in *E. superba*.

I. PRINCIPLE OF MEASUREMENT

The principle of measurement is that of time-of-flight of an acoustic pulse through a mixture of seawater and presumed uniform distribution of krill. For a nominal acoustic wavelength that is small compared to the projected length of krill along the sound path, the travel time t for the mixture is equated to the sum of the respective travel times due to water, t_0, and krill, t_1,

$$t = t_0 + t_1. \tag{1}$$

In terms of the corresponding sound speeds,

$$\frac{1}{c} = \frac{1-V}{c_0} + \frac{V}{c_1}, \tag{2}$$

where V is the volume fraction of krill.

Because the speed of sound in krill, c_1, is only slightly greater than that in seawater, c_0, it is difficult to make precise measurements directly from an oscilloscope. Adjusting a precision potentiometer to control 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

$$R = (1 - V)R_0 + VR_1. \tag{3}$$

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 simple relation

$$c_1/c_0 = R_0/R_1. \tag{4}$$

The problematical elements of the measurement are ensuring the uniformity of krill distribution in the sound path and accounting for variations in the ambient water temperature during the course of measurements in a laboratory with-

out temperature control. These issues are addressed in the following sections.

II. MATERIALS AND EXPERIMENTAL METHODS

The physical apparatus for measuring sound speed closely resembles that used by Køgeler et al.,[11] 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 setting accomplishes the equalization process, allows precise determination of the travel time, hence sound speed. A standard Kikusui COS 5100 oscilloscope with bandwidth of 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-liter tub half-filled with seawater. 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 were 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 these additions. 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.

Because the temperature of the laboratory—a bench in a cavernous and drafty building with holes in the roof—was not controlled, and because the volume of the horizontal section of the T-tube was only 130.5 ml, the temperature of seawater-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 of each krill 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.[16] Density was measured occasionally by means of density bottles.

III. ANALYSIS

Several conditions must be fulfilled for Eqs. (2)–(4) to be applicable. First, the acoustic wavelength must be small compared to the projected length of the krill along the sound path. This is satisfied since the nominal acoustic wavelength is 3 mm and typical krill lengths are 30–40 mm, while most of the animals are aligned with the axis of the tube, hence the sound path. A second condition is that the krill must be essentially uniformly distributed across the cross section of the sound path, hence the cross section of the horizontal section of the T-tube. Because of the tendency of the krill to sink, possibly due to limitation of swimming movements 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 29%–40%, the krill appeared to fill the horizontal tube uniformly. As mentioned, 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 third 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_0-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

$$1/R = aT + b, \qquad (5)$$

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 °C to the ambient laboratory temperature. Least mean squares regression of $1/R$ on T determined the value $a = 4.5 \times 10^{-5}$ $(k\Omega \cdot °C)^{-1}$. For the particular velocimeter, this describes a temperature gradient in sound speed of 2.9 m(s·°C)$^{-1}$, which agrees closely with Wilson's finding as cited by Urick.[17] The resistance $R_0(T)$ is determined by solving the equation pair:

$$1/R_0(T_i) = aT_i + b,$$
$$1/R_0(T) = aT + b,$$

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

$$R_0(T) = R_0(T_i)/[1 - aR_0(T_i)(T_i - T)]. \qquad (6)$$

For n measurements of R_0, the average is

$$\overline{R_0}(T) = \frac{1}{n} \sum_{i=1}^{n} \frac{R_0(T_i)}{1 - aR_0(T_i)(T_i - T)}.$$

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 Eq. (4), viz.,

$$c_1/c_0 = \overline{R_0}(T)/R_1(T), \tag{7}$$

in this elaborated form. The quantity $R_1(T)$ is determined by solving Eq. (3) where R_0 is replaced by $R_0(T)$. The volume fraction V in this 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

$$v = 0.9432m - 0.0034,$$

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

$$m = 9.60 \times 10^{-6} l^{2.94},$$

where l is the total krill length in millimeters.[18]

IV. RESULTS

A total of 51 measurement series were performed. This number includes series where the technique was being established and refined, and others where 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 h of the subjects' 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 Table I. Included are the number of krill specimens and associated length statistics, the time of measurement reckoned from the time of removal of animals from the sea, the measurement temperature and salinity, and volume fraction. The sound speeds were recomputed for the three cases with missing data on salinity. The respective results were identical at the extrema of the measured salinity range, namely 32.5 and 33.87 ppt, and bracketed value of 33.18 ppt shown in Table I.

The increased sound speed in krill relative to seawater is characterized by the mean 2.79% and s.d. 0.24%. That is, the determined sound-speed contrast is 1.0279 ± 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 length 31.4 ± 1.0 mm. The applicable density for krill of mean length 31 mm, as determined by J. L. Watkins, is 1.0647 ± 0.0069 g/cm^3.

V. DISCUSSION

Some krill samples were measured more than 48 h 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.[19]

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.

In the case of related data spanning a long time period

TABLE I. Relative increase in sound speed in krill over that of seawater at the same temperature, Δc_1, for 17 measurement series performed at Stromness on South Georgia. Measures of the length distribution for each sample are expressed in millimeters. The standard deviation of the length distribution is denoted Δl, and the root-mean-square length by $\overline{l^2}^{1/2}$. The elapsed time refers to the time from removal of the animals from the sea to their sound-speed measurement. The measurement temperature is denoted by T, salinity by S, volume fraction by V, and sample size by n_s. Missing data on S are indicated by brackets, which embrace the average of the immediately prior and subsequent measurements.

Series number	Date (1988)	Δc_1(%)	$\overline{l}$	Δl	$\overline{l^2}^{1/2}$	l_{min}	l_{max}	Elapsed time (min)	T(°C)	S(ppt)	V(%)	n_s
19	22/1	2.57	33.8	4.8	34.2	25	45	623	7.5	33.6	33	150
20	23/1	2.63	29.4	2.1	29.5	23	34	454	7.0	32.75	31	225
24	26/1	2.65	30.4	2.0	30.5	26	35	200	6.2	[33.18]	34	225
25	26/1	2.67	30.9	2.6	31.0	24	37	255	6.2	[33.18]	40	250
27	28/1	2.85	30.1	2.2	30.2	24	35	535	7.2	[33.18]	36	225
30	1/2	2.76	31.8	2.5	31.9	26	39	167	5.6	33.6	35	200
31	1/2	2.47	31.7	3.0	31.8	25	41	202	5.9	33.6	35	200
32	1/2	2.96	32.1	3.2	32.2	24	42	241	6.4	33.6	36	200
34	2/2	2.46	31.7	2.8	31.8	25	39	194	5.3	33.5	27	150
41	8/2	3.16	31.0	2.2	31.1	24	36	347	8.6	32.5	32	200
42	8/2	2.86	31.9	3.0	32.1	25	42	375	8.1	32.5	35	200
44	12/2	3.06	38.9	3.8	39.1	28	50	89	5.5	33.4	32	101
45	12/2	3.13	37.9	3.7	38.0	29	49	201	6.3	33.5	29	99
46	15/2	2.80	30.8	3.6	31.0	22	41	349	12.1	33.4	32	200
47	15/2	3.08	32.2	3.6	32.4	27	41	396	11.4	33.3	34	186
48	17/2	2.94	31.1	3.1	31.2	25	41	371	11.8	33.86	34	200
49	17/2	2.46	31.6	3.5	31.8	24	44	421	11.6	33.87	34	200

being 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 nonuniform distribution might be surmounted by sound-speed measurement in individual specimens by means of Gytre's fork-probe.[19]

The accomplishment of this study is measurement of the sound speed in living *E. superba*. Future sound-speed measurements, to support collateral modeling 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.[3–5] That differences are to be expected is a consequence of observed variations in krill biology between[20] and even within[21] swarms and variations in biochemical composition,[22] including especially fat and protein.[23,24]

Frequency dispersion of the sound speed has not been investigated in the present work. It has not been considered as causing significant differences between the nominal measurement frequency of 500 kHz and typical frequencies of application, e.g., 38–200 kHz or other sub-megahertz frequencies. Dispersion is believed to be entirely negligible for several reasons. First, the biological tissue is mostly composed of water—witness the low value of observed sound-speed contrast—and water has a dispersivity that is less than 10^{-4}. For seawater the quantity $c_\infty^2 / c_0^2 - 1$ is less than 10^{-4}, where c_∞ is the infinite-frequency limit of sound speed and c_0 is the zero-frequency limit.[25] In other words, the phase velocity differs from the group velocity by less than 1%.[26] Second, measurements of sound speed in soft tissues, e.g., mammalian muscle and liver, do not show significant effects of dispersion,[27–29] at least apropos of the magnitude of measurement errors and natural sources of variation characterizing the present data.

An attempt was made to measure absorption, at 500 kHz, simultaneously with the sound-speed measurements. These data have not yet been analyzed, for being of lesser importance than sound-speed and density contrasts for modeling single-krill scattering.

ACKNOWLEDGMENTS

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