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Measurements of sound-speed and density contrasts of zooplankton in Antarctic waters

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Sound-speed and density contrasts (h and g, respectively), two important acoustic material properties, of live zooplankton were measured off the western Antarctic Peninsula during a Southern Ocean GLOBEC cruise conducted from 9 April to 21 May 2002. The work included *in situ* sound-speed contrast and shipboard density-contrast measurements. The temperature and pressure (depth) dependence of the sound-speed contrast of *Euphausia superba* and *E. crystallorophias* as well as that of some other zooplankton species were investigated. The size range of *E. superba* used in the measurements varied from about 20 mm to 57 mm, with mean length of 36.7 mm and standard deviation of 9.8 mm, which covered life stages from juvenile to adult. For *E. superba*, there was no statistically significant depth dependence, but there was a moderate dependence of sound-speed and density contrasts on the size of the animals. The measured sound-speed contrast varied between 1.018 and 1.044, with mean value 1.0279 and standard deviation 0.0084, while the measured density contrast varied between 1.007 and 1.036, with mean value 1.0241 and standard deviation 0.0082. For *E. crystallorophias* and *Calanus* there was a measurable depth dependence in sound-speed contrast. The *in situ* sound-speed contrasts for *E. crystallorophias* were 1.025 ± 0.004 to 1.029 ± 0.009. For *Calanus*, they were variable, with one set giving a value of 0.949 ± 0.001 and the other giving 1.013 ± 0.002. Shipboard measurements of other taxa/species also showed substantial variation in g and h. In general, values of g ranged from 0.9402 to 1.051 and h ranged from 0.949 to 1.096. The variation of the material properties is related to species, type, size, stage, and in some cases depth of occurrence. The uncertainty of the estimates of zooplankton biomass attributable to these variations in g and h can be quite large (more than 100 fold). Improvements in making biological inferences from acoustic data depend strongly on increased information about the material properties of zooplankton and the biological causes for their variation, as well as a knowledge of the species composition and abundance.

Keywords: acoustic scattering, Antarctic krill, density contrast, krill, material properties, sound-speed contrast, western Antarctic Peninsula, zooplankton.

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Introduction

It is well known that in addition to the geometric shape and orientation of zooplankton, their material properties are also very important parameters for interpreting acoustic-backscattering data from zooplankton when using acoustic-scattering models. *Euphausia superba*, the Antarctic krill, a major marine biological resource and a key element in the Antarctic food chain (Everson, 2000), can be treated acoustically as a weakly scattering fluid object. This means that its body has negligible elastic properties. As a result, for this species and other weakly scattering zooplankton, the sound-speed contrast (h) and density contrast (g) of an individual relative to the surrounding seawater are the two dominant acoustic parameters of the material properties. It has been shown that errors of a few per cent in these parameters can cause an order of magnitude error in estimates of either abundance or biomass, or both factors for that matter, of zooplankton (Chu *et al.*, 2000a, b). However, few measurements have ever been made of g and h on plankton (Køgeler *et al.*, 1987; Foote, 1990), and the reported measurements were all conducted *ex situ*, which could not address the potential influence of ambient temperature and pressure or depth on the material properties. *In situ* measurements of these properties on live zooplankton in Antarctic have not been reported and little is known about how they vary for any species with depth, season, or life stage. One of the major difficulties in

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measuring these properties is to keep the animal alive during the sound-speed measurement since the material properties of dead individuals are substantially different (Greenlaw, 1977). An instrument deployable to several hundreds of metres was used to conduct *in situ* sound-speed contrast measurements on live Antarctic krill and other species on one of the cruises of the Southern Ocean Global Ecosystem Dynamics (SO GLOBEC) program (Hofmann *et al.*, 2002). Another major difficulty is to conduct shipboard density measurements. Since most of the zooplankton species are nearly neutrally buoyant, the accuracy and precision required for reliable measurements are extremely difficult to achieve because of the ship's motion. A specially designed system for measuring the acoustic properties of zooplankton was used on the same SO GLOBEC cruise. The primary objective of this paper is to describe the temporal and spatial variability of the material properties of Antarctic krill.

Methods and instruments

Sound-speed contrast measurements

The sound-speed contrast (h) is defined as the ratio of the sound speed in animals to that in the surrounding water. The biggest challenge in measuring the temperature and pressure dependence of the sound-speed contrast of live krill or other zooplankton species under natural conditions is that the measurements should be done at varying depths in the ocean. To our knowledge, such measurements on live Antarctic krill or any other live Antarctic zooplankton species have never been made. To conduct these types of measurements, a specially designed instrument named "Acoustic Properties Of zooPlankton" (APOP) was used (Chu *et al.*, 2000a). The system was modified from the previous version in order to make a series of measurements during a single cast or a deployment from the surface to as deep as 220 m. The deployment rate for both down and up casts was about 0.07 m s^{-1}, which is comparable to the speed of migrating zooplankton (Wiebe *et al.*, 1992; Luo *et al.*, 2000). The sound-speed contrast measurements were made at different depths on both the down cast when the APOP was lowered to depth and the up cast. Sound-speed contrast estimates are determined with APOP by measuring the time difference for acoustic waves or sounds travelling directly from one acoustic transducer (transmitter) to another transducer (receiver) with and without animals in the acoustic path (Chu *et al.*, 2000a, 2003). If sound travels faster in animal bodies than in water, the travel time with animals present in the acoustic path will be shorter and vice versa.

A dual-chambered acoustic apparatus was used in the modified APOP, with one being a primary acoustic chamber and the other a secondary or a reference chamber that provided information on the relative sound-speed changes at different depths. Each acoustic chamber contained two identical broadband transducers with a centre frequency around 500 kHz and a bandwidth of about 300 kHz (Figure 1). The two chambers were mounted next to each other in a bucket-shaped container to protect them during deployment. Two temperature- and pressure-measuring systems, MicroCat (SBE model 37) and Minilog (VEMCO, model 6977A), were used to record the depth and water temperatures inside and outside the container. The former was attached to the winch cable, measuring the water temperature outside of the APOP bucket, while the latter was tied to the APOP mounting frame, providing

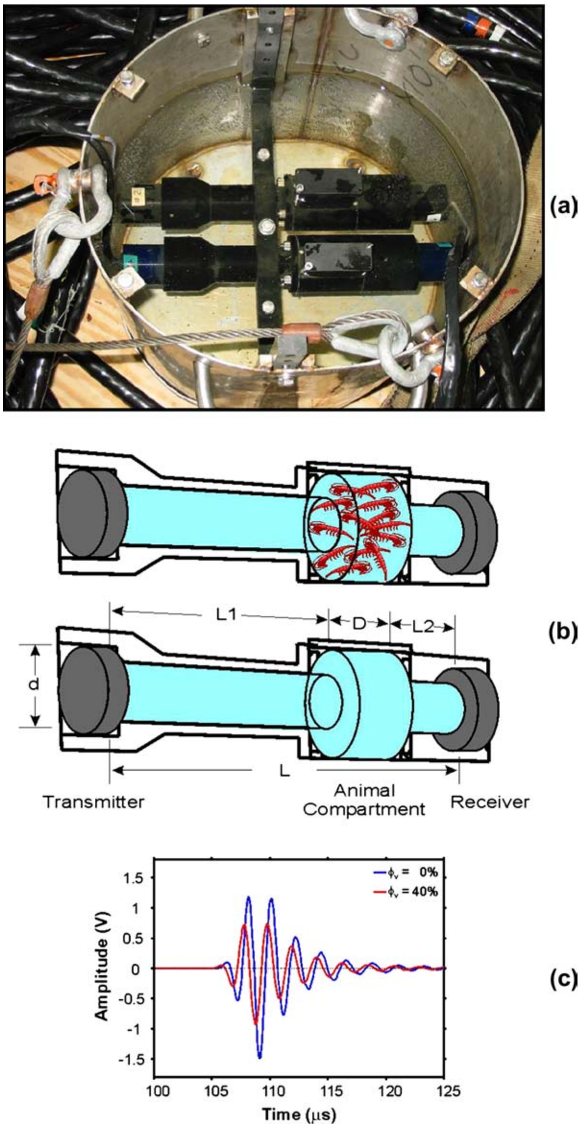

Figure 1. (a) Experimental APOP chambers in the stainless-steel bucket in which they were deployed on casts to 200 m, (b) the schematic drawing of how sound-speed contrast of live zooplankton is measured with APOP, and (c) the received waveforms from the two acoustic chambers with and without the presence of animals, respectively (c).

the water temperature inside the APOP bucket. Generally, the temperature difference varied by less than 0.2 °C during a cast.

The data-acquisition system included a Pulser/Receiver (Panametrics, model PR5800), a PC with an A/D data-acquisition board (Chase Scientific, CS210), and a digital oscilloscope (LeCroy, Model 9310C). The Pulser/Receiver provided an impulse (pulsewidth of about 30 ns) and an output trigger signal to synchronize the data acquisition and the display on the digital oscilloscope. In addition, it also amplified the received acoustic signals and sent them to the data-acquisition board. The 10 MHz maximum sampling rate of the A/D board provided roughly 20 samples per wavelength.

The sound-speed contrast can be determined by measuring the travel time (time-of-flight) difference (Δt) between the two received waveforms, one is from the pair with the animal in the acoustic path and the other is from the reference chamber with no animals present (Chu *et al.*, 2000a):

$$h = 1 + \frac{\Delta t}{\Phi_z t_D}, \tag{1}$$

where Φ_z is the volume fraction of animals in the animal compartment and t_D is the travel time for the acoustic wave propagating through the compartment (time-of-flight), which can be calculated by $t_D = D/c$, where D is the length dimension of the animal compartment and c is the sound speed in seawater determined from the temperature, salinity, and pressure measured with the CTD. The uncertainty or potential error resulting from Equation (1) can be estimated with

$$\left|\frac{\delta(\Delta h)}{\Delta h}\right| \le \left|\frac{\delta(\Delta t)}{\Delta t}\right| + \left|\frac{\delta\Phi_z}{\Phi_z}\right| + \left|\frac{\delta t_D}{t_D}\right|, \tag{2}$$

where $\Delta h = h - 1$ is the difference of the sound-speed contrast from unity. The reason why we estimate $\delta(\Delta h)/\Delta h$ not $\delta(\Delta h)/h$ is that the former quantity more directly reflects the error in predicting the target strength (TS) of the scattering objects. The estimated values for $|\delta(\Delta t)/\Delta t|$, $|\delta\Phi_z/\Phi_z|$, and $|\delta t_D/t_D|$ based on our measuring devices used in the cruise were 0.001%, 5%, and 2%, respectively. Hence, the dominant source of error was the uncertainty in total net volume of zooplankton in the animal compartment. The overall sound-speed contrast "uncertainty" was estimated to be less than 10% of Δh.

Density-contrast measurements

The density contrast (g), another important parameter used in describing acoustic scattering by weakly scattering objects, is defined as the ratio of the density of the animals to that of the surrounding water. To measure the density, or density contrast of the zooplankton on board the ship, a motion-compensated, dual-density method was used.

The ship motion was compensated for by using an additional electrical balance in a way similar to that described by Childress and Mickel (1980). The difference between our apparatus and theirs is that they used analogue signals from the same balance to be the feedback signal, while we used a different, but calibrated device to provide the digital numbers as a feedback signal to compensate the motion. Two identical electrical balances (Ohaus, AP210), with an accuracy of 0.1 mg, were mounted on the same table next to each other, with one as a primary balance and the other as a reference. The latter had a calibration mass (50 g, 100 g, or 150 g) on its weighing platform throughout the measurement. Since both balances experienced the same accelerations, the ratio of the weight readings from the two balances were, theoretically, the same and hence could be used to infer the actual weight or mass of the objects. The output digital readings from the two balances were received by a computer through a serial link (RS 232), and then the actual weight of the object being weighed on the primary balance could be calculated. The relative accuracy of this motion-compensated, weighing system was usually better than 0.02%.

The density of the krill or other zooplankton was measured using a dual-density method, in which two fluids of different densities were used. By measuring the densities of the two fluids without animals being present and their mixtures with animals present, as well as the appropriate weights associated with the density measurements when animals are in a container of a known weight and volume, the density of the animals can be determined uniquely (Chu *et al.*, 2000b). This dual-density method is able to measure the density of live animals without anaesthetizing them as is usually done in other density-measuring methods (Lowndes, 1942; Køgeler *et al.*, 1987; Foote, 1990; Knutsen *et al.*, 2001). During the cruise, we used seawater as fluid 1 (ρ_1) and distilled water as fluid 2 (ρ_2). The reason why we used distilled water was because its density was more stable and the accuracy was better than 3×10^{-5} g cm^{-3}. A densitometer with an accuracy of 3×10^{-5} g cm^{-3} (Anton PAAR 4000) was necessary for the dual-density measurement. The readings from density measurements were not affected by ship motion, even with strong pitch and roll. There were four steps in completing the measurement:

(i) Measuring the densities of the seawater, ρ_1, and the distilled water, ρ_2;
(ii) Adding the seawater and live zooplankton mixture to a pre-weighed, empty container of known volume (v_T) to make it about half full (v_1); measuring the total weight of the container plus the mixture and obtaining the net weight of the mixture, (w_1) by subtracting the weight of the container;
(iii) Adding the distilled water (v_2) to the container until it is full and measuring the total net weight of the new mixture, (w_2);

(iv) Mixing the solution well and then pouring the mixture through a fine mesh to obtain a well-mixed solution, and measuring the density of the mixture ρ_m.

The average density of the zooplankton ρ_z can be determined by solving the following linear equations:

$$\begin{cases} w_1 = (v_1 - v_z)\rho_1 + v_z\rho_z \\ w_2 = (v_T - v_z)\rho_m + v_z\rho_z \\ v_T = v_1 + v_2 \\ v_2 = (w_2 - w_1)/\rho_2 \end{cases}, \qquad (3)$$

where v_z is the net volume of zooplankton. The solutions for v_z and ρ_z can be obtained

$$\begin{cases} v_z = v_T - \left(\frac{w_2 - w_1}{\rho_2}\right)\left(\frac{\rho_1 - \rho_2}{\rho_1 - \rho_m}\right) \\ \rho_z = \rho_m + \frac{w_2 - \rho_m v_T}{v_z} \end{cases}. \qquad (4)$$

The uncertainties or potential errors resulting from the above equations can be estimated with:

$$\begin{aligned} |\delta v_z| \le |\delta v_T| &+ \left|\frac{\rho_1 - \rho_2}{\rho_1 - \rho_m}\right| \frac{|\delta w_2| + |\delta w_1|}{\rho_2} \\ &+ \left|\frac{w_2 - w_1}{\rho_2}\right| \frac{|\delta\rho_1| + |\delta\rho_2|}{|\rho_1 - \rho_m|} + \left|\frac{w_2 - w_1}{\rho_2}\,\frac{\rho_1 - \rho_2}{\rho_1 - \rho_m}\right| \frac{|\delta\rho_2|}{\rho_2} \\ &+ \left|\frac{\rho_1 - \rho_2}{\rho_1 - \rho_m}\,\frac{w_2 - w_1}{\rho_2}\right| \frac{|\delta\rho_1| + |\delta\rho_m|}{|\rho_1 - \rho_m|}, \end{aligned} \qquad (5)$$

$$\begin{aligned} |\delta\rho_z| \le |\delta\rho_m| &+ \frac{|\delta w_2| + |\delta\rho_m| v_T + |\delta v_T| \rho_m}{v_z} \\ &+ \left|\frac{w_2 - \rho_m v_T}{v_z}\right| \frac{|\delta v_z|}{v_z}, \end{aligned} \qquad (6)$$

where δv_T, δw_1, δw_2, $\delta\rho_1$, $\delta\rho_2$, and $\delta\rho_m$ are respective errors in measuring volume, weights, and densities. The estimated quantities for these measuring uncertainties were 0.01 cm^3 for δv_T, 5.0 mg for δw_1 and δw_2, 4×10^{-5} g cm^{-3} for $\delta\rho_1$ and $\delta\rho_2$, and 8×10^{-5} g cm^{-3} for $\delta\rho_m$. Using these numbers and the typical values for other parameters in Equations (5) and (6), the quantity $|\delta\rho_z|$ was estimated to be less than 0.004 g cm^{-3}, which leads to $\delta g \le 0.004$ for the uncertainty in density-contrast estimate since the density of seawater is always greater than unity.

Data collection

The cruise was conducted from 9 April to 21 May 2002 off the western Antarctic Peninsula. It was one of the SO GLOBEC's four cruises over 2 years to conduct broad-scale surveys (two in austral spring and two in austral fall) covering 92 stations distributed over an area of about 60 000 km^2 centred on Marguerite Bay and extending just beyond the shelf break (Figure 2). The survey was a two-ship operation with the RVIB "N. B. Palmer" and ASRV "L. M. Gould". The RVIB N. B. PALMER, on which the APOP experiments were done, conducted a broad-scale survey of the area while the ASRV L. M. GOULD concentrated on process-orientated projects. The water temperature was about −1.5 °C at the sea surface and increased to between 0.5 °C and 2 °C near the bottom or at a depth of 200 m. Net tows were conducted to catch live zooplankton and APOP casts were made to measure the sound-speed contrast of live Antarctic krill and a few other live zooplankton *in situ*. Shipboard measurements of density and sound-speed contrasts were also made on Antarctic krill and a number of other live zooplankton species.

Biological sampling with nets

Live krill, as well as other live zooplankton, were primarily collected with a 1-m diameter "Reeve" net (Reeve, 1981; Wiebe and Benfield, 2003). The codend bucket of the Reeve net is more than four times larger in volume than those of MOCNESS, thus insuring better survival of the live animals. The mesh size of the Reeve net was 333 µm. Twelve Reeve-net tows were taken on or near 11 broad-scale survey stations (Figure 2) from 15 April to 15 May. Some of the net tows caught significant numbers of live zooplankton for APOP measurements (Table 1). The largest catch was made on May 15 when a Reeve-net tow was taken in Martha Strait at the entrance to Crystal Sound. The net tow went as deep as 100 m, being targeted at a strong scattering layer observed on the Simard EK500 echograms between 70 m to 100 m. More than 200 krill were caught, including juvenile, sub-adult, and adult life stages.

The krill caught on 15 and 22 April and 15 May were almost exclusively *Euphausia superba*. Besides krill, animals that were caught with the Reeve net were copepods, mostly *Calanus*, amphipods (*Themisto*), and mysids; diatoms were also collected. (Table 1). Most of the *Calanus* were 3 mm in length and the main amphipods were *Themisto,* with a mean length around 20 mm.

In addition to the live animals caught with the Reeve net on N. B. PALMER, the krill ecology and physiology group led by Dr Kendra Daly on the L.M. GOULD provided us with some of their live animals at rendezvous periods on 23 and 30 April. These included a large number of live krill (*E. superba* and *E. crystallorophias*) and other zooplankton (mysids, amphipods, and copepods), as well as about 25 differently sized fish (*Pleuragramma*). Length measurements were made of all the zooplankton used in the experiments. For the krill, the measurement was made from the anterior tip of the rostrum to the posterior end of the uropod, excluding their terminal setae (standard 1 in Mauchline, 1980). For the copepods, it was the prosome length.

Shipboard and *in situ* measurements of sound-speed contrast

The live krill and other zooplankton caught with our Reeve net and provided by the Daly group on the "L. M. Gould" were used in a combination of more than

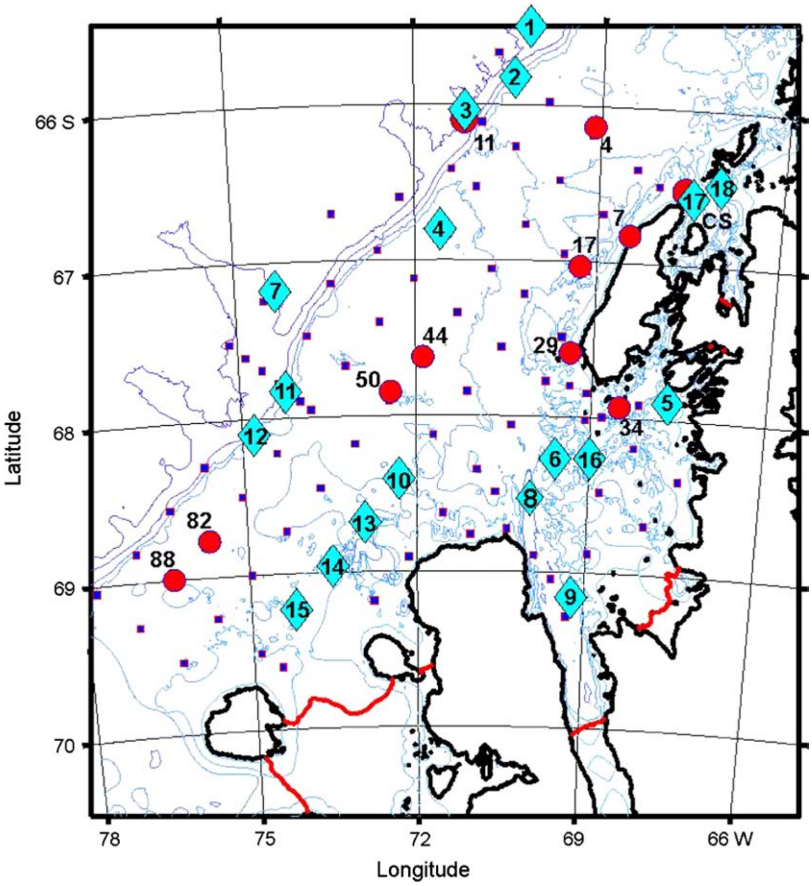

Figure 2. Map of the broad-scale survey for SO GLOBEC off the western Antarctic Peninsula. Circles are station locations where Reeve-net collections were made (see Table 1 for additional detail) and diamonds indicate where the APOP casts were made (see Table 2 for additional detail). The small squares are other survey-station locations.

30 shipboard and APOP-cast, sound-speed measurements (Tables 2 and 3). The shipboard measurements were originally made with the APOP in a container (50.8 × 40.64 × 30.48 cm). Since sound-speed measurements are very sensitive to temperature, the measurements had to be made very carefully with temperature values being recorded frequently. Later, the APOP was placed in a larger container, with surface water running through the

Table 1. Summary of Reeve-net tows made to collect live animals for the material-properties estimates and APOP casts to conduct *in situ* sound-speed contrast measurements. The positions of the net tows and APOP casts are given on Figure 2.

Cast #	Date	Station	Cast depth (m)	Catch
1	15 April 2002	4	300	Diatoms
2	15 April 2002	7	100	A few adult and juvenile krill
3	15 April 2002	7	100	More than 30 adult and 70 juvenile krill
4	17 April 2002	11	400	Diatoms
5	18 April 2002	17	350	A few juvenile krill
6	22 April 2002	29	165	A dozen adult and a number of juvenile krill
7	22 April 2002	34	150	About ten adult and a few juvenile krill
8	25 April 2002	44	60	Diatoms, copepods
9	27 April 2002	50	360	More than 100 amphipods and thousands of copepods
10	7 May 2002	82	435	Lots of copepods, a few juvenile krill
11	9 May 2002	88	375	Copepods
12	15 May 2002	Crystal Sound	100	More than 200 juvenile, sub-adult, and adult krill

Table 2. Summary of *in situ* sound-speed contrast measurements with APOP and associated shipboard density-contrast measurements. ⟨h⟩ and σ_h are the mean (over depth) and the standard deviation from the APOP-cast measurements. The uncertainties are estimated by using the following parameters: $\delta v_T = 0.01$ cm^3, $\delta w_1 = \delta w_2 = 5.0$ mg, $\delta\rho_1 = \delta\rho_2 = 4\times10^{-5}$ g cm^{-3}, and $\delta\rho_m = 8\times10^{-5}$ g cm^{-3}. The specimens used for the casts made on the dates with an asterisk were also used for the shipboard measurements. Temperatures for all APOP casts were between −1.7°C and 2.0°C. The positions of the APOP-cast locations are plotted on Figure 2.

Date	Animal	# of animals	⟨L⟩ (mm)	σ_L(mm)	⟨h⟩/σ_h	g	φ
16 April*	*E. superba*	15	50.9	2.7	—	1.026 ± 0.004	—
17 April	*E. superba*	12	51.9	2.2	1.024/0.004	1.029 ± 0.004	0.22
19 April	*E. superba*	> 30	26.9	6.1	1.018/0.006	1.007 ± 0.004	0.20
23 April	*E. superba*	17	43.24	8.8	1.022/0.007	1.027 ± 0.006	0.17
24 April	*E. superba*	14	50.4	4.7	1.036/0.007	1.026 ± 0.005	0.23
26 April*	*E. superba*	36	36.6	5.4	1.048	1.027 ± 0.006	0.16
27 April	*E. superba*	33	34.9	5.4	1.020/0.007	1.027 ± 0.006	0.16
28 April	*E. superba*	15	52.7	5.5	1.040/0.008	1.026 ± 0.004	0.27
29 April*	*E. superba*	72	25.4	5.3	1.032	1.023 ± 0.008	0.12
29 April*	*E. crystallorophias*	51	32.3	3.3	1.027	1.009 ± 0.003	0.26
29 April	*E. crystallorophias*	51	32.3	3.3	1.025/0.004	1.009 ± 0.003	0.26
1 May*	*E. superba*	13	50.5	4.0	1.039	1.036 ± 0.007	0.19
1 May	*E. superba*	13	50.5	4.0	1.044/0.004	1.036 ± 0.007	0.19
2 May*	*Calanus*	> 1 000	4.1	0.3	0.959	0.995 ± 0.001	0.47
2 May	*Calanus*	> 1 000	4.1	0.3	0.949/0.014	0.995 ± 0.001	0.47
3 May*	*E. crystallorophias*	32	31.7	3.4	1.026	1.000 ± 0.006	0.15
3 May	*E. crystallorophias*	32	31.7	3.4	1.029/0.009	1.000 ± 0.006	0.15
4 May*	*E. superba*	39	34.3	3.6	1.021	—	0.28
4 May	*E. superba*	39	34.3	3.6	1.021/0.002	—	0.28
5 May*	*E. superba*	81	28.1	8.7	1.028	1.022 ± 0.002	0.38
5 May	*E. superba*	81	28.1	8.7	1.024/0.004	1.022 ± 0.002	0.38
7 May*	*Calanus*	> 1 000	3.2	0.3	1.012	0.996 ± 0.002	0.35
7 May	*Calanus*	> 1 000	3.2	0.3	1.013/0.003	0.996 ± 0.002	0.35
15 May*	*E. superba*	129	27.1	2.9	1.034	1.017 ± 0.002	0.47
15 May	*E. superba*	129	27.1	2.9	1.030/0.005	1.017 ± 0.002	0.47

Table 3. Summary of shipboard material-property measurements. Asterisks stand for measurement using graduated cylinder to estimate the volume first. Uncertainties for density measurements were estimated using the same parameters as used for Table 2.

Date	Animal	T (°C)	# of animals	⟨L⟩ (mm)	σ_L(mm)	h	g	φ
16 April	*E. superba*	0.5	50.9	2.7	—	1.026 ± 0.004	—	—
23 April	*Pleuragramma antarcticum*	0.2−0.4	9	60−75	—	1.017	1.018 ± 0.003	0.33*
23 April	*Pleuragramma antarcticum*	−1.0−0.5	11	69	4.9	1.013	1.007 ± 0.003	0.32
23 April	Amphipods (*Eusirus*)	3.6	7	47.9	2.4	1.096	—	0.12
24 April	Amphipods (*Eusirus*)	0.8−1.3	7	47.9	2.4	1.038	1.051 ± 0.005	0.12
25 April	*Mysid arctomysis*	1.1−2.0	14	50.4	4.7	1.077	1.041 ± 0.008	0.23
26 April	*Mysid arctomysis*	−0.5 to −0.4	15	48.3	8.0	1.078	1.024 ± 0.008	0.15
26 April	*E. superba*	−0.4 to −0.1	36	36.6	5.4	1.048	1.027 ± 0.006	0.16
28 April	Amphipods (*Parathemisto*)	−1.1	~150	19.2	0.47	0.984 0.914	1.042 ± 0.005	0.21
29 April	*E. superba*	−0.2 to −0.1	72	25.4	5.3	1.032	1.023 ± 0.008	0.12
29 April	*E. crystallorophias*	−0.5 to −0.3	51	32.3	3.3	1.027	1.009 ± 0.003	0.26
1 May	*E. superba*	−0.9 to −0.6	13	50.5	4.0	1.039	1.036 ± 0.007	0.19
2 May	*Calanus*	−0.8 to −0.4	> 1 000	4.1	0.3	0.959	0.995 ± 0.001	0.47*
3 May	*E. crystallorophias*	−0.8 to −0.7	32	31.7	3.4	1.026	1.000 ± 0.006	0.15
4 May	*E. superba*	−1.4 to −1.3	39	34.3	3.6	1.021	—	0.28
5 May	*E. superba*	−1.3 to −1.2	81	28.1	8.7	1.028	1.022 ± 0.002	0.38*
7 May	*Calanus*	−1.4 to −1.3	> 1 000	3.2	0.3	1.012	0.996 ± 0.002	0.35*
15 May	*E. superba*	−1.3 to −1.2	129	27.1	2.9	1.034	1.017 ± 0.002	0.47*

tank. The APOP measurements on board the ship were made after the APOP-bucket temperature was equilibrated with the temperature in the tank.

Another major difficulty in conducting the sound-speed measurement was to prevent any bubbles from being trapped in the acoustic chambers. When there was a large temperature gradient, many bubbles were naturally generated on the surface of the acoustic chambers and the transducers. A light coating of detergent was applied to the transducer and the surfaces of the acoustic chambers to help prevent bubbles from attaching themselves to the chamber surfaces.

During the first APOP trial cast, a strong narrowband signal around 35 kHz was observed. The signal was coherent with the APOP signal, which was around 500 kHz. The amplitude of the signal increased as the APOP was deployed deeper and peaked around 165 m. The signal disappeared if the APOP was out of water. We were unable to eliminate this apparently self-induced interference signal before data were acquired, but in post-processing a digital filter was applied that removed it. Consequently the signals of interest were relatively very clean.

The APOP casts were made from the surface to 205-m depth for almost all the casts except for that on 5 May at Station 77, where the water depth was only 180 m, with measurements at 20-m intervals from 5 m to 205 m during both down and up casts. The received signal at each depth was averaged over 100 pings and was sampled at 10 MHz, well above the Nyquist frequency (~ 1.5 MHz). A total of 18 APOP casts was made. There were 16 casts with animals inside the APOP acoustic chambers (Table 2), including ten with *E. superba*, two with *E. crystallorophias*, and two with copepods (*Calanus*). There were also two calibration casts and two test casts made at the beginning of the cruise.

Before 1 May, the shipboard measurements and APOP casts were conducted separately. After the enlarged tank was acquired for the shipboard measurements, they and the APOP casts for sound-speed contrast measurement were combined. The shipboard measurements were always made before the cast. The animals stayed in the animal compartment after the shipboard measurements and until the APOP casts were completed. At each depth, the measurement was made after about 1 min of waiting time for the temperature in the APOP bucket to equilibrate with the water temperature outside the bucket. Ideally, the longer the waiting time, the smaller the temperature difference. However, waiting too long might kill the animals. In addition, time to conduct the measurements was also limited by the survey schedule. A 1-min waiting time proved reasonable. A complete APOP cast took about 1.5 h. Almost all the krill were still alive after the casts. In contrast, about 50% to 60% of copepods were still alive after the casts.

Calibration

Two APOP calibration casts were performed towards the end of the cruise on 12 May and 15 May (Figure 3). The first was conducted in the mouth of Marguerite Bay between Alexander Island and Adelaide Island and the second was conducted in Crystal Sound. The object of the calibration was to compare the differences in travel times between the two sets of transducer pairs that make up the APOP system. One set of transducers was used for the primary acoustic chamber, which is filled with animals during a normal cast, and the other set was used for the empty reference chamber. During the calibration casts, both chambers were empty.

The results from two calibrations were quite consistent. A small but noticeable phenomenon is that generally the up cast results in smaller h values than the down cast. This indicates a possible delayed response of the transducers to the effects of the pressure and temperature. However, the overall difference is small with the maximum deviation $|\delta h|_{\max} < 0.003$. Theoretically, if the calibration curve was absolutely correct, by applying the calibration results to the data from the previous APOP casts, the errors in sound-speed contrast estimates would be corrected. However, direct application of the calibration results to the data might result in bias since the difference in arrival time is a function of depth and temperature. In other words, different temperature profiles may result in different calibration curves. Since the bias attributable to the depth dependence was small, the calibrated results used here ignore the depth dependence. In spite of uncertainties in calibration results, the error analysis is valid.

Measurements of density contrast

The density-contrast measurement was always conducted right after the shipboard or *in situ* sound-speed measurements were made. The dual-density method was used throughout the cruise except for five measurements (see Table 3). The container we used had a volume of 54.57 cm^3 (v_T in Equations 3−5). The temperature at which the densities were measured was set to 5.5°C, which was chosen to compensate the increase of temperature in animal bodies after they were taken out of the cold seawater (about −1.5°C) and went through the density measurement. The two fluids used in the density measurements were natural seawater and distilled water. The use of distilled water killed the animals after the density measurements. However, the animals slowly died after the container was filled with the distilled water, and consequently there should only be a limited effect on the weight measurements, but it could have had some influence on those relating to density (ρ_m in Equation 4). Attempts to use dilute seawater were unsuccessful because of the errors in the volume and density measurements. These arose because of precision limitations of the available volume- and density-measuring devices, and in the weight measurements as a result of ship motion that sometimes could not be fully compensated for by the motion-compensating weighing system in use.

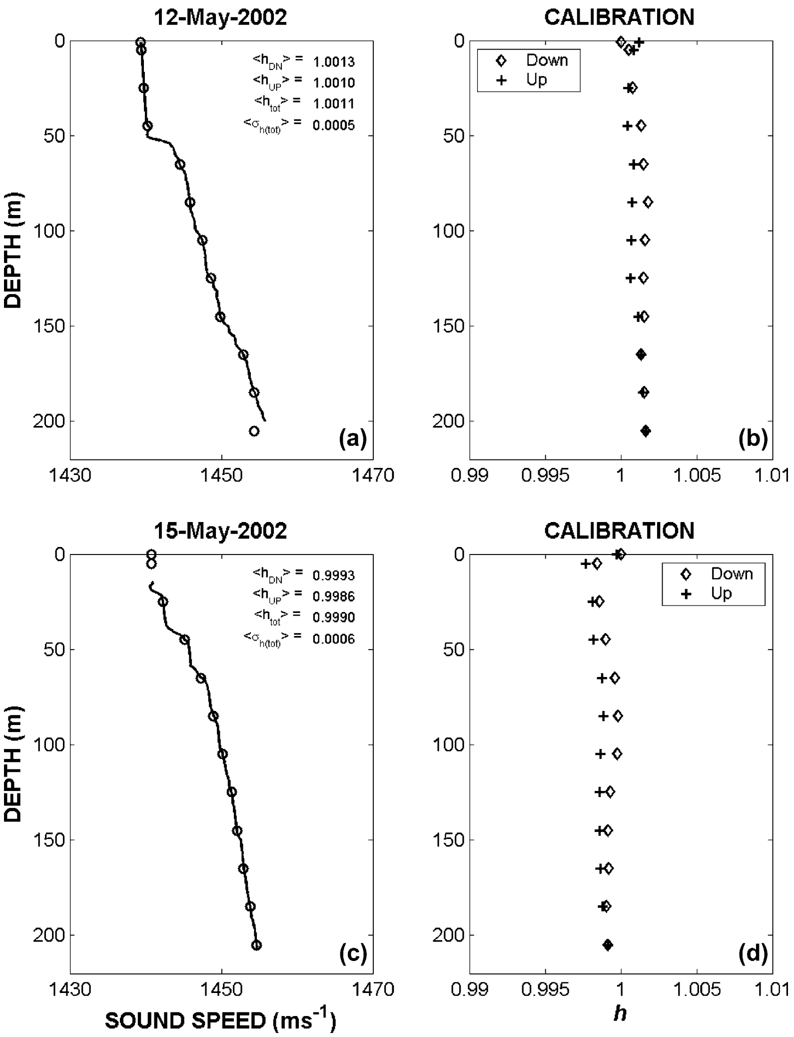

Figure 3. Sound-speed in seawater (left) and sound-speed contrast (h) in calibration mode (right) vs. depth. (a) and (b) are measurements taken on 12 May 2002 (Figure 2, cast 16), (c) and (d) are measurements taken in Crystal Sound on 15 May 2002. The open circles in (a) and (c) correspond to the depths where the sound-speed contrast measurements were made. The diamond and plus symbols correspond to down and up casts, respectively. The mean values of h over depth for down and up casts, and the overall mean and the standard deviation are given in the legend.

As with the sound-speed measurements, bubbles were generated during the transfer of the animal from the beaker to the measuring container, but they were carefully removed when the container was filled to the designated volume. There were some occasions when some trapped bubbles were still visible after the container was filled (only for *Themisto*). In these cases, the volume occupied by the trapped bubbles was estimated and subtracted in the processing software that computed the density contrast.

Another potential source of error for the shipboard density measurement comes from the fact that the temperature and pressure for the shipboard measurements and actual *in situ* ocean conditions are different. This could result in bias in g and h attributable to the differences in compressibility and thermal-expansion properties between the zooplankton and seawater. Although we cannot quantitatively estimate the potential bias in g, the measured h values for various species shown in Table 2 indicate that the difference between shipboard and *in situ* measurements is insignificant.

Results and discussion

There were 16 APOP casts and a number of shipboard measurements that measured the sound-speed and density contrasts of krill and other zooplankton species, as well as some Antarctic fish that have no swimbladders (Tables 2 and 3).

Krill

One of the primary objectives of our project during this cruise was to study the temperature and pressure (depth) dependence of the sound-speed contrast of krill. The target species was *E. superba*. Ten out of 12 krill casts were made using *E. superba*. The size range of the animals used in the casts varied from about 20 mm to 57 mm, which covered life stages from juvenile to adult. The mean length of the *E. superba* was 36.7 mm and the standard deviation was 9.8 mm. Two additional APOP casts with another krill species, *E. crystallorophias*, were also made. This species has an adult size smaller than that of *E. superba* (Everson, 2000). The size distribution of the *E. crystallorophias* used in the two casts varied from 21 mm to 38 mm, with a mean size of 31.9 mm and a standard deviation of 3.0 mm, a much narrower size distribution than that of *E. superba*.

For the sound-speed contrast, both shipboard and APOP-cast measurements were made on *E. superba*. The difference in the sound-speed contrast between the two types of measurements was statistically insignificant as long as the measurements on board ship were made when the temperature inside the APOP chamber and outside the chamber were the same. There was no significant depth dependence observed from the data sets (Figure 4a–d). In contrast, there was a depth-dependence in sound-speed contrast for *E. crystallorophias* (Figure 5), especially for up casts. This species also showed noticeable differences between down and up casts. The sound-speed contrasts were minimal at around 85 m and 105 m for the two casts, respectively. The difference between the two krill species in their response to vertical position in the water column could be due to their different biochemical composition or measurements error. The fact that patterns similar to those shown in Figure 5 were not observed in most of the ten APOP casts with *E. superba* suggests that these two krill species could indeed have different depth responses, resulting from either temperature change, or pressure change, or both.

For density contrast, all measurements were made in the ship's laboratory. The mean density of 13 measurements made on *E. superba* was 1.025, with a standard deviation of 0.008. However, the density contrasts of *E. crystallorophias* from two measurements were 1.009 and 1.000, respectively, and were significantly smaller than the mean value of *E. superba*. Both the density and sound-speed contrasts of the two krill species were relatively small compared with those of a coastal (Woods Hole) decapod shrimp (*Palaemonetes vulgaris*), whose sound-speed and density contrasts are almost always greater than 1.04 (Chu *et al.*, 2000a).

Almost all of our measurements were performed 1 day or more after the animals were collected. They were kept in buckets (5 gal.), which were put in two aquariums with surface seawater running through to keep the temperature at about −1.2°C to −1.5°C. Near the end of the cruise, however, a Reeve-net tow was made along the Martha Strait. The net tow was successful and caught more than 200 live krill (*E. superba*), including juveniles, sub-adults, and adults. The mean size was about 27 mm with a standard deviation of 7 mm. An APOP cast was made almost immediately – within an hour – after the animals were collected. The mean sound-speed contrast was 1.030 (Figure 4e and f), a little higher than observed for this size group earlier in the cruise, but still within a reasonable range of the sound-speed contrast obtained during this cruise. The standard deviation of 0.004 was no different from the previous measurements on *E. superba*. On this cast, a mild depth dependence and noticeable differences between down cast and up cast were observed and seemed to be associated with the temperature gradient.

Although there were no statistically significant differences in measured sound-speed and density contrasts between the freshly caught *E. superba* and those kept alive in aquariums for a longer time, there were slight size dependences observed in the data, which maybe attributable to the maturity of the animals. Linear regressions showed that the density and sound-speed contrasts had gradients of $5.4 \times 10^{-4}(\mathrm{mm}^{-1})$ and $5.0 \times 10^{-4}(\mathrm{mm}^{-1})$, respectively (Figure 6). This means that the difference in the target strength between a juvenile krill of size 27 mm and an adult krill of size 54 mm would be about 6 dB more than that resulting purely from size difference. The standard errors of both the sound-speed and density contrasts between the measured values and the predictions using regression curves are 0.003. The correlation coefficients are 0.48 and 0.67, respectively.

Copepods

Two APOP casts were made to measure the sound-speed and density contrasts of copepods, which were mainly *Calanus* sp. These animals are much smaller than krill and have a large proportion of lipids in their body. The mean prosome length and standard deviation of the copepods used in the measurements were around 3 mm and 0.3 mm, respectively. The sound-speed contrast of these copepods from the first APOP cast conducted on 2 May was less than unity (0.949), but slightly greater than unity from the second APOP cast conducted on 7 May (Table 2). The accurate density measurement was difficult using the current dual-density method, since the density of the seawater and distilled water mixture contained many micro-particles broken off from the copepods (exoskeleton), which may have altered the density of the pure fluid mixture significantly. To obtain a reasonable estimate of the density contrast, error analysis and numerical simulations were performed. Even with the extreme values using the maximum possible measuring errors, the density contrast was still less than unity. The best estimates from our analysis for the density contrast of the *Calanus* sp. were

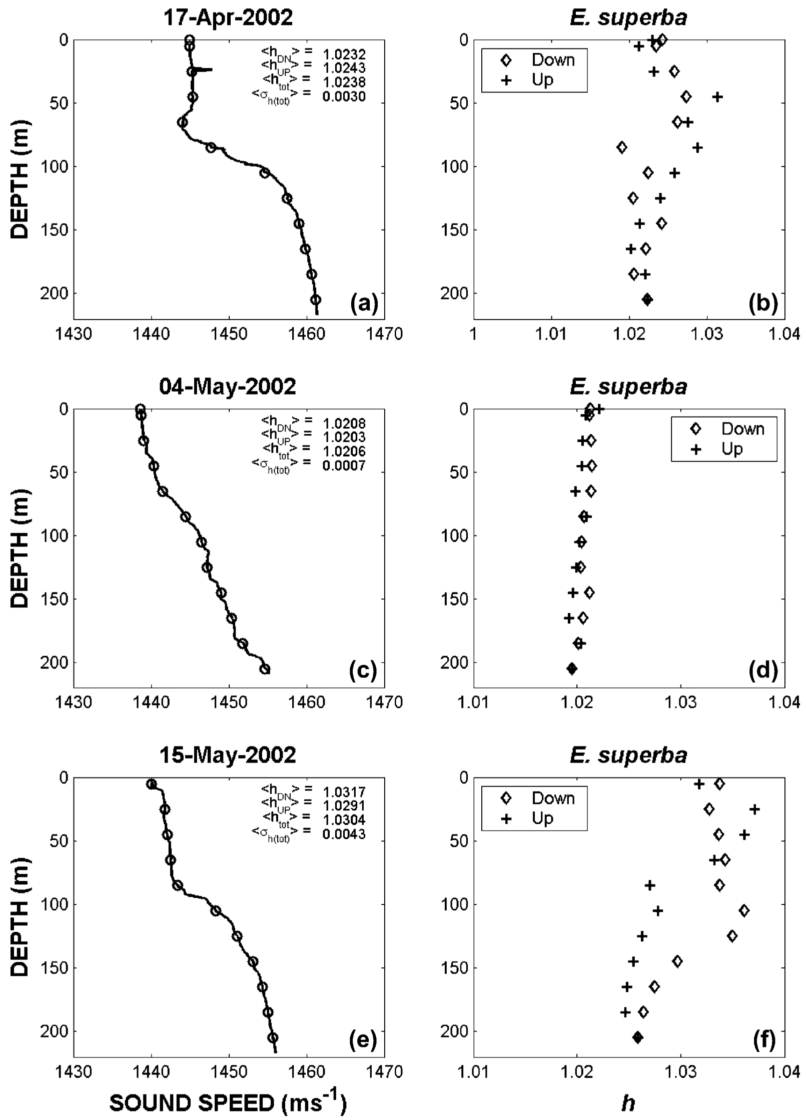

Figure 4. Sound-speed in seawater (left) and sound-speed contrast (h) of *E. superba* (right) vs. depth. (a) and (b) are measurements taken near Station 12 (Figure 2) on 17 April 2002: (c) and (d) are measurements taken near Station 74 (Figure 2) on 4 May 2002: (e) and (f) are measurements taken in Martha Strait on 15 May 2002. The open circles in (a), (c), and (e) correspond to the depths where the sound-speed contrast measurements were made. The diamond and plus symbols correspond to down and up casts, respectively. The mean values of h over depth for down and up casts, as well as the overall mean and the standard deviation are given in the legend.

0.995 and 0.996 for the two sets of measurements, respectively. Such results were consistent with what we observed before the density measurement: most animals floated on the surface of the beaker, indicating that these animals were positively buoyant.

The mean value of the copepod sound-speed contrast from the shipboard measurement was 0.959 and the mean value from the cast conducted on 2 May near Station 66 was 0.949, with a standard deviation of 0.013 (Figure 7a and b). The difference in mean value between down and up casts was 0.0055, which was a reasonable value. There was no obvious bias between the down and up casts. However, there was a distinct pattern observed in the sound-speed contrast for both down and up casts, with it being more or less a constant from the surface to about 100 m, corresponding to a basically constant sound speed in water or temperature, within the same depth range. Below 100 m, however, the sound speed in water increased as a function of depth, with a gradient of 0.12 m s^{-1} per metre, or 0.08% per metre, while the corresponding sound-speed contrast of copepods decreased (negative gradient) with a rate of 0.03% per metre. This negative gradient was very clear and definitive, especially for the down cast (monotonic). This result is consistent with and also confirms what we observed with

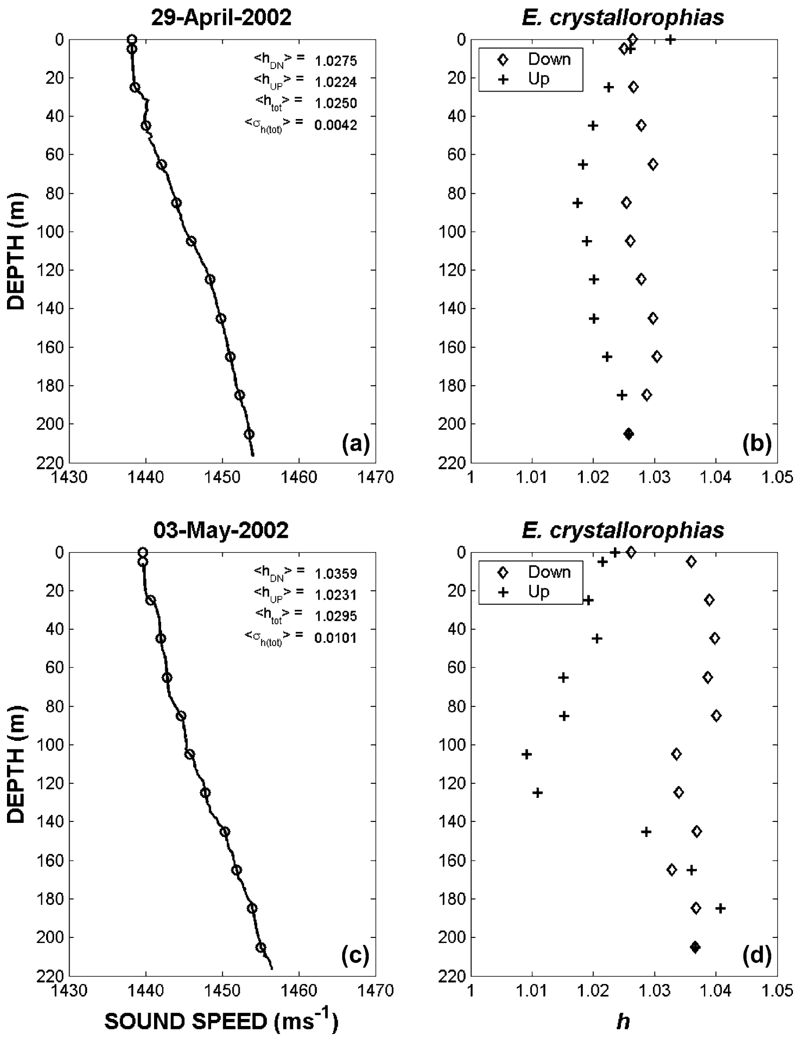

Figure 5. Sound-speed in seawater (left) and sound-speed contrast (h) of *E. crystallorophias* (right) vs. depth. (a) and (b) are measurements taken between Stations 55 and 56 (Figure 2) on 29 April 2002, while (c) and (d) are measurements taken near Station 71 (Figure 2) on 3 May 2002. The open circles in (a) and (c) correspond to the depths where the sound-speed contrast measurements were made. The diamond and plus symbols correspond to down and up casts, respectively. The mean values of h over depth for down and up casts, as well as the overall mean and the standard deviation are given in the legend.

Calanus finmarchicus on a different cruise to Wilkinson Basin, Gulf of Maine, in August 1999 (Chu *et al.*, 2000b).

However, the second APOP cast conducted on 7 May (Station 84) using the same species provided different results. The mean sound-speed contrast from the cast was 1.023, with a standard deviation of 0.002. The mean value being greater than unity indicates that the sound travelled faster in the animals than in the surrounding seawater, instead of slower as was found earlier. In addition, the depth-dependence was not observed this time (Figure 7c and d), which perhaps due to the lack of a sudden change in the slope of the sound-speed (temperature) profile. The inconsistency in these two casts suggested that acoustic estimates of biomass or abundance or both factors of this species (*Calanus* sp.) could be very challenging and may potentially result in estimates with a much larger bias than we previously believed.

Other species of zooplankton and fish

Only shipboard measurements were made on other species of zooplankton and fish (Table 2). It was much more difficult to conduct sound-speed measurements on amphipods (*Eusirus* sp. and *Themisto* sp.) and mysids (*Mysid arctomysis*) since bubbles were always attached to their bodies. To get reasonable and reliable measurements, we had to transfer the animals in the chamber without exposing them to the air. This was a difficult task with

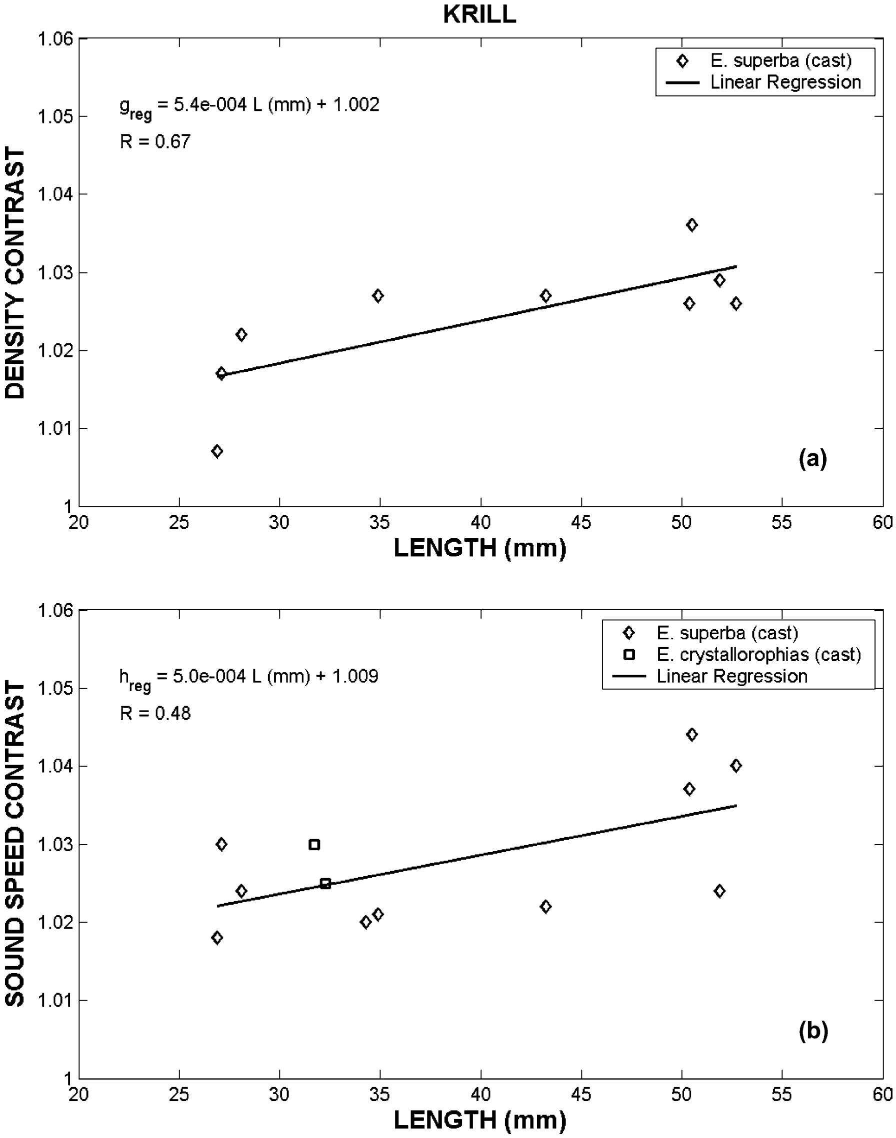

Figure 6. Density and sound-speed contrasts of krill as a function of length. (a) Density contrast as a function of length. (b) Sound-speed contrast as a function of length.

a water temperature of −0.5 °C or lower. The sound-speed contrasts for the two amphipod species were quite different. One was greater than 1.035 (*Eusirus* sp.) and the other was less than unity (*Themisto* sp.). The measured sound-speed and density contrasts of juvenile fish (*Pleuragramma antarcticum*) were all less than 1.020. This was not too surprising since this fish species has no swimbladder.

Conclusions

In situ measurements of sound-speed contrast and the shipboard measurements of density contrast, two parameters that strongly affect the acoustic scattering of zooplankton, were made off the western Antarctic Peninsula on live animals including krill (*E. superba* and *E. crystallorophias*), copepods, and other zooplankton species. The size range of *E. superba* varied from about 20 mm to 57 mm, spanning life stages from juvenile to adult. The mean length of the krill was 36.7 mm and the standard deviation was 9.8 mm. For *E. superba*, there was no statistically significant depth dependence for sound-speed contrast, but there was a noticeable depth dependence for *E. crystallorophias* and *Calanus* sp. There was a moderate dependence of sound-speed and density contrasts on the size of *E. superba*:

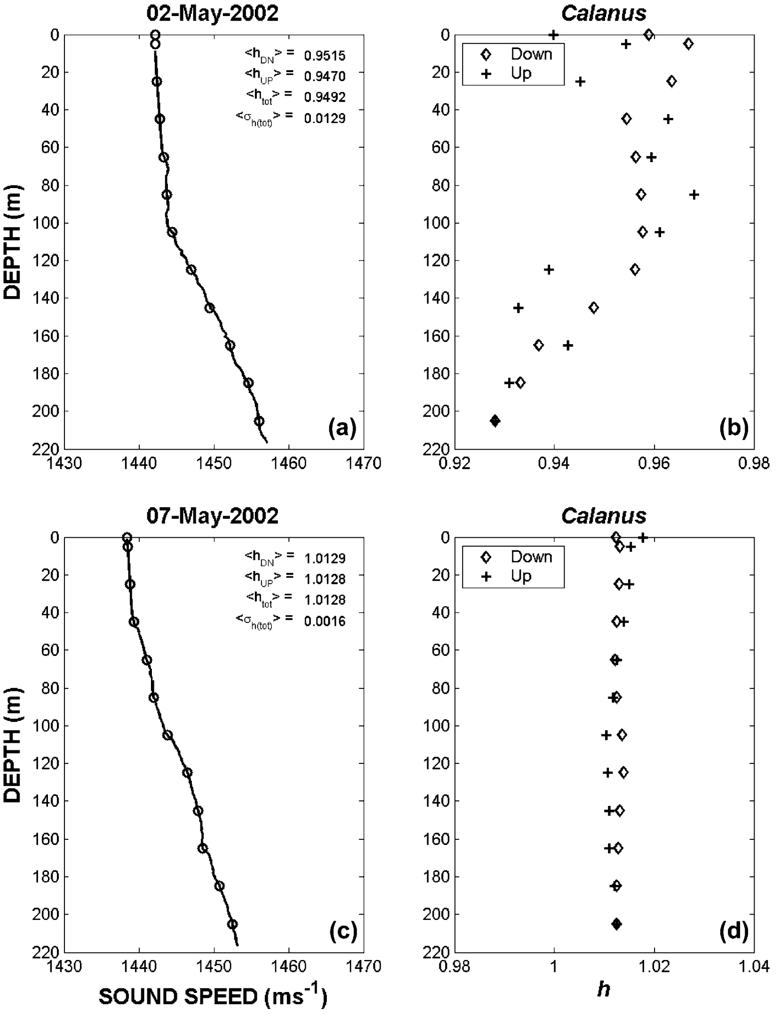

Figure 7. Sound-speed in seawater (left) and sound-speed contrast (h) of *Calanus* sp. (right) vs. depth. (a) and (b) are measurements taken near Station 66 (Figure 2) on 2 May 2002, while (c) and (d) are measurements taken near Station 84 (Figure 2) on 7 May 2002. The open circles in (a) and (c) correspond to the depths where the sound-speed contrast measurements were made. The diamond and plus symbols correspond to down and up casts, respectively. The mean values of h over depth for down and up casts, as well as the overall mean and the standard deviation are given in the legend.

$$g = 5.4 \times 10^{-4} L\ (\mathrm{mm}) + 1.002, \tag{7}$$

$$\text{and } h = 5.0 \times 10^{-4} L\ (\mathrm{mm}) + 1.009, \tag{8}$$

where L is the length of the krill, excluding their terminal setae (standard 1 in Mauchline, 1980). The measured density contrast varied between 1.007 and 1.036, with a mean value of 1.0241 and a standard deviation of 0.0082, while the measured sound-speed contrast for *E. superba* varied between 1.018 and 1.044, with a mean value of 1.031 and a standard deviation of 0.0084.

Applying these linear regressions to the Antarctic krill with a mean length of 32.2 mm, the sound-speed and density contrasts are 1.0247 and 1.0195, respectively. These values are smaller than those reported by Foote (1990) based on *ex situ* measurements (h = 1.0279 and g = 1.0357) for individuals of the same length. After taking into account the variability associated with the regression curve (Figure 6b), the difference in h is small and can be ignored, but the difference in g is quite significant and might result in about 3-dB difference in TS estimates. This indicates that the influence of material properties on the TS is comparable to that of the animal orientation, especially when an average over orientation and size distribution is involved (Stanton *et al.*, 1993).

From this research work, we found that no single set of density contrast or sound-speed contrast measurements is sufficient to characterize the material properties of zooplankton since they vary between species as well as taxa. A more comprehensive study is needed to evaluate the seasonal, spatial, and life-history variation in the material properties of zooplankton and the relationship with their biochemical composition.

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