An estimate of error for the CCAMLR 2000 estimate of krill biomass

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Abstract

Combined sampling and measurement error was estimated for the CCAMLR 2000 acoustical estimate of krill abundance (B_0) in the Antarctic Peninsular Area of the Southern Ocean (Food and Agriculture Organization's statistical area 48). First, some potential sources of uncertainty in generic echo-integration surveys are reviewed. Then, specific to CCAMLR 2000, some of the primary sources of measurement error are explored. The error in system calibration is evaluated in relation to the effects of variant water temperature and salinity on sound speed, sound absorption, and acoustical beam characteristics. Variation in krill target strength (*TS*) is estimated using a distorted-wave Born approximation model fitted with measured distributions of animal lengths and orientations. The variable effectiveness of two-frequency species classification methods are also investigated through the same scattering model. Most of these components of measurement uncertainty are frequency-dependent and covariant. Ultimately, the total error in the CCAMLR 2000 estimate of B_0 is estimated from a Monte Carlo simulation which assumes independent estimates of krill biomass are derived from acoustical

backscatter measurements at three frequencies (38, 120, and 200 kHz). The overall coefficient of variation ($10.2 \le CV \le 11.6\%$; 95% confidence interval) is not significantly different from the sampling variance alone ($CV = 11.4\%$). That is, the measurement variance is negligible relative to the sampling variance due to the large number of measurements averaged to derive the ultimate biomass estimate. Some potential sources of bias (e.g. stemming from uncertainties in the *TS* model, the krill length-to-weight model, the species classification method, bubble attenuation, signal thresholding, and survey area definition), may be more appreciable components of measurement uncertainty.

1. Introduction

In the austral summer of 2000, the Commission for the Conservation of Antarctic Marine Living Resources sponsored a survey (CCAMLR 2000; Trathan *et al.,* 1999) to estimate the biomass (B_0) and distribution of Antarctic krill in the Antarctic Peninsular area (Food and Agriculture Organization's statistical area 48). The multi-national, multi-ship survey included: 1) multi-frequency echosounders having their acoustical-beam axes aimed vertically downwards (Forbes and Nakken, 1972); 2) the application of echo integration methods to data collected along transects (MacLennan and Forbes, 1986; and Simmonds *et al.,* 1992); 3) the conversion of integrated echo energy to biomass density (Hewitt and Demer, 1993; and Stanton, *et al.,* 1995); and 4) the interpolation (or extrapolation) of the density estimates to the area sampled by the transect lines (Jolly and Hampton, 1990; Simmonds, *et al.,* 1992; and Foote and Stefansson, 1993). Each of these components can affect the overall accuracy and precision of the survey estimates (Taylor and Kuyatt, 1993; and Demer, 1994). An estimate of the total random error in B_o is necessary to quantify change in the standing stock of krill, and to set the fishery catch limits. In the remainder of the **Introduction**, the CCAMLR 2000 survey methods are summarized as they pertain to the subsequent measurement uncertainty analysis.

1. Echosounder measurements

To conduct CCAMLR 2000, four research vessels were used (*Kaiyo Maru*, *Atlantida*, *James Clark Ross*, and *Yuzhmorgeologiya*), from four nations (Japan, Russia, the United Kingdom (U.K.), and the United States of America (U.S.A.), respectively). Significant efforts were made to use identical equipment and protocols on each participating ship (Demer, 1998). Simrad EK500 echosounders (Bodholt *et al.,* 1989) were used, each fitted for synchronous transmissions at three frequencies (38, 120, and 200 kHz), every two seconds.

1.1 Sound speed and absorption

The mean sound speed ($\bar{c}$; m/s) and mean absorption coefficients ($\bar{\alpha}_{38kHz}$, $\bar{\alpha}_{120kHz}$, and $\bar{\alpha}_{200kHz}$; dB/km) were estimated for use during the entire survey area from measurements of salinity (*S*) and temperature (*T*) versus depth (*r*) from surveys conducted the previous year (Austral summer 1998/99; see **Figure 1** for station locations). Using conversion algorithms from Mackenzie (1981) and Francois and Garrison (1982), respectively, values of $c, \alpha_{38kHz}, \alpha_{120kHz}$, and α_{200kHz} were first calculated for each station at 10 m depth increments. Because krill reside mostly in the upper 150 m (Miller and Hampton, 1989), weighted-means (weight=$1/r^2$) were then calculated for each of these variables. For example:

$$\bar{c} = \frac{\sum_{i=1}^{N} c(r_i)/r_i^2}{\sum_{i=1}^{N} 1/r_i^2}, \tag{1}$$

where r_i is the mid-point of the *i*-th depth bin and N =50 is the total number of 10 m bins from 10 to 500 m. These values of $\bar{c}, \bar{\alpha}_{38kHz}, \bar{\alpha}_{120kHz}$, and $\bar{\alpha}_{200kHz}$ were ultimately used and kept constant for the entirety of the cruise (**Table 1**).

1.2 Equivalent two-way beam angle

Considering first order effects, the nominal equivalent two-way beam angles (ψ) were reduced for the survey by a factor approximately equal to the square of the ratio of $\bar{c}$ (=1449 m/s) and the sound speed during Simrad's transducer calibrations (nominally

1473 m/s). That is, the survey protocols specified that the values used for ψ were 0.14 dB less than the values in Simrad's transducer specifications.

1.3 System calibration

System calibrations for each frequency were performed before and after the survey in protected bays on South Georgia and King George Islands, respectively. Standard targets were identically prepared 38.1 mm diameter tungsten carbide spheres with 6% cobalt binder. Theoretical target strength (*TS*) values were referenced from Foote (1990a). Judging from Foote (1983b) and Foote and MacLennan (1984a and b), calibrations with the standard sphere method are precise to apx. 0.1 dB. The precision of the EK500 transceivers reduces the calibration precision from 0.1 to 0.3 dB, depending upon the receiver bandwidth (Simrad, 1993).

The initially very precise system calibrations were likely degraded over time and space, however, due to changes in *T* and *S,* throughout the survey. Variations in *T* effected the transducer characteristics (Demer and Hewitt, 1992; Demer, 1994; and Brierley *et al.*, 1998), and variations in $\bar{c}$, $\bar{\alpha}_{38kHz}$, $\bar{\alpha}_{120kHz}$, and $\bar{\alpha}_{200kHz}$ increased the uncertainty in models of sound propagation and thus measurements of echo energy. To evaluate these effects, measurements of *T*, *S*, c, and α versus *r* were made throughout the survey.

1.4 Diel vertical migration

Krill migrate vertically, generally moving from depth during the day, to the surface at night (Everson, 1982; and Godlewska and Klusek, 1987). Miller and Hampton (1989) estimated that about 40% of the krill biomass could be concentrated in the uppermost 5 m at night. Demer and Hewitt (1993) estimated that krill surveys conducted in the Elephant Island area and irrespective of the time of day could be negatively biased by an average of 49.5%. Consequently, CCAMLR 2000 was conducted exclusively during daylight hours.

2. Echo integration

So that all possible data were retained, measurements of volume backscattering strength (*Sv*) and *TS* were thresholded at the minimum values of -100 dB. For effective multiple-frequency data analyses (Greenlaw *et al.,* 1980; and Demer *et al*., 1999), the insonified volumes at each frequency were designed similarly, to the extent physically and fiscally possible. That is, most of the transducers had 7° beamwidths, were effectively collocated, and the echosounders were modified for 1 ms pulse durations at all three frequencies (atypical for 200 kHz operation).

2.1 Species classification

A two-frequency method (Madueira *et al*., 1993; and Watkins and Brierly, submitted) was used to identify and delineate acoustic backscatter from krill and other sources. After averaging *Sv* at 120 and 200 kHz (Sv_{120kHz} and Sv_{38kHz}) over cells 50 pings wide (apx. 500 m) by 5 m depth, differences in mean volume backscattering strengths ($\Delta MVBS = Sv_{120kHz} - Sv_{38kHz}$) between 2 and 16 dB were used to indicate krill. The integrated echo energy from krill aggregations (s_a; m^2/km^2) was assumed to be equivalent to the sum of energies that would have been received from the same number of individuals in isolation (Johannesson and Mitson, 1983; and Foote, 1983a). However, the relationship between s_a and the true animal density (ρ_n) is affected by many factors which are understood to varying degrees (MacLennan and Forbes, 1984). For a group of identical animals that are randomly distributed within the beam, an estimate of the animal density ($\hat{\rho}_n$; animals/m^2) is proportional to s_a or volume backscattering coefficients integrated between depths r_1 and r_2 and averaged over some trackline distance (MacLennan and Simmonds, 1992). Following Simrad (1993):

$$\hat{\rho}_n = \frac{4\pi r_0^2}{\hat{\sigma}} \left\langle \int_{r_1}^{r_2} \left(\frac{\hat{p}_r 32\pi^2 \hat{r}^2 10^{2\hat{\alpha}\hat{r}}}{\hat{p}_t \hat{g}_o^2 r_o^2 \hat{\lambda}^2 \hat{c} \hat{\tau} \hat{\psi}} \right) dr \right\rangle, \tag{2}$$

where p_t is the transmit power (W), p_r is the receive power (W), g_o is the calibrated on-axis system gain (Blue, 1984; and Foote *et al.,* 1987), r is the range (m), r_o is the reference distance (generally 1 m), λ is the acoustical wavelength of the transmitted pulse (m), c is the sound speed (m/s), α is the absorption coefficient (W/m), ψ is the equivalent beam angle (Simmonds, 1984a and b; and Foote, 1990c) and σ is the backscattering cross-sectional area representative of the animals in the surveyed area, at the time of the survey (m^2; Greenlaw *et al.,* 1980; Foote *et al.,* 1990b; Greene *et al.,* 1991; Hewitt and Demer, 1991; and Chu *et al.,* 1993). The mean is designated by $< >$.

2.2 Target strength

Krill *TS* $(= 10\log(\sigma/4\pi))$ depends upon the acoustic frequency (Chu *et al.*, 1992), and animal size, shape, density, sound speed, and its orientation within the acoustic beam (Stanton 1989a and b). Estimates of *TS* are derived from models based on scattering physics (e.g. Chu *et al.,* 1993; and Stanton *et al.,* 1993) or linear regressions of empirical *TS* data and euphausiid lengths (e.g. Wiebe *et al.,* 1990; and Greene *et al.,* 1991). Although the Greene *et al.* model has been corroborated by *in situ* measurements of

Euphausia superba (Hewitt and Demer, 1991), and has been adopted by CCAMLR (Tranthan *et al.* 1992), it does not account for *TS* variability due to animal density, sound speed, shape and orientation, and acoustical wavelength. Demer (1994) demonstrated the potential errors in using linear models of *TS* versus animal length (*L*) to approximate scattering from zooplankton (a highly non-linear phenomenon). Additionally, several investigators have shown that animal behavior has a dominant effect on the *TS* of zooplankton (Greenlaw *et al.*, 1980; Stanton, 1989a; and Demer and Martin, 1995). For example, Everson (1982) observed an 8 dB difference between the daytime and nighttime *Sv* of krill aggregations and attributed this to diel changes in orientation. McGehee *et al.* (1998), offered a *TS* model based on the distorted-wave Born approximation (DWBA) that explicitly accounts for acoustical frequency, animal shape, orientation, and material properties. The DWBA was validated using measurements of live krill in a tank, but only near broadside incidence.

Deemed accurate at 120 kHz, if not precise, the Greene *et al.* model (1991) was used to estimate mean *TS* for CCAMLR 2000. To convert $\hat{\rho}_n$ to an estimate of biomass density ($\hat{\rho}$; g/m^2), another model (see Hewitt *et al.*, this volume) provided estimates of wet weight per animal (*w*; g/animal):

$$\hat{\rho} = \hat{\rho}_n \hat{w}. \tag{3}$$

3. Measurement error

Application of this theory necessitates estimations of all the variables in Eq (2) and Eq (3) (eg, estimated x $= \hat{x}$), each introducing some uncertainty (Demer, 1994). More realistically, these variables are represented by their respective probability density functions (PDFs). Because most of these variables are covariant, an analysis of all of the individual-components of measurement uncertainty is daunting.

Considering some of these potential sources as independent variables, Tesler (1989) and MacLennan and Simmonds (1992) estimated the systematic and random components of uncertainty for generic echo integration surveys (**Table 2**). According to Tesler, the primary sources of survey bias are system calibration (±0.5 to ±1.0 dB) and the values assumed for *TS* (±1.5 dB). Although MacLennan and Simmonds stated that the calibration bias is relatively inconsequential (±0.3 dB), they agreed that *TS* could be a significant source of error (±1.8 dB) as well as species identification (±2.6 dB) (see Greenlaw and Johnson, 1983; Holliday *et al.*, 1989; and Stanton *et al*., 1995), vertical migration (0 to -1.5 dB) (see Everson, 1982; Godlewska and Klusek, 1987; and Demer and Hewitt, 1993), and possibly bubble attenuation (0 to -2.8 dB) (see Dalen and Lovik, 1981).

Although it is correct to consider the uncertainties associated with system calibration, species identification, *TS*, and animal behavior as systematic for point-measurements, the magnitudes and signs of the associated biases are often variable over the time- and space-scales of a survey. Thus, they contribute *random errors* to the biomass estimate. Moreover, each of these sources of uncertainty manifest different errors for biomass estimates derived from acoustical backscatter at different acoustical frequencies. For example: 1) System calibrations performed on separate transceiver-transducer pairs are temperature dependent to varying degrees (Demer, 1994; and Brierly *et al.,* 1998), and are subject to different sound absorption values (Francois & Garrison

1982); 2) The relative sensitivity of acoustical backscatter to krill orientation is dependent on the relationship between the animal size and the acoustic wavelength (ie. whether Rayleigh, Mie, or Geometric scattering; Demer and Martin, 1995); and 3) The transmit power, ambient noise, bubble attenuation, receive sensitivity and thus detection probabilities of each echosounder frequency are unique. Support for the latter point will be presented in the **Methods** section on **Detection Probability**.

4. Sampling error

CCAMLR 2000 was conducted using randomly spaced parallel-line transects. Following the method proposed by Jolly and Hampton (1990), each transect provided a single sample of $\hat{\rho}$. Within a stratum, mean biomass density $\left(\hat{\bar{\rho}}\right)$ was weighted by the number of averaging intervals along each transect. The total biomass ($\hat{B}$; mt) was simply estimated by multiplying $\hat{\bar{\rho}}$ by the estimated total survey area ($\hat{A}$; m^2). The coefficient of variation (*CV*; %), usually used to summarize the variance in $\hat{B}$, was derived from the ratio of the standard deviation of $\hat{B}$ (std($\hat{B}$)) and $\hat{B}$. The equations used for the CCAMLR 2000 analysis are tabulated in Hewitt *et al.* (this volume). Calculated in this way, the *CV* only accounted for the sampling variance. The aim of this study is to estimate the total error in the CCAMLR 2000 krill biomass estimate -- that is, the combination of both the measurement and sampling errors.

II. Methods

Some of the potential sources of measurement uncertainty in CCAMLR 2000 were explored in a variety of ways. The actual environmental values affecting sound propagation were compared to the constants picked before the survey. The validity of the empirical *TS* model adopted from Greene *et al.* (1991) was explored relative to a physics-based DWBA model. Expected values for $\Delta MVBS$ were also derived and compared using the two aforementioned scattering models and krill length distributions measured during the survey. Relative detection sensitivities of the echosounders aboard each ship, at each frequency, were quantified using the respective system parameters. Each of these studies identified potential errors that are frequency dependent, generally covariant, and thus difficult to quantify. Ultimately, the total error in the CCAMLR 2000 estimate of B_0 was estimated from a Monte Carlo simulation which assumed that independent estimates of krill biomass were derived from acoustical backscatter measurements at each of the three frequencies (38, 120, and 200 kHz).

1. Sound speed and absorption

At the conclusion of CCAMLR 2000, weighted mean values of $c, \alpha_{38kHz}, \alpha_{120kHz}$, and α_{200kHz} were re-estimated using Eq (1) and 10 m averages of T and S for each of 140 CCAMLR 2000 stations (sampled by UK, Japan, and USA; **Fig. 1**). The results (**Table 1**) are more representative of the actual survey conditions.

As sound propagation is affected by the values of c and α only between the transducer and the scatterers, and the mean values of c and α are dependent upon the propagation time spent in each incremental depth, these variables are more accurately calculated as harmonic means ($\bar{c}_h$, and $\bar{\alpha}_h$; Weinberg, 1971), weighted by the PDF of krill density versus depth. That is, the sound speed and absorption coefficients are best calculated by weighting the depth dependent variables $c(r_i)$ and $\alpha(r_i)$ by the incremental time (Δt_i; s) spent in the i-th depth bin (Δr_i = r_i -r_{i-1}; m) and the krill distribution probability P(Δr_i) in each Δr_i. For example:

$$c_{h_i} = (r - r_0)\left[\sum_{i=1}^{N} \frac{1}{g(r_i)} Ln\left(1 + \frac{g(r_i)}{c(r_i)} \Delta r_i\right)\right]^{-1}, \text{ and} \tag{4}$$

$$\overline{c}_h = \frac{\sum_{i=1}^{N} P(\Delta r_i) c_{h_i}}{\sum_{i=1}^{N} P(\Delta r_i)}, \tag{5}$$

where $g(r_i)$ is the gradient dc/dr in Δr_i, and r and r_0 are the maximum and minimum depths, respectively. A Rayleigh distribution ($\Re(r_i$, 40 m)) was used to closely approximate a PDF of the vertical krill distribution ($P(\Delta r_i)$). For comparison with the survey constants, the harmonic means for sound speed and absorption are tabulated and plotted (**Table 1** and **Fig. 2**).

2. Target strength

Krill *TS* were predicted using the DWBA model (generic shape (McGehee *et al.*, 1998); g=1.0357, and h=1.0279 (Foote 1990b); **Fig. 3**). Note that the scattering directivity of krill increases dramatically with animal length and frequency (90˚ = normal or dorsal incidence). In fact, the model predicts *TS* to change by 10 to 60 dB versus animal orientation angles, sometimes not too distant from normal incidence. However, McGehee *et al.* noted that their *TS* data from live *E. superba* only matched the model on the main lobe; *TS* measurements at steeper angles were elevated relative to predictions.

Using the RMT-8 net samples from each ship, three clusters of krill length-frequency distributions were identified for different portions of the CCAMLR 2000 survey area (Siegel *et al.*, this volume). Cluster one was comprised of small krill with a narrow length distribution centered at 26 mm; Cluster 2 had a broad and somewhat bi-modal length distribution peaking at 46 mm; and Cluster 3 was comprised of large krill having a positively-skewed length distribution centered at 52 mm. The DWBA model was therefore plotted versus the general range of krill lengths (20 to 55 mm), and versus acoustical frequency, and incidence angle (**Fig. 4**). The model indicates that a wide range of *TS* (apx. 5-30 dB, depending upon incidence angle) is expected for this range of animal sizes.

Choosing a very narrow distribution of angles about normal incidence (N(90,3)), *TS* distributions were estimated for each length-frequency distribution (**Fig. 5**). For comparison, also plotted are the *TS* distributions estimated from the Greene *et al.* (1991) model using the same length-frequency distributions.

3. Species classification

Again using the DWBA model (generic shape; g=1.0357; h=1.0279; density = N(600m^3, 150m^3); and a distribution of krill orientations from Kils, 1981; N(45.3˚, 30.4˚), *Sv* were predicted for each frequency and each size cluster (**Fig. 6**). The objective was to estimate the expected distributions of S_v and $\Delta MVBS$ at the survey frequencies, for the size distributions of krill in the area (**Fig. 7**).

4. Detection probability

The transmit power, ambient noise, bubble attenuation, receive sensitivity and thus the PDF of krill detection versus depth are unique for each echosounder and frequency. Detection probabilities were explored for the echosounders aboard each ship by calculating the signal-to-noise ratio (SNR; dB) versus range for various levels of *Sv*:

$$SNR = P_t + S_v + 2G_0 + \psi + 10\log 32\pi^2\lambda^2 c\tau - 20\log r - 2\alpha r - P_n, \qquad (6)$$

using the values, units, and nominal background noise levels recorded during CCAMLR 2000 listed in **Table 3**. The results for each frequency for each ship are plotted in **Figure 3**. Assuming a worst-case situation where the noise and signal are coherently additive, the SNR provides some metric of the percent bias at each detection range and level of Sv:

$$\frac{Noise}{Signal} = \left(\frac{1}{10^{SNR/10}}\right) * 100 \quad (\%). \qquad (7)$$

From Eq.(7), a 10 dB SNR in **Figure 3** indicate a 10% bias.

5. Total random error

Because the components of measurement uncertainty are generally covariant, a Monte Carlo simulation was used to quantify overall variance specific to CCAMLR 2000. Assuming each of the three frequencies provided independent estimates of krill biomass, average densities were randomly selected for each interval from one of the three frequencies and a survey biomass was simulated (equations defined in Hewitt *et al.*, this volume). Repeating this process 10,000 times, a PDF of *CV*'s was estimated for the survey biomass. Because the 38 kHz provided an estimate of krill biomass (29.41 mt) that was about 33% less than that of 120 kHz and 200 kHz (44.29 mt and 44.82 mt, respectively), the interval densities at 38 and 200 kHz were normalized to the 120 kHz estimate ($S_{Ai} f_i(W_I)_i$* 44.82/29.41 for 38 kHz and $S_{Ai} f_i(W_I)_i$*44.29/44.82 for 200 kHz), and the simulation was repeated. The PDF of *CV*s was again calculated for the survey biomass.

III. Results

1. Sound speed and absorption

At the conclusion of CCAMLR 2000, estimated means for *T*, *S*, *c*, and α versus *r* were compared to the 1998/99 data (**Table 1**). Of note: 1) the weighted-mean *T* was 1.5° warmer than that of the previous year; and 2) correspondingly, the harmonic means for *c* and α were each approximately one standard deviation higher than the pre-selected survey constants. In both cases, the inaccuracies in sound propogation parameters result in an unquantified negative bias in B_0.

2. Equivalent two-way beam angle

During the survey, the minimum sound velocity (harmonic mean) was 1447 and the maximum was 1468 m/s. These correspond to equivalent 2-way beam angle corrections (relative to Simrad specifications) of -0.16 and -0.03 dB, respectively. Therefore, relative to the survey-constant equivalent 2-way beam angles (Simrad specified Ψ -0.14 dB), the bias in equivalent 2-way beam angles is estimated as -0.02 to +0.11, or 0.04 dB with a standard deviation of 0.03 dB. The effect was an almost negligible negative bias in B_0.

3. Target strength

The *TS* predicted by the DWBA and Greene *et al.* models are quite similar for larger krill size clusters (2 and 3) and higher frequencies (120 and 200 kHz; **Fig. 5**). In contrast, the modal *TS* predicted for smaller animals (Cluster 1) and low frequency (38 kHz) are 5-8 dB different between the two models. Similarly, the DWBA model indicates virtually the same *TS* values at 200 and 120 kHz and a large difference (apx. 16 dB) between *TS* at 120 versus 38 kHz. In contrast, the Greene *et al.* model predicts constant differences of 10log(200/120)=2.2 dB and 10log(120/38)=5 dB, respectively. All this suggests that the Greene *et al.* model is not applicable for Rayleigh scattering and the DWBA model may therefore be better suited for predicting differences in mean volume backscattering strengths (e.g Sv_{120kHz}-Sv_{38kHz}). This finding is supported by the close aggreement between the B_0 estimates at 120 and 200 kHz and the 33% lower estimate at 38 kHz, derived using the Greene *et al. TS* model.

4. Species classification

For clusters 1, 2, and 3, the modes of S_v are -64, -52, and -54; -62, -51, and -52; and -62, -51, and -52 dB for 38, 120, and 200 kHz, respectively (**Fig. 6**). The distributions of S_v vary little between clusters 2 and 3, and more between cluster 2/3 and 1 (much smaller animals). Values of Δ*MVBS* show consistent modes for all three clusters (*Sv120-Sv38* = 11dB; *Sv200-Sv120*= -1dB; and *Sv200-Sv38* = 10dB; **Fig. 7**). The distributions of $Sv_{120\ kHz}$-$Sv_{38\ kHz}$ range from 9-12, 9-13, and 9-13 dB for Clusters 1, 2, and 3, respectively. Recalling that the CCAMLR 2000 window of Δ*MVBS* indicating krill was 2 to 16 dB, it is reasonable to assume that few krill were rejected with the chosen algorithm. On the other hand, the survey limits were wide so as to possibly allow other species to be counted as krill. The latter uncertainty is most certainly frequency dependent.

5. Diel vertical migration

Despite the effort to survey only during daylight hours, there was some variation in detection probability versus time-of-day. **Figure 8a** shows a non-uniform distribution of total s_a at 120 kHz, normalized to observation effort, versus time-of-day. Peak detections occured at 0700, 1000, and 2300 hr GMT or apx. noon, 3 PM, and 4 AM, local time, respectively. A detection minimum occurred between 1500 and 1600 hr GMT or between apx. 10 and 11 PM local time. The latter suggests that the survey effort may have continued slightly longer than it should have to avoid bias due to diel vertical migration. Total s_a at 120 kHz versus depth for the entire survey describes a Rayleigh-type distribution with 90% of the biomass detected in the upper 100 m (**Fig. 8b**). Also plotted were the mean and median *Sv* at 120 kHz for krill detected during CCAMLR 2000 (averaged over interval size; **Fig. 8c and d**). The distributions of S_v averaged over cells apx 5 m by 500 m peak at apx -83 and -80 dB, respectively. In view of the shallow distribution of krill (**Fig. 8b**) and the expected S_v values for the krill caught during the survey (**Fig. 6**), CCAMLR 2000 was generally not noise-limited, except possibly when surveying low density krill aggregations **(Fig. 9**). However, the detection probabilities are very frequency dependent, and worst for the 38 kHz echosounder on *R/V Atlantida* and the 120 and 200 kHz echosounders on *R/V James Clark Ross*.

6. Total uncertainty

Assuming each of the three frequencies provided independent estimates of krill biomass, combined measurement and sampling errors were quantified with a Monte Carlo simulation. Results indicate an overall variance: *CV* of B_0 = 11.3 %, std = 0.42 %.

When mean biomass values are normalized to that of 120 kHz, the overall variance is somewhat smaller: *CV* of B_0 = 10.9 %, std = 0.37 %.

IV. Discussion

During CCAMLR 2000, the weighted-mean *T* was 1.5° warmer than that of the previous year, and harmonic mean values *c* and *α,* and *ψ* were therefore higher than the survey constants. The combined effect is a small negative bias in B_0.

The Greene *et al.* model may provide accurate *TS*(*L*) values for larger krill at 120 kHz and 200 kHz, but it appears to yield erroneously high values at 38 kHz and thus causes an appreciable negative bias in B_0 at that frequency. The two-frequency method employed to delineate krill from other scatterers appeared to be quite effective, but is more likely to contribute a positive bias to B_0, if any.

Despite efforts to survey only during daylight hours, there is some evidence that diel vertical migration of krill may have also contributed a minor negative bias to B_0. The tendency for krill to reside mostly in the upper 100 m of the water column kept most echosounders from being noise limited and subject to thresholding. However, for low density krill aggregations, a small negative bias could have resulted at 38 kHz for R/V *Atlantida*.

Clearly, numerous components of an echo-integration survey can contribute uncertainty to the estimate of biomass. Individually, the magnitudes of these components of uncertainty are in reasonable agreement with the values estimated by Tesler (1989) and MacLennan and Simmonds (1992) (**Table 2**). However, most of the components of uncertainty are frequency-dependent and covariant. Consequently, a practical and robust way to estimate the overall error in the survey estimate is introduced here. This method includes a simulation that assumes each frequency provides an independent estimate of biomass.

V. Conclusion

The error in B_o is requisite for measuring change in the standing stock of krill (Hewitt and Demer, 1994), and for setting fishery catch limits. The overall *CV*, accounting for measurement and sampling error (10.2 to 11.6%, 95% confidence interval), is not significantly different from the sampling *CV* (11.4%). That is, the measurement variance is negligible relative to the sampling variance due to the large number of measurements averaged to derive the ultimate biomass estimate.

Some potential sources of bias (eg. stemming from uncertainties in sound propagation parameters, *TS*, species classification, bubble attenuation, thresholding, area definition, conversion of number density to biomass density, etc.), may be more appreciable components of measurement uncertainty and should be investigated further. TS appears to be the largest of these components of measurement uncertainty. Almost all of the potential biases in B_0 are shown to be negative, with the exception of species classification. Therefore, judging from this analysis, the CCAMLR 2000 estimate of B_0 is quite precise and possibly a bit conservative.

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VII. References

J.E. Blue, **1984**, "Physical Calibration," *Rapp. P. -v. Reun. Cons. int. Explor. Mer.*, **184**, 19-24.

H. Bodholt, H. Nes, H., Solli, **1989**, "A new echo-sounder system," Proc. IOA, **11**(3), 123-130.

A.S. Brierley, C. Goss, J.L. Watkins, and P. Woodroffe, **1998**, "Variations in echosounder calibration with temperature and some possible implications for acoustic surveys of krill biomass," *CCAMLR Science*, 5:273-282.

D. Chu, T.K. Stanton and P.H. Wiebe, **1992**, "Frequency dependence of sound backscattering from live individual zooplankton," *ICES J. Mar. Sci.*, **49**, 97-106.

D. Chu, K.G. Foote, and T.K. Stanton, **1993**, "Further analysis of target strength measurements of Antarctic krill at 38 and 120 kHz: Comparison with deformed cylinder model and inference of orientation distribution," J. Acoust. Soc. Am. **93**(5), 2985-2988.

J. Dalen and A. Lovik, **1981**, "The influence of wind-induced bubbles on echo integration surveys," *J. Acoust. Soc. Am.* **69**, 1653-59.

D.A. Demer and R.P. Hewitt, **1992**, "Calibration of an acoustic echo-integration system in a deep tank, with system gain comparisons over standard sphere material, water temperature and time, *CCAMLR Selected Scientific Papers*, CCAMLR, Hobart, Tasmania 127-144.

D.A. Demer, **1993**, "Bias in acoustic biomass estimates of *Euphausia superba* due to diel vertical migration," *Deep-Sea Res. I.* 42(4):455-475.

D.A. Demer, **1994**, “Accuracy and precision of acoustic surveys of Antarctic krill,” Ph.D. thesis, UCSD, 144p.

D.A. Demer and L.V. Martin, **1995**, “Zooplankton target strength: volumetric or areal dependence?,” *J. Acoust. Soc. Am.* 98(2,Pt.1):1111-1118.

D.A. Demer, 1998, "Some suggestions for acoustic protocols for the synoptic survey of FAO Area 48," WG-EMM-98/47.

D.A. Demer, M.A. Soule, and R.P. Hewitt, **1999**, "A multiple-frequency method for potentially improving the accuracy and precision of in situ target strength measurements," J. Acoust. Soc. Am., 105(4):2359-2376.

I. Everson, **1982**, “Diurnal variations in mean volume backscattering strength of an Antarctic krill (*Euphausia superba*) patch,” *Journal of Plankton Research*, **4**(1), 155-162.

K.G. Foote, **1983a**, “Linearity of fisheries acoustics with addition theorems,” J. Acoust. Soc. Am. **73**, 1932-1940.

K.G. Foote, **1983b**, “Maintaining precision calibrations with optimal copper spheres,” J. Acoust. Soc. Am. **73**(3), 1054-1063.

K.G. Foote, and D.N. MacLennan, **1984a**, “Comparison of copper and tungsten carbide calibration spheres,” *J. Acoust. Soc. Am.* **75**(2), 612-616.

K.G. Foote, and D.N. MacLennan, **1984b**, "Use of elastic spheres as calibration targets," FAO Fish. Rep., **300**, 52-58. (Contribution No. 31)

K.G. Foote, Knudsen, H.P., Vestnes, G., MacLennan, D.N., Simmonds, E.J., **1987**, "Calibration of acoustic instruments for fish density estimates: A practical guide," ICES Co-op. Res. Rep. 144.

K.G. Foote, **1990a**, “Spheres for calibrating an eleven-frequency acoustic measurement system,” *J. Cons. int. Explor. Mer,* **46**, 284-286.

K.G. Foote, **1990b**, “Speed of sound in Euphausia superba,” *J. Acoust. Soc. Am.* **87**(4), 1405-1408.

K.G. Foote, **1990c**, “Equivalent beam angles for several standard transducers,” ICES CM B:21.

K.G. Foote, I. Everson, J.L. Watkins and D.G. Bone, **1990**, “Target strengths of Antarctic krill (*Euphausia superba*) at 38 and 120 kHz,” *J. Acoust. Soc. Am.* **87**, 16-24.

K.G. Foote and G. Stefansson, **1993**, “Definition of the problem of estimating fish abundance over an area from acoustic line-transect measurements of density,” *ICES J. Mar. Sci.*, **50**, 369-381.

S.T. Forbes and D. Nakken, **1972**, “Manual of methods for fisheries resources survey and appraisal, Pt 2, The use of acoustical instruments for fish detection,” FAO Manuals Fish Science.

R.E. Francois and G.R. Garrison, **1982**, "Sound Absorption based on ocean measurements. Part II: Boric acid contribution and equation for total absorption," *J. Acoust. Soc. Am.*, 72:1879-1890.

M. Godlewska and K. Klusek, **1987**, "Vertical Distribution and Diurnal Migrations of Krill - *Euphausia superba Dana* - from Hydroacoustical Observations, SIBEX, December 1983/January 1984," *Polar Biol.*, **8**, 17-22.

C.H. Greene, T.K. Stanton, P.H. Wiebe, and S. McClatchie, **1991**, "Acoustic estimates of Antarctic krill," *Nature*, **349**: 110.

C.F. Greenlaw, R.K. Johnson, and T. Pommeranz, **1980**, "Volume scattering strength predictions for Antarctic krill (*Euphausia superba* Dana)," Meeresforsch **28**, 48-55.

C.F. Greenlaw and R.K. Johnson, 1983, "Multiple-frequency acoustical estimation," *Biol. Ocean.*, **2**, 227-252.

R.P. Hewitt and D.A. Demer, **1991**, "Krill Abundance," *Nature*, **353**, 310.

R.P. Hewitt and D.A. Demer, **1993**, "Dispersion and abundance of Antarctic krill in the vicinity of Elephant Island in the 1992 austral summer," *Mar. Ecol. Prog. Ser.*, **99**, 29-39.

R.P. Hewitt and D.A. Demer, **1994**, "Acoustic estimates of krill biomass in the Elephant Island area: 1981-1993," *CCAMLR Science*.

D.V. Holliday, R.E. Pieper, and G.S. Kleppel, **1989**, "Determination of zooplankton size and distribution with multifrequency acoustic technology," *Jour. Mar. Sci.*, **46** (1), 52-61.

K.A. Johannesson and R.B. Mitson, **1983**, "Fisheries Acoustics: A practical manual for aquatic biomass estimation," FAO Tech. Paper, **240**, 249 pp.

G.M. Jolly and I. Hampton, **1990**, "A stratified random transect design for acoustic surveys of fish stocks," *Can. J. Fish. Aquat. Sci.* **47**, 1282-1291.

U. Kils, **1981**, "The swimming behavior, swimming performance and energy balance of Antarctic krill, *Euphausia superba*", BIOMASS Scientific Series No. 3, 122 pp.

L.S.P. Madureira, P. Ward and A. Atkinson, **1993**, “Differences in backscattering strength determined at 120 and 38 kHz for three species of Antarctic macroplankton,” *Mar. Ecol. Prog. Ser.* **93**, 17-24.

K.V. Mackenzie, **1981**, "Nine-term equation for sound speed in the oceans," *J. Acoust. Soc. Am.,* 70:807-812.

D.N. MacLennan and S.T. Forbes, **1984**, “Fisheries acoustics: a review of general principles,” ICES Res. Rep. **184**, 7-18.

D.N. MacLennan and S.T. Forbes, **1986**, “Acoustic methods of fish stock estimation,” in *Developments in Fisheries Research in Scotland*, R.S. Bailey and B.B. Parrish eds., Fishing News (Books), London.

D.N. MacLennan, and E.J. Simmonds, **1992**, *Fisheries Acoustics*. Chapman and Hill, London, 325 pp.

D.E. Mcgehee, R.L. O'Driscoll and L.V. Martin Traykovski, **1998**, "Effects of orientation on acoustic scattering from Antarctic krill at 120 kHz," *Deep-Sea Res. II,* **45**(7):1273-1294.

D.G.M. Miller and I. Hampton, **1989**, “Biology and ecology of the Antarctic krill,” Biomass Scientific Series **9**, 1-166.

E.J. Simmonds, **1984a**, “A comparison between measured and theoretical equivalent beam angles for seven similar transducers,” *J. Sound and Vibration*, **97**:117-128.

E.J. Simmonds, **1984b**, “The effect of mounting on the equivalent beam angles of acoustic survey transducers,” ICES CM1984/B:32, 5pp.

E. J. Simmonds, N.J. Williamson, F. Gerlotto, and A. Aglen, **1992**, “Acoustic Survey Design and Analysis Procedures: A Comprehensive Review of Good Practice,” ICES Res. Rep. **187**, 1-127.

Simrad, **1993**, “Simrad EK500 Scientific Echo Sounder Instruction Manual”, Simrad Subsea A/S, Horten, Norway.

T.K. Stanton, **1989a**, “Sound scattering by cylinders of finite length. III. Deformed cylinders,” *J. Acoust. Soc. Am.* **86**(2), 691-705.

T.K. Stanton, **1989b**, “Simple approximate formulas for backscattering of sound by spherical and elongated objects,” *J. Acoust. Soc. Am.* **86**, 1499-1510.

T.K. Stanton, D. Chu, P.H. Wiebe, and C.S. Clay, **1993**, “Average echoes from randomly oriented random-length finite cylinders: Zooplankton models,” *J. Acoust. Soc. Am.* **94**(6), 3463-3472.

T.K. Stanton, P.H. Wiebe, D. Chu, M.C. Benfield, L. Scanlon, LV. Martin, and R.L. Eastwood, **1995**, “On acoustic estimates of zooplankton biomass,” *ICES, J. Mar. Sci.*.

B.N. Taylor and C.E. Kuyatt, **1993**, “Guidelines for evaluating and expressing the uncertainty of NIST measurement results,” NIST Tech. Note 1297, U.S. DOC, 15pp.

W.D. Tesler, **1989**, “Bias and precision in acoustic biomass estimation,” *Proc. Inst. Acoust.*, **11**(3), 202-211.

P.N. Trathan, J.L. Watkins, A.W. A. Murray, A. Brierley, D..A. Demer, I. Everson, C. Goss, S. Hedley, R. Hewitt, S. Kawaguchi, S. Kim, M. Nagnaobu, T. Pauly, J. Priddle, K. Reid, and P. Ward, **1999**, "The CCAMLR 2000 Krill Synoptic Survey: a description of the rationale and design", WG-EMM-99/39, 25 pp.

J.L. Watkins and A.S. Brierley, **submitted**, "Verification of acoustic techniques used to identify Antarctic krill," *Jour. Mar. Sci.*

H. Weinberg, **1971**, "A Continuous-gradient curve-fitting technique for acoustic-ray analysis," *J. Acoust. Soc. Am.* **50**(3), 975-984.

P.H. Wiebe, C.H. Greene, T.K. Stanton, and J. Burczynski, **1990**, "Sound scattering by live zooplankton and micronekton: empirical studies with a dual-beam acoustical system," *J. Acoust. Soc. Am.* **88**(5), 2346-2360.

VIII. Table Captions

Table 1. Average sound speed and absorption values calculated both pre- and post-cruise from data collected at the 1998/99 and CCAMLR 2000 stations shown in **Figure 1**, respectively**.** Averages were calculated over the ranges of 10 to 250 and 10 to 500 m. Also, weighted-means (weight=1/range^2) were calculated for the 10 to 500 m ranges. These latter pre-cruise values of $\bar{c}$ and $\bar{\alpha}$ (shaded) were used for the entirety of CCAMLR 2000. Note that the post-cruise weighted-mean values, and the more accurate harmonic mean values (bold) are similar to each other, and higher than the survey constants by approximately one standard deviations ( ).

Table 2. Uncertainty in generic echo integration surveys for aquatic biomass estimation. The magnitudes of systematic and random sources of error were estimated by Tesler (1989) and MacLennan and Simmonds (1992). Some categories were not explicitly considered by the authors (*) and some effects were considered negligible (-).

Table 3. Parameters for determining detection probabilities versus range for each ship and frequency. G_{Sv} is the on-axis system gain, P_n is the ambient noise power, P_t is the transmit power, and ψ is the equivalent 2-way beam angle. Other parameters were common to all ships.

IX. Tables

Table 1:

Summary	Temp (deg.C)	Salinity (psu)	$\bar{c}$ (m/s)	$\bar{\alpha}_{38\,kHz}$ (dB/km)	$\bar{\alpha}_{120\,kHz}$ (dB/km)	$\bar{\alpha}_{200\,kHz}$ (dB/km)
Pre-cruise 10-250 m average	0.5	34.1	1452	10	26	40
Pre-cruise 10-500 m average	1.1	34.3	1457	10	27	40
Pre-cruise 10-500 m weighted average	0.4	33.8	1449	10	26	40
Post-cruise 10-500 m weighted average	1.9 (1.2)	34.0 (0.2)	1456 (5.0)	10.4 (0.1)	27.9 (1.2)	41.4 (1.0)
Post-cruise weighted harmonic average	**1.4 (1.2)**	**34.0 (0.2)**	**1456 (5.1)**	**10.4 (0.1)**	**27.7 (1.2)**	**41.3 (1.0)**

Table 2.

	Tesler (1989)		MacLennan and Simmonds (1992)	
Source of error	**Random**	**Systematic**	**Random**	**Systematic**
Physical calibration	-	±0.5 to ±1.0 dB	±0.1 dB	±0.3 dB
Transducer motion	-0.1 dB	-	-	0 to -1.1 dB
Bubble attenuation	-	-0.5 dB	-	0 to -2.8 dB
Hydrographic conditions	*	*	±0.1 to ±0.2 dB	0 to ±0.2 dB
Target strength	-	±1.0 to ±1.5 dB	±0.2 dB	0 to ±1.8 dB
Species identification	*	*	-	0 to ±2.6 dB
Random sampling	*	*	±0.4 to ±1.5 dB	-
Fish migration	*	*	-	0 to ± 1.5 dB
Diurnal behavior	*	*	0 to -1.0 dB	-
Avoidance reactions	*	*	-	uncertain
Integrator error	±0.2 dB	-	*	*
Attenuation coefficient	-	±0.2 dB	*	*
Time-varied gain	-	±0.4 dB	*	*
Equivalent beam angle	-0.6 to -0.8 dB	-	*	*

Table 3.

Ship	Atlantida	James Clark Ross	Kaiyo Maru	Yuzhmorgeologiya
G_{Sv} 38 kHz (dB)	23.32	25.51	27.06	22.36
G_{Sv} 120 kHz (dB)	24.49	20.20	24.74	25.26
G_{Sv} 200 kHz (dB)	23.26	22.91	25.76	25.96
P_n 38 kHz (dB re 1W)	-112.0	-142.0	-140.0	-133.0
P_n 120 kHz (dB re 1W)	-141.0	-132.5	-146.0	-145.0
P_n 200 kHz (dB re 1W)	-146.0	-136.0	-143.0	-148.0
P_t 38 kHz (kW)	2	2	2	1
P_t 120 kHz (kW)	1	1	1	1
P_t 200 kHz (kW)	1	1	1	1
ψ 38 kHz (dB)	-21.3	-20.8	-20.9	-15.9
ψ 120 kHz (dB)	-21.0	-18.4	-20.6	-20.4
ψ 200 kHz (dB)	-20.3	-20.8	-20.5	-20.5

X. Figure Captions

Figure 1. Locations of 11 stations sampled for salinity (*S*) and temperature (*T*) versus depth (*r*) by the UK and the USA during their 1998/99 field seasons (white dots), and 140 stations sampled for *S* and *T* versus *r* by the UK, Japan, and USA during CCAMLR 2000 (black dots).

Figure 2. Temperature (*T*), salinity (*S*), and harmonic means of sound speed and absorption (α) at each survey frequency, averaged with a Rayleigh weighting-factor ($\mathcal{R}$(*r*,40m)) and plotted for each of 140 stations.

Figure 3. Predicted target strengths (*TS*; dB) calculated from the DWBA model (McGehee *et al.*, 1998), using a generic krill shape, g=1.0357; and h=1.0279.

Figure 4. Mean krill *TS* (-)and ± 2 std (--) as predicted by the DWBA model for variable krill lengths (*L*=20 to 55 mm) and incidence angle (0-179º) for acoustical frequencies of 38, 120 and 200 kHz.

Figure 5. *TS* distributions estimated for each length-frequency distribution (bars), using the DWBA and a very narrow distribution of angles about normal incidence (N(90°,3°)). For comparison, also plotted are the *TS* distributions estimated from the Greene *et al.* (1991) model using the same length-frequency distributions (lines).

Figure 6. Volume backscattering strengths (*Sv*) calculated from the DWBA model (McGehee *et al.*, 1998; generic shape; *g*=1.0357; *h*=1.0279; density = N(600m^3, 150m^3); and Kils' (1981) orientation distribution =N(45.3˚, 30.4˚)).

Figure 7. *Sv* differences calculated from the DWBA model (McGehee *et al.*, 1998; generic shape; *g*=1.0357; *h*=1.0279; density = N(600m^3, 150m^3); and Kils' (1981) orientation distribution =N(45.3˚, 30.4˚)), for all three krill length clusters.

Figure 8. Total integrated volume backscattering coefficients (s_a) normalized to the observation effort (**a**); total s_a versus depth for the survey (**b**); and distributions of mean (left) and maximum (right) volume backscattering strengths (*Sv*) for the krill detected during CCAMLR 2000 survey (averaged over interval size; **c and d**).

Figure 9. Signal-to-noise ratio (SNR; dB) versus range for research vessels *Atlantida* (**-**), *James Clark Ross* (-.), *Kaiyo Maru* (..), and *Yuzhmorgeologiya* (--), at *Sv*=-70 dB for 38 kHz (**a**) and *Sv*=-60 dB for 120 and 200 kHz (**b** and **c**). See **Table 3** for background noise levels and other parameters used.

XI. Figures

Figure 1.

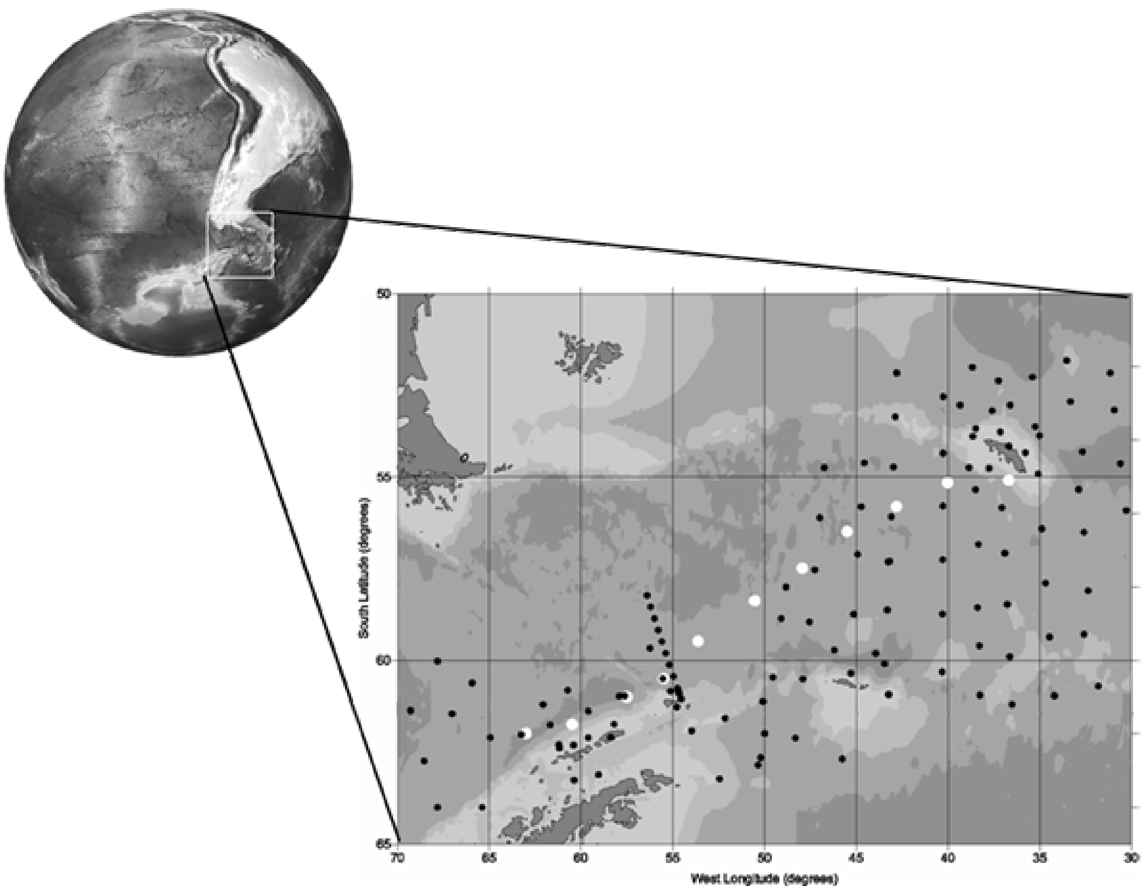


Figure 2.

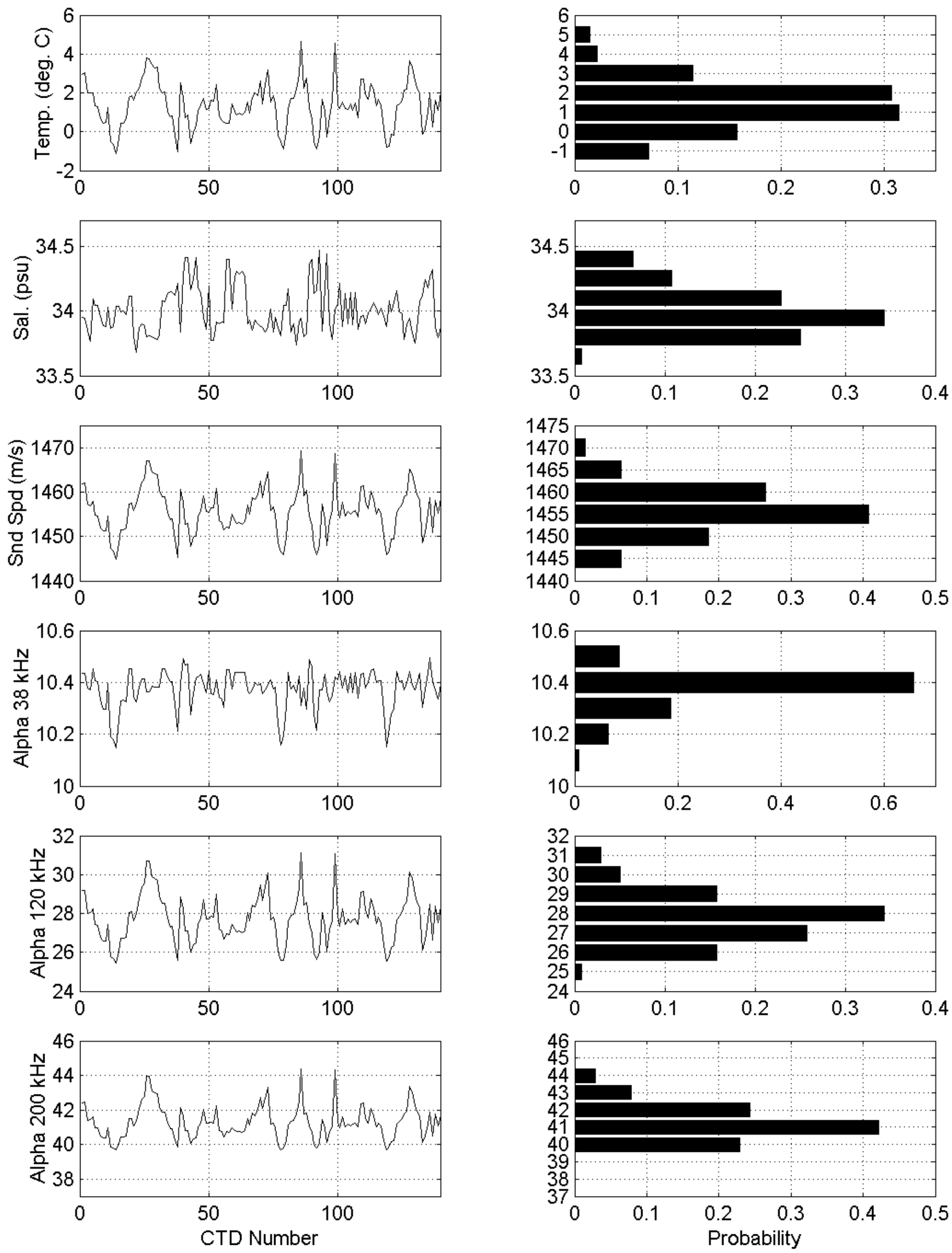

Temp. (deg. C)
Sal. (psu)
Snd Spd (m/s)
Alpha 38 kHz
Alpha 120 kHz
Alpha 200 kHz
CTD Number
Probability

Figure 3.

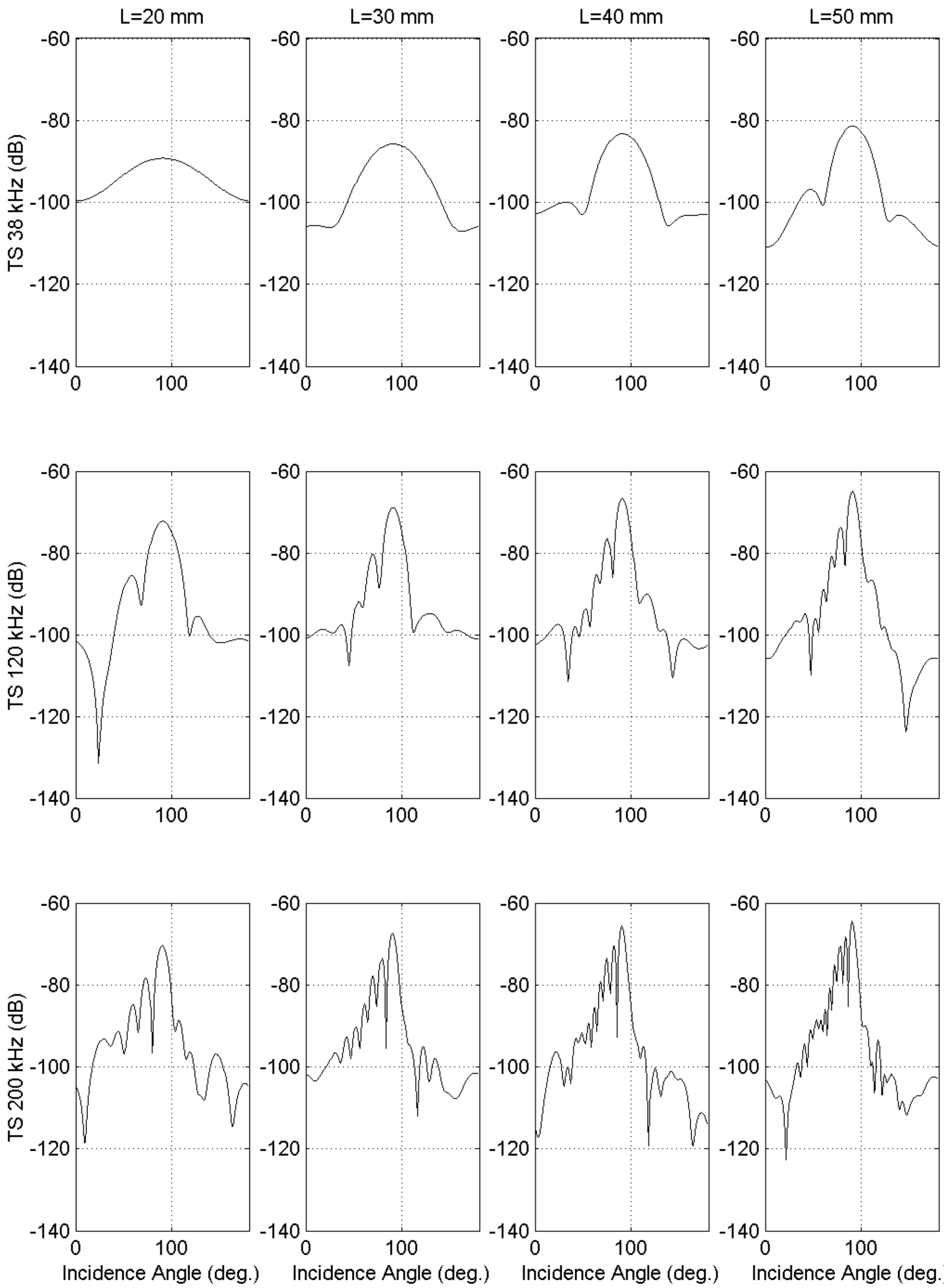

L=20 mm
L=30 mm
L=40 mm
L=50 mm
TS 38 kHz (dB)
TS 120 kHz (dB)
TS 200 kHz (dB)
-60
-80
-100
-120
-140
0
100
Incidence Angle (deg.)

Figure 4.

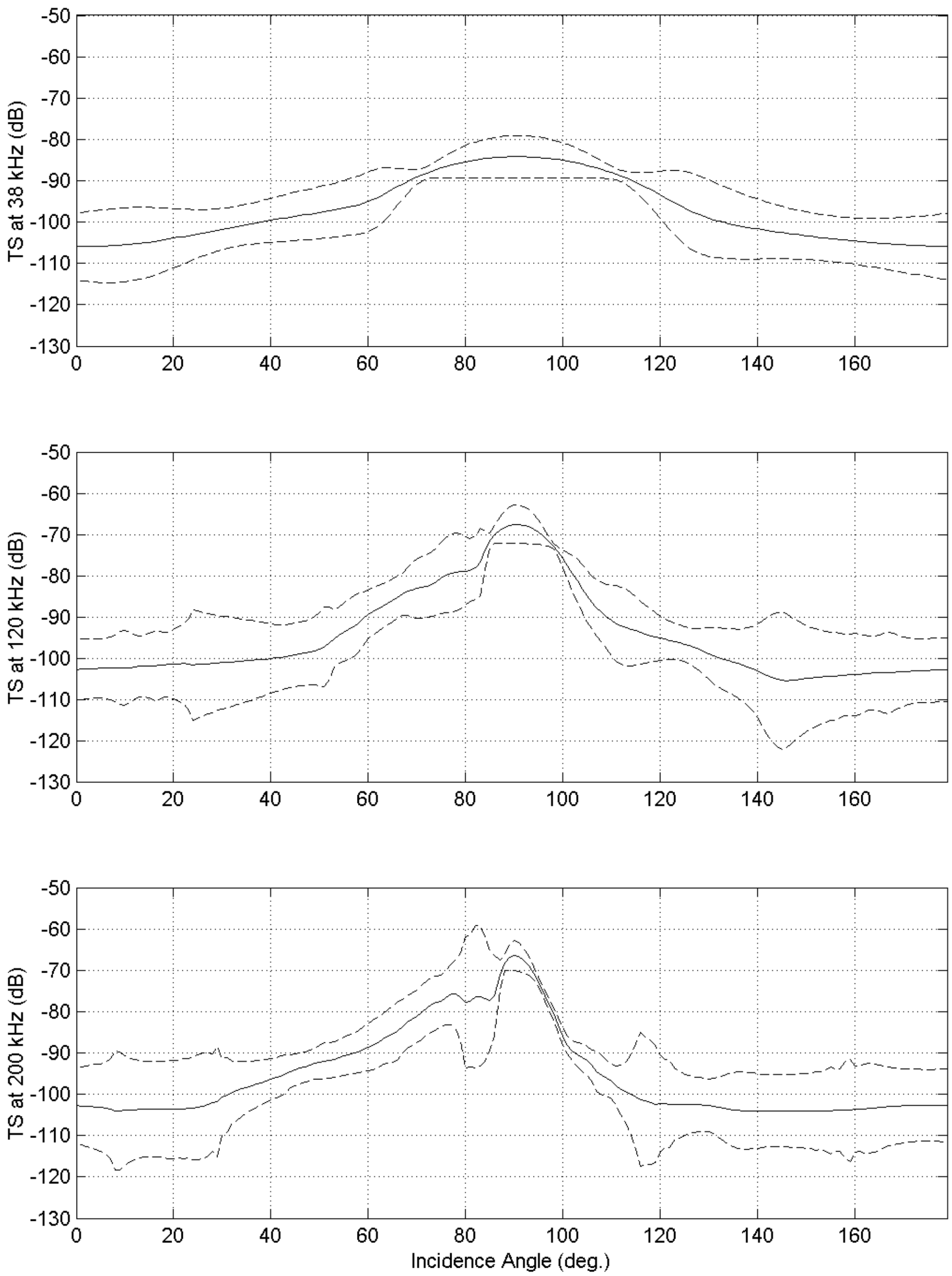
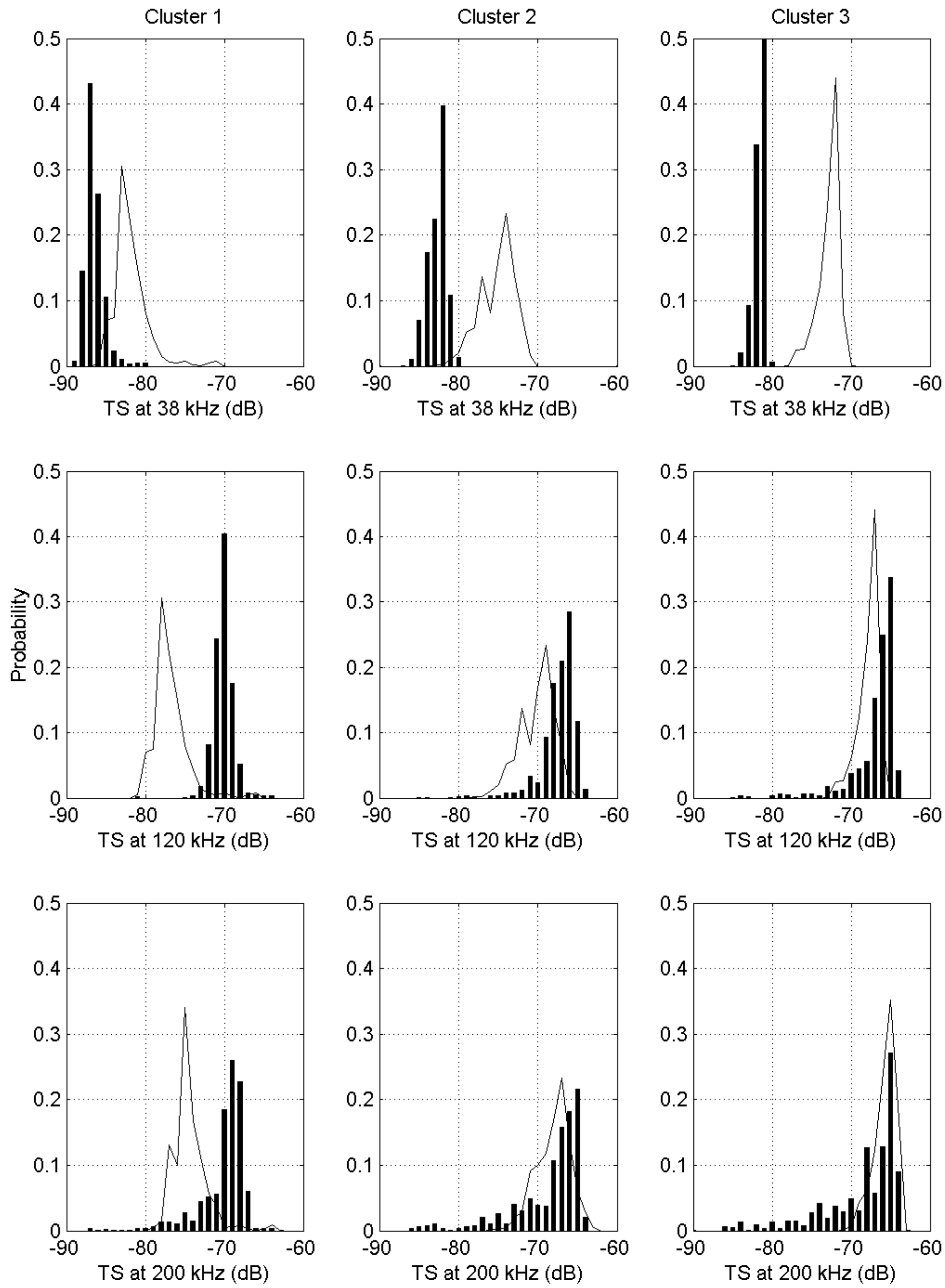
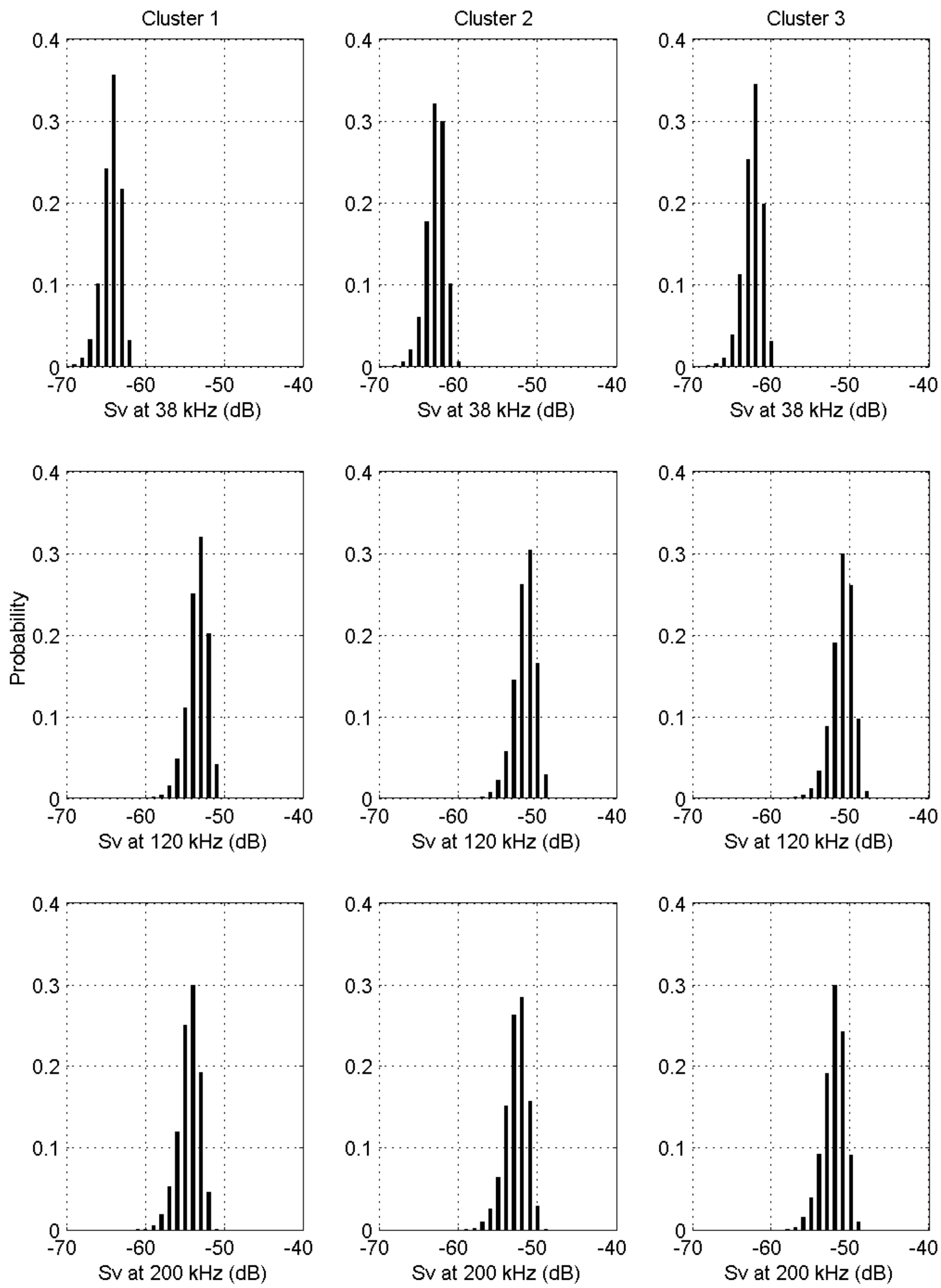

TS at 38 kHz (dB)
TS at 120 kHz (dB)
TS at 200 kHz (dB)
-50
-60
-70
-80
-90
-100
-110
-120
-130
0
20
40
60
80
100
120
140
160
Incidence Angle (deg.)

Figure 5.



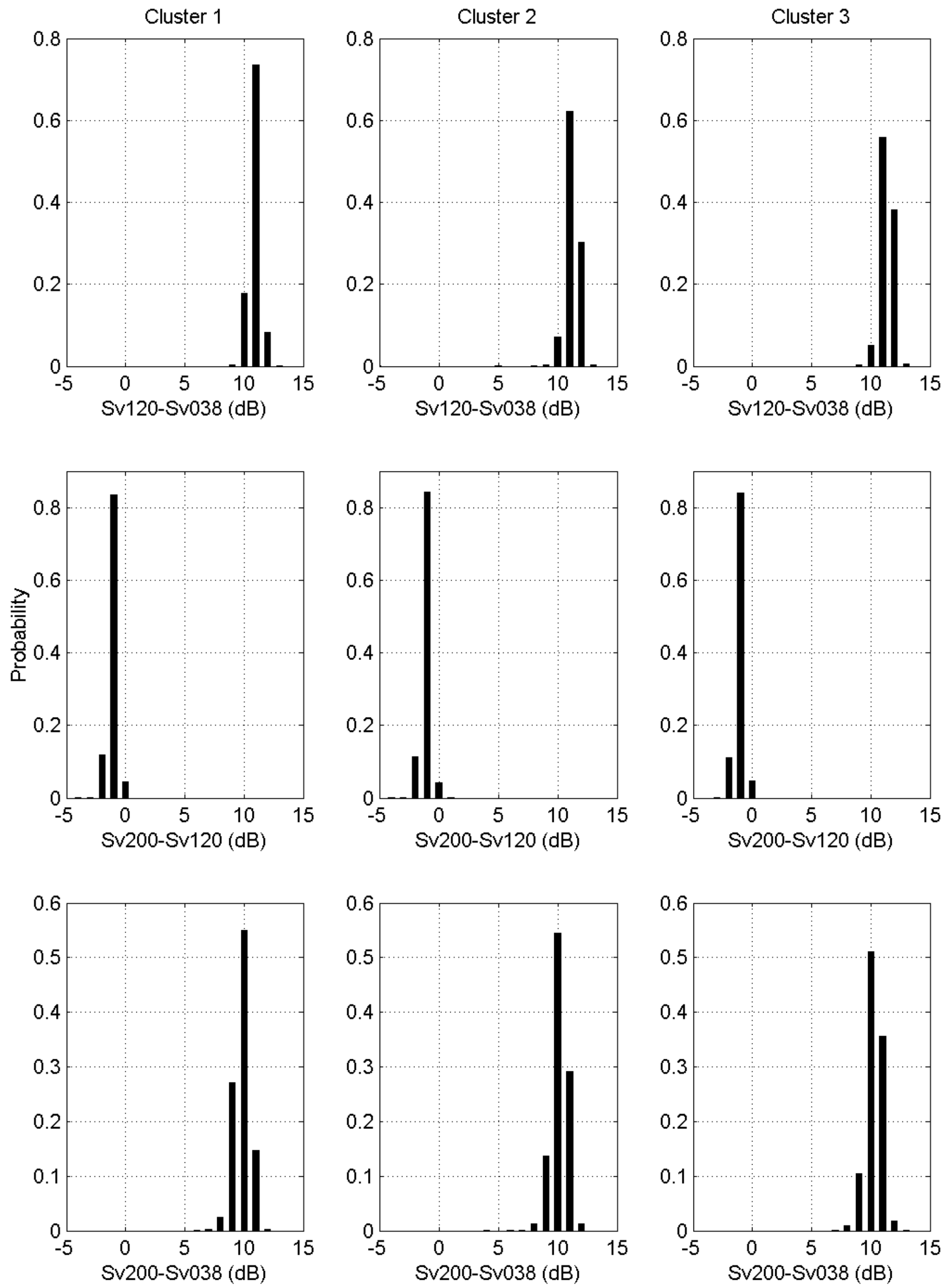
Cluster 1
Cluster 2
Cluster 3
Probability
TS at 38 kHz (dB)
TS at 38 kHz (dB)
TS at 38 kHz (dB)
TS at 120 kHz (dB)
TS at 120 kHz (dB)
TS at 120 kHz (dB)
TS at 200 kHz (dB)
TS at 200 kHz (dB)
TS at 200 kHz (dB)

Figure 6.



Cluster 1
Cluster 2
Cluster 3
Probability
Sv at 38 kHz (dB)
Sv at 120 kHz (dB)
Sv at 200 kHz (dB)
0.4
0.3
0.2
0.1
0
-70
-60
-50
-40

Figure 7.



Cluster 1
Cluster 2
Cluster 3
Probability
Sv120-Sv038 (dB)
Sv200-Sv120 (dB)
Sv200-Sv038 (dB)

Figure 8.

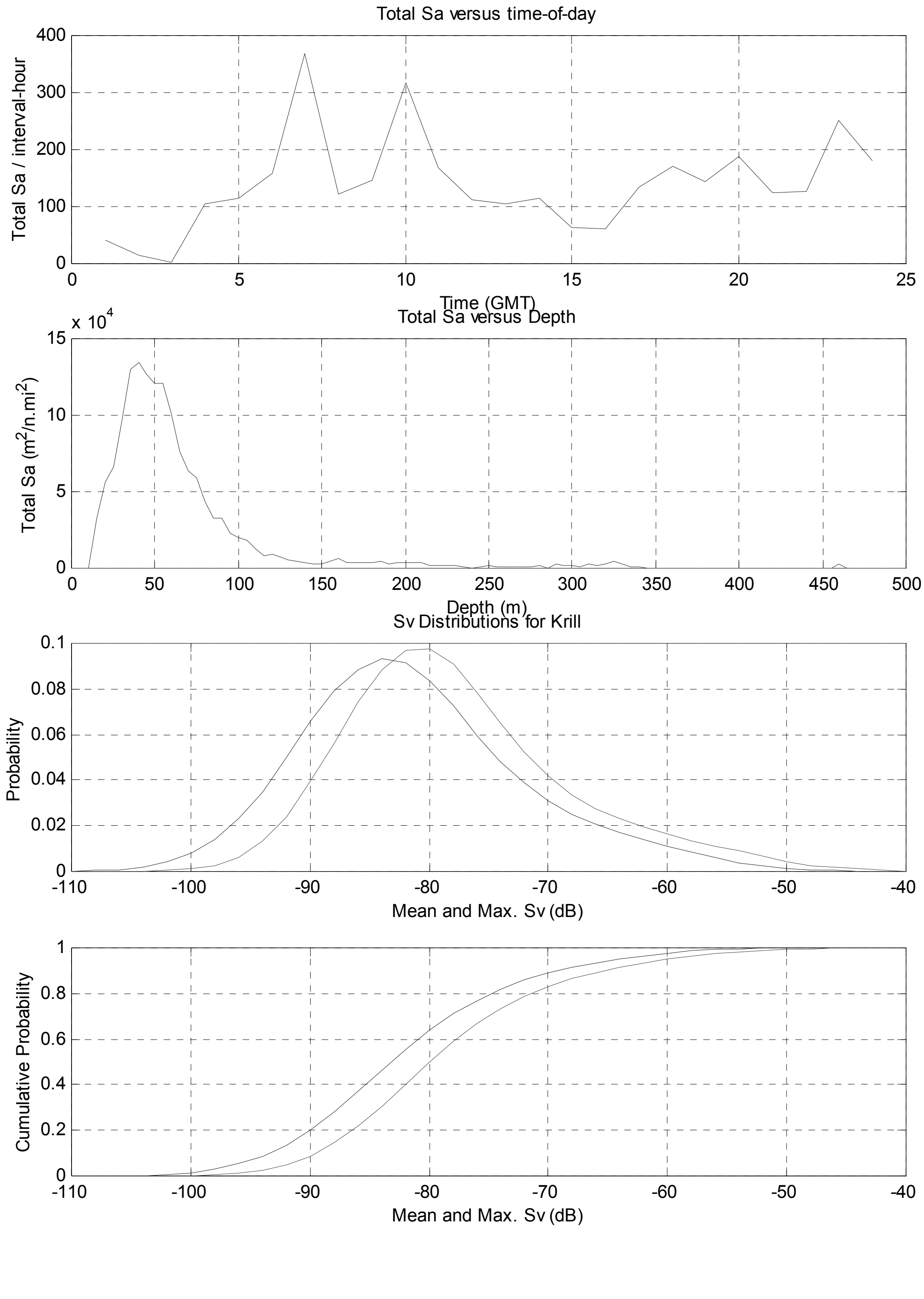
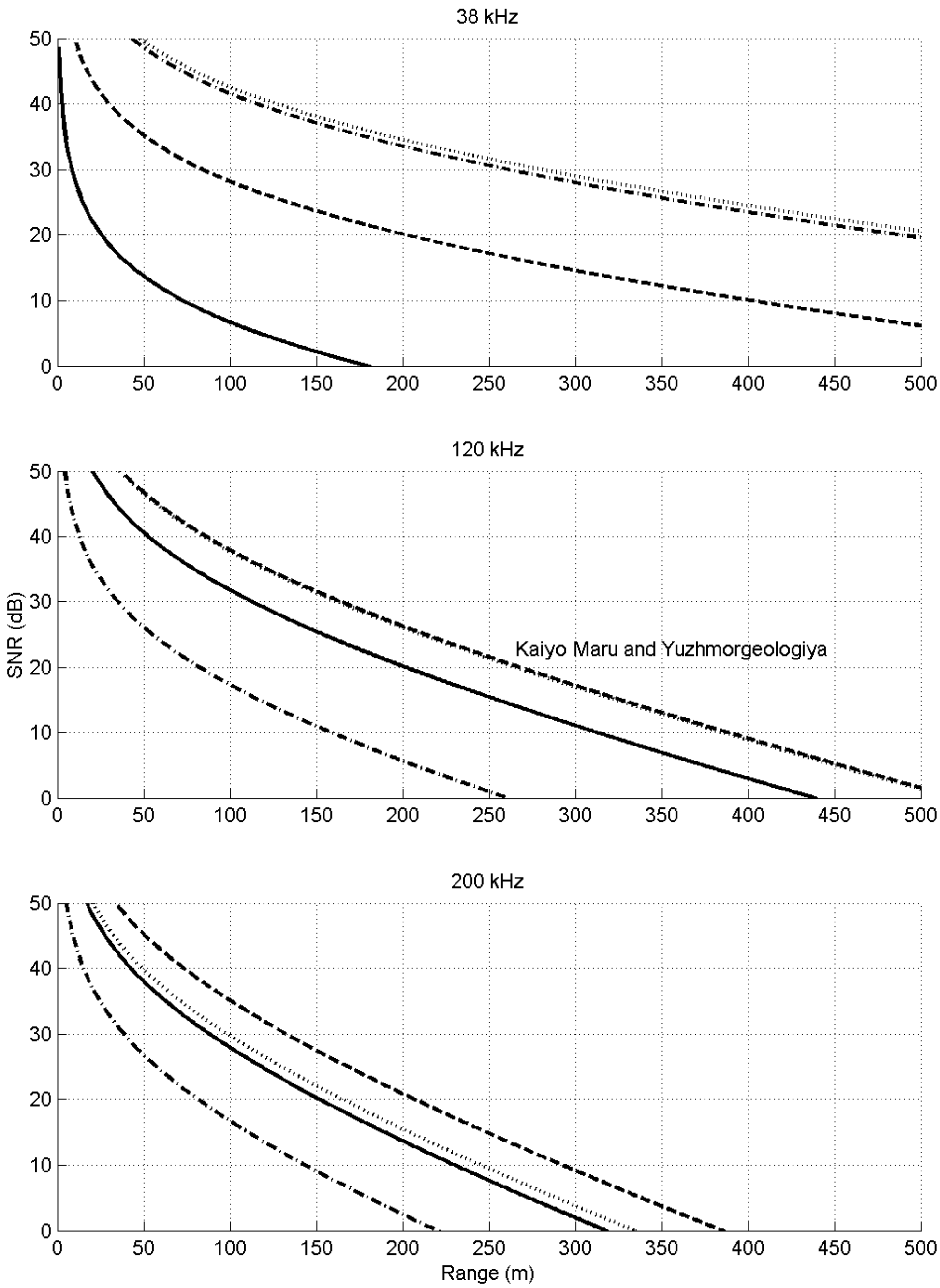

Total Sa versus time-of-day
Total Sa / interval-hour
Time (GMT)
Total Sa versus Depth
x 10^4
Total Sa (m^2/n.mi^2)
Depth (m)
Sv Distributions for Krill
Probability
Mean and Max. Sv (dB)
Cumulative Probability
Mean and Max. Sv (dB)

Figure 9.



38 kHz
120 kHz
Kaiyo Maru and Yuzhmorgeologiya
200 kHz
SNR (dB)
Range (m)
0
50
100
150
200
250
300
350
400
450
500
10
20
30
40