Verification of the acoustic techniques used to identify Antarctic krill

J. L. Watkins and A. S. Brierley

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Acoustic surveys to estimate krill biomass require that the sound backscattered by krill can be identified and distinguished from all other types of backscatter. Sampling acoustic targets with nets to verify their identity have achieved this traditionally. More recently backscattered sound has been partitioned into krill or non-krill groups using differences in mean volume backscattering strength (MVBS) at two acoustic frequencies ($\Delta MVBS = MVBS_{120} - MVBS_{38}$; where $\Delta MVBS$ between 2 and 12 dB indicated krill). Here we compare net and acoustic data from two cruises around South Georgia in 1996 to assess the reliability of acoustic-based, target-identification techniques. MVBS data at 120 and 38 kHz were collected with a Simrad EK500 echosounder and net samples were collected with an RMT8. Around 80% of the echo integration cells from targets believed to be krill on the basis of their appearance on echo-charts, were also identified as krill from their difference in backscatter at 38 and 120 kHz. Krill biomass estimated from acoustic targets identified using echo-chart appearance or Δ MVBS were broadly similar (regression: Δ MVBS=0.94 visual classification, $r^2=0.99$). Krill size was calculated from scattering models using the two frequency data and compared with that obtained in net hauls. This comparison revealed that a simplified bent-cylinder model was a better predictor of krill length than a fluid-filled, sphere model. We conclude that use of $\Delta MVBS$ to identify Antarctic krill is advantageous because it is more objective than using echo-chart appearance.

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Introduction

Acoustic surveys to estimate standing-stock biomass require that the species of interest may be reliably identified and that sound backscattered by those animals can be separated from backscattering caused by other sources. Traditionally, target identification net hauls are carried out during surveys to provide information on the size or species composition or both these aspects of the backscattering sources. However, net hauls are relatively time consuming and so it is impractical to obtain net samples from every target detected by the echosounder. It is, therefore, necessary to strike a balance between the number of net hauls needed to ensure accurate target identification and the time spent on acoustic transects. If information derived predominantly from the echosounder could be used to identify target species then the number of net hauls could be reduced. This would decrease the overall survey time or make the survey more comprehensive through the increased time available for acoustic transects.

In the case of Antarctic krill, *Euphausia superba* Dana, there has been considerable development in acoustic identification techniques. The tendency of krill to aggregate in swarms often produces characteristically compact, dense marks on single frequency echosounders and these marks have enabled subjective visual identification of krill targets (see for instance Madureira *et al.*, 1993a; Murray *et al.*, 1995). However, krill also occur in more irregular aggregations and layers (Kalinowski and Witek, 1985; Watkins and Murray, 1998). Within such irregular aggregations it is less easy to distinguish krill from other scattering organisms, which may range from squid and myctophid fish through to various macrozooplankton assemblages comprising other euphausiids, amphipods or salps (Brierley et al., 1998b). More recently multi-frequency techniques have been used to partition acoustic backscatter into putative biological categories to overcome such problems. Madureira et al. (1993a, b) found that the difference between backscatter at 120 and 38 kHz ($\Delta MVBS = MVBS_{120} - MVBS_{38}$) could be used to separate several species of euphausiid and amphipod. Brierley and Watkins (1996) and Brierley et al. (1997) used this technique in making estimates of krill biomass around South Georgia. Modifications of the technique described by Madureira et al. (1993a, b) to partition acoustic backscatter into krill and non-krill fractions are also being developed (Azzali et al., 1996; Brierley et al., 1997; Goss and Everson, 1996; Hewitt et al., 1996; Ichii et al., 1996; Kasatkina et al., 1996; Pauly et al., 1996). There is as yet no consensus on the most appropriate technique and, as a result, biomass estimates determined by different groups are not always directly comparable.

From the results of field sampling around South Georgia in 1986 and 1991, Madureira et al. (1993b) considered that AMVBS values between 2 and 12 dB were indicative of Antarctic krill. The krill sampled in these two years varied considerably in size: in 1986 the mean length was very large (54.2 mm) but in 1991 it was substantially smaller with a mean length of 38.7 mm. A number of different sound-scattering models have demonstrated a significant relationship between length and target strength (TS) of krill, and this relationship is dependent also upon the insonifying frequency (see for instance Stanton, 1989; Chu et al., 1993). Developments based upon these findings have used the difference in scattering at two frequencies to predict krill length (Mitson *et al.*, 1996). However, the average Δ MVBS values derived from the two years (1986, 1991) sampled by Madureira et al. (1993b), 5.1 dB (s.e. ± 0.39) and 4.6 dB (s.e. \pm 0.43), show only a small change with changing krill length and the direction of change (increasing Δ MVBS with increasing length) was not consistent with that predicted by modelling (cf. Stanton et al., 1994). Thus, while the Δ MVBS technique appears to offer great potential for target identification, there is still a requirement for net data to verify target identification based on dual-frequency acoustics.

In this paper we compare the information derived acoustically with that derived from precisely targeted net hauls in order to assess the functionality of acoustic target-identification techniques. In particular we

- (i) compare targets classified on the basis of echo-chart visual appearance and $\Delta MVBS$
- (ii) examine the distribution of Δ MVBS values>12 dB which are associated with aggregations of krill

- (iii) compare biomass estimates for krill identified by $\Delta MVBS$ and echo-chart visual appearance and
- (iv) compare krill lengths obtained in net samples with those calculated from scattering models.

Materials and methods

Net sampling

During two cruises (JR11, JR17) of RRS "James Clark Ross" to South Georgia in 1996 net samples were collected using a three-net multiple RMT8 (Roe and Shale, 1979) with a mouth opening of 8 m². During each cruise samples were taken in two survey areas off the eastern and western ends of the island (Brierley et al., 1997b). The net was towed at $1-1.3 \text{ m s}^{-1}$ and the volume filtered was calculated from the flowmeter data using the formulae of Pommeranz et al. (1982). Two different sampling strategies were used for net sampling. First, at two stations each day during the hours of darkness, 1-h, double-oblique tows to investigate the macrozooplankton community structure were carried out from just below the surface to 250 m (or within 15 m of the bottom if the bottom depth was less than 250 m). Such net samples are referred to as "station hauls" (Table 1). Second, up to two targeted hauls each day were directed specifically at dominant acoustic targets encountered anywhere within the upper 250 m of the water column. Such samples are referred to as "target hauls". Net catches were sorted immediately after each haul. The total volume of each net catch and the volumes of all the major taxonomic groups were measured. A sample of 100 krill was taken from each net where available. The total length of each krill was measured from the anterior edge of the eye to the tip of the telson and the length rounded to the nearest mm below. Krill maturity stages were assessed using the classification of Makarov and Denys (1981) and the nomenclature of Morris et al. (1988).

Acoustic sampling

During each net haul acoustic data were collected using a fully calibrated Simrad EK500 echosounder (software version 4.01) with hull-mounted, split beam 38 and 120 kHz transducers and a single beam 200 kHz transducer. A ping rate of 1 ping every 2.5 s was used throughout. Mean volume backscattering strength (MVBS) data were integrated for each frequency over 2 m depth intervals from 2 to 250 m below the transducers (transducer 6 m below the sea surface) for time intervals of 100 s. This corresponded to a horizontal distance (Elementary Sampling Distance Unit: ESDU) of ~125 m during net hauls.

Table 1. The RMT8 net hauls used to validate the acoustic identification of krill in January 1996 (JR11) and December 1996 (JR17). S and T identify station or target hauls respectively. Target types found on the echo-chart during each haul are given, along with a subjective assessment of the likelihood that the target was caught by the net (targets close (Y) or well off (N) estimated net trajectory).

Cruise	Event	Net number	Haul type	Krill density (g m ⁻³)	% krill in catch	Target type and likelihood of being sampled in net
JR11	136	1	S	0.02	21.5	Discrete – N
JR11	139	1	Т	3.89	96.6	Irregular – Y
JR11	146	1	S	0.39	99.2	Discrete – Y
JR11	152	1	S	0.06	19.5	Irregular – Y Discrete – N
JR11	155	1	Т	0.08	26.0	Diffuse – Y
JR11	163	1	S	0.24	57.8	Discrete – Y
JR11	177	2	Т	0.39	90.0	Irregular – Y Diffuse – Y
JR11	184	1	Т	22.20	99.9	Discrete – Y
JR11	184	2	Т	11.50	99.9	Discrete – Y
JR11	184	3	Т	2.27	99.0	Discrete – Y
JR11	193	2	S	0.25	90.0	Discrete – Y Diffuse – N
JR11	206	1	S	0.24	98.5	Irregular – Y
JR11	207	1	S	< 0.01	<1.0	Discrete – N
JR11	215	1	S	< 0.01	4.0	Discrete – N Diffuse – N
JR11	294	1	Т	6.57	99.7	Irregular – Y Diffuse - Y
JR11	294	2	Т	5.06	99.7	Irregular – Y Diffuse - Y
JR11	299	3	Т	13.04	99.6	Discrete – Y
JR17	243	1	Т	0.51	99.9	Discrete – Y
JR17	243	2	Т	< 0.01	65.5	No obvious marks
JR17	257	1	S	0.56	98.0	Irregular – Y
JR17	257	2	S	< 0.01	15.8	No obvious marks
JR17	279	1	S	0.37	93.3	Diffuse – Y Irregular – N
JR17	279	3	S	1.00	98.4	Discrete – Y
JR17	288	1	Т	0.28	67.0	Irregular – Y
JR17	288	2	Т	0.21	70.0	Irregular – Y
JR17	288	3	Т	0.92	97.6	Irregular – Y

Data processing

Following the examples of Madureira *et al.* (1993a) and Murray *et al.* (1995), a visual classification of the acoustic targets on the 120 kHz echo-charts was carried out. Acoustic targets were identified as

- (a) discrete aggregations which were assumed to be almost exclusively krill
- (b) irregular aggregations (see also Kalinowski and Witek, 1985) which were considered likely to be krill but which may also have contained other organisms, and
- (c) diffuse targets which were much less dense than those classified as discrete and were assumed to contain mainly zooplankton other than krill.

Acoustic data were visualized using a custom-written analysis system (Socha *et al.*, 1996) based on AVS (Upson *et al.*, 1990). Final calibration correction, flagging spurious data values, marking different types of target and the application of a TVG-based, noisecompensation algorithm (Watkins and Brierley, 1996) were undertaken with this system. Within the AVS system, a minimum $MVBS_{120}$ threshold value of -70 dB was applied to the integrated data from the discrete and irregular aggregations to allow rapid delineation of the edges of aggregations. Typical target types are illustrated in Figure 1.

Krill biomass was calculated from acoustic data within AVS using a custom-designed module that allowed the selection of data on the basis of marks, acoustic threshold or dB difference or both the latter two factors (Socha *et al.*, 1996). The target strength (TS) values of -39.13, -39.03 dB kg⁻¹ (January 1996) and -38.89, -38.59 dB kg⁻¹ (December 1996) used in the calculations of biomass were determined from the weighted length frequency of krill within the eastern and western South Georgia survey areas respectively (see Brierley *et al.*, 1997, 1999 for further details).

For each net haul the trajectory of the net was calculated by trigonometry using the wire-out and net depth to determine the time delay of the net behind the echosounder transducers. This trajectory was then superimposed onto the echo-chart so that the acoustic targets potentially sampled by the net could be identified.



Figure 1. Echo-chart (120 kHz) showing classification of target types.

The mean length of krill within the acoustic targets was estimated from the dB difference between 120 and 38 kHz using two basic models:

- (i) the Johnson highpass version of the fluid-sphere model described by Greenlaw (1979) and by Mitson *et al.* (1996), and
- (ii) the bent-cylinder model as described by Stanton *et al.* (1993).

In the former, an equivalent spherical radius is derived and converted to a krill length using a regression relationship derived by Greenlaw (1977).

To refine the relationship for Antarctic krill, *Euphausia superba*, the relationship of length to mass given by Morris *et al.* (1988) was used viz.

WW= $3.86 \times 10^{-6} \times L^{3.2}$

where WW is wet mass (g) and L is total length (mm).

WW was converted to volume using a density of 1.065 g cm^{-3} (Foote, 1990) which was derived for krill caught around South Georgia in 1988. A sound-speed contrast of 1.010 and a density contrast of 1.044 (Greenlaw, 1977) were used in all model calculations.

Results and discussion

Net catches

In general where discrete or irregular aggregations were fished, net catches were high (Table 1). The proportion of krill by volume found in the hauls where discrete targets were sampled was generally >90%; the one exception was haul JR11E163 where nearly 40% of the catch comprised salps and other zooplankton. The proportion of krill found in irregular targets was also high (from 67–99%). In contrast where only diffuse targets were seen on the echo-chart or where there were no visible targets, the total density and proportion of krill in the catches were low. We therefore conclude that, in this study at least,

- (a) discrete targets are most likely to be krill,
- (b) irregular targets are likely to contain a high proportion of krill, and
- (c) that few krill occur outside the dense aggregations.

The identity of diffuse targets remains much less certain but, in the one case where only diffuse targets were

Table 2. The classification of acoustic integration cells, from all net hauls combined, using visual classification based on appearance on echo chart: (a) for the entire data set where non-missing values were recorded and (b) for all integration cells where $MVBS_{120} > -70 \text{ dB}$

Visual classification	Total no	Cells where Δ MVBS between 2–12 dB					
of aggregations	of cells		Number			%	
Discrete	1399		1014		72	.5	
Irregular	2325		1696		72.9		
Diffuse	6754		5852		86.6		
Surface	2806		835		29.8		
Bottom	10 644		322		3.0		
Entire data set	162 632		80 189		49.3		
(b)							
	Total no	% of cells where Δ MVBS between					
Classification	of cells	2–12 dB	2–13 dB	2–14 dB	2–15 dB	2–16 dB	
Discrete	1395	72.6	78.3	82.4	85.2	87.0	
Irregular	2050	80.1	84.3	88.0	90.2	92.0	
Diffuse	641	89.4	91.6	94.5	95.8	96.3	
Discrete & Irregular	3445	77.1	81.8	85.7	88.3	90.0	
All MVBS ₁₂₀ > -70	6858	58.9					

sampled, the proportion of krill in the catch was low (26%).

Comparison of targets classified as krill by Δ MVBS and through visual assessment of echo-chart characteristics

Here we consider all the echo-integration cells (each equivalent to a 2 m depth bin and a horizontal distance of ~125 m) associated with the "net hauls" described in Table 1. From a total of 162 632 integration cells where valid data at both 120 and 38 kHz were recorded, 49% of cells (80 189) had a Δ MVBS of between 2 and 12 dB. Of these, only 8562 cells (<10%) corresponded with targets that were classified on the basis of their visual appearance as discrete, irregular or diffuse (Table 2a). Moreover, cells that were identified as discrete, irregular or diffuse targets did not always have a Δ MVBS of between 2 and 12 dB though between 72 and 87% of targets assumed to be biological from appearance on the echo chart did (Table 2a).

After thresholding i.e. discarding MVBS₁₂₀ values < -70 dB, the total number of cells in the analysis was reduced to 6858, of which 59% had a Δ MVBS from 2 to 12 dB. Now nearly 80% of the cells with a Δ MVBS between 2 and 12 dB were also classified visually as discrete, irregular or diffuse targets. In contrast, parts of the echo-chart identified as either surface noise or as bottom integration had less than 25% of the cells with a

 Δ MVBS of between 2 and 12 dB (Table 2b). Although 90% of the diffuse target integration cells were excluded by the threshold applied during the post-processing, the Δ MVBS of the remaining diffuse targets, which were likely to contain a low percentage of krill, appeared to cover the same range of values as both the discrete and irregular, high-probability krill targets.

Distribution of extreme dB difference values within aggregations

It would seem, therefore, that between 10 and 30% of the integration cells within visually classified discrete or irregular krill aggregations had a Δ MVBS either higher or lower than the 2 to 12 dB range, the range suggested as indicative of krill (Madureira *et al.*, 1993b) and in this paper from now on we will refer to such outlying values as "extreme Δ MVBS".

We examined the spatial location of extreme Δ MVBS in individual aggregations. In general there was little evidence that integration cells with extreme Δ MVBS values occurred in groups that were separated from groups of integration cells with Δ MVBS values between 2 and 12 dB. Thus the extreme values do not represent separate aggregations of organisms distinct from those with that property. Rather, the extreme values were found dispersed within aggregations where most cells had values in the range of 2 and 12 dB (Table 3). In the discrete aggregations significantly more extreme values

(a)

Table 3. The assessment of the position of *extreme* dB difference values (Δ MVBS >12 dB) in discrete and irregular aggregations. Because some aggregations extend over a number of integration time periods (Elementary Sampling Distant Unit) we examine each ESDU separately. Only aggregations where the vertical extent of the aggregation exceeded 8 m are included here – the position of the extreme value is classified as "at the edge" (within 2 m of top or bottom of aggregation) or "in the middle" of the aggregation.

	Extreme ΔMVBS in aggregations (%)		
Position of extreme $\Delta MVBS$	Irregular	Discrete	
Large (>4 cells) – edge	60	70	
Large (>4 cells) – middle	40	30	
Number of ESDU	70	89	

were found at the edge of the aggregations than were found in the middle of them (χ^2 test, p<0.01). The occurrence of these extreme values more often near the edges than in the denser central areas of the aggregations may be due to variation in the size, type or behaviour of organisms across the aggregation or may be a sampling artifact. However, the length of the integration periods (ESDU ~125 m) should tend to eliminate any differences caused by spatial mismatch between transducer sampling volumes. Unfortunately the spatial resolution of net sampling was not fine enough to pick out any variations between the size of krill in different parts of the aggregations.

Comparison of biomass of krill identified by Δ MVBS and from echo-chart characteristics

The biomass of the krill was estimated acoustically in two separate and independent ways, firstly, by assuming that all targets with a Δ MVBS between 2 and 12 were krill and, secondly, by calculating the biomass of all aggregations visually classified as discrete or irregular. Overall these two different assessments produced very similar results (Figure 2; dB classification=0.94 visual classification, r²=0.99).

Differences between these two techniques may arise because of either the exclusion of krill from the estimation or the inclusion of other scatterers that are not krill. The relative effects of these two biases are addressed below.

The krill biomass estimated by Δ MVBS may be low because some krill will be discarded by the 2–12 dB filter (Table 4). The scattering models of Stanton *et al.* (1994) suggest that krill smaller than 27 mm would have a dB difference greater than 12 dB (Figure 3). If the range of dB differences assumed to be krill – the targets classified visually as discrete and irregular – was increased in 1 dB increments from 2–12 dB to 2–16 dB then the pro-



Figure 2. The comparison of biomass (g m⁻²) of krill classified by dB difference (MVBS₁₂₀ – MVBS₃₈) and by visual classification of targets on echo-chart (discrete and irregular target types). The line represents equal biomasses estimated by both methods. Regression analysis produced a best fit of dB difference biomass=0.94 visual classification, r^2 =0.99.

portion of visually classified cells that were accounted for by the dB difference range also increased (Table 2b). Considering only those cells classified visually as discrete, irregular or diffuse, more than 90% of the cells had a Δ MVBS between 2 and 16 dB. The dB difference of the various target classifications was examined for each net haul and representative hauls are shown in Figure 4. The discrete aggregations with a high probability of being krill always had modal Δ MVBS values within the 2 to 12 dB range, although a number of individual cells did fall outside this range. Similarly irregular aggregations also contained some individual cell values greater than 12 dB. To assess the likelihood that this could have been caused by visual misclassification of targets, the $\Delta MVBS$ of aggregations that were sampled directly by the net were also examined. Even in these instances parts of some aggregations had Δ MVBS greater than 12 dB (Table 5) suggesting that some small krill did fall outside the 2–12 dB Δ MVBS range. However, if all the integration cells for an aggregation were combined arithmetically to give a mean Δ MVBS then the aggregation mean always fell between 2 and 12 dB in all the examples that were available in this study. Thus it is likely that there is an optimal integration cell size for Δ MVBS target identification which is a compromise between being large enough to contain an average krill aggregation but small enough so that other types of target are excluded. In this study we used an ESDU of 125 m and a vertical resolution of 2 m. Given that most targets spanned a number of depth strata we suggest that a vertical integration distance of 10 m might be more appropriate.

Conversely the estimated biomass of krill may be increased if other non-krill targets have been included in the 2 to 12 dB difference range (Table 4). This is

	Estimate based on all targets with a dB difference of 2–12 dB	Estimate based on visually classified discrete or irregular targets
Exclusion of krill	Small krill may have dB difference >12 dB	Not all krill may occur in aggregations
Inclusion of other targets	Other species may produce a similar dB difference	Irregular aggregations may be a mixture of species rather than just krill Aggregations close to surface may include some surface noise

Table 4. The matrix of biases in the estimate of krill biomass based on identification using dB difference or using echo-chart classification.



Figure 3. The calculated size of krill derived from dB difference $(MVBS_{120} - MVBS_{38} \text{ kHz})$ generated by three krill-scattering models. \bullet indicates fluid-sphere model with spherical radius-to-length relationship as derived by Greenlaw (1979) and described by Mitson *et al.* (1996). \bigcirc indicates fluid-sphere model with spherical radius-to-length relationship derived from Morris *et al.* (1988). \lor indicates the bent-cylinder model described by Stanton *et al.* (1994).

illustrated by reference to Figure 4 and particularly the "background" values, where, in most cases, the peak values fall within the 2 to 12 dB difference range. These values by definition are low density targets, which can be excluded by thresholding during analysis and would not therefore contribute greatly to the total biomass. Brierley *et al.* (1998b) have shown that in the South Georgia area a number of zooplankton species other than krill may fall within the 2 to 12 dB difference. Brierley *et al.* (1997), however, indicated that excluding all MVBS₁₂₀ < -70 dB would only result in a 10% decrease in biomass on an average transect.

When using a visual aggregation classification the biomass may be underestimated if some krill are not classified as such (Table 4). This may have occurred if krill existed outside the acoustically detected and classified aggregations. While such a scenario has been suggested a number of times (see for instance Miller and Hampton, 1989; Makarov, 1996), it appears unlikely to be a major source of error here. Examination of the net trajectories show that in all cases where substantial numbers of krill were caught an acoustically "obvious" aggregation was sampled (Table 1). A few krill were caught in hauls where there was no obvious acoustic target but, in these cases, the estimated krill density was <0.01 krill m⁻³ (hauls JR11-207 and JR11-215; Table 1). It appears, therefore, that most of the krill biomass is contained within the classified aggregations.

Finally the krill biomass may have been over estimated if other species were included erroneously in the visual aggregation classification (Table 4). Where all large dense aggregations were sampled with the net it was found that the proportion by volume of krill in the catch was >95%. Thus it appears that these aggregations contained no substantial quantities of zooplankton other than krill. Note, however, that in some cases the aggregations occurred very close to the surface and so the separation of surface noise and biological aggregation was not always easy. This was not a problem when using the dB difference classification because surface noise and false bottom echoes had a very characteristic dB difference with higher values at 38 kHz than at 120 kHz (Figure 4).

Comparison of krill length obtained in the net hauls and that calculated from scattering models

Large catches of krill (>11) occurred in 17 nets from 11 hauls (Table 1). In these catches over 95% of the catch volume was attributed to Antarctic krill. More importantly, in all cases the reconstructed net trajectory passed through large and obvious targets on the echo chart. It was therefore possible to directly link small groups of integration cells from these aggregations with krill caught in the net. MVBS values for all aggregation cells that were within $\pm 5 \text{ m}$ of the net trajectory were averaged, weighted by arithmetic abundance in fact, to form a single overall estimate of the MVBS for each separate net sample of krill. The resulting Δ MVBS was used to calculate the length of krill using the models described in the "Materials and methods" section. The lengths calculated by the fluid-sphere model using Greenlaw's relationship for euphausiid length to spherical radius (Mitson et al., 1996) were substantially



Figure 4. Example of histograms of dB difference (MVBS₁₂₀ – MVBS₃₈) for different target types in selected individual net hauls during cruise JR11 and JR17. The dotted vertical lines represent the 2–12 dB difference range.

less than the actual length of krill caught in the net (Figure 5). Using the same model, but with the length–weight relationship from Morris *et al.* (1988), resulted in calculated lengths that were still always smaller than the net lengths but somewhat closer than those derived using the Greenlaw (1977) relationship. The values of

Table 5. The percentage occurrence of dB differences from integration cells within \pm 5 m of net trajectory for net hauls where the proportion of krill in catch >95% in cruises JR11 and JR17

	% frequency			
dB differences	JR11	JR17		
0–2	2.7	3.6		
2–4	0	14.9		
4–6	0.9	33.3		
6–8	8.2	26.1		
8-10	31.8	9.2		
10-12	30.9	6.0		
12–14	16.4	1.6		
14–16	7.3	0.8		
16–18	0.9	1.2		
18-20	0.9	0.8		
>20	0	2.4		
Number in distribution	110	249		
% within 2–12 dB	71.8	89.6		
% >12 dB	25.5	6.8		
% within 6–16 dB	94.6	43.7		

krill length in the net calculated using the Greenlaw (1977) fluid-sphere model were considerably less than expected given the generally good agreement reported by Mitson et al. (1996) for comparisons between acoustic and net estimates of a variety of North Atlantic and Antarctic euphausiid species. Mitson et al. (1996) found that krill lengths were underestimated by 15-25% whereas in our data they were underestimated by 10-44%. Our acoustic-based estimates of length improved when a length-weight regression and sound velocity and density contrasts specific to E. superba were used (Figure 5). As Mitson et al. (1996) point out, there are theoretical and practical limitations inherent in using two frequencies to estimate krill length. In our case particular attention was paid to ensuring that the acoustic system was well calibrated, with multiple calibrations at the beginning and end of both cruises and also during the second cruise. Only data from dense aggregations were used thus maintaining a high signalto-noise ratio. In addition, carefully targeted net sampling linked to a reconstruction of the net trajectory, enabled us to select those parts of the aggregations that were most likely to have been sampled by the net. This should have ensured that the congruence between the acoustic and net sampling was very close. Analysis of the net catches indicated that there were very few organisms apart from krill in these samples so that it is unlikely that significant signal return was due to targets other



Figure 5. A comparison of the length of krill caught in net hauls with the length of krill calculated from different krill-scattering models. The continuous line indicates a 1:1 relationship between the calculated length and "length in net". \bullet indicates a fluid-sphere model with a spherical radius-to-length relationship as derived by Greenlaw (1979) and described by Mitson *et al.* (1996) (calculated length=3.70+0.589 net length; r²=0.64). \bigcirc indicates a fluid-sphere model with a spherical radius-to-length relationship derived from Morris *et al.* (1988) (calculated length=12.52+0.415 net length; r²=0.66). \checkmark indicates a bent-cylinder model described by Stanton *et al.* (1994) (calculated length=8.79+0.685 net length; r²=0.77).

than krill. Finally, the length frequency distribution of the net samples suggested that the samples were basically unimodal.

The calculated lengths obtained from the simplified bent-cylinder model described by Stanton *et al.* (1994) straddled the line where calculated length was equal to that observed in the net and could be described by the regression:

Calculated length (mm)=8.79+0.685 net length (mm), $r^2=0.77$, for animals between 25 and 50 mm total length.

However, there was substantial scatter around the expected values. The reasons for this are not clear, although a contributing factor could be the different behaviour patterns exhibited by the different aggregations, for instance in swimming angles. Chu *et al.* (1993) have used the difference between calculated and measured target strengths to infer the orientation distribution. The model used here was tuned for Antarctic krill by using the relevant sound speed and density contrasts but appears to be sensitive to krill width. We estimated krill width by deriving the radius of a cylinder with a volume equivalent for each size of krill using the length-to-weight relationship of Morris *et al.* (1988), density and length of the krill (length-to-width ratio

~16). However, this estimated krill width gave a worse fit to the observed data than using the fixed length-towidth ratio as shown in Stanton *et al.* (1994). However, Brierley *et al.* (1998a) recently used this model, with a length-to-width ratio ~16, to predict the expected dB differences between backscatter from an ADCP and an EK500, and found good agreement between the observed and expected scattering at 120, 153 and 200 kHz.

The implications for krill biomass assessment

Errors in the acoustic estimation of biomass may occur because krill targets are falsely excluded or because non-krill targets are erroneously included in the estimates. This is similar to the problem facing statisticians with Type 1 or Type II errors, decreasing the likelihood of one error can increase the likelihood of the other. In the particular case of Antarctic krill one approach has been to assume that all acoustically detected biomass is krill (SC-CAMLR, 1996).

The results of this study reveal that no substantially sized krill aggregations had an overall dB difference outside the range 2-12 dB. However, we found many aggregations that were predominately krill where a proportion of the integration cells within the aggregation had a dB difference greater than 12 dB. Given that very few other organisms were found in the targeted net hauls, but that extreme dB values were still found, we attribute this to the occurrence of small krill within the aggregations. This effect was more marked in data from cruise JR11 (mean krill sizes 29.7 and 32.0 mm in survey boxes) than in JR17 (krill sizes 36.1 and 45.4 mm; Table 5). Many of the previous studies have used larger integration units than those used in this study so that the extreme values observed here may be related to the small-scale structure of the krill aggregations and would not have been detected previously.

Recent work using three frequencies to separate common zooplankton species (Brierley *et al.*, 1998b) has revealed that several of these species have a 120–38 dB difference between 2 and 12 dB. In the case of that particular study, however, the organisms appeared in low-density layers (MVBS₁₂₀ < - 80 dB) which are likely to be similar to the diffuse targets identified here. Similarly in studies by Madureira *et al.* (1993b) the smaller euphausiids and other zooplankters tended to occur in low densities. Therefore it would appear that a combination of post-processing threshold (e.g. MVBS₁₂₀ > - 70 dB) and dB-difference partioning techniques would allow the best separation of krill from these other acoustic targets if only two frequencies are available.

The regression of biomass estimates arising from dB difference and visual classification techniques suggests that, on average, there was a slight under-estimation of

krill biomass using dB difference In this study, therefore, exclusion of small krill would appear to have been a greater problem than erroneous inclusion of other zooplankon species. One solution may be to adjust the Δ MVBS krill acceptance window according to the size of krill in the study area. On the basis of the results obtained on these two cruises we would suggest that an appropriate range for JR11 krill would have been 6-16 dB, while an appropriate range for JR17 krill would have been 2-12 dB (Table 5). Inspection of Figure 3 reveals that the Δ MVBS value for krill of mean length 30 mm, the approximate size of krill in JR11, is $\sim 10 \text{ dB}$ which is close to the midpoint of the suggested 6-16 dB range. Similarly the Δ MVBS value for krill of mean length 40 mm, the approximate size of krill in JR17, is \sim 7 dB which, in turn, is close to the midpoint of the suggested 2-12 dB range. It may be possible, therefore, to derive a suitable Δ MVBS range from model expectations once the mean size of krill in the survey area is determined.

Although use of dB difference alone may lead to some mis-classification of krill and zooplankton there are certain clear advantages in using such a method. In particular, the derivation of Δ MVBS is objective and therefore lends itself well to multi-operator comparisons where many different people may be processing the data. Traditional visual echo-chart classification techniques are quite subjective and standard procedures are difficult to describe (Kalinowski and Witek, 1985). We would therefore encourage the use of a standard dB difference to partition the total acoustic biomass into several categories for all studies but especially those where comparisons are made between data sets collected by different workers. Such a standardized technique has been used very effectively in the recent CCAMLR-2000 multi-ship, international survey (SC-CAMLR, 2000).

In the present paper we have concentrated on the validation of a simple, objective dual-frequency classification method as a way of eliminating dependence upon subjective echogram inspection techniques. However other techniques have been developed to identify the species of pelagic fish schools (see for instance Haralabous and Georgakarakos, 1996; Scalabrin et al., 1996; Lawson et al., 2001). To date these objective echogram inspection techniques use single-frequency acoustic measurements of morphometric, energetic and bathymetric features of the schools to derive a set of school descriptors. The species identification of the schools can then be obtained from these school descriptors through the use of discriminant function analysis (Lawson et al., 2001) or an artificial neural network (Haralabous and Georgakarakos, 1996). While such techniques have achieved a high level of successful identification for a variety of fish species they have needed an increased level of complexity of analysis over the simple dualfrequency method proposed in this paper. For Antarctic krill it remains to be demonstrated that such additional complexity adds significantly to the accuracy of the acoustic biomass estimates derived through the application of the simple dual-frequency approach. However, given the importance of developing robust speciesidentification techniques for reliable biomass estimates, we encourage further testing and comparison to assess the relative costs and benefits of these different acoustic-identification methods.

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