# Differences in backscattering strength determined at 120 and 38 kHz for three species of Antarctic macroplankton

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ABSTRACT: The ability to acoustically separate zooplankton species is an important requirement for ecological studies and to improve biomass estimates. In order to distinguish between *Euphausia superba* and other swarm-forming macroplankters we used a dual frequency echo-sounder (120 and 38 kHz) and echo-integrator during a series of Longhurst Hardy Plankton Recorder (LHPR) hauls near South Georgia. We compared the acoustic parameter Mean Volume Backscattering Strength (MVBS) according to the equation:  $\Delta$ MVBS (dB) = MVBS 120 kHz - MVBS 38 kHz. Mean values of  $\Delta$ MVBS for *E. superba, Themisto gaudichaudii* and *E. frigida* were 4.6, 9.7 and 15.6 dB, respectively, and were significantly different, allowing the 3 species to be separated acoustically.

# INTRODUCTION

Acoustic methods have been used for fish stock estimation and ecological studies for more than 20 yr (Sund 1935, Greenlaw 1979, Johannesson & Mitson 1983). The so-called echo-integration method has had a worldwide application and the merit of using acoustics relative to nets has frequently been discussed (Greenlaw 1979, Pieper & Holliday 1984, Everson & Bone 1986a).

In the Southern Ocean the Antarctic krill *Euphausia superba* (hereafter called 'krill') has been surveyed acoustically since the early 1970s in order to determine its distribution and estimate its biomass (Everson 1988). However, not all targets in the water column are krill. Other species swarm in Antarctic waters, for example the amphipod *Themisto gaudichaudii* (Kane 1966, Everson & Ward 1980), salps (Foxton 1966) and other euphausiids, such as *E. crystallorophias* (Everson 1987). It is difficult to distinguish acoustically between these different scatterers (Masson 1989, Miller & Hampton 1989) and hence make precise estimates of krill abundance.

A problem with acoustics is the choice of frequency. Low frequencies are efficient for long ranges and for large targets but are 'blind' to small organisms. Conversely, high frequencies necessary to detect small species suffer from greater attenuation and consequently have a very short range. For fish studies, the most common frequencies vary from 12 to 200 kHz (Clay & Medwin 1977, Farquhar 1977, Saville 1977, Genin et al. 1988, Eckmann 1991) while for studying zooplankton 20 kHz to more than 1 MHz have been used (Clay & Medwin 1977, Pieper 1979, Sameoto 1980, Richter 1985, Everson & Bone 1986b, Genin et al. 1988, Greene et al. 1991).

In many studies in the Antarctic single-frequency echo-sounders and net sampling are employed. Echotraces generally are assumed to be due to krill if krill are caught nearby. However, dual- and multi-frequency echo-sounders have recently become available. The comparison of backscattering strength measurements at different frequencies enables inferences to be drawn about the sizes of the targets (Greenlaw 1977), therefore allowing separation of species or groups of species of different dimensions.

In order to investigate this, Madureira et al. (in press) analyzed acoustic data from the South Georgia area, collected with a dual frequency echo-sounder (120 and 38 kHz) and echo-integrator. They visually classified 3 different types of echo-traces and compared Mean

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Volume Backscattering Strength (MVBS) for 120 kHz relative to 38 kHz for each type. Each echo-type had a characteristic range of MVBS differences which they attributed to targets of different sizes. By applying thresholds to the MVBS data they clearly separated krill type echoes from fish type ones. A third category, thought to be due to zooplankton, was identified but proved difficult to discriminate due to their low echosignal level.

This paper presents data collected during January and February 1991, in the vicinity of South Georgia. We intended to locate the targets identified by Madureira et al. (in press) as Type 1 (krill) and Type 2 (zooplankton), establish their identity using a net and estimate MVBS values at 120 and 38 kHz to further characterize them acoustically.

### **METHODS**

Acoustics. For this study we used a SIMRAD EK-400 echo-sounder operating at 120 and 38 kHz and a QD echo-integrator. The transducers were hull-mounted at 5 m depth and 1.24 m apart. The system was calibrated in Leith Bay (South Georgia) with a 38.1 mm tungsten carbide sphere, according to the standard target method (Foote 1982). Calibration set ups are shown in Table 1.

Acoustic data were collected from 9 surface referenced integration layers which were operated with depth limits varying according to target location. Signals were integrated over intervals of 0.1 nautical mile, corresponding to 1.5 min, at a ship speed of 4 knots.

Spreading and attenuation loss corrections were applied, the latter according to absorption coefficients from Francois & Garrison (1982). The values used were interpolated to be appropriate to the water temperature and salinity in the study area. Corrections were also applied to layers deeper than the maximum Time Varied Gain (TVG) depth for 120 kHz.

MVBS differences were calculated according to the equation proposed by Madureira et al. (in press):

 $\Delta$ MVBS (dB) = MVBS (dB) 120 kHz - MVBS (dB) 38 kHz.

Table 1. EK-400 calibration data at 120 and 38 kHz. SL: source level; VR: voltage response

Calibration	38 kHz	120 kHz
SL + VR (dB)	129.8	107.4
Pulse duration (ms)	1.00	1.00
Equivalent ideal beam factor (dB)	-21.0	-18.0
Adsorption coefficient (dB km <sup>-1</sup> )	9.8	28.1

Thresholds were used in order to avoid very low MVBS readings where, although scatterers were present, the MVBS was close to the intrinsic minimum for the system. Values were chosen based on integration intervals where no marks were seen on the echocharts. The highest MVBS readings for no-trace intervals were applied as threshold values and only values above those were used in subsequent analyses.

Net sampling. A Longhurst Hardy Plankton Recorder (LHPR; see Longhurst & Williams 1976, Williams et al. 1983) equipped with a 500  $\mu m$  mesh net was employed to sample the acoustic targets. This was fished at a nominal ship's speed of 4 knots using a sampling interval of 30 s, which means that a haul of 10 min would contain 20 samples. Each sample generally represented a filtered volume of 6 to 8 m³, over a horizontal distance of approximately 65 m.

Transects were run along and transversely to the shelf break to locate suitable targets. Once echo-traces were recorded a haul was made, aimed at the location and depth of the echo-traces. To do this the ship was sent back on a reciprocal course, the integration layers were adjusted to include the targets and the net was launched. As well as net depth, wire out was monitored so that distance of the net behind the ship could be calculated. This allowed the net trajectory to be directly related to the acoustic trace.

Acoustic data collection was synchronised with the net operations. Echo-sounder minute marks were switched on and the QD echo-integrator was started with the fist gauze advance, as the net was going overboard. Time was recorded (1) when the net was in the water, (2) when stable at desired depth, (3) on the commencement of hauling and (4) when the net was back at the surface. A total of 9 hauls were carried out, which averaged 30 to 45 min each.

Once onboard the net trace was read to determine the number of samples taken during the haul. The gauzes were then cut into individual sample lengths and deep frozen at -60 °C. In the UK, gauzes were thawed in seawater and the contents of each determined. The main taxa identified were Euphausia superba, E. frigida, Thysanoessa spp., Themisto gaudichaudii and total large Copepoda (i.e. Rhincalanus gigas and Calanoides acutus). Adults and juveniles were counted and where numbers were greater than 10 per sample their displacement volumes were measured.

Problems were experienced with dense concentrations of plankton occasionally jamming the confluence of the gauzes and preventing a clean wind-on after 30 s. This periodically happened with *Euphausia superba* and *Themisto gaudichaudii* which, due to their large size and occurrence in high density patches, were held up in the net and only entered the codend

slowly, some time after they had entered the LHPR. This is clearly seen for *E. superba* (see Fig. 1A) where their acoustic detection and presence on the gauzes were coincident as the swarm was first encountered. Thereafter krill appeared in samples to the end of the haul, even though the echochart indicated that the net had left the swarm behind.

With *Themisto gaudichaudii*, blockages were only partial and cleared themselves, the net effect being that the fishing time of each sample was generally extended and variable (up to a maximum of 2.5 min or 300 m horizontal distance). Blockages of *Euphausia frigida* never occurred. Despite these problems the LHPR clearly resolved the presence and identity of the target organisms concerned.

#### RESULTS

During the course of our study we located and sampled aggregations with acoustic characteristics corresponding to Types 1 and 2 as described by Madureira et al. (unpubl.). Type 1 proved to consist entirely of *Euphausia superba*, whereas those in Type 2 were either predominantly *E. frigida* or *Themisto gaudichaudii*.

Copepods were present in all hauls although concentrations in the net were not consistent with any marks on the echochart. Sameoto (1980) also found no significant correlation between the biomass of copepods and backscattering strength at 120 kHz. Lower frequencies, such as 38 kHz, would be even less sensitive to their presence. The very low estimates of target-strength for copepods predicted at 120 and 38 kHz, –101 and –113 dB respectively (Greenlaw 1977), would mean that even at the densities recorded in the nets (up to 60 m<sup>-3</sup>) the MVBS would be close to the threshold values. They were therefore disregarded as an important cause of the differences in backscattering strength.

We have selected 35 acoustic intervals positively sampled by 7 LHPR hauls to demonstrate best the differences in  $\Delta$ MVBS for each species (Fig. 1). The other 2 hauls were not used because no single species dominated.

The net trajectory has been superimposed on the acoustic trace (Fig. 1) so that the catch data can be related to the acoustic targets. It must be borne in mind, however, that the net sampling and acoustic sampling systems were remote, with the net passing through the insonified layer up to 3 min after the acoustic data had been collected. Also, the net only passed through a small part of the insonified volume. Despite this, there was a good correspondence between net catches and MVBS fluctuations at both frequencies.

Fig. 1A is an example of a haul where a single krill swarm was sampled; the resultant build up of krill in the net ahead of the codend resulted in the smearing of the krill catch, as described above. Fig. 1B shows a net haul where Euphausia frigida was sampled. The percentage catch of this species, calculated relative to the combined volume of E. frigida and Themisto gaudichaudii, was 87 %. Echo-traces occur over about half the distance where the net operated at a depth between 105 and 110 m. Fig. 1C presents a net haul where T. gaudichaudii dominated the catch (99.4 %, calculated relative to the combined volume of T. gaudichaudii and E. frigida). Echo-traces can be seen before and after the net entered the depth layer between 30 and 50 m. The net undulation in the initial part of the operation was due to an adjustment of the amount of cable in the water.

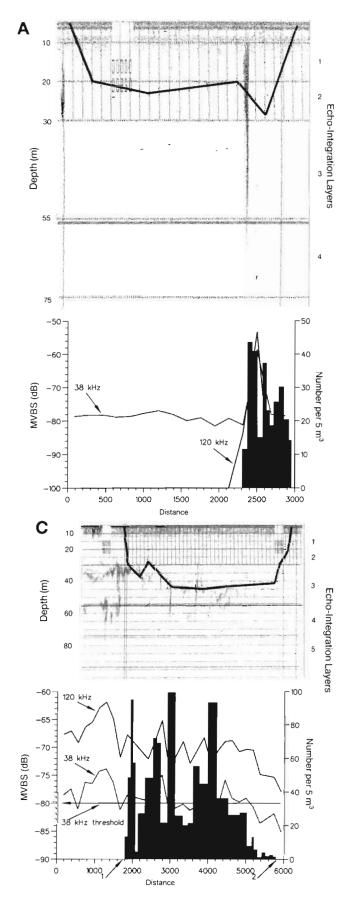
Threshold values used were variable between transects (Fig. 1B, C) because of different factors which can affect the noise level, such as sampling depth (distance from surface), sea state and bathymetry.  $\Delta MVBS$  above and below thresholds were significantly different, indicating that areas without the main targets did not have the same acoustic characteristics.

The acoustic data for the 3 target species are summarised in Table 2. These data are restricted to the portion of the transects sampled with the LHPR, when the net was operating at the desired depth.

It can be seen in Fig. 1 that the echo-traces extended beyond the strata sampled by the net, i.e. continuing from the sampled layer into adjacent ones, above and below the net trajectory. We therefore assumed that those closest to the layer the net passed through would be due to the same target species. If the MVBS from these layers exceeded the specified thresholds they were included in a new set of results (Table 3). Euphausia frigida and Themisto gaudichaudii had their sample size enlarged in this way and E. superba data were also included for comparison.

As can be seen, MVBS at the 2 frequencies and the resulting differences varied little between the original data verified by the net (Table 2) and the enlarged version (Table 3). The enlarged data set allowed us to look for relationships between  $\Delta$ MVBS and individual frequencies, in order to check whether MVBS differences were associated with swarm density (Fig. 2). Spearman's rank correlation coefficient was only significant (negatively correlated) for *Euphausia frigida* when tested against 38 kHz ( $\rho = -0.864$ ) (see 'Discussion').

The mean length and volume of the 3 species are different (Table 4). This is likely to have an important effect on the  $\Delta$ MVBS differences detected because of the relationship between wave number (k), the animal's spherical radius (a) and the target-strength (see 'Discussion'). Calculations of a were carried out according to Greene et al. (1991).



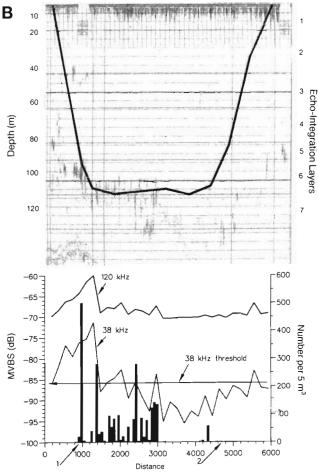


Fig. 1. Comparison between acoustic data and LHPR catches for 3 hauls dominated by (A) Euphausia superba, (B) Euphausia frigida and (C) Themisto gaudichaudii. Horizontal scales for the sections of each figure are identical. The net trajectories and the integration layers are indicated on the upper echo-traces. The integration layers for (A), (B) and (C) were respectively 20 to 30 m, 95 to 115 m and 35 to 55 m. In (B) and (C), the points where the net entered and then left these layers are indicated by 1 and 2, respectively. Catch details (no. per 5 m³) refer in each case only to these layers

# DISCUSSION

Madureira et al. (in press) identified 2 types of echoes which they attributed to krill and smaller zooplankton. They classified those echoes as Type 1 (krill) and Type 2 (smaller zooplankton) and suggested that  $\Delta\text{MVBS}$  ranges of 2 to 12 dB (Type 1) and greater than 12 dB (Type 2) could be used to separate them.

In this study we sampled targets with acoustic characteristics similar to those described by Madureira et al. (in press) and confirmed that krill was responsible for Type 1 and also that other zooplankton could constitute a significant proportion of Type 2 echoes.  $\Delta$ MVBS for Euphausia frigida and Themisto gaudi-

Table 2. Comparison of Mean Volume Backscattering Strength (MVBS) for 120 and 38 kHz for the 3 species obtained during LHPR hauls. N MVBS is number of integration intervals where MVBS were above the thresholds and SE is the standard error of the mean, calculated across the whole table

Species	N MVBS	MVBS (db) 120 kHz	SE (dB)	MVBS (dB) 38 kHz	SE (dB)	Mean ΔMVBS (dB)	SE (dB)
Euphausia frigida	7	-65.6	1.57	-81.2	1.58	15.6	1.58
Themisto gaudichaudii	13	-67.9	1.15	-77.6	1.16	9.7	0.46
Euphausia superba	15	-57.7	1.07	-62.3	1.08	4.6	0.43

Table 3. Comparison of Mean Volume Backscattering Strength (MVBS) for 120 and 38 kHz for the 3 species obtained during LHPR hauls and adjacent integration intervals with the same characteristics. N MVBS is number of integration intervals where MVBS were above the thresholds and SE is the standard error of the mean, calculated across the whole table

Species	N MVBS	MVBS (dB) for 120 kHz	SE (db)	MVBS (db) for 38 kHz	SE (db)	Mean ΔMVBS (dB)	SE (dB)
Euphausia frigida	19	-65.7	0.51	-81.1	0.9	15.4	0.55
Themisto gaudichaudii	18	-67.7	0.83	-77.9	0.89	10.2	0.46
Euphausia superba	15	-57.7	0.91	-62.3	0.98	4.6	0.5

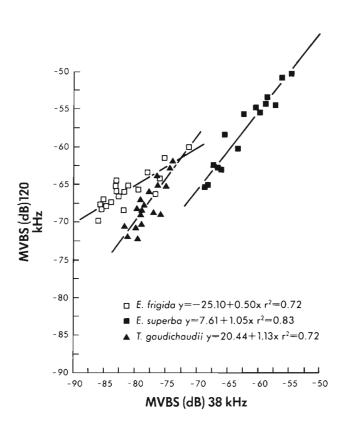


Fig. 2. MVBS values at 120 and 38 kHz for Euphausia superba, E. frigida and Themisto gaudichaudii

chaudii were different from *E. superba* and from each other (Table 3), indicating differences in the target strength of all 3 species at the 2 acoustic frequencies.

Our  $\Delta$ MVBS for krill agree well with that of Greene et al. (1991) if we apply their equation to calculate target strength values of our largest animals (58 mm total length). In this case the difference between the 2 frequencies obtained in the field work, a mean difference of 4.6 dB (Table 2), is very close to the 5 dB predicted by their equation. Also, Madureira et al. (in press) observed ΔMVBS for Euphausia superba in the range of 4.5 to 5.5 dB, obtained during a survey in 1986. However, Greene et al. (1991), criticised the use of 38 kHz for surveying krill abundance. The reason for that is associated with the 'Rayleigh scattering region'. In this region, where acoustic wavelength is larger than target size  $(k \cdot a < 1)$ , target strength rapidly decreases with reducing target size (see Caruthers 1977, Clay & Medwin 1977). In the geometric scattering region, where wavelength is smaller than the target  $(k \cdot a > 1)$ , target strength is a complicated function of frequency (Kristensen 1983) but a linear regression can explain a high proportion of the variance (Greene et al. 1991). We calculated target strength value at 120 and 38 kHz using the equation proposed by Greene et al. (1991) because our largest krill would be within the geometric scattering region for both frequencies.

All 3 species had  $k \cdot a < 1$  for 38 kHz, with the exception of the largest krill as mentioned above, and  $k \cdot a > 1$  for 120 kHz (Table 4).  $\Delta$ MVBS decreased with increasing target proportions (increasing a). This indicated

Table 4. Mean length and volume for the 3 species sampled with the LHPR. SD is the standard deviation of the mean. $a = animal$ 's
equivalent spherical radius, calculated according to Greene et al. (1991); $k = \text{wave number}$

Species	n	Mean length (mm)	SD (mm)	Mean vol. (ml)	a (mm)	<i>k∙a</i> for 38 kHz	<i>k∙a</i> for 120 kHz
Euphausia frigida	300	18.5	1.9	0.045	2.21	0.35	1.11
Themisto gaudichaudii	300	17.4	1.4	0.062	2.46	0.39	1.23
Euphausia superba	82	38.7	4.8	0.440	4.72	0.75	2.37

that the reductions in the  $\Delta MVBS$  are associated with 38 kHz because of the increased sensitivity to target size at this frequency. It was probably this sensitivity which separated the 3 species acoustically (Fig. 2). However, it is interesting to note that whilst mean length of Themisto gaudichaudii was less than Euphausia frigida (Table 4) its volume was 1.4 times greater. This is a likely reason why T. gaudichaudii had a smaller  $\Delta MVBS$  although the values for both species were not significantly different at 120 kHz (Tables 2 & 3). Our results therefore confirm the observation of Wiebe et al. (1990) that crustacean macroplankton backscatter sound as a function of volume. As far as we know this is the first confirmation of such phenomena with field data at 2 frequencies.

Results of a krill target strength experiment undertaken by Everson et al. (1990) showed a mean difference between 120 and 38 kHz of 9.2 dB for *Euphausia superba*. We suggest that the differences between their experiment and ours are due to the differing size ranges of the krill involved. Our krill length varied from 33 to 58 mm (mean 38.7 mm) while their krill were smaller (23 to 45 mm; mean 31 mm). The effect on  $\Delta$ MVBS would be in the right direction, i.e. their smaller krill would backscatter less than ours at 38 kHz, making  $\Delta$ MVBS greater.

Hampton (1990) reported MSBS (Mean Surface Back-scattering Strength) differences of about 7 dB higher at 120 kHz relative to 38 kHz for *Euphausia superba* swarms. He assumed that because of the transducers' closeness 'essentially the same targets would have been insonified at both frequencies'. In discussing factors which potentially could have caused the differences he concluded that neither calibration nor any other experimental artifacts explained the observations and that higher MSBS at 120 kHz relative to 38 kHz was due to krill target strength frequency dependence.

There are no published target strength results for live Euphausia frigida or Themisto gaudichaudii. Also, the equation of Greene et al. (1991) does not fit either, because of their  $k \cdot a$  being <1 at 38 kHz (Table 4). However, Suzuki (1969), working in the laboratory with live Themisto sp. and E. pacifica of mean length 4.4 and 19.4 mm respectively, found 10 and 5 dB higher readings at 200 kHz relative to 28 kHz for the

amphipod and the euphausiid, respectively. Our experiment and Suzuki's differ in species, size of the animals, frequencies, field and laboratory. Such distinctions make it difficult to compare his work with ours but some points can be addressed. *Themisto* sp. values would not be far from ours for *T. gaudichaudii* despite the difference in the length of the animals. But the results of his experiments with *E. pacifica* of 19.4 mm are very close to our values for *E. superba* of 38 mm and far from those for *E. frigida* of 18.5 mm. Regardless of differences, both investigations found higher back-scattering strength values at high frequencies relative to low ones.

There are some indications that  $\Delta MVBS$  can be affected by swarm density. For Euphausia frigida MVBS values at 38 kHz are negatively correlated with  $\Delta$ MVBS (Spearman's rank correlation  $\rho = -0.864$ ) but this was not the case for the other 2 species. Netcaught E. frigida densities, i.e. numbers per m<sup>2</sup>, were higher than those of the other 2 species and variation between adjacent samples was higher, implying that maximum packing density achieved within swarms will be even greater than those integrated over the 30 s intervals. We further explore this in Fig. 2, which plots MVBS at 120 kHz against MVBS at 38 kHz, with straight lines fitted for the 3 species using the least square method. The regression equations in this figure explain a high proportion of the observed variance at 120 kHz when related to 38 kHz ( $r^2 = 0.72$ , 0.93 and 0.72 respectively for E. frigida, E. superba and Themisto gaudichaudii).

It is evident from the figure that Euphausia superba and Themisto gaudichaudii can be clearly separated at these frequencies. The same is also true for E. frigida and E. superba within the limits of this dataset. However, there is some overlap between E. frigida and T gaudichaudii at the highest densities encountered (see above). We suggest that densely packed E. frigida might have been detected as a single target at 38 kHz, therefore affecting target strength and consequently  $\Delta$ MVBS (Hewitt & Demer 1991). However, Everson et al. (1990) did not notice any effect of density during an E. superba target strength experiment where they had equivalent densities of up to 16 000 m<sup>-3</sup>, much higher than our maximum numbers in the LHPR. T gaudi-

Table 5.  $k \cdot a$  values for the 3 target species. Mean length and a as in Table 4

Species	200 kHz	120 kHz	70 kHz	50 kHz	38 kHz
Euphausia frigida	1.85	1.11	0.65	0.46	0.35
Themisto gaudichaudii	2.06	1.23	0.72	0.51	0.39
Euphausia superba	3.95	2.37	1.38	0.99	0.75

chaudii may not behave in the same way. Distance between animals is probably larger because their density is lower, possibly allowing acoustic recognition of individuals at the full range of the densities which we detected. In light of this it is pertinent to consider how alternative acoustic frequencies might perform in identifying our targets.

Table 5 shows  $k \cdot a$  values for the 3 species calculated at a range of frequencies from 200 to 38 kHz. We have seen that at 120 kHz  $k \cdot a$  for all 3 species was > 1 and < 1 at 38 kHz and that under these circumstances the increased sensitivity to target size allows the 3 species to be separated acoustically.  $k \cdot a$  values at 200 kHz are all > 1; accordingly there would be little value in comparing 200 kHz with 120 kHz since the strong target strength dependence on target size would decrease as  $k \cdot a$  would be > 1 for both frequencies. However, 200 kHz would be good for quantifying abundance.

120 and 38 kHz work well for Euphausia superba and Themisto gaudichaudii but 38 kHz is probably too low for E. frigida. A frequency of 50 kHz would have  $k \cdot a$  values for E. superba too close to 1 and the frequency is also too close to 38 kHz; therefore it is not the ideal. A frequency of 70 kHz would maintain  $k \cdot a < 1$  for both E. frigida and T gaudichaudii, making a more useful comparison with 120 kHz. At the same time the shortest wavelength should alleviate the 'packing problems', allowing recognition of 2 separate target individuals at higher densities than 38 kHz would be capable of. Clearly the choice of frequencies can be critical in such comparisons, especially in acoustic systems where 3 frequencies are the operational maximum.

## CONCLUSIONS

We have separated acoustically 3 swarm-forming macroplankters in the Southern Ocean with an echosounder operating at 2 frequencies. Biomass estimations of macroplankton species using acoustics can therefore be more accurate if multifrequency systems are employed and appropriate target strength values are applied to each species. 120 and 200 kHz would be better for quantifying abundance, although 38 and 120 kHz seem to be effective for distinguishing swarm-types. Target-strength determinations for macroplank-

ton species over a wide band of frequencies are clearly necessary.

Miller & Hampton (1989) suggested that the most reliable information on the vertical migration of krill can be obtained from acoustic records. This, however, is dependent on the ability to separate krill from the other accoustic targets. More general ecological surveys, for example those

investigating predator-prey relationships (SC-CAMLR 1986), can also benefit from separating organisms whose acoustic target strength is frequency dependent. In this way a better understanding of predator-prey interaction will result.

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