



PERGAMON

Deep-Sea Research II 45 (1998) 1155–1173

DEEP-SEA RESEARCH
PART II

Acoustic discrimination of Southern Ocean zooplankton

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Received 12 March 1997; received in revised form 26 September 1997; accepted 15 October 1997

Abstract

Acoustic surveys in the vicinity of the sub-Antarctic island of South Georgia during a period of exceptionally calm weather revealed the existence of a number of horizontally extensive yet vertically discrete scattering layers in the upper 250 m of the water column. These layers were fished with a Longhurst-Hardy plankton recorder (LHPR) and a multiple-opening 8 m² rectangular mid-water trawl (RMT8). Analysis of catches suggested that each scattering layer was composed predominantly of a single species (biovolume > 95%) of either the euphausiids *Euphausia frigida* or *Thysanöessa macrura*, the hyperiid amphipod *Themisto gaudichaudii*, or the eucalaniid copepod *Rhincalanus gigas*. Instrumentation on the nets allowed their trajectories to be reconstructed precisely, and thus catch data to be related directly to the corresponding acoustic signals. Discriminant function analysis of differences between mean volume backscattering strength at 38, 120 and 200 kHz separated echoes originating from each of the dominant scattering layers, and other signals identified as originating from Antarctic krill (*Euphausia superba*), with an overall correct classification rate of 77%. Using echo intensity data alone, gathered using hardware commonly employed for fishery acoustics, it is therefore possible to discriminate *in situ* between several zooplanktonic taxa, taxa which in some instances exhibit similar gross morphological characteristics and have overlapping length–frequency distributions. Acoustic signals from the mysid *Antarctomysis maxima* could also be discriminated once information on target distribution was considered, highlighting the value of incorporating multiple descriptors of echo characteristics into signal identification procedures. The ability to discriminate acoustically between zooplankton taxa could be applied to provide improved acoustic estimates of species abundance, and to enhance field studies of zooplankton ecology, distribution and species interactions. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Acoustic survey techniques are used throughout the Southern Ocean to determine standing stock estimates of the Antarctic krill *Euphausia superba*, and to investigate aspects of the distribution and ecology of the species (e.g., McClatchie et al., 1994; Demer and Hewitt, 1995; Murray et al., 1995; Brierley and Watkins, 1996). The ability to determine accurate biomass estimates using these techniques is dependant upon a number of factors, including detailed knowledge of the target strength of the species under investigation and the ability to distinguish echoes originating from the target species from those caused by other sound scatterers. Much progress has been made toward the evaluation of krill target strength (e.g., Everson et al., 1990; Foote et al., 1990), and a standard relationship has been widely adopted (CCAMLR, 1991; Greene et al., 1991a). There is, however, as yet no accepted standard method to discriminate between acoustic signals originating from krill and those from other sources, apart from time-consuming (and hence costly) target fishing. The use of different echo-classification criteria by different groups can prevent direct comparisons between biomass estimates from being made (Brierley et al., 1998).

The increasing use of more than one frequency during routine acoustic surveys offers the potential to infer more from survey data than just signal intensity. Madureira et al. (1993a), for example, recognised three distinct biological echo classes on acoustic traces collected around South Georgia, and demonstrated that these classes—which were assumed to correspond to broad size categories of scatterer—could be separated on the basis of their characteristic differences in backscattering strength at 38 and 120 kHz. Demer (1994) has also developed a two-frequency (120 and 200 kHz) technique for distinguishing Antarctic krill from salps (also see Demer and Hewitt, 1994). The physical principles underlying such differences in echo intensity at different frequencies have been used to derive a number of mathematical models describing sound scattering by zooplankton (e.g., Greenlaw, 1979; Stanton, 1990), and predictions made by these models often have been supported by experimental measurements of scattering at different frequencies (e.g., Chu et al., 1993; Stanton et al., 1994). Furthermore, it has proved possible to invert a number of these models and obtain biologically meaningful information on the size (Mitson et al., 1996) and anatomical class (e.g., Martin et al., 1996) of scatterers from multi-frequency acoustic signals.

The realization that multi-frequency acoustic techniques potentially could contribute to many areas of biological oceanography (Holliday, 1977) led to the development of several novel bioacoustical oceanographic instruments operating at a wide ranges of frequencies (see Holliday and Pieper, 1995). These specialised instruments have been deployed in many of the world's oceans and have provided valuable data on zooplankton size, distribution and abundance, clearly demonstrating the utility of such approaches to zooplankton studies. Multi-frequency acoustic data collected using specialised transducers have also been used to discriminate between several commercially important fish species in tanks (Simmonds et al., 1996).

Inspired by these achievements, this paper describes how we have used simple multi-frequency acoustics to enhance our studies of zooplankton in the Southern

Ocean. During the course of a zooplankton community study to the north of the sub-Antarctic island of South Georgia, we encountered an extensive series of discrete horizontal sound scattering layers. High-resolution netting revealed that in many instances each of these layers contained only one macrozooplankton species. Sections of these layers thus provided the opportunity to obtain multiple *in situ* acoustic measurements from aggregations of single macrozooplankton species, and to link positively acoustic signals to individual species.

Here we present an empirical demonstration of an occasion when, with the use of three frequencies commonly employed during fisheries acoustic surveys, a number of Southern Ocean zooplankton species, some with overlapping size distributions and similar morphology, could be discriminated on the basis of their acoustic signatures alone. Application of this technique could provide a readily accessible avenue to study ecological interactions and behavioural segregations within zooplankton communities, to investigate the distribution of zooplankton species relative to oceanographic features, and could potentially increase the accuracy of acoustic estimates of krill biomass.

2. Materials and methods

The data described here were collected during two cruises aboard RRS *James Clark Ross* to the north of the sub-Antarctic island of South Georgia, in the Atlantic sector of the Southern Ocean. These cruises took place during January/February 1996 and December 1996/January 1997.

2.1. Acoustics

Mean volume backscattering strength (mvbs or S_v) data were collected using a Simrad EK500 echo-sounder operating hull-mounted split-beam 38 and 120 kHz transducers and a single-beam 200 kHz transducer. The echo-sounder was configured to ping simultaneously at each frequency every 2.5 s, with pulse lengths of 1.0, 1.0 and 0.6 ms at 38, 120 and 200 kHz, respectively (i.e. configured to produce pings at each frequency with as similar characteristics as the EK500 control software would allow). The performance of the echo-sounder was monitored regularly throughout the course of the studies using standard-target calibration techniques (Foote et al., 1987). On survey, data were integrated in 2 m depth bins from 2 to 250 m beneath the transducers, over a time period of 30 s (= 12 pings), which at a speed of 9 km h⁻¹ corresponded to a horizontal distance of 75 m. A 12-ping integration period was chosen as a compromise between the desire to maintain fine-scale spatial resolution and the necessity to compensate within an ensemble for the expected stochastic between-ping variation in echo intensity (see, for example, Martin et al., 1996). Integrated data were recorded to a Unix workstation using custom-written logging software. The depths of scattering layers were determined in real time with reference to the 38 kHz echo-trace, which was displayed on screen at the maximum sensitivity possible with the EK500 (S_v colour minimum = -100 dB, noise margin = 0).

2.2. Net sampling

The majority of net samples were taken during January 1996 using a Longhurst-Hardy Plankton Recorder (LHPR; see Longhurst and Williams, 1976; Williams et al., 1983). Sampling formed part of a zooplankton community study centred upon an off-shelf location at 39.403°W, 53.039°S. The LHPR was rigged with a 38 cm diameter nose-cone and 200 µm mesh gauze. Gauze advance interval was set at 30 s (corresponding with the echo-integration period), and was controlled using a custom-built down-wire net monitor (DWNM) commanded along the conducting towing cable by a shipboard PC (Woodroffe, 1997). In addition to recording time of gauze advance, the DWNM also recorded net depth, flow rate, water temperature, conductivity, fluorescence, light level, tow-angle and net altitude above bottom. Typically the LHPR was fished obliquely down to the depth at which the echo-sounder display indicated the layer of interest was located. The depth of the LHPR was then adjusted (by altering the length of towing cable out) to maintain it within the layer for the majority of the haul, before the net was finally fished obliquely back to the surface. During the descent and ascent phases of each haul the net often passed through shallower scattering layers, providing additional samples from these and information on the between-layer interfaces. The net also fished regions of empty water where nothing appeared on the echo-trace. The analyses reported here are based upon six LHPR hauls, which together comprised over 300 discrete and independent plankton samples. Additional net samples, including samples from layers exhibiting upward migration at dusk, from krill swarms, and from near-bottom high density zooplankton aggregations, were obtained using a multiple-opening 8 m² rectangular mid-water trawl (RMT8; Roe and Shale, 1979), which was also controlled via the down-wire net monitor. Echo-sounder configuration remained the same during all fishing events, and so acoustic data collected, for example, from swarms of *Euphausia superba* identified by RMT8 hauls had the same sampling resolution and were hence directly comparable with data collected from layers identified by LHPR to be comprised, say, of *E. frigida*.

2.3. Net sample analysis

On retrieval, LHPR gauzes were cut into lengths corresponding to discrete 30 s fishing samples, and frozen for transport to the UK. In the laboratory, gauzes were defrosted and their contents washed into separate specimen bottles and preserved in buffered formalin. Individual plankton samples were then examined to identify those in which one single zooplankton species contributed > 95% to the total sample volume: these samples were subsequently considered to be effectively monospecific. Where numbers of individuals within these samples permitted, length–frequency distributions were determined by measuring the lengths of 100 individuals chosen at random; for euphausiids total length (Morris et al., 1988) was measured, L₁ (Pakhomov and Perissinotto, 1996) was measured for amphipods, and prosome length was measured for copepods. All RMT8 catches were sorted aboard ship immediately and, where appropriate, total lengths of 100 randomly chosen

euphausiids and mysids were measured. The total catch volume and that attributable to the dominant component species were recorded.

2.4. Reconstruction of net trajectories.

The positions of the nets at the beginning and end of each discrete sampling period (i.e., every 30 s for the LHPR) were determined relative to the acoustic transducers by trigonometry. Net depth at time was recorded by the down-wire net monitor, and length of towing cable out was logged continually to the central shipboard computing system. These two parameters were used in a manner similar to that described by Zhou et al. (1994) to determine the distance of the net behind the transducers. This distance was converted into a time offset with reference to the speed at which the net was being towed (typically 9 km h^{-1} for the LHPR), and this time offset was then used in conjunction with the net depth record to replot the net trajectory over the echo chart. This enabled each zooplankton sample to be associated directly with the corresponding integrated echo data. Distance of the net aft of the transducers ranged, for example, from 100 m with the net at a depth of 10 m, to 900 m with the net at 250 m. At a towing speed of 9 km h^{-1} these distances corresponded to time offsets of 40 s and 6 min, respectively.

2.5. Acoustic data processing

Acoustic data were calibration-corrected and edited to remove erroneous points due to surface noise, bottom integration or false bottom echoes, using a custom-written AVS-based data editor (Socha et al., 1996). TVG-amplified background noise also was removed from each frequency using this software (Watkins and Brierley, 1996).

2.6. Alignment of acoustic and net data

Processed acoustic data were loaded into an Oracle database. All data corresponding to zooplankton samples where one single species dominated the catch were then selected from this database on the basis of depth and time, with reference to the reconstructed net trajectory. This enabled us to compile separate subsets of acoustic data corresponding to each macrozooplankton species caught in isolation, and ensured that each subset included all such data available for each species. Species-specific data sets therefore were not simply comprised of replicate measurements from within single layers.

2.7. Echo discrimination

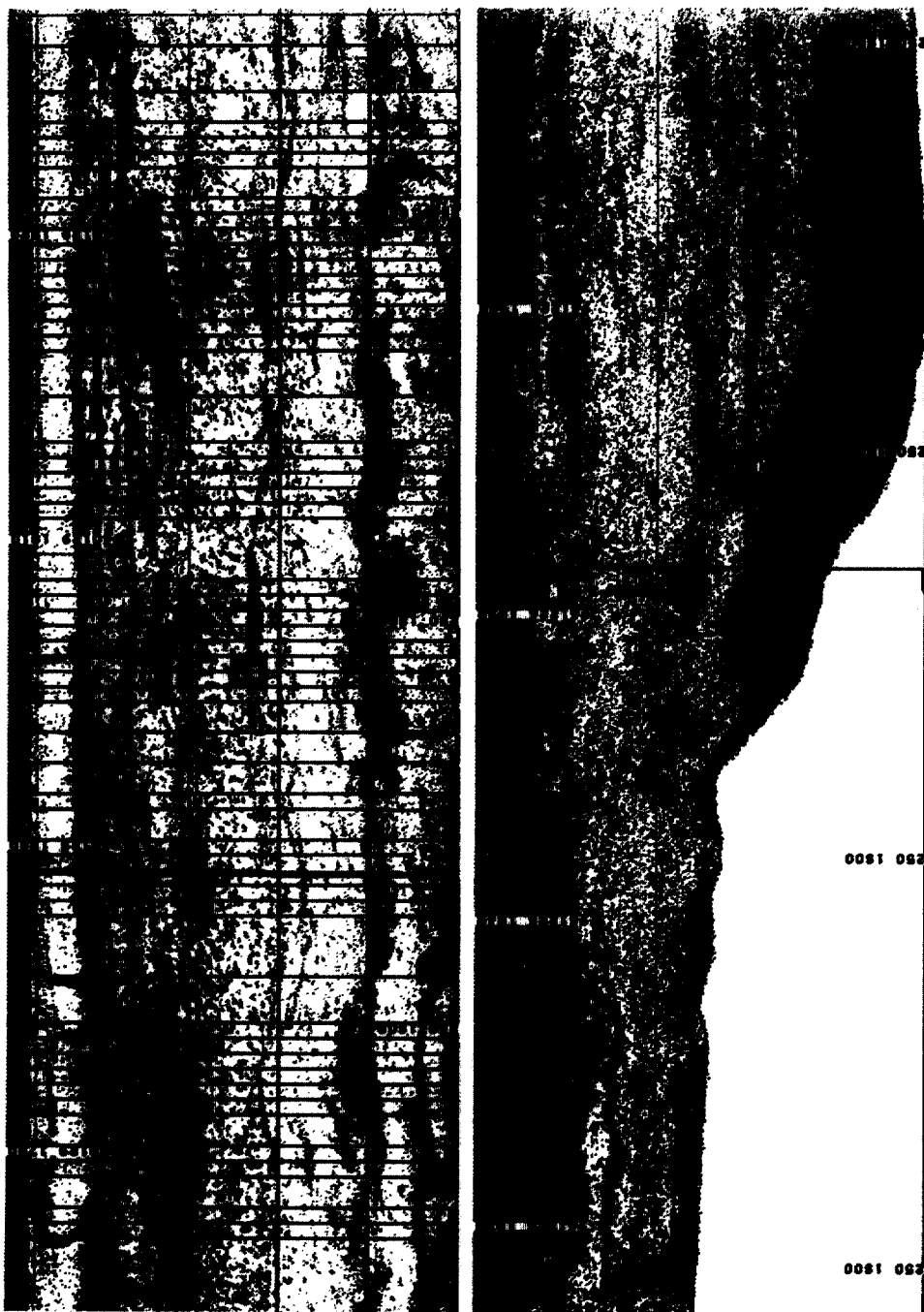
Madureira et al. (1993b) suggested that echoes from *Euphausia superba*, *Themisto gaudichaudii* and *Euphausia frigida* could be distinguished by the difference between their backscattering strengths at 38 and 120 kHz (δmVbs 120–38 kHz). They also presented regression relationships between 38 and 120 kHz signals for each

species. In order to compare our data with these findings, and to further investigate the extent to which additional species could be separated on this basis, we first constructed plots of 120 kHz data against corresponding 38 kHz signals for each of six macrozooplankton species caught from essentially mono-specific aggregations. Linear regression equations with 95% confidence limits for the slopes and intercepts were calculated for each species. To investigate the extent to which species could be further discriminated following the additional inclusion of data at 200 kHz, we performed discriminant function analysis (DFA) on all species-specific integration data where values for all three frequencies were available. Signals at 200 kHz are attenuated rapidly in sea water, and the effective range of our 200 kHz transducer is not much greater than 50 m; beyond this range the signal becomes dominated by noise. All data originating from targets below 50 m were therefore discarded. This reduced the number of data points available for analysis, and totally excluded data from the mysid *Antarctomysis maxima* because this species was only caught in near-bottom regions below 180 m. The smallest remaining data set was that relating to *Thysanöessa macrura*, which comprised 97 points. To produce data sets of equal size for further analysis, 97 points were chosen at random from the data relating to the other four species. Each three-frequency data set was then used to derive $\delta mvbs$ 200–120 kHz and $\delta mvbs$ 200–38 kHz for each species. Calculation of these $\delta mvbs$ parameters removed any absolute intensity effect from the signals, and enabled information from all three frequencies to be incorporated into the DFA without problems of multicollinearity. Each of the resulting 10 $\delta mvbs$ data sets was tested for departure from normality using the Anderson–Darling test in Minitab (Minitab Inc., 1994). Species-specific data sets were then further divided at random into two parts, designated training and testing. Mahalanobis Distance (squared distance) linear discriminant function analysis (see Manly, 1986) was then performed in Minitab, using discriminant functions developed from the training sets to classify data in the testing sets. The overall effectiveness of the discriminant functions was assessed as the proportion of all data correctly classified. The size ratio of training to testing data sets was varied to investigate the sensitivity of the analysis to sample size.

3. Results

Multiple standard-target calibrations performed before and after the acoustic surveys reported here indicated that the echo-sounder calibration remained consistent throughout the study period (38 kHz – number of calibrations = 7, standard error = 0.06 dB; 120 kHz – $n = 6$, se = 0.04 dB; 200 kHz – $n = 6$, se = 0.17 dB).

Fig. 1. Example 38 kHz echo charts from (a) the zooplankton community study area and (b) an on-shelf location to the north of South Georgia. (a) shows discrete scattering layers composed predominantly of (A) *Themisto gaudichaudii*, (B) *Rhincalanus gigas*, (C) *Thysanöessa macrura* and (D) *Euphausia frigida*. (b) shows dense, mono-specific aggregations of (E) *Euphausia superba* and (F) *Antarctomysis maxima*. Depth range is from 0 to 250 m, time stamps are every 10 min (approx. 1.5 km). Vertical bars (demarking some integration intervals) occur at a maximum frequency of once every 30 sec.



The zooplankton community study during which the majority of data presented here were collected coincided with a period of exceptionally calm weather and flat seas. These sea conditions were optimal for acoustic studies since surface noise and bubble entrainment were minimal, and pitching and rolling of the research vessel were much reduced. Under these conditions, reducing the colour minimum on the echosounder's 38 kHz display to the lowest value attainable (–100 dB) revealed a highly structured pattern of backscatter in the upper 250 m of the water column (Fig. 1a). On the 120 and 200 kHz echo-traces this level of biological detail was obscured by noise that was amplified increasingly by time-varied gain with depth, and dominated these displays if the colour minima were reduced to the same level. Signal post-processing enabled valid data to be obtained from the 120 kHz channel to 250 m, but for provision of the information necessary in real-time for positioning the net the 38 kHz channel was most effective.

The 38 kHz display showed clearly the existence of a series of horizontally continuous yet vertically discrete scattering layers (Fig. 1a). These layers began just below the thermocline (at approximately 40 m; detected by the LHPR-mounted temperature sensor) and continued to at least 250 m, the maximum depth of our survey. The horizontal layers often included gentle undulations (see Fig. 1a), probable biological manifestations of physical internal ocean waves (Clay and Medwin, 1977; Brandon et al., in prep). Horizontal scattering layers were detected periodically throughout the January/February 1996 zooplankton community study and, during hours of daylight, appeared to be persistent. Repeated fishing of these layers with the LHPR during the day enabled us to establish that, at that time, each was composed predominantly of single macrozooplankton species, since one species alone accounted typically for more than 95% of the total catch volume on all LHPR gauzes fished solely within a single layer. In the example in Fig. 1a these species were the hyperiid amphipod *Themisto gaudichaudii*, the eucalaniid copepod *Rhincalanus gigas* and the euphausiids *Thysanöessa macrura* and *Euphausia frigida*. The discrete depth distribution of each layer enabled us to ascribe acoustic signals to individual zooplankton species with a high degree of confidence, and to obtain replicated acoustic measurements from each species. Acoustic data also were obtained from dense, mono-specific aggregations of the Antarctic krill *Euphausia superba* (Fig. 1b) and the mysid *Antarcromysis maxima*, which were identified using the RMT8 net.

Length-frequency distribution histograms for each macrozooplankton species encountered within essentially monospecific aggregations during this study are given in Fig. 2.

Plots of 120 kHz signal strength against corresponding 38 kHz values are given in Fig. 3 for each of the six macrozooplankton species that our netting procedures were able to capture in effective isolation. Regression equations and 95% confidence limits for the slopes and intercepts of these relationships are given in Table 1.

Anderson–Darling tests suggested that six of the 10 δ mvbs 200–120 kHz and δ mvbs 200–38 kHz data sets deviated significantly from normality at the 95% level. Discriminant function analysis requires that data sets be normal in order to establish the statistical significance of any group differences. However, effective discrimination still can be achieved between data from non-normal distributions (Manly, 1986) and, in

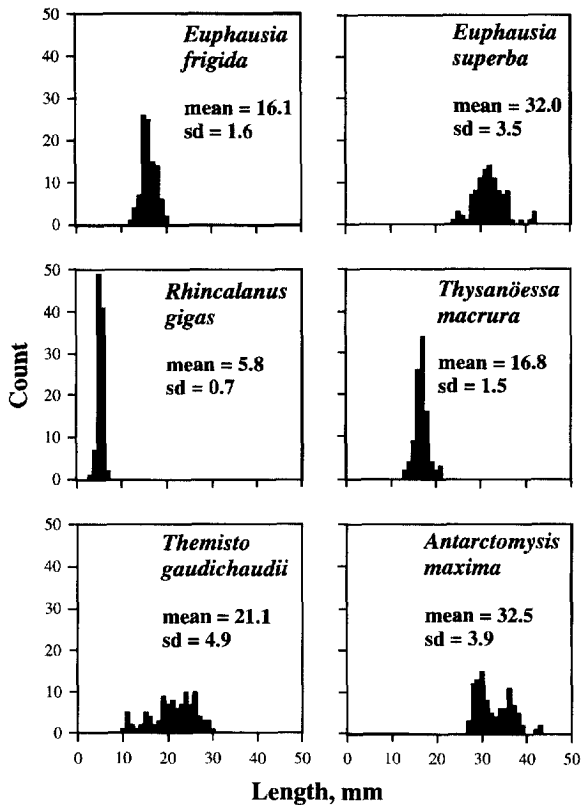


Fig. 2. Typical length frequency histograms for the six macrozooplankton species encountered in essentially monospecific aggregations. Sample sizes for all distributions are 100.

these cases, the rate at which individuals are misclassified can be used as a pragmatic evaluation of the effectiveness of the discriminant function (Krzanowski, 1988). Unbiased estimates of error rates can be obtained by splitting the data sets at random into two portions and using one part to train the discriminant function, i.e. to establish the allocation rule, and one portion to assess the performance of the rule by determining the proportion of individuals misclassified. Table 2 shows the confusion matrix for classification of 33 individuals from each species group on the basis of a Mahalanobis distance linear discriminant function developed from training sets of 64 individuals from each group. Other sample-splitting ratios (training:validation) also were tested (1:1 and 3:1), but did not differ significantly in their outcomes, indicating that the discriminant function was not subject to large sampling fluctuations (see Krzanowski, 1988). Table 2 shows that on the basis of $\delta mvbs$ 200–38 kHz and $\delta mvbs$ 200–120 kHz values alone, Mahalanobis distance linear discriminant function analysis classified five Southern Ocean zooplankton species with an overall success rate of 77%.

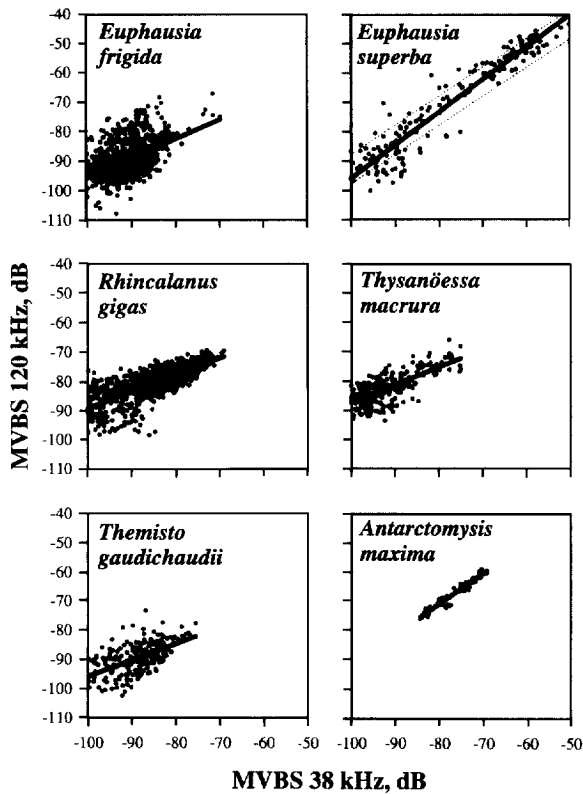


Fig. 3. Scatter plots showing the relationships between mean volume backscattering strength at 38 and 120 kHz for six species of Southern Ocean zooplankton. The dotted lines on the plot for *Euphausia superba* demarcate the 2–12 dB $\delta mvbs$ 120–38 envelope suggested by Madureira et al. (1993a) as being diagnostic of krill.

Table 1

Regression equations and 95% confidence intervals for slopes and intercepts of relationships between mean volume backscattering strengths at 120 kHz (y) and 38 kHz (x) for six Southern Ocean zooplankton species.

	n	Regression equation	r^2 , %	Intercept \pm 95%	Slope \pm 95%
<i>Euphausia frigida</i>	939	$y = -30.3 + 0.66x$	25.9	-35.70 to -24.90	0.60 to 0.71
<i>Euphausia superba</i>	180	$y = 16.8 + 1.13x$	91.5	13.06 to 20.54	1.08 to 1.18
<i>Rhincalanus gigas</i>	1086	$y = -33.2 + 0.56x$	55.7	-35.58 to -30.82	0.53 to 0.59
<i>Thysanöessa macrura</i>	273	$y = -27.6 + 0.60x$	50.9	-32.35 to -22.85	0.55 to 0.65
<i>Themisto gaudichaudii</i>	218	$y = -40.1 + 0.56x$	32.0	-46.51 to -33.69	0.49 to 0.63
<i>Antarctomysis maxima</i>	88	$y = 13.7 + 1.06x$	95.8	11.80 to 15.60	1.04 to 1.08

Table 2

Confusion matrix. Percentage successful recognition using discriminant functions. Successful classifications in bold.

Predicted species	Actual species				
	<i>Euphausia frigida</i>	<i>Euphausia superba</i>	<i>Rhincalanus gigas</i>	<i>Thysanöessa macrura</i>	<i>Themisto gaudichaudii</i>
<i>Euphausia frigida</i>	76	3	3	3	21
<i>Euphausia superba</i>	0	76	0	0	0
<i>Rhincalanus gigas</i>	6	21	85	24	0
<i>Thysanöessa macrura</i>	18	0	12	73	0
<i>Themisto gaudichaudii</i>	0	0	0	0	79

4. Discussion

The horizontally continuous and vertically discrete nature of scattering layers lends these features well to studies attempting to link acoustic data with net samples: persistence of aggregations of single species serves to reduce the degree of error likely when sampling a dynamic oceanic environment with two techniques, of necessity at slightly different times. High-resolution netting studies revealed that each of the discrete sound-scattering layers detected here in the upper 250 m at the zooplankton community study site was predominantly mono-specific in composition. Netting also revealed that the scattering layers were separated by strata essentially devoid of macrozooplankters. Patchiness in the horizontal distribution of zooplankton is a well recognised phenomenon (e.g., Haury et al., 1978) and is a major feature of acoustic surveys of Antarctic krill (e.g., Murphy et al., 1988; McClatchie et al., 1994; Brierley and Watkins, 1996). Acoustic investigations also have revealed heterogeneity to be a common feature of the vertical distribution of organisms in the ocean (Clay and Medwin, 1977). Such vertical heterogeneity ranges in scale from hundreds of metres for the so-called deep scattering layers (e.g., McCartney, 1976) to less than ten metres, as here, amongst near-surface scatterers (e.g., Richter, 1985; Greene et al., 1992; 1994). Mechanisms causing these vertical stratifications are likely to be both physical (e.g., Wiebe et al., 1996) and behavioural (Bergström and Strömberg, 1997), and predator-prey interactions within zooplankton communities may also promote species segregation (Levin, 1990; Levin et al., 1993). Acoustics is an ideal tool for the study of zooplankton distribution (e.g., Macaulay, 1994; Wiebe and Greene, 1994; Holliday and Pieper, 1995) and, in conjunction with contemporaneous underway physical measurements, can be used to relate distribution to hydrography (e.g., Webber et al., 1986).

Fundamental to the study of species distributions and interactions is the ability to identify those species involved. Although it is comparatively easy to obtain acoustic measurements from scattering layers, it has traditionally proved difficult to obtain direct samples from these features (Clay and Medwin, 1977). Improvements in

sampling technology, however, have enabled simultaneous acoustic and biological sampling on increasingly finer scales (e.g., Greene et al., 1991b; Ricketts et al., 1992; Zhou et al., 1994), and hence for specific acoustic signals to be directly associated with the zooplankton aggregations responsible for them. Using such techniques in the Southern Ocean, Madureira et al. (1993b) have shown that three common swarm-forming macroplankters can be separated on the basis of their relative echo-strengths at 38 and 120 kHz. Improvements in the spatial resolution attainable with standard fisheries echo-sounders since that study, and the additional availability of data at 200 kHz, have enabled us to build here upon that work through analysis of larger data sets. We also have been able to include data from other zooplankton species commonly encountered in the region, and consequently to investigate further the utility of acoustic signal characteristics as species descriptors.

The y -axis intercept of the linear regression relationship between backscattering intensity at 120 and 38 kHz for *Euphausia frigida* given by Madureira et al. (1993b) (-25.10 dB) falls within the 95% confidence interval of the intercept derived here from our data for the species (see Table 1). Madureira et al. (1993b) do not provide error estimates for their regression parameters, but the very close proximity of their slope value for *E. frigida* to ours makes it seem probable that the 95% confidence limits for the slopes of both data sets would also overlap. The mean length of *E. frigida* from net samples during the present study was 16.1 mm (SD = 1.6); Madureira et al. (1993b) reported a mean length for *E. frigida* of 18.5 mm (SD = 1.9), and this similarity in size between studies accounts for the congruence in acoustic data. It is likely that the slopes for the *Euphausia superba* data in both studies are likewise not statistically separable. The y intercept reported by Madureira et al. (1993b) for *E. superba* however, falls below our estimate of the 95% confidence limits for this parameter. Theory predicts (Stanton et al., 1994) and field measurements have demonstrated (Watkins and Brierley, 1997) that the difference between backscattering at 120 and 38 kHz caused by krill is dependent upon krill size. The krill caught here were somewhat smaller (mean length = 32.0 mm, SD = 3.5) than those observed by Madureira et al. (1993b) (mean = 38.7 mm, SD = 4.8), and the relative increase in δm_{vbs} 120–38 kHz we have observed is consistent with the expectations of sound scattering by smaller euphausiids. The estimates of the slope and intercept for *Themisto gaudichaudii* published by Madureira et al. (1993b) both fall outside our 95% confidence intervals for these parameters. We are unable to make direct comparisons of length between studies for this species since Madureira et al. (1993b) do not specify which length measurement they employed (see Pakhomov and Perissinotto, 1996). However, inspection of coefficients of variation of length measurements suggests that the *T. gaudichaudii* we encountered exhibited greater variation in size than those of Madureira et al. (1993b) and this may have some influence on the overall relationship between 38 and 120 kHz. The high variability within our 38 and 120 kHz data for *T. gaudichaudii* is further illustrated by the low r^2 value for the species (Table 1).

Although Madureira et al. (1993b) were able to partially distinguish three Antarctic zooplankton species on the basis of their respective backscattering intensity values at 120 and 38 kHz, inspection of Fig. 3 and Table 1 clearly shows that after the inclusion

here of additional species, none remains uniquely identified by these parameters. None of the data presented by Madureira et al. (1993b) had mvbs 38 kHz values less than -90 dB. The improved dynamic range offered here by the EK500 compared to the previous generation of echo-sounder used in their study, and the finer-scale spatial resolution of our integrated acoustic data, however, has enabled us to detect and include echoes of much lower intensity in our analysis. Many of the 38 kHz signals considered here fall between -90 and -100 dB, and it is within this region that differences in echo characteristics at 38 and 120 kHz between species become most obscured (see Fig. 3). Since a greater proportion of our data for *Euphausia frigida* fall towards the lower end of the signal intensity range this may explain why, for example, the r^2 value associated with the 38–120 kHz relationship for this species is greater than those for *E. superba* or *Antarctomysis maxima*. The increased scatter observed in the relationships between 38 and 120 kHz signals for all species with decreasing signal strength is most probably caused by an increasing contribution of noise to total signal at low signal strength. Despite the inability of the 38 and 120 kHz relationships to distinguish unambiguously between all species throughout the range of signal strengths detected here, some echoes from *Euphausia superba* could be separated from those from all other species sampled here by the imposition of a -70 dB threshold on the 38 kHz data, and subsequent application of the 2–12 δ mvbs 120–38 kHz envelope advocated by Madureira et al. (1993a) as being indicative of krill. Thresholding at -70 dB would eliminate the majority of the non-krill targets and, because of the tendency for *Euphausia superba* to form high density aggregations in hours of daylight, would only reject signals from the data set displayed in Fig. 3 that contribute $\approx 1.5\%$ to total krill biomass. This procedure may therefore offer a sufficiently robust identification criterion for krill standing stock surveys, for which variance estimates are commonly far greater than 1.5% (e.g., Brierley et al., 1997).

Whilst the 38–120 kHz regression relationships given in Table 1 are themselves insufficient to delineate between species, further inclusion of data gathered at 200 kHz, and application of simple linear discriminant techniques to parameters derived from all three frequencies, has enabled five Southern Ocean zooplankton species to be classified with a success rate of $> 77\%$ (Table 2). A scatter plot of δ mvbs 200–38 kHz against δ mvbs 200–120 kHz (Fig. 4) illustrates how these species are separable by these parameters. These species include three euphausiids with broadly similar anatomy (*Euphausia superba*, *E. frigida* and *Thysanoessa macrura*—see Martin et al., 1996), two of which had overlapping length frequency distributions (*T. macrura* mean length = 16.8 mm, SD = 1.5, and *E. frigida* length = 16.1 mm, SD = 1.6; see Fig. 2). Although one would expect animals exhibiting greatly different size distributions (as here, for example, when comparing *Rhincalanus gigas* mean length = 5.8 mm, SD = 0.7 and *Themisto gaudichaudii* mean = 21.1 mm, SD = 4.9; see Fig. 1) to elicit different acoustic responses at a number of frequencies (see Greenlaw, 1979), one would not necessarily expect to be able to distinguish in the same way between samples of species with such similar lengths as, for example, *T. macrura* and *E. frigida*. Echo strength from individual animals at any given frequency, however, is likely to be determined by a complex, non-linear interaction of factors and not simply by the animal's length alone. These factors may include shape, size, density and orientation.

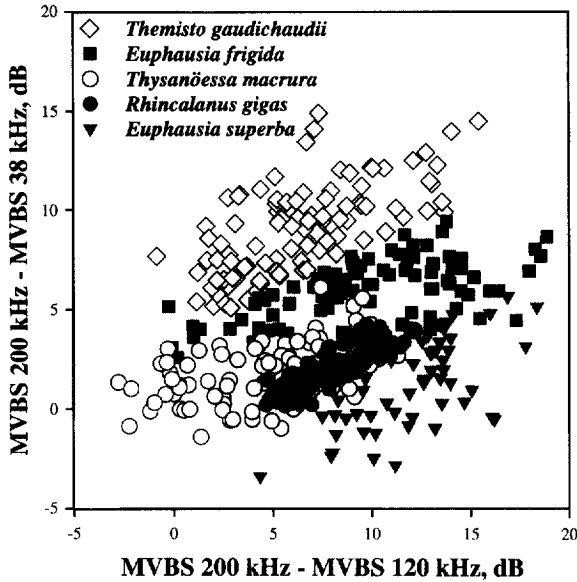


Fig. 4. Scatter plot of $\delta mvbs$ 200–38 against $\delta mvbs$ 200–120 for five Southern Ocean zooplankton species.

It is probable therefore that the curves describing the scattering response (target strength) over a range of frequencies will differ between species (e.g., Stanton et al., 1996), even if these species have similar lengths. The relative positions of peaks and nulls in backscattering strength along such curves are likely to occur at different frequencies for different species, and it is for this reason that calculation of $\delta mvbs$ values over a range of frequencies will result in differences between species becoming apparent. When considering a limited number of discrete frequencies (as here), the occasional coincidence of nulls in backscattering strength for a particular species with these frequencies may lead to deviations from the generally expected trend of increasing target strength with increasing frequency (e.g., *E. superba*—see Fig. 4), and these deviations may become strong species prognosticators. In addition, whilst echo strength over a range of frequencies can be modelled well for idealised engineering shapes (e.g., Stanton, 1990), biological forms have complex arrangements of appendages, and are subject to physiological variables such as maturity and state of nutrition, all of which are likely to complicate scattering patterns such that echoes from zooplankton are unlikely to conform exactly to expectations based upon idealised shapes. Although these biological nuances may be the cause of consternation to modelers, it would appear that their influences upon sound scattering may be exploited by the biologist seeking pragmatic mechanisms to discriminate *in situ* between zooplankton species. Here we do not seek to explain the physical processes underlying the differences in sound-scattering exhibited by the species we have observed, only to point out that the different responses can be used to distinguish between them.

The fact that *Antarctomysis maxima* had to be excluded from the three-frequency DFA because it was only encountered in deep water is in itself instructive, and illustrates the value of including as wide a range of information as possible in the development of species discrimination techniques. Mysids could, for example, be described uniquely here as those aggregations located close to the bottom where the backscatter at 38 kHz exceeds -70 dB. This type of multi-character approach, combining information on aggregation dimension and location with backscattering strength, has been adopted with much success to discriminate between fish shoals (e.g., Haralabous and Georgakarakos, 1996; Scalabrin et al., 1996; Swartzman, 1997). Such analyses also have demonstrated that artificial neural networks (ANN) provide superior powers of discrimination compared to DFA. Simmonds et al. (1996) also found ANN analysis to be more effective than DFA at classifying multi-frequency echoes from fish into species groups. Application of ANN analysis to the data presented here, and increasing the depth range over which multi-frequency data can be obtained through the use of deep-towed transducers (and consequently reducing the potential range-dependence of signals characteristic of species found only in deep water; see Kloser, 1996), would be the next logical progression for this work.

The ability to discriminate between species using non-specialised hardware and simple linear processing provides a powerful and accessible tool with which to investigate *in situ* the distribution of single zooplankton species, and hence offers a means to investigate ecological and physical mechanisms driving species segregation. In the Southern Ocean, where macrozooplankton species diversity is generally low (McGowan and Walker, 1993), and on an occasion when prevailing sea conditions were unusually calm, this simplistic approach to species discrimination appears to have functioned well. In other oceans, where the numbers of species with similar gross morphological characteristics may be higher, and in situations where individual zooplankton are found within more heterogenous aggregations of species, the likelihood that data from three frequencies alone would resolve species identity unambiguously is reduced. Ultimately, it may be possible to invert multi-frequency acoustic signals in real time to classify automatically animals into species and size-classes, and estimate the abundance of each (Holliday and Pieper, 1995; Martin et al., 1996). Although we recognise that it may not be possible to extrapolate directly from our findings into other less ideal situations, we have shown here that, with standard technology, progress toward this goal can be made.

Acknowledgements

We should like to thank A. Atkinson, R. Shreeve, D.G. Bone, H.I. Daly and R. Snýder for assistance with net sampling, P. Woodroffe for technical support of the echo-sounder and down-wire net monitor, and A.W.A. Murray for helpful discussion of statistical techniques. We are also grateful for the efforts of the Master, Officers and crew of RRS *James Clark Ross* during cruises JR11 and JR17.

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