Improving acoustic estimates of krill: experience from repeat sampling of northern krill (*Meganyctiphanes norvegica*) in Gullmarsfjord, Sweden

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A series of eight replicated acoustic surveys, four by day and four by night, was undertaken in Gullmarsfjord on the Swedish west coast during two 24-h periods on 8 and 10 September 2003, using a calibrated echosounder operating at 120 and 38 kHz. The difference in signal strength (ΔS_v) was used to distinguish northern krill (*Meganyctiphanes norvegica*) from other acoustic scatterers. The approach is concluded to be very effective, but it can be improved greatly by applying the following series of simple extensions to current protocols: first, set a very low threshold on both frequencies to minimize sampling bias; second, undertake tests to confirm that the data extracted from each acoustic frequency apply to the same scatterers; third, ensure that the range of ΔS_v is not greater than the TS_{range} at either frequency; and finally, when abundance estimation is the primary aim, arrange for sampling at the time of day and using the acoustic frequency that together provide the least variance.

Keywords: acoustic surveys, dB difference, *Meganyctiphanes norvegica*, northern krill.

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Introduction

There are several difficulties associated with sampling pelagic species in an open ocean environment. In addition to the three-dimensional structure of the water body, it is continually moving, and within it, zooplankton themselves move. Consequently, repeat sampling at the same location is no guarantee that the same part of a population is being sampled. To investigate this topic, sampling needs to be restricted to a known and constrained part of the population.

An ideal site for such a study is in the Gullmarsfjord on the west coast of Sweden; it provides a semi-enclosed environment containing significant numbers of northern krill (*Meganyctiphanes norvegica*). The bathymetry is typical of a fjord, with a deep inner basin and shallow sill at the entrance (Lindahl and Hernroth, 1988). The depth of the sill means that there is a frequent exchange of surface water, but the exchange of deepwater is infrequent and only during winter or spring (Lindahl and Perissinotto, 1987). In this environment, calanoid copepods remain in the deeper waters of the main fjord for most of the year, but are washed out and replaced during the major water-exchange events (Lindahl and Hernroth, 1988). A similar process has been noted for *M. norvegica*, which spends much of the time in deep water below the thermocline (Bergström and Strömberg, 1997). Arising from this, providing that there has not been a major exchange of water in the fjord, the "population" of *M. norvegica* within the fjord can be used to investigate variability in the short term.

The primary aim of the study was to investigate variation in the distribution of *M. norvegica* in the short term by repeat sampling along a pre-determined track. At the same time, we used the sampling programme to examine the effectiveness of current analytical protocols. Our primary sampling tool was a calibrated echosounder, supported by environmental sampling using a conductivity, temperature, and depth sensor (CTD) and net hauls to identify zooplankton. The application of acoustics to the study of zooplankton distribution and abundance has increased in recent years, as reviewed by Foote and Stanton (2000), who noted that much recent work has centred on the use of multi-frequency systems of identifying the main taxa. In this study, we used a dual-frequency system to investigate the three key aspects of thresholding effects, the selection of dB difference ranges, and the estimation of density, with a view to improving the quality of estimates of krill distribution and abundance.

The planned programme embodied similar sampling protocols to that specified for the large-scale acoustic survey for krill organized by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR; SC-CAMLR, 2000). During two day and night periods during the same week, we sampled the krill using acoustics and nets.

Material and methods

Field sampling was undertaken in the Gullmarsfjord on the west coast of Sweden (Figure 1) on board the RV "Arne Tiselius" of the Kristineberg Marine Station.

CTD profiles were made during each voyage at a single site at 58°19.2′N 11°32.7′E, the deepest part of the fjord. Water bottle samples were taken on the first deployment, but not subsequently. Profiles of salinity and temperature are shown in Figure 2.

Acoustic equipment and data logging

A Simrad EK60 scientific echosounder operating at 38 and 120 kHz with split-beam transducers, installed and maintained according to the manufacturer's specification, was used for the study. It was interfaced to a GPS to provide accurate information on vessel position and speed. It was calibrated according to the Simrad calibration protocol on 3 September 2003, in a sheltered bay on the eastern side of Gullmarsfjord, with the vessel moored fore and aft to the cliff face. The following environmental information was derived from a CTD cast in the fjord earlier the same day: temperature, 17°C; salinity, 32; sound velocity, 1510 m s^{-1}. A standard tungsten carbide sphere with theoretical target strengths (TSs) of −42.35 (38 kHz) and −39.62 (120 kHz) was used for the calibration (Simmonds and MacLennan, 2005). Table 1 lists the parameter values from this calibration that were set as default values on the echosounder for the duration of the project.

Raw data were logged onto a PC, and backup files were made after each half-day voyage.

Net sampling

An Isaacs-Kidd Midwater Trawl (IKMT) with a mouth opening of 1 m^2 and mesh size of 1.5 mm was used for most net sampling. A few surface hauls were also carried out using a WP-2 ring net of 80 cm diameter and 1 mm mesh. The IKMT net was monitored with a Scanmar trawl sensor to determine net depth and rate of change of depth. The volume sampled by the IKMT was determined by a calibrated flowmeter.

It was not feasible to carry out net tows along all acoustic transects within the time available. Therefore, a position, indicated in Figure 1, midway along the survey area was used for net hauls under the assumption that it contained an average representation of the principal backscattering fauna.

Two double oblique hauls were made with the IKMT near the centre of the deepest part of the fjord and close to the centre of the survey area. One haul covered the depth range from the surface to as close to the seabed, ~110 m, as practicable, and the other covered the depth range from the surface to some 10 m above the main acoustic-scattering layer, generally down to 50 m. The WP-2 net was deployed at the surface, close to the boat, as the IKMT hauls were taking place. Details of all net deployments are set out in Table 2.

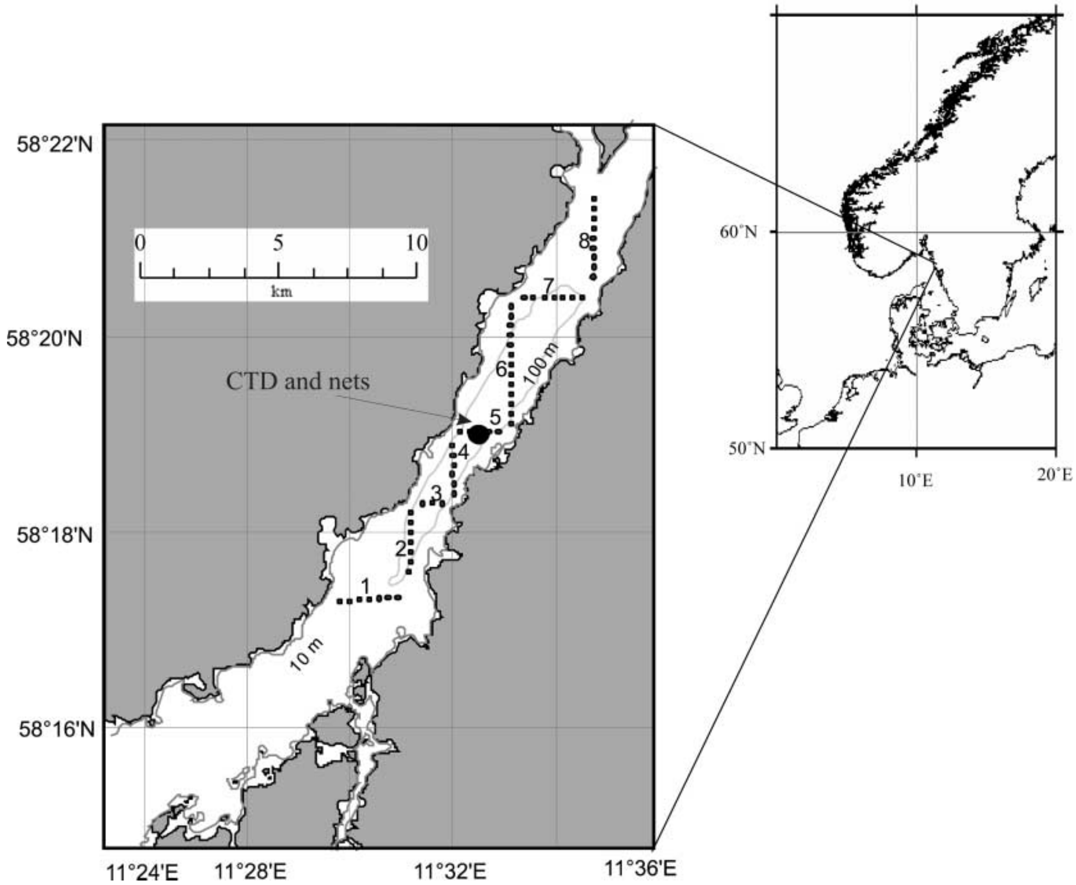

Figure 1. Gullmarsfjord, showing the paths of the sampling transects. Net hauls and CTD samples were undertaken where shown, in the deepest part of the fjord between the centres of transects 4 and 6.

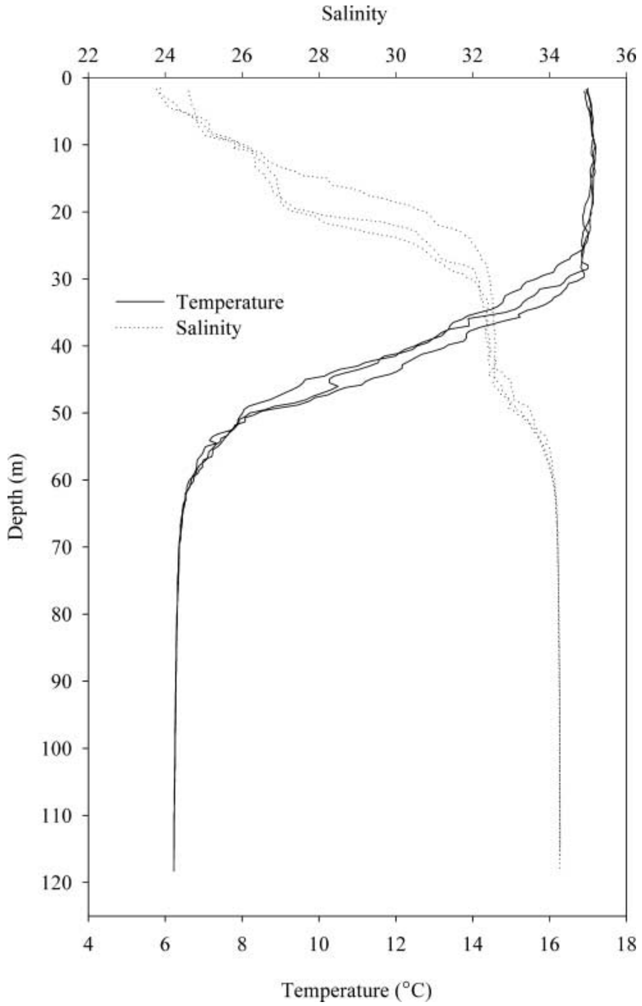

Figure 2. CTD profiles to show the physical characteristics of the water column in Gullmarsfjord on 8 and 10 September 2003, the days of the experimental sampling, and 3 September, the day on which the echosounder was calibrated.

Species composition and abundance of macrozooplankton were determined from these catches. In addition, the body lengths of all krill were measured to provide demographic information.

Sampling design

The Gullmarsfjord is a narrow fjord with its main axis orientated approximately northeast–southwest. The aim of the acoustic survey design was to provide a series of parallel transects running approximately normal to the depth contours across the fjord. Owing to the fjord's narrowness a design whereby all the series of transects were parallel would have resulted in transect lengths that would have been too short for practical navigation.

The chosen design was a zig-zag pattern of eight transects comprising four transects orientated approximately north-south and four approximately east-west. This provides for two independent series of near parallel transects for each survey. The design is shown in Figure 1. Transects 1, 3, 5, and 7 constituted the east-west series, and transects 2, 4, 6, and 8 the north-south series on each voyage.

The sampling programme for an individual voyage consisted of:

- acoustic transects in order 1 to 8 (referred to as the "Outbound" series);
- CTD and net hauls in the centre of the sampling area;
- Acoustic transects in order 8 to 1 (referred to as the "Return" series).

The timings of each survey are set out in Table 3.

Table 1. Calibration results from 3 September 2003.

Parameter	Units	38 kHz	120 kHz
Gain	dB	26.53	26.25
Absorption coefficient	dB km^{-1}	7.68	41.07
Estimated sound velocity	m s^{-1}	1 510	1 510
Sample interval	m	0.1933	0.1933
3 dB beam width	°	7	7
Pulse duration	ms	1.024	1.024
Transmit power	W	2 000	1 000
Draught correction	m	3.60	3.60

Acoustic-data analysis

Recent interest in the acoustic properties of zooplankton has involved modelling and direct measurements. The results of modelling studies have been reviewed recently by Foote and Stanton (2000) and Holliday and Stanton (2005). They highlight the strong frequency dependence of TS allied to size, shape, orientation, and the presence of gas inclusions as being the features of greatest influence. The models use information such as density and sound speed through the body components to estimate TS and have been corroborated largely through direct estimates on living animals.

Because of the difficulties in maintaining zooplankton in appropriate conditions, fewer studies provide direct estimates of TS. For *M. norvegica*, there are no direct measurements of TS, although Conti *et al.* (2005) suggested that, owing to the close similarity between the morphology and biochemical composition of *Euphausia superba* (Antarctic krill) and *M. norvegica*, the TSs of specimens of each species of the same size are likely to be more or less the same. The first series of direct measurements of TS of *E. superba* was reported by Foote *et al.* (1990), who found a TS of −76.1 dB (range 6.2) at 120 kHz and −85.1 (range 6.5) at 38 kHz during a series of cage experiments. The krill in that study were in the size range 30–39 mm total length (Foote *et al.*, 1990), a range almost identical to that in this study (Figure 3). Subsequent results have broadly agreed, although recent work by Demer (2004) suggests that the true TS should be lower.

There is a growing literature, mainly on Antarctic krill, using the difference in signal strength at two frequencies (referred to as ΔS_v hereafter) to identify echo traces likely to arise from krill (Everson *et al.*, 1993; Madureira *et al.*, 1993; Brierley *et al.*, 1998; SC-CAMLR, 2000; Woodd-Walker *et al.*, 2003).

We used the ΔS_v to identify the backscatter probably attributable to *M. norvegica.* The basic assumption behind the ΔS_v approach is that acoustic targets of a particular type have a generally predictable TS at a given frequency, such that the difference between the levels of returned echoes at two frequencies is

Table 2. Net deployments.

Net	Date (September 2003)	Time in	Time out	Mean latitude	Mean longitude	Maximum depth (m)	Volume filtered (m^3)
IKMT	8	09:36	09:50	58°19.8′N	11°33.3′E	55	602
IKMT	8	10:39	10:05	58°20.3′N	11°33.6′E	99	1 034
IKMT	8	20:58	21:17	58°18.9′N	11°32.1′E	99	823
WP-2	8	21:00	21:10	58°18.7′N	11°31.9′E	1	Not measured
IKMT	8	21:28	21:58	58°19.2′N	11°32.6′E	106	1 268
WP-2	8	21:37	21:45	58°19.2′N	11°32.7′E	1	Not measured
IKMT	8	20:20	20:37	58°19.0′N	11°13.2′E	106	Device failure
IKMT	10	10:10	10:34	58°19.5′N	11°32.8′E	97	981
IKMT	10	11:27	11:40	58°19.7′N	11°33.1′E	43	553
IKMT	10	21:28	21:45	58°20.0′N	11°33.3′E	56	689
WP-2	10	21:36	21:47	58°20.0′N	11°33.3′E	1	Not measured
IKMT	10	21:59	20:35	58°18.8′N	11°32.5′E	78	1 543
IKMT	10	20:54	21:11	58°19.4′N	11°32.8′E	64	Device failure

All times are universal time coordinated (UTC).

reasonably constant. In terms of backscatter, this is represented by

$$\Delta S_v = S_{v120} - S_{v38}, \quad (1)$$

where S_{v120} and S_{v38} are the backscatter at 120 and 38 kHz, respectively, the two frequencies used in this study. Conceptually, because volume backscatter can be attributable to a variety of organisms of varying size, it is easier to understand the principles in terms of individual scatterers and their respective TSs. Using the same subscripts as in Equation (1), this can be represented as

$$\Delta TS = TS_{120} - TS_{38}. \quad (2)$$

TS itself is dependent not only on frequency, but also on the size of the scatterer and its behavioural characteristics (Foote and Stanton, 2000; Holliday and Stanton, 2005). Thus, for a group of scatterers of the same type and exhibiting the same behaviour, the variance of the TS for a given frequency is likely to be small. The same is likely to be true for other frequencies used in fisheries research. The relationship between the variation in TS at one frequency, i.e. range TS, is compared with the range of differences between the frequencies, ΔTS, in Figure 4. Bearing in mind that for this study we are dealing with large numbers of individuals of similar size, the conditions under which the TS_{120} is greater than the overall mean are likely to be similar for TS_{38}. Also, because we are considering groups of krill that are free to move, it is unlikely that, through their behaviour, a high TS_{120} will be associated with a low TS_{38} or the converse. In short, a high TS_{120} is likely to be associated with a high TS_{38} and *vice versa*. Therefore, the ΔTS will vary around the mean difference, as indicated in Figure 5. The extreme cases shown above the tails of the distribution are very unlikely to occur because, simply and as noted above, a very low TS at one frequency is unlikely to be associated with a high value at the other frequency. Hence the range of ΔTS values is likely to reflect the TS range of each frequency. Hence, the ΔTS range is likely to be similar to the mean range of the TS ranges at the two frequencies.

If we define a mean TS range as

$$\frac{TS_{Range120} + TS_{Range38}}{2},$$

then a suitable range of values for ΔS_v would be

$$\text{Mean}\,\Delta TS \pm 0.5 \times (\text{mean TS range}). \quad (3)$$

This can be expressed, alternatively and probably more simply, by stating that the range of values for ΔS_v should not exceed the TS_{range} at either frequency.

In the absence of empirical experimental data on *M. norvegica*, we use the results of Foote *et al.* (1990) on *E. superba*, summarized in Table 4 (−76.1 dB at 120 kHz, −85.1 dB at 38 kHz), as being

Table 3. Survey timings between each “Outbound” and “Return” series of transect samples.

Survey	Date (September 2003)	Day or night	Transect direction	Time start	Time end	Duration (min)
1	8	Day	Outbound	07:28:01	08:28:24	60:23
2	8	Day	Return	13:32:00	14:37:00	65:00
3	8	Night	Outbound	19:17:30	20:19:10	61:40
4	8	Night	Return	23:02:00	00:06:00	64:00
5	10	Day	Outbound	07:31:30	08:38:15	66:45
6	10	Day	Return	12:01:00	13:05:49	64:49
7	10	Night	Outbound	19:31:11	20:39:28	68:17
8	10	Night	Return	23:37:32	00:52:00	74:28

Net haul and CTD stations were sampled in the deepest part of the fjord between the centres of transects 4 and 6 (Figure 1). All times are GMT. Local times are ahead of GMT by 46 min. Sunrise was at 04:30 and sunset at 17:50.

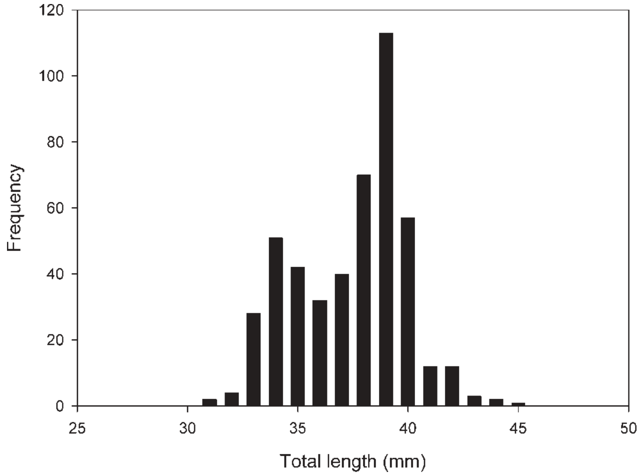

Figure 3. Length frequency of adult krill in Gullmarsfjord from depth-integrated samples taken on 8 and 10 September 2003.

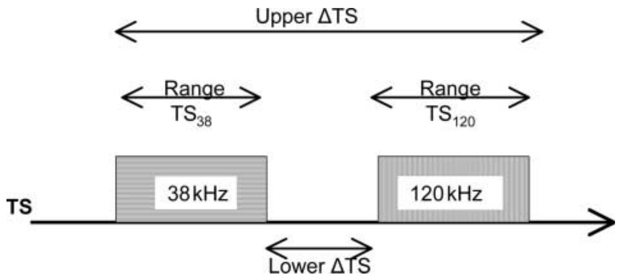

Figure 4. Schematic representation of ΔTS. The blocks labelled 38 and 120 kHz represent the expected range of TS values for a given type of scatterer.

the best "nearest" equivalents. Applying these results to Equation (3) yields a ΔS_v range of 5.8–12.2 dB. We have rounded these to a range of 6–12 dB for our analyses and compare our results with those using the range 2–12 dB used by Watkins and Brierley (2002) and SC-CAMLR (2000), a range approaching the value of the upper ΔTS from our Figure 4.

There is one further point that needs mentioning here, namely that the analysis is aimed at identifying particular types of scatterer, following on from which must be the assumption that the estimated numerical densities at the two frequencies should not be significantly different.

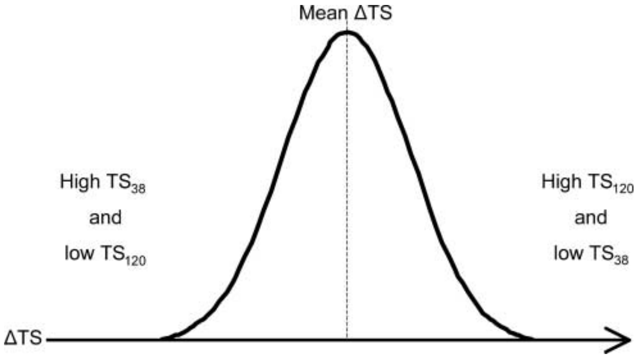

Figure 5. Distribution of ΔTS based on distributions and ranges in Figure 4, discussed in text.

Table 4. Target-strength ranges for *E. superba*, after Foote *et al.* (1990, Tables I and II).

Parameter	38 kHz	120 kHz	ΔTS
Mean TS	−85.1	−76.1	9.0
Maximum TS	−82.6	−74.5	–
Minimum TS	−89.1	−80.7	–
Range (maximum–minimum)	6.5	6.2	–
Upper ΔTS	–	–	14.6
Lower ΔTS	–	–	1.9

Terminology is given in Figure 4.

The data were analysed using the proprietary software Echoview Version 3.40.47.1551 (Sonardata; www.sonardata.com). The following basic sequence of activities, outlined in Figure 6, was implemented in Echoview.

(1) *Filter* 1. Designation of "Bottom" and "2 m" lines. The bottom line was set at 1 m above the "sounder-detected bottom" and was edited to smooth the line over regions where the bottom had not been correctly detected. The 2-m line was set at a fixed range of 2 m to exclude data from the near field.

(2) *Filter* 2. Transects were designated as sampling "regions". The criteria for selection were that the cruise track should be along the pre-determined line and that the course changes at the start should have been completed and, approaching the end, not commenced. In addition, the vessel should have settled into its cruising speed along the transect. Periods where obviously bad data were being collected were identified for exclusion.

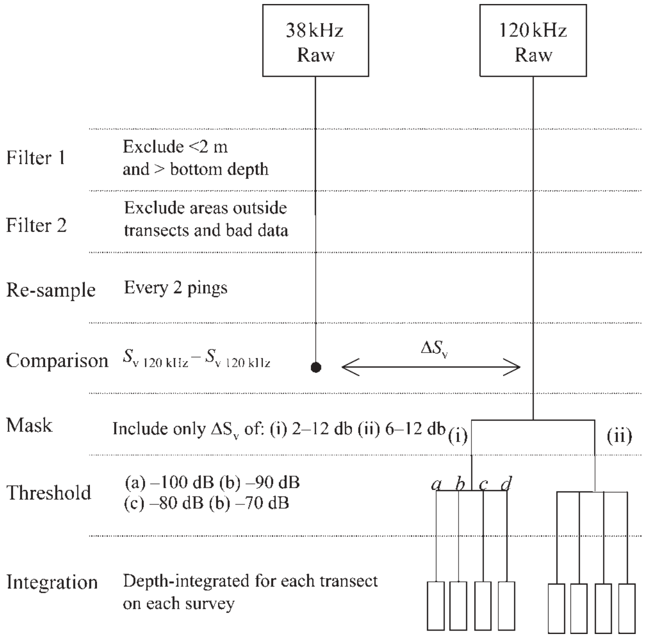

Figure 6. Processing procedure for echograms using Echoview software (Sonardata; www.sonardata.com).

(3) *Re-sample*. The data were re-sampled to restrict subsequent analyses to data that satisfied the criteria of the preceding steps.

(4) *Comparison*. A ΔS_v algorithm was implemented.

(5) *Masks*. Two masks were set up to exclude data that fell outside the designated ΔS_v ranges, 2–12 and 6–12 dB.

(6) *Threshold*. Four threshold levels were set to exclude very low echo levels.

(7) *Integration*. The data were integrated over the depth range in step (1) and over distance in step (2).

Examples of echograms before and after applying this ΔS_v process are given in Figure 7. The raw-data echograms are densely coloured because of the low threshold level of −85 dB that was set. Much of the backscatter was, therefore, likely to be attributable to small zooplankton, such as the copepod *Euchaeta*, which was abundant in the fjord at that time. Data were exported from Echoview for analysis using Excel spreadsheets. Here, the basic distance unit for the analyses was the transect, and a set of eight transects was designated as a survey.

In applying the ΔS_v method to the analysis, it is important to take account of threshold effects. If the same threshold level is set for both frequencies, then the frequency for which the TS is lowest will not detect the lowest numerical densities detectable using the other frequency. However, if a very low threshold level, equivalent to a very low numerical density, is set on both frequencies, then the differences are likely to be acceptably small. We undertook tests to determine the effect of setting different threshold levels on the analyses by estimating numerical densities with both frequencies set to the same value, ranging from −70 to −100 dB.

Results

Environment and zooplankton

The CTD profiles made are displayed on Figure 2. They had a high level of consistency with a thermocline being found over the depth range from 30 to 40 m. Throughout the sampling period, there were no major meteorological perturbations, from which we conclude that there were no significant changes in the deep water at that time.

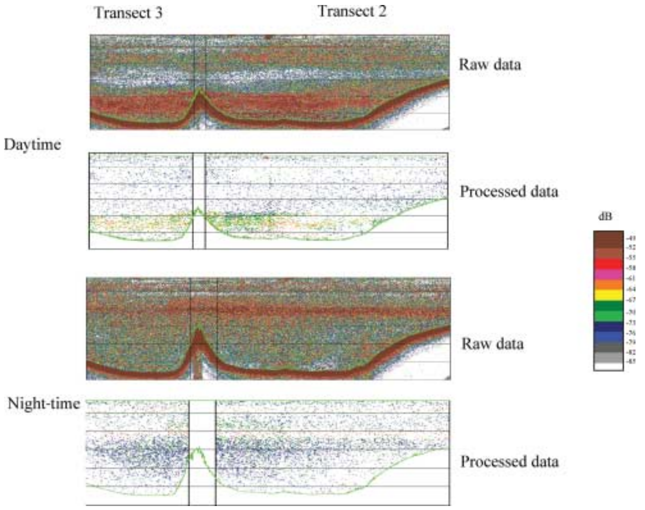

Figure 7. Echograms from 10 September 2003 at 38 kHz, showing the effect of processing on the raw data. The data were filtered with a ΔS_v of 6–12 dB, and the threshold was set to −85 dB.

Few adult krill were present in the shallow oblique IKMT hauls by day ($<0.001\ \mathrm{m}^{-3}$). More krill were present in the night-time shallow and deep hauls (mean $0.7\ \mathrm{m}^{-3}$, maximum $1.0\ \mathrm{m}^{-3}$) and daytime deep hauls (mean $1.0\ \mathrm{m}^{-3}$, maximum $1.7\ \mathrm{m}^{-3}$). No krill were caught in the WP-2 net surface deployments by either day or night.

There was no significant difference between the length frequency distributions of adult krill in any of the nets, so we combined the results for all hauls into a single distribution (Figure 3).

Acoustic data threshold level and mean density

With the threshold set to −99 dB for 120 kHz, the mean density was calculated for several threshold levels from −70 to −100 dB at 38 kHz, using the mean TS values in Table 3. A similar series of analyses with the 38 kHz threshold set to −99 dB and the 120 kHz varied was also run. The results are shown in Figure 8. With the threshold set to −80 dB or lower, there is very little variation in the mean density at either frequency. Therefore, all subsequent analyses were undertaken using a threshold of −85 dB. Using the TS measurements of Foote *et al.* (1990), that threshold is equivalent to a numerical density of around one krill per cubic metre at 38 kHz and one krill per 5 m^3 at 120 kHz.

The mean S_v along each transect was converted to density per cubic metre using the mean TS from Table 4 at the appropriate operating frequency. Analyses were undertaken using the two ΔS_v values described earlier. The mean densities are set out in Table 5, and the analysis of variance (ANOVA) results in Table 6. At the transect level, there are significant differences that could be attributable to either the short length of the transects or the spatial distribution of targets around the fjord.

For each frequency, there were no significant differences at a survey level, leading us to conclude that in each case the survey provided a representative estimate of mean numerical density. Because there was no significant difference at a survey level, we attribute the variations between transects to be due much more to the spatial distribution of the targets within the fjord than to the transect lengths.

The above analyses, although using the two frequencies to apply the ΔS_v procedure, were used also to estimate the mean density at each frequency. The results from these statistical analyses are shown in Tables 7 and 8 and in Figure 9. Using the 2–12 dB difference, there are significant differences between the day and night estimates of mean density at each frequency, although this is not the case for the 6–12 dB difference. Moreover, the difference between frequencies is significant when using the 2–12 dB difference, but not so using the 6–12 dB difference. The implications of these results are discussed later.

Discussion

The sampling design applied in a fjordic situation has allowed a series of important analyses concerning thresholding, the ΔS_v method, and the choice of echosounder frequency to be undertaken. We now consider each of these in turn.

Threshold effects

The ΔS_v method is designed as a tool with which to identify particular acoustic scatterers. The method assumes that there are consistent differences in TS at the two frequencies being considered. Effective implementation relies on the assumption

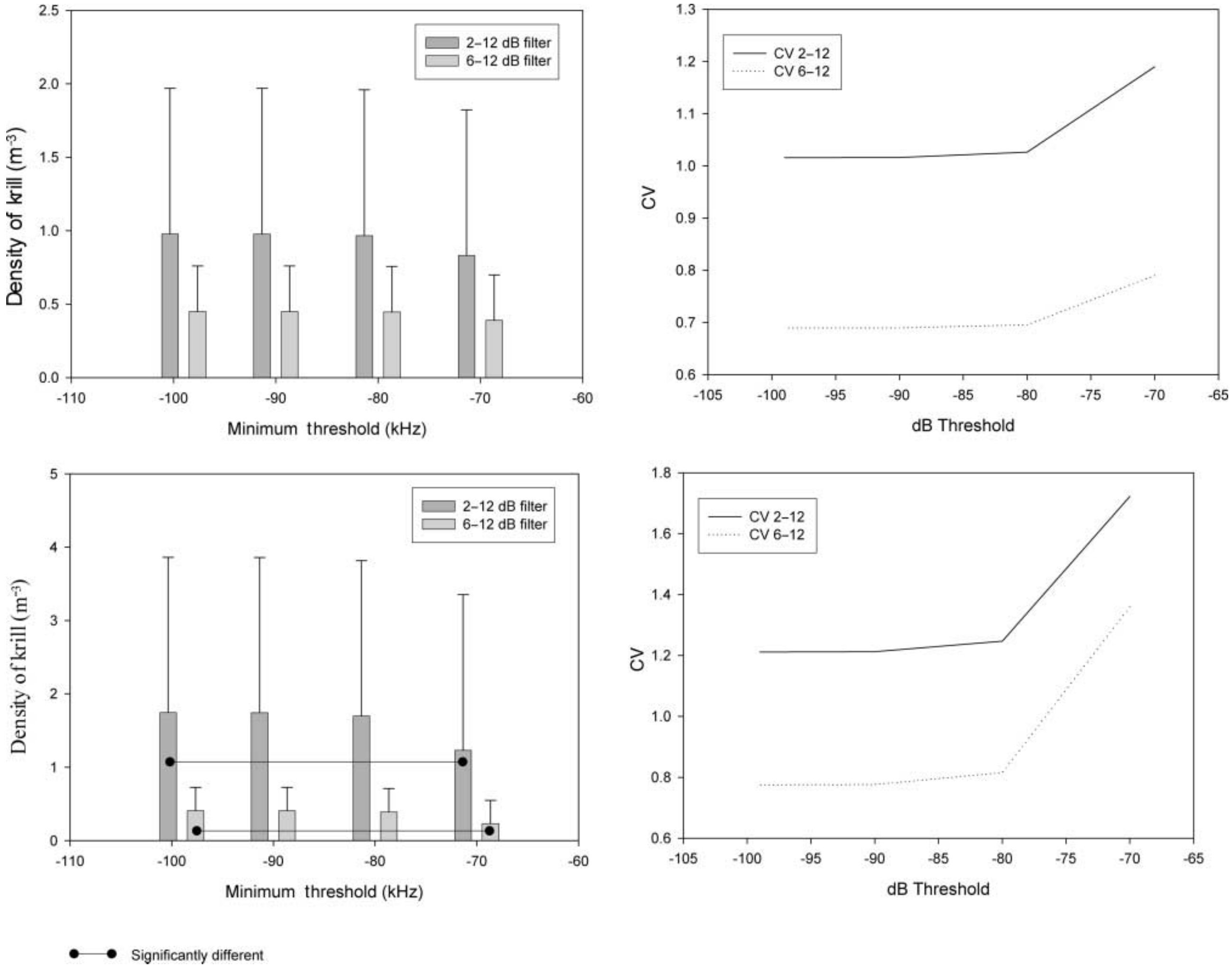

Figure 8. Results demonstrating the effect of different thresholds on average density estimates using 120 kHz (above) and 38 kHz (below).

that the same series of targets are being insonified at both frequencies. In theory, this assumption might be addressed by setting different threshold levels for each frequency. However, this would require knowledge of the *in situ* TS properties of those particular targets. Although this may be possible for individual large fish, it is not possible currently for small schooling species such as krill. The alternative is to use a single threshold level. In this case, if that is set too high, it would exclude several targets at the frequency of the weaker TS. In this study, we addressed this problem by determining a threshold level below which consistent results are obtained irrespective of frequency or ΔS_v applied. Our choice, supported by statistical analysis, is equivalent to a numerical density of $<0.2\ \mathrm{m}^{-3}$. Given that krill occur at much higher densities, of the order of thousands per cubic metre (Everson, 2000), such an approach is realistic. We would advocate a similar analytical approach being applied to the choice of threshold for other acoustic surveys, particularly on krill.

ΔS_v analyses

There are large differences, summarized in Table 9 from the results in Tables 7 and 8, between the results when analysed using the ΔS_v 2–12 dB compared with the 6–12 dB range.

The significant differences present in the comparisons of the day observations at the two frequencies, which are reflected also in the night-time observations with the 2–12 dB range, might be due to systematic differences in the TS at the two frequencies. However, we discount this explanation because there was no significant difference noted using the 6–12 dB range for the same comparison.

The acoustic sampling extended from near the surface down to 1 m from the seabed. Very few krill were found near the surface by day or night and, following from the conclusions of Lindahl and Hernroth (1988) and Lindahl and Perissinotto (1987), our assumption was that there was very little water exchange into or out of the fjord. Consequently, unless there was some behavioural difference to influence the TS, we would expect the estimated mean density over the water column to be the same by night and by day. Again we have a situation where the 6–12 dB difference indicates no significant difference, from which we conclude that the same group of scatterers was being sampled at that ΔS_v throughout the study. We conclude that some other scatterers were responsible for the day–night differences from the 2–12 dB ΔS_v analysis. With the sampling equipment available to us, we were unable to determine the precise nature of these scatterers. However, as they fall into the range

Table 5. Numerical density (individuals per cubic metre) of krill along each transect sampled on (surveys 1–4) 8 September and (surveys 5–8) 10 September 2003.

		ΔS_v 6–12 dB		ΔS_v 2–12 dB	
Survey	**Transect**	**120 kHz**	**38 kHz**	**120 kHz**	**38 kHz**
1	1	0.071	0.065	0.273	0.406
	2	0.354	0.328	1.119	1.649
	3	0.284	0.256	1.059	1.658
	4	0.237	0.212	0.749	1.096
	5	0.187	0.169	0.677	1.042
	6	0.235	0.197	0.643	0.863
	7	0.566	0.437	1.172	1.235
	8	0.412	0.324	0.852	0.904
2	1	0.044	0.037	0.175	0.275
	2	0.812	0.669	1.795	2.104
	3	0.232	0.206	0.831	1.279
	4	0.205	0.171	0.657	0.954
	5	0.168	0.150	0.540	0.810
	6	0.237	0.201	0.665	0.900
	7	0.645	0.504	1.312	1.374
	8	1.018	0.755	1.840	1.645
3	1	0.417	0.369	1.198	1.686
	2	0.442	0.386	1.245	1.731
	3	0.265	0.231	0.819	1.202
	4	0.293	0.279	0.896	1.293
	5	0.280	0.244	0.816	1.160
	6	0.296	0.273	0.826	1.175
	7	0.366	0.304	0.956	1.220
	8	0.318	0.282	0.842	1.113
4	1	0.791	0.718	2.205	2.994
	2	0.647	0.573	1.921	2.699
	3	0.461	0.411	1.254	1.713
	4	0.456	0.394	1.178	1.511
	5	0.320	0.289	0.942	1.338
	6	0.362	0.317	0.955	1.263
	7	0.231	0.198	0.641	0.859
	8	0.248	0.210	0.656	0.858
5	1	0.093	0.085	0.358	0.575
	2	1.379	1.404	6.189	10.317
	3	0.659	0.575	1.694	2.210
	4	0.667	0.557	1.574	1.874
	5	0.332	0.296	0.893	1.203
	6	0.301	0.260	0.807	1.077
	7	0.408	0.337	0.970	1.165
	8	0.269	0.225	0.674	0.852
6	1	0.056	0.051	0.197	0.313
	2	0.734	0.727	1.881	2.478
	3	0.465	0.439	1.418	2.056
	4	0.492	0.436	1.363	1.855
	5	0.361	0.308	1.022	1.361
	6	0.323	0.295	0.945	1.332
	7	0.360	0.301	0.854	1.043
	8	0.305	0.252	0.746	0.927
7	1	0.335	0.287	0.933	1.243
	2	0.743	0.659	2.328	3.357
	3	0.855	0.793	2.552	3.690
	4	0.668	0.574	1.751	2.278
	5	0.429	0.365	1.175	1.561
	6	0.292	0.257	0.865	1.219
	7	0.236	0.201	0.678	0.932
	8	0.325	0.278	0.870	1.139
8	1	0.378	0.343	1.287	1.900
	2	0.801	0.732	2.622	3.913
	3	0.762	0.678	2.191	3.052
	4	0.651	0.565	2.128	3.137
	5	0.463	0.445	1.433	2.125
	6	0.415	0.369	1.215	1.739
	7	0.382	0.314	0.946	1.172
	8	0.358	0.303	0.922	1.178

Krill target strength was −76.1 dB at 120 kHz and −85.1 dB at 38 kHz. The densities were calculated for echograms filtered by two different dB difference windows: 6–12 and 2–12 dB.

Table 6. ANOVA results for numerical density (individuals per cubic metre) estimates.

dB difference	Transects or surveys	120 kHz	38 kHz
2–12	Transects	$p = 0.00043$	$p = 0.00060$
2–12	Surveys	NS ($p = 0.16$)	NS ($p = 0.16$)
6–12	Transects	$p = 0.00127$	$p = 0.00040$
6–12	Surveys	NS ($p = 0.27$)	NS ($p = 0.20$)

From the design, there were eight surveys each consisting of eight transects. NS, not significant.

of ΔS_v close to 1, perhaps the backscatter may have been caused by fish.

In the context of krill surveys in Gullmarsfjord, we advocate the use of the 6–12 dB range for ΔS_v. In a wider context, though, we would advocate restricting the range of ΔS_v to the estimated TS_{range} at both frequencies. In the absence of such information, we suggest that the results from the use of several ΔS_v ranges be

Table 7. Statistical tests for differences in average numerical densities (for the 2–12 dB difference filter).

Comparison	Factor	T	p
	Frequency		
Day/night	120 kHz	892	0.048
	38 kHz	865	0.019
	Time of day		
Frequency (38: 120 kHz)	Day	1 295	<0.001
	Night	865	0.019

Mann–Whitney rank sum test; n (small) = 32, n (large) = 32.

Table 8. Statistical tests for differences in average numerical densities (for the 6–12 dB difference filter).

Comparison	Factor	T	p
	Frequency		
Day/night	120 kHz	922	0.015
	38 kHz	916	0.097
	Time of day		
Frequency (38: 120 kHz)	Day	1107	0.372
	Night	1151	0.138

Mann–Whitney rank sum test; n (small) = 32, n (large) = 32.

examined to identify a range that appears plausible in terms of numerical density and its associated variance.

Estimation of numerical density

Having determined the most appropriate range for the ΔS_v to identify *M. norvegica*, we can use either of our two operating frequencies, 120 and 38 kHz, to estimate density. We have three options: either of the two frequencies or the mean of the two estimates.

The difference between TS for a given size of krill at the two frequencies is due to the intrinsic scattering properties and the behavioural characteristics, particularly orientation, of the individual animals. Evidence suggests that the two frequencies are likely to have different scattering properties, with one potentially being more stable than the other. In terms of estimating numerical density, there are clearly advantages in selecting the frequency with the least response variation in the targets.

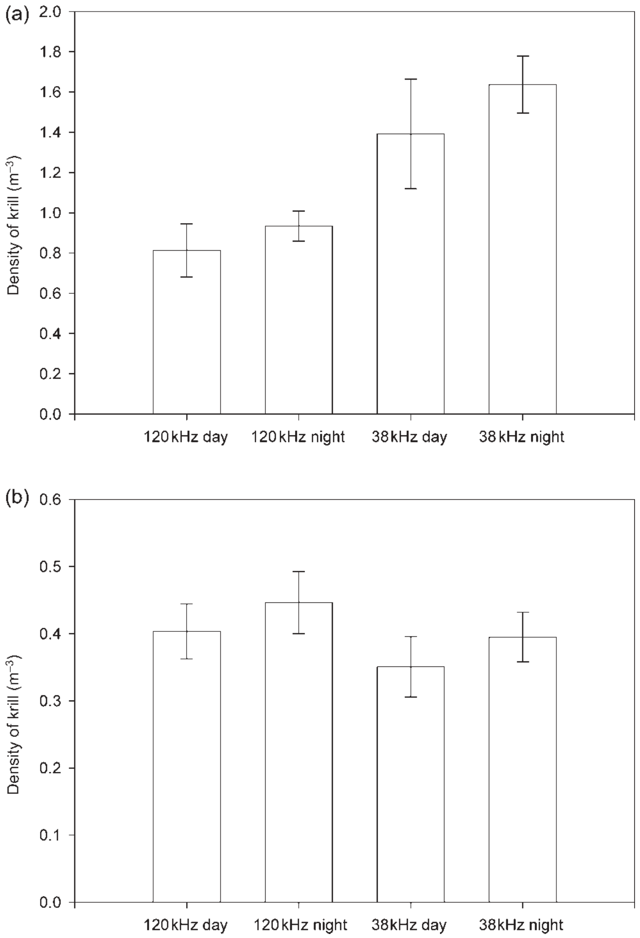

Figure 9. The difference between day and night estimates using 120 and 38 kHz extracted with the (top panel) 2–12 dB window and (bottom panel) the 6–12 dB window.

Table 9. Significance levels for comparisons of day and night-time results for each frequency and between frequencies.

ΔS_v	2–12 dB	6–12 dB
120 kHz day/38 kHz day	++	NS
120 kHz night/38 kHz night	++	NS
120 kHz day/night	+	NS
38 kHz day/night	+	NS

NS, not significant; +, significant at the 5% level; ++, significant at the 1% level.

Table 10. Statistical tests for equal variance in densities (6–12 dB filter).

Comparison	Factor	F	p
	Frequency		
Day/night	120 kHz	2.44	0.016
	38 kHz	1.17	0.661
	Time of day		
Frequency (38: 120 kHz)	Day	1.17	0.661
	Night	1.18	0.640

F-test for a normal distribution, with $n = 32$.

We investigated this by testing for equal variances in the density estimates. The results are shown in Table 10, and they indicate no significant difference between the variances at the two frequencies. We conclude from this that there is no advantage in one frequency over the other. There is no reason to suppose that this will apply to all surveys, so we recommend for abundance estimation the use of the acoustic frequency giving the lowest variance.

The same analysis provided a comparison of the differences in variance between day and night at each frequency. The results from both frequencies indicated that the variance from the night observations was significantly lower than that observed by day. The coefficients of variation (CVs) of each survey are listed in Table 11. Bearing in mind the shortness of the transects, the constraint of working in a narrow fjord, the results in Table 10 represent an acceptable level of variation in relation to the spatial scale of the sampling.

The diurnal difference is most likely attributable to the behaviour of the krill, which by day tend to aggregate into swarms but after dark likely disperse. We conclude from this observation

Table 11. CV (%) for each survey (6–12 dB difference filter).

Comparison	120 kHz	38 kHz	120 kHz	38 kHz
Day/night	Day	Day	Night	Night
8 September (Out)	18.2	16.3	7.0	6.6
8 September (Back)	29.9	28.1	15.7	16.4
10 September (Out)	27.5	31.2	17.1	18.1
10 September (Back)	17.6	19.5	12.3	12.8

that, in terms of reducing the variance of an abundance estimate, there are advantages in making use of data collected at night. This assumes that all krill are present within the sampled layer, a factor that can be tested by a comparison of the mean values.

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