Supplementary Material

High Latitude Epipelagic and Mesopelagic Scattering Layers - A reference for future Arctic ecosystem change

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1 Echogram categorization and interpretation

Echogram categorization (sometimes called scrutinization or interpretation) are based on the shape of the echo-traces, their scattering strength, their relative frequency responses, and are supported by biological samples from trawl and plankton nets. The team of two that usually carries out the echogram categorization have access to historical knowledge of catches from the waters, e.g. from previous scientific surveys, and to some knowledge of the behavior and distribution of the species commonly found in the investigated or nearby waters. Below is a description of some of the key techniques and tools available in LSSS – the Large Scale Survey System (Korneliussen et al., 2016), and how they are applied in the current work.

1.1 Multifrequency acoustic data from the north-eastern survey area

A 4.5-nmi echogram from the shallow shelf to deep water in Sofiadjupet is used to illustrate the acoustic analysis procedures used in this paper (Figure S1A). The acoustic data has in this situation been interpreted by dividing the echogram into three different depth regions, visualized by the horizontal blue lines in the echogram. These are the depth regions from 0-80 m, 80-340 m and >340 m. The upper layer 0-80 m is very faint at 38 kHz (threshold, -82 dB), with s_A-values in the range 6-16 $m^2 \cdot nmi^{-2}$ per 0.5 nautical mile. The 0-80 m layer has a frequency response (Figure S1E) where the backscatter at 18 and 120 kHz is twice what recorded for 38 kHz. This can be interpreted as a mixture of scatterers, a weaker scattering component being strong at 120 kHz, such as euphausiids or amphipods, and a stronger scattering component responsible for the high backscattering at 18 kHz, most likely the 0-group fish present in the area. This interpretation is supported by the trawl catches that do show the presence of some 0-group *Sebastes*, cod, and haddock (St19-st26, Table 5) and substantial amounts of krill and amphipods in the upper 0-60 m of the water column (St23-st24). With a switch activated in LSSS, the TS-detector recognizes stronger regularly distributed individual targets at 38 kHz in this layer (Figure S1B, a subset of the data in Figure S1A). Combining this information with the TS-distribution in same layer (Figure S1F), the presence of stronger targets having a peak TS around -56 to -57 dB at 38 kHz are highlighted, although all TS data of this type should be interpreted with caution. Backscattering in this upper layer is clearly different from what was observed on the Fram Strait North and Fram Strait South transects, where the abundance and backscattering from small swimbladder 0-group fish (e.g. *Sebastes*, cod, and haddock) were much higher.

The second layer from 80-340 m is particularly interesting since it is partly on the shelf and partly over deep water. Around 200 m there are scattered and diffuse, but still well-defined schools over the shelf and deeper waters. Focusing on individual schools using the LSSS school feature, reveals a frequency response with low values at both 18 and 38 kHz and considerably stronger at 120 kHz; when the whole layer is considered the relative frequency response (Korneliussen and Ona, 2003), with respect to 38 kHz, $r(f) = s_v(f)/s_v(38kHz)$, r(120kHz) ≈12, meaning that the backscattering is about twelve times stronger at 120 kHz compared to 38 kHz, and within the School region shaped as a box in Figure S1C, r(120kHz)≈10. The noise at 120 kHz in this case is insignificant even when the range from the transducer is close to 300 m and the system is close to the range-limit where TVG-amplified noise could normally be important. When a School box of the same size as the rectangular blue box in the 80- 340 m stratum is overlaid the shelf schools, the r(120kHz) ≈11. In addition, school structure, weak backscattering at 18 and 38 kHz, and species information from trawl data (St19-St23, see MS Table 5 and 6) strongly suggest that these schooling scatterers are krill or amphipods or a mixture of these groups, and that they completely dominate the acoustic backscatter in this depth interval. This layer can be traced within the approximate same depth interval and over large areas in the north-easternmost regions of the survey. At a S_v-threshold of -82 dB the 80-340 m layer has an average area backscattering coefficient, $\hat{s}_A \approx 31$ $m^2 \cdot nmi^{-2}$ over the approximately 5-nmi echogram shown in Figure S1A. If the S_v-threshold is set to around -60 dB, the area backscattering coefficient is $\hat{s}_A \approx 0$ $m^2 \cdot nmi^{-2}$, which means that all weaker scatterers are eliminated, but also that there are no stronger scatterers that remain detectable in the echogram. It also means that the whole layer can be assigned to the category Weak_SC at a threshold of -82 dB.

Deeper in the water column and below approximately 300 m depth, a typical DSL is registered. A couple of School boxes are shown, and within these, the frequency response shows that the backscattering at 18 and 38 kHz are close to equal, exemplified by Figure S1D, being representative of the red polygon region between 400 and 500 m depth (Figure S1A). This indicates a lack of resonance at these two frequencies, e.g. due to gas-filled swimbladders or siphonophoran pneumatophores with gas-inclusions.

1.2 Multifrequency acoustic data from the Fram Strait area

A 2.5 nmi section of the 38 kHz acoustic registrations from the western side of the Fram Strait South transect (Figure S2A) provides a contrast to the backscattering in Sofiadjupet basin. The 120 kHz acoustic registrations in the upper 200 m for the same section with a S_V-threshold = -90 dB are in Figure S2B. These registrations are over very deep water although the backscatter was only recorded to approximately 800 m. A narrow and distinct band of strong scattering is evident in the upper 50 m of the water column. That the frequency response r(f) of targets in the uppermost School box in Figure S2A is high at 18 kHz, lower at 38 kHz and the lowest at 120 kHz (Figure S2C) is interpreted theoretically and based on the embedded LSSS feature library (Korneliussen et al., 2016) as fish having a swimbladder. 0-Group fish, particularly *Sebastes* sp., were recorded during nearly every trawl haul over the entire transect, but also west of Svalbard in general (MS, Table 5). A fainter structure is visible below, particularly at 120 kHz between 50 and 100 m depth (Figure S2B). This secondary structure was less distinct if the echogram S_V-threshold was set to -82 dB at 120 kHz and was most likely a plankton component. The frequency response of this layer, derived from the second School box from the surface (Figure S2A-B), is very low at 18 and 38 kHz and about 6.5 times higher at 120 kHz (Figure S2F). This corresponds well with the scatterers being an elongated crustacean scatterer (krill or amphipods; LSSS feature library, Korneliussen et al, 2016) or other similar shaped small crustaceans if sufficiently abundant. This location was however some nautical miles from the nearest trawl station, and no “targeted haul” was conducted in the upper 0-60m, hence the acoustic registrations cannot in this case be verified. However, there were high densities of copepods in the upper 50 m of the water column several places along the Fram Strait transects as documented by stratified Multinet tows (see MS Figure 2 for Multinet stations, although results not shown in MS), and integrated WP2-hauls that showed very high biomasses in the range 40-60 g $DW \cdot m^{-2}$ (MS, Figure 8).

Deeper in the water column there are two LSSS School boxes (blue around 300 m depth) and a red box at around 350-480 m (Figure S2A). For both boxes the frequency response at 18 and 38 kHz are quite similar (Figure S2D-E), although 18 kHz data is slightly higher than 38 kHz for the School box centered around 300 m depth. The average area backscattering coefficient in the range 100-500 m for the ~2 nmi's shown in Figure S2A with the two School boxes removed, is very low, $\hat{s}_A \approx 12$ $m^2 \cdot nmi^{-2}$ and echotraces show signs also indicating fish. However, since total backscattering is very low and these individual targets nearly disappear when sequentially thresholding is applied, moving the adjustable S_v-threshold from -90 dB, step-wise to -85 dB, these are very weak scatterers, most probably fish or other organisms without any gas inclusion or swimbladder. which indicate that they are not fish, and certainly not fish with swimbladder.

1.3 The larger fish component and scrutinizing

In Figure S3A-B are presented two echograms where it has been zoomed in on part of the DSL to a depth range of approximately 340-480 m representing a snapshot located in the northernmost regions of the survey with the upper panel at an S_v-threshold of -82 dB and the lower echogram of -65 dB. What can be considered "weaker" scatterers (faint blue dots) are distributed all over echogram, but some regions have higher densities (Figure S3A). The Integration line (see Korneliussen et al., 2016) increases smoothly from the lower left to the upper right corner of the echogram and does not indicate any significant and abrupt changes in scattering structures, that could indicate stronger scatterers are also present. However, some typical fish echoes can be observed as half-moon shaped structures relative to the background, also being somewhat stronger with respect to coloration. If the S_v-threshold is increased to -65 dB, most of the weaker "faint blue" dots disappear and what could be considered "stronger" fish echoes remains still visible (see echoes marked with red rings in both Figure S3A and Figure S3B). From the Integration line in Figure S3B when thresholding at -65 dB it is also evident which of the fish echoes are contributing most to the overall s_A after thresholding, as visualized by the jumps in the Integration line. The LSSS "Threshold Window" is an additional feature used to inform the operator during scrutinizing. In this window, the nautical area scattering coefficient $s_{A,}$ versus $S_{v,}$ is plotted for the active part of the echogram, which in this case is the whole echogram shown. It is observed that the blue curve (s_A vs S_v at 38 kHz), falls off very fast as the S_v approaches about -70 dB. This indicates that most of the s_A is significantly dominated by weaker scatterers (Korneliussen et al., 2016). There is certainly subjectivity embedded in the scrutinizing process, because it is difficult to assess the precise threshold were the larger fish can be "correctly" separated from the weaker micronekton and macroplankton. For regions where these latter categories are abundant, more thresholding might be needed to detect the fish, compared to regions where they are less abundant, since larger individual fish might be masked by high abundance of weaker scatterers. An alternative approach would be to use fixed and static thresholds of i.e. -60 dB and -82 dB or even -90 dB. Such an alternative method, being more straight forward and sound from a methodic perspective, hence understandable, could also include too many of the weaker scatterers with the "stronger" scatterers, in our case Strong_SC, hence also introducing a bias in the processed data and in its ecological interpretation.

2 Information on the Åkra and Harstad trawls being used during the investigations

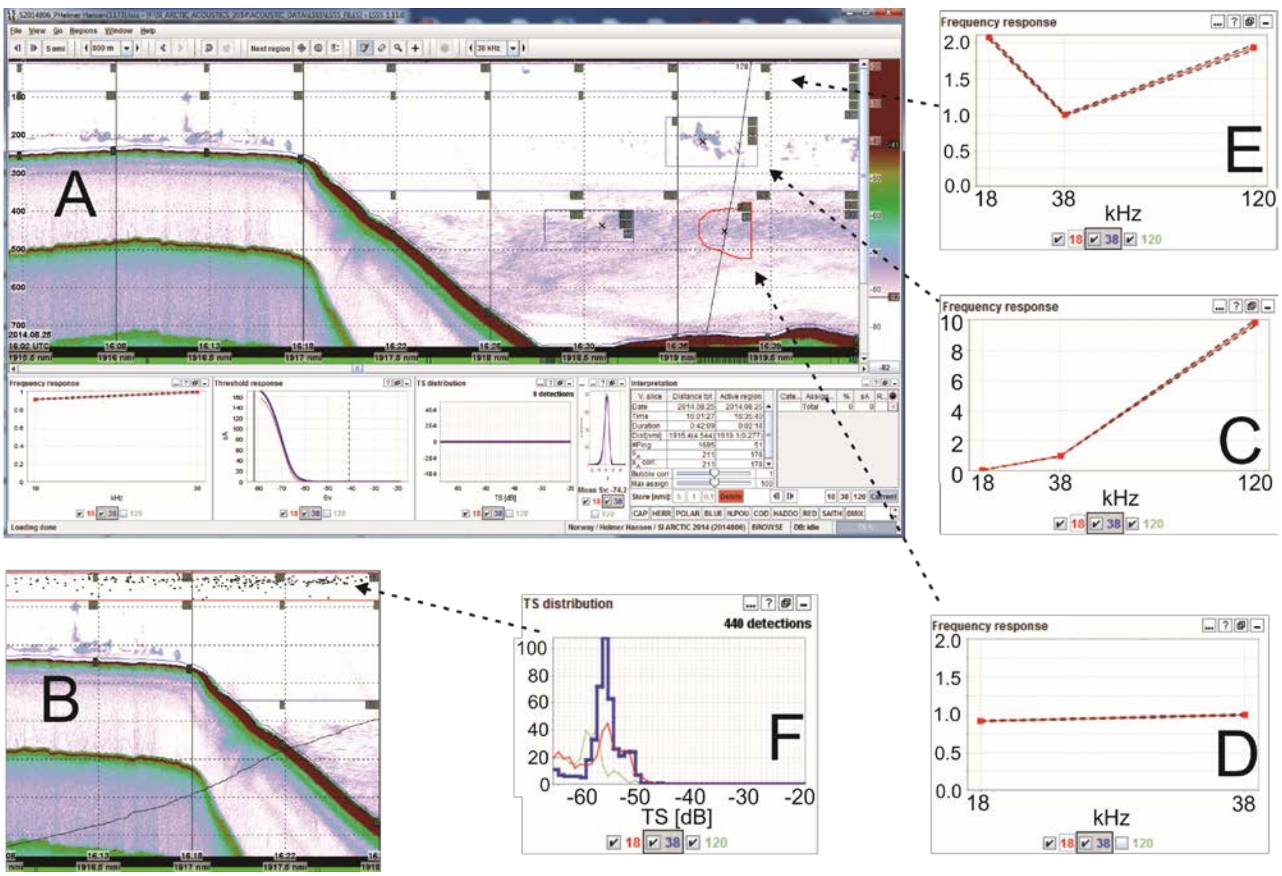
Additional information on trawl design, including detailed drawings are found in the Institute of Marine Research Quality system and can be downloaded via the links below:

Harstad trawl: (https://kvalitet.imr.no/EKWeb/docs/pub/dok01811.pdf)

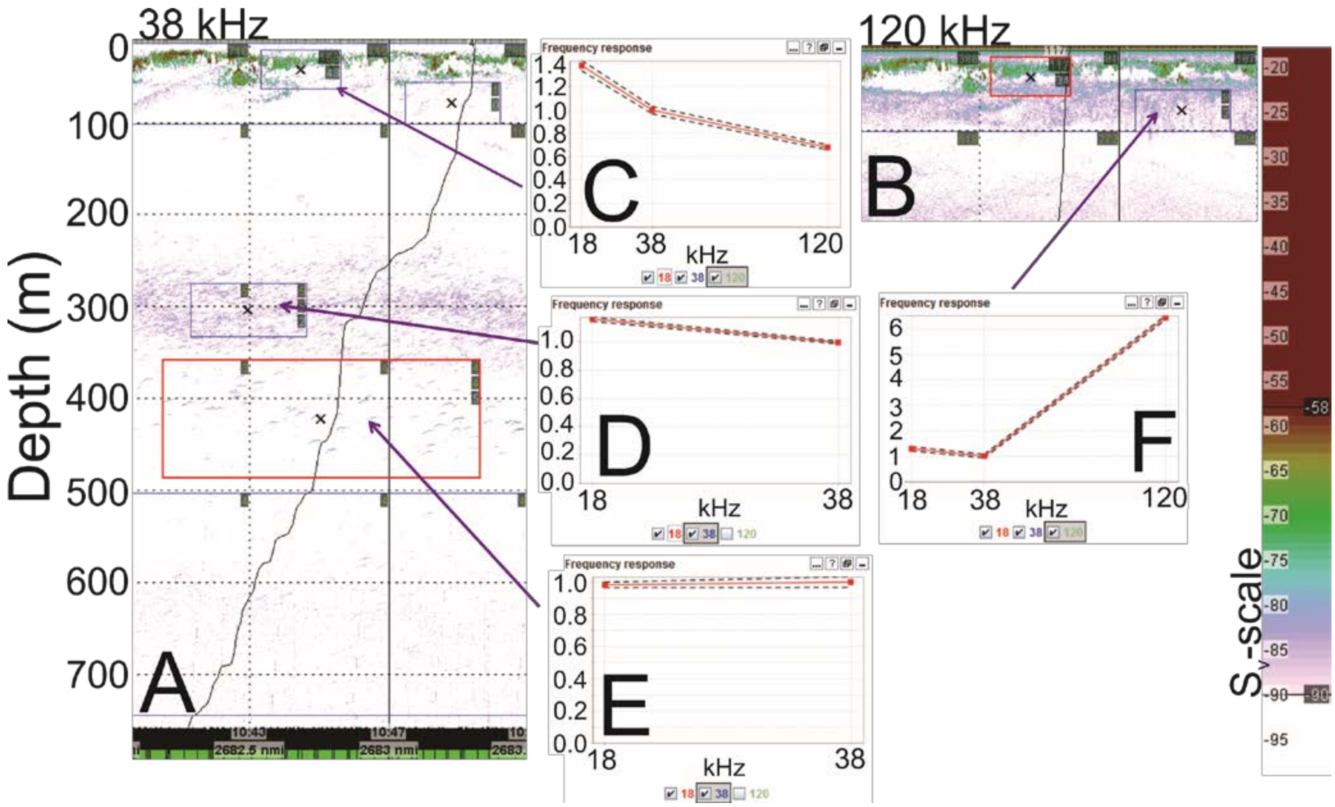
Åkra trawl: (https://kvalitet.imr.no/EKWeb/docs/pub/dok01835.pdf)

3 Supplementary Figures and Tables

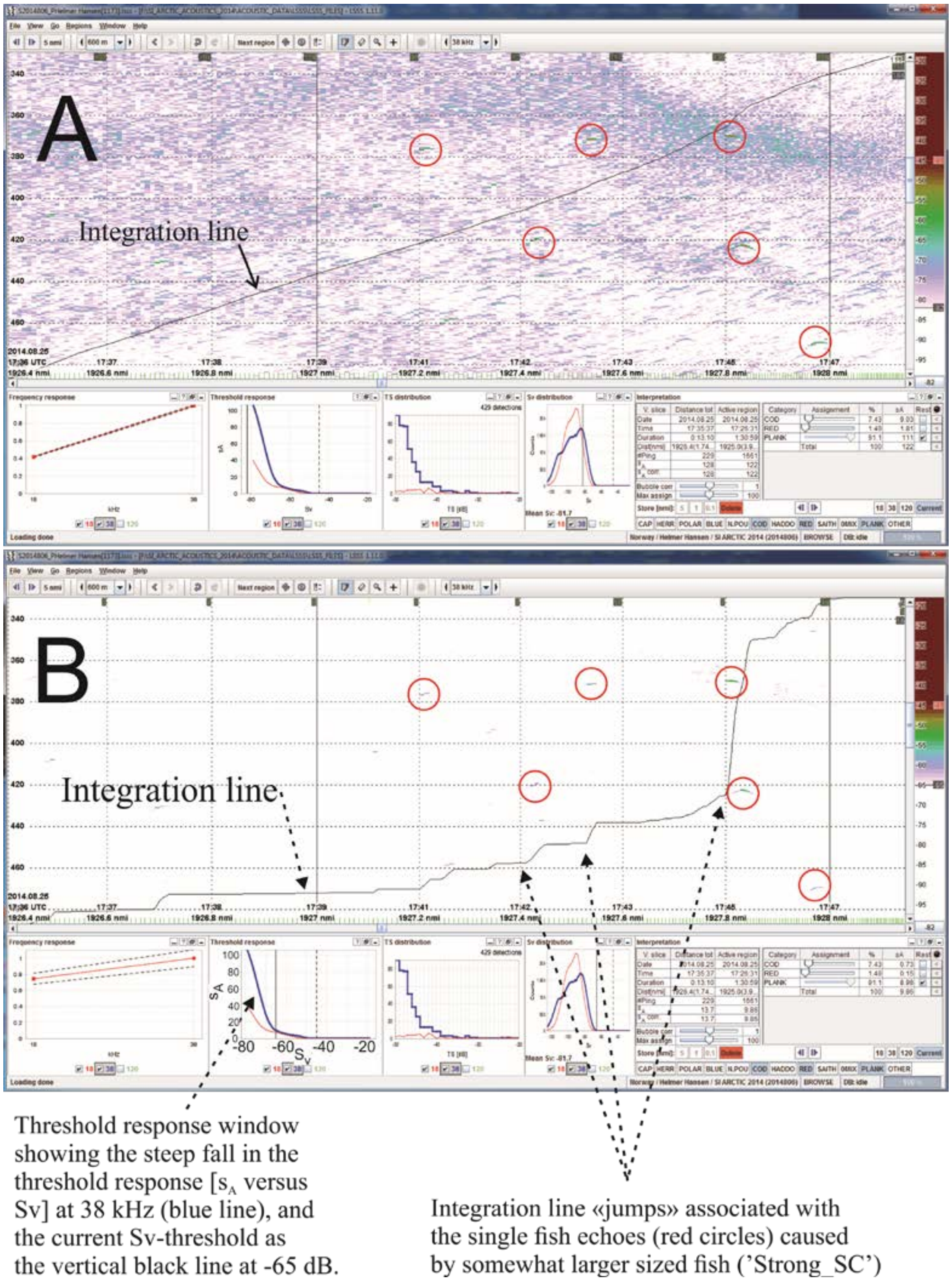
3.1 Supplementary Figures



Supplementary Figure 1. A) An exemplary 4.5-nmi echogram from the northern part of the Hinlopen transect showing typical acoustic registrations at 38 kHz from shelf to deep water (Sofiadjupet). B) A subset of the echogram shown in A), with the option that accepted targets as determined by LSSS are turned on and shown as black dots within the 0-80 m layer. C) The relative frequency response r(f) in the 80-340 m layer. D) The relative frequency response r(f) within the red polygon between ~400-500 m depth. The 120-kHz data not included due to range restrictions and noisy data. E) The frequency response in the 0-80 m layer. F) The Target Strength (TS) distribution at 18, 38, and 120 kHz in the 0-80 m layer.



Supplementary Figure 2. A) An exemplary 1.5-nmi echogram from the western side of the Fram Strait South transect showing typical acoustic registrations at 38 kHz. Note the S_v-scale that has been set to -90 dB to visualize the very weak DSL in this region. B) Acoustic registrations at 120 kHz in the upper 200 m for the same region as depicted in A). C) The frequency response from the School box drawn close to the surface, which includes the strong scattering structure seen in the upper 50 m of the water column. D) The frequency response within the blue School box centered ~300 m depth in A). E) The frequency response within the red School box centered ~420 m depth in A). F) The frequency response within the blue School box (shown in both Fig. S2A and B) that extends between 50 and 100 m depth.



Supplementary Figure 3. A) Zoomed in echogram of part of the DSL from approximately 340-480 m at a threshold of -82 dB, for a distance ~2 nmi in the northern part of the study area. B) Zoomed in echogram of part of the DSL from approximately 340-480 m at a threshold of -65 dB.

4 Supplementary Material References

Korneliussen, R.J., Ona, E. (2003). Synthetic echograms generated from the relative frequency response. *ICES Journal of Marine Science* 60, 636–640.

Korneliussen, R.J., Heggelund, Y., Macaulay, G.J., Patel, D., Johnsen, E., Eliassen, I.K. (2016). Acoustic identification of marine species using a feature library. *Methods in Oceanography* 17, 187–205.