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## Executive Summary

This deliverable reports on work undertaken on evaluating the performance of scientific sampling gear used by the MEESO partners. It constitutes an important supplement to MEESO deliverable 2.1: “Report on the implementation of acoustical, optical and catch methods for enhanced identification and biomass estimation of mesopelagic species”.

The report is split into 6 chapters based on the specific questions addressed. Chapters 1-3 all deal with quantification of performance of equipment physically catching mesopelagic organisms, whereas chapters 3-6 deal with performance of acoustic equipment. No work that has been carried out so far has been aimed at explicitly evaluating bias in measurements from optical equipment, but none of the partners use optical gear alone for abundance or biomass estimation.

An alternative way of dividing up the work would be to look at whether the work is relevant to “standard” practices/methods (Chapters 1-5), or to practices/methods being developed in MEESO (Chapter 1-3,6); most of the work is relevant to both.

MEESO sampling equipment quantification work covers many of the topics needed in order to improve our ability to accurately measure biomasses in the mesopelagic zone. However, for a variety of reasons several different types of equipment and methods are used to quantify biomasses of mesopelagic micronekton, even within the MEESO project. In a perfect world these methods should be intercalibrated, but a direct equipment comparison is hard to carry out in practice, and would also be prohibitively expensive.



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## Introduction:

There is no such thing as an unbiased biological sampling gear, and in order to interpret the results of each type of equipment, it is necessary to understand both how that particular gear works, as well as to have a basic understanding of the organisms being sampled. The MEESO project is explicitly focused on mesopelagic micronekton, which present some unique challenges to the sampling.

First of all (P1.1), the organisms tend to inhabit deep waters (at least during daytime), making it relatively physically challenging to reach their habitat, regardless of type of equipment. Secondly (P1.2), the organisms in this size range tend to occur at low densities. While the size distribution of mesopelagic fishes must still be considered largely unknown, the fact that many of these fishes can reach weights well above 10 g WW ind<sup>-1</sup> implies that huge volumes must be sampled in order to get representative numbers for “average” abundances, if the surface integrated sum of all mesopelagic fishes averages 1 g WW m<sup>-2</sup> globally (Lam and Pauly, 2005). Thirdly (P1.3), defined as micronekton, most of these organisms are quite mobile, and avoidance of sampling gear has been suggested to be important (Gjørseter and Kawaguchi 1980, Kaartvedt et al. 2012). This last point probably interacts with the first: our sampling gear constitute an exceptionally large disturbance in the otherwise relatively featureless mesopelagic zone, and may be expected to influence the behaviour of organisms found there.

Acoustic equipment has the capacity to efficiently sample large volumes, and at least for low frequency equipment the two primary challenges to physical sampling gear has little relevance, a low frequency echosounder can sample the entire vertical extent of the mesopelagic zone in a single sound emission, and the sampled volume is no longer a restricting factor. Studies of mesopelagic distribution therefore often include data from hull-mounted echosounders, often as their primary source of data. However, there are at least 4 main obstacles that makes the use of acoustics problematic wrt. mesopelagic biomasses. Firstly (P2.1), there is a general lack of data on the acoustic properties of mesopelagic organisms. For hull-mounted data, this means that even if it is easy to measure the total amount of sound (e.g. often given as Nautical Area Scattering Coefficient, NASC) scattered back from the mesopelagic community, converting the total sound to a total number of organisms' present requires some large assumptions as to how much sound an average organism scatter (target strength, TS). This is related to the second main issue (P2.2), which goes on the identification of the scatterers. Since the observation volumes are large, the total backscattered signal is usually the combination of signals from many individuals, and in the deep mesopelagic usually a mixture of taxonomic and scatterer types: finding suitable conversion functions for going from a mixed total acoustic signal to total organismal abundance is obviously non-trivial. The third issue (P2.3) can be seen as a combination of the preceding two: organisms without gas-filled inclusions (e.g. Crustaceans, jellyfish, but also cephalopods, whose main backscatter originates from the beak, as well as fishes without gas-filled swimbladders) scatter little sound at the lower frequencies needed to cover the mesopelagic zone: consequently they are hard to detect using hull-mounted echosounders, and in practice current acoustic based surveys are likely to be biased towards species with gas-inclusions.

The final main obstacle to obtaining biomasses from acoustic data (P2.4) combines issues with both physical catches and acoustics: acoustic results are normally



assigned to different taxonomic groups based on contents in physical catches (e.g. “ground-truthing”), and average weights or lengths used to compute both TS and the final biomass is also typically taken from the catches. Biases in taxonomic and size distributions from the catches must be corrected for before using these data for “ground-truthing”.

Even if methodologies utilized for mesopelagic sampling differs between the partners, work undertaken in the MEESO project has sought to address several of these issues. This report highlights efforts taken under WP2 to quantify some of the biases inherent in sampling mesopelagic biomasses, and is a part of a 3-report series from MEESO WP2. This report is not intended as a full summary of results obtained during this effort, but rather to highlight what has been addressed, by what means, and by which partner. Details on the results of the different approaches can, when analysis is complete, either be found in the MEESO data repositories, or in published literature.



## 1. Gear monitoring/filtered volumes/gear performance

Standardizing the trawling procedure and monitoring the trawl's performance is needed to compare biomass estimates. Sampling trawls used by IMR were towed obliquely at  $1 \text{ ms}^{-1}$  from the surface to 1000 m. The performance of the trawl was monitored during the haul. This included the speed of the trawl moving through the water, trawl geometry (height and width of the trawl opening), and the descent/ascent rate of the trawl. The volume of water filtered by each of the four different IMR trawls was calculated using the trawl's geometry and water flow (measured by an Acoustic Doppler current profiler) at the entrance of the trawl. The smallest trawl filtered  $\sim 34 \text{ m}^3\text{s}^{-1}$  of water, while the largest trawl filtered over 20 times the water with  $\sim 792 \text{ m}^3\text{s}^{-1}$ .

These detailed measurements of trawl geometry and water flow enable us to estimate and assess volumes filtered at certain depths.

## 2. Size distributions (P1.2, 2.4)

Mesopelagic densities can either be assessed as abundances (e.g. the number of individuals per unit volume or area), or in terms of biomass (e.g. the weight per unit volume or area). What metric is more common depends partially on subject field, the most common metric in zooplankton studies are abundances, while biogeochemical studies typically operate with biomass (or more specifically carbon) densities. The *in situ* size distribution links the two density metrics, but at least for mesopelagic micronekton these size distributions require specialized equipment to obtain: common fisheries trawls have large sampling volumes, but due to the typical use of graded meshes in the front of the trawls, the effective sampled volumes are virtually impossible to assess. Gear upscaled from zooplankton gear, i.e. typically "midwater trawls" (i.e. Tucker, RMT8, IKMT, MOHT, MOCNESS, BIONESS) with small, even mesh-size along the entire net (and thus better-defined sampling volumes) tend to be small, and thus suffer from "organism quantization" issues (see next paragraph), as well as perceived avoidance issues (P1.3, see section on Interactions/Avoidance from gear).

"Organism-quantization" issues arise when mapping the continuous values of actual organism densities to our smaller set of discrete estimations. The catch estimations are discrete since the sample volume is always finite, and the sampling gear catch organisms in multiples of 1, e.g. normal sampling depends on catching 0, 1 or more individuals. It follows logically that the minimum sampling volume that on average will result in a non-zero estimate is inversely related to the organismal densities. For common species the consequences of this "effect" is trivial for numerical abundances (e.g. you can only count what you catch), but since for most species abundances tend to drop rapidly off with size (and even more so for weight), the consequence is that even if a smallish net could get a reasonably good estimate of abundance for a species, biomass densities may be severely biased (e.g. if overall biomasses are driven by the larger, rarer fishes, see for instance Gartner Jr et al. 1989). If assessed in terms of biomass, this bias will also extend to the size distribution, so the bias has implications for the use of net catches as input for acoustic TS modelling/"ground-truthing" (P2.4). The only way to minimize the impact of the "organism-quantization" bias is to increase the sampled volume, either by increasing net size or tow-duration, or by pooling multiple tows.



An example is given in Fig. 2.1, for the genus *Cyclothone*, caught during the 2019 Norwegian cruise from Cabo Verde to Norway. The upper panel shows numerical density distribution (bars) and cumulative biomass distribution (black line) of *Cyclothone* spp. from a Multinet Mammoth, the middle panel shows the same metrics from the 6x6 Macroplankton trawl, and the lower panel shows a combined, best estimate size and biomass distribution based on a combination of the data from the 2 nets. The take home message is that close to 50% of the total “population” biomass was found outside of the size range of the *Cyclothone* spp. caught in the small net (e.g. few, but larger individuals drove ~half the population biomass), even if a population abundance estimate based on catches from this net would have been reasonably accurate.

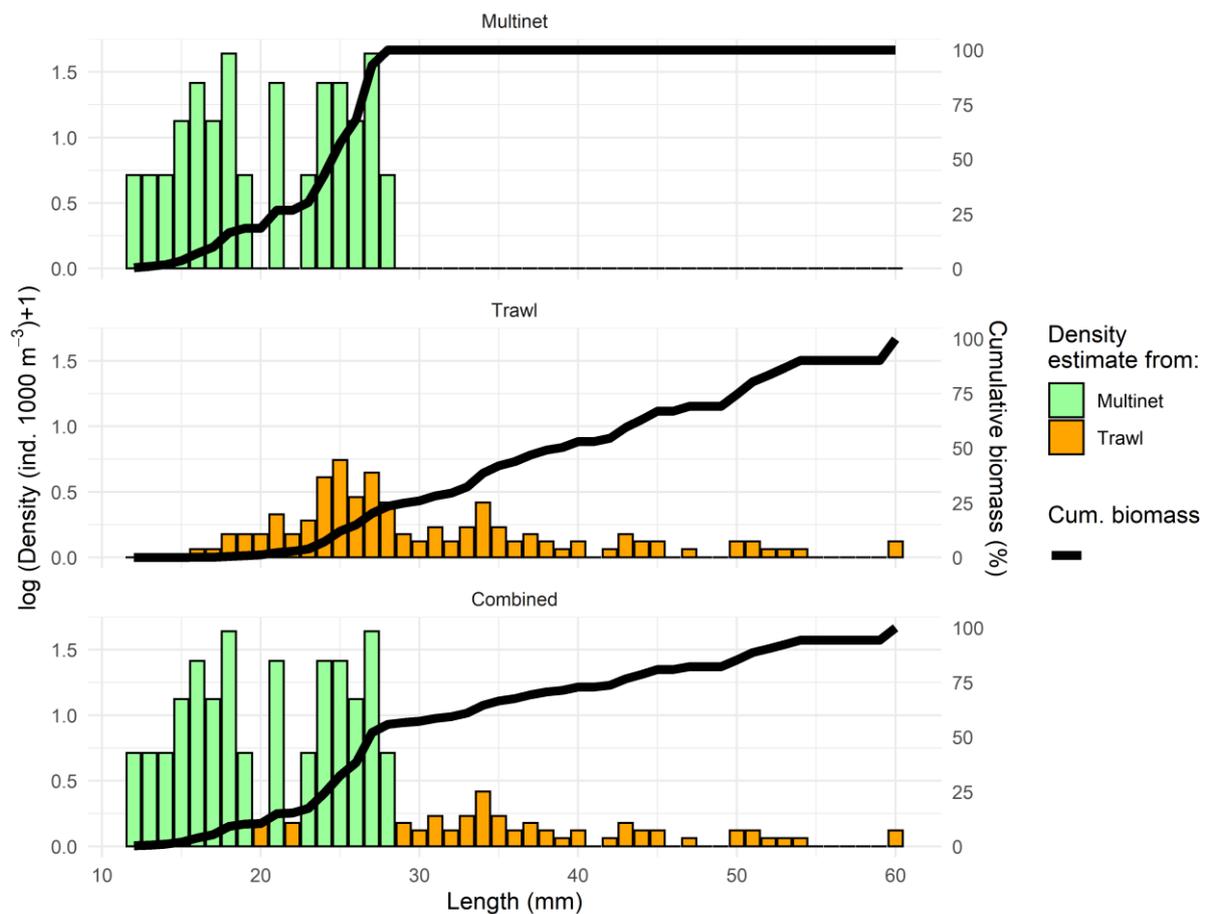


Fig. 2.1 Density estimates (histogram bars) for *Cyclothone* spp. from different gears: Multinet Mammoth (1 m<sup>2</sup>, 180 μm mesh net, upper), Macroplankton trawl (92 m circumference, 34 m<sup>2</sup>, 3x3 mm mesh opening), and combined. Black line indicates cumulative proportion of total biomass by size class. Modified from Agersted et al.(submitted)

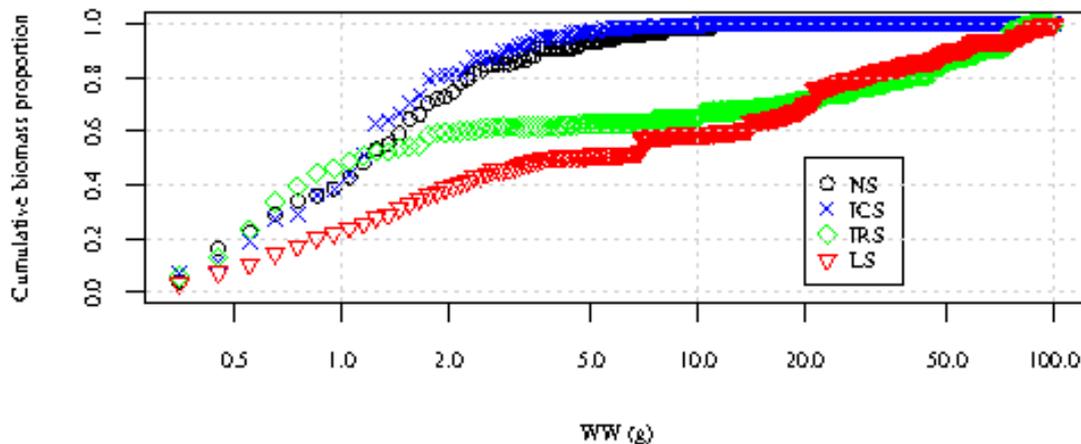


Fig. 2.2. Cumulative biomass spectra in 4 North Atlantic basins (Klevjer *et al.* 2019), catches from Macroplankton trawl (92 m circumference, 34 m<sup>2</sup>, 3x3 mm mesh opening).

Previous studies across the North Atlantic (with identical gear) have documented relatively major differences in the size distributions across these basins (Fig. 2.2, Klevjer *et al.* 2019), with individuals larger than ~10 g wet weight largely absent in the Norwegian and Icelandic Seas, yet making up 40-50% of mesopelagic biomass in the samples from Irminger and Labrador Seas. A study using an acoustic camera to size organisms (Giorli *et al.* 2018) have suggested that large organisms are more common than the current paradigm of “the small, mesopelagic fishes” would suggest, so better quantitative data on size and biomass distributions should be a high priority for mesopelagic studies.

IMR is working to provide improved data on size and biomass distributions by increasing the size of the trawls used, leading to higher volumes filtered. All trawls used in this work are based on the principle of even mesh-sizes along the entire trawls, but for logistical reasons the larger trawls have coarser mesh sizes. To supply data for biomass densities over the entire size spectrum of mesopelagic micronekton, we depend on using multiple nets/trawls. Point of contact: Shale Rosen, Webjorn Melle, Thor Klevjer, IMR

### 3. Avoidance/Interaction with sampling gear

#### 3.1 Avoidance from towed gear

As there is a move to include more sensors and equipment on submerged platforms, it is important to also assess the extent to which the presence of these platforms at depth also leads to changes the distribution and behaviour of mesopelagic organisms. Vertically profiling platforms deployed from the ships also cause a disturbance in the water column underneath the vessels, and may themselves elicit a reaction from the organisms (Koslow 1995; Benoit-Bird *et al.* 2010). Similar effects have been observed also for plankton nets, where it is suspected that avoidance of especially larger planktonic forms may cause a severe bias in net catches (Sameoto 1993; Wiebe *et al.* 2004). Using acoustic moorings constitutes an obvious way of assessing this (Rostad *et al.* 2006), but requires dedicated ship-time for deployment/retrieval. One can also use sensors on the deployed platforms itself (Dias Bernardes *et al.* 2020).

None of the avoidance experiments performed/work done under MEESO so far (3.1,3.2) have been explicitly aimed at evaluating the effects of artificial light on

behaviour, though recent works have highlighted the importance for mesopelagic distribution (Kaaertvedt et al. 2019, Underwood et al. 2020a). There are plans to include experiments with and without artificial light sources in future work at IMR, and such studies are especially important where optical systems are used for identification or enumeration of mesopelagic components, due to the potentially large biases induced by the use of artificial lights. The presence of sampling equipment at mesopelagic depths may stimulate bioluminescence (Kampa & Boden, 1957), thus while bioluminescence in itself is not artificial, the levels of bioluminescence may be artificially high in the vicinity of lowered gear. At mesopelagic depths the visualisation distances for single bioluminescent flashes may be up to 30 m for myctophids (Turner et al. 2009), presumably detection distances for bioluminescent “clouds” associated with sampling gear will be even greater, but none of the partners are presently equipped to evaluate this effect.

For mesopelagic trawl hauls, IMR routinely monitors avoidance ahead of the trawls using methodology outlined in Underwood et al. 2020b. In short, a forward-looking autonomous echosounder (Simrad WBAT) attached to the headrope of the trawl measures relative velocities and densities of organisms directly ahead of the trawl (Fig. 3.1.1 shows data from a forward looking setup on a tow-fish).

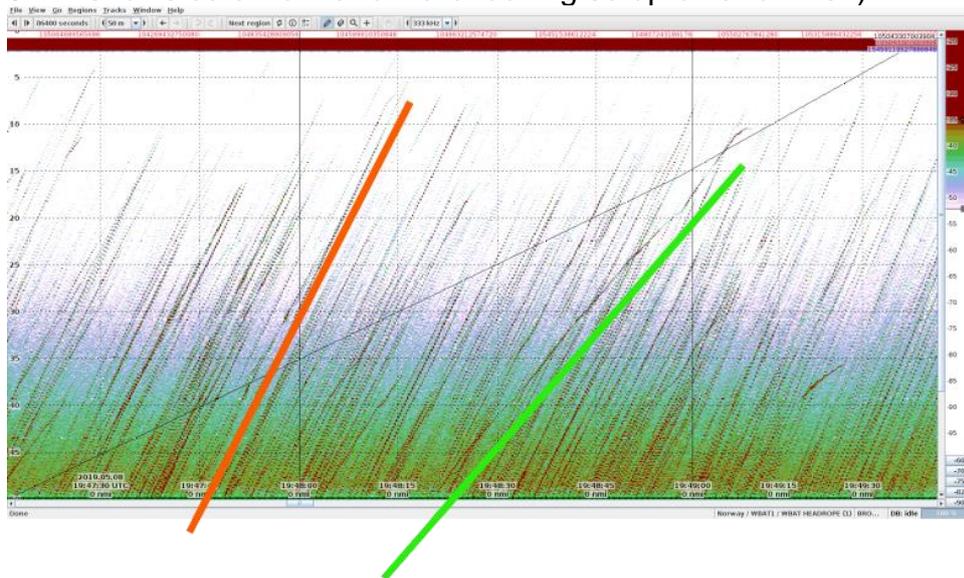


Fig. 3.1.1: Data from a forward looking echosounder deployed in the front of a tow-fish showing a situation where most organisms have very little behaviour relative to the approaching object, but with a few organisms apparently swimming away. The orange line is approximately aligned with the traces of most organisms, and indicates they approach the tow-body at a speed of  $\sim 1.3 \text{ m s}^{-1}$ , equivalent to the tow-fish' speed through water. The green line tracks an organism that approaches the tow-body at  $\sim 0.8 \text{ m s}^{-1}$ , i.e. in practice swimming away from the approaching tow-fish at  $\sim 0.5 \text{ m s}^{-1}$ .

In addition to monitoring the volumes ahead of the trawls, IMR has also conducted experiments with pulling different sampling gears over acoustic moorings (Fig. 3.1.2), in order to obtain timeseries of vertical distribution and scattering levels around perturbations at mesopelagic depths.

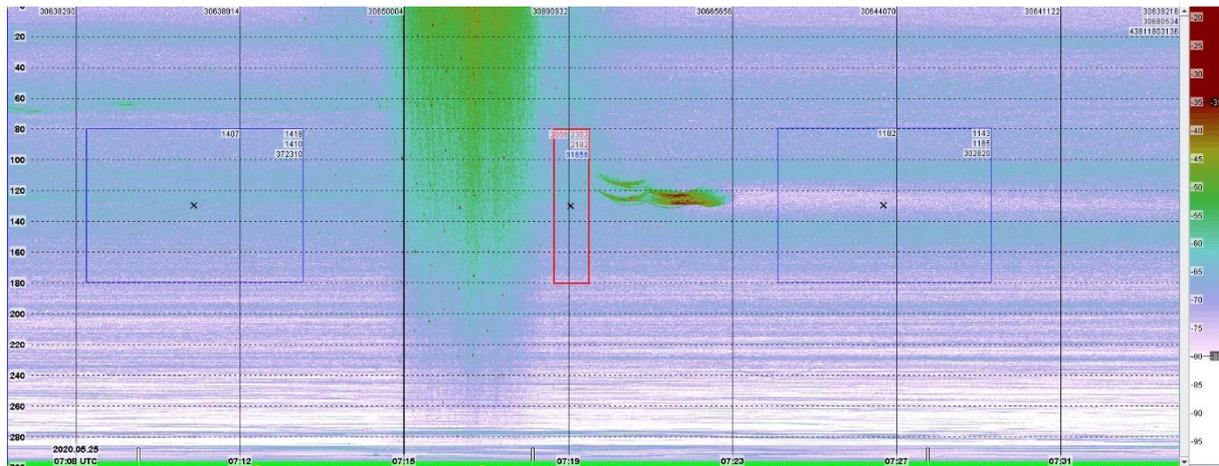


Fig. 3.1.2. Echogram from acoustic mooring showing the passing of the vessel (noise band starting at 07:15) and the trawl (strong echo in midwater around 07:20), and periods before and after. Boxes in midwater depths show locations of integration bins used in analysis.

The results show differences in vertical distribution consistent with a downward avoidance (Fig. 3.1.3), with some of the differences apparently occurring over the timeframe between the passing of the vessel and the trawl, a more detailed analysis is in progress.

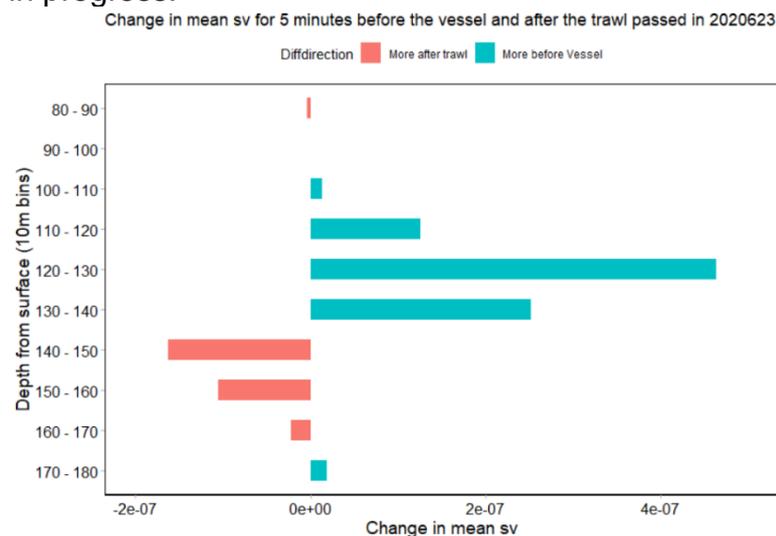


Fig. 3.1.3. Linear change in backscattering levels between the integration boxes before vessel passage and after trawl passage, in Fig. 3.1.2.

### 3.2 Avoidance from vertically lowered gear (P1.3)

The increased availability of compact submersible echosounders should enable more studies using echosounder output directly to assess organism behaviour in relation to submersed platforms (e.g. Dias Bernardes et al. 2020), but this is still quite costly equipment that is not universally available on research cruises. However, also other, less specialized, more generally available equipment can also potentially be used for this purpose. We are in the process of publishing a report where we use data from LADCP equipment to evaluate interactions between mesopelagic organisms and lowered CTDs (Klevjer et al, in prep). LADCPs are designed to estimate currents using the doppler shift of acoustic signals, but each LADCP used in this study also constitutes 4 primitive echosounders. Compared to a scientific echosounder the signal strength recorded by the LADCP for each beam has a reduced dynamic range, typically a much-

reduced vertical resolution, and the backscatter output of the LADCP beams are also usually not calibrated, but LADCPs are more widely available, and additionally collect hydrographical data.

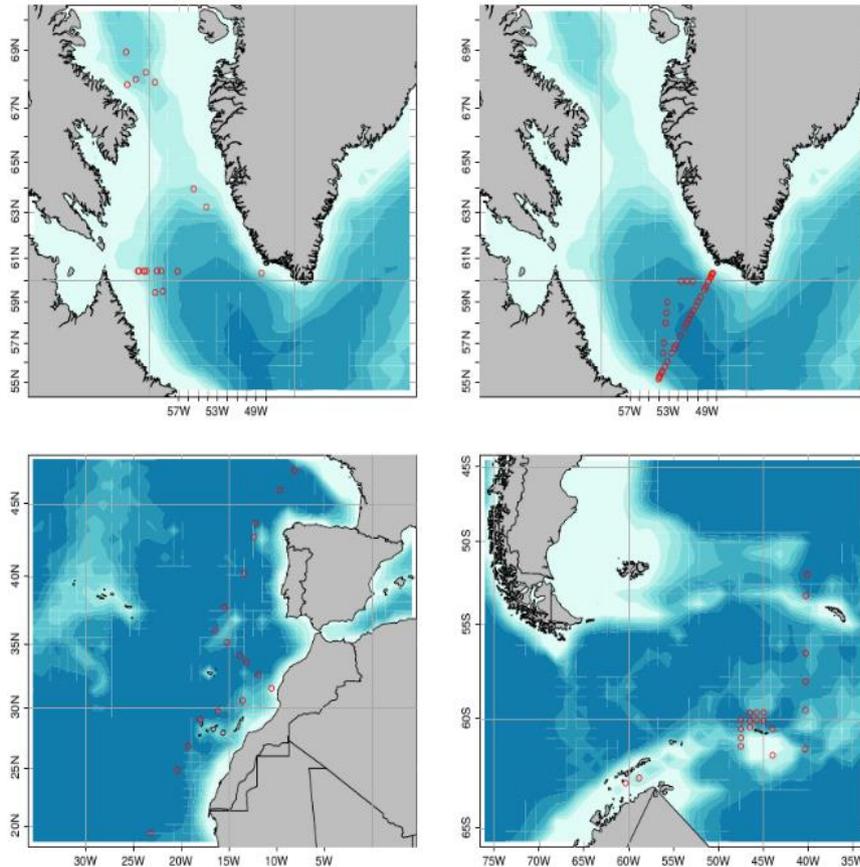


Fig 3.2.1. Location of data sampling points used for avoidance studies (Klevjer et al., in prep). Right panels: Uncalibrated mean volume backscattering strengths (MVBS) from LADCP downcasts plotted as a function of depth (Y) and range away from the LADCP (X), averages for the 4 cruises.

A method that should be insensitive to the lack of LADCP calibration is to compare paired backscattering levels (depth and range bin) between downcasts and upcasts of the CTD. In the absence of any reaction to the CTD, the expected difference in backscattering for a depth and range bin between down- and upcast is zero. The ocean is a dynamic environment, where organisms are advected horizontally and migrate vertically, so we expected a fair amount of stochastic variation, but this analysis has the advantage that it does not depend on either a calibrated output, or even a correct range correction of the signal, only that the transducer performance is the same on the down- and upcast (i.e. that effects of transducer hysteresis is not dominating). The results show that in all datasets average backscatter dropped from downcast to upcast for some depth-range bins (e.g. indicating avoidance), but also indicated that attraction to the CTD might occur at some depths.

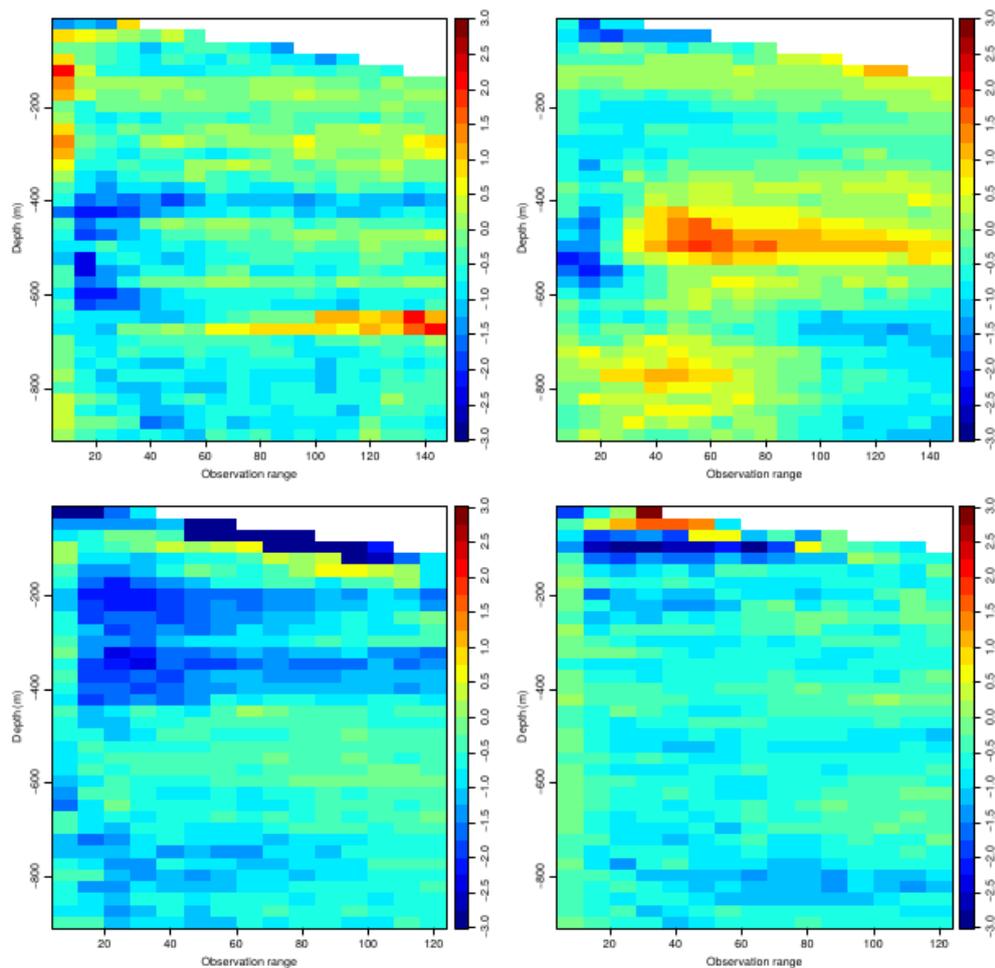


Fig. 3.2.2: Average estimated median difference in backscatter (dB) between down- and upcasts for the different datasets in Fig.3.2.1, based on all available profiles, plotted as a function of depth (Y) and range away from the LADCP (X), averages for the 4 cruises. Actual depths have been binned into 25 m vertical depth horizons. Positive values indicate avoidance, negative attraction to the CTD. From Klevjer et al., in prep.

## 4. Assessing impacts of lowered ping-rates on precision for other stocks (AZTI)

### *Impact of an increasing ping rate on acoustic abundance*

The recent increase in interest in mesopelagic species of acoustic surveys traditionally targeting epipelagic species has caused some surveys to increase their detection range. Also, the availability of different, mutually interfering, acoustic sensors onboard “acoustic vessels” cause the ping rate to increase by the typical synchronization strategy of sequential pinging of different sensors.

This has raised concern about the potential impact that the increase of ping rate, i.e., reduction of longitudinal resolution, might have on the acoustic estimates. The purpose of this work is to study whether ping rate affects the mean acoustic energy (NASC). The analysis has been carried out to determine whether the increase of range in a regular trawl acoustic survey for small epipelagic species (to assess mesopelagic

ones, which distribute deeper in the water column) may affect their assessment of biomass.

The objective of this study is to test the effect of a successive ping rate reduction on the average of NASC values collected in real conditions. The analysis tested only the effect of a reduced sampling scheme on the average based on statistical means. It was not measured, for example, the potential incidence the reduced resolution view of the targets might have on the assessment through the scrutinization process.

The analysis consisted in the echointegration of a number of transect segments targeting pure anchovy (Figure 3), selected based on high ping rates (~0.3 s) at the maximum resolution (1 ping). It followed a sequence of resamples on the echointegrated data to simulate the increasing of the ping rate. The mean NASC was calculated for each of the (simulated) increasingly spaced ping-rates, to evaluate potential uncertainty and bias in terms of sampling resolution.

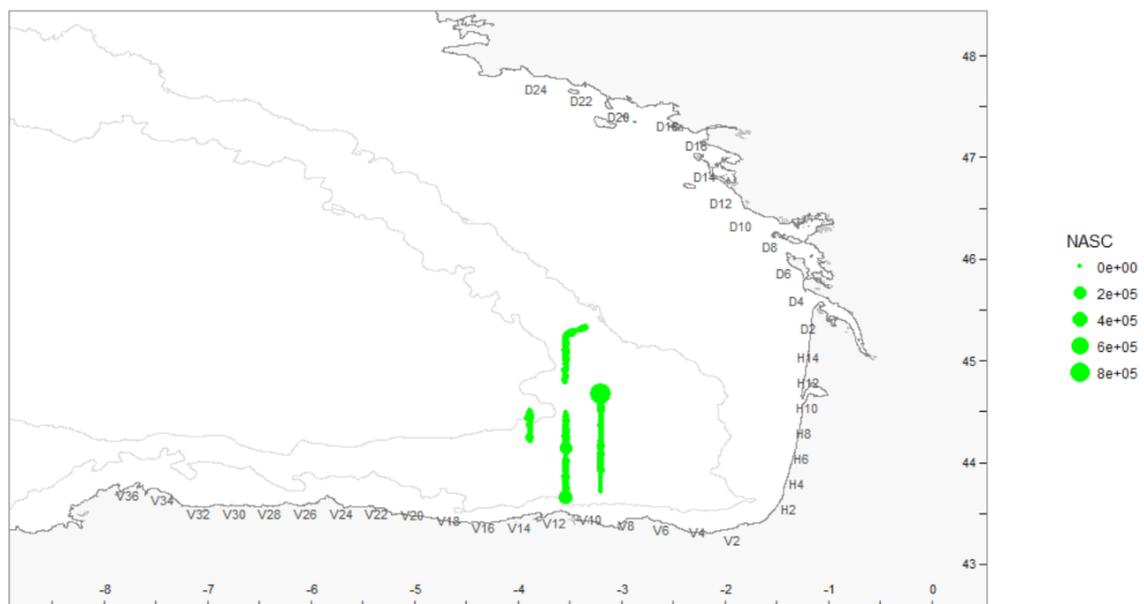


Figure 4.1 Transect acoustic segments from JUVENA 2010 used for the analysis of incidence of ping rate on acoustic abundance. The circles represent the mean NASC value per ping, with diameters proportional to the NASC value.

The transects were echo-integrated by cells of 1 ping x 50 m, with a minimum threshold of -60 dB, covering the vertical distribution of anchovy in that particular stratum. To simulate the reduction of ping rate, we performed random resampling without replacement on the vector of NASC values, using a decreasing number of samples (110000, 109000, ..., 1000). The ping rates simulated ranged 0.3 s to 30 s. For each sampling resolution, we computed resampling 1000 times and computed the average of the NASC each time. Then we computed boxplots of those 1000 average NASCs and plotted the boxplots for the different sampling resolutions.

The preliminary results showed a clear increase of uncertainty values with the increased ping rate (the trumpet shape in Figure 4).

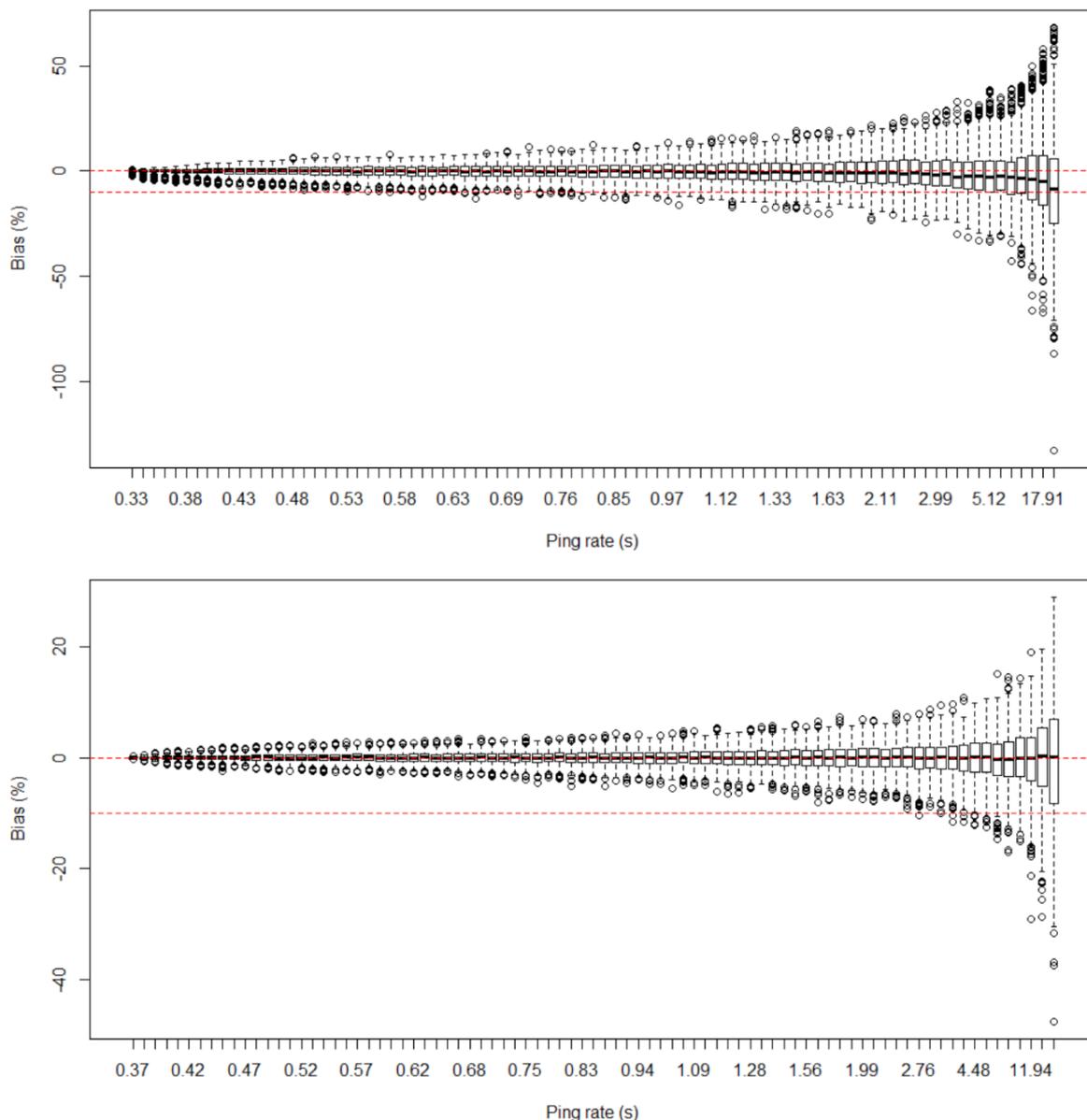


Figure 4.2. Top: Boxplot of NASC values as a function of simulated ping rate for a series of transects targeting pure anchovy in the JUVENA survey in year 2010. The increase of the upper and lower quartiles (height of the boxes) with ping rate reveals the increase of uncertainty caused. And the decrease of the median values (horizontal line inside the boxes) shows the increase of bias. Bottom: The same graph after removing the highest 1% of the data. The uncertainty still increases with ping rate (although at a slower pace; note the reduced y-axis limits), but the bias is basically removed.

An increase in bias was also noted (a decrease of averaged NASC values). The level of the bias seems low (<2%) for ping rates < 2 s. The bias was especially noticeable for extremely high ping rate values. The increase of uncertainty was expected, as is a logical consequence of a reduction of sampling resolution. But the reason for the increase of bias was not that obvious. So, we made an additional analysis to try to explain it.

The data structure indicated a highly skewed distribution with most values close to zero but dominated by a few extremely large ones (Figure 5). We repeated the boxplot of NASC against ping rate after removing the highest values. When we removed the 12 highest values (from N = 108,000) the bias was reduced to less than 1 % even for the highest ping rate (~30 s). When we removed the highest 1% of the values, the bias practically disappeared. The uncertainty increase (trumpet-like shape) remained in both cases. According to this result, we interpret that the bias is likely caused by the skewed distribution of this type of data (acoustic backscattering of fish), made of a majority of low values and a few extremely high ones. When we reduce the ping rate, the probability of missing one of the scarce high values increases, thus causing an underestimation bias of the mean value.

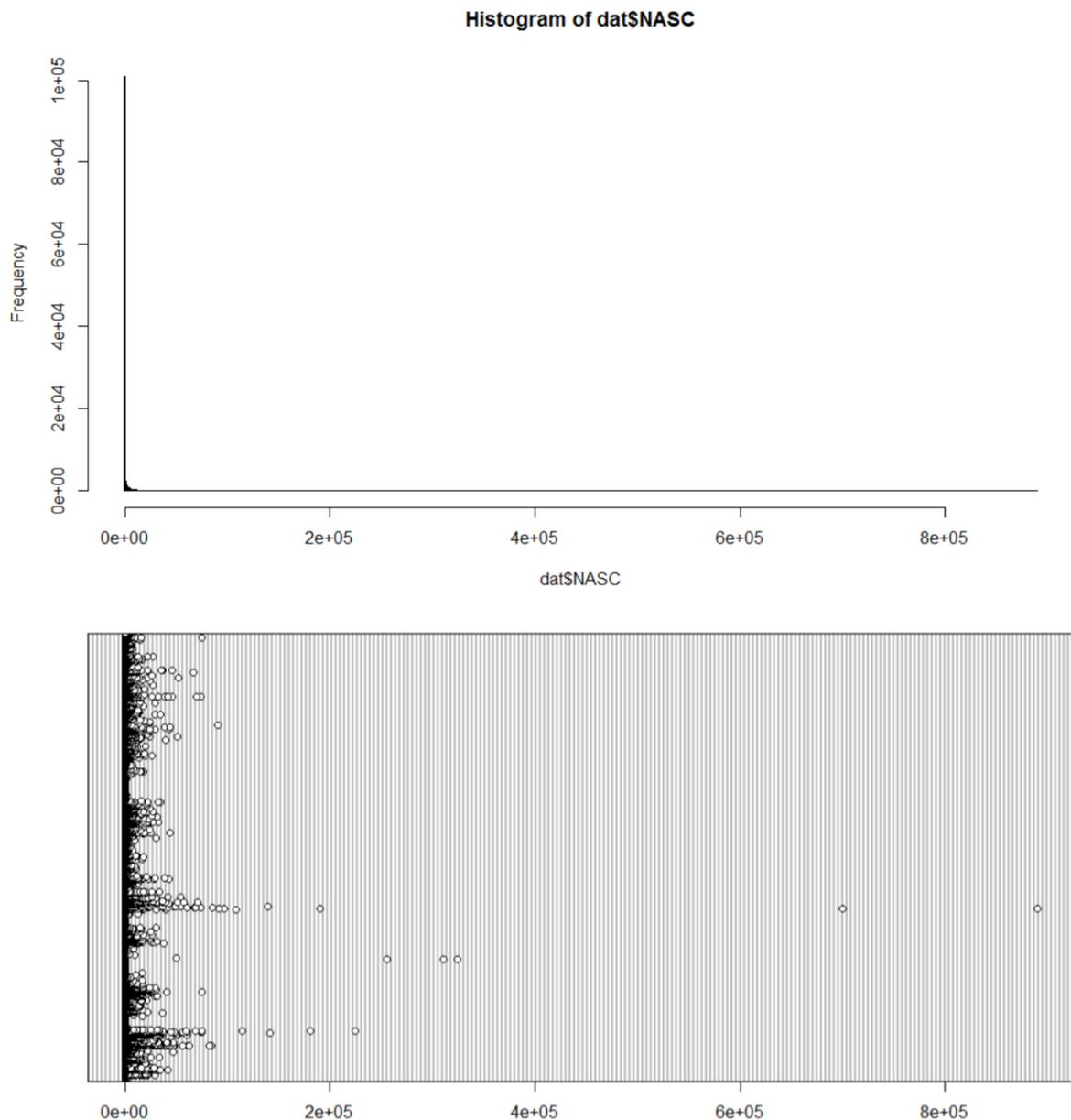


Figure 4.3 Diagrams indicating the structure of the data. The top panel shows a histogram of NASC values, highlighting the skewed nature of fisheries acoustics data, with the great majority of values close

to zero and a few extremely large ones. The dot plot (lower panel) spots the extremely large values that dominate the distribution.

This analysis is currently being refined by (1) adding a more sophisticated resampling scheme and (2) accounting for acoustic data of different heterogeneity grades, to study the possible incidence of heterogeneity on the increase of uncertainty and bias with decreased sampling resolution. Contact person: Guillermo Boyra (gboyra@azti.es)

## **5. Assessing uncertainties in the application of the echo integration method (Identification/ Acoustic properties/Target strengths)**

One of the factors that has limited the use of acoustic data for mesopelagic abundance estimation is the physical phenomenon of resonance: when the wavelength of the transmitted acoustic pulse is matched to the physical size of an organisms' gas-inclusion, the gas-inclusion will tend to resonate, which will result in a large amplitude in the backscattered signal. Since the magnitude of this resonant effect will vary with the size of the gas-inclusion, pressure at organism depth and e.g. properties of the organisms' tissues, it is a severely complicating factor for estimation of target strengths (TS) of organisms at depth (P2.1, P2.2). Recent efforts aimed at estimating biomasses/densities from acoustic measurements have attempted to use characteristics of organisms from the net-catches to parametrize acoustic models (E.g. Davison et al. 2015, Pena et al. 2014), an approach that is heavily dependent on unbiased net catches (e.g. P1.2, P1.3, P2.4), as well as the parameters used for the model. At present, the approach of using net-catches for providing details for acoustic modelling is likely to fail at larger scales due to a general lack of appropriate data on taxonomy and size distribution, an alternative method is to model these data (Proud et al. 2019).

Through MEESO, high quality data on *in situ* target strength and acoustic properties, as well as vertical profiles of micronekton densities are being collected, and these values will in the future provide improved input to the TS estimation process. These data can also be used to directly assess the accuracy/validity of current methods/assumptions for estimating mesopelagic densities. For instance output from hull-mounted echosounders is often used as a proxy for mesopelagic biomass, but as Fig. 5.1 below shows, both magnitude and vertical distribution of backscatter can depend on the frequency of observation, highlighting that a "deep scattering layer" is just that, a vertical layer of increased backscatter, rather than a layer with increased organismal densities, as it is often interpreted.



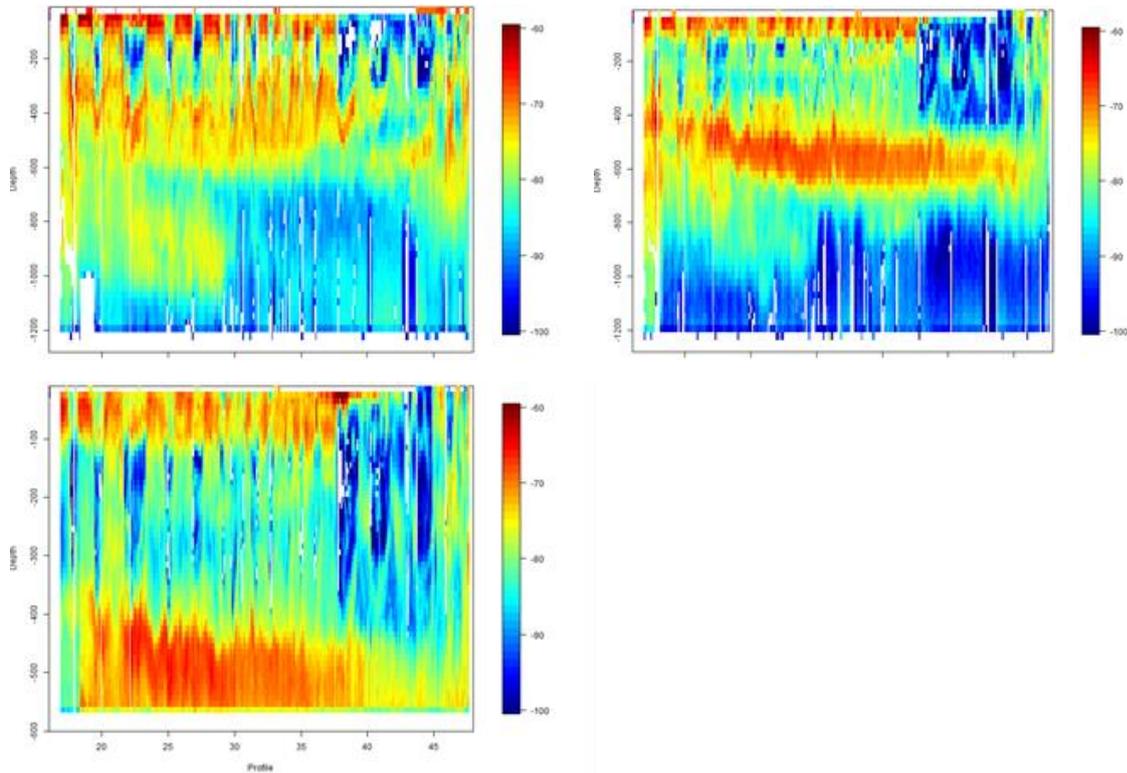


Fig. 5.1. What deep scattering layer are you referring to, Sir? Echograms from the hull-mounted acoustics showing that both relative strengths and vertical positions of “deep scattering layers” depend on the acoustic frequency used for observing them. Echograms recorded along the 2019 KPH cruise track (Cabo Verde to Bay of Biscay) from south to north showing backscattering strength ( $S_v$ , dB) both day and night at 18 (upper left), and 38 (upper right) and 70 (lower left) kHz.

Detailed vertical profiles of organism densities allow evaluation of how useful the different frequencies are in different areas. The example (Fig. 6.3) below shows estimated densities of mesopelagic micronekton (estimated from submerged 70 kHz FM data, and split into different acoustic categories by a clustering algorithm) and simultaneous backscatter measured from the hull-mounted echosounders. The vertical profile of backscatter at 18 kHz (grey dashed line in right panel) shows little overlap with the organismal densities estimated from the 70 kHz data. The vertical distribution of backscatter from the 70 kHz hull-mounted transducers (blue dashed line) shows reasonably good overlap over the effective observational range (~550 m), whereas the backscatter from the hull-mounted 38 kHz echosounder peaks ~100 m below the peak in organismal estimated densities. This means, that if using hull-mounted acoustic data measured at 18 and/or 38 kHz (which is the normally used frequencies in pelagic fishery studies), a substantial part of the mesopelagic community might be overlooked, which will lead to possibly under-estimation of “true” densities of mesopelagic organisms.

## 6. Assessing uncertainties in echo counting methods:

The use of echo counting for abundance estimation is largely impervious to effects of resonance, but it suffers from its own issues. Since the method largely operates at shorter ranges, there is high potential for effects of interactions between the sampling equipment and the organism’s distribution (e.g. avoidance). In practice, uncertainties

in the density estimates are introduced also by the echo detection algorithms and sampling volume effects.

The split-beam echo detection algorithms were developed primarily to give high quality TS estimates, rather than to obtain high detection probabilities. Effective observation volumes are however SNR dependent (Fig. 6.1), and it is necessary to ensure that SNR of detected echoes are high enough that observation volumes are not reduced (Soule et al. 1997).

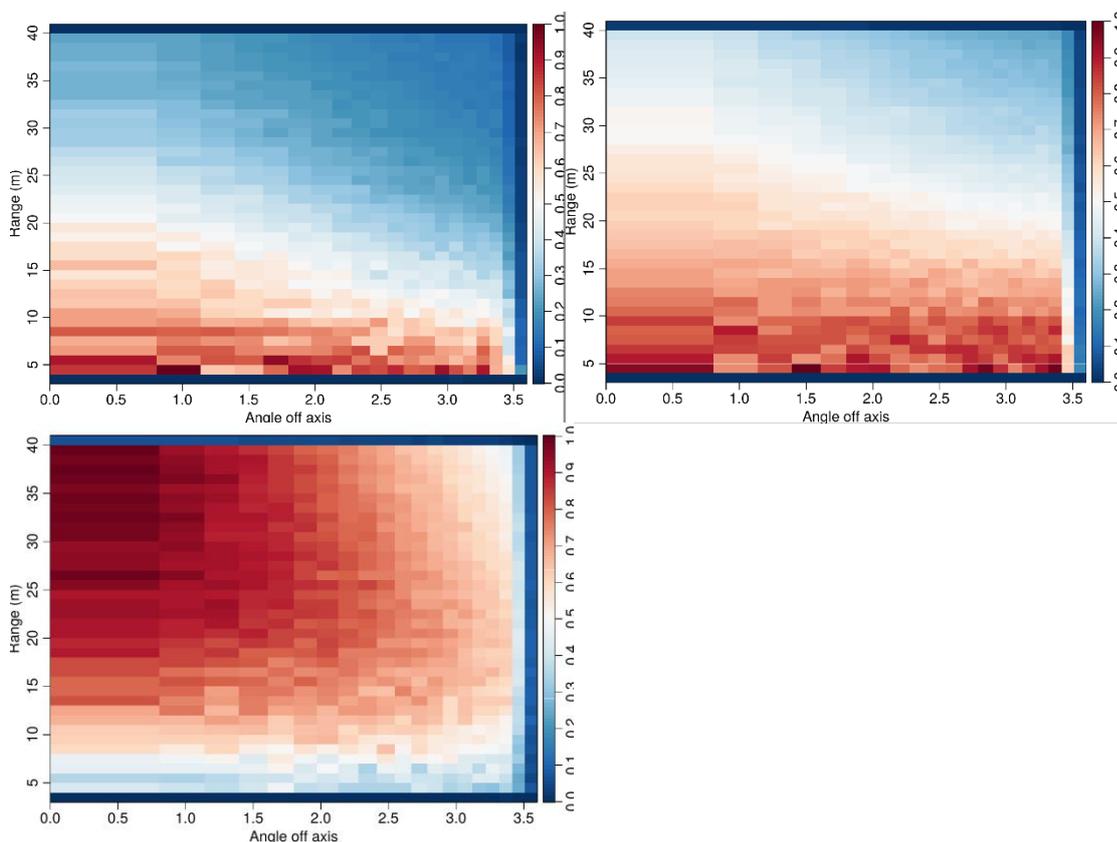


Fig. 6.1: Echo detection densities (echoes vol-1) for echoes detected in 70 kHz FM data (MESSOR) as a function of TS, range and angle off-axis. Upper left panel: Relative densities of echoes (# vol-1) with TS in  $[-90, -70]$  dB plotted against range and angle off axis (aoa), upper right panel: Relative densities echoes with TS in  $[-70, -60]$ , bottom panel: relative densities of echoes with TS in  $[-60, -50]$ . Notice drop in densities at longer ranges and off-axis positions for weaker echoes. Reduced densities of strong echoes at short ranges (lower panel) an indication of avoidance?

One way of evaluating the echo counting method, is by computing the NASC values the given density of echoes produce, this can be either compared to NASC values seen by the submerged transducer (Fig. 6.2), or NASC values recorded from hull-mounted transducers (Kloser et al. 2016, Fig. 6.3). In a perfect world there is perfect correspondence, but errors in echo-detections, poorly defined volumes and imperfect calibrations leads to deviations from perfect correlations (Fig. 6.2). However, these comparisons offer a rapid and easy ways to spot potential errors.

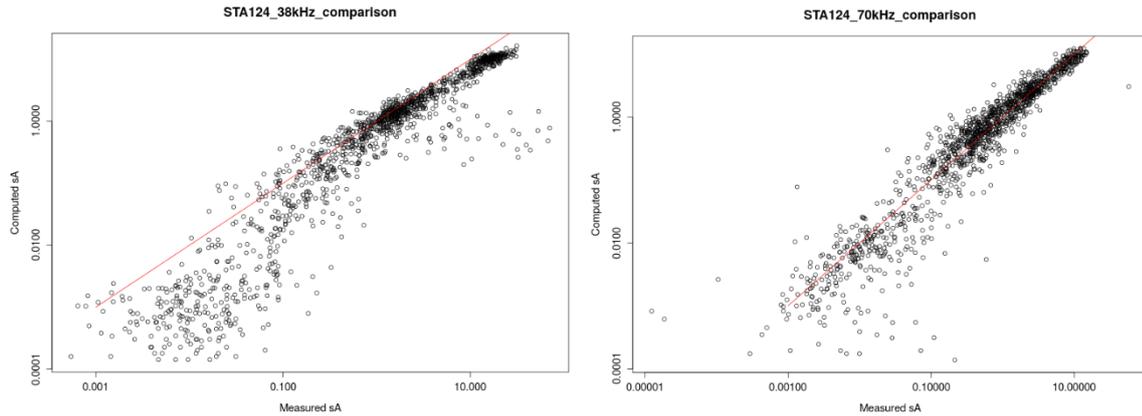


Fig. 6.2: Diagnostic plots of measured (x-axis) to back calculated (density x TS, y-axis) NASC for submerged transducers at a single station. Significant departures from the 1:1 line (red) indicates potential issues. At low NASC values, measured NASC tends to exceed computed, as the echoes have been thresholded, i.e. targets with low TS will have contributed to the measured NASC but will be excluded from the computed NASC. At high backscattering levels, estimated densities of echoes are reduced in the 38 kHz CW data (left) since echoes are only detected where there is 1 scatterer per reverberation volume, 70 kHz FM data from the same station does not appear to be affected by this.

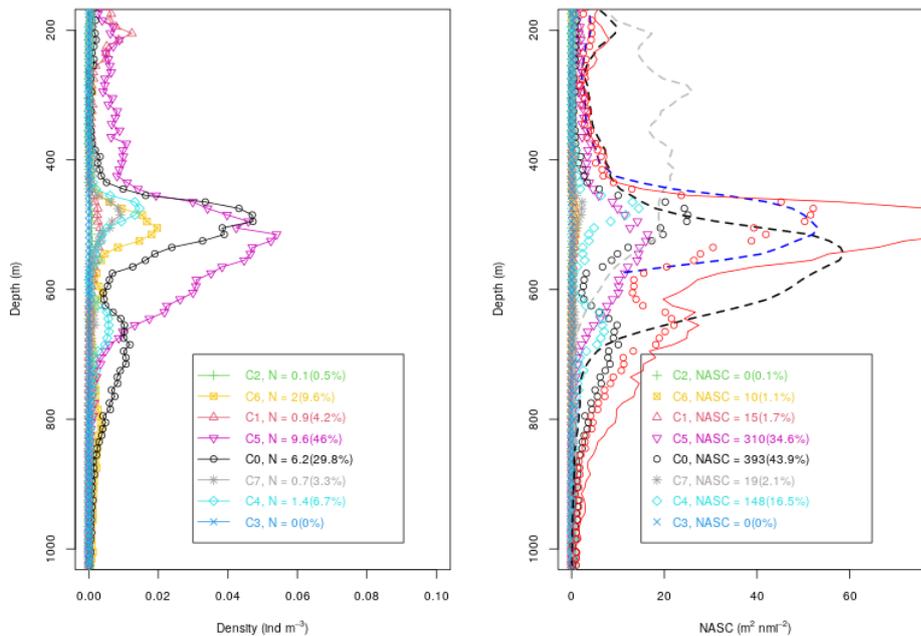


Fig. 6.3. Nighttime distribution of densities ( $\text{ind. m}^{-3}$ , left panel) based on counts from MESSOR and backscatter (nautical area scattering coefficient (NASC,  $\text{m}^2 \text{nmi}^{-2}$ ), right panel) for eight cluster types. In the right panel, the black dashed line shows vertical distribution of 38 kHz backscatter seen from the hull-mounted transducer, in the same timeframe as the MESSOR deployment. The gray dashed line is 18 kHz data, and the blue dashed line 70 kHz data. The red circles are a computed 70 kHz NASC estimate based on *in situ* densities and TS, whereas the red line shows 70 kHz NASC values measured from MESSOR. Like observed in Fig. 6.2, the measured NASC are higher than the computed, possibly due to thresholding away weaker scatterers in the computed but not in the measured NASC. Legend in left panel shows the different clusters, their integrated densities (200 – 1000 m), and their relative importance. Legends in right panels show cluster absolute and relative contribution to total NASC in the depth range.

## Conclusion/Summary:

MEESO sampling equipment quantification work covers many of the topics needed in order to improve our ability to accurately measure biomasses in the mesopelagic zone. However, for a variety of reasons several different types of equipment and methods are used to quantify biomasses of mesopelagic micronekton, even within the MEESO project. In a perfect world these methods should be intercalibrated, but a direct equipment comparison is hard to carry out in practice, and would also be prohibitively expensive.

One possible way to address this issue would be for the MEESO partners to settle on at least one method that can both be employed by all the partners, and that can be reasonably be used to enumerate mesopelagic micronekton densities. IMR suggests that submerged echosounders, used to directly count targets, can be used for this purpose and has for instance successfully deployed WBAT models in trawls and moorings (both on the seabed and floating in the water column).

On a larger scale the methods employed by the global scientific community to enumerate the mesopelagic zone are likely to have different strengths and weaknesses, and the outputs produced therefore have different levels of precision and accuracy. At present there is a move towards converting these estimates, spanning all trophic levels, into a common currency. This conversion is likely lead to increased obfuscation of the underlying differences in precision and accuracy of the different data sources. There is therefore a need for an effort to standardize mesopelagic sampling procedures, and in that process the scientific community should aim towards using methods that are quantitative.



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