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Executive Summary

This report documents the development and deployment, within the MEESO project, of methods capable of providing new insights into the behaviour of mesopelagic micronekton in relation to sampling platforms. The overarching goal of all these efforts is to provide a better understanding of how avoidance and attraction to the sampling platform itself distorts the results of methods used to quantify mesopelagic abundances and biomasses. Achieving this goal is perceived as a necessity for achieving higher accuracy in our current mesopelagic estimates: current global estimates of mesopelagic fish biomass roughly span an order of magnitude, largely because we don't know what we're measuring acoustically, nor how big they really are. This high level of uncertainty again has a detrimental effect on our overall understanding of open ocean ecology.

While some results from the MEESO efforts have already been published, more is under way, and the MEESO project is expected to make a significant contribution to the scientific knowledge on this topic. The behaviour of mesopelagic organisms, and the influence of this behaviour on our ability to quantify them, has complicated the work of the scientific community for a long time, and will continue to complicate our efforts long after the conclusion of the MEESO project. However, as the work presented in this report highlights, we're now at a stage where technologies are available that allow us a better understanding, and parametrization, of the effects of mesopelagic behaviour on our efforts to quantify them. This is timely, as the mesopelagic biota is a potential target for commercial harvesting, and the mesopelagic biota also play a role in the biological carbon pump, acting as mediators of the transport of carbon to great depths. Understanding the actual abundance levels and importance of their role is however dependent on proper quantification of the mesopelagic biota, hence the importance of the work presented here.

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Introduction:

This report summarises work undertaken under MEESO WP2 to study behaviour of mesopelagic organisms in relation to sampling gear. The title may be slightly misleading; the goal of the work is to determine how much influence behaviour has on our estimates of abundance and biomass, rather than to come up with a description of behaviour per se. This focus is also reflected in some of the approaches, for instance comparison of gear, where "selectivity curves" or other gear specific, rather than organism-specific, estimates may be the end-product of an analysis. While avoidance behaviour is normally not discussed in the context of acoustic mapping of mesopelagic resources, bias in the physical samples have consequences also for these works, either directly for our use of acoustic sensors on submerged platforms (e.g. platform avoidance), or as

"… 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". " (MEESO D2.2)

A previous deliverable from MEESO WP2 focused primarily on work undertaken in the project to assess the accuracy of presently used methodology for abundance and biomass estimation (D2.2: "Report on the use of enhanced catch and optical methods for accurate quantification of mesopelagic sampling tools"). In that report we summarised:

"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. " (MEESO D2.2)

While many factors contribute to the uncertainties in present estimates of mesopelagic biomass, mesopelagic micronekton avoiding sampling gear is perceived as a major contributor (Gjøsæter and Kawaguchi 1980, Kaartvedt et al. 2012), and in the introduction to D2.2 we stated:

"...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." (MEESO D2.2)

This deliverable is the last of the deliverables in a 3-report series from MEESO WP2 focusing on the work performed under that WP, and will be followed by a more technical protocol outlining a protocol for standardization of the estimation of mesopelagic abundance and biomass (D2.4). Like for previous WP2 deliverables in the MEESO project, this deliverable does not constitute a final report on the findings of the project, as analysis efforts are still underway, but rather reports on philosophy, approaches and methods that are or will be utilized by the partners for studying behaviour of mesopelagic organisms in relation to sampling gear. 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. In this report we have chosen to organize the approaches into methods that directly observe behaviour of individuals, or methods where behaviour is inferred indirectly through observations of population level attributes, such as population vertical distribution etc.

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It is resource intensive to deploy equipment to the mesopelagic zone, so many of the approaches utilized aim to “piggyback” on existing deployments, to remove the need for additional ship-time, platforms etc., and also by reusing standardized fisheries research tools. However, some studies are impossible to carry out without dedicated ship-time and equipment.

An important aspect of studying effects of gear on mesopelagic behaviour is to establish a baseline of undisturbed distribution or behaviour. This may or may not be readily achievable: it is often assumed that the distribution and behaviour of marine animals that we are observing represents organisms in their undisturbed or natural state. However, the presence of a research vessel constitutes a massive disturbance, and there is ample evidence in literature that large floating objects, such as a research vessel, affects the distribution and behaviour of pelagic organisms (e.g. FAD litterature), even at mesopelagic depths (Kaartvedt et al. 2004).

This effect is often implicitly disregarded in literature, and work carried out under MEESO has not had an explicit focus on it, since it is at present very hard to circumvent this issue (e.g. you can't trawl for mesopelagic organisms without a vessel). However, when assessing behaviour it is necessary to keep in mind that the presence of the research vessel constitute a detectable disturbance at mesopelagic depths.

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1. Direct observations of behaviour

From MEESO, D2.2:

“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 in 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 *et al*. 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).”

Imaging and video methods offer the most direct way of observing the behaviour of mesopelagic organisms, however these methods work over short distances (i.e. close to the observation platform due to the rapid degradation of visual cues in water), and in general need large amounts of light to work. Since the defining characteristic of the mesopelagic zone (more correctly called the “twilight zone”, Kaartvedt et al. 2019) is it's low ambient light-intensities, many imaging technologies traditionally deployed in shallower waters may not be appropriate for studying mesopelagic behaviour, as there is ample evidence that deploying artificial lights to mesopelagic depths may affect behaviour or distribution there (Klevjer et al. 2012, Kaartvedt et al. 2019, Underwood et al. 2020a).

It is fortunately possible to use sound to directly observe behaviour of mesopelagic organisms, and in general acoustic methods allow observation of behaviour at longer ranges, however generally without the ability to identify the taxonomy of the observed organism.

1.1 Direct acoustic observations of behaviour in relation to towed gear

For mesopelagic trawl hauls, IMR routinely monitors avoidance ahead of the trawls using the methodology described in Underwood et al. 2020b. In short, the method consists of a forward-looking autonomous echosounder (Simrad WBAT) attached to the headrope of the trawl, which measures relative velocities and densities of organisms directly ahead of the trawl (Fig. 1.1.1 shows data from a forward looking setup on MESSOR, a towed platform equipped with an array of sensors, including echosounders).

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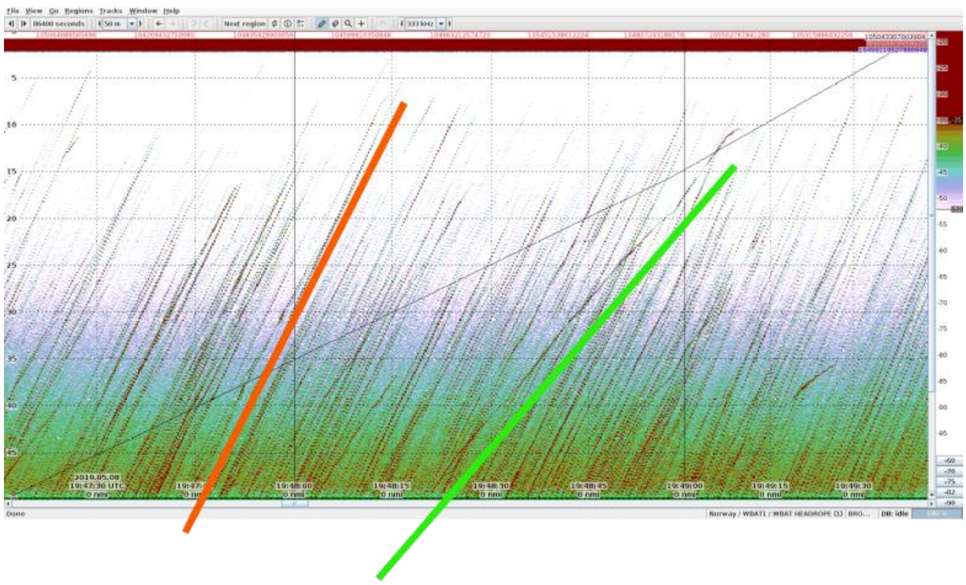

Fig. 1.1.1: Data from a forward looking echosounder (333 kHz) deployed in the front of a towed underwater platform (MESSOR) at mesopelagic depths, showing a situation where most organisms have very little behaviour relative to the approaching object, but with a few organisms apparently swimming away. The orange line is approximately aligned with the traces of most organisms, and indicates they approach of the towed platform at a speed of ~1.3 m s-1, e.g. approach speeds are equivalent to the towed platforms speed through water. The green line tracks an organism that approaches the towed platform at ~0.8 m s-1, i.e. in practice swimming away from the approaching towed platform at ~ 0.5 m s-1.

At present analysis of behaviour in relation to towed gear (E.g. towed bodies and trawls) is restricted to analysis of the approach speeds (e.g. assuming that most organisms show little behaviour in relation to an object, an organism with a swimming velocity deviating heavily from the typical is assumed to be showing behaviour relative the approaching object, see Fig. 1.1.1). Since the echosounder is capable of determining the position of the echoes in 3 dimensions, the collected echosounder data can also be used to detect spatial effects of behaviour, e.g. if organisms react by swimming downwards there should be higher number of echoes in the “lower” part of the horizontal acoustic beam. Target tracking of echoes, reconstructing the 3 dimensional swimming paths of individual organisms by combining echoes from consecutive pings, extend the current simplistic approach speed analysis to 3 dimensional speeds, and is needed in order to see if there are lateral components to reactions.

It may be possible to study other reactions as well, if for instance organisms re-orient themselves in reaction to an approaching object, a change in acoustic properties (E.g. for instance echo strength) is likely. However, current IMR efforts are centered on providing an estimate of how high a proportion of organisms are likely to evade an oncoming sampler.

Whereas organisms in the deeper mesopelagic in general appear relatively dispersed (see Fig. 1.1.1 above), some mesopelagic species form social aggregations. One of the theoretically assumed benefits of social aggregation is increased vigilance, e.g. an increased ability to detect danger, it is therefore possible that socially aggregating organisms are more efficient at avoiding our sampling gear. Fig. 1.1.2 shows a collage of 2 aggregations of krill approaching a midwater trawl, whereas one of these apparently successfully detects and avoids the approaching danger, the other aggregation shows no apparent change in swimming behaviour.

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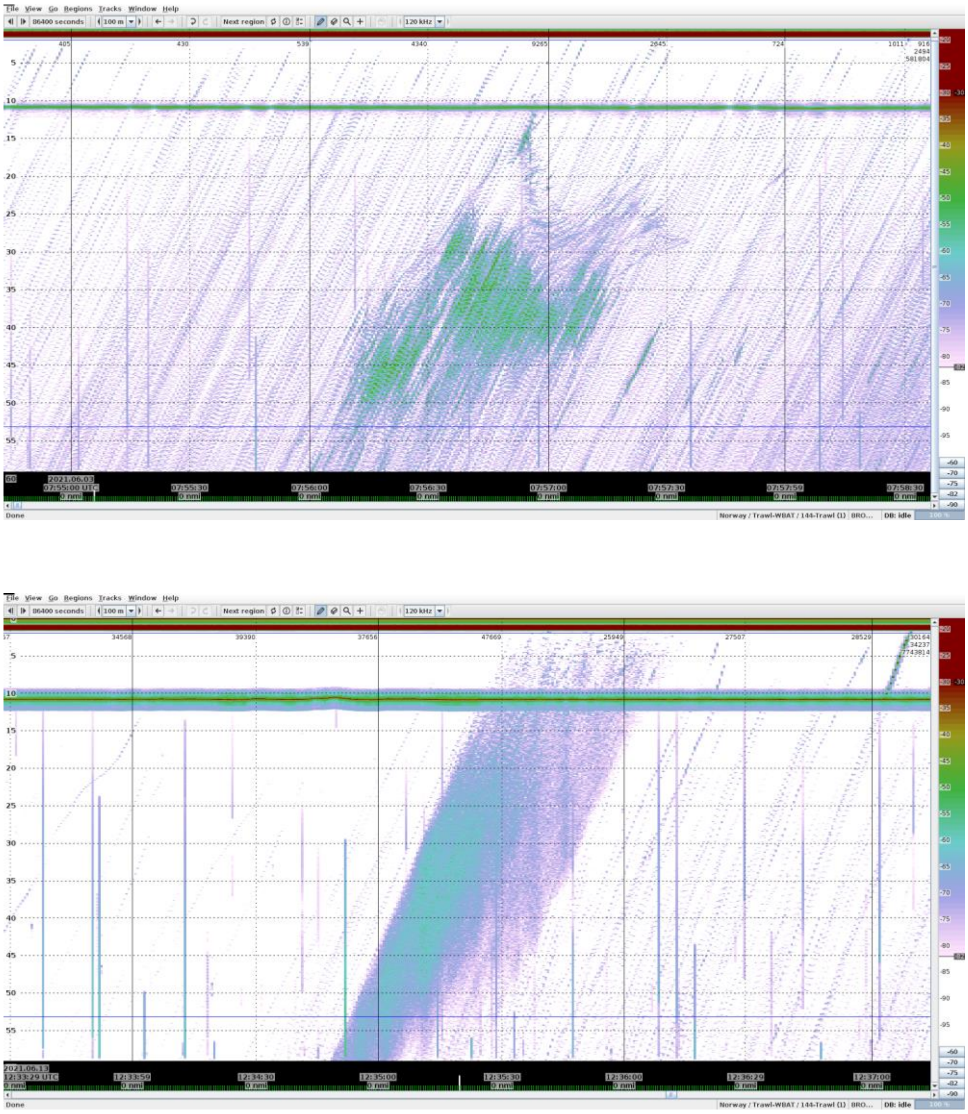

Fig. 1.1.2: Both figures show data from a forward looking echosounder (120 kHz) mounted on the headrope of the "144" trawl. Upper: Avoidance of aggregated organisms. The figure shows clear signs of avoidance for a school of organisms approaching the trawl in the upper mesopelagic zone. Strong echo at ~10 m range is the constraining rope for the "144" trawl. Lower: No real signs of avoidance for this krill swarm encountered in the upper mesopelagic zone.

The ultimate goal of these studies is to obtain parameter estimates of avoidance velocities and directions, as well as detection distances. Using simple theoretical avoidance frameworks developed in the 60's (Barkley, 1964, 1972), these parameters can then be used to estimate overall ahead of trawl avoidance.

2. Inference of behaviour:

While we have access to some methods capable of «directly observing behaviour», i.e. to follow over time the detailed swimming paths of individual organisms, more commonly behaviour is inferred through estimated changes in population level attributes/characteristics, such as for

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instance changes in organism densities or the vertical distribution of organisms, or comparison of catch characteristics such as size. Under MEESO, IMR have used several such indirect methods to assess behaviour in relation to sampling gear.

None of the avoidance experiments performed/work done under MEESO so far have been explicitly aimed at evaluating the effects of artificial light on behaviour, though recent works have highlighted the importance for mesopelagic distribution (Kaartvedt 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.

2.1 Dedicated experiments:

During several cruises in the coastal waters of Norway, IMR has conducted experiments with pulling different sampling gears over acoustic moorings (Fig. 2.1.1). These results from these experiments provide timeseries of vertical distribution and scattering levels around perturbations at depth, the overarching goal has been to assess avoidance behaviour in relation to equipment used by IMR for mesopelagic abundance and biomass estimation. In practice, these experiments were performed shallower than 200 m, but in Norwegian waters, where the experiments were performed, light attenuation in the murky water is high enough that light-intensities at 200 m corresponds to levels found much deeper in the open ocean. As a consequence, mesopelagic organisms here are frequently found at depths usually considered epipelagic in other settings. Similar vertical displacement of the mesopelagic zone to the upper 200 m can sometimes be found also in the open ocean (see for instance Klevjer et al. 2019), the 200-1000 m delineation often used is a rule of thumb that does not always apply, as the classic definition of the mesopelagic zone is based on light levels (Kaartvedt et al., 2019).

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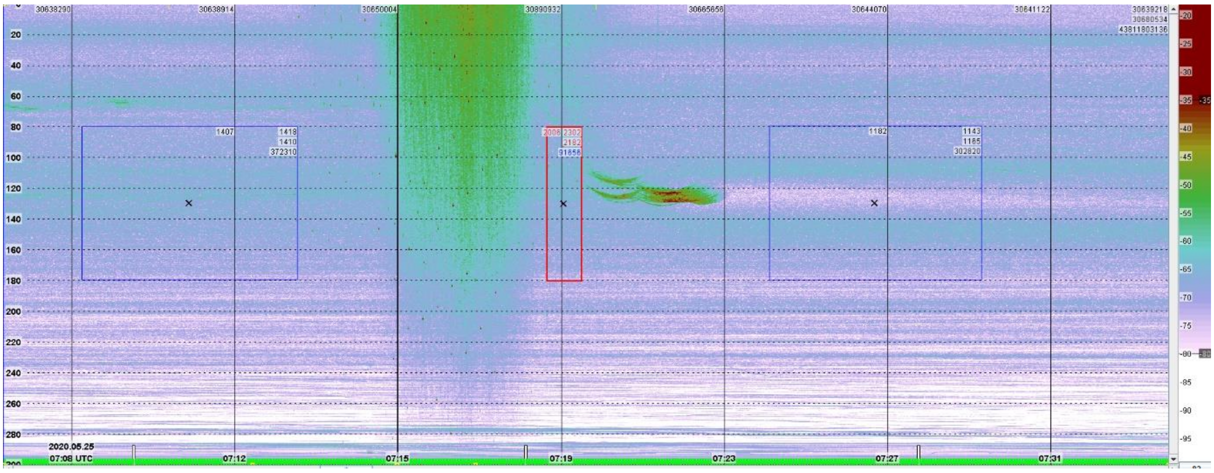

Fig. 2.1.1. From MEESO D2.2: Echogram from acoustic mooring (depth?) showing the passing of the vessel (noise band starting at 07:15) and the trawl (strong echo in midwater around 07:20), and periods before and after. Boxes in midwater depths show locations of integration bins used in analysis. Depths on the left-hand axis are meters from the surface.

The results from experiments where our trawls have been directed over the moorings, show differences in vertical distribution consistent with a downward avoidance (Fig. 2.1.2), 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.

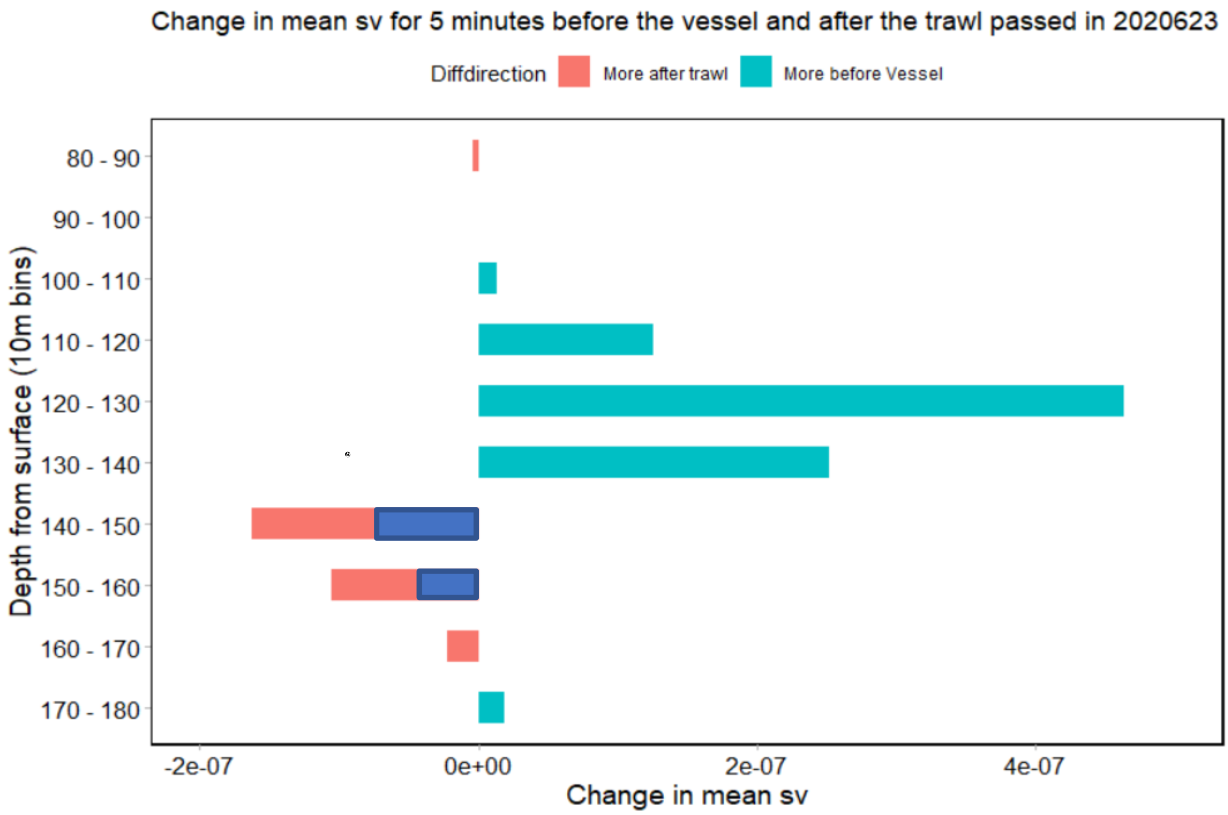

Fig. 2.1.2. Linear change in backscattering levels between the integration boxes before vessel passage and after trawl passage, in Fig. 2.1.1. Bars highlighted in dark blue show changes in backscatter from "initial" condition after passage of the ship, but prior to passage of the trawl,

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suggesting that a "ship-effect" may occur even in the absence of submerged equipment, but in this case this effect may be exacerbated due to the shallow distribution of the scattering layer.

In additional experiments the towed body MESSOR was hauled over the same mooring setup, with the results showing less pronounced differences before and after for the towed body, but again with the results consistent with the disturbance causing a downward shift in distribution.

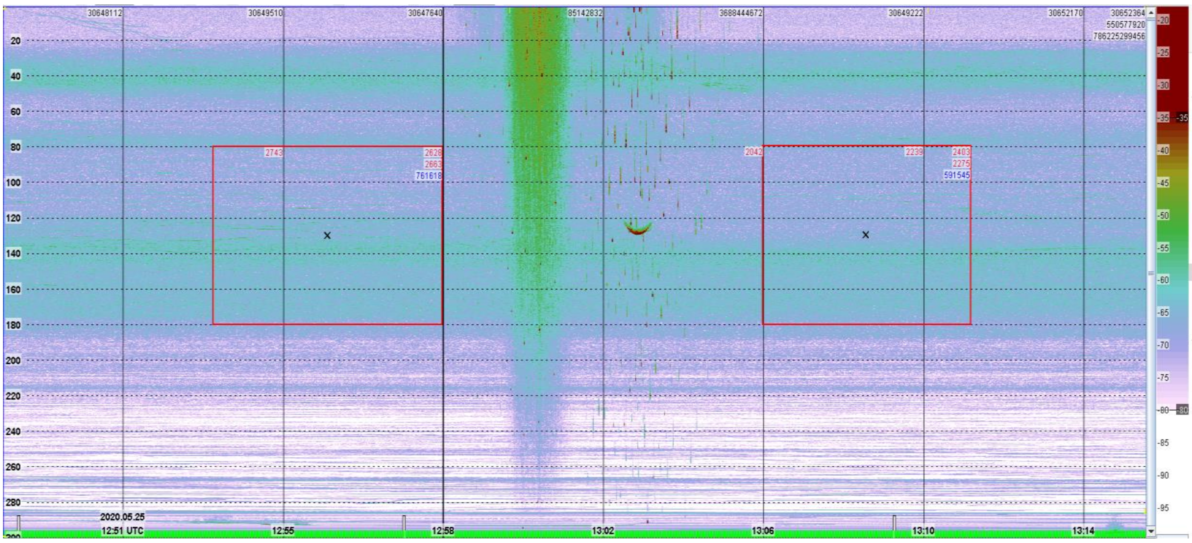

Fig. 2.1.3. Like Fig. 2.1.1, but showing the passage of ship and towed body MESSOR («red curved shape»), as observed from a submerged acoustic rig. A vertical shift in distribution was detectable also after the passage of the towed body, but relative changes in scattering levels were lower than for the trawl.

These dedicated experiments supplement more opportunistically collected data on «trawl tracks» (vertical segments of the watercolumn with reduced backscattering levels after the passage of a trawl), similar to those previously used to estimate avoidance levels for mesopelagic fish (e.g. Kaartvedt et al. 2012), and in combination IMR has collected a large dataset also at greater depths that could be used to further refine avoidance levels for mesopelagic organisms.

2.2 Catch comparisons:

Recognizing that no single catch equipment covers all situations and organism groups and sizes, it is important to have an estimate of how catches from the different types of equipment compare. In practice catch comparisons are challenging to carry out, as for instance spatial and temporal effects may render "paired samples" invalid, and there is in general high sample-to-sample variability even given identical equipment. These studies therefore tend to demand a very high sampling effort, and are resource intensive. Several large studies have however been attempted, see Skjoldal et al. 2013 for a zooplankton oriented study and Pakhomov &

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Yamamura (2010) for a micronekton oriented one, though none of these covers the catch equipment used under the MEESO project.

Using catch comparisons it may be hard to separate effects of actual avoidance behaviour from other effects of equipment bias and sampling artifacts (e.g. net escapement, herding, spatial variation etc, see D2.1/D2.2). Usually this is not an important issue, as it is the combined effect that is interesting in our context, though sometimes the combined effect in practice ends up being ascribed to "avoidance" (Kaartvedt et al., 2012), which may or may not be correct.

During MEESO, IMR has conducted several equipment comparisons, to establish a baseline for intercomparison between the micronekton equipment used in the project by IMR, as equipment opening areas used for macroplankton/micronekton catches by IMR under MEESO has ranged from ~1 m^2 (for *Cyclothone* net escapement is an issue even with 3x3 mm meshsizes, necessitating the inclusion of data from "plankton" equipment to estimate densities) to 345 m^2, with mesh sizes ranging from 0.180 to 7 mm (square opening).

An extreme example is given in MEESO D2.2 (Fig. 2.2.1 below), showing estimated densities of fish of the genus *Cyclothone*, caught with two very different gears (a 1 m^2 Multinet Mammoth and a ~34 m^2 Macroplankton trawl, during the 2019 Norwegian cruise from Cabo Verde to Norway. In Fig. 2.2.1, copied from D2.2, the upper panel shows numerical density distribution (bars) and cumulative biomass distribution (black line) of *Cyclothone spp.* from a 1 m^2 opening Multinet Mammoth (mesh size 180 µm), while the middle panel shows the same metrics from catches in the 6x6 m (~ 34 m^2 opening area, 3x3 mm mesh opening) Macroplankton trawl, and the lower panel shows a combined, best estimate size and biomass distribution based on a combination of the data from these 2 nets. About 40% 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 make up roughly 40% the population biomass), while the population abundance estimate is dominated by the smaller individuals. The comparison is only possible as both types of catches can be normalized to per unit volume, if information on volume filtered is not available, it is not possible to weigh the contribution of each equipment, and the histograms can at best be qualitatively assessed (see also Fig. 2.2.3).

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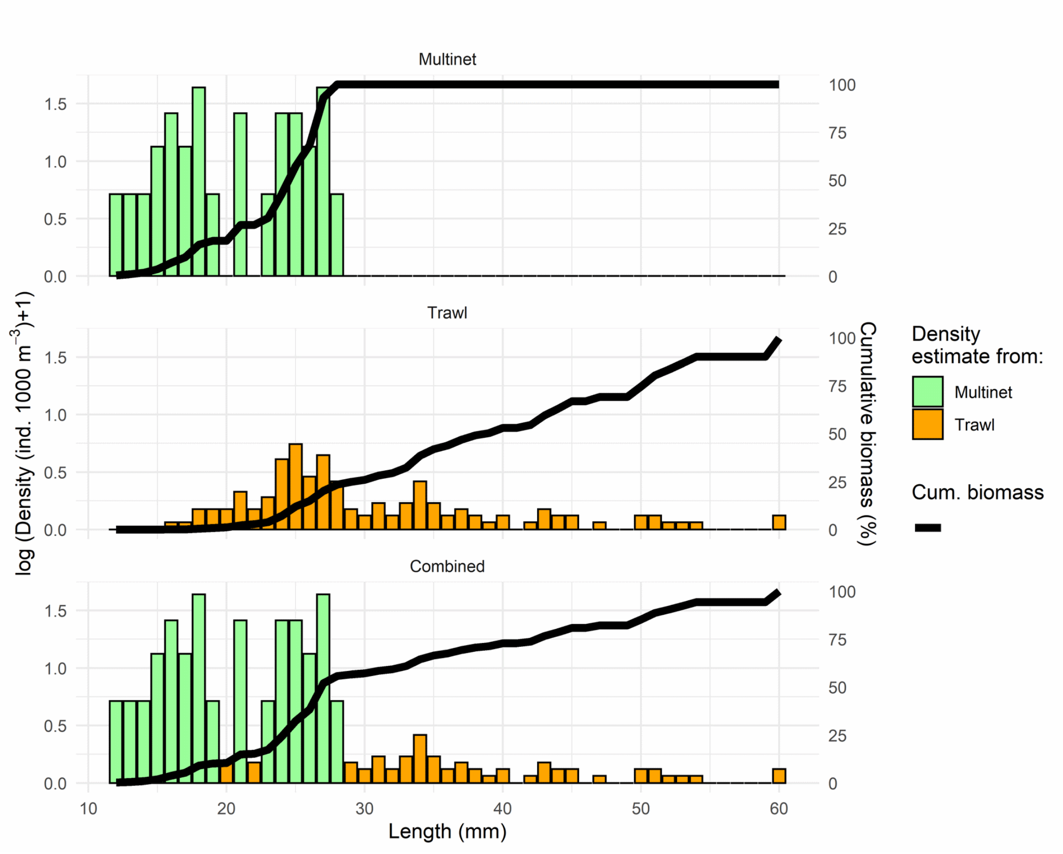

Fig. 2.2.1: From MEESO D2.2: Density estimates (histogram bars) for Cyclothone spp. from different gears: Multinet Mammoth (1 m2, 180 µm mesh net, upper), Macroplankton trawl (92 m circumference, 34 m2, 3x3 mm mesh opening), and combined. Black line indicates cumulative proportion of total biomass by size class. Modified from Agersted et al.(submitted)

IMR has also devoted a lot of effort into comparing catches from the 3 models of non-graded trawls that it currently uses in the project. Opening mouth geometry and towing resistance for the three (four) trawls tested by IMR is provided in figure 2.2.2, below.

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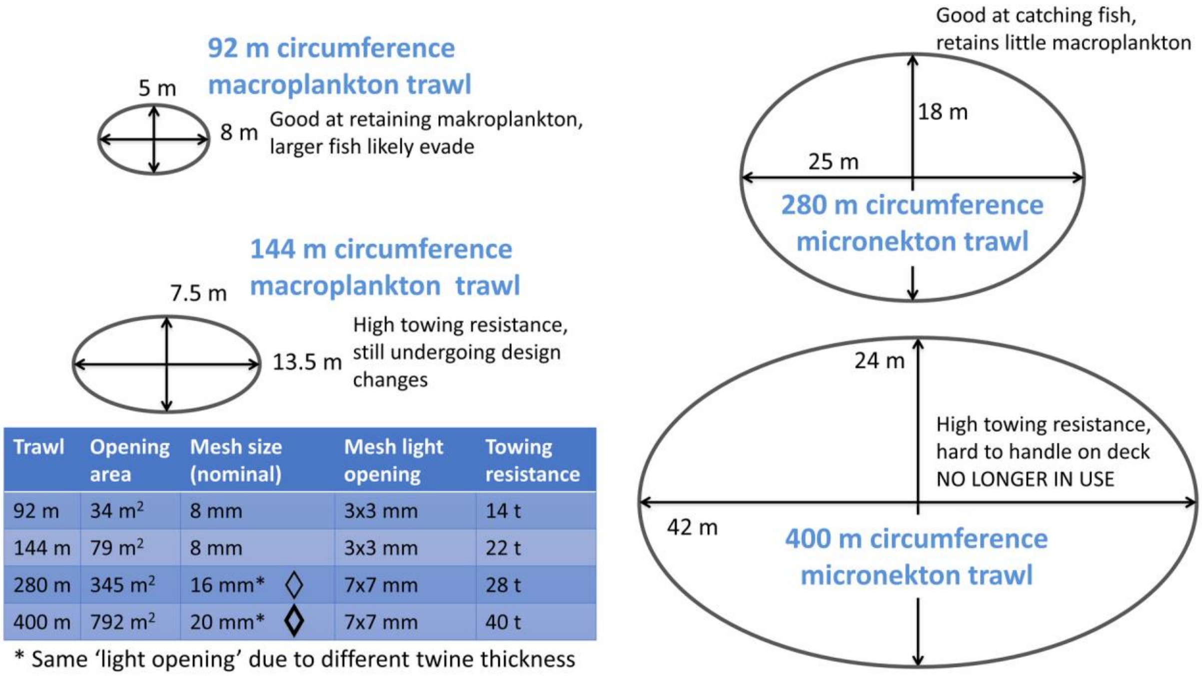

Trawl	Opening area	Mesh size (nominal)	Mesh light opening	Towing resistance
92 m	34 m^2	8 mm	3x3 mm	14 t
144 m	79 m^2	8 mm	3x3 mm	22 t
280 m	345 m^2	16 mm* ◊	7x7 mm	28 t
400 m	792 m^2	20 mm* ◊	7x7 mm	40 t

* Same 'light opening' due to different twine thickness

Figure 2.2.2. From MEESO D2.1: Opening geometry, mesh size (liner), and towing resistance for the four trawls tested by IMR to sample in the mesopelagic zone.

Preliminary analysis show that toward the smaller end of the size spectrum, estimated micronekton densities for the different trawls follows the mesh size (Fig. 2.2.3), as can be expected if extrusion through the net depends on the ratio between body size and mesh opening. Catches of micronekton toward the larger end of the micronekton size spectrum appears to be larger in the larger trawl (e.g. the "280" trawl in the table above), indicating larger specimens are able to avoid the smaller gears, but final results await quantitative analysis of the results from the 2021 IMR MEESO cruise in the North Atlantic.

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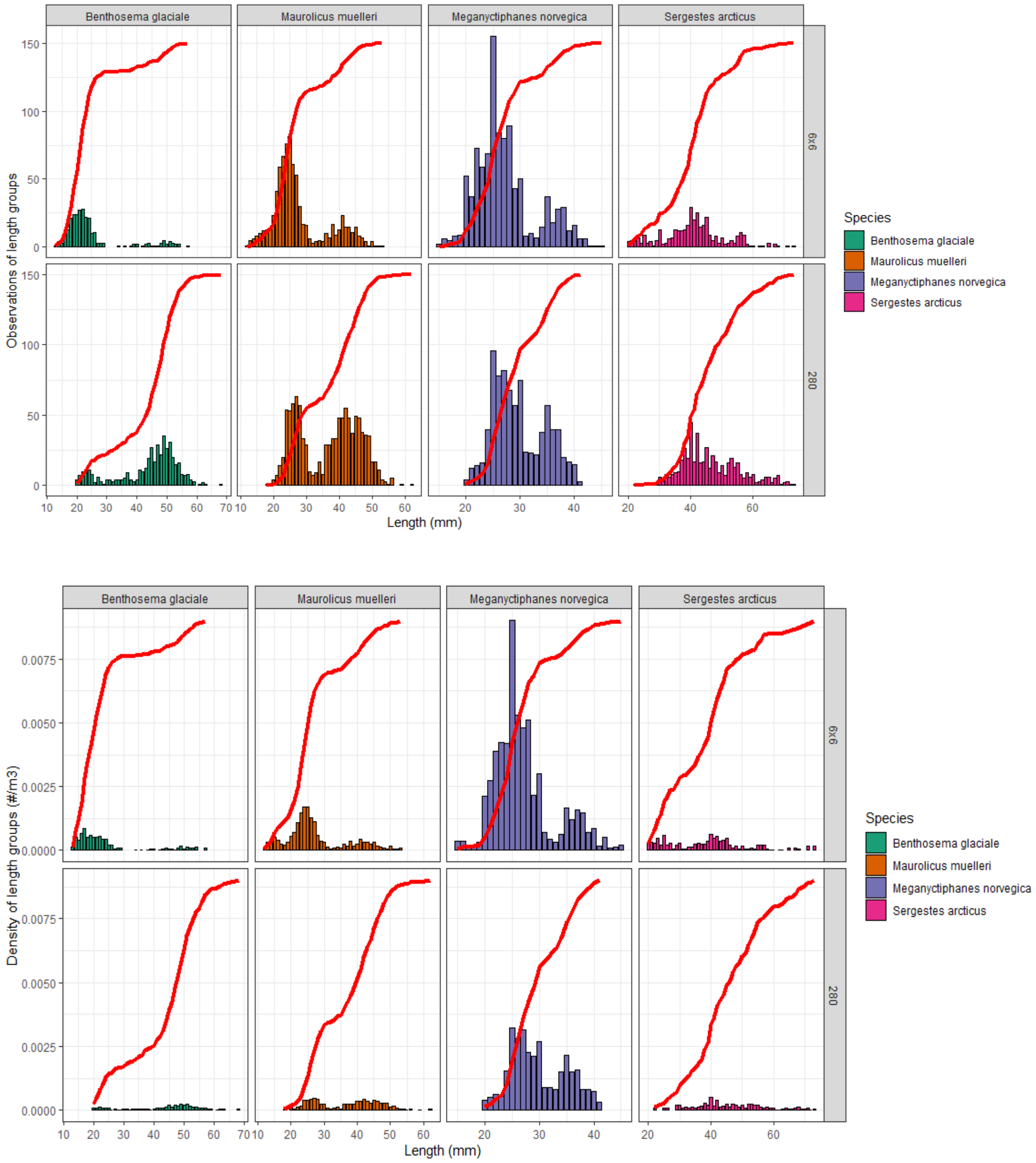

Fig. 2.2.3: Interpreting catch comparisons from different trawls. Upper panel shows raw histograms of captured individuals per length class and species from two trawls (upper row («6x6») is the 92 m circumference trawl, N= 6 (3x3 mm mesh size) in Fig. 2.2.2, lower row the 280 m circumference trawl (7x7 mm mesh size), N=5). Lower panel shows counts converted to organismal densities. While the catches show that the «92» trawl caught fewer large individuals of especially Benthosema and Maurolicus, conversion to organismal densities show that the actual dominating pattern in these data is that the «280» trawl has reduced capture efficiency for organisms below ca. 30 mm body lengths, and in these data there is no reason to conclude that avoidance has skewed the results.

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2.3 Inferences utilizing hull-mounted acoustics:

Comparison between results obtained from hull mounted echosounders and submerged instruments could theoretically allow the study of the influence of the submerged platform itself on the distribution of organisms, but such a comparison necessitates «a common currency» for the comparison. Previous studies have compared vertical profiles of backscatter at 38 kHz between a ship and a lowered probe, finding excellent overlap between the two (Kloser et al., 2016)

2.3.1 Hull vs submerged acoustics:

Discrepancies in backscattering levels recorded from the hull-mounted transducers with those recorded from a submerged body (at the same frequency), could indicate that organisms respond to the presence of the submerged body. In Fig. 2.3.1 70 kHz bacscattering levels recorded from MESSOR shows reasonable overlap with hull-mounted results, except in the region between ~150 to 400 m. Inside this depth range the sign of the differences varied, in the depth-range 200-300 m the levels recorded by MESSOR were lower, but from 300 to 400 m they were higher, which could be caused by a downward swimming reaction by the organisms in response to stimuli from the passing ship and towed platform (e.g. see Fig. 2.1.1 and 2.1.2). One complication in comparing such results is that the sampling volumes of echosounders depend on observation ranges, so that the hull-mounted echosounders observe a much greater volume at those depths. A second complicating factor is that the submerged echosounder is generally offset in time and (horisontal) space from the hull-mounted one, spatial variation may therefore affect the results, but both scales and levels of spatial variation in the mesopelagic is in general poorly known.

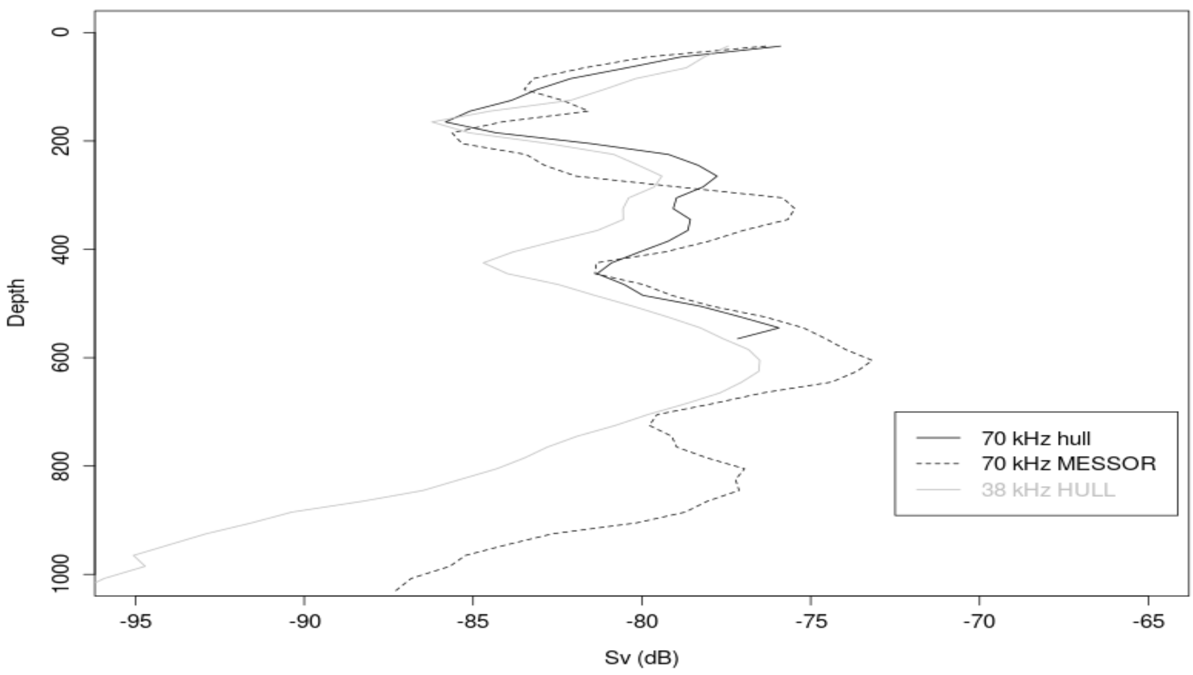

Fig. 2.3.1: Comparing backscattering levels from hull-mounted equipment with backscattering levels measured at close ranges from submerged equipment. The figure shows vertical profiles

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of backscattering levels measured with hull-mounted (whole lines) and submerged acoustic equipment, at comparable time frames (hull-mounted data were cleaned from noise-spikes etc., and averaged over 60 minutes around the towed platform deployment).

2.3.2 Inference from trawl attached sensors

A better parametrization of the avoidance of capture equipment is the overarching goal for these studies, in previous studies comparisons of biomass in trawl (catches) with expected biomass estimated from hull acoustic data have been used for estimating levels of avoidance (for instance Kaartvedt et al., 2012). The "catch" or "disturbed-state" levels can also be estimated from acoustic sensors attached to the trawl, e.g. this by simply interchanging acoustic measurements for the catch measurements in the calculations.

Comparison of hull mounted acoustic data with abundance data estimated from a forward mounted echosounder on the trawl (e.g. see Chapter 1.1) is complicated by the fact that the frequency of the echosounder used on the trawl is generally too high (due to size/weight requirements for the transducer) to allow simultaneous coverage of the mesopelagic zone for the same frequency from the hull-mounted echosounders. Comparison with a lower frequency hull-mounted echosounder (Fig. 2.3.2.1) highlights the frequency dependence of acoustic backscatter: data from a 38 kHz echosounder does not scale linearly with densities estimated at the higher frequency (120 kHz), as for instance pelagic crustaceans have very low backscatter at 38 kHz. A natural common currency for such studies might be to convert the acoustic backscatter to organismal densities, or one might also restrict the analysis to vertical ranges also covered by the high frequency hull-mounted echosounders, though this would lead to poor coverage in the lower mesopelagic. Again, changes (or lack thereof) in the estimated distribution of organisms (as opposed to backscatter) might indicate behaviour (or lack thereof) in relation to the trawl, and since

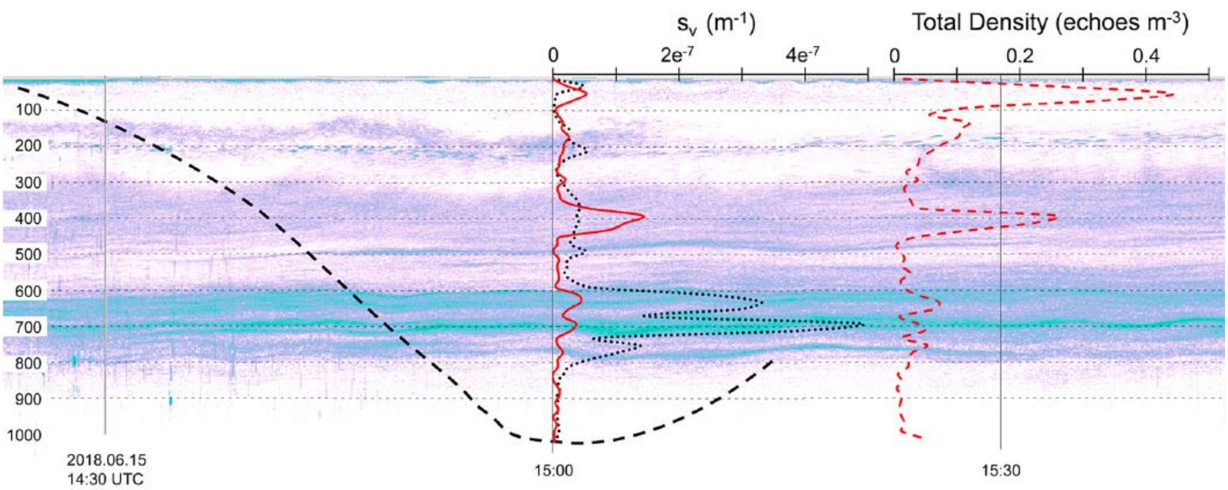

Fig. 2.3.2.1: Figure from Underwood et al. 2020: Hull mounted acoustics vs. submerged acoustics. Figure shows track of trawl headrope (black dashed line) overlaid on echogram recorded from hull-mounted 38 kHz data, covering the period of collection of data with a 120 kHz autonomous, forwardlooking echosounder attached to the headrope of the trawl. Insets show vertical profiles of volume backscattering coefficients (sV, center inset) for the 120 kHz

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submerged data (red line) and the 38 kHz hull-mounted data (black dotted line), and estimated density of micronekton (right inset, red dashed line) based on counts of single echoes from the 120 kHz submerged echosounder.

While IMR during the MEESO project typically has mounted the headrope-attached echosounders to monitor the volume ahead of the trawl, the ADCP's mounted inside the trawls, primarily to monitor flow in the trawl entrance (see MEESO D2.1), can be used to monitor and record the backscatter entering the trawl (Fig. 2.3.2.2).

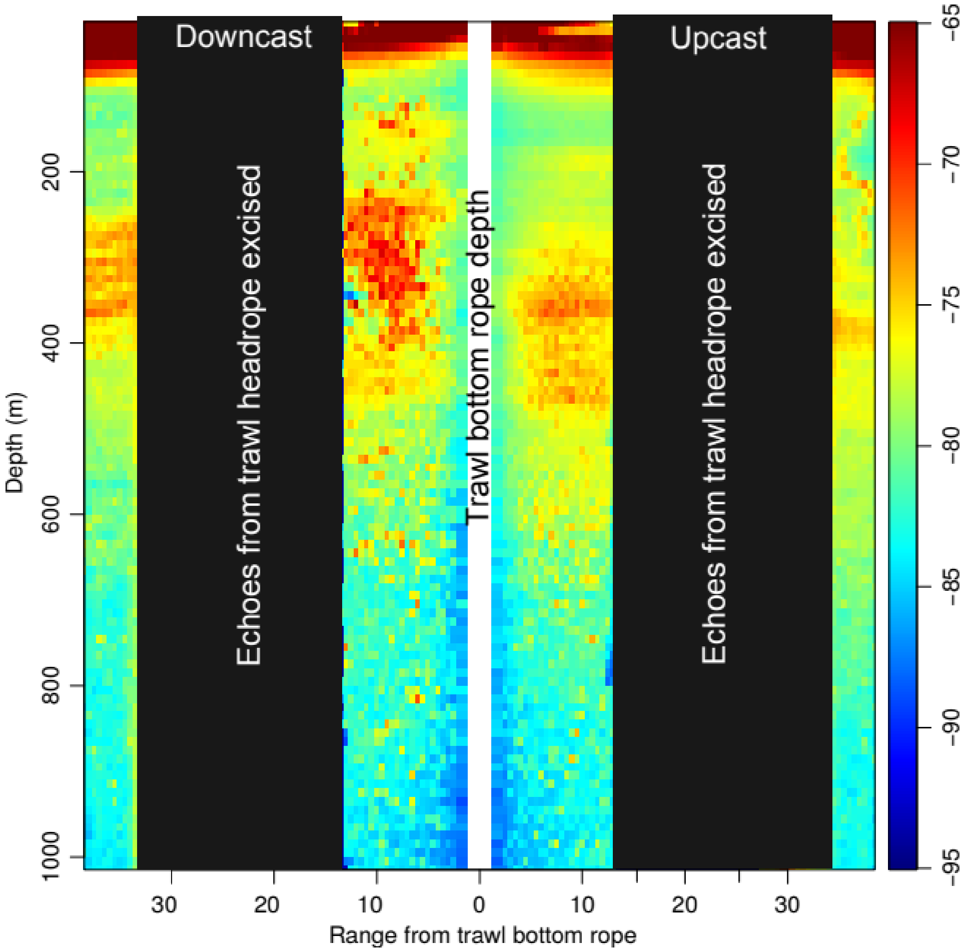

Fig. 2.3.2.2: Vertical profiles of output from a trawl attached ADCP (500 kHz). The figure shows an «echogram» of data from a trawl-mounted ADCP, originally used to measure flow into the trawl, colours give relative volume backscattering strengths, plottet against actual depth of backscatter and range from trawl bottom (ADCP was mounted on the trawl bottom). Ranges dominated by backscatter from the headrope, floats and mesh of the trawl has been omitted (black bars), the plot demonstrates that depths where backscatter enters the trawl may differ between downcast and upcast, and the difference in backscatter seen above the trawl (e.g. outside of trawl mouth area, range > 30 m) between downcast and upcast may indicate that organisms avoid trawls.

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Since the ADCP concurrently estimates flow, it is possible to estimate flux of backscatter into the trawl, for the trawl haul exemplified in Fig. 2.3.2.2 vertical profiles of flux of backscatter (e.g. catch) into the trawl is shown in Fig. 2.3.2.3. For this haul it is obvious that the catch entered the trawl at slightly shallower depths during the downcast (weighted mean depth of backscatter flux (omitting the upper 100 m) 406 m vs 438 m for upcast), but overall the upcast caught ~10% more than the downcast (backscatter flux based, again omitting data for the upper 100 m). Using this kind of in-trawl data for comparison with other concurrent data on vertical distribution, it should be possible to obtain a better understanding of vertical patterns in trawl capture efficiencies.

Behaviour of the organisms does not cease to be important once an organisms passes the mouth of the trawl, as transport from the mouth of the trawl to the cod-end can also depend on behaviour. Fast swimming species are known to be able to enter and exit the trawl more or less at will, and the degree of herding by the mesh will also determine where and if a species is more likely to «hit the mesh», and be dependent on the mesh being capable of transporting the organisms aft to the cod-end. Species who do interact with the net ahead of the cod-end

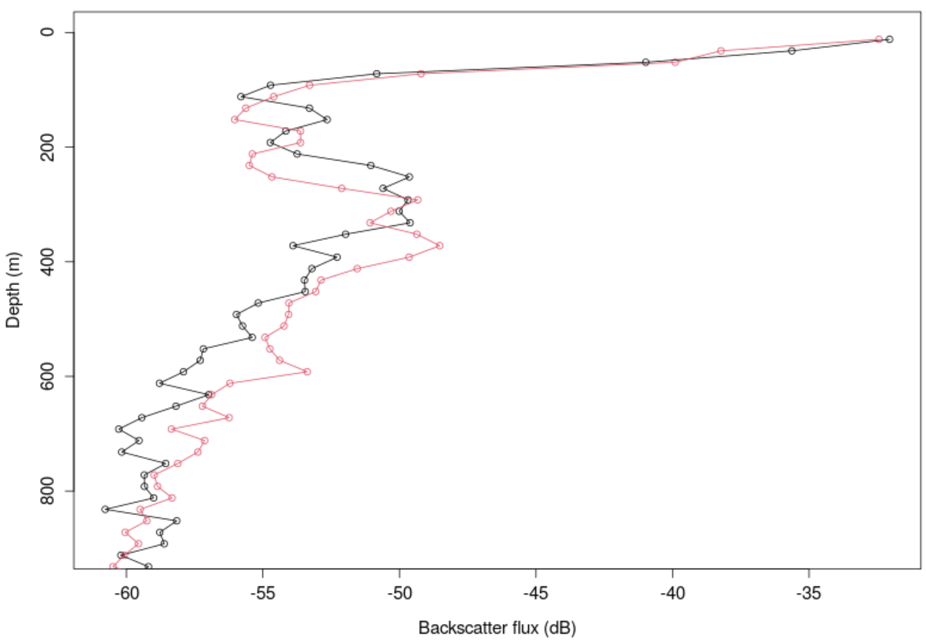

Fig. 2.3.2.3: Vertical profile of trawl «capture» of backscatter, split into downcast (black points and line) and upcast (red points and line), from the trawl haul shown in Fig. 2.3.2.3. Backscatter flux into trawl was calculated as the product (in the linear domain) of mean 500 kHz backscattering levels, water flow into the trawl (from the ADCP), and time spent at each depth (20 m vertical resolution).

may end up as «stickers», e.g. organisms attached to the net ahead of the cod-end (these may or may not be included for quantification purposes, though they are often selectively picked for taxonomic studies). The propensity for an individual to either escape through or get stuck in the trawl mesh depends on size, body shape and appendages, but is also likely to depend on species

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behaviour inside the trawl. IMR has over several years used camera systems to monitor the mesh in different portions of our macroplankton and micronekton trawls, mainly because organisms interacting with the mesh affects the results of our cod-end sampling systems (due to delayed arrival of organisms), such as DeepVision (see Rosen et al., 2013 and Rosen and Holst, 2013, as well as MEESO D2.1).

Another way of monitoring the effect of net-retention/extrusion inside the trawl (which is at least partially dependent on organisms behaviour, Krag et al. 2014), is by comparing temporal patterns in organisms ahead of (or entering) the trawl and in the cod-end. Examples of such data collected by IMR is shown in Fig. 2.3.2.4, where patterns in densities (and echo strengths) recorded by a forward looking echosounder is compared to counts of organisms entering the DeepVision system attached to the cod-end. In general, coarse acoustic categories/classes (e.g. krill vs fish) can be aligned with counts from the cod-end, though some delay is suggested, and correlations between acoustic densities and counts appear to be less than perfect.

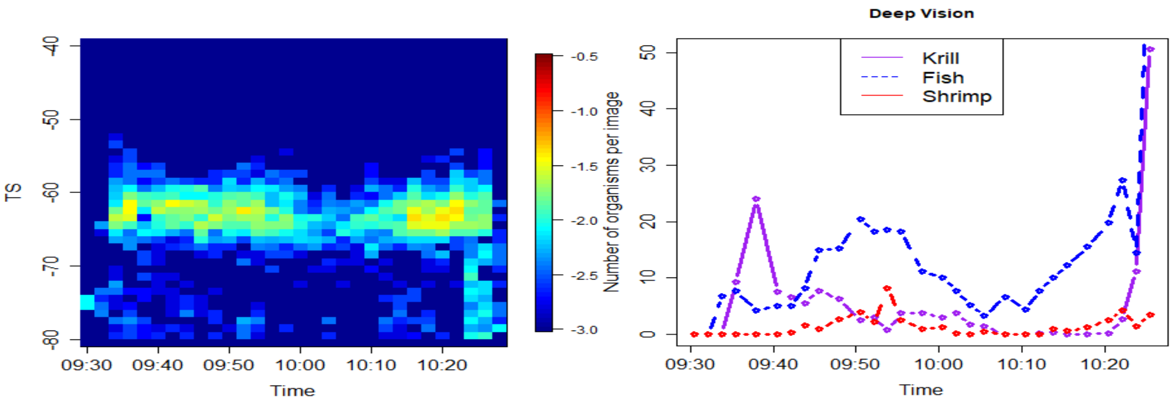

Fig.2.3.2.4: Comparing cod-end catches with echoes ahead of the trawl. Left panel shows densities of echoes plotted against echo-strength (TS, y-axis) and time (x-axis). Echo densities were estimated from data collected from a forward-looking echosounder mounted on the headrope of the trawl. Right panel shows densities of different types of organisms in images recorded by a cod-end camera system on the same trawl.

2.4 Inferences using other equipment

2.4.1 MESSOR TS distribution

Also acoustic single echo detections from submerged acoustics can potentially be utilized to assess behaviour relative to platforms. Fig. 2.4.1.1 shows the volumetric densities of echoes per range and angle off-axis for two different target strength classes (TS, a measure of signal strength), larger organisms are largely assumed to have higher target strengths. For the weaker echoes (left panel), volumetric densities of echoes are reduced at larger ranges and for echoes further off-axis, as can be expected when echo-strengths come closer to the system noise-level. Stronger echoes does not show a similar decrease with range, but the estimated densities close to the transducer (e.g. inside ~ 10 m) is reduced compared to densities estimated from ~20 m out. This could indicate that larger organisms (or at least organisms with higher TS) avoids the towed body.

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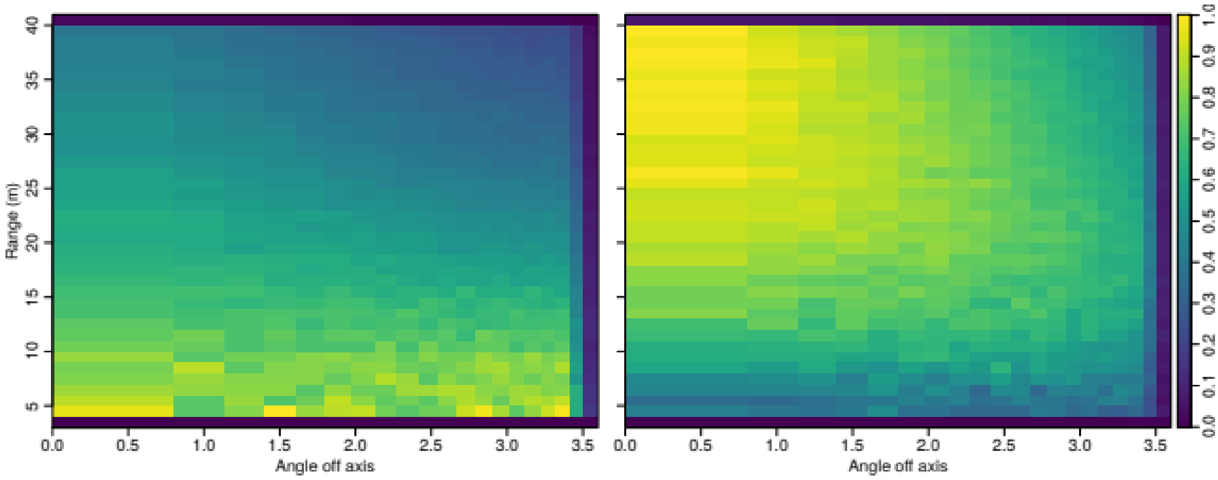

Fig. 2.4.1.1: Echo detection densities (relative echoes per volume) for echoes detected in 70 kHz FM data (MESSOR) as a function of TS, range and angle off-axis. Left panel: Relative densities of echoes with TS in [-70,-60>, right panel: relative densities of echoes with TS in [-60,-50>. Notice drop in densities at longer ranges and off-axis positions for weaker echoes, and reduced densities of strong echoes at short ranges (right panel), which could be an indication of avoidance.

2.4.2 Avoidance from vertically lowered gear

From MEESO D2.2: "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.

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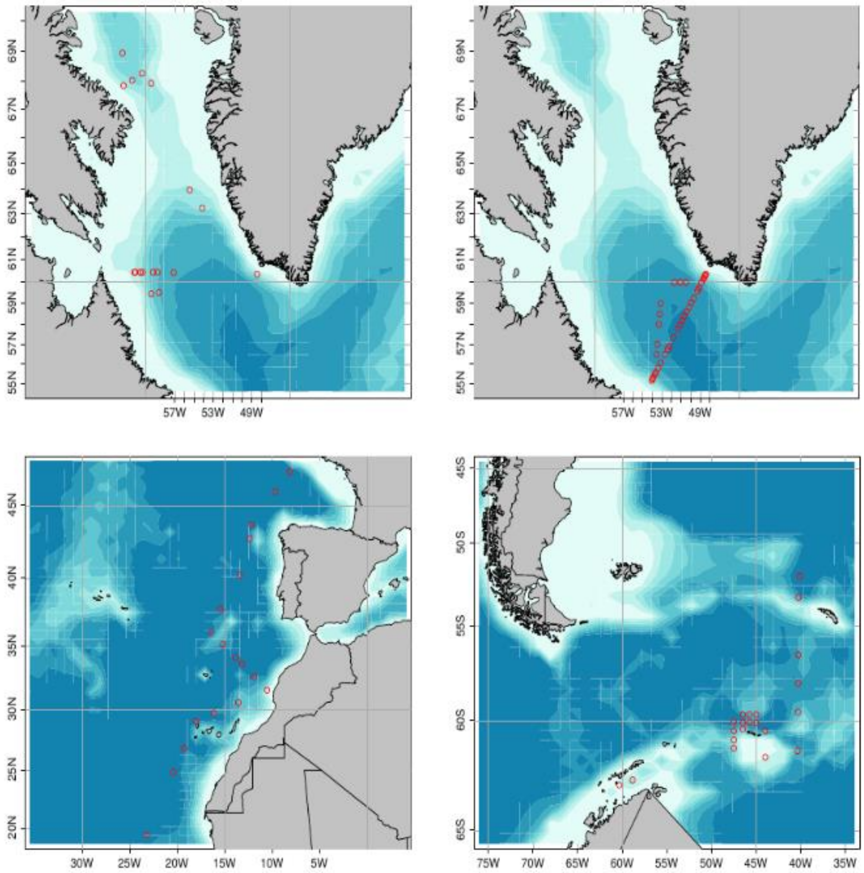

Fig 2.4.3.1. From MEESO D2.2: Location of data sampling points used for avoidance studies (Klevjer et al., in prep).

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/accumulation relative to the CTD might occur at some depths.

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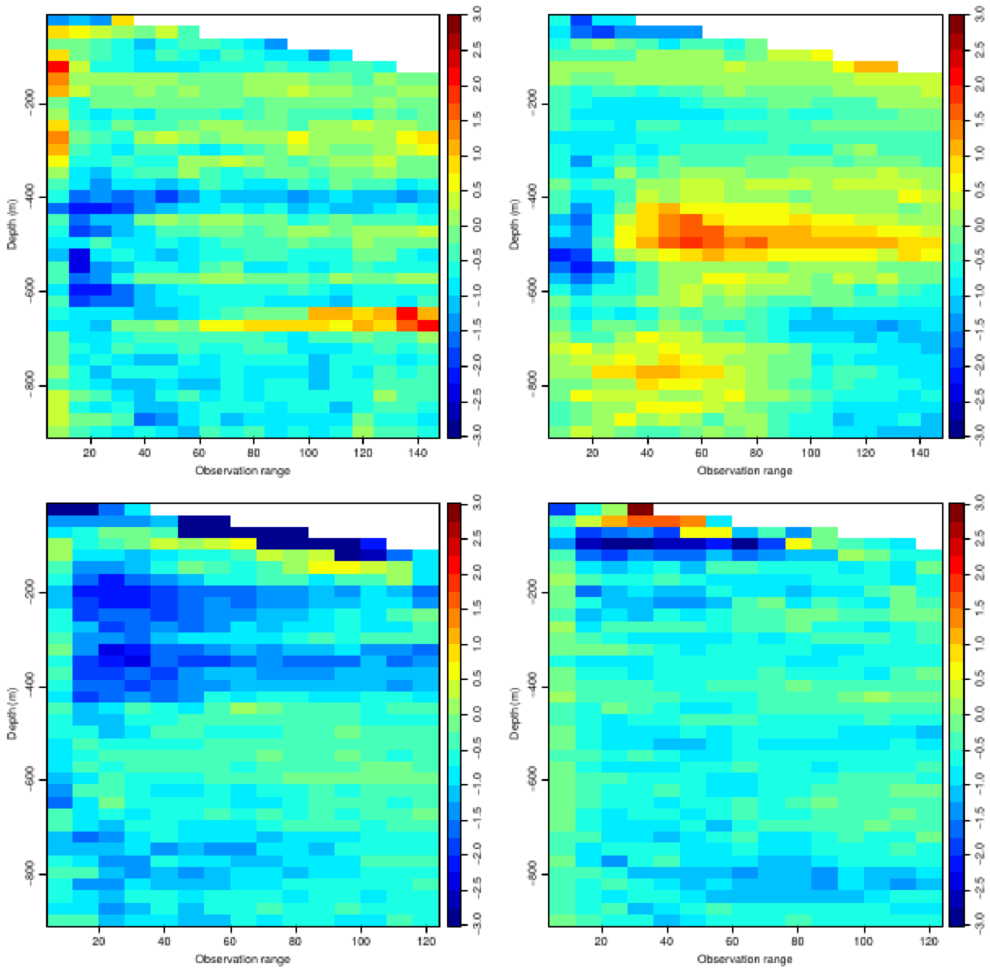

Fig. 2.4.3.2: Average estimated median difference in backscatter (dB) between down- and upcasts for the different datasets in Fig.2.4.3.1, based on all available profiles, plotted as a function of depth (Y) and range away from the LADCP (X), averages for the 4 cruises. Actual depths have been binned into 25 m vertical depth horizons. Positive values indicate avoidance, negative attraction to the CTD. From Klevjer et al., in prep.

3.1 Macrozooplankton and nekton composition- MFRI

During MEESO, MFRI sampled macrozooplankton and nekton samples in 2020 (Gislason et al., 2021) at deep scattering mesopelagic layers (DSML) with a pelagic trawl (opening ~27m^2, knot-less nylon net with 4 mm meshes throughout the trawl body, 6 mm stretched). The trawl is equipped with relatively short lastridge lines that causes the trawl net to undulate when towed through the water, preventing animals from being entangled in it. The trawl has brief 800 mm mesh size wings with floats at the upper wing linings and chains as weight at the lower linings. Floats are also fastened to the headline and chains to the bosom (i.e. the centre portion). The trawl is spread by trawl doors that are attached to the wing ends by 30 m long dynice towing ropes (Silva et al., 2017). The trawl deployments focused on two targets DSML observed in the 18 and 38kHz hull-mounted acoustics (see WP2 D2.1), and in addition, an integrated trawl was deployed down to 1000 m.

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Catch by the macroplankton trawl varied from appr. 5 kg to 49.3 kg with an average of 20 kg per nautical mile (Table 3.1). On average, catches in the integrated tows were highest (30 kg). The catch in upper- and lower-layers tow was on average 12 and 20 kg, respectively. In general, jellyfish (mainly *Periphylla* sp. and *Atolla* sp.) made up more than a half of the catch in terms of weight, on average 62% of integrated tows, and 57% and 51% of upper layer tow and lower layer tow, respectively (Table 1). Of other catch, approximately 130 animals were identified on board, belonging to seven animal classes. Fish (about fifty species) and crustaceans (approximately forty species of decapods, euphausiids and copepods) were the most specious animals in the catch. Other animals, besides jellyfish, were cephalopods, wing snails, chaetognaths and salps. Several species still need a taxonomic identification.

Table 3.1. Tows with macroplankton trawl. Date, Time of day (TOD), tow type, tow depth, total catch, the proportion of jellyfish in the catch, preliminary number of fish species, mean size and range and preliminary number of crustacean species.

Station	Date	TOD	Tow type	Depth (m)	Total catch (kg/nm)	Jelly fish (%)	Species fish (n)*	Mean size fish (cm)	Range size fish (cm)	Species crustaceans (n)*
A7-2020-439	24. July 2020	23:16	Intergrated	1000-0	49.3	63.45	16	13.6	2.3-74.0	7
A7-2020-440	24. july 2020	6:00	Target_upperlayer	325	9.1	69.51	10	5.0	2.1-26.0	4
A7-2020-441	24. july 2020	7:30	Target_lowerlayer	440	20.2	37.22	7	6.1	2.5-25.0	3
A7-2020-451	27. July 2020	11:30	Intergrated	1000-0	13.3	70.00	21	8.0	1.0-58.0	15
A7-2020-448	27. July 2020	6:49	Target_upperlayer	280	14.0	68.57	10	4.0	1.0-19.0	3
A7-2020-449	27. July 2020	8:02	Target_lowerlayer	570	20.0	53.00	23	6.8	2.4-38.0	16
A7-2020-456	28. July 2020	2:00	Intergrated	1000-0	24.3	48.11	21	15.1	2.8-102.0	19
A7-2020-458	28. July 2020	7:00	Target_upperlayer	260	5.2	38.46	13	6.5	2.5-45.0	4
A7-2020-459	28. July 2020	8:00	Target_lowerlayer	400	13.0	64.62	9	5.7	2.5-21.0	8
A7-2020-462	30. july 2020	2:00	Intergrated	1000-0	31.7	64.47	19	10.6	1.0-56.0	16
A7-2020-464	30. july 2020	6:00	Target_upperlayer	250	20.0	50.00	11	9.1	2.4-47.0	5
A7-2020-465	30. july 2020	7:00	Target_lowerlayer	470	25.0	48.00	13	9.6	2.5-82.0	9

*Preliminary results

3.1.1 Mesopelagic fish

Preliminary results showed that the number of fish species ranged from seven to twenty-three species per tow. In general, the highest catch of fish was obtained in the integrated tows. The most frequent species in all tows and areas was the lanternfish *Benthosema glaciale*. The main species (in number) besides *B. glaciale* were *Bathylagus euryops*, Bristlemouths such as *Cyclothone*, *Serrivomer beani*, *Lampanyctus macdonaldi* and *Protomyctophum arcticum*. The relative proportion of species varied among tows and areas. *B. glaciale* dominated the catch of tows taken near Reykjanes ridge (Fig. 3.1.1.1). *Lampanyctus* and *Protomyctophum* were mainly caught in the Irminger ocean. *Lampanyctus* was caught only in an integrated tow, but *Protomyctophum* was frequent in all tows in that area. *Bathylagus* and *Serrivomer* were mainly detected in integrated tows (Fig. 3.1.1.1).

Preliminary findings indicate that number of species, weight and the size distribution of mesopelagic catches seem to depend on the type of trawl deployment (Fig. 3.1.1.2). The average size of fish was higher in the integrated tows than the other two, mainly due to the high

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proportion of large species such as *Serrivomer*, *Bathylagus* and *Lampanyctus* in these types of tows (Table 3.1 and Fig. 3.1.1.2).

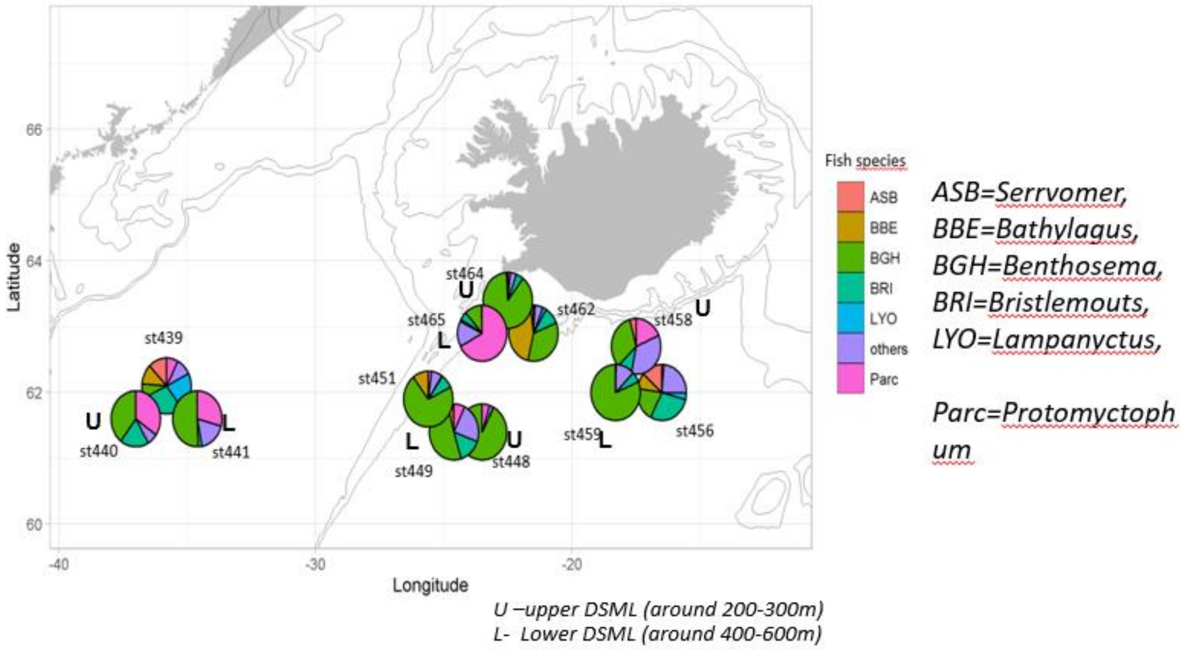

Figure 3.1.1.1. Map showing approximate location of trawl sampling stations for the MEESO project (see Fig. 1 in Gislason et al., (2021) for the exact position of tows). The pie charts show the proportion of main fish species in each tow of the macrozooplankton trawl. Size of circle indicates the relative size of catch.

Some variability in length distribution modes of most abundant mesopelagic fishes can be observed (e.g. *B. glaciale*), which could influence the net deployment or variability in the size distribution of the fishes in the mesopelagic layers (Fig. 3.1.1.2).

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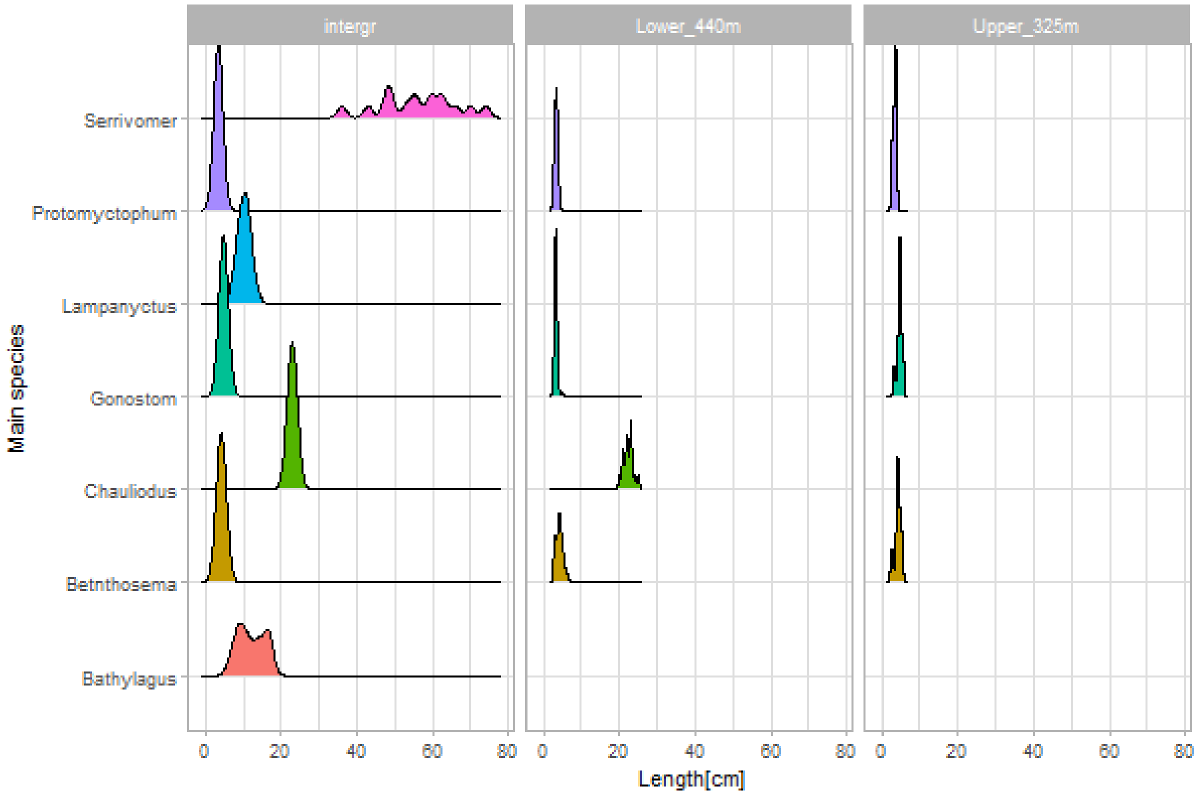

Figure 3.1.1.2. Length frequency distribution of main fish species caught by the pelagic midwater trawl at the first MEESO-01 station. Three deployments were conducted, an integrated tow from 0-1000 m, one target layer at 440 m and a second target layer at 325 m. The target tows were chosen based on the DSML found in the 18 and 38kHz hull-mounted acoustics.

3.1.2 Acoustics comparison: position of the mesopelagic organisms in the water column

Acoustic layers were scrutinized to groups/species level based on each species frequency response and catch composition of the pelagic midwater trawl. Deep scattering mesopelagic organism layers are further categorized using D´Elia et al. (2016) classification size and categorization of scatters. The categorization of the mesopelagic organism was done into four groups (see Fig. 3.1.2), from crustaceans to swimbladder fish such as the myctophids we found most abundant was *B. glaciale*. Therefore, we can then classify the groups based on the threshold sizes or dB difference windows using the difference in the mean volume backscattering strength at 18 to 38 kHz within each cell. Here we show preliminary results from LSSS from this automatization exercise using KORONA post-processing tool. Preliminary results using this method sort of confirms our findings from the trawl samples that the first mesopelagic layer observed was mainly *Benthosema* and mesopelagic fishes with swimbladder indicated here by the red colour gradient (Fig. 3.1.2). The pink and yellow colour (Fig. 2.2.6) could indicate the distribution of other groups such as gelatinous zooplankton, Cephalopoda and Pteropods. The green and blue colours (Fig. 3.1.2) indicate the distribution of crustaceans and small and large non-swimbladder fish, the latter mainly in deeper layers larger than 350m

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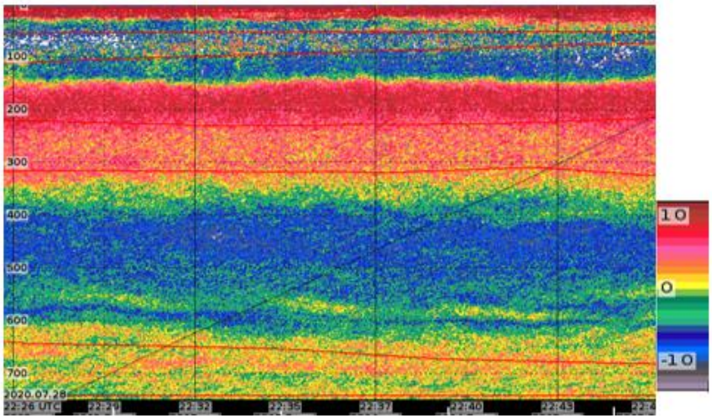
in this case. Further work will be conducted combining acoustic and trawl data to evaluate the pelagic midwater trawl's gear efficiency.

ID	Groups	ΔdB sizes
1	Crustaceans and **Small** non-swimbladder fish	-14 dB to -3dB
2	**Large** non-swimbladder fish	-3 dB to 0dB
3	Other	0 dB to 3dB
4	Swimbladder fish	3 dB to 12dB



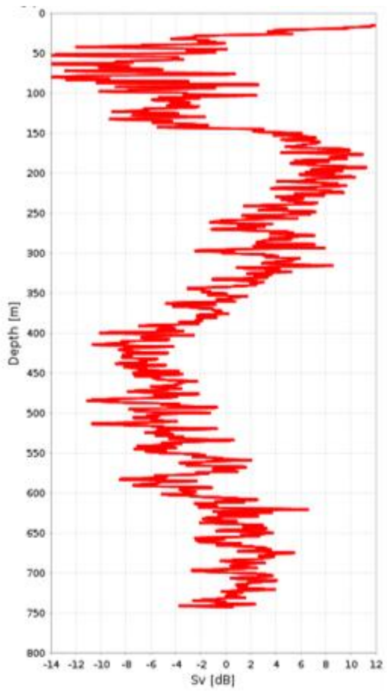


Figure 3.1.2. Size categorization of the mesopelagic organism into four groups (table left) using the difference in the mean volume backscattering strength at 18 to 38 kHz within each cell (echogram lower left) and a vertical profile of the size categorization (lower right) of the selected echogram (lower left) are shown—preliminary findings.

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Conclusion/Summary:

This report documents the development and deployment, within the MEESO project, of methods capable of providing new insights into the behaviour of mesopelagic micronekton in relation to sampling platforms. The overarching goal of all these efforts is to provide a better understanding of how avoidance and attraction to the sampling platform itself distorts the results of methods used to quantify mesopelagic abundances and biomasses. Achieving this goal is perceived as a necessity for achieving higher accuracy in our current mesopelagic estimates: current global estimates of mesopelagic fish biomass roughly span an order of magnitude, largely because we don't know what we're measuring acoustically, nor how big they really are. This high level of uncertainty again has a detrimental effect on our overall understanding of open ocean ecology.

While some results from the MEESO efforts have already been published, more is under way, and the MEESO project is expected to make a significant contribution to the scientific knowledge on this topic. The behaviour of mesopelagic organisms, and the influence of this behaviour on our ability to quantify them, has complicated the work of the scientific community for a long time, and will continue to complicate our efforts long after the conclusion of the MEESO project. However, as the work presented in this report highlights, we're now at a stage where technologies are available that allow us a better understanding, and parametrization, of the effects of mesopelagic behaviour on our efforts to quantify them. This is timely, as the mesopelagic biota is a potential target for commercial harvesting, and the mesopelagic biota also play a role in the biological carbon pump, acting as mediators of the transport of carbon to great depths. Understanding the actual abundance levels and importance of their role is however dependent on proper quantification of the mesopelagic biota, hence the importance of the work presented here.

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