DOES SEISMIC HAVE AN EFFECT ON ZOOPLANKTON?

Field study at Ekofisk with RV Kristine Bonnevie

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TOKTRAPPORT
Nr.9 2022

Tittel (norsk og engelsk):

Does seismic have an effect on zooplankton?

Påvirker seismikk dyreplankton?

Undertittel (norsk og engelsk):

Field study at Ekofisk with RV Kristine Bonnevie

Felt studie på Ekofisk med RV Kristine Bonnevie

Rapportserie: Toktrapport
ISSN:1503-6294

År - Nr.: 2022-9

Dato: 18.08.2022

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Distribusjon:

Åpen

Toktnr:

2022611

Prosjektnr:

15653

Oppdragsgiver(e):

Norges Forskningsråd

Oppdragsgivers referanse:

Prosjektnummer: 302675

Program:

Nordsjøen

Forskningsgruppe(r):

Økosystemakustikk

Plankton

Fangst

Antall sider:

41

Samarbeid med

ConocoPhillips Skandinavia AS (hereinafter referred to as “ConocoPhillips”)

Sammendrag (norsk):

Effects of seismic sound on zooplankton (ZoopSeis) is a project funded by the Norwegian Research Council, aiming at uncovering the effect of seismic on zooplankton. An experiment to test the effect of a real seismic survey on mortality and behaviour at Ekofisk was made possible by a collaboration between the ZoopSeis project and the Glider II project which is led by Akvaplan-niva and funded by ConocoPhillips. The study was conducted with the research vessel RV Kristine Bonnevie in the period 29 April to 8 May 2022. During the survey we conducted three separate experimental runs. During the experimental runs the research vessel sampled zooplankton at a fixed location at the end of a shooting line while the seismic vessel shot seismic along the shooting line until it passed the research vessel. Further, we used autonomous USV's (Otter and a kayak) holding acoustic transducers, to follow the exposed zooplankton as it drifted off from the fixed position at the end of the shooting line. In addition, landers with upward looking echosounders (WBAT's) were moored at the end of the three shooting lines before the seismic survey started. Wild zooplankton was sampled with a WP2 net over the entire depth range (~70 m). The largest distance between the sampling net and the airguns was ~16 km at the start of the line, and the distance at passage varied between 46-116 m for the three runs. There was a 48 hour "before" period before each run, in which no seismic was shot within 2 km of the experimental transect. Plankton samples were stained to assess mortality. Stained samples were split into three parts one for photographing, one was frozen, and one was preserved in formalin. In addition to net sampling and acoustic data, we deployed bags with either cultured *Calanus finmarchicus* or wild-caught zooplankton, both at the start of the transect when the seismic vessel was far away, and at the moment the seismic vessel passed. These plankton were followed individually over time to study delayed mortality after exposure. At the same time, a cage was deployed to document zooplankton behaviour during exposure on video. The sound of the seismic survey was recorded with omnidirectional hydrophones. One hydrophone recorded at 10 m depth at the sampling locations during sampling, and a hydrophone array recorded at 8 and 35 m depth for a 24-hour period at a single location. One day during the cruise the wave height was too high to shoot seismic, this day was used to take control samples and run experimental controls for the bag and cage experiment. In addition to the in-situ sampling done on the cruise, will the Glider II project by Akvaplan-niva provide acoustic baseline data from before during and after the seismic activity with their Sail-buoy Echo 333Khz. Further, will the Glider II project provide data from a lander with hydrophone (one year) and a glider Slocum G3 (water column CT profile and JASCO Observer hydrophone), the later was deployed at the beginning of the cruise. The results and findings from this field study will be published in separate peer review papers after further scrutinizing of the collected data.

Sammendrag (engelsk):

Effekter av seismisk støy på dyreplankton (ZoopSeis) er et prosjekt finansiert av Norges Forskningsråd, som har som mål å avdekke effekten av seismikk på dyreplankton. Dette felt eksperimentet, hvor vi tester effekten av seismisk på dødelighet og atferd hos dyreplankton under reelle forhold på Ekofisk, ble muliggjort av et samarbeid mellom ZoopSeis-prosjektet og Glider II prosjektet som ledes av Aquaplan-niva og finansieres av ConocoPhillips. Studeit ble gjennomført med forskningsfartøyet RV Kristine Bonnevie i perioden 29 april til 8 mai 2022. Under undersøkelsen gjennomførte vi tre separate replikate eksperiment. Eksperimentene bestod i at forskningsfartøyet plasserte seg ved enden av en seismisk skytelinje. Fra enden av skytelinjen tok vi dyreplankton prøver mens seismikkfartøyet skjøt seismikk langs skytelinjen opp mot oss og til det passerte forskningsfartøyet. Dette for å få et mål på hvordan avstanden til seismikk skipet / luft kanonen påvirker dødelighet og atferd til dyreplanktonet. Videre brukte vi autonome USV-er (Otter og en kajakk) med akustisk, som fulgte det eksponerte dyreplanktonet mens det drev av fra den faste posisjonen ved enden av skytelinjen. I tillegg ble det brukt bunn fortøyd ekkolodd (WBAT), de ble plassert i enden av de tre skytelinjene før den seismiske undersøkelsen startet. Det ble ikke skutt seismikk i en omkrets på ca 2 km fra det eksperimentelle skytelinjen de siste 48 timene før forsøkene startet. Det ble tatt dyreplankton prøver over hele vannsøylen (~70 m) ved hjelp av WP2 plankton håv. Den største avstanden mellom prøvetakingsnettet og luftkanonene var ~16 km ved starten av linjen, og avstanden ved passasje varierte mellom 46-116 m for de tre eksperimentelle kjøringene. Vi farget planktonprøvene med Red Staining for å synligjøre mengde levende og dødt dyreplankton. Fargede prøver ble delt i tre deler en for fotografering, en ble frosset og en ble konservert i formalin. I tillegg til nettprøvetaking og akustiske data, satte vi ut poser med kultiverte hoppekreps *Calanus finmarchicus* eller viltfanget hoppekreps. Dette ble gjort både ved starten av de tre seismikk skytelinjene, når seismikkfartøyet var langt unna, og i det øyeblikket seismikkfartøyet passerte oss. Disse hoppekrepsene ble fulgt individuelt over tid for å studere forsinket dødelighet etter eksponering. Samtidig ble det satt ut bur laget av plankton-nett med 3D kamera. I disse filmet vi hoppekrepsens adferd når den ble eksponert for seismikk. Lyden fra den seismiske undersøkelsen ble registrert med omnidireksjonelle hydrofoner. En hydrofon hang under forskningsskipet og registrere lyd på 10 m dyp under de tre eksperimentelle kjøringene. En annen hydrofongruppe registrert lyd på 8 og 35 m dybde i en 24-timers periode hvor det både ble - og ikke ble skutt seismikk. Et døgn under toktet var bølgehøyden for høy til at en kunne skyte seismikk. Dette døgnet ble brukt til å kjøre kontroll eksperiment. I tillegg til in-situ prøvetakingen gjort på toktet, vil Glider II prosjektet til Akvaplan-niva, med sin seilbøye Echo 333Khz bidra med akustisk data fra før under og etter den seismiske aktiviteten. Videre vil Glider II

prosjektet bidra med lyd data fra en bunnsatt lander med hydrofon (ett år) og en glider Slocum G3 (vannsøyle CT-profil og JASCO Observer-hydrofon). Sistnevnte satte vi ut i begynnelsen av toktet. Resultatene fra toktet vil bli publisert i fagfellevurderingsartikler når de innsamlede data er ferdig analysert.

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1 - Introduction

This report is from a cruise (cruise nr. 2022611) with RV Kristine Bonnevie to Ekofisk North sea 29th of April to 8 th of May 2022 were we looked at the affect of seismic on zooplankton short and long term survival and behaviour. This study was a collaboration with Akvaplan-niva and ConocoPhillips.

The Institute of Marine Research (IMR) is responsible for giving scientific advice on the seismic activity in Norway, with the objective to prevent negative effects on marine ecosystems. Current practice does not consider the effects of seismic blasts on zooplankton, as any potential effects have been assumed to be highly localized, based on studies of fish larvae (Booman et al. 1996). However, a study from Australian waters, McCauley et al. (2017), reported zooplankton mortality up to 1200 m from a seismic transect and infer a causal relationship. The peak abundance of copepods coincides with the period of highest seismic activity. If similar mortality occurs in Norwegian waters, the impact of seismic explorations could have serious consequences for the food web, and thereby for the fisheries, in these highly productive areas.
On the other hand, a recent study performed in Norwegian waters (Fields et al. 2019), found 9 % higher instantaneous mortality at distances closer than 5 m from the source and no effect on escape performance at any distance from the seismic blast. The effects of seismic airgun blasts on *Calanus finmarchicus* reported by Fields et al. (2019) are much less than reported by McCauley et al. (2017) and those used in models to assess the broader impacts of seismic surveys on zooplankton (such as Richardson et al., 2018). However, the airgun used in Fields et al. (2017) was a small airgun and not a real airgun arry, like we tested in our Ekofisk filed study.

1.1 - OBJECTIVES AND GOALS OF THE SURVEY

Effects of seismic sound on zooplankton (ZoopSeis) is a project funded by the Norwegian Research Council, aiming at uncovering the effect of seismic on zooplankton. This cruise is part of the ZoopSeis project. The aim of the cruise was to find at what distance a full-scale seismic array impacts zooplankton in terms of immediate mortality, delayed mortality and behaviour (e.g. swarming and avoidance).

In addition to this experiment on the zooplankton response to a seismic survey we conducted an acoustic transect on Vikingbanken on our way back. The transect lasted 10 hour covering ~93 nm. The methods followed standard acoustic survey coverage. The acoustic data was scrutinised following the IMR standard sandeel protocol.

1.2 - CRUISE PARTICIPANTS

Name	Institute	Country	Status
Josefin Titelman	University of Oslo	Sweden	Researcher
Thor Klevjer	Institute of Marine Research	Norway	Researcher
Geir Pedersen	Institute of Marine Research	Norway	Researcher
Espen Strand	Institute of Marine Research	Norway	Researcher
Karen de Jong	Institute of Marine Research	Norway	Researcher
Atle Totland	Institute of Marine Research	Norway	Technician
Sigurd Hannaas	Institute of Marine Research	Norway	Technician
Marina Mihaljevic	Institute of Marine Research	Norway	Technician
Rune Strømme	Institute of Marine Research	Norway	Technician
Reidar Johannesen	Institute of Marine Research	Norway	Technician
Håvard Johnsen Buschmann	Akvaplan-niva	Norway	Technician

Name	Institute	Country	Status
Saskia Kühn	Kiel University	Germany	PhD student
Emilie Hernes Vereide	University of Oslo / IMR	Norway	PhD student
Anne Christine Utne Palm	Institute of Marine Research	Norway	Researcher, Cruise leader

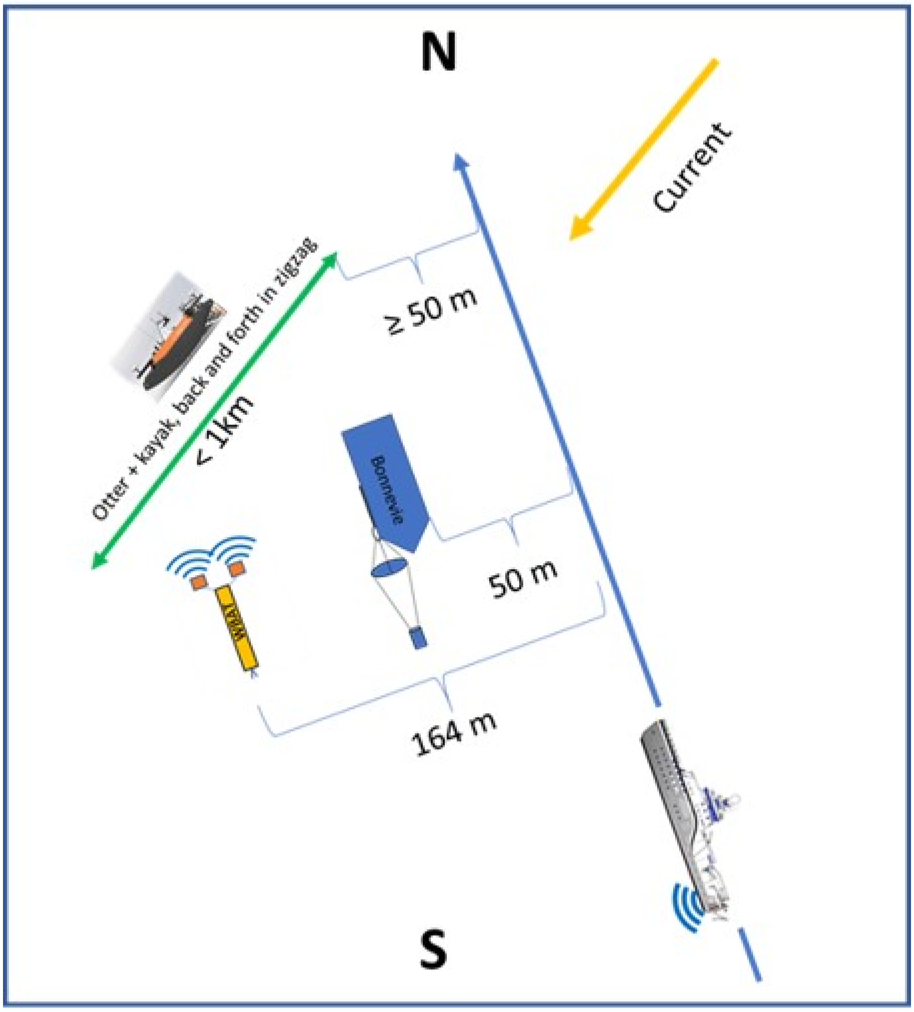
2 - Methods and Results

The experimental approach consisted of acoustic monitoring and net sampling during the approach of a seismic vessel, *Skandi Nova*. Three landers (WBATs) were deployed at Ekofisk by a suply ship of ConocoPhillips prior to the survey (April 12th). Each one of them at the end of separate seismic shooting lines. The three WBATs were holding upward looking echosounders (see section 2.1.1). During the survey three replicate experiments were performed by positioning the IMR research vessel RV Kristine Bonnevie close to each of these three WBATs, while the seismic vessel was approaching us along the shooting line at a speed of 4.5 knots (Figure 1).

The sampling during an approach allowed for the documentation of effects of seismic at different distances (~16 km to ~50 m). Plankton net samples (WP2) were taken continuously as the seismic vessel approached RV Kristine Bonnevie, and with increasing sampling frequently as the seismic vessel got closer.

The set-up used in McCauley et al. (2017) did not allow for testing whether zooplankton mortality could have been caused by other sources of disturbance, such as the propeller wake of the ships used in the experiment or variation in natural mortality over the day (Bickel et al. 2011; Tang et al. 2019). We therefore used autonomous USV’s (Otter and a kayak) holding acoustic transducers, to follow the potentially affected plankton. As an additional control for the effect of propeller we placed zooplankton in plastic bags and lowered them to 10 m for 15 sec. This plastic bag exposure was also done in an increasing frequency as the seismic vessel was getting closer.

Furthermore, we looked at effect of seismic on zooplankton behaviour. This was done by placing zooplankton in a plankton net cage holding a 3D camera set-up (see Fig. 11). Zooplankton behaviour was filmed as the seismic vessel approached and during no shooting period as a control.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Current
≥ 50 m
Otter + kayak, back and forth in zigzag
< 1km
Bonnevie
50 m
164 m
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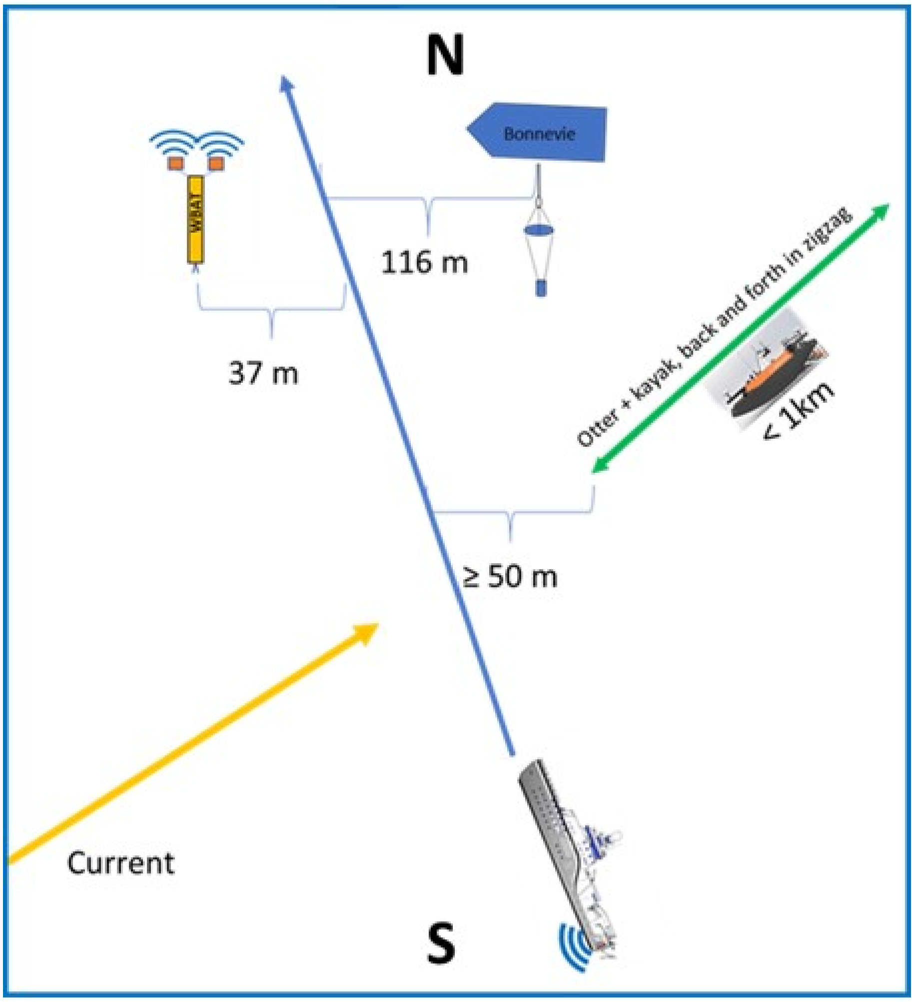
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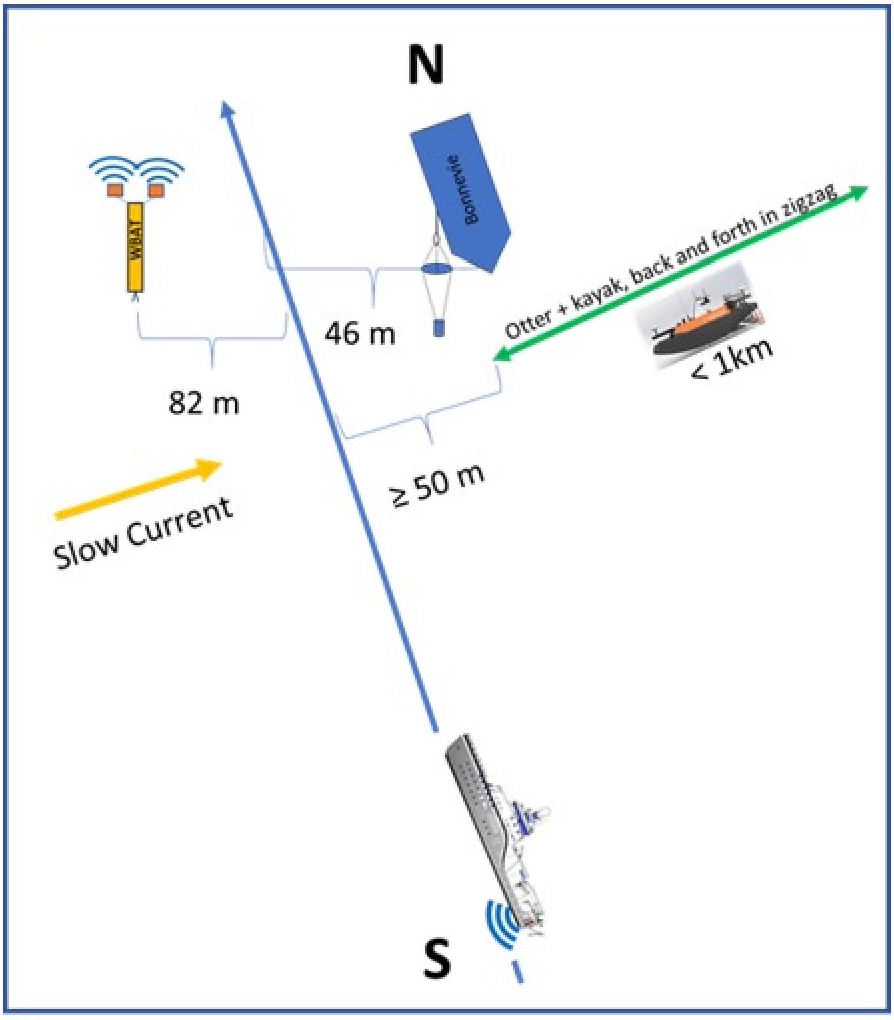


Figure 1. WBAT 1 station top, WBAT 2 station middle, WBAT 3 station bottom. Green line shows the transect of autonomous USV's (Otter and a kayak) going up and down current (yellow line). WBAT is positioned next to the shooting line at a distance of 37 to 164 m. Kristine Bonnevie positioned adjacent to the shooting line at a distance of 46 to 116 m (measure between seismic vessel hull and the position of plankton net hauls).

Current was predicted using the North West Shelf model and comparing these with ADCP measurements of the vessel. The two autonomous USV's (Otter and kayak) was following exposed zooplankton down current as it was drifting off from the seismic shooting line. This was done to look for any sinking or changes in the acoustic plankton layers after seismic exposure – an behavioural phenomenon observed in the acoustic data of McCauley et al. (2017).

2.1 - MULTIFREQUENCY ACOUSTIC SAMPLING AND ANALYSIS

(Geir Pedersen, IMR)

Three main experiments were performed at the site of three pre deployed landers (WBAT 1, 2 and 3) (see section 2.1.1.). In addition to experiments and sampling of copepods, acoustic measurements were performed by multiple platforms.

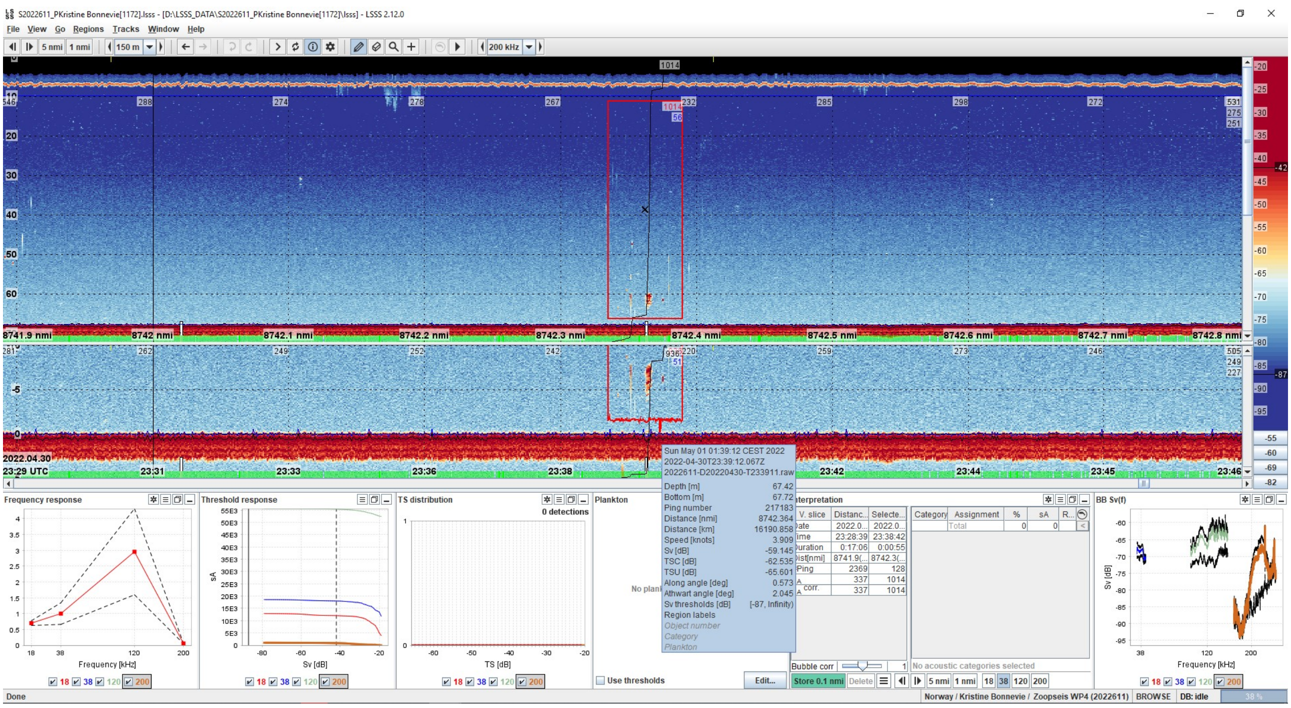
In addition to the three WBATs RV Kristine Bonnevie collected acoustic data from a fixed position, while two autonomous USV's vessels (Otter and kayak) performed 500 m transect in parallel with the current direction (see section 2.1.2.) at the three WBAT stations (see Fig. 1). Furthemore, acoustic baseline data from before during and after the seismic activity was collected by Akvaplan-niva's Sail-buoy Eco 2 equiped with an EK80 (333kHz) in adition to CTD and oxygen sensor. The Sail-Buoy run transects in the northern part of the seismic area, near our three WBAT stations.

2.1.1 - The WBAT's

WBAT is based on the same technology as the Wide Band Transceiver (WBT), however as with the Simrad EK80, the WBAT is capable of split-beam operation, which means that it can be calibrated to the same standards and with the same methods as the Simrad EK80. The WBATs continuously collected data from a fixed position from 5 m above the seafloor. We located the position of the 3 WBATs using the acoustics of Kristine Bonnevie (Table 1, and Fig 2). The three WBAT's holds EK80's for details see Table 1. The weight of the three landers were ~200 kg (Fig. 3).

Table 1. The three WBAT's positions at Ekofisk. Located by acoustics (see Fig. 2).

	Latitude	Longitude	Transceiver
WBAT 1:	56° 34,952 min	003° 8,269 min	70 and 200 kHz
WBAT 2:	56° 36,416 min	003° 13,362 min	200 kHz
WBAT 3:	56° 36,124 min	003° 15,476 min	200 and 333 kHz



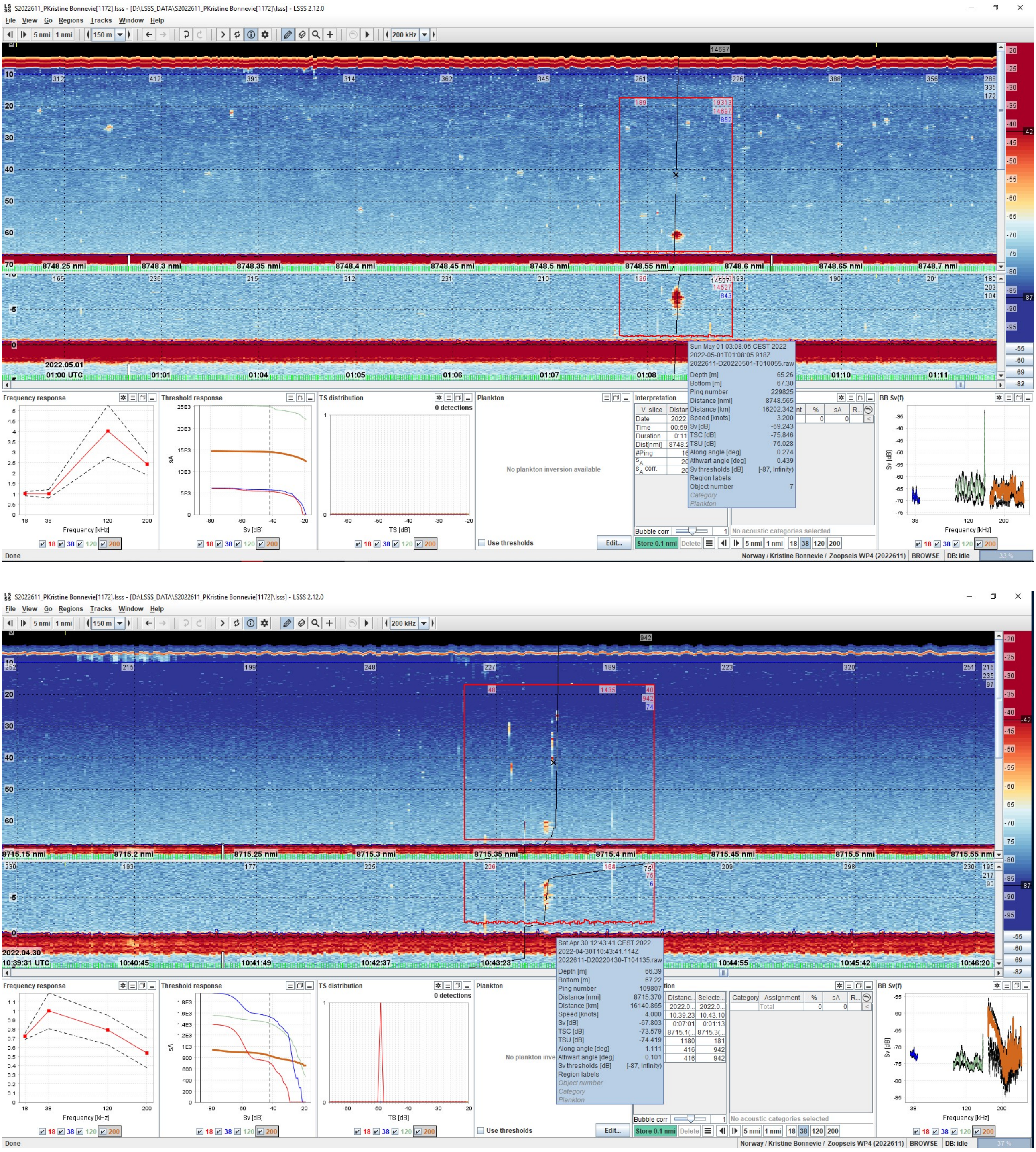


Figure 2. Showing the acoustic picture of the WBAT (within the red frame) as seen by the echosounder on RV Kristine Bonnevie. Top figure WBAT rig 1 (WBAT 1), middle figure WBAT rig 2 (WBAT 2) and bottom figure WBAT rig 3 (WBAT 3).

Figure 3. WBAT (wide band transceiver) with to upwards looking transducers. Picture by Anne Christine Utne Palm.

We were not able to retrieve WBAT 1 and 3 at the end of the cruise due to issues with the acoustic release. ConocoPhillips retrieved the last two WBATs by the use of ROV on the 11th of May.

2.1.2 - The USV's

The two autonomous vehicles were also equipped with Simrad EK80 echosounders. The Kayak hold an ES200-7CD, and the Otter a ES38/200, 333CD. Both vehicles employ electric propulsion.

Otter

The Otter, owned by Akvaplan-niva, is the smallest member of the Maritime Robotics USV family. Dimensions 200 x 108 x 106.5 cm and weight of 65kg. Akvaplan-niva's Otter holds a EK80 (200 - 38 kHz and 333 kHz transducers). The ES38 and 333 are installed on a pole just below the waterline. For more details see https://www.maritimerobotics.com/otter and Fig. 4

Kayak

The kayak is build by IMR. It is 700 cm long and weighs 300 kg. It holds an EK80 200 kHz transducer. For more details see Fig. 4. The ES200 is installed on a protruding keel (~0.5 m bellow water).

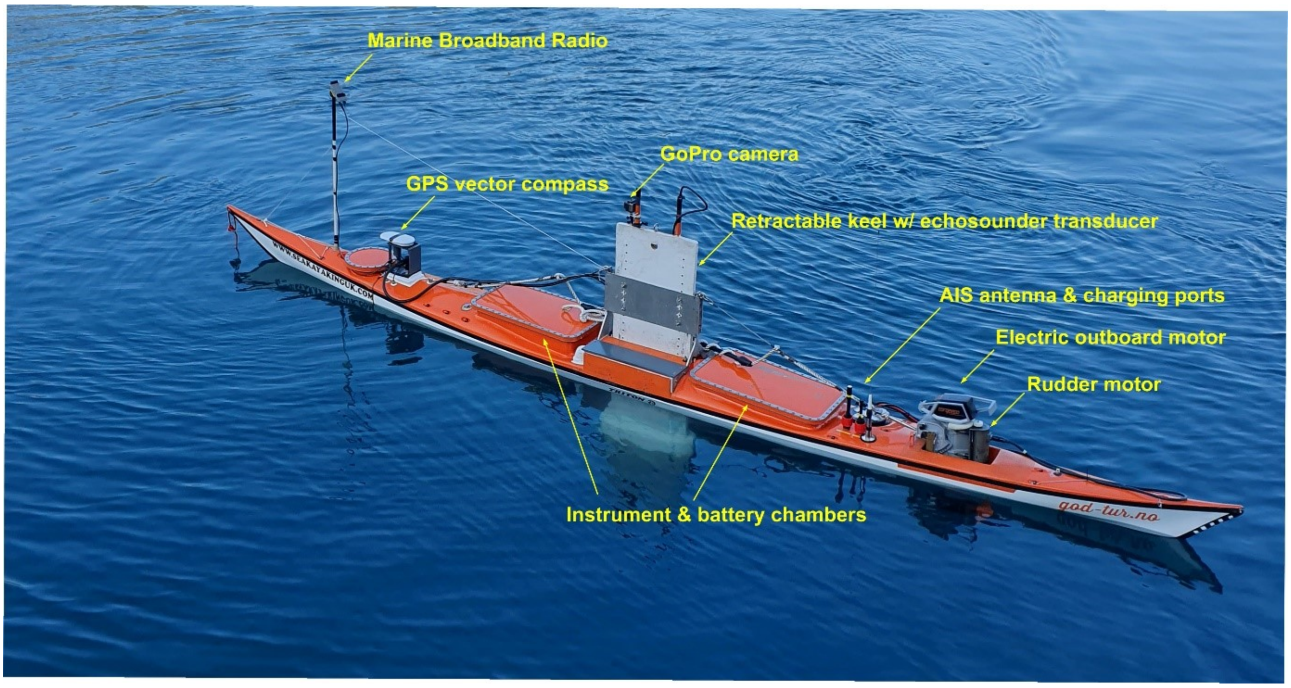


Figure 4. The two autonomous vehicles. Top: Otter (picture from Maritime Robotics), the smallest member of the Maritime Robotics USV family. Dimensions 200 x 108 x 106.5cm and weight of 65kg. Akvaplan-niva's Otter holds an EK80 (200 - 38 kHz and 333 kHz transducers). For more details see https://www.maritimerobotics.com/otter. Bottom: kayak made by IMR. It is 700 cm long and weighs 300 kg. It holds an EK80 200 kHz transducer on its retractable keel. Further it has a GPS vector compass and an AIS. It holds a GoPro camera both below and above water. It has an electrical outboard engine. Pictures of Otter from Maritime Robotics web page, of Kayak by Atle Totland.

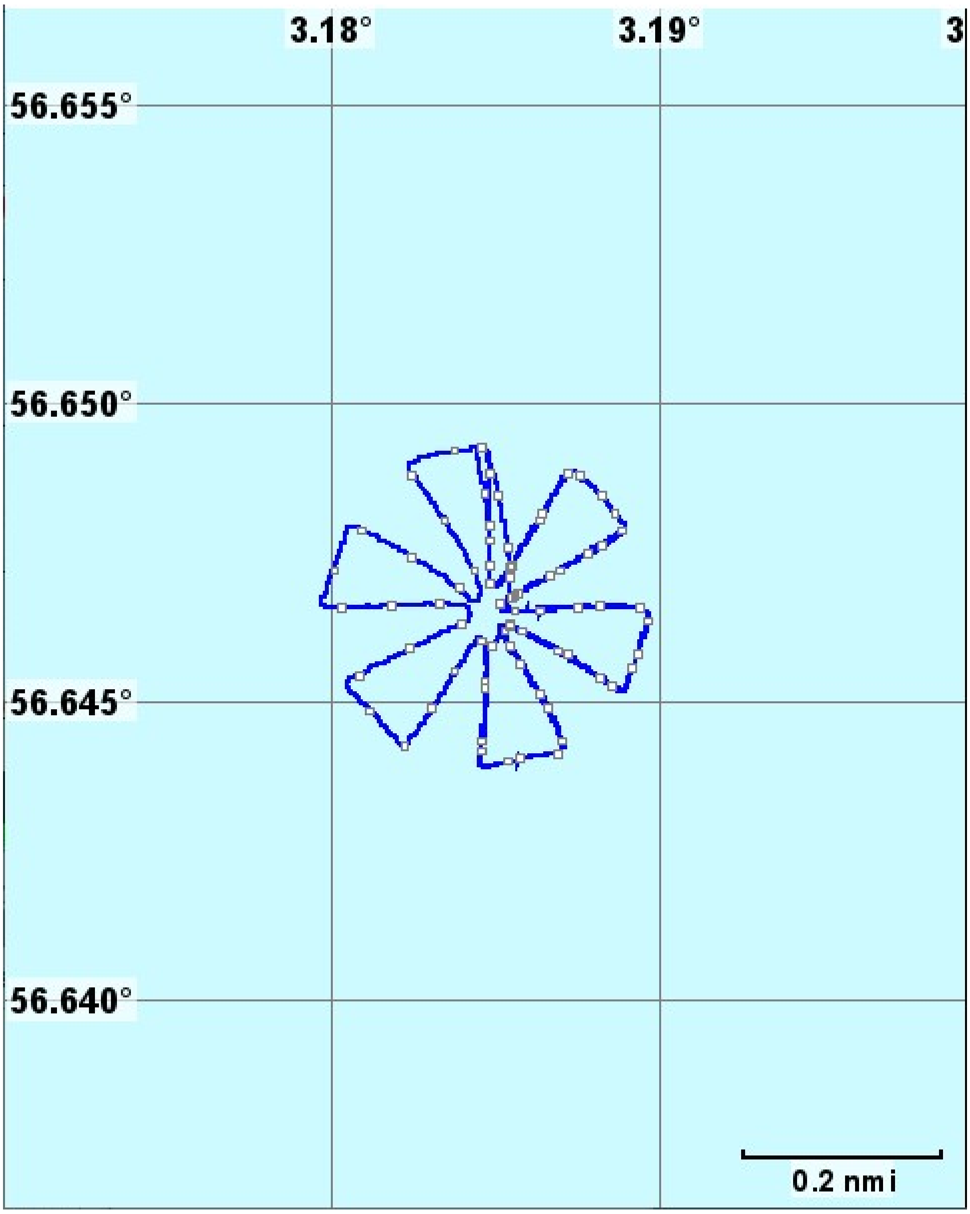
2.1.3 - Seismic experiment

For the seismic experiments (WBAT1-3) each transect was isolated and acoustic data from each transect visualized. In addition, metrics were calculated, including acoustic density, abundance, center of mass, spread. The acoustic data was further split into four layers, surface (small fish/larvae, misc plankton), thermocline/plankton, fish and deep (plankton). The acoustic density of each layer was estimated as a function of time when the seismic vessel Skandi Nova was closest to the WBATs.

Additional preprocessing will be performed later, e.g. filtering (noise, interference).

2.1.4 - FAD experiment

An additional experiment was conducted where Kayak and Otter performed transects around RV Kristine Bonnevie in a flower like pattern (Fig. 5). These transects were conducted to assess whether RV Kristine Bonnevie potential worked as fish attraction device (FAD). During the transects Kayak, Otter and RV Kristine Bonnevie collected acoustic data at all frequencies.



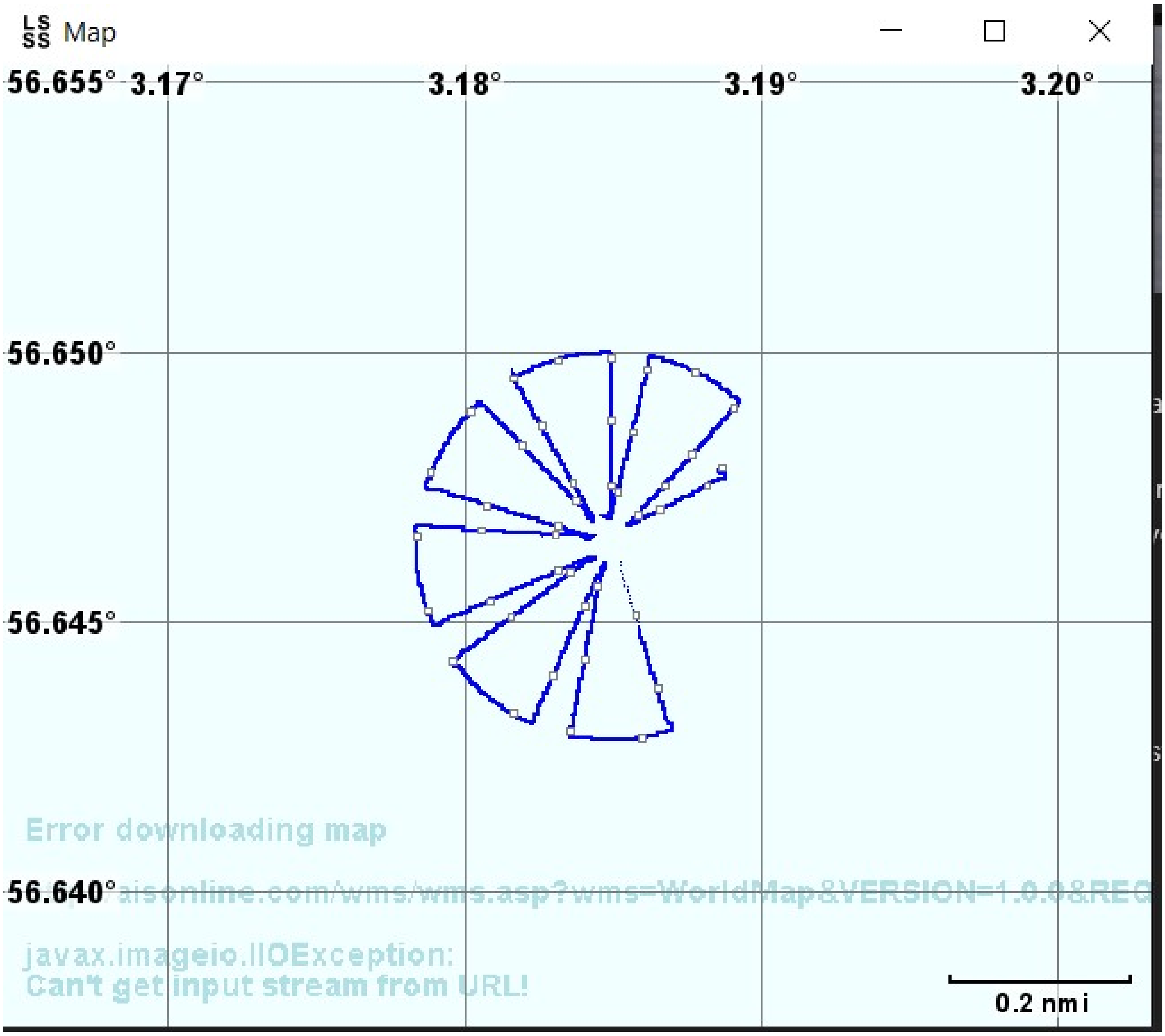
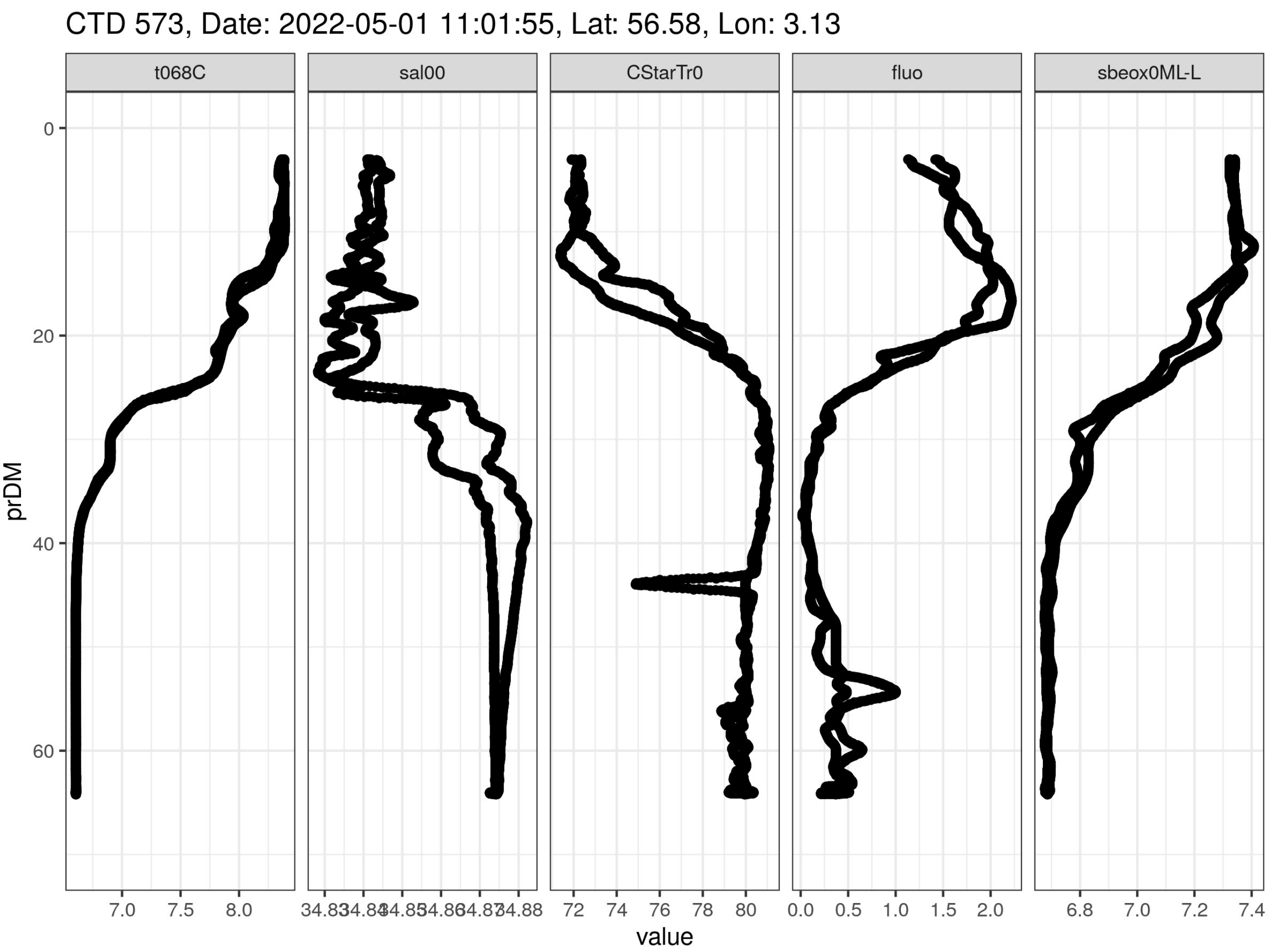


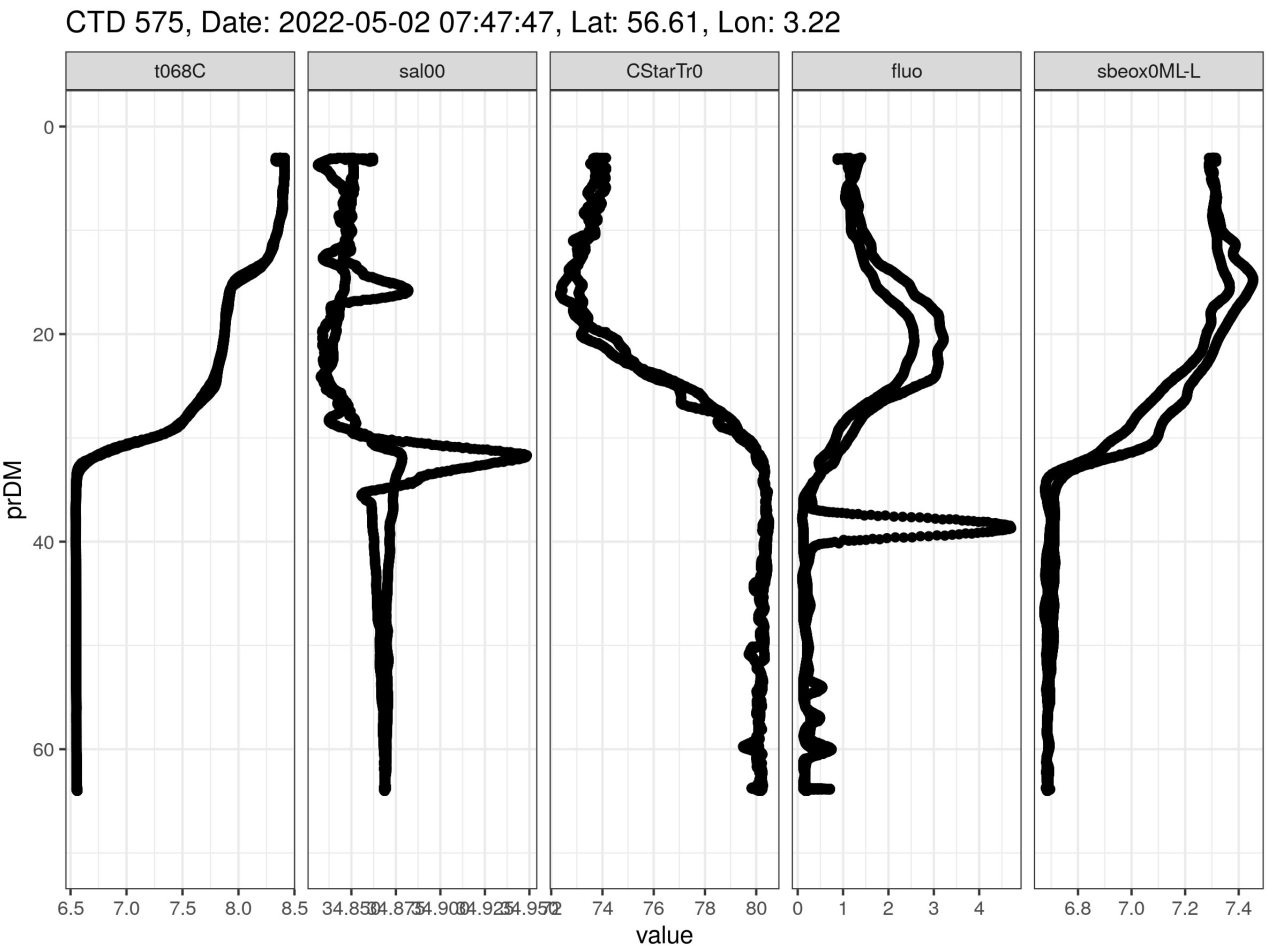
Figure 5. FAD test making flower-formed transect Kayak (top) and Otter (bottom). RV Kristine Bonnevie was positioned in the centre of the flower-formed transect, which stretched to a distance of 300 m from the vessel.

2.2 - HYDROGRAPHICAL DATA

(Thor Klevjer, IMR)

To collect data on the hydrographical conditions at the three WBAT stations and the control station, a CTD (measuring conductivity, temperature and depth) was deployed. The CTD was additionally equipped with a fluorometer (measuring fluorescence), transmissometer (measuring light transmission), oxygen sensor (measuring dissolved oxygen) and also had NISKIN bottles for water sampling, these were used to take samples for chlorophyll and nutrient concentrations.

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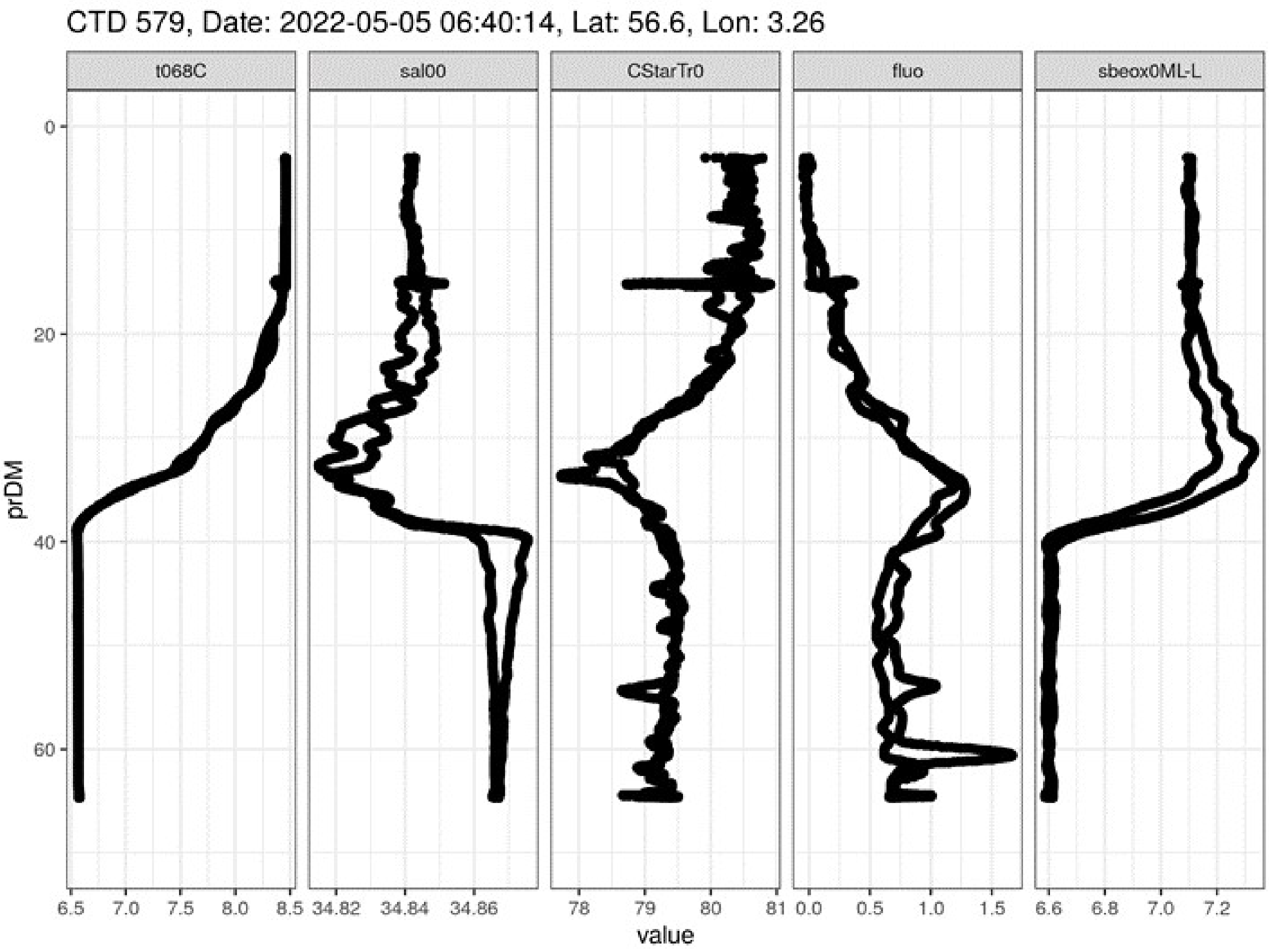


Figure 6. Figures shows temperature, salinity, transmission, fluorescence and oxygen (left to right) at station by WBAT 1 (top), WBAT 2 (middle) and WBAT 3 (bottom).

2.3 - HYDRODYNAMIC MODEL

(Geir Pedersen, IMR)

For the daily planning of the main experiments we reviewed multiple data products, and ultimately used the North West Shelf (NWS) oceanographic model predictions of current speed and direction obtained from Copernicus Marine Data Store (NORTHWESTSHELF_ANALYSIS_FORECAST_PHY_004_013). A custom script programmed in Python, using MOTU API, accessed and downloaded the daily predicted speeds and directions in the area where the experiments were taking place. An informal validation of the model predictions is shown in Figure 7 where the predicted U and V component of the current at the surface was compared with measurements from the vessel mounted ADCP (RDI 150 kHz ADCP).

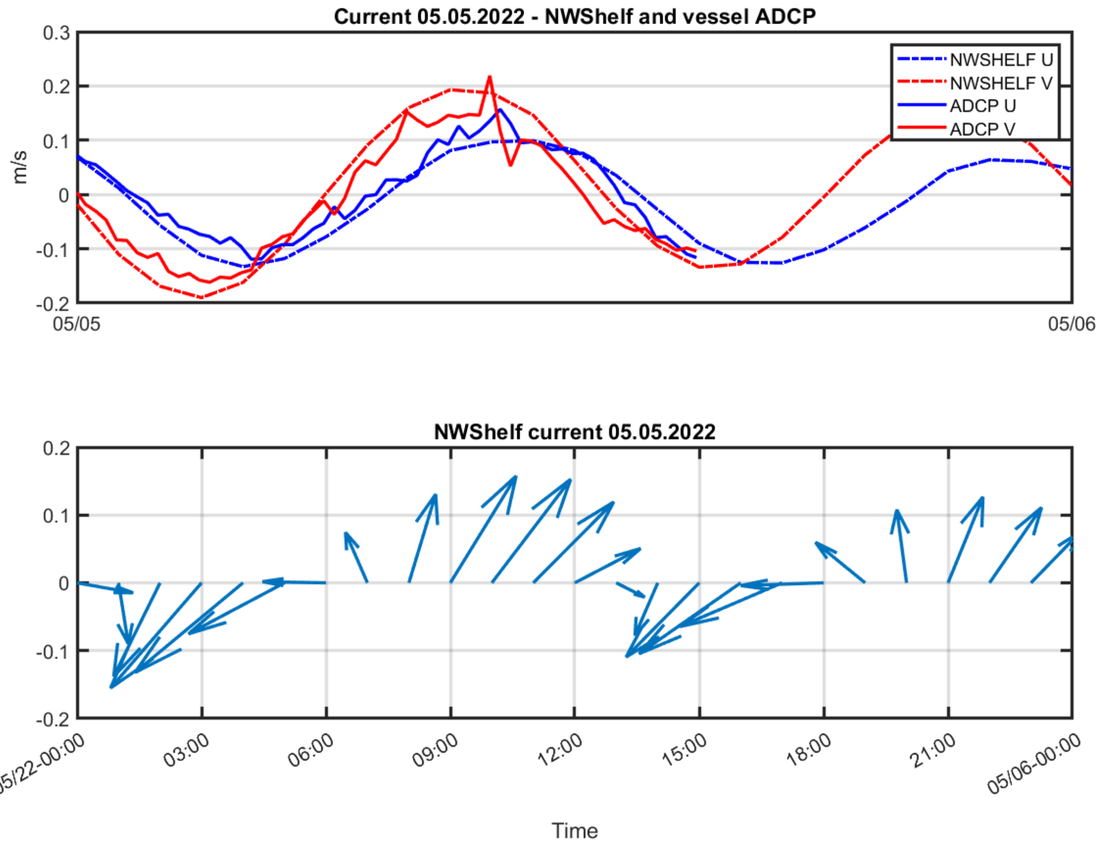


Figure 7. This figure shows the U and V components of the predicted current (upper panel) and current direction and magnitude (lower panel) using the North West Shelf (NWS) model on the 05.05.2022 at WBAT 3 station. The measured current (ADCP) data are also given in the upper panel.

2.4 - PLANKTON SAMPLES

2.4.1 - Quantitative zooplankton samples

(Thor Klevjer, IMR)

Vertical distributions of zooplankton were estimated through vertical deployments of a 0.25 m^2 Hydrobios Multinet equipped with 180 µm mesh size nets, sampling mesozooplankton in the discrete depth intervals 60-45, 45-30, 30-20, 20-10 and 10-0 m. Upon retrieval the nets were washed down on deck, and samples were worked up according to IMR standard protocols. The cod-end samples were split using a Motdo plankton splitter, and half the final sample split (usually the whole sample, but lower splits when large samples) were split in 3 size-fractions (>2 mm, 1-2 mm and 1 mm-180 µm) with sieves, rinsed in freshwater and dried. From the 2 mm fraction important categories according to standard IMR protocols (during this cruise Cheatognaths and fish larvae, and a very few krill and amphipods) were picked, enumerated and dried. The other half of the final sample split was fixed in 4% borax buffered formalin, for later taxonomy, enumeration and staging. On the 3 main stations these multinets were deployed on arrival and at departure, i.e. before and after the nets used for the staining experiments, on the first station (area away from seismic activity) a single multinet was deployed. An additional multinet was deployed at night to assess night-time vertical distribution. On two stations a single 180 µm WP2 net was also deployed from 60-0 m, providing an additional estimate of surface integrated abundances of mesozooplankton.

Echosounder data suggested the presence of fish, in order to sample teleost larvae, we also deployed a WP3 net (1 mm mesh size) vertically from 60-0 m during daytime. Additionally, a single 1m^2 Multinet Mammoth (180 µm mesh size) was deployed obliquely, sampling the same vertical strata as the vertical Multinet hauls. These samples were also worked up according to IMR standards.

Table 2. Overview of quantitative zooplankton samples taken. The positions are the position of RV Kristine Bonnevie.

Station	Lat	Long	Mon	Day	Time (UTC)	Bottom depth	Station	Sampling
571	56 ° 40.00	03 ° 09.99	4	30	13:14	68.0	Control	CTD
571	56 ° 40.00	03 ° 09.99	4	30	13:19	68.0	Control	WP II
571	56 ° 40.00	03 ° 09.99	4	30	14:04	68.0	Control	Multinet_MIDI, 5 strata sampled
572	56 ° 40.00	03 ° 09.99	5	1	7:25	66.7	Control	CTD
573	56 ° 35.02	03 ° 08.08	5	1	10:32	66.7	WBAT1	Multinet_MIDI, 5 strata sampled
573	56 ° 35.02	03 ° 08.08	5	1	11:01	69.4	WBAT1	CTD
574	56 ° 35.02	03 ° 08.08	5	1	16:35	69.4	WBAT1	Multinet_MIDI, 5 strata sampled
575	56 ° 36.43	03 °13 .49	5	2	7:26	69.4	WBAT2	Multinet_MIDI, 5 strata sampled
575	56 ° 36.43	03 °13 .49	5	2	7:47	68.4	WBAT2	CTD
575	56 ° 36.43	03 °13 .49	5	2	13:23	68.4	WBAT2	Multinet_MIDI, 5 strata sampled
577	56 ° 36.43	03 °13 .49	5	3	6:08	69.0	Control	CTD
577	56 ° 36.43	03 °13 .49	5	3	7:00	69.0	Control	WP II
578	56 ° 36.13	03 °11.21	5	3	20:57	68.8	Control (night)	CTD
578	56 ° 36.13	03 °11.21	5	3	21:19	69.0	Control (night)	Multinet_MIDI, 5 strata sampled
578	56 ° 36.13	03 °11.21	5	4	8:38	69.0	Control	WP3
579	56 ° 36.12	03 °15.48	5	5	6:40	69.0	WBAT3	CTD

Station	Lat	Long	Mon	Day	Time (UTC)	Bottom depth	Station	Sampling
579	56 ° 36.12	03 °15.48	5	5	7:28	69.0	WBAT3	Multinet_MIDI, 5 strata sampled
579	56 ° 36.12	03 °15.48	5	5	13:32	69.0	WBAT3	Multinet_MIDI, 5 strata sampled
579	56 ° 36.12	03 °15.48	5	5	16:53	69.0	WBAT3	Multinet_MAMMOTH, 5 strata sampled

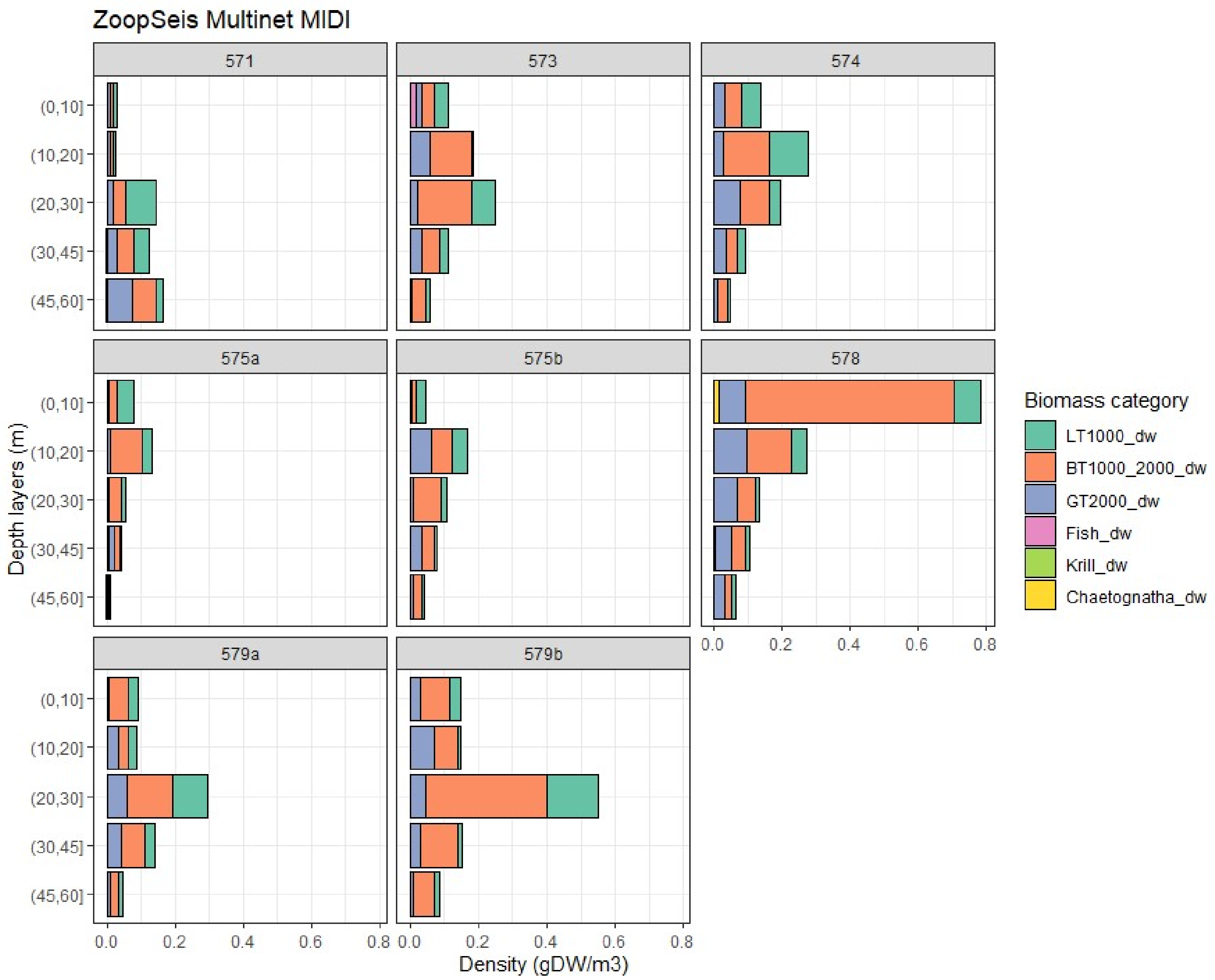


Figure 8. Results of the Multinet midi, sampling different depth layers of the water column. Biomass is given as gram dry weight per cubic meter (gDW/m 3), within different size fractions (≤1000 µm, 1000-2000 µm and ≥2000 µm) and animal categories (Fish, Krill and Chaetognaths). Multinet number is given on top of each diagram.

2.4.2 - Discrimination of live and dead zooplankton:

(Josefin Titelman, University of Oslo)

Zooplankton mortality was analyzed from samples from vertical net tows (WP2) taken at different distances from the seismic shooting (Table 4). Samples were stained with a live stain and photographed (Elliott and Tang 2009). At all times dead controls were also stained. Images will be analyzed using an automated procedure that efficiently assigns each copepod to either live or dead. Preliminary scanning by eye suggests limited (if any) effects of the seismic activity.

Table 3 . Summary of staining conditions for all experiments where staining was applied. Animals in net tows were predominately Calanus finmarchicus / C. helgolandicus . Time in UTC.

Purpose	Date	Position	CTD station	Animals	Stain conc. (ml stock/L)	Staining time (min)	Killing method
WBAT1	1 May	56 ° 35.02N 03 ° 08.08E	573	WP2 (0-60m)	0.3/1	15	Fresh water 3 min
WBAT2	2 May	56 ° 36.43N 03 ° 13.49E	575	WP2 (0-60m)	0.3/1	15	Fresh water 5 min
WBAT3	5 May	56 ° 36.12N 3 ° 15.48E	579	WP2 (0-60)	1.5/1	15	Heat, 40 °C 10 min
Control	3 May	56 ° 36.13N 03 ° 11.21 E	578	WP2, 0-58m	1.5/1	15	Heat 40 °C 10 min
Bags 1, instant mortality	3 May	56 ° 36.13N 03 ° 11.21 E	NA	From WP2	1.5/1	15	Heat 40 °C 10 min
Bags 2, instant mortality	4 May 10:45-13:32	56 ° 36.477N 03 ° 12.301E	NA	From WP2	1.5/1	15	Heat 40 °C * 10min
Bags 3, instant mortality	4 May 15:35-16:35	56 ° 36.476N 03 ° 12.402E	NA	From WP2	1.5/1	15	Heat 40 °C * 10min
Test staining time	4 May	NA	NA	From WP2	1.5/1	15, 30, 60, 120	Heat 40 °C 10min

* *Dead controls from “Staining time”, at 15 min, conducted in the same photo session*

Table 4. Overview of qualitative zooplankton samples taken to look for live dead ratio – using WP2

WP2 number	Dato	SS	LocID	Depth	Start_UTC	Stop_UTC	ES_guess
1	30/4/2022	572	A		7:36:00		TEST
2	30/4/2022	572	B		7:43:00		TEST
3	1/5/2022	573	A	60	13:21:45	13:23:32	WBAT1
4	1/5/2022	573	DEAD	35	13:25:00	13:27:00	WBAT1
5	1/5/2022	573	B	60	13:34:55	13:36:55	WBAT1
6	1/5/2022	573	C	60	13:50:01	13:52:05	WBAT1
7	1/5/2022	573	D	60	14:05:00	14:08:08	WBAT1
8	1/5/2022	573	E	60	14:11:45	14:13:47	WBAT1
9	1/5/2022	573	F	60	14:17:06	14:18:59	WBAT1
10	1/5/2022	573	G	60	14:22:04	14:24:00	WBAT1
11	1/5/2022	573	H	60	14:27:11	14:29:08	WBAT1
12	1/5/2022	573	I	60	14:45:26	14:47:26	WBAT1
13	1/5/2022	573	J	60	14:50:50	14:52:36	WBAT1
14	1/5/2022	573	K	60	14:55:25	14:57:25	WBAT1
15	1/5/2022	573	L	60	15:00:20	15:02:16	WBAT1
16	1/5/2022	573	M	60	15:05:05	15:07:10	WBAT1
17	2/5/2022	575	A	60	10:15:00	10:17:00	WBAT2
18	2/5/2022	575	B	60	10:30:00	10:32:30	WBAT2
19	2/5/2022	575	C	60	10:45:00	10:47:08	WBAT2
20	2/5/2022	575	D	60	11:00:00	11:02:05	WBAT2
21	2/5/2022	575	E	60	11:15:00	11:17:05	WBAT2
22	2/5/2022	575	F	60	11:30:00	11:32:15	WBAT2
23	2/5/2022	575	G	60	11:45:00	11:46:55	WBAT2
24	2/5/2022	575	H	60	11:55:00	11:57:20	WBAT2
25	2/5/2022	575	I	60	12:00:00	12:02:10	WBAT2
26	2/5/2022	575	J	60	12:04:35	12:06:30	WBAT2
27	2/5/2022	575	K	60	12:09:15	12:11:10	WBAT2
28	2/5/2022	575	L	60	12:13:45	12:15:45	WBAT2
29	2/5/2022	575	M	60	12:18:20	12:20:05	WBAT2
30	2/5/2022	575	N	60	12:22:30	12:24:32	WBAT2
31	2/5/2022	575	O	60	12:27:19	12:29:15	WBAT2
32	3/5/2022	577	A	58	7:10:00	7:12:00	STAINING CONTROLS
33	3/5/2022	577	B	58	7:35:15	7:37:08	STAINING CONTROLS
34	3/5/2022	577	C	58	7:40:32	7:42:25	STAINING CONTROLS
35	3/5/2022	577	D	58	7:46:20	7:48:17	STAINING CONTROLS
36	3/5/2022	577	Dead 1	58	7:51:40	7:53:26	STAINING CONTROLS
37	3/5/2022	577	Dead2	58	7:57:55	7:59:45	STAINING CONTROLS
38	3/5/2022	577	Dead3	58	8:03:15	8:05:08	STAINING CONTROLS
39	5/4/2022	578	A	58	13:05:00	13:07:00	STAINING CONTROLS

WP2 number	Dato	SS	LocID	Depth	Start_UTC	Stop_UTC	ES_guess
40	5/4/2022	578	B	58	13:11:00	13:13:00	STAINING CONTROLS
41	5/4/2022	578	C	58	13:17:00	13:19:00	STAINING CONTROLS
42	5/5/2022	579	A	NA	11:40:40	11:42:40	WBAT3
43	5/5/2022	579	B	NA	11:55:00	11:57:00	WBAT3
44	5/5/2022	579	C	NA	12:10:00	12:12:00	WBAT3
45	5/5/2022	579	D	NA	12:15:15	12:17:30	WBAT3
46	5/5/2022	579	E	NA	12:20:00	12:22:00	WBAT3
47	5/5/2022	579	F	NA	12:24:50	12:27:15	WBAT3
48	5/5/2022	579	G	NA	12:30:05	12:32:05	WBAT3
49	5/5/2022	579	H	NA	12:35:10	12:37:05	WBAT3
50	5/5/2022	579	I	NA	12:40:00	12:42:02	WBAT3
51	5/5/2022	579	J	NA	12:44:50	12:47:05	WBAT3

2.5 - PLANKTON EXPERIMENTS - SURVIVAL

2.5.1 - Bag experiments – methods

(Emilie Hernes Vereide, IMR and University of Oslo)

Preparation

Live cultured *Calanus finmarchicus* were kept at 10ºC in a container, 25 L buckets with filtered water.

Figure 9. The bag experiment with cultured Calanus finmarchicus. Top picture: three netting bags holding a 1 litre ziplocked plastic bag. Each ziplocked bag were holding 10 cultured C. finmarchicus. The three bags were placed at 80 m distance from one another. The rope holding the bags was lowered by the starboard rear winch on RV Kristine Bonnevie until the top bag stopped at 9.2 m depth, the one in the centre at 10 m and the bottom at 10.8 m depth. A 3.8 kg weight was at the end of the rope. People on the picture from the right, Emilie Vereide Hernes, Saskia Kühn and Sigurd Hannaas. Bottom picture: experiment with cultured C. finmarchicus in 3 bags when lowered to the water. Pictures by Karen de Jong (top) and Anne Christine Utne Palm (bottom).

2.5.1.1 - *Calanus bags*

We carefully transferred 10 cultured *Calanus finmarchicus* to 1L ziplock plastic bags, holding filtered seawater at a temperature of 10ºC. Further, we placed each plastic bag in nylon netting bag (laundry bags). Then 3 netting bags, holding 10 copepods each, were tied onto a line and lowered to the water. For every transect, 1 line was deployed at the very beginning, and four lines were deployed the last 8 minutes, or as close as possible to the seismic vessel. For every line, the bags were lowered to a depth of 10 m, kept there for 15 seconds, before pulled up (Fig 9).

Here, one control of no shooting was conducted, in addition to 3 rounds of exposure (3 WBAT transects). Also, we had one semi control Saturday 30th of April, with shooting from a far distance.

After deployment, every individual *C. finmarchicus* was investigated live/dead and transferred to 50 mL bottles for further investigations of delayed mortality. Dead individuals were taken out and photographed to determine size and stage. The bottles were kept in the container at 10ºC.

2.5.1.2 - *Wild zooplankton*

Net hauls (WP2) were taken Tuesday 3rd of May, when there was no shooting in the area. The zooplankton were kept in 25 L buckets at 10ºC with light aeration. Bulks of the sample were put in bags and attached to a line along with the zooplankton cages (2.5) for 1 hour of deployment (Figure 10). Here, one control with no shooting was conducted, in addition to 3 rounds with exposure. After exposure, the zooplankton were stained to determine mortality.

Table 5. Overview of bag experiments

Date	Treatment	Cultured/wild	# Lines per approach	# bags per line	# animals per bag	Time in water	Description
30.04.2022	Control (3nm)	Cultured	4	3	10	45s	
01.05.2022	WBAT1 transect	Cultured	5	3	10	45s	1 line at furthest distance, 4 lines last 8 min before passage
01.05.2022	Picking control	Cultured	1	6	10	-	Stayed in container
02.05.2022	WBAT2 transect	Cultured	5	3	10	45s	1 line at furthest distance, 4 lines last 8 min before passage
02.05.2022	Picking control	Cultured	1	6	10	-	Stayed in container
03.05.2022	Control no shooting	Cultured	4	3	10	45s	
03.05.2022	Control no shooting	Wild	4	3	> 100	45s	On same lines as above
04.05.2022	Passage	Wild	1	12	> 100	60 min	From far to close passage, on cage
04.05.2022	Passage	Wild	1	12	> 100	60 min	From far to close passage
05.05.2022	WBAT3 transect	Cultured	5	3	10	45s	1 line at furthest distance, 4 lines last 8 min before passage
05.05.2022	Picking control	Cultured	1	6	10	-	Stayed in container
05.05.2022	WBAT3 transect	Cultured	1	4	50	45s	50 individuals per bags
05.05.2022	WBAT3 transect	Wild	1	6	> 100	40 min	At stat of transect, On cage
05.05.2022	Picking control	Wild	1	6	> 100	-	Stayed in container

Figure 10. Experiment with wild caught zooplankton in three bags (white bags) and two cages (black boxes). To lessen the wave action, we used a drift anchor (orange cone) and a 5 kg weight at the end of the rope. Picture by Anne Christine Utne Palm.

2.6 - COPEPOD BEHAVIOUR

(Saskia Kühn, Kiel University, Coastal Ecology Research and Technology Centre West Coast Büsum)

In this experiment we have exposed wild caught zooplankton, cultured adult *Calanus finmarchicus*, to real-time seismic air gun blasts. Copepod behavior was filmed and will be analyzed before, during and between the exposures.

Figure 11. The cage setup used to study behaviour of cultured Calanus finmarchicus and wild caught copepods. Upper picture: The inside view of the cage (40x40x40 cm), showing the position of the two GoPro cameras and light source (a diving torch) hanging from the ceiling. The torch was used to make the plankton active – as plankton are known to swim towards the light (phototactic). Lower picture: The outside view of the cage, with the designer, Saskia Kühn adding plankton to the cage, before. The cage was covered with a thin sheet of black plastic to shoot out the light (make it dark inside the cage) enhancing the effect of the torch. Picture by Karen de Jong.

Cage experiment

Two 40*40*40 cm cages, based on an aluminum frame enclosed with a 100 µm mesh net and a black plastic bag to darken the cage, were each equipped with a stereo-camera setup (Figure 11). The cameras were pointing towards a black PVC plate on which a white diving light was connected. The two cages were connected with a rope so that one cage was 2 meters on top of the other cage (Figure 10). The upper cage was connected to a buoy with a 10 m long rope. We connected a floating plus weights to the lower cage in order to stabilize the setup. After the preparation of the setup we put 100 copepods into a small holding tanks inside the cages.
The holding tank opened automatically when the cages were lowered into the water, and released the experimental animalssmall to the cages. Then, the lights and cameras were turned on. The setup was thereafter put into the water with a crane and stayed there for approximately 1 hour. The 1 hour was chosen to study the animal behavior during increasing seismic exposure (during an a approach of the seismic vessel). We timed the deployment so that the seismic ship would pass exactly after 60 minutes of deployment. Beside the exposure we additionally included controls at times without any shooting from the seismic ship. The data will be analyzed upon individual copepod behavior (see Table 6).

Table 6. Overview Cage Experiments

Date	Code	Control/ Exposure	Cage	Copepods Culture/ Wild	Num. of Copepods	Time IN	Time OUT	Comments
01.05.2022	ZS_1	Control	1	Culture	100	08:15	09:15	
02.05.2022	ZS_2	Exposure	1	Culture	100	14:42	15:12	
03.05.2022	ZS_3	Control	1	Wild	100	12:36	13:36	
03.05.2022	ZS_4	Control	2	Wild	100	12:36	13:36	
03.05.2022	ZS_5	Control	1	Wild	100	16:15	17:15	
04.05.2022	ZS_6	Exposure	1	Culture	100	12:38	13:45	
04.05.2022	ZS_7	Exposure	2	Culture	100	12:38	13:45	All times from watch Saskia Kühn Synchronized at klokka.no
04.05.2022	ZS_8	Exposure	1	Culture	100	17:39	18:45	
04.05.2022	ZS_9	Exposure	2	Culture	100	17:39	18:45	
05.05.2022	ZS_10	Exposure	1	Culture	100	13:56	14:50	
05.05.2022	ZS_11	Exposure	2	Culture	100	13:56	14:50	
06.05.2022	ZS_12	Control - Exposure	1	Culture	130	11:49	12:35	
06.05.2022	ZS_13	Control - Exposure	2	Culture	130	11:49	12:35	

2.7 - HYDROPHONE DATA

(Karen de Jong, IMR)

To measure ambient sound and the sound from the seismic survey, we deployed an Ocean Sonic Eth-X2 hydrophone (sensitivity 205 dB *re* 1 µPa) (Figure 12 topp) on a rope midships from the port side of the research vessel. The hydrophone was deployed on a steel bracket hanging from a rope at 10m depth. The hydrophone recorded while the seismic ship was approaching and at three occasions it recorded until it had passed.

Figure 12. Hydrophones used on the cruise. Top: The Ocean Sonic Eth-X2 hydrophone, used for measuring sound during the Seismic shooting approach at the three WBAT stations. Middle and bottom: The hydrophone buoy, that contained two omnidirectional hydrophones (Naxys 02345, frequency range: 5 Hz to 300 kHz, sensitivity: -179 dB re V/μPa). The hydrophone buoy was sat out for 24h during which there were hours with and without seismic shooting. Picture top from web page, middle by Karen de Jong and bottom by Anne Christine Utne Palm.

Table 7. Description of recordings

Date	Time start (UTC)	Time stop (UTC)	Approximate time of passage*	Description
30.04.2022	17:57:50	18:13:46		Control station (no passage)
01.05.2022	08:32:00	10:47:00		Control station (no passage)
01.05.2022	13:10:00	14:31:15	14:56	WBAT1 transect
02.05.2022	09:20:00	12:06:10	12:12	WBAT2 transect
03.05.2022	12:26:00	13:00:00		Control on site without shooting
04.05.2022	10:55:04	11:50:00	10:37	Extra pass
04.05.2022	15:47:00	16:36:00	16:36	Pass on port side
05.05.2022	11:42:00	12:36:00	12:34	WBAT3 transect
06.05.2022	11:47:00	12:30:00	12:27	Pass at the start of a shooting transect, port side, hydrophone in water before shooting started.

* Taken from when last bags were taken out of the water

In addition, an anti-heave surface buoy with a vertical hydrophone array (Figure 12 middel and bottom) was deployed on northern end of the Ekofisk area, close to the position of WBAT 3. This buoy contained an UNO-2483G embedded computer with an internal flash drive for data-logging and instrumentation control and two calibrated omnidirectional hydrophones (Naxys 02345, frequency range: 5 Hz to 300 kHz, sensitivity: -179 dB re V/μPa) hydrophones that can be deployed at variable depths. One of the hydrophones was deployed at 8 m depth (sampling rate: 96 kHz, gain 0 dB) and one at 35 m depth (768 kHz, gain: 0 dB). The hydrophones recorded from 3.5.2022 17:50 till 04.05.2022 16:57:41, with a duty cycle of 28s on, 2s off. A GPS receiver enabled the buoy to be tracked and a radio Ethernet link allowed remote control and monitoring of the system. The GPS position were logged to document the exact location of the buoy over the 24-hours deployment period.

In addition to these two hydrophones we deployed a hydrophone glider, Slocum, for Akvaplan-niva on the 30th of May. Ambient sound record will also be provided from a lander rigged with JASCO hydrophone deployed at Ekofisk in March 2022 and will be retrieved in March 2023 (position of lander: Long: 3.171969 - Lat: 56.537685 (WGS 84)).

2.8 - ACTIVITY MAP

Below (Figure 13) is a map showing the cruise activity at Ekofisk. Tracking both Kristine Bonnevie and the seismic vessel Skandi Nova from the 30th of April to the 6th of May. Two of the WBAT stations (WBAT 2 and 3) are marked by name in orange. WBAT 1 is located west in the shooting area where you see the dens tracks of the kayak (orange) and Otter (purple). We had two control stations, one ca 5 nm to the north (the most northern stations on the map) and one control station between WBAT1 and WBAT2. The FAD station was North of the shooting area, between the two control stations visible as a flower track. For latitude and longitude position see Table 2, for date and time see Table 7.

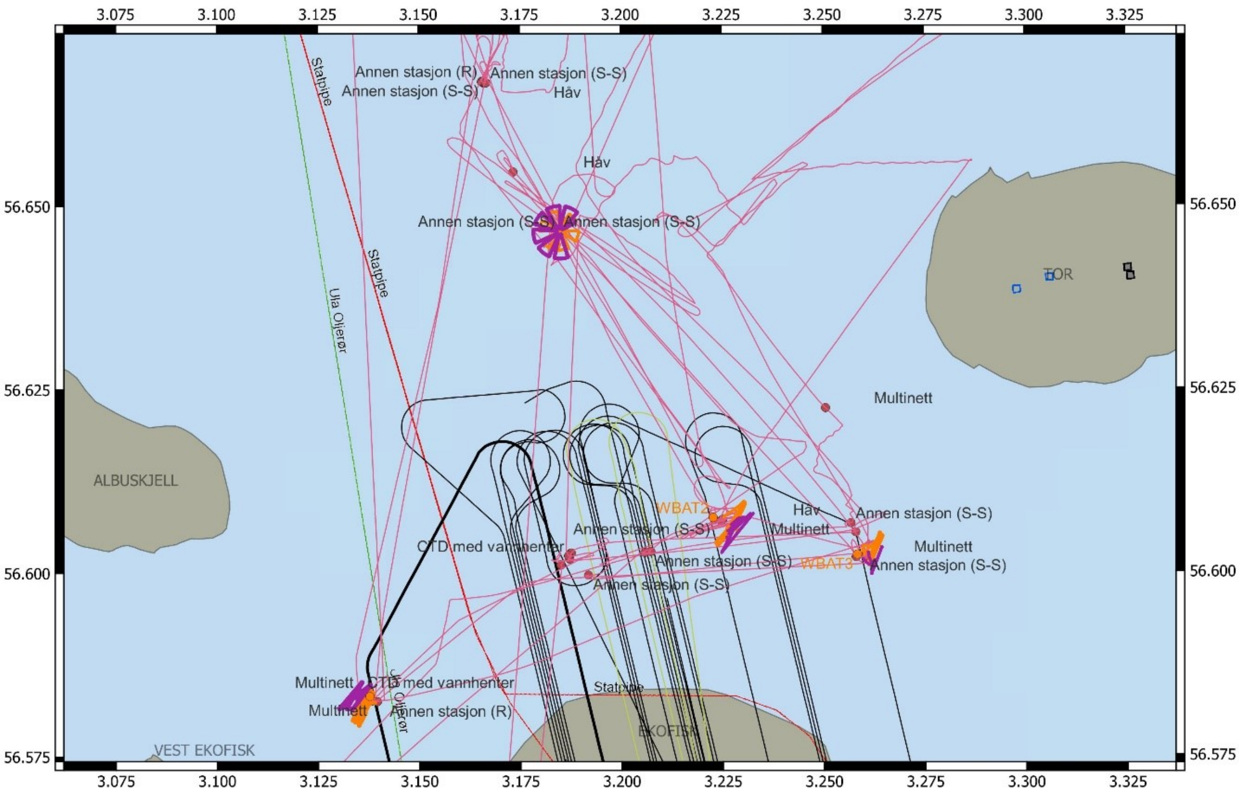


Figure 13. Tracking of relevant activity at Ekofisk. Red tracks are RV Kristine Bonnevie, black tracks are Skandi Nova (seismic vessel), orange tracks Kayak and purple the Otter. Red dots are stations, where multinet, WP2 or CTD were taken.

2.9 - SEISMIC AIRGUN

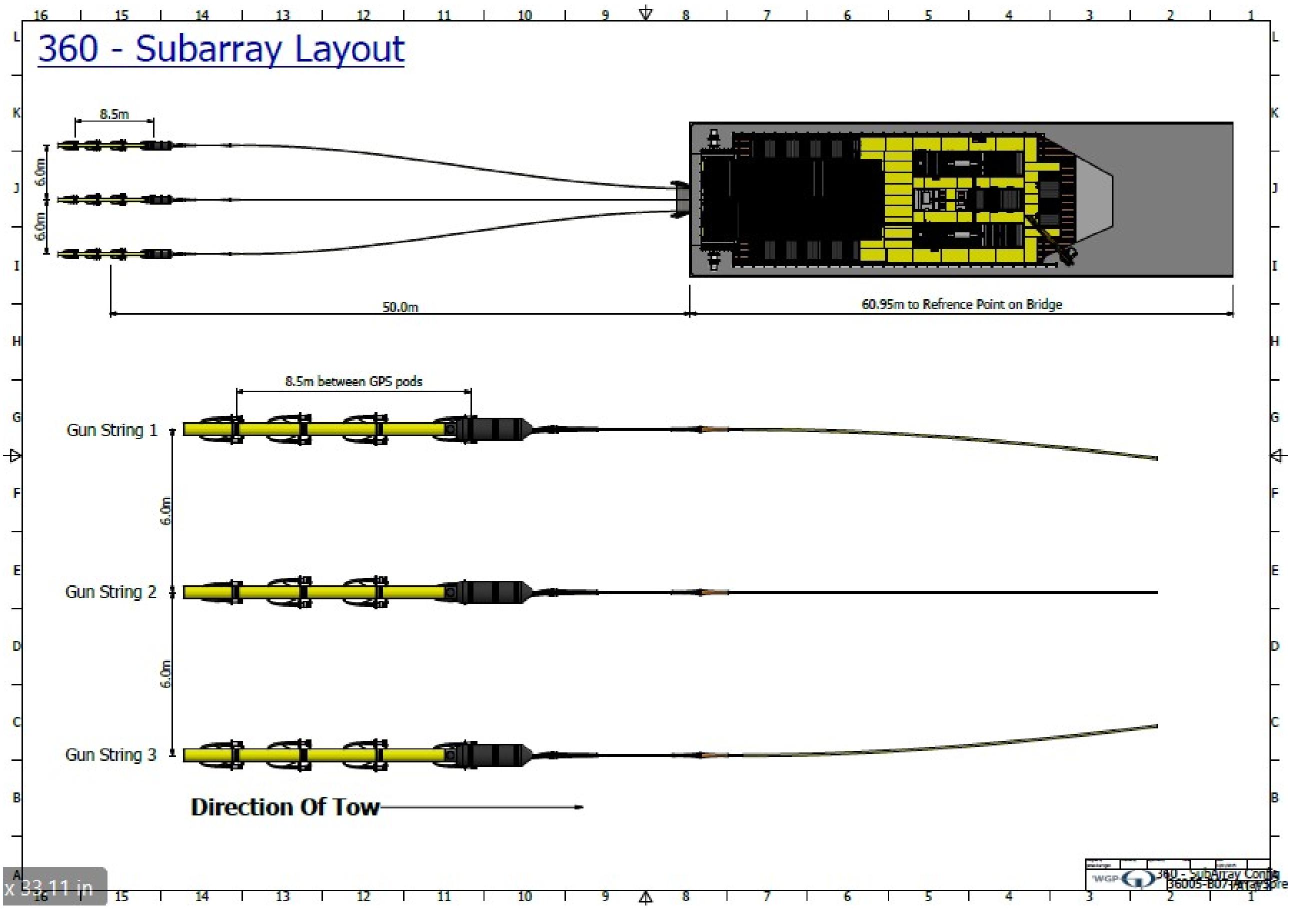
Below (Table 8) follows a description of the properties and positioning of the seismic air gun used in the experiment.

Table 8. Properties of air gun and the array

Source Parameters	
Number of Sources	1
Number of Sub-Arrays (Strings) per Source	3
Array Length	8.55 m
Sub-Array Separation	6 m
Source Width	12 m
Source Wolum	3060 Cubic inches
Number of Hydrophones per String	4
Number of Depth Transducers per String	3
Number of Pressure Transducers per String	1
Number of Guns per String (all Clusters)	String 1 = 8 (4 Clusters)

Source Parameters	
	String 2 = 8 (4 Clusters)
	String 3 = 8 (4 Clusters)
Airgun Type	Sercel G-Gun II
Operating Pressure	2000 PSI
Depth of Guns	5.0 m +/- 0.5 m
Peak to Peak Amplitude	82.06 bar m
Primary to Bubble Ratio	29.14

Figure 14. Showing the seismic vessel, Skandia Nova, and its airgun. Picture by Anne Christine Utne Palm.



Skandi Nova Towed Configuration

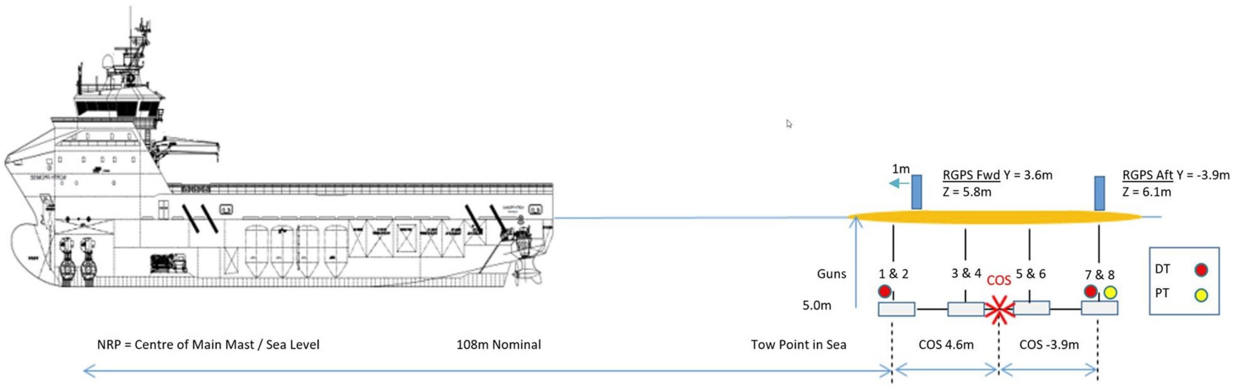


Figure 15. The two drawings show the dimension of the seismic air gun and its positioning in the water and in relation to the seismic vessel.

3 - Referances

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