

# **Field Report**

### Integrated Studies and Ecosystem Characterization of the Labrador Sea Deep Ocean (ISECOLD)



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#### **Introduction and objectives**

The Government of Canada has committed to protecting 10% of Canada's marine and coastal areas by 2020 as part of its commitment to achieve international (the Convention on Biological Diversity 2011 20 Strategic Plan for Biodiversity's Aichi Targets) and domestic (2020 Biodiversity Goals and Targets for Canada) biodiversity conservation goals. In 2017, a three year study was initiated for a deep offshore portion of the northern Labrador Sea that was under consideration for a large offshore MPA. From an oceanographic perspective, the area is well studied and of global significance as it is one of the few areas of the world where deep-water convection occurs. However, at depths beyond 750 m, virtually no data was available regarding the biota. Consequently, the Integrated Studies and Ecosystem Characterization of the Labrador Sea Deep Ocean (ISECOLD) was initiated. A CSAS meeting in 2017 (Cote et al. 2018) highlighted the need for characterization efforts related to benthic and pelagic communities, demersal fish communities, seabed mapping and habitat characterization and seabird and marine mammal observations. The Amundsen 2019, Leg 1B Expedition extends collections conducted in 2018 (Amundsen Leg 2C) and addresses these target areas with the exception of demersal fish; a program component for which an alternative vessel and sampling techniques are required. In addition to the scientific objectives of DFO, Leg 1B addresses the scientific objectives of several key academic, government, Indigenous and international collaborators.

The 2019 program (June 23-July 5, 2019) features many elements of the 2018 program including drop camera surveys, box core collections, Isaac Kidd Midwater Trawls, Hydrobios plankton sampling, various hydro-acoustic assessments (WBAT, EK60), water collections, bottom mapping, visual observations of marine mammals and seabirds, and the deployment and retrieval of environmental sensors on moorings/landers. While the ROV program was not conducted in 2019, the program was supplemented by rock dredge collections and Moving Vessel Profiler surveys of oceanographic fronts. Furthermore, the 2019 program includes surveys of more southerly areas of the study zone, previously sampled during fish characterization cruises in 2017 and also includes follow up visits to targeted areas characterized in 2019 (Figure 1). In total, 102 operations were planned for Leg 1B, of which over 95% were accomplished. Program elements, their rationale, methods and preliminary results (where possible) are highlighted in greater detail below.



**Figure 1**: Cruise track of the 2019 ISECOLD program during Leg 1B of the 2019 Amundsen cruise.

#### Water Sampling (Chen)

Seawater samples were collected at each DFO-full station as well as some mooring/lander stations using a CTD-rosette sampling system (Table 1). These samples will be analyzed for nutrients by Dr. Kumiko Azetsu-Scott at Bedford Institute of Oceanography, d15N-NO3 isotope analysis by Dr. Owen Sherwood, and compoundspecific isotope analysis of amino acids by Shao-Min Chen at Dalhousie University.

#### Nutrients

Acid-washed 10-ml tubes and caps were rinsed with sample water for three times. Sample was directly drawn from the Niskin bottle until <sup>3</sup>/<sub>4</sub> full and placed in a dark bag. All the samples were stored upright in a -20°C freezer after sampling.

#### D15N-NO3 isotope analysis

For depths that were 200 m or shallower, samples were filtered through 0.2 µm filters into the 60-ml bottles. Syringe was rinsed twice and filled with sample water using a tubing. Filter and bottle were rinsed twice with filtered sample water using the syringe. Filtered sample was drawn into the bottle until the shoulder (60 ml). For depths that were deeper than 200 m, bottle was rinsed for three times with sample water and filled up directly from the Niskin bottle. All the samples were stored at -20°C after sampling. Help was received from David Cote (Fisheries and Oceans Canada), Catherine Young (Memorial University), Rebecca Evans and Amy McAllister (Memorial University), Lauren O'Dell and Kanae Komaki (Amundsen Science) for both nutrients and NO3 isotope sampling.

#### Size-fractionated phytoplankton

During the CTD cast at each station, deep chlorophyll maximum (DCM) depth was determined. Ten or twenty liters of seawater from the surface, half-DCM depth, and the DCM were collected for size-fractionated filtration. Sample from each depth was filtered through 180  $\mu$ m and 20  $\mu$ m nylon filters, and 3  $\mu$ m and 0.2  $\mu$ m polycarbonate filters in sequence using a pump (Cole-Parmer) for a maximum period of 10 hours (Figure 2). The approximate filtered volume of each sample was recorded at the end of the filtration. After the filtration, each filter was removed from the filter holder, packed into a piece of tin foil, placed in a plastic bag and kept frozen at -20°C. The filtration system as well as the sample jugs was rinsed with fresh water after each filtration.



Figure 2: Overview of the filtration system (A). The setup of the pump for filtering 3 samples at the same time (B) and the 4 different-size filters in the filter holders for each sample with 180  $\mu$ m filter at the near-pump end and 0.2  $\mu$ m filter at the near-bucket end (C). Samples were covered by a dark bag during the filtration.

**Table 1**. Nutrients, d15N-NO3, size-fractionated phytoplankton samples collected from the CTD-Rosette and core-top sediment samples collected from the box cores during the expedition. Depth here is the bottom depth from the rosette log. Samples taken are marked by symbol "x".

Station ID	Date	Depth (m)	Nutrients	D15N- NO3	Size- fractionated phytoplankton	Core-top sediment
ISECOLD 1-500	20190625	599	Х	X	Х	Х
ISECOLD 1-1000	20190625	1013	X	X		Х
ISECOLD 1-1500	20190626	1464	X	x	Х	Х
ISECOLD 1-2000	20190627	1993	X	x		
ISECOLD 1-2500	20190628	2490	х	х	Х	Х
ISECOLD 2-2500	20190628	2396	X	x	Х	Х
ISECOLD 2-2000	20190629	1945				Х
ISECOLD 2-1500	20190630	1515	X	x	Х	Х
ISECOLD 2-1000	20190630	1021				Х
ISECOLD 2-500	20190630	529				Х
ATLAS Non- Sponge 3 Lander	20190701	573	X	Х	X	
HiBio-B	20190701	1914				Х
HiBio-A	20190702	1055	X	X	Х	

### **Drop Camera**

Drop cameras are a useful tool to characterize benthic fauna and habitat. The drop camera was deployed routinely at stations along two transects in addition to a few additional stations that were mapped with multibeam sonar in 2018. The drop camera was used in 2019 to: 1. To extend the study extent further south along the Labrador shelf and slope across a depth gradient (500m-2500m); 2. Characterize benthic fauna at targeted ridge and canyon habitats surveyed in 2018; 3. Survey a station to characterize sponge distribution at a shallow site (~350 m) on the shelf for ATLAS (A trans-Atlantic assessment and deep-water ecosystem based spatial management plan for Europe) collaborators; and 4. To test a malfuntioning HIPAP sensor. This section describes activities related to the drop camera for the ISECOLD project (objectives 1 and 2).

The deep-sea camera system was comprised of two cameras (a SubC deep-water camera and Sony 4K camera), LED lights and a HIPAP sensor, which were attached to a box core frame (Figure 3). The latter was used to provide the camera team with the real-time data of the camera position (relative to the vessel) as well as exact position of the camera relative to the seabed. Specific GPS coordinates of sampling stations for drop camera surveys can be seen in Table 2.

A modified box corer apparatus containing the drop camera setup was attached to a winch cable system and lowered from the vessel at 80 m/min. When the drop camera was within ~50 m from the last reported depth, it was lowered at 20 m/min until it touched bottom. The camera lead would communicate if the drop camera was on the seabed via observation of the HIPAP software but generally the deckhand operating the winch could determine if the camera was on bottom by examining the tensiometer on the winch, which would show a drop in tension when the drop camera system touched the bottom. From there on, a "yo-yo" method was employed whereby the camera would be raised ~2 m off the bottom (as measured by the length of winch cable retracted), and dropped on the bottom again, and this procedure was repeated for 30-40 minutes.

A record was kept of the time of the camera deployment, time on bottom, time removed from bottom, and time that the camera was lifted back on the deck. Once the camera was back on deck, the camera apparatus was rinsed with fresh water, removed from the box core frame, and taken to the foredeck lab to have the video footage from both the SubC camera and the Sony 4K camera downloaded and saved to an external hard drive. Drop camera footage was also used to inform the suitability of bottom habitats for other sampling devices (e.g. box corer).

Fourteen drop camera deployments were conducted during Leg 1B of the 2019 Amundsen Expedition, which will be analyzed for the ISECOLD project. Footage from the SubC drop camera has been preliminarily viewed for all sampling stations. Technical difficulties related to off-loading and viewing recorded video from the SubC camera system beyond ISECOLD 1-2000 station required the remaining stations to be viewed for the Sony camera only. Also, another Sony camera was installed in the camera box to get supplementary downward facing footage in some later stations.

Camera deployments were successful though some deployments were challenged by the camera view being obscured by sediment plumes, and ocean swell causing the camera to move too fast or high off the seabed. However, there were a few stations which provided very good observational conditions. There were also issues with the HIPAP signal beyond 1500 m depth and associated HIPAP data was lost in the deep stations. Drop camera surveys for the ISECOLD project ranged in depth from 369 to

2,494 m. In general, the sampling stations that occurred on hard bottom tended to have higher epifauna productivity in comparison to the soft bottom stations as observed by the abundance and distribution of marine megafauna/flora from those drop camera video transects. Transect 1 was typically soft bottomed but had the unique characteristic of more dropstones and hard substrate, with increasing depth, which will allow for a valuable comparison to last year's transect. Stations on Transect 2 by comparison were mainly soft bottom with few large rocks or boulders except for the shallow (ISECOLD 2-500) and more intermediate (ISECOLD 2-1500) sampling depths. The revisited stations for the northern 2018 transect (HiBio-B and DFO-1200) and the ATLAS station (Sponge 4) were primarily hard bottom.

Generally, anemones, urchins, sponges, corals, and brittle stars tended to dominate the epifauna of several soft bottom sites as well as the majority of hard bottom sites (Table 3; Figure 4). There were many different species of sponges (e.g. *Geodia* sp., *Asconema* sp.) and corals (*Anthoptilum* sp., *Anthomastus* sp., *Acanella* sp.) encountered throughout the study, however many more coral and sponge species remain to be identified in the aftermath of this survey. These taxa were observed out to the deepest sites surveyed (~2500m).

Fish species were also encountered during the survey. The primary species identified were grenadier and blue hake and other yet to be identified fish species were also observed. Cephalopods, including species of squid and octopus were seen in some video transects, and two decapod species (crabs and squat lobsters) were also sighted at some sampling stations. Other organisms (some shown in Figures 4-6 for ISECOLD Transects 1 and 2, and the ATLAS Transect respectively) that were observed throughout the sampling period include: Greenland Halibut, anemones, sea stars, corals (including unidentified gorgonian corals, sea pens, soft coral, black-wire coral), *Polymastia* sp. sponges (and other unidentified species of sponges), large urchins, eels, eelpout, skates, benthic siphonophore, crinoids, gastropods tunicates, and bryozoans.



**Figure 3**: The drop camera system attached to a modified box core frame utilized in Leg 1b of the 2019 Amundsen Expedition.



**Figure 4**. Photo captures of drop camera video from stations on ISECOLD 1 video transects. A: Greenland Halibut (ISECOLD 1-500); B: Dominant Anemone, seas star and *Polymasta* sp. (ISECOLD 1-500); C: Anemone and Soft coral? (representative photo of bad video quality) (ISECOLD 1-1000); D: Mushroom coral, Blue Hake and possibly dead Black-wire coral (ISECOLD 1-1500); E: *Geodia* sp., unidentified sponge (*Lissodendoryx*?), anemone, soft coral, *Hymedesmia* sp. (ISECOLD 1-1500); F: *Acanella* arbuscula. (ISECOLD 1-1500); G: Black-wire coral (Antipatharian, probably *Bathypathes* sp.) (ISECOLD 1-2000); H: Bamboo coral and unidentified sponges (ISECOLD 1-2500).



**Figure 5**. Photo captures of drop camera video from stations on ISECOLD 2 video transects. A: Redfish, grenadier, and seapen (ISECOLD 2-500); B: *Asconema* sp. sponge with crinoid (ISECOLD 2-500); C: Mushroom coral (possibly *Anthomastus* sp. and/or *Heteropolypus* sp.) (ISECOLD 2-1000); D: Glass sponge and soft coral (ISECOLD 2-1500); E: Various sponges and anemone (ISECOLD 2-1500); F: *Geodia* sp. sponges (ISECOLD 2-1500); G: Blue hake (ISECOLD 2-2000); H: Skate sp. (ISECOLD 2-2500). Note: Photos A and B acquired from downward-facing Sony camera. Photos C – H acquired from side-mounted Sony camera.



**Figure 6**. Photo captures of drop camera video from revisited ISECOLD 2018 stations (HiBio-B and DFO-1200) and ATLAS station (Sponge 4) video transects. A: Stalked crinoid (possibly *Hyocrinus* sp.), anenome, and potentially *Acanella arbuscula* (HiBio-B); B: Crinoids, anenomes, and *Anthoptilum* sp. (HiBio-B); C: Sponge (*Asconema* sp.) and *A. arbuscula* (DFO-1200); D: Various sponges (Sponge-4); E: *Geodia* sp. and various sponges; F: Crab (Sponge-4).

Station ID	GPS Coordinates on Bottom (Start)	GPS Coordinates on Bottom (End)	Date	Time Deployed	Approximate Time on Bottom (min)	Approximate Bottom Depth (m)
ISECOLD 1-500	57.7068835, 59.5290038	57.7030288, 59.5275508	25/06/2019	10:28:51	30	591
ISECOLD 1-1000	57.7100253, 59.3740387	57.7053722, 59.3706400	26/06/2019	23:24:19	30	1,038
ISECOLD 1-1500	57.7191068, 59.0786512	57.7117585, 59.0684750	26/06/2019	11:24:02	37	1,513
ISECOLD 1-2000	57.7259615, 58.6891100	57.7203665, 58.6675213	27/06/2019	02:02:42	36	2,030
ISECOLD 1-2500	57.7406145, 57.8818823	57.7434900, 57.8829188	28/06/2019	05:35:29	35	2,494
ISECOLD 2-2500	58.9054073, 58.8485567	58.8994120, 58.8509968	28/06/2019	16:02:59	42	2,393
ISECOLD 2-2000	58.8454638, 59.3721752	58.8405242, 59.3837662	29/06/2019	09:19:06	41	1,936
ISECOLD 2-1500	58.8186780, 59.6736115	58.8127792, 59.6800665	30/06/2019	22:52:46	40	1,689
ISECOLD 2-1000	58.7832133, 59.9260380	58.7795398, 59.9243578	30/06/2019	09:45:33	36	984
ISECOLD 2-500	58.7739433, 60.0435330	58.7695830, 60.0493267	30/06/2019	19:45:41	30	536
DFO-7 (HiBio B)	60.4754100, 60.3834953	60.4765185, 60.3979660	01/07/2019	19:23:21	31	1,854
DFO-1200	60.4506983, 61.0166160	60.4513527, 61.0010560	03/07/2019	00:01:45	40	1,213
Sponge 4	60.4642737, 62.1262203	60.4692672, 62.1333895	03/07/2019	16:52:21	30	369

**Table 2.** List of Drop Camera Sampling Stations for Leg 1B of the 2019 Amundsen Expedition.

**Table 3.** General Description of Drop Camera Sampling Stations by Bottom Depth, Bottom Type, Video Quality, BiologicalProductivity, and Megafauna/flora observed from preliminary observation of Drop Camera Footage for Leg 1b of the 2019 AmundsenExpedition.

Deployment #	Station ID	Approximate Bottom Depth	Bottom Type	Video Quality	Biological Productivity	Megafauna/flora observed
1	ISECOLD 1- 500	591 m	Soft bottom; muddy sediment; very few small pebbles; very few medium and large rocks/boulders.	<b>Poor:</b> Visibility was poor due to rough seas and sediment plumes and camera height off bottom was adequate.	<b>Low:</b> Very low diversity and abundances of organisms and sparse distribution throughout video transect.	<b>Dominant organisms:</b> Anemones. <b>Other organisms</b> <b>observed:</b> Polymastia and other sponge species, sea star, flat fishes, Greenland Halibut, grenadier sp., jelly fish, and octopus.
2	ISECOLD 1- 1000	1,038 m	Soft bottom; muddy sediment; very few small pebbles; very few medium and large rocks/boulders.	<b>Poor:</b> Visibility was poor due to rough seas and sediment plumes and camera height off bottom were very poor and often too far away to identify organisms. Did not touch bottom very often.	<b>Low:</b> Very low diversity and abundances of organisms and sparse distribution throughout video transect.	<b>Dominant organisms:</b> Urchins and anemones. <b>Other organisms observed:</b> brittle stars, squid, eel, and unidentified fish.
3	ISECOLD 1- 1500	1,513 m	Soft bottom; muddy bottom with many small rocks, pebbles, cobble, with some medium/large rocks and	<b>Good:</b> Visibility and camera height off bottom were adequate.	<b>Medium:</b> High diversity though low abundances of organisms and sparse distribution throughout video transect.	<b>Dominant organisms:</b> Corals ( <i>Acanella arbuscula.</i> , Soft coral and sea pens), sponges (including <i>Geodia</i> sp.) and anemones. <b>Other organisms</b> <b>observed:</b> Anemones, sponges ( <i>Asconema</i> sp., vase sponge and unidentified), yellow

			boulders throughout.			gorgonian coral, blue hake, unidentified fishes, sea stars, and brittle stars.
4	ISECOLD 1- 2000	2,030 m	Soft bottom; many small rocks, pebbles, cobbles, with medium and large rocks/boulders throughout.	<b>Good:</b> Visibility and camera height off bottom were adequate.	<b>Low:</b> Low diversity and abundances of organisms and sparse distribution throughout video transect.	<b>Dominant organisms:</b> Brittle stars. <b>Other organisms</b> <b>observed:</b> Sponges ( <i>Geodia</i> sp., glass sponges, and other unidentified sponges), corals (sea pens, <i>Acanella arbuscula</i> , soft coral, Black-wire coral), sea cucumbers, crab, and unidentified fish.
5	ISECOLD 1- 2500	2,494 m	Soft bottom; muddy sediment, some medium and large rocks/boulders.	<b>Poor:</b> Visibility good but camera height off bottom was poor and the camera touched bottom very few times.	<b>Low:</b> Very low diversity and abundances of organisms and sparse distribution throughout video transect.	<b>Dominant organisms:</b> Yellow, round sponge (possibly <i>Craniella</i> sp.) <b>Other</b> <b>organisms observed:</b> Bamboo coral and unidentified sponges.
6	ISECOLD 2- 2500	2,393 m	Soft bottom; muddy sediment, very few medium and/or large rocks/boulders.	<b>Poor:</b> Camera contact mainly on bottom with no movement, dragging along bottom, or too high in water column due to difficulty finding bottom.	<b>Low:</b> Very barren site overall. Low diversity and abundances of organisms and sparse distribution throughout video transect.	<b>Dominant organisms:</b> N/A. <b>Other organisms observed:</b> Sponges ( <i>Asconema</i> sp., and other unidentified sponges), skate sp., blue hake.

7	ISECOLD 2- 2000	1,936 m	Soft bottom; muddy sediment, very few medium and/or large rocks/boulders.	<b>Good:</b> Visibility and camera height off bottom were adequate.	Low: Fairly barren site overall. Low diversity and abundances of organisms and sparse distribution throughout video transect.	<b>Dominant organisms:</b> N/A. <b>Other organisms observed:</b> Corals ( <i>Acanella arbuscula</i> and unidentified soft corals), sponges ( <i>Asconema</i> sp., sea pens, glass sponges, and other unidentified sponges), anemones, sea stars, benthic siphonophore, urchins, skate sp., blue hake, grenadier sp., crinoid sp.
8	ISECOLD 2- 1500	1,689 m	Variable bottom type; muddy, silty sediment initially which changed with slope to a harder bottom with many small rocks, pebbles, cobble, with some medium/large rocks and boulders throughout.	Medium: Difficulty determining bottom, however visibility and camera height off bottom were adequate in portions of the video.	Medium-High: Wide diversity of organisms with an intermediate abundance and moderate distribution throughout video transect.	<b>Dominant organisms:</b> Corals (sea pens, and unidentified soft corals and gorgonians) and sponges ( <i>Geodia</i> sp., <i>Asconema</i> sp., glass sponges, and other unidentified sponges). <b>Other organisms</b> <b>observed:</b> Anemones, sea stars, sea urchins, blue hake, crab, grenadier sp.
9	ISECOLD 2- 1000	984 m	Soft bottom; muddy sediment, some small rocks, few medium and/or large rocks/boulders.	<b>Good:</b> Visibility and camera height off bottom were adequate.	Medium: Moderate diversity and abundance of organisms with moderate distribution throughout video transect.	<b>Dominant organisms:</b> Corals ( <i>Anthomastus</i> sp. and/or <i>Heteropolypus</i> sp., <i>Acanella</i> <i>arbuscula</i> and unidentified soft corals and gorgonians), sponges ( <i>Asconema</i> sp., and other unidentified sponges),

						and urchins. <b>Other organisms</b> <b>observed:</b> Sea stars, anenomes, eel, eelpout, unidentified fish species, and
						squid.
10	ISECOLD 2- 500	536 m	Hard bottom; gravel/sandy/silty sediment, some medium and/or large rocks/boulders.	<b>Good:</b> Visibility and camera height off bottom were adequate.	Medium: Moderate diversity and abundance of organisms with moderate distribution throughout video transect.	<b>Dominant organisms:</b> Grenadier sp., tunicates, anenomes, and squat lobsters. <b>Other organisms observed:</b> Corals ( <i>Anthomastus</i> sp., sea pens, and unidentified soft corals and gorgonians), sponges ( <i>Asconema</i> sp., and other unidentified sponges), gastropods, crab, eel, bryozoans, skate sp., redfish, and crinoids.
11	HiBio B (DFO- 7)	1,854 m	Hard bottom; gravel/sandy/silty sediment, many medium and/or large rocks/boulders. Sloping environment.	<b>Good:</b> Visibility and camera height off bottom were adequate.	Medium: Moderate diversity and abundance of organisms with moderate distribution throughout video transect.	<b>Dominant organisms:</b> Grenadier sp., crinoids, corals ( <i>Antomastus</i> sp., <i>Acanella</i> <i>arbuscula</i> , sea pens, and other soft corals and gorgonians). <b>Other organisms observed:</b> Anenomes, brittle stars, sponges ( <i>Geodia</i> sp., glass sponges and other unidentified sponges).
12	DFO-1200	1,213 m	Hard bottom; gravel/sandy/silty sediment, very few medium and/or large rocks/boulders.	<b>Poor:</b> Camera height was high off the bottom and into the water column for the majority of the	<b>Low:</b> Overall diversity of organisms moderate however abundances low and sparsely distributed in available bottom video.	<b>Dominant organisms:</b> N/A. <b>Other organisms observed:</b> Anenomes, brittle stars, sponges ( <i>Geodia</i> sp., <i>Asconema</i> sp., glass sponges and other unidentified

				video. Difiiculty finding bottom.		sponges), corals ( <i>Paragorgia</i> <i>arborea</i> and unidentified soft corals) crinoids, squid, blue hake and unidentified fishes.
13	Sponge 4	369 m	Hard bottom; gravel/sandy/silty sediment, many medium and/or large rocks/boulders.	Medium: Difficulty determining bottom, however visibility and camera height off bottom were adequate in portions of the video.	<b>Medium-High:</b> Moderate diversity of organisms with a high abundance and moderate distribution throughout video transect.	<b>Dominant organisms:</b> Sponges ( <i>Geodia</i> sp., <i>Asconema</i> sp., and other unidentified sponges), anenomes. <b>Other organisms</b> <b>observed:</b> Grenadier, crab, crinoids.

#### **Box Coring (Herder)**

Box core samples were collected to characterize the sediment grain size and associated benthic infaunal community along a depth gradient from 500 m to 2500 m water depth. Personnel involved in the collection of box core samples include Erin Herder (DFO, Newfoundland), Margaret Cramm (University of Calgary), Rebecca Evans (Memorial University) and Janet Ferguson-Roberts (Memorial University).

Samples collected by DFO-NL include 200 mL of sediment for grain size characterization and sediment from half of each box core to a depth of 15 cm to characterize the infaunal benthic community. Core-top sediments were collected from the undisturbed top 1-cm surface of each box core and stored at -20°C for stable carbon isotope of amino acids ( $\delta^{13}C_{AA}$ ) by Shaomin Chen at Sherwood's Stable Isotope Biogeochemistry Lab (Dalhousie University). These results will allow an estimation of the relative contribution of phytoplankton and sea ice algae to export production and to characterize the spatial variability of  $\delta^{13}C_{AA}$  signatures of export production in the Labrador Shelf. The Hubert lab at the University of Calgary collected surface sediment from the box core and stored them at -80°C for future DNA extraction of the surface sediment microbial community. Microbial community analysis is intended to support the GENICE\* assessment of hydrocarbon-degrading microbial communities in the Canadian Arctic and sub-Arctic. Additionally, the top 10 cm of the sediment surface was collected and stored at 4°C for microbial germination of thermophilic endospore-forming bacteria which have previously been found in Arctic sediments and may be associated with the deep-to-surface movement of geologic fluids. Fisheries and Oceans (DFO), St. John's, NL collected surface sediment samples on behalf of the Centre for Environmental Genomics Applications for eDNA analysis. Three replicates of approximately 5 g each of undisturbed sediment surface were collected and sediment samples were placed in clean labelled Whirl-pak bags and immediately frozen at -20°C. The Mercier Lab (Memorial University) collected deep sea phyla for opportunistic investigations of reproduction, biodiversity, and feeding ecology. Phyla sampled included poriferans, cnidarians, annelids, molluscs, echinoderms, and bryozoans. Sediment samples collected by the Mercier Lab will be used for stable isotopes, lipids, DNA barcoding, and eDNA analysis.

The box core was lowered to the sea bottom at a rate of 50 m per minute. Once close to the bottom, the rate of descent was slowed to 30 m per minute. Once each boxcore was back on-board the vessel, a photograph was taken of the surface of the box core. Environmental DNA (eDNA) samples were collected first to reduce the chance of contamination and the samples were immediately frozen. The remaining sediment samples were then collected and lastly, half of the box core was collected and retained for biota. This sample was sieved over a 0.5 mesh screen and all organisms were retained. Samples were fixed in 10% formalin for 24 hours before being transferred to 70% ethanol for preservation. A summary of the stations sampled can be found in Table 4.

Station	Date	Depth (m)	Latitude (DD)	Longitude (DD)	Successful/ Unsuccessful
ISECOLD-1-500	25/6/19	583	57.7021558	-59.5283480	Successful
ISECOLD-1-1000	26/6/19	1010	57.7140428	-59.3795900	Successful
ISECOLD-1-1500	26/6/19	1474	57.7202133	-59.0831135	Successful
ISECOLD-1-2000	27/6/19	1981	57.7295857	-58.6936458	Unsuccessful attempt. Cable wire wrapped around boxcore causing non-closure.
ISECOLD-1-2500	28/6/19	2492	57.7406032	-57.8813743	Successful
ISECOLD-2-500	30/6/19	527	58.7742730	-60.0474715	Successful on second attempt.
ISECOLD-2-1000	30/6/19	1038	58.7868608	-59.9300187	Successful
ISECOLD-2-1500	30/6/19	1496	58.8196233	-59.6731710	Successful
ISECOLD-2-2000	29/6/19	1937	58.8468195	-59.3684132	Successful
ISECOLD-2-2500	28/6/19	2395	58.9069095	-58.8513527	Successful
HiBio-B	2/7/19	1914	60.4754512	-60.3748297	Successful

Table 4. Summary of sample stations where samples were collected by box core.

Box core samples were successfully collected at 9 of 10 stations sampled ranging in depths from 500 m to 2500 m (Table 4) along the two ISECOLD transects. Overall, the sediments in the box core samples ranged in consistency from very fine mud to muddy gravel (Figure 7).



## ISECOLD-1-500

ISECOLD-1-1000

ISECOLD-1-1500



**Figure 7.** Photo plate showing box core samples collected along two ISECOLD station transects from shallow (500 m) to deep (2500 m).

ISECOLD-1-500 consisted of fine, sticky mud. Biota observed in this sample included polychaetes and their tubes, small bivalves and formainifera. The sediment at ISECOLD-1-1000 was a similar consistency with the surface of the boxcore housing hydroids and one small brittle star. ISECOLD-1-1500 was much more gravelly and the surface sediments contained a tunicate, encrusting sponge, and hydroids on some of the large rocks. ISECOLD-1-2000 was unsuccessful as the box core did not close due to cable wrapped around the box core. Re-deployment was not possible at this site due to time restrictions. ISECOLD-1-2500 contained small sponge fragments, polychaetes, bryozoan fragments and a brittle star. All residue (gravelly mixture) left after picking organisms was retained for further inspection for biota under a dissecting scope (Figure 8).



Figure 8. Photo plate of biota kept by Mercier Lab from ISECOLD-1.

ISECOLD-2-500 contained many more organisms on the surface of the mud compared to previous box cores and was very gravelly. Biota observed included bryozoans (erect and encrusting), soft corals, other unidentified soft corals, tunicates, sponges, brittle stars, and polychaetes. Nine 500 mL jars of residue (gravelly sand mixture with biota) were collected but only 2 jars were retained for further examination of biota. ISECOLD-2-1000 contained mushroom corals (*Anthomastus* sp. or *Pseudoanthomastus* sp.) and two sea pens (possibly *Kophobelemnon* sp.). Other biota included polychaetes, sponge, and hydroids. ISECOLD-2-1500 was very gravelly and contained hydroids, polychaetes, Buccinidae gastropods, amphipods, sponges and encrusting tunicates. ISECOLD-2-2000 showed evidence of 2-3 species of corals. Fragments of bamboo corals were observed along with an unidentified skeleton of what may be a sleractinian coral, and the sea pen *Distichoptilum* sp. was also observed. Other species observed and retained by Mercier lab include brittle stars, polychaetes and a small sponge fragment. ISECOLD-2-2500 consisted of softer mud to a greater depth compared to 2500 m depth at ISECOLD-1. Organisms observed in this sample included brittle stars, a small sea cucumber or tunicate (to be identified by Mercier Lab), bryozoan fragments and worm tubes (Figure 9).



Unidentified sponge (ISECOLD-2-500)

Distichoptilum sp. and unid. Scleractinian coral (TBD) (ISECOLD-2-2000)

Bamboo coral fragments (ISECOLD-2-2000)

Figure 9. Selection of biota observed along ISECOLD-2 transect.

HiBio-B was the last box core collected. This station was located at approimately 2000 m depth and the sediment was very gravelly. Organisms observed included a stony coral skeleton attached to a small rock, polychaete tubes, sponges, brittle stars and bivalves and gastropods (Figure 10).



Sediments at HiBio-B



Biota found in sediment at HiBio-B

Figure 10. Sediment and biota observed at station HiBio-B.

#### Rock Dredge (de Moura Neves and Vad)

Samples of benthic megafauna were collected for a general assessment of biodiversity, species identification, ground-truthing of drop camera imagery and DNA, and stable isotope analyses. Most samples were kept for further analyses by DFO-NL, except for ATLAS Sponge-3 and Non-Sponge-3 stations, for which samples were kept by the ATLAS team onboard (J. Vad). Subsamples from all stations were also kept by the Mercier lab team aboard (E. Montgomery and J. Ferguson-Roberts).

Samples were collected using a rock dredge (7 mm mesh; Figure 11) at 18 stations, at depths ranging between 422-2399 m (Table 5). Most of the deployments were in "drift" mode, with the ship moving at a maximum speed of 2 knots for 10-20 minutes (Table 5). At one site (Non-Sponge-3), the dredge was deployed in "tow" mode, at a speed of 1 knot for 10 minutes. At most sites, the amount of extra cable length was 120% of the water depth (cable length limitations of the Amundsen's main winch prevented conventional deployments of 2:1 cable to depth ratios across all sites). At one station we used 10%, and this deployment turned out to be unsuccessful. Two additional deployments had 20% plus 100 m of cable (DFO-1200) and 20% plus 50 m of cable (DFO-Ridge-1000, following a previous deployment where a small yielded was obtained at similar depths using only 20% of extra cable. See note about the DFO-1200 deployment under the section *Notes on the dredge deployment* below.

Two deployments were unsuccessful (ISECOLD-1-1500 and DFO-1000), however samples were collected after a second attempt at ISECOLD-1-1500. Once on deck, the dredge was rinsed, and the catch deposited in fish totes (volume capacity: 64 L). Most catches were subsampled due to the large amount of material collected by the dredge, particularly muddy material. Where subsampled, the amount kept ranged between 1/8 and ½ of the catch. The remaining material (i.e. extra material) was also partly or completely sieved and checked for potential specimens of interest.

The material was sieved through a 2 mm mesh and sorted for invertebrates and fish. Retained gravel and larger rocks were weighed and photographed before being discarded. The total catch was photographed and preserved for later species identification and quantification. Only taxa known to the team aboard were readily identified to lower taxonomic levels. Both DFO-NL and ATLAS samples were fixed in either 4% formalin (for morphological identification), 100% ethanol (for DNA analyses), or frozen at -20 °C (DNA/stable isotopes).

Invertebrate/fish diversity and presence varied across stations. Most stations at the ISECOLD transect lines had a muddy substrate, with heavy silt/clay material (to be assessed through grain size analyses from box-core samples). Polychaetes and Foraminifera were the most common organisms found at the ISECOLD-1 transect stations (Figure 12). Diversity at ISECOLD-2 stations seemed higher compared to ISECOLD-1, with some fish, a few coral species and echinoderms not seen in the latter (Figure 14).

HiBio-B station was mainly characterized by a somehow high density of stalked crinoids, a large bryozoan, and a grenadier (Figure 15). Non-Sponge site 3 was characterized by small sponge samples including *Polymastia* sp., *Axinella* sp., *Craniella* sp., and possibly *Mycale* sp. (Figure

14). At Sponge-Site-3 large *Geodia* sp., *Phakellia* sp., *Asconema* sp. sponges, the gorgonian *P. resedaeformis*, and a squat lobster (Munidae sp.) were collected (Figure 15). At DFO-3 the dredge yielded very small amounts of fauna, including some bivalves and the isopod (*Aega* sp.), and some remnants of the previous trawl (e.g. *Primnoa resedaeformis* fragments, Figure 14). DFO-1200 was mainly characterized by Hexactinellid sponges and polychaetes (Figure 14). At DFO-Ridge-1000 some ophiuroids, hexactinellid sponges, bryozoans, soft corals, and polychaete tubes were collected (Figure 14).

#### Notes on the dredge deployment

The rock dredge was deployed at sites with varied substrate types, ranging from muddy to rocky areas, from depths of 500 to >2000 m. Dredge efficiency on the seafloor is therefore difficult to evaluate. At one station (~1500 m), where the amount of extra cable released was only 10% of water depth, the dredge was unsuccessful, and we believe that the amount of cable was not enough for the dredge to touch the seafloor at that depth.

At one site (DFO-1000) the amount of cable was 20% of water depth, and still the deployment was unsuccessful. Variable seafloor relief at that location, and possibly currents, might have had an impact on this deployment. At some sites, water depth was variable across the transect, and the dredge might have been going in both up and down-slope directions. For future deployments, the water depth should be more constantly checked throughout the deployment, so that the amount of cable released can be adjusted accordingly. At the DFO-Non-Sponge-Site-3 (~500 m) we added an extra 1000 m of cable (50% of water depth) during the deployment, and this dredge yielded five totes (64 L) full of soft sediment. Similarly, at DFO-1200 the extra 100 m of cable might have been too much, as another 5 totes of soft sediment were collected. The amount of extra cable to be released should therefore consider bottom type, considering time and space limitations on onboard post-processing of the samples collected.

Furthermore, the weight supported by the winch cable should be noted once the dredge reaches bottom, so that these values can be used as reference for the amount of material being collected during the deployment. Because the weight of the cable will be greater at deeper sites, the weight should be compared across areas of similar water depth.



Figure 11. Rock dredge deployment during leg 1b of the CCGS Amundsen 2019 expedition.



**Figure 12.** Example of benthic samples collected using the rock dredge on transect ISECOLD-1 during Leg 1B of the CCGS *Amundsen* 2019 expedition. A) ISECOLD-1-1000, B) ISECOLD-1-1500, C) ISECOLD-1-2000, D) ISECOLD-1-2200.



**Figure 13.** Example of benthic samples collected using the rock dredge on transect ISECOLD-2 during Leg 1B of the CCGS *Amundsen* 2019 expedition. A) ISECOLD-2-500, B) ISECOLD-2-1000, C) ISECOLD-2-1500, D) ISECOLD-2-2000, E) ISECOLD-2-2500.



**Figure 14.** Example of benthic samples collected using the rock dredge on DFO and ATLAS stations during leg 1b of the CCGS *Amundsen* 2019 expedition. A) HiBio-B, B) Non-Sponge-3, C) Sponge-3 (note large *Geodia* sp. sponge -inset, and the gorgonian *Primnoa resedaeformis*), D) DFO-3, E) DFO-1200, F) DFO-Ridge-1000.

Station Name	Station type	Start Lat	Start Long	End Lat	End Long	Logged bottom depth (m)	Time at bottom (min)	Length of cable out (m)	Max vessel speed (knots)	Comments
ISECOLD 1-500	DFO Full	57.710	-59.531	57.698	-59.525	596	10	100	2	
ISECOLD 1-1000	DFO Full	57.714	-59.378	NA	NA	1018	10	1200	2	
ISECOLD 1-1500	DFO Full	57.722	-59.085	57.705	-59.077	1470	10	1630	2	Unsuccessful
ISECOLD 1-1500	DFO Full	57.723	-59.083	57.703	-59.070	1867	10	1835**	2	Redeployment
ISECOLD 1-2000	DFO Full	57.730	-58.695	57.712	-58.640	2007*	10	2247	2	
ISECOLD 1-2200	Rock Dredge Only	NA	NA	57.704	-58.552	2172*	20	2714	2	
ISECOLD 2-2500	DFO Full	58.909	-58.849	58.893	-58.855	2390	20	2713	2	
ISECOLD 2-2000	DFO Full	58.846	-59.369	58.828	-59.378	1924	20	2309	2	
ISECOLD 2-1500	DFO Full	58.820	-59.674	58.819	-59.695	1497	20	1797	2	
ISECOLD 2-1000	DFO Full	58.788	-59.931	58.770	-59.931	1038	20	1250	2	
ISECOLD 2-500	DFO Full	58.773	-60.045	58.759	-60.050	566	20	600	2	
Non-Sponge Site 3	Mooring/ATLAS site	59.379	-60.272	59.370	-60.288	552	10	1500	1	
HiBio-B	DFO Full	60.474	-60.375	60.488	-60.391	1830*	20	2300	2	
Sponge Site 3	Mooring/ATLAS site	60.469	-61.285	60.482	-61.298	404	20	507	2	Small catch
DFO-3 (1200)	DFO Benthic	60.469	-61.104	60.485	-61.104	1157	20	1391	2	
DFO-1200	DFO Benthic	60.451	-61.030	60.445	-61.009	1202	20	NA	2	
DFO-Ridge 1000	DFO Benthic	60.453	-61.134	60.457	-61.164	934	20	NA	2	
DFO-1000	DFO Benthic	60.466	-61.171	60.475	-61.217	936	10	1193	2	Unsuccessful

**Table 5**. Rock dredge deployment stations and parameters during leg 1b of the CCGS Amundsen 2019 expedition.

\*Depth communicated from bridge during deployment. \*\*Estimation (20% extra cable).

Station	Totes collected	Kept	Tote depth (cm)	Lrg rocks (kg)	Gravel (kg)	Main organisms	Mercier Lab
ISECOLD-1- 500	1	1/4	NA	NA	0.65	Foraminifera, polychaetes, scaphopods, sea urchins.	
ISECOLD -1- 1000	1 and 1/2	1/2	NA	NA	0.43	Mainly Foraminifera and pebbles.	1 sipunculid worm.
ISECOLD -1- 1500	1	1	8.7	6.6	NA	Sponges, hydroids, zoanthids, mushroom corals (Anthomastus sp.?), cerianthid, soft coral (Duva florida), polychaetes, brittle stars.	3 brittle stars, 1 Stegophiura sp., 1 hydroid, 1 "stony coral"
ISECOLD -1- 2000	1		27.5	19	7.5	Lantern fish and Brittlemouths, hydroids, polychaetes, crustaceans, brittle stars.	1 brittle star, 1 hydroid. One crustacean from extra sieve.

**Table 6.** Rock dredge deployments and main preliminary findings during Leg 1B of the 2019 CCGS Amundsen expedition.

Station	Totes collected	Kept	Tote depth (cm)	Lrg rocks (kg)	Gravel (kg)	Main organisms	Mercier Lab
ISECOLD -1- 2200	2	2 and 1/2	T2 = 11, T3=7.5, T5=14.5-7 (11)	6	5.9	Samples in 4% formalin, 1 sponge in ethanol 100% (2ml vials, in freezer). Extra material also kept: broken purple urchin, broken crustaceans, fish, crinoid?, polychaetes.	2 sea cucumbers, and 1 priapulid.
ISECOLD -2- 2500	1/2	1/2	14	5.5	2.2	Sponges, fish, polychaetes, sea cucumbers, ophiuroids, sea stars.	2 sea stars, 1 brown fat worm, one ophiuroid disk, and 1 unidentified potential sea cucumber/ascidian
ISECOLD -2- 2000	3/4	3/4	8.7	1.02	1.03	Zoanthids, the gorgonian <i>Acanella</i> <i>arbuscula</i> (tissue-less), sea pen (cf. <i>Protoptilum carpenteri</i> ), scaphopods, ophiuroids, polychaetes, lantern fish (Myctophydae).	1 large Scaphopoda, 4 brittle stars
ISECOLD -2- 1500	1/2	1/2	9	11.2	5.32	Sponges, hydroids, <i>Pseudodrifa</i> sp., stoloniferous octocoral, sea pen sp., gastropod, scaphopod, polychaetes, crustaceans, ophiuroids.	
ISECOLD -2- 1000	1/2	1/2	12	23.75	2.77	Duva florida, Heteropolypus sp., sea pen Kophobelemnon sp., Pennatula aculeata, bivalves, gastropods, quitons, scaphopods, polychaetes, pycnogonids, crustaceans.	

Station	Totes collected	Kept	Tote depth (cm)	Lrg rocks (kg)	Gravel (kg)	Main organisms	Mercier Lab
ISECOLD -2- 500	1/2	1/2	7	2.3	2.59	Sponges, mushroom corals ( <i>Anthomastus</i> sp.),	
DFO-Non- sponge-3- Lander	5	1/4	12.8	26.94	12.7	Sponges, hydrocorals, mushroom corals ( <i>Anthomastus</i> sp.), <i>Duva florida</i> , bivalves, gastropods, polychaetes, priapulids, sea stars, brittle stars, bryozoans, potential kelp material.	
HiBio-B	3	1/2	9.33	6.31	8.33	Acanella arbuscula (intact colonies), mushroom corals (e.g.Anthomastus), sea pens (Anthoptilum sp.), octopus (Bathypolypus), quiton, crustaceans, stalked crinoids (e.g. potentially Hyocrinus sp.), ophiuroids, grenadier, ?rockling, bryozoans.	
Sponge site 3	1	1	NA	NA	NA	One large <i>Geodia</i> sp., fragments of the gorgonian <i>Primnoa resedaeformis</i> , soft corals, mushroom corals, <i>Asconema</i> sp. and other smaller sponges, bryozoans, crustaceans, including one squat lobster (Munidae), ophiuroids, hydroids, <i>Anthoptilum</i> sp. sea pens.	Primnoa fragments.
DFO-3	1/3	1/3	NA	7.8	NA	Gravel and <i>Primnoa</i> fragments (possibly contamination from previous deployment). <i>Aega</i> sp., small sponges, lantern fish, zoanthids, crustaceans, bivalves.	
DFO-1200	5	1	17	4.6	4.5	<i>Primnoa</i> fragments (possibly contamination from previous deployment). Mushroom corals, brittle stars, sponges, gastropods, bivalves and shell fragments, lantern fish, polychaetes and tubes, scaphopods.	<i>Primnoa</i> fragments, and sponges.

Station	Totes collected	Kept	Tote depth (cm)	Lrg rocks (kg)	Gravel (kg)	Main organisms	Mercier Lab
DFO-Ridge- 1000	1	1	14	27.82	21	Soft corals ( <i>Duva florida</i> ), mushroom corals ( <i>Anthomastus</i> sp.), <i>Funiculina</i> <i>quadrangularis</i> sea pen.	
DFO-1000	unsuccessful	NA	NA	NA	NA	NA	NA

Phylum	Lower taxa	Species	ISECOLD-1-500	ISECOLD -1-1000	ISECOLD -1-1500	ISECOLD -1-2000	ISECOLD -1-2200	ISECOLD -2-500	ISECOLD -2-1000	ISECOLD -2-1500	ISECOLD -2-2000	ISECOLD -2-2500	DFO-NS-3-Lander	HiBio-B	Sponge site 3	DFO-3	DFO-1200	DFO-Ridge-1000
Foraminifera	Undetermined	Foraminifera sp.																
		Axinella sp.?																-
		Craniella sp.																
		Encrusting yellow sponge																
		Encrusting sponge																
	Demospongiae	Ficiform sponge																
		Geodia sp.																
		Mycale sp.?																
		Polymastia sp.																
		Sponge? with single spike																
		Unidentified sponge spp.																
	Hexactinellida	Asconema sp.																
	Hydrozoa	Hydroids																
		Hydrocoral																
		Jellyfish																
	Zoantharia	Zoanthid																
	Actiniaria	Large sea anemone																
Cnidaria		Sea anemone sp.																
	Ceriantharia	Cerianthid																
	Scleractinia	Small stony coral																
		Acanella arbuscula																
	Octocorallia	Anthomastus sp.																
		Anthoptilum sp.																

**Table 7**. List of taxa encountered in benthic samples collected using the rock dredge during leg 1b of CCGS Amundsen 2019 expedition.Gray cells denote presence while black cells denote the presence of the taxon in the extra sieved material.

			500	1000	1500	2000	2200	500	1000	1500	2000	2500	nder		3		(	000
			D-1-	-1-0	-1-0	0 -1-	0 -1-	D -2-	0 -2-	-2-0	) -2-	) -2-	-3-La	io-B	e site	<b>D-3</b>	-1200	lge-1
			COL	OLL	OLL	OLL	OLL	COL	OLL	OLL	OLL	OLL	-SN-	HiB	onge	DF	OFO-	)-Ric
			SE(	EC	EC	EC	EC	SEC	EC	EC	EC	EC	Ŏ		$\operatorname{Sp}$		П	ЪГС
Phylum	Lower taxa	Species	Ι	IS	IS	IS	IS	I	IS	IS	IS	IS	D					Ц
		Anthothela sp.?																
		Duva florida																
		Drifa glomerata																
		Funiculina																
		quadrangularis																
		Heteropolypus sol																
		Kophobelemnon sp.																
		Pennatula aculeata																
		Primnoa resedaeformis																
		Protoptilum carpenteri																
		Pseudodrifa sp.																
		Stloniferous octocoral																
		Sea pen sp.																
		Yellow polyps																
		Unknown octocoral																
	Bivalvia	Astarte sp.																
		Bivalve sp.																
		Musculus sp.																
	Cephalopoda	Bathypolypus sp.																
	Gastropoda	Unidentified shiny																
Mollusca		gastropod																
		Gastropoda sp.																
		Tooth-like mollusc											_					
	Scaphopoda	Siphonodentalium lobatum?																
		Scaphopoda sp. 1																
	Polyplacophora	Polyplacophora sp. 1(hairy)																
Phylum	Lower taya	Species	ISECOLD-1-500	ISECOLD -1-1000	ISECOLD -1-1500	ISECOLD -1-2000	ISECOLD -1-2200	ISECOLD -2-500	ISECOLD -2-1000	ISECOLD -2-1500	ISECOLD -2-2000	ISECOLD -2-2500	DFO-NS-3-Lander	HiBio-B	Sponge site 3	DF0-3	DFO-1200	DFO-Ridge-1000
---------------	--------------	--------------------------------------	---------------	-----------------	-----------------	-----------------	-----------------	----------------	-----------------	-----------------	-----------------	-----------------	-----------------	---------	---------------	-------	----------	----------------
1 Hylum	Lower taxa	Polyplacophora sp. 2																
		Polyplacophora sp. 2																
	Shell hash	Shell hash																
		Polychaeta																
		Polychaete soft tubes																
Annelida	Polychaeta	Polychaete sandy tubes																
		Polychaete "eyelashes"																
		Thin transparent tubes																
Sipuncula	Undetermined	Sipunculid																
Priapulida	Undetermined	Priapulid																
Р	Pycnogonida	Pycnogonid																
		<i>Aega</i> sp.																
		Amphipod																
		Barnacle sp. 1																
		Barnacle sp. 2																
		Caprellid																
		Eualus sp.																
Arthropoda	Crustacea	Unidentified large red crustacean																
		Unidentified red decapod																
		Unidentified shrimp																
		Unidentified orange crustacean																
		Squat lobster																
		Unidentified pink crustacean																
Echinodermata	Asteroidea	Ctenodiscus sp.																

Phylum	Lower taxa	Species	ISECOLD-1-500	ISECOLD -1-1000	ISECOLD -1-1500	ISECOLD -1-2000	ISECOLD -1-2200	ISECOLD -2-500	ISECOLD -2-1000	ISECOLD -2-1500	ISECOLD -2-2000	ISECOLD -2-2500	DFO-NS-3-Lander	HiBio-B	Sponge site 3	DFO-3	DFO-1200	DFO-Ridge-1000
		Henricia sp.																
		Poraniomorpha (P.) hispida																
		Unidentified white sea																
		star (gray disk)																
	Cuin ai la a	Hyocrinus sp.?																
	Crinoidea	Unidentified white																
		stalked crinoid																
		Undentified red sea																
	Echinoidea	Unidentified numble sea																
	urchin																	
		Unidentified red sea			-													
	Holothumoidae	cucumber																
	Holothuroidea	Unidentified white sea cucumber																
		Amphiura sundevalli?																
		Amphiura sp.?																
		Ophiopleura sp.?																
		Unidentified small white ophiuroid																
	Ophiuroidea	Unidentified small																
		orange ophiuroid																
		Unidentified large beige ophiuroid																
		Stegophiura sp.																
		Unidentified ophiuroid																
		Grenadier																
Chordata	Pisces	Myctophydae																
		(Benthosema glaciale)																

Phylum	Lower taxa	Species	ISECOLD-1-500	ISECOLD -1-1000	ISECOLD -1-1500	ISECOLD -1-2000	ISECOLD -1-2200	ISECOLD -2-500	ISECOLD -2-1000	ISECOLD -2-1500	ISECOLD -2-2000	ISECOLD -2-2500	DFO-NS-3-Lander	HiBio-B	Sponge site 3	DFO-3	DFO-1200	DFO-Ridge-1000
		Gomostomatidea (Brittlemouths)																
		Rockling? Look for Four-bear																
		Skate egg case																
Bruozoa	Undetermined	Bryozoan																
Bryozoa	Undetermined	Large ?bryozoan																
Chaetognatha	Undetermined	Chaetognatha																
Other	Undetermined	Kelp material?																
Undetermined		Unknown invertebrate																

### Pelagic Fish and Plankton (Chawarski, DeZutter, McAllister)

The mesopelagic fish and mesozooplankton community of the northern Labrador Sea is poorly described. Forming dense mid-water aggregations across the global oceans known as deep sound scattering layers (DSLs), mesopelagic organisms are hypothesized to be responsible for the largest biomass aggregations of animal life on the planet and are crucial to the energy flow of the deep ocean (Proud et al 2017). In the Labrador Sea, myctophids (lanternfishes) and invertebrate zooplanktivores feed predominantly on calanoid copepods, but their effect on primary and secondary surface grazing zooplankton mortality is still unclear. While some studies attribute most of the biomass in the DSL to myctophids, the true diversity and abundance of taxa as well as foraging behavior in this region is poorly described. In the deep-water basins of the North Atlantic, seasonal differences in the diurnal vertical migration of these organisms has been observed (Anderson et al 2005). In the Arctic, the diel behavior of mesopelagic organism was associated with scattering layers originating from the Atlantic water mass (Gjøsæter et al 2017). Furthermore, differential diurnal vertical migration behavior among and within taxa in the mesopelagic zone has been observed and may be attributed to different adaptations to light conditions (Knutsen et al 2017). As an example, due to low metabolic demand of myctophids, only a portion of the population may be feeding at once, and stomach content analysis revealed some fish were feeding only every other day (Pepin 2013). On the other hand, other pelagic fish, such as Arctic cod, display vertical segregation and feeding strategies based on age and size class. In this study component, we aim to describe the behavior, spatial variation, and biodiversity of mesopelagic fishes and macroinvertebrates of the Labrador Sea.

Our understanding of the biodiversity of midwater scattering may be biased by traditional net sampling techniques which introduce selectivity bias based on avoidance behavior and size. In many cases, gelatinous zooplankton and fast-swimming mesozooplankton avoid capture and thus may be underestimated. Therefore, in this study we combine high resolution acoustic imaging (Wideband Autonomous Transceiver - WBAT), zooplankton imaging (Underwater Visioning Profiler - UVP5) with traditional midwater (Isaac-Kidd Midwater Trawl –IKMT), depth-stratified plankton net sampling (Hydrobios plankton net), and eDNA (described above) to better understand the biodiversity and forage dynamics of the DSL in the Labrador Sea. By closing this knowledge gap, we can elucidate surface to deep ocean pelagic food webs along the continental slope and their relationships to changing oceanographic conditions in the North Atlantic.

Deployments of these complimentary methods were co-located at all ISECOLD stations, except when technical problems limited the deployment of UVP5 to only the last three stations (Table 7). In addition to biological measurements described above, physical oceanographic parameters and light attenuation from a prototype sensor were measured at each station. Methods for each sampling approach are described below.

Station	Sampling date	Multinet	IKMT	eDNA	WBAT	UVP 5	LOL
ISECOLD_1_500	25-June-2019	Х	Х	Х	Х		Х
ISECOLD_1_1000	25-June-2019	Х	Х	Х	Х		Х
ISECOLD_1_1500	26-June-2019	Х	Х	Х	Х		Х
ISECOLD_1_2000	27-June-2019	Х	Х	Х	Х		Х
ISECOLD_1_2500	28-June-2019	Х	2X	Х	Х		Х
ISECOLD_2_2500	29-June-2019	Х	2X	Х	Х		Х
ISECOLD_2_2000	29-June-2019	Х	2X	Х	Х		Х
ISECOLD_2_1500	30-June-2019	Х	Х	Х	Х	Х	Х
ISECOLD_2_1000	30-June-2019	Х	Х	Х	Х	Х	Х
ISECOLD_2_500	30-June-2019	Х	2X	Х	X	X	Х

**Table 7.** Pelagic sampling activities related to the ISECOLD project. X's indicate the use of a particular sampling method. 2X indicates the method was deployed twice at a single station.

### Wideband Autonomous Transceiver (WBAT)

Complementary to the traditionally used hull-mounted EK60 scientific echosounder, the broadband echosounder, an autonomous EK80 platform, offers wide bandwidth frequency measurements of acoustic backscatter. While the hull-mounted EK60 operates at three discrete frequencies (38-, 120-, and 200- kHz), the WBAT can be outfitted with two split-beam transducers. For this study, ES38-18DK-split and ES333-7CDK-split transducers were operated at 35-45 kHz and 320-420 kHhz bandwidths, respectively. In combination, both transducers provide frequency response curves and high-resolution target detection of fish and zooplankton.

In contrast to 2018 ISECOLD operations, this year the WBAT was mounted to the CTD-rosette and deployed in autonomous mode during each station cast. For each deployment, the WBAT was programmed for timed deployments of each transducer to coincide with downcast and upcasts. During the downcast, the 333 kHz transducer pinged to a range of 50 m every 0.5 seconds with 2.048 ms pulse length. During the upcast the 38 kHz transducer pinged to a range of 200 m every 0.5 seconds with a 2.048 ms pulse length. This sequence was chosen to maximize the likelihood of capturing true vertical distribution of targets in case of avoidance behavior in response to the rosette during operations.

At each ISECOLD sampling station the WBAT was deployed to a maximum depth of 1500 m due to limitations of the pressure casing. At stations deeper than 1500 m, a separate rosette cast was performed before removal of the WBAT and transduers. The rationale for this change in methods is three-fold: First, by mounting the transducers horizontally, we can ensonify a larger portion of the water column and therefore measure discrete targets many times. Secondly, combining WBAT measurements in both space and time with physical measurements of the water columns can help strengthen our understanding of relationships between biological and physical structuring of the pelagic zone. Finally, by mounting the WBAT system to the rosette, we saved at least one operational hour per station and advanced future methods of including the WBAT a multi-sensor package.

## Observations:

Vertical casts of the WBAT on the rosette were successfully timed to capture full vertical profiles of the upper 1500 m of the water column (where depths allowed). At first glance of the first deep station, ISECOLD-1-1500, the 333 kHz transducer produces integrated scattering peaks at expected depths: subsurface cholorphyll max, epipelagic grazing zone, mesopelagic or deep-scattering layer, and some individual large targets (most likely *P. Periphylla*) spread throughout (Figure 15). 38 kHz data is not presented as it will require filtering of rosette events such as stops and fired bottles during the upcast to truly capture vertical distribution. Upon visual inspection, the 38 kHz produces backscattering peaks for swim-bladdered fish in the deep-scattering layer (400-600m). At present, backscattering values are uncalibrated and an accurate calibration of the WBAT and transducers will take place in a test tank at the Marine Institute in October 2019. After calibrated TS values are achieved, echo-counting will be done to calculate densities of sound-scattering organisms. Further analysis may include an investigation of patterns in frequency response curves along the measured bandwidths.

For all organisms the echo strength (TS) will depend both on the size and the acoustic properties of the organisms. Animals with equivalent size (radius) much smaller than the wavelength will give very weak echoes (Rayleigh scattering). A weak target is therefore likely to be a small organism, the nominal wavelength at 320 kHz is ~4.7 mm, at 420 kHz it is ~3.6 mm. For the strong echoes it is not

possible at present to say whether echo strengths relates to acoustic properties or size, but by comparing the vertical WBAT profiles with the hull mounted 38 kHz data, one can usually distinguish the depths where organisms with gas-inclusions (e.g. swim bladders) are present, as the scattering at 38 kHz is usually dominated by organisms with gas inclusions. During the cruise a strong scattering layer was evident in the depth range from ~300 to ~600 m, scattering from this layer is dominated by various fishes with gas-inclusions (e.g. Myctophids, Gonostomatids, and other mesopelagic fishes). This pattern included nightly vertical migrations to the surface.



**Figure 15**. ES333-7CDK Mean volume backscatter during downcast of the CTD-rosette at ISECOLD-1-1500. Range is equivalent to distance from transducer face. Solid horizontal lines are noise artefects resulting from mild interference from the LADCP, also mounted on the rosette.

#### Light Sensor

For mesopelagic organisms light has been identified as the major driver of global-scale vertical distribution (Aksnes et al. 2017, Kaartvedt et al. 2019), and estimation or measurement of light at relevant depths is probably needed to fully understand both vertical distributions and migrations. Projects studying the mesopelagic region therefore have a need for sensors more sensitive than the ones currently available to us.

During the cruise, a prototype light sensor (LOw Light integrating sensor, LOL; Figure 16) developed by colleauges at the Institute for Marine Research (IMR) in Norway, was tested out and deployed on all CTD casts. The rationale for development of the sensor is 2-fold: first, mesopelagic projects have a need for an interim sensor solution to assess mesopelagic light levels until a permanent, CTD attached sensor solution is devised. Secondly, projects deploying stationary equipment (e.g. acoustic landers, ADCP's) at depth also have a need to assess light levels at depth, and the suitability of the planned CTD attached sensor for long term battery operation is uncertain.

PAR sensors are by now standard on many research vessel CTDs, but these sensors are geared towards the needs of the groups studying primary production. These sensors rarely have sensitivity enough to reach beyond the very upper parts of the ocean, and as their spectral responsivity by definition spans at least the region 400 - 700 nm, it is problematic to use the results from these sensors to estimate light levels at depth: a PAR sensor with low sensitivity will always overestimate the attenuation coefficients relevant to calculating light at depth. While these sensors may be sufficient for mapping light levels where there is net primary production, light also has a profound effect on higher trophic levels. It is perhaps the major proximate driver for the distribution of animals ranging in size from zooplankton to micronekton, and it directly affects trophic interactions all the way up the food-chain. *In situ* and surface light levels relevant to animals are the most influential environmental parameters that are typically not measured. Furthermore it has far greater implications for pelagic ecology than all but one of the hydrographical parameters we currently do measure, the exception being chlorophyll *a*.



Figure 16. LOw Light integrating sensor (LOL) mounted on the rosette frame.

#### Underwater Vision Profiler 5 (UVP5)

The UVP5 is an imaging platform that captures images of both living and non-living particles in the water column (Figure 17). It can provide a wide range of measurements including particulate size, number, and density. The platform is integrated with an image classification program known as Ecotaxa. Using a machine learning image classification algorithm, it can identify individuals by taxa, such as copepoda and metazoa, and in some cases down to the species level.

Technical difficulties limited the use of the UVP5 to only 3 rosette sampling stations. At each of these stations, as the rosette was lowered to its rinsing depth, the pressure sensor on the UVP5 initiates an image capture sequence. The UVP5 continuously captures images as particles moved through its light field on the downcast. A live-read out of particulate density is displayed on the rosette control screen, plotted alongside other variables such as temperature and salinity. Data was captured and downloaded with each rosette cast. Metadata was entered at the end of each day into the zooprocess program and raw files were processed for future input into Ecotaxa.

Data will be sent to an experienced Ecotaxa user and reviewed for misclassification. A portion of the data will be used to train future classification models. All post-processing will be conducted by Marc Picheral, at IFREMR Villefranche. Classified data will be delivered to Julek Chawarski for further vertical and spatial analysis.



**Figure 17**. Examples of UVP5 images, processed using Zooprocess software and ready for image classification in Ecotaxa.

#### Multi-net plankton sampler (Hydrobios)

Plankton community characterization was done at various depth zones with a Hydrobios multi-net plankton sampler. The net is equipped with nine  $200\mu m$  mesh nets (opening  $0.5m^2$ ) allowing for depth specific sampling of the water column (Table 8). The Hydrobios is also equipped with a CTD to record water column properties while collecting biological samples.

The net is deployed vertically from 1000m (or 15m off the bottom in depths shallower than 1000m) to the surface. The nets open and close one by one as the pressure decreases while the net is going up in the water column. The depth at which the different nets open and close is programmed before deployment. Once retrieved, the zooplankton samples (Figure 18) were preserved in 10% formalin solution a n d stored for further taxonomic identification at Laval University.

Station	Sampling date	Sampling time (UTC)	Net#	Sampling depth max	Sampling depth min
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	1	965/945	800
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	2	800	600
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	3	600	400
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	4	400	250
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	5	250	100
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	6	100	75
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	7	75	50
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	8	50	25
ISECOLD-1-1000/2-1000	2019-06-26	6:46:30 AM	9	25	2
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	1	1 000	800
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	2	800	600
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	3	600	400
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	4	400	250
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	5	250	100
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	6	100	75
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	7	75	50
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	8	50	25
ISECOLD-1-1500/1-200/1-2500/2-2500/2-2000/2-1500	2019-06-26	9:07:31 PM	9	25	2
ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	1	500/565	400
ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	2	400	300
ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	3	300	200

**Table 8.** Hydrobios sampling date, time and maximum and minimum sampling depth for each net.

ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	4	200	150
ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	5	150	100
ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	6	100	75
ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	7	75	50
ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	8	50	25
ISECOLD-2-500/1-500	2019-07-01	3:13:14 AM	9	25	2



**Figure 18**. An example of the depth-specific samples collected by the Hydrobios net, with vial 1 containing the deepest samples and vial 9 containing the shallowest samples.

#### Isaac-Kidd Midwater Trawl (IKMT)

The IKMT (Figure 19) was deployed to capture pelagic juvenile and adult fish and macro-zooplankton. The net is rectangular in shape with a 9m<sup>2</sup> mouth aperture and mesh size of 11 mm in the first section, 5 mm in the last section. The net was lowered at a target depth (Table 8) which was determined by the echosounder EK-60 signal and towed at that depth for 15-30 minutes at a speed between 2.5 -3 knots. A novel technique was applied during this cruise to capture vertically migrating species during the brief hours of darkness. At three stations a 'double-dip' method was employed. First, the net was deployed from the surface to depths between 75 and 100 m. The net was towed for 10 minutes and hauled back to the surface. Without removal of the frame, the cod end was collected by hand and emptied. The net was briefly rinsed and redeployed to the deep-scattering layer depth of ~500 m, where it was towed for 15-20 minutes. Collections were sorted in the laboratory by species, counted and weighed.

In the laboratory, each species was photographed and whole specimen were stored in ethanol. For large catches of single species like myctophids, subsamples of 50 were subsampled for length and frozen whole. For nearly all specimens, samples were frozen for stable isotope analysis and tissue samples were preserved for genetic identification and marker design for metagenomic sequencing of environmental DNA (eDNA).

Compound specific isotope analysis of amino acids (CSIA-AA) will be conducted on animal samples taken from both the IKMT and Hydrobios multi-net. Tissue samples were taken from fish and larger

invertebrates, while entire bodies of smaller invertebrates and zooplankton were kept, and all samples were frozen for later analysis. Carbon (d13C) and nitrogen (d15N) isotopic signatures will be measured through continuous-flow gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) with existing instrumentation in the Department of Earth Sciences at Dalhousie University.

Nitrogen isotopes will be measured to look at signatures of 'trophic amino acids' (ie, glutamic acid) which undergo significant isotopic frationation between diet and consumer. These will be compared to signatures of 'source amino acids' (ie. phenylalanine) which show minimal isotopic fractionation, and will be used as a proxy for the isotopic signatures of primary producers at the base of the food web. Together, the trophic and source amino acid signatures will be used to estimate consumer trophic positions, while accounting for differences in baseline isotopic signatures.

Carbon isotopes will also be measured from consumer tissues to examine for patterns of essential amino acids which are passed from resource to consumer virtually unmodified. These patterns are particularly diagnostic of the phylogenetic identity of source carbon, and provide an 'isotopic fingerprint' of the type of primary producers at the base of the food web. The isotopic fingerprints will be used to infer the types of primary producers that support the food web through comparison with unique amino acid fingerprints of known primary producers.

Table 9.	IKMT	sampling	date	time a	and s	ampli	ng denti	h.
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Station	Sampling date	Sampling time	cast number	Sampling depth
ISECOLD-1-500	2019-06-26	5:20:00 PM	1	400
ISECOLD-1-1000	2019-06-26	8:19:20 AM	1	360
ISECOLD-1-1500	2019-06-26	10:39:00 PM	1	440
ISECOLD-1-2000	2019-06-27	1:56:56 PM	1	500
ISECOLD-1-2500	2019-06-28	5:17:50 AM	1	100
ISECOLD-1-2500	2019-06-28	5:48:39 AM	2	480
ISECOLD-2-1000	2019-06-30	6:54:12 PM	1	470
ISECOLD-2-1500	2019-06-30	9:13:00 AM	1	450
ISECOLD-2-2000	2019-06-29	9:18:00 PM	1	80
ISECOLD-2-2000	2019-06-29	9:49:00 PM	2	430-350
ISECOLD-2-2500	2019-06-29	3:41:00 AM	1	100
ISECOLD-2-2500	2019-06-29	4:17:00 AM	2	536
ISECOLD-2-500	2019-07-01	4:12:00 AM	1	75
ISECOLD-2-500	2019-07-01	4:37:06 AM	2	480

In total, roughly 12 fish and 25 invertebrate species were captured in the net. Lanternfish (Myctophidae) were present at each station and trawl with the exception of the control event, where the aforementioned 'double-dip' was employed during daytime. Some large species of lanternfish were captured in low numbers along with the occasional larger predators such as barracudina (*Arctozenus risso*) and dragonfish (*Stomias boa*). There was a high occurrence of bristlemouths (*Gonostomatidae*) although numbers were typically low (< 5 per tow). Among the invertebrates, arrow worms (*Chaetognathae*), jellyfish, and arthropods such as shrimp and krill were the most common. Nearly every tow contained gammarid amphipods of the genus *Themisto* and some unidentified, possibly rare species were captured.



Figure 19. IKMT being deployed off the Amundsen, Leg 2C, 2018.

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# Benthic and Pelagic Community Characterization from eDNA from Water Samples (Young and Chawarski)

Seawater was collected at all ISECOLD full transect stations as well as two stations that were revisited during mooring recoveries using a CTD-Rosette water sampling system comprised of twenty-four 12L Niskin bottles. A variety of scientific analyses were conducted on these samples, and with the exception of environmental DNA, these activities (e.g. nutrient analyses) are covered in the water sampling section.

## eDNA Analysis of Water

Environmental DNA is an emerging scientific tool that uses DNA fragments shed from animals into the water column to characterize biotic community composition. The technique has promise as a non-invasive approach that is complimentary to other conventional methods, particularly in the deep sea where specimens are very difficult to collect. To characterize benthic and pelagic faunal communities water samples were collected from the surface, 250m, 500m, 750m, 1000m and the ocean bottom, where station depths allowed. These depths were selected to match other sampling activities (hydroacoustics, bottom camera, box core, plankton nets and IKMT trawls) that could be used to validate/compare results.

Prior to the CTD-Rosette deployment, the inside and upper and lower lids of the Niskin bottles were sprayed first with a DNA removal solution and then rinsed with distilled water. The bottles were also closed up after they were cleaned until deployment to prevent contamination.

Once the vessel reached the selected sampling station, the CTD-Rosette was lowered from the vessel on a winch system and Niskins were closed at programmed depths to collect a water sample. The CTD-Rosette was brought back on board the vessel and eDNA sampling took place prior to other water collection activities to prevent accidental contamination by other study team members. Once again, latex gloves were used to collect three replicate samples from each sample depth in pre-labeled sterile 2 L Whirl-pak bags.

Water filtration was subsequently completed onboard the vessel in a dedicated labratory immediately following the collection of the water samples. Prior to each station, the work area was decontaminated with DRS, a DNA removal solution, and between each sample replicate gloves were changed. A new, pre-labelled, Sterivex filter was used for each replicate sample and attached to one end of the filtration tube, which was changed between sample depths. At the other end of the tube was a pipette (changed for each sample replicate) which was also placed in the sample bag to pump the water through the filter (See Figure 20 for set-up). Approximately 1.5 L of water was pumped through each filter and the pre-labelled Sterivex filter for each sample was removed once filtration was complete and stored in a pre-labelled Whirl-pak bag. The 3 replicates were then placed in a ziploc bag and stored in the fridge until all filtration was complete and were then moved to the -80°C freezer.

In total, 12 stations were sampled for eDNA water sample collection (Table 10). The frozen Sterivex filters will be sent to Centre for Environmental Genomic Applications for analysis and the resulting data will augment and be compared to pelagic and benthic community characterization data collected with conventional methods.



Figure 20. eDNA sample filtration setup.

Station	Date	Time	Latitude	Longitude	Max depth (m)	Sample depth (m)
ISECOLD- 1-500	2019-06-25	8:50	57.705050	-59.526940	600	surface, 250, 500, bottom
ISECOLD- 1-1000	2019-06-25	21:35	57.708790	-59.377520	1000	Surface, 250, 500, 750, bottom
ISECOLD- 1-1500	2019-06-26	9:45	57.717970	-59.087130	1500	Surface, 250, 500, 750, 1000, bottom
ISECOLD- 1-2000	2019-06-27	0:05	57.729370	-58.695580	2000	Surface, 250, 500, 750, 1000, bottom
ISECOLD- 1-2500	2019-06-28	3:05	57.740650	-57.883870	2500	Surface, 250, 500, 750, 1000, bottom
ISECOLD- 2-2500	2019-06-29	2:19	58.907280	-58.846410	2500	Surface, 250, 500, 750, 1000, bottom
ISECOLD- 2-2000	2019-06-29	11:17	58.846680	-59.368320	2000	Surface, 250, 500, 750, 1000, bottom
ISECOLD- 2-1500	2019-06-30	2:04	58.819940	-59.673430	1500	Surface, 250, 500, 750, 1000, bottom
ISECOLD- 2-1000	2019-06-30	12:05	58.786760	-59.929590	1000	Surface, 250, 500, 750, bottom
ISECOLD- 2-500	2019-06-30	20:45	58.774330	-60.046970	500	Surface, 250, Bottom
HiBio-A	2019-07-02	16:30	60.4677955	-61.1460023	1000	Surface, Bottom
DFO-1	2019-07-02	20:00	60.4690913	-61.2903132	500	Surface, Bottom

**Table 10.** List of Sampling Stations for eDNA Water Sampling for Leg 1b of 2019 Amundsen

 Expedition.

#### Seabird and Marine Mammal Surveys

#### **Seabirds**

Seabirds are an integral part of marine ecosystems; their distribution is influenced by biological, chemical and physical oceanography. Changes in seabird distribution can therefore be an indicator of oceanographic variability. It is critically important to monitor seabird abundance and distribution patterns in the arctic, in order to monitor changes that are happening in response to the rapid environmental changes induced by global warming. Collecting data in the remote regions of the arctic and subarctic are extremely expensive and all opportunities to fill data gaps are very important. Seabird data collected since 1980 show population trends for significant seabird colonies in the Canadian arctic (Gaston et al. 2009), including Thick-billed Murres and Northern Fulmars. Thick-billed Murre populations are apparently stable, but this species relies heavily on the sea ice-dependent Arctic Cod during the breeding season. Changes in sea ice and therefore prey availability may become a serious issue for this species in the future, potentially effecting population size and distribution throughout the eastern North Atlantic. Northern Fulmars have been in steady decline over the last decade. Data on breeding colonies and at-sea distribution is required to understand this decline.

Seabird surveys provide important information on pelagic seabird distribution throughout the year, including patterns of dispersal from breeding areas, migration routes and wintering areas. Over time, these data show not only patterns of dispersal, but also trends in species abundance, diversity and distribution. This information helps inform decisions regarding protecting sensitive marine areas, environmental assessment of proposed development projects, and appropriate response to catastrophic events (e.g. oil spills).

Surveys were conducted using a standardized fixed-width survey area over a 90<sup>0</sup> scanning arc as per the Environment Canada Seabirds at Sea (ECSAS) protocols (Gjerdrum et al. 2012). These protocols were developed in a manner that is compatible with methods used by north Atlantic European countries. Surveys are conducted by the Canadian Wildlife Service (CWS), Department of Environment and Conservation Canada to address management and conservation responsibilities under the Migratory Bird Convention Act (MBC Act 1996). The Canadian Wildlife Service places seabird observers on multiple ships of opportunity throughout the year. Data are consolidated, summarized and analyzed from a central database maintained by the Atlantic Region office in Dartmouth, Nova Scotia. The data are open and shared with other departments and jurisdictions.

Last year, the ISECOLD sampling program was conducted during Leg 2C, (July 24-August 16 2018). This period represents the late- and post-breeding period for arctic seabirds, when they are starting to distribute away from the colonies and toward winter feeding areas. This year, the sampling program occurred during the height of breeding season (June 23-July 5, 2019), when bird distribution is limited to foraging areas closer to the breeding colonies. Distribution between years will be interesting to compare.

A summary of the distance, effort and species observed is provided in Table 11. More detail with distribution maps will be provided by CWS in a timely manner upon return.

#### Marine Mammals

Marine Mammal surveys are generally conducted using protocols involving multiple observers, covering a 180<sup>0</sup> arc at an infinite distance. There was neither the manpower nor expertise onboard to fulfill these requirements. However, marine mammal data were collected opportunistically; primarily during seabird survey efforts. Marine mammal observations made outside of seabird surveys were added to the database as "incidental observations". All marine mammals seen by the seabird observer or other personnel were recorded in the ECSAS database. Species identity was either confirmed by the seabird observer or given a more general designation (e.g. "unidentified whale") prior to data entry. Coverage was incomplete and likely underestimates marine mammal species composition and abundance. A summary is provided in Table 11.

Seabirds			
English	Latin	Percentage	Number seen
Northern Fulmar	Fulmarus glacialis	61.9	459
Thick-billed Murre	Uria Iomvia	27.9	207
Black-legged Kittiwake	Rissa tridactyla	1.8	13
Northern Gannet	Morus bassanus	1.5	11
Genus: Murres	Uria	1.5	11
Atlantic Puffin	Fratercula arctica	1.5	11
Red Phalarope	Phalaropus fulicaria	1.2	9
Herring Gull	Larus argentatus	0.7	5
Wilson's Storm Petrel	Oceanites oceanicus	0.4	3
Pomarine Jaeger	Stercorarius pomarinus	0.4	3
Great Black-backed Gull	Larus marinus	0.4	3
Arctic Tern	Sterna paradisaea	0.4	3
Black Guillemot	Cepphus grylle	0.3	2
Dovekie	Alle alle	0.1	1
Marine Mammals			
Long-finned Pilot Whale (Blackfish)	Globicephala melas	48.4	15
Order: Whales and Dolphins	Cetacea	6.5	2

Table 11.	Seabird and marine	mammal summary	for Amundsen	Leg 1b:	June 23-July4.	Surveys
represent 2	2302 minutes of obse	ervation over 783.5	kilometers			

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#### Moving Vessel Profiler (MVP) surveys (Dukhovskoy)

The Labrador Sea plays a critical role in the Global Ocean Thermohaline Circulation producing dense water masses that feed deep water currents driven by the Atlantic Meridional Overturning Circulation (Yashayaev et al., 2007). The importance of the Labrador Sea is related to the fact that several water masses of different thermohaline (and geochemical) properties carried by the mesoscale currents meet in the basin. Warm and salty Atlantic water is carried by the Irminger and West Greenland Currents, the polar water leaving the Arctic Ocean via the Fram Strait is transported by the East Greenland Current that merges with the West Greenland Current. The polar water from the Canadian Arctic Archipelago feeds the Baffin Current continuing as the Labrador Current on the southwestern shelf of the Labrador Sea. The outflow from Hudson Bay contributes ~50% of the freshwater transport of the Labrador Current (Straneo and Saucier, 2008).

The role of the Labrador Sea in the regional and global climate is more apparent in light of the present climate changes amplified in the polar regions. Accelerating Greenland melt yields additional ~250 km<sup>3</sup>/yr of surplus freshwater adding to the mean freshwater flux of 900 km<sup>3</sup>/yr from the Greenland Ice Sheet. Greenland melt water is mixed into the boundary currents flowing along the coast of Greenland (Bamber et al., 2018; Dukhovskoy et al., 2019). The substantial part of the Greenland freshwater flux is carried to the Labrador Sea, mainly with the West Greenland Current branch that turns west following the continental shelf break south of Davis Strait. Increasing storage of fresh water in the Arctic Ocean (Haine et al., 2015) will eventually be fluxed into the subpolar North Atlantic (Proshutinsky et al., 2015). One of the routes of the freshwater fluxes from the Arctic Ocean is through the Canadian Arctic Archipelago and Baffin Bay to the Labrador Sea. In order to understand present and predict the nearfuture changes caused by rapid increase of the freshwater content in the Arctic climate system, it is important to study the freshwater pathways in the Arctic and subpolar basins. The monitoring of the characteristics of the ocean currents provides information about changes in their dynamics and thermohaline structure, which is used to evaluate freshwater transport from the polar regions. Planned station locations of the Amundsen Expedition Leg 1B provides a great opportunity to conduct detailed hydrographic observations of the oceanic fronts on the southwestern Labrador Sea shelf using an MVP. The MVP is equipped with salinity and temperature sensors, pressure gauge, and a fluorometer.

MVP surveys were conducted during transits between the stations. The instrument was towed several hundred meters behind the vessel in order to reduce the impact of the vessel wake on the measurements. The cruising speed of the vessel was kept at ~8 kt. During the measurements, the instrument descended (downcast) down to 300 m (or 15 m off the bottom) and then ascended to the depth of ~15-20 m. Only downcast observations are considered in future analyses. The instrument provides measurements of the hydrographic fields at a high vertical (~0.2 m in vertical) and relatively high horizontal (~1-2 km between the downcasts) resolution in the upper 300 m.

It would be challenging to accurately observe such a narrow oceanic front using other instruments without a-priori knowledge of the front location. Locating the front based on surface temperature and salinity measurements could also be difficult as the front only has distinct temperature manifestations on the surface and the surface temperature front does not always coincide with that below the surface. As such, multiple conventionaln point sample measurements (like CTD) would be needed to capture the subsurface front structure. The MVP observations provide valuable information about high-resolution structure of the upper ocean on the Labrador shelf. This information will be used to improve our knowledge about dynamics of the boundary currents in the region. The data are also useful for assessing numerical simulations of the Labrador Sea in terms of representation of the ocean fronts and structure of the boundary currents in the area. Finally, these data may provide some high resolution

biophysical nature that will be useful for explaining variability in the biological data collected on this cruise.



**Figure 21.** Maps of the study region showing ISECOLD Transect 1 and ISECOLD Transect 2 with the MVP data points (downcast locations).



**Figure 22.** Vertical distribution of Temperature (top) and Salinity (bottom) along ISECOLD Transect 1 from the MVP observations.



**Figure 23.** Vertical distribution of Temperature and Salinity along ISECOLD Transect 2 from the MVP observations.



**Figure 24.** Vertical distribution of Temperature and Salinity in the Davis Strait area from the MVP observations.

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### Sea Ice Sampling (Chen)

Sea ice algae was collected for compound specific isotope analysis of amino acids (CSIA-AA). The samples for CSIA-AA will contribute to the development of a new biomarker to quantify the relative contribution of sea ice algae and phytoplankton to export production. The results of CSIA-AA will be compared with those of nutrients and NO<sub>3</sub> isotope analyses (see Rosette sampling section), which links the sea ice algae productivity to nutrient and nitrogen (N) dynamics in the environment.

Sea ice pieces were collected directly from the surface in a cage (Figure 25) and melted at room temperature in dark. About 500 mL melted ice was filtered through 3  $\mu$ m and 0.2  $\mu$ m polycarbonate filters each time. Filters were stored at -20°C.



Figure 25. Sea ice sampling in the cage with the help from a crew member. © Alex Ingle

## **ATLAS Lander Recovery (Tulloch)**

Two sea bed landers were deployed as part of the ATLAS project in August 2018. The deployed instruments included a sediment trap, Aquadopp current meter, flourometer and an Aanderaa system comprising turbidity, oxygen, pressure, conductivity and current sensors. The landers were deployed in the Labrador Sea, one each on a sponge and non-sponge location.

The location for the landers had been previously selected from video data collected during a 2016 CCGS *Amundsen* expedition. Although locations had been agreed and published in the "2018 Amundsen Expedition Plan" and later in an updated version of the "2018 CCGS Amundsen Expedition ROV dive plans", the precise location of the sites was planned to be finalised by viewing predeployment ROV footage. Although this was possible for site 1 (Non-sponge Site 3), due to a mechanical issue with the ROV it was not possible for the second site (Sponge site 3) and therefore the 2016 footage was reviewed and a location selected from that.

#### Lander Recoveries

The weather was appropriate to recover both landers, with good visibility and calm seas. The Non-Sponge Site lander was recovered at 8:35 local time on Monday July 1st (-59 22.8924N 060 16.6939W) in 554 m of water (Figure 26). For this recovery, the vessel's helicopter was deployed to enable Alex Ingles (ATLAS videographer) to capture outreach footage. The Sponge Site lander was recovered at 18:00 local time on Tuesday July 2nd ( 60 28.1008N, 61 17.2645W) in 410 m of water. Despite some challenges communicating with the landers using the ATLAS deck box, both units were recovered successfully. Upon retrieval, larval settlement plates were removed, placed in ethanol and provided to MUN researchers (Emy Montgomery) for further processing. Other instruments were removed, cleaned and packed away for transport.



Figure 26. Lander recovery using the CCGS Amundsen's RHIB at the Non-Sponge Site.

## Long Term Deployments of Environmental Sensors (Michaud/Meredyke)

### Recovery Operations Summary

Deck box communications were successful with the acoustic releases of both moorings deployed in 2018 (Table 12) but only HiBioC-18 (Figure 27) was successfully recovered. The other mooring (HiBioB-18; Figure 28) did not surface after it was released, nor was it observed in the water column using multibeam. It was therefore believed to have broken free from the mooring weights prior to the arrival of the Amundsen, leaving the acoustic releases on the bottom.

All instruments from the successfully recovered mooring were removed and downloaded. The sediment trap collected the maximum possible number of sediment trap samples and were processed and stored for further analysis. The remaining data will be reviewed upon return to shore.

	Table 12. Mooring	Deployment Summary	y 2018 from the CC	CGS Amundsen
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	Mooring			Latitude	Longitude	Depth	Status
Leg	ID	Latitude	Longitude	( <b>DD</b> )	( <b>DD</b> )	( <b>m</b> )	
2c	HiBioC-18	60°27.7893' N	61°09.564' W	60.46316	-61.1594	1020	Recovered
2c	HiBioB-18	60° 28.356' N	60°22.5408' W	60.4726	-60.3757	1983	Not Recovered

## 2019 Deployments

HiBioC-19 (Figure 29; Table 13) was deployed with a new AZFP (Marine Institute), new EdgeTech Port LF releases (Marine Institute) and other equipment such as an AMAR hydrophone (DFO), a sediment trap (Amundsen Science) and larval settlement plates (MUN).

HiBioA-19 (Figure 30; Table 13) was to be deployed using equipment from the recovered HiBioB-18 mooring. The unfortunate loss of this equipment during the HiBioB-18 deployment required that HiBioA-19 was deployed without a current profiler and an AMAR.

Both 2019 moorings were deployed with satellite beacons (Marine Institute) and also with single point current meters with auxiliary oceanographic sensors (Amundsen Science).

**Table 13**. 2019 HiBio Project Mooring Deployments from the CCGS Amundsen.

Leg	Mooring ID	Latitude	Longitude	Latitude (DD)	Longitude (DD)	Depth (m)
1b	HiBioC-19	60°27.843' N	61°09.469' W	60.46405	-61.1578	1025
1b	HiBioA-19	60°28.2738' N	60° 16.1043' W	60.471216	-61.2684	516



Instrument Target Depth

Component Details



Figure 27. Mooring schematic for HiBioB-18.

## HiBioC-18 Lat: 60° 27.7998' N Long: 61° 09.543' W

Site Depth : 1020 m Mooring Length : 182 m



Figure 28. Mooring schematic for HiBioC-18.

#### HiBioC-19 Lat: 60° 27.7998' N Long: 61° 09.543' W

Instrument Target Depth

Component Details

Site Depth : 1017 m Mooring Length : 469 m



Figure 29. Mooring schematic for HiBioC-19.

# HiBioA-19 Lat: 60° 28.254' N Long: 61° 15.620' W

Site Depth : 516 m Mooring Length : 167 m



Figure 30. Mooring schematic for HiBioA-19.

### Multi-beam Habitat Mapping (O'Dell, Michaud)

The mapping of the seabed is an important objective of the ISECOLD program. Multi Beam Echo Sounding (MBSE) data was continuously acquired on a Kongsberg EM302 during all activities (Figure 31) within the Labrador Sea study area, except in cases where operational requirements required that it be turned off (i.e. when HIPAP was in use for drop camera activities). When in transit, routes were selected strategically in order to complement existing multibeam coverage. Extensive sea ice (Frobisher Bay) and rough seas affected the quality of the MBES for portions of the expedition. Overall, however, the multibeam worked well and generated new data in previously uncharted areas. Post-processing of these data will continue into 2020, after which these data will be shared with the Canadian Hydrographic Service (CHS) to update marine charts and will be available to guide future scientific activities.


**Figure 31:** Multibeam Transit Lines from in the Labrador Sea. Leg 1b data collection is indicated by multi-colored tracks whereas previous mapping tracks are displayed in blue.