

Analysis of underwater benthic images obtained from ROV ROPOS Cruise in the Cape Breton Trough in 2017

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ABSTRACT

Joseph, V., Dinn, C., Méthé, D. and Côté, G. 2024. Analysis of underwater benthic images obtained from ROV ROPOS Cruise in the Cape Breton Trough in 2017. Can. Manuscr. Rep. Fish. Aquat. Sci. 3296: vii + 39 p. <https://doi.org/10.60825/gpmw-8p67>

A collaborative scientific expedition between Fisheries and Oceans Canada (DFO) and Oceana Canada was undertaken in August 2017 to explore the benthic ecosystems in the Cape Breton Trough (CBT), an area within the Gulf of St. Lawrence that is not well-known as trawl sampling is difficult. The CBT, which lies within the boundaries of the Western Cape Breton Ecologically and Biologically Significant Area (EBSA), was explored using a remotely operated underwater vehicle, ROPOS (Remotely Operated Platform for Ocean Science). Benthic images from underwater video recorded along transects were annotated to characterize species and substrate type. Sediment and water samples were collected for biogeochemical analysis. Overall, the objectives were to describe communities of epibenthic species, collect samples, and identify potential habitat sites for the Atlantic wolffish (*Anarhichas lupus*) which is currently listed as a species of special concern in the Species at Risk Act (SARA) Public Registry. The key findings were describing taxa density along four transects, the identification of 13 sponge taxa from sampled material, the observation of areas with dense sea anemone aggregations, and the identification of habitats suitable for wolffish, although no individuals were seen during the mission. This work increases our knowledge of the benthic fauna and communities of the Cape Breton Trough area.

RÉSUMÉ

Joseph, V., Dinn, C., Méthé, D. and Côté, G. 2024. Analysis of underwater benthic images obtained from ROV ROPOS Cruise in the Cape Breton Trough in 2017. Can. Manuscr. Rep. Fish. Aquat. Sci. 3296: vii + 39 p. <https://doi.org/10.60825/gpmw-8p67>

Une expédition scientifique collaborative entre Pêches et Océans Canada (MPO) et Oceana Canada a été entreprise en août 2017 pour explorer les écosystèmes benthiques de la zone de la cuvette du Cap-Breton (CCB) dans le golfe du Saint-Laurent, une zone peu caractérisée dû à la difficulté d'obtenir des échantillons par chalutage. La CCB, qui se situe dans les limites de la zone d'importance écologique et biologique (ZIEB) de l'ouest du Cap-Breton, a été explorée à l'aide d'un véhicule sous-marin télécommandé ROPOS (« Remotely Operated Platform for Ocean Science »). Les images benthiques obtenues par vidéos sous-marine le long des transects ont été annotées pour caractériser les espèces et le type de substrat. Des échantillons de sédiments et d'eau ont été prélevés à des fins d'analyse biogéochimique. Dans l'ensemble, les objectifs étaient de décrire les communautés d'espèces épibenthiques, obtenir des échantillons et d'identifier des sites d'habitats potentiels du loup atlantique (*Anarhichas lupus*), qui est actuellement inscrit sur la liste des espèces préoccupantes du registre public de la Loi sur les espèces en péril (LEP). Les principaux résultats ont été la description de la densité des taxons le long de quatre transects, l'identification de 13 taxons d'éponges à partir du matériel échantillonné, l'observation de zones présentant des agrégations denses d'anémones de mer et l'identification d'habitats propices au loup de mer, bien qu'aucun individu n'ait été vu au cours de la mission. Ce travail a augmenté nos connaissances sur la faune et les communautés benthiques de la région de la cuvette du Cap-Breton.

INTRODUCTION

The Cape Breton Trough (CBT) lies within the boundaries of the Western Cape Breton Ecologically and Biologically Significant Area (EBSA) and is recognized as an area of importance for primary and secondary production, an important migration corridor for several fish species and an area of high demersal fish diversity and biomass (DFO 2007). The CBT is also known for the aggregation and high abundance of benthic invertebrate species and feeding grounds for several marine mammal species (Savenkoff et al. 2007).

Sites within the CBT are assessed annually by the stratified random sampling DFO multi-species September trawl survey in the southern Gulf (Ricard and Swain 2018) and the fixed station Snow Crab trawl survey (Wade et al. 2018; Hébert et al. 2021), but some areas are inaccessible to trawling due to bottom type and slope, therefore some areas of the CBT have remained unexplored or incompletely studied. A collaborative scientific expedition between Fisheries and Oceans Canada (DFO) and Oceana Canada, a non-profit organization dedicated to protection of the oceans, was carried out in 2017 to explore the CBT. The expedition also surveyed two other sites in the western Gulf of St. Lawrence, as summarized in the cruise report by (Faille et al. 2019). The main research goals for the CBT dive sites were to do exploratory work and obtain preliminary baseline information of the study area, specifically to:

1. Describe biodiversity and density of the epibenthic communities through quantitative analysis of imagery.
2. Provide qualitative data on the distribution and density of wolffish and their potential habitat sites, a species listed as special concern in the Species at Risk Act Public Registry, if present.
3. Collect preliminary data on biogeochemical conditions of sediment and water.

MATERIALS AND METHODS

CAPE BRETON TROUGH STUDY AREA

The CBT study area lies on the eastern margin of the Magdalen Shallows in the southern Gulf of St. Lawrence (Figure 1). The study area is characterized by deep valleys reaching the Laurentian Channel at depths of ~200 m and shallow areas (60–100 m) on its southern boundary. More details about the bathymetry and oceanography of the area are described in (Coomber et al. 2021).

The CBT area was surveyed from August 28–29, 2017 during a cruise aboard the CCGS *Martha L. Black* (Faille et al. 2019). Video imagery and samples were collected using ROPOS (Remotely Operated Platform for Ocean Science; www.ROPOS.com). ROPOS is a remotely operated vehicle (ROV) capable of operating at depths of up to 5,000 m (Figure 2). It is owned and operated by the Canadian Scientific Submersible Facility (CSSF, North Saanich, BC, Canada). During the survey, ROPOS was equipped with 2 Shilling Robotic TITAN manipulators with the dexterity and accuracy to efficiently

collect biological samples (live organisms, sediment cores, water), 2 High Definition (2 MP resolution, 1920 x 1080, 30 FPS) underwater color zoom video cameras: Mini Zeus (downward facing), and Zeus (forward facing, Insite Pacific Inc. San Diego, CA) with lasers 10 cm apart for calibration, a conductivity, temperature, and depth (CTD) profiler, sediment corer, and NISKIN bottle samplers. ROPOS was run in exploratory mode for opportunistic sampling at 1 m above the seafloor. A total of 6 transects were carried out (Figure 3):

- 2 transects in the shallow area (CBT-7 [Dive R2022] and CBT-9 [Dive R2023]);
- 2 transects along the steep vertical slope (CBT-4 and CBT-5 [Dive R2024]); and
- 2 transects in the deepest area (CBT-2, CBT-3 [Dive R2025]).

Each transect measured approximately 1 km in length, and when deemed appropriate ROPOS remained operational between transects providing additional coverage of the study area.

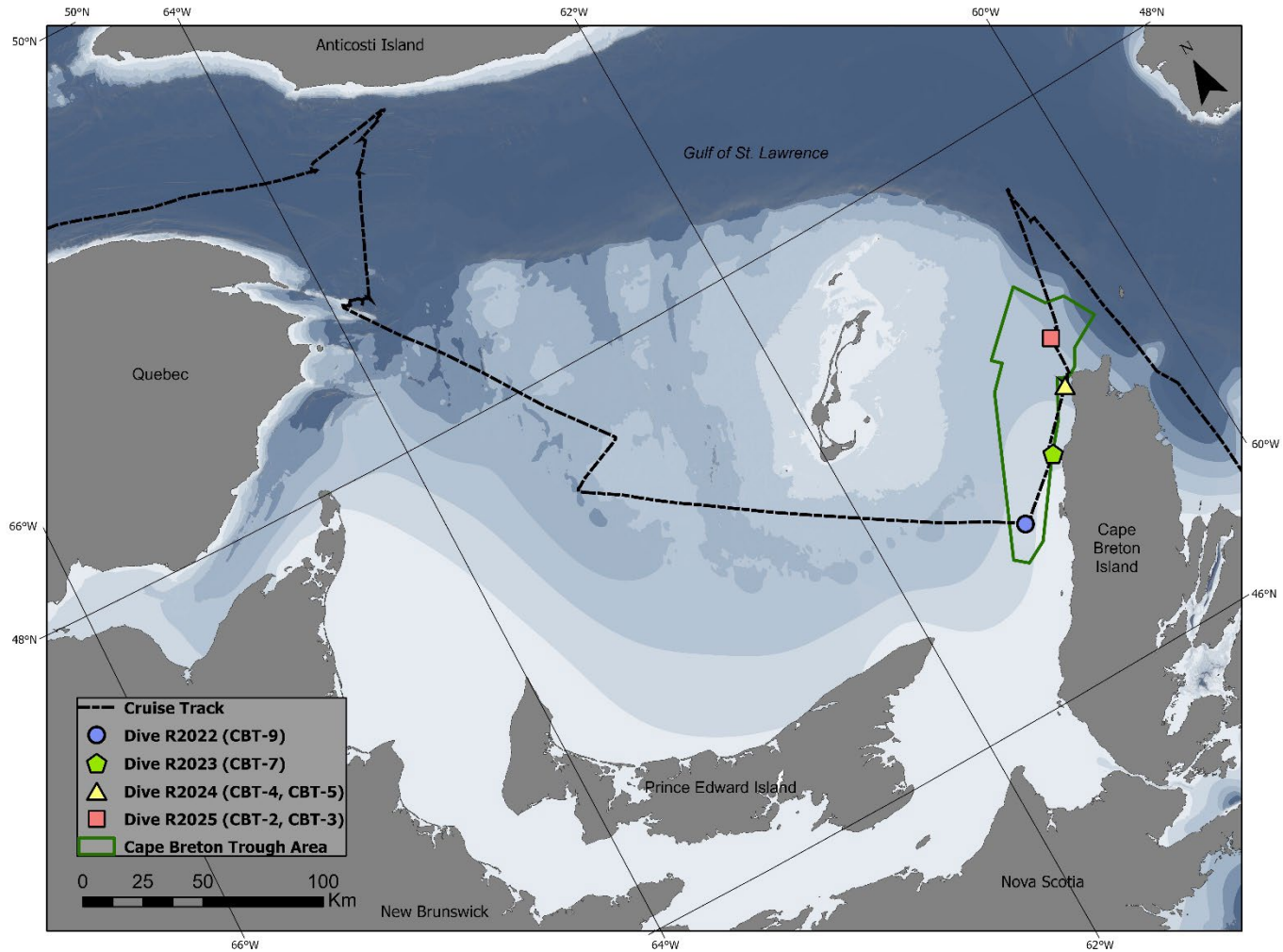


Figure 1. Location of the Cape Breton Trough study area within the Southern Gulf of St. Lawrence (solid line polygon) with the ROPOS dive locations.

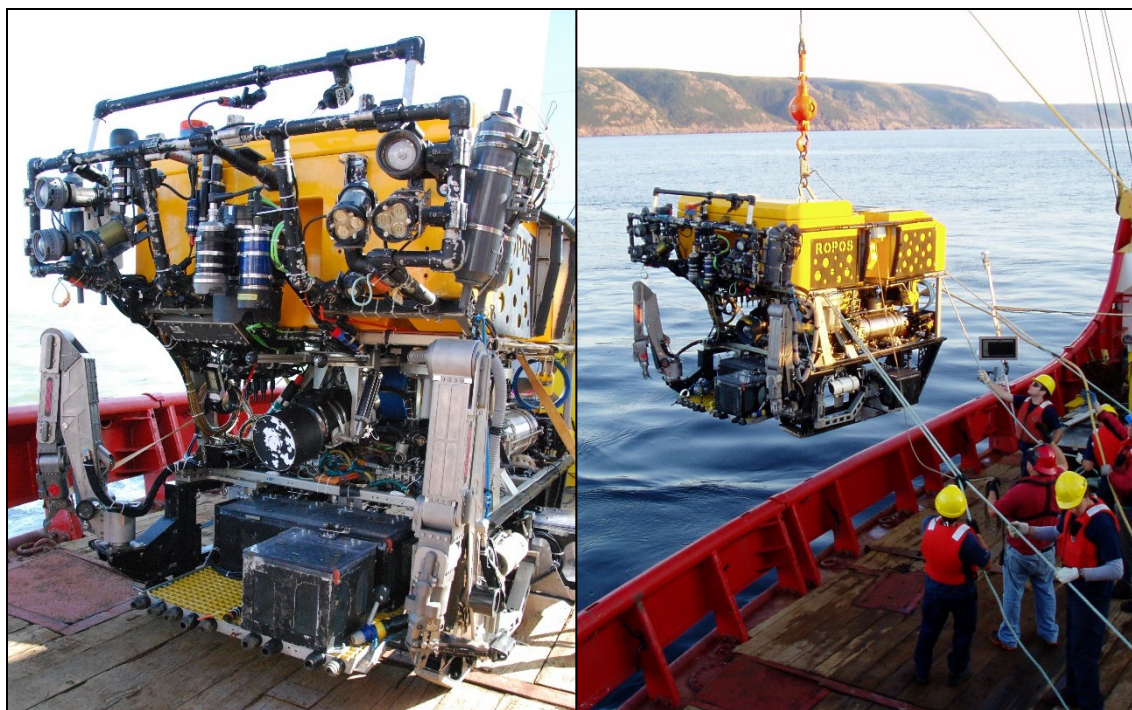


Figure 2. Images of ROPOS during deployment from the CCGS *Martha L. Black* (Remotely Operated Platform for Ocean Science; www.ropos.com). Photo Credit: DFO.

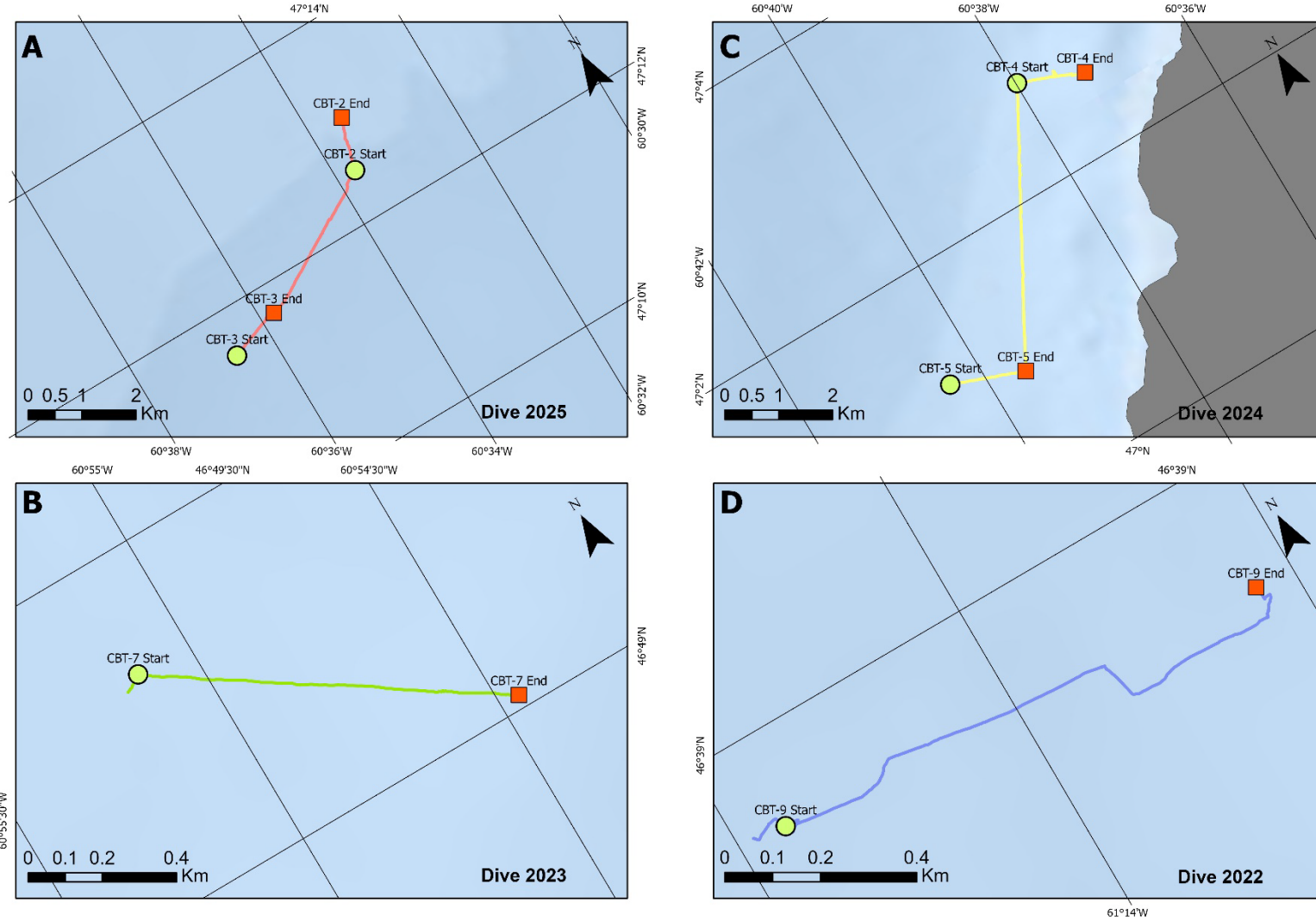


Figure 3. Transects performed during ROV dives with ROPOS in the Cape Breton Trough. A. Dive R2022, Transect CBT-9. B. Dive R2023, Transect CBT-7. C. Dive R2024, Transects CBT-4 and CBT-5. D. Dive R2025, Transect CBT-2. Transect start points denoted by circles and end points denoted by squares, dive path is represented by solid line.

VIDEO ANALYSIS

QUANTITATIVE ANALYSIS

A quantitative dataset, used for taxa and bottom type identification, was created using the video collected by the downward facing Mini Zeus camera during transects CBT-2, CBT-4, CBT-5, and CBT-7. Image analysis was adapted from a technique described in Larocque and Thorne (2012). Annotation databases were created in Microsoft Access using VideoMiner Software, version 3.0.8.0 developed by DFO (Available from: downloads.crmltd.ca/f/Crm1335/). To limit interpretation errors, screen capture image analyses were carried out by the same observer. The quantitative dataset consisted of taxon identifications from screen capture images, georeferenced and taken every 30 ± 5 m of each transect, while dominant and sub-dominant substrate type, topography and slope were noted from continuous video footage. All animals that were visible on the surface of the sediment, including mobile and living organisms closely associated with the bottom such as shrimp and fish, were identified at the lowest possible taxonomic level and counted, except for animals that could not be treated as individuals due to sprawling habit or density (e.g. encrusting sponges). For animals that grow in non-discrete formations such as some sponges, a colony count was performed for taxa richness purposes only. Photo guides from Gulf of St. Lawrence survey captures (Nozères et al. 2010a, 2014) were used to help identify taxa, following methods from and analyzed concurrently with Côté et al. (2021) and Thorne et al. (2022), although underwater ID guides for the region are not widely available. Sponges were identified visually according to morphotypes (Côté et al. 2021; Thorne et al. 2022). Individuals that were too small or which did not fit one of the eight morphotypes were counted as “Unknown Porifera”. Non-identifiable fauna were noted as “Animalia”. Taxa density was standardized as number of individuals per m^2 with the use of the lasers and ImageJ software v.1.4.3.67 (Schneider et al. 2012). Bryozoa, Hydrozoa, Ophiuroidea, and Porifera_7 (encrusting sponges) were recorded as presence/absence and not as individuals per m^2 . Taxon richness and average density (# ind./ m^2) were calculated for each transect. Presence/absence data was used for calculating taxon richness.

For each screen capture, the dominant (greater than 50%) and subdominant substrates were noted according to five categories of particle size: mud sand and fine sediments (< 4 mm), pebbles (4–64 mm), rocks (64–256 mm), boulders (> 256 mm), and bedrock. A percentage class was then assigned to the dominant and subdominant substrates based on their approximate percentage coverage in the image (> 75%, 51-75%, 26–50%, 5–25% and < 5%).

STATISTICAL ANALYSES

Analysis of the quantitative data from transects CBT-2, CBT-4, CBT-5 and CBT-7 was carried out with the multivariate software package PRIMER v.7.0 (Clarke and Gorley 2015) to investigate taxon assemblages. Taxon density (ind./ m^2) was square root transformed to reduce the importance of dominant taxa and make allowance for the less abundant taxa (Clarke and Gorley 2015). Bray-Curtis similarities were calculated and the resulting matrix was used for unconstrained non-metric multidimensional analysis

(nMDS), which made it possible to project the groupings on two axes and to visualize the differences between the groupings in the form of distances. To visualize the drivers of the difference in taxa or morphotypes between transects, a constrained ordination Canonical Analysis of Principal coordinates (CAP) was performed to reveal broad patterns across the data (Anderson and Willis 2003). To test if there were statistical differences between community assemblages in the four transects, Bray-Curtis similarities based on ranks were used in the analysis of similarities (ANOSIM). This statistical analysis is analogous to an analysis of variance (ANOVA) and makes it possible to compare the similarity between the replicates of the groupings (analyzed images). Subsequently, SIMPER (Similarity Percentage) analysis was applied to compare the contribution of each taxon to the mean of the similarities within groups and dissimilarity between groups. The taxa or types contributing the most to the similarities of each assemblage were determined.

QUALITATIVE ANALYSIS

Presence and/or potential habitat for the Atlantic wolffish was annotated in transects CBT-2, CBT-3, CBT-4 and CBT-5 and during the transit between CBT-4 and 5. Ideal habitat types for wolffish include holes, caves, and rock shelters that can accommodate a fish of at least 30cm (Larocque et al. 2010).

Qualitative datasets created from the video recording of the Zeus forward facing camera were used to evaluate dense sea anemone cover and associated fauna located between transects CBT-4 and CBT-5. Lasers were not operational during the transit, so percent cover of taxa was assessed rather than calculating density based on known area. Percent cover observations, taken from 11 selected screen capture images, were approximately 30 m apart.

SAMPLE COLLECTION

WATER SAMPLES

NISKIN bottles attached to ROPOS were used to collect water samples. Samples were taken at three depths (~1 m from the surface, ~25 m from the surface, ~1 m from the bottom) at the end of transects CBT-2 and CBT-9. An additional sample was taken at the end of transect CBT-3 (~1 m from the bottom).

A one litre sample bottle was rinsed twice with ~100 mL of water taken directly from the flexible drawing tube that extended from the NISKIN spigot to the bottom of the sample bottle. The sample bottle was filled and left to overflow by a full volume prior to inserting the glass stopper. Once in the mobile laboratory, the stopper was removed and approximately 5 mL of water was removed with a plastic syringe, and 100 μ L of mercuric chloride (HgCl_2) solution added to stabilize the sample. The stopper was dried and a streak of Apiezon grease was applied around its circumference. To form a perfect seal, the stopper was pressed and rotated by a full turn and secured using a rubber band and a plastic hose clamp. The bottle was inverted to distribute the mercuric chloride and stored at room temperature for a week prior to pH analysis. Water samples

were analyzed at the Maurice Lamontagne Institute laboratories using the method described by (Mucci et al. 2011).

SEDIMENT SAMPLES

Sediment samples were taken using core tubes maneuvered by the manipulator arms of ROPOS at the end of transects CBT-2 (duplicate), CBT-3, CBT-5, CBT-7 and CBT-9. No sediment samples were taken in CBT-4 because it was too rocky. Sediment core tubes were removed from ROPOS and brought into the mobile laboratory. For particle size analysis, samples with particles > 2 mm (gravel) were left to dry for 24h at 40°C prior to weighing (dry weight). Samples were then sieved through 16 different sieve sizes (2–26.5 mm). The analysis was done through a service contract to l'Institut des sciences de la mer de Rimouski (ISMER) at the Université du Québec à Rimouski (UQAR).

BIOLOGICAL SAMPLES

Biological samples were taken opportunistically throughout and between transects and were placed in the ROPOS bio-boxes with the aid of the suction apparatus or the ROPOS manipulator arms.

Samples were removed from the ROPOS bio-boxes and moved to the mobile laboratory for tagging. For sponges, subsamples were taken of each morphologically distinct area (i.e., ectosome, choanosome) and preserved in 95% non-denatured ethanol for spicule and genetic analyses. Sponge remnants were also fixed in 4% formaldehyde. A detailed summary of samples collected can be found in Table 10 in (Faille et al. 2019).

PROCESSING OF SPONGE SAMPLES FOR SPECIES IDENTIFICATION

Sponge collected from the CBT area by ROPOS in addition to samples collected from benthic trawl surveys elsewhere in the Gulf of St. Lawrence (Dinn 2020; Dinn et al. 2020a) were analyzed taxonomically. Spicule analysis and DNA barcoding follow methods described in (Dinn et al. 2020a). Continuous video from all transects was reviewed to compile a list of possible sponge taxa. Inferences were made from previous work in eastern Canadian waters to attempt to identify some sponge morphotypes seen in video collected in the CBT area.

RESULTS

VIDEO ANALYSIS

A total of 137 images were analyzed and cover a total area of 467.7 m² (Table 1). Transects CBT-2 and CBT-3 were located in the deep part of the trough in the north with a minimum depth of 148 m and a maximum depth of 165 m, while transects CBT-7 and CBT-9 were located in the shallow parts in the southern part of the trough with depths ranging from 70–98 m (Table 1). A steep slope was present along transect CBT-4. Depth profiles are presented in Figure 4. Analysis was not able to be completed for transect CBT-3 because ROPOS was too far from the bottom, or the camera was

orientated poorly such that it was impossible to calculate the field of view. Analysis was not attempted for CBT-9 because the lasers were absent the first 650 m of the transect and in the last segment, and visibility throughout the dive was poor which would greatly affect the quality of observations.

QUANTITATIVE ANALYSIS

There were 44 taxa and morphotypes identified and used in subsequent analyses from benthic imagery collected during the 2017 CBT ROPOS (Appendix 1). Example screenshots displaying the different habitat types encountered during quantitatively analyzed transects are shown in Figure 5. For transect CBT-2, located in the deep waters in the north of CBT, the most abundant phylum was Porifera (55%) (Figure 6). Animals counted in transect CBT-4 were composed primarily of Cnidaria (25%), and Arthropoda (24%) (Figure 6). In the beginning and end of transect CBT-4, sea anemones (Cnidaria) were dominant on rocky substrates, while the middle of the transect the dominant taxa were snow crab (*Chionoecetes opilio*, Phylum: Arthropoda) occurring on finer substrate. Animals counted in transect CBT-5 were mainly Cnidaria (40%), Brachiopoda (18%), and Mollusca (17%) (Figure 6). Transect CBT-7 in the shallow southern end of the trough, consisted mostly of Arthropoda (37%), Mollusca (17%) and Echinodermata (14%) (Figure 6). Average density of taxa (ind./m²) found in transects CBT-2, CBT-4, CBT-5 and CBT-7 is presented in Figure 7. The highest number of taxa was observed in CBT-4 (32); however, average density was greatest in CBT 5 (0.77 ind./m²) (Table 2). Colony counts of sprawling/encrusting taxa observed in the benthic imagery that could not be counted as individuals for the quantitative analysis are presented in Appendix 2.

Table 1. Summary of analyzed ROPOS transects using video images showing transect, depth (m) (min and max), area analyzed, number of images analyzed and type of analysis (quantitative vs. qualitative).

Transect	Date	Latitude (start/end)	Longitude (start/end)	Depth (m)		Area analyzed (m ²)	Number of images analyzed	Type of Analysis		
				Min	Max			Quantitative	Qualitative	
								Sea Anemones	Wolffish	
CBT-2	2017-08-29	47.2085 47.2171	-60.5618 -60.5580	148	155	150.5	32	X		X
CBT-3	2017-08-29	47.1923 47.1952	-60.6095 -60.5965	164	165	-	-			X
CBT-4	2017-08-29	47.0470 47.0437	-60.6331 -60.6211	54	137	135.2	35	X		X
CBT-5	2017-08-28	47.0182 47.0147	-60.6730 -60.6595	107	142	86.4	35	X		X
CBT-7	2017-08-28	46.8221 46.8170	-60.9187 -60.9076	70	98	95.6	35	X		
CBT-9	2017-08-28	46.6482 46.6476	-61.2403 -61.2261	81	84	-	-			
Transit between CBT-4 and CBT-5	2017-08-29	47.0147 47.0470	-60.6595 -60.6331	-	-		11		X	X

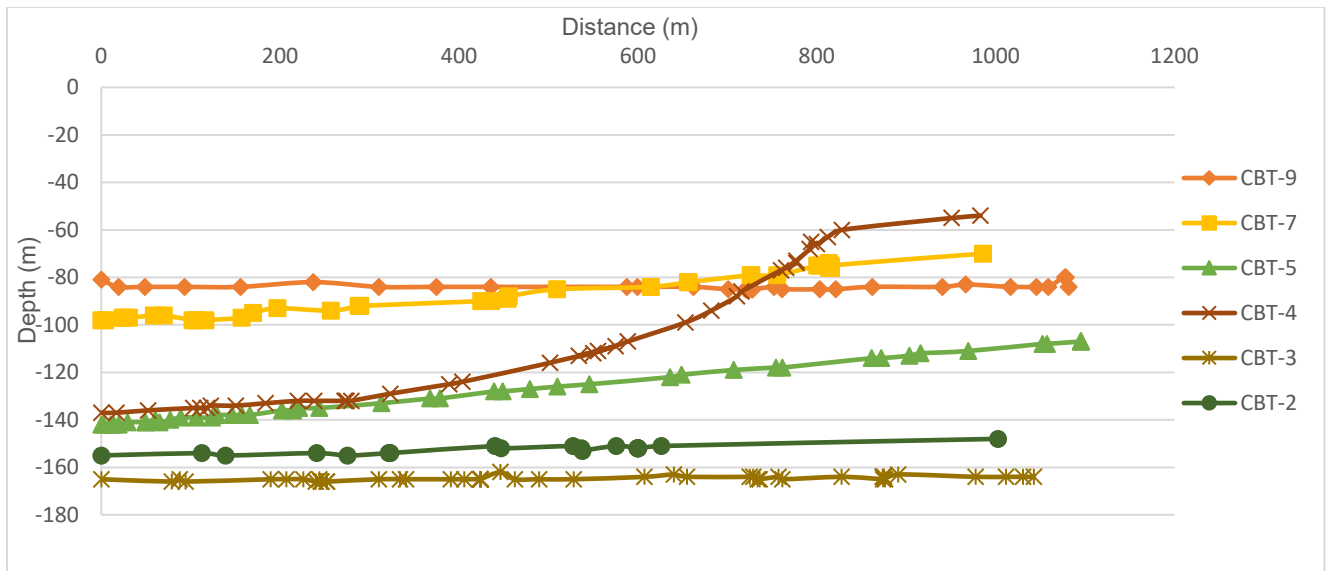


Figure 4. Depth (m) profiles and distance covered during the ROV dives for transects CBT-2, CBT-3, CBT-4, CBT-5, CBT-7, and CBT-9.

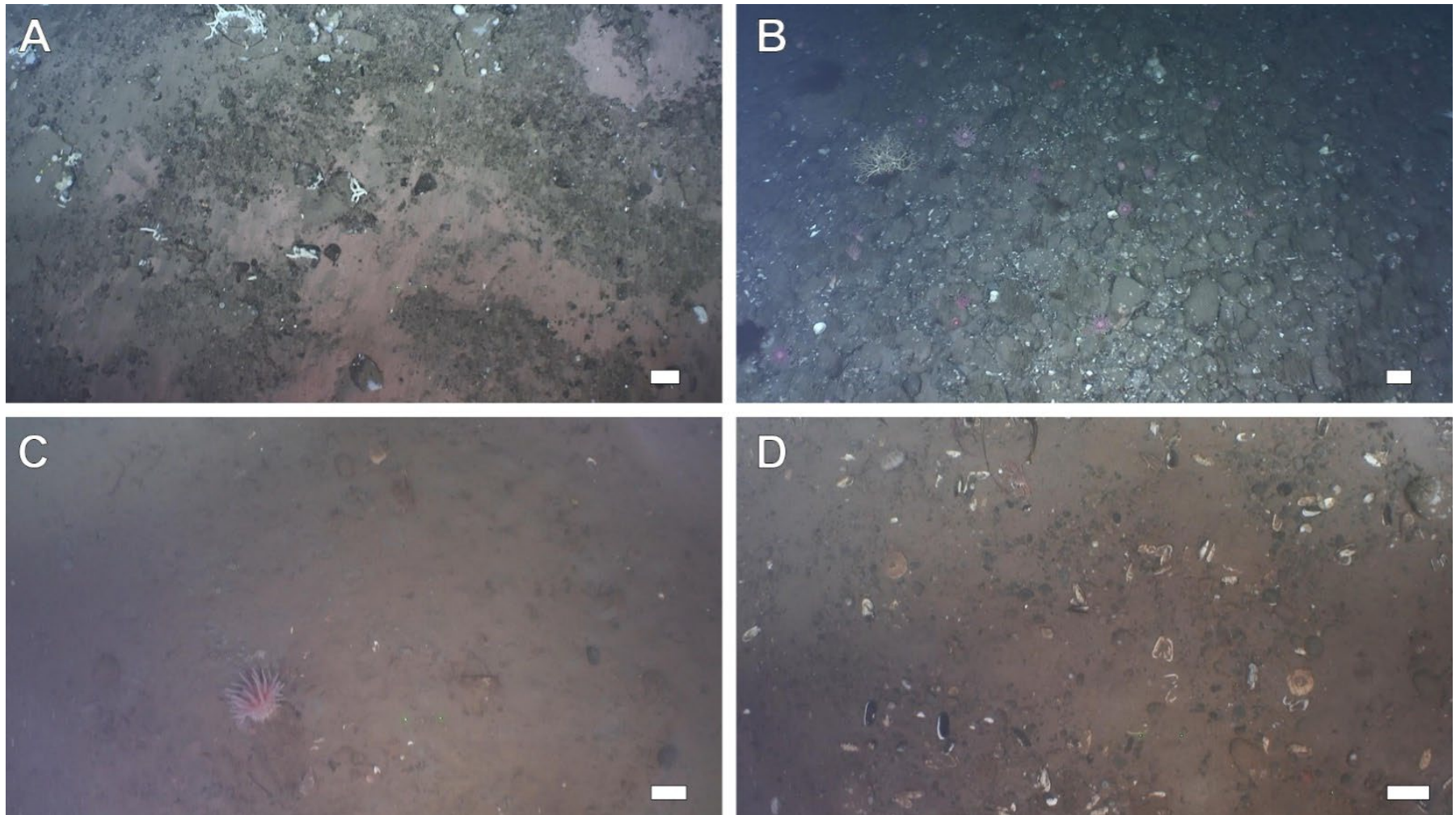


Figure 5. Examples of mini-Zeus camera screenshots of downward facing video used for quantitative image analysis from the four analyzed transects. A. CBT-2. B. CBT-4. C. CBT-5. D. CBT-7. Scale bar 10 cm.

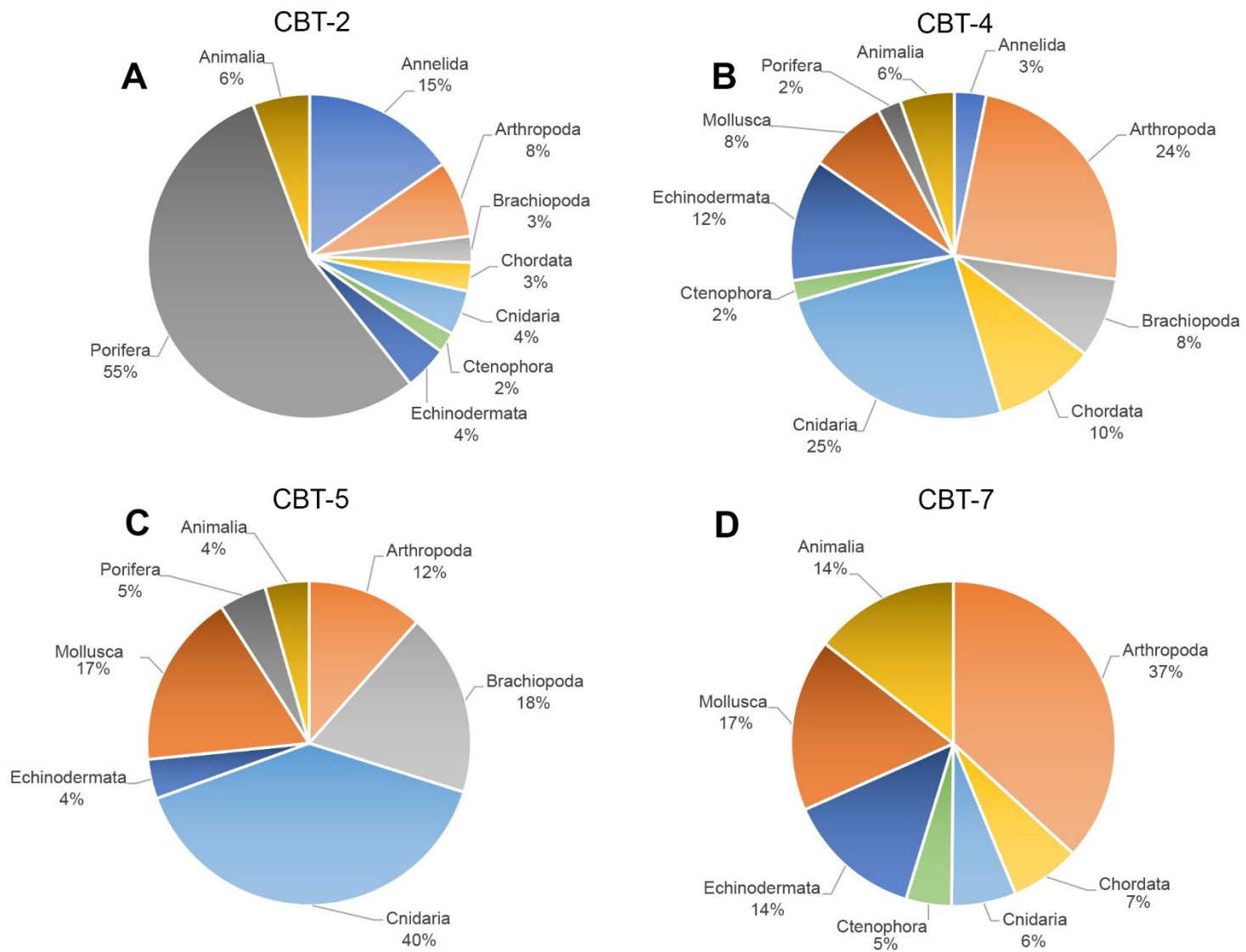


Figure 6. Percent composition of phyla in each transect, based on morphotype counts. A. CBT-2, B. CBT-4, C. CBT-5 and D. CBT-7 based on video analysis.

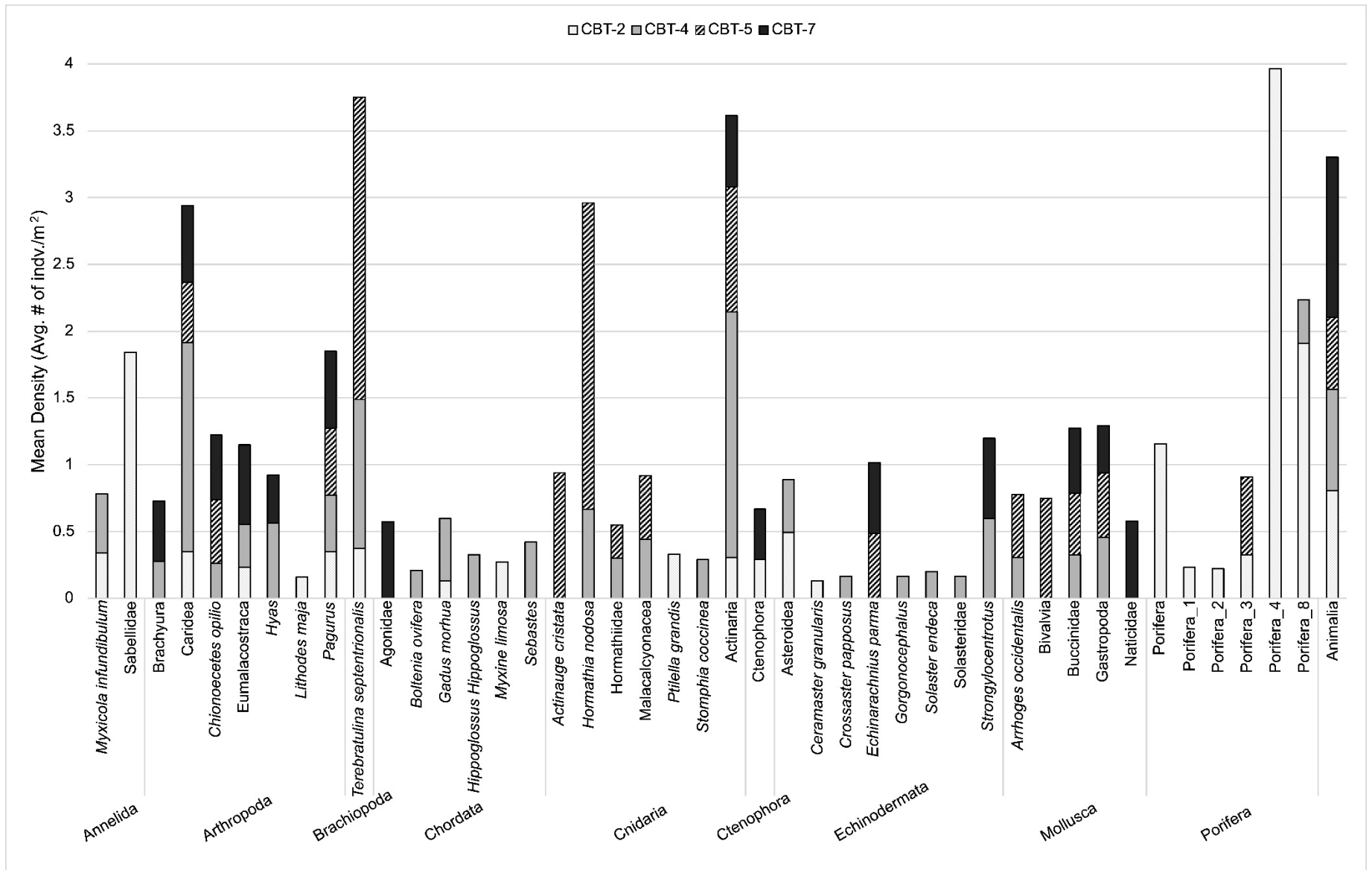


Figure 7. Mean density of taxa found in all transects based on video analysis. Taxa noted as presence/absence are not included in this histogram.

Table 2. Taxa/Morphotype Richness (R) and mean density of individuals identified in the image analyses. Richness includes all taxa even those noted as present/absent. Density includes those counted as individuals and excludes all taxa noted as presence only (i.e., Bryozoa, Hydrozoa, Ophiuroidea and Porifera_7)

Transect	R	Mean density ind./m ²
CBT-2	25	0.68
CBT-4	32	0.49
CBT-5	19	0.77
CBT-7	18	0.55

STATISTICAL ANALYSIS

The results from the nMDS analysis show that taxa from transects CBT-4 and CBT-5 group together and are most similar. Taxa from transect CBT-2 resemble the taxa seen in CBT-4 and CBT-5 while the taxa in CBT-7 appear to differ from the other 3 transects (Figure 8). Three screenshots analyzed from CBT-7 did not include the taxon driving the difference, SP 11 (*Chionoecetes opilio*), which caused those images to group closer to CBT-2. The CAP analysis revealed the taxa driving the differences between transects: (Actiniaria) for CBT-4 and CBT-5, SP 11 (*Chionoecetes opilio*) for CBT-7, and SP 4 (Asteroidea) SP 29, 32 and 33 (Porifera) for CBT-2 (Figure 9).

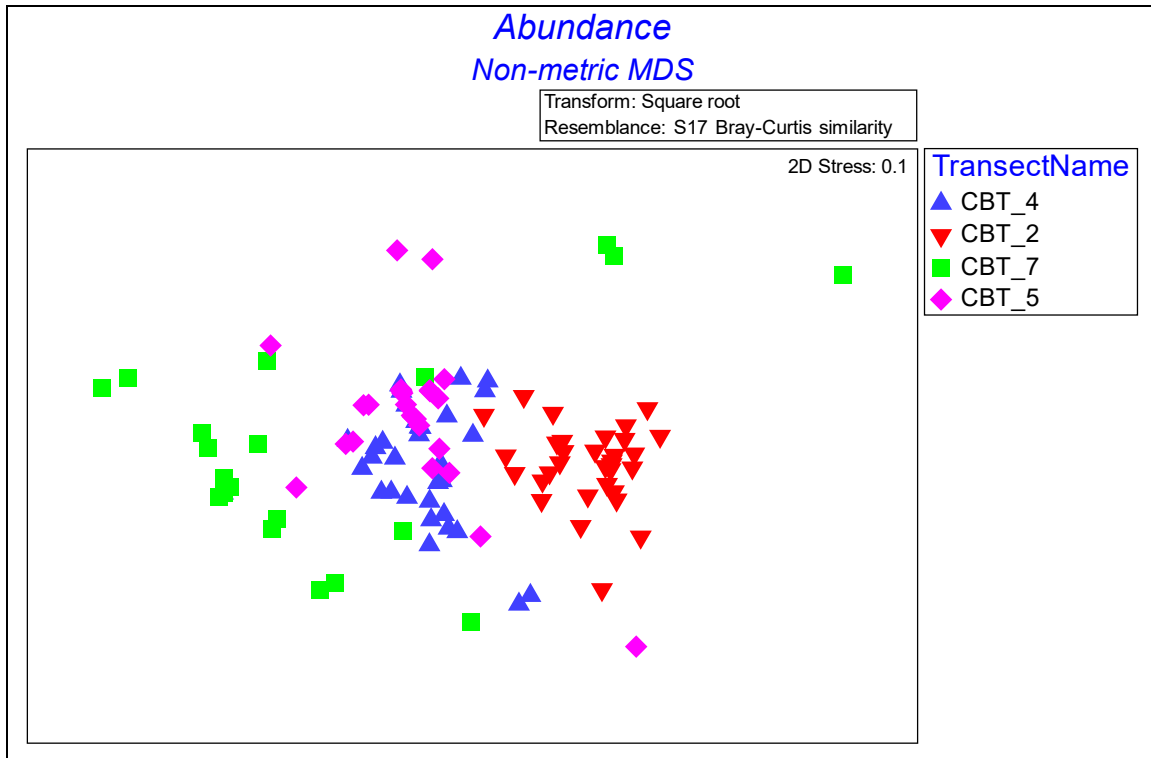


Figure 8. A non-metric multidimensional analysis (nMDS) of square-root transformed taxa abundances on Bray Curtis similarities matrix.

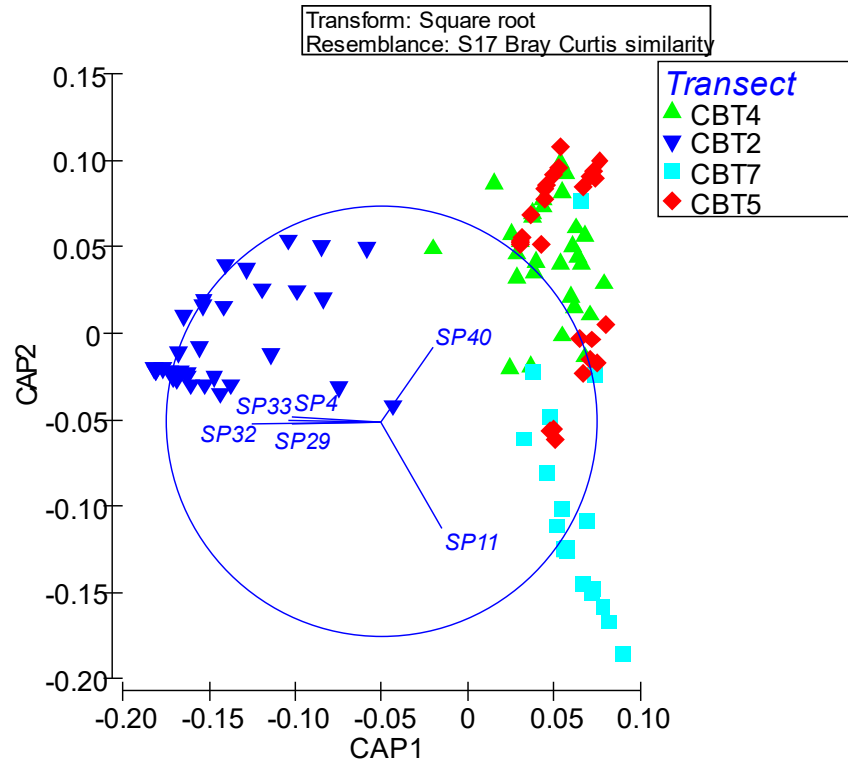


Figure 9. CAP analysis of taxa driving the differences in transects. SP 40 (Actinaria) is driving the grouping between CBT-4 and CBT-5. SP 11 (*Chionoecetes opilio*) is driving the grouping in CBT-7. CBT-2 is driven by SP 4 (Asteroidea) SP 29, 32 and 33 (Porifera).

To determine if these differences were significant, a unidirectional similarity analysis (ANOSIM routine) was performed with 9999 permutations on the same Bray–Curtis similarity matrix to verify the null hypothesis that benthic assemblages do not differ between groups (Appendix 3). All transect comparisons were significant at the $p \leq 0.001$ level except for CBT-4 and CBT-5 which was not significantly different ($p = 0.013$). The R statistic closer to 1 indicates a clear separation of sites which is the case for all transect comparisons except CBT-4 and CBT-5 which had a R statistic of 0.07 indicating similarity between sites since both had a high proportion of Cnidaria. Comparisons between CBT-2 and CBT-4, CBT-5 and CBT-7 had high R values indicating a clear separation in the taxa that were found in those transects. Comparisons between CBT-7, CBT-4 and CBT-5 indicate less of a separation between taxa (R-value 0.525 and 0.428 respectively) however this difference was significant as seen in the p-values. SIMPER analysis was then performed on the density of taxa in transects to determine the percentage of similarity between the assemblages and the contribution of each taxon to this similarity. In transect CBT-2, Porifera_4 contributed 55% of the 46% similarity within CBT-2. Dissimilarity between CBT-4 and CBT-2 are 92% and Porifera_4 and Actiniaria contributed 42% cumulatively to this between group dissimilarity. CBT-4 and CBT-5 has the lowest dissimilarity 68% (Table 3). This can also be visually seen through the CAP analysis.

Table 3. SIMPER table of taxa responsible for the similarity and dissimilarity between transects.

	CBT-2		CBT-4		CBT-5		CBT-7	
CBT-2	Avg. similarity = 46%							
	Porifera_4	55.1						
	Porifera_8	26.7						
CBT-4	Avg. dissimilarity = 92%		Avg. similarity = 35%					
	Porifera_4	26.9	Actiniaria	81.9				
	Actiniaria	15.4						
	Porifera_8	14.9						
CBT-5	Avg. dissimilarity = 94%		Avg. dissimilarity = 68%		Avg. similarity = 36%			
	Porifera_4	30.7	Actiniaria	24.7	Actiniaria	91.4		
	Porifera_8	17.1	Caridea	10.9				
	Actiniaria	13.1						
CBT-7	Avg. dissimilarity = 99%		Avg. dissimilarity = 92%		Avg. dissimilarity = 93%		Avg. similarity = 21%	
	Porifera_4	31.3	Actiniaria	31.2	Actiniaria	31.3	Actiniaria	91.4
	Porifera_8	17.3	<i>Chionoecetes opilio</i>	12.9	<i>Chionoecetes opilio</i>	17.3		

QUALITATIVE OBSERVATIONS

POTENTIAL HABITAT FOR WOLFFISH

Habitat suitable for wolffish was annotated during transects CBT-3 (Dive R2025) and CBT-4 (Dive 2024), and during the transit between transects CBT-4 and CBT-5 (Dive R2024). Ideal habitat types for wolffish include holes, caves, and rock shelters that can accommodate a fish of at least 30cm (Larocque et al. 2010). Most suitable habitat for wolffish ($n=18$) was noted during the transit between CBT-4 and CBT-5 ($n=17$), and during transect CBT-4 ($n=1$) where the habitat consisted of a steep slope and complex habitat (Figure 10). During transect CBT-3, fewer potential wolffish habitat sites ($n=5$) were seen, though this dive site was deeper (150–180 m) and is an area where wolffish have been collected in the past (Coomber et al. 2021). Examples of annotated potential wolffish habitat are seen in Figure 11. Annotators did not record potential habitat for wolffish during other dives and transects in the CBT study area.

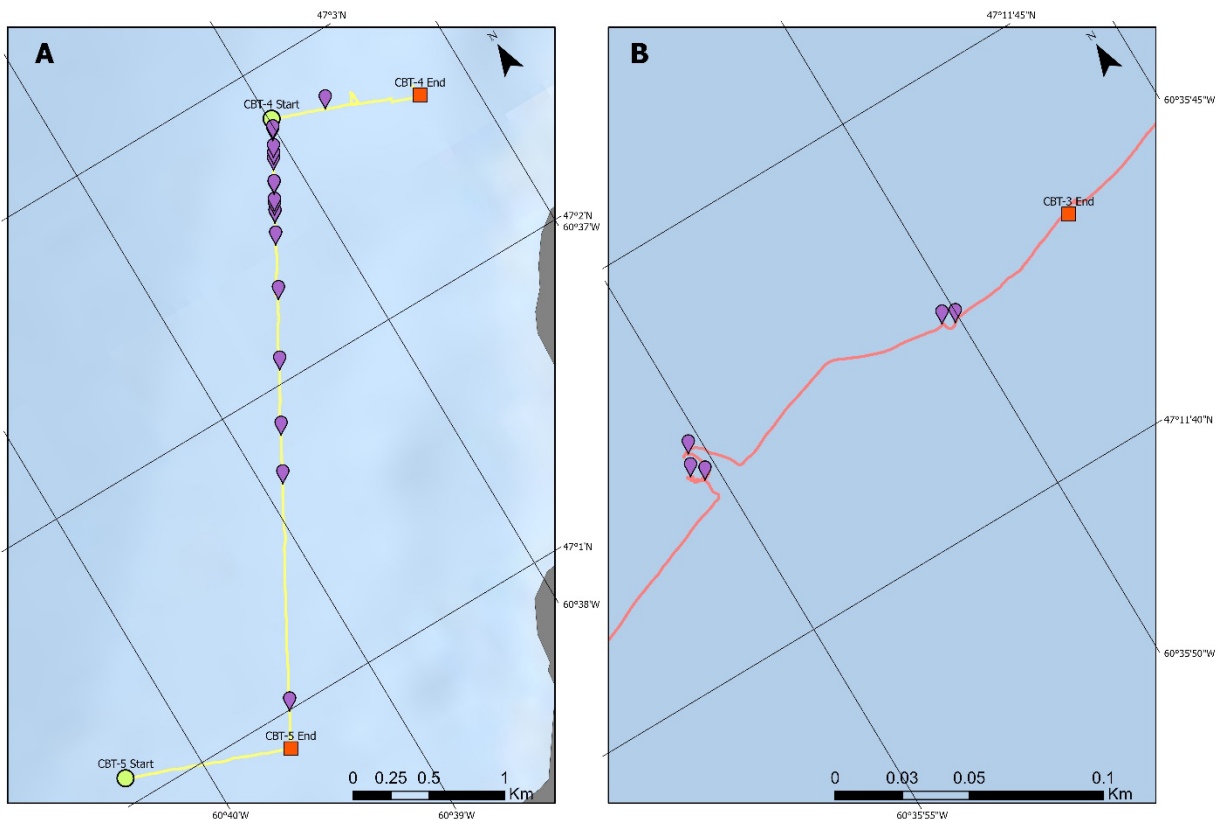


Figure 10. Habitats (purple markers) annotated as suitable for wolffish from ROV dives in the CBT area. A. suitable habitats noted during dive R2024 during transect CBT-4 and the transit between CBT-4 and CBT-5. B. Suitable habitats noted during dive R2025 in transect CBT-3.

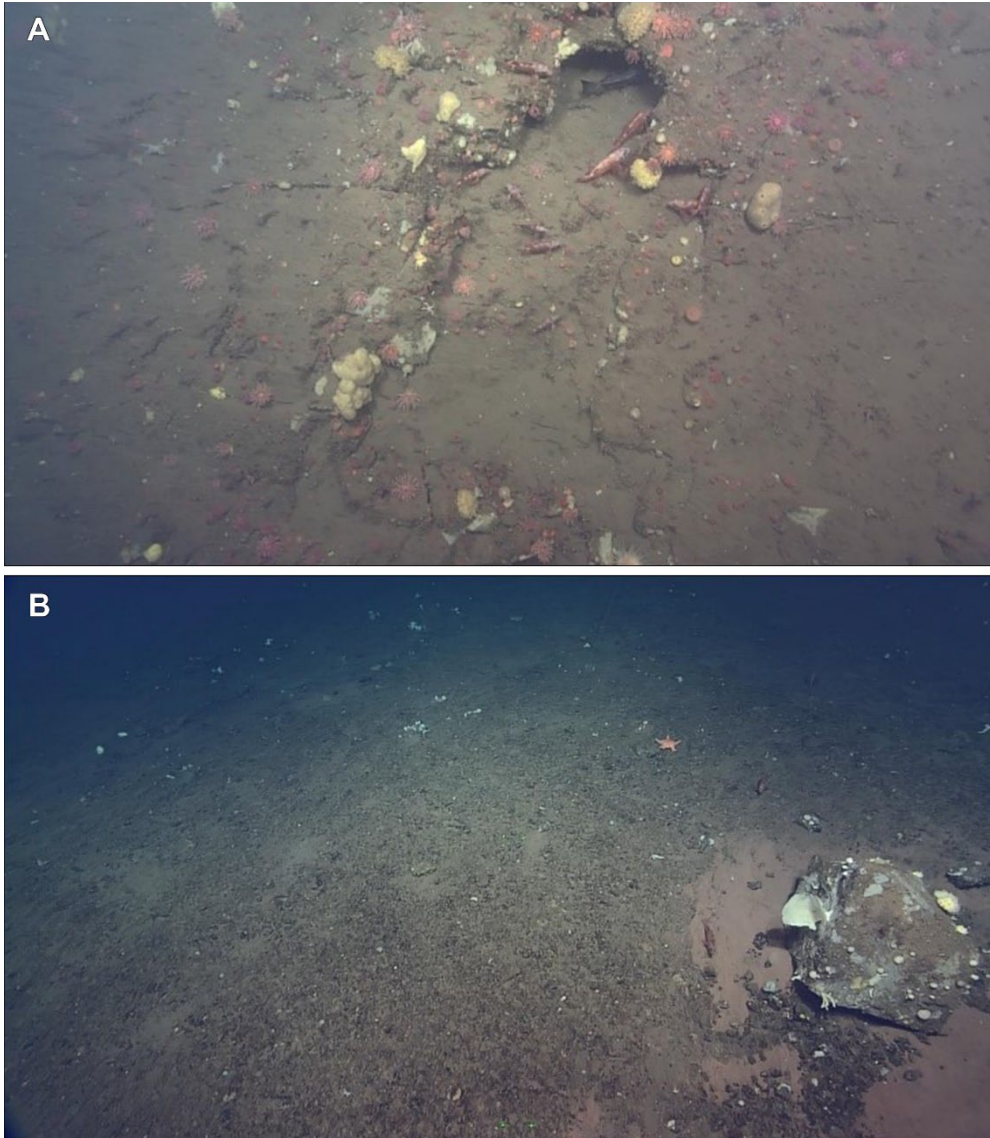


Figure 11. Examples of potential wolffish habitat annotated from CBT area. A. During transit between CBT-4 and CBT-5. B. Transect CBT-3.

SEA ANEMONE DENSE AREAS

A distinct bedrock outcrop along a sloped area was present during part of the transit between transects CBT-4 and CBT-5. Sea anemones (Order Actiniaria) were the dominant organism in this area (Table 4). Although the ROV lasers were turned off during the transit, 11 images were analyzed for percent cover of taxa. Sea anemones covered more than 75% of the visible bottom in 5 of the 11 images. Sponges were also found adjacent to the sea anemones but were far less abundant (percent cover <5–25%). Examples of other animals found with sea anemones are shown in Figure 12.

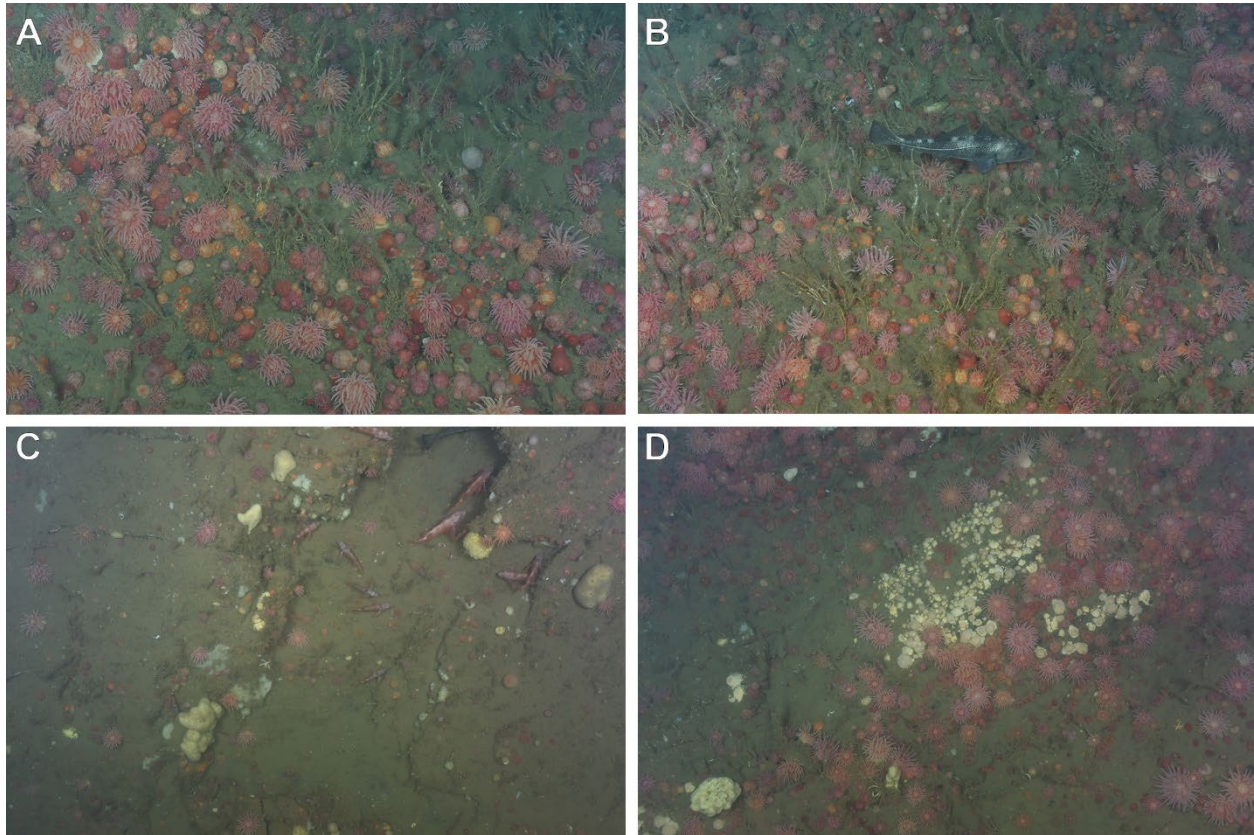


Figure 12. Examples of sea anemone-rich communities seen during Dive 2024 (transit between CBT-4 and CBT-5). A. Dense sea anemones. B. Cod amongst sea anemones. C. Redfish in bedrock caves. D. Sponges surrounded by sea anemones.

Table 4. Percent cover of annotated taxa from 11 images taken in sea anemone-rich areas during transit between transects CBT-4 and CBT-5. Animals seen but representing <5% cover are noted as present.

Screen Capture	Latitude	Longitude	Depth	Taxon	Percent Cover
1	47.03671	-60.6415	114.13	Bryozoa	Present
				Porifera_7	5–25%
				Porifera_3	Present
				Porifera_1	Present
				Hydrozoa	Present
				Actiniaria	>75%
2	47.03677	-60.6414	110.91	Hydrozoa	5–25%
				Bryozoa	Present
				Ctenophora	Present
				Porifera_1	Present
				Actiniaria	>75%
3	47.03679	-60.6413	110.11	Asteroidea	Present
				Bryozoa	Present
				<i>Gadus morhua</i>	5–25%
				Hydrozoa	5–25%
				Porifera_1	Present
				Asteroidea	Present
				Actiniaria	>75%
				<i>Hyas</i>	Present
4	47.03679	-60.6414	110.77	<i>Pagurus</i>	Present
				Asteroidea	Present
				Bryozoa	Present
				Hydrozoa	5–25%
				Actiniaria	>75%
				Porifera_1	5–25%
				<i>Gadus morhua</i>	Present
				Porifera	Present
				<i>Hyas</i>	Present
5	47.03682	-60.6413	110.02	Caridea	Present
				Henricia	Present
				<i>Pagurus</i>	Present
				Bryozoa	Present
				Asteroidea	Present
				Porifera_1	Present
				Actiniaria	>75%
				Hydrozoa	5–25%
6	47.03699	-60.6412	110.91	Ctenophora	Present
				Caridea	Present
				Brachyura	Present
				Porifera_7	Present
				Porifera	Present
				Hydrozoa	5–25%
				Bryozoa	Present
				Actiniaria	26–50%
				<i>Hormathia nodosa</i>	Present
				Porifera_1	5–25%
				<i>Sebastes</i>	Present
Bryozoa	Present				

Screen Capture	Latitude	Longitude	Depth	Taxon	Percent Cover
				Hydrozoa	51–75%
				Porifera_1	5–25%
				Actiniaria	26–50%
7	47.03715	-60.6411	112.12	<i>Sebastes</i>	Present
				Caridea	Present
				Porifera_7	Present
8	47.03726	-60.641	112.87	<i>Hyas</i>	Present
				Asteroidea	Present
				Bryozoa	Present
				<i>Sebastes</i>	Present
				Hydrozoa	26–50%
				Porifera_7	Present
				Actiniaria	51–75%
				Porifera_1	5–25%
9	47.03735	-60.6409	114.23	Porifera_7	5–25%
				<i>Hormathia nodosa</i>	Present
				<i>Sebastes</i>	Present
				Actiniaria	51–75%
				Hydrozoa	26–50%
				Caridea	Present
				Bryozoa	Present
				Porifera_3	Present
				Porifera_1	5–25%
				Asteroidea	Present
				Ophiuridae	Present
10	47.03757	-60.6407	120.18	Bryozoa	Cannot be determined
				Actiniaria	26–50%
				Porifera_7	5–25%
				Asteroidea	Present
				Hydrozoa	Cannot be determined
				Porifera_1	5–25%
				<i>Sebastes</i>	Present
11	47.03767	-60.6406	122.77	Porifera_7	Present
				<i>Sebastes</i>	5–25%
				<i>Gadus morhua</i>	Present
				Actiniaria	5–25%
				Bryozoa	Present
				Porifera_3	Present
				Porifera_1	Present
				Caridea	Present
				Hormathiidae	Present
				Asteroidea	Present
				Hydrozoa	Present

SAMPLE COLLECTION ANALYSIS

WATER CHARACTERISTICS

Temperature in surface waters of transects CBT-2 and CBT-9 were 17.1 °C and 19.3 °C respectively. Bottom temperatures in CBT-2 and CBT-9 were 4.0 °C and 1.3 °C. Surface salinity values in CBT -2 and CBT-9 were 28.0 and 26.9 PSU. Bottom salinity was higher in all three transects CBT-2 (33.4 PSU), CBT-3 (33.6 PSU) and CBT-9 (32.2 PSU) (Table 5). The bottom water pH values in CBT-2 and CBT-3, were identical (pH 7.5) as the sample taken in the shallower part of the trough in CBT-9 (Table 5). Calcite values were supersaturated ($\Omega_c > 1$) in all samples. Aragonite values were near saturation ($\Omega_a = 1$) for the northern sites and undersaturated ($\Omega_a < 1$) for CBT-9.

Table 5. Water parameters collected during transects CBT-2 (bottom, mid and surface), CBT-3 (bottom), and CBT-9 (bottom, mid and surface). Highlighted cells represent bottom water samples.

Transect	Date	Lat (°)	Long (°)	Depth (m)	Salinity (PSU)	Temp (°C)	pH	Calcite (Ω_c)	Aragonite (Ω_a)
CBT-2	29-Aug-2017	47.2172	60.5580	148	33.4	4.0	7.5	1.61	1.01
		47.2169	60.5588	25	30.7	5.8	7.6	1.80	1.13
		47.2165	60.5584	2	28.0	17.1	7.9	2.82	1.78
CBT-3	29-Aug-2017	47.1952	60.5965	165	33.6	N/A	7.5	1.49	0.93
CBT-9	28-Aug-2017	46.6475	61.2261	80	32.1	1.3	7.5	1.28	0.80
		46.6478	61.2262	22	27.8	7.0	7.8	2.41	1.50
		46.6479	61.2262	4	26.9	19.3	7.9	3.09	1.96

SEDIMENT CHARACTERISTICS

The percent dominant substrate observed in the video transect for CBT-2 was quantified as mostly pebbles (4–64 mm) and sand (<4 mm) (Figure 13). In transect CBT-4, three distinct areas were noted: Area 1 had soft substrate, mostly mud/silt with some rocks and boulders, Area 2 was a mixture of mud and sand and pebbles and area 3 consisted of mostly rock, pebbles, and fine sediment. Transect CBT-5 was soft bottom with mostly mud, some rocks and boulders. Transect CBT-7 was observed to consist of mud, sand, and silt along with some shells and pebbles. The transit between CBT-4 and CBT-5 at depths of 107 m, had three distinct areas: Area 1 was soft bottom, mostly mud

with some rocks and boulders, Area 2 was a dense sea anemone area atop bedrock, and Area 3 was soft bottom of mainly sand and mud with some rocks and boulders.

Fine sediment analysis from sediment cores found that samples taken from CBT-2 (duplicate), 5 and 7 consist of mostly sand (63–2000 μ m), whereas CBT-3 is composed of mostly silt (2–63 μ m) and CBT-9 is a mixture of sand and silt (Figure 14).

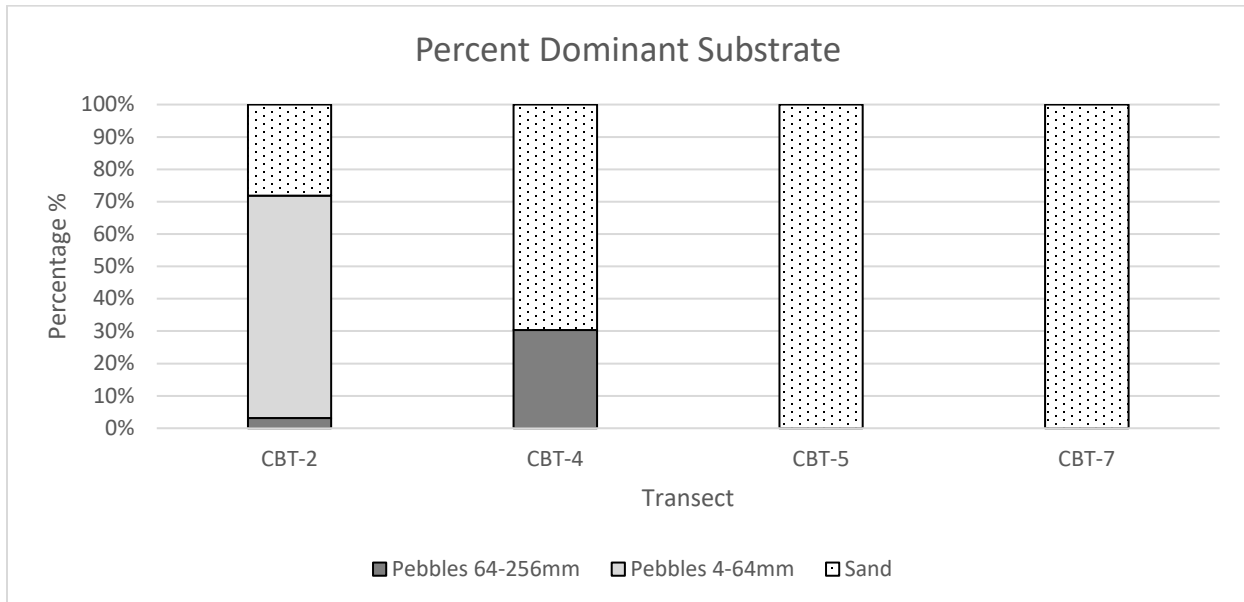


Figure 13. Dominant substrate noted from video annotation of benthic screen captures by transect.

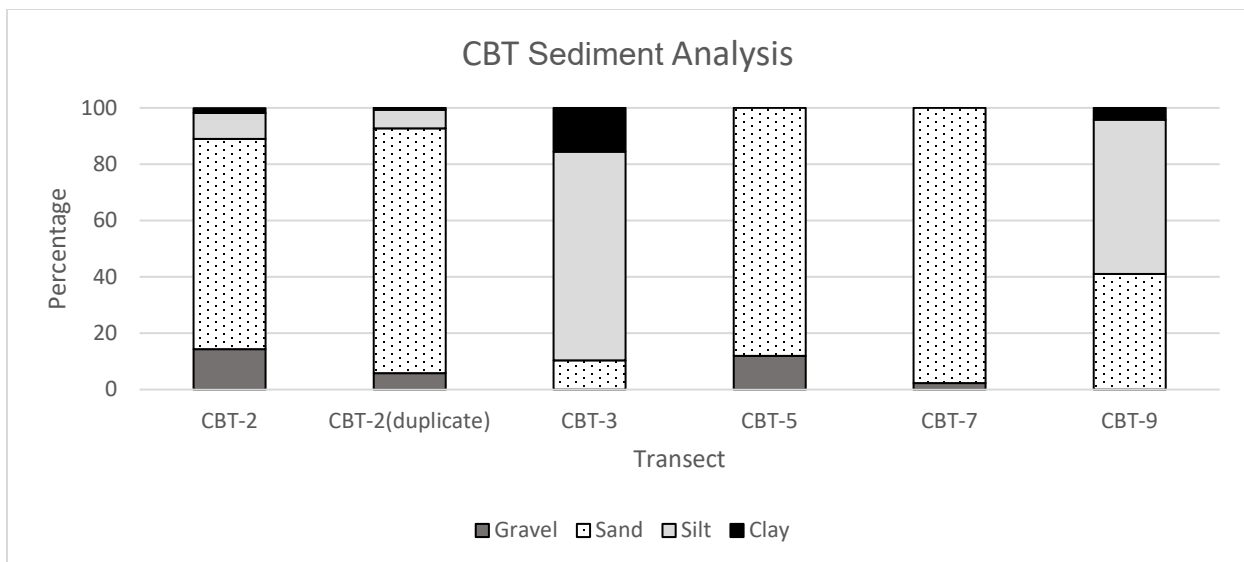


Figure 14. Percentage of gravel (>2mm), sand (63–2000 μ m), silt (2–63 μ m), and clay (<2 μ m) in sediment core sample analysis from each transect.

SPONGE SPECIES IDENTIFICATION

Morphotypes annotated during the video analysis could represent several taxa. The same eight sponge morphotypes were used to be consistent with previous video analysis reports from the American Bank (Côté et al. 2021) and the Laurentian Channel (Thorne et al. 2022). Although only eight morphotypes were used for the quantitative video analysis, 13 total sponge taxa were noted from taxonomic review of collected material and imagery analysis separate from the quantitative dataset where whole transects were reviewed. From laboratory analysis of samples taken by ROPOS, video review, and through inferences made from nearby collections, possible taxa representing the eight morphotypes are presented (Table 6). Where specimens were collected, lower-level identifications were possible through spicule and DNA analysis, but further taxonomic investigation is required to accurately name each specimen to species. Morphotypes that were not collected could not be confidently identified from HD video as sponge species can be incredibly polymorphic, thus difficult to identify from gross morphology alone.

Plicatellopsis bowerbanki (Vosmaer, 1885) (Figure 15 A) was less common during dives in the CBT than on the hard rock walls of the American Bank (Faille et al. 2019). *P. bowerbanki* is a cup-shaped or flabelliform species that grows to an impressive size and is generally attached to rocks. Previous records of this sponge have been reported as members of the genus *Phakellia*, however DNA and morphological analysis of specimens confirmed that the sponge belongs in the genus *Plicatellopsis* (Dinn et al. 2020a).

Encrusting sponges were very common on hard surfaces (Figure 15 B,C). Blue encrusting sponges were identified as *Hymedesmia* (*Hymedesmia*) cf. *paupertas* (Bowerbank, 1866). Yellow encrusting sponges were collected but they do not have spicules making the identification of the specimens difficult. As several sponge orders do not have spicule skeletons, lower taxonomic identification requires extra attention. Yellow encrusting species such as *Hexadella* sp. and *Aplysilla* sp. can form yellow encrustations and are common in the eastern Atlantic (Buhl-Mortensen et al. 2010; McIntyre et al. 2016). White encrusting sponges were not collected. From work in the North Labrador Sea white encrustations could represent several species such as *Janulum spinispiculum* (Carter, 1876), *Antho* (*Acarnia*) *signata* (Topsent, 1904), and *Phorbas microchelifer* (Cabioch, 1968) among others (Dinn and Leys 2018). It is clear from the HD video that there are several white encrusting morphotypes, so it is likely that there are multiple species represented by this broad identification. Green encrusting specimens are unknown, but may also be members of the genus *Hymedesmia*.

Suberitida unknown 1 (Figure 15 D) is an arborescent or finger-shaped sponge. COI DNA and spicules suggest the family, but a species identity remains unknown (Dinn 2020). From spicule and DNA analysis of collected specimens, this arborescent

morphotype may also represent another species, most closely related to the genus *Protosuberites*.

Sphaerotylus capitatus (Vosmaer, 1885) (Figure 15 E) was also frequently encountered and was easily identified by the many large papillae on the upper surface. This species was confirmed by COI DNA analysis. Several smaller sponges which appear similar but have fewer and smaller papillae likely represent different members of the family Polymastiidae, though only *S. capitatus* specimens were collected.

Mycale (Mycale) lingua (Bowerbank, 1866) (Figure 15 F) specimens were very common during dive 2025, living amongst sea anemones on a rocky outcrop. The species is distinctive due to the furrowed appearance of the outer portions of the sponge, and spicule analysis of specimens from other dive sites confirm the identification.

Tentorium semisuberites (Schmidt, 1870) is a cosmopolitan species that can easily be identified by its small toadstool-like appearance. There are often several short papillae on the upper distal portion of the sponge.

Iophon sp. (Bowerbank, 1858) was collected and seen forming a white encrustation, later becoming brown either on contact with air or after preservation. The spicule complement of acanthostyles, tylotes, two sizes of anisochelae, and small rounded bipocilles suggests the species to be *Iophon* cf. *nigricans*, however several specimens representing members of the genus remain to be identified in the Gulf of St. Lawrence (Dinn 2020). As only a small fragment was collected and the sponge is not well represented from the HD video, the sponge is identified only to the genus level.

Tedania (Tedania) cf. *suctorica* Schmidt, 1870 collected from the CBT has unique vermiform styles which do not fit the description of the species; thus the species designation is not assured. COI DNA results suggest the sponge is most similar to *Tedania (Tedania) pilarriosae* Cristobo, 2002 from the Iberian Peninsula, Spain but the diagnostic stylotornotes are not present in our specimens. *Tedania (Tedania) suctorica* is commonly collected during trawl surveys in the Gulf of St. Lawrence (Dinn 2020), however additional work on this specimen may reveal differences between the two species.

Table 6. Sponge morphotypes annotated in quantitative analysis (from Côté et al. 2021; Thorne et al. 2022). Some possible taxa represented by each morphotype were determined through sponge collections made by ROPOS, and from other surveys in the Gulf (Dinn 2020). Taxa collected by ROPOS in the CBT are noted in bold.

Morphotype	Body Type	Possible Taxa Predictions (based on body type and nearby collections)
1	Massive	<i>Mycale (Mycale) lingua</i> (Bowerbank, 1866), <i>Tedania (Tedania) cf. suctoria</i> Schmidt, 1870, <i>lophon</i> sp. (Bowerbank, 1858)
2	Vase	<i>Plicatellopsis bowerbanki</i> Vosmaer, 1885 , <i>Mycale (Mycale) lorea</i> Dinn, Ott, Marmen, Steeves, Côté, Hayes, Nozères, Everett, Powell & Chu, 2023
3	Sphere with Projections	<i>Polymastia</i> spp., <i>Sphaerotylus capitatus</i> (Vosmaer, 1885)
4	Sphere without projections	<i>Tentorium semisuberites</i> (Schmidt, 1870) , <i>Tethya</i> spp., <i>Polymastia</i> spp.
5	Glass Sponges	<i>Asconema foliatum</i> (Fristedt, 1887)
6	Stalked, Erect	<i>Cladocroce spatula</i> (Lundbeck, 1902), <i>Plicatellopsis bowerbanki</i> Vosmaer, 1885)
7	Encrusting	<i>Aplysilla cf. sulfurea</i> Schulze, 1878 , <i>Hymedesmia (Hymedesmia) cf. paupertas</i> (Bowerbank, 1866)
8	Other Forms	Unknown “<i>Protosuberites</i>” , Suberitida unknown 1

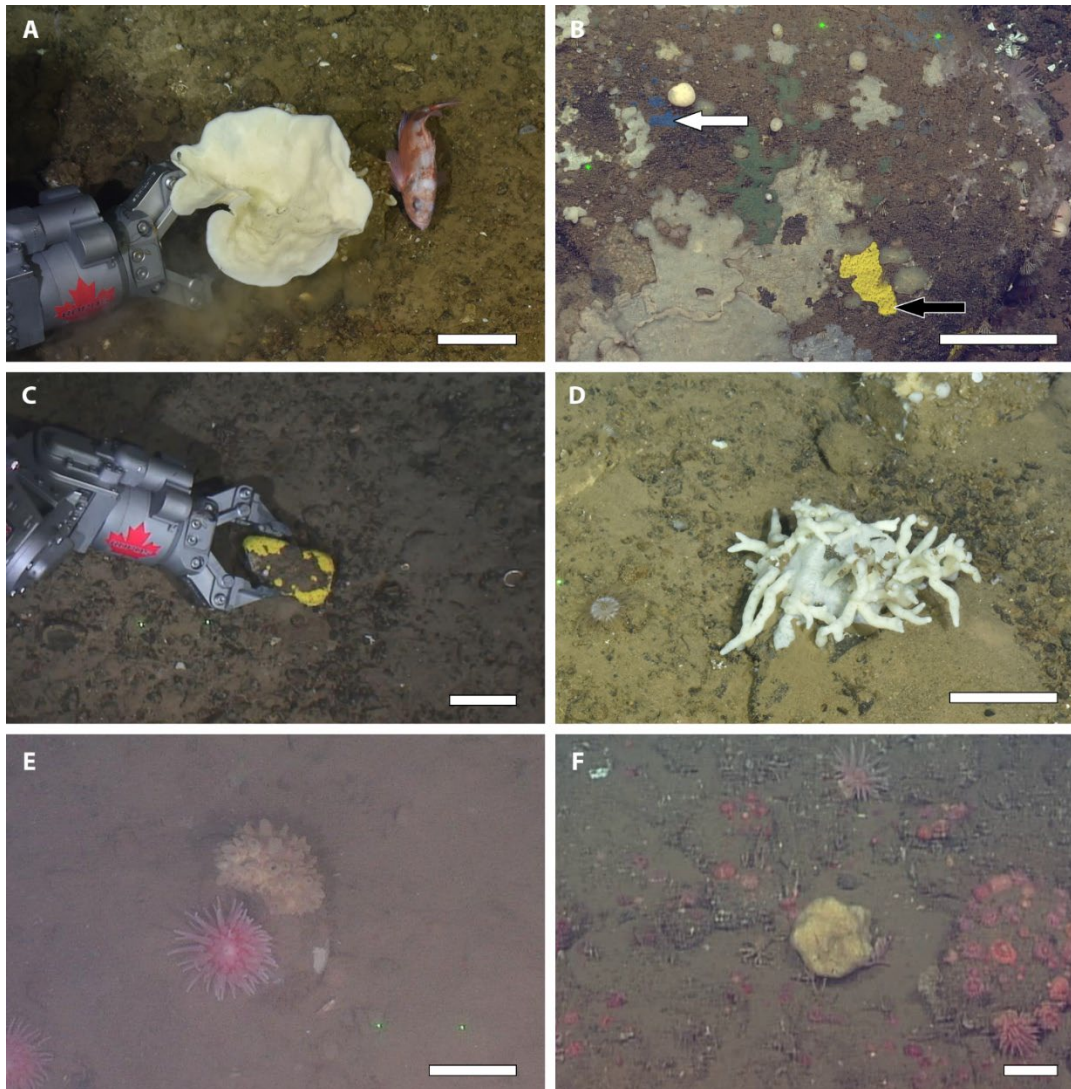


Figure 15. Examples of sponges seen and collected during Cape Breton Trough area dives and identified in the lab. A. *Plicatellopsis bowerbanki*. B. Several encrusting sponge species, white arrow – *Hymedesmia (Hymedesmia) cf. paupertas*, black arrow – Yellow encrusting, white encrusting species unknown. C. Yellow encrusting. D. Suberitida unknown 1. E. *Sphaerotylus capitatus*. F. *Mycale (Mycale) lingua*. White scale bar indicates 10 cm.

DISCUSSION

VIDEO ANALYSIS

QUANTITATIVE ANALYSIS

Porifera (sponges) dominated the northern transect CBT-2. This dive revealed the most varied substrate consisting of mostly pebbles (4-64 mm), but also included large rocks and areas with sand as the dominant substrate. Generally, substrate heterogeneity can increase benthic biodiversity (Buhl-Mortensen et al. 2010, 2012) and the increased surface area of hard structures such as boulders can also increase taxonomic and

functional richness (Franz et al. 2021). Many sponges preferentially settle on hard bottom substrates (Bergquist and Sinclair 1968; Ginn et al. 2000). In a study of sponge species richness in the eastern Canadian Arctic, species richness was higher in heterogeneous habitats (Dinn et al. 2020b), though some sponge species may specialize in soft bottom habitats (Cerrano et al. 2007). Some regional sponge species, such as *Cladocroce spatula* are found mostly in soft bottom habitats. *C. spatula* is very common in the Gulf of St. Lawrence, and was previously collected near the CBT study area (Dinn 2020) and fits Morphotype 6 (stalked, erect), however a representative sample was not collected to confirm that the species occurred along this transect.

The substrate in the CBT-4 transect was composed of mostly sand, but one area consisted of rocks and pebbles along a slope, and this was where high amounts of sea anemones and some sponges were seen. Harder surfaces underwater are often home to higher biodiversity than nearby muddy and sandy habitats, especially for suspension feeding organisms like anemones and sponges (Haedrich and Gagnon 1991). The higher range in depth of this dive (57-137 m, Figure 4) may have led to this increased taxa richness due vertical zonation of benthic communities where different habitats along a depth and sediment gradient are colonized by varied organisms (Haedrich et al. 1975; Gasbarro et al. 2018).

Transect CBT-5 was mostly composed of sand and had a lower animal abundance overall. CBT-4 and CBT-5, in the mid trough, were expected to be most similar because of their proximity to each other (Faille et al. 2019). The dominant phylum seen in both transects was Cnidaria (i.e., sea anemones). CBT-7 also consisted of mostly sand substrate and had the lowest taxon richness and abundance of all transects. The most abundant phylum in CBT-7 was Arthropoda, with large numbers of snow crab present along the transect. This is a snow crab fishing area (SFA 19) (DFO 2023) and anecdotal aggregations of crab (podding behavior) was expected but was not observed.

Overall the species richness and density in the CBT area was lower than the Laurentian Channel (Thorne et al. 2022) and American Bank (Côté et al. 2021) dives that were analyzed using similar methods.

QUALITATIVE ANALYSIS

POTENTIAL WOLFFISH HABITAT

Atlantic Wolffish (*Anarhichas lupus*), is currently listed as a species of special concern in the Species at Risk Act Public Registry due to declines in both abundance and distribution range across the Northwest Atlantic from the 1980's to mid-1990's (DFO 2013). The northern tip of the Cape Breton peninsula was predicted to be a hot spot for relative occurrence of the species (Dutil et al. 2013; Coomber et al. 2021). Atlantic Wolffish generally occur in waters less than 200 m depth (min. depth 90 m) and have habitat associations with outcrops of bedrock and sand at salinities below 34 PSU and temperatures from 1.3°–10.2°C (Kulka et al. 2007; Nozères et al. 2010; DFO 2013; Dutil et al. 2013). Although no wolffish were recorded in the CBT area ROV video, potential wolffish habitat delineated as holes, caves, and rock shelters that can accommodate a

fish of at least 30 cm (Larocque et al. 2010) were noted in the north (CBT-2 and CBT-3) and mid transects (CBT-4 and CBT-5), and when in transit between CBT-4 and CBT-5. The most suitable habitat for wolffish was noted during the transit between transects CBT-4 and CBT-5. The habitat here consisted of a wall of bed rock and areas with sand, mud and some rocks and boulders. Transect CBT-4 where the habitat consisted of a steep slope with complex topography could also be suitable. Since no water samples were taken in the mid transects of the trough (CBT-4, CBT-5), we do not have information on temperature or salinity where most potential dens were annotated. Water samples were taken nearby in CBT-2 at a similar depth. The temperature taken at 1 m from the bottom in CBT-2 was 4°C and salinity was 33.6 PSU. These conditions would have been appropriate for finding Atlantic wolffish since wolffish have a preferred temperature range of 1.5 to 4.5°C and are known to adjust their distribution to stay within their temperature preference (Kulka et al. 2007; DFO 2013; Dutil et al. 2013).

SEA ANEMONE-DENSE AREAS

Dive 2024 (transects CBT-4 and CBT-5) occurred in areas where bedrock outcrops were present. This area was observed to have dense sea anemone coverage. The sea anemone species were not able to be identified, as several species in the region are often confused and require laboratory investigation to determine species (Sanamyan et al. 2020; Isabel et al. 2024). Portions of the transit between CBT-4 and CBT-5 were analyzed qualitatively and, 5 of 11 analyzed images showed more than 75% coverage of sea anemones. Scaling lasers were not turned on during the transit between CBT-4 and CBT-5, so area covered by sea anemones was not estimated. Nearby in Newfoundland and Labrador waters, anemones could reach densities of 5 to 9 ind./m² (Mercier et al. 2017). The bedrock-dominated areas surveyed in the CBT appear to have a very high sea anemone density which could exceed that amount. Most sea anemones are sessile, limited in their ability to colonize a given habitat due to features of their physico-chemical environment (Fautin 1989). Sea anemones are often opportunistic suspension feeders, but may also actively seek out food (Sun et al. 2022). In mesocosm experiments of sea anemones from eastern Canada, three sea anemone species were shown to eat shrimps, amphipods, sea stars, brittle stars, basket stars, and sea urchins, and were also seen to have crustacean parts, sponge spicules, foraminifera, and fish scales in their gastrovascular cavities, though species differed in their food preferences (Sun et al. 2022).

COLLECTED SAMPLES

WATER SAMPLE ANALYSIS

Physio-chemical characteristics of water were similar between the northern transects (CBT-2 and CBT-3) and the southern transects. However, bottom temperatures were warmer in the northern (4°C) compared to the southern transect (1.3°C). Levels of Calcite (Ω_c) taken from bottom water in the three sampled transects were supersaturated ($\Omega_c > 1$) while Aragonite (Ω_a) was near saturated ($\Omega_a = 1$) or undersaturated ($\Omega_a < 1$). In regions where the degree of saturation of aragonite and/or calcite is greater than one, the formation of shells or skeletons of organisms is favored,

while in places where values are less than one, the dissolution of calcareous skeleton occurs gradually (Fabry et al. 2008). A decrease in pH can also alter the composition and abundance of sponge communities present (Goodwin et al. 2014). These conditions may affect sponge reproductive and regeneration success (Goodwin et al. 2014). The Gulf of St. Lawrence has experienced a decrease of 0.04 pH units (8.8% increase in acidity) per decade since 1934 (Bernier et al. 2018).

SEDIMENT CHARACTERISTICS

The percent dominant substrate in the north (CBT-2) was the most diverse, with a mixture of pebbles, rocks and sand. CBT-4 also showed heterogeneity, with rocks, pebbles, and fine sediments. CBT-5 and CBT-7 were the most homogenous of the four transects, with sand as the dominant substrate observed. The type of sediment may influence the distribution of sponges (Chimienti et al. 2018, Wilborn et al. 2018). Lacharité and Metaxas (2017) established a significant relationship between substrate complexity (determined by the presence of pebbles), and the diversity of benthic megafauna. Even a patchy presence of boulders or pebbles in a homogeneous environment can support a very rich and abundant benthic megafauna (Lacharité and Metaxas 2017), as seen in the CBT-2 transect.

SPONGE COMMUNITIES

Porifera_4 (Sphere without projections type) and Porifera_8 (Other forms) morphotypes used in the quantitative analysis were the most abundant morphotypes seen in the Cape Breton Trough area (Figure 7), however most of the sponge abundance for the area was concentrated in CBT-2 (**Error! Reference source not found.**). Species like *Tentorium semisuberites* (Schmidt, 1870), *Tethya* spp., and *Polymastia* spp. which likely represent morphotype Porifera_4 (Table 6) were the most abundant organisms seen in the dive sites. *T. semisuberites* is a very small sponge which was seen growing on boulders (Figure 15A), so although the number of individuals of this morphotype was high, the percent cover of the species throughout the dive may not have been as high as other, larger species and mobile animals that are not anchored to hard substrates. *Polymastia* sponges were often seen as small spheres with few or no noticeable projections (oscula) and were likely recorded as Porifera_3 (Sphere with projections) or Porifera_4 (Sphere without projections). Sponges can contract or close their oscula (Kumala et al. 2017), so it's possible that the same species of spherical sponge was recorded as more than one morphotype. All members of the family Polymastiidae are considered to be Vulnerable Marine Ecosystem (VME) representative taxa which includes groups of animals that form ecosystems that are easily disturbed, slow to recover, or may never recover from damage, such as damage from trawling (ICES 2020). *Mycale (Mycale) lingua* (possibly represented by Porifera_1, massive body-type sponges) is also a VME representative taxon that was seen in the CBT area. Prior to being re-combined into a separate genus in 2020, *Plicatellopsis bowerbanki* (possibly represented by Porifera_2 vase-shaped sponges or Porifera_6

stalked, erect sponges) was considered to be within the genus *Phakellia* (Dinn et al. 2020a), and *Phakellia* spp. is also considered a VME representative taxon (ICES 2020).

Encrusting sponges (Porifera_7) seen during dive transects could include several species (Table 6). Encrusting sponges often grow attached to hard substrates, but counting and delimiting individuals is difficult due to the sprawling habit of these growth forms. Few encrusting sponges are collected in trawl surveys in the southern Gulf of St. Lawrence (Dinn 2020), mostly owing to the fact that trawl surveys are biased to trawlable, soft-bottom seafloor with limited large rocks and boulders (Chimienti et al. 2018).

Since morphotypes were used to count and separate sponges seen during dives, the number of species present in the CBT area is still unknown. Limited samples were collected by ROPOS, and the confirmation of sponge species presence requires laboratory analysis. For these reasons, although sponges were very common in CBT-2, the area requires additional study to determine community structure at the species level.

LIMITATIONS AND UNCERTAINTIES

Although benthic imagery collected with ROPOS made it possible to explore previously un-trawled sites in the CBT in a non-intrusive manner, this sampling method also has limitations. Underwater visibility can be reduced if ROPOS is too far from the seafloor or from the orientation of lights in a given habitat (Thorne et al. 2022). These image quality constraints may limit the ability to observe fauna and, consequently, the accuracy of the identifications. In future missions with ROPOS, it would be important to ensure that the submersible is kept at a distance of three metres or less from the bottom, such that the mini-Zeus camera is at all times oriented in a vertical axis and the area being surveyed is well lit, all while the laser dots are always visible on the bottom. These elements are of crucial importance when it comes to image analysis for annotating organisms (Thorne et al. 2022). Future missions should also have higher resolution (image size) making it easier to see details and textures, when lighting and visibility are ideal.

As screen captures were used to inform the quantitative analysis, biodiversity could be over- or underestimated for a given transect depending on the image analyzed. It is possible that mobile species could move out of the field of view before a screen shot is taken, and habitat for fixed species such as boulders may not have been captured in screen captures. Analysis of video rather than screenshots would be more time intensive, but would give a more accurate understanding of benthic biodiversity, removing the uncertainties of screenshot-based analysis.

CONCLUSION

The data collected from the ROPOS 2017 expedition provided baseline information on the benthic habitats and biodiversity in previously unexplored areas of the Cape Breton Trough. The use of ROPOS provided an opportunity to collect biological samples for identification in the laboratory. It also provided an opportunity for image analysis of the benthic environment. This report describes the taxa annotated from benthic imagery

taken from four transects in the CBT study area. Sponges dominated the northern transect (CBT-2) while Cnidaria (e.g., sea anemones) were dominant in CBT-4 and CBT-5, and Arthropoda (Snow Crab) were most abundant in CBT-7. Potential wolffish habitat was identified in CBT-3 and CBT-4, and between CBT-4 and CBT-5. Another unique feature observed was the coverage of sea anemones on dense bedrock outcrops between CBT-4 and CBT-5. Overall, additional knowledge of the benthic landscape in the CBT has been described in this report, however the results show the need for further exploration of the area, to better identify species and their role in their associated habitats.

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APPENDICES

Appendix 1. List of animal taxa identified from video analysis in the CBT area.

Scientific Name	Phylum	Class	Order	Family	Genus	Species
Animalia						
Porifera	Porifera					
Thenaria	Cnidaria	Anthozoa	Actiniaria			
<i>Stomphia coccinea</i>	Cnidaria	Anthozoa	Actiniaria	Actinostolidae	<i>Stomphia</i>	<i>coccinea</i>
Hormathiidae	Cnidaria	Anthozoa	Actiniaria	Hormathiidae		
<i>Actinauge cristata</i>	Cnidaria	Anthozoa	Actiniaria	Hormathiidae	<i>Actinauge</i>	<i>cristata</i>
<i>Hormathia nodosa</i>	Cnidaria	Anthozoa	Actiniaria	Hormathiidae	<i>Hormathia</i>	<i>nodosa</i>
Nephtheidae	Cnidaria	Anthozoa	Alcyonacea	Nephtheidae		
<i>Pennatula aculeata</i>	Cnidaria	Anthozoa	Pennatulacea	Pennatulidae	<i>Pennatula</i>	<i>aculeata</i>
<i>Pennatula grandis</i>	Cnidaria	Anthozoa	Pennatulacea	Pennatulidae	<i>Pennatula</i>	<i>grandis</i>
Hydrozoa	Cnidaria	Hydrozoa				
Ctenophora	Ctenophora					
Sabellidae	Annelida	Polychaeta	Sabellida	Sabellidae		
<i>Myxicola infundibulum</i>	Annelida	Polychaeta	Sabellida	Sabellidae	<i>Myxicola</i>	<i>infundibulum</i>
Bryozoa	Bryozoa					
<i>Terebratulina septentrionalis</i>	Brachiopoda	Rhynchonellata	Terebratulida	Cancellothyrididae	<i>Terebratulina</i>	<i>septentrionalis</i>
Bivalvia	Mollusca	Bivalvia				
Gastropoda	Mollusca	Gastropoda				
<i>Arrhoges occidentalis</i>	Mollusca	Gastropoda	Littorinimorpha	Aporrhaidae	<i>Arrhoges</i>	<i>occidentalis</i>
Naticidae	Mollusca	Gastropoda	Littorinimorpha	Naticidae		
Buccinidae	Mollusca	Gastropoda	Neogastropoda	Buccinidae		
Eumalacostraca	Arthropoda	Malacostraca				
Caridea	Arthropoda	Malacostraca	Decapoda			
Brachyura	Arthropoda	Malacostraca	Decapoda			
<i>Chionoecetes opilio</i>	Arthropoda	Malacostraca	Decapoda	Oregoniidae	<i>Chionoecetes</i>	<i>opilio</i>

Scientific Name	Phylum	Class	Order	Family	Genus	Species
<i>Hyas</i>	Arthropoda	Malacostraca	Decapoda	Oregoniidae	<i>Hyas</i>	
<i>Lithodes maja</i>	Arthropoda	Malacostraca	Decapoda	Lithodidae	<i>Lithodes</i>	<i>maja</i>
<i>Pagurus</i>	Arthropoda	Malacostraca	Decapoda	Paguridae	<i>Pagurus</i>	
Asteroidea	Echinodermata	Asteroidea				
<i>Henricia</i>	Echinodermata	Asteroidea	Spinulosida	Echinasteridae	<i>Henricia</i>	
<i>Ceramaster granularis</i>	Echinodermata	Asteroidea	Valvatida	Goniasteridae	<i>Ceramaster</i>	<i>granularis</i>
Solasteridae	Echinodermata	Asteroidea	Valvatida	Solasteridae		
<i>Crossaster papposus</i>	Echinodermata	Asteroidea	Valvatida	Solasteridae	<i>Crossaster</i>	<i>papposus</i>
<i>Solaster endeca</i>	Echinodermata	Asteroidea	Valvatida	Solasteridae	<i>Solaster</i>	<i>endeca</i>
<i>Echinarachnius parma</i>	Echinodermata	Echinoidea	Clypeasteroidea	Echinarachniidae	<i>Echinarachnius</i>	<i>parma</i>
<i>Strongylocentrotus</i>	Echinodermata	Echinoidea	Camarodonta	Strongylocentrotidae	<i>Strongylocentrotus</i>	
<i>Gorgonocephalus</i>	Echinodermata	Ophiuroidea	Euryalida	Gorgonocephalidae	<i>Gorgonocephalus</i>	
Ophiuridae	Echinodermata	Ophiuroidea	Ophiurida	Ophiuridae		
<i>Boltenia ovifera</i>	Chordata	Ascidiacea	Stolidobranchia	Pyuridae	<i>Boltenia</i>	<i>ovifera</i>
<i>Gadus morhua</i>	Chordata	Actinopterygii	Gadiformes	Gadidae	<i>Gadus</i>	<i>morhua</i>
Agonidae	Chordata	Actinopterygii	Scorpaeniformes	Agonidae		
<i>Hippoglossus Hippoglossus</i>	Chordata	Actinopterygii	Pleuronectiformes	Pleuronectidae	<i>Hippoglossus</i>	
<i>Sebastes</i>	Chordata	Actinopterygii	Scorpaeniformes	Sebastidae	<i>Sebastes</i>	
<i>Myxine glutinosa</i>	Chordata	Myxini	Myxiniformes	Myxinidae	<i>Myxine</i>	<i>glutinosa</i>

Appendix 2. Counts (number of discreet individuals or colonies) of sprawling/encrusting taxa observed in the benthic imagery video screen captures.

Taxon	CBT-2	CBT-4	CBT-5	CBT-7
Bryozoa	32	10	1	1
Hydrozoa	25	34	19	1
Ophiuroidea	5	3	2	1
Porifera_7	30	7	0	0

Appendix 3. Analysis of Similarities (ANOSIM) and significance between transects. The value of R statistic close to 1 indicates a clear separation between transects. P-value is at 0.1% which is significant at less that $p < 0.001$.

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
CBT-4, CBT-2	0.807	0.1	Very large	999	0
CBT-4, CBT-7	0.525	0.1	Very large	999	0
CBT-4, CBT-5	0.071	1.3	Very large	999	12
CBT-2, CBT-7	0.754	0.1	Very large	999	0
CBT-2, CBT-5	0.768	0.1	Very large	999	0
CBT-7, CBT-5	0.428	0.1	Very large	999	0