NOTE:

Monitoring of Ecology Friendly Rock Berms Cables HKz

Data and Field results - 2022

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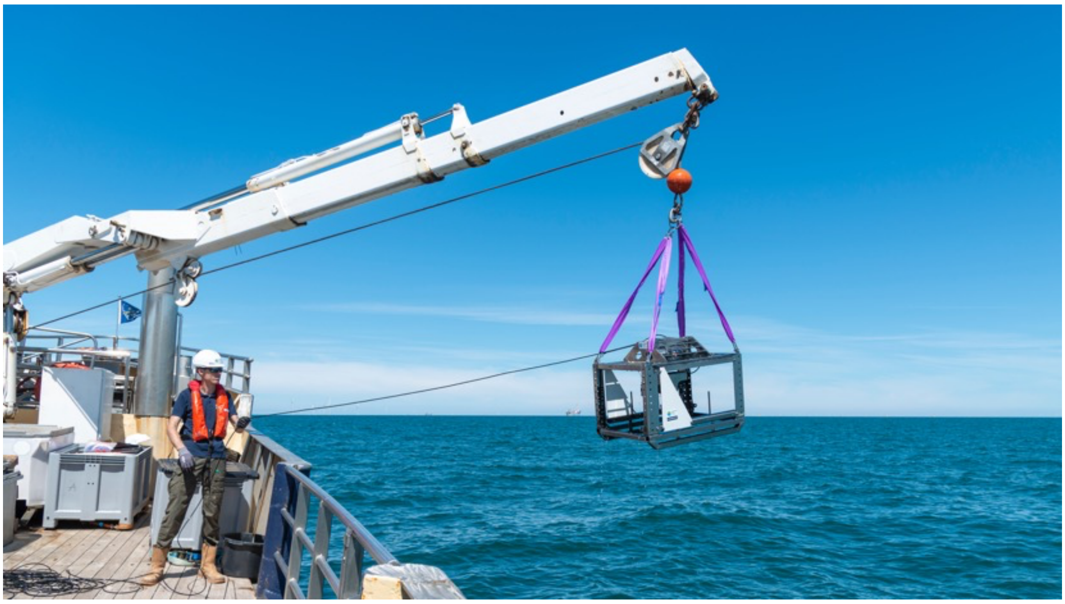

Deployment of the dropcam system from the MS Tender I (Photo: Udo van Dongen - WBE)

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Summary

Tennet constructed a first pilot of eco-friendly cable crossings in Hollandse Kust (zuid): the pilot area contains a top layer of marble instead of granite which commonly used. In the researched area both types of sprinkler layers are present. The general research question for this project is:

Do eco-friendly cable crossings (pilot design) have a higher biodiversity and/or abundance of reef (associated) species, compared to the conventional design both on the crossing works and in the direct vicinity (~10-20 m)?

The research question requires 2 different approaches for several species-groups:

1. Drop-cam (benthic sessile and slow-moving species, lots of detail)
2. Metabarcoding of environmental eDNA (semi-quantitative, highly sensitive for detection of all classified fish species)

In July 2022 the first survey (of three successive years) was performed, and the brief results are reported in this note.

Drop-cam survey: The dropcam system was acquiring video footage during a controlled drift over the sprinkler layers. At each location footage was acquired of the substrate (granite, marble or sand) which was analyzed off site for biodiversity (semi-quantitative). In this study 11 different taxa of marine life were observed. In total 41 different families and/or species of marine life were found. The highest number of species was observed on the sandy bottom, followed by the granite sprinkler layer and finally the marble sprinkler layer (29, 26 and 21 respectively).
The amount of individuals or colonies per still shows the highest number in the granite layer (4.2) , followed by the marble layer (3.65) and the sandy bottom (2.84).

Metabarcoding of environmental eDNA: Fish community assessment in the vicinity of the underwater cables was assessed with environmental DNA analysis. The fish species were identified using 12S and 16S mitochondrial genomic markers. For each marker gene (12S and 16S) two primersets were used, one fish optimized and one shark/ray optimized primer set. A total of twelve fish species were found: six benthic species and six pelagic species. No relation between stone type (G or M) and fish community composition was found based on the species composition. Three out of the four species that varied between locations were found both in location 2 and 3, which were located further offshore. The three-spined stickleback, which is considered an estuarian species, was only detected in location 1 (closest to shore).

Since the layers have been deployed recently (2021) further evolution of the communities might diverge in upcoming years. The planned surveys in 2023 and 2024 might give insight in the developments of this still fairly young habitats

1 Background and Scope of work

1.1 1.1 Purpose of research and general methods

Tennet constructed a first pilot of an eco-friendly cable crossings at cable crossings in Hollandse Kust (zuid): the pilot area contains a top layer of calcareous stone, meaning the entire sprinkler layer of a cable crossing is replaced with a layer of calcareous type of stone, such as marble or limestone, instead of conventional quarry stone such as granite. This pilot construction was applied to half of the crossings (see *table* 1).
As the application of marble is a pilot, it is essential to assess the impact on nature. The ecological development is monitored in 2022 for the first time and will be compared to the conventional design.

Table 1 Pilot setup of the cable crossings. Crossings are listed from south to north.

CDS location	Alpha 1 Sea conventional design	Alpha 2 Sea pilot design
TAQA 26-inch Gas P15D to Maasvlakte Pipeline	22-90mm Granite	22-90mm Marble
TAQA 10-inch Oil P15C to Hoek van Holland Pipeline	22-90mm Granite	22-90mm Marble & 22-90mm Granite1
Neptune 8-inch Oil Q13a-A to P15C Pipeline	22-90mm Granite	22-90mm Marble

1.1.1 Research question

The general research question for this project is:

Do eco-friendly cable crossings (pilot design) have a higher biodiversity and/or abundance of reef (associated) species, compared to the conventional design both on the crossing works and in the direct vicinity (~10-20 m)?

For a quick and semi-quantitative determination of the differences in biodiversity on the different sprinkler layers, video and stills will be acquired.

The research question requires 2 different approaches for several species-groups:

3. Drop-cam (benthic sessile and slow-moving species, lots of detail)
4. Metabarcoding of environmental eDNA (semi-quantitative, highly sensitive for detection of all classified fish species)

The sprinkler layer with marble is expected to hold a more bio-diverse marine community then the conventional granite sprinkler layer.

1.2 Geographical area and position of equipment

The operations took place at cable crossings with three life pipelines; from South to North that are the TAQA (P15-D to Maasvlakte) Gas pipeline, the TAQA (P15-C to Hoek van Holland) oil pipeline and the Neptune (formerly GDF Suez) (Q13a-A to P15-C) Oil pipeline. Tennet constructed (or will construct) a first pilot of an eco-friendly cable crossing at cable crossings in Hollandse Kust (zuid). In figure 1, the three target cable crossings are indicated with a red rectangle.

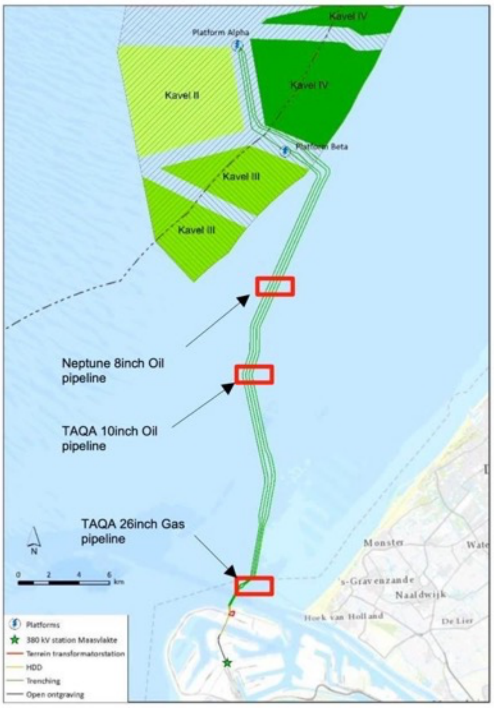


Figure 1: Locations of the cable crossings

1.3 Outline of this note

This note shows the results of the first field campaign (T1) which was performed during summer 2022 from the MS Tender I (*Photo* 1). The note is divided in three chapters which describe the performed field work (chapter 2), the results of the analysis of the acquired imagery including a brief interpretation of the results (chapter 3) and finally a species list determined from different eDNA samples that were collected during the field work (chapter 4).

An overall and more thorough and sensible analysis will be performed for the final report after the third and final field campaign in 2024.

Photo 1: The MS Tender departing from the Stellendam harbour (Udo van Dongen - WE)

2 FIELD WORK 2022 (3-6 July 2022)

The 2022 field work campaign was executed July 3-6th. Below a day by day description is presented.

July 3rd, 2022

Equipment and crew mobilized around 10 p.m. to the MS Tender in the Stellendam harbor (NL). All expedition members embarked that same night.

July 4th, 2022

Weather: mostly sunny, wind: 3 Bft WNW, wave height: 0.5-0.8 m

Departure from Stellendam harbor around 8:00 a.m., steaming towards the Alpha cable crossings HKZ. During the sail the toolbox meeting and Last Minute Risk Assessment (LMRA) were conducted and all equipment was prepared for operation. The Neptune Oil Pipeline (RWS ID: PL0228_PR; KP [km] 25.9) crossing was the first stop where deployment of the dropcam system with simultaneously water sampling for eDNA analysis was planned. The second stop was planned to be the TAQA Energy B.V. Oil pipeline (RWS ID: PL0039_PR; KP [km] 17.4) The third stop was the crossing at TAQA Energy B.V. Gas pipeline (RWS ID: PL0099_PR, KP[km] 2.4). On each location an initial sonar scan was performed in order to localize the sprinkler layer. Prior to the deployment of the camera system a drifting plan was prepared depending on the direction of the sprinkler layer, the actual tidal current, wave action and wind direction. A number of drifts over the sprinkler layers was performed to acquire sufficient and qualitative footage. During camera deployment water samples for eDNA analysis were taken for analysis at Wageningen University. At each location a separate eDNA sample was taken by filtering 50 l of sea water over a single use filter cartridge according to the prescribed protocol by Datura BV.

First camera deployment was performed at **location 3, Marble** (3M; Neptune Oil Pipeline, HKZ_RPL-D14_Alpha2) at 12:45. Second camera deployment was performed at **location 3, Granite** (3G; Neptune Oil Pipeline, HKZ_RPL-D14_Alpha1) at 14:15. Both locations were at 20 m depth during acquisition. During acquisition, the visibility was moderate but approriate for image acquisition and analysis.

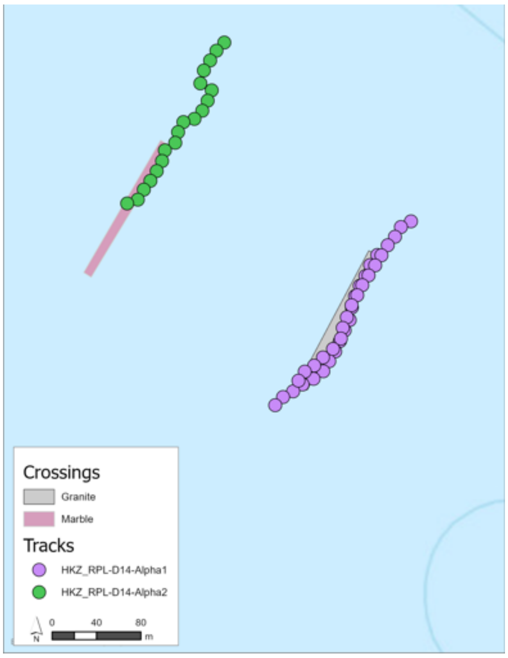


Figure 2: GPS-tracker data at Neptune Oil Pipeline

During acquisition it was possible to acquire proper footage for detailed analysis and several marine species already could be observed. Figure 2 shows the GPS tracker information which was recorded on deck during deployment. *Photo* 2 shows the 4K videocamera system in the frame that was used during this survey.

Photo 2: The 4K camera system which was used for image acquisition

The third and fourth camera deployments were conducted at **location 2 Marble** (2M, TAQA Energy B.V. Oil pipeline HKZ_RPL-D14_Alpha 2) and **location 2 Granite** (2G, TAQA Energy B.V. Oil pipeline HKZ_RPL-D14_Alpha 1) at 15:32 and 16:33 respectively. Both cable crossings at location 2 were at 20 m depth during acquisition but most marine life on the sprinkler layers was not visible due to coverage by a massive layer of Bryozoa (most likely *Electra Pilosa*). Visibility during acquisition was moderate. Figure 3 shows the GPS tracker information which was recorded on deck during deployment.

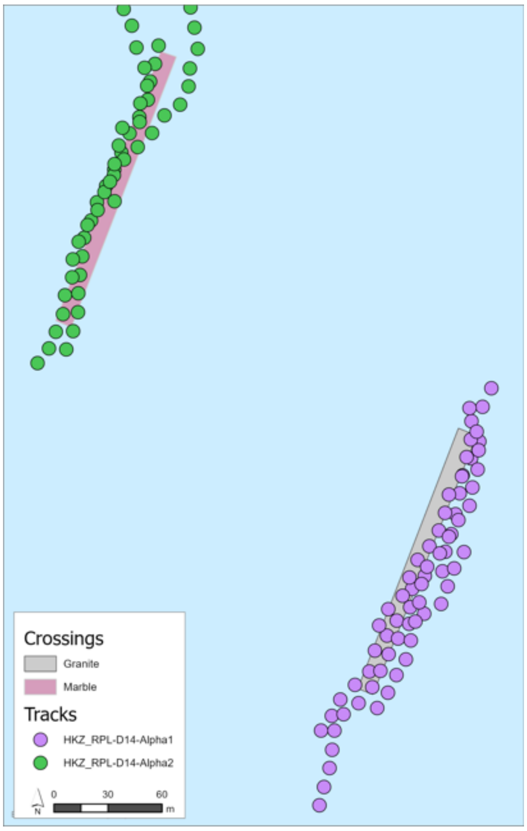


Figure 3: GPS- tracker data at TAQA Energy B.V. Oil Pipeline

At each location a separate eDNA sample was taken with a Niskin bottle for external investigation as well as by filtering 50 l of sea water over a single use filter cartridge for this research.

Photo 3: eDNA sampling with a Niskin bottle water sampling device.

July 5th, 2022

Weather: mostly sunny, wind: 3 Bft. NW,wave height 0.5-0.8 m

Departure from Pistoolhaven (Port of Rotterdam, NL) at 8:00 towards **location 1 Marble** (1M, TAQA Energy B.V. Gas Pipeline HKZ_RPL-D14_Alpha 2) and **location 1 Granite** (1G, TAQA Energy B.V. Gas Pipeline HKZ_RPL-D14_Alpha 1). At this location the sites of both sprinkler layers are within 20 meters of each other. Because the tidal current was nearly perpendicular to the sprinkler layer's direction, the plan was to gather video footage by drifting over both layers during each transect. Therefore, it was not possible to collect separate eDNA sample for each sprinkler layer. Dropcam footage of both sprinkler layers was acquired between 9:20 and 10:54. The visibility at this location was good for gaining video footage. During acquisition the depth of the sprinkler layers was between 14 and 15 meters and a large diversity of marine life could be observed. In several spots the armour layer of rocks was sticking out of the sprinkler layer.

Because of the direction of the current during deployment several attempts had to be conducted. In figure 4 the sampling locations are shown as they were recorded on deck with GPS during acquisition.

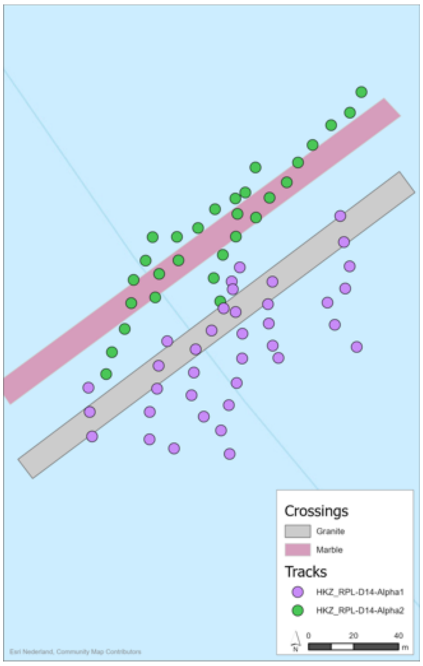


Figure 4: GPS-tracker data at TAQA Energy B.V. Gas Pipeline

July 6th, 2022

Demobilisation of equipment and departure back to Bureau Waardenburg headquarters in Culemborg.

3 Analysis of acquired imagery

All acquired footage was recorded as video at a resolution of 4K (3840 x 2160 pixels). The dropcam system was acquiring video footage during a controlled drift over the sprinkler layers. During acquisition the camera was controlled and monitored from the surface on a laptop computer. When necessary the crane line length was adjusted. All footage was backed up on external harddrives.

Table 2 shows a description of all investigated locations during the 2022 survey. The first column shows the location name as it was used during this research.

Table 2: investigated locations for this research

location	Sprinkler layer (tonnes)		3rd party asset name cf RPL	RPL	RWS ID	Product	Size	KP [km]	Depth [m LAT]	WGS84	
	Granite (t)	Marble (t)								Lat	Long
1G	1557		TAQA Energy B.V. Gas Pipeline	HKZ_RPL-D14-Alpha1	PL0099_PR	Gas	26"	2,397	-15,507	52°0'7.159"N	4°2'30.852"E
1M		1681	TAQA Energy B.V. Gas Pipeline	HKZ_RPL-D14-Alpha2	PL0099_PR	Gas	26"	2,446	-15,228	52°0'8.348'N	4°2'30.591'E
2G	597	0	TAQA Energy B.V. Oil pipeline	HKZ_RPL-D14-Alpha1	PL0039_PR	Oil	10"	17,351	-21,474	52°7'50.489"N	4°3'9.644"E
2M	114	569	TAQA Energy B.V. Oil pipeline	HKZ_RPL-D14-Alpha2	PL0039_PR	Gas	10"	17,479	-21,376	52°7'56.729'N	4°3'0.515'E
3G	722		Neptune Oil Pipeline	HKZ_RPL-D14-Alpha1	PL0228_PR	Oil	8"	25,867	-22,183	52°12'6.275"N	4°5'40.152"E
3M		1034	Neptune Oil Pipeline	HKZ_RPL-D14-Alpha2	PL0228_PR	Oil	8"	25,9	-22,106	52°12'9.442'N	4°5'29.999'E

All videos were analysed by extracting stills from the video footage. All stills that were extracted were stored in the lossless .DNG format (Digital Negative Graphics) which provides maximum options for post-processing. Furthermore stills were analysed that were acquired in the surrounding sand bed of each sprinkler layer. Each still was carefully determined by our marine specialist. All visible and recognizable species were analysed to the highest taxonomic level possible which is determined and limited by the visible layer in the photo and its quality.
Table 3 shows the total amount stills that were extracted from all acquired video footage that were used for analysis.

Table 3: Number of stills per locaton and per substrate

Location	Total # of stills	# of stills per substrate		
		granite	marble	sand
1	71	23	23	25
2	69	19	22	28
3	70	18	17	35

Photo 4 A-D and *Photo 5 A-D* show some examples of stills that were analysed for this study. Obviously, not all stills are of equal quality as conditions (visibility, current, waves, clouds, etc.) may vary during the day. Furthermore, at locations 2M and 2G massive layers of bryozoa (*Electra pilosa*) piled up around the sprinkler layers. These massive abundance of the organisms made it difficult to get a good view on the sprinkler layer, which might result in an underestimation of the amount of species. Most likely these bryozoa did not grow on the sprinkler layer but arrived at the sprinkler layers with tidal currents. *Photo 6* shows a still of this massively abundant bryozoa.
Furthermore, in all locations the underlying armour layer was visible, possibly due to mechanical disturbance or heavy storms.

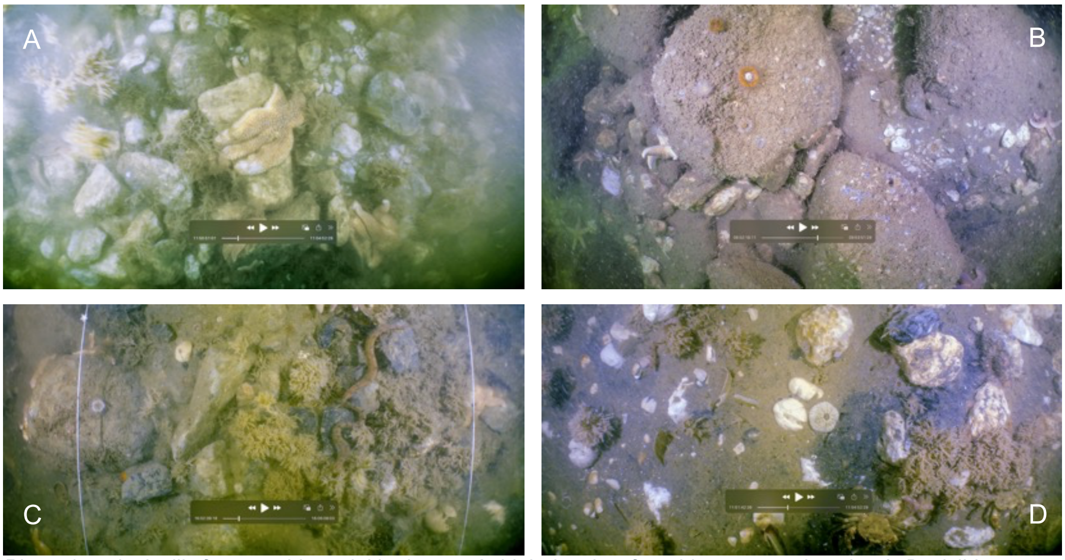


Photo 4: typical stills from marble sprinkler layers. A: high density of marble stones at location 3. B: visible armour layer inhabited by some Sagartia sp. *at location 3. C: Pholis gunnelus in a mixed environment of marble and granite stones at location 2. D: marble stone sprinkler layer at the border with the sandy bed.*

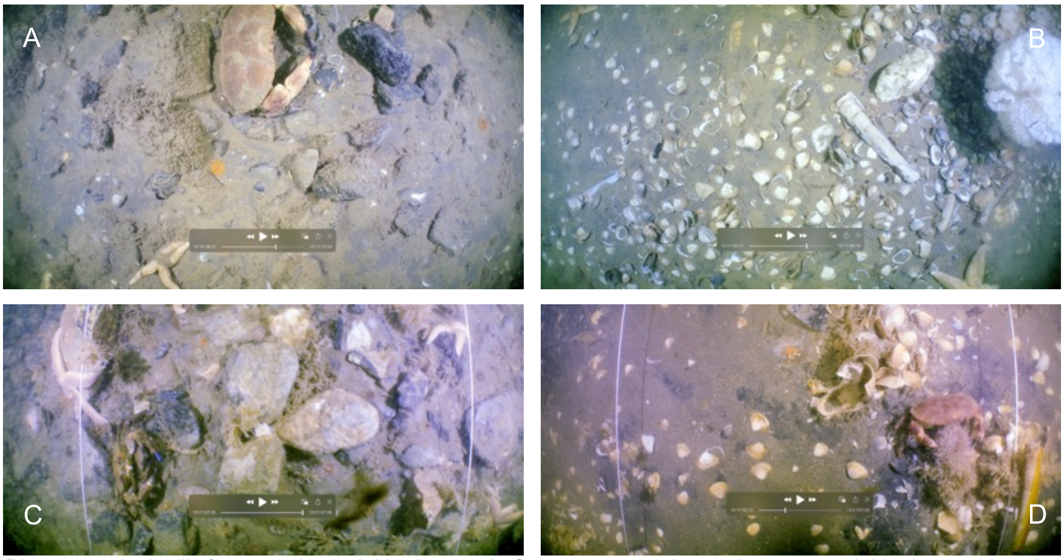


Photo 5: typical stills from granite sprinkler layers. A: Cancer pagurus in granite stone sprinkler layer at location 1. B: Granite sprinkler layer at border with sandy bottom, with Metridium senile *and* Asterias rubens. *C: granite sprinkler layer and some underlaying armour protection rocks at location 3. D: marine life on sandy bottom near granite sprinkler layer at location 3.*

Photo 6: Massive abundance of bryozoa Electra Pilosa, as observed on top of both sprinkler layers of marble and granite at location 2.

Species diversity

During this study 11 different taxa of marine life were observed. In total 41 different families and/or species of marine life were found. *Table 4* shows a list of all species and taxa that were observed in this study.

*Table 4: species list and their numbers on different substrates in the 2022 study (**green**: least abundant; **red**: most abundant)*

Species	Common name	Taxa	Granite (total #)	Marble (total #)	Sand (total #)
Lanice conchilega	sand mason worm	Annelida	0	0	2
Spionidae sp.	marine worms	Annelida	1	1	0
Anthozoa sp.	corals and sea anemones	Anthozoa	1	5	0
Metridium senile	frilled anemone	Anthozoa	5	5	1
Sagartia sp.	sea anemones	Anthozoa	47	50	7
Sagartia troglodytes	cave-dwelling anemone	Anthozoa	10	6	1
Sagartiogeton undatus	small snakelocks anemone	Anthozoa	1	1	0
Urticina felina	northern red anemones	Anthozoa	1	0	0
Cancer pagurus	edible crab	Arthropoda	8	7	1
Liocarcinus holsatus	flying crab	Arthropoda	0	0	2
Liocarcinus sp.	swimming crab	Arthropoda	1	0	2
Macropodia sp.	spider crabs	Arthropoda	0	0	1
Necora puber	velvet crab	Arthropoda	28	18	1
Pagurus bernhardus	common hermit crab	Arthropoda	3	3	10
Sessilia sp.	barnacles	Arthropoda	2	1	0
Bivalvia sp.	clams	Bivalvia	0	1	5
Chamelea striatula	clamstriped venus	Bivalvia	0	0	1
Spisula sp.	surf clams	Bivalvia	2	0	9
Bryozoa sp.	moss animals	Bryozoa	14	5	0
Electra pilosa	thorny sea mat	Bryozoa	47	42	38
Asterias rubens	common starfish	Echinodermata	49	51	49
Asteroidea sp.	starfish	Echinodermata	0	0	1
Ophiura albida	serpent's table brittle star	Echinodermata	0	0	8
Ophiuridae sp.	brittle star	Echinodermata	0	0	6
Psammechinus miliaris	green sea urchin	Echinodermata	0	0	1
Euspira sp.	necklace shells	Gastropoda	0	0	1
Gastropoda sp.	snails and slugs	Gastropoda	1	0	1
Nassarius sp.	dog whelks	Gastropoda	0	0	6
Ectopleura larynx	ringed tubularia	Hydrozoa	2	0	0
Hydractinia echinata	snail fur	Hydrozoa	2	1	2
Hydrozoa sp.	hydralike animals	Hydrozoa	9	4	6
Tubularia indivisa	oaten pipes hydroid	Hydrozoa	2	3	0
Discodoridae sp.	dorid nudibranch	Nudibranchia	0	1	0
Agonus cataphractus	hooknose	Pisces	0	0	1
Gobiidae sp.	goby	Pisces	0	0	4
Myoxocephalus scorpius	shorthorn sculpin	Pisces	2	0	0
Pholis gunnellus	butterfish	Pisces	6	13	2
Syngnathus sp.	pipefish	Pisces	0	0	1
Taurulus bubalis	long-spined bullhead	Pisces	2	0	0
Trisopterus luscus	pout whiting	Pisces	6	4	0
Cyanea lamarckii	blue jellyfish	Scyphozoa	1	4	3

In *table 5* the total number of different species per habitat type is shown.

Table 5: number of species per habitat type

habitat type	# species	# of stills
Granite	26	60
Marble	21	62
Sand	29	88

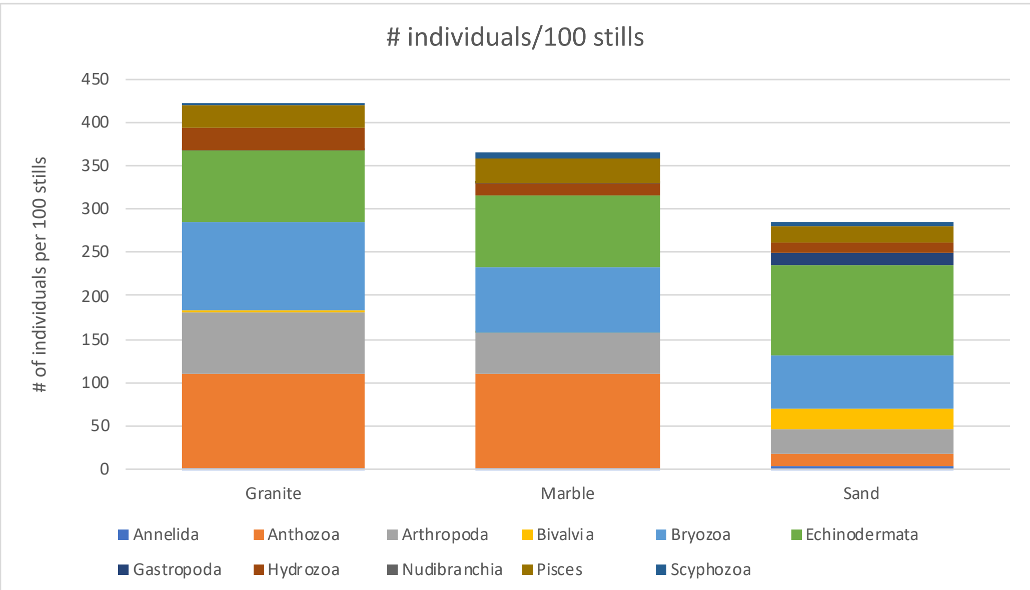
The highest number of species was observed on the sandy bottom, followed by the granite sprinkler layer and finally the marble sprinkler layer.

Abundance of species

In total 11 different taxa were observed in this study. All taxa were present in all habitats, but some taxa are more present in one habitattype then in the other. *Graph 1* and *table 6* show the total counted number of individuals or colonies of the different taxa per habitat type. To correct for the difference in the amount of stills analysed, the number of species is converted to # individuals / 100 stills.

Table 6: number of individuals per 100 stills.

Taxa	# individuals/100 stills		
	Granite	Marble	Sand
Annelida	1,7	1,6	3,2
Anthozoa	108,3	108,1	14,5
Arthropoda	70,0	46,8	27,4
Bivalvia	3,3	1,6	24,2
Bryozoa	101,7	75,8	61,3
Echinodermata	81,7	82,3	104,8
Gastropoda	1,7	0,0	12,9
Hydrozoa	25,0	12,9	12,9
Nudibranchia	0,0	1,6	0,0
Pisces	26,7	27,4	17,7
Scyphozoa	1,7	6,5	4,8
Total	**422**	**365**	**284**



Graph 1: number of individuals/colonies per habitat type.

Graph 1 shows that the differences in density and variety in taxa between the marble and granite sprinkler layer and sandy bottom. Both sprinkle layers are more or less comparable when it comes to composition and numbers of the organisms present. The sandy bottom shows the lowest number of individuals/colonies per 100 stills.
Echinodermata and bryozoa are present in comparable numbers in all habitat types, which can be explained by the motility of many species in these groups (autonomically or by current). The major difference between the hard and soft substrate habitats can be found in the anthozoa group: species in this group are benthic and have strong dependence on hard subtrates to attach to. Also arthropoda are more present in the sprinkler layer environment: many species in this group seek shelter under and around hard substrates.
On sandy bottom bivalves are obviously more visible and seem therefore more present: many bivalves prefer soft substrate to dig themselves in to hide.

No clear conclusions can be drawn, the biodiversity and species numbers in marble sprinkler layers is not obvious higher then in the granite sprinkler layers. Moreover, the species diversity in sandy areas exceed the number of species found om the rocky berms. On the other hand the number of individuals is higher on the scour layers when compared to the sandy parts.

Since the layers have been deployed recently (2021) further evolution of the communities might diverge in upcoming years. The planned surveys in 2023 and 2024 might give insight in the developments of this still fairly young habitats.

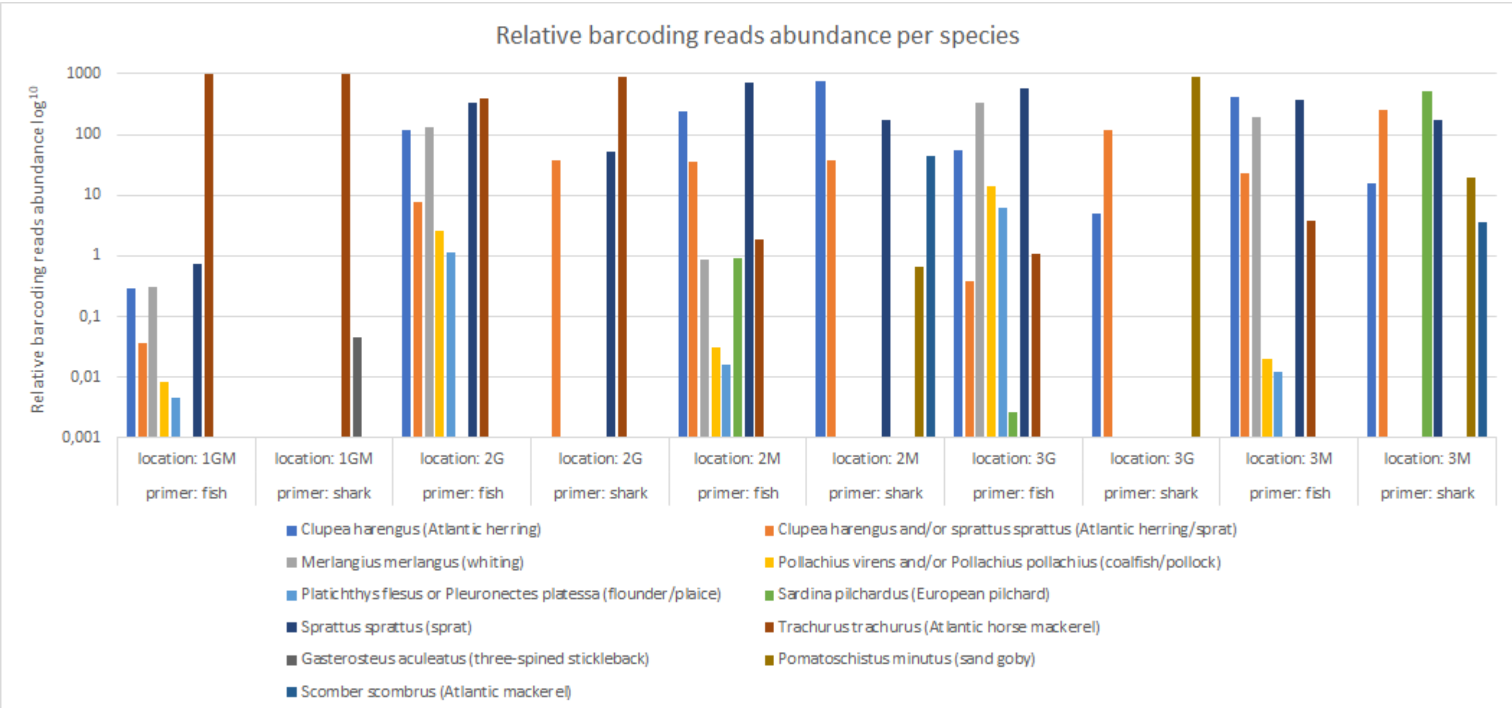
4 eDNA analysis

Fish community assessment in the vicinity of the underwater cables was assessed with environmental DNA analysis. For location 1MG, 2M, 2G, 3M and 3G a 50L seawater sample was taken approximately 1 meter above the sea bottom. Because of the close proximity between location 1M and 1G (20m) only a single eDNA sample was taken for both these locations (Location 1MG). To concentrate the eDNA, seawater was passed through a capsule filter (0,45 µm cellulose nitrate cellulose acetate). eDNA samples were immediately preserved on-site by addition of lysis buffer to the filter capsules. Subsequent fish eDNA analysis was performed by Datura BV using metabarcoding. The fish species were identified using 12S and 16S mitochondrial genomic markers. For each marker gene (12S and 16S) two primersets were used, one fish optimized and one shark/ray optimized primer set.

The sequencing analysis yielded a total of twelve fish species, six benthic species and six pelagic (*Table 7*). Eigtht of these fish species were detected at all locations, these included all benthic species exept the sand goby which was not detected in 1MG. No relation between stone type (G or M) and fish community composition was found based on the species composition. Three out of the four species that varied between locations were found both in location 2 and 3, which were located further offshore. The three-spined stickleback, which is considered an estuarian species, was only detected in location 1. This was to be expected since it is the sample point closest to the shore. Based on reads abundance pelagic fish (eg Atlantic herring, sprat and Atlantic horse marckerel) comprise the majority of barcoding reads (*Graph 2*). Note that the reads abundance can not directly be interpreted as species-biomass abundance data. Metabarcoding primer binding affinity differences between species introduces abundance biases during PCR amplification known as primer bias. Therefore the abundance patterns observed should only be interpreted roughly as indicative for abundance.

Table 7: Species presence/abdsence based on eDNA analysis

			1GM	2G	2M	3G	3M
Clupea harengus	Atlantic herring	pelagic	present	present	present	present	present
Merlangius merlangus	whiting	benthic	present	present	present	present	present
Pollachius virens	coalfish	benthic	present	present	present	present	present
Pollachius pollachius	pollock	benthic	present	present	present	present	present
Platichthys flesus	flounder	benthic	present	present	present	present	present
Pleuronectes platessa	plaice	benthic	present	present	present	present	present
Sprattus sprattus	sprat	pelagic	present	present	present	present	present
Trachurus trachurus	Atlantic horse mackerel	pelagic	present	present	present	present	present
Gasterosteus aculeatus	three-spined stickleback	pelagic	present	absend	absend	absend	absend
Pomatoschistus minutus	sand goby	benthic	absend	absend	present	present	absend
Scomber scombrus	Atlantic mackerel	pelagic	absend	absend	present	absend	absend
Sardina pilchardus	European pilchard	pelagic	absend	absend	present	present	present



Graph 2: Relative fish metabarcoding reads per species