NOTE:

Monitoring of Ecology Friendly Rock Berms Cables HKz

Data and Field results - 2023

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

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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 June 2023 the second 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 13 different taxa of marine life were observed. In total 47 different families and/or species of marine life were found. In 2023 the highest number of species was observed on the granite sprinkler layer, followed by the marble sprinkler layer and the sandy layer (39, 27 and 27 respectively).
The amount of individuals or colonies per still shows the highest number in the Marble layer (33.55) , followed by the granite layer (32.55) and the sandy bottom (6.85).

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.
The sequencing analysis yielded a total of eleven fish species, five benthic species and six pelagic. Sand goby (*Pomatoschistus minutus)* was the only species that was consistently found on only one substrate type (marble). Other species were detected on both substrates or just once on a single substrate. Atlantic mackerel (*Scomber scombrus*) and sand-eel (*Ammodytes spec.*) were only detected in the location furthest offshore (location 3).

Since the layers have been deployed in 2021 further evolution of the communities might diverge in upcoming years. The surveys in 2022, 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 2023 for the second 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 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.

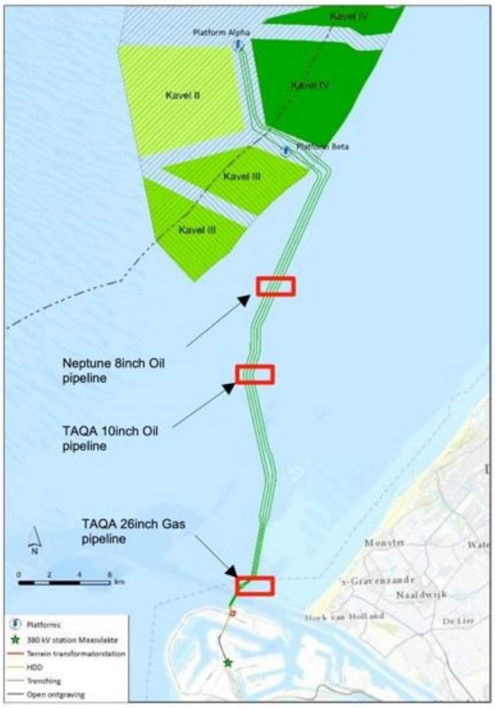


Figure 1: Locations of the cable crossings

1.3 Outline of this note

This note shows the results of the second field campaign (T1) which was performed in June 2023 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.

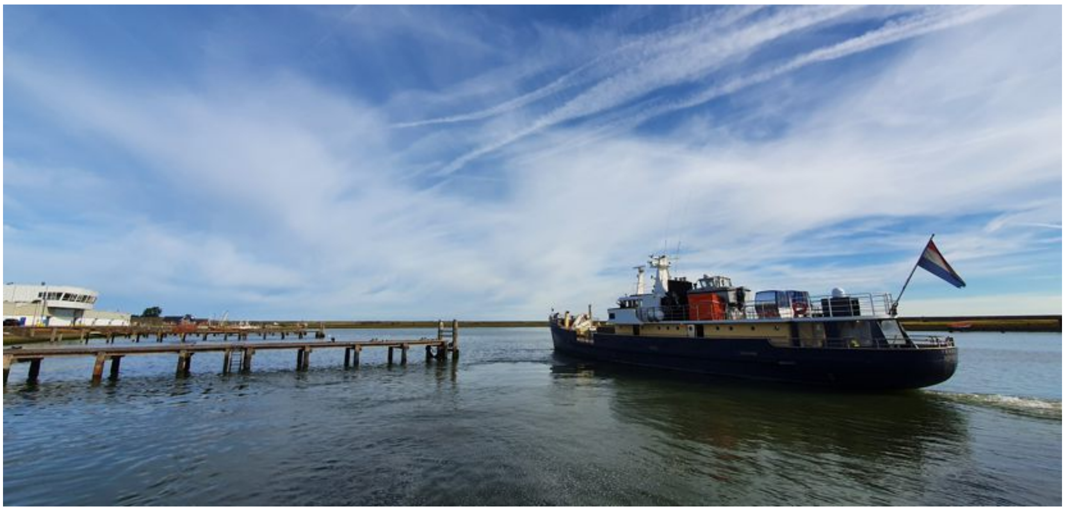


Photo 1: The MS Tender (Udo van Dongen - WE)

2 FIELD WORK 2023 (16-18 June 2023)

In 2022 the first survey was executed from July 3rd-6th. The second field work campaign was executed June 16-18th 2023. Below a day by day description of the 2023 survey is presented. On each day a daily progress report was sent to the client and other stakeholders (See appendix 1).

June 16th, 2023

Equipment and crew mobilized around 9 p.m. to the MS Tender I in Scheveningen harbor (NL). All expedition members embarked that same evening. That evening, the RAMS Briefing confirmation form was conducted with the entire crew and a planning was made for the following day (See appendix 2).

June 17th, 2023

Weather: sunny (morning), mild fog (afternoon), wind: 1-2 Bft NO (~3 at the end of the day), wave height: 0.0 - 0.5 m.

Departure from Scheveningen harbour around 8 a.m. First destination was the Alpha cable crossing HK(z). During the sail the equipment was prepared for operation.

Before deployment of the dropcam system with the ship's crane (Photo 2), sonar scans were executed to localize the cable and the sprinkle layer. Prior to each 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.

Photo 2: the dropcam system just before deployment

Simultaneous with camera deployment, 60 L of water for eDNA analysis sampled for analysis afterwards. For this a dedicated eDNA filter cartridge was attached to the dropcam frame (Photo 3). The outlet of the eDNA filter cartridge was connected to a vacuum hose, which was connected to a vacuum pump onboard. The inlet of the filter cartridge was open during sampling but kept closed until the latest moment before deployment to prevent contamination with foreigh DNA. At each location 60 L of seawater was sampled over the eDNA filter cartridge.

Photo 3: eDNA filter cartridge mounted to the dropcam frame. The filter cartridge shown is vacuum sealed and sterile. Just before deployment the cartridge is opened and the outlet was connected to the vacuum pump hose.

First stop was the Neptune Oil Pipeline (RWS ID: PL0228_PR; KP [km] 25.9) crossing: location 3, marble (3M) HKZ_RPL-D14_Alpha2, at 11:37).
The second deployment was **location 3, granite** (3G, HKZ_RPL-D14_Alpha1, at 13:42). Both locations were at approximately 20 m depth during acquisition. During acquisition, the visibility was very good, making the dropcam footage very suitable for image analysis. In photo 4 an example of the acquired footage for image analysis is shown. The photo was taken at location 3M (location 3, marble (3M) HKZ_RPL-D14_Alpha2).

During acquisistion of the video footage and the collection of the eDNA sample the position of the ship was logged with a GPS tracker. Figure 1 shows the GPS tracking data of both samplings (video footage and eDNA).

Photo 4: Example of the acquired footage for image analysis at location 3M

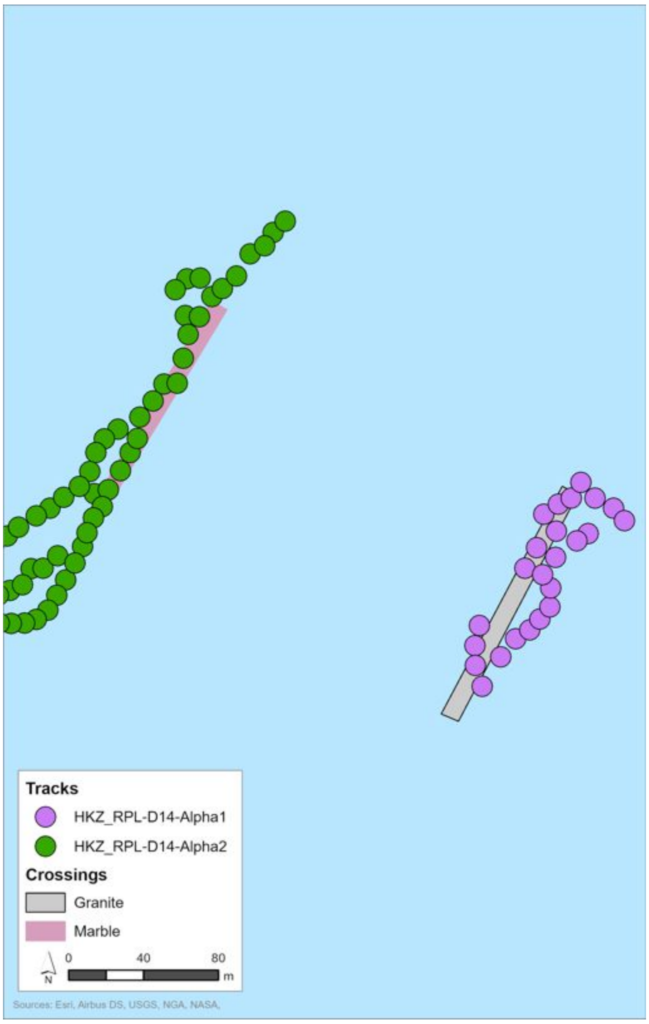


Figure 1: GPS tracking data of video en eDNA sampling at location 3Marble en 3Granite

The third deployment was at the **TAQA Energy B.V. Oil pipeline** (RWS ID:PL0039_PR; KP [km] 17.4): **location 2 Marble** (2M, HKZ_RPL-D14_Alpha 2, at 15:05). Visibility was worse at this location and because of the higher tidal currents the vessel drifted faster then during the first two deployments. However, the visibility was still sufficient, but because higher drifting speed it was more difficult to acquire in-focus, stable footage.
Figure 2 shows the GPS tracking data of the third deployment.

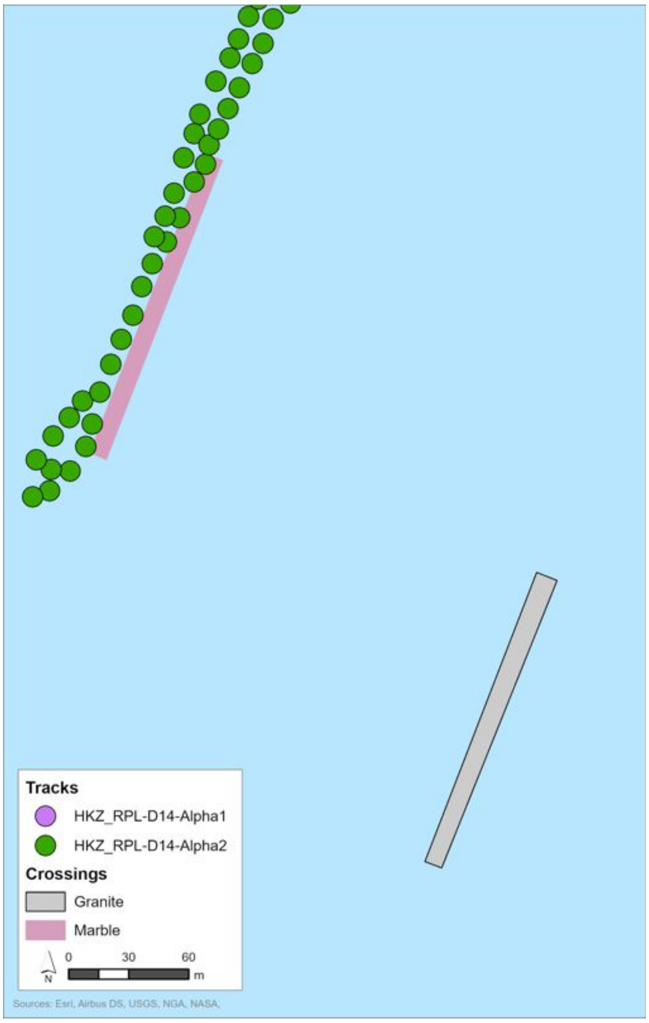


Figure 2: GPS tracking data of video en eDNA sampling at location 2 Marble

Because of the timing of the tides, **location 1 Granite** (1G, TAQA Energy B.V. Gas Pipeline HKZ_RPL-D14_Alpha 1, at 17:10) was scheduled next, followed by **location 1 Marble** (1M, TAQA Energy B.V. Gas Pipeline HKZ_RPL-D14_Alpha 2, at 18:05). At these locations, both situated at ~15 meters depth, the sites of the sprinkler layers are within 20 meters of each other. Still, two separate eDNA samples have been taken which, given the close proximity of the sites, in practice serves as a duplication. The visibility was lower than the previous locations because of silty sediments and turbidity. Figure 3 shows the GPS tracking data of the fourth and fifth deployment that were executed at location 1.

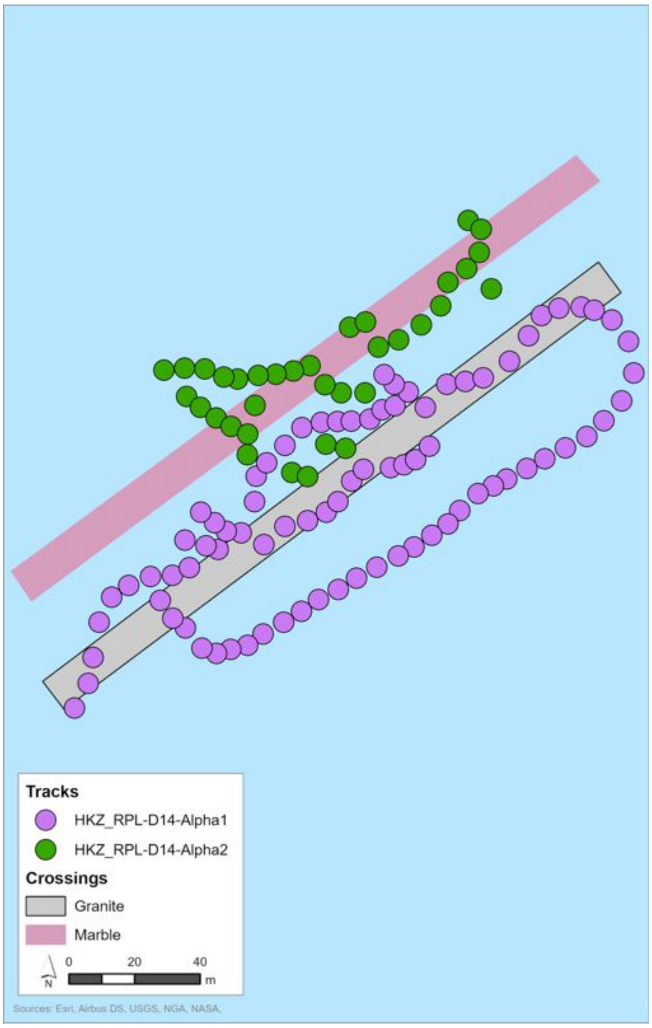


Figure 3: GPS tracking data of video en eDNA sampling at location 1 Marble en 1 Granite

Location 1 was the final location of the day and after the final deployment the ship sailed to the Pistoolhaven at the Rotterdam harbour to stay overnight.

June 18th

Weather: cloudy, wind 2-3 Bft O, wave height 0.0 - 0.5 m.

Departure from Rotterdam Pistoolhaven at 5 a.m. Final destination of the monitoring campaign was the TAQA Energy B.V. Oil pipeline (RWS ID:PL0039_PR; KP [km] 17.4): **location 2 Granite** (2G, HKZ_RPL-D14_Alpha 1, at 07:28). Visibility was very good - therefore the location at the TAQA Energy B.V. Oil pipeline (**Location 2 Marble** (2M, HKZ_RPL-D14_Alpha 2) which was sampled on June 17th, was surveyed with the dropcam again at 08:09. The second run resulted in much better footage quality because of the more calm currents. In Figure 4 the GPS tracking data is shown the the final two deplyments at location 2.

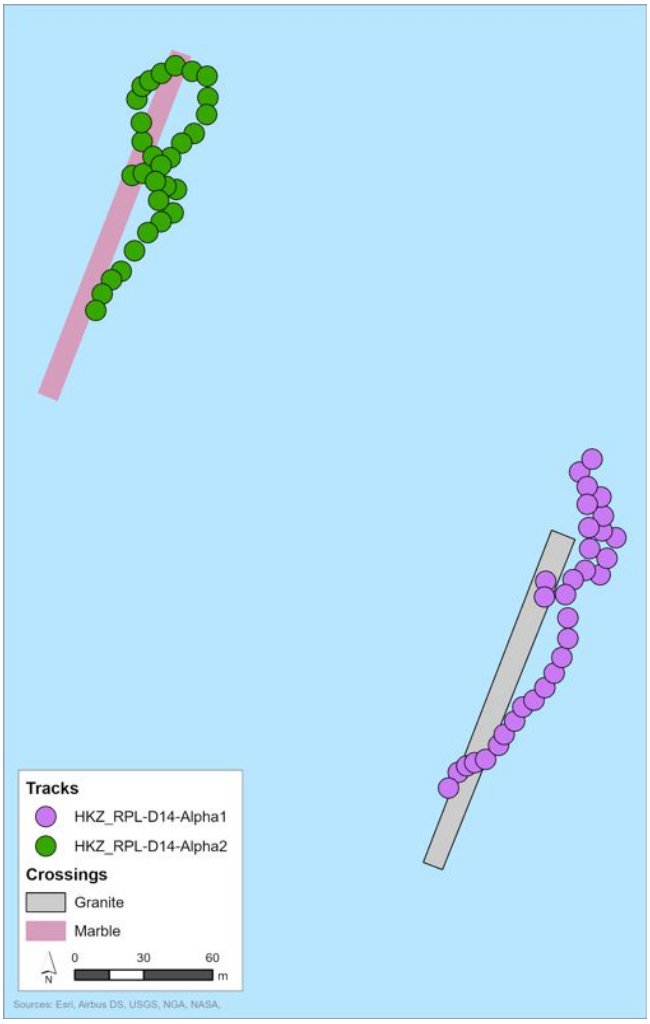


Figure 4: GPS tracking data of video en eDNA sampling at location 2 Marble en 2 Granite

The final deployment was finished around 08:30, after which the Tender I returned to harbor Scheveningen to arrive around 09:45.
During the morning all equipment was demobilized from the ship and loaded for transport.

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)			RPL	RWS ID	Product	Size	KP [km]	Depth [m LAT]	WGS84	
	Granite (t)	Marble (t)	3rd party asset name cf RPL							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

Due to very good sea conditions during acquisition an abundance adequate quality footage was available. In total 1121 stills were extracted and stored from all acquired video footage. Despite the good conditions at sea, varieties in quality of the selected stills still occurred. For analysis 60 stills per location were selected; 20 stills per substrate. During selection of these stills several parameters (rock size, substrate, abundance/coverage of life were taken in consideration in order to obtain the most diverse possible selection from each substrate. 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. Of each substrate a selection of 20 stills was analysed

Table 3: Number of stills per locaton and per substrate

Location	Total # of stills	# of stills per substrate extracted from video		
		granite	marble	sand
1	232	63	61	108
2	436	85	93	258
3	453	124	84	245

Photos 4 A-D and *Photo 5 A-D* exemplify the stills analyzed in this study. *Photo 4* llustrates typical scenes of the granite and marble sprinkler layers, while *photo 5* shows the visible armor layers. The underlying armor layer was visible at all locations, possibly attributed to mechanical disturbance or severe storms.

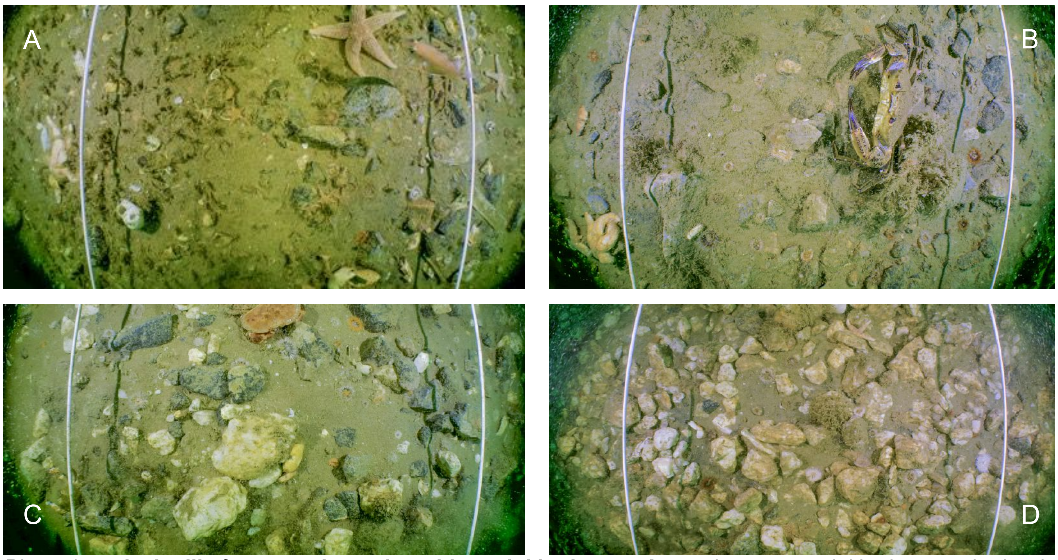


*Photo 4: **typical stills from granite and marble sprinkler layers**. **A**: granite stone sprinkler layer at the border with the sandy seabed, predominantly inhabited by Lanice conchilega and Ophiura ophiura at location 3G. **B**: Necora puber spotted near a hydrozoa colony with some nudibranchia on the granite stone sprinkler layer at location 3G. **C**: Cancer pagurus in a mixed environment of marble and granite stones at location 2M. **D**: an area with a high density of marble stones at location 3M.*

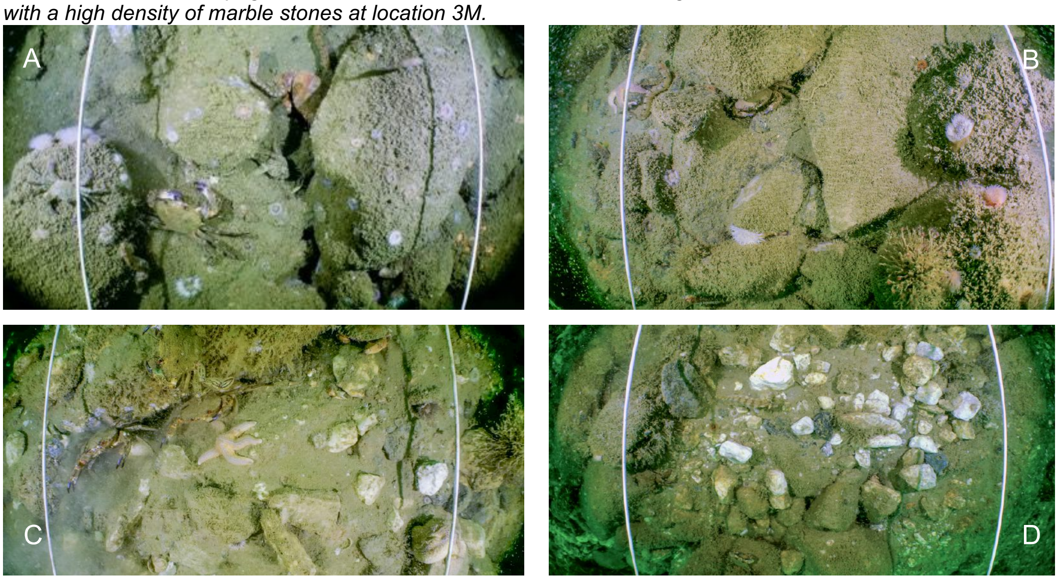


*Photo 5: **typical stills from armor layers**. **A**: location 2G, Taurulus bubalis and Pholis gunnelus among other species. **B**: Metridium senile and Tubularia indivisa at location 3G. **C**: armor layer at the boarder and sprinkled with marmer stones at location 2M. **D**: armor layer with marmer stones at location 3M, with Parablennius gattorugine present.*

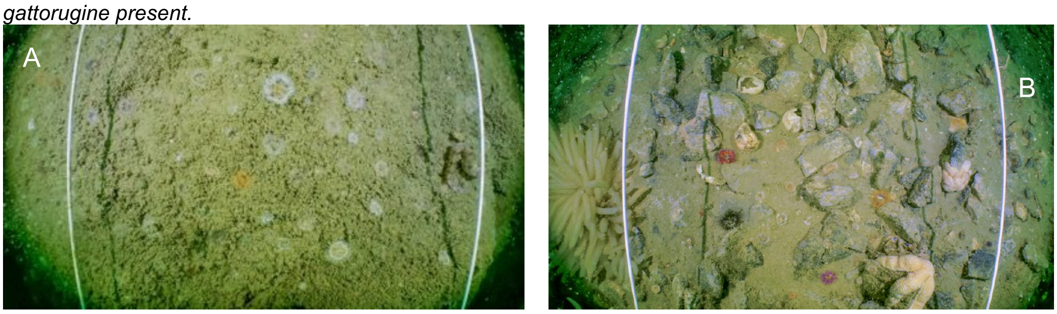


*Photo 6: **Additional observations from the stills. A**: massive abundance of amphipoda/polychaeta, evident in the formation of numerous tubes, observed on both the sprinkler layers and the armor layer across all locations. This image also shows the abundance of anthozoa. **B**: eggs from Loligo vulgaris, found outside the quadrat in granite area at location 3G, were similarly observed within the quadrat on the sandy area and were documented.*

Species diversity

During this study 13 different taxa of marine life were observed. In total 47 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 2023 study (**green**: least abundant; **red**: most abundant). For species without a common name, "N/A" (Not Available) is indicated.*

Species	Common name	Taxa	Granite (total #)	Marble (total #)	Sand (total #)
Lanice conchilega	sand mason worm	Annelida	11	23	58
Phyllodocidae	paddle worms	Annelida	4	0	2
Sabellidae	feather duster worms	Annelida	35	0	5
Serpulidae	sabellids	Annelida	5	0	0
Actiniaria	sea anemones	Anthozoa	0	2	0
Cylista	N/A	Anthozoa	948	1134	25
Cylista elegans	elegant anemone	Anthozoa	114	90	0
Cylista troglodytes	cave-dwelling anemone	Anthozoa	334	425	85
Metridium senile	frilled anemone	Anthozoa	37	51	0
Sagartia undata	small snakelocks anemone	Anthozoa	2	1	2
Amphipoda/Polychaeta	scuds/bristleworms	Anthozoa/Annelida	45	30	0
Brachyura	crabs	Arthropoda	5	9	6
Cancer pagurus	edible crab	Arthropoda	10	6	0
Caridea	caridean shrimp	Arthropoda	1	0	0
Liocarcinus	swimming crab	Arthropoda	1	0	0
Liocarcinus holsatus	flying crab	Arthropoda	0	1	0
Necora puber	velvet crab	Arthropoda	34	21	4
Pagurus bernhardus	common hermit crab	Arthropoda	1	0	1
Sessilia	barnacles	Arthropoda	5	7	0
Bryozoa	moss animals	Bryozoa	44	23	1
Electra pilosa	thorny sea mat	Bryozoa	13	19	1
Loligo vulgaris	common squid	Cephalopoda	0	0	1
Chlorophyta	green algae	Chlorophyta	1	0	0
Asterias rubens	common starfish	Echinodermata	160	108	73
Ophiura	serpent stars	Echinodermata	12	4	46
Ophiura albida	serpent's table brittle star	Echinodermata	0	0	34
Ophiura ophiura	serpent star	Echinodermata	18	0	37
Psammechinus miliaris	green sea urchin	Echinodermata	0	0	1
Euspira catena	large necklace shell	Gastropoda	1	0	1
Gastropoda	slugs and snails	Gastropoda	0	0	2
Tritia	dog whelks	Gastropoda	1	0	5
Callionymus lyra	common dragonet	Gnathostomata	1	2	8
Gnathostomata	jawed vertebrates	Gnathostomata	2	0	1
Gobiidae	goby	Gnathostomata	2	0	2
Parablennius gattorugine	tompot blenny	Gnathostomata	0	4	0
Pholis gunnellus	rock gunnel	Gnathostomata	17	6	1
Taurulus bubalis	long-spined bullhead	Gnathostomata	1	0	0
Trisopterus	small cods	Gnathostomata	2	1	0
Trisopterus luscus	pout whiting	Gnathostomata	17	7	4
Anthoathecata	athecate hydroids	Hydrozoa	4	1	0
Hydractiniidae	snails fur	Hydrozoa	0	0	3
Hydrallmania falcata	sickle hydroid	Hydrozoa	2	0	0
Hydrozoa	hydralike animals	Hydrozoa	47	31	2
Sertularia cupressina	whiteweed	Hydrozoa	1	0	0
Tubularia indivisa	oaten pipes hydroid	Hydrozoa	11	4	0
Nudibranchia	nudibranchs	Nudibranchia	2	1	0
Porifera/Ascidiacea	sponges/sea squirts	Porifera/Ascidiacea	2	1	0

In *table 5* the total number of species/groups per habitat type is shown. This number is based on the numbers in Table 4.

Table 5: number of species per habitat type

Habitat type	# species	# stills
Granite	39	60
Marble	27	60
Sand	27	60

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

Abundance of species

In total, 13 distinct taxa were observed during this study. Among them, two taxa were categorized as anthozoa/annelida and porifera/ascidiacea. These classifications were introduced due to inherent limitations of image analysis, as detailed information is not retrieveable. While the majority of taxa were observed across all habitats, chlorophyta was exclusively found in the granite area *photo 7*. Additionally, porifera/ascidiacea were present in all hard substrate areas and not on the sandy seabed. Some taxa are more present in one habitat type 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. It is important to mention that colony forming organisms like *Lanice conchilega*, sessilia, amphipoda/polychaeta, porifera/ascidiacea, hydrozoa, and bryozoa are counted as a single individual. This method may lead to a potential underestimation of species abundance.

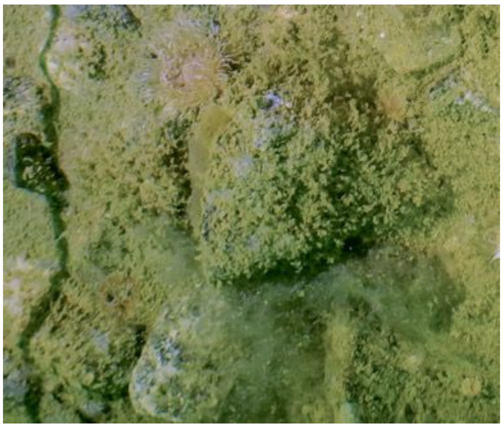
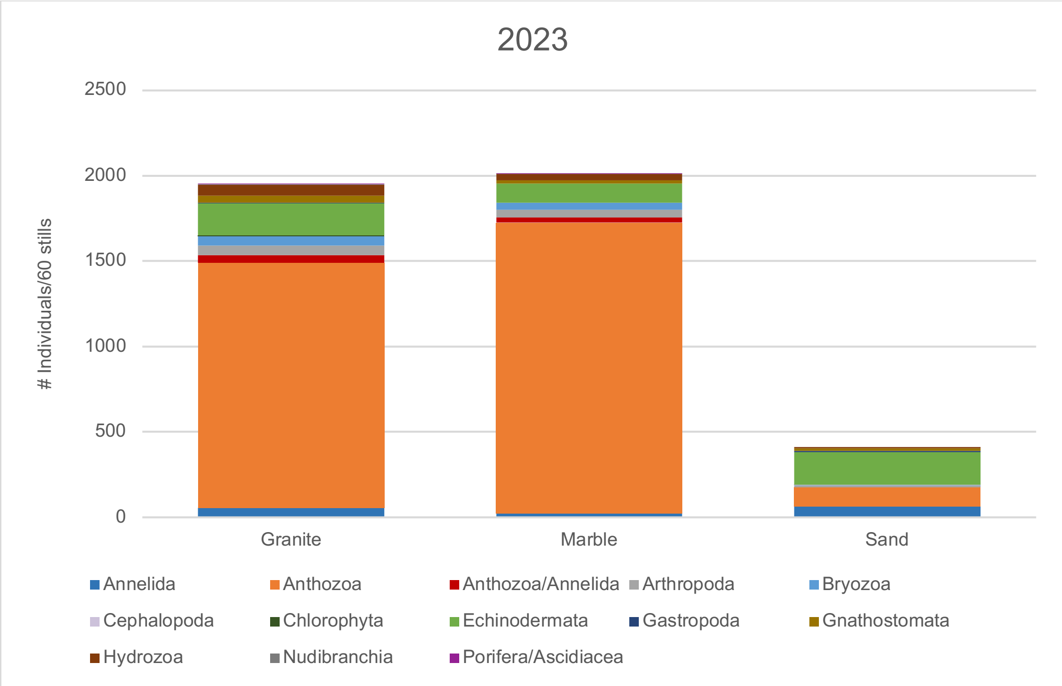


Photo 7: A cropped still capturing chlorophyta at location 3G.

Table 6: number of individuals/colonies per 60 stills.

Taxa	# individuals/60 stills		
	Granite	Marble	Sand
Annelida	55	23	65
Anthozoa	1435	1703	112
Anthozoa/Annelida	45	30	0
Arthropoda	57	44	11
Bryozoa	57	42	2
Cephalopoda	0	0	1
Chlorophyta	1	0	0
Echinodermata	190	112	191
Gastropoda	2	0	8
Gnathostomata	42	20	16
Hydrozoa	65	36	5
Nudibranchia	2	1	0
Porifera/Ascidiacea	2	1	0
Total	**1953**	**2012**	**411**



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

Graph 1 shows the differences in density and variety in taxa between the marble and granite sprinkler layer and the sandy bottom. Both sprinkler 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 60 stills.

While the sandy bottom area generally exhibits fewer visible species compared to the other two areas, it does stand out for the higher species abundance in the taxonomic groups annelida, echinodermata, gastropoda, and mollusca. While this might initially appear substantial, it's important to note that the actual number of species seems to be relatively low, which is not nescesarily true. Many species, belonging to for example the Annelida or Asteroidea class, inhabit the lower layers of the sandy bed. They are not visible and therefore they cannot be observed.

Photo 8: a cropped still capturing the eggs of Loligo vulgaris at location 3Z.

The variations in the number of individuals across taxonomic groups in this region can be attributed to specific preferences and behaviors. Many gastropoda species prefer to inhabit soft substrates and therefore they seem to be more present on sandy bottoms. The same effect applies to annelida like *Lanice conchilega*: they exclusively inhabit soft sediment beds. *Loligo vulgaris* eggs, categorized under Cephalopoda, were spotted on both the sandy bottom (*photo 8*) and in the granite area at location 3, outside the quadrant (*photo 6B*). Despite appearing on the sandy area, they must have settled onto a form of hard substrate. Based on the footage it cannot be determined what hardsubstrate it was. There is a minimal disparity of one individual between the sandy bottom and granite, where the deviation of 79 individuals is present in the marble area. This might be due to the autonomous movement or transportation by (tidal) currents, rather than the type of substrate.

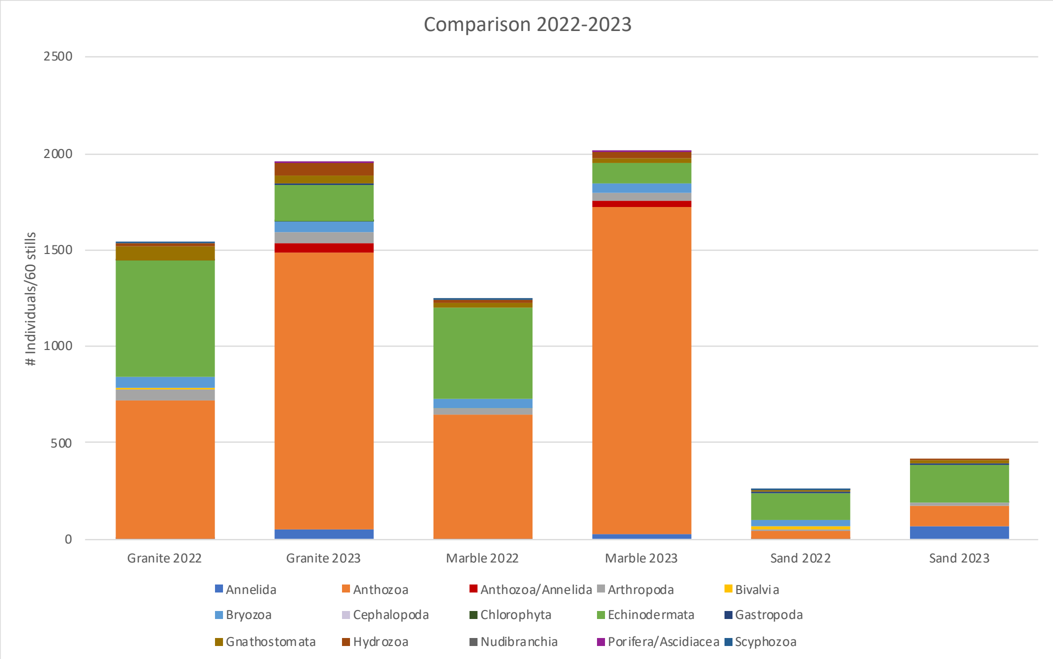
The primary distinction between the hard and soft substrate habitats lies within the Anthozoa group. Species in this category are predominantly benthic, and many of them rely heavily on hard substrates for attachment. The bryozoa and hydrozoa observed in this year’s survey also predominantly rely on hard substrate. Nudibranchs often consume species associated with hard substrate, such as sea anemones, hydrozoans, and bryozoans. This explains why they are mostly observed in this area. Additionally, arthropoda are more prevalent in the sprinkler layer environment, with numerous species seeking shelter beneath and around these rigid surfaces.

The substantial differences observed in gnathostomata populations (formerly listed as pisces) across the three different substrates are challenging to explain. The observed variation might arrise from the diverse habitat preferences among species, but this cannot be concluded. In contrast, for chlorophyta and porifera/ascidiacea, which have been observed only infrequently, there is little information available.

The biodiversity and species counts in marble sprinkler layers do not distinctly differ from those in granite sprinkler layers and therefore at this point no conclusion can be drawn. Given the recent deployment of the sprinkler layers (2021), the communities may undergo further evolution in the coming years. The planned survey in 2024 possibly offers the potential to gain valuable insights into the development of these relatively young habitats.

Brief comparison 2022-2023

In total, the 2023 study observed 13 distinct taxa. In 2022 11 different taxa were found. Some taxa are more present in one habitattype then in the other. *Graph 2* and *Table 7* show a comparison of the total counted number of individuals/colonies of the different taxa per habitat for the years 2022 and 2023.



Graph 2: comparison of the number of individuals/colonies per habitat type for the years 2022 and 2023.

*Table 7: The numerical difference in the number of individuals/colonies per habitat type for the years 2022 and 2023 (**green**: positive difference; white: minimal to no difference; **red**: negative difference).*

Taxa	# individuals/60 stills						Difference 2022-2023		
	Granite 2022	Granite 2023	Marble 2022	Marble 2023	Sand 2022	Sand 2023	Difference Granite	Difference Marble	Difference Sand
Annelida	1	55	1	23	1	65	54	22	64
Anthozoa	719	1435	642	1703	40	112	716	1061	72
Anthozoa/Annelida	0	45	0	30	0	0	45	30	0
Arthropoda	60	57	38	44	14	11	-3	6	-3
Bivalvia	2	0	1	0	16	0	-2	-1	-16
Bryozoa	61	57	45	42	26	2	-4	-3	-24
Cephalopoda	0	0	0	0	0	1	0	0	1
Chlorophyta	0	1	0	0	0	0	1	0	0
Echinodermata	605	190	476	112	143	191	-415	-364	49
Gastropoda	1	2	0	0	6	8	1	0	2
Gnathostomata	71	42	26	20	5	16	-29	-6	11
Hydrozoa	15	65	10	36	5	5	50	26	0
Nudibranchia	0	2	1	1	0	0	2	0	0
Porifera/Ascidiacea	0	2	0	1	0	0	2	1	0
Scyphozoa	1	0	4	0	2	0	-1	-4	-2
Total	**1536**	**1953**	**1244**	**2012**	**259**	**411**	**417**	**768**	**152**

In *Graph 2*, an overall increase in the number of counted individual/colonies is evident. However, upon closer examination of the numerical differences presented in *Table 7*, there are exceptions in certain taxonomic groups, including bivalvia, bryozoa, echinodermata, gnathostomata, and scyphozoa. Although both surveys were conducted at the same season, it is likely that seasonal differences per year may vary. Between 2022 and 2023 this could account for these anomalies within certain taxa in this group. Within gnathostomata, the most notable variation is observed in the population of Trisopterus luscus. The abundance of this pelagic fish fluctuates due to migration patterns, potentially leading to a decrease within this year's gnathostomata group. For the bivalvia, no significant differences between 2023 and 2022 were observed.

In 2022, Echinodermata and Bryozoa (mostly *Electra pilosa*) were present in comparable numbers across all habitat types. This can possibly be attributed to the mobility of many species within these groups, either through autonomous movement or by (tidal) currents. In 2023, this is only evident in the case of echinodermata. This change is likely linked to the substantial decrease in *Electra pilosa*, a seasonal Bryozoa which travelled to the area by tidal currents. In this year’s survey extensive layers of bryozoa (*Electra pilosa*) were absent around the sprinkler layers. Compared to 2022 there was a reduction of -74.02%, resulting in a notably improved visibility of the sprinkler- and amour layer. Therefore, typical benthic species like anthozoa were more often observed during this years survey (*photo 6A*). Bryozoa observed in this year’s survey were found in all three locations but are mostly benthic.

An increase in species abundance and variety has been observed over the past two years for both sprinkler layers. Whether this increase is due to natural evolution of the ecosystem remains to be seen. Due to good weather and visibility conditions the 2023 footage was more diverse and generally of better quality then in 2022. The difference in numbers and diversity between granite versus marble sprinkler layers is in 2023 also not signicant.

4 eDNA analysis

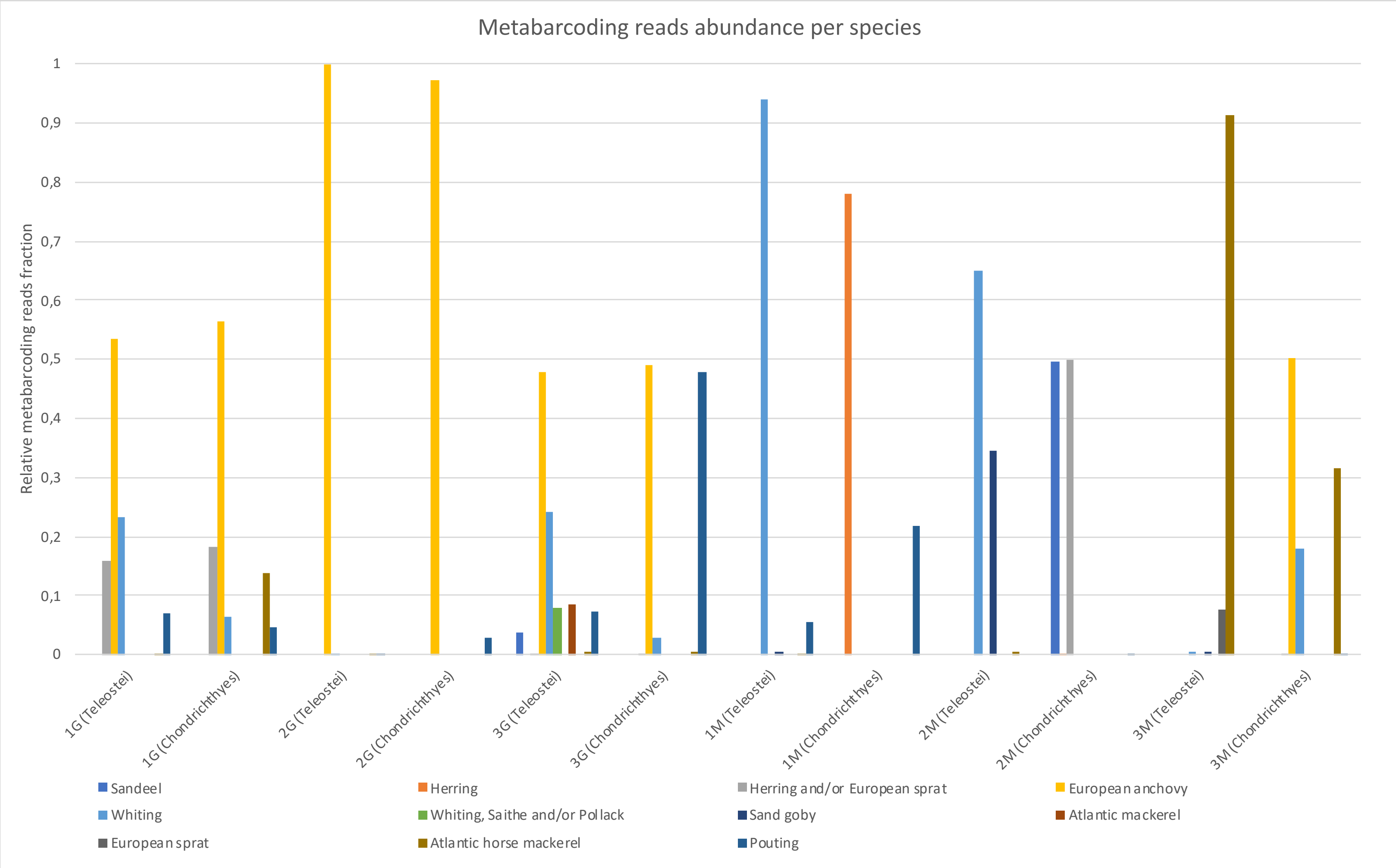
Fish community assessment in the vicinity of the underwater cables was assessed with environmental DNA analysis. For location 1MG, 2M, 2G, 3M and 3G a 60L seawater sample was taken approximately 0,5 meter above the sea bottom. To concentrate the eDNA, seawater was passed through a filter capsule filters (0,45 µm pore size, filter material cellulose nitrate cellulose acetate). EDNA samples were immediately preserved on-site by addition of preservation buffer to the filter capsules. Subsequent fish eDNA analysis was performed by the company Datura using metabarcoding. The fish species were identified using 12S and 16S mitochondrial genomic markers. Two custom developed primersets were used by Datura, one optimized for bony fish (Teleostei) and one primerset optimized for sharks and rays (Chondrichthyes).

The sequencing analysis yielded a total of eleven fish species, five benthic species and six pelagic (*Table 8; Graph 3*). No shark or ray species were detected with either the Teleostei and Chondrichthyes optimized primersets. Although the majority of fish species were detected with Teleostei optimized primers, in some cases Chondrichthyes optimized primers yielded detection of additional species that were not picked up by the other primerset. This can be explained by the fact that the Chondrichthyes optimized primers are not designed to exclusively amplify sharks and rays, but rather modified bonyfish primers that were optimized to preferentially amplify these species. Therefore these primers are still capable of amplifying bony fish species. Additionally, both primersets have different DNA target binding affinity for different fish species (primers bias). This binding affinity affects the rate of species target amplification during PCR. Translating into variable levels of detectability between species within a metabarcoding analysis. These differences in binding affinity can explain why some species are detected by one primerset but not by the other.

Table 8: Species presence absence based on eDNA detection

Common name	Scientific name		1G	2G	3G	1M	2M	3M
Sandeel	*Ammodytes spec*	Benthic	Absent	Absent	Present	Absent	Absent	Absent
Herring	*Clupea harengus*	Pelagic	Absent	Absent	Absent	Present	Absent	Absent
Herring and/or European sprat	*Clupea harengus* and/or *Sprattus sprattus*	Pelagic	Present	Absent	Present	Absent	Present	Present
European anchovy	*Engraulis encrasicolus*	Pelagic	Present	Present	Present	Absent	Absent	Present
Whiting	*Merlangius merlangus*	Benthic	Present	Present	Present	Present	Present	Present
Whiting, Saithe and/or Pollack	*Merlangius merlangus* or *Pollachius virens* or *Pollachius pollachius*	Benthic	Absent	Absent	Present	Absent	Absent	Absent
Sand goby	*Pomatoschistus minutus*	Benthic	Absent	Absent	Absent	Present	Present	Present
Atlantic mackerel	*Scomber scombrus*	Pelagic	Absent	Absent	Present	Absent	Absent	Absent
European sprat	*Sprattus sprattus*	Pelagic	Absent	Absent	Absent	Absent	Absent	Present
Atlantic horse mackerel	*Trachurus trachurus*	Pelagic	Present	Present	Present	Present	Present	Present
Pouting	*Trisopterus luscus*	Benthic	Present	Present	Present	Present	Present	Present

The metabarcoding reads were clustered in both single species and multispecies OTUs (Operation Taxonomic Units). Whiting (*Merlangius merlangus)*, pouting (*Trisopterus luscus)* and Atlantic horse mackerel (*Trachurus trachurus*) were present on all locations. Sand goby (*Pomatoschistus minutus)* was the only species that was consistently found on only one substrate type (Marble). Other species were detected on both substrates or just once on a single substrate. Atlantic mackerel (*Scomber scombrus*) and sand-eel (*Ammodytes spec.*) were only detected in the location furthest offshore (location 3).
European anchovy (*Engraulis encrasicolus)*, sprat (*Sprattus sprattus*) and herring (*Clupea harengus)* made up the largest part of the detected pelagic fish reads (*Graph 3*). For the benthic species whiting and pouting were most detected. Note that the reads abundance cannot 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, only large differences in reads abundance can be interpreted as indicative of species biomass abundance.



Graph 3 Relative reads abundance based on filtered metabarcoding reads for bony fish optimized primers (Teleostei) and cartilaginous fish (Chondrichthyes).

5 Appendixes

Appendix 1: Daily reports and toolbox/LMRA

Appendix 2: RAMS Briefing confirmation form

Appendix 1: Daily reports and toolbox/LMRA

WAARDEN BURG Ecology

DAILY PROGRESS REPORT

GENERAL		PROVIDED TO:
Project number	21-0569	TenneT: Wijnand Mellegers
Project name	ring of ecology friendly rock berms cables hollandse ku	WE: Edwin Kardinaal
Date	17-06-2023	WiBo: Annmiek Hermand
Location	Scheveningen - cable crossings HK(z)	Tennet: Saskia Jaarsma
Surveyors	Udo van Dongen	
	Joost Bergsma	
	Britte Schilt	
Vessel	MS Tender I	

DAILY CHECKS
Weather
Toolbox meeting / LMRA
Equipment

ACTIVITY

Time (local)	Activitity	Comments
08:00	Departure Scheveningen	
11:37	First dropcam & eDNA samples (3M)	
13:42	Dropcam & eDNA samples (3G)	
15:05	Dropcam & eDNA samples (2M)	
17:10	Dropcam & eDNA samples (1G)	
18:05	Dropcam & eDNA samples (1M)	
19:15	Arrival Rotterdam	

GENERAL COMMENTS

According to plan.

WEATHER

Time (local)	Wind direction	Windspeed (Kts)	Sea State	Visibility
08:00	from N/NO	1-2 Bft	calm (1)	high
14:00	from N/NO	2 Bft	calm/smooth (2)	high
18:00	from NO	3-4 Bft	slight (3)	medium

PROGRESS SUMMARY

Sampling (Dropcam and eDNA) of 5 locations:
3M, 3G, 2M, 1M and 1G

HSE OBSERVATIONS

Type	Description	Immediate action(s) taken	Reported to QHSE manager

Bijlage I Toolboxformulier: Varen/werken langs het water

Project: 21-0569
Locatie: Noordzee
Datum: 17-6-2023
Door: UDO VAN DONGEN

Onderwerpen toolboxmeeting:
(Na behandelen afvinken)

	Onderwerp	
1	Waar gaan we varen/werken langs het water, wat gaan we doen	✓
2	Welke technieken/ gereedschappen gaan we toepassen	✓
3	Wie is er aanwezig	✓
4	Wie heeft welke taken en verantwoordelijkheden	✓
5	Stilstaan bij risico's *(aanvullingen n.a.v. veldsituatie)* en veiligheidsmiddelen, inclusief PBM's (Persoonlijke Beschermingsmiddelen)	✓
6	Bij varen met eigen vaartuig: Doornemen "werkinstructies varen met eigen vaartuig" en uitleg van schipper aan opvarenden hoe boot werkt	n.v.t.
7	Voorzorgs-/ beheersmaatregelen omgaan met motorboten en werken op het water, zie ook werkinstructie	n.v.t.
8	Scheepvaart en andere gebruikers	✓
9	Weersomstandigheden	✓
10	Noodnummers en noodprocedures (check)	✓
11	Aangemeld bij verkeerscentrale	✓
12	Zijn er persoonlijke gezondheidsrisico's	✓

Aanwezig

	Naam aanwezigen:	Handtekening
1	Udo van Dongen	[signature]
2	Joost Bergsma	[signature]
3	Britte Schilt	[signature]
4	Niek Laze	[signature]
5	Frank Loonstra	[signature]
6	Piero Tundo	[signature]
	Siebren Jansma	[signature]

DAILY PROGRESS REPORT

GENERAL		PROVIDED TO:
Project number	21-0569	TenneT: Wijnand Mellegers
Project name	ring of ecology friendly rock berms cables hollandse ku	WE: Edwin Kardinaal
Date	18-06-2023	WiBo: Annemiek Hermans
Location	Scheveningen - cable crossings HK(z)	Tennet: Saskia Jaarsma
Surveyors	Udo van Dongen	
	Joost Bergsma	
	Britte Schilt	
Vessel	MS Tender I	

DAILY CHECKS
Weather
Toolbox meeting / LMRA
Equipment

ACTIVITY		
Time (local)	Activitity	Comments
05:00	Departure Rotterdam Pistoolhaven	
07:28	Dropcam and eDNA sample (2G)	
08:09	Dropcam (duplicate yesterday (2M)	
09:45	Arrival Scheveningen	

GENERAL COMMENTS
According to plan.

WEATHER				
Time (local)	Wind direction	Windspeed (Kts)	Sea State	Visibility
08:00	from O/ZO	variable, 2-3	slight (2-3)	high

PROGRESS SUMMARY
Sampling (Dropcam and eDNA) of 1 location: 2G
Repeating sampling (Dropcam) of 2M,
because of high visibility

HSE OBSERVATIONS			
Type	Description	Immediate action(s) taken	Reported to QHSE manager

Bijlage I Toolboxformulier: Varen/werken langs het water

Project: 21-0569
Locatie: Noordzee
Datum: 18-6-2023
Door: UDO VAN DONGEN

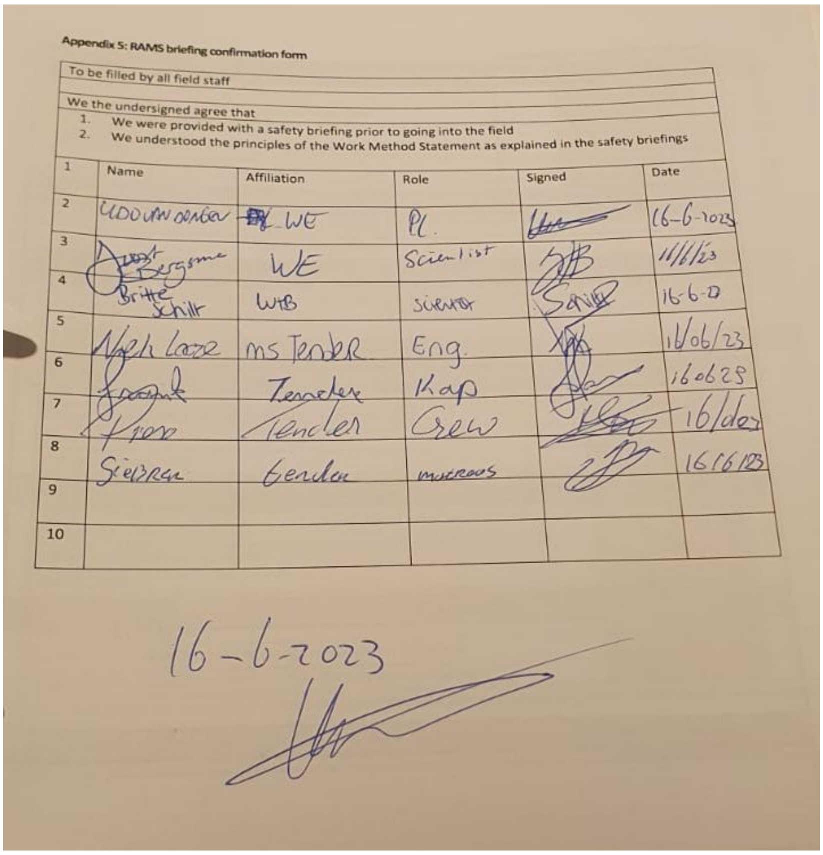
Onderwerpen toolboxmeeting:
(Na behandelen afvinken)

	Onderwerp	
1	Waar gaan we varen/werken langs het water, wat gaan we doen	✓
2	Welke technieken/ gereedschappen gaan we toepassen	✓
3	Wie is er aanwezig	✓
4	Wie heeft welke taken en verantwoordelijkheden	✓
5	Stilstaan bij risico's *(aanvullingen n.a.v. veldsituatie)* en veiligheidsmiddelen, inclusief PBM's (Persoonlijke Beschermingsmiddelen)	✓
6	Bij varen met eigen vaartuig: Doornemen "werkinstructies varen met eigen vaartuig" en uitleg van schipper aan opvarenden hoe boot werkt	n.v.t.
7	Voorzorgs-/ beheersmaatregelen omgaan met motorboten en werken op het water, zie ook werkinstructie	n.v.t.
8	Scheepvaart en andere gebruikers	✓
9	Weersomstandigheden	
10	Noodnummers en noodprocedures (check)	✓
11	Aangemeld bij verkeerscentrale	✓
12	Zijn er persoonlijke gezondheidsrisico's	✓

Aanwezig

	Naam aanwezigen:	Handtekening
1	UDO VAN DONGEN	[signature]
2	Niek Looze	[signature]
3	Joost Bergsma	[signature]
4	Britte Schilt	[signature]
5	FRANK COONSTRA	[signature]
6	Siebren Jonsma	[signature]
7	Piero Tundo	[signature]

Appendix 2: RAMS Briefing confirmation form



Appendix 5: RAMS briefing confirmation form

To be filled by all field staff

We the undersigned agree that
1. We were provided with a safety briefing prior to going into the field
2. We understood the principles of the Work Method Statement as explained in the safety briefings

1	Name	Affiliation	Role	Signed	Date
2	UDO VAN DALEN	WE	PL.	[signature]	16-6-2023
3	Joost Bergsma	WE	Scientist	[signature]	16/6/23
4	Britte Schilt	WMR	Scientist	[signature]	16-6-23
5	[illegible]	ms Tender	Eng.	[signature]	16/06/23
6	[illegible]	Tender	Kap	[signature]	160623
7	[illegible]	Tender	Crew	[signature]	16/06
8	Siebren	Tender	[illegible]	[signature]	16/6/23
9					
10					

16-6-2023