DNA-BASED MONITORING: A PILOT STUDY ON AUTONOMOUS REEF MONITORING STRUCTURES (ARMS) IN THE NORTH SEA REGION

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GEANS– Genetic tools for Ecosystem health Assessment in the North Sea region

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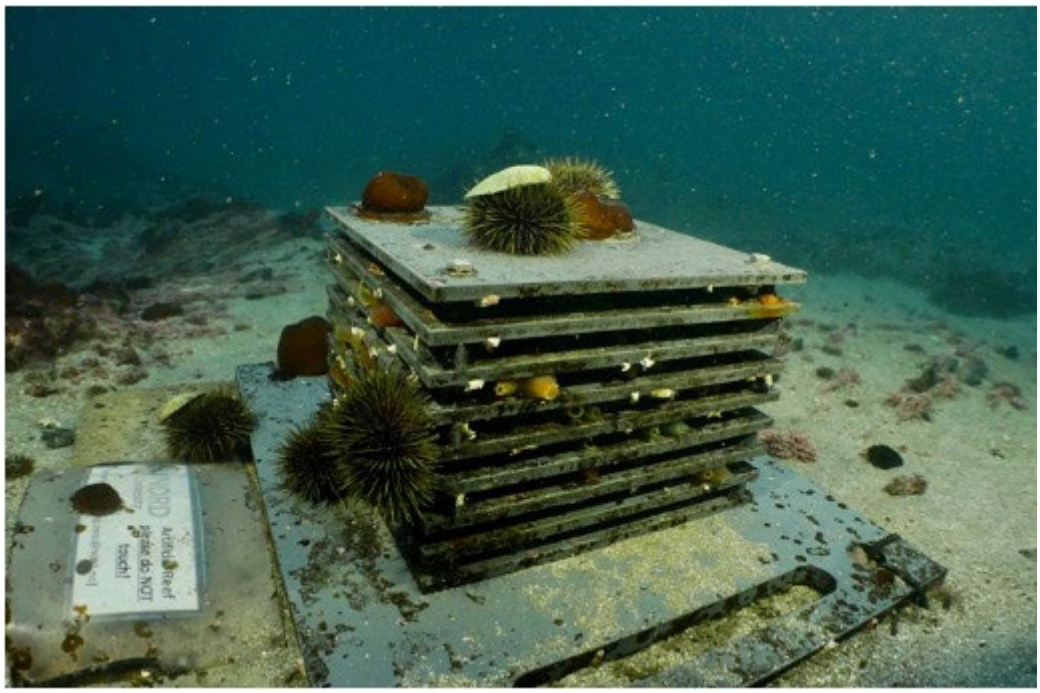

Autonomous Reef Monitoring Structures (ARMS) deployed on the coast of Norway. Photo credits: Henning Reiss.

Contents

1. Summary

Here we evaluated a genetic monitoring protocol for potential applications in regional level status assessments of benthic diversity across in the North Sea Region (NSR). For this study, we chose an established method for passive sampling of hard-bottom communities using Autonomous Reef Monitoring Structures (ARMS). We created thirteen sample events in five coastal observatories throughout Denmark, Norway, Sweden, and Belgium. Field work was carried out by each partner, while the laboratory work, data management as well as analyses were performed centralized. Sequence analysis of the collected samples resulted in identification of 599 species across the five observatories. Sixty percent of the identified species belonged to metazoans, while the remaining forty percent consisted of plants, fungi as well as single- and multicellular eukaryotic taxa. A scan against the ecological checklists for sensitive, alien, and red-listed species resulted in observations of 76 sensitive species, 24 alien species, and 4 vulnerable or near-threatened species across the five observatories. Our results show that benthic communities can be monitored by independent parties while data can be analyzed on a regional scale. Cost comparison with diver-based monitoring methods showed that the tested method is not more expensive than conventional monitoring. We conclude that ARMS provide a mature and cost-effective method for genetic monitoring of benthic hard bottom habitats in the North Sea Region and recommend implementation of the method in national monitoring programs as well as environmental impact assessments in order to better assess the health status and change of coastal ecosystems and the biological response to human activities in the ocean.

2. Introduction

Healthy ecosystems and the biodiversity they harbor are a prerequisite for the sustainable future of our oceans. Scientists are now more than ever forced to provide evidence to understand, and where possible counteract, the factors causing severe change in the biological composition of marine ecosystems. Such knowledge is critical as human pressures increase and accumulate, especially in coastal zones. Currently our understanding of the impact of human activities on marine ecosystems is limited due to lack of comparative biological time-series data at a large spatial scale (Kissling et al., 2018; Muller-Karger et al., 2018).

To make biological monitoring programs more effective, several new methods have been proposed recently, one of them being DNA metabarcoding (Danovaro et al., 2016, Bourlat et al., 2018, Staehr et al 2022). In principle, DNA-based techniques are capable of sampling and identifying biological communities at high temporal and spatial frequency, and with fine taxonomic resolution (Staehr et al., 2022). But despite the frequent application of metabarcoding in marine ecological research, only few genetic protocols have so far been implemented in marine long-term surveillance programs. This is partly because sample processing, data management and analytics is more complex compared to conventional methods.

Biological monitoring programs for hard bottom fauna typically monitor the sea floor using direct observations from divers, photography and filming with drop cams or remotely operated vehicles (ROVs), or by passive sampling. The latter is usually achieved by settlement plates for which important standards have been developed in the past years. Examples include the HELCOM guidelines for monitoring non-indigenous species (HELCOM, 2013) as well as Artificial Substrate Units (ASU) (Gobin

and Warwick, 2006) and Autonomous Reef Monitoring Structures (ARMS) units (Leray & Knowlton, 2015). ARMS are particularly effective in capturing hard-bottom communities as they create three-dimensional substrates with crevices and microhabitats for benthic organisms.

Here we test the implementation of a genetic monitoring protocol developed by the ARMS MBON program (Obst et al., 2020) across multiple countries in the North Sea Region (NSR) to evaluate if samples can be collected in concert and if resulting genetic data can be analyzed on a regional level to assess status and changes in benthic diversity. For the present pilot study, we chose the ARMS protocol available at https://github.com/arms-mbon because it is a published standard and because there is already an existing monitoring program which allows for comparisons between countries and geographic areas (Obst et al., 2020).

3. Pilot design

Field sampling

Five sampling sites were selected based on existing biodiversity monitoring sites across NSR member states (Fig. 1). A total of 18 ARMS settling panels were deployed in 5 observatories during two consecutive deployment cycles in 2018 and 2019 during which ARMS were submerged between 1-18 months at depths between 2-37 m (Fig. 1; Table 1). Following retrieval of the panels from the water, ARMS were disassembled and each individual plate was photographed from both sides for documentation and identification of species (Fig. 2). These photographs were not further analyzed, but are made publicly available as part of this study (Table 3). The next step was to scrape off all organisms from the plates and sieve them through a 40 µm net to create a sessile fraction (SF40). The remaining seawater in the crate used to store the units until processing in the lab was sieved through 500 µm and further 100 µm nets to create two motile fractions (MF500, MF100). Large bulks of organisms (like tunicates and mussels) were removed before sieving. Organisms were fixed in DMSO (Obst et al. 2020) and stored in a freezer at -20 °C until DNA extraction. A detailed field protocol (handbook) is available on the ARMS MBON GitHub site https://github.com/arms-mbon.

Figure 1. *Sampling sites along the NSR coastline (above). Assembled ARMS unit before and during deployment (below). Photos. Peter Staehr.*

Table 1. Overview over observatories, sample locations, dept, date, and deployment period.

Sequencing

All samples were shipped to and processed by one partner institute of the ARMS MBON network, the Hellenic Centre for Marine Research (HCMR), following the Molecular Standard Operating Procedures (MSOP) available on the GitHub site https://github.com/arms-mbon. DNA was extracted from the three fractions of each ARMS unit. Amplicon libraries were prepared for two molecular markers, the gene cytochrome c oxidase I (COI) and the nuclear gene 18S rRNA (18S). In addition, negative controls were created in the same way for control of contamination. All raw sequence files are available for download from the European Nucleotide Archive (ENA) through the accession numbers that are available on the ARMS MBON website (Table 3) for marker genes cytochrome c oxidase I (COI), 18S

Observatory-ID	ARMS-ID	Latitude	Longitude	Depth (m)	Monitoring area	Antropogenic pressure category	First deployment IN	First deployment OUT	Second deployment IN	Second deployment OUT	Duration first (months)	Duration second (months)
Koster (SE)	VH1	58.875155	11.103194	24	MPA	protected	2018-04-18	2019-05-27	2019-05-27	2020-07-16	13	14
Koster (SE)	VH2	58.87633	11.111884	22	MPA	protected	2018-04-18	2018-09-06	2019-05-27	2020-07-16	5	14
Koster (SE)	VH3	58.859877	11.080491	25	MPA	protected	2018-04-18	2018-09-06	2019-05-27	2020-07-16	13	14
Limfjord (DK)	Yellow8	56.902366	9.055797	4	LTER site	LHI	2019-06-18	2019-10-29	2019-10-29	2020-11-10	4	13
Limfjord (DK)	Green33	56.901246	9.058189	4	LTER site	LHI	2019-06-18	2019-10-29			4	
Limfjord (DK)	Red2	56.89985	9.05663333	4	LTER site	LHI	2019-06-18	2019-10-29	2019-10-29	2020-11-10	4	13
Laeso (DK)	Laeso1	57.25689	11.14193	10	LTER site	LHI			2019-08-19	2020-08-11		12
Laeso (DK)	Laeso2	57.25692	11.14197	10	LTER site	LHI			2019-08-19	2020-08-11		12
Laeso (DK)	Laeso3	57.25692	11.14199	10	LTER site	LHI			2019-08-19	2020-08-11		12
Bodo (NO)	GStraM	67.240217	14.711533	2	MPA	protected			2019-06-21	2020-12-07		18
Bodo (NO)	GStraB	67.24035	14.711733	2	MPA	protected			2019-06-21	2020-12-07		18
BelgiumCoast (BE)	AAZFPin	51.66522	2.82558	37	Wind farm	LHI	2018-07-12	2018-08-18			1	
BelgiumCoast (BE)	AZBE1	51.364298	3.20701	7	Marina	semi-industrial			2019-09-24	2020-03-03		6

rRNA (18S), as well as for negative control samples.

Bioinformatic processing

Sequence data were processed with the Pipeline for Environmental DNA Metabarcoding Analysis, PEMA (Zafeiropoulos et al., 2020). PEMA consists of 4 main steps: sequence pre-processing, OTUs clustering or ASVs inference, their taxonomic assignment and optionally the performance of biodiversity analysis based on the taxonomic inventory retrieved. It has been shown that parameter settings in the framework of workflows such as PEMA can lead to rather different outcomes (Zafeiropoulos et al., 2020). Therefore, for comparison reasons, a specific set of parameters (available at https://github.com/arms-mbon/data_workspace) was used for each marker gene for every sampling period. For the 18S rRNA marker gene, Operational Taxonomic Units (OTUs) were clustered using the VSEARCH algorithm (Rognes et al., 2016) with a similarity threshold of 0.97 against the PR2 database (v.4.14.0) (Guillou et al., 2013) for taxonomy assignment. Species level identifications are summarized in Table 2, while all sequence data will be made available through the Github site (https://github.com/arms-mbon). For the COI marker gene, the Swarm v2 algorithm (Mahe et al., 2015) was used to infer Amplicon Sequence Variants (ASV) followed by taxonomic annotation using the MIDORI database version 2.0 (Machida et al., 2017) and the RDP classifier by Wang et al (2007). We kept all singletons or potential contaminants other than those showing up in the negative controls.

Table 2. *Overview over the processed samples and results from the sequence analysis.*

	Overall	**COI**	**18S**
Sample events (deployed ARMS)	18	-	-
Resulting fractions (sequenced samples)	108	56	52
Sequencing effort (reads)	1,974,939	689,474	1,285,465
Overall number of unique ASVs/OTUs	-	14,202	4,910
Species identified	599	463	170
Overlap in identified species	-	34	

Table 3. Overview over the published data from ARMS pilot.

Data type	**Link**
Observatories	https://arms-mbon.github.io/old-arms-mbon-website/#info/ObservatoryData
Sample events	https://arms-mbon.github.io/old-arms-mbon-website/#info/SamplingEventData
Images	https://arms-mbon.github.io/old-arms-mbon-website/#info/ImageData
Genetic data	https://arms-mbon.github.io/old-arms-mbon-website/#info/OmicsData

Figure 2. *ARMS sampling and processing; a: Retrieval of the units with divers; b-c: Disassemblage of the units into individual plates; d-e: Photographic set-up for documentation and species identification. Photos: Maria Asplund (a), Matthias Obst (b-e).*

Ecological analyses

We performed a series of data visualization and analysis tasks to evaluate if the collected genetic data can be analyzed on a regional level to assess status and changes in benthic diversity. To this end, we excluded all taxonomic assignments below a confidence value of 0.8 in the COI data. Thereafter, we collapsed all ASV and OTU reads to presence/absence observations of species per ARMS unit and calculated taxonomic representations and species richness across the five observatories. In order to test the application potential of the derived species observation data, we performed a scan against

reference checklists for ecological key species, searching for species very sensitive to disturbance according to the AMBI index (Borja & Muxika, 2005), species with alien status at the place of observation according to the World Register of Introduced Marine Species (Rius et al., 2023), and species registered as vulnerable or near to threatened according to the International Union for Conservation of Nature (IUCN). To this end, we used the web services provided by the World Register of Marine Species (WoRMS Editorial Board, 2023). All data exploration and visualization tasks were performed in R (R Core Team, 2018).

Cost and time calculations

We calculated cost and time effort for all activities in field sampling, laboratory and analytical work separately for each observatory. We then calculated the average for each activity for the DNA-based study across all five observatories. For comparison against conventional monitoring programs, we estimated the cost and time effort investigating similar habitats with direct observations using divers in two countries, namely in Sweden and Denmark.

Data publication

All original data collected by this study are made publicly available (Table 3), while all species observations derived from the bioinformatic analysis will be submitted to the Ocean Biogeographic Information System (www.obis.org) during 2023.

4. Results

Species richness

The sequence analysis resulted in 1,974,939 reads which corresponded to 14,202 amplicon sequence variants (ASVs) from COI and 4,910 OTUs from 18S (Table 2). These could be assigned to 599 species (463 species identified by COI, 170 species identified by 18S, with 34 species overlapping between both markers). Sixty percent of the identified species belonged to metazoans, while the remaining 40% consisted of plants, Fungi as well as other single- and multicellular eukaryotic taxa (Fig. 3). Within metazoans most of the detected species belong to arthropods, annelids, and mollusks, although all of the common marine animal phyla were represented in the samples. Species richness per sample unit was highest in Bodö (Norway) with 160 species identified on a single ARMS unit (Fig. 4). However, the species richness per observatory was highest in Koster (Sweden) where we identified 246 species with six ARMS units (data not shown).

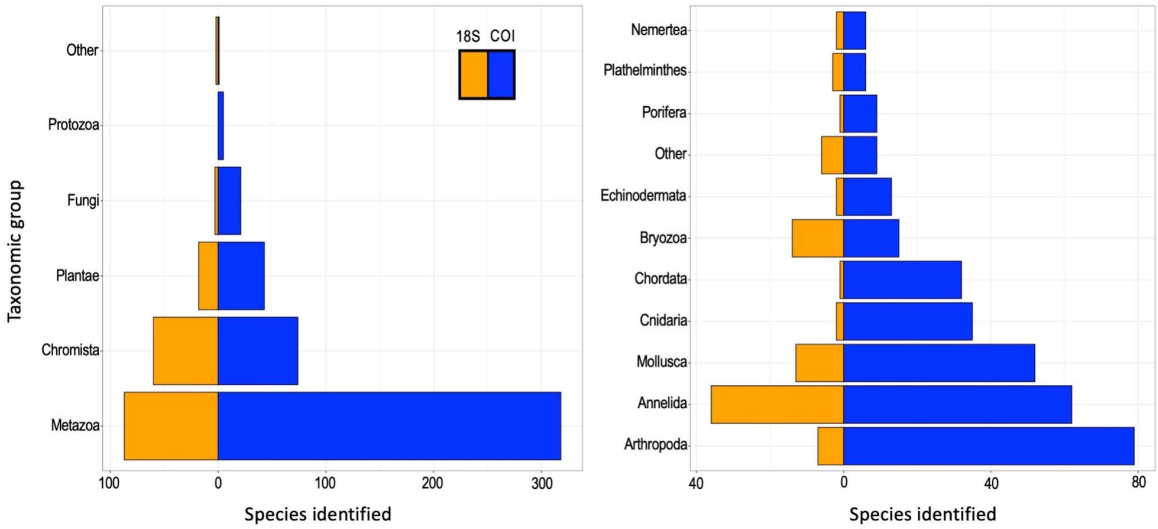

Figure 3. *Distribution of identified species across various kingdoms (left) and with metazoan phyla (right), separately for two markers.*

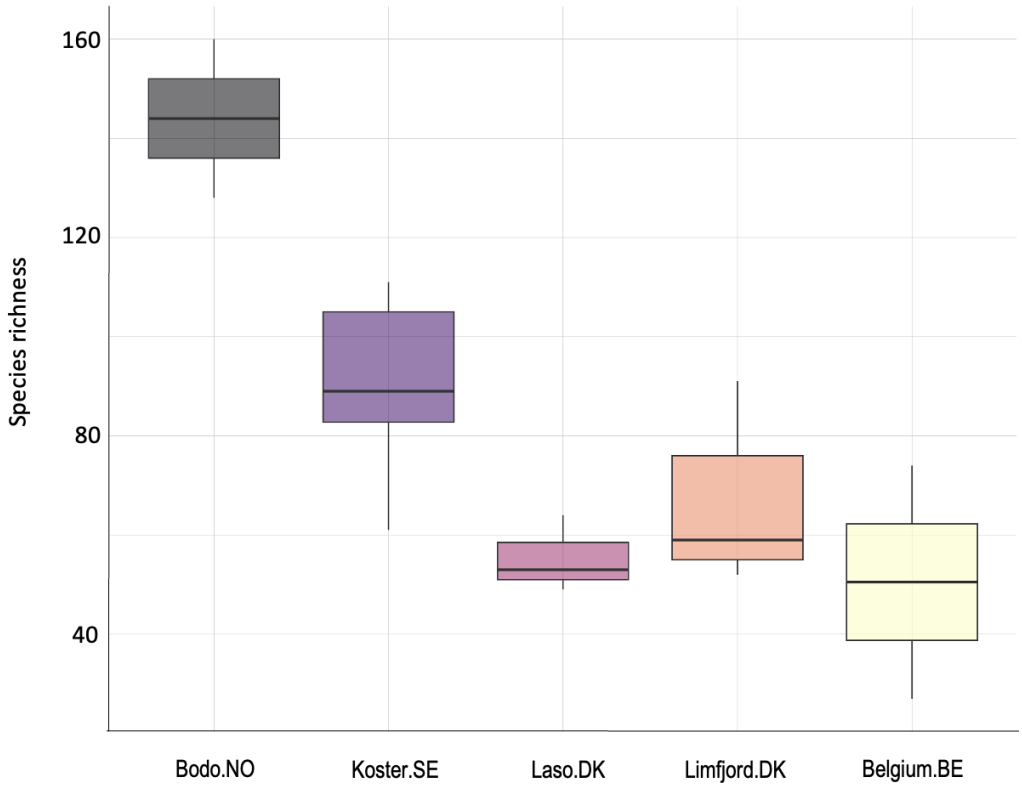

Figure 4. *Species richness observed across the five observatories in number of identified species per ARMS unit based on the two marker genes.*

Ecological indicator species

The scan against the ecological checklists for sensitive, alien, and red-listed species resulted in observations of species in all three categories across the five observatories (Fig. 5). Overall, we observed 24 alien species, 76 sensitive species, and four vulnerable or near-threatened species across the five observatories. The observatory at Koster (Sweden) detected the highest number of ecological indicator species in all three categories, which is likely due to the large sample size (n=6).

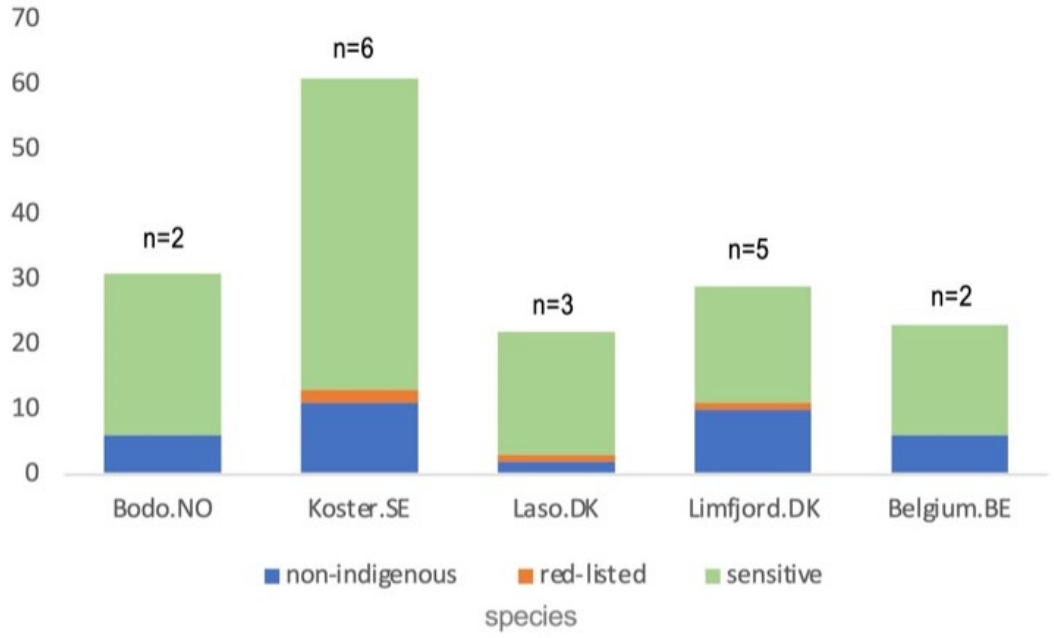

Figure 5. *Application potential of the ARMS data for biological monitoring. The diagram shows the number of identified species for three ecological trait categories across the five observatories. Number of ARMS units at the respective observatory (n) shown on top of the bars.*

Cost comparison with conventional methods

Our cost-time estimates showed expenses for monitoring benthic fauna with DNA-based approaches are in the same range as expenses for conventional diver-based surveys. Comparing the methods in detail showed that conventional surveys are likely to be more expensive in field work and diving, while DNA-based methods are likely to be more expensive in sample processing, analysis, and data publication (Table 4).

Table 4. Cost and time estimates for obtaining the presented results calculated per observatory and for alternative survey methods.

Item per observatory (3 ARMS)	**cost ARMS**	**cost alternatives (e.g. diver-based transects)**

Field work	3000 € (boat time, divers to recover/replace ARMS)	6000 € (boat time, divers to film transect and/or make direct observations)
Sample processing	600 € (0.5 days wet lab work per unit), 1200 € (libraries and sequencing)	200 € (basic video film analysis)
Data management	600 € (4 hrs)	100 € (1 hr)
Analysis	600 € (4 hrs)	600 € (4 hrs)
Sum	**6000 €**	**6900 €**

5. SWOT analysis

Strengths

- Currently there are no consistent hard-bottom monitoring programs and ARMS may be a good candidate
- We see indications that DNA-based methods become time and cost-effective if they are scaled
- Identifications can be done without expert taxonomists, who may only need to revise specific findings
- Standardized method enabling to scale up
- Data management is already addressed, see Obst 2023a,b, Obst et al., 2020
- False positives are likely less common than in eDNA methods

Weaknesses

Specific

- Passive sampling takes at least 3-6 month
- Results are not immediate and usually take several months

General

- Reference databases are still incomplete
- Fast shifting technology (primers, sequencing platforms)
- Abundance measures are still challenging, needs combination with photographic analysis which takes more time/effort
- Needs a learning curve for ecologists and taxonomists to analyze molecular data
- False positives are likely more common than in conventional methods

Opportunities

- much more information provided for many different purposes
- provides additional data (e.g. images) with opportunities to estimate abundance
- genetic data available for studying intraspecific diversity (e.g. Martaeng et al., 2023)

Threats

- no legal framework exists currently that motivates use of hard bottom monitoring
- linkage to MSFD and WFD indicators missing, ie., current indices for hard-bottom diversity can not be calculated with ARMS data

6. Discussion

Our pilot study presents a test case for monitoring benthic fauna by independent parties in a standardized and centralized way and thereby allows data to be compiled and analyzed on a regional scale. The method offers well-tested field protocols and established standards for data processing and publications.

Our results show that metazoans are especially easy to observe and monitor as genetic identification within this group is supported by well-populated reference libraries. In addition, we find that also other taxa are well-represented in our samples, such as brown algae. This result stands in sharp contrast to the lack of genetic observations for brown algae in eDNA samples in Danish waters reported by Staehr et al (2022), indicating that ARMS may have an advantage over eDNA sampling for monitoring benthic flora. However, many DNA-based surveys do have false positive rates higher than in conventional surveys and this may also be the case here. For this reason, especially new species observations should be treated with caution and cross-checked with other sources of information as well as with expert taxonomists.

ARMS monitoring on a regional scale is especially powerful for tracking the distribution and range shift of alien species as part of the Marine Strategy Framework Directive (MSFD) descriptor D2 on non-indigenous species (Bourlat et al., 2013, Obst 2023a,b). Here the method allows for consistent assessments across a larger biogeographic region and identification of early stages of invasions (Martaeng et al., 2023). In this context, ARMS observatories are particularly useful for effective alien species matches between ports as part of same risk area assessments (SRAs) under the Ballast Water Management Convention (BWMC) (Stuer-Lauridsen et al., 2018). However, the risk of false positives must also be taken into account as DNA or tissue can be swept in from afar (e.g. insect contamination) or spilled from a ship that has emptied ballast water.

It also must be pointed out that many public databases with DNA sequences (for example GenBank) are not quality assured. This means that there are errors where the published sequence does not belong to the species specified. This may lead to erroneous species identifications and it is therefore important to have a critical mind when interpreting the results of the bioinformatic analysis and use additional sources of information such as photographs to manually confirm dubious identifications. Also, the choice of bioinformatic pipeline, database, and parameters may affect the list of identified species.

We show that ARMS can identify a large number of ecological indicator species, which are typically used in environmental monitoring for the MSFD or the Water Framework Directive (Duarte et al., 2023) as well as in various environmental impact and risk assessments. ARMS data are likely to improve such assessments since they provide information on both species and genetic diversity, which can be analyzed in relation to anthropogenic pressures in an area or in relation to protective measures. As such, ARMS may be deployed continuously across industrial sites such as ports, marinas, offshore wind farms, aquaculture sites, and protected areas to assess positive and negative impacts of human activities on marine biodiversity. Based on related studies (Obst et al., 2020), we recommend deploying three ARMS units for at least three to six months during the growth season to be sure to capture a representative community.

In conclusion, this report shows that ARMS provide a mature and cost-effective method for genetic monitoring of benthic hard bottom habitats in the North Sea Region. We recommend implementation of ARMS in national monitoring programs in order to better assess the health status and change of coastal ecosystems and the biological response to human activities in the ocean. The highly standardized method and data provided by ARMS deployments offer cross-comparison between regular and sporadic assessments and would allow to relate sample events around e.g. aquacultures, wind parks, marine protected areas or ports to background data from national monitoring programs which would allow to study the impact of these activities on marine diversity.

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1 ANNEX

Protocols

www.arms-mbon.eu (will phase out in 2023)

https://data.arms-mbon.org/old-arms-mbon-website/ (currently phasing in)

Publications

Peer reviewed articles

Obst M, Exter K, Allcock AL, Arvanitidis C, Axberg A, Bustamante M, et al (2020) A marine biodiversity observation network for genetic monitoring of hard-bottom communities (ARMS-MBON). Frontiers in Marine Science 7: https://doi.org/10.3389/fmars.2020.572680

Sundberg P, Axberg A, Daragmeh N, Panova M, Obst M (2022) Genomics-based methods in environmental monitoring - Identification and detection of alien invasive species based on DNA. Swedish Agency for Marine and Water Management (SwAM). Report 2022:4. ISBN: 978-91-89329-32-4. https://www.havochvatten.se/data-kartor-och-rapporter/rapporter-och-andra-publikationer/publikationer/2022-08-16-genomik-baserade-metoder-i-miljoovervakningen.html

Martaeng R, Obst. M., Kuklinski P (2023). Phylogeographic study using Autonomous Reef Monitoring Structures indicates fast range expansion of the invasive bryozoan *Juxtacribrilina mutabilis* (Ito, Onishi & Dick, 2015). In press

MSC theses

Aljoša Gračner (2021) Assessing fouling communities in the Northern Adriatic through photo-analysis of ARMS plates. MSc thesis UNIVERSIDADE DO ALGARVE. Faculdade de Ciências e Tecnologia

Daniël van Berkel (2022) Early detection and diversity of benthic alien species along the northeast Atlantic Ocean coastline. MSc thesis Wageningen University

Alizz Axberg (2021) Comparing genetic methods. Detecting and monitoring Non-Indigenous Species. MSc thesis Department of Marine Sciences, University of Gothenburg

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