ARMS workflow
photographic and genetic data from *A*utonomous *R*eef *M*onitoring *S*tructures to track NIS colonisation of European waters and monitor long-term changes of marine hard-bottom communities

(The “what is this all about” part)

Katrina Exter (VLIZ), Matthias Obst (UGot), Christina Pavloudi (HCMR)

- What is an ARMS unit?
- What is the ARMS MBON project?
- What samples and data do we have?
- Where are the data archived and how are they linked?
- What science can we do with these data?

- What would we like the workflow to do, and why?

What is an ARMS unit?

An Autonomous Reef Monitoring Structure

- A stack of settlement plates, off-the shelf with a standardised construction and setup, and reusable
- Emplaced on hard underwater surfaces (the sea bottom, a harbour wall, ...) to encourage the surrounding colonising species to settle on and between the plates
- Left in place for months at a time: shorter periods to look for NIS, longer periods for biological monitoring
- Once retrieved, the plates with their colonists and the surrounding water samples are photographed, filtered and preserved, and sequenced (COI, ITS, 18S)
- ARMS units were developed under the *Census of Marine Life* project and the Global ARMS project has expanded since then

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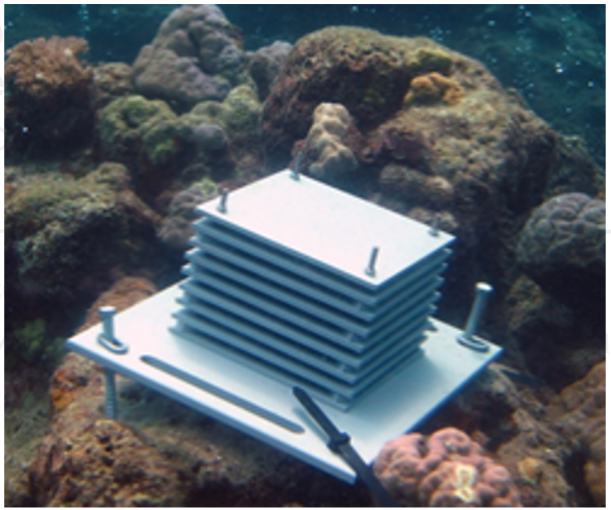

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What is the ARMS MBON project?

- Part of the EMBRC/ASSEMBLE Plus Joint Research Activity on Genomics Observatories (https://www.assembleplus.eu/research/ARMS-MBON)
- Is a network of ~20 partners placing ARMS units in the vicinity of marine stations, ports, marinas, and LTER sites distributed over Europe and polar regions.
- The aim is to assess the status of, and changes in, hard-bottom communities of near-coast environments, using genetic methods supplemented with image analysis and visual inspection methods
- Explained in detail in https://www.frontiersin.org/articles/10.3389/fmars.2020.572680/full

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What samples and what data do we have?

Material samples

- Plates are scraped, filtered, preserved, and sent to HCMR for sequencing
- Backup samples are kept in the partner labs

Observation data – standardised spreadsheets with species identifications

- Visual observations made during the retrieval of the plates
- Visual inspection of the plates made in the lab while processing
- Manual analysis of the plate photographs

Digital data

- Photographs of the plates taken in the lab
- Metabarcoding data (raw sequences) (HCMR)

Plagioecia patina

Ciona intestinalis

Ascidiella sp.

Cryptosula pallasiana

What samples and what data do we have?

Processed data

- Processed sequences (cleaned and clustered, OTU tables/ASV files with taxonomic assignments matched to WoRMS)
- (Semi-)automatic analysis of the images (species identifications matched to WoRMS, measure of quantity)

Up to today, we have

- 1 test year, 2 full years, and we are in our 3rd year now
- 21 observatories
- Per observatory, per each sampling event
  - 3 material samples (sessile and motile fractions)
  - dozens of images (ARMS plates, interesting specimens, etc
  - 9 fastq files

Archiving and linking the data

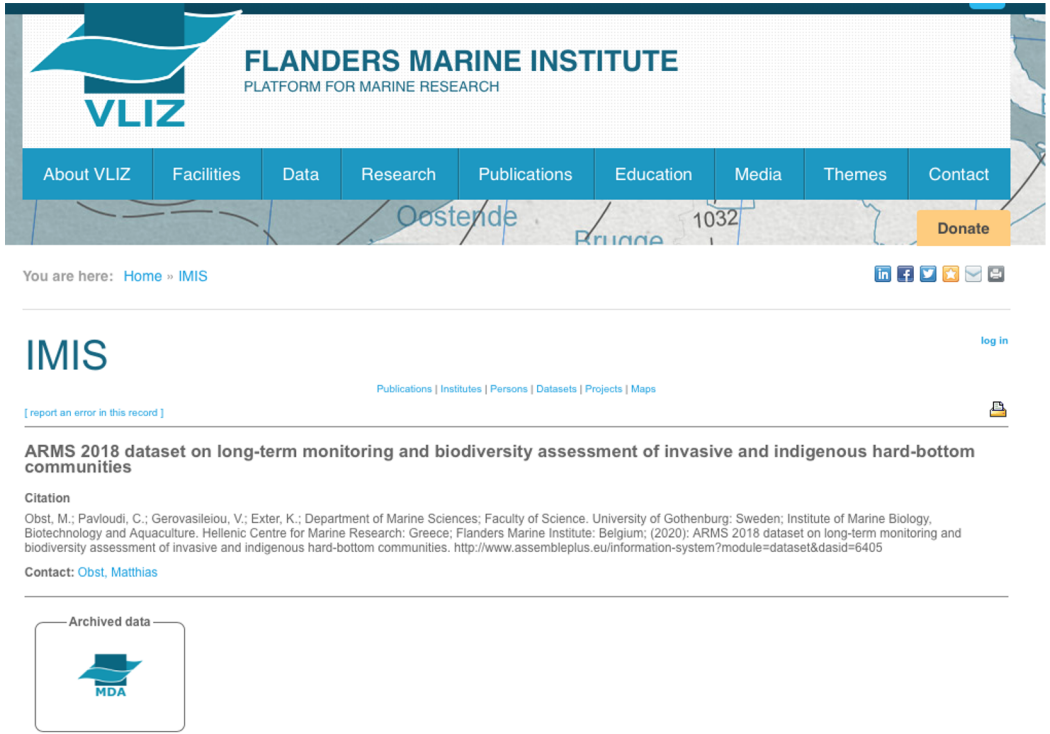
Archiving and data storage

- PlutoF data management platform (internal project use)
- VLIZ's Marine Data Archive
- ENA for raw sequences
- Looking for an appropriate image archive and an archive for ASV/OTU data

Linking the data

- Data are linked to each other via a standardised "logsheet" made FAIR and open access via its metadata record in the Integrated Marine Information System (https://www.vliz.be/en/imis?module=dataset&dasid=6405)
- Species identifications made from photographic, visual, and sequence data to be published via EurOBIS; within the DwC-A data format we need to link these multiple lines of evidence for each species, to the data they were found from

Archiving and linking the data



Archiving and linking the data

	A	B	C	D	E	F	G	H	I	J	K	L
1	Country	Observator	ARMS	MaterialSampleID	longitude	latitude	depth	Deploy_D	Collect_D	physicalSamp	Fraction	Preserv
2	NA	NA	NA	NA	decimalDeg	decimalDeg	mete	NA	NA	NA	NA	NA
3	Greece	Crete	1HERP	ARMS_Crete_1HERP_180928-190128_	25.136605	35.343153	5	2018-09-2	2019-01-2	ARMS_14	sessile	DMSO
4	Greece	Crete	1HERP	ARMS_Crete_1HERP_180928-190128_	25.136605	35.343153	5	2018-09-2	2019-01-2	ARMS_15	sessile	EtOH
5	Greece	Crete	1HERP	ARMS_Crete_1HERP_180928-190128_	25.136605	35.343153	5	2018-09-2	2019-01-2	ARMS_16	motile	DMSO
6	Greece	Crete	1HERP	ARMS_Crete_1HERP_180928-190128_	25.136605	35.343153	5	2018-09-2	2019-01-2	ARMS_17	motile	EtOH
7	Greece	Crete	1HERP	ARMS_Crete_1HERP_180928-190128_	25.136605	35.343153	5	2018-09-2	2019-01-2	ARMS_18	sediment	frozen
8	Greece	Crete	1HERP	ARMS_Crete_1HERP_180928-190128	25.136605	35.343153	5	2018-09-2	2019-01-2	ARMS_19	plankton	EtOH

M	N	O	P	Q	R	S	T
Filter	gene_COI	gene_18S	gene_ITS	negativeControl_ger	negativeControl_ger	negativeControl_ger	OtherData_m2mlink
microme	ENA_RunAcces	ENA_RunAcces	ENA_RunAcces	ENA_RunAccession	ENA_RunAccession	ENA_RunAccession	NA
40	ERR3460466	ERR4018423	ERR4018429	ERR3460471	ERR4018474	ERR4018475	NA
40	ERR3460467	ERR4018424	ERR4018430	ERR3460471	ERR4018474	ERR4018475	NA
500	ERR3460468	ERR4018425	ERR4018431	ERR3460471	ERR4018474	ERR4018475	NA
500	ERR3460469	ERR4018426	ERR4018432	ERR3460471	ERR4018474	ERR4018475	NA
NA	ERR3460470	ERR4018427	ERR4018433	ERR3460471	ERR4018474	ERR4018475	NA
250	NA	ERR4018428	ERR4018434	ERR3460471	ERR4018474	ERR4018475	NA
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What science can we produce from ARMS data?

From a pilot study (2 sites, 1 year):

- ~70 identified species with only 4-8% overlap between genetic- and image-based species for each site
- 16 of the detected species were NIS (alien or cryptogenic to the region of detection)
- High number of NIS on Crete ARMS (Eastern Mediterranean is a region of substantial biological invasion), and low number on Swedish ARMS (Marine Protected Area)
- Taxonomic shift between genetic fractions: sessile fractions dominated by chordates; motile fractions by arthropods, nematodes, mollusks and single cellular eukaryotes

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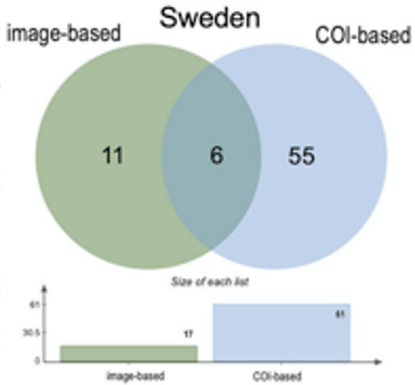

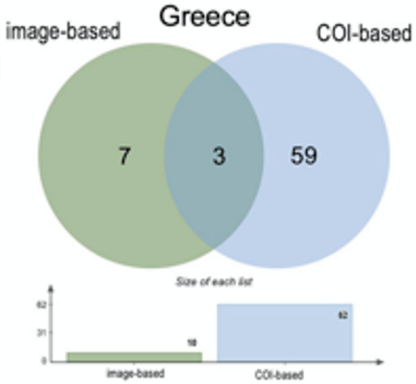

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- Taxonomic chordates; cellular eukaryotes

Species	NIS status	Taxon	Source	Sequence reads	Confidence	ARMS
Clytia linearis (as *C. hemisphaerica*)	AL	Hydrozoa	ERR13:415770.613156	1	1.00	Greece
Cephalothrix simula	CR	Nemertea	ERR6:336120.637271	380	1.00	Greece
Bugula neritina	CR	Bryozoa	ERR3:1130880.648428	17	1.00	Greece
Bugulina stolonifera (as *Bugula stolonifera*)	CR	Bryozoa	ERR4:1011990.601062	30	1.00	Greece
Amphibalanus amphitrite	CR	Crustacea	ERR3:111700.649742	321	1.00	Greece
Balanus trigonus	AL	Crustacea	ERR9:501910.609480	32	1.00	Greece
Monocorophium acherusicum	CR	Crustacea	ERR5:433720.605205	96	1.00	Greece
Anteaeolidiella lurana	CR	Mollusca	ERR3:1097540.663621	56	1.00	Greece
Pinctada imbricata radiata (as *Pinctada radiata*)	AL	Mollusca	Image	NA	NA	Greece
Botryllus schlosseri	CR	Tunicata	ERR3:1093300.604240	97	1.00	Greece
Ascidiella aspersa	AL	Tunicata	Image	NA	NA	Greece
			ERR16:281940.641233	6	1.00	
Ciona robusta	AL	Tunicata	Image	NA	NA	Greece
Clavelina lepadiformis	CR	Tunicata	Image	NA	NA	Greece
Herdmania momus	AL	Tunicata	ERR3:387270.604124	15	1.00	Greece
Phallusia nigra	AL	Tunicata	Image	NA	NA	Greece

AL, alien species; CR, cryptogenic species; NA, Non-applicable. Non-accepted names in the PEMA output were replaced with accepted synonyms.

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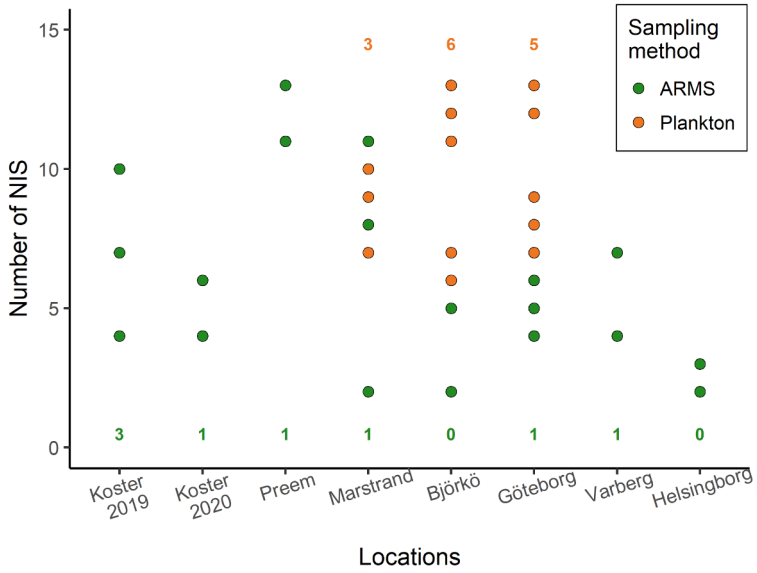
Explorative studies using more sites and years

- 35 alien species detected across 19 ARMS in 2020

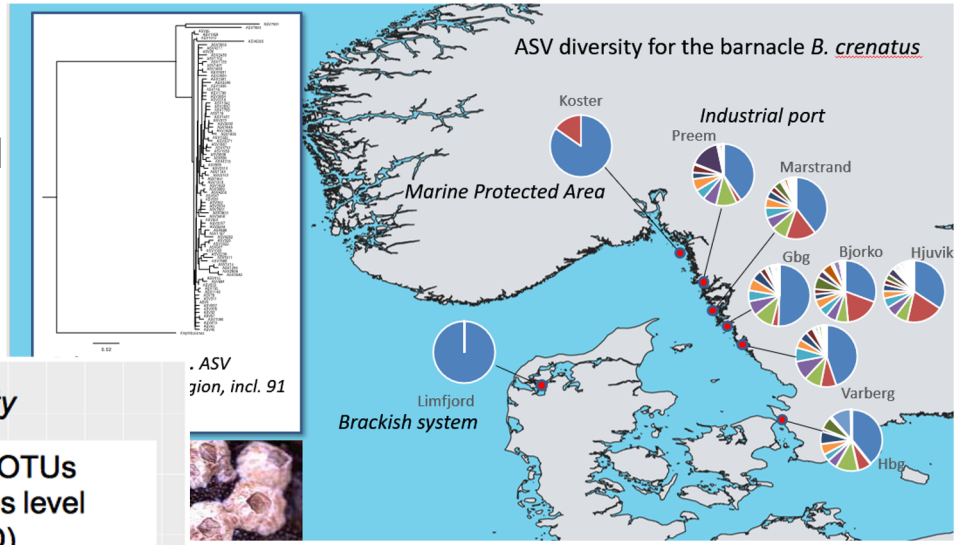


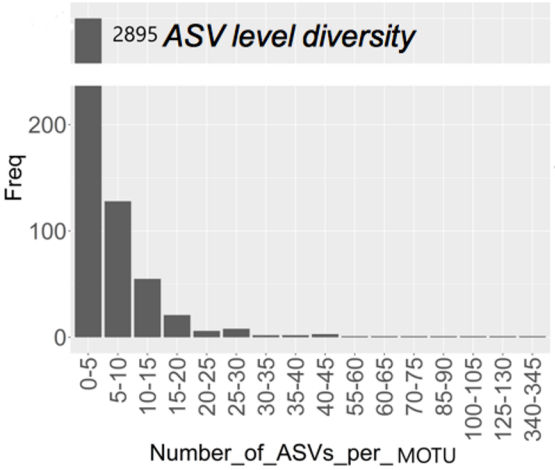
Vetenskapligt namn	Fylum	Klass	Max likhet (%)
Acartia clausi	Arthropoda	Copepoda	100
Acartia tonsa	Arthropoda	Copepoda	100
Acrochaetium moniliforme	Rhodophyta	Florideophyceae	100
Aglaothamnion hallae	Rhodophyta	Florideophyceae	100
Amathia imbricata	Bryozoa	Gymnolaemata	99.67
Amphibalanus amphitrite	Arthropoda	Thecostraca	100
Amphibalanus eburneus	Arthropoda	Thecostraca	100
Amphibalanus improvisus	Arthropoda	Thecostraca	100
Antithamnionella spirographidis	Rhodophyta	Florideophyceae	98.71
Bonnemaisonia hamifera	Rhodophyta	Florideophyceae	100
Calanus euxinus	Arthropoda	Copepoda	100
Caprella mutica	Arthropoda	Malacostraca	100
Crepidula fornicata	Mollusca	Gastropoda	100
Dasya baillouviana	Rhodophyta	Florideophyceae	98.00
Dasysiphonia japonica	Rhodophyta	Florideophyceae	100
Ercolania viridis	Mollusca	Gastropoda	99.35
Gonionemus vertens	Cnidaria	Hydrozoa	100
Haminoea solitaria	Mollusca	Gastropoda	99.68
Hydroides elegans	Annelida	Polychaeta	98.00
Hymeniacidon sinapium	Mollusca	Gastropoda	99.00
Jassa marmorata	Arthropoda	Malacostraca	100
Lyrodus pedicellatus	Mollusca	Bivalvia	99.00
Mnemiopsis leidyi	Ctenophora	Tentaculata	99.80
Monocorophium acherusicum	Arthropoda	Malacostraca	100
Monocorophium sextonae	Arthropoda	Malacostraca	100
Mya arenaria	Mollusca	Bivalvia	100
Mytilus trossulus	Mollusca	Bivalvia	100
Penilia avirostris	Arthropoda	Branchiopoda	100
Petricolaria pholadiformis	Arthropoda	Copepoda	99.00
Pileolaria militaris	Annelida	Polychaeta	100
Proceraea cornuta	Annelida	Polychaeta	100
Pseudochattonella verruculosa	Ochrophyta	Dictyochophyceae	98
Pseudodiaptomus marinus	Arthropoda	Copepoda	99.68
Sparus aurata	Chordata	Actinopteri	100

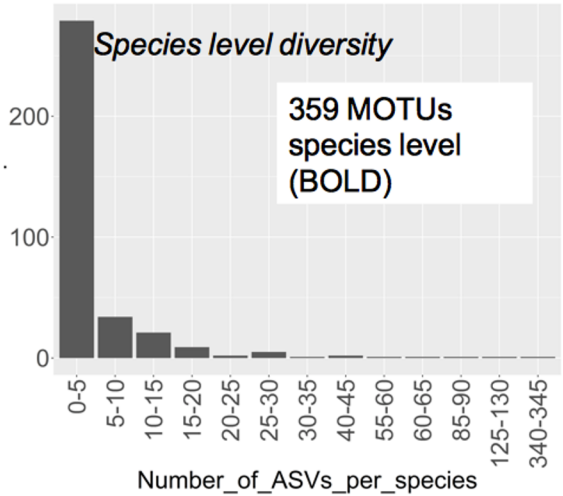
What science can we produce from ARMS data?

Explorative studies using more sites and years

- ASV diversity between Swedish sites







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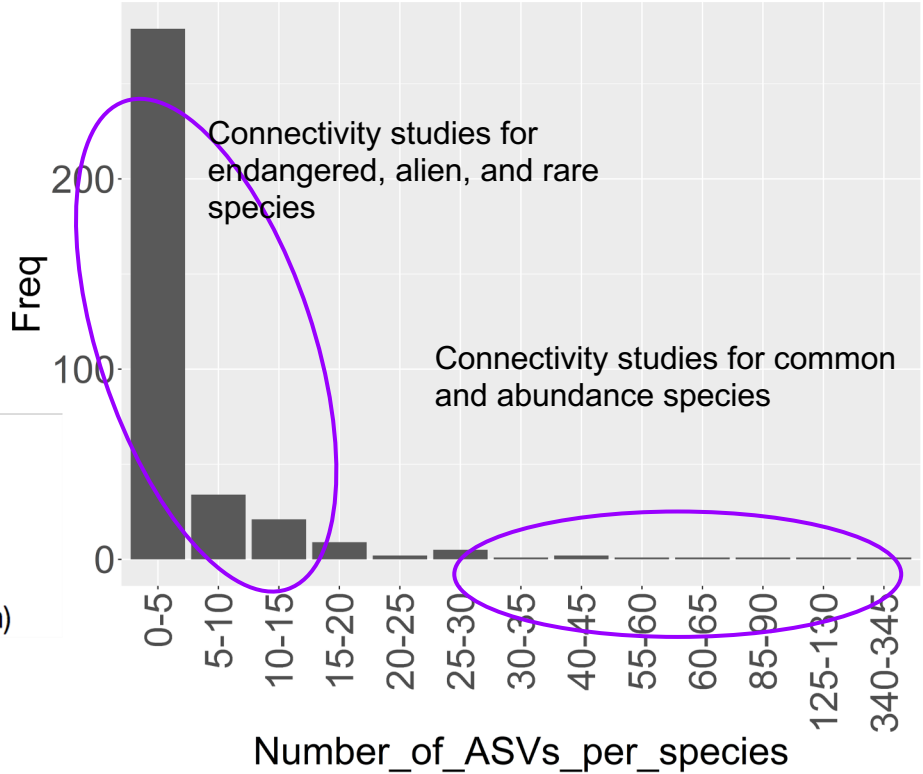
Explorative studies using more sites and years

- Connectivity studies for endangered species, alien species, and rare species



Hypotheses testing

- Natural and human-mediated connectivity on a continental scale
- Evenness in benthic habitats in time and space
- Stepping-stone hypotheses for Marine Protected Areas (MPAs)
- Effectiveness of preventive measures (e.g. Ballast Water Mgmt Convention)

What would we like the LW workflow to do?

1. Access the Master logsheet from the IMIS metadata record
2. Inspect the ARMS data availability via the logsheet
3. Chose which parts of the ARMS data you want to work on ♺
    a. Date(s)
    b. Location(s)
    c. Sequence file(s)
    d. Image(s)
4. Chose which workflow “path” you want to follow ♺
    a. Raw sequence cleaning and taxonomic assignments using PEMA
    b. Cleaned sequence taxonomic assignments via BOLD or other
    c. Image analysis via [as yet to be determined] software

What would we like the LW workflow to do?

5. Outputs from step 4 are always run through a taxonomic standardisation via WoRMS and NIS check using WRIMS webservices
6. Outputs from step 5 can be pushed through biological statistical analysis using RvLab ♺
7. Outputs from steps 4 and 5 can be formatted for publishing on EurOBIS
8. Outputs can be downloaded to be archived where the user choses, or direct to the LW catalogue via the workflow environment