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Integrating ROV ship hull cleaning robots and eDNA analysis for monitoring alien marine species

A novel method for early warning of new introductions

Frode Fossøy, Vivian Husa, Markus Majaneva, Tor Mikal Østervold, Abigail Robinson, Erwann Legrand, Siri Aaserud Olsen, Vanessa Solvang Klocke, Ida Pernille Øystese Molander, Hege Brandsegg

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Norwegian Institute for Nature Research

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Abstract

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Non-indigenous marine species (NIMS) represent a major threat to biodiversity, ecosystem functioning, and economic activities in coastal environments worldwide. Human-mediated pathways, particularly shipping, are the primary drivers of NIMS introductions, with biofouling material on vessel hulls now considered the dominant vector following ballast water management regulations. Increased globalization and climate change have amplified the frequency of marine invasions, underscoring the need for early detection and integrated management strategies. In Norway, a national monitoring program for marine alien species is currently being implemented, incorporating DNA-based methods for efficient and accurate species identification. DNA-based approaches offer significant advantages, enabling large-scale and cost-effective detection of a broad range of taxa, including cryptic and early-stage invaders.

To address sampling challenges on large commercial vessels, we tested a novel method combining ROV hull cleaning with DNA analyses of the collected biofouling material. We collected both environmental DNA (eDNA) from the waste water and biofouling waste from ROV hull cleaning operations of marine vessels in two Norwegian ports, Tananger and Bergen, including cargo vessels, cruise ships and coastal steamers.

DNA-metabarcoding detected a large range of species both in the wastewater and biofouling waste. In total, the analyses detected 2199 genetic taxa (OTUs), of which 784 were multicellular eukaryotes. The most species-rich groups were Arthropoda, Annelida, Mollusca and Rhodophyta, including 129, 111, 87 and 67 species, respectively. The recorded species included 19 species listed as invasive in Norway. Among these, 8 species were listed as door-knockers, suggesting that this method can provide information on early warning of new introductions of marine alien species. Most of the door-knocker species were detected on the vessels in international traffic. The 8 door-knockers species were not found in the port control water samples, indicating that the DNA were originating from the hull biofouling. We also detected two NIMS that are included in the EASIN European alien species list but that have not been considered by the Norwegian lists so far. Finally, we detected several species where either the alien status is unknown, or the species is not recorded in Europe previously, indicating the need for further assessments of NIMS. Some alien species had nearly identical DNA-sequences with their local sister species in Norway, and the genetic markers included in this study were not able to discriminate between the species. Hence, some alien species were potentially not recorded.

The approach of combining ROV hull cleaning and DNA-analyses offers a unique opportunity to assess biofouling material communities and the invasion pressure of alien species in local ports, and can help inform management strategies to prevent their establishment and further spread. Overall, ROV-assisted hull cleaning combined with eDNA analysis represents a promising addition to the suite of tools available for NIMS monitoring. It allows for standardized, repeatable, and efficient sampling of large vessels, provides comprehensive insight into biofouling community composition, and can be incorporated into routine cleaning operations with minimal disruption. If integrated into the national monitoring program, this method could significantly strengthen Norway's capacity to detect invasive species early, assess biosecurity risks associated with incoming vessels, and design targeted management strategies to prevent secondary spread.

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Foreword

Non-indigenous marine species (NIMS) pose an increasing threat to biodiversity, ecosystem functioning, and socio-economic values in coastal environments worldwide. Shipping has become the primary pathway for marine species introductions, with biofouling on vessel hulls now recognized as the dominant vector following the implementation of ballast water management regulations. Ongoing globalization and climate change are further intensifying invasion pressure, highlighting the importance of effective monitoring systems, early detection, and integrated management strategies.

In Norway, a national monitoring programme for marine alien species is currently being implemented, with a strong emphasis on the use of DNA-based tools to improve efficiency, accuracy, and spatial coverage. DNA-based approaches offer significant advantages over traditional morphological methods, enabling large-scale and cost-effective detection of a broad range of taxa, including cryptic and early-stage invaders.

This report presents the results of a pilot study testing a novel approach that combines ROV-assisted hull cleaning with DNA analyses of biofouling material and wastewater originating from the cleaning process. Our results demonstrate that DNA metabarcoding provides detailed insights into biofouling community composition and enables the detection of a wide diversity of taxa, including several species listed as invasive or considered “door-knockers” in Norway. The detection of door-knocker species primarily on vessels engaged in international traffic, and their absence in local control samples, highlights the potential of this method as an early warning tool for new marine introductions. If incorporated into Norway’s national monitoring programme, it could substantially strengthen national capacity for early detection, biosecurity risk assessment, and the development of targeted management measures aimed at preventing the establishment and secondary spread of non-indigenous marine species.

The pilot project was funded by the Norwegian Environment Agency, whose support is gratefully acknowledged. We would also like to extend our thanks to Egil Postmyr for project assistance and being a very patient project contact. The field work was funded by the Institute of Marine Research (IMR).

Trondheim, May 2025

Frode Fossøy

1 Introduction

Non-indigenous marine species (NIMS) pose a large threat to biodiversity and ecosystem services in many coastal environments globally (Katsanevakis et al. 2023, Tsirintanis et al. 2023). NIMS are primarily introduced through human-mediated pathways such as ship ballast water discharge and ship hull fouling. NIMS can eradicate native species and disrupt local food webs, leading to cascading ecological effects and significant economic losses in fisheries and tourism (Diagne et al. 2021). The occurrence of marine invasions has increased dramatically in recent decades due to increased globalization and climate change (Seebens et al. 2017), making early detection and integrated management strategies essential for mitigating their impacts. Over the past three decades, global marine traffic has increased tenfold, and projections indicate a potential increase of 240-1200 % by 2050, anticipating a 3-20-fold rise in global marine introductions (Sardain et al. 2019).

Historically, ballast water in ships has been the main pathway for introductions of NIMS along the Norwegian coast, but with the implementation of the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM), this risk has been strongly reduced. Biofouling material on ships and vessels is therefore currently considered to be the most important vector for introductions of new marine species to Norway (Husa et al. 2022a). An analysis of the frequency and origin of 158 000 vessel arrivals to Norwegian ports in the period 2020-2021 shows that the Oslofjord and the western coast have the highest risks for new introductions by shipping traffic in Norway (Husa et al. 2022a). While there is extensive documentation on species travelling in ballast water, our understanding of the species assembly travelling long distances as biofouling material on vessels is limited. Previous studies have mainly focused on recreational vessels or vessels in specific areas. The challenge lies in the difficulty of sampling large commercial vessels that follow busy operational schedules and in obtaining permission to sample such ships.

Early detection and warning of new introductions is crucial for implementing necessary mitigation measures. Early warning requires robust and standardised monitoring, and a national monitoring program for NIMS is currently being developed and implemented in Norway (Husa et al. 2022b, Husa et al. 2024). As part of this work, DNA-based methods such as environmental DNA (eDNA) have proven an important method for species identification of invasive species (Husa et al. 2024). DNA-based species identification methods are rapidly changing the way we monitor biodiversity, including the detection of red-listed species and unwanted alien species of concern in both terrestrial and aquatic environments (Taberlet et al. 2018). DNA-based detection and monitoring of NIMS is currently being tested and evaluated across many countries (Duarte et al. 2021, Katsanevakis et al. 2023). Importantly, DNA-based methods can process a higher number of samples compared to morphological species identification within a reasonable time and cost frame. DNA-based species identification includes both targeted approaches for detection of single species such as qPCR and ddPCR as well as more general methods such as DNA-metabarcoding which can simultaneously identify hundreds of species.

One such example of an invasive species being monitored using eDNA is *Didemnum vexillum*, commonly known as the Japanese sea squirt, or more popularly named sea vomit. It is classified as an invasive species with a very high ecological risk (SE) in the Norwegian alien species list (Artsdatabanken 2023). This colonial ascidian, originally endemic to Japan, has spread extensively across global maritime networks as a biofouling material organism transported on ship hulls. Regional surveys using a combination of eDNA of port water samples and visual surveys have mapped its distribution in Norway and confirmed its presence in several harbours, including Stord, Bergen, Fensfjorden, Florø, and Måløy (Fossøy 2022, Fossøy et al. 2023). Its rapid growth and ability to form dense mats can suffocate native benthic communities, alter habitat structure and interfere with aquaculture operations, and the ecological and economic impacts of *D. vexillum* are expected to be considerable (VKM 2023). Furthermore, its presence on ship hulls can facilitate secondary spread, increasing the risk of introduction to new regions. Effective

monitoring and management strategies are therefore critical to prevent further dispersal and mitigate large ecological effects.

Here, we present a novel method for monitoring early spread of NIMS, by integrating ROV-based ship hull cleaning and eDNA analysis of the collected biofouling material. The Norwegian company Ecosubsea (www.ecosubsea.com) has developed an innovative ROV designed to remove biofouling material from ship hulls. Unlike conventional cleaning methods, this technology incorporates collection of all the cleaned material for onshore disposal, thereby preventing the release of invasive species into harbour waters, which otherwise poses a significant risk of further spread. Ecosubsea currently operates in multiple Norwegian and international ports, including Haugesund, Stavanger, and Bergen. Where *D. vexillum* is already established. This approach could provide critical insights into the prevalence of *D. vexillum* and other invasive species, while simultaneously informing management strategies aimed at reducing marine introductions of door-knocker species.

These cleaning operations provide a unique opportunity to get access to biofouling material communities on large vessels and develop easy sampling methods for advanced analysis. Such methods can be useful to evaluate the risk of new alien species, give early warning of pest species, and inform management strategies aimed at reducing marine introductions of door-knocker species.

2 Material and methods

2.1 Field work

Sampling of marine vessels was performed in the period 18th of March to 17th of May 2024 in the Tananger port in Rogaland county and Bergen port in Vestland county. Five petroleum service vessels, one cargo vessel, one cruise vessel and four coastal steamers (Hurtigruten) were sampled during cleaning operations. I addition, port water from the harbour in the different localities were sampled, to help distinguish between DNA-detections of species present in the local environment from the species present on the vessel. Each vessels history over the last 360 days was tracked by the help of the Marine Traffic Database (**Table 1**). All of the vessels had been sailing in the North Sea and Norwegian Sea area during this period.

***Table 1**. Overview of vessels sampled during the survey in the period 18th March and 17th may in 2024 in Tananger and Bergen ports. Vessel history source: Marine Traffic.*

Date	Port	Vessel ID	Vessel category	Vessel history last year
18.03.24	Tananger	PSV 1	Petroleum service vessel	Oilfields North Sea
20.03.24	Tananger	PSV2	Petroleum service vessel	Oilfields North Sea and Norwegian Sea
21.03.24	Tananger	PSV3	Petroleum service vessel	Oilfields North Sea and Norwegian Sea
25.03.24	Bryggen, Bergen	PSV4	Petroleum service vessel	Oilfields North Sea and Norwegian Sea, Hamburg, Baltic, UK
03.04.24	Tananger	PSV 5	Petroleum service vessel	Oilfields North Sea and Norwegian Sea
05.04.24	Laksevåg, Bergen	CAR	Cargo/Icebreaker	Greenland, Iceland
06.05.24	Jekteviken	CRU	Cruise	North Sea
08.05.24	Nøste-terminalen	PASS 1	Coastal steamer	The Norwegian coast, North Sea south to France
09.05.24	Nøste-terminalen	PASS 2	Coastal steamer	The Norwegian coast, North Sea south to France
10.05.24	Nøste-terminalen	PASS 3	Coastal steamer	The Norwegian coast
17.05.24	Nøste-terminalen	PASS 4	Coastal steamer	The Norwegian coast
20.03.24	Tananger	Port-water		
05.04.24	Laksevåg, Bergen	Port-water		
11.04.24	Bryggen, Bergen	Port-water		
10.05.24	Jekteviken, Nøstet	Port-water		

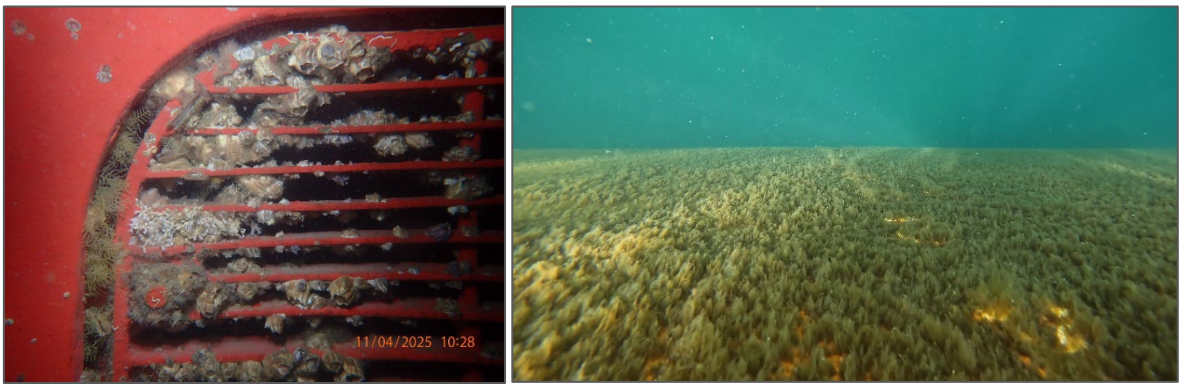

***Figure 1**. Examples of biofouling on marine vessels before cleaning (Foto: Ecosubsea).*

The in-water hull-cleaning system contains a large ROV with rotating water-jets and suction openings. Fouling organisms and cleaning water are transported from the ROV to a container unit located on shore where the material first goes into sedimentation tanks, and then through different stages of mechanical filtration. The biofouling waste is collected in two bags, and the wastewater is cleaned by UV radiation before being released in the sea (**Figure 2**, **Figure 3**).

***Figure 2**. To the left: Petroleum service vessel and container unit for in-water hull cleaning. To the right: Cleaner ROV in action under water (Foto: Vivian Husa and Ecosubsea).*

Water samples were collected from two different places in the container unit: one from the outlet of the sedimentation tank (tank water) and one from the water sieving out under the biofouling material bags (biofouling water), approximately every hour during the cleaning operation (**Figure 3**). The water samples were filtered *in situ* using a syringe filter (Sterlitech 5.0 µm GF/0.8 µm PES filter) and a handheld peristaltic pump (Bürkle Vampire). Care was taken to avoid contamination by using single-use clean buckets, and pre-packed kits for each site with gloves, tubing, buffer, filters and caps. Samples were preserved by adding ATL-buffer (Qiagen) to the filters after filtration. Filters were stored at room temperature until genetic analysis. The maximum volume of water that could be filtered varied substantially from 0.5 to 7 liters, depending on the concentration and size of various particles (organic and paint) in the water. Port water control samples were collected in duplicates per locality, and filtration volume ranged from 7.5 to 10 liters.

Approximately 150 ml of biofouling material were collected from different parts of each biofouling material bag once the bag was full and then fixated in 350 mL of 96% ethanol (**Figure 4**).

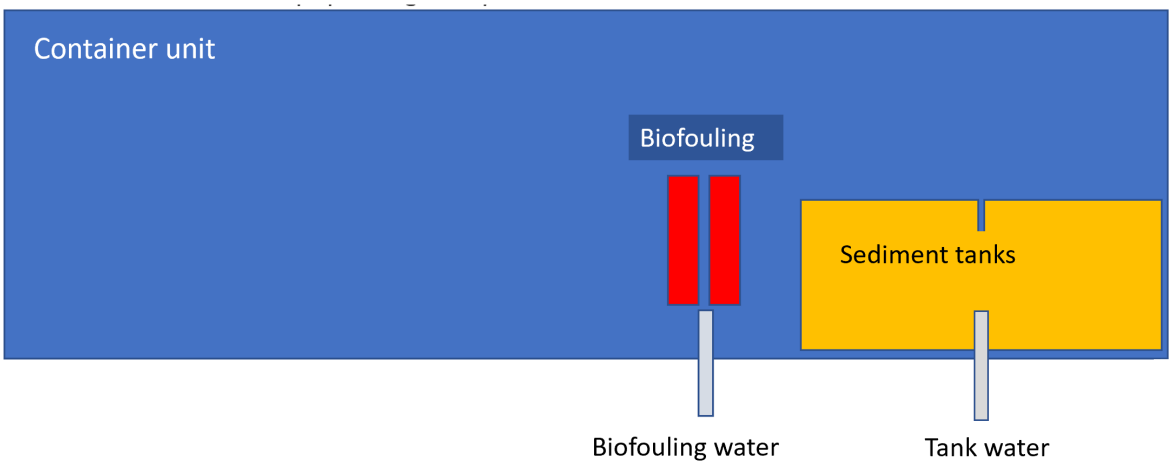

***Figure 3**. Overview of container unit for in-water cleaning with sampling points for biofouling material and wastewater.*

***Figure 4**. Example of bags with biofouling material after in-water cleaning. Foto: Vivian Husa.*

2.2 Laboratory analyses

2.2.1 DNA extraction

For DNA extraction of the eDNA filters, 130 µL proteinase-K (1:10 dilution, Qiagen) was first added to the eDNA filters containing ATL buffer and incubated at 56°C overnight. DNA was extracted from filters using Nucleospin Plant II Midi kit columns (Macherey–Nagel GmbH, Düren, Germany) in combination with lysis and wash buffers from the Qiagen Blood & Tissue kit (1000 µL buffer AL, 1000 µL EtOH, 1 mL wash buffer AW1 and 3 mL wash buffer AW2). DNA was eluted in 200 µL of pre-heated AE buffer (Qiagen). Purity and DNA concentration were measured in the samples using a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

To extract DNA from the biofouling material samples, the samples were homogenized in 250 mL flasks (MP Biomedicals) with 100 g of Matrix D and one tube of Matrix A at 1600 rpm for 2 min

using a FastPrep-96 homogenizer (MP Biomedicals) in total volume of 175 mL of 96% ethanol. A 500 µL aliquot of the homogenized material was transferred from each sample to 2 mL tubes, and the tubes were dried in a heat oven at 56°C. Once the samples were dry, 540 µL of buffer ATL (Qiagen) and 60 µL of proteinase-K (Merck) were added to the aliquots, which were then incubated at 56°C overnight with shaking. DNA extraction of the samples was performed the next day using the King Fisher MagMax Tissue 2.0 kit (Thermo Fisher) in a KingFisher Apex extraction robot (Thermo Fisher) according to the manufacturer's instructions. DNA was eluted with AE buffer (Qiagen).

2.2.2 qPCR analyses

A species-specific genetic marker for *D. vexillum* (Gargan et al. 2022) was analysed using quantitative PCR (qPCR). The qPCR analysis amplifies a small fragment of target DNA, defined by the genetic marker, using a heat-sensitive enzyme. The cycle threshold (C_T) value indicates the number of PCR cycles required for the DNA quantity to generate a detectable fluorescence signal. Lower C_T values therefore indicate higher DNA concentrations. All analyses were run in triplicates together with positive and negative controls. To classify a sample as positive, at least two out of three technical replicates were required to be positive. Results with only one out of three positive replicates are considered inconclusive and highlighted in yellow.

All qPCR analyses were conducted twice, including either 1 or 5 µL of template DNA. The qPCR analyses were run in 30 µL total volumes including 15 µL Fast Advanced Master Mix (Applied Biosystems, Foster City, CA), 2.7 µL each of forward and reverse primers (10 µM), 0.08 uL probe (100 µM), ddH_2O and DNA template. The PCR program was initated with 50°C for 2 min, 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec and 60°C for 90 sec.

2.2.3 DNA-metabarcoding

Two different gene regions were amplified: an approximately 372 bp fragment (TAReuk) of the hypervariable V4 region of the nuclear 18S rRNA gene, using the universal primers TAReuk454FWD1 and TAReukREV3r (Stoeck et al. 2010), and a 313 bp fragment (Leray-XT) of the mitochondrial COI gene, using the universal primers mlCOIintF2-XT and jgHCO2198 (Wangensteen et al. 2018 and Geller et al. 2013). These markers target eukaryotes (TAReuk) and invertebrates (Leray). The amplifications followed the two-step Illumina (San Diego, CA, USA) 16S protocol (Illumina, 2013). The first-stage TAReuk PCR reactions had a final volume of 25 µL containing 2.5 µL DNA (each sample was diluted to have approximately 10 ng/µL DNA), 12.5 µL 2 × KAPA HiFi HotStart ReadyMix (Roche Sequencing Solutions, Pleasanton, CA, USA), 5.0 µL forward primer (2 µM), and 5.0 µL reverse primer (2 µM). The PCR conditions were, with a heated lid, 94 °C for 5 min, followed by a total of 25 cycles of 94 °C for 40 s, 53 °C for 1 min, and 72 °C for 30 s, and a final extension at 72 °C for 3 min. The Leray PCR reactions in the first step had a final volume of 25 µL containing 2.5 µL DNA (each water sample was diluted to 10 ng/µL DNA, while biofouling material was diluted to 1 ng/µL DNA), 12.5 µL 2 × KAPA HiFi HotStart ReadyMix, 1.25 µL forward primer (5 µM), 1.25 µL reverse primer (5 µM), and 7.5 µL molecular grade H_2O. The PCR conditions followed a touchdown protocol, with a heated lid and 5 min preheating at 95°C followed by 16 cycles of 10 sec at 95°C, 30 sec at 62°C and 1 min at 72°C with a 1°C reduction in annealing temperature in each new cycle, followed by 25 cycles of 10 sec at 95°C, 30 sec at 46°C and 1 min at 72°C, and a final extension of 5 min at 72°C. All PCRs included negative control reactions (no DNA).

In the second PCR step, the amplicons were dual-indexed using the Illumina DNA/RNA UD-Index Kit A-D under PCR conditions with a heated lid, 95°C for 3 minutes, followed by a total of 8 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The second-stage PCRs had a final volume of 50 µL containing 5 µL of the first-stage PCR product, 25 µL of 2 × KAPA HiFi HotStart ReadyMix, 10 µL of Index Dual Primer, and 10 µL of molecular grade H_2O. The PCR products were visualized on a Tape Station (Agilent 4200, Agilent, Santa Clara, CA, USA) to check if the amplification was successful and purified

with magnetic beads (Mag-Bind® TotalPure NGS, Omega Bio-Tek Inc., Norcross, GA, USA) after each PCR step. Finally, the indexed amplicons were normalized based on values from Tape Station and assembled into a library. The library was sequenced using a 2x250bp flow cell on the Illumina NovaSeq 6000 platform at the Norwegian Sequencing Center in Oslo.

2.3 Bioinformatic analyses

Primers were removed from both the 5' and 3' ends of the sequences using cutadapt 3.7 (Martin 2011) with a minimum length of 17 bp and 0.15 expected errors in the primer region. Quality filtering, error correction, merging, and chimera detection of the sequence data were performed using DADA2 1.9 in R (Callahan et al. 2016). Sequences with ambiguous bases, sequences with >2 expected errors in the forward and reverse directions, or sequences with length <50bp after truncation at the first occurrence of a base with a quality score <10 were removed from the dataset. Error rate models with monotonicity were estimated per marker for both forward and reverse sequences. After correction, the sequences were merged with a minimum overlap of 30 bp. Amplicon sequence variants (ASVs) were generated for each sample and chimeric sequence variants were assessed per sample. If a sequence variant was flagged as chimeric in more than 90% of the samples in which it occurred, it was removed.

TAReuk ASVs were first identified using DADA2 with the PR2 5.0.0 reference database (Guillou et al. 2012). Second, ASVs were identified using blastn in BLAST+ 2.6.0 (Zhang et al. 2000) to search the PR2 database, and third, using blastn to search the NCBI GenBank (Clark et al. 2015) (Clark et al. 2016). An ASV was retained if it was associated with a eukaryotic species, was classified using PR2, and had a BLAST match of >97% and >80% coverage to a sequence in PR2 or GenBank. Leray ASVs were first identified using the RDP classifier implemented in mothur 1.48 (Schloss Patrick et al. 2009) with the MetaZooGene v2023-m07-15 global reference database (Bucklin et al. 2021) and then, using blastn to search the MetaZooGene and GenBank reference databases. An ASV was retained if it was associated with a metazoan species, was classified using mothur, and had a BLAST match of >97% and >80% coverage to a sequence in MetaZooGene or GenBank.

2.4 Data analyses

The species lists were cross-checked against the Norwegian Red List from 2021 (Artsdatabanken 2021) and the alien species list from 2023 (Artsdatabanken 2023). In addition, we cross-checked the species lists against the European Alien Species Information Network (EASIN) list of invasive species in Europe (European Commission 2025). Statistical analyses were performed using R 4.4.1 (R Core Team 2024) and the R packages phyloseq 1.48.0 (McMurdie & Holmes 2013), VennDiagram 1.7.3 (Chen & Boutros 2011), eulerr 7.0.4 (Larsson 2018), iNEXT 3.0.2 (Hsieh 2016), glmmTMB 1.1.13 (Brooks et al. 2017) and ggplot2 3.5.1 (Wickham 2016).

3 Results

In total, we collected 161 samples, including 42 water samples from the sediment tank, 48 water samples from the biofouling bags, 63 samples from the biofouling material and 8 samples from the port water as control samples.

3.1 Detection of *Didemnum vexillum*

Two water samples (one from water tank and one from biofouling water) from PASS4 sampled at Nøsteterminalen in Bergen tested positive for *D. vexillum* and another biofouling water sample was unconclusive (**Table 2**). The control port water sample was negative, indicating that this signal was originating from *D. vexillum* on the ship.

The qPCR analyses for the detection of *D. vexillum* were positive in one biofouling water sample and inconclusive in another sample collected from PSV3 in Tananger (**Table 2**). However, one of the port water samples also tested positive, suggesting that the positive could have originated from *D. vexillum* present in the port environment rather than from biofouling associated with the vessel.

***Table 2**. Results from qPCR analyses for detection of Didemnum vexillum. The analyses were run twice using two different DNA concentrations, with either 1 µL or 5 µL of DNA included. The column "qPCR" shows the proportion of positive replicates where at least 2 out of 3 PCR replicates are required to classify a sample as technically positive. Results with 1 out of 3 positive replicates are considered inconclusive and are highlighted in yellow. The column "C_T Mean" indicates the average number of PCR cycles required for the DNA to produce a defined fluorescence signal. Lower C_T values therefore indicate higher DNA concentrations. The column "C_T SD" shows the standard deviation of C_T values among replicates.*

			1 µl DNA			5 µl DNA		
Port	**Sample type**	**Vessel ID**	**qPCR**	**C_T Mean**	**C_T SD**	**qPCR**	**C_T Mean**	**C_T SD**
Bergen	Biofouling water	CAR	0/3			0/3		
Bergen	Biofouling water	CAR	0/3			0/3		
Bergen	Biofouling water	CAR	0/3			0/3		
Bergen	Biofouling water	CAR	0/3			0/3		
Bergen	Water tank	CAR	0/3			0/3		
Bergen	Water tank	CAR	0/3			0/3		
Bergen	Water tank	CAR	0/3			0/3		
Bergen	Water tank	CAR	0/3			0/3		
Bergen	Biofouling water	PSV4	0/3			0/3		
Bergen	Biofouling water	PSV4	0/3			0/3		
Bergen	Biofouling water	PSV4	0/3			0/3		
Bergen	Biofouling water	PSV4	0/3			0/3		
Bergen	Water tank	PSV4	0/3			0/3		
Bergen	Water tank	PSV4	0/3			0/3		
Bergen	Water tank	PSV4	0/3			0/3		
Bergen	Water tank	PSV4	0/3			0/3		
Bryggen, Bergen	Port water		0/3			0/3		
Bryggen, Bergen	Port water		0/3			0/3		
Bryggen, Bergen	Port water		0/3			0/3		
Bryggen, Bergen	Port water		0/3			0/3		
Laksevåg, Bergen	Port water		0/3			0/3		

			1 µl DNA			5 µl DNA		
Port	**Sample type**	**Vessel ID**	**qPCR**	**C_T Mean**	**C_T SD**	**qPCR**	**C_T Mean**	**C_T SD**
Laksevåg, Bergen	Port water		0/3			0/3		
Tananger	Biofouling water	PSV1	0/3			0/3		
Tananger	Biofouling water	PSV1	0/3			0/3		
Tananger	Biofouling water	PSV1	0/3			0/3		
Tananger	Water tank	PSV1	0/3			0/3		
Tananger	Water tank	PSV1	0/3			0/3		
Tananger	Water tank	PSV1	0/3			0/3		
Tananger	Water tank	PSV1	0/3			0/3		
Tananger	Water tank	PSV1	0/3			0/3		
Tananger	Biofouling water	PSV2	2/3	41.18	1.09	0/3		
Tananger	Biofouling water	PSV2	0/3			0/3		
Tananger	Biofouling water	PSV2	1/3	40.92		0/3		
Tananger	Biofouling water	PSV2	0/3			0/3		
Tananger	Water tank	PSV2	0/3			0/3		
Tananger	Water tank	PSV2	0/3			0/3		
Tananger	Water tank	PSV2	0/3			0/3		
Tananger	Water tank	PSV2	0/3			0/3		
Tananger	Biofouling water	PSV3	0/3			0/3		
Tananger	Biofouling water	PSV3	0/3			0/3		
Tananger	Biofouling water	PSV3	0/3			0/3		
Tananger	Biofouling water	PSV3	0/3			0/3		
Tananger	Water tank	PSV3	0/3			0/3		
Tananger	Water tank	PSV3	0/3			0/3		
Tananger	Water tank	PSV3	0/3			0/3		
Tananger	Water tank	PSV3	0/3			0/3		
Tananger	Port water		0/3			0/3		
Tananger	Port water		2/3	40.13	0.40	3/3	39.20	0.27
Tananger	Biofouling water	PSV5	0/3			0/3		
Tananger	Biofouling water	PSV5	0/3			0/3		
Tananger	Biofouling water	PSV5	0/3			0/3		
Tananger	Biofouling water	PSV5	0/3			0/3		
Tananger	Water tank	PSV5	0/3			0/3		
Tananger	Water tank	PSV5	0/3			0/3		
Tananger	Water tank	PSV5	0/3			0/3		
Tananger	Water tank	PSV5	0/3			0/3		
Jekteviken	Water tank	CRU	0/3			0/3		
Jekteviken	Biofouling water	CRU	0/3			0/3		
Jekteviken	Biofouling water	CRU	0/3			0/3		
Jekteviken	Water tank	CRU	0/3			0/3		
Jekteviken	Biofouling water	CRU	0/3			0/3		
Jekteviken	Water tank	CRU	0/3			0/3		
Jekteviken	Biofouling water	CRU	0/3			0/3		
Jekteviken	Water tank	CRU	0/3			0/3		

			1 µl DNA			5 µl DNA		
Port	Sample type	Vessel ID	qPCR	C_T Mean	C_T SD	qPCR	C_T Mean	C_T SD
Jekteviken	Biofouling water	CRU	0/3			0/3		
Jekteviken	Water tank	CRU	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS1	0/3			0/3		
Nøsteterminalen	Water tank	PASS1	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS1	0/3			0/3		
Nøsteterminalen	Water tank	PASS1	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS1	0/3			2/3	41.35	0.08
Nøsteterminalen	Water tank	PASS1	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS2	0/3			0/3		
Nøsteterminalen	Water tank	PASS2	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS2	0/3			0/3		
Nøsteterminalen	Water tank	PASS2	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS2	0/3			0/3		
Nøsteterminalen	Water tank	PASS2	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS2	0/3			0/3		
Nøsteterminalen	Water tank	PASS2	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS2	0/3			0/3		
Nøsteterminalen	Water tank	PASS2	0/3			0/3		
Nøsteterminalen	Water tank	PASS3	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS3	0/3			0/3		
Nøsteterminalen	Water tank	PASS3	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS3	0/3			0/3		
Nøsteterminalen	Water tank	PASS3	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS3	0/3			0/3		
Nøsteterminalen	Water tank	PASS3	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS3	0/3			0/3		
Nøsteterminalen	Water tank	PASS4	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS4	2/3	43.78	0.51	0/3		
Nøsteterminalen	Water tank	PASS4	3/3	42.76	1.68	0/3		
Nøsteterminalen	Biofouling water	PASS4	1/3	44.33		0/3		
Nøsteterminalen	Water tank	PASS4	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS4	0/3			0/3		
Nøsteterminalen	Water tank	PASS4	0/3			0/3		
Nøsteterminalen	Biofouling water	PASS4	2/3	41.92	0.11	0/3		
	Negativ PCR kontroll		0/2			0/2		
	Negativ DNA kontroll		0/3			0/3		
	Positiv DNA kontroll		2/2	23.38	0.09	2/2	20.97	0.31

3.2 DNA metabarcoding

We detected a total of 2199 genetic taxa (OTUs) across the 161 samples. Single-celled eukaryotes was a major group in term of species richness (**Figure 5**). However, because their alien/native status is not well known, this group was excluded from further analyses, leaving 784 species remaining. The most diverse groups detected were Arthropoda, Annelida, Mollusca and Rhodophyta, including 129, 111, 87 and 67 species, respectively. In most groups, species could be classified as either native or alien (on average, 80% classified). In contrast, for several meiofaunal groups such as Nematoda, Platyhelminthes and Tardigrada, most species could not be classified as native or alien due to limited knowledge of their natural distributions and because they have not yet been included in the Norwegian assessments. Similarly, most Porifera species remained unclassified. Arthropoda and Rhodophyta contained the highest number of alien species in the dataset (**Figure 5**).

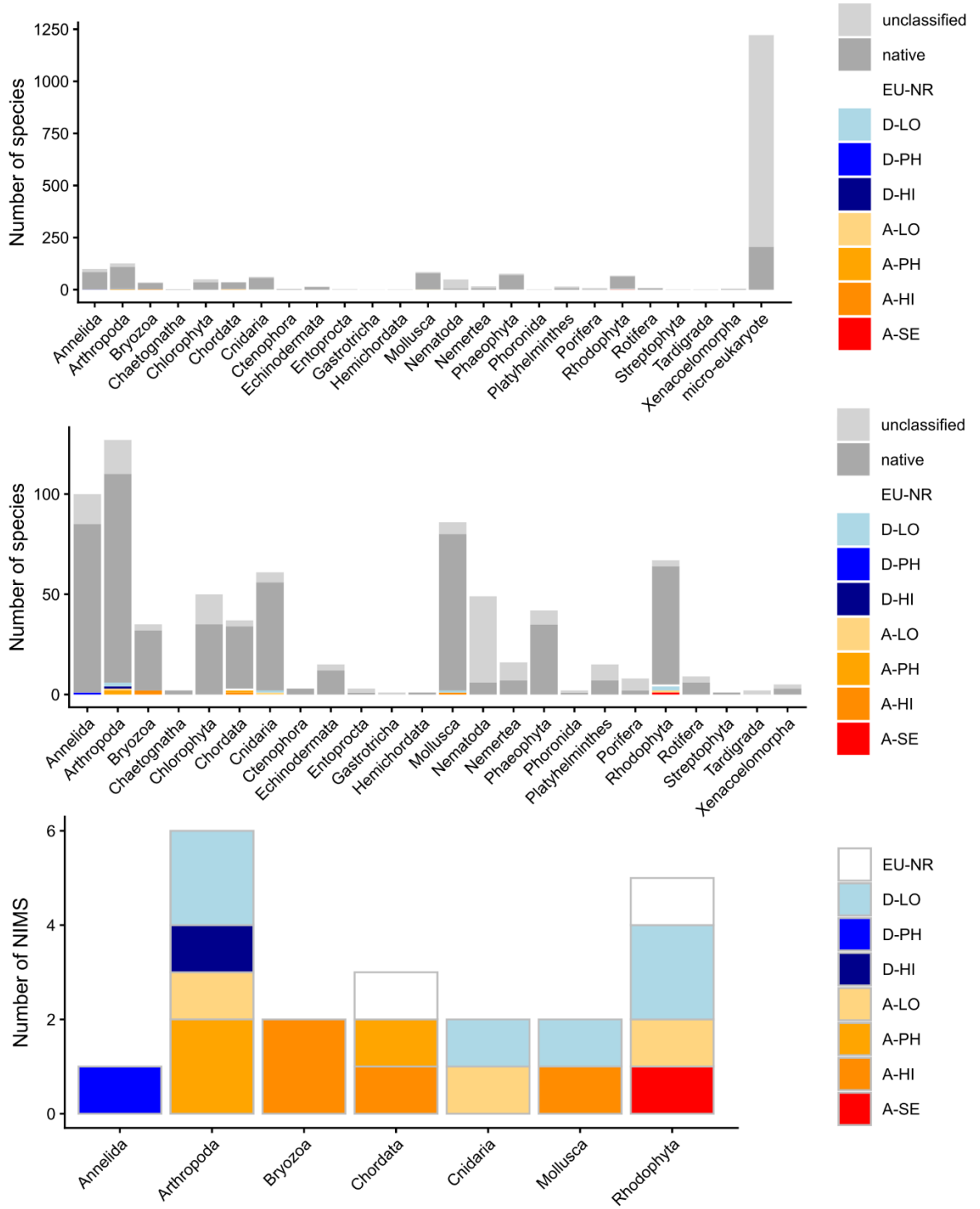

***Figure 5**. The total number of species (upper panel), the number of species excluding single-celled eukaryotes (middle panel), and the number of alien species only (lower panel) recorded in the analyses, coloured by their alien assessment risk category. Door-knocker species are represented by blue shades and "D- "preceding their risk category, while alien species are represented by yellow to red shades and an "A- "preceding their risk category". LO=Low, PH=Potential High, HI=High, SE=Severe. Species listed only in the European EASIN list are in white and "EU- "preceding their risk category NR=Not Risk Assessed".*

In total, we detected 19 NIMS included in the Norwegian alien species list (**Figure 6**, **Figure 7**). In addition, two NIMS listed in the EASIN European alien species list, but not yet included in the Norwegian list, were also detected. The red alga *Bonnemaisonia hamifera* was the only species classified as a severe impact (SE) alien species recorded in this study. High impact (HI) alien species included the bryozoans *Schizoporella japonica* and *Juxtacribrilina mutabilis*, the mollusk *Crepidula fornicata* and rainbow trout *Oncorhynchus mykiss*. We also detected several doorknocker species, including the high risk (HI) barnacle *Austrominius modestus*. *Asterocarpa humilis* and *Grateloupia* sp. are not currently included in the Norwegian list but are listed in the European EASIN database. The genus *Grateloupia* includes several invasive alien species relevant to Norway, but species-level identification was not possible because the genetic marker used (TAReuk) showed insufficient variability to distinguish among species.) The alien species *Grateloupia turuturu* was first recorded in Norway in 2019 and is now present at several marinas in Rogaland County (Husa et al. 2024). In addition, we recorded several potential alien species that are either not yet included in the existing alien species, not previously recorded in Europe, or whose taxonomic affiliation remains uncertain based on the selected genetic markers (**Table 2**, **Appendix**).

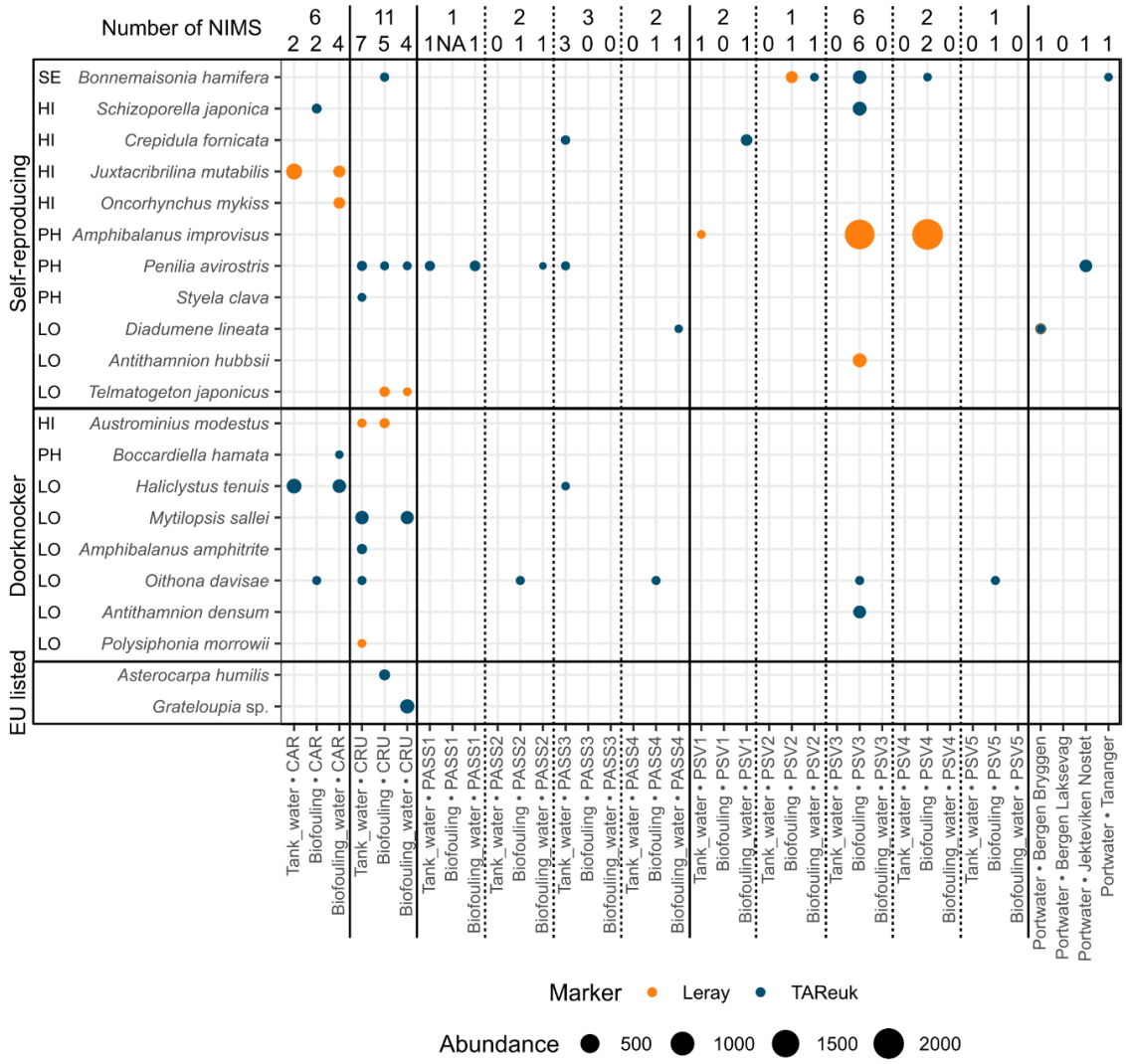

***Figure 6**. NIMS recorded that are included in the Norwegian alien species list, as self-reproducing or doorknocker species, or included in the EASIN European alien species list (EU-listed). Risk categories of the Norwegian listed species precede their name. LO=Low, PH=Potential High, HI=High, SE=Severe. Different genetic markers are represented by different colours, and the size of the circle represents the total number of DNA-copies recorded. Vertical solid lines differentiate different types of ships (CAR = cargo ship, CRU = cruise ship, PASS = passenger ferry, PSV = supply vessel) and dotted lines for different ships. Total number of NIMS is given above for each sample type; PASS1 was not sampled for biofouling material (NA).*

***Table 3**. A list of potential alien species detected. However, these were not included in the analyses as NIMS because most of these species have not been included in the official lists or have likely not been detected in Europe before.*

Species	Group	Origin	Marker	Vessel	Comment
Ercolania felina	Gastropoda		TAReuk	PSV3	
Acartia hudsonica	Copepoda		Leray TAReuk	Portwater, CAR, CRU, PASS1-4, PSV2, PSV5	Sequence identical to NIMS *Acartia tonsa*
Tortanus discaudatus	Copepoda	EU	Leray	PSV2	
Cauloramphus magnus	Bryozoa	Japan	Leray	CAR	
Gnathopleustes pachychaetus	Amphipoda	NE Pacific	Leray	CAR, CRU, PSV4	
Pseudocalanus mimus	Copepoda		Leray	Portwater	
Nematoflustra flagellata	Bryozoa	Antartica	TAReuk	CAR, PSV3, PASS3, PASS4	Sequence identical to NIMS *Juxtacribrilina mutabilis*
Zaus unisetosus	Copepoda	Korea	TAReuk	CAR, CRU, PSV1-4, PASS4	
Liponema brevicorne	Cnidaria	NW and NE Pacific	TAReuk	PSV2	
Amonardia coreana	Copepoda	Japan	TAReuk	Portwater, CAR, CRU, PSV2, PSV4, PASS1, PASS3, PASS4	
Salvatoria koorineclavata	Polychaeta		TAReuk	CRU	
Primavelans insculpta	Bryozoa	NW and NE Pacific	Leray TAReuk	CAR, PSV3	1 supporting record only (Leray), 1 base difference in TAReuk
Porphyra corallicola	Rhodophyta	W Canada	Leray	CRU, PASS2	1 supporting record only
Perlophiura profundissima	Echinodermata	Southern hemi-sphere	TAReuk	Portwater, CAR, PSV2, PSV4, PASS2-4	GBIF record from Bay of Biscay
Saccharina japonica	Phaeophyta	W Pacific	TAReuk	Portwater, CAR, CRU, PSV2-4, PASS1-4	3 bases difference to native species *Chorda filum*, but identical to alien species *Agarum clatharum*, *Alaria fistulosa*, *Ecklonia cava*, *Costaria costata*

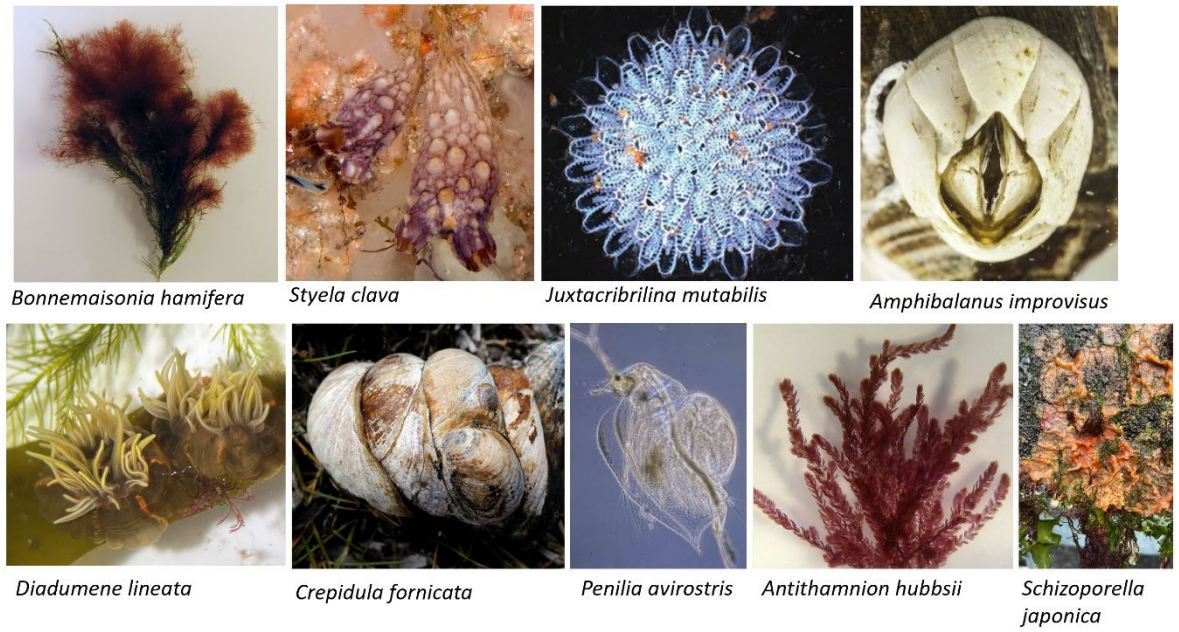

***Figure 7**. Species detected in this study and registered as self-reproducing alien species in Norway. Photos from various online sources CC BY-SA 3.0.*

The red alga *Bonnemaisonia hamifera*, the copepod *Penilia avirostris* and the barnacle *Amphibalanus improvisus* were the most frequently detected self-reproducing NIMS. The rest of the self-reproducing NIMS were only recorded from one or two vessels each (**Figure 6**). Three of these species were also detected in port water samples collected from three different ports. Thus, we can not exclude the possibility that some detections came from individuals established within the port rather than from individuals associated with the ships. The sea anemone *Diadumene lineata* has so far only been recorded sporadically in Norway.

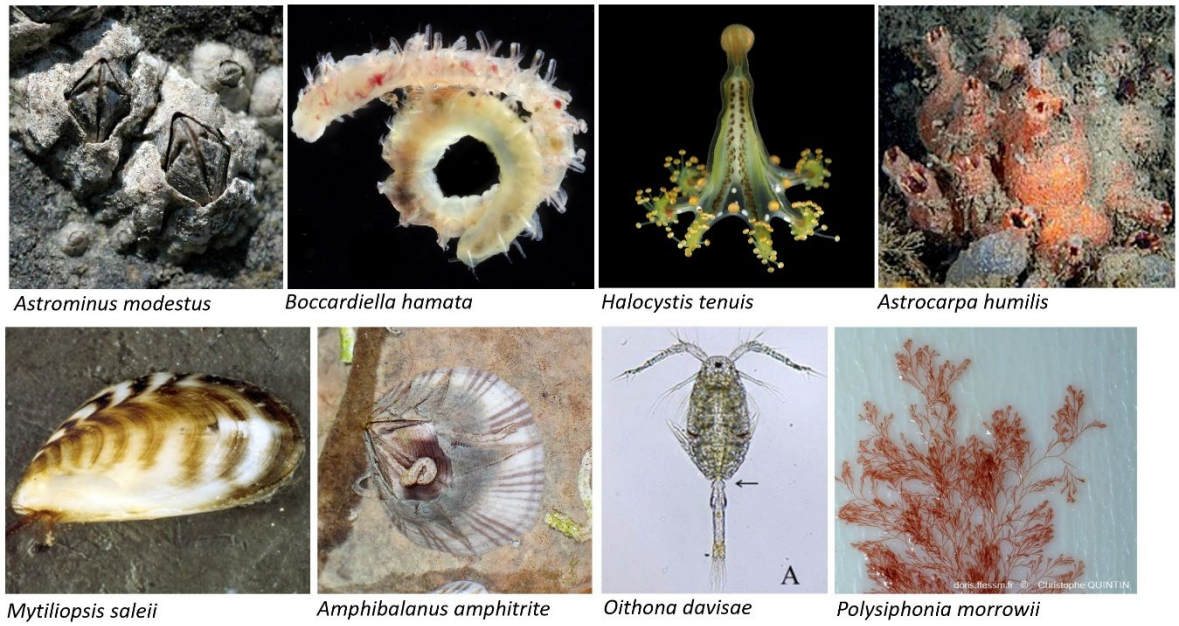

***Figure 8**. Species detected in this study and registered as door-knocker species in Norway. Photos from various online sources CC BY-SA 3.0.*

A total of 8 door-knocker species registered in Norway were detected in this study (**Figure 8**). None of these species were detected in the port water samples, indicating that their DNA were most likely originating from the ship hulls rather than being established in the ports. Most door-knocker species were detected on the two vessels engaged in international traffic (one cargo and one cruise vessels), with the exception of the cnidarian *Haliclystus tenuis*, the copepod *Oithona davisae* and the red algae *Antithamnion densum*, which were recorded on vessels operating along the Norwegian coast and the North Sea area (**Figure 6**). *H. tenuis* and *A. densum* have previously been recorded from Norwegian eDNA samples and are likely already established in Norwegian waters.

Approximately 12–18 % of all species were detected in all sample types and in all ships (**Figure 9**). Biofouling water samples contained the highest overall species richness (387 Metazoa and 127 macroalgae in 42 samples), followed by tank water samples (363 Metazoa and 128 macroalgae in 44 samples) and biofouling material samples (331 Metazoa and 123 macroalgae in 60 samples). Port water samples recorded the lowest number of species (185 Metazoa 54 macroalgae in 8 samples). Across vessel categories, the four passenger ferries presented the highest species richness (311 Metazoa and 110 macroalgae), followed by the cruise ship (271 Metazoa and 106 macroalgae), the five petroleum service vessels (265 Metazoa and 91 macroalgae) and the cargo vessel (267 Metazoa and 81 macroalgae).

Among the NIMS, only the ctenopod *Penilia avirostris* was recorded in all sample types, while the red algae *Bonnemaisonia hamifera* was detected in all sample types except tank water. All remaining NIMS were recorded in only one or two sample types (**Figure 9**). A total of 11 NIMS were recorded in both tank water and biofouling water samples, 10 in biofouling material samples and three in port water samples. The highest number of NIMS was recorded on the singe cruise ship, with 11 species detected in total, of which 8 were unique to that vessel. We detected 6 NIMS on the single cargo vessel, 6 NIMS across the five petroleum service vessels, and 5 NIMS across the four passenger ferries.

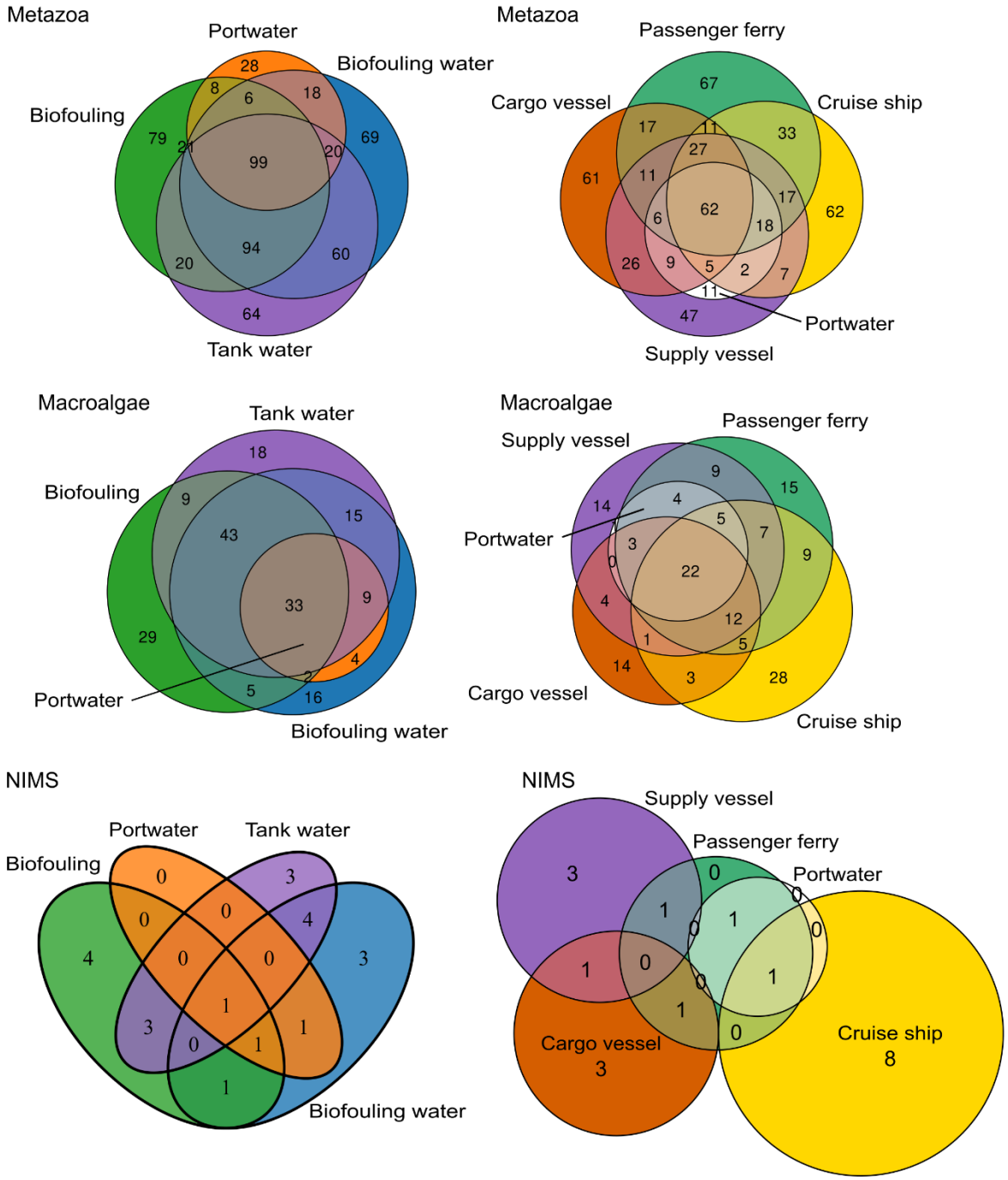

***Figure 9**. Venn diagram showing the number of species recorded for Metazoa (upper panels), macroalgae (middle panels) and NIMS (lower panels), coloured by sample material (left panels) and by vessel type (right panels). Note: Because there were more than three groups, the R package eulerr is unable to model all overlaps accurately. As a result, the number of shared species between the groups may deviate slightly from the actual number. For example, the NIMS Venn diagram showing the different vessel types is missing two species that could not be included in the diagram because they were shared among portwater, cruise ship and supply vessel (Bonnemaisonia hamifera) and among all vessels but not with portwater (Oithona davisae).*

To test whether species richness differed among sample types and in relation to fouling density, we used generalized linear mixed-effects models with vessel ID as a random effect to account for between-vessel variability. Separate models were fitted for each species group using a negative binomial distribution.

For both metazoan and macroalgal species, the models showed no significant differences in the number of detected species among sample types (biofouling material, biofouling water, tank water) after accounting for vessel ID and fouling density. However, both Metazoa and macroalgae showed clear between-vessel heterogeneity (random-intercept SD = 0.45 and 0.30, respectively). This implies that vessel ID accounted for 58% of the variation detected in Metazoan richness and 34% of the variation in macroalgal richness. In both datasets, fouling density had a significant negative effect on the species richness ($p = 0.016$ and $p = 0.007$), demonstrating a consistent decline in detected richness with increasing fouling density (**Figure 10**).

For NIMS, no significant differences in species richness were detected in relation to sample type ($p>0.1$). However, biofouling material samples were associated with a 63% higher number of detected NIMS, and biofouling water samples showed a 2% lower number of NIMS compared with tank water samples. Between-vessel heterogeneity was also observed (random-intercept SD = 0.443; variance = 0.194 on the log scale), implying that vessel ID accounted for 56% of the variation in NIMS richness. Fouling density showed marginally negative effect on the number of detected NIMS ($p = 0.098$).

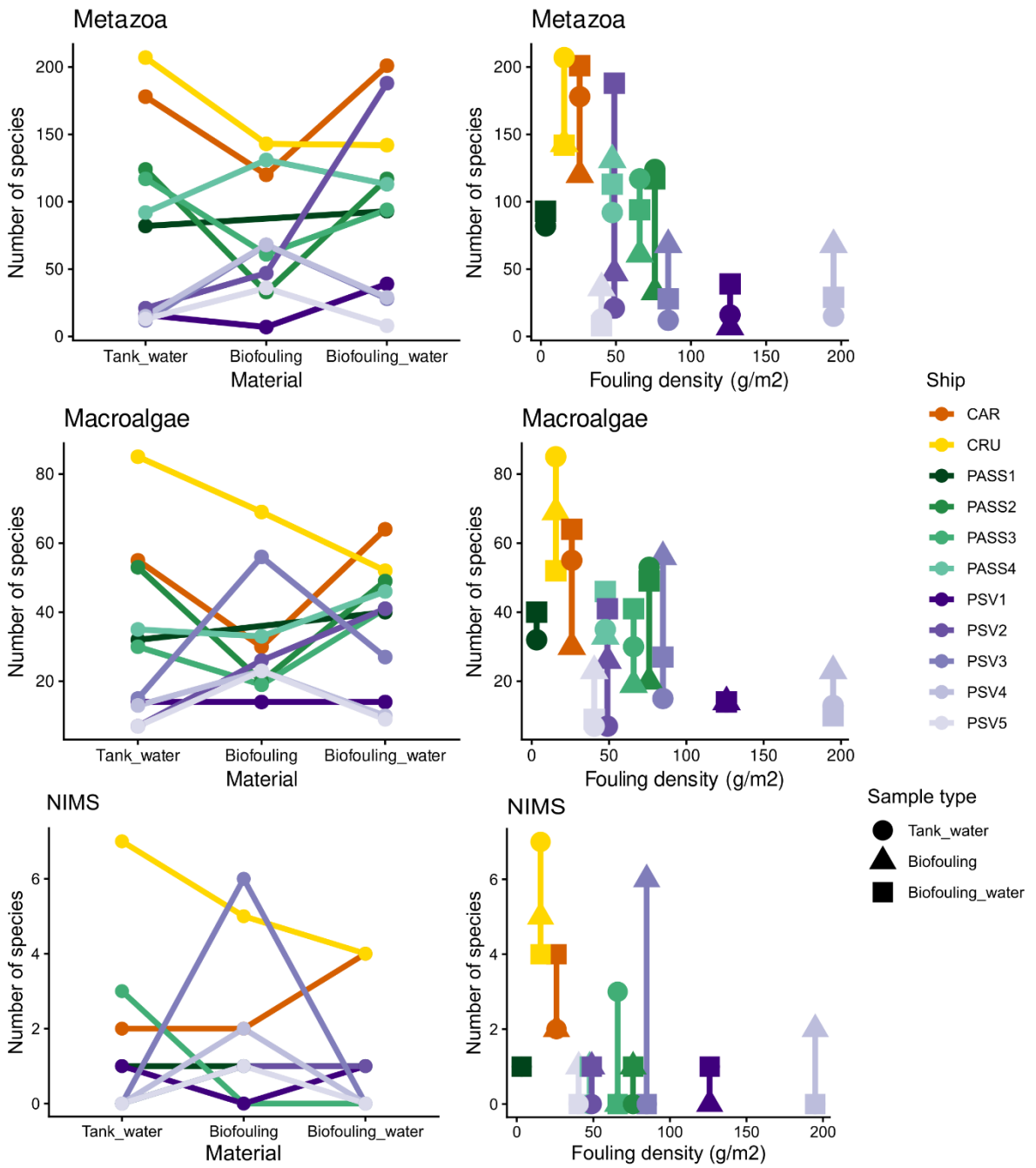

***Figure 10**. Line diagrams showing the number of species detected in different sample types (left panels) and the number of species detected in different sample materials in relation to fouling density (g/m^2, right panels). Data is divided into number of total metazoan species (upper panels), macroalgae (middle panels) and NIMS (lower panels). Fouling density showed a significant negative effect in the case of Metazoa and macroalgae (p = 0.016 and p = 0.007) and a non-siginificant negative effect in the case of NIMS (p = 0.098).*

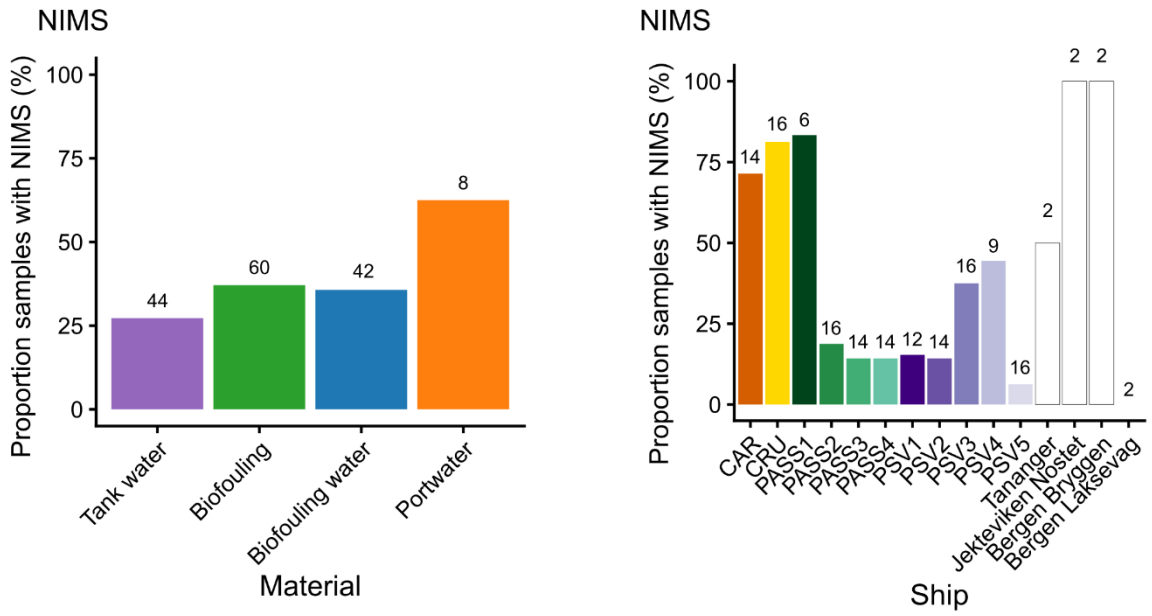

***Figure 11**. Proportion of samples that contained at least one NIMS recorded grouped by sample material (left) and vessel ID (right). The number of samples is given above the bars. CAR = cargo vessel, CRU = cruise ship, PASS = passenger ferry, PSV = petroleum service vessel.*

Approximately one third of the tank water (27%), biofouling material (37%) and biofouling water samples (36%) contained at least one NIMS, while five out of eight port water samples (63%) recorded at least one NIMS (**Figure 11**).

At the vessel level, samples collected from the cargo vessel (CAR: 71%), the cruise ship (CRU: 81%) and passenger ferry 1 (PASS1: 83%) most frequently contained at least one NIMS. In contrast, samples from other passenger ferries and petroleum service vessels recorded a NIMS in less than half of the samples (6-44%).

Species accumulation curves generally reached a plateau, suggesting that the sampling effort was sufficient to detect most species present on the vessels (**Figure 12**). In general, higher species richness was recorded on the cargo vessel and cruise ship compared with the passenger ferries and petroleum service vessels. The number of detected NIMS was insufficient to construct robust species accumulation curves specifically for this group. Nevertheless, the cruise ship harboured the highest number of NIMS, with almost twice as many as the second most affected vessels (the cargo vessel and petroleum service vessel 3). Samples from the cruise ship consistently showed high numbers of NIMS across tank water, biofouling material, and biofouling water samples.

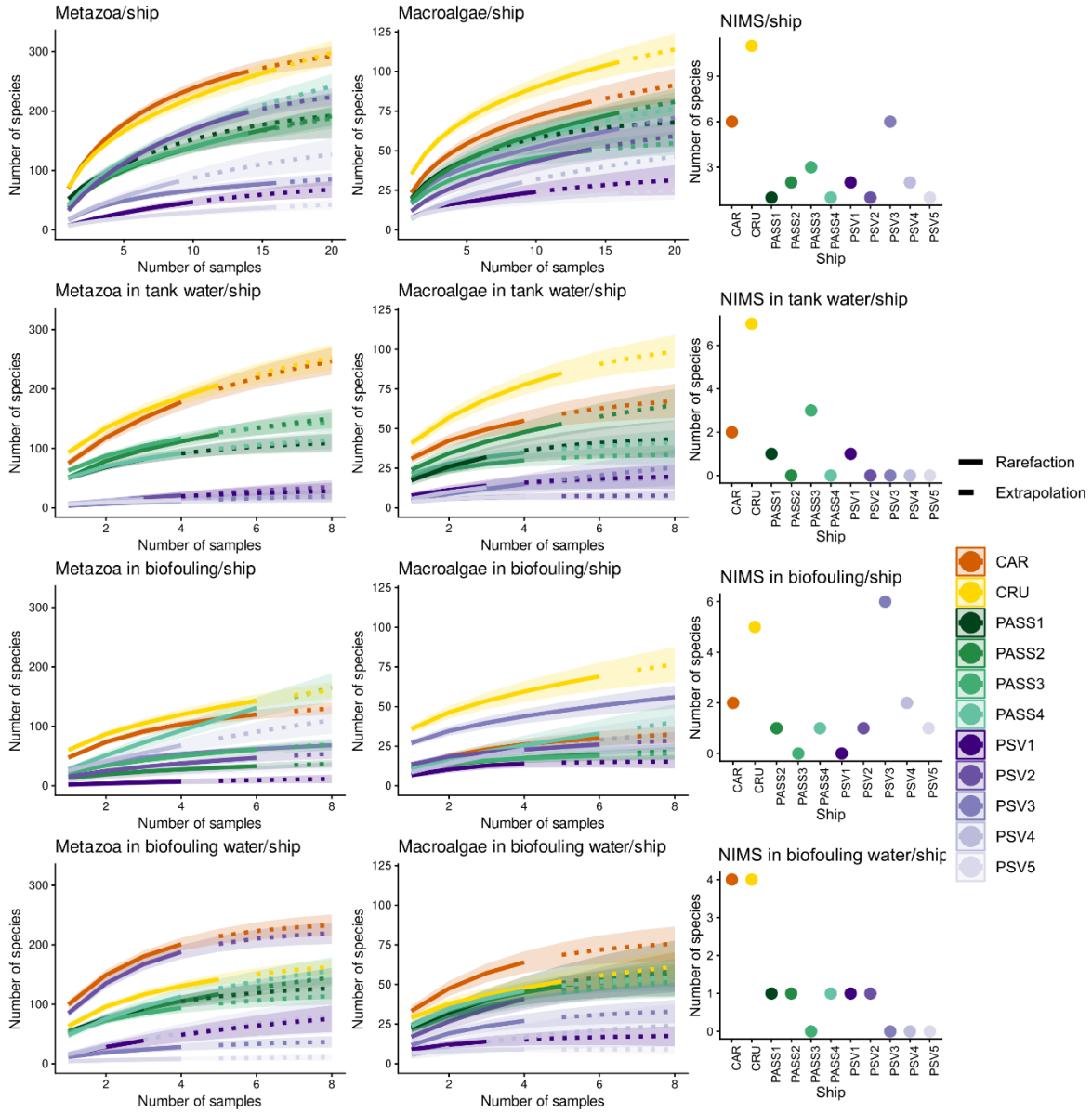

***Figure 12**. Species accumulation curves showing the total number of species detected across samples per ship for Metazoa (left panel) and Macroalgae (middle panel) with 95 % confidence intervals. The solid lines show the actual number of species detected and the stippled lines show a model prediction of the total number of species if more samples had been analysed. The right panel show the number of NIMS detected in the different vessels. The top row shows number of species in all samples/vessel, while the second row shows number of species in samples taken from tank water/vessel, third row in biofouling material samples/vessel and the lowest row the number of species in samples taken from biofouling material water/vessel. Non-overlapping 95-% confidence intervals indicate significantly different number of species.*

4 Discussion

Non-Indigenous Marine Species (NIMS) pose a growing threat to coastal ecosystems worldwide (Seebens et al. 2017). Their establishment can lead to severe biodiversity loss, alteration of ecosystem functioning, and disruption of key ecological processes (Katsanevakis et al. 2023, Tsirintanis et al. 2023). In addition to ecological impacts, NIMS can impose significant economic costs through damage to fisheries, aquaculture, marine infrastructure, and tourism (Diagne et al. 2021). Human activity is the primary driver of these biological invasions, with maritime transport recognized as the dominant pathway. While ballast water historically has represented the major vector, recent international regulations and improved treatment systems have reduced this pathway's relative importance. As a result, biofouling has emerged as the most influential vector of new introductions. Globalization and increased maritime traffic have increased the diversity and frequency of species being transported across biogeographical boundaries. Climate change further interacts with these processes by expanding the range conditions suitable for non-native species survival and establishment. These trends underscore the urgency of developing monitoring frameworks capable of early detection, which is crucial for preventing establishment and reducing management costs.

Obtaining representative biofouling samples from large commercial vessels remains a logistical and technical challenge. Direct sampling of hull surfaces is often restricted by vessel size, operational schedules and safety considerations. To overcome these limitations, we tested a novel approach combining ROV hull cleaning with eDNA sampling of the resulting biofouling wastewater and biofouling material.

Our pilot study, conducted in the ports of Tananger and Bergen in Norway, included cargo vessels, cruise ships, and coastal steamers mostly operating in Norwegian waters. By collecting both wastewater from the ROV cleaning system and the biofouling debris itself, we were able to compare the detection performance across sample types. In addition, we also collected eDNA water samples from the ports to help exclude species likely to be found in the harbour rather than on the vessels. DNA-metabarcoding revealed a high diversity of taxa in general, with 784 multicellular species detected in total, including 19 species listed as NIMS and 8 door-knocker species. Three of the recorded NIMS were also detected in a single port water sample each. Two of these species were recorded on several different vessels and in different ports, suggesting that they were likely found both on the vessels and in the harbour. Importantly, all the 8 door-knocker species were only recorded in the biofouling samples, with no detections in the port water, suggesting that they were all originating from the vessel hulls, and thus represented a real threat for new introductions to the Norwegian coast. The presence of door-knockers in this pilot study provides strong evidence that the method can function as an early warning tool, identifying species at the stage of arrival before they become established or spread. Early detection of such species is critical, as management interventions are most effective and cost-efficient during the initial introduction stage. The approach tested here has the potential to become an efficient and non-intrusive monitoring tool, especially in busy ports where direct inspection of vessel hulls is difficult.

Most of the door-knockers were detected on the vessels in international traffic (cargo and cruise ships), with the exception of the cnidarian *Haliclystus tenuis*, the copepod *Oithona davisae* and the red algae *Antithamnion densum*, that were recorded on vessels operating in the Norwegian coast and in the North Sea area. *H. tenuis* and *A. densum* have previously been recorded from Norwegian eDNA samples and is probably already established in Norwegian waters. Our pilot study only included two international vessels, and hence a future screening of more international vessels could potentially reveal a much higher occurrence of door-knockers.

The red alga *Bonnemaisonia hamifera*, the copepod *Penilia avirostris* and the barnacle *Amphibalanus improvisus* were the most frequently found self-reproducing NIMS in Norway. The other self-reproducing NIMS were only recorded from one or two vessels each. Three of the species were also recorded in the port water. The anemone *Diadumene lineata* has only been

sporadically recorded in Norway so far, and hence the occurrence of this species on ship hulls indicates that the vessels pose a major threat for further spreading.

The number of NIMS detected by the three different sampling materials, sedimentation tank water, biofouling waste water, and biofouling material, was relatively similar. Importantly, they did detect different species, and no material detected more than ca. 50% of the NIMS recorded in this study. Hence, we recommend that future studies collect all three sample types in order to maximise detection probability of alien species. Based on the accumulation curves of all species detected we would recommend at least 5 samples per material to find most species on a vessel. However, different vessels vary greatly in size, and the number of samples should be adjusted based on the cleaning area.

We also included the level of biofouling, represented as fouling density of the vessel hull. Surprisingly, we found a negative relationship between fouling density and the number of species detected. Hence, vessels with more biofouling actually contained fewer species. However, our pilot study only included two international vessels, and despite low fouling density they contained the highest number of NIMS. Thus, we need more data on international vessels to properly assess the relationship between fouling density and the number of alien species.

By aligning emerging molecular technologies with practical operational workflows in ports, this approach contributes to a more proactive and evidence-based marine biosecurity framework. As maritime traffic continues to intensify and environmental conditions shift, innovative monitoring solutions such as this will be essential for preventing spread of marine alien species and supporting sustainable coastal management.

Overall, ROV-assisted hull cleaning combined with eDNA analysis represents a promising addition to the suite of tools available for NIMS monitoring. It allows for standardized, repeatable, and efficient sampling of large vessels, provides comprehensive insight into biofouling community composition, and can be incorporated into routine cleaning operations with minimal disruption. If integrated into the national monitoring program, this method could significantly strengthen Norway’s capacity to detect invasive species early, assess biosecurity risks associated with incoming vessels, and design targeted management strategies to prevent secondary spread.

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6 Appendix.

***Appendix table 1**. A list of potential alien species detected, but where the genetic species determination is uncertain due to closely related native species and low species discrimination ability for the TAReuk genetic marker.*

Species	Latin	Origin	Marker	Comment
Loimia ingens	Polychaeta	Australia, NE Pacific	TAReuk	identical to native species *Scoloplos armiger*
Vesicomya galatheae	Mollusca	NW and NE Pacific	TAReuk	Sequence identical to native species *Arctica islandica*
Ceriantheopsis americana	Cnidaria	NE Pacific	TAReuk	Sequence identical to native species *Cerianthus lloydii*
Capitella teleta	Polychaeta		TAReuk	Sequence identical to native species *Capitella capitata*
Phallusia ingeria	Ascidiacea		Leray	Sequence identical to native species *Ascidia mentula*
Euspira gilva	Mollusca	Japan, Korea	TAReuk	Sequence identical to *Cryptonatica* sp. including several native species
Sphaeronectes christiansonae	Cnidaria	NE Pacific	TAReuk	Sequence identical to native species *Agalma elegans*
Balanus rostratus	Thecostraca	NW and NE Pacific	Leray	Sequence identical to native species *Balanus balanus*
Calanus euxinus	Copepoda	Black sea	Leray	Sequence identical to native species *Calanus helgolandicus*
Cirratulus spectabilis	Polychaeta		TAReuk	Sequence identical to native species *Cirratulus cirratus*
Coryphella amabilis	Mollusca	Korea, West US	TAReuk	Sequence identical to native species *Coryphella verrucosa*
Lepidonotus sublevis	Polychaeta	Gulf of Mexico	Leray	Sequence identical to native species *Gattyana amondseni*
Gorgoniapolynoe caeciliae	Polychaeta	France	TAReuk	Sequence identical to native species *Harmothoe imbircata*
Vrijenhoekia balaenophila	Polychaeta		TAReuk	Sequence identical to native species *Hesiopsina aurantiaca*
Metridia curticauda	Copepoda	Sørlige halvkule, spredt ett funn i Nordsjøen	TAReuk	Sequence identical to native species *Metridia lucens*
Ophiothrix oerstedii	Echinodermata	Gulf of Mexico	TAReuk	Sequence identical to native species *Ophiopholis aculeata*
Pasiphaea telacantha	Decapoda	New Caledonia	TAReuk	Sequence identical to native species *Pasiphaea sivado*
Spirobranchus latiscapus	Polychaeta		TAReuk	Sequence identical to native species *Pomatoceros triqueter*
Tetraclitella divisa	Thecostraca	South America, Africa, Indonesia	TAReuk	Sequence identical to native species *Semibalanus balanoides*
Hexelasma velutinum	Thecostraca	Indonesia	TAReuk	Sequence identical to *Balanus perforatus*, native species?
Amphicorina mobilis	Polychaeta		TAReuk	Sequence identical to *Chone infundibuliformis*, native species?
Protodrilus ciliatus	Polychaeta	Faroe Islands	TAReuk	Native species?
Polycirrus carolinensis	Polychaeta	Gulf of Mexico	TAReuk	1 base difference to native species *Polycirrus norvegicus*
Eubranchus rustyus	Mollusca		TAReuk	1 base difference to native species *Eubranchus rupium*
Ascidia zara	Ascidiacea	NW NE pacific	TAReuk	1 base difference to native species *Phallusia mammilata*

Stomopneustes variolaris	Echinodermata	Africa, Australia	TAReuk	1 base difference to native species *Spatangus raschi*
Urechis unicinctus	Polychaeta	NW Pacific	TAReuk	2 bases difference to native species *Echiurus echiurus*
Barantolla lepte	Polychaeta	Australia	TAReuk	3 bases difference to native species *Heteromastus filiformis*
Dactylopusia pauciarticulata	Copepoda		TAReuk	<99% match, might be a related species
Quinqulaophonte aurantius	Copepoda		TAReuk	<99% match, might be a related species
Apistobranchus typicus	Polychaeta		TAReuk	98% match, might be a related species
Boccardia pugettensis	Polychaeta	NE, Pacific	Leray	No match in GenBank or BOLD, potential misidentification in MetaZooGene

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NINA Report

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