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## Looking for alien invertebrates in Norwegian ports, extensive sampling, and precise identification

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# Abstract

Alien species, together with climate change, habitat loss to land and sea use, overexploitation and pollution are the major threats to biodiversity globally. In the marine environment, species are spread through different pathways, international shipping and aquaculture being some of these. One important step in managing alien species is to monitor areas susceptible to receive alien species for early detection. Such areas are, among others, commercial harbors that receive international shipping and marinas where recreational crafts may contribute to secondary spread of species. In this study, the occurrence of 21 alien marine invertebrates were investigated in 22 harbors and marinas along the coast between Trondheim and Oslo. International harbors along the coast between Trondheim and Oslo were surveyed following sampling procedures from the HELCOM/OSPAR sampling protocol. Marinas for recreational crafts in the same area were sampled with a Rapid Coastal Survey approach. Alien species were found in all surveyed sites. More alien species was found in the southern part of the study area than further north. Four species, the amphipod *Caprella mutica*, the cirriped *Amphibalanus improvisus*, the bivalve *Crassostrea gigas* and the ascidian *Styela clava*, were recorded. All the species found are already known to be established in Norwegian waters, no doorknockers, species that are established in neighboring countries or expected to establish in Norway within 50 years, were found. Correct species identification is crucial for monitoring of alien species. Tools to aid precise identification are provided for the four species found in this study, with special regard to distinguishing the alien species from native congruences. Identification sheets for these species are provided. An identification key for commonly found caprellids in Norwegian waters was developed.

# Sammendrag

Fremmedarter er sammen med klimaendringer, tap av habitat, overhøsting og forurensning de mest alvorlige truslene globalt mot biologisk mangfold. I det marine miljø spres arter blant annet gjennom internasjonal skipstrafikk og introduksjoner for akvakultur. Et viktig steg for å kunne effektivt forvalte fremmedarter er å overvåke områder som er spesielt sårbare for å motta nye arter. Kommersielle havner som mottar internasjonal skipsfart, og småbåthavner hvor fritidsfartøy kan bidra til sekundærspredning er eksempler på områder som bør overvåkes for tidlig oppdagelse av nyankomne fremmedarter. I denne studien ble forekomst av 21 fremmede marine virvelløse dyr undersøkt. Prøver ble tatt i 22 havner langs kysten mellom Trondheim og Oslo. Internasjonale havner i dette området ble undersøkt med metoder fra HELCOM/OSPAR protokollen for havneundersøkelser. Småbåthavnene ble studert etter Rapid Coastal Survey metodikk. Fremmedarter ble funnet på alle de undersøkte stedene. Det ble funnet flere fremmede arter i den sørlige delen av studieområdet enn lenger nord. Fire fremmedarter ble funnet, spøkelseskrepsen *Caprella mutica*, ruren *Amphibalanus improvisus*, muslingen *Crassostrea gigas* og sekkedyret *Styela clava*. Alle disse artene er allerede kjent fra norske farvann. Ingen dørstokkarter, arter som er etablert i naboland eller forventet å komme til norske kystområder innen 50 år, ble funnet. Presis artsbestemmelse er viktig i arbeidet med å overvåke fremmedarter. I denne studien ble det lagd bestemmelsesverktøy for de artene som ble funnet. Bestemmelsesverktøyene har spesielt søkelys på å skille fremmedartene fra hjemmehørende forvekslingsarter. En bestemmelsesnøkkel for vanlige spøkelseskreps i norske farvann ble også utviklet.

# Preface

A thesis project is a long process. I started out with no idea about how to do such a project, I did not know up from down on a pacific oyster and *Styela clava* could just as well be a strange ball game for all I knew. Now, one and a half year later I am approaching the end of a process of wet hands, painstaking investigation of tiny creatures in the lab, hours of research on how to identify the specimens in front of me and months of reading, writing and rewriting.

As I reached the end phase of this project, the world changed as the Covid-19 pandemic spread. Almost overnight, the NTNU University Museum closed and everyone had to do their work from home. I was supposed to do the final sorting, preservation and cataloguing of my material during these last months of my project. Such work is not possible to do in my home office, so it will have to wait until things are settling into a new normal state. I would prefer to refer to the NTNU University Museum sample IDs for the specimens I photographed for my identification sheets and the appendix species list, but it was not possible to prepare them before the finish of my thesis project.





# Acknowledgements

This project would not have been possible without all the help, discussions, and guidance from my knowledgeable and committed supervisors Torkild Bakken, Eivind Oug and Maria Capa. Thank you so much for the hours spent together in field, in the lab and discussing this project. And thank you for reading through my drafts and giving feedback as I struggled to find out how to write this thesis. Amphipod enthusiast and expert Anne Helene Solberg Tandberg spent many hours in the lab with me, helping to sort out how to deal with the Caprellids. Thank you very much for sharing your time, great humour, and passion for amphipods. Luis Martell joined much of the fieldwork, thank you for all the interesting discussions and for sharing all the curious facts and insights into the strange world of hydrozoans. During the 2019 fieldwork, I stayed at NIVAs facilities in Grimstad for sampling and working on my samples, thank you to all the NIVA staff I met for making me feel very welcome and helping out with all the practical issues.

All the fellow master students at the NTNU University Museum, thank you for all the discussions, lunch breaks and digressions, I wish we could spend the last months of writing thesis together also, instead of hiding in our home offices. Thank you to the NTNU University Museum colleagues for including us, the master students in the museum community and Friday cake lunches. Thank you, Tuva for your considerate advices through this process. Thank you, Rachel, Siv Elin, Maren and Maja for all the outdoor adventures to ease my mind from the thesis work and reminding me that life is a bit more than marine invertebrates. Thank you, mum and dad for sparking my interest for the living things that surrounds us, and for giving me warm dinner and some muddy work at the farm when the thesis work got to frustrating sometimes. Thank you Simen for always standing by my side through this wave ride, both in the troughs and on the crests, your support has been unwavering.



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# List of Abbreviations

|           |                                                               |
|-----------|---------------------------------------------------------------|
| BaS       | Baltic Sea                                                    |
| BS        | Black Sea                                                     |
| CBD       | Convention on Biological Diversity                            |
| GBIF      | Global Biodiversity Information Facility                      |
| HELCOM    | Helsinki Convention                                           |
| HI        | High Impact (NBIC risk category)                              |
| IMO       | International Maritime Organization                           |
| LO        | Lo Impact (NBIC risk category)                                |
| MS        | Mediterranean Sea                                             |
| NAO       | North Atlantic Ocean                                          |
| NBIC      | Norwegian Biodiversity Information Centre<br>(Artsdatabanken) |
| NEAO      | North-East Atlantic Ocean                                     |
| NK        | No Known Risk (NBIC risk category)                            |
| NOBANIS   | European Network on Invasive Alien Species                    |
| NPO       | North Pacific Ocean                                           |
| NTNU      | The Norwegian University of Science and Technology            |
| NWAO      | North-West Atlantic Ocean                                     |
| OSPAR     | Oslo and Paris Convention                                     |
| PH        | Potential High Impact (NBIC risk category)                    |
| Polyports | Polychaetes in Norwegian Ports Project                        |
| RCS       | Rapid Coastal Survey                                          |
| SAO       | South Atlantic Ocean                                          |
| SE        | Severe Impact (NBIC risk category)                            |
| SIO       | South Indian Ocean                                            |
| SO        | Southern Ocean                                                |
| SPO       | South Pacific Ocean                                           |
| TAO       | Tropic Atlantic Ocean                                         |
| TIO       | Tropic Indian Ocean                                           |
| TPO       | Tropic Pacific Ocean                                          |
| WoRMS     | World Register of Marine Species                              |

# 1 1. Introduction

## 1.1 Marine alien species as a global concern

In a modern globalised world, people, goods, cultural phenomenon and living organisms are moving around the globe at an unprecedented speed. Concerns about this leading to cultural homogenization, at the expense of local traditions have been raised (Greig, 2002). The same concerns can be raised in biogeography, with biotic homogenization where alien species spread around the world at the expense of local endemic species (Mckinney and Lockwood, 1999). Invasive alien species are, along with land and sea use, overexploitation, climate change and pollution, among the main drivers of global biodiversity loss according to the Intergovernmental Science-Policy Platform for Biodiversity and Ecosystem Services (Díaz et al., 2019). Marine alien species are a problem of international nature which is receiving attention from global actors. The International Union for Conservation of Nature (IUCN) refers to marine alien species as a major threat to biodiversity in the world's oceans (Poorter et al., 2002). Alien species can cause harm by displacing native species, alter interactions and food webs and influence ecosystem processes such as sedimentation and nutrient cycling (Molnar et al., 2008).

Many different terms are used in the conversation about invasion ecology (Colautti and Macisaac, 2004). In the following text, the term *invasive* will be used when discussing organisms outside of their natural geographic range that cause ecological or economical damage (Lockwood et al., 2007), while the term *alien species*, used and recommended by the Norwegian Biodiversity Information Centre (Artsdatabanken, 2018b), contains all species outside their natural geographic range by means of anthropogenic transport.

Alien species in marine environments are spread intentionally, for example for aquaculture, or accidentally, for example as stowaways in ballast water or attached to ships hulls (Drake and Lodge, 2007). International shipping, aquaculture and secondary transport from nearby areas by ocean currents are the most important mechanisms of transport of species in the marine environment (Norling and Jelmert, 2010). The coastal cities of the world are connected in a complex network of cargo transport routes. Through these shipping routes, species are transported out of their natural range, and if they can survive in their new environment, they may establish non-native populations. Shipping routes between harbours with similar environmental conditions are important vectors of introductions (Keller et al., 2011). International ship transport and organisms attached to oysters introduced for shellfish farming, are among the most important vectors of marine introductions (Fofonoff et al., 2003).

Climate change is also an important driver of change in global biogeography (Oviatt, 2004). Warming of the oceans may favour biotic invasions by increasing disturbance in native ecosystems and facilitate survival of propagules (introduced individuals) with more favourable environmental conditions (Occhipinti-Ambrogi and Galil, 2010). In addition to favouring establishment of alien species, ocean warming facilitates natural range expansion poleward by species that previously was restricted by temperature requirements (Oviatt, 2004). When established in an area, alien species can spread and increase their range. In the marine environment, the patterns of ocean currents will

influence the spread of organisms, both from their natural area of origin, and from a site of introduction (Lockwood et al., 2007).

Given the international nature of the problem of alien species, joint international efforts are necessary to minimize the spread of species. The spread of species through ballast water exchange has been known for several decades and the International Maritime Organization (IMO) made guidelines for ballast water treatment in order to prevent the transfer of organisms in ballast water in the 1990's. In 2004, the legally binding *International Convention for the Control and Management of Ship's Ballast Water and Sediment* (the Ballast Water Convention) was adopted (Gollasch et al., 2007) and entered force in 2017 with ratification from 81 nations (IMO, 2019). Even though an international cooperation is essential to minimize spread of alien species, the implementation must happen at a regional or local scale (Gollasch et al., 2007). At the regional level, those nations bordering the Baltic Sea are cooperating through the *HELCOM Baltic Marine Environment Protection Commission*, and those nations bordering the North-East Atlantic are joined in the *OSPAR Commission for Protecting and Conserving the North-East Atlantic and its Resources*. OSPAR and HELCOM cooperate to meet the Ballast Water Convention in the harbours in their areas. The HELCOM/OSPAR cooperation have developed a set of guidelines for granting exemptions to the convention's ballast water treatment requirements, and a protocol for port surveys for monitoring alien species in harbours (HELCOM/OSPAR, 2015).

## 1.2 Monitoring and management of marine alien species

Even though problems associated with alien species are receiving increasing academic and public attention (Lockwood et al., 2007), there is still a lack of knowledge concerning many aspects of invasive species (Streftaris et al., 2005).

The Aichi Targets from the Convention on Biological Diversity (CBD) has one target specifically addressing the management of invasive alien species. The Aichi Target 9 aims to prevent biological invasions by managing pathways of spread and control or eradicate prioritized invasive alien species by 2020 (Convention on Biological Diversity, 2018). Early detection of alien species populations is key to increase the efficiency of measures for eradication or mitigation (Minchin, 2007). Early detection can be facilitated by monitoring areas susceptible to alien species establishment (Rinde et al., 2017), such as harbours, marinas and aquaculture facilities (Minchin, 2007). In the IMO Ballast Water Management Convention, monitoring of species diversity in harbours is highlighted as a key to early detection of alien species. The HELCOM/OSPAR cooperation provides procedures for monitoring and management of alien species in the Baltic and North Sea. Their port survey protocol gives detailed description of how international ports should be sampled in order to monitor the species composition present in the port. The HELCOM/OSPAR port survey procedure is based on extensive sampling of different habitat types, and environmental factors. It yields quantitative data suitable for statistical analysis (HELCOM/OSPAR, 2015), but monitoring through extensive harbour surveys is very costly, labour intensive and time consuming. In order to increase detection rate and decrease monitoring costs, specialized alien species detection methods have been developed, Rapid Coastal Survey (RCS) is one of them (Campbell et al., 2007). RCS for alien species are usually carried out in areas of high anthropogenic influence, like harbours or aquaculture facilities, with structures that are submerged in the sea most of the time, such as floating pontoons, ropes and buoys (Minchin, 2007). Following the RCS



procedure, personnel trained to identify species on a target list, surveys the biodiversity on floating pontoons, submerged ropes and other structures for targeted species. Organisms can be identified in field, or in a lab facility if they are hard to identify or need microscopy for identification. Organisms are removed from the substrate by hand or with light handheld equipment like a scraper and a pocket net. The depth possible to investigate is usually limited to < 0.5 meter. In some cases, other sampling techniques are employed, such as small hand operated bottom grabs and benthic sleds (Campbell et al., 2007). RCS surveys are based on selective sampling of both sampling area and organisms sampled. The data sampled with the RCS methods are qualitative and not suitable for statistical analysis (Campbell et al., 2007).

Geographic data about the occurrence of marine alien organisms and making this available at both national and international level, is a necessary contribution to the studies of invasion ecology. This provides a tool for management purposes (Katsanevakis et al., 2013). Precise species identification is necessary in management of alien species (Heggøy et al., 2008). If the identification aids used does not compare the alien species to easily confused native species, it can lead to misidentification. Therefore, it is very important to assess the existing identification literature, and to develop identification tools that compare the target species to native species.

### 1.3 Marine alien species in Norway

The knowledge about marine alien species is limited. In the 2012 assessment of alien species in Norway, it was pointed out that the general knowledge about alien marine invertebrates is poor in Norway (Gederaas et al., 2012). One important reason for this, is that many species are taxonomically difficult, and identification must be confirmed by experts. This may lead to certain species being overlooked or underreported (Gederaas et al., 2012). The available literature about many of the marine alien species is international and often does not compare alien species to similar looking species in the native fauna. There are some European sources available that can be useful, like NOBANIS, but there are no easily available identification aids for alien species specifically in Norwegian waters. The low availability of identification aids that compares to native fauna, can lead to misidentifications and can be an obstacle for performing precise alien species surveys.

In a 2013 Official Norwegian Report, a governmentally appointed commission pointed out that invasive alien species are among the major threats to Norwegian ecosystems. There is a need for more knowledge, especially good time series of biodiversity monitoring and early detection strategies in Norway (NOU, 2013). At present, there is no national program for monitoring biodiversity change in the waters along the Norwegian coast. Extensive alien species surveys have been carried out in some areas, for example in Rogaland (Husa et al., 2012a) and Hordaland (Husa et al., 2012b). Even though the general alien marine diversity is poorly mapped, the populations of a few species are monitored more closely for different reasons. The king crab *Paralithodes camtschaticus* (Tilesius, 1815), is monitored and managed as an economical resource, and is very important for the coastal fisheries in Troms & Finnmark (Sundet and Hoel, 2016). The pacific oyster *Crassostrea gigas* (Thunberg, 1793) is closely monitored as it has altered the structure and habitat in many areas, that way devaluing popular recreational beaches in addition to influencing the local ecosystems (Bodvin et al., 2014).

In Norway, the Norwegian Biodiversity Information Centre (NBIC/Artsdatabanken) is responsible for managing occurrence data reported to the species map service, artskart.no. they also provide an assessment of alien species and make the national alien species list. The alien species list contains alien species that are already present in Norway and so-called door knockers, species that are established in neighbouring countries or are expected to establish in Norway within 50 years. Also, species that are regionally alien, occurring naturally in parts of the country, but are alien in other areas, are included in the alien species list. The risk assessment of alien species in Norway is done by evaluating criteria concerning the organism's ability to establish viable populations, spread and invade ecosystems, and multiple criteria considering possible impact on endemic species and ecosystems (Artsdatabanken, 2018c). Based on the ecological effect and invasion potential, the species are appointed a risk category in a standard matrix for NBIC alien species risk assessment (figure 6 in Sandvik et al. (2017)). The NBIC alien species risk assessment operate with five risk categories: No Known Risk (NK), Low Impact (LO) Potentially High Impact (PH), High Impact (HI) and Severe Impact (SE). In the 2018 alien species assessment for mainland Norway (not considering the waters around Svalbard and other oceanic islands of Norwegian territory), a total of 71 species of marine invertebrates was included, 26 of these are established species and 45 are assessed as door knockers. To keep the knowledge about alien species in Norway updated, the NBIC alien species risk assessment is carried out approximately every 6<sup>th</sup> year.

In Norway, the number of established alien species decrease with latitude, leaving the southern part of the country most crowded with alien species (Sandvik et al., 2019). The coastal areas of southern Norway are the areas with the highest human population, and the highest density of international cargo harbours, and thus these areas are susceptible to receive alien species from international shipping (Norling and Jelmert, 2010). These areas have the highest number of naturalised alien species in Norway (Sandvik et al., 2019). Also, these areas are located close to the coastal waters of the neighbouring countries and are expected to be the first areas to receive many of the "door-knocking" species (Johnsen et al., 2010). A door-knocking species is a species that is expected to arrive in Norwegian waters within 50 years (Artsdatabanken, 2018b). Due to a multitude of environmental and anthropogenic factors such as temperature, salinity, currents and marine traffic, the coast of Skagerrak is expected to receive many of the new marine alien species first (Sandvik and Sæther, 2012). Alien species may spread to the Norwegian coast of Skagerrak through secondary spread from established populations in the Baltic Sea or further south in Europe, or by human transport (Rinde et al., 2017). The major international ports in southern Norway are especially susceptible to receive newcomers. The ocean currents in the North Sea and along the Norwegian west coast can also facilitate establishment of alien species by moderating temperatures and contributing to transport of organisms (Johnsen et al., 2010). Recreational crafts may be an important contributor in secondary spread of alien species, as they often travel along the coast in short legs, staying in marinas overnight or longer.

## 1.4 The present study

In this study, international harbours, and marinas along the coast between Trondheim and Oslo where surveyed for alien invertebrate species. Standardized grab, dredge, diver and fouling plates samples were collected in the international harbours following the

HELCOM/OSPAR port survey protocol and the Rapid Coastal Survey was carried out in the marinas. The sampling effort was mainly carried out with the project *Polychaetes in Norwegian Ports: Uncovering Diversity in Coastal Anthropogenic Environments and Assessing Cryptogenic and Non-indigenous Species (PolyPorts for short)* at the NTNU University Museum. A target list of alien species based on the alien species list from the NBIC was developed for this study totalling eight established and 13 door knocking species of marine invertebrates.

## 1.5 The aim of this project

This project aims to map the occurrence of a selection of alien species in some of the biggest international harbours and some marinas in Norway south of Trondheim and develop suitable identification tools for a selection of the target list species. Based on available occurrence data and recent inventories, it can be expected that the spatial distribution of marine alien species follows certain patterns. Therefore, assessing the spatial distribution of alien species is also an aim of this study. Of the species on the project target list, several are known to be established in Norway and these are expected to be found in this study. Further, it is expected that more alien species occur in the southern part of the study area than further north (Sandvik et al., 2019). Another spatial pattern that might be detected is that alien species are associated with anthropogenically modified environments (Turcotte and Sainte-Marie, 2009). The following goals are the basis for this project:

1. Map occurrence of targeted marine alien species in selected Norwegian ports.
2. Assess the spatial distribution of alien species within the study area.
3. Develop precise and easy to use identification tools for a selection of the marine alien invertebrate species in Norway, with special regard to comparisons to native species

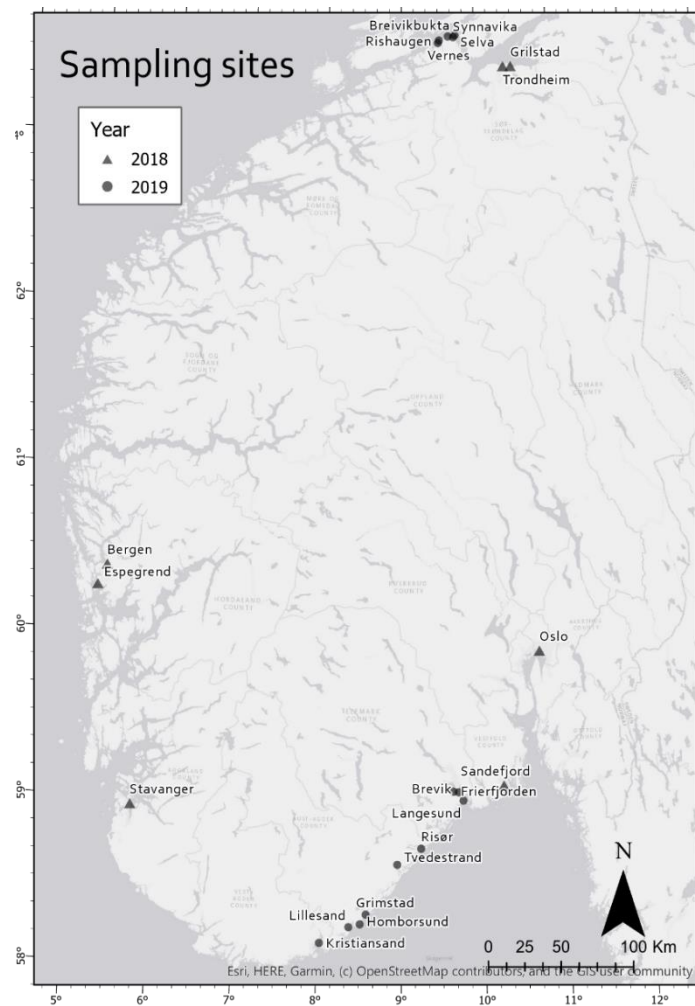
## 2 Methods

### 2.1 Study area

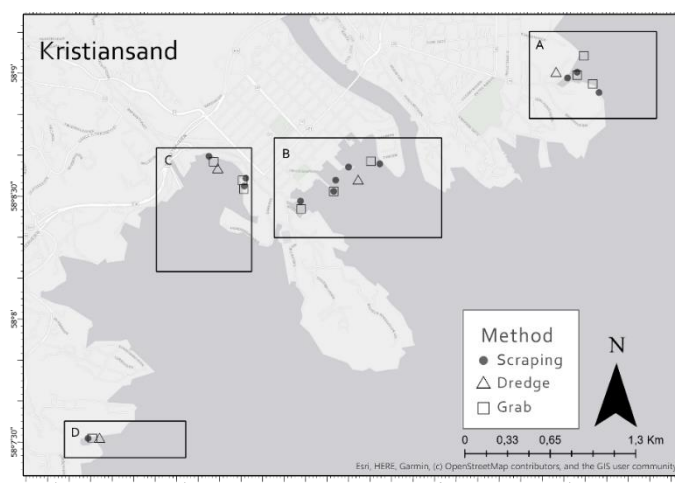
Location of the surveyed ports and marinas is shown in Figure 2.1. The sites included anthropogenically modified areas utilized for different maritime activities including recreational boating, industry and transport of goods and passengers. The sites were selected to include harbours used for different purposes. Oslo, Kristiansand, Stavanger, Bergen and Trondheim were all included in this study. They are among the biggest international ports in Norway, and they harbour much international shipping activity.

Sampling in the harbours of Grillstad, Trondheim, Bergen, Espesvåg, Stavanger, Sandefjord and Oslo was done in August and September 2018 as part of the sampling for the PolyPorts project. Sample collection in the ports of Kristiansand, Lillesand, Homborsund, Grimstad, Tvedestrand, Risør, Brevik, Frierfjorden and Langesund was done in August 2019 under the framework of the *PolyPorts* project, and as part of the fieldwork for this thesis. In addition, marinas in Agdenes (Figure 2.4) was sampled in October 2019 for this project. Samples from a total of 22 sites was included in the analysis for this project (Table 2.1).

In the large harbours, an extensive sampling scheme of scrape, dredge and grab was applied. The harbour was divided into smaller areas according to what anthropogenic activities they are utilized for. In Kristiansand area A is an old industrial harbour, area B is a marina for recreational crafts, area C harbours the passenger ferry to



**Figure 2.1: Location of sampling sites. Year of main sampling event is given in legend**



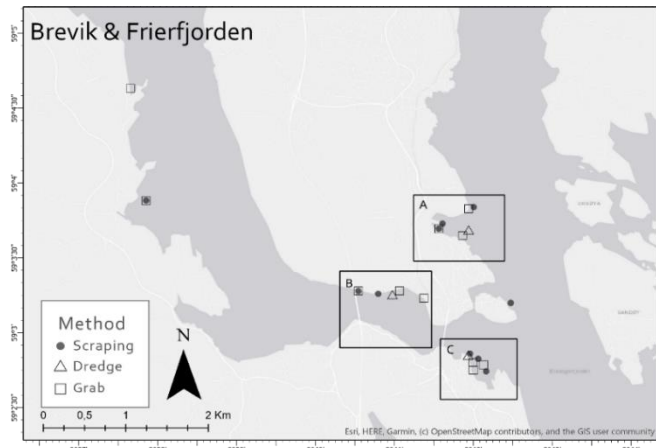
**Figure 2.2: Sampling methods in Kristiansand. The sub-areas A, B, C and D are used for different purposes, and are anthropogenically altered in different ways.**

Denmark and area D is an industrial area (Figure 2.2). Near area B in Kristiansand is a river outlet, adding freshwater to the area. In Brevik, area A is an industrial area and international cargo harbour, area B is an industrial area and area C is a marina for recreational crafts (Figure 2.3). Samples was also collected in Frierfjorden (Figure 2.3), an area of high freshwater influence. The Brevik and Frierfjorden area is strongly influenced by freshwater runoff from nearby rivers. In each of the sub-areas, multiple grab, dredge and scrape samples were collected.

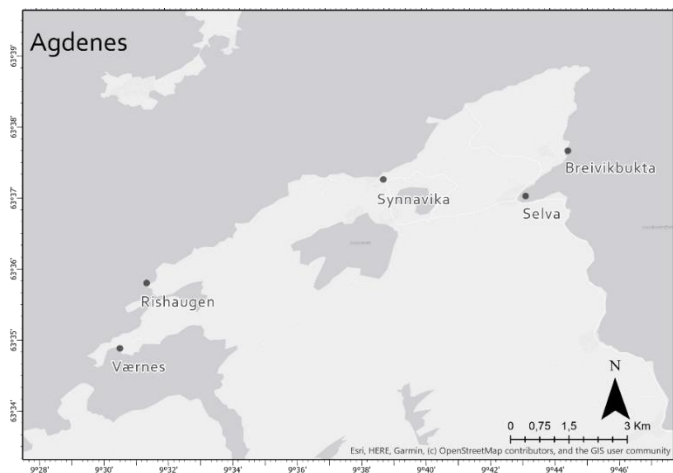
Along the coast between Brevik and Kristiansand, seven marinas for leisure crafts was sampled (Figure 2.1). These were Langesund, Risør, Tvedestrand, Holvika, Grimstad, Homborsund and Lillesand. Along the coast of Agdenes in Trøndelag, four marinas, Værnes, Synnavika, Breivikbukta and Selva, and one natural habitat, Rishaugen, was sampled (Figure 2.4). In the marinas, the Rapid Coastal Survey sampling procedure was applied.

## 2.2 Sampling methods

To sample as many harbours and marinas as possible and obtain as many samples from different environment as feasible, two different sampling strategies was applied, one for large harbours, and one for marinas. In large harbours, parts of the HELCOM/OSPAR joint harmonized protocol for port sampling was applied. This sampling protocol includes standardized diving samples, fouling plates, grab samples, scraper samples and dredge samples (Table 2.1). In some of the large harbours, divers were employed to sample at depths not available from land and plastic fouling plates were deployed and left suspended for 10-12 months to study fouling communities. In the larger ports, a small boat was deployed to sample soft and mixed bottom with hand operated dredge and grab and hard substrates with a handheld scraper. In the marinas, qualitative samples from a variety of different shallow water habitats were taken following the RCS procedure described by Minchin (2007), with some adjustments for this project. A team of 2-4 persons spent 10-30 minutes in each marina collecting samples with handheld gear like a small scrape and pocket net and by handpicking organisms. Instead of in-field identification as Minchin (2007) did, the samples were taken to the lab for identification and preservation in the present study. Multiple different environments were sampled, including artificial hard substrates, natural hard substrate, and sediments. Samples collected following the RCS procedure yield qualitative data, sampling by dredge, grab



**Figure 2.3: Sampling methods in Brevik and Frierfjorden. Brevik is subdivided into areas used for different purposes.**



**Figure 2.4: Sampling sites at Agdenes, Trøndelag. Small leisure craft marinas were sampled. Rishaugen is a natural habitat.**

and scraper give semi-quantitative data, while the HELCOM/OSPAR procedures for diver samples, grab samples and fouling plates returns quantitative data. A total of 284 samples was collected (Table 2.1).

**Table 2.1: Overview of sampling techniques applied at the sampling sites. Number of samples obtained at each site by each method is given. Coordinates for one of the samples at each site is given. Details of the methods are given in the main text.**

| Sampling Site | Type            | Latitude/ Longitude       | Number of samples and sampling technique                                             | Sampling date                       |
|---------------|-----------------|---------------------------|--------------------------------------------------------------------------------------|-------------------------------------|
| Grillstad     | Marina          | N 63.43781<br>E 10.50624  | 1 Dredge<br>7 Grab<br>3 Scraper<br>2 Intertidal walks                                | 4. Sept. 2018                       |
| Trondheim     | Harbour         | N 63.43234<br>E 10.37914  | 8 Dredge<br>8 Grab<br>10 Scraper<br>1 Intertidal walk<br>9 Diver<br>6 Fouling plates | 5. & 7. Sept. 2018<br>26. jun. 2019 |
| Selva         | Marina          | N 63.616042<br>E 9.719692 | 1 Rapid Survey                                                                       | 19. Oct. 2019                       |
| Breivikbukta  | Marina          | N 63.626553<br>E 9.741837 | 1 Rapid Survey                                                                       | 19. Oct. 2019                       |
| Synnavika     | Marina          | N 63.620300<br>E 9.645996 | 1 Rapid Survey                                                                       | 19. Oct. 2019                       |
| Rishaugen     | Natural habitat | N 63.596491<br>E 9.522819 | 1 Intertidal walk                                                                    | 18. Oct. 2019                       |
| Værnes        | Marina          | N 63.581212<br>E 9.508716 | 1 Rapid Survey                                                                       | 16. Oct. 2019                       |
| Bergen        | Harbour         | N 60.38077<br>E 5.34076   | 11 Dredge<br>9 Grab<br>9 Scraper<br>11 Diver<br>25 Fouling plates                    | 10.-11. Sept. 2018<br>9. sept. 2019 |
| Espegrend     | Harbour         | N 60.25660<br>E 5.23780   | 4 Grab<br>5 Scraper                                                                  | 13. Sept. 2018                      |
| Stavanger     | Harbour         | N 58.95835<br>E 5.76056   | 14 Grab<br>13 Scraper                                                                | 14.-15. Sept. 2018                  |
| Kristiansand  | Harbour         | N 58.14232<br>E 8.00309   | 4 Dredge<br>11 Grab<br>12 Scraper                                                    | 23. Aug. 2019                       |
| Lillesand     | Marina          | N 58.24725<br>E 8.37979   | 1 Rapid Survey                                                                       | 25. Aug. 2019                       |
| Homborsund    | Marina          | N 58.26484<br>E 8.51268   | 1 Rapid Survey                                                                       | 27. Aug. 2019                       |
| Grimstad      | Marina          | N 58.34061<br>E 8.59692   | 2 Rapid Survey                                                                       | 25. Aug. 2019                       |
| Holvika       | Marina          | N 58.323348<br>E 8.580955 | 1 Rapid Survey                                                                       | 22. Aug. 2019                       |
| Tvedestrand   | Marina          | N 58.62487<br>E 8.95345   | 2 Rapid Survey                                                                       | 28. Aug. 2019                       |
| Risør         | Marina          | N 58.72174<br>E 9.23818   | 1 Rapid Survey                                                                       | 28. Aug. 2019                       |
| Langesund     | Marina          | N 59.01067<br>E 9.74821   | 1 Rapid Survey                                                                       | 26. Aug. 2019                       |
| Brevik        | Harbour         | N 59.05341<br>E 9.68988   | 3 Dredge<br>9 Grab<br>9 Scraper                                                      | 26. Aug. 2019                       |
| Frierfjorden  | Marina          | N 59.06459<br>E 9.63139   | 2 Grab<br>1 Scraper                                                                  | 26. Aug. 2019                       |
| Sandefjord    | Harbour         | N 59.11402<br>E 10.23026  | 7 Grab<br>6 Scraper                                                                  | 17. Sept. 2018                      |
| Oslo          | Harbour         | N 59.90942<br>E 10.69936  | 7 Dredge<br>17 Grab<br>10 Scraper<br>11 Diver<br>5 Fouling plates                    | 18-20. Sept. 2018<br>19. Jun. 2019  |

**Dredge** - A 50 x 20 cm bottom scraping Hydrobios™ dredge with 6 mm net was towed 5-20m to collect bottom dwelling organisms and infauna (Figure 2.5). The dredge samples were rinsed with seawater in a 1 mm sieve. Rocks with no visible fouling was removed.

**Grab** - Bottom sediments with infauna was collected using a 0.028 m<sup>2</sup> van Veen (model KC Denmark) hand operated grab (Figure 2.6). The grab samples were rinsed with seawater in a 1 mm sieve. Rocks with no visible fouling were removed.

**Scraper** - Handheld scrapers with a 25 cm wide metal frame and 250 µm fine meshed pocket net was used to collect samples from submerged infrastructure like pontoons, poles and other harbour structures (Figure 2.7).

**Intertidal walk** - A team of 2-3 persons spent 15-20 minutes in the tidal zone collecting organisms.

**Diver** - Divers scraped a 30x30cm square at shallow depth below the reach of land-based methods for fouling communities. Sampling by divers was only carried out at selected locations (Table 2.1).

**Fouling plates** - At some sites fouling plates of 15x15cm was left submerged to different depths and was retrieved after 10-12 months.

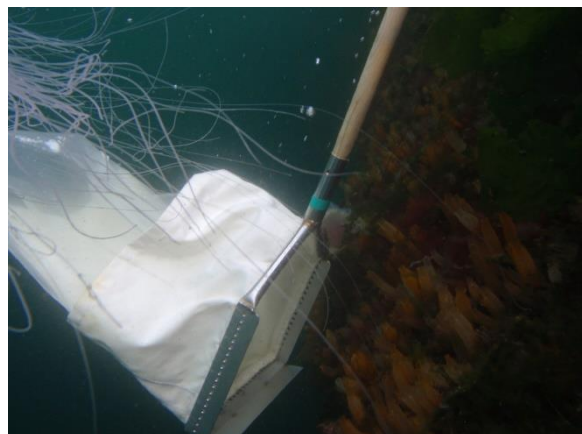
**Rapid Survey** - In marinas, Rapid Coastal Survey was conducted. Teams of 2-4 persons spent 10-30 minutes in each marina investigating living organisms on submerged structures such as ropes, buoys and floating pontoons. Samples were collected with the scrape described above or by handpicking. The content of the scrape was assessed, and all the content or a subsample was brought to the lab for identification and preservation. Handpicked samples were collected by picking organisms by hand or using a small scraper and pocket net to obtain organisms of interest.



**Figure 2.5: A Hydrobios™ dredge was used to sample bottom dwelling organisms. Photo Torkild Bakken**



**Figure 2.6: Bottom sediments with infauna was collected with a hand operated grab. Photo: Marte Svorkmo Espelien**



**Figure 2.7: A handheld scrape with pocket net was used to collect samples from submerged infrastructure. Photo: Marte Svorkmo Espelien**

## 2.3 Target species

A list of target species was developed based on the 2018 alien species list from NBIC (Artsdatabanken, 2018a). The target species list contains marine alien invertebrates known to be established or door-knocking along the Norwegian coast between Trondheim and Oslo. The NBIC alien species list contain 23 species established in this area and 45 species assessed as door knockers to the areas along the coast between Oslo and Trondheim. Selection of species for the target list was needed to limit the number of species to fit the scope of this thesis. Selection of target species was done based on the sampling methods and the identification expertise available. Only those species that could be detected with the present sampling methods and could be identified with certainty by the present project team, were included. With these specifications, the target species list for this project contain eight species known to be established (Table 2.2) in the study area and 13 door-knocking species (Table 2.3). The target list contains five molluscs, five chordates (tunicates), four crustaceans, five annelids, one cnidarian and one bryozoan.

**Table 2.2: Target list of alien species known to be established in the study area, with Norwegian Biodiversity Information Centre alien species risk category, year of first record and area of current distribution range in Norway. Risk categories: SE= very high risk, HI= high risk, LO=low risk, PH= potentially high risk and NK= no known risk.**

| Species name                                  | Taxonomic group | 2018 risk category | First observed in Norway | Known range in Norway         |
|-----------------------------------------------|-----------------|--------------------|--------------------------|-------------------------------|
| <i>Crassostrea gigas</i> (Thunberg, 1793)     | Mollusca        | SE                 | 1980                     | Swedish border to Møre        |
| <i>Botrylloides violaceus</i> Oka, 1927       | Asciacea        | HI                 | 2007                     | Egersund-area                 |
| <i>Styela clava</i> Herdman, 1881             | Asciacea        | LO                 | 1990                     | Rogaland to Grimstad          |
| <i>Caprella mutica</i> Schurin, 1935          | Crustacea       | SE                 | 1999                     | From Swedish border to Troms  |
| <i>Amphibalanus improvisus</i> (Darwin, 1854) | Crustacea       | PH                 | 1900                     | Oslofjord, Skagerrak          |
| <i>Marenzelleria viridis</i> (Verrill, 1873)  | Annelida        | SE                 | 2008                     | Drammensfjorden               |
| <i>Goniadella gracilis</i> (Verrill, 1873)    | Annelida        | PH                 | 1968                     | Unknown                       |
| <i>Gonionemus vertens</i> A. Agassiz, 1862    | Cnidaria        | LO                 | 1921                     | Southern west coast of Norway |

**Table 2.3: Target list of door-knocking species, with Norwegian Biodiversity Information Centre Risk category 2018, natural range and present range. Abbreviations: TAO=Tropic Atlantic Ocean, NAO=North Atlantic Ocean, SAO=South Atlantic Ocean, NWAO=North-West Atlantic Ocean, NEAO=North-East Atlantic Ocean, BaS=Baltic Sea, BS=Black Sea, NPO=North Pacific Ocean, SPO=South Pacific Ocean, TPO=Tropic Pacific Ocean, SO=Southern Ocean, TIO=Tropic Indian Ocean, SIO= South Indian Ocean, MS=Mediterranean Sea**

| Species name                                  | Taxonomic group | 2018 Risk category | Natural range | Present range, marine      |
|-----------------------------------------------|-----------------|--------------------|---------------|----------------------------|
| <i>Rangia cuneata</i> (G. B. Sowerby I, 1832) | Mollusca        | LO                 | TAO           | NWAO, NEAO, BaS            |
| <i>Mytilopsis leucophaeata</i> (Conrad, 1831) | Mollusca        | HI                 | TAO, NWAO     | NWAO, TAO, BaS, BS, NEAO   |
| <i>Dreissena polymorpha</i> (Pallas, 1771)    | Mollusca        | SE                 | BS            | BaS, Europe, North Amerika |
| <i>Ocinebrellus inornatus</i> (Récluz, 1851)  | Mollusca        | HI                 | NPO           | NPO, NEAO, NWAO            |
| <i>Corella eumyota</i> Traustedt, 1882        | Asciacea        | HI                 | SPO, SAO, SO  | SAO, SPO, SO, NEAO, NWAO   |



|                                                     |           |    |          |                                                        |
|-----------------------------------------------------|-----------|----|----------|--------------------------------------------------------|
| <i>Didemnum vexillum</i> Kott 2002                  | Asciacea  | SE | NPO      | NWAO, NEAO, SPO, NPO                                   |
| <i>Perophora japonica</i> Oka, 1927                 | Asciacea  | PH | NPO      | NPO, NEAO                                              |
| <i>Amphibalanus amphitrite</i> (Darwin, 1854)       | Crustacea | LO | SPO      | NEAO, SPO, NWAO, SAO, MS, BS, NPO, SIO                 |
| <i>Austrominius modestus</i> (Darwin, 1854)         | Crustacea | HI | SPO      | NEAO, MS, NPO, SPO                                     |
| <i>Ficopomatus enigmaticus</i> (Fauvel, 1923)       | Annelida  | HI | TIO, SIO | NWAO, NEAO, TAO, SAO, BaS, Ms, NPO, TPO, SPO, TIO, SIO |
| <i>Hydroides dianthus</i> Verrill, 1873             | Annelida  | LO | NWAO     | NWAO, TAO, SAO, MS, BS, NPO, NEAO                      |
| <i>Marenzelleria neglecta</i> Sikorski & Bick, 2004 | Annelida  | PH | NWAO     | NEAO, BaS, NPO                                         |
| <i>Watersipora subatra</i> (Ortmann, 1890)          | Bryozoa   | HI | Unknown  | British Isles                                          |

## 2.4 Specimen preservation and identification:

Samples were taken to lab and sorted to the lowest taxonomic level possible. *Handbook of the marine fauna of north-west Europe* (Hayward, 2017) was used for sorting and identification of the organisms. In addition, specialized literature for each species or taxonomic group was used for precise identification (Table 2.4). Specimens were preserved in 96% ethanol. Samples with taxa relevant for the target species list was then examined thoroughly for target species. The organisms were examined using stereo microscopes in the lab at the NTNU University Museum. All specimens are curated and stored in the scientific collections at the NTNU University Museum (Bakken et al., 2020). Identification of target species, except for the polychaetes was done by the author, but during the process I received help and support from specialists with expertise in different taxonomic groups. The polychaetes were identified morphologically by specialists in the *PolyPorts* project. Species nomenclature follow the system used by Norwegian Biodiversity Information Centre (NBIC) and World Register of Marine Species (WoRMS). Details of literature and diagnostic characters used in the identification work for the established target species is given in Table 2.4. All specimens collected during the work is kept in the scientific collections at the NTNU University Museum, and the identified species will be registered in the NBIC species mapping service which share their data with the Global Biodiversity Information facility (GBIF).

**Table 2.4: Literature and diagnostic characters used for species identification of the established species on the target list.**

| Species                       | Litterature                                              | Diagnostic character                                                                                                                                                                 |
|-------------------------------|----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Crassostrea gigas</i>      | Hayward (2017), Mortensen et al. (2019)                  | Elongated oval irregular shape<br>Outer structure of layers of sharp flakes<br>Dark colour                                                                                           |
| <i>Botrylloides violaceus</i> | Hayward (2017), Salem Sound Coastwatch (unknown year)    | Bright yellow or orange colour<br>Individual zooids arranged in long chains                                                                                                          |
| <i>Styela clava</i>           | Jensen (2010c), Hayward (2017)                           | Stalk structure at the base of the organism                                                                                                                                          |
| <i>Caprella mutica</i>        | Guerra-García (2012)<br>Turcotte and Sainte-Marie (2009) | Pereonite 1 and 2 are elongated, hairy (male)<br>Dorsal spines organized in four rows along the length of the animal (females and males)<br>Very elongated gills (females and males) |

|                                |                                                                                        |                                                                                      |
|--------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <i>Amphibalanus improvisus</i> | Hayward (2017), Southward (2007), Nilsson-Cantell (1978), Noever (2019), Jensen (2015) | Ridge on the internal view of the scutum<br>Fin keel shape of the spur on the tergum |
| <i>Marenzelleria viridis</i>   | Didžiulis (2006), Oug et al. (2019c)                                                   | Identification to species level based on external morphology is almost impossible*   |
| <i>Goniadella gracilis</i>     | Oug et al. (2019b), (Worsfold, 2007)                                                   | Distribution of transitional parapodia and different types of chaetae*               |
| <i>Gonionemus vertens</i>      | Couwelaar (unknown year)                                                               | Tiny umbrella, four lines from centre of organism                                    |

\*All the annelids were identified by experts and DNA barcoded as part of the PolyPorts project.

For the door knocking species on the target list a short, general description of each species was made based on available identification literature (Table 2.5). When looking through the samples collected in this project, if any specimens fitting the general description was found, more thorough identification work and literature search would be done.

**Table 2.5: Descriptions used when prospecting for the door-knocking target list species.**

| Species name:                  | Description:                                                                                                                                                                                                 |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Rangia cuneata</i>          | Bivalve. Thick and heavy valves, brown, thin outer layer. Shells are equivalve, but valves are not symmetrical. Oval shape with prominent curved umbo (tip). Source: U.S. Fish and Wildlife Service (2010)   |
| <i>Mytilopsis leucophaeata</i> | Bivalve. Shell shape similar to <i>Mytilus edulis</i> , size 15-20mm, adults are brown, juveniles are striped brown and cream. Source: Global Invasive Species Database (2015)                               |
| <i>Dreissena polymorpha</i>    | Bivalve. Shell shape similar to <i>Mytilus edulis</i> but sharper edges. Striped brown and yellow. Up to 5 cm. Source: Birnbaum (2011)                                                                       |
| <i>Ocenebrellus inornatus</i>  | Gastropod. Elongated shape, clear edge on the spiralling shell. Size about 2.5 cm. Source: Global Invasive Species Database (2020)                                                                           |
| <i>Corella eumyota</i>         | Solitary tunicate. Often found in high densities, attached to substrate on right side. Transparent body, coils of spiral stigmata on oral siphon, 40 or more longitudinal vessels. Source: Bilewitch (2009)  |
| <i>Didemnum vexillum</i>       | Colonial tunicate. Forms extensive sheets, yellow cream colour. Zooids distinctive and arranged in elongated or circular systems, area between zooids grey. Source: Hayward (2017)                           |
| <i>Perophora japonica</i>      | Colonial tunicate. Individual zooids united by basal "stalk". Longitudinal vessels, star-like buds, yellow and transparent. Source: Hayward (2017)                                                           |
| <i>Amphibalanus amphitrite</i> | Barnacle. Six outer plates. Grouped stripes down the outer plates. Source: Southward (2007)                                                                                                                  |
| <i>Austrominius modestus</i>   | Barnacle. Four outer plates. Source: Hayward (2017)                                                                                                                                                          |
| <i>Ficopomatus enigmaticus</i> | Annelid. Calcareous tubes with "rings" corresponding to the organism's growth. Can be confused with the native species <i>Pomatoceros triqueter</i> and <i>Hydroides norvegicus</i> . Source: Jensen (2010b) |
| <i>Hydroides dianthus</i>      | Annelid. Calcareous tube. Identification by experts                                                                                                                                                          |
| <i>Marenzelleria neglecta</i>  | Annelid. Unsolved taxonomy, identification based on morphology impossible at present. Source: Didžiulis (2006)                                                                                               |
| <i>Watersipora subatra</i>     | Bryozoa. Bright orange or red. Flat or foliose, can develop big lobed mass. Source: Global Invasive Species Database (2008)                                                                                  |

## 2.5 Developing species descriptions

Species diagnoses with identification sheets with special regard to similar native species were developed for the species on the target species list that were found in the sampled material. Thorough morphological investigation of the specimens in the sampled material provided the basis for the species descriptions. In order to make good comparisons to native caprellids, samples containing *Caprella linearis* (Linnaeus, 1767) and *Caprella septentrionalis* Krøyer, 1838, from the NTNU University Museum and recent samples from Saltstraumen were examined and photographed. Specimens of *Crassostrea gigas* and *Ostrea edulis* Linnaeus, 1758, from the scientific collections at the NTNU University Museum (Bakken et al., 2020) were photographed for the identification sheets. Taxonomical training was given by experts in different taxonomical groups (A.H. Tandberg, pers. com., T. Bakken pers. com. & E. Oug, pers. com.). In addition, a variety of different literature was used to develop precise identification aids, see details for each species in Table 2.4. Individuals of species on the project target list found in the study were photographed in the field with a waterproof compact pocket camera or in the lab with a Leica MZ 16A stereomicroscope with Leica DFC420 camera and the Leica Application Suite LAS 4.5. ArcGIS was used to develop maps of where the species were recorded in this study.

## 3 Results

### 3.1 Species found

Of the eight known established species on the target species list, four were found in the sampled locations (see Appendix I for full species-site list). None of the 13 door-knocking species on the target list was found. The Japanese skeleton shrimp, *Caprella mutica* was the most widespread species and was found within the whole study area. *C. mutica* was found in 18 of the 22 sites sampled, including the northernmost part of the study area in Trøndelag. The second most widespread species was also a crustacean, the bay barnacle, *Amphibalanus improvisus*. It was found at seven sites, and had a more southern range, occurring along the coast between Oslo and Kristiansand. The Pacific oyster, *Crassostrea gigas*, was found at six sites between Oslo and Kristiansand. The tunicate *Styela clava* was found in four sites along the southern coast, in Grimstad, Holvika, Homborsund and Stavanger. Alien species was found in all the sites sampled (Table 3.1).

**Table 3.1: Occurrence of alien species at sampling sites**

|              | <i>Caprella mutica</i> | <i>Amphibalanus improvisus</i> | <i>Crassostrea gigas</i> | <i>Styela clava</i> |
|--------------|------------------------|--------------------------------|--------------------------|---------------------|
| Grillstad    | X                      |                                |                          |                     |
| Trondheim    | X                      |                                |                          |                     |
| Selva        | X                      |                                |                          |                     |
| Breivikbukta | X                      |                                |                          |                     |
| Synnavika    | X                      |                                |                          |                     |
| Rishaugen    | X                      |                                |                          |                     |
| Værnes       | X                      |                                |                          |                     |
| Bergen       | X                      |                                |                          |                     |
| Espegrend    | X                      |                                |                          |                     |
| Stavanger    | X                      |                                |                          | X                   |
| Kristiansand |                        | X                              | X                        |                     |
| Lillesand    | X                      |                                |                          |                     |
| Homborsund   | X                      |                                | X                        | X                   |
| Grimstad     | X                      |                                |                          | X                   |
| Holvika      | X                      |                                |                          | X                   |
| Tvedestrand  | X                      | X                              | X                        |                     |
| Risør        | X                      |                                | X                        |                     |
| Langesund    |                        | X                              |                          |                     |
| Brevik       |                        | X                              | X                        |                     |
| Frierfjorden |                        | X                              |                          |                     |
| Sandefjord   | X                      | X                              |                          |                     |
| Oslo         | X                      | X                              | X                        |                     |

### 3.1.1 *Caprella mutica* Schurin, 1935

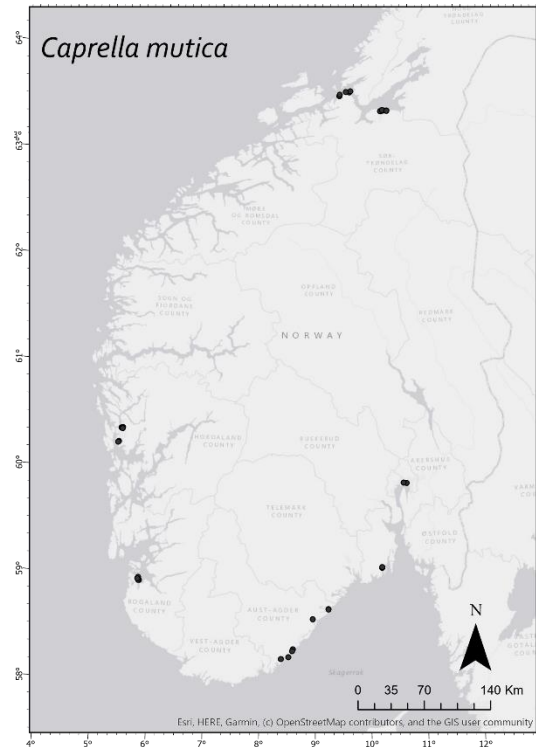
**Common names:** Japansk spøkelseskreps (NO), Japanese skeleton shrimp (GB)

**Records:** The Japanese skeleton shrimp, *Caprella mutica* was found at sampling sites in the whole study area (Figure 3.1). In most of the samples containing *C. mutica*, no other *Caprella* Lamarck, 1801, species was recorded. However, in two of the samples containing *C. mutica* another caprellid species *Phtisica marina* Slabber, 1769, was found. The Japanese skeleton shrimp occurred in samples dominated by macroalgae, often red algae or blue mussels, and the individuals were hidden in between the substrate. New occurrence locations were recorded for *C. mutica* in Trøndelag County, Værnes, Rishaugen, Breivikbukta, Synnavika, Selva, Trondheim and Grillstad.

**Distribution:** The Japanese ghost shrimp, *C. mutica*, was first recorded in Norway in 1999. It is now occurring along most of the Norwegian coast north to Troms (Jelmert et al., 2018c). Rapid growth, short generation time, high reproductive capacity and high tolerance of different environmental conditions are traits that make *C. mutica* an efficient invader (Boos et al., 2011). Caprellids are poor swimmers, therefore *C. mutica* has low potential for spreading on its own, but it spreads efficiently by clinging to ship hulls and floating algae and debris (Guerra-García, 2012).

**Ecological impacts:** Previous studies found that locations with high densities of *C. mutica* have reduced growth of *Mytilus* for mussel aquaculture in Canada and Scotland (Turcotte and Sainte-Marie, 2009). *Caprella mutica* is a strong competitor to other caprellids and organisms with similar feeding strategies or preferred habitat (Turcotte and Sainte-Marie, 2009, Jensen, 2010a). It has been found to show aggressive behaviour toward native *Caprella* sp. such as *C. linearis* (Jensen, 2010a). The Norwegian Biodiversity Information Centre places *C. mutica* in the highest risk category, SE. It is assessed to pose a medium ecological risk but have a high potential for spread (Jelmert et al., 2018c).

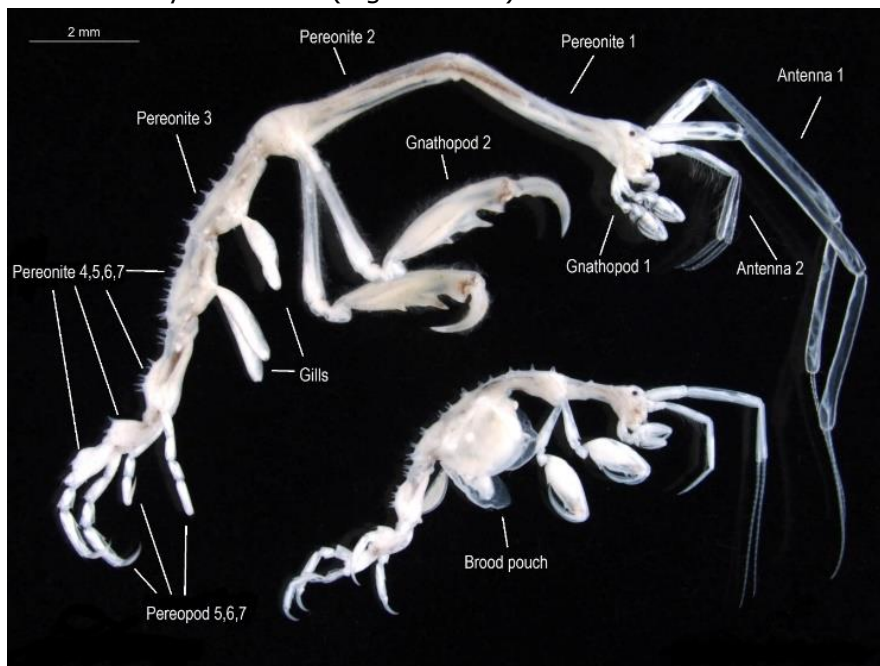
**Morphological description:** Body comprises of seven body segments, called pereonites (Figure 3.2). The antennae, mouthparts and gnathopod 1 are situated at pereonite 1. Gnathopod 2 is attached to pereonite 2. The gnathopods are pereopods modified for grasping and feeding aid. On pereonite 3 and 4 the gills are attached. The pereopods 3 and 4 are so reduced that they are not visible, and not possible to detect on *Caprella* species. On pereonite 5, 6 and 7, pereopods 5, 6 and 7 are attached. The pereopods 5, 6 and 7 are used to attach to the substrate. Distinguishing between the sexes is done by looking for oostegites (the brood pouch) on females. There are many other morphological differences between the sexes, but the brood pouch develops early in females and is a good character to distinguish between male and female. In adult males, pereonite 1 and 2 and gnathopod 2 are elongated and covered with setae, giving a hairy look (Figure 3.3). Pereonites 3-7 have spines arranged in four rows along the dorsal side on males.



**Figure 3.1: Records of *Caprella mutica* in the study area.**

On adult females, spines are present at the back of pereonites 4, 5 and 6, sometimes on pereonites 2 and 3 also (Figure 3.2 and 3.3). The distribution of the spines varies a lot between individuals. The antennae of male *C. mutica* are slender and longer than half of the body length. *Caprella mutica* is a big caprellid, male body length reaching up to 35 mm and females 11 mm (Jensen, 2010a), measuring from the head to the last body segment, not including the antennae. Both sexes have very elongated gills.

**Remarks:** Precise morphological identification of *C. mutica* is difficult without fully developed male specimens. In the North East Atlantic, there are three native species that can be confused with *C. mutica*, namely, *C. linearis* (Linnaeus, 1767), *C. septentrionalis* Krøyer, 1838, and *C. equilibra* Say 1818 (Jensen, 2010a). Adult males of *C. mutica* are distinguished from these by looking for several characters (Table 3.2). Adult *C. mutica* are larger than the other three species. *Caprella equilibra* has a projection on the ventral side between gnathopods 2 and can be distinguished from *C. mutica* by the presence of this. The exterior morphology of *C. septentrionalis* can vary greatly, but the presence of a small projection on the head and the round, just slightly elongated shape of the gills separates it from *C. mutica*. *Caprella linearis* can appear similar to *C. mutica* as it also has elongated pereonite 1 and 2 and gnathopod 2, but *C. linearis* lacks the dense setae on these body parts and the gills are less elongated than on *C. mutica*. The diagnostic characters to identify *C. mutica* and separate it from the other *Caprella* species are different or absent at early developmental stages. Therefore, juveniles are not possible to identify to species level by looking at the exterior morphology of the specimen. As it grows, many of the characters appear gradually until the individual is adult and the characters are fully developed. Some characters develop early and can be used to identify young individuals. For instance, the spines on the back of both males and females start developing early, as do the elongation of the gills in both sexes and development of setae on pereonite 1 and 2 and gnathopod 2 in males. A young *Caprella* specimen with very elongated gills and spines on the dorsal side of pereonites 4,5 and 6, is most likely *C. mutica* (Figure 3.3 d).



**Figure 3.2: General anatomy of *Caprella* sp. The upper left individual is a male and female bottom right. *Caprella mutica*. Photo: Marte Svorkmo Espelien**

**Material examined:** All caprellids collected during this project were examined. In addition, *C. linearis* and *C. septentrionalis* in samples from Saltstraumen were examined and photographed for comparison between the species.

**Identification of *Caprella mutica*:** The organism comprises of seven body segments, called pereonites. The antennae, mouthparts and gnathopod 1 is situated at pereonite 1. Gnathopod 2 is attached to pereonite 2. On pereonite 3 and 4 the gills and strongly reduced pereopods 3 and 4 are attached. The pereopods 3 and 4 are so reduced that they are not visible. On pereonite 5, 6 and 7, pereopods 5, 6 and 7 are attached (Figure 3.2). *Caprella mutica* is a big caprellid, male body length reaching up to 35 mm and females 11 mm, measuring from the head to the last body segment, not including the antennae. Selected diagnostic characters to distinguish *C. mutica* from *C. linearis*, *C. septentrionalis* and *C. equilibra* are given in Table 3.2. Pictures of juvenile and adult *C. mutica* males and females are given (Figure 3.3 A-D) and adult males of *C. linearis* and *C. septentrionalis* (Figure 3.3 E-F).

**Table 3.2: Comparison of characters on adult specimens of *Caprella mutica*, *C. linearis*, *C. septentrionalis* and *C. equilibra*.**

|                                                                                                                 | *Pereonite 1 & 2 (males) | *Setae on gnathopod 2 (males) | *Dorsal projection on head | *Dorsal projection pereonites                  | Gills              | Ventral projection |
|-----------------------------------------------------------------------------------------------------------------|--------------------------|-------------------------------|----------------------------|------------------------------------------------|--------------------|--------------------|
| <i>C. mutica</i>                                                                                                | Elongated and hairy      | Covered in setae              | Absent                     | Spines in rows<br>Males: P4-7<br>Females: P2-7 | Very elongated     | Absent             |
| <i>C. linearis</i>                                                                                              | Elongated not hairy      | Setae on "palms"              | Very tiny                  | Variable                                       | Slightly elongated | Absent             |
| <i>C. septentrionalis</i>                                                                                       | Short not hairy          | No setae                      | Distinct projection        | Variable                                       | Slightly elongated | Absent             |
| <i>C. equilibra</i>                                                                                             | Short not hairy          | Setae on "palms"              | Visible rostrum            | Absent                                         | Slightly elongated | Between G2         |
| * characters that are clear in fully developed males but may vary at earlier development stages and in females. |                          |                               |                            |                                                |                    |                    |

**Key to adult males of common caprellids in Norwegian coastal waters:**

1a. Pereopods 3 and 4 are clearly visible.

.....*Phtisica marina*

1b. Pereopods 3 and 4 so reduced that they are not visible.

.....2

2a. Ventral projection between gnathopods 2

.....*Caprella equilibra*

2b. Ventral projection between gnathopods 2 absent

.....3

3a. Dorsal projection on head, pereonites 1 and 2 short, base of gnathopod 2 short

.....*Caprella septentrionalis*

3b. Dorsal projection on head absent, pereonites 1 and 2 elongated, base of gnathopod 2 elongated

.....4

4a. Setation on pereonites 1 and 2 and gnathopod 2. "Hairy" look on upper body, projections organised in four distinct rows on pereonites 3-7, gills very elongated

.....*Caprella mutica*

4b. No setation on pereonites 1 and 2, setation on the palm of gnathopod 2 only, smooth body surface or few rounded projections on pereonites, gills slightly elongated

.....*Caprella linearis*





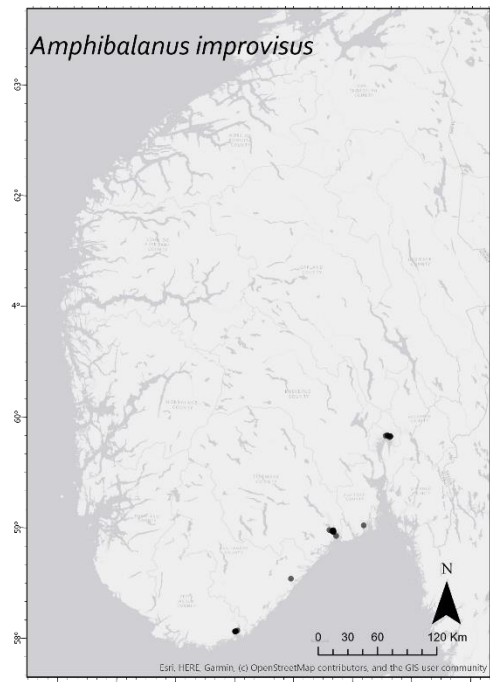
**Figure 3.3: Stereomicroscope pictures of *Caprella mutica* (a-d), a: adult female of *C. mutica*, b: adult male of *C. mutica*, c: juvenile female of *C. mutica* at late development state, d: Juvenile male of *C. mutica* at late development state, e: adult male of *C. linearis* and f: adult male of *C. septentrionalis*. Spines on the dorsal side of *C. mutica* females may vary (a & b). Length of pereonites 1 and 2 varies with developmental stage in *C. mutica* males. Photo: Marte Svorkmo Espelien**

### 3.1.2 *Amphibalanus improvisus* (Darwin, 1854)

**Common names:** Brakkvannsrur (NO), Bay barnacle (GB)

**Records:** The bay barnacle *Amphibalanus improvisus* was found in the southern part of the study area along the coast between Oslo and Kristiansand (Figure 3.4). *Amphibalanus improvisus* occurred in port areas near estuaries with freshwater influenced water. No new locations were recorded for occurrence of *A. improvisus*. The species map service provided by the Norwegian Biodiversity Information Centre shows that *A. improvisus* is likely occurring in a bigger portion of the study area than recorded by this study.

**Distribution:** The bay barnacle, *A. improvisus*, has native range along the northwest coast of the Atlantic Ocean (Jelmert et al., 2018a, Jensen, 2015). It has been present in Norway since the early 1900's. It is present in estuary systems in the Oslofjord and Skagerrak.



**Figure 3.4: Records of *Amphibalanus improvisus*.**

**Ecological impact:** *Amphibalanus improvisus* has high tolerance for low salinity levels and is often found in estuary systems. The bay barnacle is placed in the risk category potential high impact (PH), with no known ecological impact and high invasion potential (Jelmert et al., 2018a). It can cover the substrate in the intertidal zone in areas where it occurs. Even though, it is assessed to having low ecological impact as it mostly settles in areas of lower salinity where few native species are present (Jelmert et al., 2018a).

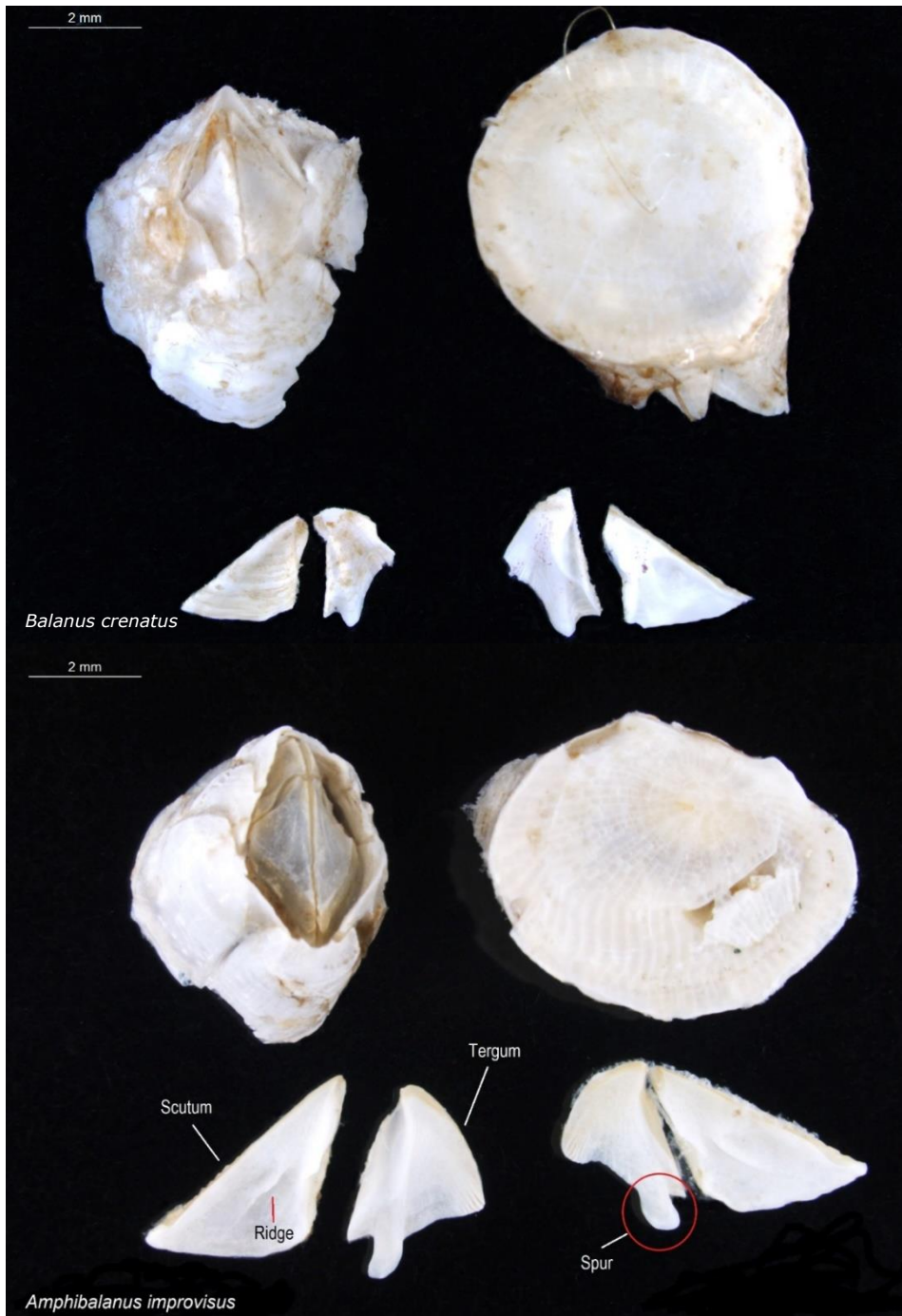
**Morphological description:** *Amphibalanus improvisus* is a white barnacle with six outer plates and four inner, movable plates. Some of the outer plates can be quite small and hard to detect. The outer plates are smooth. The inner plates join to form a diamond shape and they close tightly, leaving no gaping when closed. When removed from the substrate, *A. improvisus* leave a calcareous baseplate with a pattern of lines radiating from a point that often is slightly of centre of the plate. This baseplate sometimes stays attached to the organism, keeping the specimen intact. *Amphibalanus improvisus* has a ridge on the internal side of the anterior moveable plates (scutum) and a distinct shape of the spur on the posterior movable plates (tergum) (Figure 3.5). The spur on the tergum has a shape that resembles the fin keel of a sailboat.

**Remarks:** The description is based on thorough investigation of the specimens present in the samples, the description by NOBANIS (Jensen, 2015) and the identification key by NBIC (Noever, 2019). This identification key is a good tool to identify *A. improvisus* by looking at exterior morphology. However, *A. improvisus* can be confused with the native *Balanus crenatus* Bruguière, 1789. For certain identification of *A. improvisus* exterior morphology is not enough, the inner plates called scutum and tergum, must be examined.

**Material examined:** All samples collected during the project containing barnacles was examined, and the bay barnacles was identified and sorted out.

## Identification of *Amphibalanus improvisus*

Cirriped crustacean attached to hard substrate. Six outer plates and four inner plates. Calcareous baseplate. The bay barnacle can be confused with the native species *Balanus crenatus*. Both species have six smooth outer plates and a calcareous baseplate. By picking out the movable inner plates and investigating their shape, the two species can be told apart with high accuracy. *Amphibalanus improvisus* has a long and defined spur and a ridge on internal side of scutum (Figure 3.5). *B. crenatus* has a broad and short spur and no ridge on internal side of scutum.



**Figure 3.5: Anatomy of *Balanus crenatus* and *Amphibalanus improvisus*. Top of specimen with movable parts scutum and tergum in the centre (top left), calcareous baseplate (top right), scutum and tergum (bottom). The scutum of *A. improvisus* has a triangular shape and a ridge in the middle of the plate. The spur on the tergum of *A. improvisus* has a distinct shape, like a fin keel under the main shape of the plate. Photo: Marte Svorkmo Espelien**

### 3.1.3 *Crassostrea gigas* Thunberg, 1793

**Common names:** Stillehavsøsters (NO), Portuguese oyster, Pacific oyster (GB)

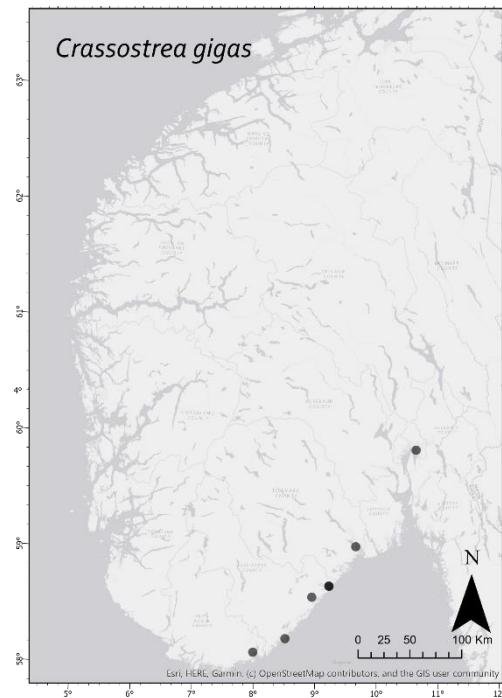
**Records:** *Crassostrea gigas* was found in six sites between Oslo and Kristiansand (Figure 3.6). The sites where *C. gigas* was recorded was in the already known range of the species. At the locations where *C. gigas* was present, it was found in high numbers and large individuals. *Crassostrea gigas* were often covered by epi growth such as algae, hydroids and tiny blue mussels growing on them. The species map service provided by the NBIC shows that *C. gigas* is likely occurring in a bigger portion of the study area than recorded by this study.

**Distribution:** The Pacific oyster, *C. gigas*, is native in the Pacific Ocean and was brought to Norway for shellfish farming in 1979 (Wrangé et al., 2010, Jelmert et al., 2018d). In 1980, the first populations outside of the farming facility was recorded. It is assumed that the present occurrence in Norway is also due to secondary spread from introduced wild populations in Sweden and Denmark, but the importance of the different introductory routes is presently not known (Bodvin et al., 2014). The Pacific oyster is now present along the Norwegian coast from the Swedish border to north in Møre & Romsdal county.

**Ecological impact:** The NBIC risk assessment places the Pacific oyster in the highest risk category, SE. It has high potential for spread, even though this can be limited by cold temperatures. With warmer climate, the potential for spread can increase further. *Crassostrea gigas* have medium ecological impact. On soft sediment bottoms, the Pacific oyster can build solid reefs, transforming the soft bottom to hard bottom. It is a competitor to the native Blue mussel *Mytilus edulis* Linnaeus, 1758, and the European flat oyster *Ostrea edulis*, (Jelmert et al., 2018d).

**Morphological description:** *Crassostrea gigas* is an asymmetrical bivalve with one valve often attached to some substrate or cemented onto hard bottom or pier pontoons. The shape of the pacific oyster is irregular, and the shape vary a lot between individuals. The shape and structure vary according to the habitat the individual grows in and how dense the population is. On individuals that are attached to hard substrate, like rocks or port structures one valve of the shell is cemented onto the substrate following the shape of the substrate, while the other valve is convex and has sharp layers of edges. The specimens found in this study measured up to 10-15 cm. The shell of *C. gigas* has multiple dark, almost violet, razor-sharp edges scaffolding in layers on the valve facing away from the substrate (Figure 3.7).

**Remarks:** There is one other oyster species in Norway that might be confused with the pacific oyster, the European flat oyster *Ostrea edilus*, but it can be distinguished from *C. gigas* by looking at shape, colour, and structure.



**Figure 3.6: records of *Crassostrea gigas* in the study area.**

**Material examined:** For this description, specimens in field was observed and photographed, and selected specimens was taken to the lab for further examination. The samples contained mostly large individuals, and these were examined while fresh before being destroyed. Specimens of *C. gigas* and *O. edulis* from the NTNU University Museum were photographed for the identification sheet.

## Identification of *Crassostrea gigas*

Asymmetrical bivalve with one valve attached to substrate or cemented to hard bottom. Shape, structure, and size can vary greatly. *Crassostrea gigas* has oval shaped valves (shape can vary greatly), the upper (right) valve has sharp scaffolding and dark colour on parts of the valve edges. The European flat oyster *Ostrea edulis* on the other hand has a round almost circular shell with a smooth, cream coloured surface compared to *C. gigas*.



**Figure 3.7: *Crassostrea gigas* (top two and lower left) and *Ostrea edulis* (lower right). *Crassostrea gigas* has layers of sharp edges and an irregular shape compared to the smoother and more circular *O. edulis*. Note the variation in shape and structure among *C. gigas* Photo: Marte Svorkmo Espelien**

### 3.1.4 *Styela clava* Herdman, 1881

**Common names:** Østasiatisk læsekkedyr (NO)  
Leathery sea squirt, Asian tunicate, Clubbed tunicate, Rough sea squirt (US, CAN, GB)

**Records:** *Styela clava* was found in Grimstad, Holvika, Homborsund and Stavanger harbours (Figure 3.8). It occurred on the floating pontoons in ports for recreational crafts. No new occurrence sites for *S. clava* were recorded. The organisms were often covered by epi growth such as hydroids, algae, blue mussels and bryozoans growing on them. The species map service provided by the NBIC shows that *S. clava* is likely occurring in a bigger portion of the study area than recorded by this study.

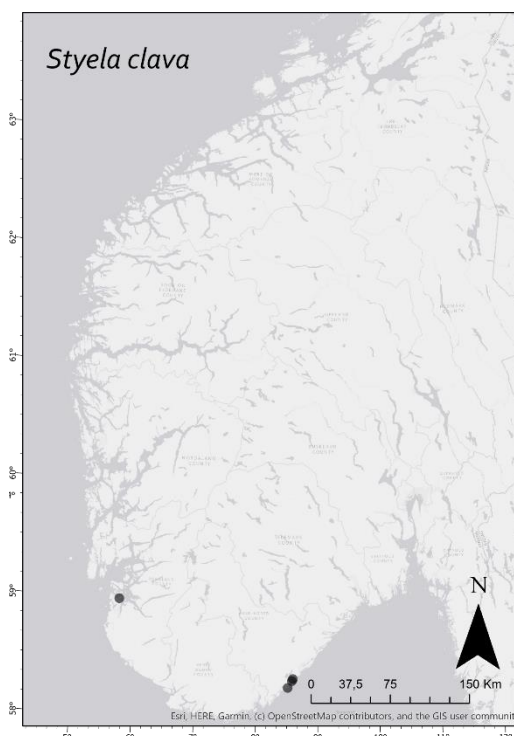
**Distribution:** *Styela clava* was first recorded in Norway near Stavanger in 1990. It is now established along the coast between Bergen and Grimstad. The distribution of *S. clava* seem to be limited by salinity (22-34 psu), which may limit the eastward spread of the species, and by temperature, which may limit the spread northwards (Jelmert et al., 2018g).

**Ecological impact:** *Styela clava* is a solitary ascidian that lives attached to hard bottoms, where it can occur in dense populations (Jensen, 2010c). *Styela clava* compete for space and nutrition in fouling communities, but it is assessed to having medium ecological effect as it does not seem to outcompete other organisms to a large extent. In the risk assessment, *S. clava* is placed at low risk LO (Jelmert et al., 2018g).

**Morphological description:** *Styela clava* is a solitary tunicate with firm texture and leather like surface like other species of the *Styela* Fleming, 1822, genus. It is brown and cream coloured in an uneven dotted pattern, with stripes around the siphons. *Styela clava* is club shaped and relatively large, often more than 10 cm long. The base of the organism is narrow, giving the impression that the organism is attached to the environment with a "stalk". *Styela clava* is often overgrown by other organisms, such as hydrozoans, algae, bryozoans and caprellids (figure 3.9).

**Remarks:** There are several *Styela* species in Norwegian waters, but *S. clava* can easily be distinguished from them by the "stalk" at the base of the organism.

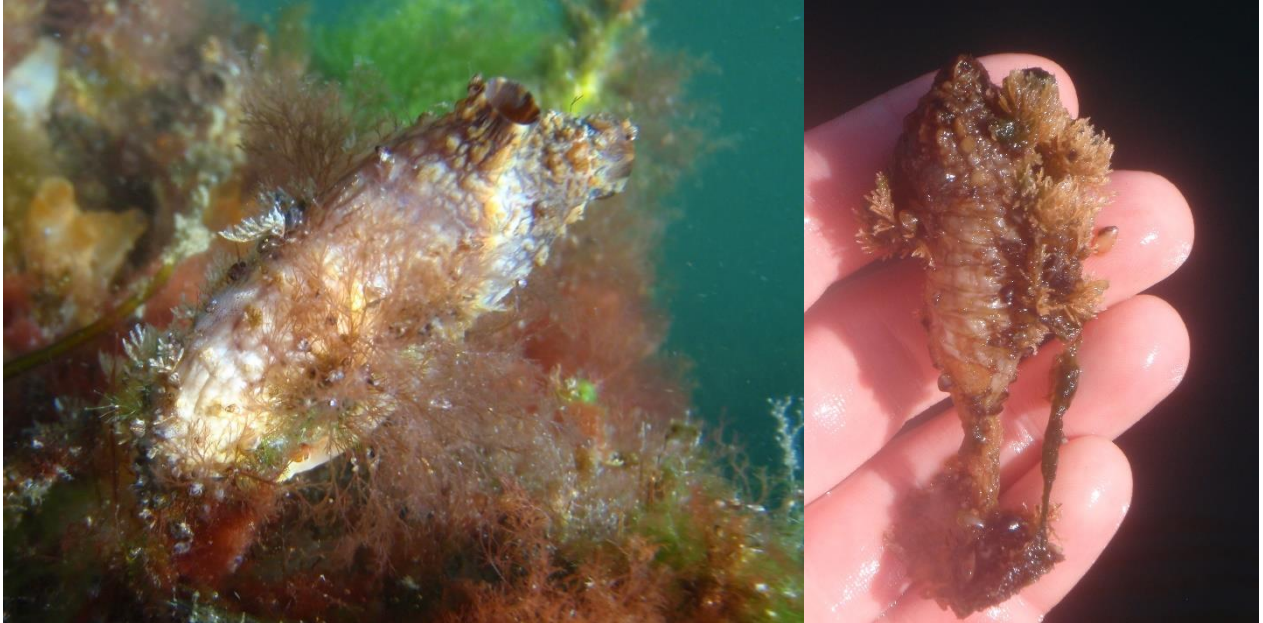
**Material examined:** The specimens present in the samples was examined in addition to observation in field and photos *in situ* and of fresh sampled specimens.



**Figure 3.8: Records of *Styela clava*.**

## Identification of *Styela clava*

Brown and beige ascidian with leathery, uneven surface. Can be covered with epi growth. Stripes of beige and brown along siphons, perpendicular to opening. Club shaped and large, up to 10 cm. Base of organism form a distinct "stalk". Distinguished from other *Styela* sp. by the presence of the stalk.



**Figure 3.9: *Styela clava* in situ and a collected specimen of *S. clava*. Note the distinct "stalk" at the base of the organism in the right picture. Individuals of *S. clava* can be densely covered by epi growth, often, only the siphons are visible. Photo: Marte Svorkmo Espelien**



## 3.2 Species not found:

Of the eight species known to be established in Norway that were included in the target list for this project, four species were not found. None of the door-knocking species were found. In the following, a short summary of the available current knowledge about each of the species not found is given.

### 3.2.1 *Botrylloides violaceus* Oka, 1927

**Description, distribution and ecological impact:** *Botrylloides violaceus* is a colonial ascidian inhabiting hard bottoms. It is native to the North-Western Pacific Ocean (Jelmert et al., 2018b, Fofonoff et al., 2018). It is recorded in several areas near Egersund on the South-Western coast of Norway since 2007. It seems to have limited natural spread, but potential for spread with boat traffic. It is often found in marinas. The ecological impact of *B. violaceus* is medium. It competes for space to attach in fouling communities and can overgrow other organisms. *Botrylloides violaceus* is placed in the risk category HI (Jelmert et al., 2018b).

### 3.2.2 *Marenzelleria viridis* (Verrill, 1873)

**Description, distribution and ecological impact:** *Marenzelleria viridis* is a soft bottom burrowing annelid. The only verified record in Norway is in Drammensfjorden in 2008. There is a lack of knowledge about the distribution of this species as it is very hard to identify by looking at morphology. It is assessed to the highest risk category (SE) with high invasion potential and medium ecological effect (Oug et al., 2019d).

### 3.2.3 *Goniadella gracilis* (Verrill, 1873)

**Description, distribution and ecological impact:** *Goniadella gracilis* is an annelid associated with soft sediment bottoms. It has been recorded in Skagerrak and near the oilfields on the Norwegian continental shelf. It is assessed to potential high impact (PH) in the Norwegian alien species risk assessment. It is expected to have minor ecological impact but high invasion potential (Oug et al., 2019a).

### 3.2.4 *Gonionemus vertens* A. Agassiz, 1862

**Description, distribution and ecological impact:** *Gonionemus vertens* is a small hydromedusa native to the Pacific Ocean. It was recorded in the North Sea in 1913 and is probably common in the waters off South-West Norway now. It is assessed to pose a low risk (LO) with minor ecological effect and restricted invasion potential (Falkenhaug et al., 2018b).

## 4 Discussion

Only four of the species on the project target list were found in this survey. These were the amphipod *Caprella mutica*, the cirriped *Amphibalanus improvisus*, the bivalve *Crassostrea gigas* and the ascidian *Styela clava*. Based on the current known distribution range of the species on the project target list, all the eight established target species could be expected to be found in this study. None of the species were found outside of their known range, but the Japanese skeleton shrimp, *C. mutica* was found at new sites in the already known range of the species. The door-knocking target species are expected to establish in the study area within 50 years (Norling and Jelmert, 2010), but none of them were found in this study.

### 4.1 Occurrence of species

#### 4.1.1 The species found

*Caprella mutica* occur along the Norwegian coast north to Troms (Jelmert et al., 2018c). The NBIC species map service, artskart.no, holds 81 records of *C. mutica* along the Norwegian coast. There are observations north to Troms & Finnmark, but most observations are south of Trøndelag (artskart.artsdatabanken.no, 2020b). It is mostly associated with anthropogenic environments such as floating pontoons in marinas and aquaculture facilities (Boos et al., 2011, Jensen, 2010a, Turcotte and Sainte-Marie, 2009), but in this study, it was also found at Rishaugen in Agdenes which is a natural environment. The bay barnacle, *A. improvisus* is present in the whole study area according to artskart.no, but only found between Oslo and Kristiansand in the current study. Two different factors could explain this, population retreat and sampling bias. The bay barnacle has shown no sign of population decline in areas where it is already established (Jelmert et al., 2018a), so it is likely that it has a larger range than detected by this study. *Amphibalanus improvisus* was found at sites with high freshwater influence, where it was the dominating species and covered the substrate. Checking the distribution range outlined by the data in artskart.no, could be done by systematic resurveying of the locations where *A. improvisus* has been found previously and specifically targeting areas with freshwater influence. The Pacific oyster, *C. gigas*, is recorded in the southern part of the study area in artskart.no, but it was not found at many sites in the present study. This can be explained by population retreat or sampling bias. The Pacific oyster is temperature sensitive and rely on warm summers for successful reproduction and mild winters for survival (Jelmert et al., 2018d). Several pathogens can influence the survival of *C. gigas* such as the herpes (OsHV-1  $\mu$ var) virus outbreak that hit pacific oysters in Sweden and Norway in 2014 (Bodvin et al., 2014, Mortensen et al., 2014). In previous work on Pacific oyster in Norway, it has been found that intertidal walks and surveys in natural habitats have yielded more records of *C. gigas* than RCSs in marinas (Rinde et al., 2017). Very few intertidal walks were conducted in the present study and the specimens of *C. gigas* that were found, were sampled in marinas and harbours. As specimens of *C. gigas* are very large, they were often recorded in the field notes only in the present study, without being collected and included in the museum collections. Therefore, it is possible that specimens were left

undetected in field and not included in the species records for the current study. According to the records in artskart.no, *S. clava* is present along the coast between Bergen and Grimstad. Artskart.no holds 68 observation records of *S. clava* in Norway (artskart.artsdatabanken.no, 2020d). The findings from the present study are all inside the area of previously recorded occurrences, but it was not found in the material collected north of Stavanger and east of Grimstad. *Styela clava* make dense local populations but has limited potential to spread on its own due to short larval stage (Jensen, 2010c). This study may have failed to detect such local populations. The knowledge about *S. clava* in Norway is limited and more studies are needed to understand its present and potential range. It is likely that eastward distribution is limited by salinity and northward distribution is limited by temperature (Jelmert et al., 2018g).

## 4.1.2 The species not found

### 4.1.2.1 The established species

Even though the project target species list contained 21 species, only four alien species were found in the study area. There may be several different reasons for finding so few of the target species, it might be that they are not present in the study area, that they are not present at the sampling sites, or that the sampling methods employed in the current study did not detect them. Some of the known established species on the project target list have very local populations, only recorded in certain areas previously. This is the case with the annelids *Marenzelleria viridis* and *Goniadella gracilis* and the ascidian *Botrylloides violaceus*. The colonial ascidian *B. violaceus* is known to occur at sites in the Egersund area south of Stavanger (Jelmert et al., 2018b). It was not found in this study. This may indicate that it still has not spread from the Egersund area, or it might be hard to detect with the sampling effort employed in the present study. The annelid *M. viridis* has been recorded in Drammensfjorden and there are some unverified reports of it from Trøndelag and Vestland (Oug et al., 2019c). The annelid *G. gracilis* has been recorded in Skagerrak and the Norwegian continental shelf (Oug et al., 2019b). It was relevant to look for it in the southern part of the study area as these sites are close to previous records of the species. The existing records of these species in Norway are from a few sites. These can be early populations which have not reached a population number that enables them to expand their range. It is important to monitor such populations and include these species on target lists for surveys in a greater area to detect changes in their range early. The hydromedusa *Gonionemus vertens* was not found, even though it is known to occur occasionally in the study area (Falkenhaus et al., 2018b), but it is strongly associated with eel grass beds (Falkenhaus et al., 2018b) which was not systematically sampled in this study. None of the door knocking species on the target list was found. It can be hard to detect the early populations of a newly arrived species as the first populations often have a limited number of individuals and are limited to a small area. Detection of new alien species is therefore often lagged in time after the initial population established (Lockwood et al., 2007).

Several of the species not found in this study are species belonging to groups of unresolved taxonomy, which need expert identification. Species of the genus *Marenzelleria* are hard to tell apart based on morphology (Kauppi et al., 2015) and two species from this genus were on the target species list for this study: *M. viridis* (considered established in Norway) and *M. neglecta* (door knocker, recorded in the Baltic

Sea), while a third species *M. arctia* is also recorded in the Baltic Sea (Oug et al., 2019c, Kauppi et al., 2015), but not included in the alien species list from NBIC. None of these species were found in this study. *Goniadella gracilis* need expert identification (Walker, 1972, Oug et al., 2019b) and was not found in the study area. The ascidian *B. violaceus* belongs to a group of ascidians of unresolved taxonomy and identification need expert judgement (Jelmert et al., 2018b). To facilitate monitoring of this species and other alien ascidians, projects to revise the taxonomy of ascidians in Norwegian waters should be implemented and good identification tools should be produced.

#### **4.1.2.2 The door-knocking species**

Including door-knocking species in target species lists in surveys for alien species is crucial to be able to detect new species that arrive (Norling and Jelmert, 2010). In this project, door-knocking species that could be detected with the applied sampling methods at the surveyed sites were included in the target list.

The molluscs *Rangia cuneata*, *Mytilopsis leucophaeata* and *Dreissena polymorpha*, and the annelids *Ficopomatus enigmaticus*, *Hydroides dianthus* and *Marenzelleria neglecta* are closely connected to estuaries and systems with high freshwater influence (Kerckhof et al., 2007, Falkenhaus et al., 2018a, Jelmert et al., 2018e, Kjærstad et al., 2018, Oug et al., 2018c, Oug et al., 2018d, Oug et al., 2018e). *R. cuneata*, *M. leucophaeata* and *M. neglecta* are present in the Baltic Sea, *D. polymorpha* is present in the Mälaren and Götekanal freshwater systems in Sweden, *F. enigmaticus* is found in Denmark and *H. dianthus* is found on the British Isles. Areas with high freshwater influence are suggested as areas that should be object to monitoring for alien species in Norway (Rinde et al., 2017). Many of the door-knocking species on the target list for this project and on the NBIC alien species list live in low salinity water and are expected to establish in estuaries and freshwater influenced areas in Norway. The present study surveyed areas with freshwater influence, but not exclusively such systems.

The mollusc *Ocenebrellus inornatus* and the ascidians *Corella eumyota*, *Didemnum vexillum* and *Perophora japonica*, originate from areas with similar climatic conditions as can be found along the southern coast of Norway (Minchin et al., 2013, Minchin, 2007, Gulliksen et al., 2018a, Gulliksen et al., 2018b, Gulliksen B, 2018, Jelmert et al., 2018f). *C. eumyota*, *D. vexillum* and *P. japonica* are established along the coast of the British Isles and *O. inornatus* have been found in Limfjord, Denmark. These species are expected to enter Norwegian coastal waters soon and it is therefore important to be aware of them in future alien species surveys.

The molluscs *R. cuneata* and *M. leucophaeata*, the crustaceans *Amphibalanus amphitrite* and *Austrominius modestus*, the annelid *F. enigmaticus* and the bryozoan *Watersipora subatra* are currently restricted by the low temperatures in Norwegian waters (Oug et al., 2018c, Oug et al., 2018f, Oug et al., 2018a, Oug et al., 2018b). The populations of *A. Amphitrite* and *W. subatra* closest to Norway are in the English Chanel, while *A. modestus* is present in England and Denmark. At current temperature regimes they may establish local populations in areas of higher temperatures such as semi closed lagoons and tidal ponds (Oug et al., 2018c, Oug et al., 2018f). With a warmer climate, they may be able to survive along the southern coast of Norway.

## 4.2 Assessment of spatial distribution

Distribution of alien species often follow certain geographical patterns (Rohde, 1992). In temperate regions, one such pattern is the latitudinal gradient of species richness (Sax, 2001). In the current study more alien species was found in the southern part of the study area than further north. The sampling effort also was higher in the southern part of the study area, but the negative correlation between alien diversity and latitude is supported by a recent alien species inventory for Norway (Sandvik et al., 2019). In Norway, two important factors for alien species distribution also follows the latitudinal gradient, temperature, and human population density (Kommunal- og moderniseringsdepartementet, 2018). Higher human population provides a higher introduction pressure with more vectors of species transport. Higher temperatures in the south facilitates survival for species that depend on warm temperatures for reproduction and survival (Occhipinti-Ambrogi, Galil, 2010). Temperature is one explanatory factor for the higher number of alien species in the southern part of the study area. Many of the species on the project target list and the NBIC alien species list have temperature requirements in a range slightly above the current temperature regime in Norwegian coastal waters (Sandvik et al., 2019 and previous sources for target list species). With a future warmer climate, the door knocking species that currently are restricted by temperature may establish along the coast of Norway. The species already established in Norway may increase their geographic range northward with increased temperature.

As previously discussed, many of the project target species have high tolerance for low salinity. Estuaries and other marine systems with high freshwater influence are especially susceptible to alien species (Nehring, 2006). The bay barnacle was found in many of the areas of freshwater influence sampled for this study. To gain more knowledge about the alien range of species with high tolerance for low salinity such as *A. improvisus* in Norway, some monitoring efforts should focus on areas with high freshwater influence.

The present study focused on anthropogenic environments, but one natural habitat was also investigated, and an alien species was found there. This might indicate that some alien species have a more widespread range in natural habitats than known. Previous alien species surveys have focused mainly on anthropogenic environments (Campbell et al., 2007). Consequently, there might be a lack of knowledge about alien species in natural habitats in Norway, so further surveys targeting alien species in natural habitats should be done. To develop more extensive knowledge about the range of alien species such as *C. mutica* and *C. gigas* in Norway, mapping in both natural and anthropogenic environments should be done. Monitoring of areas that are especially susceptible to marine alien species, such as areas with freshwater influence and harbours that receive international marine traffic along the southern coast of Norway should be done to detect new arriving species early (Rinde et al., 2017).

## 4.3 The importance of precise identification

There are two major error categories in scientific studies: type I error or false positives and type II error or false negatives. When assessing alien species, a type I error is to misidentify a specimen of a native species as an alien species (Campbell et al., 2007). Type II error is failing to detect a species that is present and can occur by several means. One of the aims of this project was to develop good identification tools to avoid misidentification of alien and native species. During this work it became clear that such

still non-existent tools were needed. Type II error can also be associated with small sample size or inappropriate sampling regime may have been applied (Campbell et al., 2007).

The NBIC species map database artskart.no provides much of the available knowledge about occurrence of species in Norway. This service is based on records provided from a great variety of contributors spanning from research institutes, consultancy businesses, citizen science and school projects. Observations of alien species are object to the same type of biases as observation data in general, connected to spatial, temporal and taxonomical biases (Ruiz et al., 2000). Geographical and spatial biases in observation data occur when some areas, habitat types and environments are sampled more than others. Alien species surveys often focus on the pathways of transport of organisms and areas of anthropogenic influence are subject to such studies (Campbell et al., 2007, Minchin, 2007, Rinde et al., 2017). Uneven availability of expertise across taxonomic groups and the overrepresentation of sensational species are important taxonomical biases (Ruiz et al., 2000, Pyšek et al., 2008). Among the marine invertebrates many successful invaders are molluscs and crustaceans, and these taxa are well studied, while the knowledge about many other taxa is inadequate (Pyšek et al., 2008)

It is a trend in invasion ecology that harmful alien species are well studied compared to other taxa (Pyšek et al., 2008). Of the species on the project target list, *C. gigas* had the highest number of observations in artskart.no, but it was not the most abundant species in the project samples. The pacific oyster is an alien species that is well known to the public and easy to identify, it is also prioritized by the government for monitoring. Artskart.no has 712 records of *C. gigas* in Norway and these observations are done by a great number of different contributors, ranging from school classes and citizen science to environmental consultancies and research institutions (artskart.artsdatabanken.no, 2020c). The Pacific oyster is a species that is easily recognized, and it has received a lot of public attention over the last years in Norway. This may explain the high number of records in artskart.no. Many of the records of *C. mutica* in artskart.no, are observations only, with no physical record or photography. Knowing how difficult precise identification of this species can be, it might be necessary to evaluate the validity of some of the records in artskart.no. A big issue with records without physical samples is that the identification cannot be assured in hindsight. Many of these identifications are done by experts, but for some for the records no name is given to verify the identification work, and no reference specimen exist. Of the 81 records of *C. mutica* in artskart.no, only six have a reference sample (artskart.artsdatabanken.no, 2020b). In artskart.no, 274 observations of *A. improvisus* are registered, only 11 of these with reference samples (artskart.artsdatabanken.no, 2020a). Given that precise identification of the bay barnacle relies on dissection, some of these observations may be misidentifications. As so few of the observation records on artskart.no have sampled reference material, and, given how complicated identification of some species can be, more focus should be given to validation of identifications and storing of reference material in the future.

Precise identification of the project target species relies on identification tools that compare the alien species to easily confused native species. A lack of such tools may lead to misidentifications. Therefore, identification sheets were made for the alien species found in this project. Few identification aids for *C. mutica* give a comparison to native species in the Northeast Atlantic, and it proved very hard to make precise identification supported by the available literature. A thorough morphological description is given in a Canadian synopsis (Turcotte and Sainte-Marie, 2009), however, they compare *C. mutica*

to similarly looking species in Canadian waters, which do not include the congruent species in the Norwegian waters. Of the literature used for the present study, only the NOBANIS fact sheet (Jensen, 2010a) compared *C. mutica* to native *Caprella* species in the Northeast Atlantic, but not specifically in Norwegian waters. Identification of *A. improvisus* can be done following the barnacle key by Noever (2019) available on the NBIC webpage, but additional investigation of the movable inner plates should be done to secure precise identification. The need for investigation of internal morphology is not specified in the NBIC identification key, which may result in misidentifications when this key is used. The Pacific oyster is an easily recognisable species, but the outer morphology can vary greatly between individuals. Therefore, identification aids for *C. gigas* should describe how to distinguish between the Pacific oyster and the native European flat oyster, *O. edulis*. Identification of *S. clava* is simple, as there are no similar looking species in Norwegian coastal areas (Husa et al., 2012a). Sampling and identification of *S. clava* can be done by non-experts with identification tools like the one provided in this paper.

Observation registration services such as artskart.no and gbif.org are open for many contributors to register species observations. This may lead to easily recognisable species being overreported while less conspicuous species are underreported. Also, such registration systems can be susceptible to misidentification errors and species being hard to identify being underreported.

#### 4.4 Assessment of the methods

The HELCOM/OSPAR sampling protocol (HELCOM/OSPAR, 2015) is an extensive sampling scheme which aims to sample a multitude of different habitats in addition to several environmental parameters. In this study, selected parts of the HELCOM/OSPAR sampling scheme was applied. Grabs, scrapes, dredges, diver samples and fouling plates are sampling techniques that are described in the sampling protocol (HELCOM/OSPAR, 2015). These give quantitative or semi-quantitative data. By dividing the big harbour sampling sites into different sub-areas, sampling in a broad variety of anthropogenic environments is done. Extensive sampling efforts such as the HELCOM/OSPAR sampling protocol can be efficient at detecting the species diversity present (Rohde et al., 2017), but they can be expensive to conduct. The HELCOM/OSPAR protocol is developed specifically to survey commercial harbours and focuses on ballast water as the vector of species transport (Kraus et al., 2019). The Rapid Coastal Survey procedure is more flexible and can be applied to survey other environments such as aquaculture facilities and marinas (Minchin, 2007). That way, the RCS method can be adjusted to search for species introduced through other pathways than ballast water.

In a 2007 review study it was found that surveys following the RCS procedure attain approximately 30% of the alien species present, while other methods, such as *Hewitt & Martin* and *Bishop museum* surveys attain up to 50% of the non-native species present (Campbell et al., 2007). However, the two latter methods are more comprehensive and includes species richness beyond alien species presence, which also make them more costly and time consuming. RCS surveys are reliant on the taxonomic expertise of the team employed at each specific survey, therefore, detection rate, comparability between sites and studies and resurveying is dependent on gathering similar expertise for each successive survey (Campbell et al., 2007). In the present study, this problem was reduced by collecting physical samples in field and bringing them back to the lab for

identification work, instead of assessing the specimens in field only. This makes the outcome of the survey less reliant on having a constant field team, but it also increases the amount of time spent on assessing the biodiversity. The RCS method is dependent on the availability of taxonomic expertise which can be limited. This challenge can be overcome by non-expert personnel being trained to recognize the species on the target list. If so is done, it is crucial that they learn how to distinguish between the alien species and similar looking native fauna and provide good tools to aid this. The data obtained from qualitative methods such as the RCS procedure give knowledge about the presence of the species found, but they cannot tell anything about the species not found (no absence data). A limitation with the RCS approach is that it will only detect species that are in the fouling community on easily available structures in the harbours. This excludes mobile fauna, planktonic organisms, benthic sediment infauna and organisms living at greater depths than 0.5-1 meter. To increase the detection rate, it is suggested to supplement the RCS procedure with video transects (Rinde et al., 2017), baited straps, grabs (Minchin, 2007), fouling plates (Rinde et al., 2017) and divers (Husa et al., 2012b).

If easy to use identification aids was available for more of the alien species, both those on the target list for this project and the others species on the NBIC alien species list, more knowledge could be generated through citizen science and local monitoring programmes. Making such tools available to the public will contribute to make it easy to do precise identifications of alien species and should be done for a greater number of alien species in Norway. A good next step now would be to develop easy to use guides on how to assess the alien biodiversity in an area with sampling procedures and identification tools. Such guides already exist in Norwegian for many terrestrial species but lack for many marine alien species (miljolare.no, 2020). With such a guide, school classes and citizen science initiatives could contribute to the development of knowledge about the distribution of alien species. This would have the potential to increase public awareness and might give valuable contributions to the knowledge about the distribution of alien species.

As the technology advance and get less costly, using environmental DNA metabarcoding from sediment and water samples (Holman et al., 2019) might be useful to combine with traditional surveys of biodiversity and alien species. Metabarcoding eDNA has the potential to detect more species than traditional methods, sometimes with higher precision (Holman et al., 2019). Molecular methods can complement morphological methods and make alien species surveys less dependent on expert identification (David, Krick, 2019).

Extensive sample collection was carried out for this study, 22 sites were sampled. In 10 of these, multiple methods were applied in a systematic sampling scheme, while 11 sites were sampled through RCS and one intertidal walk. Despite all the sampling effort, only four alien species were found. Other alien species surveys have recorded a higher number of alien species with similar amount of sample collection (Husa et al., 2012b, Husa et al., 2012a, Rinde et al., 2017). These studies targeted a wider list of species, including macroalgae and mobile fauna which was excluded from the target species list of the current study. In the surveys done in Rogaland and Hordaland, the majority of the alien species found were macroalgae, while the only invertebrates found were *C. gigas* (only in the Hordaland survey), *S. clava* and *C. mutica* (Husa et al., 2012b, Husa et al., 2012a). *Amphibalanus improvisus* was not on the target list for those studies (Husa et al., 2012b, Husa et al., 2012a). In the Oslofjord study, *C. gigas* and *C. mutica* were



found in addition to several macroalgae (Rinde et al., 2017). Since the same invertebrate species were found in the studies discussed above as in the present study, they are probably well established in the southern part of the study area for this project. When no other target species was found, it may be because they are not established in the study area, they have small local populations in sites not sampled, or that the methods deployed were not able to detect them. Despite the massive sampling effort, not enough quantitative data was sampled to make statistical analysis possible. Statistical analysis of differences in distribution range and latitudinal range would make the results of this study more powerful.

## 4.5 Conclusion

Alien species are considered a threat to global biodiversity as well human society's prospect of exploiting ecosystem resources in a sustainable way. Some important measures for combatting alien species include early detection facilitated by precise identification, managing pathways of introduction, and controlling or eradicating alien populations (Convention on Biological Diversity, 2018, Pagad et al., 2015). This study found that marine alien invertebrates are present along the Norwegian coast between Oslo and Trondheim. As expected, based on previous studies (Sandvik et al., 2019), more species of alien organisms were found in the southern part of the study area. This indicate that the number of alien species decreases with latitude in the study area, but further studies should be done to investigate this more systematically. Previously it has been suggested that many marine alien species are strongly associated with anthropogenically modified environments, and this is where most of the alien species surveys are done (Campbell et al., 2007). In the current study an alien species was also found in a natural environment. Further studies to gain more knowledge about spatial distribution and presence of alien species in different environments are recommended. In Norway, the general lack of knowledge about marine invertebrate biodiversity (Gederaas et al., 2012) is a limiting factor for management of alien species. Inadequate identification guides may hamper the process of gaining more knowledge about alien species as taxonomical expertise often is needed to make precise identifications. This study contributes to the knowledge about alien marine invertebrates by adding occurrence data to the databases and identification tools to facilitate precise identification and distinguish alien species from similar looking native species.

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## 6 Appendix

Appendix I: Stations where the alien species was found. At many of the locations, multiple samples were taken from different substrates, therefore some locations have several entries with the same species. Number of individuals in collected samples are given for some entries.

| Species         | Location             | Date       | VM station# | Remarks                                           |
|-----------------|----------------------|------------|-------------|---------------------------------------------------|
| Caprella mutica | Tvedestrand          | 28.08.2019 | 2019115     | 30 + ind (+uncertains)                            |
| Caprella mutica | Homborsund           | 27.08.2019 | 2019112     | 20 + ind (+uncertains)                            |
| Caprella mutica | Risør                | 28.08.2019 | 2019113     | 30 + ind (+uncertains)                            |
| Caprella mutica | Risør                | 28.08.2019 | 2019114     | 5 + ind (+uncertains)                             |
| Caprella mutica | Bergen               | 11.09.2018 | 2018105     |                                                   |
| Caprella mutica | Bergen               | 11.09.2018 | 2018108     |                                                   |
| Caprella mutica | Bergen               | 11.09.2018 | 2018109     |                                                   |
| Caprella mutica | Bergen               | 11.09.2018 | 2018110     | 1 ind                                             |
| Caprella mutica | Bergen               | 10.09.2018 | 2018092     | 2 ind                                             |
| Caprella mutica | Grimstad, Østerbugt  | 25.08.2019 | 2019084     | 35+ ind (+uncertains)                             |
| Caprella mutica | Grimstad, Vesterbugt | 25.08.2019 | 2019085     | 4 ind (+uncertains)                               |
| Caprella mutica | Holvig marina        | 22.08.2019 | 2019056     | 10+ ind (+uncertains)                             |
| Caprella mutica | Lillesand            | 25.08.2019 | 2019086     | 15+ ind (+uncertains)                             |
| Caprella mutica | Stavanger            | 14.09.2018 | 2018131     |                                                   |
| Caprella mutica | Stavanger            | 15.09.2018 | 2018155     | 15+ ind (+uncertains)                             |
| Caprella mutica | Stavanger            | 14.09.2018 | 2018138     | 4 ind (+uncertains)                               |
| Caprella mutica | Stavanger            | 14.09.2018 | 2018135     | 2 ind (+uncertains)                               |
| Caprella mutica | Stavanger            | 15.09.2018 | 2018151     | 4 ind (+uncertains)                               |
| Caprella mutica | Stavanger            | 15.09.2018 | 2018159     | 8 ind (+uncertains)                               |
| Caprella mutica | Stavanger            | 15.09.2018 | 2018153     | 1 ind                                             |
| Caprella mutica | Trondheim            | 05.09.2018 | 2018047     | 15+ ind (+uncertains)                             |
| Caprella mutica | Trondheim            | 05.09.2018 | 2018053     | 2 ind (+ <i>Phtisica marina</i> )                 |
| Caprella mutica | Trondheim            | 05.09.2018 | 2018051     | 1 ind (+2 uncertain, + 3 <i>Phtisica marina</i> ) |
| Caprella mutica | Trondheim            | 05.09.2018 | 2018059     |                                                   |
| Caprella mutica | Espegrend area       | 13.09.2018 | 2018126     | 2 ind                                             |
| Caprella mutica | Espegrend area       | 13.09.2018 | 2018124     | 3 ind (+uncertains)                               |
| Caprella mutica | Espegrend area       | 13.09.2018 | 2018123     | 15+ ind (+uncertains)                             |
| Caprella mutica | Oslo                 | 18.09.2018 | 2018187     | 2 ind (+ 1 uncertain)                             |
| Caprella mutica | Oslo                 | 18.09.2018 | 2018179     | 5+ ind (+uncertains)                              |
| Caprella mutica | Grilstad             | 04.09.2018 | 2018034     | 4 ind (+uncertains)                               |
| Caprella mutica | Grilstad             | 04.09.2018 | 2018036     | 1 ind                                             |
| Caprella mutica | Grilstad             | 04.09.2018 | 2018030     | 2 ind                                             |
| Caprella mutica | Grilstad             | 04.09.2018 | 2018038     | 2 ind                                             |
| Caprella mutica | Vernes ferjekai      | 16.10.2019 | 2019144     | 10 + ind                                          |
| Caprella mutica | Rishaugen            | 18.10.2019 | 2019149     | 2 ind                                             |
| Caprella mutica | Selva                | 19.10.2019 | 2019150     | 15+ ind (+uncertains)                             |

|                         |              |            |         |                                                  |
|-------------------------|--------------|------------|---------|--------------------------------------------------|
| Caprella mutica         | Breivikbukta | 20.10.2019 | 2019151 |                                                  |
| Caprella mutica         | Synnavika    | 21.10.2019 | 2019152 | 1 ind                                            |
| Caprella mutica         | Sandefjord   | 17.09.2018 | 2018164 |                                                  |
| Caprella mutica         | Sandefjord   | 17.09.2018 | 2018166 |                                                  |
| Amphibalanus improvisus | Oslo         | 18.09.2018 | 2018186 | 2 ind                                            |
| Amphibalanus improvisus | Oslo         | 18.09.2018 | 2018173 | 10 + ind                                         |
| Amphibalanus improvisus | Oslo         | 18.09.2018 | 2018213 | 2 ind                                            |
| Amphibalanus improvisus | Oslo         | 18.09.2018 | 2018176 | 4 ind                                            |
| Amphibalanus improvisus | Oslo         | 18.09.2018 | 2018217 | 2 ind                                            |
| Amphibalanus improvisus | Oslo         | 18.09.2018 | 2018179 | 1 ind                                            |
| Amphibalanus improvisus | Oslo         | 18.09.2018 | 2018215 | 3 ind                                            |
| Amphibalanus improvisus | Sandefjord   | 17.09.2018 | 2018161 | 1 ind                                            |
| Amphibalanus improvisus | Langesund    | 26.08.2019 | 2019111 | 8 ind                                            |
| Amphibalanus improvisus | Tvedestrand  | 28.08.2019 | 2019115 | 2 ind                                            |
| Amphibalanus improvisus | Frierfjorden | 26.08.2019 | 2019109 | 10 + ind                                         |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019094 | 10 + ind                                         |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019099 | 15 + ind                                         |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019106 | 5 ind                                            |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019103 | 15 + ind                                         |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019088 | 13 ind                                           |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019093 | 16 ind                                           |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019105 | 10 ind                                           |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019098 | 8 ind                                            |
| Amphibalanus improvisus | Brevik       | 26.08.2019 | 2019090 | 30+ ind                                          |
| Amphibalanus improvisus | Kristiansand | 23.08.2019 | 2019061 | 2 ind                                            |
| Amphibalanus improvisus | Kristiansand | 23.08.2019 | 2019072 | 1 ind                                            |
| Amphibalanus improvisus | Kristiansand | 23.08.2019 | 2019076 | 3 small ind. Sample of mostly <i>B. crenatus</i> |
| Amphibalanus improvisus | Kristiansand | 23.08.2019 | 2019064 | 1 small ind. On <i>Zostera</i>                   |
| Amphibalanus improvisus | Kristiansand | 23.08.2019 | 2019065 | 20 ind                                           |
| Amphibalanus improvisus | Kristiansand | 23.08.2019 | 2019069 | 15 ind                                           |
| Amphibalanus improvisus | Kristiansand | 23.08.2019 | 2019066 | 20 ind                                           |
| Amphibalanus improvisus | Kristiansand | 23.08.2019 | 2019070 | 15 ind                                           |
| Crassostrea gigas       | Oslo         | 20.09.2018 | 2018204 |                                                  |
| Crassostrea gigas       | Homborsund   | 27.08.2019 | 2019112 |                                                  |
| Crassostrea gigas       | Risør        | 28.08.2019 | 2019113 |                                                  |
| Crassostrea gigas       | Risør        | 28.08.2019 | 2019114 |                                                  |
| Crassostrea gigas       | Tvedestrand  | 28.08.2019 | 2019115 |                                                  |
| Crassostrea gigas       | Kristiansand | 23.08.2019 | 2019078 |                                                  |
| Crassostrea gigas       | Brevik       | 26.08.2019 | 2019088 |                                                  |
| Styela clava            | Homborsund   | 27.08.2019 | 2019112 |                                                  |
| Styela clava            | Grimstad     | 25.08.2019 | 2019084 |                                                  |
| Styela clava            | Grimstad     | 22.08.2019 | 2019056 |                                                  |
| Styela clava            | Stavanger    | 15.09.2018 | 2018157 |                                                  |