UNIVERSITY OF GOTHENBURG

DEPARTMENT OF MARINE SCIENCES

COMPARING GENETIC METHODS

Detecting and monitoring Non-Indigenous Species

Alizz Axberg

Degree project for Master of Science (60 hp) with a major in Marine Sciences
MAR702, Degree Project for master in Marine sciences – Biology
Second Cycle

Semester/year:	Spring 2021
Supervisor:	Matthias Obst, Department of Marine Sciences
Examiner:	Sten-Åke Wängberg, Department of Marine Sciences

Photo of an ARMS deployment site, by Alizz Axberg

Acknowledgements

I would like to thank my supervisor Matthias Obst for helping me through this project, as well as Per Sundberg who has given me valuable input and guided me into new projects and ideas. I am also thankful for Leon Green, and Charlotta Kvarnemo who let me use their ethical permit to be able to conduct parts of the studies, which never would have been possible otherwise. And lastly thank you to, Thomas Dahlgren, Yannic Wocken, and Nauras Daraghmeh, who all have contributed with important parts to make all of these projects come together.

Abstract

The spread of Non-Indigenous Species, NIS, is a global issue, and is taking a toll on both ecosystems and economies. Anthropogenic vectors like ship transports, live bait trade, and hull fouling are considered some of the main ways of NIS to spread. Taxonomic expertise is on the decline and even the most experienced taxonomists are have trouble keeping up with all the new species that are being introduced. This coupled with the fact that many new species are being transported during the resting stage of their life cycles making them even harder to identify. Which is why using genetic methods to detect NIS is on the rise, and in this study I have tested different genetic methods to both detect new, and monitor previously known NIS. To test the reliability of eDNA analysis from water samples, the detectability of eDNA from the invasive species, Round goby, was tested *in vitro*. Autonomous Reef Monitoring Structures, ARMS, were deployed along the west coast of Sweden to allow NIS to settle, and DNA samples of the settled species were taken. Plankton samples were taken outside of Gothenburg, and metabarcoding was used to analyse both ARMS and plankton samples. To detect the most amount of NIS both the COI and 18S gene was targeted. Monitoring of known NIS was done by running target genetic analysis, dPCR, on water samples taken at sites where the presence of Round goby is known, as well as further out to test how far from the source Round gobies can be detected. The experiments showed both ARMS and plankton being effective methods to monitor known NIS as well as detecting completely new ones. A total of nine previously unreported species were detected, and two rare ones, including the Clinging jellyfish *Gonionemus vertens*. eDNA from Round goby is detectable for up to 80h, and does not seem to travel far from the source, indicating genetic methods of water samples to be an effective method to monitor known NIS.

Popular scientific summary

Non-Indigenous Species, or NIS, are species that have entered a water body of which they are not native, not all NIS become harmful and many can co-exist in new ecosystems without any issues. However, those species that do cause harm are considered invasive, and can have severe effects on the native species, surrounding ecosystems and can cause major economic losses for the countries they have invaded. For these reasons it is very important to detect NIS well before they start to become invasive, since once they have settled, it is almost impossible to get rid of them. Historically what have been done to monitor species compositions is by taken physical samples and to have experts identify as many species as possible. This method has its limitations since this type of expertise is on the decline, coupled with the fact that many experts are very well versed in the native species, but can have a harder time identifying completely new ones, especially if the species have many life stages where they look almost identical, also called cryptic species. This is why genetic methods are on the rise where bulk samples are taken from the environment, like plankton samples, and DNA is extracted from those samples and either matched to existing databases to find as many species as possible, or to target one, or a few specific species. In my study I tested the accuracy and effectiveness of these methods to see if they can compliment of even replace traditional methods. What I found was that using genetic methods is a very effective way to both monitor known NIS and to find new ones. However, further research need to be made within this field to be able to use it on larger scales, these methods also need to be more standardised so viable comparisons can be made between studies as well as countries.

Contents

Introduction

The problem with Non-Indigenous Species

The global decline of biodiversity (Halpern et al., 2008) can partly be explained by anthropogenic environmental disturbances (Quijón, Kelly, & Snelgrove, 2008; Olsgard, Schaanning, Widdicombe, Kendall, & Austen, 2008; Norderhaug et al., 2015). It is therefore important to monitor human activities that might have environmental impacts (Halpern et al., 2008) as well as biodiversity and ecological changes (Stachowicz, Bruno, & Duffy, 2007). Since marine environments are often shared between countries, having mechanisms of collaborations such as the OSPAR, HELCOM commission are of great value.

The number of non-indigenous species (NIS) has increased dramatically in the last decade according to the European environmental agency. Although not all are harmful, the ones that do become invasive are considered one of the largest threats to ecological well being and global biodiversity (CBD, 2000; EEA, 2015; Gollasch, 2006), as well as having a huge economic impact on many countries (Diagne et al., 2021). Just as many other things within the field of biology, the definition of an invasive species differs between researchers. Some are of the opinion that it has to have entered a new area via direct or indirect human activity, and that it has a geographical discontinuity (Boudouresque & Verlaque, 2002; Williamson & Fitter, 1996). Others use it as a synonym for non-indigenous species (Goodwin, McAllister, & Fahrig, 1999). A more widespread and commonly used outlook is however that NIS is the predecessor, so when a species has arrived and settled in a new area they are considered a NIS, and they become invasive if/when they cause harm, ecologically, economically or otherwise (Colautti & MacIsaac, 2004). The definition I will be referring to in this study is that a non-indigenous species, is a species, in any life stage or form, that has been introduced with anthropogenic assistance beyond its natural habitat (Clout & De Poorter, 2000). While an invasive species is one who's introduction is threatening to native species or surrounding environments (Naturvårdsverket, 2008), and are able to survive and reproduce (Zenetos et al., 2010). Invasive species can affect its new found habitat in multiple ways, even though it might seem counter-intuitive they often cause a decrease in biodiversity through competition for resources, predating on native species, or bringing in new diseases etc. But also through physical changes of the environment that can affect native species (Wallentinus & Nyberg, 2007), and once a species has become established the chances of eradicating them are extremely low (Summerson et al., 2013). It is therefore important to find NIS before they become invasive.

There are many ways humans can introduce new species into an area, such as live bait trade (Mahon, Nathan, & Jerde, 2014), aquaculture, or the more commonly studied, ship transport. This is a significant vector in transporting species across oceans, both in the ballast water that ships picks up in one part of the world, and releases it in another (Gollasch, 2006; Williams, Griffiths, Van der Wal, & Kelly, 1988). And as hull fouling (Minchin & Gollasch, 2003), where unwanted organisms attach to the hull of the ship. Ports with frequent international traffic are therefore considered potential hotspots for the introduction of NIS, and are areas of interests in my study.

Sampling methods for NIS

Hard bottom sampling

Monitoring soft bottom substrates are considered fairly straight forward, it can be done from beaches and shores as well as there being many ways to standardise sampling (Schönfeld et al., 2012). Hard bottom substrates, on the other hand, have shown to be much more difficult to monitor, even though these environments are just as important since epibenthic communities are good indicators of the environmental conditions. However, it has proven to be difficult and expensive to monitor hard-bottom substrates because of their three dimensional structures and the fact that they are often fragile. Divers

are another common way of monitoring, though, they can only be used in safe diving conditions ie. little to no currents and during a calm sea state. Deeper samples, > 20m, results in shorter dive times, requiring more divers which quickly becomes more expensive (Beisiegel, Darr, Gogina, & Zettler, 2017). Imaging of the sea floor is another feasible option for hard bottom monitoring, it allows for more liberties when analysing since it can be done at any time, as well as using ROVs for low-risk sampling. Additional data softwares, such as PhotoQuad, can also be utilised (Gomes-Pereira et al., 2016). However, imaging often leads to lower taxonomic resolution since the pictures or videos are generally taken in low-light conditions. And the species in question are only seen from one angle, making identification of cryptic species difficult, leading to lower confidence and less identified species (Beisiegel et al., 2017). Physical samples allow for higher number of taxa being identified (Borja et al., 2013) but can be difficult to obtain without damaging surrounding substrates.

Artificial substrates are a good way to go about studying hard-bottom communities without causing damage. It has been done for some time (Gobin & Warwick, 2006; Menge et al., 2002), and are already being used in some European monitoring programs in the form of settling plates (HELCOM, 2013). However, more standardized monitoring programs are being developed (Leray & Knowlton, 2015), like the Autonomous Reef Monitoring Structures, ARMS (www.oceanarms.org). These structures yields information on multiple layers, without being harmful to the surrounding environment. ARMS are three dimensional PVC structures composed of nine layers of 22.5 x 22.5cm plates and a 25 x 45 cm bottom plate (David et al., 2019), see Figure 1. They are submerged and left in the water, from weeks to years, to allow organisms to settle or crawl in between the layers. The ARMS are retrieved and can be analysed using photography, metabarcoding, target analysis, and/or morphological identification (e.g (Pearman, Anlauf, Irigoien, & Carvalho, 2016; Obst et al., 2020; Carvalho et al., 2019; Hurley et al., 2016)), all of which will be utilised in my experiments. Pearman et al. (2016) showed that the different methods complement each other well, but that metabarcoding yielded, by far, the highest number of OTUs. It still is important to find a standardized way of using and analysing ARMS as Ransome et al. (2017) showed in their study.

Many studies regarding ARMS are heavily focused on coral reefs, as the name suggests, and use them as a way of monitoring them without causing harm to existing structures (Pearman et al., 2016; Ransome et al., 2017). In this study, however, I will investigate the possibility of using them as an early detection method for NIS. Many marine organisms start of their lives as pelagic larvae and live in a planktonic state until they find a substrate to settle, at this stage they can become either a settling species like a barnacle, or a sessile bottom dwelling one (Crisp, 1976). Many of these organisms have preferences on where to settle, especially if it is a species that spends its entire adult life attach to a single spot, and studies have shown that some species tend to settle on man-made structures more frequently than others (Chase, Dijkstra, & Harris, 2016). Lambert and Lambert (2003) showed in their study that some NIS settled more frequently on anthropogenic habitats than on natural hard-bottom substrates, which is something that has been a consistent observation among studies (Simkanin, Davidson, Dower, Jamieson, & Therriault, 2012). One potential reason for this could be that anthropogenic structures, such as docks, are in direct contact with some of the most common ways of new species being introduced, boat traffic (Floerl & Inglis, 2005). Which is why placing ARMS in heavily trafficked ports and harbours are great prerequisites of finding NIS.

Plankton samples

As previously mentioned, the first life-form of many marine organisms is in a planktonic state, so taking plankton samples is another suitable way to monitor marine ecosystems. Especially since they also fill an important roll of indicating environmental changes (Taylor, Allen, & Clark, 2002). However, previously used biodiversity assessments in the form of morphological identification has proven to have its limitations, since a lot of planktonic organisms are very cryptic and is therefore challenging or even impossible to positively identify on a species level, but also because of a lack of

expertise in recent years (Chen & Hare, 2008). New alternatives are becoming more common and next generation sequencing allow for metabarcoding of plankton samples, which is a powerful tool to detect NIS. Especially considering that plankton can easily be picked up in the ballast water of ships and transported across oceans. It is also a powerful tool in overall biodiversity monitoring (Abad et al., 2016; *Generic*, 2012), and the simple sampling techniques makes it very cost-effective (Lindeque, Parry, Harmer, Somerfield, & Atkinson, 2013).

Environmental DNA from water samples

The definition of environmental DNA can differ slightly between researchers. Throughout this paper I have based my studies on the definition of Taberlet, Coissac, Hajibabaei, and Rieseberg (2012) who refers to eDNA as "DNA that can be extracted from environmental samples, such as soil water or air". And makes the important distinction between that and target organism sampling such as fur traps or plankton samples.

When investigating biodiversity and the presence of non-indigenous species, there is always a risk for human errors and varying results can come from the same water body (Hering, Feld, Moog, & Ofenböck, 2006; Borja et al., 2013). It is therefore important to find reliable methods of investigating the presence of NIS that greatly reduces the room for human error. Haase, Pauls, Schindehütte, and Sundermann (2010) showed that >30% of the taxa differed between the analyst and the auditors, and that 29% of the specimens had been overlooked when used traditional morphological methods. Taxonomic expertise has been declining for the last few decades (Hopkins & Freckleton, 2002), and differentiating between zooplankton species is challenging even for experienced morphologists. Especially considering many marine zooplankton cannot survive in ballast water tanks for a long period of time unless they are in a resting stage such as diapausing eggs, which is even more of a challenge to identify without genetic methods (Cabrini et al., 2019; Briski, Cristescu, Bailey, & MacIsaac, 2011). In a study by Sundberg, Obst, Bourlat, Bergkvist, and Magnusson (2018), only about 15% of 61 unique taxa could be morphologically identified to a single species, while a sample taken simultaneously but analysed using DNA methods could identify 143 taxa, 66%. Furthermore, working with environmental DNA coupled with barcoding and metabarcoding could solve many of these issues (Lodge et al., 2006). Leese, Kahlert, Drakare, and Zimmermann (2016) showed eDNA and metabarcoding to be a very useful tool in finding recently introduced NIS, as well as monitor overall community composition. They also found that sediment samples contain three times the amount of DNA compared to in the free water mass. However, when detecting NIS water samples works just as well as any other sample type, since sediment samples tend to have an accumulation of DNA dating further back in time. Thus, the best assessment of biodiversity is given when taking samples from multiple environmental types. They believe replicated eDNA metabarcoding surveys should be conducted over time to enhance existing monitoring programmes. They also bring up the importance of investigating how DNA degrades over time in marine environments, which is something that has been investigated in a few studies, both in mesocosms(Seymour et al., 2018), and *in situ* (Collins et al., 2018). Several studies have now shown eDNA to have greater sensitivity than traditional methods (Jerde, Mahon, Chadderton, & Lodge, 2011; Creer et al., 2016; Bohmann et al., 2014), it is also important to consider the economic pros and cons, a Danish study showed that genetic methods from water samples is more cost efficient as a monitoring tool than traditional fishing methods (Sigsgaard, Carl, Møller, & Thomsen, 2015). However, it is still important to keep the limitations of eDNA sampling in mind, Roussel, Paillisson, Tréguier, and Petit (2015) brings up the risks of false positives, i.e. finding DNA from a species that is not actually present, and might only have been brought in with ballast water. As well as stating non-detection does not equal absence of a certain species, and the importance of investigating the strengths and weaknesses of genetic methods. Wolff (2005) showed that eDNA using digital PCR methods gave a good indication of the presence of the invasive species crown-of-thorns sea star and deemed it a promising method for early detection of this invasive species, which is the same method I will be using in my experiments.

Genetic detection methods for NIS

There are many different approaches to genetic methods for species detection, and it is important to carefully consider what the desired outcome is before committing to a certain method. Metabarcoding, or community-wide characterization methods, use universal primers to detect entire groups of organisms (Thomsen et al., 2012; Wood et al., 2019), while more targeted methods such as quantitative PCR, qPCR, or Digital PCR, dPCR use species-specific primers.

Metabarcoding is becoming a widely used method of monitoring community composition, as well as a way of detecting rare or new NIS (Wood et al., 2019; Bean et al., 2017; Borja et al., 2016; Bourlat et al., 2013). Many researchers consider it a valuable complement or even a replacement to traditional morphological methods (Cahill et al., 2018; Couton, Comtet, Le Cam, Corre, & Viard, 2019; Kelly et al., 2017; Pearman et al., 2016). Metabarcoding allows for multiple organisms to be detected from a single sample by targeting species wide genes like 18S or COI. However, distinctions between methods for detecting NIS and more traditional monitoring need to be made. When looking for NIS a wide range of taxon is to be expected, but perhaps certain groups or species are of greater interests. Biodiversity monitoring, however, is often intended to pick up as many species as possible, and primers need to be made with these constraints in mind.

There are, however, limitations to metabarcoding methods as well. For example, databases used as references tend to be incomplete (Hestetun et al., 2020; Meiklejohn, Damaso, & Robertson, 2019; Weigand et al., 2019), and "universal" primers are not always completely universal, leading to mismatches and species being missed. This, paired with the variety of bioinformatic pipelines (von Ammon et al., 2018), can lead to wrongful assignment of taxa. Though, some argue that this is seldom a big issue when using metabarcoding as a way of detecting and monitoring NIS, considering the fact that problematic NIS are often well represented in said databases (Briski et al., 2011). The most common marker genes when targeting NIS are either the 18S rRNA or the mitochondrial cytochrome c oxidase subunit I, COI, using both together is not as common (Duarte, Vieira, Lavrador, & Costa, 2021). The two target genes have complementary strengths and weaknesses, 18S cover many groups of invertebrates, algae among others. However, has been shown to have low resolution e.g. for some molluscs and tunicates (Huhn et al., 2020; Couton et al., 2019). COI, on the other hand is optimal for species-level identification within the animal kingdom, and because of the high abundance of mitochondrial DNA within a cell, it is well suited for detecting species of low abundance (Bucklin, Steinke, & Blanco-Bercial, 2011; Galtier, Nabholz, Glémin, & Hurst, 2009). Though, it can have low detection rates for some groups like copepoda or nematoda (Duarte et al., 2021). Duarte et al. (2021) shows twelve studies that have used both genes in a complementary way, which is what is being done in my study as well.

Targeted methods have a higher sensitivity in finding specific species with low DNA abundance compared to both metabarcoding and traditional taxonomy (Wood et al., 2019). Furthermore, quantitative PCR methods, such as dPCR, are even more sensitive to low abundance of target species DNA than conventional PCR. DNA metabarcoding yielded almost double the amount of NIS compared to traditional taxonomy when investigating diapausing eggs (Briski et al., 2011). However, DNA barcoding requires species specific primers to be developed so that no other organisms are being picked up.

Hypotheses

1. Unknown NIS can be detected at an early stage of introduction using genetic methods
2. The number of detected NIS relate to anthropogenic introduction sources
3. Known NIS can be monitored using genetic methods

   I There is a relation between the eDNA signal and time of release from the source

   II There is a relation between the eDNA signal and the biomass of the source

   III There is a relation between the eDNA signal and the distance to the source

Methods

Early detection of NIS

To test hypothesis 1, a combination of ARMS and plankton sampling coupled with metabarcoding was done.

A total of 19 Autonomous Reef Monitoring Structures, ARMS, were placed in five areas along the Swedish west coast, see Figure 2. The locations were chosen based on the likeliness of NIS being introduced. Such as ports with frequent international traffic, where the risk of primary introduction of NIS is high, and marinas, where the risk secondary spread is higher (Molnar, Gamboa, Revenga, & Spalding, 2008; Roche et al., 2015). Three ARMS are deployed year round in the marine protected area, Koster, and serve as a "control" to compare the settled indigenous species as well as the number of NIS. It also gives an insight in suitable deployment time frames. If they are not deployed long enough, several species may not have had the opportunity to settle, but if they are out for too long, one, or a few groups, may take over completely. The remaining structures were deployed for three to four months during spring (31/1/2020 - 1/4/2020) to allow as many species as possible to settle while also considering the time frame. The ARMS were either placed on the bottom attached to a 35 kg concrete paver at a depths between 5-25 m, or hung from jetties at 1-2 m, more detailed data in table 3 in the appendix.

Figure 1: An ARMS, including the concrete paver.

To decrease the risk of DNA degradation and an excessive loss of species, all preparations and fixations were made in the field. The ARMS were dismantled and both sides of all 9 layers were photographed as methodically as possible to allow for a morphological assessment of the settled species using PhotoQuad (Trygonis & Sini, 2012) at a later time. Any larger animals that were hiding in the structures or fell off into the barrel upon retrieval were photographed separately. Furthermore, smaller organisms of interest were either more thoroughly examined at site with a stereo microscope if available, or added into a falcon tube with

95% ethanol for later identification, morphological and/or genetic. All the organisms were scraped off of the plates using a putty knife and mixed together with a blender. The mixture was sieved through mesh strainers into three different fractions; 40 µl, 100-500 µl, 500-µl - 2 mm. The three fractions were then fixated in i falcon tubes in DSMO (Obst et al., 2020) and stored in freezer until sent away for DNA extraction and sequencing.

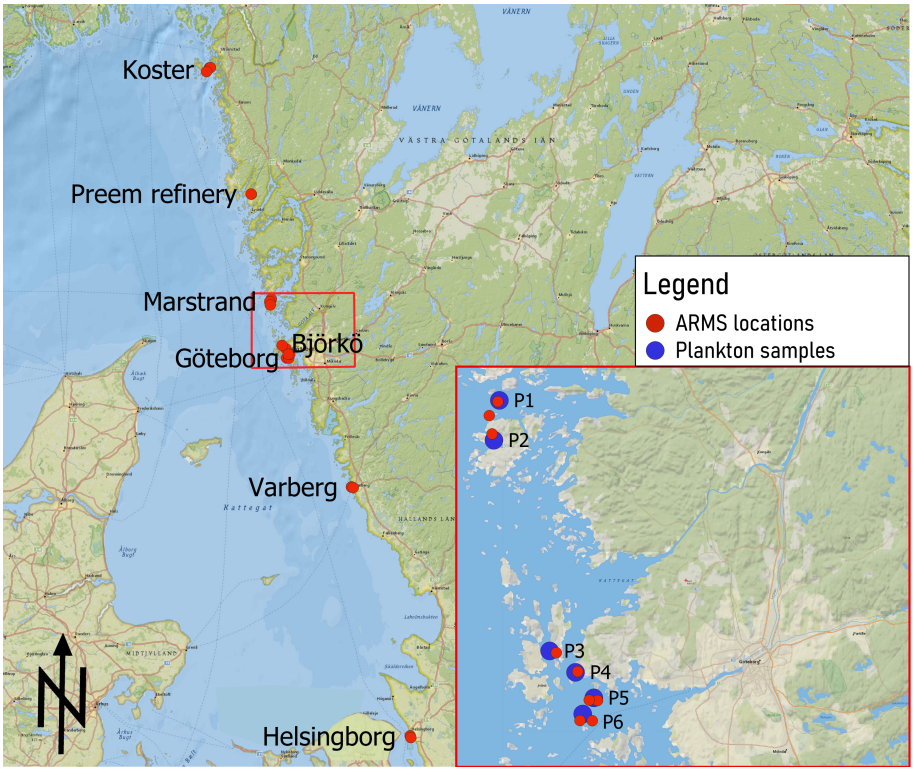


Figure 2: Map showing sample sites, Red points are indivual ARMS deployment sites and blue points are plankton sample sites.

Analysis: The photos were analysed in the software PhotoQuad, with 300 randomized points per picture. Each point represents a location on the plate of which a species was determined, this is to get a quantification of how much space every species take up on each plate. For detailed DNA analysis of the sieved organisms see: http://www.arms-mbon.eu/ under "Documentation". To get most amount of relevant data both the COI and 18S was used as target genes for analysis, and the assays used was developed by Knudsen and Møller (2020); Andersen et al. (2018), for Danish invasive species.

Plankton samples

To further test the first hypothesis, plankton samples were taken at three localities close to ARMS deployment sites, at Marstrand, Björkö, and Gothenburg, see Figure 2. The samples were taken using a Hydro-Bios Apstein 90 µl net during May - July 2020. Three samples were taken from every location at three different times. The samples were taken at a depth of 10 m, and were fixated in sterile single-use containers with 60-70% ethanol. The samples were then filtered through a 80 µl sieve and fixated again in 98% ethanol and stored in a freezer until extraction.

Monitoring known NIS

To test hypothesis 3.1 and 3.2, the known Invasive species Round goby, *Neogobius melanostomus*, was used to investigate the lifespan of eDNA in the free water mass *in vitro* and how it relates to the biomass of fish.

Collection of fish: The 12 fish from the first experiment (F3/n) were retrieved from another researcher, Leon Green, who had used crab cages at the harbour of Arendal using shrimp as bait and left them over night and retrieved them the next morning. The fish for the second experiment were collected close to Klippans färjeläge, Gothenburg, using the same method as well as hook fishing. For the third experiment, with five round gobies in each mesocosm. Only ten Round gobies were caught at Klippan with the crab cages, so the additional 10 needed retrieved from Leon Green at Kristineberg. However, these fish were kept at a salinity (20/30 psu), so an acclimation period of an additional 24h was needed to safely keep them in 15 psu.

Preperations: Four mesocosms with 30 litres of artificial saltwater and a salinity of 15‰ were placed in an evenly tempered room at 15°C with no direct sunlight and left for 24h after mixing to avoid any unwanted reactions from the salt. The fish were weighed and placed in the oxygenated mesocosms for 24 hours to allow a sufficient amount of DNA to be released. After 24h the first sample (T0) was taken with the fish still in the mesocosms, they were then euthanised using a lethal dose of MS-222 followed by decapitation, some fish tissue was saved in 95% ethanol as a positive control. This was repeated three times, with three, one and five fish in each mesocosm. Sampling was done at intervals of six(x5), 12(x1) and 24(x3) hours, see table 1. However, these three sampling occasions were combined and concentration / gram was used for analysis.

Table 1 Time intervals of which water samples were taken. T0 is with the Round Gobies still in the water.

	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9
time(h)	0	6	12	18	24	30	42	66	90	114

Sampling procedure: One litre of water was extracted from the mesocosms using a plastic bucket, it was then filtered through 0.22 µm Sterivex filters using 60 ml syringes. Once the full litre had been filtered through, 99.5% Ethanol was added to the filter with 25 ml syringes to fixate the DNA. Caps were attached to the filters and put in labelled falcon tubes and stored in a freezer. To decrease the risk of contamination between samples, the buckets used for water sampling were thoroughly cleaned with bleach after each use, and the syringes were discarded.

Analysis: All mathematical calculations were made using R (Team, 2021). The equation used to calculate decay rate was:

$$C(t) = C_0 e^{-\alpha t} \tag{1}$$

where C(t) is the concentration after t hours, C_0 is the concentration at t=0, and α is the decay factor. To fit the model to exponential decay model the 'nls' method of the "drc" (Gerhard, Baty, Streibig, & D., 2015) package in R was used.

Detection range of known NIS

To test the third hypothesis, four samples of one litre each were taken with a 5l Ruttner type water sampler at five harbours around Gothenburg where Round goby had been detected a few months prior using crab cage fishing as well as being reported at ArtDatabanken. The samples were kept in single use plastic buckets until filtration which occurred within 3 hours. As much of the water as possible was filtered through 0.45 µl Stervex filters up until the full litre or the filter got clogged, it was then fixated with 95% ethanol and stored in a freezer until extraction. A negative field control was taken from

Toredammen to ensure that the sampling equipment was uncontaminated (Toredammen is a small fresh water pond where Round goby should not be present).

Additional water samples were taken at five locations outside of Gothenburg, where the presence of Round goby is unknown, using the CTD on board R/V Skagerak. Two samples were taken at each location at different depths, one as close to the bottom as possible, where Round goby usually resides, without stirring up sediment, and one in the middle of the water column. These sampling sites were chosen based on the distance from locations of which the presence of Round goby is known. The first sample, 1 in Figure 7, were taken farthest out from known sources, and as far away from any typical habitats, such as rocky shores. Samples were then taken progressively closer to potential Round goby habitats, so sample 5 is taken as close to known sources as possible, where the presence Round gobies had been reported. The water samples were filtered through 0.22 µl Sterivex filterers directly on the ship, they were then fixated using 95% ethanol.

Extraction: The DNA was extracted using the DNeasy power water kit from Qiagen with the original protocol (https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powerwater-kit/).

Analysis: The samples were analysed using the QiAcuity One digital PCR system, with a cycling profile of 1x: 95°C for 2 min, 40x: 95°C for 30s, 68°C for 1 minute. A 12S mitochondrial assay was developed for the Round gobies.

Results

Early detection of NIS

Taking genetic samples from both ARMS and plankton proved to be an effective method for detecting previously unreported NIS at an early stage of introduction, as well as monitoring known NIS.

The number of NIS detected from DNA samples from the ARMS differed between sites, however, a total of 25 NIS was found, see Figure 3. Preem refinery had the highest number of NIS out of all localities with ten unique species, followed by Marstrand which had eight, most of these were, however, previously known species. Two ARMS picked up two rarely detected species, the Clinging jellyfish *Gonionemus vertens* at Koster in 2019, and the bryozoan *Amathia imbricata* at Preem 2020. Four completely new species were also detected, see Figure 4, which have not been reported before in Swedish waters; *Hydroides elegans*, *Hymeniacidon sinapium*, *Lyrodus pedicellatus*, and *Pileolaria militaris*.

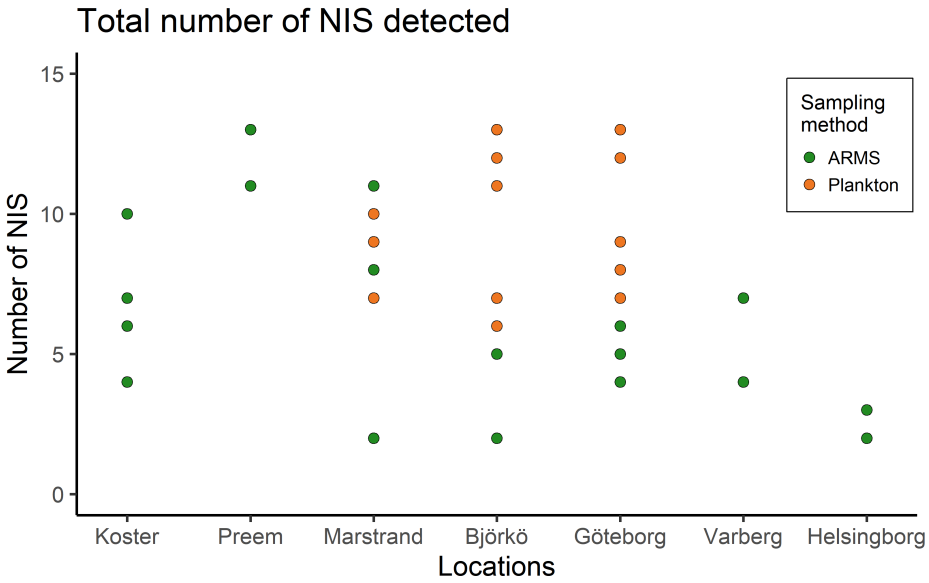


Figure 3: Total number of NIS detected, each point represents one sampling occation, i.e. one ARMS or one Plankton sample, colour indicate sample method.

Metabarcoding from the plankton samples picked up a total of 20 NIS, one rarely reported species, the same Bryozoan that was detected in the ARMS, *Amathia imbricata*, though this time it was detected in Gothenburg. The plankton samples overall had high numbers of unique NIS at all sampling locations, Marstrand, Björkö and Gothenburg, at 10, 13, and 13, respectively. They also picked up, 3, 6, and 5 previously undetected NIS respectively, see Table 2.

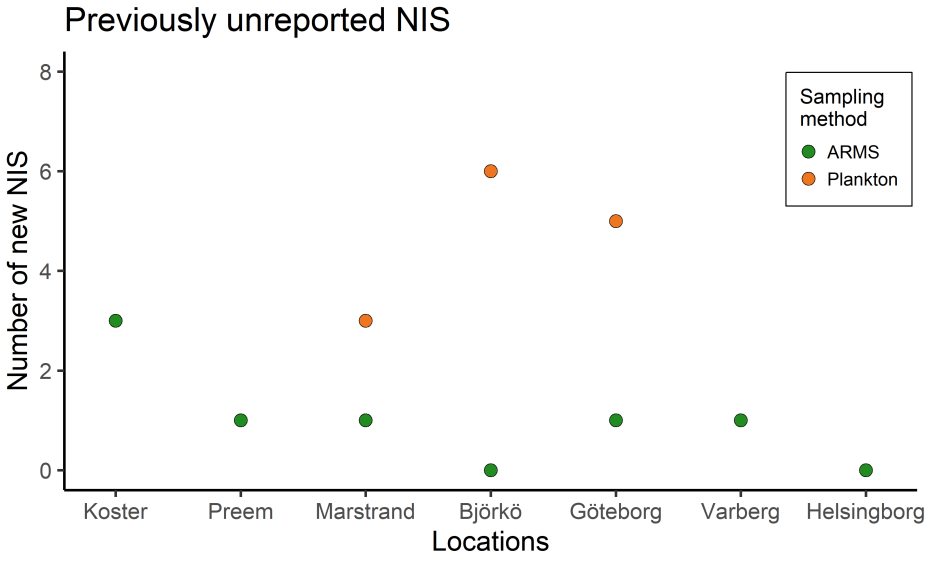


Figure 4: Number of newly detected NIS from all localities, points represents the number of previously unreported NIS detected at that location, colours indicate sampling method (ARMS or Plankton).

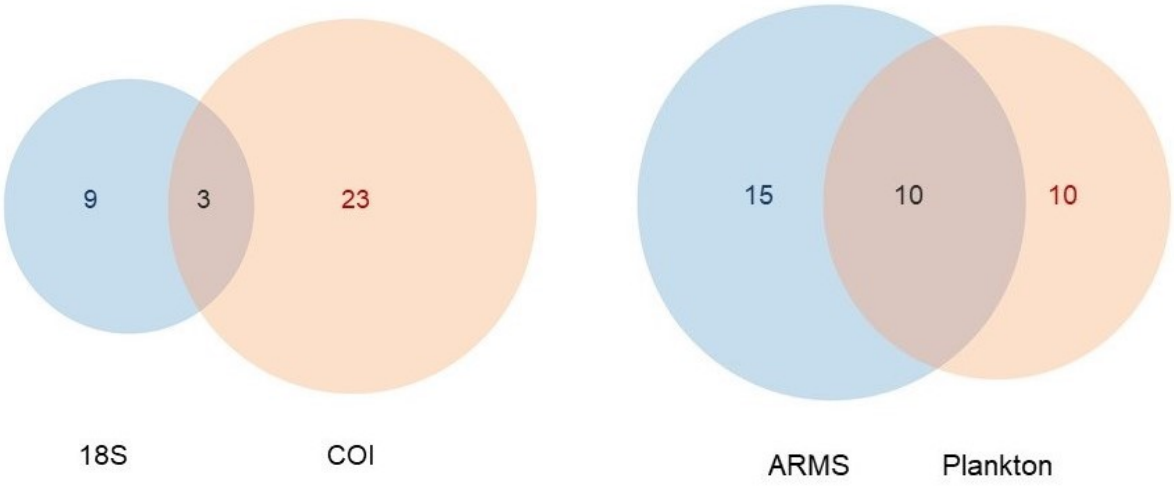
Comparing the sample methods, the ARMS picked up 25 unique NIS and the plankton samples 20, and had an overlap of 10 species, see Figure 5b. No NIS could be positively identified using morphological assessments from image analysis, and only few specimens could be determined on species level.

No clear correlation could be seen between the different locations and their introduction vectors. Koster, which is a marine protected area but with strong oceanographic connectivity, e.g. currents coming in from the Atlantic, had surprisingly high numbers of NIS, at 10 previously known ones and three unreported, compared to the Low amounts of NIS in Helsingborg, which is a port with large amounts of international traffic. However, Gothenburg, Björkö, Marstrand had many new NIS from far away indicating a connection between the large port of Gothenburg as a primary introduction and secondary spread via currents and leisure boats from guest harbours along the coast.

Table 2 Shows which introduction vectors are the most prevalent in the different locations, the distribution of NIS that are previously unreported, and sampling method (ARMS or Plankton(PL)). Species marked with * is rarely reported.

		Koster	Preem	Marstrand		Björkö		Gothenburg		Varberg	Helsingborg
		ARMS	ARMS	ARMS	PL	ARMS	PL	ARMS	PL	ARMS	ARMS
Introduction Vectors											
Industrial ships (e.g. Tankers)			X					X			X
Leisure boats		X		X		X		X		X	X
Oceanographic connectivity		X	X	X							
New NIS (scientific name)	**Phyla**										
Dasya baillouviana	Rhodophyta					X					
Ercolania viridis	Mollusca					X			X		
Haminoea solitaria	Mollusca					X					
Hydroides elegans	Annelida	X									
Hymeniacidon sinapium	Mollusca	X	X	X	X		X	X	X	X	
Lyrodus pedicellatus	Mollusca				X		X		X	X	
Pileolaria militaris	Annelida	X									
Pseudochattonella verruculosa	Ochrophyta				X		X		X		
Pseudodiaptomus marinus	Arthropoda						X		X		
Amathia imbricata*	Bryozoa		X						X		
Gonionemus vertens*	Cnidaria	X									

A total 35 NIS was detected targeting both COI and 18S, 26 were found using COI, and 12 for 18S, See Figure 5a, there was an overlap of three species when using the reference libraries, ArtDatabanken (AdB), OSPAR/HELCOM (OSP-HEL), Hav och Vattenmyndigheten (HAV), and Aqua-NIS.



(a) Target genes

(b) Sampling methods

Figure 5: The venn diagrams shows the overlap of detected NIS between the (a) two target genes 18S and COI, and (b) sampling methods of ARMS and plankton.

Monitoring known NIS

eDNA from Round goby can be positively detected using genetic methods for up to 80 hours. It has a decay rate, α, of 0.055 and a half-life of 12.4 hours, see Figure 6. The lifespan of eDNA from Round goby followed, as expected, an exponential decay model, with a standard error of the regression, σ, of 1.25, indicating this model to be a good fit.

However, a spike of high DNA concentration came at the end of the second trial (F1) which has been considered outliers and excluded from any calculations, see Table 3 in appendix. No correlation in DNA concentrations could be seen between the studies with 3, 1, and 5 Round gobies, leading to the trials being combined and concentration / gram of fish was used instead.

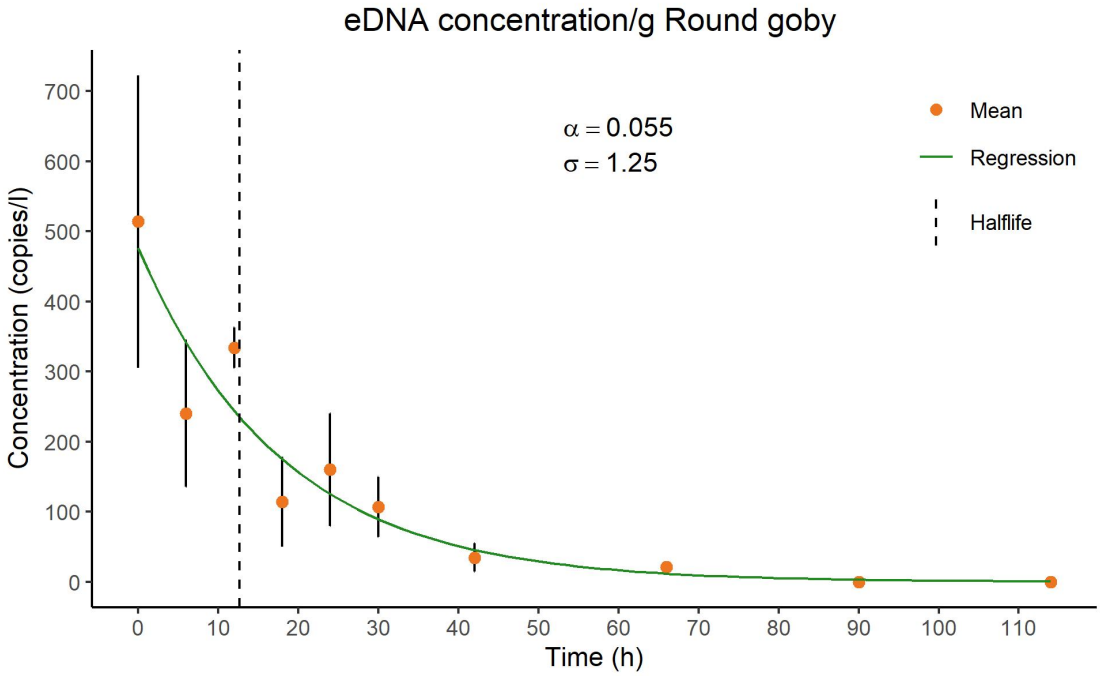


Figure 6: Exponential decay of eDNA from Round Goby, *Neogobius melanostomus*, in mesocosms. Orange points are mean concentration, error bars signify Standard Error, green line is the exponential decay model, and halflife (dashed vertical line) was met after 12.4 hours.

Detection of NIS using genetic methods

DNA from the invasive species, Round goby, *Neogobius melanostomus*, can be detected using genetic methods from water samples if taken at a site which they currently occupy.

Round goby could be positively detected at Arendal, Klippan, and Långedrag. However, Klippan had only one positive sample out of four, and the concentration is not much higher than Toredammen (4.31 at Klippan vs. 3.59 at Toredammen), which was the negative field control, indicating possible contamination. Additionally, the samples taken further out with R/V Skagerak showed no positive hits for Round goby, indicating risks for false positives to be low, i.e. DNA does not seem to travel far from the source and positive hits seem indicate presence of the target species. Figure 7 shows the sample sites, if Round goby was detected using eDNA, and where they have been detected using traditional methods in the past taken from Artdatabanken.

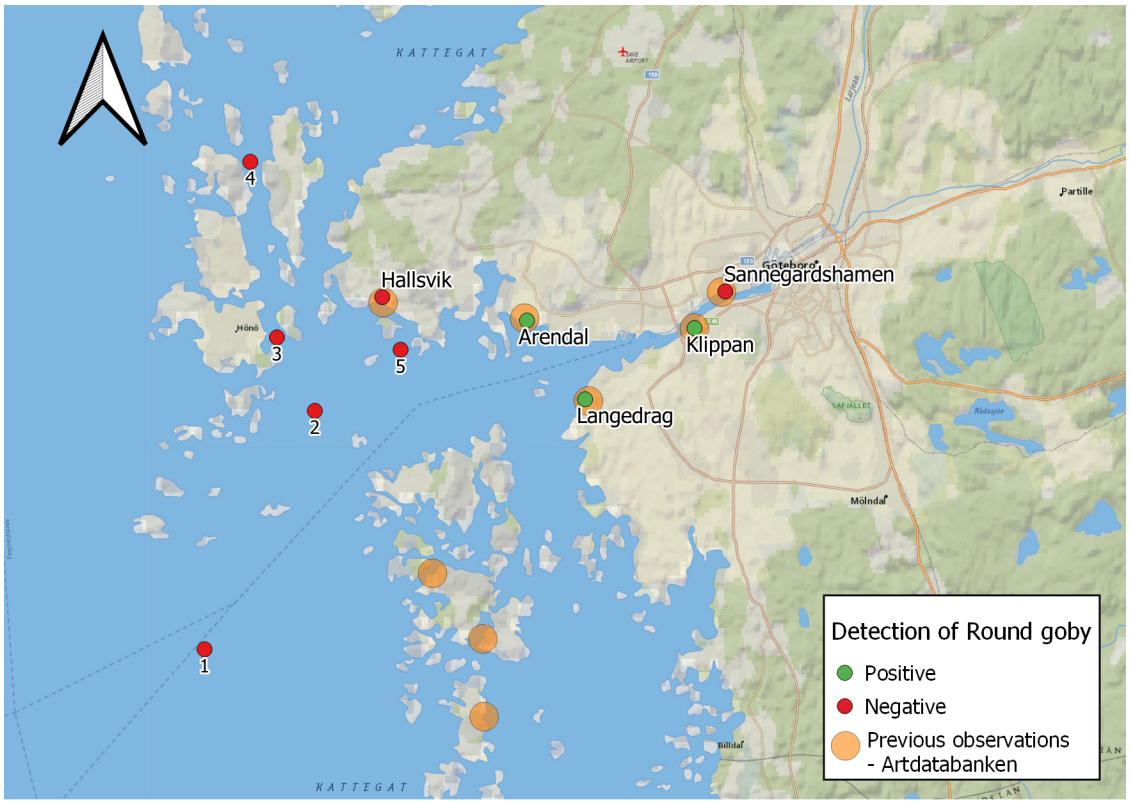


Figure 7: Sample sites of Round Goby detection, numbers indicate samples taken on R/V Skagerak, location names are samples taken from land, and orange circles are previously reported Round gobies from Artdatabanken.

Discussion

Early detection of new NIS with genomic sampling

Genetic analysis from the ARMS yielded the highest number of NIS, and with great accuracy. The ARMS deployed at Preem refinery in Lysekil which is part of the largest refinery in Sweden (together with Gothenburg), had the highest number of Non-indigenous species, 10 unique NIS between the two deployed structures. Which is not very surprising considering the amount of international traffic that goes through there. Ballast water discharges is recognised to be one of the main vectors for translocating NIS (Cabrini et al., 2019), so this together with the high amounts of anthropogenic structures in these areas, such as docks, make for an optimal place for NIS to settle.

Furthermore, Marstrand had the second highest numbers at eight settled NIS between the three deployed structures. This could be explained by the popularity this area has among tourists. According to their website, (https://www.marstrandsgasthamn.se/en/), the guest harbour with surrounding areas are visited by thousands of tourists and sailors each year. So even if these tourist boats are not the primary cause of bringing in new species, the secondary spread from places like Preem in Gothenburg where we, unfortunately, were not able to deploy any ARMS at this time, or any other major international port, could be an explanation. Another reason could be the currents that move through this area could easily facilitate transport of organisms in their pelagic states across large areas, where

they can finally settle on the ARMS, or other hard-bottom substrate. A more surprising result is the amount of NIS detected at the marine protected area, Koster, as well as the detection of the rare Clinging jellyfish. Even though there is a limited amount of industrial ships at Koster, there are quite a bit of leisure boat traffic, since it is such a popular tourist area, which could cause secondary spread from other international docks. However, the Koster sea has a fairly high salinity (30 PSU) compared to other parts of the Swedish west coast, a relatively mild climate, and has great connectivity to the Atlantic through currents. Koster has, overall, a unique and varying topography, with everything from rocky hard-bottom substrates, sand bottoms to the 200m deep Koster fjord, which gives optimal living conditions for many NIS. Another important aspect to consider about the Koster samples is that they are deployed year round, and were retrieved for analysis much later than the other samples due to diving restrictions as a result of Covid-19. Most ARMS were retrieved mid-may to early June, while Preem and Koster were retrieved mid-July. This extra month of deployment during the summer could have significant effects on the number of settled species as well, and is something that would be interesting to investigate further in future studies.

Helsingborg had the lowest number of NIS out of all locations, with only three unique species between the two ARMS, *Acartia clausi*, *Amphibalanus eburneus*, and *Mya arenaria*. All of which are commonly occurring in Swedish waters. This is somewhat surprising considering the port of Helsingborg being the second largest container terminal and has a lot of marine industrial traffic. However, some potential explanations for this could be the relatively low salinity (<10 PSU) in the southern parts of Sweden, which makes for a more hostile environment for many marine organisms.

Interestingly, some completely new species were found that is not present on any NIS list used, (ArtDatabanken (AdB), OSPAR/HELCOM (OSP-HEL), Hav och Vattenmyndigheten (HAV), Aqua-NIS). Most of the new species were picked up in the plankton samples around Gothenburg, which could be an indication that these species frequently enter Swedish waters, but cannot survive here, and therefore never settles. Gothenburg also have a lot of international traffic in the form of tankers and other industrial ships, ferry traffic, and leisure boats which all have contributing factors to bringing in NIS with both primary, and secondary spread. Thus, it is of great value to continue to monitor these influxes of organisms, and in the future do further comparative studies between the species detected in plankton samples, and how many settles onto ARMS. Since once they settle on the ARMS there is reason to believe they have the ability to survive and reproduce here, and are on the way of becoming invasive. Which is why the fact that some rare species, like the Clinging jellyfish, and some completely new ones were detected on the ARMS is of great interest. The Clinging jellyfish in particular is of high significance, and have been a hot topic in Sweden for the last few years since it is considered to be highly toxic, and can have severe effects on humans. In the summer of 2018, a sting from the clinging jellyfish was reported in Sweden, which is the first one in the North Atlantic since the 1970s. It is believed that one vector of spread is via ship hulls (Govindarajan, Källström, Selander, Östman, & Dahlgren, 2019), since polyps of this species are usually found attached to hard-bottom substrates (Marchessaux et al., 2017). Which is why ARMS is an ideal monitoring method for early detection of NIS like this one.

Another interesting aspect to investigate further is the presumed distance the NIS have travelled, this can give an indication of which introduction vector they most likely came with. For example, *Dasya baillouviana*, *Lyrodus pedicellatus*, *Pseudochattonella verruculosa*, *Pseudodiaptomus marinus*, and *Gonionemus vertens* have all been observed in the North sea and could therefore have been swept in by oceanographic connectivity, i.e. currents and natural movements. Especially planktonic species like the copepod, *Pseudodiaptomus marinus*, but also possibly by leisure boats from neighbouring countries like the Algae, *Dasya baillouviana*, which have been observed in both Denmark, and Norway. Furthermore, Other species have a native range which is much further away, and is likely to have been transported by tankers or other industrial ships. The two species *Ercolania viridis*, and *Haminoea solitaria* originate from italy and the USA, respectively, and were both only detected around Gothenburg

(including Björkö), which is an indication that these have strong linkage to the major ports of Gothenburg. However, explaining the route of entry for *Hymeniacidon sinapium*, and *Pileolaria militaris* are a little more challenging, since they both come from afar, western North America, and Northwest Africa, respectively. Coupled with the locations they were detected in here, where *Hymeniacidon sinapium* was detected using both ARMS and Plankton sampling, and was found in all locations except Helsingborg. While *Pileolaria militaris* was only detected in the MPA, Koster. Which is why further research with this aspect in mind is highly relevant. *Amathia imbricata* is another interesting species to investigate further since it has not been reported in Sweden in over 80 years, and was detected in both Preem using ARMS, and Gothenburg using Plankton sampling. Occurrence data obtained from: https://www.gbif.org/ (GBIF.org, 2020).

An additional aspect to take into account is the substrate. Studies on Ascidians have shown that they seem to have preferences on what to attach themselves to (Chase et al., 2016). This was also observed on our ARMS which were attached to concrete pavers as weights, see Figur 1. The concrete paver had often many more barnacles attached than the ARMS itself. This could be due to a multitude of things. The individual plates of the ARMS had a very smooth surface, which could make it difficult for some organisms to settle. Especially if they have another substrate to choose from that is more course. A way around this could be to thoroughly sand the plates prior to deployment. Another potential reason it might be a chemical response from the PVC plates that some organisms have an aversion to (Chase et al., 2016).

Taking pictures and analysing them in PhotoQuad was a waste of time here on the Swedish west coast when looking for NIS, only a few organisms had time to settle during spring time, and those who did were very difficult to identify to a species level. Lighting conditions and different sizes of organisms made photographing with good results unattainable. However, the three plates in Koster, which are continuously deployed all year, had a lot of settled species, and there were noticeable differences between the years of 2019 and 2020. Where 2019 had higher number of species, and were overgrown with mostly sea squirts. So as a method of biodiversity assessments and monitoring, image analysis can be a very useful tool for long term deployments, however, not in finding NIS.

Considering sample effort, running genetic analysis on ARMS and plankton samples is a highly effective way of finding NIS. And to combine them seem to be a powerful way to both detect new, and monitor known NIS, since they both but seem to pick up varying taxon and at different life stage. Which gives a broader picture of which NIS to focus efforts on. Both ARMS and Plankton are relatively cost effective if you compare to other methods of hard bottom monitoring (Pearman et al., 2016), and is less invasive and damaging to surrounding environments.

Monitoring known NIS with water samples

The decay of eDNA from Round goby in mesocosms followed, as expected, an exponential decay model, see equation 1 and had a relatively short lifespan of 80h. Which gives valuable information on what positive hits in the field means. Knowing the lifespan of the DNA of a certain species, allows for more precise interpretation of data. It is now known that if water samples are tested positive for Round goby, that they have been in that area within the last 80h.

However, the first trial, F3, rendered normal looking data which followed the decay model. F1, and F5, however, both had plenty of outliers (See, table 3 in appendix), that were discarded from any calculations. F5, which started off with five fish in each mesocosm, had the lowest concentrations already at T1, which is considered undetectable. F1, which started off with one fish, had normal looking data up until T8 and later, where a huge spike occurred with as high, or higher concentrations than at T0. The latter was considered an outlier since it is impossible for more DNA to have been created after the fish were removed. Some possible explanations for this could be that as larger pieces of tissue or faecal matter decayed, more DNA was released. Another could be that when taking water

with the bucket, sediment from the bottom that contained high concentrations of DNA whirled up and got sampled. The latter is quite likely since the start volume of water in the mesocosms was only 30l, one litre was taken at each sample time, with the mesocosms being quite wide, the water became very shallow, making it difficult to not accidentally get sediment into the sample. In the future, I suggest filtering out all sediment or faecal matter that was left by the fish prior to sampling.

In other studies the DNA was undetectable in mesocosm experiment after two weeks using the same extraction method. However, they used other species, and took samples over several weeks, both with and without a target species. And in that study they could find species with genetic methods that was not found with conversional ones. They also showed that, for amphibians, there was a positive correlation between population density and DNA concentration (Thomsen et al., 2012).

Water samples taken far away from known sources did not pick up any Round goby DNA, indicating positive hits portray an accurate picture of where target organism resides. However, we also got very low concentrations, which were considered negative, of target DNA in areas where the presence of Round goby is known. Both from reports form ArtDatabanken, but also having detected them a few weeks earlier using traditional methods (hook, and crab cage fishing). Furthermore, it is important to take volume of water that was filtered through into account. Obviously the more water that is pushed through the filter the higher the probability of finding DNA from the target species gets. And at both Sannegårdshamnen and Klippan, very little water was able to be filtered through since the filters got clogged up very easily. However, it is concerning that Toredammen, the negative field control, gave, although low, a positive hit. This could be a case of DNA having been transported there by, e.g. a bird. Or, more likely, some sort of field contamination. Which is why I decided to mark Klippan and Sannegårdshamnen as negative. Even though small amounts of DNA were detected, since the concentrations were lower than at Toredammen, so contamination cannot be ruled out.

Overall, using eDNA seems to be a time, and cost effective way to monitor known NIS, like the Round goby. The DNA itself does not seem to travel far from the source, coupled with the short lifespan that the DNA has in the water indicates low risks of false positives. I.e. if a target species is detected it is likely to reside in the area of sampling, and to have been present within a few days.

Future Research

I believe that as of today, we have only scratched the surface of possibilities that is genetic monitoring methods.

More studies need to be made with the aim of standardising monitoring methods on a national level, as well as internationally. Especially in countries like Sweden, Norway, Denmark, and Finland which are in close proximity of one another and share huge water bodies. The ARMS protocol is a great way of monitoring NIS as well as overall community composition, but more research needs to be made in temperate climates which have completely different species compositions than tropical ones. More tests regarding optimal time frames and deployment seasons of ARMS are needed in order to obtain the NIS as they settle rather than before or after. Another interesting aspect to study more thoroughly is the best way to deploy them and at what depths, i.e. hung from jetties or attached to weights and left at the bottom. In addition, explore whether it would be effective to leave them for longer periods of time, like at Koster, but check on them more frequently using divers or ROVs.

Plankton sampling is not a new method of monitoring, but using genetic methods as an addition or substitute might be. I would like to see studies conducted over a longer periods of time, as well as coupled with other methods of monitoring like ARMS to see which species tend to have higher risks of actually settling and surviving in Swedish waters.

The behaviour of eDNA and the optimal ways of handling it, is important aspects to consider if we want to be able to use genetic monitoring on a larger scale and draw reasonable conclusions from

them. Lifespan and distance investigations are important as well as how the release and spread of DNA works between taxon. For example, can crayfish or other arthropods be monitored as effectively as Round gobies or other fish? How does DNA travel in different water bodies, like moving streams or open oceans? And how we tackle the issues with filtering water from murky samples? Taking water samples from closed systems with known species is a good way to start, especially if those systems contain a variety of taxa.

References

Abad, D., Albaina, A., Aguirre, M., Laza-Martínez, A., Uriarte, I., Iriarte, A., ... Estonba, A. (2016). Is metabarcoding suitable for estuarine plankton monitoring? a comparative study with microscopy. *Marine Biology*, *163*(7), 149. Retrieved from https://doi.org/10.1007/s00227-016-2920-0 doi: 10.1007/s00227-016-2920-0

Andersen, J. H., Kallenbach, E., Thaulow, J., Hesselsøe, M., Bekkevold, D., Hansen, B. K., ... Knudsen, S. W. (2018). Development of species-specific edna-based test systems for monitoring of non-indigenous species in danish marine waters. *NIVA-rapport*.

Bean, T. P., Greenwood, N., Beckett, R., Biermann, L., Bignell, J. P., Brant, J. L., ... Righton, D. (2017). A review of the tools used for marine monitoring in the uk: Combining historic and contemporary methods with modeling and socioeconomics to fulfill legislative needs and scientific ambitions. *Frontiers in Marine Science*, *4*(263). Retrieved from https://www.frontiersin.org/article/10.3389/fmars.2017.00263 doi: 10.3389/fmars.2017.00263

Beisiegel, K., Darr, A., Gogina, M., & Zettler, M. L. (2017). Benefits and shortcomings of non-destructive benthic imagery for monitoring hard-bottom habitats. *Marine Pollution Bulletin*, *121*(1-2), 5-15. Retrieved from https://dx.doi.org/10.1016/j.marpolbul.2017.04.009 doi: 10.1016/j.marpolbul.2017.04.009

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., ... de Bruyn, M. (2014). Environmental dna for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution*, *29*(6), 358-367. Retrieved from https://www.sciencedirect.com/science/article/pii/S016953471400086X doi: https://doi.org/10.1016/j.tree.2014.04.003

Borja, A., Elliott, M., Andersen, J. H., Berg, T., Carstensen, J., Halpern, B. S., ... Rodriguez-Ezpeleta, N. (2016). Overview of integrative assessment of marine systems: The ecosystem approach in practice. *Frontiers in Marine Science*, *3*(20). Retrieved from https://www.frontiersin.org/article/10.3389/fmars.2016.00020 doi: 10.3389/fmars.2016.00020

Borja, A., Elliott, M., Andersen, J. H., Cardoso, A. C., Carstensen, J., Ferreira, J. G., ... Zampoukas, N. (2013). Good environmental status of marine ecosystems: What is it and how do we know when we have attained it? *Marine Pollution Bulletin*, *76*(1-2). doi: 10.1016/j.marpolbul.2013.08.042

Boudouresque, C. F., & Verlaque, M. (2002). Biological pollution in the mediterranean sea: invasive versus introduced macrophytes. *Marine Pollution Bulletin*, *44*(1), 32-38. Retrieved from https://www.sciencedirect.com/science/article/pii/S0025326X01001503 doi: https://doi.org/10.1016/S0025-326X(01)00150-3

Bourlat, S. J., Borja, A., Gilbert, J., Taylor, M. I., Davies, N., Weisberg, S. B., ... Obst, M. (2013). Genomics in marine monitoring: New opportunities for assessing marine health status. *Marine Pollution Bulletin*, *74*(1), 19-31. Retrieved from https://www.sciencedirect.com/science/article/pii/S0025326X13002890 doi: https://doi.org/10.1016/j.marpolbul.2013.05.042

Briski, E., Cristescu, M. E., Bailey, S. A., & MacIsaac, H. J. (2011). Use of dna barcoding to detect invertebrate invasive species from diapausing eggs. *Biological Invasions*, *13*(6), 1325-1340. Retrieved from https://doi.org/10.1007/s10530-010-9892-7 doi: 10.1007/s10530-010-9892-7

Bucklin, A., Steinke, D., & Blanco-Bercial, L. (2011). Dna barcoding of marine metazoa. *Annual review of marine science*, *3*, 471-508.

Cabrini, M., Cerino, F., de Olazabal, A., Di Poi, E., Fabbro, C., Fornasaro, D., ... David, M. (2019). Potential transfer of aquatic organisms via ballast water with a particular focus on harmful and non-indigenous species: A survey from adriatic ports. *Marine Pollution Bulletin*, *147*, 16-35. Retrieved from https://www.sciencedirect.com/science/article/pii/S0025326X18300857 doi: https://doi.org/10.1016/j.marpolbul.2018.02.004

Cahill, A. E., Pearman, J. K., Borja, A., Carugati, L., Carvalho, S., Danovaro, R., ... Olenin, S. (2018). A comparative analysis of metabarcoding and morphology-based identification of benthic communities across different regional seas. *Ecology and evolution*, *8*(17), 8908-8920.

Carvalho, S., Aylagas, E., Villalobos, R., Kattan, Y., Berumen, M., & Pearman, J. K. (2019). Beyond the visual: using metabarcoding to characterize the hidden reef cryptobiome. *Proceedings of the Royal Society B*, *286*(1896), 20182697.

CBD, C. o. b. d. (2000). Invasive alien species - global strategy on invasive alien species.

Chase, A. L., Dijkstra, J. A., & Harris, L. G. (2016). The influence of substrate material on ascidian larval settlement. *Marine Pollution Bulletin*, *106*(1), 35-42. Retrieved from `https://www.sciencedirect.com/science/article/pii/S0025326X16301795` doi: https://doi.org/10.1016/j.marpolbul.2016.03.049

Chen, G., & Hare, M. P. (2008). Cryptic ecological diversification of a planktonic estuarine copepod, acartia tonsa. *Molecular ecology*, *17*(6), 1451-1468.

Clout, M., & De Poorter, M. (2000). Iucn guidelines for the prevention of biodiversity loss caused by alien invasive species. *Aliens*, *11*.

Colautti, R. I., & MacIsaac, H. J. (2004). A neutral terminology to define 'invasive' species. *Diversity and Distributions*, *10*(2), 135-141. Retrieved from `https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1366-9516.2004.00061.x` doi: https://doi.org/10.1111/j.1366-9516.2004.00061.x

Collins, R. A., Wangensteen, O. S., O'Gorman, E. J., Mariani, S., Sims, D. W., & Genner, M. J. (2018). Persistence of environmental dna in marine systems. *Communications Biology*, *1*(1), 185. Retrieved from `https://doi.org/10.1038/s42003-018-0192-6` doi: 10.1038/s42003-018-0192-6

Couton, M., Comtet, T., Le Cam, S., Corre, E., & Viard, F. (2019). Metabarcoding on planktonic larval stages: an efficient approach for detecting and investigating life cycle dynamics of benthic aliens. *Management of Biological Invasions*, *10*(4), 657-689.

Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W. K., ... Bik, H. M. (2016). The ecologist's field guide to sequence-based identification of biodiversity. *Methods in Ecology and Evolution*, *7*(9), 1008-1018. Retrieved from `https://doi.org/10.1111/2041-210X.12574` doi: https://doi.org/10.1111/2041-210X.12574

Crisp, D. (1976). *Settlement responses in marine organisms* [Book]. Butterworths, London.

David, R., Uyarra, M. C., Carvalho, S., Anlauf, H., Borja, A., Cahill, A. E., ... Chenuil, A. (2019). Lessons from photo analyses of autonomous reef monitoring structures as tools to detect (bio-)geographical, spatial, and environmental effects. *Marine Pollution Bulletin*, *141*, 420-429. Retrieved from `https://www.sciencedirect.com/science/article/pii/S0025326X19301663` doi: https://doi.org/10.1016/j.marpolbul.2019.02.066

Diagne, C., Leroy, B., Vaissière, A.-C., Gozlan, R. E., Roiz, D., Jarić, I., ... Courchamp, F. (2021). High and rising economic costs of biological invasions worldwide. *Nature*, *592*(7855), 571-576. Retrieved from `https://doi.org/10.1038/s41586-021-03405-6` doi: 10.1038/s41586-021-03405-6

Duarte, S., Vieira, P. E., Lavrador, A. S., & Costa, F. O. (2021). Status and prospects of marine nis detection and monitoring through (e)dna metabarcoding. *Science of The Total Environment*, *751*, 141729. Retrieved from `https://www.sciencedirect.com/science/article/pii/S004896972035258X` doi: https://doi.org/10.1016/j.scitotenv.2020.141729

EEA, E. E. A. (2015). Trends in new records of non-indigenous species introduced by human activities.

Floerl, O., & Inglis, G. J. (2005). Starting the invasion pathway: the interaction between source populations and human transport vectors. *Biological invasions*, *7*(4), 589-606.

Galtier, N., Nabholz, B., Glémin, S., & Hurst, G. (2009). Mitochondrial dna as a marker of molecular diversity: a reappraisal. *Molecular ecology*, *18*(22), 4541-4550.

GBIF.org. (2020). Gbif home page. available from: https://www.gbif.org.

[Generic]. (2012).

Gerhard, C. R., Baty, F., Streibig, J. C., & D. (2015). Dose-response analysis using r. *PLOS ONE*, *10*(12). Retrieved from `http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0146021`

Gobin, J. F., & Warwick, R. M. (2006). Geographical variation in species diversity: A comparison of marine polychaetes and nematodes. *Journal of Experimental Marine Biology and Ecology*, *330*(1), 234-244. Retrieved from `https://www.sciencedirect.com/science/article/pii/S002209810500612X` doi: https://doi.org/10.1016/j.jembe.2005.12.030

Gollasch, S. (2006). National checklist for aquatic alien species in germany. *Aquatic Invasions*, *1*(4), 245-269. doi: 10.3391/ai.2006.1.4.8

Gomes-Pereira, J. N., Auger, V., Beisiegel, K., Benjamin, R., Bergmann, M., Bowden, D., ... Santos, R. S. (2016). Current and future trends in marine image annotation software. *Progress in Oceanography*, *149*, 106-120. Retrieved from `https://www.sciencedirect.com/science/article/pii/S0079661116301240` doi: https://doi.org/10.1016/j.pocean.2016.07.005

Goodwin, B. J., McAllister, A. J., & Fahrig, L. (1999). Predicting invasiveness of plant species based on biological information. *Conservation Biology*, *13*(2), 422-426. Retrieved from `https://conbio.onlinelibrary.wiley.com/doi/abs/10.1046/j.1523-1739.1999.013002422.x` doi: https://doi.org/10.1046/j.1523-1739.1999.013002422.x

Govindarajan, A. F., Källström, B., Selander, E., Östman, C., & Dahlgren, T. G. (2019). The highly toxic and cryptogenic clinging jellyfish gonionemus sp.(hydrozoa, limnomedusae) on the swedish west coast. *PeerJ*, *7*, e6883.

Haase, P., Pauls, S. U., Schindehütte, K., & Sundermann, A. (2010). First audit of macroinvertebrate samples from an eu water framework directive monitoring program: human error greatly lowers precision of assessment results. *Journal of the North American Benthological Society*, *29*(4), 1279-1291. doi: 10.1899/09-183.1

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., Agrosa, C., ... Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, *319*(5865), 948. Retrieved from `http://science.sciencemag.org/content/319/5865/948.abstract` doi: 10.1126/science.1149345

HELCOM. (2013). Helcom aliens 2- non-native species port survey protocols, target species selection and risk assessment tools for the baltic sea. , 34 pp.

Hering, D., Feld, C., Moog, O., & Ofenböck, T. (2006). Cook book for the development of a multimetric index for biological condition of aquatic ecosystems: Experiences from the european aqem and star projects and related initiatives. *Hydrobiologia*, *566*(1), 311-324. doi: 10.1007/s10750-006-0087-2

Hestetun, J. T., Bye-Ingebrigtsen, E., Nilsson, R. H., Glover, A. G., Johansen, P.-O., & Dahlgren, T. G. (2020). Significant taxon sampling gaps in dna databases limit the operational use of marine macrofauna metabarcoding. *Marine Biodiversity*, *50*(5), 70. Retrieved from `https://doi.org/10.1007/s12526-020-01093-5` doi: 10.1007/s12526-020-01093-5

Hopkins, G. W., & Freckleton, R. P. (2002). Declines in the numbers of amateur and professional taxonomists: implications for conservation. *Animal Conservation*, *5*(3), 245-249. Retrieved from `https://doi.org/10.1017/S1367943002002299` doi: https://doi.org/10.1017/S1367943002002299

Huhn, M., Madduppa, H. H., Khair, M., Sabrian, A., Irawati, Y., Anggraini, N. P., ... Setiamarga, D. H. (2020). Keeping up with introduced marine species at a remote biodiversity hotspot: awareness, training and collaboration across different sectors is key. *Biological Invasions*, *22*(2), 749-771.

Hurley, K. K., Timmers, M. A., Godwin, L. S., Copus, J. M., Skillings, D. J., & Toonen, R. J. (2016).

An assessment of shallow and mesophotic reef brachyuran crab assemblages on the south shore of o 'ahu, hawai 'i. *Coral Reefs*, *35*(1), 103-112.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "sight-unseen" detection of rare aquatic species using environmental dna. *Conservation Letters*, *4*(2), 150-157. Retrieved from https://doi.org/10.1111/j.1755-263X.2010.00158.x doi: https://doi.org/10.1111/j.1755-263X.2010.00158.x

Kelly, R. P., Closek, C. J., O'Donnell, J. L., Kralj, J. E., Shelton, A. O., & Samhouri, J. F. (2017). Genetic and manual survey methods yield different and complementary views of an ecosystem. *Frontiers in Marine Science*, *3*, 283.

Knudsen, S. W., & Møller, P. R. (2020). Development of species-specific edna-based test systems for monitoring of non-indigenous decapoda in danish marine waters. *NIVA-rapport*.

Lambert, C. C., & Lambert, G. (2003). Persistence and differential distribution of nonindigenous ascidians in harbors of the southern california bight. *Marine Ecology Progress Series*, *259*, 145-161.

Leese, F., Kahlert, M., Drakare, S., & Zimmermann, J. (2016). Dnaqua-net: developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in europe. *Research Ideas And Outcomes*, *2*. doi: 10.3897/rio.2.e11321

Leray, M., & Knowlton, N. (2015). Dna barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences*, *112*(7), 2076. Retrieved from http://www.pnas.org/content/112/7/2076.abstract doi: 10.1073/pnas.1424997112

Lindeque, P. K., Parry, H. E., Harmer, R. A., Somerfield, P. J., & Atkinson, A. (2013). Next generation sequencing reveals the hidden diversity of zooplankton assemblages. *PloS one*, *8*(11), e81327.

Lodge, D. M., Williams, S., MacIsaac, H. J., Hayes, K. R., Leung, B., Reichard, S., ... McMichael, A. (2006). Biological invasions: Recommendations for u.s. policy and management. *Ecological Applications*, *16*(6), 2035-2054. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-33845473138&doi=10.1890%2f1051-0761%282006%29016%5b2035%3aBIRFUP%5d2.0.CO%3b2&partnerID=40&md5=ebdabfd3c3394dfb41fac45079b8008c doi: 10.1890/1051-0761(2006)016[2035:BIRFUP]2.0.CO;2

Mahon, A. R., Nathan, L. R., & Jerde, C. L. (2014). Meta-genomic surveillance of invasive species in the bait trade. *Conservation Genetics Resources*, *6*(3), 563-567. Retrieved from https://doi.org/10.1007/s12686-014-0213-9 doi: 10.1007/s12686-014-0213-9

Marchessaux, G., Gadreaud, J., Martin-Garin, B., Thiéry, A., Ourgaud, M., Belloni, B., & Thibault, D. (2017). First report of the invasive jellyfish gonionemus vertens a. agassiz, 1862 in the berre lagoon, southeast france. *BioInvasions Records*, *6*(4), 339-344.

Meiklejohn, K. A., Damaso, N., & Robertson, J. M. (2019). Assessment of bold and genbank – their accuracy and reliability for the identification of biological materials. *PLOS ONE*, *14*(6), e0217084. Retrieved from https://doi.org/10.1371/journal.pone.0217084 doi: 10.1371/journal.pone.0217084

Menge, B. A., Sanford, E., Daley, B. A., Freidenburg, T. L., Hudson, G., & Lubchenco, J. (2002). Interhemispheric comparison of bottom-up effects on community structure: Insights revealed using the comparative-experimental approach. *Ecological Research*, *17*(1), 1-16. Retrieved from https://doi.org/10.1046/j.1440-1703.2002.00458.x doi: 10.1046/j.1440-1703.2002.00458.x

Minchin, D., & Gollasch, S. (2003). Fouling and ships' hulls: How changing circumstances and spawning events may result in the spread of exotic species. *Biofouling*, *19*(sup1), 111-122. Retrieved from https://doi.org/10.1080/0892701021000057891 doi: 10.1080/0892701021000057891

Molnar, J. L., Gamboa, R. L., Revenga, C., & Spalding, M. D. (2008). Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, *6*(9), 485-492.

Naturvårdsverket. (2008). Nationell strategi och handlingsplan för främmande arter och genotyper.

Norderhaug, K. M., Gundersen, H., Pedersen, A., Moy, F., Green, N., Walday, M. G., ... Hjermann, D. y. (2015). Effects of climate and eutrophication on the diversity of hard bottom communities on the skagerrak coast 1990-2010. *Marine Ecology Progress Series*, *530*, 29-46.

Obst, M., Exter, K., Allcock, L., A. Louise, Arvanitidis, C., Axberg, A., Bustamante, M., ... Pavloudi, C. (2020). A marine biodiversity observation network for genetic monitoring of hard-bottom communities (arms-mbon). *Frontiers in Marine Science*, *7*. Retrieved from `https://hal.archives-ouvertes.fr/hal-03033264` doi: 10.3389/fmars.2020.572680

Olsgard, F., Schaanning, M. T., Widdicombe, S., Kendall, M. A., & Austen, M. C. (2008). Effects of bottom trawling on ecosystem functioning. *Journal of Experimental Marine Biology and Ecology*, *366*(1), 123-133. Retrieved from `http://www.sciencedirect.com/science/article/pii/S0022098108003377` doi: https://doi.org/10.1016/j.jembe.2008.07.036

Pearman, J. K., Anlauf, H., Irigoien, X., & Carvalho, S. (2016). Please mind the gap – visual census and cryptic biodiversity assessment at central red sea coral reefs. *Marine Environmental Research*, *118*, 20-30. Retrieved from `http://www.sciencedirect.com/science/article/pii/S0141113616300617` doi: https://doi.org/10.1016/j.marenvres.2016.04.011

Quijón, P. A., Kelly, M. C., & Snelgrove, P. V. (2008). The role of sinking phytodetritus in structuring shallow-water benthic communities. *Journal of Experimental Marine Biology and Ecology*, *366*(1-2), 134-145.

Ransome, E., Geller, J. B., Timmers, M., Leray, M., Mahardini, A., Sembiring, A., ... Meyer, C. P. (2017). The importance of standardization for biodiversity comparisons: A case study using autonomous reef monitoring structures (arms) and metabarcoding to measure cryptic diversity on mo'orea coral reefs, french polynesia. *PloS one*, *12*(4), e0175066.

Roche, R., Monnington, J., Newstead, R., Sambrook, K., Griffith, K., Holt, R., & Jenkins, S. (2015). Recreational vessels as a vector for marine non-natives: developing biosecurity measures and managing risk through an in-water encapsulation system. *Hydrobiologia*, *750*(1), 187-199.

Roussel, J., Paillisson, J., Tréguier, A., & Petit, E. (2015). The downside of edna as a survey tool in water bodies. *Journal of Applied Ecology*, *52*(4), 823-826. doi: 10.1111/1365-2664.12428

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., Korsun, S., & Spezzaferri, S. (2012). The fobimo (foraminiferal bio-monitoring) initiative—towards a standardised protocol for soft-bottom benthic foraminiferal monitoring studies. *Marine Micropaleontology*, *94-95*, 1-13. Retrieved from `https://www.sciencedirect.com/science/article/pii/S0377839812000552` doi: https://doi.org/10.1016/j.marmicro.2012.06.001

Seymour, M., Durance, I., Cosby, B. J., Ransom-Jones, E., Deiner, K., Ormerod, S. J., ... Creer, S. (2018). Acidity promotes degradation of multi-species environmental dna in lotic mesocosms. *Communications Biology*, *1*(1), 4. Retrieved from `https://doi.org/10.1038/s42003-017-0005-3` doi: 10.1038/s42003-017-0005-3

Sigsgaard, E. E., Carl, H., Møller, P. R., & Thomsen, P. F. (2015). Monitoring the near-extinct european weather loach in denmark based on environmental dna from water samples. *Biological Conservation*, *183*, 46-52. Retrieved from `https://www.sciencedirect.com/science/article/pii/S0006320714004480` doi: https://doi.org/10.1016/j.biocon.2014.11.023

Simkanin, C., Davidson, I. C., Dower, J. F., Jamieson, G., & Therriault, T. W. (2012). Anthropogenic structures and the infiltration of natural benthos by invasive ascidians. *Marine Ecology*, *33*(4), 499-511.

Stachowicz, J. J., Bruno, J. F., & Duffy, J. E. (2007). Understanding the effects of marine biodiversity on communities and ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 739-766. Retrieved from `https://doi.org/10.1146/annurev.ecolsys.38.091206.095659` doi: 10.1146/annurev.ecolsys.38.091206.095659

Summerson, R., Skirtun, M., Mazur, K., Arthur, T., Curtotti, R., & Smart, R. (2013). Economic evaluation of the costs of biosecurity response options to address an incursion of mytilopsis

sallei (blackstriped mussel) into australia. *ABARES Report to client prepared for Plant Health Australia, Canberra*.

Sundberg, P., Obst, M., Bourlat, S. J., Bergkvist, J., & Magnusson, M. (2018, 2018). *Utvärdering av ny övervakning av främmande arter : Metodjämförelse mellan traditionell och dna-baserad identifiering* (Report No. 978-91-88727-15-2 (ISBN)). Havs- och vattenmyndigheten. Retrieved from http://urn.kb.se/resolve?urn=urn:nbn:se:havochvatten:diva-188

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental dna. *Molecular Ecology*, *21*(8), 1789-1793. Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-294X.2012.05542.x doi: https://doi.org/10.1111/j.1365-294X.2012.05542.x

Taylor, A. H., Allen, J. I., & Clark, P. A. (2002). Extraction of a weak climatic signal by an ecosystem. *Nature*, *416*(6881), 629-632. Retrieved from https://doi.org/10.1038/416629a doi: 10.1038/416629a

Team, R. C. (2021). R: A language and environment for statistical computing. Retrieved from https://www.R-project.org

Thomsen, P. F., Kielgast, J. O. S., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., ... Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental dna. *Molecular Ecology*, *21*(11), 2565-2573. Retrieved from https://doi.org/10.1111/j.1365-294X.2011.05418.x doi: https://doi.org/10.1111/j.1365-294X.2011.05418.x

Trygonis, V., & Sini, M. (2012). photoquad: a dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. *Journal of Experimental Marine Biology and Ecology*, 424-425, 99-108. doi: 10.1016/j.jembe.2012.04.018

von Ammon, U., Wood, S. A., Laroche, O., Zaiko, A., Tait, L., Lavery, S., ... Pochon, X. (2018). Combining morpho-taxonomy and metabarcoding enhances the detection of non-indigenous marine pests in biofouling communities. *Scientific reports*, *8*(1), 1-11.

Wallentinus, I., & Nyberg, C. D. (2007). Introduced marine organisms as habitat modifiers. *Marine Pollution Bulletin*, *55*(7), 323-332. Retrieved from https://www.sciencedirect.com/science/article/pii/S0025326X0600498X doi: https://doi.org/10.1016/j.marpolbul.2006.11.010

Weigand, H., Beermann, A. J., Čiampor, F., Costa, F. O., Csabai, Z., Duarte, S., ... Ekrem, T. (2019). Dna barcode reference libraries for the monitoring of aquatic biota in europe: Gap-analysis and recommendations for future work. *Science of The Total Environment*, *678*, 499-524. Retrieved from https://www.sciencedirect.com/science/article/pii/S0048969719317760 doi: https://doi.org/10.1016/j.scitotenv.2019.04.247

Williams, R. J., Griffiths, F. B., Van der Wal, E. J., & Kelly, J. (1988). Cargo vessel ballast water as a vector for the transport of non-indigenous marine species. *Estuarine, Coastal and Shelf Science*, *26*(4), 409-420. Retrieved from http://www.sciencedirect.com/science/article/pii/0272771488900212 doi: https://doi.org/10.1016/0272-7714(88)90021-2

Williamson, M., & Fitter, A. (1996). The varying success of invaders. *Ecology*, *77*(6), 1661-1666. Retrieved from https://esajournals.onlinelibrary.wiley.com/doi/abs/10.2307/2265769 doi: https://doi.org/10.2307/2265769

Wolff, W. J. (2005). *Non-indigenous marine and estuarine species in the netherlands* [Book]. Nationaal Natuurhistorisch Museum.

Wood, S. A., Pochon, X., Laroche, O., von Ammon, U., Adamson, J., & Zaiko, A. (2019). A comparison of droplet digital polymerase chain reaction (pcr), quantitative pcr and metabarcoding for species-specific detection in environmental dna. *Molecular Ecology Resources*, *19*(6), 1407-1419. Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1111/1755-0998.13055 doi: https://doi.org/10.1111/1755-0998.13055

Zenetos, A., Gofas, S., Verlaque, M., Cinar, M., Garcia raso, J., Bianchi, C., ... Streftaris, N. (2010). Alien species in the mediterranean sea by 2010. a contribution to the application of european union's marine strategy framework directive (msfd). part i. spatial distri-

bution. *2010*, *11*(2). Retrieved from https://ejournals.epublishing.ekt.gr/index.php/hcmr-med-mar-sc/article/view/12060 doi: 10.12681/mms.87

Appendix

Round Goby eDNA concentration table

Table 3 DNA concentrations from Round goby eDNA lifespan trial, bold numbers marked with "*" indicate outliers that are excluded from calculations

time(slot)	time(h)	F3	F1	F5
T0	0	3385,073	326,4	3080,0
T0	0	745,5548	1834,3	873,4
T0	0	165,2457	131,1	**28,2***
T0	0	737,0098	704,1	**0,4***
T1	6	1633,627	289,7	**0,8***
T1	6	197,5767	36,5	**0,5***
T1	6	1395,143	19,5	**2,9***
T1	6	221,8032	36,2	**7,0***
T2	12	1635,419	0,5	0,1
T2	12	2645,152	2,1	68,4
T2	12	1817,598	0,3	0,2
T2	12	1851,056	76,0	18,5
T3	18	215,5267	35,0	**2,2***
T3	18	288,5843	18,8	**0,3***
T3	18	1129,609	0,1	**0,5***
T3	18	90,81535	0,0	**1,2***
T4	24	20,88921	30,7	**2,6***
T4	24	999,9094	4,1	**2,7***
T4	24	663,5754	**0,2***	**4,0***
T4	24	94,20694	89,5	**2,4***
T5	32	245,5142	**0,1***	703,1
T5	32	346,2049	**0,3***	171,2
T5	32	167,0378	43,4	0,1
T5	32	268,6645	5,6	2,5
T6	44	0,818633	8,2	40,6
T6	44	27,78959	75,0	25,9
T6	44	82,64254	13,9	4,3
T6	44	346,0314	-	1,5
T7	56	5,436379	137,1	1,1
T7	56	33,6562	207,5	1,5
T7	56	15,24304	0,0	6,2
T7	56	124,8442	0,1	11,5
T8	80	0,021585	**289,9***	0,1
T8	80	0,130258	2,6	0,1
T8	80	1,939519	**1173,0***	0,2
T8	80	0,02152	**1733,1***	0,2
T9	104	0,261975	**1822,0***	0,1
T9	104	1,315164	**1427,3***	0,1
T9	104	0,344205	**415,2***	0,0
T9	104	0,08626	**667,5***	1,6

NIS found in ARMS and Plankton, table

Table 4 NIS detected using ARMS and Plankton, and which reference library they correlate to. Species marked with green are rarely reported in Sweden, and those marked with red have never previously reported in Sweden.

Scientific name	Phyla	Class	References				Max likeness %	Sample Method
			HAV	OSP-HEL	ADb	Aqua NIS		
Acartia clausi	Arthropoda	Copepoda				x	100	AP
Acartia tonsa	Arthropoda	Copepoda		x		x	100	PL
Acrochaetium moniliforme	Rhodophyta	Florideophyceae				x	100	ARMS
Aglaothamnion halliae	Rhodophyta	Florideophyceae	x		x	x	100	ARMS
Amathia imbricata	Bryozoa	Gymnolaemata				x	99.67	AP
Amphibalanus amphitrite	Arthropoda	Thecostraca				x	100	AP
Amphibalanus eburneus	Arthropoda	Thecostraca				x	100	AP
Amphibalanus improvisus	Arthropoda	Thecostraca	x		x	x	100	AP
Antithamnionella spirographidis	Rhodophyta	Florideophyceae				x	98.71	ARMS
Bonnemaisonia hamifera	Rhodophyta	Florideophyceae	x		x	x	100	AP
Calanus euxinus	Arthropoda	Copepoda				x	100	PL
Caprella mutica	Arthropoda	Malacostraca	x	x	x	x	100	ARMS
Crepidula fornicata	Mollusca	Gastropoda	x	x	x	x	100	ARMS
Dasya baillouviana	Rhodophyta	Florideophyceae	x		x	x	98.00	PL
Dasysiphonia japonica	Rhodophyta	Florideophyceae	x		x	x	100	AP
Ercolania viridis	Mollusca	Gastropoda				x	99.35	PL
Gonionemus vertens	Cnidaria	Hydrozoa				x	100	ARMS
Haminoea solitaria	Mollusca	Gastropoda				x	99.68	PL
Hydroides elegans	Annelida	Polychaeta		x		x	98.00	ARMS
Hymeniacidon sinapium	Mollusca	Gastropoda				x	99.00	AP
Jassa marmorata	Arthropoda	Malacostraca				x	100	ARMS
Lyrodus pedicellatus	Mollusca	Bivalvia				x	99.00	AP
Mnemiopsis leidyi	Ctenophora	Tentaculata	x	x	x	x	99.80	PL
Monocorophium acherusicum	Arthropoda	Malacostraca				x	100	ARMS
Monocorophium sextonae	Arthropoda	Malacostraca				x	100	ARMS
Mya arenaria	Mollusca	Bivalvia				x	100	AP
Mytilus trossulus	Mollusca	Bivalvia				x	100	ARMS
Penilia avirostris	Arthropoda	Branchiopoda	x		x	x	100	PL
Petricolaria pholadiformis	Arthropoda	Copepoda			x	x	99.00	PL
Pileolaria militaris	Annelida	Polychaeta				x	100	ARMS
Proceraea cornuta	Annelida	Polychaeta				x	100	ARMS
Pseudochattonella verruculosa	Ochrophyta	Dictyochophyceae	x	x	x	x	98	PL
Pseudodiaptomus marinus	Arthropoda	Copepoda				x	99.68	PL
Sparus aurata	Chordata	Actinopteri				x	100	ARMS
Sum species			10	6	11	34		

ARMS coordinates table

Table 5 Table over ARMS deployment Including if they were located at the bottom (B), or hung from a jetty (J)

Location	plate nr.	lat	long	Date in	Date out	Local J/B	Depth (m)
Hjuvik	1	57.703221	11.711363	31/01/2020	18/05/2020	J	2
Björkö	1	57.717984	11.679989	31/01/2020	18/05/2020	J	1.5
GBG	1	57.664805	11.714735	06/02/2020	20/05/2020	B	5
GBG	2	57.664608	11.732876	06/02/2020	20/05/2020	B	5
GBG	3	57.680533	11.740578	06/02/2020	20/05/2020	B	5
GBG	4	57.680805	11.728331	06/02/2020	20/05/2020	B	5
Varberg	1	57.1133741	12.2299832	13/02/2020	03/06/2020	J	2
Varberg	2	57.1126278	12.23027320	13/02/2020	03/06/2020	J	2
Varberg	3	57.1107004	12.2439775	13/02/2020	03/06/2020	J	2
Marstrand	1	57.914434	11.594154	28/02/2020	27/05/2020	B	6
Marstrand	2	57.903512	11.581548	28/02/2020	27/05/2020	B	6
Marstrand	3	57.889278	11.585722	28/02/2020	27/05/2020	J	1.5
Helsingborg	1	56.026272	12.695728	05/03/2020	04/06/2020	B	2
Helsingborg	2	56.018061	12.700541	05/03/2020	04/06/2020	B	2
Preemraff	1	58.3533	11.43486	01/04/2020	15/07/2020	B	3
Preemraff	2	58.35405	11.43394	01/04/2020	15/07/2020	B	3
KOSTER	VH1	58.875155	11.103194	27/05/2019	16/07/2020	B	24
KOSTER	VH2	58.876330	11.111884	27/05/2019	16/07/2020	B	22
KOSTER	VH3	58.859877	11.080491	27/05/2019	16/07/2020	B	25

Plankton sampling coordinates

Table 6 Sample dates and location for plankton samples

Location	Date	Lat	Long
Göteborg 1	20/05/2020	57.6699	11.7188
Göteborg 1	26/06/2020	57.6720	11.7401
Göteborg 1	13/07/2020	57.6712	11.6773
Göteborg 2	29/05/2020	57.6826	11.7351
Göteborg 2	26/06/2020	57.6842	11.7372
Göteborg 2	13/07/2020	57.6811	11.7311
Hjuvik	29/05/2020	57.7026	11.7082
Hjuvik	26/06/2020	57.7020	11.7088
Hjuvik	13/07/2020	57.7016	11.7111
Björkö	29/05/2020	57.7194	11.6702
Björkö	26/06/2020	57.7191	11.6702
Björkö	13/07/2020	57.7191	11.6772
Marstrand	27/05/2020	57.8843	11.5884
Marstrand	26/06/2020	57.8825	11.5918
Marstrand	12/07/2020	57.8830	11.5870
Marstrandsfjorden	27/05/2020	57.9154	11.5964
Marstrandsfjorden	26/06/2020	57.9102	11.6021
Marstrandsfjorden	12/07/2020	57.9045	11.5945

eDNA Round goby data

Table 7 Raw data from the Round goby detection using eDNA, including the filter volumes.

Location	Lat	long	Salinity %	Filtered Volume (ml)	Conc (copies/l)
Arendal 1	11.8163	57.6943	20	1000	5,87
Arendal 2				1000	4,53
Arendal 3				1000	0,00
Arendal 4				1000	13,77
Hällsvik 1	11.7351	57.7013	10	1000	0,22
Hällsvik 2				1000	1,08
Hällsvik 3				1000	0,43
Hällsvik 4				1000	0,00
Sannegårdshamen 1	11.9276	57.7030	0.5	175	0,00
Sannegårdshamen 2				225	2,88
Sannegårdshamen 3				300	0,72
Sannegårdshamen 4				225	0,00
Klippan 1	11.9103	57.6920	7	420	0,00
Klippan 2				450	0,00
Klippan 3				400	0,00
Klippan 4				1000	4,31
Långedrag 1	11.8489	57.6707	21	1000	3,23
Långedrag 2				1000	13,78
Långedrag 3				1000	10,55
Långedrag 4				1000	3,23
Toredammen	11.8802	57.6794	0	60	3,59
Skagerrak_1B	11.63536	57.59572	33.5	1000	0
Skagerrak_1M			31.7	1000	0
Skagerrak_2B	11.69726	57.66724	31.9	1000	0
Skagerrak_2M			29	1000	0
Skagerrak_3B	11.67598	57.68924	31.9	1000	0
Skagerrak_3M			29.7	1000	0
Skagerrak_4B	11.66106	57.74177	31.5	1000	0
Skagerrak_4M			29.4	1000	0
Skagerrak_5B	11.74538	57.68554	27	1000	0
Skagerrak_5M			21.3	1000	0