Options in dealing with marine alien species

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Summary

Invasive species can have strong impact on the local ecosystem, not only substantial impact on the local ecosystem, but also on economy and human health. This review on marine alien species outlines aspects of prevention, eradication and control strategies. When managing invasive species, prevention is preferable and less costly than controlling species. Especially for marine environments, invasive species can disperse rapidly and can be particularly hard to detect.

Commercial and recreational shipping, aquaculture and aquarium trade are important vectors for the introduction marine invasive species (AIS). Here, we describe the current prevention methods, related to shipping, aquaculture and trade. Followed by an overview of monitoring methods and on-site surveys that are required to check the effectiveness of prevention measures. Specifically, the possibilities, advantages and disadvantages of molecular monitoring are evaluated.

When prevention methods are not successful, complete removal (eradication) of AIS from the newly colonized area is the preferred option. For marine AIS, successful eradication programs are described. However, complete eradication of an establishment invader is rare.

When prevention fails and eradication is not possible, the final option is to control the situation which means reducing the change of further spreading of the unwanted species. Depended on the species similar methods as described to prevent an invader to enter can be used. Other options are to exploit the economic value of the species or to setup specific catch programmes with the public.

We conclude the study with suggestions and recommendations on how a marine AIS management program could function.

Samenvatting

Invasieve soorten kunnen een grote impact hebben op een lokaal ecosysteem, naast verstoringen van het ecosysteem kan er ook economische schade zijn of schadelijk zijn voor de gezondheid van de mens. Dit rapport geeft een overzicht over de status van mariene exoten met betrekking tot aspecten van preventie, uitroeiing en strategieën op beheersen van soorten. Bij het beheer van invasieve soorten blijkt dat preventie van exoten het meest effectief en kosten efficiënt is ten opzichte van het uitvoeren van beheersmaatregelen. Specifiek voor het mariene milieu is dat invasieve soorten zich snel kunnen verspreiden en tegelijkertijd ook nog moeilijk te detecteren zijn.

Uit verschillende andere studies blijkt dat commerciële en recreatieve scheepvaart, aquacultuur en aquariumhandel belangrijke routes zijn waarlangs mariene invasieve soorten (AIS) in Nederland worden ingevoerd. Deze studie geeft een overzicht over de huidige preventiemethoden voor de belangrijkste routes scheepvaart, aquacultuur en handel. Hiervoor worden zowel internationale en nationale methoden beschreven. Vervolgens wordt ingegaan op de mogelijkheden rondom monitoring en vroegtijdige detective van mariene soorten. Extra aandacht is gegeven aan de mogelijkheden, voordelen en nadelen van moleculaire monitoring.

Wanneer preventiemethodieken niet succesvol en een mariene exoot toch gevonden wordt is een volledige verwijdering (uitroeiing) van de AIS in het recent gekoloniseerde gebied de geprefereerde optie. De succesvolle uitroeiingsprogramma’s waar mariene exoten werden bestreden zijn beschreven. Het werd duidelijk dat volledige uitroeiing van een indringer zeer zeldzaam is.

Op het moment dat preventie faalt en uitroeiing niet meer mogelijk is, blijft beheersen van de soort als enige optie over. Hiermee wordt getracht verdere verspreiding van de ongewenste soort tegen te gaan. Afhankelijk van de soort kunnen hiervoor dezelfde methoden worden gebruikt als gebruikt om de soort preventief te weren. Andere mogelijkheden zijn de economische waarde benutten van een soort of specifieke programma’s opzetten waarin het publiek betrokken wordt.

Tot slot geven we aanbevelingen over hoe een marien AIS beheersprogramma zou kunnen functioneren.

1 Introduction

Various human activities have facilitated the distribution of species around the world for many decades, allowing that regions have been colonised by species that would have never been able to reach these places by natural ways. With increasing global transportation the change of introduction of these so called 'alien' species to regions, either intentional or as 'stowaway', also increases. It is believed that as long as the key components are not affected by the new inhabitants an ecosystem can cope with this situation. This changes when the newly introduced species affect the native species by competition, predation, by transferring pathogens and parasites, or through hybridisation, enabling the alien species to become harmfull/nuissance (EEA, 2012).

Invasive alien species (IAS) can have strong impact on the local ecosystem, not only with substantial impact on ecology (Salvaterra et al., 2013); (Bax et al., 2003), but also on economy (EEA, 2012; Lodge et al., 2006; Streftaris and Zenetos, 2006).

Since 1991, for instance, South America has been struggling with the Asian golden mussel *Limnoperna fortunei*, which has changed the biodiversity and crippled fishing in parts of the continent (e.g. Crosier et al.); (Darrigran et al., 2011; Darrigran et al., 2012; Molina et al., 2012; Ricciardi, 1998). In the Blach Sea and Caspian Sea, fishermen contend with similar problems due to an invasion by an Asian jellyfish species *Mnemiopsis leidyi* that eats both the food of fish and their spawn (e.g. WWF International, 2009); (Antajan et al., 2014; Roohi et al., 2010). This jellyfish is also present in Dutch coastal waters.

As an example of potential economic impact it was estimated that a bloom of harmful algae that could be released with ballast water in the harbour of Rotterdam can result in a welfare loss of over 300 million euro's due to loss of recreational use and marine ecosystem benefits (Nunes and Markandya, 2008). The World Wide Fund for Nature (WWF) estimated that the global economic losses from the damage caused by harmful invasive aquatic species exceed 7 billion US$ (ca. 5.9 billion EURO) per year (WWF, 2009). However, in the same year Kettunen et al. (2009) estimated these costs to be at least 12 billion EURO per year already for Europe alone.

These estimates indicate that it is hard to predict the actual costs involved. However, it is clear that introduction of invasive alien species can result in tremendous economic and other consequences. For this reason the prevention of introduction and spreading as the management of invasive species is high on the European agenda (EEA, 2012).

Apart from a negative impact, several alien species also have benefits at least for specific user functions such as recreation (sport fishing, hunting), economy (e.g. the manila clam in the Venice Lagoon, (Nunes and Markandya, 2008)), or simply because they are regarded as 'lovely creatures' (e.g. grey squirrels and hedgehogs; Genovesi, 2005). This, in combination with the problem of recognition of potentially invasive species, the detection of their presence, the limited possibilities to remove them without ruining the local environment, and the global scale of the problem makes management of invasive alien species a real challenge for policy makers.

This report focusses on the management of marine alien species, and follows in general the scheme as indicated in Figure 1 (words between brackets refer to boxes in figure 1): Measures to prevent [prevention] the introduction of invasive alien species should be installed and/or maintained, and monitoring [monitoring] of sensitive areas should be performed in order to be able to detect the presence of a 'new' alien species [detection] as soon as possible after its introduction. Ideally this monitoring should be performed in areas that serve as entrance to the EU, like harbours. In this situation it should be focussing on unwanted species, which are not yet established in the EU.

In addition monitoring for the presence of unwanted species that are already locally present in the EU waters could be performed in non-invaded areas that in some way are linked (e.g via shipping, shell fish transport) with invaded areas.

In case an alien species is detected, the risk that this species can become invasive must be assessed [risk assessment]. When this risk is estimated as being substantial it is important to know the distribution of the species in the area. If this is still in a restricted area eradication could be an option [eradication]. If the species has already spread over a larger area the chance for successful eradication becomes low, and the remaining management options are trying to control [control] the impact and further distribution [prevention] and eventually acceptance of the presence of the species. The fact that a new alien species was introduced indicates that the preventive measures already in place [prevention] where not effective in this case. The prevention program should therefore be adapted where possible.

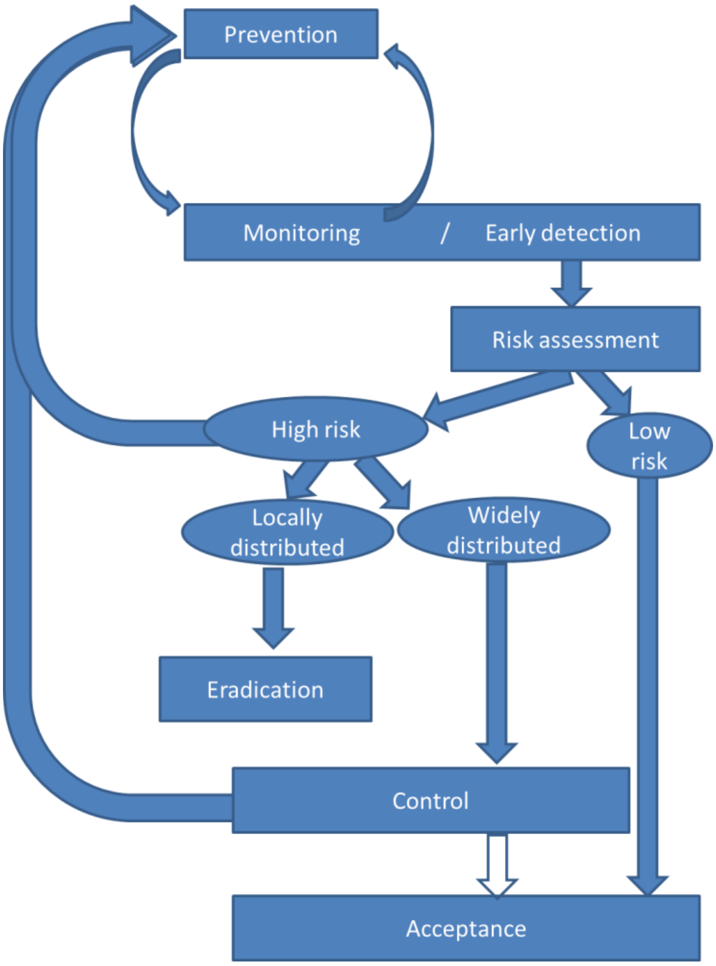

Figure 1 Presentation of a possible scheme for management of (marine) invasive alien species.

In the following chapters an overview is given on experiences with different management options that are published on governmental websites, in (mostly) public available reports and scientific literature. In the final chapter recommendations are made how the insights from these chapters can be included in the scheme presented in Figure 1.

1.1 Terminology

There are multiple terms being used in this work of field, often mixed up and misapplied (Galil et al., 2014). A description of the definitions used in any document is, therefore, crucial. In this report we use the terms alien species and invasive alien species and use the description of these definitions as described in the EU regulation 1143/2014:

"'*alien species' means any live specimen of a species, subspecies or lower taxon of animals, plants, fungi or micro-organisms introduced outside its natural range; it includes any part, gametes, seeds, eggs or propagules of such species, as well as any hybrids, varieties or breeds that might survive and subsequently reproduce;*

(2) 'invasive alien species' means an alien species whose introduction or spread has been found to threaten or adversely impact upon biodiversity and related ecosystem services;

(3) 'invasive alien species of Union concern' means an invasive alien species whose adverse impact has been deemed such as to require concerted action at Union level pursuant to Article 4(3);

(4) 'invasive alien species of Member State concern' means an invasive alien species other than an invasive alien species of Union concern, for which a Member State considers on the basis of scientific evidence that the adverse impact of its release and spread, even where not fully ascertained, is of significance for its territory, or part of it, and requires action at the level of that Member State;

(5) 'biodiversity' means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems;

(6) 'ecosystem services' means the direct and indirect contributions of ecosystems to human wellbeing;

(7) 'introduction' means the movement, as a consequence of human intervention, of a species outside its natural range;

(8) 'research' means descriptive or experimental work, undertaken under regulated conditions to obtain new scientific findings or to develop new products, including the initial phases of identification, characterisation and isolation of genetic features, other than those features which make a species invasive, of invasive alien species only insofar as essential to enable the breeding of those features into non-invasive species;

(9) 'contained holding' means keeping an organism in closed facilities from which escape or spread is not possible;

(10) 'ex-situ conservation' means the conservation of components of biological diversity outside their natural habitat;

(11) 'pathways' means the routes and mechanisms of the introduction and spread of invasive alien species;

(12) 'early detection' means the confirmation of the presence of a specimen or specimens of an invasive alien species in the environment before it has become widely spread;

(13) 'eradication' means the complete and permanent removal of a population of invasive alien species by lethal or non- lethal means;

(14) 'population control' means any lethal or non-lethal action applied to a population of invasive alien species, while also minimising the impact on non-targeted species and their habitats, with the aim of keeping the number of individuals as low as possible, so that, while not being able to eradicate the species, its invasive capacity and adverse impact on biodiversity, the related ecosystem services, on human health or the economy, are minimised;

(15) 'containment' means any action aimed at creating barriers which minimises the risk of a population of an invasive alien species dispersing and spreading beyond the invaded area;

(16) 'widely spread' means an invasive alien species whose population has gone beyond the naturalisation stage, in which a population is self-sustaining, and has spread to colonise a large part of the potential range where it can survive and reproduce;

(17) 'management' means any lethal or non-lethal action aimed at the eradication, population control or containment of a population of an invasive alien species, while also minimising the impact on non-targeted species and their habitats."]

2 Prevention strategies

When managing invasive alien species, prevention is preferable and less costly than controlling species (Mack et al., 2000). Especially for aquatic and marine environments, invasive species can be particularly hard to detect and many aquatic/marine species can disperse rapidly which makes eradication and control extremely difficult (Genovesi, 2005). Prevention begins with understanding how alien species are introduced and how they spread once they are introduced. Most marine invasive alien species known in Europe are introduced via the pathways shipping (ballast water and hull fouling) including floating structures, canals and wild fisheries/culture activities (Galil et al., 2014; Katsanevakis et al., 2014; Ojaveer et al., 2014; Olenin et al., 2010). The spread may occur through a combination of natural dispersal and human-associated transport mechanisms. Especially when planning new activities in the marine environment, like choosing locations for aquaculture, algae cultivation, wind farming or oil and gas exploration, one should also be aware that a network of structures in the marine environment may also create new corridors for alien species to reach previous unconnected areas (Ling et al., 2012; Lopez-Duarte et al., 2012).

In Galil et al. 2014, the cumulative number of alien species by likely vector in the Netherlands is presented and shipping and aquaculture are considered as the main vectors (Galil et al., 2014). Although there is a large network of canals in the Netherlands, the introduction of marine invasive alien species via this pathway is minor. The reason for this is that the canals in the Netherlands do not function as portal from one specific marine environment to another specific marine environment like the Suez Canal. Other potential pathways for with numerous vectors as described by Olenin and co-workers like aquarium and live food trade, leisure activities, research and education including pilot projects, biological control, alterations to natural water flow and habitat management (Olenin et al., 2010), seems less relevant for the Dutch marine situation.

In the following paragraphs prevention methodologies for the best regulated pathways and vectors relating to shipping and aquaculture followed by a list of other prevention methods used worldwide.

2.1 Shipping related prevention

The problem of invasive alien species travelling on ships has been with us for centuries. While originally related primarily to organisms attached to the hulls of vessels, the introduction of steel hulls later created ballast water as a new vector for their dissemination. It is generally recognized that around 90% of our commercial trade goes via shipping. The commercial shipping industry is a growing market with a world fleet in 2014 of around 1.7 billion dry weight tons. Total cargoes have increased from 2,605 million of tons loaded in 1970 till 9,548 million of tons loaded in 2013. Although the number of ships seems to have stabilised since the last decade, the average ship size has almost doubled (UNCTAD, 2014). The intensification of shipping means that this issue is becoming ever-more problematic.

Beside transportation of alien species via commercial shipping, the unwanted stowaways can also travel via recreational boating. When dealing with management for prevention of alien species via shipping the focus should not only be on commercial shipping, but also on recreational boating. In this paragraph an overview is giving of the current prevention methods related to commercial and recreational shipping including biofouling, ballast water and sediments.

2.1.1 *Biofouling*

Biofouling is the accumulation of micro-organisms, plants, algae, or animals to surfaces. Hull fouling is a specific type of biofouling or biological fouling on the surface of any type of vessel, including recesses built into the surface of a vessel like the sea chest. To prevent organisms to attach to a ship's hull, anti-fouling paints are developed. Generally, there are three types of antifouling products which can be defined as products with a soluble matrix, an insoluble matrix and self-polishing (European Chemicals Agency, 2014). Paints with a soluble matrix are mixtures of a resin matrix with active substance(s) that are freely associated, meaning that they leach from the paint into the water. The efficacy of soluble matrix antifouling products is active until the active substance(s) have leached and follows a release rate curve which decays exponentially. Typical lifetime of such coatings is 12-36 months. Antifouling products with an insoluble matrix contain a mixture of resins that together form an insoluble binder phase. Active substance(s) are mixed into this matrix. After application of the paint, seawater penetrates the paint film and the active substance(s) are released insight the paint by dissolution and diffusion. This type of paint also shows an exponential release rate curve and typical lifetime is 12-36 months. Self-polishing paints are currently the most common and cover a range of different technologies like ion-exchange and hydrolysis. These types of paints have a gradual depletion/ablation of the active substance(s) throughout the lifetime of the coating. These types of coatings are often custom made to the requirements from the vessel or the structure. Very active vessels can be applied with a paint that also makes the surface of the ship slippery so that when the ship moves through the water the organisms are washed off. Lifetime of these paints range from 24-60 months, but some of these systems are also specified for longer lifetimes.

Antifouling coatings

Antifouling paints are not only applied on hulls of ships, but also used in aquaculture, in harbours and on other static structures placed in the marine environment. Lifetime of antifouling paints has increased over time, but is still limited depending on the painted surface in combination with activities. For instance, a combination of products is commonly used to paint a commercial ship to cope with different hydrodynamic forces and light conditions (European Chemicals Agency, 2014). Antifouling paints are only effective when applied and maintained correctly. Damage to the paint and wrongly applied paint facilitate the growth of biofouling.

In the past it was very common to use anti-fouling paints that contained metallic compounds like tributyltin (TBT). The metallic compounds in these antifouling paints slowly leach into the water killing organisms that have attached to the ship, the so-called paints with a soluble matrix. These leaching paints, however, remained toxic in the environment inducing environmental pollution issues. In 2001 the International Maritime Organization (IMO) adopted the "International Convention on the Control of Harmful Anti-Fouling Systems in ships (AFS Convention) whereby member states agree to prohibit the use of harmful antifouling paints and other anti-fouling systems that contain harmful substances. The AFS convention was enforced in September 2008. The European Union regulates the organotin compounds on ships since July 2003 (Regulation No. 782/2003). As alternative to TBT coatings copper-based coatings are now being used that are based on the specific toxic effect of copper on molluscs. Silicon-based paints form another alternative that are not making use of a toxic agent. These types of paint make the surface of the ship slippery so that the organisms are washed off when the ship moves through the water. So far, most of these non-toxic compounds are less effective in preventing biofouling and other preventive measures are also needed to limit the potential introduction and spread of marine alien species (Al-Juhni, 2006). European money is invested to encourage the development of non-toxic antifouling systems (Genzer and Efimenko, 2006); (Lejars et al., 2012)).

Additional measures

Only using antifouling paint is currently not sufficient to prevent the introduction of marine alien species and, therefore, several countries and international bodies have developed additional measures for recreational and commercial shipping. These additional measures include vessel inspections, cleaning of hulls (in water and/or in dry docks) and communication programs to raise the awareness of the public. IMO has developed and adopted guidelines for survey and certification of anti-fouling systems on ships - resolution MEPC.102 (48), guidelines for brief sampling of anti-fouling systems on ships - resolution MEPC.104(49) and guidelines for inspection of anti-fouling systems on ships resolution MEPC.105(49). Member states who signed the convention agree to these guidelines and implemented these in national and/or regional regulations.

Public Awareness programs

Several countries have developed websites to increase the public awareness and to motivate people to take action. As one example, in the USA, the government has launched a "Grant Program" in 2015, where projects can be submitted to raise awareness to prevent the introduction of invasive species (AIS Prevention Grant Program: Public Awareness Projects Application Deadline: Monday, February 17, 2015). An example of a website to increase public awareness from Michigan State in the United States is presented in Figure 2. Australia, Canada and New Zealand have built similar websites. The websites can also serve as portal for all alien species issues including links to relevant legislation. Links to some example websites are:

- http://www.marinepests.gov.au;
- http://wildlife.utah.gov/quagga/pdf/boat_inspection.pdf;
- http://www.habitat.noaa.gov/pdf/best_management_practices/CleaningWatercraftandEquipment.pdf

Awareness programs are a useful tool to detect new alien species, lower the distribution of alien species for instance when directly connected to an eradication option and to prevent new alien species to be introduced. Although awareness programs can help preventing introductions, this method should not be used solely, but needs to be part of a broader management plan.

Cleaning Boats and Equipment to Prevent AIS

For more information about signs for boating access sites, please contact Kevin Walters (DEQ Water Resources Division) at 517-284-5473 or waltersk3@michigan.gov

Did you know it's illegal to launch a boat in Michigan that has aquatic plant material attached to the boat or trailer? Follow these simple steps to prevent the introduction and spread of aquatic invasive species in Michigan's waters:

- Clean boats, trailers and equipment before and after launch
- Drain water from bilges and livewells at the ramp before leaving
- Dry trailers, boats, equipment and storage areas thoroughly before using in a different body of water
- Dispose of unused bait in the trash
- Don't transfer live fish to waterbodies other than where they were originally caught
- Disinfect livewells and bilges with a bleach solution (1/2 cup bleach to 5 gallons of water)

Figure 2 Example of a website page to increase public awareness of the risk of spreading unwanted species. Source: http://www.michigan.gov/deq/0,4561,7-135-3313_8314-317692--,00.html

2.1.2 Ballast water and sediment

Cargo vessels move billions of tons of ballast water and sediment around the world every year, allowing organisms such as plankton, mussels, crabs and jellyfish to travel as stowaways. When released at the port of arrival, exotic plants and animals can cause severe damage to both the local ecosystem and the economy, with coastal areas being especially vulnerable. The WWF International stated that every hour, an estimated 7,000 marine and coastal species travel across the world's oceans as stowaways in ships' ballast water tanks. The vast majority of marine species carried in ballast water do not survive the journey and even for those that do survive, the change of surviving in the new ecosystem is further reduced. However, when all factors are favourable, an alien species may become invasive, out-competing native species and multiplying into pest proportions (WWF International, 2009; http://Globallast.imo.org).

Ballast water treatment requirements
The International Maritime Organization (IMO) has taken the global lead in controlling the transfer of aquatic invasive species via ship's ballast water tanks and adopted the ballast water management convention in 2004 aiming to prevent the spread of harmful aquatic organisms from one region to another, by establishing standards and procedures for the management and control of ships' ballast water and sediments (IMO, 2004).

Countries that have signed the convention directly agree on the D-1 regulation which states that ships need to perform ballast water exchange to ensure that at least 95% volumetric exchange of ballast water. One year after ratification of the convention D-2 regulation becomes active. D-2 regulation states the requirement of ballast water discharge quality. The discharged ballast water must satisfy the five main criteria: (i) less than 10 cells per m3 for plankton with more than 50μm in dimension; (ii) less than 10 cells per ml for plankton with 10 to 50μm in dimension; (iii) less than 1 CFU (colony forming units) per 100ml of toxicogenic *V. cholerae*; (iv) less than 250 CFU per 100ml of E. coli; and (v) less than 100 CFU per 100ml of enterococci". Sediments from the ballast tanks are considered waste and need to be discarded as such. Currently, the convention is pending and waits for sufficient members to sign to become active.

Ballast water treatment system
In order to meet the D-2 discharge standard, treatment systems need to be installed on board of all ships to prevent transport of various organisms with ballast water. Mostly such ballast water treatment system includes two-stages firstly a mechanical treatment like filtration followed by a chemical or physical treatment like chlorination or UV light. (Van den Brink et al. 2013) When systems use chemicals, there must be guarantees that the ballast water will be safe for the ecosystem after discharge. Unstable chemicals must therefore be used, or the active ingredients neutralised before discharge. The Netherlands has signed the Ballast water and sediment convention of IMO.

Additional Regional or national BW treatment requirements
Beside the international IMO convention on ballast water countries, regions and local areas have developed ballast water requirements. Lloyd's Register compiled a synopsis of these other national and regional ballast water management requirements (Lloyd's Register, 2014). These include requirements on ballast water exchange, treatment on board with an approved system, delivery to a shore reception facility, non-allowance of discharge ballast water (e.g. Panama canal) and quality on uptake of ballast water (e.g. de-ballast in Novorossiysk, Russia is only allowed with ballast water from the Black Sea).

The Netherlands is one of the members of OSPAR and IMO and have implemented a regional voluntary requirement for ballast water management for the North East Atlantic and the Baltic Sea area. The ballast water management requirements are based on exchange of all ballast tanks at least 200 nautical miles from the nearest land in water at least 200 metres deep prior to entering their waters for vessels transiting the Atlantic, or entering the areas of the OSPAR and HELCOM Conventions from routes passing the West African Coast, except for vessels entering the area from the Mediterranean Sea. If exchange has not been undertaken as above, vessels will be expected to exchange in waters at least 200 nautical miles from the nearest land in water at least 200 metres deep within the North East Atlantic. If this is not possible for operational reasons then such exchange should be undertaken as far from the nearest land as possible, and in all cases in waters at least 50 nautical miles from the nearest land and at least 200 metres deep. Ships must not release sediments during the cleaning of ballast tanks within 200 nautical miles of the coastline of the North East Atlantic or within the Baltic Sea.

Exchanging ballast water at open sea reduces the amount of potential invasive alien species to be transported via this vector, but is still not a perfect method. Species are still being allowed to enter and exit the ballast tanks. Only the location of discharge is moved to a less susceptible area at open sea. However, still at open sea alien species may become invasive. When checking estimates for ratios of alien species versus native species, rough approximations may be 1:40 in majority of the European waters, 1:20 at open coasts and 1:5 in estuaries or lagoons (Olenin et al., 2010). Ballast water treatment would be a better solution as the water is then sterilised not allowing species to be discharged. However, the majority of the current requirements do not demand active ballast water treatment yet.

In the recent years many ballast water management systems have been developed and approved and some have been installed on board of ships. However, these ballast water management systems might not be fully effective under any condition with relation to for instance water salinity, turbidity and organism density. Using ballast water treatment to prevent alien species to be spread around the world is currently the best available method, especially when the efficacy of a system is being tested under a range of challenging conditions, and that approval is given for tested conditions.

2.2 Aquaculture related prevention

Due to a rising demand for fish and shellfish the global aquaculture production is increasing. In the Netherlands aquaculture is carried out in closed systems on land, and on cultivation plots in open sea, like Oosterschelde.

Many of the cultured species are not native to the areas in which they are farmed (Goldberg et al., 2001; The World Conservation Union, 2006). Cultured species can be released or escape, and in addition species can be introduced as "by-product" (associated species) of the cultured species. The preparation of life seafood and bait is also responsible for new introductions of alien species. There have been many escapes introducing farmed species into the wild. For marine species, escapes from aquaculture plants have been reported for pacific white leg shrimp, pacific oyster and Atlantic salmon. A study in the United States revealed that of the 126 alien species related to aquaculture alone, an enormous 106 species has become established, indicating a success rate of 84% for establishment (Grosholz et al., 2012).

Fish aquaculture
Fish aquaculture carried out in cages in the open sea is not applied in the Netherlands and only takes place in closed recirculation aquaculture systems (RAS) on land. RAS are land based fish production systems in which water from the tanks is re-used after purification. Compared to the cages placed in open sea, RAS have the advantage to safe energy and water use, control the water quality and waste production, low nutrient emission to the environment and a high level of biosecurity. According to Dutch Agrofood website the current production of fish is approximately 7,000 MT at circa 15 farms (www.dutchagrofood.com).

In the USA bighead carp and silver carps, imported from Asia for food production, have been escaped/introduced in the Mississippi basins with substantial impact on local biodiversity (U.S. Fish and Wildlife Service, 2005).

Also farmed salmons have frequently escaped. Around 40% of Atlantic salmon and more than 90% caught in the Baltic Sea are of farmed origin (Hindar at al., 2006). There are also concerns about the impact on the local salmon population of escaped transgenic salmon, containing growth hormones genes that mix with the populations that are evolutionary adapted to the local situation (Hallerman et al., 2007). Canada approved the production of these transgenic salmon eggs for commercial purposes in 2013. However, other relevant regulatory bodies still need to provide approval before these eggs can be sold. To prevent the establishment of escaped farmed species, sterile triploids are produced and the species are cultivated in environments that are not suitable for reproduction as an extra safety measurement. In some occasions like in Canada salmon aquaculture at sea is solely allowed to work with infertile females. The males are only allowed to be kept on land. However, there are studies describing escaped triploid farmed fish reproduced in the wild (Papoulias et al., 2011). So these prevention methods are not full proof.

Shellfish aquaculture
Farming of the Pacific Oyster has led to establishment of this species in the wild in many countries, under which the Netherlands. Most often not only the cultured species is introduced, but also other alien species living with or inside this species. Waldichuk and co-authors describe the introduction of six species of bivalves, seven species of snails (gastropods), four polychaete worm species and assorted other invertebrates having been associated to be accidentally introduced with imported oyster spat (Waldichuk et al. 1994).

Annually 38,000 MT of blue mussels, 19 million Pacific oysters and 1 million of European oysters are being produced in the Netherlands (www.dutchagrofood.com). Most of the production is through farming on bottom plots either in combination with seed collectors via suspension methods or via mussel seed collection from natural beds. The majority of the shellfish aquaculture is carried out in the Wadden Sea (mussels) and Zeeland (mainly mussels and oysters) area. Not all shellfish produced in the Netherlands has its origin in the Netherlands. Mussels and oysters can be imported from but not limited to different regions like Ireland, Norway or France (Wijsman & Smaal, 2006). In the province Zeeland, an artificial lake called the Veerse Meer is being used for aquaculture and related research for instance with the alien manilla clam. This lake was separated by the Delta Works dam system and has a transmission structure called the "Katse Heule" that allows salt water from the Oosterschelde estuarine to enter the lake resulting in stable salinity levels. For us it is unclear if this construction prevents transport of clam larvae from the Veerse Meer to the Oosterschelde.

Plant aquaculture
Plant aquaculture is a relative new aquaculture method in the Netherlands. Cultivation of non-native species holds a potential risk for the local environment. For example, seaweed has been introduced in Hawaii in 1973 and has spread across coral reefs ({Neushul, 1992 #524}; http://www.reefresilience.org/case-studies/hawaii-invasive-species).

Wageningen UR has developed a concept of sustainable seaweed-based seafarms (Van den Burg et al., 2013) and tested this concept for three years in the Oosterschelde region resulting in the first private seafarm for cultivation of seaweeds, Hortimare (www.hortimare.com). The research was performed using endemic North Sea seaweed species as the researchers were aware of the risk of introducing non-native species (www.dutchagrofood.com, www.wageningenur.nl). Since 2014, a second seaweed research station has opened its doors in the North Sea area on Texel (http://www.nioz.nl/zeewiercentrum).

This type of aquaculture is still very new for the Netherlands, but can be expected to increase in intensity in the upcoming years than becoming a potentially relevant vector for introductions of alien species. It is however unclear if restrictions are formulated on what species may be used for these activities in the future.

Control and management policies
Australia and New Zealand have established national frameworks to prevent and manage invasive species associated with aquaculture. Australia has developed a "National System for Prevention and Management for Marine Pests". The importation of a new species for the use in aquaculture should be approved by Biosecurity Australia. If approval is given, the operator is required to submit and have an approved Emerging Marine Pest Plan that describes options for action on case of escape or disease outbreak. Additionally, ongoing monitoring would be required with mandatory reporting to State authorities. In New Zealand, legislation for prevention and management of invasive species is recorded in "The New Zealand's Hazardous Substances and New Organisms Act (1996)". Importers of non-native species must apply to an independent regulatory authority. Moreover, all species are considered potentially invasive and therefore prohibited unless proven otherwise.

In many other countries there is not one centralized competent authority that regulates the import oe entry of new non-native organisms. Mostly, there are multiple permit processes and several agencies with overlapping or different competences. Recommendations how to set-up national guidelines are based on the FAO Code of Conduct in responsible fisheries (1995) and the ICES code of Practice on the Introduction and Transfers of Marine organisms (2005). Application is non-binding and voluntary. ICES provides its working Group on Introductions and Transfer of Marine Organisms as an approved body to evaluate the importation of non-native species, however this is unacceptable to non-ICES member nations.[also IMO guidelines on ballast water, Port sampling an survey (Joint OSPAR/HELCOM) en Regulation 1143/2014/EU on invasive species.]

The major production of shellfish via aquaculture in the Netherlands in combination with the introduction of marine alien species has led to the development of national requirements on import and translocations of shellfish (Policy framework on shellfish import and transport, (Bleker, 2012)). A license to import or translocate shellfish is given to the applicant when they meet several requirements. When imported, the origin of the shellfish is within coastal areas from an OSPAR region II (North Sea) or region III (Celtic Sea). Translocation of shellfish from south to north (Oosterschelde to Wadden Sea) within the Netherlands also requires a license. The license can be given if the applicant has demonstrated that adequate measures have been taken to prevent *problem species* are being introduced alive into the Oosterschelde (or Wadden Sea, in case of mussel seed) and a control and management plan is written including an integrated risk assessment, arrangement of critical control points, execution of a retrospective monitoring to selected species being imported, and potential corrective actions.

As a response to these requirements, a shellfish import protocol was developed (Bleker, 2012). The basis of the protocol is producing up to date species lists from the export area. For potentially problematic species a risk assessment should be performed based on best available knowledge, including the changes for introduction, transportation, establishment, dispersal and ecological impact (i.c. on 'Natura 2000 waarden'). The outcome of these analyses defines if a species is regarded as problematic or not. Lack of information about species could thus result in underestimation of the potential risk (Mack et al., 2000). The protocol describes that when these problem species are discovered, the imported batch of shellfish is put under quarantine and the area where the shellfish is imported is cleaned. This means that the imported batch may already be spread in the import area before results from the monitoring is available. This protocol has the intention to minimise the changes that new invasive species are being introduced but cannot fully prevent new invasions to occur. For that adopting a "guilty until proven innocent" approach would be needed as proposed by Mack et al. (2000), however implementation of such an approach might not be possible without large practical and economic consequences.

The United States seem to have chosen for the strategy to separate prevention into two groups: prevention of introductions and prevention of establishment (NISC, 2008). The shellfish import protocol may be classified as a method to prevent establishment of target species and is a first step.

2.3 Other prevention methodologies

The aquarium trade represents a 1 billion USD a year global industry and introduced alien species in the marine environment related to aquarium trade seem to be highly successful in becoming established in some areas. A study in the United States discovered that 9 out of 13 (69%) introduced alien species through aquarium trade became established (Williams et al., 2012). Even though the introduction rate and the success for establishment for this vector is not at high as for biofouling, ballast water or aquaculture, the impact of an introduction can be as high as for the other vectors. This is also the reason that countries included this vector in their legislation and have started awareness programs.

An interesting campaign in the United States is called "Habitattitude" which aims at adopting a conservation mentality of the public to protect the environment by not releasing unwanted fish and aquatic plants (http://www.habitattitude.net/). They claim to be different from other campaigns because of involvement of both the pet and aquarium trade industry and the nursery and landscape industry. It is not clear how active this campaign is as no dates are mentioned on the website. Australia gives special attention to aquarium as vector for introduction of their marine pests on the web (www.marinepests.gov.au/aquarium/Pages/default.aspx). Here they explain what aquarium owners need to do when exposing of material and link to lists of species which are allowed to import (white list). Species not on the list are not allowed to be imported. The idea behind a white list is the "guilty until proven innocent" theory.

As especially tropical marine species are kept in aquarium the chance of survival and establishment in the Dutch coastal zone may be considered low or even absent for most of these species. However the potential risk has been given little attention so far.

3 Monitoring and early detection

In order to formulate prevention measures and to check their effectiveness detection of the presence of alien species is a critical step. Monitoring and surveys of alien species are key elements for managers. In an ideal situation, monitoring for the detection of alien invasive species should be performed in representative batches of material (e.g. ballast water, aquaculture products) that are imported from other regions, and in the field at locations with high risk (e.g. harbours, marinas, aquaculture plants).

In the Netherlands at this moment batches of shellfish are visually inspected for the presence of unwanted species, and occasionally rapid species inventories are performed in aquaculture facilities (Foekema et al., 2014) and source locations. However, a substantial number of new alien species in the marine environment are first reported by recreational scuba-divers.

A brand new paper, written by Lehtiniemi et al. (2015), presents an overview of different types of monitoring and surveys. In total 13 types of monitoring and surveys are described including information on the studied environment, current advantages/disadvantages and relative costs. The types of monitoring and surveys included are:

1. Harmful aquatic organisms and pathogens for IMO BWMC
2. Export licensing requirements, veterinarian services
3. Rapid assessment surveys
4. Target species
5. Port surveys
6. At border customs/consignment service
7. Long-term monitoring, hitchhiking on other surveys
8. Surveys of navigation buoys/offshore structures
9. Marina surveys
10. Shellfish biosecurity
11. Genetic monitoring
12. Risk assessment profiles of species/areas
13. Diver surveys, involving public participation

In this paper from Lehtiniemi et al. (2015), the authors recognize the lack in monitoring and surveys and highlight the importance of early detection and of long-term monitoring. Except for one, all of the types of monitoring and surveys described are based on visual observations and need high taxonomic expertise. Most of these types of surveys are time consuming and therefore costly making them unfeasible for high-throughput biodiversity analysis. When using rapid methodologies focussing on a hot spot or a target list to reduce costs and time, the major disadvantage is then that species can be missed. Moreover, specimens with a small body size including early life stages of unwanted species, toxic algae or disease agents are often not detected using the visual methods.

An alternative approach is to use molecular technologies for monitoring and early detection of alien species. In this chapter a description is given of the different types of technologies and examples how these technologies can be a useful addition to the conventional monitoring methods of invasive alien species. The advantages and disadvantages of two different techniques are described including an insight in the costs. Additionally, the availability of molecular barcodes present in public databases for relevant marine AIS was exanimated (Annex 2, NCBI accession numbers for relevant marine AIS).

3.1 Molecular techniques to identify species

Environmental DNA or eDNA refers to DNA that can be extracted from air, water, or soil, without isolating any specific type of organism beforehand. Environmental DNA does not refer to a specific molecular method for qualification or quantification of species. Since till now, in most cases only one specific species has been identified in an eDNA sample, the idea arose that eDNA is a method to identify one specific species in environmental samples. However this is incorrect. Total eDNA contains cellular DNA (living cells and organisms) and extracellular DNA, includes faeces, urine, mucous, skin, hair and carcasses (http://www.environmental-dna.nl/). eDNA from an environmental sample can be used to identify the presence of DNA from either a specific species using barcoding or DNA from all species simultaneously using metabarcoding.

3.1.1 DNA Barcoding

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species (Hebert et al., 2003). DNA barcoding can only be used if the between-species genetic variation in the barcode between species is greater than the variation within species.

The golden standard for DNA barcoding of animals and other eukaryotes is using a fragment of the mitochondrial cytochrome c oxidase, subunit 1 (COI) gene. For terrestrial plants, the concatenation of two chloroplast genes is used. The nuclear ribosomal internal transcribed spacer (ITS) region is used for fungi (Hollingsworth et al., 2009; Schoch et al., 2012; Smith et al., 2006). All three DNA regions have a relatively fast mutation rate, which results in enough variation in DNA sequences between species and relatively small variance within species.

The Barcode of Life Consortium (BOLD) is a worldwide initiative, to assembly reference libraries of these barcode sequences (Ratnasingham and Hebert, 2007).

To copy the DNA barcode (target sequence) from the genome, a set of primers and a DNA polymerase (enzyme) is needed. Primers are short DNA fragments, containing sequences complementary to the target region. These primers combine with sequences in the target region and serve as starting point for DNA amplification of the barcode (see Figure 3). Ideally, the same primers ("universal primers") are used to amplify the barcode region for all animals. In this case, the short DNA fragment, where the primers bind to the target sequence should have identical sequences in all animals. However, the copied DNA barcode needs to be unique for a species to be able to identify and especially for marine species it is harder to accomplish, due to the broad diversity in their barcodes. For marine species "universal primers", complementary to the target DNA region are not easily found. As a consequence, alternative primers have to be developed to amplify the barcode of specific groups of species. For marine species, "universal primers" are available for alternative barcodes, like 12S, 16S, 18S and 28S ribosomal RNA fragments (Che et al., 2012; Fonseca et al., 2010; Ivanova et al., 2007; Machida et al., 2012).

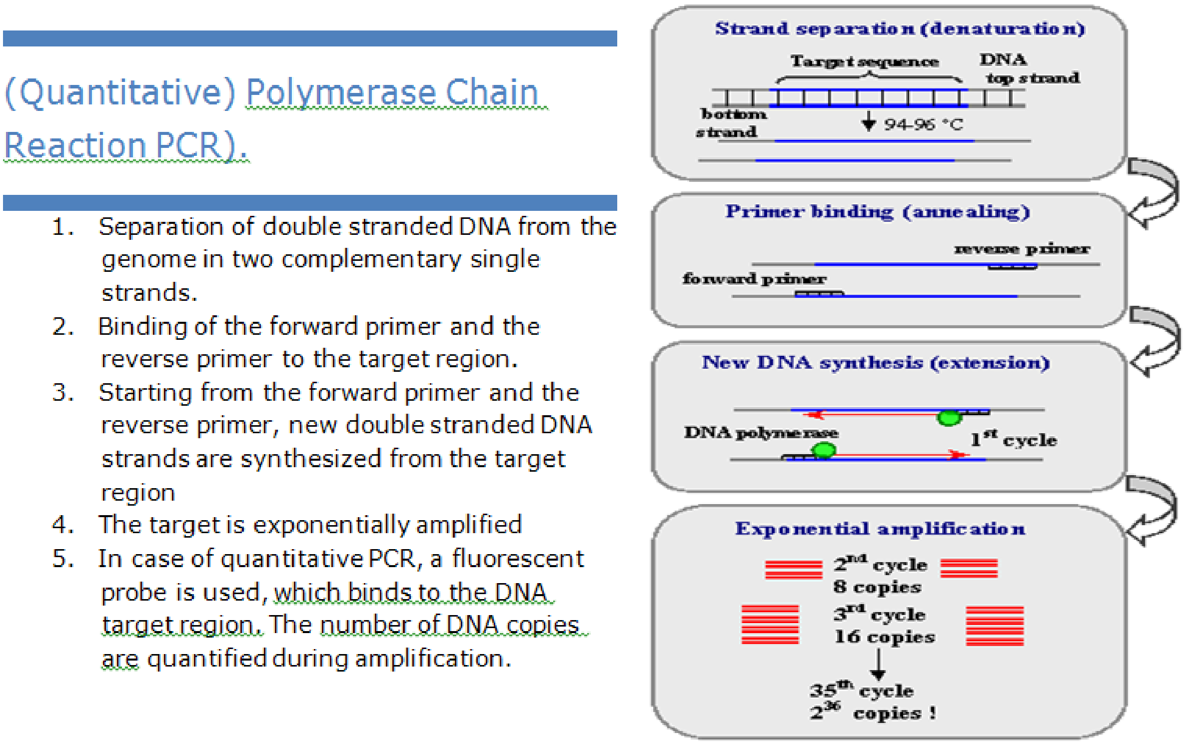

Figure 3 Schematic representation of the processes in a (quantitative) polymerase chain reaction.

For the identification of a single species in an eDNA sample, quantitative PCR (qPCR) methods are preferable to conventional PCR because they are more sensitive and give quantitative information on the target DNA concentration (Real Time QPCR Data Analysis Tutorial www.youtube.com/watch?v=GQOnX1-SUrI). As an eDNA sample contains DNA from multiple species, species-specific primers and probes (fluororecent reporter molecules) have to be designed. To design the primers/probes, genetic information is needed from the target species. Additionally, genetic information is needed from related non-target species to avoid binding of the primers to non-target DNA, leading to false positives. If sequences from these non-target species are not available in public databases, these species should first be collected and sequenced. For many species sufficient information is already available, if not it can be time-consuming to develop a reliable test.

Curent costs for DNA barcoding of individual species for which single species have been identified, range from 440-880 euro's for analysis of 1 to a maximum of 12 species in a an eDNA sample. Identification of additional species will cost a multiplication of the 440-880 euro's (100 species in 1 sample can add up to ~8000 euro's). Additional costs to develop the method to identify a single species in an e DNA sample depend highly on the amount of false positives and the labor associated, typically range from 2-6 months (H. van Pelt-Heerschap, personal communication).

3.1.2 DNA Metabarcoding

The DNA metabarcoding technology couples the principles of barcoding with high-throughput parallel sequencing. In contrast to barcoding, where just one single species can be sequenced, thousands of barcodes can be sequenced in parallel.

In this case, species in an environmental sample can be identified by comparing obtained sequences to a standard reference library of sequences from known organisms. Such DNA barcode libraries will lead to molecularly based analysis of known species in environmental samples. Compared to DNA barcoding and conventional visual observation monitoring, DNA metabarcoding is a high-throughput approach to obtain biodiversity data as many samples can be sequenced simultaneously producing a wealth of information (Aylagas et al., 2014; Bik et al., 2012; Creer, 2010; Epp et al., 2012; Yu et al., 2012).

When using metabarcoding as technique, "universal primers" can be used, since all target DNA molecules are sequenced in parallel creating unique identification options. Species in the sample can then be identified by comparing obtained sequences to a standard reference library of sequences from known organisms. In addition, variation in nucleotide sequence composition of the barcode of a species, can give information on the population structure (different haplotypes) of that species. In most cases, "unknown barcodes", not available in the reference standard library, can classify organisms on phylum level. Efforts should be made to extent the reference database in cooperation with taxonomists. Great advantage of this technique is that samples containing unknown barcodes can later be checked again when the reference database is filled with new barcodes.

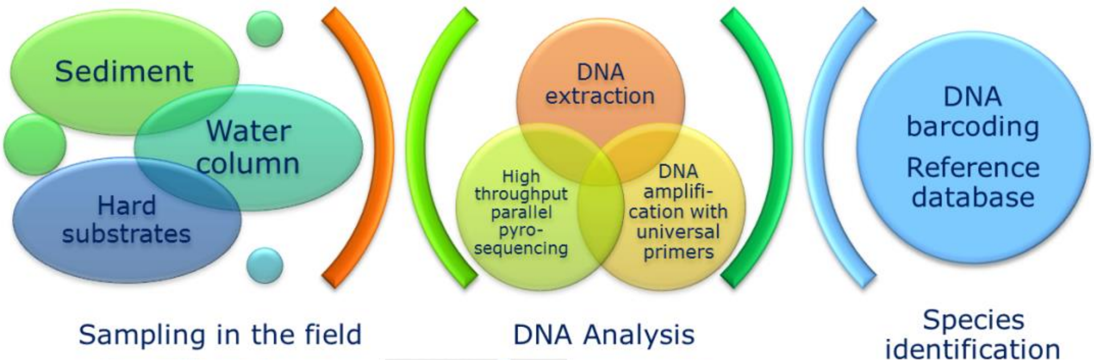

Figure 4 Schematic overview of DNA metabarcoding processes for monitoring of species.

For this technology, the relationship between the eDNA concentration and relative abundance of species in an environmental sample has to be investigated, to validated if eDNA metabarcoding tools can be used for reliable quantitative assessment of ecological communities.

Quantification of DNA concentration as measure for biomass is currently not possible with the metabarcoding technique. This technology is currently under development.

Costs for metabarcoding analysis of a single sample, with unlimited species, is currently around 6500 euros. However, a batch of 48 samples can be analyzed simultaneously lowering the price to around 300 euros per sample.

3.1.3 Pros and cons of the use of DNA (meta)barcoding tools

Species identification and abundance
Metabarcoding allows identification of rare species due to sequencing depth, providing millions of sequences from an environmental sample and has the potential to assess whole community diversity (Cristescu, 2014). Although, some studies failed to find a correlation between the number of sequences assigned to a species (Porazinska, 2009), others found positive relations between sequences and relative abundance of species (Hajibabaei, 2011). Further improvements of this technology are necessary before relative abundance can be assigned to species with high fidelity.

Limitations in taxonomic assignments
The availability of reference sequences in public databases is a limitation to the assignment of sequences to species. Furthermore, if primers are used, that are not truly universal, some taxa may not be amplified and as a consequence not identified.

DNA and detecting living organisms
Detecting DNA does not necessary mean detecting living organisms. In case quantitative PCR is used for barcoding of species, the concentration of DNA in an eDNA sample can be calculated. However, till now little is known about the degradation rate of eDNA and transport in marine environments. Moreover, the different life stages of a species cannot be identified separately by (meta) barcoding, yet.

3.1.4 Application options for molecular techniques

Early detection of invasive species increases the feasibility of rapid response to eradicate the species or contain its spread. In fresh water environments, current tools such as netting, traps, electrofishing, and visual surveys are not effective to identify early life stages and low-abundant species. For marine species it is even harder to control and eradicate species. In addition, the costs associated with early detection and response are far less than those for long-term management programs for species that have already become established and spread (Higgins and Vander Zanden, 2010), (Genovesi and Shine, 2004); (Langor and Sweeney, 2009). Environmental DNA (eDNA) analysis is increasingly being used in the early detection of invasive species. Environmental DNA refers to DNA that can be extracted from air, water, or soil, without isolating any specific species.

Molecular detection and monitoring of invasive species has already been demonstrated to be useful in ponds and lakes. eDNA detection was first demonstrated in fish and amphibians, because they have a permeable skin and can slough (to peel skin), so that they can contribute to eDNA presence in the water. The American bullfrog was the first species detected in freshwater samples (Ficetola et al., 2008); (Dejean et al., 2012). Here, eDNA analysis demonstrated a higher level of sensitivity compared to standard methods for the detection of this species in ponds. eDNA analysis was also more sensitive than traditional methods to track the invasion front of Asian carp in the north American Great lakes. eDNA tools revealed that these species have spread beyond barriers that were designed to prevent there passage (Jerde et al., 2011). For invertebrates, with non-permeable exoskeletons and shells it was expected they would be less detectable than vertebrates in aquatic environments. However, later on gastropods and arthropods were identified with success in aquatic environments ((Thomsen et al., 2012); (Goldberg et al., 2013)). The widely distributed invasive aquatic invertebrate, the New Zealand mudsnail (*Potamiopyrus antipodarum*) can be detected using eDNA tools. Moreover, a relationship was detected between the mudsnail population density and quantity of eDNA. Such a quantitative relationship was also found for the biomass of fish in ponds. For these species eDNA surveys have a high potential in early detection.

To identify species in lotic (flowing water) systems, like marine aquatic environments, it has to be investigated first how long eDNA remains detectable and how it is transported. Furthermore, the timing of sampling need to coincidence with the life history or behaviour of the target species. For two invertebrate species it has been demonstrated how far away from the source population in a lake their DNA could be detected at different times of the year in an outflowing river. The results indicate that there may be species-specific transported distances for eDNA (Jane et al., 2014).

To study the expansion of invasive species around the Australian coastline eDNA tools are developed to identify *Maoricolpus Roseus* (New Zealand Screwshell) larvae in plankton.

Specific primers for eDNA analysis are also developed to detect larvae of *Musculista senhousia* (Asian mussel), *Corbula gibba* (Basket shell), *Sabella spallanzii* (European fan worm) in ballast water and *Undaria pinnatifida* (Japanese seaweed) in hull fouling samples (See "Marine pests, Australian Government, Department of Environment ")

In conclusion, for many species molecular tools have been developed for identification, eradication and control of invasive species. In almost all cases species-specific primers are developed for quantitative identification of a species DNA (qPCR). Disadvantage of this technology is that genetic information is needed from related non-target species to avoid binding of the primers to non-target DNA, leading to false positives. Moreover, if different populations are present from one species, there is a chance that the primers do not bind to the DNA of all populations, leading to false negatives. The advantage of the metabacoding technology is that all species can be identified simultaneously in an environmental sample if their barcode is available in databases, avoiding false positives. Moreover, variability in the barcode within the species can be identified, avoiding false negatives. For this technology, it has still to be investigated if there is a relationship between the eDNA concentration and relative abundance of species in an environmental sample.

3.1.5 Molecular projects to develop methods for control of invasive species.

In Australia several national molecular projects are running to develop methods to prevent or control the establishment of invasive species. For the invasive marine algae, *Caulerpa taxofolia*, causing dramatic declines in local biodiversity, molecular techniques are developed to deliver a species-specific toxin molecule into the colonies, to kill the species. Experimental work is undertaken to develop a prototype toxin that influences osmoregulation and photosynthesis and is water-soluble for direct uptake (http://www.environment.gov.au/resource/feasibility-study-genetic-control-caulerpa-sa-and-nsw). Additionally, new genetic techniques are developed to create "daughter-less" carp. Daughterless carp are produced by blocking the gene that produces aromatase, which prevents the development of female embryos and leads to an all-male population. Release of these genetically modified animals could control the abundance of this species. At the Auburn University in Alabama, USA this approach is tested in controlled specialized facilities (http://theconversation.com/male-only-gene-trick-could-leave-invasive-fish-species-floundering-26370).

3.2 Availability of molecular barcodes for alien species on the selected list

An alien species list containing 1,425 records that was compiled by Radbout University was provided. The species list did not include specific information on habitat, but was a full list including marine, terrestrial and aquatic alien species that could potentially enter the Netherlands. A selection was made by screening the list to search for marine species using the database of the World Register of Marine Species (WoRMS) and the list of Wolff (2005). The screening resulted in a selection of 197 species which are marine species and/or can survive in brackish or saline conditions. Some freshwater species might be included as result of the extraction method used. The selected species were then checked on availability of molecular barcode sequences using a worldwide database called GenBank. This list is included as Annex 2 of this report.

From the 197 species which were extracted from the full alien species list, a total of 142 species have molecular barcodes published in GenBank. For 53 species, no molecular barcode was found. The selection of species were summarized into informal taxonomic groups that were used in the original list and is presented in Table 1, including information on the number of species representing this group and the number of molecular barcodes found. For all hydroids, mammals, mosses, sea spiders, sea squirts and sea stars, molecular barcodes were available.

The other “informal groups” show some gaps where specifically the lack of barcodes in the phytoplankton group is worth mentioning. The lack of data is possibly because it can be difficult to identify phytoplankton to the species level by microscopy. In conclusion, for 73% of the species on the list, the molecular barcodes are already available, which is interesting for the development of a metabarcoding tool for alien species.

Table 1 “Informal groups” of species including the selected number of species represented by this group and the number of molecular barcodes available in GenBank.

Informal group	**Number of species**	**Number of molecular barcodes**
Bacteria	1	0
Birds	5	4
Comb jellies	2	1
Crustaceans	40	28
Fish	35	33
Hydroids	3	3
Mammals	2	2
Molluscs	23	18
Mosses	3	3
Phytoplankton	2	0
Plants	42	27
Reptiles	1	0
Sea spider	1	1
Sea squirts	5	5
Sea stars	1	1
Viruses	1	0
Worms	19	8
Zooplankton	11	8

4 Eradication

When preventive measures were not successful to prevent an alien species to reach a new area additional measures can be considered to prevent the species from becoming established, spreading over a wider area and/or having a strong impact on ecology and economy. Complete removal (eradication) of the unwanted species from the newly colonized area is, when possible, the preferred option.

Most marine environments experience continuous exchanges of water and biota between locations and the majority of potentially invasive species have great dispersal potential by producing large numbers of pelagic larvae. This facilitates colonization of large areas, as long as environmental circumstances suite the needs of the species. Marine systems can therefore be considered particularly vulnerable to biological invasions (Salvaterra et al., 2013). In addition these features hamper successful eradication of unwanted marine species once they have become established at locations with large connectivity to other areas.

Until 2005, at least 37 eradication programs were successfully performed in Europe, all involving terrestrial species, mainly rats and rabbits at Islands (Genovesi, 2005). In that literature review no records were found of European eradication programs (before 2005) that focused on marine species. The only record we know of today about a European program in the marine environment focused on the eradication of the alien ascidian ***Didemnum vexillum*** that was first observed in Britain in a marina in Wales in 2008 (Sambrook et al., 2014). In the following two years full eradication attempts were made. Artificial structures in the marina were packed in plastic restricting availability of food and oxygen, resulting in death of the fouling organisms after 10 days. Sodium hypochloride was used to spray surfaces that could not be packed. Although initially effective, re-colonization occurred, as the authors suggest with recruits from small colonies that were treated after spawning had already taken place. The total costs of the program were about ₤800.000.

4.1 Success stories

From outside Europe other successful marine eradication programs are recorded, although the number is still limited. In California the sabellid polychaete (***Terebrasabella heterouncinata***) knowns as a pest organism from other regions, that was introduced with abalone from South Africa to an aquaculture facility was successfully eradicated (Culver C.S., 2000). Besides isolation of the source by a screen that was installed at the aquaculture facility and removal of potentially infected material, also the most highly susceptible and preferred host the black turban snail, *Tegula funebralis* was removed from the local environment. In total 1.6 million individual snails were removed from the intertidal area. Monitoring indicated that this combination of measures was effective and that the established population sabellid had been eradicated.

In New Zealand the invasive seaweed ***Undaria pinnatifida*** was succesfully eradicated from a from a sunken trawler, after salvage attempts had failed, by applying a heat treatment (Wotton et al., 2004). By means of plywood boxes attached to the hull and heated by elements and a flame torch for surfaces that could not be covered by these boxes the full outside of the ship was heated to a temperature of 70°C for 10 minutes. The operation took 4 weeks to complete, and monitoring for three years showed that Undaria was removed successfully.

Another invasive marine seaweed ***Caulerpa taxifolia*** was successfully eradicated from a lagoon in California (Anderson, 2005). The accidental discovery of this species that is present on the US Federal Noxious Weed list was rapidly followed by containment of the beds with 1 to 500 m2 PVC enclosures and treatment with hypochlorite by applying solid chlorine-releasing tablets.

As alternative for this treatment dredging of the seaweed beds was considered. It was however rejected since it was anticipated that the turbidity caused by the dredging would hamper the identification of other beds.

Dredging was successfully applied in New Zealand to prevent the further spread of the alien mussel ***Perna perna*** from a rig defouling site (Hopkins et al., 2011). An area of 13 ha was dredged. As the authorities realized that it would be impossible to remove all individual mussels the goal was to reduce the final density at least three orders of magnitude lower than the density necessary for successful reproduction. For this species dredging was thus an appropriate strategy, however, for other species that can reproduce by fragmentation from one single individual, like sponges or colony forming tunicates like *Didemnum* sp (Bullard et al., 2007) it is not.

The eradication programs reviewed above all are based on the principle that the unwanted species is detected and removed/killed. This approach requires a lot of practical effort and can only be applied for species with a relatively low mobility. Alternative methods that involve less active manipulation such as biological control by predators (e.g. herbivorous mollusks and sea urchins for seaweed), has been investigated without being applied in practice, as the introduction of a new alien species as a remedy might have long term impact on the ecosystem that cannot be foreseen (Anderson, 2007).

4.2 Other techniques for eradicate an unwanted alien species

Some researchers believe that the application of so-called Trojan sex chromosomes (TSC) could form a good measure against alien fish (Senior et al., 2013). For this, fish of the target species are cultivated with manipulated sex chromosomes (TSC) and released into the environment. After mating these fish will only produce male offspring causing a male skewed population sex ratio, which eventually should result in a population collapse. This method that (in the future) might also be applicable for other taxonomic groups than fish has as far as we are aware of until now never been tested in a marine field situation. The drawback is that to be successful a large quantity of manipulated fish must be introduced, which on its own might have ecological consequences.

4.3 Collateral damage of eradication programs

Collateral ecological damage as a result of an eradication program cannot always be avoided In most situations this will not be considered a large problem as the area will normally be recolonized within one season. When however the 'treated' community supplies important features (food and/or shelter) for other species special care is required. Such a situation consisted in the San Francisco Bay where an invasive plant, a hybrid **Spartina**, replaced the native Spartina in large areas. The eradication program of the invasive plant resulted in a more than 50% decline of the numbers of an endangered bird (the California clapper rail, *Rallus longirostris obsoletus*), that uses Spartina (either what species) for nesting (Lampert et al., 2014). Model calculations revealed that with this knowledge another eradication strategy that would ensure a minimum area of native or alien Spartina available would have benefitted the birds.

As mentioned above relatively little has been published about eradication of alien species in the marine environment. But the far majority of such studies published in peer reviewed literature describe that the eradication was successful. This is remarkable knowing that of the 136 terrestrial eradication programs that were reviewed by (Pluess et al., 2012) only 53% was indicated as successful, and (Drolet et al., 2014) identified 52% programs to be successful from a selection of 143 aquatic or marine case studies. (Drolet et al., 2014) further indicates that the factors that are related with the successfulness of the programs will be reported later.

For the 136 terrestrial studies however Pluess (Pluess et al., 2012) identified that a limited distribution area of the alien species at the moment that the eradication program started was the most significant predictor for success. Reaction time after first discovery, specific knowledge about the target species and the isolation of the area were of less relevance for the success. Before this study it was already suggested that successful eradication of an established invader is rare (Mack et al., 2000) (Lodge et al., 2006).

The reported eradication programs in the marine environment indeed all have in common that the infested area was limited. Apparently only in these cases the authorities see the advantages of such expensive programs in the marine environment, which seems justified given the high success rate that are reported. In this respect however it is good to realize that it is not always easy to determine when the eradication was 100% successful (Rout et al., 2014).

Genovesi and co-workers (Genovesi, 2005) argue that eradication programs in Europe are difficult to perform due to the scarce awareness, a legal basis that often automatically protects alien species by national laws, and the opposition of the public and animal rights groups. The latter of course being more relevant when the program involves ‘lovely’ vertebrate species such as grey squirrels and hedgehogs, which will be less relevant for the marine mainly invertebrate species that are nowadays known as potentially invasive. The invertebrate species are also less protected by national laws. Lack of funding forms the major reason for delayed execution of the programs or no execution at all (Anderson, 2005) (McCann et al., 2013).

5 Control

When prevention failed and eradication is not possible, the most obvious remaining management option is to reduce the chance of further spreading of the unwanted species. In fresh water systems barriers have been created to prevent alien fishes (Lamprey) to reach their spawning grounds. Nets have been used to prevent alien fish such as largemouth bass (***Micropterus salmoides***) from escaping over the spillway in the Colorado River, and an electrical grid system was developed to prevent Asian carp spreading over the Great Lake basins in the USA (Rahel, 2013). These measurements were not always effective, and must have impacted the local natural ecosystem as well, are not applicable in the marine environment, due to its open nature. Isolation of an area without substantially changing the ecosystem seems or is no option.

The systems that are available for controlling further distribution of marine species are the same as applied to prevent introduction to the region in the first place, and that are described in chapter 2. Of course once a species is present in the region these measures are only useful to prevent transport to locations that are more or less isolated (e.g. upstream) from the infected locations. According to (Mack et al., 2000) successful control of biotic invasions depends more on commitment and continuing diligence than on the efficacy of specific tools themselves and is most effective when it employs a long-term, ecosystem-wide strategy rather than a tactical approach focused on battling individual invaders.

Experimental results indicate that disturbed communities or new structures, like artificial reefs, are more welcoming for invasive species than systems that contain a stable developed ecosystem (Ceccherelli et al., 2014) (Simkanin et al., 2013). Preserving the quality of the ecosystem as good as possible might therefore reduce the spreading of invasive species.

Most effort with respect to the control of established non-invasive species can be found where the new species have a substantial economic impact. In these cases special measures are being taken to reduce this impact at the specific site. A clear example of this are the freshwater mussels; the zebra mussels reached the Western European waters already decades ago, while the related quagga mussel was more recently detected for the first. Their settling larvae can block cooling water inlets of power plants and have to be removed by periodic chlorination. Although this results in considerable maintenance costs, as far as we are aware of no action has ever been taken to controlling or eradicate these mussels in the natural environment.

Although the harmful algae (dinoflagellate) ***Alexandrium ostenfeldii*** is not considered as an alien species, the successful repression of a local bloom in the Netherlands is worth mentioning here. The bloom was detected, after a dog was poisoned by ingesting brackish surface water, in a canal that discharges water into the Oosterschelde that is used for shellfish aquaculture. The canal and surrounding areas which also contained the algae were isolated by dams and the water was treated with hydrogen peroxide (H_2O_2). The algal bloom and related dioxin concentrations disappeared rapidly after this treatment. Some impact on fish and zooplankton was observed (Burson et al., 2014). In Burson and co-workers, the involved costs are not reported. With this action, the algae have not been eradicated completely, but local negative effects were controlled.

Some alien species have a commercial value, which often is the reason for their introduction in the first place, such as the Pacific oyster (***Crassostrea gigas***) and the Manilla clam (***Tapes phillipinarum***). The Venice lagoon is now the main production site for the Manila clam in Europe. The economic value is evident while the negative impact of this species on the lagoon's ecosystem seems limited, although the increased bottom disturbing fishing effort related to the presence of this valuable species has a negative impact on the benthic ecosystem (Nunes and Markandya, 2008).

The Pacific oyster, although also introduced in Europe for aquaculture purposes developed more into a pest, overgrowing large areas with hard substrate, and thus altering the habitat, especially soft sediment habitats. The commercial value of the wild Pacific oyster is low and harvesting plays no role in the control of this species. In 2006, a pilot started in the Netherlands to remove wild Pacific oysters from the Oosterschelde. After four years, it was concluded not feasible to eradicate the oyster completely, but only possible to control the species in specific locations (Wijsman et al., 2008).

An interesting approach to help controlling invasive species is the '**Lion fish derbies'** that are being organized in the South western coast of the USA. The public is encouraged to participate in such derbies by catching as much as possible of lion fishes that invaded the local reefs. As part of this derby the fishes are prepared as food in order to stimulate the public to continue fishing for them. The organization claims that this is an effective way to control the impact of these alien fishes (http://www.reef.org/reef_files/REEF%20Sanctioned%20Lionfish%20Derbies%20Packet%20Info.pdf).

6 Recommendations

The continuous spreading of species to regions where they are considered as alien indicates that the attempts to prevent this management failed at several occasions. Mack and co-workers (Mack et al., 2000) suggest the following reasons that are also valid for marine invaders, for this:

- Efforts to identify general attributes of future invaders have often been inconclusive;
- Eradication of an established invader is rare, and control efforts vary enormously in their efficacy;
- Control of biotic invasions often is focused on battling individual invaders, where it would be more effective when it employs a long-term, ecosystem-wide strategy.

In addition they indicate that in general it is problematic to predict susceptible locales for future invasions. They suggest that this is less the case when focus is on marine species for which harbours, marinas and aquaculture facilities can be identified as susceptible locales. Monitoring for new species should thus focus on these locales.

The scheme presented in the introduction of this report (fig 1) indicates how a complete management program could look like. Based on the insight gained from the literature review, for each of the management steps within this scheme (prevention, monitoring/detection, risk assessment, eradication, control) specific recommendations will be given that could reduce the reasons for failure as suggested by (Mack et al., 2000), with special emphasis on the situation in the Netherlands. Mack and co-workers do also conclude that despite the efficacy of specific management tools successful control of alien invasive species depends more on commitment and continuing diligence.

<u>Early detection</u> is crucial for effective management. Not only to be able to avoid introduction of stowaways in batches of (ballast)water or aquaculture products, but also for effective monitoring of locations with a potential portal function for alien species such as harbours, marinas and aquaculture facilities. Since, the use of taxonomical methods for AIS monitoring is time-consuming and costly, they are unfeasible for high-throughput biodiversity analysis. Moreover, early life stages, toxic algae, disease agents and species with a small body size are not detected. For now, the use of metabarcoding methods for monitoring of ballast water, harbours, and species transports (aquaculture) has to be validated in conjunction with conventional methods. New developments in meta-barcoding analysis can result in reduction of the costs for these analyses and for the majority of the marine potentially invasive species the molecular barcode is already available.

These multispecies early detection techniques will also be profitable in the verification of the effectiveness of <u>prevention</u> measures, such as ballast water disinfection or freshwater treatment of marine shellfish batches. For all treatments currently available, and probably those that will be developed in the future, it is proven that they will not be effective in 100% removal of unwanted species. The intention of these measures is therefore to reduce the change that unwanted species are being introduced in new areas.

Prevention of import of non-native species via aquaculture is best optimised by exclusion of import from regions where unwanted species have been detected. However, this would have strong economic consequences. Second best could be to exclude only those regions where species that could potentially survive the freshwater treatment are present and to perform the fresh water treatment on all shellfish batches imported. Finally, it must be realised that prevention measures can only be fully effective if all potentially invasive species can be detected. With respect to Ballast water this thus includes micro-organisms smaller than 10 µm which are until now not taken into account.

Successful eradication from the marine environment is only possible before the unwanted species has invaded a larger area. Apart from early detection, also a fast response is thus essential. As eradication programs are very costly (although often considered far less expensive than post-entry control (Mack et al., 2000)) funding forms the most important reason of delay of actions after detection. Allocation of emergency funding that can be made rapidly available would facilitate a fast response when required (Lodge et al., 2006) (Anderson, 2005). Eradication programs should be tailored to the local situation and the target species. Manual removal could be an option for larger specimens that have only invaded a small area, dredging or enclosure and treatment with a degradable toxicant could be applied for smaller species or larger areas. In most eradication programs one must take into account that some collateral damage cannot be avoided, especially when chemicals are applied. In such cases special care should be taken that the public is informed correctly about the potential risk of the alien species that is targeted.

7 Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

Justification

Report Number: C082/15
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The scientific quality of this report has been peer reviewed by a colleague scientist and the head of the department of IMARES.

Approved: Dr. ir. J.W.M. Wijsman
Researcher

Signature:

Date: 1 June 2015

Approved: Drs. F.C. Groenendijk
Head of department Maritime

Signature:

Date: 1 June 2015

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Annex 1 Definitions

Alien species: see 1.1

Aquarium (= ornamental) species: All species imported or transferred into confinement for ornamental indoor or outdoor use.

Bait organisms: Live specimens used (e.g., on a hook or in a trap) to allure target species.

Biocontrol species: The intentional release of an organism that is intended to consume, infect, or debilitate a selected species to decrease its population size. Note: The possible limited specificity of biocontrol species is of concern as native species might be negatively affected.

Broodstock: Specimens of a species in any life stage from which a first or subsequent generation/ growth may be produced for possible introduction to the environment.

Current commercial practice: Established and ongoing cultivation, rearing, or placement of an introduced or transferred species in the environment for economic or recreational purposes, which has been ongoing for a number of years.

Disease agent: Any organism, including parasites and prions which causes or contributes to the development of a disease.

Donor location (= source localities): Specific localities in a country or zone from which the import or transfer originates.

Genetic diversity: All of the genetic variation in an individual population, or species.

Genetically modified organism (GMO): An organism in which the genetic material has been altered anthropogenically by means of recombinant DNA technologies. This definition includes transgenic organisms, i.e., an organism bearing within its genome one or more copies of novel genetic constructs produced by recombinant DNA technology, but excludes chromosome manipulated organisms (i.e., polyploids), where the number of chromosomes has been changed through cell manipulation techniques.

Indigenous (= native) species: A species or lower taxon living within its natural range (past or present) including the area which it can reach and occupy using its natural dispersal systems (modified after CBD, GISP).

Introduced species (= non-indigenous species, = exotic species): Any species transported intentionally or accidentally by a human-mediated vector into aquatic habitats outside its native range. Note: Secondary introductions can be transported by human-mediated or natural vectors.

Marine species: Any aquatic species that does not spend its entire life cycle in fresh water.

Native range: Natural limits of geographical distribution of a species (modified after Zaitsev and Ozturk, 2001).

New introduction: The human-mediated movement of a species outside its present distribution.

Non-target species: Any species inadvertently accompanying in, on, or with the species intended for introduction or transfer.

Polyploidy: An organism or cell having more than two haploid sets of chromosomes.

Progeny: Next generation(s) of an organism. Also included are new stages/fragments of seaweeds, protists, and clonal organisms.

Quarantine: The facility and/or process by which live organisms and any of their accompanying organisms can be held or reared in isolation from the surrounding environment.

Release: Voluntary or accidental dissemination of an organism, or its gametes, outside its controlled area of confinement.

Tetraploid: An organism or cell having four haploid sets of chromosomes.

Transferred species = transplanted species): Any species intentionally or accidentally transported and released within areas of established populations, and continuing genetic flow where it occurs.

Triploid: An organism or cell having three haploid sets of chromosomes.

Vector: Any living or non-living carrier that transports living organisms intentionally or unintentionally.

Zone: Part of a coastal area or an estuary of one or more countries with the precise geographical delimitation that consists of a homogeneous hydrological system (modified after OIE).

Annex 2 NCBI accession numbers for relevant marine IAS

List of potentially invasive species for the marine environment (and some freshwater species that were included in WorMs) with the NCBI accession number (http://www.ncbi.nlm.nih.gov/) where information on the DNA sequence can be found, if available.

Scientific name	Informal group	Accession number NCBI	
Novirhabdovirus sp. genotype IV *sublineage b*	Viruses		
Cordylophora caspia	Hydroids	KC489509.1	
Chelicorophium curvispinum	Crustaceans	KC559527.1	
Acartia tonsa	Zooplankton	KM458087.1	
Alexandrium catenella	Zooplankton	GQ501132.1	
Alexandrium minutum	Zooplankton	GQ501158.1	
Alexandrium tamarense	Zooplankton	GQ501183.1	
Glugea hertwigi	Zooplankton		
Karenia mikimotoi	zooplankton	GQ501252.1	
Mytilicola intestinalis	Zooplankton	HM775197.1	
Prorocentrum minimum	Zooplankton	GQ501297.1	
Psammonobiotus communis	Zooplankton	KC855628.1	
Psammonobiotus linearis	Zooplankton		
Sphaeromyxa sevastopoli	Zooplankton		
Anguillicola crassus	Worms		
Teredo navalis	Worms		
Ficopomatus enigmaticus	Worms		
Gyrodactylus salaris	Worms	AF540905.2	
Pileolaria berkeleyana	Worms		
Pseudodactylogyrus anguillae	Worms		
Gianius aquaedulcis	Worms		
Hydroides dianthus	Worms		
Hydroides elegans	Worms	JQ885939.1	
Hydroides ezoensis	Worms	JQ885951.1	
Ichthyocotylurus pileatus	Worms	HM064726.1	
Janua brasiliensis	Worms		
Marenzelleria neglecta	Worms	EF137727.1	
Potamothrix moldaviensis	Worms	KF366633.1	
Potamothrix vejdovskyi	Worms	KF366632.1	
Ripistes parasita	Worms	AF534856.1	
Scolex pleuronectis	Worms		
Spirorbis marioni	Worms		
Timoniella sp.	Worms		
Asterias amurensis	Sea stars	DQ992558.1	
Didemnum vexillum	Sea squirts	JF738069.1	
Styela clava	Sea squirts	GU328035.1	
Corella eumyota	Sea squirts	EU140818.1	
Didemnum sp.	Sea squirts	KM411613.1	
Microcosmus squamiger	Sea squirts	KJ944390.1	
Ammothea hilgendorfi	Sea spider	DQ390091.1	
Iguanidae	Reptiles		
Spartina anglica	Plants	1	rbcl chloropast genen
Sargassum muticum	Plants	KC782903.1	

Scientific name	Informal group	Accession number NCBI	
Undaria pinnatifida	Plants	KC782897.1	
Codium fragile	Plants	1	rbcl chloropast genen
Cotula coronopifolia	Plants	1	rbcl chloropast genen
Chladophora aegagrophila	Plants		
Acrothamnion preisii	Plants		
Asparagopsis armata	Plants	KJ960344.1	
Asparagopsis taxiformis	Plants	JN642177.1	
Bonnemaisonia hamifera	Plants	KJ960353.1	
Caulerpa racemosa	Plants		
Caulerpa taxifolia	Plants		
Chaetoceros muelleri	Plants		
Chattonella verruculosa	Plants		
Chroodactylon ornatum	Plants		
Codium fragile fragile	Plants		
Codium fragile spp. atlanticum	Plants		
Codium fragile spp. Scandinavicum	Plants		
Coscinodiscus wailesii	Plants	GQ844250.1	
Gracilaria vermiculophylla	Plants	JQ794759.1	
Grateloupia doryphora	Plants	1	rbcl chloropast genen
Grateloupia filicina	Plants	HQ422590.1	
Halophila stipulacea	Plants	1	rbcl chloropast genen
Juncus gerardii	Plants	1	rbcl chloropast genen
Nitellopsis obtusa	Plants	1	rbcl chloropast genen
Odontella sinensis	Plants	1	rbcl chloropast genen
Phaeocystis pouchetii	Plants		
Pleurosira laevis	Plants	1	rbcl chloropast genen
Polysiphonia morrowii	Plants	HM573540.1	
Skeletonema subsalsum	Plants	1	rbcl chloropast genen
Sphacelaria fluviatilis	Plants		
Sphacelaria lacustris	Plants		
Stephanodiscus subtilis	Plants		
Stypopodium schimperi	Plants	1	rbcl chloropast genen
Thalassiosira baltica	Plants	1	rbcl chloropast genen
Thalassiosira pseudonana	Plants	1	rbcl chloropast genen
Thalassiosira weissflogii	Plants	GQ844277.1	
Ulva (Enteromorpha) flexuosa ssp. flexuosa and *flexuosa ssp. paradoxa*	Plants	1	rbcl chloropast genen
Ulva intestinalis	Plants	1	rbcl chloropast genen
Ulva prolifera	Plants	1	rbcl chloropast genen
Womersleyella setacea	Plants	1	rbcl chloropast genen
Zostera japonica	Plants	1	rbcl chloropast genen
Chattonella antiqua	Phytoplankton		
Chattonella marina	Phytoplankton		
Watersipora subtorquata	Mosses	DQ417457.1	
Tricellaria inopinata	Mosses	AY061750	
Victorella pavida	Mosses	JN200653.1	
Rapana venosa	Molluscs	JX503056.1	
Crepidula fornicata	Molluscs	KF643526.1	
Potamopyrgus antipodarum	Molluscs	AB703677.1	
Crassostrea gigas	Molluscs	KF644048.1	
Ensis americanus	Molluscs	KF643876.1	

Scientific name	Informal group	Accession number NCBI
Mytilopsis leucophaeata	Molluscs	U47649.1
Anadara spp. Inaequivalvis / demiri	Molluscs	
Aulacomya ater	Molluscs	JF301757.1
Brachidontes pharaonis	Molluscs	AJ865787.1
Ceratostoma inornatum	Molluscs	
Corbula amurensis	Molluscs	HG005370.1
Ensis directus	Molluscs	KF643876.1
Mercenaria mercenaria	Molluscs	KF644183.1
Monodacna colorata	Molluscs	
Musculista senhousia	Molluscs	JN991305.1
Mya arenaria	Molluscs	KF644343.1
Petricola pholadiformis	Molluscs	
Pinctada radiata	Molluscs	GQ355878.1
Potamocorbula amurensis	Molluscs	
Tapes philippinarum	Molluscs	AB040840.1
Urosalpinx cinerea	Molluscs	KF644187.1
Viviparus georgianus	Molluscs	AF120634.1
Alopex lagopus	Mammals	AY894421.1
Axis axis	Mammals	KF983349.1
Blackfordia virginica	Hydroids	JQ716116.1
Rhopilema nomadica	Hydroids	HF930518.1
Neogobius melanostomus	Fish	KC501012.1
Ameiurus melas	Fish	HQ557269.1
Salvelinus fontinalis	Fish	HQ961028.1
Proterorhinus marmoratus	Fish	EU524310.1
Salmo salar	Fish	HQ961026.1
Oncorhynchus mykiss	Fish	HQ961049.1
Catostomus commersoni	Fish	KF929688.1
Oncorhynchus kisutch	Fish	KF918886.1
Acipenser baerii	Fish	GQ328816.1
Oncorhynchus gorbuscha	Fish	HQ712701.1
Morone americana	Fish	HQ024972.1
Coregonus peled	Fish	HQ960663.1
Huso huso	Fish	FJ809719.1
Neogobius gorlap	Fish	
Alosa aestivalis	Fish	KC015129.1
Alosa pseudoharengus	Fish	KF929583.1
Anguilla rostrata	Fish	HQ339978.1
Apeltes quadracus	Fish	KF929609.1
Aphanius dispar	Fish	KJ552732.1
Atherina boyeri	Fish	KJ709477.1
Clupeonella cultriventris	Fish	NC_015109.1
Fistularia commersoni	Fish	
Gasterosteus aculeatus	Fish	KJ204898.1
Knipowitschia caucasica	Fish	HQ600736.1
Liza haematocheila	Fish	JN813098.2
Oncorhynchus keta	Fish	HQ712702.1
Oncorhynchus nerka	Fish	KF930200.1
Oncorhynchus tshawytscha	Fish	HQ712706.1
Osmerus mordax	Fish	JQ354253.1

Scientific name	Informal group	Accession number NCBI
Petromyzon marinus	Fish	KF930255.1
Salmo trutta	Fish	KM287120.1
Saurida undosquamis	Fish	KC501292.
Seriola fasciata	Fish	KF930429.1
Siganus rivulatus	Fish	KF930442.1
Sphoeroides pachygaster	Fish	KJ709636.1
Cercopagis pengoi	Crustaceans	AF320014.1
Eriocheir sinensis	Crustaceans	AF317336.1
Balanus improvisus	Crustaceans	AJ319878.1
Gammarus tigrinus	Crustaceans	EF570348.1
Marsupenaeus japonicus	Crustaceans	KC789233.1
Callinectes sapidus	Crustaceans	KC789113.1
Elminius modestus	Crustaceans	
Hemimysis anomala	Crustaceans	EU029170.1
Homarus americanus	Crustaceans	U29717.1
Corophium curvispinum	Crustaceans	
Bosmina coregoni	Crustaceans	AY075057.1
Bythotrephes longimanus	Crustaceans	AF435131.1
Caprella mutica	Crustaceans	DQ466518.1
Charybdis japonica	Crustaceans	HM237603.1
Charybdis longicollis	Crustaceans	
Chionoecetes opilio	Crustaceans	AB211154.1
Cornigerius maeoticus maeoticus	Crustaceans	AY189516.1
Daphnia cristata	Crustaceans	
Echinogammarus ischnus	Crustaceans	AY326126.1
Echinogammarus warpachowskyi	Crustaceans	
Ectinosoma abrau	Crustaceans	
Eurytemora affinis	Crustaceans	HM474028.1
Gammarus fasciatus	Crustaceans	
Gammarus pulex	Crustaceans	JF965944.1
Hemigrapsus penicillatus	Crustaceans	JX502904.1
Hemimysis anomala	Crustaceans	EU029170.1
Heterocope caspia	Crustaceans	
Heteropsyllus nr. nunni	Crustaceans	
Katamysis warpachowsky	Crustaceans	
Obesogammarus crassus	Crustaceans	KF478539.1
Paraleptastacus spinicaudus triseta	Crustaceans	
Paramysis intermedia	Crustaceans	DQ889119.1
Paramysis lacustris	Crustaceans	DQ779845.1
Paramysis ullskyi	Crustaceans	DQ779878.1
Percnon gibbesi	Crustaceans	JQ306102.1
Podonevadne trigona ovum	Crustaceans	
Portunus pelagicus	Crustaceans	KF604896.1
Rhithropanopeus harrisii	Crustaceans	GQ332606.1
Schizopera borutzkyi	Crustaceans	EU029170.1
Mnemiopsis leidyi	Comb jellies	KF435121.1
Beroe cucumis	Comb jellies	
Alopochen aegyptiacus	Birds	
Aix galericulata	Birds	JN703260.1
Threskiornis aethiopicus	Birds	JQ176468.1

Scientific name	Informal group	Accession number NCBI
Netta rufina	Birds	GU571988.1
Tadorna ferruginea	Birds	GQ482749.1
Aeromonas salmonicida	Bacteria	