Marine aliens in Svalbard

eDNA barcoding as innovative monitoring tool for alien species in Svalbard

FINAL REPORT

Project 17/25 - Marine aliens in Svalbard

RiS ID: 10731 - Indicators for marine invasive species in the Arctic

Martine van den Heuvel-Greve, Anneke van den Brink, Sander Glorius (Wageningen Marine Research)
Arjen de Groot, Ivo Laros (Wageningen Environmental Research)

DISCLAIMER: this report is confidential as the detailed results of this project will be submitted as scientific manuscript to Polar Biology soon. The scientific paper will become publicly available.

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Summary

The introduction and potential settlement of marine alien species in Svalbard is increasing due to climate change related declines in sea ice coverage and the consequential opening up of areas for shipping. This project aimed to monitor the presence and distribution of marine alien species in west Svalbard using innovative metabarcoding techniques. Monitoring is essential to detect and manage the introduction of alien species. In this study, metabarcoding of sediment samples was applied in the coastal marine system of Svalbard using 18S and COI as barcodes. New barcodes were developed for several species to enlarge the existing international DNA barcode database, including eight species identified as potential Arctic alien species. In sediment samples of Svalbard ten potential Arctic alien species were observed. Two of these were identified to species level: the harpacticoid copepod *Euterpina acutifrons* and the colonial tunicate *Botrylloides violaceus*. Both have not been reported in Svalbard waters before. Metabarcoding techniques were shown to be highly suitable to assess the presence of alien species in Arctic marine waters. A factsheet was developed to inform the general public on the presence, impact and mitigation of marine alien species in Svalbard with emphasis on children, students and tourists.

Contents

Cover photo: Martine van den Heuvel-Greve

Background

Invasive species are non-indigenous or alien species that have been able to settle and reproduce in areas where they did not use to occur. Invasive species are considered the second most important threat to biodiversity after habitat loss, and they can have strong ecological and economic impact on local ecosystems (http://caff.is/invasive-species). Initial effects may be subtle, but consequences can be as large as impacting ecosystem stability or threatening the collapse of fisheries (Bax et al., 2003). Therefore, early detection and recognition of potentially invasive species are crucial tools for their management.

The number of documented established alien species, including invasive species, in the Arctic is currently low (Lassuy & Lewis, 2013). Ruiz and Hewitt (2009) described the Alaskan king crab as single invasive species known to have an established population in the Arctic Ocean, while Molnar et al. (2008) raised the number to nine species. The threat of invasive species is however high in environments where shipping traffic and the discharge of ballast water present ideal vectors for their spread (Brown et al., 2016). This is the case in the Arctic, with shipping activity increasing in recent years as a result of climate change induced declines in sea ice coverage. With the Arctic experiencing the highest rate in global warming over the past decades, this also enables alien species to settle into regions in which they previously could not survive and reproduce (figure 1)(Walther et al., 2009).

Marine alien species a mainly arriving on and around Svalbard via biofouling of ship hulls (e.g. tourist vessels) and ballast water discharges (of coal bulk carriers)(Ware et al., 2014). At least 23 alien species were recently recorded in ballast water samples of ships discharging their ballast water near Svalbard ports (Ware et al., 2016). However, none of these species have as yet been recorded as established in Svalbard waters (Ware, 2013). Increased shipping from tourism, scientific research, and mining in the past decades provide a high risk of the introduction of alien species in Svalbard. Additionally, recent warming of the Svalbard area may result in more suitable habitat conditions for introduced species, increasing the chance of settling invasive species (Førland et al, 2011).

Traditionally, visual methods are used for identification of invasive species. These methods are labour intensive and require specialist knowledge. Juvenile life stage are often not covered due to a lack of distinguishing features, but are critically important in the establishment and spread of invasive populations. The accuracy of traditional methods of species detection is also limited for species with low population densities (Brown et al., 2016).

Figure 1. Concept diagram relating invasion opportunity to anthropogenic greenhouse gas emissions, global climate change, ocean acidification, arctic shipping/vectors, and natural resource exploration/extraction (Fernandez, 2014). Arrows indicate directionality of effect, but not strength of interactions. Environmental effects of greenhouse gas emissions are depicted in white, human activities in light grey and invasion opportunity in dark grey.

An alternative approach to efficient species detection can be the use of molecular technologies. 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). When used to detect multiple species in a single sample based on high-throughput sequencing, this method is called DNA metabarcoding. This is particularly useful in monitoring the presence of species in water, soil or air samples, by the identification of so-called environmental DNA (eDNA), i.e. the DNA fragments that animals release into their living environment. The analysis of eDNA in environmental samples enables a quick and easy identification of possible invasive species living in the direct vicinity of the sampled site without the need for specialist taxonomic knowledge.

This project focused on a more comprehensive first overview of the occurrence of marine invasive species in coastal areas of western Svalbard by using eDNA metabarcoding as an innovative monitoring tool, and consisted of the following steps:

1) Update the worldwide DNA barcode library with currently identified Arctic marine invasive species;

2) Publish new data on the potential presence of Arctic marine invasive species in environmental samples of the western coast of Svalbard using eDNA techniques;

3) Develop an factsheet on marine invasive species at Svalbard for educational purposes.

Field work and methods

Barcoding – marker selection and sample collection

Two barcodes were selected based on the potential to differ between taxa and the availability of online reference data: 18S and COI. 18S was applied as broad screening identifying the main taxonomic groups among eukaryotes, whereas COI enables an identification of animals to a species level.

A list of potential Arctic aliens was derived from the 'SeaBasin Checkpoint – Arctic' webpage (http://www.emodnet-arctic.eu/alien-species). A first screening of the online barcoding libraries (e.g. Genbank, BOLD) of these species resulted in 23 species having full coverage, 48 species having partial coverage and 32 species having no coverage of these markers (either C01 or 18S).

22 Species were collected from locations where the species are known to be well established (Table 1). European specimens were collected by hand in various locations in the Eastern Scheldt in the Netherlands in August-September 2017. 15 Arctic species were collected from the Kongsfjorden, northwest Svalbard, in July 2017, and barcoded to serve as positive controls of native species in the environmental samples. Each collected specimen was morphologically identified to species level, stored in 97% ethanol in a plastic tube and transported to the laboratory of Wageningen Environmental Research for DNA sequencing.

eDNA – sample collection

Samples for eDNA analysis were collected in four locations in Kongsfjorden, northwest Svalbard, in July 2017 (Figure 2). A total of 30 marine sediment samples were collected using a Van Veen grab on-board the Teisten research vessel. Sampling was conducted nearshore in relatively shallow water (up to 52 meters in depth). Each 50-ml sample tube was filled with approximately 40 g of surface sediment (wet weight from the upper 1 cm layer) and was kept cool until return to the Kings Bay Marine Laboratory at the end of each sampling day, where it was stored frozen (-20°C). Samples were transported frozen to the environmental lab of Wageningen Environmental Research for DNA sequencing.

Table 1. Sample details of species collected for the production of COI and 18S barcodes. Potential Arctic alien species are highlighted (http://www.emodnet-arctic.eu/alien-species). No successful barcodes could be produced for names in red.

Area	Location	Site	Collection date	Phylum	Species
Arctic	Svalbard	Kongsfjord	July 2017	Arthropoda	*Monoculodes borealis*
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Nuculana pernula*
Arctic	Svalbard	Kongsfjord	July 2017	Annelida	*Nephtys sp.*
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Macoma calcarea*
Arctic	Svalbard	Kongsfjord	July 2017	Annelida	*Euchone analis*
Arctic	Svalbard	Kongsfjord	July 2017	Echinodermata	*Pelonaia corrugata*
Arctic	Svalbard	Kongsfjord	July 2017	Annelida	*Pectinaria hyperborea*
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Euspira sp.*
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Astarte borealis moerchi*
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Buccinum sp.*
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Mytilus sp.*
Arctic	Svalbard	Kongsfjord	July 2017	Arthropoda	*Gammarus setosus*
Arctic	Svalbard	Kongsfjord	July 2017	Rhodophyta	*Polysiphonia arctica*
Arctic	Svalbard	Kongsfjord	July 2017	Annelida	*Unknown*
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Mya sp.*
Europe	Netherlands	Oosterschelde	September 2017	Annelida	*Scoloplos armiger*
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Palaemon longirostris*
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Hippolyte varians*
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Gammarus locusta*
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Ammothea hilgendorfi*
Europe	Netherlands	Oosterschelde	September 2017	Mollusca	*Cerastoderma edule*
Europe	Netherlands	Oosterschelde	September 2017	Arthropoda	*Hemigrapsus takanoi*
Europe	Netherlands	Oosterschelde	September 2017	Cnidaria	*Actinia equina*
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Caprella mutica*
Europe	Netherlands	North Sea	September 2017	Arthropoda	*Cancer pagarus*
Europe	Netherlands	Oosterschelde	August 2017	Chordata	*Ciona intestinalis*
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Austrominius modestus*
Europe	Netherlands	Oosterschelde	August 2017	Mollusca	*Macoma balthica*
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Gammarus obtusatus*
Europe	Netherlands	Oosterschelde	August 2017	Annelida	*Nereis diversicolor*
Europe	Netherlands	Oosterschelde	August 2017	Annelida	*Arenicola marina*
Europe	Netherlands	Oosterschelde	August 2017	Tracheophyta	*Cotula coronopifolia*
Europe	Netherlands	Oosterschelde	August 2017	Ochrophyta	*Sargassum muticum*
Europe	Netherlands	Oosterschelde	August 2017	Chordata	*Botryllus schlosseri*
Europe	Netherlands	Oosterschelde	August 2017	Chlorophyta	*Codium fragile*
Europe	Netherlands	Oosterschelde	August 2017	Ochrophyta	*Fucus serratus*
Europe	Netherlands	Oosterschelde	August 2017	Rhodophyta	*Caulacanthus okamurae/ustulatus*

Generation of reference barcodes

DNA extraction for barcoding purposes (species) was conducted using the DNeasy Blood & Tissue Kit of Qiagen.

Primers and PCR protocols were identical for the reference barcoding and eDNA metabarcoding samples. For 18S, primers were based on Stoeck et al. (2010), amplifying a ~270bp fragment of the V4 region of the eukaryote SSU rRNA gene. We adopted their forward primer TAReuk454FWD1 while using an optimized version of their reverse primer TAReukREV3_(TAReukREV3_v1; 5'-ACTKTCGYTCWTGAYYRA-3'). For COI, primers were based on Leray et al. (2013), aplifying a ~bp fragment. Here, we adopted their reverse primer jgHCO2198, while using an optimized version of their forward primer mlCOIintF (mlCOIintF_v2; 5'-GGIACIGGITGRACWGTNTAYCCNCC-3'). Optimization consisted in both cases of creating a more degenerate version to increase amplification success for specific taxonomic groups (particulary Annelida, Arthropoda and Mollusca). In a direct comparison for the same eDNA samples,

these versions showed to detect a clearly higher taxon diversity than the original primers for the same samples. Forward and reverse primers were augmented with CS1 and CS2 tag sequences (Fluidigm, South San Francisco, CA, USA) to be used as sequence primers in Sanger sequencing (for reference barcodes, see below) and to allow the indexing PCR for multiplexed analysis via high-throughput sequencing (see below). PPCR reactions were performed in a 25µl reaction volume, consisting of 1U Platinum Taq (Fisher Scientific), 1x PCR buffer, 2.5 mM MgCl2, 5%(m/m) Trehalose, 200ng/µl BSA, 200µM dNTP and 250µM of each primer. The cycling program was as follows: 2 minutes at 94°C followed by 15 cycles of 30 seconds at 94°C, 3 minutes at 56°C reduced by 1°C each cycle and 1 minute at 72°C, followed by 20 additional cycles of 30 seconds at 94°C, 3 minutes at 42°C and 1 minute at 72°C and ended by a 10 minutes hold at 72°C.

PCR products were then sent to Macrogen for Sanger sequencing (F + R per sample) and final consensus sequences per sample were determined by hand using Seqman (DNAStar).

eDNA metabarcoding

DNA extraction from sediments (for metabarcoding purposes) was performed using the Powermax soil DNA isolation kit (Mobio) with the following adaptation from the kit protocol: dispersing of the sediment samples by vortexing with beads was replaced by a dispersing using an Ultra Turrax T25 High dispersing instrument equipped with an in house constructed titanium dispersing shaft, while keeping the samples cooled on ice.

PCR reactions were conducted following the above mentioned protocols for reference barcodes, The produced amplicons were then sent to Genome Quebec (Canada) for high-throughput sequencing. Here, a second PCR reaction was conducted to add sample-specific index barcodes and Illumina adaptor sequences, and the resulting amplicons, indexed amplicons were normalized and pooled per marker, and then sequenced in a 250bp paired-end run on a Illumina Miseq flow cell platform.

Raw sequence data was processed in R programming environment (R Core Team, 2017) and making use of functions available in the DADA2 packages (Callahan et al., 2016). Primer sequences were removed from the raw sequence reads and the read quality was inspected by plotting the quality scores per base position for each sample. The following filtering steps were carried out; sequences with undetermined nucleotides, exceeding the expected number of errors of two and reads contaminated with the Phix genome were removed. All reads were trimmed at the point where read quality dropped below a score of two. The minimum size for the forward and reverse read lengths were set by inspecting the quality plots and varied between 202 and 230 depending on gene and forward/reverse read). The DADA error model parameter learning logarithm was carried out on the sequences passing the filtering steps, followed by a dereplication step and inferring of the sequence variance using standard settings of the DADA functions 'derepFastq' and 'dada'. Forward and reverse reads were merged and a table containing the number of reads per unique sequence variant per sample was constructed. In a final step, chimeras were identified and removed.

All obtained unique sequence variants were blasted against NCBI nucleotide database for identification purpose using the BLAST® program. The top 50 best results (highest similarity between the unique sequence variant and the reference sequence) were stored for further identification purposes. Sequences were only identified when blast results contained records with an identity of 97% or higher. When in the selection of blast results with a minimum identify level of 99% contained records of just a single species the sequences was identified to that species. When more species were present in this selection identification to genus level was attempted. In this case, the selection of blast results with identify of 97% or higher were assigned to only one genus. If species from multiple genera were present, the sequences remained unidentified.

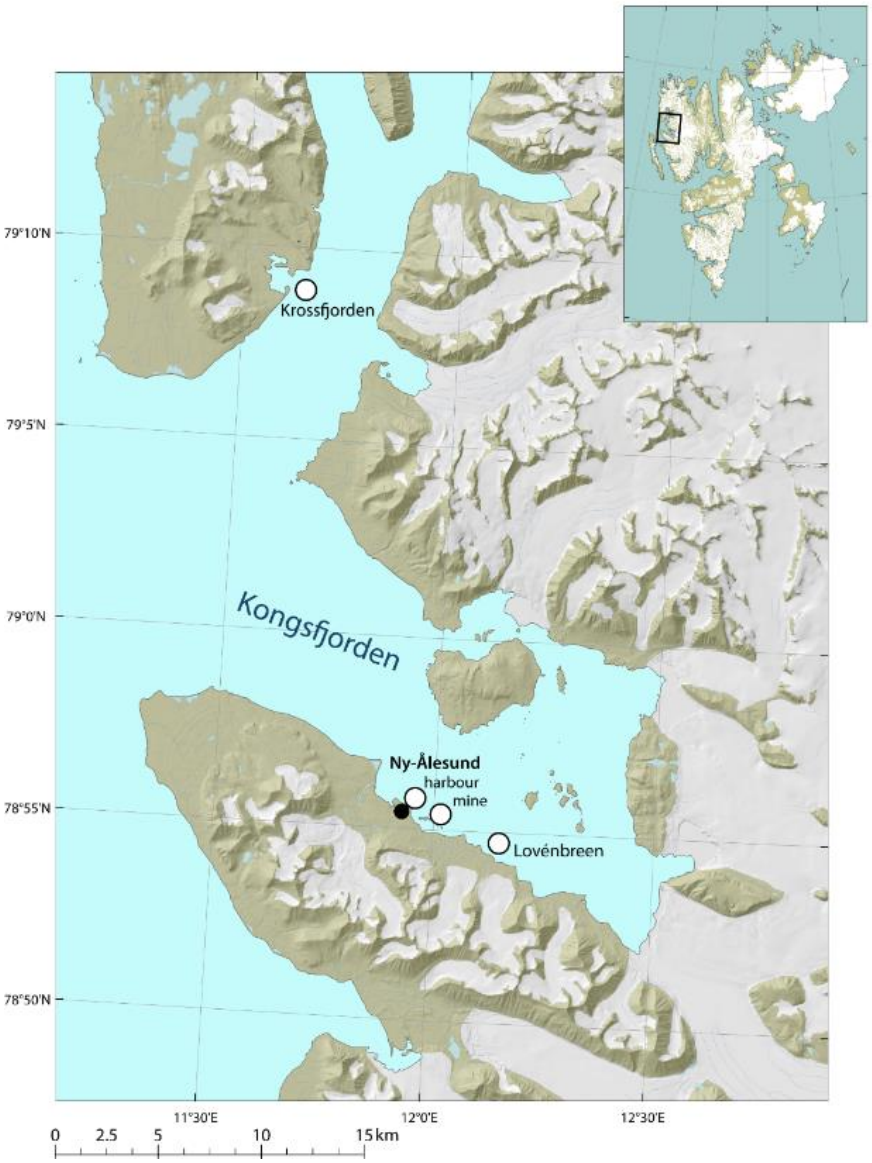

Figure 2. Sediment sampling sites (open circles) in Kongsfjorden and Krossfjorden, Svalbard, in July 2017.

Figure 3. Collection of sediment and species on-board the Teisten vessel, Kongsfjorden, 2017.

Results and discussions

Barcoding

New barcodes were developed for 31 of the 37 species collected for the 18S marker, and 28 of the species for the COI marker (Table 1). These barcodes were uploaded to the NCBI database. Successful barcodes could not be produced for the following six samples due to DNA or lipid interference: an unknown tube worm and Mya sp. from Kongsfjorden, the tunicate *Botryllus schlosseri* and the macroalgal species *Codium fragile*, *Fucus serratus* and *Caulacanthus okamurae/ustulatus*, collected from the Oosterschelde (see red coloured names in Table 1). Five of the freshly barcoded Arctic species were positively identified in the sediment samples collected in the Kongsfjorden. This included four species based on the COI marker (*Macoma calcarea*, *Astarte borealis moerchi*, *Mytilus* sp. and *Polysiphonia arctica*) and one species using the 18S marker (*Pelonaia corrugata*).

Of the initial potential Arctic alien species list (http://www.emodnet-arctic.eu/alien-species), ten species were identified in 30 sediment samples collected in the Kongsfjorden; five using the 18S marker, and five using the COI marker (Table 2). One identified species on this list, *Oithona similis*, was considered an alien species for Canadian ports (Brown et al. 2016). It is however ubiquitous and widespread throughout other parts of the Arctic, and occurs naturally in Svalbard (Ormanczyk et al., 2017). It was therefore omitted from our (potential) Arctic alien species list.

Most of the potential Arctic alien species were identified to a higher taxonomic level than specified in the list of potential Arctic alien species (Table 2). Based on genus level no confirmation on the presence of potential Arctic alien species could be made as many of the these taxa include species native to Svalbard (Gulliksen et al. 1999, Varpe, 2012, Sikorski & Pavlova, 2015). This highlights the need for species-level identification when identifying alien species. Incomplete identification of species was generally due to an incomplete DNA barcode references in the barcode library, and/or to the 18S and COI markers being inadequate to distinguish between species within a genus. Further development of the DNA database, and refining of the metabarcoding method will allow more detailed identification so that alien species sharing a genus with native species can be successfully distinguished in the future.

Two potential alien species were identified to species level in the sediment samples: the harpacticoid copepod *Euterpina acutifrons* and the tunicate *Botrylloides violaceus* (Table 2). *E. acutifrons* has not previously been recorded in the Svalbard region. It was earlier detected as an alien species in Canadian East Arctic-West Greenland and Hudson Bay, with vessels as single possible vector and an unknown source region (Brown et al. 2016; Chan et al 2018). There is little information available on the potential impacts of *E. acutifrons*. As a planktonic copepod its impact is likely to be limited to minor changes in the zooplankton species assemblage, but its ecological function is not known.

Botrylloides violaceus was found in only one sediment sample in Ny-Ålesund harbour. *B. violaceus* is an invasive colonial tunicate that may cause significant negative impacts, both environmentally and economically (Carver et al., 2006). *B. violaceus* was thought to be introduced to North America as 'hitchhiker' in the shellfish aquaculture trade, and tunicates can be transported by vessel hull fouling (Lejeusne et al., 2011). It can also be travel on floating debris, making floating plastic a potential vector of introduction (Carver et al., 2006). It can overgrow existing hard substrates and become a nuisance fouling organism on equipment and ships, increasing maintenance costs. It can smother other species, compete with filter feeders for food, and potentially lead to changes in the local biodiversity (Carver et al., 2006). Water temperature, also in the near future, may not favour the sexual reproduction of *B. violaceus* in the Svalbard area, but temperatures in west Svalbard fjords are within its survival range (Reimer et al., 2017). Impact of *B. violaceus* in other invasion areas indicates that targeted action to prevent further introduction and spreading of *B. violaceus* is needed on Svalbard.

Table 2. Potential Arctic alien species observed in sediment samples of Kongsfjorden, Svalbard, in July 2017 (based on species listed by EMODnet Arctic).

Phylum	**Species name in samples**	**18S**	**COI**
Arthropoda	*Acartia sp.*	X	
	Euterpina acutifrons	X	
	Calanus sp.	X	
	Balanus sp.		X
Chordata	*Molgula sp.*	X	
	Botrylloides violaceus		X
Nematoda	*Daptonema sp.*	X	
Mollusca	*Mytilus sp.*		X
Ochrophyta	*Fucus sp.*		X
Annelida	*Scolelepis sp.*		X

Conclusions

The project resulted in:

1) the development of 31 new barcodes for the 18S marker, and 28 new barcodes for the COI marker.

2) the identification of two potential Arctic alien species (*B. violaceus* and *E. acutifrons*) in sediment samples of Svalbard waters. Based on the impacts of *B. violaceus* in other areas, potential effects on the coastal ecosystem on Svalbard needs to be evaluated and possible vectors for introduction to Svalbard identified. In the meantime, targeted actions are advised for this species (e.g ballast water treatment and cleaning of hulls of vessels to prevent *B. violaceus* from further entering the area).

3) the development of a factsheet on marine invasive species at Svalbard for educational purposes (see Appendix A).

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Appendix A - Flyer

MARINE ALIENS ON SVALBARD

Climate change and increasing human activity in the Svalbard region raise the risk of the introduction of marine alien species.

Alien species are organisms that have been introduced into an environment where they do not occur naturally...

...most marine alien species are introduced to Svalbard by shipping or floating debris:

Organisms can hitch a ride on the hulls of ships

Organisms can attach to discarded floating (plastic) objects and travel hundreds of kilometres on ocean currents

Larvae and microscopic organisms can travel long distances in the ballast water of ships and be released into Svalbard waters

SVALBARD ENVIRONMENTAL PROTECTION FUND

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Alien species can put native species at risk and disrupt the local food web. Some can threaten local ecotourism and fishing industries

The tunicate *Botrylloides violaceus* is a marine alien that grows on hard surfaces like ship hulls and floating objects

Preventing the introduction of marine alien species will help preserve the delicate ecosystem of Svalbard

The crab *Hemigrapsus takanoi* is a potential marine alien that can hitch a ride in equipment, among shellfish or as larvae in ballast water

Scientists at WMR are developing efficient monitoring methods to identify marine alien species around Svalbard. With this knowledge, effective prevention methods can be developed

WHAT CAN YOU DO TO PREVENT THE INTRODUCTION OF MARINE ALIEN SPECIES TO SVALBARD?

Are you a local?

If you see a marine species that you suspect to be alien, report it to us!

Observation.org
Together you'll discover more!

- Take a picture
- Record the date and place
- Submit this information (incl. photo) to: http://svalbard.observation.org
  Observations are easy to add online or via the available apps (ObsMapp or iObs)
- See instruction video on https://youtu.be/xGksc6xHrd4

Are you a visitor?

Keep your equipment and clothing clean to avoid the accidental transfer of (microscopic) organisms.
Dry equipment thoroughly before you use it on Svalbard.

Are you a boat owner or user?

Clean the hull of your vessel and treat your ballast water in an effective way prior to discharge, before you arrive in Svalbard waters.

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For more information:
Wageningen Marine Research (WMR)
www.wur.eu/arctic-aliens

WMR PROJECT (2017-2018)
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DEVELOPING EFFECTIVE NOVEL DNA METHODS TO DETECT MARINE ARCTIC ALIENS

Step 1:
Collection of local and potential Arctic alien species

Step 2:
DNA analysis of collected organisms to build a DNA database of relevant species

Step 3:
Scanning DNA in environmental (sediment) samples from Svalbard to detect marine aliens using the DNA database

Our results so far:

- Addition of 31 species to the DNA database (of which 8 potential aliens for Svalbard)
- Identification of at least one marine alien species in sediment at Kongsfjorden in 2017

This project was funded by the Svalbard Environmental Protection Fund and the Knowledge Base Program 'System Earth Management' of Wageningen University and Research.

Appendix B – Communication & outreach

Communication of the project:

-Project page on the WMR website: https://www.wur.nl/en/project/Arctic-Aliens.htm

-Poster presentation Arctic Frontiers, January 2018, Tromsø, Norway (see next page)

-Flyer distribution on the following locations on Svalbard in August 2018:

- Hall UNIS – university centre Longyearbyen
- Entrance Svalbardbutikken – supermarket Longyearbyen
- Entrance Longyearbyen Public Library
- Office Port of Longyearbyen
- Plancius vessel, Oceanwide Expeditions

-Tourist presentation on Oceanwide Expeditions cruise on Plancius, 29 August - 5 September 2018

-Research interview on website Oceanwide Expeditions: https://oceanwide-expeditions.com/blog/arctic-invaders-interview-with-a-marine-ecotoxicologist

Poster presented at the Arctic Frontiers conference in Tromsø, Norway, January 2018.

Arctic Aliens:

mapping the presence of marine alien species in west Svalbard

Martine van den Heuvel-Greve[1], Anneke van den Brink[1], Frits Steenhuisen[3], Michiel Klaassen[1], Mare van den Heuvel, Sander Glorius[1], Ivo Laros[2], Jan Bovenschen[2], Arjen de Groot[2]

WAGENINGEN UNIVERSITY & RESEARCH

Background

Globally, alien species are considered the third most important threat to biodiversity after habitat loss and fishery. Alien species have the potential to impact the environment and economy by disrupting the ecological system. To better understand what species are, or could be introduced to the Arctic, it is necessary to have a fast, efficient and accurate monitoring method for identifying alien species. We developed and tested a DNA technique that allows an easy detection of the presence of multiple species in a water or sediment sample.

Objectives

- To enlarge the current DNA barcoding database with species that are listed as Arctic invasive species;
- To assess the presence of currently described invasive species in the coastal area of western Svalbard;

Methods

DNA barcoding

A list of 101 species identified as alien species for the Arctic was used as background (http://www.emodnet-arctic.eu/alien-species).

Specimens of 37 species were collected from Svalbard and origin locations for potential Arctic alien species (including the Netherlands). Various methods were used in different habitats to target a variety of species. The specimens were sent to the lab for DNA barcoding to supplement the existing international DNA barcode reference library.

Metabarcoding

32 Sediment samples were collected in the Kongsfjorden, Svalbard. These will be used to assess the presence of alien species *en masse* using a metabarcoding technique.

Figure 1. Specimen collection in Kongsfjorden, Svalbard, (left) and from origin locations of alien species in the Arctic (right: Oosterschelde, the Netherlands) for DNA barcoding.

Figure 2. Collection of sediment samples in Kongsfjorden, Svalbard, for analysis of presence of alien species *en masse*.

Results

DNA barcoding

Of the 37 collected species new barcodes could be developed for 31 species for 18S and 28 for CO1 (Table 1). This includes eight species identified as Arctic aliens.

Table 1. Number of successful development of new barcodes (18S and CO1) for Arctic and temperate marine species.

Area	Location	Site	Barcode 18S	Barcode CO1	Identified as Arctic aliens
Arctic	Svalbard	Kongsfjorden	13	11	-
Europe	Netherlands	North Sea	1	1	1
Europe	Netherlands	Oosterschelde	17	16	7
	Total		31	28	8

Figure 3. Examples of specimens of reported Arctic aliens for which successful new barcodes were developed. From left to right: *Hemigrapsus takanoi*, *Cotula coronopifolia* and *Capreila mutica*.

Metabarcoding

The metabarcoding of sediment samples is still in progress. These will be used to determine the presence of marine aliens in Kongsfjorden, Svalbard, by comparing metabarcoding results with the updated DNA barcode reference library.

Conclusions

- New barcodes have been developed for 31 species, of which eight are known Arctic marine alien species.
- With this information we are in the process of further developing the metabarcoding technique for identifying alien species in environmental samples.

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[1] **Wageningen Marine Research**
P.O Box 77, 4400 AB Yerseke, the Netherlands
Contact: martine.vandenheuvel-greve@wur.nl
T +31 317 483823
www.wur.eu/marine-research

[2] **Wageningen Environmental Research**
PO Box 47, 6700 AA Wageningen, the Netherlands
Contact: g.a.degroot@wur.nl
T +31 317 485926
www.wur.eu/environmental-research

[3] **Arctic Centre – University of Groningen**
PO Box 716, 9700 AS Groningen, the Netherlands
Contact: f.steenhuisen@rug.nl
T +31 50 36 36832
https://www.rug.nl/research/arctisch-centrum/