Miljøministeriet
Miljøstyrelsen

Application of DNA-based tools for monitoring and management of NIS in the Danish seas

GEANS workshop
26. October 2022
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NIS monitoring obligations

EU - Regulation on Invasive Alien Species 2014

- The aim of the regulation is to provide member states with a set of measures to combat invasive alien species

UN - The ballast Water Management Convention 2017

- The aim is to prevent the spread of potentially harmful aquatic organisms and pathogens in ships' ballast water
- Standards for treatment and management of ballast water
- Monitoring is needed for management purposes in order to assess potential dispensations

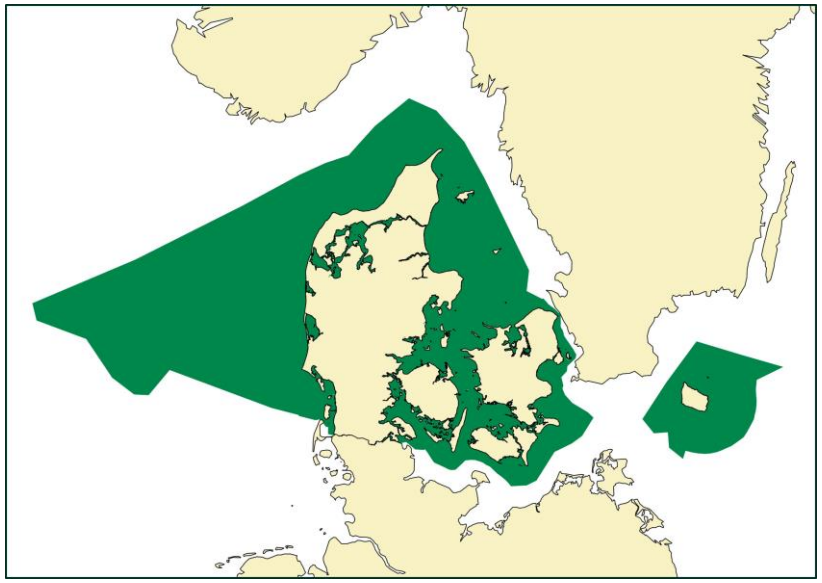
EU Marine Strategy Framework Directive 2008

- The aim of the directive is to achieve or maintain Good Environmental Status (GES) in the marine environment, while allowing for sustainable use of marine ressources
- Member states are required to implement monitoring programmes that makes it possible to assess GES

Marine Strategi Framework Directive

Descriptor

1. Biodiversity
2. **Non-indigenous species**
3. Commercial fish and shellfish
4. Food webs
5. Eutrophication
6. Sea-floor integrity
7. Hydrographical conditions
8. Contaminants
9. Contaminants in seafood
10. Marine litter
11. Energy incl. underwater noise



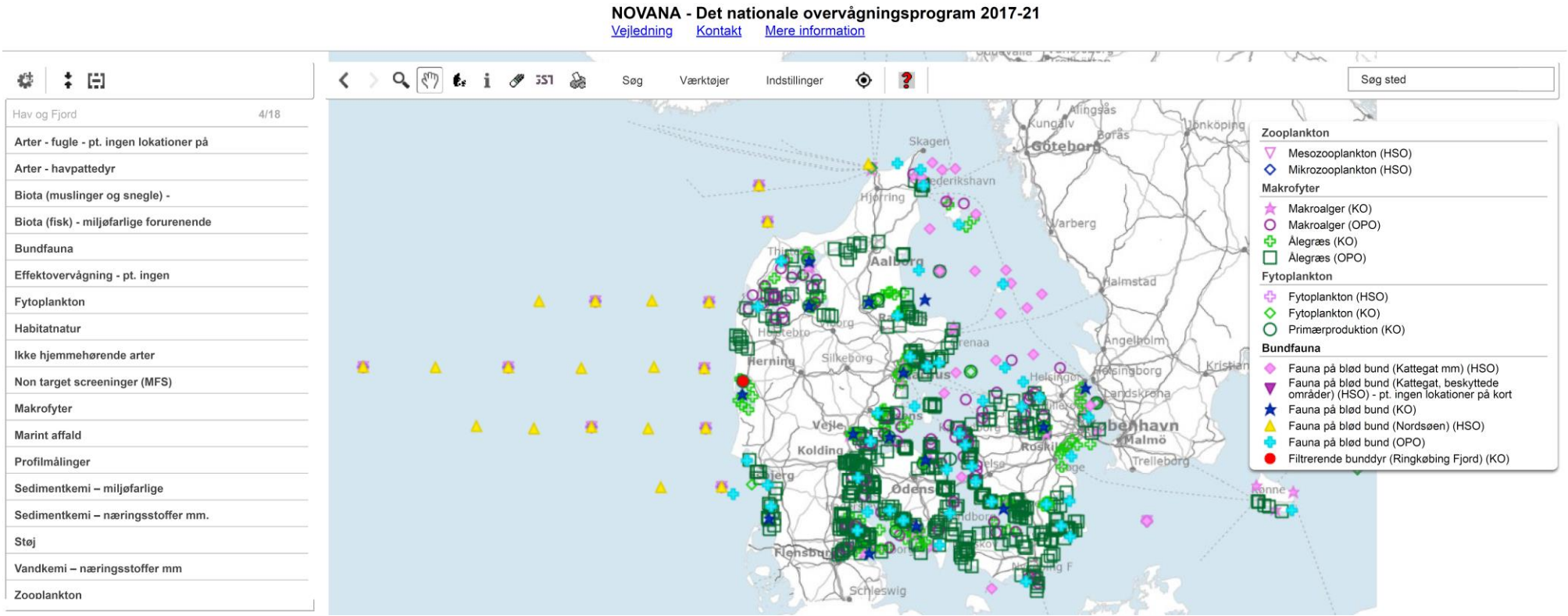
2017 revision – Commision Decision on Good Environmental Status (GES-Decision)

- Defines a set of criteria and standards for determining GES
- Primary and secondary criteria

NIS Criteria

- D2C1 – Primary: Number of newly-introduced NIS
- D2C2 – Secondary: Abundance and spatial distribution of established NIS
- D2C3 – Secondary: Negative effects of NIS

Developing a strategi for NIS monitoring



NOVANA station map: https://miljoegis.mim.dk/cbkort?profile=novana2017-21

- Macrophytes
- Phytoplankton
- Zooplankton
- Bottom Fauna

Fish monitoring by the Ministry of Food, Agriculture and Fisheries

Developing a strategi for NIS monitoring

2014 - Tender to analyse data and design a cost-effective monitoring programme

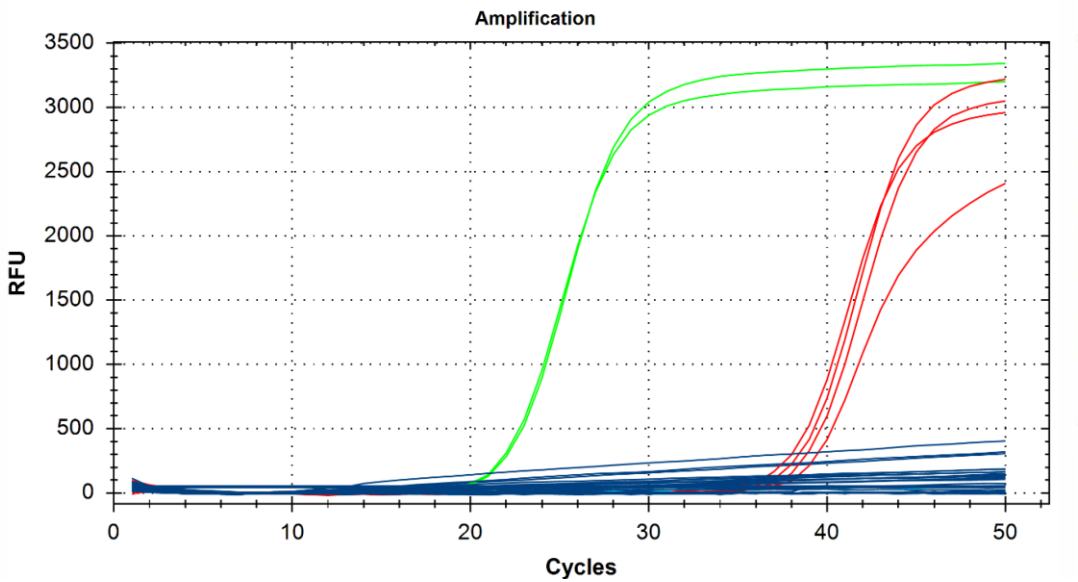
Published by the EPA in 2014 ”*Monitoring of non-indigenous species in Danish marine waters. Background and proposals for a monitoring strategy and a monitoring network”. MONIS 1 report.*

- Conventional NOVANA monitoring methods should be improved to target NIS (if possible)
- Conventional monitoring should be supplementet with eDNA monitroing – Water sampling at NOVANA stations and hot spots (habours).

Developing eDNA based monitoring

MONIS 2 (2016) / MONIS 3 (2018)

- National Target Species list based on five criteria (Established NIS, Potential NIS, Invasiveness, Ease of determination, Ease of determination by eDNA) (50 species)
- Draft technical guidlines for sampling, storage and analysis
- In vitro and in vivo test of in sillico developed detection systems on extracted DNA from non-target and target species (22 species).



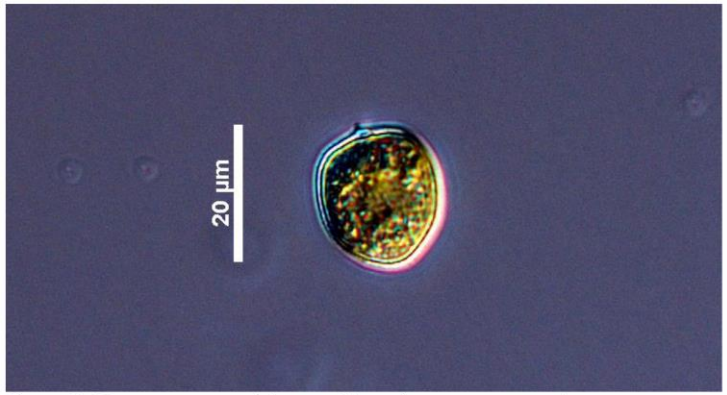


Figure 2.1 Prorocentrum minimum. *Photo from www.eoas.ubs.ca.*

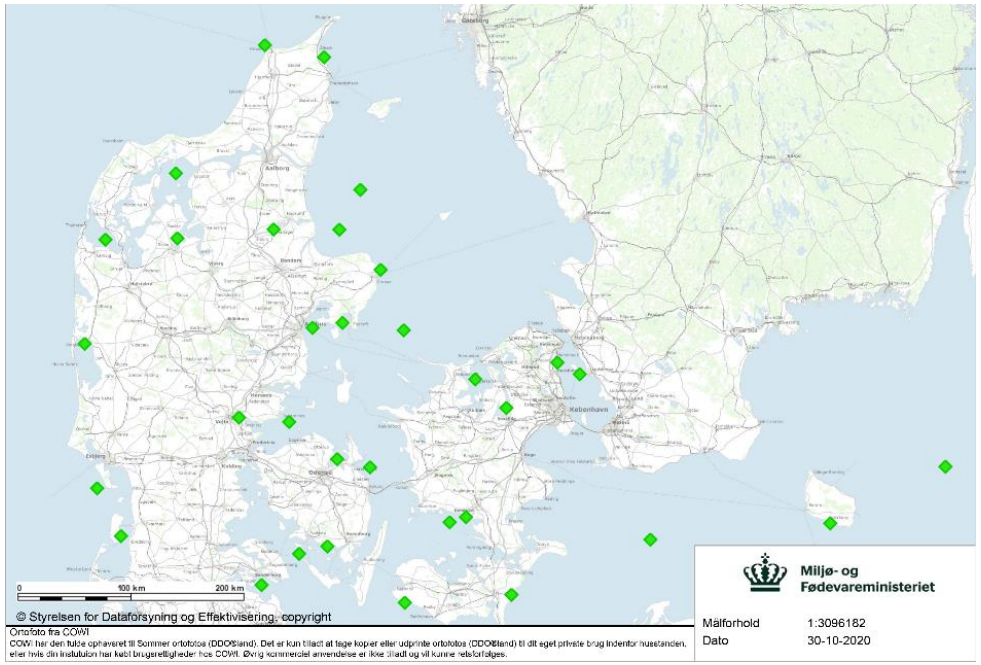
Amplification of Prorocentrum species using the F3R3P3 assay. Target species P. minimum is shown in green and non-target sister species in blue. P. triestinum showed amplification with assay (marked in red).

Developing eDNA based monitoring

Since 2017 the EPA has been collecting water samples for eDNA

- 33 Annual stations, of which 16 are sampled in both spring and fall

- Macrophytes (10 stations)
- Phytoplankton (5 stations)
- Phyto- and zooplankton (8 stations)
- Bottom Fauna (10 stations)
- Eelgrass (2 stations)



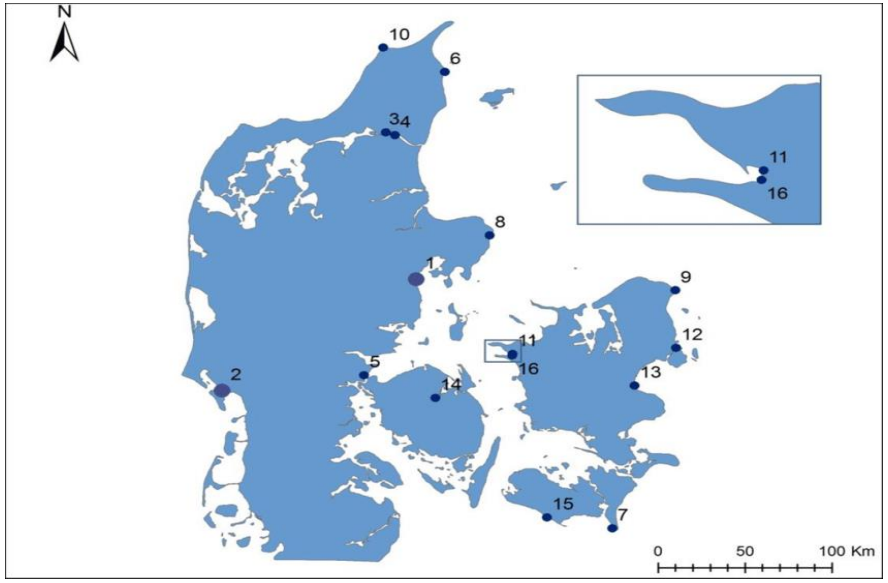
Developing eDNA based monitoring

MONIS 4 (2017-2018) (EHFF funded)

- Test and prof of concepts for 21 of the developed detection systems in 16 Danish ports
- Results available here: https://niva.brage.unit.no/niva-xmlui/handle/11250/3024342
- In addition, JHP Port Survey protocol at 2 ports.
- Detection of 8 NIS with eDNA

Table 4.11: *Monitoring by eDNA in Sep-Oct 2017. The numbers divided by slashes indicates the number of technical qPCR replicates that resulted in: No Cq / Below LOD / Above LOD below LOQ / Above LOQ. The color coding reflects the highest amplifcation level, and thereby reflects the level of eDNA detected. White equals 'NoCq' - i.e. no amplification in any of the replicates, which reflects no target eDNA present in the water sample. Yellow equals at least one replicate below LOD. Orange equals at least one replicate above LOD but below LOQ. The yellow and orange coloring reflects there is an inadequate amount of target DNA to obtain conclusive detection. Red equals at least one replicate above LOQ. Black equals all three replicates above LOQ. The red and black colorings reflect a sufficient level of target DNA to confirm the detection of the invasive species by eDNA.*

Species	Assay IDNo	AalborgHavn	Aalborgportland	Aarhus	Esbjerg	Fredericia	Frederikshavn	Gedser	Grenå	Helsingør	Hirtshals	Kalundborg	KalundborgStatiol	København	Køge	Odense	Rødby
Bonnemaisonia hamifera	1	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'0/2/0/1'
Prorocentrum cordatum	2	'0/3/0/0'	'0/3/0/0'	'3/0/0/0'	'2/1/0/0'	'0/3/0/0'	'0/3/0/0'	'0/0/0/3'	'0/3/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'0/0/0/3'	'0/0/0/3'	'0/2/0/1'	'0/0/0/3'
Pseudochattonella farcimen	3	'0/1/0/2'	'0/0/0/3'	'0/3/0/0'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/3/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'2/1/0/0'
P. verruculosa	4	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/2/0/1'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/1/0/2'	'3/0/0/0'
Karenia mikimotoi	5	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'
Carassius auratus	6	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Cyprinus carpio	7	'3/0/0/0'	'2/1/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Colpomenia peregrina	8	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'2/1/0/0'	'0/0/0/3'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Neogobius melanostomus	09	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'0/3/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'0/3/0/0'
Oncorhynchus mykiss	10	'1/2/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Oncorhyncus gorbuscha	13	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Crassostrea gigas	14	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/2/1/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Mya arenaria	15	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'1/2/0/0'
Rhithropanopeus harrisii	16	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'
Eriocheir sinensis	18	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Cordylophora caspia	21	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Mnemiopsis leidyi	22	'0/0/0/3'	'0/0/0/3'	'2/1/0/0'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'0/1/0/2'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'0/3/0/0'	'0/3/0/0'	'0/0/0/3'
Acipenser baerii	23	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'



Developing eDNA based monitoring

MONIS 5 (2018-2020)

- Analysis of NOVANA samples collected from 2017-2020
- Further development of technical guidelines for sampling and eDNA analysis (**https://www.researchgate.net/publication/338740378_Tekniske_anvisninger_for_eDNA-baseret_overvagning_af_ikke-hjemmehorende_marine_arter)**)
- Testing of new detection systems, and revisiting old systems (Now 24 system in operation)
- Results from 2017-2018 reported in the NISAR project

MONIS 6 (2021-2023)

- Work continues with analysing samples from 2021-
- Developing new detection systems

Port monitoring (2021-2022)

- 6 harbours (spring/fall)
- Water samples and eDNA analysis
- Reduced JHP setup

Future perspectives for eDNA in NOVANA

- A database and a quality control system are still under development
- The conventional methods in the NOVANA programme are still the backbone in NIS monitoring, however, eDNA based monitoring show good synergy with the conventional methods
- It is likely that eDNA monitoring will be used more broadly in NOVANA over time.
- eDNA based monitoring have a potential in monitoring related to other MSFD descriptors e.g. Biodiversity (D1), Food webs (D4), and Sea-floor integrity (D6)
- The current species specific approach have pros and cons
  - Considered to have high qualtity and low detection levels
  - You can only screen for select species (more suited for D2C2)
  - Limitations for the development of new detection systems
- Therefore, the EPA is interested in metabarcoding, as it is perhaps more suited for D2C1 monitoring, and has fewer of the above mentioned limitations. However, high quality of detection is paramount.
- Still fairly expensive to use eDNA, and as such we are interested in cost effective proof of concept

List of marine detection systems

Common name (Danish)	Species	Habitat
sortmundet kutling	Neogobius melanostomus	marine
regnbueørred	Oncorhynchus mykiss	marine
pukkellaks	Oncorhyncus gorbuscha	marine
Siberisk stør	Acipenser baerii	marine
Torsk	Gadus morhua	marine
rødspætte	Pleuronectes platessa	marine
skrubbe	Platichthys flesus	marine
Almindelig makrel	Scomber scombrus	marine
Atlantisk sild	Clupea harengus	marine
trepigget hundestejle	Gasterosteus aculeatus	marine
rødtot alge	Bonnemaisonia hamifera	marine
dinoflagelat	Prorocentrum minimum	marine
heterokont flagelat	Pseudochattonella farcimen	marine
heterokont flagelat	Pseudochattonella verruculosa	marine
dinoflagelat	Karenia mikimotoi	marine
østerstyv	Colpomenia peregrine	marine
sortmundet kutling	Neogobius melanostomus	marine
regnbueørred	Oncorhynchus mykiss	marine
pukkellaks	Oncorhyncus gorbuscha	marine
stillehavsøsters	Magallana gigas	marine
sandmusling	Mya arenaria	marine
mudderkrabbe	Rhithropanopeus harrisii	marine
kamtjatka krabbe	Paralithodes camtschaticus	marine
kinesisk uldhåndskrabbe	Eriocheir sinensis	marine
amerikansk hummer	Homarus americanus	marine
Brakvandskrølle_polyp	Cordylophora caspia	marine
amerikansk ribbegoble	Mnemiopsis leidyi	marine
blå svømmekrabbe	Callinectes sapidus	marine
Stribet klippekrabbe	Hemigrapsus sanguineus	marine
pensel klippekrabbe	Hemigrapsus takanoi	marine