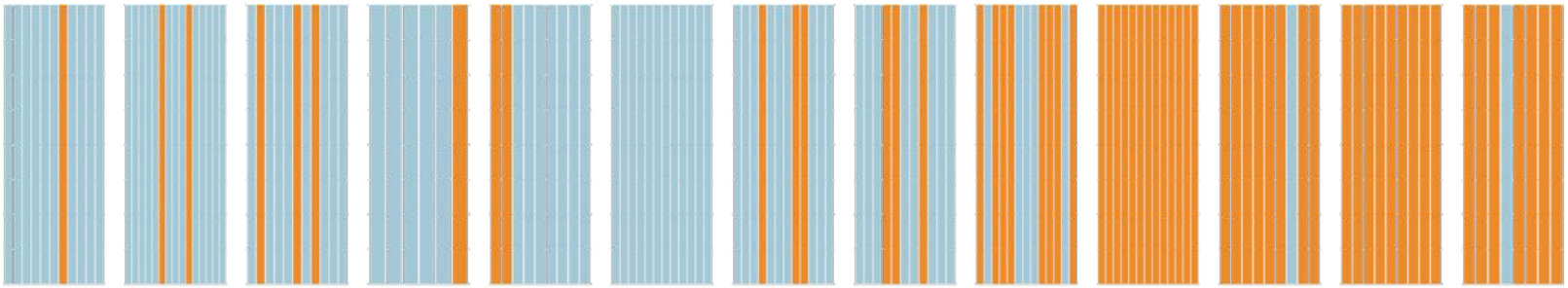
Genetics in support of fisheries and aquaculture management

17-19 September
Faro, Portugal

ICES CIEM | UAlg | CCMAR Centro de Ciências do Mar



Challenges and perspectives for application of e-DNA to marine monitoring and fisheries management

18 September
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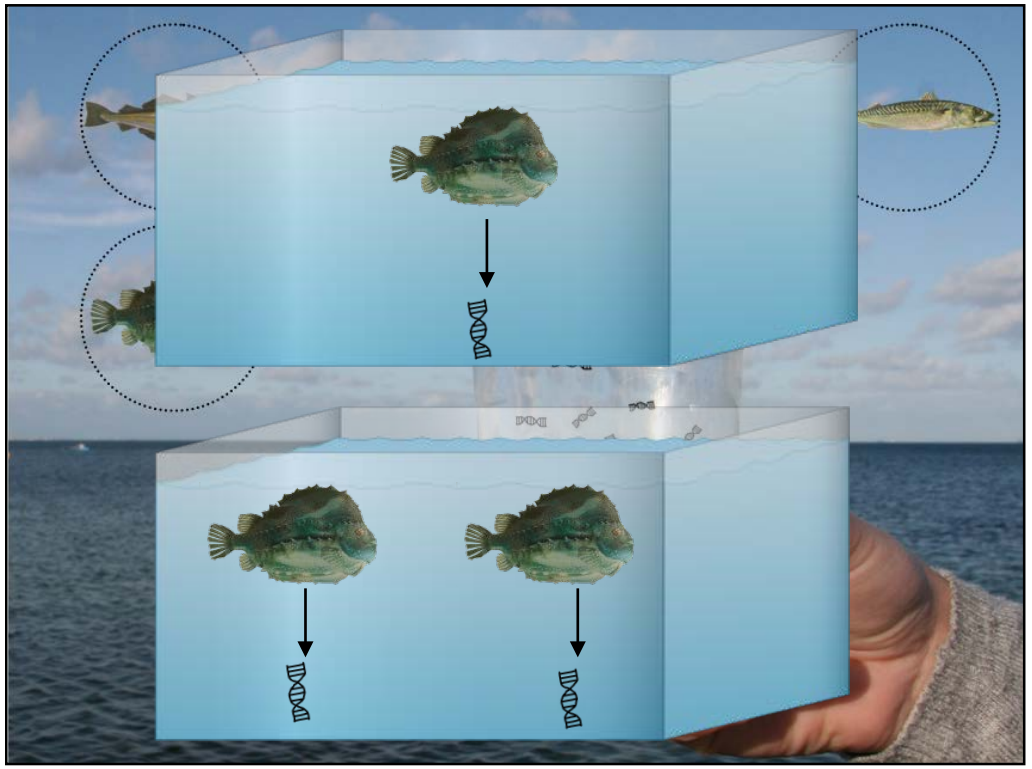
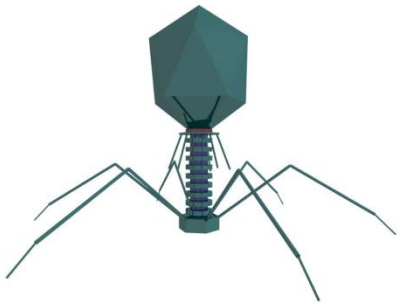
What is environmental DNA, “eDNA”?

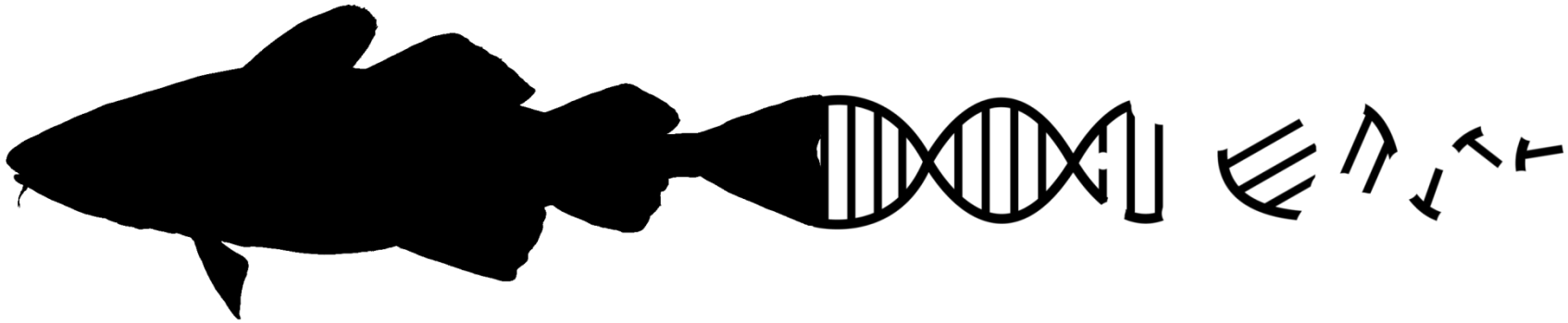
- Genetic material retrieved from an environmental sample (soil, air, water) without obvious traces of the organism(s) subject to monitoring



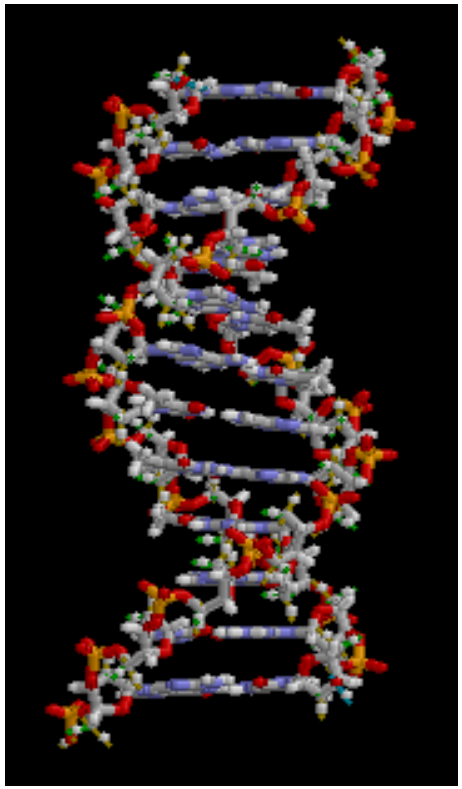
What can be monitored?

- Potentially all organisms from “virus to whales”





Procedure for e-DNA analysis



Two approaches:

Next generation sequencing and “meta-barcoding”

- Which species are present?

Quantitative PCR “qPCR” with species specific primers

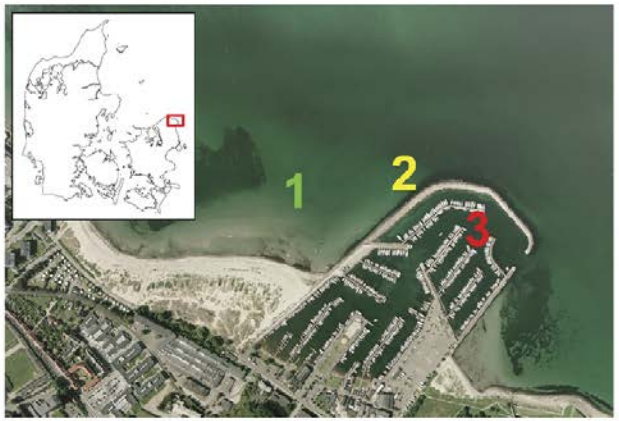
- Is this (are these) species present?

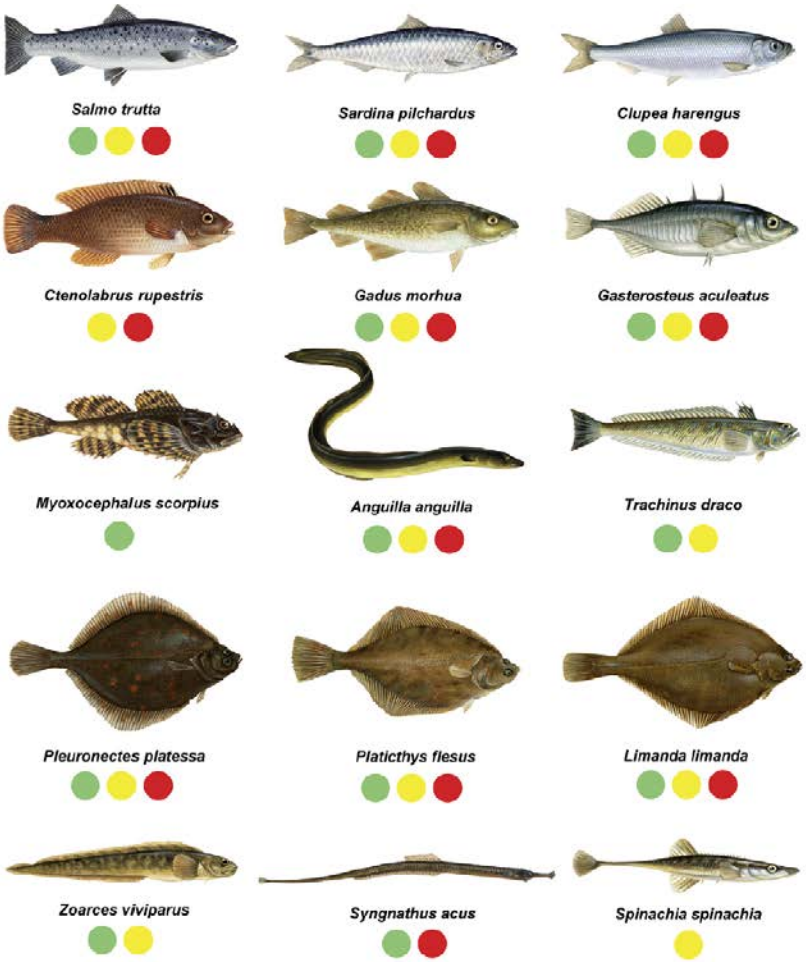
Technical challenges not the focus of this presentation

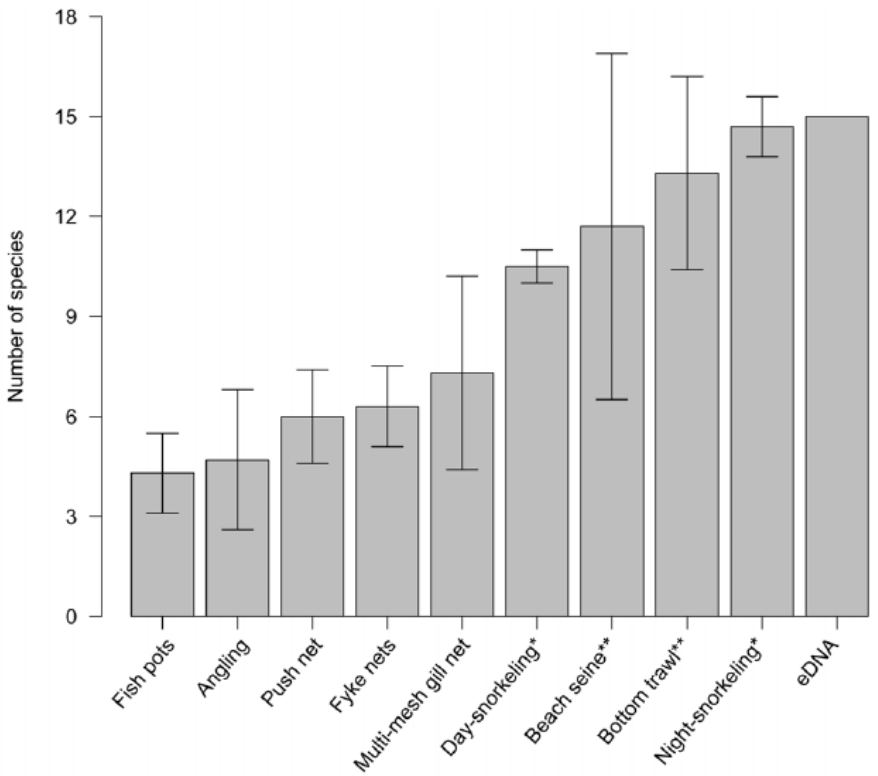
Why use eDNA?

- **Sensitivity**
  - Better than traditional monitoring methods?
- **Non-invasive**
- **Easy to standardise**
  - Fixed volume of water (Coke bottle)
  - Anyone can collect water (citizen science)
- **DNA is universal**
  - High taxonomic resolution
  - Objective species identification without specialised expertise
- **Costs**
  - (Dana ≈ *200.000 DKK per day)*

The beginning in DK







Thomsen et al. 2012

Marine eDNA monitoring – ”the new black”

Title:

Assessing Vertebrate Biodiversity in a Kelp Forest Ecosystem using Environmental DNA

Jesse A. Port[1*], James L. O'Donnell[2], Of

Litvin[3], Kerry J. Nickols[3,4], K

MiFish, a set of universal PCR primers for metabarcoding

ental DNA from

more

Environmental DNA as a 'Snapshot' of Fish Distribution: A Case Study of Japanese Jack Mackerel in Maizuru Bay, Sea of Japan

Satoshi Yamamoto[1*‡*], Kenji Minami[2*‡*], Keiichi Fukaya[3*‡*], Kohji Takahashi[4], Hideki Sawada[4], Hiroaki Murakami[4], Satsuki Tsuji[5], Hiroki Hashizume[1], Shou Kubonaga[6], Tomoya Horiuchi[4], Masamichi Hongo[5], Jo Nishida[5], Yuta Okugawa[5], Ayaka Fujiwara[7], Miho Fukuda[7], Shunsuke Hidaka[7], Keita W. Suzuki[4], Masaki Miya[8], Hitoshi Araki[9], Hiroki Yamanaka[5], Atsushi Maruyama[5], Kazushi Miyashita[10], Reiji Masuda[4], Toshifumi Minamoto[1], Michio Kondoh[5]

Science & Environment

Shark eDNA study could be 'game-changer'

By Mark Kinver
Environment reporter, BBC News

18 March 2015 | Science & Environment

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Marine Fishes in a

and

ONE

Scientists Identify Marine Life From DNA in Seawater

January 15, 2014
By Liz Hambleton, Science Communications Early Career Fellow

Innovative tool that examines tissue and cells left in water promises new way to monitor ocean species

Scientists used the Monterey Bay Aquarium's Open Sea tank to test new DNA sequencing technology to identify marine animals.
Photo credit: *Randy Wilder, Monterey Bay Aquarium*

Investigating

(eDNA) for G

Using

Large

of Marine Mammals

Ryan P. Kelly[1,2*], Jesse

1 Center for Ocean Solutions, Woods Institute

Environmental Affairs, University of Washington, Seattle,

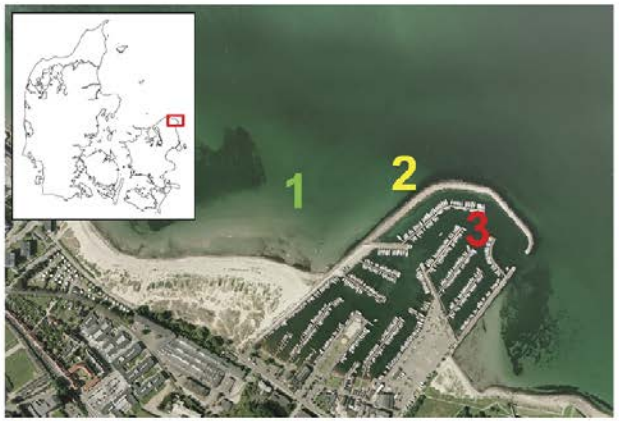
Andrew D. Foote[1*§], Philip

Anders Galatius[2], Ludovic Orlando[1], M. Thomas P. Gilbert[1]

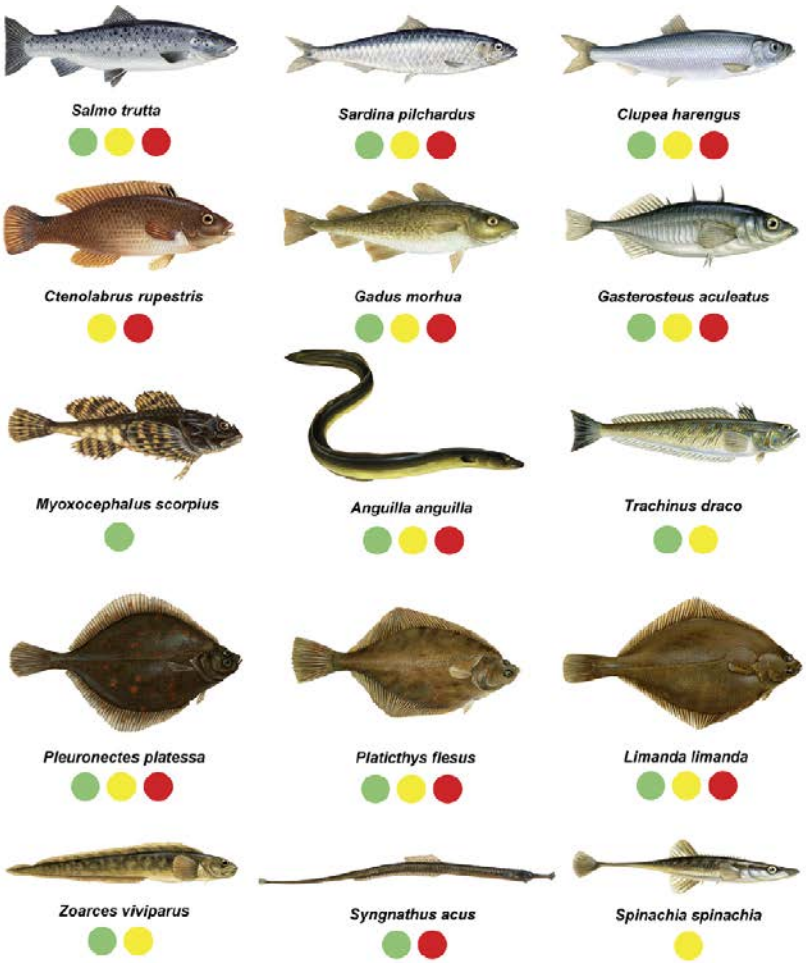
Signe Sveegaard[2], Magnus Wahlberg[3,4], Jos Kielgast[1], Line A. Kyhn[2], Andreas B. S

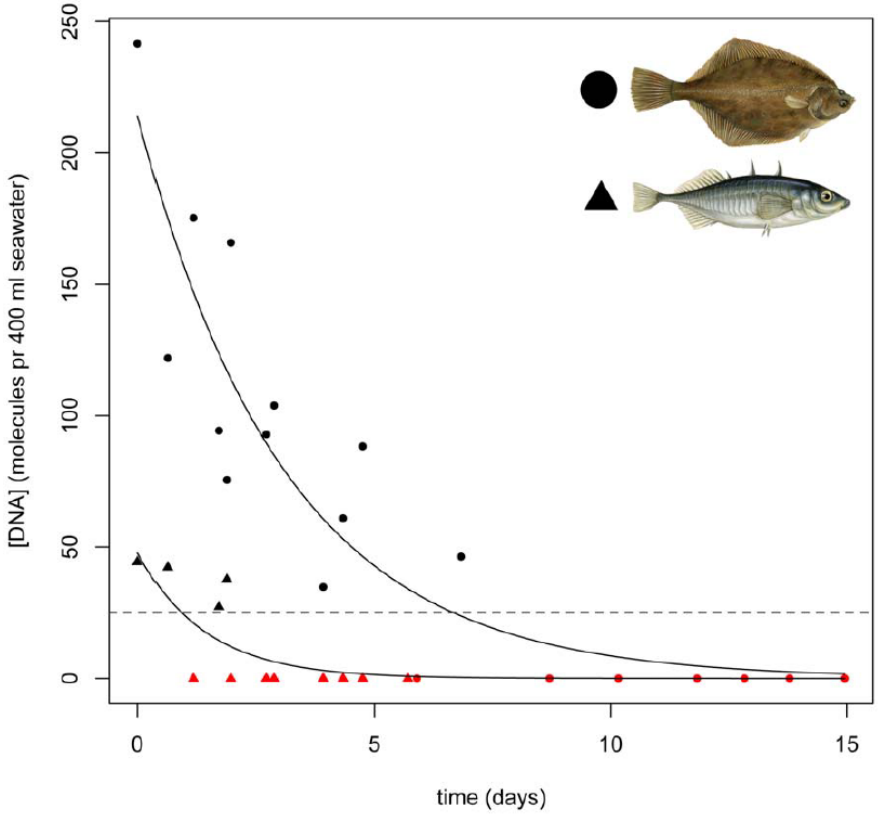
1 Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark, **2** Department of Bioscience, Aarhus University, Roskilde, Denmark, **3** Fjord&Bælt, Kerteminde, Denmark, **4** Marine Biological Laboratory, University of Southern Denmark, Kerteminde, Denmark

Practical applications: eDNA monitoring of (50!) invasive marine species in Danish waters – MONIS (Ministry of Environment)



But where was the fish?





Thomsen et al. 2012

When a simple method becomes complex

Production

Biomass

Concentration/density

Surface area

Season

Diet

Sex

Condition

Degradation

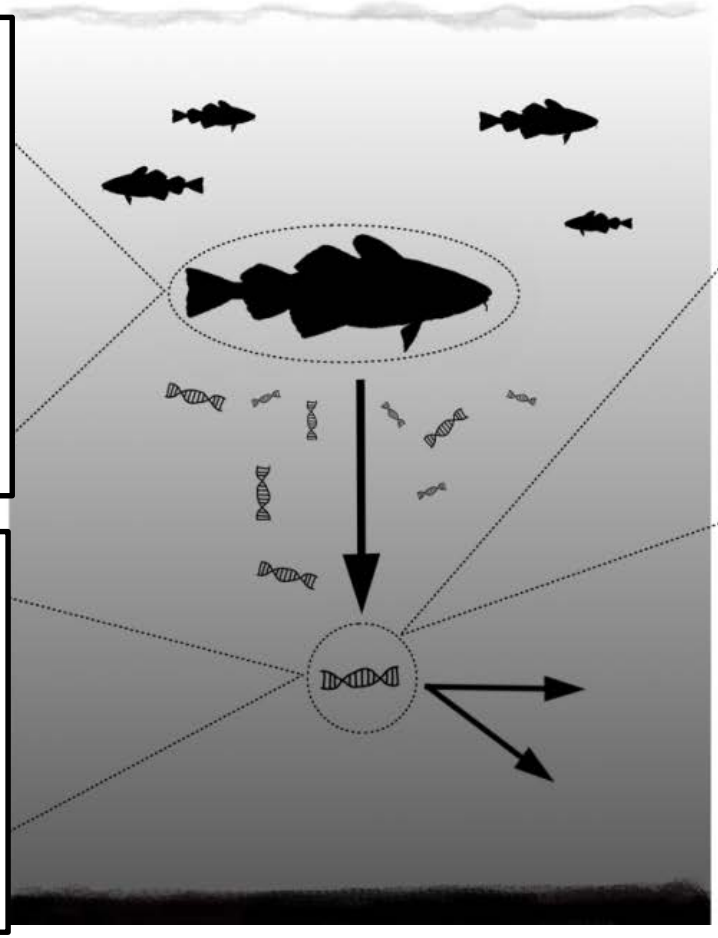
Microbial

Temperature

UV light

pH

Form of DNA (free, attached or sediment)



Transport

Diffusion

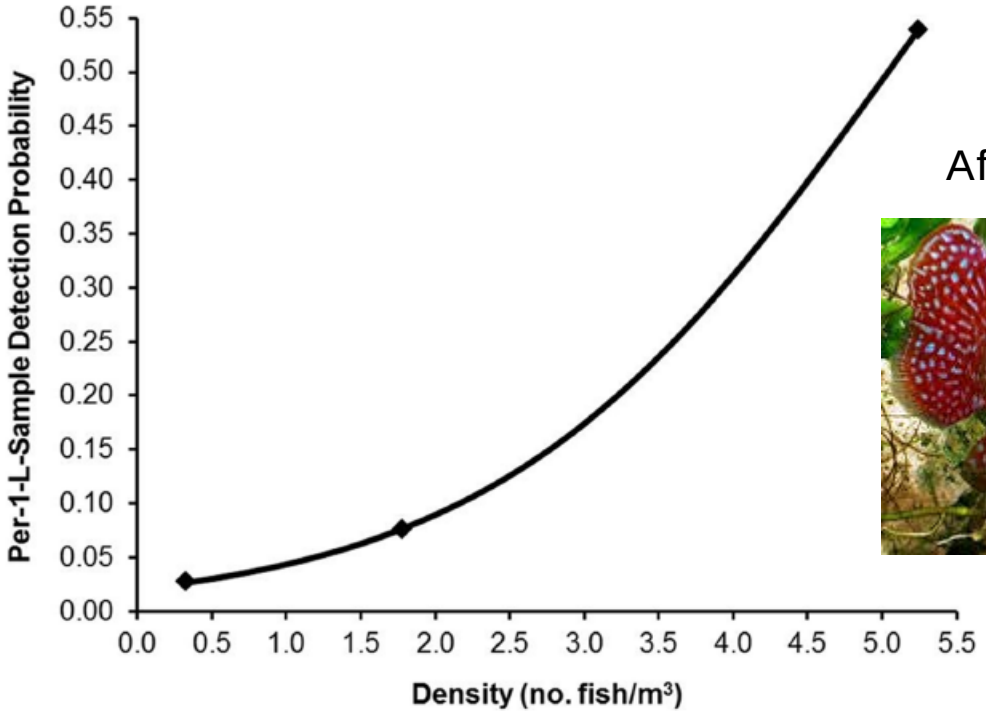
Advection

Sedimentation

Resuspension

Transport with other organisms

Challenge I: Can we find what we're looking for?



African Jewelfish

Moyer et al. 2014

Figure 2. Predicted per-1-L water sample DNA detection probability with increasing densities of African jewelfish in experimental ponds. Detection estimates are based on parameter estimates from the best-approximating hierarchical logistic regression models relating African jewelfish eDNA detection/non-detection data to pond- and sample-level covariates. Filled diamonds represent the low (0.32 fish/m^3), moderate (1.75 fish/m^3), and high densities (5.24 fish/m^3) used in this study.
doi:10.1371/journal.pone.0103767.g002

Challenge I: Can we find what we're looking for?

Table 1. Detection of harbor porpoise DNA using qPCR at a controlled site (Fjord&Belt pen) and at natural sites.

	Acoustic detection	Genetic detection	
Location	**% Porpoise positive days**	**Positive PCRs**	**Cycle threshold**
Positive control (DNA extracted from skin)		3/3	18, 18, 18
Fjord&Bælt pen		3/3	34, 35, 35
<10 m from F&B pen		1/3	49
>10 m from F&B pen		0/3	-
Site 1	94	1/3	49
Site 2	42	0/3	-
Site 3	63	0/3	-
Site 4	6	0/3	-
Site 5	0	0/3	-
Site 6	0	0/3	-
Site 7	0	2/3	38*, 50*
Site 8	79	0/3	-

Genetic detections at the eight natural sites are compared with acoustic detection rates based on data from static acoustic monitoring devices over the three months prior to eDNA sampling.
*sequencing of PCR clones indicates these were genetic detections of long-finned pilot whale and not genetic detection of harbor porpoise at this site.
doi:10.1371/journal.pone.0041781.t001

45 ml water!

Foote et al. 2012

Challenge II: Where does the DNA come from?

- Degradation rates and transport!

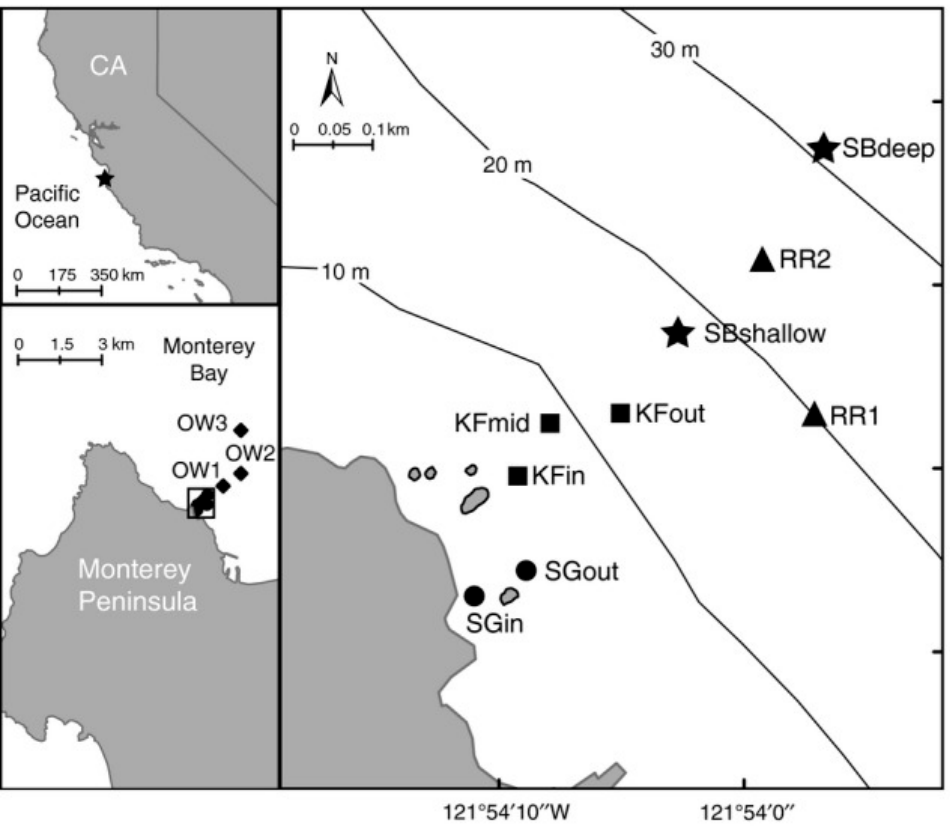
- DNA persistence: reported to be between 1 and 58 days (Dell'Anno and Corinaldesi 2004; Pilliod et al. 2014; Strickler et al. 2014).

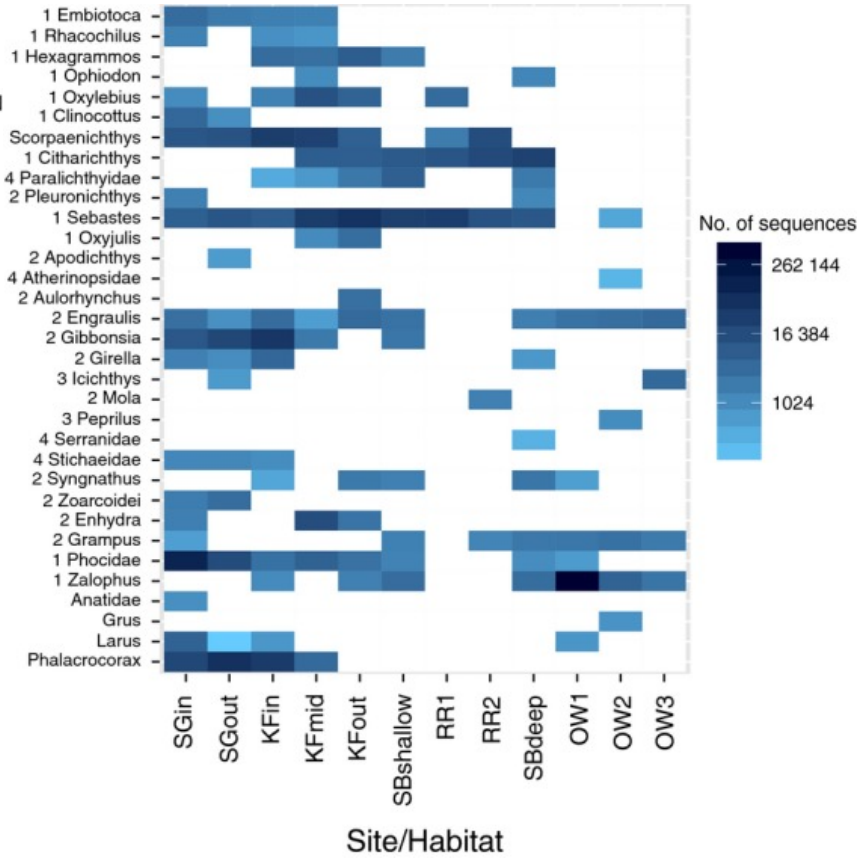
- Time for degradation to below detection level: 0.9-7.8 days (Thomsen *et al.* 2012, Sigsgaard *et al.* 2016)

- Degradation rates: 0.5-15.9% reduction per hour (Maruyama *et al.* 2014; Sassoubre *et al.* 2016)

- Potential transport of DNA over many hundred kilometers dependent on currents and degradation rates (Barnes *et al.* 2014; Pilliod *et al.* 2014; Strickler *et al.* 2014)

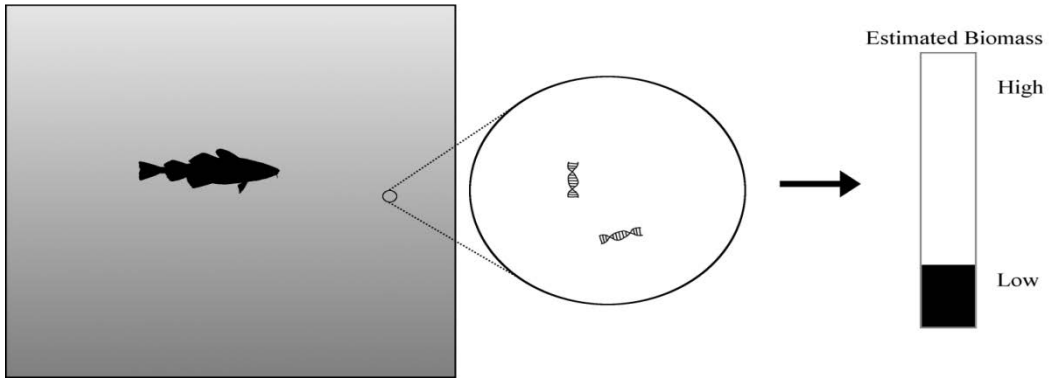
Vertebrate eDNA data compared to simultaneous visual dive surveys in a kelp forest transect (2.5 km)

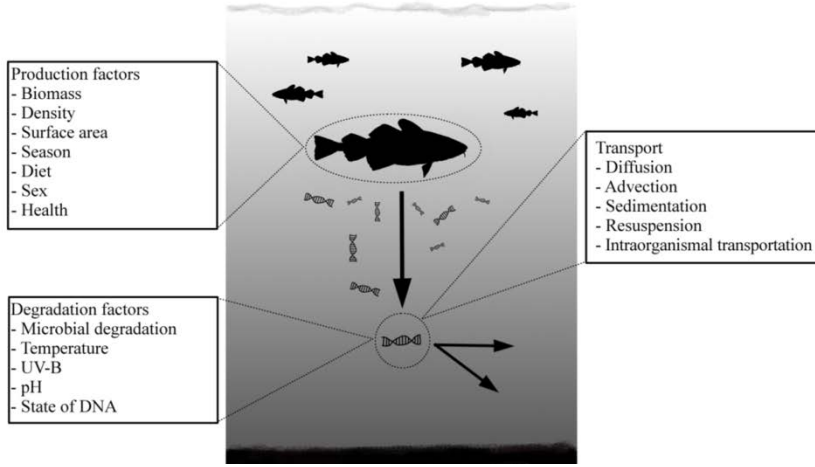


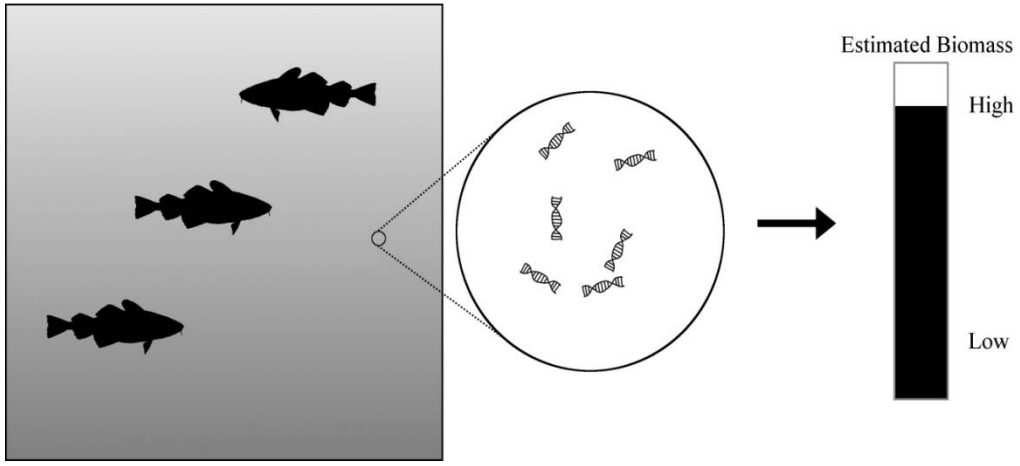


Port et al. 2015

Challenge III: Relationship between eDNA and numbers/biomass







100 fold day to day variation in release of DNA from the same individual (Piliod et al. 2014)

Correlations often significant but weak in both nature and experimental setups

The overall validity of eDNA for biomass estimation has been seriously questioned (Iversen et al. 2015)

Relationship between fish survey catches and eDNA abundance in Greenland

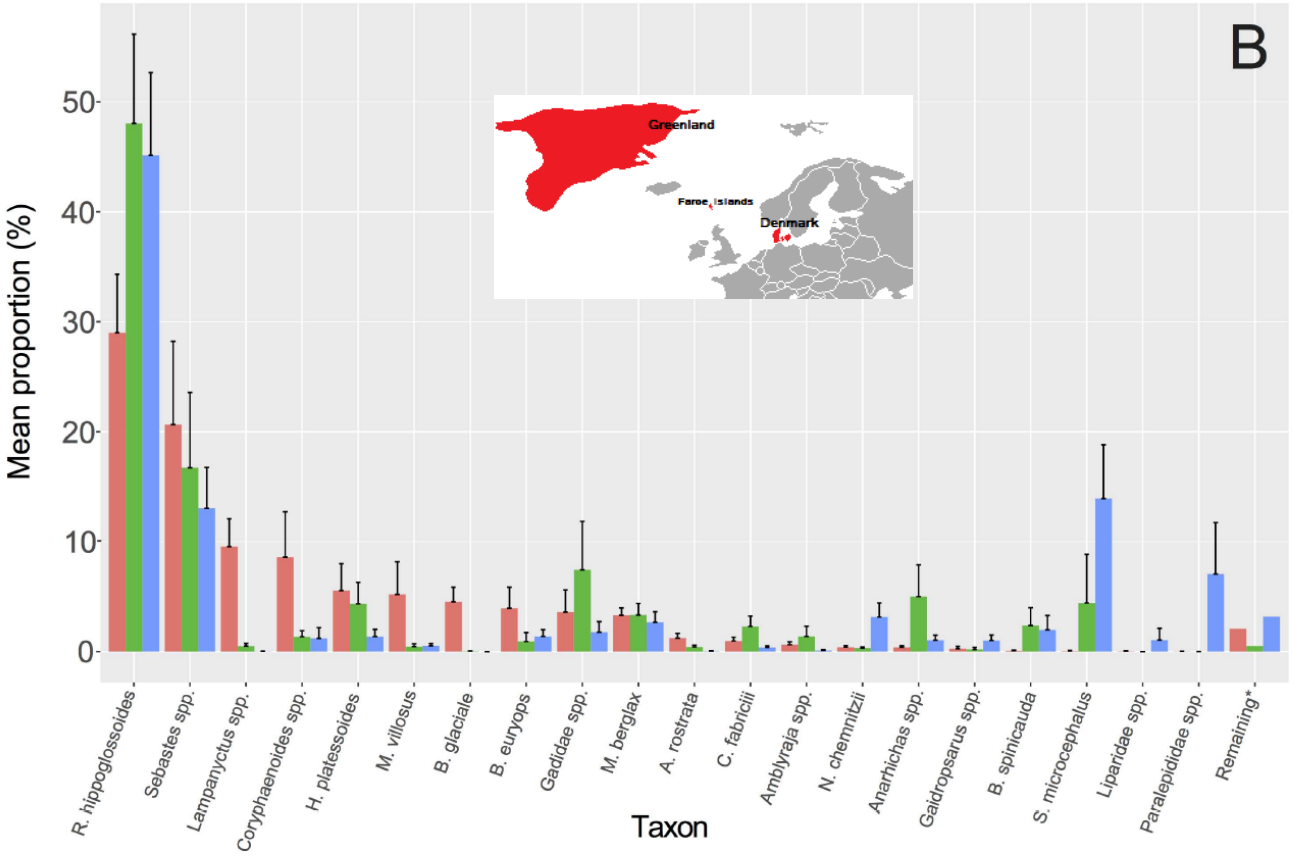


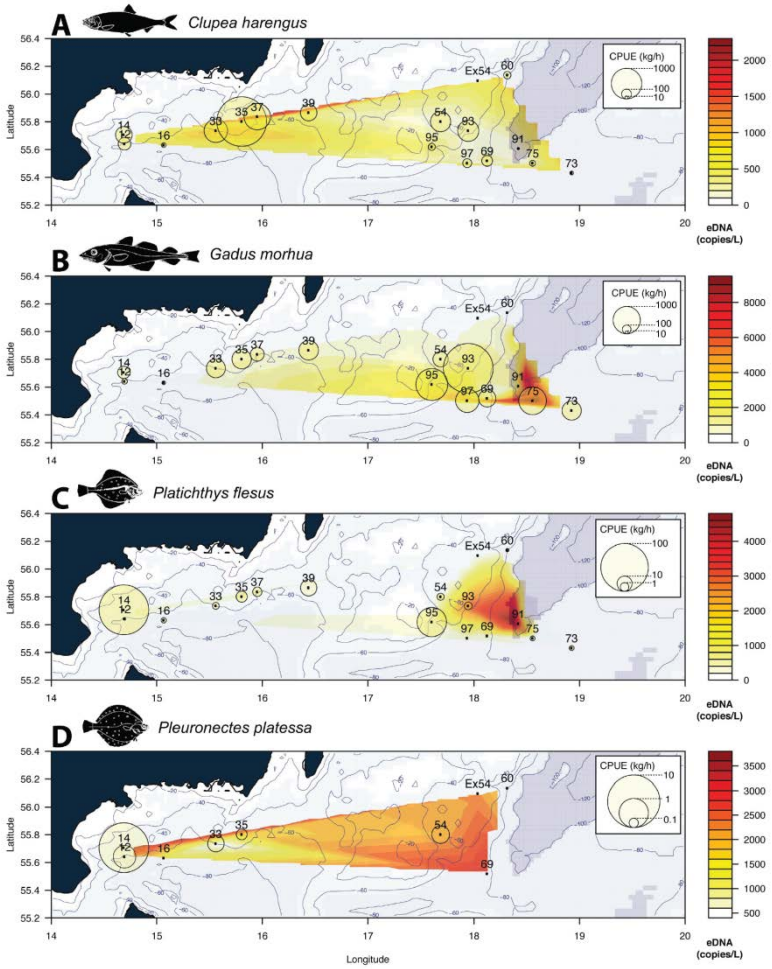
Fig 4. Overview of quantitative results. Barplot of mean + SE of relative fish abundance (red), biomass (green) and eDNA read abundance (blue) across all samples. Data shown for families (A) and lower taxonomic resolution (B). Taxa for which the means of all three variables are <1% are shown together (remaining*) as summed means. Only taxa that are found using both methods are included. Full list of taxa is given in Table 1.

doi:10.1371/journal.pone.0165252.g004

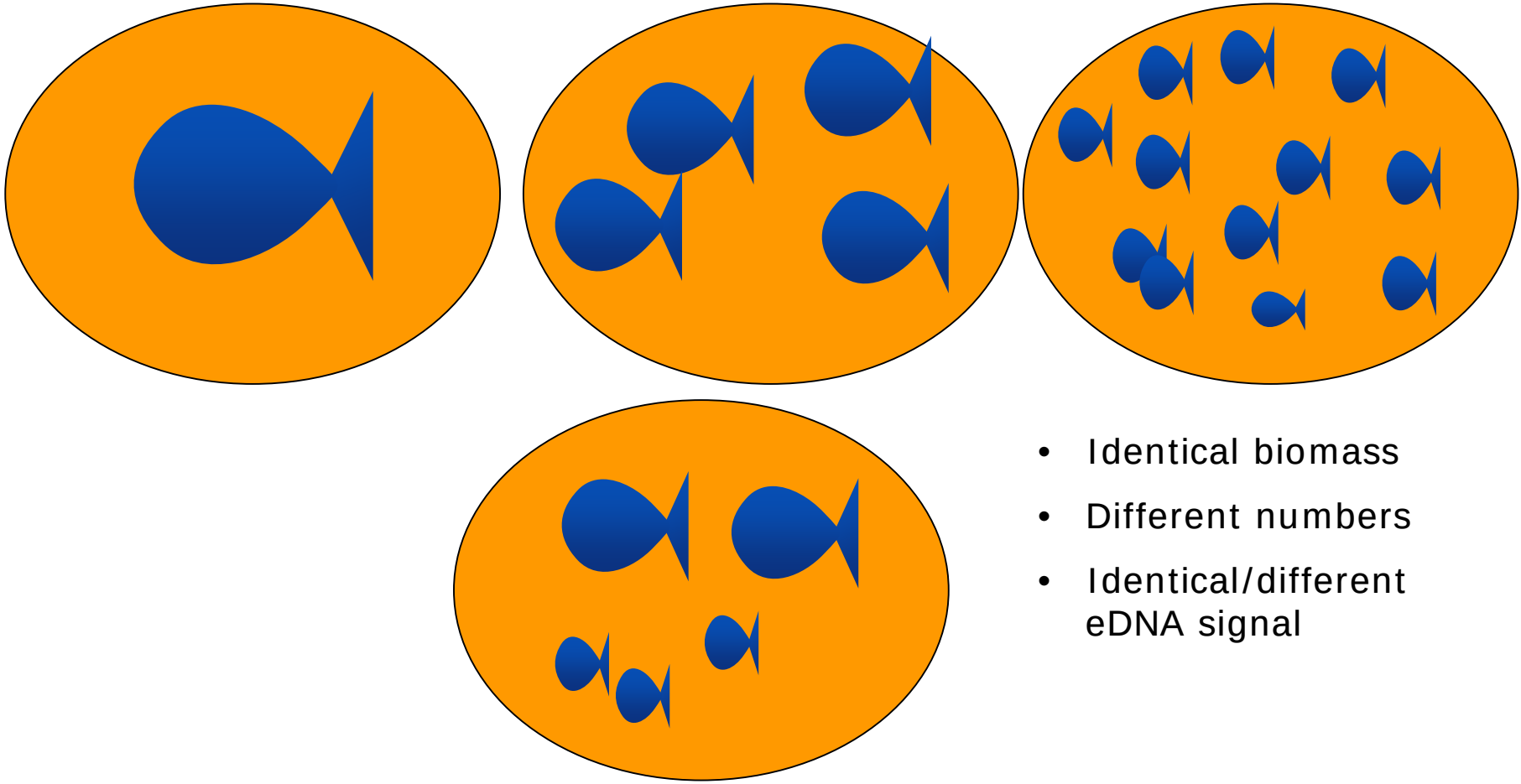
Thomsen et al. 2016

Relationship between fish survey catches and eDNA abundance in the Baltic sea

Fig. 1. heat maps



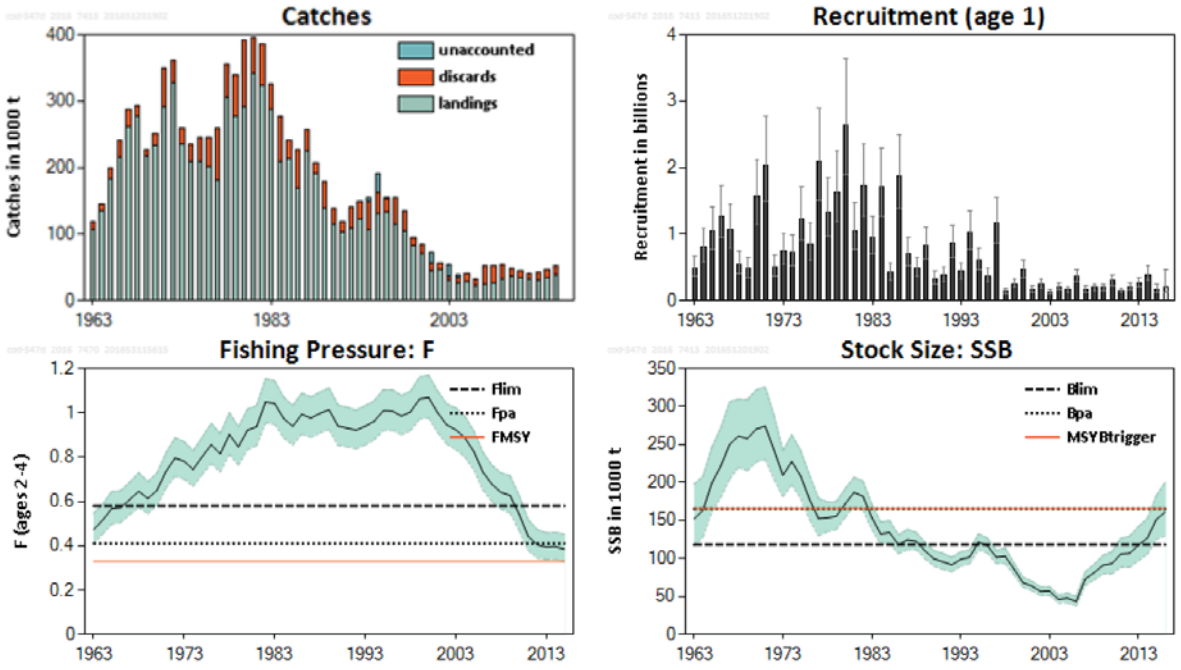
Challenge IV: Application to fisheries management



- Identical biomass
- Different numbers
- Identical/different eDNA signal

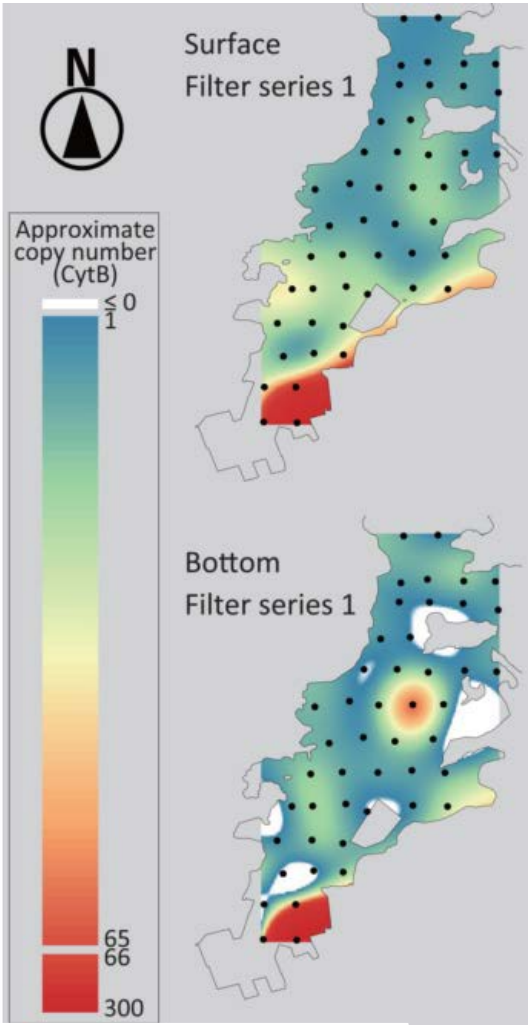
Data typically used in stock asessment of marine fish

- Recruitment (age 1)
- Growth
- Spawning biomass
- Fisheries mortality
  - Require: Age, weight/lenght, maturity



Challenge V: Other sources of eDNA

- eDNA from dead/discarded fish (Merkes et al.2014)
- eDNA from fish markets/factories/harbors
- DNA from sediments (1800X) (Turner et al 2014)



Yamamoto et al. 2016

Conclusions

- There is very little knowledge about the origin of DNA in marine eDNA studies, but it appears that most of it comes from nearby sources

- The relationship between numbers/biomass and eDNA concentration is relatively weak and affected by many factors

- It is difficult to envision how current eDNA methodology can replace traditional marine monitoring for fisheries and conservation management – at least in the short run (paradigm shift?)

- There are a number of other sources of eDNA than the apparent organisms you want to monitor

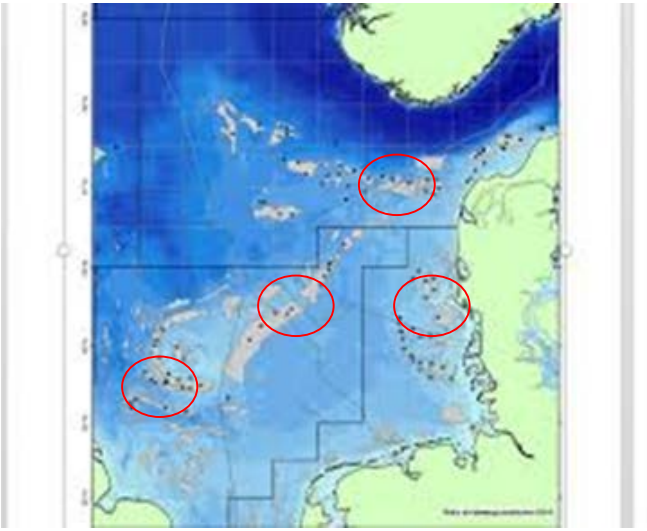
Suggestions

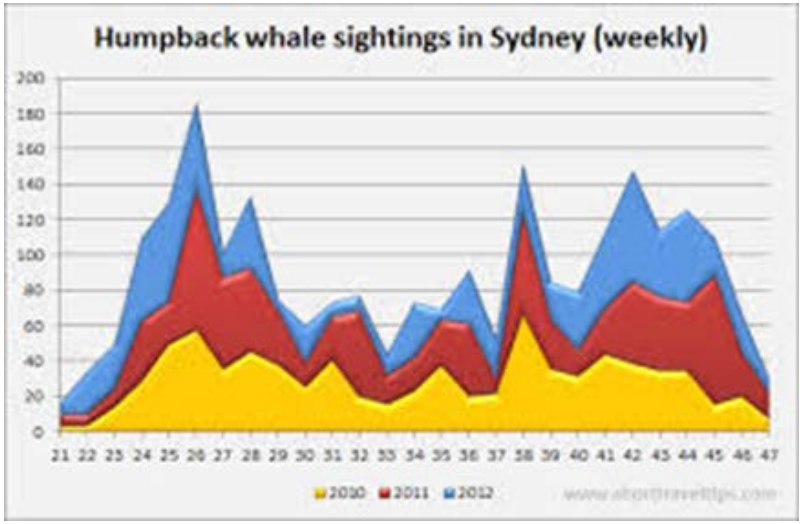
- I: Think carefully about whether your eDNA approach is likely to be sensitive enough to monitor your target organism(s)

- II: Evaluate the likely spatial origin of your eDNA - hydrographic modelling (backtracking)?

- III: Abundance estimates from marine eDNA are (at best!) difficult – focus on presence/absence, aggregations or temporal changes

Quantitative eDNA applications

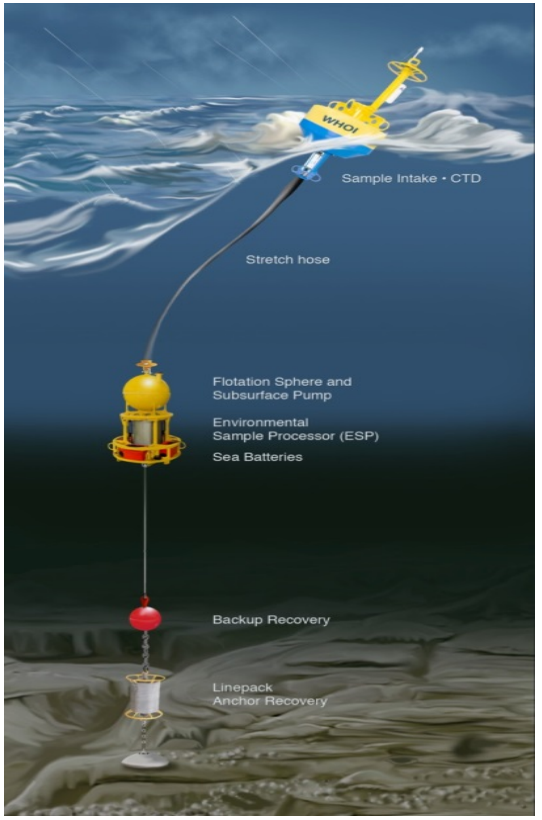




Danish Sandeel fishery

New technology

- In situ eDNA monitoring: Environmental Sample Processor (DTU/DHI collaboration)



New technology

- Portable 3rd generation sequencing unit SmidgION

Suggestions

- I: Think carefully about whether your eDNA approach is likely to be sensitive enough to monitor your target organism(s)
- II: Evaluate the likely spatial origin of your eDNA - hydrographic modelling (backtracking)?
- III: Abundance estimates from marine eDNA are (at best!) difficult – focus on presence/absence, aggregations or temporal changes
- IV: eDNA can be a supplement but not replace traditional marine monitoring
- V: Evaluate other potential sources of DNA for your target organism(s)

What do we need?

- More basal knowledge about eDNA and the processes governing production, degradation and transport
- More controlled experiments – and relatively less collection of water
- More studies combining eDNA with hydrographic modelling
- More long-term monitoring of temporal changes in eDNA concentrations
- Focus on the classical strengths of eDNAs for species detection, identification and ecosystem description


Miljø- og
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