

Using microbial eDNA to solve the hard-bottom biomonitoring problem

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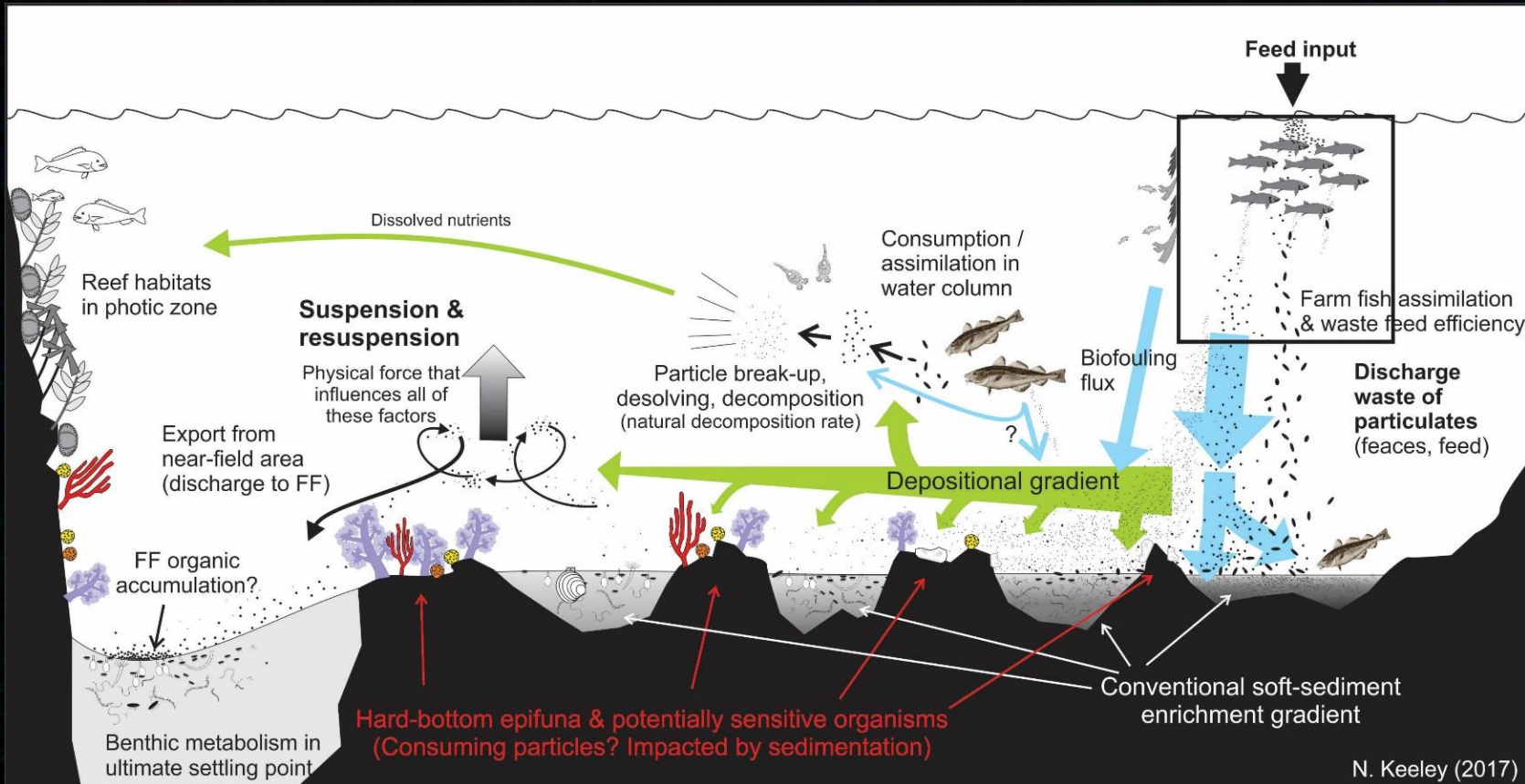
Olivier Laroche (HI, Tromsø)

Murray Birch (Marine engineer, New Zealand)



See: Keeley, NB, Laroche O, Birch M, Pochon X. Substrate Independent Benthic Sampler (SIBS) for hard & mixed-bottom habitats: a proof of concept study. *Frontiers in Microbiology*. In Review.

Can eDNA solve 'the hard-bottom problem'?



Marine sediments contain microbial communities that can be used to reliably describe benthic enrichment using metabarcoding (eDNA)

OXFORD FEMS JOURNALS investing in science

FEMS Microbiology Ecology, 91, 2015, fiv089

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Research Article

2015

RESEARCH ARTICLE
Assessing the effects of salmon farming seabed enrichment using bacterial community diversity and high-throughput sequencing

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Research paper

Development and preliminary validation of a multi-trophic metabarcoding biotic index for monitoring benthic organic enrichment

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ARTICLE INFO ABSTRACT

Keywords: Sea-based fish farms are associated with benthic organic enrichment. This has the potential to impact on the benthic community structure and function. We developed and validated a multi-trophic metabarcoding biotic index for monitoring benthic organic enrichment. The index was based on the relative abundance of 10 bacterial taxa. The index was validated using sediment samples from 10 sites with varying degrees of organic enrichment. The index was found to be a good indicator of benthic organic enrichment and was able to distinguish between sites with different levels of enrichment. The index was also found to be a good indicator of benthic community structure and function. The index was found to be a good indicator of benthic community structure and function.

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Environmental DNA metabarcoding of benthic bacterial communities indicates the benthic footprint of salmon aquaculture

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ARTICLE INFO ABSTRACT

Keywords: Environmental monitoring
Salmon aquaculture
eDNA metabarcoding
Bacterial communities

We evaluated benthic bacterial communities as bioindicators in environmental impact assessments of salmon aquaculture, a rapidly growing sector of seafood industry. Sediment samples (n = 72) were collected from below salmon cages towards distant reference sites. Bacterial community profiles inferred from DNA metabarcodes were compared to reference data from standard macrofauna biomonitoring surveys of the same samples. Deltaproteobacteria were predominant in immediate vicinity of the salmon cages. Along the transect, significant shifts in bacterial community structures were observed with Gammaproteobacteria dominating the less-im-

frontiers in Microbiology

ORIGINAL RESEARCH
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Temporal Bacterial Surveillance of Salmon Aquaculture Sites Indicates a Long Lasting Benthic Impact With Minimal Recovery

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Coastal aquaculture sites and associated impacts. Understanding both the effect of recovery during fallow and environmentally sustainable

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Translational Molecular Ecology in practice: Linking DNA-based methods to actionable marine environmental management

Eva Aylagas^{a,*}, Angel Borja^b, Xavier Pochon^{c,d}, Anastasija Zaiko^{c,d}, Nigel Keeley^e, Kat Bruce^f, Peiyong Hong^g, Gregory M. Ruiz^{h,i}, Eric D. Stein^j, Susanna Theroux^j, Nathan Gerdali^a, Alejandra Ortega^a, Laura Gajdzik^a, Darren J. Coker^a, Yasser Katan^k, Was Hikmawan^k, Ammar Saleem^l, Sultan Alamer^l, Burton H. Jones^a, Carlos M. Duarte^a, John Pearman^m, Susana Carvalho^a

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MOLECULAR ECOLOGY WILEY

2020

Ecosystems monitoring powered by environmental genomics: A review of current strategies with an implementation roadmap

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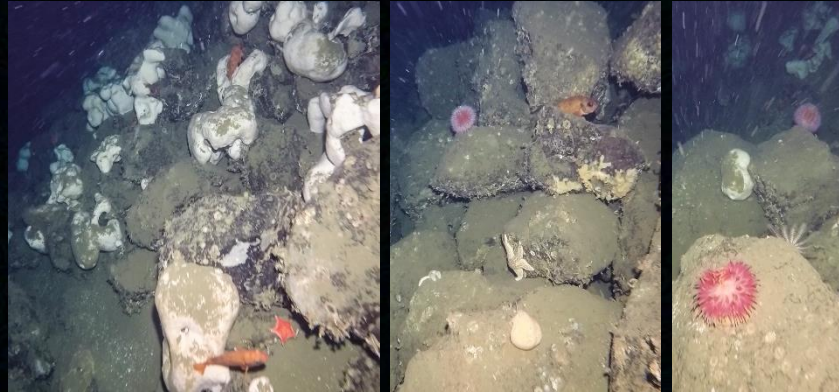


Challenging mixed habitat environments

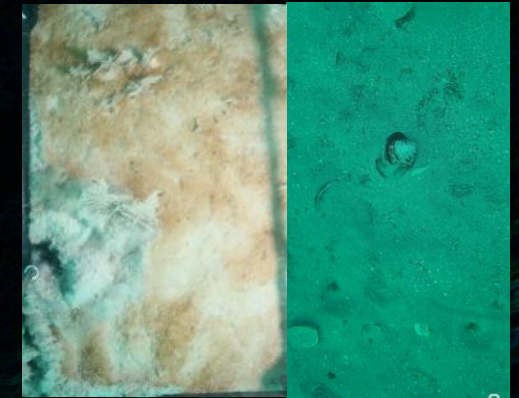
Bedrock



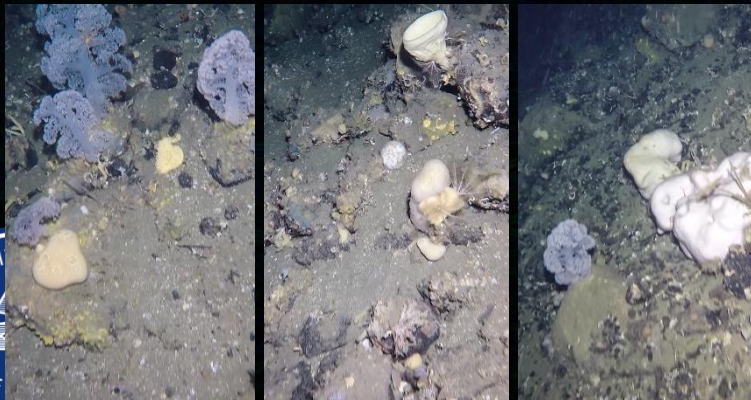
Boulder



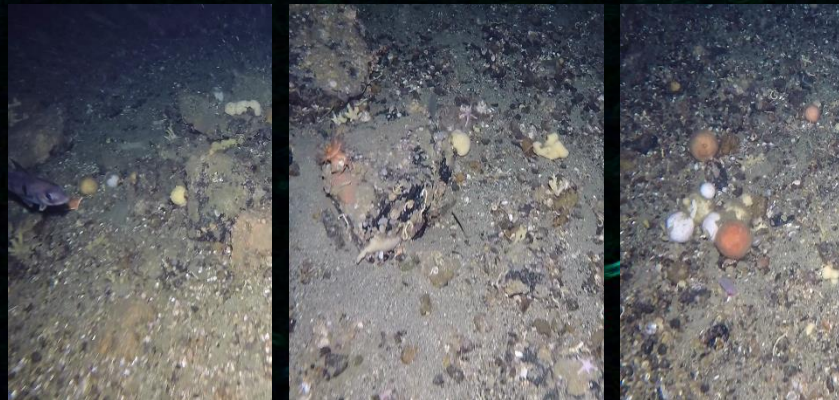
Sand and mud



Broken rocks, gravel & cobbles on sediment



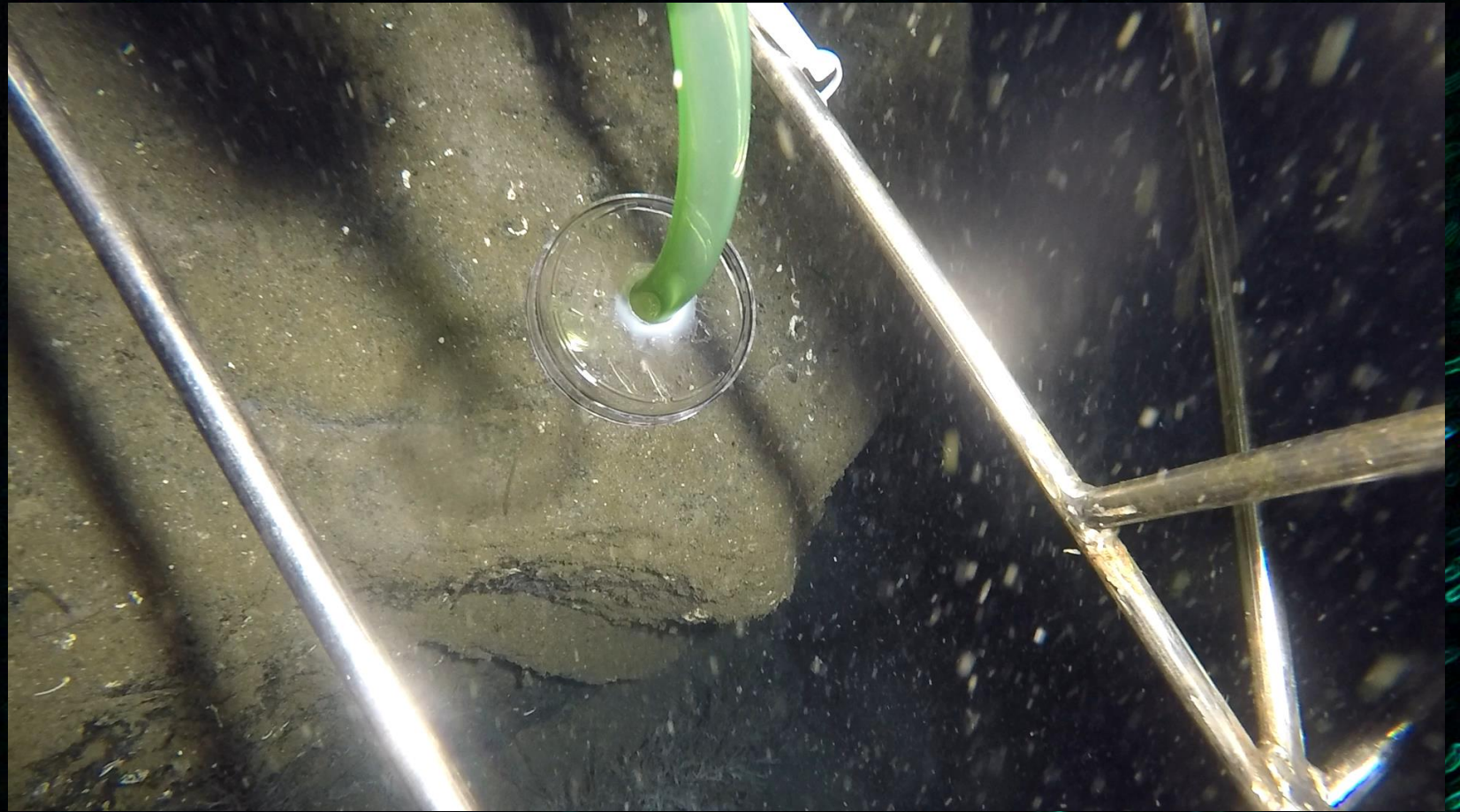
Gravel & cobbles, medium sand



Coarse sand with stones

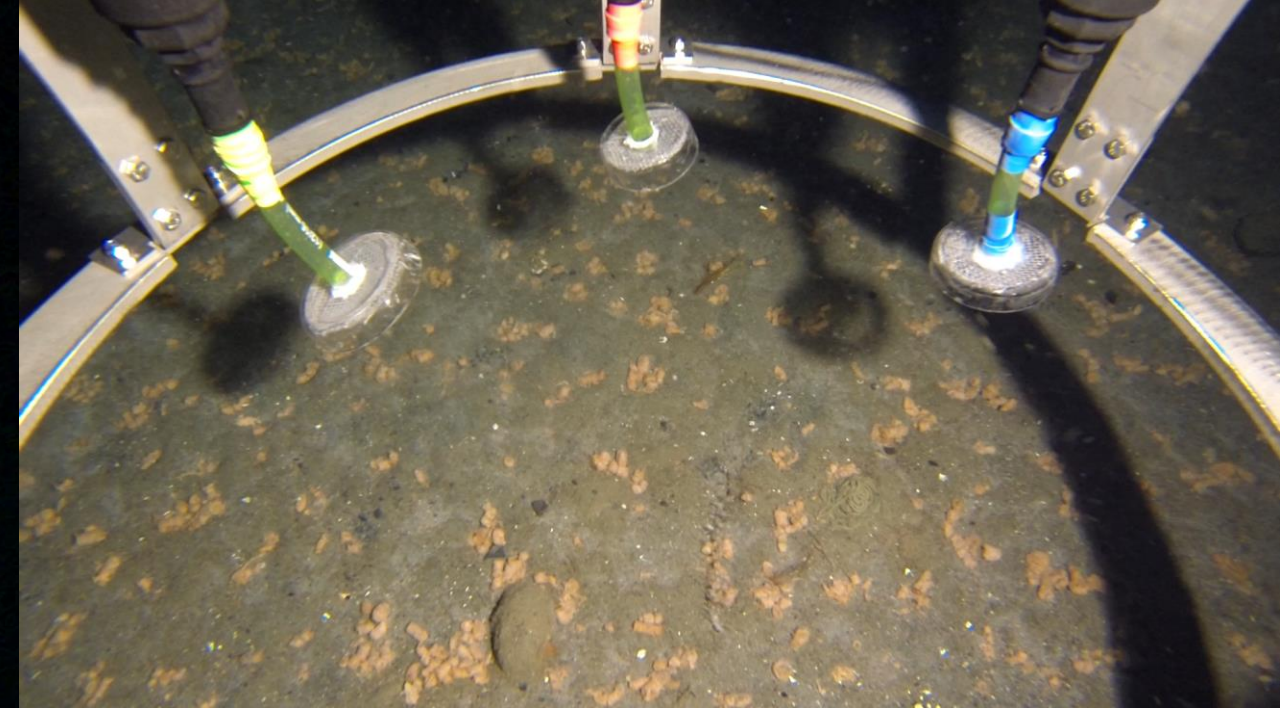
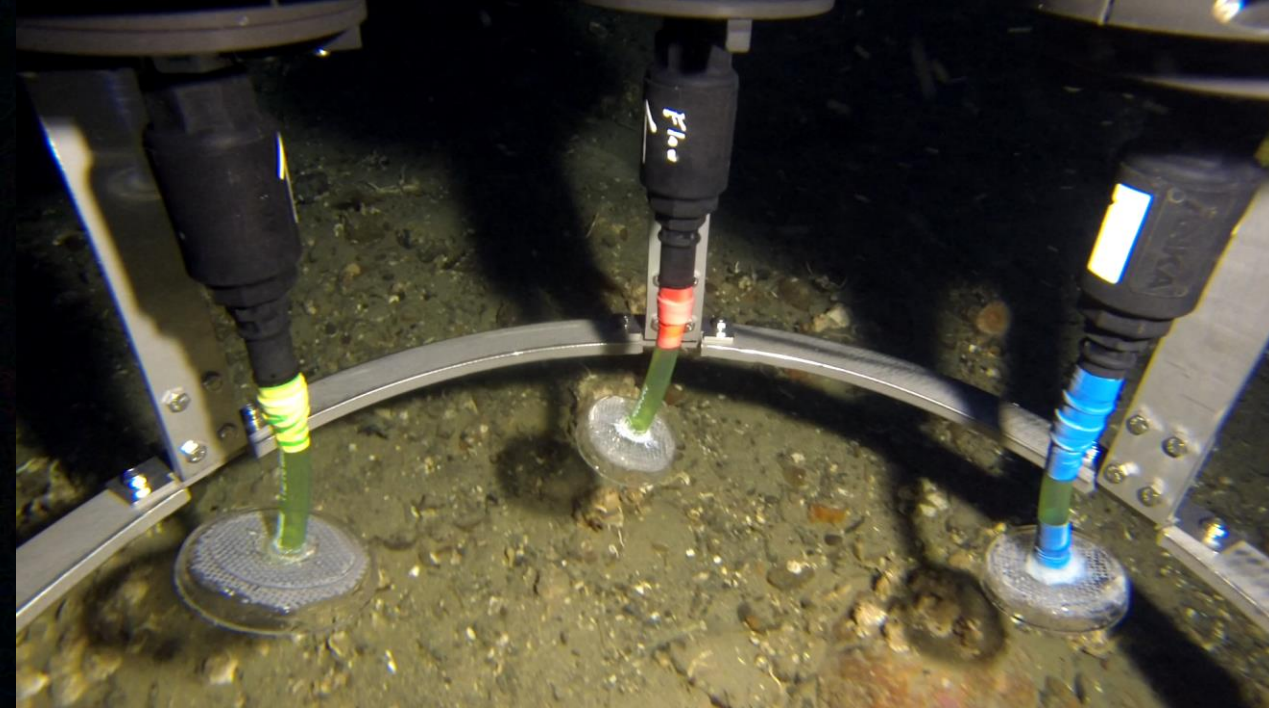


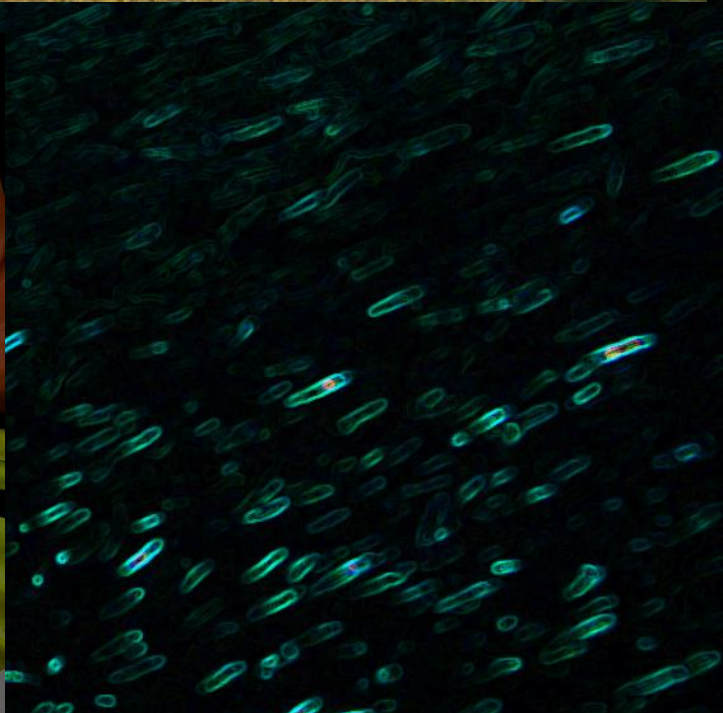
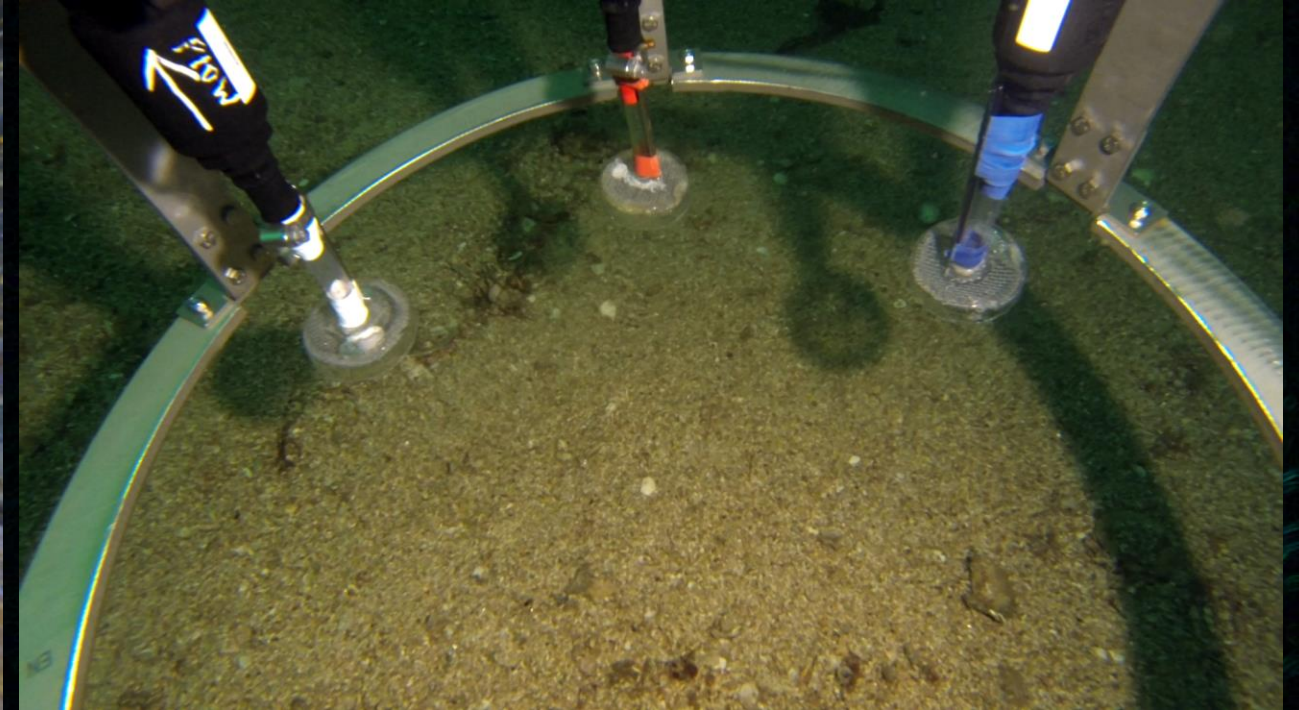
SIBS ('floc sampler') development



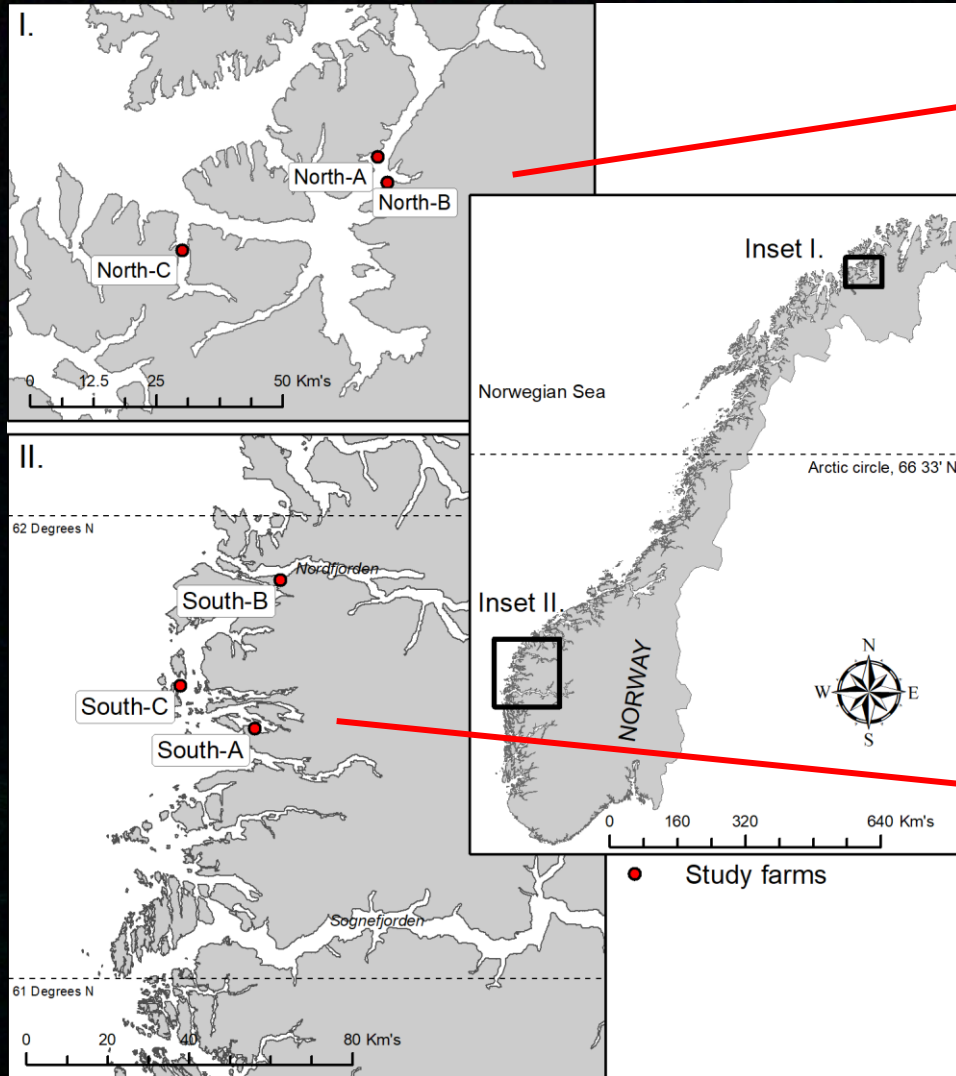


Generation 2 'Substrate Independent Benthic Sampler' (SIBS)



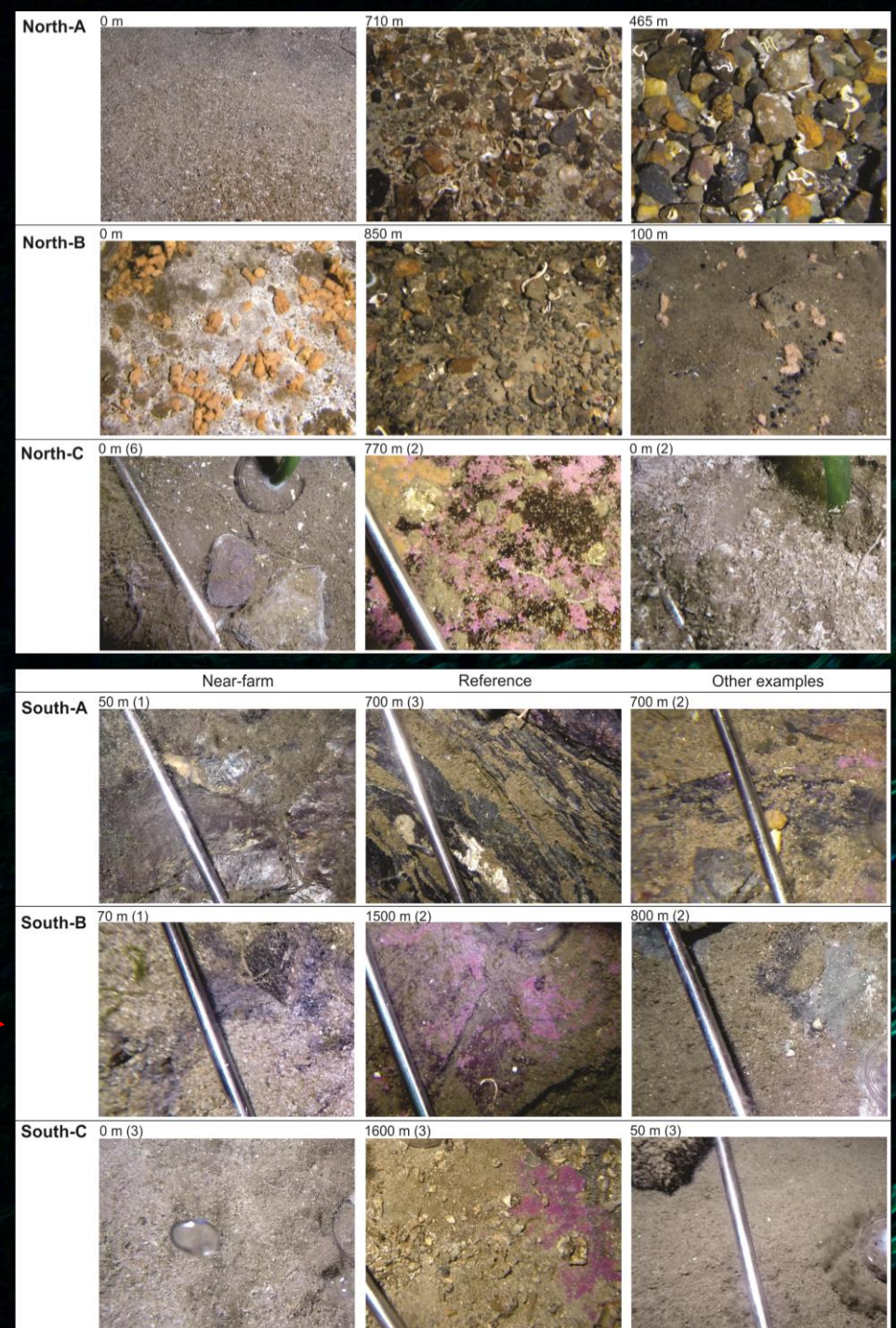


The study sites:



-Mixed bottom
-Rock, gravel,
sand

-Bedrock
-No sediment



Analytical methods



Conventional sediment grab

'Grab-MF'

Marcocofauna

- Conventional, established 'gold standard' enrichment indicator
- Full taxonomy
- Species count data & indices
- Used to validate enrichment status (where possible)
- Very difficult to obtain samples. Impossible at Southern sites.

'Grab-eDNA'

Microbial eDNA

- 5g sediment from core surface
- DNeasy PowerSoil kit

- V3-V4 region of 16s RNA
- Illumina MiSeq sequencing
- Amplicon purification & normalization
- Quality filtering & denoising
- Microbial ASV read abundance data



SIBS sampler

'SIBS-eDNA'

Microbial eDNA

- 500ml sample 'hoovered' up
- Contained in sterile bag
- Filtered on to 47mm GFF
- Zymo Quick DNA mini-prep kit



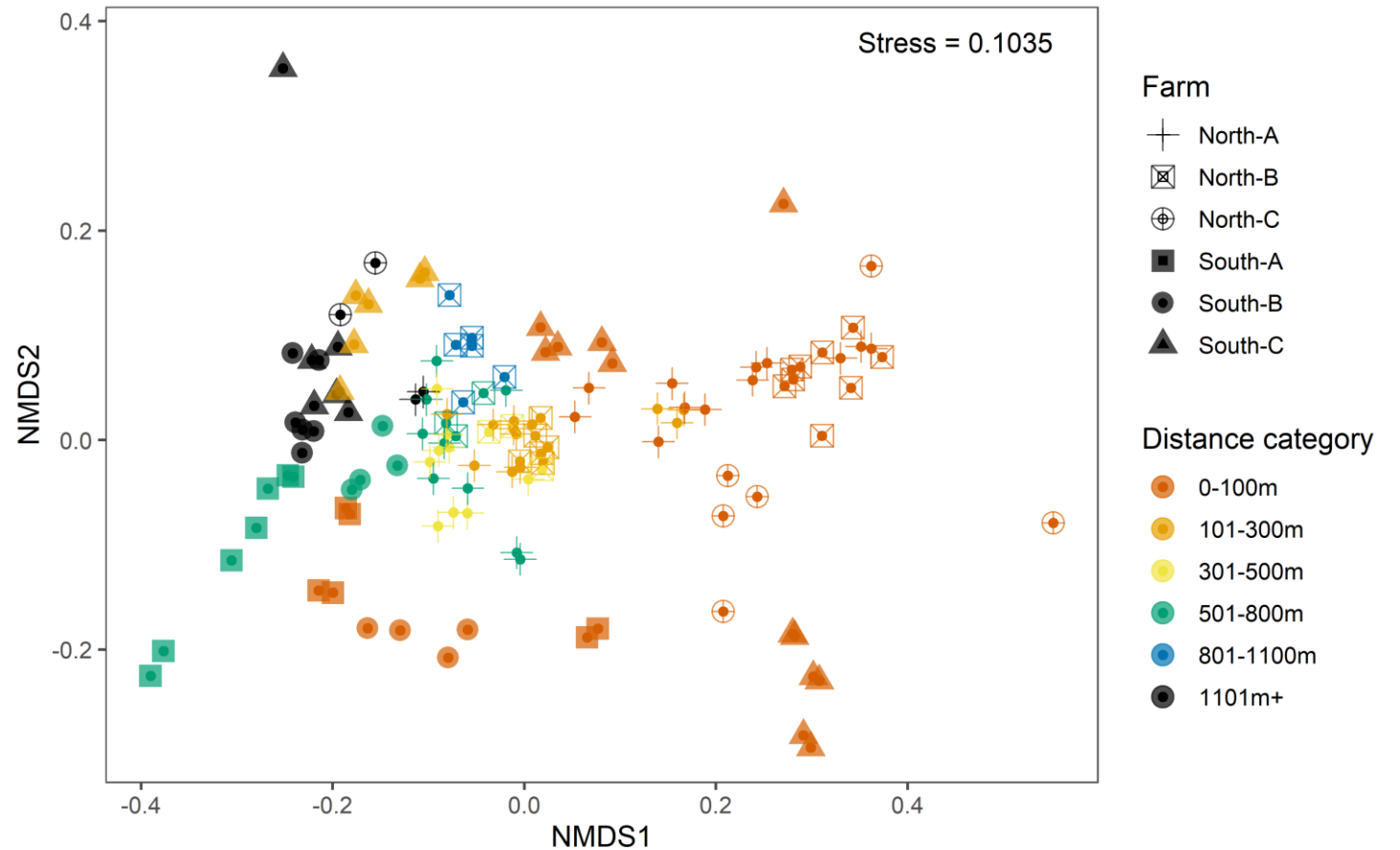
Substrate assessment categories:

- **Distance:** distance of station from farm (m)
- **Base substrate type:** Underlying substrate, e.g. gravel, bedrock, sand
- **Mobile substrate type:** type of flocculent material, e.g., sand, shell-sand, organic silt etc
- **Extent of flocculant cover:** extent of loose inorganic and organic layer overlying base substrate
- **Visual impact category:** visual assessment of organic enrichment based on expert judgement
- **Enrichment stage (ES):** Measure of benthic enrichment level based on environmental parameters (in this case by b-MBI – explained shortly...)



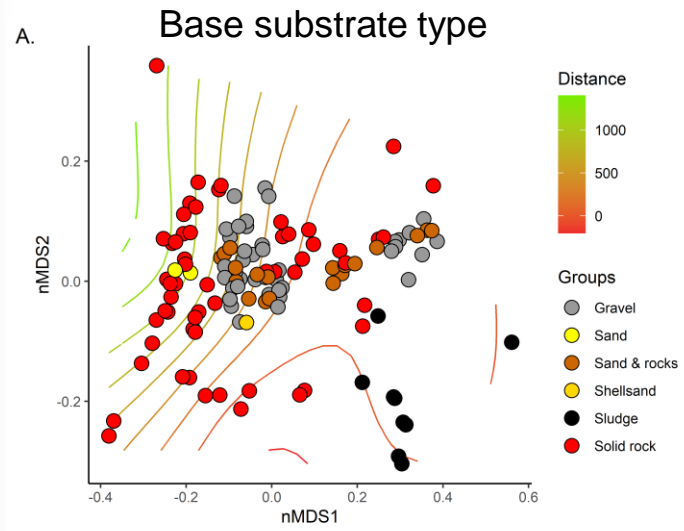
- Microbial eDNA from SIBS – read abundance of ASV's
- Differences are well described by distance from farm
- Not perfect – but distance is not necessarily a good proxy for enrichment...
- Need to dig a little deeper to see what is responsible for the variances

'SIBS-eDNA'



Factors	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
A. Region	1	2.3028	2.3028	39.320	0.13261	0.001	***
log(Distance + 1)	1	4.7807	4.7807	81.630	0.27531	0.001	***
Base Subst Type	5	1.4536	0.2907	4.964	0.08371	0.001	***
Region:Farm	4	1.9172	0.4793	8.184	0.11041	0.001	***
Residuals	118	6.9107	0.0586		0.39797		
Total	129	17.3650			1.00000		

'SIBS-eDNA'



'Grab-MF'

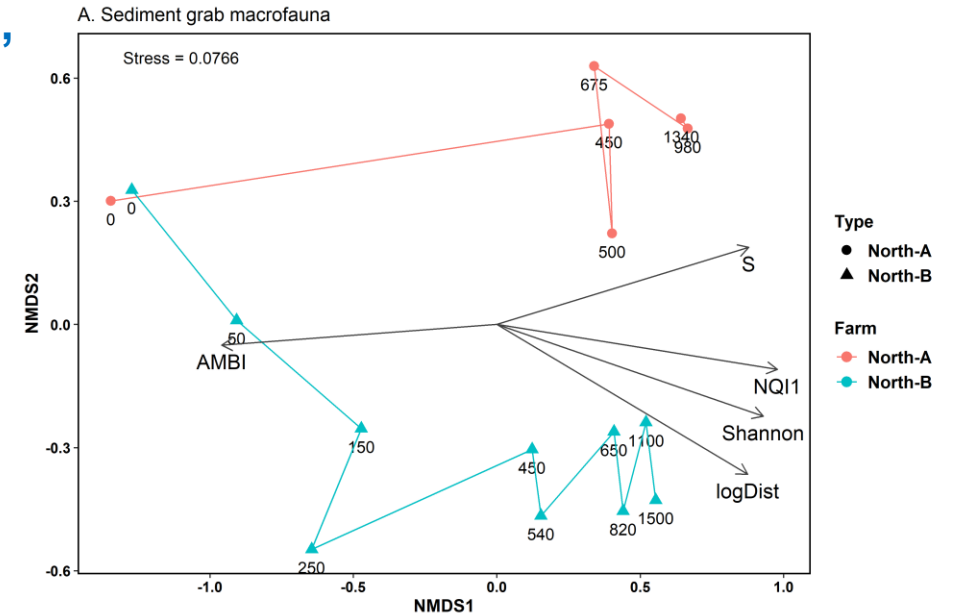


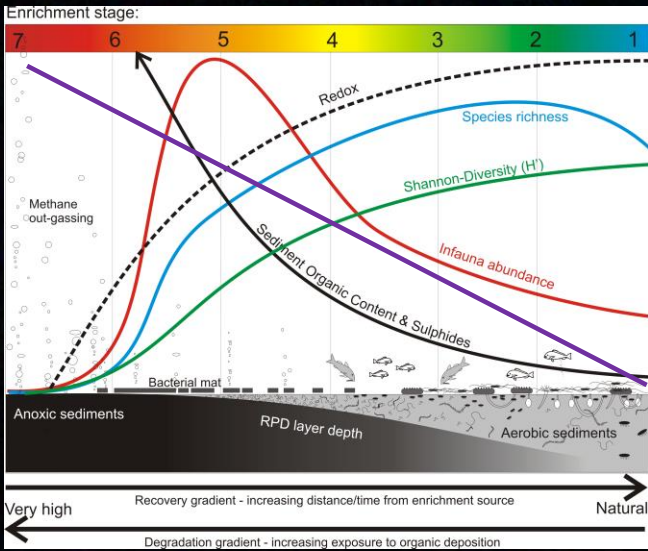
'Grab-eDNA'

- Procrust Protest correlation:
Cor = 0.757, P<0.0001
- ... Grab-sourced microbial eDNA shows similar differences between samples as benthic macrofauna
- They are different things, so it is unlikely to ever be perfect



'Grab-MF'





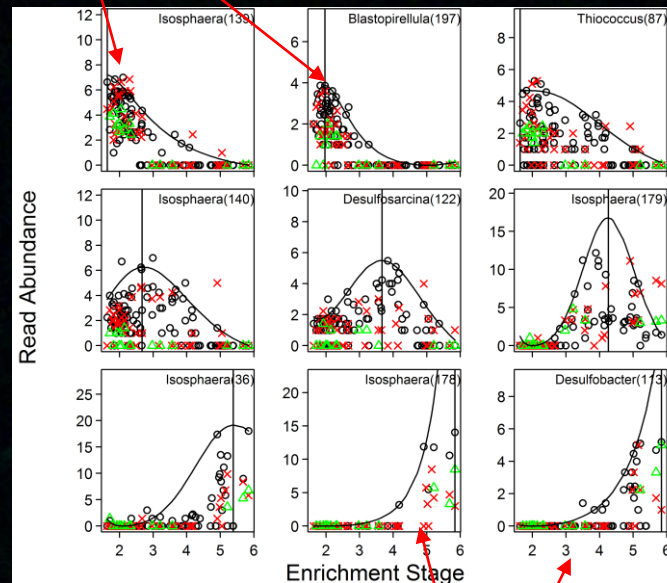
The b-MBI index

('bacterial-metabarcoding biotic index')

Sensitive species? → EG I

b-MBI

Bacteria (eDNA)



Tolerant opportunistic species? → EG V

AMBI – Broja et al. (2000)

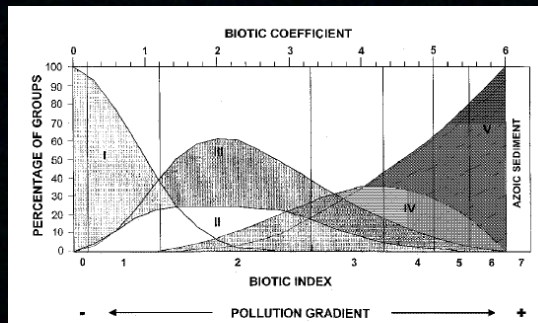


Fig. 2 Theoretical model, modified from Hily (1984), Hily *et al.* (1986) and Majeed (1987), which provides the ordination of soft-bottom macrofauna species into five ecological groups (Group I: species very sensitive; Group II: species indifferent; Group III: species tolerant; Group IV: second-order opportunistic species; Group V: first-order opportunistic species), according to their sensitivity to an increasing pollution gradient. The relative proportion of abundance of each group in a sample provides a discrete BI with eight levels (0-7) and an equivalent continuous BC (values between 0 and 6).

$$\text{AMBI Biotic Coef.} = [(0 \times \%GI) + (1.5 \times \%GII) + (3 \times \%GIII) + (4.5 \times \%GIV) + (6 \times \%GV)] / 100$$

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Research paper

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ARTICLE INFO ABSTRACT

Keywords: Aquaculture, Bacteria, Benthic enrichment, Eukaryotes, High throughput sequencing, Macrofauna, 18S ribosomal RNA gene

Sea-based fish farms are associated with strong, benthic enrichment gradients and routine monitoring is usually required by regulation. This has traditionally been based on benthic macrofaunal communities, which is reliant on taxonomic expertise, and turn-around times can be slow, limiting opportunities for adaptive management. Environmental metabarcoding is a powerful high-throughput sequencing-based technique that can identify and quantify benthic assemblages. Previous studies have demonstrated relationships between specific taxonomic groups (e.g., bacteria, foraminifera) and anthropogenic effects. However, the absence of fixed categorical scales makes the use of data challenging for routine bio-monitoring. In this study, we analysed 105 sediment samples collected over three years from three salmon farms spanning two separate bioregions. Environmental DNA and RNA (eDNA/eRNA) metabarcoding of three taxonomic groups (foraminifera, bacteria, and general eukaryotes)

- Only 395 bacterial ASV's
- Derived from New Zealand sediments

'GeneCode-ID' database (EG's assigned to bacteria ASV's)

$$b\text{-MBI} = 0 \cdot \text{EGI} + 1.5 \cdot \text{EGII} + 3 \cdot \text{EGIII} + 5 \cdot \text{EGIV} + 12 \cdot \text{EGV}$$



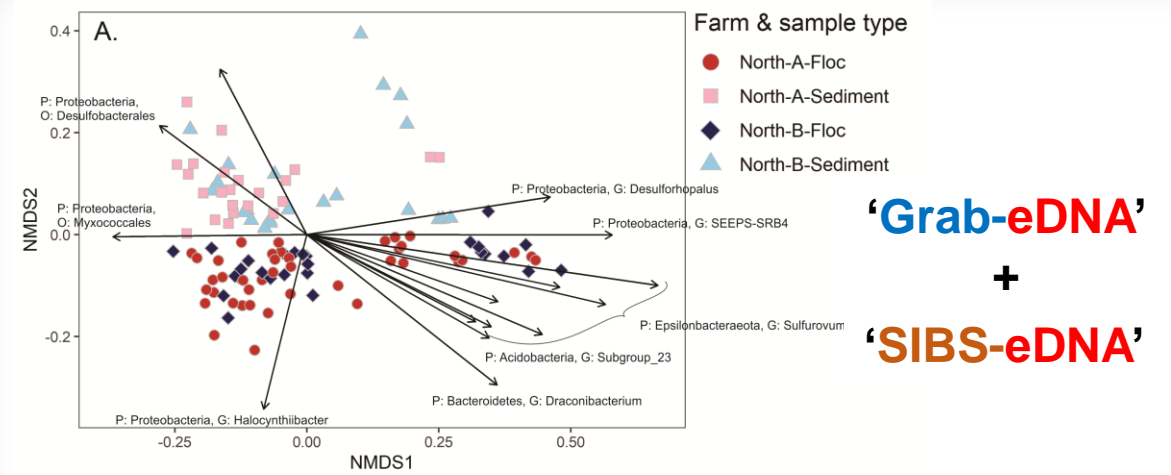
‘Grab-eDNA’



‘SIBS-eDNA’

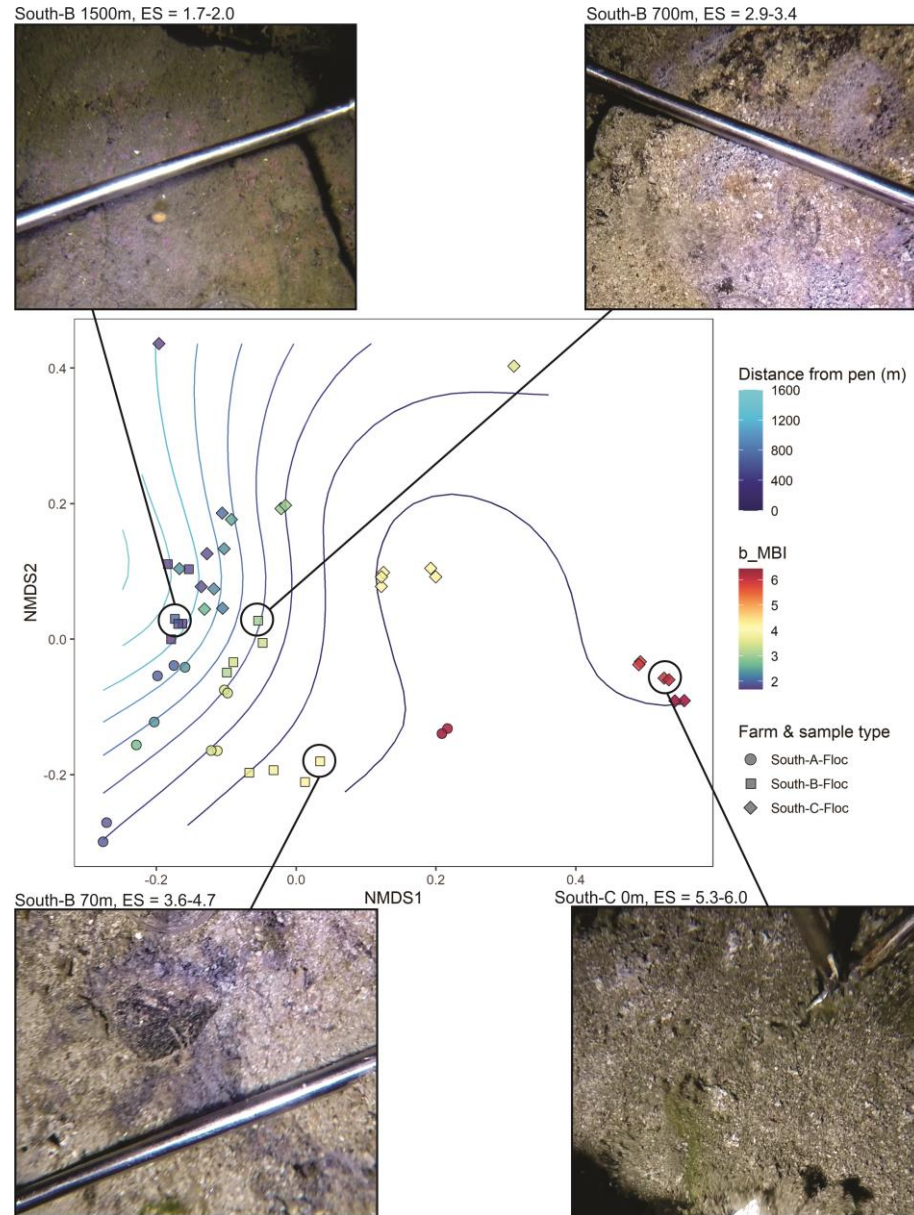
Table 4 Test for significance of (A) distance-based factors (‘Farm’ and ‘Distance’ from farm) in relation to Source of sample (SIBS versus grab) and Base substrate type, and (B) replacing Distance with b-MBI (assessed Enrichment Stage) using permutational analysis of variance (PERMANOVA) based on distance matrices from all SIBS obtained microbial eDNA samples.

A.	Df	SumsOfSqs	MeanSqs	F.Mod	R ²	Pr(>F)	
				el			
Farm	1	0.3378	0.33780	6.043	0.03130	0.0008	***
log(Distance)	1	2.3529	2.35285	42.088	0.21799	0.0001	***
Source of sample (SIBS/Grab)	1	1.5523	1.55228	27.767	0.14382	0.0001	***
Base Substrate type	3	0.4036	0.13453	2.406	0.03739	0.0088	**
Farm:log(Distance)	1	0.2769	0.27688	4.953	0.02565	0.0028	**
Residuals	105	5.8698	0.05590		0.54384		
Total	112	10.7932			1.00000		



‘Grab-eDNA’
+
‘SIBS-eDNA’

- Challenging steep bedrock sites
- Previously impossible to assess waste prevalence / organic enrichment
- b-MBI from SIBS provides invaluable information about waste distribution and enrichment status
- Raises interesting philosophical questions about what it actually means...?



Take home messages

- Microbial eDNA extracted from sediment layers overlying almost all marine substrates contain valuable information
- Microbial assemblages contain the same 'species' that are being used to assess benthic enrichment in conventional soft sediments
- A new device (SIBS) has been developed to sample these challenging substrates
- The few microbial ASV's for which we have assigned EcoGroupings (based on New Zealand sediments) already permit us to calculate a meaningful biotic index in Norway – this will only get better – fast!
- The tool also has a lot of potential for mapping the distribution ('footprint') of farms irrespective of substrates – a tracer style approach
- The potential is still relatively untapped (still a lot to learn)

