Nye metoder for miljøovervåking for fremtidens oppdrett

Nigel Keeley, Kathy Dunlop, et al.

Havforskningsinstituttet

✓ = Quantitative and useful
~ = Qualitative / some relevance
? = Potential relevance / not well understood

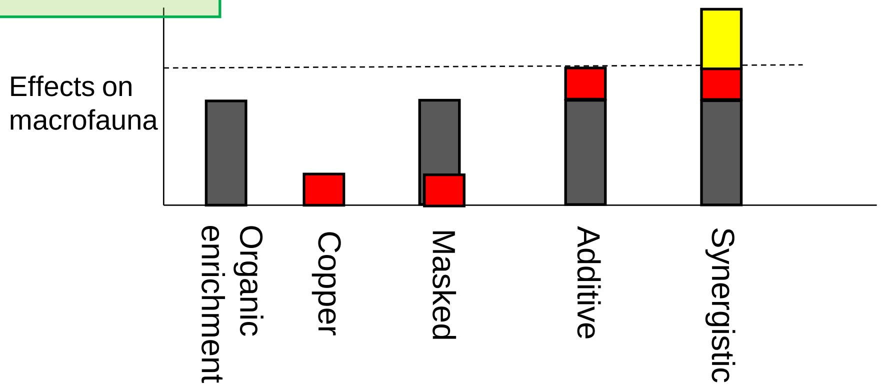
NS: 9410:2016

Analytical types

Ecological compartment		**B-investigation**						**C-investigation**											
		Sensory (Farge, luft, konsistens etc.)						**Macrofauna**					Kjemiske parametere (in sediments)						
Discharge type		Farge	Luft	Konsistens	Grabvolum	Tykkelse	Redox /pH	S	N	NQI1	NSI	H'	TOM	nTOC	N	C:N	P	Zn	Cu
Soft sediment footprint																			
Organic enrichment		~	~	~		~	✓/?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Biofouling drop off				~				~	~	~	~	~	~			~			
Antifouling chemicals:	Cu							~	~	~	~	~							✓
	All new products							?											
Infeed therapeutants	Zn							~	~	~	~	~						✓	
	All others							?											
New feed composition (eg terrestrial)								?								~			
Bath treatments								?											

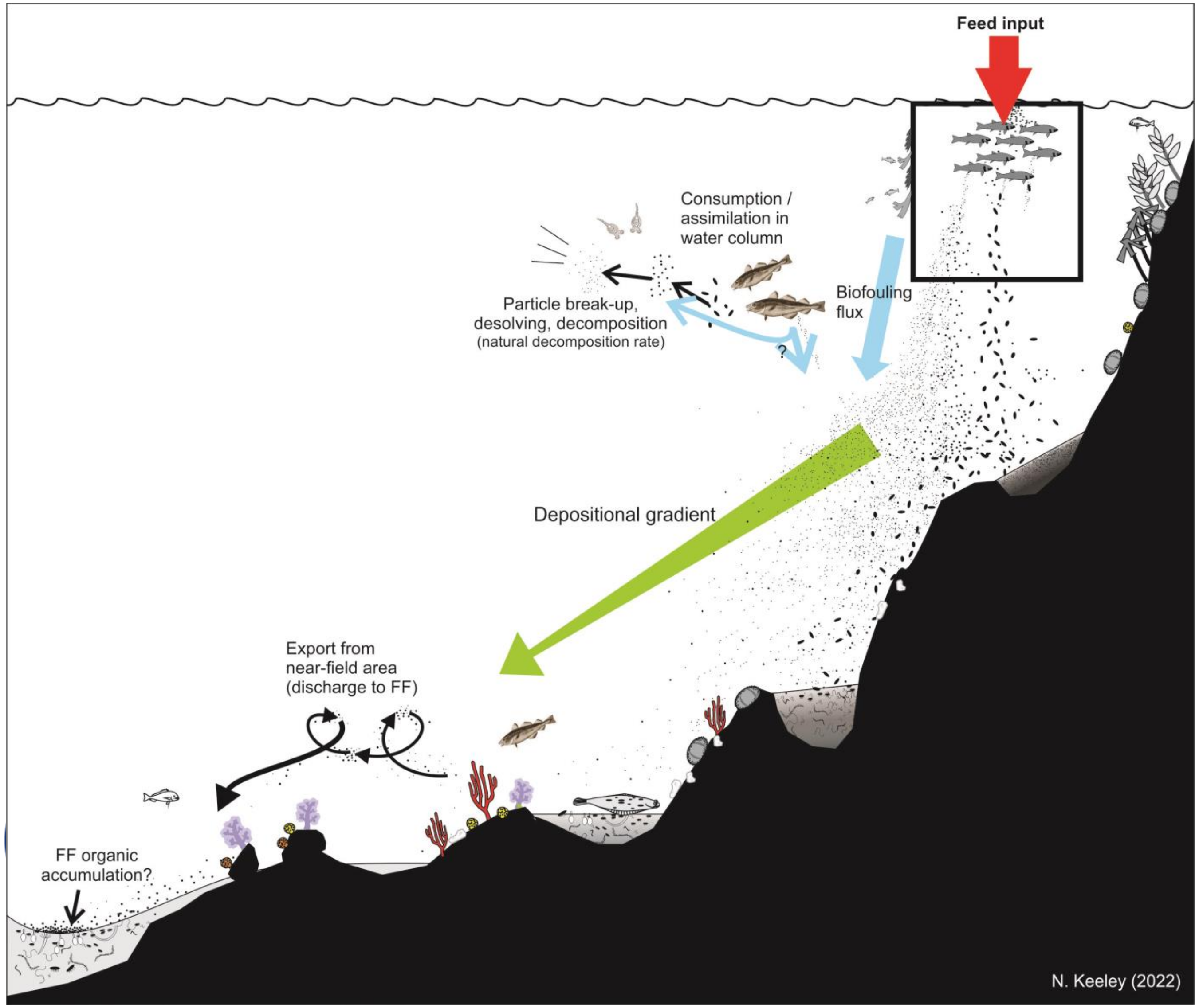
Lacking (W.I.P.)

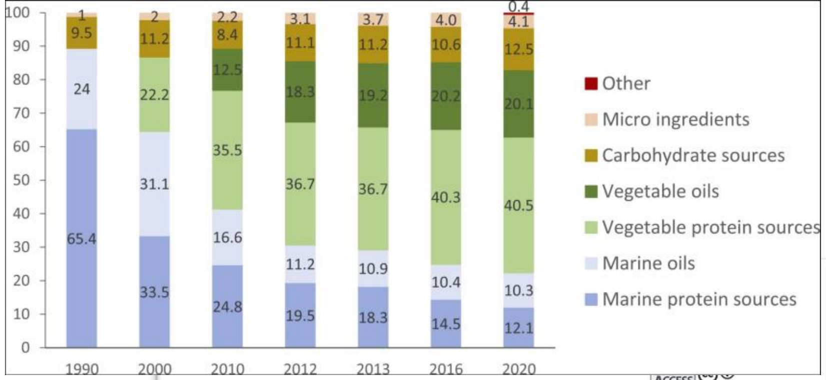
Additional /independent /synergistic effects?

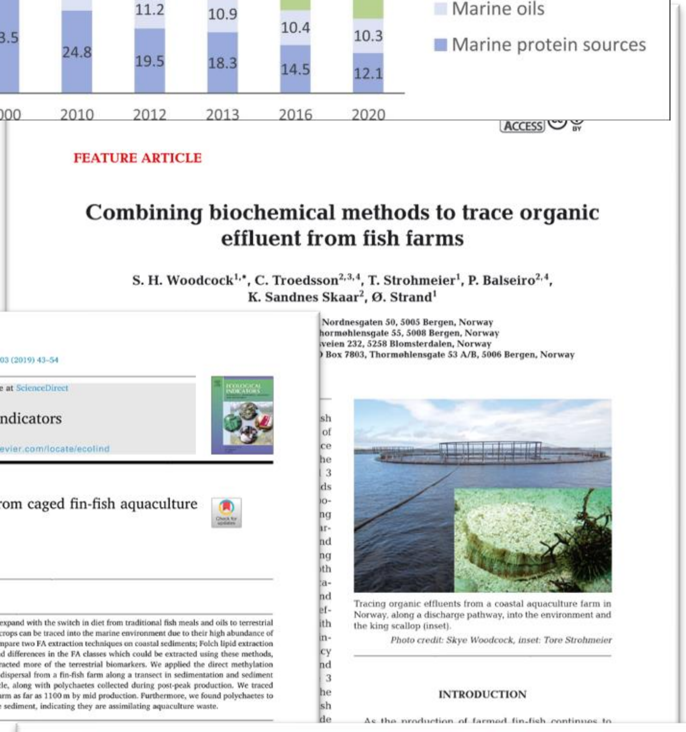


Aas et al. (2022) AquaReports 26

Avfallssporingsmetoder







FEATURE ARTICLE

Combining biochemical methods to trace organic effluent from fish farms

S. H. Woodcock[1,*], C. Troedsson[2,3,4], T. Strohmeier[1], P. Balseiro[2,4], K. Sandnes Skaar[2], Ø. Strand[1]

Tracing organic effluents from a coastal aquaculture farm in Norway, along a discharge pathway, into the environment and the king scallop (inset).
Photo credit: Skye Woodcock, inset: Tore Strohmeier

INTRODUCTION

Ecological Indicators 103 (2019) 43–54

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Fate and longevity of terrestrial fatty acids from caged fin-fish aquaculture in dynamic coastal marine systems

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Institute of Marine Research, Postboks 1870 Nordnes, 5817 Bergen, Norway

ARTICLE INFO

Keywords:
Fin-fish aquaculture
Sedimentation
Sediment
Polychaetes
Method development

ABSTRACT

Fin-fish production continues to expand with the switch in diet from traditional fish meals and oils to terrestrial sourced components. Terrestrial crops can be traced into the marine environment due to their high abundance of C18 fatty acids (FA). Here we compare two FA extraction techniques on coastal sediments; Folch lipid extraction and direct methylation. We found differences in the FA classes which could be extracted using these methods, and that direct methylation extracted more of the terrestrial biomarkers. We applied the direct methylation method to detect organic waste dispersal from a fin-fish farm along a transect in sedimentation and sediment samples across a production cycle, along with polychaetes collected during post-peak production. We traced organic waste from the fin-fish farm as far as 1100 m by mid production. Furthermore, we found polychaetes to have FA profiles reflective of the sediment, indicating they are assimilating aquaculture waste.

1. Introduction

REVIEWS IN Aquaculture

Reviews in Aquaculture (2019) 11, 133–148 doi: 10.1111/raq.12230

Terrestrial fatty acids as tracers of finfish aquaculture waste in the marine environment

Camille A. White[1,2], Skye H. Woodcock[3], Raymond J. Bannister[3] and Peter D. Nichols[4]

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2 Sustainable Aquaculture Laboratory – Temperate and Tropical, School of BioSciences, University of Melbourne, Parkville, Vic., Australia
3 Institute of Marine Research, Bergen, Norway
4 Oceans and Atmosphere, Commonwealth Scientific and Industrial Research Organization, Hobart, TAS, Australia

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Received 11 July 2017; accepted 30 October 2017.

Abstract

Waste from open-cage aquaculture flows directly into the marine environment from uneaten feeds, faecal material and dissolved nutrients. Sustainable management outcomes are regularly based on the dispersal patterns of the waste, with biochemical tracing a key tool in understanding the footprint of aquaculture. We examined the use of fatty acid (FA) analysis to trace aquaculture waste for this purpose, with the aim of identifying specific biomarkers for environmental applications, as well as identifying challenges that are regularly encountered. Overall, the widespread use of terrestrial-based oils in the production of marine aquaculture feeds has increased the use of FA biomarkers to trace aquaculture waste across benthic and pelagic systems, in vertebrates, invertebrates and environmental samples such as sediment and seston. A combination of linoleic acid (LA), oleic acid (OA) and α-linolenic acid (ALA), which are dominant C_{18} FA in terrestrial seed and animal oils, is the most commonly used biomarkers, along with

Avfallssporingsmetoder

Tracing feed signatures into environment

- Terrestrial fatty acids (LA, OA, ALA)
- Soy DNA (other candidates?)
- Stable isotopes (δN:δC)
- Others? – new feed compositions…

Ecological Indicators 103 (2019) 43–54

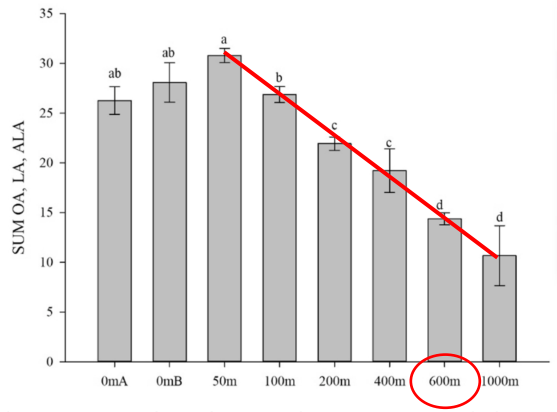
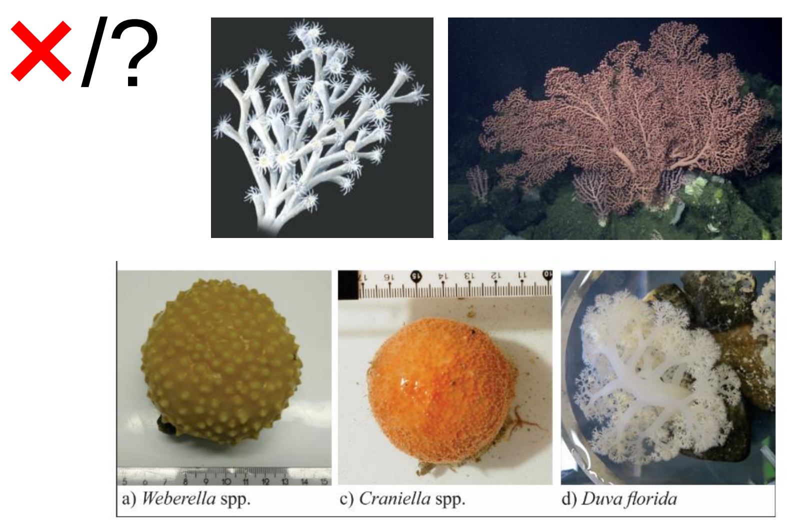


Fig. 7. Comparison of sum of OA, LA and ALA (average ± standard error) of infauna polychaetes sampled during peak production along a transect moving away from a fin-fish farm. Different letters represent significant difference in the sum of OA, LA and ALA.

Sediments (sinks)
Bivalves
Crustaceans
Echinoderms
Fish
…

Corals
Sponges
…



Bentisk mikrobiell eDNA – sediments

Dowle, E., Pochon, X., Keeley, N., Wood, S.A., **2015**. Assessing the effects of salmon farming seabed enrichment using bacterial community diversity and high-throughput sequencing. FEMS Microbiology and Ecology 91.

'Sustain Aqua': NFR No. 267829

Received: 21 September 2020 | Revised: 31 March 2021 | Accepted: 4 May 2021
DOI: 10.1111/1755-0998.13426

ORIGINAL ARTICLE

MOLECULAR ECOLOGY RESOURCES WILEY

Beyond taxonomy: Validating functional inference approaches in the context of fish-farm impact assessments

Olivier Laroche[1,2] | Xavier Pochon[2,3] | Susanna A. Wood[2] | Nigel Keeley[1,2]

frontiers in Microbiology
ORIGINAL RESEARCH
published: 29 April 2021
doi: 10.3389/fmicb.2021.637811

Global Trends of Benthic Bacterial Diversity and Community Composition Along Organic Enrichment Gradients of Salmon Farms

Larissa Frühe[1], Verena Dully[1], Dominik Forster[1], Nigel B. Keeley[2,3], Olivier Laroche[2], Xavier Pochon[2,4], Shawn Robinson[5], Thomas A. Wilding[6] and Thorsten Stoeck[1]*

frontiers in Marine Science
ORIGINAL RESEARCH
published: 14 April 2021
doi: 10.3389/fmars.2021.627687

A Substrate-Independent Benthic Sampler (SIBS) for Hard and Mixed-Bottom Marine Habitats: A Proof-of-Concept Study

Nigel Keeley[1], Olivier Laroche[1], Murray Birch[2] and Xavier Pochon[3,4]*

IR Inter-Research Science Publisher
Aquaculture Environment Interactions
Volume 17:1–20
Published January 23, 2025
https://doi.org/10.3354/aei00489
OPEN ACCESS

Disaggregation rates of salmon feces and microbial inoculation of sediments: new insight for particle dispersion modelers

Nigel Keeley[1,*], Katherine Dunlop[1], Olivier Laroche[2], Ellie Watts[1], Pål Sævik[3], Jon Albretsen[3]

Wood, S.A., Tremblay, L.A., Laroche, O., Pochon, X., Lear, G., Ellis, J.I., 2018. Incorporating molecular-based functional and co-occurrence network properties into benthic marine impact assessments. FEMS Microbiology Ecology 94.

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Pearman, J.K., Keeley, N., Wood, S.A., Laroche, O., Zaiko, A., Thomson-Laing, G., Biessy, L., Atalah, J., Pochon, X., 2020. Comparing sediment DNA extraction methods for assessing organic enrichment associated with marine aquaculture. PeerJ 8:e10231.

Frühe, L., Dully, V., Forster, D., Keeley, N.B., Laroche, O., Pochon, X., Robinson, S., Wilding, T.A., Stoeck, T., 2021. Global Trends of Benthic Bacterial Diversity and Community Composition Along Organic Enrichment Gradients of Salmon Farms. Frontiers in Microbiology 12.

- New Zealand*
- Scotland (WIP)
- Switzerland*
    - (ASC -> Norge)
- Canada (WIP)
- Chile (WIP)

'AQUAed': NFR No. 320076

Marine Genomics 66 (2022) 100991
Contents lists available at ScienceDirect
Marine Genomics
journal homepage: www.elsevier.com/locate/margen
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Bimodal distribution of seafloor microbiota diversity and function are associated with marine aquaculture

R. Pettersen[a], I. Ormaasen[b], I.L. Angell[b], N.B. Keeley[c], A. Lindseth[d], L. Snipen[b], K. Rudi[b,*]

Philip et al. BMC Bioinformatics (2024) 25:237
https://doi.org/10.1186/s12859-024-05837-z
BMC Bioinformatics

RESEARCH Open Access

METASEED: a novel approach to full-length 16S rRNA gene reconstruction from short read data

Melcy Philip[1*], Knut Rudi[1], Ida Ormaasen[1], Inga Leena Angell[1], Ragnhild Pettersen[2], Nigel B. Keeley[3] and Lars-Gustav Snipen[1]

ISME
ISME Communications, 2024, 4(1), ycae071
https://doi.org/10.1093/ismeco/ycae071
Advance access publication: 9 May 2024
Original Article

Swarm and UNOISE outperform DADA2 and Deblur for denoising high-diversity marine seafloor samples

Tonje Nilsen[1], Lars-Gustav Snipen[1], Inga Leena Angell[1], Nigel Brian Keeley[2], Sanna Majaneva[3], Ragnhild Pettersen[3], Knut Rudi[1,*]
[1]Faculty of Chemistry, Biotechnology and Food Science (KBM), Norwegian University of Life Sciences (NMBU), Chr. M. Falsensvei 18, Biotechnology Building

Nilsen T, Pettersen R, Keeley NB, Ray JL, Sanna Majaneva S, Stokkan M, Hervik A, Angell IL, Snipen LG, Sundt MØ, Rudi K. **In Review**. Key roles of microbial sulfur and ammonium oxidizers for the coastal seafloor ecological state.

Keeley N*, Dunlop K, Laroche O, Hansen PK, Rudi K. In Prep. **An approach for quantifying the influence of fish farm waste on hard-bottom habitats.**

Mikrobiell eDNA

- Converged on microbial
- Repeatedly proven for organic enrichment
- Sensitive / tracer equivalent
- Uptake for compliance is slow*

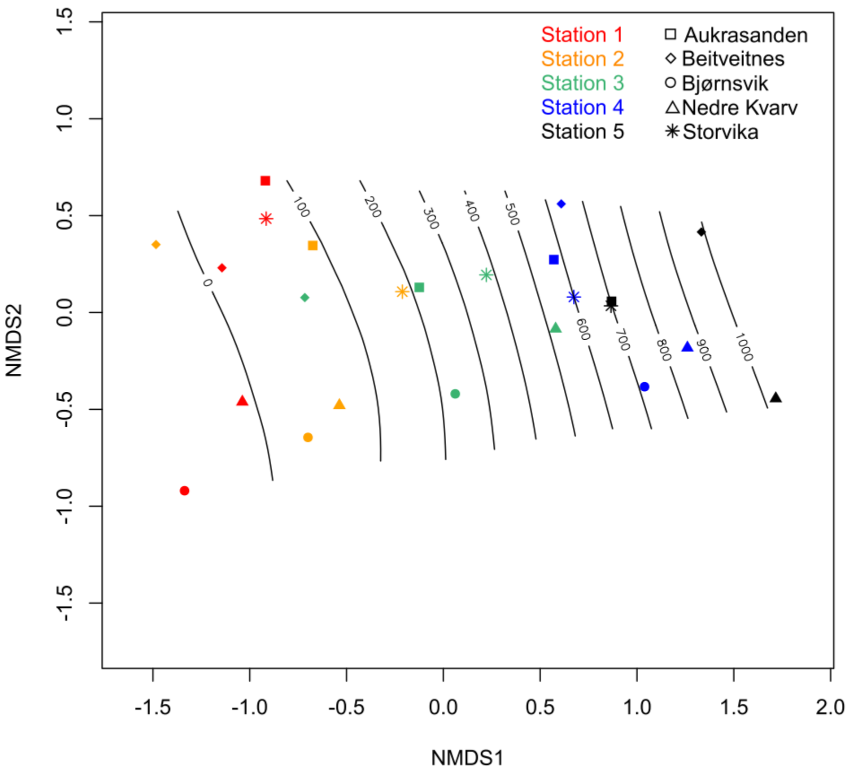
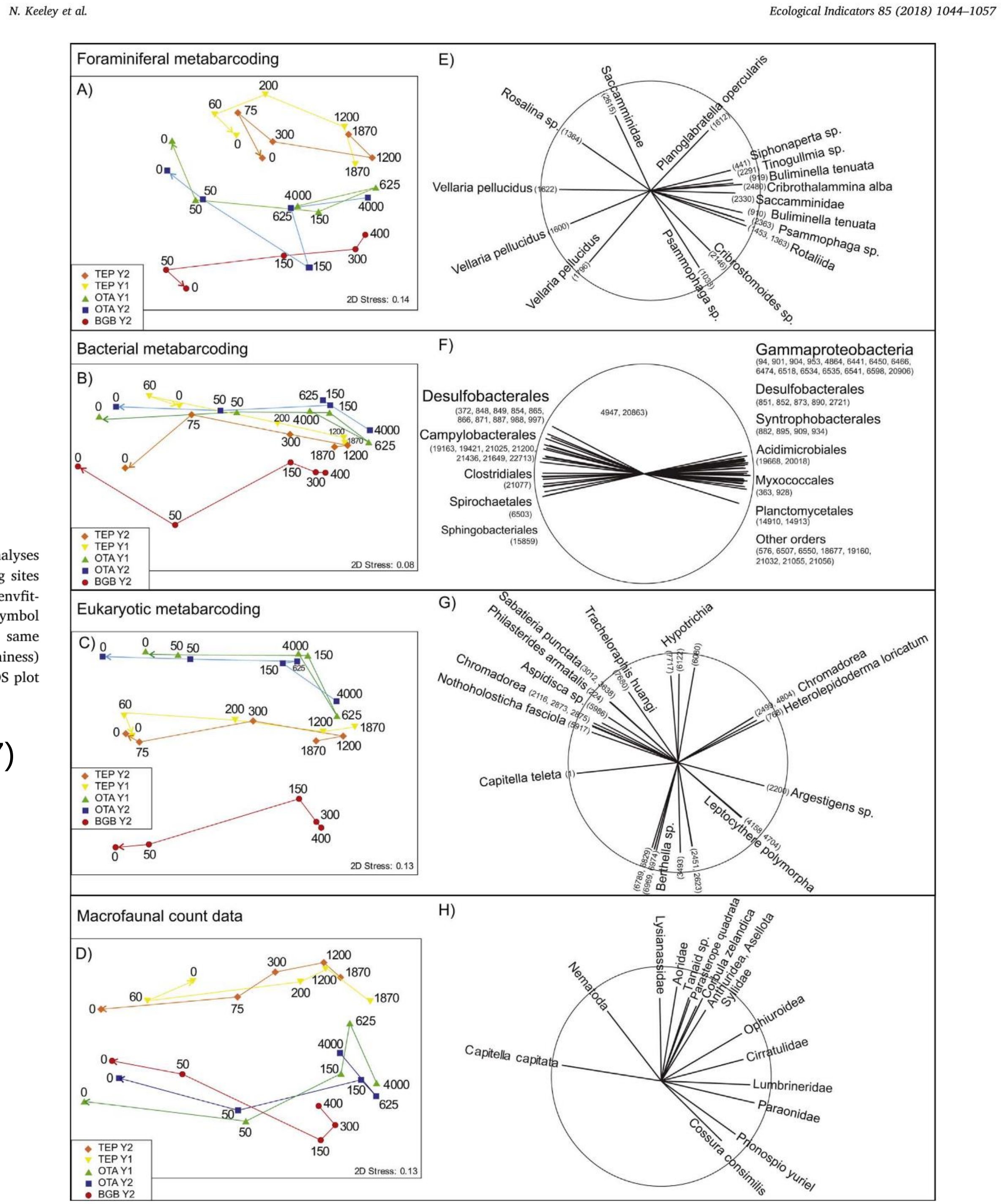


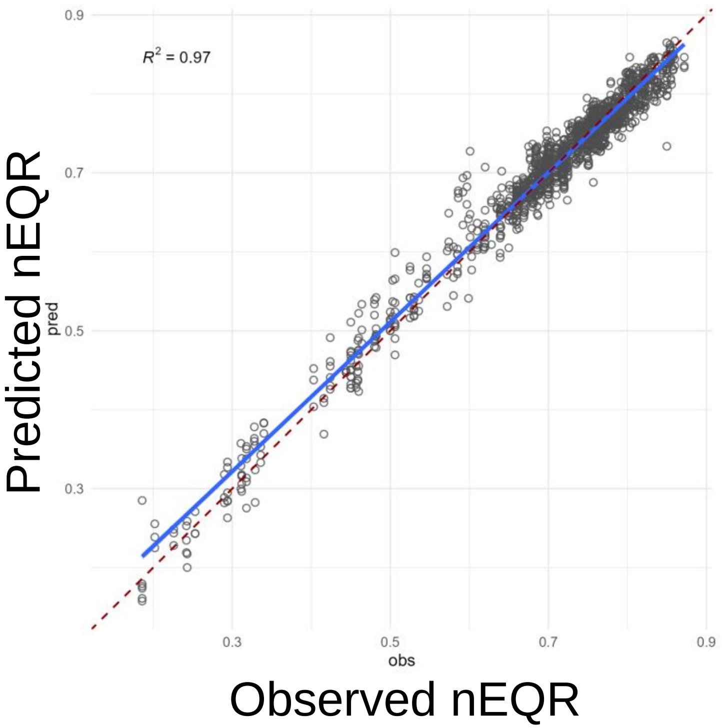
Fig. 5. Plot of non-metric multidimensional scaling (NMDS) analyses of bacterial communities at the different farms and sampling sites with model-fitted distance contour. Correlation results of envfit-analyses for NMDS axes 1 and 2 are shown in Table 2. Each symbol in the plot originates from three pooled replicates of the same sampling sites, accounting for potential spatial variation (patchiness) of bacterial communities at a specific sampling site. An NMDS plot for all individual replicates is provided as Supplemental File.

(Stoeck et al. 2018, MPB127)

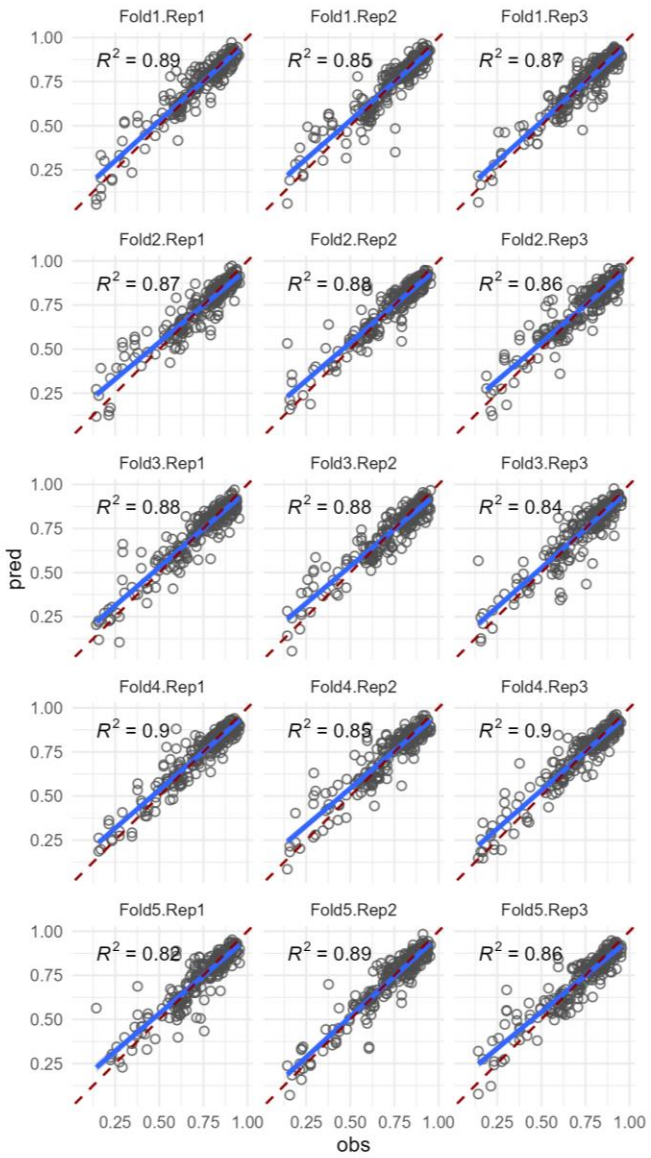


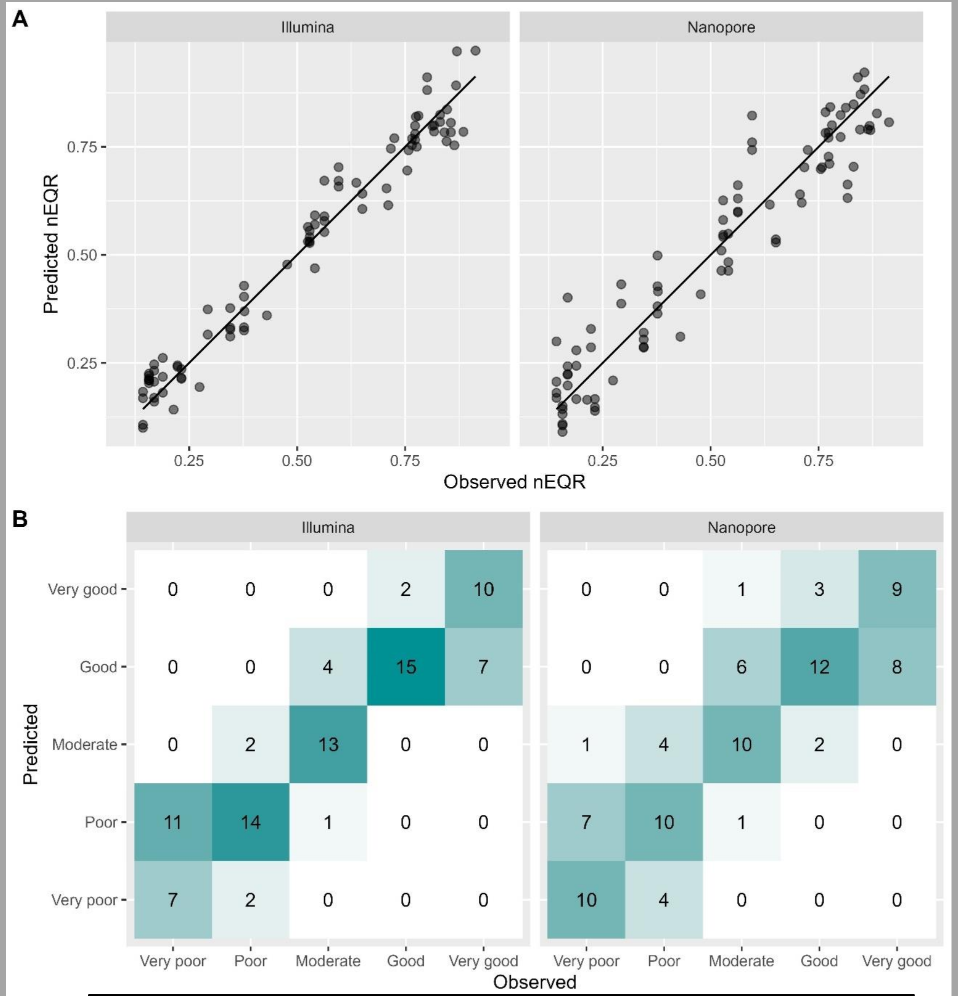
Mikrobiell eDNA

- Norway-specific AQUAed database
- Can be used to assess benthic enrichment *now*
- Question of exact method and how to implement



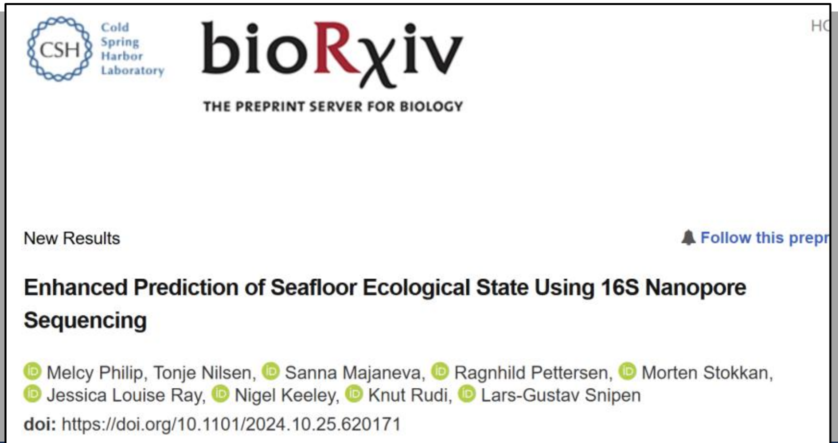
nEQR





Predicted \ Observed (Illumina)	Very poor	Poor	Moderate	Good	Very good
Very good	0	0	0	2	10
Good	0	0	4	15	7
Moderate	0	2	13	0	0
Poor	11	14	1	0	0
Very poor	7	2	0	0	0

Predicted \ Observed (Nanopore)	Very poor	Poor	Moderate	Good	Very good
Very good	0	0	1	3	9
Good	0	0	6	12	8
Moderate	1	4	10	2	0
Poor	7	10	1	0	0
Very poor	10	4	0	0	0


CSH Cold Spring Harbor Laboratory bioRxiv THE PREPRINT SERVER FOR BIOLOGY

New Results

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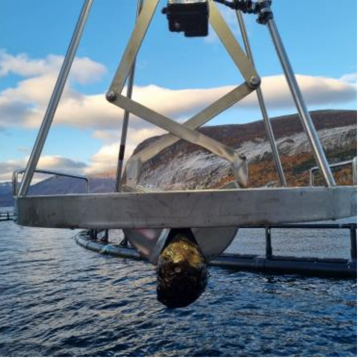
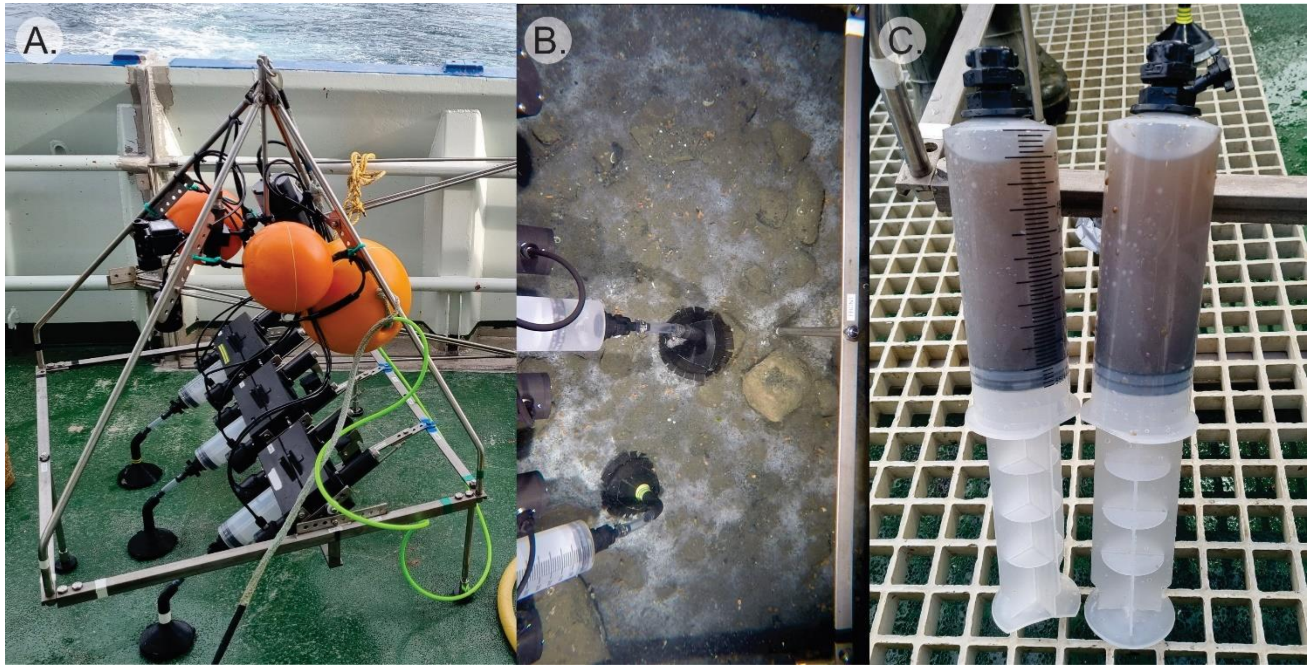
Enhanced Prediction of Seafloor Ecological State Using 16S Nanopore Sequencing

Melcy Philip, Tonje Nilsen, Sanna Majaneva, Ragnhild Pettersen, Morten Stokkan, Jessica Louise Ray, Nigel Keeley, Knut Rudi, Lars-Gustav Snipen

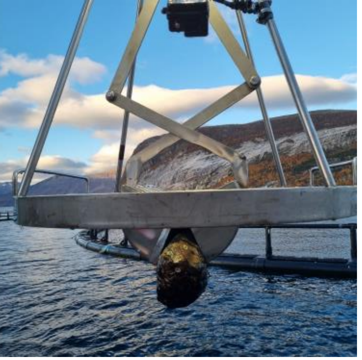
doi: https://doi.org/10.1101/2024.10.25.620171



Hardbunnsovervåking

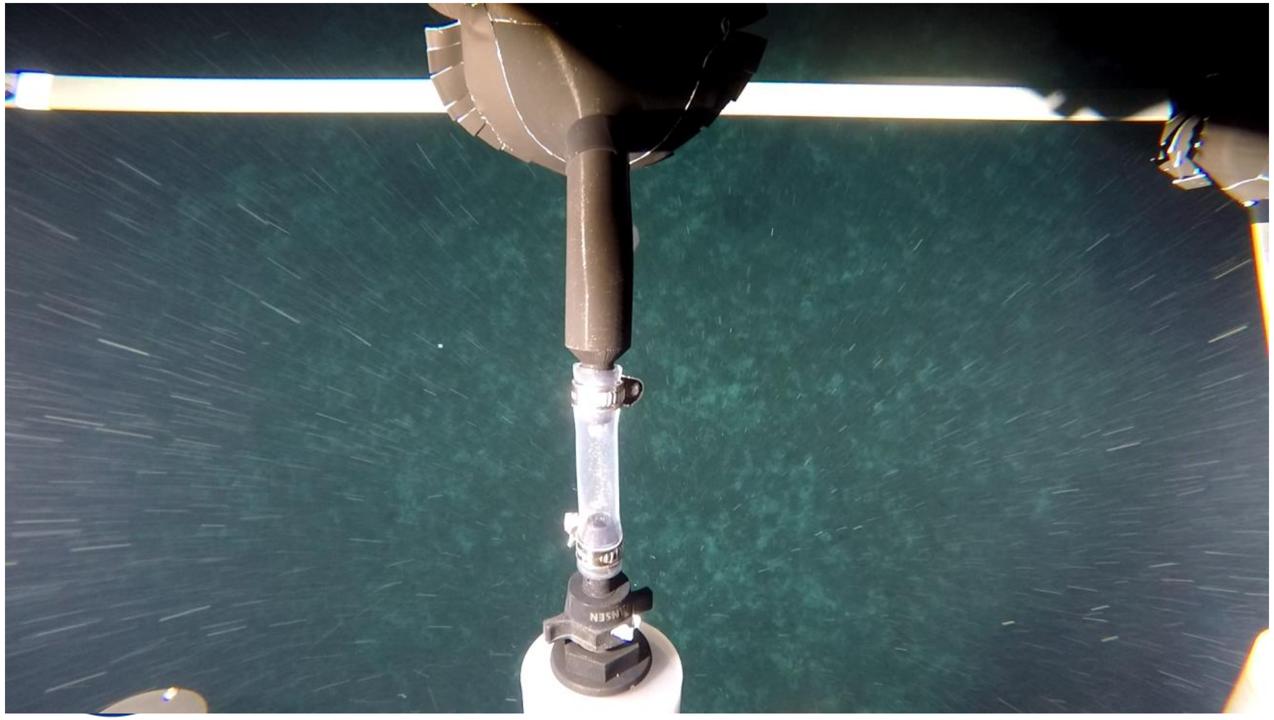


Funded by: Akvakyst (HI), ‘Sustain Aqua’: NFR No. 267829, ‘AQUAed’: NFR No. 320076



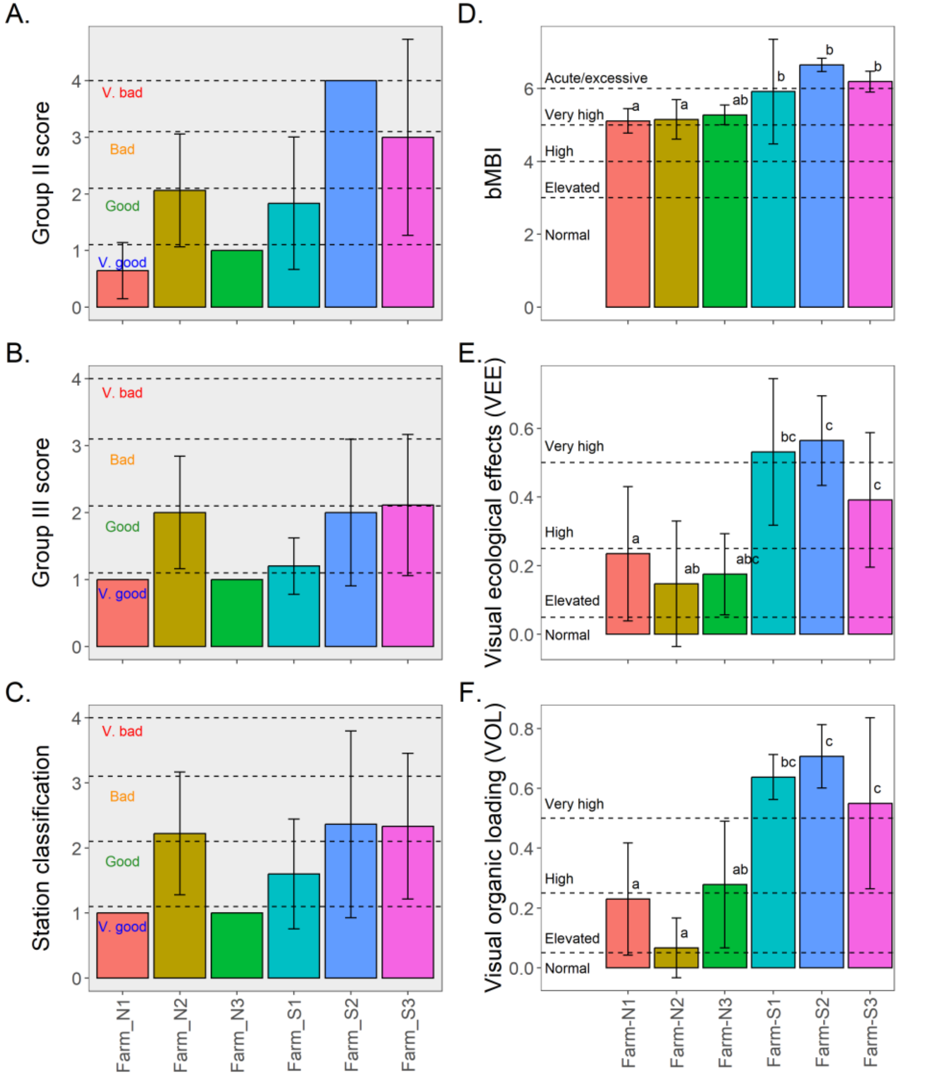
Hardbunnsovervåking

- Combining 16S microbial eDNA + visual indicators
- Cable mounted sample 0-170m depth
- Triplicate samples
- Relatively rapid, quantitative, sig. cheaper than macrofauna

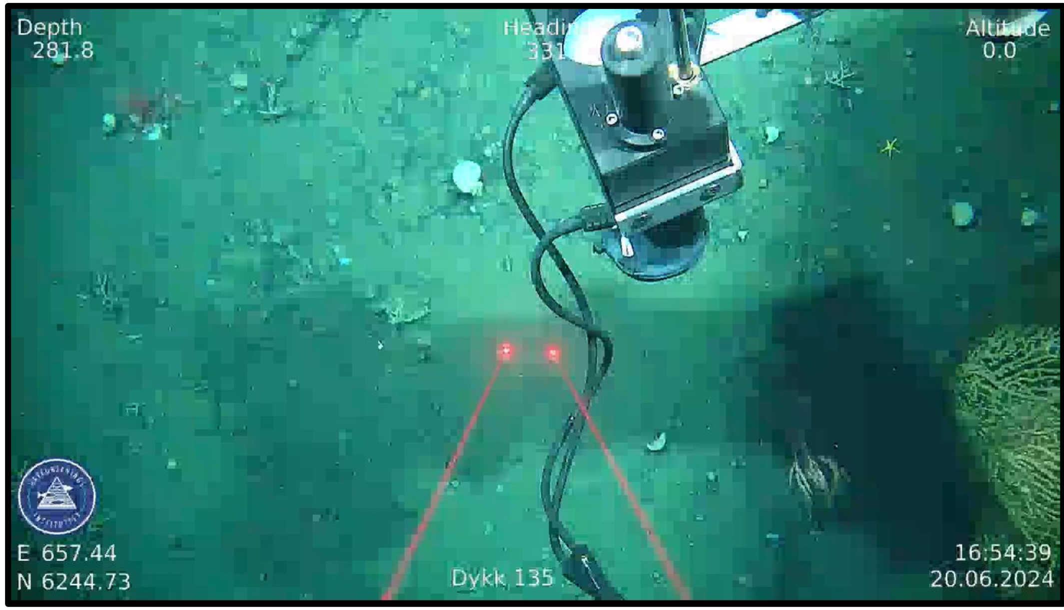


Keeley et al. In Review. An approach for quantifying the influence of fish farm waste on hard-bottom habitats. Ecological Indicators

Existing (b-investigation) | New (eDNA bMBI + visual)



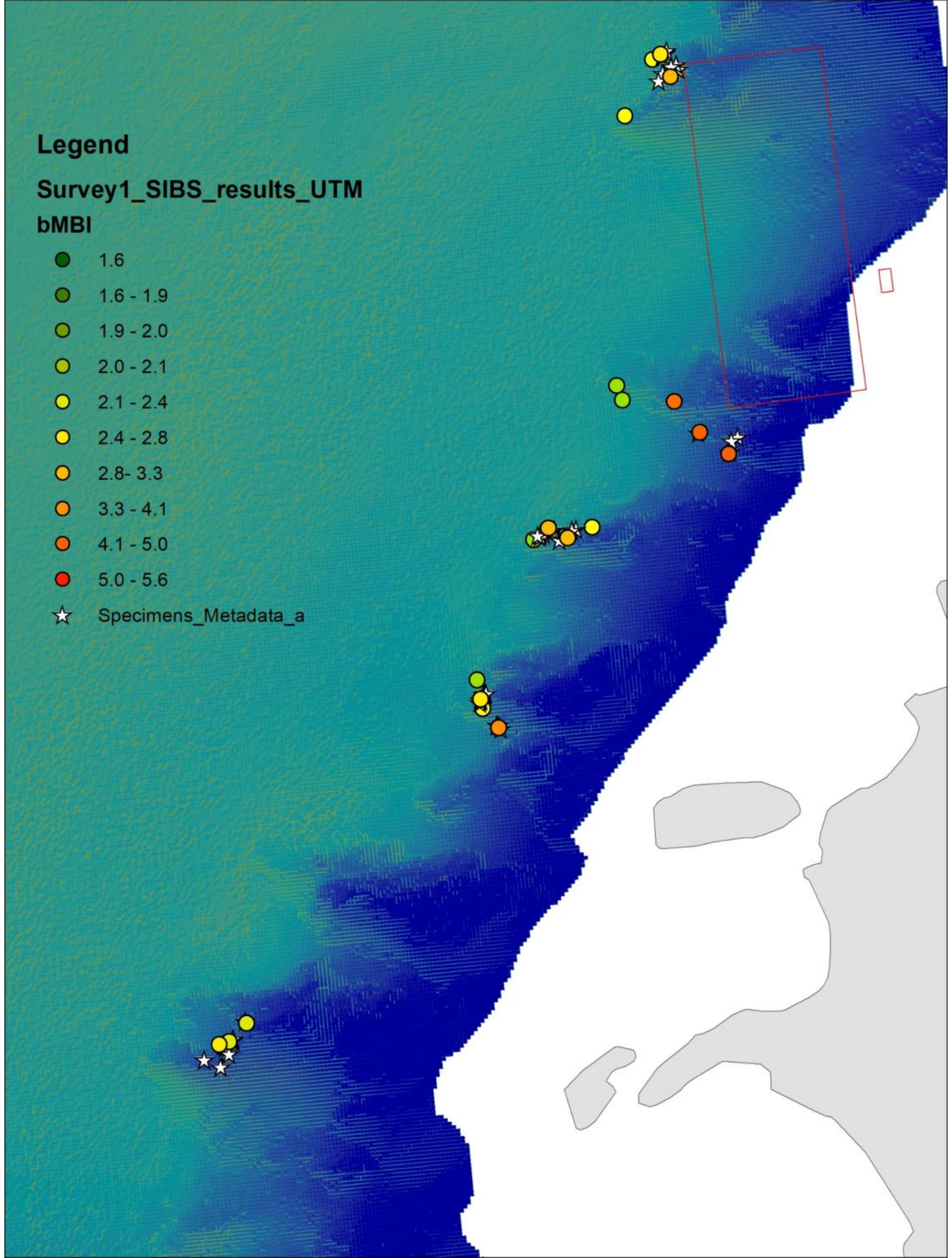
Hardbunnsovervåking - dype lokaliteter



Hardbunnsovervåking - dype lokaliteter

(FHF: VDWS Transition)

- Triplicate samples per dive
- Control over positioning
- Potential for benthic enrichment monitoring at depth
- Scope for properly mapping waste distribution
- Scope for studying waste exposure on sessile reef species



UV-sulfider

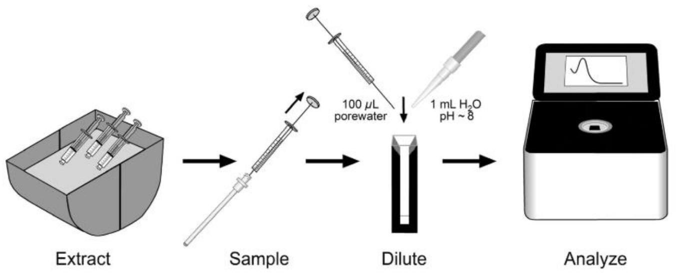
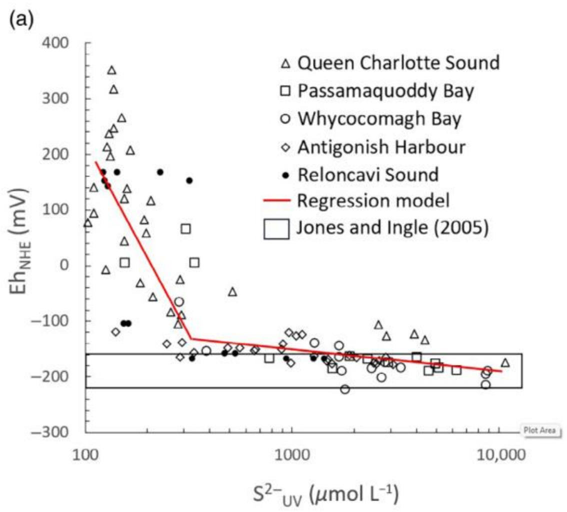


Fig. 1. Illustration of the steps involved in the collection and analysis of sediment porewater for total free sulfide analysis by simplified direct UV spectro-photometry. The depicted materials are described in the text.

Cranford et al (DFO, Canada)

- New UV sulfides method
- Resolved some historical issues with TFS method
- Rapid, practical, needs more testing in Norway
- Arguable 'better' than redox

Cranford



LIMNOLOGY and OCEANOGRAPHY: METHODS

ASLO

Limnol. Oceanogr.: Methods 22, 2024, 608–617
© 2024 Association for the Sciences of Limnology and Oceanography.
doi: 10.1002/lom3.10619

A simple and rapid method for measuring total free sulfides in marine sediments

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Emeritus Marine, St. Andrews, New Brunswick, Canada

Abstract

The quantitatively most important process by which organic matter in marine sediments is mineralized is performed by sulfate-reducing bacteria, resulting in the accumulation of total dissolved (free) sulfide ($S^{2-} = H_2S + HS^- + S^{2-}$) in porewater. S^{2-} is toxic to benthic animals and vascular plants and measurements serve as a proxy for the deleterious effects of organic enrichment on benthic habitat, biodiversity, and ecosystem function. Methodologies for measuring S^{2-} in water have been pursued for at least a century, and standard approaches employ colorimetry (methylene blue and iodometric titration) and potentiometry. These standard methods require between 1 and 200 mL of porewater, which can be laborious to obtain. The ion-selective electrode method is widely employed as a practical approach for sediment S^{2-} analysis but lacks analytical robustness and is highly prone to measurement biases that misinform research and environmental management decisions. A technically simple method is described, based on direct UV spectrophotometry, for the near real-time field analysis of small porewater samples. The procedure prevents known measurement biases associated with particulate sulfide interference, S^{2-} volatilization and oxidation, and represents a practical approach for monitoring organic enrichment and classifying benthic ecological quality status. Porewater concentrations between 200 and 15,000 μmol L^{-1} can be measured and instrument calibration is highly stable. The method has the capacity to rapidly process and analyze sediment samples at low cost, which helps resolve the problem of chronic under-sampling associated with the use of traditional S^{2-} methods.

Anoxic conditions occur at some depth in all marine sediments. In areas where the vertical flux of organic matter exceeds the supply of oxygen required for aerobic carbon mineralization, the anoxic zone can reach the sediment surface and even into the water column. Sulfate is plentiful in seawater and its reduction to sulfide by heterotrophic bacteria is the quantitatively dominant process in the diagenesis of organic matter in marine sediments (Fenchel 1987). Sulfide accumulates in porewater primarily in the form of HS^- but also includes H_2S and S^{2-} depending on pH. These three dissolved sulfide forms are collectively referred to as total free sulfide or S^{2-}.

The ecological implications of sediment organic enrichment from coastal eutrophication and industry effluents (e.g., aquaculture, wood pulp, seafood waste, and offshore drilling) are of interest to scientists and managers worldwide. S^{2-} is toxic to benthic macrofauna (Bagarinao 1992; Grieshaber and Völkel 1998; Wang and Chapman 1999; Gray et al. 2002; Hargrave et al. 2008) and some vascular plants that provide critical coastal habitat (Lamers et al. 2013). S^{2-} measurements have been recommended as a more practical indicator of sediment toxicity than traditional benthic community taxonomic analysis, which requires greater technical expertise, much higher analysis and interpretation costs, and long delays to obtain results (Wildish et al. 2001). Subsequently, S^{2-} measurements are used in several countries to monitor and classify habitat quality and the health status of benthic communities exposed to organic enrichment (Hargrave et al. 2008; Cranford et al. 2020, 2022).

The measurement of S^{2-} in water and sediments has been the topic of research for many decades. Established analytical methods for S^{2-} analysis of water include methylene blue colorimetry, potentiometry with an ion-selective electrode, and iodometric titration (APHA 2017; Methods 4500-S^2-D, 4500-S^2-G, and 4500-S^2-F). These classical methods require between 5- and 200-mL sample volumes although laboratory adaptations of the methylene blue method can reduce the required sample volume to 1 mL for measuring sulfide concentrations below 30 μmol L^{-1}. Extraction of this volume of porewater from sediments is often impractical for research and environmental management applications that require large numbers of samples. The ISE method was adapted for measuring S^{2-} directly in 5-mL sediment/porewater slurries and is relatively simple and inexpensive to perform compared with the

*Correspondence: peter.cranford@outlook.com

Sensitive deepwater species monitoring

- SustainAqua (NFR 2017-2020)
- Akvakyst (HI, on-going)
- VDWS Transition (FHF, 2023-2025)

Desmophyllum pertusum

Paramuricea placomus

Paragorgia arborea

Primnoa resedaeformis

Phakellia ventilabrum

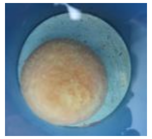
Geodia barretti

Acesta excavata

Isidella lofotensis

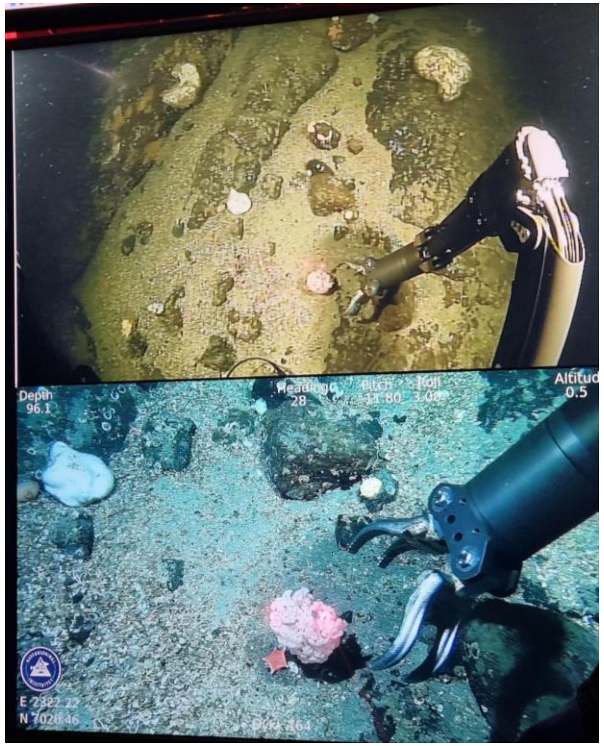
Duva florida

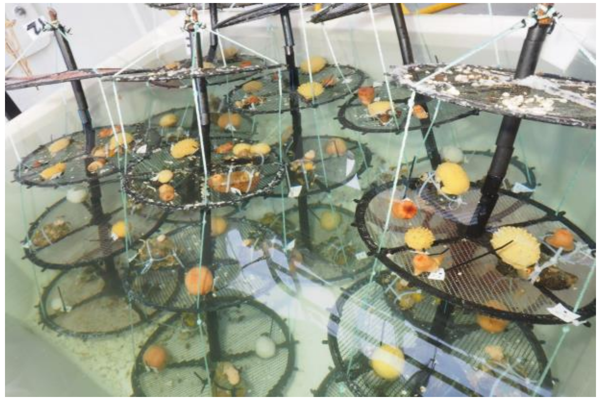
Craniella sp.

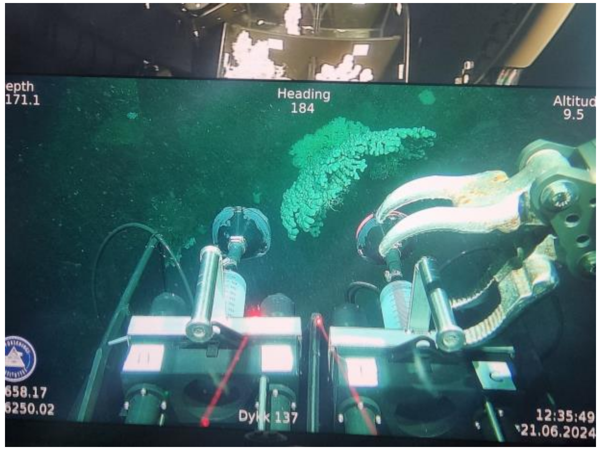


Weberella sp.

Hormathia diitata







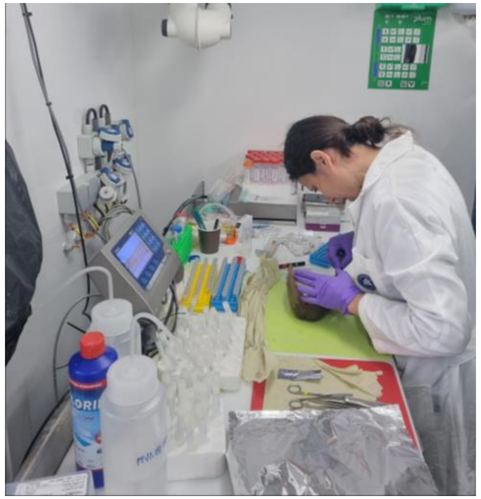
Analytical processes

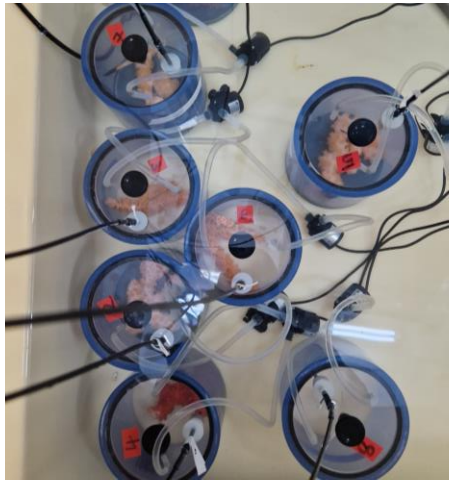
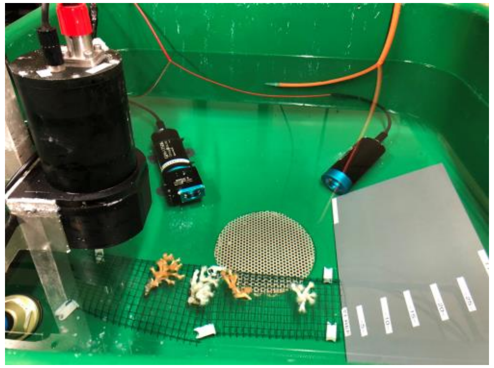
(VDWS Transition + Akvakyst)

Culled specimens:
- In-situ visual assessment
- On-board respirometry
- On-board LMS
- Multiple types of tissue samples for in-depth analysis for stress indicators* ('scatter gun' approach)

Live specimens:
- Laboratory exposure trials
- Visual / hyper spectral assessment

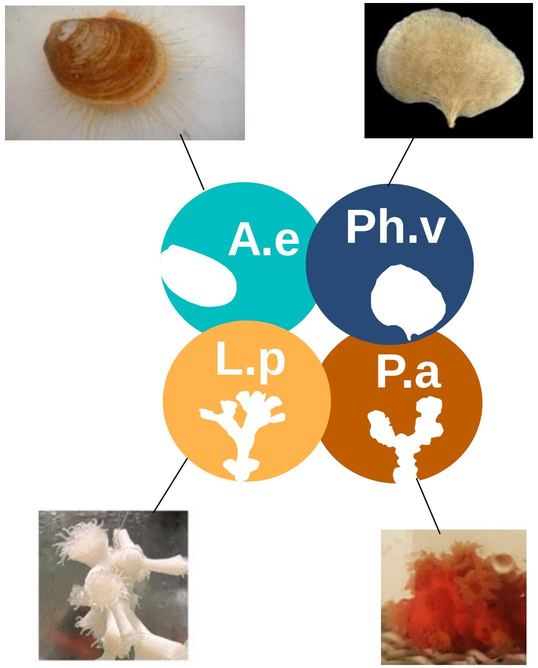
Evaluate 'specimen-specific' wase exposure
- Distance from farm (as the crow flies)
- Sedimentation measurements
- Modelled particle dispersion
- Waste influence on seabed (microbial eDNA)

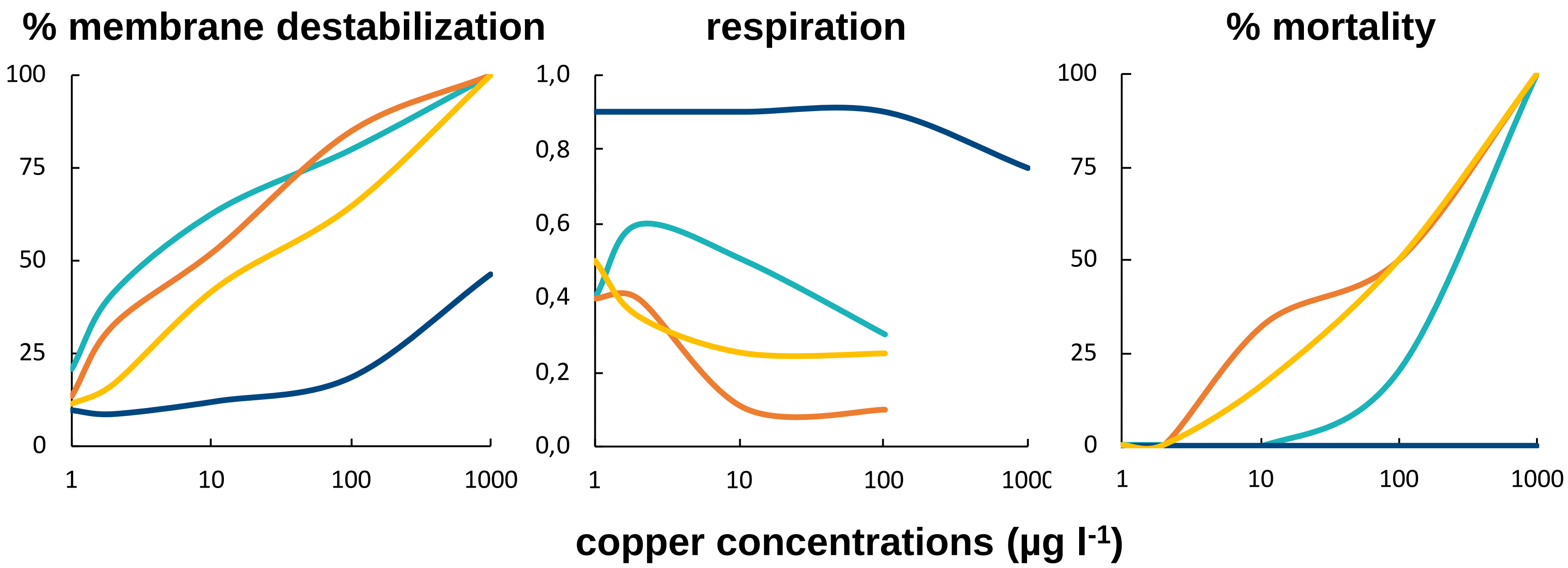



Sensitive deepwater species monitoring

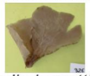
Effects of antifouling agents on selected deep-sea foundation species - Exposure of 4 deep-sea foundations species to dissolved copper. **Tina Kutti**, Vivian Husa, James Kar Hei Fang, Gry Hunvik, Bjørn Einar Grøsvik, Ketil Hylland





- Large differences in lethal and sub-lethal thresholds for Cu exposure between exposed species
- High sensitivity to Cu detected in the two corals, likely linked to limited mucus production capacity and high metabolic rates
- Rapid mobilization of cellular defence system in the bivalves, possibly causing enhanced tolerance to copper exposure
- No mortality and high tolerance to copper detected in the sponge *Phakellia*

Stressors / contaminants

Deepwater species	In-situ exposure	Organic enrichment*	Zn	Cu	Hydrogen-peroxide	Azametifos	Diflubenzuron	Florfenicol	Teflubenzuron	Praziquantel	Emamectin	New-a	New-b
Desmophyllum pertusum 	✓/~			✓							✓		
Paramuricea placomus 	✓/~												
Paragorgia arborea 	✓/~			✓							✓		
Primnoa resedaeformis 	✓/~												
Phakellia ventilabrum 	✓/~			✓							✓		
Geodia barretti 	✓/~			✓							✓		
Acesta excavata 	✓/~			✓									
Duva florida 	✓/~												
Craniella sp. 	✓/~												
Isidella lofotensis 	✓/~	✓		✓							✓		

W.I.P.

For each:

- Relative sensitivity (eg LC50, waste tolerance)
- Species specific stress indicators
- General knowledge of distributions
- General knowledge of functions and ecological value

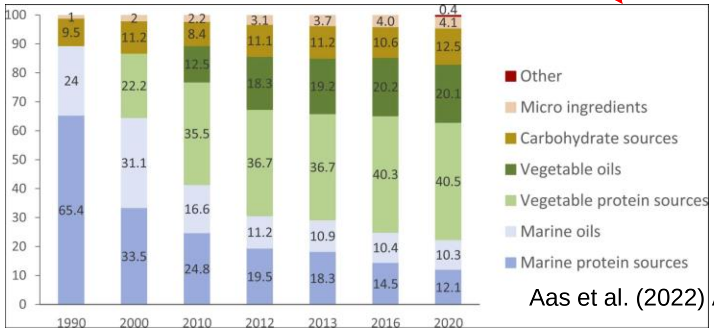
Clear need to prioritize important stressors based on level of use and anticipated use in future

✓= Quantitative and useful
~ = Qualitative / some relevance
? = Potential relevance / not well understood

Potential of new technologies

Analytical types

Ecological compartment		B-investigation						C-investigation															
		Sensory						Macrofauna					Kjemiske parametere (in sediments)										
Discharge type		Farge	Luft	Konsistens	Grabvolum	Tykkelse	Redox /pH	S	N	NQI1	NSI	H'	TOM	nTOC	N	C:N	P	Zn	Cu	Microbes (eDNA)	Targeted, adaptive contaminant monitoring (>> toxicity testing)	Stress indicators in sensitive species	Specific tracers FA / isotope /genetics
Soft sediment footprint																							
Organic enrichment		~	~	~		~	✓/?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓			
Biofouling drop off				~				~	~	~	~	~	~			~							✓
Antifouling chemicals:	Cu							~	~	~	~	~							✓	?	✓		
	All new products							?												?	✓		
Infeed therapeutants	Zn							~	~	~	~	~						✓		?	✓		
	All others							?												?	✓		
New feed composition (eg terrestrial)								?								~				✓	✓		✓
Bath treatments								?													✓		
Subtle far-field effects								~	~	~	~	~								✓			✓
Information from hard substrates?																				✓			
Deep water, hard substrata fauna (corals?)																				✓/?		✓	?
Mobile, demersal, pelagic species?																						?	✓



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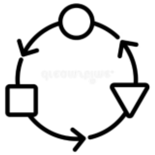
NB: Fish feed does not start and end with the salmon!

More sensitive, farm-specific options available



Adaptive monitoring

Recommendations for future emphasis

1. Expand toxicity testing for new and emerging contaminants – *don't wait for the natural environment to be the testing ground*…

2. Continue multi-species eco-tox & stress indicator work with 'sensitive' species.

3. Continue to develop new waste tracers based on new feed compositions.

4. Attempt to separate effects from organic enrichment per se, versus that of associated contaminants.

5. Obtain consensus on 16s eDNA monitoring methods. Decide on optimum method and implementation pathway. Implement.

6. Revision of existing monitoring strategy to ensure it is optimally focused and using best available (and cost effective) methods