

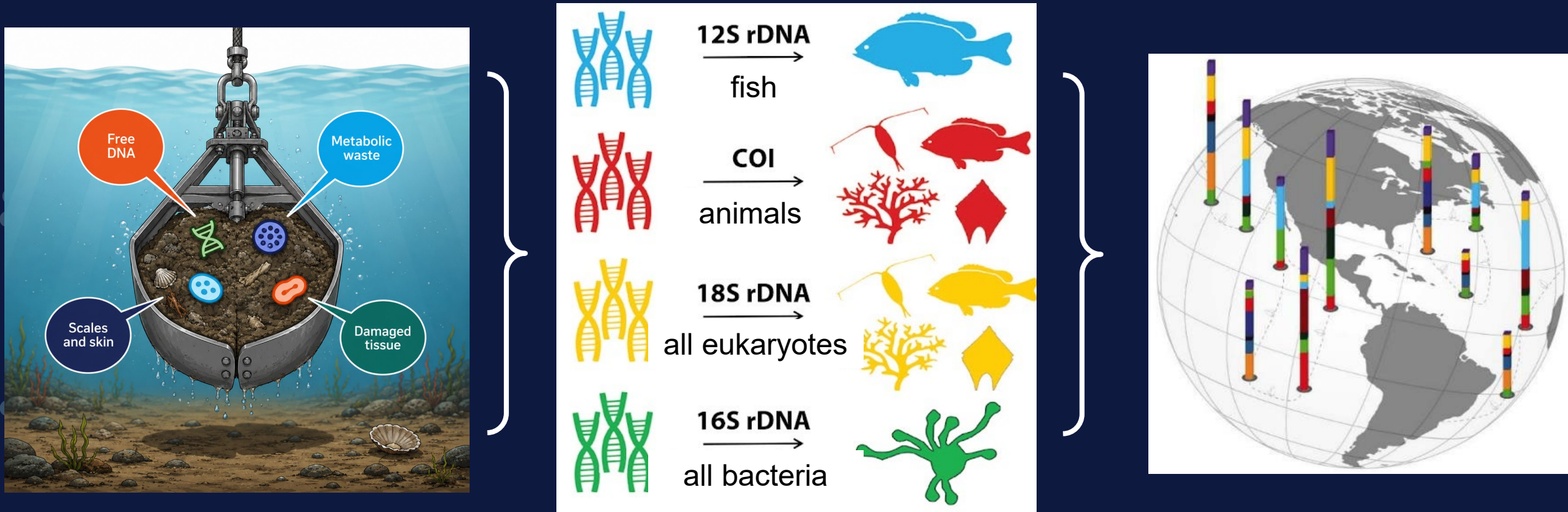
Integrating eDNA tools into benthic monitoring: from macrofauna to bacteria

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Environmental DNA tools

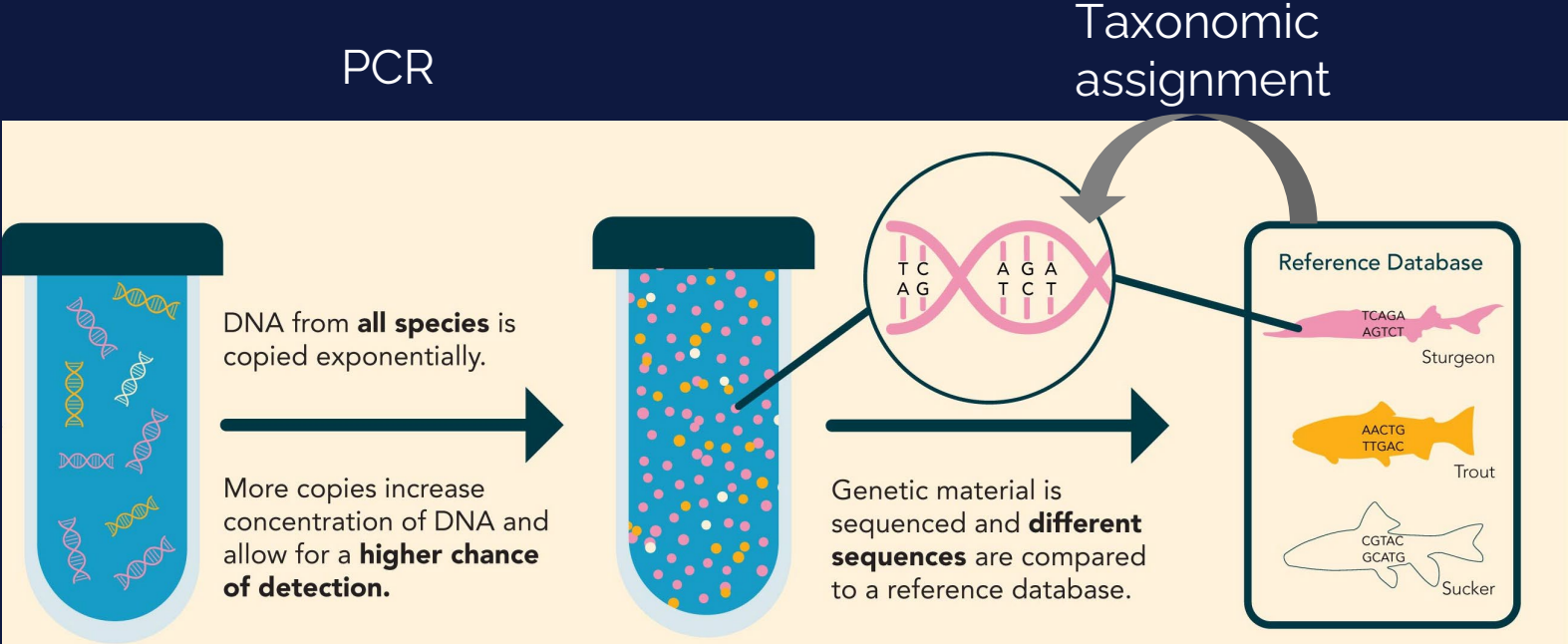


From Chavez et al. 2021

- Description of biodiversity in samples containing 100's of organisms
- Comprehensive: all life compartments
- Scalable: small sample volumes, fast processing (weeks vs. months)
- Cost-effective (~7000+ NOK vs. ~2500 NOK per sample, 64% cheaper)

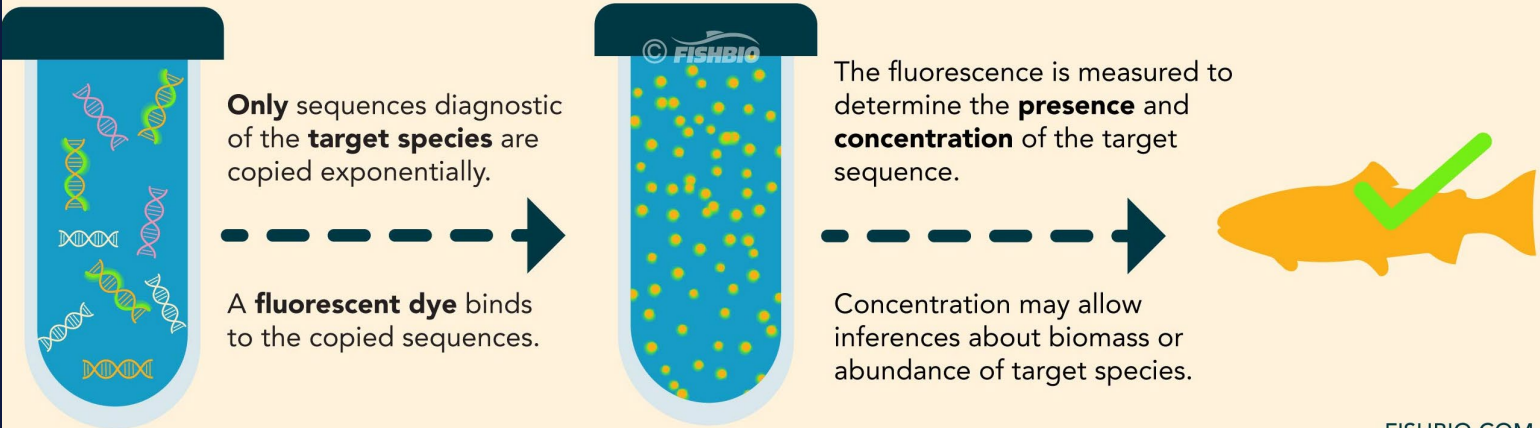
Environmental DNA tools

Metabarcoding

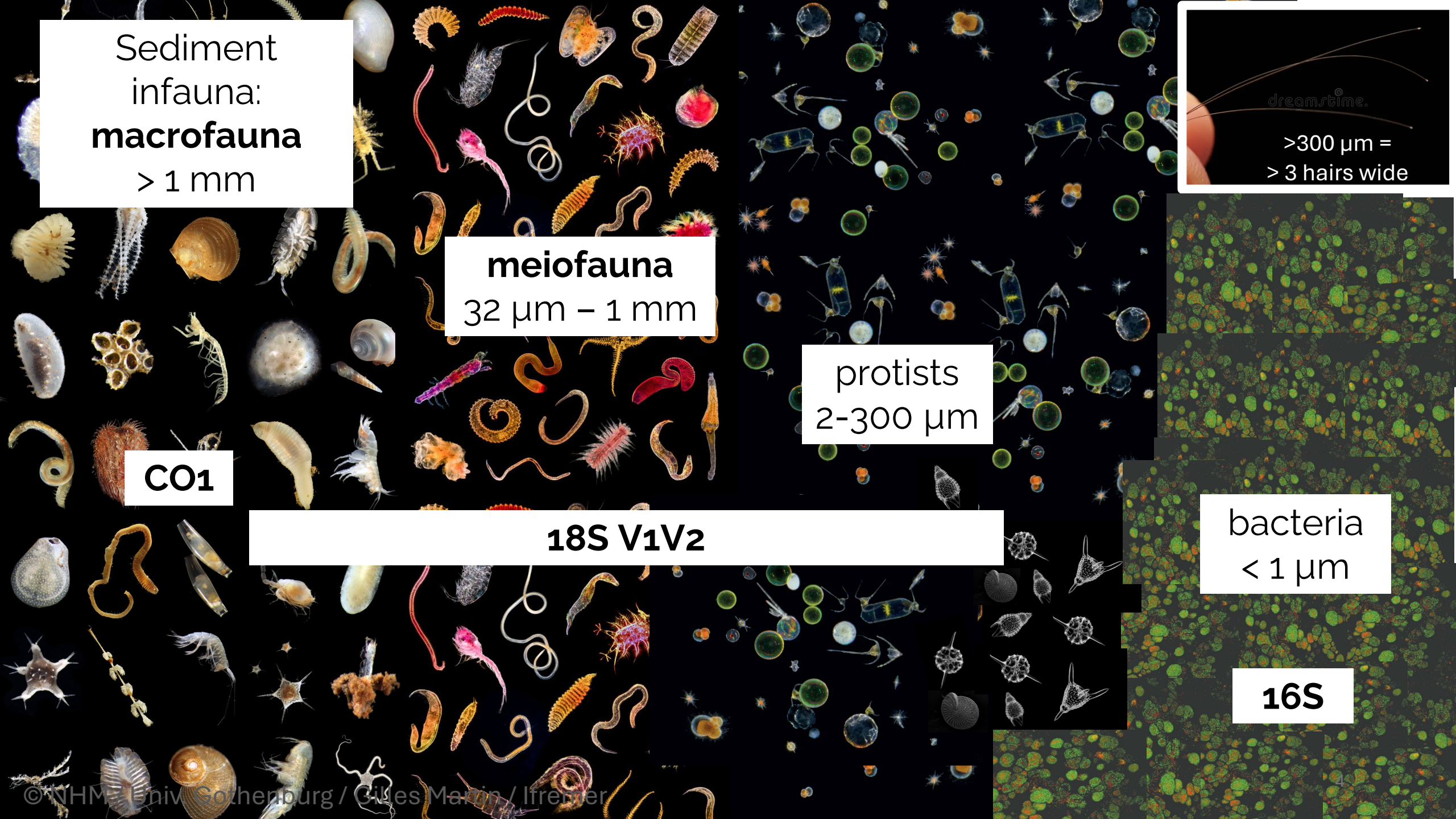


Community description

Quantitative PCR



Target species quantification



Sediment
infauna:
macrofauna
> 1 mm

meiofauna
32 μm – 1 mm

protists
2–300 μm

CO1

18S V1V2

bacteria
< 1 μm

16S

dreamstime.

>300 μm =
> 3 hairs wide

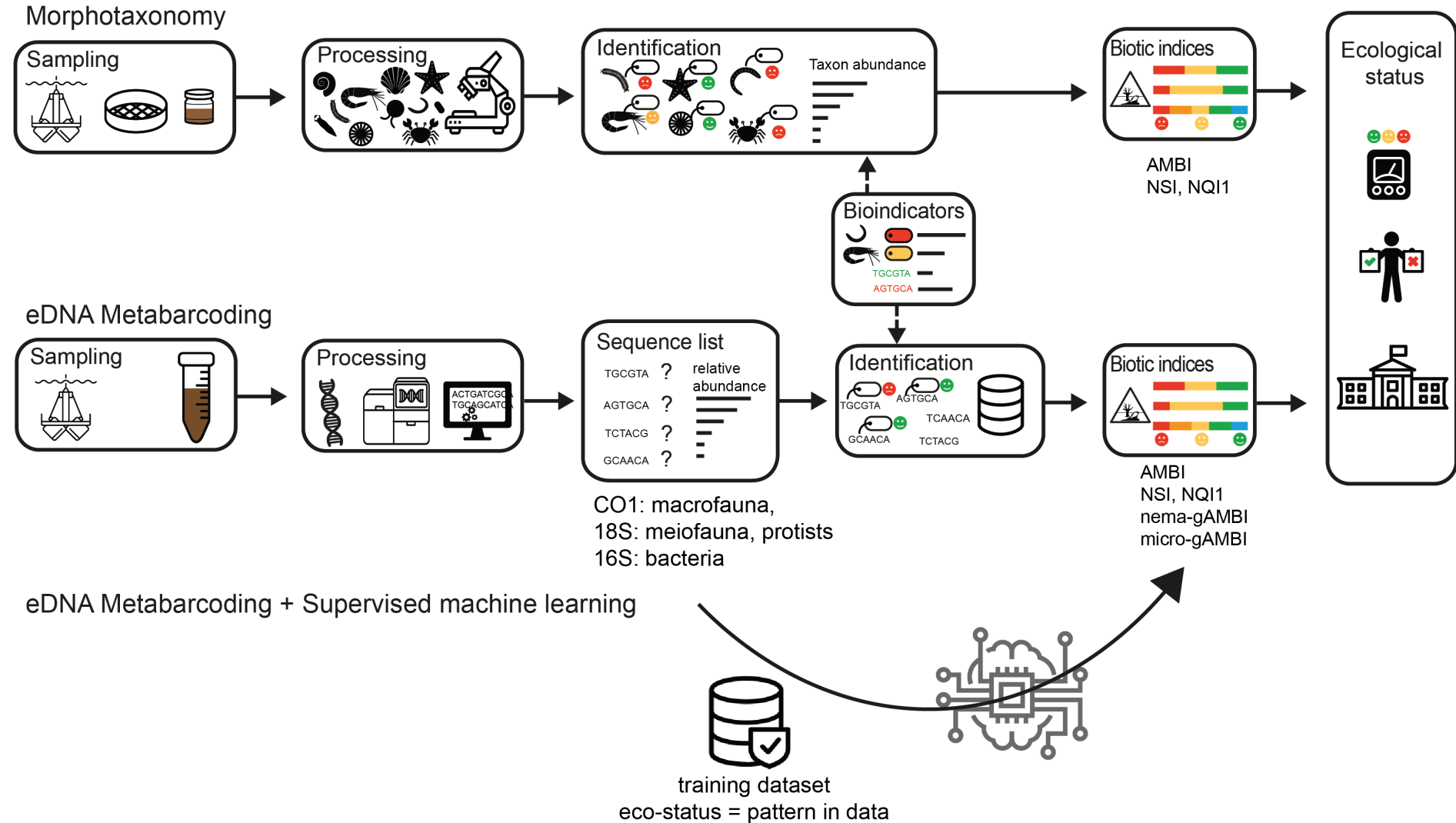


Benthic Biotic Index (BI) calculation via eDNA metabarcoding

Biomonitoring: from morphotaxonomy to eDNA

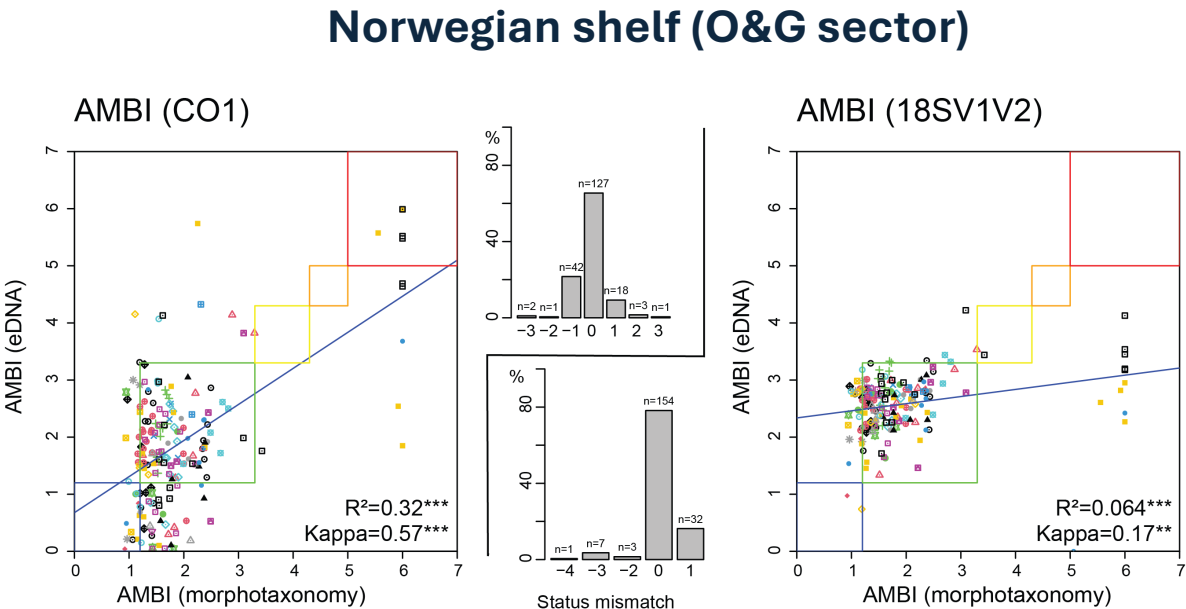
Three approaches:

- Biotic indices based on macrofauna
- Biotic indices based on meiofauna and bacteria
- Biotic indices predicted from machine learning



Direct BI calculation from metabarcoding data

- Weak to moderate agreement between eDNA and morpho-taxonomy BI values
- CO1 > 18S



- Very good
- Good
- Moderate
- Bad
- Very bad

Norwegian coast (aquaculture sector)

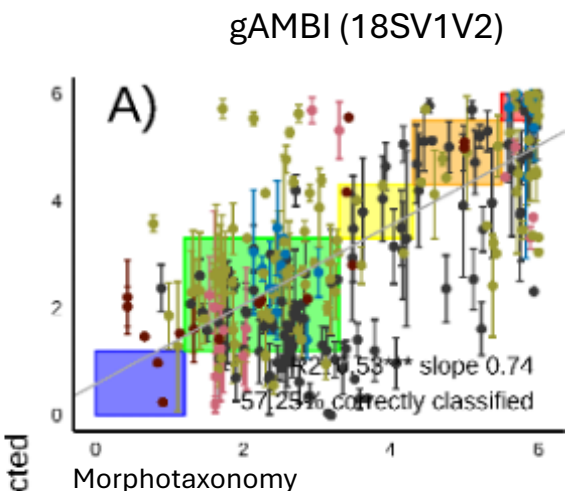


TABLE 3. Interpretation of Cohen’s kappa. **McHugh, 2012.**

Value of Kappa	Level of Agreement	% of Data that are Reliable
0-.20	None	0-4%
.21-.39	Minimal	4-15%
.40-.59	Weak	15-35%
.60-.79	Moderate	35-63%
.80-.90	Strong	64-81%
Above .90	Almost Perfect	82-100%

- Balder FPU
- Brage
- Gina Krog
- Goliat-TB
- Goliat-TG
- Heidrun
- Heidrun E
- Heidrun F
- Iris
- Ivar Aasen
- Njord
- Ormen Lange
- Oseberg C
- Oseberg F
- Oseberg Sor
- Regional02
- Regional03
- Regional06
- Regional09
- Ringhorne
- RoseBank
- Snohvit G
- Troll W1
- Troll W2
- Veslefrikk

Ecoregions

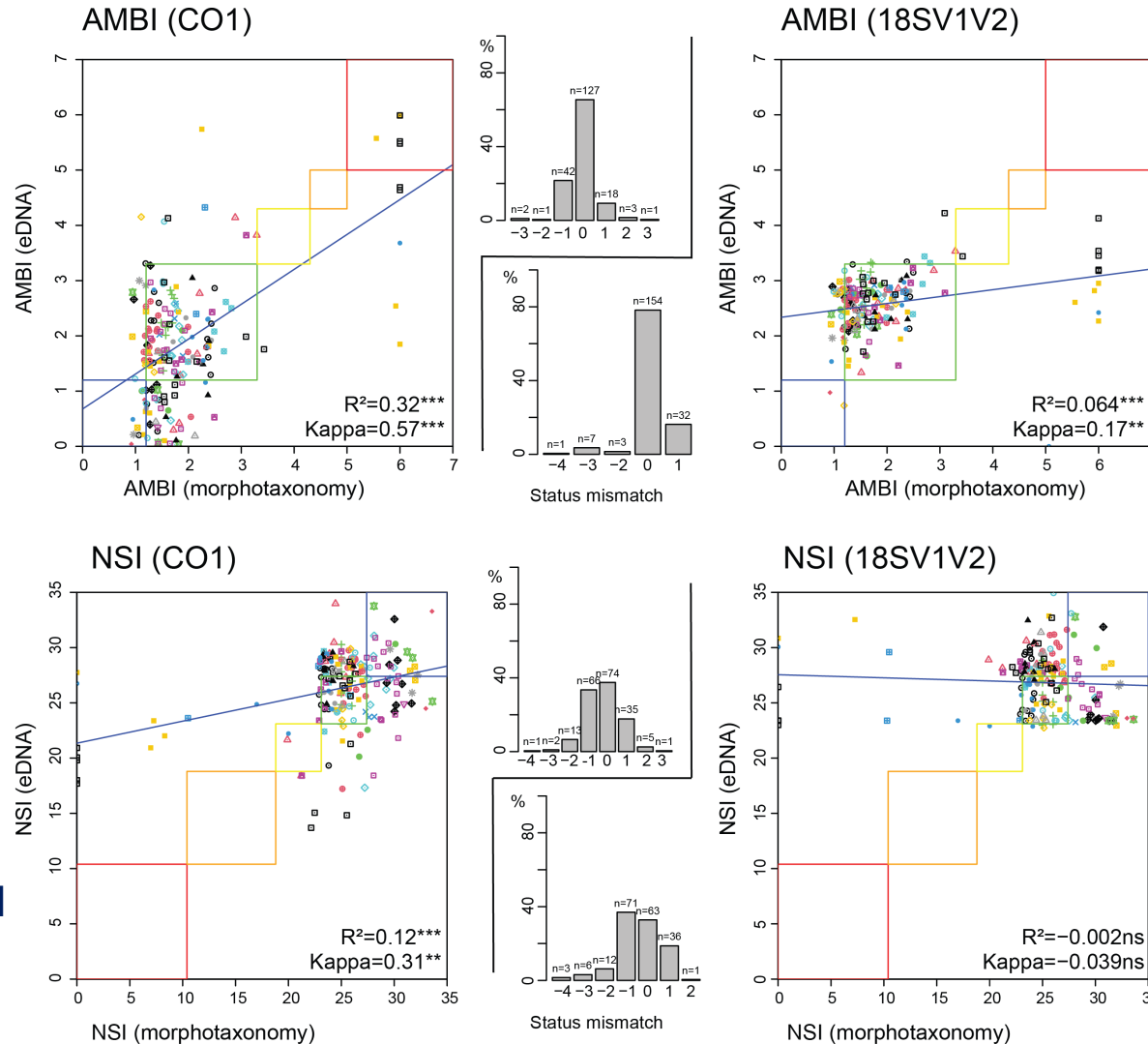
- C1
- C2
- C3
- C4
- C5

~200 shelf samples
~140 coast samples

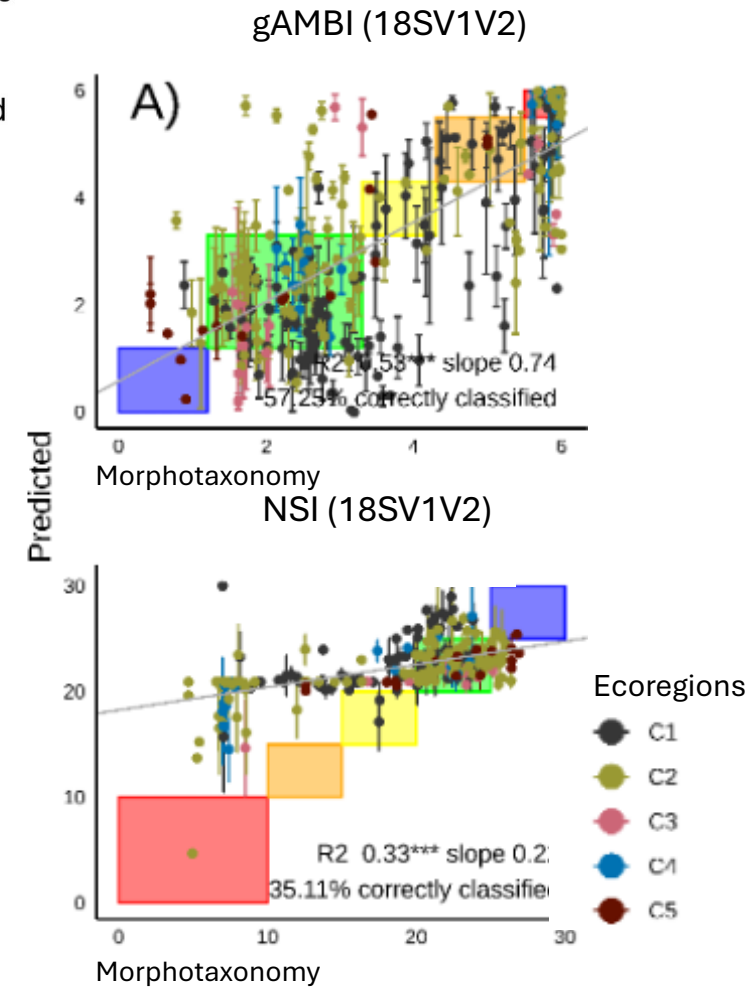
Direct BI calculation from metabarcoding data

- Weak to moderate agreement of eDNA and morpho BI values
- CO1 > 18S
- AMBI is calculated with greater accuracy than NSI
- Problem of taxonomic sufficiency and missing barcodes

Norwegian shelf (O&G sector)



Norwegian coast (aquaculture sector)



Antich, Cordier et al. in prep.

Extended results from Cordier et al. 2018. *MER*

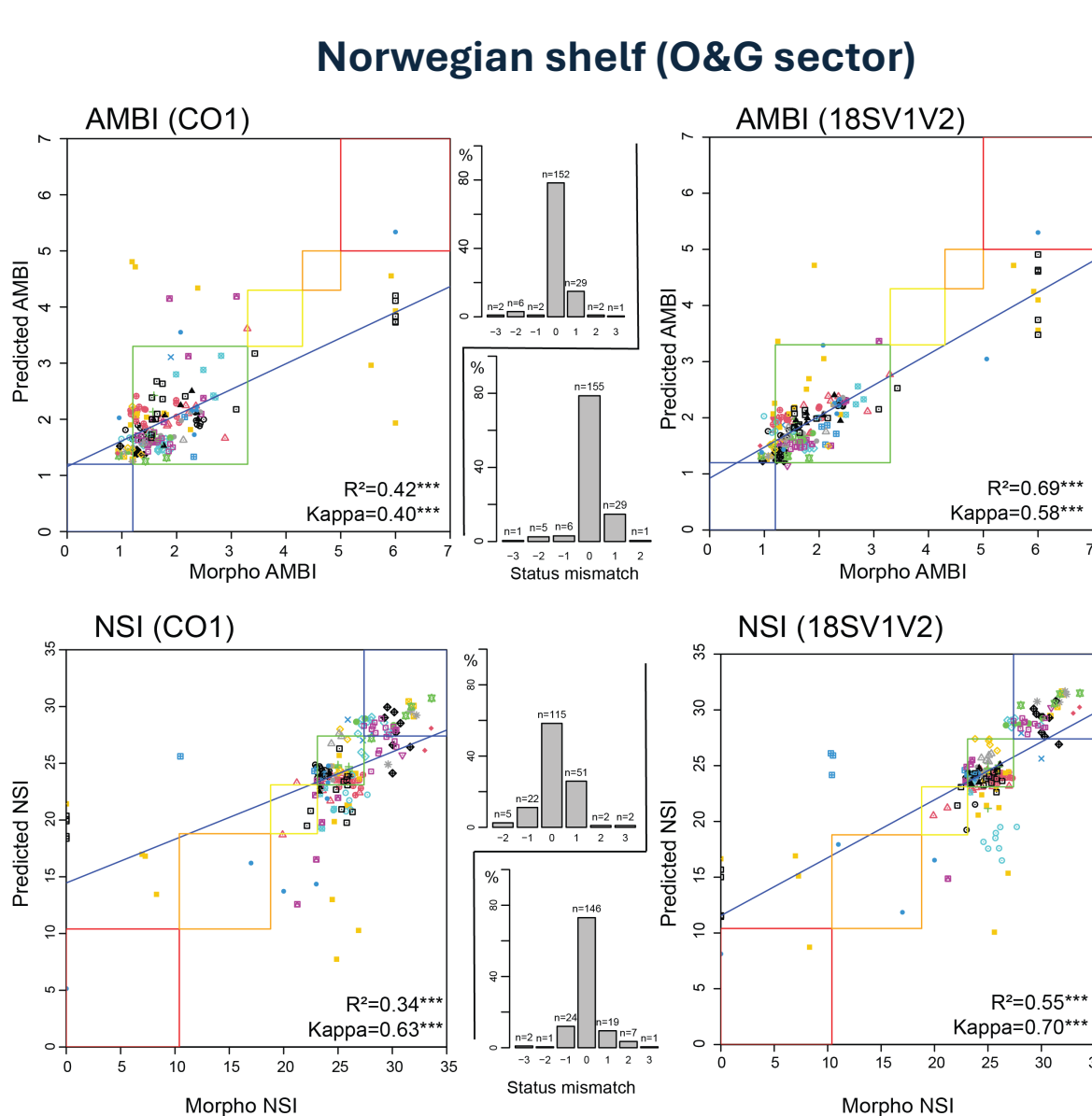
Brandt et al. in prep. ; extended results from Lanzén et al. 2021. *Ecol. Indic.*

BI prediction using RF-ML: eukaryotes (CO1 + 18S)

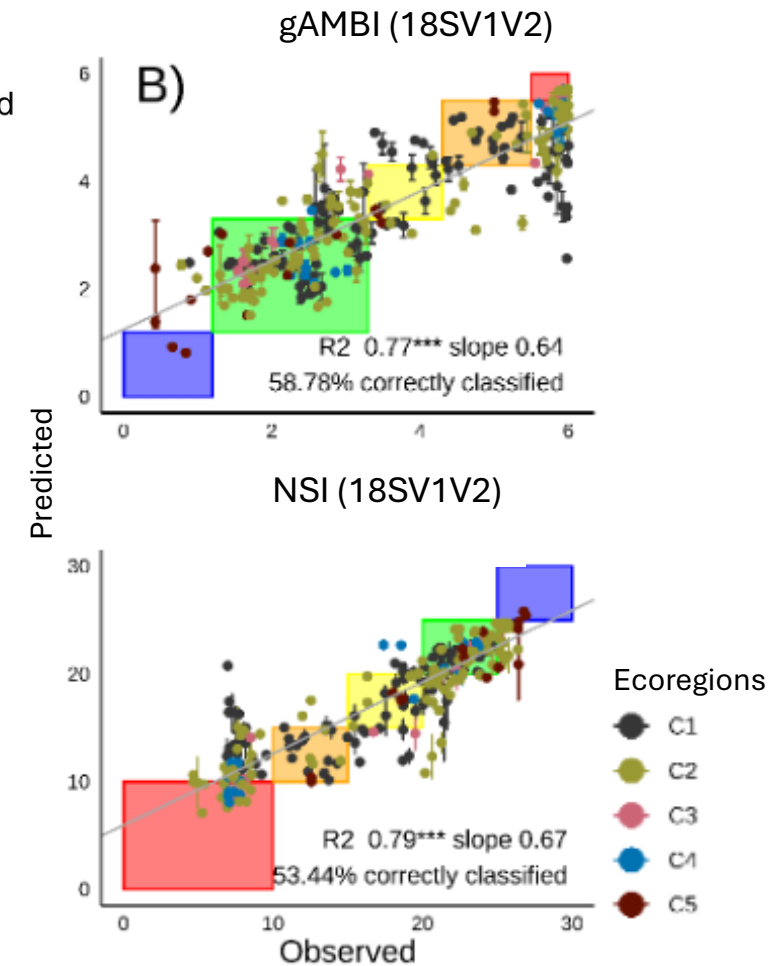
- Weak to moderate agreement between predicted and morpho BI values

- 18S > CO1

- NSI slightly better predicted than AMBI



Norwegian coast (aquaculture sector)



~200 shelf samples
 ~140 coast samples

Lanzén et al. in prep. ; extended results from Lanzén et al. 2021. *Ecol. Indic.*

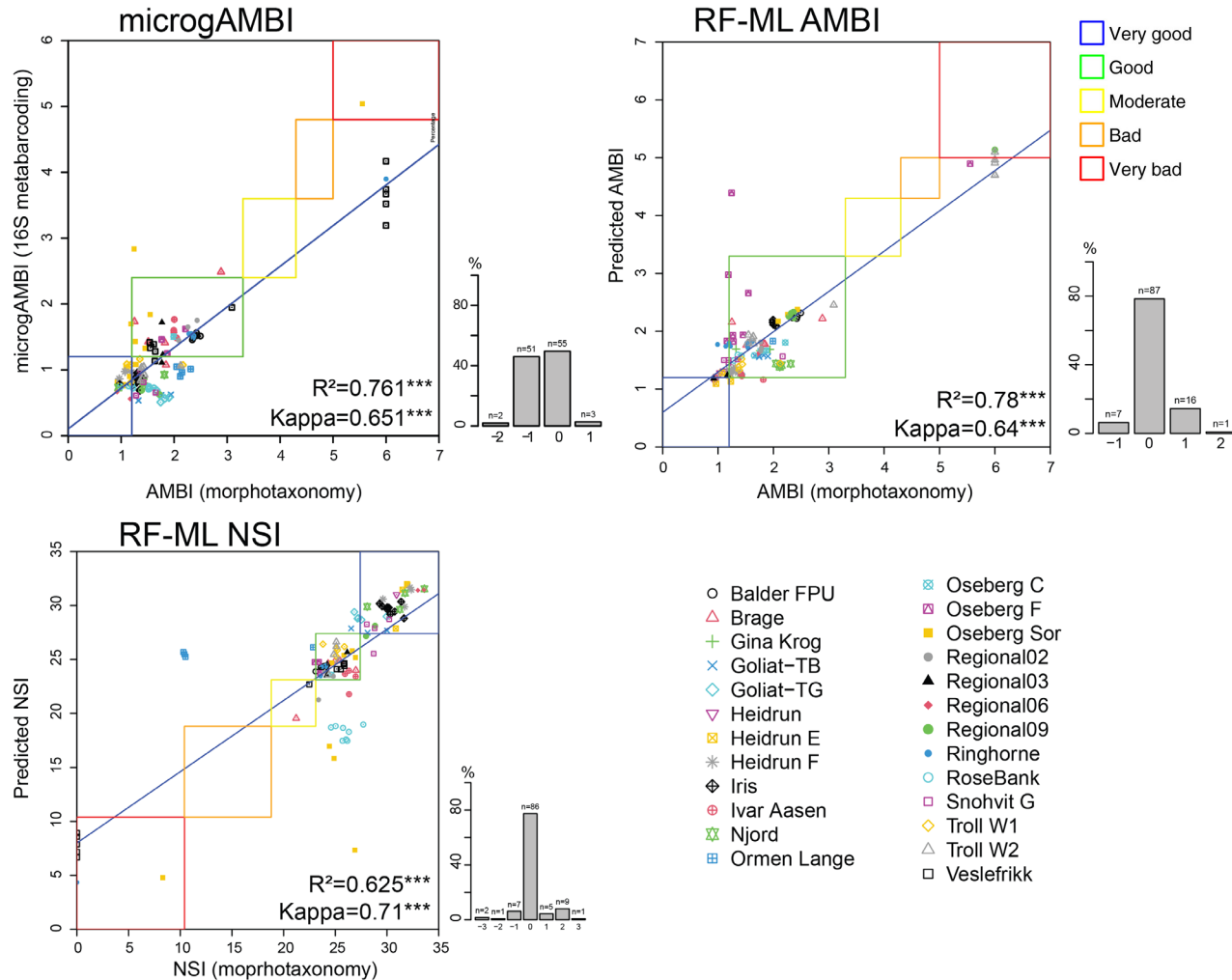
Antich, Cordier et al. in prep.

Extended results from Cordier et al. 2018. *MER*

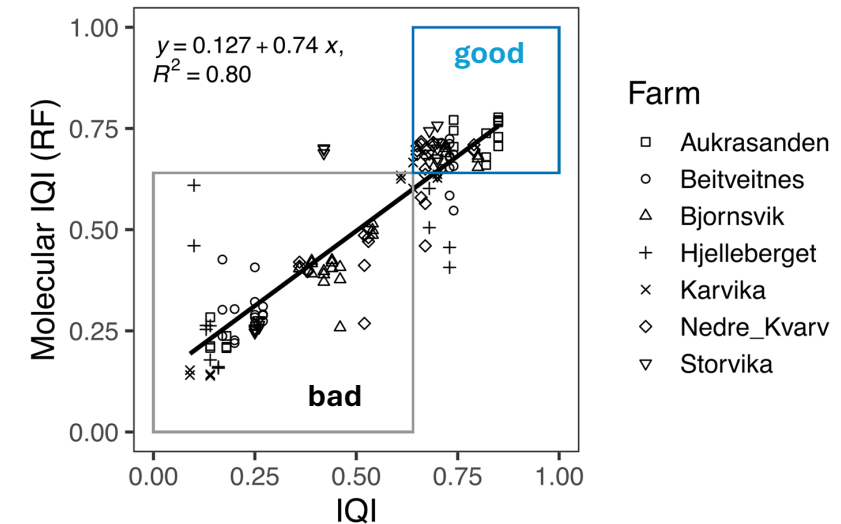
BI prediction using RF-ML: bacteria (16S)

- Bacterial signal well correlated to macrofauna
- 5 status classes: moderate agreement between predicted and morpho BI values
- NSI slightly better predicted than AMBI
- 2 status classes: up to 88% accurate predictions (*almost perfect agreement*)

Norwegian shelf (O&G sector)



Norwegian coast (aquaculture sector)



Leontidou et al. 2023. *PeerJ*

$$IQI \sim \frac{AMBI}{AMBI_{Ref}} + \frac{Evenness}{Evenness_{Ref}} + \frac{Richness}{Richness_{Ref}}$$

~110 shelf samples

~140 coast samples

Lanzén et al. in prep. ; extended results from Lanzén et al. 2021. *Ecol. Indic.*

Use of eDNA in Scotland

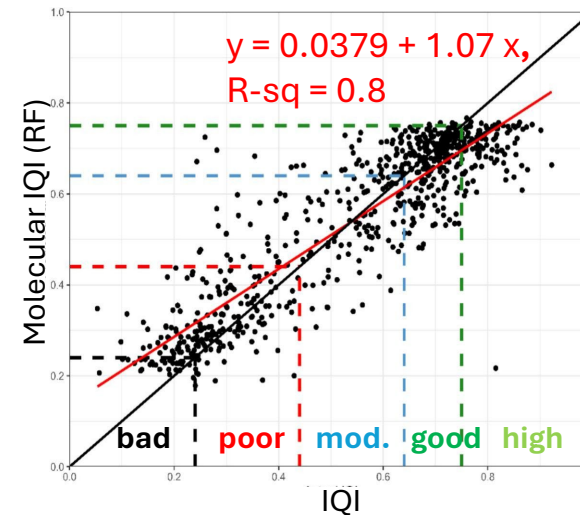
- Machine learning model trained on bacterial (16S) sequence data for predicting on morphotaxonomy (macrofauna) IQI
- Performs moderately well at predicting 5 impact categories (61.4%) and very well at differentiating between impacted vs unimpacted habitats (92%)
- Scottish Environmental Protection Agency (SEPA) have begun incorporating 16S eDNA sequencing with RF ML model to estimate IQI



Marine Finfish Farms

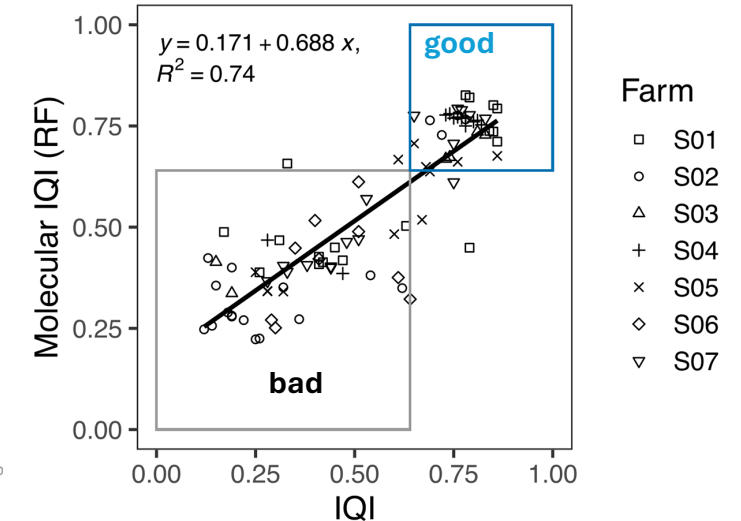
Guidance on the use of DNA in monitoring compliance with seabed mixing zone area limits

Scottish coast (aquaculture sector)



Wyness et al. 2026. *Aquaculture*

- 745 samples,
- 61.4% accurate predictions (5 categories)



Leontidou et al. 2023. *PeerJ*

- 92 samples,
- Strong agreement between predicted and actual values (Kappa score 0.84, 92% accurate predictions, 2 categories)

$$IQI \sim \frac{AMBI}{AMBI_{Ref}} + \frac{Evenness}{Evenness_{Ref}} + \frac{Richness}{Richness_{Ref}}$$

Use of eDNA in Scotland

- SEPA has generated a standardized pipeline for eDNA analysis using “eDNA2IQI” R package

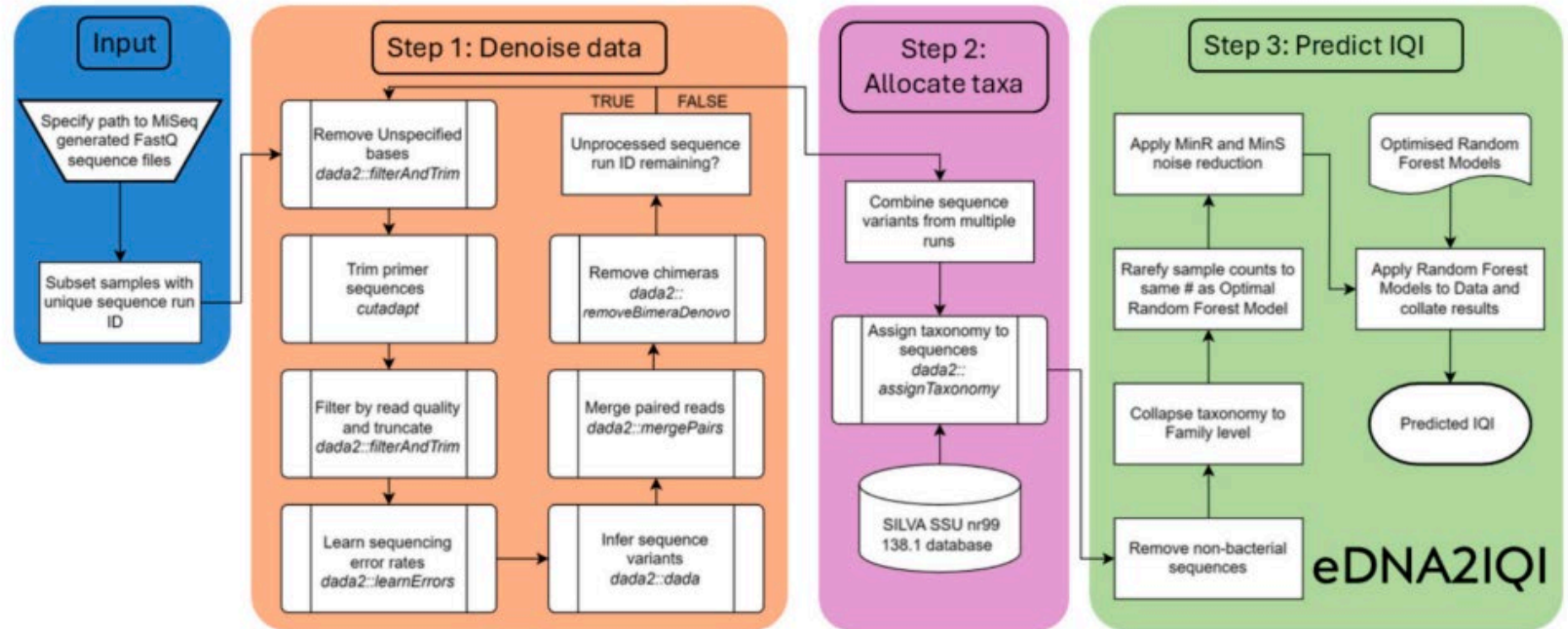
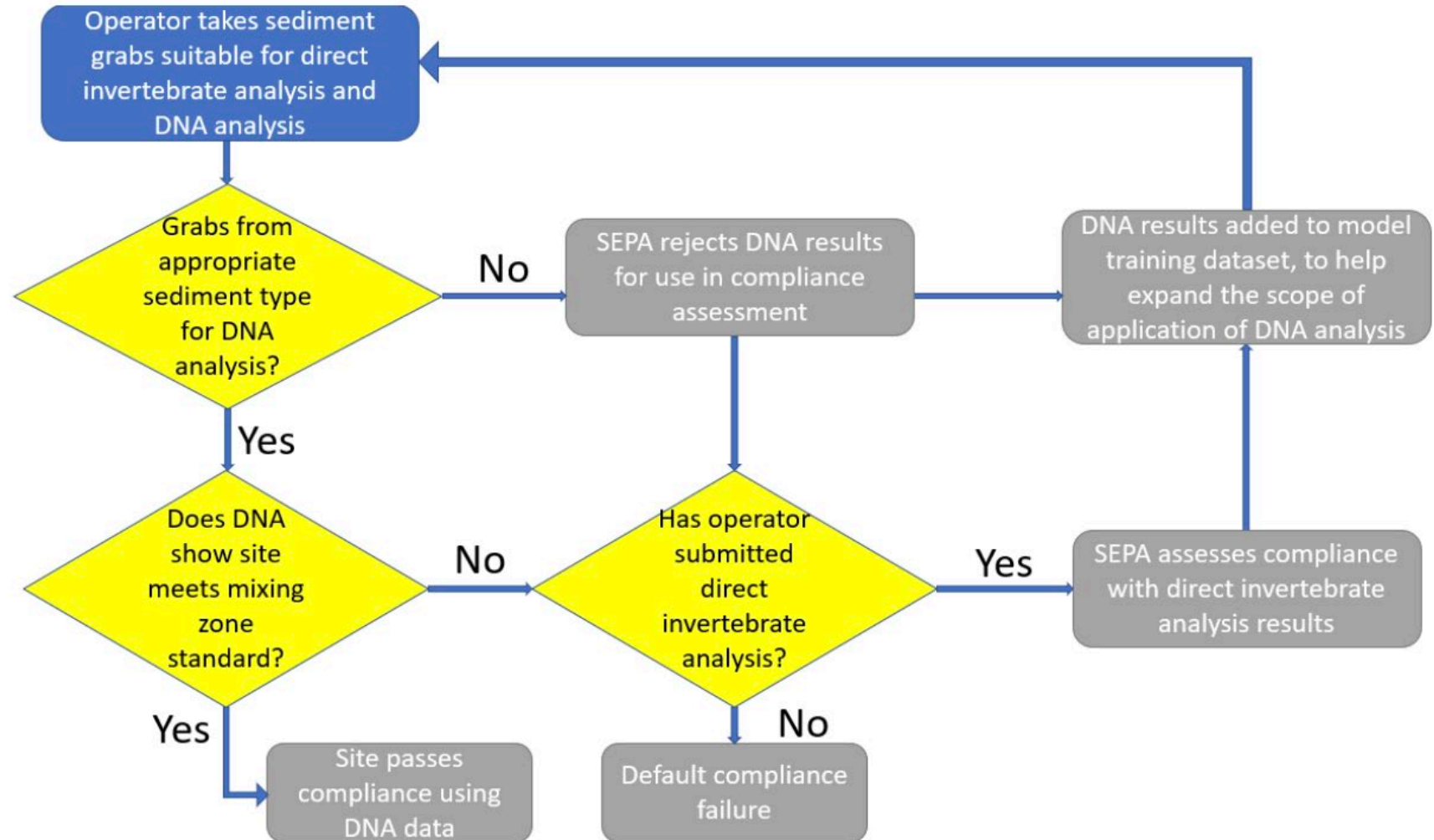


Fig. 1. Flow diagram describing eDNA2IQI process for predicting IQI values from raw eDNA sequence data. Chart symbols follow American National Standards Institute (ANSI)/International Organization for Standardization (ISO) standards.

Wyness et al., 2026. *Aquaculture*

Use of eDNA in Scotland

- SEPA has generated a standardized pipeline for eDNA analysis using “eDNA2IQI” R package
- And a decision tree outlining circumstances where eDNA vs. morpho-taxonomy are used for benthic habitat scoring



SEPA, 2023

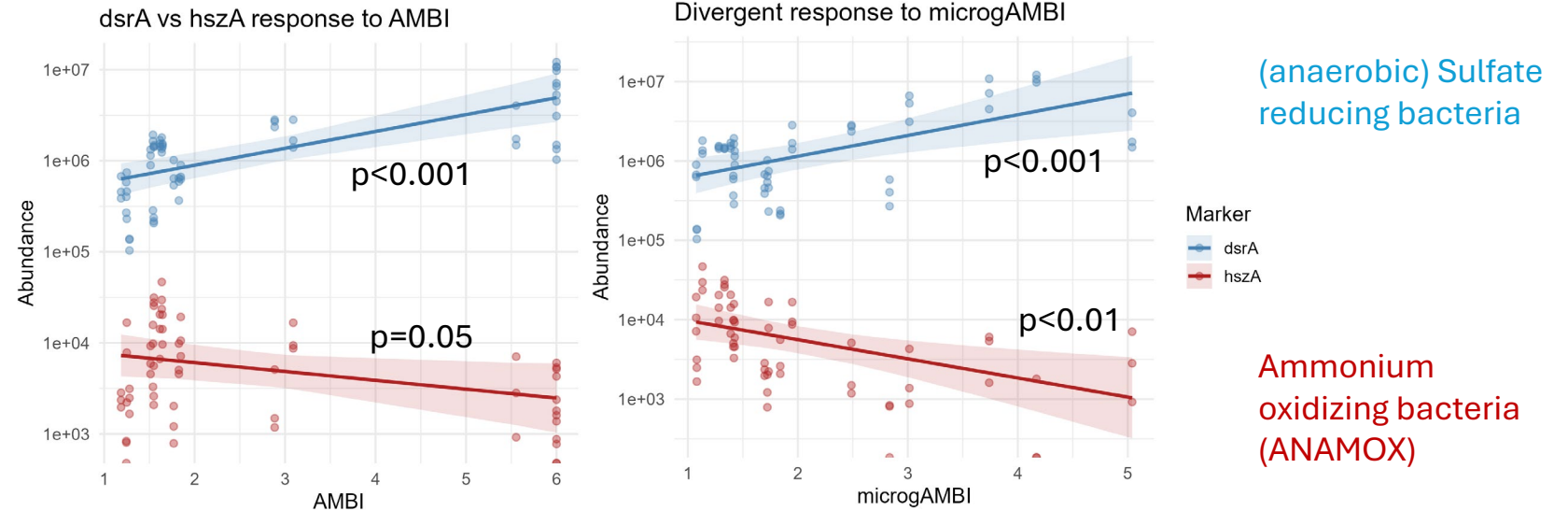
Figure 1. Use of DNA analysis results in assessing compliance with seabed mixing zone area standard



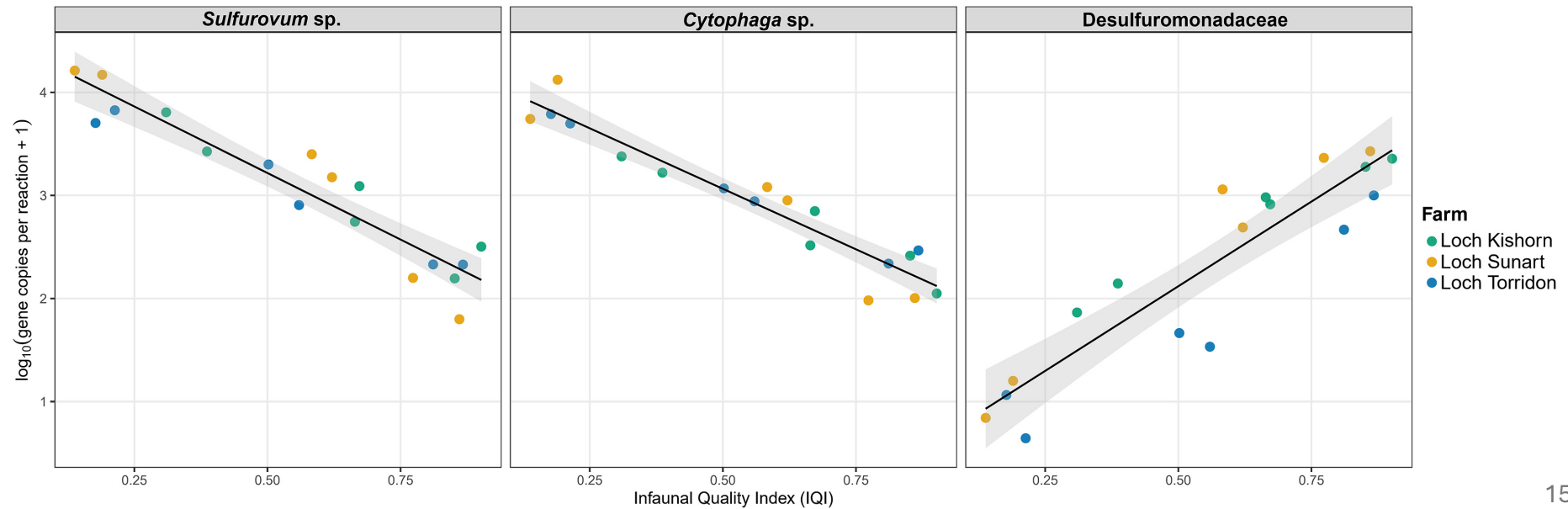
Indicator species quantification with eDNA

Quantification of indicators for early detection ?

- In Norway, assays targeting “bacterial bioindicators” show strong association with AMBI and microgAMBI



- In Scotland, assays have been designed targeting “bacterial bioindicators” strongly associated with IQI scores



Conclusions

Benthic Innovation

Scope	Approach	Barcode Marker	Pros	Cons	Future directions
Macrofauna	Direct BBI calculation	CO1 > 18S	• Most comparable to current standards	• Limited use of eDNA data • Only accounts for taxa that have been categorized with respect to EQS	• Needs development of eDNA-proof BIs
Expanded Diversity	Machine Learning assisted BBI calculation	16S > 18S > CO1	• Makes best use of eDNA data • Does not require a priori assumptions of species ecological niches	• Requires robust ML training datasets • Less intuitive interpretation relative to current BI calculations	• Large-scale paired training data • Develop BIs including microfauna
Holistic Biotic Assessment: Linking biotic and abiotic signals	Machine Learning assisted Pressure Index calculation.	16S > 18S > CO1	• Links biotic community to abiotic measurements • Ground-truths relationship between specific abiotic stressors and biotic response • Compatible with expanded suite of chemical stressors	• No comparable historical data so needs ground truthing to standard BIs	• Construct pressure index including abiotic stressors • Compare predictive performance between markers (16S to 18S)
Targeted Bioindicator Assessment	Quantitative (qPCR/ddPCR) Bioindicator Assays	CO1+18S+16S	• Rapid and cost-effective • Minimal infrastructure • Could facilitate more frequent biotic assessments	• Requires identification of reliable indicators + assay design/validation	• Identify indicator taxa • Assay design for micro-, meio-, and macrofauna



MEMBER OF
BASQUE RESEARCH
& TECHNOLOGY ALLIANCE



DNV

PHARMAQ
Analytiq

Thank you. Takk.

Merci. Gracias. Obrigado.



FISKERI- OG HAVBRUKSNÆRINGEN
FORSKNINGSFINANSIERING

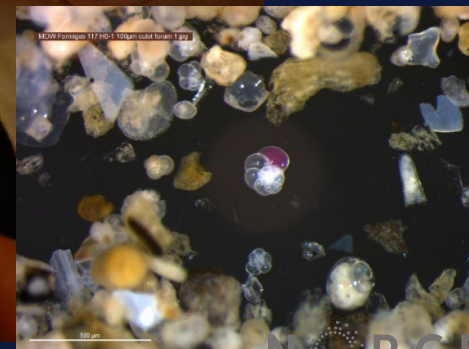
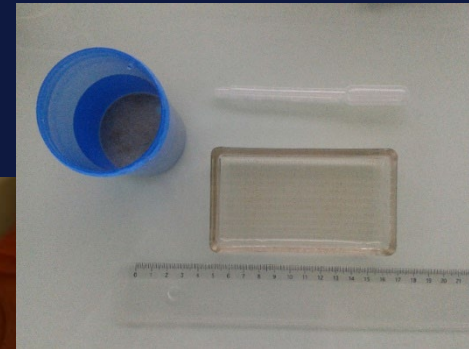
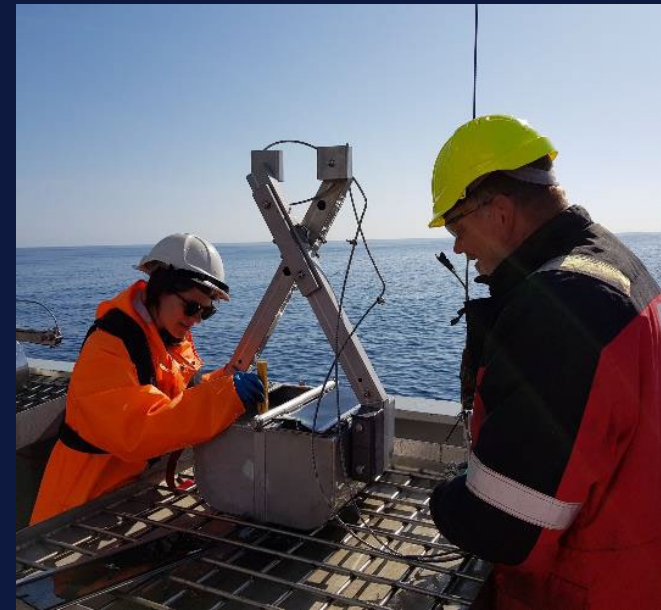


Institute of Oceanology
Polish Academy of Sciences

NORCE

BenthicInnovation
Project number: 910477

Norwegian marine biomonitoring





Linking biotic and chemical signals

Linking biotic data to chemical pressure

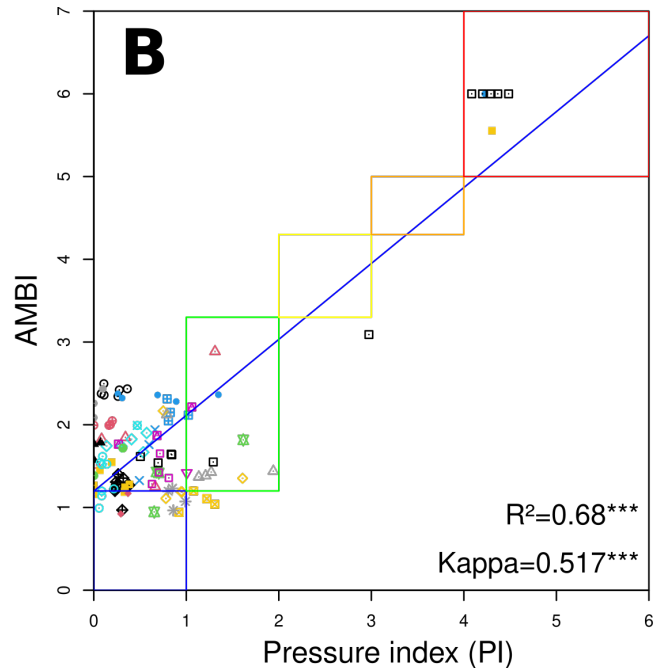


- Macrofauna BBIs: one way to integrate chemical impacts
- Chemical pressure (PI) can be used to ground-truth eDNA-based biotic signals
- Bacterial eDNA better reflects chemical pressure gradients

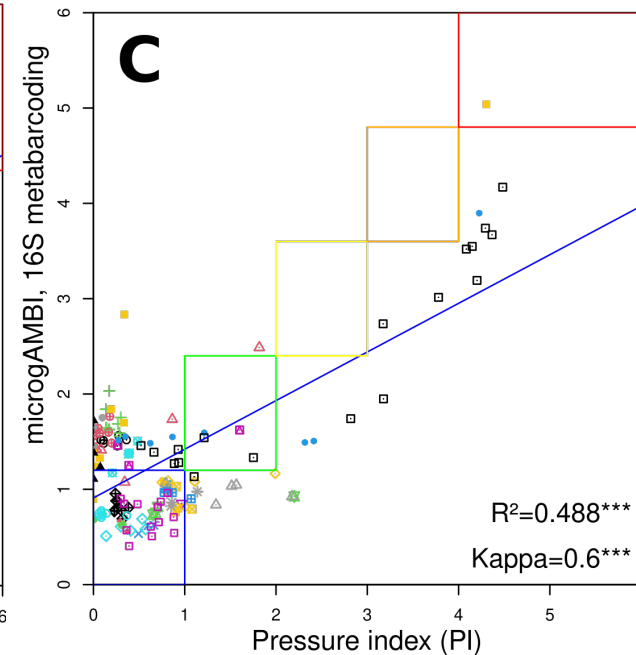
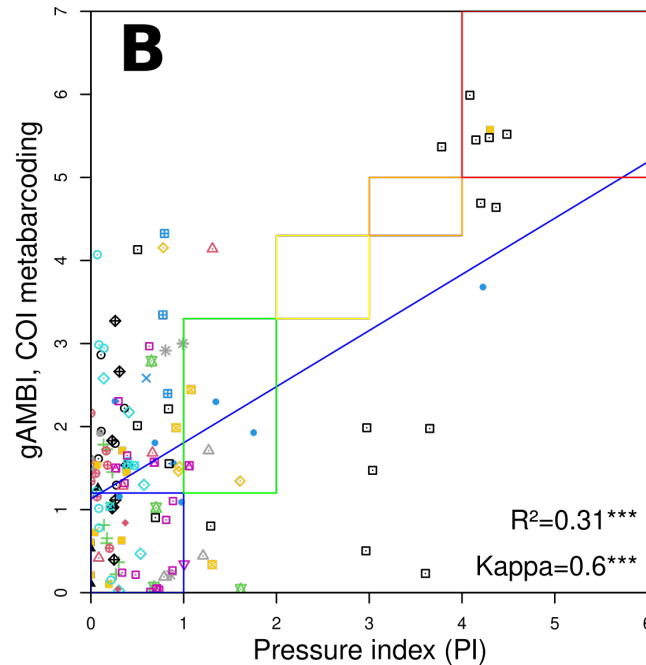
$$PI = \Sigma(\log_2(2 \times \text{conc}_p / \text{limit}_p) + 2) / N_p$$

Parameters (p): THC, TotalPAHs, Ba, Cd, Hg, Pb, Cu
Limit values from Norwegian directives or (Cd, Hg, Pb, Cu) 2x max. conc. in reference sites

Morphotaxonomy

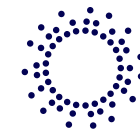


eDNA

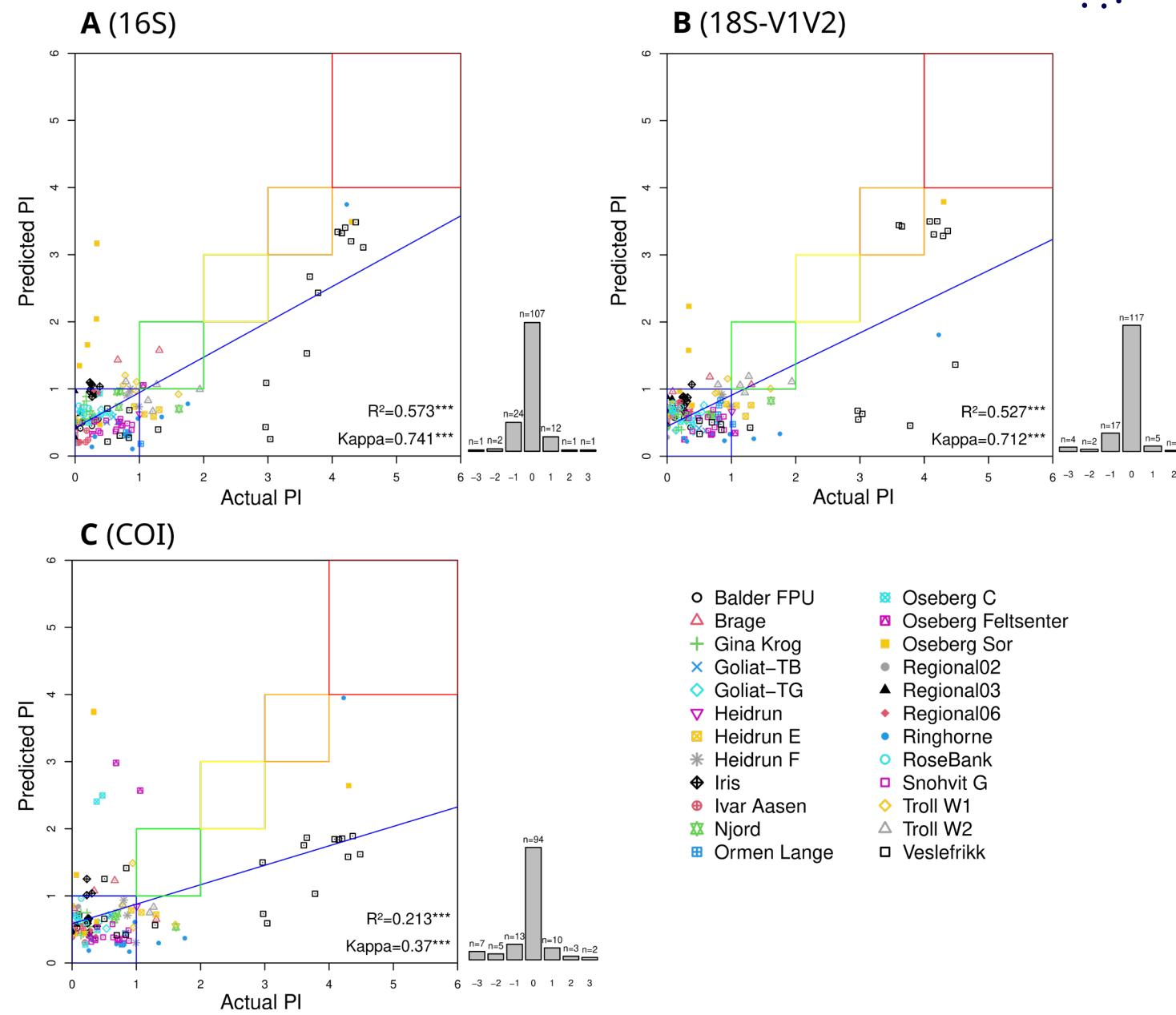


- Balder FPU
- △ Brage
- + Gina Krog
- × Goliat-TB
- ◇ Goliat-TG
- ▽ Heidrun
- Heidrun E
- * Heidrun F
- ◆ Iris
- Ivar Aasen
- ✱ Njord
- Ormen Lange
- Oseberg C
- Oseberg Feltseier
- Oseberg Sor
- Regional02
- ▲ Regional03
- ◆ Regional06
- Ringhorne
- RoseBank
- Snohvit G
- ◇ Troll W1
- △ Troll W2
- Veslefrikk

RF-ML prediction of PI in the offshore sector



- Datasets of ~140 samples
- 16S/18S: Moderate agreement of predicted and observed PI values (kappa = 0.6-0.79)
- CO1: Weak agreement of predicted and observed PI values
- 16S (and 18S?) better suited for RF-ML regression along chemical gradients



Future Directions

Scottish system diagram:

And example of how eDNA can be implemented to efficiently supporting morphotaxonomy methods
DNA analysis can be eDNA metaB, eDNA metaB+SML, ddPCR . This choice is to do by regulators

What is Benthic Innovations addressing: RF on 16S and 18S, further developing eDNA-suited biotic indices like nema-gAMBI, ddPCR assays on macrofauna

Key in implementing eDNA approaches for monitoring will be to standardize

1. at the identification phase: same markers, reference databases
2. at the prediction phase: choice of parameters in RF regression models (LOO cross-validation, feature selection, sample weighing)

