Sampling & Methods for MIC Determination

By Tom Jack & Lisa Gieg

Biological Sciences

UNIVERSITY OF CALGARY

Sampling SOPs
(Standard Operating Procedures)

- Microbial/genomics and corrosion data are only as good as samples taken!

- NACE (e.g., TM0194-2014; TM0212-2018) and other standards (DNV GL RPs, etc.)

⇒ consistent, high quality sampling

Sampling SOPs

- Important to have sampling SOPs in place for:
  - Liquids (e.g., produced water)
  - Solids (e.g., pig solids, corrosion coupons, pipeline scrapings)
  - **Ensure as high quality samples as possible**

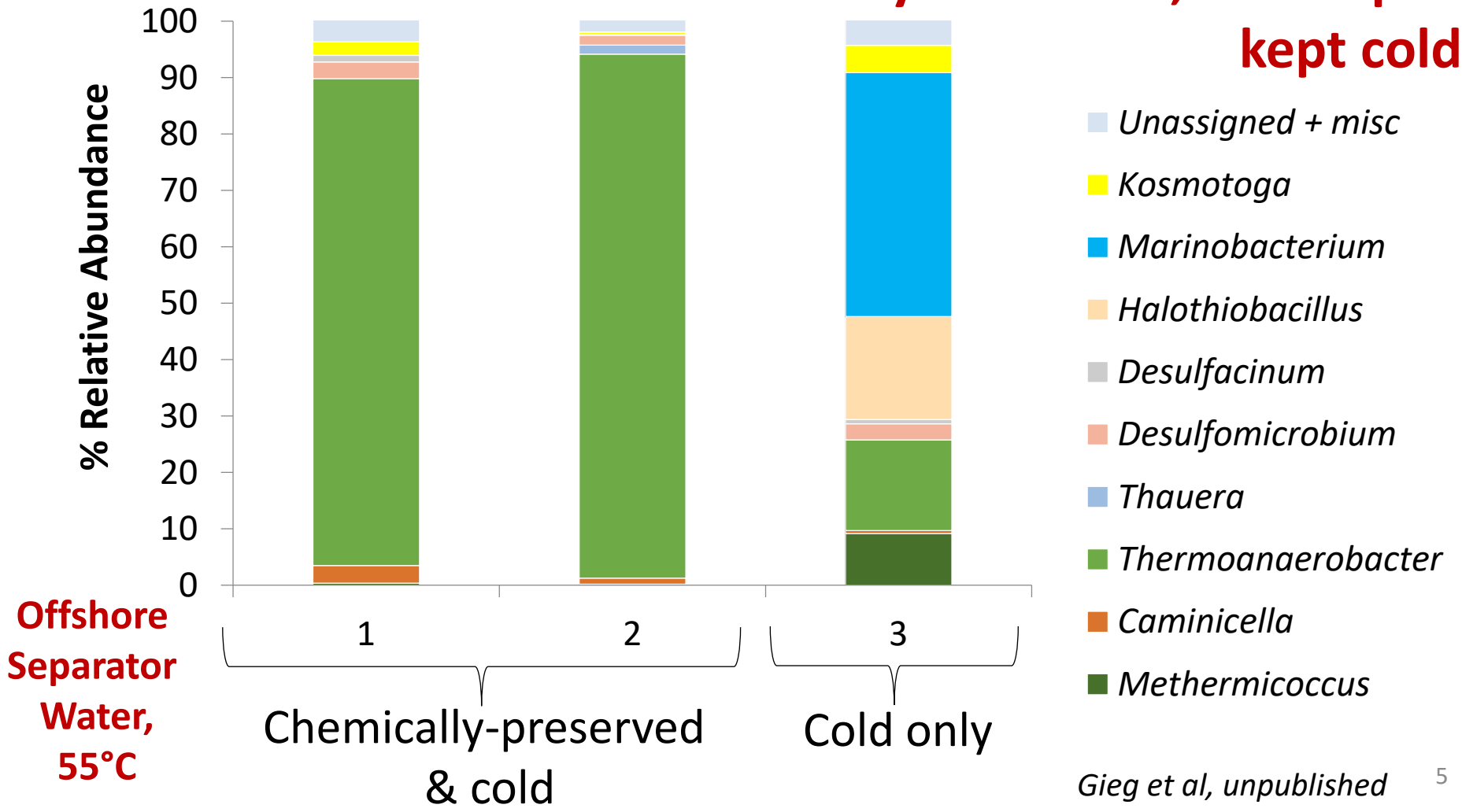
- **Recommended materials and preservation methods**
  - **Sterile vessels and sampling tools**
  - Filled to brim to minimize air
  - Proper preservation and timely shipment to lab for further analysis are **critical**

Sampling SOPs

- **Samples for conducting lab experiments/microbial corrosion experiments**
    - keep cold (on ice, do not freeze)
    - can feasibly add samples to anoxically-prepared serum bottles already containing carbon steel beads or coupons (good for long transport times)
- **Samples for genomic analysis**
    - can stay on ice for short time (24-48 h)
    - add chemical preservative
        - DNAzol™, RNAlater™, ethanol, isopropanol, etc.
    - filter in-field and add preservative or keep cold

Microbial community composition can change dramatically during transport if samples not chemically-preserved after sampling

8 days in transit, all samples kept cold



Offshore Separator Water, 55°C

Gieg et al, unpublished

Where to Sample?

- Corrosion occurs at the metal surface therefore surface-attached/sessile microorganisms are the best samples to get

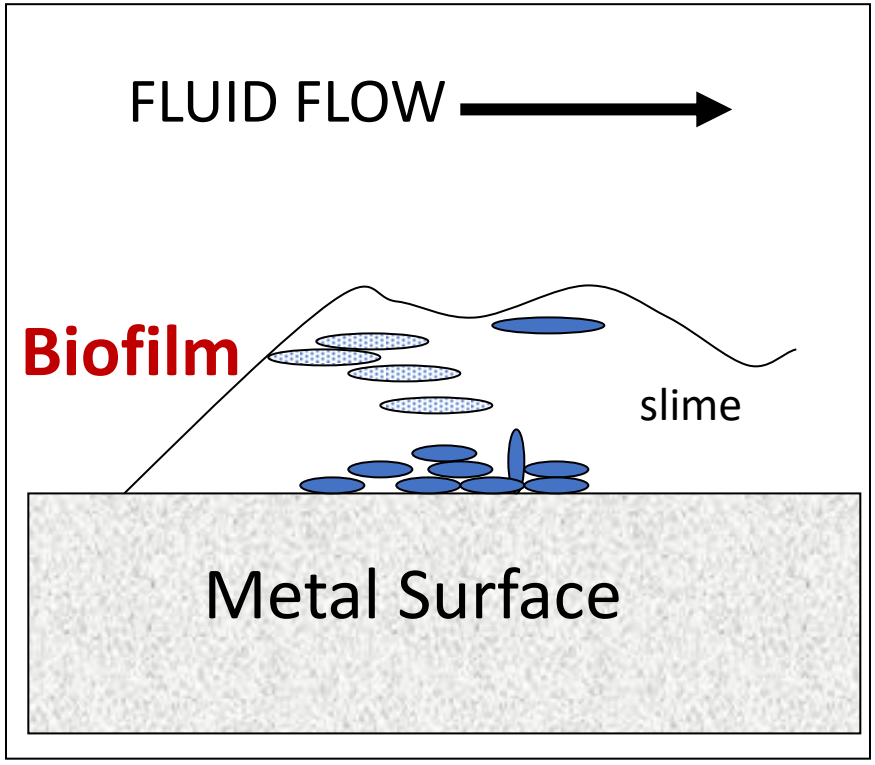


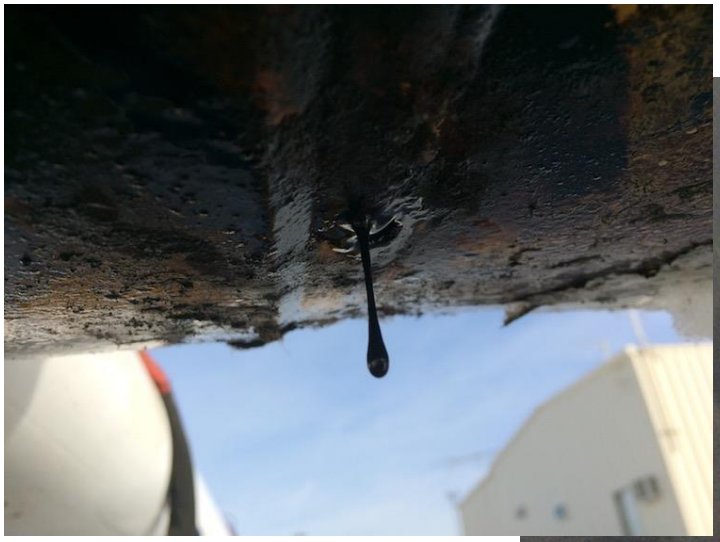
Photo: Torben L. Skovhus

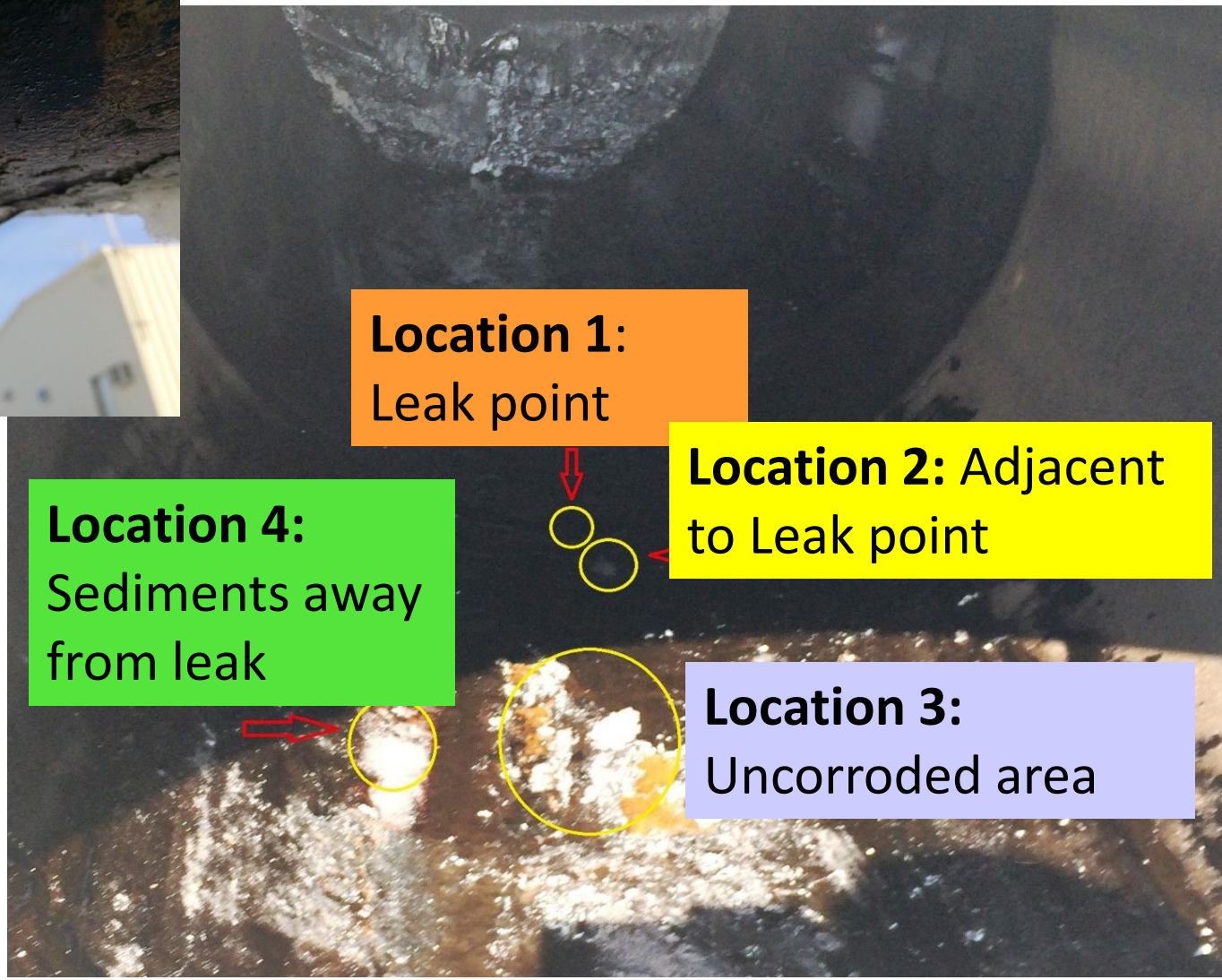
Where to Sample?

- Sessile samples can be challenging to obtain but provide the most relevant information
  - *Sampling corroded & uncorroded areas helps to assess importance of MIC*
  - *Liquid samples more common and sometimes are the only possible samples*

To help understand MIC – need to obtain samples from lines/operations with history of MIC, and compare with those with no history

Where to Sample?

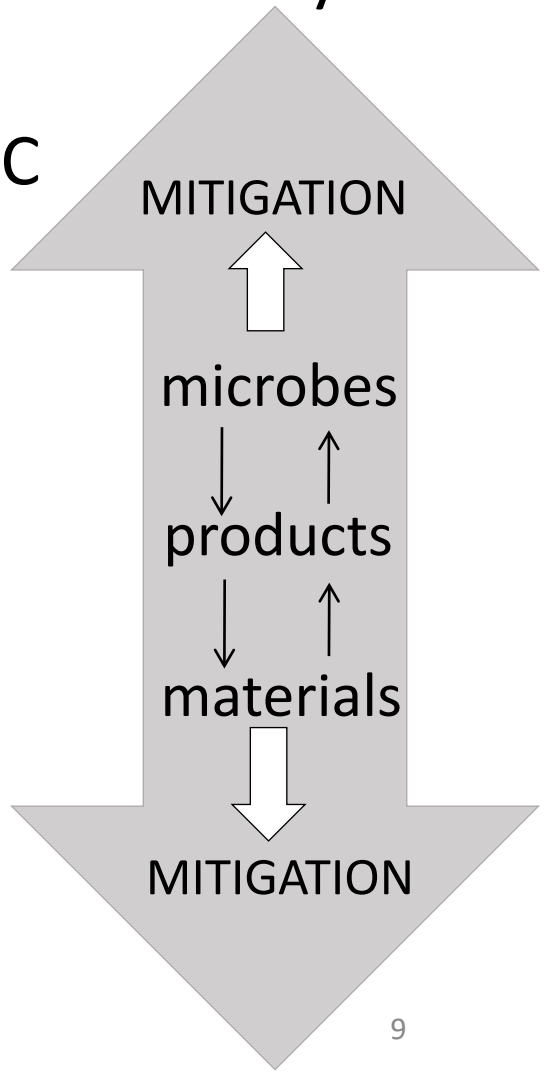




Measuring & Diagnosing MIC

- Diagnosing the root cause of corrosion can be very challenging
- No single measurement can identify MIC
- **<u>Three types of evidence needed:</u>**
  - **Microbiological**
  - Chemical
  - Metallurgical

All should be consistent with MIC

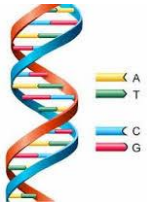


Microbiological Monitoring

ATP Assay

Bug bottles

Molecular Microbiological Methods (MMM)

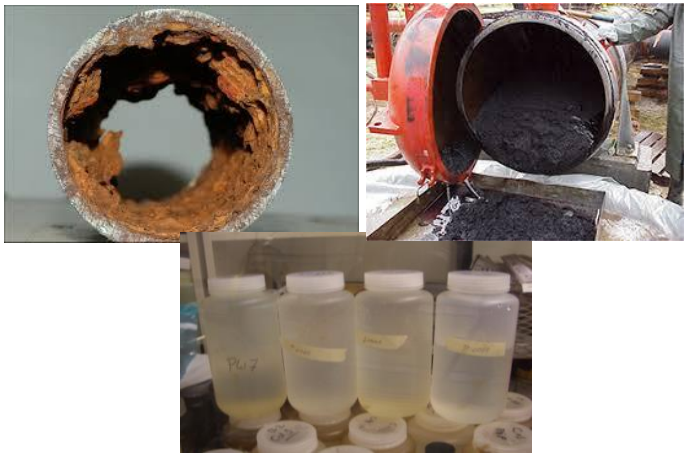


Genomics, qPCR

Microbiological Monitoring

ATP Assay

Metabolic Activity ≈ microbial counts



Bug bottles

Microbial counts (SRB, APB, etc.)

Advantages:

- Most widely used, especially for baseline monitoring/KPI's
- Easy to use/interpret (if done right)

Disadvantages:

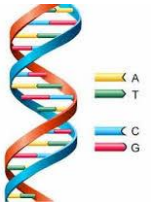
ATP assay: no information on types of microorganisms present

Bug bottles: only counts those organisms that can be cultured (targets <1% of all microorganisms)

Microbiological Monitoring



Molecular Microbiological Methods (MMM)



Genomics, qPCR

Advantages:

- No cultivation – based on genetic material
- **Total microbial community!**
- Some methods give microbial counts (qPCR) – common KPI

Disadvantages:

- Data interpretation more complex

Genomics (MMM) as a Monitoring Tool

(1) 16S rRNA genes

- 'Fingerprint' molecule that identifies all microorganisms in a sample
- *'Who' is there*?

Can quantify to get total numbers

(2) 'Functional' genes

- Genes that encode for specific kind of microorganism (or metabolism) in a sample
  - e.g., gene for sulfide production

Can quantify to get total numbers

(3) Metagenomic sequencing

- Total DNA sequenced in a sample
- Complete genetic potential

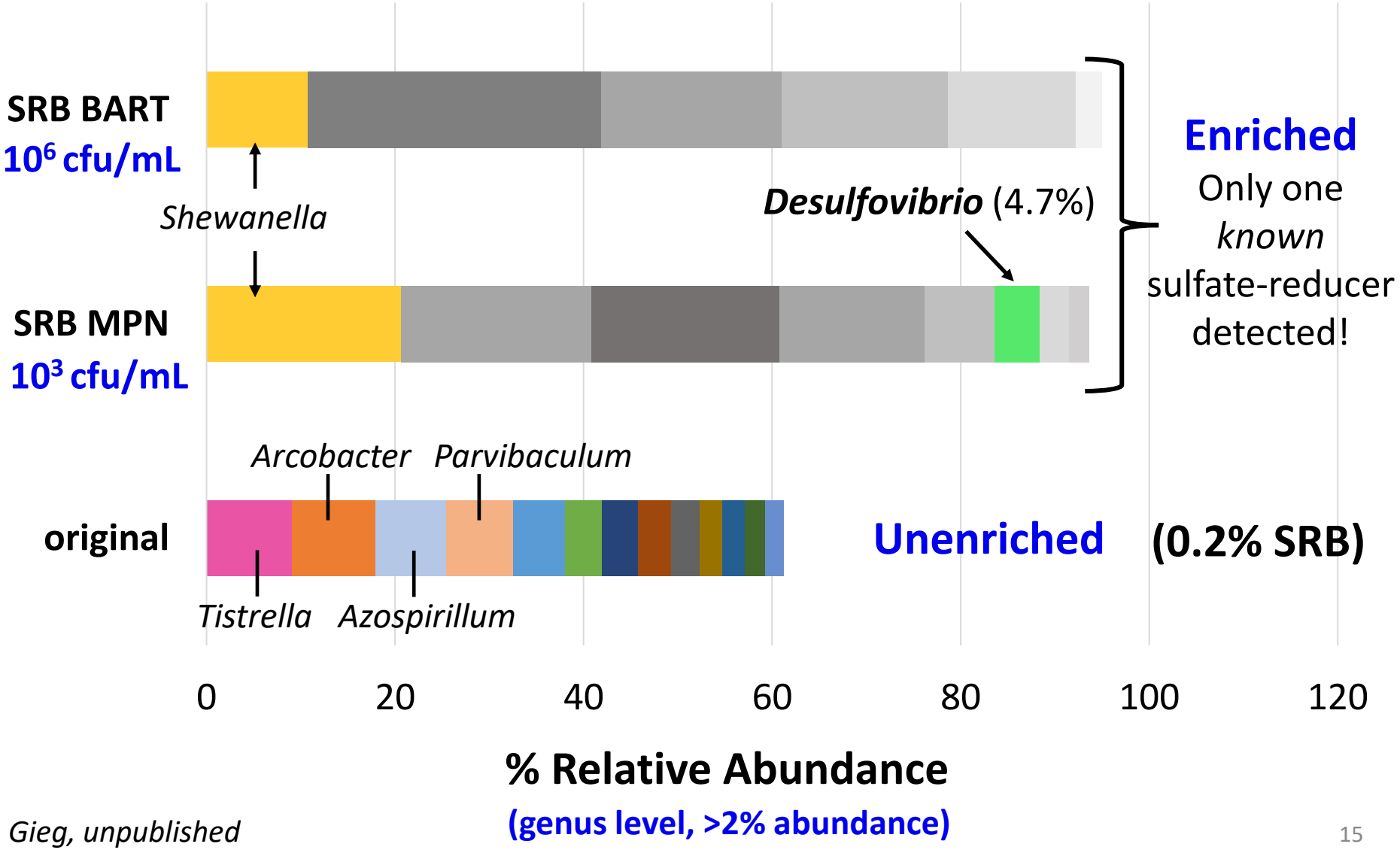
Genomic Analysis (qPCR) vs. Bug Bottles (MPN)

Analysis	Location[a]			
	Eider Production Manifold	Otter Production Pipeline	Inlet to Crude Oil Coalescer V-1100	Crude Oil Coalescer PW outlet V-1100
qPCR (gene abundance per cm^2)[c]				
Sessile samples from corrosion coupons				
Total bacteria	$< 4.0 \times 10^2$	4.6×10^5	$< 4.0 \times 10^2$	2.9×10^3
SRB	$< 4.0 \times 10^2$	2.9×10^4	$< 4.0 \times 10^2$	3.0×10^3
SRA	$< 4.0 \times 10^2$	1.9×10^4	$< 4.0 \times 10^2$	1.1×10^4
Methanogens	1.4×10^5	4.2×10^{11}	5.0×10^5	4.2×10^5
MPN (cells per cm^2)[b]				
Sessile samples from corrosion coupons				
mSRB (30 °C)	5.9×10^0	3.3×10^1	0.5×10^0	0.5×10^0

⇒***Lower numbers!***

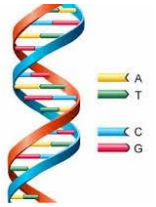
Modified from Skovhus et al. 2017, J. Biotechnol. 256: 31-45.

Bug Bottles vs. Genomics Analysis



Gieg, unpublished

Microbiological Monitoring



KPI – 'number of bacteria' for system monitoring

'High' numbers don't necessarily mean
MIC will be a problem
'Low' numbers don't necessarily mean
MIC won't be a problem

You need to get the whole story $\Rightarrow$
multiple lines of evidence

MIC

Holistic approach

Microbiological Data

- **Samples**
  - Preserved samples: MMM - who is there?
  - Unpreserved samples → lab tests – what are they doing?
- **Choice of primers for 16S MMM?**
  - A: 926F, 1392R (V6-V8)
  - B: 515F, 926R (V4-V5)*
  - C: 341F, 785R (V3-V4)

Context

- **Chemical and operating parameters**
  - Water chemistry: organic acids, cations, anions, pH, total soluble iron etc.
  - Solid deposits: X-ray diffraction (XRD) etc.
  - Flow rates, temperature, operational changes etc.
- **Corrosion damage**
  - Field: pitting profiles, sampling locations etc.
  - Lab Tests: corrosion damage under various conditions

Summary

- Data are only as good as your samples!
  - Important to have SOPs in place for sampling and preservation for field samples
  - Take samples from appropriate locations (corroded vs. non-corroded area)
- Diagnosing MIC requires multiple lines of evidence
  - Chemical
  - Metallurgical
  - Microbiological
    - **Genomics – cultivation-free!**
    - ATP/bug bottles

Thank you!