

DISCOVERY ISLANDS BENTHIC REMEDIATION STUDY: SPATIAL AND TEMPORAL CHANGES IN BACTERIAL COMMUNITY COMPOSITION AND DETECTION OF ANTIMICROBIAL RESISTANCE GENES IN SEDIMENTS

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Conclusions:

- There was limited recovery of bacterial communities in sediments following farm removal over a 3-year period.
- There were few differences in the numbers and types of ARGs detected between the farm-impacted and reference sites.
- Results from different ARG detection methods vary. This is likely due to difference in the relative sensitivities of these methods.



Figure 1. Locations (red stars) of the decommissioned Atlantic Salmon farms in the Discovery Islands used in this study. Triplicate sediment samples were taken along transects in the subdominant and dominant current directions (0m, 30m, 60m, 125m, 250m, 500m and Reference Stations).

Introduction:

There is limited understanding of the process by which the benthic environment recovers following salmon farm removal, including rates of recovery and the recovery potential of benthic communities. The removal of farms from the Discovery Islands area of British Columbia has provided a unique opportunity for us to characterize sediment bacterial communities that represent different stages in the recovery process, as well as to determine whether past antimicrobial use on these farms is associated with increased abundance and/or diversity of Antimicrobial Resistance Genes (ARGs) in the sediments (Fig. 1). Preliminary results from the first 3 years of the project are reported here.

Methods:

Characterization of Sediment Bacterial Communities:

MinION™ amplicon-based full-length 16S sequencing using Dorado super accurate basecalling model and metagenomic analysis with Kraken 2 using the SILVA 138.1 16S rRNA database.

Identification and Quantification of Sediment ARGs:

- 1) Bait-capture targeted metagenomics (>4,000 ARG targets)¹
- 2) Resistomap qPCR panel² of 51 ARGs selected based on the antimicrobials which are licenced for use in Canada and were identified in bait capture results obtained for sediments from active salmon farms.
- 3) MinION metagenomic sequencing using Dorado's super accurate basecalling model and *de novo* annotation of sequences for their antibiotic resistance using the Resistance Gene Identifier (RGI) method of the Comprehensive Antibiotic Resistance Database (CARD) database.

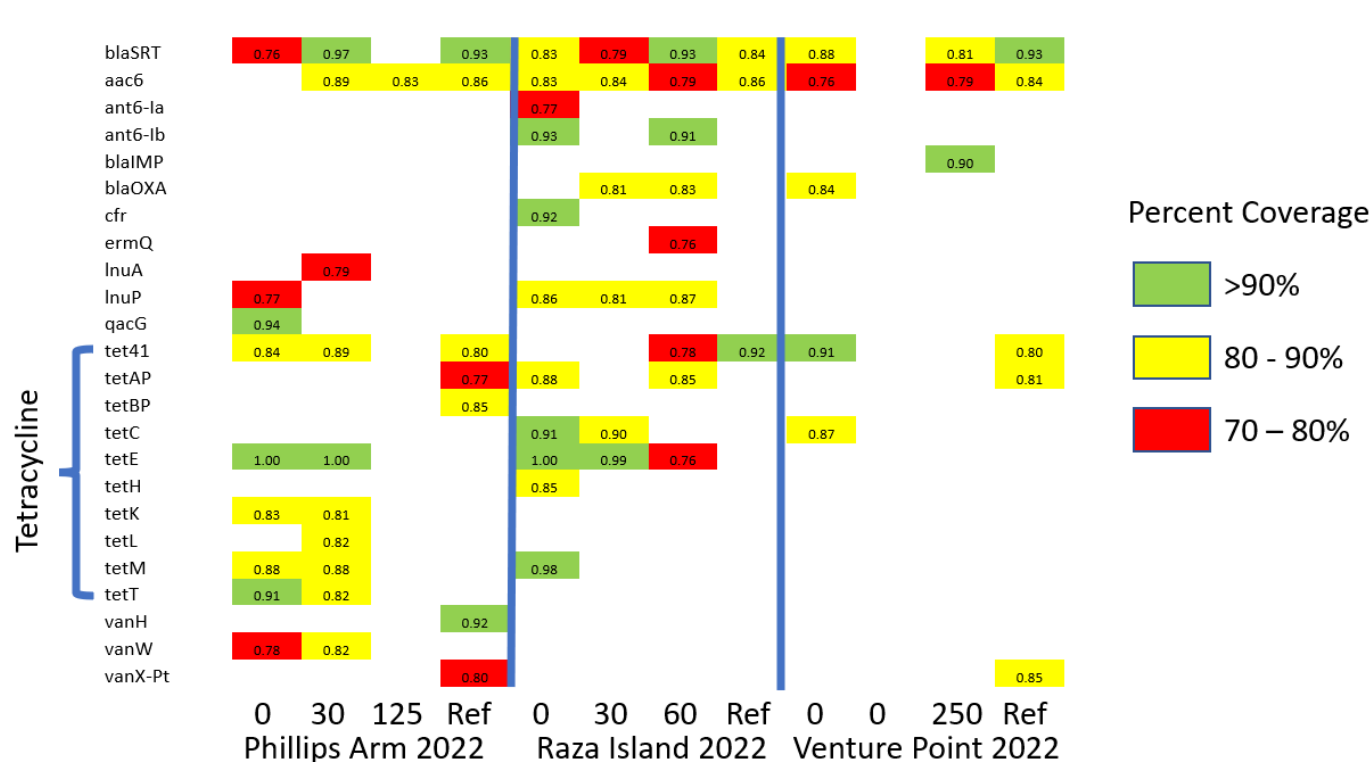


Figure 2. Using a bait-capture enrichment method, that enriches for >4000 ARGs, we detected relatively few ARGs (highlighted in green) in sediment samples collected at salmon farms 18 – 19 months post-decommissioning. Oxytetracycline residues were detected in all the sediment samples with the highest levels in Phillips Arm samples. No other antimicrobial residues were detected. Note: a limit of > 90% coverage was set as the threshold for ARG detection. Distances are in meters and all samples are from the dominant current direction.

Acknowledgments and References:

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(1) Shay, J. A. *et al.* 2023. Exploiting a targeted resistome sequencing approach in assessing antimicrobial resistance in retail foods. *Environ. Microbiome* 18, 1–17; (2) Stedtfeld, R. D. *et al.* 2018. Primer set 2.0 for highly parallel qPCR array targeting antibiotic resistance genes and mobile genetic elements. *FEMS Microbiol. Ecol.* 94;

Results and Discussion:

Bacterial Community Composition

We have investigated spatial and temporal changes in the sediment bacterial communities at all 3 sites over a 3-year period. The results from Raza Island illustrate the types of changes we are seeing at all 3 sites (Figure 1). Differences in the deposition pattern of farm wastes between the dominant (A) and subdominant (B) current directions are reflected in the relative abundances of bacteria and sediment chemistry (data not shown). The sulfur oxidizing genus *Sulfurovum* is the most common genus found in immediate vicinity of the farms. This genus is highly correlated with organic loading and sulfides in our samples.

There is evidence for only limited recovery of the sediment bacterial community following farm removal.

Antimicrobial Resistance Genes (ARGs)

To date we have only examined sediment samples collected 18 – 19 months after farm removal in 2022 for ARGs.

Eighteen ARGs and 1 replicon were detected in 12 of our sediment samples by bait-capture, including 5 tetracycline-resistance (*tet*) genes, a single phenicol-resistance (*cfr*) gene, and 5 ARGs for other antibiotics not used on salmon farms (Figure 2). These genes were found sporadically at both farm-impacted and reference sites.

Resistomap analysis detected between 27 and 40 ARGs in 32 samples, including the 12 used for bait-capture (Figure 3). There were few differences in the numbers and types of ARGs detected between the farm-impacted and reference sites. With respect to antimicrobials used in Canadian aquaculture our analysis detected 4 phenicol, 5 tetracycline, 7 macrolide, lincosamide, streptogramin (MLS_B), and 3 sulfonamide resistance genes.

Metagenomic sequencing detected a variety of ARGs such as *VanT*, *VanW*, *VanZ* (vancomycin), *rsmA* (phenolic) and *nimE* (nitroimidazole). These genes were associated with genera such as *Desulfococcus*, *Novosphingobium*, *Pseudomonas* and *Variovorax*. These genes were not detected by bait-capture or Resistomap Analysis.

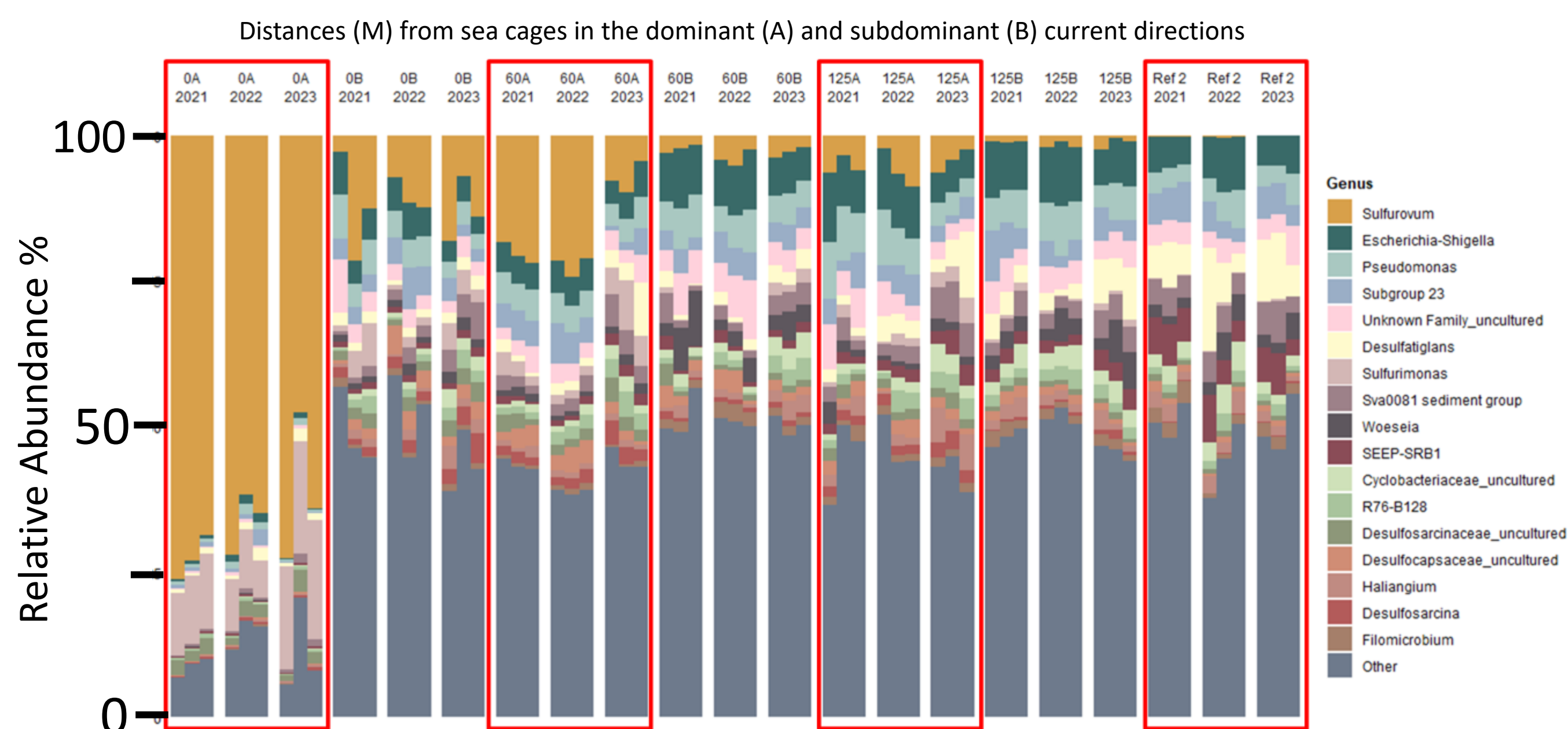


Figure 1. Relative abundances of bacteria genera in sediment samples collected at a decommissioned salmon farm, Raza Island. Samples were collected along transects in the dominant (A) and subdominant (B) current directions. Others includes genera present at 1 to 10% abundance.

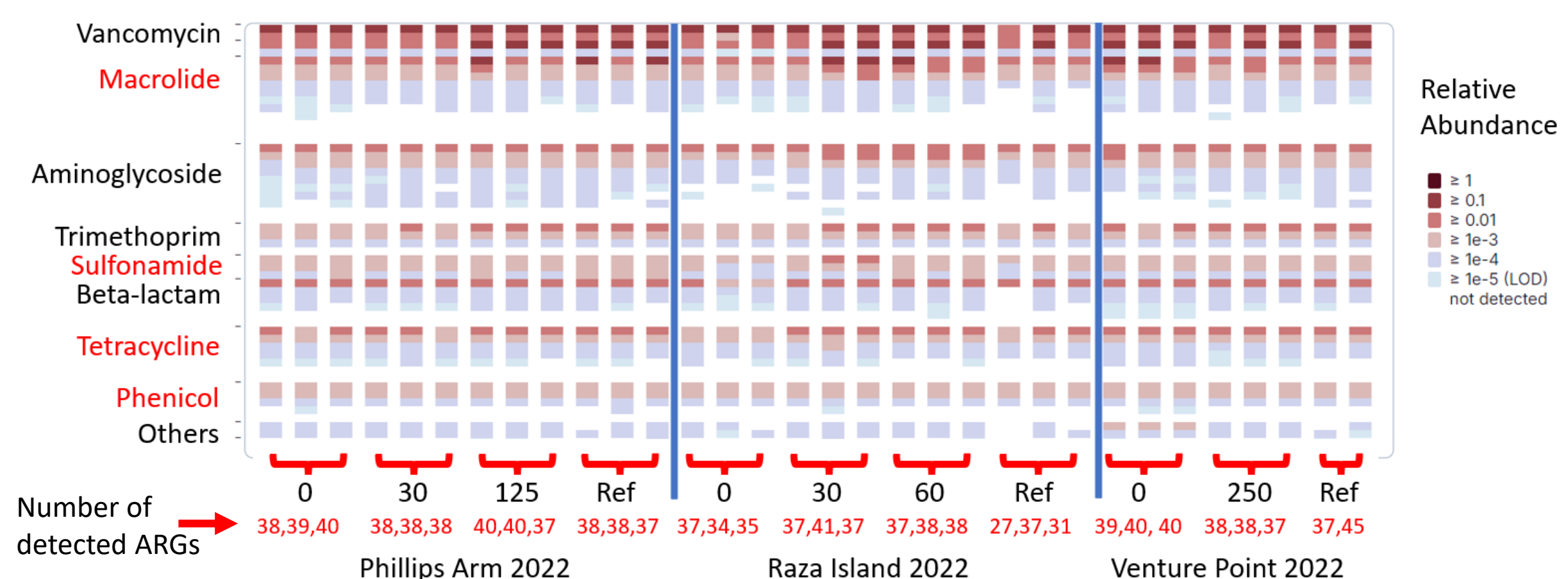


Figure 3. Relative abundances of the ARGs in sediment samples collected at salmon farms 18 – 19 months post-decommissioning. High-throughput qPCR analysis of 49 ARGs, 2 mobile genetic elements and the 16 S rRNA gene was conducted under contract with RESISTOMAP™. Classes of antimicrobials licenced for use in Canadian aquaculture are shown in red text. Between 54 and 78 % of the analyzed ARGs were detected in these samples. Only oxytetracycline residues were detected in these samples although other licenced antimicrobials were used at these farms when they were in production. Distances are in meters and all samples are from the dominant current direction.