# Impacts of Radioactive $^{137}$Cs on Marine Bacterioplankton: Effects of the Fukushima Disaster on Hawaii's Kaneohe Bay Bacterial Communities

**Introduction** Marine bacteria are unmatched in their diversity and abundance. They exhibit mutualism with economically significant organisms, synthesize life-saving natural products, and play a vital role in oceanic nutrient cycling. Despite our dependence on marine bacteria, very little research has been conducted on how they respond to large-scale disasters.

One such catastrophe, a tsunami off the coast of Japan, occurred on March 11, 2011. The tsunami caused the Fukushima-Daiichi Nuclear Power Plant to emit 10 PBq of radiation[2], the largest ever release of anthropogenic radionuclides into the ocean[4]. The main pollutant, $^{137}$Cs, has a half-life of 30 years and will first hit the US territories at the Hawaiian Pacific Islands in early 2014, diluted by only three orders of magnitude[2] (figure 1).

While 63 marine species have already exceeded the Japanese limit for radioactive Cs (100 Bq/kg), the impacts of radioactive waste on marine microorganisms are largely unknown[6]. Due to their short reproductive lifecycle and unicellularity, bacteria evolve faster than most eukaryotes when exposed to radiation, so much so that radiation is used in laboratories to induce mutagenesis.

This project aims to assess the impacts of radiation on the bacterioplankton community of Kaneohe Bay in Oahu, Hawaii. The bay is in the direct path of Fukushima's radioactive waste and has a bacterioplankton community that was well-characterized pre-disturbance[1], making it the ideal case study for the microscopic impacts of radioactive pollution. I will compare trends after radiation exposure to previously documented annual/seasonal fluctuations. This is possible because Fukushima bacterial populations were catalogued bimonthly over an 18-month period.
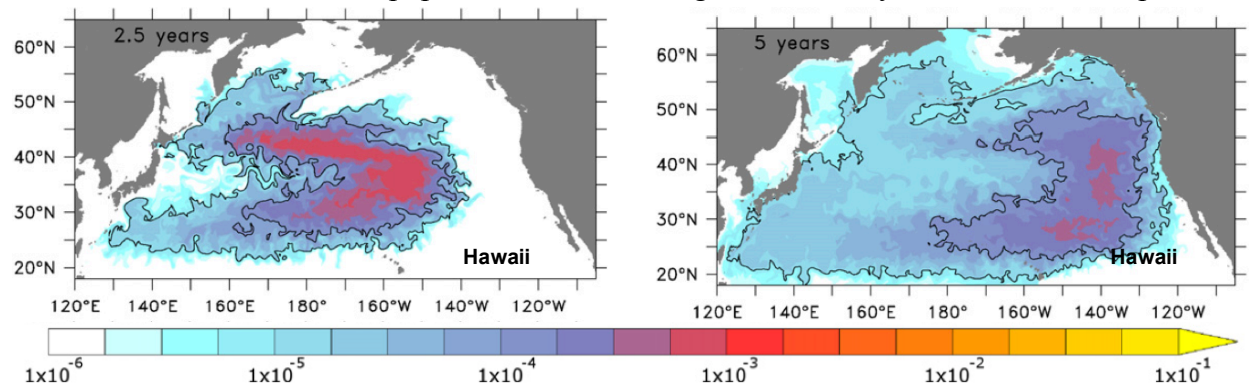


Figure 1: Predicted spread of $^{137}$Cs after 2.5 and 5 years[2]; color scale shows dilution factor

**Research Questions**

1. How has the bacterioplankton species composition in Kaneohe Bay (as determined by 16S small-subunit ribosomal RNA (SSUrRNA) barcodes) changed since the Fukushima leak?

2. Has there been a significant increase in single nucleotide polymorphisms (SNPs) since the radiation event, as compared to mutation rates that would occur due to random chance?

**Methods** I will work within the Rappé laboratory for aquatic microbial ecology at Hawaii Institute of Marine Biology (University of Hawaii at Manoa), which is equipped with all necessary instruments and sampling materials. Rappé is at the forefront of bacterioplankton ecology, and having established the 2006-2007 baseline[1], his lab will provide an excellent knowledge base for collecting comparable data.

Seawater will be sampled at a depth of 1m at 2 sites (reef flat and lagoon) separated by 600m near Coconut Island in southern Kaneohe Bay. Samples will be taken twice monthly from

January 2015 to July 2018 between 07:00 and 08:30h. In situ measurements of temperature, salinity and pH will be taken at 1m depths using a multi-parameter sonde, and radiation levels will be monitored with a scintillation probe. Dissolved inorganic nutrient concentrations ($NH_4^+$, $NO_2^-$, $PO_4^{3-}$, silicate) will be measured using a continuous segmented flow system. Bacteria will be isolated by filtering 1L of water through a 1.6 μm microfiber membrane pre-filter followed by a 0.2 μm polyethersulfone membrane and stored at –80°C in DNA lysis buffer.  Genomic DNA will be extracted using the DNeasy Tissue kit[1].

Bacterioplankton will be characterized by PCR of SSUrRNA and sequenced in a barcoded Illumina HiSeq run. The bacterial primers 27F-B-FAM and 519R will be used[1]. OCTUPUS and UC-LUST will be used to process raw reads, which will then be clustered into operational taxonomic units using MegaBLAST[3]. Mutation and species compositional shifts due to random chance will be determined from the 2006-2007 data[1] using a Poisson distribution and extrapolated to determine the number of mutations that should occur from 2015 to 2018. The experimental 2015-2018 community structure and SNP prevalence will be compared against these values to identify changes that are due to radiation.

**Anticipated Results**
1.  The bacterial community structure will change significantly more than due to random chance.
2.  Post-Fukushima species will have significantly more nonsense and missense mutations in non-essential genes and neutral mutations in housekeeping genes than would have accumulated due to random chance.

**Broader Impacts** This research will help characterize the full repercussions of radioactive pollution at its first outset, providing insight that will allow us to prepare for future radiation leaks and the arrival of the contaminants to the California coast[6]. It will reduce the knowledge gap of what potential harm radioactivity causes marine microbial communities, and give policy makers the information they need to manage affected ecosystems. In light of the recent shift towards increased nuclear power reliance, this research will inform the tradeoffs of pursuing various energy sources in future development, as well as allow policy makers to establish and enforce adequate safety standards for nuclear power plants. In doing so, this research will protect the ecosystem services that marine bacterioplankton provide for humanity, including the nutrient cycling that supports economically important fisheries and large-scale oceanic biodiversity. Resultant policies will protect the biodiversity of marine microbes, which has already proven itself a priceless source of natural products that combat neurological disorders, infections, and cancer[5]. This study will also characterize the impact of radiation on pathogenic bacteria in coastal communities, which is crucial to fully assessing the impact of radioactive waste on human and environmental health.

**Literature Cited**

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