

# A 'Natural' Experiment in Santa Monica Bay, California: Potential Changes in the Microbial Loop in Response to Anthropogenic Nutrient Input

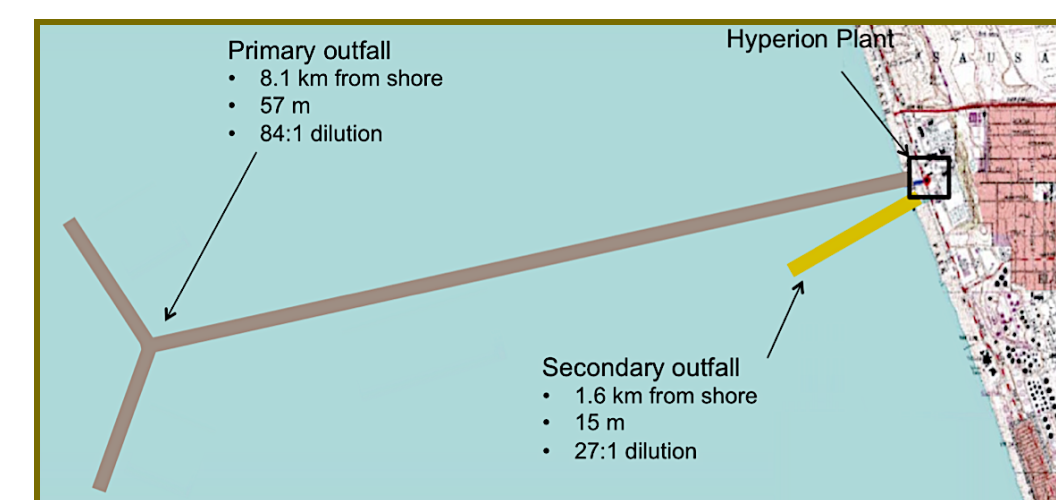
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## Introduction

- Hyperion Water Reclamation Plant discharges ~1.06 x 10<sup>9</sup> L/day of 2<sup>o</sup>-treated effluent into Santa Monica Bay, CA through a 8.1 km outfall pipe (effluent nutrient content >100x above ambient).
- 8.1 km outfall pipe required extensive repairs – effluent flow was diverted to a secondary 1.6 km outfall pipe for 6 weeks in autumn 2015.
- Previous work on an effluent diversion on the San Pedro Shelf, CA in 2012 showed a surprisingly muted phytoplankton response to effluent discharged through a nearshore pipe.
- The lack of phytoplankton response in 2012 was hypothesized to be a result of:
  1. Immobilization of effluent nutrients due to rapid bacterial response<sup>1</sup>
  2. Disinfection by-products inhibiting phytoplankton response<sup>2</sup>



Schematic of Hyperion's Ocean Outfalls



Locations of the 2015 effluent diversion (this study) and the 2012 effluent diversion (~70km south)

## Study Questions

- Will the diverted Hyperion effluent cause a phytoplankton bloom in Santa Monica Bay?
  - If phytoplankton do respond, what groups responded and what was the magnitude of the response?
  - If phytoplankton do not respond, what inhibited the response?

## Methods

### WHAT

**Field Surveys:** Weekly cruises monitored phytoplankton response in the environment at 10 stations in-situ

**Experimental:** Water was collected near the 1.6 km pipe and away from the 1.6km pipe for experiments to examine how effluent impacted the community over time:

- 4-Day Bottle Incubations:** Water from each location was incubated in triplicate in 4-L polycarbonate bottles. Water was incubated without amendment or filtration. Bottles were sampled daily.

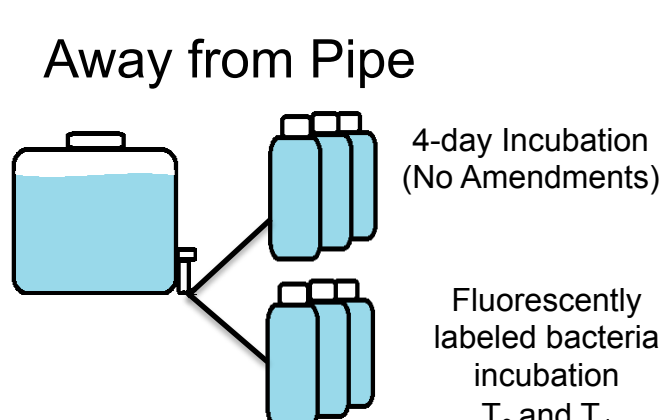
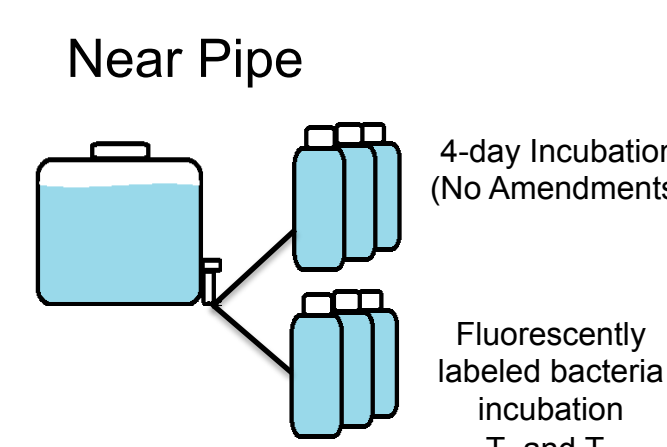
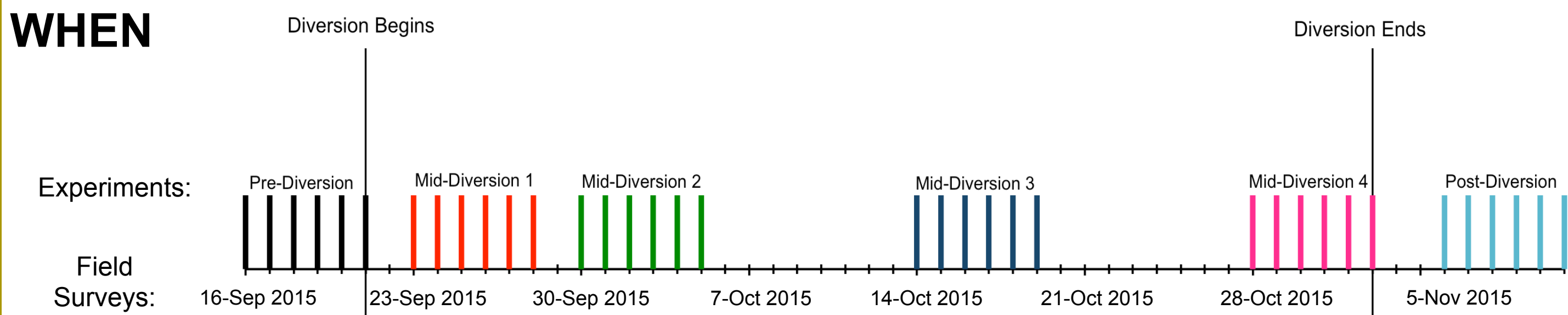
- FLB Disappearance<sup>3</sup> Incubations:** 24-hour incubations were conducted at the time of water collection (T<sub>0</sub>) and after 4 days of incubation (T<sub>4</sub>). Changes in the abundance of DTAF labeled, heat killed bacteria (~1µm diameter) was tracked in whole seawater treatments and a 0.2-µm filtered control (both in triplicate).

### HOW

**Phytoplankton Response:** Chlorophyll *a* concentrations (Trilogy Fluorometer, Turner Designs), Microscopy (Utermöhl method<sup>4</sup>)

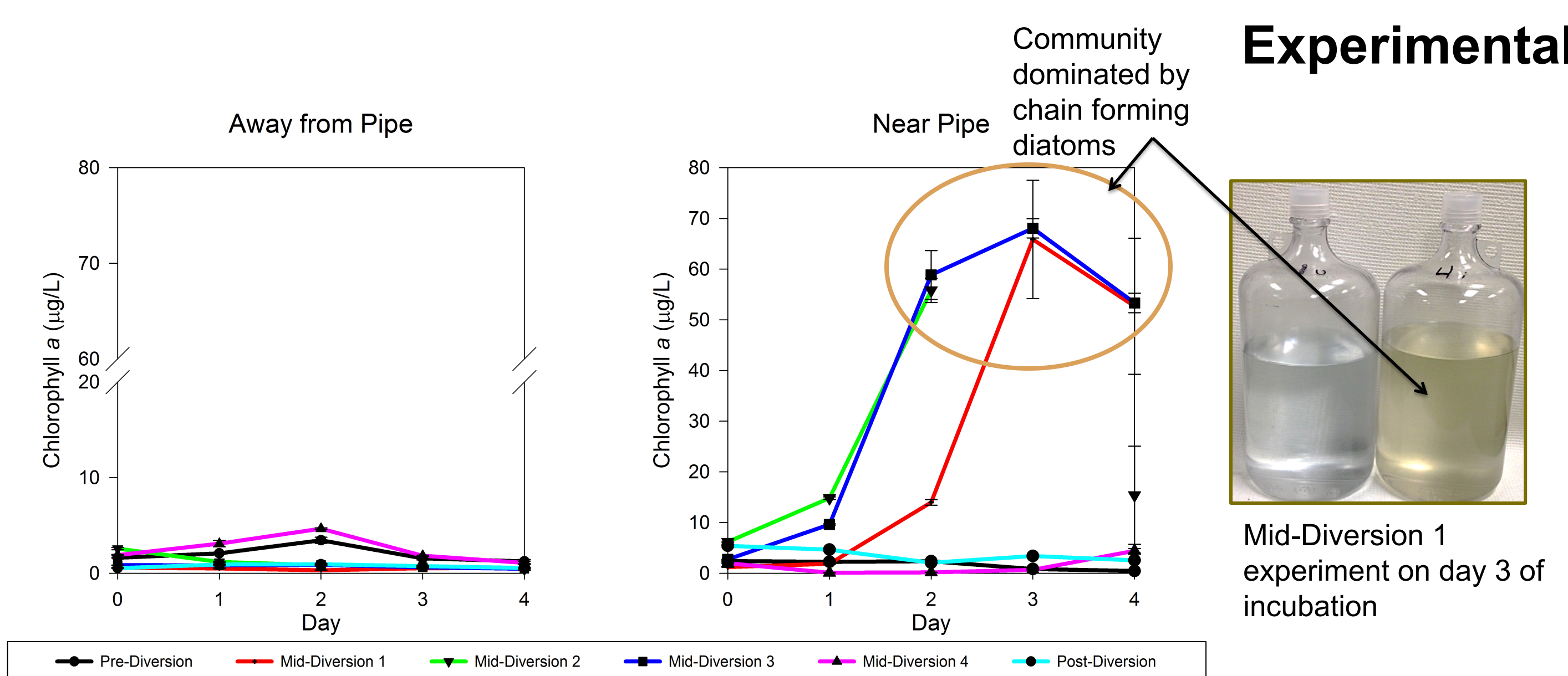
**Bacterial Abundances and FLB Disappearance:** Flow cytometry (FACScalibur, BD)

### WHEN



Schematic of experimental set up for the 4-Day Bottle incubation and the FLB Disappearance incubations

## Phytoplankton Response



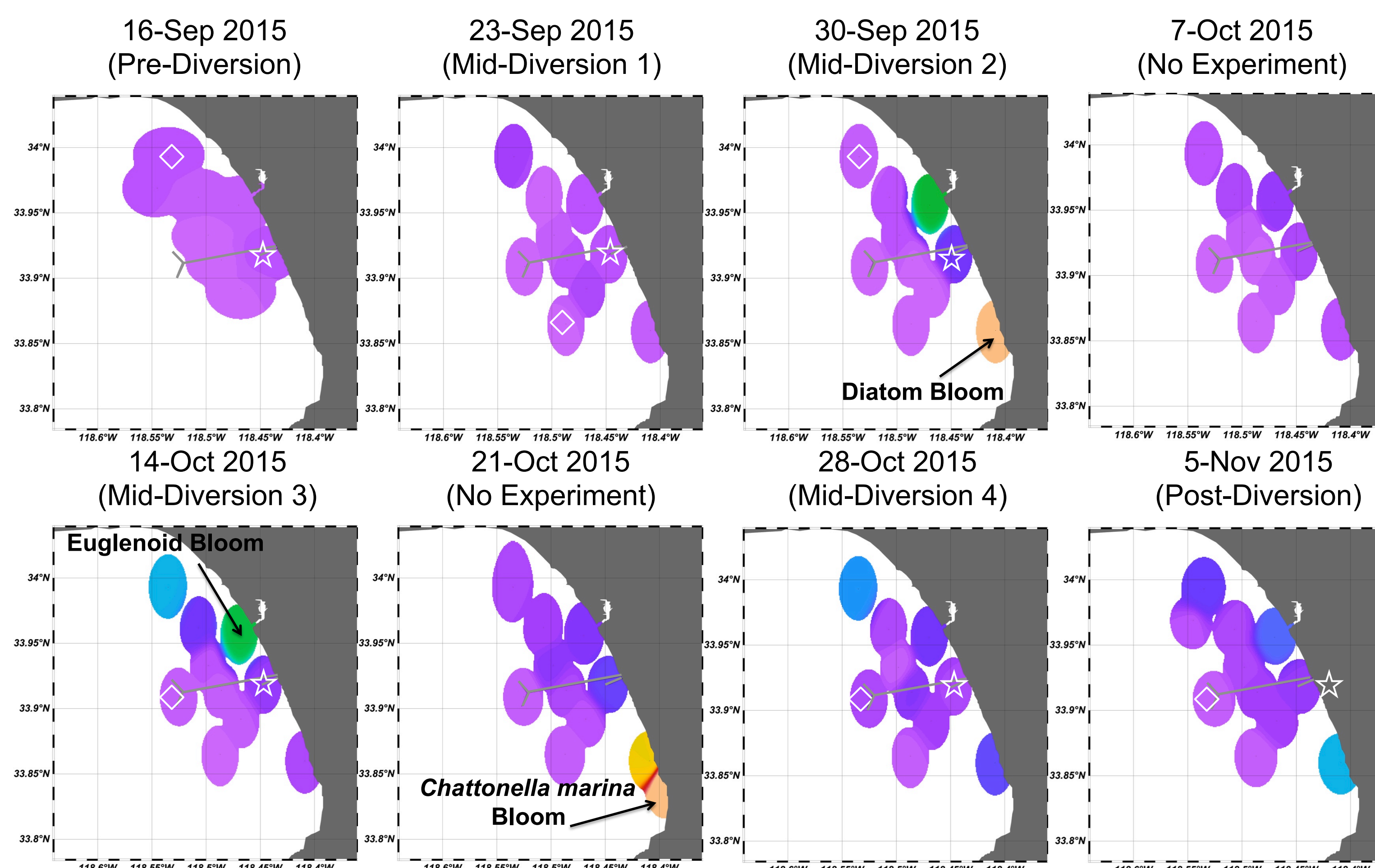
Chlorophyll *a* (µg/L) from the 4-day incubation experiments. A large phytoplankton response was observed in the Mid-Diversion experiments 1-3 in the water collected near the pipe.

## Experimental

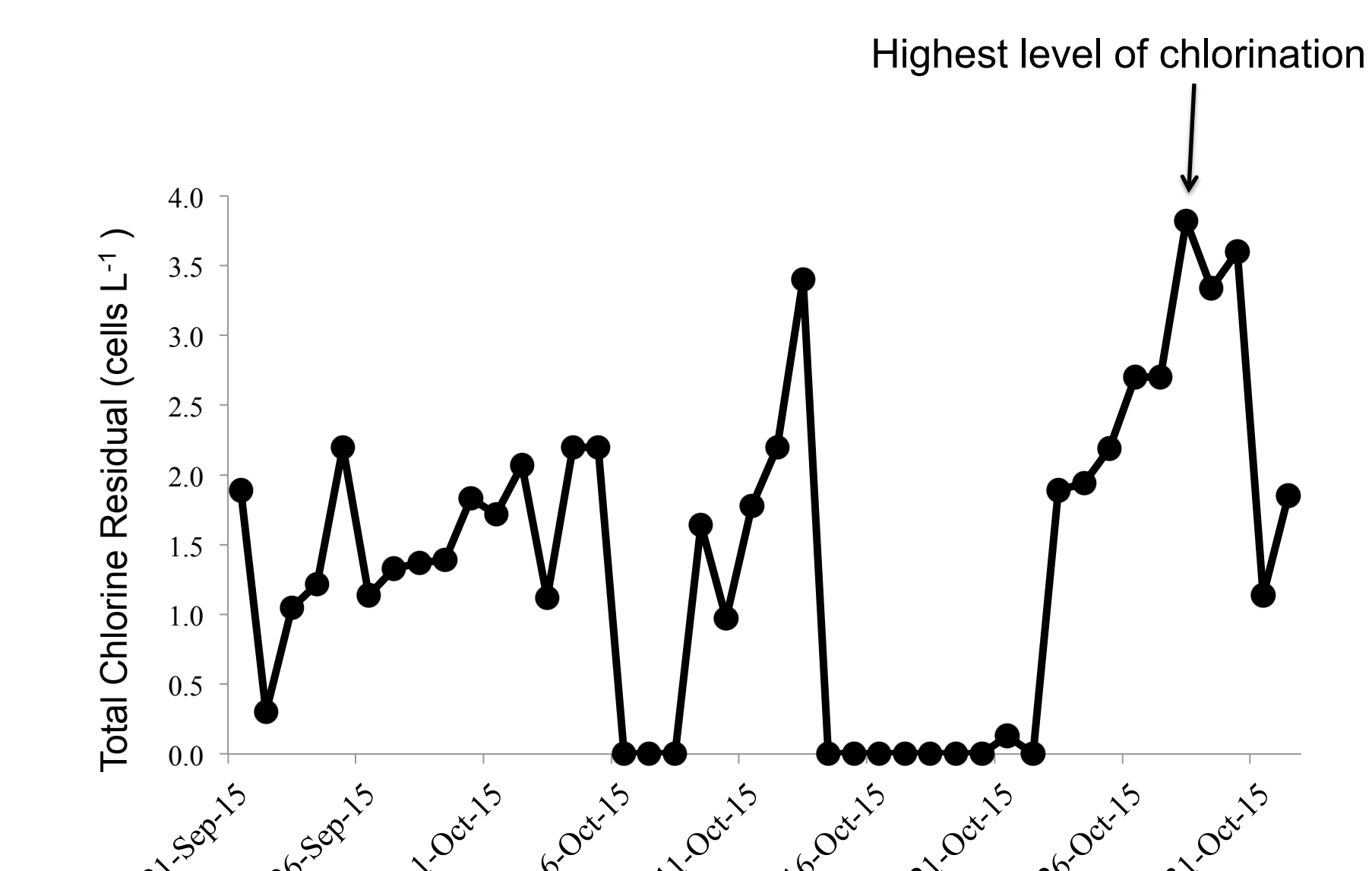
Experiment	Location	PO <sub>4</sub> <sup>3-</sup> (µM)	SiO <sub>4</sub> <sup>4-</sup> (µM)	NO <sub>2</sub> (µM)	NO <sub>3</sub> <sup>-</sup> +NO <sub>2</sub> <sup>-</sup> (µM)	NH <sub>4</sub> <sup>+</sup> (µM)
Pre-Diversion	Away from Pipe	0.35	5.98	0.10	bd	3.92
Pre-Diversion	Near Pipe	0.30	3.00	0.13	1.01	0.89
Mid-Diversion 1	Away from Pipe	0.19	2.45	bd	bd	0.19
Mid-Diversion 1	Near Pipe	4.16	20.0	0.87	2.16	189
Mid-Diversion 2	Away from Pipe	0.12	1.58	bd	bd	3.65
Mid-Diversion 2	Near Pipe	3.24	13.4	0.22	0.81	131
Mid-Diversion 3	Away from Pipe	0.16	2.59	bd	bd	0.21
Mid-Diversion 3	Near Pipe	2.70	12.3	0.57	1.39	119
Mid-Diversion 4	Away from Pipe	0.28	3.91	bd	0.51	1.75
Mid-Diversion 4	Near Pipe	4.85	22.0	0.45	1.27	184
Post-Diversion	Away from Pipe	0.23	2.85	0.12	0.20	1.00
Post-Diversion	Near Pipe	0.12	2.93	0.17	0.31	bd

Dissolved nutrient concentrations of incubation water at the beginning of the incubation. Ammonium concentrations in the water collected near the pipe were 100 – 1000X higher than water collected away from the nearshore pipe.

## Environmental



Surface chlorophyll *a* concentrations from weekly field surveys. Hyperion's ocean outfalls are indicated by grey lines. The locations of incubation water collection are indicated by white stars (near pipe treatment) and diamonds (away from pipe treatment). Incubation water was not collected during all field surveys.

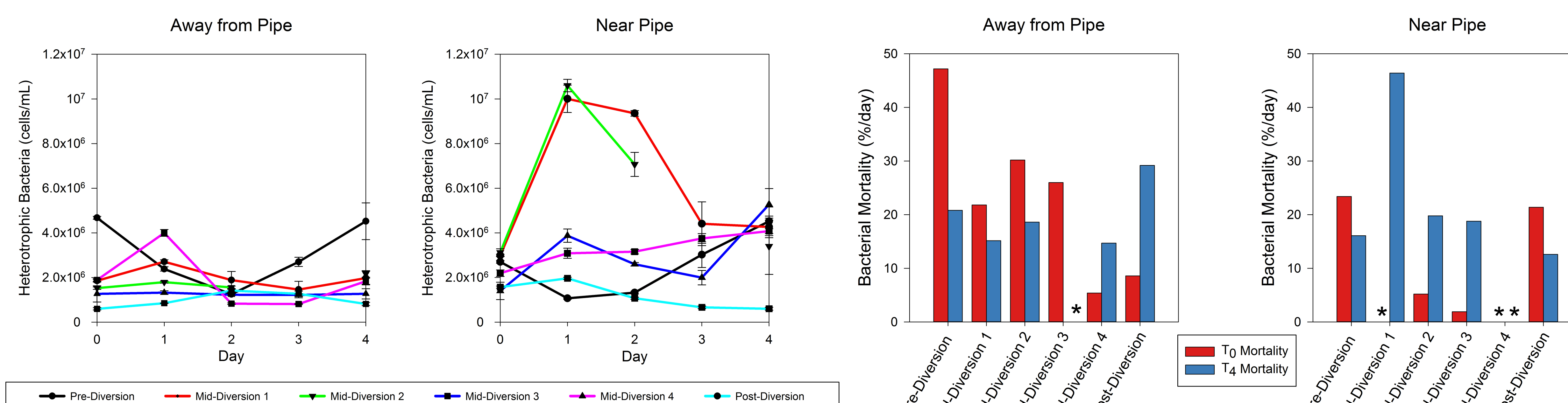


Total chlorine residual (mg L<sup>-1</sup>) measured in the 1-Mile Wet Well (location where effluent was chlorinated prior to discharge) for the period of the diversion spanning 21 Sep to 1 Nov 2.



The effluent plume was fresh and created a "boil" near the site of "near-pipe" incubation water collection

## Bacterial Response



Bacterial abundances from the 4-day incubation experiments. Bacterial abundances generally increased over the 1-2 days of incubation in the near pipe treatments, particularly in Mid-Diversion experiments 1 and 2.

Bacterial mortality rates (%/day) at T<sub>0</sub> and T<sub>4</sub> from the FLB disappearance incubations. Asterisks indicate the measurement of a negative grazing rate on FLBs, which are assumed to represent non-significant grazing. Grazing rates generally increased between T<sub>0</sub> and T<sub>4</sub> near pipe experiments during the diversion.

## Discussion

- Phytoplankton generally **responded positively** to diverted effluent, *unlike* 2012 environmental observations on the San Pedro Shelf.<sup>1,2</sup>
- 4-day incubations grew chain forming diatoms in near-pipe treatments – field surveys showed three taxonomically distinct phytoplankton blooms in SMB.
- Mid-Diversion experiments 1 and 2 showed concurrent, rapid growth of phytoplankton and bacteria over the first few days of incubation – this suggests that increased bacterial abundances did not negatively impact phytoplankton growth in these experiments *contrary* to hypothesis 1 from the 2012 study.<sup>1</sup>
- Mid-Diversion experiment 4 showed a muted phytoplankton response and non-significant bacterial mortality – we hypothesize this to be a result of inhibition by disinfection by-products (*supporting* hypothesis 2 from 2012 study<sup>2</sup>) as the total chlorine residuals on 28-Oct 2015 were the highest of the entire diversion.

## References

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