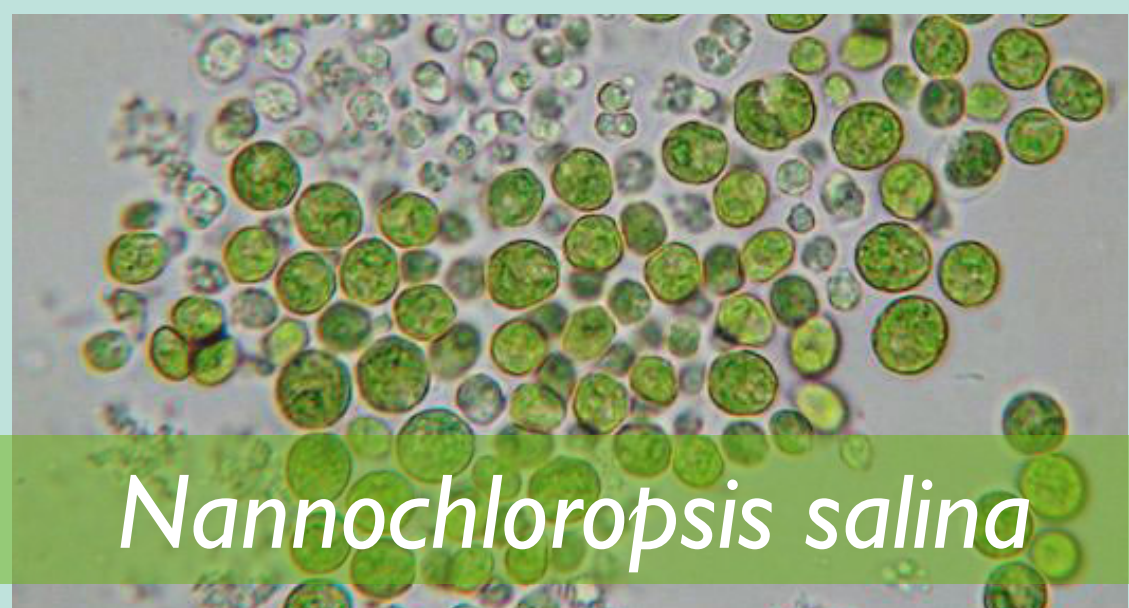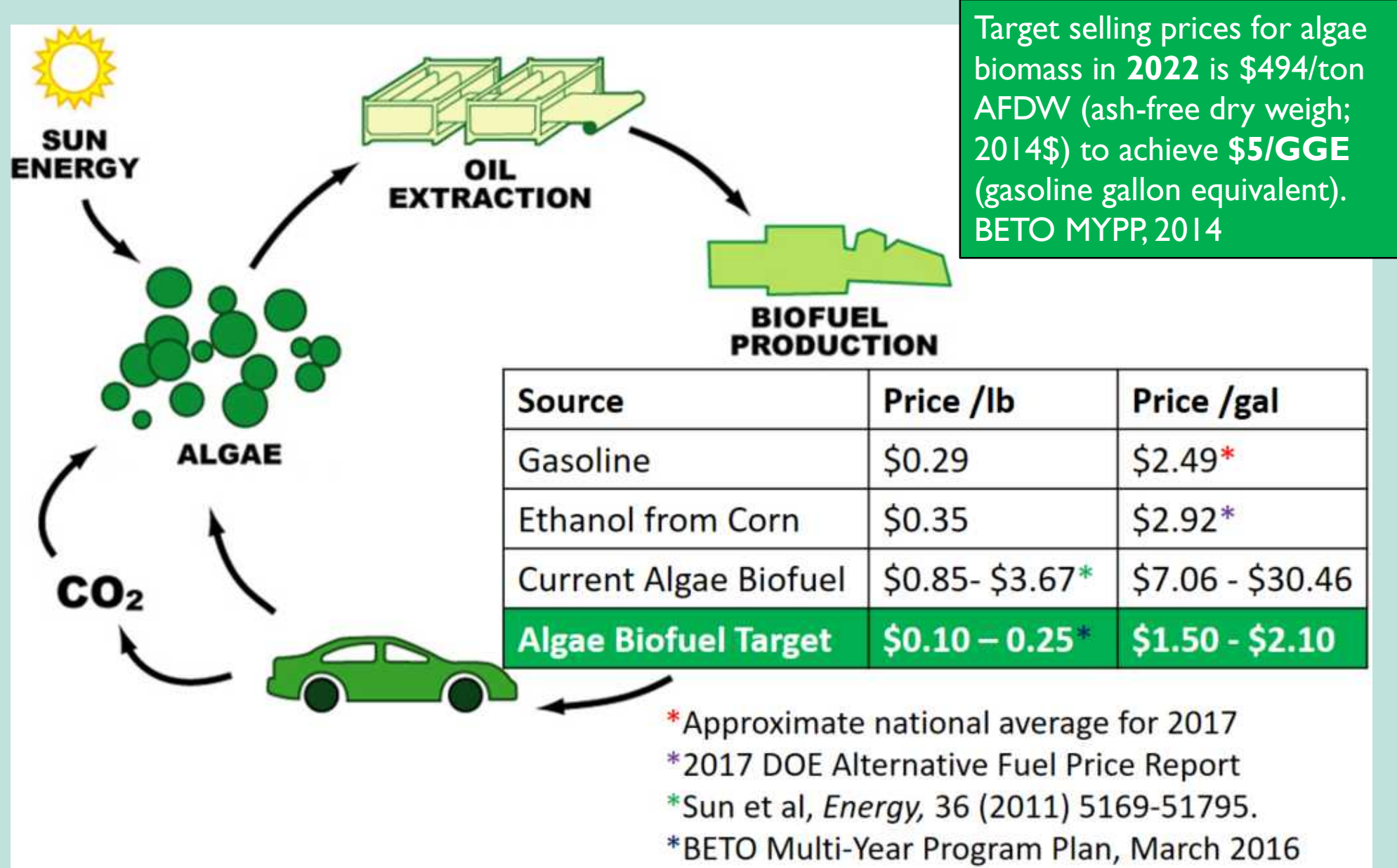SAND2018-8059C

Field Trials for Microalgae Cultures with Probiotic Bacteria

Frances D. Carcellar, Carolyn L. Fisher, Pamela D. Lane, Todd W. Lane
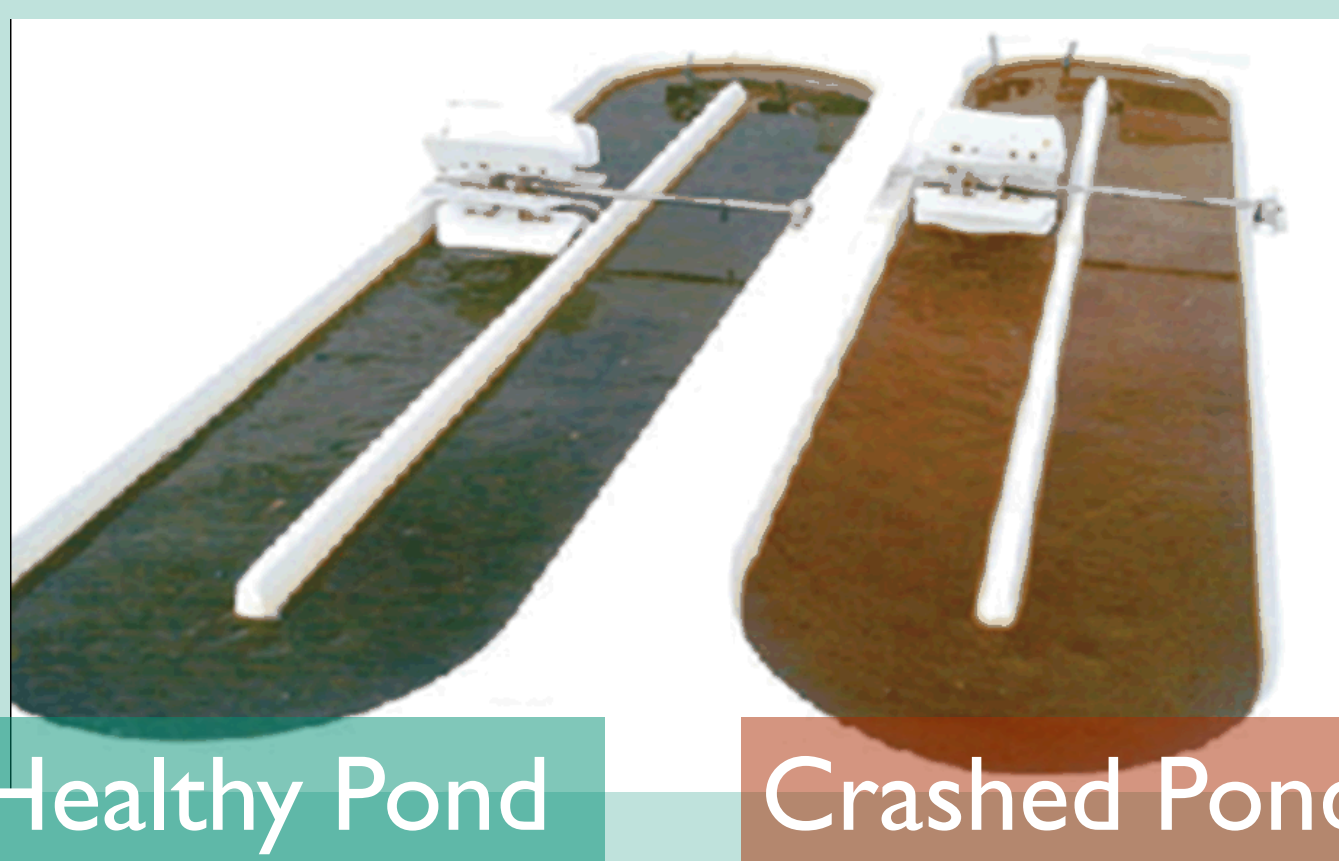
Introduction

The introduction of biofuels would create a drastic shift in both national and global policies towards an increasingly **environmentally aware future**. One of the main obstacles of mass microalgal biofuel production is the threat of pond crashes by foreign predators and bacteria. One way Sandia has tackled this problem is with the use of **probiotic consortium** that protect algae from foreign bacteria.

Target selling prices for algae biomass in **2022** is $494/ton AFDW (ash-free dry weigh; 2014$) to achieve **$5/GGE** (gasoline gallon equivalent). BETO MYPP, 2014

SUN ENERGY
OIL EXTRACTION
ALGAE
CO_2
BIOFUEL PRODUCTION

Source	Price /lb	Price /gal
Gasoline	$0.29	$2.49*
Ethanol from Corn	$0.35	$2.92*
Current Algae Biofuel	$0.85- $3.67*	$7.06 - $30.46
Algae Biofuel Target	$0.10 – 0.25	$1.50 - $2.10

*Approximate national average for 2017
*2017 DOE Alternative Fuel Price Report
*Sun et al, *Energy,* 36 (2011) 5169-51795.
*BETO Multi-Year Program Plan, March 2016
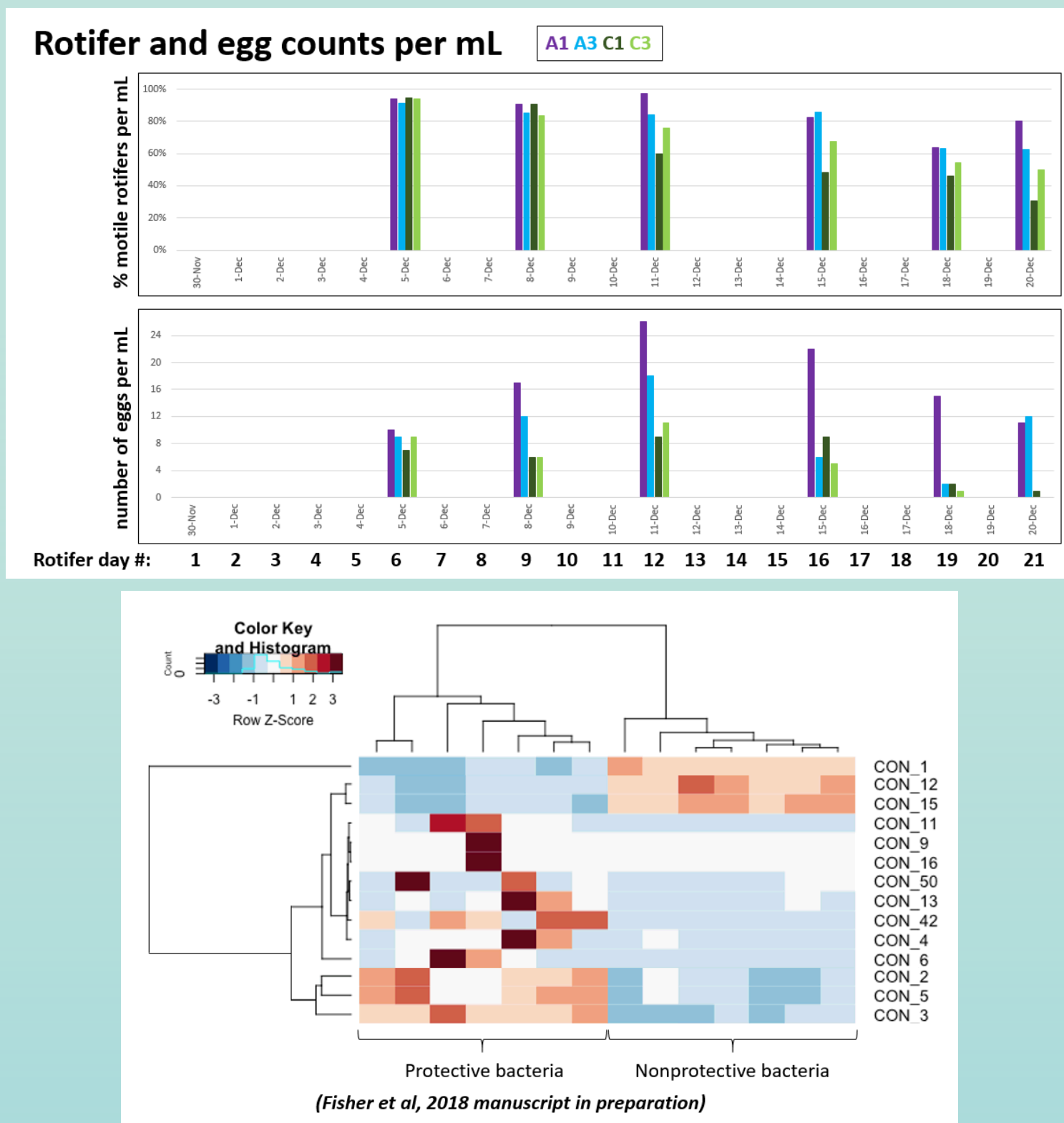
Brachionus plicatilis

Nannochloropsis salina

- Microalgae contain 25-60% lipids and do not require arable land, they not a source of food, and do not cause environmental harm
- Adaptable/ found in many systems: freshwater, marine, high pH, high salinity, and high nutrient municipal wastewater systems.
- Microalgae utilize atmospheric CO_2 and are responsible for up to 40% carbon fixation worldwide, reducing harmful greenhouse gas.

Hannon et al, 2010; Douskova et al, 2009.

Algal population crashes cause losses of up to 30% of annual crop production from the typical open raceway system. Richardson et al, 2014.

Healthy Pond
Crashed Pond

Future Work

Rotifer and egg counts per mL   A1 A3 C1 C3

% motile rotifers per mL

number of eggs per mL

Rotifer day #: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Future work will include:

- Ash-Free Dry Weight (AFDW) and Nutrient Analysis

- Dailey rotifer and egg counts (top)

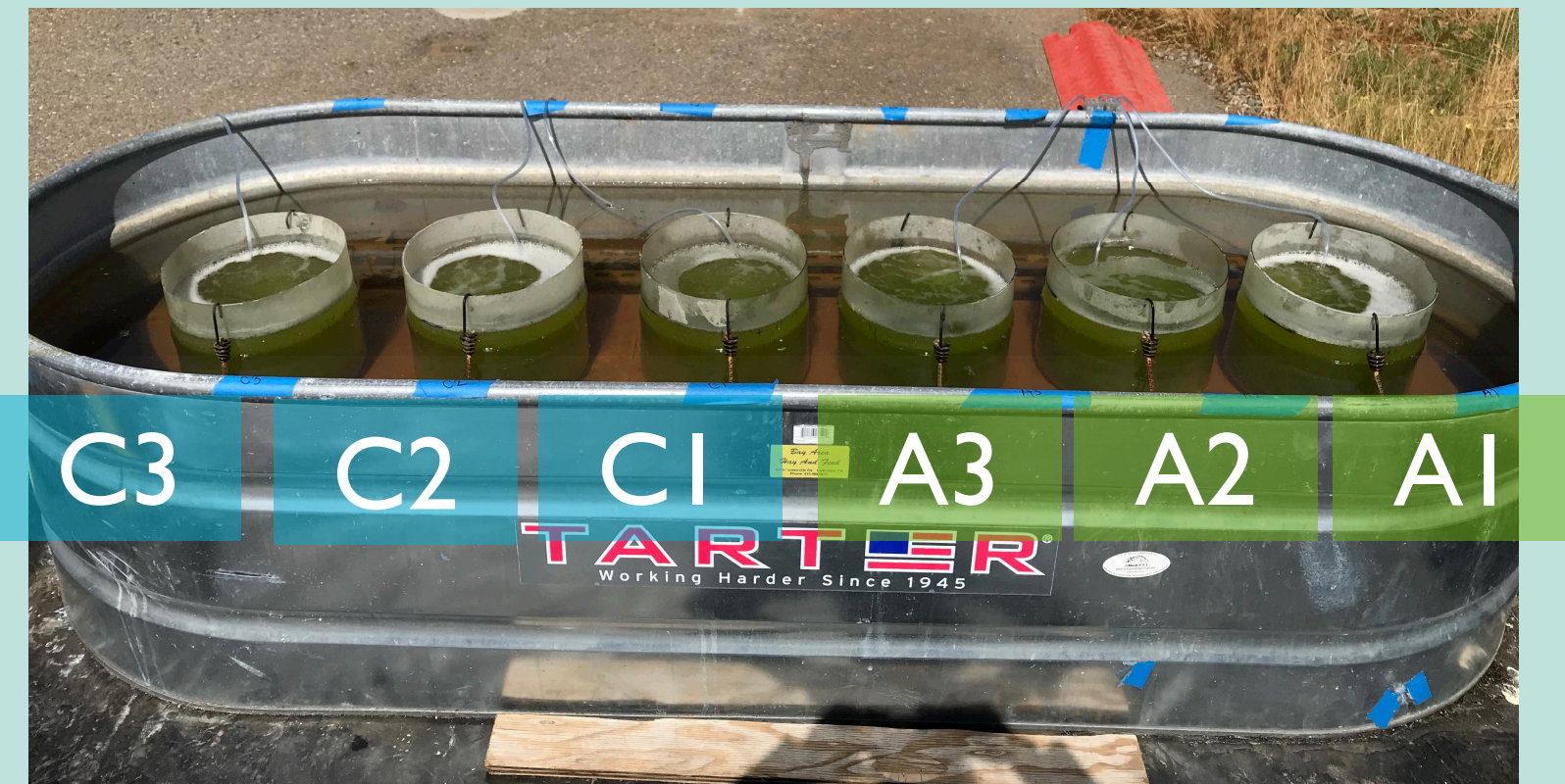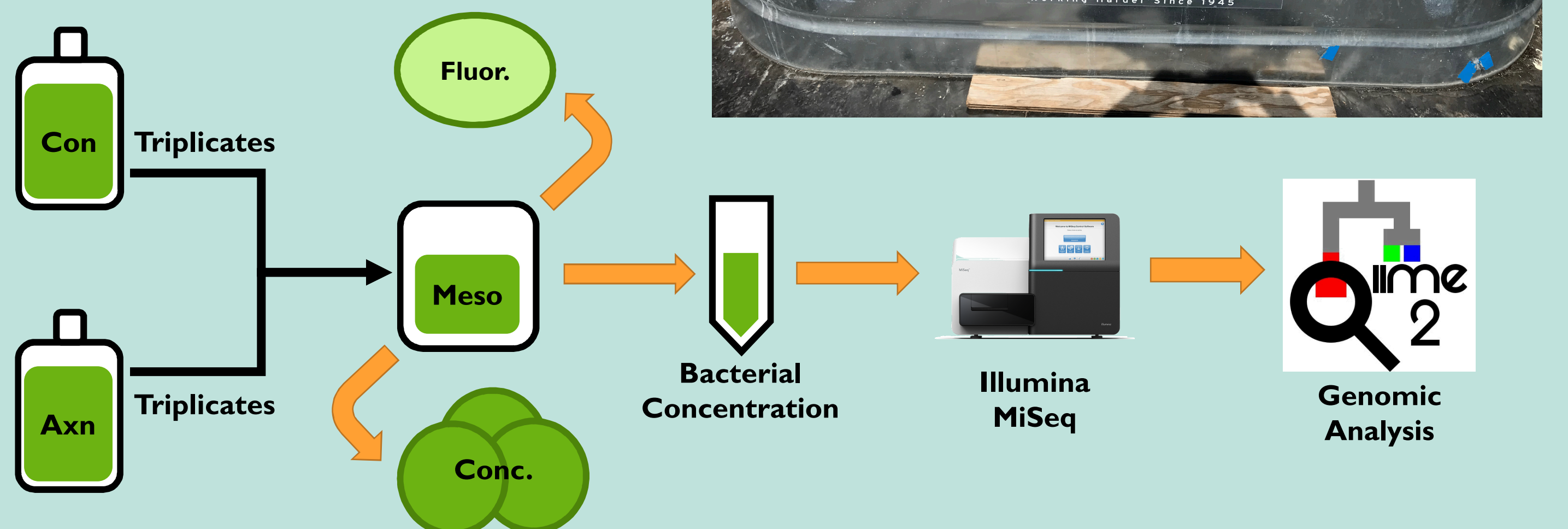As well as further metagenomic analysis and generation of microbial community heatmap. (bottom)

Color Key and Histogram
Row Z-Score

CON_1
CON_12
CON_15
CON_11
CON_9
CON_16
CON_50
CON_13
CON_42
CON_4
CON_6
CON_2
CON_3

Protective bacteria   Nonprotective bacteria
(Fisher et al, 2018 manuscript in preparation)

Experimental Methods

3 sets of consortium algae culture and 3 sets of axenic algae culture were placed in outdoor mesocosms. Field trials of laboratory grown bacterial consortia test whether or not the consortia are still protective uncontrolled conditions.

Mesocosm data collection methods:

- Daily sampling of cell counts and fluoresce
- Nutrient and Sequence samples were collected every 3-4 days
- 90% harvests were done on **Day 11** and **Day 18**
- Mesocosm run lasted 25 days

C3   C2   C1   A3   A2   A1

Con   Triplicates
Axn   Triplicates
Fluor.
Meso
Conc.
Bacterial Concentration
Illumina MiSeq
Genomic Analysis

Results

Outdoor mesocosm trials were conducted to test the protectiveness of laboratory grown consortia samples in an environment similar to industry-wide outdoor algae raceways. These consortia mesocosms were compared with a set of axenic, non-bacterial algae culture. The DNA extracted from each mesocosm was amplified in the V3/V4 region of the 16s ribosome. Samples were sequenced using **Illumina MiSeq** and were further analyzed using **QIIME 2** microbiome bioinformatics platform.
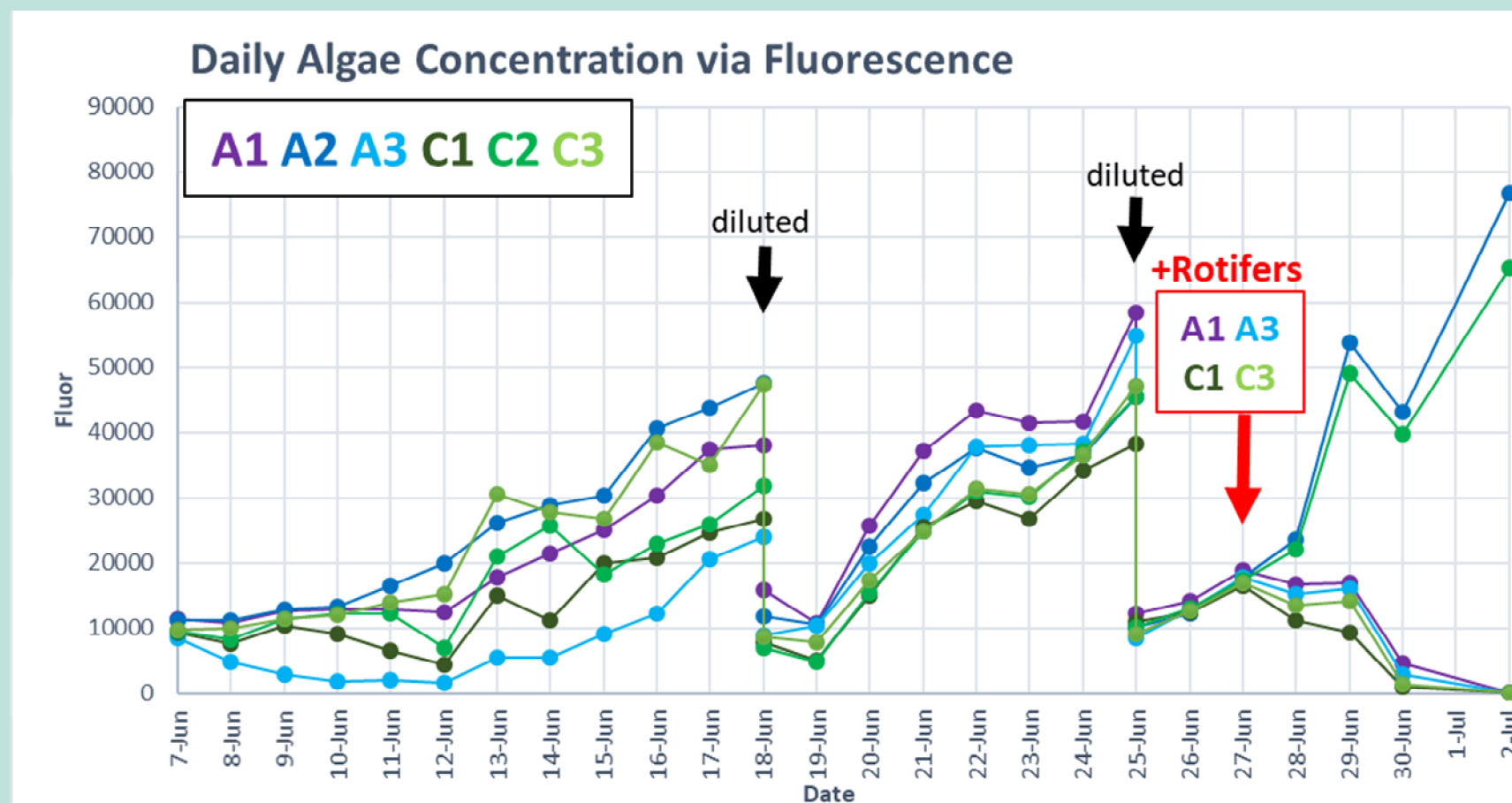
Daily Algae Concentration via Fluorescence
A1 A2 A3 C1 C2 C3
diluted   diluted
+Rotifers
A1 A3
C1 C3

Fig. 1 Algae concentration via fluorescence illustrated the relative fluorescence unit of chlorophyll in algal cells.

Daily Algae Concentration via Cell Counts
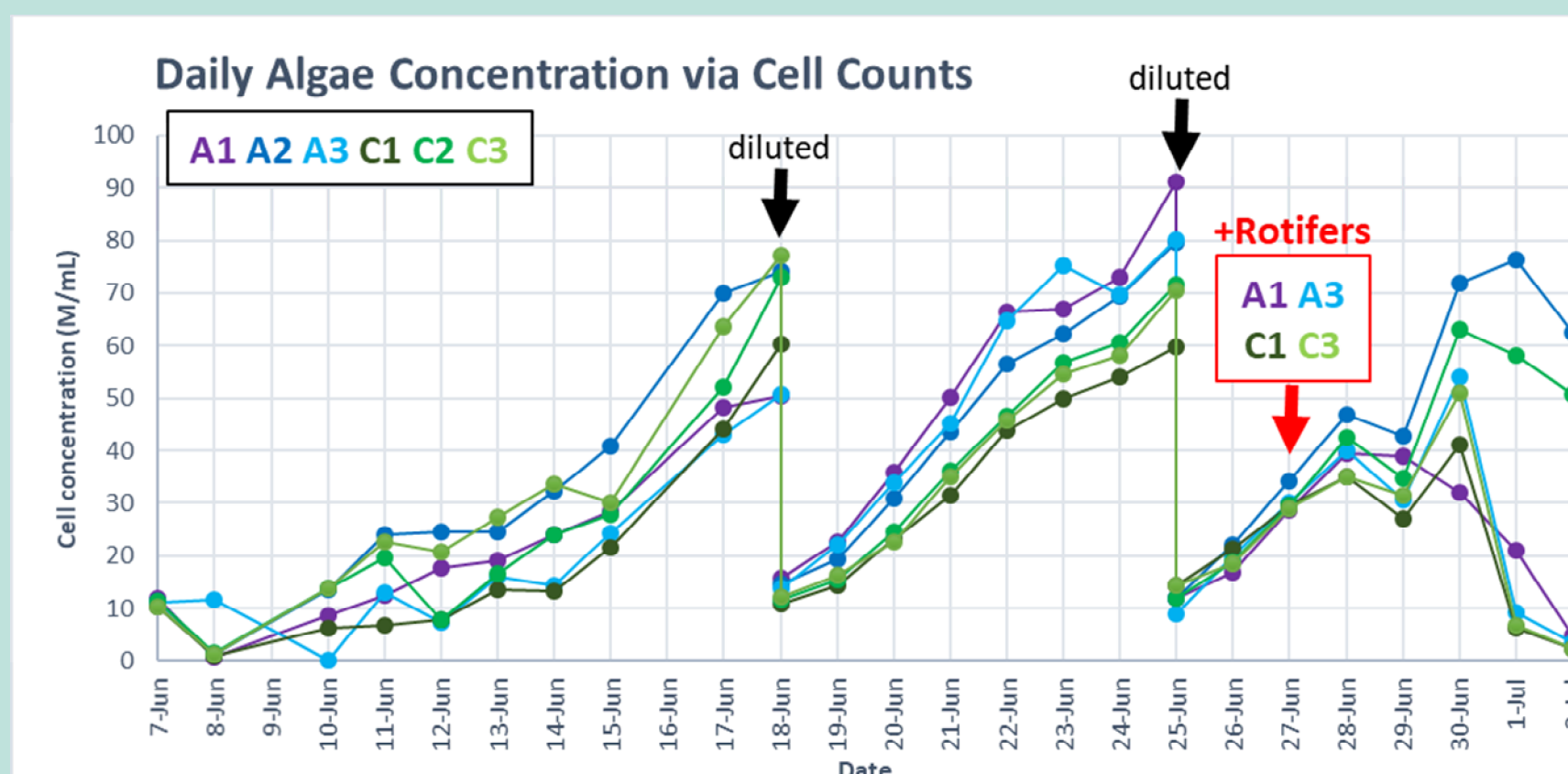A1 A2 A3 C1 C2 C3
diluted   diluted
+Rotifers
A1 A3
C1 C3

Fig. 2 Algae concentration via cell counts illustrated how many cells between 1.807-3.849μm were present in each mesocosm.

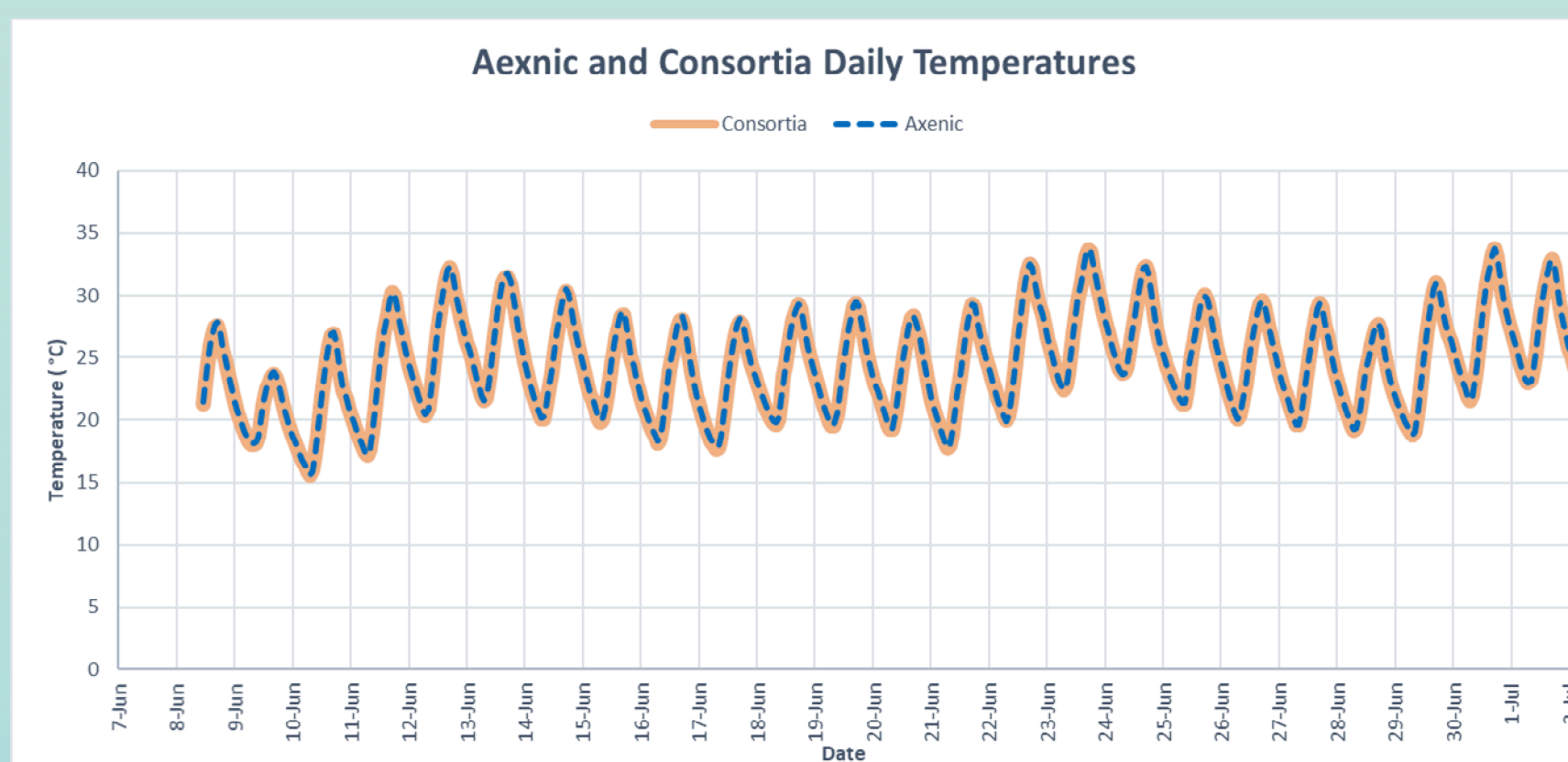**Aexnic and Consortia Daily Temperatures**
Consortia   Axenic

Fig. 3 HOBO temperature sensor data. Sensors were placed in mesocosm A2 to record axenic temperatures and in C2 to record consortia temperatures.
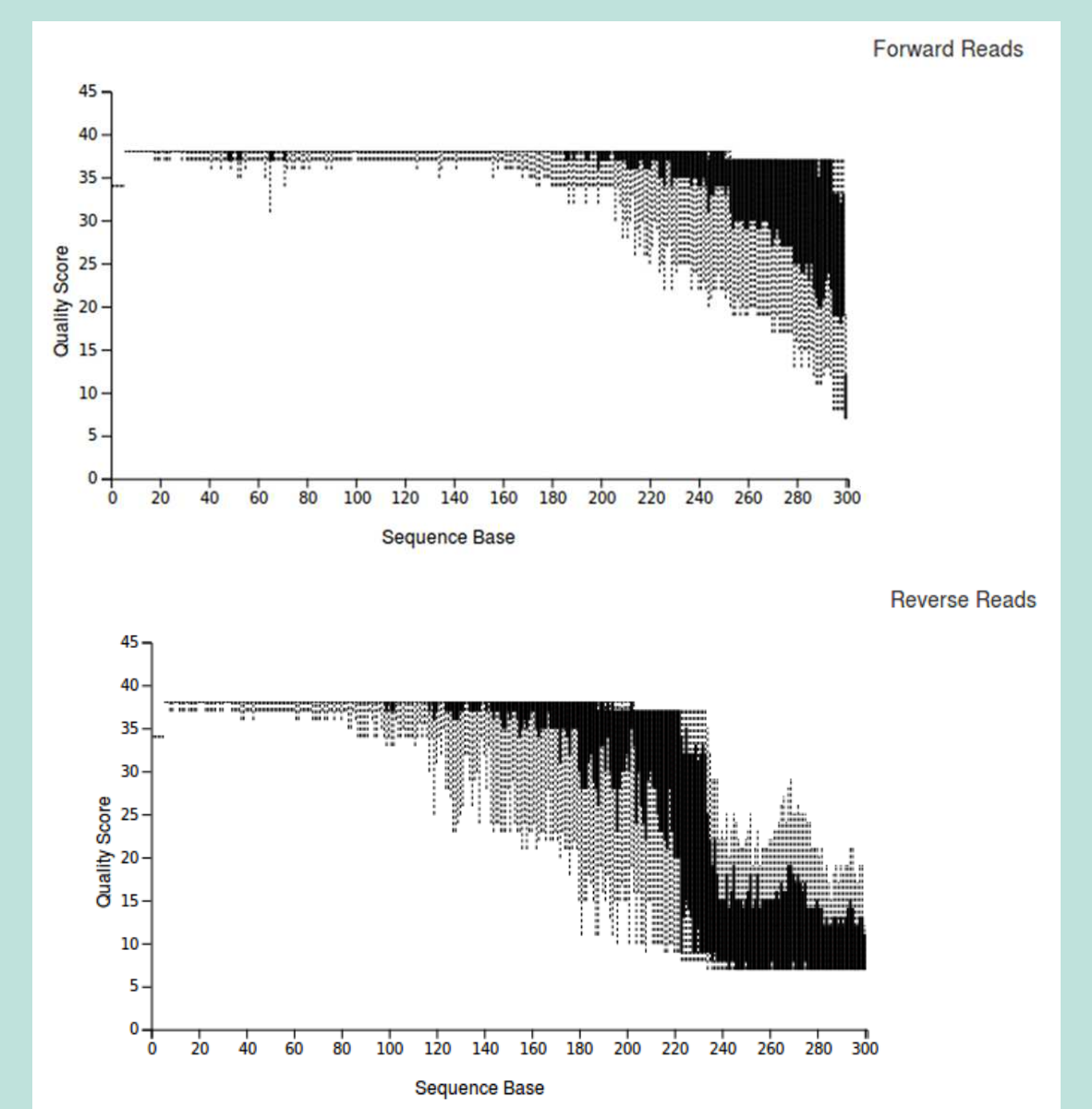
Forward Reads
Reverse Reads

Fig. 4 Quality plots generated in QIIME 2 from Illumina MiSeq data for both forward and reverse sample reads.
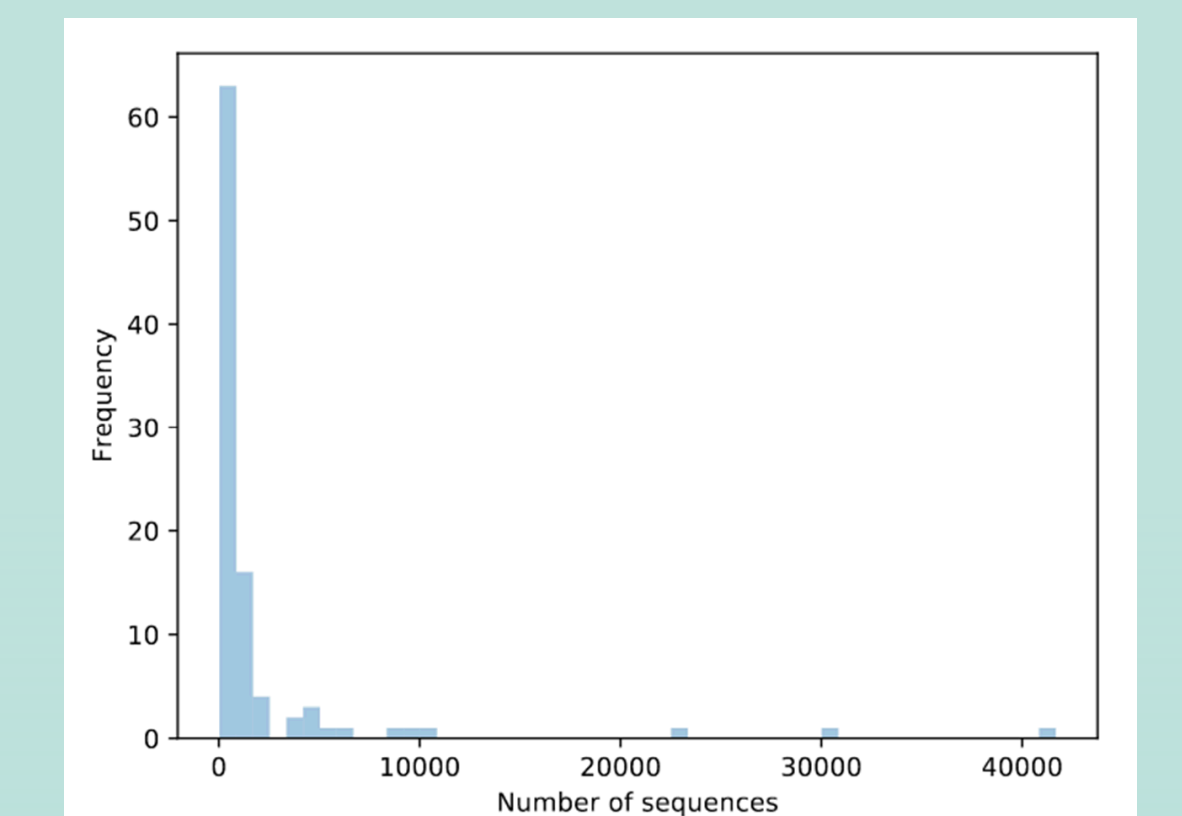
Fig. 5 Demultiplexed sequences generated in QIIME 2. Shows number and range of sequences generated across samples.

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