Pacific Northwest
NATIONAL LABORATORY

PNNL-XXXXX

# TEAMER-3newable LLC. Data Guide

## Data Summary and Compendium

October 2022

Linnea Weicht

U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
under Contract DE-AC05-76RL01830

**DISCLAIMER**



PACIFIC NORTHWEST NATIONAL LABORATORY
*operated by*
BATTELLE
*for the*
UNITED STATES DEPARTMENT OF ENERGY
*under Contract DE-AC05-76RL01830*

PNNL-XXXXX

# TEAMER-3newable LLC. Data Guide

Data Summary and Compendium

October 2022

Linnea Weicht

Prepared for
the U.S. Department of Energy
under Contract DE-AC05-76RL01830

Pacific Northwest National Laboratory
Richland, Washington 99354

# Abstract

This guide describes data products produced during the 3newables LLC. TEAMER project entitled Initial Testing of Wave Energy Powered UV-C LED Anti-Biofouling System. Data are primarily comprised of imagery, quantitative biofouling mass, and scanning electron microscopy (SEM) analysis. Temperature was also recorded for the experimental campaign.

# Biofouling Analysis

## Coupon Nomenclature

Twenty-eight coupons were provided for this experiment, resulting in four sets of triplicates for each control and treatment sets, as well as four coupons allocated for initial process testing. Coupons were formed of 1 inch by 1 inch by 1/8 inch platinum-coated titanium and weighed approximately 9.4 grams each.

Coupons were assigned identifiers based on location in 3D printed coupon trays to assist in data organization. As depicted in Figure 1, the tray's rows are lettered A through D, and the tray's columns are numbered 1 to 4. Additionally, a '-T' is added to coupons in the treated sample set, and a '-C' is added to coupons in the control sample set. Trays were also printed in different colors (blue for treated and black for control) to assist in keeping control and treated coupons separate.


Figure 1: Coupon identifiers based on tray location

## Coupon Weight Data

Dry weights were collected for each coupon before the experimental campaign began. Wet weights were collected for triplicate treated and control test coupons using a microbalance to compare growth inhibition. Weight data was calculated as initial dry weight subtracted from the final wet weight for each biofouled coupon. Compiled weight-gain per coupon is organized in the Excel document 'Triplicate_Weights.xlsx in four separate tabs. The tabs contain information as follows:

Table 1. Triplicate Weight Gain Excel Overview

| *Tab* | *Information* |
|---|---|
| Pre-Experiment | This tab contains the initial recorded weights for all coupons. Information includes date, time, the scientist weighing coupons, coupon ID, coupon weight, and the scientist inputting data and taking photos. |
| Month 2<br>Month 4<br>Month 6 | These three tabs contain date, time, scientist weighing coupons, coupon ID, coupon weight, and scientist inputting data and taking photos for each monthly triplicate analysis respectively. |
| Analysis | This tab pulls data from the previous four to calculate weight gain per coupon. An average and standard deviation is calculated for each monthly triplicate set. |

## Image Data

Coupons were photographed in triplicate after wet weights were taken in order to provide another semi-quantifiable metric to track biofouling growth. After initial photographing, coupons were stained using a tri-mix dye containing Erythrosine B, Rhodamine, and Coomassie Brilliant Blue, causing biofouling to pick up red, blue, and purple hues. This dye was allowed to sit on the coupons for one minute and they were photographed again. The coupons were then rinsed in filtered seawater to remove excess dye and photographed a third time. The stain enhances contrast of biological growth on the surface of a coupon as compared to the coupon itself, which can be processed through a software to determine the percentage of gray, red, green, and blue hues found on a coupon, quantitatively indicating amount of biofouling buildup.

### Raw Image Data

Raw image data contains photographs of all three steps of the staining process as described above. Images are labeled with the coupon identifier and step of the process the photograph was taken during. These images were taken with a Canon 70D camera and the files contain metadata including camera settings.

Table 2. Raw image nomenclature

| *Identifier* | *Meaning* |
|---|---|
| “pre” | Image was taken of the coupon pre-staining. Wet weights have already been collected, and this process may have minimally disturbed biofouling. |
| “stain” | Image was taken after the coupon had been stained with trimix dye, which was allowed to set for one minute. |
| “post” | Image was taken after stained coupon had been rinsed in filtered seawater. These are the final images to be analyzed. |

### Processed Image Data

The third step of the staining process resulting in photographs of stained coupons after rinsing with filtered seawater was compiled and converted to .JPG format for processing. Images are labeled only with coupon identifiers. The resulting library of images was processed using PNNL’s BGI software, and the resulting quantitative data is saved in an excel file called ‘ImageAnalysis.xlsx.’

The BGI software additionally outputs four image files representing the biofouled coupon in gray, red, green, and blue scales. Each image is labeled with coupon identifier and the colored image. For example, the coupon A3-C's blue-scale image is labeled "A3CBGIblue."

### Additional Images

Photos were taken throughout the process of installing the test setup, collecting samples at bi-monthly intervals, and removing the test setup. These images are labeled according to the date they were taken and the general contents of the photograph.

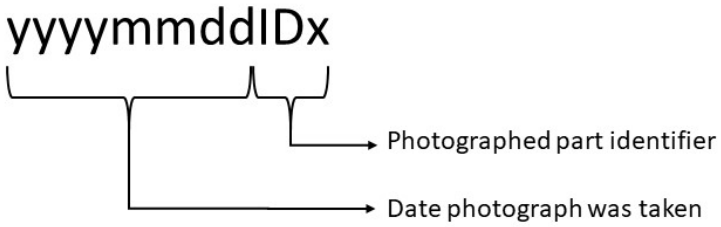


Figure 2: Photograph Identifier Nomenclature

Table 3. Additional image identifiers and descriptions

| *Identifier* | *Description* |
|---|---|
| CAMERAS | Image contains camera enclosures after the end of the experiment. |
| CONTROL | Image contains control coupon tray after removal from test chamber. |
| COUPONS | Image contains individual coupons after removal from trays. |
| DISHES | Image contains plastic dishes used to hold individual coupons in filtered seawater after removal from trays and awaiting analysis. |
| LIDC/LIDT | Image contains the 3d printed tray lid. "C" indicates control tray lid, "T" indicates treated tray lid. |
| ORGANISM | Image contains organism found on a coupon or on the tray itself. |
| SEM | Image contains coupons chosen for SEM analysis in shipping petri dishes. "SEM" identifier is preceded with the coupon identifier, A1T for example. |
| TANK | Image contains the tank with test rig in place. |
| TANKEMPTY | Image contains the tank after water has been drained. The test rig is still in place, and biofouling growth is photographed on the tank and test rig. |
| TREATED | Image contains treated coupon tray after removal from test chamber. |
| TUBEWORM | Image contains a tube worm found on a coupon. |

## Scanning Electron Microscopy (SEM)

Scanning electron microscopy analysis was performed on a triplicate coupon set at the final timepoint (6 months) by PNNL's Environmental Molecular Sciences Laboratory (EMSL). Samples were shipped to EMSL in petri dishes sealed with parafilm. Samples were stabilized

with custom 3d-printed coupon trays and arrived in undisturbed condition. Upon arrival at EMSL, the samples were fixed in 2.5% glutaraldehyde to stabilize the biological portion on the surface and processed using standard SEM protocol. This included dehydrating samples by gentle washing with an ascending ethanol series (33%, 50%, 75%, 100%), placing them in the chamber of a critical point dryer (CPD, Tousimis, Autosamdri-815), and processing according to an automated CPD scheme, with CO2 as the transitional fluid. The samples were then mounted on standard carbon tape-covered aluminum SEM stubs (Ted Pella, Redding, CA), and sputter-coated with carbon. The samples were then imaged using an FEI Helios Nanolab DualBeam SEM (Thermo Fisher Scientific, Hillsboro, OR) at 2KeV (kiloelectron volts). The ultrastructural detail of the sample surface was compared with a blank control coupon.

SEM imaging data is sorted by coupon and provided in .jpg format. Samples had varying numbers of unique features to image, so the quantity of photos varies by coupon depending on the features selected for imaging. Additionally, coupons were photographed in profile to assist in evaluating height of biofouling buildup, and these images are provided in summary pdfs with the SEM analyst's comments.

## Water Temperature

Water temperature was recorded with a Hobo U20L-01 temperature logger. Data is stored in an Excel file titled "3newables_temp.xlsx." Raw data is presented as a list of datetimes and stored water temperature values. Data is also presented with temperature spikes over 100°F filtered out and again smoothed for two-hourly averages.

# Pacific Northwest National Laboratory

902 Battelle Boulevard
P.O. Box 999
Richland, WA 99354

1-888-375-PNNL (7665)

***www.pnnl.gov***