DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Algal Productivity Enhancements by Rapid Screening and Selection of Improved Biomass and Lipid Producing Phototrophs (APEX) EE0008904

Advanced Algal Systems

Matthew Posewitz
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DE-FOA-0002029: FY19 BIOENERGY TECHNOLOGIES OFFICE MULTI-TOPIC FUNDING OPPORTUNITY ANNOUNCEMENT
Period of Performance: 10/2020 – 9/2024

Collaboration with Pacific Northwest National Laboratory, Queensland University of Technology and Global Algae Innovations

Project Overview

- *Global Algae Innovations (GAI) has state-of-the-art algal culturing facilities and media formulations for robust algal growth at the Kauai growth facility. This project aims to attain high-biomass AND high-lipid productivity strains.*
- *Specifically, lipid yields >31% with biomass productivities at >23 g/m^2/day are targeted. >20% increase in lipids and >50% increase in biomass from current baseline.*
- *Atmospheric and room temperature plasma (ARTP) mutagenesis will be used to generate a mutant library of GAI high-productivity strain (e.g. Nitzschia sp.). ATRP yields insertions and deletions versus base mutations – more stable phenotypes with fewer reversions.*
- *Algal breeding will be pursued using Nitzschia sp. to generate genetic diversity for the isolation of high-lipid AND high biomass strains. Very high-risk/reward.*
- *Nannochloropsis mutants will be screened for high-lipid and high biomass strains.*
- *Bioprospecting and "survival-of-the-fittest" selection strategies will be used to isolate the fastest-growing, high-lipid strains that thrive in the GAI high pH media.*
- *Demonstrate scalability of high-lipid AND high-productivity strains from the lab bench to the green house to the outdoor algal farm.*

1 - Management

Investigator	Roles
Matthew Posewitz Colorado School of Mines	Overall responsibility for ensuring that project obligations are realized. Coordinates routine (weekly) project meetings. Responsible for assembly and submission of DOE reports. Responsible for integrating peer-review feedback into the project. Responsible for communication of all research results among team members, identifying/mitigating risk and enabling all project participants to contribute towards project objectives. Responsible for communication and collaboration where possible with related projects and advisory boards.
Jesse Traller/Aga Pinowska Global Algae Innovations (GAI)	Responsible for managing project activities at GAI facilities. Leverages collaborative synergies with other GAI projects when possible. Shapes research thrusts and objectives to focus project on areas that are most likely to improve farm yields. Critically evaluates datasets and formulates experimental design. Overseeing bioprospecting and algal breeding efforts at GAI.
Alexander Beliaev Pacific Northwest National Laboratory (PNNL)	Responsible for coordinating mutagenesis efforts with QUT and library screening/mutant selections via cell sorting approaches. Performs strain characterization studies using mini-raceway systems in controlled environmental chambers. Responsible for helping to shape project goals and directions and ensuring open communication across the project.
Robert Speight Queensland University of Technology (QUT)	QUT is responsible for constructing plasma mutagenesis libraries, aspects of high-lipid screening, evaluating research progress and shaping project goals.

Goal Statement

- *Use mutagenesis, breeding and/or bioprospecting to isolate high-biomass and high-lipid strains.*
- *Use flow cytometer to find high lipid strains.*
- *Use custom laboratory photobioreactors that effectively mimic outdoor pond parameters to enrich high-lipid pool for the best overall biomass producers.*
- *Demonstrate test-bed to farm scalability using custom bioreactors and mini-raceways.*
- *Ultimately select algal strains that attain >23 g/m^2/day and >31% lipid at the Kauai* testbed*.*

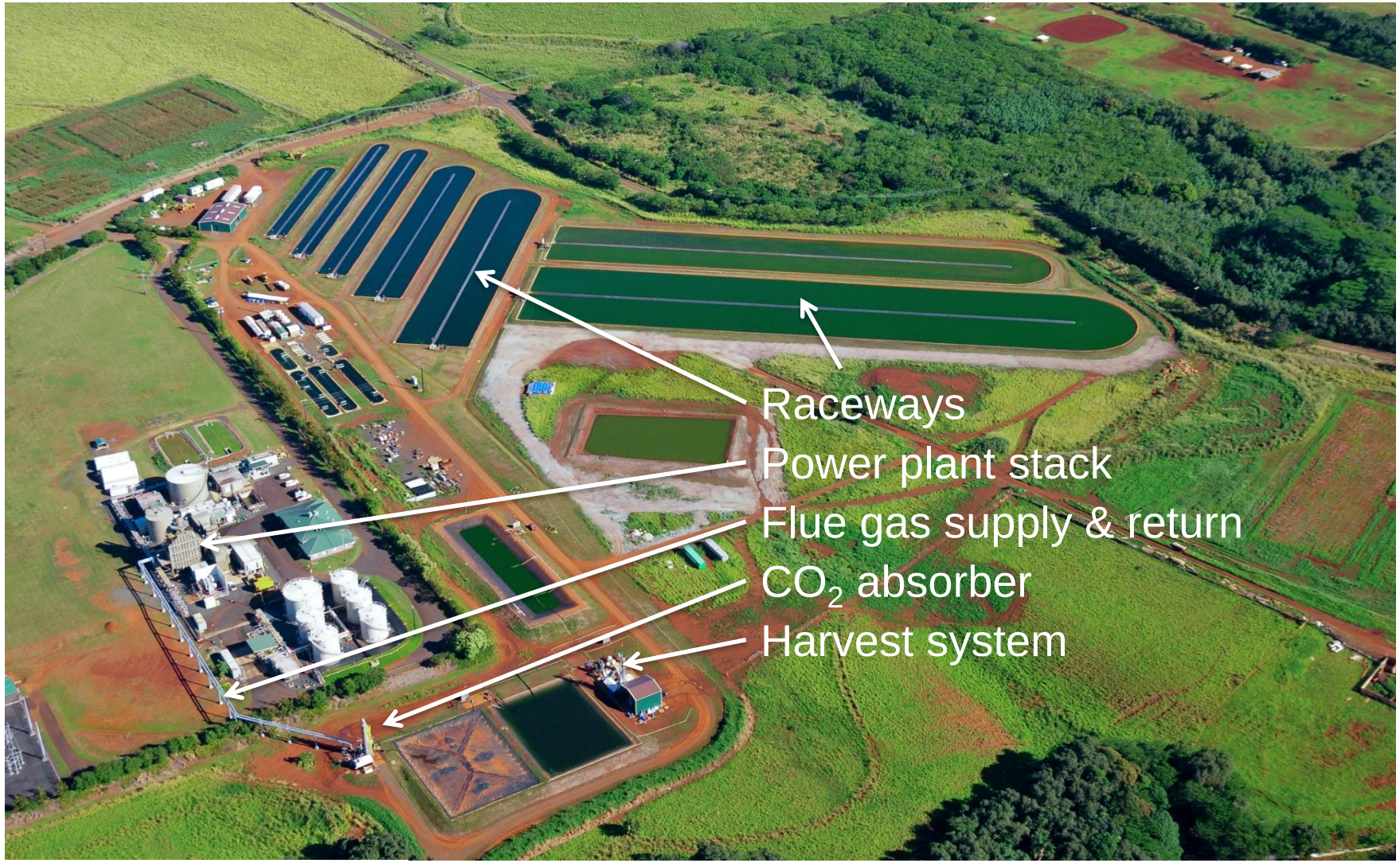
2 – Approach

- *Strains of GAI Nitzschia and species of Nannochloropsis are mutagenized using atmospheric and room temperature plasma (ARTP) mutagenesis. This approach generates high levels of insertions and deletions that are more stable to reversion relative to single base mutations.*
- *High-lipid strains will be selected using flow cytometry and cell sorting. This pool will then be tested for high growth in custom-built environmental bioreactors to the best growing high-lipid strains. This process may iterate depending on results. The reverse approach may also be explored where robust growth in bioreactors is first selected followed by lipid screening from this population.*
- *Bioprospecting will be used to isolate high-productivity AND high-biomass strains.*
- *The largest challenges include the ability generate/isolate strains for interest and to maintain mutants without reversion.*
- *The Go/No-Go metrics included the ability to reach 23 g/m^2/d and 31% lipid from a strain and show that this can be scaled from laboratory bioreactors to small raceways and then at the GAI farm facility.*
- *The primary technical metric is algal productivity (g/m^2/d) and quality (gallons of gasoline equivalent).*

3 – Impact

- *The overarching goal is to use directed evolution to improve algal lipid yields in high productivity strains at the GAI algal growth facility. Specifically, we are targeting productivities of >23 g/m^2/day and >31% lipid; 50% and 20% respective improvements over baseline.*
- *High lipid biomass is preferable for conversion to fuel.*
- *ARTP mutagenesis is cutting edge technology not yet applied to algal systems to our knowledge – powerful tool to apply for stable, improved strains.*
- *Environmental strain selection using GAI medium and unique survival of the fittest enrichment strategies for the isolation of new high lipid/biomass strains from bioprospecting.*
- *Exploration of novel mating strategies to improve Nitzschia diversity. Very high-risk approach but advances in breeding are potentially high reward.*
- *GAI is already an algal biomass provider – advances in lipid yield productivity will benefit their existing technologies.*
- *Research team is actively engaged in publishing and presenting research at international conferences and top-tier publications.*

Algae cultivated on CO_2 supplied from power plant flue gas since June 2014

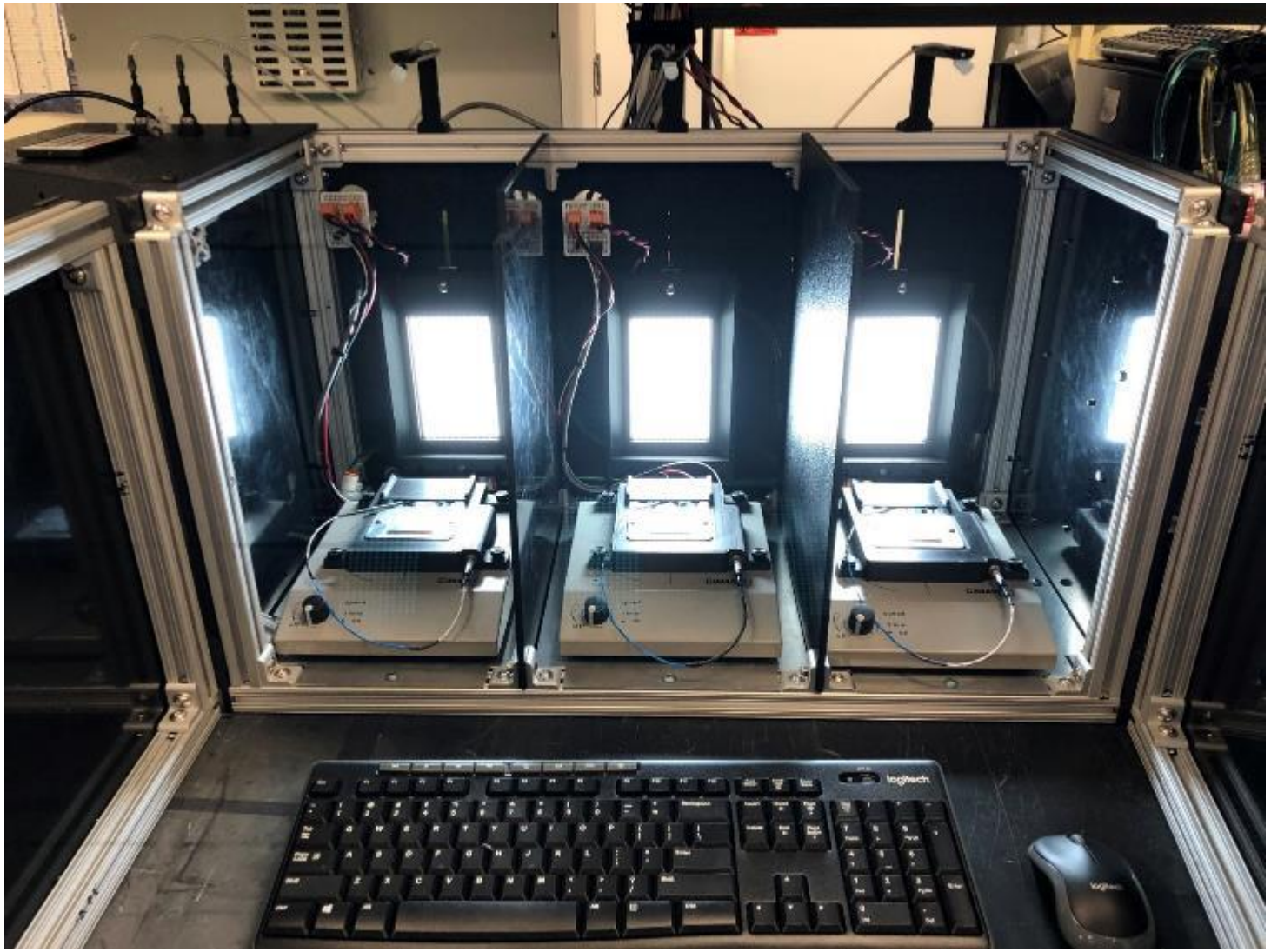


GLOBAL ALGAE

4 – Progress and Outcomes

- *Project launched in October 2020*
- *Extensive Nitzschia and Nannochlropsis growth expertise established*
- *Strains shipped to QUT for library generation*

4 – Progress and Outcomes
Laboratory Photobioreactors



- Custom built environmental photobioreactors
- All software and electronics custom built
- Maintained and improved as undergraduate capstone projects
- Each station controls lights and temperature across diel cycle
- Gas mixing/flow options

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Laboratory Photobioreactors

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- Gas mixing/flow options
- CO_2 on demand and OD

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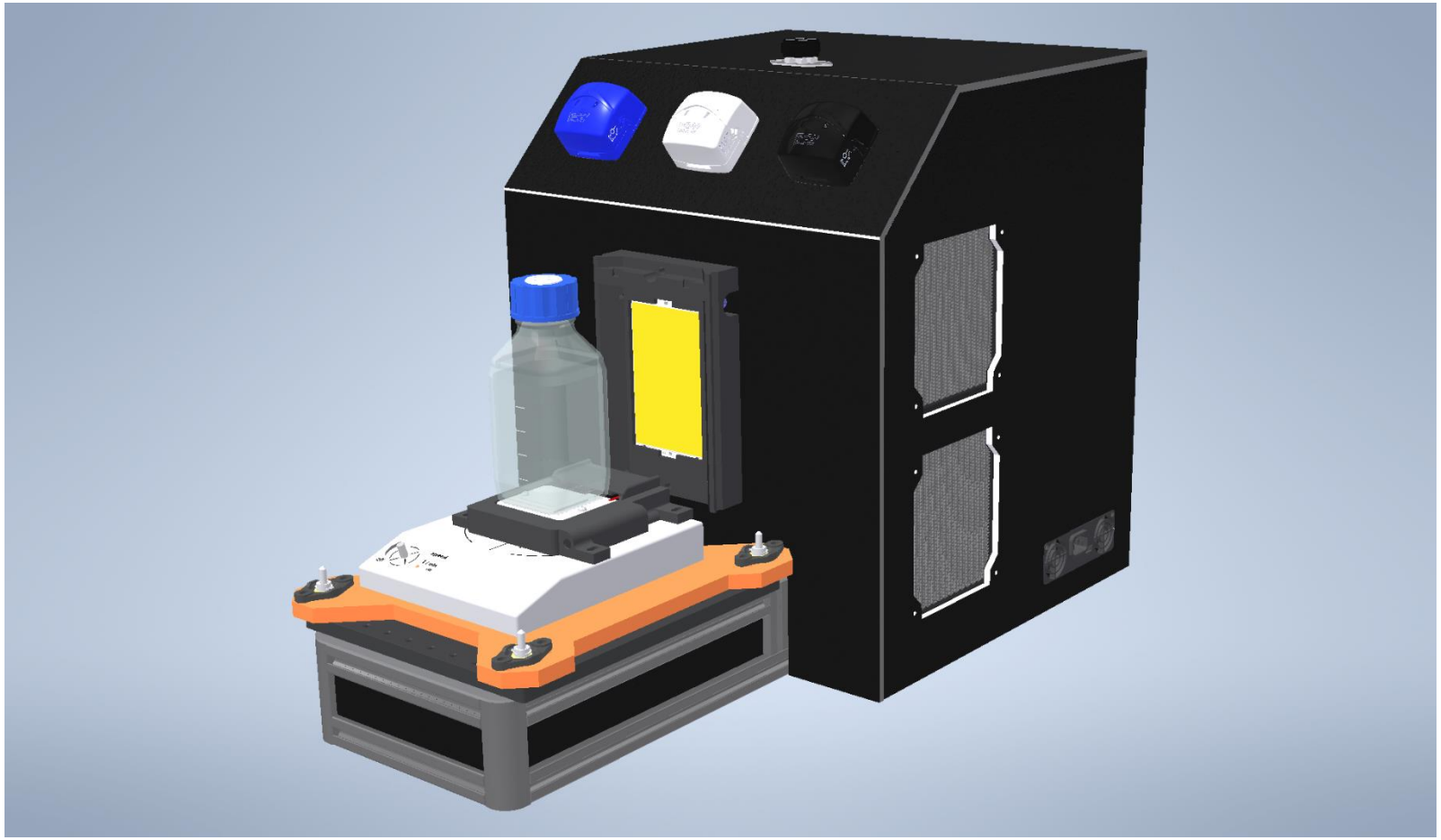
Laboratory Photobioreactors

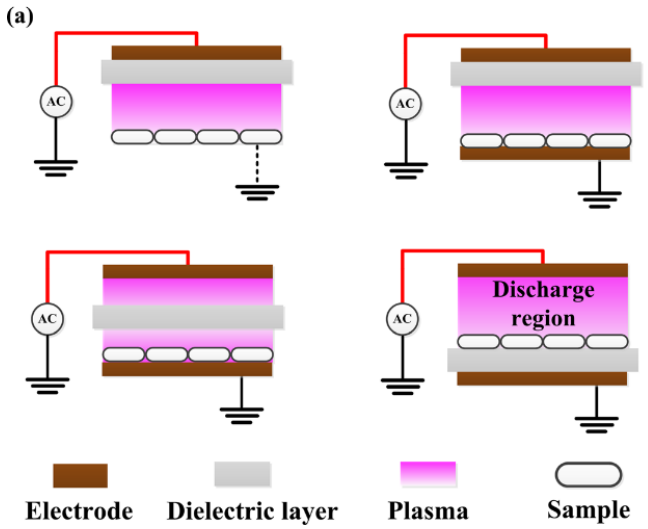


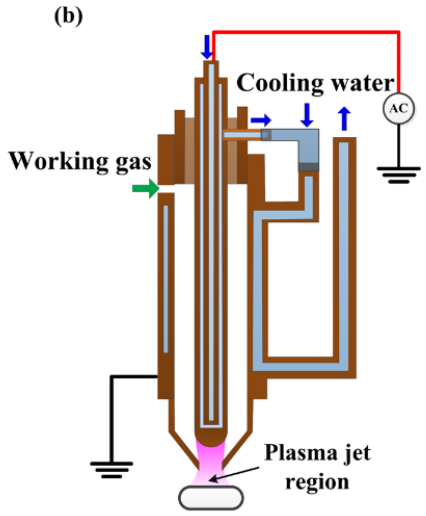
- Custom built environmental photobioreactors
- All software and electronics custom built
- Maintained and improved as undergraduate capstone projects
- Single station units under construction
- Fully automated media delivery, sample harvest, and collection
- Gas mixing/flow options
- CO_2 on demand and OD

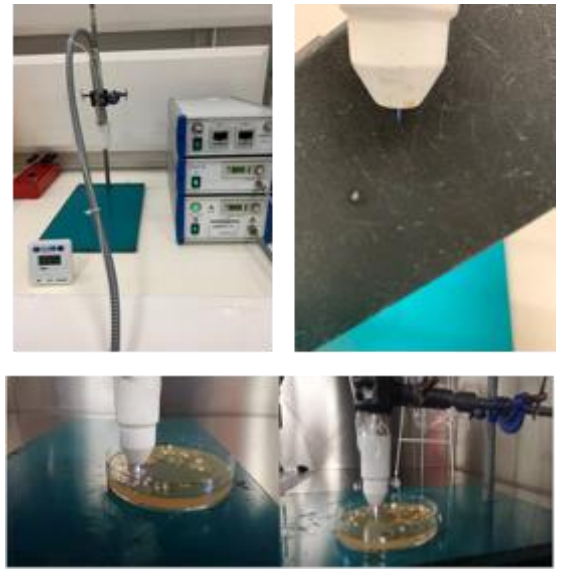
4 – Progress and Outcomes
Environmental Rooms

- Two 80-ft^2 walk-in Conviron BDW80 units are available at Biological Science Facility at PNNL (located next to the lab housing the photobioreactors)
- Enables simulation of different climate conditions: programmable temperature (+ 10°C to +40°C), photon fluxes (up to full sun light), humidity, and CO_2 concentrations with 90-min steps to simulate diurnal cycles.

4 – Progress and Outcomes
Plasma Mutagenesis and Directed Evolution





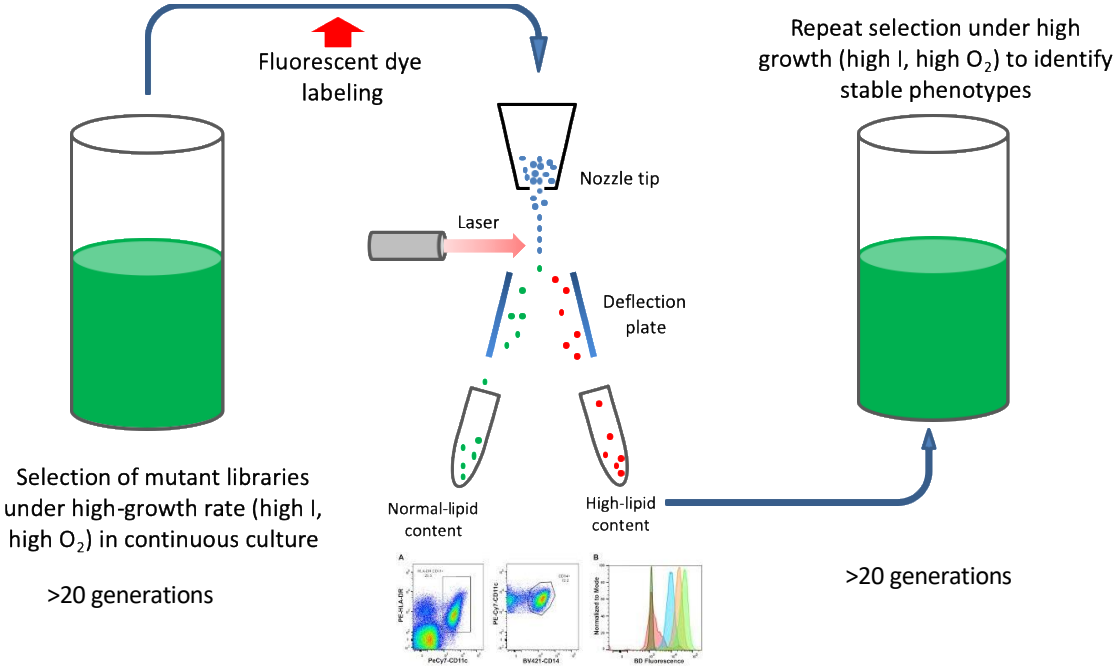


Plasma pen

Multi-pin plasma plate

- **Atmospheric and Room Temperature Plasma (ARTP) mutagenesis uses the radio-frequency glow discharge plasma jets to generate mutations**
- **Superior to traditional mutagens because of low and controllable gas temperatures, abundant chemically reactive species (UV radiation, charged particles, neutral reactive species, electromagnetic frequency, heat), rapid mutation, high operation flexibility**
- **Theoretical expertise in cold plasma as well as experience with biological applications at QUT**
- **Three ARTP machines available: with indirect action pattern (plasma pen), with direct action (multipin plate), and bubble pen**

4 – Progress and Outcomes
Strain Selection and FACS capabilities



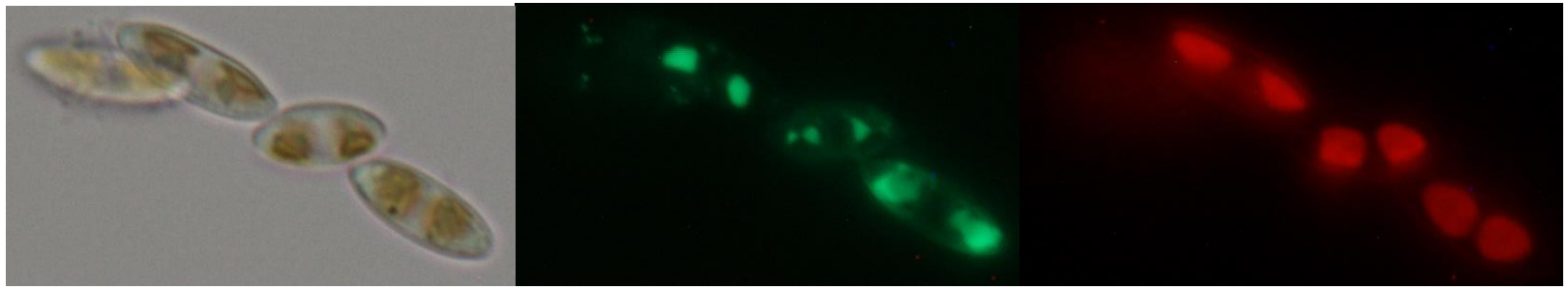
- The PNNL team has access to three Fluorescence Activated Cell Sorters: SH800S (Sony Biotechnology), BD Influx and BD Aria II (BD Biosciences)
- Experience with a broad range of biological systems
- Cell sorting capabilities are located next to the cultivation lab enabling rapid screening and instant transfer of samples from and to photobioreactors

4 – Progress and Outcomes

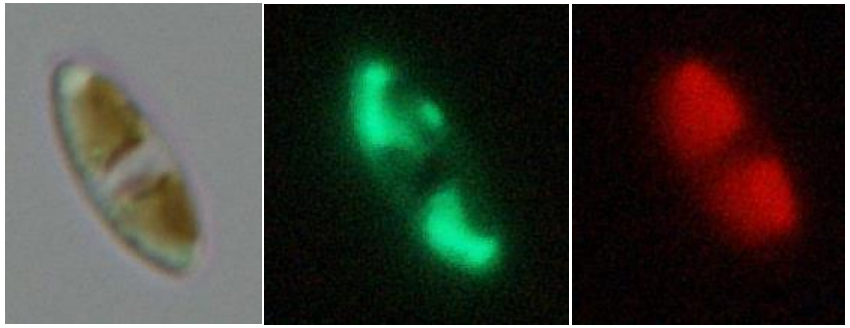
Lipid Accumulation Culturing

Optimization of diel cycle growth parameters for high productivity and high lipid in GAI 229

Day 0
Replete medium

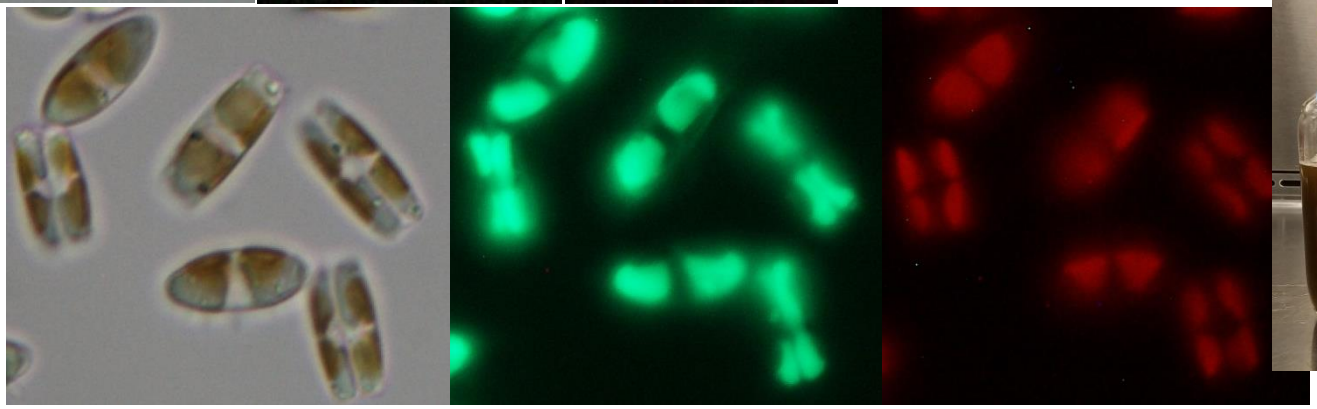


Day 1



Green = Bodipy lipid fluorescence
Red = Chlorophyll fluorescence

Day 3



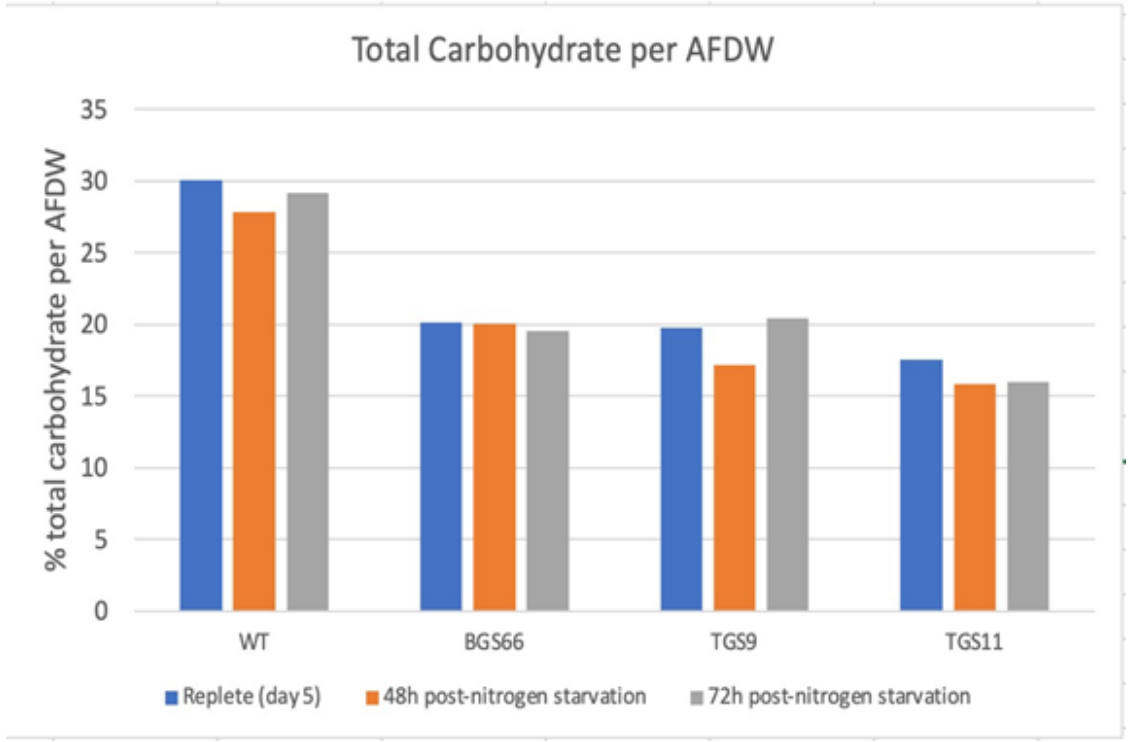
4 – Progress and Outcomes
Bioprospecting

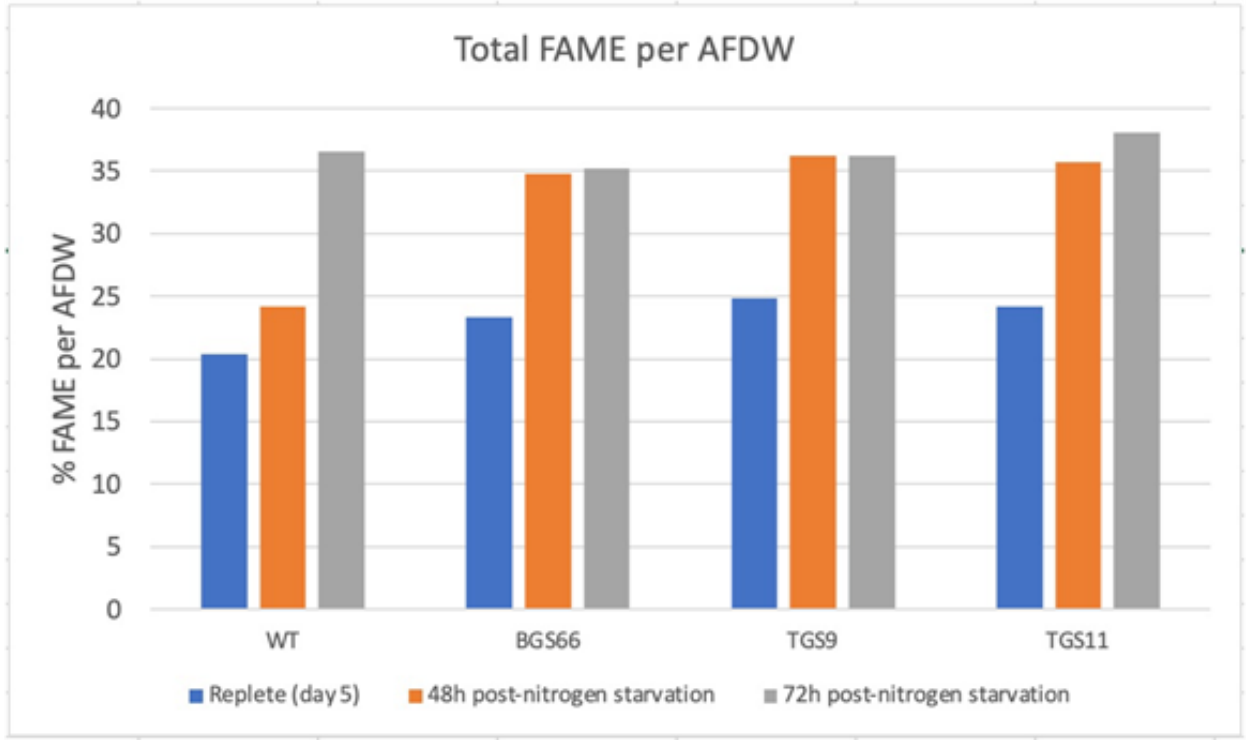



- GAI personnel travelled to the Eastern Sierras to sample alkaline regions.
- Primary goal was to obtain strains of *Nitzschia* that may be possible mating types to GAI-229 or new strains of *Nitzschia* capable of mating and good production candidates.
- Additonal effort to isolate other algae species that may be of interest.
- 15 sites in California and Nevada were sampled from 11/5 – 11/8/2020 with a total of 33 distinct samples collected. Sites varying in pHs from 7.39 – 9.94 and conductivities from 0.1 – 49.3 mS/cm and temperatures from 7.5 – 37.5C.
- All samples were plated onto high pH GAI media with or without Si and thus far have resulted in significant algae growth. Isolation efforts are underway, but already there are a few good diatom contenders.

4 – Progress and Outcomes

Characterize Carbohydrate mutants in Nannochloropsis gaditana generated by Cas9 prior to award





Soluble carbohydrates substantially reduced and lipids increase in the chrysolaminarin mutants of Nannochloropsis. However, biomass yields are also reduced.

Summary

- **Overview**

Strong outdoor production strains are in hand. Efforts to improve lipid yields using mutagenesis and selection and screening are underway. Bioprospecting is likely to provide promising new strains. High risk/reward breeding approaches are being pursued.

- **Approach**

State-of-the-art ARTP mutagenesis techniques are being applied. FACS cell sorting, custom bioreactors and out growth facilities are being used to identify high-lipid production strains. Ability to test from bench to farm is available.

- **Technical Accomplishments/Progress/Results**

Bioprospecting efforts are underway and new strains are in hand. High-lipid mutants of Nannochloropsis are being characterized. Nitzschia and Nannochloropsis strains have been transferred to QUT and ARTP mutagenesis efforts are being initiated.

- **Relevance**

Improved photoautotrophic lipid yields will improve biofuel potential of algae.

- **Future work**

Mutant library screening, expanded bioprospecting, initial breeding efforts, and testing from lab to field yields will be prioritized in the coming year.

Project in Initial Stages

Project 4 months into period of performance →

Task; Go/No-Go	Project Quarter (Q)
Task 1: Project Validation	
Go/No-Go #1: Successfully pass validation	
Task 2: GAI-229 mutagenesis and screening	
Task 3: *Nannochloropsis gaditana* high-lipid mutants	
Task 4: Bioprospecting for high biomass, lipid strains	
Task 5: Scaling from ePBR to farm	
Go/No-Go #1: Successfully attain 23 g/m^2/d biomass and 31% lipid in ePBR	
Go/No-Go #1: Successfully attain 23 g/m^2/d biomass and 31% lipid in greenhouse or raceway	
Task 6: Transcriptome/genome from high biomass/lipid strains	
Task 7: LCA/TEA	

Quad Chart Overview

Timeline
- Project start date: October 1, 2020
- Project end date: September 30, 2024
- Percent complete: 2%

	FY20 Costed	Total Award
DOE Funding	$0	$3,936,302
Project Cost Share	$0	$984,076 (20%)

Project Partners*
- PNNL
- Global Algae Innovations
- Queensland University of Technology

Project Goal

The goal of this project is to increase lipid levels in highly promising production strains and retain/improve overall biomass yields.

End of Project Milestone

Attain >23 g/m^2/day algal biomass with >31% lipid at the GAI facility in Kauai.

Funding Mechanism

DE-FOA-0002029 (2019)