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Outdoor annual algae productivity improvements at the pre-pilot scale through crop rotation and pond operational management strategies

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ABSTRACT

The Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR) collaborative consortium operated pre-pilot scale outdoor ponds to deliver much-needed multi-year, long-term and consistent, algae cultivation data relevant to understanding the current state of technology in terms of expected seasonal algae biomass productivity. Over the course of four years from 2018 to 2021, twelve identical 4.2 m^2 mini-ponds were run in triplicate sets to test strains and operational strategies demonstrated in small-, indoor photobioreactors, in pursuit of increasing overall algae areal productivity and projected farm yield. Fourteen different cultivars derived from a strain screening pipeline were tested. Through deliberate seasonal crop rotation and improvements in operational strategies, annual biomass productivity increased from 11.6 to 17.6 g m^{-2} day^{-1}, a > 50 % increase over the 2018 baseline. Both brackish and marine strains were included and four out of the fourteen strains consistently yielded high productivity across multiple years; brackish strains *Monoraphidium minutum* (26BAM) and *Scenedesmus obliquus* (UTEX393), and marine strains *Tetraselmis striata* (LANL1001) and *Picochlorum celeri* (TG2). These freely available datasets, which represent nearly complete annual daily coverage of cultivation metrics including weather, pond temperature and pH, nutrients, and productivity, are unique in the public domain and seek to fill agronomic and operational knowledge gaps to help in the eventual commercialization of algal biofuels and bioproducts.

1. Introduction

Algae-based biofuels have the potential to substantially contribute to renewable fuel needs however further research and development are needed to improve algal productivity and outdoor pond performance, reduce risk and uncertainty in large-scale deployment, and inform the data gap between assumed and actual long-term agronomic values [1–4]. There is a dearth of published research on long-term (spanning several years), multi-season, outdoor algal cultivation in shallow (10–35 cm depth), paddlewheel driven ponds also known as high-rate algal ponds (HRAP). Dedicated research into algae-based biofuels began decades ago with the U.S. Department of Energy's Aquatic Species Program (ASP). The closeout report [5] presents results of several large-scale open ponds studies summarized here; Outdoor, parallel ponds in California and Hawaii and ponds of up to 1000 m^2 surface area in Roswell, New Mexico, were operated non-continuously for 6 years. Experiments at the University of California's Richmond Field Station, generated productivities of 8.5 to 15 g m^{-2} day^{-1} for a week at a time when growing filamentous *Oscillatoria* strains. These cultures would however be replaced frequently by native *Micractinium* and *Scenedesmus* strains. For these studies, 1/3 of the pond volume was typically harvested daily with a 40 % recycle of the harvested biomass. Productivity of *S. quadricuada* grown in sequential-batch mode was harvested every few days and averaged 15 g m^{-2} day^{-1} over an 8-month period from March to October with continuously diluted (during the day) cultures increasing by 20 %. At Roswell, productivities of 3.5 to 30 g m^{-2} day^{-1} were observed with various species in ponds having 3 m^2 in surface area from winter to summer respectively. While the productivities were very low during the winter, it is interesting to note that even though the ponds froze over occasionally, there was still some biomass produced. Productivities were typically lower in the large ponds (1000 m^2) fluctuating from 3 to 18 g m^{-2} day^{-1} from winter to summer, respectively. Single-day productivities of 50 g m^{-2} day^{-1} were observed but long-term productivity was approximately 10 g m^{-2} day^{-1}. In other early studies, *Cyclotella gracilis* reached a maximum of 40 g m^{-2} day^{-1} for the period of June, July, and August in 0.35 m^2 microponds and *Isochyris*

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galbana reached a productivity of 23.6 g m^{-2} day^{-1} after dilutions were started for a period of 2 weeks in a 100 m^2 pond.

More recently, productivities for two algal strains in a hybrid PBR (25 m^3 volume)/open pond (400 m^2 surface area) configuration in Kona, Hawaii were reported [6]. Harvest-based ash-free dry weight (AFDW) productivities of 15.1 and 12.7 g m^{-2} day^{-1} were reported for *Staurosira* sp. and 11.9 and 12.8 g m^{-2} day^{-1} were reported for *Desmodesmus* sp. C046 in a 2-day or 3-day batch harvest cycles respectively in a low nitrogen fertilization case. In a high nitrogen fertilization case, AFDW productivities of 23.9 and 19.7 g m^{-2} day^{-1} were reported for *Staurosira* sp. and 22.6 and 18.6 g m^{-2} day^{-1} were reported for *Desmodesmus* sp. C046 in a 2-day or 3-day batch harvest cycle respectively. These experiments were performed over a 4-month period starting in April and ending in July. The open ponds were 400 m^2 of lighted surface area and operated at a depth of 15 cm though it was noted that there was no change in areal productivity with a depth increase to 30 cm. Sapphire Energy at the Columbus Algal Biomass farm, reported in-situ productivities of approximately 10, 14, and 8 g m^{-2} day^{-1} and 13, 20, and 9 g m^{-2} day^{-1} for 2012 and 2014 respectively during the primary growing season starting in April, peaking in July, and ending in October [7]. A 50 % year over year increase was noted in harvest yield productivity culminating in the 2014 growing season however detailed harvest metrics were not given. Open algae culturing ponds were either 1.1 or 2.2 acres in surface area and operated as semi-continuous where up to 40 % volume was removed and replaced daily with water recycled from the harvesting process. A wastewater treatment demonstration was conducted in 5-ha (14,000 m^2 including central berm), 35 cm deep, open ponds over 15 months. Continuous daily harvesting reached 4.4 to 11.5 g m^{-2} day^{-1} seasonally of colonial algal species that naturally developed and were dominated by *Micractinium* and *Desmodesmus* sp. [8]. Over one year of continuous cultivation in 3.5 m^2 raceway ponds fed by reclaimed municipal wastewater with a novel strain *Tribonema minus*, cultures were shown to be more productive than a native algal polyculture, and achieved an annual average productivity of 15.9 ± 0.3 versus 13.4 g m^{-2} day^{-1} for the polyculture [9]. During a 16-month outdoor open raceway pond study of *Nannochloropsis oculata* in 2.5 m^3 (12.5 m^2) ponds of 20 cm depth operated using batch harvests at early stages of culturing followed by semi-continuous mode to maintain a constant biomass concentration, productivities of 0.72 to 3.61 g m^{-2} day^{-1} were measured in winter and summer respectively with an overall maximum of 14.0 g m^{-2} day^{-1} [10].

Between 2012 and 2017, the U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO) has funded several large collaborative efforts having as a primary goal, long-term outdoor algae cultivation. One objective of the National Alliance for Advanced Biofuels and Bioproducts (NAABB) was to screen algae strains and develop low-cost culture media with validation in outdoor ponds [11]. NAABB investigated 9 strains outdoors at mid- and large- scale (800–23,000 L) generating productivities of 11 to 25.2 g m^{-2} day^{-1}. Similarly, the Regional Algal Feedstock Testbed (RAFT) project was tasked with identifying new algae strains for outdoor pond cultivation and demonstrate high seasonal and annual biomass productivities via crop rotation. RAFT achieved 0.9 to 35.2 g m^{-2} day^{-1} seasonal productivities across 7 strains in 600–1000 L raceway ponds with production runs of 66–106 days [12]. The objective of a third consortium, the Algae Testbed Public-Private Partnership (ATP^3) was to generate a robust dataset of algal growth metrics in outdoor open ponds with a focus on the comparison of harvested biomass productivity in identical ponds under different seasonal, climatic, and operational conditions at a small-scale (1025 L, 4.2 m^2) by controlling the non-geographical variables of inoculum seed growth, biomass production systems, processes and protocols, system scale, and algae strain. The Unified Field Studies (UFS) were set up as the baseline upon which later experiments would build upon and as such, no attempts at optimization of growth or lipid accumulation were made. Rather, the primary focus of the UFS were to cultivate algal biomass under consistent conditions and harvesting operations to provide data on year-round outdoor algal biomass production that could be directly compared between one site and another and thus represent a conservative baseline of non-optimized algal growth that could be expected at these sites [13–15]. Throughout the course of 1.75 years (October 2013 – July 2015) of monthly cultivation across three algae strains, productivities of 1.6 to 14 g m^{-2} day^{-1} were observed during winter to summer respectively [13].

Despite the above described results, with a few exceptions, achieving consistent and high biomass productivity remains a limiting factor in the commercial success of algae cultivation for the purpose of biofuels and bioproducts. Publicly available, long-term, outdoor algae cultivation data at a suitable scale is needed to inform techno-economic analysis (TEA), life cycle (LCA), and resource assessments (RA), predictive growth modeling, and crop protection strategies to guide and de-risk the development of algae agronomic practices. Identifying suitable algae species for a given application, understanding their optimal growth conditions and dependence on weather (e. g., temperature and solar insolation) and water chemistry (e. g., salinity, pH), susceptibility to biotic failure-inducing organisms, and response to operational strategies (e. g., culture depth, harvest frequency) are all critical to successful outdoor cultivation. Recently, computational process simulations and modeling efforts (e.g. TEA, LCA, and RA) to predict metrics (e.g., biomass productivity, evaporative losses, pond temperatures, biofuel productivity, and baseline cost) have been important to better understanding the risks and opportunities associated with outdoor open pond algae cultivation have been reported [16–19]. Two recent studies developed and validated algae growth models using geospatial data (e.g. local weather, evaporation rates), reactor geometry (open pond, PBR) inputs, and strain specific parameters (e. g., temperature and light intensity tolerance, nightly respiration rate) to predict metrics such as biomass and biofuel productivity. Using publicly available datasets from the ATP^3, such models showed a 0.9 ± 2.35 % and − 4.59 ± 8.13 % relative accuracy in respect to productivity thus validating this approach [18,19]. Though the data for these modeling efforts is largely provided by mid-scale ponds, through the use of both mid-scale (800 L) and large scale (23,000*L*) ponds, the NAABB concluded that results generated at mid-scale translated to large scale thus validating that mid-scale production systems can act as appropriate research tools to predict expected results at larger scales [11].

The U.S. DOE-BETO uses an annual State of Technology (SOT) analysis to quantify improvements in productivity and help project future trends in algae cultivation [20–22]. From 2014 to 2017 data for the SOT was generated from seasonal cultivation data by ATP^3 with the initial SOT baseline established in 2015 using *Nannochloropsis oceanica* KA32 achieving an annual average productivity of 8.5 g m^{-2} day^{-1} and increasing to 10.3 g m^{-2} day^{-1} primarily through the addition of new cultivars and a crop rotation strategy for both cool and warm seasons. Here we describe the continuation of year-over-year outdoor algae cultivation trials with the goal of improving algae productivity first started under the guidance of the ATP^3 and now as part of the Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR) consortium ([23] this issue). These year over year data from January 2018 through December 2021 were obtained under mainly semi-continuous cultivation conditions in 4.2 m^2 open raceway ponds operated simultaneously year-round under various operational conditions. This continuing data pipeline for the an annual SOT analysis reports, is used as a measure of progress relative to an established target. t The same data also supports year over year algae agronomics useful to the broader algae research community. We explored the primary drivers of phototrophic biomass production, light and temperature, and assessed seasonal effects on productivity for top-performing strains identified in DISCOVR's three-tiered strain screening pipeline ([24–27] this issue). A strain rotation strategy to maximize annual productivity through cultivation of the best seasonal strains was utilized, though we have also identified cultivars that perform well year-round.

2. Materials and methods

2.1. Standard cultivation operations and algae biomass compositional analysis

The establishment of the Arizona Center for Algae Technology and Innovation (AzCATI) testbed and standard operational framework for conducting SOT cultivation trials under ATP^3 was previously described [13–15,28] and this same framework was utilized for DISCOVR. Briefly, outdoor fiberglass ponds (Commercial Algae Professionals, http://www.commercialalgae.com) of 820 L nominal volume at a depth of 20 cm and a total surface area of 4.2 m^2 are operated in triplicate for each experimental condition (e.g., strain, media, dilution rate, etc.). Pond mixing is with a stainless-steel paddlewheel driven by a 1/3 hp. motor (Leeson model # 191201) and gearbox (IPTS model IBLCS050, 80:1 gear ratio) controlled by a variable-frequency drive (KB Genesis, Model KBDA-24D) operated at 20 Hz. Ponds were inoculated after seed scale-up in indoor flat panel reactors and operated as described previously [15]. The primary mode of operation for the majority of the cultivation trials was to operate the ponds in a semi-continuous fashion, with experiments beginning with an inoculation target density ≥ 0.05 g AFDW L^{-1} and subsequent grow out to 0.3–0.5 g AFDW L^{-1} to trigger harvesting operations. Ponds were then harvested one to three times a week depending on the season. Higher productivity led to more frequent harvests with corresponding higher dilution rates in the summer while the opposite was true in the winter. On harvest days, ponds are sampled to determine biomass density (g AFDW L^{-1}), the target percent of pond volume was removed, ponds were re-filled with fresh media, allowed to mix, and sampled again to quantify the change in various metrics due to the harvest (e. g., decrease in OD_{750} and AFDW, added macronutrients of nitrogen and phosphorous). An estimated daily dilution rate was calculated as the percentage of harvested pond volume multiplied by the number of harvests in a week divided by 7 days. The majority of pond operations followed this standard semicontinuous protocol of 1×–3× weekly morning harvests under nutrient replete conditions, but other operational modes were explored. Changes in operational mode including pond depth, differing harvest/dilution rates, different pH set points, and for select strains, different crop protection strategies, were compared. While some limited work on nutrient source impact (e.g., ammonia versus nitrate) and overall media formulation were conducted, primarily involving salinity levels for marine strains, media optimization and media recycling were not the focus for the SOT cultivation trials to date.

Routine daily samples were taken for optical density, AFDW, nutrients (N and P), pH, salinity, and microscopy. Water quality monitoring and pH control was through the use of a YSI 5200A-DC (YSI Inc., Yellow Springs, OH, USA) water quality monitoring system simultaneously measuring pH, pond water temperature (°C), dissolved oxygen saturation (%), and salinity (g L^{-1}) recorded at 15-minute intervals. The pH was maintained by on-demand sparging with CO_2 through a ceramic micro-bubbler diffuser (Sweetwater® Model# DYPFP4, www.pentairaes.com) triggered by the YSI pH probe. The CO_2 supply was turned off at night. Weather data was collected on site using a HOBO RX3000 Weather Station (Onset Computer Corporation, MA), including sensors for air temperature (°C), relative humidity (%), rainfall (mm), photosynthetically active radiation (PAR), solar radiation (W m^{-2}), wind speed (m s^{-1}) and direction (degrees), and was collected at 5-minute intervals. In addition, samples were collected and preserved for pond metagenomic analysis and identification of algal pests, as well as for the establishment of indoor failure assays for crop protection research as new pests arise ([29,30] this issue). Finally, samples were also collected at harvest points for proximate analysis of biomass composition. For biochemical biomass sampling, approximately 1 L of culture volume was collected and centrifuged at 4200 rpm for 10 min to collect pelleted biomass, which was preserved by freezing at −20 °C prior to bulk lyophilization. Proximate composition of the harvested algae biomass was performed following rigorous methods and sample quality control protocols as previously described [14,31–34].

2.2. Strain selection, sourcing, and cultivation media

The primary source of strains for the SOT cultivation trials performed at AzCATI since the summer season of 2018 were from the DISCOVR consortium's strain screening and down-selection pipeline which identified candidate cultivars for outdoor evaluation ([25–27] this issue). The primary strains run between 2018 and 2021 under DISCOVR as part of the annual fiscal year (FY) SOT trials are shown in Table 1. The strains that performed best within a given month/season were included in formal calculations of the seasonal and annual average productivity and are highlighted. The media used for a given cultivar is indicated in Table 1. The source of each strain is listed and all strains cultivated since 2018 are publicly available or available under material transfer agreements. The majority of outdoor cultivation was performed using either a modified BG-11 media adjusted to 5 ppt salinity for brackish strains or a modified f/2 media adjusted to 35–50 ppt salinity for marine strains. All media used for the SOT were either brackish (5 ppt) or full marine salinity (35 ppt) up to 1.5× full marine salinity (50 ppt salinity). No freshwater cultivation was performed for the SOT trials. The modified f/2 media is as previously reported [37]. The modified BG-11 media was composed of: 5 mM NH_4HCO_3, 0.31 mM K_2HPO_4, 46.3 μM H_3BO_3, 0.77 μM $ZnSO_4{\cdot}7H_2O$, 9.15 μM $MnCl_2{\cdot}4H_2O$, 0.17 μM $Co(NO_3)_2{\cdot}6H_2O$, 1.78 μM $NaMoO_4{\cdot}2H_2O$, 0.316 μM $CuSO_4{\cdot}5H_2O$, 0.304 mM $MgSO_4{\cdot}7H_2O$, 0.245 mM $CaCl_2 \cdot 2H_2O$, 22.8 μM $C_6H_9FeNO_7$, 0.189 mM Na_2CO_3, 31.2 μM $C_6H_8O_7$, 3.42 μM $C_{10}H_{16}N_2O_8$. To adjust salinity in the brackish media, 5.2 g of Instant Ocean Sea Salt (www.instantocean.com) was added to 1 L of media. Additional media formulations utilized included brackish and marine versions of DISCOVR media ([26] this issue) as well as a modified artificial seawater media (MASM) [34]. Water source for outdoor cultivation was municipal, potable water (City of Mesa, AZ) which was used through May 2019. A reverse osmosis (RO) system with in-line ultraviolet (UV) sterilization was installed and has been in use since June 2019.

2.3. Data analysis and calculations

Data were quality checked and compiled as described previously [13]. Briefly, primary cultivation and composition data were collected in Excel spreadsheets which were then compiled based on fiscal year into one comprehensive file using R scripts [36]. The files contained either cultivation and composition data, water chemistry data, or weather data. All primary datasets are freely available (https://apps.openei.org/DISCOVR/) and the relevant summarized datasets used in this manuscript are provided as Supplemental File 1. These compiled yearly data files were then used for analysis. Areal harvest yield productivities (AHYP) in g algal biomass produced m^{-2} pond surface area day^{-1} were calculated based the amount of harvested algal biomass that was removed from the ponds as determined by the volume harvested and the AFDW at the time of harvest thus representing the actual amount of biomass that would be available for downstream processing. In order to determine the seasonal and annual averages, we first calculated AHYP on a month-by-month basis for each strain/condition by calculating the sum total biomass harvested during the month, divided by the surface area of the ponds and total days within that month over which the biomass was harvested (harvested biomass (g)/4.2 m^2 * days). The following conventions were used for the seasons: Fall = September, October, November; Winter = December, January, February; Spring = March, April, May; and Summer = June, July, August. The seasonal average for a given strain/condition reporting are the average of the three months within that season. The annual average is calculated based on the average of the four seasons within a fiscal year. For any given month or seasonal value used to represent the best performing data for that month or season, there is no overlap between strains or conditions

Table 1

Species and strain (Strain ID) grown during the FY SOT trails at AzCATI from 2018 to 2021. Strains in bold font contributed at least one month of productivity in a given season and FY year to the formal SOT. The particular season and year a strain was included as part of the SOT calculation is highlighted in green.

Species	Strain ID*	Strain Source	Target Salinity (ppt)	Season	2018	2019	2020	2021
Nannochloropsis oceanica	**KA32[a]**	Cellana, LLC	35	Fall	X			
***Desmodesmus* sp.**	**C046[a]**	Cellana, LLC	35	Summer	X			
				Fall		X		
Desmodsesmus armatus	**SE00107[b]**	Sapphire Energy	18	Summer	X			
Scenedesmus acutus	**LRB0401[c]**	ASU	5	Winter	X			
Monoraphidium minutum	**26BAM[c]**	NAABB	5	Winter	X	X	X	x
				Spring	X	X	X	X
				Fall	X	X	X	X
Scenedesmus obliquus	**UTEX393[c]**	UTEX	5	Winter		x	x	x
				Spring		X	X	x
				Summer		X	x	x
				Fall	x	X	x	
Picochlorum soloecismus	DOE101[a,d]	LANL	35-50	Summer	x		x	x
				Fall			x	x
Scenedesmus rubescens	46DB3[d]	NREL	5	Summer	x			
Picochlorum renovo	39A8[d]	NREL	35 - >50	Summer		x		
Picochlorum celeri	**TG2[a,d]**	CSM	35 - >50	Spring				x
				Summer		x	X	X
				Fall			X	X
Tetraselmis striata	**LANL1001[a,d]**	LANL	35-50	Winter			x	x
				Spring			x	X
				Summer			x	
				Fall			X	x
Micractinium reisseri	14F2[d]	NREL	15-35	Fall			x	
				Winter			x	
Porphyridium cruentum	CCMP675[a]	NCMA	35	Summer				x
Chlamydomonas sp.	PATC1[d]	PNNL	5	Winter				x
				Spring				x
				Summer				
				Fall				x
Phaeodactylum tricornutum	UTEX646[d]	UTEX	35	Winter			x	x
				Spring				x

Notes: *Cultivation media indicated with superscript: a) AzCATI modified f/2, b) modified artificial seawater media, c) AzCATI modified BG-11, d) DISCOVR medium. See Materials and Methods for specific formulations. The strains *N. oceanica* KA32, *Desmodesmus* sp. C046, *M. minutum* 26BAM (2018 winter/spring), and *S. acutus* LRB0401 (2018 winter/spring) were run under the Algae Testbed Public-Private Partnership (ATP^3) [15,28], *D. armatus* SE00107 (Summer 2018) was run under Rewiring Algal Carbon Energetics for Renewables (RACER) [35] and *S. obliquus* UTEX393 (Fall 2020 thru 2021) was run under Decision Model Supported Algae Cultivation Process Enhancements (DMSACPE, DOE funding award number DE-0008906).

within the month or season when calculating the total days for month or season, the overall biomass harvest yield, and thus the AHYP. We calculate this metric starting in the Fall season of the previous calendar year and running through the Summer season of the current year to allow for reporting of an annual productivity number that aligns with the federal fiscal year reporting requirements. Thus, formal SOT annual averages cross calendar years (CY). When making any seasonal/annual summary calculations it is indicated if it is a function of FY running from September of the previous year through August of the current year, or CY, from January through December of the same year.

3. Results and discussion

3.1. Outdoor pond cultivation

Outdoor algae cultivation experiments with the goal of setting a baseline for algal AHYP and developing a framework within which to improve AHYP were begun under the ATP^3 and generated productivities for *N. oceanica* KA32 during all four seasons across a single year ranging from 2 to 14 g m^{-2} day^{-1} for winter and summer respectively with an annual average of 8.5 g m^{-2} day^{-1} as the baseline AHYP for the first year of the SOT in 2015 [13–15,28]. Over the course of the next two years, this was improved to 10.3 g m^{-2} day^{-1} in 2017, an increase of 21 % through strain rotation. More productive cultivars relative to *N. oceanica* KA32 were found for both seasons, *Desmodesmus* sp. C046 in the summer and *M. minutum* 26BAM in the winter [13,20,28]. This performance baseline and experimental framework was continued as part of the DISCOVR consortium starting in the year 2018 with outdoor cultivation at the AzCATI testbed site.

Using the DISCOVR strain screening pipeline ([25–27] this issue), outdoor algae cultivation at AzCATI was used as a final testing arena for strains showing promise across multiple stages for a given selection strategy. Different species were run in a given season and focused on the optimal climate conditions for a given strain to determine the best performing strains and to develop a crop rotation strategy for maximal annual productivity. The actual feasibility or need for a crop rotation strategy in a commercial algae cultivation facility is not yet fully clear but as with terrestrial crops, which are grown in specific climates based on optimal light and temperature, it will likely be similar for many commercial scale algae farms depending on their location and seasonal climates. In our case at AzCATI, given the northern geographic location relative to the equator and thus experiencing yearly seasons, crop rotation was necessary because there was no single strain that effectively covered the range of temperatures experienced at the site across the year ([26] this issue). In contrast, a gulf coast site or sites further south closer to the equator, may not require crop rotation from a seasonal temperature tolerance aspect.

A primary goal of the SOT trials under DISCOVR is the selection of best seasonally performing strains. The main objective was to cultivate those strains under real-world, varying weather conditions, and work to improve their outdoor performance while simultaneously verifying if newly identified cultivars or operational concepts in the DISCOVR pipeline which demonstrate improved performance indoors, hold up once taken out to the field. A summary of the seasonal and overall annual average productivities for the top performing strains for the FY SOT from 2018 to 2021 are shown in Table 2. Under ATP^3, annual average AHYP were 8.5, 9.1, and 10.3 g m^{-2} day^{-1} for 2015, 2016, and 2017, respectively [22]. Under DISCOVR (2018–2021), annual AHYP was advanced from 11.6 g m^{-2} day^{-1} in 2018 to 17.6 g m^{-2} day^{-1} in 2021, ***a 52 % increase***. Since the establishment of the U. S. DOE SOT benchmark for AHYP FY cultivation metrics in 2015, annual AHYP has more than doubled, improving on the 2015 baseline of 8.5 g m^{-2} day^{-1} to 17.6 g m^{-2} day^{-1} in 2021, ***a 108 % increase***. In the first 6 years since the inception of the DOE SOT cultivation trials in FY 2015, productivity improvements of 7 %, 13 %, 14 %, 36 %, and 16 % (2016–2020) relative to each preceding year were achieved. However, in 2021 there was 4 % reduction in annual AHYP relative to 2020. The decrease in productivity in 2021 was driven primarily by a significant decrease in summer productivity relative to 2020 of 25 %. The reasons for that decrease remain unclear, though they are actively being investigated by DISCOVR and will be briefly discussed in Section 3.3.

A benchmark, technical and economical, performance target was set for demonstrating an average productivity of 25 g m^{-2} day^{-1} by 2030 with an intermediate goal of 20 g m^{-2} day^{-1} by 2025, against which the annual SOT will be compared and reported [37]. It is unlikely the substantial year over year improvements observed from 2015 to 2020, even at the smaller, pre-pilot scale being run for DISCOVR, can be sustained moving forward as evidenced by the drop in 2021, but fortunately a more modest 4–5 % improvement year over year is all that must be demonstrated to achieve both the 2025 and 2030 targets. By way of comparison, agricultural crop yield improvements for soy and corn have shown an average of 2 % increase year over year for the last 30 years (https://www.nass.usda.gov).

Fig. 1 shows the minimum and maximum water temperature (°C) for 20 cm deep ponds along with the daily light integral (DLI, mol m^{-2} day^{-1}, calculated as the number of photosynthetically active photons (PAR, photons in the 400–700 nm wavelength range) accumulated in a square meter over the course of a day) from January 1, 2018 through December 31, 2021. The Mesa, AZ AzCATI site offers a unique and effective testbed location for exploring the effects of natural light and temperature. Though there are routinely over 300 days of sunshine per year, there is significant seasonality in solar insolation and temperature. That light and temperature are the primary drivers is evident in Fig. 2 showing the monthly AHYP for CY 2018–2021. Seasonal transitions from fall to winter and again from winter to spring show the fastest changes in both light and temperature and correlates to rapid increases and decreases in productivity for spring and fall, respectively. This is reflected in the higher variability in the seasonal average AHYP for fall and spring seasons relative to winter and summer across the short three-month transitional seasons where harvest yields can almost double from March to May and decrease by a similar amount from September to November (Fig. 2, Table 3).

When the SOT was first established under the ATP^3, the trials were conducted on a seasonal basis but would often include multiple strains and or operational conditions being tested with only six replicate ponds available for a given testbed site. Thus, cultivation trials within a season were typically on the order of 30–45 days allowing for two rounds of experimentation in a season usually with different strains, and resulted in gaps in seasonal coverage for a given strain/condition [13]. To limit gaps in monthly/seasonal data which can increase the uncertainty in extrapolation of productivity estimates across a full season or year, DISCOVR set an explicit goal to maximize seasonal and thus annual coverage throughout the full calendar year, limiting strain turnovers during any given month or season. For DISCOVR, the cultivation trials were expanded to twelve ponds to allow for more conditions to be tested side-by-side (e.g., strains, operational set points, etc.) with an explicit operational target of maximizing the overall number of days of experimental uptime across the year. This resulted in three benefits for the annual SOT cultivation trials; 1) better temporal resolution in particular across the rapid change in light and temperature for spring and fall

Table 2
FY SOT seasonal and annual average AHYP for 2018 through 2021.

FY	CY	Season	No. months	AHYP (g/m^2 day)	Total days season	Annual average AHYP	Total days year	Percent annual increase
2018	2016	Fall	2[a]	9.0 ± 2.1	42			
2018	2018	Winter	2[b]	7.7 ± 1.6	46			
2018	2018	Spring	3	14.8 ± 3.1	78			
2018	2018	Summer	3	14.9 ± 1.3	56	**11.6 ± 3.8**	**222**	**14%**[c]
2019	2018	Fall	3	11.3 ± 1.6	66			
2019	2019	Winter	3	6.4 ± 0.08	94			
2019	2019	Spring	3	18.6 ± 6.0	88			
2019	2019	Summer	3	27.1 ± 2.8	87	**15.9 ± 9.0**	**335**	**37 %**
2020	2019	Fall	3	15.0 ± 3.6	87			
2020	2020	Winter	3	8.4 ± 1.2	93			
2020	2020	Spring	3	18.4 ± 4.5	93			
2020	2020	Summer	3	31.6 ± 3.9	81	**18.4 ± 9.8**	**354**	**16 %**
2021	2020	Fall	3	19.3 ± 8.3	92			
2021	2021	Winter	3	8.3 ± 1.2	90			
2021	2021	Spring	3	19.4 ± 3.7	88			
2021	2021	Summer	3	23.8 ± 0.9	90	**17.6 ± 6.6**	**360**	**−4 %**

[a] Fall data for FY 2018 was carried forward from fall CY 2016 as no cultivation work was conducted for the SOT in fall CY 2017.

[b] The Fall and Winter FY2018 seasons included data from only 2 months, October/November CY 2016 and January/February CY 2018, respectively. The remaining SOT years had cultivation from all 12 months of the year. AHYP is the average ± 1 standard deviation of the mean. Total days represents the total number of cultivation days within the season that contributed to the seasonal average.

[c] Percent increase over FY 2017 SOT under the ATP^3.

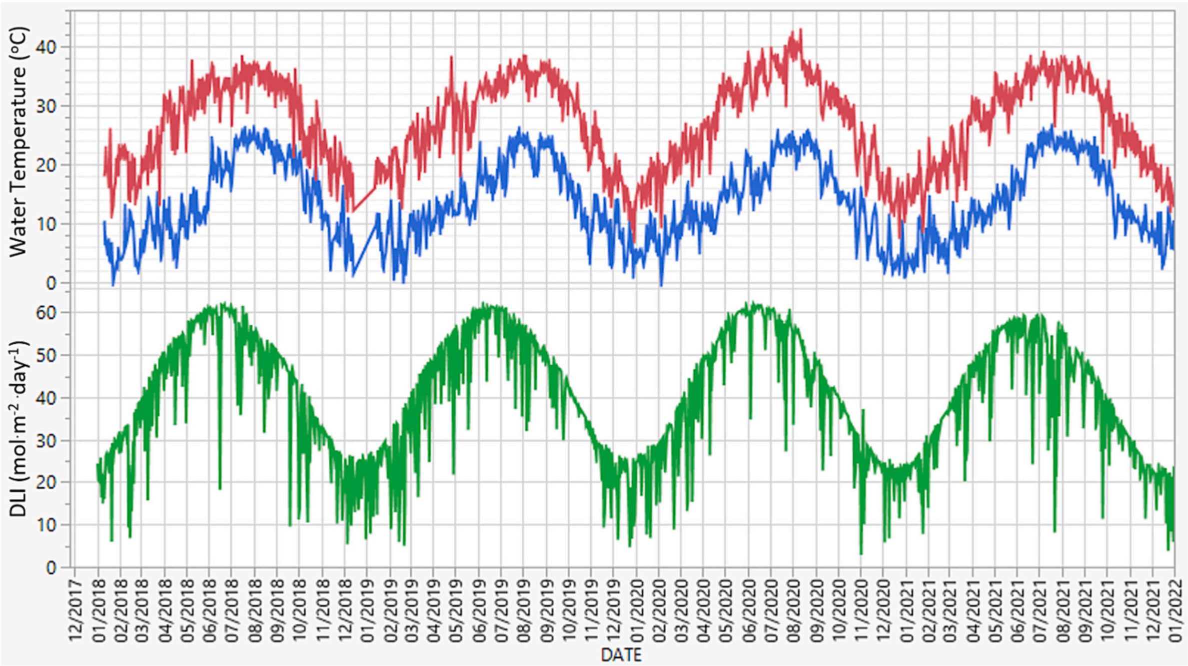


Fig. 1. Daily pond water temperatures (°C) (maximum (red), minimum (blue)) and DLI (mol m^{-2} day^{-1} (green)) from January 1, 2018, through December 31, 2021. Note that no ponds were running on site from December 18, 2018, to January 7th, 2019, producing a minor gap in the water temperature data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

seasons improving certainty for calculations of seasonal and annual average productivities, 2) the agronomic comparisons became more robust with monthly resolution as we generate year over year comparisons for the best performing strains within the SOT and, 3) the ability to extrapolate data (e. g., productivity) to other geographic locations as different parts of the year (e. g., winter and transitional seasons) may simulate closely other locations (e. g., more Northern locations) as well as allow for more robust, validated models of biomass productivity within the algae research and development community with some use of this data for such modeling already demonstrated [18,19].

3.2. Strain selection and crop rotation

Between 2018 and 2021 fourteen different strains vetted in the DISCOVR strain selection pipeline were tested outdoors at AzCATI (Table 1, Supplemental Fig. 1). DISCOVR's overall strategy for improving AHYP on an annual basis implies a crop rotation strategy and incudes cultivars shown to perform better in cool weather (e.g., *M. minutum* 26BAM, *T. striata* LANL1001, *M. reisseri* 14F2, *Chlamydomonas* sp. PATC1, *P. tricornutum* UTEX646) and those that perform better in warm weather (e.g., *S. obliquus* UTEX393, *P. celeri* TG2, *P. renovo* 39A8, *P. soloecismus* DOE101, *P. cruentum* CCMP675). The monthly and annual average AHYP for the top performing SOT strains on a CY basis (2018–2021) are shown in Fig. 2 and summarized in Table 3. Since the start of DISCOVR, assuming crop rotation and using the single best performing strain in a given month, an increase from 12 g m^{-2} day^{-1} in 2018 to 17.0 g m^{-2} day^{-1} was achieved, an improvement of 41.6 % on a CY basis. Of all the strains tested to date, four have consistently been the top performers across multiple years; brackish strains *M. minutum* 26BAM and *S. obliquus* UTEX393, and marine strains *T. striata* LANL1001 and *P. celeri* TG2 (Fig. 3).

The top performing cool weather strain to date has been *M. minutum* 26BAM and while we have observed better performing cool weather strains indoors ([26] this issue), we have not as of yet found another strain that once taken outside to AzCATI has outperformed *M. minutum* 26BAM. *S. obliquus* UTEX393 has proven to be a versatile, all-season, brackish strain which has been tested year-round for three years from 2019 to 2021 showing its best productivity in CY 2019 (annual AHYP of 15.5 g m^{-2} day^{-1}). However, cool season performance for *S. obliquus* UTEX393 considerably lags behind that of *M. minutum* 26BAM, with a three-year average productivity of 5.8 and 15.9 g m^{-2} day^{-1} for winter and spring seasons, respectively, versus 7.8 and 17.4 g m^{-2} day^{-1} for *M. minutum* 26BAM. Comparing two-year averages for 2020 and 2021, *T. striata* LANL1001 had an average AHYP of 7.9 and 15.6 g m^{-2} day^{-1} for winter and spring seasons, respectively, versus 8.2 and 18 g m^{-2} day^{-1} for *M. minutum* 26BAM. From 2018 to 2021 we improved on the baseline productivity of *M. minutum* 26BAM year over year in winter, spring, and fall seasons of 7.5 %, 30.9 %, and 10.2 %, respectively relative to 2018.

T. striata LANL1001 (marine, 35 ppt), close in outdoor performance to *M. minutum* 26BAM, was selected as the second-best cool weather strain and the best cool weather marine strain tested, outperforming two other cool weather marine strains *M. reisseri* 14F2 and *P. tricornutum* UTEX646, all showing comparable performance to *M. minutum* 26BAM in the DISCOVR indoor testing pipeline ([26,27] this issue). While no cool weather strains matched or exceeded the productivities for *M. minutum* 26BAM outdoors, *T. striata* LANL1001 demonstrated superior robustness with no culture crashes in 2020 or 2021, and no need for any active crop protection measures. We have not yet optimized nor expended a similar amount of effort on *T. striata* LANL1001 as with *M. minutum* 26BAM, but expect to be able to improve productivity through additional cultivation optimization in the future, in particular as it relates to dilution rate (see Section 3.4).

Broad, seasonal gains in productivity have been achieved from 2018 to 2021, with the largest gains occurring in the warmer seasons. With the introduction of *P. celeri* TG2, first cultivated in August and September of 2019, we obtained the highest productivities at AzCATI for summer and early fall in 2020 with a four-month average (June – September) of 31 g m^{-2} day^{-1}, and a single month high of 36 g m^{-2} day^{-1} for August. As reported, *P. celeri* TG2 has significant potential for biofuel production, with high productivity, and demonstrated genetic engineering toolkits available [38,39]. It is now the current benchmark warm weather marine strain for the DISCOVR SOT exhibiting high temperature and salinity tolerance (majority of data in 2020 and 2021

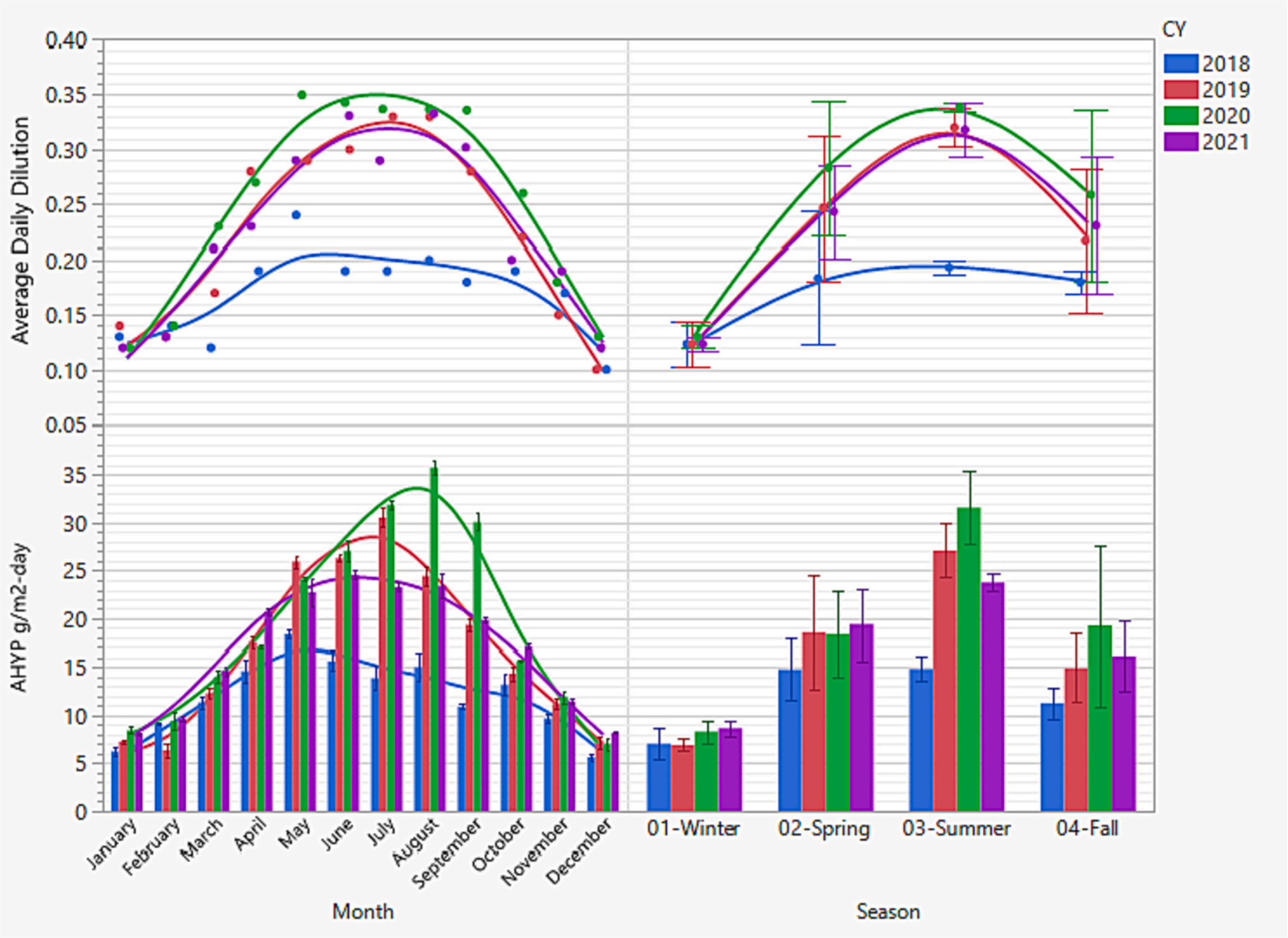


Fig. 2. Monthly and seasonal AHYP using best performing strains for annual SOT by calendar year (January 2018 through December 2021), solid line through discrete points represents a spline fit to the data. Estimated monthly and average seasonal daily dilution rates (top graphs, left and right respectively) and monthly and seasonal AHYP (bottom graphs, left and right respectively). For the monthly AHYP values, each bar graph represents the single best performing strain for the month/year (n = 3 ponds). For seasonal summary, each bar graph represents the average values for three months within a season (n = 9). Error bars are ±1 standard deviation from the mean.

was at 50 ppt salinity). It also was quite robust with no pond crashes in 2019–2021, including running over 143 days from mid-June thru the end of October 2020 without a pond crash or need to re-inoculate ponds due to a drop in productivity. However, one issue observed with *P. celeri* TG2 was contamination by diatoms, which was common in all three years, in particular for late summer and early fall of 2021 leading to several restarts once the diatom population exceed roughly 10 % (by cell count). The presence of diatoms can lead to an increase in auto-flocculation and buildup of biomass at the water's edge and on the paddlewheels and cause increased settling of biomass. The effect of diatom contamination on primary productivity for *P. celeri* TG2 is as of yet undetermined.

3.3. *Media selection: brackish versus marine strains*

DISCOVR has looked at both brackish and marine strains typically assuming a nominal 5 ppt or 35 (and higher) ppt salinity, respectively (see "target salinity' in Table 1). Strains are evaluated for salinity tolerance under the Tier 1 screening for DISCOVR ([26] this issue). Higher salinity tolerance as a function of absolute value as well as tolerance to changing salinity, is a key performance metric due to expected increases in salinity over time during continuous/semi-continuous cultivation due to evaporation. The expectation is that at scale, no fresh water use for make-up, and thus there is a need to minimize environmental and economic impacts of blow down and strains that can tolerate higher salinity are favored [16–19]. Media recycle or allowing for increasing salinity during cultivation due to evaporation has not been a part of routine SOT cultivation trials to date. In addition, for the formal reported productivity calculations shown in Table 2 and Table 3, the calculation of the seasonal values is not segregated by media type as the current intent of the formal annual reporting is to provide the best monthly/seasonal productivities, regardless of media type. However, the formal calculation of the key metric, minimum biomass selling price (MBSP), does account for the actual salinity for a given cultivar and the expected costs of blow-down to maintain a strain at its optimal salinity target [21,22].

A given algae cultivation facility will more likely only have one type of water available of a narrow salinity range and so it is more commercially relevant to look at trends in AHYP based on media type. Fig. 4 shows the monthly AHYP values for 2019, 2020, and 2021 based on either a brackish strain rotation or a marine strain rotation scenario with the top four DISCOVR strains. Seasonal and annual averages based on a brackish or marine media are summarized in Table 4. The most productive year for brackish strains was 2019 with an annual average productivity of 16.9 ± 8.4 g m^{-2} day^{-1} and 2020 for marine strains with an annual average productivity of 18.6 ± 10.1 g m^{-2} day^{-1}. In both

Table 3
Seasonal and annual average CY AHYP for the best performing strains for 2018 through 2021.

Calendar year	Season	AHYP (g m^{-2} day^{-1})	Total days/ season	Annual average AHYP	% Annual increase AHYP	Total days/ year
2018	Winter	7.1 ± 1.7	84			
2018	Spring	14.8 ± 3.1	78			
2018	Summer	14.9 ± 1.3	56			
2018	Fall	11.3 ± 1.6	66	**12.0 ± 3.7**	N/A	**284**
2019	Winter	6.9 ± 0.6	90			
2019	Spring	18.6 ± 6.0	88			
2019	Summer	27.1 ± 2.8	87			
2019	Fall	15.0 ± 3.6	87	**16.9 ± 8.4**	41 %	**352**
2020	Winter	8.3 ± 1.2	88			
2020	Spring	18.4 ± 4.5	93			
2020	Summer	31.6 ± 3.9	81			
2020	Fall	19.3 ± 8.3	92	**19.4 ± 9.5**	15 %	**354**
2021	Winter	8.7 ± 0.8	89			
2021	Spring	19.4 ± 3.7	88			
2021	Summer	23.8 ± 0.9	90			
2021	Fall	16.2 ± 3.7	91	**17.0 ± 6.4**	−12 %	**358**

cases significant improvement in seasonal and thus annual productivities was achieved with the largest % increase in summer and fall seasons for both media types relative to 2018.

While steady progress was made over the last 4 years, periodic declines were experienced year over year for particular seasons. In summer and fall of 2020, a significant decline in productivity of 23 % was observed for *S. obliquus* UTEX393 relative to 2019. This decline was attributed to a new bacterial pest that appeared on site in the spring of 2020 and significantly decreased productivity for this cultivar (Fig. 4) and also significantly decreased robustness with a sharp decrease in mean time to failure (MTTF) relative to 2019 (see Section 3.5). In 2021, we also observed a year over year drop in the summer and early fall for *P. celeri* TG2 relative to its peak in summer of 2020 with a decline of almost 25 % for the summer season in 2021. The reasons for this year over year drop are less clear than the year over year decline observed for *S. obliquus* UTEX393 as we did not see any significant difference in contamination and little to no grazing or other weedy algae in *P. celeri* TG2 cultures. The monsoon season of 2021 was more active relative to 2020 with higher dust levels and more clouds leading to decreased solar insolation especially for July 2021. Fig. 5 shows the maximum and minimum water temperatures and DLI for June thru September for 2019–2021 as a function of calendar day and month. The monthly average DLI for July 2021 was 10–15 % below that for 2020 and morning water temperatures were slightly higher in the morning with a tighter range indicating overall warmer overnight temperatures relative to 2020. The combination of lower overall available light and warmer nighttime temperatures may be expected to decrease biomass productivity through lower daytime productivity, and has the potential for increased nighttime biomass loss due to increased respiration [40]. However, August and September 2021 had the largest year over year decreases in productivity (~34 %) but light and temperature differences were much more modest relative to 2020. DISCOVR continues to explore the reasons for this large drop in productivity including exploring a combination of; 1) retrospective biomass productivity modeling via the PNNL Biomass Assessment Tool (BAT) [41] using the actual temperature and light observations for 2020 and 2021, 2) biotic differences through evaluation of preserved pond samples for pond metagenomic analysis, and 3) indoor lab experiments with climate-simulation photobioreactors running water temperature and DLI scripts for specific seasons. Understanding year over year fluctuations in biomass productivity and the drivers of those fluctuations are a key aspect to developing best agronomic practices and gaining the experience to manage long term algae cultivation at scale.

3.4. *Operational strategies improve AHYP*

While strain selection has driven a significant portion of the gains in productivity, successfully cultivating algae at commercial scales will require the development of best agronomic practices in order to overcome the yield gap between indoor lab and outdoor field performance and requires identifying optimal operating conditions for a given strain/season to yield improvements in productivity and robustness [3,5,7]. There are many parameters that need to be managed in a rapidly changing environment where the key drivers of productivity, light and temperature, remain largely uncontrollable. The DISCOVR SOT trials have focused primarily on understanding the performance of different strains when run outdoors in varying environmental conditions under non-limiting conditions for nutrients and CO_2. The annual cultivation trials operate under a recurring cycle of comparing a benchmark strain for a given season (i.e., the best performing strain in the previous year for that season) side-by-side with any new strain that has made it through the DISCOVR pipeline while also adjusting operational parameters that can improve performance (e.g., depth, pH, dilution rate), and as needed, the implementation of crop protection strategies. This year over year experience with a given strain allows valuable experience to be gained in developing best agronomic practices and improving productivity.

It has been shown that culture depth and dilution rate are two factors that need to be optimized in algal biomass production. Depth and dilution rate can be a means to regulate light availability and pond temperature providing a mechanism to keep a particular strain as close to its optimal production rate as a function of seasonal changes [5–8,42–44]. Fig. 6 shows examples of comparisons for some of the key control parameters that are routinely optimized under the SOT for a given strain/season. Culture depth comparisons, such as shallower depths in the winter to improve light availability, were evaluated for *M. minutum* 26BAM in winter as well as spring seasons with significantly higher biomass concentration at harvest observed at shallower culture depths (1.8-2× higher for 10 cm vs 20 cm). However, there was no statistically significant impact on areal productivities (i.e., AHYP g m^{-2} d^{-1}, Fig. 6a). In warmer seasons with more available light, this relationship held (i.e., higher biomass concentration at harvest, but similar overall harvest yields), but as the risk of culture overheating can increase at shallower culture depths relative to a deeper water column [42], these comparisons were not explored in summer months with warm season strains. Given that the temperature profiles of small, pre-pilot raceways do not mimic well the temperature profiles at large scale (i.e., >1 acre ponds) [18,19], a standardized depth of 20 cm was set for the SOT runs in 2020 for experimental simplification. Culture depth may continue to be explored in future SOT trials.

Another major operational parameter evaluated was pH. That biomass productivity for algae are highly dependent on pH is well known and has been studied in natural systems as well as in open ponds and photobioreactors [45–47]. When the SOT cultivation trials were first begun, the majority of cultivation in 2018 through Spring of 2019 were conducted at a pH setpoint of 7.9. In spring of 2019, DISCOVR began screening for effects of pH on productivity and showed for both *M. minutum* 26BAM and *S. obliquus* UTEX393 that a lower pH setpoint of

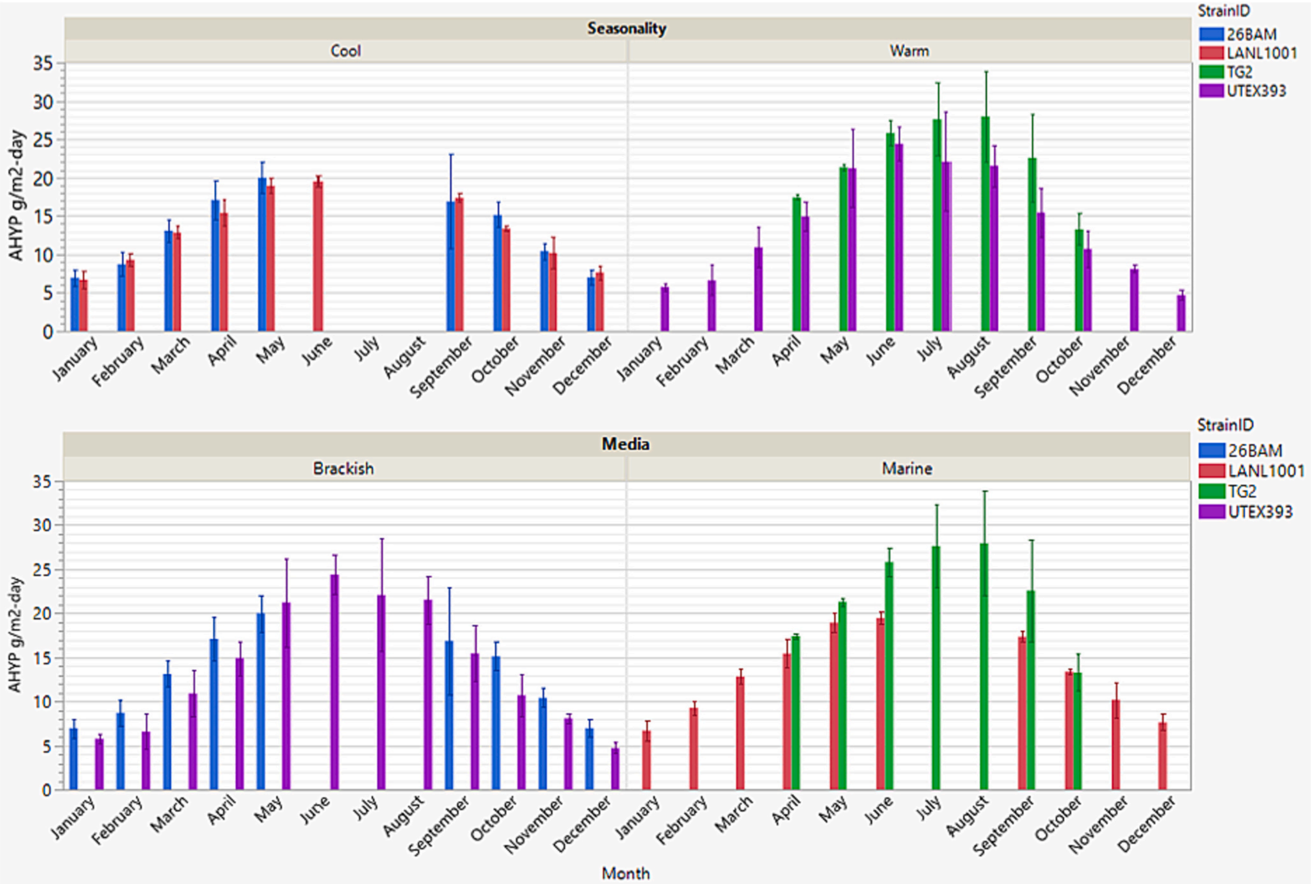


Fig. 3. Monthly average AHYP (g m^{-2} day^{-1}) by seasonality of strain (top graph) and media salinity (bottom graph). Monthly averages are the average across three years for *M. minutum* 26BAM and *S. obliquus* UTEX393 (2019–2021) and the average across two years for *T. striata* LANL1001 and *P. celeri* TG2 (2020–2021, except June for LANL1001 which is CY 2020 only). Error bars are ±1 standard deviation from the mean (n = 9 for *M. minutum* 26BAM and *T. obliquus* UTEX393, and n = 6 for *T. striata* LANL1001 and *P. celeri* TG2.

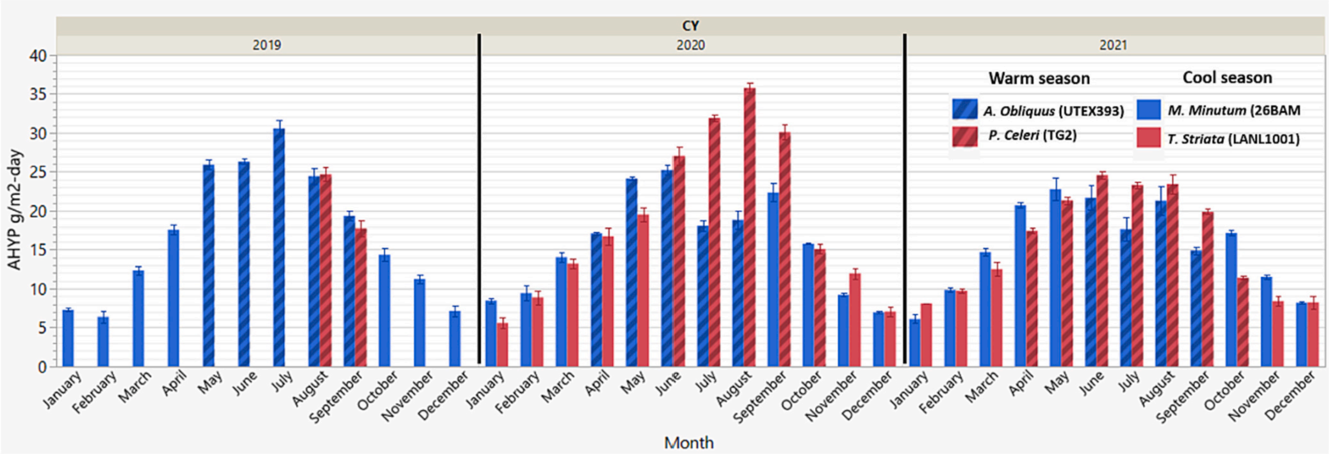


Fig. 4. Monthly AHYP from January 2019 through December 2021 for brackish (blue) and marine (red) top four cultivars. Warm weather strains indicated by bars with cross hatching. For the monthly AHYP values, each bar graph represents the single best performing strain for the month/year (n = 3 ponds) and for a given media condition (either brackish, 5 ppt salinity, or marine, 35–50 ppt salinity). Error bars are ±1 standard deviation from the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

7.0 had a significant effect improving AHYP 20–30 % (Fig. 6b). In addition to higher growth rates, greater robustness for many strains was observed at the lower pH setpoints. While lower pH did greatly improve productivity for top performing DISCOVR strains, running at lower pH has limitations, in particular for carbon utilization efficiency as at these lower pH set points, significant outgassing of CO_2 will occur [46] and thus strategies to maintain higher productivity at conditions with lower CO_2 losses to increase carbon utilization are important to develop.

Daily dilution rate is another operational parameter that affects productivity. As shown previously in Fig. 2, the estimated daily dilution

Table 4
Seasonal and annual calendar year average AHYP for the best performing strains used in annual performance improvement calculation for 2018 through 2021.

Media type	CY	Season	No. months	Avg. AHYP (g m^{-2} day^{-1})	Avg. AFDW at harvest (g L^{-1})	Avg. est. daily dilution	Harvests / week	Total days/ season	Annual avg. (g m^{-2} day^{-1})	Total days/year
Brackish	2019	Winter	3	6.9 ± 0.6	0.426 ± 0.09	0.12 ± 0.02	1×-2×	90		
Brackish	2019	Spring	3	18.6 ± 6.0	0.602 ± 0.12	0.25 ± 0.07	2×-3×	88		
Brackish	2019	Summer	3	27.1 ± 2.8	0.430 ± 0.05	0.32 ± 0.02	3×	87		
Brackish	2019	Fall	3	15.0 ± 3.6	0.355 ± 0.00	0.22 ± 0.07	2×-3×	87	16.9 ± 8.4	352
Brackish	2020	Winter	3	8.3 ± 1.2	0.536 ± 0.22	0.13 ± 0.01	1×-2×	88		
Brackish	2020	Spring	3	18.4 ± 4.5	0.431 ± 0.17	0.28 ± 0.06	2×-3×	93		
Brackish	2020	Summer	3	20.7 ± 3.5	0.377 ± 0.02	0.28 ± 0.04	3×	93		
Brackish	2020	Fall	3	15.8 ± 5.7	0.330 ± 0.06	0.23 ± 0.06	2×-3×	77	15.8 ± 5.4	351
Brackish	2021	Winter	3	8.1 ± 1.6	0.330 ± 0.05	0.12 ± 0.02	1×-2×	89		
Brackish	2021	Spring	3	19.4 ± 3.7	0.408 ± 0.04	0.24 ± 0.04	2×-3×	88		
Brackish	2021	Summer	3	20.2 ± 2.4	0.349 ± 0.10	0.22 ± 0.00	2×-3×	28		
Brackish	2021	Fall	3	14.5 ± 2.5	0.347 ± 0.07	0.20 ± 0.01	2×-3×	63	15.6 ± 5.6	268
Marine	2020	Winter	3	7.2 ± 1.6	0.376 ± 0.08	0.10 ± 0.04	1×-2×	84		
Marine	2020	Spring	3	16.5 ± 2.9	0.324 ± 0.02	0.26 ± 0.07	2×-3×	90		
Marine	2020	Summer	3	31.6 ± 3.9	0.497 ± 0.08	0.34 ± 0.00	3×	81		
Marine	2020	Fall	3	19.1 ± 8.4	0.354 ± 0.10	0.27 ± 0.08	2×-3×	92	18.6 ± 10.1	347
Marine	2021	Winter	3	8.7 ± 0.9	0.318 ± 0.06	0.14 ± 0.02	1×-2×	89		
Marine	2021	Spring	3	17.1 ± 3.9	0.354 ± 0.02	0.24 ± 0.05	2×-3×	84		
Marine	2021	Summer	3	23.8 ± 0.9	0.378 ± 0.02	0.32 ± 0.02	3×	90		
Marine	2021	Fall	3	13.2 ± 5.2	0.306 ± 0.01	0.21 ± 0.08	2×-3×	74	15.7 ± 6.4	337

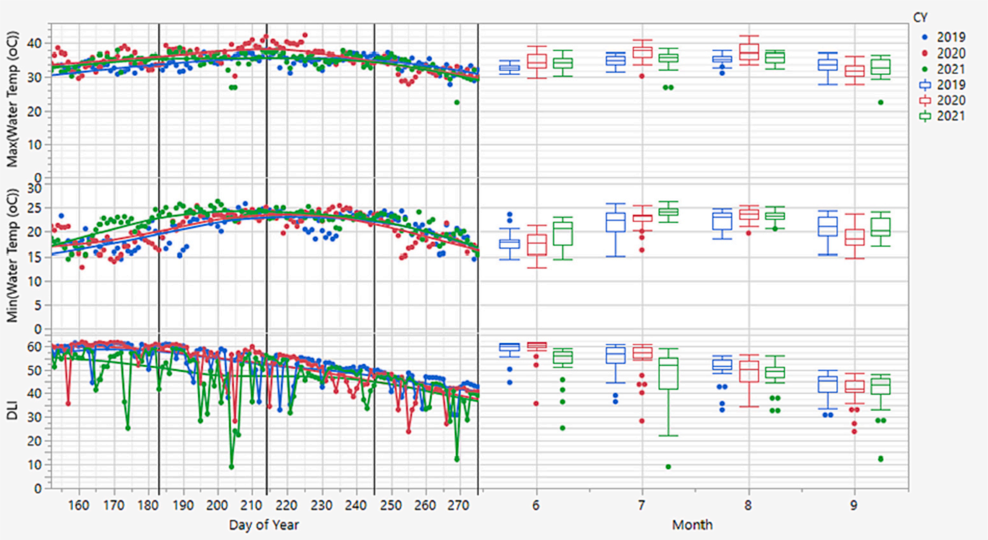


Fig. 5. Daily maximum (red) and minimum (blue) water temperature (°C) and DLI (green) (mol m^{-2} day^{-1}) by calendar day (left plot) and month (right plot). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rate has a strong correlation with productivity with higher dilution rates during higher productivity seasons and lower dilution rates during lower productivity seasons. For the DISCOVR SOT trials, dilution rate is a parameter that is primarily managed empirically with minor adjustments based on the observed growth rate and culture density at harvest. Our biomass density targets were to keep harvest density in the range of 0.3 to 0.5 g L^{-1} at harvest and ≥0.1 g L^{-1} post-harvest. On a seasonal basis, optimization of dilution rate for a given strain is determined directly by comparing higher or lower dilution rates side by side. Fig. 6c shows examples for three of the top performing strains showing the effect of dilution rate on AHYP for *T. striata* LANL1001 in the winter, *P. celeri* TG2 in early fall, and *S. obliquus* UTEX393 in the spring. All showed an increase in AHYP at higher dilution rates. More optimization is certainly needed, in particular the ability to move away from an empirical, trial and error driven approach to managing dilution rate more dynamically [5–8,11,48]. This remains a primary focus of the DISCOVR annual performance, in particular for the warmer season strains.

3.5. Crop protection and pest management

One of the most challenging aspects of achieving and maintaining high algae biomass productivities at scale is controlling or avoiding completely weedy algae, microzooplanktonic grazers, and fungal and bacterial parasitoids, all which can have a devastating effect on biomass quality and quantity. Thus, successful large-scale algae cultivation at commercial scales requires the development of effective crop protection and integrated pest management [3,5,7,11,12,49–52]. Under ATP^3, frequent culture crashes with both marine and freshwater strains were reported including from grazers, weedy algae strains, and fungal parasitoids, with contamination and thus the risk of pond crashes highest in the warmer seasons. Metrics were established to better quantify pond failure and thus reliability, and allow for quantitative tracking to provide insight into potential pond management and contaminant mitigation [53]. Beginning in late Spring through the Summer and early Fall of CY 2018, significant culture crashes with brackish strains *S. acutus* LRB0401, *M. minutum* 26BAM, *S. obliquus* UTEX393, and *D. armatus* SE00107 (18 ppt), and the marine strain *Desmodesmus* sp. CO46 were

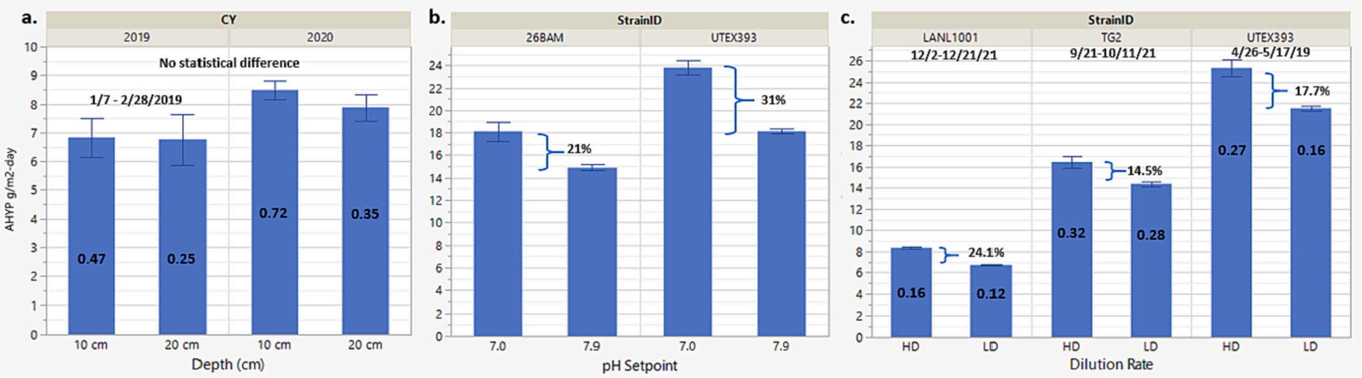


Fig. 6. Average harvest yield productivity (AHYP) comparisons for different operational setpoints (n = 3 ponds for each bar graph/condition) a.) *M. minutum* 26BAM cultivated side by side at two culture depths of 10 cm or 20 cm from January 7, 2019 thru February 28th, 2019 and again from December 31, 2019 thru January 31, 2020 (n = 3 ponds per depth). Error bars represent ±1 standard deviation from the mean, the average AFDW at harvest is listed on each bar graph. b.) *M. minutum* 26BAM and *S. obliquus* UTEX393 cultivated at two different pH setpoints (SP) in April 2020 for 14 days (*M. minutum* 26BAM) and May 2019 for 18 days (*S. obliquus* UTEX393). Error bars represent ±1 standard deviation from the mean with the percent improvement of lower pH relative to higher pH setpoints. c.) three strains cultivated under two different dilution rate regimes, high dilution rate (HD), low dilution rate (LD). Harvest frequency was 3× per week for all conditions changing only the volume of harvested culture at each set point. Average daily dilution is listed in each bar graph along with percent improvement of HD versus LD. Cultivation dates for each strain are shown at top of each graph). Error bars represent ±1 standard deviation from the mean.

observed which limited overall annual productivity. In all cases of pond crashes in CY 2018, the main contaminant appeared to be algal parasitoids which were assumed to be fungal or fungal-like based on the morphology observed via microscopy. These algal parasitoids caused a rapid decline in productivity with cultures turning brown within a few days of first observations of infection. The morphology of the crashed cultures looked different depending on strain, but specifically for *S. acutus* LRB0401, *S. obliquus* UTEX393, and *M. minutum* 26BAM, crash morphology was similar to examples of algal parasitoids isolated and identified from ponds in New Mexico which infected *S. obliquus* and *D. armatus* SE00107 cultures and reported to be in the phylum Aphelida [50,51].

Chemical treatment protocols using commercially available fungicides has been reported in the literature and shown to be effective at reducing fungal infections allowing for improved and sustained productivity with *S. dimorphous* [52] and *D. armatus* SE00107 [54]. The active agent in the broad-spectrum fungicide used for *D. armatus* SE00107 was fluazinam (3-chloro-N-(3-chloro-2,6-dinitro-4-trifluoromethylphenyl)-5-trifluoromethyl-2-pyridinamine). A version of this fungicide, Secure® (manufactured by Syngenta) has been utilized at AzCATI since 2018, beginning with *D. armatus* SE00107 and then expanding to both *S. obliquus* UTEX393 and *M. minutum* 26BAM in 2019. Fig. 7 shows the initial trials with fluazinam outdoors with *S. obliquus* UTEX393 where an active fungal parasitoid infection observed via microscopy early in a cultivation trial led to a sharp decline in biomass productivity. Two out of three replicate ponds were treated with Secure® at an application rate of 1 ppm of the active agent every seven days (4 applications across 3 weeks). The third pond was left untreated.

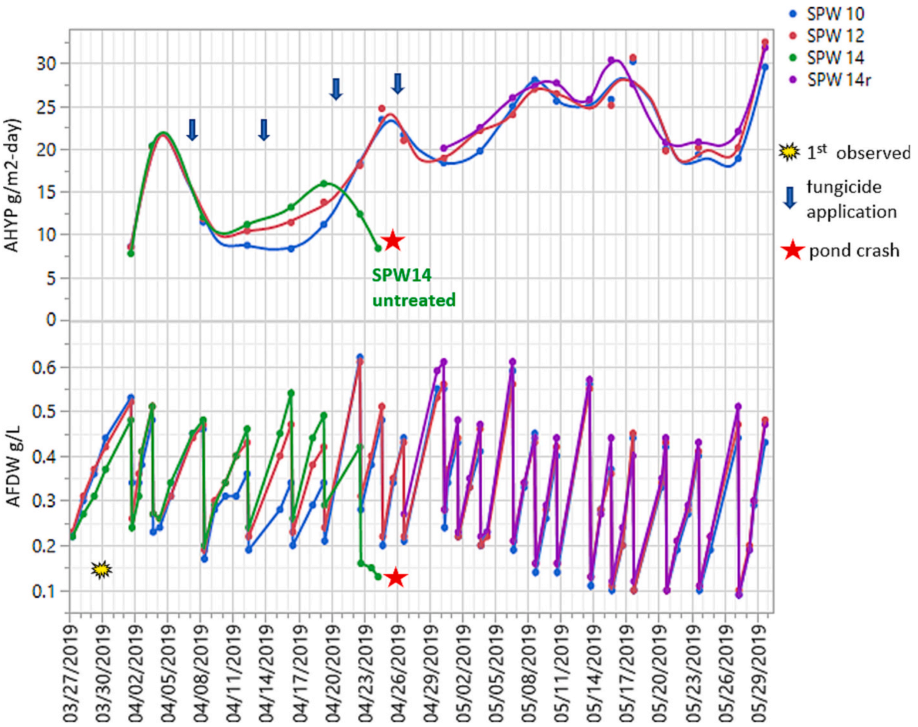


Fig. 7. AHYP (top) and AFDW (bottom) for *S. obliquus* UTEX393 spring 2019 cultivation trial. Parasitic fungal infection was first observed via microscopy on 3/30/2019. Ponds treated with (SPW10 and SPW12) and without (SPW14) 1 ppm of fluazinam. Fungicide application began on 4/8/2019 (post-harvest) and continued weekly for 3 weeks (4 applications total). The untreated pond crashed on 4/24/2019. SPW14 was restarted from SPW12 on 4/26/2019. The treated ponds fully recovered and did not experience any further infection for >30 days from last fungicide application. Decline in productivity in late May was due to cloudy/cool weather.

As shown in Fig. 8, *S. obliquus* UTEX393 ponds were heavily contaminated by early April with classic signs of fungal parasitoid infection (e.g., ghost cells and residual bodies) and the infection had progressed significantly by time of first application. Within 2 weeks treated ponds fully recovered and the untreated pond turned brown and crashed. Dosing was stopped at the end of April and cultivation continued with the recovered culture through the end of May 2019. Productivity for May 2019 was the highest sustained productivity ever observed at AzCATI to date achieving >20 g m^{-2} d^{-1}. Ponds were restarted with fresh inoculum for the summer 2019 season with implementation of a fungicide-based pest management routine initiated at the first sign of infection in ponds. With this implementation, the highest summer productivities at AzCATI to date were achieved with *S. obliquus* UTEX393 with a summer seasonal average of 27.1 g m^{-2} d^{-1}, *an **82 % increase*** year over year 2018 to 2019 with no culture crashes from May through October demonstrating the significant impact of pest mitigation. Ponds were restarted with fresh culture at the start of summer season and then again in August due to a cyanobacterial contamination (when contamination exceeded ~10 % of cells), but no decline in productivity was observed.

S. obliquus UTEX393 showed significant tolerance to fluazinam with no apparent trade-off in productivity (at doses up to 2 ppm) unlike *D. armatus* SE00107, *Desmodesmus* sp. C046, and *M. minutum* 26BAM which all show significant productivity declines upon application of 0.5–1.0 ppm. Despite the lower tolerance to fungicide, we were successful at developing dosing regimes for both *D. armatus* SE00107 and, in particular *M. minutum* 26BAM, where protection from fungal parasitoid infections for 1–2 months would be observed but much more care had to be used, including lower application amounts and frequency, in order to minimize productivity losses. An example of the successful implementation of fluazinam with *M. minutum* 26BAM is shown in Fig. 9a. A dosing regime was established to minimize productivity loss and maintain protection against fungal parasitoids. Replicate sets of ponds with and without treatment were cultivated side-by-side in April and May 2021. The ponds that were treated with fluazinam had an average harvest productivity of 21.1 g m^{-2} d^{-1} versus 15.6 g m^{-2} d^{-1} for untreated ponds, a 29 % improvement. In addition, treated ponds lasted almost 3× longer with a MTTF of 41 versus 14 days, for treated and untreated ponds, respectively.

Outdoor cultures were tested using the polymerase chain reaction (PCR) with primer sequences of known parasitoid strains in the literature, in particular those identified and isolated at Sapphire Energy, including parasitoid strains *A. protococcarum* FD95, *A. occidentale* FD01, and *A. desmodesmi* FD104 [50,51]. The progression of infection and life cycle for the fungal parasitoids we observed in *S. obliquus* UTEX393 and *M. minutum* 26BAM were very similar to those observed at Sapphire Energy and thus these pests were our first target for identification. An example of a fungal parasitoid lifecycle in *M. minutum* 26BAM is shown in Fig. 9b-g. This is typical for what has been observed in *M. minutum* 26BAM and *S. obliquus* UTEX393 with zoospores that appear to be amoeboid (as opposed to flagellated). Through early 2020, outdoor cultures of both *S. obliquus* UTEX393 and *M. minutum* 26BAM routinely tested positive for FD01, but negative for FD95 and FD104. In addition, *D. armatus* SE00107, the original host for FD104 isolation from outdoor ponds in New Mexico and which routinely crashed when cultivated at AzCATI, were negative for FD104 (as well as FD01 and FD95) and thus the algal parasitoid infecting in *D. armatus* SE00107 cultures at AzCATI, while controllable by fungicide, as of yet remains unidentified. Since spring of 2020, cultures that are positive for FD95, FD104, or FD01 have not been observed via PCR, yet cultures continued to crash due to apparent fungal parasitoids and are controllable through the use of fungicides which indicates one (or more) as yet unidentified strains of fungal parasitoids may be present. Work to isolate and identify these fungal parasitoids is ongoing.

An active pest management program for algal cultivation needs to include a robust program of surveillance for known pests, active mitigation based on established thresholds for action, and surveillance for new threats [7,52–54]. An example of the process of developing a mitigation strategy for one pest/pest type, only to be challenged with the appearance of a new pest threat, can be seen in the year over year results for *S. obliquus* UTEX393. We identified a fungal parasitoid issue in 2019, developed a mitigation strategy (e.g., fungicide treatment) that essentially eliminated the threat of that pest only to be faced with a new pest threat in 2020 that was different in nature (i.e., non-responsive to fungicide). This lead to significant year over year declines in summer and fall productivity for 2020 and 2021 relative to 2019 (Fig. 4). More

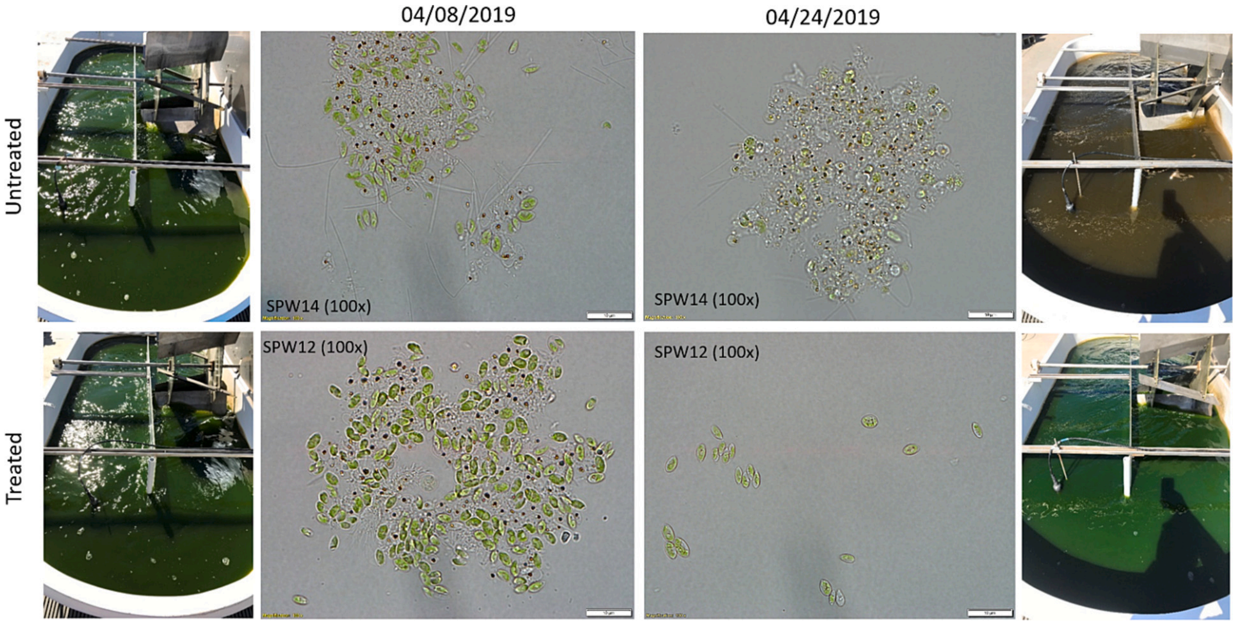


Fig. 8. Photographs and optical microscopy of *S. obliquus* UTEX393 during Spring 2019 cultivation. Top panel shows pictures of untreated ponds and corresponding optical microscopy (100×, measurement bar 10 μm) showing badly infected culture on 4/8/2019 and proceeding to full culture collapse on 4/24/2019. Bottom panel shows pictures of treated ponds (as described in Fig. 6) and corresponding optical microscopy (100×, measurement bar 10 μm) after first dose showing a similar level of infection as the untreated pond on 4/8/2019 and full culture recovery by 4/24/2019.

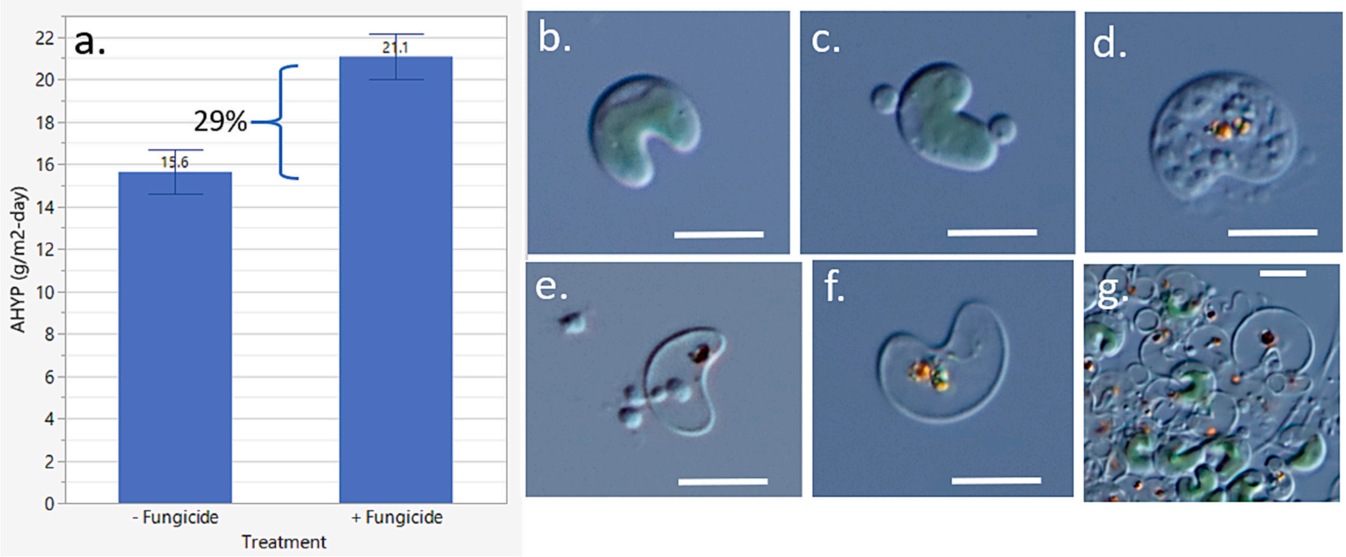


Fig. 9. Comparison of fungicide application with *M. minutum* 26BAM cultures in April and May 2021. a.) AHYP comparison for triplicate ponds with (+ fungicide) and without (− fungicide). Error bars are ±1 standard deviation from the mean. Typical lifecycle of fungal parasitoid contaminant in *M. minutum* 26BAM cultures showing b.) healthy *M. minutum* 26BAM cell, c.) zoospore attachment encysted on cell surface, d.) zoospore replication and segmentation inside the host cell, e.) amoeboid zoospores leaving the host cell, f.) empty host cell with residual body, and g.) completely crashed culture of *M. minutum* 26BAM (measurement bars on panels b-g is 5 μm).

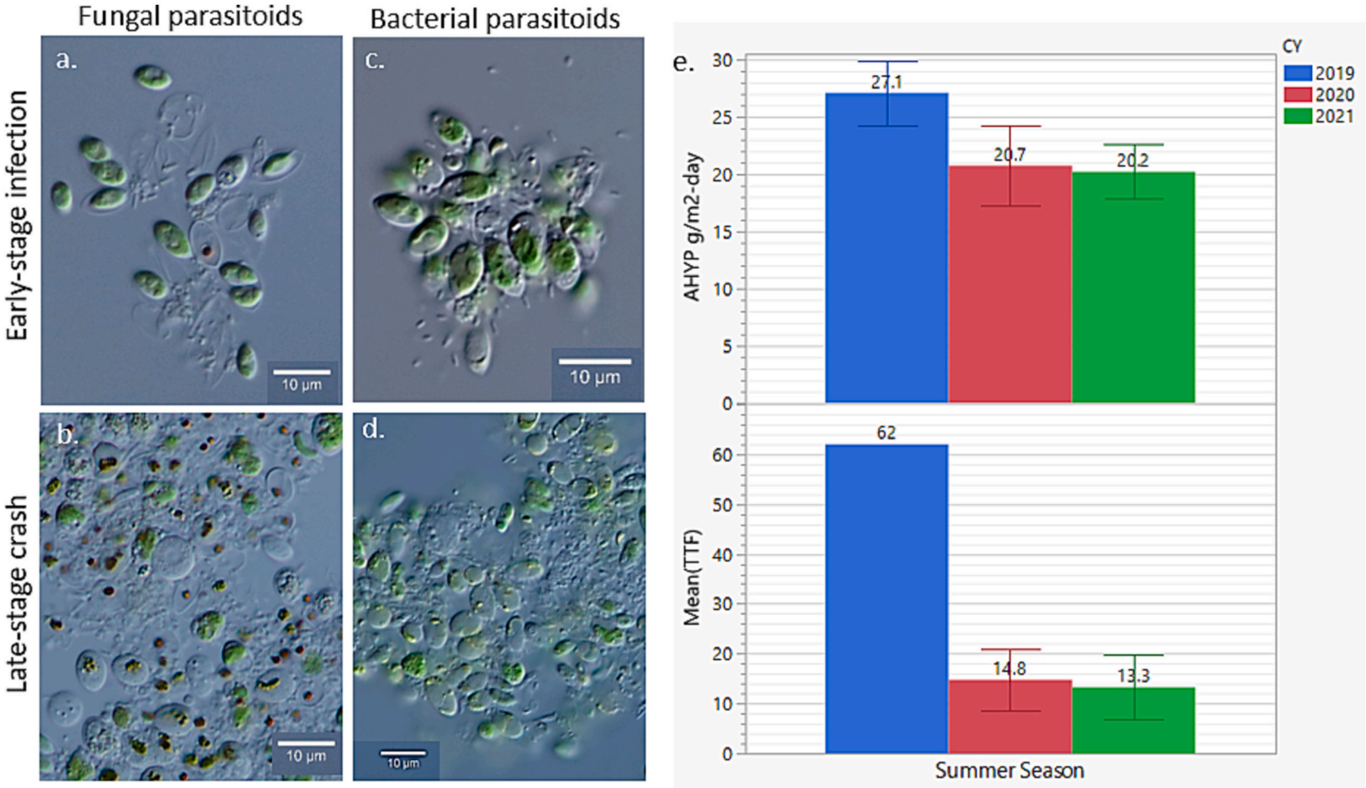


Fig. 10. Optical microscopy of two different pest types that routinely infect *S. obliquus* UTEX393 cultures at the AzCATI field site. Fungal parasitoids which were the main contaminant observed in 2019 for *S. obliquus* UTEX393 showing typical early-stage (a) and late-stage(b) phenotypes and a new bacterial parasitoid (endobiotic) that started infecting *S. obliquus* UTEX393 cultures in summer 2020 showing early-stage (c) and late-stage infection (d). Overall summer season productivity for CY 2019, 2020, and 2021 showing AHYP g m^{-2} d^{-1} (e., upper right panel) and MTTF in days (e., lower right panel). Error bars are ±1 standard deviation from the mean. Total days of cultivation for each summer season were 87, 72, and 40 for 2019, 2020 and 2021, respectively. Summer 2019 had only one reseeding event at beginning of August due to contamination by cyanobacteria (*Stanieria* sp.) but was counted as a crash event for the MTTF analysis. Summer 2020 and 2021 were restarted from seed 5 times and 3 times, respectively.

specifically, what was observed for *S. obliquus* UTEX393 going into summer of 2020 was a new pest with a culture crash morphology very different from that observed for fungal based crashes. Fig. 10a-b shows the microscopy from 2019 *S. obliquus* UTEX393 ponds infected with a fungal parasitoid and its progression to complete pond failure with typical morphology showing parasitoid attachment, penetration tubes, and eventually empty ghost cells with residual bodies (Fig. 10a-b). This is in contrast to the infection and ultimate crash morphology of *S. obliquus* UTEX393 beginning in summer of 2020 where increased bacterial presence was observed along with cells going chlorotic, yellowing slightly with loss in pigmentation, partial or complete disappearance of cell contents, and most importantly, no residual bodies as seen in all our confirmed fungal infections (Fig. 10c-d). Rapid decline in productivities were observed within 7–10 days of inoculating ponds outside in 2020 and decreased to <7 days in 2021. Even with repeated restarts from fresh indoor seed culture, and enhanced sterilization protocols in ponds, high harvest productivities could not be maintained. Fig. 10e shows the AHYP for the summer seasons for 2019–2021 with 2020 and 2021 both showing a 25 % decline in AHYP relative to 2019 as well as a significant decline in MTTF from 62 days in 2019 dropping to an average of 14.8 and 13.3 in 2020 and 2021 respectively. While the specific bacterial pathogen has not yet been isolated or identified from *S. obliquus* UTEX393, it has been confirmed that it is likely a predatory bacterium. Using aliquots of crashed field cultures (at 1 % v/v), they were shown to re-infect indoor cultures relative to controls. The crash phenotype could be prevented by filtering crashed pond samples through a filter pore size of 0.45 μm or smaller prior to introduction into clean cultures. In addition, cultures treated with antibiotic (25 μg/mL ampicillin) also showed no signs of infection when challenged with 1 % aliquot of infected outdoor culture.

The overall phenotype of crashed *S. obliquus* UTEX393 culture looked similar to that reported for *Nannochlorposis* sp. caused by a novel bacterial pest (designated as FD111) which was shown to have similarity to bacteria in the order *Bdellovibrionales* [49]. However, using the PCR sequences reported, we did not get a match for FD111 and additional work is ongoing to isolate and or identify this extremely harmful bacterial pest of *S. obliquus* UTEX393. Mitigation was attempted similar to that described for FD111 based on sodium hypochlorite addition however *S. obliquus* UTEX393 was too sensitive to chlorine addition and immediately bleached out even at concentrations 4-fold lower than those described (0.5 versus 2 mg L^{-1}) for *Nannochloropsis* that showed efficacy for controlling FD111 infection [49]. One mitigation step that proved marginally successful but did not fully eliminate pond infection and culture collapse was increasing salinity from 5 ppt to 10–15 ppt which improved MTTF from 14 to 24 days but only partially restored productivity. This illustrates the significant knowledge gap around algae crop pest susceptibility and the challenges for large-scale deployment of algae cultivation including the development and implementation of robust crop protection methodologies.

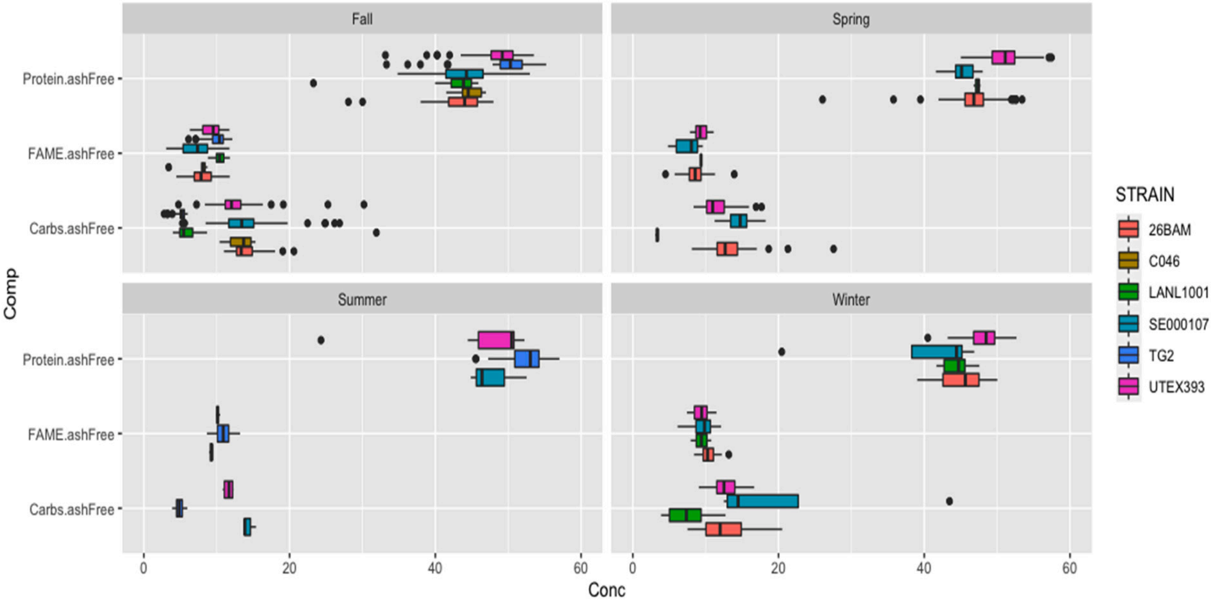
3.6. Biomass composition

Biomass samples from a subset of harvests (between 4 and 66 points for a given strain, year, and season) were collected for proximate analysis for each of the reported outdoor production runs. Compositional analysis for carbohydrate (measured as monomers after acid hydrolysis), lipid (measured as total fatty acid methyl ester (FAME) after whole biomass transesterification), protein (via nitrogen-to-protein conversion), and ash content determination [14], as well as elemental carbon and nitrogen of the biomass, were determined. The data shown in Table 5 illustrates the overall compositional profiles, separated by season, year, and strain. A total of 768 individual samples were collected and analyzed over the course of the outdoor production runs, creating an unprecedented depth of algae composition data, unique in the current literature. Cultivation experiments were focused on maximizing biomass productivity, and thus did not target a biochemical shift in the biomass to a more attractive biorefinery-ready composition (e. g., higher carbohydrate and lipid content), which is reflected in the >40 % protein content for almost all samples analyzed. However, when looking at the data in aggregate (Table 5, Fig. 11), we can observe trends between deployed strains that point to an inherent capacity of some of the strains to accumulate higher storage carbon content (primarily reflected in total carbohydrates) during active growth without the need for a purposeful shift. The strains *S. obliquus* UTEX393, *D. armatus* SE000107, and

Table 5

Proximate biomass composition for strains grown during the 2018–2021 SOT outdoor cultivation experimental trials. All data shown as the mean ± 1 standard deviation from the mean of triplicate ponds cultivated over the course of multiple harvests throughout the season, on either a dry weight (% DW) or on an ash-free dry weight basis (% AFDW) for the number of samples collected and analyzed for that season/strain/year combination (N = number of samples analyzed for a given season/strain).

Season	Year	Strain	N	Ash (%DW)	Carbohydrates (% AFDW)	FAME (% AFDW)	Protein (% AFDW)	C (% AFDW)	N (% AFDW)
Fall	2018	26BAM	30	12.2 ± 7.1	14 ± 2.2	7.9 ± 1.7	43 ± 4.6	52.4 ± 2.8	9 ± 1
Fall	2018	C046	11	17.5 ± 2.3	13.3 ± 1.7	7.8 ± 1.5	44.8 ± 1.7	49.2 ± 0.5	9.4 ± 0.4
Fall	2018	SE000107	80	33.7 ± 14	13.9 ± 4	7 ± 2.1	44 ± 3.9	49.3 ± 3.8	9.2 ± 0.8
Fall	2018	UTEX393	29	11.2 ± 4.7	12.2 ± 1.7	8 ± 1.2	47.7 ± 3.1	50.9 ± 2.5	10 ± 0.6
Winter	2018	SE000107	4	17 ± 6.4	21.2 ± 14.9	9.5 ± 2.5	39 ± 12.4	51.3 ± 2.7	8.2 ± 2.6
Fall	2019	UTEX393	66	10.5 ± 2.8	12.7 ± 3.4	9.8 ± 0.9	49 ± 3.3	52.3 ± 1.2	10.3 ± 0.7
Spring	2019	26BAM	32	8.3 ± 1.1	14.3 ± 1.3	9.7 ± 0.8	46.2 ± 1.3	54.2 ± 1.1	9.7 ± 0.3
Spring	2019	SE000107	21	18.1 ± 13.7	14.7 ± 1.8	7.5 ± 1.6	45 ± 2	51.5 ± 1.5	9.4 ± 0.4
Spring	2019	UTEX393	35	8.7 ± 2.4	12.7 ± 1.9	8.8 ± 0.4	49.4 ± 1.8	52.8 ± 0.8	10.3 ± 0.4
Summer	2019	SE000107	3	10.6 ± 0.3	14.3 ± 0.9	9.2 ± 0.2	47.9 ± 4.1	52.6 ± 1.3	10 ± 0.8
Summer	2019	UTEX393	6	8.4 ± 0.3	11.6 ± 0.6	10.1 ± 0.2	45.5 ± 10.7	48.3 ± 11.2	9.5 ± 2.2
Winter	2019	26BAM	21	11.4 ± 6.4	14.5 ± 2.7	10.5 ± 1.1	43.4 ± 2.4	53.2 ± 1.6	9.1 ± 0.5
Winter	2019	UTEX393	21	14.5 ± 7.5	13.2 ± 1.7	8.8 ± 1	47.3 ± 2.5	50.9 ± 1.9	9.9 ± 0.5
Fall	2020	TG2	65	20.5 ± 8.8	5.2 ± 0.6	10.1 ± 1.3	49.6 ± 3.8	48.5 ± 4	10.4 ± 0.8
Fall	2020	LANL1001	21	18.5 ± 1.1	7 ± 5.9	10.4 ± 0.8	42.6 ± 4.7	48.2 ± 1.1	8.9 ± 1
Spring	2020	26BAM	24	6.4 ± 0.5	12.2 ± 4.2	9.2 ± 1.2	48.5 ± 5.9	53.2 ± 0.7	10.1 ± 1.2
Spring	2020	UTEX393	32	9.2 ± 0.6	10 ± 0.7	10 ± 0.8	52.7 ± 1.9	52.2 ± 0.5	11 ± 0.4
Summer	2020	TG2	4	17.5 ± 2.5	5.5 ± 0.1	11.7 ± 0.7	51.5 ± 0.8	52.4 ± 1	10.8 ± 0.2
Winter	2020	26BAM	14	6.2 ± 0.8	9.7 ± 1.4	10 ± 0.8	47.8 ± 1.1	52.8 ± 0.7	10 ± 0.2
Winter	2020	LANL1001	18	19.7 ± 1.6	8.3 ± 3.3	9 ± 0.4	43.7 ± 1.8	47.8 ± 0.7	9.1 ± 0.4
Winter	2020	UTEX393	14	9 ± 0.9	11.7 ± 1.8	10.2 ± 0.5	49.4 ± 2.1	51.3 ± 0.6	10.3 ± 0.4
Spring	2021	26BAM	58	6.8 ± 1.2	12.5 ± 2	7.9 ± 0.9	46.4 ± 2.5	51.5 ± 1.4	9.7 ± 0.5
Spring	2021	LANL1001	3	21.4 ± 0.2	3.3 ± 0.2	9.4 ± 0	47.3 ± 0.5	49.4 ± 0.3	9.9 ± 0.1
Summer	2021	TG2	62	22 ± 9.3	4.9 ± 0.5	10.8 ± 1.1	52.8 ± 2.5	51 ± 2.5	11 ± 0.5
Winter	2021	LANL1001	24	20.7 ± 0.8	7 ± 2	9.8 ± 0.9	44.8 ± 1.7	48.5 ± 1.3	9.4 ± 0.4



Figs. 11. Proximate biomass composition for strains grown during the 2018–2021 SOT outdoor cultivation experimental trials.

M. minutum 26BAM all consistently present higher carbohydrate content of 15–25 % on an ash-free dry weight basis while *P. celeri* TG2 and *T. striata* LANL1001 consistently across years and seasons do not accumulate much storage carbon (<10 % carbohydrates or lipids). When looking at the lipid content, across all samples, there are no statistically significant differences between strain and or seasons and remains constant and modest around 10 % of the biomass. It remains to be tested whether there is a relationship between the environmental conditions (e. g., light, temperature, etc.) that were particularly conducive to the higher storage carbon conditions in some of the outlier points.

4. Conclusions

Though there has been significant effort with both public and private research programs in the last several decades, cultivation of algae for the purpose of conversion to biofuels remains challenging. The cost of production is mainly driven by biomass productivity demanding that improvements be made in year over year outdoor productivity to consistently deliver low-cost algae biomass. Our objective as part of the DISCOVR consortium was to validate algae strains vetted in the 3-tiered strain screening pipeline showing promise for highly productive outdoor cultivation. To this end, in aggregate, we examined a total of 14 algae species/strains, through 1348 days of triplicate pond cultivation, in all seasons across a period of 4 years. Though of no surprise, we observed that for algae cultivation sites in geographic areas that experience significant seasonal climatic variations, such as the Phoenix, AZ, area, the weather (e. g., temperature, daylength, and daily solar insolation) is the main driver for algae productivity over the year. Maximum AHYP during the summer is >5× that in the winter and experiences very rapid increases/decreases during the transition seasons. Given these large and relatively rapid changes in temperature and insolation leading to such large changes in AHYP, and typically relatively narrow temperature tolerances of algae, we have shown that a strategy of cultivating different seasonally optimized strains (i.e., crop rotation) will be necessary for maximizing annual productivity. Crop rotation notwithstanding, fluctuations in the weather in a given season will still influence productivity. To wit, after three years of ever-increasing AHYP during the summer, minor fluctuations in the weather in the summer of 2021 brought about a precipitous decline (-7.8 g m^{-2} day^{-1} (-25 %)) in AHYP for the summer season.

The next major productivity driver is susceptibility to pond failure inducing organisms. These organisms can be broad host range as well as strain specific and can change overtime for a given cultivar. Thus, developing effective crop protection strategies effective against a variety of organisms is crucial. Other minor drivers of productivity are pH and salinity but these drivers will largely be determined by choice of cultivation site and associated water supply. These drivers can be manipulated at an additional cost, though algae cultivation for biofuels will be required to use water that is not useful for other purposes (e. g., high salinity, wastewater, produced water, brackish). In addition, the operational strategies of dilution rate and pond depth have potential in increasing AHYP though further study is needed and is strain and season dependent. Finally, though our studies show multi-season year over year improvements in outdoor algae cultivation productivity, these studies were done at a pre-pilot scale of 800 L. Though other studies have shown that experiments at this scale are in fact relevant to larger scales, much work remains to be done to validate our productivity and pond management strategies on scales relevant to commercialization.

In summary, the last 4 years have seen a maximum increase of 62 % for CY 2018–2020, but dropping to 42 % for CY 2018–2021 due to summer 2021 weather and other as of yet unidentified impacts. With these successes, nominal 4 % annual increases year over year for the next several years will need to be realized to achieve the 2030 goal of 25 g m^{-2} day^{-1}. Through future work in the DISCOVR consortium, our successful strategy of crop rotation using seasonally-selected best strains combined with optimal operational strategies can continue to be improved and the publicly available datasets can be utilized for biomass productivity modeling and concomitant TEA, LCA, and RA modeling to enable future commercialization of renewable, sustainable, algae-based biofuels and bioproducts and position innovative algae agronomics on par with terrestrial crop production.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2023.102995.

Informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this project.

CRediT authorship contribution statement

John McGowen: Conceptualization, Writing – Review & Editing, Data Curation, Formal Analysis, Project Supervision; Eric Knoshaug: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft; Lieve Laurens: Writing – Review & Editing, Data Curation, Formal Analysis; Jessica Forrester: Data Curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] R. Davis, D. Fishman, E.D. Frank, M.S. Wigmosta, A. Aden, A.M. Coleman, P. T. Pienkos, R.J. Skaggs, E.R. Venteris, M.Q. Wang, Renewable Diesel From Aglal Lipids: An Integrated Baseline for Cost, Emissions, and Resource Potential From a Harmonized Model, NREL/TP-5100-554341, 2012.

[2] R. Davis, J. Markham, C. Kinchin, N. Grundl, E.C.D. Tan, D. Humbird, Process Design and Economics for the Production of Algal Biomass: Algal Biomass Production in Open Pond Systems and Processing Through Dewatering for Downstream Conversion, NREL/TP-5100-64772, 2016.

[3] EERE-BETO, Bioenergy technologies office national algal biofuels technology review. https://energygov/sites/prod/files/2016/06/f33/national_algal_biofuels_technology_reviewpdf, 2016.

[4] J.C. Quinn, R. Davis, The potentials and challenges of algae based biofuels: a review of the techno-economic, life cycle, and resource assessment modeling, Bioresour. Technol. 184 (2015) 444–452.

[5] J. Sheehan, T. Dunahay, J. Benemann, P. Roessler, A Look Back at the U.S. Departments of Energy's Aquatic Species Program–Biodiesel From Algae, NREL/TP-580-24190, 1998.

[6] M.E. Huntley, Z.I. Johnson, S.L. Brown, D.Sills, L. Gerber, I. Archibald, S. C. Machesky, J. Granados, C. Beal, C.H. Greene, Demonstrated large-scale production of marine microalgae for fuels and feed, Algal Res. 10 (2015) 249–265.

[7] R.L. White, R.A. Ryan, Long-term cultivation of algae in open-raceway ponds: lessons from the field, Ind. Biotechnol. 11 (2015) 213–220.

[8] R. Craggs, D. Sutherland, H. Campbell, Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production, J. Appl. Phycol. 24 (2012) 329–337.

[9] A.K. Davis, R.S. Anderson, R. Spierling, S. Leader, C. Lesne, K. Mahan, T. Lundquist, J.R. Benemann, T. Lane, J.E.W. Polle, Characterization of a novel strain of Tribonema minus demonstrating high biomass productivity in outdoor raceway ponds, Bioresour. Technol. 331 (2021), 125007.

[10] A. Parsy, L. Bidoire, M. Saadouni, M. Bahuaud, T. Elan, F. Perie, C. Sambusiti, Impact of seasonal variations on nannochloropsis oculata phototrophic productivity in an outdoor pilot scale raceway, Algal Res. 58 (2021), 102375.

[11] P.J. Lammers, M. Huesemann, W. Boeing, D.B. Anderson, R.G. Arnold, X. Bai, M. Bhole, Y. Brhanavan, L. Brown, J. Brown, et al., Review of the cultivation program within the National Alliance for advanced biofuels and bioproducts, Algal Res. 22 (2017) 166–186.

[12] K. Ogden, D. Anderson, S. Simpson, W.Van Voorhies, J. Brown, M. Huesemann, M. Kacira, R.J. Skaggs, P. Waller, Regional Algal Feedstock Testbed (RAFT) Final Report, DOE-UAZ-0006269, 2019.

[13] E.P. Knoshaug, E. Wolfrum, L.M.L. Laurens, V.L. Harmon, T.A. Dempster, J. McGowen, Unified field studies of the algae testbed public-private partnership as the benchmark for algae agronomics, Sci. Data 5 (2018), 180267.

[14] L.M.L. Laurens, S. Van Wychen, P.T. Pienkos, V.L. Harmon, J. McGowen, Harmonization of experimental approach and data collection to streamline analysis of biomass composition from algae in an inter-laboratory setting, Algal Res. 25 (2017) 549–557.

[15] J. McGowen, E.P. Knoshaug, L.M.L. Laurens, T.A. Dempster, P.T. Pienkos, E. Wolfrum, V.L. Harmon, The algae testbed public-private partnership (ATP3) framework; establishment of a national network of testbed sites to support sustainable algae production, Algal Res. 25 (2017) 168–177.

[16] Q. Bechet, N. Coulombier, C. Vasseur, T. Lasserre, L. Le Dean, O. Bernard, Full-scale validation of an algal productivity model including nitrogen limitation, Algal Res. 31 (2018) 377–386.

[17] J.R. Cruce, A. Beattie, P. Chen, D. Quiroz, M. Somers, S. Compton, K. DeRose, B. Beckstrom, J.C. Quinn, Driving towards sustainable algal fuels: a harmonization of techno-economic and life cycle assessments, Algal Res. 54 (2021), 102169.

[18] J.M. Greene, D. Quiroz, S. Compton, P.J. Lammers, J.C. Quinn, A validated thermal and biological model for predicting algal productivity in large scale outdoor cultivation systems, Algal Res. 54 (2021), 102224.

[19] D. Quiroz, J.M. Greene, J. McGowen, J.C. Quinn, Geographical assessment of open pond algal productivity and evaporation losses across the United States, Algal Res. 60 (2021), 102483.

[20] R. Davis, L.M.L. Laurens, Algal Biomass Production Via Open Pond Algae Farm Cultivation: 2019 State of Technology and Future Research, NRE:/TP-5100-76569, 2020.

[21] R. Davis, B. Klein, Algal Biomass Production via Open Pond Algae Farm Cultivation: 2020 State of Technology and Future Research, NREL/TP-5100-79931, 2021.

[22] B. Klein, R. Davis, Algal Biomass Production via Open Pond Algae Farm Cultivation: 2021 State of Technology and Future Research, NREL/TP-5100-82417, 2022.

[23] M.H. Huesemann, E.P. Knoshaug, L.M.L. Laurens, T. Dale, T.W. Lane, J. McGowen, Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR): a coordinated research-driven approach to improve microalgal productivity, composition, and culture stability for commercially viable biofuels production, Algal Res. (2022) this issue.

[24] N. Beirne, S. Edmundson, S. Gao, J. Freeman, M. Huesemann, A streamlined approach to characterize microalgae strains for biomass productivity under dynamic climate simulation conditions, Algal Res. (2022) this issue.

[25] S. Gao, S. Edmundson, M. Huesemann, A. Gutknecht, K. Pittman, M. Greer, L.M. L. Laurens, S.Van Wychen, B. Panczak, H. Alt, DISCOVR strain pipeline screening - Part III: strain evaluation in outdoor raceway ponds, Algal Res. (2022) this issue.

[26] M. Huesemann, S. Edmundson, S. Gao, S. Negi, T. Dale, A. Gutknecht, H. E. Daligault, C.K. Carr, J. Freeman, S.R. Starkenburg, DISCOVR strain pipeline screening - Part I: specific growth rate as a function of temperature and salinity for 38 candidate microalgae strains for biofuels production, Algal Res. (2022) this issue.

[27] M. Huesemann, S. Gao, S. Edmundson, L.M.L. Laurens, S.Van Wychen, N. Beirne, A. Gutknecht, R. Kruk, K. Pittman, M. Greer, DISCOVR strain pipeline screening - Part II: winter and summer season areal productivities and biomass compositional shifts in climate-simulation photobioreactor cultures, Algal Res. (2022) this issue.

[28] J. McGowen, Final Closeout Report the Algae Testbed Public Private Partnership - ATP3, DE-EE0005996, 2019.

[29] P.D. Lane, A. Hagerstrand, T.W. Lane, Resistance of DISCOVR algae strains to deleterious species, Algal Res. (2022) this issue.

[30] K. Poorey, P. Rai, H. Kolla, D.J. Curtis, J. McGowen, P.D. Lane, T.W. Lane, Characterizing industrial pond ecology timeline in DISCOVR cultivation trials for early detection of pond crashes, Algal Res. (2022) this issue.

[31] M. Lane, S. Van Wychen, A. Politis, L.M.L. Laurens, A data-driven comparison of commericially available tesing methods for algae characterization, Algal Res. 53 (2021), 102134.

[32] S.Van Wychen, L.M.L. Laurens, Determination of total solids and ash in algal biomass - Laboratory Analytical Procedure (LAP). http://wwwnrelgov/docs/fy16osti/60956pdf, 2015.

[33] S.Van Wychen, K. Ramirez, L.M.L. Laurens, Determination of total lipids as fatty acid methyl esters (FAME) by in situ transesterification - Laboratory Analytical Procedure (LAP). http://wwwnrelgov/docs/fy16osti/60958pdf, 2015.

[34] S.Van Wychen, L.M.L. Laurens, Determination of total carbohydrates in algal biomass - Laboratory Analytical Procedure (LAP). http://wwwnrelgov/docs/fy16osti/60957pdf, 2015.

[35] D. Douchi, M. Mosey, D.P. Astling, E.P. Knoshaug, A. Nag, J. McGowen, L.M. L. Laurens, Nuclear and chloroplast genome engineering of a productive non-model alga desmodesmus armatus: insights into unusual and selective acquistion mechanisms for foreign DNA, Algal Res. 53 (2021), 102152.

[36] R. C. Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2021.

[37] B. T. Office, 2019 R&D State of Technology, DOE/EE-2082, 2020.

[38] A. Krishnan, M. Likhogrud, M. Cano, S. Edmundson, J.B. Melanson, M. Huesemann, J. McGowen, J.C. Weissman, M.C. Posewitz, Picochlorum celeri as a model system for robust outdoor algal growth in seawater, Sci. Rep. 11 (2021) 11649.

[39] A. Krishnan, M. Cano, T.A. Burch, J.C. Weissman, M.C. Posewitz, Genome editing using Cas9-RNA ribonucleoprotein complexes in the high-productivity marine alga picochlorum celeri, Algal Res. 49 (2020), 101944.

[40] S. Edmundson, M. Huesemann, The dark side of algae cultivation: characterizing night biomass loss in three photosynthetic algae, Chlorella sorokiniana, nannochloropsis Salina, and picochlorum sp, Algal Res. 12 (2015) 470–476.

[41] M. Huesemann, B. Crowe, P. Waller, A. Chavis, S. Hobbs, S. Edmundson, M. S. Wigmosta, A validated model to predict microalgae growth in outdoor pond cultures subjected to fluctuating light intensities and water temperatures, Algal Res. 13 (2016) 196–206.

[42] Q. Bechet, A. Shilton, B. Guieysse, Maximizing productivity and reducing environmental impacts of full-scale algal production through optimization of open pond depth and hydraulic retention time, Environ. Sci. Technol. 50 (2016) 4102–4110.

[43] B.H. Kim, J.E. Coi, K. Cho, Z. Kang, R. Ramanan, D.G. Moon, H.S. Kim, Influence of water depth on microalgal production, biomass harvest, and energy consumption in high rate algal pond using municipal wastewater, J. Microbiol. Biotechnol. 28 (2018) 630–637.

[44] S. Yadala, S. Cremaschi, A dynamic optimization model for designing open-channel raceway ponds for batch production of algal biomass, Processes 4 (2016) 10.

[45] J.C. Goldman, Y. Azov, C.B. Riley, M.R. Dennett, The effect of pH in intensive microalgal cultures. I. Biomass regulation, J. Exp. Mar. Biol. Ecol. 57 (1982) 1–13.

[46] K.R. Hinga, Effects of pH on coastal marine phytoplankton, Mar. Ecol. Prog. Ser. 238 (2002) 281–300.

[47] N.R. Moheimani, Inorganic carbon and pH effect on growth and lipid productivity of Tetraselmis suecia and chlorella sp (Chlorophyta) grown outdoors in bag photobioreactors, J. Appl. Phycol. 25 (2013) 387–398.

[48] A. Malek, L.C. Zullo, P. Daoutidis, Modeling and dynamic optimization of microalgae cultivation in outdoor open ponds, Ind. Eng. Chem. Res. 55 (2016) 3327–3337.

[49] P.A. Lee, K.J.L. Martinez, P.M. Letcher, A.A. Corcoran, R.A. Ryan, A novel predatory bacterium infecting the eukaryotic alga nannochloloropsis, Algal Res. 32 (2018) 314–320.

[50] P.M. Letcher, S. Lopez, R. Schmieder, P.A. Lee, C. Behnke, M.J. Powell, R. C. McBride, Characterization of Amoeboaphelidium protococcarum, an algal parasite new to the Cryptomycota isolated from an outdoor algal pond used for the production of biofuel, PLoS ONE 8 (2013), e56232.

[51] P.M. Letcher, M.J. Powell, S. Lopez, P.A. Lee, R.C. McBride, A new isolate of Amoeboaphelidium protococcarum, and Amoeboaphelidium occidentale, a new species in phylum Aphelida (Opisthosporidia), Mycologia 107 (2015) 522–531.

[52] R.C. McBride, S. Lopez, C. Meenach, M. Burnett, P.A. Lee, F. Nohilly, C. Behnke, Contamination management in low cost open algae ponds for biofuels production, Ind. Biotechnol. 10 (2014) 221–227.

[53] V.L. Harmon, E. Wolfrum, E.P. Knoshaug, R. Davis, L.M.L. Laurens, P.T. Pienkos, J. McGowen, Reliability metrics and their management implications for open pond algae cultivation, Algal Res. 55 (2021), 102249.

[54] A.A. Corcoran, M.A. Saunders, A.P. Hanley, P.A. Lee, S. Lopez, R. Ryan, C.B. Yohn, Iterative screening of an evolutionary engineered desmodesmus generates robust field strains with pesticide tolerance, Algal Res. 31 (2018) 443–453.