U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

Exploring the Utilization of Complex Algal Communities to Address Algal Pond Crash and Increase Annual Biomass Production for Algal Biofuels

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Executive Summary

Algal biomass for biofuels presents an opportunity to contribute significantly to expansion of domestic resources for advanced biofuel production. Algae can be grown on non-arable land with non-potable water, reducing both land-use change and water resource footprints.

Additional benefits of algal biofuels include the following:

1. Utilization of carbon dioxide (CO_2) by algae for growth, providing a carbon sink leading to relative reductions in CO_2 emissions (relative to fossil fuels), or a carbon-neutral footprint
2. Potential for utilization of waste resource streams, leading to water remediation and reduced fertilizer pollution while simultaneously avoiding utilization of limited "clean" water for energy production.

The number one barrier faced by the emerging algae biomass industry is reducing the cost of the finished fuel to be competitive with petroleum-based fuels. This barrier will require creating a new agricultural system with algae at its core.

Significant challenges to creating this agricultural system include the following:

1. Increasing the per quantum productivity, biomass yields, lipid content (>60% at commercial scale),
2. Reducing or removing pond crash occurrences or poor pond performance, as one means to address final cost goals of $3 gasoline per gallon equivalent (gge) by 2030 (Table 1)
3. Developing inexpensive (capital and operating cost expenditures), commercial-scale, outdoor pond systems capable of producing sequential batches and continuous, annual operations
4. Increasing yields of stable biofuel intermediates capable of conversion to advanced biofuels
5. Developing optimal conversion technologies in terms of end product quality and quantity including bi-products (bioproducts)
6. Developing optimal conversion technologies for which the optimal is defined in terms of the final gge price as well as environmental impacts
7. Increasing harvest and processing efficiency
8. Reducing requirements for resources such as fresh water, nutrients, and energy for biomass cultivation in outdoor, commercial scale systems
9. Transferring government-funded technologies to the private sector for continued development, to facilitate rapid commercialization and lead to reduced gge costs, making algal biofuels cost competitive
10. Stimulating workforce development by creating bioindustry green jobs in both rural and urban areas
11. Establishing the United States as a leading producer and exporter of biobased fuels and biobased products.

An example of a major challenge to algal biofuel production at competitive prices is the recurrent issue of pond crash (Box 1) or pond collapse. Pond collapse is typified by total or near total loss of algal biomass due to the natural introduction of a predator or pathogen as well as abiotic (Box 1) phenomena. Culture instability under open-pond conditions is due to increased predation susceptibility, as well as abiotic variation retarding algal growth rates and/or biomass accumulation, e.g., temperature and salinity variation. Algal monocultures are favorable feeding grounds for predators and other pests because they provide an abundant supply of a readily available, high-quality food. "Readily available" means easily obtained with little energy exertion, and for predators, the trade-off between cost of obtaining prey and consuming it can determine where predation occurs (this is important to consider when comparing monocultures with polycultures). Culture stability is not exclusive to the open pond scenario for biofuels production. To date, the production technologies for an algae-based system are not sufficiently developed to meet cost targets set by fuel markets or the U.S. Department of Energy's Bioenergy Technologies Office (BETO) goals. A significant proportion of operational cost impacts on the final dollar/gge value of algal based fuels is due to variation in biomass production losses, whether one is discussing biomass for hydrothermal liquefaction or oil yields, open ponds, or photobioreactor (PBR) systems (Davis *et al.* 2011, Richardson *et al.* 2014). Thus, stabilizing biomass yields for either lipid production or total biomass (the final value product being determined by conversion processes and co-products) is a critical step toward ensuring algal based biofuel's market competitiveness.

The basic requirements to produce competitive, sustainable algal-based fuels include the following:

1. Consistent, stable production with either consecutive batches or continuous cultures
2. Increasing economic cost competitiveness with fossil fuels and terrestrial biofeedstock-based fuels (Box 1) or fuel blends
3. Requiring little or no land-use change and associated potential for increased biodiversity loss or carbon emissions
4. Reducing greenhouse gas emissions and potentially providing carbon-sequestering
5. Requiring minimal to zero fresh water usage in the production process(es).

In this white paper, we briefly review the research literature exploring complex algal communities as a means of increasing algal biomass production via increased tolerance, resilience, and resistance to a variety of abiotic and biotic perturbations occurring within harvesting timescales. This paper will identify what data are available and whether more research utilizing complex communities is needed to explore the potential of complex algal community stability (CACS) approach as a plausible means to increase biomass yields regardless of ecological context and resulting in decreased algal-based fuel prices by reducing operations costs. By reviewing the literature for what we do and do not know, in terms of CACS methodologies, this report will provide guidance for future research addressing pond crash phenomena. We ensure CACS is considered within BETO's Algae Program goals and milestones, briefly addressing both economics and sustainability.

Box 1. Definitions

Word	Definition
Abiotic	Non-biologically based ecological components such as temperature, salinity, and ultraviolet radiation exposure.
Biofeedstock	Any type of biomass utilized to produce biofuels (including intermediates), e.g., short rotation woody crops, perennial grasses, algae.
Biotic	Biologically based ecological phenomena such as predator-prey relationships, competition within and between taxa, and disease.
Community	A group of multiple species (multiple populations of species) interacting directly or indirectly within a defined geographical unit, limited and defined by species tolerances.
Complementarity	Considered causal to biodiversity-ecosystem function and expressed as a shift in plant species biomass present at the community level due to natural selection as opposed to neutral phenomena; i.e., probability of sampling X over Y. An example is the accumulation of nitrogen in community biomass as a result of increased numbers or contribution from nitrogen-fixing plants.
Extremophiles	Organisms capable of living in conditions of temperature, pH, salt, or other chemical extreme.
Niche	Multiple-dimension hyper-volume includes microhabitats, abiotic factors, resources and predators determining species response (physiological, growth, fitness, etc.).
Niche complementarity	Condition when species interactions at the community level are a result of niche differentiation, retarding competitive interactions and increasing species diversity along with ecosystem function through full resource utilization by the community and positive feedbacks between resources used and made available (facilitation).
Niche differentiation/partitioning	Result of natural selection driving species into unique patterns of resource use or ecological tolerances caused by competition for resources including space (as defined by abiotic tolerance).
Over-yielding	Condition when polycultures yield more biomass compared to monocultures of the same species in the polyculture.
Pond crash (collapse)	Natural introduction of predator or pest to algal monoculture typically following high algal biomass accumulation resulting in significant to total algal biomass losses.
Redundancy	Multiple (>1) species within a community/system providing similar or identical system services. Such redundancy can stabilize a system because the loss of one species does not mean the loss of the service provided. An example is the presence of multiple legume species (nitrogen-fixing plant-bacterial associations) present in a community serving to increase the amount of nitrogen available to all community members.
Resilience	The ability to return to a stable state. For example, communities are resilient when they return to a pre-defined stable state following perturbation.
Resistance	The ability to resist perturbation from a stable state.

There is a role in aquatic systems for sustaining productivity via linkages between resilience and niche partitioning (Box 1), as found in plant communities. Aquatic ecosystems researchers and resource managers are utilizing ecological theory and empirical support for the relationship between system stability and species richness/diversity (Hughes *et al.* 2005). Increased community complexity (resulting from increased biodiversity or species richness) includes the development of facilitative relationships between different species, direct or indirect insurance against predator grazing efficiency, and the consequences to predator-prey interactions complicated and mediated by relatively complex prey communities (Corcoran and Boeing 2012). Behl *et al.* (2012) found a positive correlation between species diversity and biomass yield in all phytoplankton examined except cyanobacteria. Increases in algal group diversity were positively correlated with complementarity (Box 1). They concluded that functional identity (defined as functional phylogenetic distance, i.e., niche differentiation) is more important in aquatic systems than in terrestrial systems. Supporting this statement is the observation that transgressive over-yielding (Box 1) occurred in more than half of the polycultures they explored.

The observation of over-yielding also supports non-random selection of community membership when the goal is to develop algal pond communities resistant and/or resilient to a variety of perturbations. Additional support for positive diversity-biomass relationships is provided by Ptacnik *et al.* (2008; see also Zhang and Zhang 2006, Schmidtke *et al.* 2010, Cole *et al.* 2014).

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A. Topic Summary: Fostering Algal Biofuels Production through Research & Development

Federal Goals (Strategic U.S. Department of Energy View)

The key challenges and potentials addressed by the U.S. Department of Energy's Bioenergy Technologies Office (BETO) are based on the dominance of transportation fuel in America's transportation sector. Nationwide, the amount of petroleum consumed for transportation is 70% of transport fuels, accounting for 30% of greenhouse gas emissions (GHGe) (Knittel 2011). Natural gas and biomass-based fuels account for 7% of transportation energy consumption (EIA 2012). Biomass-based fuel and product supplies provide enormous opportunities for job creation nationally through growth of biofeedstocks, as well as production of refined fuels and biomass-derived materials (e.g., plastics), which could potentially increase economic security and development. Both urban and rural economies will benefit from job creation fostered by a novel, production-oriented, and national, biomass-based industry (BETO 2013a). Algal biofuel production can positively impact both rural and urban economies because algal biofuels can be produced in either region depending upon the design system utilized (open ponds versus photobioreactors [PBRs]). Ponds require more space, not unlike large agronomic systems, and thus, would be rurally located. Urban, industrial settings could house PBR-based systems.

By definition, advanced biofuels must reduce carbon dioxide emissions by 50% relative to fossil fuels (EISA 2007). Algal biofuels can contribute to GHGe reductions because algal growth depends on CO_2. Co-locating algal biomass production sites with CO_2-emitting facilities provides the production site with a source of CO_2 required for algae cultivation (Darzins *et al.* 2010) and provides an opportunity to decrease the relative GHGe (relative to fossil fuels) and meet the life-cycle standards required by EISA (2007).

BETO Mission and Goals

The mission of BETO is to "*Develop and transform our renewable biomass resources into commercially viable, high-performance biofuels, bioproducts, and biopower through targeted research, development, demonstration, and deployment supported through public and private partnerships*" (BETO 2013b).

BETO's Overarching Strategic Goal is to "*Develop commercially viable biomass utilization technologies to enable the sustainable, nationwide production of biofuels that are compatible with today's transportation infrastructure and can displace a share of petroleum-derived fuels to reduce U.S. dependence on oil and encourage the creation of a new domestic bioenergy industry, supporting the Energy Independence and Security Act of 2007 goal of 36 billion gallons per year of renewable transportation fuels by 2022*" (BETO2013b).

Milestones for the Algae Program

The goals and milestones for BETO's Algae Program can be found in the Office's Multi-Year Program Plan (BETO 2013b) as well as on the Office's Peer Review Portal (BETO 2013c).

Table 1. Key Algae Program Targets from BETO's Multi-Year Program Plan 2013. Source: BETO 2013b.

Year	Gallons/Acre/Year	Dollar Price (2011$) per Gasoline Gallon equivalent (gge) Algal Oil Intermediate
2014	1,500	$13.13
2018	2,500	$6.30
2022	5,200	$3.27
2030	> 5,200	$3.00

The following are key areas for research and development:

1. Productivity of large-scale algae cultivation
2. Harvest and processing efficiency
3. Resource efficiency (water, nutrient, electricity, and land use)
4. Yield of stable biofuel intermediates capable of conversion to advanced biofuels

The focus of this white paper is operational productivity increase in large-scale algae cultivations. In September 2012, BETO established two initial priority pathways for lipid upgrading to fuel quality from algal biomass: (1) algal lipid extraction and upgrading through biochemical and thermochemical technologies and (2) whole algae hydrothermal liquefaction (HTL). The latter, HTL (Biddy *et al.* 2013, Elliott *et al.* 2013), is ideal for complex communities of algae as it avoids potential necessities of separating algal strains according to cell size, lipid content, and other phenotypic characteristics (e.g., chemical variation) of potential importance in upgrading technologies. The resulting production of an intermediate from HTL processes provide opportunities to upgrade to a variety of fuel types, i.e., gasoline equivalent, bioethanol, biodiesel, and jet fuel (Biddy *et al.* 2013). Much of research suggested herein is considered with the assumption HTL conversion processes will be employed.

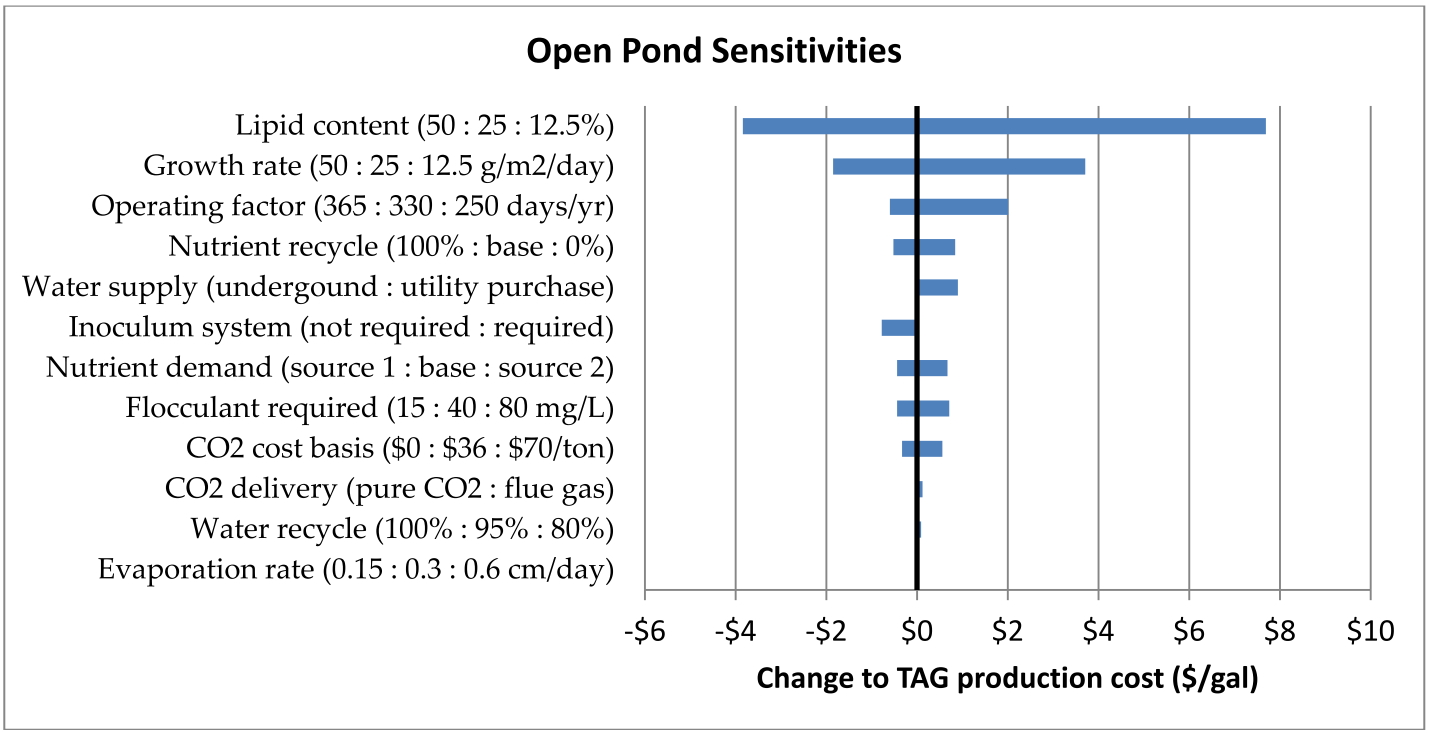

Figure 1. Sensitivity analysis of change to triacylglyceride (TAG) production costs. Source: Davis *et al.* 2011.

The cost of poor, large-scale algae production losses due to phenomena such as pond crash has been quantified via techno-economic models (Davis *et al.* 2011) and is indicated in Fig. 1. Algal biomass growth rates can change production costs from -$2/gal to +$4/gal (Fig. 1). This finding means ecological conditions conducive to rapid, stable biomass accumulation and successful conversion of that biomass to oil can reduce the price by as much at $2 per gallon equivalent (gge). On the other hand, unfavorable ecological conditions can result in pond crash phenomena, altering the cost by -$2/gge to +$4/gge (Davis *et al.* 2011, Fig. 1). This type of sensitivity analyses provides engineers, researchers, and funding agencies with information about where high impacts and opportunities may be realized. The goal is to reduce the variation (sensitivity to causal phenomena) and thus reduce price variation and production costs.

Based on Fig. 1, the two foci showing the highest sensitivity, and thus the highest impact on final costs, are lipid content and growth rate. Focusing on research trajectories reducing pond crash phenomena, increasing and stabilizing growth rate and/or biomass accumulation ultimately lead to reduced production costs. Strain development is an active research topic in algal biofuels; we offer a novel approach with a nascent research history in aquatic systems and utilize a long, abundant research history in terrestrial systems.

B. Empirical & Theoretical Background to Support Polyculture Research—Terrestrial Systems

Plant Systems

A community is defined to include diverse taxonomic groups structured in a complex trophic web or as multiple genera or species from a single taxonomic entity coexisting within a defined space and time. The importance of species or functional group diversity, in terms of community stability and/or ecosystem function, is illustrated by numerous reports. Diverse communities tend to be more resilient and/or resistant to perturbation due to a variety of mechanisms, including niche complementarity, niche differentiation, and functional redundancy (Box 1), to name a few (Tilman 1999, Cardinale *et al.* 2013, Dzialowski and Smith 2008, Zeller *et al.* 2012). An excellent example of the theoretical underpinnings for niche differentiation and improved system function is through classic game theory, e.g., the Prisoner's Dilemma. As Jim Lane recently pointed out in *Biofuels Digest* (Lane 2013), if two algae, A and B, can optimize light harvesting by cooperating, then their individual and combined biomass will be maximized. However, in the real world, such optimization is confounded by incomplete information, cheating, and other phenomena. As will be discussed in more detail later, this is one reason why utilization of extremophilic algae—engineered algae in concert with polycultures—may present the best strategy.

Traditionally, complex agroecology has assumed that complex systems contain multiple species and/or genera (Picasso *et al.* 2008). Nevertheless, fundamental to the argument in favor of complex assemblages is, polycultures provide a means of utilizing feedbacks inherent in complex systems to retain agricultural production in the face of pest pressures and resource shortages. For example, Altieri (1999) discusses the reduced use of noxious chemicals and regulation of microclimates to increase soil moisture via agroecosystem management designed to enhance functional biodiversity in crops. In terrestrial systems, diversity can be accomplished via cover crops, intercropping, agroforestry, and crop/livestock mixtures, to name a few (Altieri 1994). The result of such approaches is optimal nutrient recycling, soil conservation, and natural control of pests (including diseases) while maintaining or increasing crop yields. Not unlike precision agroecosystem management in terrestrial systems, algal crop planning can include utilization of strains or species specific to intra-annual variation, geographic variation, and utilization of polycultures exhibiting facilitation (one species enables another to grow) or other phenomena resulting in stable cropping systems (Jensen *et al.* 2005, Hauggaard-Nielsen *et al.* 2008, Kole 2013).

Resilience and Resistance

Resilience relates to the stability of a community faced with stress/perturbation arising from redundancies of function and differential adaptability to perturbation exhibited by distinct taxa. Thus, the community can return to a previous stable state of productivity (e.g., steady state birth and death rates). This is because there is sufficient functional redundancy (be it species, genera, taxa, or genes) to accommodate the loss of one or more taxa and still retain the community or system stability. Perhaps Elmqvist *et al.* (2003) summarized it best by describing higher resilience as “higher-response diversity.” Complex communities have a greater probability of including strains or species with an optimal response to biotic and abiotic variation, i.e. diurnal variation, salt concentration changes, and pests. Increased community phenotypic variation can positively correlate with increased environmental variation as measured by resistance and resilience to the variation. Thus, variation in diurnal or annual temperatures, salt concentrations, etc., will not, on average, reduce biomass production. Resistance to perturbation is the ability to resist movement away from a stable state (Figure 2). More diverse communities often possess greater resistance to stress because phenomena such as synergistic, mutualistic, or facilitative interactions have a higher probability of occurrence. Since complexity seems to be a prerequisite for community stability in broad taxa ranging from microalgae to trees, it is reasonable to expect complex algal communities may be better suited to traverse the vicissitudes inherent in open pond systems, e.g., temporal temperature changes or salinity variation.

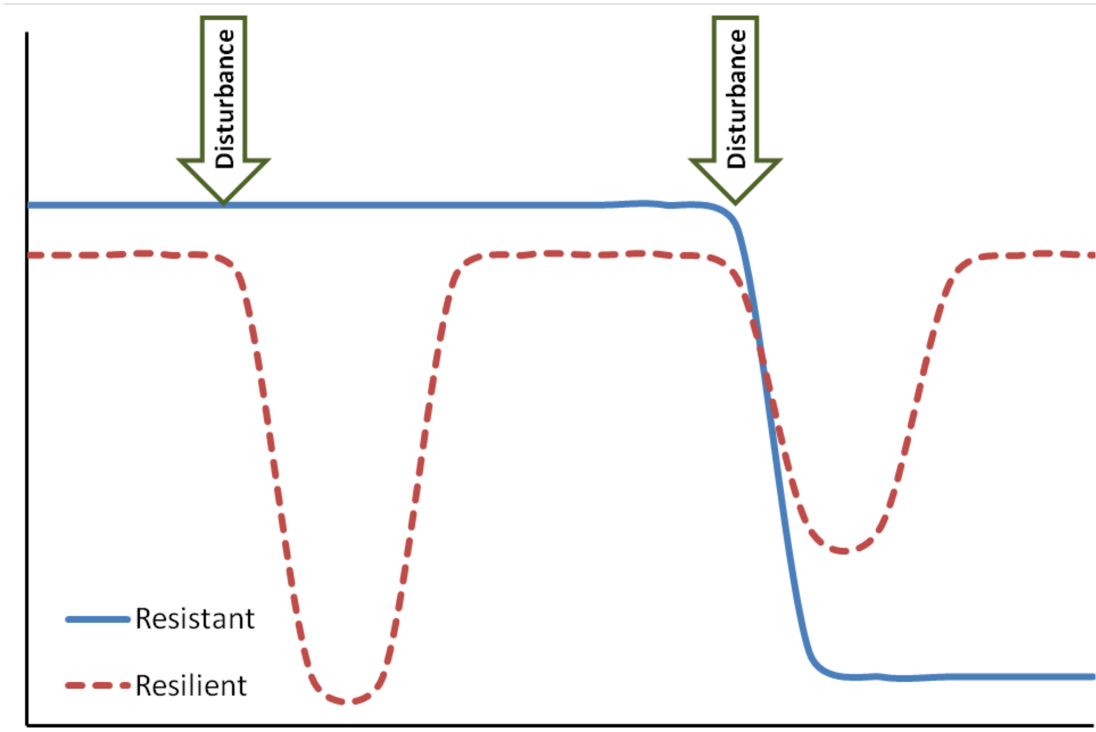

Figure 2. Graphical depiction of resistance versus resilience in response to functional service provided (y-axis) and time (x-axis). Source: Stovall 2012.

C. Background & Brief Research Review of Algal Polycultures

Pond Crash Phenomena and Losses

As suggested by Dzialowski (2008), algal monocultures are likely to be inherently unstable because too many interactions remain possible due to unoccupied niches (Box 1). Communities in general are dominated by two major phenomena, top-down (e.g., predators and pests) or bottom-up (e.g., resource availability) drivers. These same phenomena are the primary factors driving pond crashes, so a potential fix is to control algal response to both top-down and bottom-up pressures by increasing resistance. Owen-Smith and Mills (2008) found high amplitudes for predator-prey oscillations leading to pond crashes. They suggested top-down controls to dampen these oscillations and decrease the probability of pond crash from predation (e.g., zooplankton) via food-web trophic manipulations. Where predator deterrence or decreased predator success is an emergent phenomenon of complex algal community stability (CACS), other means of stabilizing biomass against various causes of pond crash are in the section D "Increasing Algal Biomass Production via Culture Stabilization—Recommendations."

Increasing Algal Biomass Production via Culture Stabilization—Background

The following are the four scale-up challenges listed by the Algal Biofuels Technology Roadmap (BETO 2010):

1. Culture stability
2. Standardized metrics for system-level productivity analysis
3. Nutrient source scaling, sustainability, and management
4. Water conservation, management, and recycling.

This report focuses on stability of large-scale cultures as they are a precursor to productivity, recognizing that a challenge of maintaining algal monocultures at large scale is due to the pervasiveness of predation by a variety of pests (Brussaard 2004, Owen-Smith *et al.* 2008). Strategies to reduce pond crash in response to predation involve utilization of complex algal communities (Paerl *et al.* 2000, Weis *et al.* 2008, Brennan and Owende 2010, Corcoran and Boeing 2012). The alternative is utilization of extremophilic algal strains (Box 1; Dismukes *et al.* 2008). A third possibility is to explore CACS with inclusion of extremophiles or community composition consisting solely of extremophiles.

Open pond systems are likely to exist in a diversity of habitats from southwestern deserts to southern coastlines. A community of temperature- and salt-tolerant extremophiles may be the best strategy in the Sonoran or Chihuahuan deserts, while in temperate ecosystems, the best strategy may be complex communities exhibiting resilience to intra-annual temperature variation in addition to predator resistance. Open ponds with limited access to clean water and high effluent usage may be best populated by extremophiles (native or engineered), for example. Algal polycultures may be more stable to a diversity of perturbations because complex communities contain organisms with tolerances for unique optima and different ranges of ecological/biological/physiological tolerance (Figure 2; Norberg 2004, Tylianakis *et al.* 2008). In addition, diverse cultures (diverse defined as multiple strains and/or algal taxa) can be more resistant to invading algal strains and alga pests. This condition results from what is termed "tighter niche packing," in which more or all possible niches are mined, and thus, invading algal strains are excluded by resource limitation via competition-based mechanisms (Table 1). An excellent example of this is the work by Weissman and Benemann (1978), in which they explored the efficacy of mixed algal cultures in response to biomass recycling. Mixed algal cultures produced steady-state coexistence on limiting resources provided from biomass or effluent recycling. The mechanism identified for this observation is the creation of unique nutritional niches developing as a result of water recycling and the consequential reduction of competition between species. Competition is reduced by different taxa preferentially feeding on distinct nutrients present in the recycled stream.

Key Ecological Phenomena

Systems in nature are not random assemblages, but rather, the result of population responses to a variety of ecological phenomena over ecological and evolutionary timescales. What happens in nature? One study by Ptacnik *et al.* (2010) suggests resource-use efficiency (defined as access to phosphorous) is positively correlated with phytoplankton diversity regardless of habitat (Fig 2). In a study by Dzialowski and Smith

(2008), fresh water zooplankton showed support for complementarity and facilitation as mechanisms to explain species richness. They found that zooplankton feeding on algae did better if their (zooplankton) cultures were mixed, but only if nutrients were high (phosphorous). This occurred regardless of whether or not algal communities were mixed or monocultures. Algae, however, showed increased total biomass declines in mixed algal communities versus monoculture communities. In summary, the study suggests that predation was not retarded by polycultures versus monocultures; it suggests high nutrients could play an important role in community dynamics.

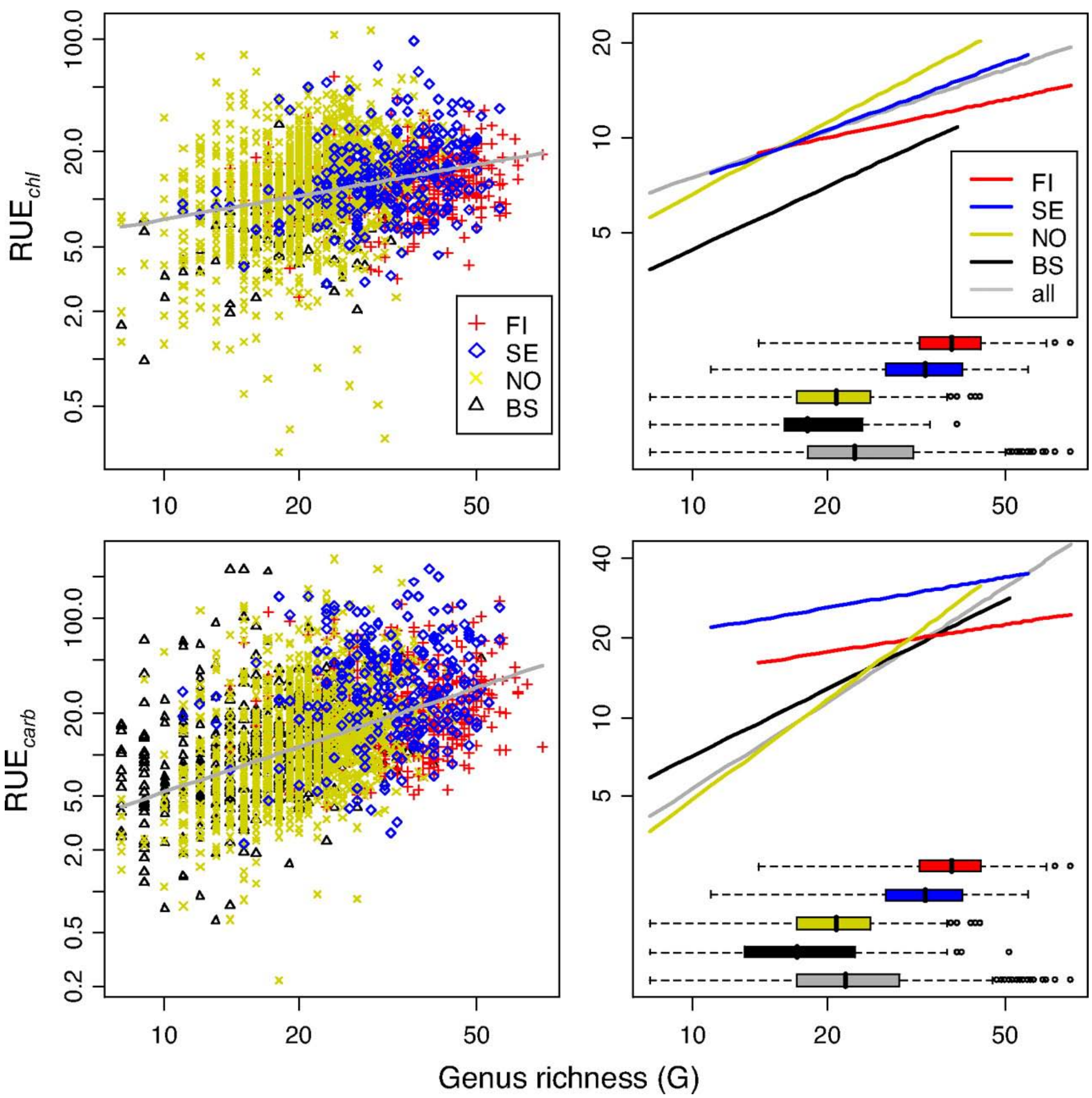

Figure 3. Resource-use efficiency (RUE) as a function of algal genera diversity. RUE$_{carb}$ indicates carbon per unit phosphorus consumed while RUE$_{chl}$ indicates measure of chlorophyll-*a* per unit phosphorus consumed. The colored lines indicate the different geographic regions to which these mixed communities are native: red = FI = Finland, blue = SE = Sweden, yellow = NO = Norway, and black = BS = Baltic Sea, gray is the average of all sites. Source: Ptacnik 2008.

It is critical to note: most polyculture studies involving random assemblages are problematic from a design perspective as well as from a biological perspective (Hooper and Vitousek 1997, Loreau and Hector 2001, Loreau 1998). Polycultures based on random species selection can introduce unnecessary complexity and even lead to erroneous results when testing whether or not *select* polyculture assemblages (designed based on plausible interspecific interactions) are able to remain resistant to pests and perturbation through phenomena such as niche complementarity and partitioning. If the goal of the experiment is to determine whether niche partitioning or complementarity leads to increased resilience and/or resistance of a community to perturbation, then the polyculture needs to reflect an assemblage of species capable of such niche partitioning (Behl *et al.* 2012). It is essentially the same as picking players for a team. Players on a soccer team are not selected at random; players are selected based on distinct skill sets because this increases the probability of a team composed of members with unique, complementary skills and some skill redundancy. Such a team is less likely to be "perturbed" or "thrown off their game." This selective polyculture (or team membership) approach was successfully approximated by the Corcoran and Boeing study (2012) (see also Behl *et al.* 2012, Striebel *et al.* 2009, Schmidtke *et al.* 2010). For example, Corcoran and Boeing (2012) chose species for their algal polycultures based on algal cell size and resulting impacts of rotifer predation. Mixed prey body size can decrease predation (Hansen *et al.* 1997, Steiner 2001) by increasing predator costs through phenomena like search time (an energy intensive process for the predator) or abundance of prey the predator is hunting.

D. Increasing Algal Biomass Production via Culture Stabilization—Recommendations

What We Know About Complex Algal Communities

To design polycultures for the desired function of optimal biomass production over time, researchers need to consider multiple ecological parameters (Pienkos and Darzins 2009), including the following:

1. Maximum growth rates within polycultures
2. Responses to nutrient and light utilization efficiency in polyculture
3. Stability of polyculture to perturbation
4. Resistance of the polyculture to pests (including invading algal strains/taxa).

Resources and considerations required for algal biomass include but are not limited to the following:

1. CO_2 (source)
2. pH control
3. O_2 (mixing regime)
4. UV (mixing regime)
5. Nutrient availability and concomitant salinity control
6. Temperature control
7. Evaporation redress
8. Pest and contamination redress
9. Ease and efficiency of harvest.

Many of these items allow for niche differentiation versus competition for limiting resources and provide opportunities for facilitation through strain/species selection and/or engineering.

Biomass in Polycultures (Complex Communities)—Recommendations

Approaches to address pond crash phenomena should utilize a breadth of theoretical knowledge and technology to develop robust resilient biomass production in response to current climate, future climate change, highly variable geographic differences, etc. This includes both breeding and reverse engineering (via synthetic biological approaches), whereby multi-species assemblages can be created and experiments can be designed based on the environmental context to which the pond is exposed.

What we do not know is more than what we do know in terms of CACS. Algal biofuel production both in the U.S. Department of Energy's Aquatic Species Program and in its current incarnation focuses on individual strain selection and monoculture cultivation systems (Sheehan *et al.* 1998). The UTEX Culture Collection at the University of Texas, Austin, (http://web.biosci.utexas.edu/utex/) currently includes 3,000 unique living, native (not engineered or cultivated) algal strains with concomitant identification of a range of environmental tolerances (phenotypic characterization). Thus, there is a rich knowledge base and library to select from for environmental requirements of specific algal strains for CACS.

We recommend the utilization of these strains, databases, and associated research literature to design specific communities with algal strains and species selected to reduce competition among algal strains. How can this be done? Designed communities can be based on niche differentiation, probability of niche complementarity, and different abiotic and biotic tolerances reflective of the geographic and climatic conditions of the experimental setting. We think this approach of non-random species selection for the community, as per Corcoran and Boeing (2012), is more likely to identify community assemblages expressing reduced competition and increased biomass production due to niche partitioning and complementarity, the desired end production of algal communities resistant to pond crash. To complement empirical work, creation and utilization of models to inform, guide, and improve research through iterative, recursive research is warranted.

Proposed Analyses Specific to Polyculture Production of Algae

Commercial-scale algal biomass production, especially in conjunction with HTL, provides a promising application for biofuel and energy generation. To achieve economical, large-scale production, resource additions need to be reduced. Utilization of brackish water, low- to no-nutrient additions, and water recycling can reduce basic resource consumption. An additional benefit of algal biomass for biofuels is the necessity of utilizing CO_2 to feed algal/cyanobacterial communities. This provides a CO_2 sink and possible GHGe mitigation strategy in the production of biofuels and energy.

Based on the findings of Dzialowski and Smith (2008), low-nutrient environments combined with polycultures may be more stable. If top-down feeding by zooplankton is inhibited by low-nutrient content, which is likely to be due to bottom-up causal phenomena, then low-nutrient environments combined with polycultures may be more stable. If algae in low-nutrient environments are less likely to be preyed upon, then their relative increase in biomass may be sufficient within harvesting time ranges of less than a week to avoid pond crash. These are questions to be addressed by research in pilot scale open pond systems.

A brief literature survey suggests a combination of industrial biotechnology, fundamental ecology, and –omics techniques can lead to creation of artificial algal/cyanobacterial communities more resilient and resistant to a variety of perturbations. What is clear from this paper is that additional research is warranted at the field scale using non-random taxonomic assemblages. Utilization of taxa with diverse nutrient requirements (from UTEX and similar culture collections/libraries), pH tolerances, salt tolerance, and ultraviolet (UV) radiation exposure needs are more likely to lead to non-competitive communities with facilitative action expressing potential to withstand unexpected, unpredictable perturbation. Exploration of libraries such as UTEX for the taxa will facilitate choice of strains/species when designing community assemblage specific to environmental context of interest/focus. Additional research efforts by national labs and academic institutions include sequencing algal genomes and generating –omics data to identify (1) candidate genes important in desired phenotypes, (2) algal species and/or strains which can be grouped functionally leading to community stability, and (3) symbiosis genes. Radakovits *et al.* (2010) identified many genes involved in algal stress tolerance, especially those related to reactive oxygen species scavengers (e.g., antioxidants). Photosynthetic light-harvesting complexes, as well as other physiological and chemical parameters, may be the best means of identifying functional groups for community assemblage (Anandarajah *et al.* 2012). As discussed above, communities designed to utilize different light wavelengths and/or intensities to express different abiotic tolerances and to possess different morphological and chemical characters are more likely to show niche differentiation and, as a result, produce communities in which over-yielding occurs. By removing the probability of competition between algal species/strains and allowing for complementarity in resource utilization, maximum resource utilization ought to occur. Communities of different shape and size and chemistry will make foraging by a common predator challenging. If, in addition, extremophiles are introduced, then the potential predator or pest pool is further reduced.

Methods for Identifying Complementary Species for Polyculture Design

According to Padilla and Allen (2000), potential "markers" for algal functional groups include, but are not limited to the following:

- Photosynthetic rate
- Nutrient use rate and/or efficiency
- Abiotic susceptibility (resistant or resilience)
- Biotic susceptibility (tolerance or resilience)
- Successional role (competition versus facilitative interactions).

Phylogenetic distance (a measure of both history and evolution) can also be utilized to select group membership based on potential functional.

More About Extremophiles and Engineered Communities

It is conceivable that algal strain development of extremophiles is capable of optimal growth in response to extreme UV exposure, annual and intra-annual, as well as diurnal temperature variation. The utilization of effluent and municipal waste water could produce robust communities with maximal biomass production (Aitken and Antizar-Ladislao 2013) in environments exposed to high predation or extreme environmental variation. We suggest considering community dynamics in concert with extremophiles. As such, long-term stability of production may benefit from a community of extremophiles, behaving in a successional or complementary fashion (Behl *et al.* 2012). An example of such phenomena can be found in terrestrial systems as reported in the Biomass Resource Assessment Report 2012 (UCS 2012). In the report, biofeedstock productivity was increased by utilizing multiple plant species, or polycultures, as a means of maximizing resource use while decreasing the resource inputs (e.g., complete nitrogen utilization with concomitant reduction in nitrogen fertilizer applications). Maximized resource use happens as a result of niche complementarity in which species "mine" most or all available niches, optimize in them leading to maximized resource utilization. This also could reduce resources available to unwanted (weedy) algal species.

Another approach, not mutually exclusive of any proposed thus far, is engineering novel properties and/or interactions between organisms to produce complex stable communities. These communities could include extremophiles and species/strains capable of niche differentiation in terms of UV tolerance/utilization, nutrients, or other basic resource. For example if a range of light-harvesting pigments can be engineered into algae to allow for utilization of a maximum range of UV, then one can plausibly expect (1) reduced photoinhibition and (2) reduced pond crash in response to salt tolerance, predation, etc. Engineering strains with a range of salt tolerance would allow for maintenance of high productivity over a wide range of salt concentrations resulting from local ecological phenomena, e.g., water evaporation rates, source water. Thus, pond evaporation could be addressed with fewer fresh water inputs. This would also facilitate utilization of water from municipal and agricultural waste, desalination plants, and coastal or otherwise brackish habitats. Dismukes *et al.* 2008 found both decreased water utilization and contamination (by pests) in communities composed of extremophiles.

Waste Water and Extremophiles in Brief

Several authors support the utilization of waste water to grow algae (Lundquist 2010, Brennan and Owende 2010, Carney *et al.* 2013). Multiple benefits include accounting for limited supplies of fresh water through water remediation, thereby reducing the cost of biofuel production and creating or increasing the market value for non-potable water supplies, and finally, removing exposure of the larger environment to contaminated water with multiple, positive consequence to habitats, the ecosystems. Utilization of extremophiles (native or engineered) may foster effluent usage and reduce overall resource requirements involved in growth algal biomass while simultaneously addressing pond crash. Assuming the extremophiles are able to utilize waste water from a variety of sources with high or highly variable parameters over time (e.g., high in metals, nutrients, and salinity, or high variation thereof), then sustainability is in the form of reduced fresh water usage. This is also a means of remediating gray or various types of waste/effluent streams. Since extremophiles, by definition, can tolerate what most organisms cannot, their utilization would reduce the probability of pond crash due to predation or other pest introduction. Presumably, most pests and predators would not survive extreme ecological conditions. This type of hypothesis can be quickly and inexpensively tested and warrants considerations.

Conversion processes and by-product development must be considered when utilizing waste water in concert with extremophiles, due to the potential challenges metal ions and other toxins present and problematic in co-product development from algae grown in waste water, e.g., feed and nutraceuticals. Though an important topic, it is outside the scope of this white paper. We mention it because it is an important consideration when designing research programs and emphasizes the importance of end product quality as well as quantity.

This is part of an all-of-the-above approach which considers context-determined challenges and opportunities (e.g., colocation to waste water treatment plant, location in Sonoran desert, location in eastern part of Washington State).

White Paper Participants and Acknowledgements

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