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Species Diversity Affects Biomass Productivity and Resistance to *Daphnia* Grazing in
Freshwater Algae Communities

A thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Xianyuan Zhang

Committee in charge:

Professor Jonathan Shurin, Chair
Professor David Holway
Professor Scott Rifkin

2018

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University of California, San Diego

2018

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ABSTRACT OF THE THESIS

Species Diversity affects Biomass Productivity and Resistance to *Daphnia* Grazing in
Freshwater Algae Communities

by

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Master of Science in Biology

University of California, San Diego, 2018

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As increasing human demand and decreasing in global reserve, fossil fuel will no longer be able to sustain human energy usage in the near future. Algae based biofuel is an alternative energy source that has the potential to replace the demand of fossil fuel due to the high productivity and minimum land usage. However, considering the high cost, algae biofuel is not

ready for practice in large industrial scale. Scientist put a lot of efforts on modifying algae genome to improve their productivity. But the ecological approach on create algae productive communities has been rarely practiced. In this study we tested how algae biomass productivity and resistance by grazers affected by algal species diversity. 11 different species of single celled algae were assigned into different combinations, and the changing in chlorophyll- α concentration and cell number for each treatment group has been recorded as the reference of cell productivity. *Daphnia* was introduced into the culture to test the ability to resist consumer grazing for each combination. There is no liner relationship among algae diversity, biomass productivity and resistance to *Daphnia* grazing. The biomass productivity and the ability to resist *Daphnia* grazing varies widely between combinations. Overyielding only been observed in some specific algae combinations. It is possible to reduce the cost of algae-based biofuel by creating a productive algae community. Extra effort is required to find the desired combination.

Species Diversity Affects Biomass Productivity and Resistance to *Daphnia* Grazing in Freshwater Algae Communities

Introduction

With increasing population and the improvement of technology, fossil fuel exploitation can hardly sustain human demand, and will become unavailable in the near future (Lee *et al.* 2014). In addition, accumulating carbon in the atmosphere threatens the stability of the climate and ecosystems. Finding alternative energy sources to replace the demand of fossil fuel is therefore an urgent imperative. One approach is converting the lipid extraction from algae into biodiesel and gasoline through process called transesterification and distillation (Amin 2009, Luo *et al.* 2010). Previous studies suggest that even though biofuel can be generated from many different organisms, single-celled algae may be favored as a bioenergy stock due to their high productivity and ability to grow in non-arable environments (Georgianna & Mayfield 2012; Shurin, Mandal & Abbott 2014). From the data provided by US Department of Energy (2010), only 4% of US land area could theoretically be used to culture enough algae biomass to satisfy the fossil energy demand of the entire US. By contrast, oil palm, which is one of the most oil productive plants, would require up to 23% of land area to generate the same amount of fuel. In addition to the minimum land usage, algae biofuel also does not require arable land, and may also be useful in bioremediation, nitrogen fixation, as well as production of valuable co-products like bio-pharmaceuticals (Chisti 2007; Georgianna & Mayfield 2012; Mayfield & Golden 2015; Suresh & Guieysse 2004).

However, the commercial potential of algae-based biofuel production has not been realized due to the high cost for maintaining algae productivity at industrial scales and the loss of energy when converting into biofuel (Georgianna & Mayfield 2012). Algae biofuel cannot compete with fossil fuels unless the rate of biomass production per area is increased. Most current studies focused on genetically manipulating algae productivity by either created a super-productive genetic hybrid (Larkum *et al.* 2012; Ajjawi *et al.* 2017), or genetically modify algae's metabolic reactions (Castruita *et al.* 2011; Radakovits *et al.* 2010). However, genetically engineered algae strains cannot be released into open environment, due to concerns about the danger of spreading from cultivation, invasion of natural habitats and potential extinction and loss of biodiversity as well as transformation of natural ecosystems. Further research into the risks and benefits of genetic modification of algae are needed before such biotechnology can be widely applied (Szyjka *et al.* 2017). On the other hand, ecological approach aims to create algae productive communities with combinations of local algae strains, which can reduce the concern of species invasion and can be practiced outside the laboratory in the local environment (Shurin *et al.* 2013). Unfortunately, unlike the genetic approach, ecological approach has been rarely practiced (Shurin, Mandal & Abbott 2014; Beyter *et al.* 2016; Rakowski and Cardinale 2016).

In the past decades, hundreds of studies showed that because different species can access different parts of the resource spectrum present in the environment, species diversity will often increase and sustain overall biomass productivity in both terrestrial and aquatic environment (Gross 2014; Ives & Carpenter 2007; Stockenreiter *et al.* 2012). However, the relationship between biodiversity and ecosystem productivity is highly variable, and some studies even suggest that biodiversity could reduce productivity by intraspecies competition (Holmgren *et al.* 1997). Therefore, whether algae species diversity enhances biomass productivity is the

theoretical premise of ecological approach to reduce the cost of biofuel production (Shurin, Mandal & Abbott 2014).

Diversity can increase productivity both by increasing the efficiency of resource use and conversion, and by dampening top-down control by consumers. In the productivity study of 147 grassland plots, Tilman *et al.* showed that both plant productivity and soil nitrogen utilization increased as plant diversity increased (1996). In addition, Liu and his colleagues demonstrated that consumer grazing had less top-down impact on plant biomass in high species diversity grassland systems (Liu *et al.* 2015). Consumers may exert weaker control of biomass in diverse resource assemblages as more defended or resistant species or strains increase in abundance in response to declines of taxa in response to grazing. Whether diversity of algae increases productivity in the presence or absence of consumers remains to be shown.

In this study, we used laboratory experiments to test the hypothesis that algae species diversity increases biomass productivity and the ability to resist *Daphnia* grazing. Seven different single-celled freshwater algae species were cultured into different combinations and in the presence and absence of *Daphnia* grazers to analyze the relationship among species diversity, maximum biomass productivity and maximum growth rate. Our goal was to ask whether diversity of algae can enhance bioenergy yield both by increasing biomass production in the absence of grazers and by mitigating the top-down impact of consumers

Methods

Preparing and culturing phytoplankton

Six different species of unicellular green algae (*Scenedesmus dimorphus*, *Botryococcus braunii*, *Chlorella vulgaris*, *Neochloris oleabundans*, *Scenedesmus obliquus*, and *Chlorella*

minutissima) and one species of diatom (*Navicula* spp.) were selected from the phytoplankton collection in Jonathan Shurin's lab, based on their phylogenetic and structural differences. Selected phytoplankton species were transplanted either from a stock cultured flask or from an isolated colony grown on solid medium in a petri dish. All seven phytoplankton species were maintained as monocultures in 1 L flasks filled with filtered COMBO medium (Kilham et al., 1998) in an incubator set to a 12-12 light cycle with a fixed temperature at 17°C. All species had been cultured for at least one month to make sure they all reached the stationary phase before the experiment.

Culturing Daphnia and Daphnia selection

The *Daphnia* involved in this experiment was collected from Helen Lake, in Yosemite National Park, California. *Daphnia* undergo asexual reproduction when maintained under favorable living condition with unlimited food supply (Decaestecker et al., 2009). We isolated each individual *Daphnia* into test tubes filled with COMBO medium. The *Daphnia* were feed with *Nannochloropsis* paste every two days and the medium was refreshed weekly. We harvested all juveniles daily and placed them into new culture tubes.

Chlorophyll-Weight regression curve

Different species of phytoplankton vary in-cellular chlorophyll- α content and average cell biomass (Reynolds, 1993). To ensure all species in this experiment had same initial biomass at the beginning of the experiment, we generated chlorophyll-weight regression curve for each species. Each species was diluted into 4 different concentrations (1:2.5:5:10) with 3 replicates each. The chlorophyll- α content of each concentration was measured using a Turner Trilogy fluorometer with an *in vivo* chlorophyll- α module. All replicates were filtered on pre-combusted

filter papers and oven dried under 45°C for 48 hours. The dry-weight biomasses were measured by weighting each filter paper before and after filtering and taking the difference. The regression curve was then generated by fitting the chlorophyll- α readings and dry weight measurements onto a linear function.

Grazing experiment

All phytoplankton species were transplanted into sterilized 50mL glass flasks with cotton plug and aluminum foil on top to prevent contamination. To ensure all experimental units started with same initial total biomass of 2.5mg/L, we used fluorometer to measure the phytoplankton concentration and the regression curve to estimate the amount of culture we need to add into each flask. Phytoplankton species were transplanted into either monoculture or a mixed culture. In this experiment, 7 monocultures (1 for each species) and 11 polycultures (9 with combination of 2 species, 1 with 3 species, and 1 with 4 species) were generated. Each culture combination consisted of 10 identical replicates filled to 25mL with filtered fresh COMBO medium. *Daphnia* juveniles were introduced two days before the experiment randomly to half of the experimental cultures. The experiment was executed in two stages with 4 species in stage one and 3 species in stage two due to the large number of replicates (Table S1, Table S2).

All cultures were grown in the same incubator with equal light input. The incubator was set to 17°C with 12-12 light cycle. Every two days, 5mL of culture was removed from each flask for analysis and replaced with 5mL fresh COMBO medium to represent a 20% renewal rate for every 2 days. We used fluorometer to measure the *in vivo* chlorophyll- α concentration in each sample and Lugol's iodine to preserve 1mL of each sample for cell counting purpose. We also used sterile micropipettes to remove all the new born *Daphnia* juveniles as the experiment

progressed to maintain a consistent grazing pressure. If a *Daphnia* died during the experiment, we removed the culture with dead *Daphnia* from the experiment.

We monitored phytoplankton growth by plotting chlorophyll concentration against time. When the curve leveled off at a steady state, we assumed that culture reached its stationary phase and that treatment was ended.

Data were analyzed by using *grofit* in R programming to fit each treatment into theoretical growth curves. The max productivity and max growth rate for each treatment had been collected.

Cell counting

In order to determine how species performed in the polycultures, we estimated cell densities by species. Algae culture samples were selected from three specific dates during the experiment which represented the lag phase shortly after introduction, exponential phase, and stationary phase were collected for cell-counting under the microscope. A 10 μ L well mixed sample from each Lugol's iodine preserved culture was transferred onto DHC-N01 hemocytometer chip for cell counting. The counting area on each chip has 9 1 mm² large squares arranged in order. The central square is divided into 25 0.04 mm² small squares. We discriminated and counted every cell present in all four corners and the center square. If a sample had more than 300 cells in one large square, we then only counted the cells on 5 small squares from the center square. The counting chamber on the chip has a depth of 0.1 mm. After counting cells from either 5 large squares or the central 5 squares, the result will be converted into cells per mL (Andersen pp240-250).

Results

Four different species of phytoplankton with 11 different combinations were involved in the 1st stage of growth experiment to evaluate how species richness will affect the biomass productivity and *Daphnia* grazing in freshwater phytoplankton community.

Table 1: Variables affect algae growth and their significance. “Algae” means the species of algae in the treatment. “Daph” means the presence or absence of *Daphnia*. “Date” means the experimental date. Values in **bold** means they are statistically significant.

variable	Df	sum of square	mean Square	F	p
Stage 1					
Algae	10	14.2	1.42	63.103	< 0.0001
Daph	1	39.7	39.72	1764.813	< 0.0001
Date	10	511	55.1	2448.194	< 0.0001
Algae:Daph	10	1.5	0.15	6.748	0.00024
Algae:Date	86	15.7	0.18	8.099	< 0.0001
Stage 2					
Algae	6	22.9	3.82	50.46	< 0.0001
Daph	1	9.9	9.93	131.242	< 0.0001
Date	10	504.2	50.42	666.575	< 0.0001
Algae:Daph	6	5.1	0.85	11.177	< 0.0001
Algae:Date	50	72.3	1.45	19.121	< 0.0001

Chlorophyll- α concentration for all 11 algae combinations throughout the experiment and are shown Figure 1. Experimental algae productivity was strongly affected by both the algae species or combination ($p < 0.0001$, Table 1) and the presence of *Daphnia* grazers ($p < 0.0001$, Table 1). Different growth curves and maximum chlorophyll- α concentrations were observed between treatments, indicating that the maximum growth rates and stationary phase density differs among species and combinations (Figure 1). Most species and pairs showed a decrease in

density in response to *Daphnia* grazing. However, in the treatment that combined *Scenedesmus dimorphus* with *Botryococcus braunii*, we observed the cultures with *Daphnia* had chlorophyll-*a* concentrations greater than the cultures without *Daphnia*. Moreover, in some of the treatments, none of the *Daphnia* survived throughout the experiment (Figure 1).

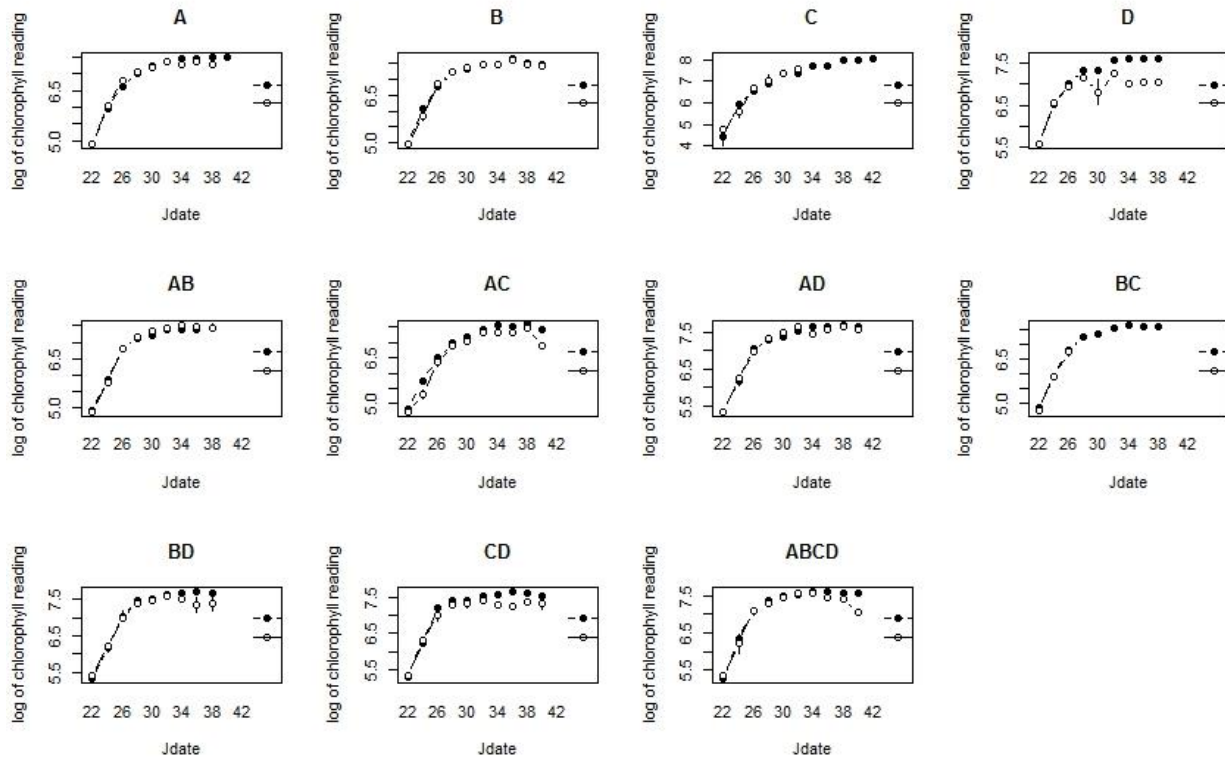


Figure 1: Growth curves for ABCD with and without *Daphnia*. A is *Scenedesmus dimorphus*, B is *Botryococcus braunii*, C is *Chlorella vulgaris*, and D is *Navicula* spp. x-axis is date, where experiment started on 22; y-axis is the log of chlorophyll concentration. Black dots represent the cultures grow without *Daphnia* grazing, and the white dots represent the cultures with *Daphnia*.

The impact of *Daphnia* on algal biomass varied among species and combinations of algae (Figure 2, $p=0.00024$, Table 1). The mixed cultures did not always show productivity close to the mean value of its component species. For instance, in the combinations of *Scenedesmus dimorphus* and *Navicula* spp., we observed the overall productivity was higher than either of the

component species grown alone. In the treatments that mixed *S. dimorphus* with *Botryococcus braunii* and mixed *Chlorella vulgaris* with *Navicula* spp., the maximum chlorophyll densities were lower than the mean of their component monocultures (Figure 2). Thus, the productivity of the mixed-species algae cultures varied widely relative to the maximum and mean of the two monocultures.

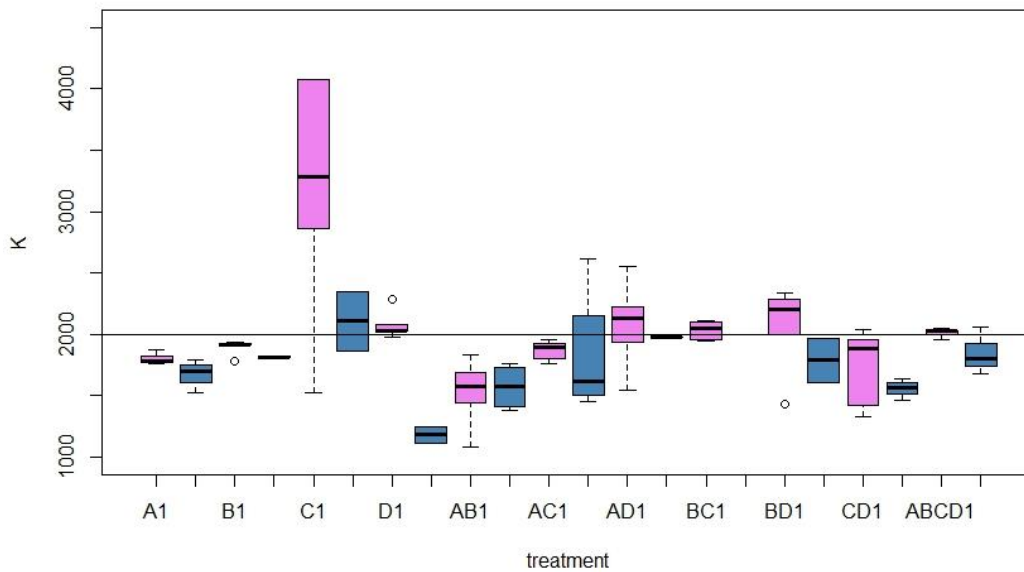


Figure 2: k-value based on chlorophyll concentration for the first set of experiment. X-axis is the treatment groups and y-axis is the carrying capacity. Two bars for each treatment group. Pink bars represent the cultures without *Daphnia* and the blue bars represent the cultures with *Daphnia*.

Daphnia also played a significant ($p < 0.0001$, Table 1) role in algae biomass productivity. The magnitude of grazing pressure varied significantly between treatments ($p < 0.00024$, Table 1). Some algae treatments showed a relatively higher resistance to the presence of *Daphnia*, while others were more vulnerable (Figure 2, Figure 3). Moreover, the *Daphnia*

effects on polycultures were less negative than the mean *Daphnia* effects of their component monocultures (Figure 3), which indicates polycultures were less vulnerable to *Daphnia* grazing than the monocultures. In addition, the treatment that mixed *Scenedesmus dimorphus* with *Botryococcus braunii*, tended to have slightly higher maximum density when *Daphnia* was present (Figure 2, Figure 3).

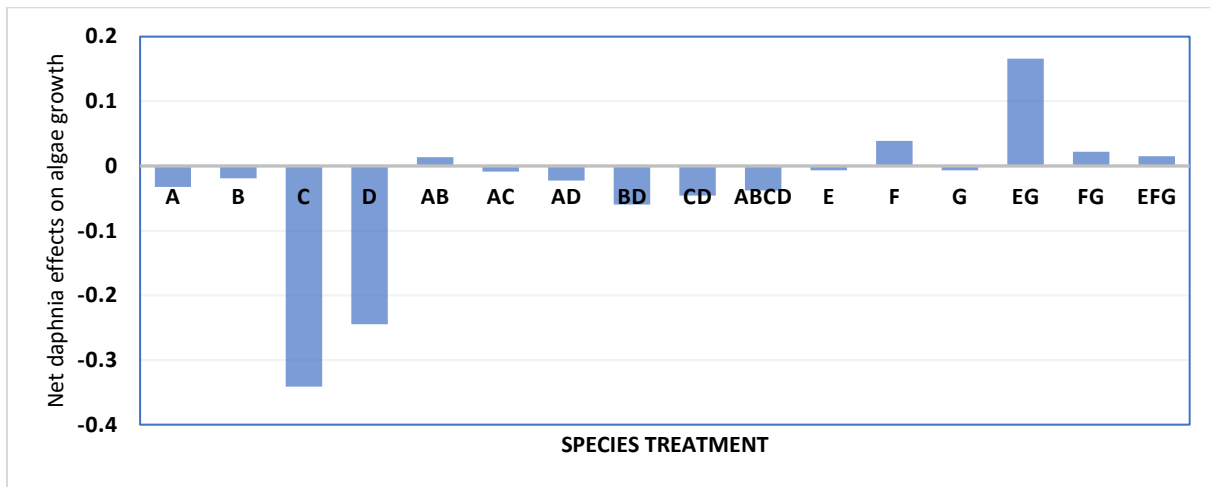


Figure 3: *Daphnia* effects on each treatment. Relative to the biomass productivity of same combination without *Daphnia*. Each bar represents one treatment group. The capital letters described the component algae species in that treatment group. Meaning of each letter can be checked in Table S1 and Table S2.

We also analyzed the max growth rate for each treatment. Species pairs tended to have higher maximum growth rates than monocultures (Figure 4). Moreover, the maximum growth rate of *Botryococcus braunii* and *Chlorella vulgaris* monocultures and the polyculture that mixed *S. dimorphus* with *Botryococcus braunii* increased in respond to the presence of *Daphnia*, while the other treatments had no respond or slightly decrease in the maximum growth rate (Figure 4).

In the 2nd stage of the experiment, like what we saw in previous experiment, Different algae combination also significantly affected algae biomass in terms of chlorophyll- α

concentration in the 2nd stage of the experiment ($p < 0.0001$, Table 1). The overall productivity of *Scenedesmus obliquus*/*Chlorella minutissima* mixture was lower than either of the component species cultured separately, while the productivity of other mixed cultures fell between the component species (Figure 5). We also found that all the mixed treatments had higher or similar maximum growth rate as the monoculture treatments, consistent with the result from the 1st stage of the experiment (Figure S1).

Although *Daphnia* still affected algae productivity significantly in the 2nd experiment ($p < 0.0001$, Table 1), they were less effective at suppressing algae growth in many treatments. Grazing only had negative effects on the monocultured *Neochloris oleabundans* and *Chlorella minutissima*, and had positive effects on monocultured *Scenedesmus obliquus* and all the polycultures (Figure 3, Figure 5). In addition, in the presence of *Daphnia*, cultures mixed *Neochloris oleabundans* with *C. minutissima* and *S. obliquus* with *C. minutissima* had higher max growth rate than the ones without *Daphnia* (Figure S1).

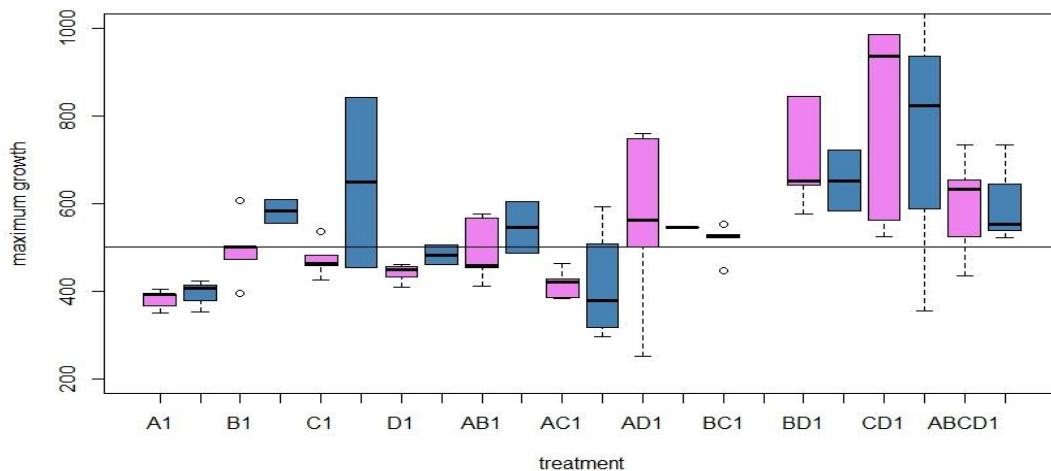


Figure 4: Max growth rate for the first set of experiment. The y-axis is the maximum growth rate for each treatment groups showed on x-axis. Two bars for each treatment group. Pink bars represent the cultures without *Daphnia* and the blue bars represent the cultures with *Daphnia*.

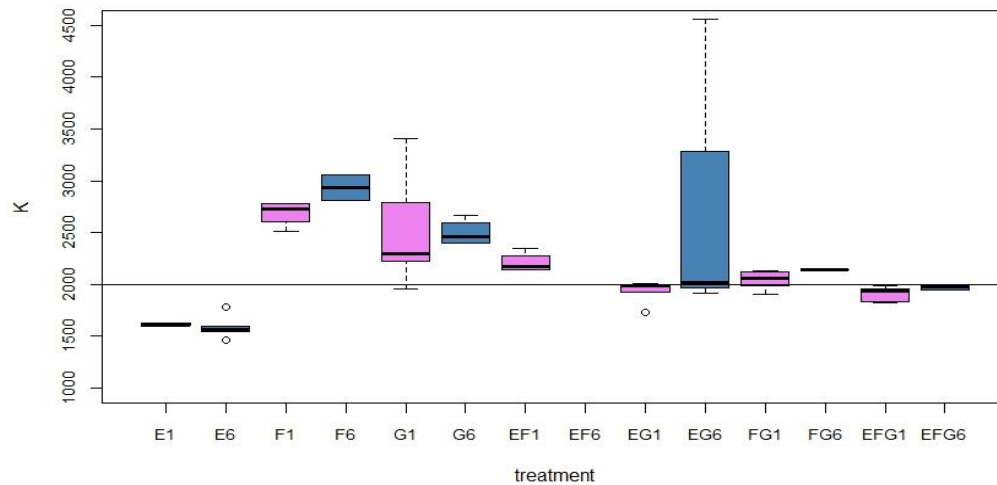


Figure 5: k-value based on chlorophyll concentration for the second set of experiment. Two bars for each treatment group. Pink bars represent the cultures without *Daphnia* and the blue bars represent the cultures with *Daphnia*.

Although all algae species that were introduced into each treatment at equal biomass at the beginning of the experiment (Table 1S), their growth varied considerably (Figure 6). In all the treatments containing *Scenedesmus dimorphus*, *S. dimorphus* tended to grow faster than when it was grown alone, and it always dominant the other species (Figure 6a, b, c, and e). By contrast, *Botryococcus braunii* tended to have relatively low competitiveness when equally mixed growth with *S. dimorphus* or *Navicula* spp. (Figure 6a and d). When four algae species were cultured together, the proportions of each species in the culture were less variable while when only two species were cultured together the difference is greater (Figure 6). Some mixed cultures had total relative cell number over 1.0 when they entered stationary phase, which suggested that in the term of cell numbers, their overall productivity is higher than each component species grew separately (Figure 6).

Based on the date from cell counting, we also found that the *Daphnia* effect on algae productivity varied strongly with algae composition. In the culture *S. dimorphus* mixed with *B.*

braunii, *Daphnia* grazing barely changed the proportion of each species, but when *S. dimorphus* mixed with *Navicula* spp., both species numbers were grazed down significantly by *Daphnia*. Moreover, in the cultures that mixed *S. dimorphus* with *C. vulgaris* and *B. braunii* with *Navicula* spp., in the presence of *Daphnia*, the proportion of each component species in the culture were less divergent than the same mix without *Daphnia* grazing, which indicated the dominance hierarchy is less distinct for certain algae species combinations when *Daphnia* exist (Figure 6).

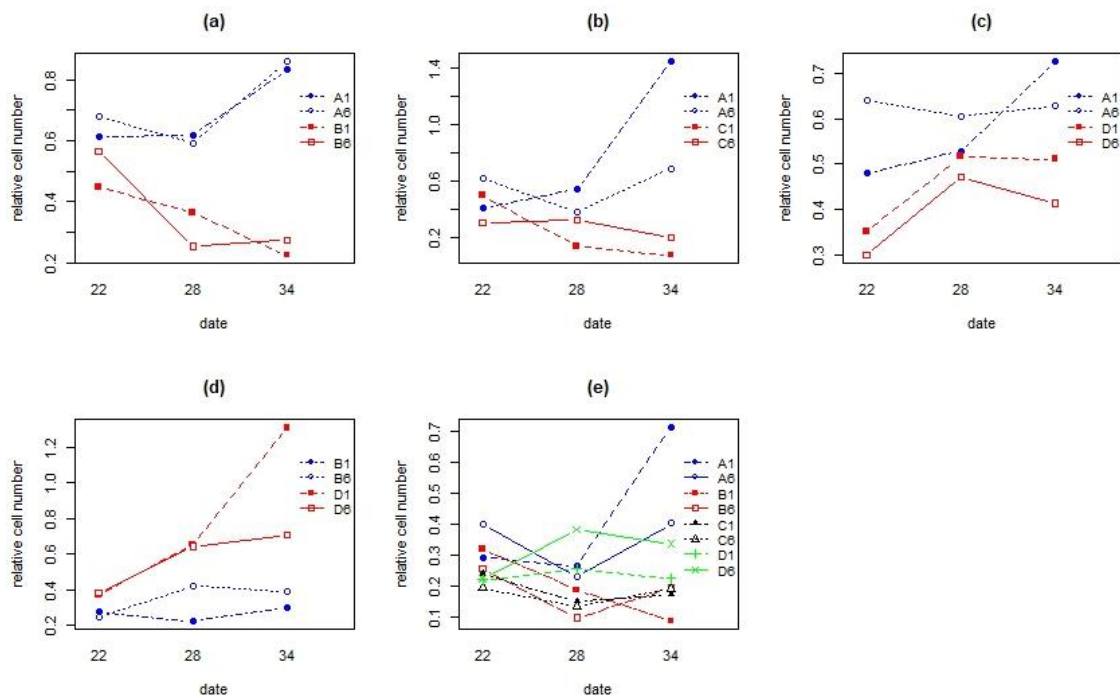


Figure 6: Cell Counting data. Number is relative to the biomass of the same species in monoculture in the same date.

Discussions

Our results indicate that mixed algae communities can sometimes show overyielding or higher biomass productivity and greater resistance to grazing than any of the component algae

species in the community by itself. However, overyielding and grazing resistance only occurred for certain species combinations. Algae involved in our study have diverse characteristics including nutrient requirements, cell size, shape, lipid ratio, replication speed, and stationary phase density (Shurin *et al.* 2014). Our results indicate that when algae from multiple taxa were cultured together, the maximum growth rate and maximum biomass productivity of the polycultures could be either much higher or much lower relative to their component. In addition, we found that *Daphnia* did not only act as a consumer in algae cultures but also could stimulate growth of some species, likely due to nutrient recycling. The positive effects of polyculture algae on biomass productivity and environmental stability indicates a well-designed polyculture may become an alternative approach to increase the yield in biofuel feedstock algae production (Beyter *et al.* 2016; Newby *et al.* 2016).

Under-yielding and overyielding of polyculture algae communities have both been demonstrated to occur in algal diversity experiments aimed at bioenergy applications (Naughton *et al.* 2015, Narwani *et al.* 2017). Our results indicate that it is common to observe a decreasing in biomass productivity in polycultures, which agreed with Schmidtke, Gaedke & Weithoff (2010), who argued that under-yielding occurs because the species that dominate in competition are often less productive than weaker competitors as a trade off. Our results also indicate that biomass productivity in some algae combinations showed a higher maximum biomass productivity than the component species cultured alone, which agreed with Liu (2016) and Stockenreiter *et al.* (2012) who argued that biomass productivity is positively associated with biodiversity in phytoplankton communities. We observed overyielding in some polyculture combinations which had higher maximum growth rate and maximum biomass productivity than either of the monoculture components (Figure 2, Figure 4). Our results indicate if one species did

extremely well in a polyculture, the growth of the other species usually will be suppressed by competition.

In both stages of our experiment, the 4-species polyculture and the 3-species polyculture did not show a clear advantage in productivity compared to our 2-species polycultures. This finding suggests that increasing species richness does not always result in increasing biomass productivity in single-celled algae community. Similar conclusions have been drawn in multiple previous studies (Shurin *et al.* 2013; Shurin, Mandal & Abbott 2014; Naughton *et al.* 2015). However, some algae combinations with obvious increasing in productivity suggesting that finding the proper algae combination with high maximum growth rate and maximum biomass productivity is still a good approach to increase biofuel algae productivity.

In addition to nutrient utilization and growth, our experiment also showed that species richness affected how algae responded to *Daphnia* grazing. The results showed enormous difference in algae biomass productivity in response to the presence of *Daphnia*. *Daphnia* effects vary with respect of each experimental treatment indicating that the impact of *Daphnia* grazing on different algal taxa ranged from negative to positive. Our results indicate that *Daphnia* grazing commonly cause a decrease in maximum algae biomass productivity and an increase in maximum growth rate. Our results suggesting that introducing *Daphnia* into algae culture usually have negative effects on algae growth, which in agreement with Kampe *et al.* (2007) who argued *Daphnia* act as a keystone species that down-regulates algae biomass in the community by grazing and infochemical releasing. Our studies also suggest species richness may help to reduce the negative effects of *Daphnia* in the term of biomass productivity. Our results indicate that in some specific combination treatments, cultures with *Daphnia* have higher biomass productivity than the same combination without *Daphnia*, which agreed with Callieri *et al.*

(2004) who showed *Daphnia* treatment increase the efficiency of *Synechococcus* sp. productivity. Analyzing the proportion shifting of each component species in the polyculture when *Daphnia* exist indicates that the increasing in biomass productivity might due to *Daphnia* grazing down regulated the population of dominant species and release more nutrients to the other species in the community which increase in productivity (Figure 6). Therefore, culturing multiple species of algae together could be a good strategy to enhance algae biomass yield and reduce the grazing effects when culturing biofuel productive algae in the field.

Our study provides solid evidence that some specific algae combinations will enhance productivity. When comparing with the monocultures of their component species, our results indicate the polyculture of *Scenedesmus dimorphus* and *Navicula* spp. showed a clear advantage in both maximum biomass production and the maximum growth rate. The maximum biomass production will affect the final cell density in the culture, which will directly determine the amount of biomass can be harvest. Higher maximum growth rate will shorten the time that algae culture needed to achieve maximum production, which will shorten the harvest cycle and reduce the potential to grazing and pathogen associated biomass lost (Georgianna & Mayfield, 2012). Even though this productive combination may not to outcompete the *Chlorella vulgaris* monoculture which has 50% higher maximum biomass productivity with slightly lower maximum growth rate, by considering the respond to *Daphnia* existence, our results indicate the polyculture might still be a wise choice due to their high *Daphnia* resistance. Furthermore, our results indicate that with extreme high maximum growth rate, a maximum biomass productivity falls in between its component species, and significant positive effects with *Daphnia*, the polyculture of *Neochloris oleabundans* and *Chlorella minutissima* might be another good combination for field biofuel algae crop culturing.

Our study reveals the possible advantage of culturing multiple biofuel algae species in same culture. We found several combinations of species that produce more biomass and better resist *Daphnia* grazing. However, our study was done in a laboratory; whether our results can be repeated in the field where more uncertainty including changing in temperature, sunlight intensity, nutrient input and weather, remains to be tested by field experiments. Our results agree well with Beyter *et al.* (2016) who found increased biomass productivity and stability in more diverse algal communities grown outdoors in production facilities over a year and exposed to both consumer invasion and environmental fluctuations. Our results also limited to the *Daphnia* grazing pressure and the length of the experiment. Because only one *Daphnia* was added into each culture, they might not be able to regulate the overwhelmed algae growth. We cannot determine whether we would observe the same result if we increase the grazing pressure by introducing more *Daphnia* into each culture. Also, it is hard to say whether a succession will happen in the later growth since we terminated the experiments when the cultures reached their stationary phase. In addition, the conclusions we drew from our results can only apply to the seven species involved in the study, the associations between productivity and other biofuel productive algae combinations remain to be tested. Nevertheless, our study allows us to understand the complex associations among algae species diversity, biomass productivity and grazing resistance. Next step we will test the association between algae species diversity and pathogenic resistance and design a field experiment to study the algae combination growth in an open environment.

Our result indicates that even though increase species diversity in algae cultures does not reliably increase overall biomass productivity, it is possible for specific combinations to show over-yielding relative to the component monocultures. We also found that polycultures are more

likely to have high resistance or even respond positively to *Daphnia* grazing but monocultures turned to be more vulnerable. Even though *Chlorella vulgaris* monoculture had the highest maximum biomass productivity in our study, the high *Daphnia* resistance and relatively high biomass yield made certain polycultures became strong competitors of better biofuel production crop. The mechanism behind the grazing resistance conferred by algal diversity remains to be shown. Our study demonstrates that polycultures may be an effective approach to simultaneously achieve both high productivity and crop protection and thereby advance the commercial potential of algae as a bioenergy crop.

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Table S1: Species involved in the first experiment and their proportion in each treatment group.

Name of Treatment Species	A	B	C	D	AB	AC	AD	BC	BD	CD	ABCD
<i>A-Scenedesmus dimorphus</i>	1	0	0	0	0.5	0.5	0.5	0	0	0	0.25
<i>B-Botryococcus braunii</i>	0	1	0	0	0.5	0	0	0.5	0.5	0	0.25
<i>C-Chlorella vulgaris</i>	0	0	1	0	0	0.5	0	0.5	0	0.5	0.25
<i>D-Navicula spp.</i>	0	0	0	1	0	0	0.5	0	0.5	0.5	0.25

Table S2: Species involved in the second experiment and their proportion in each treatment group.

Name of Treatment Species	E	F	G	EF	EG	FG	EFG
<i>E-Neochloris oleabundans</i>	1	0	0	0.5	0.5	0	0.25
<i>F-Scenedesmus obliquus</i>	0	1	0	0.5	0	0.5	0.25
<i>G-Chlorella minutissima</i>	0	0	1	0	0.5	0.5	0.5

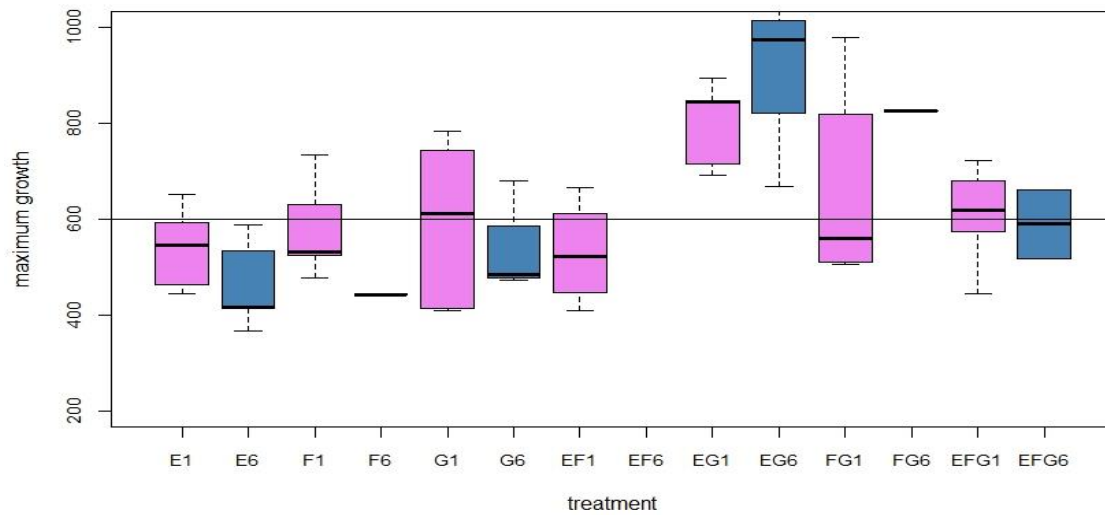


Figure S1: Max growth rate for the second set of experiment. Two bars for each treatment group. Pink bars represent the cultures without *Daphnia* and the blue bars represent the cultures with *Daphnia*.

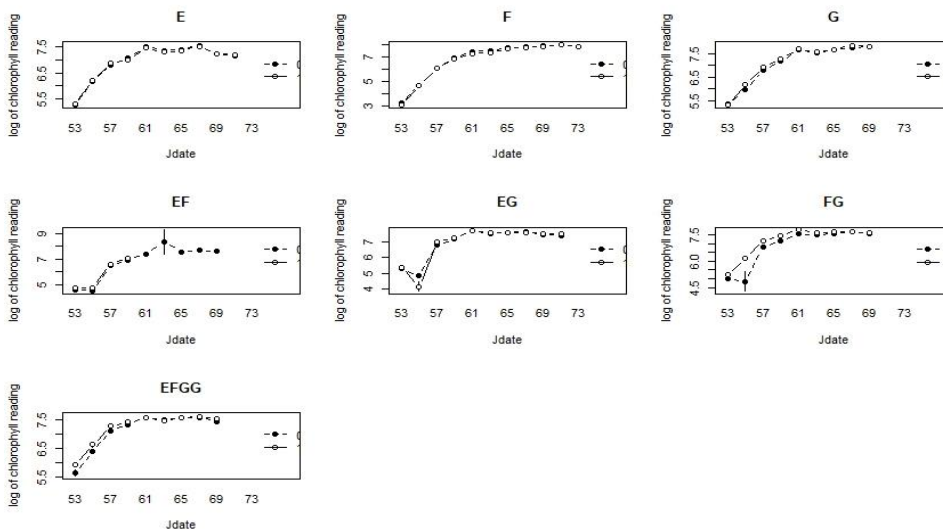


Figure S2: growth curve for EFGG with and without *Daphnia*. E is *Neochloris oleabundans*, F is *Scenedesmus obliquus*, G is *Chlorella minutissima*. x-axis is date, where experiment started on 53; y-axis is the log of chlorophyll concentration. Black dots represent the cultures grow without *Daphnia* grazing, and the white dots represent the cultures with *Daphnia*.

