EVALUATION OF STRUVITE AS A REPLACEMENT FOR TRADITIONAL SOURCES OF NITROGEN AND PHOSPHORUS IN THE PRODUCTION OF MICROALGAE IN OUTDOOR RACEWAYS

By

Nathan D. Huysman

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Fisheries and Mariculture Program

Corpus Christi, Texas

APPROVED: _____ DATE:_____

Dr. Anthony J. Siccardi, Co-Chair

_____

Dr. Paul V. Zimba, Co-Chair

_____

Dr. Joe M. Fox, Member

_____

Dr. David J. Moury, Interim Chair
Department of Life Sciences

_____

Dr. Frank L. Pezold, Dean
College of Science and Engineering

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Abstract

Fossil fuels are a finite resource with no true consensus as to remaining reserves. The production of microalgae to generate biofuel is a way to help alleviate the ever growing demand on fossil fuels. A persistent challenge with growing microalgae is the continued availability of nutrients required for its production in mass quantities. Phosphorus is an essential nutrient required by all plants, however, it is a finite resource whose global depletion is expected to occur at earliest estimates by 2050. If methods to conserve and cost effectively recycle phosphorus are not developed, depletion could eventually lead to a food vs. fuel crisis. A possible means of conserving phosphorus lies in struvite, a crystal compound with a 1:1:1 molar ratio of Mg^{2+}:NH_4^+:PO_4^{3-} formed by the combination and reaction of magnesium, phosphate, and ammonia. Struvite is typically derived from waste streams and has good potential for use as a microalgae fertilizer. Eight 15-day outdoor trials and two 90-day semi-continuous microalgae culture trials using *Nannochloropsis salina* (CCMP 1776), *Phaeodactylum tricornutum* (local isolate), or a mixture of both evaluated struvite's ability to replace or supplement nitrogen and phosphorus. All trials were conducted outdoors in 557 L raceways. Control raceways (n=3) were supplemented with ammonium sulfate and phosphoric acid at a 1:1 ratio for N replacement trials and a 16:1 ratio for P replacement trials. Experimental raceways (n=3) were supplemented with struvite to replace 100, 67, or 33% of the N and/or P in the control. Struvite supplemented raceways performed statistically similar or better than control raceways. Maximum productivity values in 15-day trials ranged from 5.02 to 19.42 g AFDW/m^2/day. Mean productivities of the 90-day trials ranged from ≈11.5 (winter) to ≈16.5 g AFDW/m^2/day (summer). Results indicate that struvite is able to completely replace N and P without negative effects on growth.

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I. Introduction

A. Alternative Energy

As world populations continue to grow, the use of fossil fuels and the need for food will increase as well. It is estimated that by 2030, 60% more food and energy will have to be produced on a daily basis to meet the requirements of a larger population (Alexandratos and Bruinsma, 2012; IEA, 2010; Khan et al., 2009). New energy sources will be needed to provide heating, lighting, and everyday essentials necessary for modern life. Many countries that do not produce fossil fuels will need to find alternative sources of fuel if they are incapable of affording the ever increasing prices associated with conventional fuels (Khan et al., 2009). In direct competition with energy production will be food production. Arable land, already a scarce commodity, will ultimately be needed to be utilized to provide food for the growing populations and not for biofuels.

Fossil fuels are largely considered a finite resource with no consensus in literature regarding the scope of reserves. Several estimates forecast peak production will occur by 2030 or under increased rate of usage by 2020 (Owen et al., 2010; Sorrell et al., 2010). Increased production of non-renewable fossil fuels will also result in an increase in undesirable greenhouse gas emissions. This is of particular concern considering that fossil fuels contribute to 61.4% of the world's total greenhouse emissions (Herzog, 2009). Although a considerable amount of effort has been undertaken to reduce negative environmental impacts of fossil fuels, a growing trend in research is focusing on identification and optimization of renewable sources of energy (Shafiee and Topal, 2009). Some of these renewable sources of energy include geothermal, wave, solar, and hydroelectric power. While these sources of energy are promising and possibly effective,

they require large-scale storage of generated power which is costly. In addition, these sources of energy cannot be refined into liquid fuels (Valentine et al., 2012).

On the other hand, renewable bioenergy energy sources can be refined into products used for transportation while sequestering atmospheric carbon (unlike fossil fuels). Carbon sequestration over the course of growing bioenergy crops results in a product with a small environmental footprint while also being an efficient energy source (Ziolkowska and Simon, 2014). In addition to carbon sequestration, renewable energy sources in wet biomass form can be digested to produce methane and hydrogen (Valentine et al., 2012).

One class of renewable energy products is known as first generation biofuels (FGB), consisting of bioenergy crops such as cereals and sugarcane. While FGB may have potential, they still compete with arable land and do not solve the food vs fuel dilemma facing biofuel crops (Juneja et al., 2013; Sánchez and Cardona, 2008). Second generation biofuels (SGB), which require reduced environmental inputs and land requirements compared to FGB, are derived from cellulosic or oil plant materials like switchgrass, jatropha or oil palms. While these types of plants typically have a fast growth rate and are not commonly used for human consumption, they still require arable land and in many situations large volumes of freshwater irrigation otherwise needed for drinking water (Juneja et al., 2013). In addition, technological and production uncertainties may preclude SGB achieving a production volume sufficient to impact the fossil fuel industry (Ziolkowska and Simon, 2014). This reduces the attractiveness of SGB, especially when considering the food vs fuel debate. Given these bottlenecks, the concept of third generation biofuels (TGB), or algae based biofuels, is gaining support.

TGB are attractive in that they do not compete with food crops making them have a positive effect on the food vs fuel dilemma while producing needed biofuel to help alleviate the strain on fossil fuels.

B. Algae Biofuels

The production of algae to produce biofuels is being examined as a possible way to help alleviate the ever growing demand on fossil fuels and reducing greenhouse emissions associated with fossil fuel refining and collection. Whereas micro- and macroalgae have both been considered potential sources of biofuel, microalgae appears to show the most promise. For the purpose of this document, the term "algae" will be used in reference to microalgae. Algae biomass can be produced indoors, outdoors, in saltwater or freshwater mediums, waste streams, and under a variety of weather conditions. Marine algae are especially useful as it does not require freshwater that could be used for human consumption (Rajkumar et al., 2014). Algae also assimilates 2 g of CO_2 for every 1 g of generated biomass classifying them as net consumers of CO_2, not producers (Pienkos and Darzins, 2009). Furthermore, algae grown for biofuels represents a renewable source of fuel. Solar, wind, and tidal energy sources may help alleviate some of the strain on fossil fuels, but require their energy to be stored and also need fuel for backup, unlike algae biofuels (Valentine et al., 2012).

Another advantage of algae as a source of fuels is that it does not require arable land for production; hence, avoiding competition with traditional agricultural crops. Algae uses less land than competing bio-energy-type crops such as corn used for ethanol production (Menetrez, 2012). The US Department of Energy estimated that algae would only need $1/7^{th}$ of the area currently used for corn to meet the US energy requirements

(US DOE 2010). On a dry weight basis, algae also produces the highest level of lipids among all biofuel sources. Compared to corn, soybeans, and palm, algae can produce from 30 to 300 times more oil per ha of land used (Ziolkowska and Simon 2014). Algae also has a faster biomass production rate compared to food crops and can be produced year round under diverse environmental conditions. Whereas land based crops can take months to reach a harvest, many algae species have 24hr doubling times.

Algae Biomass Production Methods

Algae can be cultured in a variety of environmental conditions using many different methods and nutrient mixes making them a very diverse group. While there are many different methods employed to culture algae in mass quantities, the two primary methods that have garnered interest are raceway/pond and closed photobioreactors**.** Raceways are typically round or oval in shape and contain paddlewheels for vertical and horizontal mixing of the water column keeping algae cells suspended. Typical depth for raceways are 20 to 30 cm as this allows for maximum light penetration and absorbance by the algae. CO_2 additions are necessary to cultures to provide carbon substrate for growth and also to maintain pH. Photobioreactors are typically constructed as closed transparent tubes filled with algae and growth medium. These culture vessels can either be situated outdoors where they are subject to the same light and temperature regimes as raceways, or indoors where light and temperature can be controlled.

There are advantages and disadvantages to both types of culture systems. In a comparison of the two types of systems, raceways were found to be relatively cheaper to operate and easier to clean compared to photobioreactors (Brennan and Owende, 2010; Ugwu et al., 2008). The cost difference was mostly due to lower energy inputs and

building costs associated with raceways. On the other hand, Brennan and Owende (2010) reported that closed photobioreactors had better productivity and had the potential to generate larger quantities of biomass due to a larger illumination surface. The closed design of photobioreactors also aids in inhibiting contamination that could occur in open systems. Closed photobioreactors are still generally considered more expensive to operate compared to open pond/raceway designs (Carvalho et al., 2006).

Factors Affecting Biomass Culture

Regardless of growing methods, there are many different factors that need to be considered for both selecting a site and growing the actual algae. The two most important environmental factors for culturing algae are temperature and light. Different species of algae have different temperature and light requirements. This makes selecting a species to match the seasonal temperatures of the growing site of extreme importance. Temperature has been found to increase the likelihood of photo-inhibition and can also negatively affect growth rates if selected species are not grown near their optimum (Juneja et al., 2013). While many species of algae have high temperature tolerances, exceeding their optimum by only 2-4 ^{o}C can result in a total algal culture die-off (Mata et al., 2010).

Light is an integral component of the algal photosynthetic process. In the natural environment, algae, in the presence of sunlight, converts environmental CO_2 into usable energy products (ATP) used for cellular growth and reproduction. Growing algae in a commercial setting will require a location with sufficient solar or artificial ultraviolet radiation to facilitate the best growth for the selected species.

While light and temperature are the most important factors to consider for algae production, required nutrients for growth are also a major consideration. Without appropriate nutrients for cell division, light and temperature conditions can be optimal, but of no consequence. The two most important nutrients are nitrogen (N) and phosphorus (P). According to Redfield (1958), calcium, magnesium, potassium and many other nutrients are readily available in seawater but N and P appear in limiting quantities. Since commercially grown algae will be grown in denser quantities than the natural environment, it is imperative that the algae be supplied with N and P. Most other macro and micronutrients can be supplied via the natural water used due to the algae only needing them in small quantities (Abdelaziz et al 2013, Anderson 2005).

Producing Fuel from Algae

While achieving maximum lipid yield is desirable for biofuel purposes, algae can be manipulated with differing culture strategies to have other desirable end products. For example, harvesting algae in an unstressed condition with maximum growth being achieved will result in a greater protein content of the cell. Protein can potentially be used as a fishmeal replacement or for some algal species, as a human supplement. Cellular lipid contents can be increased by nutrient depriving algal cells in batch culture before harvest.

Upon harvesting, the first step in the biodiesel refining process is extraction of the lipids from the harvested algae. Lipid extraction generally requires an organic solvent mixture such as hexane/isopropanol or chloroform/methanol (Halim et al., 2012). Algae generally has to be dried to have the lipid extracted; however, wet extraction methods are currently being explored to make the process more economically feasible (Halim et al.,

2011). Other lipid extraction processes currently being investigated include ultrasound and microwave assisted (Cravotto et al., 2008).

Upon extraction, two main methods are employed for refinement depending on the quantity of biomass, economic considerations, and end product desired: thermochemical or biochemical conversion (Brennan and Owende, 2010; Mckendry, 2002). Pyrolysis, a type of thermochemical conversion involving high temperatures and lack of oxygen, can yield high oil amounts but requires the algae to be of very low moisture content which is expensive (Juneja et al., 2013). Fermentation can be utilized for an ethanol end product (Ueno et al., 1998). The fermentation process requires large amounts of time and algae feedstock has to be preprocessed to be available for use (Juneja et al 2013).

One of the most desired end products of algal biomass is biodiesel. Biodiesel is generated using a procedure called transesterification. This process occurs in three steps and involves the conversion of triglycerides to esters (biodiesel) with glycerol as a by-product (Mata et al., 2010). The resulting biodiesel product has similar physical and chemical properties compared to petroleum-based diesel which should allow it to be comparative in terms of functionality (Brennan and Owende, 2010).

Nutrient Availability

A major challenge to biomass production is the availability of nutrients required for growing algae in quantities conducive to large scale production of fuel feedstocks. Limitation of nutrients during growout stages will adversely affect production and harvestable quantities of the algae. N and P are required as nutrients for algae culture and

also required nutrients for plant growth and included in many agricultural fertilizers. Phosphorus is a finite resource with global depletion expected to occur at earliest estimates by 2050 (Van Vuuren et al., 2010). The depletion of P can make a food vs. fuel crisis as P will be needed for both crop fertilizers and biofuel production units.

Algae will need P inserted into the growth media to be able to be grown effectively. A lack of P can result in reduced productivity and overall biomass production (Abdelaziz et al., 2013). Furthermore, it is estimated that in order to completely replace all fossil fuels in the United States, 53 million tonnes of P would be required for biomass production while less than 40 million tonnes are actually mined in the United States yearly (Hannon et al., 2010). In order for algae biomass production to resolve the demand on fossil fuels and not compete with food security demands, a renewable source of P is required.

C. Struvite - an Alternative Source of Phosphorus

Struvite is currently being considered as an effective tool to help mitigate the P problem that could be facing many who require P as a fertilizer. Struvite is a crystal compound that builds up naturally in wastewater treatment plants (WWTP) in the course of normal operation. WWTP have to remove struvite from their pipes and other equipment as it accretes in order to maintain effective operations. Effective crystallization and removal before accretion in the WWTP pipes would benefit the WWTP and also create a renewable source of fertilizer that could have many applications. Forced formation of struvite can aid the WWTP in reduced N and P loads released into streams and even turn into an income generator for the WWTP (Cullen et al., 2013).

Struvite is formed by the combination of magnesium, phosphate, and ammonia resulting in a white crystallized substance with a 1:1:1 molar ratio of Mg^{2+}:NH_4^+:PO_4^{3-}. The reaction formula is as follows;

$$Mg^{2+} + NH_4 + H_nPO_4^{n-3} + 6H_2O \leftrightarrow MgNH_4PO_4\ 6H_2O + nH^+$$

Struvite dissolves in water and has slow release characteristics of its nutrients. These slow release characteristics make struvite a viable plant crop nutrient source, even in areas prone to large amounts of rainfall.

Struvite can also be commercially manufactured in association with WWTP. There are many factors associated with its commercial production including pH, chemical composition of wastewater, mixing energy, and temperature (X. Liu et al., 2013). Struvite will crystallize at a pH between 7 and 11 but lower pH values will result in a purer form of struvite. Formation trials utilizing actual wastewater have revealed that a pH higher than 8.0 should not be utilized to get a high purity struvite (Hao et al., 2009).

Finding an effective Mg source is also a consideration in the forming of struvite as commercial Mg sources can be expensive. Seawater and brine, both of which are inexpensive, are being tested as possible sources of Mg. Struvite can be formed with purity levels up to 95% when using seawater and brine (B. Liu et al., 2013). This makes coastal areas a very attractive location for struvite generation. In addition, the brine leftover from desalination plants is very high in Mg and can also be recycled to make struvite.

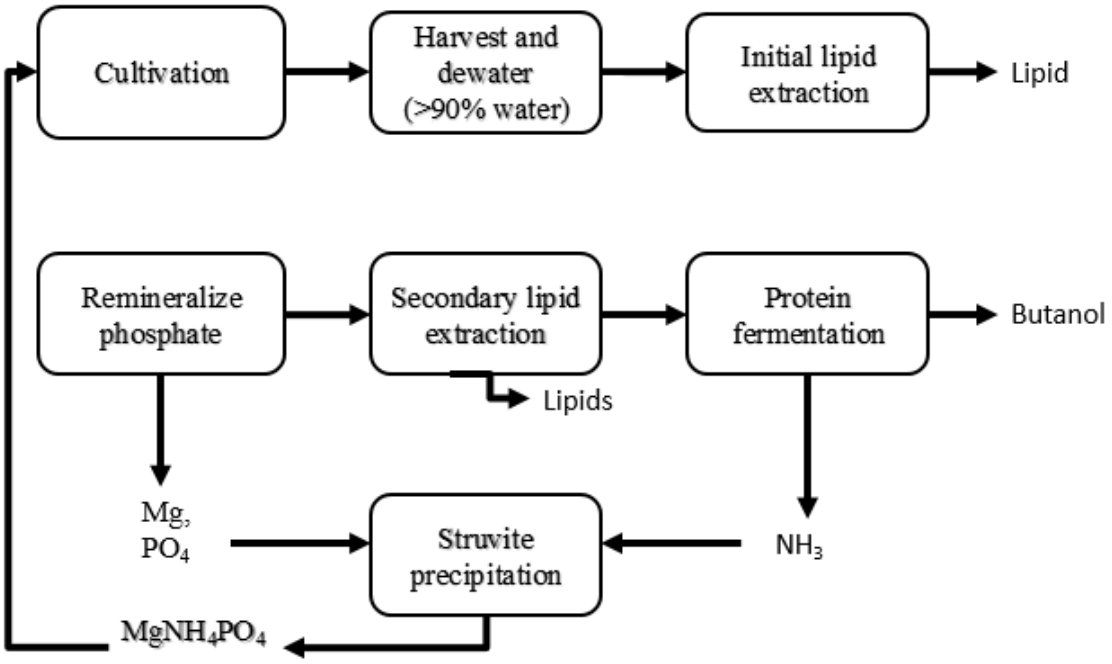
Commercial production of struvite is generally made using wastewater or urine from pig or cattle farms. Wastewater and urine contain relatively high concentrations of

N and P but are lacking in sufficient quantities of Mg to complete the crystallization in larger quantities. WWTP's are required to reduce the amounts of N and P released to the environment which is typically a cost intensive process and a potential cause of eutrophication of receiving streams and surrounding environments. Producing struvite would benefit the WWTP, reduce the N and P in their effluents, and potentially allow them to recoup costs through the sale of struvite.

Apart from mitigating wastewater issues, struvite can be used as a fertilizer for agricultural crops. As previously mentioned, struvite's slow nutrient release properties make it an effective nutrient source even in areas prone to large amounts of rainfall (Rahman et al., 2014). In addition, struvite formation can be beneficial for peoples who live in low socio economic societies who do not have access to quality fertilizers (Schneider et al., 2013). For example, farmers in Nepal are learning to collect their urine and make struvite in homemade reactors for use as fertilizer for food crops (Etter et al., 2011).

Furthermore, another avenue of struvite formation being explored utilizes the "left-over" biomass after harvesting and removal of lipid from algae (Fig. 1). This process utilizes remineralized phosphate with magnesium addition (supplying Mg and PO_4) combined with fermented proteins (supplying NH_3) to create struvite (Lane, 2015). This struvite can then be recycled back into the cultivation process.

Figure 1. Process of struvite precipitation from algal biomass (Adapted from Siccardi, 2015)



D. Rationale of Study

Since algae have a similar nutrient requirement as land plants, struvite is currently being considered as a possible nutrient source for the mass culture of algae for biofuels. . The production of biofuel using algae is still not economical compared to fossil fuels; however, the use of struvite could provide biomass producers with a more cost effective production process. With ever increasing interest into biofuels, commercial sized operations need to find cost effective and available nutrient sources. Struvite can help to fill that gap. A review of the literature prior to conducting this study indicated that few research investigations have been undertaken to evaluate the use of struvite for algal

production for biofuels. For this reason, the aim of this research is to add to current scientific knowledge on the subject.

E. Study Objectives

The primary objectives of this study were 1) to evaluate struvite as a nutrient supplement or replacement for both N and P in the culture of *Nannochloropsis salina* (*N. salina*) (CCMP 1776) and *Phaeodactylum tricornutum* (*P. tricornutum*) (local isolate); 2) to optimize the ratio of N and P replacement with struvite in these same cultures; and 3) to apply results from Objectives 1 and 2 to a long-term (90-day) semi-continuous commercial culture scenario.

III. Materials and Methods

A. 15-Day Trials

1. Selected Species of Algae

Two different species of algae were used in trials; *N. salina* (CCMP 1776) and *P. tricornutum* (local isolate).

N. salina (Fig. 2), a member of the C. Eustigmatophyceae which is characterized by yellow-green unicellular algae that can be found in freshwater or saltwater, contain an eyespot, and only have chlorophyll *a* (Lee 2008).

The diatom, *P. tricornutum* (Fig. 2) is a member of C. Bacillariophyceae. The diatoms are an extremely diverse group that can be found in just about every habitat. One of the key characteristics of the diatoms is that their frustule is composed of silica (Lee 2008). One advantage of *P. tricornutum* compared to other diatoms is its reduced silica

requirement compared. Growth curves of *P. tricornutum* were unaffected when comparing silica starved cultures vs those supplied with silica (Zhao et al., 2014).

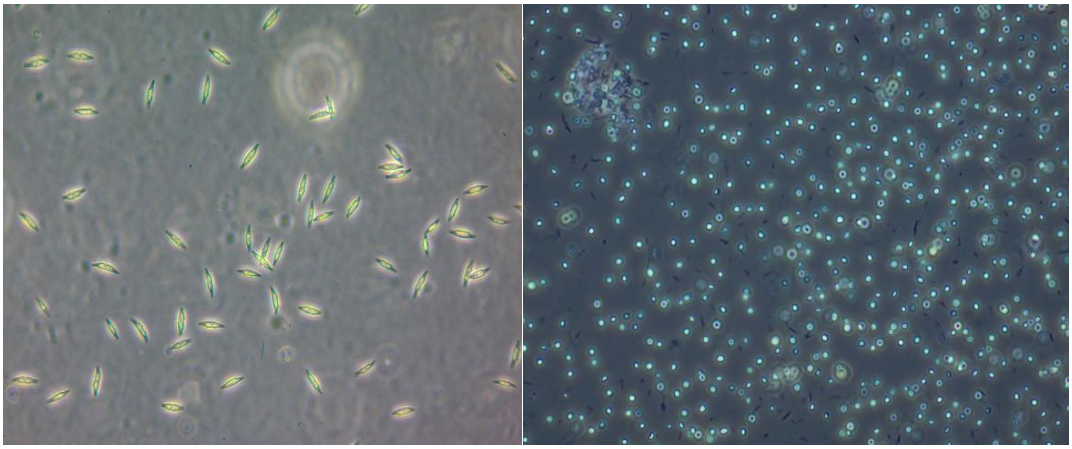


Figure 2. *P. tricornutum* (Author photo); *N. salina* (deepblue-marine.de)

Diatoms are thought to be suitable candidates for biofuels due to their ecological success in natural environments, contaminant resistivity, and lipid storage capacity (Levitan et al., 2014; Taguchi et al., 1987). *P. tricornutum*, along with many other diatoms, has been shown to outcompete other species of algae in mixed cultures and have good productivity in outside pond cultures (Goldman et al., 1975; Levitan et al., 2014). The other alga included in this study, *N. salina*, has also been identified as a suitable biofuel candidate due to its ability to grow well under outdoor conditions and achieve high biomass production numbers (Ma et al., 2014).

While being able to grow well in outdoor systems and contaminant resistivity are important features to consider, one of the main criteria for selecting an algal candidate for biofuel is lipid productivity followed by growth rates (Griffiths and Harrison, 2009). *Nannochloropsis sp.* and *P. tricornutum* have both been identified to have average to

above average growth rates (g/L/day) and lipid content (% dw) (Griffiths and Harrison 2009).

2. Experimental Systems, Start-up

Cultures of *N. salina* (obtained from Bigelow Laboratory for Ocean Sciences, CCMP 1776) and *P. tricornutum* (local isolate) were grown on-site, indoors, and scaled up from 125mL flasks to 1500 L floor tanks. Upon reaching densities ≥ 0.15 g/L ash free dry weight (afdw), cultures were transferred to the outdoor raceways. Outdoor raceways were filled with the algal inoculum and treated seawater to an initial stocking density of ≈0.15 g/L afdw at 5cm depth (≈140L). Raceway culture seawater (Laguna Madre, Corpus Christi, TX) was chlorinated (15 ppm) and diatomaceous earth filtered (Pentair Pool Products, Sanford, NC) prior to use. Seawater salinity was also adjusted with domestic water to 30-32 ppt. Mixed culture experiments were stocked with a 50/50 mix of *N. salina* and *P. tricornutum* based on AFDW. A 50/50 mix results in ≈4:1 cell ratio of *N. salina* to *P. tricornutum* based on hemocytometer cell counts.

All trials were conducted outdoors in twelve 557 L raceways located at Texas A&M AgriLife Research Mariculture facility in Flour Bluff, TX (Fig. 3). Each raceway was outfitted with a paddlewheel and center partition circulating the algae at 50-60 cm/second. Raceways were also provided with a Pinpoint pH probe (American Marine Inc. Ridgefield, CT) attached to a solenoid for CO_2 delivery based upon change in pH. Raceways were maintained at a pH of ≈7.8 by injecting CO_2 when cultures reached a pH of 8 and turned off at culture pH of 7.6. Raceways were stocked initially to 5 cm and gradually increased to a maximum depth of 20 cm at 5, 10, and 20 cm intervals based on productivity and weather related events. Nutrients were added proportionally as depth

increased. Trial duration was 15 days to assess if the slow release of nutrients by struvite had any effect productivity or contamination. Depending on experiment and treatment, algae was supplemented with ammonium sulfate, phosphoric acid (pH adjusted), iron sulfate, and struvite. Each raceway was monitored daily for AFDW, pH, temperature, salinity, solar radiation, NH_3, Phosphate, and rainfall.

Figure 3. Texas AgriLife Research Microalgae Production Site, Flour Bluff, TX

3. Experimental Design

Four 15-day nitrogen replacement trials were conducted, each consisting of four different treatments among the 12 raceways. The control treatment (n=3) was supplemented with a standard nutrient blend of 2.0 mM N from ammonium sulfate, 2.0 mM P from pH balanced phosphoric acid, and 0.07 mM iron from iron sulfate at a 1:1 N:P ratio. Struvite was used to replace 100, 67, and 33% of the N in the experimental treatments (n=3) with supplementation by ammonium sulfate and phosphoric acid as necessary to maintain the same nutrient levels as the control. Mixed culture trials were conducted with seasonal temperature considerations to favor one algae species over the other.

Trials were conducted as follows:

a. *N. salina* mono-culture trial
b. *P. tricornutum* mono-culture trial
c. Mixed culture trial using *N. salina* and *P. tricornutum* with seasonal temperature considerations to favor *N. salina*
d. Mixed culture trial using *N. salina* and *P. tricornutum* with seasonal temperature considerations to favor *P. tricornutum*

Four 15-day phosphorus replacement trials were conducted, each consisting of four different treatments among the 12 raceways. The control treatment (n=3) was supplemented with a standard nutrient blend of 2.0 mM N from ammonium sulfate, 0.13 mM P from pH balanced phosphoric acid, and 0.07 mM iron from iron sulfate at a 16:1 N:P ratio. Struvite was used to replace 100, 67, and 33% of the P in the experimental treatments (n=3) with supplementation by ammonium sulfate and phosphoric acid as

necessary to maintain the same nutrient levels as the control. As with the N replacement trials, mixed culture trials were conducted with seasonal temperature considerations to favor one algae species over the other.

Trials were conducted as follows:

a. *N. salina* mono-culture trial
b. *P. tricornutum* mono-culture trial
c. Mixed culture trial using *N. salina* and *P. tricornutum* with seasonal temperature considerations to favor *N. salina*
d. Mixed culture trial using *N. salina* and *P. tricornutum* with seasonal temperature considerations to favor *P. tricornutum*

4. Nutrient Analyses of Algae Biomass

Algae samples were obtained from raceways in a standard 100mL Nalgene bottle on a daily basis (≈ 9AM) for analyses. Biomass (TSS/VSS) was performed according to Standard Methods (1998). Daily TSS/VSS measurements (g/m^2) were used to calculate daily productivity (g AFDW/m^2/day) over the course of the 15 day trials. FIAlab® (FIAlab Instruments Inc., Bellevue, WA) was used to determine total phosphate (determined as orthophosphate) in the water samples. Total N in the daily water samples was determined using the ammonia for seawater method (Bower & Bidwell 1978; Solarzano 1969; Spotte 1972a,b). Nutrient data was used to determine algal biomass (dry weight) per 1 g N or P added for all trials.

5. Statistical Analysis

Productivity and algal biomass values were tested for normality and equality of variance prior to ANOVA with R-Studio (V. 0.98.1091). Significant differences were determined by the Tukey's HSD inequality with the experiment-wise error rate set at ≤

0.05. 90-day semi-continuous trials were tested for significant differences in productivities over time using repeated measures ANOVA.

B. 90-day Semi-continuous Mixed Culture Trials

These trials were conducted over two 90-day periods during winter and summer months, using mixed species of *N. salina* and *P. tricornutum*. Seasonal timing of trials were designed to have weather characteristics favoring one algal species. Most of the methodologies used in the 15-day trials were applied to these trials. Control raceways (n=6) were supplemented with a blend of ammonium sulfate, pH-balanced phosphoric acid, and iron sulfate at an 8:1 N:P ratio. Treatment raceways (n=6) were fertilized to provide 100% of P from struvite and the remaining N required to match that of the control was supplemented with ammonium sulfate. Raceways were partially harvested (75% total volume or ~ 418 L) every three to four days in order to maintain productivity levels above 15 $g/m^2/day$. Depending on experiment and treatment, algae cultures were fertilized with ammonium sulfate, phosphoric acid (pH adjusted), iron sulfate, and struvite. Raceways were monitored daily for level of productivity (i.e., g $AFDW/m^2/day$), pH, temperature, salinity, solar radiation, NH_3, phosphate, and rainfall. Daily N and P determinations were used to adjust nutrient levels in raceways to avoid nutrient limitation between harvests.

III. Results

A. 15-day Nitrogen Replacement Trials

Results of N replacement of the control nutrient mix with N from struvite are shown in Table 1. For cultures of *N. salina*, final biomass productivity ranges from 3.28 ± 0.49 to 7.77 ± 0.43 g AFDW/m^2/day, with the replacement treatments showing significantly higher rates of productivity (P = 0.0003) than the control (ammonium sulfate N source). Although not significantly different, maximum biomass productivity was numerically lower for *N. salina* cultured outdoors using the control nutrient than that of struvite replacement mixes (11.05 vs. 16.12-13.21 g AFDW/m^2/day). For *P. tricornutum* cultures, replacement of control N with struvite N yielded no significant trend in final biomass productivity values (P = 0.07) with values in the range of 6.21 ± 0.40 - 7.27 ± 0.60 g AFDW/m^2/day. Results from the mixed-species trial favoring *N. salina* (Table 1) showed no significant difference (P = 0.42) in final biomass productivity levels among treatments. Values ranged from 4.11 ± 1.20 to 5.83 ± 1.41 g AFDW/m^2/day. There was also no significant difference in maximum biomass productivity values (P = 0.48), with values ranging from 12.56 ± 3.44 to 15.91 ± 3.59 g AFDW/m^2/day. This trial experienced a total of 15.75 cm of rain over the course of the trial which resulted in decreased mean salinity and solar radiation (Table 2) Results from the mixed-species trial favoring *P. tricornutum* (Table 1) showed significantly higher (P = 0.002) final biomass productivity of algae cultures receiving N supplementation with struvite (7.71 ± 0.87 - 8.45 ± 1.07 g AFDW/m^2/day) vs. the control nutrient mix (4.19 ± 2.12 g AFDW/m^2/day). A similar relationship was shown for maximum biomass productivity cultures receiving 100, 67 and 33% replacement of N

with struvite showed 10.10 ± 1.00, 7.91 ± 1.16, and 7.71 ± 0.87 g AFDW/m^2/day vs. 5.01 ± 1.00 g AFDW/m^2/day for the control nutrient mix (P = 0.02). In all four experiments, struvite performed as well as, or better, than a traditional ammonium sulfate N source.

Nutrient usage data is presented in Table 3. For comparison purposes, all values were converted to g algal biomass (dry weight) per 1 g N added. The nitrogen replacement trials 1:1 molar ratio resulted in excess P throughout the duration of the trial so P utilization data is not presented. The mono-culture *N. salina* trial resulted in significantly greater (P = 0.001) N utilization by the struvite treatment compared to control treatments (2.12 vs. 12.04 -9.60 g algal biomass per 1 g N added). The mixed culture trial favoring *N. salina* resulted in no significant differences (P = 0.11) between treatments with values ranging from 1.43 ± 1.06 to 3.95 ± 1.54 g algal biomass per 1 g N added. The mixed culture trial with weather favoring *P. tricornutum* featured the highest amount of N usage compared to all other trials (19.34 ± 2.47 to 26.38 ± 3.80 g algal biomass per 1 g N added) although there was no significant differences among treatments (P = 0.11).

Table 1. Maximum and final biomass productivity (g AFDW/m^2/day) of four different experiments with four treatments each of *N. salina* (CCMP 1776), *P. tricornutum* (local isolate), or a mix of both cultures with nitrogen replacement using commercial struvite[a,b,c]

	N. salina				*P. tricornutum*				*N. salina* and *P. tricornutum* with weather favoring *N. salina*				*N. salina* and *P. tricornutum* with weather favoring *P. tricornutum*			
% N replacement with Struvite	Control	100	67	33	Control	100	67	33	Control	100	67	33	Control	100	67	33
Maximum Biomass Productivity (g AFDW/m^2/day)	11.05 ± 3.15[a]	16.12 ± 1.68[a]	14.84 ± 1.96[a]	13.21 ± 4.88[a]	9.72 ± 0.32[a]	12.20 ± 0.06[a]	11.75 ± 0.57[a]	9.87 ± 3.51[a]	12.56 ± 3.44[a]	15.91 ± 3.59[a]	12.98 ± 2.56[a]	12.98 ± 0.67[a]	5.01 ± 1.01	10.10 ± 1.00[b]	7.91 ± 1.16[b]	7.71 ± 0.87[b]
Mean Biomass Productivity (g AFDW/m^2/day)	3.28 ± 0.49[a]	7.49 ± 0.55[b]	7.64 ± 1.04[b]	7.77 ± 0.43[b]	6.21 ± 0.40[a]	7.03 ± 0.37[a]	7.27 ± 0.60[a]	6.59 ± 0.34[a]	5.83 ± 1.41[a]	4.11 ± 1.20[a]	4.27 ± 0.93[a]	5.29 ± 1.85[a]	4.19 ± 2.12[a]	8.45 ± 1.07[b]	7.91 ± 1.16[b]	7.71 ± 0.87[b]

[a]Means with similar superscript in same column are not statistically different (p>0.05)

[b]N = 3 raceways

[c]Standard Deviation

Table 2. Mean water quality parameters for 12 outdoor raceways and environmental conditions at the Texas A&M AgriLife microalgae production site for 4 experiments featuring N replacement with struvite

	N. salina	*P. tricornutum*	*N. salina* and *P. tricornutum* with weather favoring *N. salina*	*N. salina* and *P. tricornutum* with weather favoring *P. tricornutum*
Mean water temp (^{o}C) A.M.	19.0±3.0	11.28±5.5	21.20±1.74	11.6±4.3
Mean water temp (^{o}C) P.M.	25.6±2.8	17.0±7.0	24.97±2.73	16.8±5.1
Mean Salinity (ppt)	29.0±5.2	30.3±0.7	24.83±3.88	31.9±0.9
Mean pH	7.7±0.2	7.56±0.1	7.54±0.25	7.5±0.1
Mean Solar Radiation (Cal/cm^2/day)	369.8±76.8	288.4±154.8	279.92±110.21	316.9±185.3
Mean Air Temperature (^{o}C)	23.8±2.6	13.8±6.0	23.3±1.3	13.8±5.1
Mean Total Precipitation (cm)	8.18	0.13	15.75	2.16
Mean Wind Speed (km/h)	8.9±2.9	11.2±3.7	8.2±2.0	11.6±4.1

Table 3. Algal biomass (dry weight) per 1 g N or P added of four different experiments with four treatments each of *N. salina* (CCMP 1776), *P. tricornutum* (local isolate), or a mix of both cultures with nitrogen replacement (1:1) using commercial struvite[a,b,c]

	N. salina				*P. tricornutum*				*N. salina* and *P. tricornutum* with weather favoring *N. salina*				*N. salina* and *P. tricornutum* with weather favoring *P. tricornutum*			
% N replace-ment with Struvite	Control	100	67	33	Control	100	67	33	Control	100	67	33	Control	100	67	33
g Algal Biomass (dry weight) per 1 g N added	2.12 ± 1.69[a]	9.60 ± 1.98[b]	10.77 ± 2.39[b]	12.04 ± 1.73[b]	14.16 ± 1.35[a]	13.65 ± 0.81[a]	14.78 ± 0.32[a]	13.59 ± 0.78[a]	3.95 ± 1.54[a]	2.33 ± 0.85[a]	1.82 ± 1.08[a]	1.43 ± 1.06[a]	19.34 ± 2.47[a]	26.38 ± 3.80[a]	20.53 ± 2.95[a]	21.33 ± 3.39[a]
g Algal Biomass (dry weight) per 1 g P added	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

[a]Means with similar superscript in same column are not statistically different (p>0.05)

[b]N = 3 raceways

[c]Standard Deviation

[*]N replacement trials ran in excess P so utilization of P not calculated

B. 15-day Phosphorus Replacement Trials

In all four trials, P-struvite performed as well as a traditional P source, phosphoric acid (Table. 4). In terms of P replacement, final biomass productivity of monocultures of *N. salina* was similar (P = 0.41) regardless of struvite-P replacement level and ranged between 5.01 and 5.73 g AFDW/m^2/day. A similar response was shown for monocultures of *P. tricornutum* (P = 0.98), with biomass productivity ranging from 3.59 - 3.95 g AFDW/m^2/day. Replacement of control nutrient mix P with struvite-P for both summer and winter mixed cultures resulted in similar levels of biomass productivity (P = 0.12 and 0.67; 5.28 - 6.72 g AFDW/m^2/day and 5.23 - 5.41 g AFDW/m^2/day, respectively). The highest maximum productivity (19.42 g/m^2/day) in all P replacement trials was observed during the course of the mixed-species trial during weather favoring *N. salina* (summer). This trial had high mean solar radiation (505 cal/cm^2/day) and mild temperatures (24 -28 °C; Table 5) throughout its duration. Biomass productivity numbers in this trial were high despite also receiving a total of 5.4 cm of rainfall.

Nutrient usage data is displayed in Table 6. The mono-culture trial of *N. salina* resulted in the 100% P replacement with struvite having a significantly increased (P = 0.03) usage of N vs. control (3.21 ± 0.04 to 2.08 ± 0.31 g algal biomass per 1 g N added). The same trial was nearly significant (P = 0.07) for differences between control and 100% struvite replacement of P (17.73 ± 3.17 to 26.85 ± 1.24 g algal biomass per 1 g P added). The mono-culture trial of *P. tricornutum* saw increased N usage for struvite treatment vs. control (5.23-7.81 vs. 2.96 g algal biomass per 1 g N added), although not significant (P = 0.07), for all treatments. Both N and P utilization were not significantly different (P = 0.61, 0.83) in the mixed culture trial with weather favoring *N. salina* for all

treatments with values ranging from 6.84 ± 1.25 to 9.68 ± 2.96 for N utilization and 55.38 ± 8.58 to 69.19 ± 20.71 for P utilization. The mixed culture trial with weather favoring *P. tricornutum* featured low productivity values (Table 4) but this trend did not carry over to the utilization values. N utilization ranged from 6.38 ± 0.42 to 6.96 ± 0.33 (P = 0.61) and P utilization ranged from 59.63 ± 6.86 to 79.60 ± 6.57 (P = 0.06).

Table 4. Maximum and final biomass productivity (g AFDW/m^2/day) of four different experiments with four treatments each of *N. salina* (CCMP 1776), *P. tricornutum* (local isolate), or a mix of both cultures with phosphorus replacement using commercial struvite[a,b,c]

	N. salina				*P. tricornutum*				*N. salina* and *P. tricornutum* with weather favoring *N. salina*				*N. salina* and *P. tricornutum* with weather favoring *P. tricornutum*			
% P replace-ment with Struvite	Control	100	67	33	Control	100	67	33	Control	100	67	33	Control	100	67	33
Maximum Biomass Productivity (g AFDW/m^2/day)	11.98 ± 2.22[a]	11.08 ± 0.50[a]	12.00 ± 1.25[a]	13.48 ± 0.70[a]	16.26 ± 0.00[a]	14.80 ± 0.38[a]	15.40 ± 3.26[a]	16.77 ± 1.32[a]	13.05 ± 1.36[a]	16.02 ± 5.93[a]	15.68 ± 0.86[a]	19.42 ± 4.41[a]	6.64 ± 0.74[a]	5.98 ± 1.22[a]	6.35 ± 1.16[a]	6.04 ± 0.78[a]
Mean Biomass Productivity (g AFDW/m^2/day)	5.18 ± 0.32[a]	5.01 ± 0.38[a]	5.16 ± 0.61[a]	5.73 ± 0.53[a]	3.70 ± 0.03[a]	3.95 ± 1.17[a]	3.59 ± 0.85[a]	3.81 ± 1.39[a]	5.28 ± 0.61[a]	5.51 ± 0.36[a]	5.98 ± 0.40[a]	6.72 ± 0.99[a]	5.34 ± 0.03[a]	5.23 ± 0.05[a]	5.26 ± 0.28[a]	5.41 ± 0.27[a]

[a]Means with similar superscript in same column are not statistically different (p>0.05)

[b]N = 3 raceways

[c]Standard Deviation

Table 5. Mean water quality parameters for 12 outdoor raceways and environmental conditions at the Texas A&M AgriLife microalgae production site for 4 experiments featuring P replacement with struvite

	N. salina	*P. tricornutum*	*N. salina* and *P. tricornutum* with weather favoring *N. salina*	*N. salina* and *P. tricornutum* with weather favoring *P. tricornutum*
Mean water temp (^{o}C) A.M.	25.1±0.4	16.2±5.5	23.2±4.0	10.9±5.8
Mean water temp (^{o}C) P.M.	29.4±1.7	24.8±4.4	30.5±2.4	12.9±7.0
Mean Salinity (ppt)	26.5±5.1	33.4±1.0	34.0±2.3	33.5±1.1
Mean pH	7.4±0.4	7.4±0.3	7.5±0.4	7.5±0.1
Mean Solar Radiation (Cal/cm^2/day)	363.9±124.8	504.9±137.1	455.1±71.0	215.6±113.5
Mean Air Temperature (^{o}C)	28.1±0.8	20.9±2.7	26.9±2.8	11.6±5.5
Mean Total Precipitation (cm)	16.43	0.33	5.4	0.05
Mean Wind Speed (km/h)	10.1±2.6	10.0±3.6	8.3±1.7	11.7±4.6

Table 6. Algal biomass (dry weight) per 1 g N or P added of four different experiments with four treatments each of *N. salina* (CCMP 1776), *P. tricornutum* (local isolate), or a mix of both cultures with phosphorous replacement (16:1) using commercial struvite[a,b,c]

	N. salina				*P. tricornutum*				*N. salina* and *P. tricornutum* with weather favoring *N. salina*				*N. salina* and *P. tricornutum* with weather favoring *P. tricornutum*			
% P replace-ment with Struvite	Control	100	67	33	Control	100	67	33	Control	100	67	33	Control	100	67	33
Algal Biomass (dry weight) per 1 g N added	2.08 ± 0.31[a]	3.21 ± 0.04[b]	2.88 ± 0.76[a]	2.26 ± 0.15[a]	2.96 ± 1.64[a]	7.81 ± 1.83[a]	5.23 ± 0.90[a]	6.04 ± 2.14[a]	6.84 ± 1.25[a]	9.68 ± 2.96[a]	8.10 ± 2.75[a]	7.52 ± 3.07[a]	6.96 ± 0.33[a]	6.38 ± 0.42[a]	6.87 ± 0.79[a]	6.94 ± 0.51[a]
g Algal Biomass (dry weight) per 1 g P added	17.73 ± 3.17[a]	26.85 ± 1.24[a]	23.75 ± 6.77[a]	17.96 ± 1.05[a]	32.10 ± 19.00[a]	66.90 ± 12.79[a]	58.53 ± 14.24[a]	57.89 ± 14.15[a]	55.38 ± 8.58[a]	69.19 ± 20.71[a]	59.86 ± 20.19[a]	58.26 ± 22.99[a]	79.60 ± 6.57[a]	59.63 ± 6.86[a]	75.25 ± 12.01[a]	72.03 ± 3.67[a]

[a]Means with similar superscript in same column are not statistically different (p>0.05)

[b]N = 3 raceways

[c]Standard Deviation

C. 90-day Semi-continuous Mixed Culture Trials

1. Summer 90-day Trial

Mean productivity values for the summer 90-day trial are shown in Fig. 4 and summarized in Table 7. A total of 23 partial harvests occurred over the course of the 90-day trial period with no significant differences in productivity (P = 0.65) between control and treatment raceways (17.1 ± 5.6 vs. 16.1 ± 5.7 g AFDW/m^2/day). Throughout the culture period, both raceways were maintained at levels of >0.50 g/L dry weight for each harvest.

Figure 4. Productivity values (g AFDW/m^2/day) for harvest days over the course of the summer 90-day trial

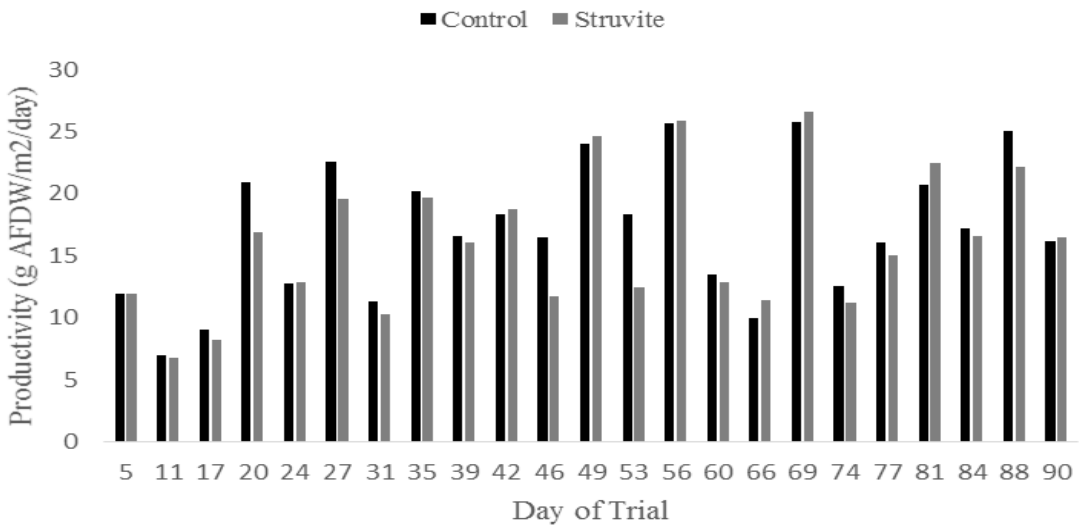


Table 7. Mean productivity (g AFDW/m^2/day) of *Nannochloropsis salina* (CCMP 1776) and *Phaeodactylum tricornutum* (local isolate) for summer 90-day trial[a,b,c]

Treatment	Mean Productivity (g AFDW/m^2/day)
Control	17.1±5.6[a]
Struvite	16.1±5.7[a]

[a] Means with similar superscript in the same column are not statistically different (p>0.05)

[b] N = 4 raceways

[c] Standard deviation

Culture water parameters are listed in Table 8 and environmental conditions are listed in Table 9. Water temperatures were warm but never averaged over 30^0C over the course of the trial. Mean solar radiation was elevated indicating good conditions for growth.

Table 8. Mean water temperature, salinity, and pH of all raceway algae cultures of *Nannochloropsis salina* (CCMP 1776) and *Phaeodactylum tricornutum* (local isolate) in twelve outdoor raceways for summer 90-day trial

	Temperature (°C)		Salinity (ppt)	pH
	A.M.	P.M.		
Raceways (1-12)	23.1±4.6	29.5±4.1	32.7±2.2	7.4±0.2

Table 9. Mean solar radiation, air temperature, precipitation, and wind speed at the Texas A&M AgriLife microalgae production site over the course of the summer 90-day trial

	Solar Radiation (Cal/cm^2/day)	Air Temperature (°C)	Total Precipitation (cm)	Wind Speed (km/h)
Texas A&M AgriLife Microalgae Production Site	535.6±132.0	26.7±2.6	9.8	10.9±2.7

2. Winter 90-day Trial

Mean productivity values for the winter 90-day trial are shown in Fig. 5 and summarized in Table 10. A total of 21 partial harvests occurred over the course of the 90-day trial period with no significant differences in productivity (P = 0.68) between control and treatment raceways (11.49 ± 6.62 vs. 11.40 ± 6.45 g AFDW/m^2/day). Throughout the culture period, both raceways were maintained at levels of >0.35 g/L dry weight for each harvest.

Figure 5. Productivity values (g AFDW/m^2/day) for harvest days over the course of the winter 90-day trial

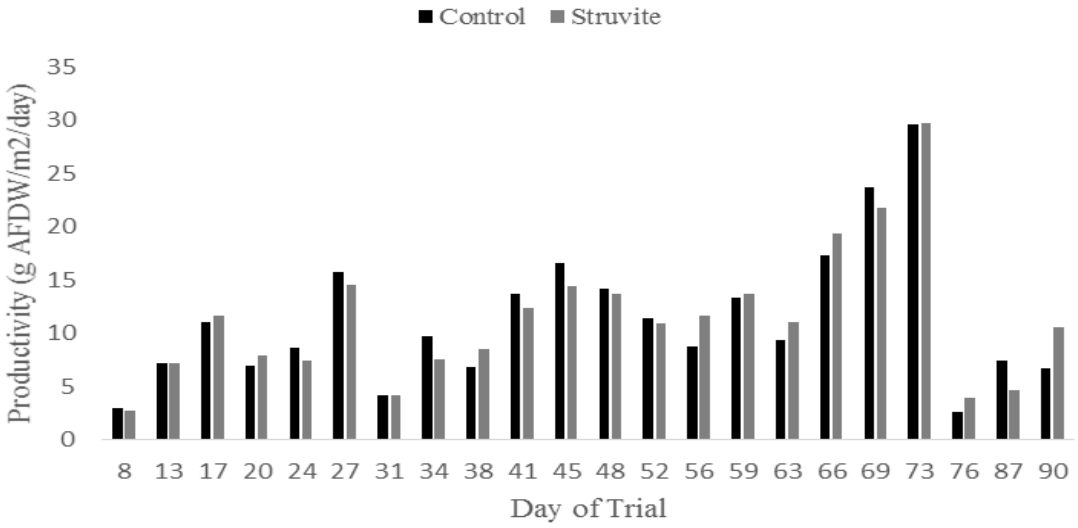


Table 10. Average productivity (g AFDW/m^2/day) of *Nannochloropsis salina* (CCMP 1776) and *Phaeodactylum tricornutum* (local isolate) for winter 90-day trial[a,b,c]

Treatment	Mean Productivity (g AFDW/m^2/day)
Control	11.49±6.62[a]
Struvite	11.40±6.45[a]

[a] Means with similar superscript in the same column are not statistically different (p>0.05)

[b] N = 4 raceways

[c] Standard deviation

Culture water parameters are listed in Table 11 and environmental conditions are listed in Table 12. Mean water and air temperatures were relatively low for maximum algal growth. Over the course of the 90 days nearly 21.6 cm of rainfall of occurred making a wide range of sustained culture salinities. Solar radiation was low indicating numerous cloudy days typical of winter weather.

Table 11. Mean water temperature, salinity, and pH of all raceway algae cultures of *Nannochloropsis salina* (CCMP 1776) and *Phaeodactylum tricornutum* (local isolate) in twelve outdoor raceways for winter 90-day trial

	Temperature (^{o}C)		Salinity (ppt)	pH
	A.M.	P.M.		
Raceways (1-12)	12.45±5.56	17.65±6.45	26.38±3.58	7.42±0.07

Table 12. Mean solar radiation, air temperature, precipitation, and wind speed at the Texas A&M AgriLife microalgae production site over the course of the winter 90-day trial

	Solar Radiation (Cal/cm^2/day)	Air Temperature (^{o}C)	Total Precipitation (cm)	Wind Speed (km/h)
Texas A&M AgriLife Microalgae Production Site	270.20±150.05	14.9±5.2	21.6	9.6±4.2

IV. Discussion

A. 15-day Trials

Both *N. salina* and *P. tricornutum* were able to utilize up to 100% struvite replacement of traditional N and P nutrient sources with no statistical differences in productivity or perceivable negative influences in culture quality. These results highlight

struvite's ability to effectively dissolve in water and release N and P into the water column which can be effectively utilized by algae. Struvite's ability to dissolve in soil and provide nutrients to land based plants has been widely reported (Münch and Barr, 2001; Rahman et al., 2014; Ryu et al., 2012). This study has shown that struvite can also be utilized as a nutrient source for the mass production of algae.

Algae has been shown to be able to be grown at many different N:P nutrient ratios other than the traditional Redfield Ratio (Rhee and Gotham, 1980). These two experiments were run at 1:1 for the N replacement and 16:1 for the P replacement. Struvite's 1:1 molar ratio of N and P necessitated that it be ran at 1:1 for the N replacement experiment so that 100% replacement of traditional nutrients could be tested vs a control. This ratio would not be ideal for "real-world" situations in that there would be excess P left over and most likely not utilized by the algae as witnessed in our studies. Excess P in the water left over after harvest would defeat the purpose of struvite being utilized as an alternate P source. Effluent water from after harvesting could be returned back into the system which could recycle excess nutrients; however, that might necessitate a more complex growth medium than utilized in the current trials potentially increasing production costs. Nevertheless, it is most likely that struvite would be utilized as a P replacement with some external N supplementation to achieve the desired N:P ratio.

Productivity numbers in most of the trials would be considered low respectively. This is due to most productivity numbers reported in literature being based on benchtop scale indoor environments where temperature and light are easily controlled or in bioreactors. Outdoor productivity numbers utilizing raceways are not easily found in

literature. In a technical report to the U.S. DOE, Benemann et al (1982) reported that algae can be grown at an average productivity of 22.5 g/m^2/day. This value is said to take into account slower winter months and higher productivity in the summer months. In another report Olaizola (2003) reported achieving an average of 13 g dry biomass m^2/day. Olaizola's (2003) dry-weight biomass numbers involve commercial scale (25,000 L) photobioreactors. In a an attempt to make a life cycle analysis and algae biomass model, Sills et al (2013) reviewed numerous literature articles on reported productivity values. Their conclusion was that productivity could be lumped into three categories; low (2.4-16 g/m^2/day), base (17-33 g/m^2/day), and high (34-50 g/m^2/day). High amounts of productivity variation could also be due to the variation in the selected species of algae. Many species may achieve high productivity numbers but have low lipid amounts.

Our results would generally be on the low side of the scale for maximum productivity. There are many explanations for our productivity results. Solar radiation along with rainfall varied from trial to trial. Our winter productivity numbers were also lower than our summer values. This could be solely related to temperature or possibly our cold weather species, *P. tricornutum*, may be suited for "cooler" weather and not be an optimum cold weather species. Levitan et al (2014) reports that diatoms can easily reach annual averages of 17 g/m^2/day when grown in outdoor ponds. Ma et al (2014) reported that *N. salina* had biomass productivity of 316.18±4.50 mg/L/day.

The reported productivity numbers of both *P. tricornutum* and *N. salina* did not exceed the energy return on investment productivity threshold number of 17 g/m^2/day. The energy return on investment threshold was modeled from a literature search by Sills et al (2013) and necessitated a wet lipid extraction technology to receive an energy return

on investment. Their exhaustive search and ensuing model simulation resulted in a 75% success rate for an energy return on investment if productivity could be maintained at 17 g/m^2/day with wet lipid extraction. Our results were below that number but achieving high productivity was not the basis of this particular study. More research with struvite and possibly different species of algae could potentially result in higher productivity numbers.

In order to show N and P utilization over time, defined growth rates with clear indicators of exponential and lag phase growth are needed. Unfortunately, outdoor trials do not always exhibit clear rates as they are subject to many fluctuations due to weather related events. Rain events can significantly lower overall biomass quickly and cloudy days followed by days with significant solar radiation can alter growth rates. Due to these effects, and for the sake of comparability and compatibility, the choice was made to display algal biomass (based on dry weight basis) per 1 g N or P added for the entire course of the trials. In addition, trials with considerable cloudy days and rain events were never nutrient replete.

Surface area to volume of algal cells plays an important role in N uptake by cells (Hein et al., 1995). Larger cells are able to uptake nutrients in greater quantities than smaller. Uptake of both ammonium or nitrate was has been found to be strongly correlated with surface area to volume ratios (Hein et al., 1995). *P. tricornutum's* size and shape compared to *N. salina* gives it a much larger surface area to volume ratio which could aid it in increased N uptake. An examination of the results of the N and P utilization numbers seems to support this hypothesis. In both the N and P replacement studies, *P. tricornutum* had a higher algal biomass per unit N or P added. Even the mixed

culture N replacement trial (weather favoring *N. salina*) resulted in reduced N utilization efficiency compared to *P. tricornutum* mono and mixed trials with weather favoring it. Due to extreme weather conditions it is hard to make conclusive decisions based on these results. For example, both the P replacement mono-culture *N. salina* and mixed-culture N replacement with weather favoring *N. salina* had over 15 cm of rain during the course of the trial. These two trials also had the lowest N utilization values.

Interestingly, in both mono-culture P replacement trials (16:1), struvite raceways had higher algal biomass values for both N and P. Although the *P. tricornutum* trial did not have significant differences, it was borderline ($p=0.055$). Under lab scale conditions struvite was found to have better late stage growth compared to struvite lacking treatments (Davis et al., 2015). It is hypothesized that struvite may contain some trace metal elements that could be limiting.

B. 90-day Semi-continuous Trials

Struvite successfully competed with a more traditional nutrient source for the duration of both 90-day trials. There were no statistical differences in productivity or average harvest weights of the algae. Struvite was able to dissolve in the water and provide nutrients to the algae effectively in the time period between harvests. These results highlight struvite's ability to be considered as a possible P replacement in commercial settings.

A mixed culture design was selected for the 90-day experiments. It is believed that in natural environments having a variety of different species traits and tolerances (biodiversity) allows an ecosystem to maintain itself and adapt to environmental changes

more efficiently (Norberg, 2013). Culturing algae for biomass purposes creates an environment that greatly differs from natural communities. Nonetheless, research has focused on polycultures versus monocultures for productivity, lipid production, and contamination resistivity. In an experiment containing 22 naturally occurring lake species of algae, high diversity of cultures resulted in increased algal biomass and lipid production compared to corresponding monocultures (Stockenreiter et al., 2012) Corcoran and Boeing (2012) found that algal polycultures exhibited greater overall stability over time in the presence of rotifers compared to monocultures. It is difficult to establish whether polycultures grown outdoors, in raceways exposed to the environment and contaminants, and at densities much greater than normal environmental conditions will be able to exhibit stability in the presence of consumers.

Over the course of the 90-day trials the cultures were subject to outside contamination by both algae and grazer such as ciliates and other protozoans. While studying the effects of mixed culture designs versus monoculture were not the purpose of these trials, raceways were able to overcome stressors from external contamination from grazers. It is possible that grazing of one species of algae over others made it possible for contamination algae to proliferate and dominate at times. The relationships of the environmental factors versus biodiversity are unclear from these experiments.

A semi-continuous design was chosen for this study as batch harvesting is not used in the research community. Batch cultures, especially those that utilize N starvation to raise lipid levels, are time consuming and require new algae cultures to be constantly prepared for re-stocking. Utilizing a semi-continuous design allows for multiple harvests from the same stock culture resulting in increased overall biomass compared to single

batches. The idea of a semi-continuous design was first espoused in literature in 1938 (Ketchum and Redfield, 1938). Ketchum and Redfield (1938) wrote that in order to maintain a population of diatoms at its greatest concentration, the diatom species maximum daily yield must be found and then maintained through a percentage harvest of cells and re-introduction of enriched water equivalent to the harvest.

Semi-continuous designs have garnered positive results in literature. Feng et al (2014) reported that *Scenedesmus obliquus* had higher productivity, dry weight, and lipid accumulation for outdoor semi-continuous cultures compared to indoor. In another experiment, Fuentes-Grünewald et al (2015) reported that *Porphyridium purpureum* had higher cell densities utilizing semi-continuous design when grown outdoors in bioreactors compared to batch cultures. *Botryococcus braunii* was shown to have increased overall lipid productivity grown in semi-continuous mode versus batch mode in outdoor raceways (Ashokkumar et al., 2014). Our results suggest that mixed cultures of *P. tricornutum* and *N. salina* can be grown over extended periods of time in a semi-continuous design while utilizing struvite as the sole P source without negative effect on productivity.

N. salina dominated the culture mix over the summer period. In our experience, *N. salina* has a higher optimum temperature rating (≈24ºC) and tolerance (≈34ºC) compared to *P. tricornutum.* The summer trial had greater algal contamination than the winter trial. Two different diatom species (loosely identified as *Amphora sp.* and *Cylindrotheca sp.*) began to proliferate and at times dominate the cultures. Productivity of the entire system did not seem to be negatively affected from the contamination. Once temperature routinely increased over 32ºC during the day, *N. salina* was the dominant

alga. *N. salina's* can tolerate more extreme weather conditions compared to other algal species possibly making it a good candidate for use in warm to hot climates.

P. tricornutum dominated the culture mix over the course of the winter trial. In our experience, *P. tricornutum* has a lower optimum temperature (≈18 to 20ºC) compared to *N. salina.* There was not nearly as much outside algal contamination over the course of the winter trial presumably due to the colder winter temperatures. It is also possible that *P. tricornutum* may be better at inhibiting growth of competing diatoms compared to *N. salina*. For example, *Amphora coffeaeformis* and *Cylindrotheca fusiformis* have both been found to produce a mucilage that is hypothesized to form floating gelatins in the sea and possibly also have allelopathic abilities to inhibit growth of other algal species (De Angelis et al 1993; Hiromi et al 1995). *P. tricornutum* will produce surface bubbles under aeration indicating it may also be a mucous producer that could also possibly aid in inhibiting other diatoms.

C. Conclusions

Struvite was successfully able to compete with a traditional nutrient formulation for growing mixed and monocultures of algae in outdoor raceways. Struvite did not exhibit any "slow release" capabilities of its nutrients in the water column. No culture quality differences or contamination resistivity were observed to be different for cultures grown on struvite compared to the traditional nutrient mix.

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