ABSTRACT

Determination of planktonic metabolism and trophic status in Texas shallow ponds

Aaron Dack

Dr. Robert D. Doyle, Ph.D.

Potential daily aerial rates of plankton production and respiration for twenty-seven shallow ponds within the Bullhide Creek watershed between January and March 2013 were determined. Photosynthetic parameters were estimated using the oxygen change method in closed biochemical oxygen demand bottles (BOD). Daily potential planktonic production and respiration were estimated using the Walsby method and incident solar radiation data for a cloudless day during the survey period. Results contrasted original predictions with plankton communities being autotrophic on average (P:R = 1.39). Although recent research has concluded that most surface waters are heterotrophic, we found eight ponds were strongly heterotrophic (P:R < 0.7) nine were strongly autotrophic (P:R > 1.3) and ten in approximate balance (P:R 0.7-1.3). Plankton photosynthesis and P:R ratios were not affected by the abundance of submerged aquatic vegetation (SAV), (p=.87). Average P:R ratio for ponds without SAV averaged 1.61, with intermediate and abundant SAV averaging 1.14 and 1.79 respectively.

APPROVED BY DIRECTOR OF HONORS THESIS:

_____

Dr. Robert D. Doyle, Department of Biology

APPROVED BY THE HONORS PROGRAM:

_____

Dr. Andrew Wisely, Director

DATE: _____

DETERMINATION OF PLANKTONIC METABOLISM AND TROPHIC STATUS IN TEXAS SHALLOW PONDS

A Thesis Submitted to the Faculty of

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In Partial Fulfillment of the Requirements for the

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By

Aaron Dack

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DEDICATION

This thesis is dedicated to my mother and sisters whom through their love, inspiration, and guidance have made me the student I am today.

CHAPTER ONE

Introduction

1.1 Aquatic Photosynthesis

Literature has established phytoplankton as a controlling factor in global environmental impacts, carbon cycling, and the order of the food chain. In fact phytoplankton is the leading producer of oxygen contributing aquatically and terrestrially over 50% to the supply on earth (Molles, Cahill, 2011). Because of its far-reaching environmental and global importance, phytoplankton has been researched extensively. Subsets of phytoplankton research have mainly been distributed into three groups: Ecology, carbon cycling, and phytoplanktonic role in agricultural systems. (Hilligsøe 2011).

1.2 Carbon Cycling in Aquatic Ecosystem

Unlike lake and pond ecosystems, the open ocean lack rooted aquatic vegetation. In such, phytoplankton is the main carbon producer in ocean ecosystems. Phytoplankton is especially useful to the ocean ecosystem because they use carbon dioxide and bicarbonate substituents to make food through photosynthesis. Then, carbon dioxide is released during respiration. This produces a vast efflux of carbon into the ocean contributing greatly to the organic portion of the carbon cycle. Considering that oceans make up the

majority of water on planet earth, the role of phytoplankton in carbon cycling is extremely important. (Gutierrez-Rodriguez et al., 2010).

Alongside oceans the role that phytoplankton plays in carbon cycling extends to enclosed bodies of water. The first, lakes, are primarily made up of both phytoplankton and macrophytes. Although in smaller lakes macrophytes are the primary producers of carbon, in larger lakes phytoplankton become the main contributor due to the depth dependence of rooted plants. In larger lakes rooted plants are subjected to limited growth, which takes part around the outer edges of the lakes. In such, phytoplankton is usually the primary contributor to carbon production in larger lakes; and in smaller lakes rooted plants and floating macrophytes become more important (Molles, Cahill, 2011).

It has long been accepted in the scientific community that phytoplankton production is of utmost importance in understanding the development of energy and carbon flow in aquatic systems. (Tilahun, Girmel, Ahlgren 2010). As the recognized base of the food chain in many aquatic ecosystems phytoplankton has far reaching effects on a global economic scale. In such, phytoplankton is the primary producer for the development of life. Not only that, phytoplankton production plays a large role in controlling the trophic status of bodies of water that affects communities living in the environment. (Gle et al., 2008).

Photosynthesis is the process of incorporating inorganic carbon dioxide into an organic form while releasing oxygen as a by-product. Using light as an

energy source for photosynthesis is the single most important process for the conversion of carbon into organic forms (Lampert et al., 2007).

In aquatic systems the role that phytoplankton photosynthesis and respiration plays determines the production yield of the system. To help determine the plankton photosynthesis several key terms need to be addressed. First, gross photosynthesis (GP) is defined as a light-dependent rate reaction that takes into account all the photosynthetic carbon produced, regardless if this be part of the immediate organism or excreted into the surrounding environment as organic carbon (Falkowski, Raven 2007). On the other hand, respiration (R) is a metabolic process that oxidizes organic carbon to carbon dioxide by the consumption of oxygen and the release of energy and carbon dioxide. Photosynthesis is not possible without light while respiration occurs throughout the day and night. We typically compute rates of GP and R for 24-hour periods to provide estimates of total daily production and respiration. By subtracting the daily respiration from the total daily gross photosynthesis we determine the Net primary production (NPP). By definition net primary production is the total amount of organic carbon produced by photosynthetic processes subtracted by the amount metabolically consumed by respiration by the photosynthetic organism over a complete day period.

$$NPP = GP - R$$

1.3 Patterns of Planktonic Production & Respiration Through the Water Column

Photosynthesis and respiration in aquatic ecosystems are usually estimated on an aerial basis as the total photosynthetic production or respiration under a square meter of the water surface (from the surface to the bottom of the water column). Because photosynthesis is a light-requiring process, it does not occur evenly through the vertical water column. Looking at figure one describes the unevenness that photosynthesis is produced at through the water column. For phytoplankton, production is limited to the euphotic zone (zone where light is present and typically computed from water surface to a depth of 1% light), which accounts for the top layer of a system. The reasons for this are simple; the light intensity is the greatest at this point so it is best to utilize this zone for the potential for phytoplankton to photosynthesize (Tundisi et al., 2012). However, with the light intensity in the euphotic zone there are confounding effects on planktonic production. In most systems we observe an initial suppression in photosynthesis at the surface. This is readily noticeable on figure 1 where there is a distinct drop in production just below the water surface where the irradiance is highest. This is due to the process of photoinhibition. Under very high light levels, the incident radiation can damage the light-sensitive photosystem II pathway and result in somewhat depressed rates of photosynthesis compared to slightly lower levels. At the other end of the light curve, photosynthesis drops as the light level drops until photosynthesis is no

longer possible. Respiration is a much less light dependent process and typically assumed to be constant through the water column (Wetzel 1983).

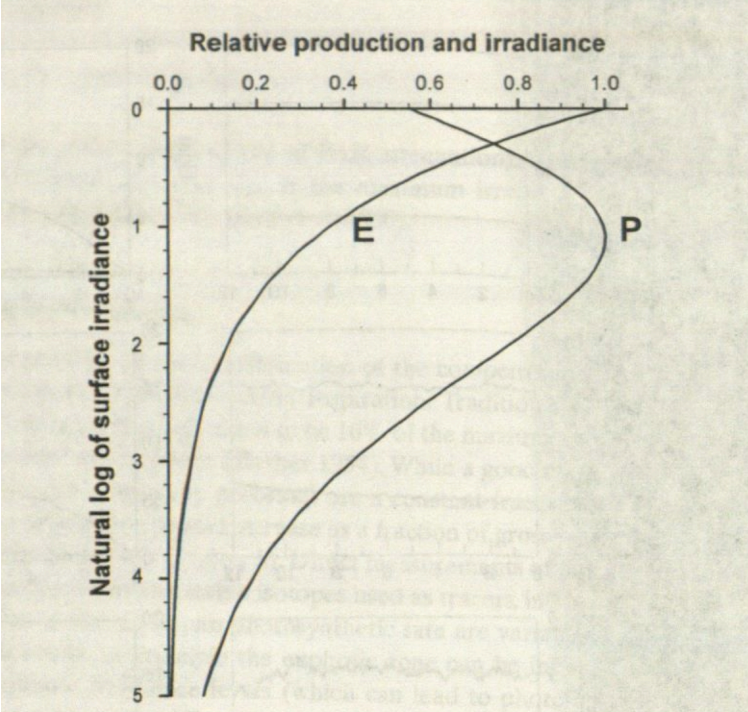


Figure 1. Diagram showing vertical representation of photosynthetic production (P) and irradiance (E) in a water column. Figure taken from (Wetzel 1983).

1.3.1 Importance of the P:R ratio

In ecological systems the relationship between production and respiration has been used to classify communities into either heterotrophic or autotrophic environments (Odum 1969). A system that is heterotrophic has a higher daily net respiration than primary production (P:R < 1). Conversely, for a system to be autotrophic its primary production exceeds its daily community respiration (P:R > 1). (Odum 1969). Stemming from that, we can discern the differing values of primary production to respiration by splitting the values up into two systems,

allochthonous and autochtonous. For an allocthonous system the main supply of organic matter comes from an outside source and is not primarily made in the system. On the other hand, autochtonous systems produce the majority of its organic matter in the system itself. (Vallino et al., 2005).

This is especially important in understanding the flow of energy in ecosystems. In ecosystems, an important factor in studying energy flow is the food web and transfer of nutrients. Essentially, all organic matter is either created or produced by photosynthetic production. On the other hand, organic matter is decomposed by respiration. Because all living organisms are dependent on organic matter it is useful to understand whether systems are allocthonous or autochtonous.

1.3.2 Trophic States of Systems

Phytoplankton photosynthesis is a key factor in helping provide numerical data to identify the trophic status of a system; and is especially useful in the beginning stages of systems that have high plankton biomass due to the large quantity of nutrients. Through a single growing season the nutrient density decreases from the surfaces mixed layer, plankton biomass starts to wane and other controlling factors come into play. Profiling the trophic status of systems is important in determining nutrient density and organic carbon production. By definition a lake that is considered oligotrophic is one that has

low nutrient input and low organic carbon production (Wetzel 1983). On the other hand, systems that are considered to be high in nutrients and also high in organic carbon production are eutrophic.

The concentration of organic material is split into two groups: (POC) particulate organic carbon and (DOC) dissolved organic carbon. The actual supplier of these carbon distributions can be either allochthonous or autochthonous. When the carbon is produced in the system (autochthonous) this is where it depends on the size, depth and turbidity to determine the primary producer for the respective body of water (Pinckey et al., 2001).

1.4 Methods for Calculating Plankton Production

Because of the importance of phytoplankton production several methods have been developed to measure this key ecological process. While numerous variations exist, these generally can be divided into the ^{14}C method, simulated incubations, and the In situ incubation.

1.4.1 ^{14}C Method

To best suit phytoplankton analysis, research is performed by enclosing a community in a container. There are several methods that can be performed once a community has been isolated (Brawley et al., 2003). The ^{14}C method is very suitable for studies on oligotrophic systems. To achieve this method, most

researchers incorporate radiolabeled CO_2 is added into the water supply. The plankton are then exposed to a known light level (either back in the lake or in the lab) and allowed time to photosynthesize, thereby incorporating ^{14}C into the cells. The water is then filtered and the radioactivity of the cells retained on the filter is measured. From there the equation is used to calculate the activity by measuring the amount of carbon that becomes assimilated into algal cells.

$$\frac{^{14}\text{C available}}{^{14}\text{C assimilated}} = \frac{^{12}\text{C available}}{^{12}\text{C assimilated}}$$

Although an accurate tool to measure plankton productivity, this method comes with several disadvantages. First, the use of the radioactive carbon creates a potential health hazard and should be handled with care. (Wetzel, Likens 2000).

1.4.2 Oxygen Change Method

Because of the strong light dependence of photosynthesis, assays must be done under varying light conditions in order to calculate the aerial rate of photosynthesis. This can be done either by placing bottles at varying depths through the water column, in situ, or by carefully exposing the bottles to varying light levels in the lab (lab incubations).

To accomplish in situ measurements water samples are collected and placed into “light” and “dark” bottles. The dark bottles are completely void of light with aluminum blocking light at the cap and the light bottles are

transparent glass bottles. Next, the bottles are hooked on to a metered suspension with the light bottles at the end and the dark bottle at the center and placed at varying depths in the water column where they were collected. The incubation period starts and should be allowed enough time for proper photosynthetic activity to occur (via the dissolved oxygen concentration changes). For this method the gross photosynthesis is determined by subtracting the final concentrations of both the light and dark bottles (Lampert et al., 2007). There are distinct advantages and disadvantages to the in situ method. The advantage is that the measurement is done on site, which gives a true environmental reading. However, disadvantages are evident in that the ease of access is extremely limited and time constraining. To get a full days cycle of production the incubation needs to start before the sun rises and be collected after, which can be time consuming for one measurement. The other confounding factor is that if the environment experiences a cloudy day, there will be limited photosynthetic activity and the measurement will have to be re-accomplished (Wetzel, Likens 2000).

1.4.3 Lab Incubation

Unlike the in situ method for measuring oxygen change, the incubation method takes place in the laboratory under controlled temperature and light conditions. These light and temperature conditions should simulate underwater

conditions in the field. The bottles are then measured using an O_2 electrode. Knowing the irradiance of light and the photosynthesis of the phytoplankton one can mathematically estimate the primary production that would happen in a given environment. To estimate this production for a day ecologists use a P vs. I curve (Wetzel 1983). There are two key pieces of information that can be attained from the P vs. I curve; they are Pmax and alpha. Pmax is the point where light-saturation plateaus the production curve. Alpha is the initial slope of the line indicating the cells ability to trap the attenuating light. (Wetzel, Likens 2000).

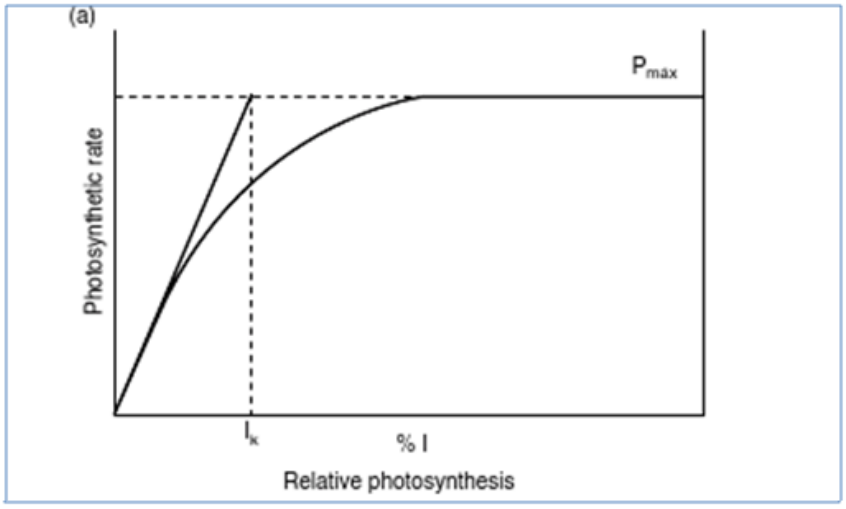


Figure 2. Graph showing the rate of photosynthesis versus irradiance (%I) adapted from (Falkowski 1997).

1.5 Role of Plankton Production and Respiration in the Net Ecosystem Production of Small Ponds

1.5.1 Net Ecosystem Production

Net ecosystem production (NEP) has been defined as total carbon within an ecological environment as the difference between gross primary production and total community respiration (Randerson et al., 2002). NEP takes into account all substances producing and respiring in a given system. The gross primary production, which is essentially the total amount of carbon accumulated in a period of time, has contributions to the ecosystem. Phytoplankton and various submerged aquatic vegetation can greatly contribute to the production through their ability to photosynthesize. In systems the amount that these producers create is affected by environmental conditions such as temperature, nutrients, irradiance and mixing all play key roles in what organism becomes the primary producer. (Woodwell, Whittaker 1968). Total community respiration is the sum of all organisms respiring in the aquatic ecosystem. This includes the plankton in the water column, SAV, and microorganisms in the sediment.

Recent evidence has concluded that many, perhaps most, aquatic ecosystems exhibit net heterotrophy despite the historical emphasis on primary production and the general expectation that aquatic ecosystems should be net autotrophic communities (Duarte and Prairie, 2005). A recent study of 19 small (mean depth 1-3.5m) natural (not man-made) Danish Lakes support this

conclusion with all of the lakes exhibiting net heterotrophy with an estimated annual community P:R ratio of only 0.6 (Sand-Jensen and Staehr, 2009). The net heterotrophic nature of aquatic ecosystems indicates that an organic carbon subsidy from the surrounding terrestrial environment is necessary to support the observed high rates of aquatic respiration.

1.5.2 Importance of Small Ponds

Little has been studied in regards to small pond environments, but the importance cannot be overlooked. Approximately 20% of standing water that exists in the United States is composed of shallow systems (Smith et al., 2002). These shallow systems are crucial to the survival of various species as well as contribute to the carbon pool. Alongside the effects that small ponds have on the carbon cycle they also affect nutrient cycling altering changes in fluxes of both nitrogen and phosphorous (Smith et al., 2002). Although shallower bodies of water appear to have a significant ecological effect, only a small subset of research has been devoted to clarifying these results.

1.5.3 Submerged Aquatic Vegetation vs. Phytoplankton

Both algae and larger aquatic plants can contribute photosynthetically to an aquatic environment. In most aquatic environments, researchers have focused on phytoplankton P:R ratios because it has been found that most reservoir production is from phytoplankton (Woodwell, Whittaker, 1968). Due to

the role of phytoplankton in these systems, extensive knowledge is known on the production capabilities of the organism. In such, plankton serves as a key base for the food chain and generation of oxygen and energy for the environment (Lindeman, 1942). However, there are reservoirs where the phytoplanktonic role is diminished, and the main producer becomes aquatic vegetation. For example, in an ocean phytoplankton is the main producer because the oceans are too deep for rooted plants and the water surface is too turbulent for floating aquatic plants. On the other hand, there are ponds and riverine environments where the primary producer has been submerged vegetation.

These conditions have been readily studied, and an idea that ecosystems can have differing stable states has been offered. In shallow aquatic ecosystems there are two differing states: Turbid and clear. In the turbid state phytoplankton dominate the lake and turbidity will increase due to the large influx or recycling of nutrients. Because of the turbidity of the system it is difficult for light to penetrate the water column and aquatic plants cannot grow. Contrary to that, there can be a clear stable state where aquatic vegetation is heavy in number reducing the turbidity and phytoplankton growth. (Scheffer 2007). In regards to small-pond ecosystems, there has been no distinction made to whether or not the sole producer in the ponds is either aquatic vegetation or phytoplankton.

1.5.4 Plankton Production in Small Ponds

There is a large literature regarding the ecological importance of small farmland ponds. The topic of interest to most of this research has been the effects that algal production has on yield of fish or crayfish in ponds (Wichelen et al., 2006). Most studies discern the relationship of phytoplankton to the number of macrophyte and fish populations (Søndergaard et al., 2005). However, few studies have been made to calculate plankton production in small ponds for the purpose of ecological awareness almost all studies focus on agricultural implications. From an ecological perspective, understanding rates of plankton production is crucial because it is known that small ponds exhibit the alternating states. Understanding whether or not a shallow system is dominated by rooted aquatic vegetation or phytoplankton will shed light onto which species is the primary producer (Allende et al., 2009). Considering small lakes and ponds make up the most of the standing water bodies on earth understanding plankton production rates is important for ecological, agricultural, and carbon cycling purposes (Christensen et al., 2013). The ecological role of plankton production and respiration as it contributes to net ecosystem production in shallow farm ponds appears to be understudied and is the focus of my research.

1.6 Factors Regulating Plankton Production

1.6.1 Light

It has been established that photosynthesis is a light-dependent process; however there are mechanisms that phytoplankton can exhibit to increase photosynthetic processes in an environment where light is scarce. For instance, in the Tagus estuary the light deprived phytoplankton exhibited extremely high photosynthetic efficiency and low saturated photosynthetic rates as a response to the light limitation (Gameiro et al., 2011). In general, phytoplankton display mechanisms to acclimate to light limiting and light-saturating circumstances, and the presence of these light variable environments controls plankton production.

1.6.2 Mixing

In shallow systems mixing is a key player in regulating plankton production. Because these systems have a limited depth, the mixing of nutrients is of a regular occurrence. Because it has been noted that phytoplankton biomass density is inversely proportional to mixing depth, one would expect that in a shallow system with large amounts of mixing phytoplankton biomass would be higher, thus increasing the net production. However, this factor alone does not control the ecological system and the potential for changing states to a system dominated by submerged aquatic vegetation is a possibility (Huisman, 1999).

1.6.3 Temperature

Phytoplankton follow the general trend that aquatic vegetation in regards to temperature. In especially cold environments such as during the winter period, phytoplankton growth and production is retarded. In periods of high temperature such as the summertime there is a mass influx of nutrients and phytoplankton production and growth. Temperature is a key regulator in phytoplankton production because algal cells can only operate at certain temperatures (Molles, Cahill, 2011).

1.7 Research Hypothesis / Objectives

Although plankton production and respiration is only a portion of the total community net ecosystem production, it is a critically important component and is the focus of my research. The main objective of the study performed is to:

1. Determine daily aerial rates of plankton production and respiration for 27 ponds in a watershed in the Waco area.
2. Compute the daily plankton P:R ratios in these ponds and determine whether or not the plankton communities are heterotrophic (P:R <1) or autotrophic (P:R > 1)
3. Determine if rates of plankton photosynthesis and P:R ratios are different in ponds with abundant macrophytes versus ponds lacking macrophytes.

This study will test the following hypotheses:

1. Ho: Plankton P:R ratios for these 30 ponds will be < 1. Current research suggests that most aquatic ecosystems are net heterotrophic (see above).
2. Ho: Plankton P:R ratios in ponds without macrophytes is the same as ponds with macrophytes.

CHAPTER TWO

Materials and Methods

2.1 Study Site

Twenty-seven small ponds in Central Texas were measured for planktonic metabolism from January to late March in this current study. The ponds were all within the Bullhide Creek watershed, which drains into the Brazos River and is located in central Texas; (Figure 1). My research is part of a larger study of small, shallow ponds being done as a Ph.D. research project by Ms. Melissa Mullins, Department of Biology. Approximately 300 off-channel ponds were identified and a random sub-sample of those identified were used for possible inclusion in the study. A final group of 28 ponds was selected based on location and ability to secure permission from private land-owners to sample the ponds. If the pond was not found another similar pond would be replaced in the study. The ponds sampled most were agricultural farm ponds, and some were for aesthetic qualities. Of the twenty-eight ponds measured in this study they ranged in size from 300 m^2 to 18,000 m^2.

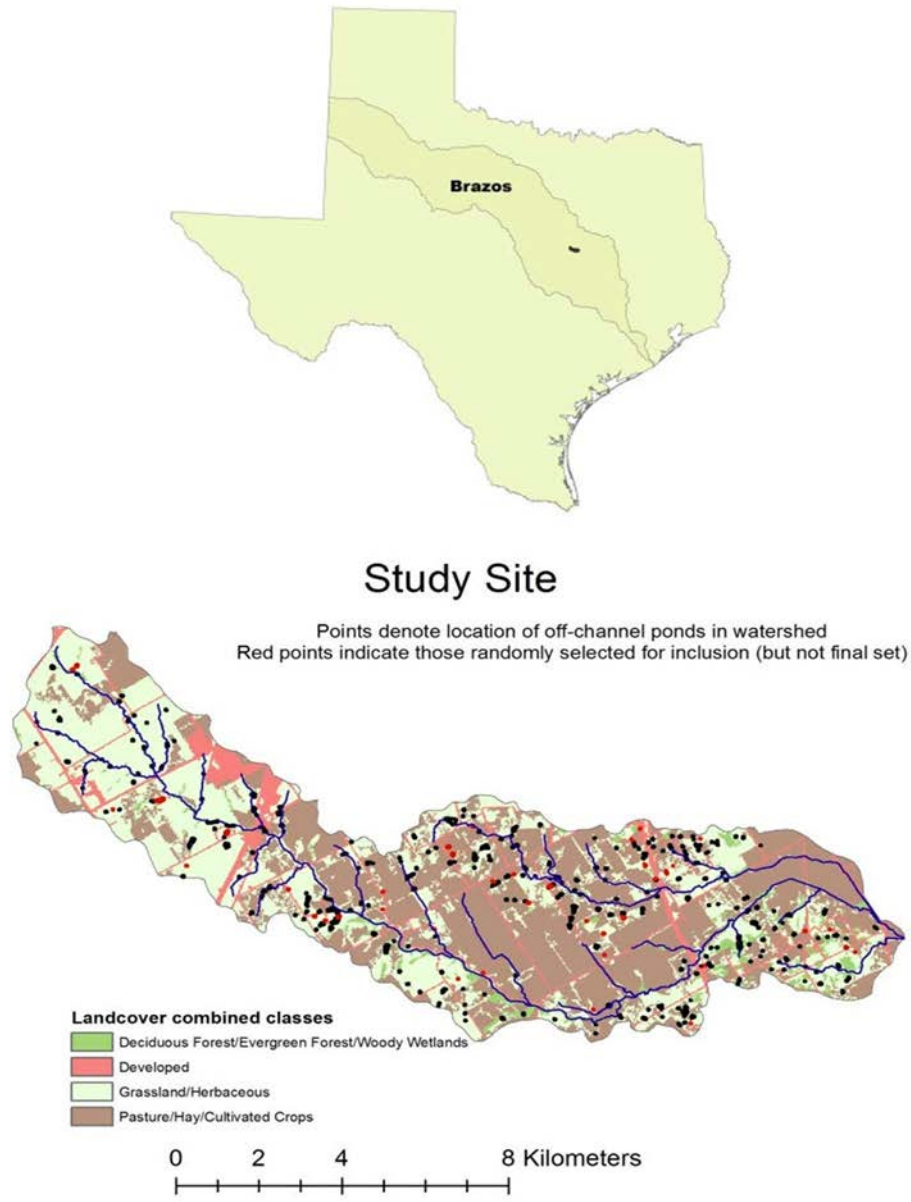


Figure 3. Bullhide Creek watershed southeast of Waco, Texas. Approximately 300 total off-channel ponds were identified in the watershed and 28 ponds were selected from a randomly selected sub-sample (red dots) for inclusion in this study based on location and land-owner permission to access and sample the ponds.

2.2 Field Data Collection

Data was collected weekly from the period from January to March of 2013. Sub-surface grab samples were collected in acid-washed containers from 1-2 ponds each sampling day and put in coolers and transported back to the laboratory at the Baylor Science Building for further measurements. If the sample needed to be stored for more than two hours before laboratory analyses began, they were kept at collection site. Turbidity was the first measurement acquired and was run on a Hach Company 2100 N bench top turbidimeter.

In-vivo fluorescence was determined by using a Trilogy Turner Design machine. Sample pond water was placed into a cuvet and wiped carefully with chem-wipes and placed into the fluorometer. Fluorescence was than measured from the machine.

2.3 Chlorophyll a

Chlorophyll a was measured by filtering a known amount of water through a GFF filter. Filtration volumes between 500 and 1000 ml were used based on sample turbidity impacting the total volume, which could be filtered through a single filter. The filter was removed from the manifold and folded in half several times, placed in foil, and frozen at -20C. Chlorophyll samples are maintained in the freezer until further extraction analysis is performed on them.

Chlorophyll a on the filter was extracted in ethanol and analyzed following CRASR standard operating procedures based on the procedure detailed

in the New Zealand Stream Periphyton Monitoring Manual (Biggs, Kilroy 2000). For extraction the filters were first opened and then placed in 15mL tubes. The filter was than unfolded so the sides containing the chlorophyll could maximize the interaction with the ethanol. Fill the tube with 5.0 mL of 90% ethanol and cap the tube. Next, I inverted the tubes and placed the racks in a water bath heated to 78C for 5 min. After the 5 minutes had passed I immediately placed the entire rack in a dark drawer at room temperature and let them stand for 24 hours. The next day I transported the rack into a room with a spectrophotometer. In each individual measurement the spectrophotometer was blanked with a control of 90% ethanol. Each individual tube was inverted again to assure proper mixing of the ethanol with the filter. The absorbances were measured at 665 and 750 nanometers respectively. 4.00mL of the supernatant is pipetted into the cuvette and then read in the spectrophotometer. Additionally, after the initial reading I acidified the sample with 0.1mL of 0.30M HCl and re-read the absorbances to correct for phaeophytin (degraded chlorophyll a).

2.4 Incubation Measurements

The photosynthesis-irradiance curve relationship of plankton was discerned by using the oxygen change method in closed biochemical oxygen demand bottles (BOD). This method is identical to the oxygen change method talked about earlier where the light bottles have an increase in dissolved oxygen

marking photosynthesis and in the dark bottles oxygen is consumed showing respiration. (Wetzel 1983). Planktonic photosynthetic parameters of P_{max}, alpha, and respiration were estimated by measuring the changes in dissolved oxygen (DO) in light-dark bottle incubations (Fee 1973). The incubations were performed in the laboratory under artificial light. Initial DO readings were taking of the entire sample in the container from the site. The container was bubbled down to 50% saturation with a 350-ppm carbon dioxide/ balance nitrogen gas mixture so there could be a sufficient change in oxygen without supersaturating the system. Oxygen supersaturation yields a poor estimation of the true photosynthesis because photorespiration occurs in the background at those oxygen concentrations. Once the DO level was lowered to about 50% saturation, nine 300mL BOD bottles were filled and capped immediately. Initial DO readings were taken for all bottles using a YSI oxygen model 5000 bench top DO meter and model 510 self-stirring BOD probe; twelve bottles from each pond were incubated at known light levels and DO changes measured over known time periods.

In addition, three control bottles were filled with high, medium, and low levels of tap water, and then topped off all the way with deionized water. These controls will also undergo an initial BOD measurement and then will be incubated and subject to varying levels of light. These controls will be used to keep the YSI probe from varying to far out of an appropriate range.

There are nine light bottles present in our experimental design. These nine bottles are split into three categories: High, medium, and low light levels. The light bottles are clear 300mL BOD bottles. Once the light bottles were filled and initially measured they were incubated in a water bath under ambient temperature of the site. I expected the high light level bottles to have the highest oxygen change, followed by the medium and low light bottles respectively. Because the oxygen change happens so quickly for the high light bottles; having the largest positive change in dissolved oxygen shows large changes in photosynthetic activity and will reach saturation faster so the high bottles are measured for oxygen change between 2-4 hours after the incubation. More often than not with such a high fluorescence that most of these agricultural ponds had; the middle bottles will have sufficient changes in dissolved oxygen as well and will be measured at the same time as the high light bottles. The low light bottles were left to incubate and were measured in the morning along with the dark bottles. Only in a couple extremely productive ponds were the low light level bottles read at the same time as the high and medium light bottles. Light was provided by two double-bulb fixtures of aquaria lights fitted with 6700K and 10,000K bulbs to provide a light quality similar to that of natural daylight; the high light bottles are placed directly under the light, the medium light bottles are placed just outside the direct light, and the low light are placed outside of the light range. Typically the high light bottles were

incubated at a PAR range of 450–600 $\mu E^{-2}s^{-1}$ while the medium and low lights were incubated at 150-200 and 20-140 $\mu E^{-2}s^{-1}$, respectively. The light measurements were taking with a LiCor Spherical underwater PAR sensor that was fully submerged under water where the bottle would be placed.

An additional three BOD bottles were used to measure planktonic respiration in dark BOD bottles. These BOD bottles will be completely shaded with no light being able to penetrate. I filled the bottles and took the initial measurement, then capped them, wrapped them in aluminum foil and incubated them in the water bath. DO levels were adjusted so that the initial Do were in the range of 50-70% oxygen saturation. For the respiration cycle, bottles were left overnight to incubate and measured in the morning, most often having a twenty-four hour incubation. The standard measurement seen was a decrease in dissolved oxygen, which is consistent with respiration in a given system.

2.5 Estimation of Potential Daily Planktonic Photosynthesis and Respiration (Walsby Method)

Daily gross primary production and respiration of the surface layer of our ponds were calculated using the Walsby method. For the Walsby calculation several values were needed for input such as light extinction coefficient, P_{max} , alpha, temperature, respiration, irradiance, and depth of the surface layer. Several photosynthetic parameters were generated by data analysis and observed

from the incubation measurements. P_{max} (max photosynthesis) was calculated as the average of all the high light bottles in a given sample. Alpha was calculated as the slope between the dark and light bottles and the respiration bottle was calculated as the average of the dark bottles. These parameters were used in graphing the photosynthesis-irradiance curve where alpha is the slope of the graph, see figure 2. Evident on figure 2 is the high light levels, which have the highest net photosynthesis followed by the middle and low light level bottles.

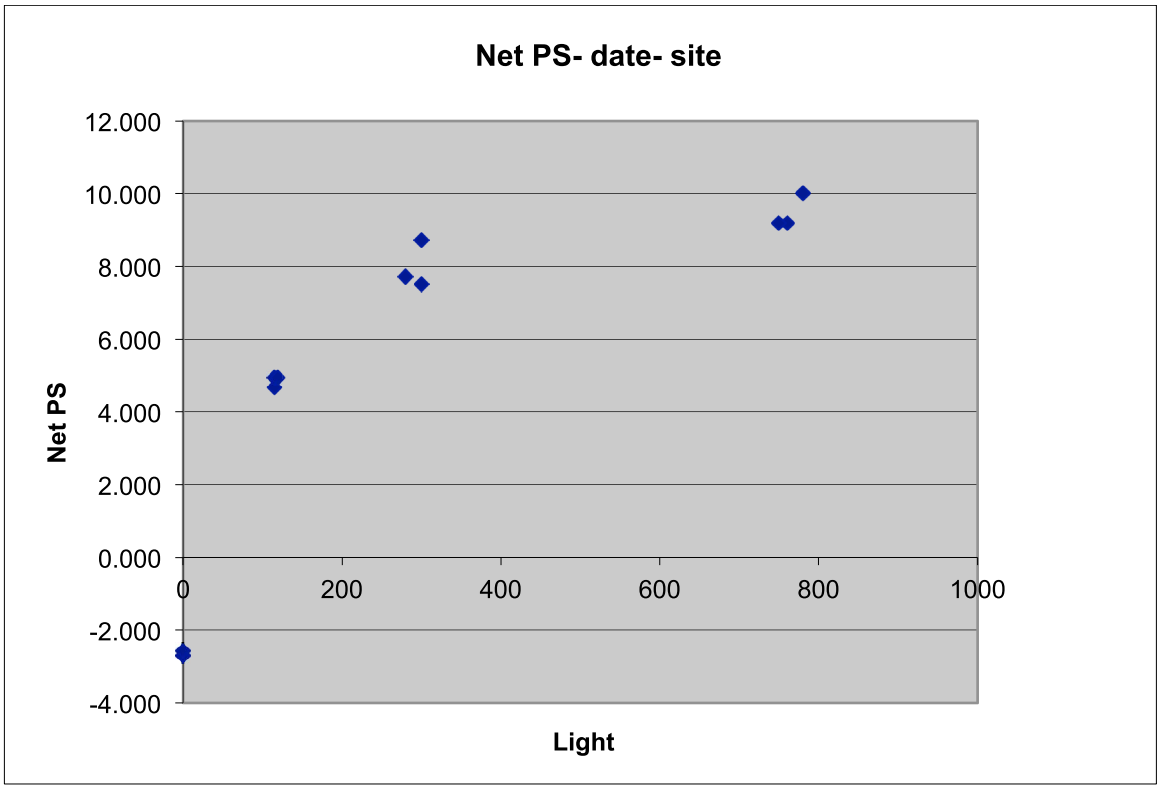


Figure 4. The photosynthesis-irradiance curve. Light is plotted against photosynthesis, the slope of the line is alpha. Taken from a pond in the current study (SG1).

Incident light was obtained from the Hobo U30 brand meteorological station maintained by Dr. Ryan King at the Lake Waco Wetlands. For the Walsby

calculations incident light was used for a time period from sun up to sun down for a 24-hour period. Light extinction coefficients were obtained from the light (LiCor) readings at varying depths in the water column. More often than not several depths were presented and to calculate the light extinction coefficients I plotted the LN light vs. depth, and k (light-extinction coefficient) was the slope of the line. Pond depth was obtained from the field. A canoe was taken to each individual pond, swam out to the middle, and a meter stick was stuck into the water column until the bottom was found and than the meter stick was measured. If a YSI was available and water was deep enough a vertical light profile was done through the water column. Field temperature was obtained from a field thermometer, which was set at the surface and measured when the DO sensors were deployed. Chlorophyll a is also needed for the Walsby method and the methods were stated earlier.

CHAPTER THREE

Results

Table 1. Physical features of study ponds including size (m^2), depth (m), the type and relative abundance of Submersed Aquatic Vegetation (SAV) and the use of the land near the ponds.

Pond	Size (m^2)	Depth (m)	SAV Present	Dominant SAV Species	Land-Use
LF1	250	1.50	Absent	N/A	Grazed Pasture
HDG	313	0.56	Intermediate	Najas	Grazed Pasture
UP1	453	0.50	Absent	N/A	Residential
GK	546	0.70	Intermediate	N/A	Residential
SG1	548	1.37	Intermediate	Extremely Sparse Chara	Pasture/Field
BN2	787	0.92	Intermediate	Potamogeton	Grazed Pasture
BF2	815	1.30	Abundant	Chara	Grazed Pasture
HH1	1,064	1.37	Intermediate	Sparse Chara	Hayfield
GP	1,583	1.20	Absent	N/A	Pasture
SG2	1,952	2.40	Absent	N/A	Pasture/Field
DJ2	2,229	5.03	Intermediate	Sparse Chara	Wooded
DC	2,398	2.37	Absent	N/A	Grazed Pasture
BN1	2,530	1.00	Abundant	Narrow Leaf Pondweed	Grazed Pasture
DG	2,558	2.60	Intermediate	N/A	Wooded
JJ1	2,587	0.41	Intermediate	Moderate Chara	Grazed Pasture
BF1	2,683	1.30	Absent	N/A	Wooded
WJC1	3,112	2.83	Absent	N/A	Grazed Pasture
DJ1	3,371	5.80	Intermediate	Sparse Chara	Wooded
HH2	3,549	1.98	Intermediate	Sparse Chara	Wooded
AB1	3,759	3.65	Intermediate	Southern Naiad	Hayfield
WJC2	4,012	2.25	Intermediate	Ludwigia	Hayfield
AB2	4,313	2.40	Intermediate	Chara	Grazed Pasture
SA1	5,743	0.60	Intermediate	Chara	Grazed Pasture
A1	7,987	0.76	Abundant	Chara	Grazed Pasture
A2	11,837	0.91	Abundant	Chara	Grazed Pasture
JT	14,208	2.07	Abundant	Abundant Coontail	Hayfield
JH	17,366	4.60	Intermediate	Moderate Chara	Hayfield

3.1 Physical and Plankton Photosynthetic Characteristics of the Ponds

Physical factors of the twenty-seven are listed above in table 1. Ms. Melissa Mullins as part of her Ph.D. research surveyed the ponds for size, depth, and presence of aquatic macrophytes during the winter 2013 period. These are all small, shallow ponds. Size varied greatly from pond to pond with the smallest being 250 m^2 and the largest being 17,366 m^2. Of the twenty-seven ponds sampled the average area of these was 3,935 m^2. The average depth was 1.94m. Depth varied from a minimum depth of 0.41m to a maximum of 5.80m. There were seventeen ponds that had submerged aquatic vegetation (SAV) and types varied per pond with the most abundant genus being *Chara*. For analysis, ponds were divided based on the amount of SAV into three groups: absent, intermediate, and abundant. Of the twenty-seven ponds there were five ponds absent of SAV, twelve with intermediate, and six with abundant SAV. Predominant land-use surrounding the pond was accessed visually by Ms. Mullins during her survey. Twelve of the twenty-seven ponds were in grazed pastures, five each were categorized as hay fields or wooded and a smaller number were found in yards or pasture/fields.

Table 2. Plankton photosynthetic parameters for each sample date.
Pmax & R (mgO_2 mg $chla^{-1}$ h^{-1}); Alpha (mgO_2 mg $chla^{-1}Ein^{-1}m^{-2}$)

Date	Pond	Pmax	Respiration	Alpha
2/13/13	LF1	17.2	-14.0	38.3
2/20/13	HDG	7.6	-1.0	10.3
2/13/13	UP1	26.3	-3.4	45.6
2/11/13	GK	20.4	-6.8	48.9
2/18/13	SG1	6.0	-1.3	13.6
2/27/13	BN2	12.9	-4.9	38.9
3/8/13	BF2	12.2	-7.2	50.0
3/13/13	HH1	15.7	-1.3	38.1
2/11/13	GP	23.5	-1.4	38.1
1/23/13	SG2	14.4	-1.3	35.0
1/28/13	DJ2	13.2	-3.1	35.0
3/15/13	DC	17.4	-1.9	38.9
2/27/13	BN1	16.5	-5.8	36.1
2/18/13	DG	11.4	-2.3	20.6
2/25/13	JJ1	25.4	-2.8	29.7
3/8/13	BF1	12.9	-3.1	48.3
2/27/13	WJC1	12.2	-6.7	19.2
1/28/13	DJ1	21.0	-3.4	26.4
3/13/13	HH2	10.2	-1.9	59.4
3/5/13	AB1	29.0	-3.9	40.8
2/27/13	WJC2	20.2	-1.8	46.4
3/5/13	AB2	64.4	-20.9	67.5
3/12/13	SA1	10.4	-6.5	100.6
3/5/13	A1	23.7	-6.8	45.6
3/5/13	A2	30.2	-2.6	26.4
3/15/13	JT	15.9	-6.5	51.4
1/23/13	JH	27.3	-4.6	84.2

Photosynthetic factors Pmax, respiration and alpha values are shown in table 2. The average Pmax (Maximum photosynthesis) was 19.34 and the average respiration was -4.84 mgO_2 mg $chla^{-1}$ h^{-1}. Maximum value for Pmax was 64.4 and

the minimum was 6 mgO_2 mg $chla^{-1}$ h^{-1}. Alpha was averaged to be 43.23 mgO_2 mg $chla^{-1}Ein^{-1}m^{-2}$. Respiration values ranged from -1.01 to -20.94.

Table 3. Chemical factors that were related to the twenty-seven ponds. Chl-a (mg/m^3), Fluorescence (FU), Turbidity (NTU), Zeu (m), DOC (mg/L), TP (ug/L).

Pond ID	Chl-a	Fluorescence	Turbidity	Extinction Coefficient	Zeu	Zeu/Z	TP	DOC
LF1	1.89	171	4.55	3.5	1.31	0.87	15.6	228
HDG	4.82	85	7.89	1.2	3.86	6.89	15.0	42
UP1	8.77	325	18	4.4	1.05	2.11	18.0	460
GK	2.58	384	4.61	3.7	1.24	1.77	16.8	305
SG1	25.8	287	12.7	1.9	2.43	1.77	8.6	32
BN2	7.02	178	11.3	2.4	1.91	2.08	11.4	48
BF2	4.24	91	4.34	0.8	5.85	4.50	13.2	30
HH1	95.95	778	77.4	6.2	0.74	0.54	27.7	512
GP	9.2	396	13.3	1.7	2.77	2.31	13.6	64
SG2	42.65	574	107.14	3.5		0.99	11.2	148
DJ2	8.25	114	1.8	0.9	0.61	0.26	11.7	181
DC	22.24	378	112	7.6	5.40	5.40	11.2	31
BN1	1.84	67	6.07	0.9	2.31	0.89	18.7	39
DG	6.28	118	4.95	2.0	1.07	2.60	9.6	53
JJ1	29.63	1355	11	4.3	0.82	0.63	13.0	147
BF1	26.31	582	52.2	5.6	1.48	0.52	9.6	75
WJC1	13.7	242	33.5	3.1	4.40	0.76	8.2	25
DJ1	7.22	232	3.6	1.0	1.91	0.96	6.3	37
HH2	12.4	161	21.5	2.4	2.82	0.77	9.7	31
AB1	9.69	195	2.78	1.6	1.18	0.52	12.7	88
WJC2	12.2	336	40.6	3.9	3.30	1.38	9.9	31
AB2	1.2	90	5.07	1.4	1.35	2.25	11.1	271
SA1	7.28	227	70.4	3.4	4.04	5.32	7.8	39
A1	3.15	69	5.37	1.1	1.23	1.35	10.3	152
A2	50.56	3998	50.1	3.7	3.16	1.53	6.6	21
JT	2.87	72	7.84	1.5	2.91	0.63	9.0	19
JH	2.97	65	30.3	1.6	1.31	0.87	15.6	228

Algal, water clarity and nutrient factors measured in the ponds are shown in Table 3. Chlorophyll-a averaged 14.54 mg Chl-a/m^3. Chlorophyll-a ranged from a maximum value of 95.95 to a minimum of 1.2 mg Chl-a/m^3. Fluorescence that was measured using the Trilogy fluorometer averaged 422.97 and had a range from 64.87 to 3998.1 FU. The turbidity average was 23.58 and had a median of 11.3 NTU. Zeu, the euphotic zone, is measured as the distance from the surface to a depth that has a light intensity equal to one percent of the surface. My measured Zeus averaged 2.47 meters. Zeu/Z is a way to measure how far the euphotic extends down through the pond. It is a measure of the Zeu divided by the entire depth. A Zeu/Z < 1 shows that light penetrates only part of the way down through the water column and the lower portion of the water column is dark (and hence unavailable for photosynthesis) even during the daytime. Likewise a Zeu > 1 shows that the entire water column is capable of photosynthesis during the day. Our Zeu/Zmix average was 1.91 with a median of 0.98 meaning that on average light penetrates throughout the water column but ~50% of the values actually are less than one suggesting at least part of the water column is dark during the daylight periods. The total phosphorous averaged 12.3 ug/L with a median of 11.2. Dissolved organic carbon (DOC) ranged from 19 to 512 mg/L with an average of 124 mg/L. The median of the DOC measurement was 53 mg/L.

Table 4. Production to respiration values as well as gross photosynthesis and respiration for each sample pond. Daily gross production integral, Daily Respiration Integral (mmol O2 m^2).

Pond ID	Daily GP Integral	Daily R Integral	P:R	P:R Code
LF1	7.9	-29.69	0.27	Heterotrophic
HDG	4.5	-1.82	2.47	Autotrophic
UP1	25.87	-11.21	2.31	Autotrophic
GK	8.91	-9.09	0.98	Balanced
SG1	21.95	-35.27	0.62	Heterotrophic
BN2	22.1	-23.56	0.94	Balanced
BF2	20.36	-29.76	0.68	Heterotrophic
HH1	219.33	-126.48	1.73	Autotrophic
GP	65.76	-11.64	5.65	Autotrophic
SG2	165.61	-98.82	1.68	Autotrophic
DJ2	77.98	-77.49	0.82	Balanced
DC	43.82	-8.07	0.57	Heterotrophic
BN1	9.81	-27.75	1.22	Balanced
DG	32.94	-112.37	1.19	Balanced
JJ1	45.97	-80.45	0.41	Heterotrophic
BF1	45.24	-195.3	0.56	Heterotrophic
WJC1	46.57	-105.22	0.24	Heterotrophic
DJ1	138.03	-34.12	1.31	Balanced
HH2	55.77	-104.41	1.63	Autotrophic
AB1	135.02	-37.68	1.29	Autotrophic
WJC2	63.74	-45.22	1.69	Autotrophic
AB2	38.17	-21.28	0.84	Balanced
SA1	6.69	-12.19	0.31	Heterotrophic
A1	15.27	-88.56	1.25	Balanced
A2	431.41	-29.18	4.87	Autotrophic
JT	27.46	-46.36	0.94	Balanced
JH	43.81	-46.36	0.95	Balanced

For the twenty-seven ponds included in the study the average P:R ratio was 1.39 (Table 4). The P:R average above 1 showed that on average these ponds were autotrophic by nature. The average gross production integral was 67.41 ranging from 3.15 to 427.42 mmol O2 m^2. Also, the average respiration integral

for the entire day was -53.82 with a median of -34.7 and ranged from -1.82 to -195.3 mmol O2 m^2. There were nine autotrophic ponds, ten balanced, and eight that were heterotrophic.

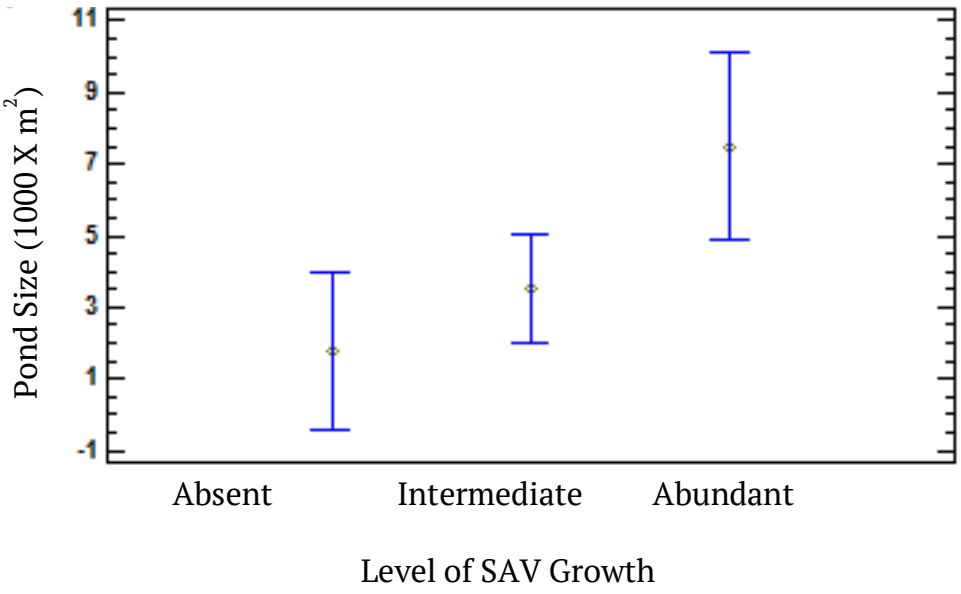
For the twenty-seven ponds measured in the winter period the minimum P:R listed was 0.24 and the maximum was 5.65 with the range being 5.41.The average P:R ratio of 1.39 had a standard deviation of 1.26. The standard error was 0.24 for all the ponds.

3.2 Relationships of photosynthetic parameters to measured physical and chemical factors.

Detailed in appendix 1 is a correlation chart showing each variable against the various parameters that were measured in the study. The p-values highlighted in red are relationships that are deemed to be significant in the chart. For my study a p-value <.05 was taken as significant.

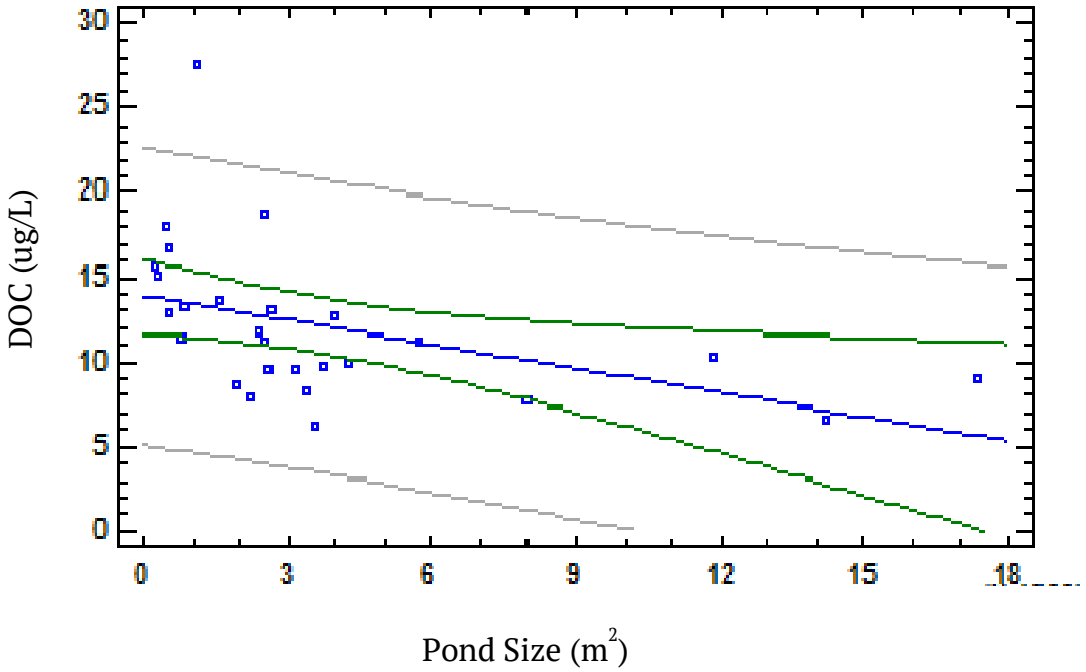
Although pond size was not related to P:R directly (p= .64), there was a distinct relationship between pond size and SAV (Figure 5). The ponds with more abundant SAV were significantly larger than ponds lacking SAV. Smaller ponds tend to have a greater inflow of sediments clouding the pond and decreasing the SAV. This is evident in the ANOVA showing an upward trend between the size and the proportion of SAV in the pond.

Figure 5. Relationship between pond size and level of SAV growth in ponds (95% C.I). The average size of ponds with abundant SAV tended to be larger than other ponds (Means and 95% LSD Intervals, One way ANOVA, p = 0.04).



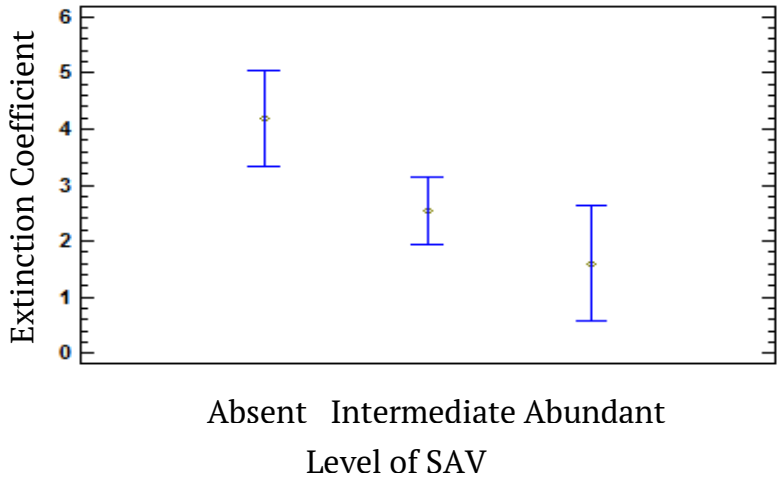
A regression performed between the amount of DOC (Dissolved Organic Carbon) and the size of the pond was also performed. There was weak but significant relationship between size and the amount of DOC in the ponds (Figure 6). The relationship showed that as size increased the amount of DOC in the pond decreased. Having a smaller pond allows more DOC to remain in the water column. The slope of the line was significant meaning that the relationship is near linear. The correlation coefficient of 0.45 describes the strength of the linear relationship between the two variables, in this case pond size alone explains only 20% of the variability in DOC indicating that many other factors influence this parameter.

Figure 6. Regression analysis of DOC vs. Pond size (p = .02 r = 0.45)



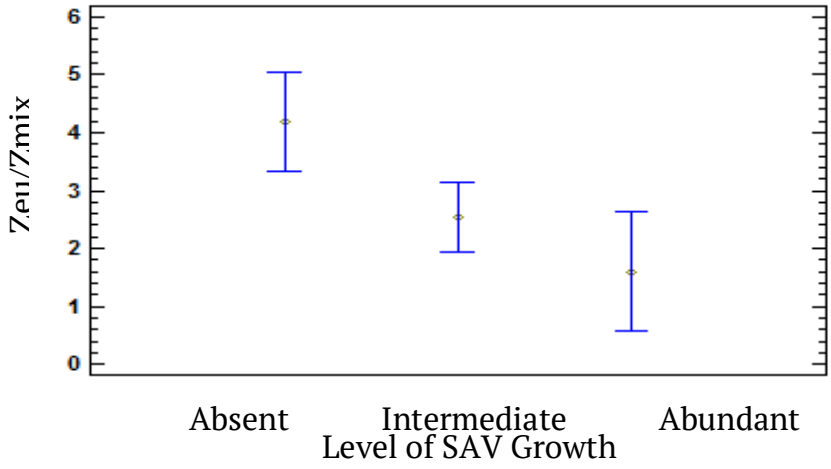
In addition submerged aquatic vegetation also has a distinct relationship with the extinction coefficient (Figure 7). In figure 7 below the relationship shows that as SAV gets denser the extinction coefficient decreases. This is consistent with ecological principles; with higher vegetation more light penetrates through the water column causing ponds to be stirred less and much clearer. The analysis had a p-value of .02 and in statistics that is deemed a significant relationship.

Figure 7. Relationship between extinction coefficient and SAV. The extinction coefficient lowers as SAV becomes more abundant (One-way ANOVA, p= .02).



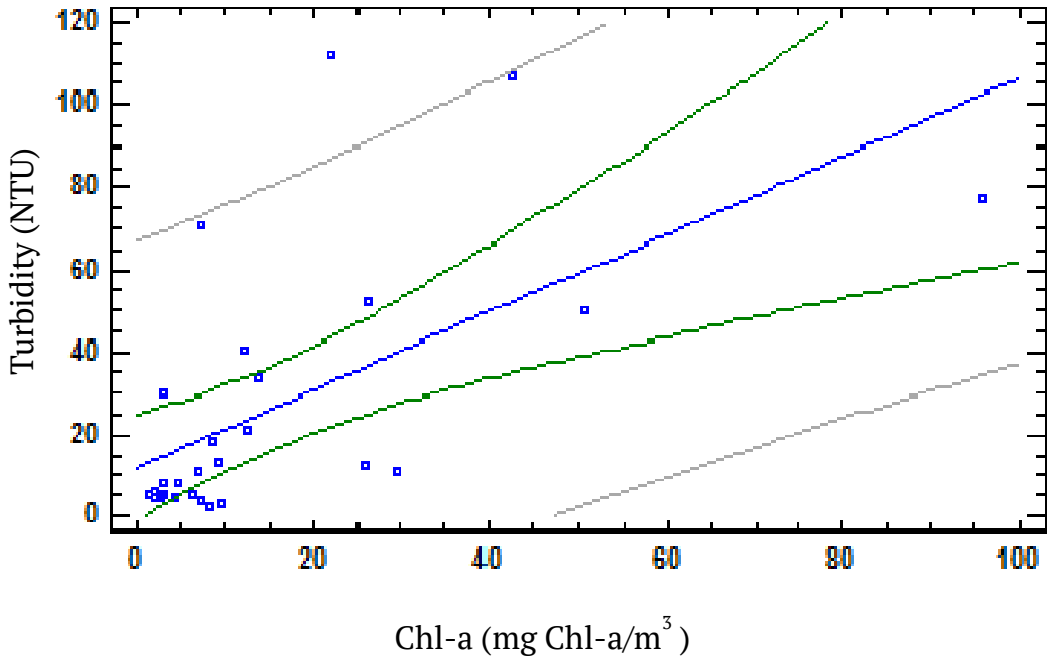
In addition there is a positive correlation between Zeu/Zmix and SAV abundance. As SAV increases Zeu/Zmix is also increased with light penetrating deeper.

Figure 8. Relationship between Zeu/Zmix and SAV. The Zeu/ Zmix increases as SAV becomes more abundant (Means, 95% Intervals One-way ANOVA, p= .02).



Chlorophyll-a and turbidity had a significant relationship according to appendix 1. There was a correlation between these two parameters when I performed a regression. Figure 9 below has the P-value of 0.0008 showing that the relationship is significant. Ecologically this relationship is supported in literature. A more turbid could mean more algal cells increasing the amount of chlorophyll-a.

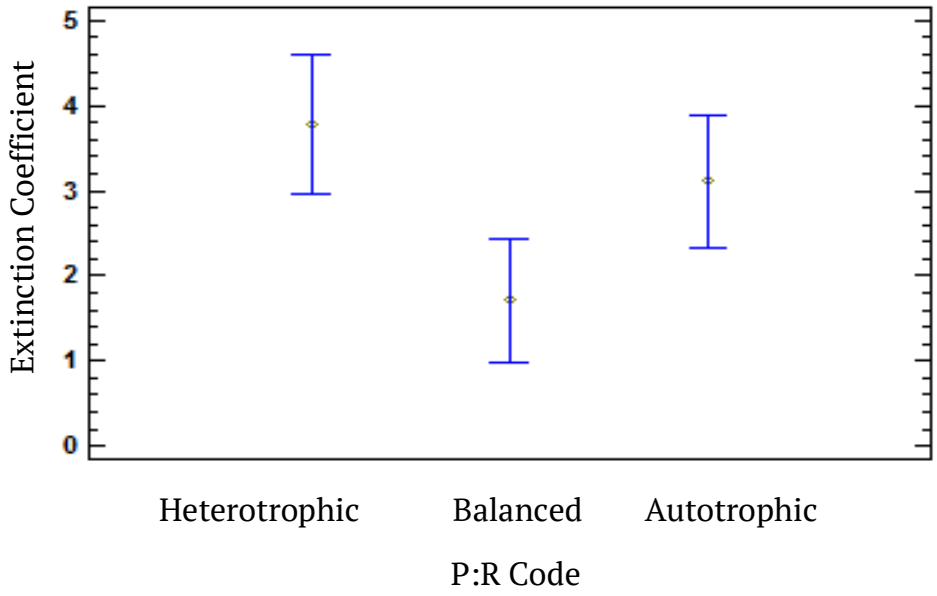
Figure 9. Regression between chlorophyll-a and turbidity (p=.0008, r =0.60).



For use in the ANOVA, P:R ratios were split into three categories. Any P:R ratio between .24-.70 was considered heterotrophic. P:R's within the range of 0.7-1.3 were considered approximately balanced. Finally, P:R ratios greater than 1.3+ were considered autotrophic. The relationship between P:R category and the extinction coefficient was significant with differing results. The data shows

that the extinction coefficient was higher for both heterotrophic and autotrophic ponds but not for balanced ponds (Figure 10). Probable causes of this disparity could be due to mud and algae affecting the water but will be discussed further in the discussion.

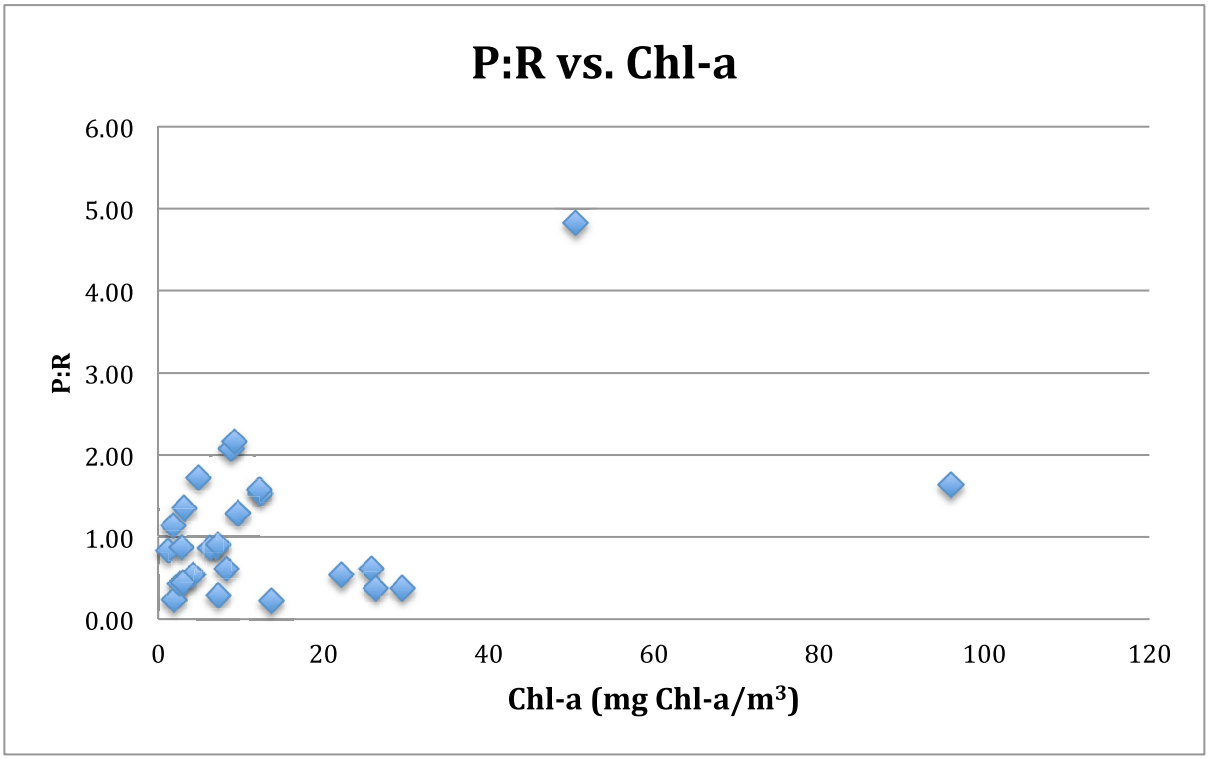
Figure 10. Relationship between extinction coefficient and PR category. The extinction coefficient was higher for both heterotrophic and autotrophic conditions (Means & 95% Intervals, One-way ANOVA, p= .03).



Although one might assume that the water column P:R ratio would be related to chlorophyll-a, we did not find a significant relationship in our study (Figure 11). Ponds with chlorophyll-a below 20 mg Chl-a/m^3 were about equally divided into our P:R classes with six heterotrophic, five balanced, and seven autotrophic ponds. Therefore ponds with 20-40 mg Chl-a/m^3 were all heterotrophic. The two ponds with abundant chlorophyll-a were strongly autotrophic. It is possible in

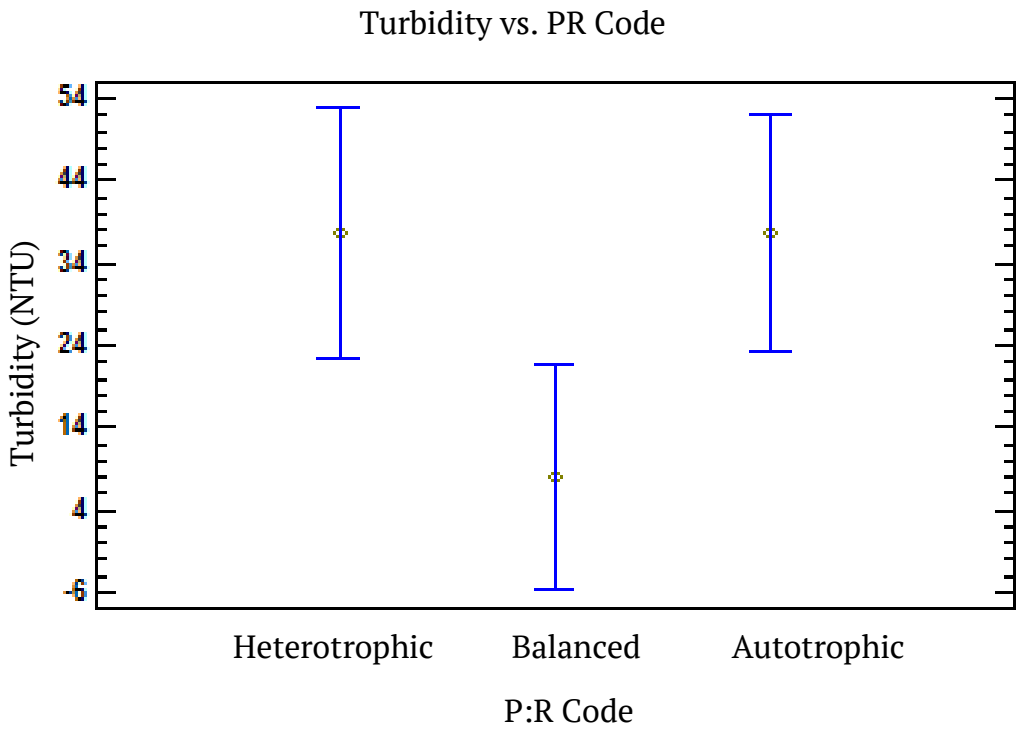
our twenty-seven ponds that a higher chlorophyll has to be reached to make a substantial change such as the data point at 55 mg Chl-a/m^3.

Figure 11. Regression between the P:R ratio and Chl-a values. (p=.27)



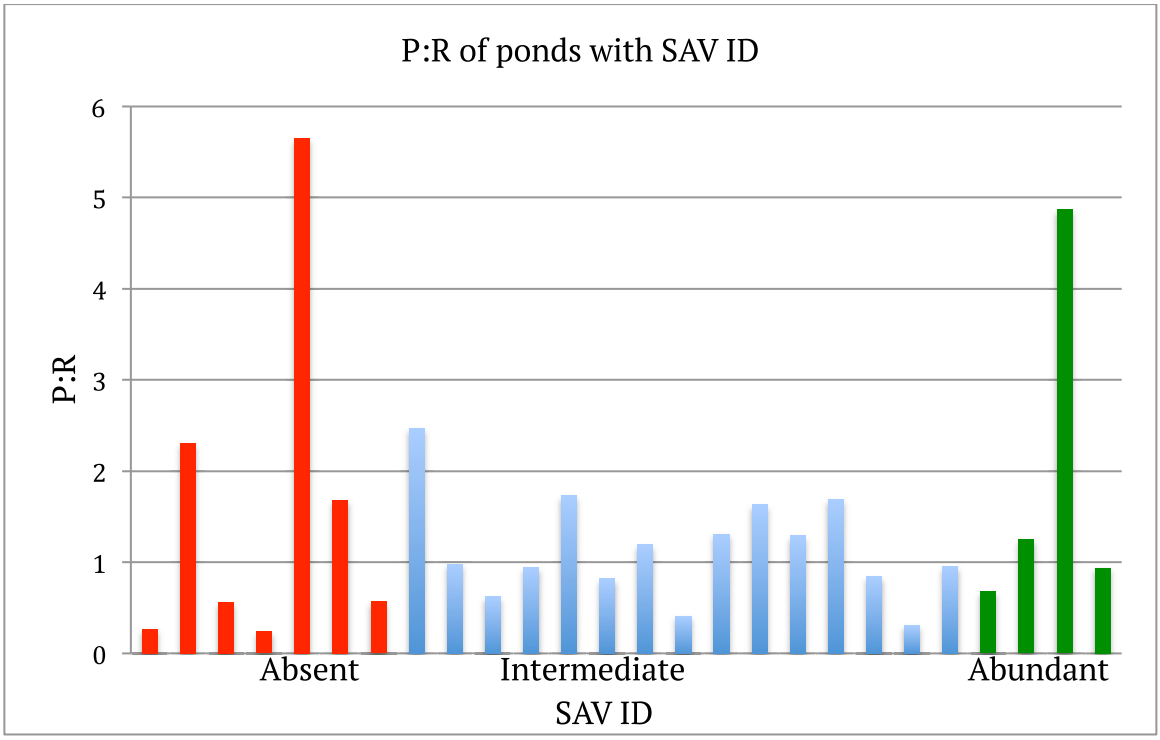
The relationship that shows up in appendix 1 that has a distinct relationship with P:R is turbidity. Shown below in figure 12 is an ANOVA of the ponds between turbidity and the P:R ratio. As stated above in relationship to the extinction coefficient, both the heterotrophic and autotrophic ponds had much higher turbidities than the "balanced" pond.

Figure 12. Relationship between turbidity and PR code. Turbidity tends to be higher for both heterotrophic and autotrophic types (Means and 95% Intervals, One-way ANOVA, p=.05).



The P:R ratio of each pond based on the SAV abundance level is shown in figure 13. Ponds with medium vegetation were much more balanced than ponds with no and high vegetation that had variable P:R values. Both absent vegetation and abundant vegetation had the greatest ranges in P:R values, but it is unknown what could cause this relationship because P:R and SAV were not correlated significantly. There were no significant similarities between P:R category and the related SAV, type of landscape, size, and depth were all checked for relationships. However, this does not account for the relatively stable intermediate SAV P:R ratios. Further research is needed to identify these disparities.

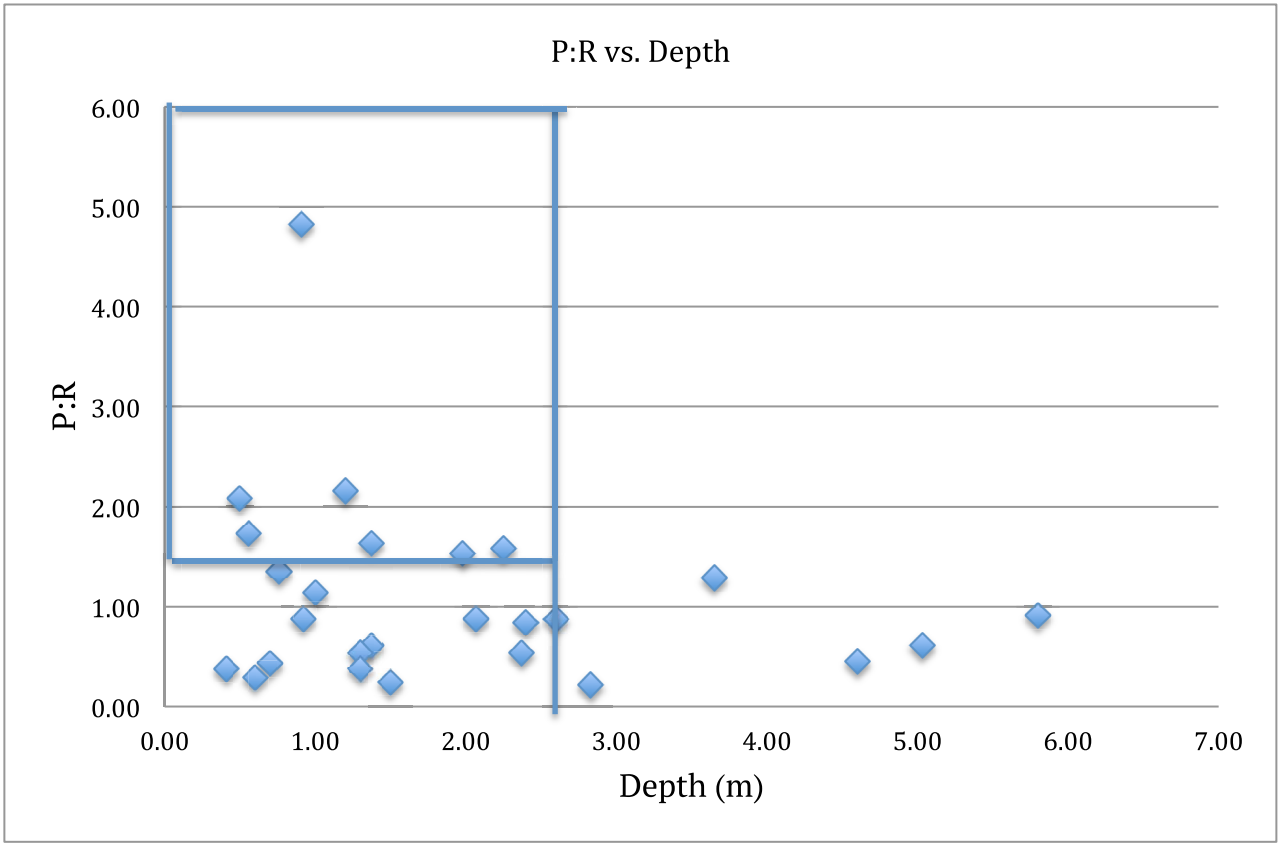
Figure 13. P:R ratio of pond groups according to SAV abundance. (red = SAV absent, blue = intermediate SAV, green = abundant SAV).



According to the data there is no clear relationship between the abundance of SAV and the P:R ratio. For our pond set it has been determined that P:R does not vary with SAV abundance. There are other underlying parameters that effect P:R and SAV is not one of them.

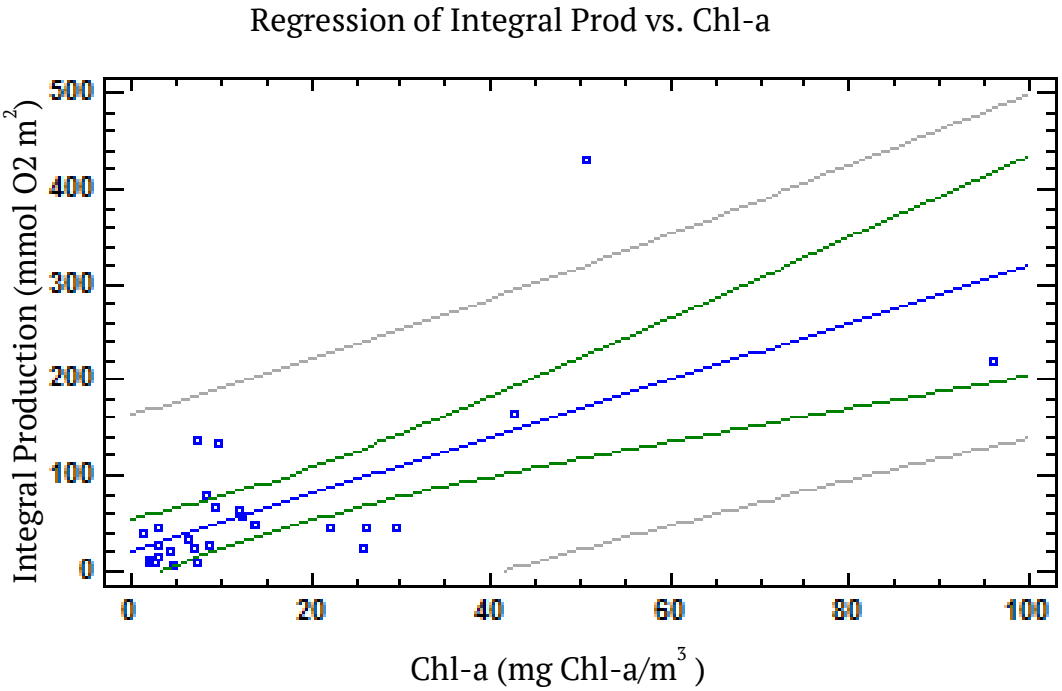
An interesting relationship shown in the boxed out section of Figure 14 is the P:R of autotrophic ponds versus the depth. All ponds that were strongly autotrophic also are very shallow ponds. Ponds that were heterotrophic ranged throughout all depths, and most balanced ponds were also independent of pond depth. We observed that none of the ponds deeper than 2.5m were strongly autotrophic.

Figure 14. Regression between P:R and pond depth. (p= .36)



Although chlorophyll-a was not directly linked to the P:R ratio it does have a distinct relationship with integral daily production. Figure 15 below shows a regression done on these two variables. The P-value at 0.0001 shows a significant relationship also paired with a high correlation coefficient of .679. Algal cells have a direct relationship with the production values that were obtained from the ponds.

Figure 15. Regression analysis between Chl-a and integral production (p= 0.0001, r = 0.68)



CHAPTER FOUR

Discussion

Substantial amounts of research have established the ecological effects that plankton has on ecosystems (Lampert et al., 2007). However, little has been established in terms of phytoplankton in small pond environments. There are large amounts of studies dealing with the agricultural importance of small farmland ponds, but not direct ecology studies on phytoplankton.

Photosynthetic parameters of Pmax, respiration, and alpha measured in this study were consistent with values reported in previous studies (Huang 2006). The phytoplankton daily production estimated in this study was based on an optimum light day (cloudless conditions). Therefore, my data represent the potential maximum production values for the plankton in the ponds. Since the daily respiration rates are not impacted by the light conditions used (since I assume respiration is constant and independent of light), the P:R ratios I report are the maximum potential P:R rates. The actual P:R ratio would in face be somewhat lower than the ratios obtained. A cloudy day would affect the P:R ratio decreasing the production.

The data set was seasonal only showing wintertime data, which potentially represents the minimum production and respiration rates compared to differing seasons. Seasonal variation of planktonic production in small pond ecosystems is yet to have many definitive studies and needs to be researched further before a clear answer is defined. (Gameiro et al., 2006).

4.1 Plankton P:R Ratios

My initial hypothesis that the ponds on average would be net heterotrophic and P:R < 1 was not supported by my results. Of the twenty-seven ponds studied fourteen of these had an average P:R < 1. According to the bounds set in the research there were nine strongly autotrophic ponds, ten relatively balanced, and eight strongly heterotrophic. There were thirteen ponds that had P:R > 1; (balanced and autotrophic) ponds that contributed to the average of the twenty-seven being net autotrophic had an average P:R ratio of 2.17. These thirteen ponds that averaged to be strongly autotrophic raised the overall average of the twenty-seven ponds to 1.39 and did not support my hypothesis.

Although most inland water sites tend to be heterotrophic (Soballe, Kimmel 1987; Sand-Jensen and Staehr, 2009; Duarte and Prairie, 2005), I found a broad mix of strongly heterotrophic, near balanced and strongly autotrophic ponds. I found that the eight ponds, which were strongly autotrophic, were all less than 2.7 m in depth while no clear depth pattern was observed for the

strongly heterotrophic or near-balanced ponds.. The shallower ponds may facilitate autotrophy by providing the potential for light to penetrate further down the water column (perhaps even to the bottom of the pond) allowing for a potential of more plankton photosynthesis. Shallower ponds aided in the ponds having higher production values according to the relationship found in the results where all autotrophic ponds had lower depth levels. Surprisingly, Zeu/Zmix was not correlated with GPP or the P:R ratio of the ponds I studied. This is perhaps because Zeu/Zmix ratio of these ponds tended to be relatively high. For the twenty-seven ponds the average Zeu/Zmix was 1.91. Zeu/Zmix may help explain why so many of these small land-locked ponds are autotrophic. A recent study investigating the potential planktonic production of the surface mixed layer in various zones of Texas reservoirs concluded that the very shallow riverine zones were unexpectedly usually net autotrophic (Forbes et al., 2012). Forbe's study found that at shallower riverine zones of man-made reservoirs production rates and chlorophyll-a concentrations were higher raising the P:R at these locations. The results obtained from the Forbe's study showed that the riverine zone was primarily autotrophic. The riverine zones that were studied in this experiment were similar in depth to the ponds in my study, strengthening that these shallow ponds are autotrophic. Other studies have been performed noting that shallower depth lead to higher production rates. (Soballe,Kimmel 1987).

4.2 Production and P:R with relationship to SAV

My second hypothesis that production and P:R in ponds would be related to the amount of macrophyte growth in the ponds was not supported by the results. Submerged aquatic vegetation was not correlated significantly to the P:R ratio production values with a p-value of .8740. Ponds with SAV the P:R average was 1.31 and without SAV the P:R was 1.61. Ponds with SAV tended to be slightly more heterotrophic than ponds without SAV, but this analysis was not directly correlated to the SAV/P:R relationship. For the ponds that lacked any SAV the P:R averaged 1.61, ponds with intermediate SAV averaged 1.14, and ponds with abundant SAV averaged 1.79. There was no clear relationship between the abundance of SAV and the P:R ratio. Daily integral production was significantly related to the P:R ratio and was the primary effector in the production of phytoplankton. Many other factors such as size, turbidity, extinction coefficient, and the Zeu/Zmix had relationships with SAV. Most relationships that were established made sense in the bounds of ecology. The more abundant the SAV the lower the extinction coefficient was and light penetrated the ponds. Having abundant SAV made the water clearer in the ponds allowing light to penetrate throughout, lowering the extinction coefficient. Likewise Zeu/Zmix increased with denser SAV having more dark areas in the pond.

Instead of SAV playing a large role on the P:R ratio, the most intriguing relationship discovered was the effects that both turbidity and extinction coefficient had on the ratio. Both the extinction coefficient and turbidity were higher in ponds found to be autotrophic or heterotrophic ponds than in ponds where the P:R ratio was balanced. Turbidity was directly related to chlorophyll-a. In the heterotrophic and autotrophic ponds that are increasingly turbid, I hypothesize that mud and algal cells are contributing to the water column turbidity. For the heterotrophic turbid ponds mud could cloud the water column not allowing light to penetrate through decreasing plankton photosynthesis and greatly reducing the P:R ratio. This explains both the increase in the extinction coefficient and turbidity. For the autotrophic turbid ponds algal cells could potentially cloud the water column leading to great amounts of photosynthesis and also raising the extinction coefficient that light would not penetrate completely. These are ideas to the relationship that I found in my data, but further research is needed to clarify these effects.

4.3 Factors Effecting Plankton Production and Respiration

The chief primary factor that influenced plankton production was chlorophyll-a. According to appendix 1, there was a significant relationship between chlorophyll-a and daily integral production with a p-value of 0.0001. In fact chlorophyll-a has a direct relationship with daily integral production, as it

was the only parameter that had a significant relationship. The data provided in the study show a direct relationship with the increase abundance of chlorophyll-a leading to an increased daily gross production integral. This is consistent with ecology literature, which for decades has used chlorophyll-a as a measurement of phytoplankton biomass (Tilahun et al., 2010).

In our study chlorophyll was correlated to several factors. Besides turbidity and extinction coefficient, which were discussed above, chlorophyll also had a relationship with total phosphorous and dissolved organic carbon (DOC). This nutrient relationship aids in showing how these nutrients relate to plankton production. Higher chlorophyll values indicated larger nutrient levels and larger integral daily production. My data supports that nutrients are essential for increased values of planktonic production.

4.4 Shallow Ponds and Carbon Cycling

The study performed found that the on average the trophic state of the water column was net autotrophic. The primary focus of the study was on the water column, but there are other factors that effect pond metabolism such as SAV. In regards to carbon cycling, our data noting that the water column was net autotrophic also indicates that it was also an autochthonous producer of carbon. This means that the ponds carbon (DOC) was produced on site. Having the water columns of these ponds autotrophic, production exceeded respiration and algal

cells utilized light and carbon dioxide to produce carbon compounds. This is especially useful data in regards to discerning the far-reaching effects of carbon cycling. Roughly 20% of all enclosed bodies of water in the United States are shallow ponds. Understanding that the water column in our ponds was autotrophic and the carbon production was autochthonous makes these shallow ponds carbon sinks. Although studying the NEP of the entire pond could eventually lead to knowing that the ponds are net heterotrophic and carbon sources, the water column is autotrophic. The understanding so far is that these act as sources, which is the point where carbon is produced and released into the atmosphere. Carbon sinks and sources are integral parts of the carbon cycle and our studied extensively (Gutierrez-Rodriguez et al., 2010). Understanding how shallow pond environments that make up ~20% of standing water affect the carbon cycle is useful in interpreting how carbon is used and recycled through the biosphere as well as its effect on other factors such as climate change.

APPENDIX

	Resp	Pmax	Alpha	IntProd	IntResp
Resp		-0.6234	-0.3725	0.2737	-0.2229
		-27	-27	-27	-27
		0.0005	0.0557	0.1672	0.2638
Pmax	-0.6234		0.2882	0.1934	0.2362
	-27		-27	-27	-27
	0.0005		0.1449	0.3338	0.2355
Alpha	-0.3725	0.2882		-0.2137	0.1022
	-27	-27		-27	-27
	0.0557	0.1449		0.2846	0.6118
IntProd	0.2737	0.1934	-0.2137		-0.1302
	-27	-27	-27		-27
	0.1672	0.3338	0.2846		0.5174
IntResp	-0.2229	0.2362	0.1022	-0.1302	
	-27	-27	-27	-27	
	0.2638	0.2355	0.6118	0.5174	
PR	0.3417	0.1657	-0.21	0.5461	0.2241
	-27	-27	-27	-27	-27
	0.0811	0.4087	0.2932	0.0032	0.2611
Chla	0.3931	-0.0897	-0.2234	0.6795	-0.4213
	-27	-27	-27	-27	-27
	0.0425	0.6564	0.2628	0.0001	0.0286
Depth	0.0183	0.1142	0.0124	0.1393	-0.115
	-27	-27	-27	-27	-27
	0.9277	0.5705	0.9509	0.4884	0.5679
Size	-0.0736	0.2645	0.4372	0.2585	-0.0033
	-27	-27	-27	-27	-27
	0.7151	0.1824	0.0226	0.1929	0.9869
SAV	-0.0632	0.0741	0.11	0.1233	0.1658
	-27	-27	-27	-27	-27
	0.7541	0.7133	0.585	0.54	0.4086
Turb	0.3156	-0.1528	0.1624	0.3561	-0.2197
	-27	-27	-27	-27	-27
	0.1088	0.4468	0.4184	0.0683	0.2708
ExtCoef	0.2282	-0.0774	0.0204	0.2175	-0.2527
	-27	-27	-27	-27	-27
	0.2524	0.7012	0.9194	0.2759	0.2034
ZeuZmix	-0.0303	-0.1251	-0.1392	-0.3222	0.3163
	-27	-27	-27	-27	-27
	0.8808	0.534	0.4886	0.1012	0.108
TP	0.1029	-0.1211	0.0295	0.0997	0.048
	-27	-27	-27	-27	-27
	0.6096	0.5473	0.8839	0.6207	0.8122
DOC	0.1254	-0.1358	-0.186	0.051	-0.0632
	-27	-27	-27	-27	-27

	PR	Chla	Depth	Size	SAV	Turb
Resp	0.3417	0.3931	0.0183	-0.0736	-0.0632	0.3156
	-27	-27	-27	-27	-27	-27
	0.0811	0.0425	0.9277	0.7151	0.7541	0.1088
Pmax	0.1657	-0.0897	0.1142	0.2645	0.0741	-0.1528
	-27	-27	-27	-27	-27	-27
	0.4087	0.6564	0.5705	0.1824	0.7133	0.4468
Alpha	-0.21	-0.2234	0.0124	0.4372	0.11	0.1624
	-27	-27	-27	-27	-27	-27
	0.2932	0.2628	0.9509	0.0226	0.585	0.4184
IntProd	0.5461	0.6795	0.1393	0.2585	0.1233	0.3561
	-27	-27	-27	-27	-27	-27
	0.0032	0.0001	0.4884	0.1929	0.54	0.0683
IntResp	0.2241	-0.4213	-0.115	-0.0033	0.1658	-0.2197
	-27	-27	-27	-27	-27	-27
	0.2611	0.0286	0.5679	0.9869	0.4086	0.2708
PR		0.2217	-0.1842	0.0947	-0.032	0.0233
		-27	-27	-27	-27	-27
		0.2665	0.3578	0.6384	0.874	0.9082
Chla	0.2217		-0.1459	-0.0738	-0.0817	0.6054
	-27		-27	-27	-27	-27
	0.2665		0.4678	0.7144	0.6852	0.0008
Depth	-0.1842	-0.1459		0.2608	-0.0176	-0.0646
	-27	-27		-27	-27	-27
	0.3578	0.4678		0.1889	0.9305	0.7489
Size	0.0947	-0.0738	0.2608		0.3889	0.0408
	-27	-27	-27		-27	-27
	0.6384	0.7144	0.1889		0.0449	0.84
SAV	-0.032	-0.0817	-0.0176	0.3889		-0.4132
	-27	-27	-27	-27		-27
	0.874	0.6852	0.9305	0.0449		0.0322
Turb	0.0233	0.6054	-0.0646	0.0408	-0.4132	
	-27	-27	-27	-27	-27	
	0.9082	0.0008	0.7489	0.84	0.0322	
ExtCoef	-0.0682	0.5891	-0.2865	-0.1871	-0.5182	0.7309
	-27	-27	-27	-27	-27	-27
	0.7353	0.0012	0.1474	0.35	0.0056	0
ZeuZmix	0.1257	-0.3032	-0.5007	-0.1292	0.4229	-0.3863
	-27	-27	-27	-27	-27	-27
	0.5323	0.1242	0.0078	0.5209	0.028	0.0466
TP	0.0072	0.4946	-0.3952	-0.3001	-0.2515	0.2987
	-27	-27	-27	-27	-27	-27
	0.9715	0.0087	0.0413	0.1283	0.2058	0.1301
DOC	0.1228	0.4684	-0.3549	-0.4487	-0.1776	0.1339
	-27	-27	-27	-27	-27	-27
	0.5417	0.0137	0.0693	0.0189	0.3755	0.5054

	ExtCoef	ZeuZmix	TP	DOC
Resp	0.2282	-0.0303	0.1029	0.1254
	-27	-27	-27	-27
	0.2524	0.8808	0.6096	0.533
Pmax	-0.0774	-0.1251	-0.1211	-0.1358
	-27	-27	-27	-27
	0.7012	0.534	0.5473	0.4993
Alpha	0.0204	-0.1392	0.0295	-0.186
	-27	-27	-27	-27
	0.9194	0.4886	0.8839	0.3528
IntProd	0.2175	-0.3222	0.0997	0.051
	-27	-27	-27	-27
	0.2759	0.1012	0.6207	0.8006
IntResp	-0.2527	0.3163	0.048	-0.0632
	-27	-27	-27	-27
	0.2034	0.108	0.8122	0.7543
PR	-0.0682	0.1257	0.0072	0.1228
	-27	-27	-27	-27
	0.7353	0.5323	0.9715	0.5417
Chla	0.5891	-0.3032	0.4946	0.4684
	-27	-27	-27	-27
	0.0012	0.1242	0.0087	0.0137
Depth	-0.2865	-0.5007	-0.3952	-0.3549
	-27	-27	-27	-27
	0.1474	0.0078	0.0413	0.0693
Size	-0.1871	-0.1292	-0.3001	-0.4487
	-27	-27	-27	-27
	0.35	0.5209	0.1283	0.0189
SAV	-0.5182	0.4229	-0.2515	-0.1776
	-27	-27	-27	-27
	0.0056	0.028	0.2058	0.3755
Turb	0.7309	-0.3863	0.2987	0.1339
	-27	-27	-27	-27
	0	0.0466	0.1301	0.5054
ExtCoef		-0.4544	0.591	0.432
		-27	-27	-27
		0.0173	0.0012	0.0244
ZeuZmix	-0.4544		-0.1797	-0.0084
	-27		-27	-27
	0.0173		0.3696	0.9668
TP	0.591	-0.1797		0.7172
	-27	-27		-27
	0.0012	0.3696		0
DOC	0.432	-0.0084	0.7172	
	-27	-27	-27	
	0.0244	0.9668	0	

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