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Factors affecting primary production and respiration in small forested pools Heiberg Forest, Tully, NY

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by

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Abstract

Small permanent to semi-permanent wetland pools were at one point very abundant in northern temperate regions. Due to habitat degradation there has been a drastic decrease in their numbers. Construction of vernal pools is becoming more common to replace lost pools and help conserve these specialized habitats for abundant and endangered amphibian species. There is little study of factors regulating ecosystem processes in pools, even though factors regulating autochthonous production or respiration levels may affect amphibian success. A subset of 26 (72 total) constructed vernal pools in a mixed second growth forest (Heiberg Forest, Tully, NY) were measured for primary productivity (GPP, NPP) and respiration. It was hypothesized that conditions which reduced light in the water column would be negatively correlated with autochtonous production as estimated by both algal biomass and primary production in the water column, and that higher respiration would be correlated with higher DOC concentrations which should promote heterotrophic bacterial growth. Surprisingly although canopy cover was relatively high and uniform (82 $\pm$ 4.2% SD), chlorophyll a was relatively high, similar to levels in mesotrophic lakes, and average GPP for all pools was positive, although variable (1.2 + 5.2 mg C m^{-3} day^{-1}). Average net primary productivity in the pools was negative, although 7 of the 26 pools had positive NPP, suggesting net autotrophy for some of the pools in summer. No simple correlations between NPP or algal biomass and any other measured parameter were significant. Similarly, higher respiration rates in pools were not correlated with DOC concentrations, perhaps because DOC was surprisingly low. Ongoing work in collaboration with other scientists is evaluating additional factors and multivariate models that may help explain differences in autochthonous production and respiration among these constructed vernal pools. Pools with net autochthony and allochthony will also be examined for differences in amphibian breeding success measured by other collaborating scientists.

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Introduction

Vernal pools are temporary to semi-permanent wetlands found in varying habitats across the globe. They provide vital breeding habitats for amphibians that have specifically adapted to breeding in these pools (McGreavy et al., 2012). In addition, they provide ecosystem services to mammals, birds, and reptiles (Mitchell et al., 2007). They also provide connectivity between wetlands and terrestrial systems (Baldwin et al 2006; Gibbs, 2000) and facilitate nutrient cycling (Gibbons et al., 2006; King, 1985).

Vernal pools have become increasingly threatened due to a lack of good management practices and an increase in development. This causes wetlands to be degraded and forests to become fragmented (Baldwin and deMaynadier, 2009; Calhoun et al., 2005; Windmiller and Calhoun, 2008). While examining the effects of urbanization on vernal pools and how the current management practices were mitigating issues associated with development, Baldwin and deMaynadier (2009) found that the management plans failed to protect 46% of potential breeding pools and 80% of the adjacent non-breeding habitat. This one example highlights the fact that the current planning and conservation strategies in place for vernal pools are not yet fully formed and continuing research is necessary to develop better management practices.

Several case studies demonstrate that wetland conservation efforts are effective in helping vernal pool ecosystems to rebound. Amphibian populations dramatically increased after a 54 year period of protection from intensive agricultural activity, land disturbance and drought when protected in a 10 ha wetland in South Carolina (Gibbons et al., 2006). While this study did not include vernal pools specifically, it did include species of amphibians commonly found in vernal pools. This shows the resilience of these amphibian species and the promise that proper management plans can be successful.

In order to help these ecosystems, more research needs to be done in understanding how these systems function. Mostly, vernal pools are defined based upon the fauna that live and breed within them. It is easy to classify them in this manner as the organisms are fairly unique to vernal pools and other similar small woodland pools. However, there has been little if any research done on water parameters in these systems or on the roles of autochtonous (within-system) versus allochtonous (outside-system) production (Colburn 2004). Little if any research has been done on primary productivity, dissolved organic carbon (DOC) content, and light characteristics for these habitat types (Calhoun and DeMaynadier 2008). Most research assumes that leaf litter and allocthonous production are important in woodland pools (Ostrofsky 1997), but it is possible that authochtonous production in the pond may at times be high, and such production is often of higher food quality for consumers (Sterner and Elser 2002), and so may be important for vernal pond communities.

While primary productivity is of the utmost importance in most aquatic habitats, other characteristics play an important role in influencing that environment as well. Amongst these other characteristics are dissolved organic carbon (DOC) and the light extinction coefficient. DOC can serve a number of functions in a system. It can be used as a food source by bacteria, invertebrates, and larval amphibians, and it also attenuates different wavelengths of light (Wetzel 2001). The effect that DOC has on light attenuation is well documented, and different concentrations and chemical species absorb and reflect varying wavelengths of light. This can have a pronounced effect on photosynthesis, heating from solar irradiation and the penetration of UV radiation into a body of water (Wetzel 2001).

The light extinction coefficient is a relative value which takes into consideration the depth and intensity of solar irradiation which enters a body of water. Importantly, DOC affects this coefficient as it attenuates certain wavelengths of light, thus, varying concentrations will affect photosynthesis and

negative effects from UV radiation differently. This has been seen in various studies looking at *Daphnia*, a common invertebrate in many vernal pools (Huovinen et al., 2001; Ribeiro et al., 2011, Huovinen 2003).

The objective of this study was to measure pelagic primary productivity (GPP, NPP) and respiration in a subset (26) of constructed small pools (72 total) in a mixed second growth forest (Heiberg Forest, Tully, NY). We hypothesized that factors reducing light in the water column (including higher canopy cover, light extinction coefficients, DOC, and turbidity) would be negatively correlated with both pond algal biomass and autochthonous primary production in the water column and that higher DOC would be correlated with higher respiration rates in the water column.

Methods

Site Description

The study site was located in Heiberg Forest, Tully, NY (Figure 1). This is a mixed second growth forest with large variation in tree assemblages throughout the entire study site. The pools were constructed in 2010 by clearing areas throughout the forest and then using backhoes; soil was excavated to create the pools. To classify the different pools a grid of hexagons was overlaid on top of the area within which the pools resided (Figure 2).

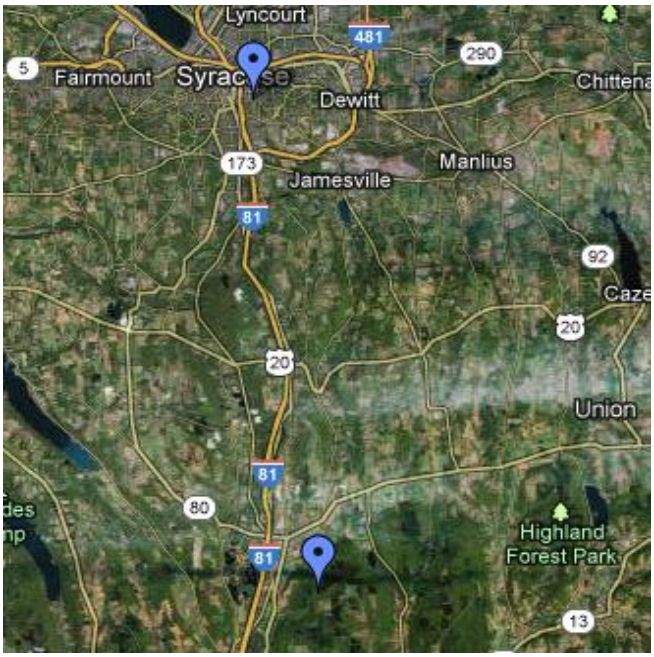

Figure 1. Map of Heiberg Forest and surrounding area. The blue pin located at the bottom of the map is Heiberg Forest.

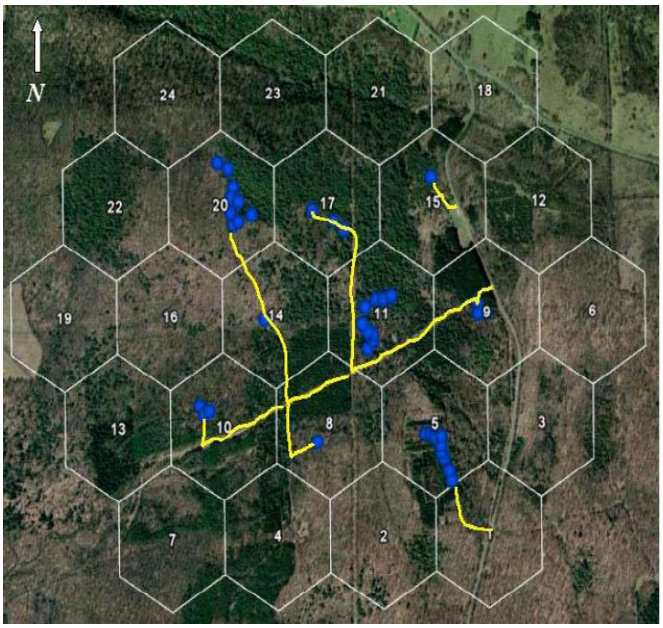

Figure 2. This map shows the hexagon classification system used to divide and classify each subset of pools. Each pool is marked by a blue dots. Pools were laid out in one, three and seven pool clusters as part of a study on amphibian pool use and dispersal led by Professor James Gibbs at SUNY ESF.

Field Sampling and Analysis using the Winkler Method

Winkler titrations were used in this study to determine the dissolved oxygen concentrations in this subset of vernal pools. To obtain samples for this analysis, 300 mL Biological Oxygen Demand (BOD) bottles were used. This method uses 1 initial bottle, 1 dark bottle and 1 light bottle. The initial bottle gives the initial dissolved oxygen concentration of the vernal pool. The dark bottle represents oxygen loss to respiration and the light bottle represents net photosynthesis as net oxygen gain over the initial after these bottles are incubated *in situ* for a known period of time. The initial bottle is filled and then immediately injected with two reagents, manganese sulfate and sodium azide. If dissolved oxygen was present, a brown precipitate of manganous hydroxide formed. Both the light and dark bottles were placed in the pools and allowed to incubate for approximately four hours (the exact times were

recorded) to estimate oxygen production per time in the known volume of water. Two replicates were used in each of the pools, allowing one set of bottles to be placed at the eastern most edge of the pool and another to be placed at the western most edge of the pool.

Due to the shallow water, a plastic pitcher with a hose attached to the bottom was used as a sampling apparatus. The apparatus was placed in the center of each pool to collect water from a constant location. Each BOD bottle was filled by placing the end of the hose into the bottom of each bottle and allowing the bottle to overflow approximately 2-3 times. This was done to prevent any atmospheric oxygen from becoming dissolved into the sample bottle due to agitation. Each bottle was filled as high as possible (to overflowing) so that when the glass stopper was placed in, there would be no air space. After each set of light and dark bottles had incubated for approximately four hours, the manganese sulfate and sodium azide were added to the bottles and brought back to the lab for analysis.

Two mL of sulfuric was added to each bottle immediately before titrations were performed. This served to dissolve the precipitate, turning the liquid inside the bottle a light to dark shade of yellow, depending on the amount of dissolved oxygen present in each sample. When the precipitate had dissolved, 100 mL of sample were added to a beaker for titration using sodium thiosulfate. Enough titrant was added to first turn the sample a pale straw yellow color. At this point 4-5 drops of starch solution were added to the sample, turning it blue. Sodium thiosulfate was added to the sample until it turned clear. The amount of titrant used was recorded and used in the following equation to determine the concentration in mg/L of O_2 in the sample:

$$\frac{8000 * (mL\ thiosulfate\ added) * (thiosulfate\ normality)}{F * (mL\ of\ sample\ titrated)}$$

$$F = \frac{(BOD\ bottle\ volume) - (volume\ of\ reagents\ added)}{(BOD\ bottle\ volume)}$$

The means of the dissolved oxygen concentrations from each pool were taken for the initial (IB), light (LB) and dark bottles (DB). These values could then be put into the following formulas to solve for respiratory activity, net photosynthesis and gross photosynthesis.:

$$Respiratation = IB - DB$$

$$Net\ photosynthesis = LB - IB$$

$$Gross\ photosynthesis = (LB - IB) + (IB - DB) = LB - IB$$

To calculate the total amount of net primary production for the day, the following equation was used:

$$\frac{Total\ light\ for\ the\ day}{Total\ light\ for\ the\ incubation\ period} * Net\ primary\ production\ during\ incubation$$

Chlorophyll a Sampling and Analysis

To obtain samples for chlorophyll analysis, a 1 L HDPE plastic bottle was used. At each of the pools, a plastic pitcher was used to obtain water. Water was then poured from the pitcher into the sample bottles. Samples were immediately placed inside a cooler to await processing at the laboratory.

The analysis of chlorophyll a required the use of a filter funnel, GF/F Whatman filters with a 0.7 µm pore size, high quality (chromatography grade) buffered acetone, and 15 mL polypropylene tubes. Each sample was run through the filter funnel with the GF/F Whatman filters. The amount of sample run through the funnel was determined by the coloration of the filter. Once a slight brown or greenish tint was noticed, no more sample was put through the filter. The volume of sample filtered was recorded, the filter was taken out of the funnel, folded, wrapped in tin foil and then placed in the freezer in a bottle with desiccant until all the samples were ready to be analyzed on the fluorometer (Turner Designs 10-AU). It is important to note that once filtered, the samples must not be exposed to light. This was important in the next part of this procedure.

Once all samples were filtered, the fluorometric analysis was performed. The fluorometer is calibrated for algal chlorophyll a in a buffered acetone using a National Institute of Standards certified sample each time the lamp is replaced, and then the instrument calibration is checked before each use with a dry standard (Turner Designs). To prepare the samples for the fluorometer, the filters were removed from the freezer and placed in a 15 mL polypropylene tube with 10 mL of buffered acetone. These were placed in a tube rack, covered in foil and allowed to incubate at 4 ^{o}C for 24 hours. After the incubation period, the samples were taken out, still in a low light environment and the acetone was poured from the polypropylene tubes into a borosilicate fluorometer tube and then placed inside the fluorometer where the chlorophyll a concentration was measured in µg/L.

Canopy Coverage and Light Extinction Sampling and Calculation

Photos of canopy coverage were collected by using a camera with a fish lens eye piece. The camera was placed in the center of each pool on a tripod and pictures were taken over the course of three days at the same times each day (12:00pm-2:00pm). Analysis of the percent canopy coverage was done using GAP Light Analyzer software.

To measure the light extinction coefficient a spherical photosynthetically active radiation (PAR) probe was used (Licor spherical sensor). This was placed on a pole with ruled measurements running down the side of it and lowered into each pool at 2cm increments where a measurement of the PAR was taken. A Licor deck cell (flat sensor) was floated on the surface of each pool to measure the incoming solar irradiance at the surface. Using these measurements the light extinction coefficient values were then calculated using the following formula:

$$I_z = I_0 e^{-kz}$$

Where:

I = irradiance,

I_0 = irradiance just below surface

I_z = irradiation at depth z

k = extinction coefficient

Turbidity and Dissolved Organic Carbon Concentrations

Turbidity was measured using a YSI probe (6600) with an optical turbidity sensor. To obtain DOC, water samples were collected in glass vials from each pool and then stored in a freezer to prevent the decomposition of DOC compounds. Preparation for analysis involved thawing the samples and then filtering them through a filter funnel with a Millipore polycarbonate filter with a pore size of 0.2 µm into specialized scintillation vials, which prevented any type of carbon contamination. The filtrate in each of the scintillation vials was then processed using high pressure liquid chromatography (HPLC).

Results and Discussion

Although all of the vernal pools were constructed at the same time (spring 2009) in the same forest, and had different canopy cover when constructed (range of 31-97%; Jim Arrigoni personal communication), by the time of this study (summer 2012) they had very similar canopy cover (mean 82.6 $\pm$ 4.2% SD) (Appendix Table 1).

However, the pools varied greatly from one another in regard to in pond factors that affect light climate. The light extinction coefficients were very high and varied widely (Figure 3; mean 10.8 $\pm$ 12.9 m^{-1}); in lakes these values usually range from 0.2-4.0 m^{-1} (Wetzel 2001), suggesting in many of the vernal pools light may limit

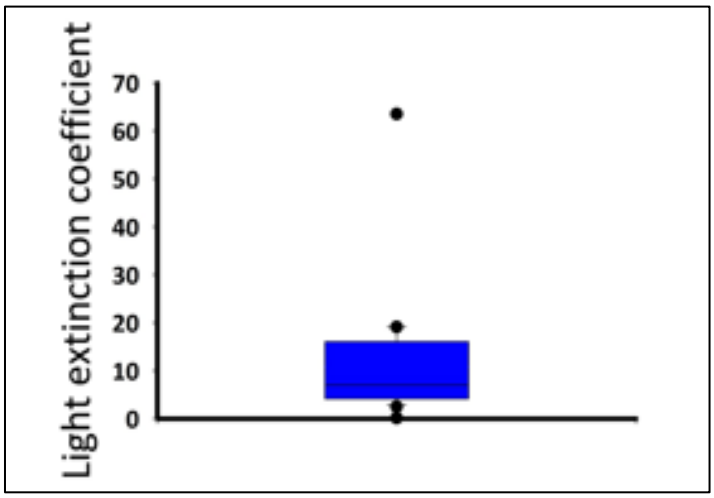

Figure 3. This box plot of light extinction coefficients shows a mean value of 10.8 m-1 with a standard deviation of $\pm$ 12.9 m-1. This value is very high when compared with values found in lakes.

primary production.

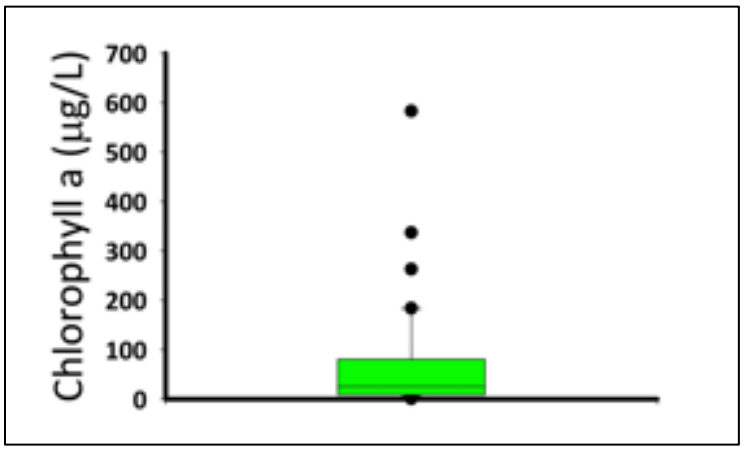

Figure 4. In this box plot chlorophyll a concentrations were found to average 65 µg/L with high variability, + 105 µg/L, averaging levels close to that of mesotrophic lakes.

Turbidity was also very high (334 ± 699 NTU), suggesting particulates play a large role in the low light penetration. Chlorophyll a, indicative of phytoplankton biomass, was relatively high (averaging levels equivalent to those in mesotrophic lakes), but highly variable among pools (65 ± 105 µg chl a/L) (Figure 4). This suggests that phytoplankton have the potential to contribute both to decreased light penetration (self-shading) and to primary production in some pools where they are blooming.

On average gross primary production (GPP) was positive (1.2 ± mg C m^{-3} day^{-1}), but net primary production (NPP) in the vernal pools was negative (-2.4 ± 5.6 mg C m^{-3} day^{-1}), although 7 of the 26 pools had positive NPP (Appendix Table 1). There were no simple correlations between NPP or GPP or any of the other variables examined in this study (Appendix Figures 1-5). Similarly, multivariate analyses revealed no significant correlates of NPP or GPP in the dataset. It is surprising that chlorophyll a was not related to NPP or GPP, perhaps suggesting grazing is high in some pools, reducing the standing stock, but not the productivity of pool phytoplankton.

DOC was averaged 151.5 ± 63.5 (Figure 5) and was less variable than other factors contributing to light extinction; the mean DOC in the pools was surprisingly much less than that reported in a study of

7,500 northern lakes (7.58 mg/L; range 0.1-332 mg/L; Sobek et al 2007). Perhaps due to this lack of variation, DOC was not correlated with pool respiration rates (p=0.802) contradicting our initial hypothesis.

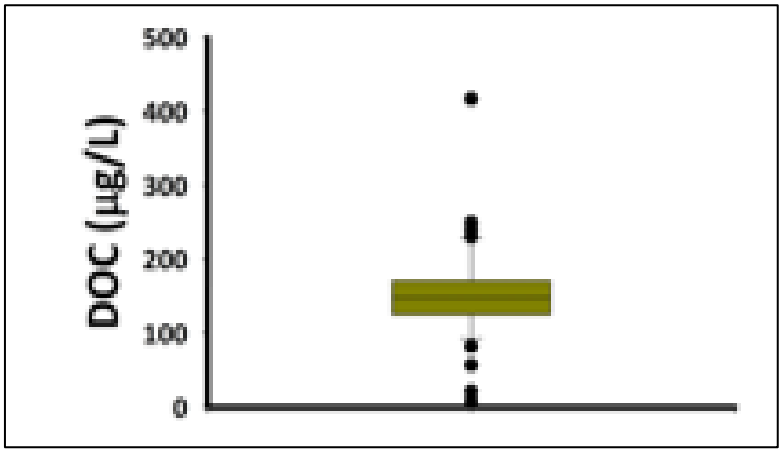

Figure 5. In this box plot the average DOC was 151.5 µg/L + 63.5 which is much less than the average found in most lake systems.

Conclusion

Although we were unable to show any correlations between NPP or GPP and the other individual variables in the dataset, or in a simple multiple regression model, we did find positive net primary productivity occurring in these pools. This means that autochonous production could play a significant role as a food source for the fauna in some of these pools. The literature on this subject is lacking for vernal pools in general. More studies such as this should be conducted to examine the role of primary production in these systems. Further evaluation of our findings in relation to the variable NPP, GPP, algal biomass, light climate, respiration rates, and oxygen concentrations and their relation to the zooplankton assemblages in the ponds (grazing) as well as the importance of these variables to successful amphibian breeding habitat and assemblages are being conducted. Multivariate statistical analyses are being performed on zooplankton abundance and amphibian success with data from this project as well as with data provided by collaborators at SUNY ESF and the University of Illinois in summer 2014.

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Appendix

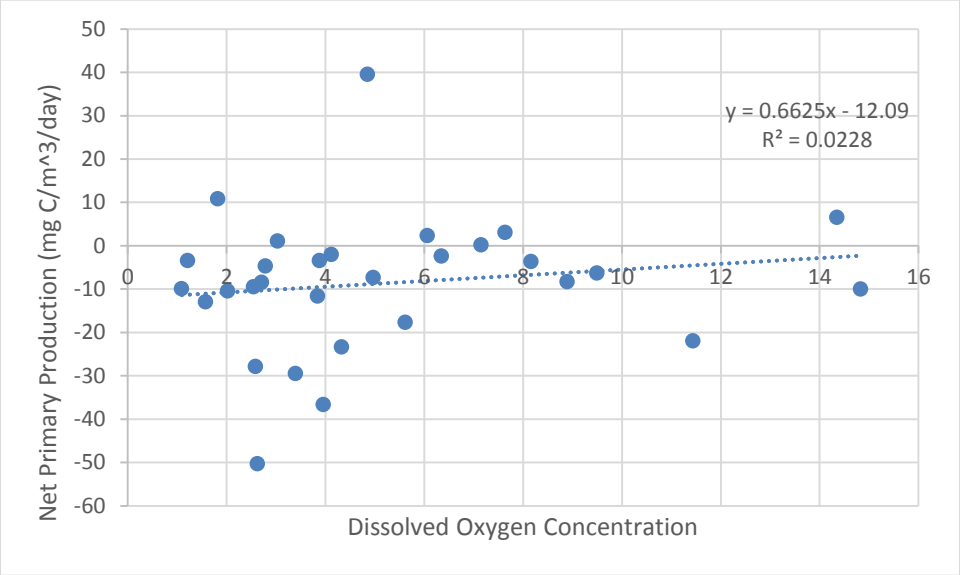

Figure 1. Regression model comparing net primary production with dissolved oxygen concentrations in the 26 vernal pools sampled.

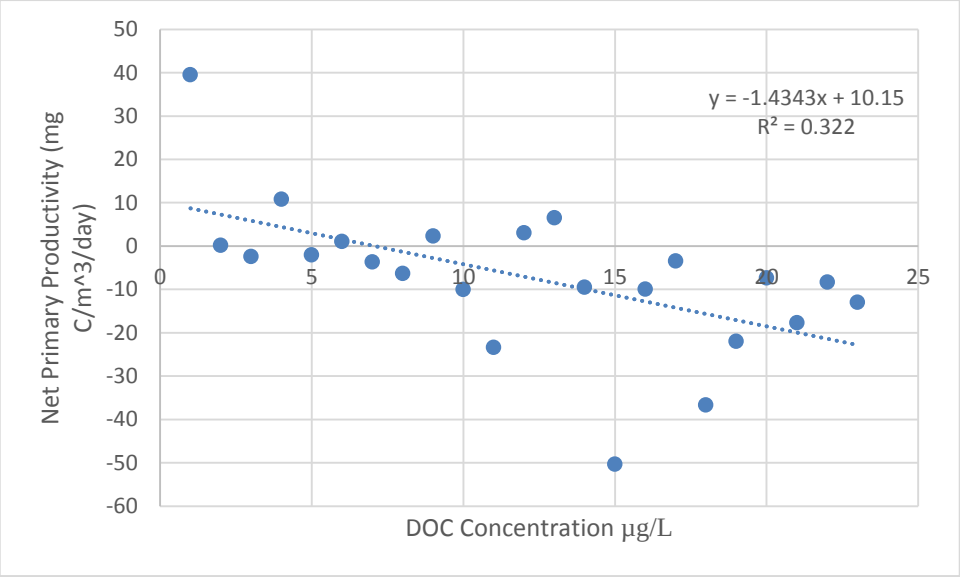

Figure 2. Regression model of net primary production and DOC concentrations in the 26 vernal pools sampled.

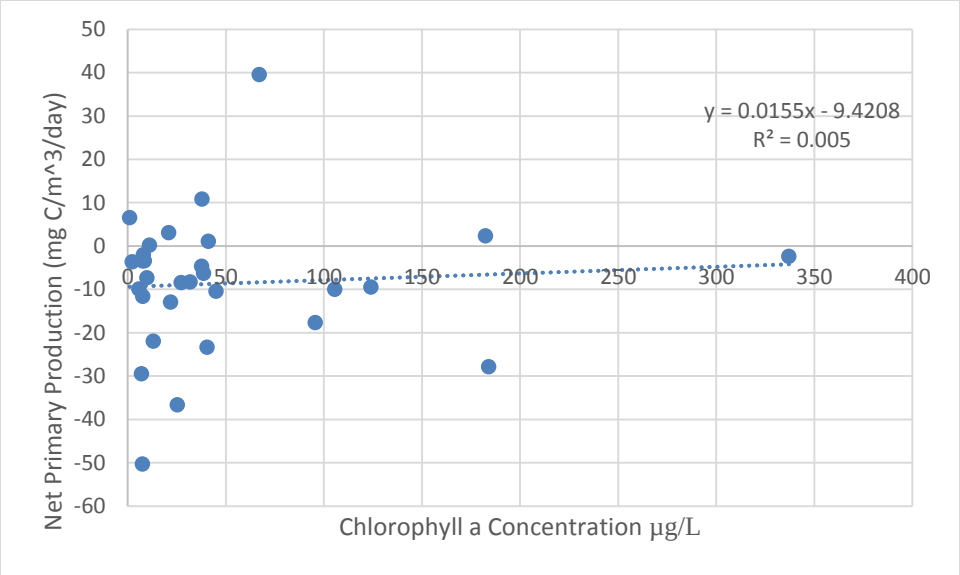

Figure 3. Regression model of net primary production and chlorophyll a concentrations in the 26 vernal pools sampled.

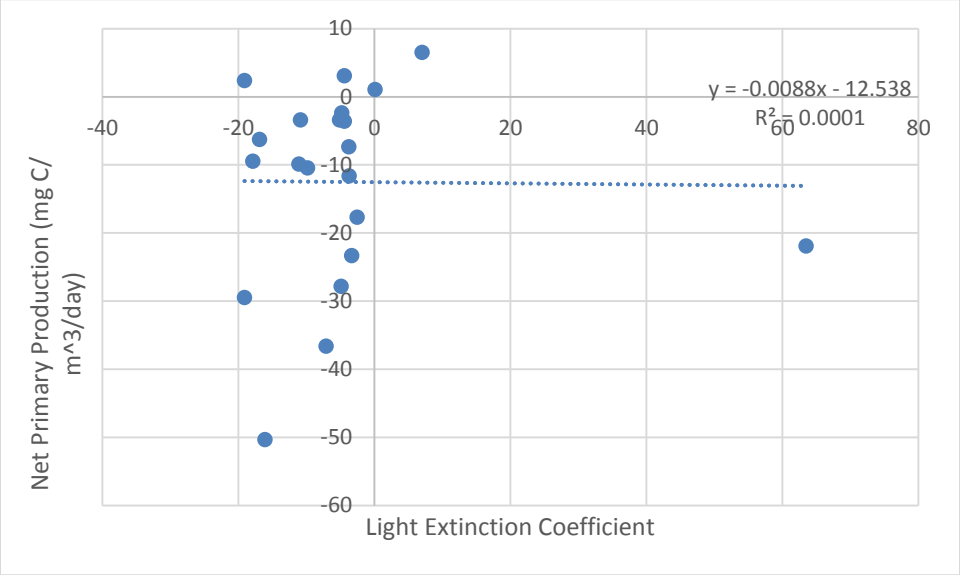

Figure 4. Regression model of net primary production and light extinction coefficients in the 26 vernal pools sampled.

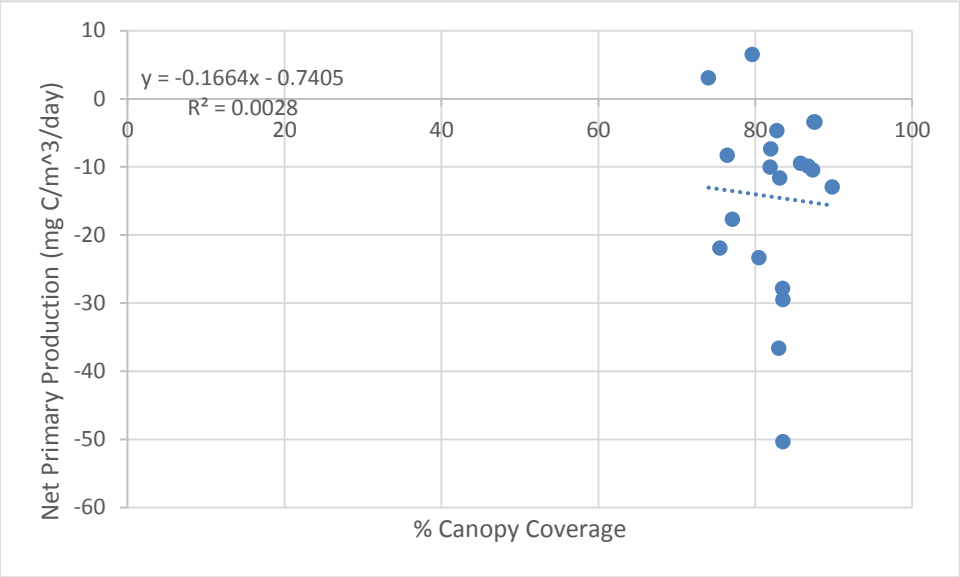

Figure 5. Regression model of net primary production and percent canopy coverage in the 26 vernal pools sampled.

Table 1. Measurements of all parameters examined in this study as well as dates of samples and analyses which are of importance.

Sample Date		August 30 2013		August 30 2013		Date		August 21-22 2013	August 21-22 2013	August 21-22 2013	Date / /
Pool	Dissolved Oxygen Concentration	DOC Concentration Averages µg/L	Sampling date Chlorophyll a concentrations	Chlorophyll a concentrations µg/L	Light Extinction Coefficient	Sampling date Canopy Coverage	Percent Canopy Coverage	Gross Primary Productivity for the day	Respiration for the day	Net Primary Production for the day	Total estimated net Primary production for the entire day
			13-14 Aug	115							
1		57	13-14 Aug	16.6							
2		162	13-14 Aug	88.33333333							
3		8.65	13-14 Aug	2.796666667							
4		140.6666667									
5		21.17									
6		81.92									
7		249.53									
8		416.7									
15					-4.13						
26			13-14 Aug	117							
30			13-14 Aug	40							
32			13-14 Aug	72.7							
5A			13-14 Aug								
5B	4.848484848	108.3	13-14 Aug	25.3				18.26047138	-0.215488215	13.30424242	39.55794747
5C		190.5	13-14 Aug	15.23333333							
5D	2.707070707		13-14 Aug	67				0.600673401	3.447811448	-2.847138047	-8.46549046
5E	7.151515152	125.2	13-14 Aug	27.2				1.561750842	-0.215488215	0.053333333	0.158577778
5F	6.343434343	140.2	13-14 Aug	11.16666667	-4.8			0.480538721	-1.292929293	-0.812390572	-2.415507969
5G	1.818181818	163.3	13-14 Aug	337				7.508417508	3.878787879	3.62962963	10.79209877
5H	4.121212121	138.5	13-14 Aug	37.9				0.180202	0.861952862	-0.681750842	-2.027072503
5I	3.030303033	183.2	13-14 Aug	8	0.132			0.780875421	0.430976431	0.34989899	1.04036633
8A	8.161616162	125.1	13-14 Aug	41.2	-4.396			3.180035651	-4.335115865	-1.155080214	-3.636464297
9A	9.494949495	130.1	15-Aug	2.256666667	-16.888			-0.675757576	0.727272727	-1.887878788	-6.314954545
9B	6.060606061	173.2	15-Aug	38.6	-19.107			0.946060606	-0.242424242	0.703636364	2.353663636
9C	14.82828283	116.7	15-Aug	182.4		5-Sep	81.9	12.02848485	-15.03030303	-3.001818182	-10.04108182
10A	4.323232323	160.3	15-Aug	105.6	-3.333	5-Sep	80.49	-1.283939394	3.030303030	-6.980909091	-23.35114091
10B	7.636363636	163.4	15-Aug	40.4	-4.412	5-Sep	79.6	4.189696970	-3.272727273	0.916969697	3.067263636
10C	14.35	155.4	15-Aug	20.92	7.02	5-Sep	74.04	5.338484848	0.969696970	1.944545455	6.504504545
11A	2.545454545	217.8	15-Aug	0.935	-17.877	5-Sep	85.76	-1.621818182	1.212121212	-2.833939394	-9.479527273
11B	2.626262626	225.6	15-Aug	124	-16.059	5-Sep	83.52	-6.081818182	8.969696970	-15.05151515	-50.34731818
11C	1.090909091	239.9	15-Aug	7.55	-11.069	5-Sep	86.76	0.675757576	-3.636363636	-2.960606061	-9.903227273
11D	3.878787879	175.27	15-Aug	5.8	-10.831	5-Sep	87.62	2.027272727	-4.848484848	-2.821212121	-3.415954545
11E	3.959595960	176.8	15-Aug	8.566666667	-7.08	5-Sep	83.01	1.892121212	-12.84848485	-10.95636364	-36.64903636
11F		109.6	15-Aug	8.533333333							
11G	11.43434343	103.4	15-Aug	13	63.505	6-Sep	75.49	3.378787879	9.939393939	-6.560606061	-21.94522727
11H	4.969696970	112	15-Aug	9.733333333	-3.762	6-Sep	81.98	2.162424242	-4.363636364	-2.201212121	-7.363054545
11I	5.616161616	150.1	15-Aug	95.6	-2.505	6-Sep	77.1	2.703030303	-8	-5.296969697	-17.71836364
14A			15-Aug	11.33333333							
15A	8.888888889	128.2	13-14 Aug	31.9		6-Sep	76.42	-11.75818182	-0.484848485	-12.72787879	-8.305454545
17A	1.575757576	145.7	15-Aug	21.84		6-Sep	89.83	-1.216363636	2.666666667	-3.883030303	-12.98873636
17B	1.212121212	139.73	15-Aug	7.5	-5.12	6-Sep	87.48	-0.810909091	0.484848485	-1.025454545	-3.430145455
17C	3.393939394	131.9	15-Aug	7.09	-19.095	6-Sep	83.51	-3.243636364	-5.575757576	-8.819393939	-29.50087273
20A		230.97	15-Aug	5.71							
20B		161.37	15-Aug	583	-10.509	6-Sep	85.28	No titrant	0.484848485	-2.424242424	-8.109090909
20C		155.93	15-Aug	263		7-Sep	81.7		-0.969696970	-0.969696970	-3.243636364
20D	2.585858586	124.97	15-Aug	184	-4.877	7-Sep	83.48	-2.027272727	5.333333333	-8.330303030	-27.86486364
20E	3.838383838	124	15-Aug	7.633333333	-3.706	7-Sep	83.12	-0.810909091	2.666666667	-3.477575758	-11.63249091
20F		150	15-Aug	20.5							
20G		144.53	15-Aug	16.3							
20H	2.787878788	157.47	15-Aug	37.65		7-Sep	82.74	-0.675757576	0.242424242	-1.403030303	-4.693136364
20I	2.020202020	152.23	15-Aug	45.1	-9.823	7-Sep	87.31	0.308917749	-3.047619048	-2.738701299	-10.46966382