Sapphire Energy®

Exp_151123_195 Water Recycle

Bi-weekly R&D meeting 4/7/16

Overview

- Background
- Proposal
- Experiment data
- Conclusions

Literature Background

8.6.1 Growth inhibitory substances and conditions

The decisive effect of growth inhibition which unfolds in UHCD is demonstrated in a recent study of Zhang *et al.* (unpublished), who compared the areal productivity of *Nannochloropsis* sp. grown in 1, 3 and 9 cm OP glass plate reactors, in which the entire growth medium was either replaced every 24 h or not replaced. In the later case, growth medium nutrients were added to the culture every 72 h (Table 8.3). If the medium was not replaced and only replenished with nutrients, highest productivity was obtained in the 9 cm OP reactor, lowest productivity being obtained in the 1 cm reactor. If the growth medium was, however, replaced every 24 h, highest areal productivity was obtained in the 1 cm OP reactor, being higher by close to 45% compared with the 9 cm reactor under identical conditions, or higher by over 100% above the productivity obtained in the 9 cm OP, in which growth inhibition was not removed or corrected (Table 8.3). Frequent replacement of the entire growth medium was also mandatory in UHDC of *Chlorococcum litoralle*, cultured in 1 cm OP plate reactors (Fig 8.5), which reached record high cell concentrations of over 80 dry cell mass l^{-1} (Hu *et al.*, 1998c). Similarly, Javanmaradian & Palsson (1991) used an online ultrafiltration unit to exchange spent with fresh medium, which was mandatory to achieve very high cell densities, i.e. up to 10^9 of *Chlorella vulgaris* cells ml^{-1}. What is the nature of the growth inhibitory substances or growth inhibitory conditions, without the removal or correction of which the very large surge in the areal output of cell mass taking place in narrow (e.g. 1 cm) OP reactors could not be expressed?

The presence of algae-inhibitors in culture filtrates of several algal species has been rather extensively reported (Pratt, 1942; Leving, 1945; VonDennffer, 1948; Rice, 1954; Steeman Nielsen, 1955; Jörgensen, 1956; Proctor, 1957a; Lefevre, 1964; Harris, 1971, 1975; Fogg, 1971; Keeting, 1978). Other studies provided evidence for the existence of algal antibiosis in situ (Vance, 1965; Proctor, 1957a; Keeting, 1977). Excreted algal metabolites were reported to inhibit their own species' growth, as well as other species': Pratt & Fong (1940) observed growth of *Chlorella vulgaris* depressed by its own product excreted into the culture medium, naming the active substance *chlorellin*. Likewise, Curl & McLeod (1961) reported that dense cultures of

Table 8.3. Effect of removing growth-inhibition in *Nannochloropsis* sp. culture on output rate of cell mass* (from Richmond *et al.* (2003)).

Optical path (OP)cm	Output Rate of Cell-mass ($mg\,m^{-2}\,h^{-1}$) Growth inhibition (GI) Present	Removed	Difference (%)
1.0	504	2184	+333
3.0	798	1764	+121
9.0	924	1512	+64

* Cultures exposed to a total of 2000 μmole photons $m^{-2}\,s^{-1}$ from both sides of the reactors. Temperature and pH are optimal. Reprinted with permission from Blackwell Scientific Publications.

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Inhibitory effects of soluble algae products (SAP) released by *Scenedesmus* sp. LX1 on its growth and lipid production

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HIGHLIGHTS

- Soluble algae products could significantly inhibit the growth of *Scenedesmus* sp. LX1.
- All of the fractions of SAP could inhibit the growth of *Scenedesmus* sp. LX1.
- Organic bases and HIA expressed the strongest inhibition on growth.
- HIA could significantly inhibit the lipid accumulation of *Scenedesmus* sp. LX1.
- Molecular weight and fluorescence spectroscopy of HIA were investigated.

ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 25 July 2013
Accepted 29 July 2013
Available online 6 August 2013

Keywords:
Soluble algal products (SAP)
Scenedesmus sp.
Organic fractions
Microalgal biodiesel
Lipid production

ABSTRACT

Soluble algal products (SAP) accumulated in culture medium via water reuse may affect the growth of microalga during the cultivation. *Scenedesmus* sp. LX1, a freshwater microalga, was used in this study to investigate the effect of SAP on growth and lipid production of microalga. Under the SAP concentrations of 6.4–25.8 mg L^{-1}, maximum algal density (K) and maximum growth rate (R_{max}) of *Scenedesmus* sp. LX1 were decreased by 50–80% and 35–70% compared with the control group, respectively. The effect of SAP on lipid accumulation of *Scenedesmus* sp. LX1 was non-significant. According to hydrophilic–hydrophobic and acid–base properties, SAP was fractionized into six fractions. All of the fractions could inhibit the growth of *Scenedesmus* sp. LX1. Organic bases (HIB, HOB) and hydrophilic acids (HIA) showed the strongest inhibition. HIA could also decrease the lipid content of *Scenedesmus* sp. LX1 by 59.2%. As the inhibitory effect, SAP should be seriously treated before water reuse.

Effect of Centrifugation on Water Recycling and Algal Growth to Enable Algae Biodiesel Production

Thomas Igou, Steven W. Van Ginkel, Patricia Penalver-Argueso, Hao Fu, Shusuke Doi, Asmita Narode, Sarasija Cheruvu, Qian Zhang, Fariha Hassan, Frazier Woodruff, Yongsheng Chen*

ABSTRACT: The latest research shows that algal biofuels, at the production levels mandated in the Energy Independence and Security Act of 2007, will place significant demands on water and compete with agriculture meant for food production. Thus, there is a great need to recycle water while producing algal biofuels. This study shows that when using a synthetic medium, soluble algal products, bacteria, and other inhibitors can be removed by centrifugation and enable water recycling. Average water recovery reached 84% and water could be recycled at least 10 times without reducing algal growth. *Water Environ. Res.*, **86**, 2325 (2014).

KEYWORDS: water recycling, algal biofuels, centrifugation, soluble algal products, algal growth rate.

doi:10.2175/106143014X14062131178871

footprint of algae biofuel production can be significantly reduced by receiving co-product substitution credits when the use of algal meal replaces traditional animal feeds and the water these feeds demand (Subhadra and Edwards, 2011). According to Yang et al. (2011) and Zhang et al. (2010, 2013), water recycling can conserve 84% of the water demand, retain nutrients, and if accomplished using membrane filtration can remove bacteria, predators, and soluble algal products (SAPs) which may inhibit algal growth (Figure 1). However, low molecular weight SAPs cause membrane fouling and may reduce algal growth if they accumulate (Zhang et al. 2010). Thus, new membrane filtration technologies or membrane filtration combined with more powerful methods such as centrifugation may be needed at the commercial scale (Figure 1).

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Sapphire Preliminary Work

- Recycled Media Flask Experiments May 2015
- Recycled Media Flas Experiments June 2015
- LC Reactors with PBR culture Slides 8 and 9
- Exp 181 Recycled Water MPs

- OD750 and DW correlation changes due to flocking

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Proposal – November 2015

Title:	Impact of fresh media on seasoned culture
Project:	Water Recycle

Express FAME on harvests didn't happen but we did collect express FAME data on pond samples monthly

Treatment	Pond Type	Number of Ponds	Culture Source	pH	PW	Media	Crop Protection	Density Range	Analytical	Notes
Controls	RW100	2	Existing	7.8-8.2	Winter	16NFL101:150/40	Standard	.2 - .5 g/L	Standard plus express FAME for first four harvests	
New pond, 1/2 fresh media	RW100	1	Existing	7.8-8.2	Winter	16NFL101:150/40	Standard	.2 - .5 g/L	Standard plus express FAME for first four harvests	
Existing pond, 1/2 fresh media	RW100	1	Existing	7.8-8.2	Winter	16NFL101:150/40	Standard	.2 - .5 g/L	Standard plus express FAME for first four harvests	

Rationale:

Literature and laboratory data have suggested that SAPs may accumulate in cultures with continually reused water. It is also possible that we are depleting an unknown nutrient. Water recycle experiments to date have not shown a clear impact on productivity and/or EPA.

Contingency:

Given season, we may not see a significant impact. Contingency is to repeat this experiment in the Spring.

Special Instructions:

Ponds have performed differently over the last few weeks. Please mix all ponds before setting up new treatments. After mixing, one pond will be split in to two.

Amendments:

Overview

- Background
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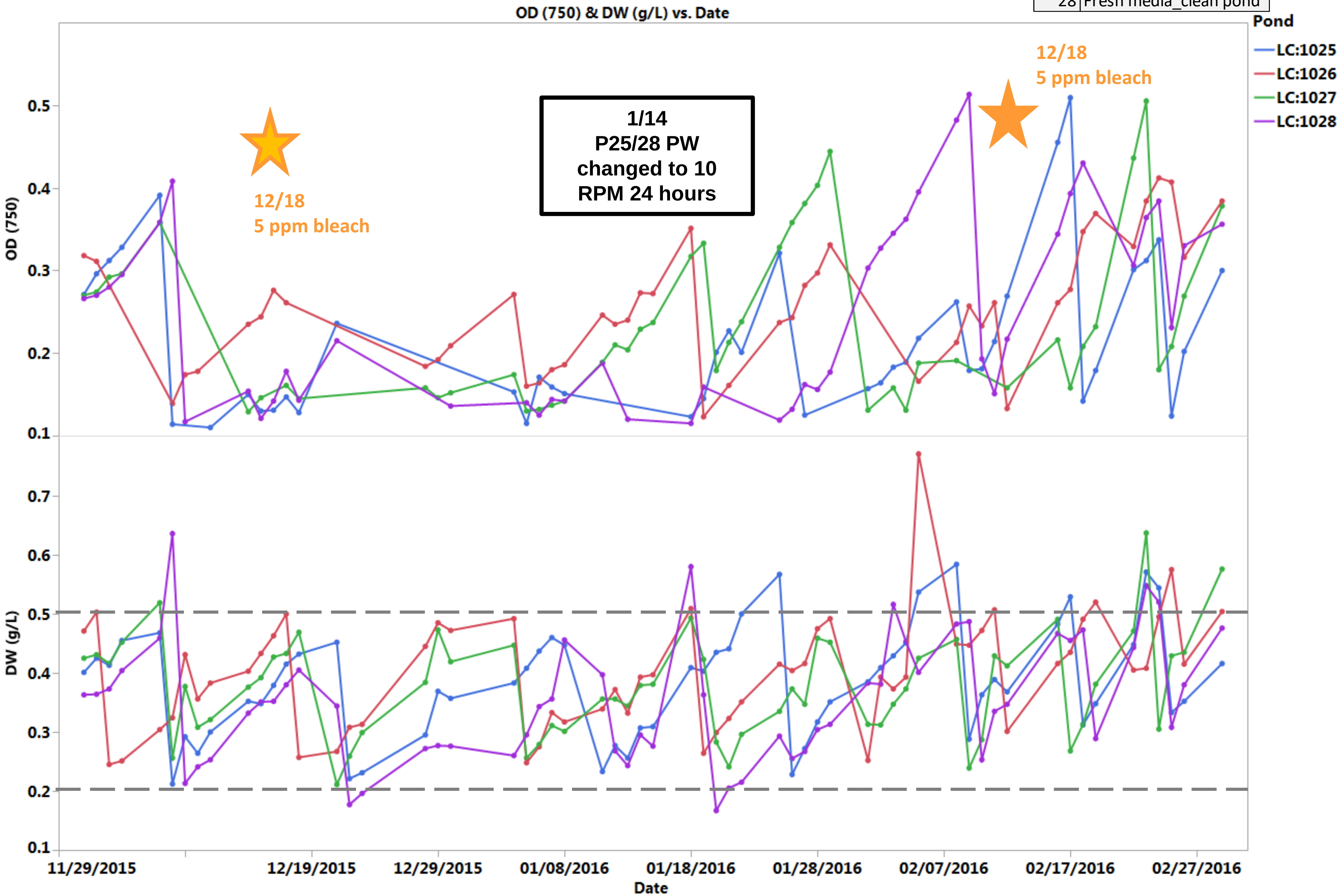
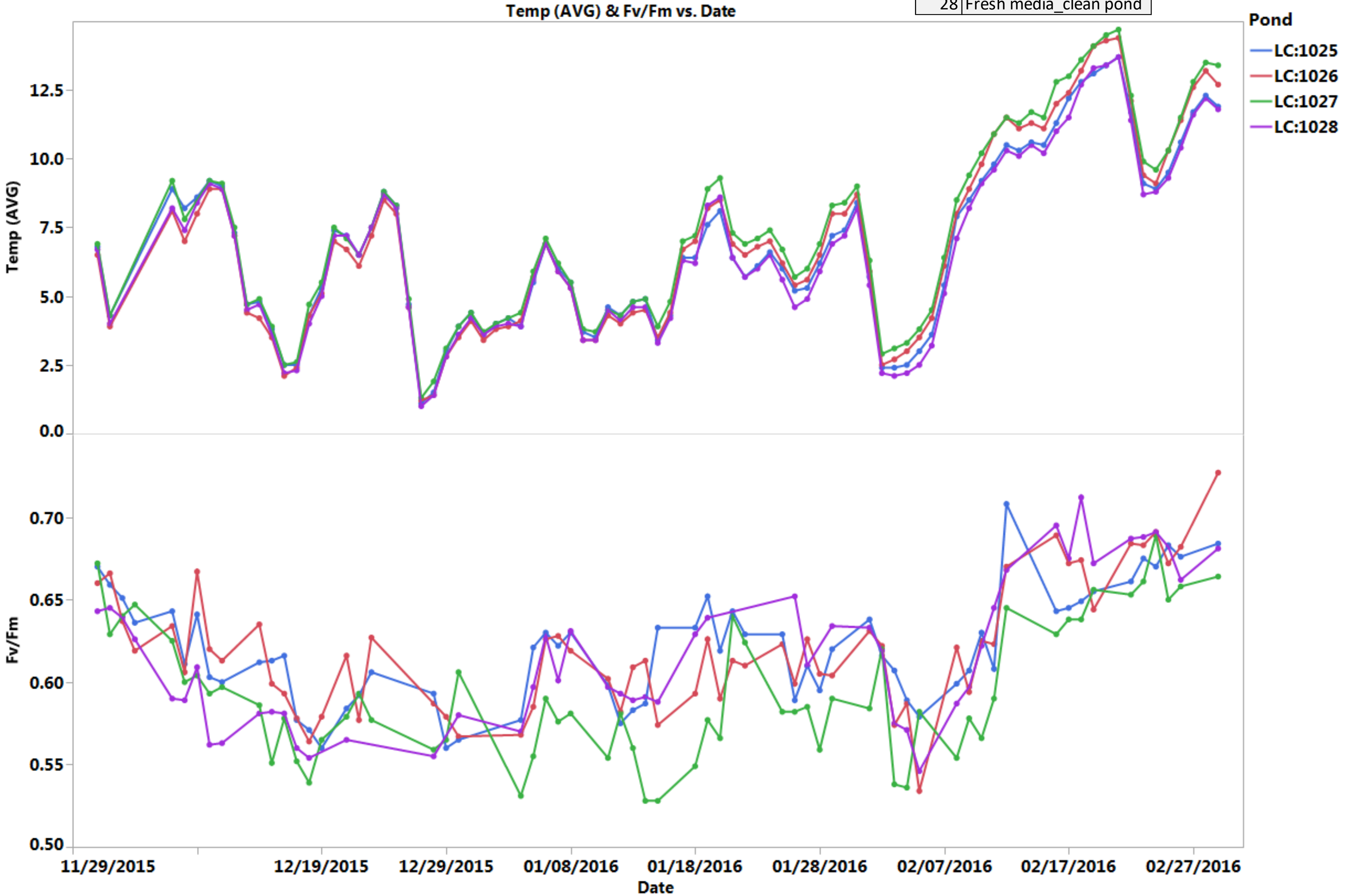
Pond Density and bleach application

Pond	Treatment
25	Control
26	Control
27	Fresh media
28	Fresh media_clean pond



Temp (°C) and PAM

Pond	Treatment
25	Control
26	Control
27	Fresh media
28	Fresh media_clean pond



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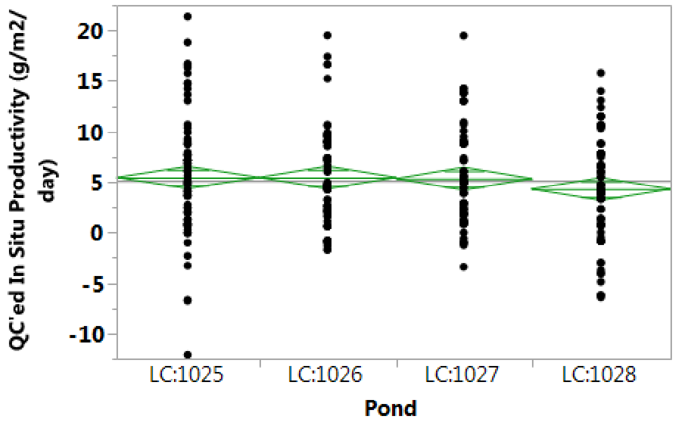
In Situ Productivity

Pond	Treatment
25	Control
26	Control
27	Fresh media
28	Fresh media_clean pond

Oneway Analysis of QC'ed In Situ Productivity (g/m2/day) By Pond



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Oneway Anova

Summary of Fit

Rsquare	0.009099
Adj Rsquare	-0.00034
Root Mean Square Error	5.022317
Mean of Response	5.13657
Observations (or Sum Wgts)	319

Analysis of Variance

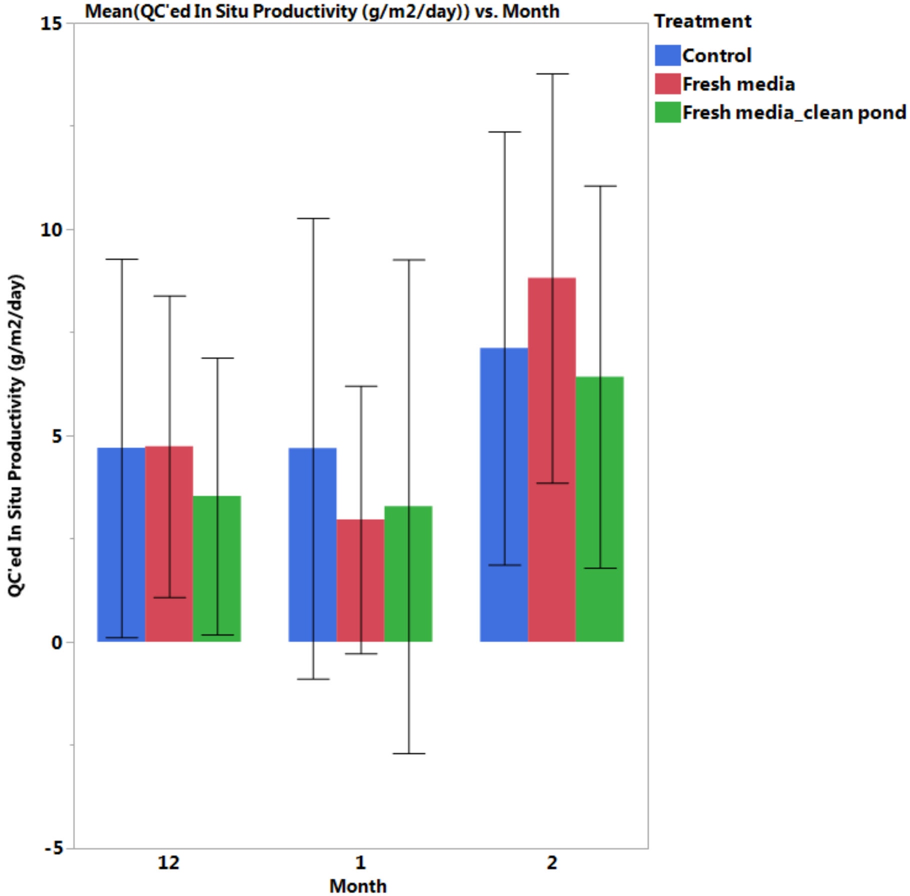
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pond	3	72.9623	24.3208	0.9642	0.4099
Error	315	7945.4567	25.2237		
C. Total	318	8018.4190			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
LC:1025	82	5.45418	0.55462	4.3629	6.5454
LC:1026	79	5.44838	0.56505	4.3366	6.5601
LC:1027	78	5.33018	0.56867	4.2113	6.4490
LC:1028	80	4.31433	0.56151	3.2095	5.4191

Std Error uses a pooled estimate of error variance

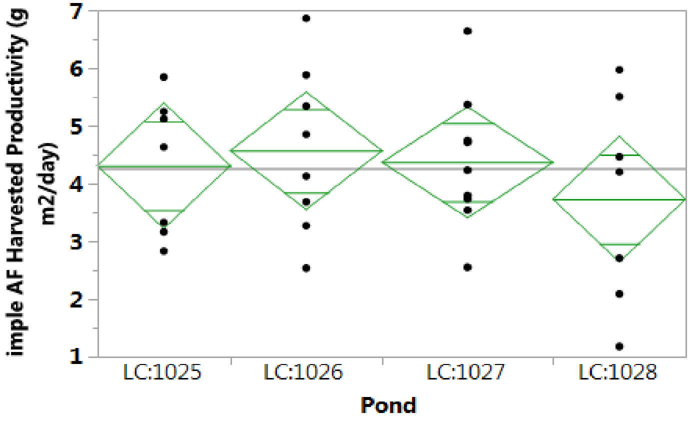
Graph Builder



Each error bar is constructed using 1 standard deviation from the mean.

Harvested Productivity and EPA

Oneway Analysis of Simple AF Harvested Productivity (g/m2/day) By Pond



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Oneway Anova

Summary of Fit

Rsquare	0.050746
Adj Rsquare	-0.05473
Root Mean Square Error	1.404646
Mean of Response	4.263044
Observations (or Sum Wgts)	31

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Pond	3	2.847838	0.94928	0.4811	0.6981
Error	27	53.271826	1.97303		
C. Total	30	56.119664			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
LC:1025	7	4.30947	0.53091	3.2201	5.3988
LC:1026	8	4.56919	0.49662	3.5502	5.5882
LC:1027	9	4.36872	0.46822	3.4080	5.3294
LC:1028	7	3.73087	0.53091	2.6415	4.8202

Std Error uses a pooled estimate of error variance

Date	Pond	% EPA on Pond (Ash corrected)	% FAME on Pond (Ash corrected)
12/14/2015	LC:1025	2.9	9.0
12/14/2015	LC:1026	3.2	10.0
12/14/2015	LC:1027	2.9	9.4
12/14/2015	LC:1028	3.2	10.0
1/7/2016	LC:1025	2.8	9.2
1/7/2016	LC:1026	1.8	4.9
1/7/2016	LC:1027	3.2	9.4
1/7/2016	LC:1028	2.4	10.5
2/8/2016	LC:1025	3.8	10.4
2/8/2016	LC:1026	3.8	12.8
2/8/2016	LC:1027	4.5	13.8
2/8/2016	LC:1028	4.9	13.4

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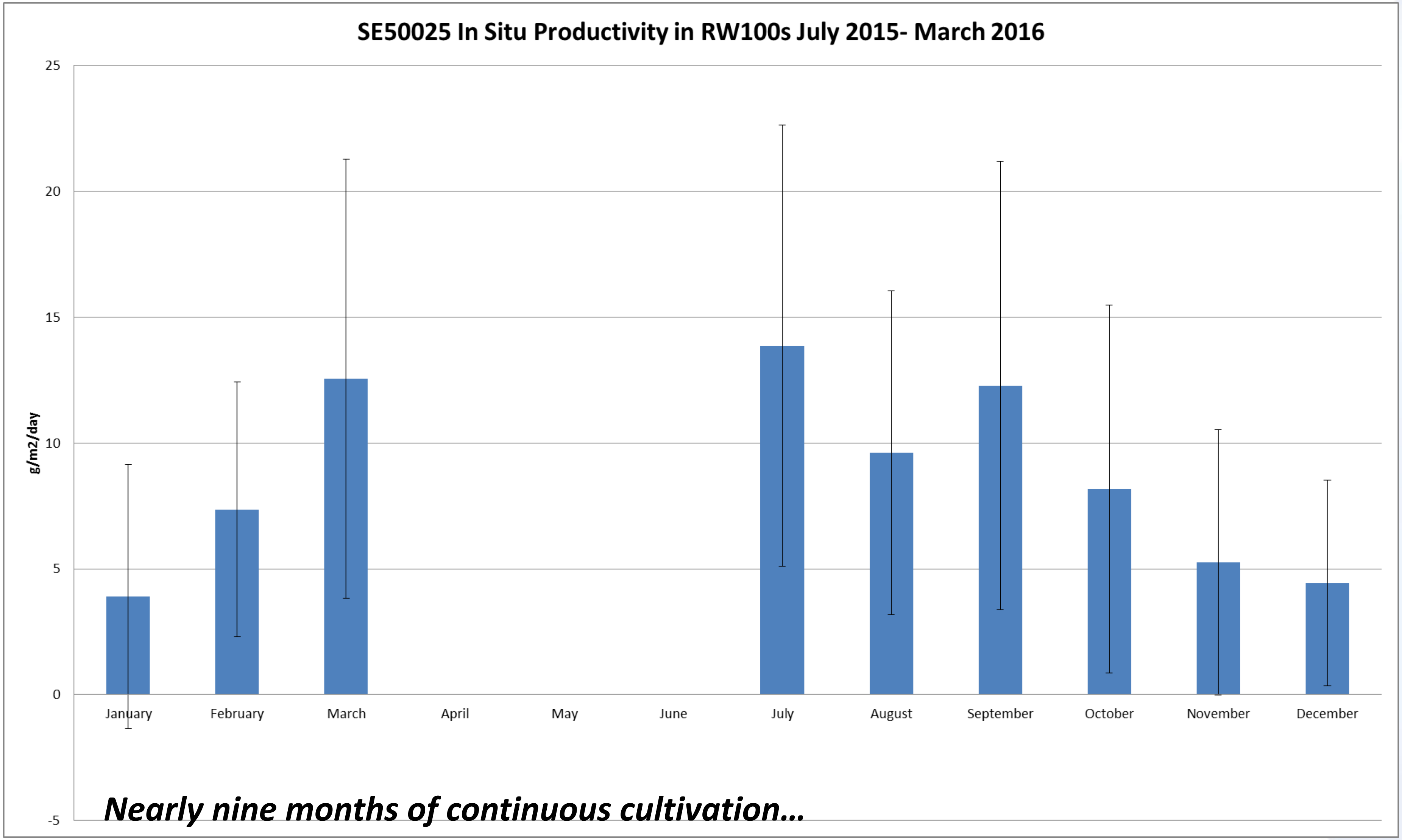
SE50025 Field Productivity - P25 - 28



Nearly nine months of continuous cultivation…

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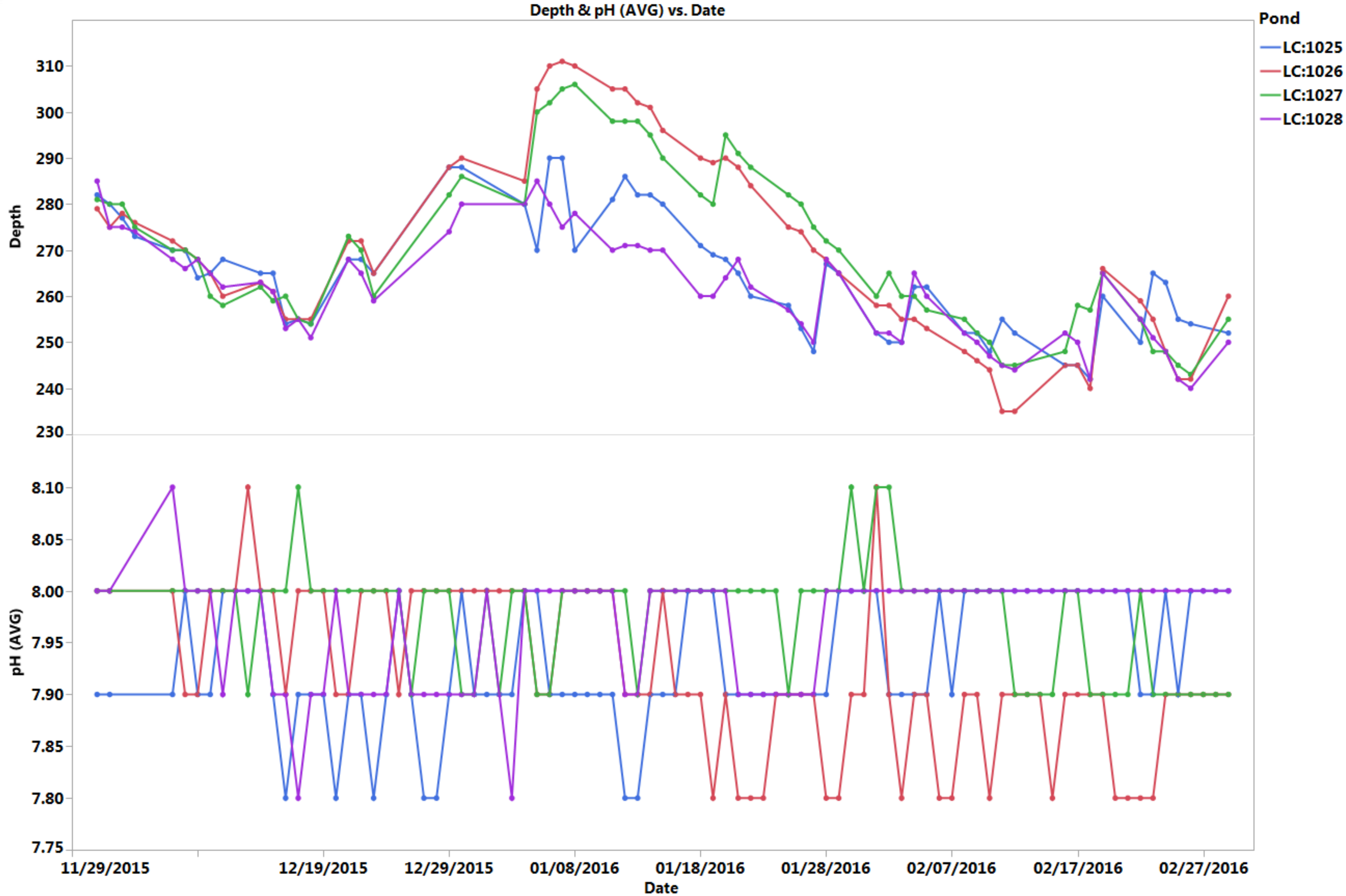
Conclusions and Next Steps

- Neither fresh media nor a clean pond increased productivity from December to February in RW100 for SE50025
- %EPA by Express FAME ran from ~3 to 4.5% over trial for most samples
- P25 has run with continuously returned water for nearly 9 months. We had planned to run this experiment again in the spring, but no longer a priority.
- Harvest process will greatly impact ability to reuse water. We will continue to work towards improved harvest efficiency; reduced flocculation in the ponds post-harvest.
- Working with sea water means new challenges with salinity management. How does this impact the recycled water question?

Supplemental Slides

Depth and pH

Pond	Treatment
25	Control
26	Control
27	Fresh media
28	Fresh media_clean pond



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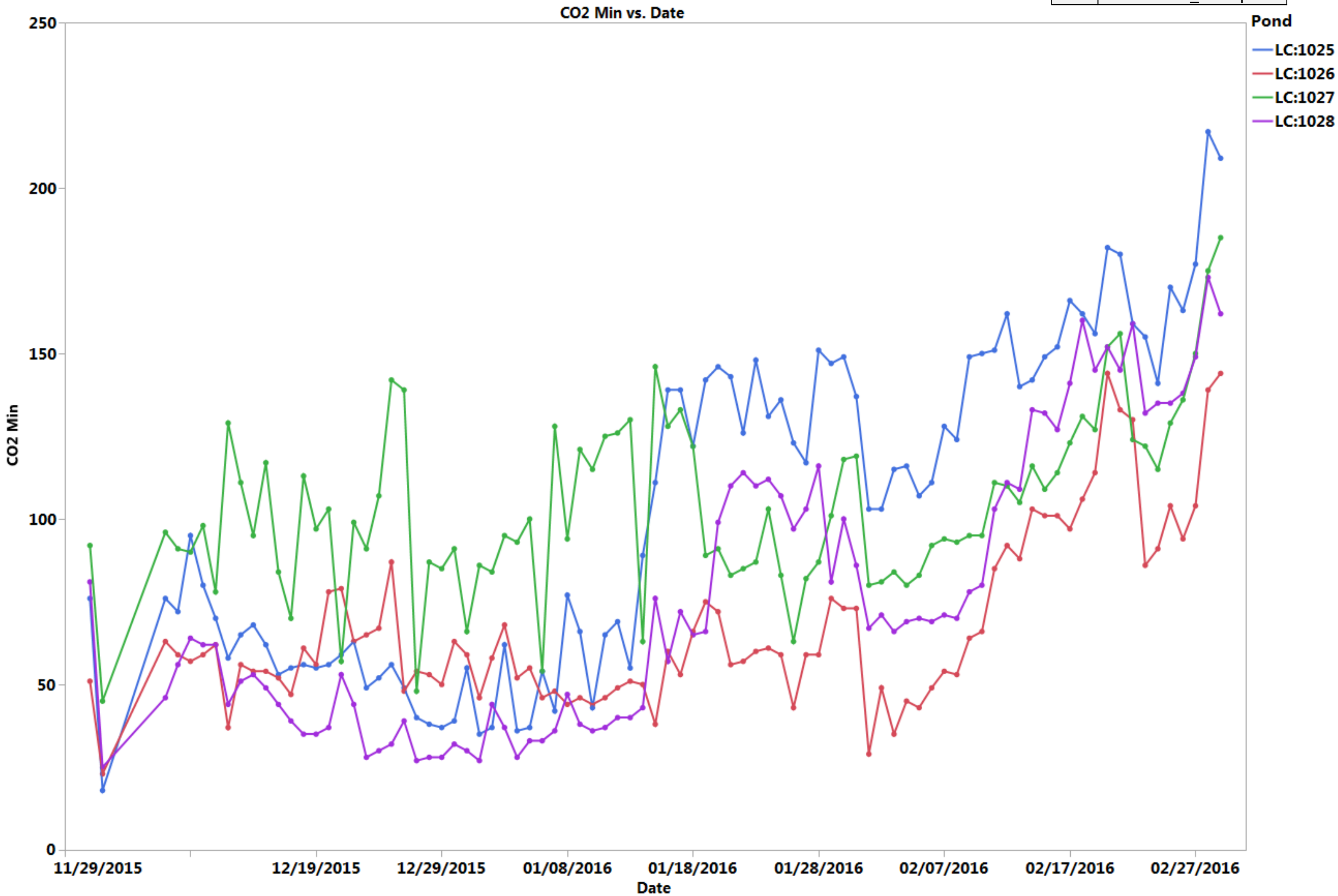
CO2 Minutes

Pond	Treatment
25	Control
26	Control
27	Fresh media
28	Fresh media_clean pond



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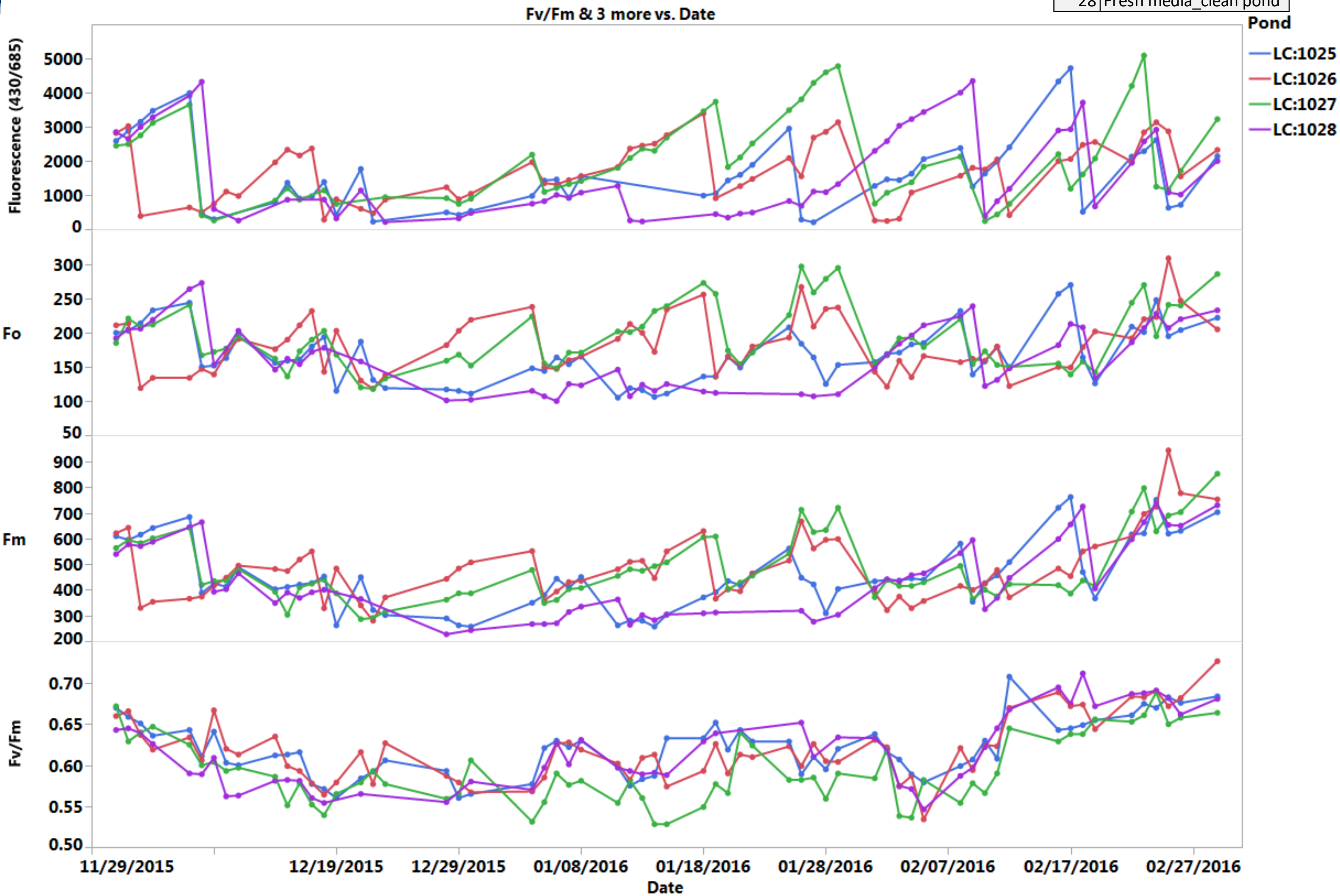
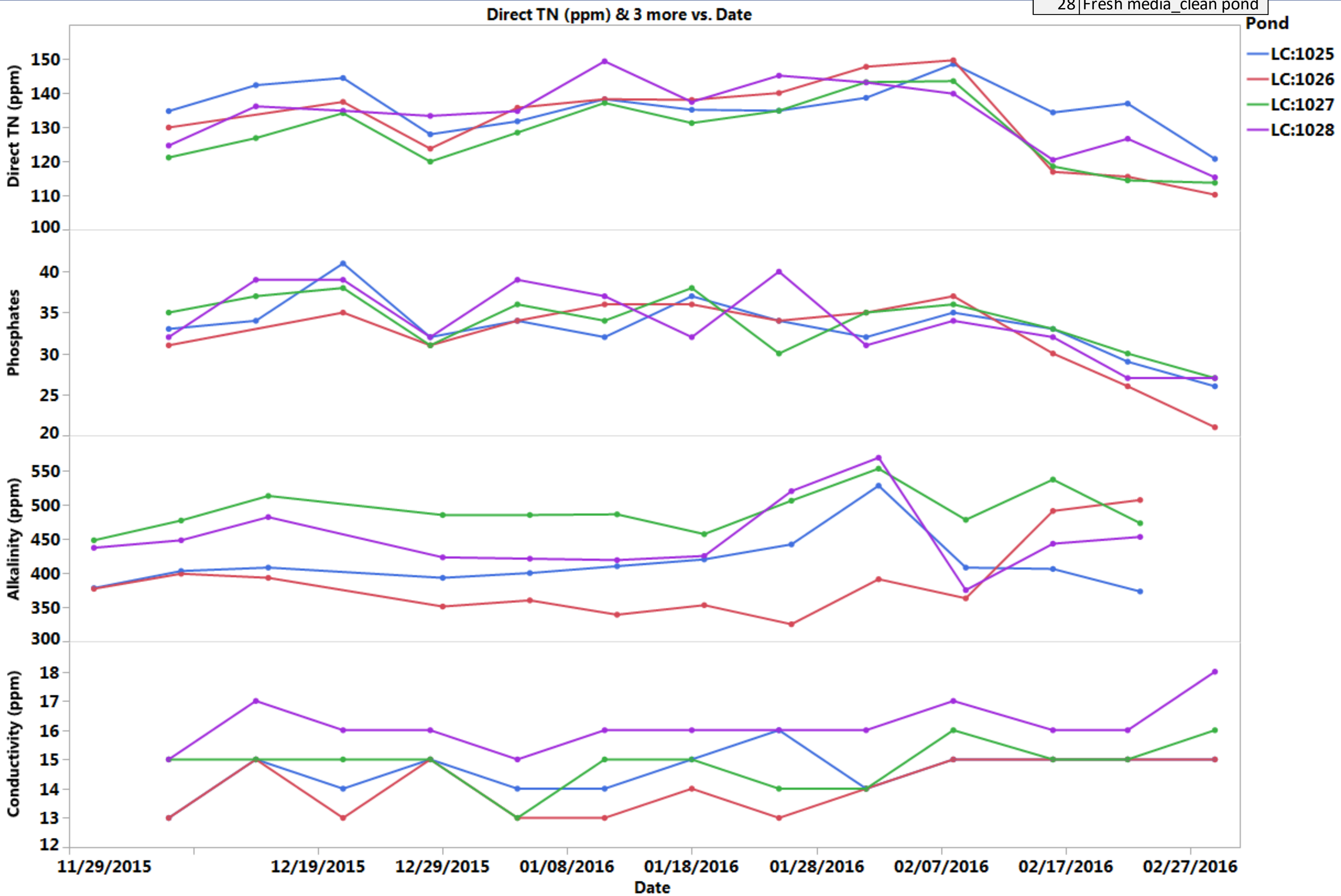
Photometrics

Pond	Treatment
25	Control
26	Control
27	Fresh media
28	Fresh media_clean pond



Nutrients and Water chemistry

Pond	Treatment
25	Control
26	Control
27	Fresh media
28	Fresh media_clean pond



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