High-rate algal ponds (HRAPs) are simple, low-energy, and low-cost wastewater treatment systems that consistently produce high quality effluent and harvestable nutrients, which can be useful for fertilizer, feed, or biofuel [1-4]. Significant research has been conducted to determine conditions (residence time, depth) that produce high-quality effluent and generate the most harvestable biomass. Independent of the number of actual samples collected, performance is commonly evaluated as productivity of algal biomass, expressed on an annual average (g/m²-day) [5]. In HRAPs, the microalgae growth rate can change rapidly in response to insulation and temperature. For every 10°C increase in temperature, the microalgae growth rate is expected to double until the optimal temperature is exceeded [6]. Therefore, diet, season, and weather changes have a significant impact on the reported productivity value depending on the time and frequency of sampling. Since March, 2014, productivity data has been gathered on three sets of triplicate pilot raceway ponds in Delhi, California using a once per week sampling frequency. The sampling time and frequency were chosen with the prospect that values would be representative of the diurnal marginal value, therefore encapsulating die, weather, and seasonal changes [6]. In order to evaluate how close reported productivity values are to the actual productivity value, we investigate Consecutive Daily and Diel sampling frequencies.

Figures 1: Samples were collected from three sets of triplicate 3.5-m², 36-cm deep raceway ponds:
- South Ponds: 2-day hydraulic residence time (HRT) fed primary clarifier effluent (PE)
- Middle Ponds: 3.7 to 4.8-day HRT fed facultative pond effluent
- North Ponds: 2 to 3-day HRT, fed PE
Influent pulses every half hour from 0700 to 1600. Mixing 50 RPM rotating paddle wheel.

Objectives

To compare the mean ash-free dry weight (AFDW) of triplicate pond sets and of three sampling frequencies to determine the effect of pond and sampling frequency on reported mean AFDW.

Methodology

Location:
- Pilot ponds were located at a full-scale wastewater treatment plant in Delhi, California.

Sample Collection:
- Instantaneous grab samples were collected for all frequency experiments. Grab samples were used instead of composite samples because grab samples may collect a more representative sample when algae is filamentous or flocculated.
- Samples were collected using a standard technique overflow using a plastic sampling cup with a diameter slightly smaller than the standpipe inner diameter. This cup was placed inside the standpipe immediately after or near the end of each influence pulse.

Data Collection:
- In pond experiments: one to two probes were installed in each pond set and measurements for hourly dissolved oxygen (DO) (percent saturation), pH, and temperature (°C) were recorded using Neptune Systems’ Apex Fusion software.
- Samples were analyzed according to Standard Methods [8] including: Total suspended solids (TSS), volatile suspended solids (AFDW), non-volatile suspended solids (NVSS).

Data Analysis:
- A General Linear Model (GLM) Analysis of Variance (ANOVA) was conducted using Minitab 17 statistical analysis software. This model tests the hypothesis that means of two or more populations are equal. In this case, the response variable being compared between factors (frequency and pond) was the AFDW. The GLM was used to compare the means between sampling frequencies (Diel, Consecutive Daily, and 1x/week) for each pond set and to compare the means between triplicate ponds in each set.
- The null hypothesis for this model was that the mean values would be statistically equal if the P-value < 0.05.

Results: Analysis of Variance

Table 2: Comparison of mean AFDW for South, Middle, and North pond sets.

<table>
<thead>
<tr>
<th>Pond Set</th>
<th>Mean AFDW (mg AFWD/L)</th>
<th>Grouping</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>281.66</td>
<td>A</td>
<td>0.131</td>
</tr>
<tr>
<td>M</td>
<td>238.73</td>
<td>A</td>
<td>0.237</td>
</tr>
<tr>
<td>N</td>
<td>219.49</td>
<td>A</td>
<td>0.083</td>
</tr>
</tbody>
</table>

Table 2: Comparison of sampling frequency to mean AFDW for South, Middle, and North pond sets.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean AFDW/L</th>
<th>Grouping</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/day</td>
<td>281.66</td>
<td>A</td>
<td>0.131</td>
</tr>
<tr>
<td>1/wk</td>
<td>238.73</td>
<td>A</td>
<td>0.237</td>
</tr>
<tr>
<td>1/wk</td>
<td>219.49</td>
<td>A</td>
<td>0.083</td>
</tr>
</tbody>
</table>

Sampling Frequency Comparison:
- Median sampling frequency using the TPC results, Table 2 and Figure 4 show that for all three pond sets, the AFDW concentration for Consecutive Daily (1/day) and Diel (4-6/day) sampling frequencies are significantly different from one another. One reason for this occurrence could be because the time periods for the Diel and Consecutive Daily sampling frequencies did not range for any one week, whereas the 1x/week sampling frequency period overlaps the other two frequencies.
- For the S and M ponds, both P-values are greater than 0.05 (Table 2), meaning the AFDW per mean sampling frequency for these pond sets can be statistically considered. For the N pond set, the P-value is 0.004 and each frequency has been placed in a different group (A, B, C), suggesting a significant difference between the three frequencies.
- Potential explanations for this difference include a lack of control for different experiments and a loss of data overall to be a smaller data set for these ponds. The study spanned operational regimes of several different HRTs, influent dosing schemes, and night aeration.

Results: Discussion

Diel vs Consecutive Daily:
- Both Consecutive Daily and Diel sampling frequencies are more consistent means between ponds could be because of the longer hydraulic residence time (HRT) especially at higher dilution rates.
- More samples at any sampling frequency are more likely to capture the true annual average productivity.
- Optimal Sampling Frequency:
- Based on the TPC results, Table 2 and Figure 4, it seems likely that the 1x/week sampling frequency per week sampling frequency for four consecutive weeks. Works were chosen in correspondence with Diez 3 and Consecutive Daily 3, 4, and 5.