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Summary of Chytrid bulk analysis

Aaron Collins

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Notes

- As a follow up to the microscopic investigations of FD01 infection of *Scenedesmus dimorphus* I wanted to see if the chytrid infection was specific or generic to host physiology particularly photosynthesis.

FD01 was used to infect *Scenedesmus* cultures in duplicate according to the following experiment time table

Colors represent unique infections (in duplicate) as we are trying to squeeze 24 hours of imaging into a 1 day experiment.

Infection conditions; ~32C, 400 uE*m^2*s^{-1} shaking on an orbital shaker.

Infected? Yes	No	Date & Time of Infection	ID	Time of analysis		PAM	Oxygraph	pH	Chl ext
	X		Control t0	12/12	9 am	X	X	X	X
X		02/13, 5 pm	Infected t16	12/12	9 am	X	X	X	X
X		02/14, 6 am	Infected t3	12/12	9 am	X	X	X	X
	X		Control t3	12/12	12 pm	X	X	X	X
X		02/14, 6 am	Infected t6	12/12	12 pm	X	X	X	X
	X		Control t7	12/12	4 pm	X	X	X	X
X		02/13, 5 pm	Infected t23	12/12	4 pm	X	X	X	X
X		02/14, 6 am	Infected t10	12/12	4 pm	X	X	X	X

*Control cells will be cultured under same conditions as infected cultures.

Measurements;

Chlorophyll fluorescence (ΔF/Fm') – Waltz Mini-PAM

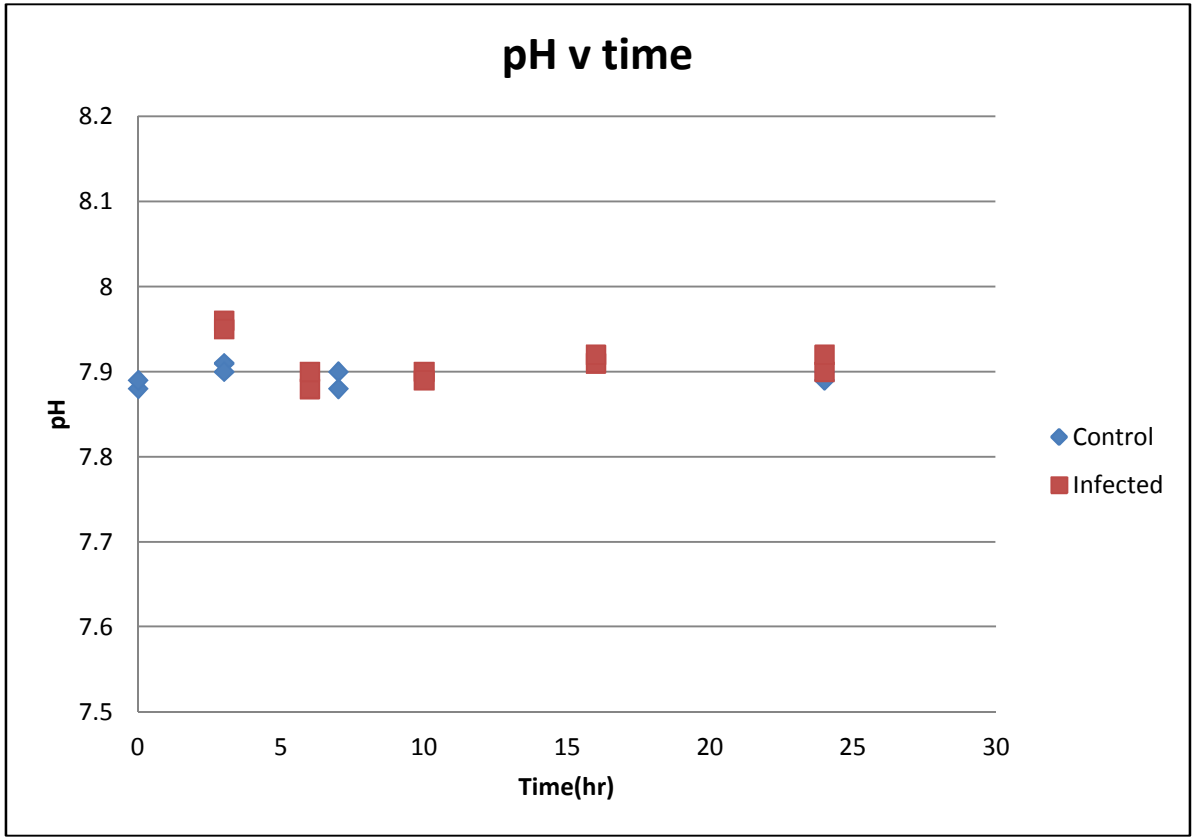
O_2 evolution – Hasatech Oxygraph – Clark style electrode, 100 uE m^2 s^{-1} fluorescent light excitation

pH – VWR temperature corrected pH electrode and meter

Chl extraction – 1 ml of culture pelleted, freeze-dried and resuspended in 1 mL of DMF. Incubated for 24 hours in the dark followed by measurement on Ocean Optics spectrophotometer

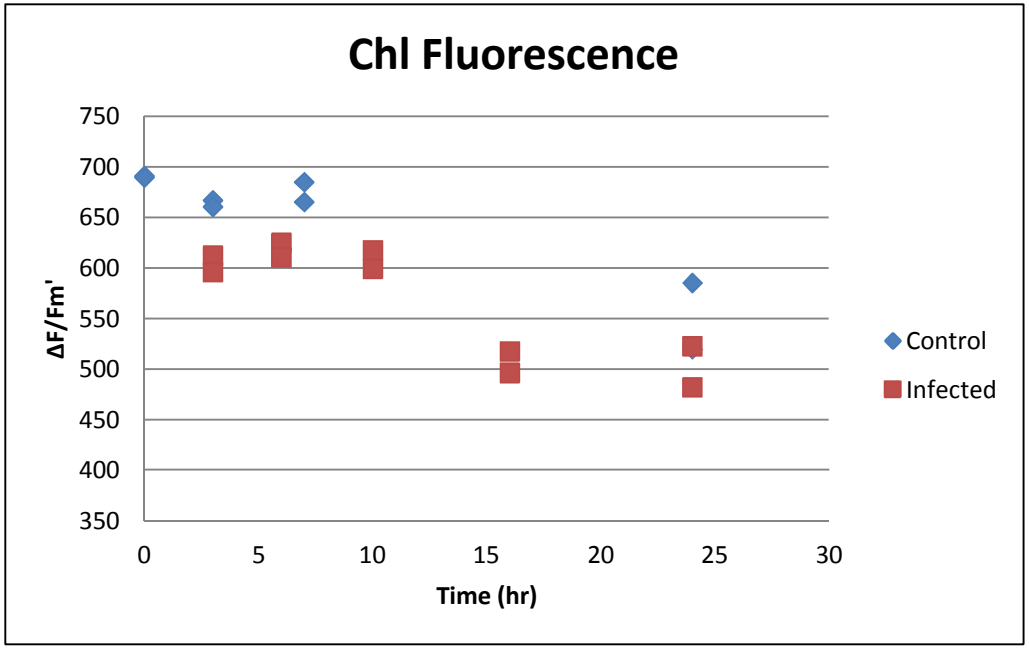
pH

I am plotting in excel for convenience however, I will make more aesthetically pleasing plots for publication purposes.



No change in control or infected cultures over the course of the investgation.

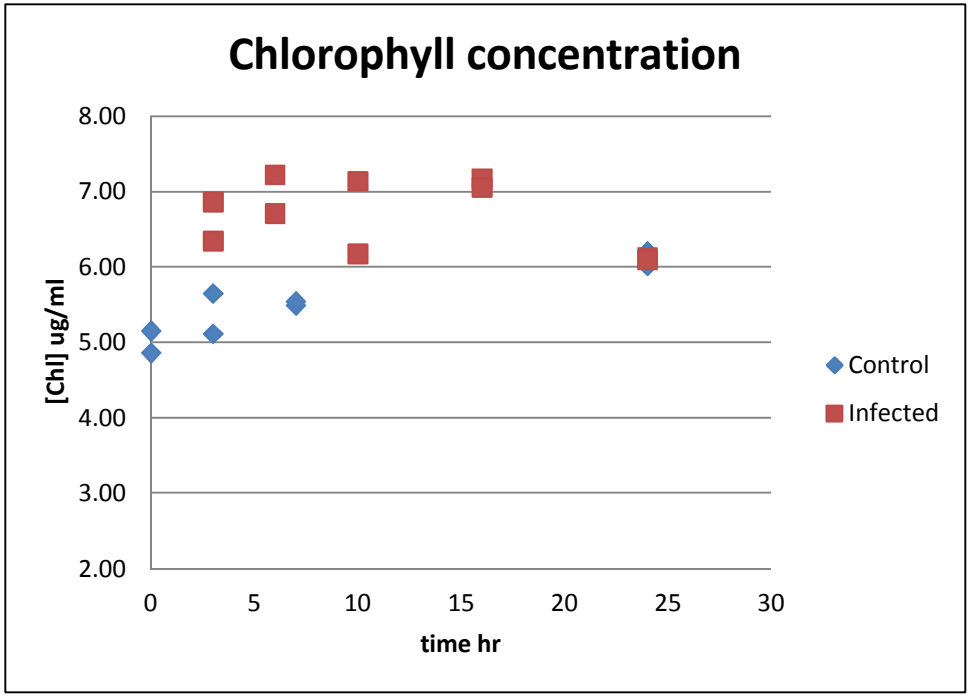
Chlorophyll fluorescence



ΔF/Fm’ is a light independent measure of PSII activity. As long as samples are measured under the sample conditions, then between-sample comparisons are valid.

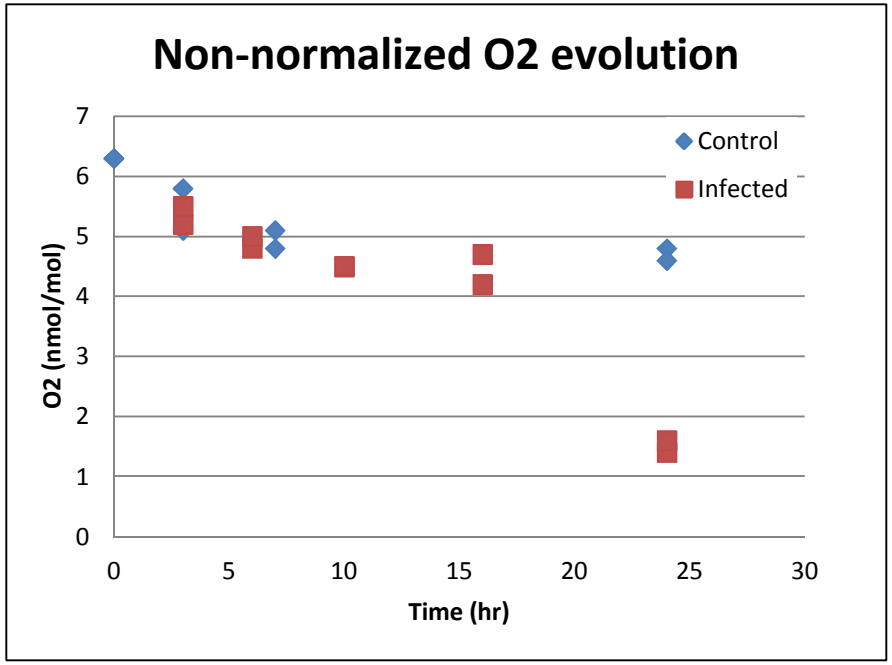
In the infected cells always lag the control cells however even the control cells are stressed after 24 hours in high-light and temperature.

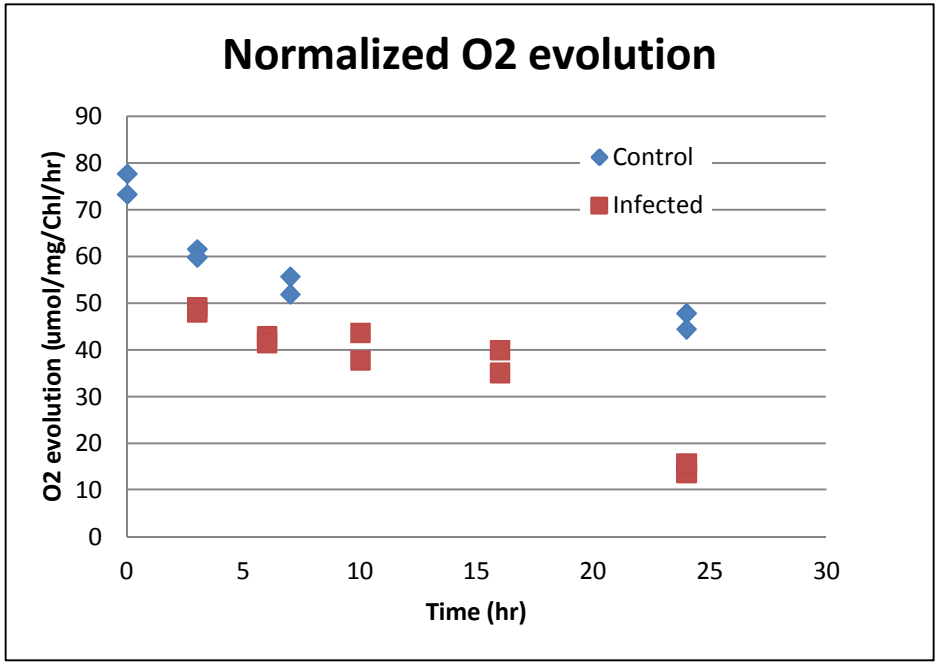
Chlorophyll concentration



The initial concentration of control cells were less than the infected samples. This matches my notes and suggests a complete extraction of Chl from the samples. The control cells increase in chl over the experiment indicating slight cell growth but it is really close to the noise. The infected cells do not change concentration over the 24 hour period.

Oxygen evolution





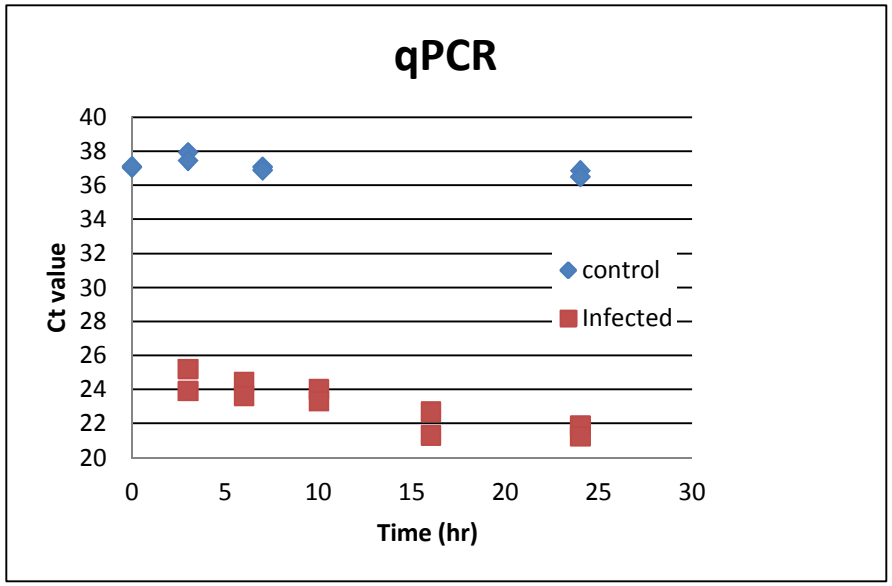
Raw O_2 evolution measured at each time point

O_2 evolution normalized to the Chlorophyll concentration

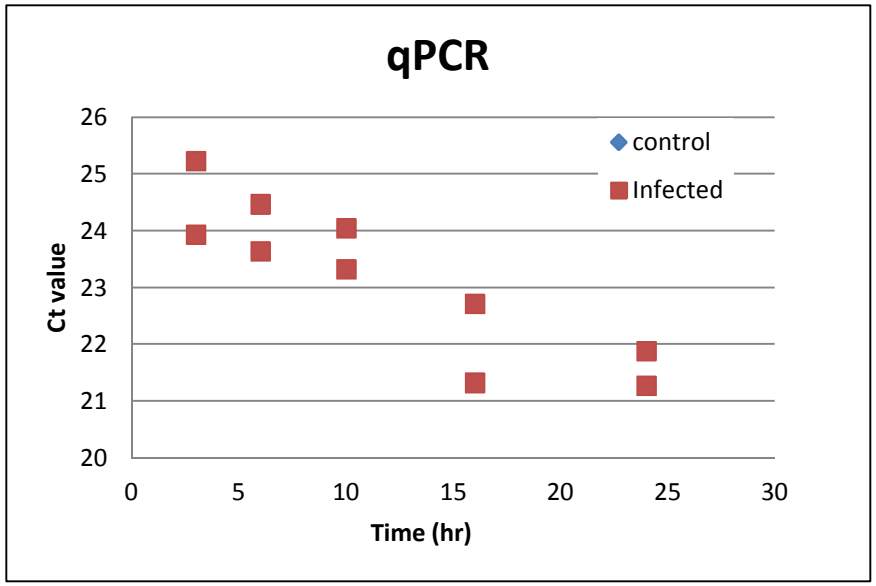
The normalized O_2 evolution data mirrors (for the most part) the PAM measurements. A reason why the infected cultures do not coincide with the control cells at early times likely results from the addition of the infection source to these samples. The infection source is a crashed culture that still have some Chl in it however that Chl fraction is not performing O_2evolution so it decreases the overall value.

qPCR – performed at Sapphire SD

Full view



Zoom view



Two infection events were utilized for these overall experiment (see slide 2). The Ct values for the two infection sources were 22.1 and 21.7 suggesting that both infections events occurred with the approximate MOI.

What does this data tell us?

I believe the PAM and normalized O2 data the most relevant for the manuscript. These data tell us that productivity has decreased but not until 24 hours post infection. At earlier time points we cannot separate stress (control cells in HL and elevated temp) from infection.

The qPCR data suggest that by t=16 hours chytrid infection is taking off but the "typical" phenotype response of the host lags by several hours (8 in this case)*.

Thus, these data (and I am sure others from Sapphire) point to the need for early detection strategies.

I think we can use the 3 different microscopic investigations (hyperspectral, unlabeled pseudo-bulk measurement, hyperspectral, unlabeled high-res investigation and Nile Red labeled high-res investigation) to

1) Show that a subtle but statistically significant changes in host fluorescence correlated with infection
2) Fluorescence and structural changes to host with infection
3) Host-chytrid interactions in living cells via Nile Red
4) Others???

If everyone is on board, then I will start drafting an outline and requesting info from others on the team.

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*The only other system I am partly familiar with is an Algal/virus pair that behaves very differently.

(Seaton et al. Plant Physiol. (1995) 108: 1431-1438). In this host/virus pair, the virus causes changes in the host cell's metabolism specifically to shunt photosynthesis in under 1 hour post-infection. The time from attachment to host-lysis is 10 hours in this case to the infection is very rapid. According to the Sapphire's PLoS paper, a chytrid infection cycle takes days so that a slow changes to the host physiology is expected. With early detection and a slower chytrid life cycle, we might actually have time for mitigation!
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