**Paper Outline**

**Working Title:** Investigating the dynamics of cryptophyte algae in the Columbia River Estuary

**Abstract** will happen eventually...

**Introduction**

Every year, in the late summer or early fall, massive non-toxic red water blooms form in the Columbia River Estuary. These blooms consist of both cryptophyte algae and the mixotrophic ciliate, *Mesodinium major,* previously referred to in past publications on the Columbia River Estuary as *Mesodinium rubrum* (*= Myrionecta rubra*)(Lohmann, 1908; Jankowski, 1976).

-explain stuff about name change? New genetic analysis and “medusa form” (Garcia-Cuetos et al., 2012)

-how much should I explain about what a mixotroph is? Is it explained well enough in the paragraph below?

-transition sentence to put the focus back on the cryptophytes

Reasons for the timing, patchiness, and persistence of the bloom are not yet fully understood. Past research on the estuary has focused largely on understanding the dynamics of the *M. major*, but it has been hypothesized that the cryptophytes may be important in controlling the bloom, as they are both a source of prey and photosynthetic ability to the ciliate. Chloroplasts originating from the cryptophyte, *Teleaulax amphioexa,* have been found inside the *M. major* in the Columbia River Estuary, conferring photosynthetic capabilities (Herfort et al., 2011b). Though free-living T. amphioexa are low in number during the red tide blooms (Herfort et al., 2011b), the presence and growth dynamics of these cryptophytes have the potential to be key factors in the proliferation of their ciliate predator.

Our major questions:

-How do the growth rates of the cryptophytes change throughout the bloom?

-Is there a relationship between cryptophyte growth rate and abundance of *M. major*?

How are we doing this?:

-continuous flow cytometry, size-structured growth model

Other potentially important stuff to include in introduction:

-cryptophyte chloroplast pigments are well adapted to dim light, turbid waters of CRE (Herfort et al., 2012)

-data showing cryptophyte counts in relation to *M. major* (Peterson, 2012)

-some of the data from (Herfort et al., 2011b) on bloom initiation:

-saltwater intrusion and subsequent temporary decrease in turbulence has recently been shown, along with coinciding neap tides, to correlate with the initiation of bloom

**Methods**

**Study Area**

The Columbia River Estuary, located in between Washington and Oregon (**fig. 1**), is a turbid and often highly stratified system. It is characterized by its dynamic physical processes and strong influence from diurnal and semi-diurnal tides (Jay, 1984). This tidal forcing, in combination with the seasonality of freshwater discharge, results in an extended summer saltwater intrusion (Chawla et al., 2008). The estuary also has a short residence time, with flushing in the range of 0.5-5 days (Neal, 1972).

**Sample Collection**

A SeaFlow continuous flow cytometer (Swalwell et al., 2011) was stationed on a dock outside of Astoria, OR (**fig. 1**) and set up to run continuous measurements of surface water for up to five days each week for four weeks in September-October 2013. 1mL samples of whole water for (how do I specify “regular” flow cytometery from SeaFlow?) flow cytometry, 45mL samples for *M. major* counts via FlowCam, and 30mL surface water samples for nutrient analysis were taken in duplicate. The samples for flow cytometery and *M. major* counts were taken at three depths, and preserved with 20uL and 1mL of 25% gluteraldehyde, respectively. The samples for flow cytometry and nutrient analysis were then stored at -20°C, the samples for *M. major* counts refrigerated at 4°C.

**Division Rate Lab Verification**

For verification of the division rates found using the size-structured growth model, cultures of the cryptophyte, *Rhodomonas sp*. (CCMP 755), were grown in flasks at 13 °C in f/2 seawater media under a (16:8 hr) light-dark cycle. The abundances of daily 1mL samples of Rhodomonas sp. were determined via cell counts using a Sedgewick-Rafter slide. Growth rates were calculated using blah equation (insert equation here).

A single replicate of the *Rhodomonas sp.* cultures was brought to a volume of 20L in a blah carboy with a concentration of 50 cells/mL and mixed using a magnetic carboy stirbar. SeaFlow was run on the culture under a (16:8) light-dark cycle for 4 days. During the first day running SeaFlow, 1mL triplicate samples of the *Rhodomonas sp.* culture were taken every 2 hours for 28 hours, preserved with 20uL of 50% gluteraldehyde, flash frozen, and stored at -80 °C for cell cycle analysis.

The 1mL samples of Rhodomonas sp. were later thawed over ice, and run on an Influx flow cytometer...

-I might need some help with describing the cell cycle analysis (mostly the processing of the flow cytometery data)

***M. major* Enumeration**

TP writes this (?)

**qPCR for *Teleaulax* abundance**

PZ writes this (?)

**Flow Cytometry Sorting – Light microscopy**

A 1mL surface water sample was run on an Influx flow cytometer, and cells from the gated population of supposed “cryptophytes” were sorted onto a glass slide. The cells were then examined under a Nikon Eclipse 80i epifluorescent microscope at 20x magnification and photographed using a Qimaging MicroPublisher 3.3 RTV camera.

**Nutrient Analysis**

not sure who did this part?

**SeaFlow Data Analysis (?)**

Talk about popcycle here? (This section can be moved.)

-SeaFlow file conversion

-evt files filtered with blah filtering parameters used throughout time course

-manual clustering of beads and “cryptophyte” population applied to single time point file and then used throughout time course

-https://github.com/uwescience/popcycle

**Size-structured Division Rate Model**

Not sure if this should also go here or not?

-based off of model from Sosik et al., 2003

-”matrix population model” based on light and cell size

-assumptions of the model

-might also need some help explaining model here...

**Results**

**Environmental Data**

Surface water temperature and salinity (**fig. 2, a**) appear to be anti-correlated and oscillate with the tidal cycle. High tide is characterized by an influx of colder, more saline water, and low tide by an increase in warmer freshwater. In the first and third weeks of the time course, the average surface water salinity is higher and the temperature lower than the rest of the time course. Week four had both the lowest surface water salinity and lowest temperature, compared to the other weeks.

Surface water concentrations of ammonium ranged from 2.3 μM-74.4 μM, concentrations of nitrate from 3.5 μM-16.1 μM, and of phosphate from 0.4μM-1.5 μM (fig. 2, c). Changes in phosphate and nitrate appeared to be coupled throughout the first three weeks of the time course. A peak in the surface water concentrations of all nutrients occurred on 9/16.

-not sure what to say about PAR data??

-after writing it, some of the stuff that I mention above about the saltier, colder water coming in with the tide now seems really obvious and I'm not sure I even need to include it?

**Flow Cytometry Sorting – Light microscopy**

The gated population was confirmed as consisting of cryptophytes after observations of the sorted cells revealed the orange autofluorescence, characteristic of the cryptophyte group, when viewed under epifluorescent microscopy (image as supplemental data?). The size of the cells viewed (<5 um) agreed with past observations of cryptophytes in the Columbia River Estuary (Peterson et al., 2012) and were teardrop-shaped.

**Abundances**

Data from SeaFlow showed the abundances of the surface water cryptophytes to be between <0.01x10⁶ - 19.2x10⁶ cells L⁻¹ throughout the time course, with some of the highest concentrations occurring within the first week (**fig. 3, a**). Cryptophyte abundance did not appear to be correlated with tidal cycle, though oscillations in abundance did occur within individual days.

**Division Rate**

Model estimates of the mean daily division rate of the surface water cryptophytes ranged from 0.023 to 9.78 (units?) (**fig. 4**), with weeks three (**fig. 4, c**) and four (**fig. 4, b**) exhibiting dramatically increased rates as compared to the first two weeks.

The model estimates of the mean daily division rates did not have a significant correlation with any of the surface water nutrient concentrations or PAR (**fig. 5**) (check p values). Additionally, there was no relationship between cryptophyte abundance and division rate (data not shown?).

***M. major* Counts**

Abundances of *M. major* ranged from between 21-323 cells mL⁻¹ (**table 1**)...

**qPCR for *Teleaulax* abundance**

The percent of the total cryptophytes that were *T. amphioexa*, as estimated from qPCR data, was very low across the entire time series, ranging from 0.0615% - 0.397% (**table 1**), and agreeing with observations made in previous years (Herfort et al., 2011b). When applied to abundances measured via SeaFlow, this percent translated to between blah and blah number of *T. amphioexa* cells mL⁻¹ in the surface waters.

**Discussion**

Discussion of high growth rates, model:

-Why do we have such high growth rates? Is it the model assumptions being violated or are these cryptophytes just growing crazy fast?

-growth rates for *M. major* found in CRE (Herfort, 2011; Peterson, 2012) are way higher than culture, does this mean that our crazy high growth rates for the cryptophytes could potentially be true?

-does this potentially have any larger implications for those studying algae growth in culture?

-explain limitations of model, how some of the assumptions may have been violated

-we could have multiple taxonomic groups of cryptophytes with different physiologies represented within the population of cryptophytes that we observe with SeaFlow

-evidence of trouble with model parameter optimization (hitting extremes during last two weeks where we are also observing the highest division rates) (parameter plot in supplemental data)

Discussion of *T. amphioexa* in relation to *M. major:*

-still low abundances of free-living *T. amphioexa*, but why?

-could be attached to cirri, can be up to 50 cryptophytes attached (Peterson et al., 2012)

-could be inside *M. major* (use FISH probe picture from PZ and cite as “unpublished data”?)

-mention that the day after the day with the highest M. major abundance has the lowest % of *T. amphioexa*, could potentially suggest selective grazing??? (**table 1**)

-but the dinos could also be selectively grazing too (we have pics of dinos)

-discuss grazing rates seen in culture for *Mesodinium sp.*

**Conclusion**

**Figure Captions**

**Fig. 1** Map of the Columbia River estuary with the sampling site location marked.

**Fig. 2** Time series of environmental data from the sampling site including (a) temperature and salinity in surface waters, overlain with a low-pass filter represented by the grey and black lines, (b) post-low-pass filtered PAR (?), and (c) measurements of ammonia, nitrate, and phosphate concentrations in surface waters. Temperature, salinity (a), and PAR measurements (b) were determined via moored sensors (?). Nutrient measurements (c) were taken from discrete water samples.

**Fig. 3** Time series of SeaFlow measurements of cryptophyte cell abundance, with each plot showing data collected for a specific week. Grey points represent individual time points and the black line overlain represents the data after a low-pass filter was applied. Shading within the plot shows the tidal cycles, with the beginning of each grey region corresponding to low tide and the beginning of each white region corresponding to high tide.

**Fig. 4** Time series of the model estimates of mean daily division rate for the cryptophyte population separated by week. Vertical bars represent the standard error of the average of 24 model estimates. Shading within the plot shows the tidal cycles, with the beginning of each grey region corresponding to low tide and the beginning of each white region corresponding to high tide.

**Fig. 5** Plots of nitrate (a), phosphate (b), ammonia (c), and PAR (d) vs. the mean daily division rates of the cryptophyte population.

**Table 1.** *M. major*, total cryptophyte, and *T. amphioexa* abundances (cells mL⁻¹ ), and the estimated percent of *T. amphioexa* as determined via qPCR. *T. amphioexa* abundances calculated using the estimated percent.