**Paper Outline**

**Working Title:** Dynamics of cryptophyte populations 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 as *Mesodinium rubrum* (*= Myrionecta rubra*)(Lohmann, 1908; Jankowski, 1976). *M. major* and *M. rubrum* are among the marine microzooplankton that have been observed to harbor the plastids of their cryptophyte algae prey, allowing these predators to function as mixotrophs, capable of utilizing both phagotrophic and photosynthetic mechanisms to acquire carbon (Crawford, 1989).

-unclear as to specifics of predator-prey relationship: type of symbiosis (Hansen et al., 2012) or karyoklepty (Johnson et al., 2007)

-transition sentence

The Columbia River Estuary is highly influenced by both tidal forcing and the seasonality of freshwater discharge, which results in an extended summer saltwater intrusion (Chawla et al., 2008). This saltwater intrusion and subsequent temporary decrease in turbulence has recently been shown, along with coinciding neap tides, to correlate with the initiation of the yearly *Mesodinium sp.* bloom in the estuary (Herfort et al., 2011a). But reasons for the timing, patchiness, and persistence of the bloom are still 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,* are found inside the *M. major* in the Columbia River Estuary, conferring photosynthetic capabilities (Herfort et al., 2011b). Weekly counts of M. major and cryptophyte cells during a red tide bloom in 2011 showed that the abundance of small (<5 um) “Teleaulax-like” cryptophytes declined just prior to an increase in *M. major* abundance, suggesting active predation by *M. major* or another zooplankton (Peterson et al., 2012). Though free-living *T. amphioexa* are overall 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:

-What are the effects of environmental conditions, such as nutrient availability, temperature, salinity, and light, on the physiology of the cryptophyte populations, and how does their physiology change over both tidal cycles, and the course of the bloom?

-Is there a relationship between cryptophyte physiology and the dynamics of *M. major*?

How are we doing this?:

-monitoring the abundances of cryptophyte populations continuously over one month during a red tide bloom

-estimate division rates of the combined populations of cryptophytes using a size-structured division rate model from Sosik et al.

-monitor *M. major* abundances with FlowCAM counts of the ciliate once per day

-estimate abundances of *T. amphioexa* using qPCR once per week

**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). 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 (should this be split up into two parts?)**

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**

person who did qPCR writes this (in PZ's lab)

**Flow Cytometry Sorting – Light microscopy**

A 1mL surface water sample was run on an Influx flow cytometer, and 100 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. 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.

**Nutrient Analysis**

JN writes this

**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**

FR writes this

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

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

-assumptions of the model

**Results**

**Lab Verification**

**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.

-compare these values for temperature and salinity to data from past red tides

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?

**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 mean PAR (**fig. 5**) (check p values), though it is important to note that nutrient concentrations were determined from single time point samples. Additionally, there was no relationship between cryptophyte abundance and division rate, pointing to the importance of loss processes (both physical and biological).

***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 5.03 and 93.18 number of *T. amphioexa* cells mL⁻¹ in the surface waters.

**Discussion**

How are abundances changing over tidal cycle?

-There isn't a clear pattern. What does this mean?

-Biological processes (growth, grazing) are obscuring the relationship between tidal cycle and abundance

-Other physical processes (non-tidal) are influencing abundance???

How are abundances changing over the month?

-weekly average of cryptophyte abundance is highest during the first week, decreases in the middle of the month, and then increases again during the last week (**table 1**)

How do the division rates change with environmental conditions, and are they limited by these?

-I think I need some help interpreting the data for this...

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, so our high growth rates for the cryptophytes are not necessarily an inaccurate estimate

-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)

-we don't actually have any evidence that these assumptions were violated, and our laboratory verification of the model worked out, so we still trust our results

-measuring growth in the field is difficult, but this new approach eliminates many of the problems associated with other methods (i.e. dilution experiments) and is less labor-intensive

1. major abudnances compared to cryptophyte data

No correlation with M. major and cryptophyte division rate, but there may be a relationship between the M. major abundances and the percent of T. amphioexa.

-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* (FISH probe picture from PZ and cite as “unpublished data”?)

-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 weekly average, 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.