Ecology and genetic analysis of *Katablepharis CRE*, a heterotrophic flagellate that ‘blooms’ in the Columbia River estuary during the spring

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1. Background

- Heterotrophic protists play important roles in the complex estuarine food web by shaping bacterial populations through predation, transferring prey carbon to higher trophic levels, and remineralization of nutrients such as nitrogen and phosphorus.
- While difficulty in their identification using light microscopy has often led to heterotrophic protists being lumped into broad classifications, there is a strong need to develop methods that increase the spatial and temporal resolution of observations of particular organisms in order to discover the drivers of population structure and ecological function.
- The Columbia River estuary contains large amounts of allochthonous detritus which is the primary source of organic matter driving ecosystem processes. This organic matter can fuel a high level of bacterial growth and productivity, which in turn can fuel propagation of grazing heterotrophic protists via the microbial loop. However, major heterotrophic protists (primary consumers and bacterivores) in the estuary are not well characterized.

2. *Katablepharis* sequences dominate mid-salinity SSU clone libraries during the spring

- Apicomplexa • Cercozoa • Chrysomonadina • Ciliophora • Dinophyceae (H) • Dinophyceae (M) • Katablepharisaceae • Other

Fig. 1. SSU sequence libraries of heterotrophic protists from April and August 2007, and in April, July and September 2008. Freshwater = 0; Mid-Salinity = 15; Plume = 25-31. “H” refers to putative heterotrophic dinoflagellates, while “M” refers to putative mixotrophic dinoflagellates.

- SSU sequence libraries from 2007 and 2008 revealed an abundance of sequences related to *Katablepharis* in spring mid-salinity waters, a non-pigmented heterotrophic flagellate that has been found to be an important primary consumer and bacterivore associated with particles in other aquatic systems. *Katablepharis* sequences comprised over 40% of all protist sequences in spring-mid salinity samples.
- Events occurring in the spring, such as upstream diatom blooms and spring runoff, deliver organic matter to the estuary that can fuel *katablepharis* proliferation.

3. Unique sequence element within the D2 region of *Katablepharis CRE* 28S rRNA gene

% similarity to *Katablepharis japonica*

**% similarity**

| % GC | 18S | ITS 1 | ITS 2 | 28S | USE | 28S

**28S USE primer** • General eukaryotic primer • *Katablepharis*-specific primer

Fig. 2. Sequencing of the 28S rRNA gene of the Columbia River Estuary *Katablepharis CRE* revealed a 332 bp region within the D2 region of the 28S rRNA gene that is unique to the CRE strain. This unique sequence element (USE) is GC-rich compared to the rest of the gene and shows no significant similarity to other *katablepharis* in the NCBI database, while the rest of 28S rRNA gene align well with *Katablepharis japonica* and other sequenced *katablepharis*. Colored arrows indicate PCR primers designed in the 28S unique sequence element (purple), to be general for all eukaryotes (teal), or *katablepharis* assemblages (including autotrophic and heterotrophic fractions) during the spring months of this study.

• 1. What is the temporal and spatial extent of this unique element amongst *Katablepharis CRE* and other *katablepharis* in the Columbia River system?

- All *Katablepharis CRE* sequences recovered from the estuary contain this region. The only other *katablepharis* detected in the system, *Leucoctrema* does not appear to have this element.

2. Is the unique element found in any other organisms in the Columbia River system or elsewhere?

- No, so far *Katablepharis CRE* is the only organism linked to positive amplification from the unique element. This region has also failed to align with the NCBI sequence database and metagenomic databases. However, several other unique sequence elements have been detected from other protist taxa, including the parasitic dinoflagellate *Eud_ISO_013* and deep-water diplomonads.

• 3. Can the unique element be used as a taxonomic marker to study the ecology of *Katablepharis CRE*?

- USE-specific primers for quantitative PCR (qPCR) and probes for fluorescent in-situ hybridization (FISH) were developed based on this element and used on estuarine samples collected across a five site river-to-ocean transect from 2013.

4. Spatial and temporal distribution of *Katablepharis CRE* in 2013 assessed through qPCR

- No, so far

5. FISH with USE-specific probes for *Katablepharis CRE*

- No, so far

6. Conclusions

- *Katablepharis CRE* dominated protist assemblages (including autotrophic and heterotrophic fractions) during the spring months of this study. qPCR suggests a wide distribution of *Katablepharis CRE* with regards to salinity, but a preference for bottom waters. Given their high abundance and repeatable temporal patterns, we hypothesize that they play an important role in estuarine biogeochemical and ecosystem function.

- The unique sequence element of *Katablepharis CRE* confers extreme variability compared to the rest of the D2 region, with no significant similarity to any sequences in the NCBI database. The discovery of the unique sequence element (USE) within the 28S rRNA gene of *Katablepharis CRE* provided an excellent template to track the distribution of *Katablepharis CRE* in the system through USE-specific probes combined with quantitative and qualitative methods.

- Unique sequence elements can increase the taxonomic resolution in studies of 28S rRNA protist diversity and be utilized to develop strain-specific probes for quantitative or qualitative monitoring of ecologically relevant heterotrophic species.

Fig. 3. Sampling locations for qPCR and FISH analyses conducted for March-July 2013. Surface and bottom samples were taken monthly for five sites.

**A.** Distribution of *Katablepharis CRE* USE gene copy numbers measured at the outfall of Bonneville Dam for 2013 (daily mean). Gray arrows indicate sampling dates for qPCR and FISH analyses.

**B.** Distribution of *Katablepharis CRE* USE in the Columbia River estuary estimated by qPCR from March-July 2013. Asterisks denote samples which were also analyzed with FISH.

- This qPCR assay confirmed the wide distribution of *Katablepharis CRE* in the Columbia River estuary with respect to salinity (0-25PSU), but with overall at each site higher abundances measured in bottom waters. High abundance of *Katablepharis CRE USE* gene copy numbers persisted until river discharge returned to pre-freshet levels.

**C.** Comparison of *Katablepharis CRE* abundance estimated by FISH and qPCR using probes specific for its Unique Sequence Element (USE) within the large subunit for both approaches.

- FISH analysis detected a ~7 µm organism, which is the approximate size of *katablepharis* in other systems.

**D.** The slope of the regression between qPCR and FISH estimates suggest a ratio of 2.5 gene copies per cell. The R² value for this correlation is low, likely because biases in DNA extraction and qPCR optimization, as well as potential variability in growth rate, causes discrepancies between the two methods.

Fig. 4. Epifluorescence micrographs of Columbia River estuary water A. DAPI-stained cells and B. the corresponding microscopic field using the USE-specific *Katablepharis CRE* probe. C. Comparison of *Katablepharis CRE* abundance estimated by FISH and qPCR using probes specific for its Unique Sequence Element (USE) within the large subunit for both approaches.

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