Determining the Optimum Method for DNA Extractions an PCR Clean Up to Monitor Microbial Eukaryote Populations in Bellingham Bay

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Introduction

Microbial eukaryotes are important parts of all ecosystems. Microbial eukaryotes are microscopic members of the domain Eukarya (all microscopic organisms that are not Archaea or Bacteria). Microbial eukaryotes are particularly diverse and dynamic in marine ecosystems and are often referred to as phytoplankton (microscopic autotrophs) and zooplankton (microscopic heterotrophs). Phytoplankton are microscopic photosynthetic organisms that use sunlight, nutrients, and warmer water temperatures to create their own energy and reproduce (Sarkar & Malchow 2005). Ideally, they help keep marine ecosystems healthy through the production of oxygen and because they act as a food source at the base of the marine food web. Due to more recent anthropogenic activities and climate change, mass reproductions of phytoplankton are becoming more prevalent. Some species of algae have harmful effects especially in high population numbers known as harmful algal blooms (HABs) (Beckmann & Hense 2004). With an increase in nutrients and warmer water phytoplankton reproduce at an expeditious rate (Sarkar & Malchow 2005). HABs can be devastating for marine ecosystems and human populations.

Figure 1. Summer 2019 Se'lhaem Buoy in Bellingham Bay, Washington. The Se'lhaem Buoy provides data such as wind turbidity, Chlorophyll, wind speed, water and air temperature, salinity, and dissolved oxygen. It was funded by the Coastal Margin Observation and Prediction (CMOP). The University of Washington, Western Washington University, the Northwest Indian College, and National Oceanic and Atmospheric Administration (NOAA) are contributing partners.

The effects of HABs can be particularly detrimental to coastal tribes and their communities because they cause closures of shellfish hatcheries, fisheries, and traditional gathering grounds (Yakoubian & Daniel 2018). Marine ecosystems are interwoven with the culture and traditions of the Pacific Northwest tribal communities. These culturally and ecologically relevant marine food webs are heavily influenced by the dynamic nature of microbial eukaryotes. Microbial eukaryote communities in estuarine systems are dynamic and variable. Bellingham Bay is a dynamic ecosystem influenced by the Salish Sea and the Nooksack River which warrants frequent monitoring of microbial eukaryote population. This project uses microscopy and meta-amplicon sequencing of samples collected weekly in Bellingham Bay at the Se'lhaem Buoy to describe the community structure of microbial eukaryotes.

Colleagues at the Western Washington University have begun working on methods to monitor microbial eukaryote communities in Bellingham Bay. This project aims to continue the WWU monitoring project, and optimize methods for long-term weekly monitoring.

We will 1) analyze and investigate three DNA extractions kits to determine which kit extracts enough DNA for downstream analyses and 2) compare PCR clean-up methods to determine which method is the most efficient and accurate.

Figure 2. Paddle to Lummi 2019. Canoe Journey holds special significance to Coast Salish Tribes as "it truly honors and nourishes the unique relationships and connections with the land, water, and one another" (Paddle to Lummi 2019).

Methods

Sample Collection

Methods for sample collection and DNA extraction have been modified from Taylor L. Clement's thesis research (Clement, 2017). Samples were collected weekly from Bellingham Bay at the Se'lhaem Buoy. Environmental data in addition to location, date, and sample number were collected. Two 500mL samples were collected at the surface in addition to two 500mL samples collected at from a depth of 1m above the sea floor (~80ft). 500 mL of whole water was filtered through sterile filter towers onto 0.22 µm filters. Samples were stored at 4°C for future use.

DNA Extraction

The manufacturer's protocol was followed for each kit (Qiagen QIAamp Fast DNA Tissue Kit, Qiagen DNeasy PowerPlant Pro Kit, and Zymo Quick DNA Fecal/Soil Microbe MiniPrep kit) with a minor modification. Filters were flash frozen with ~500 ul of liquid Nitrogen onto the filter. The filter was then cut with sterile scissors. We used an Invitrogen Qubit 4 Fluorometer to determine the DNA concentration of each sample and ultimately the effectiveness of each kit.

PCR Clean-Up

I used PCR to amplify the small ribosomal subunit (18S) known to be marker gene for species. The PCR product was split in half to compare the two PCR clean-up kits; Beckman Coulter XP AMPure Kit and Qiagen QIAquick PCR Purification Kit. There were two different PCR clean-up methods used to help identify which one would be the best in analyzing the PCR product.

Figure 3. PCR Clean-Up Process. Both the Beckman Coulter XP AMPure Kit and Qiagen QIAquick PCR Purification Kit removed primers, DNTPs, and genomic DNA from the final PCR product (Figure modified from: New England Biolabs).

Results- Extraction

The DNA concentration from these extractions can be found in Table 1, and visualized in Figure 4. Both Qiagen kits only extracted less than 3 ng/ul for all extractions and the Zymo kit had much better results (Table 1, Figure 4). In all, I have found that the Zymo Quick DNA Fecal/Soil Microbe MiniPrep Kit is the best kit to use to extract DNA from phytoplankton.

Table 1. Sample Data & DNA Concentration.

Name	Depth	Date	ng/ul	Kit
BBB1	S1	4/3/2019	1.59	FTK
BBB3	S1	4/9/2019	2.4	FTK
BBB13	S1	5/9/2019	0	PPP
BBB15	D1	5/9/2019	0	PPP
BBB30	S2	6/6/2019	0.562	PPP
BBB32	D2	6/6/2019	0.511	PPP
BBB33	S1	6/14/2019	1.59	PPP
BBB35	D1	6/14/2019	0.0433	PPP
BBB41	S1	6/26/2019	0	PPP
BBB44	D2	6/26/2019	0.72	PPP
BBB46	S2	7/1/2019	0.555	PPP
BBB48	D2	7/1/2019	0.7	PPP
BBB53	S1	7/16/2019	27.7	Zy
BBB55	D1	7/16/2019	1.35	Zy
BBB58	S2	7/25/2019	1.47	Zy
BBB60	D2	7/25/2019	22.4	Zy
BBB62	S2	7/31/2019	19.2	Zy
BBB64	D2	7/31/2019	4.2	Zy
BBB65	S1	8/6/2019	17.5	Zy
BBB67	D1	8/6/2019	4.85	Zy

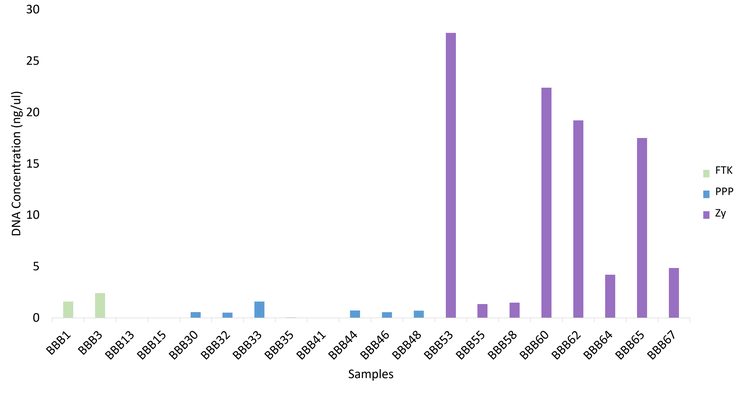

Figure 4. DNA Concentration From Extracted Samples. Samples from Qiagen QIAamp Fast DNA Tissue Kit (FTK) are shown in green, Qiagen DNeasy PowerPlant Pro Kit (PPP) are shown in blue, and Zymo Quick DNA Fecal/Soil Microbe MiniPrep kit (Zy) shown in purple.

Results –PCR Clean Up

To compare the effectiveness of the Beckman Coulter XP AMPure Kit and Qiagen QIAquick PCR Purification Kit I used both a Invitrogen Qubit 4 Fluorometer and an Agilent 2100 Bioanalyzer. Optimizing PCR clean-up provides a more efficient protocol for future projects and more accurate sequence reads for downstream analysis. The Qubit Fluorometer was used to determine the concentration of PCR amplicons before and after PCR. The average loss of PCR product concentration for each clean up method was compared using a paired-samples t-test to determine if there was a significant difference between the two options of the PCR clean-up.

These findings support the notion that there is a significant difference between the average loss of PCR product from the bead group (M=0.94) compared to the Qubit group (M=0.90). Though there was a significant difference in the average loss of PCR product, analysis using the Agilent 2100 Bioanalyzer (Figure 5) shows that the desired band at ~500 bp is absent following PCR clean up.
Due to complications with PCR, only 4 samples produced any usable PCR product. It is difficult to arrive at any conclusions regarding data within the PCR clean-up due to the small volume of observations. From this standpoint, the findings can be considered as insufficient.

t-Test: Two-Sample Assuming Unequal Variances		
	Bead	**Qubit**
Mean	0.944	0.901
Variance	1.300053	1.238333
Observations	4	4
df	6	
t Stat	0.053978	

Table 1. t-Test Two Sample Assuming Unequal Variances. using a paired-samples t-test to determine if there was a significant difference between the two options of the PCR clean-up.

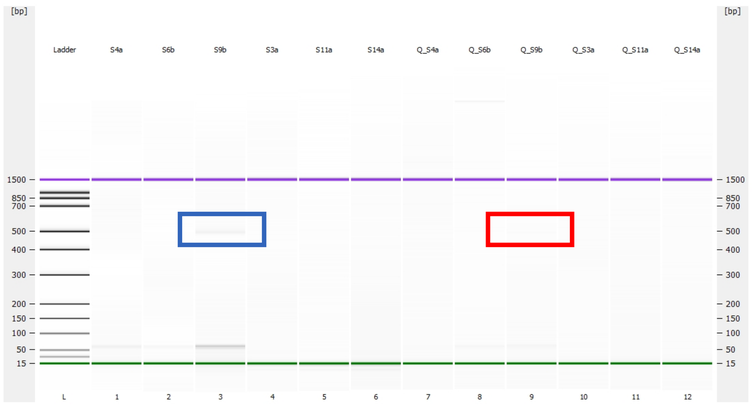

Figure 5. Agilent 2100 Bioanalyzer Results. The Bioanalizer shows successful PCR (blue box), and lost amplicons following use of Qiagen QIAquick PCR Purification Kit.

Next Steps

- Continue to work on PCR and PCR Cleanup
- Final steps before sequencing; Index PCR and Index PCR Cleanup quantification.

Sequencing of whole water samples will help describe the community structure of microbial eukaryotes in Bellingham Bay. Future research will help native communities' practice tribal sovereignty.

References

Beckmann, A., and I. Hense. "Torn between Extremes: The Ups and Downs of Phytoplankton." Ocean Dynamics, vol. 54, no. 6, 2004, pp. 581-592.

Clement, T. L. (2017). *Phylogenetic-based characterization of microbial eukaryote community structure and diversity of an estuary in the Salish Sea*. Bellingham, WA: Western Washington University.

Sarkar, Ram R., and Horst Malchow. "Nutrients and Toxin Producing Phytoplankton Control Algal Blooms -- a Spatio-Temporal Study in a Noisy Environment." Journal of Biosciences, vol. 30, no. 5, 2005, pp. 749-60.

Paddle to Lummi 2019. "Paddle to Lummi 2019." Paddle to Lummi - July 24-28, 2019, paddletolummi.org/.

Raymond-Yakoubian, Julie, and Raychelle Daniel. "An Indigenous Approach to Ocean Planning and Policy in the Bering Strait Region of Alaska." Marine Policy, Pergamon, 10 Sept. 2018.

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