

Investigating Temporal Variation of eDNA in a Marine Environment

URSP

Student Research Support
provided by
The Edith and Lew Wasserman
Fund for Undergraduate Support

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Introduction

eDNA is increasingly used to determine the biodiversity in marine ecosystems¹. Despite recent advances, much remains to be learned about the potential biases of eDNA techniques, limiting its implementation as a biomonitoring tool. In particular, few studies have investigated eDNA degradation rates of marine fishes. In the laboratory, decay rates range from 0.5 and 7 days^{2,3}. However, these studies fail to capture *in situ* marine physical and biological conditions and thus may not provide accurate estimates of eDNA decay rates in marine environments³. In addition, little is known of how eDNA signatures of marine vertebrate diversity change over a short time period⁴.

Hypotheses

- 1) We expect the foreign eDNA signature to completely degrade after 3.5 days once it has been introduced to a coastal temperate kelp forest based on results from past studies^{2,3}.
- 2) We do not expect changes in community structure to be driven by tidal or temporal variation in a coastal temperate kelp forest because this variation has not been found in other ecosystems⁴.

Methods

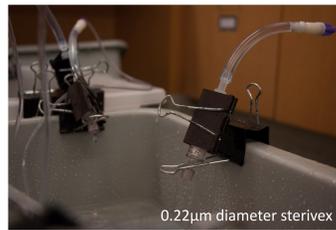
We generated and traced known concentrations of eDNA from a foreign freshwater fish species, *Ctenopharyngodon idella*. This was released off the dock at USC Wrigley Marine Science Center at 3 locations 19 meters apart. Samples collected at 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 24, 48, and 96 hours after *C. idella* was introduced. Time point 0 was collected before introduction to quantify baseline foreign DNA concentration. One 1L sample collected at A,B,C at each time point using a hand held niskin at 10m depth.



Sampling Water



Gravity Filtering the Samples



0.22µm diameter sterilix

Extraction

Qiagen DNeasy Blood and Tissue Kit with modifications for sterilix

Amplification

Universal Teleost 12S primer (MiFish)⁵ and triplicate touchdown PCR reactions

Indexing

Illumina Nextera Primers

Metabarcoding Sequencing

Illumina MiSeq sequencing

Bioinformatics

Anacapa eDNA metabarcoding Tool Kit and CRUX 12s mtDNA reference library⁶

Statistical Analysis

Exponential Fit, ANOVA, PERMANOVA on Bray Curtis Dissimilarities in R

eDNA Disappears Rapidly in Marine Environments

The eDNA signature of *C. idella* was no longer detectable after 7.5 hours after introduction (Fig. 1). However eDNA disappearance was inconsistent resulting in a discrepancy between predicted and observed *C. idella* detection rates. Additionally, further from shore locations (B,C) always had more reads of *C. idella* than the closest to shore location (A). This variation in location and time is most likely due to water movement driven by either currents or tides.

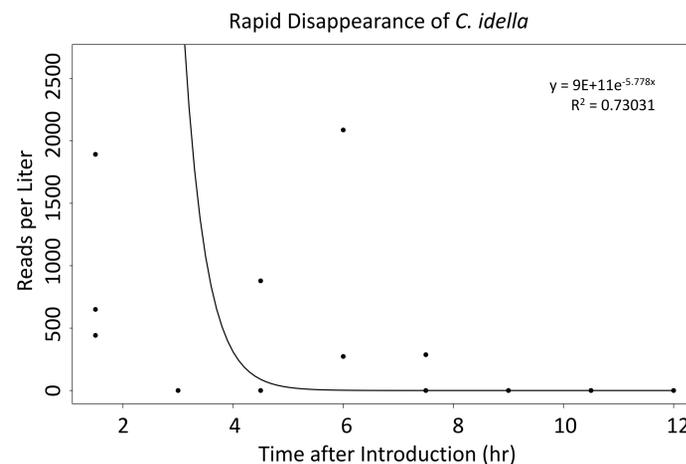


Figure 1: The number of reads of *C. idella* are over time and exponential curve fit to mean reads.

eDNA Detected a Wide Diversity of Vertebrate Species Monitored by Management Agencies

eDNA detected four classes, 36 orders, 71 families, 108 genera, and 107 vertebrate species, including 21 species monitored by National Park Service (NPS), Reef Check, or Partnership for Interdisciplinary Studies of Coastal Oceans (PISCO). eDNA detected 4 out of the 5 species that Reef Check visual surveys observed at our field site, but also detected an additional 4 monitored species that were not observed during visual surveys. Interestingly, we did not detect every monitored species or even all the most abundant species (>10,000 reads) at every time point (Fig. 2).

eDNA Recovers Temporal Variation in Kelp Forest Communities

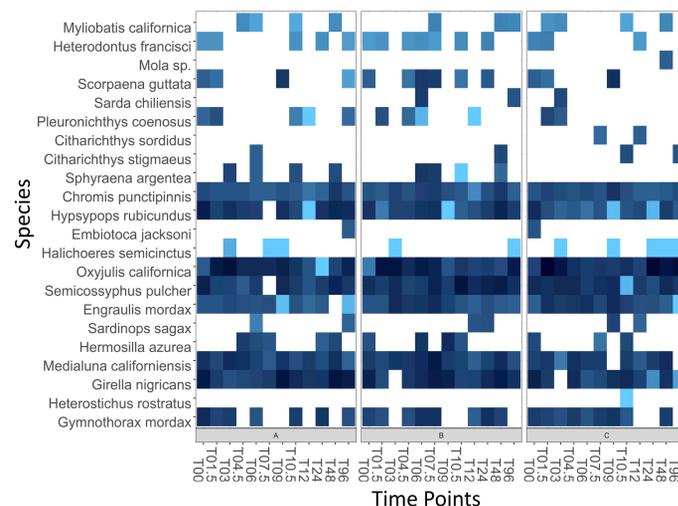
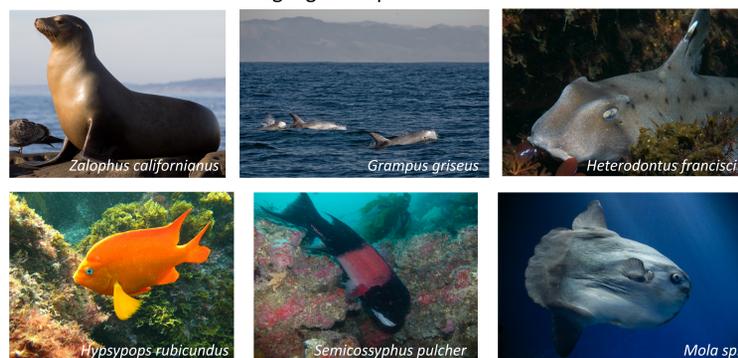


Figure 2: Heat map showing number of reads per monitored kelp forest species ordered by time point and faceted by location replicate. Darker Blue indicates more reads.

Highlight of Species Detected



Detecting Maximum Diversity Requires Multiple Samples

Species accumulation curves for all taxa did not reach saturation even after 12 sampling points, indicating increased sampling efforts are needed to capture total vertebrate diversity (Fig. 3A). However, when sampling managed species saturation is reached at 9 time points (Fig. 3B), indicating the utility of eDNA in monitoring kelp forest communities.

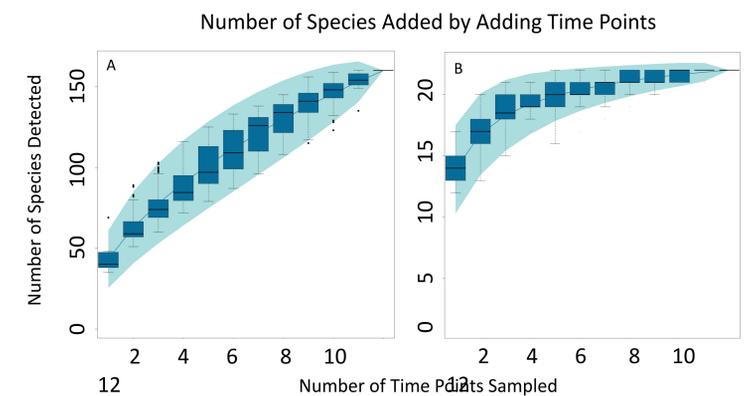


Figure 3A: Species accumulation curve considering all species detected; 3B: Species accumulation curve only considering species that are managed by Reef Check, PISCO, and NPS.

Despite variation in taxonomic diversity among samples, there were no distinct consistent patterns between communities over time (NMDS ordination plot of Bray-Curtis dissimilarities and heat map of species). The largest source of variation in community composition is unaccounted for by the 5 factors analyzed may result from PCR amplification biases⁴ (Fig. 4). Thus, we hypothesize that variation in laboratory steps has a more significant effect on recovered community structure than tidal amplitude or temporal variation.

Multiple Processes Influence Temporal Variation in Community Structure

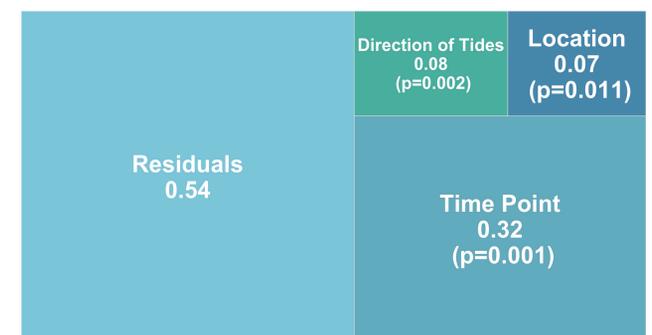


Figure 4: Percentage that each process accounts for the variation in community structure observed across the samples found from a PERMANOVA with Bray-Curtis dissimilarities

Conclusion

Our results suggest that eDNA persists for only a short time (7.5hrs) in natural marine environments, indicating that eDNA methods capture highly localized communities. Thus, effective monitoring with eDNA techniques will require multiple temporal and/or spatial replicates to detect all species within a given marine environment. Our results also suggest that three replicate samples taken over 9 time points are needed to successfully detect all monitored species within a given kelp forest, indicating the need to substantially increase current sampling efforts.

Acknowledgements

I would like to thank the Wasserman Family, URSP, and the Whitcome Research Undergraduate Summer Fellowship. I would also like to thank everyone in the Barber Lab for always helping me. Special thanks to Erick Zerecero and Onny Marwayana for helping with field collections and laboratory work.

- References
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 6. Curd et al. (2018). Anacapa: an environmental DNA toolkit for processing multilocus metabarcoding datasets. in prep.
 7. Pictures: Sea Lion (Rhododendrites), Garibaldi (stars112/John), Risso's Dolphin (Mike Baird), Horn Shark (NOAA), Mola mola (Tom Bridge), CA sheephead (Ed Bierman)

Field

Laboratory

Analysis