



JANUARY 18, 2023

**MONITORING  
BIODIVERSITY  
RETURNS OF  
KELP  
RESTORATION  
USING  
ENVIRONMENTAL  
DNA (E-DNA)**

**REPORT OF PHASE 1**

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Cite this document as: Lang-Wong A., Robinson C., Warner A., Glover R. & Drews C. (2023). Monitoring biodiversity returns of kelp restoration using environmental DNA (e-DNA). Report of phase 1. Ocean Wise, Vancouver.

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## BASELINE ASSESSMENT OF BIODIVERSITY USING E-DNA IN KELP RESTORATION TRANSECTS – AN EXPLORATION

### Background

Kelp restoration is a major pillar of Ocean Wise's Seaforestation Initiative. Ocean Wise and Canadian Kelp Resources (CKR), and Rendezvous Dive Adventures worked together to establish a kelp restoration demonstration site in Rainy Bay, Barkley Sound. Specifically, CKR produced and out-planted green gravel for three species of kelp in Rainy Bay, during the latter half of 2021. Study species include: giant kelp (*Macrocystis pyrifera*), bull kelp (*Nereocystis luetkeana*) and sugar kelp (*Saccharina latissima*). The success of this experiment will be assessed during 2022 and 2023 with a focus on understand the biodiversity benefits of kelp restoration. We used eDNA in a pilot for this purpose in Barkley Sound, Vancouver Island, Canada. We assess here the effectiveness and efficiency of this method, in comparison with diving transects to identify species composition.

### Gravel Planting & eDNA Methods

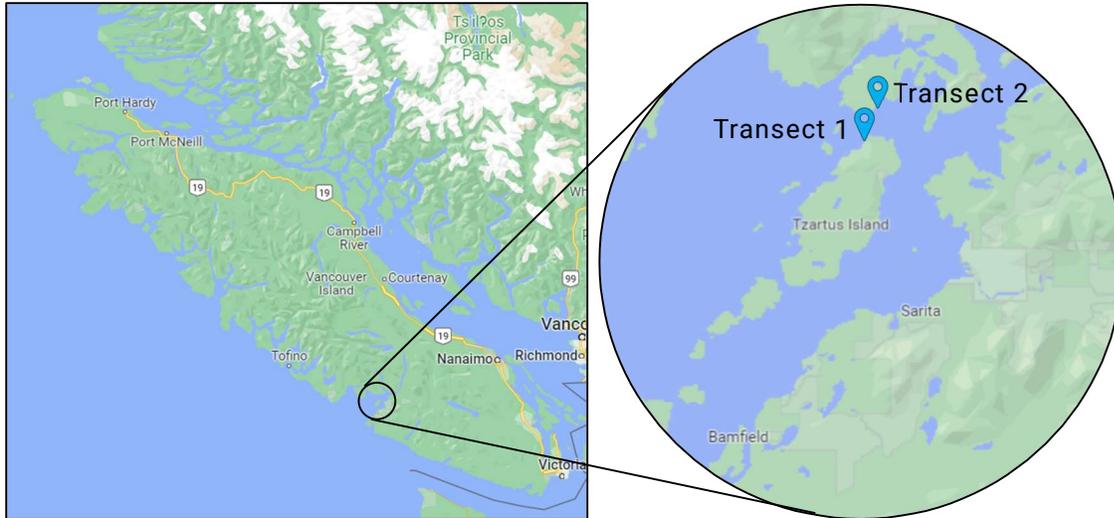
Divers from Rendezvous Dive Adventures identified suitable sites to conduct in-situ green gravel trails, selecting two transects close to Rainy Bay, British Columbia (Fig. 1) These transects (100m x 1m each) spanned multiple substrates, from large boulders to small pebbles and sandy bottomed areas. They were selected considering depth, freshwater outflow, local knowledge of the area, and convenience for monitoring. Kelp was by-and-large absent in both transects at the onset of the experiment. Green gravel was deployed between late November and early December, 2021

Transect 1 (T1) is located in a moderately sheltered shelf on Cross Point (48.952453, -125.067471). This transect was seeded with bull kelp and sugar kelp.

Transect 2 (T3) is located along the northwestern tip of Rainy Bay (48.968656, -125.047997). This transect was seeded with giant kelp and sugar kelp.

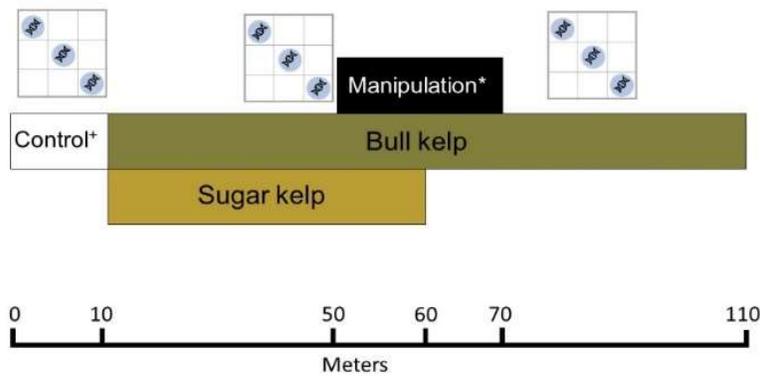
By December 2021, over 148,000 pieces of gravel seeded with bull kelp, sugar kelp and giant kelp were dispersed onto the two 100 m<sup>2</sup> transects. An additional 10 m of this transect serves as a control section, where no green gravel was deployed



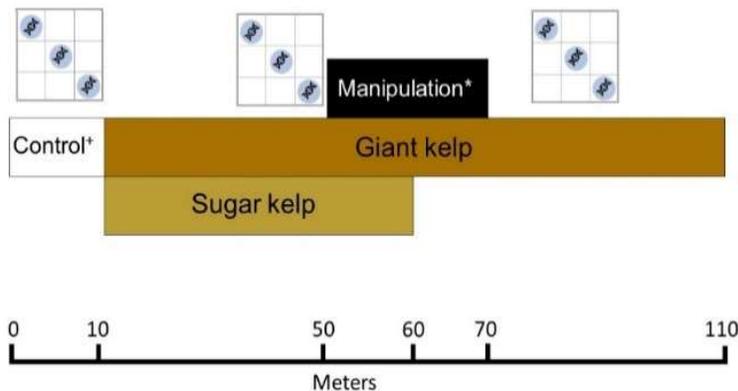


**Figure 1.** *Transect locations in Barkley Sound, British Columbia.*

Transect 1:



Transect 2:



### GREEN GRAVEL

The seeding of kelp propagules onto small rocks that are easily spread on the ocean floor is an emerging and promising technique for kelp forest restoration.

This method has gained recent attention across the world due to its cost effectiveness, scalability, and social acceptance. Ocean Wise is participating in a global knowledge sharing network through the [Green Gravel Action Group](#)

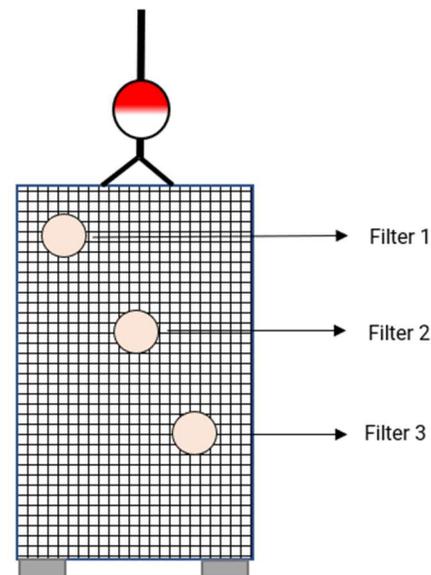
**Figure 2 & 3.** *Experimental design of green gravel transects and eDNA frame placement.*

## Generating Visual Information on Biodiversity

- On April 7<sup>th</sup>, 2022, two SCUBA divers conducted [roving diver fish counts](#) of the two transects two weeks prior to the planned eDNA sample collection
- Fish counts, invertebrate and macroalgae abundances were recorded using REEF survey denotations of S = single (1) F = few (2-10) M = many (11-100) A= abundant (100+)
- Data was compiled into a community composition matrix for each transect

## eDNA Collection

- On April 21<sup>st</sup>, 2022, three eDNA ‘frames’ were deployed per transect (transect replicates)– frames are sealed wire mesh (50cm x 150cm) containing eDNA filters (biological replicates, 47mm in diameter) - [Bessey et al., 2021](#). The led-weighted frames were positioned on the ocean floor and kept vertically by Styrofoam floats. A long rope and surface buoy allowed an easy retrieval.
- These frames were deployed on the 0, 25, and 75 meter marks along transect lines
- Frames were deployed and left for 24 hours prior to retrieval (following [Bessey et al., 2021](#))
- Filters were removed from the frames, preserved in 95% ethanol and shipped to PSEC for analysis by Ocean Wise staff



## eDNA Extraction and Analyses

- Standard eDNA extraction (following current Ocean Wise eDNA protocols)
- DNA metabarcoding of [18S gene](#) (mitochondrial DNA) to generate fish DNA sequences (total of 18 samples for sequencing)
- Use [18S FishDB database](#) to create an artificial genome containing DNA barcodes for ~6000 fish and additional marine species such as urchins, snails, and anemones
- Utilization of the EPI2ME bioinformatics pipeline to map DNA sequence reads to specific species and generate reports

## Intended Outcomes of this Approach

- Determine baseline fish and invertebrate community composition within transects containing a variety of seeded kelp beds
- Determine similarity between visual and eDNA monitoring approaches for fish community assemblages
- Investigate changes in fish and other biodiversity over time with seaforestation activities and kelp ecosystem growth
- Establish the feasibility and cost-efficiency of eDNA to monitor biodiversity returns associated to kelp restoration and cultivation.





## PRELIMINARY RESULTS

### DNA Extraction

In order to test the feasibility of using eDNA to monitor ocean biodiversity in sites prior to kelp out-planting areas – the baseline - and post planting, six eDNA filter frame deployments, with three filters each, distributed over two sites were analyzed for a total of 18 eDNA samples. Each filter had DNA extracted using current Ocean Wise protocols, involving a modified protocol for the Qiagen Blood and Tissue DNA extraction kit. DNA was then amplified at the 18s rRNA loci with the universal barcode primer MARVER3 (Valsecchi et al., 2020). This primer set is able to efficiently amplify the 18s rRNA region from most vertebrate species as well as many invertebrate species, which is close to optimal for analyzing marine eDNA samples for biodiversity. After amplifying total DNA at the 18s loci, all DNA within a given sample was tagged with a unique barcode via PCR to allow for the mixing of all amplified DNA together for DNA sequencing.

As a preliminary test, we used an Oxford Nanopore SpotON flow cell that had previously been used to genotype killer whale fecal samples. While mostly exhausted of sequencing capability it was washed with a DNase solution to destroy any remaining killer whale DNA and refresh the nanopores remaining for sequencing. Of note, any killer whale DNA remaining, if any, would not be detected using the downstream bioinformatics pipeline used as the killer whale DNA was nuclear DNA, not mitochondrial DNA. A total DNA library was pooled with all eDNA samples and loaded onto the Ocean Wise MinION MK1C DNA sequencer and run for 48 hours. This yielded ~5 million reads of ~300 bases in length across all 18 eDNA samples, giving sufficient depth of DNA sequencing to assay the quality of the eDNA sampling process and demonstrate feasibility of this method, while saving approximately \$3,500 from the initial budget for outsourcing due to sufficient data from in-house sequencing.

During data analysis of the whole sample set using the program EPI2ME (<https://github.com/nanoporetech/epi2me-api>), two recurring issues persisted across all samples – a high level of human DNA contamination and a high level of bacterial DNA from strains associated with water treatment/sewage. However, filtering out these unwanted DNA sequences allowed for in depth analysis of marine species present.



## 1. Species Detected

There were 30 species identified in the diving surveys, 10 of which (33%) were also identified in the eDNA samples (Table 1). In addition, various species of rockfish identified by divers may have shown up as “Snapper” generically in the eDNA analysis. However, an additional 10 species were detected with significant amounts of DNA which were not observed via the dive survey including: North Pacific Hake, Pacific Herring, Pacific Mackerel, Medaka (or highly related species of small brackish fish), Snapper (or highly related species of various types of rockfish), Bull Kelp (likely seen but not noted and eDNA did pick up significant amounts), Spiny Chromis Damselfish, Bullhead minnow, Catfish (or highly related species) and Large-finned Icefish (or highly related species). The total number of species detected in both diving surveys and eDNA samples was 40.

When comparing with the dive transect, ~50% of the species identified in the dive transect with mitochondrial genome data available were also identified via eDNA sequencing, with some species not possible to identify due to lack of a DNA sequence for the specific species such as Northern kelp crab and Fish-eating anemone among others.

The two methods, diving and eDNA, are complementary when the objective is to generate an inventory of species. Any one of the two methods in isolation is likely to miss a considerable number of detectable species. For relative comparisons over time and/or between locations, the method of eDNA, once simplified as recommended here, offers a cost-effective way of sampling marine biodiversity.

The breakdown of species identified is shown in Figure 1 for each transect (Transect 1 = bull kelp transect, Transect 2 = giant kelp transect), sampling site along each transect (0, 25 and 75 m), and specific filter positions. The log value of DNA read numbers is used as a way of showing the relative abundance of DNA per species. Many downstream factors may affect the levels of DNA sequenced due to the exponential nature of PCR, primer binding efficiency among others, but it reflects the relative levels of DNA sequenced for each species at each timepoint, and between each replicate.

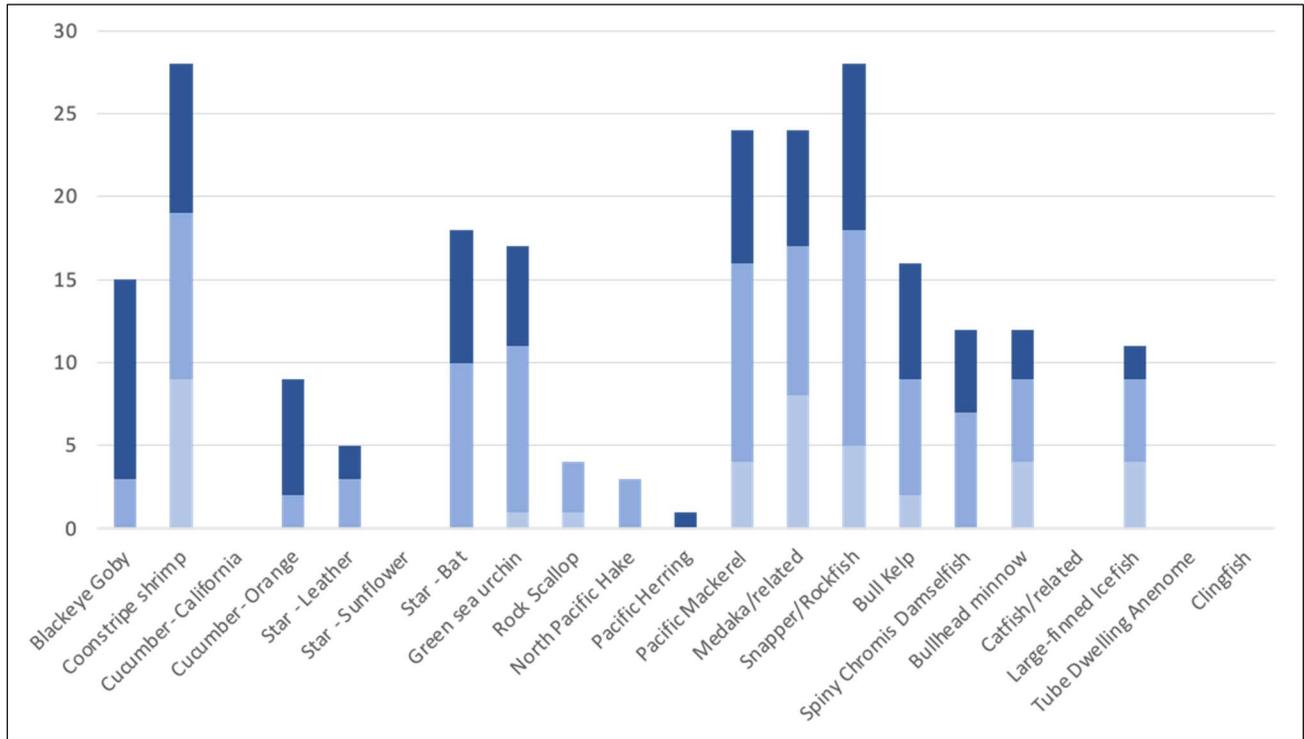


**Table 1** Species identified by dive survey and eDNA analysis. REEF survey denotations were used to record species abundance.

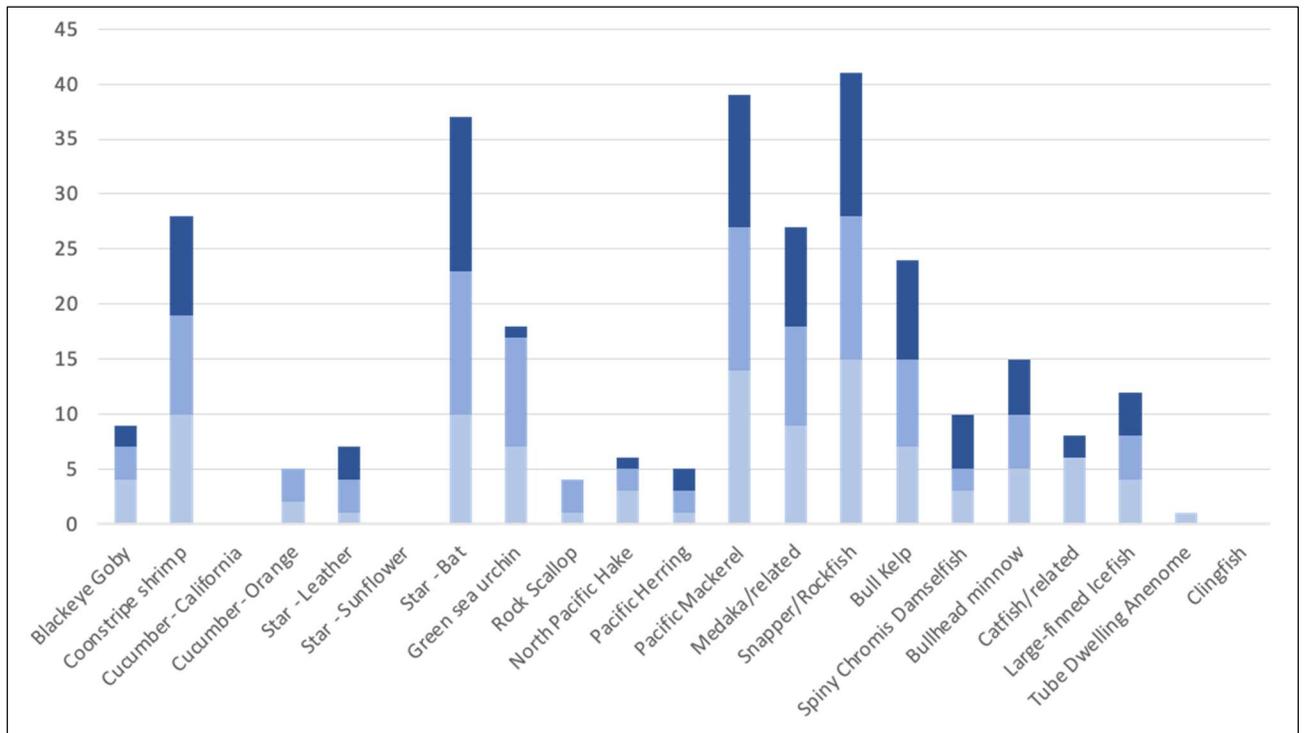
- S = single (1)
- F= few (2-10)
- M=many (11-100)
- A=abundant (>100)

Rainy Bay Survey April 7, 2022 - transect dive							
	Shallow			Deep			
Species seen	P	K	Difference	P	K	Difference	eDNA
			between			between	detected?
Kelp Greenling	F		5	C	F	8	NO
Lingcod		S		S	F	1	NO
Painted greenling		S					NO
Rockfish - Black	F	F					Possibly showing up as snapper
Rockfish - Copper	F	F					Possibly showing up as snapper
Rockfish - Quillback							Possibly showing up as snapper
Rockfish - Yellowtail	S	S					Possibly showing up as snapper
Rockfish -yoy (<5cms)		F	2				Possibly showing up as snapper
Grunt sculpin	S	S					NO
Scalyhead Sculpin		S	1				NO
Kelp Perch	F		3	C	C		NO
Shiner Perch							NO
Blackeye Goby	A	A		C	F	?	YES
Coonstripe shrimp							YES
Northern Kelp Crab	F		3	S		1	NO
Red Rock Crab	S	F	2	F	F		Not in database
Fish-eating anemone							Not in database
Tube-dwelling anemone	C	C		A	A		YES
White-spotted anemone							NO
Cucumber- California	F	F					YES
Cucumber- Orange	A	C	?				YES
Star - Leather	C	C		C	C		YES
Star - Sunflower	F	F		F	F		YES
Star - Bat	C	C		C	F	7	YES
Green sea urchin							YES
Giant nudibranch					F	3	NO
Leafy Hornmouth	F	F					NO
Northern Abalone							
Rock Scallop	F	S	1	F	S	1	YES
Turban snails	A	A		A	A		Not in database

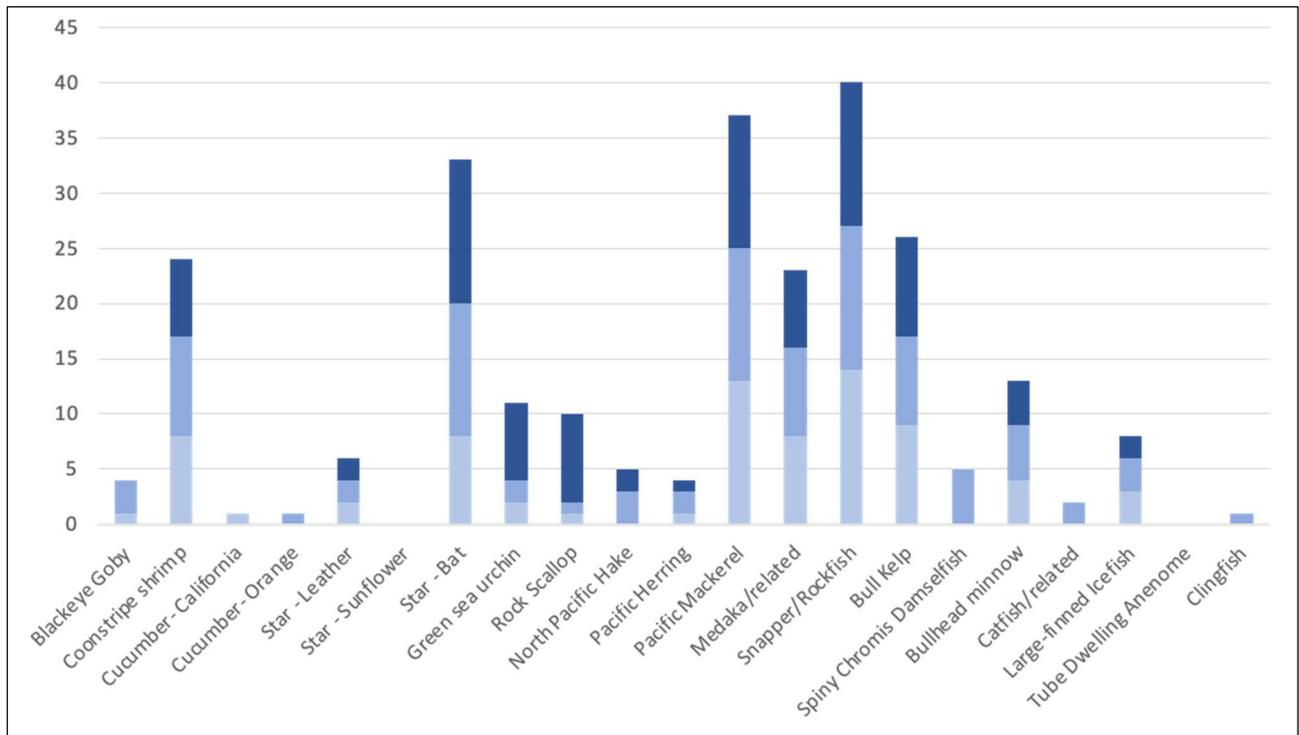
### Transect 1 at 0 meters



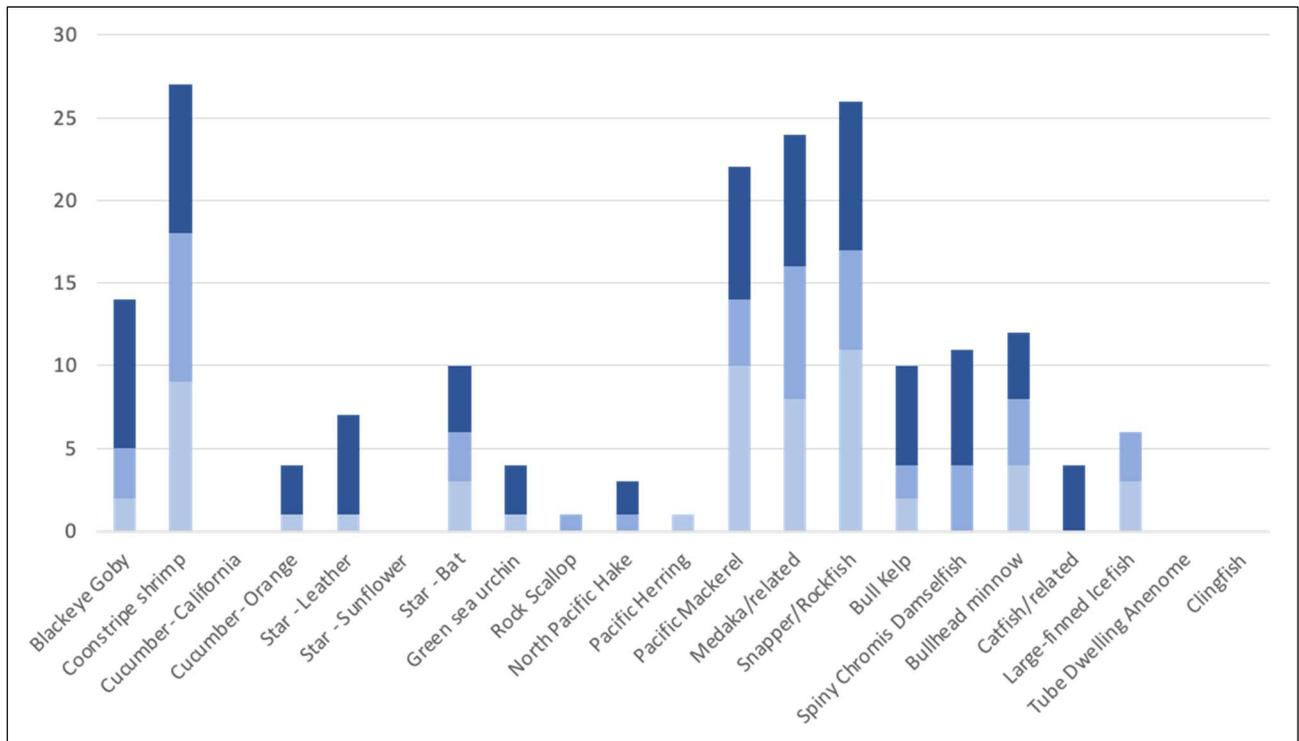
### Transect 1 at 25 meters



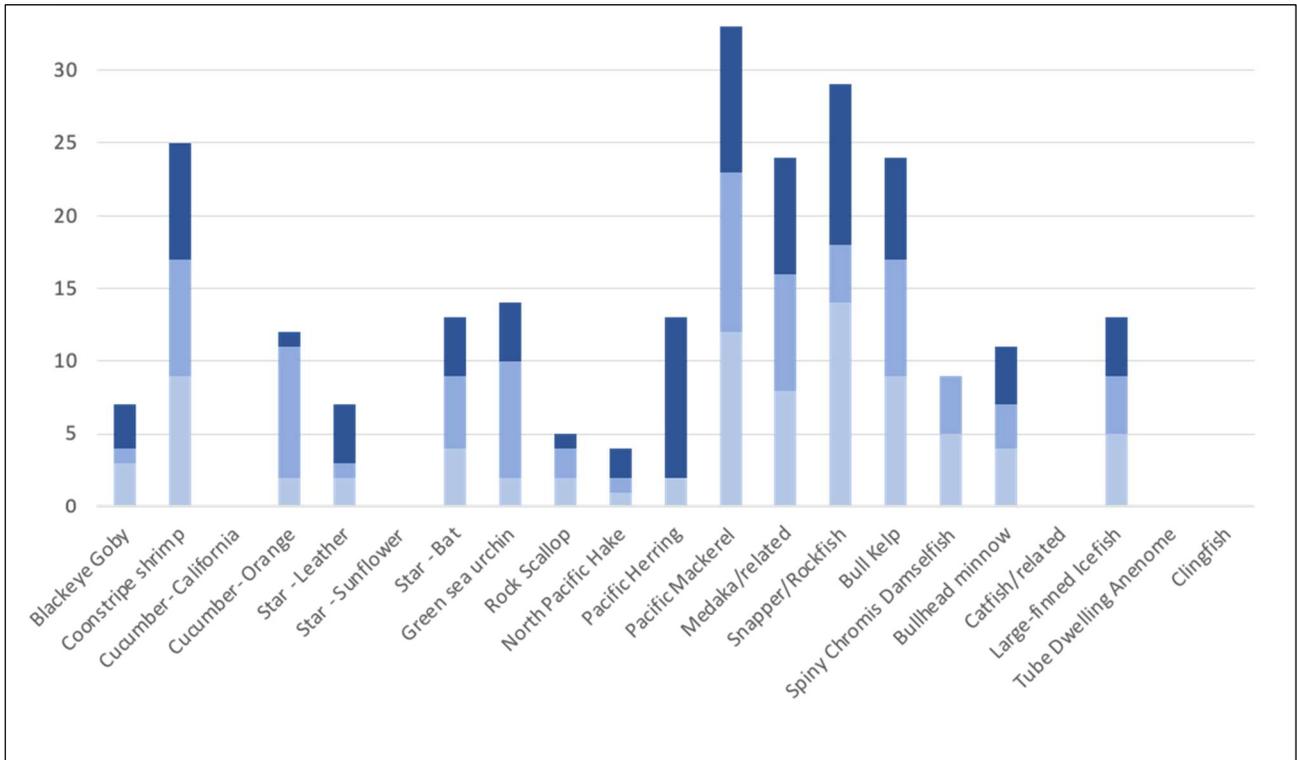
### Transect 1 at 75 meters



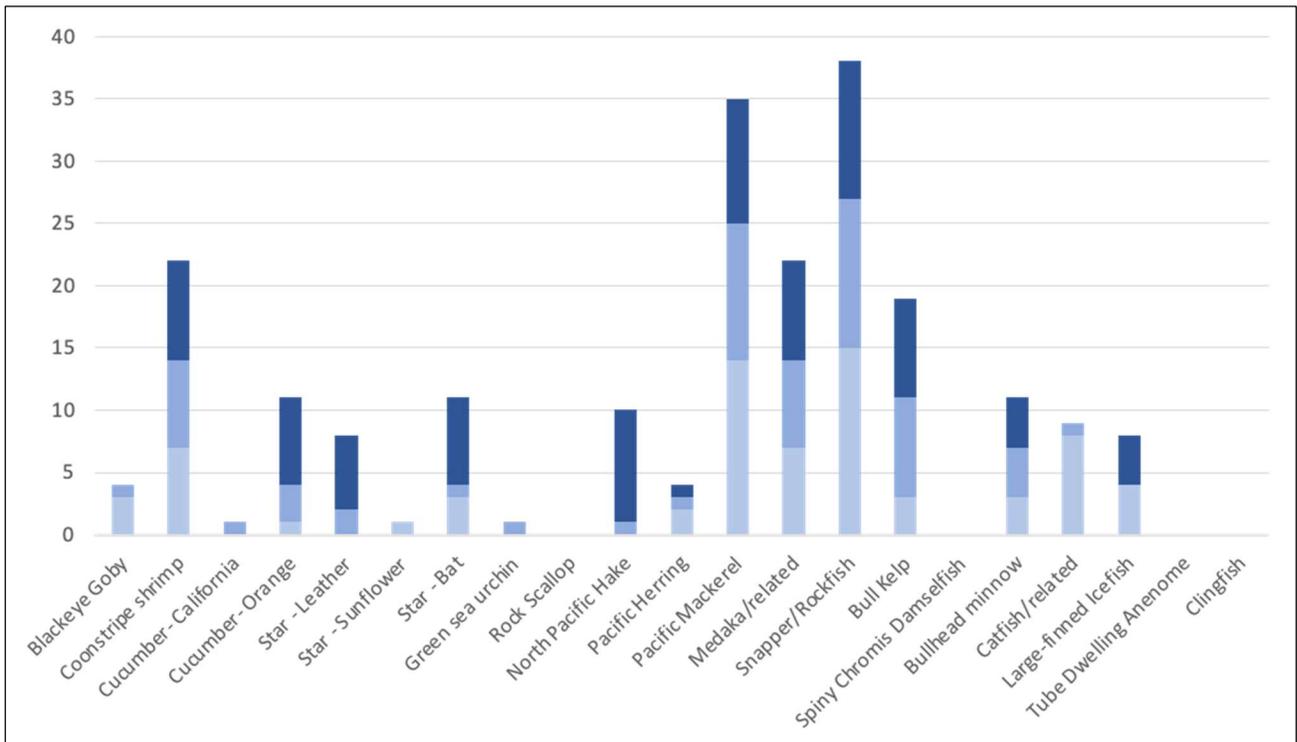
### Transect 2 at 0 meters



### Transect 2 at 25 meters



### Transect 2 at 75 meters



#### Figure 4. Species Detected on Each Transect and Filter Replicate

Species were identified and quantified from eDNA samples by creating an artificial genome using ~6000 fish species' mitochondrial DNA (Yang et al., 2020) represented as individual chromosomes, and an additional selection of species such as urchins, snails, and anemones. DNA reads were then mapped to regions of the artificial genome using the Oxford Nanopore program EPI2ME, with results archived at: <https://epi2me.nanoporetech.com/report-350933> (Username and password available upon request). Read levels were then scaled via log scale to represent relative abundance and plotted. **Light blue** represents the first filter (top position in the frame), **medium blue** represents the second filter (medium position in the frame), and **dark blue** represents the third filter (low position in the frame, closest to the ocean floor) at each site. Species are listed on the X-axis and log scale DNA sequencing read levels are represented on the Y-axis. Transects are labelled "Transect 1" (Bull kelp) and "Transect 2" (Giant kelp).

#### 2. Differences Between Sampling Sites Along the Transect

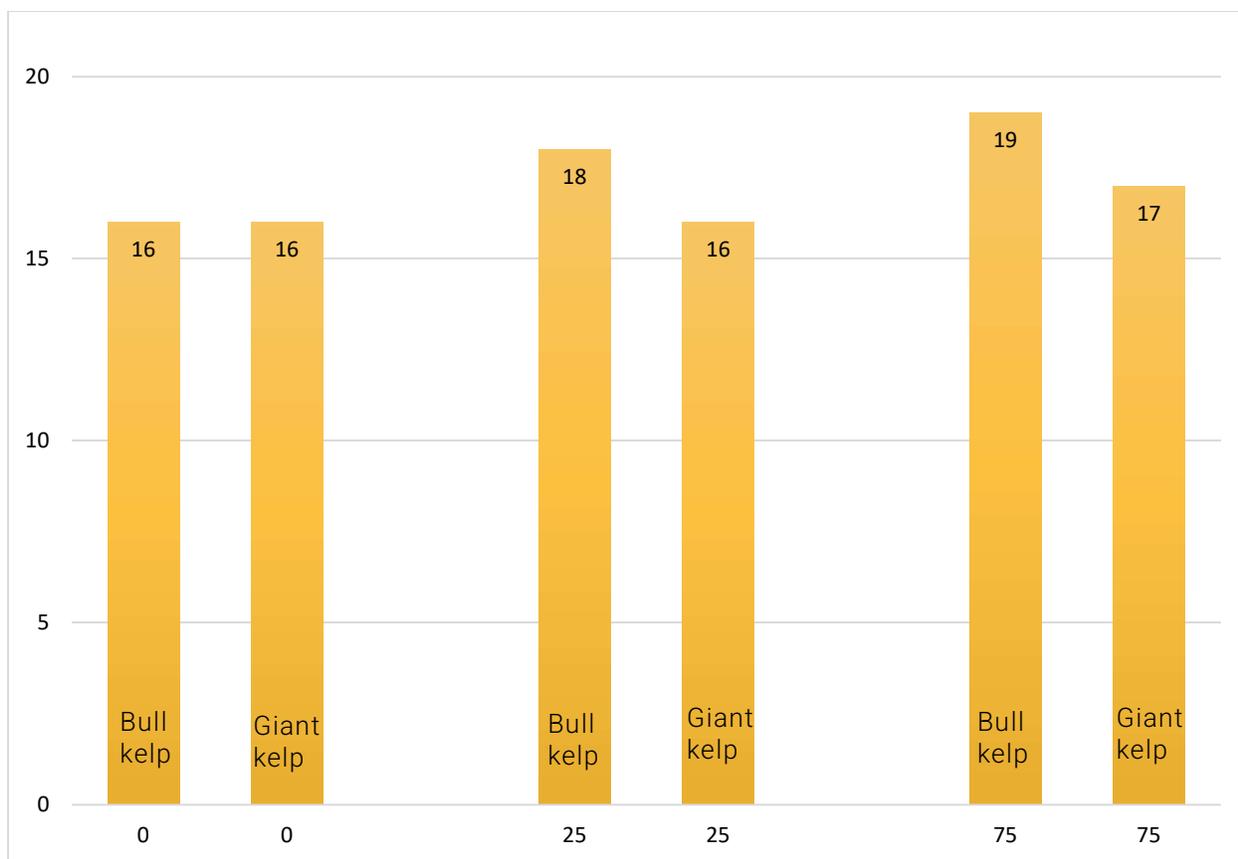
21 species were found in the full sample of three sites. 95% (max) was found in one site (75 m) and 81% (min) in site 0 m (Table 2). If we had used only one site, we would have picked up at least 81% of all species in the samples. The average number of species detected across all sites was 17 (81% of the total number of species in the sample).

**Conclusion** Using one site only – centrally located along the transect – seems sufficient to get a representative list of species for comparative purposes.

#### 3. Differences Between Two Transects

21 species were found in the full sample of three sites per transect, on two transects (Fig. 2). There seems to be no significant difference between transects 1 (Bull kelp) and transect 2 (Giant kelp) with respect to the overall yield in species at the time of taking this baseline when kelp was by-and-large absent, with 19 species in the Bull kelp transect and 17 species in the Giant kelp transect. There were no salient differences in species composition between the two transects.





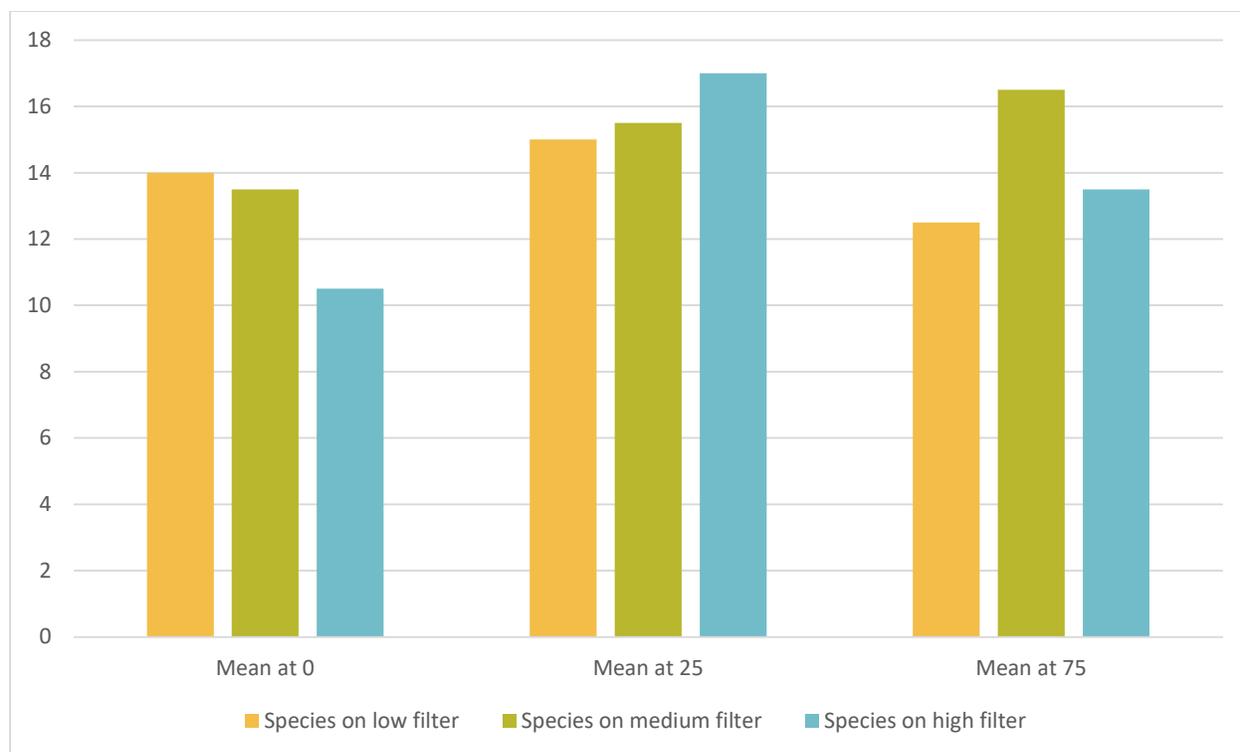
**Figure 5.** Number of species detected in eDNA samples by transect (Bull kelp, Giant kelp) and site along the transect (0, 25 and 75 m)

#### 4. Differences Between Three Filter Positions on the Sampling Frame

In general, there was good concordance between filter replicates at each site. No single filter position stands out above the others in terms of number of species detected. The highest number detected in site 0 was in the dark filter (closest to the ocean sediment), whereas in site 25 it was in the light filter and in site 75 in the medium filter. Sampling sites (0, 25 and 75m) were pooled across transects to calculate means (Fig. 3).

Conclusion: Using one filter position per sampling grid – centrally located on the vertical sampling frame – seems sufficient to get a representative list of species for comparative purposes.





**Figure 6.** Mean number of species detected by position of the eDNA filter on the sampling frame. Low filter is the one closest to the ocean sediment.

## 5. Effect of Number of Filters Per Sampling Grid on Species Detection

21 species were found in the full sample of three sites. A mean of 10.8 (51%) species were found in all and each of the three filters. Having three filters increased by almost twice the probability of detecting the totality of species of the sample.

**Conclusion** The total collective area of three filters is much better at detecting more species than the surface area of one single of the filters deployed. Taking all the results of this analysis together, it is recommended to use one larger filter, placed centrally on the sampling grid device and deployed in the center of the transect. This will reduce significantly the costs of the analysis.

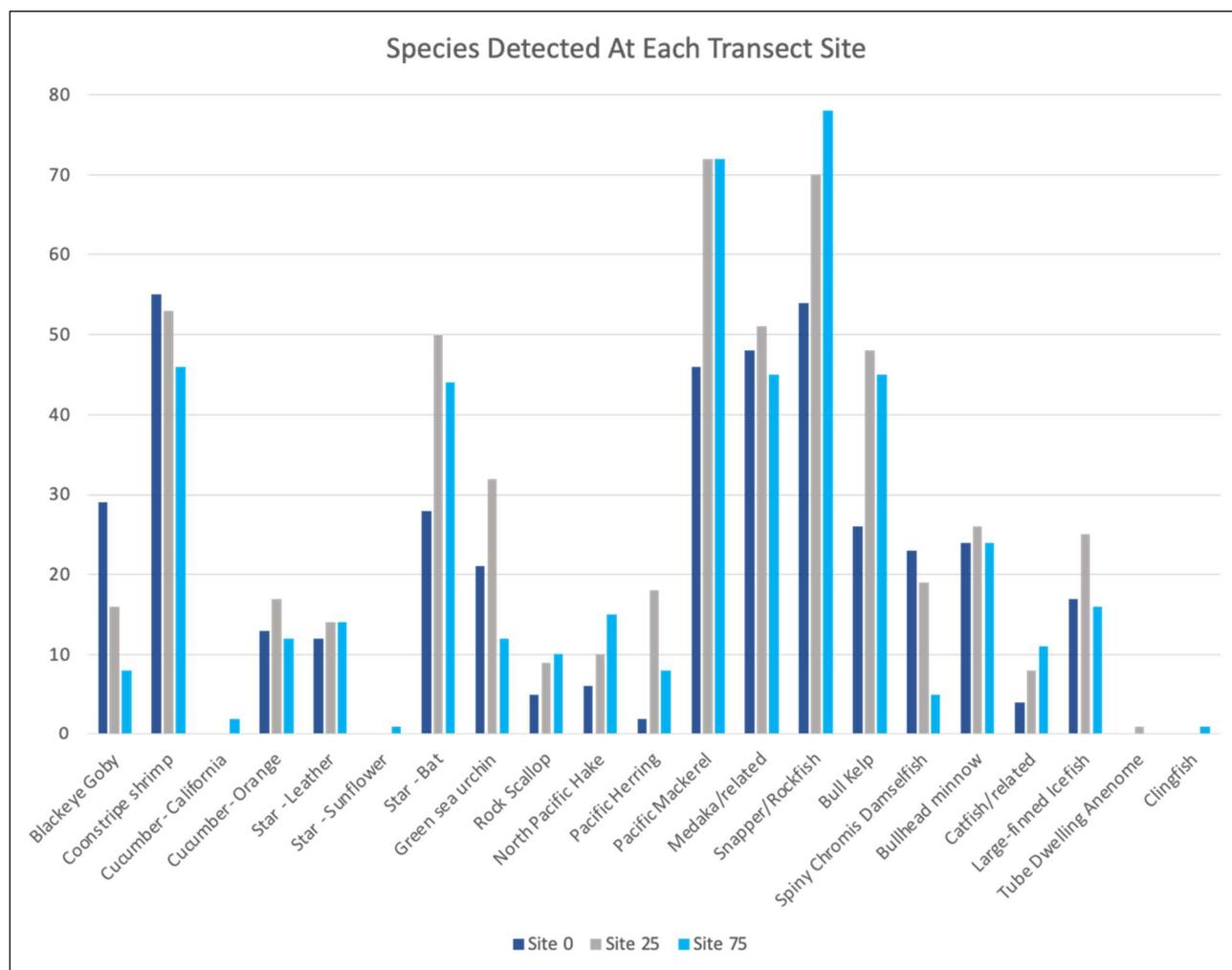
Site	Transect	Species on 1 filter	Species on 2 filters	Species on 3 filters	Species missed	Total species on sample	Total Species present	% Species detected
0	Bull	2	6	8	5	16	21	76
	Giant	3	5	8	4	16	21	76
<b>Mean at 0</b>		<b>2.5</b>	<b>5.5</b>	<b>8</b>	<b>4.5</b>	<b>16</b>	<b>21</b>	<b>76</b>
25	Bull	1	3	14	3	18	21	86
	Giant	0	2	14	4	16	21	76
<b>Mean at 25</b>		<b>0.5</b>	<b>2.5</b>	<b>14</b>	<b>3.5</b>	<b>17</b>	<b>21</b>	<b>81</b>



75	Bull	5	2	12	2	19	21	90
75	Giant	3	5	9	4	17	21	81
Mean at 75		4	3.5	10.5	3	18	21	86
Mean across all sites		2.3	3.8	10.8	3.7	17.0	21	81

**Table 2.** Number of species concurrently detected in 1, 2 and 3 filters, by site of sampling along the transects (0, 25 and 75 m).

In Figure 7, the species detected, and relative abundance are shown in a comparison across transect sites, which shows some variation in the species detected as well as their abundance.



**Figure 7** Species detected at each transect site. Species identified at each transect site are displayed, with species listed on the X-axis and log scale DNA sequencing read levels represented on the Y-axis. Sites (0, 25 and 75 m) were pooled across the two transects.





## FUTURE DIRECTIONS

### **Future Directions**

This methodology has clearly shown to be promising for the detection of species either alongside dive analysis or on its own. To improve the process, reducing human DNA contamination during the filter deployment and collection process is essential. By doing so this will increase the magnitude of sequencing resources going towards identifying species rather than background noise. In addition, minor improvements/additions to the DNA database used to compare DNA sequences obtained to known sequences would be beneficial. This would increase the sensitivity of the process as well as provide greater confidence in results for species of low abundance and likely reannotate some of the species identified as a closely related species. Lastly, the use of Illumina sequencing at higher depth than what was obtained via Nanopore DNA sequencing would be beneficial for future sample sets. For this initial run, Nanopore sequencing was cost-effective and efficient at obtaining good breadth of species present, but once the eDNA sampling protocol is optimized, the use of Illumina sequencing with higher accuracy and only slightly higher cost would increase the identification of lower abundance species.

In terms of in-water methodological adjustments, we recommend deploying a single frame on a central location on each 100m transect (instead of three). The number of filter replicates per frame should be reduced to a single filter of at least three times the size (surface area) of the currently used filters. This should be placed centrally on the sampling frame. This should reduce substantively the costs of analysis.



**Project Design**

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**Field Deployment**

Andrew Lang Wong, Carlos Drews

**Laboratory Analysis**

Robin Glover, Adam Warner, PhD

**Bioinformatic Analysis**

Adam Warner, PhD, Robin Glover

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