PNNL-32310

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Michelle Fu
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Prepared for the U.S. Department of Energy
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Pacific Northwest National Laboratory
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Summary

The installation of marine energy systems may affect marine environments, and by extension, marine fish communities. Therefore, biomonitoring is an integral part of assessing impacts on species. Environmental DNA (eDNA) provides a noninvasive alternative to conventional monitoring surveys and the possibility of a more accurate assessment of species richness. Yet, its cost efficiency compared to traditional methods of monitoring is relatively unknown, especially when applied to monitoring around tidal, wave, and offshore wind energy installations.

For this study, 202 peer-reviewed journal articles were dissected to inventory the diversity of supplies used for collecting and processing eDNA samples and to compile the average cost of eDNA surveys. Information collected included the type, volume, and brand of containers used in sampling; material, size, and brand of filters; and extraction methods. Cost information was gathered for the most common supplies, and a total cost was estimated for a hypothetical eDNA survey in Sequim Bay, WA, to compare with traditional methods of surveying such as beach seining and scuba surveys.

The results showed a higher-than-expected diversity of supplies to collect and process eDNA samples. The most common supplies were 1 L Nalgene bottles at an average cost of 7.96 USD for collecting samples, 0.45 µm glass fiber Merck Millipore filters at an average cost of 1.51 USD for filtering samples, and the Qiagen DNeasy Blood and Tissue kit at 3.54 USD per sample for extracting DNA. When compared to beach seine and scuba surveys, eDNA surveys undertaken by senior researchers are less expensive for both initial surveys with all new materials as well as for follow-up surveys reusing some of the supplies. However, when surveys are done solely by students, eDNA surveys are more expensive than scuba surveys when no prior supplies are available and more than both beach seine and scuba surveys for follow-up surveys reusing supplies.

In a professional sphere, where surveys are less often conducted by teams of students only, eDNA surveys are an effective and less-costly alternative to conventional methods. We anticipate that the development and refinement of eDNA methodology will continue to decrease surveying costs.

Acknowledgments

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1.0 Introduction

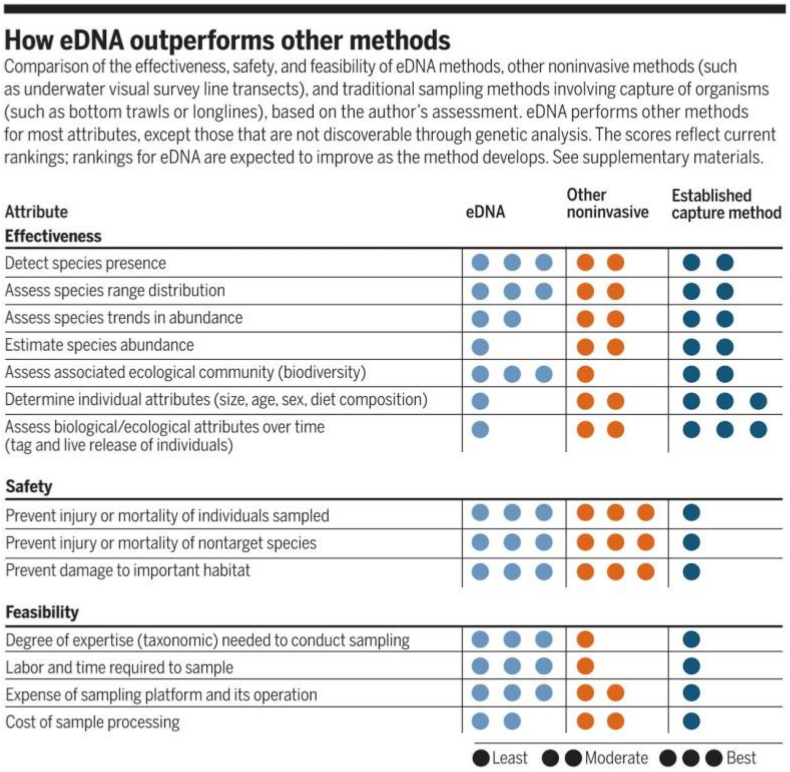
The health of our marine systems dictates the quality of many integral ecosystem services such as fishing and tourism (Pimm et al., 2014). Biodiversity indicators are often used as proxies for the health of an ecosystem (Helfman, 2007). Therefore, precise and efficient monitoring of aquatic biodiversity is essential for ecosystem management and the sustainable use of marine resources, as well as ecological conservation. Traditional monitoring methods for aquatic biodiversity are often invasive and ecologically destructive (e.g., trawls) or demand considerable manpower and resources (Bayley and Peterson, 2001; Jones, 1992). Furthermore, given the vast spatial scale of aquatic habitats such as oceans, accurate assessments of aggregate biodiversity are virtually impossible.

Marine energy is generated through harnessing hydrokinetic energy from ocean currents, tides, or wave motion using various turbines and energy converters. The energy is cleaner than conventional fossil fuel energy sources. Deployments have already occurred in the United States (U.S.), such as a turbine in the tidal strait of Long Island Sound in New York (Roosevelt Island Tidal Energy), an instream river turbine to power a remote Alaskan community (RivGen), and various technology tests at the Wave Energy Test Site in Hawaii and at the PacWave North Test Site in Newport, OR. Despite these deployments, the potential effects of the installation and operation of marine energy devices on marine ecosystems is not yet fully understood. According to the 2020 State of the Science Report by Ocean Energy Systems – Environmental (Copping and Hemery, 2020), marine energy deployments can affect marine ecosystems in the following ways: the production of underwater noise that could harm hearing abilities of marine mammals and fish, the emission of electromagnetic fields by export cables, the risk of animal collision with moving turbine blades, changes in water quality and other oceanographic systems, changes in benthic and pelagic habitats, and the risk of marine animals getting entangled in mooring lines. However, marine energy projects with single devices or small numbers of devices are unlikely to cause harm to marine animals, to significantly alter habitats on the seafloor or in the water column, or to modify oceanographic processes (Copping and Hemery, 2020; Copping et al., 2020). Ultimately, however, the report often cites the need for further monitoring actions to gauge possible deleterious effects.

Environmental DNA (or eDNA) metabarcoding consists of using discarded genetic material in the form of scales, skin, blood, etc. to simultaneously determine a broad range of taxonomic groups in an ecosystem (Rees et al., 2014). eDNA surveys broadly consist of three steps: sampling, filtering, and extracting DNA. Sampling is done by collecting an environmental sample (e.g., water) using a container (such as a vial or bottle). Sampling can be performed in a variety of environments, such as but not limited to lakes, streams, marine environments, coral reefs, off a pier, from a boat, etc. The collected water is filtered to concentrate the biological material. Lastly, the DNA present in the biological materials is extracted using extraction kits.

eDNA could provide an alternative approach to conventional survey methods for monitoring aquatic biodiversity. Indeed, previous studies indicate that eDNA provides a higher resolution for detecting species than conventional methods such as dip netting (Ruso et al., 2019) and electrofishing (McColl-Gausden et al., 2020). Furthermore, as shown in Figure 1, eDNA outperforms traditional sampling methods in terms of non-invasiveness and decreased dependence on taxonomic expertise while simultaneously increasing sensitivity to species detection (Pikitch et al., 2018). Indeed, a study using volunteers to collect eDNA samples to assess the presence of great-crested newts yielded a 99.3% detection rate, higher than rates found through traditional bottle traps (Biggs et al., 2015). Both studies by Biggs et al. (2015) and

Pikitch et al. (2018) conclude that eDNA surveying is less expensive than traditional surveying, with Figure 1 showing that eDNA surveying provides the best cost efficiency in terms of labor as well as operation.



How eDNA outperforms other methods

Comparison of the effectiveness, safety, and feasibility of eDNA methods, other noninvasive methods (such as underwater visual survey line transects), and traditional sampling methods involving capture of organisms (such as bottom trawls or longlines), based on the author's assessment. eDNA performs other methods for most attributes, except those that are not discoverable through genetic analysis. The scores reflect current rankings; rankings for eDNA are expected to improve as the method develops. See supplementary materials.

Attribute	eDNA	Other noninvasive	Established capture method
Effectiveness			
Detect species presence	●●●	●●	●●
Assess species range distribution	●●●	●●	●●
Assess species trends in abundance	●●	●●	●●
Estimate species abundance	●	●●	●●
Assess associated ecological community (biodiversity)	●●●	●	●●
Determine individual attributes (size, age, sex, diet composition)	●	●●	●●●
Assess biological/ecological attributes over time (tag and live release of individuals)	●	●●	●●●
Safety			
Prevent injury or mortality of individuals sampled	●●●	●●●	●
Prevent injury or mortality of nontarget species	●●●	●●●	●
Prevent damage to important habitat	●●●	●●●	●
Feasibility			
Degree of expertise (taxonomic) needed to conduct sampling	●●●	●	●
Labor and time required to sample	●●●	●	●
Expense of sampling platform and its operation	●●●	●●	●
Cost of sample processing	●●	●●	●

● Least ●● Moderate ●●● Best

Figure 1. Comparison of eDNA surveys with other noninvasive survey methods and established capture methods (Pikitch et al., 2018).

Two common conventional monitoring methods are beach seine surveying and visual surveys conducted by scuba divers. Beach seine surveying involves deploying a net at a coastal site to trap fish. Once collected, the fish are placed into buckets where researchers identify the organisms to the lowest taxonomic level possible by staff with advance training and expertise in species identification. Beach seine surveys can only be undertaken in favorable conditions; that is, when currents and wave conditions support effective and safe deployment and retrieval of the net. Beach seine surveys have, in the past, found comparable diversity when compared to eDNA surveys; however, beach seine surveys take substantial time to set up compared to eDNA surveys, which mainly consist of collecting water samples (Shelton et al., 2019). Furthermore, beach seine surveys have documented limitations, such as smaller and faster species evading capture, as well as how the short duration of sampling provides a limited snapshot of community assemblages (Bayley and Herendeen, 2000).

Scuba surveying is a type of visual survey where scuba divers identify and count the organisms that they can see while swimming along a transect. Similarly, scuba surveys, and visual surveys methods as a whole, either perform on-par or worse than eDNA surveying while taking considerably more manpower and resources, as well as being limited to favorable conditions such as low wave intensity and high visibility (Fernandez et al., 2020). Like beach seine surveys, trained and experienced staff are necessary to identify species to the lowest taxonomic level of footage recorded by scuba divers. Scuba surveys are also limited in spatiotemporal scale because there is a physical limit to the distance that can be covered by scuba divers. eDNA can overcomes the issues presented by beach seine and scuba surveys because eDNA sampling does not have the same limitations. In addition, because the biological material needed for eDNA analysis remains in the water for some time, the spatiotemporal scale of eDNA sampling is larger and more indicative of the ecosystem assemblage present (Beng and Corleet, 2020).

As shown above, several studies have compared the efficacy of eDNA over traditional survey methods. Yet, there has been little research that compares the cost of carrying out eDNA surveys vs. beach seine and scuba surveys. Assessment of whether eDNA surveys are more or less costly than traditional survey methods would help entities assigned with surveying and monitoring potential effects of marine energy projects on local biodiversity decide which methodology would be best for their project. Thus, the intent of our study was to thoroughly assess the cost of carrying out an eDNA survey, then compare that cost to two common traditional surveying methods (beach seine and scuba surveys). We conducted a literature review of eDNA surveys in aquatic environments, documented the most common supplies used in the three initial steps associated with eDNA surveys (i.e., sampling, filtering, and extraction), and identified the cost of these supplies. To compare the total costs of undertaking eDNA, beach seine, and scuba surveys, we set up hypothetical fish surveys in Sequim Bay, WA, and listed all the costs associated with these surveys.

2.0 Methods

The cost analysis was carried out in three successive phases: (1) a thorough literature review to compile an exhaustive list of supplies used for eDNA surveys; (2) an assessment of the cost of each supply; and (3) a cost comparison of a hypothetical eDNA fish survey with two hypothetical fish surveys using conventional methods

2.1 Literature Review

The goal of this literature review was to gather a representative sample of journal articles describing eDNA surveys in freshwater and marine environments.

2.1.1 Sources of Literature

Peer-reviewed articles detailing eDNA surveys in marine and riverine ecosystems were selected from generalist scientific journals, such as *PLoS ONE* and *Scientific Reports*, and additional articles were derived from online searches in knowledge bases. To conduct a thorough search of relevant scholarly articles, several keywords were used across the academic search engines Web of Science, Google Scholar, and Wileyplus. Keyword combinations included: “environmental DNA”, “environmental DNA + freshwater”, “environmental DNA + marine” and “environmental DNA + aquatic”. All searches were repeated using “eDNA” instead of “environmental DNA”. Furthermore, all articles from the 12 issues of the open-access journal *Environmental DNA* available as of July 2021 were also evaluated because of the journal’s direct interest in the field. Only studies assessing biodiversity in aquatic environments (e.g., streams, lakes, pelagic environments, coastal environments, coral reefs, etc.) were retained. All scholarly articles that included a description of the methodology used in eDNA surveying were also included in the analysis.

2.1.2 Extraction of Data from Literature

Once the relevant literature was identified, 11 categories were created to organize the information extracted from each article (Table 1). For the purpose of this review, eDNA surveys were broken down into three general steps: sampling, filtering, and extraction. While some categories were created for identification purposes and only contained metadata (such as the name of a paper or citation), those that were used for analyzing the cost of the eDNA process contained data related to supplies necessary for the sampling (e.g., deployment method, type of container), filtering (such as filter type and pore size), and extraction steps.

After the information was extracted from the literature, entries were sorted to compile a list of supplies as exhaustive as the data allowed, and proportions were calculated to identify the most commonly used methods and supplies.

Table 1. Information extracted from the literature.

Category		Description
Metadata	PDF name	Name of the document for internal purpose
	Citation	Journal citation with author names, article title, and publication information
Sampling	Deployment method	Whether sampling was conducted on the shore, with a boat, or by some other method of reaching the sampling site
	Type of container	The brand and type of sampling container used, as well as the volume of the container
	Reused supply	Whether the sampling containers and other supplies were used for multiple sampling events or not
Filtering	Filter brand	The brand of the filter
	Type of filter	The material of filter used (cellulose nitrate, etc.)
	Peristaltic pump	Whether peristaltic pumps were used to pump water directly through a filter or not
	Filter size (mm)	The overall size of each filter
	Pore size (µm)	The pore size of each filter
Extraction	Extraction kit	The DNA extraction method used

2.2 Cost Review

Cost was researched for each supply identified in the literature review. This step included type and brand of containers for the sampling category, type (i.e., material, filter size, and pore size) and brand of filters for the filtering category, and extraction kits for the extraction category. Several sources were used to search for supply cost to compute an average cost when possible, including manufacturer websites (e.g., Masterflex, Qiagen), laboratory supply providers (e.g., Millipore Sigma, Thermo Fisher Scientific, Thomas Scientific), or non-specialized websites (e.g., Amazon). Supplies had to be omitted from the analysis when their cost was not publicly available, or if the item was no longer in production. Several items (e.g., sampling bottles, filter cups) are sold in large quantities, and a cost per unit was obtained from averaging across packs of various sizes. The average cost for the most common supply was identified for each category. Some supplies, such as water samplers and collection bottles, can be reused multiple times as long as steps are taken to avoid contamination between samples (e.g., sterilization), which decreases the overall cost. Whether containers were being reused or discarded between sampling events was noted for each study.

2.3 Cost Comparison with Conventional Survey Methods

To compare the costs of surveying marine organisms using either eDNA methods, a beach seine, or scuba diver video transects, a hypothetical case study was designed to survey fish communities in the nearshore habitat of Sequim Bay, WA, where no such direct and standardized comparison has been undertaken between the three methods yet. Sampling conditions in Sequim Bay can be challenging for scuba divers, with some areas of strong current and, in the spring and summer, heavy phytoplankton blooms that drastically decrease visibility, thus hindering visual surveys by divers. Furthermore, Sequim Bay is surrounded by high, unstable bluffs and a narrow shoreline, which makes sampling with a seine challenging at some locations and during high tidal conditions. Using eDNA for fish surveys provides an alternative to beach seine or scuba surveys in this area.

The hypothetical surveys assumed four sites where data were collected over the course of a single day during daylight hours. The eDNA survey considered two staff (one boat operator and one water sampler), the scuba survey included three staff (one boat operator and two scuba divers), and the beach seine survey included four staff (one boat operator and three field biologists to deploy the seine and process the catch). Parameters considered were cost of supplies and labor costs to obtain necessary environmental permits, collect data in the field, and process and analyze field-collected data (including by potential subcontractors). The data processing and analyzing portion only included steps to convert raw, field-collected data (with quality control checks) and did not include statistical analyses. Labor costs incurred to projects (i.e., including overhead and other related costs) were calculated based on a nationwide average of undergraduate student hourly rate ($20/hour) and a senior researcher hourly rate ($150/hour).

Supplies considered for each type of the hypothetical survey are outlined in Table 2. The supply list for the eDNA survey was compiled from the literature review by selecting the most commonly used item for each supply category. Cost of eDNA supplies were identified during the cost review step. Supply list and cost for the hypothetical beach seine and scuba surveys were provided by subject matter experts.

Table 2. Materials used for each type of survey.

Beach Seine Survey	Scuba Survey	eDNA Survey
Bucket	Transect tape	Sampling container
Bucket aerator	Underwater video camera	Filter
Aquarium net	Air tank fill	Extraction/sequencing
Measuring board	Permitting labor cost	Permitting labor cost
Beach seine	Sampling labor cost	Sampling labor cost
Permitting labor cost	Video review labor cost	Filtering labor cost
Permit fees		
Sampling labor cost		
Data processing labor cost		

In this cost comparison, it was assumed that the divers and beach seiners provided their own gear (e.g., waders, boots, dry suits), so this cost was not factored into the analysis. In addition, costs associated with vessel use and operation (moorage, maintenance, gasoline) were not included.

3.0 Results

We evaluated the cost differences between hypothetical eDNA, beach seine, and scuba surveys. We first identified the cost of most common supplies and methods used for each step of eDNA surveying (i.e., sampling, filtering, and extraction), then compared this cost to those of conducting beach seine and scuba surveys.

3.1 Literature and Cost Reviews

A total of 202 scholarly articles was found, published between 2010 and 2021 in 50 different journals (Supplementary Material 1). The literature consisted of studies carried out on all continents except Antarctica and Africa. A range of aquatic ecosystems were surveyed—freshwater systems from lakes to streams, and saltwater environments from marshes to the open ocean and deep-sea ecosystems. Target species in these studies included amphibians, mammals, fish, coral, and other invertebrates.

While some articles did not provide much detail regarding the methods and supplies used for the sampling, filtering, and/or extraction steps, others thoroughly described their methods, and some used different items for conducting the same steps of their experiment. For example, the literature review of 202 articles found 221 entries for the sampling container type. Some articles merely described the methodologies of eDNA surveying and did not focus on a description of the actual experiment. This resulted in less entries; for example, there were 188 entries for the filter type. Furthermore, because some supplies (such as outdated water samplers) may not be available for purchase, these were also excluded from our analysis.

3.1.1 Sampling

The literature review identified three deployment methods for collecting eDNA water samples: boat, shore, and paddleboard sampling (Figure 2, Table 3). 35 studies (17%) did not mention any deployment method. Sampling using a boat and without using a boat (sampling from shore) were equally prevalent in the literature (85 studies each, 41%).

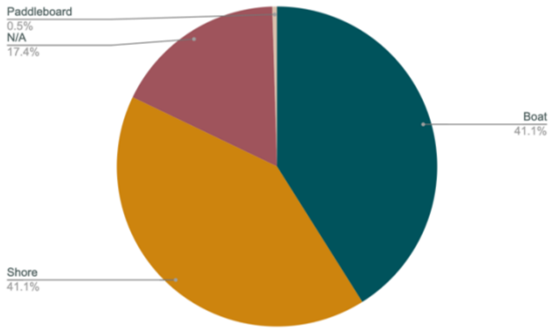


Figure 2. Proportions of methods of deployment.

Table 3. Methods of deployment. Counts denote the number of studies reviewed that reported using the corresponding deployment type.

Method of Deployment	Count
Boat	85
Shore	85
Paddleboard	1
N/A	36

As shown in Figure 3, the most common water collection method was with a bottle of an unspecified brand, with a total of 49 articles (23.8%) mentioning sampling with this method. The second most common method of sampling was with a container of an unspecified brand (12.6%). The third most common method of collection, a Nalgene bottle (12.1%), was used to determine the cost of the most commonly used sampling container. Table 4 provides the full list of collection methods identified in the literature review.

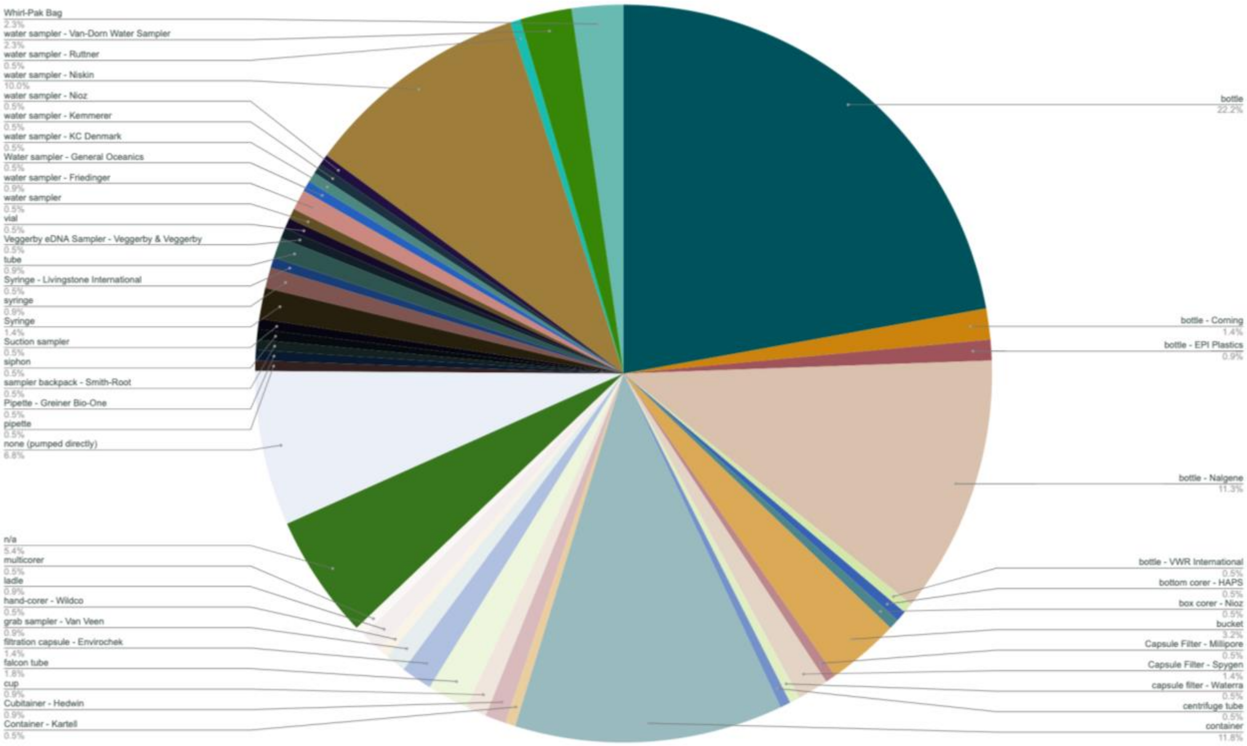


Figure 3. Proportions of types of collection methods.

Nalgene bottles come in different volumes, and determining the most common volume was important because prices vary widely. The most common volume used for sampling was 1 L, which was used in 75 of the studies (Figure 4, Table 5).

Table 4. Types of collection methods. Counts denote the number of studies reviewed that reported using the corresponding type of collection method.

Type of container	Count	Type of container	Count
Bottle	49	Siphon	1
Bottle – Corning	3	Suction Sampler	1
Bottle – EPI Plastics	2	Syringe	5
Bottle – Nalgene	25	Syringe – Livingstone International	1
Bottle – VWR	1	Tube	2
Bucket	7	Veggerby eDNA Sampler	1
Centrifuge Tube	1	Vial	1
Container	26	Water Sampler	1
Container – Kartell	1	Water Sampler – Friedinger	2
Cubitainer – Hedwin	2	Water Sampler – General Oceanics	1
Cup	2	Water Sampler – KC Denmark	1
Falcon Tube	4	Water Sampler – Kemmerer	1
Ladle	2	Water Sampler – NIOZ	1
n/a	12	Water Sampler – Niskin	22
None (pumped directly)	15	Water Sampler – Ruttner	1
Pipette	1	Water Sampler – Van-Dorn	5
Pipette – Greiner Bio-One	1	Water Sampler – Whirl-Pak Bag	5

Table 5. Volumes used in sampling.

Volume (L)	Count	Volume (L)	Count
0.01	1	2	21
0.015	4	3	5
0.025	1	4	6
0.05	8	5	10
0.1	1	7	1
0.18	1	10	4
0.2	1	12	1
0.25	10	15	1
0.4	4	20	4
0.5	16	30	2
0.75	1	40	1
1	75	60	1
1.5	3	150	1
1.7	1	n/a	28

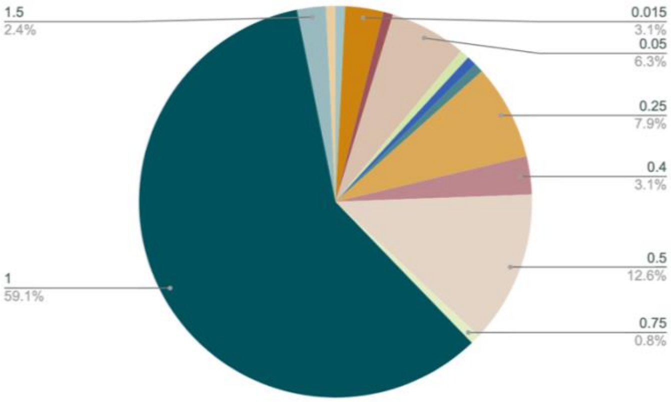


Figure 4. Proportions of sampling volumes.

Figure 5 reports the costs pertaining to sampling 1 L of water using different brands and types of sampling containers. For sampling methods that did not come in the 1 L size, the cost of the container was converted to a 1 L unit; for example, the cost of a 4 L container would be divided by 4 to get the price of a 1 L sample. Some sampling methods and containers noted in the literature review were not included in this analysis because prices were not publicly available. Also not included were methods of collection that, when scaled to 1 L, resulted in excessively expensive prices (for example, a 1 mL pipette is around 1 USD—converting this price to the 1 L equivalent would result in 1,000 USD). As shown in Figure 5, all bottles are close in price regardless of brand, with more specialized sampling containers such as ladles and centrifuge tubes exhibiting a higher average cost. The average cost of a 1 L Nalgene bottle was 7.96 USD, higher than a 1 L Corning bottle but cheaper than a 1 L VWR bottle (Figure 5).

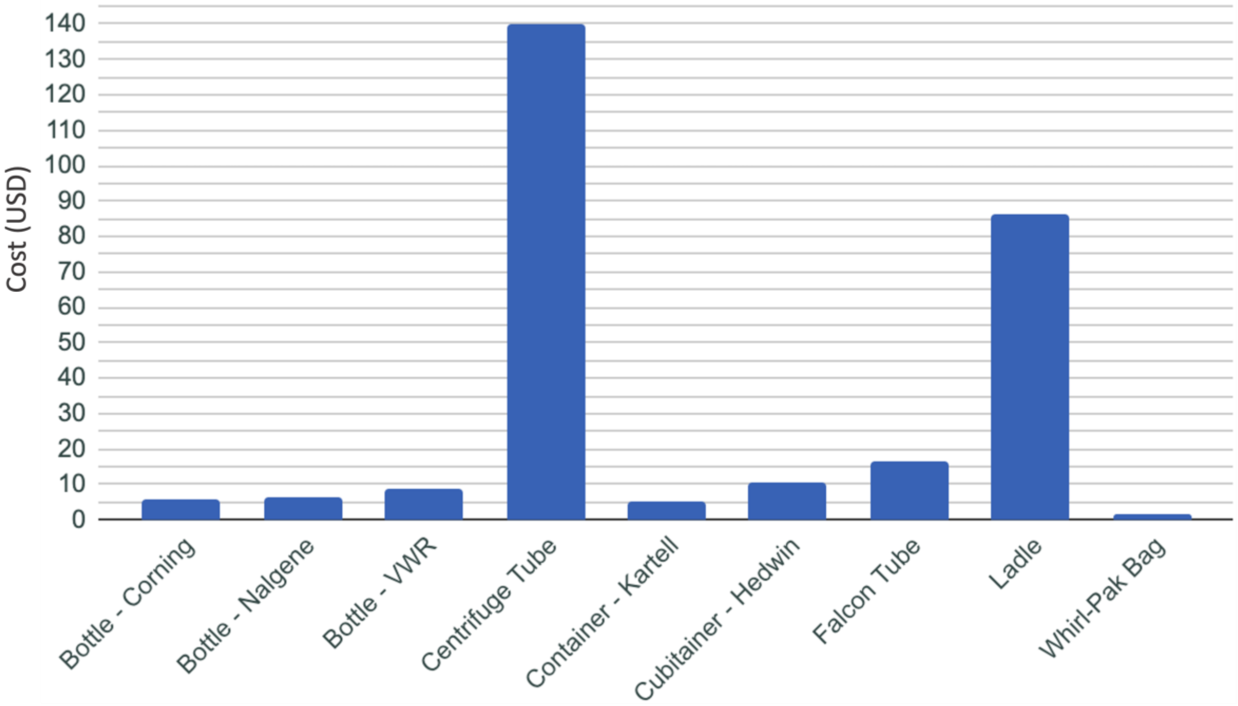


Figure 5. Average sampling costs (USD) of types of collection methods per liter.

3.1.2 Filtering

To determine the most common filter used, we identified (1) the most common pore size of the filter, (2) the material/type of the filter, and (3) the brand of filter used. Filters are available with pore sizes ranging from 0.1 µm to 20 µm. The most common filter pore size was found to be 0.45 µm, used in 35% of the studies (Figure 6, Table 6). Filters can be made of cellulose acetate, cellulose nitrate, glass fiber, polyethersulfone, and many other materials (Figure 7). The most common type of filter used was a glass fiber filter (used in 25% of the studies), although cellulose nitrate filters were also commonly used (24%; Figure 7, Table 7). The most common filter brand was Merck Millipore filters, used in 36% of the studies (Figure 8, Table 8). We found that the most common type of filter was the 0.45 µm glass fiber Merck Millipore filter.

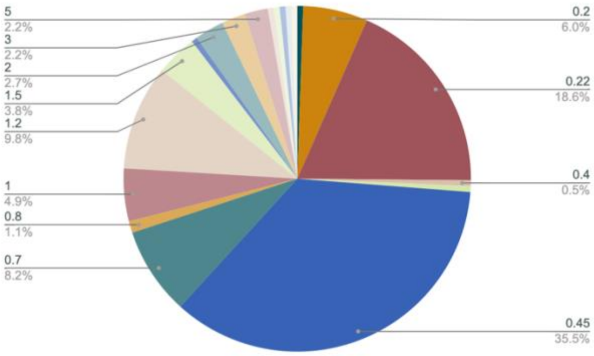


Figure 6. Proportions of filter pore sizes.

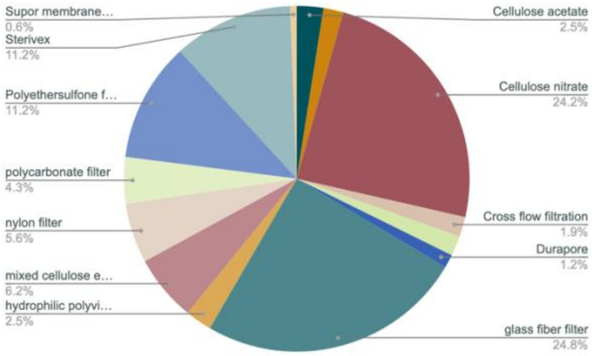


Figure 7. Proportions of filter types.

Table 6. Pore sizes of filters. Counts denote the number of studies reviewed that reported using the corresponding filter pore size.

Pore Size	Count	Pore Size	Count
0.1	1	1.5	7
0.2	11	1.6	1
0.22	34	2	5
0.4	1	3	4
0.44	1	5	4
0.45	65	10	1
0.7	15	12	1
0.8	2	20	1
1	9	30	1
1.2	18	100	1

Table 7. Types of filters. Counts denote the number of studies reviewed that reported using the corresponding filter type.

Filter Type	Count	Filter Type	Count
Cellulose acetate	4	Hydrophilic polyvinylidene fluoride filter	4
Cellulose	3	Mixed cellulose ester	10
Cellulose nitrate	39	Nylon filter	9
Cross-flow filtration	3	Polycarbonate filter	7
Capsule	3	Polyethersulfone filter	18
Durapore	2	Sterivex	18
Glass fiber filter	40	Supor membrane filter	1

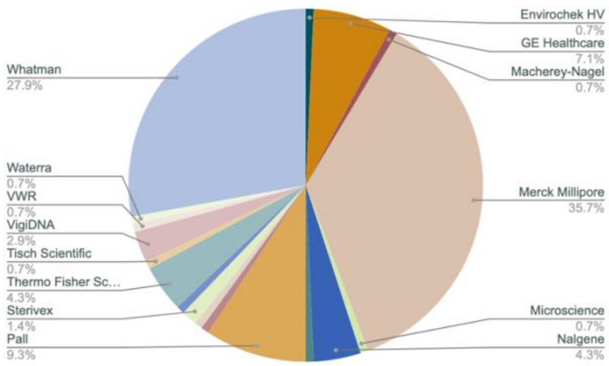


Figure 8. Proportion of filter brands.

Table 8. Filter brands. Counts denote the number of studies reviewed that reported using the corresponding filter brand.

Brand of Filter	Count	Brand of Filter	Count
Advantec	1	Sartorius	1
Envirochek HV	1	Smith-Root	1
GE Healthcare	10	Sterivex	2
Macherey-Nagel	1	Sterlitech	1
Merck Millipore	50	Thermo Fisher Scientific	6
Microscience	1	Tisch Scientific	1
Nalgene	6	VigiDNA	4
Osmonics	1	VWR	1
Pall	13	Waterra	1
Whatman	39		

The cost review showed that the 0.45 µm pore size exhibited the largest range in cost of all the filter pore sizes. Overall, the average cost of a filter was 1.51 USD, with the upper bound of the cost being 4.46 USD and the lowest cost being 0.57 USD.

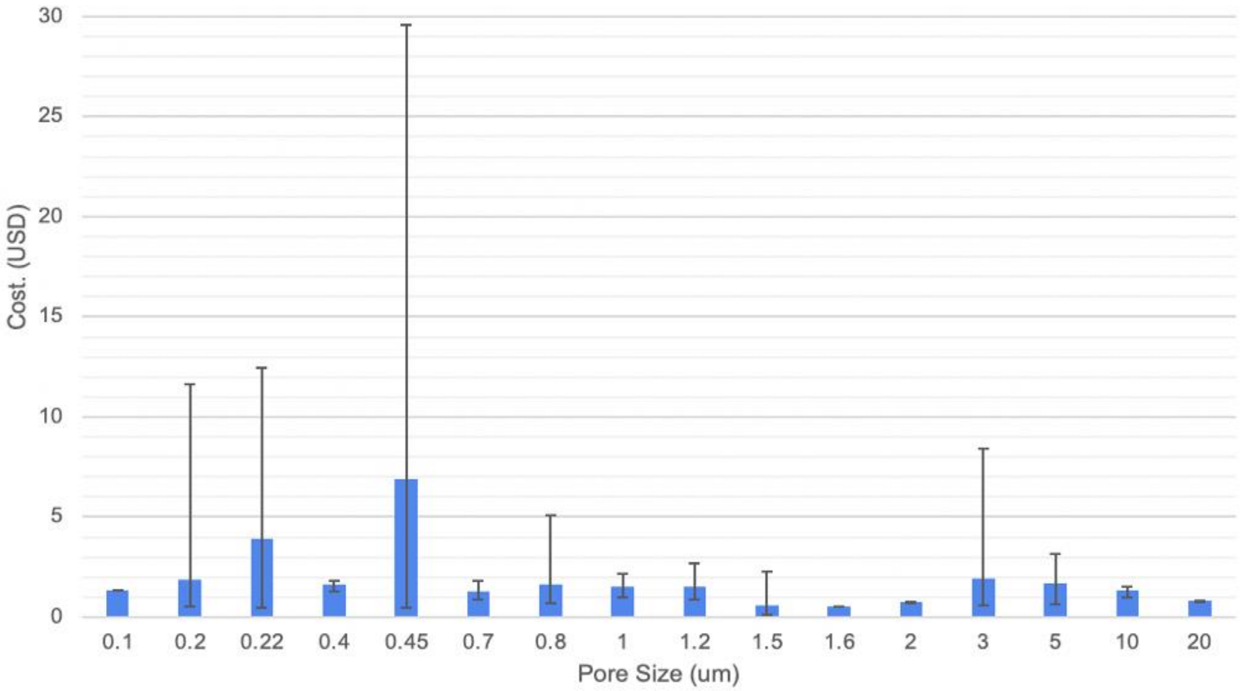


Figure 9. Average cost of filter by pore size. The upper bound represents the most expensive filter in each pore size, while the lower bound represents the least expensive.

3.1.3 Extraction

Fifteen different methods of DNA extraction were identified through the literature review, and most of them included extraction kits from various brands and targeted different types of tissue or organisms (Figure 10, Table 9). The most common extraction kit used was the Qiagen DNeasy Blood and Tissue kit. Extraction kits are typically sold in large quantities (e.g., 50, 100, or 250). Averaging over all available kit sizes, the cost of the Qiagen DNeasy Blood and Tissue kit was 3.54 USD per sample, half as much as the average cost of 7.41 USD (Figure 11).

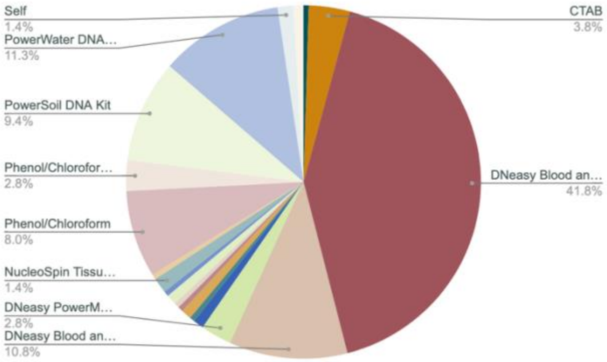


Figure 10. Proportions of DNA extraction methods.

Table 9. List of kits for DNA extraction. Counts denote the number of studies reviewed that reported using the corresponding extraction kit type.

Type of Kit	Count	Type of Kit	Count
Accuprep Genomic DNA Extraction Kit	1	n/a	2
CTAB	8	NucleoSpin Soil Kit	1
DNeasy Blood and Tissue Kit	89	NucleoSpin Tissue System	3
DNeasy Blood and Tissue Kit	23	Omega Biotek E.Z.N.A. Water Kit	1
DNeasy PowerMax Soil Kit	6	Phenol/Chloroform	17
E.Z.N.A. Tissue DNA Kit	2	Phenol/Chloroform (modified)	6
GeneMATRIXBio-Trace DNA Purification Kit	1	PowerSoil DNA Kit	20
gMAX Mini Genomic DNA Kit (IBI Scientific, Peosta, Iowa)	2	PowerWater DNA Isolation Kit	24
M1 Sample Prep Kit	1	Self	3
Mu-DNA	1	ZR-DuetTM DNA/RNA MiniPrep Kit Plus	2

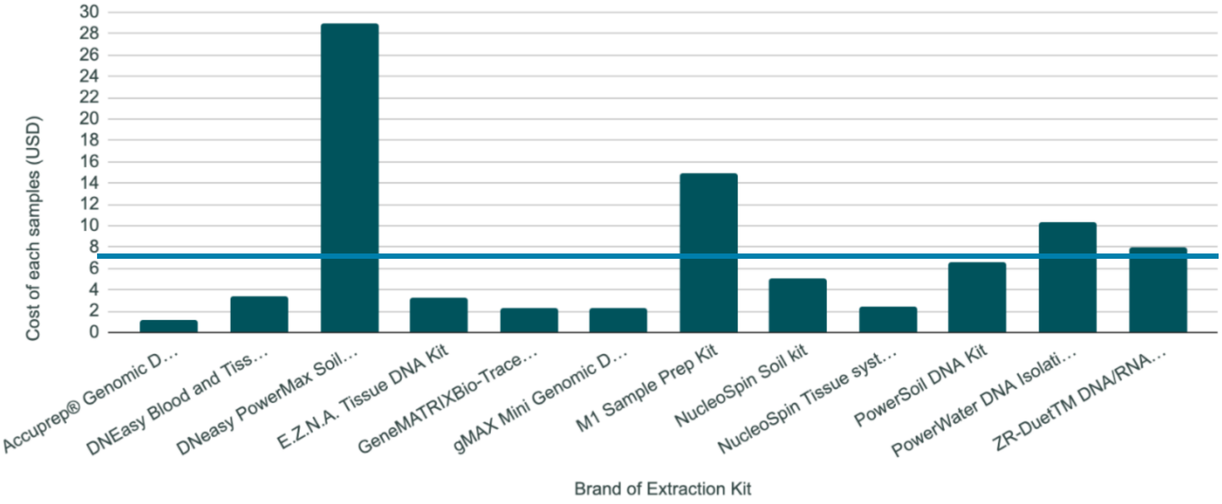


Figure 11. Cost of each extraction kit model. The average cost of 7.41 USD is shown as the horizontal line across the graph.

3.2 Cost Comparison with Conventional Methods

The most common eDNA supplies and their costs were identified from the literature and cost reviews (see section 3.1) and used for the hypothetical eDNA survey in Sequim Bay. The time required for the sampling and filtering steps was estimated based on experience from subject matter experts. Labor hours for completing permitting requirements were estimated by consulting with environmental permitting experts. Due to the high cost of performing DNA extractions and sequencing in small laboratory facilities, the hypothetical survey assumed that these steps were conducted by a subcontractor charging 200 USD a sample. The cost of undertaking this hypothetical eDNA survey by teams of students (2,760 USD) and senior researchers (4,450 USD) is shown in Table 10. Because the sampling containers (e.g., Nalgene bottles) can be cleaned and sterilized between surveys, a follow-up survey would cost 2,665.40 USD for a team of students and 4,355.40 USD for a team of senior researchers.

Table 10. Cost (in USD) of a hypothetical eDNA survey carried out by a team of either two students or two senior researchers.

		Student team			Senior researcher team		
	Quantity	Cost per unit	Total initial cost	Total follow-up cost	Cost per unit	Total initial cost	Total follow-up cost
Sampling container (1 L Nalgene bottle)	12	7.96	95.52	0	12	95.52	0
Filter (0.45 µm glass fiber Merck Millipore)	12	0.45	5.4	5.4	12	5.4	5.4
Extraction/sequencing (subcontractor)	12	200	2,400	2,400	12	2,400	2,400
Sampling labor cost	4	20	80	80	4	600	600
Filtering labor cost	6	20	120	120	6	900	900
Permitting labor cost	3	20	60	60	3	450	450
Total cost			**2,760.92**	**2,665.4**		**4,450.92**	**4,355.40**

Similarly, the cost of undertaking the hypothetical beach seine survey by a team of students (4,033 USD) and senior researchers (14,953 USD) is shown in Table 11. In both scenarios, the supplies (buckets, bucket aerator, aquarium net, measuring board, beach seine) can be reused, so only the labor and permit costs would be factored into follow-up surveys. These follow-up surveys would cost 1,800 USD and 12,720 USD for teams of students and senior researchers, respectively.

Table 11. Cost (in USD) of a hypothetical beach seine survey carried out by a team of either four students or four senior researchers.

		Student team			Senior researcher team		
	Quantity	Cost per unit	Total initial cost	Total follow-up cost	Cost per unit	Total initial cost	Total follow-up cost
Bucket + lid	6	10	60	0	10	60	0
Bucket aerator	6	8	48	0	8	48	0
Aquarium net	1	20	20	0	20	20	0
Permit fees	1	120	120	120	120	120	120
Measuring board	3	35	105	0	35	105	0
Beach seine	1	2,000	2,000	0	2,000	2,000	0
Sampling labor cost	16	20	320	320	150	2,400	2,400
Review labor cost	8	20	160	160	150	1,200	1,200
Permitting labor cost	60	20	1,200	1,200	150	9,000	9,000
Total cost			**4,033**	**1,800**		**14,953**	**12,720**

Table 12 displays the costs of undertaking the hypothetical scuba diver video transect survey by a team of students (2,740 USD) and senior researchers (11,580 USD). Follow-up surveys would reuse the transect tape and underwater video camera but would require new air tank fills for divers. Therefore, the cost of follow-up surveys is 1,480 USD and 10,320 USD for teams of students and senior researchers, respectively.

Table 12. Cost (in USD) of a hypothetical scuba diver video transect survey carried out by a team of either three students or three senior researchers.

		Student team			Senior researcher team		
	Quantity	Cost per unit	Total initial cost	Total follow-up cost	Cost per unit	Total initial cost	Total follow-up cost
Transect tape	1	60	60	0	60	60	0
Underwater video camera	1	1,200	1,200	0	1,200	1,200	0
Air tank fill	8	15	120	120	15	120	120
Sampling labor cost	36	20	720	720	150	5,400	5,400
Review labor cost	24	20	480	480	150	3,600	3,600
Permitting labor cost	8	20	160	160	150	1,200	1,200
Total Cost			**2,740**	**1,480**		**11,580**	**10,320**

Overall, the cost of surveys done without prior material as well as follow-up surveys in Figure 12. When it comes to students conducting the surveys, initial eDNA and scuba surveys presented very similar costs, but scuba was the least expensive follow-up survey. For both scenarios, beach seine and scuba surveys carried out by senior researchers were more expensive than those carried out by students. The difference was much smaller for eDNA surveys. For teams of senior researchers, eDNA surveys were less expensive than both beach seine surveys and scuba surveys by more than half.

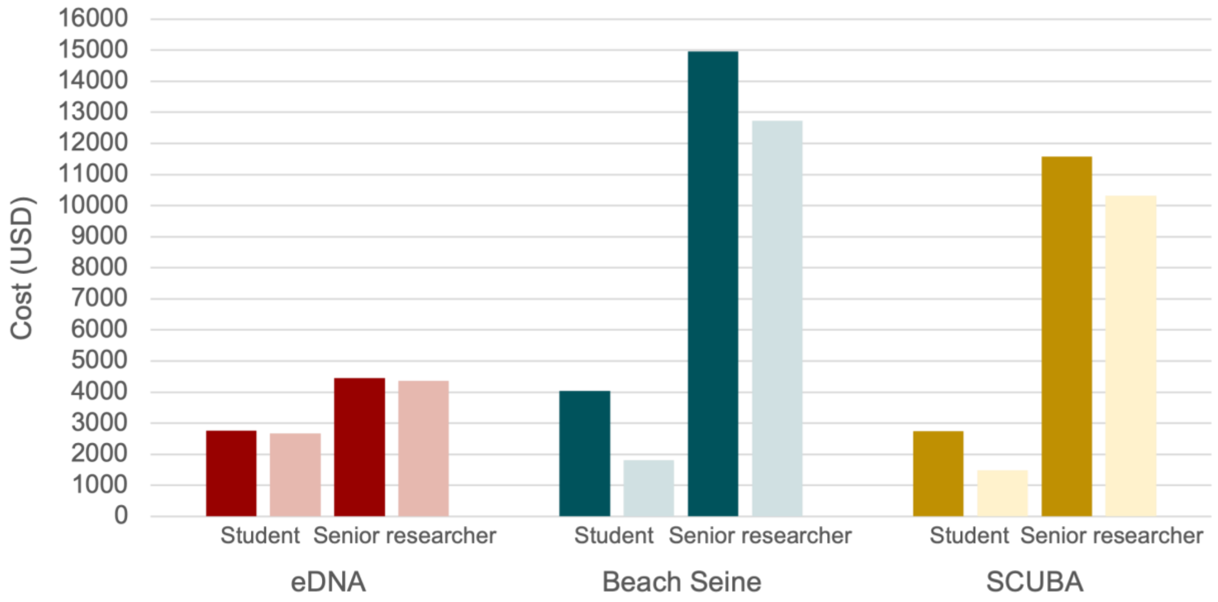


Figure 12. Cost comparison of eDNA, beach seine, and scuba surveys undertaken by teams of students or senior researchers with all new supplies (indicated by the darker colors), as well as follow-up surveys that would reuse part of the supplies (lighter colors).

4.0 Discussion

The cost of eDNA surveys was determined through cost analysis of sampling, filtering, and extraction procedures observed in 202 peer-reviewed journal articles across 50 journals. A review of the literature revealed a diverse array of supplies and techniques; for example, 42 distinct sampling containers were identified. The diversity of supplies and techniques reflects the absence of a standardized procedure. This could be due to the relative novelty of the field but also to the fact that different aquatic environments require different sampling techniques to optimize the collection of eDNA genetic material, especially in the presence of high turbidity (Sanches and Schreier 2020).

A hurdle faced in carrying out this literature review was the absence or inconsistent documentation of materials and methods used in several studies. For example, 36 articles did not specify how sampling sites were accessed or what collection methods were deployed. Another 29 studies did not include the type of filter used. Knowing that the first four types of filters identified in this review (i.e., glass fiber, cellulose nitrate, polyethersulfone, and sterivex) are within 22 counts of one another, a greater identification of the filter type could tip the balance in any direction, which in turn would modify the costs of the filtering step and the overall eDNA hypothetical survey. Among suggested best practices for eDNA surveys, the research community would benefit from authors providing more detail in published studies on the specific types of materials and methods used.

The supply prices presented here are representative of extensive searches made at the time of writing this report; therefore, the availability and pricing may vary over time. In some instances, the brands or kinds of supplies listed in several studies were no longer available for purchase, or a price was unable to be ascertained. For example, bottles produced by EPI Plastics are only available to purchase through large orders via email, and thus a cost could not be established. Furthermore, several supplies (of the same brand and type) often exhibited a large range of costs due to many market options, as shown in the cost variance in Figure 9. Therefore, the results gained from this study should be a rough estimate of the costs associated with each surveying type and not taken as a concrete number. The same caution should also be applied to the hourly salary assigned to each staff group in the hypothetical surveys. Research teams are likely to be more diverse than represented in the hypothetical surveys, and the hourly rates may be higher or lower for different organizations and may change over time.

The cost comparison between the three hypothetical fish surveys revealed that eDNA surveys provide cost advantages over beach seine surveys and scuba surveys due to the high labor needs and associated costs of obtaining the necessary permits for beach seining (e.g., federal permit Section 10(a)(1)(A) of the Endangered Species Act, and state scientific collection permit) and for collecting and processing the data for both beach seine and scuba. Especially when upfront expenses are required to purchase the essential material for initial surveys, eDNA surveys are much cheaper than conventional methods. While the total costs decrease more significantly for beach seine and scuba follow-up surveys where some of the equipment can be reused, follow-up eDNA surveys still present a large cost saving over conventional methods due to the high labor costs of senior researchers. This substantial difference in cost makes eDNA surveys a sustainable option for biodiversity surveys.

While eDNA surveys may be the less costly choice for senior researchers, initial scuba surveys appear slightly cheaper than eDNA surveys if undertaken by teams of students. Follow-up scuba and beach seine surveys by teams of students are almost two times cheaper than eDNA surveys because of the few supplies being reused for follow-up eDNA surveys and the cost of subcontracting eDNA sample processing (i.e., extraction, sequencing, and bioinformatics). However, this conclusion should be regarded conservatively because surveys done completely and only by students are rare given the specialization needed for both beach seine surveys and scuba surveys, especially to identify species. In general, research teams are not completely composed of students but a mix of junior to senior researchers, lab assistants, and students at various levels of education. A realistic cost would be somewhere between the lower bound (team of students) and upper bound (team of senior researchers).

The main drivers of the cost difference between the least expensive and most expensive methods are the labor needs, especially with high labor rates. The drastic difference in total cost exhibited is largely the result of the difference in labor hours between eDNA surveys and conventional surveys. In the hypothetical case studies, eDNA surveys required the least effort at 13 labor hours, followed by scuba surveys with 68 labor hours, and beach seine surveys with the greatest effort at 84 labor hours. This difference is mainly explained by the need to secure the required environmental permits for beach seine surveys and the longer time needed for scuba divers to collect and process the underwater videos. Because of its non-invasiveness (i.e., fish are not collected, only discarded genetic material), eDNA sampling does not require a lengthy environmental permitting process. Personnel needs for sampling also increase the total cost: in the hypothetical case studies, the eDNA surveys only required two staff members, compared to the four staff members necessary for beach seine surveying and the three divers needed for the scuba survey. As the cost per labor hour increases, the more drastic the difference becomes. Overall, eDNA surveys appear more cost-efficient than conventional methods for fish surveying.

As eDNA becomes more prevalent in detecting and monitoring aquatic biodiversity, it has been considered an effective, noninvasive, and cost-efficient alternative to conventional surveying methods such as beach seine and scuba surveys because it often provides higher resolution as well as detection over a larger spatiotemporal scale (Beng and Corleet, 2020; McColl-Gausden et al., 2020; Ruso et al., 2019). Compared to these methods, eDNA provides a serious cost advantage. Through our comparison of hypothetical eDNA, beach seine, and scuba surveys in Sequim Bay, we determined that eDNA is cheaper for initial surveys, where all supplies need to be purchased up-front. eDNA is potentially more expensive for follow-up surveys if carried out by teams of students, but drastically cheaper if conducted by teams of professionals. High energy environments, associated with marine energy deployments, are difficult to adequately sample using any survey method (Hemery et al. sub.). No survey technique is without caveats, and this analysis of eDNA methods and costs has provided insights into the economic viability of this approach for marine biodiversity surveys.

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6.0 Supplementary Material

Supplementary Material 1. List of peer-reviewed journal articles used in the literature review.

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