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DISCUSSION PAPER

The Ocean as a Living Sensor:
Environmental DNA and Acoustics for Detecting Marine Life

Alison W. Watts and Jennifer Miksis-Olds

University of New Hampshire

Concept Summary

The Ocean as a Living Sensor: Environmental DNA and Acoustics for Detecting Marine Life

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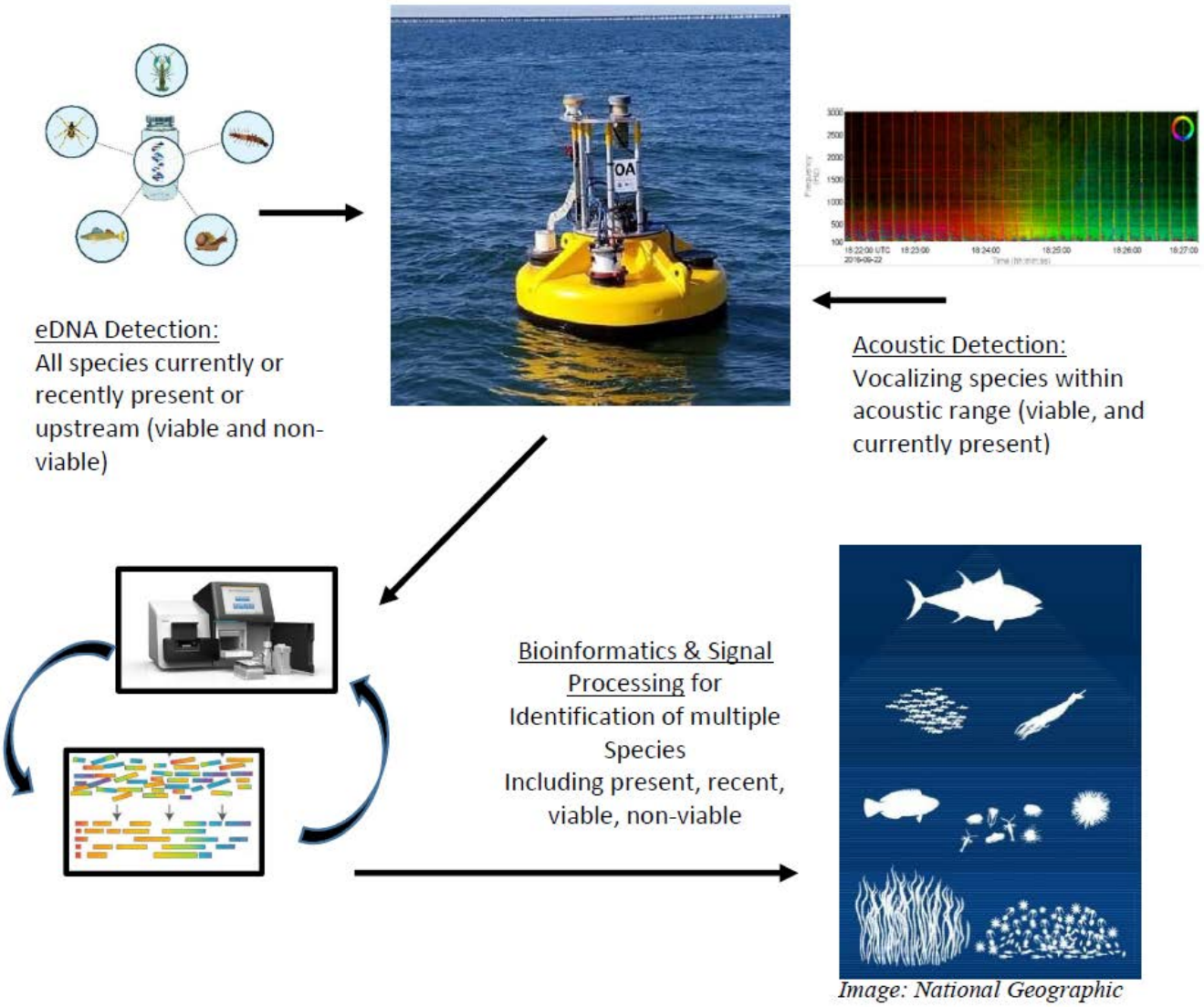

Image: National Geographic

As eDNA and acoustic technology and methodologies continue to develop, it will be possible to develop an ocean observation system systematically addressing questions of ecosystem connectivity from estuarine habitats to deep ocean environments far offshore. We envision the development of autonomous platforms, both tethered and mobile, which will collect data through multiple technologies including eDNA and acoustics. eDNA analyses and acoustic signals will be analyzed on board or transmitted to a signal processing station, and will detect and identify multiple species in the plant, animal, and microbial systems.

The Authors

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Dr. Jennifer L. Miksis-Olds is the Associate Director of Research and Research Professor in the School of Marine Science & Ocean Engineering at the University of New Hampshire. She is a member of the Scientific Committee of the International Quiet Ocean Experiment Program and serves as a Scientific Advisor to the Sound and Marine Life Joint Industry Programme (International Oil & Gas Producers), which is devoted to the study of effects of sound on marine organisms. Dr. Miksis-Olds was the recipient of an Office of Naval Research Young Investigator Program award in 2011 and the Presidential Early Career Award in Science and Engineering in 2013. She is also a newly elected Fellow in the Acoustical Society of America. Dr. Miksis-Olds received her A.B. cum laude in Biology from Harvard University, her M.S. in Biology from the University of Massachusetts Dartmouth; she was a guest student at Woods Hole Oceanographic Institution, and then received her Ph.D. in Biological Oceanography from the University of Rhode Island.

University of New Hampshire

The Ocean as a Living Sensor: Environmental DNA and Acoustics for Detecting Marine Life

Alison W. Watts and Jennifer Miksis-Olds, University of New Hampshire

Introduction

Exploring, understanding, and ultimately managing our ocean resources require us to identify the living organisms present. Most commonly used monitoring methods target a specific animal family or size range (e.g., visually detected marine mammals, fish caught in nets, or plankton captured in trawls). Orthogonal to direct or invasive measurements, there are several marine technologies that can detect organisms based on signals transmitted through the water itself. Organisms inhabiting an environment broadcast their presence, intentionally or inadvertently, through many signals. They communicate with each other vocally, generate sound through movements, broadcast gametes, and shed cells and other genetic material. In aquatic systems, this information is transmitted or dispersed through the water, creating unique, detectable signals that can be identified through field or lab-based technology. Here, we suggest the ocean water itself as a ***living sensor***, which contains both biologic and physical signatures identifying an aquatic community. Modern genetic and acoustic tools provide complementary data identifying organisms at a range of distances, to comprehensively detect aquatic species. Environmental DNA (eDNA) and passive acoustic monitoring are evolving technologies that may transform our understanding of marine communities.

What can eDNA tell us about the oceans?

Advances in DNA methods and rapid reductions in analytical costs present an opportunity to harness a new technology and fundamentally improve our capacity to monitor biological communities and individual species. Environmental DNA (eDNA) includes whole microorganisms (microalgae, bacteria, etc.) and fragments of tissue, reproductive and waste products, and other cellular material in an environmental sample (**Figure 1**). eDNA methods allow researchers and resource managers to identify species in an ecosystem without having to actually capture and identify individual organisms. As such, they have the capacity to identify a wide range of species and record both baseline biodiversity and changes over time. Over the past 2-3 years, eDNA techniques, which have been used more in freshwater ecosystems, are now being explored in marine environments (Andruszkiewicz et al., 2017; Bakker et al., 2017; Gargan et al., 2017). eDNA monitoring protocols are currently being developed for monitoring selected marine species, including invasive species targeted for control, and for native species that may be in decline for special management attention. High throughput sequencing supports analysis of complex micro and macro communities, such as zooplankton, algae, and microbes, but we are only beginning to appreciate the wide array of potential applications. Newly developing real-time sequencing technologies, combined with improved bioinformatics pipelines, support rapid identification of target species or communities in a field setting.

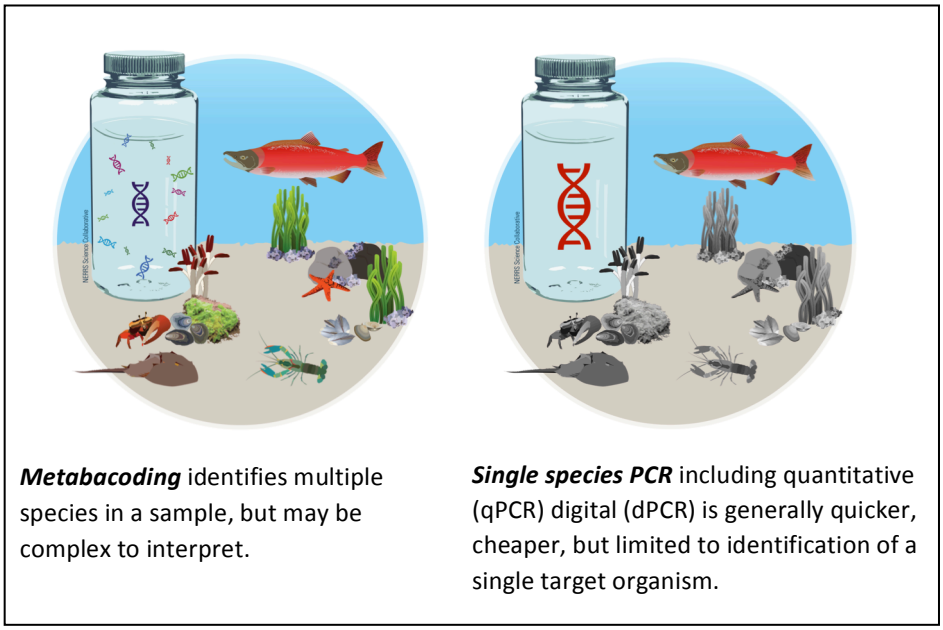

Figure 1. Metabarcoding and single species PCR are common eDNA analytic methods.

The most common approaches to eDNA analysis are metabarcoding, also called amplicon analysis, which involves the amplification of short DNA or RNA sequences. Sequence "primers" are designed to target general kingdoms, such as eukaryotes, or specific families, such as whales. Amplified sequences are compared to reference databases to match sequences to known species. Single-species PCR amplifies a DNA segment that is unique to the target species, eliminating the need for bioinformatics and reference databases, but limiting the amount of information obtained.

The power of high-throughput sequencing. The development of lower cost, lab-based instruments has increased accessibility to high-throughput sequencing of DNA, while advances in direct read and field instruments may eventually eliminate the necessity of large-scale lab instrumentation. As the cost of sequencing decreases, and more studies are conducted, capacity and acceptance improve. Bioinformatics pipelines and packaged software are now available to support data analysis, which further reduces the cost of analysis and interpretation.

Moving beyond presence/absence. Most eDNA studies to date have focused on presence/absence; is this rare species present? Or what communities of fish are in the vicinity? We have not yet been able to reliably link the number of animals (or plants) with DNA quantity. Enumeration beyond a very qualitative level is complicated by both the heterogeneity of environmental DNA, and by nonlinearity in amplification. Newer technologies such as digital PCR or direct sequencing may better reflect the amount of DNA present in a sample (Lafferty et al., 2018; Thomsen et al., 2016), but fundamental heterogeneity of DNA sources in the environmental will be difficult to overcome; is there a high concentration of DNA in the water because there are many fish? Or is one individual shedding large amounts of DNA because it is spawning, wounded, or even decaying? Distinguishing between viable/nonviable organisms (measured by comparing degraded loci) may be possible, but is not well developed.

Increasingly, eDNA is being used to understand relationships and movement within an ecosystem. Analysis of multiple loci, including haplotypes, can be used to infer family

relationships, genetic diversity and stock structure within detected species (Baetscher et al., 2018; Stat et al., 2017; Parsons et al., 2018). We can use this information to better understand how groups of animals are related, and how far they may travel. We can explore relationships between eggs, larvae, and adults to understand how far organisms move in each stage, and processes that effect recruitment from one stage to another (e.g., Burghart et al., 2014).

Barriers and weaknesses. eDNA analysis is improving rapidly, but there are inherent challenges, which will need to be overcome before the application becomes more widely applied. Some of these challenges, including resource-intensive bioinformatics, will be addressed as the research field advances. However, some of the challenges are inherent in the method and are better addressed through co-deployment of complementary monitoring tools. Much of the DNA in aquatic systems is in particulates shed by organisms. The particles are transported and decay at rates that are highly variable, depending on currents, water temperature, UV exposure, etc. Particles may also be transported by other organisms, including humans, through ingestion or physical transport. A DNA-based detection of an organism, therefore, indicates either that the organism is in the vicinity, or that there is a transport pathway to a distant source. If transport to a distant source is eliminated, there remains the question of how close is "in the vicinity?" Pairing molecular tools with technologies that provide potential transport information (e.g., shipping vessels, currents) significantly strengthens eDNA application.

What can acoustics tell us about the oceans?

Physical signals in the form of acoustic energy have historically been and presently are the state-of-the-art for efficiently imaging the marine environment over large spatial scales from the smallest plankton to large-scale anthropogenic activities (**Figure 2**). Ocean sound is a national and international focus because it crosses borders unimpeded. Acoustic signals, as opposed to visual or biological signals, can propagate long distances in the ocean and provide a means for marine life and humans to gain information about the environment. Passive acoustic technology is used noninvasively to assess environmental sound levels, surface conditions, human activity, and the distribution and biodiversity of vocalizing marine life. Vocalizing species include a wide range of organisms, from marine mammals to fish and invertebrates. Discovery of pests, invasive species, and animal distribution shifts to areas outside traditional documented ranges have been detected with passive acoustic monitoring systems both on land and in the water (Martinez et al., 2018; Seger and Miksis-Olds, in press).

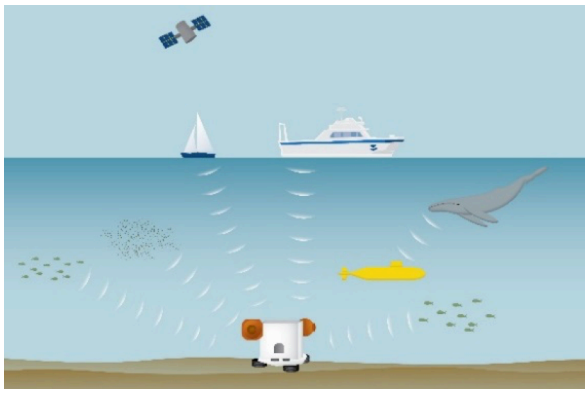

Figure 2. Acoustic monitoring detects vocalizing species (fish, mammals, crustaceans etc.) up to several miles away.

Passive acoustic monitoring (PAM) is performed using hydrophones deployed as single sensors or system arrays composed of multiple sensors. Depending on the temporal and spatial scale of

the question being addressed, and the level of available resources, passive acoustic sensors can be deployed as a fixed or mobile system. Advances in power, onboard data storage, and data telemetry capabilities have resulted in a recent increase in mobile PAM platforms ranging from unmanned underwater vehicles (e.g., ocean gliders) to animal-borne acoustic tags (Johnson et al., 2009). Passive acoustic sensors have long been an integral part of regional, national, and international ocean observation.

A great deal of information related to ocean dynamics and ocean use can be gained simply by listening to the ambient sound field, or soundscape. The soundscape, or auditory landscape, is a combination of the traditionally measured physical sound signal and the dynamically changing acoustic environment. It is composed of multiple sound sources, the perception of which depends upon the relative contribution of each source, its direction, the propagation of the signals, behavioral context of the listener, and history of the listener with similar sounds (**Figure 3**). Marine animals and humans both heavily rely on acoustic cues contributing to soundscapes to gain information about their surroundings. A large number of aquatic species use sound cues contained in local soundscapes to navigate, forage, select habitat, detect predators, and communicate information related to critical life functions (e.g., migration, breeding, etc.). More important for monitoring, however, is the wealth of knowledge humans can gain from the sounds produced by animals in a region. Acoustic monitoring can inform the timing and location of fish spawning, shifting distribution of animal populations.

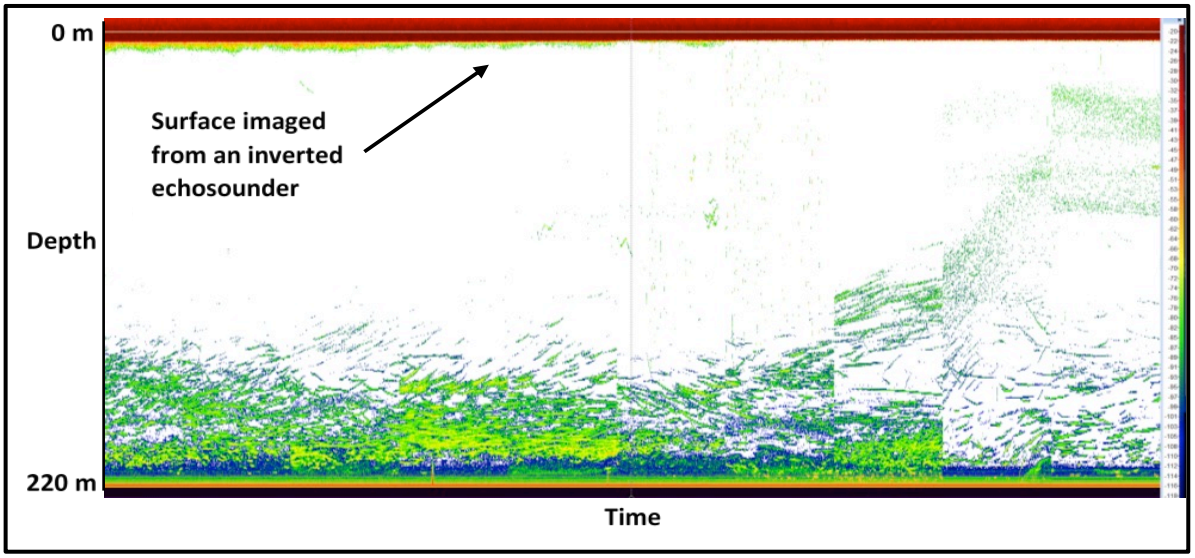

Figure 3. A 38 kHz four-hour echogram from 30 Nov. 2017 in the eastern Atlantic Outer Continental Shelf off Virginia showing the vertical migration of fish and zooplankton from depth to the surface.

As powerful as passive acoustics can be in detecting, localizing, and providing information about local marine life, passive acoustic technology fails when sources are not producing sound. Future innovation related to passive acoustic technology and applications is likely to come in the form of a combination with other non-acoustic sensing methods.

Active echosounder technology provides a time series of acoustic backscatter information that not only provides critical information on biology but also physical components of the water

column (Lavery et al., 2009, 2010; Benoit-Bird and Lawson, 2016). The integration of multifrequency echosounders in cabled and remotely deployed observation systems have contributed invaluable knowledge on marine life community structure, distribution and size of marine organisms, oceanic microstructure, and suspended sediments. By recording acoustic backscatter from at least two frequencies, the differences in backscatter between the two frequencies can be used to distinguish between different scatterers in the water column (Watkins and Brierley, 2002; Warren et al., 2003).

Successful incorporation of upward looking, single beam echosounders in moorings at Ocean Station Papa (Trevorrow, 2005) and in the Bering Sea (Stauffer et al., 2015; Miksis-Olds and Madden, 2014; Miksis-Olds et al., 2013) demonstrate the maturity of this technology in providing time series of acoustic backscatter used to investigate the abundance and behavior of zooplankton and fish, predator-prey relationships, and community structure. Success of using multiple single-frequency echosounders to study ecosystem dynamics has led to the evolution to broadband systems (e.g., Lavery et al., 2010; Stanton et al., 2010; Benoit-Bird and Lawson, 2016), which are now being used in both cabled and mooring configurations for inferring species composition. Broadband data have the advantage of improved spatial resolution, allowing better target isolation and noise suppression through the use of pulse compression techniques (Chu and Stanton, 1998; Ross et al., 2013). Broadband measurements and theoretical physics-based approaches for classifying zooplankton have been combined successfully to classify biological scattering layers from the Victoria Experimental Network Under the Sea (VENUS) mooring in Saanich Inlet, British Columbia (Ross et al., 2013). Two years of broadband data (85-155 kHz) was collected from the VENUS system; data processing classified scattering layers based on their assemblages into four animal groups – (1) diel migrating euphausiids, (2) chaetognaths, (3) fish, and (4) a mix of pteropods and bottom-to-oxycline migrating amphipods. Data generated from active acoustic systems provides biological information on trophic levels containing fish and zooplankton (**Figure 3**). When combined with the information obtained from passive acoustic systems related to physical conditions (e.g., surface conditions, ice cover, etc.), upper trophic level dynamics of marine mammals and other top predators, and even human use factors, underwater acoustics becomes a valuable tool in monitoring ecosystems in terms of overall function, biodiversity, and health.

Better together – Deploying multiple technologies to recover overall ecosystem character

eDNA and acoustics are both powerful methods for identifying and describing the organisms present in the marine environment. No survey can fully represent the “real” condition, but understanding and supplementing the gaps within each method allows us to more closely approach a true understanding of our oceans. As these technologies mature they are evolving towards more autonomous, accessible and affordable systems. We envision a future where remote platforms can gather and relay data in near-real time, and can deploy a targeted response to information, such as launching a drone to survey additional locations, or increasing sampling frequency when key species are nearby.

There a few targeted studies where eDNA and acoustics have been deployed together: The Hawaiian Islands Cetacean Assessment and Ecosystem Survey used acoustics to locate beaked whales, and eDNA was used to more clearly identify the species (Jacobsen, 2017). In a 2017 ADEON cruise active acoustics was used to direct a deepwater trawl. Water samples collected for a pilot eDNA program confirmed the presence of a target fish species (**Figure 4**). This type of analysis could be used to identify closely related fish or mammal species that travel together and are difficult to distinguish by sound. eDNA from targeted marine mammal species has been shown to be detectable and comparable to both visual and acoustic surveys 2 hours after an animal has swum through a volume of water (Baker et al., 2018). For example, in the New York Bight, the Wildlife Conservation Society, City University of New York, and partners are currently conducting an eDNA study of cetaceans and co-localized prey species in order to contrast eDNA detections with acoustic detections of vocalizing species. In the coming year, the eDNA work will also encompass a broader marine biodiversity census that can also be paired with any other detections of acoustic and soniferous species in the New York region. As part of these ongoing and planned projects in New York, efforts will be made to explore factors affecting the detectability of eDNA across temporal and spatial scales, the relationship to acoustic detectability, and ways in which to consider these factors for remote applications. In future studies plankton masses located with acoustics (as in Figure 3, above), could be sampled for eDNA to determine community composition, possibly by a drone, which would then return the sample to a buoy-based automated lab for analysis. Recent eDNA analysis of samples collected from ocean sites in the ADEON network identified up to 467 unique eukaryote sequences in a sample (Watts and Miksis-Olds, 2018, unpublished data). This depth of information will support much deeper understanding of biodiversity and ecosystem function.

Figure 4. A Bristlemouth fish caught in an IKMT trawl at the Wilmington ADEON site in December 2017. The trawl location was directed by active acoustics, and this fish species was identified both in the trawl and in an eDNA sample collected at the site. (Photo credit – Joseph Warren, Stony Brook University; eDNA data - Stoeckle 2017, unpublished data).

One of the weaknesses of eDNA analysis, that it cannot (currently) distinguish between DNA from live or dead organisms, may be a strength when coupled with acoustics, which does not detect dead or non-vocalizing animals. If DNA is present, but the animal does not generate sound, it may indicate a recent mortality, a distant source beyond hearing range, or that the animal is present but not vocalizing. Both methods suffer from incomplete databases, and the

need to ground truth new or rarely detected species. Concurrent deployment to confirm and document rare or cryptic species is particularly useful in remote areas where visual identification is not possible.

Challenges and opportunities – Where do we go from here?

Several advances in technology are needed to support widespread deployment of coordinated eDNA and acoustic systems: Both methods require "big data" and the infrastructure capacity to transmit and process large data sets. Satellite transmission and core computing power needs will only increase as acoustic measurements reach higher resolution and frequency. eDNA analysis is currently focused on short amplicon sequencing, but as sequencing technology advances, longer reads and full genome analysis will become possible in remote locations, stretching the cyberinfrastructure even further. Digital data, although challenging to manage, does have the advantage of potentially supporting automated reporting.

When signals, either genomic or acoustic, are used to classify a species, the field data must be matched to a database to identify the specific organism. The existing databases for both technologies are incomplete. Genomic data for most mammals and fish is available through several international databases, including the International Barcode of Life (IBOL.org) and the National Institute of Health GenBank, which incorporates data from other international databases. Of the estimated 1 million marine species, the majority do not have reference sequences. Appendix 1 shows currently available genomic data within marine clades; although progress has been made over the last several years, there are still entire clades with little to no reference information. Acoustic reference data is also limited, particularly for rarer species, and the libraries are not always easy to access. The International Bioacoustics Council lists 14 sound libraries, including the Cornell Bioacoustics Research Program, but we are not aware of any global databases incorporating information from major libraries.

Arguably, for certain types of analyses, such as biodiversity indices or ecosystem indicators, absolute species identification is not necessary. For these uses, high-throughput sequencing can distinguish operational taxonomic units (OTUs) at a much lower cost and greater precision than traditional taxonomic methods even when reference data is incomplete or absent.

Autonomous acoustic systems are already deployed in multiple observations systems, but development of autonomous or remote eDNA sampling and analysis systems is in its infancy. Field-deployable instruments with near real-time analysis of short DNA sections are being developed. For example, Oxford Nanopore's MinION supports sequence reads on an instrument about the size of a cell phone, while USGS's loop-mediated isothermal amplification (LAMP) assay provides rapid field detection of target species (Williams et al., 2017). To date, these technologies do not provide the reliability and capacity of lab-based sequencers and PCR instruments, but sequencing technology is improving rapidly, in part driven by investments in biomedical research. Autonomous sampling systems are also being developed for DNA sample collection (**Figure 5**). An Environmental Sample Processor developed by the Monterey Bay Aquarium Research Institute collects and filters water samples, then applies a molecular probe to provide real-time identification of harmful microorganisms (MBARI, 2017). Other platforms have been proposed (e.g., MarinEye, 2017) but are not yet available for ocean deployment.

Figure 5. The MBARI Environmental Sample Processor (ESP), heads out to sea for the launch of a long-range autonomous underwater vehicle carrying the latest version of the instrument. Photo: Todd Walsh © MBARI 2017

Acoustic monitoring is already accepted as a powerful method for exploring and understanding complex ocean ecologies. eDNA is an emerging and rapidly developing technology that will yield an unprecedented depth of detail about biologic communities. Widespread deployment of observing systems that integrate these complementary technologies across the world's oceans will allow us to understand, manage, and protect our global marine resources for future generations.

Acknowledgements

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Appendix 1. Status of DNA reference databases

Figure A.1. The number of described and estimated marine species for various clades along with the total number of molecular sequences available from each group. Adapted and updated from Dunn and Ryan, 2015. The total number of draft genomes and available molecular sequences were updated based on Table A.1.

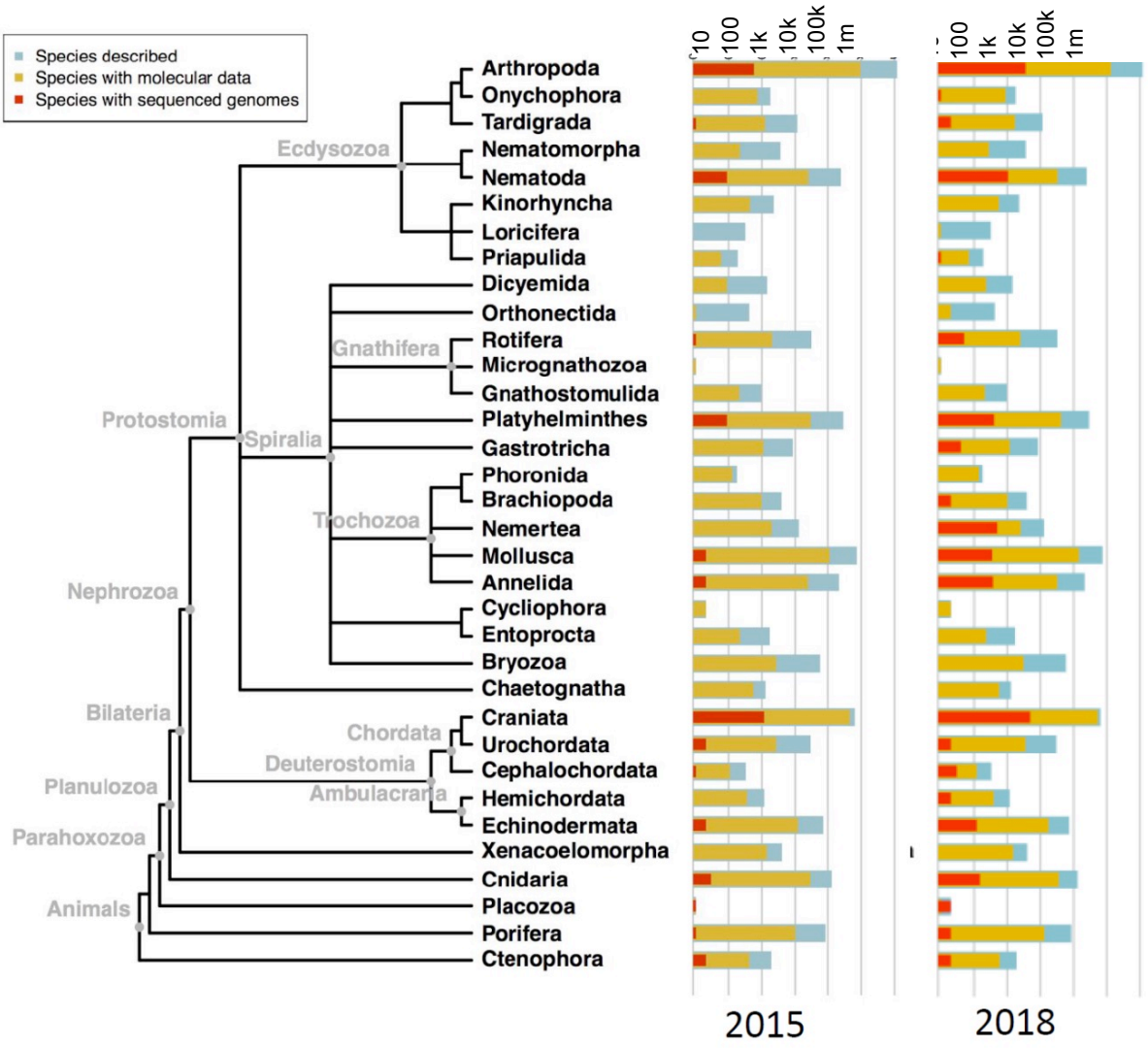

Table A.1. Estimated number of reference genomes for marine species. The total number of estimated and described species was obtained from Appeltans et al. (2012) supplemental data. The total number of sequences available from NCBI was retrieved using the "entrez_search" in the R package rentrez. The total number of mitochondrial genomes was obtained NCBI (ftp://ftp.ncbi.nlm.nih.gov/refseq/release/mitochondrion/). The total number of 18S sequences was obtained from SILVA release 132. Data compiled by Joseph Sevigny, University of New Hampshire, November 2018.

Phylum	Total_Described	Total_Estimated	Genomes	Mitochondria	SILVA	NCBI
Acanthocephala	450	720	0	10	57	132
Annelida	13721	31554	7	65	1236	3230
Polychaeta	12632	18952	2	38	899	1786
Hirudinea	179	292	2	7	-	378
Oligochaeta	910	12310	3	20	159	1003
Arthropoda	55013	293100	699	1616	8667	133120
Chelicerata	2685	6201	40	139	1442	9833
Decapoda	12029	22639	4	174	-	5405
Peracarida	17115	180264	5	27	239	2388
Other Crustacea	21086	81599	-	-	-	-
Hexapoda	2037	2147	633	1206	5367	111800
Myriapoda	61	251	1	15	212	1000
Brachiopoda	388	388	3	4	69	101
Bryozoa	5900	9900	0	7	104	310
Kinorhyncha	228	2028	0	2	42	56
Loricifera	32	1155	0	0	2	1
Nematomorpha	5	43393	0	0	13	28
Priapulida	19	-	2	2	8	7
Chaetognatha	129	309	0	5	45	57
Chordata	3906	5879	1130	4523	1025	53800
Cephalochordata	33	33	7	9	6	12
Tunicata	3020	4850	9	25	127	353
Mammalia	135	140	591	897	496	6816
Reptilia	110	135	0	0	-	8216
Aves	641	721	200	572	9	13045
Cnidaria	10163	16497	24	129	1188	3594
Hexacorallia	3152	4541	13	86	162	1137
Octocorallia	3171	4871	1	27	118	826
Cubozoa	37	87	0	0	13	26
Hydrozoa	3426	6251	3	9	308	853
Siphonophorae	176	286	0	0	45	105
Scyphozoa	201	361	1	3	41	85
Staurozoa	48	101	1	1	-	25
Ctenophora	190	408	2	2	23	59
Cycliophora	2	73	0	0	1	2
Echinodermata	7291	11434	16	49	120	1749
Asteroidea	1922	2435	6	9	34	436
Echinoidea	999	1825	5	22	42	281
Ophiuroidea	2064	2769	2	7	14	609
Crinoidea	623	723	0	4	14	150
Holothuroidea	1683	3683	3	9	15	273

Phylum	Total_Described	Total_Estimated	Genomes	Mitochondria	SILVA	NCBI
Echiura	175	218	0	2	14	21
Entoprocta	193	1223	0	2	17	23
Gastrotricha	434	2744	0	1	141	119
Gnathostomulida	98	316	0	2	24	21
Hemichordata	118	128	3	4	31	39
Mesozoa	134	1229	0	0	9	25
Mollusca	47689	149997	30	312	934	14573
Bivalvia	9000	14000	15	127	347	2320
Caudofoveata	133	633	0	2	2	13
Cephalopoda	761	1411	2	39	52	420
Gastropoda	95000	190000	13	134	475	11556
Monoplacophora	30	83	0	2	1	5
Polyplacophora	930	1055	0	6	43	213
Scaphopoda	572	1127	0	2	14	27
Solenogastres	263	688	0	0	-	19
Myxozoa	700	8495	0	0	551	640
Nematoda	11400	61400	151	153	1947	3289
free-living	6900	56900	-	-	-	-
parasitic	4500	-	-	-	-	-
Nemertea	1285	2335	1	17	155	254
Phoronida	18	18	0	0	-	14
Placozoa	1	74	3	5	4	1
Platyhelminthes	11690	54369	50	100	1786	4173
Cestoda	1393	3693	19	46	474	858
Monogenea	1626	17126	2	14	185	994
Aspidogastrea	18	24	0	0	9	13
Digenea	6000	13500	21	34	304	1503
Catenulida	12	37	0	0	35	19
Rhabditophora	2641	19989	0	0	779	789
Porifera	8553	26203	7	60	372	1319
Rotifera	114	1534	11	3	123	248
Sipuncula	150	287	0	3	39	64
Tardigrada	183	1303	4	3	114	171
Xenacoelomorpha	401	4501	0	6	160	151
Acoela	391	4491	0	1	136	128
Nemertodermatida	8	8	0	0	23	16
Xenoturbellida	2	-	0	5	1	7