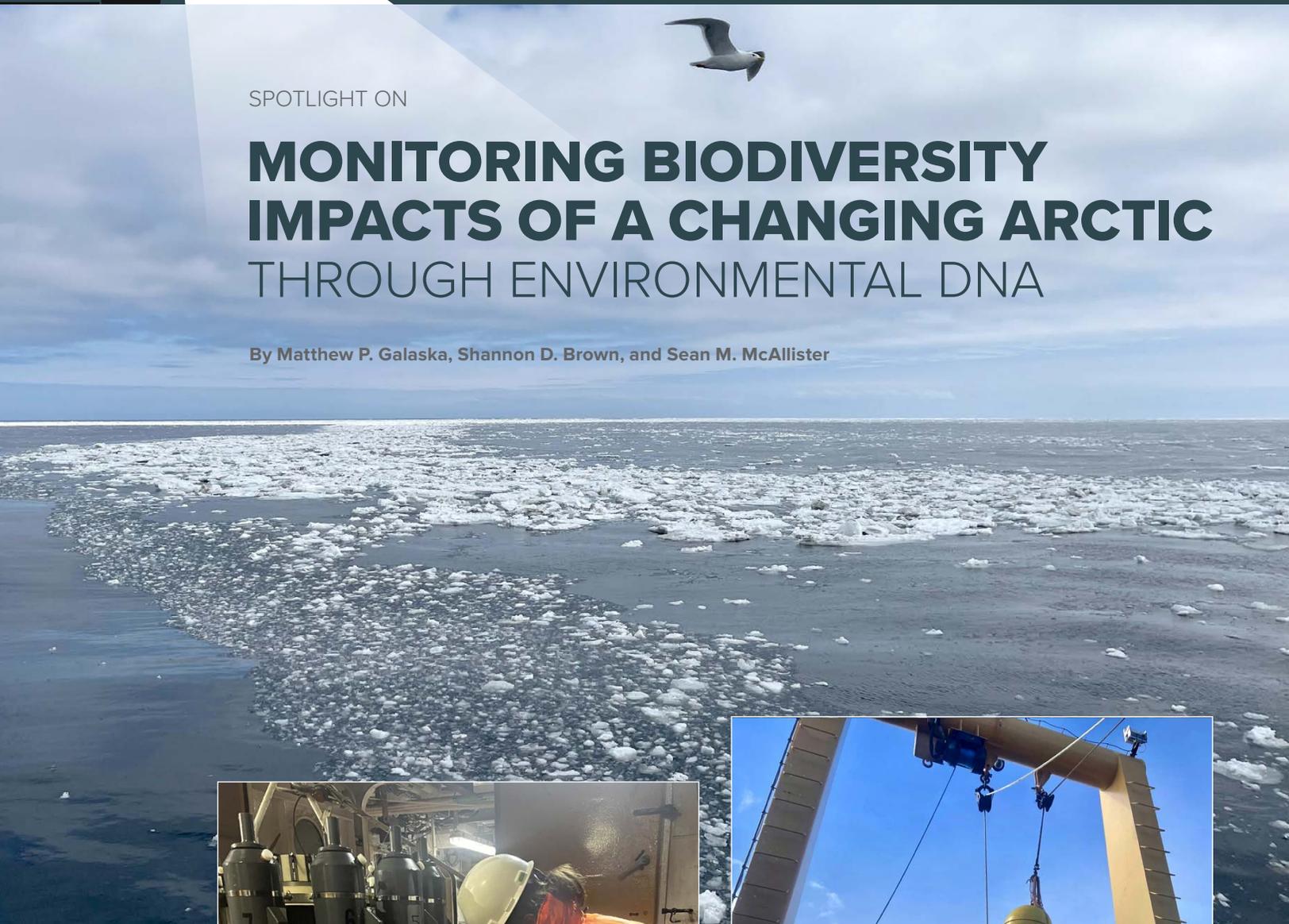SPOTLIGHT ON

MONITORING BIODIVERSITY IMPACTS OF A CHANGING ARCTIC
THROUGH ENVIRONMENTAL DNA

By Matthew P. Galaska, Shannon D. Brown, and Sean M. McAllister

The Ocean Molecular Ecology (OME) program at the NOAA Pacific Marine Environmental Laboratory (PMEL) is a recently established research group that partners with several PMEL and Alaska Fisheries Science Center (AFSC) programs including Ecosystems & Fisheries -Oceanography Coordinated Investigations (EcoFOCI), Ocean Carbon, and Earth-Ocean Interactions (EOI). The OME program utilizes a suite of molecular tools to support long-term research initiatives through genomics applications, and their development and application as novel environmental (e)DNA approaches has been key for facilitating collaborations within PMEL and NOAA Oceanic and Atmospheric Research, and across NOAA line offices.

Environmental DNA is the genetic material of multicellular and single-celled eukaryotes, Bacteria, and Archaea that can be extracted from an environmental sample without isolating a stand-alone species (Taberlet et al., 2012). Utilizing eDNA from water collected for other research (e.g., water chemistry, phytoplankton measurements) provides a complementary tool for characterizing ecosystem biodiversity down to species level for some taxa (Kelly et al., 2014) and for detecting endangered inconspicuous species (Jerde et al., 2011); in addition, it yields information regarding temporal variability and spatial distribution of target species (Sutter and Kinziger, 2019). Compared to traditional approaches (e.g., trawling, surveys), eDNA sampling can be both less costly and less environmentally damaging. When used in tandem with established monitoring methods, eDNA can provide data on community composition as well as biodiversity shifts over time through repeated sampling (Goldberg et al., 2015). Further, eDNA extracts can often be cataloged for future use to address additional, complementary questions, such as quantitative estimates of species abundance (Spear et al., 2020). The integration of eDNA into routine ecosystem monitoring has the potential to be transformative by providing managers with novel additional resources to address NOAA's goal of predicting, conserving, and managing the impacts of a changing ocean on US marine resources.

At high latitudes, the impacts of climate change are among the most extreme in the world (Walsh, 2008). Climate change and other environmental impacts attributed to anthropogenic inputs include ocean warming, loss of seasonal sea ice cover, hypoxia, ocean acidification, and noise pollution. These cumulative stressors impact ecosystem dynamics and directly influence the distribution and diversity of key species at all trophic levels (Cheung et al., 2009). The Arctic Ocean and Bering Sea, known for their high productivity, are home to abundant and diverse populations of benthic invertebrates, seabirds, marine mammals, and commercially significant fish (Hood and Calder, 1981). PMEL has monitored the Bering, Chukchi, and Beaufort Seas for over 25 years (Tabisola et al., 2022). Mooring measurements and multidisciplinary sampling at a set of observatories have allowed scientists to detect and predict environmental changes in this region. Additional tools that can help to quantify a rapidly changing ecosystem, such as moored automated environmental samplers, would be welcome additions. Within the last decade, PMEL scientists have begun employing eDNA metabarcoding in the Arctic to expand on previous efforts that mainly employed remote sensing, fisheries surveys, and moorings (Leduc et al., 2019). In 2020, eDNA sampling was added to PMEL's extensive Arctic sampling effort to complement ongoing work (Stabeno et al., 2023, in this issue) and to provide further information on species distribution and abundance.

BIODIVERSITY STANDARDS FOR THE ARCTIC OBSERVATORIES

From September through October 2020, 249 eDNA samples were collected in partnership with EcoFOCI. These efforts had additional support through the Arctic Marine Biodiversity Observation Network (AMBON), and the samples will be shared with the AFSC to address complementary scientific questions related to fish. In total, 51 sampling sites in the Bering, Chukchi, and Beaufort Seas were selected to be co-located with long-term EcoFOCI moored sites and the established Distributed Biological Observatories. One-liter seawater samples were collected by Niskin bottles on CTD casts and were subsequently filtered onto single-use Sterivex capsule filters following sterile procedures (Figure 1), preserved with molecular grade ethanol, and frozen at –20°C for further analysis.

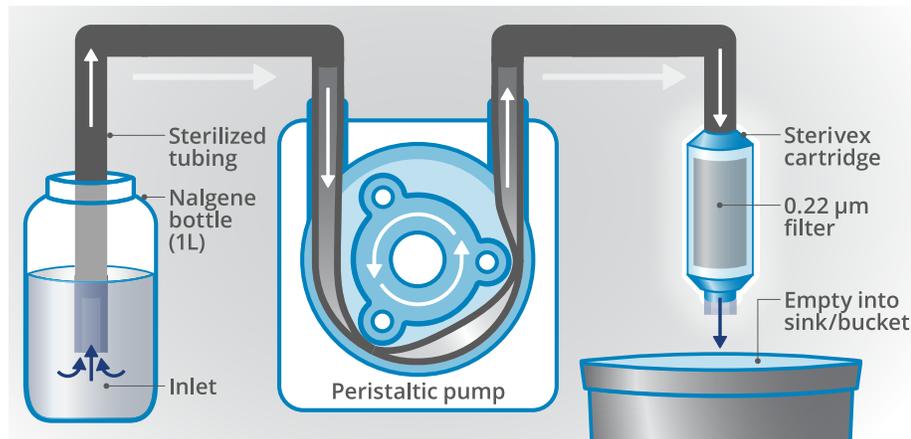Preserved eDNA samples were sent to a contracted laboratory (Center for

FIGURE 1. The shipboard environmental DNA sampling scheme used by the PMEL Ocean Molecular Ecology program.

Quantitative Life Sciences, Oregon State University) for DNA extraction following Spens et al. (2017). Two complementary loci, the 18S nuclear rRNA locus (Machida and Knowlton, 2012) and the 16S rRNA mitochondrial locus (Parada et al., 2015), were amplified using the source literature's published protocols and sequenced on an Illumina MiSeq platform, using 2 × 300 bp paired-end sequencing. Each sample was processed in triplicate to detect potential amplification bias, and both positive and negative controls were integrated throughout the sample processing protocol. Raw sequences were analyzed using the bioinformatic pipeline, REVAMP (McAllister et al., 2023, in this issue), which quality-control reads (Cutadapt, DADA2; Martin, 2011; Callahan et al., 2016), merges paired reads and assigns sequence variants (DADA2), assigns taxonomy

(BLASTn; Camacho et al., 2009), and conducts initial data analyses. Data analysis includes estimation of alpha-diversity metrics as well as non-metric multidimensional scaling (NMDS) to visualize multivariate data (phyloseq; McMurdie and Holmes, 2013) and fit environmental observations (vegan; Oksanen et al., 2020). DNA extractions are stored at PMEL, while all raw sequence data are deposited in the National Center for Biotechnology Information (NCBI) system for public dissemination.

RESULTS AND DISCUSSION

This initial assessment of the Alaskan/Arctic eDNA captured substantial biodiversity from microbes to phytoplankton in addition to detecting many important metazoans. The 18S marker captured 1,536 unique amplicon sequence variants (ASVs, or DNA sequences) from

557 unique Eukaryotic and 25 unique Bacterial taxa. Due to within-species genetic variation, multiple ASVs can be assigned to a single species. Through the 16S primer, we captured 2,326 ASVs from 260 unique Eukaryotic, 318 Bacterial, and six Archaeal taxa. While the two genetic markers applied are complementary, some species were detected by both assays. Between the two markers, 722 unique Eukaryotic, 340 Bacterial, and 6 Archaeal lineages were detected, an overlap of only 8.4% identical taxa between markers.

Applying NMDS to the eDNA metabarcoding sequence data showed structuring of the biological community (Figure 2), differentiating the northernmost samples (Beaufort Sea) from the rest, particularly along NMDS2 axis (Figure 2a,d). Along the latitudinal sample gradient in the Bering Sea, pelagic and benthic
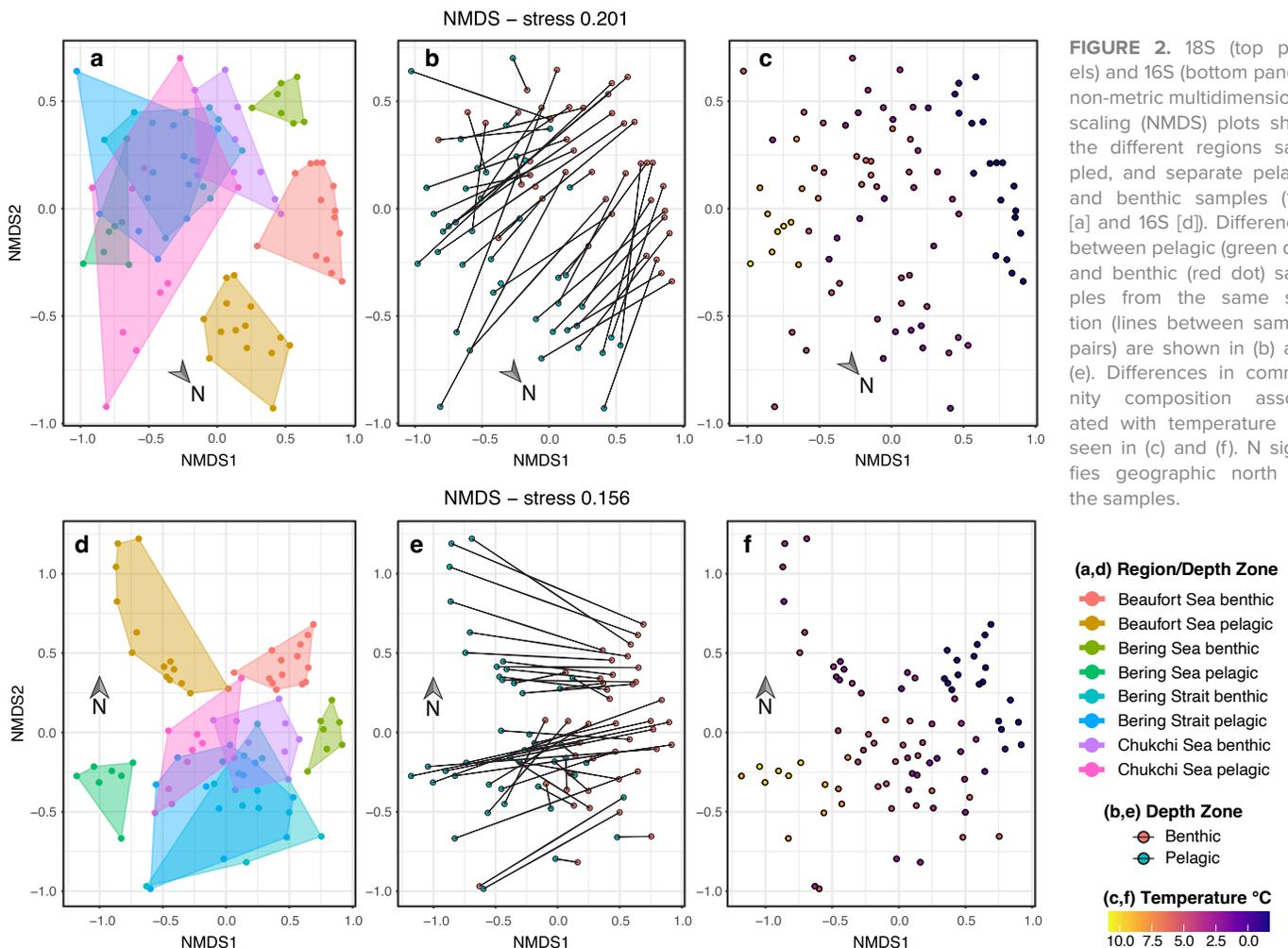

FIGURE 2. 18S (top panels) and 16S (bottom panels) non-metric multidimensional scaling (NMDS) plots show the different regions sampled, and separate pelagic and benthic samples (18S [a] and 16S [d]). Differences between pelagic (green dot) and benthic (red dot) samples from the same station (lines between sample pairs) are shown in (b) and (e). Differences in community composition associated with temperature are seen in (c) and (f). N signifies geographic north for the samples.
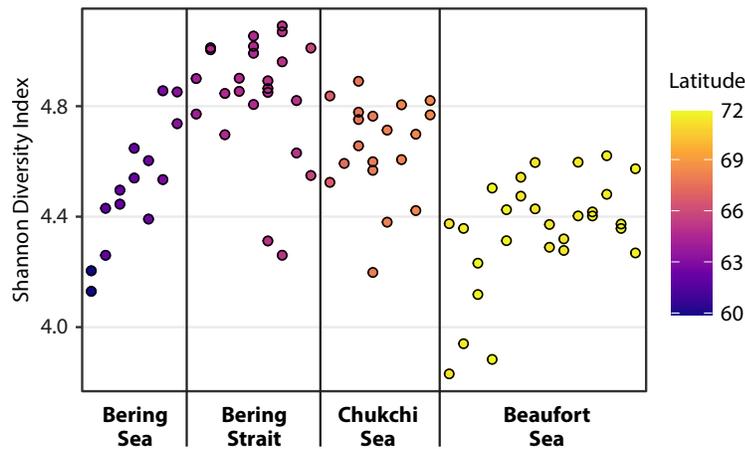
FIGURE 3. Shannon diversity indices for each station, arranged from south to north and colored by degrees latitude. Diversity is highest in the samples from Distributed Biological Observatory-2, which is south of the Bering Strait.

communities were clearly differentiated, while these communities overlapped in the Bering Strait, and were only slightly differentiated in the Chukchi Sea before differentiating again in the Beaufort Sea. This effect is more pronounced with the 16S marker (bottom) compared to the 18S marker (top). Further, depth trends are also clear along the NMDS1 axis (Figure 2b,e).

The Shannon diversity index (alpha-diversity measurement; Shannon, 1948) considers both the number (richness) and relative abundance (evenness) of the ASVs detected in each sample. Here, a clear trend in Shannon diversity was identified in the 16S marker data set, where high diversity was associated with Bering Strait samples compared to regions either to the south or the north (Figure 3). This trend appears to be related to the constriction of benthic and pelagic samples in the NMDS space.

Environmental fitting, applied to the observations in the NMDS plots, provided unique insights into the factors affecting the observed patterns of bio-diversity. In order of statistical signifi-cance, the following variables impacted structuring of the biological diversity found in Alaskan waters: degrees latitude (p-value = 0.001), temperature (p-value = 0.001), salinity (p-value = 0.001), depth (p-value = 0.001), fluorescence

(p-value = 0.003, 18S only), and oxygen (p-value = 0.005 for 18S; p-value = 0.003 for 16S). Additionally, these models high-lighted the most significant ASVs that differentiated the sampled communities (see Figshare 18S and 16S links in Data Availability below: statistically signifi-cant ASVs for each marker). For the 18S marker, 359 ASVs were attributed to sig-nificant (p-value ≤ 0.05) differentiation among communities, the majority asso-ciated with phytoplankton, protists, and dinoflagellates. Data from the 16S marker revealed 589 statistically significant ASVs, with 328 associated with Bacteria, 255 with Eukaryota, and six with Archaea. We detected representatives across a wide array of marine phyla, including Annel-ida, Chaetognatha, Cnidaria, Copepoda, Crustacea, Ctenophora, Echinodermata, Mollusca, Rotifera, and Tunicata. Only nine metazoan ASVs were significant to community differentiation within the 18S data (none for the 16S data), including four annelids (*Nephyts* sp., *Phyllodoce* sp., *Trochochaeta* sp., and a member of family Capitellidae), two copepods (*Oithona* sp. and *Pseudocalanus* sp.), a chaetognath (order Aphragmophora), a cnidarian (family Pelagiidae), and a ctenophore (*Dryodora glandiformis*).

The base of the food web (primary productivity, primary consumers, and the microbial loop) were particularly

well represented by both markers. Primary consumers included the pro-tists in Ciliophora and dinoflagellates in Dinophyceae. From the microbial com-munity, we detected substantial signals from Bacteroidetes taxa, suggesting a pro-lific heterotrophic community for organic carbon degradation (Thomas et al., 2011). Due to warming trends, there is increas-ing concern within Alaska about marine species responsible for harmful algal blooms (HABs; Anderson et al., 2022). We detected genera and species that may be associated with toxic HABs, though they were primarily found in very low relative abundance. This included known HAB species—*Aureococcus anophagefferens, Karenia mikimotoi, Karenia papilionacea, Karlodinium veneficum, Margalefidinium fulvescens, Pseudo-nitzschia delicatis-sima*, and *Pseudo-nitzschia multiseries*—in addition to several toxic HAB genera, including *Alexandrium*. We also detected HAB associated parasites from the genus *Amoebophrya*, an endoparasite that can infect a wide range of dinoflagellates and has been associated with the termina-tion of blooms (Velo-Suárez et al., 2013; Nishitani et al., 2021). The detection of this endoparasite in the water column may indicate a recent bloom, and detec-tions such as this exemplify the poten-tial use of eDNA for tracking HAB fre-quencies in time and space via key indicator species.

From microbes to metazoans, this work reflects the PMEL OME program's inaugural eDNA biodiversity assessments in Alaska and the Arctic, in collaboration with EcoFOCI, AFSC, and AMBON. By expanding the scope of species detected through the use of additional biodiversity markers and standardizing eDNA as an integrated routine protocol in field sea-son surveys by PMEL and our partners, we can build on the work conducted at these observatories with a highly infor-mative, complementary, biodiversity-focused toolset. Further, building a long-term spatial data set will provide a fundamental record of how the Alaskan and Arctic ecosystems are responding

to a rapidly changing marine environment. To this end, large geographic surveys are critical to our understanding of the system as a whole, but the inclusion of moored automated samplers, a PMEL staple, are key for detecting seasonal and annual variability. These efforts are critical to the mission goals of NOAA, PMEL, our local and academic partners, and stakeholders. 🔁

REFERENCES

Anderson, D.M., E. Fachon, K. Hubbard, K.A. Lefebvre, P. Lin, R. Pickart, M. Richlen, G. Sheffield, and C. Van Hemert. 2022. Harmful algal blooms in the Alaskan Arctic: An emerging threat as the ocean warms. *Oceanography* 35(3–4):130–139, https://doi.org/10.5670/oceanog.2022.121.

Callahan, B.J., P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, and S.P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13:581–583, https://doi.org/10.1038/nmeth.3869.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T.L. Madden. 2009. BLAST+: Architecture and applications. *BMC Bioinformatics* 10:421, https://doi.org/10.1186/1471-2105-10-421.

Cheung, W.W.L., V.W.Y. Lam, J.L. Sarmiento, K. Kearney, R. Watson, and D. Pauly. 2009. Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries* 10(3):235–251, https://doi.org/10.1111/j.1467-2979.2008.00315.x.

Goldberg, C.S., K.M. Strickler, and D.S. Pilliod. 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation* 183:1–3, https://doi.org/10.1016/j.biocon.2014.11.040.

Hood, D.W., and J.A. Calder. 1981. *The Eastern Bering Sea Shelf: Oceanography and Resources.* Two volumes, University of Washington Press, Seattle, 1,339 pp.

Jerde, C.L., A.R. Mahon, W.L. Chadderton, and D.M. Lodge. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters* 4(2):150–157, https://doi.org/10.1111/j.1755-263X.2010.00158.x.

Kelly, R.P., J.A. Port, K.M. Yamahara, and L.B. Crowder. 2014. Using environmental DNA to census marine fishes in a large mesocosm. *PLoS ONE* 9(1):e86175, https://doi.org/10.1371/journal.pone.0086175.

Leduc, N., A. Lacoursière-Roussel, K.L. Howland, P. Archambault, M. Sevellec, E. Normandeau, A. Dispas, G. Winkler, C.W. McKindsey, N. Simard, and L. Bernatchez. 2019. Comparing eDNA metabarcoding and species collection for documenting Arctic metazoan biodiversity. *Environmental DNA* 1(4):342–358, https://doi.org/10.1002/edn3.35.

Machida, R.J., and N. Knowlton. 2012. PCR primers for metazoan nuclear 18S and 28S ribosomal DNA sequences. *PLoS ONE* 7(9):e46180, https://doi.org/10.1371/journal.pone.0046180.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17:10–12, https://doi.org/10.14806/ej.17.1.200.

McAllister, S.M., C. Paight, E.L. Norton, and M.P. Galaska. 2023. REVAMP: Rapid Exploration and Visualization through an Automated Metabarcoding Pipeline. *Oceanography* 36(2–3):114–119, https://doi.org/10.5670/oceanog.2023.231.

McMurdie, P.J., and S. Holmes. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* 8:e61217, https://doi.org/10.1371/journal.pone.0061217.

Nishitani, G., K. Yamamoto, M. Nakajima, Y. Shibata, W. Sato-Okoshi, and M. Yamaguchi. 2021. A novel parasite strain of *Amoebophrya* sp. infecting the toxic dinoflagellate *Alexandrium catenella* (Group I) and its effect on the host bloom in Osaka Bay, Japan. *Harmful Algae* 110:102123, https://doi.org/10.1016/j.hal.2021.102123.

Oksanen, J., G.L. Simpson, F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara, P. Solymos, M.H.H. Stevens, E. Szoecs, and others. 2020. vegan: Community ecology package. R package version 2.5-7, https://CRAN.R-project.org/package=vegan.

Parada, A.E., D.M. Needham, and J.A. Fuhrman. 2015. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology* 18(5):1,403–1,414, https://doi.org/10.1111/1462-2920.13023.

Shannon, C.E. 1948. A mathematical theory of communication. *The Bell System Technical Journal* 27:379–423, https://doi.org/10.1002/j.1538-7305.1948.tb01338.x.

Spear, M.J., H.S. Embke, P.J. Krysan, and M.J. Vander Zanden. 2020. Application of eDNA as a tool for assessing fish population abundance. *Environmental DNA* 3(1):83–91, https://doi.org/10.1002/edn3.94.

Spens, J., A.R. Evans, D. Halfmaerten, S.W. Knudsen, M.E. Sengupta, S.S.T. Mak, E.E. Sigsgaard, and M. Hellström. 2017. Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: Advantage of enclosed filter. *Methods in Ecology and Evolution* 8:635–645, http://doi.org/10.1111/2041-210X.12683.

Stabeno, P.J., S. Bell, C. Berchok, E.D. Cokelet, J. Cross, R.M. McCabe, C.W. Mordy, J. Overland, D. Strausz, M. Sullivan, and H.M. Tabisola. 2023. Long-term biophysical observations and climate impacts in US Arctic marine ecosystems. *Oceanography* 36(2–3):78–85, https://doi.org/10.5670/oceanog.2023.225.

Sutter, M., and A.P. Kinziger. 2019. Rangewide tidewater goby occupancy survey using environmental DNA. *Conservation Genetics* 20(3):597–613, https://doi.org/10.1007/s10592-019-01161-9.

Taberlet, P., E. Coissac, M. Hakibabaei, and L.H. Rieseberg. 2012. Environmental DNA. *Molecular Ecology* 21(8):1,789–1,793, https://doi.org/10.1111/j.1365-294X.2012.05542.x.

Tabisola, H., J. Duffy-Anderson, C. Mordy, and P. Stabeno. 2022. EcoFOCI: A generation of ecosystem studies in Alaskan waters. Pp. 34–35 in *Frontiers in Ocean Observing: Documenting Ecosystems, Understanding Environmental Changes, Forecasting Hazards.* E.S. Kappel, S.K. Juniper, S. Seeyave, E. Smith, and M. Visbeck, eds, A Supplement to *Oceanography* 34(4), https://doi.org/10.5670/oceanog.2021.supplement.02-15.

Thomas, F., J.H. Hehemann, E. Rebuffet, M. Czjzek, G. Michel. 2011. Environmental and gut *Bacteroidetes*: The food connection. *Frontiers in Microbiology* 2:93, https://doi.org/10.3389/fmicb.2011.00093.

Velo-Suárez, L., M.L. Broshnahan, D.M. Anderson, and D.J. McGillicuddy Jr. 2013. A quantitative assessment of the role of the parasite *Amoebophrya* in the termination of *Alexandrium fundyense* blooms within a small coastal embayment. *PLoS ONE* 8(12):e81150, https://doi.org/10.1371/journal.pone.0081150.

Walsh, J.E. 2008. Climate of the arctic marine environment. *Ecological Applications* 18(sp2):S3–S22, https://doi.org/10.1890/06-0503.1.

TITLE PAGE PHOTOS

(top) Sea ice encountered during a spring EcoFOCI mooring cruise in the Bering Sea. (bottom left) Shannon Brown collects water samples for environmental DNA filters on NOAA Ship *Oscar Dyson*. (bottom right) Natalie Monacci and David Strauz assist with the deployment of the McLane Particle and Phytoplankton Sampler from NOAA Ship *Oscar Dyson*. Photo credits: (top) Shannon Brown; (bottom left and right): Iris Ekmanis

DATA AVAILABILITY

Raw sequence reads were deposited at the National Center for Biotechnology Information's Sequence Read Archive under bioproject PRJNA982176. Additional data and analyses were deposited at Figshare and can be found there for the 18S (https://figshare.com/articles/dataset/18S_Supplemental_Files/22330783) and 16S (https://figshare.com/articles/dataset/Microbial_16S_Supplemental_Files/22330462) data.

AUTHORS

Matthew P. Galaska (matt.galaska@noaa.gov) is Interdisciplinary Research Biologist, NOAA Pacific Marine Environmental Laboratory (PMEL), Seattle, WA, USA. **Shannon D. Brown** is Research Scientist, and **Sean M. McAllister** is Research Scientist, both at Cooperative Institute for Climate, Ocean, and Ecosystem Studies, University of Washington, and NOAA PMEL, Seattle, WA, USA.

ARTICLE CITATION

Galaska, M.P., S.D. Brown, and S.M. McAllister. 2023. Monitoring biodiversity impacts of a changing Arctic through environmental DNA. *Oceanography* 36(2–3):109–113, https://doi.org/10.5670/oceanog.2023.221.