Best practices for monitoring biological effects of ocean acidification with environmental DNA (eDNA) and other 'Omics approaches

March 17-18, 2025
Workshop Proceedings

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SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT

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EXECUTIVE SUMMARY

Climate change is impacting marine environments on a global scale. Along the California Current System (CCS), organisms at all trophic levels are at risk due to ocean acidification (OA). Understanding these impacts on biological communities is critical for developing and implementing effective environmental management strategies. While we routinely monitor ocean chemistry, the extent and magnitude of impacts of OA on biological communities are more difficult to assess.

Environmental DNA (eDNA) methods provide an opportunity to match the scale of our biological observations to our monitoring needs. eDNA is the DNA shed by living organisms into their environment. eDNA methods allow for querying environmental samples for multiple biological assemblages in a non-invasive and sensitive manner. In recent years, eDNA tools have been piloted in several marine monitoring programs throughout California and the northeast Pacific for surveilling fish, phytoplankton, microbes, zooplankton, and mammals. However, there is a lack of clarity on how these data sets can be used to report on the status and trends of biological impacts of OA. In addition, consensus lacks on how to best incorporate eDNA methods into routine monitoring programs, including both field and analytical methods, as well as priority taxa to survey as indicators or predictors of OA impacts.

To help expedite the integration of DNA-based monitoring to generate datasets that can be scaled across space, time, and monitoring programs, a two-day workshop was held in Costa Mesa, California on March 17-18, 2025. The goal of the workshop was to identify best practices for monitoring of the biological effects of OA using eDNA and other “omics” approaches. It included three sessions focused on identifying key metrics of biological response to OA stress and best practices for eDNA sampling, laboratory methods, and data analysis. In the final session, participants synthesized the conclusions from the three previous sessions and identified the top actionable items needed to lay the groundwork for scalable, accurate, and informative biomonitoring of OA stressors on biological communities.

Significant Workshop Findings and Recommendations

Among CCS regional monitoring program participants, strong motivation existed to incorporate eDNA into routine monitoring; several of these programs have already conducted pilots or have ongoing studies. Given this interest, an important opportunity exists to coordinate these programs to develop a standardized suite of eDNA-based indicators that can be adopted across the region. Two basic metric types are a priority: 1) population estimates (i.e., abundance measures) of targeted calcifying species as the natural complement to OA-specific measures (e.g., pCO2, alkalinity) and physiological responses to OA such as shell dissolution, and 2) community measures that provide a broader understanding of ecosystem change. To enhance

our ability to adopt these metrics into routine practice, workshop participants prioritized three actions:

1. **Develop assays for priority OA-indicator taxa.**

Genetic assays to quantify organismal relative abundance exist and methods for their development are well-known, but currently there are very few that target OA-indicator taxa such as calcifying organisms. Other approaches, such as quantitative polymerase chain reaction (qPCR) and digital PCR (dPCR), can be used to detect and track population abundances across space and time. Assay development should be prioritized for ecologically and commercially important bioindicator taxa such as Dungeness crab (*Metacarcinus magister*), spiny lobster (*Panulirus interruptus*), krill (*Euphausia pacifica*), or those known for their high degree of sensitivity to OA (e.g., pteropods). Development of these assays may require additional investments in DNA reference sequence generation for individual taxa and closely related taxa to ensure adequate assay specificity.

2. **Standardize eDNA tools and integrate datasets to assess biological community response to OA.**

Several marine biomonitoring programs already have existing OA-relevant eDNA data sets (i.e., DNA metabarcode sequencing data) that profile plankton communities, but the studies cannot be synthesized into a comparative assessment of the status of those communities because the bioinformatic pipelines used to interpret eDNA data are not standardized. With minimum investment, these datasets could form the foundation for time series that will yield insights to investigate how biological communities vary with environmental stressors, OA-relevant chemical parameters and other co-occurring climate stressors (dissolved oxygen, temperature).

To achieve this standardization, an eDNA taxonomic library of CCS species must be developed and linked to physiological traits that indicate the sensitivity of OA stress (for example, shelled vs non-shelled). Disparate datasets could be compiled and a working group supported to conduct a hackathon that would standardize the bioinformatics pipeline and analysis methods to synthesize these data.

3. **Genetically analyze the wealth of archived samples that already exist from long-term ocean monitoring programs.**

Workshop participants recognized that if regional monitoring program archival samples were analyzed, they could provide important insights into how global change is restructuring habitat for target sentinel taxa and marine communities in general. For example, CalCOFI has a 75-year archive of quarterly monitoring archival samples; this dataset could be a high priority for analysis of both qPCR/dPCR abundance of targeted species and eDNA community analysis.

Investing in these three key recommendations will help lay the foundation for a robust eDNA monitoring strategy to support environmental management decision making.

Finally, the workshop participants agreed much is to be gained by scaling from genes to genomes. Many empirical studies currently focus on understanding the metabolic underpinnings of sensitivity or resilience to OA stressors (e.g., calcification genes) via other 'omics approaches. However, these studies have yet to produce operationalized monitoring tools that can inform management decision-making. Future workshops could be focused on how genomic, or even transcriptomic, pathways can be leveraged to create OA early-warning tools based on detection of known biological stress responses.

INTRODUCTION

The effects of OA are becoming globally apparent and are particularly pronounced in upwelling-dominated coastal regions, such as the California Current System (CC*S*). Marine ecosystems are affected both by long-term OA trends as well as discrete events triggered by upwelling, nutrient input, or other phenomena. Monitoring these impacts has become a priority for coastal resource managers in California, who need to understand how different members of the ecosystem will respond differently to OA to better protect and manage the invaluable biodiversity of the CC*S*.

Current approaches to biological monitoring the effects of OA are limited. Pteropods, a group of pelagic sea snails, have emerged as key sentinel taxa for OA monitoring, with measurements of shell dissolution from declining aragonite saturation state being piloted among *CCS* regional monitoring programs. However, this method does not provide information about how pteropod populations are trending nor insight into community responses to this biological change. There is therefore an urgent need to develop more comprehensive approaches to assessing biological response at the ecosystem level.

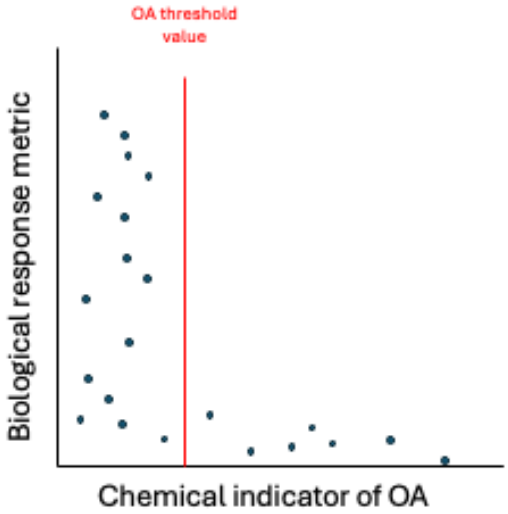
Environmental DNA ("eDNA") provides a scalable, accurate, noninvasive approach to biomonitoring that holds great potential to assess OA impacts on marine ecosystems. In a biomonitoring context, eDNA is used in one of two main ways: 1) for marker gene sequencing ("metabarcoding") to generate community composition data or 2) in single-species assays (using quantitative or digital PCR) to get quantitative information for individual taxa. Metabarcoding data is compositional and provides a snapshot of the relative abundance of multiple species. In contrast, qPCR and digital PCR are highly sensitive and quantitative for a single species or taxonomic group. Both techniques rely on primers designed to amplify the target molecules, which in turn rely on accurate, comprehensive reference sequence databases.

The choices of marker gene, target species, and assay can all be customized for specific research questions, making eDNA a valuable tool for measuring OA-specific impacts. However, the methodologies employed at each step of the workflow (including sample collection, preservation, processing, and bioinformatic analysis) can affect the results and their interpretation. Moreover, there is no consensus on the most appropriate biological metric to measure, whether it is the abundance of a single taxon, a diversity metric, or another index of community composition.

The California Ocean Protection Council (OPC) has been supporting science to pave the way for consistent OA-relevant biological monitoring; identification of optimal strategies and early priorities to incorporate eDNA into OA biomonitoring are urgently needed to better inform future monitoring efforts across California Coastal Waters.

Workshop Goals

To accelerate the development of methods and measurements that address key biomonitoring needs for OA impacts, the Southern California Coastal Water Research Project (SCCWRP) convened a 2-day workshop of thirteen leading researchers in ocean acidification, eDNA methods, and California Current Large Marine Ecosystem (CCLME) regional monitoring program managers. Participants were tasked with the goal of identifying which methods and measurements could provide a CCLME-wide synthesis of biological responses to OA, as shown in the conceptual graphic (right).



Towards this end, the goals of the workshop were to:

1. Determine which eDNA measurements should be used as indicators of OA impacts in biomonitoring efforts
2. Generate recommendations for eDNA sampling, processing, and analysis best practices in support of OA-focused biomonitoring
3. Identify knowledge gaps in both OA and eDNA research that need to be filled for the appropriate design of OA-focused biomonitoring surveys

Workshop Structure and Session Summaries

The first workshop session focused on identifying the appropriate biological response metrics (y-axis) for this graph. The second and third sessions addressed the sampling and analysis methods that should be used to generate the biological data. Participants assessed the commonalities among all represented monitoring programs as well as differences in methodology that would need to be resolved for effective data synthesis. The discussion included the relevant OA chemical indicator (x-axis), largely in the context of how that metric would need to be expressed depending on the habitat and targeted communities being sampled. Emerging tools like eRNA ("metatranscriptomics") and environmental metabolites ("metabolomics") were discussed as potential additional tools to generate alternative data streams for scientists and resource managers; however, there was a consensus that these tools were not ready for near-term implementation; more pilot testing and documentation of efficacy is needed.

The fourth and final session engaged participants to prioritize the scientific gaps in order to form the basis for a strategy to accelerate adoption of eDNA for OA biomonitoring.

Session 1. What are the key metrics of biological response and OA stress that we want to measure?

All biomonitoring programs include indicators that reflect the variable(s) or condition(s) of interest. In this session, participants discussed the most appropriate biological indicators for ocean acidification. Dr. Zack Gold presented on the state of the science of OA assessment in the context of biomonitoring and highlighted key aspects to consider, including the scale of measurements (i.e., molecular, cellular, organismal, or population-level) and the level of confidence required to quantify effects. The phenomenon of coral bleaching in response to water temperature was provided as a case study of a biological response that has been studied at the molecular and organismal level and can now be forecasted at the population level. OA stressors could be scaled similarly with the appropriate metric.

A few participants had personal experience conducting studies to identify OA impacts and measure them in the environment. The best-known impact was identified as the dissolution of calcium carbonate body parts, which characterize many marine invertebrate taxa. This is a result of the low aragonite and/or calcite saturation states of increasingly corrosive seawater. However, other effects have been documented including physiological stress responses and participants agreed that organisms highly affected by changing pH have yet to be identified.

Target indicators for biomonitoring could belong to one of two categories: 1) organisms for which the impacts of OA are well-studied or 2) organisms of high priority for economic or conservation reasons, such as spiny lobster, Dungeness crab, or krill. The impact of shell dissolution has been well studied in pteropods; thus, they are a potential target of molecular assays. However, pteropods compose a small fraction of the marine biological community, so community-based surveys may not capture the population-level effects. Animals like spiny lobster, Dungeness crab, and krill are important for recreational and commercial fisheries and form important prey sources for megafauna like whales, but the specific impacts of OA on these taxa are largely unknown.

The taxa listed above currently do not have well-established, species-specific primer sets that could be used in a targeted assay. Therefore, the design of new assays was identified as a high priority for any future OA biomonitoring work. An important prerequisite for primer design is high-quality reference sequences generated from voucher specimens of the indicator species and closely related taxa; these efforts must therefore also be prioritized for taxa that lack reference genomes or barcode sequences.

While individual organisms may be useful targets for biomonitoring, participants agreed that there are likely community-level effects of OA that have yet to be documented. Future work could include the development of a community index that incorporates multiple taxa. Such an index could be defined by a physiological trait, for example a "calcifier index," or a wider variety of taxa including multiple trophic levels. To develop an appropriate index, a trait-based database should be developed to link relevant physiological features, like shell building, to species names. Such a database would enable an assessment of functional diversity as well as taxonomic diversity, which participants identified as an important component of any OA biomonitoring.

Multiple regional monitoring programs are already generating metabarcoding data sets, with the most common markers targeting microbes (16S rRNA) and phytoplankton and other protists (18S rRNA). Participants agreed that these data sets could be used immediately for correlation analyses and identification of target taxa from these planktonic groups. However, species-specific assays for organisms with known OA vulnerabilities would be more quantitative and provide faster data turnaround. While krill and Dungeness crab assays are under development, other species like spiny lobster and the pteropod *Limacina helicina* would be highly valuable and these efforts need more resources to become fully operational.

Participants reached the following conclusions on biological indicators for OA:

- Community-based assays are ready for coordinated analysis to identify potential indicator taxa or taxonomic groups. This analysis could be facilitated relatively quickly with a coordinated effort like a hackathon.
- Species-specific assays need to be developed and should target calcifying plankton and invertebrate taxa.
- A trait database linking taxonomy with relevant physiological features would be highly valuable to assess functional diversity and could be used with both existing and future metabarcoding data sets.

Session 2. What are best practices for eDNA sampling?

Interoperability of eDNA data relies on harmonized sampling methodology following recommended best practices. Several regional biomonitoring programs were represented at the workshop, including the California Cooperative Oceanic Fisheries Investigations (CalCOFI), the Bight Regional Monitoring Program, the Hakai Institute Biomolecular Observing Network (HI-BON), the City of San Diego's Ocean Monitoring Program, Orange County Sanitation District's Ocean Monitoring Program, NOAA West Coast Ocean Acidification biomonitoring, and

NOAA Sanctuaries/Point Blue ocean monitoring. Together, these programs employ a range of sampling methodologies. Participants discussed the commonalities and discrepancies among the methods and where more coordination and standardization may be needed for data sets that can be compared across space and time.

This session began with a presentation by Dr. Nastassia Patin on eDNA sampling approaches, which fall into two major categories: 1) water column and 2) net tow. Water column sampling captures eDNA in its environmental proportions and can be used for analysis of all trophic levels. In contrast, net tows enrich for the organisms captured by the net mesh size and are generally suitable for zooplankton and invertebrates because these organisms are highly concentrated in the net.

For water column (or "bottle") sampling, water volume, filter pore size, and preservation method were identified as being the most important variables affecting captured eDNA. The most common filter pore size used across programs was 0.22 µm, which captures all cell sizes and trophic levels. However, this size also captures the highest proportion of bacterial:eukaryotic DNA, which presents more challenges for generating surveys of zooplankton, fish, or mammals. In contrast, a 1.0 µm or larger pore size excludes many bacteria and allows for higher water volumes to be filtered, which can improve recovery of higher trophic level eDNA. This approach can also be valuable in coastal or eutrophic waters which will clog small pore size filters more quickly than oligotrophic water. Filter preservation method has been shown in the literature to affect eDNA recovery, with buffers such as Longmire's and DNA/RNA Shield improving yields over ethanol or dry storage, but the methods used by the represented programs were in relatively good alignment. Participants agreed on the following water column sampling best practices:

1. Existing time series should maintain their historical filter pore size for continuity. However, where possible, higher water volumes and more biological replicates should be sampled.
2. While filter preservation method is unlikely to affect final data, an intercalibration study to test this hypothesis would be worthwhile.

In contrast to water column sampling, net tows varied widely across represented programs by mesh size, targeted depth, and tow method (e.g., oblique vs. vertical). CalCOFI was identified as having the longest time series and most robust approach for zooplankton collection using net tows, and this biorepository is currently being interrogated for historical zooplankton community dynamics. Thus, the CalCOFI approach should be considered the gold standard for net tow eDNA. However, participants agreed that it would be very challenging to align net tow methods across all programs. Therefore, participants reached a consensus that water column

sampling rather than zooplankton net tows should be a primary focus of cross-regional standardization of eDNA biomonitoring.

Session 3. What are best practices for laboratory and data analysis?

An integrated approach to eDNA-based biomonitoring entails running comparable assays on water samples and harmonizing methodologies for each assay. This harmonization applies to both the chemical variable measured as a proxy for OA as well as the biological (eDNA) variable. This session featured two presentations: one by Dr. Susie Theroux on molecular methods and the associated choices that affect data generation and accuracy, and one by Dr. Christina Frieder on generating appropriate OA metrics from water chemistry.

Christina Frieder presented aragonite saturation state (Ω_{ar}) as the community standard for assessing OA state in the ocean. However, the method for calculating Ω from other carbonate chemistry measures and the appropriate sampling resolution can vary. The California Current Acidification Network (C-CAN) recommendations provide two guidelines: a) to directly measure carbonate chemistry variables used for calculating Ω_{ar} , such as pH or pCO_2, and b) to limit the uncertainty to +/- 0.2. Participants agreed that adherence to these guidelines is important and emphasized the importance of replication and capturing a latitudinal and depth gradient to fully understand baseline OA conditions. Moreover, nutrient measurements were agreed upon as an important additional chemical data stream. Chemical oceanography model data was discussed as an alternative to empirical data when none is available. While participants agreed model data should not replace chemical measurements, they can be used as part of the assessment regime when *in situ* data are not available to pair with biological data.

The link between organism habitat and associated Ω_{ar} was a critical point of discussion in this session. Two questions were raised: 1) If eDNA data is generated from a discrete depth, is it necessary for the associated water chemistry to be taken at the same depth? 2) If target organisms vertically migrate, should they be associated with a discrete measurement or in integrated Ω index of the water column? Participants agreed these approaches may depend on the organism's physiology, and the importance of a trait database was reiterated to better understand how to interpret data and infer correlations.

The discussion on molecular biology methods first addressed the choice of quantitative PCR (qPCR) or digital PCR (dPCR) for species-specific assays. qPCR is cheaper and more commonly used and understood by the community; however, dPCR is more quantitative, less prone to inhibition, and does not rely on the generation of a standard curve. Participants agreed that

either method could be used effectively and any differences in methodology could be statistically accounted for when combining or comparing data.

Community-based assays include metabarcoding, shotgun metagenomics, and metatranscriptomics. Participants agreed that metabarcoding is the most appropriate approach for biomonitoring and harmonization among programs should include primer sets, PCR protocols, and sequencing chemistry. Several participants highlighted existing efforts to develop standardized protocols (in particular, the Better Biomolecular Ocean Practices initiative, or "BeBOP") and the development of controlled vocabulary and ontologies for data and metadata (FAIR eDNA or FAIRe terms). The 18S rRNA and CO1 marker genes were highlighted as particularly applicable for OA-related questions because the taxonomic breadth captured includes shelled organisms and other ecologically relevant taxa.

Regardless of assay choice, the first step of all molecular processing is DNA extraction. This step varies among programs, but participants agreed it did not merit a high level of concern for data interoperability based on the literature and personal laboratory experience. In contrast, participants agreed upon the importance of including negative controls at multiple stages of sampling and processing.

Finally, participants discussed the prospect of new sequencing technologies generating more data at lower cost and how this might change the eDNA landscape. For example, long-read benchtop sequencing could potentially generate full-length marker gene sequences requiring entirely different primer sets than the ones currently in use. However, while technological advances should be assessed, participants agreed that existing data sets using validated methods are highly valuable and should remain a focus of current research.

The following conclusions were reached at the end of this session:

1. Aragonite saturation (Ω_{ar}) or other carbonate saturation state should be estimated following the C-CAN guidelines and should be linked to associated eDNA samples according to target organism physiological traits.
2. Molecular methods may vary among programs, but all steps should be well documented using standardized terminology and easily interpretable protocols.
3. Either dPCR or qPCR can be used for species-specific assays. As discussed in previous sessions, these assays require dedicated resources and personnel for development and validation.
4. Existing metabarcoding data, in particular data sets using 18S rRNA or CO1 marker genes, should be integrated and analyzed across monitoring programs.

Session 4. Synthesis and recommendations for future investments

In this session, participants voted on their top three recommendations for future investments in eDNA-based OA monitoring. The following action items received the most votes:

1. Development of new species-specific marker gene assays targeting high priority taxa, including Dungeness crab, pteropods, spiny lobster, and krill species.
2. A hackathon-style workshop to integrate existing metabarcoding data sets across biomonitoring programs.
3. The development of a trait database linking taxonomy with OA-relevant features like calcification.

Other action items that received votes included an intercalibration experiment to test the effect of filter preservation method on eDNA recovery and data quality, and an analysis and potential re-design of the existing CO1 marker gene to maximize its capture of ecologically relevant taxa.

APPENDIX A. WORKSHOP AGENDA

Day 1

8:00 am: Light breakfast and welcome

8:30 am: Introduction

- Workshop goals, key products and process to get there
- Management needs: Opening remarks from Steve Weisberg
- Facilitated Q&A on key products and outputs from the workshop to inform monitoring strategies.

9:00 am: What are the key metrics of biological response and OA stress that we want to measure?

- Charge: The intent behind eDNA-OA monitoring is to measure OA stress (on the x axis), relative to a measure of biological response (on the Y axis). This session will focus on the appropriate 'omics measures of biological responses to OA and what OA metrics and environmental metadata are needed to inform the relationship between OA stress and biological response.

12:00 pm: Lunch

1:00 pm: Session 2: Best practices for sampling

- Charge: Once we understand what key metrics should be monitored, the next step is to identify the best practices for sampling eDNA and OA. This session will produce those recommendations.

5:00 pm: Summary of progress and prospectus for the next day

6:00 pm: Group dinner

Day 2

8:30 am: Light breakfast and welcome

9:00 am: Best practices for laboratory and data analysis

- Charge: This session will focus on recommendations for best practices for laboratory and data analysis of eDNA, OA chemistry and environmental metadata.

12:00 pm: Lunch

1:00 pm: Session 4: Synthesis and recommendations for future investments

- Charge: During this session, participants will reflect on what we produced to date, then focus on identifying key gaps and recommendations for future investments in science and implementation support of regional monitoring programs.

2:00 pm: Wrap up and next steps

3:00 pm: Adjourn

APPENDIX B. WORKSHOP PARTICIPANTS

Ashton Bandy, UC Irvine

Christina Frieder, SCCWRP

Zachary Gold, Natural History Museum of Los Angeles County

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Jaime Jahncke, Point Blue

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Sean McAllister, UW CICOES

Megan Hepner, Southern California Coastal Ocean Observing System

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