Title: Enhancing biodiversity monitoring efficiency through CRISPR-driven depletion and enrichment of aquatic environmental DNA

Running Title: Biomonitoring marine ecosystems using CRISPR-Cas

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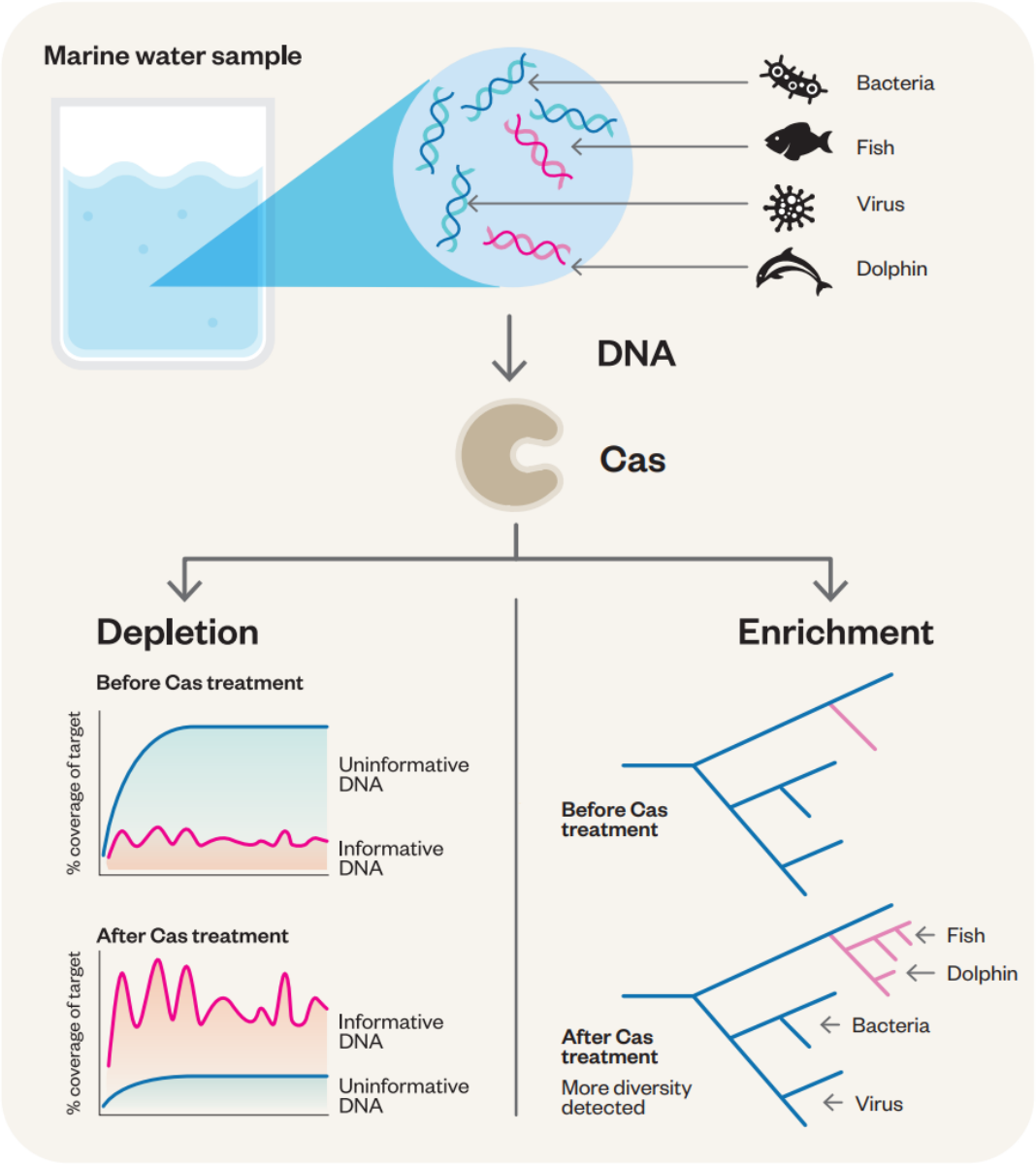
41 # Graphical Abstract



42

Abstract

Characterising biodiversity using environmental DNA (eDNA) represents a paradigm shift in our capacity for biomonitoring complex aquatic environments. However, eDNA biomonitoring is limited by biases towards certain species and low taxonomic resolution of current metabarcoding-based approaches. Shotgun metagenomics of eDNA enables the collection of whole ecosystem data by sequencing all molecules present in a sample, allowing them to be characterised and identified. Ongoing enhancements of whole genome reference databases are improving the resolution of shotgun metagenomics, reducing database related limitations in species and individual identification for metagenomic studies. However, shotgun metagenomics-based insights are constrained by preferential sequencing, favouring more abundant organisms in the water column like bacteria and viruses over less abundant target species like vertebrates. To improve the probability of detecting low abundant target organisms, methods such as target species enrichment or the removal of non-target DNA prior to sequencing can be employed. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the CRISPR-associated proteins (Cas) have recently emerged as a novel technology that can achieve both enrichment and depletion. CRISPR-Cas-based methods have the potential to improve the efficiency of eDNA metagenomic sequencing and simplify data analysis by reducing non-target data filtration steps, however, they have not been widely implemented due to a lack of interest and support in the past, as well as limited number of studies demonstrating they can be applied in a robust and cost-effective manner. Here, we review current approaches of CRISPR-Cas to study underrepresented aquatic taxa. We advocate that further optimization of depletion and enrichment methods of eDNA using CRISPR-Cas holds great promise for the rapidly evolving field of eDNA biomonitoring through refining monitoring approaches, overcoming PCR bias, and enabling efficient high-throughput applications.

Introduction

Assessing the health of the vast marine biome poses a significant challenge. Currently, most biomonitoring techniques implemented in aquatic environments rely on visual identification of species or measurements of physico-chemical water quality attributes as a proxy for ecosystem health. While visual methods provide valuable estimates of population health and size, they often miss or underestimate cryptic taxa, are frequently unable to identify juveniles and "damaged" specimens, and rely on SCUBA diving and/or video surveys as well as taxonomic specialists for visual species identification (Glaviano et al., 2022; Zhai et al., 2023). Likewise, physico-chemical properties offer valuable real-time insights into ecosystem health, but are not capable of measuring organism presence or abundance (Smale et al., 2011). Molecular-based monitoring techniques using environmental DNA (eDNA) for biodiversity detection have grown rapidly over the past decade (Bayer et al., 2024; Blackman et al., 2024; De Brauwer et al., 2023; Ficetola et al., 2008; Garlapati et al., 2019; Taberlet et al., 2018; Takahashi et al., 2023). Environmental DNA biomonitoring has recently been applied to health indices (Borja, 2018; Pawlowski et al., 2022; Wilkinson et al., 2024), as the increased reliability and accuracy of eDNA biomonitoring through continuous methodological innovation has been shown to be comparable to, and even surpassing, previous monitoring records with regards to efficiency and species detection (Baetscher et al., 2023; Elbrecht et al., 2017; Shea et al., 2023; Wilkinson et al., 2024). To accommodate eDNA biomonitoring in regional, national, and global surveillance programmes, best practice guidelines have been developed for consistent eDNA sampling and analysis (De Brauwer et al., 2023; Minamoto et al., 2021). With the ever pressing need for faster, more comprehensive, and consistent monitoring of marine environments driven by challenges such as invasive species incursions and range shifts due to climate change (Rahel et al., 2008); habitat loss and degradation; and unsustainable anthropogenic activities such as overfishing and pollution (Duda & Sherman, 2002; Pratchett et al., 2011; Trainer et al., 2020), scientists and end-users are currently exploring advancements in molecular methods to address these challenges more effectively compared to traditional monitoring techniques.

Metabarcoding is a widely adopted technique that uses taxonomically broad eDNA assays to target specific groups, such as fish, elasmobranchs, or corals (Alexander et al., 2020; Leray et al., 2013; West et al., 2021), or broader 'universal' targets such as eukaryotes (Geller et al., 2013; Leray et al., 2013). Metabarcoding provides the opportunity to assess biodiversity across the tree of life (Stat et al., 2017). However, metabarcoding relies on PCR amplification of barcoding gene regions for species detection, thereby introducing PCR amplification bias through variable thermodynamic binding affinities of primers for different taxonomic groups, leading to incomplete or unrepresentative results

with no correlation to species relative abundance (Baetscher et al., 2023; Elbrecht et al., 2017). Primer set and bioinformatic pipeline choice can further influence the accuracy of metabarcoding results, with the same primer set behaving differently and not always predictably based on the complexity of an eDNA sample and what species are present, and with different bioinformatic pipelines each having different criteria the data is tested against, thus causing variable results from the same data (Baetscher et al., 2023; Bayer et al., 2024; De Brauwer et al., 2023; McCauley et al., 2024; Pearce et al., 2023; Shea et al., 2023). While metabarcoding holds promise for rapid ecosystem monitoring, reliance on PCR amplification is resulting in false-negative species detections challenges in accuracy and limiting quantitative measurements of species abundance and biomass, thereby presenting challenges for widespread adoption as a monitoring method for management and the knowledge-transfer to end-users (Elbrecht et al., 2017; Ruppert et al., 2019).

Hybridisation capture enrichment methodologies offer an alternative to PCR-based approaches and address many of the current drawbacks of metabarcoding. Capture enrichment uses biotinylated probes to hybridise with the target DNA which can then be pulled out to separate the target DNA bound to the probes from the rest of the DNA. Capture enrichment eliminates the need for PCR, allowing for more quantitative measurements than metabarcoding (Giebner et al., 2020; Wilcox et al., 2018). However, as with any technique, capture enrichment methods have specific limitations. Capture efficiency generally relies on high probe-target similarity (~150bp), restricting assays to targeting a single, or very closely related, species (Jimenez-Mena et al., 2022), though family-specific probes are feasible for conserved DNA regions (Dickson et al., 2021) and capture across greater divergences is possible with a more time-intensive relaxed hybridisation approach (Li et al., 2013). Furthermore, capture enrichment protocols require lengthy incubation (24- 48 hours), limiting scalability (Wilcox et al., 2018). Despite the advantage of capture enrichment approaches, these mentioned limitations hinder their widespread adoption in aquatic biomonitoring applications.

Metagenomics, i.e., shotgun sequencing of native eDNA, sequences genomic DNA of all species in a sample. Due to the lack of target enrichment through PCR amplification or bait capture, limitations in the abovementioned techniques are circumvented. The comprehensive information gathered can enable monitoring of species at the individual level through population genetics and Single Nucleotide Polymorphism (SNP) analysis (Afshinnekoo et al., 2015; Cowart et al., 2018; Deiner et al., 2017; Williamson et al., 2008). However, incomplete reference databases for whole genomes of eukaryotes (Singer et al., 2020) and the 4.5-fold higher cost to run the assay compared to metabarcoding (Bell et al., 2021) limits the utility and scalability of shotgun sequencing (de Jong et al., 2024; Pollie, 2023). These two limitations are gradually reducing through continual efforts to

expand reference databases (de Jong et al., 2024) and sequencing costs will continue to decrease with expanding technology and uptake by industries (Pollie, 2023). Still, data dominated by uninformative repeats and highly abundant non-target species DNA is the largest obstacle of shotgun sequencing in routine biomonitoring (Singer et al., 2020; Stat et al., 2017; Tessler et al., 2017). For example, Stat et al. (2017) found that in marine eDNA samples, most reads were of bacterial origin (94.5%), followed by viruses (3.0%), with only 2.4% of reads originating from eukaryotes, of which only 0.00004% of reads were fish (class Actinopterygii, Chondrichthyes, and Cyclostomata). Hence, depletion of over-abundant organisms and sequences must be carried out by, for example, removing the mitochondria and chloroplast organelles (Miller et al., 2017) or by removing RNA sequence types (Wahl et al., 2022). However, these methods cannot remove data in a sequence specific way.

Here, we explore two options for Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the CRISPR-associated proteins (Cas) technologies to circumvent current drawbacks of metagenomic sequencing: (1) the removal of non-target sequences to limit DNA exploration to target species, and (2) selectively enriching for taxa of interest. Unlike PCR amplification and hybridisation capture approaches, CRISPR-Cas in conjunction with shotgun sequencing provides the precision of probe enrichment and non-target depletion without the need for lengthy laboratory protocols to capture genome-wide data of whole ecosystems from environmental DNA samples.

CRISPR-Cas mechanisms and Diversity

CRISPR-Cas was discovered as part of an adaptive immune system in bacteria and archaea (Jinek et al., 2012). Due to its ability to precisely target DNA, CRISPR-Cas has since been adapted to selectively target both nucleic and non-nucleic acids for various applications such as cancer treatment (Z. Liu et al., 2023), agriculture (Zaidi et al., 2020), or controlling pest populations (Bier, 2022). The system uses a short guide RNA (sgRNA) probe in complex with a Cas protein to precisely cleave the complementary DNA to the sgRNA (~20 bp long) downstream of a protospacer-adjacent motif (PAM) site.

CRISPR-Cas-based technologies leverage the Cas enzymes programmability through their sgRNAs and their cleavage properties for both '*cis* cleavage' (e.g., Cas9; Jinek et al. (2012)) and *cis*-triggered '*trans*-cleavage' (e.g., Cas12, Cas13; Abudayyeh and Gootenberg (2021)). Directed evolution in CRISPR-Cas systems (Koonin & Makarova, 2022; Mohanraju et al., 2022) and bioengineering have generated variants with nearly no PAM requirements, the option for no enzymatic cleavage activity (Kong et al., 2023; Tang, 2020; Walton et al., 2020), and, recently, the possibility of generating new CRISPR-Cas systems (Nguyen et al., 2024). Despite the early stage the field is in, the expanding

repertoire of commercial Cas enzymes (e.g., dCas9, mutated Cas9 nickases, Cas9 fused with reverse transcriptase enzyme, and recombinant Cas12a [IDT Technologies]), the discovery of new enzymes (Hu et al., 2022; Koonin et al., 2023; Tang, 2020), and the significant private investment (i.e., JumpCode genomics, Mammoth biosciences, Sherlock biosciences) has resulted in diverse CRISPR-Cas based studies. However, Cas:sgRNA:target interaction efficiency can be influenced by factors such as GC content (Yu et al., 2017), optimal sgRNA lengths for different enzymes (Y. Liu et al., 2023; Lv et al., 2019), preferences of nucleotides in specific positions in the sgRNA for enhanced DNA binding, and distinct regions within the sgRNA that have varying abilities to accept mismatches (Y. Liu et al., 2023; Molina Vargas et al., 2023; Specht et al., 2020). Addressing these factors is crucial for effective CRISPR-Cas deployment in environmental studies. Consequently, ongoing efforts include the development of deep learning models to systematically account for these factors (Duran-Vinet et al., 2023; Mantena et al., 2023; Metsky et al., 2022). In conjunction with more accurate sgRNA design capabilities, research into the generation of RNA guides is underway (Gilpatrick et al., 2023), allowing for assays that can be customised in a matter of hours (Metsky et al., 2022) without compromising the outcome quality of the desired assay.

The promise of CRISPR-Cas deployment in environmental DNA studies

Depleting over-abundant DNA

One of the first approaches to deplete non-target sequences using CRISPR-Cas is the Depletion of Abundant Sequences by Hybridization (DASH, Figure 1a) method developed by Gu et al. (2016). DASH works by cleaving target DNA in a sequencing library so that only non-targeted sequences with adapters at both ends remain to be sequenced. Rossato et al. (2023) demonstrated the effectiveness of using the DASH method by using 566,766 sgRNAs to deplete repetitive elements in a lentil genome to improve genotyping methods. DASH-depletion resulted in a 37.7%–41.2% reduction of repetitive DNA sequences, with an increase of up to 160% in target DNA reads sequenced. This led to the identification of ~4.5–to ~18-fold more variants in the DASH-depleted samples when compared to non-depleted samples. Underscoring the success and effectiveness of the approach, commercialisation of this technique has already occurred with a similar technology called "CRISPRclean" developed by JumpCode genomics. CRISPRclean has been used in microbiome analyses (Uranga et al., 2022), single cell transcriptomics (Pandey et al., 2023), and ribosomal RNA (rRNA) depletion (Cerón et al., 2022). Other CRISPR-Cas based depletion methods have also been used in immunotherapy for cancer treatment by down-regulating the methionine transporter

SLC43A2 and restricting the methionine uptake by tumor cells (Huang et al., 2023), used as an antimicrobial agent by selectively depleting antibiotic resistant strains of bacteria (Rodrigues et al., 2019), or used to deplete eukaryotic host DNA for amplicon sequencing in microbiome studies (Zhong et al., 2021). The diverse range of demonstrated applications of DASH and other CRISPR-Cas technologies provide an encouraging foundation for its potential use in eDNA monitoring of aquatic environments.

For the use of DASH and other CRISPR-Cas depletion technologies to be useful, eDNA samples must first be shotgun sequenced to find the most abundant sequences to be targeted. Once this is done however, marine eDNA samples are similar, comprising common bacteria, viruses and repeats with predictable patterns of most abundant species (King et al., 2023; Munson-McGee et al., 2022). Meaning the same set of guides can be used in multiple areas reducing most of the cost to develop these depletion assays. CRISPR-Cas depletion can fill a gap in sequence specific depletion methods that has not been possible yet without taking advantage of structural differences in unwanted DNA (i.e., rRNA) or removing organelles to remove their unwanted DNA.

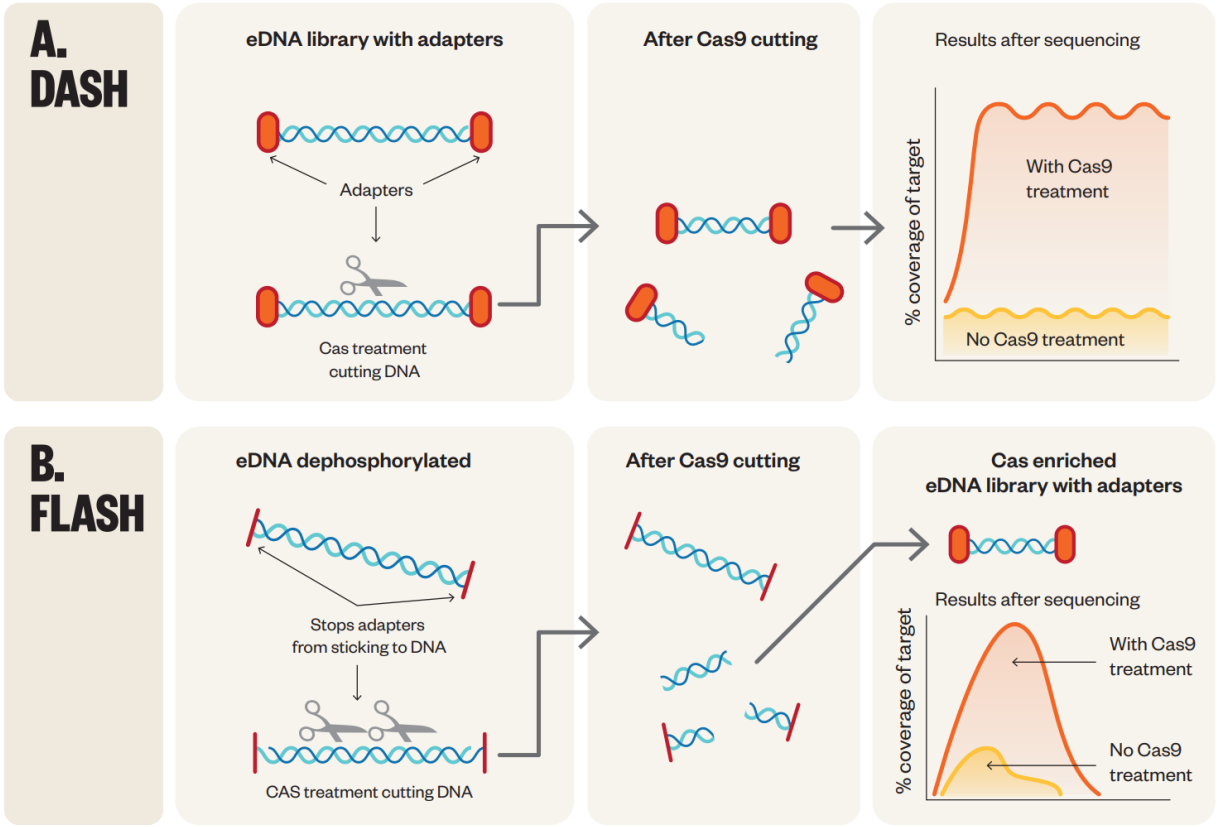


Figure 1. DASH and FLASH basic principle. A) DASH uses Cas9 is to target and cleave unwanted sequences after library preparation, where the cleaved libraries will not be amplified in later steps

enriching low-abundant target sequences. B) Using DNA that has dephosphorylated ends which will block sequencing adapter ligation, FLASH enriches DNA fragments that are cut by Cas9 to allow adapters to be ligated onto the Cas9-cut DNA ends, while non-target sequences will have no adapters ligating and so cannot be sequenced.

Enrichment of low-abundant DNA

Conversely to CRISPR depletion, CRISPR enrichment techniques offer a promising means of selectively targeting and enriching DNA sequences in an eDNA sample. By using Cas enzymes to treat the DNA and isolate it in various ways, as extensively detailed by Schultzhaus et al. (2021), CRISPR-Cas-based enrichment becomes a powerful and highly adaptable tool to enrich almost any sequence of target taxa before sequencing, thereby enabling enhanced species and individual identification with ultra-conserved elements (Pierce, 2019) or SNP analysis (Afshinnekoo et al., 2015; Cowart et al., 2018; Williamson et al., 2008). Quan et al. (2019) demonstrated the use of CRISPR-Cas enrichment through a method called "Finding Low Abundance Sequences by Hybridisation" (FLASH, Figure 1b), a simple way of targeting specific DNA sequences for enrichment. FLASH first dephosphorylates the ends in DNA libraries preventing adapters from ligating onto these ends, and then uses CRISPR-Cas9 to cut target DNA to allow for sequencing adapters to be ligated onto the cut ends. FLASH treatment of simulated clinical dried blood spot samples targeting drug resistant malaria sequences produced far higher on-target sequencing efficiency with 85.6% on-target sequence reads, compared to <0.02% on-target reads without FLASH enrichment (Quan et al., 2019). Moreover, with costs of <$1 US per library, FLASH also provides a cost-effective option for detection of rare sequences (Quan et al., 2019). Targeted single species enrichment using other CRISPR-Cas technologies has been demonstrated in several studies, for example in detecting SARS-CoV-2 (Broughton et al., 2020), endangered delta smelt (Nagarajan et al., 2024), harmful algal blooms (Pal et al., 2023), and Atlantic salmon (Williams et al., 2023; Williams et al., 2021). Although multiplex species enrichment and detection with FLASH is possible and comparable to metabarcoding studies in freshwater bulk eDNA samples (Kardailsky, 2023), and despite FLASH's capability to detect very rare and low abundance species, its application beyond human disease studies remains largely unexplored.

CRISPR-Cas enrichment offers distinct advantages compared to other enrichment techniques allowing for a high degree of multiplexing. For example Quan et al. (2019) used 5,513 sgRNAs to target 127 genes, and Ackerman et al. (2020) used more than 4,500 sgRNAs in one assay to detect 169 different species with no detectable reduction in reaction efficiency. Additionally, CRISPR-Cas enrichment and depletion offer solutions to several challenges encountered in current eDNA monitoring methods, including the potential for quantitative assessment by avoiding PCR limitations,

streamlining lengthy laboratory stages, and by increasing specificity/accuracy allowing for portable and isothermal assessment (Cai et al., 2024; Islam & Kasfy, 2023; Vargas et al., 2023).

eDNA limitations and CRISPR opportunities

CRISPR-Cas based methods have shown promise in eDNA studies (Kardailsky, 2023; Nagarajan et al., 2024; Wei et al., 2023; Williams et al., 2023; Williams et al., 2021). So far, we have explored the possible improvements that can be made to eDNA monitoring through selective depletion of highly abundant DNA using DASH and selective enrichment of low-abundant DNA using FLASH. Besides FLASH/DASH, we explore three more promising further avenues of CRISPR-based biomonitoring approaches.

Detecting eDNA interactions and dynamics

The accuracy of eDNA studies is limited by our knowledge of factors including eDNA decay (Lamb et al., 2022), exogenous DNA interactions (e.g., biofilms from bacteria; Panlilio and Rice (2021)), and endogenous DNA interactions (e.g., nucleosomes inhibiting protein binding and cleavage; Yarrington et al. (2018)). The vast diversity in organism types in an eDNA sample (e.g., vast diversity in all Bacteria, Archaea, Eukarya domains) means that DNA condition or DNA interactions within the eDNA collected can be hard to predict (Panlilio & Rice, 2021), which may impact the ability to isolate the DNA of interest. CRISPR-based screens of extracellular DNA can shed light on the mechanisms of DNA release, like those in cell free DNA in human studies (Davidson et al., 2024) and as a result inform targets for eDNA based monitoring if certain DNA fragments are more readily released than others.

The degradation of eDNA and eRNA are difficult to quantify (Scriver et al., 2023), but leveraging CRISPR-Cas-based techniques can allow for more accurate estimates of degradation rate. By sequencing specific species to a higher read depth than is possible without enrichment or depletion we can simplify the high variability of biological systems and more easily estimate degradation rates. From known degradation rates we could estimate the time since an animal may have been present in the environment, possibly uncovering distinct degradation or methylation patterns which may exist between different domains or stages of life (Zhao et al., 2023), which could be exploited to remove unwanted species or predict age ranges.

The benefits of identifying and quantifying all organisms and their interactions in an environmental sample are becoming more apparent (Duda & Sherman, 2002; Rahel et al., 2008) and CRISPR-Cas methods can assist this process. Specifically, CRISPR-Cas-based depletion of uninformative sequences

from non-target taxa can increase the depth of information gathered on low-abundant organisms which would include organisms that have not been studied before. These rare or understudied organisms can shed further light onto inter-species interactions, for example polymicrobial interactions creating biofilms are common in disease (Peters et al., 2012), they may also be common in harmful algal bloom biofilms. Targeting a single species will miss these interactions. In this scenario, once interacting species are identified, enrichment of the specific species can then further show what DNA interactions are occurring by avoiding PCR (which removes epigenetic marks on the DNA) and in turn let us learn more about the abiotic/biotic factors influencing persistence, and toxin production in harmful algal blooms. Though other CRISPR-based enrichment methods such as CAPTURE (Slesarev et al., 2019) must be used instead of FLASH, as FLASH does have a short PCR amplification step to attach barcode primers. Additionally, CRISPR-Cas-based biomonitoring can potentially be applied for accurate and sensitive early detection of blooms by sampling eDNA instead of relying on spectrophotometry methods (Durán-Vinet et al., 2021).

eRNA-based biodiversity mapping

In addition to eDNA, environmental RNA (eRNA) is a growing field in environmental biomonitoring, offering distinct advantages, but also some disadvantages, over eDNA due to its faster degradation rate. Its rapid degradation means that eRNA can signal the presence of live organisms at the time of sampling (i.e., if it is found present in a sample then it can be assumed that the organism was still alive at the time of sample collection), and its decay rate can be used to estimate a more precise location of an organism (Scriver et al., 2023). Comparatively, eDNA persists longer in the environment with eRNA potentially having 4-5 times faster half-life than eDNA depending on many environmental factors (Scriver et al., 2023), potentially leading to false positives if an organism has left the area or organism DNA has been carried into the area by predators. Furthermore, as reviewed in Scriver et al. (2023) eRNA may offer improved correlations with fish abundance estimates compared to eDNA due to the tendency of eDNA to disperse via water currents to areas where the target animal may not be present. Knowledge on eRNA also aids in predicting eDNA age since being released from the organism (Marshall et al., 2021), helping to refine species distribution estimates. However, sampling and sequencing eRNA presents challenges because of RNA's rapid degradation, sampling difficulties associated with RNA sampling, and the need for a cDNA conversion step for most sequencing technologies (Scriver et al., 2023) excluding nanopore direct RNA sequencing (Jain et al., 2022). Moreover, eRNA is highly repetitive due to the common transcripts being released (e.g., rRNA), and so requires treatment to target informative sequences.

CRISPR-Cas methods such as DASH and FLASH can further address the limitations of RNA analyses. While RNA depletion kits exist to optimise RNA analysis, for example by removing abundant rRNAs (Wahl et al., 2022), these kits lack the sequence-specific targeting capability of CRISPR-Cas technologies. This limitation restricts depletion to only certain sequences and limits the efficiency of RNA analysis. This presents an opportunity for DASH, which has already been successfully adapted to RNA sequencing by Prezza et al. (2020) and in MAD-DASH by Hardigan et al. (2019), to remove adapter dimer and abundant miRNAs respectively. RNA enrichment methods are similar to capture enrichment approaches (Chung et al., 2018), and consequently they also share the same limitations. FLASH represents a promising alternative for RNA enrichment, underscoring the potential for CRISPR-Cas to contribute to the advancement of eRNA techniques. We believe that with the use of CRISPR-Cas based enrichment and depletion methodologies, RNA-based biomonitoring represents a promising avenue for further exploration.

In-field detection challenges and solutions

For eDNA detection and monitoring methods to be most effective, there is a growing demand for in-field detection capabilities in all environments regardless of the resources available. Advancements such as SHERLOCK – Specific High Sensitivity Enzymatic Reporter UnLOCKing (Baerwald et al., 2023) or DETECTR – DNA Endonuclease-Targeted CRISPR Trans Reporter (Wei et al., 2023) which use Cas13a and Cas12a respectively for florescence readings have paved the way for such developments. Many CRISPR technologies have been harnessed for targeted single species detection in a portable and compact way (e.g., SARS-CoV-2, Dengue, Zika), exemplified by lateral flow assays (Li et al., 2023), fluorescence-based detection (Ackerman et al., 2020; Baerwald et al., 2023; Wei et al., 2023), and automated systems using robots for sampling and detection in the environment (Zhang et al., 2023). Some devices for multiplexed testing have been developed, for example through microfluidic devices outputting fluorescence signals when sgRNAs can bind to their target, achieved by Welch et al. (2022) for SARS-CoV-2 variants, by Xu et al. (2022) for HPV subtypes, and Gootenberg et al. (2018) for Zika and Dengue virus detection.

Both the DASH and FLASH methods require a thermocycler and sequencing machine, posing logistical challenges for in-field monitoring. Despite these challenges, ongoing advancements in in-field DNA extraction methods (e.g., PDQeX (Nguyen et al., 2022), ExCad (Jeunen et al., 2022; Stanton et al., 2019), and HUDSON (Qiu et al., 2023)), hand-held lab equipment such as PCR machines (Myhrvold et al., 2018), portable sequencers (Truelove et al., 2019), and other technological advancements compatible with CRISPR-Cas offer potential solutions for more accessible in-field monitoring where fast and accurate detections are needed (e.g., remote locations for biosecurity

monitoring). Both DASH and FLASH methods can be used at sea if molecular laboratories are available on board. These methods both have relatively short lab protocols as the most time-consuming step is a two-hour incubation. As with most initially expensive technologies, the cost of CRISPR-Cas assays can reduce dramatically when used in repeated monitoring (Rossato et al., 2023). The adaptability of CRISPR-Cas to other technologies, its relatively short protocols, and its scalability are major advantages over other capture enrichment and depletion methods and can empower users to undertake eDNA studies in a variety of environments with greater ease and efficiency.

Summary

Environmental DNA promises to be an effective method to acquire whole ecosystem information in a cost- and time-effective way (Blackman et al., 2024; De Brauwer et al., 2023; Garlapati et al., 2019; Taberlet et al., 2018; Takahashi et al., 2023). We propose that CRISPR-based approaches can significantly improve the efficiency of eDNA data analysis and reduce computational burden. Techniques such as DASH and FLASH, in combination with shotgun sequencing, show promise in improving eDNA sequencing by removing non-target DNA and streamlining enrichment, thereby enhancing the overall accuracy and quality of results. Integration of CRISPR-Cas technologies offer avenues for refining monitoring approaches, overcoming PCR bias, and enabling efficient high-throughput applications. Moreover, the adaptability and scalability of CRISPR-Cas systems provide a customizable toolset to meet diverse research needs and study limitations. Broader interest and support for more accurate monitoring methods may lead to wider adoption of CRISPR-Cas techniques, revolutionizing our capacity to monitor natural systems on a global scale.

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