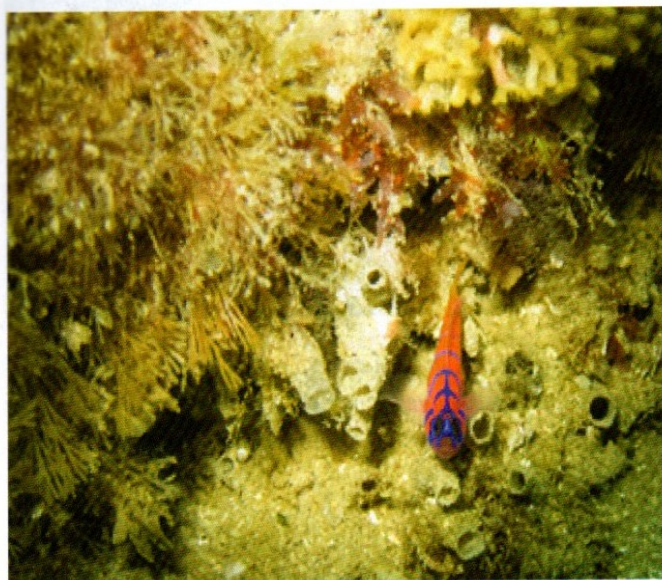


The Emergence of eDNA

LESLEY EVANS OGDEN

An interdisciplinary tool helps monitor biodiversity and health.



Lythrypnus dalli, the bluebanded goby, is a cryptic fish characterized by eDNA, found in the kelp forests of the Long-Term Ecological Research site off the Santa Barbara coast. Photographs: Thomas Lamy.

B Biologists have long struggled with the limitations of conducting physical, visual, or acoustic surveys as a means to identify life's richness and abundance. Visual identification has traditionally required specialized taxonomic skills and has been prone to identification errors, and physically sampling live organisms can have a destructive environmental impact, with the small size or cryptic behavior of some species making them hard to find and easy to miss. That has led to a quest for alternative methods to monitor biodiversity qualitatively and quantitatively. One new and promising tool is environmental DNA.

The use of environmental DNA (eDNA) is growing across biological fields, from ecology and conservation to fisheries, environmental

engineering, and public health. As an analysis technique, eDNA (or eRNA for viruses) capitalizes on the idea that every individual of each species leaves behind genetic traces of itself in its environment. As organisms move through time and space, they excrete urine and feces; shed tiny fragments of skin, hair, fur, feathers, slime, or mucus; deposit eggs, sperm, pollen, or seeds; and after death, leave behind scraps of decomposed tissues. These traces can be sampled, with bits of DNA extracted and sequenced, using primers to amplify sections of interest or performing bulk analyses of pooled material using techniques known as *metabarcoding* and *metagenomics*. Methodological protocols for eDNA analysis are now widely available and rapidly improving as the technologies

available for genetic sequencing grow ever faster. So eDNA has become an increasingly valuable tool for taking advantage of these formerly ignored and invisible castoffs to answer a range of research questions and meet monitoring goals.

Capturing the ghost of presence past

"It's very trendy to use eDNA right now," says Thomas Lamy, former post-doctoral researcher with the Marine Science Institute at the University of California, Santa Barbara, now a marine ecologist at the University of Montpellier, in France. Contributing to research on the natural and human drivers that affect kelp forest dynamics off the Santa Barbara coast, Lamy and colleagues piggybacked on the

long-term ecological research site running there since 2000 to test the utility of using eDNA for biodiversity monitoring. “For at least 20 years, we had a good understanding of how fish species change over space and time,” via diver-collected visual transect surveys, says Lamy. With well-established baseline data available, this was an ideal location for testing the utility of eDNA. Collaborating with the Monterey Bay Aquarium and its extensive experience in metabarcoding, Lamy’s team took triplicate water samples at 11 shallow subtidal rocky reef sites, filtering them before extracting and analyzing the captured DNA.

Beginning as a pilot study, says Lamy, “it really blew my mind to see how well eDNA was doing at trying to capture the diversity of the fish.” From a few samples taken in 2017, “we captured and detected more species than had been recorded for decades,” he says, with eDNA analysis identifying many species absent from visual surveys such as pelagic species that likely quickly swam away from divers or those cryptic in appearance or microhabitat choice. That is not to say there are not detection gaps when using eDNA, notes Lamy. For example, in rockfishes, genus *Sebastes*, an ancient and fast evolving clade of fishes, the database of sequences available was not well enough resolved at the time of their study to distinguish between species.

Indeed, for species identification via eDNA, a well curated local and global database is key. The development of new primers to target particular fragments of the genome is improving eDNA’s scientific power. Lamy is also working in Madagascar, in scientifically understudied marine ecosystems crucial to a region reliant on fish for food security. More than 2000 fish species occur there, with many gaps in the reference database. Lamy is working with Madagascan fishers to access specimens that can be sequenced and added to the genetic library.

Beyond establishing what species are in a region, one of the challenges of keeping tabs on the status of



In Brazil’s Atlantic Coastal Forest Carla Martins Lopes filters water that will be analyzed for eDNA. Her analyses have identified rare frog species including some not detected for decades. Photograph: Célio F. B. Haddad.

endangered and threatened species is their rarity. That makes it difficult and often undesirable to invasively seek them out. Sampling noninvasively has made eDNA a welcome addition to the conservation scientist’s tool kit. In Brazil’s Atlantic Coastal Forest and Cerrado grasslands, for example, a team of conservation biologists led by Carla Martins Lopes, postdoctoral researcher at Brazil’s Universidade Estadual Paulista, collected water samples during the wet season from six sites to examine amphibian diversity. Her team found DNA traces of four declined species (*Hylodes ornatus*, *Hylodes regius*, *Crossodactylus timbuhy*, and *Vitreorana eurygnatha*), two species thought to have locally disappeared (*Phasmahyla exilis* and *Phasmahyla guttata*), and one frog species (putatively *Megaelosia bocainensis*) not seen since 1968. Now, her team is expanding their eDNA sampling to additional regions of Brazil to examine whether frog declines match up with the presence of disease.

Another project is focused on using eDNA to examine oyster reef

communities. In the Port Royal Sound area of South Carolina, oysters establish in intertidal reefs, settling onto a solid surface after their free-floating larval stage. This aggregation of oysters and their shells provides shelter for a diversity of other microscopic and macroscopic creatures, including mussels, barnacles, sea anemones, and fish. “When the tide’s out, the oyster reef is a buffet for birds to come and pick things off, ... and when the tide comes back in, ... it’s a nursery ground for another set of small organisms,” says marine geneticist Mercer Brugler, at the University of South Carolina Beaufort, who oversees the Tidal Marsh Task Force, a training and research opportunity led by undergraduate students aimed at increasing diversity and inclusion in marine science.

Undergraduate Iesha Whittaker is part of this team, aiming to elucidate the diversity, stability, and age-related patterns of oyster reefs. Oyster reef three-dimensional structure reminds student team member Yessenia Becerra of the throne on *Game of Thrones*. And the reefs not only bolster biodiversity



Yessenia Becerra (left) in the intertidal habitat of Port Royal Sound, South Carolina. She is part of a team of undergraduate students working with marine geneticist Mercer Brugler (right) using eDNA to study natural and artificially established oyster reefs. Photograph (left): Delilah Brielle Bledsoe-Becerra. Photograph (right): Mercer R. Brugler.

but also provide coastal resilience against erosion during extreme weather events, such as storms and hurricanes, she explains. Traditional visual surveys with quadrats—“very much the Godzilla approach,” says recent graduate Jicayla Johnson-Rosemond—probably miss many species as they flee, hide, or burrow. In the lab, Rosemond, Whittaker, and Becerra lead their team in developing primers, targeting certain gene regions to identify from eDNA samples a host of bacteria, archaea, metazoans, fungi, viruses, and protists. Their aim is to compare visual and eDNA surveys to look for indicator species for oyster reef health, determine how biodiversity changes with reef age, and examine diversity effects of storm-water runoff. Better understanding the biology—including the microbiology—of oyster reefs is enormously important because these ecosystems also improve water clarity via filtration and act as a carbon sink due to the oyster calcium carbonate housing.

Monitoring a small fish species

Further north and west, at Oregon State University, environmental

geneticist Taal Levi became aware of eDNA as it emerged as an ecological tool in the early 2000s. He has since used it for monitoring bears from their saliva; Amazonian vertebrates from their biting flies; martens, wolves, and other carnivores from their feces; and fish from aquatic eDNA. “It was obvious pretty early on that [eDNA] worked for presence-absence,” says Levi. What was less clear was whether the technique could indicate abundance. So Levi has been testing eDNA’s usefulness for monitoring eulachon, a fish culturally important to coastal Indigenous peoples, including Alaska Natives, who formerly rendered the fish oil for trade. In collaboration with the Chilkoot Indian Association, Levi began taking water samples for eDNA analysis in 2014. A mark-recapture project for the same fish was already in place, allowing a direct comparison.

Eulachon are anadromous—moving from ocean to freshwater rivers to breed, similar to salmon but with their migratory pulse more concentrated in time. For five years, Levi’s team took daily water samples before, during, and after the eulachon run. He wanted

to determine whether it was possible to get a signal of eulachon abundance and run timing based on eDNA. It worked. The correlation between mark-recapture and eDNA estimates was high, even before correcting for water flow. That early success led to expanded eDNA monitoring for eulachon on many more rivers. Now his team is testing how well their model works from one river to another. With community elders concerned about physical handling of eulachon given its declined numbers, it is powerful, says Levi, to use eDNA to monitor species across a broad spatial range.

Eulachon uniquely lend themselves to monitoring with eDNA, explains collaborator Meredith Pochardt, consultant with the Chilkoot Indian Association. “The eulachon run is relatively short,” lasting about 10–15 days, “so we just shoot for daily sampling,” says Pochardt. Now sampling 7–10 rivers across a vast, remote, and mainly roadless region, “In a lot of those rivers, a mark-recapture or other kind of traditional sampling method wouldn’t be feasible,” she says, heralding the huge benefit of eDNA.

At an Alaskan weir where fish are counted, Levi is also testing eDNA for estimating in-season salmon abundance. Though salmon runs are months long, fitting eDNA concentration against water flow, his team found that eDNA provided a useful signal. One uncertainty, says Levi, was whether DNA would accumulate as fish entered the river or whether, as they moved upstream, their signal would dissipate. His data, sampled low in the watershed, determined that fish traveling to lakes and further upstream do leave a detectable signal, but the dominant signal comes from fish of the past 24 hours, “like the ghost of DNA passing our sampling site,” says Levi. Promisingly, their eDNA data jibed with sockeye and coho counts, the trends going up and down together. “It’s super expensive to count salmon,” says Levi. His hope for the future of salmon monitoring is to deploy auto-sampling robots that collect eDNA.

When sampling eDNA from water, understanding flow rates and temporal distribution of fish is critical in interpreting data in meaningful ways. As Lee Hrenchuk, senior biologist at the International Institute for Sustainable Development’s Experimental Lakes Area explains, this research site, where whole lakes are manipulated with fish populations closely monitored for decades, is ideal for testing questions about eDNA. In work led by Joanne Littlefair at Queen Mary University of London, Hrenchuk was part of a team addressing the question *When an eDNA sample is collected, what does it mean about the fish?* Are they actually there, or might the DNA come from an upstream lake? “eDNA, depending on the environment, degrades in a matter of days or weeks, unless it’s in the sediment,” says Hrenchuk. That is important to know when drawing conclusions about connectivity across the landscape. But northern lakes are complex habitats for water movement. In summer, temperature differences between surface and deeper waters result in vertical layers that



Paul Fafard, a technician with the International Institute for Sustainable Development’s Experimental Lakes Area, retrieves a receiver used to acoustically track fish in the lake. The receiver collects locational data on the date, time, identification, and depth of individually tagged fish. By knowing the depth locations of the fish, researchers can test the validity of their eDNA results.

Photograph: Lee Hrenchuk.

do not mix. In contrast, in fall and spring, thermal shifts cause water turnover, with water mixing because of shifting water density.

Does this matter for eDNA distribution? That is what they wanted to find out. By sampling five lakes and examining eDNA distribution relative to known lake trout (*Salvelinus namaycush*) locations from acoustic telemetry, the researchers revealed that during summer, when cold-loving trout spend time in deep waters, they could only be detected from eDNA sampled in deep water. In summer, lake trout eDNA was hardly ever detected in the top one-sixth of the lake despite this species being the top predator, important to the food web, and abundant. In contrast, during fall lake turnover, lake trout eDNA was found everywhere. “The biggest take home,” says Hrenchuk, “is that if you dipped a sampling bottle in the surface waters of a lake in summer, you’d miss lake trout, ... and that’s a big hole.”

Out of the water

While eDNA has been extensively used in the ocean and freshwater, it has also been increasingly used on land. In sampling sometimes referred to as iDNA—invertebrate-derived DNA—researchers such as entomologist Eleanor Slade, at the Asian School of the Environment at Nanyang Technological University in Singapore, focuses on dung beetles and their role in tropical forests. These insects feed on animal dung and are therefore uniquely linked to mammal populations. Slade was curious to know whether dung beetles can be messengers of mammalian diversity. By sequencing gut contents from large and small dung beetle species in Malaysian Borneo, her team, led by Rosie Drinkwater, successfully identified six mammalian species from the samples, including bearded pig (*Sus barbatus*) and sambar deer (*Rusa unicolor*)—both considered vulnerable species. Interestingly, for dung beetle-sampled eDNA, they found that

the time window representing dung beetle-mammal interactions was about one to two days. “These kind of time stamps can be quite useful,” Slade says.

One challenge was contamination from human DNA swamping the samples, says Slade. Her lab is exploring the use of human-blocking primers. Their innovative research used dung beetle eDNA to sample Southeast Asian vertebrate fauna and highlights the potential for dung beetles to aid future mammal biodiversity monitoring surveys.

Other invertebrates that can provide genetic messages include blood sucking ground leeches. Rosie Drinkwater wanted to explore whether leech DNA was a mechanism for rapidly sampling for mammal presence. In Sabah, Malaysian Borneo, an area fast being converted to oil palm plantations, degraded habitats between forests and plantations often get disregarded as potential mammal habitat, says Drinkwater. “But actually, the animals use them quite a lot.” Her thinking: If researchers could quickly sample mammal presence across a gradient of forest degradation using leeches, they could later follow up with camera traps. Leeches are not everyone’s favorite organism, but as useful biological messengers, Drinkwater says she “learned to love them.”

By mashing up blood-feeding leeches (*Haemadipsa picta*) to glean eDNA, “We consistently got a higher diversity of mammals in the higher quality forest,” she says, but the highly degraded forest had important mammalian diversity too. Revealing differences in diversity across a human-modified tropical landscape, with traces of mammals from porcupines and elephants to civets, sun bear and pangolins, she demonstrated that eDNA from “leech soup” can provide a valuable noninvasive biomonitoring approach for conservation.

Alex Greenwood, at the Leibniz Institute for Zoo and Wildlife Research and the Free University of Berlin, has taken leech learnings further. He has found it possible to simultaneously sample the diversity of viruses and



Dung beetle of the Catharsius genus taking flight. Eleanor Slade, Rosie Drinkwater, Elizabeth Clare, and their team analyzed eDNA (or iDNA) from dung beetles in Sabah, Malaysian Borneo, finding the insects’ diversity reflects a wide assortment of vertebrates and is a valuable indicator of habitat heterogeneity and forest disturbance. Photograph: Dave Hemprich-Bennett.



By sampling eDNA from water holes such as this one in Tanzania, Alex Greenwood’s team is gaining an understanding of a diversity of viruses and their wildlife hosts. Photograph: Peter Seeber.

their wildlife hosts. “For biodiversity studies, a lot of the animals you’re interested in—you just can’t get to them,” he says. They may flee, live high up in trees or in dense jungle.

Collecting eDNA from leeches and biting insects allows the scientists to sample animals that are inaccessible. “You let evolution do all the work for you,” says Greenwood. Leeches have evolved

to find, bite, and suck animal blood. The disadvantage, he says, is that it is much harder to link virus to host. For example, he found a new coronavirus from leeches that fed on Southeast Asian sambar deer (*Rusa unicolor*), “but we don’t really have a statistical association that says it *has* to be them.” And leeches, says Greenwood, while useful, have limitations. As ground dwellers, they are unlikely to indicate anything about the diseases of bats, for example. But sampling blood suckers is a nice opportunity, says Greenwood, bringing together experts in ecology, biodiversity, and infectious disease medicine, and making scientists realize, he says, that “we kind of need each other.”

The analysis of liquids—seawater, freshwater, or a soup of ground-up leeches—is not the only useful medium for gleaning eDNA. In preliminary research, Elizabeth Clare, at York University, in Toronto, Canada, had found examples of extracting eDNA from honey, snow, rain, watersheds, and soil but couldn’t find anything in the scientific literature about extracting it from air. So she set to work. Although her university (she then worked in the United Kingdom) was under lockdown in 2020, she found that lab animal housing provided an environment accessible for air sampling. Inside a captive naked mole rat colony, and in the room that housed their enclosed burrow, she obtained air samples. After the samples were vacuumed through a fine filter, Clare was then able to chemically isolate and amplify DNA. “We’re talking about forensically small quantities of DNA,” she says, but the mole rat DNA they expected to find—along with unexpected genetic traces of the technician’s pet dog—was there, demonstrating a proof of concept for extracting eDNA from air.

Public health

During the COVID-19 pandemic, interest in using eDNA and eRNA as a monitoring tool for public health has focused on wastewater. At Syracuse University, David Larsen realized early



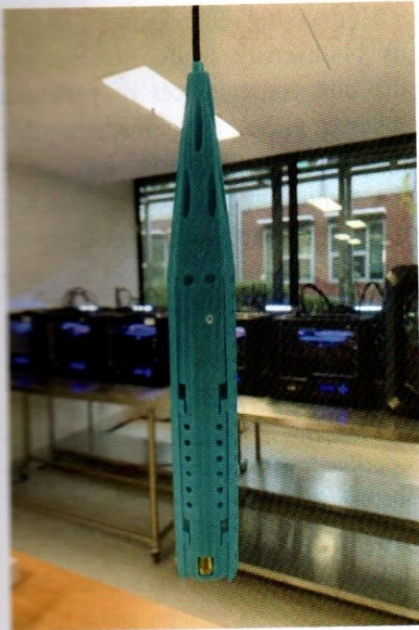
Elizabeth Clare demonstrated proof of concept for the ability to sample air for the eDNA it contains, using a captive colony of naked mole rats as her test site. Photograph: Lorna Ellen Faulkes Photography.

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on that such monitoring could be a game changer. “Sanitation engineers at treatment plants regularly take samples,” says Larsen. It was an obvious next step to collaborate on collecting samples of wastewater that could be sent to the lab and analyzed and the

data relayed to the public through the health department. Working on a system for New York State since May 2020, Larsen says “we’re making progress.” Going forward, he explains, wastewater surveillance using genetic methods is likely to be valuable for lots



This “torpedo,” (left) an open-source, 3D-printed design, houses an electro-negatively charged filter that allows collection of DNA and RNA fragments from wastewater, enabling identification of important pathogens including SARS-COV-2. Photograph: Miao Wang, Victoria, Australia.

A lab technician (right) analyses wastewater for its genetic fragments that enable identification of pathogens. Sampling wastewater is one of the ways community-level infectious diseases such as COVID-19 can be monitored. Photograph: Syracuse University.

of different infectious diseases, including Zika, Ebola, fecal-oral transmitted diseases, HIV, tuberculosis, and malaria. “Previously, epidemiologists like myself never thought about wastewater as a way to surveil an infectious disease that was respiratory transmitted, ... but my mind is shifting around the idea that we can use this as a very broad public health tool,” perhaps for monitoring tick-borne illnesses too.

In Australia, Monash University’s David McCarthy has been tackling how to make wastewater sampling for infectious disease more sensitive and effective even when community rates are very low. At small scales, such as monitoring presence of COVID-19 at individual buildings, McCarthy explains that “depending on when you or I go to the toilet, [when sampling] I may not have caught your poop,” since grab sampling typically represents wastewater collected over a 15-second window in time. After testing various absorbent materials, including tampons and ear buds, he developed a torpedo, a hollow plastic device with holes allowing wastewater to permeate

its inner electrostatic membrane, capturing genetic traces of a pathogen during its 24-hour deployment. The enlarged time window of this torpedo provides the potential to find pathogens that grab sampling might miss. Now in use in many places, including Australia, Canada, the United States, and South Africa, this open-source-designed tool can be a valuable early warning system, says McCarthy. He knows of at least 15 occurrences where torpedoes enabled detection of SARS-CoV-2 in wastewater before a single clinical case was observed.

Limitations

Although it is an increasingly popular tool, eDNA analysis is not without its limitations. One is that eDNA is only as powerful as its reference databases, which are missing or incomplete for many regions of our planet, explains Clare Adams, doctoral student at the University of Otago, in New Zealand. She has used eDNA to examine the ecology of New Zealand fur seals (*Arctocephalus forsteri*) and pāua (*Haliotis iris*), a commercially

important marine mollusk also culturally significant to the Māori people. Sequenced eDNA must be matched up to genetic codes in a database. “Once we get a match, we can tell what organism we have,” explains Adams. “But oftentimes with the understudied southern ocean, we can only assign that organismal trace to a family or order, not always the species.” Doing the basic science to get the genetic barcodes is important, she notes, but finding funding can be difficult.

Many questions also remain about the ecology of DNA in the environment. How far does it travel? What are the limits of detection? These ecological factors have been highlighted in many recent studies including in *Environmental DNA*, a new journal solely dedicated to eDNA studies, first published in 2019.

It is important to recognize, says marine geneticist Kevan Yamahara, at the Monterey Bay Aquarium Research Institute, that eDNA samples represent only snapshots in time. “Whether it’s an ocean, river, or lake, there’s a lot of temporal-spatial variability, so

understanding your system is important for making any really conclusive statements about what you're finding in your samples." Rivers flow. Oceans have currents and temperature differences, and some organisms make vertical migrations. With so many influential factors, "you really have to put bounds on your system before you can really make ecological sense of what your samples are telling you," he says. Another concern, Lamy notes, is that, with many companies getting into fast processing eDNA analyses, there is a risk of lack of

standardization of protocols. He and others underline that eDNA is likely to remain complementary to—and not a replacement for—other survey techniques.

Nevertheless, says Clare, "This whole field—environmental DNA—has huge potential." She sees it very quickly expanding into society and foresees a future where continuous automated eDNA samplers are deployed around the world, extracting and sequencing DNA on the spot, bouncing their data to the cloud to tell researchers where and how life is changing.

Humankind increasingly imperils biodiversity while experiencing the dire consequences of pathogen spillover. eDNA, a tool made possible by increasingly powerful and sensitive technology, is emerging as a key method for biological monitoring to expand understanding and mitigate risk. "I think it's exciting," says Clare.

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