Environmental DNA Detection as a Survey Tool for the Endangered Tidewater Goby

Requested by
Amy Golden, Division of Environmental Analysis

January 16, 2015

The Caltrans Division of Research, Innovation and System Information (DRISI) receives and evaluates numerous research problem statements for funding every year. DRISI conducts Preliminary Investigations on these problem statements to better scope and prioritize the proposed research in light of existing credible work on the topics nationally and internationally. Online and print sources for Preliminary Investigations include the National Cooperative Highway Research Program (NCHRP) and other Transportation Research Board (TRB) programs, the American Association of State Highway and Transportation Officials (AASHTO), the research and practices of other transportation agencies, and related academic and industry research. The views and conclusions in cited works, while generally peer reviewed or published by authoritative sources, may not be accepted without qualification by all experts in the field.

Table of Contents

Executive Summary

Background

The goal of this investigation is to provide information to support Caltrans' possible development and implementation of environmental DNA (eDNA) survey methods to detect the endangered fish species *Eucyclogobius newberryi*, commonly called the tidewater goby. Traditional survey methods are invasive and potentially harmful to this species, motivating Caltrans' interest in this alternative eDNA approach.

This Preliminary Investigation is centrally concerned with identifying appropriate eDNA sampling protocols, assay methods and statistical modeling for establishing species presence/absence. Together, this information will provide guidance on feasibility of eDNA-based surveying for the tidewater goby.

Because the tidewater goby is native only to California, this Preliminary Investigation necessarily looks beyond this species to eDNA testing of other aquatic vertebrates. Our interviews with experts include university researchers as well as government practitioners involved with species that have advanced implementation of eDNA testing: the Asian carp in the Great Lakes region of the United States and the great crested newt in the United Kingdom.

This Preliminary Investigation also surveys research on eDNA testing, particularly for aquatic species. Use of polymerase chain reaction (PCR) to amplify and identify a small sample of DNA is a rapidly growing field, and extensive research has been published in the past few years, with new citations appearing frequently.

Summary of Findings

Tidewater Goby Conservation

Findings throughout this Preliminary Investigation indicate that the design of an eDNA survey for any given species will depend on the unique characteristics of that species. For this reason, it is important to understand the tidewater goby's biology, habitat and life cycle as well as established plans for its conservation.

Resources from both the U.S. Environmental Protection Agency and the U.S. Fish and Wildlife Service (USFWS) provide such details for the tidewater goby. The USFWS is responsible for the recovery plan for the tidewater goby. Of particular interest for this Preliminary Investigation is the Programmatic Letter of Concurrence for Routine Maintenance and Repair Activities from USFWS's office in Arcata, CA, to Caltrans in **Tidewater Goby Conservation**. This document notes that DNA may be an acceptable means of identifying the presence of the tidewater goby; it provides detailed protocols for traditional field surveys but not for eDNA surveys.

Consultation with Experts

Because Caltrans' interest in this topic involves understanding how practice-ready this technology may be for its purposes, we spoke with experts who could provide guidance on implementation and answer questions relevant to the tidewater goby in particular. Interviewees included:

- Two USFWS practitioners: Steve Kramer, a biologist from the Arcata, CA, office closely involved with tidewater goby and Caltrans issues, and Emy Monroe, a molecular geneticist from the Whitney Genetics Lab in Onalaska, WI, that conducts eDNA testing for the invasive Asian carp.
- Two U.S. professors: Andrew Kinziger, Humboldt State University who is leading efforts on eDNA detection for tidewater goby, and Caren Goldberg, Washington State University, who is developing eDNA survey protocols.
- Neil Boonham, a molecular biologist practitioner representing the British Food and Environment Research Agency (FERA), which developed eDNA protocols for the endangered great crested newt.
- Robin Allaby, a professor from the University of Warwick (United Kingdom) with an alternative perspective on the appropriate type of PCR testing for the great crested newt.

Key points and repeated themes from the interviews follow.

Prospects for eDNA Testing

Kramer said that eDNA testing is of interest to USFWS and shows promise, particularly given the invasive nature of traditional methods (also noted by Kinziger). Monroe, however, stated that eDNA detection is farther along as a lab technique than as a tool for making management decisions.

Tidewater Goby Progress

For the tidewater goby, Kinziger has established a quantitative PCR (qPCR) assay that meets the two criteria of species specificity and sensitivity, as proved through field tests. The next step now in progress by his team is the development of a statistical occupancy model to determine what kind of sampling and results are needed to establish presence/absence at a desired confidence level.

Kramer envisions working with Humboldt State University and Caltrans to develop eDNA protocols, similarly to how traditional field protocols were developed. Given the genetic variation of the tidewater goby by geographic region, Kinziger noted a future step for statewide implementation of tidewater goby eDNA testing would be to develop an assay (or multiple assays) applicable for tidewater gobies across the state, and not just in the three northern California counties studied so far.

qPCR Versus Endpoint PCR

All but one interviewee acknowledged qPCR as the most common method in place for testing for target eDNA. qPCR is seen as cheaper, faster and more sensitive than traditional—or endpoint—PCR. However, Allaby questioned whether qPCR is truly more sensitive than PCR or if it simply yields more false positives. The United Kingdom's move to a performance-based specification for eDNA survey of the great crested newt, as described by Boonham, will help shift focus away from the government need to specify a given technology.

eDNA Field Test

Several individuals noted that protecting against sample contamination in the field is critical. In fact, carefully protecting against contamination through a controlled lab environment is one of the barriers to a field-based test using current PCR methods. The time required for filtering DNA samples is another. Other possible options for a field test—notably, the use of nanopores to

detect single DNA molecules—were viewed as not yet technologically mature and cost-prohibitive.

eDNA Vectors

False positive vectors—which would cause eDNA to test as present when the species in question is absent—are of interest to Caltrans. Kinziger did not see DNA longevity as a factor, with eDNA only lasting and remaining detectable for a few weeks. Monroe noted that the Asian Carp Regional Coordinating Committee (ACRCC) is calibrating for false positive vectors such as eDNA in bird feces and barge leachate; not all such vectors will be applicable to the tidewater goby.

Research and Resources

Tidewater Goby eDNA

Researchers at Humboldt State University are actively conducting lines of investigation on eDNA detection for the tidewater goby. Citations on published and in-progress research at Humboldt State University provide additional details to complement Kinziger's comments in **Consultation with Experts**. The latest findings, which include paired study of traditional and eDNA field detection and statistical occupancy modeling, have not yet been published.

Asian Carp

U.S. government agencies are also using eDNA to detect the invasive Asian carp (a term that represents multiple fish species). State, regional and national agencies work together through the ACRCC to best implement eDNA as a tool to detect Asian carp. Key publications include the annually updated Quality Assurance Project Plan (QAPP) that spells out testing protocols, the ongoing calibration study to improve the methodology and manage uncertainty, and the summary of the USFWS' Great Lakes eDNA Monitoring Program. All government testing is conducted by the USFWS's Whitney Genetics Lab; comments from lab manager and molecular geneticist appear in the **Consultation with Experts** section of this report. Two additional research citations address recent work on validating and improving eDNA testing for Asian carp.

Great Crested Newt

Another species with government-established eDNA testing is the endangered great crested newt in the United Kingdom. Several resources provide further documentation to complement Boonham's comments in **Consultation with Experts**. These include a web page from the U.K. Department for Environment, Food & Rural Affairs (DEFRA) with an overview on national research to develop the protocols as well as the protocols themselves. Also included is National England's guidance for developers on surveying for the newt. A web site from the University of Warwick provides an alternative viewpoint on the comparative effectiveness of endpoint versus qPCR.

Application of eDNA

Several publications explore eDNA as a tool for detecting species. Among these, one that FERA's Boonham suggested for review is a Dutch white paper that addresses many of Caltrans' areas of interest, including persistence of DNA, factors on the amount of DNA, sampling strategies, reliability and detection probability. A U.S. Geological Survey fact sheet from 2012 is similarly instructive, providing "biologists and resource managers understand emerging methods for detecting environmental DNA and their potential application for inventorying and monitoring aquatic species."

In addition to two recent reviews of this technology, we present an extensive list of research citations dating from 2011 to 2014 that address the range of scientific, technological and management issues related to eDNA testing for water-based species.

PCR

Questions regarding the most appropriate type of PCR for eDNA surveying prompted the callout of two citations that address this issue: One compares endpoint and qPCR; the other addresses validation of PCR.

Nanopore Technology

Several interviewees mentioned nanopore technology as a possible next step for field testing. Though practical and affordable application is likely years away, this technology presents the possibility for testing a single DNA strand in the field.

Gaps in Findings

- We collected several figures related to the costs of eDNA: field setup ($300); per-sample costs (ranging from $45 to $225); assay development costs ($3,000 to $10,000); and PCR equipment purchase ($50,000 to $60,000). However, we were unable to establish comparative costs for eDNA survey compared with traditional field survey. This is likely due in part to the time-intensive nature of eDNA testing, which is not easily captured in dollars, and in part to commercialization of eDNA testing, which is in its early stages.
- The interviewees for this Preliminary Investigation shared Caltrans' desire for feasible eDNA field test. However, as their comments bear out, this testing remains impractical at this time.
- For Caltrans' purposes in developing a plan to implement eDNA testing, it would have been helpful to identify road agencies making use of eDNA testing. We were unable to identify state departments of transportation (DOTs) involved in this practice. We found that the state-level partners in the ACRCC are not state DOTs but state departments of natural resources and environmental conservation.
- Interest in eDNA is high, leading to a large number of citations and frequent new ones. It is impossible to present a comprehensive listing in the scope of a Preliminary Investigation. We sought to include a sample that represents the most recent and relevant citations for Caltrans' purposes.

Next Steps

Given the mutual interest for exploring tidewater goby eDNA testing among key stakeholders at the USFWS (Steve Kramer) and in the California State University system (Andrew Kinziger), a possible next step for Caltrans would be to open a dialogue among these individuals to discuss steps for developing protocols and field tests. Kramer indicated that in the past, traditional protocols were likewise developed as a joint effort among the stakeholders involved. Caltrans may also wish to inquire about the latest research findings at Humboldt State University that are not yet ready for publication.

More can be learned about management issues and the applicability of eDNA test results from USFWS personnel involved with the Asian carp. Both Emy Monroe and Kelly Baerwaldt may be able to answer specific questions or concerns that Caltrans may have about how to develop action plans based on eDNA test results.

Detailed Findings

Tidewater Goby Conservation

The following resources provide a background on the species of interest for this Preliminary Investigation, the tidewater goby (*Eucyclogobius newberryi*). These include fact sheets about the species as well as the latest governing documents on its conservation.

Endangered Species Facts: Tidewater Goby, U.S. Environmental Protection Agency, February 2010.
http://www.epa.gov/espp/factsheets/tidewater-goby.pdf
This fact sheet provides information about the tidewater goby's description; ecology (range, habitat, reproduction/life cycle); and federal recovery plan.

Tidewater goby (Eucyclogobius newberryi), Environmental Conservation Online System, U.S. Fish & Wildlife Service, undated.
http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=E071
This page includes the most up to date Federal Register documentation on the tidewater goby (including designation and reclassification documents) as well as federal recovery plans, critical habitat documents, conservation plans and petitions. The "12-Month Finding and Proposed Rule Reclassifying the Tidewater Goby from Endangered to Threatened" was registered on March 13, 2014.

Recovery Plan for the Tidewater Goby (Eucyclogobius newberryi), U.S. Fish & Wildlife Service, December 2005.
http://www.fws.gov/pacific/ecoservices/endangered/recovery/documents/TidewaterGobyfinalRecoveryPlan.pdf
This recovery plan outlines the current species status of the tidewater goby, the recovery objective to reclassify the tidewater goby from endangered to threatened or delisted, and the recovery criteria.

Programmatic Letter of Concurrence for Routine Maintenance and Repair Activities, and Small Projects Program: California Red-Legged Frog, Marbled Murrelet, Northern Spotted Owl, Western Snowy Plover, Tidewater Goby, and Point Arena Mountain Beaver, Memorandum from the Arcata Field Office, Fish and Wildlife Service of the U.S. Department of the Interior, to Caltrans, April 2014.
http://www.dot.ca.gov/ser/downloads/MOUs/arcata_fws_concurltr.pdf
From page 14:

> Tidewater Goby—The following avoidance and minimization measures apply to action areas where tidewater gobies have been detected using the Service's recommended presence\absence survey protocol (Service 2005a: Appendix F; Attachment F), when water samples taken from the water body detect tidewater goby DNA, or when Caltrans presumes goby presence. For ground-disturbing activities conducted within unoccupied (based on the aforementioned surveys) suitable habitat within designated tidewater goby critical habitat, Caltrans must ensure that the primary constituent elements (Service 2005a) of goby critical habitat are maintained.

Attachment F begins on page 181 of the PDF and details the complete protocol for traditional field survey of the tidewater goby.

Consultation with Experts

Below we summarize our conversations with experts and practitioners about the feasibility of using eDNA to survey for the presence/absence of the tidewater goby.

U.S. Fish & Wildlife Service

USFWS Arcata Fish and Wildlife Office

Contact: Steve Kramer, Fish and Wildlife Biologist, USFWS Arcata Fish and Wildlife Office, 707-822-7201, steve_kramer@fws.gov.

We interviewed Steve Kramer, who is listed as the office's primary contact for issues related to the tidewater goby.

(Note: Caltrans asked us also to speak with Greg Schmidt, USFWS fish and wildlife biologist, whose job function includes working with Caltrans. Kramer advised that Schmidt is on extended field assignment; Kramer works closely with him and is currently handling much of the Caltrans-related work.)

Outlook for eDNA Testing: Comparison to Traditional Methods

Kramer said that eDNA shows a lot of promise and he is "very much in favor of it." He noted the great work being done at Humboldt State University (see our interview with Andrew Kinziger below). He also mentioned the extensive work being done on the Asian carp (see our interview with USFWS's Emy Monroe below) as well as other aquatic species.

He noted that compared with collecting water samples for eDNA, the traditional survey methods (such as seines and dip nets) are invasive and particularly detrimental when tidewater gobies are constructing their burrows. Moreover, traditional methods aren't always as good at locating specimens: A site that yields no specimens with traditional survey methods may mean that the species is present but difficult to find, as supported by positive eDNA tests and historical records that indicate gobies were present in the past.

Kramer sees eDNA testing as complementing the traditional method. For clearly confirming the presence of the species, Kramer said nothing beats having species in hand. However, eDNA testing provides useful data to help support the conclusion that tidewater gobies are present. Like others we interviewed, Kramer emphasized the importance of ensuring that there is no contamination during the DNA collection process.

Protocol Development

The detailed USFWS protocol for surveying tidewater gobies in the field using traditional methods, as outlined in the 2014 programmatic letter to Caltrans (see the **Tidewater Goby Conservation** section), was put together with the input of several stakeholders and experts. That letter allows for eDNA evidence for detecting tidewater gobies but does not specify a protocol. Kramer said that such a protocol would probably be developed as a coordinated effort among his office, Caltrans, Humboldt State University and others.

Future Items: Field Testing, Detecting Population Size and Other Species

Like Caltrans, Kramer would like to see a field test for detecting tidewater goby eDNA, but he doesn't think the technology is there yet. Another future goal is using eDNA testing to determine tidewater goby population size. This would require correlating species counts from traditional methods with eDNA results for different kinds and sizes of water bodies; more data would need to be collected and correlated first. Kramer also sees potential value of eDNA testing as a powerful tool for other kinds of species Caltrans may encounter, including amphibians and reptiles.

USFWS Midwest Region—Whitney Genetics Lab

Contact: Emy Monroe, Lab Manager and Molecular Geneticist, USFWS Whitney Genetics Lab, 608-783-8402, emy_monroe@fws.gov.

USFWS is one of several partners in the ACRCC (http://www.asiancarp.us) seeking to stop the spread of the Asian carp through the Great Lakes ecosystem. The term "Asian carp" refers to any of four distinct species; among these, ACRCC tests for eDNA for the bighead and silver carp.

USFWS's Whitney Genetics Lab conducts all of the agency's eDNA testing for the invasive Asian carp. We interviewed molecular geneticist Emy Monroe, the lab's manager. USFWS Asian Carp/eDNA Program Coordinator Kelly Baerwaldt reviewed the following interview notes as well.

Outlook for eDNA Testing

Monroe said that eDNA detection has been refined as a lab technique, but as a tool for making management decisions, it is not as far along. Questions remain on the most appropriate policy for reacting to positive eDNA findings—particularly given a trend where field tests (netting or shocking) triggered by eDNA evidence often do not yield any specimens.

Monroe thinks of eDNA testing as "one tool in the tool kit" for monitoring. Current plans call for eDNA testing twice per year in high-risk locations and once per year in medium- and low-risk locations, with repeated monitoring events when eDNA is detected. eDNA surveys are also paired with intensive field surveys using traditional fishery techniques. More frequent regular eDNA testing would be helpful to establish baseline data and to identify spikes in eDNA detections.

Protocols

Monroe noted that detailed eDNA testing protocols for the Asian carp appear in USFWS' Quality Assurance Project Plan: eDNA Monitoring of Bighead and Silver Carp, published annually (see **Research and Resources** for the 2014 edition). The protocols detail sample collection, processing, shipping, DNA assay, internal quality control and checks for data precision, accuracy and completeness.

She also outlined the timeline for developing the current protocols, which earlier were based on endpoint PCR before current qPCR protocols were established.

Monroe said that biology and hydrology must be considered when developing field collection protocols for any species. These will determine the best location (surface, benthic or pelagic zone) and time (possibly during spawning) to collect samples.

Factors for Using eDNA Results

One confounding factor for testing Asian carp DNA is that it can be detected when the fish themselves are not present, such as in bird feces, through fish market sewers and leached from barges. These vectors for false positives (meaning the DNA is present when the organism is not) must be calibrated and accounted for. Environmental inhibitors that prevent detection of eDNA, such as humic acids and digestive enzymes, must also be taken into account.

Research is ongoing with paired studies of eDNA and traditional fieldwork. New calibration models are also soon to be published (see the eDNA Calibration Study (ECALS) page at http://www.asiancarp.us/ecals.htm for the latest peer-reviewed report). Monroe also referenced research by Notre Dame (see Improved Methods for Capture, Extraction, and Quantitative Assay of Environmental DNA from Asian Bigheaded Carp (*Hypophthalmichthys spp.*) in **Research and Resources**) and others that is helping to refine and determine what a positive eDNA detection means and how to avoid false negatives and positives. It is an ongoing refinement process and part of an adaptive management program.

Costs

The most recently calculated cost is $110 per sample, which includes only lab consumables. This cost is based on the 2013 Qiagen brand kit for extraction and endpoint PCR, and does not include the field portion (such as field staff time, travel costs, eDNA trailers and trailer lab consumables to filter in a clean environment).

Humboldt State University

Contact: Andrew Kinziger, Professor and Chair, Department of Fisheries Biology, Humboldt State University, 707-826-3944, andrew.kinziger@humboldt.edu.

We corresponded with Kinziger by email and followed up with a telephone interview. Discussion highlights follow.

Step 1. Development of Assay

The first step for Kinziger's team was to develop a qPCR assay for the tidewater goby presence/absence detection using eDNA in water samples. The test was designed to be applicable to tidewater goby in three northern California counties: Mendocino, Humboldt and Del Norte. The qPCR assay needed to be:

- Species-specific so the testing wouldn't produce false positives for similar species, such as the arrow goby (Clevelandia ios).
- Sensitive to eDNA at very low concentrations.

Kinziger said that the team's qPCR assay meets these criteria of specificity and sensitivity. He also noted that qPCR tests, in addition to determining species presence/absence, give estimates of concentration that relate to abundance.

Step 2. Field Test

Next, Kinziger's team conducted field testing at 27 sites in northern California. At each location, they took paired samples from water collected for eDNA testing (2-liter sample) and with a traditional seine haul (10 feet long and 1/8-inch mesh, inspected for species presence/absence).

- At each site, the number of paired samples depended on the size of the water feature, ranging from just a few samples to as many as 23. At each site, sampling was systematic at evenly spaced (200- to 300-meter) intervals.
- The primary objective was to compare the detection rates of eDNA and traditional methods.
    - For eDNA, 141 of 240 water samples detected tidewater goby— a detection rate of about 59 percent.
    - For seine hauls, 91 of 240 water samples detected the tidewater goby—a detection rate of about 38 percent.
- The results clearly demonstrate that eDNA is a more sensitive test.

Step 3. Occupancy Model

The next significant step, now in progress, is data analysis to produce an occupancy model framework. According to Kinziger, "If eDNA is in the lagoon, what's the probability that we're going to detect it? How many water samples do you need to take to detect tidewater gobies at 90 percent probability?" Kinziger described this step as an evaluation of the qPCR itself—his team knows it's working and has evidence that it's more sensitive than the seine haul. These efforts are necessary to develop a protocol and should eventually determine how many replicate water samples (at systematic points around a water feature) are needed.

Considerations

Advantages of eDNA Testing

Compared with traditional testing, eDNA testing is noninvasive and it's possible to get into hard-to-access habitats. There is also less red tape since permits to directly handle animals are not required. The higher sensitivity makes eDNA approaches better at detecting rare and elusive species.

Longevity of eDNA

Under normal environmental conditions, tidewater goby eDNA typically lasts on the order of seven to 20 days. Extracted DNA samples may be frozen and kept indefinitely for future testing.

Quality Assurance and Control

Because the approach is very sensitive (involving very small amounts of source DNA), the process requires a great deal of quality assurance and quality control. Kinziger's team has detailed water sampling protocols and lab techniques. The major phases of the procedure are conducted in different rooms under sterile conditions. Water filtration, DNA extraction and the qPCR test itself are conducted in three independent labs; they couldn't all be done in one facility.

Time and Costs

Kinziger estimated that a test could be turned around in 24 to 48 hours if someone wanted to pay for it. It's difficult to put a firm number on costs at this time. The significant piece of equipment involved is the qPCR machine, which runs $50,000 to $60,000. Other considerations are dedicated lab space and labor.

eDNA Field Test

Kinziger is unaware of any practical "litmus test"-like eDNA field test. A mobile qPCR laboratory might be conceivable, but the quality assurance and quality control concerns to avoid contamination as described above would remain.

Scaling Up Across California

To scale up to tidewater goby testing at the state level, it would be necessary to evaluate the applicability of the current test across the range of the species. The tidewater goby exhibits substantial genetic differentiation across its geographic range (some believe it might constitute more than one species). If this test based on northern California tidewater gobies proved not to work for specimens found further south, it would not be problematic to develop multiple region-specific tests. According to Kinziger, once the water sample and extract DNA are obtained, multiple tests can be used.

Additional Contacts

Kinziger suggested we contact Caren Goldberg at Washington State University (see the next interview). He recommended against contacting Molly Schmelzle, who was on Caltrans' original contact list, since Schmelzle is a graduate researcher on Kinziger's team who would report much the same information as Kinziger.

Washington State University

Contact: Caren Goldberg, Assistant Professor, School of the Environment, Washington State University, 509-335-3673, caren.goldberg@wsu.edu.

We spoke with Caren Goldberg by phone; she outlined her work in developing species-specific tests and implementation protocols for eDNA qPCR and provided other thoughts on this topic.

Protocols in Development

Field staff members are the targeted end users of the protocols, which will provide guidance on sampling technique, site selection for sampling, equipment needed and related information. Protocols will address field testing design and how to account for variation in the field (such as running versus standing water). For field staff, learning how to properly collect clean, high-detection samples is straightforward. However, trained specialists perform lab testing.

Applicability of Other Tests and Protocols to the Tidewater Goby

The same basic collection and detection processes are used for amphibians (Goldberg's primary focus) and for fish such as the tidewater goby. However, fish are more likely than amphibians to have genetically similar species in the same habitat. This requires additional consideration in developing an assay to detect just the target species.

Management and Risk

Goldberg said that eDNA detection in the United States is farthest along for the Asian carp (see the **Research and Resources** section of this Preliminary Investigation). All technical aspects of eDNA testing are the same for the Asian carp as they are for endangered species. However, management decisions are necessarily based on risk tolerance, and there may be different consequences for making a detection mistake for an invasive species compared with an endangered one.

On the topic of management, Goldberg recommended "From Molecules to Management: Adopting DNA-Based Methods for Monitoring Biological Invasions in Aquatic Environments" (see **Research and Resources**).

eDNA Compared with Field Surveys

While eDNA has higher detection probability than traditional field surveys, it also varies depending on eDNA diffusion, time of sampling and other factors. For either method, tests must be designed based on the system in question. Goldberg suggested pairing field surveys with eDNA testing because each can pick up things the others cannot. eDNA, for example, indicates presence of an animal, but doesn't provide counts or reproductive status.

Field Testing

Goldberg said threats of contamination would make conducting qPCR in the field extremely difficult, adding that with current techniques, an eDNA lab in the field that didn't produce false positives is almost impossible. It would also be time-consuming, as current extraction techniques have an overnight incubation step.

Beyond qPCR, Goldberg said that nanopores are very promising for field detection but as far as she knows, not close to ready for field application. Other innovations in eDNA are likely to come in the field of medicine, given the intense interest in bedside tests for diseases.

qPCR Versus Endpoint PCR

Goldberg's team still conducts endpoint PCR to sequence DNA on a proportion of samples to make sure the qPCR is performing as expected. However, endpoint PCR is significantly more expensive and time-consuming. qPCR has its own challenges (it's important to be certain that qPCR is detecting only the target DNA), but the best way to address those challenges is through validation.

Costs

Goldberg provided eDNA costs for illustration purposes. She expects companies to get involved in eDNA testing as it becomes more common.

- Developing and validating an assay for one species can run from $3,000 to $10,000 or more.
- It costs agencies approximately $300 to get set up for field testing, including hand pumps and other equipment. The combined costs for a single sample are $45, which include a single-use water filter, shipping and lab testing.
- Some additional cost is incurred to conduct periodic field tests on "field negatives," which are samples known not to contain the target DNA (distilled water, for example) that are used to ensure field collection equipment is not contaminated.

Food and Environment Research Agency

Contact: Neil Boonham, Head of Detection Surveillance Technologies, Food and Environment Research Agency, United Kingdom, 011 44 (0) 1904-462332, neil.boonham@fera.gsi.gov.uk.

We interviewed Neil Boonham, a molecular biologist with FERA.

Great Crested Newt Testing

Boonham said that spring 2014 was the first time eDNA testing for detecting the endangered great crested newt was widely used and endorsed by Natural England. (Natural England and FERA are both subbodies of DEFRA.) He said that a significant amount of time, resources and research went into the method that Natural England ultimately endorsed.

The protocols are detailed in "Technical Advice Note for Field and Laboratory Sampling of Great Crested Newt (*Triturus cristatus*) Environmental DNA" (see **Research and Resources**). These protocols include lab and field methods, quality assurance and quality control, and data recording and reporting requirements.

Proficiency Testing and Performance-Based Standards

Boonham noted that Natural England is taking steps to set up a proficiency test in 2015 for alternative eDNA testing protocols. The current testing methods, used typically by third-party consultants, are prescriptive and require strict adherence. This is hard to sustain, particularly given ongoing changes in techniques and equipment.

In 2015, Natural England plans to supply labs with standardized samples that can be tested using any prospective method. Users will submit results and may or may not receive a certificate of proficiency based on how well they perform. This will allow innovators to vary parts of the test to make it better, faster or cheaper—as long as the method works. In effect, the protocol will become performance based with strict proficiency requirements.

qPCR and Endpoint PCR

In comparing qPCR and endpoint PCR, Boonham said endpoint PCR won't be as sensitive as needed, which will lead to false negatives. Like all prospective methods, Natural England's proficiency testing program will demonstrate whether endpoint PCR will be a viable eDNA detection method for the great crested newt.

Field Testing

As with others we interviewed, Boonham would be interested in a field test and said it might be possible in the future. The challenge now is that because the amounts of eDNA available in the field are so low, elaborate purification and concentration are required before testing. This is difficult to do in the field.

Spygen

Among private firms leading the field of eDNA testing, Boonham noted the French company Spygen (http://www.spygen.fr/en) as a front-runner in research and implementation.

University of Warwick

Contact: Robin Allaby, Associate Professor, School of Life Sciences, University of Warwick, United Kingdom, 011 44 (0) 247-657-5059, r.g.allaby@warwick.ac.uk.

We spoke with Robin Allaby, who provided an alternative perspective on FERA's testing protocols for the great crested newt.

qPCR Versus Endpoint PCR

Allaby's main concern with FERA's protocols is that qPCR commonly results in false positives—indicating the presence of the target DNA when, in fact, none is present. The additional positive readings could be misinterpreted as qPCR being more sensitive than endpoint PCR. He said that qPCR has a chance of amplifying the wrong thing, and this is compounded by the minute amount of initial eDNA typically present in a sample. He added, "It's dangerous to be seeing newts everywhere if you haven't got any way to verify what you're looking at."

Allaby described the University of Warwick's alternative testing regimen, which uses the same collection methodology as FERA but a different testing that produces a DNA sequence at the end. This is outlined further on the University's web page about newts (see **Research and Resources**).

Field Testing

When asked about possible new technologies for eDNA field testing, Allaby said techniques are coming along and could be possible in the future. He described nanopore technology with the potential to sequence a single DNA molecule. A water sample would be entered into a single use "key" and the DNA would draw down through a nanoscale pore. A computer would look to match the target DNA. The costs now are prohibitively expensive, but a 10-year horizon is conceivable.

Research and Resources

Research and resources are divided into the following six areas:

- **Tidewater goby eDNA.** Citations are specific to research conducted on eDNA testing for the tidewater goby.
- **Asian carp.** Citations address policy, procedures and research related to eDNA detection of Asian carp in the Midwest.
- **Great crested newt.** Citations similarly address policy and procedures related to eDNA detection of the great crested newt in the United Kingdom.
- **Application of eDNA.** Currently there is great interest in eDNA detection for endangered and invasive species. These citations include several recent reviews of the technology, a U.S. Geological Survey fact sheet and an extensive sampling of recent research findings.
- **PCR.** Because questions remain regarding the most appropriate type of PCR to use for eDNA surveying, this section cites two additional reports on the topic. The first is a research study—not specifically addressing aquatic species—that compares endpoint and qPCR. The second is a white paper on PCR validation.
- **Nanopore technology.** Several of our interview subjects mentioned nanopore technology as a possible future field method for identifying DNA. While the consensus is that this technology to identify individual samples of eDNA is not yet mature enough for Caltrans' use, the agency might find these resources instructive.

Tidewater Goby eDNA

Conservation Genetics of the Federally Endangered Tidewater Goby (*Eucyclogobius Newberryi*) in Northern California, W. Tyler McCraney and Andrew P. Kinziger, Humboldt State University, Project No. RWO 79, June 2009.
http://www2.humboldt.edu/cuca/documents/reports/RWO79Tidewater%20Goby%20Population%20Genetics%20Final%20Report.pdf
From the report summary: The objective of this project was to estimate levels of genetic differentiation, genetic diversity, and migration among geographically isolated North Coast tidewater goby (*Eucyclogobius newberryi*) populations. The data set consisted of 621tidewater goby sampled from 13 populations including eight Humboldt Bay populations and five coastal lagoon populations. All individuals were genotyped at nine microsatellite loci and a subset of 103 individuals was sequenced at the mitochondrial control region.

Based on the genetic data, natural and artificial habitat fragmentation has caused marked divergence among North Coast tidewater goby. Thus all populations warrant conservation because they may contain unique genetic material not replicated elsewhere within the species. Additionally, the genetic structure in Humboldt Bay versus coastal lagoon populations is very different and we recommend different management approaches at the two scales.

The Humboldt Bay populations exhibited very high levels of among population genetic differentiation, extremely low levels of within population genetic diversity, and no among population migration making them vulnerable to extirpation. We recommend habitat restoration activities that would increase the potential for between population migration among Humboldt Bay populations. Migration would likely erase existing among population genetic differentiation which would potentially restore Humboldt Bay tidewater goby to the presumptive historical

population structure for this system. Restoration of among population migration would also allow for re-colonization and (or) colonization of suitable habitats. Lastly, migration should also increase within population genetic diversity which could potentially increase fitness of the Humboldt Bay populations.

Coastal lagoon populations also exhibited very high levels of among population genetic differentiation, but in contrast, contained substantial levels of within population genetic diversity with infrequent migration among lagoons. All coastal lagoon populations appear to be stable and genetically healthy with the exception of Lake Earl, which exhibited reduced levels of genetic diversity in comparison to similar coastal lagoon populations. The reduced genetic diversity observed within Lake Earl is consistent with repeated population bottlenecking. In Lake Earl population bottlenecks are most likely caused by artificial breaching. We recommend institution of breaching methods in Lake Earl that do not cause mass mortality of tidewater goby.

Research in Progress
http://www2.humboldt.edu/cuca/research.html

- **Genetic Analysis of Tidewater Goby Tissue Samples**, RWO 83.
- **Tidewater Goby Monitoring**, RWO 86.
- **Phase II: Monitoring the Endangered Tidewater Goby (*Eucyclogobius Newberryi*) Using Environmental DNA in Water Samples: Field Tests**, RWO 87.

Asian Carp

[Note: Some online literature and web sites state that eDNA testing for Asian carp is conducted at the U.S. Army Corps of Engineers (USACE) Engineer Research and Development Center in Vicksburg, MS, or at USFWS's Whitney Genetics Laboratory in Onalaska, WI. A transition plan report (http://www.fws.gov/midwest/fisheries/eDNA/Transition-Plan-Report-Final.pdf) published in August 2013 details the transfer of eDNA processing from the USACE facility to the USFWS facility, which opened in April 2013 and currently processes samples as part of the Great Lakes eDNA Monitoring Program.]

Government Efforts

A number of agencies work in concert to improve the use of eDNA for Asian carp detection as a way to help prevent the spread of the species.

Asian Carp Regional Coordinating Committee, undated.
http://www.asiancarp.us
From the About Us page: The Asian Carp Regional Coordinating Committee with support from federal, state, and local agencies, and other private stakeholder entities, will create a sustainable Asian carp control program to prevent introduction and implement actions to protect and maintain the integrity and safety of the Great Lakes ecosystem from an Asian carp invasion via all viable pathways. The goals and actions of the ACRCC are outlined in the annual Asian Carp Control Strategy Framework and the Monitoring and Response Plan.

The "Environmental DNA" page (http://www.asiancarp.us/edna.htm) outlines how ACRCC uses eDNA as one of its detection tools:

> eDNA has been used as an early detection surveillance tool since 2009. It provides information about whether Asian carp DNA is present in water samples. What it doesn't tell researchers is if the genetic material came from a live or dead fish, one fish or several, or if the eDNA may

have been transported from other sources (e.g., navigation vessels or fish-eating birds). Due to the two-week sample processing time, eDNA cannot yet provide precise, real-time, information about where Asian carp might be.

So if eDNA can't answer all these questions, why use it? Asian carp are notoriously difficult to find in waterways if the population is very low. The eDNA technique is much more sensitive than other standard fishery sampling gear, and is useful for early Asian carp DNA detection and to identify distribution patterns of DNA when the fish are low in abundance. A positive eDNA result tells researchers if Asian carp genetic material is present in an area, then that area may be a good place to use other sampling tools, such as netting, to look for signs of live Asian carp. It is important to note though, that despite over two years of eDNA sampling, hundreds of hours of monitoring efforts and tons of fish harvested, only one Asian carp has been captured in the Chicago Area Waterways above the electric barriers in the Chicago Sanitary and Ship Canal.

Quality Assurance Project Plan: eDNA Monitoring of Bighead and Silver Carps, Midwest Region, U.S. Fish and Wildlife Service, September 2014.
http://www.fws.gov/midwest/fisheries/eDNA/QAPP-eDNA-2014.pdf
The QAPP outlines the detailed procedures for the planning, collection, filtering, processing and reporting of eDNA samples for the ACRCC.

eDNA Calibration Study, Asian Carp Regional Coordinating Committee, undated.
http://www.asiancarp.us/ecals.htm
ACRCC's eDNA Calibration Study (ECALS) is a multiphase study to "improve the application of eDNA methodology to assess and manage uncertainty. ECALS will investigate alternate sources of Asian carp DNA, improve existing genetic markers and investigate the relationship between the number and distribution of positive eDNA samples with the density of Asian carp populations. The results of this study will allow project managers to better interpret eDNA results as well as investigate ways to make the eDNA process more efficient (decrease processing time and cost)."

Among other project milestones, this web page includes a link to a 2014 final report:

A Probabilistic Analysis of Environmental DNA Monitoring Results in the Chicago Area Waterway System, Martin Schultz, Carl Cerco, Brian Skahill, Richard Lance, Mark Noel, Patricia DiJoseph, David Smith, Michael Guilfoyle, U.S. Army Corps of Engineers, November 26, 2014.
http://www.asiancarp.us/documents/FINAL_REPORT-ALL_ACRCC_Framework_Item_2.6.3.Probabilistic_Model_120314.pdf
From the web site: This report summarizes efforts to resolve some of the ambiguity that surrounds interpretation of eDNA monitoring results in the Chicago Area Waterway System (CAWS). This has been accomplished by developing a model that supports probabilistic statements about the source(s) of eDNA detected in water samples and the presence of live bighead carp and silver carp upstream of the electric fish barrier, which is located at Romeoville, Illinois. Numerous other methods and models developed in the course of this effort are described in the report. These include methods to estimate the probability of detecting bighead carp and silver carp eDNA in the water column, methods to make inferences about target marker concentrations from eDNA monitoring results, methods to assess the probability of target species presence using data on conventional fishing effort, and hydrodynamic fate and transport models to simulate target marker concentrations. The major take-away messages with respect to using eDNA for early detection of invasive

species is that, at lower concentrations, Asian carp target markers are difficult to detect in the water column and the polymerase chain reaction (PCR) assay has a high false negative rate.

Summary of the 2014 Great Lakes eDNA Monitoring Program, U.S. Fish and Wildlife Service, 2014.
http://www.asiancarp.us/documents/2014GreatLakeseDNAMonitoringProgram.pdf
This report describes the "comprehensive, basin-wide Great Lakes eDNA Monitoring Program targeted to detect the genetic presence of two species of Asian carp: bighead carp and silver carp." It also mentions the QAPP as a tool to ensure "continuity among all agencies involved in eDNA sampling activities by setting the same protocols for the collection and processing of eDNA samples."

Environmental DNA (eDNA) Surveillance, **Whitney Genetics Lab**, U.S. Fish and Wildlife Service, 2014.
http://www.fws.gov/midwest/WGL/programs.html
The U.S. Fish & Wildlife Service's Whitney Genetics Lab "processes water samples from the Great Lakes and Mississippi River Systems, including the Chicago Area Waterway, as part of efforts to detect and monitor for Silver and Bighead carp." The lab "provides information to the ACRCC and our state partners so that decisions can be made to prevent the spread of invasive carp."

Research

Two citations address recent research on validating and improving eDNA testing for Asian carp.

"Improved Methods for Capture, Extraction, and Quantitative Assay of Environmental DNA from Asian Bigheaded Carp (*Hypophthalmichthys* spp.)," Cameron R. Turner, Derryl J. Miller, Kathryn J. Coyne and Joel Corush. *PLOS ONE*, December 4, 2014.
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0114329
From the abstract: Indirect, non-invasive detection of rare aquatic macrofauna using aqueous environmental DNA (eDNA) is a relatively new approach to population and biodiversity monitoring. As such, the sensitivity of monitoring results to different methods of eDNA capture, extraction, and detection is being investigated in many ecosystems and species. One of the first and largest conservation programs with eDNA-based monitoring as a central instrument focuses on Asian bigheaded carp (*Hypophthalmichthys* spp.), an invasive fish spreading toward the Laurentian Great Lakes. However, the standard eDNA methods of this program have not advanced since their development in 2010. We developed new, quantitative, and more cost-effective methods and tested them against the standard protocols. In laboratory testing, our new quantitative PCR (qPCR) assay for bigheaded carp eDNA was one to two orders of magnitude more sensitive than the existing endpoint PCR assays. When applied to eDNA samples from an experimental pond containing bigheaded carp, the qPCR assay produced a detection probability of 94.8% compared to 4.2% for the endpoint PCR assays. Also, the eDNA capture and extraction method we adapted from aquatic microbiology yielded five times more bigheaded carp eDNA from the experimental pond than the standard method, at a per sample cost over forty times lower. Our new, more sensitive assay provides a quantitative tool for eDNA-based monitoring of bigheaded carp, and the higher-yielding eDNA capture and extraction method we describe can be used for eDNA-based monitoring of any aquatic species.

The article includes a protocol, "CTAB DNA Extraction Protocol for PCTE or PES Filters," http://www.plosone.org/article/fetchSingleRepresentation.action?uri=info:doi/10.1371/journal.pone.0114329.s003, in Word format.

"Validation of eDNA Surveillance Sensitivity for Detection of Asian Carps in Controlled and Field Experiments," Andrew R. Mahon, Christopher L. Jerde, Matthew Galaska, Jennifer L. Bergner, W. Lindsay Chadderton, David M. Lodge, Margaret E. Hunter and Leo G. Nico, *PLOS ONE*, March 5, 2013.
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0058316
From the abstract: In many North American rivers, populations of multiple species of non-native cyprinid fishes are present, including black carp (*Mylpharyngodon piceus*), grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys* nobilis), silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), and goldfish (*Carassius auratus*). All six of these species are found in the Mississippi River basin and tracking their invasion has proven difficult, particularly where abundance is low. Knowledge of the location of the invasion front is valuable to natural resource managers because future ecological and economic damages can be most effectively prevented when populations are low. To test the accuracy of environmental DNA (eDNA) as an early indicator of species occurrence and relative abundance, we applied eDNA technology to the six non-native cyprinid species putatively present in a 2.6 river mile stretch of the Chicago (IL, USA) canal system that was subsequently treated with piscicide. The proportion of water samples yielding positive detections increased with relative abundance of the six species, as indicated by the number of carcasses recovered after poisoning. New markers for black carp, grass carp, and a common carp/goldfish are reported and details of the marker testing to ensure specificity are provided.

Great Crested Newt

Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt, and Other Pond Vertebrates, WC1067, U.K. Department for Environment, Food & Rural Affairs, 2014.
http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18650
This web page provides background about the United Kingdom's efforts to develop eDNA testing for the great crested newt, including rationale based on advances in eDNA testing and the low detection rate for the species using traditional methods. It outlines research conducted by DEFRA to:

- Establish the performance of eDNA techniques to determine the presence of great crested newt in a wide variety of pond habitats across Great Britain.
- Investigate the performance of eDNA techniques to predict great crested newt population sizes within ponds.
- Document a technical advice note for the use of eDNA field and laboratory techniques for the great crested newt.
- Produce surveillance sampling options for the great crested newt across Great Britain to determine baseline and trend data.

Key publications include:

- **Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt**, Final Report, WC 1067, Freshwater Habitats Trust, January 2014.
  http://randd.defra.gov.uk/Document.aspx?Document=11973_WC1067_FinalReport.pdf
  This final report summarizes the work to develop surveillance monitoring for the great crested newt.

- **"Technical Advice Note for Field and Laboratory Sampling of Great Crested Newt (*Triturus cristatus*) Environmental DNA,"** Appendix 5, Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt, WC 1067, Freshwater Habitats Trust, September 30, 2014.
  http://randd.defra.gov.uk/Document.aspx?Document=12287_WC1067_Appendix_5_TechnicalAdviceNoteUpdatedSept2014.docx
  This appendix to the final report serves as the government's eDNA survey protocols.

Great Crested Newts: Protection, Surveys and Licenses, Natural England and the U.K. Department for Environment, Food & Rural Affairs, October 2014.
https://www.gov.uk/great-crested-newts-protection-surveys-and-licences
This resource from the British government gives advice to developers: "Find out what you must do to avoid harming great crested newts and when you need a license." For conducting presence/absence surveys of water bodies, the ecologist should:

- Use three methods per visit (preferably netting, torch survey, bottle trapping and egg searching).
- Make at least four visits.
- Visit between mid-March and mid-June with at least two visits between mid-April and mid-May.

The ecologist could instead do an eDNA survey, which involves collecting a sample of water and testing it for traces of DNA to see whether newts are present.

Newts, School of Life Sciences, The University of Warwick, June 2014.
http://www2.warwick.ac.uk/fac/sci/lifesci/research/archaeobotany/ecological_forensics/newts/
This web page details the university's eDNA testing program for the great crested newt, including costs. *From the web page*:

> In the wake of Natural England's reporting of the DEFRA study on eDNA tests to establish the presence of great crested newts in water bodies we have had numerous enquiries wishing to know whether this is a service we would be able to offer. We had in fact been working on a similar test, and can offer it for this coming season. The methodology we have developed is slightly different to that of the DEFRA project in that we produce DNA sequences that verify the taxonomic assignation of amplified DNA signals rather than the quantitative PCR approach the study employed. The sensitivities of the two approaches should be much the same. The sampling procedure is similar to the one described in the DEFRA study.
>
> The cost of the test is £144 [approx. $225] (including VAT). On notification, we will send out sampling kits so you can sample a water way and return the kit to us for testing. The price includes a shipping protocol (which accounts for £40 of the cost). This is necessary because it is not possible to ship laboratory alcohol through the Royal Mail, we have to use DHL who themselves will have to approve you. This is easily done, and instructions are included in the instructions we send out with kits. Note that you will be required to arrange with DHL to pick up the package when you have completed your sampling. We expect the test to take about three weeks after receipt of the samples from the field, currently.
>
> Alternatively, if your operation is local to the University of Warwick you can pick up and drop off kits, in which case we can arrange a cheaper system (£96, including VAT).

The test is prepaid. Please understand that request of a kit is a commitment to the £144 (or £96) charge.

Application of eDNA

Environmental DNA: A Review of the Possible Applications for the Detection of (Invasive) Species, Jelger Herder, Alice Valentini, Eva Bellemain, Tony Dejean, Jeroen van Delft, Philip Francis Thomsen and Pierre Taberlet, Bureau Risicobeoordeling & Onderzoeksprogrammering, part of the Netherlands Food and Consumer Product Safety Authority, 2014.
http://www.environmental-dna.nl/Portals/7/Herder%20et%20al%202014%20-%20Environmental%20DNA%20review.pdf

(Note: This publication was recommended by FERA's Neil Boonham.)

This white paper provides practical guidelines and considerations for eDNA testing. Topics that may be of particular interest for this Preliminary Investigation include:

- Checklist of factors impacting the reliability of an eDNA assay (page 8).
- Section 2.2, Persistence of eDNA in Different Environments (page 15).
- Section 2.3, Factors Influencing the Amount of eDNA (page 16).
- Section 3.2, Sampling Methods and Strategies (page 22).
- Chapter 7, What is the Reliability of the eDNA Method? (page 49).
- Chapter 8, What is the Detection Probability with the eDNA Method? (page 57).

Caltrans may also be interested in Chapter 5, What Other Potential DNA-Based Techniques Exist? (page 41), which discusses laser transmission spectroscopy and microarray technology. PCR remains the main technology of interest for this report.

Application of Environmental DNA for Inventory and Monitoring of Aquatic Species, David S. Pilliod, Caren S. Goldberg, Matthew B. Laramie and Lisette P. Waits, U.S. Geological Survey, Fact Sheet No. 2012–3146, November 2012.
http://pubs.usgs.gov/fs/2012/3146/
From the introduction: This fact sheet was created to help biologists and resource managers understand emerging methods for detecting environmental DNA and their potential application for inventorying and monitoring aquatic species. It is a synthesis of published information.

The main sections of this fact sheet are:

- What is Environmental DNA?
- Use of eDNA for Inventory and Monitoring.
- Developing eDNA Protocols for Species Monitoring.
- Sources of Error.

This publication also includes a summary of design of species-specific primers and probes for qPCR and design of molecular assay for aquatic organisms in freshwater environments.

"Review: The Detection of Aquatic Animal Species Using Environmental DNA—A Review of eDNA as a Survey Tool in Ecology," Helen C. Rees, Ben C. Maddison, David J. Middleditch, James R.M. Patmore and Kevin C. Gough, *Journal of Applied Ecology*, Vol. 51, Issue 5, pages 1450-1459, October 2014.
http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12306/abstract
From the abstract:

1. Knowledge of species distribution is critical to ecological management and conservation biology. Effective management requires the detection of populations, which can sometimes be at low densities and is usually based on visual detection and counting.
2. Recently, there has been considerable interest in the detection of short species-specific environmental DNA (eDNA) fragments to allow aquatic species monitoring within different environments due to the potential of greater sensitivity over traditional survey methods which can be time-consuming and costly.
3. Environmental DNA analysis is increasingly being used in the detection of rare or invasive species and has also been applied to eDNA persistence studies and estimations of species biomass and distribution. When combined with next-generation sequencing methods, it has been demonstrated that entire faunas can be identified.
4. Different environments require different sampling methodologies, but there remain areas where laboratory methodologies could be standardized to allow results to be compared across studies.
5. Synthesis and applications. We review recently published studies that use eDNA to monitor aquatic populations, discuss the methodologies used and the application of eDNA analysis as a survey tool in ecology. We include innovative ideas for how eDNA can be used for conservation and management citing test cases, for instance, the potential for on-site analyses, including the application of eDNA analysis to carbon nanotube platforms or laser transmission spectroscopy to facilitate rapid on-site detections. The use of eDNA monitoring is already being adopted in the UK for ecological surveys.

"Review: Environmental DNA for Wildlife Biology and Biodiversity Monitoring," Kristine Bohmann, Alice Evans, M. Thomas P. Gilbert, Gary R. Carvalho, Simon Creer, Michael Knapp, Douglas W. Yu and Mark de Bruyn, *Trends in Ecology & Evolution*, Vol. 29, Issue 6, pages 358-367, June 2014.
http://www.cell.com/trends/ecology-evolution/abstract/S0169-5347(14)00086-X?_returnURL=http%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS016953471400086X%3Fshowall%3Dtrue
From the abstract:

- eDNA is driving rapid advances in ecology, evolution, and conservation.
- eDNA provides mechanistic insights into ecological and evolutionary processes.
- Foremost among these is an improved ability to explore ecosystem-level processes.
- We examine current frontiers of eDNA, outlining key aspects requiring improvement.
- We suggest future developments and priorities for eDNA research.

Extraction and identification of DNA from an environmental sample has proven noteworthy recently in detecting and monitoring not only common species, but also those that are endangered, invasive, or elusive. Particular attributes of so-called environmental DNA (eDNA)

analysis render it a potent tool for elucidating mechanistic insights in ecological and evolutionary processes. Foremost among these is an improved ability to explore ecosystem-level processes, the generation of quantitative indices for analyses of species, community diversity, and dynamics, and novel opportunities through the use of time-serial samples and unprecedented sensitivity for detecting rare or difficult-to-sample taxa. Although technical challenges remain, here we examine the current frontiers of eDNA, outline key aspects requiring improvement, and suggest future developments and innovations for research.

"Moving Environmental DNA Methods from Concept to Practice for Monitoring Aquatic Macroorganisms," Caren S. Goldberg, Katherine M. Strickler, David S. Pilliod, *Biological Conservation*, Special Issue Article, December 2014.
http://www.sciencedirect.com/science/article/pii/S0006320714004650
From the abstract: The discovery that macroorganisms can be detected from their environmental DNA (eDNA) in aquatic systems has immense potential for the conservation of biological diversity. This special issue contains 11 papers that review and advance the field of eDNA detection of vertebrates and other macroorganisms, including studies of eDNA production, transport, and degradation; sample collection and processing to maximize detection rates; and applications of eDNA for conservation using citizen scientists. This body of work is an important contribution to the ongoing efforts to take eDNA detection of macroorganisms from technical breakthrough to established, reliable method that can be used in survey, monitoring, and research applications worldwide. While the rapid advances in this field are remarkable, important challenges remain, including consensus on best practices for collection and analysis, understanding of eDNA diffusion and transport, and avoidance of inhibition in sample collection and processing. Nonetheless, as demonstrated in this special issue, eDNA techniques for research and monitoring are beginning to realize their potential for contributing to the conservation of biodiversity globally.

Articles in this special issue of *Biological Conservation* relevant to this Preliminary Investigation include:

Characterizing the Distribution of an Endangered Salmonid Using Environmental DNA Analysis, M. B. Laramie, D. S. Pilliod and C. S. Goldberg.

Choice of Capture and Extraction Methods Affect Detection of Freshwater Biodiversity from Environmental DNA, K. Deiner, J.-C. Walser, E. Mächler and F. Altermatt.

Effects of Sample Processing on the Detection Rate of Environmental DNA from the Common Carp (*Cyprinus carpio*), T. Takahara, T. Minamoto and H. Doi.

Environmental DNA—An Emerging Tool in Conservation for Monitoring Past and Present Biodiversity, P. F. Thomsen and E. Willerslev.

Monitoring the Near-Extinct European Weather Loach *Misgurnus fossilis* in Denmark by Combining Traditional Fishing Surveys and Environmental DNA from Water Samples, E. E. Sigsgaard, H. Carl, P. R. Møller and P. F. Thomsen.

Quantification of eDNA Shedding Rates from Invasive Bighead Carp (*Hypophthalmichthys nobilis*) and Silver Carp (*Hypophthalmichthys molitrix*), K. E. Klymus, C. A. Richter, D. C. Chapman and C. Paukert.

Quantifying the Effects of UV, Temperature, and pH on Degradation Rates of eDNA in Aquatic Microcosms, K. M. Strickler, A. K. Fremier and C. S. Goldberg.

Using Environmental DNA Methods to Improve Detectability in a Hellbender (*Cryptobranchus alleganiensis*) Monitoring Program, S. Spear, J. D. Groves, L. A.Williams and L. P. Waits.

"Assessing Environmental DNA Detection in Controlled Lentic Systems," Gregory R. Moyer, Edgardo Díaz-Ferguson, Jeffrey E. Hill and Colin Shea**,** *PLOS ONE*, July 31, 2014.
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0103767
From the abstract: Little consideration has been given to environmental DNA (eDNA) sampling strategies for rare species. The certainty of species detection relies on understanding false positive and false negative error rates. We used artificial ponds together with logistic regression models to assess the detection of African jewelfish eDNA at varying fish densities (0, 0.32, 1.75, and 5.25 fish/m^3). Our objectives were to determine the most effective water stratum for eDNA detection, estimate true and false positive eDNA detection rates, and assess the number of water samples necessary to minimize the risk of false negatives. There were 28 eDNA detections in 324, 1-L, water samples collected from four experimental ponds. The best-approximating model indicated that the per-L-sample probability of eDNA detection was 4.86 times more likely for every 2.53 fish/m^3 (1 SD) increase in fish density and 1.67 times less likely for every 1.02 C (1 SD) increase in water temperature. The best section of the water column to detect eDNA was the surface and to a lesser extent the bottom. Although no false positives were detected, the estimated likely number of false positives in samples from ponds that contained fish averaged 3.62. At high densities of African jewelfish, 3–5 L of water provided a >95% probability for the presence/absence of its eDNA. Conversely, at moderate and low densities, the number of water samples necessary to achieve a >95% probability of eDNA detection approximated 42–73 and >100 L, respectively. Potential biases associated with incomplete detection of eDNA could be alleviated via formal estimation of eDNA detection probabilities under an occupancy modeling framework; alternatively, the filtration of hundreds of liters of water may be required to achieve a high (e.g., 95%) level of certainty that African jewelfish eDNA will be detected at low densities (i.e., <0.32 fish/m^3 or 1.75 g/m^3).

"Harnessing DNA to Improve Environmental Management," Ryan P. Kelly, Jesse A. Port, Kevan M. Yamahara, Rebecca G. Martone, Natalie Lowell, Philip Francis Thomsen, Megan E. Mach, Meredith Bennett, Erin Prahler, Margaret R. Caldwell, Larry B. Crowder, *Science*, Vol. 344, Issue 6191, pages 1455-1456, June 2014.
http://www.sciencemag.org/content/344/6191/1455.summary
From the abstract: Responsive environmental policy demands a constant stream of information about the living world, but biological monitoring is difficult and expensive. For many species and ecosystems—especially in aquatic and marine environments—practical monitoring methods are lacking; even where methods do exist, they may be inefficient, highly destructive, or dependent on diminishing taxonomic expertise.

"Environmental Conditions Influence eDNA Persistence in Aquatic Systems," Matthew A. Barnes, Cameron R. Turner, Christopher L. Jerde, Mark A. Renshaw, W. Lindsay Chadderton and David M. Lodge, *Environmental Science & Technology*, Vol. 48, Issue 3, pages 1819-1827, January 14, 2014.
http://pubs.acs.org/doi/abs/10.1021/es404734p
From the abstract: Environmental DNA (eDNA) surveillance holds great promise for improving species conservation and management. However, few studies have investigated eDNA

dynamics under natural conditions, and interpretations of eDNA surveillance results are clouded by uncertainties about eDNA degradation. We conducted a literature review to assess current understanding of eDNA degradation in aquatic systems and an experiment exploring how environmental conditions can influence eDNA degradation. Previous studies have reported macrobial eDNA persistence ranging from less than 1 day to over 2 weeks, with no attempts to quantify factors affecting degradation. Using a SYBR Green quantitative PCR assay to observe Common Carp (*Cyprinus carpio*) eDNA degradation in laboratory mesocosms, our rate of Common Carp eDNA detection decreased over time. Common Carp eDNA concentration followed a pattern of exponential decay, and observed decay rates exceeded previously published values for aquatic macrobial eDNA. Contrary to our expectations, eDNA degradation rate declined as biochemical oxygen demand, chlorophyll, and total eDNA (i.e., from any organism) concentration increased. Our results help explain the widely divergent, previously published estimates for eDNA degradation. Measurements of local environmental conditions, consideration of environmental influence on eDNA detection, and quantification of local eDNA degradation rates will help interpret future eDNA surveillance results.

"Using Environmental DNA to Census Marine Fishes in a Large Mesocosm," Ryan P. Kelly, Jesse A. Port, Kevan M. Yamahara and Larry B. Crowder, *PLOS ONE*, January 15, 2014. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0086175
From the abstract: The ocean is a soup of its resident species' genetic material, cast off in the forms of metabolic waste, shed skin cells, or damaged tissue. Sampling this environmental DNA (eDNA) is a potentially powerful means of assessing whole biological communities, a significant advance over the manual methods of environmental sampling that have historically dominated marine ecology and related fields. Here, we estimate the vertebrate fauna in a 4.5-million-liter mesocosm aquarium tank at the Monterey Bay Aquarium of known species composition by sequencing the eDNA from its constituent seawater. We find that it is generally possible to detect mitochondrial DNA of bony fishes sufficient to identify organisms to taxonomic family- or genus-level using a 106 bp fragment of the 12S ribosomal gene. Within bony fishes, we observe a low false-negative detection rate, although we did not detect the cartilaginous fishes or sea turtles present with this fragment. We find that the rank abundance of recovered eDNA sequences correlates with the abundance of corresponding species' biomass in the mesocosm, but the data in hand do not allow us to develop a quantitative relationship between biomass and eDNA abundance. Finally, we find a low false-positive rate for detection of exogenous eDNA, and we were able to diagnose non-native species' tissue in the food used to maintain the mesocosm, underscoring the sensitivity of eDNA as a technique for community-level ecological surveys. We conclude that eDNA has substantial potential to become a core tool for environmental monitoring, but that a variety of challenges remain before reliable quantitative assessments of ecological communities in the field become possible.

American Fisheries Society meetings.
Both in 2014 and 2013, more than a dozen papers were presented each year at the American Fisheries Society meetings. The web pages listed here include links to abstracts.

> **Environmental DNA: A New Tool for Aquatic Conservation and Fisheries Management**, American Fisheries Society 2014 Meeting, Quebec City, Quebec, Canada.
> Part 1: https://afs.confex.com/afs/2014/webprogram/Session2959.html
> Part 2: https://afs.confex.com/afs/2014/webprogram/Session3153.html
> Fifteen presentations are listed. *From the session summary:* Molecular ecologists can now take advantage of great advances in sequencing technologies and analytical methods, but the potential of genetic tools in aquatic management and conservation is still underexploited. In particular, environmental DNA (eDNA) is one of the latest tools in applied molecular

ecology with a huge, still untapped potential for faunal monitoring. The eDNA method is a novel sampling approach for macro-organism that detects traces of cellular or extracellular DNA in the soil and water from sources such feces, secreted mucous membranes, gametes and skin cells. The method has great potential to increase the power of detection and quantification, spatial coverage and frequency of sampling for aquatic ecosystems. Large-scale studies are often limited by the lack of broad spatial and temporal data. The analysis of eDNA could be a revolutionary tool to overcome this problem without physically manipulating the organisms. At local scale, eDNA has been shown to be a promising method to early detection of invasive species, but the method is also gaining interest for broader scopes for a large number of marine and freshwater ecosystems such as collecting data for species at risk, species that are difficult to capture and fisheries management in general. However, integrating eDNA with management strategies has important scientific challenges: (1) developing molecular techniques (specific markers and probes design, DNA extraction, traditional PCR and quantitative PCR), (2) developing and standardizing sampling methods among species and habitats and (3) developing new statistical analyses. In this context, the goal of the symposium is to advance the field by uniting researchers working on both improving eDNA methods as well as showing its potential for fisheries management.

Environmental DNA (eDNA) Analysis—A New Genetic Tool for Monitoring, Managing, and Conserving Fishery Resources and Aquatic Habitat, American Fisheries Society 2013 Meeting, Little Rock, AR.
https://afs.confex.com/afs/2013/webprogram/Session2539.html
Nineteen presentations are listed. *From the session summary:* Rapid advances in the field of molecular genetics continue to provide new tools for research, management and conservation. One such genetic tool is environmental DNA (eDNA) analysis. eDNA refers to DNA that organisms leave behind or shed as they pass through the environment. This shed DNA can be detected using routine molecular techniques such as the polymerase chain reaction (PCR) to amplify species-specific genes, potentially linking the organism to the environment without actually observing the organism. eDNA analysis is currently being evaluated and applied for uses such as surveillance and control of aquatic invasive species, identification and monitoring of endangered species, and analysis of biodiversity. In this symposium we will explore the methodologies and potential uses of eDNA analysis for monitoring, managing and conserving fishery resources and aquatic habitat.

"Robust Detection of Rare Species Using Environmental DNA: The Importance of Primer Specificity," Taylor M. Wilcox, Kevin S. McKelvey, Michael K. Young, Stephen F. Jane, Winsor H. Lowe, Andrew R. Whiteley and Michael K. Schwartz, *PLOS ONE*, March 26, 2013.
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0059520
From the abstract: Environmental DNA (eDNA) is being rapidly adopted as a tool to detect rare animals. Quantitative PCR (qPCR) using probe-based chemistries may represent a particularly powerful tool because of the method's sensitivity, specificity, and potential to quantify target DNA. However, there has been little work understanding the performance of these assays in the presence of closely related, sympatric taxa. If related species cause any cross-amplification or interference, false positives and negatives may be generated. These errors can be disastrous if false positives lead to overestimate the abundance of an endangered species or if false negatives prevent detection of an invasive species. In this study we test factors that influence the specificity and sensitivity of TaqMan MGB assays using co-occurring, closely related brook trout (*Salvelinus fontinalis*) and bull trout (*S. confluentus*) as a case study. We found qPCR to be substantially more sensitive than traditional PCR, with a high probability of detection at concentrations as low as 0.5 target copies/µl. We also found that number and placement of

base pair mismatches between the TaqMan MGB assay and non-target templates was important to target specificity, and that specificity was most influenced by base pair mismatches in the primers, rather than in the probe. We found that insufficient specificity can result in both false positive and false negative results, particularly in the presence of abundant related species. Our results highlight the utility of qPCR as a highly sensitive eDNA tool, and underscore the importance of careful assay design.

"Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples," Philip Francis Thomsen, Jos Kielgast Lars Lønsmann Iversen, Peter Rask Møller, Morten Rasmussen and Eske Willerslev, *PLOS ONE*, August 29, 2012.
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0041732
From the abstract: Marine ecosystems worldwide are under threat with many fish species and populations suffering from human over-exploitation. This is greatly impacting global biodiversity, economy and human health. Intriguingly, marine fish are largely surveyed using selective and invasive methods, which are mostly limited to commercial species, and restricted to particular areas with favourable conditions. Furthermore, misidentification of species represents a major problem. Here, we investigate the potential of using metabarcoding of environmental DNA (eDNA) obtained directly from seawater samples to account for marine fish biodiversity. This eDNA approach has recently been used successfully in freshwater environments, but never in marine settings. We isolate eDNA from ½-litre seawater samples collected in a temperate marine ecosystem in Denmark. Using next-generation DNA sequencing of PCR amplicons, we obtain eDNA from 15 different fish species, including both important consumption species, as well as species rarely or never recorded by conventional monitoring. We also detect eDNA from a rare vagrant species in the area; European pilchard (*Sardina pilchardus*). Additionally, we detect four bird species. Records in national databases confirmed the occurrence of all detected species. To investigate the efficiency of the eDNA approach, we compared its performance with 9 methods conventionally used in marine fish surveys. Promisingly, eDNA covered the fish diversity better than or equal to any of the applied conventional methods. Our study demonstrates that even small samples of seawater contain eDNA from a wide range of local fish species. Finally, in order to examine the potential dispersal of eDNA in oceans, we performed an experiment addressing eDNA degradation in seawater, which shows that even small (100-bp) eDNA fragments degrades beyond detectability within days. Although further studies are needed to validate the eDNA approach in varying environmental conditions, our findings provide a strong proof-of-concept with great perspectives for future monitoring of marine biodiversity and resources.

"Monitoring Endangered Freshwater Biodiversity Using Environmental DNA," Philip Francis Thomsen, Jos Kielgast, Lars L. Iversen, Carsten Wiuf, Morten Rasmussen, M. Thomas P Gilbert, Ludovic Orlando and Eske Willerslev, *Molecular Ecology*, Vol. 21, Issue 11, pages 2565-2573, June 2012.
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2011.05418.x/full
From the abstract: Freshwater ecosystems are among the most endangered habitats on Earth, with thousands of animal species known to be threatened or already extinct. Reliable monitoring of threatened organisms is crucial for data-driven conservation actions but remains a challenge owing to nonstandardized methods that depend on practical and taxonomic expertise, which is rapidly declining. Here, we show that a diversity of rare and threatened freshwater animals—representing amphibians, fish, mammals, insects and crustaceans—can be detected and quantified based on DNA obtained directly from small water samples of lakes, ponds and streams. We successfully validate our findings in a controlled mesocosm experiment and show that DNA becomes undetectable within 2 weeks after removal of animals, indicating that DNA traces are near contemporary with presence of the species. We further demonstrate that entire

faunas of amphibians and fish can be detected by high-throughput sequencing of DNA extracted from pond water. Our findings underpin the ubiquitous nature of DNA traces in the environment and establish environmental DNA as a tool for monitoring rare and threatened species across a wide range of taxonomic groups.

"Estimation of Fish Biomass Using Environmental DNA," Teruhiko Takahara, Toshifumi Minamoto, Hiroki Yamanaka, Hideyuki Doi and Zen'ichiro Kawabata, *PLOS ONE*, April 26, 2012.
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0035868
From the abstract: Environmental DNA (eDNA) from aquatic vertebrates has recently been used to estimate the presence of a species. We hypothesized that fish release DNA into the water at a rate commensurate with their biomass. Thus, the concentration of eDNA of a target species may be used to estimate the species biomass. We developed an eDNA method to estimate the biomass of common carp (*Cyprinus carpio* L.) using laboratory and field experiments. In the aquarium, the concentration of eDNA changed initially, but reached an equilibrium after 6 days. Temperature had no effect on eDNA concentrations in aquaria. The concentration of eDNA was positively correlated with carp biomass in both aquaria and experimental ponds. We used this method to estimate the biomass and distribution of carp in a natural freshwater lagoon. We demonstrated that the distribution of carp eDNA concentration was explained by water temperature. Our results suggest that biomass data estimated from eDNA concentration reflects the potential distribution of common carp in the natural environment. Measuring eDNA concentration offers a non-invasive, simple, and rapid method for estimating biomass. This method could inform management plans for the conservation of ecosystems.

"From Molecules to Management: Adopting DNA-Based Methods for Monitoring Biological Invasions in Aquatic Environments," J. A. Darling and A. R. Mahon, *Environmental Research*, Vol. 111, Issue 7, pages 978-988, October 2011.
http://www.ncbi.nlm.nih.gov/pubmed/21353670
From the abstract: Recent technological advances have driven rapid development of DNA-based methods designed to facilitate detection and monitoring of invasive species in aquatic environments. These tools promise to improve on traditional monitoring approaches by enhancing detection sensitivity, reducing analytical turnaround times and monitoring costs, and increasing specificity of target identifications. However, despite the promise of DNA-based monitoring methods, the adoption of these tools in decision-making frameworks remains challenging. Here, rather than explore technical aspects of method development, we examine impediments to effective translation of those methods into management contexts. In addition to surveying current use of DNA-based tools for aquatic invasive species monitoring, we explore potential sources of uncertainty associated with molecular technologies and possibilities for limiting that uncertainty and effectively communicating its implications for decision-making. We pay particular attention to the recent adoption of DNA-based methods for detection of invasive Asian carp species in the United States Great Lakes region, as this example illustrates many of the challenges associated with applying molecular tools to achieve desired management outcomes. Our goal is to provide a useful assessment of the obstacles associated with integrating DNA-based methods into aquatic invasive species management, and to offer recommendations for future efforts aimed at overcoming those obstacles.

"Persistence of Environmental DNA in Freshwater Ecosystems," Tony Dejean, Alice Valentini, Antoine Duparc, Stéphanie Pellier-Cuit, François Pompanon, Pierre Taberlet and Claude Miaud, *PLOS ONE*, August 8, 2011.
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0023398
From the abstract: The precise knowledge of species distribution is a key step in conservation biology. However, species detection can be extremely difficult in many environments, specific life stages and in populations at very low density. The aim of this study was to improve the knowledge on DNA persistence in water in order to confirm the presence of the focus species in freshwater ecosystems. Aquatic vertebrates (fish: Siberian sturgeon and amphibian: Bullfrog tadpoles) were used as target species. In control conditions (tanks) and in the field (ponds), the DNA detectability decreases with time after the removal of the species source of DNA. DNA was detectable for less than one month in both conditions. The density of individuals also influences the dynamics of DNA detectability in water samples. The dynamics of detectability reflects the persistence of DNA fragments in freshwater ecosystems. The short time persistence of detectable amounts of DNA opens perspectives in conservation biology, by allowing access to the presence or absence of species e.g. rare, secretive, potentially invasive, or at low density. This knowledge of DNA persistence will greatly influence planning of biodiversity inventories and biosecurity surveys.

"Molecular Detection of Vertebrates in Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant Salamanders," Caren S. Goldberg, David S. Pilliod, Robert S. Arkle and Lisette P. Waits, *PLOS ONE*, July 26, 2011.
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0022746
From the abstract: Stream ecosystems harbor many secretive and imperiled species, and studies of vertebrates in these systems face the challenges of relatively low detection rates and high costs. Environmental DNA (eDNA) has recently been confirmed as a sensitive and efficient tool for documenting aquatic vertebrates in wetlands and in a large river and canal system. However, it was unclear whether this tool could be used to detect low-density vertebrates in fast-moving streams where shed cells may travel rapidly away from their source. To evaluate the potential utility of eDNA techniques in stream systems, we designed targeted primers to amplify a short, species-specific DNA fragment for two secretive stream amphibian species in the northwestern region of the United States (Rocky Mountain tailed frogs, *Ascaphus montanus*, and Idaho giant salamanders, *Dicamptodon aterrimus*). We tested three DNA extraction and five PCR protocols to determine whether we could detect eDNA of these species in filtered water samples from five streams with varying densities of these species in central Idaho, USA. We successfully amplified and sequenced the targeted DNA regions for both species from stream water filter samples. We detected Idaho giant salamanders in all samples and Rocky Mountain tailed frogs in four of five streams and found some indication that these species are more difficult to detect using eDNA in early spring than in early fall. While the sensitivity of this method across taxa remains to be determined, the use of eDNA could revolutionize surveys for rare and invasive stream species. With this study, the utility of eDNA techniques for detecting aquatic vertebrates has been demonstrated across the majority of freshwater systems, setting the stage for an innovative transformation in approaches for aquatic research.

"'Sight-Unseen' Detection of Rare Aquatic Species Using Environmental DNA," Christopher L. Jerde, Andrew R. Mahon, W. Lindsay Chadderton and David M. Lodge, *Conservation Letters*, Vol. 4, Issue 2, pages 150-157, April/May 2011.
http://onlinelibrary.wiley.com/doi/10.1111/j.1755-263X.2010.00158.x/full
From the abstract: Effective management of rare species, including endangered native species and recently introduced nonindigenous species, requires the detection of populations at low density. For endangered species, detecting the localized distribution makes it possible to

identify and protect critical habitat to enhance survival or reproductive success. Similarly, early detection of an incipient invasion by a harmful species increases the feasibility of rapid responses to eradicate the species or contain its spread. Here we demonstrate the efficacy of environmental DNA (eDNA) as a detection tool in freshwater environments. Specifically, we delimit the invasion fronts of two species of Asian carps in Chicago, Illinois, USA area canals and waterways. Quantitative comparisons with traditional fisheries surveillance tools illustrate the greater sensitivity of eDNA and reveal that the risk of invasion to the Laurentian Great Lakes is imminent.

PCR

"Comparison of a Quantitative Real-Time Polymerase Chain Reaction (qPCR) with Conventional PCR, Bacterial Culture and ELISA for Detection of *Mycobacterium avium* subsp. *paratuberculosis* Infection in Sheep Showing Pathology of Johne's Disease," Ganesh G. Sonawane and Bhupendra N. Tripathi, *Springerplus*, Vol. 2, No. 45, February 11, 2013.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3604594/
From the abstract: A quantitative real-time PCR (qPCR) assay employing IS*900* gene specific primers of *Mycobacterium avium* subsp. *parartuberculosis* (MAP) was compared with conventional PCR, bacterial culture and enzyme-linked immunosorbent assay in 38 sheep showing granulomatous enteritis and lymphadenitis with and without demonstration of acid-fast bacilli (AFB). The lesions were classified as multibacillary (MB) (n□=□23), which had diffuse granulomatous lesions with abundant AFB, and paucibacillary (PB) (n□=□15), which had focal or multifocal granulomatous lesions with few or no AFB. In the multibacillary group (MB), IS*900* PCR detected 19 (82.6%), and qPCR detected all 23 (100%) sheep positive for MAP in the intestine and lymph node tissues. In the paucibacillary group (PB), IS*900* PCR detected 2 (13.3%), and qPCR detected all 15 (100%) sheep positive for MAP in tissues. When results of both groups were taken together, IS*900* PCR detected 21(55.2%), and qPCR detected all 38 (100%) animals positive for MAP genome either in the intestine or lymph node tissues. On Herrold egg yolk medium, tissues of 14 (60.9%) MB and 5 (33.3%) PB sheep were found to be positive for MAP. Out of 27 sheep (PB□=□8, MB□=□19) tested by an ELISA, 21 (77.7%) were found to be positive for MAP antibody, of which 25% (2/8) and 100% (19/19) sheep were from PB and MB sheep, respectively. Based on the results of the present study, it was concluded that qPCR was a highly sensitive test in comparison to conventional PCR, ELISA and bacterial culture for the diagnosis of paratuberculosis on infected tissues especially from paucibacillary sheep.

Validation of PCR-Based Assays and Laboratory Accreditation for Environmental Detection of Aquatic Invasive Species, Invasive Species Advisory Committee, U.S. Department of the Interior, May 2012.
http://www.invasivespecies.gov/ISAC/White%20Papers/ISAC_PCR_WHITEPAPER_FINAL.pdf
From the introduction: This white paper provides:

a) Background information on the use, accuracy and reliability of PCR-based assays such as environmentally sampled DNA (eDNA) for early detection of aquatic invasive species (AIS) and;
b) Recommendations for establishing a system for validating assays and accrediting laboratories that report on the presence or absence of AIS.

This white paper was developed by the members of ISAC and discusses the need for developing validation requirements for Polymerase Chain Reaction (PCR) and other DNA-based molecular assays that are increasingly being used to detect AIS. It does not provide a

simplified checklist for evaluation of their ability to detect AIS. Rather, it is intended to demonstrate the need for a required and regulated framework to validate these molecular assays. A regulated framework for validation would greatly increase confidence in the utility of DNA-based assays and better enable decision-makers and managers regarding AIS detection, prevention, monitoring and control.

Nanopore Technology

"Nanopores: A Journey Towards DNA Sequencing," Meni Wanunu, *Physics of Life Reviews*, Vol. 9, Issue 2, pages 125-158, June 2012.
http://www.ncbi.nlm.nih.gov/pubmed/22658507
From the abstract: Much more than ever, nucleic acids are recognized as key building blocks in many of life's processes, and the science of studying these molecular wonders at the single-molecule level is thriving. A new method of doing so has been introduced in the mid 1990's. This method is exceedingly simple: a nanoscale pore that spans across an impermeable thin membrane is placed between two chambers that contain an electrolyte, and voltage is applied across the membrane using two electrodes. These conditions lead to a steady stream of ion flow across the pore. Nucleic acid molecules in solution can be driven through the pore, and structural features of the biomolecules are observed as measurable changes in the trans-membrane ion current. In essence, a nanopore is a high-throughput ion microscope and a single-molecule force apparatus. Nanopores are taking center stage as a tool that promises to read a DNA sequence, and this promise has resulted in overwhelming academic, industrial, and national interest. Regardless of the fate of future nanopore applications, in the process of this 16-year-long exploration, many studies have validated the indispensability of nanopores in the toolkit of single-molecule biophysics. This review surveys past and current studies related to nucleic acid biophysics, and will hopefully provoke a discussion of immediate and future prospects for the field.

Oxford Nanopore Technologies, 2015.
https://www.nanoporetech.com/
From the web site: Oxford Nanopore Technologies® is developing a new generation of nanopore-based electronic systems for analysis of single molecules, including DNA, RNA and proteins. The MinION™ device, the PromethION™ and GridION™ systems are designed to provide novel qualities in molecular sensing such as real-time data streaming, improved simplicity, efficiency and scalability of workflows and direct analysis of the molecule of interest. The devices may be used in scientific research, personalized medicine, crop science, security and defence and environmental applications. Supported by a broad patent portfolio, the Oxford Nanopore pipeline includes multiple generations of nanopore-based sensing technologies, including those based on both biological and solid-state nanopores.

Contacts

CTC contacted the individuals below to gather information for this investigation.

U.S. Fish & Wildlife Service

Steve Kramer
Fish and Wildlife Biologist
USFWS Arcata Fish and Wildlife Office
707-822-7201, steve_kramer@fws.gov

Emy Monroe
Lab Manager and Molecular Geneticist
USFWS Whitney Genetics Lab
608-783-8402, emy_monroe@fws.gov

Kelly Baerwaldt
Asian Carp/eDNA Program Coordinator
U.S. Fish & Wildlife Service
309-757-5800 ext. 208, kelly_baerwaldt@fws.gov

Food and Environment Research Agency, United Kingdom

Neil Boonham
Head of Detection Surveillance Technologies
Food and Environment Research Agency
011 44 (0) 1904-462332, neil.boonham@fera.gsi.gov.uk

Universities

Andrew Kinziger
Professor and Chair, Department of Fisheries Biology
Humboldt State University
707-826-3944, andrew.kinziger@humboldt.edu

Caren Goldberg
Assistant Professor, School of the Environment
Washington State University
509-335-3673, caren.goldberg@wsu.edu

Robin Allaby
Associate Professor, School of Life Sciences
University of Warwick
011 44 (0) 247-657-5059, r.g.allaby@warwick.ac.uk