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Information Management

Gaining decision-maker confidence through community consensus: developing environmental DNA standards for data display on the USGS Nonindigenous Aquatic Species database

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Abstract

To advance national efforts for the detection and biosurveillance of aquatic invasive species (AIS), we employed a community consensus process to enable the incorporation of environmental DNA (eDNA) detection data into the U.S. Geological Survey's (USGS) Nonindigenous Aquatic Species (NAS) database (https://nas.er.usgs.gov/eDNA/). Our goal was to identify minimum standards and best practices for the verification of eDNA data by working closely with AIS eDNA community practitioners and natural resource managers across government, private and academic sectors. To better inform management decisions, verified AIS eDNA data will be displayed on a separate mapping layer alongside visual sighting data with the inclusion of additional information on the eDNA methods employed to collect and produce the data. To allow for eDNA data display, we produced consensus derived online documents including a submission application and data submission template and are developing a guidance document for detailing the eDNA data submission process. We also developed a communication plan including a mechanism for reporting detections to appropriate managers for consideration prior to display. The products of these efforts are an application and data submission process that will be used in the new environmental DNA data layer on the Nonindigenous Aquatic Species (NAS) database. Herein, we detail how we engaged the eDNA community for consensus of our standards, share lessons learned from the process, and describe the benefits of such an approach at instilling confidence among the research and decision-maker community.

Key words: aquatic invasive species (AIS), best practices, data standards, detection, mapping application

Introduction

Aquatic invasive species (AIS) are organisms that are not native or indigenous to the aquatic system in which they are found and can be harmful to local ecosystems, economies, and human health. In the United

States, a diverse community of scientists, managers, stakeholders, and decision-makers rely on accurate AIS detection data to inform their efforts to effectively address new and established invasive organisms. The acceptance and utilization of invasive species detections are greatest when the data are standardized to meet the varied needs and expectations across this community. Though visual detection methods of AIS have well established protocols, newer molecular approaches, such as the use of environmental DNA (eDNA) sampling are still defining best practices (Goldberg et al. 2016; Helbing and Hobbs 2019; Thalinger et al. 2021). Environmental DNA is genetic material that can originate from the sloughing of skin, mucus, feces, gametes, etc. of an organism into the environment. Uses of eDNA sampling methods for the management of AIS include early detection, range delimitation, and evaluating eradication efforts, and can be performed across large spatial scales (Dunker et al. 2016; Davis et al. 2018; Dorazio and Erickson 2018; Chen and Ficetola 2019; Darling 2019; Da Silva Neto et al. 2020; Keller et al. 2022). For example, recent studies indicate eDNA data are valuable sources of information for consideration of landscape scale range delimitation of aquatic species, including investigations of Burmese python presence in Florida (Hunter et al. 2019), round goby in New York (George et al. 2021), and northern pike in the Columbia River basin (Carim et al. 2019). Also, researchers have been effective at performing high sensitivity monitoring efforts of cryptic or low abundance AIS by testing water, soil, or even air samples for their DNA with increased sensitivity relative to traditional visual sighting methods (Rees et al. 2014b; Thomsen and Willerslev 2015; Clare et al. 2022; Lynggaard et al. 2022). However, due to the evolving nature of this nascent field, there are few published examples of the direct use of eDNA data for specific management decision-making.

Groups and individuals within government, academia, and private industries have been increasingly developing eDNA assays targeting aquatic invasive species. Due to their high sensitivity, stringently validated and controlled eDNA sampling protocols and assays can have low false-negative detection rates relative to visual surveys, which are the current gold-standard for determining species presence, particularly when sampling aquatic environments (Hunter et al. 2015; Tucker et al. 2016; Burian et al. 2021). Use of eDNA sampling methods often results in higher efficiency in cost and personnel time needed for invasive species detection (Evans et al. 2017; Darling 2019; Sepulveda et al. 2020b; Jerde 2021). Additionally, the use of visual sighting to detect emerging species introductions can be difficult or impossible due to low detection probabilities associated with small population size (Victorian Government 2010). Therefore, eDNA data is valuable to decision makers as it can indicate areas to focus visual surveys after genetic material is detected in the field, or it can be integrated with data from visual detections to improve species distribution

models and decision science frameworks (Burian et al. 2021; Morisette et al. 2021). Finally, in addition to detection data, non-detection eDNA data (analyses indicating an absence of genetic material from a target species) provide unique information generally not reported with visual sampling efforts and may be used to de-prioritize visual surveys in that sampling area.

A key component to the usefulness of eDNA data for decision-making relates to an early detection and rapid response (EDRR) framework wherein both historic and novel data are able to be verified and made accessible to researchers and managers (Reaser et al. 2020a, b; Wallace et al. 2020). Therefore, we proposed establishing standards for eDNA data verification and display on a centralized repository, the U.S. Geological Survey (USGS) Nonindigenous Aquatic Species (NAS) database. The NAS database currently displays visual sighting data for AIS in the United States (described in Methods). Since its inception, the NAS program has developed a broad user-base of managers and researchers that regularly access the database to monitor invasive species.

Data and metadata standards for sample collection, processing, analysis, and reporting for eDNA research are still being established by practitioners, and recent publications have begun to lay out proposed standards (Goldberg et al. 2016; Klymus et al. 2020; Loeza-Quintana et al. 2020; Thalinger et al. 2021). To determine the most appropriate standards for adding eDNA data to the NAS database, we sought a community consensus approach. The synergistic use of community consensus for the approval of standards has proven fruitful in a variety of fields, and a useful model is described for the translational ecological scientific community in Enquist et al. (2017). The community-development approach has been applied to produce the Darwin Core biodiversity data standards (https://dwc.tdwg.org/), (Wallace et al. 2020), and by the North American Invasive Species Management Association, whose mapping standards were recently updated with input from data integrators, scientists, and managers in the community (Wallace et al. 2020). In medical research, international standards were established via community input to report the origin of human embryonic stem cells (Stephenson et al. 2007) and for data sharing and reporting on mitochondrial diseases (Karaa et al. 2021). Furthermore, it has been argued that this type of translational research and collaborative discussion is critical to advance the protection of global biodiversity (Morelli et al. 2021). The common factor across these studies is the involvement of people from all aspects of the decision-making process including stakeholders, federal and international agencies, researchers, and input from the public.

We selected appropriate standards through an iterative process of consulting subject matter experts including technical experts, decision-makers, and stakeholders within the AIS eDNA community in the United States. We asked them to review proposed standards, make suggestions of standards to include or reject, incorporated their feedback and requested

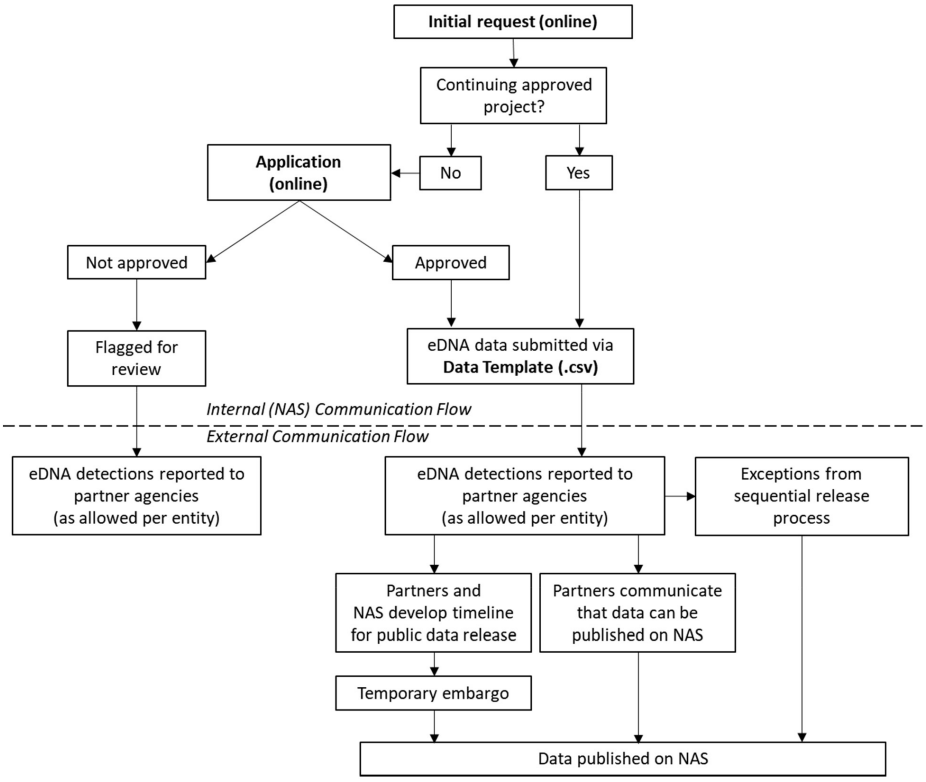

Figure 1. Communication plan for environmental DNA (eDNA) data submission and display on the U.S. Geological Survey Nonindigenous Aquatic Species (NAS) database. Bold text denotes required online forms to be filled out by an applicant. Below the dotted line describes the notification of partner agencies (as allowed by the entity represented by the applicant) that a detection may have occurred in their jurisdiction. The left side of the plan shows the process for applications not approved, while the right side shows possible outcomes for those that were approved. Partner agencies include the local, tribal, state, and/or federal agencies with jurisdiction in the area(s) of the detection. Communication among partners is encouraged, along with the offer to provide additional information or communication support.

additional reviews. Through this process, we gained consensus on the minimum required controls and best study practices for data to be displayed on the NAS database. Additionally, stringent criteria were established to provide increased confidence in the displayed data that excluded any data not meeting the identified controls and best practices. We also created a communication plan outlining the process for datasets to be approved and hosted by the database, including the plan to transmit eDNA detections to appropriate stakeholders prior to public display (Figure 1).

Our intention to host eDNA data on the NAS database required meeting management needs to ensure that the hosted data are produced using best practices, but not be so prescriptive as to dictate protocols that can impede the process. Herein, we describe our process for obtaining consensus from the AIS eDNA community, discuss the outcomes of the methods employed including brief examples of arriving at consensus for some questions, and describe the benefits of such an approach at improving confidence among the community of researchers and decision-makers alike. We plan to follow this report with a thorough review of individual standards and feedback received during this process, as these topics are extensive and are not pertinent to our community consensus approach described here.

Methods

Phase 1

A national viewer for eDNA data

Detection and non-detection from species-specific eDNA sampling represent the most recent advancement in early detection. The display of eDNA data on the USGS Nonindigenous Aquatic Species database (https://nas.er.usgs.gov) helps to meet one of the database's central goals – the dissemination of timely information about the presence and distribution of nonindigenous aquatic species. The incorporation of eDNA detection data creates an integrated view of eDNA data alongside visual sightings and advances national efforts for detection and biosurveillance of aquatic invasive species. For this layer, eDNA data will not be permitted from surveys associated with private industry (i.e. aquaculture facilities, commercial stores, tanks and the like) or from single sample data (e.g. citizen science). Synthesis of eDNA data in a database allows for the improved ability to quantify and characterize the spatial extent of new data points. Environmental DNA and observational data combined in a centralized location provide different temporal and spatial resolution and when viewed together, can help to create a more complete picture of AIS extent and spread. For example, the U.S. Fish and Wildlife Service (USFWS) Asian Carp eDNA monitoring program generates thousands of data points a year (https://fws.maps.arcgis.com/apps/dashboards/52b22abe9c4d4575adfe851a946f444d). Furthermore, in addition to eDNA detection data we also include eDNA non-detect data, which is generally not a data type reported by physical sighting efforts.

Currently, the NAS database maps the distribution of > 1,380 introduced and invasive aquatic species and contains over 680,000 records obtained from published manuscripts, state and federal observation and monitoring programs, museums, and verified citizen sightings across the United States and its territories. The platform itself includes an existing visual component (map layer) and the capacity to hold large datasets. Additionally, the NAS database visual sighting communication plan successfully notifies a network of stakeholders and subscribers of novel species introductions and range extensions. Information displayed on the NAS website is shared freely and available for viewing by any public entity and is a source for managers and scientists of historical and early detection information.

Incorporating guidance from managers

We began our collaborative discussions with natural resource managers and decision-makers in the community, as they are likely to be the primary end-users of these data. We based our discussions on data validation and standards to better aid management decisions and with a priority of working to ensure confidence in the data that would be housed and displayed on the site. Also discussed was a communication plan for eDNA

data, which was based on the existing plan used for visual observations in the NAS database. There were initial concerns about the quality of eDNA data, particularly whether possible false-positives (a positive result when no target eDNA was present at the sampling site) or false-negatives might be displayed, and over the public interpretation of eDNA detections alongside visual observations. This resulted in a request that new eDNA detections not be published or released on the database prior to timely notification of the relevant jurisdictional managers. To improve the management of potentially harmful invasives species through Early Detection and Rapid Responses (EDRR), the timely release of information is necessary. The process developed here assists with the verification of the data quality for rapid public display, however, individual institutions should follow approved guidelines for releasing unpublished data to third parties. If preferentially/sequentially released data are not permitted (for unpublished data), the relevant points of contact will be informed of the data when published on the site.

There have been recent situations that have affected how the community perceives eDNA data as a stand-alone source of data for decision-making. While some situations have been positive, such as the national efforts in the UK to monitor the Great Crested Newt for directed management (Rees et al. 2014a; Biggs et al. 2015; Buxton et al. 2022), others have been more controversial (Jerde et al. 2013; Mahon et al. 2013). A notable example involved the early application of new eDNA detection methods for invasive carp in the Great Lakes, beyond the electric barrier meant to keep them out (Jerde et al. 2013). Recently, Jerde described the history of the incident and outlines the inherent uncertainty that comes with eDNA detection methods (Jerde 2021). In this early application of the eDNA detections a federal level response was initiated, although concerns remained at the local level that the results were false-positives and the response had been unwarranted. The eDNA field has incorporated this information and evolved extensively since its initiation, and it is recommended to interpret detections carefully by employing repeated sampling, or visual confirmation.

A plausible consideration is that the database may display the results of preliminary research efforts or of allochthonous eDNA (eDNA originating from a place other than where the sampling occurred), and that a few positive eDNA detections could be interpretated as species invasion and result in costly management efforts (Goldberg et al. 2016; Tucker et al. 2016; Jerde 2021). In consideration of this concern, the application questions include a significant focus on the use of proper controls, sampling best practices, and validated assay standards to address false-positive tests (i.e. sample level error due to poor assay optimization or contamination). Furthermore, PCR replicates were deemed a critical component to help reduce false-negative occurrences in any given sample.

To further protect against false-positive results, we greatly support the use of recognized best-practices in molecular laboratories including for general PCR experiments and the broad use of MIQE guidelines (Bustin et al. 2009; Huggett et al. 2013). We also strongly encourage the recommendations for careful study design, pilot data, repeated sampling and interpretation of eDNA found in recent publications (Goldberg et al. 2016; Mathieu et al. 2020; Burian et al. 2021; Langlois et al. 2021; Thalinger et al. 2021). While guidelines are in place for molecular biology studies like these, each laboratory is ultimately responsible for identifying and upholding these practices for the production of quality data. Additionally, this site will not be interpretive, but rather will provide the viewer with data to consider in the context of the system from which it was collected. We strongly encourage the provision and careful consideration of associated metadata and consultation with eDNA methodological experts to enrich and inform data interpretation in a biological context.

Convening a Core Advisory Panel

To facilitate discussions and ensure that multiple perspectives were taken into consideration, we convened a core advisory panel (CAP) made up of eDNA and AIS technical experts to guide the application and submission process in an objective and balanced way. Due to the specificity of the requirements for production and dissemination of data in our agency (USGS) and the Department of the Interior (DOI), the CAP was restricted to DOI subject-matter experts. This panel was designed to ensure that we comprehensively considered the numerous methodological validation (e.g., qPCR assay standard curve meeting minimum efficiency, R^2) and experimental controls (e.g., replicates, negative and positive controls) that are necessary to allow for repeatability and confidence in eDNA data for AIS detections. It also served as an initial discussion group for the consideration of multiple viewpoints on specific concepts that we received from reviewers. After discussion, the group would diplomatically gain consensus on the next iteration of standards to be provided to the broader community for feedback. The CAP also served as a final referee of the questions and language selected for the application.

Phase 2

Proposed best practices and standards

The best practices for eDNA analysis have benefited from multiple scientific fields that use PCR-based molecular tools, including the fields of soil microbiology, microbial source-tracking, ancient DNA, non-invasive genotyping, and forensics (Martellini et al. 2005; Pedersen et al. 2015; Fløjgaard et al. 2019; Stewart and Taylor 2020). These best practices can be divided into assay development, field collection and analytical categories.

To ensure our application process could assess the validity of the eDNA data submitted, we worked to develop a set of minimum standards focused on quality assurance and control of collection, extraction, and analytic processes as described in the previous section. The issue of detection confidence and uncertainty in eDNA is long standing due to the high sensitivity and impacts of environmental and/or laboratory-based stochasticity on the method. Numerous studies have discussed ways to improve detection using eDNA assays and reduce uncertainty in these studies (Hunter et al. 2015; Sepulveda et al. 2020a; Darling et al. 2021; Jerde 2021; Langlois et al. 2021). The primary recommendations include optimization of methods in the laboratory and field, efforts to reduce contamination, repeated sampling and carefully planned study design and the use of multiple negative and positive controls at each stage of the process.

We began the process of developing standards by incorporating the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) and digital PCR experiments (dMIQE) standards as well as other highly referenced published guidelines for the collection and processing of eDNA samples (Bustin et al. 2009; Huggett et al. 2013; Goldberg et al. 2016; Wilcox et al. 2018; Baillie et al. 2019; Klymus et al. 2020). Consistently, it was recommended that minimum standards should be built into the study design to reduce the potential for reporting false-positive or false-negative results (Carim et al. 2016; Goldberg et al. 2016; Wilcox et al. 2018; Helbing and Hobbs 2019; Morisette et al. 2021). These recommended protocols and standards are accepted within the community and describe appropriate methods to follow in the field and laboratory. With the assumption that researchers follow best practices in field including these MIQE guidelines, we then focused on the details necessary to report for each eDNA processing step.

Due to the nuanced and complicated nature of various eDNA sampling methods, we chose to first develop minimum standards for the broadly used hydrolysis probe-based (i.e., TaqMan) quantitative or digital PCR (qPCR and dPCR, respectively) assays for targeted species or genus detection (Holland et al. 1991). We exclude data produced by non-probe-based qPCR (SYBR), traditional PCR confirmed by Sanger sequencing, and metabarcoding until we can properly identify best practices and standards associated with each method. Additionally, targeted probe-based methods are widely used in both scientific and management applications, and thus have greater acceptance and confidence among the community. Probe-based assays amplify a single species or genus to the exclusion of all others and comprise the largest percentage of eDNA detection data, making then the most applicable for initial inclusion on the NAS platform. They provide quantitative data that can be used to evaluate assay sensitivity and utilize a DNA primer pair and probe to improve specificity (Wilcox et al. 2013). Best practices for probe-based assay development are intended to improve

specificity and sensitivity, while avoiding contamination, and are discussed in detail in the literature (Goldberg et al. 2016; Langlois et al. 2021; Thalinger et al. 2021).

Successful eDNA detection can be subject to many variables, including those associated with sampling (e.g., sample volume, frequency, season, sample replication, spatial coverage, processing method, filter type, filter pore size, or extraction kit), the organism (e.g., the number of individuals, time from shedding, the shedding rate of eDNA), and environmental factors (e.g., water depth, flow rate, pH) (Strickler et al. 2015). Environmental DNA data will be displayed alongside visual survey data to assist with avoiding false-positive or false-negative inferences (i.e. when only one or the other indicates a detection of the eDNA or organism sighting), a priority when addressing management concerns. Specifically, defining a detection of eDNA as a false-positive due to a lack of visual confirmation of a target species (a false-positive inference) is a misuse of the term and leads to unnecessary uncertainty in the use of eDNA data (Darling et al. 2021).

That said, it is important to consider true sources of false-positive "detect" and negative "non-detect" results that are a product of poor study design (not including enough samples or geographic coverage), assays that are not properly validated or optimized, contamination, and inhibition of PCR amplification. We encourage careful consideration of the extensive reviews, recommendations, and methods that have been developed to avoid these types of results and increase the confidence in the data (Miller et al. 2011; Wilcox et al. 2013; Chambert et al. 2015; Lahoz-Monfort et al. 2016; Sepulveda et al. 2020a; Darling et al. 2021). Also, it is critical that eDNA data are produced using methods and best practices which ensure that proper experimental controls and validation procedures are in place to optimize sensitivity, specificity, repeatability and reproducibility (Bustin et al. 2009; Huggett et al. 2013; Goldberg et al. 2016; Baillie et al. 2019; Klymus et al. 2020; Abbott et al. 2021). The standards implemented here to allow for display of eDNA data on the NAS database may serve as a basis for the future standardization of eDNA protocols in a broad sense.

Phase 3

Community input leading to consensus

Many benefits arise from approaches that embody an intentional and inclusive process in which researchers, decision makers, and stakeholders each contribute their expertise to the development and implementation of science. These benefits include improved outcomes, transparency, and trust in the final product (Enquist et al. 2017; Morelli et al. 2021), all goals for the emerging field of eDNA science. As we began, the ability for high quality face-to-face engagement at conferences and planned workshops were abruptly halted by the COVID-19 pandemic. We quickly pivoted to

virtual meetings to provide a forum for AIS eDNA community input, which ultimately broadened our reach across the community. Our goal was to follow the best practices found in current consultative approaches to standards development in working towards community engagement with a diverse population of people associated with AIS eDNA research and management. We focused on getting buy-in from participants to provide iterative feedback and reviews, a communication plan that ensures transparency, and the commitment by both ourselves and the users/co-developers that we will provide feedback to improve the database and submission process in the future.

To create both broad awareness of our goal for eDNA data integration into the NAS database and to solicit feedback from a wide variety of stakeholders, we convened a series of seven town hall webinars. Notifications for these town hall webinars were emailed broadly to the community and advertised through the six Aquatic Nuisance Species Task Force (ANSTF) Regional Panels, composed of AIS coordinators and researchers from federal, tribal, and state natural resource management agencies (our target users of the eDNA reporting and alerting system), non-profits and regional user groups, and academic researchers. Each town hall webinar consisted of a short presentation describing our goals, and the proposed process for eDNA data integration into NAS. We had 164 total participants, and each presentation was followed by an extensive listening session dedicated to answering questions, addressing general concerns, and soliciting feedback on how to best display eDNA detection data to aid in interpretation and use for natural resource management. We incorporated common or impactful questions from each webinar into a frequently asked questions (FAQ) section (Supplementary material Appendix 1) included in subsequent webinar presentations. To encourage support from the AIS management community, we also solicited professional scientists directly to voluntarily peer review the proposed process, data standards and metadata, and documentation.

Following the town hall webinars, a concerted effort was made to broadly survey the field of eDNA research and AIS management communities for review of the documents. To ensure the review process was well coordinated and organized, the application underwent four consecutive rounds of expert peer-review and was iteratively updated in each round. After developing a framework of questions and standards with the CAP, we solicited reviews from panels of scientists who perform eDNA research. After the documentation was emailed to the participants for initial review, we held a meeting to explain the process and allow for verbal discussion and questions. Iterative panels included members from universities and from within the Government eDNA Working Group (GeDWG), managers of invasive species programs and partner organizations, and a final review by members of the National Invasive Species Council (NISC)

eDNA Working Group and ANSTF. Beyond those who expressed interest during the town halls, we directly invited key governmental, private, and academic researchers who produce highly cited manuscripts in the field, and active decision-makers of AIS at various levels of government. Overall, fifty-four individuals were invited to provide feedback on the data submission application (many who provided verbal feedback in the meeting), and we received comprehensive written reviews from eighteen, not including repeated reviews by the project leaders and five members of the CAP.

The diverse perspectives of researchers and managers led to reviews that were primarily focused on detailed feedback regarding experimental processes versus high-level feedback on application of the data by decision-makers, respectively. The researchers in the first group addressed much of the minutiae related to each specific question on the application, leading to important discussions and decisions about the inclusion of certain metrics. These included many of the questions that related to which experimental controls should be required and which specific best practices were going to be essential (i.e., number of replicates analyzed, sample collection and preservation). The managers in the second group returned mainly high-level feedback, choosing to focus on comments about how the data will be visualized, the importance of educating website visitors on interpretation of eDNA data, advocating for the use of best practices by way of questions on the application, the use of website disclaimers, and the importance of an effective communication plan between NAS and local managers.

Documentation development

The documentation associated with this process evolved as community engagement grew. The initial documentation was made up of questions related to field and lab methodology, the implementation of proper controls, and the adherence to certain standards and best practices from the current literature. It also included a basic .csv data template for submitting the dataset, including columns for metadata such as sample collection dates and location, sample type, and number of replicates to go along with the detect/non-detect data. Considering feedback from the community, supplemental information was identified as a need to bolster the initial data submission questionnaire and data template form. Thus, the final forms include: an (1) *initial online request* (Figure S1) (https://nas.er.usgs.gov/eDNA/prescreening.aspx), (2) *online application* (Appendix 2) (3) *data template* (Figure S2), and (4) associated *guidance document* that will be housed on the website https://nas.er.usgs.gov/eDNA/ which will function as a living document to allow for modifications requested by the community.

The website is structured to contain the *initial online request* with introductory questions that establish where the study was performed, what species were targeted, and a few other key questions to verify that the dataset is appropriate for the site (e.g., the study targets aquatic invasive

species, employs hydrolysis probe qPCR or dPCR assays, etc.). This is also where the applicant indicates whether their data are subject to non-preferential release rules and unable to be shared with management POC's prior to publication on the website. If the request meets the appropriate basic requirements listed above, the applicant is provided a link to the *online application*. The application is designed to ensure the study meets the minimum consensus criteria via an extensive questionnaire. Each question is formatted as a Yes or No response with room for comment. Each question, however, must be answered in the affirmative, or the site will flag it as "not approved" and alert both the applicant and the NAS team. The NAS team can work with the applicant, if they wish, to determine how their data may become compliant with the minimum requirements. After applications are approved, a *data template* (in a .csv format) will be provided for the applicant to enter the data for display. The template will allow for detailed eDNA data and metadata to be uploaded to the NAS database. The goal for breaking the process into multiple, shorter steps, is to allow for improved management of the application approval process and simplification of the experience for the applicant. As eDNA data and metadata sets tend to be quite large, the template data entry may take a longer time to complete than the other forms, and thus will not be required until after application approval.

Throughout the process, several key concerns emerged across the community. In particular, some application questions initially structured as a voluntary disclosure of information, such as having dedicated lab space for various steps in the process, were pressed to be reclassified as requirements by the practitioners. One reviewer stated, *"Post-PCR needs its own dedicated lab. Labs that have tried to use separate hoods in the same lab have eventually ended up with contamination"*. This example shows how an initial suggestion, stringently interpreted, could exclude other methods, such as field-based research where PCR is done on-site. In the end, the question was removed in lieu of a focus on the inclusion of focused controls to address contamination and not where the samples were processed per se, although we point the applicant to review and apply the current best practices for the field. Other operational protocol discussions, such as if and when to collect field equipment blanks, found consensus within the community, and the wording was changed to accommodate that agreement. Ultimately, this application does not require the use of certain tools or methodologies (filter size and type, extraction method, etc.) to account for the nuanced nature and technical complexity of eDNA methods. Therefore, some metadata and covariates will be deemed voluntary, but which the applicant will be encouraged to provide (see guidance document). In all cases, positive and negative control samples and sample replicates were foundational to the best practices and therefore incorporated as application questions (Rees et al. 2014a; Sepulveda et al.

2019, 2020a; Darling et al. 2021). The questions were grouped into the following categories: A) Basic study information; B) Sampling and Processing (to include Sampling, Contamination controls, and Processing methods); C) PCR Assay (to include Validation and Optimization); and D) Reporting. As more is understood about eDNA fate and transport, certain data fields may adjust, becoming more detailed, or less restrictive, to ensure the data can be interpreted accurately. It is encouraged that decision-makers reach out to experts to ensure the data are understood and interpreted in the context of each study's parameters.

As mentioned above, the *guidance document* will provide additional resources and context for the numerous aspects of the methodological standards addressed in the application questions. It will aid in completing the application and preparing data for submission by further defining key terms and concepts while providing topical information about each standard or best practice. The structure of our guidance document is partially informed by a recently-published guidance document produced by the Canadian Science Advisory Secretariat (CSAS) to support study design between researchers and managers for aquatic invasive species and species at risk which guides decision making using eDNA sampling results (Abbott et al. 2021). This document is also where we will describe a protocol for the correction of submitted data that may include an error, explaining the process we will employ to edit datasets by facilitating corrections provided by the owners of the data, should such an incident occur. That said, the original dataset would have been accepted based on the use of all appropriate required controls and methodological standards, so we expect this type of incident to be rare.

Phase 4

Communication plan for informing management agencies

In keeping with the existing stakeholder communication plan employed by NAS for visual sighting reports, the new NAS eDNA detection communication plan (Figure 1) formally 1) defines an updated and maintained list of points of contact (POC) by job title within natural resource management agencies that will receive specific information regarding an eDNA detection record, 2) when that information will be delivered to the POC(s), 3) the communication channels and methods that will be used to notify stakeholders, and 4) a decision tree for when the eDNA data will be made publicly available. An individual POC would include regional Federal AIS coordinators and natural resource management staff (if a detection occurs on federally owned lands, e.g., National Wildlife Refuge), the AIS coordinator for the state in which a detection occurs, and other entities that have management authority within the jurisdiction of the detection. For detections that occur in shared boundary waters, POCs from

all bounding jurisdictions will be included. Before an eDNA detection is entered into the NAS database, a geographic filter allows for rapid automatic identification of POCs within their area of concern. An email will be sent to the list of pertinent POCs and including a predeveloped notification email identifying the taxon identified by eDNA study, the date and location of the detection, and a mechanism for POCs to acknowledge receipt of notification and sign-off on public release. If requested by managers, eDNA detection data may be hidden from public view via a temporary visibility embargo to provide time for a coordinated response. Exceptions to this process are for entities providing data that are not permitted to preferentially disclose unpublished data. Data from applicants who fall under this category will not have their information communicated to POC's whether their application is approved or not, rather POC's will be notified at the time of publication on the database when publicly available.

This communication plan was designed with Early Detection and Rapid Response (EDRR) efforts in mind to meet the needs and requests of AIS managers regarding eDNA data (Reaser et al. 2020b). This attains our goal to ensure that each stakeholder will have the best up-to-date information to formulate any management responses that may be necessary by coordinating the release of eDNA detection information visible in and pertinent to their jurisdiction. Notably, while data from unapproved applications will not be displayed in the NAS database, the data will be communicated to pertinent AIS managers following the plan so that they can choose whether to follow up on these potential observations.

Displaying the eDNA data

At present, NAS displays visual sighting observations on an interactive map that allows the user to customize the base map, include various thematic overlays (such as watershed or Congressional district boundaries), and alternate between individual points and geographic clusters of records. The integration of eDNA detection data for display alongside traditional visual observation data presents several challenges for information design and interpretation. First, given the ability of eDNA assays to yield both positive and negative information (compared to the positive-only nature of the sighting data currently in NAS), symbology that clearly represents detections and non-detection while being visually distinct from existing visual observations will be important. Second, given the potential for interpretation issues around false-positive inferences defined above (i.e., detection of genetic material in a sample without visual detection of the organism at the site), it is important that the user is made aware of the nature of eDNA detection data and that such data is only presented to users after they signify their understanding. These two points lead us to use an "opt-in" model for display of eDNA detection data on the species distribution map. By default, a user will only be presented with the visual

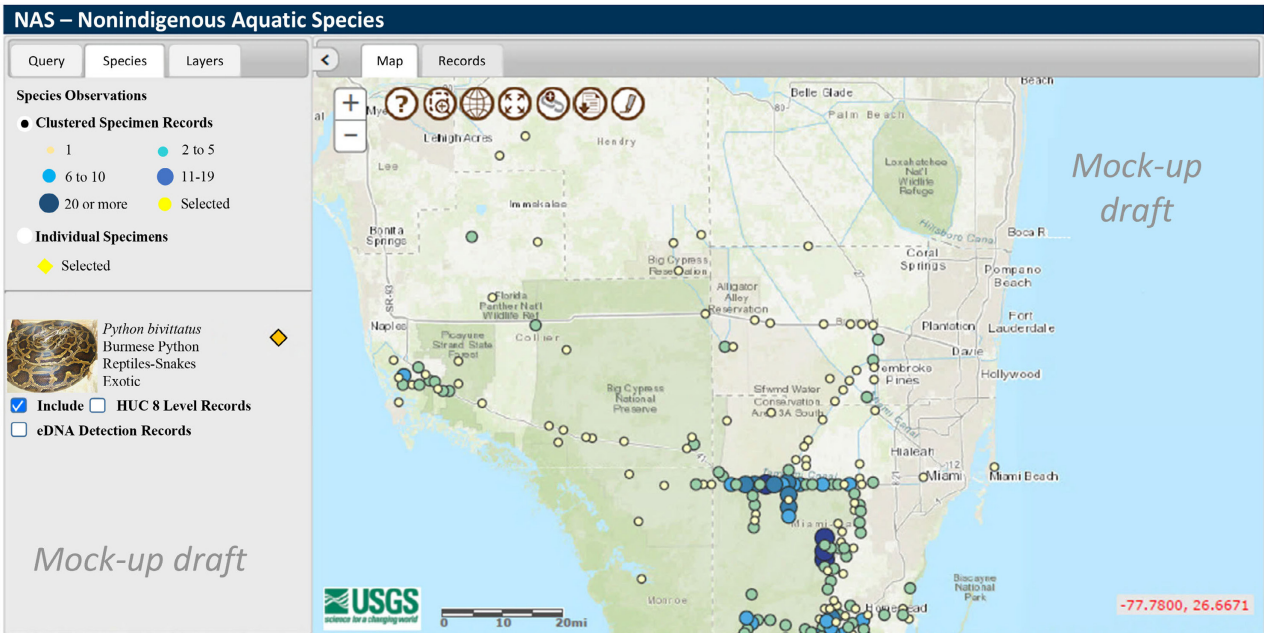

Figure 2. U.S. Geological Survey Nonindigenous Aquatic Species (NAS) database display with visual sightings indicated by the circle icons. to display the environmental DNA (eDNA) data, the visitor will need to select the opt-in check box on the lower left labeled "eDNA Detection Records". Screenshot of visual observations for Burmese python (*Python bivittatus*) (https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2552; accessed 2022-06-09) modified to show placement of opt-in checkbox.

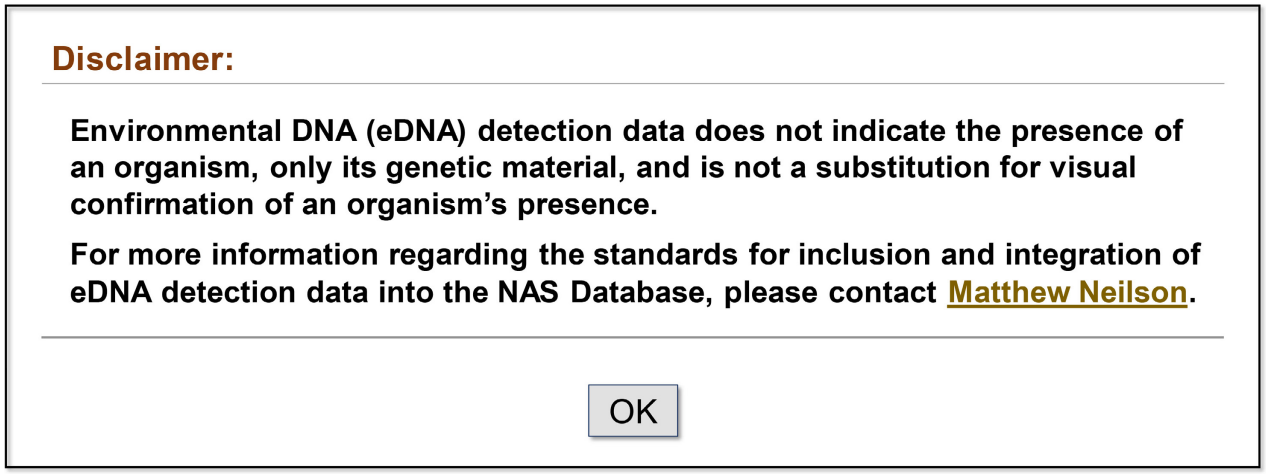

Figure 3. U.S. Geological Survey Nonindigenous Aquatic Species (NAS) database disclaimer will appear for opting-in to include environmental DNA data in the display viewer.

observation data when the map opens. If eDNA detection data are available for a species, a checkbox will appear in the existing species box (where users can control visibility of observation data, native range polygons, or watershed-level distribution polygons (Figure 2). When checked, the user will be presented with a pop-up disclaimer describing some of the limitations of eDNA detection data and how it may be interpreted, along with contact information for a NAS staff member (Figure 3). Notably, this "opt-in" approach had strong support by AIS managers and eDNA researchers alike during the community review. Once the disclaimer has been acknowledged, the eDNA data layer will then be displayed on the

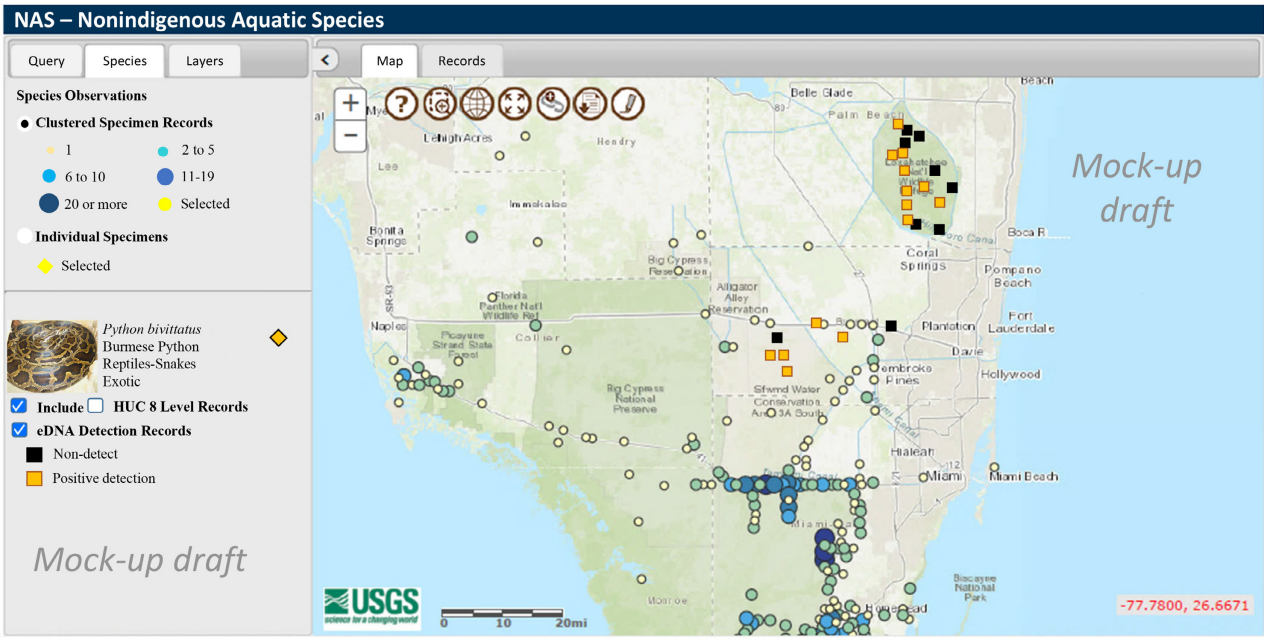

Figure 4. Mock-up of visual observations with environmental DNA (eDNA) detection data (Hunter et al. 2019) for Burmese python (*Python bivittatus*) as would be displayed in the optional eDNA layer of the U.S. Geological Survey Nonindigenous Aquatic Species (NAS) database once corresponding species data are submitted.

distribution map (Figure 4). Finally, the archiving of eDNA data in a publicly accessible database also allows for the data to meet FAIR (Findable, Accessible, Interoperable, and Reusable) Data Principles and be utilized in secondary analyses for risk assessments, and distribution modeling (Wilkinson et al. 2016; Berry et al. 2020).

There are multiple potential ways to symbolize eDNA detection data. The various reviewing groups discussed several different options to symbolize eDNA detections as outlined here. A qualitative scheme (i.e., positive, or null detection) could help to inform interpretation (e.g., the target eDNA was either detected or not detected) but lacks any information about signal strength and intensity or temporal changes. Incorporating signal strength (i.e., symbolizing target eDNA copy number in a quantitative gradient color ramp) adds information that may allow users to help inform organism movement, presence, or location (based on water flow), but is likely dependent on environmental covariates and may be difficult to interpret among various assay types and/or sample designs. Displaying the time since the last eDNA detection event may allow for better biosurveillance and EDRR efforts, but is highly dependent on rapid sample processing, analysis, and data submission. Calculating and displaying probability of the organism's presence through occupancy modeling may help inform occurrence and detection rates but could become computationally difficult over large spatial scales (Tingley et al. 2020). Ultimately, a simple detect/non-detect reporting using binary colors and symbols was agreed upon for the initial display on the web-viewer. In this way, the results (detect or non-detect) from the combined analysis of all PCR replicates sampled from each station on a given effort will be plotted. Data from

samples collected between nearby stations will not be presented in an aggregated manner (the current default view for visual sightings). This simpler approach ideally will result in less confusion among reviewers related to visual understanding of the data and interpretations.

The datapoints will be displayed at the resolution of individual samples, whether one or more is taken at a station (i.e. geographically distinct sampling point/ location). This means if more than one sample is collected at a station, multiple detect or non-detect reports will be reported. This allows the end-user to assess individual data points and provides the ability for downstream occupancy modeling (Miller et al. 2011; Mordecai et al. 2011; Schmelzle and Kinziger 2016; Dorazio and Erickson 2018). Due to the requirement that all detections are verified by two or more PCR replicates, some individual detections may be categorized as *inconclusive data*, meaning target eDNA was detected with only one PCR replicate. Although the community did not feel these results were appropriate to be mapped on the viewer, as they were not reproduced, it was agreed that inconclusive data could be stored and made available via downloadable data tables so that they may be included in temporal tracking of low replicate or low concentration eDNA detection datasets, as appropriate. Such partitioning of the data can help to avoid confusion and reduce the chances for misinterpretation until such detections can be confirmed with further survey data. This particular topic was discussed at length over the development of the data template, and for good reason. Aquatic invasive species managers expressed hesitancy to respond too quickly after a positive eDNA detection of a species of concern. Still, most participants indicated they would rather know if a putative detection was made, even if not replicated in that specific effort, as opposed to such data being excluded from the dataset. Follow up eDNA or visual studies to verify a detection helps to reduce concerns about false-positive sample or site level inferences (Goldberg et al. 2016; Thalinger et al. 2021).

Another topic that influenced our use of an opt-in display was the potential for spatial and temporal mismatches of target DNA and the organism source in eDNA surveys. This can lead to false-positive inferences (discussed above in *Proposed best practices and standards*) if there is detection of genetic material (eDNA) from the target species in a sample, but no associated visual detection of the organism at the same location (Darling et al. 2021). While this mismatch may be due to the greater sensitivity of molecular eDNA sampling methods to detect target eDNA relative to visual surveys, there are also alternative mechanisms by which a positive eDNA detection might occur despite a lack of visual confirmation. These include contamination during the analytical process, or lack of assay specificity, termed by Darling et al. (2021) as a false positive test in which error occurs at the sample level. Mitigation of these variables is achieved by the design of eDNA surveys employing stringent experimental controls and

validation procedures that optimize sensitivity, specificity, repeatability, and reproducibility. Other mechanisms include allochthonous transport (DNA originating from a place other than where the sampling occurred), anthropogenic transport (human mediated), predator transport/dead organisms, which lead to a site level false positive inference (Darling and Mahon 2011; Carim et al. 2016; Goldberg et al. 2016; Sepulveda et al. 2020a).

Discussion

The benefits of a community consensus approach

The goal for this project was to create a centralized space to share eDNA data for the benefit of researchers and decision-makers. Achieving this goal, however, was best accomplished by including the broader AIS eDNA community and by gaining consensus on the best way to standardize the application and data display process; thus, increasing confidence in the mapped data as a result. The goal of this approach was greater utilization and trust in the data displayed on the NAS website throughout the community due to the diverse community members that were a part of its development.

Lessons learned

Iterative incorporation of feedback on the application questions led to some interesting results. We structured our reviews to primarily include researchers who are experts in the technology for the first review, followed by a round of reviews by individuals in natural resource management and decision-maker roles. As detailed earlier, certain aspects of the application were focused on comments by each group. When we sent the revised documents out for a second round of review, we informed the reviewers the previous review was performed by researchers in the community. The result was that the managers tended to express trust in the opinions of the researchers and did not contribute many suggestions or questions associated with best practices or minimum requirements. Likewise, following managerial review, a tertiary review by a mix of both researchers and managers resulted in consensus on the questions posed with comments on ideas for clarification and expansion of descriptions of the questions to facilitate effective interpretation by applicants. This iterative process and wide invitation for input was designed to increase trust in the final product by engaging the end-users in its design. Early confidence in this process may be driven by the small and interconnected nature of the eDNA AIS community, and many of the individuals involved in this effort have direct knowledge of the work of the other participants.

Future work

Probe-based assays targeting a specific species or taxon are often used for molecular assessment of AIS presence. They require specific best practice

guidelines for the optimization and assessment of sensitivity and specificity of qPCR or dPCR (Bustin et al. 2009; Huggett et al. 2013). From the minimum standards identified in this effort, a strong foundation has been laid for the minimum guidelines developed here to apply to other eDNA detection methods, which could be incorporated into the database later. In the near future, we plan to integrate the best practices being developed for multi-species or community assessment using metabarcoding sequencing. Metabarcoding is the simultaneous identification of multiple taxa from a single sample, and its data present unique challenges for inclusion in NAS due to the large quantity of information generated, as well as variability in what species are detected. Comprehensive discussions with practitioners, bioinformaticians, and statisticians will be required to determine how best to qualify and integrate these data into the NAS database. However, it is anticipated that many of the standards for field and laboratory sample processing developed herein can be applied to metabarcoding data.

Following the launch of the eDNA database, we plan to continue to regularly engage with the various members (researchers, managers, etc.) of the AIS eDNA community to encourage two-way dialogue, and to work with stakeholders to ensure our shared goals are being met. This will be accomplished by holding regular mixed-format (virtual/live) workshops and seminars at conferences to illustrate the data submission process, answer questions, and gather feedback to help improve the process moving forward. We will solicit feedback on how the application process could be improved to continue optimizing the efficient and accurate use of the database for management and policy decisions (Shackleton et al. 2019; Morelli et al. 2021). As new technical details on eDNA methodology are discovered, and new standards and best practices are adopted, we plan to iteratively incorporate the best available science into these living documents. We recognize that there is a national and international movement to develop eDNA methodological standards, and we will consider and integrate any developments into future NAS database best practices. In addition to our communication plan, we plan to facilitate discussions on eDNA data quality and any concerns between natural resource managers and eDNA practitioners, and to include the data submitters themselves.

There is also interest in the number of datasets submitted in the first year and longer term, and the timeframe of eDNA detections prior to visual detection at a site. This would inform the degree to which eDNA detection functions as an "early warning system" as related to visual sightings. As AIS eDNA data and metadata populate the database, this centralized source may help to improve the response time to new invasions, enable managers to make informed resource allocation and management decisions, and inform researchers as to which methods, kits, filter types, etc. are being used. Further, these efforts may help to facilitate

communication among managers in the region, and to provide situational awareness (i.e., downstream, across state boundaries, etc.) for the region. Ongoing goals include improvement of the data submission, viewing and communication process to allow for an efficient, easy to use, effective, and useful resource for the community.

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Authors' contribution

JAF, MEH, and WMD were responsible for the conception of this study; JAF was responsible for the management of the project and the preparation of the original draft; ALL authors contributed to the development of the products, as well as the writing of the manuscript and review for accuracy.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. USGS NAS eDNA Webinar Frequently Asked Questions (FAQ) from town hall presentations.
Figure S1. USGS NAS eDNA initial online request.
Appendix 2. Planned NAS eDNA Application.
Figure S2. Planned NAS eDNA Template.

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