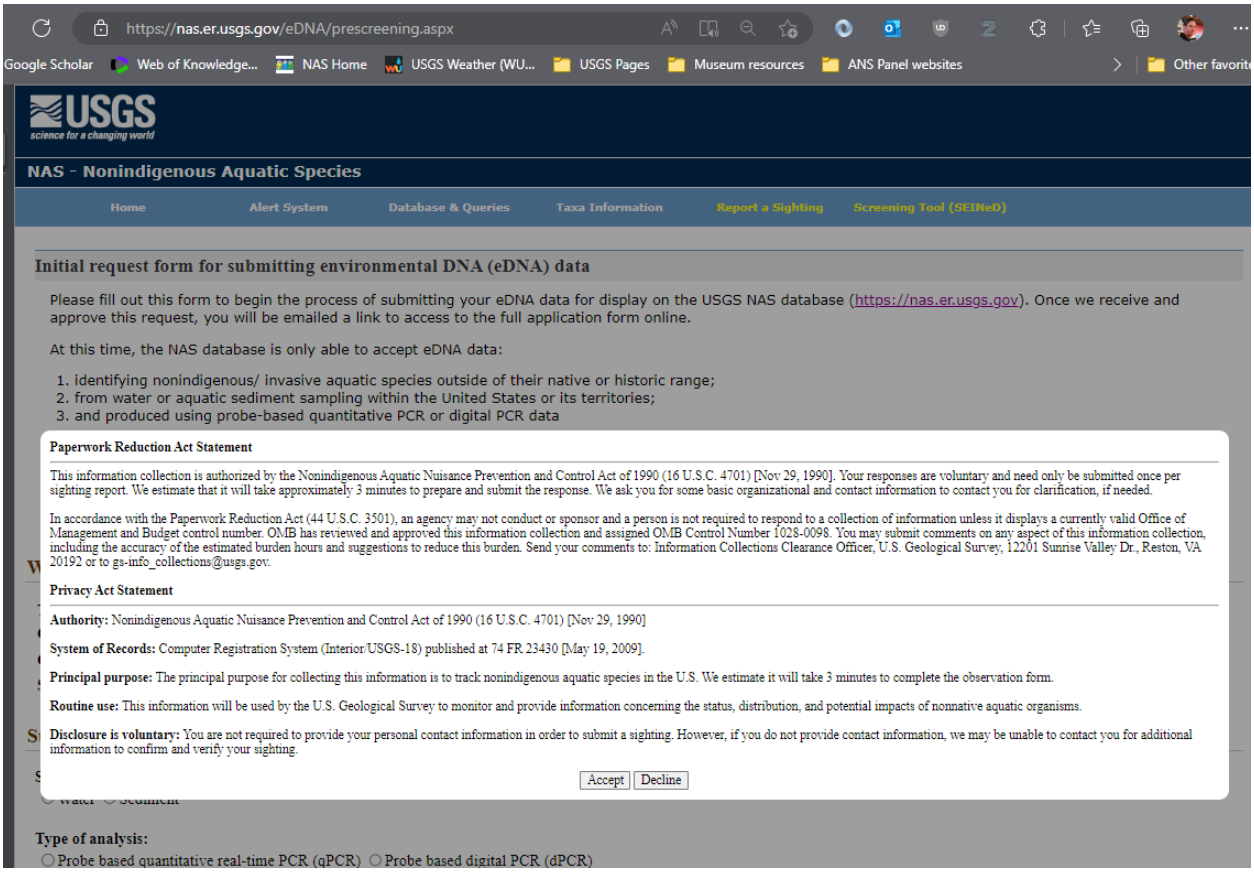
Submission of eDNA data to the USGS Nonindigenous Aquatic Species (NAS) database is a two-step process. Users interested in the process can review the database's goals for incorporation of eDNA data at https://nas.er.usgs.gov/eDNA/default.aspx. Users interested in submission of eDNA data will be directed to the pre-screening form (https://nas.er.usgs.gov/eDNA/prescreening.aspx), which gathers very basic information about the study and eDNA data the user would like to contribute. On initial page load of the pre-screening form, the user is presented with a pop-up notification containing the Paperwork Reduction Act and Privacy Act statements (see below; number contained in the pop-up is currently a placeholder)



This pop-up contains the OMB control number, but not the expiration date. When the user clicks on the 'Accept' button, the user can then interact with the form. The following screenshots show the content of the form. When the user completes the form and scrolls to the bottom of the page, the OMB Control Number and the expiration date (both placeholders currently) are visible:

https://nas.er.usgs.gov/eDNA/prescreening.aspx

Google Scholar | Web of Knowledge... | NAS Home | USGS Weather (WU... | USGS Pages | Museum resources | ANS Panel websites | Other favorite

USGS science for a changing world

NAS - Nonindigenous Aquatic Species

Home | Alert System | Database & Queries | Taxa Information | Report a Sighting | Screening Tool (SEINeD)

Initial request form for submitting environmental DNA (eDNA) data

Please fill out this form to begin the process of submitting your eDNA data for display on the USGS NAS database (https://nas.er.usgs.gov). Once we receive and approve this request, you will be emailed a link to access to the full application form online.

At this time, the NAS database is only able to accept eDNA data:

1. identifying nonindigenous/ invasive aquatic species outside of their native or historic range;
2. from water or aquatic sediment sampling within the United States or its territories;
3. and produced using probe-based quantitative PCR or digital PCR data

Additionally, it is not a requirement to have positive detections to submit your data, but proper controls are paramount (see Guidance Document). Please fill in all entries then click the **Send Request** button below. Further details and descriptions about your submission, or questions you have for the NAS team, can be included within the Comments entry area.

Do you have multiple target species data to submit? *If so, please fill out a request for each species independently. If you have questions, please email a NAS staff member for assistance.*

Which aquatic invasive taxa/species are you reporting on?

Type: (Select type)
Common Name:
Genus:
Species:

Study details

Sample Medium:
○ Water ○ Sediment

Type of analysis:
○ Probe based quantitative real-time PCR (qPCR) ○ Probe based digital PCR (dPCR)

Do you have positive target detections to report?
○ Yes ○ No

Is this part of a continuing project from which you have previously submitted eDNA data?
○ Yes ○ No

Study dates and location

Date range of the study: from _____ to _____

Please state the location where the study was performed as accurately as possible (list names of states, water bodies, national or state parks, management areas, etc.):

Draw a polygon (or rectangle) encompassing your study area:

☐ Draw Polygon
☐ Draw Rectangle
☐ Polygon from WKT

Unselect all three options to pan map.

Requestor Information

Your personal information will NOT be shared on the website but may be shared with aquatic invasive species (AIS) managers and partners responsible for the AIS in the location where your study took place.

First Name:
Last Name:
Email:
Telephone Number (optional):
Address or Institution Name:
Important: non-preferential release: ○ True ○ False

My data are subject to non-preferential release: Select 'True' if your data cannot be preferentially released (e.g., USGS data cannot be released to invasive species managers prior to public release). Please check with your institutional regulations to determine the status of your data prior to answering this question.

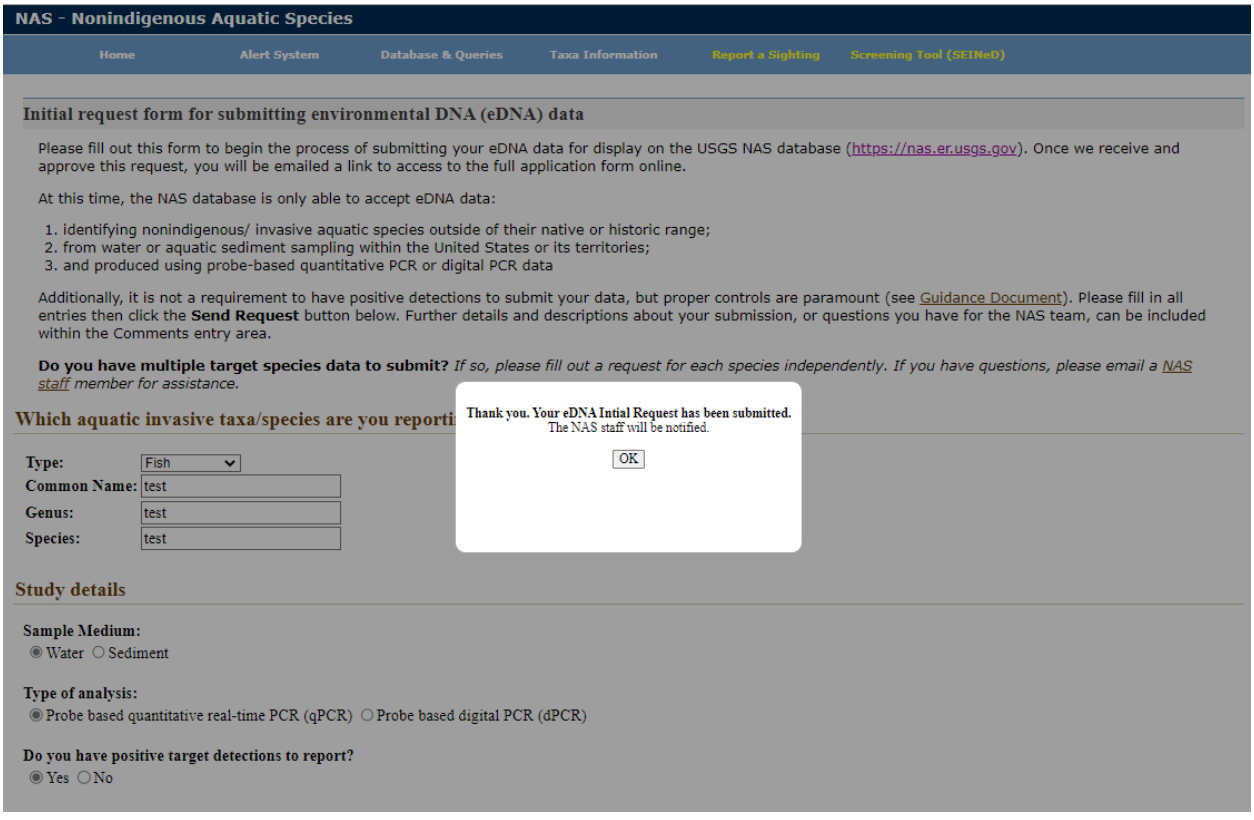
Additional Comments

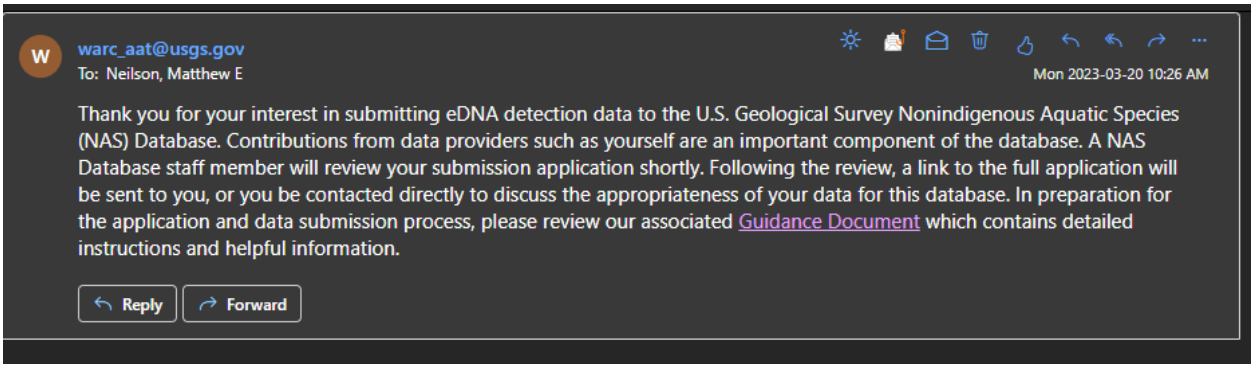
Please leave any comments here:

Submit

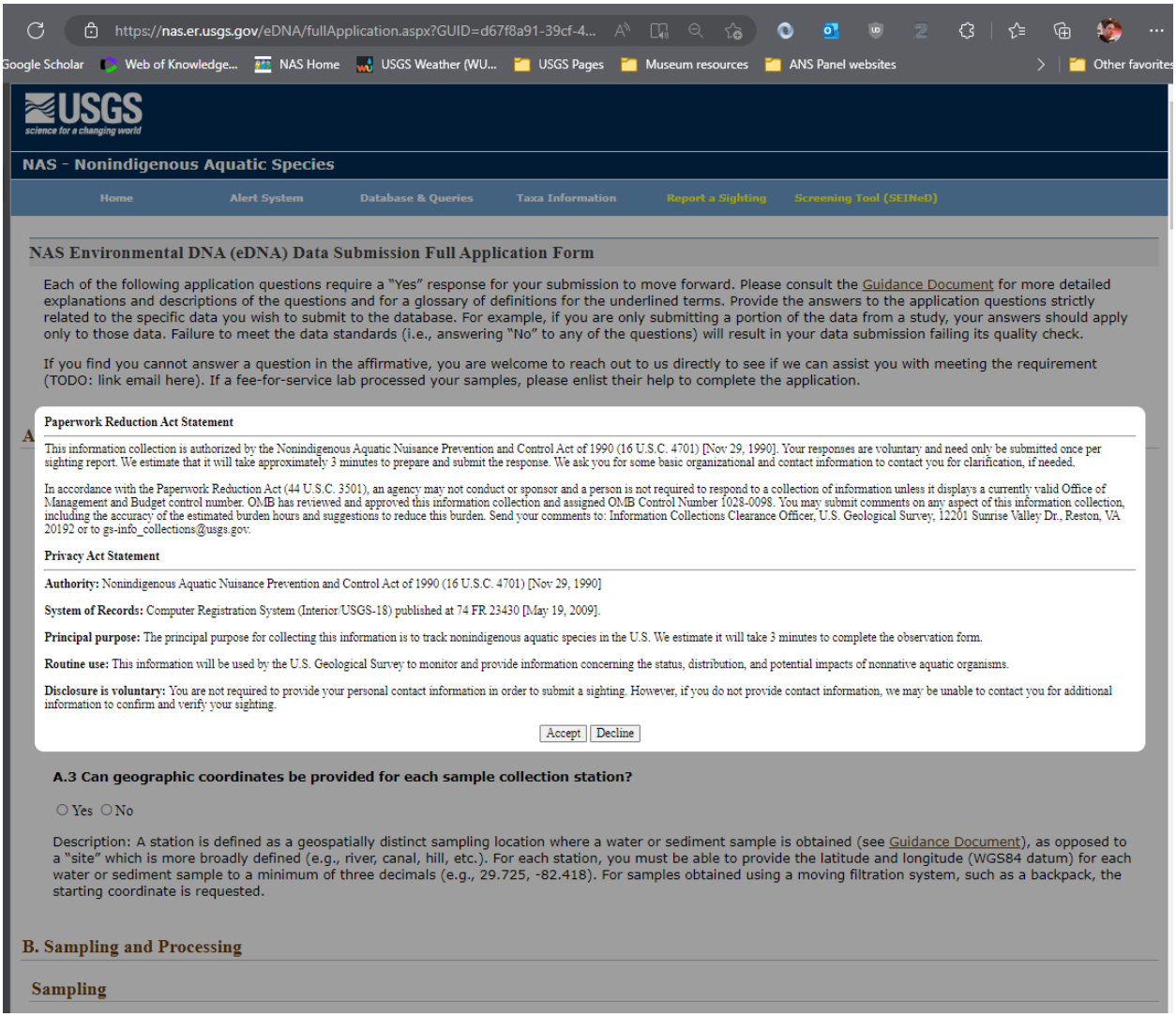
OMB Control Number: 1028-0098
Expiration Date: 01/31/2023

Upon successful completion and submission of the prescreening form, the user is presented with a notification, and receives an automated email describing the review and submission process. Data from this form is entered into a table in the NAS Database for a staff member to quickly review and approve/deny for the second, full application form.





After the pre-screen form is reviewed by a NAS staff member the user then receives a second email containing a unique link to the full submission form (https://nas.er.usgs.gov/eDNA/fullApplication.aspx), which captures detailed metadata about the eDNA data the user would like to submit. The user is again presented with a pop-up notification containing the Paperwork Reduction Act and Privacy Act statements (number is screenshot is currently a placeholder)



This pop-up contains the OMB control number, but not the expiration date. When the user clicks on the 'Accept' button, the user can then interact with the form. The following screenshots show the content of the form. When the user completes the form and scrolls to the bottom of the page, the OMB Control Number and the expiration date (both placeholders currently) are visible:

NAS Environmental DNA (eDNA) Data Submission Full Application Form

Each of the following application questions require a "Yes" response for your submission to move forward. Please consult the Guidance Document for more detailed explanations and descriptions of the questions and for a glossary of definitions for the underlined terms. Provide the answers to the application questions strictly related to the specific data you wish to submit to the database. For example, if you are only submitting a portion of the data from a study, your answers should apply only to those data. Failure to meet the data standards (i.e., answering "No" to any of the questions) will result in your data submission failing its quality check.

If you find you cannot answer a question in the affirmative, you are welcome to reach out to us directly to see if we can assist you with meeting the requirement (TODO: link email here). If a fee-for-service lab processed your samples, please enlist their help to complete the application.

A. Basic Study Information

A.1 Was either probe-based quantitative PCR (qPCR) or digital PCR (dPCR) performed for all sample data being reported?

○ Yes ○ No

Please indicate which: ○ qPCR ○ dPCR

Description: Only data produced via hydrolysis, probe-based (i.e., TaqMan, etc.) quantitative PCR or digital PCR assays are applicable. This does NoT include data produced using methods such as non-specific, fluorescent dye-based qPCR (e.g., SYBR Green, EtBr, LAMP, etc.), metabarcoding, or conventional/end point PCR.

A.2 Can the collection date be provided for each water or sediment sample for which data are being reported?

○ Yes ○ No

Description: The date that the sample was collected, preferably using the ISO 8601 date format of Full Year-Month-Day (e.g., 2019-07-26), must be available for each sample reported.

A.3 Can geographic coordinates be provided for each sample collection station?

○ Yes ○ No

Description: A station is defined as a geospatially distinct sampling location where a water or sediment sample is obtained (see Guidance Document), as opposed to a "site" which is more broadly defined (e.g., river, canal, hill, etc.). For each station, you must be able to provide the latitude and longitude (WGS84 datum) for each water or sediment sample to a minimum of three decimals (e.g., 29.725, -82.418). For samples obtained using a moving filtration system, such as a backpack, the starting coordinate is requested.

B. Sampling and Processing

Sampling

B.1 Can the volume of the water sample or mass of sediment sample be provided?

○ Yes ○ No

Description: A water volume (in liters) or sediment mass (in grams) must be reported for each sample leading to an individual data point.

B.2 Were the initial field samples protected from light and (1) immediately preserved using a DNA stabilizing buffer then stored on ice/refrigeration (4°C) until processed or (2) frozen (0°C minimum) within 24 hours of collection? Alternatively, were the samples (3) filtered on site and then preserved (using stabilizing buffer, desiccant, etc.) for analysis off-site or (4) filtered and immediately analyzed on-site? Answer Yes if ANY of the methods were employed.

○ Yes ○ No

Please indicate which method(s) was/were used:

☐ (1) stabilized with preservative on site and stored at 4°C
☐ (2) frozen for future processing or filtered within 24 hours
☐ (3) filtered and/or preserved (e.g., desiccant, freezing, etc.) on site
☐ (4) PCR analyzed on site

Description: Evidence shows rapid degradation of eDNA from water or sediment samples at warmer temperatures (Strickler et al. 2015). A maximum short-term storage temperature of 4°C is needed to preserve eDNA yield and quality, prior to and after concentration (i.e., filtration or centrifugation). If no preservation method listed was used, samples must have been analyzed through the PCR step on location for approval of the application.

Contamination Controls

Negative controls, or "blanks," are required for approval of the application. The next questions center on controls which account for possible contamination sources. For each type of control, you will need to verify that the PCR did not amplify DNA. This result applies to negative controls collected and associated with the specific samples you wish to report.

B.3 Field Equipment blanks: Was at least one equipment blank (DNA-negative water or sediment) prepared at each site (per day) using the field sampling methods to ensure neither the equipment, nor site, were contaminated by DNA introduced prior to the field effort? Please indicate the frequency and timing of field blank preparation (i.e., daily, upon arrival at each new site, after finishing at each station, for each sampling event at a station, etc.) in comments.

○ Yes ○ No

Description: We require, at a minimum, one field blank at each site (broadly defined as a river, canal, hill, etc.), and if multiple days at a site, a field blank must be collected for each day. We recommend doing so prior to the beginning of sampling (i.e., at the field vehicle, in boat before launching, before collecting any sediment, etc.). Please indicate the frequency (daily, at each site, at each station, etc.) of field blank collection in the comments. A field blank is collected to ensure the site or equipment is not contaminated by DNA introduced from the lab, or a previous sampling site, etc. It involves pouring DNA-free water or dispensing DNA-free sediment into a collection receptacle (e.g., bottle, tube, bag, etc.) in the field. When using a field filtering apparatus (mentioned above), this would mean pulling DNA-free water through the equipment system and collecting the blank as if it were a sample. Optimally, this is done before field sampling begins and using decontaminated equipment.

B.3a Did all the field equipment blanks result in no amplification of DNA?

○ Yes ○ No

Description: Please verify that the PCR did not amplify target or non-target DNA. This result applies to blanks collected and associated with the specific samples you wish to report.

B.4 Method blanks: Were DNA-negative water or sediment blanks processed using the eDNA concentration (isolation from substrate) methodology (i.e., did you filter/centrifuge negative controls)?

○ Yes ○ No

Description: A method blank is collected to ensure the equipment used for isolation is not contaminated as a result of processing. It involves preparing and analyzing DNA-free water or sediment using the same protocol as the field samples including the concentration equipment (i.e., filtration systems, centrifuge, etc.). Concentration, or isolation, here refers to the step wherein the biological material is concentrated or separated from the medium (water or sediment) and does not refer to the extraction of the DNA from the biological material (see Q. B.5).

B.4a Did all the method blank(s) result in no amplification of DNA?

○ Yes ○ No

Description: Please verify that the PCR did not amplify target or non-target eDNA. This result applies to blanks collected and associated with the specific samples you wish to report.

B.5 DNA extraction blanks: Were DNA-negative water or sediment samples processed using the same eDNA extraction (purification) method and/or kits?

○ Yes ○ No

Description: Here potential contamination of the eDNA extraction step is tested by running a target DNA-negative water (e.g, distilled) or sediment sample, using the DNA extraction kit/method. An extraction blank is processed along with your samples to ensure the eDNA extraction (purification) process does not contaminate the samples while obtaining the final purified DNA product.

B.5a Did all the extraction blank(s) result in no amplification of DNA?

○ Yes ○ No

Description: Please verify that the PCR did not amplify target or non-target eDNA. This result applies to blanks collected and associated with the specific samples you wish to report.

Processing methods

B.6 Was a peer-reviewed, published method for extraction (purification) of eDNA samples used in the study?

○ Yes ○ No

Please select the product type and include a reference and/or commercial product name in the comments.

○ (1) Silica column
○ (2) Phenol-chloroform
○ (3) Enzyme
○ (4) Bead
○ (5) Other published method (MUST include reference in comments)

Description: At this time, only extraction methods validated for eDNA samples published in peer-reviewed journal articles are accepted. Studies which use novel, unpublished methods are unable to be accepted by our process at this time. Please include a published reference in the comments for the method you employed, and the specific product name.

C. PCR Assay

Validation and Optimization

PCR assay validation

Description: In this section, validation indicates the detection of target eDNA and not that of non-target eDNA. For some questions, you will be asked to distinguish in-house efforts versus reporting the results of a previously published study used as the reference for your study. We require that answers based on another lab's work are applicable ONLY if they included species (in silico) or samples (in vitro/situ) from your study area at the regional scale (defined as a larger geographic unit encompassing numerous sites, such as a park or preserve, drainage basin, county/state, etc.). Otherwise, we require that these validation steps be assessed by your lab. Please cite the referenced study from which your assay is derived in the comments. At this time, only qPCR or dPCR methods validated for eDNA samples and published in peer-reviewed journal articles are accepted. Studies which use novel, unpublished methods are unable to be accepted at this time.

C.1 Has the specificity of the PCR primers and probes been validated for the exclusion of non-targeted taxa in silico?

○ Yes ○ No

Description: Were in silico tools/software used to validate the specificity of primers and probes to the taxa's target sequence? Any appreciable homology to similar or unexpected targets that may be in the study area (regional scale) should be noted in the comments. This is often specific to the target organism as evolutionary rates and the number of species in a genus will vary significantly.

If yes, are you reporting based on

○ (1) published information from another lab
○ (2) results from your own lab

C.2 Has the PCR assay been validated for the exclusion of non-targeted taxa specificity in vitro?

○ Yes ○ No

Description: Was this assay tested against genetic samples (e.g., DNA extracts, etc.) from non-target taxa? This question seeks to ensure the assay did not cross-react with an organism that could occur within the system being sampled (regional scale). This is often accomplished by performing a PCR (end-point, qPCR or dPCR) to test genetic samples from closely related, non-target species of the same genera and non-related genera. If this test resulted in a positive detection for a non-target species, please list the non-target species (scientific names) in the comments.

If yes, are you reporting based on:

○ (1) published information from another lab
○ (2) results from your own lab

C.3 Has the PCR assay been validated for taxa specificity in situ against co-occurring, non-target taxa?

○ Yes ○ No

Description: Please indicate whether water or sediment samples from a site within the study region where the target taxa are known to be absent have been tested using the assay and that the confirmed target taxon was not detected. For this control, the sample is processed like a blank and assayed with an internal positive control (IPC) not derived from the target species to ensure the PCR reaction occurred, but that no non-specific amplification occurred.

If yes, are you reporting based on:

○ (1) published information from another lab
○ (2) results from your own lab

C.4 Has the PCR assay been validated for target taxa in situ using target eDNA positive water or sediment samples obtained from the geographic region of your study?

○ Yes ○ No

Description: This question addresses the possibility of false negatives based on genetic differences within a species. For example, using an assay designed for target taxa in the Great Lakes to test for the same target taxa in the Pacific Northwest would not meet this criterion. Here we ask if the qPCR or dPCR assay was tested by sampling in an area where the target taxa are known to be present. Alternatively, this can be done by spiking water or sediment from the study area with target taxa DNA and observing a positive detection for the target DNA. Spiking the sample is acceptable for samples from study areas where a species has not yet invaded or in rare cases, suitable samples of similar habitat type are not accessible.

If yes, are you reporting based on:

○ (1) published information from another lab
○ (2) results from your own lab

C.5 Has the PCR assay been validated in situ to test for inhibitors and other environmental cofactors in the study area that may affect PCR amplification of your target eDNA?

○ Yes ○ No

Description: Although the chemical and physical makeup of a water body or area of sediment can change daily, seasonally, etc., this test shows that the assay is functional in the area being sampled. One way to assess inhibitors is to run tests of water or sediment samples with internal positive controls (IPCs) to determine if inhibitors may be affecting amplification.

If yes, are you reporting based on:

○ (1) published information from another lab
○ (2) results from your own lab

PCR Assay Optimization

Description: The assays used to produce the data must meet specific standards from the Minimum Information for Publication of Quantitative Real-Time Experiments (MIQE) or digital PCR experiments (dMIQE) to include standard curves and serial dilutions within the acceptable MIQE parameters (Bustin et al. 2009; Huggett et al. 2013). The NAS objective is to host eDNA data that are repeatable. Standard curve data provide critical information regarding repeatability and quality assurance. For lab-based qPCR analysis, a standard curve must be run at least once with the same protocol and equipment (qPCR machine, reagents, etc.) used to analyze the samples. If a commercial field qPCR system (instrument, reagents, etc.) was used, the company's validation metrics for that assay can be used to answer the questions that follow.

While the following metrics (C.6, C.6a-C.6b) do not directly apply to dPCR assays, we still require that you report the metrics based on a published work that validated the primers/probes for the dPCR assay you used (please cite your reference(s) in the comments). If such a publication does not exist, at this time we require the applicant needs to have performed a validation qPCR standard curve in their own lab and may use the results of that to answer the questions below.

C.6 Were standard curves (qPCR) or dilution series (dPCR) run using a minimum of three technical replicates per standard/dilution with a minimum of five standards/dilutions covering the expected dynamic range, OR can you report such metrics from an approved source (see description)?

○ Yes ○ No

Description: A minimum of three (amplified) replicates for each of five standards are required for statistical analysis of the curve following MIQE guidelines (Bustin et al. 2009). If data are produced via a qPCR assay, a standard curve must be run on your system with your reagents at least once prior to analyzing samples for reporting below. It is not required that standard curves be run with each set of samples (i.e., on each plate, etc.). If multiple curves are run, the minimum requirements below would apply to the dataset associated with each curve.

An appropriate material used for standards would be custom or synthetic DNA, to include gene fragments, oligonucleotides, plasmids, etc. Genomic DNA or PCR amplicons may be used if able to accurately quantify starting concentration using fluorometry.

C.6a Was the assay efficiency between 90 and 110%?

○ Yes ○ No

Description: Efficiency values are calculated from the standard curve and should fall within the 90-110% range. Efficiency is a measure of how effectively the assay amplifies the target sequence and optimally is as close to 2-fold each cycle (Cq), or 100%. You are welcome to enter your efficiency value in the comments.

For dPCR, are you reporting based on:

○ (1) published information from another lab (please cite reference in comments)
○ (2) results from your own lab
○ (3) I did not run dPCR

C.6b Was the R2 of the standard curve ≥ 0.95?

○ Yes ○ No

Description: R2 is the coefficient of determination (i.e., goodness-of-fit) of a linear regression model. This is calculated from the standard curve, generally by the qPCR software or by a statistical analysis program. The closer the value to 1.00, the better the regression is predicting the approximate real data points.

For dPCR, are you reporting based on:

○ (1) published information from another lab (please cite reference in comments)
○ (2) results from your own lab
○ (3) I did not run dPCR

C.6c Was the limit of detection (LOD) determined?

○ Yes ○ No

Description: The limit of detection is the lowest standard concentration with at least a 95% detection rate across all replicates (see Guidance Document). For lower concentration standards (those that fall within the double digits), it is recommended to run 10 or more replicates to ensure accuracy, as more replicates may help to decrease the effective LOD (Klymus et al. 2020).

For dPCR, are you reporting based on:

○ (1) published information from another lab (please cite reference in comments)
○ (2) results from your own lab
○ (3) I did not run dPCR

C.7 Were negative controls, or no-template controls (NTCs) run with each batch of samples or PCR plate?

○ Yes ○ No

Description: It is required that negative controls (i.e., NTCs, instrument blanks) are analyzed with each batch of samples (i.e., each plate, run, etc.). This is appropriately accomplished if DNA-free water or buffer is used to replace template (samples, standards, etc.) in a minimum of two reactions (for validation by reproducibility of results) during the analysis of each batch of samples. For the purposes of this application, a batch could mean one 96-well plate, or for assays not performed on a plate, such as field qPCR systems, a minimum of two negative controls may be analyzed across two separate runs (as some systems only analyze a few reactions per run) for each batch of samples (samples collected from the same site on the same day) to account for reagent differences or potential on-site contamination.

C.7a Did the negative controls result in no amplification of DNA?

○ Yes ○ No

Description: Please verify that the negative control PCR reaction did not amplify target or non-target eDNA. This result applies to negative controls analyzed with each specific batch of samples you wish to report. Note: All negative controls associated with a batch must result in no amplification to permit the data from the batch to be accepted. At this time, subtracting positive detection copy numbers from the experimental samples (often defined as 'limit of blank') is not allowed.

C.8 Were positive controls run in the PCR assay?

○ Yes ○ No

Please identify which type:

○ (1) Genomic DNA
○ (2) PCR amplicon
○ (3) Custom/ Synthetic DNA (i.e., gene fragment, g-Block, oligonucleotide, plasmid, etc.)

Description: It is required that positive controls are run with each batch of samples (i.e., each plate, PCR, etc.). This is appropriately accomplished if a standard curve is included in the run, or by the addition of target DNA (such as from a tissue sample) to several wells (minimum two) of the plate/strip/etc. to verify PCR amplification occurred (Q. C.4). Genomic DNA could be extract from the tissue, blood, etc. of the target organism. PCR amplicons and synthetic DNA strands should have at least five nucleotides 5' of ('flanking' or 'upstream of') the binding sites of the forward and reverse primers (e.g., the DNA fragment should not begin or end at the first nucleotide of the primer binding site). For assays not performed on a plate, such as field qPCR systems, the minimum two positive controls must be run with each batch of samples (samples collected from the same site on the same day) to account for reagent differences or local sample source variation (pH, inhibitors, etc.), but do not necessarily need to be analyzed in the same run.

For dPCR: If dPCR was run, the positive control should have been run to function as a calibrator (i.e., a DNA control containing the target sequence) (Huggett et al. 2013). This positive control should be used at the same volume and concentration throughout the study and must have been run on each plate, or with each round of analyses, as appropriate. Each reaction should yield a consistent number of copies for the calibrator/ positive control.

C.8a Did the positive controls result in (qPCR) detection of target DNA or did your positive control (calibrator) yield similar copy numbers and amplitude from dPCR run to run?

○ Yes ○ No

Description: Please verify that the qPCR/dPCR did amplify target DNA. This result applies to positive controls analyzed with each specific batch samples you wish to report. If running dPCR, please verify that similar copy numbers and amplitude were observed from run to run.

C.9 Were at least three PCR technical replicates run for each sample in the PCR analysis?

○ Yes ○ No

Description: We require at least three technical replicates per sample (at this time) to ensure repeatability of results, reduce the chances of false negatives, and to most accurately report true positives.

D. Reporting

D.1 Are the data reportable for each sample in one of these standardized formats: Qualitative detection (non-detect or detection) or quantitative detection (concentration in copies/Liter for water or copies/gram for sediment)?

○ Yes ○ No

Please indicate which or both.

☐ (1) Qualitative
☐ (2) Quantitative (you must report the limit of quantification in the comments)

Description: (see Guidance Document)

Non-detection: For qualitative or quantitative reporting, this is a result of no detections of target eDNA among all PCR replicates.

Qualitative detection: All reported detections must be replicated either in 2 or more technical replicates or among two or more station replicates (not just in one reaction/replicate). For qPCR, detection must occur with value(s) above the LOD but are below the LOQ in two or more of all qPCR reactions for a station sample.

Quantitative detection: For qPCR, the concentration value of at least 2 replicates with positive detection must be above the assay limit of quantification (LOQ). This is defined as the lowest standard concentration that resulted in less than 35% coefficient of variation. For dPCR at least two (2) reactions must have one or more positive droplets with an amplitude within the amplitude range of the positive control/ calibrator run on that plate. Calculations should use the metrics: copies/Liter for water or copies/gram for sediment.

Note about Inconclusive detection (see Guidance Document): Defined as a detection in only 1 of the PCR technical replicates for a station sample. These data will be permissible to submit with your dataset, but will not be displayed on the map viewer. They will be retrievable in the table format with the rest of your submitted data.

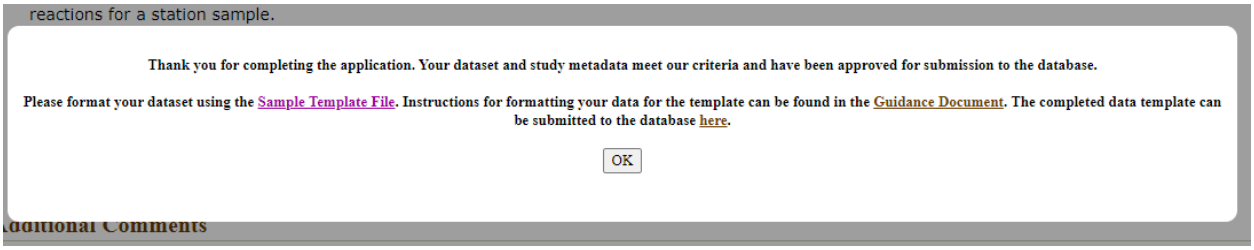
Additional Comments

Please leave any comments here:

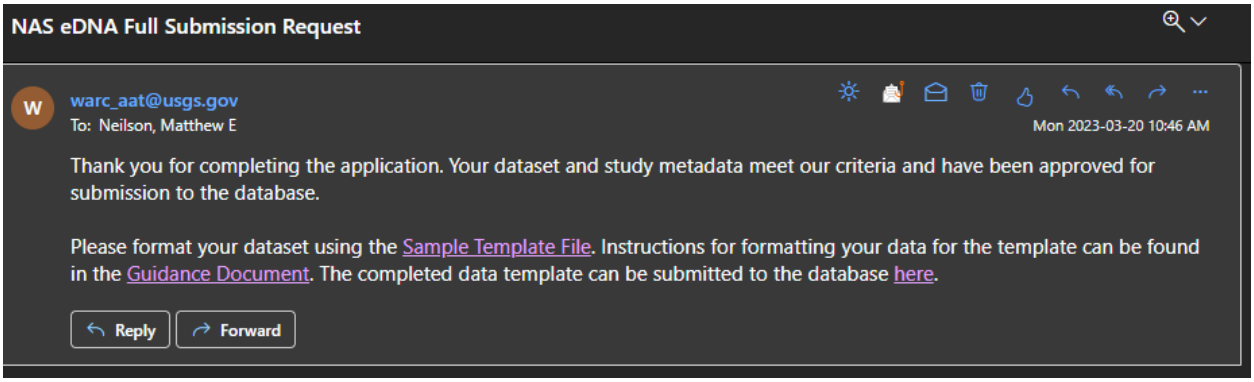
Submit

OMB Control Number: 1028-0098
Expiration Date: 01/31/2023

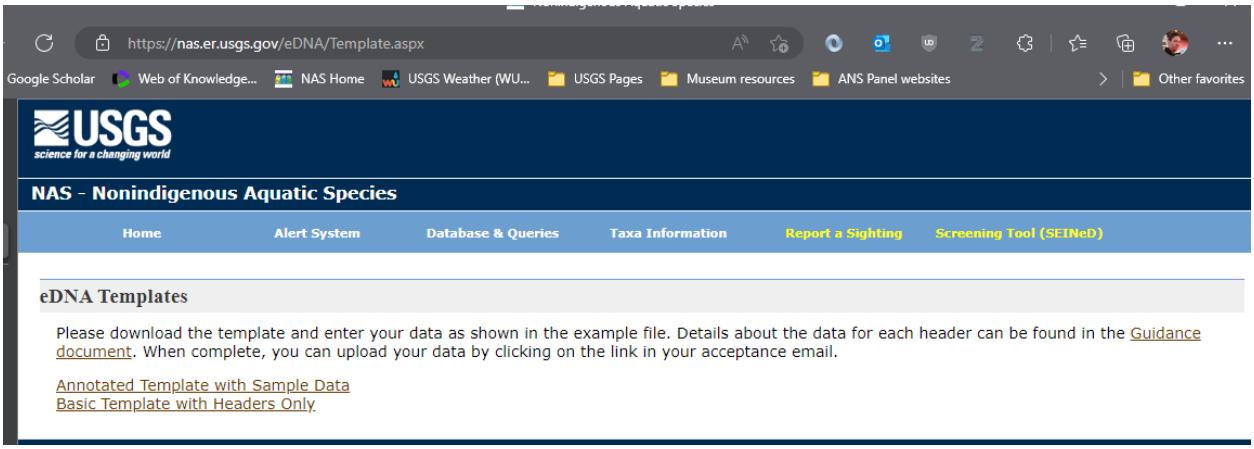
Upon submission of full application form, the form data is evaluated based on automated rules: if all questions are answered in the affirmative (indicating that the eDNA data meets with the previously established standards for submission), the user is shown a pop-up notification containing a link to the data template file, the submission guidance document, and a unique URL for submission of their dataset



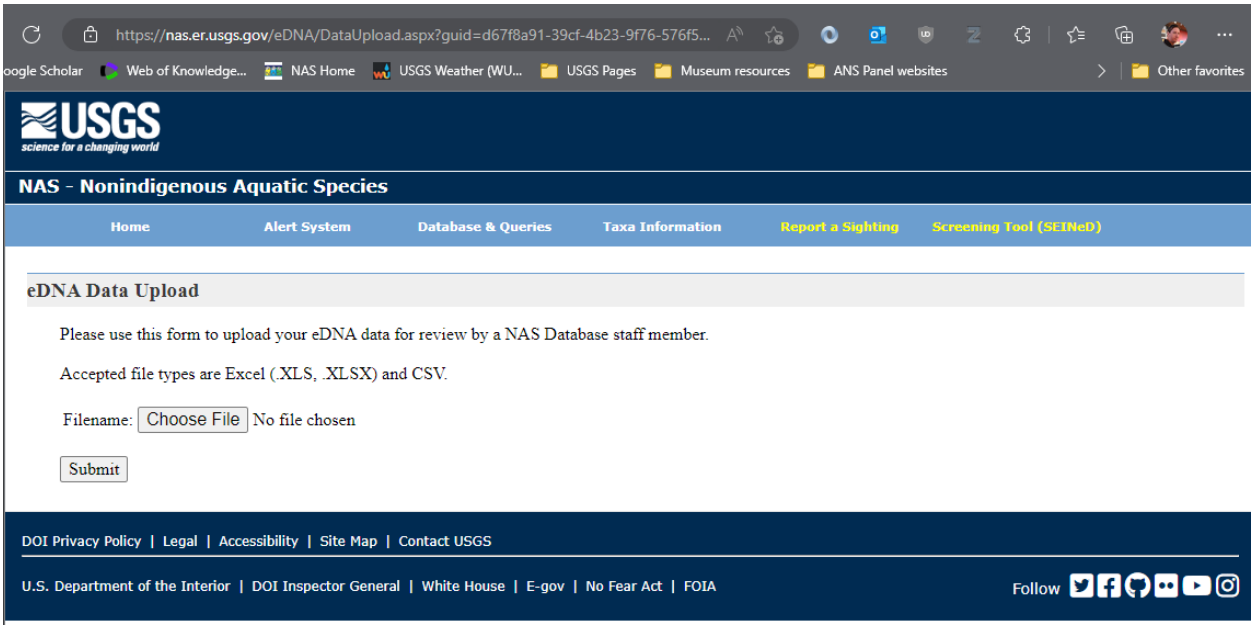
The user also receives an email containing the same links



The sample template file page contains two versions of the template: one with annotations on the field names and sample data, and one just containing field names. No OMB control number is displayed on this page, nor is a pop-up containing the PRA and Privacy Act statements presented (as the user has been shown this information twice during the submission process).



After the user formats their data according to the data submission template, the user can upload their dataset to the NAS database using a unique URL present in the acceptance email (see above)


https://nas.er.usgs.gov/eDNA/DataUpload.aspx?guid=d67f8a91-39cf-4b23-9f76-576f5...
oogle Scholar
Web of Knowledge...
NAS Home
USGS Weather (WU...
USGS Pages
Museum resources
ANS Panel websites
Other favorites
USGS
science for a changing world
NAS - Nonindigenous Aquatic Species
Home
Alert System
Database & Queries
Taxa Information
Report a Sighting
Screening Tool (SEINeD)
eDNA Data Upload
Please use this form to upload your eDNA data for review by a NAS Database staff member.
Accepted file types are Excel (.XLS, .XLSX) and CSV.
Filename:
Choose File
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