Systematic Map Protocol

Title
What evidence exists on the use of environmental DNA as an operational method for rivers studies? A systematic map protocol.

Citation:

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Keywords

Background
Monitoring of water bodies provides information about a particular site and its changes across time, as well as the effects of different impacts on biodiversity, which is essential to support management of freshwater environments. These assessments use physicochemical characterization and sometimes bioindicators. However, current approaches are dependent on suitable field conditions, a particular set of skills, special equipment, and thus, monitoring can be invasive, time-consuming, and financially expensive. An approach that has been used recently and that promises to be an effective method to improve monitoring is the use of environmental DNA (eDNA), which consists of analyzing a mixture of DNA that originates from the feces, saliva, urine, and skin cells of animals from multiple taxa and can be taken from a wide variety of sample types such as sediments, soil, feces, or marine and fresh waters. Although eDNA and Next Generation Sequencing approaches have been widely used, their application is more restricted to marine environments and lentic systems, despite the unique characteristics and capability of interconnection between ecosystems, making the study of riverine systems a priority in the application of this approach. This study was conducted in order to: analyze the trends in the application of eDNA, identify geographic and taxonomic biases as well as information gaps; discuss future and potential investigation niches that consider the use of eDNA in lotic water bodies; and identify possible paths to access this kind of information on available platforms. Finally, with the information generated, we hope that the systematic map generated by the implementation of this protocol will enable stakeholders, the scientific community, and decision makers to facilitate the implementation of this technique to fill the data gaps in the areas where there is a lack of information about river systems.

Theory of change or causal model
See attached figures named "eDNA_monitoring" and "Conceptual map".

Stakeholder engagement
The formulation of this research question and the scoping of this systematic map was discussed with different Mexican research institutes from different perspectives (ecological, biological, biotechnology, marine sciences, and limnology) and a Non-Governmental Organization specializing

in citizen science (Global Water Watch Mexico), in order to enhance knowledge, improve monitoring design, and scale-up the use of eDNA approaches to generate data relevant for stakeholders and decision makers.

Objectives and review question
The objective of this systematic map is to identify, map, and describe evidence of eDNA investigations in rivers. -Primary question What is the existing evidence that the environmental DNA approach has been applied in river systems? -Secondary questions What are the spatiotemporal trends of eDNA studies in rivers? Which taxonomic groups have been studied with this approach? What are the most commonly used methods? Which are the most commonly used sequencing platforms in the study of rivers? How much of the data generated in the studies is available for public access?

Definitions of the question components
Population: Lotic water bodies, particularly rivers (except estuaries). Intervention: use of the eDNA approach. Outcome: All outcomes related to the studied population, including data about taxonomic groups studied, sequencing platforms, environmental matrix used (water, sediment, biofilm, mixed sample), biodiversity, community structure, detected pathogens, type of technique applied (amplicon, WGS, transcriptomic), conserved sequences employed (if applicable), and public availability of sequencing data.

Search strategy
The strategy designed for this systematic map protocol and its corresponding systematic map is designed in accordance with the Guidelines and Standards for Evidence Synthesis in Environmental Management, following the ROSES format for Reporting Standards for Systematic Map Protocols. A search in 2 bibliographic databases and 1 web-based search engine will be conducted. For the searches, a word string will be used. The selected search strings will be reviewed and tested by the whole team in order to secure a good "specifity" level of returned studies that allows us to identify relevant studies. In the platform, the field "topic" that includes title, abstract, and keywords will be used. The scoping search string will use the Web of Science format, considering only English-language studies, using the following Booleans (AND, OR) and the wildcards: (environmental DNA OR eDNA OR genom* OR metagenom*) AND (river* OR stream OR lotic OR watershed OR catchment OR basin OR riparian OR watercourse OR waterway OR brook OR tributary OR channel OR creek) For the other bibliographic databases and the search engine (SCOPUS and Google Scholar), this search string will be adapted in accordance to the format of the database, as long as the search includes the title, abstract, and keywords.

Bibliographic databases
A search in two bibliographic databases will be conducted (Web of Science and SCOPUS). These databases were selected because of their renowned relevance as databases for this type of studies. Also, we count with institutional subscriptions to those platforms, those are provided by the Universidad Nacional Autónoma de México digital library and digital database (comprises publications since 1900). Searches will consider full text, English, and the search strings provided in the previous search strategy.

Web-based search engines
The search engine Google Scholar will be used to identify additional literature that can not be found in the bibliographic databases. We will focus only on the grey literature launched by this search engine.

Organisational websites

N/A

Comprehensiveness of the search
The comprehensiveness of our search string was tested using 10 papers considered relevant (by the whole team) as an indicator of a successful search. If those key papers, or the majority (at least 8), were returned by the search string, it was considered an optimum. However, if that search string did not return the majority of papers, it was modified. Watch the "Key_papers" document.

Search update
There are plans to update the launched results in the searches, during the conduct of the review, in order to improve the quantity and spatiotemporal resolution of the systematic map, once the present protocol is submitted to this repository and accepted as valid (April 2022). Actual results cover until January 2022.

Screening strategy
In our screening strategy, there will be two stages of screening: The first one is focused on a review of the title and abstract presented in the studies, in order to determine their inclusion or exclusion, based on a decision tree that was designed by all the review team (see image "screening stage I"), in concordance with the proposed objectives of the study. Prior to determine if the documents passed the first screening stage, the whole team reunited for a general training focused on the review of articles. After that, each study will be reviewed by double-screening. The studies that were considered with "uncertainty" about their inclusion/exclusion, will pass to stage two of the screening. Stage two of the screening process involves a full text review of the articles that passed the first stage and those that are categorized as "uncertain". However, if those studies cover at least one of the exclusion criteria, they will be excluded, even if they pass the first stage. For those studies where the uncertainty continues, a second review by another two members of the team will proceed.

Eligibility criteria
-Inclusion criteria: Type of study: Original articles, studies presented in theses and conferences. Language: English. Population: Lotic water bodies, particularly rivers (except estuaries). Intervention: use of the eDNA approach. Outcome: All outcomes related to the studied population, including data about taxonomic groups studied, sequencing platforms, environmental matrix used (water, sediment, biofilm, mixed sample), biodiversity, community structure, detected pathogens, type of technique applied (amplicon, WGS, transcriptomic), conserved sequences employed (if applicable), and public availability of sequencing data. Study design: -Experimental studies that included sampling of eDNA through some environmental matrix such as: water, sediment, or biofilm. -Study that used eDNA for modeling persistence, resistance, or its distribution. -Studies that used eDNA for detection of species focused on monitoring species at risk, exotic or invasive species, or those of human health importance. -Comparison of the use of eDNA versus conventional techniques for monitoring and identifying organisms. -Studies that focus on determining a baseline for biodiversity. Geography: no limit. Period: no limit. -Exclusion criteria: Type of study: Books, chapters, letters to the editor, review studies (systematic reviews, meta-analysis), modeling studies that did not take environmental samples. Language: Non-English Population: Wastewater Treatment Plants, sewage, lakes, microcosm experiments, estuarine or marine systems Intervention: no use of eDNA approach Outcome: Studies in which there was only a Draft Genome Complete Genome Sequencing Isolated Watch image: "eligibility criteria"

Consistency checking
10% of the articles screened by a reviewer will be selected randomly and screened by two other reviewers in order to check the consistency of eligibility. All discrepancies regarding screened

articles will be discussed by the 3 members of the review team, and if there is no final consensus about it, the whole team will be consulted if necessary. Consistency of reviewers' screening will be measured by the Kappa coefficient.

Reporting screening outcomes
Screening outcomes will be reported in a ROSES diagram, a list of eligible articles and the list of full text articles excluded with the reasons of their exclusion (watch the "ROSES example" diagram).

Study validity assessment
We will not be critically appraising the validity of robustness of the included articles. First, due to the big number of studies that will be encountered in the searches; secondly, because the included articles will be reviewed by all the team in order to identify if there is a study that did not fulfill the criteria; third, because the aim of this study is to describe the location of existent studies and not to analyze the results; and finally, because of the great variability in design, approach, and objectives of the several studies.

Consistency checking
N/A

Data coding strategy
For each of the studies that passes the screening stages, data extraction and codification will proceed. Any of the members of the team will have a data sheet where meta-data and information about relevant variables will be placed. Once a reviewer finishes the review of the articles, 10% of those studies will be reviewed by the other two members of the team in order to ensure that the data extraction was done correctly. Also, in these formats, a commentary section will be available, so the rest of the team can review and decide if data remains on the data sheet or will be removed.

Meta-data to be coded
Data extracted from each of the studies will be included: -Bibliographic details (author affiliations, keywords, etc.) -Study location -Intervention -Comparators -Outcome -Study design -Year of publication -Data availability -Taxonomic groups studied

Consistency checking
Once a reviewer finishes the review of the articles, 10% of those studies will be reviewed by other two members of the team in order to ensure that the data extraction was done correctly.

Type of mapping
The data set generated via data extraction will be analyzed in R in order to provide a narrative synthesis that summarizes searchable databases and visual outputs reflecting the actual evidence of the use of the eDNA approach in the study of rivers. Data extracted for any of the studies will be available in a database, so that users can filter, analyze and evaluate the existent evidence.

Narrative synthesis methods
The findings will be summarized in the form of tables, graphs (comparison of variables), and maps (geographic distribution of actual evidence) that allow visualizing the generated evidence. These representations will allow the identification of gaps, thematic synthesis, major taxonomic groups that are studied, sequencing platforms used, etcetera.

Knowledge gap identification strategy
Generated evidence will be analyzed and discussed in order to find evidence gaps, geographic bias of generated evidence, and try to synthesize the presented evidence to understand where this

approach has been applied globally. The trends shown by the data will serve as a preliminary tool in the form of a narrative synthesis that helps the scientific community, non-governmental organizations, stakeholders, and decision makers to take better action in the way rivers are studied or monitored.

Demonstrating procedural independence
In case there is a member of the review team who could be listed as an author on an article considered for the review, that member will not be involved in the review process or any of the decisions of inclusion or exclusion related to that article.

Competing interests
All authors declare they have no competing interests.

Author's contributions
RICC, MK, RASV and LBD developed the concepts and framework for the systematic map. RICC wrote the manuscript. All authors contributed for decision tree construction based on their expertise and discussion of ideas. AAC, NBCC and MRE contributed with ideas, proof-reading, and review, assuring the protocol applicability. All authors have read and approved the information embodied in the document.

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