Collaboration for Environmental Evidence

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Systematic Map Protocol

Title
What evidence exists on the study of pathogenic microorganisms in reef-building coral species? A systematic mapping protocol

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Background
The incidence of coral diseases is believed to be related to human activities, especially in coastal areas subject to various disturbances. Diseases are a natural process, caused by both biotic and abiotic stressors, but certain factors can exacerbate them and cause outbreaks. Research suggests that global warming and other anthropogenic stressors such as land pollution, sedimentation, overfishing and human use are important factors for increased incidence of coral diseases. The causes of coral disease outbreaks are complex and not well understood. It is known that biotic coral diseases are caused by a multitude of microorganisms and macroorganisms, including pathogenic bacteria, microbial consortia, ciliates and parasites dominated by cyanobacteria. Disease outbreaks can cause significant changes in reproduction rates, growth rates, community structure, species diversity, and abundance of reef-associated organisms. To identify microbial communities and pathogens, there are different techniques available, including conventional ones like visual identification, sampling of coralline tissue, stains, cultures, microscopic observations, and molecular techniques ranging from PCR to NGS of environmental DNA. There are several reviews discussing the geographic and taxonomic gaps or bias, that occur in the study of coral diseases; however, some of them are not recent, or did not consider the different methodological approximations to solve their research questions, such is the case of methods focused on coral tissue culture, NGS sequencing, specific stains, among others. Thus, the information in these reviews has some limitations, and they may not include the latest advancements that originated from the SARS-CoV-2 pandemic, where new methods, approaches, and studies particularly in the detection, isolation and processing of viral and bacterial samples have emerged. As a result, the available information may have significant biases; so, this study aims to provide an updated review of the state of the art of the existent approaches.

Theory of change or causal model
Despite the information that has been generated so far regarding the increasingly common issue of diseases in reefs around the world, it is important to consistently update and synthesize this information. This is necessary to gain a proper understanding of the best methods for detecting, isolating, and identifying these diseases. The goal is to optimize efforts aimed at monitoring coral

pathogens, which will allow the information generated to be used in making informed decisions to contribute towards the conservation of these vulnerable ecosystems. See attached figures "Conceptual_model" and "mapping_model"

Stakeholder engagement
The formulation of this research question and scoping of systematic map was discussed with different Mexican research institutions from different perspectives (ecological, biological, biotechnology, marine sciences and limnology) in order to improve study design, and generate data relevant for stakeholders and decision makers. The aim is to provide a solid and up-to-date foundation for future research in this area, enabling better-informed decision-making, in identifying and characterizing coral pathogens. Additionally, it aims to develop more effective strategies for environmental monitoring that will enable the optimization of conservation efforts in coral reefs.

Objectives and review question
Primary question: How much information exists globally on the microorganisms that cause diseases in reef-building corals? Secondary questions: What microorganisms are considered pathogenic for corals? What group of microorganisms are the most studied and reported in corals? What are the most studied and reported diseases in corals? What techniques or methodological approaches are most used for the study of diseases in corals? Which coral species are most vulnerable to diseases? What are the spatio-temporal trends regarding the information generated on this topic?

Definitions of the question components
Population: Studies in corals where pathogenic agents are reported, identified or studied specifically or community-wise. Intervention: Use of molecular tools (metabarcoding, sequences, cultures, use of PCR, etc.) in tissue, coral mucus or water samples. Some other type of technologies or methods applied to the detection, isolation and identification of pathogens in corals (census, visual identification, video or photographic reports, etc). Outcome: Identification, characterization and reports of coral pathogenic organisms, as well as the diseases they cause.

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. Those guidelines aim to capture a wide range of sources and ensure its replicability and transparency. A search in 2 bibliographic databases and 1 web-based search engine will be conducted. Also for the searches, in each of the databases and search engines, a general word string will be used. The selected search string 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 English-Spanish language studies, using the following Booleans (AND, OR) and the wildcards: ((coral* OR reef*) AND (disease* OR health* OR bleach* OR band* OR plague OR pox OR spot* OR necro* OR syndrome* OR anomal* OR lesion* OR mortality*) AND (identification* OR detection)) For the other bibliographic database and search engine (SCOPUS and Google Scholar), the search string will use the same terms, only to 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, SCOPUS). These databases were selected because of their renowned relevance as databases for this type of studies. It is worth to mention that 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 or Spanish language, 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 cannot be found in the bibliographic databases. We will focus only on the grey literature launched by this search engine. As in the Bibliographic databases section, our searches will consider full text, English language, and the search strings provided in the search strategy.

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. See the "key_papers_corals" document.

Search update
There are plans to update the launched results in the searches, during the conduct of the review, to improve the quantity and spatiotemporal resolution of the systematic map, once the present protocol is submitted to this repository and accepted as valid (November 2023). Actual results cover until August 2023.

Screening strategy
In our screening strategy, there will be two stages: 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 "decision_tree"), 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, as well that those who did pass it 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
Eligibility criteria are showed in the "Criteria" document. Inclusion criteria - Type of studies: Articles, preprint and thesis - Language: English and Spanish - Population: Coral studies where pathogenic agents are reported, identified or studied specifically or community-wise - Intervention/Exposure: Use of molecular tools (metabarcoding, sequencing, use of PCR, etc) and other types of technologies applied to the detection, isolation and identification of pathogens in corals , as well as manual techniques (cultures,stains,etc) or conventional survey methods. - Outcome: Identification, characterization or report of coral pathogenic organisms - Study design: In situ and experimental studies that included taking samples and coral mucus in which they applied a technology for the identification of pathogens or field studies. -Geography: There was no limitation for geographic areas -Period: There was no time limit for studies Exclusion criteria - Type of study: Abstracts of conferences , books, book chapters, journalistic notes, models, systematic reviews and

meta-analysis. - Language: Non-English and Spanish papers - Population: Studies where no work is done with corals, nor are pathogens reported, in addition to studies that do not generate data in the field. - Intervention/Exposure: Simulations and models. Studies that do not take samples in situ. - Outcome: Report of non- pathogenic organisms for corals. - Study design: Studies that involve the analysis of databases or bibliographic reviews.

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 2 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 flow diagram_corals" 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. However, considering the first and prospective review of results launched by the databases, an estimated number of studies that meet the inclusion criteria and could be used for the database construction ranges between 300-400 studies.

Consistency checking
A Kappa value of 0.61 to 0.80 can be regarded as substantial, according to the research that is currently available. Nevertheless, the team determined that a value of 0.69 would be the proper threshold to assess consistency.

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: country, geographic coordinates -Intervention: method employed for the study of coralline diseases (visual, conventional, molecular, census, etc.) -Outcome: type of pathogen studied, studied coral species, identified pathogen species -Study design: type of sample studied (tissue, water, etc.), number of samples used, used volume, cultured pathogens, etc. -Year of publication -Data availability: repositories, primers used, etc. -Taxonomic groups of pathogens studied These variables are captured in a standardized Excel form (View "BD_corals_data" file) that the reviewers will use to fill with the corresponding information.

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 of different methods in the study of pathogens of coral reef constructing species. Data extracted for any of the studies will be available in a database, so that users can filter, analyze and evaluate the existent evidence. R code used for the construction of graphs will also be shared in the Supplementary Material of the systematic mapping.

Narrative synthesis methods
We will adhere to the ROSES guidance for reporting systematic maps. In addition to the narrative synthesis of the systematic maps, information gathered from eligible studies will be documented in an Excel spreadsheet and provided as a supplementary file. 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, main methods 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 and how different methods have been applied in order to study diseases in coral species. 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 coralline communities can be studied in a diseases incidence approach.

Demonstrating procedural independence
In case there is a member of the review team who could be listed as an author of 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.

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Author’s contributions
AGG, RICC, DSO and HAPM developed the concepts and framework for the systematic map. AGG, RICC and HAPM wrote the manuscript. All authors contributed for decision tree construction based on their expertise and discussion of ideas. RICC, DSO and HAPM 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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