Review Methods to Address Issues in Environmental Health Epidemiology

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Anisha Singh

August 2022

Acknowledgements

I am eternally grateful to my advisor, Dr. Kristina W Kintziger, for patiently guiding and supporting my academic and professional development, and for making my doctoral program an enriching experience. I am also thankful to Dr. Christy Leppanen, Dr. Jiangang Chen, Dr. Evah Odoi, and Dr. Agricola Odoi for their continuous guidance and support, and I would like to acknowledge the unwavering dedication of Dr. Jiangang Chen that has enabled me to complete the dissertation.

I am deeply grateful to the Department of Public Health for supporting my academic pursuits and thank Ms. Wendy Smith for her support and assistance with the logistics of the doctoral program.

I am deeply thankful to the Graduate School and to the College of Education, Health, and Human Sciences for financial support via Tennessee Fellowship for Graduate Excellence (2017-2021) and Julius D McElroy Fellowship (2021-2022) respectively. The support of the library at the University of Tennessee, Knoxville is greatly appreciated for successful completion of this dissertation.

I am thankful to my family in India for their endless emotional support and love, and to Nitin for holding me through these very intense academic years.

Abstract

The advent of evidence-based practice in health has led to widespread acceptance of reviews in clinical and healthcare fields. Similarly, review methods in environmental epidemiology offer a unique opportunity of collating and analyzing highly heterogenous data, i.e., human and animal data, for unbiased decision making. Thus, this dissertation focuses on the use of systematic review and scoping review (a type of systematic review) methods to understand specific issues in environmental health.

The aims of this dissertation were to investigate: (i) the associations between chemical nutrients/contaminants, temperature, and harmful algal blooms due to *Karenia brevis* in the North American region; (ii) the environmental effects of a novel insecticide, cycloxaprid (CYC), on target and non-target insect species; and (iii) the effect of land use, land cover, and climate change on incidence of Japanese Encephalitis (JE) disease among Asian populations.

We performed a scoping review of the existing literature to get an overview of the scientific advancements and status quo of emerging evidence related to the associations between temperature, chemical nutrient/contaminants, and *K. brevis* blooms. We recommended increasing the availability and accessibility of contaminants data in aquatic systems and enhancing contaminant data coverage to account suggestive shift in the distribution of *K. brevis* blooms.

Further, we aimed to find answers to specific questions related to JE infection and CYC. Our objective was to identify and retrieve international research, assess the quality of evidence to

confirm if the current knowledge is based on relevant evidence, and to address any uncertainties or conflicting evidence to draw meaningful conclusions for future research and unbiased decision making. Findings of the CYC systematic review suggested conducting additional research to clarify the mechanism of action of cycloxaprid and its metabolites on target and non-target insect species. The findings from the JE systematic review suggested that future research must focus on conducting high-quality ecological and individual-level studies.

In conclusion, use of systematic and scoping review methods unveils the quality of evidence and informs future environmental health research.

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Abbreviations

AChE	Acetylcholinesterase
AES	Acute Encephalitis Syndrome
AIC	Akaike Information Criteria
CAT	Catalase
CI	Confidence Interval
CYC	Cycloxaprid
°C	Degree Celsius
EC50	Effective Concentration 50
ENSO	El Niño–Southern Oscillation
EPA	Environment Protection Agency
GLP	Good Laboratory Practices
HAB	Harmful Algal Bloom
IMI	Imidacloprid
IRR	Incidence Rate Ratio
IPCC	Intergovernmental Panel on Climate Change
JE	Japanese Encephalitis
JEV	Japanese Encephalitis Virus
K. brevis	*Karenia brevis*
LC50	Lethal Concentration 50
LD50	Lethal Dose 50
LRE	Loxahatchee River Estuary

NASA	National Aeronautics and Space Administration
NHS	National Health Service
NOS	Newcastle-Ottawa Scales
nAChR	Nicotine Acetylcholine Receptors
nAChR	Nicotinic Acetylcholine Receptor
OR	Odds Ratio
OHAT	Office of Health Assessment and Translation
OSF	Open Science Framework
OECD	Organization for Economic Co-operation and Development
PICO/PECO	Population, Intervention, Comparator, and Outcomes/Population, Exposure, Comparator, and Outcomes
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
RCT	Randomized Control Trials
RR	Relative Risk
SST	Sea Surface Temperature
SOD	Superoxide Dismutase
SYRINA	Systematic Review and Integrated Assessment
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
VTG2	Vitellogenin2 Gene

Chapter I

Introduction

Background and Statement of Problem

The notion of synthesizing results across similar studies is not new. James Lind in 1753 conducted systematic review of current evidence to design clinicals trials to find the potential cure for scurvy [1]. Lind's work is revered as the first reported systematic review study [2]. In 1904, Karl Pearson designed a study to investigate the association between typhoid and mortality by inoculation status among British soldiers living in different geographical locations [3]. He calculated the mean of the correlation coefficients obtained from five two-by-two contingency tables and thus, founded the first formal technique for data pooling [3]. Later, in 1931, Tippett used ordered *p*-values observed across studies to describe an approach for estimating the likelihood of a significant effect [4]. Similarly, in 1932, Fisher identified a method to merge *p*-values across studies investigating a similar question [5]. To reach a definitive conclusion about the treatment effect, Fisher analyzed an alternative research hypothesis of pooled treatment effect against a null hypothesis of no treatment effect across all studies [5].

Such relatively isolated examples are common for more than a couple of centuries. However, in the early 1990s, with the advent of evidence-based practice, there has been widespread acceptance of reviews [2]. This has led to an increase in the undertaking of literature reviews, and consequently, a plethora of terminologies exist to describe different approaches for conducting literature reviews. Grant and Booth [2] conducted a review to provide a descriptive account of the most common reviews in the health domain. This scoping search identified 14 review types such as critical review, literature review, mapping review/systematic map, meta-analysis, mixed studies review/mixed methods review, overview, qualitative systematic review/qualitative evidence synthesis, rapid review, scoping review, state-of-the-art review, systematic review, systematic search and review, systematized review, and umbrella review [2]. Recently, Moher et al. [6] identified systematic review methods as a family that includes four other types of review, i.e., rapid review, scoping review, evidence map, and realist review. The variety of these reviews have emerged to accommodate the needs for information of the policymakers and other stakeholders where the systematic review methodology does not fit [6]. We acknowledge the importance of all the review methods, and for the purposes of this research, we consider scoping review as a type of systematic review method. However, in this section, we will describe the systematic review and scoping review methods separately, as we acknowledge that the aim, scope, and method of conducting a systematic and scoping review are different.

Overview of systematic and scoping review methods

A systematic review is a comprehensive and exhaustive search of primary studies on a focused research question, selection of studies using clear and reproducible eligibility criteria, critical appraisal of studies for quality, and synthesis of results according to a pre-determined and explicit method [7-9]. A systematic review is the best-known type of review, and it has the highest level of quality of evidence [2,10]. It allows for a more objective evaluation of evidence as compared to other reviews and thus, it might provide a more certain conclusion when the primary research, reviews, and editorials do not agree [7]. Often adhering to the guidelines of the National Health Service Centre for Reviews and Dissemination or the Cochrane Collaboration, a systematic review is transparent in reporting its methods and thus, facilitates others to replicate the process [8,9]. Systematic reviews might include a meta-analysis component. A meta-analysis is the statistical technique to data pooling across studies to summarize estimates of effects [11]. However, a meta-analysis does not include the complete methodological techniques which define a systematic review process such as the evaluation of quality assessment of included studies. Thus, the use of the terms meta-analysis and systematic review interchangeably is ambiguous [12].

A meta-analysis is possible when all included studies are sufficiently similar and more importantly, there is similarity in outcome measure i.e., homogeneity of effect/outcome measures [2,11]. However, effect measures can be heterogenous, and meta-analysis cannot be conducted. Thus, there are instances when a meta-analysis is clearly not appropriate [11]. In such cases, systematic reviews without meta-analyses are an acceptable way of summarizing the evidence. Hence, systematic reviews and meta-analyses are not the same [7]. Additionally, in practice, there are instances when a meta-analysis can be performed without a preliminary systematic review, for example, individual patient data (IPD) meta-analysis, in this case, reviewers retrieve individual patient data for the primary study authors and combine the raw data to get a pooled estimate [11]. The basic steps for conducting a systematic review are framing a focused review question; comprehensive, thorough search and study selection based on eligibility criteria; data extraction and study quality assessment; synthesis of study results; and interpreting the results and reporting [7-9]. The fundamental goal of a systematic review is to summarize the evidence on a specific review question [7-9]. Secondary goals are to assess the study quality, identify heterogeneity in results across included studies, and identify future research directions [7-9].

The strength of systematic reviews lies in its explicit bias limiting methods and its ability to summarize the evidence to identify both best and redundant practices [8]. In other words, one study is not enough to make a clinical or health policy decision, so systematic reviews provide an unbiased review of available evidence to facilitate a sound clinical or health policy decision [13]. Further, systematic review methods typically provide accurate and reliable results by performing a comprehensive review of existing literature [7-9]. Another potential strength of systematic reviews is the transparency in each step, allowing the readers to focus on the merits of each conclusion made in summarizing the evidence rather than a simple comparison of different studies as in other types of reviews [13]. Systematic reviews are also instrumental in identifying gaps in the existing literature [13]. Moreover, a systematic review with a meta-analysis of similar studies conducted with high methodologic rigor, such as randomized control trials (RCT), provides the highest level of evidence in evidence-based decision making [14,15].

Systematic reviews and meta-analyses have a few potential limitations. First, the reliability of results and conclusions provided in a systematic review and meta-analysis depends on the methodologic rigor of the included studies, which is defined by the quality of study design, statistical methodology, and reporting of the primary studies. Thus, conducting a systematic review and meta-analysis will not resolve the problems inherent in the design and implementation of the included primary studies [13]. Second, merging estimates of poor quality studies with high quality ones might not be useful and it might lead to false estimates of the underlying fact or lead to a false precision around the true estimates [16]. Finally, there have been instances where a study labelled as systematic review or meta-analysis was not conducted and reported with methodologic rigor and thus, researchers may use the label loosely [17,18]. A few authors have critically evaluated and described methodologic flaws of systematic reviews published in peer-reviewed journals, and the most common flaws were related to failure to assess study quality and avoid bias in inclusion of studies [19-21]. Systematic reviews may be plagued by publication bias which is an overrepresentation of significant associations due to the tendency to publish only those studies that have significant results [22]. If publication bias is not adequately assessed and addressed, it can lead to inaccurate conclusions in the systematic review [23]. However, systematic reviews with 10 or less primary studies are difficult to assess for publication bias, and it is suggested that a more reasonable count would be more than 10 studies combined using meta-analysis [23]. Also, industry-supported reviews often fail to acknowledge the methodologic limitations of included studies and pool the estimates of less rigorously conducted studies with the more rigorous studies [24]. In the context of the ever-increasing list of published literature, the estimated time to conduct a systematic review is six to eighteen months [25] or in other words it is large and uncertain [26]. Thus, new forms of reviews, such as the scoping review, are emerging to rapidly fulfill the need for information specifically when a rapid overview of the research area is needed and not a detailed answer to a research question [6].

Scoping reviews are a form of knowledge synthesis that are embraced because of their relevance to both emerging and established fields. Before 2009, less than ten scoping reviews were published annually; however, after 2009, there has been a consistent increase in the number of scoping review published annually, such as 85 reviews in 2013 [27]. However, in 2019, the publication rate of scoping reviews has further increased, and it reached almost 80 scoping reviews published per day [28]. In 2005, Arksey and O'Malley defined scoping reviews as, "scoping studies aim to map rapidly the key concepts underpinning a research area and the main sources and type of evidence available, and can be undertaken as standalone projects in their own right, especially where an area is complex or has not been reviewed comprehensively before" [18]. Unlike a systematic review, a scoping review does not analyze data to answer a focused research/review question, and it addresses broader topics where a variety of study designs are included [18,27]. The key steps involved in scoping review evidence synthesis are identifying a research question; systematic searching of literature; study selection based on inclusion and exclusion criteria; charting the data; and collating, summarizing and reporting the results using clearly defined methods based on a priori questions [27,29]. Unlike a systematic review, the scoping review does not assess the quality of included studies [18]. However, it should be highlighted that a scoping review emphasizes comprehensive coverage and not the standard of the evidence on a particular topic of interest [30].

Scoping reviews are undertaken to explore the scope, range, and nature of research activity in an area of interest and mapping the existing research where it is difficult to visualize the range of research activity; determine if a full systematic review is possible; collate and disseminate research findings to policy makers and practitioners; conclude the overall research activity in an area of interest, and identify research gaps [18]. Scoping review methods share similarity with systematic reviews and thus, it is considered systematic, replicable, and transparent [2]. Thus, a key strength of scoping review lies in providing a transparent and rigorous method for exploring and mapping a research area [18]. Further, scoping reviews facilitate the reviewer to understand the extent, and nature of primary research in a short span of time [18]. The scoping review rapidly maps the key concepts of a research area and provides the study findings in an accessible and summarized format to policy makers and practitioners, and thus, they are ideally placed to make efficient use of the review results [18].

It is necessary to acknowledge and understand the limitations of a scoping review, so the findings of a scoping review are interpreted with caution. First, study quality is not assessed in any formal sense, and thus, it can affect the precision with which research gaps are identified and any recommendations are proposed [18,31]. Also, the scoping reviews have the potential for bias [2]. The broad research questions will lead to a considerable amount of data extraction; thus, the reviewers might face the challenge of deciding if the coverage of the breadth of research activity is more important than in-depth coverage of research activity [18]. Second, scoping reviews do not synthesize data and consequently, they provide a descriptive account of the existing literature. Thus, this lack of rigor of the scoping review precludes it from being considered as the final output [2]. Finally, a scoping review often identifies a research gap and proposes recommendations about the research that should be conducted in the future, rather than adding to the body of evidence on a research question.

Use of reviews to address environmental health problems

Initially, systematic review techniques were developed in the field of medicine, social science, psychology, and have served as an essential tool for evidence-based decision making since the 1980s across different disciplines [32]. In medicine, systematic reviews have provided an unbiased synthesis and interpretation of conflicting primary studies leading to better scientific decision making in health care [12]. Like clinical research, systematic reviews play a transformative role in being a robust and transparent method for synthesizing the existing evidence into more meaningful conclusions and recommendations related to environmental health [33].

Systematic reviews have the potential of addressing the shortcomings in the quality of execution and reporting of the methods in evidence synthesis of environmental health research [34]. Systematic reviews not only facilitate evidence-based decision making, but they also channel the resources to identify and address research gaps, thus, preventing waste in environmental research [12,35,36].

Since the 1990s, systematic reviews and meta-analyses in ecology have offered synthesis of scientific evidence to facilitate formulation evidence-based environmental policy [37]. The recent establishment of the Collaboration for Environmental Evidence promotes evidence synthesis on issues related to environmental policy and practice [38]. The application of systematic review methodology in chemical exposure-related environmental health research is recognized widely [12]. The review methodology is now frequently used in assessing the association between chemical exposures and health outcomes through systematic synthesis of the available evidence, and to inform the policy decisions and recommendations [12]. In the case of chemical risk assessment research, systematic review methodology can address complex data synthesis by extrapolating evidence from human studies and animal studies [12]. Additionally, human health data comes from observational studies which are more prone to bias and confounding than RCTs in medical research. Therefore, systematic review methodology can be one potential remedy to synthesizing data from a more heterogeneous database in environmental health research [12].

Contribution of review methods to address environmental health epidemiology

In environmental health research, a systematic review is considered a powerful and critical tool to evaluate and communicate about the health impact of environmental exposures such as chemicals and inform policy decisions [12]. Furthermore, the application of meta-analysis can further increase the accuracy in drawing conclusions from the existing evidence [39]. More recently, systematic review methodology has become more widely used in the environmental health research and has expanded to synthesize research related to climate change and adaptation [40]. A few instances in the past, such as the criticism of Intergovernmental Panel on Climate Change (IPCC) report, necessitated research synthesis that is robust, transparent, and not limited by reviewer bias [41,42]. Similar to medical research, systematic review methodology provides solution to the problems in the field of climate change research like lack of transparent methods for searching, selection and data synthesis, and possibility for reviewer or funder bias [42].

The application of systematic review methodology is well established in the field of clinical research, but its application in environmental health is relatively novel and the methods mostly mirror that of medical research [12,43]. Recently, the systematic review methodology has been adapted both by academic and federal organizations to collate and appraise data related to the effects of environmental exposures on human health [12]. In 2014 at the University of California, scientists developed a *Navigation Guide* to systematically review environmental health related research [43]. Similarly, the US Office of Health Assessment and Translation (OHAT) at the US National Toxicology Program contributed a new framework, known as *OHAT Framework*, for systematically reviewing environmental health research to synthesize evidence for identifying hazards to human health [44]. These two approaches include the basic characteristics of standard systematic review methods to answer questions related to environmental exposures associated with human health, and the risk of bias appraisal methods of these approaches were adapted from the Cochran Collaboration tool for assessing risk of bias of primary studies [12,45]. In addition , these two approaches include a key element of combining animal and human research data to understand the associations between environmental exposures and human health [12].

In environmental health, reviewers are challenged as they often need to collate evidence from different disciplines such as cell-based and bio-chemical research, studies related to laboratory animals, wildlife, and epidemiological and exposure science studies to reach to a meaningful conclusion about a single environmental exposure [46]. Therefore, the initiatives to systematically review environmental exposures are ongoing and a few examples of this initiative are systematic review methods used by European Food Safety Authority [47,48], systematic and rapid synthesis of evidence method by UK Joint Water Evidence Group [49], Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) [50], Evidence-Based Toxicology Collaboration [51], the Collaboration for Environmental Evidence [52,53], and the Systematic Review and Integrated Assessment (SYRINA) to review evidence related to endocrine disrupting chemicals and human health [54].

The systematic review methods provide a unique opportunity by addressing the unmet need to shorten the time between scientific breakthrough and better health outcomes. Environmental exposures affecting human health are ubiquitous and the adverse health outcomes due to these exposures are prevalent and emerging [43]. The transparent and robust methods of systematic review have contributed to making environmental health research actionable [43]. Moreover, the body of environmental health science is voluminous, of variable quality and mostly not familiar to decisionmakers or policymakers [43], but systematic reviews offer to synthesize the often-conflicting evidence into more meaningful recommendations and policy decisions. Also, the use of systematic review methodology may prevent wasteful expenditures on research that are duplicative or unwarranted for decision making in the field of environmental health [43]. Therefore, reviews of scientific evidence in the field of environmental health are considered integral to decision making now and the methods will continue to improve and adapt to more efficient and transparent synthesis of environmental health research.

Current study applications

The above evidence highlights the importance of review methods in addressing issues in environmental health. Thus, the present dissertation explores specific issues in environmental health using systematic review methodology i.e., systematic review and scoping review, conducted when the aim is to rapidly summarize the evidence to get an overview of a particular area of research. The three issues/themes this study explores are related to harmful algal blooms (HAB) due to *Karenia brevis*; a novel insecticide, cycloxaprid (CYC); and Japanese Encephalitis (JE) infection among Asian populations. To our knowledge, the specific environmental health research questions posed in this dissertation have not been examined using systematic review methods. Additionally, we identify specific areas where systematic review methods need improvement for broader applicability in environmental health fields.

Karenia brevis and environmental exposures

Karenia, a toxic dinoflagellate, exists throughout the oceans of the world, and we now know of 12 different species of this dinoflagellate that are present throughout the year even when not in blooming phase [55]. In the North American region, *K. brevis* mostly inhabits the Gulf of Mexico [56]. The unchecked toxic blooms of *K. brevis* are predominantly associated with the Florida red tide/red tide [57]. *K. brevis* produces brevetoxin, a potent neurotoxin [57]. Brevetoxin, released in water, is known to cause morbidity in humans and mortality or morbidity in marine birds, mammals, and other marine organisms [57,58]. Further, brevetoxin can aerosolize through bubble-mediated transport, and this aerosolized neurotoxin can cause respiratory health problems in humans [59,60].

Globally, there has been an increase in the frequency of algal bloom events [61-63], with more dramatic increases in *K. brevis* bloom cell concentrations over the past 50 years [64]. Different physical, chemical, and biological factors that may impact blooms include wind pattern, ocean currents, hydrodynamics, sea surface temperature (SST), climate change, chemical nutrient fluxes, phytoplankton and zooplankton species' trophic interaction, and eutrophication [65-68]. However, our understanding of the global distribution of the *Karenia* species is still not complete because most research was initiated in response to an obvious bloom event [55]. Furthermore, influence of temperature change and chemical nutrients/contaminants on HABs due to *K. brevis* are an active area of ongoing research, as these are most amenable to environmental policy interventions [65]. Thus, for a comprehensive understanding of the associations between temperature and chemical nutrients/contaminants with *K. brevis* blooms and find avenues for future research, it is necessary to summarize the breadth of existing evidence to get an overview of this association. This overview would lead to understanding of recent advancements and research gaps to predict or guide the trajectory of future research. Thus, we used scoping review methodology to rapidly explore the evidence to understand the associations between temperature, chemical nutrients/contaminants, and HAB events due to *K. brevis*, and identify future avenues of research.

Environmental effects of cycloxaprid

Neonicotinoids are the most widely used class of insecticides worldwide owing to their broad range of insecticidal activity [71,72], primarily against insect pests in animal health and crop protection, often by treating plants, soil, and seeds [73]. However, neonicotinoid use is also problematic, e.g., the neonicotinoid imidacloprid (IMI) negatively affects behavior (foraging such as trips per bee, number of workers foraging, predation, parasitism, movement), condition (colony mass, individual mass, mass of cocoons, size of individuals, and overall biomass), abundance (post-treatment counts, seasonal sums, final sampling mean, overall sum, and experimental sum), reproductive success (brood production, fecundity, cocoon production, emergence, egg production), and survival (adult survival rate, longevity, larval survival, and brood survival) of pollinators and other non-target arthropods [74]. Therefore, replacements with similar efficacy but lesser non-target effects are desirable and under development. Recently, cycloxaprid (CYC), a novel neonicotinoid [75], was discovered by researchers at the East China University of Science and Technology [76]. The researchers propose that while its neurotoxicant mechanism of action is similar to IMI, the novel cis-configuration of CYC might involve a differential action [76,77].

Some researchers propose CYC as an appropriate replacement for IMI, e.g., "… cycloxaprid is an alternative to imidacloprid for the management of resistant *A. gossypii*." [78] and "… cycloxaprid is a good alternative for the targeted control of this mirid bug" [79]. However, studies researching CYC efficacy are recent and ongoing, and empirical evidence has not yet been collectively considered. Since CYC is a new insecticide, additional study is needed to determine if it harms natural enemies and other non-targets, if resistance is developed, and if it exhibits cross-resistance with other insecticides [80]. Thus, it is essential to collate and synthesize the present evidence to understand the effect of CYC on target and non-target arthropod species. The current study is, therefore, the first review of target and non-target effects of CYC aimed at synthesizing existing data and informing using systematic review methodology to achieve an in-depth understanding of the environmental effects of CYC on non-target species and reach a conclusion if we have a complete comprehension of the environmental effects of CYC on non-target species.

Japanese Encephalitis (JE) infection and environmental factors

JE, a mosquito-borne single-stranded RNA virus in the genus flavivirus, is a significant public health problem in Asia and many western Pacific countries [81]. The virus originated in Southeast Asia and has evolved from an ancestral virus to a highly mutated form [82]. This virus is mostly transmitted by a night-time biting mosquito, *Culex tritaeniorhynchus.* The virus is transmitted in an enzootic cycle involving water birds (the natural host), domestic pigs, rice paddy-breeding mosquitos and other non-avian vertebrates [83]. Humans are a dead-end host of JE virus due to short duration and the low titer viremia [84]. In endemic countries, JE is mainly a disease of children, as adults have acquired immunity through previous exposure, but individuals of any age may be affected [81]. Most cases of JE are asymptomatic. However, if clinical illness develops, the symptoms range from nonspecific fever to aseptic meningitis that might lead to irreversible neurological sequelae [85]. According to the World Health Organization, more than 3 billion people living within 27 Asia-Pacific countries are at risk of JE infections [86]. Each year, approximately 68,000 new cases are diagnosed with a total morbidity rate of 1.8 per thousand [86,87].

JE virus has two epidemiological patterns, i.e., endemic sporadic cases and outbreaks of JE [86,88]. Asian countries have played a major role in JE virus transmission due to changing agricultural practices such as increased irrigation and animal husbandry, and migratory birds [82]. The emergence of JE involves a complicated interplay of hosts, vectors, climatic variables such as precipitation and humidity, and anthropogenic factors such as proximity to rice fields and animal husbandry [89,90]. Since the identification of the first clinical case of JE, the major exposures are change in land use pattern (i.e., from dry land to wetland crops); change in agricultural practices (i.e., increase in rice paddy cultivation); change in socioeconomic factors and animal husbandry (i.e., promotion of pig breeding and duck farming, park and bird sanctuaries, and bird migration in the region); and change in climate (i.e., increased unpredictability of temperature and rainfall) [91].

A fundamental feature of JE virus transmission is the physical environment. Land cover and land use changes have been driving JE exposure and incidence [92]. Environmentally, the biggest risk factor for JE infection is the proximity to irrigated agricultural lands, especially rice fields. Thus, JE incidence is highest in rice-growing countries. In Asia, paddy field surfaces have increased since 1960 and will continue to expand to meet increased production demands [92]. For decades, studies have examined the epidemiology of JE virus, but it continues to be a poorly understood disease. There are multiple factors like socioeconomic status, environment, and population distribution, that make JE infection a complex phenomenon. Of these, environmental factors are less studied. The most relevant environmental factors are climate change, land use, and land cover [92]. Despite ample research to support that climate change, land use, and land cover are associated with increasing emergence and reemergence of JE infection, these associations are still not completely understood, and no systematic review or meta-analysis has been done exploring the strength of the association between theses exposures and JE incidence. Thus, this study reviews the association between land use, land cover, climate change, and incidence and outbreaks of JE infection among Asian populations.

Purpose and Specific Objectives

The overall purpose of this study was to better understand the benefits and scope of using systematic review methodology to address issues related to environmental health epidemiology, to identify research gaps, and find avenues for future research. More precisely, we reviewed literature related to HAB events due to *K. brevis*, CYC association with different arthropod species, and environmental factors associated with JE infection among Asian populations. These environmental exposures and related outcomes are associated with human health, and there was, therefore, an urgent need to collect the heterogenous data, i.e., human research data, animal/arthropod research data, lab/field study data, to get a comprehensive understanding of the exposures and outcomes in question and identify the gaps in the existing literature to inform future research. The specific objectives of this study were:

Part 1: *Understanding the interrelationships between chemical contaminants, harmful algal blooms, and temperature: A scoping review*. The aim of this review was to investigate the association between chemical contaminants, temperature, and HAB events due to *K. brevis*. The variables of interest were chemical nutrients or contaminants, temperature and frequency and distribution of HAB events. In this study, we considered nutrients such as nitrogen and phosphorus, present above natural or intended/beneficial concentrations as contaminants. Thus, the term nutrient and contaminant were used interchangeably in this review. We intended to rapidly review the existing literature to get an overview of the scientific advancements and status quo of emerging evidence related to the associations between temperature, chemical nutrient/contaminants, and *K.*

brevis bloom. Thus, we selected the scoping review methodology, and the scoping review methodology framework by Arksey and O'Malley [18] was followed for reporting this study. The study protocol was registered and made available at the international database Open Science Framework (OSF) since December 9, 2020 [93].

Part 2: *Known target and nontarget effects of the novel neonicotinoid cycloxaprid to arthropods: A systematic review.* The aim of this study was to review the target and nontarget effects of CYC to understand environmental effects of CYC on different arthropod species. The variables of interest were arthropod test species, CYC concentration, and the endpoint and outcome of CYC on test species. We aimed to find answers to specific questions related to environmental effects of CYC. Here, our objective was to identify and retrieve international evidence, assess the quality of evidence to confirm if the current knowledge is based on relevant evidence, and to draw meaningful conclusions for unbiased decision making about the effects of CYC on non-target insect species. Thus, we used systematic review methodology to conduct this review. Furthermore, Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) methodology and checklist were followed for reporting this systematic review [94]. This study focused on arthropods, so we could not register the study protocol at PROSPERO. A quality assessment was performed to determine if methods reported in each study aligned with international testing standards and good laboratory practices (GLP) [95].

Part 3: *The effect of land use, land cover, and climate change on the incidence and outbreak of Japanese Encephalitis disease among Asian populations: A systematic review*. The aim of this review was to explore and describe existing research to provide a more comprehensive synthesis of the associations between climate change, land use, and land cover on incidence and outbreaks of JE infection among Asian populations. The variables of interest for land use were agricultural lands, paddy/rice fields, cultivation, irrigated land, and land covered for pig farming; for land cover were human habitation, wet land, vegetation cover, grassland, and forest; for climate change were temperature, humidity/relative humidity, and rainfall. Like CYC review, we intended to answer a specific research question related to association between JE and environmental exposures. Our goal was to identify and retrieve evidence, assess the quality of evidence, and to address any uncertainties or conflicting evidence to draw meaningful conclusions for future research. Thus, we used systematic review methodology for this review. We used the PRISMA methodology and checklist for reporting this systematic review [94]. The study protocol was registered with the international database PROSPERO and the registration number is CRD42019122203 [96].

Organization of the Manuscripts

This dissertation comprises three separate reviews that are unrelated studies thus, it is organized as a multi-part manuscript format and a consistent format is used for the three manuscripts. The first part of this dissertation is an introductory chapter, which provides an overview of the background and statement of problem of the study and the purpose and study objectives. Chapters 2, 3, and 4 are the three reviews that describe aims, methods, and results of each review. The last chapter summarizes the major contributions of the three reviews and discusses future research directions related to the specific environmental health issues, and the limitations we identified in systematic review methods in environmental health research.

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Chapter II

Understanding the Interrelationships Between Chemical Contaminants, Harmful Algal Blooms, and Temperature: A Scoping Review

As the first author, I identified the research question, designed search strategy, performed data extraction and wrote the manuscript. Dr. Kristina Weis Kintziger contributed to conducting this review and reviewed and edited the manuscript. Dr. Agricola Odoi, Dr. Jiangang Chen, and Dr. Evah Wangui Odoi critically reviewed the research question and search strategy and revised and edited the manuscript.

Abstract

Karenia brevis, a toxic dinoflagellate, mostly inhabits the Gulf of Mexico. Unchecked population growth of *K. brevis* results in harmful algal blooms and are predominantly associated with red tide. *K. brevis* produces a potent neurotoxin called brevetoxin. Brevetoxin, released in water, is known to cause morbidity in humans and mortality or morbidity in marine birds, mammals, and other marine organisms. Different physical, chemical, and biological factors contribute to *K. brevis* blooms, and the influences of temperature changes and chemical nutrients/contaminants on *K. brevis* blooms is an active area of research. The purpose of this review was to summarize existing literature on the association between temperature and chemical nutrients/contaminants, and the frequency and distribution of *K. brevis* blooms, and to identify avenues for further research. The scoping review methodology framework by Arksey and O'Malley was followed for reporting this study. The study protocol was registered and available on Open Science Framework. Web of Science, Academic Search Complete, and PubMed databases were searched for English-language articles published between 2000 and 2021. The keyword search in the databases yielded 2276 citations. After title/abstract and full text screening, eight studies were included in the review. We found that temperature above 30°C inhibited the growth of *K. brevis* bloom, both in the environment and under laboratory conditions, and nitrogen was crucial in persistent growth and maintenance of *K. brevis* bloom. However, field studies suggested no association between phosphorus and *K. brevis* bloom abundance. We recommend increasing the availability and accessibility of contaminant data in aquatic systems as well as enhancing contaminant data coverage in the aquatic systems to accommodate the suggestive shift in the distribution of *K. brevis* bloom in North American water. These steps will result in better understanding of the environmental factors driving red tide blooms.

Keywords

Karenia brevis, harmful algal bloom, red tide, nutrients, contaminants, temperature.

Introduction

Karenia, a toxic dinoflagellate, exists throughout the oceans of the world, and we now know of 12 different species of this dinoflagellate that are present throughout the year even when not in blooming phase [1]. However, our understanding of the global distribution of these *Karenia* species is still not complete because most research was initiated in response to an obvious bloom event [1]. Of the 12 different species of *Karenia*, *Karenia brevis*, formerly known as *Gymnodinium breve* and *Ptychodiscus brevis*, is known to have the largest impact on human health [1]. In the North American region, it mostly inhabits the Gulf of Mexico [2]. The unchecked toxic blooms of *K. brevis* are predominantly associated with the Florida red tide/red tide [3]. These harmful blooms of *K. brevis* are most frequent on the west coast of Florida, between Tampa and Sanibel Island [4]. Recent evidence suggests that the Gulf Stream transports these blooms out of the Gulf of Mexico, and the blooms can extend up the east coast of the U.S. as far as North Carolina [1-3].

Red tide occurs regularly in the Gulf of Mexico mostly during the fall season [1]. The blooms may occur as small, low abundance ($\leq 10^5$ cells/L), short-lived patches or as large, high cell abundance blooms ($>10^6$ cells/L) spanning thousands of square miles [5,6]. Globally, there has been an increase in the frequency of algal bloom event [7-9], with more dramatic increases in *K. brevis* bloom cell concentration over the past 50 years [4].

K. brevis produces brevetoxin, a potent neurotoxin [3]. Brevetoxin is a tasteless, odorless, and heat-stable lipid-soluble cyclic polyether compound [1]. *K. brevis* produces two primary brevetoxin compounds, i.e., PbTx-1 and PbTx-2 [10,11]. PbTx-2 is present in higher concentration than PbTx-1, but PbTx-1 is more toxic [12,13]. Brevetoxin, released in water, is known to cause morbidity in humans and mortality or morbidity in marine birds, mammals, and other marine organisms [3,14]. Further, brevetoxin can aerosolize through bubble-mediated transport and this aerosolized neurotoxin can cause respiratory health problems in humans [15,16].

The increase in frequency and magnitude of *K. brevis* algal blooms has increased research efforts geared towards understanding the factors and their complex interactions that are responsible for these increases. Different physical, chemical, and biological factors that may impact blooms include wind pattern, ocean currents, hydrodynamics, sea surface temperature (SST), climate change, chemical nutrient/contaminant fluxes, phytoplankton and zooplankton species' trophic interaction, and eutrophication [17-20]. Of these, influence of temperature change and chemical nutrients/contaminants on HABs due to *K. brevis* are an active area of research, as these are most amenable to environmental policy interventions [17].

An increase in agricultural activities introduces chemical nutrients/contaminants into the water bodies and alters the biodiversity of aquatic ecosystems [21]. Nitrogen-based fertilizers are widely used to increase agricultural productivity, and this non-point source of nitrogen was found to be the predominant source of overall nitrogen in the Gulf of Mexico [22,23]. Additionally, changing weather conditions, specifically global mean temperature, might cause either extreme precipitation or drought [24]. Extreme precipitation will lead to a decrease in salinity and concentration of chemical nutrients/contaminants, and present evidence suggests that such conditions are not ideal for *K. brevis* bloom growth [25,26]. On the contrary, a drought will increase the salinity and concentration of chemical nutrients/contaminants, and these conditions are known to favor the growth of *K. brevis* blooms [26]. Based on the above arguments, we understand that temperature and level of chemical nutrients/contaminants in water bodies are important determinants of prevalence and abundance of red tide blooms, and thus these should be important variables in monitoring the distribution and frequency of red tide blooms. However, no review has been conducted to explore and understand the importance of this association within the North American region. Therefore, we conducted a scoping review to summarize existing information on the association between temperature, chemical contaminants, and frequency and distribution of *K. brevis* blooms within the North American coastal region.

Methods

The scoping review methodology framework by Arksey and O'Malley [27] was followed for reporting this study. The study protocol was registered and made available at the international database Open Science Framework (OSF) since December 9, 2020 [28]. We selected the scoping review methodology to rapidly review the existing literature on the associations between temperature, chemical contaminants, and harmful algal blooms (HABs) due to *K. brevis* to get an overview of the existing scientific knowledge base and identify future research avenues.

Identification of research question

We did a quick initial review of the literature on HABs due to *K. brevis*, and the following research question was identified: What are the associations between temperature change, chemical contaminants, and the frequency and distribution of HAB events related to *K. brevis*? In this study, we focused on the HABs due to *K. brevis* in the North American region. We considered nutrients, such as nitrogen and phosphorus, present in the aquatic environment above natural or intended concentrations as contaminants. Thus, the term nutrient and contaminant were used interchangeably in this review. Table 2.1 summarizes the PICO/PECO approach to frame the research question for this study [29]. To avoid duplication, we searched the OSF database to

Table 2.1. Systematic search concepts and keywords

Concept	**Keywords**
Problem (P): HABs in Florida/Gulf of Mexico	*"harmful algal bloom" OR HAB OR "Florida red tide" OR "Karenia brevis" OR Karenia OR "Gymnodinium breve" OR "Ptychodiscus brevis" OR brevetoxin* OR phycotoxin* OR "red tide" OR "K. brevis"*
Exposure (E): Chemical contaminants and temperature	*chemical* OR "chemical contaminant" OR pesticide* OR insecticide* OR fertilizer* OR pollut* OR toxicant* OR xenobiotic OR "global heating" OR "global warming" OR "warming" OR climat* OR meteorolog* OR weather OR temperature* OR “air temperature” OR “sea surface temperature” OR “ambient temperature”*
Outcome(O): frequency, distribution and other characteristics of HABs in Florida	*frequency OR distribut* OR density OR prevalence OR recurrence* OR regularity OR abundance OR intensit**
Databases • Web of Science • Academic Search Complete • PubMed	Final search query = *(((((((((((("harmful algal bloom") OR (HAB)) OR ("Florida red tide")) OR ("Karenia brevis")) OR (Karenia)) OR ("Gymnodinium breve")) OR ("Ptychodiscus brevis")) OR (brevetoxin*)) OR (phycotoxin*)) OR ("red tide")) OR ("K. brevis")) AND ((((((((((((((((((chemical*) OR ("chemical contaminant")) OR (pesticide*)) OR (insecticide*)) OR (fertilizer*)) OR (pollut*)) OR (toxicant*)) OR (xenobiotic)) OR ("global heating")) OR ("global warming")) OR ("warming")) OR (climat*)) OR (meteorolog*)) OR (weather)) OR (temperature*)) OR ("air temperature")) OR ("sea surface temperature")) OR ("ambient temperature"))) AND ((((((((frequency) OR (distribut*)) OR (density)) OR (prevalence)) OR (recurrence*)) OR (regularity)) OR (abundance)) OR (intensit*))*
Limits 2000 - 2021; English	

**PICO/PECO approach of framing a research question. However, we did not include a comparison/control (C) group in this study. Abbreviations: HAB = harmful algal bloom* ensure that this research question and scoping review was unique.

Identification of relevant studies

Web of Science, Academic Search Complete, and PubMed databases were searched for English-language articles published between 2000 and 2021 using the keywords listed in Table 2.1. Based on the objective of this scoping review, we included the following types of studies: 1) studies related to the HAB events caused by *K. brevis/G. breve/P. brevis* only; 2) field and laboratory studies; 3) experimental and observational studies; 4) studies exploring the relationships between temperature, chemical contaminants, and HAB from an environmental health perspective; 5) all peer-reviewed published studies; 6) all primary research studies and systematic reviews; 7) studies published between 2000 and 2021 to include only those studies which focused on the recent scientific advances in the field of climate change and harmful algal bloom; and 8) studies focusing on the North American region. The search terms included Florida red tide, but the search was not limited to Florida.

We excluded the following types of studies: 1) narrative reviews which do not follow explicit systematic review methodology; 2) gray literature such as blogs, conference papers, or dissertations; 3) studies focusing on the microbiological and genetic aspect of HABs; 4) studies related to HABs not caused by *K. brevis/G. breve/P. brevis*; and 5) studies focusing on the impact of *K. brevis* bloom on human health only. The search strategy is reported in Table 2.1.

Study selection

We used Microsoft Excel 2016 (Microsoft Corporation; Redmond, WA) to import all the identified studies from three databases in one spreadsheet. Next, duplicate studies were removed. Screening included three steps: title, abstract, and full-text screening. The inclusion/exclusion criteria defined above were applied at each step. The decision about each study was reported in the Excel spreadsheet, along with remarks for excluding a study. The stepwise selection of studies is summarized in Figure 2.1

Charting of data

One investigator reviewed the articles and entered and summarized data in a Microsoft Excel 2016 (Microsoft Corporation; Redmond, WA). Data were organized by title, author and year, journal, study location, study period, types of exposure, statistical analysis, *K. brevis* cell count and/or *K. brevis* bloom events, and important results.

Summarizing and reporting of results

Unlike a systematic review, a scoping review neither synthesizes evidence nor assesses the quality of the evidence, but it provides a narrative overview of the existing literature [27]. In the present review, after charting the data collected from the included studies, we provided a narrative account of the exposures of interest, i.e., temperature and chemical contaminant and outcome, i.e., frequency and distribution of *K. brevis* bloom.

Results

The keyword search in the databases yielded 2276 citations. The following exclusions were made at each step of screening: 631 duplicate records and 1429 titles and 150 abstracts that did not

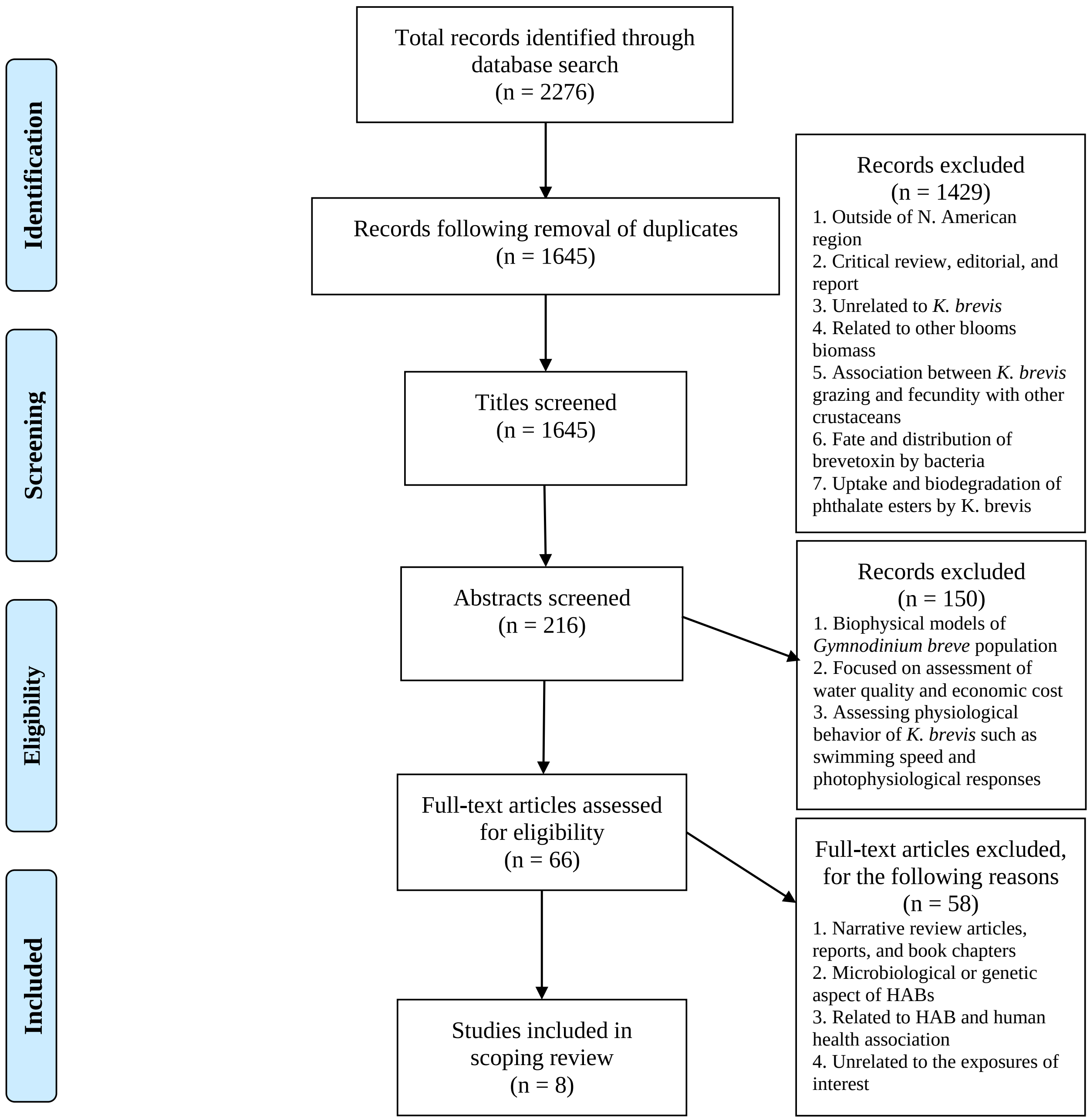

Figure 2.1 PRISMA study selection flow chart and reasons for exclusion.

meet the inclusion criteria. At the final screening step, 66 full-text articles were assessed for eligibility, and eight studies were included in the review. Reasons for exclusion at full-text review are shown in Figure 2.1.

A summary of the included studies is provided in Table 2.2. The included studies were published between 2006 and 2020 and were conducted either in the state of Florida (n = 5) or

Texas (n = 2). One study was conducted both in Florida and Texas (n = 1). The included studies were a mix of laboratory and field study designs. The focus of most of these studies was to assess trends and frequency of *K. brevis* bloom event, understand the associations between environmental factors and *K. brevis* bloom, and mapping and forecasting of *K. brevis* bloom events. The study period of included citations ranged from 1955 to 2018. Additionally, some of the studies used proxy measures such as chlorophyll-a and turbidity index, i.e., a measure for clarity of water through scattering of light caused by suspended particles, to determine phytoplankton blooms and biomass [21,30].

The studies included a variety of exposures. Temperature-related exposures in the included studies were sea surface temperature (SST), water temperature, and average daily air temperature. Chemical nutrient/contaminant-related exposures included colored dissolved organic matter, total organic carbon, nutrients [ammonia-nitrogen (NH_3-N) nitrate + nitrite nitrogen (NO_2+NO_3), total organic and inorganic nitrogen, total Kjeldahl nitrogen, total nitrogen, orthophosphate, and total phosphorous, and total nitrogen to total phosphorus ratios], and salinity. Of the eight studies, six studies included both chemical nutrients/contaminants and temperature as the variables of interest [21,26,30-33] (Table 2.2), and the remaining two included chemical nutrients/contaminants only [4,34]. Here, we provide an overview of the associations between *K. brevis* bloom, chemical

Table 2.2. Summary of included studies, types of exposure, and important results

Author	Geographic location	Study period	Types of exposure	Statistical analyses	*K. brevis* cell count & number of bloom events	Important results
Harris et al. 2020	Southeast Florida	Oct 2018 - Nov 2018	Water quality: CDOM, TOC, nutrients/contaminants: NH3-N, NO2+NO3, Org. N, TKN, TN, OP, and TP, TN:TP, and water temperature	Permutation ANOVA, Stepwise distance-based linear models using corrected AICc	In marine water: 10,000 to 1,000,000 cells/L & in river water: 1000 to 1,000,000 cells/L	Significant positive association between multivariate measure of chlorophyll and *K. brevis* abundance during bloom condition
Medina et al. 2020	Eastern Gulf of Mexico, and Central and Southern Florida	Dec 2012 - Feb 2018	NOx, TP, and Gulf SST	NLTS analysis	9356 bloom events	*K. brevis* bloom exhibit temporal regularity, and the blooms were systematically affected by NOx concentration.
Tominack et al. 2020	South Texas coast	1955-2016	Average daily air temperature, and salinity	ANOVA, linear and logistic regression	5,000 to 100,000 cells/L	Increase in red tides on Texas coast in past 60 years Significant negative association with temperature and positive association with salinity
Karki et al. 2018	Charlotte County, Florida	2010 - 2017	SST, and chromophobic dissolved organic matter	MLR modelling, and GIS framework	>300 cells/L and 128 bloom events	Significant positive association between Chl-a, and turbidity index with *K. brevis* bloom event. Significant negative association between SST and *K. brevis* bloom event was found.
Dixon et al. 2014	North of Tampa Bay to south of the mouth of Caloosahatchee River, Florida	2007 - 2010	NH_4^+, NO_{2+3}-, PO_4^{3-}, and Si $(OH)_4$), Urea-N, DTN, DTP, and PC, PN, an PP, DON and DIN.	Spearman *rho*, Kruskal-Wallis ANOVA, and Principal Component Analysis	<10,000 to 2,10,000 cells/L	Significant correlation of *K. brevis* with DON and salinity, but inverse correlation of *K. brevis* with DIN:Si

Table 2.2. Continued

Author	Geographic location	Study period	Types of exposure	Statistical analyses	*K. brevis* cell count & number of bloom events	Important results
Lekan et al. 2010	Florida and Texas (laboratory study)	Florida clone from 1953 and Texas clone dated 1999	Water salinity and water temperature	Heteroscedastic and non-parametric tests, and logarithmic transformations	1000 cell/L to 100,000 cells/L	Comparing data from 1954-1963 and 1994-2002, *K. brevis* bloom abundance has by increased 13 to 18 times
Brand et al. 2007	Southwest Florida coast	Jan 1954-May 2002	Nutrients/contaminants in water	Two-tailed heteroscedastic t-test and logarithmically transformations, Mann-Whitney nonparametric test, and Chi-square test	Between 1954 to 1963: 2.5 million cells/L; between 1994 to 2002: 34.7 million cells/L	Significant increase in the cell density in more recent years i.e., 1994-2002 as compared to 1954-1963 The research suggested that increased nutrient-rich land runoff from human activities in South Florida were the most plausible explanation for additional nutrients and resulting, increased bloom density.
Magana et al. 2006	Texas coast (laboratory study)	2006	Water salinity and water temperature	Non-linear least-squares regression		Adequate growth of *K. brevis* between a salinity range of 25-40 and at temperature range of 20-25°C.

Abbreviations: ANOVA = Analysis of variance, AICc = Akaike information criterion, CDOM = Colored dissolved organic matter, Chl-a = Chlorophyll-a, DTN = Dissolved total nitrogen, DTP = Dissolved total phosphorus, DON = Dissolved organic nitrogen, DIN = Dissolved inorganic nitrogen, GIS = Geographical Information System, MLR = Multivariate linear regression, NH_4^+ *= Dissolved ammonium,* NO_{2+3}*/ NOx = Nitrate+nitrite, NLTS = Non-linear time series,* NH_3*-N = Ammonia-nitrogen,* NO_2+NO_3 *= Nitrate+nitrite nitrogen, Org. N = organic nitrogen, OP = orthophosphate,* PO_4^{3-} *= Reactive phosphate, PC = Particulate carbon, PN = Particulate nitrogen, PP = Particulate phosphorus, SST = sea surface temperature,* $Si(OH)_4$ *= Silica species, TKN = Total Kjeldahl nitrogen, TN = Total nitrogen, TOC = total organic carbon, TP = Total phosphorous* nutrients/contaminants, and temperature (air temperature/SST/water temperature).

Association between chemical nutrients/contaminants, temperature, and K. brevis blooms: laboratory studies

Magaña et al. [31] conducted a laboratory study to investigate the effect of environmental factors, i.e., salinity and water temperature, on the growth of a Texas clone of *K. brevis*, labelled as SP3. The authors measured salinity using Ficher Scientific refractrometer calibrated with a seawater salinity standard of 35, and *K. brevis* cell growth rates (division/day or div/day) were calculated as relation between cell counts and time [35]. The maximum growth rate at all water temperature/salinity combinations was 0.36 div/day at salinities of 30 and 35 at 20 and 25°C, respectively [31]. Additionally, a rapid death of *K. brevis* cells was reported at water temperatures above 30°C, and the upper limit of salinity tolerance was 40-45. Further, the researchers found that *K. brevis* cells did not perefer higher salinities (≥45) but it tolerated salinity better between 20-25°C ranges. This might explain the lack of blooms in Launga Madre, a hypersaline lagoon in Texas, during summer when water temperature exceeds 30°C [31]. This study revealed similarity in the range of environmental conditions, such as water temperatre tolerance, for both Texas and Florida clones of *K. brevis.*

Similarly, Lekan et al. [33] conducted a laboratory study to investigate the growth rate of three separate clones of *K. brevis* form Florida (Wilson clone) and Texas (SP3 S-tox and SP3 N-tox) at different salanities and water temperatures. This study reported *K. brevis* growth rates as cell counts each day. Overall, the study reported a minimal growth below a salinity of 25 that rarely exceeded 0.16 day^{-1}. The investigators observed the highest growth rate of ≥0.25 day^{-1} exclusively for Texas clones (SP3), and Wilson clone had low growth rates of <0.18 day^{-1} [33].

Particularly, SP3 N-tox reported the highest growth of 0.29 day^{-1} in N-limited condition at a alinity of 30 and temperature of 25°C [33]. The growth patterns of SP3 and Wilson clone were similar to the growth patterns reported by Magana et al. [31], i.e., Wilson clone had higher growth rates 0.30 day^{-1} at 20°C and salinity of 30, growth rate of 0.36 day^{-1} at salainity of 30, and growth rate of 0.32 day^{-1} at a salinity of 35 [31].

Association between chemical contaminants/contaminants, temperature, and K. brevis blooms: field studies

Medina et al. [32] performed preliminary diagnostics on anthropogenic drivers of nearshore blooms of *K. brevis* using nonlinear time series analysis. In this study, *K. brevis* data were aggregated to a semimonthly time series by computing mean of all observations for each two-week period [32]. The study was conducted in the eastern Gulf of Mexico and southern and central Florida [32]. These anthropogenic drivers consisted of chemical nutrients/contaminants, and the other predictor was SST [32]. The investigators concluded that anthropogenic drivers explained >50% of total variance in *K. brevis* bloom density. Chemical nutrients/contaminants and SST were together associated with the temporal patterns of *K. brevis* log-density dynamics. *K. brevis* blooms exhibited an irregular periodicity with maxima between October and January, and smaller maxima between April and May of 2014 and 2015 [32]. Further, the authors applied cross-convergent mapping to test causality and concluded that phosphorus was not a driver of *K. brevis* bloom, but terrestrially sourced nitrogen played an essential and persistent role in growth and maintenance of *K. brevis* blooms except in the upstream basin [32]. This study suggested that a strong temporal pattern of blooms was nitrogen limited.

Tominack et al. (2020) found a statististically significant increase in the red tide events for the Texas coast for the environmental parameters studied such as average daily air temperature (used as a proxy for water temperature), salinity, wind speed, precipitation, and monthly indices for El Niño–Southern Oscillation (ENSO). Salinity was positively associated with red tide event [$\beta = 0.54$, 95% confidence interval (CI) = 0.17, 1.14, odds ratio (OR) = 1.72], indicating odds of red tide event for higher salinity level were 1.72 times the odds of red tide events for lower salinity level [26]. The authors further studied the association beween average daily air temperature and presence or absence of *K. brevis* red tide event. Three logistic regression models were chosen based on the stage of red tide event which were: 14 days before red tide (B), during red tide event presence (D), and 14 days after red tide event (A). Model with all stages of red tide event, i.e., A, B, D, temperature was negatively associated ($\beta = -0.12$, 95% CI = -0.19, -0.06, OR = 0.88) with *K. brevis* red tide event. Model with stages B and D only, temperature was negatively associated ($\beta = -0.52$, 95% CI = -0.74, -0.34, OR = 0.74, and Nagelkerke pseudo-$R^2 = 0.75$). This study concluded that red tide significantly increased on the Texas coast between 1955 and 2016, and a positive ENSO, i.e., a phase of increased rainfall and warming SST, was not favorable for *K. brevis* bloom due to decreased salinity [26].

Karki et al. [21] performed linear regression analyses to identify the factors controlling *K. brevis* bloom events, utilizing data on the days leading up to when the blooms were reported, i.e., same day and one, two, and three days in advance and their relative significant association were reported in percentages. Thus, the authors chose three models that were based on same-day, one-, two-, and three-day predictions. For all four models, SST and *K. brevis* events were negatively associated ($\beta_{\text{same day}} = -0.0188$ (0.8%), $\beta_{\text{one day}} = -0.2164$ (0.2%), $\beta_{\text{two day}} = -0.1514$ (0.7%), $\beta_{\text{three day}}$

= -0.2002 (1.3%)). However, chlorophyll-a ($\beta_{same\ day}$ = 0.512 (3.4%) , $\beta_{one\ day}$ = 0.542 (7.6%), $\beta_{two\ day}$ = 0.901 (5.4%), $\beta_{three\ day}$ = 1.087 (7%)) and turbidity index ($\beta_{same\ day}$ = 73.093 (2.4%), $\beta_{one\ day}$ = 143.033 (1.9%), $\beta_{two\ day}$ = 135.869 (2.4%), $\beta_{three\ day}$ = 202.167 (2.8%)) were positively associated with *K. brevis* bloom events. The study reported same day nowcasting of *K. brevis* events acuracy of 90%. Similarly, one, two and three days advance forecasting *K. brevis* events had accuracies of 65%, 72% and 71%, respectively [21].

Harris et al. [30] studied *K. brevis* blooms in the estuarine systems of Loxahatchee River Estuary (LRE). The researchers did not statistically test the associations between *K. brevis* bloom and nutrients/contaminants, but they explored the variation in the concentration of nutrients/contaminants and water temperature berfore, during, and after bloom events by assessing the water quality of LRE [30]. During *K. brevis* bloom event on the east coast of Florida (450,000 cell count/L), Harris et al. [30] found positive relationships between measures of chlorophyll and *K. brevis* cell abundance, i.e., on average, the concentrations of chlorophyll-a (37%), chlorophyll-c (23%), and carotenoid (63%) were high during blooms. However, during non-bloom conditions, this association between *K. brevis* abundance and chlorophyll disappears [30]. The investigators hypothesized that terrestrial-derived nutrients/contaminants circulating through estuaries might play a vital role in promoting and sustaining algal blooms and increase the average duration of algal blooms. Regardless of sufficient nutrient/contaminant availability and water quality conditions, the *K. brevis* bloom was short-lived, which suggested that dilution of nutrients/contaminants due to tidal exchange, rather than lack of nutrients/contaminants, caused early dissipation of the *K. brevis* blooms [30]. Furthermore, this study used the data from September, October, and November 2018 to understand water temperature changes before, during, and after bloom events. The average water temperature decreased from 29 to 26°C from September to November 2018. This water temperature range coincides with the water temperature needed to support optimum growth of *K. brevis* bloom, i.e., 22-28°C [36].

Association between chemical nutrients/contaminants and K. brevis blooms: field studies

Dixon et al. [34] performed a large-scale investigation to identify associations between annual blooms of *K. brevis* and dissolved nutrients/contaminants and phytoplankton communities in estuaries and coastal waters of the West Florida Shelf. During the study period, investigators observed a *K. brevis* event when the salinity was 37-37.2 in 2007, 35.7-36.1 in 2009, and found a positive correlation between *K. brevis* cell concentration and salinity overall (Spearman's *rho* (Sr) $p = 0.002$). Further, *Karenia* cell counts were found to be significantly correlated with several nutrients/contaminants, such as (i) with decreased dissolved inorganic nitrogen (DIN) ($p = 0.002$), dissolved organic phosphate (DOP) ($p = 0.010$), and dissolved inorganic nitrogen (DIN): dissolved inorganic phosphorus (DIP) ($p = 0.011$), DIN: dissolved silica (DSi) ($p<0.001$), (ii) with increased dissolved orthosilicic acid ($DSi(OH_4)$) ($p<0.001$), dissolved organic nitrogen (DON) ($p = 0.024$), and DON:DOP ($p<0.001$). However, investigators could not find a direct association between algal bloom event or severity of bloom and the terrestrial contribution of nutrients/contaminants [34]. Also, the association between *K. brevis* event and phosphorus were inconsistent during the study period and no conclusion could be drawn about this association [34].

Brand et al. [4] studied the possible causes of increase in *K. brevis* bloom cell concentrations during two time periods, i.e., 1954-1963 and 1994-2002. The possible causes considered were sampling bias, increase in nutrients/contaminants from land runoff, temperature/seasonality, and increase in iron-stimulated nitrogen fixation. The investigators concluded that (i) increase in water temperature caused a shift in the water ecosystem leading to increase in *K. brevis* blooms; (ii) the 13-18 fold increase in *K. brevis* abundance between 1954-1963 (36,000 cells/L) and 1994-2002 (474,200 cells/L) implied an increase in the nutrient/contaminant availability, as no known natural source of nutrients/contaminants increased to 13-18 fold during the study period [4]; and (iii) in the past 50 years, increased nutrient-rich land runoff from human activities, such as increase in human population, in South Florida was the most possible source of additional nutrients/contaminants.

Strengths and limitations of included studies

In this part, we summarize the strengths and limitations of each study. The studies by Magana et al. [31] and Lekan et al. [33] were conducted as experimental studies. Thus, the researchers were able to precisely control the nutrients/contaminants and water temperature to study *K. brevis* cell growth under different conditions, simulating field environmental conditions to draw conclusions about the factors that favor the *K. brevis* cell growth. However, the limitation of this study was that the physiology of the *K. brevis* clones may vary between field and laboratory studies [33]. Thus, caution should be practiced when interpreting the results of laboratory studies along with studies conducted under field environmental conditions. In the field study by Medina et al. [32], the authors applied advanced statistical methods, such as non-linear time series and convergent cross-mapping methods, to provide evidence supporting the association between anthropogenic nitrogen inputs and nearshore *K. brevis* blooms. However, this study had data limitations related to low spatial resolution of water quality data and limited most recent continuous period of record. Also, another potential limitation was related to water sampling, i.e., sampling protocols and devices varied due to a variety of sample collectors [32]. The major strength of Tominack et al. [26] study was the use of non-traditional data sources as it permitted the quantitative analysis of bloom trends during data poor periods, and it also facilitated in identifying weather and local environmental factors associated with HABs due to *K. brevis*. The potential limitation of this study was the use of average daily air temperature as a proxy for water temperature since the linear relationship between air and water temperature was not substantially strong at air temperature <15°C.

Karki et al. [21] used both satellite data, i.e., the National Aeronautics and Space Administration (NASA) MODIS Aqua satellite data, and corresponding field data to understand the factors associated with *K. brevis* bloom events. However, the study methodology had an important potential limitation due to the assumption that algal blooms are stationary and lie on the surface. This assumption might have introduced discrepancies, as in real conditions, a time lag is expected between reporting of bloom event and the capture the of image by the satellite [21]. Dixon et al. [34] for the first time used a large data set and conducted multivariate statistical analysis to understand the association between *K. brevis bloom* and nutrients/contaminants in the West Florida Shelf coastal waters. A potential bias in the study could be due to the samples being collected only during the October months of 2007, 2008, and 2009.

The major strength of Brand et al. [4] study was in analyzing historical bloom data, i.e., from 1954-2002, and provide an overview of the increasing trend of *K. brevis* bloom events, and potential key elements associated with *K. brevis* blooms. However, study conclusions were limited due to the biased sampling of bloom events, since most of the sample were collected during a bloom event which could potentially overestimate the *K. brevis* cell count during the study period. Finally, the Harris et al. [30] study was the first study to address *K. brevis* cell encroachment into the inshore LRE segments and provided a unique opportunity to understand *K. brevis* bloom dynamics within estuarine systems. However, this study did not test the association of nutrients/contaminants with *K. brevis* bloom, but evaluated the change in water quality data before, during, and after *K. brevis* bloom events. The change in water quality was measured as any substantial changes in nutrient/contaminant concentrations and water temperature. Thus, we cannot infer any causal associations between the water quality parameters and *K. brevis* cell growth.

Discussion and Conclusions

This scoping review examined the existing literature to obtain a better understanding of the interrelationships between chemical nutrients/contaminants, temperature, and frequency and distribution of *K. brevis* blooms along the North American coast. This scoping review is unique in summarizing associations between chemical nutrients/contaminants, temperature, and the frequency and distribution of *K. brevis* blooms that are specific to the North American region. The studies showed that temperature above 30°C negatively affected *K. brevis* bloom both in the field/natural and under laboratory conditions, and nitrogen was crucial in persistent growth and maintenance of *K. brevis* bloom. However, the included studies agreed that phosphorus was not associated with *K. brevis* bloom abundance [30,32]. Nevertheless, we should acknowledge that the understanding of this association could be limited by the nature of the studies included in this review. Additionally, nutrient/contaminant dilution was suggested as one of the factors limiting growth of *K. brevis* blooms. Further, we emphasize that more research is needed to understand the association between *K. brevis* blooms and temperature with changes in environmental nutrients/contaminants such as nitrogen or phosphorus.

This review suggested that the *K. brevis* bloom events had increased enormously over the past 50 years, and 1955 was the year of change point from less to more recurring events of red tide. Brand et al. [4] emphasized that the enormous growth of *K. brevis* blooms in the past 50 years cannot not be attributed to naturally present nutrients/contaminants but can only be explained by the chemical nutrients/contaminants introduced into the water bodies due to human activities. However, none of the studies specifically described the human activities which had increased the availability of nutrients/contaminants in water bodies. Data from a laboratory study showed that the Texas and Florida clones of *K. brevis* had similar environmental characteristics, such as water temperature and salinity, affecting growth of *K. brevis* blooms [31].

Based on the existing evidence of anthropogenic-induced climate change, we can hypothesize that changes in weather conditions will increase summer-fall temperatures in the future, which may delay the initiation of red tides, whereas increases in winter temperatures will delay the dissipation of red tide blooms [26]. Also, anthropogenic activities will continue to affect the nutrient/contaminant concentration in aquatic systems, and consequently, affect *K. brevis* cell growth. Thus, the future trends predict that there will be an overall shift in the red tide bloom distribution and abundance toward more abundant and prolonged duration of blooms. Consequently, researchers will need new strategies to increase the availability and accessibility of nutrients/contaminants data in aquatic systems, as well as enhancing nutrient/contaminant data coverage in the aquatic systems to accommodate the suggestive shift in the distribution of *K. brevis* bloom in the North American water. These steps will result in better understanding of the environmental factors driving red tide blooms. A few included studies used proxies, such as turbidity indices to account for the nutrient/contaminant content in the aquatic systems. However, statistical models would have improved predictions if data on daily nitrogen and phosphorus compounds were available [21]. Thus, we recommend improving data quality and availability of nutrients/contaminants in aquatic systems, which could lead to major advancements in HAB research. Also, Tominack et al. [26] highlighted the challenges in understanding the long-term trends in bloom of interest due to limited data related to bloom events and duration and associated key environmental factors driving bloom growth. This further emphasizes the need to improve data quality and availability of the HAB of interest.

This scoping review had a few limitations. First, we only searched the published literature, so this might cause an overrepresentation of significant findings. Second, due to lack of translation services, we restricted our search to studies published in English language only. Another limitation of this review was scarcity of studies examining the associations between chemical contaminants, temperature, and *K. brevis* blooms. However, we included all studies meeting inclusion criteria, demonstrating the need for further research in this area.

We chose to do a scoping review rather than a systematic review or meta-analysis, as scoping reviews are a relatively quick method of extracting information [27] to get an overview of the scientific advancements and status quo of emerging evidence and were best suited to the purpose of our research question: to understand the current scientific evidence base of *K. brevis bloom* research with respect to the environmental factors of interest, and it provided the authors with a better understanding of existing/available chemical nutrient/contaminant databases for use in future studies.

In conclusion, current empirical evidence was suggestive of associations between temperature, chemical nutrients/contaminants, and *K. brevis* blooms and an increasing trend in the red tide bloom in the North American region. However, better quality and availability of data on daily nutrients/contaminants and HAB events would allow for better understanding of the environmental factors driving red tide bloom events and would also accommodate the uncertainty related to future climatic conditions and anthropogenic sources of nutrients/chemical contaminants which will affect our ability to predict and respond to *K. brevis* blooms. Thus, there remains an urgent need to better measure and understand the environmental factors driving red tide events.

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Chapter III

Known Target and Non-target Effects of the Novel Neonicotinoid Cycloxaprid to Arthropods: A Systematic Review

A version of this chapter was originally published by Anisha Singh and Christy Leppanen:

Singh, A., Leppanen, C. Known Target and Nontarget Effects of the Novel Neonicotinoid Cycloxaprid to Arthropods: A Systematic Review. Integr Environ Assess Manag 2020, 16, 831-840, doi:10.1002/ieam.4305.

As the first author, I identified the research question, designed search strategy, performed data extraction, and wrote the manuscript. Dr. Christy Leppanen contributed to conducting this review, and critically reviewed and edited the manuscript.

Abstract

Neonicotinoids are the most widely used insecticide class worldwide, and unfortunately, the widely used neonicotinoid imidacloprid is problematic for pollinators and other non-target organisms. These non-target impacts and the development of resistance prompt the ongoing development and testing of new neonicotinoids. The novel neonicotinoid cycloxaprid was described in 2011 and registered in China in 2015. Studies investigating its use and effect on target and non-target species are recent and ongoing, and empirical evidence has not yet been collectively considered. Therefore, a systematic review was performed to identify and summarize data associated with target and non-target, lethal and sublethal impacts of cycloxaprid for its use as a new insecticide. We performed keyword literature searches in Web of Science, PubMed, Academic Search Complete, and Google Scholar and explored citations used in identified articles. The search strategy yielded 66 citations; 25 citations fulfilled eligibility criteria and were included in the review. Under experimental conditions, cycloxaprid reduced populations of plant-feeding insect pests, suppressed populations of sucking and biting insect pests, and affected reproduction, development time, longevity, growth, gene regulation and expression, and phloem-feeding behavior of various life stages of certain insects. Studies focus on pest control efficacy and comparison with imidacloprid. Five non-target organisms have been evaluated: *Apis mellifera*, *Chrysoperla sinica*, *Harmonia axyridis*, *Daphnia magna*, and *Eisenia fetida*. Variation in study design, to-date, precludes a meta-analysis. However, these results provide valuable insight into possible effects of cycloxaprid to target and non-target arthropods. As a new insecticide, additional research is needed to clarify the mechanism-of-action of cycloxaprid and its metabolites, and to determine if it harms natural enemies or other non-target organisms, if resistance develops, and if it exhibits cross-resistance with other insecticides. Although research on target arthropods will inform some effects on non-target organisms, studies focusing explicitly on impacts to non-target organisms are needed.

Keywords

cycloxaprid, imidacloprid, neonicotinoid, non-target, pesticides, systematic review.

Introduction

Neonicotinoids are the most widely used insecticides worldwide owing to their broad range of insecticidal activity [1,2], primarily against coleopteran, dipteran, and lepidopteran insect pests in animal health and crop protection, often by treating plants, soil, and seeds [3]. However, neonicotinoid use can also be problematic, e.g., the neonicotinoid imidacloprid (IMI) negatively affects behavior, condition, abundance, reproductive success, and survival of pollinators and other non-target arthropods (reviewed in [4]). Studies increasingly indicate negative impacts of IMI to other non-target taxa such as aquatic macroinvertebrates (e.g., [5,6] and plankton (then subsequently water quality) [7], crayfish [8], and mollusks [9]). Cascading population and ecosystem level effects are now known, e.g., delayed songbird migration [10] and decreased fishery yields [11]. Therefore, replacements with similar efficacy but lesser non-target effects are desirable and under development.

Cycloxaprid (CYC) is a novel neonicotinoid [12]. It was discovered and patented by researchers at the East China University of Science and Technology [13,14]. Recently, researchers have proposed that while its neurotoxicant mechanism of action is similar to IMI, the novel cis-configuration of CYC might involve a differential action [15]. CYC has an NO_2 group in cis-configuration. Other commercial neonicotinoids have NO_2 groups in trans-configuration [16].

Conventional neonicotinoid insecticides like IMI act as agonists to the insect nicotinic acetylcholine receptor (nAChR) [17-19]. Nicotine extracted from tobacco and used in pest control since the late seventeenth century set the premise for modern insecticides that exploit the same mechanism-of-action (see reviews [20,21]. Nicotine and neonicotinoids are "cholinergic,", meaning that their activity involves the chemical neurotransmitter acetylcholine. Acetylcholine released by a nerve cell transfers an impulse and thus activates the next cell when it binds to receptors on that cell. The enzyme acetylcholinesterase (AChE) subsequently converts acetylcholine into an inactive form, causing the impulse to cease. Neonicotinoids are often referred to as "anticholinergic" because they bind to nicotine acetylcholine receptors (nAChRs) but resist breakdown facilitated by acetylcholinesterase, thus activity does not cease. Continuous nerve firing leads to a cascade of dysfunction, from seizure-like activity to paralysis and cell death, killing insects or altering their behavior and thus, reducing or eliminating damaging effects of pests.

Studies researching CYC efficacy are recent and ongoing, and empirical evidence has not yet been collectively considered. Some researchers propose CYC as an appropriate replacement for IMI, e.g., "… cycloxaprid is an alternative to imidacloprid for the management of resistant *Aphis gossypii*." [22] and "… cycloxaprid is a good alternative for the targeted control of this mirid bug" [14]. Because CYC is a new insecticide, additional study is needed to determine if it harms natural enemies and other non-target organisms, if resistance is developed, and if it exhibits cross-resistance with other insecticides [23]. Therefore, the current paper is the first review of target and non-target effects of CYC aimed at synthesizing existing data and informing understanding of environmental effects of CYC on non-target species.

Methods

Eligibility criteria

The review included only studies published after 1 January 2008 because cycloxaprid was first reported in 2008 [24] and first named in 2011 [25]. Eligible studies (i) determined lethal or sublethal outcomes of cycloxaprid exposure to test organisms; (ii) compared experimental and control treatments; (iii) described appropriate source populations and handling of test organisms; (iv) used homogenous sample populations; (v) described appropriate chemical preparation and dosing/exposures; (vi) described appropriate chemical analyses methods; (vii) included replication; and (viii) included appropriate statistical analyses. Inclusion was not restricted by language or country. Systematic reviews, critical reviews, observational studies, and studies with qualitative methods were excluded.

Information sources

The literature search was performed using four conventional electronic databases (Web of Science, PubMed, Academic Search Complete, and Google Scholar). We also performed a pointed review of literature published in six journals where such studies are often published (*Environmental Science and Pollution*, *Environmental Science and Technology*, *Environmental Health Perspectives*, *Science of Total Environment*, *Environmental Pollution*, and *Ecotoxicology*). We acknowledge that this systematic review might be limited by publication bias because only published literature is included.

Search strategy

The search strategy involved two steps. The first step was to select words or combinations of words to conduct a systematic search. Keywords included in the systematic search were *P (problem) = non-target*, non target*, off target*, off-target*, and native; E (exposure) = cycloxaprid*; C (comparison) = efficacy, effective, control, pest, and invasive; and O (outcome) = reproduction, feed*, behavior, mortality, sublethal, and lethal.* The second step was to perform the literature search. Both authors used these keywords to independently perform a literature search using the conventional databases and specific journals listed above. Thus, the search was duplicated. The final literature search for this systematic review was conducted on 31 August 2019.

Study records

After the two authors independently performed the literature search, digital versions of the identified studies were downloaded and saved in a folder. After this, the authors were responsible for data management.

Study selection

Abstracts, full journal articles, conference papers, and dissertations yielded by the keyword search were reviewed for possible inclusion in the review. Titles, abstracts, and full-text of the articles were independently and systematically screened by each author based on eligibility criteria. Further, each author performed a manual bibliographical search of selected articles for possible additional studies, and systematically reviewed each possible additional study using the same screening and review process. The authors compared findings and resolved discrepancies about articles excluded or included through discussion and consensus. For instance, authors prevented reporting of redundant results by tracing each study outcome to the original study that generated each result. Therefore, only unique results were included. The authors kept a log of all reviewed studies with reasons for exclusion.

Quality assessment of the studies

A quality assessment was performed to determine if methods reported in each study aligned with international testing standards and good laboratory practices (GLP). This tool [26] was designed to systematically evaluate data quality and application in hazard and risk assessment, using separate criteria for studies with acute and chronic exposure duration. A study was coded 1 through 4 based on quality of methods: (1) reliable without restriction, (2) reliable with restriction, (3) not reliable, and (4) not assignable. Studies meeting code 1 criteria are classified as 'high quality,' code 2 as 'moderate quality,' and code 3 and 4 as 'low quality.' Each author independently assessed the quality of the included studies and resolved any conflicting results by discussion.

Data collection process

Following a calibration exercise to ensure consistency using a group of unrelated studies, from each eligible study each author independently extracted data: test species and life stage, CYC concentration, IMI concentration (if applicable), application/treatment type and duration, replication, endpoint, outcome, authors, study title, study location, and publication date. Authors resolved disagreements by discussion.

Results

The systematic keyword search yielded 66 citations. Screening and removal of duplicates yielded 20 citations, and the bibliographical search yielded five additional citations (Fig.3.1). Thus, a total of 25 original citations met eligibility criteria, and a summary is provided in Table 3.1. Studies were published between 2012 and 2019 in English and Chinese language, and all studies were conducted in China. Results of 455 experimental tests were included in the 25 studies: 306 conducted on targets and 149 on non-target organisms, and 209 and 246 evaluating lethal and sublethal endpoints, respectively.

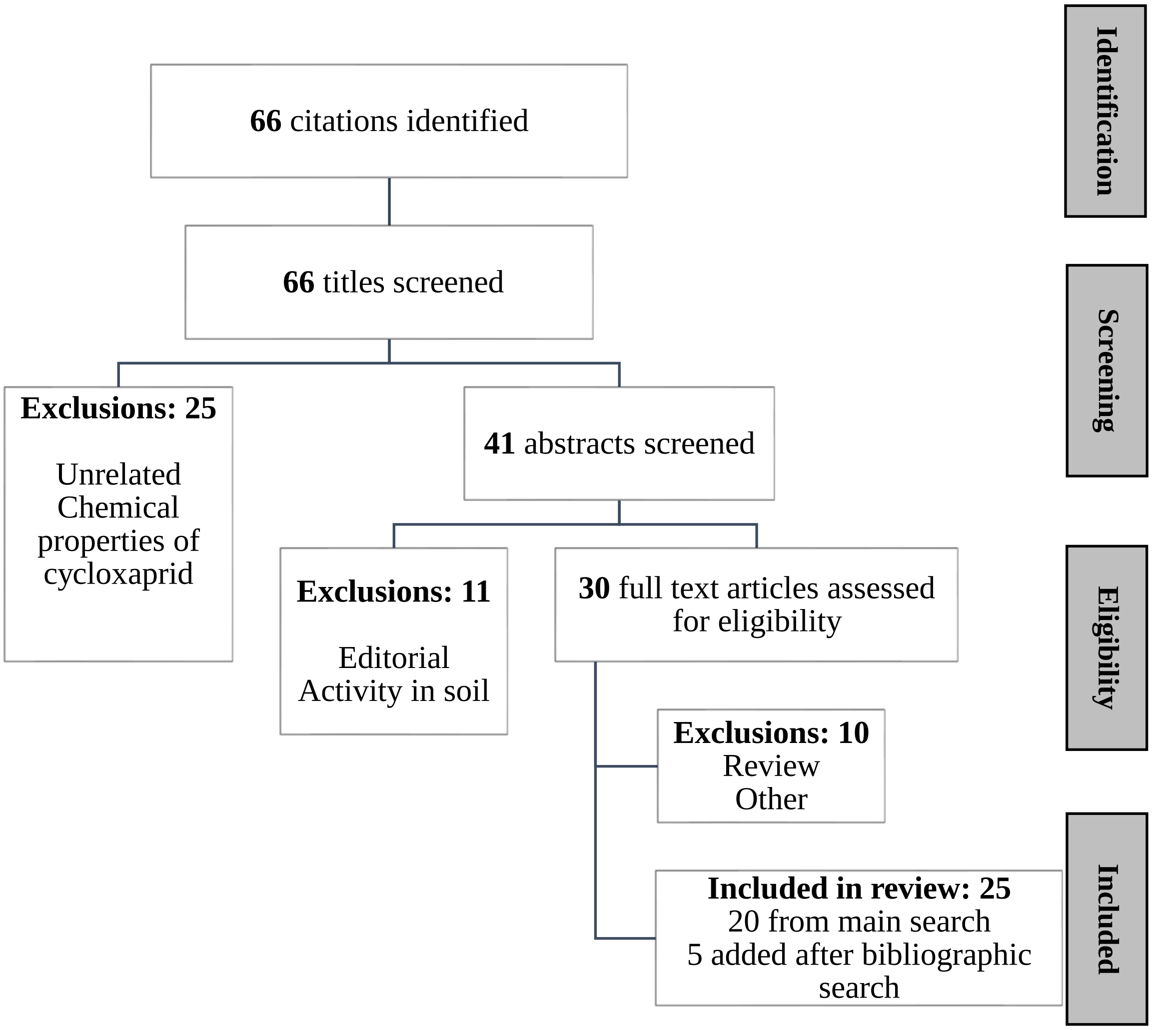

Figure 3.1. Study selection flow chart, including reasons for exclusions.

Table 3.1. Summary of included studies, measurement endpoints/effects (ME), and quality assessment (QA) score of each study.

Title	**Author**	**Study Location**	**Journal**	**Test Organism** Species T =target, NT =non-target	**Test Organism** Common Name	**ME ***	**QA ****
Baseline susceptibility and cross-resistance of cycloxaprid, a novel cis-nitromethylene neonicotinoid insecticide, in *Bemisia tabaci* MED from China	Wang et al. 2018	China	Crop Protection	*Bemisia tabaci* (T)	tobacco whitefly	M	2
Cross-resistance and baseline susceptibility of brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae) from China to cycloxaprid	Fang et al. 2018	China	Journal of Economic Entomology	*Nilaparvata lugens* (T)	brown planthopper	M	2
Cycloxaprid insecticide: Nicotinic acetylcholine receptor binding site and metabolism	Shao et al. 2013	China	Journal of Agricultural and Food Chemistry	*Musca domestica* (T)	house fly	GE	2
Cycloxaprid: A novel cis-nitromethylene neonicotinoid insecticide to control imidacloprid-resistant cotton aphid (*Aphis gossypii*)	Cui et al. 2016	China	Pesticide Biochemistry and Physiology	*Aphis gossypii* (T)	cotton aphid	M	2
Design, synthesis, crystal structures, and insecticidal activities of eight-membered azabridge neonicotinoid analogues	Xu, Xia, et al. 2014	China	Journal of Agricultural and Food Chemistry	*Aphis craccivora* (T) *Nilaparvata lugens* (T)	cowpea aphid brown planthopper	M	2
Divalent and oxabridged neonicotinoids constructed by dialdehydes and nitromethylene analogues of imidacloprid: Design, synthesis, crystal structure, and insecticidal activities	Shao et al. 2010	China	Journal of Agricultural and Food Chemistry	*Aphis craccivora* (T) *Nilaparvata lugens* (T)	cowpea aphid brown planthopper	M	2
Effects of a novel neonicotinoid insecticide cycloxaprid on earthworm, *Eisenia fetida.*	Qi et al. 2018a	China	Environmental Science and Pollution Research	*Eisenia fetida* (NT)	earthworm	B Mo GE	1
Effects of cycloxaprid, a novel cis-nitromethylene neonicotinoid insecticide, on the feeding behaviour of *Sitobion avenae*	Cui et al. 2012	China	Pest Management Science	*Sitobion avenae* (T)	English grain aphid	M RD B Mo	2
Isoxazole-containing neonicotinoids: Design, synthesis, and insecticidal evaluation	Lei et al. 2018	China	Bioorganic and Medicinal Chemistry Letters	*Aphis craccivora* (T) *Nilaparvata lugens* (T)	cowpea aphid brown planthopper	M	2
Lethal and sublethal effects of a novel cis-nitromethylene neonicotinoid insecticide, cycloxaprid, on *Bemisia tabaci*	Wang et al. 2016	China	Crop Protection	*Bemisia tabaci* (T)	tobacco whitefly	M RD	2

Table 3.1. Continued

Title	**Author**	**Study Location**	**Journal**	**Test Organism** Species T =target, NT =non-target	**Test Organism** Common Name	**ME** *	**QA** **
Lethal and sublethal effects of cycloxaprid, a novel cis-nitromethylene neonicotinoid insecticide, on the mirid bug *Apolygus lucorum*	Pan et al. 2014	China	Journal of Pest Science	*Apolygus lucorum* (T)	mirid bug	M RD	2
Lethal, sublethal and transgenerational effects of the novel chiral neonicotinoid pesticide cycloxaprid on demographic and behavioral traits of *Aphis gossypii* (Hemiptera: Aphididae)	Yuan et al. 2017	China	Insect Science	*Aphis gossypii* (T)	cotton aphid	M RD B Mo	2
Midgut transcriptome of the cockroach *Periplaneta americana* and its microbiota: Digestion, detoxification and oxidative stress response	Zhang et al. 2016	China	PLOS ONE	*Periplaneta americana* (T)	cockroach	M GE	2
Neonicotinoid insecticides imidacloprid, guadipyr, and cycloxaprid induce acute oxidative stress in *Daphnia magna*	Qi et al. 2018b	China	Ecotoxicology and Environmental Safety	*Daphnia magna* (NT)	water flea	M RD GE B Mo	2
Resistance to cycloxaprid in *Laodelphax striatellus* is associated with altered expression of nicotinic acetylcholine receptor subunits	Zhang et al. 2018	China	Pest Management Science	*Laodelphax striatellus* (T)	brown planthopper	M	1
Seven-membered azabridged neonicotinoids: Synthesis, crystal structure, insecticidal assay, and molecular docking studies	Xu, Luo, et al. 2014	China	Journal of Agricultural and Food Chemistry	*Aphis craccivora* (T) *Nilaparvata lugens* (T) *Paravilla separata* (T)	cowpea aphid brown planthopper armyworm	M	1
Specific synergist for neonicotinoid insecticides: IPPA08, a cis-neonicotinoid compound with a unique oxabridged substructure	Bao et al. 2016	China	Journal of Agricultural and Food Chemistry	*Nilaparvata lugens* (T) *Periplaneta americana* (T) *Aphis gossypii* (T) *Bemisia tabaci* (T) *Musca domestica* (T) *Apis mellifera* (NT)	brown planthopper cockroach cotton aphid tobacco whitefly house fly western honeybee	M	1
Sublethal effects of the novel cis-nitromethylene neonicotinoid cycloxaprid on the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae)	Cui et al. 2018	China	Science Reports	*Aphis gossypii* (T)	cotton aphid	M RD GE	1
Synthesis and insecticidal activities of novel bridged-neonicotinoids	Zhang et al. 2017	Chinas	Chinese Chemical Letters	*Aphis craccivora* (T)	cowpea aphid	M	2

Table 3.1. Continued

Title	**Author**	**Study Location**	**Journal**	**Test Organism** Species T =target, NT =non-target	**Test Organism** Common Name	**ME** *	**QA** **
Synthesis and insecticidal evaluation of tetrahydroimidazo[1,2-a]pyridin-5(1H)-one derivatives	Liu et al. 2016	China	Chinese Chemical Letters	*Aphis craccivora* (T) *Nilaparvata lugens* (T)	cowpea aphid brown planthopper	M	1
Tetrahydroindeno[1′,2′:4,5]pyrrolo[1,2-a]imidazol-5(1H)-ones as novel neonicotinoid insecticides: Reaction selectivity and substituent effects on the activity level	Chen et al. 2015	China	Journal of Agricultural and Food Chemistry	*Nilaparvata lugens* (T) *Aphis craccivora* (T) *Mythimna separata* (T)	brown planthopper cowpea aphid armyworm	M	1
The binding properties of cycloxaprid on insect native nAChRs partially explain the low cross-resistance with imidacloprid in *Nilaparvata lugens*	Zhang et al. 2019	China	Pest Management Science	*Nilaparvata lugens* (T)	brown planthopper	M	2
The influence of temperature on the toxicity of insecticides to *Nilaparvata lugens* (Stål)	Mao et al. 2019	China	Pesticide Biochemistry and Physiology	*Nilaparvata lugens* (T)	brown planthopper	M	1
The toxicity and detoxifying mechanism of cycloxaprid and buprofezin in controlling *Sogatella furcifera* (Homoptera: Delphacidae)	Chang et al. 2015	China	Journal of Insect Science	*Sogatella furcifera* (T)	whitebacked planthopper	M	2
Transcriptomic responses to different doses of cycloxaprid involved in detoxification and stress response in the whitebacked planthopper, *Sogatella furcifera*	Yang et al. 2016	China	Entomologia Experimentalis et Applicata,	*Sogatella furcifera* (T)	whitebacked planthopper	M GE	1

** M = Mortality, GE = Gene expression and/or enzyme activity, RD = Reproduction and/or development, B = Behavior, and Mo = Morphology*
*** 1 = Reliable without restriction, 2 = Reliable with restriction*

Non-target organisms were evaluated in only four of the 209 tests of mortality. Sublethal tests evaluated gene expression or enzyme activity (175 tests, 40 target, 135 non-target), reproduction and development (60 tests, 58 target, 2 non-target), and behavior or morphology (11 tests, 3 target, 8 non-target). A detailed overview of included study characteristics is presented as Table 3.1 and summarized to follow. Based on the quality assessment scoring criteria Klimisch et al. [26], nine studies were classified as 'high quality' as these scored 1, and the rest of the 16 studies were classified as 'moderate quality' as these scored 2. Table 3.1 summarizes the quality assessment of each study included in the review. The majority of the studies followed OECD guidelines, EPA protocol, or good laboratory practices.

CYC data are available for five non-target species: *Apis mellifera*, *Chrysoperla sinica*, *Harmonia axyridis, Daphnia magna*, and *Eisenia fetida*, and for four of these effects of comparable IMI concentrations were also determined. One study evaluated a pollinator, *A. mellifera,* and documented 48-h CYC (3.116 x10^{-2} μg/worker) and IMI (1.542 x10^{-2} μg/worker) LD50s administered via oral feeding to adult workers [27]. One study determined 24-h LC50s (after 1-h exposure) for third instar lacewings, *C. sinica*: CYC (58.37 mg/l) and IMI (87.64 mg/l) [22]. 24-h LC50s (also third instars after 1-h exposure) for the coccinellid, *H. axyridis*, were 28.22 mg/l for CYC and 31.28 mg/l for IMI [22]. One study [28] evaluated various sublethal gene expression, enzyme activity, reproductive, developmental, and behavioral endpoints in the water flea, *D. magna*, described below. Likewise, Qi et al. [29] evaluated similar gene expression and enzyme activity endpoints as well as morphology in the earthworm, *E. fetida,* but without IMI comparisons.

Mortality following exposure to CYC was evaluated in 15 arthropod species: 11 target species (*Aphis craccivora*, *Aphis gossypii*, *Apolygus lucorum*, *Bemisia tabaci*, *Laodelphax* *striatellus*, *Musca spp.*, *Mythimna separata*, *Nilaparvata lugens*, *Periplaneta americana*, *Sitobion avenae*, *Sogatella furcifera*) and 4 non-target organisms (*A. mellifera*, *C. sinica*, *E. fetida*, *H. axyridis*). IMI was evaluated under the same conditions in 24 of 40 studies and 57 different assessments of individual species (Table 3.2). CYC concentrations causing mortality in the arthropod species tested were generally lower than or similar to concentrations of IMI causing mortality under the same conditions.

Twenty-eight gene expression and/or enzyme activity endpoints were evaluated in six studies with target species *A. gossypii*, *P. americana*, and *S. furcifera*, and non-target organisms *D. magna* and *E. fetida*. CYC and IMI were evaluated in one study: Qi et al. [28] used juvenile *D. magna* in 48-h exposures to assess nine endpoints at three similar* CYC and IMI concentrations. IMI increased AChE activity, VTG2 (vitellogenin2 gene)-SOD (super-oxide dismutase) expression, VTG1-SOD expression, and CAT (catalase) activity, while the former two were reduced and latter two were unchanged by CYC. On the other hand, CAT expression and SOD activity were reduced by IMI, but were increased or unchanged by CYC. IMI and CYC reduced AChE expression. SOD-Cu/Zn and SOD-Mn expression was unchanged with IMI but reduced with CYC. *Note: CYC concentrations in exposure water reduced to a greater extent than IMI. However, CYC effect concentrations in the same study, e.g., described below, were also somewhat lower than IMI concentrations.

Reproduction and/or development were evaluated at 14 endpoints in four target and one non-target species. One of these studies evaluated CYC and IMI and established a 11.3 mg/l and 16.2 mg/l EC50 for CYC and IMI, respectively, for embryo hatching in the non-target *D. magna* [29]. Reductions were found in the adult preoviposition period of *A. gossypii* at its 24-h CYC LC20

Table 3.2. Number of publications evaluating CYC mortality, that also evaluate IMI under the same conditions, and the total number of individual CYC and IMI assessments in all studies subsequently compared to the right. Comparison of outcomes of assessments evaluating CYC and IMI are considered inconclusive when all test organisms died in CYC and IMI test concentrations. CYC concentrations causing mortality are represented as less than, greater than, or equal to IMI concentrations according to an absolute or conservative comparison of values, the latter assigning values as equal if CYC and IMI concentrations are within an order of magnitude (LD and LC) or differ by less than 10% (percent mortality).

Test Organism T =target NT =non-target	**Publications**	**w/IMI**	**w/CYC & IMI assessments**	**Inconclusive**	**CYC < IMI**	**CYC > IMI**	**CYC = IMI**	**CYC < IMI**	**CYC > IMI**	**CYC = IMI**
					absolute			**conservative**		
Aphis craccivora (T)	7	5	10	5	5	0	0	3	0	2
Aphis gossypii (T)	4	2	17	0	15	2	0	13	0	4
Apis mellifera (NT)	1	1	1	0	0	1	0	0	0	1
Apolygus lucorum (T)	1	1	1	0	1	0	0	0	0	1
Bemisia. tabaci (T)	3	2	3	0	3	0	0	3	0	0
Chrysoperla sinica (NT)	1	1	1	0	1	0	0	0	0	1
Eisenia fetida (NT)	1	0	0	0	0	0	0	0	0	0
Harmonia axyridis (NT)	1	1	1	0	1	0	0	0	0	1
Laodelphax striatellus (T)	1	0	0	0	0	0	0	0	0	0
Musca spp. (T)	2	1	1	0	1	0	0	0	0	1
Mythimna separate (T)	3	2	3	0	3	0	0	1	0	2
Nilaparvata lugens (T)	10	6	13	3	10	0	0	7	0	3
Periplaneta americana (T)	2	1	1	0	0	1	0	0	0	1
Sitobion avenae (T)	1	1	5	0	3	2	0	0	0	5
Sogatella furcifera (T)	2	0	0	0	0	0	0	0	0	0
TOTAL	40	24	57	8	43	6	0	27	0	22

(0.81 mg/l) [30], in adult fecundity and longevity, and fecundity and net reproductive rate of their offspring at its 48-h CYC LC10 (0.55 mg/l), and in adult fecundity and longevity, and fecundity, longevity, development time, and net reproductive rate in their offspring at its 48-h CYC LC40 (4.58 mg/l) [31]. Pan et al. [14] documented a reduction in oviposition period, fecundity, and longevity in *A. lucorum* at 24-h CYC LD40 (1.98 ng ai/adult). Wang et al. [32] found a reduction in *B. tabaci* oviposition period, egg hatching rate, fecundity, and survival from egg to second and third instars, but increased development time from the egg to the first instar, second instar, third instar, pseudopupae, and adult at its 48-h CYC LC25 (0.16 mg/l). Cui et al. [33] observed a reduced change in weight (i.e., reduced growth) in first-instar *S. avenae* nymphs at the 24-h CYC LC15 (1.4 mg/l) determined for 4 to 5-d old adults.

Behavior was evaluated in three studies, and one of these studies also evaluated IMI. The CYC EC50 for immobilization in the non-target *D. magna* was 14.7 mg/l and in the same study 16.5 mg/l for IMI [29]. Feeding by target species *A. gossypii* was unaffected at 0.55 mg/l CYC but reduced at 4.58 mg/l CYC [31] and likewise reduced in *S. avenae* at 1.4 mg/l CYC [33]. Only one study evaluated morphology: CYC ranging from 0.3 to 2.0 mg/kg caused damage to the epidermis and intestine of the non-target *E. fetida* [28].

Discussion and Conclusions

We conducted a systematic review of 25 laboratory and field studies to inform understanding of the possible environmental effects of CYC on non-target species. Data from studies of non-target effects of CYC to arthropods indicate that under experimental conditions, CYC has lethal and sublethal effects on non-target species, i.e., *A. mellifera*, *C. sinica*, *H. axyridis*, *D. magna*, and *E. fetida*. Data indicate that CYC has potential for insect pest control. However, CYC readiness for insect pest control (e.g., [14,22]) is premature because studies are too limited to predict effects to non-target organisms such as pollinators and natural enemies (e.g., lacewings and coccinellids), the latter also important for insect pest control. Of 246 tests exploring sublethal effects of CYC, 145 involved non-target species. However, 135 of those tests were from only two studies by Qi et al. published in 2018. These studies explored CYC effects to enzyme activity in *D. magna* [29] and *E. fetida* [28,29], important in aquatic and soil communities, respectively. Including these two species, only five non-target species have been evaluated, each in only one study. Only one study evaluated a pollinator and that study considered only mortality [27], despite increasing attention to sublethal impacts of neonicotinoids, in particular to pollinators (e.g., [34-38].

Parallel studies of IMI in four non-target organisms as well as in target organisms suggest some effects of CYC might be similar to those associated with IMI. However, CYC concentrations causing effects are generally lower than IMI concentrations evaluated under the same experimental conditions, suggesting CYC toxicity is higher. IMI resistance reported, e.g., in *N. lugens* [39] and *A. gossypii* [22], demonstrates the need for its replacement according to some researchers [22,39] and might create an urgency to adopt a novel alternative such as CYC. Zhang et al. [39] found a resistant strain of *N. lugens* with high IMI resistance (168-fold), but very low CYC resistance (six-fold). In the field, *N. lugens*, cross-resistance to CYC was also low, i.e., between 2.3 and 4.4-fold [40]. Cui et al. [22] found that CYC efficacy increased with increased CYC concentrations against resistant *A. gossypii*.

Available data and empirical evidence indicate it is early to consider CYC an appropriate replacement to IMI. Thus, at this stage, our findings recommend conducting studies on non-target species that explore different laboratory and field settings, e.g., all studies to-date have occurred in China, as well as wide-ranging endpoints to understand toxicity among non-target species. Because field efficacy and effects to non-target organisms can differ considerably with variable field conditions and practices, future studies should evaluate expected exposure levels in the field under such varying conditions and practices. Such studies will aid development of informed risk. Interestingly, Mao et al. [41] found *N. lugens* sensitivity to CYC increased with increasing temperature and discussed the importance of understanding chemical efficacy in the context of climate change. Non-target impacts should be similarly considered. Future research could further evaluate acute and chronic toxicity, resistance, and cross-resistance among target and non-target arthropods under different climatic conditions and geographical locations. Additionally, field studies could explore changes in arthropod population density and behavior, as well as the outcomes of those changes.

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Chapter IV

The Effect of Land Use, Land Cover, and Climate Change on the Incidence and Outbreak of Japanese Encephalitis Disease Among Asian Populations: A Systematic Review

A version of this manuscript was submitted to Environmental Health Perspectives on May 16, 2022, and it is currently under review. As the first author, I identified the research question, designed search strategy, performed data extraction and wrote the manuscript. Dr. Pragya G Poudel and Dr. Kristina W Kintziger contributed to conducting this review by reviewing the search strategy, study screening, and quality assessment of the included studies, and reviewed and edited the manuscript.

Abstract

Japanese Encephalitis (JE), which is caused by the Japanese Encephalitis virus (JEV), is a mosquito-borne disease. It is prevalent in Southeast Asia and Asian Pacific Rim. Each year 67,900 cases of JE are reported with an overall incidence rate of 1.8/100,000 per year globally. There are multiple factors that increase the risk to JE infection like poverty, socio-cultural practices, environment, and population distribution. Among these, the impacts of certain environmental factors, are not completely understood. The aim of this systematic review was to explore and understand the associations between land use, land cover, and climate change with JE infection among Asian populations. PubMed, Web of Science, Academic Search Complete, and hand searched three other journals specific to environmental health were searched for publications from January 1950 through February 2021. The systematic search strategy yielded 1,118 citations, and based on the inclusion criteria, 15 citations were included. Fourteen studies were ecological designs, and the one study was a case-crossover design. An increase in JE cases were reported with increasing rainfall and summer temperature. Also, pig rearing and paddy/rice cultivation were significantly associated with JE presence. However, land covered with forest had a protective effect, probably due to less human activity. Based on the findings, we concluded that, at the population level, there is a significant association between land use, land cover, climate change, and JE incidence. However, most studies were of low (n = 6) or acceptable (n = 7) quality. Thus, future research must include high-quality individual-level studies.

Keywords: Japanese Encephalitis, land cover, land use, Asia, climate change, systematic review.

Introduction

Japanese Encephalitis (JE), a mosquito-borne single-stranded RNA virus in the genus flavivirus, is a significant public health problem in Asia and many western Pacific countries [1]. The virus originated in Southeast Asia and has evolved from an ancestral virus to a highly mutated form [2]. This virus is mostly transmitted by a night-time biting mosquito, *Culex tritaeniorhynchus.* The virus is transmitted in an enzootic cycle involving water birds (the natural host), domestic pigs, rice paddy-breeding mosquitos, and other non-avian vertebrates [3]. Humans are a dead-end host of JE virus due to short duration and the low titer viremia [4]. JE is endemic to rural agricultural areas, such as farms and rice paddies, but with changing ecological conditions, transmission also occurs in urban and peri-urban areas. In endemic countries, JE is mainly a disease of children, as adults have acquired immunity through previous exposure, but individuals of any age may be affected [1]. Most cases of JE are asymptomatic. However, if clinical illness develops, the symptoms range from nonspecific fever to aseptic meningitis that might lead to irreversible neurological sequelae [5].

According to the World Health Organization, more than 3 billion people living within 27 Asia-Pacific countries are at risk of JE infections [6]. Each year, approximately 68,000 new cases are diagnosed with a total morbidity rate of 1.8 per thousand [6,7]. JE fatality rates range from 20% to 30%, and 30% to 50% of surviving patients experience paralysis, seizures, and behavioral changes, with some patients remaining severely disabled with permanent neurological or psychiatric sequelae [8]. Globally, in 2002, JE accounted for 709,000 disability-adjusted life years [9]. However, this might be an underestimation of the disease, as JE surveillance is not effectively implemented in many endemic countries [9]. There is no cure for the disease, but there is a vaccine available for prevention. The JE vaccination was introduced in India in 2006, and in 2014, it became a part of routine immunization. In 2016 in India, over 125 children were reported to have died in one hospital in one district from acute encephalitis, mostly due to JE infection [10]. Thus, the JE vaccination intervention alone has failed to reduce the incidence of Acute Encephalitis Syndrome (AES)/JE in India because of poor vaccine coverage, AES cases of unknown etiology that might be caused either by an infectious agent or toxin, and poor disease surveillance [10,11]. Thus, the actual disease burden, its distribution and trends are still not completely understood.

Geographical distribution of Japanese Encephalitis

JE virus has two epidemiological patterns. Endemic sporadic cases of JE are observed in Southern India, Vietnam, Southern Thailand, Malaysia, Philippines, Indonesia, Cambodia, Lao People's Democratic Republic, Sri Lanka, Myanmar, Brunei Darussalam, Papua New Guinea, Australia, and Timor. The second pattern, outbreaks, are reported most frequently in Northern India, Northern Thailand, Nepal, Burma, Bhutan, Northern Vietnam, Bangladesh, China, Korea, Taiwan, Southern Russia, Pakistan, Afghanistan, Madagascar, and Oriental Africa [6,12]. Figure 4.1 and 4.2 shows the distribution of JE infection in 2008 and 2018, based on the data provided by the Global Health Observatory, WHO [13]. We can see the change in disease distribution over ten years. In countries like India, Nepal, Chia and Thailand, there is a significant increase in the JE case per 100,000 population from 2008 to 2018. Asian countries have played a major role in JE virus transmission due to changing agricultural practices such as increased irrigation and animal husbandry, and migratory birds [2].

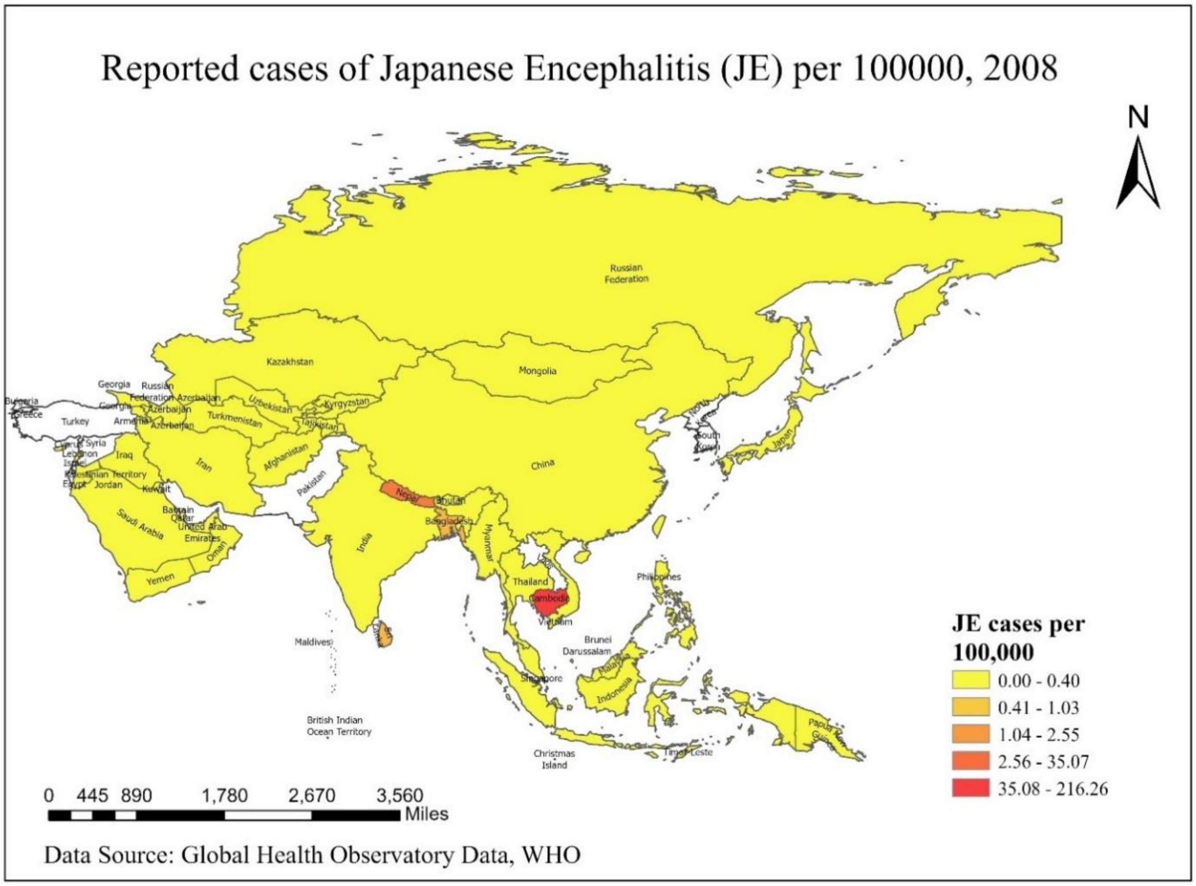

Figure 4.1. Japanese Encephalitis case distribution, 2008

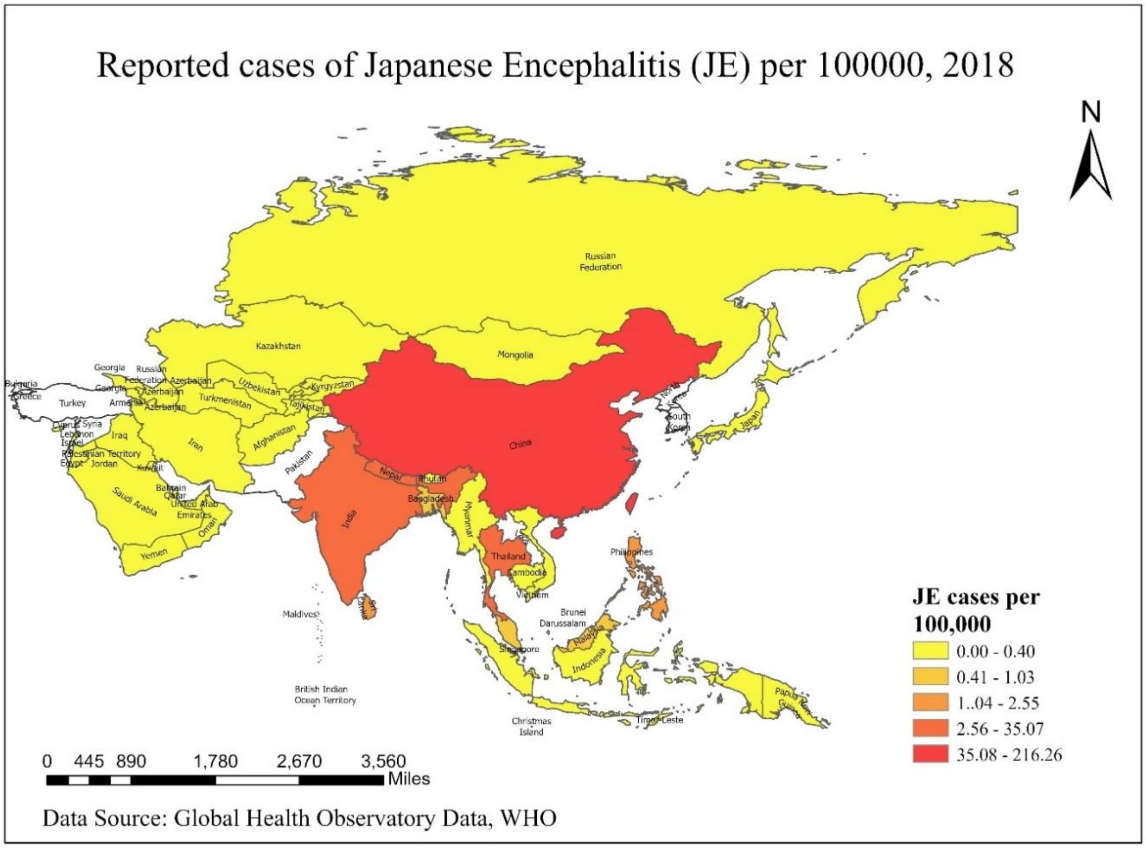

Figure 4.2. Japanese Encephalitis case distribution, 2018

Environmental risk factors

The emergence of JE involves a complicated interplay of hosts, vectors, climatic variables such as precipitation and humidity, and anthropogenic factors such as proximity to rice fields and animal husbandry [14,15].

There are several individual-level risk factors related to the disease including low awareness, change in social behavior, and failure to get the JE vaccination [16]. Here, we focus on environmental exposures. Since the identification of the first clinical case of JE, the major exposures are change in land use pattern (i.e., from dry land to wetland crops); change in agricultural practices (i.e., increase in rice paddy cultivation); change in socioeconomic factors and animal husbandry (i.e., promotion of pig breeding and duck farming, park and bird sanctuaries, and bird migration in the region); and climate change (i.e., increased unpredictability of temperature and rainfall) [17].

A fundamental feature of JE virus transmission is the physical environment. Land cover and land use changes have been driving JE exposure and incidence [18]. Environmentally, the biggest risk factor for JE infection is the proximity to irrigated agricultural lands, especially rice fields. Thus, JE incidence is highest in rice-growing countries. The JE vector breeds mainly in rice fields [19]. In Asia, paddy field surfaces have increased since 1960 and will continue to expand to meet increased production demands [18]. Additionally, researchers have found that increased water availability during early growth stage of paddy cultivation is a sign of increased mosquito breeding and abundance [19,20].

Once the JE virus is introduced, its ecological sustainability depends on the susceptible host population and transmission between hosts and vectors. Further, the risk of incidental infection to humans relies on the probability of disease transmission within the surrounding landscape [21]. Its transmission to humans needs competent mosquito vectors, reservoir amplifying hosts, and non-immune human hosts. In this process, three major landscape elements are paddy fields, pig farms, and human habitation, and these are distributed in habitat patches. Thus, the density, size and spatial arrangements are the major factors influencing the degree to which landscape enables or precludes the movement of vector [22].

Additionally, climatic factors like temperature, relative humidity, and rainfall are closely related to JE infection. These climatic factors have a direct influence on JE primarily through their effect on virus cycle, and vector's maturation in the stages of larvae and pupae [23,24]. Therefore, it is evident that land use, land cover, and climatic factors complicate JE transmission. Thus, it is crucial to explore and understand the intricacies of these environmental factors on the incidence and outbreak of JE infection.

Study aim

For decades, studies have examined the epidemiology of JE virus, but it continues to be a poorly understood disease. There are multiple factors like socioeconomic status, environment, and population distribution, that make JE infection a complex phenomenon. Of these, environmental factors are less studied. The most relevant environmental factors are climate change, land use, and land cover [18]. In the context of changing climate, it is suggested that the meteorological factors, such as change in local temperature and rainfall, will keep modifying the distribution of the vector and the risk of JE infection [25,26]. Despite ample research to support that climate change, land use, and land cover are linked to increasing emergence and reemergence of JE infection, these associations are still not completely understood, and no systematic review has been done exploring the association between theses exposures and JE incidence. Thus, this review aims to explore and describe existing research to provide a more comprehensive synthesis of the impact of climate change, land use, and land cover on incidence and outbreaks of JE infection among Asian populations.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) methodology and checklist were followed for reporting this systematic review. [27]

Protocol

The study protocol is registered with and available on the international database PROSPERO with the registration number CRD4201912220 [28].

Study eligibility criteria

A systematic search was performed for studies examining the environmental predictors, i.e., land use, land cover and climate change, of JE infection. We included original observational research studies, such as ecological, cohort, case-control, and cross-sectional, that examined the effect of any of the predictors, i.e., land use/land cover/climate change on JE infection among Asian population. We excluded studies that were conducted only within healthcare settings such as hospital-based studies, studies assessing travel-related JE cases, studies exploring the JE infection in vectors only, studies only investigating the role of vaccination in JE infection, studies focusing on viral encephalitis other than JE, and studies focused on populations other than Asian countries.

Search strategy

We conducted the literature search over the period from 1 January 1950 to 15 February 2021 in three databases: PubMed, Web of Science, and Academic Search Complete. In addition, we hand searched three specific environmental health and infectious disease journals, i.e., Journal of Environmental Health, International Journal of Infectious Disease, and Environmental Health Perspectives. We did not include gray literature to avoid challenges in systematic search of evidence and ensure the reproducibility of search strategy. [29,30] We enlisted the help of an experienced public health librarian to finalize our search terms, and the search strategy was implemented by the reviewers (AS and PGP) separately. Reviewers (AS and PGP) validated the search by comparing the results yielded by each database. The complete search strategy can be found in the supplementary material (Appendix A).

Study selection

We used Microsoft Excel 2016 (Microsoft Corporation; Redmond, WA) to import all the studies obtained from different databases in one sheet. Next, two independent reviewers (AS and PGP) removed duplicate studies and reviewed all studies, applying inclusion and exclusion criteria in three steps: title screening, abstract screening, and full-text screening. Reviewers documented their decision in the Excel spreadsheet along with a reason for rejecting each study. Disagreement between the investigators was discussed and settled by consensus, or in case discussion failed, a third investigator (KWK) resolved any disagreements.

Data extraction

Based on the study objective, we designed a data extraction form in Excel, and two reviewers (AS and PGP) extracted data. The reviewers compared their data and discussed discrepancies in the collected data. Any disagreements were solved by consensus, and we consulted the third reviewer (KWK), when required. For missing or unclear data in primary studies, we contacted the authors for clarification. The following data were extracted when available: citation, study design, database and data collection, study period, study site/country, exposure, number of JE cases, incidence rate, results, and statistical analysis.

Risk of bias assessment of included studies

Two investigators (AS and PGP) evaluated each study included in the review for risk of bias using standard tools: the Newcastle-Ottawa scales (NOS) [31] for quality reporting of observational studies, i.e., case-control and cohort studies and their variations (e.g., case-crossover). NOS consists of seven (case-control) or eight (cohort) multiple choice questions which addresses subject selection, comparability, exposure (case-control), and outcome (cohort). A high quality response on each question earns a star, totaling up to nine (case-control) or ten (cohort) stars (the comparability question earns two stars for both designs). The higher the number of stars, the higher the quality of the study [31]. However, most included studies were ecological. There is no validated tool to assess the quality of ecological studies. Thus, we used an adapted checklist which was originally proposed by Dufault [32-34]. Each study was assessed on 15 items with a maximum score of 21: 12 points for study design, 6 for statistical methodology, and 3 for quality of reporting [32]. Studies with a total score below 13 were low quality, between 13 to 18 were acceptable quality, and studies above 18 were high quality studies [32]. The studies included in the systematic review used spatial epidemiological methods. Thus, in analytical methodology section, we gave a score of 2 if the statistical methods included spatial statistics along with linear regression. However, scoring spatial statistical methods was not a part of the scale used in earlier studies [32]. We had to make this exception based on the sophistication of the methods used in the included studies and to provide an appropriate score to studies that used advanced spatial epidemiological methods. A full description of the scale used to assess the risk of bias of the ecological studies is included in the supplementary material (Appendix B). Any disagreement was settled by discussion, or in case discussion failed, we had a third investigator (KWK) resolve our disagreements. We assessed the heterogeneity of included studies based on the study design, outcome measures and estimates.

Results

Selected studies

The search strategy yielded 1,118 citations from six different databases/journals. The number was reduced to 1,013 unique citations after removing 105 duplicates. Out of 1,013 citations, 125 citations were included, and 888 were excluded based on the title screening. Further, on abstract screening, 34 citations were selected for full-text screening, and 91 citations were excluded. During initial screening of titles and abstracts, the investigators (AS and PGP) had disagreement on 26 citations. However, this was settled on discussion. Reasons for exclusion included not related to JE infection or environmental factors and focused on vaccination and other preventive measures. Also, a few of the studies were excluded based on their focus on non-Asian populations, non-human infection, and inpatient care. Next, the reviewers selected 34 citations for full-text evaluation. Of these, 19 citations were excluded due to reasons such as study explores JE infection and vector association, focused on other viral encephalitis, and a few of the citations were either a review or case study. Finally, the authors included 15 citations in the systematic review. Figure 4.3 flowchart summarizes the study selection process.

Description of the included studies

A total of 15 citations representing 15 original studies met the eligibility criteria. Included studies were published between 2003 and 2020. These studies focused on a variety of exposures. Related to land use, exposures included irrigated lands, agricultural lands, paddy/rice fields, and pig farming. Land cover was usually measured as human habitation, wetland, grassland, and forest. The climatic variables were measured as minimum-maximum temperature, humidity/relative humidity, and rainfall. The outcome of interest for this review was measured as JE incidence rate and total number of JE cases. The included studies used either Bayesian or inferential statistics along with spatial epidemiological methods to analyze the data and multi-level modeling was done to understand the association between different exposures and JE. Data from 1,515,522 participants with JE were included across all studies, with a median of 1,197 participants with JE per study (range: 53-1,400,000). Studies were conducted in China (n = 10), India (n = 1), Taiwan (n = 2), Laos (n = 1), and Nepal (n = 1). All studies were observational, including ecological (n = 13), case-crossover (n = 1), and one study used both ecological and cross-section study design (n=1). A detailed account of the included studies is presented in Table 4.1.

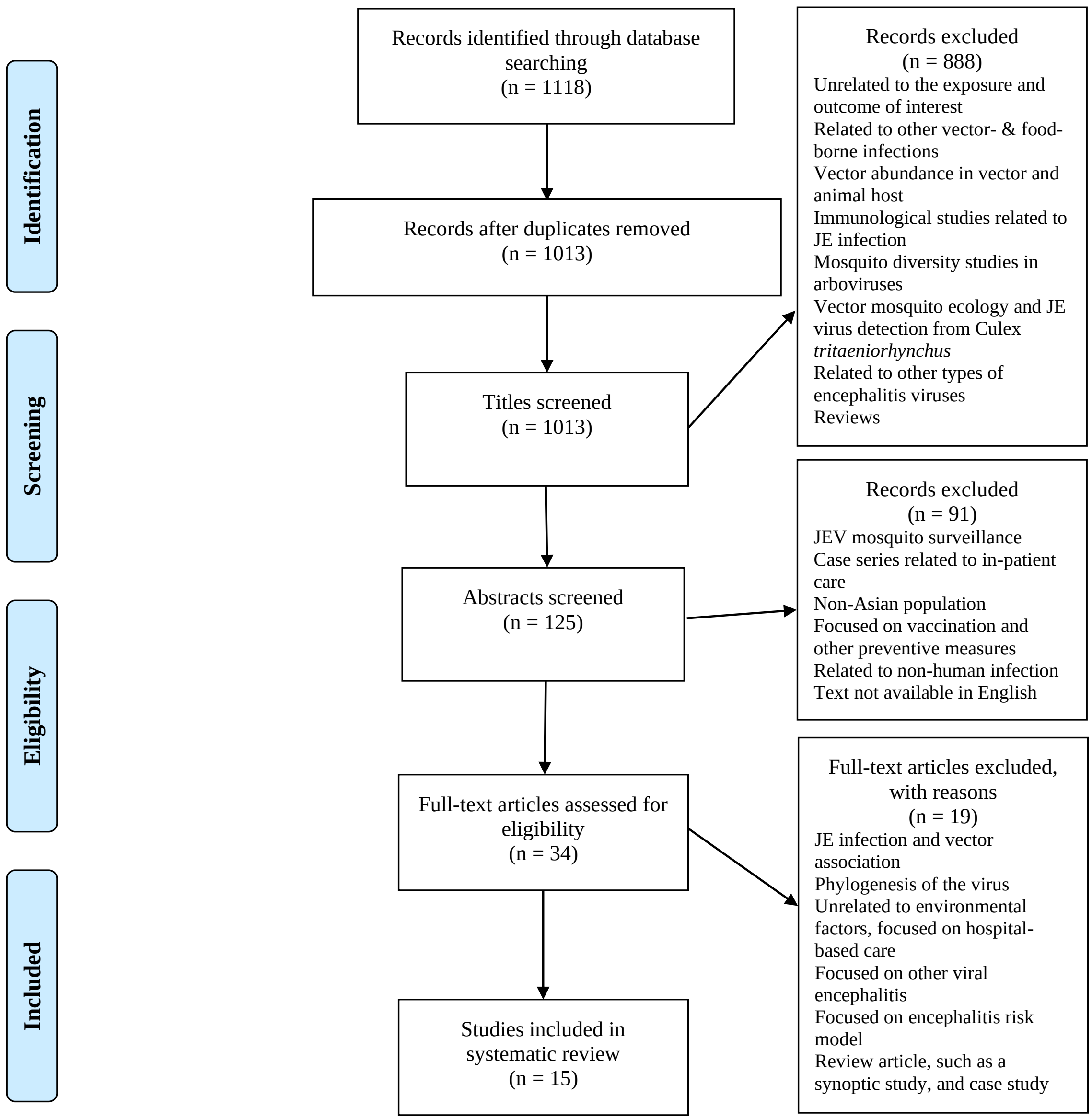

Figure 4.3. Study selection flow chart, including reasons for exclusion

Table 4.1. Overview of the 15 studies included in the systematic review

Title	Author	Study design	Study period	Country	Exposure	No. of cases & incidence rate	Results	Statistical analysis
Epidemic changes and spatiotemporal analysis of Japanese Encephalitis in Shaanxi Province, China, 2005-2018	Song et al. 2020	Retrospective ecological and spatial epidemiologic study	2005-2018	China	Weather variables: relative humidity (RH), temperature, and ENSO (El Niño-Southern Oscillation); land cover data	2242; 0.08/100,000 in 2011; 1.38/ 100,000 in 2006	Maximum JE cases between Jun-Sep; Significant associations of JE cases with temperature, RH, wind velocity, and ENSO; Two protective factors—coniferous forest coverage, and urban areas	Negative binomial regression, descriptive spatial epidemiologic methods, and spatiotemporal Scan Statistics
Spatial epidemiology of Japanese encephalitis virus and other infections of the central nervous system infections in Lao PDR (2003–2011): A retrospective analysis	Rattanavong et al. 2020	Spatial epidemiological, ecological analysis, and cross-section study design	2003-2011	Laos	Normalized vegetation index (NDVI), and enhanced vegetation index (EVI); vegetation comes under land cover operational definition	94	JE cases maximum between Jul-Sep, mostly among children (5-14 years), and in villages with flooded lands. No significant association between JE cases and vegetation.	Exploratory spatial data analysis, and logistic regression
How Socio-Environmental Factors Are Associated with Japanese Encephalitis in Shaanxi, China-A Bayesian Spatial Analysis	Zhang et al. 2018	Ecological	2006-2014	China	Meteorological (rainfall, temperature, and socio-demographical factors (gender, age, county, home address, date of symptom and hospital admission & JE vaccination)	1,197	Highest JE cases between Jun-Oct; Rainfall was positively associated with JE incidence, and minimum temperature had inverse association with JE incidence between 2012 and 2014	Spearman correlation analysis, Bayesian Conditional Autoregressive model.

Table 4.1. Continued

Title	Author	Study design	Study period	Country	Exposure	No. of cases & incidence rate	Results	Statistical analysis
Seasonal Patterns of Japanese Encephalitis and Associated Meteorological Factors in Taiwan	Lin et al. 2017	Case cross over	2000-2014	Taiwan	Climatic variables: rainfall & temperature	379; 1.105 (0.59-1.61)/ 1000,000 individual per year; Men vs women - 1.366 vs 0.839 per 1000,000	JE incidence increased with increase in patient's age. Highest cases in Jun-Aug, and significant association of JE cases with mean temperature (<22°C) and mean RH (70-74%)	Poisson regression model
How environmental conditions impact mosquito ecology and Japanese encephalitis: An eco-epidemiological approach	Tian et al. 2015	Ecological	2004-2009	China	Temperature, rainfall, agricultural, and land use: rice paddy and land cover: vegetation	53; 0.15/100,000	Most JE cases in Jul-Aug; Significant association of JE cases with temperature (25-26°C), absolute humidity, and paddy.	Cross-correlation analysis, Poisson regression
A Spatial and Temporal Analysis of Japanese Encephalitis in Mainland China, 1963–1975: A Period without Japanese Encephalitis Vaccination	Li et al. 2014	Ecological	1963-1975	China	Climatic factors (rainfall and temperature)	1.4 million; 150,000 in 1966 & 170,000 in 1971; 1963 to 1975 - 1.2 million cases & 15 to 22/100,000	More JE cases in geomorphic unit with abundant rainfall and higher summer temperature	Cluster Analysis: Local Indicators of Spatial Association (LISA)

Table 4.1. Continued

Title	Author	Study design	Study period	Country	Exposure	No. of cases & incidence rate	Results	Statistical analysis
Japanese Encephalitis Risk and Contextual Risk Factors in Southwest China: A Bayesian Hierarchical Spatial and Spatiotemporal Analysis	Zhao et al. 2014	Ecological	2004-2009	China	Agricultural and climatic variables	17,007 cases (male= 10,489 & female=6,518)	JE cases peak in Jul-Aug; Significant association of JE cases with mean temperature, mean rain and mean pig-rearing number	Spatial Autocorrelation Analysis, , Bayesian Hierarchical Spatial and Spatiotemporal Modeling
Regional Impact of Climate on Japanese Encephalitis in Areas Located near the Three Gorges Dam	Bai et al. 2014	Ecological	1997-2008	China	Climatic drivers: temperature, rainfall, and relative humidity	2,552 (range 23 to 510, median = 151); 0.4 to 5.7/100,000 with a median of 1.6/100,000	JE cases peaked in Jun-Aug; Significant association of JE cases with temperature and rainfall	Zero Inflated Poisson Regression (ZIPR)
The role of environmental factors in the spatial distribution of Japanese encephalitis in mainland China	Wang et al. 2014	Ecological	2005-2011	China	Temperature, humidity, rainfall, human population, pig density, irrigated land and crop land.	23,026	Model predicted contribution of min temperature, human population density, mean temperature, mean rainfall, and mean humidity to JE incidence	Spatial Autocorrelation Analysis
Association of weather and anthropogenic factors for transmission of Japanese encephalitis in an endemic area of India	Borah et al. 2013	Retrospective ecological study	2008-2010	India	Meteorological and anthropogenic: housing, contact with animals and birds, occupational factors	178	Highest JE cases between Jun-Oct, significant associations of JE cases with rain, min and max temp, relative humidity, and presence of rice paddy and pig farm	Linear regression analysis

Table 4.1. Continued

Title	Author	Study design	Study period	Country	Exposure	No. of cases & incidence rate	Results	Statistical analysis
Time series analysis of Japanese encephalitis and weather in Linyi City, China	Lin et al. 2012	Ecological	1956-2004	China	Weather variables: rainfall, temperature	45,317; 93.69/100,000	Significant association between JE cases and temperature	Autoregressive Integrated Moving Average models
The spatial heterogeneity between Japanese encephalitis incidence distribution and environmental variables in Nepal	Impoinvil et al. 2011	Ecological	2004-2009	Nepal	Land use: irrigated and crop land, pig number; land cover: tree and grassland cover, and climate change	669	Temperature and irrigated land were significantly associated with JE incidence but no significant association between JE cases and pig: human ratio	Spatial Autocorrelation Analysis
Contextual risk factors for regional distribution of Japanese encephalitis in the People's Republic of China	Cao et al. 2010	Ecological	2004-2007	China	Rice planting, pig rearing, & proportion of rural population	22,334 (in 2004-07, 13,707= male & 8,627=female); 0.01/100,000-4/100,000	JE cases peaked between Jun-Oct; Rice-planting area, extent of pig-rearing and proportion of rural population were significantly associated with JE incidence	Spearman's rank correlation, Poisson regression.
The impact of climate on Japanese encephalitis	Hsu et al. 2008	Ecological	1991-2005	Taiwan	Temperature, rainfall, and pig rearing	287	JE cases peaked between May-Aug, and JE incidence was significantly associated with temperature, rain, and pig density	Generalized Autoregressive Poisson Regression model
Climate variability and transmission of Japanese encephalitis in eastern China	Bi et al. 2003	Ecological	1980-1996	China	Climatic variables: rainfall & temperature	1/100,000-12.89/100,000 in 1990	Most JE cases in Jul-Sep, Significant association between JE incidence, and temperature and rainfall	Spearman's rank correlation, multiple regression and time series analysis

Association of land use and JE incidence

We defined land use as agricultural lands, paddy/rice fields, cultivation, irrigated land, and land covered for pig farming. Seven studies were found to explore the association between land cover and JE (Table 4.1). Impoinvil et al. [35] reported a positive association between irrigated land and JE incidence, i.e., $\beta = 0.033$, p = 0.005, and 95 % CI = 0.010 - 0.056. However, the pig to human ratio was not significantly associated with JE incidence, i.e., $\beta = 0.637$, $p = 0.217$, and 95% CI = -0.374 – 1.648 [35]. Tian et al. [36] reported that paddy cultivation and a one-month lag in JE incidence were significantly associated, i.e., $\beta = 0.92$, $p < 0.01$, and this might be due to the capability of paddy fields to provide optimum habitat for vector growth [36]. Borah et al. [37] found that both paddy cultivation (OR = 1.93, 95% CI = 1.01-3.68, p = 0.05) and proximity to pigs were significantly associated with JE incidence (OR = 3.6, 95% CI = 1.34-9.7, and p = 0.011).

Further, Cao et al. [38] found that paddy cultivation (3^{rd} quartile: Adjusted RR = 2.924, 95% CI = 1.370 – 6.801) and pig rearing (2^{nd} quartile: Adjusted RR = 12.962, 95% CI = 7.221 – 20.396) were significantly associated with JE incidence. Zhao et al. [39] found high-risk areas of JE cases in southwest China had significant positive association with pig to human ratio when modelled with monthly mean minimum temperature ($\beta = 0.242$; 95% Credible Interval = 0.067 – 0.41) and when modelled with monthly mean maximum temperature ($\beta = 0.333$; 95% Credible Interval = 0.177 – 0.485). Similarly, Hsu et al. [40] found significant association between pig density and JE incidence ($\beta = 1.31$, $p < 0.01$). Additionally, Wang et al. [41] used ecological niche model to investigate the percentage contribution of each predictor from 2005 t0 2011 and found that irrigated and rain-fed croplands (0.58-1.77%), and pig density (0.11-0.16%) were associated with JE cases.

Association of land cover and JE incidence

We defined land cover as human habitation, wet land, vegetation cover, grassland, and forest. Four studies were found to explore the association between land cover and JE (Table 4.1). Wang et al. [41] reported that urban/built-up areas were strongly associated with JE presence as these added to the man-made breeding sites for mosquitoes. The probability of JE presence increased with the increasing human population or pig density, and then remained stable above 2,500 per km^2 and 400 per km^2, respectively [41].

Song et al. [42] conducted a univariable analysis and found that human population density (Crude Incidence Rate Ratio(IRR) = 0.99 [0.99-1], $p < 0.001$), broad-leaved forest coverage (Crude IRR = 1.01 [1.00-1.01], $p = 0.067$), coniferous forest coverage (Crude IRR = 1.03 [1.00-1.06], $p = 0.08$), shrub or herbaceous coverage (Crude IRR = 0.85 [0.74-0.99], $p = 0.032$), urban areas (Crude IRR = 0.98 [0.98-0.99], $p < 0.001$), and water bodies were significantly associated with JE incidence.

Unlike Wang et al [41], Song et al. [42] found negative association between urban areas (multivariable model: Adjusted HR = 0.98 [0.98-0.99]) and JE incidence and explained it an outcome of better sanitation and awareness leading to self-protective behavior among urban population resulted in lesser JE spread [42]. Further, final multivariable model indicated that coniferous forest coverage (Adjusted HR = 0.95 [0.91-0.98]) was a protective factor and inversely associated with JE disease incidence, and this might be related to less human activity in the forest [42]. Impoinvil et al. [35] studied association between grassland and deciduous cover, and JE cases, the Pearson coefficient correlation analysis showed negative association between grassland ($r = -0.69$, $p < 0.001$) and JE incidence, however, positive association was found betwee deciduous tree cover ($r = 0.442$, $p < 0.001$) and JE incidence. Further, Impoinvil et al. [35] applied parsimonious model to different years and found that grassland cover was significantly associtaed with JE incidence in 2004 ($\beta = -0.05$, $p < 0.05$) and 2006 ($\beta = -0.03$, $p < 0.01$).

Unlike other studies, Rattanavong et al. [43] conducted multivariable regression analysis at village level and individual level. The village level study was focused on the villages of JE diagnosed cases. They found that a high vegetation index, i.e., an indicator of land surface covered with green vegetation, was associated with JE patient home villages i.e., the home villages of JE patient had a higher vegetative index during the month of JE case detection and hospitalization. Further, Rattanavong et al. [43] conducted a village level analysis and reported that the odds of a larger population among the villages with JE cases was 1.74 times the (Adjusted OR = 1.74 [1.55-1.96]) the odds of villages without JE cases, i.e., significant association between villages with large population and JE cases. Additionally, the individual level analysis showed that season was a major predictor of JE infection (Adjusted OR = 7.40 [1.45-37.67]), i.e., the odds of a patient to be diagnosed with JE during the months of Jul-Sep was seven time the odds of the patient to be diagnosed with JE during the months of Jan-Mar [43]. Also, JE infection was most commonly found among children between ages of 5 to 14 years (Adjusted OR = 2.74 [1.31-5.69]), and a significant association was found between JE infection and villages with flooded lands (Adjusted OR = 3.06 [1.04-8.96]) [43].

Association of climatic variables and JE incidence

We defined climatic factors as the variation in temperature, humidity/relative humidity, and rainfall during the study period. Thirteen studies explored the association between weather variables and JE cases/incidence (Table 4.1). Nine studies conducted in China found significant positive associations between temperature, humidity/relative humidity, rainfall, and JE incidence [14,16,36,39,41,42,44-46]. However, the study conducted by Zhang et al. [46] in Shaanxi province of China found an inverse association between temperature and JE incidence. Wang et al. [41] found that places with mean precipitation of 80-120 mm had a high probability of JE incidence in mainland China. However, excessive precipitation destroyed mosquito habitat through flooding. They also found that minimum and mean temperatures were associated with the spatial distribution of JE in China [41]. Similarly, Zhao et al. [39] found that the model with monthly mean minimum temperature (DIC = 5470.98) had a greater fit as compared to the model with monthly mean maximum temperature (DIC = 5702.9). They also found that changes in temperature plays a key role in JE variation and more crucial as compared to precipitation, and these results are consistent with the earlier studies [23,47].

Song et al. [42] investigated the association between environmental factors and spatiotemporal distribution of JE disease at county level. The final multivariable model found significant association between temperature (Adjusted Hazard Ration [HR] = 1.50 [1.39-1.61] and relative humidity (Adjusted HR = 1.05 [1.02-1.08]) with spatiotemporal distribution of JE incidence [42]. This study suggested that temperature had the most significant association with JE disease, and global warning contributed to the gradual spread of JE infection towards the northern part Shaanxi province [42]. The cross correlation analysis conducted by Tian et al. [36] found a positive association between monthly mean absolute humidity (ρ(lag 0) = 0.48, ρ(lag 1) = 0.39, $p < 0.05$), mean temperature (ρ(lag 0) = 0.44, ρ(lag 1) = 0.35, $p < 0.05$) , and JE cases. Additionally, the human-JE model predicted a key role of absolute humidity ($\beta = 0.61$, $p < 0.05$) in JE transmission, and reported a threshold of 25-26°C temperature for increase in JE cases [36]. This increase in JE cases above the threshold temperature might be due to the increased ability of mosquitoes to obtain blood meal from other animas when environmental temperature reaches above breeding temperature and humidity further prolongs the survival of mosquitoes and thus, rapid spread of JE infection [36]. A study conducted in mainland China found that between 2005-2011 mean relative humidity had a moderate proportional contribution (0.49%-6.43%) to the distribution of JE cases [41].

A study conducted in India found statistically significant associations between temperature [β (min temp) = 0.231, $p = 0.018$; β (max temp) = 0.071, $p = 0.042$], rainfall (β = 0.342, $p = 0.005$), and JE incidence [37]. Generally, more cases of JE occurred in geomorphic units with more abundant summer rainfall and higher summer temperature, and vice versa [45]. Li et al. [45] explored the JE incidence data before the JE vaccination was enrolled in Mainland China and found a higher JE incidence between 1963 to 1975. This was attributed to high summer rainfall (average JE incidence between 1963 and 1975 was 22.14/100,000). Increasing summer rainfall and high summer temperature were consistently correlated with an upward tendency of JE incidence during the epidemic period. However, in the plateau with less summer rainfall, JE cases ranged between 0 to 9.29 per 100,000, and in plains with higher summer rainfall, JE cases ranged between 0.19 to 22.14 per 100,000 [45]. Another study in Taiwan by Hsu et al. [40] reported similar findings as in China and India, and found temperature and precipitation significantly associated with JE incidence [β(temp) = 0.188, $p < 0.01$; β(rain) = 0.117, $p = 0.05$]. Lin et al. [48] used Taiwan data to conduct case-crossover analysis and found significant association between JE incidence, temperature (IRR = 1.30 [1.06, 1.60] and $p = 0.013$) and relative humidity (IRR = 1.10 [1.04-1.18], $p = 0.002$). Further, the authors reported that the JE cases started increasing at temperature of 22°C and at a relative humidity of 70-74% [48]. The study conducted in Nepal reported that temperature (β = -0.032, 95% CI= -0.05, -0.01, and $p < 0.001$) was inversely associated with JE incidence [35], similar to the Zhang et al. [46] study in China.

Risk of bias assessment of included studies

The results from the 14 ecological studies are summarized in Figure 4.4. The case-crossover study was found to be of high quality. Rattanavong et al. [43] used cross-sectional study design along with retrospective ecological study design. We assessed the study quality for the cross-sectional study using NOS scale adapted for cross-sectional study design [49]. This cross-sectional study was found to be of high quality. All ecological studies scored between 13 to 18 on a maximum of 21 points. Out of 14 ecological studies, seven studies were of acceptable quality, and one study was of high quality. However, the remaining six studies were low quality, with

scores were below 13 [32]. Among the acceptable and high quality studies, the average score on study design was 9 (max = 11). Similarly, the average score on statistical methodology and quality of reporting was 4.5 (max = 6) and 1.6 (max = 3) respectively. The most common domain of weakness among the ecological studies was quality of reporting. Most of the studies did not mention the key elements of ecological study design and did not caution the readers about the limitations such as ecological fallacy. However, the strength of the studies was their statistical methodology. All the studies scored an average of 4 (median = 4, max = 6). Most authors detailed their statistical methods and used sophisticated analytical methods. Further, under the statistical methodology domain, only four studies adjusted for socioeconomic variables, and none adjusted for clinical variables. A detailed risk of bias assessment of the included ecological studies is included in supplementary file (Appendix C).

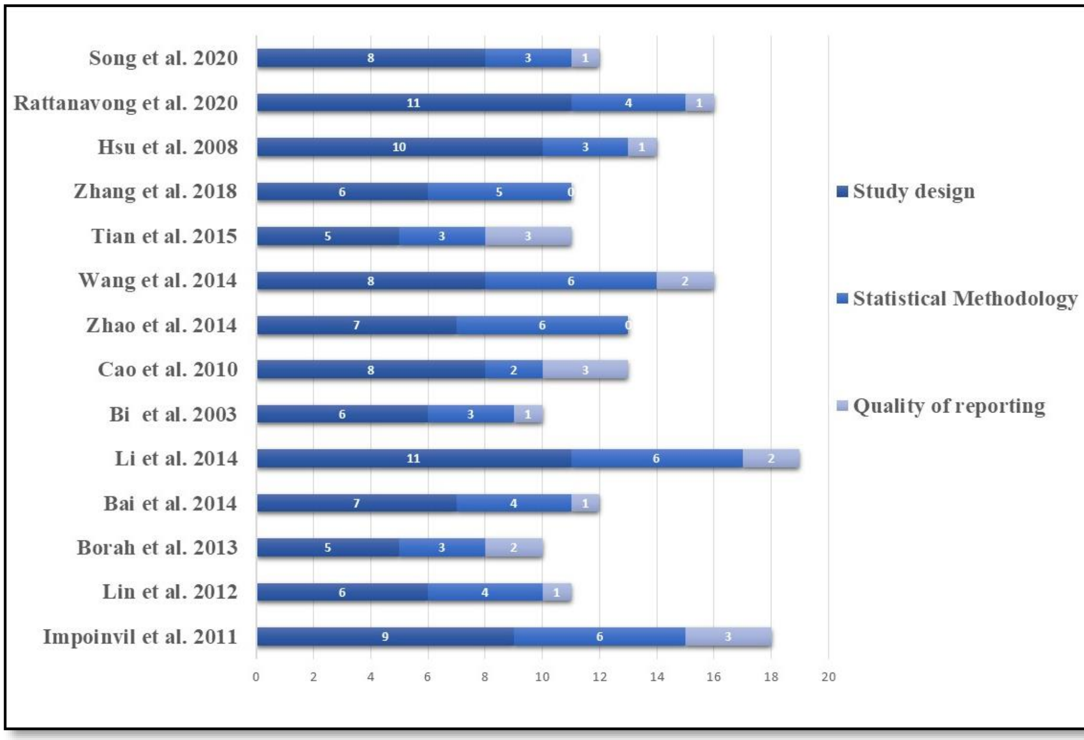

Figure 4.4. Risk of bias assessment of included studies

Discussion

This study aimed to systematically review the literature to understand how land use, land cover, and climate change factors are associated with the incidence of JE infection among Asian populations.

Presence of irrigated lands, paddy cultivation, and pig-rearing were found to be significantly associated with JE incidence. The studies included in the review found a positive association between land use, land cover, and JE incidence. Findings were consistent across geographies. Most of the studies (n = 14) were ecological designs, with inferences made at the population level. Thus, at the population level, the presence of irrigated fields, paddy cultivation, and pig-rearing increased JE incidence.

For climate change factors, the overall association between temperature variation, rainfall, and JE incidence were mostly consistent among the included studies. However, the mechanism underlying the effects of humidity and temperature are still unclear [48]. Findings were similar regardless of the different geographical regions studied within China, India, and Taiwan, except for the Shaanxi province of China which found an inverse association between minimum temperature and JE incidence between 2012 and 2014, and rainfall was inversely associated with JE incidence between 2006 and 2008. [46] The authors proposed that the period between 2012 and 2014 in Shaanxi province was marked by excessive precipitation so rainfall had a higher impact on JE incidence during this period than other meteorological factors such as temperature [46].

However, it is important to note at present there are a dearth of individual-level studies, and the included studies were population-level ecological studies except two individual-level study, i.e., case-crossover and cross-sectional [43,48]. These individual-level studies stated that JE incidence showed significant seasonality. Most cases occurred during summertime, and number of JE cases started to increase at a temperature of 22° C ($r^2 = 0.88$, $p < 0.001$) [48]. Based on the included ecological studies, we concluded that, at the population level, temperature change and rainfall were positively associated with JE incidence. For example, many studies (n = 11) reported that the JE incidence increased in summer months, during monsoon season (June - October) or periods of increased rainfall [14,37,45]. However, the study conducted in Nepal was an exception [35]. This study found a negative association between precipitation and JE incidence i.e., at population-level, the incidence of JE increased with decreased precipitation. To explain this anomaly, authors suggested that low precipitation precluded the immatures developmental stages of mosquito from being washed away in high water currents and thus, resulted in higher JE incidence [35].

The results of risk of bias assessment yielded seven studies of acceptable quality and three studies of high quality. Six studies were found to be of low quality as they scored very low on the study design and quality of reporting. Also, 14 out of 15 studies were ecological and have limitation of cross-level bias and ecological fallacy. We could only include two high quality individual-level studies, and remaining studies were population-level studies of low or acceptable quality. Additionally, a major limitation of included primary studies was related to their ecological nature. Ecological associations are difficult to interpret as they do not imply causality. Also, confounding is an important source of bias in the included studies [32]. Consequently, we could not conclude substantially about the exposures and JE incidence associations even at the population-level.

Based on the findings from this systematic review, it is recommended that future research must focus on conducting high-quality studies at the population and individual-levels. Planning a high-quality population-level study should focus on representative sample size, confounding factors, unbiased inclusion of ecologic units, selection of ecologic units should be based on study hypothesis and not influenced by convenience or necessity, and quality of reporting. However, most of the research conducted to date examines the association of environmental factors and JE incidence at the population-level. Despite the existence of a vaccine, there is still a high incidence of JE. The primary reason for high JE incidence is lack of understanding of individual-level factors that spread JE infection. At present, there is dearth of individual-level high-quality studies exploring this association. Thus, there is an urgent need to understand the individual-level factors to control and eliminate JE infection.

Furthermore, this review highlighted the current limitations in systematic review methodology in assessing the risk of bias related to ecological studies. We acknowledge that ecological studies are important tools in environmental health studies. In fact, many environmental health research questions may be best addressed using aggregate-level data, and many potentially meaningful associations can only be studied using ecological study designs, such as between neighborhood disparities and human health outcome, or evaluating the impact of community level interventions for example, seat belt laws, water fluoridation, mass media health campaigns. Additionally, ecological studies are very useful in scenarios where variability of an exposure is limited, such as community-level exposure to a pollutant through air or water, so here, ecological correlations provide more valid inferences as compared to individual level studies [50]. However, there is no established tool to assess the quality of ecological studies included in a systematic review. Thus, current risk of bias or study quality assessment tools automatically downgraded the quality of evidence of ecological study designs because of inherent challenges and biases in using aggregate data. Thus, this review recommends designing a new tool or adapting an existing tool to assess the quality of ecological studies to appropriately appraise the biases in primary studies in environmental health research.

This review had a few limitations that should be acknowledged. First, we only searched published literature. Thus, this might lead to an overrepresentation of significant findings (publication bias). However, we included several databases and hand-searched several journals, so the likelihood of missing published literature is minimal. Second, many of the articles were not available in the English, Hindi, and Nepali languages. The authors team included two multi-lingual individuals so only articles not in English, Hindi, and Nepali were excluded. Thus, due to the lack of translation services we had to exclude articles in other languages that the authors could not translate themselves. This might have resulted in loss of valuable information on related research published in other languages.

Conclusions

We identified clear associations between land use, land cover, climate change, and JE incidence. Specifically, increasing temperatures and precipitation, presence of rice paddies and pig farms were positively associated with JE incidence across most studies. However, inferences must be made with caution as these associations are valid only at the population-level, as most identified studies were ecological. More individual-level studies are needed to make individual-level inferences.

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Appendix A.

Table 4.2. Search strategy

Query	**Results found**	**Remarks**
(Encephalitis,Japanese OR Encephalitis,Japanese OR Encephalitis virus, Japanese OR Japanese encephalitis virus* OR JEV OR JE) AND (Climate change OR Climate* OR Geography* OR Agriculture* OR Land us* OR Land cove* OR Environmental monitoring OR Environmental exposure OR Environmental restoration and remediation OR Environment OR Climatic processes OR Droughts OR Floods OR Rain OR Weather OR Environ* OR Environmental factor* OR Landscap*) AND (Disease outbreak* OR Incid* OR Outbreak* OR Infectious disease outbreak* OR Disease outbreak, infectious OR Infection rate* OR New case* OR Incidence rate* OR Number of outbreak* OR Outbreak disease* OR Endemic* OR Epidemi*)	1118	Search limited to humans and years from 1950 to 2021

Appendix B.

Table 4.3. Quality assessment of ecologic studies: template and definitions

Evaluation criterion	Categories	Definition	Points (max=21)
STUDY DESIGN (max=12)			
Design	Cross-sectional Longitudinal	If it is a multi-level design (e.g., ecologic + individual), the study is upgraded one point (e.g., cross-sectional + multi-level receives 2 points)	1 2
Sample size	< 80% units ≥ 80% units	Number of ecologic units included in the analysis as proportion of the total number of units, e.g., 119 countries of a total of 180 worldwide would be 66%.	0 1
Unbiased inclusion of units	No Yes	Were the units included representative of the group for which inferences are being drawn? For example, for worldwide inferences, inclusion of only developed countries would be biased.	0 1
Level of data aggregation	Other than below Regional, State National	Population to which the units refer to. "Other" may be city, race groups.	1 2 3
Level of inference	Individual or unclear Ecologic	Use of the results of the analysis of the study's sample data to draw inferences for individuals or groups (ecologic).	0 1
Pre-specification of ecologic units	No Yes	Where the ecologic units selected to suit the hypothesis? (as opposed to selection motivated by convenience or necessity)	0 1
Outcomes of interest included	Some All	Inclusion of all relevant outcomes (i.e., maternal and neonatal mortality and morbidity) or only of some outcomes.	1 2
Source of data	Inadequate Adequate	Validity of the sources of data to represent the level that it refers to (e.g., the CS rate for one single hospital in one city would be an inadequate source of data to represent the national CS rate).	0 1

Table 4.3. Continued

Evaluation criterion	Categories	Definition	Points (max=21)
STATISTICAL METHODOLOGY (max=6)			
Analytic methodology	Spearman's rank correlation, Linear least square regression models, Quadratic model, Exponential model, LOWESS, Fractional polynomial regression Piecewise regression, **Spatial statistics**	All statistical methods are acceptable if they are used appropriately. We assign a score based on the sophistication and flexibility of the method. 1 = Spearman's rank correlation, Linear least square regression models, Quadratic model, Exponential model 2 = LOWESS, Fractional polynomial regression, Piecewise regression, **spatial statistics**	1 2
Validity of regression	No Yes	Did the adjustment have at least 10 units per covariate?	0 1
Use of covariates	None Socio-economic Socio-economic + clinical	Authors adjusted the analysis for desirable variables or not. Examples of socio-economic covariates: GDP or HDI. Examples of clinical covariates: proportion of women with diabetes or hypertensive disorders or BMI.	0 1 2
Proper adjustment for covariates (yes)	No Yes	Are the outcomes standardized or adjusted for certain factors before model adjustment? For standardized or adjusted outcomes, the standardized or adjusted factors should be included in the adjustment model. If standardized/adjusted outcomes are not used, this criterion is considered to have been met.	0 1
QUALITY OF REPORTING (max=3)			
Statement of study design (yes)	No Yes	Did the authors present key elements of study design in the paper?	0 1
Justification of study design (yes)	No Yes	Did the authors justify the ecologic analysis, the rational and the specific objectives, including any prespecified hypotheses?	0 1
Discussion of cross-level bias and limitations (yes)	No Yes	Did the authors caution readers about the limitations of the ecologic design, the ecologic fallacy, the impossibility of extrapolating to a different level?	0 1

Appendix C.

Table 4.4. Risk of Bias Assessment of included ecological studies

ROB for Ecological Studies															
Study design: Ecological															
Citation		**Impoinvil et. al 2011**	**Lin et al. 2012**	**Borah et. al. 2013**	**Bai et al. 2014**	**Li et al. 2014**	**Bi et. al 2003**	**Cao et. al. 2010**	**Zhao et. al 2014**	**Wang et al. 2014**	**Tian et al. 2015**	**Zhang et al. 2018**	**Hsu et al. 2008**	**Rattanavong et al. 2020**	**Song et al. 2020**
	Study Design (max=12)														
	Design	1	1	1	2	2	1	1	1	1	1	1	1	1	1
	Sample size	1	0	0	0	1	0	1	0	1	0	0	1	1	0
	Unbiased inclusion of units	1	0	0	0	1	0	1	0	1	0	1	1	1	0
	Level of data aggregation	1	1	1	1	3	1	1	2	1	1	1	3	3	2
	Level of inference	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Pre-specification of ecologic units	1	1	0	1	1	1	1	1	1	0	0	1	1	1
	Outcome of interest included	2	1	1	1	1	1	1	1	1	1	1	1	2	2
	Source of data	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Statistical Methodology (max=6)														
	Analytical methodology	2	2	1	2	2	1	1	2	2	1	2	1	2	2
	Validity of regression	1	1	1	1	1	1	1	1	1	1	1	1	0	0
	Use of covariates	2	0	0	0	2	0	0	2	2	0	2	0	1	0
	Proper adjustment for covariates (yes)	1	1	1	1	1	1	0	1	1	1	0	1	1	1

Table 4.4. Continued

ROB for Ecological Studies															
Study design: Ecological															
Citation		**Impoinvil et. al 2011**	**Lin et al. 2012**	**Borah et. al. 2013**	**Bai et al. 2014**	**Li et al. 2014**	**Bi et. al 2003**	**Cao et. al. 2010**	**Zhao et. al 2014**	**Wang et al. 2014**	**Tian et al. 2015**	**Zhang et al. 2018**	**Hsu et al. 2008**	**Rattanavong et al. 2020**	**Song et al. 2020**
	Quality Of Reporting (max=3)														
	Statement of study design (yes)	1	1	1	0	1	0	1	0	1	1	0	1	1	1
	Justification of study design (yes)	1	0	0	1	1	0	1	0	1	1	0	0	0	0
	Discussion of cross-level bias and limitations (yes)	1	0	1	0	0	1	1	0	0	1	0	0	0	0
	Total score	18	11	10	12	19	10	13	13	16	11	11	14	16	12
		Impoinvil et al. 2011	**Lin et al. 2012**	**Borah et al. 2013**	**Bai et al. 2014**	**Li et al. 2014**	**Bi et al. 2003**	**Cao et al. 2010**	**Zhao et al. 2014**	**Wang et al. 2014**	**Tian et al. 2015**	**Zhang et al. 2018**	**Hsu et al. 2008**	**Rattanavong et al. 2020**	**Song et al. 2020**
	Study design	9	6	5	7	11	6	8	7	8	5	6	10	11	8
	Statistical Methodology	6	4	3	4	6	3	2	6	6	3	5	3	4	3
	Quality of reporting	3	1	2	1	2	1	3	0	2	3	0	1	1	1
	Total score	**18**	**11**	**10**	**12**	**19**	**10**	**13**	**13**	**16**	**11**	**11**	**14**	**14**	**14**

Chapter V

Conclusions

Summary of Dissertation Research

This dissertation addresses three different issues related to environmental health epidemiology, i.e., associations between temperature, chemical nutrients/contaminants, and HABs due to *K. brevis;* effects of cycloxaprid on target and non-target species; and associations between environmental factors and JE infection among Asian populations, using scoping and systematic review methods. Understanding these environmental exposures using review methods has public health relevance specifically related to the 'one health' concept, i.e., the health of the population is interconnected with the health of the animals and their shared environment [1]. Therefore, this approach offers unique opportunities to synthesize heterogenous evidence and inform mitigation and future research strategies to health issues at the human-animal-environment interface. Further, systematic review methods are instrumental in making policy recommendations due to its unique element of systematically collating data from primary studies to attain unbiased actionable conclusions, and scoping reviews are mostly conducted to summarize the breadth of existing evidence on variables of interest to find avenues of future research.

The innovative contribution of this dissertation is the use of review methods to critically apprise the existing literature to (i) explore the breadth of recent research related to the associations between temperature, nutrients/contaminants, and *K. brevis* blooms in the North American region to identify research gaps for guiding future research questions; (ii) understand if the environmental effects of novel insecticide CYC are less harmful than the existing neonicotinoid class of insecticides; and (iii) conduct a comprehensive synthesis of less understood association between the environmental factors and JE infection among Asian populations. This dissertation is an ensemble of three themes guided by review methodology and organized as a multi-part thesis.

Theme 1: Understanding the interrelationships between chemical contaminants, harmful algal blooms, and temperature: A scoping review

Exploring and summarizing the breadth of the existing literature about associations between temperature, chemical nutrients/contaminants, and HAB due to *K. brevis* is the initial step in understanding this association and identifying future research strategies and needs. The use of scoping review methodology was important to rapidly review the currently available literature and obtain an overview of the existing scientific evidence. We used the scoping review methodology framework by Arksey and O'Malley [2] for reporting this study as Chapter 2 of this dissertation. In this study, we focused on the *K. brevis* events in the North American region only. We did a keyword search in Web of Science, Academic Search Complete, and PubMed databases. This search yielded 2276 citations. However, after multilevel screening based on our inclusion and exclusion criteria, we were able to include eight studies in the scoping review. The potential reason for finding only eight citations to include in this review could be partially due to our search being restricted to finding published literature only and did not include grey literature; searched only for articles published in English language; and the overall scarcity of studies examining the associations between temperature, chemical nutrients/contaminants, and *K. brevis* blooms. The included studies were a combination of field and laboratory studies, and overall, the included studies explored the red tide events due to *K brevis* between 1955 and 2018.

This review concluded that the included studies, except for the Dixon et al. [3] study, were suggestive of associations between water temperature (optimum range 22-28°C), and chemical nutrients/contaminants, i.e., salinity and nitrogen were important in terms of limiting the growth of *K. brevis* blooms. Further, included studies emphasized that the source of the nitrogen might be anthropogenic, however, none of the studies descried the human/anthropogenic activities that led to an increase in chemical nutrients/contaminants in the aquatic system. Moreover, proxy indicators were used to understand the chemical nutrient/contaminant concentration in the water body, thus highlighting the need to increase chemical nutrient/contaminant data availability in the aquatic systems. Some of the included studies were also challenged due to the poor quality of HAB data. Besides, this review also suggested that the changes in the future weather conditions will affect the summer-fall temperature which might cause a potential shift in the frequency and distribution of the *K. brevis* bloom. Consequently, this review recommends that researchers will need new strategies to increase the availability and accessibility of nutrients/contaminants data in aquatic systems as well as enhancing nutrient/contaminant data coverage in the aquatic systems to better understand the suggestive shift in the distribution of *K. brevis* bloom in North American water.

Regardless of the nature of this review being rapid and summarizing only the breadth of evidence, the findings of this review were valuable in highlighting future research needs. First, this review captured the increase in the frequency of HAB events in the past 50 years and suggested a further shift due to change in future weather conditions and anthropogenic drivers of chemical nutrients/contaminants. Next, this review highlighted how researchers were challenged due to data scarcity. Thus, this review could be crucial in providing direction to the future research trajectory related to *K. brevis* bloom events in the North American region. However, we acknowledge that the findings of this review are limited due to the scarcity of studies exploring the association between these variables of interest and *K. brevis* bloom. Therefore, we recommend conducting more focused studies exploring this association in the North American region.

Theme 2: Known target and nontarget effects of the novel neonicotinoid cycloxaprid to arthropods: A systematic review

Chapter 3 of this dissertation focused on the environmental effects of a novel insecticide, of neonicotinoid class, CYC. This insecticide was recently discovered and the effect of this insecticide on target and non-target species are under development. Imidacloprid (IMI) is the most widely used neonicotinoid insecticide globally. However, the recent evidence claim that IMI has harmful environmental effects and negatively impacts non-target species, such as aquatic micro-invertebrates, plankton, crayfish, mollusks, and effects water quality. Thus, replacement with a more potent and less harmful neonicotinoid insecticide is desired. Hence, CYC gained popularity as a potential replacement of IMI as some of the studies claimed that CYC is specifically effective against IMI-resistant pests [4].

In this case, the use of systematic review methods to synthesize available literature to answer a focused research question was crucial and most appropriate. We did keyword searches in four conventional databases, i.e., Academic Search Complete, Google Scholar, PubMed and Web of Science, and hand-searched six relevant journals where related research are frequently published. Based on the inclusion and exclusion criteria screening, we included 25 studies in this review. The included studies were conducted either in the field or in the laboratory. This review found that under experimental conditions, CYC has lethal and sub-lethal effects on non-target species such as *Chrysoperla sinica, Apis mellifera, Harmonia axyridis, Eisenia fetida*, and *Daphnia magna*. Additionally, the data also suggests the potential efficiency of CYC in controlling insect pests. However, the research related to the effects of CYC on pollinators and other non-target species was found to be premature. Thus, based on the available data and empirical evidence this review suggested that it is too early to consider CYC as a suitable replacement of IMI.

This review serves a valuable purpose of practicing caution before introducing a new insecticide into the environment by conducting a comprehensive synthesis of existing evidence to provide an in-depth understanding of its environmental effects. This review recommended that further research on CYC should be conducted testing its effect on non-target species under different laboratory and field conditions to inform risk exposure of non-target species under changed climatic conditions. Additionally, future research should focus on investigating acute and chronic effects, resistance and cross-resistance to CYC among target and non-target species in different geographical locations. This review included only published studies, so we acknowledge that this review might be limited due to publication bias.

Theme 3: The effect of land use, land cover, and climate change on the incidence and outbreak of Japanese Encephalitis disease among Asian populations: A systematic review

Under this theme, we focused on understanding the effect of environmental factors on JE infection among Asian populations. Regardless of ample research to understand the epidemiology of JE diseases, JE disease in Asian populations continues to be a poorly understood and controlled disease. Recent evidence suggests role of socioeconomic and environmental factors in the spread and JE disease distribution. The understanding of the environmental factors is still premature to the complex nature of vector-host-environment interactions. The use of systematic review methods was most appropriate in synthesizing the evidence related to the effects of land use, land cover, and climate change on the incidence of JE disease among Asian populations. In chapter 4 of this dissertation, we reported this review using PRISMA methodology and the associated checklist [5].

We conducted literature search in Web of Science, Academic Search Complete, and PubMed, and hand-searched three related journals where such studies are published often. After multilevel screening, 15 original studies met the eligibility criteria and were included in this review.

Of the 15 studies, 14 studies followed an ecological study design with the remaining study following case-crossover study design. This review suggested statistically significant association between the presence of irrigated lands, paddy cultivation, and pig-rear farming, and JE infection. The findings were consistent across geographical locations. The overall associations between temperature variation, rainfall, and JE incidence were mostly consistent among the included studies, except for one study conducted in the Shaanxi province of China where minimum temperature had an inverse association with JE incidence between 2012 and 2014, and rainfall was inversely associated with JE incidence between 2006 and 2008 [6]. The authors proposed that the period between 2012 and 2014 in the Shaanxi province was marked by excessive precipitation, so rainfall had a higher impact on JE incidence during this period than other meteorological factors such as temperature [6]. Further, this review concluded that, at the population level, temperature change and rainfall were positively associated with JE incidence. For example, many studies reported that the JE incidence increased in summer months, during monsoon season (June - October) or periods of increased rainfall [7-9]. However, the study conducted in Nepal was an exception [10], this study found a negative association between precipitation and JE incidence. To explain this anomaly, authors suggested that low precipitation precluded the immature developmental stages of mosquitos being washed away in high water currents and thus, resulted in higher JE incidence [10].

Study quality assessment found only nine studies of high and acceptable quality. Also, most of the included studies were ecological so we could not draw meaningful conclusions about JE incidence association and environmental exposures of interest, as ecological studies do not imply causality. This review is important as it revealed that there is dearth of individual-level high quality studies exploring the associations in question, and the understanding of individual-level factors is crucial for JE infection control and mitigation activities. Thus, this review underscores the unmet need of individual-level high quality studies to inform JE infection mitigation strategies. Furthermore, this review recommended that future research must focus on conducting high quality population-level and individual -level studies to attain comprehensive and in-depth understanding of association between environmental factors and JE disease among Asian populations.

However, this review also highlighted the current limitations in systematic review methodology in assessing the risk of bias related to ecological studies. Ecological studies are important tools in environmental health studies. In fact, many environmental health research questions may be best addressed using aggregate level data, and many potentially meaningful associations can only be studied using ecological study designs, such as between neighborhood disparities and human health outcome or exposure related to certain communities and occupations, for example, vector-borne diseases affecting rural or agricultural communities. Furthermore, ecological studies have great importance in scenarios where variability of an exposure is limited, such as community-level exposure to a pollutant through air or water, so here, ecological correlations provide more valid inferences as compared to individual-level studies [11]. However, there is no established tool to assess the quality of ecological studies included in a systematic review. Thus, current risk of bias tools automatically downgrade evidence based on ecological study designs because of inherent challenges and biases in using aggregate data. Thus, this review identifies an urgent unmet need to design a tool or adapt an existing tool to assess the quality of ecological studies to appropriately appraise the biases in primary studies in environmental health research.

In sum, the use of systematic review methods in addressing the three themes related to environmental epidemiology contributes not only to the in-depth understanding of these important environmental exposures on human and environmental health, but also highlights the importance of systematic and scoping review in environmental health research. This dissertation further acknowledges that the use of systematic review methods in environmental health research is wide and essential. This dissertation focused on the use of two types of systematic review methodologies only. However, we affirm that there is no single 'ideal type' of systematic literature review, and all literature review methods offer a set of tools that should be used appropriately by researchers [2]. Appropriately conducted systematic reviews are powerful tools which add timely and impactful evidence to the existing body of literature and therefore, are necessary tools in guiding evidence-based environmental health interventions and policy recommendations.

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Vita

Anisha Singh is a resident of Lucknow, Uttar Pradesh, India. She received her PhD in Public Health Sciences from the University of Tennessee, Knoxville in 2022, MPH in Social Epidemiology from Tata Institute of Social Sciences, Mumbai MH, India in 2013 and her Bachelor of Dental Surgery from the RML Avadh University, Lucknow UP, India in 2010. Her public health practice experience includes working with the Ministry of Health and Family Welfare, Government of India, and USAID, India, and her academic experience is in the field of environmental epidemiology. Her research interest focuses on understanding the interrelationships between climate, chemical exposure, and human health.