

Faculty of Biology, Jagiellonian University

METHODOLOGICAL WORKSHOP IN EVOLUTIONARY BIOLOGY FOR PHD STUDENTS

practical part devoted to systematic review



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Photos & cover by Joanna Rutkowska

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General Information

Participants of the course carry out systematic review of the literature of their interest. Systematic review is the first step of meta-analysis, but does not have to end-up with statistical analyses of the effects sizes. Instead, you stop at the point of coding the selected aspects of the full-texts. The resulting reports are written according the requirements of Biology Letters, reviewed by experienced external reviewers and corrected.

More information on the course is available in USOS
(https://www.usosweb.uj.edu.pl/kontroler.php?_action=katalog2/przedmioty/pokazPrzedmiot&kod=WB.SDSP.B%2FPhD-5).

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Topics initially suggested by course participants

1. If obesity taken into account in studies relating fertility to steroid hormones secretion (EM)
2. Role of leptin in female fertility. (EM)
3. Effect of adipokines in human reproduction. (EM)
4. The physiological effects of western diet on different rodent species (AH)
5. Non-alcoholic fatty liver prevalence comparison between humans and mice. (AH)
6. Western diet and reproduction: The sex-specific effect (AH)
7. Overview of papers that compared quality of open source geodata (MH)
8. Anthropogenic factors influencing forest biodiversity (MH)
9. Relationship between impervious areas and local temperature (MH)
10. Different behaviors exhibited by *Drosophila* males before mating (GB)
11. Temperature variation and life span in *Drosophila* (GB)
12. Satellite data and local biodiversity (GB)

What aspects of forest biodiversity are studied when there is a road barrier?



First version of the project

What aspects of forest biodiversity are studied when there is a road barrier?

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Summary

Worldwide forest biodiversity is degrading and declining at a rapid pace. The causes of this decline are diverse and cannot be simply explained. Most species survival depends on forest ecosystems, which is why forest biodiversity is an integral component of many ecosystem services. Forests serve in many different capacities, they counteract climate change, protect watersheds, prevent soil erosion, and provide habitat for animals and livelihoods for humans. Although infrastructure development remains a necessity in the modern world, the impact on nature that it causes is devastating. This issue has been studied for over a decade, and the damage of road construction to biodiversity and associated environmental degradation is well documented. Our review shows in which geographical scale and system research was conducted. It was assessed which species and which type of road were studied.

Keywords

Forest biodiversity, roads, infrastructure, forest function, systematic review

Introduction

Forest ecosystems and biodiversity around the globe decline at a rapid rate. The causes for the decrease are manifold and cannot be answered straightforwardly [1]. But the survival of many species depends on forest ecosystems, consequently forest biodiversity is an integral element of many ecosystem services [2]. Forests not only provide habitat for animals and livelihoods for

people, they also protect watersheds, prevent soil erosion, and mitigate climate change [3]. The rate at which forests are disappearing is alarming. Many factors contribute to the decline of forests. Although infrastructure development remains a necessity in the modern world, the impact on nature that it creates is devastating. This topic has been studied for over a decade and the damage of road construction on biodiversity and associated environmental degradation is well documented [4]. Roads act as barriers, impeding the movement of animals, fragmenting the landscape, and leading to the loss of animal and plant species [5,6].

In this study, we aim to identify which impacts have been most studied on the topic of road infrastructure on forest biodiversity. We will examine which species or environmental systems were considered and how the studies were conducted. The time span and data collection of the studies will be analysed, as it can provide information about the dynamics of changes in forest biodiversity.

Materials and Methods

We designed and conducted this study in a summer workshop (June 2022) during which we chose the broader theme to understand the pattern of studies on the forest biodiversity upon the road infrastructure. As a first step, we outlined the study in the PECO (Population, Exposure, Comparators, and Outcomes) framework (Supplementary Material, **Table S1**). Relevant keywords were sorted, and the published records were identified using *Scopus* and *Web of Science* databases. Search Strings

The following search strings were coded to fetch the articles.

Scopus: ((road* OR highway* OR motorway* OR route* OR speedway*) W/4 (construction* OR building* OR infrastructure* OR barrier*)) AND ((wood* OR forest*) W/4 (biodiversity OR diversity OR richness OR abundance* OR density OR stand* OR health OR function*))

Web of Science: road* OR highway* OR motorway* OR route* OR speedway*) Near/4(construction* OR building* OR infrastructure* OR barrier*) AND (wood* OR forest*) Near/4(biodiversity OR diversity OR richness OR abundance* OR density OR stand* OR health OR function*)

Initial literature screening

We could obtain a total of 550 articles from the databases (312 from *Scopus* and 238 from *Web of Science*). All the articles were imported to the Zotero reference manager (<https://www.zotero.org/>) for removing the repeated articles. After this deduplication, there were 347 articles. These unique records were uploaded to the software Rayyan (<https://rayyan.ai/>) to perform the initial screening based on the title, abstract, and keywords. The screening was carried out independently by the two authors. During this initial screening, we excluded records that did not fulfil all the criteria under the PECO framework. A detailed flowchart of the criteria and process of the initial literature screening is presented in **Figure S1** (Supplementary Material). A total of 110 articles were selected upon the completion of the initial screening.

Full-text screening

The selected articles after the initial screening were uploaded to the Zotero reference manager to retrieve full texts. While most of the full texts were available, 8% of the articles could not be accessed and were not part of the systematic review. 23.2% of the articles that were fully scanned were excluded at this stage. The articles for full-text screening were divided equally among the authors for independent analysis, however, the first 10% of articles were mutually screened. A *Google Form* was created with parameters to be taken from each paper to facilitate effective full-text screening. There were few articles where the full text was not available, sometimes the language of the manuscript was not in English, and non-peer-reviewed (mainly conference articles), and reviews. We excluded the articles under these categories and ended up screening a total of 99 literature. A detailed flowchart is presented in the PRISMA diagram (**Figure S2**).

Data Extraction

We extracted data on the year of publication of the respective articles, and the acceptance year was considered, and not the article submission date/year. The study species data were collected under the categories ‘plants’/ ‘animals’/ ‘fungus’/ ‘plants and animals’. The environmental system, the duration of the study, the geographical location (from regional to global), and the type of data used for the study were extracted directly from the literature (at the time the supplementary materials were also screened). The road and its type were also classified.

Results

Study species

With the completion of full-text screening and from the inclusion of studies under the theme of this review, we found that both plants and animals are almost equally studied (33 and 36 respectively). However, there are significantly less articles that are conducted to study both plants and animals (**Figure 1a**). During the full-text screening, we also had kept an option of fungus, however we found no studies on fungus.

Studied environmental system

We accounted for the most studied environmental system, and our data indicates that there are very few studies on specified systems like soil (6.6%), fire (1.35) and water (2.6%). Most of the studies were in general looking at the effects and patterns on ecosystems and not single resources (89.5%). The frequency of studies on the environmental systems are presented in **Figure 1b**.

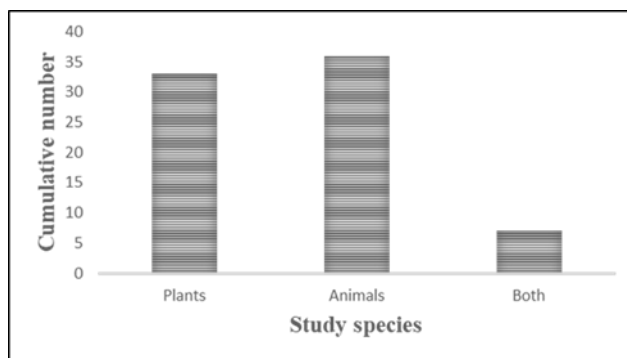


Figure 1a

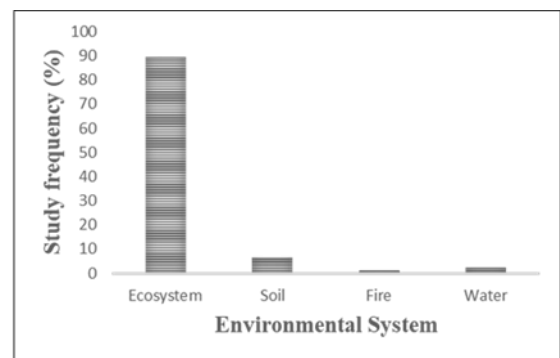


Figure 1b

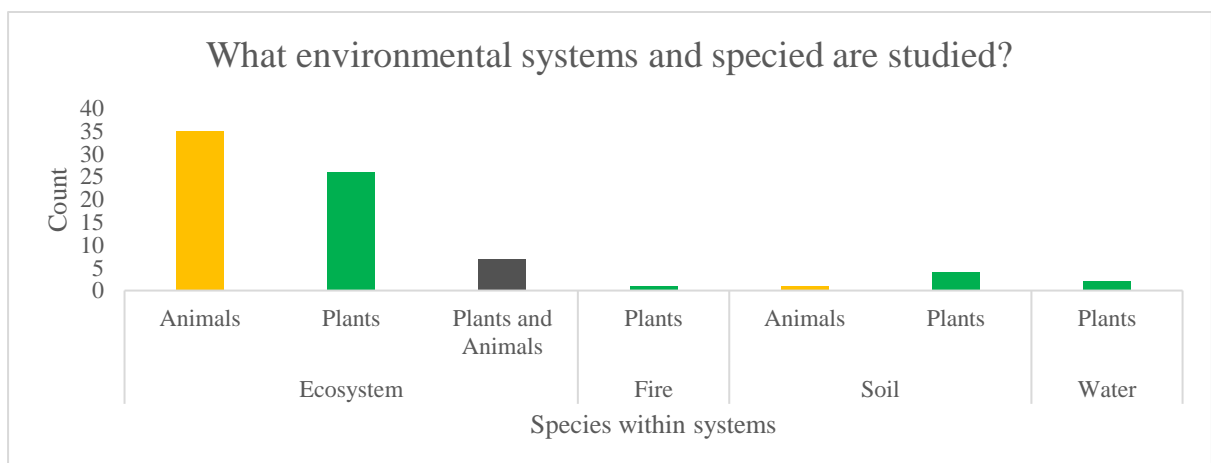


Figure 1: The number of articles that reports the study on plants, animals and or both (Figure 1a); frequency of studies on the environmental systems (Figure 1b), which species were studied in which system (Figure 1c).

The study year and frequency of publications

We found that the studies that fit in the spectrum of this review range from the year 1982 – 2022. Our data suggests comparatively there are less studies happened in the 20th century (over the span of 18 years). Long term research is necessary when looking at forests and also on road impacts, over 50% of the conducted research was done over a period of years or decades. There is an evident spike in the number of articles especially from 2005, and in the last three years the numbers have almost doubled (**Figure S3**). This shows the importance of the research field and its growing interest in the scientific community.

Discussion

Forest biodiversity impacted by roads is mostly researched on regional scale. Only 12% of the research was conducted on country, continental or global scale. In most of the studies the roads were not defined. Road surface on the other hand was mentioned in over 50% of the articles. In these researches spatial data is essential and a variety of data was used from satellite images to camera traps to assess the forest biodiversity in relation to anthropogenic factors e.g. roads. Satellite images and ground-truthed data was used over 60% of the cases. Especially in the field of remote sensing data is increasing in quality and can therefore provide valuable insight in regions which are not easily accessible or show changes over time. The decrease in forest cover is a threat to many species. In this systematic review the search criteria could have been defined more precisely to get a better understanding of which systems were studied and how they correlate to roads and species.

Acknowledgements

We would like to thank our course coordinator Prof. Dr. Joanna Rutkowska, for providing constructive advice and guidance for the successful completion of this review. GB thank the Doctoral School of Exact and Natural Sciences, Jagiellonian University, Krakow, and the National Science Centre (NCN), Poland for the financial support.

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Reviews

Małgorzata Łagisz

The authors conducted a systematic review of literature focusing on the effects of roads on forest biodiversity. The manuscript requires further work to improve logic and clarity of the writing. I have the following minor technical concerns and suggestions (no line numbering provided):

1. The first search string misses the field code, the second misses the field code and opening brackets. For WoS, you should also note which sub-databases were searched (this varies between subscribing institutions). Search date not reported.
2. Data extraction: More details on data coding should be provided (meta-data, in a table in the main text or as SI files). It should be replicable.
3. Figure 1 legend: change “species” to “organism types”. Striped bars are distracting.
4. Discussion – looks like some of the data discussed has not been presented in the Results section.
5. No section/acknowledgement of study limitations.
6. No list of excluded full-text studies (with reasons), no list of included studies, no extracted data, no meta-data and analytic code.

SI Table1: “Knowledge gap in research” is not Outcome in PECO

Figure S2: Numbers in the topmost box do not match

Figure S2: description too vague – all articles globally?

Piotr Zieliński

In this article the authors want to answer the question: which aspects of forest biodiversity are studied in respect to road barrier. I agree that the topic chosen by the authors is interesting, timely and might reveal which “aspects” require more attention and which might be already over studied thus further studies might be focused on the area that truly require more work. However, my expectations are just partially met. To me one of the major findings of the study is that the fungi seem to be heavily understudied in respect to road barriers while studies on animals and plants seem to be equally numerous. Nevertheless, I don’t see any discussion of this finding in the discussion section! To me it is a pity that, there is nothing about the distribution of studies across phyla or classes which could reveal some interesting patterns within plants and animals. Unfortunately, to me the meaning of the word “aspects” in the title is not clear and is has not been clarified within the manuscript. It was not clear if the “aspects” of biodiversity are plants, animals and fungi or rather something else? Actually I’m missing an explanation why classification to plants, animals and fungi was chosen?

Below I put some more specific comments:

Line 2: explanation for affiliation 4 and 5 is missing

Summary section: actually no result and any outcome is given there. To me it is a summary of the introduction rather than a whole manuscript.

Keywords: change dots to commas

Line 23: manifold

Introduction: I’m missing more citations on: the rate at which forests are disappearing (line 27), impact of infrastructure on nature (lines 28 and 29).

Line 33: authors say: “we aim to identify which impacts have been most studied on the topic of road infrastructure on forest biodiversity”. First it is not clear what do mean by “impacts”? Second what do you mean by “forest biodiversity”. Do you mean here which organisms or rather which biological process have been studied in respect to roads?

In general, in the introduction but before aims I’m missing a short explanation why you want to tackle this issues? I mean I was missing a paragraph about possible uneven distribution of focus in the studies and the importance of it. So the reader has the notion that such a review as yours is really needed.

Line 34: “environmental systems” meaning of this is not clear

Line 35-37: authors say: “The time span and data collection of the studies will be analyzed, as it can provide information about the dynamics of changes in forest biodiversity”. It does not convince me that there is a clear relation between the date of study being published and the dynamics of changes in forest biodiversity. To me it might be also related to the change of scientific focus through years.

Line 40: I would remove “the broader theme” since it brings nothing to the sentence

Line 62-63: authors say: “During this initial screening, we excluded records that did not fulfil all the criteria under the PECO framework”. To me it would be clearer if you would exactly say which criteria, since this step seems pretty important for the results as it removes 2/3 of the articles. Figure S2 is also not helping to understand this step.

Line 70-71 and 75: it seems that this is a repetition: “8% of the articles could not be accessed” & “There were few articles where the full text was not available”

Line 76: remove “in” before English

Line 78: “articles” instead of “literature”

Line 84: “plants” and “animals” are plural here while “fungus” is singular - change to “fungi”

Line 86: what do you mean “type of data” please make it clear

Line 87: “road and its type were also classified” – classified to what categories? Be more clear here. What does it mean that the “road and its type”?

Line 90: consider changing “theme” to “topic”

Line 91: I would change “equally studied” to “equally often studied”

Line 92: you use a word “significantly” do you refer here to statistical significance (as it is usually assumed in scientific articles)? If yes, then you should write which statistical test you used and what was the p value.

Line 94: consider changing “fungus” to “fungi” or “funguses”

Line 96: authors say: “We accounted for the most studied environmental system”. First it is not clear to me what you mean by “environmental system” – please clarify. Second it is not clear how did you “accounted for” it? This also requires clarification. Third I don’t see much biological relevance in system classification to: ecosystem, soil, fire and water. I wonder why such a classification was performed. Maybe authors can explain it better and/or change it?

Line 97-99: authors say: “Most of the studies were in general looking at the effects and patterns on ecosystems and not single resources (89.5%).” To me it is really hard to grasp what authors really want to say here. What are “the effects and patterns” and what are “single resources”? Here I think it requires more clarification. I also think that manuscript would benefit from use of one, common terminology throughout the text.

Figure 1c: change “specied” to “species”

Line 109: change “spectrum” to “scope”

Line 110-111: First, “less” in comparison to what? Second, “happened” change to done / performed / undertaken. Third, can you provide a p value to support such conclusion?

Line 111: consider changing “research” to “studies”

Line 112-114: To support your statement you can provide some correlation coefficient. To me it seems that from 2006 not 2005.

Line 117-118: authors say: "Only 12% of the research was conducted on country, continental or global scale". First, consider changing "country" to "national". Second, and most importantly, this information is first said in discussion. I don't see such a result nowhere in the results section (in none of the figures or tables or text). I think that such a result should find their place in the results section and later just be discussed in the discussion section.

Line 118-119: explain what do you mean by saying: "In most of the studies the roads were not defined". Is it about classification to a certain category of road?

Line 119: "Road surface on the other hand was mentioned in over 50% of the articles" – once more this is rather a result and should be given in the results section.

Line 122: "Satellite images and ground-truthed data was used over 60% of the cases" – this is also rather a result. Besides "ground-truthed" is not clear to me. Finally, the questions arise: if 60% of the data were satellite images and these "ground-truthed" data then what was the other 40%?

Line 125-127: I definitely agree! I would like also to see more discussion of the results in the discussion section, possibly with a broader perspective. Now discussion section mostly consists of results.

Ewa Młyczyska

The reviewed article concerns the impact of roads as barriers on forest biodiversity. The authors tried to determine how the aspect of forest biodiversity has been studied so far in the context of the effects of the road formation crossing forests. It was determined what part of the environmental system was studied and whether research focused on the effects on plant and/or animal species. In addition, the authors checked the time frame of the studies, trying to answer the question of whether there were studies analyzing long-term effects. Considering how the natural environment is changed by humans nowadays, including also disruption of forest integrity which might lead to global changes, the systematic review proposed by the authors is very interesting and necessary.

The introduction is clearly presented and the main problems are outlined. The methods used and the PECO model correspond to the assumptions made. The text is stylistically correct and clearly written. Nevertheless, it was not without a few errors and aspects that can be improved to make the work better.

The main aspect that could be improved is the discussion section. The authors did not discuss exhaustively the obtained results. Additionally, I did not notice any citations in this part of the text. Therefore, I suggest extending the discussion and referring to the analyzed articles. The whole work has only 6 references, I think mainly because of a little extensive discussion. It should definitely have more articles cited. Subsequently, the references section should be changed as required by the journal to which the authors submitted the article. There should definitely include doi number.

Apart from these comments, I have minor remarks for a quick correction:

- Affiliation for one of the authors was not given
- I also suggest changing the captions within Figure 1 instead of Figure 1a, Figure 1b just enter the letters A, B, and C in the upper left corner.
- Authors should provide the number of funding received from the Doctoral School of Exact and Natural Sciences, Jagiellonian University, Krakow and the National Science Center (NCN).

Forests are dramatically lost modifying its biodiversity. Anthropogenic effects are the main drivers of forest biodiversity loss. In this review, the authors focuses on the available literature that specifically studied the impacts of road barrier infiltrating the forests on its biodiversity. The authors showed that the majority of the articles considered how roads disturb plant and animal biodiversity one species at a time, while non or only few articles studied fungi biodiversity or the interaction between two or more species. It also showed that although researchers work on studying the pattern generally on the ecosystem, more studies are conducted nowadays and probably the more specific the articles will be. This increase in the number of publications highlight on the detrimental effects of road barriers on the forest biodiversity, making this review an interest for most of the ecologists.

Major comments:

- 1) The abstract and the introduction are written in a similar way. Sentences were written in one part and just paraphrased in the other part without any modification. The authors should keep one part, preferably the abstract, and completely or partially modify the other part.
- 2) The discussion part is only represented by one paragraph, and it should be extended more. This would allow to include more references making the discussion easier to understand.

Minor comments:

- 1) The title is written as a question, and it would be better to be written as an informative sentence.
- 2) The affiliation of one of the author is missing.
- 3) The title of the main parts example “SUMMARY...” should be written in capital letter to differentiate the major titles from the minor ones.
- 4) In “Data Extraction”, the environmental system as well as the classified types of roads were not written clearly enough.
- 5) In figure 1, figure 1c is not attached to figure 1a and figure 1b. Both the three images in addition to figure S3 should be combined all together to represent only one image.
- 6) Figure 1a, figure 1b, and figure 1c should be replaced by only A, B and C respectively.
- 7) The title of figure 1c, contains a spelling mistake. it should be “Species” instead of “specied“. In addition to that, the title of this graph should not be a question.
- 8) The references are not written according to the author’s guideline.
- 9) Figure S2 and Table S1, would be better if included in the article, and not put in the supplementary materials.

Final version of the project

What aspects of forest biodiversity are studied when there is a road barrier

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SUMMARY

Forest biodiversity is declining at a rapid pace worldwide. One of the causes of this decline in biodiversity is infrastructure development. Although infrastructure development remains a

necessity in the modern world, the impact on nature that it causes is devastating. Most species survival depends on forest ecosystems, which is why forest biodiversity is an integral component of many ecosystem services. Forests serve in many different capacities: they counteract climate change, protect watersheds, prevent soil erosion, and provide habitat for animals and livelihoods for humans. The goal of this systematic review is to understand the impact of roads on forest biodiversity. We investigated which species were studied in forests related to road construction or road existence, which environmental system was examined, and the types and surface of roads that are studied. Furthermore, we looked at what types of data are used and how they evolve by the year the article was published. We searched Scopus and Web of Science and screened 550 articles, 110 of which were selected for the full-text screening. The results indicate that both plants and animals are studied almost equally. For environmental systems, most studies focused on ecosystems. Paved roads were the most studied road surface; however, there was an equal representation of undefined surface of roads in the articles. We found that there is an evident knowledge gap in the field of mycology.

KEYWORDS

Forest biodiversity, roads, infrastructure, forest function, systematic review

INTRODUCTION

Forests around the world are critical to both biodiversity conservation and climate change mitigation. The survival of many species depends on forest ecosystems, consequently forest biodiversity is an integral component of many ecosystem services [1]. Forests not only provide habitat for animals and livelihoods for people, they also protect watersheds, prevent soil erosion, and mitigate climate change [2]. The rate at which forest ecosystems and biodiversity are declining globally is alarming. FAO states that 420 million hectares of forest have been lost since 1990 [3]. The causes for the decrease are manifold and cannot be answered straightforwardly [4]. Forest decline is a complex socioeconomic, cultural, and political process. Therefore, it is wrong to attribute forest decline to a simple cause-and-effect relationship or to expect that this relationship will not change over time. A single driver, such as road building, can have different effects depending on the context of other variables and circumstances in a given situation. There are no simple solutions to this complex phenomenon [5]. Therefore, it is important to understand which anthropogenic factors are contributing to the forest decline and how. One of the major drivers of deforestation in the Amazon is road construction and its following contagious development [6,7]. Although infrastructure development remains a necessity in the modern world, the negative side effects on nature it entails are devastating [8]. The negative impacts of roads and road construction are particularly destructive in forests. They can alter the soil, hydrological and aquatic ecosystems. The opening of normally closed tree cover canopy can allow invasive species to enter [9]. Increases in poaching, mining, wildlife-vehicle collisions, and deforestation follow, once a road is built. These impacts have been studied in detail for over three decades, and the impact of road construction on biodiversity and associated environmental degradation are well documented [10]. Roads act as barriers, impeding the movement of animals, fragmenting the landscape, and leading to the loss of animal and plant species [11,12]. Even though fungi are an important component of ecosystems and are widely ignored in ecosystem research in relation to road building.

In this study, we aim to determine which impacts of road infrastructure on forest biodiversity have been most studied. We will examine which species have been investigated in relation to the ecosystem and road that surrounds them. We also want to know how the studies were conducted, what data were collected, and the time period over which the studies were conducted. Whether the data was collected at one point in time or whether the studies observed the forest and roads over months, years, or decades. The time period and data collection of the studies will be analysed as they can provide insight into the dynamics of biodiversity change in the forest.

MATERIALS AND METHODS

We designed and conducted this study in a summer workshop (June 2022) during which we tried to understand the pattern of studies on the forest biodiversity upon the road infrastructure. As a first step, we outlined the study in the PECO (Population, Exposure, Comparators, and Outcomes) framework (Supplementary Material, Table S1). Relevant keywords were sorted, and the published records were identified using *Scopus* and *Web of Science* databases.

Search Strings

The following search strings were coded to fetch the articles.

Scopus: ((road* OR highway* OR motorway* OR route* OR speedway*) W/4 (construction* OR building* OR infrastructure* OR barrier*)) AND ((wood* OR forest*) W/4 (biodiversity OR diversity OR richness OR abundance* OR density OR stand* OR health OR function*))

Web of Science: (road* OR highway* OR motorway* OR route* OR speedway*) Near/4 (construction* OR building* OR infrastructure* OR barrier*) AND (wood* OR forest*) Near/4(biodiversity OR diversity OR richness OR abundance* OR density OR stand* OR health OR function*)

Initial literature screening

We obtained a total of 550 articles from the databases (312 from *Scopus* and 238 from *Web of Science*) on June 1st, 2022. All the articles were imported to the Zotero reference manager (<https://www.zotero.org/>) to remove the repeated articles. After this deduplication, 347 articles remained for further analysis. These unique records were uploaded to the software Rayyan (<https://rayyan.ai/>) to perform the initial screening based on the title, abstract, and keywords. The screening was carried out independently by the two authors. During this initial screening, we excluded records that did not fulfil all the criteria under the PECO framework (mainly based on keywords, and content). A detailed flowchart of the criteria and process of the initial literature screening is presented in **Figure S1** (Supplementary Material). A total of 110 articles were selected upon the completion of the initial screening.

Full-text screening

The articles selected after the initial screening were uploaded to the Zotero reference manager to retrieve the full texts. While most of the full texts were available, 8% of the articles could not be accessed. 23.2% of the articles that were fully scanned were excluded at this stage (Table S2). The articles for full-text screening were divided equally among the authors for independent analysis, however, the first 10% of articles were mutually screened. A *Google Form* was created with parameters to be taken from each paper to facilitate effective full-text screening. There were few articles where the full text was not available, sometimes the language of the manuscript was not English, and non-peer-reviewed (mainly conference articles), and reviews. We excluded the articles under these categories and ended up screening a total of 99 literature. A detailed flowchart is presented in the PRISMA diagram (Figure S2).

Data Extraction

We extracted data on the most studied species, the environmental system, and the types and surface of roads. Further, to better understand the range/depth of each studies the time span, spatial scale and the type of data used in these studies were quantified.

RESULTS

Study species and environmental system

With the completion of full-text screening and from the inclusion of studies under the theme of this review, we found that both plants and animals are almost equally studied (33 and 36 respectively).

However, there are less articles that are conducted to study both plants and animals (Figure 1A). During the full-text screening, we also had kept an option of fungi, however we found no studies on fungi. Our data indicates that there are very few reports that studied exclusively soil (6.6%), fire (1.35) or water (2.6%). Rather, the substantial amount of study was focused on the ecosystem in general (89.5%). The frequency of studies on the environmental systems are presented in Figure 1B.

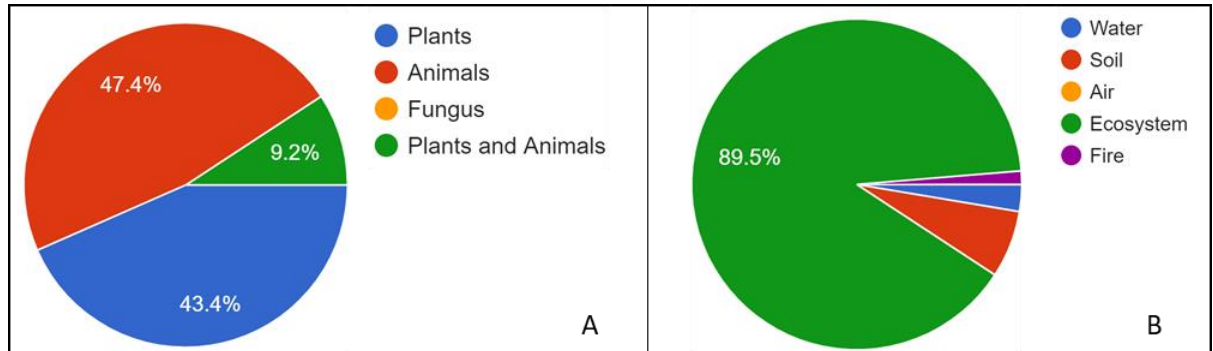


Figure 1: A) The percentage of articles that reports the study on plants, animals and or both; B) percentage frequency of studies on the environmental systems.

Different types of roads and surface

We have limited the study to 3 types of roads. The data show that there are relatively few studies that mention “highway” (13 articles), “forest roads” are mentioned in 16 articles and the broad category “road” is named in 46 articles. It is possible that most of the investigations generally looked the effects of roads than addressing to specific road types. To better understand the trend, we also investigated the patterns of the road surface. The number of studies on unpaved roads were comparatively low (12 articles). Paved roads were the most studied road surface with equal number of undefined road types (30 articles). This not defined road surfaces might have proximate correlation with the investigations that looked the road effects in general. Interestingly, there were minimal studies that covered both paved and unpaved road types (4 articles). The road type, their surface along with their corresponding article numbers are presented in Figure 2A and B respectively.

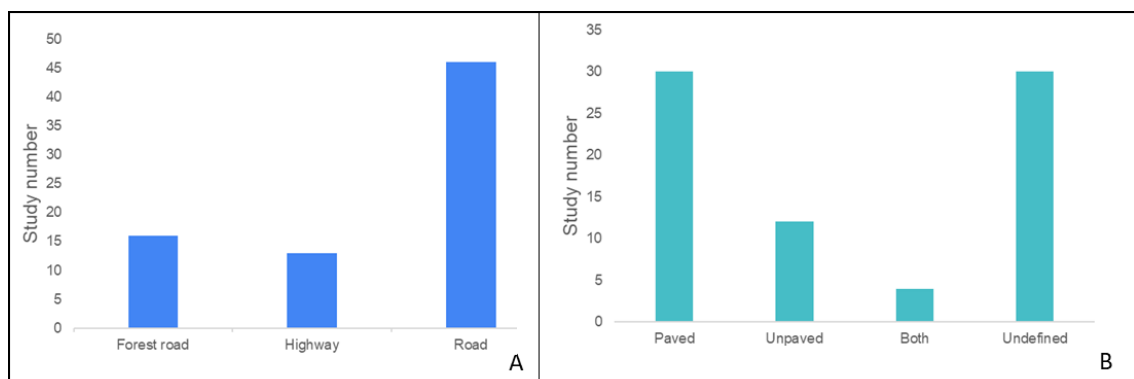


Figure 2: A) Three types of roads and the number of articles that investigate the road types; B) road surface and the respective study numbers.

Data type

We checked the different types of data used in the studies, as the availability and feasibility of such studies is highly dependent on the type of data. We found spatial data is the most utilized source

(44%). Satellite and ground-truthed data were almost equally contributed to the studies (24% and 22% respectively). The usage of invasive techniques like capture method, traps, sampling for data collection were minimal. The different types of data sources and their use in the publications studied are shown in Figure 3.

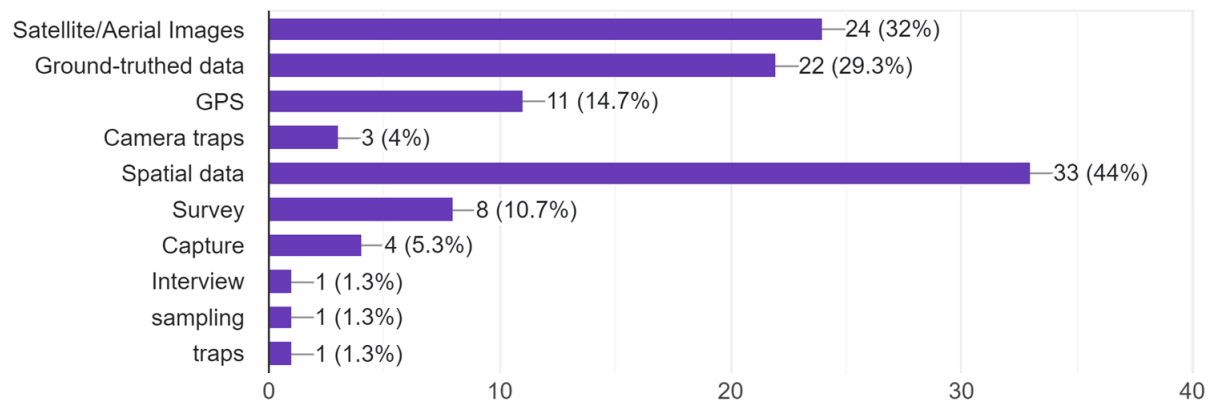


Figure 3: Geodata used in the studies

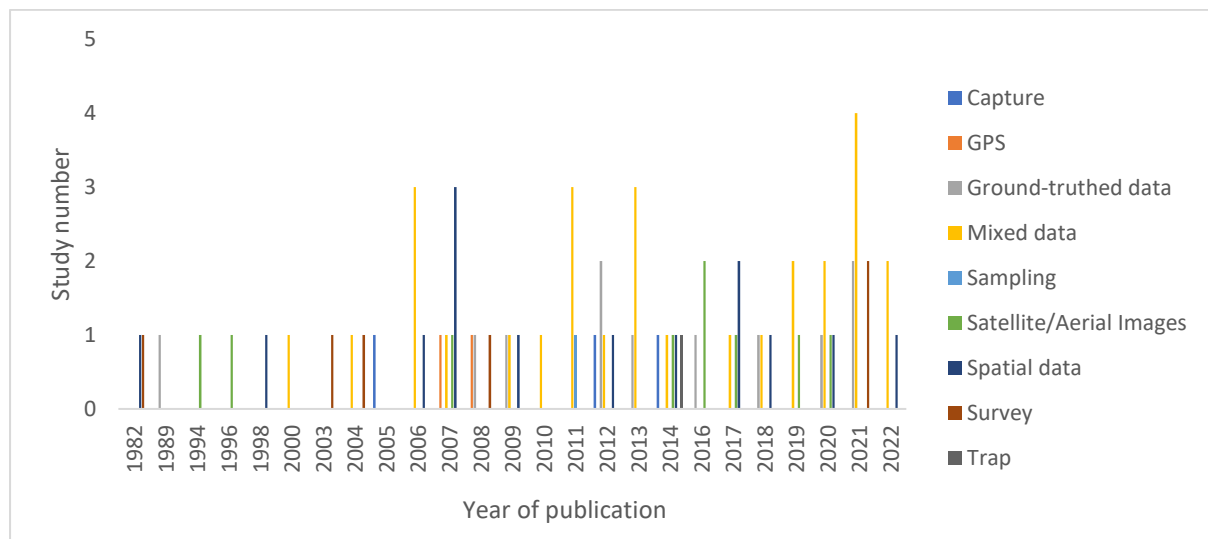


Figure 4: Geodata used in the studies classified by year of publication (articles that used several methods are listed under mixed data)

Study scale

To determine the magnitude and intensity of the published studies, we extracted the information on the time span and spatial structure of each study. The data clearly suggests that most studies were conducted over several years (45.9% of studies), and 27% of studies lasted several months. Only 14.9% studies ranged to decades. Surprisingly, there were 12.9% of studies where there was no indication regarding the study time span (Figure 4A). The spatial scale data show that most of the studies were conducted at the regional level. (88.2%). Studies on biomes, multiple countries, global scale were rare (1.3%). There were very few articles (3.9%) that reported results based on country and continental scale (Figure 4B).

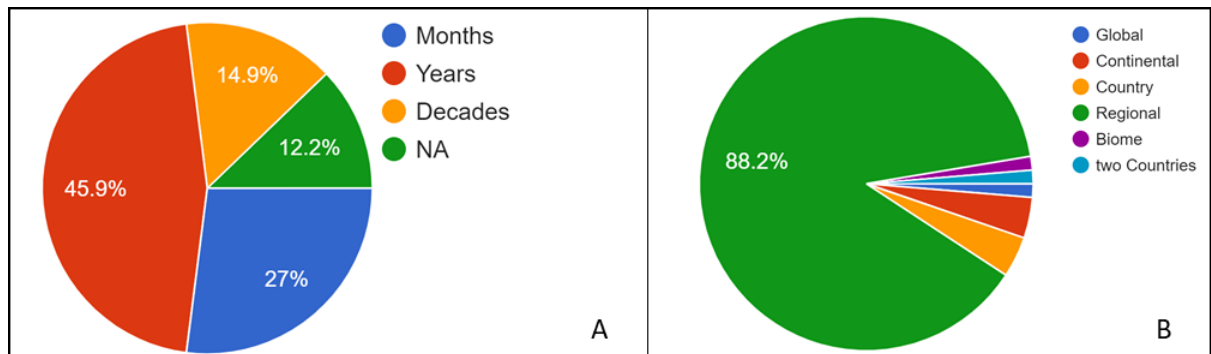


Figure 4: A) Percentage of articles with different time spans; B) studies with different spatial scale.

The study year and frequency of publications

We found that the studies that fit in the spectrum of this review range from the year 1982 – 2022. Our data suggests that there have been comparatively fewer studies in the 20th century (over an 18-year period). Long term research is necessary when looking at forests and on road impacts, over 50% of the conducted research was done over a period of years or decades. There is an evident spike in the number of articles especially from 2005, and in the last three years the numbers have almost doubled (Figure S3). This shows the importance of the research field and its growing interest in the scientific community.

DISCUSSION

The results of this systematic review show that in the field of forest biodiversity and roads as barriers, research in the field of mycology is not existing. This is a clear knowledge gap that should be addressed in future research. The importance of fungi to forest biodiversity is well known, and the fact that roads can alter fungal populations in forest soils should receive more attention [13]. We focused on animal and plant species, but the results would have been much more precise if we had distinguished between phyla or classes. This was not possible due to time constraints. Our results show that forest biodiversity impacted by roads is mostly researched on regional scale. Only 12% of the research was conducted on country, continental or global scale. These results show us that there is a knowledge gap when it comes to measuring the impact of roads on a broader scale. Especially in countries with high tree cover density, studies at the country, biome or even continental level would be beneficial to understand the impacts of roads on large forest ecosystems. In most of the studies the roads were not specified. Perhaps we should have defined our search terminology better here. However, it also shows us that most researchers do not specify the road category that was analysed. Road surface on the other hand was mentioned in over 50% of the articles. This is a very useful information, although the results show that few studies were conducted that examined both forest and paved roads. Most forest have logging roads and asphalted roads, but in only four articles analysed both paved and unpaved roads. Spatial data are critical in this type of research, and a variety of data have been used, from satellite imagery to camera traps, to assess forest biodiversity in relation to anthropogenic factors, such as roads. Satellite images and ground-thruthed data was used over 60% of the cases. Especially in the field of remote sensing, data is increasing in quality and can therefore provide valuable insight in regions which are not easily accessible or show changes over time. Figure 4 shows the increase in mixed data usages over time. This can be explained by the fast development in spatial equipment. Every phone has a GPS sensor and satellite images are accessible for scientific research, computers can process big data and drones can access previously inaccessible areas. This trend is therefore predictable. The decrease in forest cover is a thread to many species. In this systematic review the search criteria could have been defined more precisely to get a better understanding of which systems were studied and how they correlate to roads and species. However, even with these very broad search terms, clear results were obtained. The knowledge gap in fungal research as well as the spatial scale of research should be expanded.

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Supplementary materials

Contents

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Figure S1: Inclusion criteria and guidelines for the initial literature screening.

Figure S2: PRISMA diagram

Figure S3: The number of articles published every year from 1982 – 2022 (May 31st).

Table S2: Reasons for full text exclusion

Table S1: Aspects of forest biodiversity studied when there is a road barrier fitted in the PECO framework.

PECO Model	Attributions	Keywords
Population	Forest	Forest, diversity, richness
Exposure	Roads and road infrastructure	Road, infrastructure, highway, construction, motorway
Comparator	<ul style="list-style-type: none"> • NA 	
Outcome(s)	<ul style="list-style-type: none"> • Biodiversity • Road impacts assessment on forest biodiversity 	

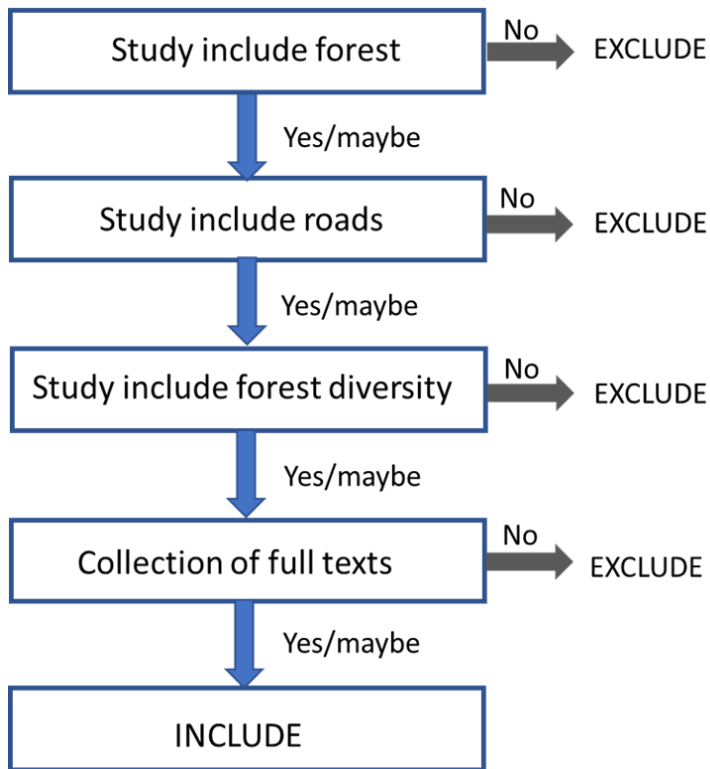


Figure S1: Inclusion criteria and guidelines for the initial literature screening.

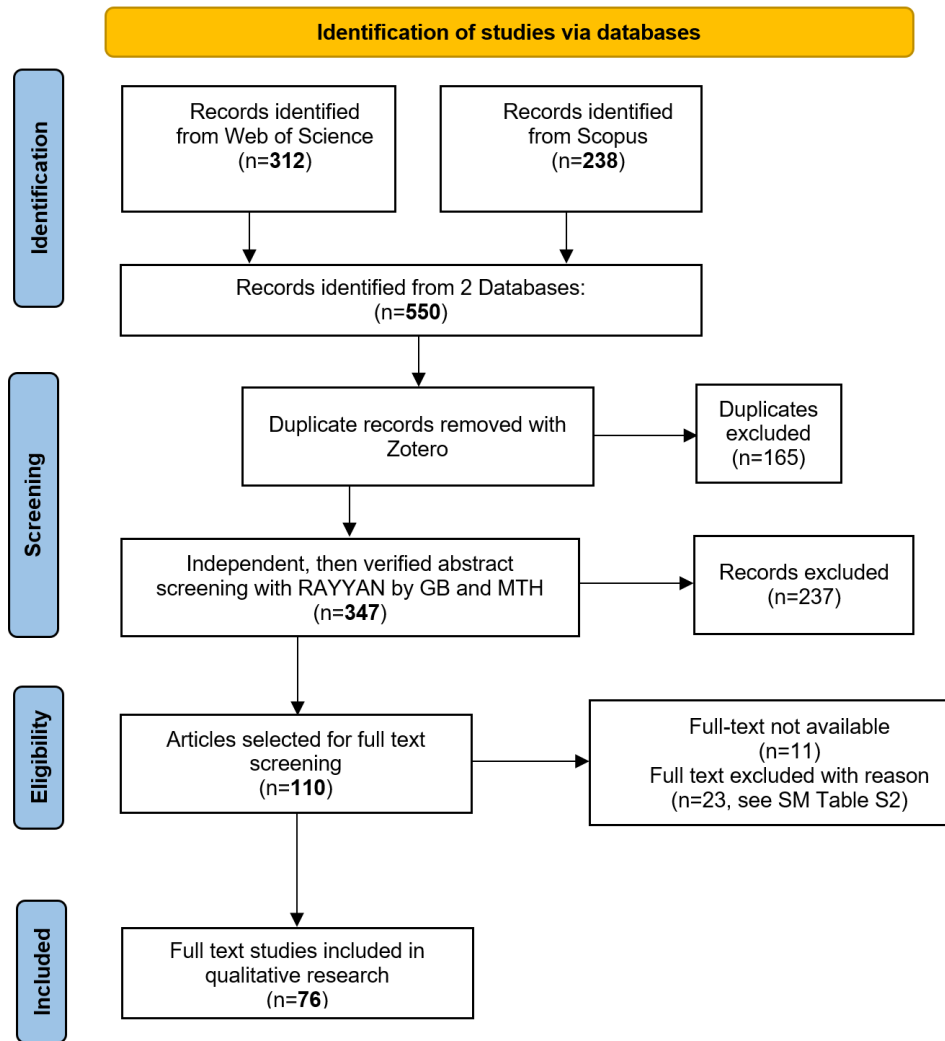


Figure S2: PRISMA diagram *modified by MTH* From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71

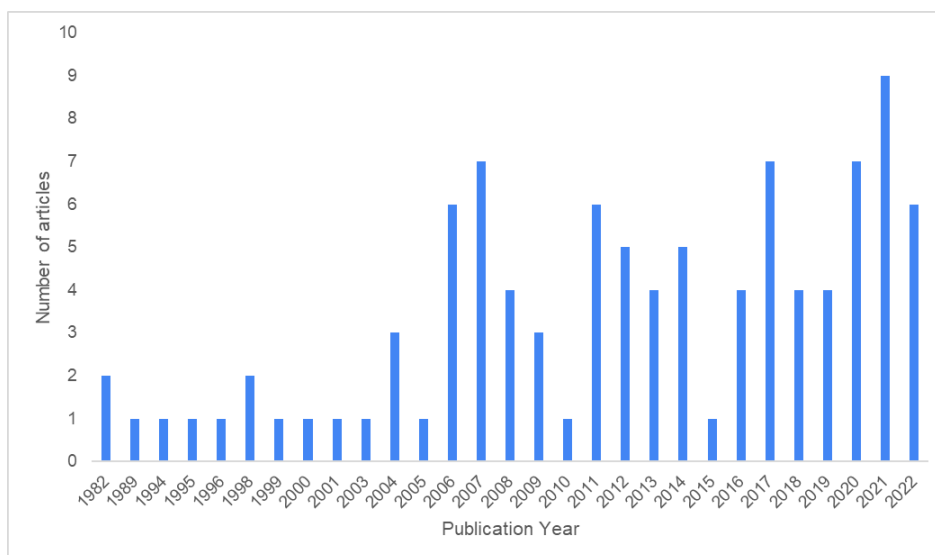


Figure S3: The number of articles published every year from 1982 – 2022 (May 31st).

Table S2: Reasons for full text exclusion

Article	Full text excluded with reason
1	About forest altitudes
2	Assessed the nature of roads constructed
3	Clear cutting in planted forest
4	Climate change is the main theme
5	Forest cover change without direct link to road
6	Management and potential solution proposing article
7	Management article
8	Management article
9	Mathematical modelling
10	Related to the life pattern of villagers
11	Review paper
12	Roads are not part of the study
13	Roads are not part of the study
14	Roads are not part of the study
15	Study Overview: two alternative routes to the proposed superhighway
16	Text not available in English
17	Text not available in English
18	Text not available in English
19	The aim of the paper is to present experiences and comprehensive solutions
20	The article deals about the forest regeneration
21	the study deals with the integration of RS, GIS and SPCA can effectively quantify and assess environmental vulnerability.
22	The study estimated the forest roads' total construction cost, the annual maintenance cost, and the skidding cost
23	Workshop recommendation

The effects of the Western diet on rodent reproduction



First version of the project

The effects of the Western diet on rodent reproduction

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ABSTRACT

Background: Western diet have detrimental effects on the organism's health and especially on reproduction. However, it is still unknown how efficient researchers are in studying these effects and especially considering the impact on steroid hormones. Thus, in this systematic review, we highlight the knowledge gap in studying the effects of the western diet on reproduction Methods: We searched Scopus and Web of Science and screened 927 abstracts and 96 full texts for studies that fed rodents western diet before reproduction or in the case of females during pregnancy. We recorded the species, sex, and generation of the studied rodents, and also assessed whether steroid hormones were studied and whether the diet groups were represented equally. Results: The obtained studies focused only on rats and mice and specifically on the mothers while neglecting fathers and other rodent species. In addition to that 32% of the articles didn't assure equal representation of the groups and this might reduce the efficiency of their conclusions. Finally, only 10% of the articles studied the important role of steroid hormones on reproduction and this was mainly studied in only one generation. In conclusion, there are many biases in conducting research that should be solved.

Keywords: Western diet, reproduction, hormone, rodent, systematic review

INTRODUCTION

Reproduction is an essential process to ensure the existence of many species. Despite the research ongoing for many years on identifying the mechanisms underlying the reproductive system,

infertility is undoubtedly one of the crucial problems. The physiological mechanisms that control energy balance are reciprocally linked to those that control the reproduction and together they optimize reproductive success under fluctuating metabolic conditions. Thus, it is difficult to understand reproductive success without understanding its links with metabolism. Currently, we know that hormones responsible for the regulation of metabolism and food intake have a vital role in controlling reproductive function [1]. For example, adipokines, hormones produced by adipose tissue, like leptin, vaspin, and chemerin have multiple effects on the reproductive system, especially in females [2,3]. Thus, it is well known that the nutritional status and the type of diet strongly influence the reproductive potential through many mechanisms. Western diet, rich in fats and simple sugars, is a risk factor leading to overweight or obesity [4]. Considering that obesity has serious consequences for fertility by affecting the secretion of steroid hormones, follicular development, oocyte maturation, and corpus luteum function [5] it is worth studying the impact of eating a popular obesogenic, western diet on the reproduction process. Although studies involving the influence of a western-style diet on the different aspects of humans and animal physiology are becoming more frequent, there are still many questions about the influence of diet, especially in the long-term perspective on the next generation of offspring.

The aim of the current systematic review was to identify the knowledge gap in the study of the effects of the western diet on rodent reproduction. Since rodents are the most common model for studying the effects of the western diet, we tried to summarize what has so far been established in terms of the impact of high-fat and high-sugar (HFHS) diets on fertility. Next, we checked the gender of which this effect was tested. We expect that due to the greater impact of females in the whole process of reproduction, including the period of pregnancy and lactation, this effect may be tested more frequently in females. We also verified whether the influence on the secretion of steroid hormones such as estradiol, progesterone, or testosterone, the correct homeostasis of which is a crucial factor for reproductive success [6], has been investigated. Another important question was to check whether giving the western diet to parents, would affect also the offspring's health and even grand offspring.

METHODS

Search Strategy

Two electronic databases were simultaneously searched on June 1st 2022: SCOPUS and the Web of Science.

Both databases were searched using the following keywords: 1) "western diet" OR western-diet OR "Western-style diet" OR "high fat high" OR "HFHS diet" OR high-fat-high OR "HFS diet" OR "high-fat/high" OR "high fat, high" OR " high fat-high" AND 2) reproduc* OR fertility OR fecundity OR mating OR pregnan* OR breed*. The selection process was summarized in Figure 1.

Eligibility Criteria

The participants, interventions, comparisons, outcomes, and study design (PICOS) criteria were as follows: participants were rodents at reproductive age; intervention was a western diet rich in fat and sugar; the comparison was control group, given the standard diet; outcomes were the effects of the western diet on reproduction; The study design was a controlled experiment in which the western diet group should be compared to any type of control diet that had a lower fat and sugar content.

We only included primary studies published in English and working on rodents as their model organism. The rodents under study should be given only one type of western diet i.e., not a cafeteria diet. In addition to that, the western diet should be given before reproduction in the case of both males and females, while during the whole pregnancy period, or only for some stages of pregnancy, in the case of females.

Study selection and data extraction.

The articles represented from both databases were exported to Zotero in which they were deduplicated and then screened using Rayyan® [7] (Fig. 1). Article screening was performed by both authors (AMH and EM) independently and any occurrence of conflict was resolved by the discussion of both parties. The full texts of the selected articles were divided randomly into two equal sets. Each set was analyzed by a different author. The data extracted during the full texts screening included: 1) the species of the rodent; 2) the sex of the parent given the western diet; 3) the generation in which the effects of the western diet were studied. Furthermore, the authors noted whether the articles worked at the hormonal (steroid hormones or other hormones) or non-hormonal level. Finally, the authors considered the number of individuals included in each group to assess the equal representation of groups. An unequal representation was assessed by having at least 2 individuals more or less than the other group.

RESULTS

In total 1368 articles were generated utilizing SCOPUS (n=787 articles) and the Web of Science (n=581 articles) databases. Out of the obtained articles, 441 were duplicates and removed using Zotero software. The remaining 927 papers had their titles and/or abstracts assessed for acceptability, with 831 being rejected owing to failing to meet preliminary eligibility criteria. During the full texts screening of the 96 articles, three articles were excluded because the effects of the western diet were not compared to a control group. Another article used cafeteria test and was also excluded. In total 92 articles were identified to match our inclusion criteria. (Fig. 1)

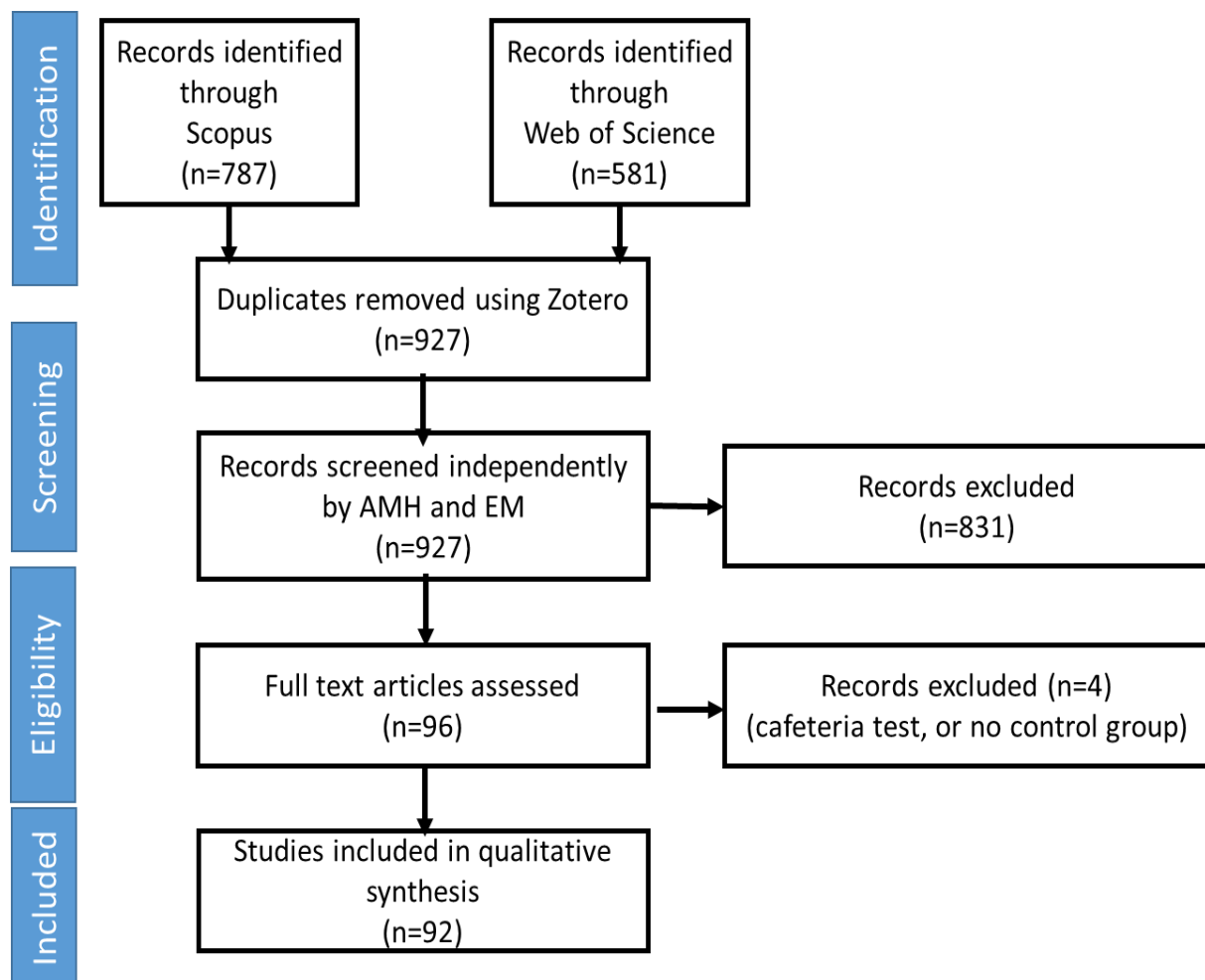


Figure 1: PRISMA flow diagram showing the search strategy of studies

Our systematic review focused on the effects of the western diet on the reproduction of all species of rodents. Whereas, while screening the articles we have found that only two model organisms were represented. 51% of the articles were studying these effects on mice, while the other 49% were considering the rats as the model organism in their studies (Fig. 2 A).

In addition to neglecting studies working on rodents other than mice and rats, only 5% of the articles studied how the western diet might affect the vital role of males in reproduction, while 8% worked on both males and females simultaneously (Figure 2 A). Visibly, females are the heroes in studying reproduction in which majority of the researchers focus on the mothers (87%). (Fig. 2 A)

Our findings also exposed another flaw in the current state of knowledge. We have realized that not just a little, but around 32% of the analyzed articles didn't have equal representation of the groups. The number of individuals representing the western diet group is different from the number of individuals representing the control group giving the standard diet.

Our review also reveals that the effects of the western diet on reproduction were almost equally distributed between hormonal and non-hormonal effects. These effects were mainly studied in both parents and offspring simultaneously (n=41 articles) with the presence of high number of research focusing only on Parents (n=16) or only on their offspring (n=27) (Fig. 2 B). Nonetheless, when considering only the hormonal effects, only 11 articles focus on the effects of the western diet on two generations (parents and offspring) simultaneously, while the majority focus on one generation, either the parents (n=16) or the offspring (n=18) (Fig. 2 C). Deeper into the hormonal effects, only 20% of the articles studying hormones measured the change in the steroid hormones and this constitutes only 10% of all the studied articles (Fig. 2 A). Knowing that steroid hormones are the key regulator of reproduction in both males and females, these percentages might be considered low.

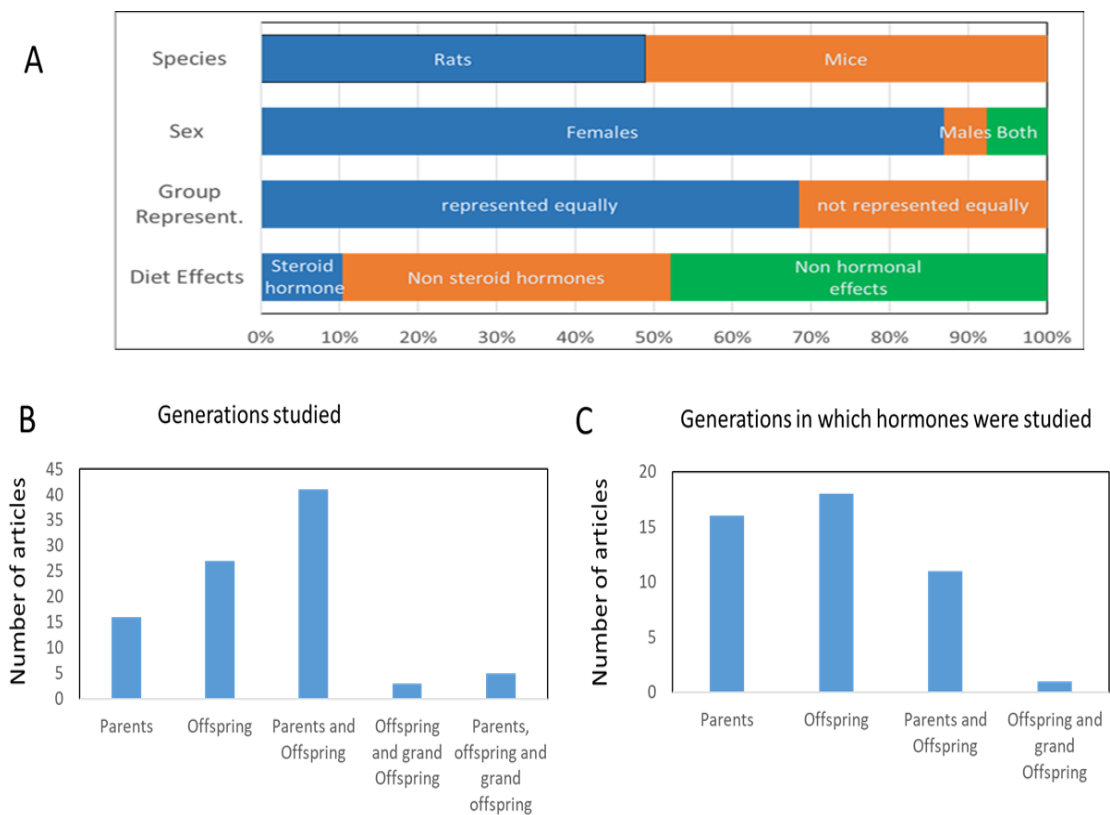


Figure 2: (A) stacked bar showing the percentage distribution of the articles between the different groups in a given criterion. The criteria are Species, Parent sex, group representation and diet effect. A bar graph showing the number of articles distributed into different categories based on the

generation(s) in which all the effects of the western diet (hormonal and non-hormonal) were studied(B), and based on the generation(s) in which only hormonal effects were studied (C).

DISCUSSION

The results of our analysis clearly indicate that the study of the effect of western diets on rodent reproduction focuses mainly on females. Almost 90% of the articles analyzed by us concerned the effects of administering the HFHS diet only to females. The vast majority of females were exposed during pregnancy, and the observed effects were most often negative. Our analysis revealed that the effects of the western diet on male fertility are very poorly understood. Especially the effect of the HFHS diet on the offspring of such fathers. As we assumed, due to the greater proportion of females in the entire reproduction process, they can be tested more often in terms of their influence on the reproductive potential, the maintenance of pregnancy, and foetus development, as well as on the quality of the offspring. The diet administered with HFHS resulted in increased maternal weight, and in extreme cases lead to obesity [8,9]. The studies mainly focused on the effects of eating the HFHS diet during pregnancy on its course, where parameters such as placental function, foetal development, number, and mortality, as well as long-term effects on the health of the offspring, were studied. Here, the effects on the development of organs such as the heart, liver, and metabolic parameters as well as behavior were most often studied [10-12]. The offspring of females eating western diets were fewer and had malformations as well, and were at risk of developing diseases such as metabolic syndrome in the future [13,14].

A review of the literature indicates that the research conducted so far focuses on understanding the impact on the quality of offspring and their health after reaching adulthood. There is a lack of data on the impact of western diets consumed by parents before mating and during pregnancy on the reproductive parameters of the offspring and their reproductive abilities, such as sperm quality, development of ovarian follicles, or the secretion of sex hormones. An exception to this we found an interesting article showing that a maternal obesogenic diet can negatively affect oocyte development for the next three generations [15]. However, this type of research is definitely missing. What is related to this, we observed that, although a lot of research focuses on the analysis of the endocrine system, only 10% of articles concern steroid hormones. These hormones regulate every stage of the reproductive process in both females and males. Estradiol is necessary for the growth of the ovarian follicles, which will be able to release the mature oocyte, progesterone maintains the function of the corpus luteum, which is necessary for the maintenance of pregnancy, and testosterone regulates spermatogenesis [6]. Therefore, we anticipated that steroid hormones would be studied more extensively in the context of the effects of western diets on fertility in rodents. It is worth investigating the future effects of the parents' diet on the levels of sex hormones in offspring. Such studies were conducted on males and females of reproductive age who directly consumed a western diet. The other tested hormones were mainly insulin, which often appeared in the context of the analysis the assessment of the proper metabolism of parents and offspring [16,17]. Leptin, or adiponectin, and resistin was frequently tested hormones [16, 18]. This is a very interesting finding showing that scientists are focusing on novel reproductive regulators such as adipokines in assessing the effects of western diets on the reproductive system. Thus, leptin has become a factor that is taken into account in the assessment of proper fertility. In addition, many studies have focused on non-hormonal effects. Such studies included, for example, histological analysis of tissues, e.g. the liver [16], or the same sexual processes as the development of the placenta, but without examining the hormonal aspect of this process [19]. A very interesting aspect was the research carried out on several generations (F1, F2, F3) [15, 20], showing the long-term effects of consuming HFHS diets, but they were very rare.

The main rodent species used in the research were mice and rats, which is not a surprising result, but it is interesting that the research on these species is almost half broken. Thus, both mice and rats are equally frequently used models for this type of research. Regarding the question of the representativeness of the number of individuals in the study groups, over 2/3 of the studies were performed so that the number of individuals in the control group and the western diet group was

the same. We observed slight deviations from this rule, where both groups differed by a maximum of 1-3 individuals. However, did not affect the statistical analysis of the data and the final conclusion. In addition, although we did not indicate this in our evaluation criteria, we observed that the vast majority of the articles analyzed were dated over the past 5 years, indicating that there has been a growing interest and need to study the effects of Western diets on reproduction.

In conclusion, despite the fact that research into the reproductive effects of Western diets is increasing, there are still many aspects that need to be developed. Above all, more emphasis should be put on studying male western diets and observing the effects on the health of the offspring. Additionally, studies are needed to show the long-term effects also on the reproductive parameters of the offspring.

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Reviews

Małgorzata Łagisz

The authors conducted a systematic review of literature focusing on the effects of the western diet on rodent reproduction. The manuscript is well written, in mostly concise and understandable style. However, I would recommend avoiding excessive use of passive voice. I have the following minor technical concerns and suggestions:

1. Lines 61-64: The search string seems to be wrong or presented in a strange way – It is best practice to present search strings exactly in the format they appear at the top of the results window, including any filters and additional restrictions. For WoS you should also note which sub-databases were searched (this varies between subscribing institutions).

2. Line 75: you did not define cafeteria diet or explain how it is different from “Western diet” (it actually can be considered a type of Western diet). Note capital letter in “Western”.
3. Lines 83-87: more details on data coding should be provided (meta-data, in a table in the main text or as SI files)
4. Line 84: you should also record the rodent strain name (they can be very distinct genetically and phenotypically, and it would be important to see which ones are being used)
5. Lines 97-89: you did not mention investigation of unequal representation in your study aims nor provided justification for studying it. Thus, it is unclear why you are extracting this data. Also, small deviations are unlikely to affect the results.
6. Lines 95-97: Excluded studies should be listed in SI or you could add references to them right in the sentences describing causes of exclusion.
7. Lines 103-105: technically rats and mice are not individual species (there are many different species of rats and mice_ - you could use Latin names here to be explicit that you only found studies using 2 most commonly used lab species (*Mus musculus domesticus* and *Rattus norvegicus*).
8. Figure 2 legend: these are not criteria, these are extracted variable/data. B and C should have same y-scale range. Potentially, these could be a single plot.
9. Lines 143-144: it is odd to mention results of a few selected studies at this point, as this suggest you actually extracted data on some outcomes, which you did not? Be explicit, these are just examples.
10. No section/acknowledgement of study limitations, missing list of included studies, extracted data, meta-data and analytic code.

Edyta Sadowska

The authors present a systematic review of current knowledge about obesity and infertility - two medical issues on a worldwide scale, especially in developed societies. They focused only on the rodents model as it is most studied. From the initial 927 abstracts, there were 96 full-text articles included in the study. This allowed for showing sex bias in such research and there are not a lot of studies on the carry-over effect of parental diet on offspring. The paper is very interesting and shows where are the potential gaps in our research on the effect of obesity on reproduction, but in the discussion, the authors made very often the conclusions that are not confirmed by the results of their own conducted systematic review. I suggest rewriting the discussion and re-submit the paper.

Detail comments:

The introductory part explains nicely the link between reproduction, metabolism, and hormones; and how the Western diet may play a role in it. However, the aim part was a little bit chaotic and some part was not understandable. For example, I am not sure how the authors wanted to achieve the last aim: “check whether giving the western diet to parents, would affect also the offspring’s health and even grand offspring”. The aim rather should be rather how often or if it is studied.

I would also like to see a rephrased title of the study. In the current version, it is misleading that it shows the original research but this is a systematic review of published research. I have doubts also if the title “the effect of western-diet on reproduction” reflects the aims of the study. Reading such a title I would expect the paper about how the western diet affects reproduction – so if they reproduced, and if yes then how many offspring and their condition in comparison to the control group.

Line 88-89 I do not agree with the authors that the criteria that difference of two individuals between groups leads always to the unbalanced design of the experiment. Two individuals out of 100 would be not any problem, but having a difference of two individuals out of five is, of course, a serious one. I would rather see this criterium as the percentage one.

Line 78: please remove the dot at the end of the title

Fig 2a and b: please make the same axis on both graphs as it helps to compare them.

Line 137-138 “The vast majority of females were exposed during pregnancy, and the observed effects were most often negative” - where does this conclusion come from? In the result parts, it was only mentioned that the studies were conducted in 87% of papers.

Line 143-151 again this is not the conclusion of the author’s study but summarizing knowledge of the effect of diet on females. The authors did not categorize the paper into anatomical/organs or behavioral effects of western diet so the conclusion “the effects on the development of organs such as the heart, liver, and metabolic parameters, as well as behavior, were most often studied” is only a “feeling” of the author without confirmation in results of its own systematic-review analysis. The same concerns many other parts of the discussion. Please rewrite the discussion taking into account the main aims that were stated in the introduction.

Monika Hoffmann

The manuscript presented by Alaa Hseiky and Ewa Mlyczyńska analyses the knowledge gap on the effects of Western diets on reproduction in mice and rats. A focus of this systematic review was on the effects of steroid hormones affecting reproduction. In my opinion, this study is timely and relevant.

The introduction provides a good overview of the research area and the goals the researchers hope to achieve with this review. The authors followed the PICOS criteria, and the methodological design and search criteria were well chosen. The results show that both rodent species were almost equally represented. However, only 5% of the studies examined the role of male reproduction. There is also a major deficiency in the representation of dietary groups. In 32% of the analysed articles, the number of individuals was different in western diet and standard diet. In only 10% of the studies the steroid hormones were considered. These results already show a large knowledge gap that should be addressed in future research. The total number of articles identified by database screening is 927 and was very high. 10% of the articles were considered for a full-text search. The search criteria were efficiently chosen. The authors demonstrate that their research is relevant in many ways, and they succeed in showing that there are several knowledge gaps that should be addressed.

Overall, this systematic review is very well written, and the research objective is justified throughout the paper. Figure 2 could be improved. I would suggest not to use the same colours in figure 2A. It can be misleading. Besides this minor remark I would recommend this research for publication. For the short period of time in which the study was prepared, the execution is of good quality and the overall synthesis is consistent.

Gokul Bhaskaran

The study provides a systematic review of the literature to understand how rodent reproduction is affected by the western diet. The authors focused on publications, which are specific for rodents and their diet. Further, which species was studied the most was identified along with the sample size distribution. One of the main focus of the review was to identify which sex is studied the most. The hormonal level impact of western diet was also checked generally and also the inter-generational effect.

The presented research is undoubtedly an important problem not just in the field of ecology and evolution – but the nutritional/diet studied here has a lot implication in the population dynamics

and behaviour. These studies can provide insights in the pharmaceutical industry for various drug development. In my opinion, these reviews are really innovative and provides a collection of previous studies to understand the trend of research and potential futuristic research ideas to fill the knowledge gap.

The abstract is very well written and clear even to a non-specialist and conveys the entire study effectively. Line 13: after reproduction stop the sentence (missing a full stop), and then start describing about the methods. The introduction part is adequate and covers the theme of the study very effectively. The length of the introduction is optimum. Line 29-30: the reciprocal linkage may be obvious for experts in the field, but it will be good add a reference there. Line 49-50: it is true that in the reproduction the greater impact is from the mothers, however there are very famous theories indicating how crucial is the fathers' condition (not a criticism but just indicating it might not be that obvious and there is change from species to species).

Line 61-64: Yes, these are keywords – but in the context of a systematic review this is the “search string”. Line 64: not “was” but “is”. The PICOS model is well justified, and the framework perfectly fits the study. Line 71-72: the western diet group is compared against any diet that is lower in fat and sugar, is taking such comparisons will negatively impact the overall results/conclusion if western diet is having any positive implications (in the context of trade-off). Line 73: is the review accounts only for peer-reviewed articles or please clearly indicate if any grey articles are used.

The PRISMA diagram is clear and the quality is nice. The graph qualities are at good standards. Line 78: the title does not need a full stop (just remove it). Line 79-80: just wondering why authors cited only Rayyan software and not Zotero. The discussion section is well narrated and touches the reasoning to all the findings. Line 173: “taken into account” may be change to “considered” which is better suited here. Line 194-195: Yes, it will be interesting to understand the offspring fitness components, given how western diet/any diet is exposed/non-exposed in their life-time (will be very interesting to look on). A general curiosity is that the authors had checked for multiple factors in a single paper, and it can be difficult that all the parameters are taken by the paper or the parameters were different. A slight opinion here is the conclusion derived taking these aspects in to concern? One minor missing is the contact (email) address of the authors, please add it. Also, sometimes it is fig, and at times it is Figure – keep the uniformity (a very minor issue). The work is very well-written and informative.

Final version of the project

The effects of the Western diet on rodent reproduction: A Systematic Review

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ABSTRACT

Background: Western diet has detrimental effects on the organism's health and especially on reproduction. However, it is still unknown how efficient researchers are in studying these effects and especially considering the impact on steroid hormones. Thus, in this systematic review, we highlight the knowledge gap in studying the effects of the western diet on reproduction. Methods: We searched Scopus and Web of Science and screened 927 abstracts and 96 full texts for studies

that fed rodents western diet before reproduction or, in the case of females, during pregnancy. We recorded the species, sex, and generation of the studied animals, and also assessed if steroid hormones were studied and whether the diet groups were represented equally. Results: Studies included in the review focused only on rats and mice and specifically on the mothers, while neglecting fathers and other rodent species. In addition, 32% of the articles did not assure equal representation of the groups and this might reduce the efficiency of their conclusions. Finally, only 10% of the articles studied the important role of steroid hormones on reproduction and this was mainly studied in only one generation. In conclusion, there are many biases in conducting research that should be solved.

Keywords: Western diet, reproduction, hormone, rodent, systematic review

INTRODUCTION

Reproduction is an essential process to ensure the existence of many species. Despite the research ongoing for many years on identifying the mechanisms underlying the reproductive system, infertility is undoubtedly one of the crucial problems. The physiological mechanisms that control energy balance are reciprocally linked to those that control reproduction and together they optimize reproductive success under fluctuating metabolic conditions. Thus, it is difficult to understand reproductive success without understanding its links with metabolism. Currently, we know that hormones responsible for the regulation of metabolism and food intake have a vital role in controlling reproductive function [1]. For example, adipokines, hormones produced by adipose tissue, such as leptin, vaspin, and chemerin have multiple effects on the reproductive system, especially in females [2,3]. Thus, it is well known that the nutritional status and the type of diet strongly influence the reproductive potential through many mechanisms. Western diet, rich in fats and simple sugars, is a risk factor leading to overweight or obesity [4]. Considering that obesity has serious consequences for fertility by affecting the secretion of steroid hormones, follicular development, oocyte maturation, and corpus luteum function [5] it is worth studying the impact of eating a popular obesogenic, western diet on the reproduction process. Although studies involving the influence of a western-style diet on the different aspects of humans and animal physiology are becoming more frequent, there are still many questions about the influence of diet, especially in the long-term perspective on the next generation of offspring.

The aim of the current systematic review was to identify the knowledge gap in the study of the effects of the western diet on rodent reproduction. Since rodents are the most common model for studying the effects of the western diet, we tried to summarize what has so far been established in terms of the impact of high-fat and high-sugar (HFHS) diets on fertility. We checked the gender of which this effect was tested. Due to the greater impact of females in the whole process of reproduction, including the period of pregnancy and lactation, we expect that this effect may be tested more frequently in females. In addition, we checked whether the group's research was equal and representative, which could affect the quality of the research. We also verified whether the influence on the secretion of steroid hormones such as estradiol, progesterone, or testosterone, the correct homeostasis of which is a crucial factor for reproductive success [6], has been investigated. Another important question was to check how often the effect of the western diet consumed by parents was a study on the offspring's health and even grand offspring.

METHODS

Search Strategy

Two electronic databases were simultaneously searched on June 1st 2022: SCOPUS and the Web of Science. Both databases were searched using the following keywords:

(("western diet" OR western-diet OR "Western-style diet" OR "high fat high" OR "HFHS diet" OR high-fat-high OR "HFS diet" OR "high-fat/high" OR "high fat, high" OR " high fat-high") AND (reproduc* OR fertility OR fecundity OR mating OR pregnan* OR breed*))

The selection process is summarized in Figure 1.

Eligibility Criteria

The participants, interventions, comparisons, outcomes, and study design (PICOS) criteria were as follows: participants were rodents at reproductive age; intervention was a western diet rich in fat and sugar; the comparison was control group, given the standard diet; outcomes were the effects of the western diet on reproduction; The study design was a controlled experiment in which the western diet group should be compared to any type of control diet that had a lower fat and sugar content (Table 1).

Table 1. Characteristics of the used PICOS model

PICOS model	Attributions	Keywords
Participants	Rodents at reproductive age	reproduc* OR fertility OR fecundity OR mating OR pregnan* OR breed*
Interventions	Western diet rich in fat and sugar	"western diet" OR western-diet OR "Western-style diet" OR "high fat high" OR "HFHS diet" OR high-fat-high OR "HFS diet" OR "high-fat/high" OR "high fat, high" OR " high fat-high"
Comparisons	Control group, given the standard diet	
Outcomes	The effects of the western diet on reproduction	
Study Design	Controlled experiment: Western diet group should be compared to any type of control diet that had a lower fat and sugar content.	

We only included primary studies, peer-reviewed articles published in English and working on rodents as their research model. The rodents understudy should be given only one type of western diet i.e., not a cafeteria diet. Cafeteria diet is when one individual is given more than one type of food at the same time, and the individual is able to choose between them. In addition to that, the western diet should be given before reproduction in the case of both males and females, while during the whole pregnancy period, or only for some stages of pregnancy, in the case of females.

Study selection and data extraction

The articles represented from both databases were exported to Zotero (<https://www.zotero.org/>) in which they were deduplicated and then screened using Rayyan® [7] (Fig. 1). Article screening was performed by both authors (AMH and EM) independently and any occurrence of conflict was

resolved by the discussion of both parties. The full texts of the selected articles were divided randomly into two equal sets. Each set was analyzed by a different author. The data extracted during the full texts screening included: 1) the species of the rodent; 2) the sex of the parent given the western diet; 3) the generation in which the effects of the western diet were studied. Furthermore, the authors noted whether the articles worked at the hormonal (steroid hormones or other hormones) or non-hormonal level. Finally, the authors considered the number of individuals included in each group to assess the equal representation of groups. An unequal representation was assessed by having at least 2 individuals more or less than the other group.

RESULTS

In total 1368 articles were generated utilizing SCOPUS (n=787 articles) and the Web of Science (n=581 articles) databases. Out of the obtained articles, 441 were duplicates and removed using Zotero software. The remaining 927 papers had their titles and/or abstracts assessed for acceptability, with 831 being rejected owing to failing to meet preliminary eligibility criteria. During the full texts screening of the 96 articles, three articles were excluded because the effects of the western diet were not compared to a control group. Another article used cafeteria test and was also excluded. In total 92 articles were identified to match our inclusion criteria (Fig. 1).

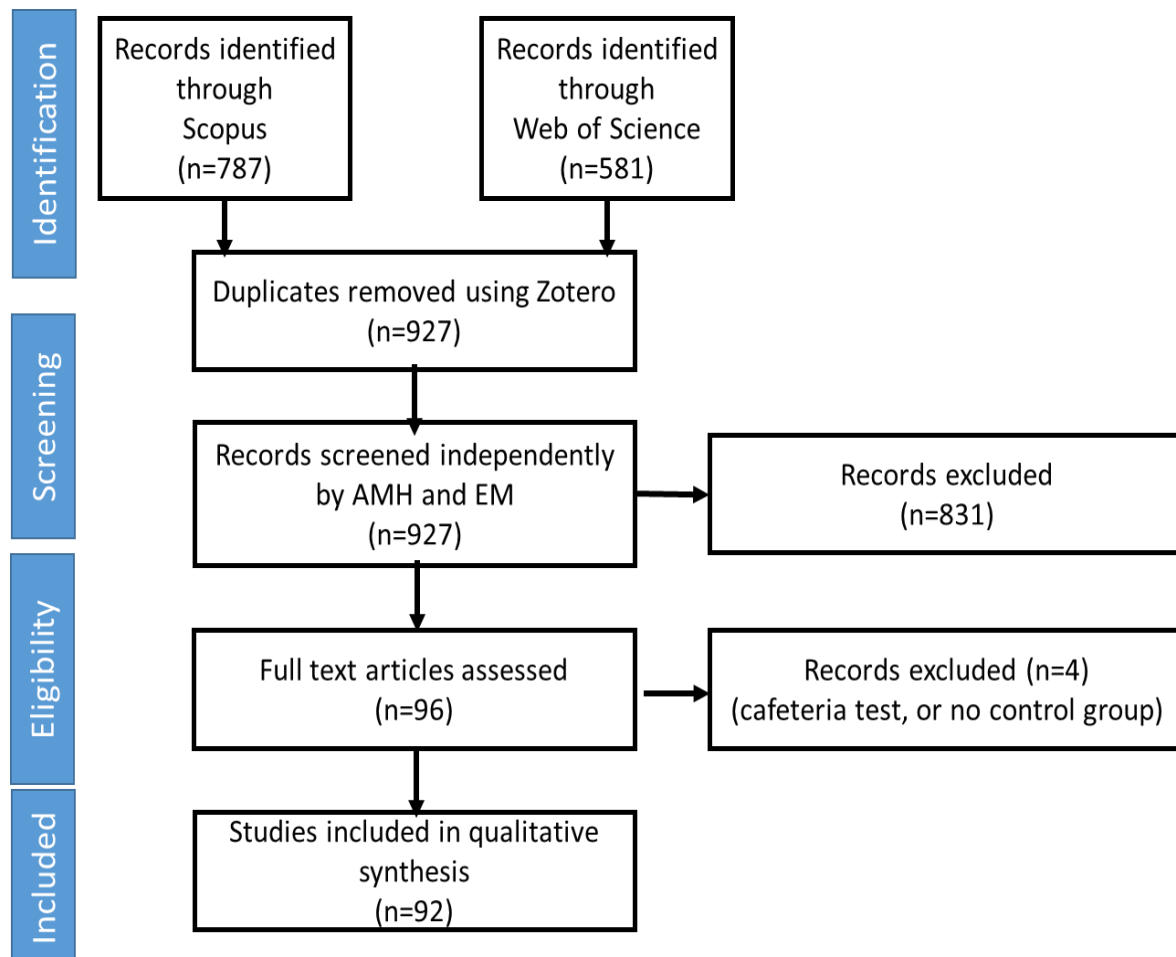


Figure 1: PRISMA flow diagram showing the search strategy of studies

Our systematic review focused on the effects of the western diet on the reproduction of all species of rodents. Whereas, while screening the articles we have found that only two model organisms were represented. 51% of the articles were studying these effects on mice (*Mus musculus*

domesticus), while the other 49% were considering the rats (*Rattus norvegicus*) as the model organism in their studies (Fig. 2 A).

In addition to neglecting studies working on rodents other than mice and rats, only 5% of the articles studied how the western diet might affect the vital role of males in reproduction, while 8% worked on both males and females simultaneously (Fig. 2 A). Visibly, females are the heroes in studying reproduction in which majority of the researchers focus on the mothers (87%) (Fig. 2 A).

Our findings also exposed another flaw in the current state of knowledge. We have realized that not just a little, but around 32% of the analyzed articles didn't have equal representation of the groups. The number of individuals representing the western diet group is different from the number of individuals representing the control group giving the standard diet.

Our review also reveals that the effects of the western diet on reproduction were almost equally distributed between hormonal and non-hormonal effects. These effects were mainly studied in both parents and offspring simultaneously (n=41 articles) with the presence of high number of research focusing only on Parents (n=16) or only on their offspring (n=27) (Fig. 2 B). Nonetheless, when considering only the hormonal effects, only 11 articles focus on the effects of the western diet on two generations (parents and offspring) simultaneously, while the majority focus on one generation, either the parents (n=16) or the offspring (n=18) (Fig. 2 B). Deeper into the hormonal effects, only 20% of the articles studying hormones measured the change in the steroid hormones and this constitutes only 10% of all the studied articles (Fig. 2 A).

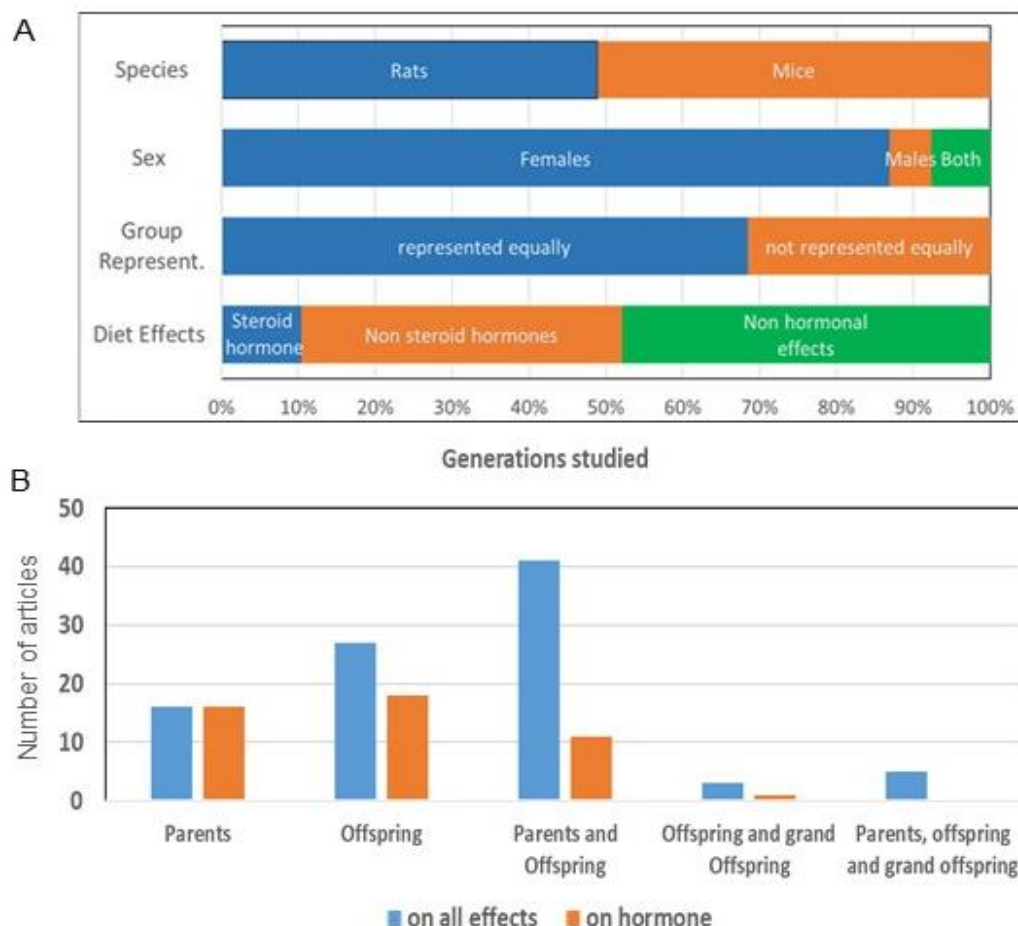


Figure 2: (A) Distribution of the articles between the different groups in a given extracted variable. The variables extracted are Species, Parent sex, group representation and diet effect. (B) Number of articles distributed into different categories based on the generation(s) in which all the effects of the western diet (hormonal and non-hormonal) were studied or only the hormonal effects).

DISCUSSION

The results of our analysis clearly indicate that the study of the effect of western diets on rodent reproduction focuses mainly on females. Almost 90% of the articles analyzed by us concerned the effects of administering the HFHS diet only to females. The vast majority of females were exposed during pregnancy. Our analysis revealed that the effects of the western diet on male fertility are very poorly understood. Especially the effect of the HFHS diet on the offspring of such fathers. As we assumed, due to the greater proportion of females in the entire reproduction process, they can be tested more often in terms of their influence on the reproductive potential, the maintenance of pregnancy, and foetus development, as well as on the quality of the offspring. The studies mainly focused on the effects of eating the HFHS diet during pregnancy on its course, where parameters such as placental function, foetal development, number, and mortality, as well as long-term effects on the health of the offspring, were studied. For instance, the effects on the development of organs such as the heart, liver, and metabolic parameters, as well as behavior, were studied [8-9].

A review of the literature indicates that the research conducted so far focuses on understanding the impact on the quality of offspring and their health after reaching adulthood. There is a lack of data on the impact of western diets consumed by parents before mating and during pregnancy on the reproductive parameters of the offspring and their reproductive abilities, such as sperm quality, development of ovarian follicles, or the secretion of sex hormones. An exception to this we found an interesting article showing that a maternal obesogenic diet can negatively affect oocyte development for the next three generations [10]. However, this type of research is definitely missing. What is related to this, we observed that, although a lot of research focuses on the analysis of the endocrine system, only 10% of articles concern steroid hormones. These hormones regulate every stage of the reproductive process in both females and males. Estradiol is necessary for the growth of the ovarian follicles, which will be able to release the mature oocyte, progesterone maintains the function of the corpus luteum, which is necessary for the maintenance of pregnancy, and testosterone regulates spermatogenesis [6]. Therefore, we anticipated that steroid hormones would be studied more extensively in the context of the effects of western diets on fertility in rodents. It is worth investigating the future effects of the parents' diet on the levels of sex hormones in offspring. Such studies were conducted on males and females of reproductive age who directly consumed a western diet. The other tested hormones were mainly insulin, which often appeared in the context of the analysis the assessment of the proper metabolism of parents and offspring [11,12]. Leptin, or adiponectin, and resistin was frequently tested hormones. This is a very interesting finding showing that scientists are focusing on novel reproductive regulators such as adipokines in assessing the effects of western diets on the reproductive system. In addition, many studies have focused on non-hormonal effects. Such studies included, for example, histological analysis of tissues, e.g. the liver [11], or the same sexual processes as the development of the placenta, but without examining the hormonal aspect of this process [13]. A very interesting aspect was the research carried out on several generations (F1, F2, F3) [10, 14], showing the long-term effects of consuming HFHS diets, but they were very rare.

The main rodent species used in the research were mice and rats, which is not a surprising result, but it is interesting that the research on these species is almost half broken. Thus, both mice and rats are equally frequently used models for this type of research. In our study we did not take into account the rodents strain. Rodents have different strains that can be genetically and phenotypically distinct. Recording such a parameter could have highlight a preference to research on few specific model organisms while neglecting the others,

Regarding the question of the representativeness of the number of individuals in the study groups, over 2/3 of the studies were performed so that the number of individuals in the control group and the western diet group was the same. We observed slight deviations from this rule, where both groups differed by a maximum of 1-2 individuals. However, did not affect the statistical analysis of the data and the final conclusion. We are aware that the criteria of deciding whether the groups were represented equally were not extracted in the proper way. Instead of just showing that the groups are not represented equally, we should have shown the exact representation of the groups or

at least a ratio calculated by dividing the number of individuals in the WD group by those of the SD. Despite this, we do not expect that the conclusion will differ that much. In addition, although we did not indicate this in our evaluation criteria, we observed that the vast majority of the articles analyzed were dated over the past 5 years, indicating that there has been a growing interest and need to study the effects of western diets on reproduction.

In conclusion, despite the fact that research into the reproductive effects of western diets is increasing, there are still many aspects that need to be developed. Above all, more emphasis should be put on studying male western diets and observing the effects on the health of the offspring. Additionally, studies are needed to show the long-term effects also on the reproductive parameters of the offspring.

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AMH and EM would like to thank Professor Joanna Rutkowska for her assistance with the crystallization of the research idea and helping in the search strategy. We would also like to thank anonymous reviewers for their valuable remarks and our colleagues: M. Hoffmann and G. Bhaskaran for an additional review of our systematic review.

DATA AVAILABILITY

To have access to the articles that were included and/or excluded after the full text screening, contact either Authors.

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