Canadian Journal of
Fisheries and
Aquatic Sciences

Review

Consequences of roads and traffic-related stressors on freshwater biodiversity

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Abstract

Road development has expanded globally, with growing recognition of the impacts on freshwater systems. We synthesized findings from 426 publications to assess (1) where and for which freshwater taxa road effects have been studied; (2) the types of roads and stressors examined; (3) the study designs and biological responses measured; and (4) the reported road effects on freshwater taxon. Most studies were conducted in North America (62.1%), with the overall evidence base focusing on amphibians (36.1%), fish (14.8%), and reptiles (10.9%) in ponds/wetlands, laboratory settings, and streams. Research primarily assessed the impacts of road density or proximity (20%), traffic (18.4%), and salt/de-icers (17.9%) stressors, mostly using impact-only (51.0%) and control-impact (46.5%) study designs, restricting causal inference. Demographic, diversity, and mortality responses were the most frequently measured outcomes. Road effects were predominantly negative, underscoring widespread road impacts on freshwater taxon. Major knowledge gaps included understudied taxa (avifauna, mammals, crustaceans, molluscs, insects, plants), life-stages (eggs, juveniles), and several stressors. Future research should focus on addressing the knowledge gaps and apply comparative approaches to improve understanding and management.

Key words: road ecology, landscape connectivity, aquatic biodiversity, road networks, land use

Introduction

Road infrastructure (including roads and their associated vehicles) was historically viewed as a benign feature of the landscape and a necessary component for promoting progress and economic productivity, reflecting a philosophy of "automobility" with little consideration for effects on wildlife (Coffin 2007; Kroll 2015). Even Aldo Leopold argued that roads should not be considered to meaningfully affect animal populations except in unusual circumstances (see Kroll 2015). Today we know differently. Far from benign, roads are ubiquitous landscape-level anthropogenic features that can adversely affect a wide range of terrestrial taxa and systems (Forman and Alexander 1998; Spellerberg 1998; Trombulak and Frissell 2000; Foley et al. 2005; Coffin 2007; Fahrig and Rytwinski 2009; Benítez-López et al. 2010; Bennett 2017). Although not intuitive, roads and traffic also have the potential to impact freshwater systems and biodiversity (Trombulak and Frissell 2000; Dixon et al. 2022).

Freshwater biodiversity is under immense threat, with substantial declines observed since the 1970s and the rate of decline outpacing that of marine and terrestrial diversity (Dudgeon et al. 2006; Reid et al. 2019; Deinet et al. 2020). Freshwater systems including lakes, reservoirs, rivers, streams, and wetlands account for ~9.1% of the Earth's surface (Lehner and Döll 2004) and are estimated to support 9.5% of described animal species (Balian et al. 2008). Land-use change is one of the most prominent drivers of global aquatic biodiversity loss (Allan 2004; Janse et al. 2015), and the impervious surfaces from road infrastructure development is a common form of land-use change (Paul and Meyer 2001; van Asselen et al. 2013; Patenaude et al. 2015). Many of the materials used to build and/or maintain roads are considered pollutants of variable toxicity to aquatic organisms (e.g., concrete, asphalt and tar, road salt; Cooke et al. 2020; Driscoll et al. 2020; Dixon et al. 2022; Szkarlek et al. 2022). Further, roads can be a vector of pollutants produced by vehicles and humans to reach freshwater systems (e.g., tire-sourced microplastics and rubber, oil and gasoline, antifreeze, waste and litter along roadways; Schuler and Relyea 2018; Azevedo-Santos et al. 2021, 2022). Roads can also facilitate or promote the introduction and/or spread of invasive species (Drake and Mandrak 2010; Strayer 2010; Kizuka et al. 2014; Chapman et al. 2020), which can have a myriad of negative effects on freshwater biodiversity (Dextrase and Mandrak 2006; Ricciardi and MacIsaac 2011).

Three major mechanisms underpin the direct and indirect effects of roads and traffic on population persistence of a species (Teixeria et al. 2020): (1) reduced connectivity (limiting immigration and access to resources) leading to reduced population size (e.g., roads and their culverts and bridges acting as barriers and altering water flows); (2) increased mortality from collisions with vehicles reducing population size; and (3) reduced habitat quality (e.g., altered water quality, contaminant exposure, light and noise pollution), leading to reduced reproductive rates and thus over time, reduced population size (Trombulak and Frissell 2000; Allan 2004; Angermeier et al. 2004; van der Ree et al. 2015; Piczak et al. 2019).

The direct and indirect road effects on freshwater biodiversity proliferate in part because road effects on freshwater systems were previously overlooked both by society and in scientific inquiry (Cooke et al. 2013, 2016; Juffe-Bignoli et al. 2016). In the past, freshwater systems and taxa were an afterthought for transportation planners (Angermeier et al. 2004; Stokes 2015). Impact assessments, aiming to avoid or mitigate harm to freshwater systems, are typically required for new road developments or repairs to existing roads in regulatory regimes (Karlson et al. 2014). With the sheer volume of expected future road infrastructure development worldwide in coming years (OECD 2024), road infrastructure development will continue to be a driver of freshwater biodiversity declines if mitigation of road effects remains overlooked or are discounted.

To curb biodiversity losses, the discipline of "road ecology" has emerged and grown considerably over the past decades, generating a large body of scientific literature on the impacts of roads at different scales and on various taxa (Forman et al. 2003; Coffin 2007; Fahrig and Rytwinski 2009; Rytwinski and Fahrig 2011; van der Ree et al. 2011, 2015; Bennett 2017). Many road ecology studies focus on the associated environmental impacts of roads (e.g., Fahrig and Rytwinski 2009; Benítez-Lopez et al. 2010; Rytwinski and Fahrig 2012; van der Ree et al. 2015) and the effectiveness of road and/traffic mitigation measures for reducing the barrier impacts of roads and road mortality (roadkill) on terrestrial wildlife (e.g., Glista et al. 2009; Taylor and Goldingay 2010; Lesbarrères and Fahrig 2012; Rytwinski et al. 2016; Markle et al. 2017; Teixeira et al. 2017; Soanes et al. 2024). However, apart from herpetofauna (especially turtles and amphibians), freshwater biodiversity has received relatively less attention in the road ecology literature. A few reviews and syntheses on the effects of roads on freshwater systems and/or taxa exist (e.g., Trombulak and Frissell 2000; Angermeier et al. 2004; van der Ree et al. 2015; Dixon et al. 2022), but none are inclusive of lotic, lentic, and freshwater transition systems and few span diverse freshwater taxa. Further, none have examined the study designs and biological response variables that researchers used to measure road effects on freshwater flora and fauna.

Our aims for this paper were to provide a synthesis of the state of knowledge on the impacts of roads on freshwater biodiversity and identify knowledge gaps and research opportunities. To this end, we conducted a rigorous evidence synthesis with the objectives to determine:

(1) Where road effects on freshwater biodiversity have been investigated (i.e., study locations and ecosystem types) and for which freshwater taxa;
(2) The phase of road development (e.g., construction, use, and maintenance), types of roads (e.g., paved, unpaved), and road-related putative stressors that researchers have investigated;
(3) The study designs and settings, and types of biological response variables that researchers used to assess road effects on freshwater biodiversity; and
(4) The number of negative, positive, and neutral road effects reported on freshwater biodiversity.

We focused on plants, invertebrates, and vertebrates that (a) complete their life cycle entirely in freshwater (facultative, true aquatic); (b) complete a portion their life cycle in freshwater (meroquatic); or (c) spend considerable time in or near freshwater (semi-aquatic/amphibious; i.e., live and grow in shoreline or riparian or margins and are periodically found in water).

In addition to lentic and lotic freshwater ecosystems, we also included the riparian zone, estuaries, and coastal wetlands in our synthesis because many freshwater flora and fauna interact with these freshwater transition ecosystems for resources (e.g., habitat, food) or transit through these areas (e.g., during migration). Laboratory- and mesocosm-based studies were included as an ecosystem type when they served as a proxy for one of the aforementioned ecosystem types.

Methods

Literature search

A systematic search of the publication database ISI Web of Science Core Collections was performed on 31 August 2021, accessed from Carleton University's institutional subscription, using the following search string:

((road* OR highway$ OR traffic OR motorway$ OR freeway$) AND (wildlife OR fauna OR flora OR animal$ OR fish* OR mammal$ OR bird$ OR avian OR amphibian$ OR reptile$ OR plant$ OR vegetation OR macrophyte$ OR invertebrate$ OR insect$ OR arachnid* OR arthropod* hexapod$ OR rotifer$ OR mollus* OR crustacea* OR dragonfl* OR mayfl* OR springtail$ OR stonefl* OR caddisfl* OR beetle$ OR diptera$ or flatworm$ OR nematod* OR polychaet* OR annelid$) AND (aquatic* OR "Fresh water" OR Freshwater OR Stream$ OR Water$ OR River$ OR Fluvial OR Lake$ OR Pond$ OR Wetland$ OR Estuar* OR Reservoir$ OR Canal$) AND (effect$ OR impact$ OR affect))

Table 1. Eligibility criteria used for screening publications.

Included	Excluded
Taxa (freshwater flora and fauna)	
Mammals, birds, reptiles, amphibians, fish, molluscs, crustaceans, microinvertebrates, macroinvertebrates, zooplankton, insects, and plants that: (i) Complete their life cycle in freshwater (facultative, true aquatic); (ii) Complete a portion their life cycle in freshwater (meroquatic); (iii) Spend considerable time in or near freshwater (semi-aquatic/amphibious; i.e., lives and grows in shoreline or riparian or margins and is periodically found in water).	Completely terrestrial or marine flora and fauna, and those with limited interaction with freshwater ecosystems.
Freshwater ecosystems	
(i) Lentic ecosystems (wetlands, ponds, lakes, reservoirs); (ii) Lotic systems (streams, rivers); (iii) Estuaries (i.e., transition zone); (iv) Coastal wetlands (i.e., transition zone); (v) Riparian zone (i.e., transition zone); (vi) Proxy for a freshwater system (i.e., laboratory or mesocosm).	(i) Terrestrial (nonriparian); (ii) Marine (nonestuarine or coastal).
Exposure (roads and/or road associated infrastructure)	
(i) Paved or unpaved roads (e.g., roadways, airplane runways, logging roads); (ii) Road infrastructure including (but not limited to): vehicles (e.g., automobiles, all-terrain vehicles) and traffic, road lights/lighting, road allowances, culverts, concrete barriers and other obstructions, contaminants produced from road use or maintenance (e.g., tire dust, salt/de-icers), excavation during road construction.	Naturally occurring trails (e.g., animal trails), buildings and other anthropogenic impervious surfaces, trains and railways, and airplanes (e.g., airplane traffic noise).
Outcome	
(i) Biological effects or impacts of roads and/or associated infrastructure on freshwater taxa.	(i) Abiotic outcomes; (ii) Biological outcomes from studies that were conducted solely to determine the effectiveness of mitigation actions, such that road effects were not examined.
Study design	
(i) Primary research studies that reported empirical results (quantitative or qualitative) that were field-, laboratory-, mesocosm-, or modelling-based; (ii) Study designs could include Before-After-Control-Impact, Before-After, Control-Impact, or Impact-only.	Studies that did not report empirical data, were purely theoretical, and review papers.
Language	
English	Other languages

Our approach was inclusive but not exhaustive and is global notwithstanding that our literature search was restricted to publications available in ISI Web of Science Core Collections database using English search terms. The comprehensiveness of the search string was tested using key publications. The freshwater fauna included in the search string were derived following biota outlined in the Freshwater Animal Diversity Assessment (Balian et al. 2008). Search terms for freshwater flora were less prescriptive (e.g., plant, vegetation) to attempt to capture a wide array of biota.

Screening

The records ("publications" henceforth) resulting from the literature search were exported into EPPI-Reviewer Web (https://eppi.ioe.ac.uk/EPPIReviewer-Web/home), where duplicates were identified and removed. Publications were screened by two reviewers (DAA and MLP) using predetermined eligibility criteria (Table 1). Screening was conducted in two distinct stages: first at the title and abstract level, and then at full text (if included after the first screening stage). The references section of literature reviews and books that were returned in the literature search were screened for relevant citations, and those publications were subsequently screened. Prior to screening all publications, a consistency check was performed at the title and abstract stage whereby each reviewer screened 50 publications (∼1% of the total publications) against the eligibility criteria; the reviewers agreed on 98% of the screening decisions. The discrepancies and uncertainties were discussed and resolved before proceeding independently with screening the full database, and adjustments to the screening criteria were made as necessary to reduce ambiguity. A consistency check was also performed at the full text stage. Each reviewer screened 15 publications (∼2%) against the criteria; there was agreement for 100% of the publications.

Data extraction and analysis

Data extraction, analysis, and visualization were performed using Microsoft Excel and R-Studio. We extracted (1) geographic region of the study, ecosystem type (e.g., wetland/pond, lake, laboratory/mesocosm, stream/river), and taxa information (e.g., genus and/or species, life stage); (2) key road predictor(s): road development phase (i.e., construction, use and maintenance, existence), type (i.e., unpaved,

Table 2. Putative stressor categories and illustrative examples.

Putative stressor category	Example(s)
Altered water flows, levels, or tables	Construction or restoration activities that increased or decreased water flows, water withdrawals
Altered water quality parameters	Water nutrient content, water temperature, conductivity, dissolved oxygen
Barrier/obstruction	Culverts, road crossings, dams, roads acting as a behavioural barrier
Contaminants	Heavy metals, polycyclic aromatic hydrocarbons, oil and grease, tire and road debris
Excavation or vegetation removal	Construction or restoration activities that result in excavation or vegetation removal for road development
Human access	Road providing human access to areas previously not frequented by humans (e.g., forest service road)
Light	Street light levels, vehicle headlights
Noise	Traffic noise exposure
Road density and proximity	Distance to road(s), total road length in a unit area (density)
Road or shoulder composition and configuration	Road, ditch, or shoulder cover, area, width, and length, road reflectivity
Road presence	Presence of the road, roads acting as a conduit for invasive species spread
Salt/de-icers	Application or use of road salt and other de-icer compounds
Sediment loss or loading, or turbidity	Construction or restoration activities causing sediment loading, measures of suspended sediments or solids in water
Traffic presence, volume, or density	Presence of traffic, level of traffic (qualitative), amount of traffic per unit time (quantitative), amount of traffic per unit area

paved), and investigated putative (i.e., presumed) stressor(s) (e.g., salt/de-icer, traffic level, road density); (3) study design (e.g., Before-After, Impact-only), study setting (e.g., field, laboratory), and biological response(s) (e.g., growth, mortality, diversity, richness) that researchers used to measure road effects on freshwater biodiversity; and (4) the direction of the biological response (i.e., positive, negative, or neutral/inconclusive).

For each unique combination of road predictors, ecosystems, taxa, biological responses, study setting, and/or study design reported in a publication, a separate "case" was created in the dataset (i.e., a new row of data in the spreadsheet). Further, when a publication examined multiple road development phases, road types, and ecosystems such that it was not possible to isolate into a single nominal value, these were combined. Other complementary data extracted included publication information (e.g., authors, year published) and year of the study.

Taxa were categorized into mammals, avifauna, reptiles, amphibians, fish, molluscs, crustaceans, macro-invertebrates, micro-invertebrates, zooplankton, insects, and plants. Micro-invertebrates, macro-invertebrates, and zooplankton were further combined into one category (MMZ) to reduce the number of taxa categories and aid in visualizing results. Taxa were assigned as invasive species according to the author's statements. As there was a wide array of putative stressors examined across publications, stressors were categorized into common themes (Table 2). Road development phase was categorized into construction (e.g., excavation, paving), use and maintenance (i.e., active use of the road (e.g., traffic levels) and maintenance activities (e.g., salting)), and road existence (i.e., the presence of the road (e.g., road density, configuration)). As there were also a wide range of biological responses examined across publications, measured biological responses were categorized into common themes, including demographic and diversity; behavioural; genetic; mortality and survival; reproduction; and sublethal physiological responses, (Table 3), acknowledging that overlap exists in what some measured responses could be categorized into (e.g., some mortality and survival responses could be categorized as demographic responses).

Road effects were qualitatively assigned as positive (i.e., putative stressor had a positive effect on the taxa), negative (i.e., putative stressor had a negative effect on the taxa) or neutral/inconclusive (i.e., stressor had no detected effect on the taxa) based on the reported statistical results and/or the authors' statements. The qualitative trends identified for each road effect were used to identify the general, overall effects of roads. However, we acknowledge that using such an approach (i.e., vote counting) does not provide a quantitative estimate of the effect of roads and ignores the uncertainty around the effect estimate. A formal meta-analysis was not conducted due to the substantial diversity in both predictor and response variables across studies, which made it impractical to derive a meaningful overall effect size (Bushman and Wang 2009; Koricheva et al. 2023).

Results

We reviewed the title and abstracts of 4811 unique publications returned from the literature search and conducted a full text review of 771 of these publications. Our final evidence base (i.e., included after full text screening) was comprised of 426 publications, which produced 1324 cases, that examined the impacts of roads and/or traffic on freshwater biological responses (evidence base available in Supplemental Materials). The earliest publication in our evidence base was published in 1966, with a steady increase in the number of annual publications starting around 2000 (Fig. A1). Henceforth, results are reported as frequency and percentage of cases in

Table 3. Biological response categories, illustrative examples, and direction of negative road effects on taxa.

Biological response category	Example(s)	Negative road effect
Demographic and diversity		
Biomass	Organism biomass, relative biomass, community biomass	Decrease in biomass
Composition (of assemblages, community, populations, etc.)	Assemblage composition, community composition, functional group composition	Change in composition deemed as disadvantageous
Density	Organism density per spatial unit, species density	Decrease in density
Distribution	Organism distribution within an area	Decrease in distribution
Diversity (biodiversity, indices)	Organism diversity within an area, species diversity, Shannon's Diversity Index, Simpson's Diversity Index	Decrease in diversity
Evenness	Species evenness	Decrease in evenness
Frequency (abundance, counts, percentages, ratios, abundance indices)	Number of organisms/individuals, catch per unit effort, percent cover, biotic index, sex ratio	Decrease in frequency
Occurrence (presence, absence, occupancy)	Site occupancy, organism presence/absence at a location, persistence over time, eDNA	Decrease in occurrence
Population size/change	Population change over time, population size over time, relative population size, recruitment	Decrease in population size
Richness	Species richness	Decrease in richness
Structure (of assemblages, communities, populations, etc.)	Population structure, community structure, age distribution	Change in structure deemed as disadvantageous
Behavioural		
Activity level	Jumping/swimming levels and rates	Decrease in activity level
Avoidance/attraction	Attraction to roads, avoidance behaviour to vehicles, traffic disturbance and/or road surfaces	Increase in attraction or decrease in avoidance
Connectivity/passage	Movement obstructed, behaviour in/at passage/drainage infrastructure or while crossing roads	Decrease in connectivity/passage
Dispersal	Inadvertent dispersal by individuals by vehicles, larval drift	Decrease in dispersal
Feeding	Feeding rates, feeding activity	Decrease in feeding
Movement patterns	Migration, in-season movements, approach angles, home range, road crossing time	Change in movement patterns deemed as disadvantageous
Preference/selection	Habitat selection, habitat use	Change in habitat selection or use deemed as disadvantageous
Vigilance	Anti-predator/parasite behaviour, flushing behaviour, alarm behavior	Decrease in vigilance
Vocalizations	Calling traits, effort/duration, structure, volume	Decrease or change in vocalizations
Genetic		
Gene expression	Gene expression related to survival and stress	Change in gene expression deemed as disadvantageous
Genetic differentiation/variation	Genetic variation of organisms within and/or between areas	Change in genetic variation deemed as disadvantageous
Genetic distance	Isolation by distance, relatedness by genetic distance	Change in genetic distance deemed as disadvantageous
Genetic divergence	Population isolation measured by genetic divergence	Change in genetic divergence deemed as disadvantageous
Genetic diversity	Allelic richness, observed/expected heterozygosity, genetic introgression	Change in genetic diversity deemed as disadvantageous
Mortality and survival		
Lethality	Lethality rates (e.g., LC50)	Increase in lethality
Mortality frequency	Number or percent of individuals killed, roadkill survey data, mortality from contaminant exposure	Increase in mortality frequency
Mortality rate	Mortality within a given timeframe due to contaminant exposure or road construction	Increase in mortality rate
Mortality risk	Risk of vehicle caused roadkill, mortality probability	Increase in mortality risk
Survival frequency	Number or percent of individuals that survive, brood survival, survival to hatching	Decrease in survival frequency
Survival probability	Population viability analysis	Decrease in survival probability

Table 3. (*concluded*).

Biological response category	Example(s)	Negative road effect
Reproduction		
Fecundity	Number of gravid females, embryo size	Decrease in fecundity
Frequency of eggs	Number of eggs laid, number of egg masses	Decrease in frequency of eggs
Frequency of nests/redds	Number of nests or redds, distribution of nests	Decrease in frequency of nests or redds
Nest or hatching success	Nest success, successful hatching of offspring	Decrease in nest or hatching success
Production rates	Recruitment, successful breeding, spawning	Decrease in production rates
Sex determination	Organism sex determination	Change in sex determination deemed as disadvantageous
Sublethal physiological		
Blood/other tissue constituents	Cortisol/corticosterone levels, lipid content, oxidative stress, molecular changes	Increase in blood/other tissue constituents deemed as disadvantageous
Contaminant uptake	Lead, phosphate, nitrogen, zinc, heavy metal concentrations in organisms	Increase in contaminant uptake
Deformities	Developmental abnormalities, malformations	Increase in deformities
Development rates	Time and/or size at metamorphosis, Gosner stage	Change in development rates deemed as disadvantageous
DNA changes	Transcriptional changes	Change in DNA deemed as disadvantageous
Growth/productivity	Organism growth, weight/body mass, change in biomass, above- or below-ground biomass	Change in growth/productivity deemed as disadvantageous
Immune function	Measures of immune function, infection vulnerability	Decrease in immune function
Parasite infection	Parasite load, parasite infection frequency	Increase in parasite infection
Toxicity	Metal, salt, physiological toxicity	Increase in toxicity

Note: Responses were deemed as disadvantageous according to the publication author's statements.

the overall evidence base (n = frequency; %), unless otherwise specified.

Geography, freshwater ecosystem types, and taxa

Over 60% of road effects cases were investigated in the United States and Canada (Fig. A2). The remaining 53 countries in our evidence base accounted for fewer than 80 cases each.

Research was most frequently conducted in ponds and wetlands ($n = 524$; 39.6%), followed by laboratories or mesocosms ($n = 284$; 21.4%) and rivers or streams ($n = 276$; 20.8%), and least frequently in lakes ($n = 36$; 2.7%; Fig. 1). Riparian ($n = 2$; 0.15%) and estuarine or brackish ($n = 42$; 3.2%) ecosystems received limited research focus in the evidence base. Most publications reported road effects on a single ecosystem type rather than multiple ecosystems.

Amphibians were the most studied taxon ($n = 478$), representing more than a third of all cases in the evidence base (36.1%; Fig. 1). Fish ($n = 196$; 14.8%) and reptiles ($n = 144$; 10.9%) were the next most studied. Avifauna ($n = 109$), MMZ ($n = 118$), insects ($n = 100$), and plants ($n = 78$) each accounted for less than 10% of the cases. Mammals, molluscs, and crustaceans were the least studied, with fewer than 60 cases each.

Across taxa, nearly half of the cases examined the impacts of roads on adults ($n = 567$; 42.8%), followed by larval ($n = 205$, 15.5%), egg ($n = 46$; 3.5%), and juvenile stages ($n = 45$; 3.4%; Fig. 2). Some research encompassed multiple life stages ($n = 88$; 6.6%). The life stage was not clearly specified for about one quarter of the cases ($n = 373$; 28.2%). Within each taxon, adults were the most commonly studied life stage, except for insects and molluscs where the larval stage was most commonly studied. Egg stages were only examined in amphibians, avifauna, and fish. Juveniles were only examined in amphibians, avifauna, crustaceans, and fish.

The impacts of roads on freshwater invasive species were infrequently examined ($n = 44$; 3.3%). Plants ($n = 13$), fish ($n = 11$), and amphibians ($n = 9$) were the most common invasive taxa studied. Results on invasive species hereafter will focus on these three taxa.

Road phases and types, and study designs and settings

Research on road effects were most frequently conducted during road use and maintenance activities ($n = 592$; 44.7%), followed by road existence ($n = 528$; 39.9%), and road construction activities ($n = 103$; 7.8%). Most research was conducted on paved roads ($n = 1157$; 87.4%) compared to unpaved roads ($n = 109$; 8.2%), with a small number of cases encompassing both paved and unpaved roads ($n = 58$; 4.4%).

Half of the research was conducted using an impact-only design ($n = 675$; 51.0%), followed by control-impact design ($n = 616$; 46.5%). Before-After ($n = 22$; 1.7%) and Before-After-Control-Impact (BACI) ($n = 11$; 0.8%) study designs were not commonly used (Fig. 3). Most research was conducted in a field setting ($n = 974$; 73.6%), followed by laboratory studies

Fig. 1. Distribution and frequency of cases ($n = 1324$) that examined the impacts of roads on different freshwater taxa and ecosystem types. MMZ = micro-invertebrates, macro-invertebrates, and zooplankton. Mammal = red bars, avifauna = orange, reptile = cyan, amphibian = dark green, fish = blue, mollusc = purple, crustacean = medium grey, MMZ = light gray, insect = black, and plant = green.

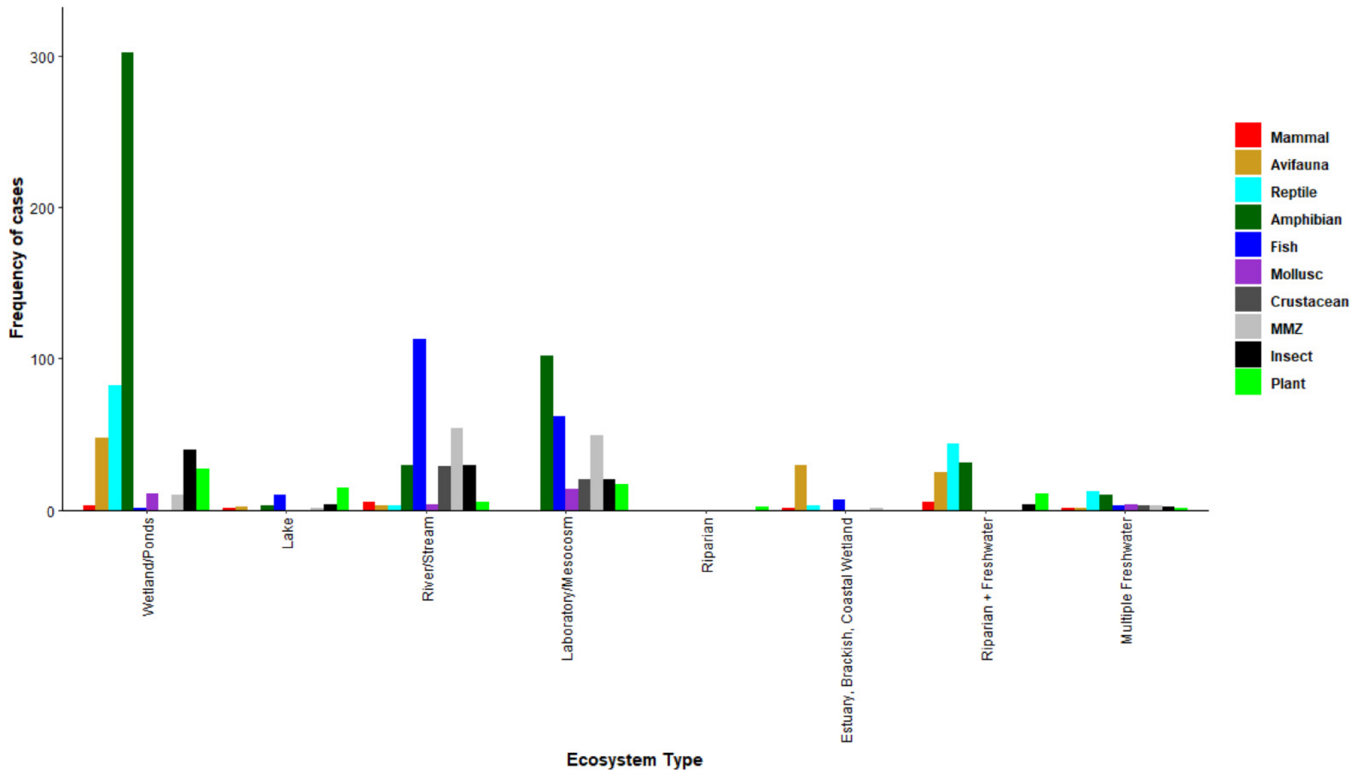


Fig. 2. Distribution and frequency of cases ($n = 1324$) that examined impacts of roads on freshwater taxa according to life stage. MMZ = micro-invertebrates, macro-invertebrates, and zooplankton. Mammal = red bars, avifauna = orange, reptile = cyan, amphibian = dark green, fish = blue, mollusc = purple, crustacean = medium grey, MMZ = light gray, insect = black, and plant = green.

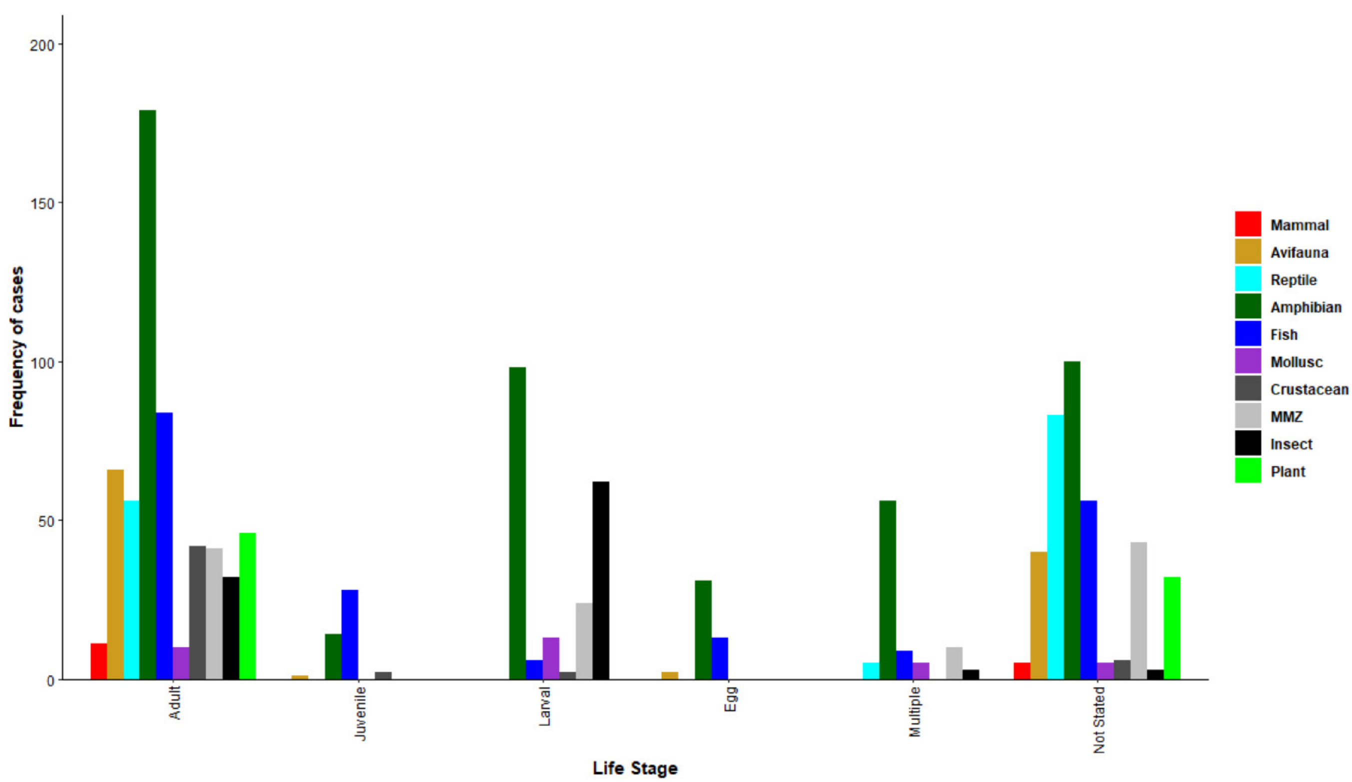
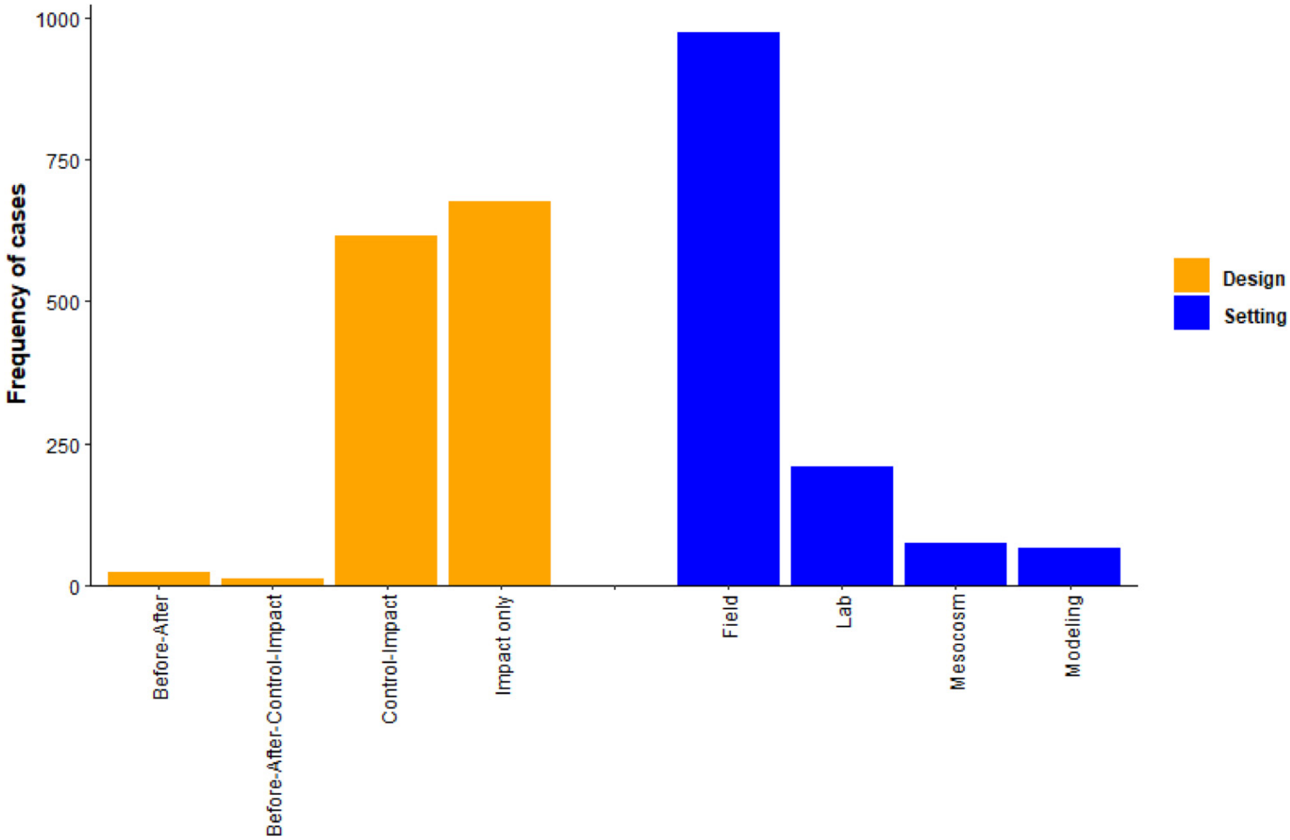


Fig. 3. Frequency of study designs (orange bars) and settings (blue bars) used to examine the effects of roads on freshwater biodiversity among publications in the evidence base. Some publications used more than one study type or design.



($n = 210$; 15.9%), modelling ($n = 29$, 6.8%), and mesocosms ($n = 23$; 5.4%).

Putative stressors and biological response variables

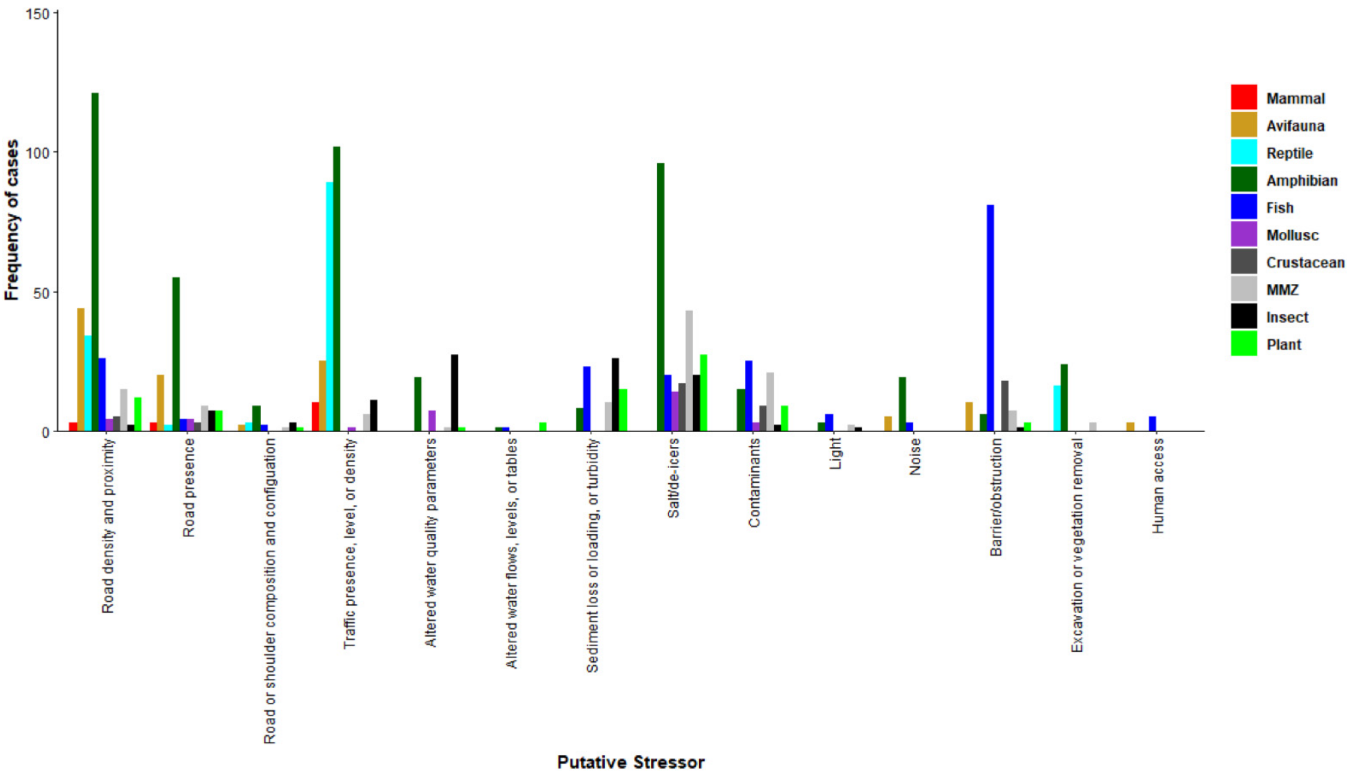
The most frequently examined putative stressor categories were road density and proximity to roads ($n = 266$; 20.0%), followed by traffic presence, level, or density ($n = 244$; 18.4%) and salt/de-icers ($n = 237$; 17.9%), with the remaining having fewer than 50 cases each.

Within taxa, for amphibians, the most frequently examined putative stressors included road density, traffic-related, and salt/de-icers, while human access, altered hydrology, and light pollution each had fewer than five cases (Fig. 4). Barriers were the most frequently examined putative stressor for fish, followed by road density, altered sediment loads, and contaminants (Fig. 4). For reptiles, traffic presence was the most frequently examined putative stressor followed by road density. Most stressors were minimally investigated for MMZ, insects, crustaceans, molluscs, plants, and mammals.

The most frequently examined biological responses included demographic and diversity accounting for half of the cases ($n = 664$; 50.2%), followed by mortality and survival ($n = 332$; 25.0%), sublethal physiological impacts ($n = 131$; 9.9%), and behavioral responses ($n = 112$; 8.5%) (Fig. 5). Reproduction- ($n = 45$; 3.4%) and genetic-related responses ($n = 40$; 3.0%) received comparatively limited focus. Of the demographic and diversity responses, measures of frequency, occurrence, and richness were the most reported response metrics, accounting for almost 75% of the demographic and diversity responses category and over one third of the evidence base (Table A1). Frequency of mortality and survival were the most reported response metrics within the mortality and survival category, accounting for almost 25% of the evidence base. For sublethal physiological responses, effects on organism growth were the most reported response metrics. For behavioural responses, movement patterns, dispersal, and vocalizations were the most reported response metrics. For reproduction responses, the number of eggs produced was the most reported response metric, accounting for over one third of the reported responses. For genetic responses, measures of genetic diversity were the most reported response metrics, accounting for almost half of the genetic category.

Within taxa, for amphibians, the most frequently recorded response category was demographic and diversity, followed by mortality and survival, sublethal physiological, and behavioural responses (Fig. 5). For fish, demographic and diversity responses were also the most frequently recorded response category, followed by behavioural, mortality and survival, and sublethal physiological responses. Mortality and survival, followed by demographic and diversity were the most frequently examined response categories for reptiles

Fig. 4. Distribution and frequency of cases ($n = 1324$) that examined the impacts of roads on freshwater diversity across taxa and putative stressors. MMZ = micro-invertebrates, macro-invertebrates, and zooplankton. Mammal = red bars, avifauna = orange, reptile = cyan, amphibian = dark green, fish = blue, mollusc = purple, crustacean = medium grey, MMZ = light gray, insect = black, and plant = green.



and mammals. For avifauna, molluscs, crustaceans, MMZ, insects, and plants, the most frequently examined response category was demographic and diversity responses.

Examining putative stressors and biological responses concurrently across all taxa, the most frequently reported stressor–biological response combinations were road density and proximity impacts on demographic and diversity responses ($n = 208$; 15.7%) and traffic presence for mortalities ($n = 207$; 15.6%; Fig. 6). Barriers were the most commonly investigated putative stressor on behavioural responses, salt/de-icers for sublethal physiological responses, and road density for genetics and reproduction responses.

Regarding invasive species, the most frequently reported putative stressors examined were road density and proximity and road presence on invasive plants ($n = 8$ cases), fish ($n = 10$), and amphibians ($n = 4$), accounting for over 60% of the putative stressor cases regarding invasive species. Demographic and diversity responses of invasive plants ($n = 11$), fish ($n = 10$), and amphibians ($n = 7$) were the most frequently investigated biological responses, also accounting for over 60% of the measured biological responses regarding invasive species taxa.

Trends in road effects

Putative road stressors had variable effects on freshwater taxa, eliciting negative, neutral/inconclusive (i.e., no observed effect), or positive biological responses (Fig. A3; Tables A2 and A3). Negative effects on freshwater taxa accounted for over 60% of cases across putative stressor and biological response categories. Neutral and positive effects on freshwater taxa were reported for most putative stressor categories and all biological response categories, but were proportionally lower (31% and 6%, respectively).

Most effects on freshwater taxa were reported in consistent stressor–biological response category combinations (Fig. A4). Negative effects were most frequently reported for traffic-related impacts on mortality and survival, accounting for over 15% of the cases in our evidence base. Positive effects, though minimal, were most frequently reported for demographic and diversity responses to road density and proximity. The negative, positive, and neutral effects of salt/de-icers and contaminants were fairly varied across taxa within demographic and diversity, mortality and survival, and sublethal physiological response categories relative to other putative stressors. Within the demographic and diversity category, taxa responses were fairly varied across most putative stressor categories relative to other biological responses.

Negative, neutral, and positive effects varied among taxa (Fig. 7). Studies on amphibians reported the most negative ($n = 328$) and neutral ($n = 135$) effects, which accounted for about 25% and 10%, respectively, of all effects reported. Fish had the second most negative ($n = 107$) and neutral ($n = 77$) effects, accounting for about 8% and 5%, respectively, of all

Fig. 5. Distribution and frequency of cases ($n = 1324$) that examined the impacts of roads on freshwater diversity across taxa and biological responses. MMZ = micro-invertebrates, macro-invertebrates, and zooplankton. Mammal = red bars, avifauna = orange, reptile = cyan, amphibian = dark green, fish = blue, mollusc = purple, crustacean = medium grey, MMZ = light gray, insect = black, and plant = green.

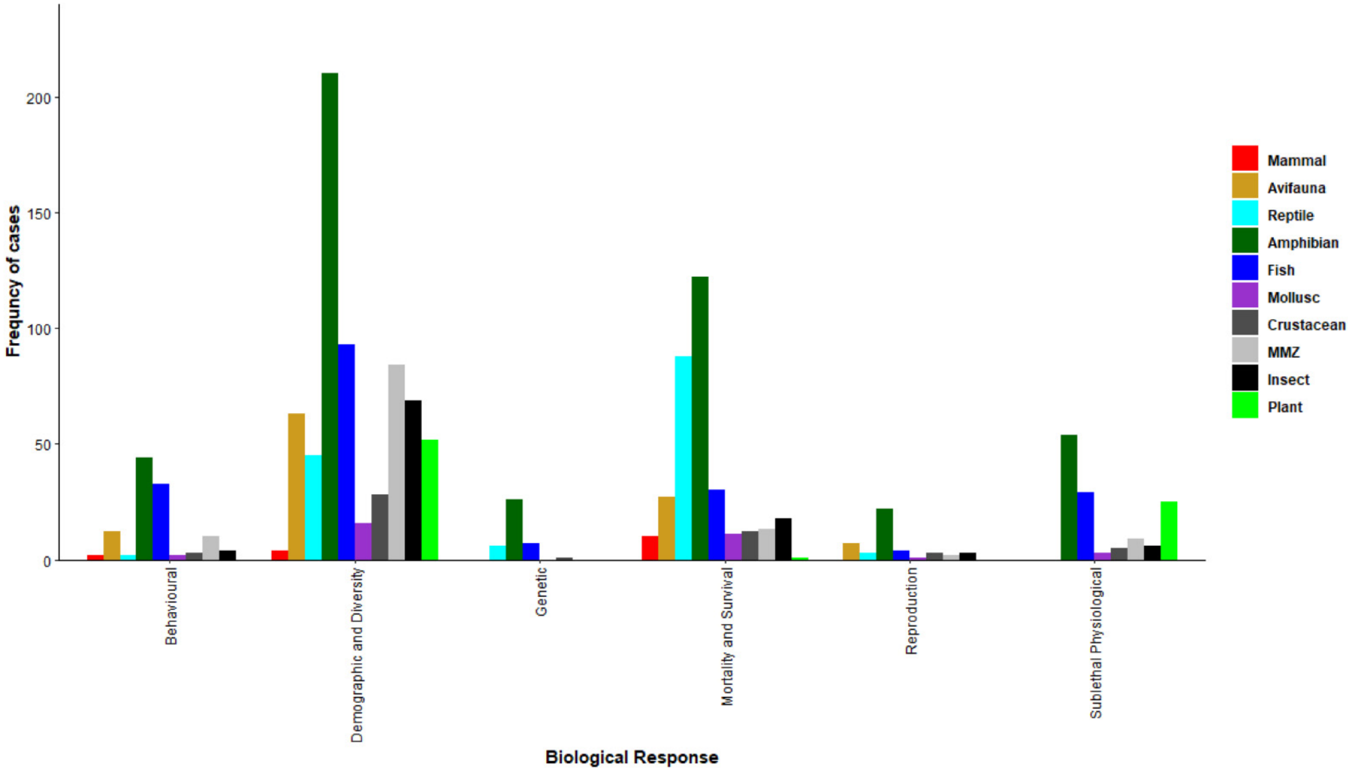


Fig. 6. Distribution and frequency of cases ($n = 1324$) that examined the impacts of roads on freshwater diversity according to putative stressors and biological responses. Behavioural = orange bars, demographic and diversity = black, mortality and survival = red, sublethal physiological = dark green, genetic = blue, and reproduction = purple.

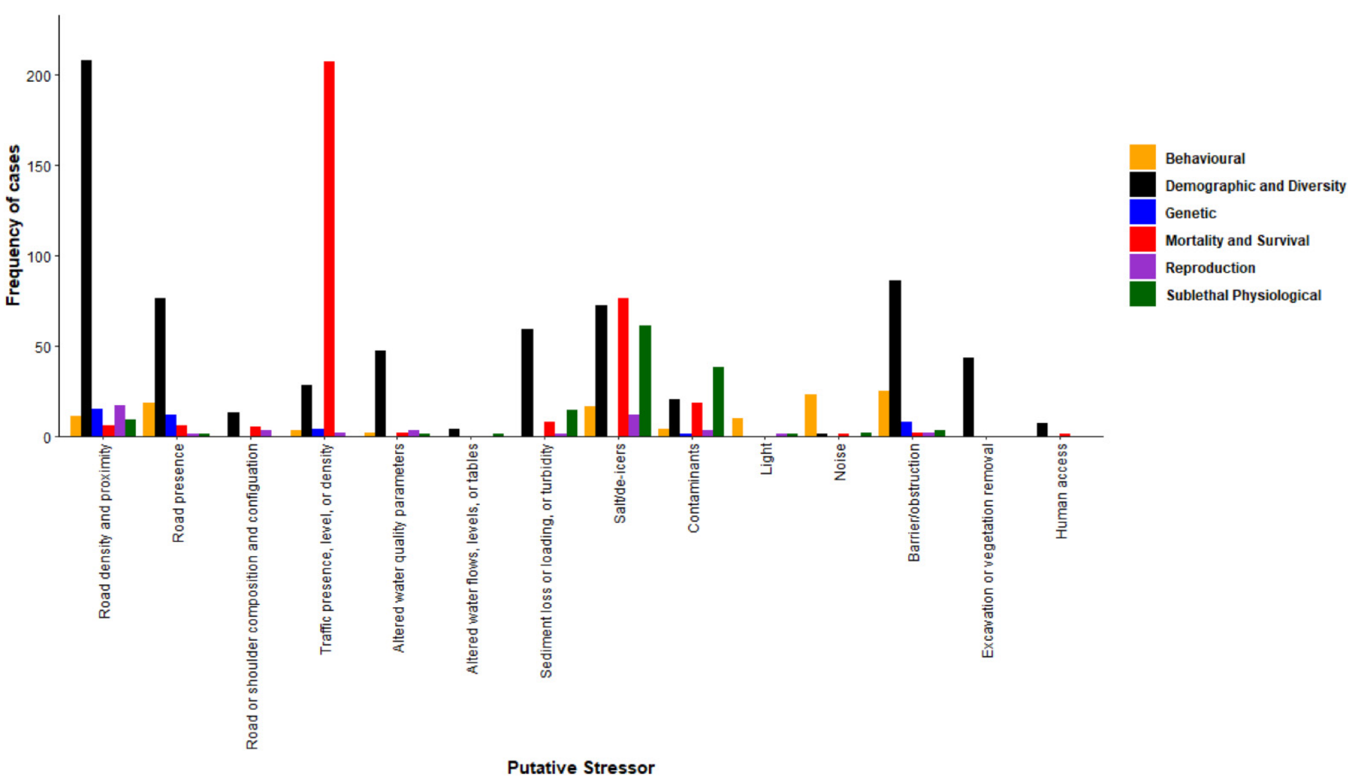
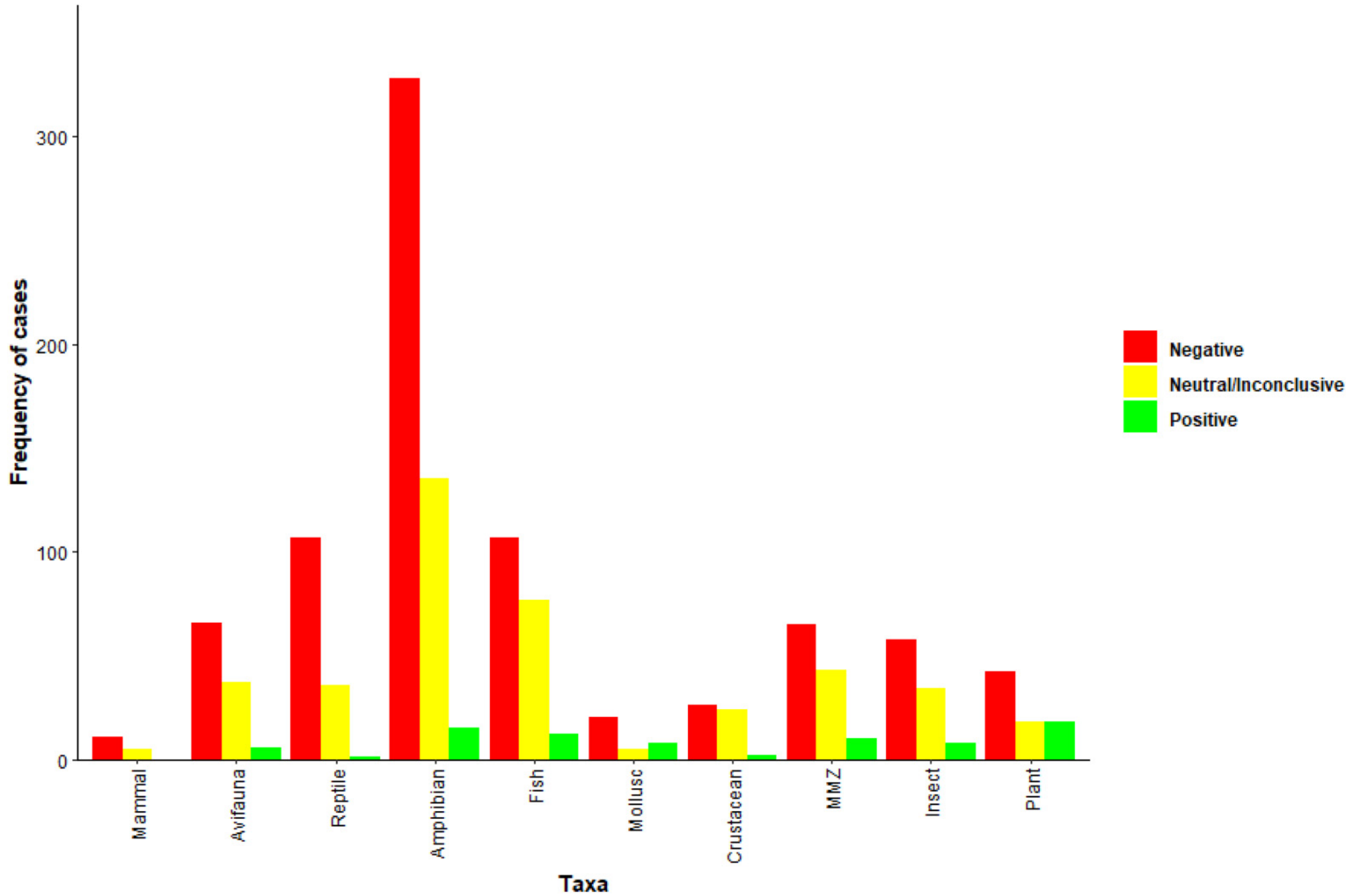


Fig. 7. Distribution and frequency of negative (red bars), neutral/inconclusive (yellow bars), and positive (green bars) effects on freshwater taxa. MMZ = micro-invertebrates, macro-invertebrates, and zooplankton.



effects reported. Negative effects on reptiles were relatively commonly reported (n = 107), accounting for about 8% of the total effects reported. Negative effects on avifauna (n = 66), MMZ (n = 65), and insects (n = 58) accounted for about 5% each of the total effects. The remaining negative, neutral, or positive effects reported accounted for less than 50 cases each. Although limited, positive effects were most reported for plants (n = 18), amphibians (n = 15), and fish (n = 12).

Some taxa level patterns were evident (Figs. A5–A8), notably for amphibians and fish, the two most-studied groups. Negative effects on amphibians were fairly prominent in many stressor–biological response category combinations, including traffic-related and salt/de-icer effects on mortality and survival, salt/de-icer effects on sublethal physiological responses, and road density and proximity and road presence effects on demographic and diversity responses. Positive and neutral effects reported for amphibians were limited. Negative and positive effects of barriers on behavioural, demographic and diversity, and genetic responses were prominent for fish relative to other taxa, with the caveat that the effects of barriers were not investigated for most taxa. Reptiles, the third most studied taxon, had the highest frequency of negative traffic-related effects on mortality and survival, which also accounted for the majority of effects reported on reptiles in the overall evidence base.

Of the studies that investigated invasive plants, fish, and amphibians, positive (n = 11) effects were more frequently reported than negative effects (n = 1). Road density, proximity, and presence were found to have positive effects on demographic and diversity (n = 7) and sublethal physiological-related responses (n = 2) of invasive plants, as well as on demographic and diversity responses of invasive fish (n = 2). The only negative effect on invasive species reported was the impact of road presence on invasive plant demographic and diversity responses. Road density and proximity were found to have no effect on demographic and diversity- and reproduction-related responses of invasive amphibians (n = 7). A variety of stressors were found to have no effects on demographic and diversity- (n = 8) and genetic-related (n = 1) responses for invasive fish, and on demographic and diversity responses for invasive plants (n = 3).

Discussion

Geography, ecosystem types, and taxa

Our goals for this evidence synthesis were to assess the state of knowledge on the impacts of roads on freshwater biodiversity, and identify knowledge gaps and research opportunities. Consistent with other syntheses (Dixon et al. 2022), we found a disproportionate focus on studies conducted in North America, particularly the United States and Canada. All other regions represented very few cases in our evidence base. While we acknowledge that this geographical imbalance could be due in part to a language bias in our search methodology, the geographic imbalance may represent a "real" knowledge gap in the current evidence base which could benefit from future primary research. This is especially important considering many of these regions, such as South America and parts of Asia and Africa, are biodiversity hotspots, rich in freshwater diversity, and are projected to see substantial road development in the coming decades (Moslen and Miebaka 2024).

In terms of ecosystem type, we found that most research was field-based and conducted in ponds, wetlands, and rivers or streams. Lacustrine ecosystems remain underrepresented in the literature, which was surprising because lakes are among the most heavily modified and degraded freshwater environments worldwide due to their association with shipping, urbanization, settlement, and resource extraction (Edgar et al. 2000). Our review also identified riparian zones and estuaries or brackish areas as among the least studied. This was surprising given the long-standing recognition that freshwater ecosystems are tightly connected to surrounding terrestrial environments (Hynes 1975), particularly in riparian zones where anthropogenic disturbances can have direct and cascading effects on freshwater health (Cooke et al. 2022). The limited attention to riparian areas is concerning given their critical role in mediating land–water interactions. Similarly, given that water runs downstream, the lack of effort to understand impacts on estuaries is also surprising. Taken together, these findings highlight the need for future research focused on underrepresented and transitional freshwater ecosystems.

Although studies investigating road and traffic effects spanned a diversity of freshwater taxa, the evidence base was dominated by three main groups: amphibians, reptiles, and fish. Amphibians were the most studied freshwater taxa in our evidence synthesis, which is consistent with a previous review focused on lentic systems (Dixon et al. 2022). Reptiles were among the most commonly studied in our evidence synthesis relative to other taxa, whereas reptiles were among the least in Dixon et al. (2022), likely owing to our ecosystem inclusion criteria expanding beyond lentic systems.

We found a comparatively higher prevalence of studies examining fish, which was consistent with Dixon et al. (2022). Fish may be studied more frequently because they provide important insights into road effects on fully aquatic freshwater taxa, and can have different physiological requirements (Jeffrey et al. 2015) than meroquatic taxa typically affected by roads (e.g., amphibians; Hall et al. 2017). Some fish are considered indicator species (Walters et al. 2009; Meland et al. 2010*b*) in determining ecosystem health (i.e., aquatic "canaries"; Lynch et al. 2016), including for determining whether roads are affecting aquatic ecosystems (e.g., Meland et al. 2010*a*). Macro-invertebrates, which are also commonly used as indicator species for aquatic ecosystem monitoring (Jeppesen et al. 2011; Carter et al. 2017), were relatively limited in our evidence base. This was surprising given the widespread use of aquatic invertebrates as indicator species for environmental monitoring purposes.

While the limited focus on mammals and avifauna in our evidence base may reflect the fewer freshwater-oriented mammalian and avian species (Dehorter and Guillemain 2008; Veron et al. 2008), the lack of research on other taxa, such as MMZ, crustaceans, molluscs, and insects, aligns with well-documented taxonomic biases in ecological research. These biases often arise from a tendency to prioritize charismatic species that attract greater public and scientific interest (Borgi and Cirulli 2016; Rosenthal et al. 2017), resulting in the underrepresentation of less charismatic taxa or those that are logistically difficult to study (e.g., Jeppesen et al. 2011). This issue may be especially pronounced in freshwater ecosystems, where many species are perceived as less appealing (e.g., Sigouin et al. 2017; Lipták et al. 2023; Li et al. 2025), reinforcing these research imbalances. The limited attention to these overlooked groups restricts our ability to fully understand the scope of road impacts on freshwater biodiversity and impedes the development of inclusive conservation and management strategies.

Roads can exert differential effects across the life stages of freshwater taxa (Cocchiglia et al. 2012; Hoffman et al. 2012; Hintz and Relyea 2019). Adult stages were the predominant research emphasis in our evidence base (e.g., Huang et al. 2018), which was not surprising because examining adult specimens poses several logistical advantages. For example, adults are typically more easily tracked (i.e., owing to their larger size) and ontologically more stable (i.e., developmentally, genetically) relative to earlier life stages. Egg and larval categories were considerably underrepresented in the evidence base across taxa, which is concerning given these early developmental life stages are particularly sensitive to perturbations in environmental conditions and toxins (Mohammed 2013). Further, a substantial number of studies did not specify the life stage studied, indicating a gap in precision that could limit the understanding of life stage-specific vulnerabilities to road impacts. Given the pervasive impacts of roads across life stages, we recommend that researchers report the life stage of study/observed species. These taxa and life stage patterns suggest both a need for broader taxonomic and developmental coverage in future road ecology studies and a reevaluation of potential life stage-specific sensitivities in freshwater ecosystems.

We found that very few studies examined the impacts of roads on invasive freshwater species, a pattern consistent with other evidence syntheses (Dixon et al. 2022). Specifically, about 3% of studies in our review explicitly focused on invasive species, with most of these addressing plants (25%), fish (25%), and amphibians (20%). This gap is striking given that roads are known to facilitate plant invasions (e.g., Gelbard and Belnap 2003; Joly et al. 2011; Mortensen et al. 2009) through multiple mechanisms, including elevated nutrient inputs from adjacent land uses such as agriculture, increased disturbance, and the greater tolerance of invasive plants to degraded or polluted habitats (e.g., Brisson et al. 2010; Gallardo 2014; Lemke et al. 2019). Interestingly, three of the invasive-focused studies identified in our review concentrated on *Phragmites australis* alone, a notorious invasive aquatic plant whose extensive rhizome networks (Juneau and Tarasoff 2013) facilitate rapid spread along linear features such as roads and associated drainage corridors. Roads are widely recognized as invasion corridors that often support higher abundances of invasive species than less degraded, more intact habitats (Crooks et al. 2011), making the paucity of studies explicitly examining road–freshwater invasion interactions particularly surprising. Collectively, these findings underscore a critical knowledge gap and highlight the need for targeted research on how road networks influence the introduction, spread, and persistence of invasive species in freshwater ecosystems, particularly beyond single-species case studies.

Road phases and types, and study design and setting

The majority of the research in our evidence base focused on impacts of paved roads during road use (e.g., by vehicles, etc.), maintenance (e.g., salting/de-icing), and the presence of the road(s) in the ecosystem. Relatively little research in our evidence base focused on examining the impacts during the road construction phase, which was unexpected because road construction related activities produce the first impacts to aquatic ecosystems and associated taxa. Further, given the objectives of Environmental Impact Assessment (EIA), specifically, to avoid or mitigate environmental harm, and their increasing application in road development projects in recent years (Karlson et al. 2014), we expected to find more studies examining road impacts during the construction phase. The relative lack of research at this stage may be due to EIAs often being published as reports or in the grey literature (Lawrence 2017), which were not captured by our search strategy. To ensure evidence syntheses include valuable data and results contained in EIAs and other environmental reports, grey literature databases should be included in search strategies.

Our evidence base encompassed a complementary mix of field- and laboratory-based research. Most research was field-based, which is advantageous in fostering ecological validity of road effects, covering large spatial scales and a variety of ecosystem types, temporally monitoring road effects, and observing organismal responses to road stressors in a natural setting (Binning et al. 2025). In most cases, laboratory studies were conducted to determine the effects of salt/de-icers and other contaminants on various taxa in a controlled environment, which is advantageous in determining thresholds of (sub)lethality (e.g., concentration, temporal) and observing behavioural responses. This mix of field- and laboratory-based settings contributes to building a more comprehensive understanding of the consequences of roads and traffic on freshwater taxa (Binning et al. 2025).

The most common study designs used to assess the impacts of roads on freshwater taxa were impact-only and control-impact. The widespread use of impact-only designs is particularly concerning because this design lacks a comparator, either in the form of pre-disturbance data (temporal comparison) or data from unaffected sites (spatial control), which limits the ability to attribute observed effects specifically to road-related stressors. To build a more reliable and informative evidence base for decision-making, we recommend the broader adoption of study designs that incorporate spatial and/or temporal comparisons, as outlined in best practices for environmental monitoring (Cooke et al. 2023*a*) and road impact/mitigation effectiveness evaluation works (e.g., Roedenbeck et al. 2007; Rytwinski et al. 2015, 2016; van der Ree et al. 2015; Soanes et al. 2024). Consistent with other evidence syntheses (Su et al. 2026), very few studies in our evidence base used a BACI design, which is widely regarded as one of the most effective approaches for detecting causal relationships in complex, real-world ecosystems (Green 1979; Conquest 2000). BACI designs are particularly well suited for evaluating road impacts, as they account for both natural temporal variability and site-level differences (Underwood 1992) helping to isolate the effects of roads from background environmental changes (e.g., Torres et al. 2011). An additional consideration of the application of BACI designs is the configuration of the years of data (e.g., even number of sampling years before and after; Smokorowski and Randall 2017). Although we did not quantify this in our review, for researchers undertaking future research on the effects of roads on freshwater taxa, it would be beneficial to have even and consistent sampling years (i.e., composition) across both before and after (Smokorowski and Randall 2017). We do acknowledge comparative study designs, especially BACIs, are often difficult to implement, especially given that timelines may be dictated by road construction or enlargement projects (Lesbarrères and Fahrig 2012; Rytwinski et al. 2015; van der Ree et al. 2015). However, to improve our ability to detect and quantify road effects on freshwater biodiversity, road agencies, environmental regulators, and land managers that strive to make evidence-informed decisions must recognize that comparative study designs are an essential component of evaluation, insist on their inclusion, and support these investigations accordingly (Soanes et al. 2024).

Putative stressors and biological response variables

The most frequently examined putative stressors in our evidence base included: road density and proximity; traffic presence, volume or density; and salt/de-icers. These road-, traffic-, and pollutant-related stressors were consistent within herpetofauna taxa (amphibians and reptiles), two of the most studied taxa in our evidence base, as well as with the most reported stressors in other evidence syntheses (Dixon et al. 2022; Su et al. 2026). Many of the other stressors appear to remain poorly investigated. Water-related (e.g., altered hydrology, water quality parameters, and/or sediments) and habitat-related stressors (e.g., barriers, excavation activities, and increased human access), although found to be the most commonly studied stressors for fish in our evidence base, were comparatively limited for other species in our evidence synthesis overall and in other syntheses (Dixon et al. 2022). Despite the pervasiveness of light and noise pollution from road- and traffic-related stressors (e.g., street lights and vehicular traffic), which can have impacts on freshwater taxa (Longcore and Rich 2004; Mickle and Higgs 2018), limited research investigated the effects of these stressors on freshwater taxa. Furthermore, although reported singly in our evidence synthesis, many of these stressors are correlated and likely produce cumulative road effects (Teixeira et al. 2020).

Our evidence base on biological responses was strongly skewed toward demographic and diversity and mortality and survival-related outcomes. For example, within avifauna, there were no studies that examined genetic or sublethal physiological responses, highlighting the limited knowledge base on the effects beyond demographic metrics (e.g., Pedrana et al. 2014). Measures of occurrence, richness, and direct mortality dominated, which may reflect the relative ease of detecting changes in abundance or roadkill rates and their clear conservation relevance to transportation managers (Hels and Buckwild 2001). There was comparatively lower representation of sublethal physiological-, behavioral-, reproductive-, and genetic-related responses; a pattern that was consistent across taxa, including for those relatively well-represented groups in our evidence base.

Although most studies measured demographic and diversity-related outcomes (i.e., population outcomes; Nash Suding et al. 2003), the vast majority of studies focused on identifying and quantifying impacts that did not scale effects to a population level. For instance, a substantial portion of our evidence base examined mortality, sublethal impacts, and animal behaviour; responses, which generally do not allow for strong inference about the impacts of roads at the population level (Rytwinski and Fahrig 2015). For effective species conservation and to improve our understanding of road and traffic effects, it is important, if not essential, to determine how roads influence population persistence, meaning the long-term viability of a group of individuals rather than effects on single organisms. Identifying the mechanisms responsible for population declines is also critical for selecting appropriate mitigation strategies. For example, if increased mortality from vehicle collisions is the primary mechanism, then interventions such as wildlife fencing or barriers should be implemented to keep animals off roads. If mitigation targets the wrong mechanism, populations may continue to decline because the root cause has not been addressed. As Teixeira et al. (2020) suggest, future research should aim to develop studies that generate predictions capable of distinguishing among different mechanisms and quantifying their relative contributions to population decline. Conversely, some argue that sublethal effects and reproductive metrics can act as early warning indicators of population-level impacts before declines are detected through abundance-based measures (Cooke et al. 2023*b*). While these metrics offer valuable insights, they are most useful when integrated into a broader framework that also assesses demographic consequences. Notably, genetic responses were the least studied in our synthesis, despite their potential to reveal important information about population structure, gene flow, and long-term evolutionary outcomes (Sunnucks and Balkenhol 2015). This represents an important opportunity for future road ecology studies to better incorporate genetic data into impact assessments.

It should also be noted that, within each of our categories of putative stressors and biological responses, the specific metrics used were highly variable. For example, within our demographic and diversity responses category, we grouped measures of frequency (e.g., organism counts, percentages, ratios, abundance indices), occurrence (e.g., presence/absence, occupancy), and diversity measures (e.g., taxonomic or species richness). This general heterogeneity in measured responses makes it challenging to synthesize findings, advance the evidence base, and draw general conclusions, ultimately limiting the feasibility of meta-analytical approaches (e.g., not all metrics within a given measured response can and should be combined in a meta-analysis) (Harrison 2011; Haddaway and Rytwinski 2018).

Road effects trends

Road- and traffic-related stressors resulted in predominantly negative consequences for freshwater taxa. More than 60% of reported effects were negative, with traffic-related stressors most frequently linked to increased mortality and reduced survival, underscoring the direct lethality of vehicle collisions and associated disturbances for freshwater-associated taxa. Salt/de-icers elicited a mixture of negative, neutral, and occasionally positive responses across biological response categories (e.g., for invasive species; Silliman and Bertness 2004), suggesting context-dependent mechanisms that may be mediated by local hydrology, road design, or species-specific tolerances (e.g., Turtle 2000; Buss et al. 2020; Lawson and Jackson 2021). For example, at a continental scale in Europe, the occurrence of invasive Canada goose (*Branta canadensis*) was positively associated with socio-economic indicators including road density, reflecting the tendency of this species to thrive in human-modified landscapes where transport infrastructure and habitat disturbance are prevalent (Gallardo 2014). Neutral/inconclusive effects (i.e., no effects) were reported in some stressor–response categories for certain taxa, potentially highlighting the complexity and context-dependence of road impacts on freshwater taxa. Using individual-based simulation models, Rytwinski and Fahrig (2013) found that populations of species with small territories and movement ranges, and high reproductive rates may not be affected by roads. Although some positive effects were reported in some categories of responses in our evidence base (e.g., abundance, Suwannatrai et al. 2011; and reproduction; Hmamouchi et al. 2020), these were limited and the dominance of negative outcomes across most stressor–response combinations highlights that roads, traffic, and associated maintenance functions act as pervasive pressures on freshwater taxa and ecosystems.

Taxon-specific patterns further illustrate how roads can affect freshwater taxa in uneven ways. Amphibians and fish, two of the most frequently studied taxa, showed contrasting sensitivities to different stressors: amphibians were especially affected by traffic and chemical stressors (e.g., Glista et al. 2008; Bommarito et al. 2010; Gu et al. 2011; Dixon et al. 2022; Su et al. 2026), while fish exhibited negative and positive responses to barriers affecting movement, demography, and genetic connectivity (e.g., Amezcua et al. 2019). Reptiles also displayed a comparatively high frequency of negative traffic-related mortality (e.g., Garrah et al. 2015; Gonçalves et al. 2018), suggesting herpetofauna in general being acutely vulnerable to direct road impacts (Fahrig and Rytwinski 2009; Piczak et al. 2019). Taxon-specific patterns further emphasized strong research biases and uneven understanding among freshwater groups. Beyond herpetofauna and fish, the rest of the taxon groups were comparatively understudied, with few reported effects and limited inference potential. These taxonomic imbalances elucidate the need to improve our understanding of the impacts on understudied taxa and to evaluate whether stressor–response relationships observed in well-studied taxon groups generalize across freshwater biodiversity.

A small number of studies reported positive effects for invasive species, indicating that roads may facilitate invasive species, with positive demographic and diversity- and physiological-related responses reported for invasive plants and fishes in relation to road presence, density, and proximity. Positive effects of roads on some freshwater invasive taxa suggest that invasive species may exploit disturbed or connected environments created by road infrastructure. For instance, invasive plant species were found to be resilient to roadside conditions relative to native species (Jodoin et al. 2008). Similarly, invasive fish have been found to be more tolerant of disturbed habitats (Larentis et al. 2022), which could result from road infrastructure installations near watercourses. Increased road presence, density, and proximity can also promote invasions of non-native freshwater fish and plants by increasing opportunities for inadvertent or intentional introductions via unauthorized stocking, pet release, bait bucket dumping, and unintentional releases through transport of boating or fishing equipment (Buchan and Padilla 1999; Johnson et al. 2001; Drake and Mandrak 2010; Strayer 2010; Kizuka et al. 2014). For example, the abundance of some non-native species, such as red-eared sliders *Trachemys scripta elegans*, is positively correlated with road density, suggesting a positive relationship between species occurrence and human activity (Buchanan et al. 2019). Collectively, these patterns imply that roads not only reduce freshwater biodiversity through direct and indirect stressors but may also shift community composition by disproportionately benefiting invasive taxa, with long-term consequences for ecosystem structure and function. Furthermore, the intersection of roads and streams ("road crossings") has been shown to have negative effects on the richness and abundance of native macroinvertebrates—including the number of protected taxa, and alien taxa tend to be more abundant at these crossings (Gál et al. 2020). Future research should aim to better understand the specific biological mechanisms by which roads may positively influence freshwater invasive species establishment and persistence, and identify context-dependent factors that mediate or mitigate these impacts across taxa and ecosystems.

Conclusions and future study

(1) Our synthesis revealed that road effects on freshwater biodiversity are predominantly negative. Most studies reported declines in demographic and diversity-related responses (e.g., abundance, diversity) and survival, increased mortality, and sublethal physiological and behavioural effects (e.g., growth and movement).
(2) Our findings highlight substantial gaps in the evidence base. Reported effects on amphibians, fish, and reptiles dominated the literature, while invertebrates, plants, molluscs, crustaceans, insects, and mammals were comparatively understudied. Similarly, riparian zones, estuaries, and lacustrine environments were underrepresented, despite their ecological significance and presumed vulnerability to road-related impacts.
(3) Road density, traffic, and salt/de-icers, the most commonly studied putative stressors in our evidence base, were most frequently associated with negative outcomes among taxonomic groups, but many other pervasive stressors (e.g., noise, light, altered hydrology) remain poorly understood.
(4) Importantly, most studies used impact-only or control-impact designs, with few undertaking rigorous comparative frameworks like BACI, limiting our ability to infer causal relationships.
(5) To strengthen the evidence base and better inform avoidance and mitigation strategies, future studies should address understudied taxa and regions, incorporate life stage-specific assessments, and adopt comparative study designs.
(6) As road infrastructure continues to grow worldwide, integrating considerations for freshwater habitats into transportation policy and environmental assessment processes will be vital. Doing so will help safeguard freshwater biodiversity and promote ecosystem resilience in an era of rapid environmental change.

Acknowledgements

The authors thank B. Hlina for producing the map and reviewers for their valuable feedback.

Article information

History dates

Received: 13 July 2025
Accepted: 3 February 2026
Accepted manuscript online: 22 April 2026
Version of record online: 4 June 2026

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Data availability

All data for this synthesis are available at dataset https://doi.org/10.5061/dryad.tdz08kqd9.

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Competing interests

The authors declare there are no competing interests.

Funding information

The authors declare no specific funding for this work beyond general support from the Natural Sciences and Engineering Research Council in the form of Discovery Grants to LF, AR, and SJC.

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Appendix A

Fig. A1. The frequency of publications per year that examined the impacts of roads on freshwater diversity.

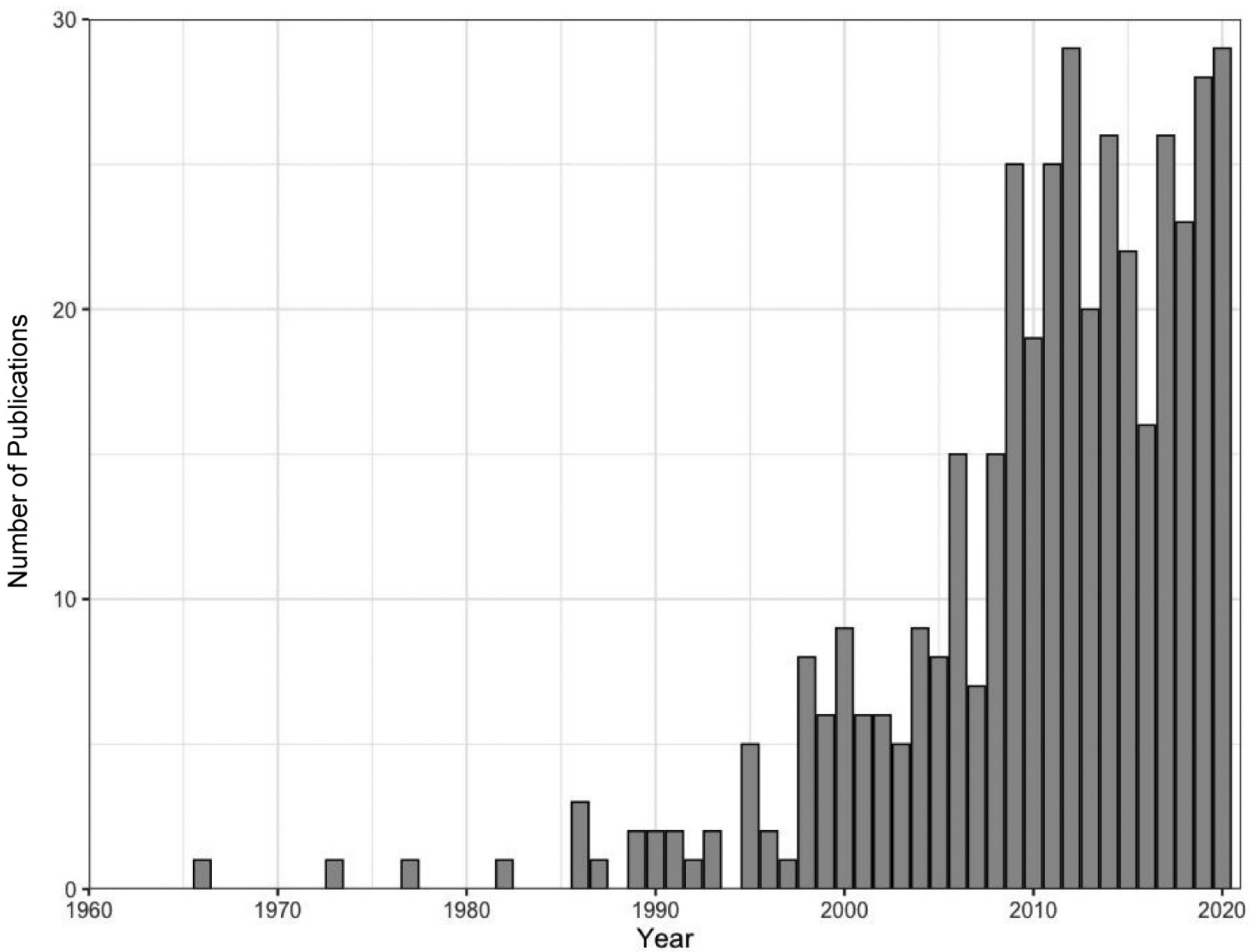

Fig. A2. Geographic distribution of the frequency of cases that examined the impacts of roads on freshwater diversity. Map created using "rnaturalearth" basemap (Massicotte and South 2026); projection and datum: WGS84; coordinate system: latitude/longitude.

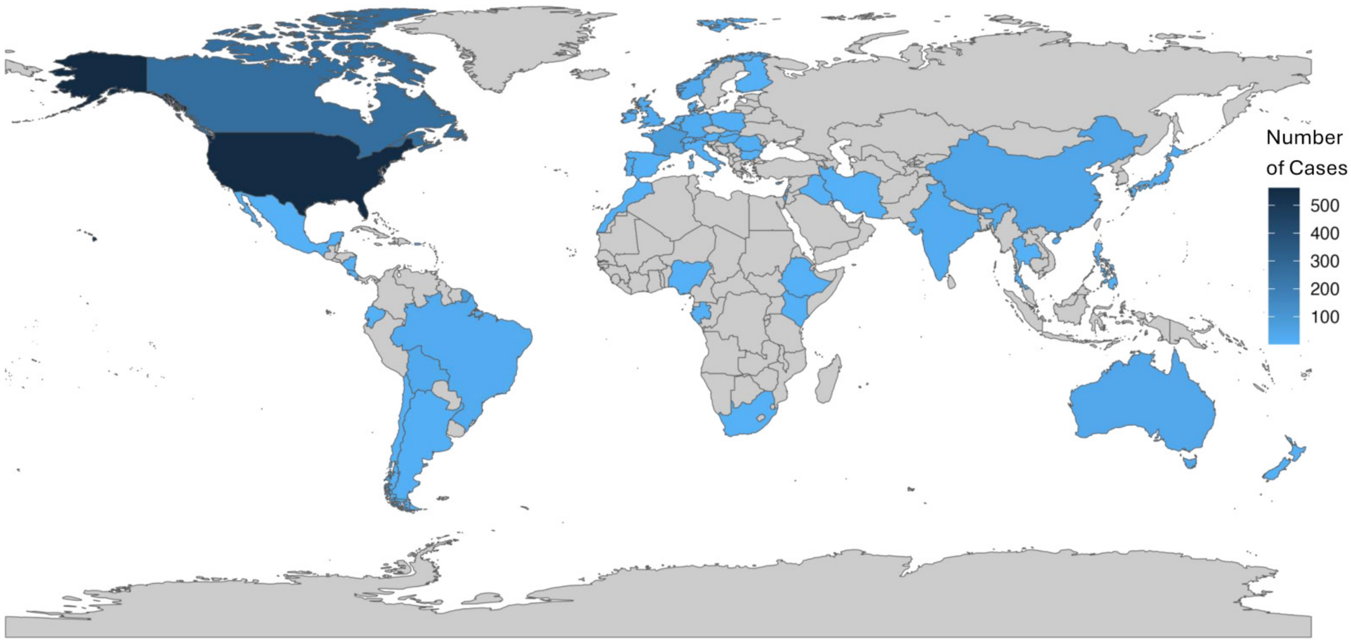

Fig. A3. The overall frequency of negative (red bars), neutral/inconclusive (yellow bars), and positive (green bars) effects on freshwater taxa reported in the evidence base.

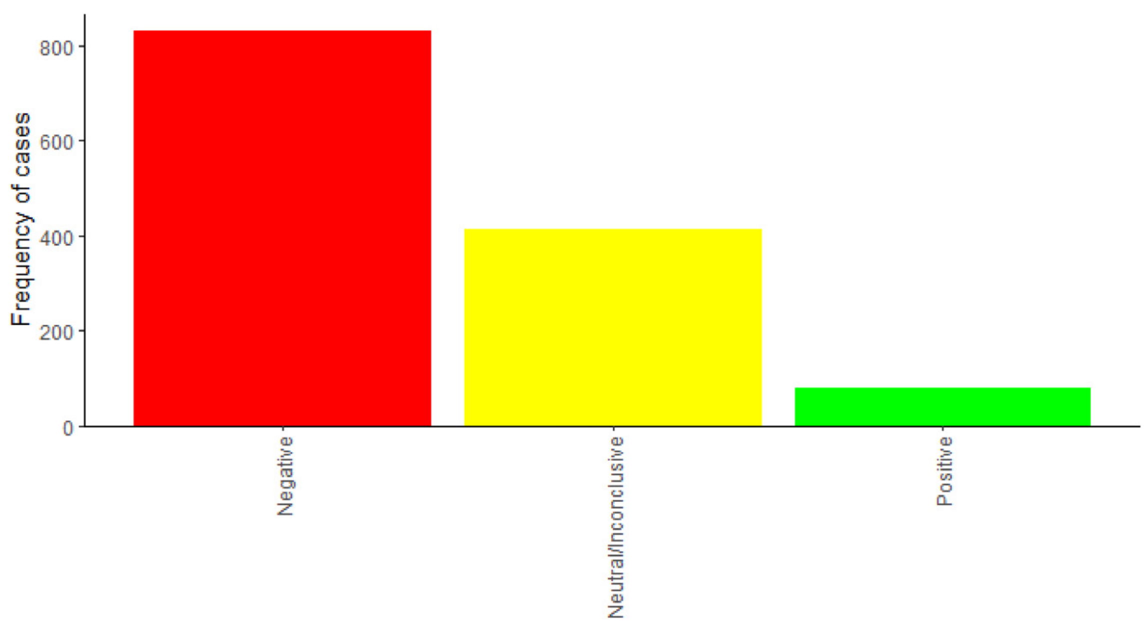

Fig. A4. Distribution and frequency of negative (red bars), neutral/inconclusive (yellow bars), and positive (green bars) effects on freshwater taxa according to stressors (x-axis) and biological response categories (vertical facets).

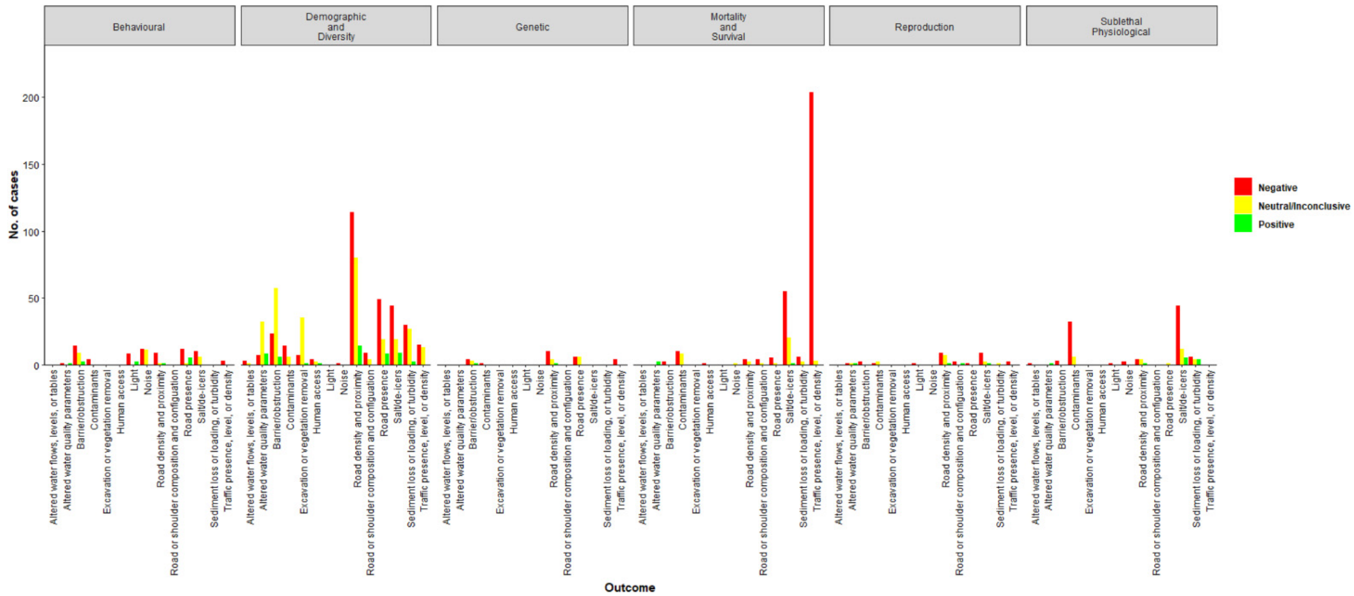

Fig. A5. Distribution and frequency of negative (red bars), neutral/inconclusive (yellow bars), and positive (green bars) effects on freshwater taxa according to stressors (horizontal facets) and biological response categories (vertical facets).

Fig. A6. Distribution and frequency of negative (red bars), neutral/inconclusive (yellow bars), and positive (green bars) effects on freshwater taxa according to stressors (horizontal facets) and biological response categories (vertical facets).

Fig. A7. Distribution and frequency of negative (red bars), neutral/inconclusive (yellow bars), and positive (green bars) effects on freshwater taxa according to stressors (horizontal facets) and biological response categories (vertical facets).

Fig. A8. Distribution and frequency of negative (red bars), neutral/inconclusive (yellow bars), and positive (green bars) effects on freshwater taxa according to stressors (horizontal facets) and biological response categories (vertical facets)

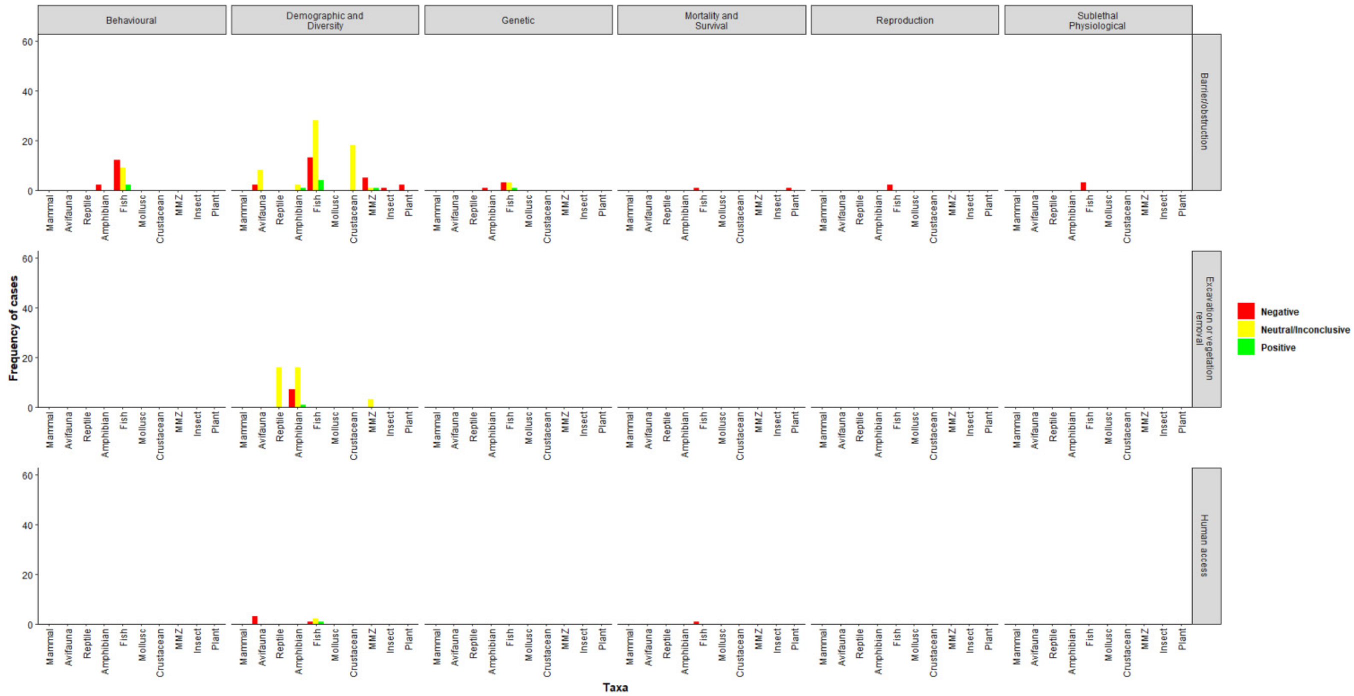

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Table A1. Frequency (number of cases) of the measured response metrics used within the biological response categories.

Response category and metric	Mammal	Avifauna	Reptile	Amphibian	Fish	Mollusc	Crustacean	MMZ	Insect	Plant	Totals
Demographic and diversity	**4**	**63**	**45**	**210**	**93**	**16**	**28**	**84**	**69**	**52**	**664**
Biomass					5			5	2	7	19
Composition		1	1	3	5			11	3	2	26
Density		4	3	12	12			7	24	1	63
Distribution	1	1	4	6	1						13
Diversity		2	1	6	4			11	2	4	30
Evenness								1		1	2
Frequency		21	29	73	28	5	8	31	7	21	223
Occurrence	2	13	3	66	24	2	20	3		5	138
Population size/change		9		1	1						11
Richness	1	12	3	42	11	8		14	30	9	130
Structure			1	1	2	1		1	1	2	9
Behavioural	**2**	**12**	**2**	**44**	**33**	**2**	**3**	**10**	**4**		**112**
Activity level				8	1						9
Avoidance/attraction		2		2					1		5
Connectivity/passage	1			1	7						9
Dispersal			2	3	3	2	3	6	1		20
Feeding				1				2	1		4
Movement patterns		1		9	21			2			33
Preference/selection	1	6							1		8
Vigilance		3		3							6
Vocalizations				17	1						18
Genetic			**6**	**26**	**7**		**1**				**40**
Gene expression							1				1
Genetic differentiation/variation				9	4						13
Genetic distance				5							5
Genetic divergence				1	1						2
Genetic diversity			6	11	2						19
Mortality and survival	**10**	**27**	**88**	**122**	**30**	**11**	**12**	**13**	**18**	**1**	**332**
Lethality					1	2					3
Mortality frequency	8	25	84	81	8	3		6	14		229
Mortality rate	1		2		1					1	5
Mortality risk	1			6							7
Survival frequency		2		35	20	6	12	7	4		86
Survival probability			2								2

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Table A1. (*concluded*).

Response category and metric	Mammal	Avifauna	Reptile	Amphibian	Fish	Mollusc	Crustacean	MMZ	Insect	Plant	Totals
Reproduction		**7**	**3**	**22**	**4**	**1**	**3**	**2**	**3**		**45**
Fecundity				2	1						3
Frequency of eggs		1		13			1		2		17
Frequency of nests/redds		2	2		2						6
Nest or hatching success		4		2		1					7
Production rates			1	4	1		2	2	1		11
Sex determination				1							1
Sublethal physiological				**54**	**29**	**3**	**5**	**9**	**6**	**25**	**131**
Blood/tissue constituents				1	7						8
Contaminant uptake				3	2		2	2			9
Deformities				10	1						11
Development rates				13	1				1		15
DNA changes					2						2
Growth/productivity				22	7			7		24	60
Immune function				1		2			1		4
Parasite infection				4	1						5
Toxicity					8	1	3		4	1	17
Totals	**16**	**109**	**144**	**478**	**196**	**33**	**52**	**118**	**100**	**78**	**1324**

Note: MMZ = micro-invertebrates, macro-invertebrates, and zooplankton.

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Table A2. Frequency (number of cases) of negative, neutral/inconclusive, and positive road effects on freshwater taxa in the evidence base according to putative stressors.

	Mammal	Avifauna	Reptile	Amphibian	Fish	Mollusc	Crustacean	MMZ	Insect	Plant	Totals
Altered water flows, levels, or tables				**1**	**1**					**3**	**5**
Negative				1						3	4
Neutral/inconclusive					1						1
Positive											0
Altered water quality parameters				**19**		**7**		**1**	**27**	**1**	**55**
Negative				5		2			2		9
Neutral/inconclusive				8		2			22	1	33
Positive				6		3		1	3		13
Barrier/obstruction		**10**		**6**	**81**		**18**	**7**	**1**	**3**	**126**
Negative		2		3	34			5	1	3	48
Neutral/inconclusive		8		2	40		18	1			69
Positive				1	7			1			9
Contaminants				**15**	**25**	**3**	**9**	**21**	**2**	**9**	**84**
Negative				8	22	3	9	12	1	7	62
Neutral/inconclusive				7	3			9	1	2	22
Positive											0
Excavation or vegetation removal			**16**	**24**				**3**			**43**
Negative				7							7
Neutral/inconclusive			16	16				3			35
Positive				1							1
Human access		**3**			**5**						**8**
Negative		3			2						5
Neutral/inconclusive					2						2
Positive					1						1
Light				**3**	**6**			**2**	**1**		**12**
Negative				3	6				1		10
Neutral/inconclusive											0
Positive								2			2
Noise		**5**		**19**	**3**						**27**
Negative				13	2						15
Neutral/inconclusive		5		6	1						12
Positive											0
Road density and proximity	**3**	**44**	**34**	**121**	**26**	**4**	**5**	**15**	**2**	**12**	**266**
Negative		24	19	73	12	1	2	10	2	7	150
Neutral/inconclusive	3	16	14	45	10	2	3	5			98
Positive		4	1	3	4	1				5	18

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Table A2. (*concluded*).

	Mammal	Avifauna	Reptile	Amphibian	Fish	Mollusc	Crustacean	MMZ	Insect	Plant	Totals
Road or shoulder composition and configuration		**2**	**3**	**9**	**2**			**1**	**3**	**1**	**21**
Negative		1		7	2			1	3	1	15
Neutral/inconclusive			3	2							5
Positive		1									1
Road presence	**3**	**20**	**2**	**55**	**4**	**4**	**3**	**9**	**7**	**7**	**114**
Negative	1	11		40	4	1	1	7	6	2	73
Neutral/inconclusive	2	8	2	14				2			28
Positive		1		1		3	2		1	5	13
Salt/de-icers				**96**	**20**	**14**	**17**	**43**	**20**	**27**	**237**
Negative				71	13	12	14	20	16	16	162
Neutral/inconclusive				24	7	1	3	19		5	59
Positive				1		1		4	4	6	16
Sediment loss or loading, or turbidity				**8**	**23**			**10**	**26**	**15**	**82**
Negative				6	10			8	15	3	42
Neutral/inconclusive					13				11	10	34
Positive				2				2		2	6
Traffic presence, level, or density	**10**	**25**	**89**	**102**		**1**		**6**	**11**		**244**
Negative	10	25	88	91		1		2	11		228
Neutral/inconclusive			1	11				4			16
Positive											0
Totals	**16**	**109**	**144**	**478**	**196**	**33**	**52**	**118**	**100**	**78**	**1324**

Note: MMZ = micro-invertebrates, macro-invertebrates, and zooplankton.

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Table A3. Frequency (number of cases) of positive and negative road effects on freshwater taxa in the evidence base according to biological response category.

	Mammal	Avifauna	Reptile	Amphibian	Fish	Mollusc	Crustacean	MMZ	Insect	Plant	Totals
Abundance/occurrence	**4**	**62**	**45**	**210**	**93**	**16**	**28**	**84**	**69**	**52**	**662**
Negative	1	30	13	122	33	8	7	47	31	28	319
Neutral/inconclusive	3	30	32	82	51	2	21	29	33	12	295
Positive		2		6	9	6		8	5	12	48
Behaviour/movement	**2**	**9**	**2**	**44**	**33**	**2**	**3**	**10**	**4**		**109**
Negative	1	3	2	33	22			6	3		70
Neutral/inconclusive	1	5		10	9		1	2			28
Positive		1		1	2	2	2	2	1		11
Genetic			**6**	**26**	**7**		**1**				**40**
Negative			3	18	3		1				25
Neutral/inconclusive			2	8	3						13
Positive			1		1						2
Mortality	**10**	**27**	**88**	**122**	**30**	**11**	**12**	**13**	**18**	**1**	**332**
Negative	9	27	86	104	21	10	10	7	16	1	291
Neutral/inconclusive	1		2	16	9	1	2	6	1		38
Positive				2					1		3
Reproduction		**9**	**3**	**22**	**4**	**1**	**3**	**2**	**3**		**47**
Negative		5	3	13	3	1	3		2		30
Neutral/inconclusive		2		8	1			2			13
Positive		2		1					1		4
Sublethal		**2**		**54**	**29**	**3**	**5**	**9**	**6**	**25**	**133**
Negative		1		38	25	1	5	5	6	13	94
Neutral/inconclusive				11	4	2		4		6	27
Positive		1		5						6	12
Totals	**16**	**109**	**144**	**478**	**196**	**33**	**52**	**118**	**100**	**78**	**1324**

Note: MMZ = micro-invertebrates, macro-invertebrates, and zooplankton.