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SYSTEMATIC REVIEW PROTOCOL Open Access

Does structural connectivity facilitate movement of native species in Australia's fragmented landscapes?: a systematic review protocol

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Abstract

Background: Habitat fragmentation and accompanying isolation effects are among the biggest threats to global biodiversity. The goal of restoring connectivity to offset these threats has gained even greater urgency under the looming spectre of climate change. While linear corridors have been the most commonly proposed solution to these issues, it has become increasingly recognised that structural connectivity exists in different forms with a variety of characteristics. We previously conducted a systematic review from 2008-2010 to collate and synthesise evidence regarding the relationship between these different types of structural connectivity and the actual movement of native Australian plants and animals (i.e., functional connectivity). Our previous review produced a number of management recommendations but also identified significant knowledge gaps. Given that empirical research into connectivity has become even more common since the original review and that it has been more than five years since the original literature searches, the time is ripe for an update of that review.

Methods: We will update our previous systematic review by repeating a thorough search for both published and unpublished evidence on the effects of structural connectivity on animal and plant movement through heterogeneous landscapes. We will slightly broaden the scope of the original review by including data on semi-aquatic species as well as terrestrial ones. Studies will be included if they: 1) contain data on a terrestrial or semi-aquatic native Australian species; 2) have at least one study site that contains some form of structural connectivity between otherwise isolated patches of habitat; and 3) include data on movement of species through the connectivity or data that allow inference of movement (or the lack thereof). We will repeat the analyses carried out for the original review which used hierarchical linear modelling to assess the effects of numerous sources of heterogeneity (e.g., type of connectivity, width of connection, ecosystem type, taxonomic group, and many other characteristics of the species, habitat, and connectivity) on the amount of movement observed in a landscape. If increased sample sizes allow we will also carry out additional meta-analyses, which were not possible with the original dataset.

Keywords: Connectivity, Corridor, Stepping stone, Fragmentation, Dispersal

Background

The modification, loss and fragmentation of natural ecosystems are among the most serious threats to global biodiversity because the resulting altered landscapes invariably support smaller, more isolated populations of native species and increasingly degraded habitats, all of which are likely to reduce population viability and increase risk of extinction for many species. Habitat fragmentation is thought to impact populations through three main effects: edge, area and isolation effects. Edge effects can include increased rates of predation and altered microclimates which may reduce survivorship and reproductive success. Due to the smaller habitat patches and thus smaller populations created by fragmentation, area effects may include increased levels of inbreeding, reduced genetic variability, and increased sensitivity to stochastic events. These area effects will be further intensified when combined with isolation effects, whereby the possibility of demographic or genetic rescue is reduced or eliminated because individuals cannot disperse between fragments through the matrix of

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unsuitable habitat. Extensive research has demonstrated these impacts of fragmentation, and numerous syntheses have been produced, so the basic problem is relatively well understood [1-8].

Fragmentation has become an even more serious concern now that global climate change is predicted to force species to locally adapt or move elsewhere in order to persist [9-11]. Smaller populations will be less resilient to altered local conditions and therefore less able to locally adapt, and isolated populations will have difficulty shifting their ranges to track changing environments. This may be of particular concern in agricultural regions of Australia, where extensive land clearing occurred following European settlement, leaving fragments of remnant native vegetation within a matrix dominated by intensive production systems [12,13]. The long-term consequences of this fragmentation are expected to be serious with at least some researchers predicting that Australia will lose half of its bird species within the next century [14].

Action is therefore urgently needed to reverse some of the effects of fragmentation—to reconnect small, isolated populations and restore their ability to function as larger, more resilient populations. Such actions need to occur at local, regional, and even continental scales to ensure benefits accrue at the population level but also that species can move to new areas as necessary under climate change. Fortunately, this need has captured the attention of government and the public. Connectivity restoration is frequently a goal of private revegetation efforts, local landcare groups, and incentive schemes administered by regional natural resource management bodies. Over two decades ago, large networks of connected habitats were first proposed in North America [15] and Australia [16], and the Australian Government and non-governmental organisations have initiated a number of major projects involving continental scale connectivity restoration such as Gondwana Link, Habitat 141, and the Great Eastern Ranges Initiative, including its component projects such as Kosciusko2Coast and Slopes to Summit.

Unfortunately, it is unclear exactly what actions should be taken to restore connectivity to our landscapes, aside from trying to recreate vast swaths of native ecosystems (which would not be practicable given the need for other land uses). By definition, a connected landscape is one in which individuals of all species (or their propagules or genes) can move or disperse from one resource patch to another ([17] see Appendix for definitions of terms used in this protocol). So how much habitat, what kind of habitat, and in what spatial configuration might be required to facilitate such dispersal? Unfortunately, the many syntheses of the problems of habitat fragmentation tell us relatively little about dispersal, and thus about the appropriate *solutions* to the problem, and new research and syntheses specifically focused on connectivity, as opposed to fragmentation, are required.

The most commonly proposed solution is to retain or restore habitat corridors. While the interpretation of this term varies (see [18] for six different definitions), we define a corridor as a relatively unbroken (contiguous) linear strip of habitat that connects two or more patches of habitat that are otherwise surrounded by unsuitable areas for the species or community in question [19,20]. We believe this matches the operational definition used by most Australian land managers and by members of the public. The theory behind corridors is that individuals will be exchanged and/or genes will flow between connected patches or populations either because the corridor is occupied by the species or community and thus the corridor creates a continuous population between the two patches, or because dispersing individuals (or seed dispersers or pollinators) will use the corridor to move from one patch to the other. However, the ability of corridors to achieve this goal, and provide for dispersal just as much as continuous habitat would, may depend very much on the dispersal behaviour of the species involved as well as many other characteristics of the corridors themselves, the habitat patches, and the surrounding matrix [21-24]. As a result, the effectiveness of corridors has been the subject of considerable debate [18,19,25-27], and there is an imperative to determine which characteristics might make them most effective across different species and different ecosystems, and whether there are viable alternatives – connections that aren't necessarily unbroken and linear but which nonetheless support dispersal and gene flow.

There are a number of ecological reasons which suggest that alternatives to corridors need to be seriously considered. First, if corridors are to provide for gene flow by providing occupied habitat, then there may be costs to the populations involved, so a thorough weighing of the balance between benefits and costs is required. In particular, edge effects in narrow habitat strips may mean that population sinks may be created when corridors are occupied [28-30]. Such sinks could potentially decrease both the likelihood of dispersal between patches and the overall viability of the population, even though the corridor might appear to be a success because it is occupied. Second, the corridor concept is based on a binary patch/matrix model of the landscape—that there are distinct, suitable parts of a landscape (patches) and unsuitable parts (matrix), but nothing in between. However, ecologists have recognised that there are other valid landscape models, including the variegated model [31,32] and continuum models [33,34], in which different parts of the landscape may vary in their suitability for any given species, resulting in different densities or patterns of use. These models are particularly important in Australia, as many of Australia's ecosystems naturally form a patchy mosaic [35], so native species may have evolved to take advantage of that heterogeneity during dispersal. This means that individuals may not require continuous strips of habitat for dispersal, and also that suitable habitat for dispersal might actually have a very different composition and structure than habitat suitable for long-term survival and reproduction.

The increased appreciation of these ecological concepts has led scientists to broaden their thinking about connectivity restoration beyond corridors and into the paradigm of structural vs. functional connectivity [36-39]. Under this paradigm, structural connectivity is anything that physically links separate populations, and it may consist of just about any kind of landscape heterogeneity in between occupied patches of habitat. Examples of structural connectivity include corridors and partially vegetated drainage lines or fence lines, but also more subtle habitat elements such as scattered trees or shrubs, or even scattered clumps of tussock grass or coarse woody debris. In contrast, functional connectivity refers to the outcome we desire from these structural features—the degree to which movement and dispersal actually occur. Research is now focused on trying to understand the relationships between structural and functional connectivity, which includes work on corridors but is more broadly focused on movement and gene flow in heterogeneous landscapes. Ideally, this research will reveal which types of structural connectivity really do provide functional connectivity (dispersal in the landscape) for the majority of species in an ecosystem.

Such general principles for connectivity restoration—recommendations for what is likely to work for most species in most systems—can only come from syntheses of many empirical studies. While the utility of corridors has been tested using theoretical modelling [40,41], and empirical evidence for use of corridors has been accumulating for a number of years [19,42-45], research on other types of structural connectivity is relatively recent. Furthermore, evidence comes from a variety of different types of studies (survey, mark-recapture, genetic, radio-tracking, etc.), which can make the resulting conclusions difficult to interpret *across* studies. The systematic review approach is especially useful, as it provides a rigorous framework in which to attempt a formal comparison of the different types of evidence produced by different types of studies.

We undertook a systematic review from 2008-2010 [46] with the aim of providing clearer, science-based information to natural resource planners and managers about how best to invest in connectivity, and to identify critical knowledge gaps that could guide future research to ensure that Australia's significant on-ground expenditures achieve their goals of restoring functional connectivity in Australian landscapes. Our review produced some preliminary recommendations for managers and identified a number of knowledge/research gaps. However it has now been over 5 years since the searches for that review were completed. Pilot searches suggest that the evidence base has nearly doubled in that time with 6,395 of 13,912 potential sources identified having been published since the original literature searches. Thus, we believe the time is ripe to update this systematic review. We will also broaden the scope of the review question slightly to incorporate data on semi-aquatic species as well as purely terrestrial ones, as the role of riverine and floodplain systems in supporting landscape connectivity is currently a topic of interest in Australia.

Objective of the Review

To evaluate whether structural connectivity (i.e., habitat elements of any sort in an otherwise unsuitable matrix) linking patches of occupied habitat facilitates functional connectivity (i.e., movement of native species) in fragmented landscapes in Australia, and to identify which characteristics of structural connectivity increase the probability of dispersal. While different characteristics may be important for different species or in different landscapes or ecosystems, we aim to identify principles for natural resource managers that will be as generally applicable as possible, while also distilling recommendations for specific taxa or communities where more general insights do not emerge.

Primary question

What is the relative effectiveness of different landscape elements that provide structural connectivity in Australian fragmented landscapes in terms of facilitating dispersal of native species between habitat patches or populations? This question is broken down into 'population-intervention-comparator-outcome' components (Table 1).

Data permitting, we will examine the relative benefits of different types of structural connections (e.g., scattered trees, shrubs, drainage lines, fallen timber, large tussocks—whatever types of heterogeneity exist between populations), not just traditional linear corridors. We will also attempt to analyse the effectiveness of structural connections based on quantitative characteristics such as length, width, vegetation density, composition and structure, and maximum gaps to be crossed. Of particular interest for the updated review, we will also examine the effectiveness of riparian connections compared to purely terrestrial connections and whether the effectiveness of riparian connections depends on hydrological characteristics such as flood regime.

Methods

Searches

We will focus our search on those databases that proved to be most useful during the original review and will not

Table 1 Components of the primary systematic review question

Population	Intervention	Comparator	Primary Outcome	Secondary Outcome
Any terrestrial, arboreal, or semi-aquatic native Australian species	Patches of occupied habitat, surrounded by a dissimilar matrix, with some form of structural connectivity between the patches	Patches of occupied habitat, surrounded by a dissimilar unoccupied matrix, without (or with less) structural connectivity between the patches	Relative movement* rates of individuals or propagules (observed or inferred) between patches	Binary variable describing whether or not there is evidence of movement* of individuals or propagules (observed or inferred) between patches

See Appendix for definitions of ecological terms.
*Note that while we are ultimately most interested in dispersal, this is difficult to observe and quantify, and not all studies of movement are explicit about why the animals are moving. In some studies, the movement is even experimentally induced. Thus, we will include all studies with evidence of inter-patch movement, regardless of whether the purpose of the movement is known. Where the purpose of the movement is known but is related to migration or even daily foraging movements, we will still retain the study in the review because elements of structural connectivity that assist with these movements are likely to also be beneficial to dispersal. Depending on the data presented in a study, movement rates may be presented in terms of proportions of individuals moving, frequencies of movements, migrants per generation, etc.

be searching those that produced relatively few unique sources (i.e., ones not found in other databases). Because our inclusion criteria have changed slightly since the original review (see below), we will not be restricting searches to any particular time period. The electronic databases shown below in bold text will be searched for studies to be included in the updated review (databases searched during the original review that will not be searched again have been italicized):

1. ISI Web of Knowledge
   i. **ISI Web of Science – Science Citation Index.**
   ii. **ISI Web of Science – Conference Proceedings Citation Index (Science).**
   iii. *Current Contents.*
   iv. *CAB Abstracts.*
   v. *Zoological Record.*
   vi. *Web Citation Index.*
2. *Directory of Open Access Journals.*
3. **Scopus.**
4. *Australian Agriculture and Natural Resources Online (AANRO).*
5. *CSPubList (via EnCompass; official CSIRO publications).*
6. *CSIRO Library Catalogue (Voyager).*
7. **Trove** (for Theses only – this search engine has replaced the *Australian Digital Theses Program*).
8. *ProQuest Dissertations and Theses.*

We will search using two-term searches: one movement-related term and one term relating to landscape context. We will conduct searches of each database using all possible two-term searches based on the movement and landscape terms below.

Movement-related terms:
1. colonisation (OR colonization).
2. dispersal.
3. migration.
4. movement*.
5. nomad* (new term that was not included in the original review).

Landscape-context terms:
6. connectivity.
7. corridor*.
8. fragment*.
9. isolation.
10. landscape*.
11. matrix.
12. paddock tree*.
13. patch*.
14. stepping stone*.

We will also search for the following single terms:
15. interpatch* (OR inter-patch*).
16. gap-crossing.

Where possible, we will use the following NOT terms: alga*, alloy*, bacteria*, brain, Campylobacter, capital, cell*, clinical, corrosion, cortex *, cultur*, deep-sea, diatom*, disease*, evangel*, eye-movement*, fish*, fluvial, gas*, Holocene, ion, larva*, medicine, molecul*, neural, neuro*, marine, motion, patient, phylogenetic, phytoplankton*, plankton*, plate*, Pleistocene, politic*, polymer*, protein*, Salmonella, scripture, sediment, shear, social movement*, soil*, speciation, stent, stygo*, thermal sensor, train*, transport*, uplift, virus*, weed*, zooplankton*. These terms have been selected based on our experience with the original searches and additional pilot searches for the update. They do not include terms (e.g., “aquatic”, “river”, and “stream”) that were used to exclude studies on semi-aquatic species from the original review.

No non-English language searches will be conducted, as we anticipate that all research on connectivity management in Australia will be published in English.

Searches will also be conducted using the internet-based search engine **Google Scholar** (but not *Alltheweb* which was searched during the original review) using both single-term searches and the ten best two-term searches as determined by the Trove search results. The first 50 hits from each search will be examined for possible inclusion in the review.

The extensive grey literature search undertaken for the original review allowed us to identify the most relevant researchers, managers and policy makers in this area. Rather than repeating that time-consuming search, we will focus our effort on those individuals who proved to be valuable sources during the original review. We will contact these individuals to request information regarding published or unpublished data produced since the original review that would be relevant to this update. Due to the relatively small size of the ecological research community in Australia, these enquiries will be quite thorough.

Study inclusion criteria

We will include in the review any and all studies that meet the following criteria. These criteria differ from those of the original review only in the inclusion of semi-aquatic species such as amphibians.

- **Relevant subject(s)**: The study provides data on any terrestrial, arboreal, or semi-aquatic native Australian species including mammals, birds, reptiles, amphibians, invertebrates or plants (including seeds or pollen).
- **Types of intervention**: The study site contains any type of structural connectivity between otherwise isolated patches of native habitat or, even more broadly, landscapes with significant spatial heterogeneity in structure and occupancy by the subject.
- **Types of comparator**: The study compares patches connected by any type of structural connectivity vs. patches with less or no connectivity. No comparator is necessary for inclusion in the review; however, comparators are required for studies to be included in many of the analyses.
- **Types of outcome**: The study contains data on relative movement rates of individuals between patches, or at least evidence (direct or inferred—see Study quality assessment section) of movement within a heterogeneous landscape.
- **Types of study**: A wide variety of types of study are often conducted in dispersal and connectivity research (see Study quality assessment section below) and we will attempt to include as many types as possible, including presence/absence studies, abundance surveys, mark-recapture or re-sighting data, genetic studies (including population-level analyses as well as more detailed analyses like assignment tests), and more direct observations of movement such as radiotelemetry studies.

References returned by all searches will be filtered in several stages to determine whether they will be included in the review or not. First, we will compare the pre-filtering library from the original review with the pre-filtering library from the new searches and remove all duplicates from the new library (to avoid the effort of filtering sources that were already found for the original review). However, because our inclusion criteria are slightly different this time, we will search the original pre-filtering library to identify references focused on semi-aquatic species which would have been filtered out for that review, and add these references to the new reference database. Next, we will perform a series of geographic filters: accepting references that contain Australian-specific geographic terms, rejecting any remaining references that contain non-Australian geographic terms, and finally accepting those that contain neither. Then, title filtering will be performed to remove references that are clearly irrelevant to this review, followed by abstract filtering to remove references that, based on the abstract, do not meet the above study inclusion criteria. Finally, full text filtering will be performed to remove references that may have appeared relevant from the abstract, but do not actually meet the study inclusion criteria.

When it is unclear whether a reference should be included in the next stage of the review, it will be included. At each of these stages, two reviewers will independently examine a subset of references (about 10% of the studies) and results will be compared via a Kappa Test. If the Kappa value is <0.6, the filtering strategy will be revised and repeated until Kappa >0.6 is achieved. Two reviewers will also independently review references whose status remains unclear and any disagreements will be resolved via consensus or by a third reviewer.

Potential sources of heterogeneity

Effects of structural connectivity may differ between studies for a number of reasons. Whether or not any type of structural connection is effective at facilitating dispersal may simply depend—on the species, the ecosystem type, the characteristics of the rest of the landscape, etc. Yet in practice, land managers are unlikely to be managing for single species in single locations. Instead, they need general principles distilled from among all this variation (e.g., habitat specialists respond similarly, or scattered trees are more effective when they connect woodlands as opposed to forests). Thus, we need to analyse whether these sources of heterogeneity among studies affect our overall conclusions. Possible sources of heterogeneity to be considered wherever possible (and analysed through meta-analysis where sufficient data exist) will include:

1. Taxonomic group of study organism

2. Ecology, behaviour, and dispersal mechanism of study organism.
3. Life history of study species and specifically the life-history stage of individuals included in the study.
4. Size of study organism and spatial scale of movements.
5. Type of community or ecosystem (e.g., temperate vs. tropical).
6. Size, number and habitat quality of patches being connected.
7. Type of connectivity – e.g., continuous corridor, disjunct corridor, stepping stones, etc.
8. Quantitative characteristics of connectivity – e.g., width, length, species composition, age, vegetation complexity, gap distances, etc.
9. For riparian sites, information on water flow, flood regime, and/or inundation history.
10. Characteristics of the intervening matrix (crop, pasture, pine plantation, etc.).
11. Whether the connectivity and/or patches are remnant or restored habitat.
12. Landscape level characteristics (e.g., total percent cover of native vegetation).
13. Disturbance history of study areas.
14. Climatic conditions during study (e.g., drought).
15. Type/purpose of movements studied (home-range movements vs. foraging trips vs. dispersal vs. migratory or nomadic movements).
16. Study design (replicated comparisons, etc.).
17. Type of study (tracking, mark/recapture, population genetic, etc).
18. Artificial barriers such as roads or dams.

Study quality assessment

Each study accepted into the final review will be assigned two scores to rank its quality according to two distinct sets of criteria. One set of scores relates to the experimental design employed in each study. We will use the hierarchy of evidence table presented by Pullin and Knight [47], which is modified from systematic reviews in medical research.

Because we are particularly interested in assessing the value of different landscape elements in terms of providing functional connectivity, our "gold standard" will be data that document movement paths between patches (so we know which landscape elements were actually used) and which document successful reproduction following successful dispersal. This first point is particularly important because data showing that individuals have transferred between patches that are connected by a corridor (e.g., through mark-recapture data) does not necessarily prove that movement has occurred *via* the corridor. The second point is important because dispersal without reproduction will not ultimately affect levels of gene flow or "rescue" populations from extinction. Very few studies will meet this gold standard by providing data on both of these aspects of dispersal (and most will have data on neither). Instead, most studies use a variety of different surrogates, including everything from following dispersal movements until settlement or observing part of the dispersal (or other movement) process, to inferring dispersal using genetic data or even presence/absence of a species. Thus, we also intend to use a hierarchy of evidence approach to distinguish between studies that fully documented paths leading to effective dispersal versus those that inferred that movement occurred using a variety of methods, some of which are better surrogates for dispersal and make fewer assumptions than others (Table 2).

Two reviewers will independently assess a random subset of accepted articles (approximately 10% of the studies accepted at full text); any disagreement on study quality will be resolved by consensus and referred to a third reviewer if necessary.

Data extraction strategy

For each study accepted into the final review, a reviewer will record data regarding the study characteristics (subject, intervention, and outcomes measured), study quality, and sources of heterogeneity (see above) in a format suitable for meta-analysis where possible on a specially designed data extraction form. We will use the data extraction form developed for the original review, but with a few additional columns to record information of particular relevance for riparian corridors (see #9 on the list of potential source of heterogeneity above). Data extraction will be repeated by a second reviewer for a random

Table 2 Hierarchy of evidence based on the type of data recorded

Quality of evidence – measuring effective dispersal	
I-1	Individuals followed directly so dispersal paths known—individuals followed until death or successful reproduction.
I-2	Individuals followed directly so dispersal paths known but only followed until settlement.
I-3	Individuals followed directly so dispersal paths known but only for part of dispersal search path.
I-4	Movement path known but unknown whether for dispersal or other purposes.
II-1	Between-patch movement known from mark-recapture data, genetic assignment tests, or radiotelemetry data but movement path not known.
II-2	Between-patch dispersal inferred from population genetic data.
III-1	Between-patch movement inferred from presence in connecting landscape element (e.g. in corridor or stepping stone between patches)
III-2	Between-patch dispersal inferred from presence/absence data in patches.

Data are generally assumed to be more reliable to the extent that dispersal (or other movement) was actually observed rather than inferred.

subset of studies (approximately 10%) to check accuracy and repeatability.

Data synthesis

We will produce a narrative synthesis of the results of all studies included in the final review based on the data extraction summary tables. Meta-analysis including random effects will be employed if suitable data are available. We will repeat our previous analyses which used hierarchical linear modelling to assess the effects of the various sources of heterogeneity (see above) on observed or inferred movement. We will also synthesise all available data regarding gap-crossing distances and inter-patch distances where movement does and does not occur, to try to identify thresholds, which emerged as important in previous empirical research [48] and in the first review [46].

Appendix: Glossary of important terms

Structural Connectivity – habitat features in a fragmented or heterogeneous landscape that physically link other features, especially discrete areas of habitat occupied by any species in question (e.g., patches).

Functional Connectivity – the degree to which organisms actually move through the landscape, especially between discrete areas of occupied habitat (e.g., patches) and especially for dispersal & gene flow.

Connectivity – we adopt the definition of landscape connectivity first proposed by Taylor et al. [17], who defined it as "the degree to which the landscape facilitates or impedes movement among resource patches"; thus, a landscape with high connectivity is one that provides functional connectivity regardless of what it looks like in terms of structural connectivity.

Corridor – a landscape element that connects two or more patches in a relatively unbroken (contiguous) line; thus, a form of structural connectivity.

Dispersal – movement of organisms or propagules that may potentially result in gene flow, including the movement of individuals from their place of birth to the site of their first breeding (natal dispersal), movement from one breeding site to another breeding site (breeding dispersal), movement of seeds (seed dispersal), and movement of pollen (pollen dispersal).

Effective dispersal – occurs when dispersal movements result in actual gene flow (e.g., natal dispersal followed by successful reproduction, seed dispersal followed by successful establishment and reproduction, etc.).

Patch – a discrete area of habitat occupied by a species, surrounded by areas not occupied by that species that are known or thought to be unsuitable as habitat (e.g., matrix); note therefore that a patch may not be clearly observable in a structural sense to researchers, and thus this definition of patch is compatible with landscape models such as the variegated model and continuum model as well as the patch-matrix model.

Matrix – unoccupied region, thought or known to be unsuitable as habitat, surrounding patches of suitable habitat.

Habitat – a place suitable for survival and/or reproduction of a particular plant or animal species; note that different structural and compositional characteristics may be associated with habitat used for long-term survival, reproduction, and short-term survival during dispersal.

Stepping stone – a landscape element that is located between but not contiguous with two or more patches; thus, a form of structural connectivity.

Gap-crossing – movement across matrix (e.g., from a patch to a stepping stone). Gap-crossing studies typically involve experimental translocation of individuals across gaps and observing their behaviour after release.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EDD, VAJD, and MJD developed the original review question and carried out the original systematic review. EDD wrote this protocol with contributions from HMM, MJD, and VAJD. All authors read and approved the final manuscript.

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