Landscape Ecology

Time-delayed influence of urban landscape change on the susceptibility of koalas to chlamydiosis

--Manuscript Draft--

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Abstract:	Context Infectious diseases are important in the dynamics of wildlife populations, but there is limited understanding of how landscape change influences susceptibility to disease. Objectives We aimed to quantify the time-delayed influence of spatial and temporal components of landscape change and climate variability on the prevalence of chlamydiosis in koala (Phascolarctos cinereus) populations in southeast Queensland, Australia. Methods We used data collected over 14 years (n = 9078 records) from a koala hospital along with time-lagged measures of landscape change and rainfall to conduct spatial and temporal analyses of the influence of landscape and environmental variables on

	prevalence of chlamydiosis and koala body condition. Results Areas with more suitable habitat were associated with higher levels of disease prevalence and better body condition, indicating that koalas were less likely to be impacted by chlamydiosis. More intact landscapes with higher proportions of total habitat are associated with a reduction in prevalence of chlamydiosis and a decrease in body condition. Increased annual rainfall contributed to a decrease in prevalence of chlamydiosis and an increase in body condition. Urbanization was associated with an increase in disease, however the effects of urban landscape change and rainfall variability on chlamydiosis may not manifest until several years later when overt disease impacts the population via effects upon body condition and reproductive success. Conclusions Our study highlights the importance of effects of landscape change and rainfall variability on disease prevalence in wildlife. This recognition is essential for long-term conservation planning, especially as disease often interacts with other threats.
Response to Reviewers:	Review: LAND-D-15-00143R1 Reviewer 1: General comments The manuscript has been improved since the initial submission and most of the comments have taken into account. There is still a need to explain how the disease was diagnosed in koalas and if the disease was diagnosed or if the infection was diagnosed. In the response to reviewer 3, you mention that this has been done through symptoms → you therefore have an indication about the disease and not about the infection. This is an important distinction. This is important as, as stipulated, not all koalas express the disease even if usually a large portion is infected. RESPONSE to Major Concerns: Chlamydia diagnosis Chlamydia disease was diagnosed using physical examination, symptoms or necropsy results. Disease is more commonly diagnosed than infection which would require serology or PCR (Polymerase chain reaction) Swab. Given that disease admissions records were not included in the analysis (to reduce the bias) a large proportion of the records would be car hits or dog attacks and the majority of the records would be from dead koalas. Consequently it would be unusual to get serology for an already dead koala, although the koala may die after undergoing a treatment and died later. Cost is also an issue (PCR or ClearView (AU$10/test)/rapid testing). Can chlamydia modify koala’s behaviour? Study by Dique et al (2003) found no significant difference in disease prevalence between wild koalas and hospital records associated with vehicle trauma. Similarly de Villiers (2015 – PhD Thesis) found the same thing with dog trauma and debunked the myth that koalas suffering from disease were more likely to hit on the roads or attacked by dogs. RESPONSE to Specific Comments In the discussion, I have 2 main comments: 1.In the "synthesis" first paragraph, you don't mention at all how the "urban extent" factor influence on your variables. RESPONSE: this has been corrected.

2.You don't refer in the discussion to the univariate models: if so, why do you present it in the M&M and result section?

RESPONSE: Univariate models represent exploratory data analyses, a step in the selection of variables to go in the final model.

I believe therefore that there is still a need for more clarity in the manuscript even if most of the reviewers' comments have been addressed.

Specific comments

Abstract
L.20-27: why don't you mention the influence of the presence of urban habitat ("Urban extent" variable (and in the first part of the discussion); adding the "urban extent" results, this section will be much clearer (e.g. both suitable habitat and urban extent had a positive effect on prevalence while total habitat and annual rainfall had a negative effect etc."

RESPONSE: this has been corrected

L.30-32: mention of "time-lags" that are not presented in the result section in the abstract

RESPONSE: this has been corrected.

Keywords
Maybe add the name of the host in the keywords list.

RESPONSE: name added

Check paragraphs first line indentation.

Introduction
L52-53: "intensification of landscape matrix"

REPSONSE: Not sure what the issue is here. Urban development does intensify the landscape matrix. Have changed to: “intensification of the land-use matrix”.

L.55: I am not sure that Cleaveland et al. 2000 is a good reference for Urban impact. Cleaveland deals with CDV in dogs in Masai populations in the Serengeti National Park. Idem for Millan et al. 2009.

RESPONSE: Thanks, these references have been removed from this sentence.

L.65: a bracket needs to be added at the end of the citation.

RESPONSE: Done.

L.65: the point about sexual transmission applies also to other modes of transmission and therefore for most infectious diseases (more contacts, more transmission).

RESPONSE: Thanks this has been clarified.

L.65-66: decrease gene flow argument → need to indicate the link with susceptibility to diseases.

RESPONSE: Thanks for this feedback. We have clarified that reduced gene flow can increase disease susceptibility.

L73-76: please rephrase. I don't understand the argument here. It is not surprising that a pathogen impact varies between 2 different hosts (even if they belong to the same family).

RESPONSE: Thanks for this feedback. This sentence has been rephrased and two contrasting examples added.

L.92-93: Please indicate how "non-venereal transmission" can occur in sub-adults (direct/indirect transmission?).

RESPONSE: We have added “non-venereal transmission also occurs in pre-pubertal sub-adults”.

L.106: "co-contribution": not very explicit. "Co-infection with other infectious agents promoting or limiting Chlamydia infection""" for example.

RESPONSE: Thanks for this. We have now clarified.

L.120: check the reference list: I did not find Hume 1990 in the list. This paper seems to be quite important for your study as it directly relates to your questions, yet you cite it only once and do not give many information about it.

RESPONSE: Reference has been added and the sentence revised to clarify the original statement by Hume (1990).

Material & Methods
- L.151: please define "NSW".

RESPONSE: Done.

- L.166: not clear "(…) with an estimated 80% decline and an estimated 54% decline (…)": we don't know to what these 2 percentages refer to.

RESPONSE: Thanks for pointing this out, we have clarified.

L.213-214: "Landscapes have a temporal as well as a spatial component (…)"; you mean landscape effect or impact.

RESPONSE: This sentence has been clarified.

- Figure 2: this figure has been greatly improved. Make sure the temporal indications are clearly allocated to each factor (not easy to understand what is the time-lag for "%landscape urban" for example).

RESPONSE: This has been corrected.

- L.239: a "IN the final dataset" is lacking.

RESPONSE: Thanks, this has been corrected.

- L.244: rephrase after "The strength of the dataset (…)"

RESPONSE: Corrected.

- L.251: I did not find the temporal trends in the Appendix 1.

RESPONSE: This has been modified to “A time series of the spatial distribution of records..”

- L.254: a bracket is missing

RESPONSE: This has been corrected.

- L.260: it is still unclear how you evaluate disease presence in individual koalas; you need to explain how disease was diagnosed in koalas by hospital and the sensitivity of

the tests. In addition, you need to specify if infection is diagnosed (e.g. serology) or if disease is diagnosed (based on infected organs or symptoms). It is important as you state in the introduction that most koalas are usually infected but few express the disease. The diagnostic was targeting C. pneumonia, C. pecorum or both?

RESPONSE: See above. Diagnosis was based on clinical signs done by physical examination under the supervision of an experienced veterinary surgeon. Disease was diagnosed based on the infected organs or symptoms such as conjunctivitis.

- L.281: reference to "(…) pre-clearing Regional Ecosystems (…)" is not clear to me. Is it a class type identified by the Reg. Eco. Mapping?

RESPONSE: This has been clarified. We are referring to regional ecosystem classes mapped by the Queensland Herbarium at 1:100000 scale.

Results
- L.375: when you say "The strongest effect was for the total habitat in the landscape following a two year time-lag (…)", you mean "The strongest effect for the total habitat in the landscape was following a two year time-lag (…)" or "The strongest effect OF ALL UNIVARIATE MODELS was for the total habitat in the landscape following a two year time-lag (…)"

RESPONSE: Thanks for this. Sentence has been corrected to “The strongest effect for the total amount of habitat in the landscape was following a two year time-lag.”

Discussion
- L.412-424: you don't give any of your results about the urban habitat in this "synthesis" paragraph, when you state in the first line of the paragraph that you "quantify the relationship between urban landscape change and the prevalence of Chlamydiosis"

RESPONSE: Our focus is on the amount of suitable koala habitat remaining in the landscape.

- L.428: "(…) a contracted area of habitat (…)": you mean of natural habitat?

RESPONSE: corrected

- L431-433: "counter intuitive argument": not very counter intuitive: it just says that body condition is not only impacted by chlamydiosis. There can be a lot of other variables that can impact body condition (gastro-intestinal worms, breeding status, quality of forage, stress etc.).

RESPONSE: corrected

- L.457: maybe give an explanation/hypothesis to explain the differences between time-lags for chlamydiosis and body condition in urban areas (4 and 1 years): can this 2 variables be linked?

RESPONSE: corrected

- L.501: "(…) small sample of koala (…)": indicate the sample size so the reader can judge by itself.

RESPONSE: corrected

- L.517: define "STD"

RESPONSE: this section has been revised and reference to STD removed.

- L.518 "Approach and limitations": you need to discuss also potential bias in the diagnostic technique (apparently based on symptoms) and provide a reference on its efficiency if they exist.

RESPONSE: We disagree, this is going beyond the scope of the paper.

Reviewer 3: The authors made good efforts to improve their manuscript and clarify their analyses. There remains however several points that need to be better discussed or interpreted more carefully.
Two main points: 1) there is still some confusion regarding the possible mechanisms by which landscape features could impact infection risk (thus some predictions/hypotheses are not clear) and 2) the new focus on the time-lagged effects of landscape and rainfalls is interesting but not enough discussed (these results are not even given in the abstract!). Below are my detailed comments

ABSTRACT
- Results on time-lags are not mentioned although it is now at the core of the revised manuscript.

RESPONSE: Thanks, this has been corrected.

INTRODUCTION
- L.61: human-modified landscapes certainly expose wildlife species to other stressors than overcrowding (which is not a stressor but rather a consequence and may not occur for any species) and nutritional deficiencies (which again may not be true for all species - think of rats thriving with human food). Other examples may be more appropriate.

RESPONSE: - L.64: lacks precision. Clumping may increase sexual contacts (although?) but may mostly increase other types of contacts (eg physical contacts around clumped food resources) .

RESPONSE: Thanks for pointing this out. We have clarified this sentence.

- L.72: precise whether you talk of variations among species of host/parasite/both

RESPONSE: As per response to comment to Reviewer 1, we have revised this sentence and provided contrasting examples of variations in species susceptibility to disease.

- L78: of INFECTIOUS disease.
This a recurrent problem throughout the manuscript - a disease is different from an infectious disease. Note also that 'disease' refers to the clinical symptoms - you never mention 'parasite', 'pathogen' or infectious agent although it is what your paper is about: how landscape features and their modifications by humans impact the dynamics of a parasite (that is a pathogen apparently when conditions degrade for its host as mentioned in the discussion).

RESPONSE: We have changed infection to disease where considered appropriate.

- L.107: you forget individual variations in behaviour (ie variations in exposure to the pathogen) and variations in physiology (ie variations in susceptibility to the pathogen - for instance variations in immunocompetence, variations in levels of stress hormones or testosterone etc)

RESPONSE: Yes this is important and we have added a small section (L123-126) dealing with differences between individuals based upon their physiological status. Sex steroid hormones in many species can affect immunocompetence. We also discuss the influence of physiological stress in the final paragraph of Urban Land Use.

- L.113: 'irreversible infertility in females' - this was already said before

RESPONSE: Thanks, this has been corrected.

- L.115-117: this should be moved around lines L.100-103 where you talk about the progression/not to clinical disease.

RESPONSE: Thanks, this sentence has been moved.

- L.120: please nuance since the link between nutritional stress and increased susceptibility to Chlamydia can only be hypothesised/suspected

RESPONSE: Thanks, this statement has been modified to indicate contention by Hume 1990.

- L.125: 'population' is misspelled

RESPONSE: Corrected.

- L.125: INFECTIOUS disease (+ which ones?)

RESPONSE: Changed to Chlamydia.

- L.133: consider reformulating: body condition cannot refer to a population - must be of individuals

RESPONSE: Corrected.

- L. 133-135: I would expect some explanations on why you expect such effects to come earlier in the paper than in the middle of the method section. What about moving the conceptual model between the introduction and the methods? I don't know whether the journal would accept any such modification in the structuring but it would look more logical to me. I usually expect the hypotheses to be formulated in the introduction - so the other option could be to move them at the end of the introduction (while sharpening them a bit).

RESPONSE: This section has been moved to between the Introductions and the Methods.

METHODS
- L. 149-155: Why do you separate males and females if you don't account for the sex of the koalas in your study?

RESPONSE: Reference to the sex of koalas has been removed.

I actually still don't understand why you do not account for the koalas' age and sex if you have these information (they surely record at least the gender of the individuals at admission to hospital?). These variables are often extremely important risk factors of infectious diseases. Age and sex influence both the likelihood of individuals to encounter the parasite (via differences in host behaviour and because an older individual simple had a longer exposure time), and their susceptibility (eg differences in immune defences) so these variables can constitute important confounding factors. You prove here that they are important factors by mentioning their effect on koalas' home range size and movements… If adding them as correcting factors decrease to much the power of your analyses you could first test separately if they have an effect (preliminary analyses).

RESPONSE: Thanks for this suggestion. In hindsight it would have been constructive to include age and sex. However, this would require major reanalysis of the data which we are no longer in a position to do.

- L. 159: where is Ipswich? - it is not on the map (Fig1)

RESPONSE: Ipswich has been added. Figure 1 is now Figure 2.

- L. 160-161: what is the time frame in which this cover loss occurred?

RESPONSE: Thanks, this has been corrected.

- L. 167: Brisbane town instead of time?

RESPONSE: Corrected.

- L. 176: […] influencing CHLAMYDIOSIS prevalence
You need to be careful when presenting this conceptual model since it may only apply to your specific system or at least similar ones. For instance, it would probably not apply to parasites transmitted differently (eg prolonged social contacts or environmentally-transmitted).

RESPONSE: Thanks, this has been corrected.

- L. 178: replace disease dynamics by parasite ecology

RESPONSE: Thanks, this has been changed.

- L. 179: you mention body condition here but it is not on Fig2.

RESPONSE: This has been corrected.

Throughout the paper - except in some parts of the discussion - you avoid talking about the link between body condition and infection risk. The link is always difficult to explain because a weak body condition may increase the risk of infection as much as an infection can decrease body condition - however you cannot ignore body condition in your conceptual model.

RESPONSE: Body condition is now included in the Conceptual Model.

- L. 181-191: Prediction 1 is not explained clearly enough. You explain how Forest area and fragmentation can influence body condition but you don't explain the possible mechanisms by which it could affect prevalence. For instance, if fragmentation increases the clumping of individuals, it should increase contacts between hosts and, if it includes increased sexual contacts, it should increase Chlamydia transmission.

RESPONSE: Thanks the mechanisms have been clarified.

- L. 219-220: Here you finally cite several important drivers that could be at play - why is it not also discussed in the other predictions?

RESPONSE: Thanks the mechanisms are now clarified in Prediction 1.
.
- Fig2: precise whether it is an increase or decrease in suitable habitat/total habitat/etc that you expect to increase/decrease prevalence

RESPONSE: Figure 2 has been redrawn to clarify these relationships.

- L. 226: You are not looking at all diseases infecting koalas - not even of all infectious diseases - so rather use Koala chlamydiosis records

RESPONSE: Thanks this has been changed.

- L. 236: is a repetition (see L232)

RESPONSE: Thanks this has been corrected.

- L.239-240: remove from 'to avoid the problem of…'. You also have possible uncertainty around Chlamydiosis diagnostic (using clinical symptoms) in the admission dataset

RESPONSE: Thanks this has been corrected. All koala hospital chlamydiosis diagnoses were done under the supervision of an experienced vet.

- L.242-244: combining the 2 sentences would make it easier to read

RESPONSE: Thanks for this suggestion. Prefer to keep the 2 sentences separate.

- L.246-247: remove from 'not a series of separate hospitals…'

RESPONSE: Done
- L. 247: 'we ALSO removed'

RESPONSE: Done

- L. 249: I still think that if you have the data it should be accounted for (see previous comment)

RESPONSE: Point taken.

- L. 256:precise what is a 'time period' (I guess 1 year but it hasn't been said yet)

RESPONSE: changed to year.

- L.260: prevalence is the proportion of infected individuals (or of individuals with clinical symptoms in your case) in the considered group - precise that you look at the proportion of symptomatic individuals in a cell each year.

RESPONSE: thanks corrected.

- L.261: move the sentence 'Records were…seasonal variation' at the beginning of the previous paragraph.

RESPONSE: thanks corrected.

- L. 265-266: inverse the order of either 'not felt, or barely felt' or of '9 or 10' to make it coherent Also, precise whether you use the numerical or categorical scoring of body condition in your analyses.

RESPONSE: thanks corrected.

- L. 281: define 'pre-clearing Regional Ecosystem'

RESPONSE: thanks this has been clarified, refers to before European settlement.

- Table1: replace 'severity' by body condition

RESPONSE: thanks corrected.

- L. 304-305: as it is prediction 2 seems only related to dog attacks - try to reformulate/precise
RESPONSE: Dog attacks are stated as a stressor in prediction 2.

- L.324: remove probability and clarify that you model the probability for an individual to be infected (prevalence is at the grid cell level whereas if I understand properly your response is at the individual level)

RESPONSE: Thanks this has been clarified.

- L. 334: Explain why you look first at univariate models. (aim?)

RESPONSE: Thanks this has been clarified.

- L.337-341: true for all models so move before the part on univariate models

RESPONSE: Thanks these sentences have been moved to before the univariate models.

RESULTS
- L. 365-367: 'Explanatory analysis…. Removed from the analysis' this could be put in the method section

RESPONSE: thanks, this has been moved to the methods section.

- Add results on the prevalence of the pathogen: what is the prevalence over all study years and grid cells? Per year? The range of values over the different areas etc?

I asked for these information in my previous review already and still think it is very important - whether the reader is interested in disease ecology or landscape ecology needs some basic descriptive information - Is the infection highly prevalent in the population?

RESPONSE: Apologies for this. We no longer have access to the data as post-doc working on this project has left the university and back-up copies are no longer stored on the system.

- L. 373-375: very heavy - cannot you just say the 'proportion of suitable habitat' instead of 'the proportion of the landscape with suitable habitat'?

RESPONSE: Corrected

- L. 383: positive effect on what - ie does it increase prevalence or decrease prevalence?

RESPONSE: Thanks, this has been corrected.

- As in my previous review, I'm missing the mention of the best models? You do a model selection but don't give use the results of it… You must also give results on the strength of the effecst - you cannot just say that there were weak or strong and a p-value - you need to give us some figures (value of the estimate/value of the statistic etc)

RESPONSE: Thanks for this comment. The p-value of the best model is now reported plus the Wald Statistic of the variable parameters. The strength of the effects and their SE are shown in Figure 4. We do not consider it necessary to include these statistics in the main text. The reporting of the multivariate models for body condition (Figure 6) have also been modified.

- L. 402: Figure 6 not 4

RESPONSE: Thanks this has been corrected.

DISCUSSION

Your paper is now centred on time lags so you must mention and discuss them i) when you summarise your results at the beginning of your discussion, and ii) when you discuss the effects of habitats (L.426-451) (& in the abstract) For instance, how do you interpret that an increase in suitable habitat leads to an increase in prevalence 3 years later?

RESPONSE: Thanks for this comment. We have strengthened reference to time-lags in the Discussion and Abstract.

- L. 428: area of suitable habitat? Total habitat? Both?

RESPONSE: We are referring here to higher quality habitat not all habitat types.

- L. 427-437: be careful in your formulation. You make hypotheses on the mechanism - you did not test causal effects

RESPONSE: thanks, we are not implying causality.

- L. 434-437: BUT if fragmentation leads to contacts that are clumped between diseased individuals then prevalence should be higher! So same effect than before your 'conversely'…

RESPONSE: this section has been revised.

To deduce potential underlying mechanisms we lack information on whether a large area of suitable habitat means higher densities of koalas and increased contacts between them & whether reduced area of suitable habitat means a more patchy landscape with clumped koalas in the patches or simply a landscape with lower densities of koalas. Because for now it looks like as if both larger and smaller area of suitable habitats mean an increase in prevalence.

RESPONSE: Thanks we have clarified this in the revised Discussion.

- L. 480-486: keep only 2-3 relevant species - you will never be exhaustive anyway and it is not the point.

RESPONSE: this has been corrected.

- L. 495: '…for disease infection risk' not disease infection and risk

RESPONSE: Thanks, this has been corrected.

- L. 545-525: I think the idea is rather that infected individuals may be more susceptible to predation and road kills because their ability to detect or escape them may be altered (infections tend to reduce movements instead of increasing them, except in some rare cases) - and that they may be more likely to be admitted in a hospital? Ie that your dataset may overestimate the proportion of infected koalas in your study site.

RESPONSE: We have explained this in the Methods section under Koala chlamydia records.

- L. 530: give a reference

RESPONSE: Hanger et al. 2000 added

- To me, the main limitations of the study are that you don't know (or don't discuss?) how changes in landscape structure (eg proportion of suitable habitat) change contact patterns between koalas and in particular mating patterns (most importantly considering the transmission mode of the pathogen) and that you don't know how changes in the proportion of suitable or total habitats translate into the patchiness of the landscape in your grid cells.

RESPONSE: This is an important point. It will require a fine-scale tracking study of koala movements to answer this question.

- L. 535: finish your sentence by 'in human-modified landscapes'?

RESPONSE: Done.

Time-delayed influence of urban landscape change on the susceptibility of koalas to chlamydiosis

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Abstract

Context

Infectious diseases are important in the dynamics of many wildlife populations, but there is limited understanding of how landscape change influences susceptibility to disease.

Objectives

We aimed to quantify the time-delayed influence of spatial and temporal components of landscape change and climate variability on the prevalence of chlamydiosis in koala (*Phascolarctos cinereus*) populations in southeast Queensland, Australia.

Methods

We used data collected over 14 years (n = 9078 records) from a koala hospital along with time-lagged measures of landscape change and rainfall to conduct spatial and temporal analyses of the influence of landscape and environmental variables on prevalence of chlamydiosis and koala body condition.

Results

Areas with more suitable habitat were associated with higher levels of disease prevalence and better body condition, indicating that koalas were less likely to be impacted by chlamydiosis. More intact landscapes with higher proportions of total habitat are associated with a reduction in prevalence of chlamydiosis and a decrease in body condition. Increased annual rainfall contributed to a decrease in prevalence of chlamydiosis and an increase in body condition. Urbanization was associated with an increase in disease, however the effects of urban landscape change and climate variability on

chlamydiosis may not manifest until several years later when overt disease impacts the population via effects upon body condition and reproductive success.

Conclusions

Our study highlights the importance of effects of landscape change and climate variability on disease prevalence in wildlife. This recognition is essential for long-term conservation planning, especially as disease often interacts with other threats.

Keywords

Wildlife disease, body condition, habitat loss, chlamydiosis, time lags, climate variability, *Phascolarctos cinereus.*

Introduction

Infectious diseases play a key role in the dynamics of natural populations (Holmes 1996; Lafferty and Holt 2003), including posing a threat to population viability (McCallum and Dobson 1995; Bairagi et al 2007; Su et al 2009). Diseases can cause population declines or extinction in wildlife as reported for amphibians, mammals and birds (de Castro and Bolker 2005; MacPhee and Greenwood 2013). A high profile marsupial example of this is the facial tumour disease in Tasmanian devils (*Sarcophilus harrisii*) (Lunney et al 2008; Grueber et al 2016). Human-induced landscape change also impacts on the health and fitness of wildlife (Cottontail et al 2009; Brearley et al 2013), with habitat loss and fragmentation changing population densities and lowering genetic diversity, thereby making individuals more susceptible to disease (Smith et al 2009; Clark et al 2011). Further, landscape change can alter ecological and evolutionary processes, including those interactions between hosts and parasites (Walsh et al 1993; de Castro and Bolker 2005; Chasar et al 2009). Increasing urban development results in loss and fragmentation of native habitats, and the intensification of the land-use matrix (Fahrig 2003). Urban landscapes present novel threats to wildlife, such as road traffic and introduced predators, as well as an increased susceptibility to persistent and new diseases (e.g. Lehrer et al 2010). The increased risk of wildlife disease in urban landscapes adds to existing threats, forcing populations into decline (Plowright et al 2008), yet there are few studies that have attempted to explicitly determine these effects.

The influence of urban landscape change on the susceptibility of wildlife populations to disease is likely to follow three pathways. Firstly, prolonged exposure to stressors, such as overcrowding or nutritional deficiencies, diminishes most immune processes (Martin 2009), with oxidative stress and inflammation among the many known physiological responses to stressors being implicated (Isaksson 2015). Secondly, fragmentation of habitats can lead to increased densities of individuals in habitat fragments, and therefore increase the probability of physical contacts and sexual transmission (Brearley et al 2013). Thirdly, habitat loss and fragmentation

reduces landscape connectivity and increases landscape resistance, thereby decreasing gene flow between populations (e.g. Dudaniec et al 2013) and potentially increasing their susceptibility to disease.

Studies of the influence of habitat loss and fragmentation on the susceptibility of wildlife populations to disease reflect a focus on agricultural, plantation or cattle-grazing areas (Chapman et al 2005; Chasar et al 2009; Evans et al 2009). They show that the influence of landscape change varies considerably (Brearley et al 2013). The few urban studies have also shown that landscape change can have both a positive and negative impact on the prevalence of disease in wildlife species (Fischer et al 2005; Gibbs et al 2006; Geue and Partecke 2008; Friggens and Beier 2010; Lehrer et al 2010). For example, urban blackbirds (*Turdus merula*) had lower blood-parasite prevalence than forest blackbirds in Munich, Germany (Geue and Partecke 2008). In contrast, the risk of avian infection of the vector-borne pathogen West Nile Virus was higher in urban areas in Georgia, USA, compared to surrounding mountainous regions. Further, the effect of the same parasite in two different hosts can vary, even between similar species such as red colobus (*Procolobus rufomitratus*) and black-and-white colobus monkeys (*Colobus guereza*) (Chapman et al 2006; Gillespie and Chapman 2008).

Understanding the dynamics of disease and landscape change interactions is becoming increasingly important for koala (*Phascolarctos cinereus*) populations in the eastern seaboard of Australia, which are declining due to urban development and the loss and fragmentation of their forest habitat (McAlpine et al 2006a, 2015; Rhodes et al 2011; Lunney et al 2016), with climate change adding to the decline (Lunney et al 2012, 2014; Santika et al 2014). Disease has been implicated as one of the drivers of the decline of some koala populations (Rhodes et al 2011; Kollipara et al 2013).

Koalas are threatened by two major infectious diseases, Chlamydia (Kollipara et al 2013) and Koala Retro Virus (KoRV) (Simmons et al 2012). Two species of Chlamydia infect koalas (*C. pecorum* and *C. pneumoniae*) but *C. pecorum* is much more common and has been linked to the majority of clinical disease in this host. While both diseases are important, Chlamydia is considered to be the major threat as it is widespread throughout the koala's range and can lead to both life-threatening disease (such as keratoconjunctivitis resulting in blindness) and infertility in female koalas (Polkinghorne et al 2013; Waugh et al 2016). While strong evidence supports venereal transmission as the most frequent mode of chlamydial transmission (Girjes et al 1988; Martin and Handasyde 1999; Griffith 2010), non-venereal transmission also occurs in pre-pubertal sub-adults (Weigler et al 1988; Jackson et al 1999; Santamaria and Schlagloth 2016; Griffith 2010).

Recent modelling suggests that, without intervention, chlamydial disease can lead to localized extinction events in relatively short time periods (Polkinghorne et al 2013). While disease levels in wild populations vary considerably, levels from 40% to 80% are not uncommon, particularly in New South Wales and Queensland koala populations (see Figure 3 Polkinghorne et al 2013). Studies have shown that over 90% of wild koalas in south-east Queensland can be infected with Chlamydia. However, levels of overt chlamydial disease are much lower, often with only 9-12% showing clinical signs of the disease (Preece 2007). As with most infectious diseases, not all infected koalas progress to overt disease, with some apparently resolving their infections, some having ongoing asymptomatic infections, and a percentage progressing to severe clinical disease. A number of factors might be responsible for this variation in the expression of the disease in koalas, both between different geographical areas and within defined koala populations. These may include: (a) genetic differences between strains of *C. pecorum* (there is evidence for avirulent as well as virulent strains in other chlamydial species/hosts); (b) co-infection with other infectious agents promoting or limiting Chlamydia infection and clinical symptoms, for example KoRV; (c) individual animal genetic differences; (d) environmental stresses (such as habitat reduction in

amount or quality, drought and heatwaves) that might result in biochemical, metabolic or immune effects, and (e) individual variations in behaviour (i.e. variations in exposure to the pathogen) and variations in physiology (i.e. variations in susceptibility to the pathogen, for instance variations in immunocompetence or variations in levels of stress hormones or testosterone). In some species, males may be more susceptible to infection than females because androgens reduce immunocompetence and sex steroid hormones affect disease resistance genes and behaviours that render males more susceptible to infection (Folstad & Karter 1992; Klein 2000). Koalas may be able to tolerate a latent chlamydial infection until something triggers the expression of the disease (Ellis et al 1993). Hume (1990) contends that habitat fragmentation is the primary threat to koalas and that the prevalence of Chlamydia is a consequence of this threat and nutritional stress, so that increase in habitat fragmentation increases susceptibility to disease. However, 26 years later, this contention has not been explicitly tested.

Qualitative comparisons of studies among koala populations in undisturbed and disturbed habitats suggest that chlamydiosis is likely to increase in populations under pressure from landscape change, particularly in urban areas (Carrick et al 1990; Ellis et al 1993). Rhodes et al (2011) quantified the mortality rates from a number of threats in a peri-urban koala population under stress of habitat loss and fragmentation in southeast Queensland, and identified Chlamydia as a major contributor to mortality. While the threats posed by habitat loss, dog attacks and vehicle collisions are relatively well understood (McAlpine et al 2006a, 2015; Rhodes et al 2011), the threat posed by disease, and its interaction with other threats, is poorly understood.

Our aim in this study was to quantify the influence of spatial and temporal components of landscape change and climate variability on the prevalence of chlamydiosis and the body condition of individual koalas in southeast Queensland, Australia. Diseased individuals with a lower body condition score were considered to be more vulnerable to chlamydiosis .We postulated that disease

prevalence would be higher in urban landscapes with the lowest proportion of suitable koala habitat as well as during dry conditions. Our approach involved analyzing koala hospital records in southeast Queensland, Australia, using generalized estimating equations models (GEE) to account for temporal correlations in the response variables.

Conceptual model

The koala is an arboreal marsupial and an obligate folivore (Martin and Handasyde 1999). Its distribution and density are influenced by numerous factors affecting habitat extent and quality, climate change and population dynamics (Melzer and Houston 2001; McAlpine et al 2006, 2015; Rhodes et al 2006). Almost all the koala populations in Queensland and New South Wales (NSW) are declining, invariably from multiple threats (Adams-Hosking et al 2016; McAlpine et al 2015). Koala home range sizes vary with habitat quality, season and koala density. In southeast Queensland, home range areas of 1 - 135 ha have been observed (Dique et al 2003a; Thompson 2006; de Oliveira et al 2013). Dispersal is usually undertaken by sub-adult koalas in the pre-mating and early mating period of the breeding season from June to December (Dique et al 2003b; Rhodes et al 2011; Matthews et al 2016).

A conceptual model of the key landscape and climate factors influencing chlamydiosis prevalence in the koala is shown in Figure 1. Embedded in the model are a number of *a priori* predictions, based on the parasite ecology of diseases, koala ecology, and the potential influences of urban landscape change and climate variability on chlamydiosis prevalence and koala body condition.

Prediction 1: The loss and fragmentation of habitat increases chlamydiosis prevalence and reduces body condition in koalas. Habitat loss is the reduction in the amount of suitable habitat while fragmentation is a landscape-scale process involving habitat loss and the breaking apart of habitat (Fahrig 2003). Collectively, we hypothesize that these processes increase the prevalence of

chlamydiosis and reduce the body condition of koalas in urbanized landscapes. Reduction in the area of forest reduces habitat quality which is linked to the nutrients in the leaves of a limited number of closely-related trees of the genera *Eucalyptus, Corymbia* and *Angophora* (e.g. Phillips 2000; Moore and Foley 2005; McAlpine et al 2006a). Habitat loss and fragmentation may also influence disease susceptibility through nutritional deficiencies or physiological stress from adjacent land-uses. Fragmented habitats increase the risk of disease transmission through changes in population density or clumping of individuals around key resources, and by increasing sexual encounters and aggressive territorial behaviour. Anthropogenic threats such as dog attacks will be likely enhanced, and intra- and inter-species competition will increase.

Prediction 2: Chlamydiosis prevalence in koalas will increase, and body condition decrease, in landscapes containing a greater urban extent. Urban development is likely to be important in wildlife disease transmission and susceptibility by increasing physiological stress and adversely influencing individual health. However, these potential influences remain to be tested for chlamydiosis in koalas. Urban areas form a relatively hostile matrix for koalas, exposing them to additional environmental stressors and hazards, such as a greater risk of harassment and attack from dogs (McAlpine et al 2006b; Lunney et al 2007) and elevated mortality from vehicles on roads (Dique et al 2003b).

Prediction 3: A decrease in annual rainfall will increase disease prevalence and decrease body condition in koalas. Variation in annual rainfall, including drought, is a key indicator of climate variability and it is linked to other effects, such as heatwaves and bushfires (Melzer et al 2000; McAlpine et al 2015). Since the expression of clinical disease (including chlamydiosis) is the result of interactions between the host, pathogen and environment, it is reasonable to predict that drought and associated stressors (e.g. dehydration, heat stress) may alter the balance between host

and pathogen, with an increase in disease susceptibility and a decrease in body condition (see Harvell et al 2009; Lunney et al 2012).

Prediction 4: Time lags will be important in the effect of the processes outlined in Predictions 1-3. Time-series assessments of landscape change provide a greater understanding of the length of time between landscape disturbance and the prevalence of wildlife disease. The effect of landscape change can have a temporal as well as a spatial component, and associated wildlife responses to disturbance may occur over long periods (Tilman et al 1994). It is likely that temporal-scale parameters will influence wildlife-disease systems (Brearley et al 2013). Thus it is reasonable to predict that in most cases Chlamydia prevalence in a koala population will not change immediately following disturbance. Rather, time-lagged processes will create situations that favour disease transmission and prevalence, including increased physiological stress and contact rates, sexual encounters and aggressive territorial behaviour. Anthropogenic threats will increase, as will intra- and inter-species competition.

#Figure 1 approximately here#

Methods

Koala chlamydiosis records

We reviewed the Queensland Government's Moggill (20 km southwest of Brisbane) koala hospital dataset of 34,598 koala records, comprising all hospital admissions and field observations of koalas collected by researchers, government employees, wildlife rehabilitators and the general public over 14 years (1997-2011) from 10 Local Government Areas in southeast Queensland (Figure 2). All data were provided by Queensland's Department of Environment and Heritage Protection. For the hospital admissions, each koala was examined for chlamydiosis symptoms (e.g. cystitis, conjunctivitis), health status (e.g. wasting, pneumonia, body condition score), and reason for

admission (e.g. dog attack, vehicle collision, disease). Chlamydia disease was diagnosed using physical examination, symptoms or necropsy results. We did not examine any koalas, but accepted the hospital records. A large portion of the dataset comprised field observations that were based solely on sightings. These data were not included in the final dataset because of potential unreliable disease diagnosis and assessment of clinical health.

#Figure 2 approximately here#

Potential biases in the koala data may exist due to higher rates of reporting in areas with higher human population densities. In addition, animals that were sick or displayed unhealthy characteristics were more likely to be taken to hospital than animals that were healthy. Balanced against these possible biases is the strength of the dataset, which is large, georeferenced and has consistent criteria for admission and recording. To minimize biases, we also removed records of koalas brought to the clinics because of disease. As a result, the final dataset contained 9078 records for 14 consecutive years. We note that by any measure, this number is far larger than in most studies of koalas, so limitations in the dataset are offset by its size. We did not separate records according to animal age or sex.

Records were on an annual basis and did not capture seasonal variation. A time series of their spatial distribution, aggregated at 3 year intervals, is shown in Appendix 1. For areas south of Brisbane, admission records peaked in the 2000-2002 period. For areas north of Brisbane, admission records increased for each time period. A 10 x 10 km raster grid representing 150 cells was overlaid onto the study area using ArcMap 10 (ESRI, Redlands, CA, USA). The grid size was selected to match an approximate maximum koala dispersal distance of 10 km (Dique et al 2003a). Of the 150 grid cells, only 42 had sufficient records (minimum 10 in any one year) to be used in the

analyses. These grid cells formed the units of analysis for quantifying the influence of landscape structure and climate variables on koala disease prevalence and body condition.

Disease prevalence was calculated as the proportion of individuals with clinical symptoms of chlamydiosis per cell per year for each year. Body condition scores ranged from 1 to 10. A score of 1 is emaciated whereas a score of 10 is well nourished and with good muscle development. The score is a widely-used assessment, mainly based on the muscle on either side of the scapular spine. In essence, if the muscle in the fossa has a concave aspect, then the score is less than 5, and if it is convex, and fills the fossa to the extent that the spine of the scapula is barely felt or not felt, then the score is a 9 or 10. Scores of 1-2 = emaciated, 3-4 = poor, 5-6 = fair, 7-8 = good and 9-10 = excellent body condition. Some studies use body scores of 1-5, which correspond exactly to the paired groupings in the 1-10 scale. Individuals with a low body condition score were considered to be more likely to be diseased (J. Hanger, veterinarian, personal communication). Scores were analyzed as ordinal data.

Explanatory variables

The area of suitable habitat (*Eucalyptus*, *Corymbia* and *Melaleuca* forests and woodlands), and total habitat (all forests and woodlands), were chosen as the key indicators of habitat loss (Prediction 1) (Table 1). The habitat suitability class for each of these vegetation communities was assigned using the proportional abundance of the identified preferred food tree species (see McAlpine et al 2006a, b). A time-series of the area of suitable koala habitat was quantified using the intersection of land cover and Regional Ecosystem mapping (scale 1:100,000) (Queensland Herbarium 2011). Time-series archived Landsat data were available from 1972-2012 for southeast Queensland (see Lyons et al 2012). This imagery allowed broad land cover types, such as forest, urban and agricultural, to be identified, but did not allow for koala habitat quality to be mapped. Areas identified by the time-series land cover mapping to contain forest cover, and pre-clearing (before European settlement)

Regional Ecosystem categories dominated by *Eucalyptus*, *Corymbia* and *Melaleuca* vegetation communities, were classified as suitable koala habitat. Areas identified by land cover mapping to contain forest cover, but pre-clearing Regional Ecosystems dominated by non-koala food trees, including *Casuarina*, notophyll vine forest and exotic pine plantations, were classified as secondary habitat that may be used for shelter refuge and cover for movement. Grassland, agriculture and open parkland/farmland identified by the land cover mapping were also classified as secondary habitat that may be used for movement, because koalas can move across open areas, especially if scattered trees are present (McAlpine et al 2006a,b; Preece 2007).

The time-series data of the area of suitable koala habitat were used as an explanatory variable in all models. In addition, suitable habitat areas were combined with secondary habitat areas to represent the total area of available koala habitat in each grid cell. To quantify habitat connectivity (Prediction 1), we applied a distance-weighted metric, determined by the connectivity of highly suitable habitat patches in a grid cell, as the key indicator of habitat fragmentation (Table 1) (see Moilanen and Nieminen 2002; Rhodes et al 2006). This metric quantifies the combined influence of the amount of habitat and its connectivity. It is essentially a distance-weighted mean of the habitat values around each patch, with the weighting following a negative-exponential decline with distance from a patch.

For Prediction 2, urban extent (derived from the time-series land-use data) was classified as unsuitable habitat because it represented a largely inhospitable matrix with limited vegetation cover, and a high risk of mortality to koalas during dispersal. Exploratory analysis confirmed that road density was highly correlated with urban extent (see Table S1), so it was removed from the analysis. Urban areas also comprise residential and commercial estates that increase koala mortality from dog attacks, so the proportion of urban extent for each time period was used as the primary predictor for

Prediction 2. It represents all urban land-use types and magnitudes, including threats associated with vehicle collisions and dog attacks (Table 1).

For Prediction 3, mean annual rainfall was calculated for the 38 climate stations in the study region (see Appendix B) (Queensland Government 2011). Mean annual rainfall data (mm) were then assigned to each grid cell based on the nearest station using ArcMap 10 (ESRI, Redlands, CA, USA).

To test the influence of time lags in the effect of the predictor variables on disease prevalence and body condition (Prediction 4), each habitat predictor variable was calculated yearly for up to five years prior to the year of the disease record. For annual rainfall, time lags for each year for two years prior to the disease record were calculated. This shorter period represents the time scale at which variation in rainfall affects vegetation growth and habitat conditions in the region (C. McAlpine personal observation).

#Table 1 approximately here#

Statistical analysis

Using univariate Generalized Estimating Equations (GEE) models using the R statistical package "geepack" and function "geeglm" (Halekoh et al 2006), we modeled how the probability an individual being infected with chlamydiosis and their body condition were influenced by the explanatory variables for each grid cell, with and without time lag effects. Analyses were performed using the R public-domain statistical package (R Project for Statistical Computing release 2.14.0 http://www.r-project.org). GEE models have proven to be useful in analyzing data that are collected in clusters, and where observations are longitudinal or have repeated measures, because they account for the lack of statistical independence between samples by adjusting regression

coefficients and variance to avoid spurious correlations (Liang and Zeger 1986; Zorn 2001; Larsen et al 2009; Zuur et al 2009). They also enable a correlation structure to be implemented in the model to account for the temporal or repeat measure correlation in the data.

Explanatory variables were first standardized to a mean of 0 and a standard deviation of 1 to allow for comparisons of model parameter estimates. Spearman's rank correlation was applied to test for correlation between explanatory variables (Table S1). If a pair of variables had a correlation coefficient of >0.5, it was considered a proxy of the other and one of the variables was removed (Booth et al 1994). Exploratory analysis found that habitat connectivity was positively correlated with the amount of suitable habitat (see Appendix A), and it was subsequently removed from the analysis. As a result, the amount of suitable habitat and total habitat was considered a good proxy for the connectivity of habitat throughout the landscape.

To select the variables with the strongest effect on the response variables, we compared the effect of all univariate models for all time periods using the Wald statistic (Engle 1984). We plotted the Wald statistic against the mean and standard deviation of each explanatory variable for each time-lag period, and examined whether the Wald statistic weight followed a similar pattern to the mean and standard deviation of the predictor variables.

For disease prevalence, multivariate modeling analyses were conducted using logistic regression (link = logit). This is particularly important in this study because the koala disease data were collected from 14 consecutive years, and this procedure enabled us to account for temporal correlation in the data. For body condition, modeling analyses were conducted using multinomial logistic regression (link = logit). These analyses allowed us to determine the effect on disease prevalence and body condition of the predictor variables at different time lags. Due to the within-grid cell temporal replication in the koala data, an auto-regressive correlation structure was used

(corAR1). Autoregressive correlation is observed when correlation both between and within subject observations can be modeled directly as a function of the 'distance' between the observations in question (Zuur et al 2009).

The best model for each response variable was selected using backwards step-wise model selection. Step-wise model selection was used because R statistical package "geepack" does not compute the Akaike Information Criteria (AIC) and is not conducive to multi-model inference (Burnham and Anderson 2002) . Starting with all possible predictor variables, the least significant variable was removed until all existing variables in the model were significant ($p = < 0.05$).

Results

Disease prevalence

The univariate models for disease prevalence showed a high level of support for the proportion of suitable habitat (WS = 6.380; Figure 3a) and the proportion of the landscape identified as urban (WS = 18.390; Figure 3b), following a three year time lag. The strongest effect for the total amount of habitat in the landscape was following a two year time lag (WS = 3.090; Figure 3c), while the highest level of support (WS = 3.040) for annual rainfall was following a one year time lag (Figure 3d).

#Figure 3 approximately here#

The best-fitting multivariate model ($p = 0.002$) for disease prevalence showed a) the proportion of highly suitable habitat (WS = 5.806; Prediction 1) and b) that urban extent (WS = 3.441; Prediction 2) increased disease prevalence ($p <0.001$) with four- and three-year lags respectively (Figure 4). Conversely, the proportion of total habitat decreased disease prevalence

(WS = 3.444; Prediction 1) following a four-year lag ($p < 0.001$). Increased annual rainfall decreased disease prevalence (WS = 2.146) following a two-year lag ($p < 0.05$).

#Figure 4 approximately here#

Body condition

For body condition (Figure 5), there was a high level of support for a stronger effect of the proportion of the landscape occupied by suitable habitat (WS = 2.51; Figure 5a) in the current year, and the proportion of the landscape occupied by urban (WS = 3.79; Figure 5b) and total habitat (WS = 15.34; Figure 5c), following a one and two year time lag respectively. The effect of rainfall in the current year had a high level of support for influencing body condition (WS = 7.08; Figure 5d).

#Figure 5 approximately here#

The best-fitting multivariate model ($p = 0.004$) for body condition showed that the proportion of urban development (WS = 2.352) reduced body condition with a lag of one year ($p < 0.05$), indicating strong support for Prediction 2 (Figure 6). Body condition was higher in landscapes with a higher proportion of suitable habitat (WS = 3.553) following a three-year lag ($p < 0.001$). Increased annual rainfall in the current year (no lag) (WS = 2.192; Prediction 3) increased body condition ($p < 0.05$).

#Figure 6 approximately here#

Discussion

In this study, we used spatio-temporal models to quantify the relationship between urban landscape change and the prevalence of the disease chlamydiosis in koalas and their body condition. We found that there were time lags in the effects of urban landscape change and climate variability on chlamydiosis, which may not occur until up to several years after the initial change occurred. The study demonstrates the importance of longitudinal studies in landscape ecological research. An increase in the area of suitable habitat was associated with an increase in disease prevalence but an improvement in body condition, indicating that koalas were less likely to be impacted by chlamydiosis. More intact landscapes with a higher proportion of total habitat, including suitable and secondary habitat, are associated with a lower prevalence of chlamydiosis. Similarly, increased annual rainfall, representing an improvement in vegetation condition and habitat resources available to koalas, contributed to a decreased prevalence of chlamydiosis.

These findings allow a number of key inferences to be made.

Amount of habitat

We predicted that landscapes with a lower amount of suitable habitat would both increase disease prevalence and reduce body condition (Prediction 1). The results support some elements of this prediction, but not all. Better body condition was associated with landscapes with a higher proportion of suitable habitat. However, landscapes with a higher proportion of suitable habitat had a higher prevalence of chlamydiosis, three years later. While this finding may appear to be counter-intuitive, it can be interpreted as koalas being more likely to occur at high densities in these landscapes due to the quality of habitat resources (Rhodes et al 2015). One explanation is that higher population densities lead to higher exposure to *Chlamydia spp.* but a greater ability to cope because of adequate nutrition. The loss of suitable habitat is likely to lead to isolated populations of koalas (McAlpine et al 2006a) and subsequent clumped reproductive contact rates between diseased individuals. This has implications for the transmission and infection rates of chlamydiosis.

Chlamydia prevalence in a koala population did not increase immediately following landscape modification. This is consistent with Prediction 4. The presence of time-lagged processes helps to explain situations that favour disease transmission and prevalence, including increased physiological stress and contact rates, sexual encounters and aggressive territorial behaviour. This is consistent with Brearley et al (2012) who postulated that the condition or quality of remnant wildlife habitats is likely to decline, whereas anthropogenic threats associated with the human-modified matrix will increase, as will intra- and inter-species competition. These processes create situations that favour disease transmission and prevalence, including increased physiological stress, increased contact rates and aggressive territorial behaviour due to resource clumping and limited availability as well as reduced dispersal opportunities.

The amount of suitable habitat was positively associated with a higher body condition score. A number of studies have noted a link between nutritional stress and changes to disease prevalence in wildlife. Studies of the red colobus and black-and-white colobus (Salzer et al 2007; Gillespie and Chapman 2008) found that the prevalence of gastrointestinal parasites was lower in the black-and-white colobus, most likely due to the willingness of this species to raid nearby agricultural crops, thereby reducing any effects of nutritional stress and maintaining immune capacity (Chapman et al 2006). Similarly, a study of Balinese macaques (*Macaca fascicularis*) found that increased anthropogenic activity led to a lessening of the intensity and diversity of gut parasites, most likely due to the high contact with tourists and consequent heavy provisioning of resources (Lane et al 2011). Despite the current understanding of the link between the nutritional status of the host and body condition and susceptibility, there is still a paucity of information on the link between nutritional deficiency and susceptibility to disease in wild settings. This link has not been tested for chlamydiosis in koalas.

Urban land-use

Our findings support Prediction 2 that urbanization is associated with an increase in the prevalence of chlamydiosis and a decrease in the body condition of koalas. This relationship was evident following time lags of four years and one year, respectively. This may well result from changes to densities, breeding patterns and disease transmission, and dispersal rates across the landscape matrix. Increased urban development creates smaller habitat fragments where food resources become clumped and isolated by a non-habitat landscape matrix. This is likely to function in a similar way to that discussed above for the amount of habitat. Further investigation on disease transmission dynamics is required to test this inference. Resource clumping has been found to be a key driver of contact rates and the prevalence of parasites in raccoons (*Procyon lotor*) in minimally disturbed environments (Wright and Gompper 2005). It was suggested that anthropogenic disturbance resulted in a decreased prevalence of parasites in raccoons because resources for this omnivorous species achieve their highest densities in human-dominated landscapes, where resources are evenly spread. Although this may be true for species that are able to exploit the urban matrix for resources, this does not apply to forest-dependent species, including the koala, which rely on specific food and shelter trees. Therefore, the decline in body condition in urban areas, following a one year time lag (between development/habitat clearing and an increase in severity), can provisionally be attributed to the rapid loss of habitat, as has occurred in south-east Queensland, and an increase in nutritional and/or physiological stress.

In addition to nutritional stress, excessive physiological stress resulting from a number of causes must also be considered when inferring changes to disease prevalence and severity in human-modified landscapes. It is logical to propose that wildlife may be faced with novel and prolonged exposure to stressors in highly modified environments, leading to long-term physiological stress. There is evidence of increased physiological stress in response to human-induced landscape change and associated stressors in numerous species, including the squirrel glider *Petaurus norfolcensis* (Brearley et al 2012), agile antechinus *Antechinus agilis* (Johnstone et

al 2011, 2012) and African elephants *Loxodonta africana* (Ahlering et al 2011), as well as koalas (Lunney et al 2012). The dynamics of physiological impacts of environmental stressors on Australian marsupials are reviewed by Narayan and Williams (2016). Although the link between stress, immunity and disease is regularly discussed because they follow a logical causal path, examination of how these systems interact to impact on survival and fitness in free-living wildlife is only starting, entering through the field of ecological immunology (Blaustein et al 2012).

Climate variability

Our results showed that higher annual rainfall was associated with reduced prevalence of chlamydiosis and a better body condition. The short time lags associated with these changes demonstrate that climatic variability creates rapid outcomes for disease infection risk. This is particularly important in Queensland which, prior to 2011, experienced almost a decade of drought. In a study on the fragmented agricultural landscape of the Liverpool Plains, northwest New South Wales, heatwaves in 2009 not only killed an estimated quarter of the koala population from dehydration, but also reversed the anecdotal status of this koala population as being Chlamydia-free, at least at a clinically-visible level (Lunney et al 2012). In 2008, although detailed pathological studies showed that 8% of a small sample of koalas was carrying *Chlamydia pecorum*, there was little evidence of clinical disease. In 2010-2011, after the heatwaves, preliminary PCR results found that 43% of the population were carrying that pathogen, with clinical disease being evident in both sexes (Lunney et al 2012). The importance of this finding has led to a more substantial study of this population, which is now underway. Since the expression of clinical disease is the result of interactions between the host, pathogen and environment, climate change may alter the balance between host and pathogen, with a particular impact on koala populations. It is possible that mechanisms relating to nutritional, hydration and physiological stress are key drivers of these changes, however this is yet to be directly tested. The adverse effect of drought and heatwaves on the quality of nutrients and moisture available in the koala's diet is documented (Gordon et al 1988,

1990; Moore and Foley 2000). The option of retreat to nest hollows or to go underground is not available to koalas during weather extremes, such as heatwaves, which are expected to increase with climate change (Hennessy et al 2007). These will have direct impacts on koala populations, with recent research identifying that tree choice is mediated by climate (McAlpine et al 2009; Crowther et al 2014) and thus confirming that koalas are directly influenced by weather extremes.

Approach and limitations

Although the data selection in this study aimed to minimize many potential sampling biases, it is impossible to eliminate all biases when using hospital data such as this in lieu of randomly sampled observations of populations. Although we were able to minimize searching and capture biases, we were not able to control for movement patterns of diseased koalas compared with healthy individuals, or young versus old. For example, are unhealthy individuals more likely to move around, resulting in a greater incidence of vehicle collisions or dog attacks in these individuals? Matthews et al (2016) found that the koalas that moved further were the ones most likely to die. Similarly, are healthy dispersing individuals over-represented in these trauma cases? We do not yet know the answers to these questions. We were also unable to incorporate true estimates of koala density. Host density is likely to be an important driver of disease prevalence, and although highly suitable habitat logically provides a good proxy for koala numbers, further studies should aim to include density values in landscape/disease assessments. We were also unable to account for the possible influence of other causal mechanisms, such as the recently-discovered Koala Retrovirus (Hanger et al 2000). While results from these studies provide insight into potential associations, further investigations using direct field based measurements are required to test these associations, and to identify and test key causal mechanisms that may drive the prevalence and severity of chlamydiosis in the koala in human-modified landscapes.

Conclusions

This study demonstrated an impact of urban landscape change on disease prevalence and body condition in a regional wildlife population, in this case, chlamydiosis in koalas. The high public profile of the koala stimulated the setting up of a wildlife hospital to rehabilitate koalas, and it was thus able to supply a valuable dataset, of exceptionally rare size, which included date, location and disease status in urban koalas. Our analyses have provided insights into how landscape changes influence a wildlife-disease system, and highlight the importance of time lags in determining the effect of landscape change and climate variability on disease prevalence. Understanding how disease and landscape change in urban areas impact on wildlife species is essential for their long-term conservation, as these factors often interact with other threats, such as introduced predators (dogs) and vehicle strikes, thereby amplifying the likelihood of local wildlife extinctions. Only when all potential threats are considered can effective management plans be prepared, implemented and monitored to maximize the long-term survival of wildlife species impacted by disease in human-modified landscapes.

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Tables and Figures

Table 1. Description of landscape change and climate predictor variables used to assess changes in koala disease prevalence and body condition within each 10 km^2 landscape (10000 ha).

Variable	Unit	Time-lag	Full description
Habitat loss and fragmentation			
Highly suitable habitat	Proportion	Each year for 5 years prior	Amount of suitable habitat available in the landscape dominated by vegetation communities containing primary koala food trees (McAlpine et al 2006a)
Total habitat	Proportion	Each year for 5 years prior	Total amount of suitable habitat + secondary habitat
Habitat connectivity	Metric	Each year for 5 years prior	Distance-weighted metric calculated by the distances to nearest suitable habitat patch (Rhodes et al 2006)
Land-use intensity			
Urban extent	Proportion	Each year for 5 years prior	Proportion of urban built-up area in the landscape
Climate Variability			
Rainfall	Millimetres	Each year for 2 years prior	Annual rainfall (mm)

Figure 1. Conceptual model of spatial and temporal scale landscape and climate factors influencing disease prevalence and body condition in urban koalas in southeast Queensland, Australia. 'Thick black arrow' indicates a hypothesized positive effect and; 'grey arrow' indicates a hypothesized negative effect.

Figure 2. Left, location of study region in southeast Queensland. Right, the distribution of koala hospital records used in this study and the 10 x 10 km grid cells used in the analysis.

Figure 3. Mean (white bar), standard deviation (error bars) and Wald Statistic (black line) of key predictor variables at increasing time lags for koala disease prevalence. (a) amount of suitable habitat as proportion of the landscape; (b) urban (developed land) as proportion of the landscape; (c) total habitat (suitable habitat & secondary habitat) as proportion of the landscape; and (d) annual rainfall (mm).

Figure 4. Path diagram of the effect of explanatory variables on disease prevalence in urban koalas in southeast Queensland, Australia. Time lags are displayed on left, and significance of the effect is shown from left to right based on p-values (* = $p < 0.05$; *** = $p < 0.001$) and parameter estimates. 'Thick black box and arrow' indicates a positive effect, and 'Thin black box and grey arrow' indicates a negative effect.

Figure 5. Mean (white bar), standard deviation (error bars) and Wald Statistic (black line) of key predictor variables at increasing time lags for koala body condition. (a) suitable habitat as proportion of the landscape; (b) urban (developed land) as proportion of the landscape; (c) total habitat (suitable habitat & secondary habitat) as proportion of the landscape; and (d) annual rainfall (mm).

Figure 6. Path diagram of the effect of explanatory variables on body condition in urban koalas in southeast Queensland, Australia. Time lags are displayed on left, and significance of the effect is shown from left to right based on p-values (* = $p < 0.05$; *** = $p < 0.001$) and parameter estimates. Thick black box and arrow' indicates a positive effect, and 'Thin black box and grey arrow' indicates a negative effect.

Figure 1

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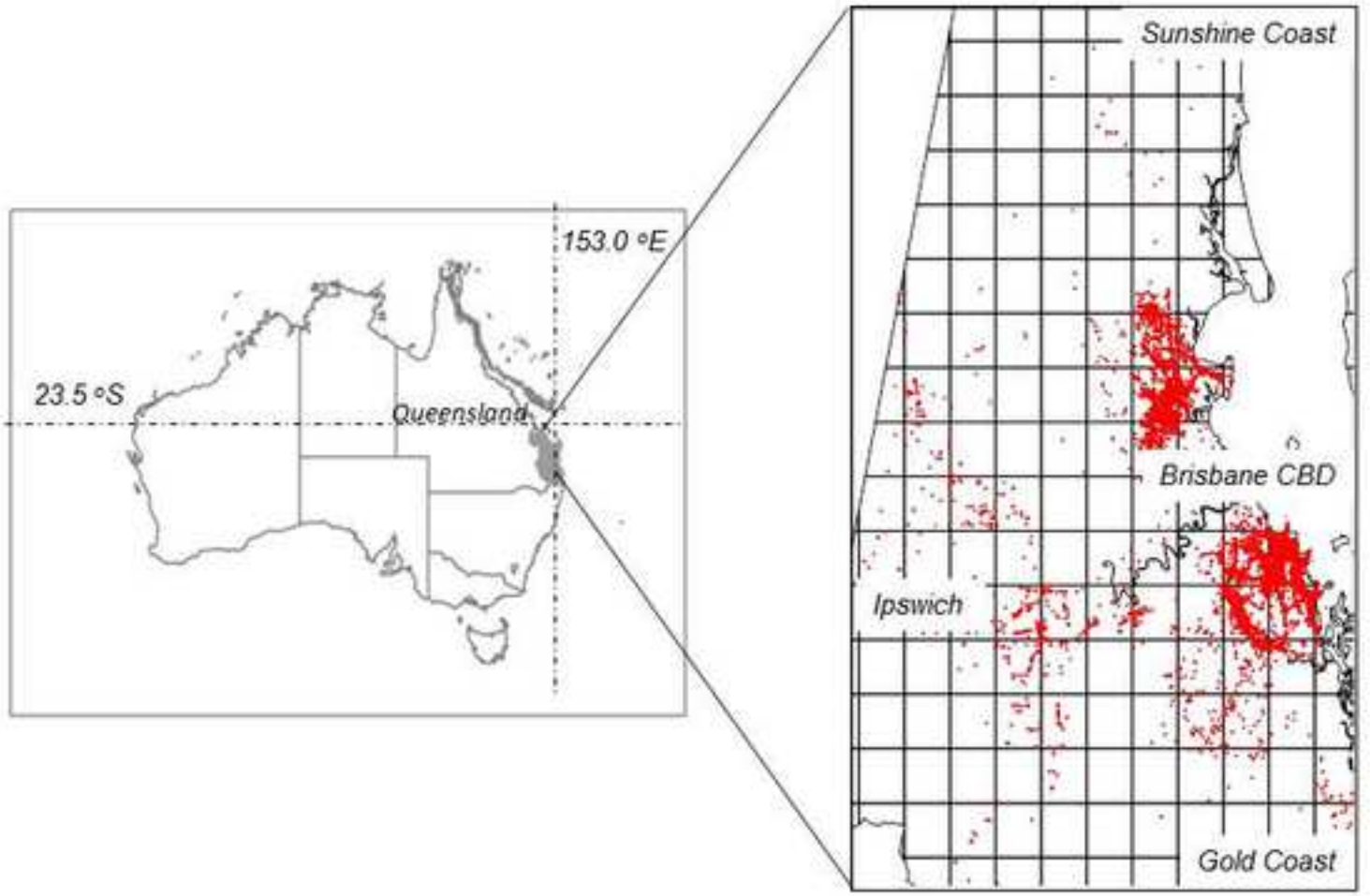
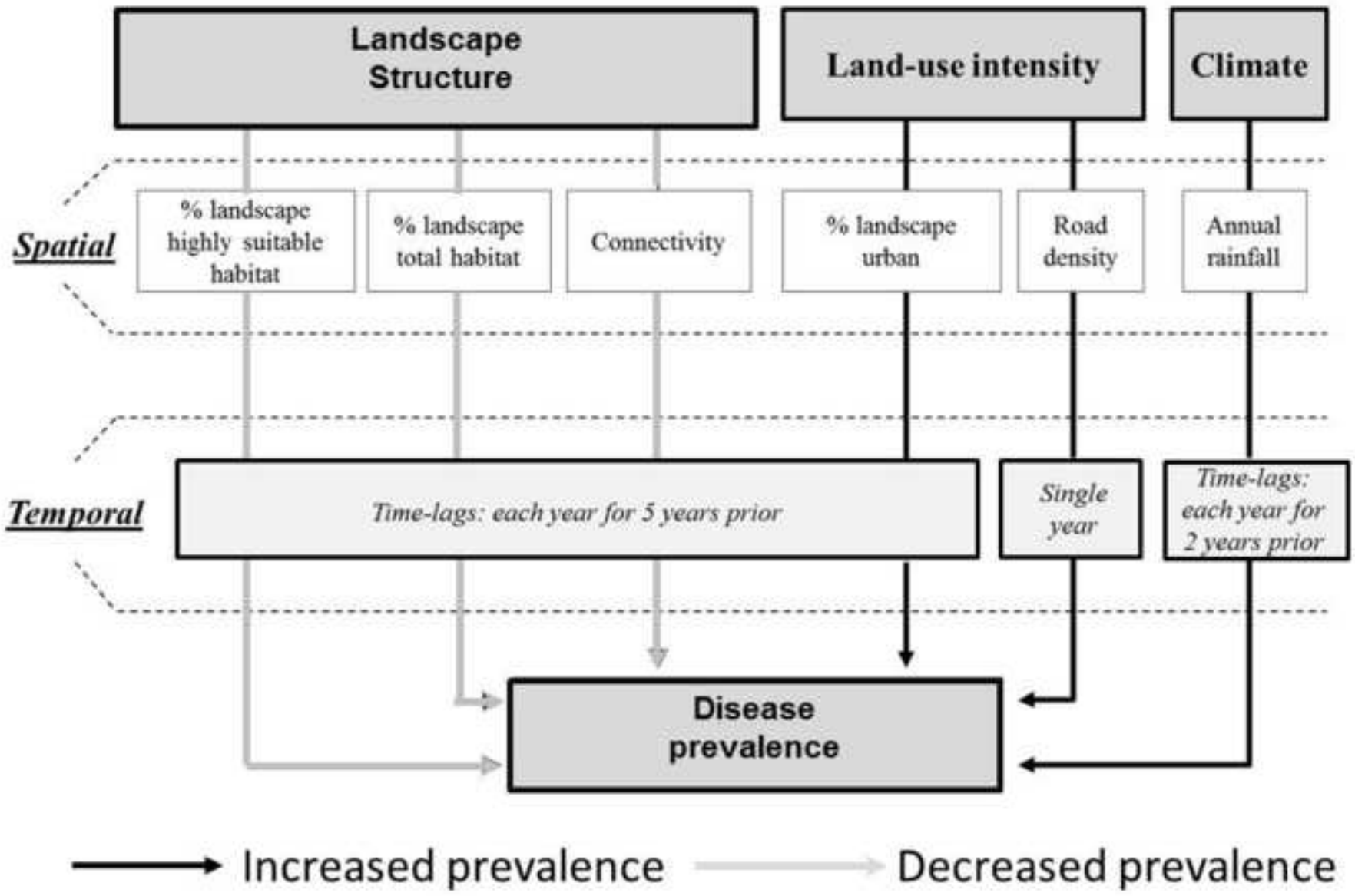


Figure 2



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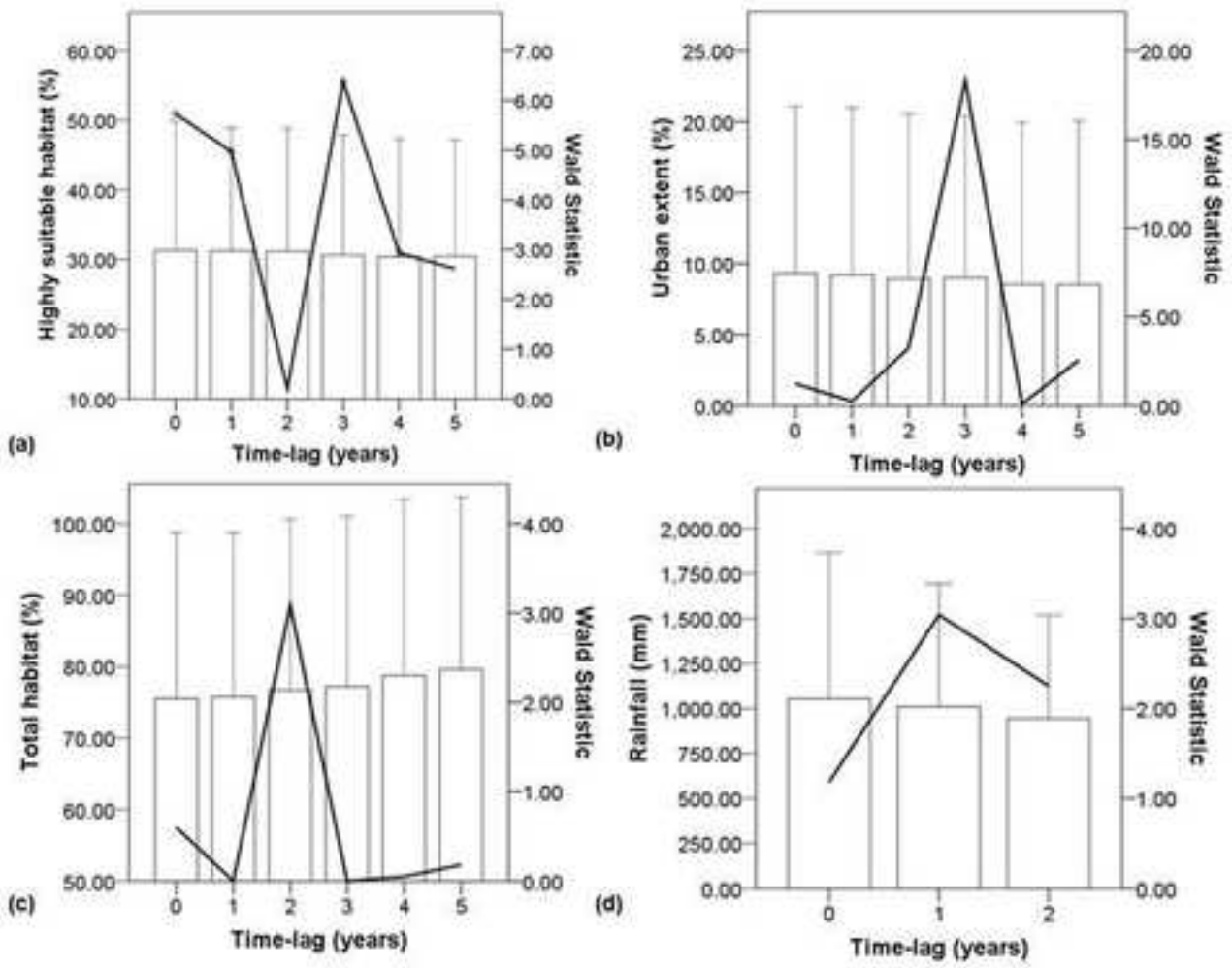

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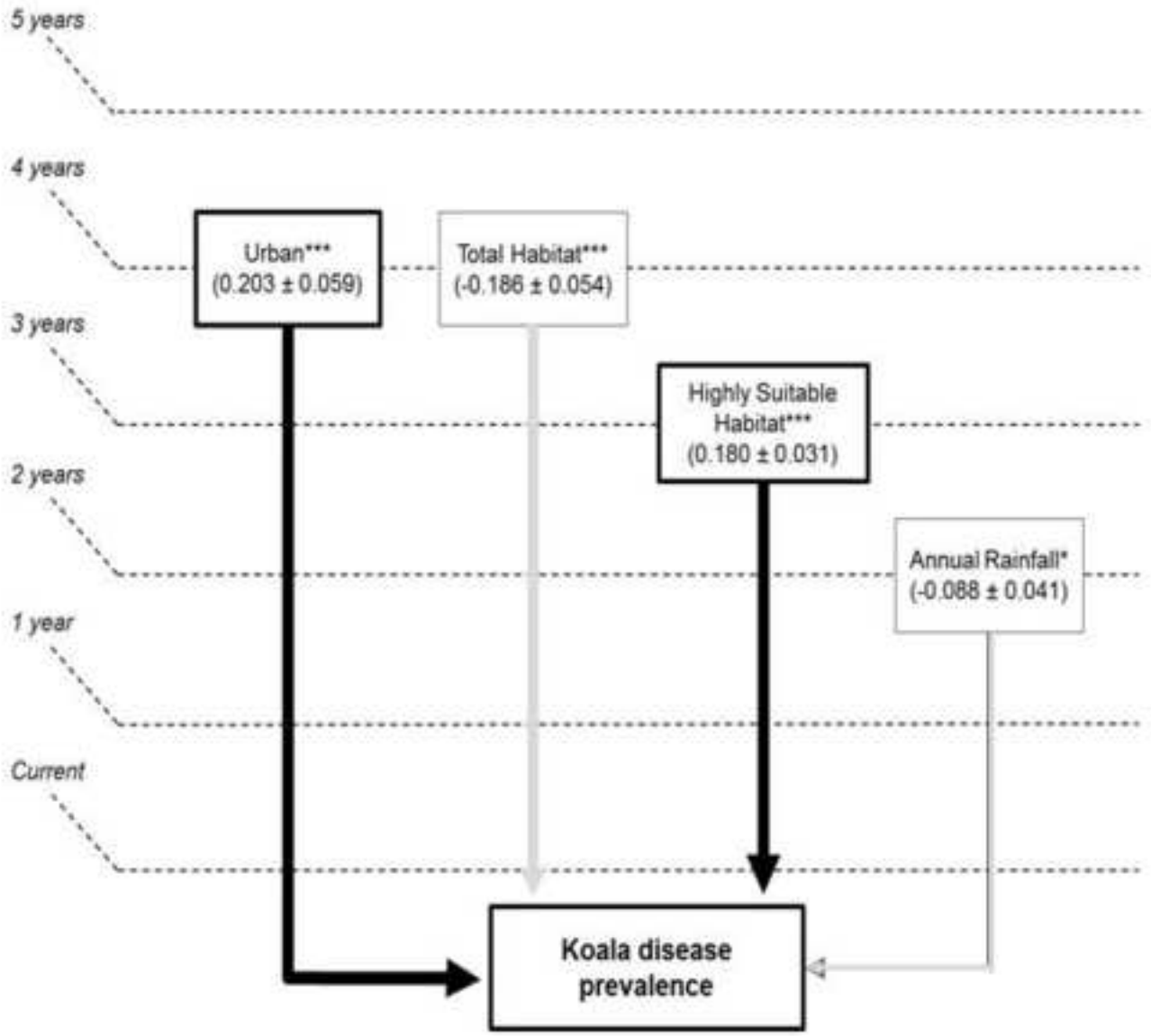

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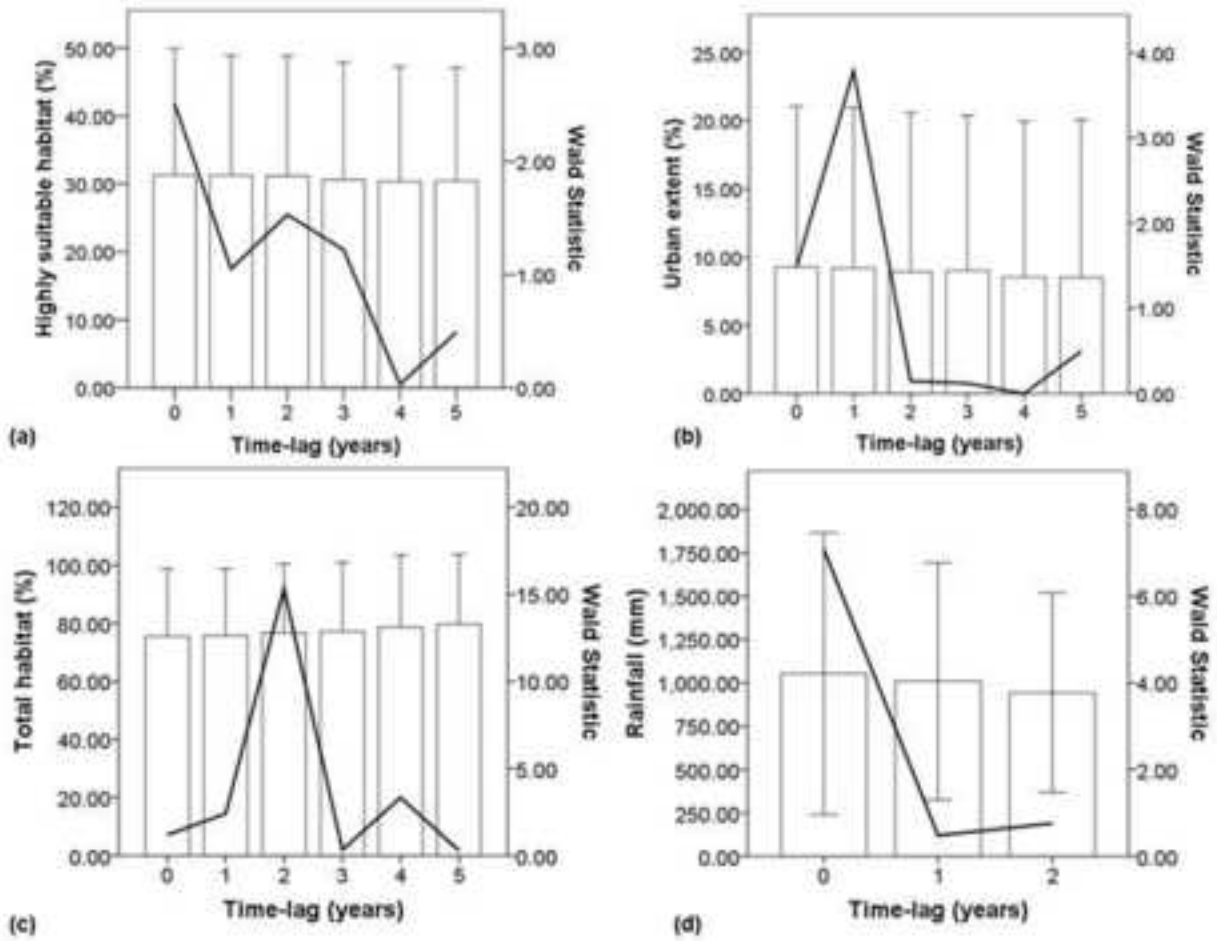

Figure 6

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