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Sub-optimal study design has major impacts on landscape-scale inference

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ABSTRACT

Landscape-scale processes (e.g. habitat loss) are major drivers of the global biodiversity crisis, but the complexity and size of landscapes makes study design at this scale difficult. However, the impact of statistical problems associated with sub-optimal study design on inferences drawn from landscape-scale studies is poorly understood. Here, we examine how three common statistical 'pitfalls' associated with sub-optimal study design – (1) using landscapes that overlap in space; (2) using only a portion of the potential range of the landscape predictor variable(s) of interest; (3) failing to account for correlations among landscape predictor variables – affect the inferred relationships between the abundances of six species of anurans and the amount of forest in the landscape using a large ($n = 1141$) empirical dataset from Wisconsin and Michigan, USA. We show that sub-optimal study design alone can be sufficient to cause a switch in the sign of the inferred relationship between a species response and landscape structure, and that using only a portion of the potential range of a predictor variable, and correlations between predictor variables, are particularly likely to affect inferences. Our results also provide the first evidence of a non-monotonic relationship between forest amount and gray treefrog abundance, and suggest that inconsistencies in the literature about the inferred relationships between anuran presence/abundance and forest amount in the Great Lakes basin are likely largely due to sampling design issues. Increased attention to study design is therefore necessary for the development of robust generalizations in landscape ecology.

1. Introduction

Over the past 20 years, landscape ecology has led to major strides in understanding the impacts of landscape-scale human activities on biodiversity (Turner, 2005). However, the complexity and size of landscapes means that study design at this scale is challenging (McGarigal and Cushman, 2002), making it difficult for investigators to avoid common statistical 'pitfalls' associated with sub-optimal study design. Spatial autocorrelation (Legendre, 1993), lack of replication or pseudoreplication (Hulbert, 1984), and multicollinearity of predictor variables (e.g. Graham, 2003) are statistical pitfalls that are problematic throughout ecology, while a failure to conduct a study at a scale (spatial extent) appropriate for the species and process being studied (Wiens, 1989; Holland et al., 2004) is an additional problem particularly relevant to landscape ecology. Finally, logistical restrictions in the choice of sampling units mean that variation in landscape-scale predictor variables of interest is often low relative to the potential range of the predictor (Brennan et al., 2002).

It is likely that sub-optimal study design has a major impact on inferences drawn from landscape ecological studies. McGarigal and Cushman (2002) reviewed the literature on empirical studies on landscape fragmentation and concluded that sub-optimal study design was a major contributor to the lack of a consensus within this field. However, the lack of consensus on the effects of habitat fragmentation may also be due to the large number of questions that can be asked about this subject and the many definitions of "fragmentation" in the literature (Fahrig, 2003). Thus, the question remains: to what extent are the conclusions of empirical studies in landscape ecology compromised by sub-optimal study design?

Here, we provide the first empirical examination of how different elements of sampling design can affect inferred relationships between landscape structure and species responses. As a test case, we evaluate the effects of three major statistical pitfalls on the inferred relationships between the amount of forest in the landscape and abundances of six species of anurans, using a large empirical dataset from the Great Lakes basin. The three pitfalls we consider are: (1) using landscapes that overlap in space (non-independence; pseudoreplication); (2) using only a portion of the potential range of the landscape predictor variable(s) of interest; and (3) failing to

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account for correlations among landscape predictor variables. We use real ecological data rather than a simulated dataset in this study, because our goal is to determine whether sub-optimal study design is likely to lead to large errors in inferences in actual field studies. We chose these three pitfalls because these relate to three of the key aspects of experimental design in landscape ecology as identified by Brennan et al. (2002) and McGarigal and Cushman (2002).

Overlapping landscapes are a form of pseudoreplication (Hulbert, 1984) because values of predictor variables from nearly the same landscapes are used as multiple observations in the dataset; the degree of pseudoreplication depends on the degree of overlap between landscapes. Pseudoreplication will result in non-independence of residuals, thus increasing the likelihood of making Type I errors by causing a systematic underestimation of confidence intervals (Legendre, 1993). Overlapping landscapes can also lead to lower variation in the predictor variable (Brennan et al., 2002), thus reducing the statistical power to detect an effect.

Overlapping landscapes are relatively common in landscape ecological studies due to logistic constraints. For example, Gibbs et al. (2005) examined the effects of (among other things) land cover change at up to 10 km radii from sampling points on population transitions in anuran populations in upstate New York by re-sampling sites in 2001–2002 that were originally surveyed 1973–1980. As the goal of this analysis was a historical comparison, site selection was constrained by the 1973–1980 survey. Sites in this first survey were clustered into short survey routes, so many sites in this analysis are located less than 20 km apart (the minimum distance required to ensure fully non-overlapping landscapes at the scale of Gibbs et al.'s analyses).

The chance of detecting an effect of a predictor will also be greatly reduced if only a portion of its potential range is considered (Brennan et al., 2002). If there is a non-monotonic effect of a predictor, then a limited range of predictor variables could additionally lead to contradictory findings, as the slope of the relationship between the response and the predictor will vary depending on the range of the predictor value.

Correlations among predictors are a problem throughout ecology (e.g. Freckleton, 2002; Graham, 2003), but particularly so for landscape ecology (McGarigal and Cushman, 2002; Smith et al., 2009). High correlations among predictors mean it is impossible to know which of the related predictors are in fact responsible for a given effect on a species, and can lead to erroneous inferences.

To control for correlations, investigators often use multiple regression models. This reduces the likelihood of making incorrect inferences, but can still lead to the erroneous conclusion that there is no effect of a predictor, because variation shared between predictors (where they co-vary) is not included in the estimates of the effect of each individual predictor, reducing statistical power. In addition, multicollinearity in predictors can still lead to inaccurate model parameterization even when a multiple regression is used (Graham, 2003).

We do not consider the other three important issues in experimental design in landscape ecology identified by Brennan et al. (2002) and McGarigal and Cushman (2002) here. These three issues are: (1) a failure to account for large-scale gradients in environmental variables (which can lead to problems of spatial autocorrelation even if landscapes are non-overlapping (Schooley, 2006)); (2) a failure to select the appropriate landscape extent for the study; and (3) small samples sizes. Preliminary analyses showed that large-scale environmental gradients have little effect on the relationship between anuran abundance and forest amount in our dataset, so we were unable to test the effects of this particular 'pitfall' here. Similarly, we were restricted to landscape extents of 500–5000 m radii because (1) the response variable was the number of anurans calling within hearing distance (approximately 500 m) of the survey site (Mossman et al., 1998) and (2) selecting non-overlapping landscapes at extents larger than 5000 m would have severely restricted site selection. The 500–5000 m range of radii corresponds to scale at which anurans are generally thought to respond to the amount of forest in the landscape (Cushman, 2006), but covers only one order of magnitude; Holland et al. (2004) showed shifts in the sign of the relationship between the abundance of a beetle and the amount of forest in the landscape when the latter was measured over 20–2000 m, i.e., two orders of magnitude. Preliminary analyses (Table A1 in the Appendix) confirmed that the scale at which the amount of forest cover in the landscape was measured, within 500–5000 m radius, had little effect on the relationships between anuran relative abundance and forest amount in our data set, so we were unable to test the effects of this 'pitfall'. Finally, we did not test the effect of sample size since, in isolation, reduced sample size simply leads to a loss of statistical power.

The relationships between forest cover and anurans in the Great Lakes basin are a particularly suitable choice for this study because for six anuran species there are 13 published landscape-scale studies in this region relating forest amount to anuran abundance. For two of these species (gray treefrog and American toad) the various studies give contradictory results, and for two other species (leopard frog and green frog) 'no effect' is the most common finding (Table 1). Are these differences and lack of effects real, or are they likely artefacts of study design?

2. Materials and methods

2.1. Data sources

For the response variables, we used relative anuran abundance data at 1141 survey sites (minimum distance between sites is 188 m; maximum distance is 839,200 m) from two large-scale volunteer-based anuran monitoring programs – the Wisconsin Frog and Toad Survey and the Michigan Frog and Toad Survey (Fig. 1). The two surveys use a very similar and well-established protocol (Mossman et al., 1998). Trained volunteers conduct nocturnal call surveys under suitable weather conditions (warm humid nights with little wind) at or near wetlands (10 sites per route) three times a year (early spring, late spring and early summer). Each call survey lasts a minimum of 3 min in Michigan and 5 min in Wisconsin, with up to 10 min allowed in both cases, so the observer can be confident that all calls were recorded. The estimated relative abundance of anurans is then assigned to one of four classes: 0 – not present; 1 – present, few individuals, no overlap among calls, individuals can be counted; 2 – several individuals, some overlap

Table 1
Summary of the results of 13 studies examining the effects of the amount of forest cover in surrounding landscapes on the presence or abundance of six anuran species in focal ponds/wetlands in the centre of each landscape. The superscript numbers next to each species indicate which species are covered by each reference.

Species	Summary of forest effects		
	Positive	Negative	No effect
Rana sylvatica (wood frog)[a]	8	0	3
Pseudacris crucifer (spring peeper)[b]	8	0	1
Rana pipiens (northern leopard frog)[c]	0	3	6
Bufo americanus (American toad)[d]	2	5	4
Hyla versicolor (gray treefrog)[e]	3	1	7
Rana clamitans (green frog)[f]	3	0	4

[a,c,d,e]Lehtinen et al. (1999), [a,c,d,e,f]Findlay et al. (2001), [a,b,c,d,f]Guerry and Hunter (2002), [a,b,c,d,e,f,]Houlahan and Findlay (2003), [a,b,c,d,e,f]Trenham et al. (2003), [a]Homan et al. (2004), [b,d,e]Knutson et al. (2004), [b,d,e,f]Price et al. (2004), [a]Porej et al. (2004), [a,b,c,d]Gibbs et al. (2005), [a,b,d,e,f]Herrmann et al. (2005), [a,b,c,d,e,f]Gagné and Fahrig (2007), [a,b,c,d,e,f,]Eigenbrod et al. (2008).