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Frog occupancy of polluted wetlands in urban landscapes

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ARTICLE IMPACT STATEMENT

That contaminated urban wetlands are readily occupied by frogs has implications for conservation.

ABSTRACT

Global urban sprawl and the rising popularity of water-sensitive urban design (WSUD) has led to a surge in the number of wetlands constructed to collect and treat stormwater run-off in cities around the world. However, contaminants such as heavy metals and pesticides present in stormwater adversely affect the survival, growth and reproduction of animals inhabiting these wetlands. A key question is whether wildlife can identify and avoid highly-polluted wetlands. We investigated this question using pond-breeding frogs across 67 urban wetlands in Melbourne, Australia, to determine if frogs are attempting to breed in wetlands that affect the fitness of their offspring. We found little evidence for any important relationships between species richness and the concentration of any of the contaminants assessed, and frogs were present and calling at even the most polluted wetlands. The proportion of fringing vegetation at a wetland had the greatest positive influence on the number of frog species present and the probability of occurrence of individual species, indicating that frogs inhabit wetlands with abundant vegetation, regardless of their pollution status. These wetlands contained contaminant levels similar to urban wetlands around the world, and at levels which reduce larval amphibian survival. These results are, thus, likely generalizable to other areas, suggesting that urban managers could inadvertently be creating ecological traps in countless cities, with significant conservation implications. Wetlands are important tools for the management of urban stormwater run-off, but we need to ensure their construction does not facilitate declines in wetland-dependent urban wildlife.

INTRODUCTION

More than half the world's population lives in urban areas (WHO 2017). As cities expand, the requirement for land, and safe, reliable and sustainable sources of water are changing the nature and distribution of wetlands within urban landscapes (Kentula et al. 2004). A rise in water-sensitive urban design (WSUD), for example, has seen wetlands constructed in cities around the world to intercept stormwater and the pollutants it carries (Kentula et al. 2004).

With tens of thousands of these wetlands built in residential, commercial and industrial areas (Tixier et al. 2011), they have become a common feature of urban landscapes.

While stormwater wetlands are inhabited by a variety of animals (Brand & Snodgrass 2010; Le Viol et al. 2012; Hassall & Anderson 2015), their ecological benefits are unclear because they progressively accumulate toxic contaminants such as heavy metals and pesticides that can adversely affect fitness of animals (i.e. survival, reproduction, growth; Bishop et al. 2000; Tixier et al. 2011; Egea - Serrano et al. 2012). Global analyses indicate that while the species richness and population densities within urban wetlands can be comparable to those in natural wetlands, the fitness of individuals is often compromised (Sievers et al. 2018a).

Amphibians are susceptible to anthropogenic alterations to wetlands (Sievers et al. 2018a), and are suffering high rates of extinction (Monastersky 2014). Many species are considered sensitive to the contaminants that urban wetlands sequester (Hamer & McDonnell 2008; Wake & Vredenburg 2008), and exhibit reduced survival and impaired growth following exposure (Lefcort et al. 1998; Snodgrass et al. 2008; Gallagher et al. 2014). Contaminants also cause physical abnormalities (Reeves et al. 2010; Ruiz et al. 2010) and affect behaviour (Shuman-Goodier & Propper 2016; Sievers et al. 2018b), with consequences for fitness related end-points. For example, copper increased tadpole activity levels, and combined with elevated temperatures, increased the frequency of predatory attacks from larval dragonflies (Hayden et al. 2015). Despite these impacts, amphibians are frequently found occupying and breeding in urban wetlands around the world (e.g. Brand & Snodgrass 2010; Scheffers et al. 2013).

Most studies of urban amphibian communities focus on how vegetation structure (Scheffers & Paszkowski 2013), hydroperiod (Hamer & Parris 2011), water quality (Ramesh et al. 2017), predators (Hamer & Parris 2013) and wetland size (Scheffers & Paszkowski 2013) influence species richness and occupancy. A recent survey found that amphibian richness and, for some species, occurrence was negatively influenced by the concentration of heavy metals in wetland sediments (Ficken & Byrne 2013).

Contaminants might influence occupancy if frogs avoid poor-quality habitats, or if population persistence is impaired at inappropriate sites.

Although frogs can modify their oviposition behaviour to avoid pesticides (Takahashi 2007; Vonesh & Buck 2007), when all available wetlands suffer moderate to high levels of pollution, such as in highly urbanized areas, frogs may be less capable of discerning wetland quality. The ecological impact of urban wetlands will be exacerbated when frogs mistakenly live and breed in wetlands containing harmful levels of contaminants, causing these to function as ecological traps (i.e. habitats that animals find equally or more attractive than other available habitat, despite animals that prefer that habitat having lower fitness than if they selected an available alternative; Robertson & Hutto 2006). We have recently shown that urban wetlands can act as ecological traps for spotted marsh frogs (*Limnodynastes tasmaniensis*), with some individuals choosing to breed in wetlands with metal and pesticide levels that reduced tadpole survival and interfered with anti-predator behaviours (Sievers et al. 2018c).

Given the increasing prevalence of stormwater wetlands around the world, and growing evidence the pollutants they accumulate impact larval amphibians, it is pertinent to assess how frequently frogs may be attempting to breed in polluted wetlands. We used 67 urban wetlands throughout the Greater Melbourne Region, Australia as a case study to explore relationships between species richness and occupancy and a range of habitat- and landscape-scale characteristics, with a focus on the concentration of metals and pesticides within sediments. As with many other cities, constructed wetlands are a common stormwater treatment option in Melbourne, and these habitats show considerable spatial variability in their contaminant loads (Marshall et al. 2016; Sharley et al. 2017). If frogs inhabit and attempt to breed at urban wetlands irrespective of contaminant levels, managers could be unintentionally creating ecological traps (Hale et al. 2015a), which have the potential to threaten conservation efforts.

METHODS

Amphibian surveys

We conducted five replicate nocturnal call surveys (runs) at 67 urban wetlands within the Greater Melbourne Region, Australia (Appendix S1) over two breeding seasons for spring/summer breeders (September 2015–March 2016 and October 2016–February 2017). The order in which we surveyed wetlands was randomized within each run, but stratified based on geographic location (i.e. clusters of sites). Following Parris et al. (1999), two people listened for advertisement calls at each wetland for 5 min, followed by a physical search of the surrounding banks and vegetation using headlamps for a minimum of 10 min.

Pollution levels

As part of a monitoring program conducted during the first year of surveys, the Centre for Aquatic Pollution Identification and Management (CAPIM; www.capim.com) measured the concentrations of 24 heavy metals and nine pesticides in urban wetland sediments (Table 1; For detailed descriptions of sediment collection and analysis see Marshall et al. 2016; Sharley et al. 2017). From these data, we calculated a heavy-metal index based on benchmark values set by The Australian and New Zealand Environment Conservation Council (ANZECC & ARMCANZ 2000; Ficken & Byrne 2013). We also compared levels of heavy metals within the sediments of our study wetlands with those from Snodgrass et al (2008) and Sievers et al (2018c), which caused significant mortality of amphibian larvae (Table 1). We chose to use an index in our models as the concentrations of most metals were highly correlated with each other.

We also examined the effects of bifenthrin, diuron and trifloxystrobin within wetland sediments as model variables. These three pesticides are relatively common in urban wetlands around the world (Brown et al. 2010; Mimbs IV et al. 2016), and based on limits of reporting, were detected at a greater proportion of our study wetlands (bifenthrin: 82%, diuron: 88%, trifloxystrobin: 27%) than the other six pesticides. Bifenthrin is a synthetic pyrethroid used in residential developments (e.g. for termite control); diuron is a photosynthesis-inhibiting herbicide used primarily for weed control; and trifloxystrobin is a strobilurin fungicide commonly used to control mildews and brown patch of turf grass (Marshall et al. 2016; Allinson et al. 2017). These pesticides can adversely affect fish and invertebrates at low concentrations (Nebeker & Schuytema 1998; Amweg et al. 2005; Zhu et al. 2015), but little is known about their impact on frogs (although see Belden et al. 2010). Given their considerable toxicity to algae, plants, and other aquatic animals, however, it is likely that frogs could experience direct (e.g. mortality, reduced growth) and indirect (e.g. reduced food availability, immunological impacts and endocrine disruption) effects across one or more life stages.

Habitat and landscape characteristics

We calculated the wetland surface area using the spatial analysis tools on www.nearmap.com. We estimated the proportion of the wetland surface area covered by fringing and emergent vegetation during the first round of surveys and these two proportions were averaged to provide a vegetation index ranging from 0 to 1. During each survey, we recorded water temperature, EC and pH.

We also included the underlying catchment geology (sedimentary or basalt), the area of wetland within a 500m radius of each survey wetland (using GIS software), and the proportion of the sub-catchment classified as urban and as road (Table 2; see Sharley et al 2017 for the calculation and specific definitions of these variables). Area-informed metrics, such as the amount of available habitat within a given radius of a wetland as used here, can predict the likelihood of immigration (Bender et al. 2003). Although information on the movement ecology of our species is limited, we assumed 500 m to be an achievable dispersal distance (Sinsch 2014).

Statistical analysis

We assessed potential explanatory variables for collinearity and only included uncorrelated variables ($r < 0.4$) in the same model (Appendix S2). We examined the influence of eleven habitat- and landscape-scale variables on the number of frog species detected as adults (i.e. species richness) using Bayesian Poisson regression modeling with uninformative priors for the intercept term ($a \sim$ dnorm[0, 1.0 × 10^{-6}]) and regression coefficients ($\beta_j \sim$ dnorm[0, 1.0 × 10^{-6}], where j is an explanatory variable) in OpenBUGS (Lunn et al. 2000; Spiegelhalter et al. 2007). We used logistic regression with the same explanatory variables to identify the influence of the variables on the occurrence of the five most common species (runs were combined to account for detectability; we considered five surveys to give a high level of confidence of detecting a species if it was present at a site; Canessa et al. 2012).

We transformed the heavy metal index (ln[x + 1]), wetland area, and water and pesticide variables (ln[x]) prior to analysis to meet statistical assumptions. We included one null model (M1; constant only), and to aid convergence we centered all explanatory variables by subtracting their means. We used OpenBUGS to generate 100,000 samples from the posterior distribution of 12 models after discarding an initial ''burn-in'' of 10,000 samples. We obtained 95% Bayesian credible intervals (BCI) from the 2.5th and 97.5th percentiles of the distribution. We assessed the relative fit of the models against model complexity using the deviance information criterion (DIC) and considered the best models to be those with a ΔDIC < 2 (Spiegelhalter et al. 2007). We assessed the relative importance of the explanatory variables by calculating multiplicative effect sizes and 95% BCIs (Appendix S3; Hamer & Parris 2011; Hamer et al. 2012).

For species that occurred at fewer than 20% of wetlands ('rare species'), we conducted principal component analysis to compare sites occupied by a rare species to sites that weren't (using all eleven model variables), and to make the same comparison for a subset of the variables (proportion of vegetation in the emergent and fringing zones, the heavy-metal index and species richness).

Finally, we used canonical correspondence analysis (CCA) to assess the relative importance of the independent variables used previously on community composition. We excluded rare species and six unoccupied wetlands. CCA was performed using the vegan package (Oksanen et al. 2007) in R 3.2.2 (R Development Core Team 2015).

Ethics and collection permits

This research was approved by the University of Melbourne Animal Ethics Committee (permit no. 1513577.1) and conducted under DELWP research permit no. 10007589.

RESULTS

Amphibian surveys

We detected nine species of frogs during field surveys (Table 3). *Crinia signifera* (common eastern froglet) was the most common species, detected in 58% of wetlands, while *Litoria peronii* (Peron's tree frog), *Litoria raniformis* (growling grass frog), *L. fallax* (eastern dwarf tree frog) *and L. verreauxii* (whistling tree frog) were detected in fewer than 20% of wetlands (Table 3). We detected an average of 2.5 species per wetland (SE: 0.2; range: 0–6), with six wetlands unoccupied.

Species richness

The best-supported model of species richness included only the proportion of the emergent and fringing vegetation at a wetland (Model 2, Table 4). Model 3, which also included the heavy-metal index (ΔDIC = 1.5), and Model 4, which also included the habitat area in a 500m radius (ΔDIC = 1.2) both had some support. The proportion of emergent and fringing vegetation had a substantial positive effect, with the number of species at the most vegetated wetland 2.1–2.4 times higher than the number of species at the least vegetated (Fig. 1). There was less evidence that heavy metals affected species richness; although multiplicative effect sizes were consistently negative, means were close to zero with considerable uncertainty surrounding estimates (Fig. 1). Similarly, although there was some evidence for positive effects of habitat area in a 500-m radius, urbanization, geology and trifloxystrobin concentration, BCIs overlapped zero (Fig. 1). There were also no clear relationships between species richness and the concentration of any of the specific metals or pesticides (Fig. 2), and seven of the nine frog species were present at wetlands with metal concentrations above probable effect concentrations identified by consensus-based sediment guidelines (MacDonald et al. 2000). These guidelines are based on biological effects on macroinvertebrates and microcrustaceans, and while these consensus-based freshwater quality guidelines provide an interesting reference, they should be interpreted with caution.

Species occurrence

Crinia signifera (common eastern froglet)

Common eastern froglets were more prevalent at wetlands with a high cover of emergent and fringing vegetation, with the probability of occurrence increasing by 66–82% (Fig. 3; percentages here and below represent the change in occurrence probability between the wetlands with the lowest and highest value for that variable, while holding all other variables constant). We also found evidence of an important positive effect the amount of wetland habitat in a 500-m radius (57–78%), and the underlying catchment geology strongly influenced occurrence, with basalt wetlands more likely to support common eastern froglets (Table 4; Fig. 3).

Limnodynastes tasmaniensis (spotted marsh frog)

The best-supported model of the occurrence of this species included the vegetation cover at a wetland and the area of wetland habitat in a 500-m radius as the explanatory variables (Table 4). Spotted marsh frogs were more prevalent at wetlands with high cover of emergent and fringing vegetation (55–67%), while the influence of wetland habitat within 500 m was less certain, but consistently positive (6–33%; Fig. 3).

Limnodynastes dumerilii (eastern banjo frog)

Models including only landscape-scale variables had the most support (Table 4). The eastern banjo frog was more likely to be present at basalt wetlands in highly urbanized sub-catchments (82–85%). The probability of eastern banjo frogs occurring at a wetland was also negatively associated with the area of wetland habitat in a 500m radius (Fig. 3). Although the prevalence of roads was included in the best supported model, it had no influence on occurrence.

Limnodynastes peronii (striped marsh frog)

DIC values were very similar across most models, indicating support for many different models (Table 4). Most notably, there was evidence that wetlands in highly urbanized sub-catchments with a high prevalence of roads were more likely to contain striped marsh frogs (46–51% and 50%, respectively; Fig. 3). There was also some evidence for a positive effect of vegetation and heavy metals on the occurrence of striped marsh frogs, albeit with considerable uncertainty (Fig. 3).

Litoria ewingii (southern brown tree frog)

Models including only landscape-scale variables had the most support (Table 4). Of these, the underlying catchment geology and the prevalence of roads had a strong influence on occurrence, with southern brown tree frogs more often inhabiting sedimentary wetlands with fewer roads (75–76% decrease; Fig. 3). We also found some evidence for a positive effect of the area of wetland habitat in a 500m radius (25–58%; Fig. 3).

Rare species

We found no evidence that the sites occupied by rare species in the study area (Peron's tree frog, growling grass frog and the whistling tree frog) had different habitat conditions based on our models (Appendix S4; S5). Although rare in the study area, we did not include the eastern dwarf tree frog as it is not native to Melbourne and is currently invading the study area.

Community responses to urban environmental gradients

The Canonical Correspondence Analysis (CCA) revealed considerable separation of species, with only the common eastern froglet and spotted marsh frog close together in the plot (Fig. 4). The first CCA axis explained 49.6% of the variability in the species occurrence data and the second axis explained 32.7%. The striped marsh frog and the eastern banjo frog were positively associated with urbanisation and the prevalence of roads, and negatively associated with the size of wetlands, the total area of habitat around wetlands and conductivity (Fig. 4). The spotted marsh frog and the common eastern froglet were positively associated with vegetation, heavy metal concentrations, the size of wetlands, and the total area of habitat around wetlands, and negatively associated with urbanisation and pesticide levels (Fig. 4). The occurrence of the southern brown tree frog was mostly influenced by geology (Fig. 4).

DISCUSSION

More than 90% of surveyed wetlands were inhabited, with vegetation cover strongly influencing both species richness and the occurrence of most species. Although there was some evidence that species richness was negatively correlated with the level of heavy metals and positively with the amount of wetland within a 500-m radius, these relationships were less clear. The proportion of the catchment classified as urban, the underlying catchment geology, the concentration of pesticides within sediments, electrical conductivity, wetland size, and the prevalence of roads had little effect, with considerable uncertainty around model estimates.

The role of pollution in driving habitat use

For the species and pollutant levels recorded in this survey area, our results suggest that urban wetlands will be readily inhabited irrespective of the concentration of heavy metals and pesticides present. Although previous studies have found amphibian richness to be influenced by heavy metals (e.g. Ficken & Byrne 2013) and pesticides (e.g. Gibbs et al. 2009), these studies typically compare wetlands with highly contrasting concentrations of contaminants. In our study, where all wetlands were in human-dominated areas and had some degree of contamination, we did not observe such strong trends, suggesting that while richness may be higher when contamination is very low (i.e. at natural wetlands), it plateaus once contamination reaches a certain level. Alternatively, there may be a wider potential pool of species to begin with in less-urban areas (such as the rural areas surveyed in Ficken & Byrne 2013).

The heavy-metal index and the pesticides were not included in any of the most supported models. We did find some evidence suggesting the occurrence of the striped marsh and eastern banjo frogs were influenced by heavy metals, with the former more prevalent and the latter less prevalent at wetlands with higher metal concentrations. Intraspecific differences in tadpole behaviour may mean tadpoles of the striped marsh frog spend less time in contact with sediments and are thus less susceptible to contaminants, but few inter-specific differences are known about larvae from this genus. Alternatively, competitive exclusion may be occurring, however, plots of the probability of species occurrence based on the presence or absence of other species show that competitive exclusion is unlikely to be a significant driver (Appendix S6).

Primarily relying on call surveys may have even underestimated the occupation of contaminated wetlands if pollution impacts vocalisations. Indirect effects could occur through reduced food availability that increases the marginal energetic cost of vocalising (such as in birds; Golabek et al. 2012), while direct effects could occur through impacts to physiology and behaviour *via* absorbed ions and ingestion. Cadmium, for example, adversely affects advertisement call duration and rate in black-spotted frogs (*Pelophylax nigromaculata*) (Huang et al. 2015), and the fungicide vinclozolin reduces calling activity in South African clawed frogs (*Xenopus laevis*) (Hoffmann & Kloas 2010). If similar effects were prevalent, individuals present at highly contaminated wetlands could be assumed to be absent due to reduced detection probability, strengthening our conclusion that frogs are selecting wetlands irrespective of contaminant levels.

Habitat and landscape characteristics

Fringing and emergent vegetation provides amphibians with shelter and protection, and a place to feed, call and breed (Tarr & Babbitt 2002; Egan & Paton 2004) and is often positively associated with the occurrence of amphibians (Hamer et al. 2012; Kruger et al. 2015). Our results are largely consistent with these findings, with species richness greatest at the most vegetated wetlands. It is also possible that vegetation has other impacts on habitat quality. For example, vegetation can mitigate the toxic effects of pesticides through their removal by sorption or alkaline hydrolysis (Brogan III & Relyea 2017). Therefore, it is possible that the relationship between vegetation and amphibian assemblages is also related to vegetation lowering the concentrations of other contaminants not measured here, making the wetland more habitable, and consequently increasing the probability of occurrence. More work examining the interactions between vegetation and different pollutants, and the consequences this has for frogs would allow this possibility to be further examined.

Although the size of wetlands may influence species richness (via well-established species-area relationships; Hanski 1994; Parris 2006), we found no substantial effect of wetland area on species richness or occurrence. Unlike natural wetlands where wetland size may be a proxy for permanence or ephemerality – which may actually be the driver of increased richness at larger wetlands – urban wetlands fed by stormwater are often permanent regardless of size, and other studies have similarly found no effect of wetland size on amphibians in urban areas (e.g. Kruger et al. 2015). On the other hand, the total wetland area within a 500-m radius was important, similar to results from other urban wetland systems (Scheffers & Paszkowski 2013). More habitat typically means greater connectivity and, thus, a greater likelihood of colonization (Bender et al. 2003).

In general, landscape-scale variables exerted a substantial influence on species occurrence. However, the strong effect of catchment geology on southern brown tree frogs and common eastern froglets likely reflects the matching of species ranges with regional differences in geology rather than a causal relationship, as southern brown tree frogs were detected almost exclusively in eastern sites that have a sedimentary geology, while common eastern froglets were detected almost exclusively in western sites that have a basalt geology. Wetlands with higher levels of surrounding urban cover were more likely to harbor striped marsh and eastern banjo frogs, two species that commonly inhabit urban wetlands and that may be well adapted to urban environments (Hamer & Parris 2011; Ficken & Byrne 2013).

Ecological and conservation implications

Urban sprawl and the rising popularity of water-sensitive urban design (WSUD) has led to the global proliferation of wetlands designed and constructed to intercept stormwater run-off. Frogs occupied urban wetlands that commonly had sediment heavy-metal concentrations above published thresholds for likely biological effects (Fig. 2; MacDonald et al. 2000), and above those within sediments that impacted tadpole survival in other studies (Snodgrass et al. 2008; Sievers et al. 2018c). However, we did not consider a range of other contaminants that are common to urban wetlands (e.g. PAHs; Gallagher et al. 2011) and also known to impact amphibians (e.g. salts and PAHs; Snodgrass et al. 2008). Further, Snodgrass et al (2008) and Sievers et al (2018c) focused on only a limited number of species and identified considerable species-specific vulnerabilities. More work is needed to explore the effects of a wider range of contaminants on frogs. Nonetheless, the presence of frogs at wetlands along a strong pollution gradient here still suggests that some urban wetlands may function as sinks for some species (Pulliam 1988).

There is also a strong *prima facie* case that some urban wetlands are ecological traps when the results presented here are considered in conjunction with our previous research, which has shown that breeding adults oviposit in a range of habitats, including those where larval fitness is significantly reduced (Sievers et al. 2018c). The Greater Melbourne Region likely represents an ideal model system that replicates what is occurring in many other urban areas. For example, average concentrations of heavy-metals within stormwater pond sediments were higher in our wetlands for 5 of 7 metals measured in South Carolina (Crawford et al. 2010) and for 3 of 12 metals in Florida (Liebens 2001). Further, levels of the pesticides bifenthrin and permethrin were higher at our sites than in urban waterways in China (Li et al. 2011) and California (Weston et al. 2005), and for diuron in France (Datry et al. 2003). This suggests urban managers could be unintentionally creating ecological traps in hundreds or thousands of cities around the world.

How much of a lasting threat these wetlands pose to population persistence in urban landscapes depends on whether frogs have sufficient phenotypic plasticity or evolutionary potential to adapt to high pollution environments. Amphibians living near agricultural areas with high levels of pesticide exhibit greater tolerance (e.g. Cothran et al. 2013; Hua et al. 2015), and induced tolerance to a single insecticide can lead to cross-tolerance to other insecticides (Hua et al. 2014). The presence of a range of contaminants, often at sublethal levels, within stormwater wetlands may be sufficient to drive evolutionary resistance in exposed populations (Major et al. 2018). Along with phenotypic plasticity, is an important avenue for future research to consider the full impact of stormwater wetlands on amphibians.

Ultimately, urban managers should monitor contaminant loads within stormwater wetlands to identify those with continuously high levels based on published benchmark guidelines (e.g. ANZECC & ARMCANZ 2000), and make efforts to reduce either their occupation by wildlife or pollution input/levels. For example, emergent and fringing vegetation could be minimized or substituted for alternate vegetation (e.g. deep submerged), wetlands in areas known to produce high contaminant loads could be isolated, or runoff could be pre-treated before entering these wetlands. Wetlands are important tools for the management of urban stormwater run-off, but we need to ensure their construction does not facilitate declines in wetland-dependent urban wildlife.

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TABLES

Table 1. Minimum, maximum and mean (± SD) sediment quality variables for the 67 wetlands, and where possible, the low and high interim sediment quality guideline (ISQG) values from ANZECC and ARMCANZ (2000), and the concentrations of metals within wetland sediments from Snodgrass et al 2008 (Snodgrass I – Owings Mills; Snodgrass II – New Cut Road), Sievers et al 2018c (Sievers I – Chandler Rd; Sievers II – Cheltenham; Sievers III Ringwood Lake), and Gallagher et al (2011).

Contaminant	Min	Max	Mean ± SE	ISQG-Low	ISQG-High	Snod I	Snod II	Sievers I	Sievers II	Sievers III	Gallagher (Mean ± SD)
Heavy metals (mg kg-1 dry weight)											
Aluminium	6850	37100	17311 ± 691					16200	20000	9570	
Antimony	2.5*	17	4.2 ± 0.4	2	25			5	2.5*	9	
Arsenic	2.5*	47	7.9 ± 1.0	5	70	0.3	0.6	39	37	10	
Barium	40	430	130 ± 9					430	150	60	
Beryllium	0.5*	2	0.73 ± 0.04					0.5*	1	0.5*	
Cadmium	0.5*	11	0.77 ± 0.16	1.5	10	0.3	0.6	2	2	0.5*	
Chromium	12	121	40.5 ± 2.3	80	370	14.9	9.4	73	48	21	55.2 ± 8.1

Cobalt	2	22	10.7 ± 0.7					19	13	3	
Copper	11	1090	64 ± 16	65	270	13.7	7	299	113	40	46.6 ± 17.6
Iron	6360	36900	23859 ± 801					29300	28000	14300	
Lead	10	456	46.1 ± 8.6	50	220	8.4	10.6	176	283	48	24.7 ± 10.0
Manganese	36	496	154 ± 13					187	136	55	
Mercury	0.05*	2.9	0.13 ± 0.04	0.15	1			0.4	0.4	0.05*	
Molybdenum	1*	13	1.5 ± 0.2					13	2	1*	
Nickel	4	159	26.7 ± 2.6	21	52	14.2	4.3	159	36	10	63.0 ± 152.4
Silver	1*	6	1.07 ± 0.07	1	3.7			1*	1*	1*	
Strontium	7	151	25.6 ± 2.8					38	37	11	
Thallium	2.5*	2.5*						2.5*	2.5*	2.5*	
Tin	2.5*	48	7.3 ± 1.0					48	11	2.5	
Titanium	20	620	150.4 ± 14.2					430	370	90	
Vanadium	14	130	41.0 ± 2.2					47	58	27	
Zinc	23	3790	458 ± 82	200	410	56.5	62.7	3790	2390	597	189.1 ± 128.5
Pesticides (mg kg-1 dry weight)											
Bifenthrin	1*	330	39.5 ± 6.6					68.4	37.2	13	
Diethyltoluamide	1*	33	8.1 ± 0.9					2.5	1*	15	
Diuron	1.25*	5316	118 ± 78					20	22	91	

Fenamiphos	5*	69.9	11.5 ± 1.3	10	10	15
Permethrin	1.5*	930.5	26.6 ± 13.9	209	39.4	32.8
Prometryn	1*	30.8	8.1 ± 1.2	2.5	5	27
Pyrimethanil	0.5*	7.6	2.0 ± 0.2	7.6	1	5
Triclosan	5*	155	21.4 ± 3.3	155	80.8	60
Trifloxystrobin	1*	49	7.1 ± 1.1	2.5	2.5	5

Table 2. Minimum, maximum and mean (± SE) wetland and landscape-level attributes for the 67 wetlands.

Variable	Min	Max	Mean ± SE
Wetland attributes			
Area (m^2)	136	97282	10127 ± 2114
Perimeter (m)	55	4781	649 ± 102
Vegetation (prop)	0.025	0.925	58.9 ± 0.03
Closest road (m)	1	425	64.1 ± 8.0
pH	6.8	8.6	7.66 ± 0.04
Electrical conductance (µS)	87	3339	526 ± 71
Landscape-level characteristics			

Urban (proportion of sub-catchment)	0	1.00	0.67 ± 0.03
Road (proportion of sub-catchment)	0	0.18	0.03 ± 0.005
Habitat 500m* (m^2)	723	323207	38966 ± 8172

* Habitat 500m refers to the amount (m^2) of wetland habitat in a 500m radius surrounding each wetland.

Table 3. Frog species detected as adults during the study and the percentage of wetlands at which they were detected.

Family/species	Common name	% occupied
Hylidae		
Litoria ewingii	Southern brown tree frog	43.3%
Litoria peronii	Peron's tree frog	17.9%
Litoria raniformis	Growling grass frog	6.0%
Litoria fallax	Eastern dwarf tree frog	4.5%
Litoria verreauxii	Whistling tree frog	3.0%
Myobatrachidae		
Crinia signifera	Common eastern froglet	58.2%
Limnodynastes dumerilii	Eastern banjo frog	40.3%
Limnodynastes peronii	Striped marsh frog	40.3%
Limnodynastes tasmaniensis	Spotted marsh frog	34.3%

Table 4. Deviance information criterion (DIC) values for the 12 regression models of species richness (Poisson) and species occurrence (Logistic) at a wetland, with boldface values indicating models for which ΔDIC<2.

		Species richness		*Crinia signifera*		*Limnodynastes tasmaniensis*		*Limnodynastes dumerilii*		*Limnodynastes peronii*		*Litoria ewingii*	
Model	Variables	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC
1	Const.	241.9	3.6	93.1	10.3	88.2	2.3	92.4	8.4	**92.4**	**0.0**	93.7	35.0
2	Const. Veg	**238.3**	**0.0**	88.3	5.6	**85.9**	**0.0**	94.2	10.2	**93.5**	**1.1**	95.8	37.1
3	Const. Veg HM	**239.8**	**1.5**	90.3	7.6	88.0	2.0	95.0	11.0	**92.7**	**0.4**	96.7	38.0
4	Const. Veg Hab	**239.5**	**1.2**	**83.9**	**1.1**	**87.2**	**1.3**	94.1	10.1	95.5	3.1	96.9	38.2
5	Const. Veg HM Hab	241.1	2.8	86.0	3.2	89.4	3.4	94.4	10.4	94.9	2.5	98.1	39.4
6	Const. Veg HM Bifen Diu Trif	242.7	4.4	94.3	11.5	91.7	5.7	95.2	11.2	97.0	4.6	89.2	30.5
7	Co V H C	2	2.	9	9.	9	4.	9	9.	**9**	**1.**	9	3

	Const.	Veg	HM	Cond			41.1	8	2.4	6	0.0	0	3.5	5	**3.9**	**5**	8.4	9.7
8	Const.	Hab	Bifen	Diu	Trif		246.7	8.4	95.9	13.2	92.9	7.0	91.8	7.8	98.6	6.3	90.3	31.6
9	Const.	Urb	Hab	Geol	Road		245.9	7.6	92.6	9.8	96.3	10.3	**84.0**	**0.0**	94.9	2.6	**59.4**	**0.7**
10	Const.	Urb	Geol	Road			244.3	6.0	94.2	11.5	94.4	8.5	**84.9**	**0.9**	**92.8**	**0.4**	**58.7**	**0.0**
11	Const.	Veg	Area	Hab	Geol		241.4	3.1	**82.8**	**0.0**	91.3	5.3	95.3	11.3	99.4	7.0	63.3	4.6
12	Const.	Veg	HM	Hab	Urb	Area	242.4	4.1	90.1	7.3	93.4	7.4	**85.6**	**1.6**	96.4	4.0	102.3	43.6

Key to model variable abbreviations: Const, model constant; Veg, total proportion of aquatic vegetation; HM, sediment heavy metal concentration index (see Ficken and Byrne 2014; ln+1 transformed); Hab, area of wetland habitat within a 500m radius (ln-transformed); Bif, sediment concentration of the insecticide bifenthrin (ln-transformed); Diu, sediment concentration of the herbicide diuron (ln-transformed); Trif, sediment concentration of the fungicide trifloxystrobin (ln-transformed); Urb, proportion of the sub-catchment classified as urban; Road, proportion of the sub-catchment classified as road; Geology, sedimentary or basalt catchment geology ; Area, wetland area (ln-transformed), and; Cond, electrical conductivity (ln-transformed).

FIGURES LEGENDS

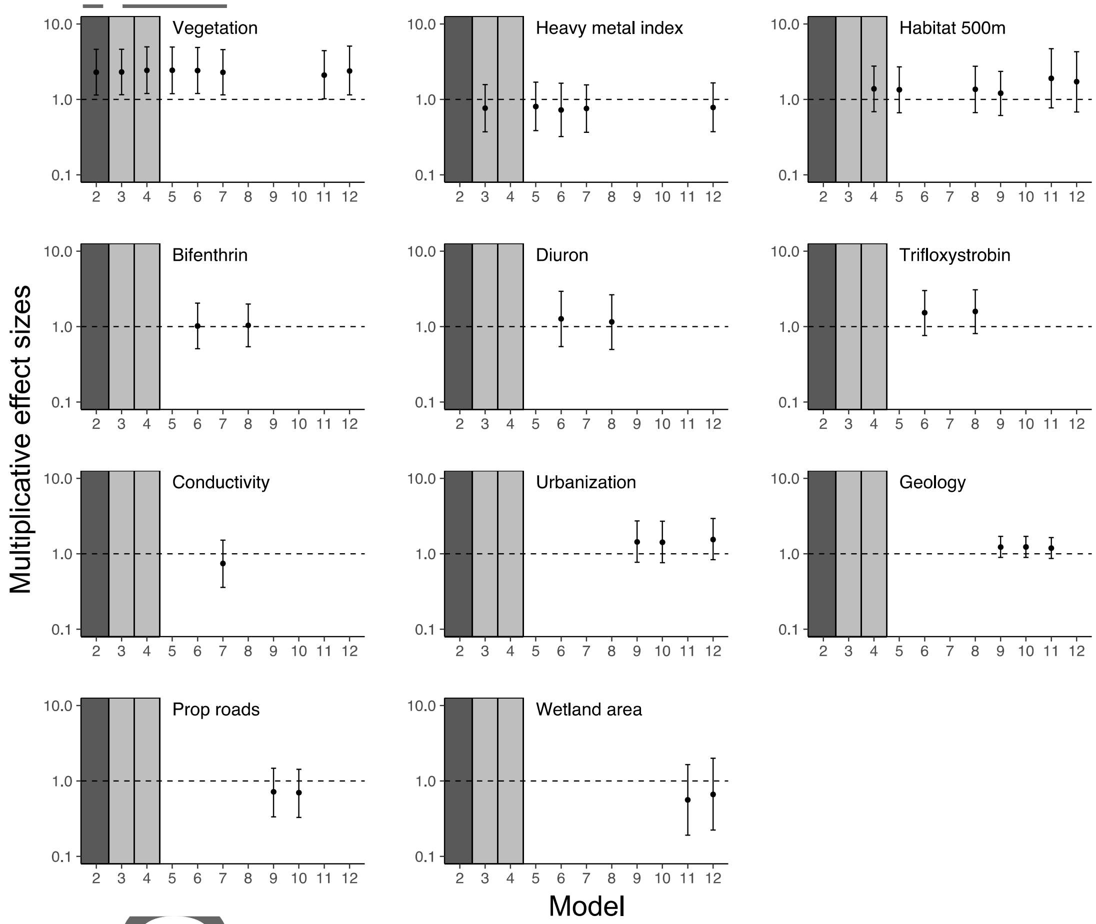

Figure 1. The multiplicative effect of the explanatory variables (mean and BCIs) on adult species richness at 67 wetlands. The dark grey band indicates the best-supported model (i.e. ΔDIC = 0), and the light grey band indicates models with some support (ΔDIC < 2). See Table 3 for model numbers and structure. Effect sizes > 1 indicate a positive effect of the explanatory variable on species richness while effect sizes < 1 indicate negative effects. Model 1 (constant) is not shown.

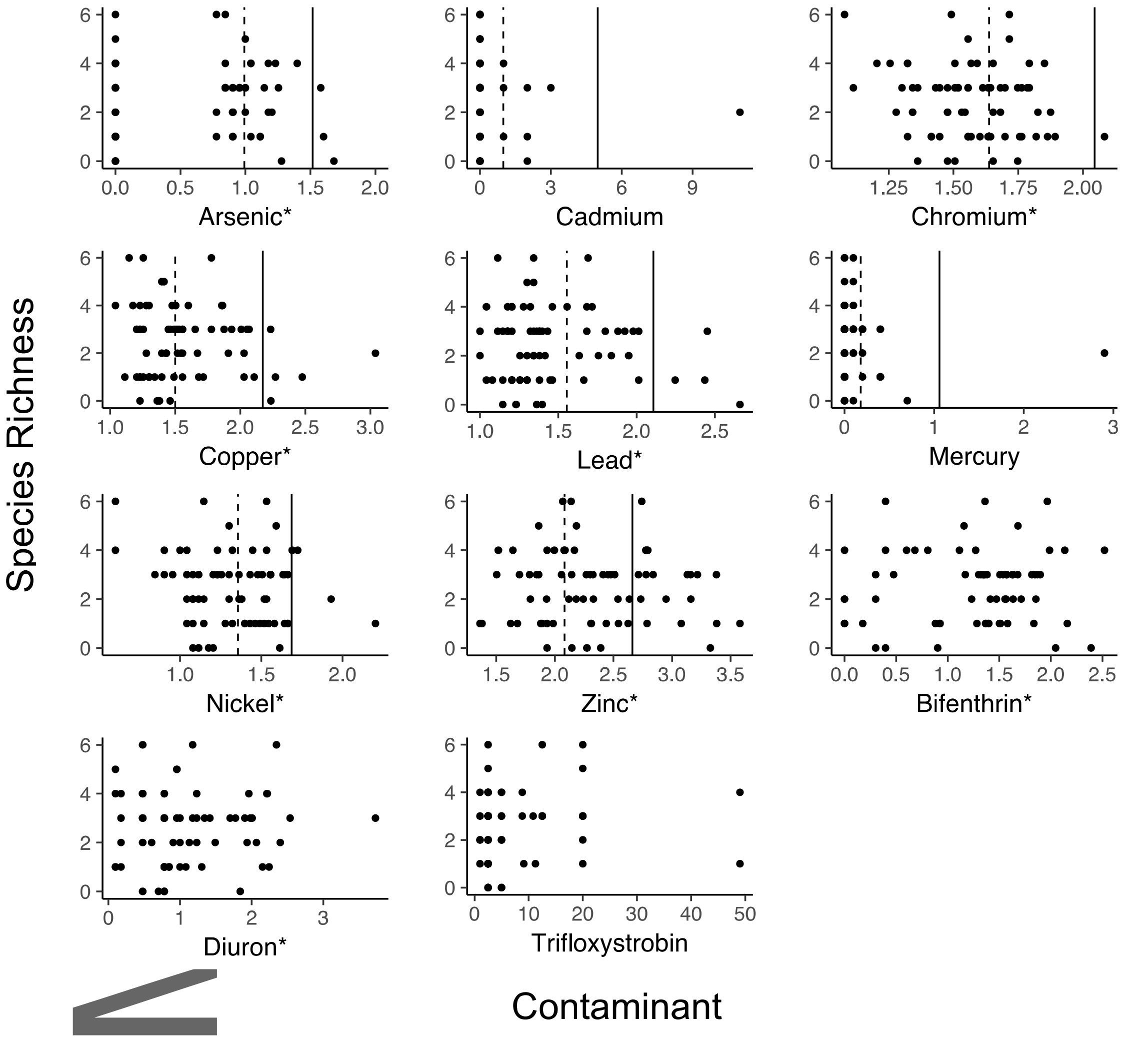

Figure 2. Frog species richness plotted against the concentration of heavy metals (mg/kg) and pesticides (mg/kg) in wetland sediments. Dashed lines represent the threshold effect concentration (below which harmful effects are unlikely to be observed) and solid lines represent the probable effect concentration (above which harmful effects are likely to be observed) based on MacDonald et al. (2000). Some contaminants are log-transformed to aid visualization (asterisks).

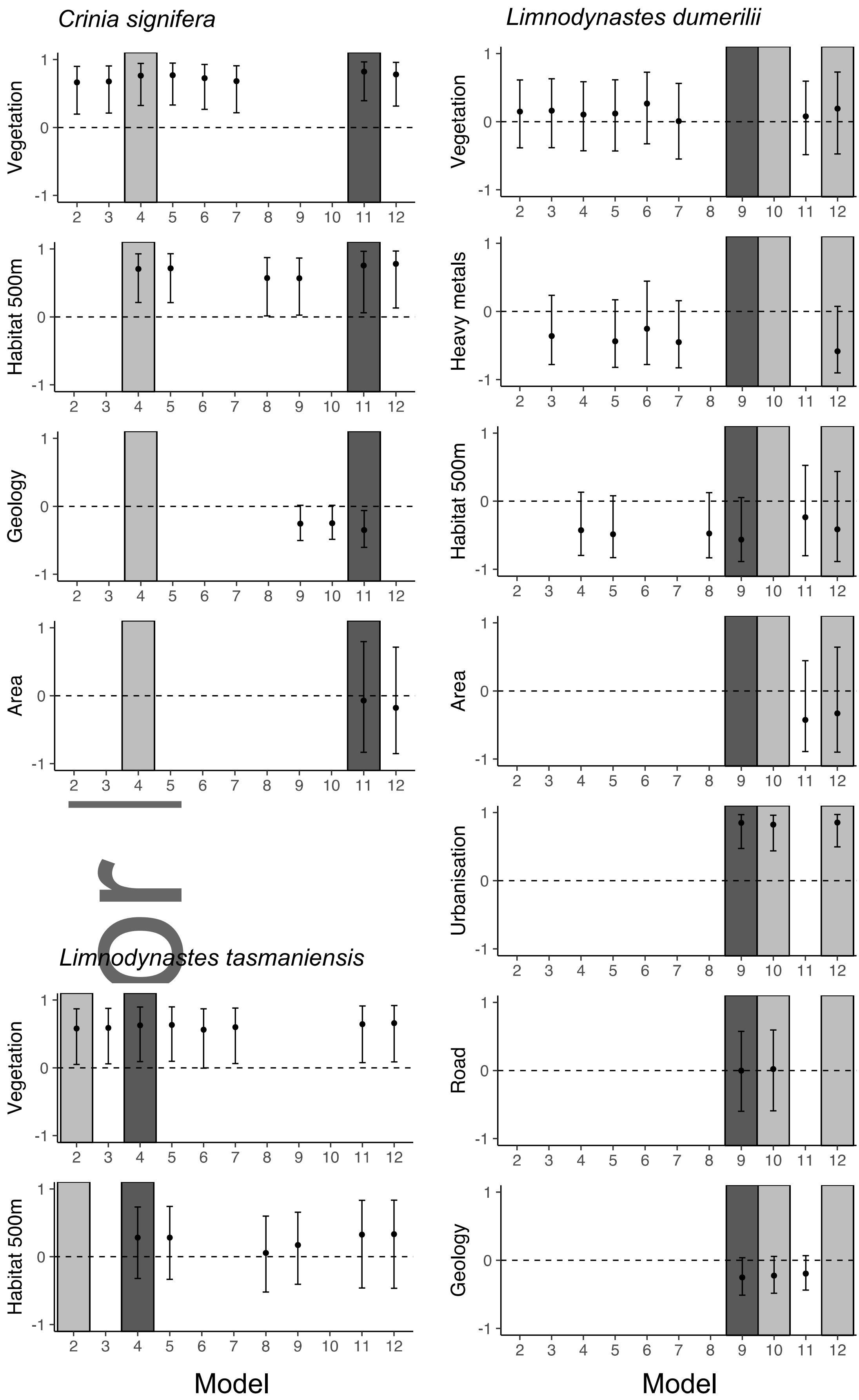
Crinia signifera
Limnodynastes dumerilii
Limnodynastes tasmaniensis
Vegetation
Habitat 500m
Geology
Area
Heavy metals
Urbanisation
Road
Model
Model

Figure 3. The multiplicative effect of the explanatory variables (mean and BCIs) on species occurrence at 67 wetlands. Only variables present in well-supported models for each species (ΔDIC < 2) are shown. The dark grey band indicates the best-supported model (i.e. ΔDIC = 0), and the light grey bands indicate models with some support (ΔDIC < 2). See Table 3 for model numbers and structure. Effect sizes > 0 indicate a positive effect of the explanatory variable on species occurrence while effect sizes < 0 indicate negative effects. Model 1 (constant) is not shown.

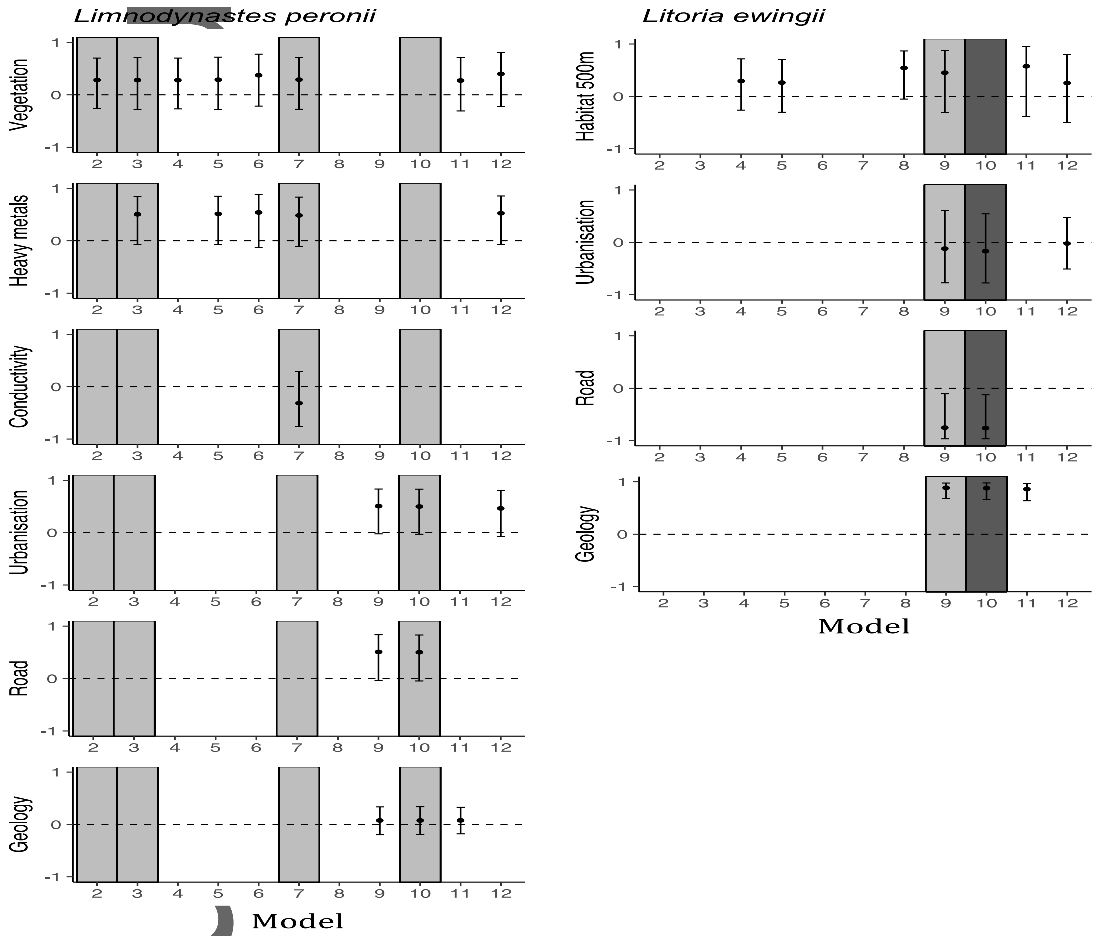

Figure 3 continued.

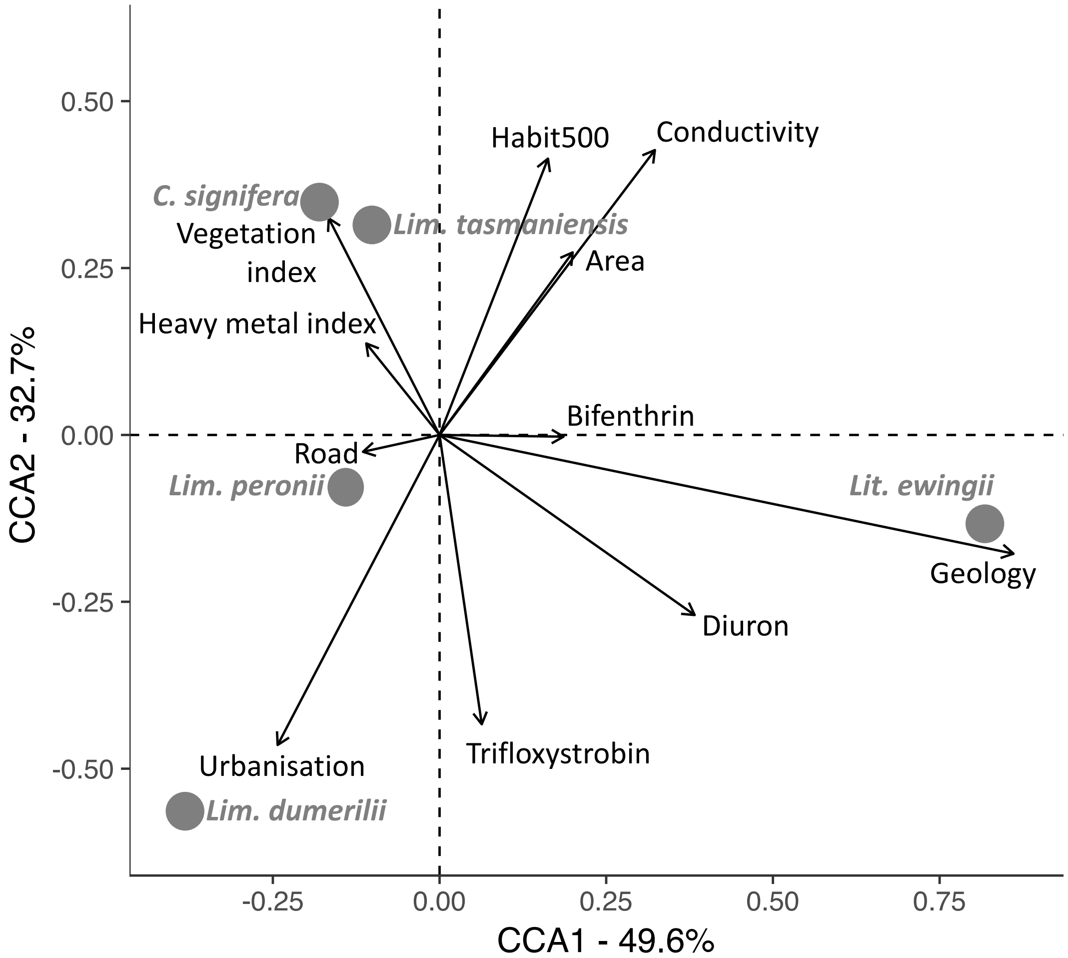

Figure 4. Ordination diagram (biplot) of the canonical correspondence analysis (CCA) for the detection/non-detection of adults according to eleven explanatory variables at 61 wetlands in the Greater Melbourne area, Australia. See Table 3 for a description of variables.