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Heavy metal pollution negatively correlates with anuran species richness and distribution in south-eastern Australia

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Abstract

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Keywords

distribution, richness, species, anuran, correlates, negatively, pollution, australia, metal, eastern, heavy, south

Disciplines

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Heavy metal pollution negatively correlates with anuran species richness and distribution in south-eastern Australia

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Abstract Heavy metal pollution has likely played an important role in global biodiversity decline, but there remains a paucity of information concerning the effects of metals on amphibian diversity. This study assessed anuran species richness and distribution in relation to sediment-metal content and water chemistry in wetlands located along the Merri Creek corridor in Victoria, south-eastern Australia. Anurans were present in 60% (21/35) of study sites, with a total of six species detected: the eastern common froglet (*Crinia signifera*), the eastern sign-bearing froglet (*Crinia parinsignifera*), the southern brown tree frog (*Litoria ewingi*), the growling grass frog *(Litoria raniformis),* the eastern banjo frog (*Limnodynastes dumerilii*), and the spotted marsh frog (*Limnodynastes tasmaniensis*). Mean species richness was 1.77 ± 0.32 per site, and species richness ranged from 0 to 6 species per site. Across sites, species richness correlated negatively with sediment concentrations of 6 heavy metals: copper, nickel, lead, zinc, cadmium and mercury. Species richness also correlated negatively with wetland water electrical conductivity (a proxy for salinity) and concentrations of orthophosphate. Distributions of the three most commonly observed frog species (*C. signifera*, *L. tasmaniensis* and *L. ewingii*) were significantly negatively associated with the total level of metal contamination at individual sites. The study is the first to provide evidence for an association between metal contamination and anuran species richness and distribution in the southern hemisphere, adding to a small but growing body of evidence that heavy-metal pollution has contributed to global amphibian decline.

Keywords: heavy metal, pollution, biodiversity, species richness, amphibian decline.

INTRODUCTION

Environmental change mediated by anthropogenic activities is responsible for unprecedented rates of species extinction, presenting a major threat to global biodiversity. Based on fossil records and current rates of species extinction, biodiversity models have projected that more than seventy-five percent of the world's organisms could face extinction within the next three hundred years, plunging the world into its sixth mass extinction (Barnosky *et al.* 2011). Such rapid changes to biodiversity are predicted to have dramatic impacts upon global ecosystem functioning and stability (Loreau *et al.* 2001). Therefore, identifying the factors responsible for population declines in species from various taxonomic groups is currently a major focus of ecological research.

Among vertebrates, high extinction rates have been reported for all classes, but the taxon that has been most severely impacted is the amphibians (Hero and Morrison 2004). Current estimates indicate that over one third of the world's amphibians are facing extinction, and that almost one half of extant species are experiencing widespread population declines (Stuart *et al.* 2004) . One factor that has been widely implicated in population declines of amphibians, and other freshwater vertebrates such as fish, is the contamination of freshwater systems with heavy metals derived from industrial and agricultural sources (Greig *et al.* 2010; Hopkins and Rowe 2010).

Anuran amphibians are particularly susceptible to the uptake of heavy metals because they have highly permeable skin, which allows the rapid absorption of metal ions. Additionally, due to the larval microphagous feeding habit of most species, tadpoles frequently ingest sediment in which heavy metals have accumulated (Hopkins and Rowe 2010). Over the past three decades, a large number of toxicology studies have reported that ecologically relevant concentrations of metals are lethal to amphibian embryos, larvae and adults (Hopkins and Rowe 2010; Linder and Grillitsch 2000). Furthermore, there is a rapidly growing body of evidence to suggest that sublethal concentrations of metals can have a range of harmful effects, including reduced growth rates, delayed metamorphosis, and impaired behavioural responsiveness (Hopkins and Rowe 2010; Hopkins *et al.* 2000). The susceptibility of amphibians to both the lethal and sublethal effects of metal exposure is known to vary between species due to differences in physiological tolerances, habitat requirements, developmental periods and breeding patterns (Snodgrass *et al.* 2004). Consequently, metal pollution can be expected to be a potent force shaping amphibian species distribution and the composition of species assemblages.

To date, only a limited number of studies have attempted to examine regional influences of metal pollution on amphibian diversity. In an investigation of the effects of chemical contaminants on amphibian distribution near a smelting operation in Sudbury Canada, Glooschenko *et al*. (1992) reported that the presence of frog species was negatively related to increasing levels of cadmium, nickel, aluminium, zinc and copper. Similarly, in a study on the effects of metals derived from paper mill operations on anuran biodiversity in the Fox River and Green Bay ecosystems in Wisconsin USA, Karasov *et al.* (2005) reported that anuran species richness declined with increasing pond concentrations of cadmium, chromium and lead. More recently, Garcia-Munoz *et al.* (2010a) reported that anuran species richness in southern Spain was significantly lower in regions where wetlands were surrounded by olive groves routinely treated with a copper-sulphate fungicide. In a successive set of toxicological experiments, Garcia-Munoz *et al*. (2010b) reported high inter-specific variance in copper tolerance, with relatively intolerant species being those that were absent from chemically altered wetlands. Taken together, these studies indicate that metal pollution is likely an important factor contributing to global amphibian decline.

Within Australia, patterns of amphibian decline mirror the global trend, with approximately 22% of extant species currently listed as endangered or vulnerable (Hero and Morrison 2004). Over the past 100 years, rapid industrialisation in Australia has resulted in many freshwater systems being contaminated with heavy metals (Thorp and Lake 1974). Moreover, there is now unequivocal evidence that metal contamination has negatively impacted the structure of aquatic invertebrate communities (Wright and Burgin 2009). Surprisingly, however, the sensitivity of Australian anurans to heavy metals has received very little empirical attention (Ferraro and Burgin 1993), and we have found no published evaluations of the effects of heavy metal pollution on anuran species assemblages. In this study we investigated the effects of heavy-metal pollution on anuran diversity in wetlands along the Merri Creek corridor in Victoria Australia. The Merri Creek flows through a highly disturbed landscape, beginning in a rural region used for agriculture, flowing through regions of heavy industry and terminating in urban Melbourne, Australia's second largest city. Based on the variety of land-use types along the Merri Creek corridor, we predicted that metal contamination would vary among wetlands and have a significant influence on anuran diversity.

The aims of the study were threefold: 1) to quantify levels of sediment metal contamination and variance in water quality in wetlands along the Merri Creek corridor 2) to determine if sediment-metal concentration, and water quality, influenced anuran species richness, and 3) to determine if the degree of sediment metal contamination influenced the distribution of individual anuran species.

METHODS

Study area and study sites

The Merri Creek corridor is approximately 73 kilometres in length and runs in a southerly direction from Wallan (60 km north of Melbourne) (37° 24' 48.54" S, 144° 58' 44.05" E) to the Yarra River in Melbourne's inner suburbs (37° 47' 47.33" S, 145° 00' 06.58" E) (Fig. 1). The study involved a total of 35 hydraulically separated wetland sites distributed along the entire length of the Merri Creek (Fig. 1, Appendix 1). In order to ensure that wetlands were approximately equidistant from the primary water source, we only chose sites that were between 0.6 and 2 kilometres from the main creek line. We also intentionally avoided sites that were i) smaller than $10m^2$, because their ephemeral nature would have excluded several frog species (Cogger 2000), and ii) larger than $40m^2$, in order to minimise variance in sampling effort between sites. Furthermore, to ensure that all study sites were suitable for anuran occupancy, we only selected sites that possessed 3 wetland characteristics known to favour the presence of Victorian anurans: 1) emergent reed vegetation 2) gradual bank decline and 3) still or slow moving sections of water (Cogger 2000; Hazell 2003).

Species surveys and tadpole collection

The number of species at each study site was estimated by conducting a combination of call surveys and tadpole surveys. Call surveys were conducted using a fixed-point manual call recording approach, whereby audio recordings were made at a set point located between five and ten metres from the edge of a wetland's main water body. Recordings were made using an Olympus audio recorder (model DS-50), with individual recordings lasting five minutes. All recordings were preceded by five minutes of observer silence to ensure frogs had resumed calling after initial observer disturbance (Lane and Burgin 2008). Recordings were made between 1900 hrs and 2300 hrs because Victorian anuran species are nocturnal breeders (Cogger 2000). To account for temporal variation in calling activity, two call surveys were conducted between October 6 and 17, 2008. These sampling times coincided with periods of peak breeding activity for Victoria anurans (Cogger 2000). Playback of audio recordings was used to identify the frog species present at each study site. Identification was validated against previous recordings of Australian frog species (D. Stewart, Nature Sound CD, *Australian Frog Calls,* http://www.naturesounds.com.au/).

Tadpole surveys were conducted using trapping and dip netting techniques. Two types of traps were used: 1) funnel traps, and 2) fish traps (Smith *et al.* 2007). At each study site, traps were set along a 10 m transect placed approximately 1 m from the water's edge, with three funnel traps (set at 5 m intervals) and one fish trap (set 1 m from the central funnel trap) per transect. The number of transects used at each site was adjusted according to the approximate size of the wetland, with one transect per 10 m^2 of wetland shoreline. On each day of sampling, traps were set in the afternoon between 1600 hrs and 1900 hrs, and collected the following morning between 0700 hrs and 1000 hrs. All traps were set, and checked, between November 5 and 18, 2008. This sampling period was selected as it was approximately one month after the peak in calling activity, and pilot sampling indicated that tadpoles had reached a developmental stage at which they could be easily identified (Anstis 2002). During trap collection, we also sampled for tadpoles using dip netting. At each site, dip netting was performed for 10 minutes per 10 m^2 of water body. A second round of dip netting was performed between November 24 and 26, 2008. After capture, tadpoles were euthanised via immersion in 200 mg of MS-222 (300 mg/L) and preserved in 80% ethanol. Tadpoles were later identified to species level using mouthpart morphology, cross checked against fin morphology and tail pigmentation patterns (Anstis 2002). During species identification, tadpoles were also examined for a range of abnormalities, including gill malformation, oral malformation, body malformation and tail malformation. All surveys and tadpole collections were approved by the Monash University Animal Ethics Committee (licence number BSCI/2008/12) and the Victorian Department of Sustainability and Environment (licence number 10004639).

Collection and analysis of sediment samples

Sediment samples were collected from each of the 35 study sites between November 5 and 17, 2008. At each site we collected three 15-20 ml sediment samples taken from the location of the first three tadpole traps (see above). Following sediment collection, samples from each site were combined and oven dried for a period of one week at 40° C. Sediment samples were then homogenized and sieved to 0.2 mm using a mortar and pestle, and the resultant samples were weighed and stored in 20 ml airtight glass sample containers (Allen *et al.* 1974). Sediment samples from each site were subsequently analysed for eight metals: arsenic, cadmium, chromium, copper, nickel, lead, zinc and mercury. In order to extract metals for analysis, the samples were initially subjected to an acid digestion for liquefaction using the US-EPA Method 3051a (1998). Metal analysis followed the US-EPA 6010C (2000) method using a Prodigy High Dispersion ICP-AES. Mercury was analysed separately using a Varian machine (model number 710-ES). Acid digestions and heavy-metal analyses were all conducted by the Australian Sustainable Industry Research Centre (ASIRC).

Collection and analysis of water samples

A number of water-borne chemical contaminants (orthophosphate, nitrate and ammonia) and water chemistry variables (pH, dissolved oxygen concentration and electrical conductivity) can influence the fitness and survival of anuran embryos and larvae, and in turn, species richness (Hecnar and M'Closkey 1996). Furthermore, these variables can also potentially influence the toxicity of heavy metals (Linder and Grillitsch 2000). Therefore, we also collected data on several water quality variables. In order to obtain water for analysis, we collected two 300 ml samples (stored in 500 ml plastic bottles) from each study site. Water samples were collected at the same time as sediment samples. Samples were stored on ice during transport to the laboratory, where they were then stored in a freezer at -5° C. Prior to freezing, one sample per site was filtered using a vacuum pump filter with 0.45 μm GF/C 4.7 cm glass microfiber filter, while the other sample was left unfiltered. Using spectrophotometry (Shimadzu Pharma Spec UV-1700 UV-Visible), concentrations of reactive phosphorus (orthophosphate) were determined in filtered samples, while concentrations of nitrate (NO_3) and ammonia (NH_3) were determined in unfiltered samples. At each site, we measured pH level, dissolved oxygen concentration

(DO), and electrical conductivity using a TPS 90-FLT portable data meter. For each of these variables we made three separate measurements, taken at the location of the first three tadpole traps (see above).

Habitat characteristics

Previous studies have shown that anuran species richness can be significantly influenced by pond size and the amount of vegetation surrounding ponds (García-Muñoz *et al.* 2010a; García-Muñoz *et al.* 2010b; Glooschenko *et al.* 1992; Hazell 2003). Therefore, at each study site we measured pond area (m^2) and estimated the amount of vegetation surrounding ponds (% cover in a 1km radius). Furthermore, because anuran species richness has been shown to decline with increasing urbanisation (Babbitt *et al.* 2009; Hamer and McDonnell 2010), we also used topographic maps to calculate the distance of each site from the Melbourne CBD, and used local-council zoning data to classify the predominant land-use type (urban, industrial, rural) within a 2km radius of each study site (Appendix 1).

Statistical analyses

Across the 35 study sites we calculated mean (±SE) values for each of the sediment metals and water chemistry variables examined, and compared these with benchmark values set by The Australian and New Zealand Environment Conservation Council (ANZEEC) (ANZECC 2000). The water-quality guidelines provide benchmark values (trigger values) indicative of typical slightly to moderately disturbed eco-systems in south-east Australia (ANZECC 2000). For non-metallic inorganic toxicants (e.g. ammonia and nitrate), the trigger values represent concentrations at which 95% of aquatic species are protected. The

ANZEEC sediment-quality guidelines provide a 'low' and a 'severe' contamination value for individual metals. The 'low' value represents a threshold concentration (mg/Kg dry weight) where the lowest toxic effects on benthic biota become apparent. The 'severe' value represents concentrations (mg/Kg dry weight) that could potentially eliminate the majority of benthic organisms (ANZECC 2000).

To test for correlations between sediment variables, water quality variables, and habitat variables, we ran a Principle Components Analysis (PCA) on eight metal variables (As, Cd, Cr, Cu, Ni, Pb, Zn, and Hg), six water quality measures (orthophosphate, NH_2, NO_3, pH, DO, and electrical conductivity) and four habitat variables (wetland area, % vegetation cover, land-use zone and distance from Melbourne CBD). To test which components predicted anuran species richness we ran a GLM analysis, using a Poisson distribution, with PC scores as the independent variables and species richness (species counts) as the dependent variable. Species richness was determined for each study site by calculating the total number of species detected in the call and tadpole surveys, with species detected in both survey types only counted once.

To test whether variance in metal contamination among wetlands predicted the distribution of individual species we used logistic-regression analysis. For analysis, presence/absence data (binomial distributions) for each species were related to a pollution score derived from an index of metal contamination (Karasov *et al.* 2005). The pollution index was constructed for each of the eight metals examined by creating six pollution categories: 1) low 2) low-moderate 3) moderate 4) moderate-high 5) high and 6) severe (see Appendix 2). The pollution categories were created using the 'low' and 'severe' heavy metal concentration values set by the ANZEEC sediment quality guidelines (ANZECC 2000). Each metal pollution category received a value between 0 and five, with one being the lowest level of contamination and five the highest (see Appendix 2). After allocating a concentration score for each metal at each site, individual scores for the eight metals were summed to give a total pollution score for each site, which ranged between 0 (least polluted) and 40 (most polluted)(see Appendix 1)**.**

All statistical analyses were conducted using the statistical package R 2.6.1 (R.D.C.T. 2007).

RESULTS

Sediment and water chemistry

Sediment concentrations of all eight metals (copper, nickel, lead, zinc, cadmium, mercury, arsenic, and chromium) were highly variable across study sites (Table 1, Appendix 3). Mean concentrations for every metal exceeded concentrations deemed to be 'low' by Australian standards (ANZECC 2000). Maximum concentrations for four metals (chromium, copper, lead and zinc) exceeded levels deemed to be 'severe' by Australian standards (Table 1). In regards to water chemistry, average orthophosphate concentrations exceeded concentrations deemed to be 'toxic' by Australian standards (ANZECC 2000). Average levels of nitrate, ammonia, pH, dissolved oxygen (DO) and electrical conductivity were all within a range deemed to be acceptable by Australian standards (Table 2, Appendix 4). Maximum levels for all water chemistry variables (except pH) exceeded acceptable limits (Table 2, Appendix 4).

A PCA on the eight metals resulted in three components with Eigen-values greater than one, which together explained approximately 76% of the variance. The PCA revealed that four metals (copper, nickel, lead and zinc) dominated component 1 (Eigen value = 3.76, % variance = 47.08), two metals (arsenic and chromium) dominated component 2 (Eigen value = 1.19, % variance = 14.88), and two metals (cadmium and mercury) dominated component 3 (Eigen value = 1.13, % variance = 14.10) (Appendix 5). A PCA on the six water chemistry variables produced three components with Eigen-values greater than one, which collectively explained approximately 74% of the variance**.** The PCA showed that component 1 was dominated by pH and dissolved oxygen (Eigen value = 1.66, % variance = 27.72), component 2 was dominated by orthophosphate and electrical conductivity (Eigen value = 1.50, % variance = 24.99) and component 3 was dominated by nitrate and ammonia (Eigen value = 1.30, % variance = 21.67) (Appendix 6). In regard to habitat variables, a PCA on four variables resulted in two components with Eigen-values greater than one, which explained 87% of the variance. Land-use zone and distance from city dominated component 1 (Eigen value = 2.42, % variance = 60.7%), while wetland size and % surrounding vegetation dominated component 2 (Eigen value = 1.05, % variance = 26.42%)(Appendix 7).

Species richness and distribution

Anurans were detected in 60% (21/35) of wetlands, with a total of six species recorded: *Crinia signifera, Crinia parinsignifera, Litoria ewingii, Litoria raniformis, Limnodynastes dumerilii,* and *Limnodynastes tasmaniensis* (Table 3)**.** Average species richness was 1.77 ± 0.32, but species richness was highly variable, ranging from 0 to 6 species per site. Of the 6 species detected, one species (*C. signifera*) was found at 60% of sites, but the other species occurred in less than 40% of sites, with two of these species **(***C. parinsignifera* and *L. raniformis***)** found in less than 15% of sites (Table 3, Appendix 8).

Species detection was more effective using call surveys than tadpole surveys. Across sites, call surveys detected 100% (6/6) of species, while tadpole surveys detected 83.33% (5/6) of species (Table 3). For each species, the percentage of study sites in which a species was detected was always substantially higher for call surveys than tadpole surveys (Table 3), and for only one species (*L. raniformis*) were tadpoles detected at a site in which frogs were not reported calling (Table 3, Appendix 8). Tadpoles were collected for every species except *C. parinsignifera* (Table 3, Appendix 8). Of the five species for which tadpoles were collected, the highest average number of tadpoles collected per site was for *C. signifera* (min = 0, max = 18, mean ± SE = 2.14 ±0.78), followed by *L. raniformis* (min = 0, max = 11, mean ± SE = 0.34 ± 0.31), *L. tasmaniensis* (min = 0, max = 2, mean ± SE = 0.07 ± 0.05), *L. ewingi* (min = 0, max = 2, mean ± SE = 0.05 ± 0.05), and *L. dumerilii* (min = 0, max = 1, mean ± SE = 0.02 ± 0.02). Tadpoles were predominantly collected using dip-netting, with only 3.15 % (3/95) of tadpoles caught in traps (see Appendix 8). None of the tadpoles collected displayed signs of morphological deformity.

Concentrations of copper, nickel, lead, and zinc (component 1 for metals), and cadmium and mercury (component 3 for metals) significantly affected anuran species richness (component 1: $\chi^2 = 49.711$, d.f. = 34, $P < 0.001$; component 3; $\chi^2 = 49.711$, d.f. = 34, $P < 0.001$)(Fig. 2). However, arsenic and chromium (component 2 for metals) did not significantly affect anuran species richness ($\chi^2 = 49.711$, d.f. = 34, $P = 0.091$). With respect to water quality, species richness was significantly affected by orthophosphate and conductivity (component 2 for water quality) ($\chi^2 = 49.711$, d.f. = 34, $P < 0.01$)(Fig. 3), but not by pH and dissolved oxygen (component 1 for water quality), or nitrate and ammonia (component 3 for water quality)(component 1; $\chi^2 = 49.711$, d.f. = 34, $P = 0.975$; component 3; $\chi^2 = 49.711$, d.f. = 34, $P = 0.122$). With respect to wetland habitat, species richness was not associated with wetland size or % surrounding vegetation (component 2 for habitat)($\chi^2 = 0.03$, d.f. = 34, $p = 0.85$), but species richness was significantly affected by surrounding land use (urban, industrial, rural) and distance from the city (component 1 for habitat) ($\chi^2 = 9.54$, d.f. = 34, $P < 0.01$). Urban and industrial sites were located closer to the city than rural sites, and had higher total heavy-metal pollution scores (Fig. 1, Appendix 1).

Distributions of the three most commonly found anuran species were all significantly negatively related to the total heavy metal pollution score (*C. signifera*; $\chi^2 = 47.111$, d.f. = 34, $p < 0.01$: *L. ewingii*: $\chi^2 = 41.879$, d.f. = 34, $P < 0.05$: *L. tasmaniensis*: $\chi^2 = 47.111$, d.f. = 34, $p < 0.05$). In contrast, distributions of the three least common species were not significantly related to the total heavy metal pollution score (*C. parasignifera* (χ^2 = 24.877, d.f. = 34, *p* <0.181), *L. dumerilii* (χ^2 = 39.903, d.f. = 34, *P* <0.077) and *L. raniformis* (χ^2 = 24.877, d.f. = 34, *P* < 0.203). Interestingly, while three species (*C. signifera, L. tasmaniensies* and *L. dumerilli*) were present in highly polluted sites (total pollution score >20/40) (Table 4), only two species (*C. signifera* and *L. dumerilli*) were present in the least polluted sites (total pollution score = 1/ 40) (Table 4). This result indicates that extremely unpolluted (relatively pristine) sites were only occupied by a small subset of species.

DISCUSSION

This study showed that six metals (copper, nickel, lead, zinc, cadmium and mercury) correlated negatively with anuran species richness throughout the Merri Creek corridor. To date, no studies have examined the lethal effects of heavy metals on Australian anurans, so it remains unknown whether any of the metals tested exceeded toxicity thresholds for any species. However, average and maximum sediment concentrations for several metals (copper, nickel, lead, zinc, cadmium and mercury) were within the range known to have toxic effects on various European and North American anurans (Hopkins and Rowe 2010; Linder and Grillitsch 2000), so it seems likely that toxic heavy metal contamination has contributed to the localised extinction of several species throughout the Merri Creek corridor.

Population declines also may have resulted from sublethal effects of heavy metal contamination. Exposure of tadpoles to sublethal concentrations of metals can have a range of detrimental effects, including impaired behavioural responsiveness, reduced growth rates and delayed metamorphosis (Hopkins and Rowe 2010; Linder and Grillitsch 2000). Critically, because these effects all have the potential to reduce individual fitness, sublethal effects may have negatively impacted recruitment rates, and in turn, the persistence of local populations. Toxicological tests are now needed to determine the lethal and sub-lethal effects of heavy metals on Australian anurans. The negative association found between heavy-metal contamination and anuran species richness also may have resulted from indirect effects of metals on anuran food resources. Heavy metals are known to kill algae and invertebrates (Clements 1991), so reduction in the abundance of these groups may have triggered trophic cascades that disrupted food-web dynamics. Such indirect effects on anuran amphibians have previously been reported in response to pesticide pollution (Relyea and Diecks 2008), but whether heavy metal pollution can initiate similar harmful trophic cascades remains unknown. Mesocosm experiments could be used to investigate this possibility.

Species richness was also negatively associated with two water chemistry variables: orthophosphate and electrical conductivity. Orthophosphate, which is generally derived from agricultural fertilisers, was present in most study sites and the average level was extreme by Australian standards. In general, phosphate has not been found to kill amphibians (Earl and Whiteman 2010), but there is some evidence that phosphate is toxic to Australian frogs. A comparative study by Hamer *et al.* (2004) reported that chronic exposure of calcium phosphate had no effect on *Crinia signifera* and *Limnodyanstes peronii* tadpoles, but significantly reduced the survival of *Litoria aurea* tadpoles. Differential species sensitivity to phosphate suggests that this pollutant could directly alter anuran species assemblages. However, the concentration (15 mg/l) at which phosphate was found to be lethal to *L. aurea* was several orders of magnitude higher than the maximum concentration (3.09 mg/l) detected across our study sites. Therefore, the reported effect of orthophosphate on species richness is likely to have been indirect. It is well established that phosphate concentrations above 0.1 mg/l can result in blooms of cyanobacteria (eutrophication) (Correll 1998), which can decimate communities of aerobic freshwater organisms by creating hypoxic conditions and/or releasing hepatotoxins or neurotoxins (Peltzer *et al.* 2008). Recently, a North American study also found that eutrophication resulting from phosphate enrichment can circuitously eradicate amphibians by promoting the production and transmission of trematode parasites that induce severe limb malformations, and in turn, elevate predation risk (Johnson *et al.* 2007). Irrespective of the causation, our study is only the second to provide evidence for a negative association between orthophosphate concentration and amphibian species richness (Bishop *et al.* 1999), highlighting the need for further empirical studies focussed on understanding the direct and indirect ecological impacts of phosphate-based pollutants.

The negative association between electrical conductivity and species richness was expected because high electrical conductivity, indicative of elevated salt levels, has previously been shown to impact the growth, development and survival of various anurans (Chinathamby *et al.* 2006). Furthermore, previous research has provided evidence for an effect of salinity on anuran diversity in Australia. Smith *et al.* (2007) investigated the impacts of salinity on anuran species richness in western Victoria, a region with a similar frog fauna to the Merri Creek corridor, and reported that electrical conductivity levels above 3000 $\mu s\ cm^{-1}$ had negative impacts on species presence (Smith *et al.* 2007). In our study, maximum electrical conductivity levels did not exceed 2295 μs cm^{-1}, so the detrimental effects of salinity appear to have manifested at a lower salt concentration. Compared to the Merri Creek corridor, western Victoria has a long history of secondary salinisation, so anuran populations in this region may have evolved increased salt tolerance (Gomez-Mestre and Tejedo 2003). Alternatively, interactive effects between salinity and chemical contaminants present in Merri Creek wetlands, but absent from western Victoria, may have exacerbated the detrimental effects caused by salinity. For example, Ortiz-Santaliestra *et al* (2010) recently reported that salt-stressed embryos of the Iberian green frog *Pelophylax perezi* had elevated mortality rates following experimental exposure to an ammonium-nitrate fertiliser (Ortiz-Santaliestra *et al.* 2010). Irrespective of the explanation for regional differences in the effects of salinity, our findings are in agreement with the popular supposition that human-induced salinisation has had widespread negative impacts upon freshwater biodiversity (Semlitsch and Bodie 1998)

In regard to the habitat variables examined, anuran species richness was not related to wetland size or the percentage of vegetation surrounding a wetland. These findings contrast with previous studies that have found these habitat variables to be reliable predictors of anuran species richness (Hazell 2003; Pillsbury and Miller 2008). However, in this study we did not expect a significant relationship between habitat characteristics and species richness because we purposely chose study sites with habitat characteristics known to support a diversity of Victorian anuran species (Cogger 2000). This approach was taken to reduce the potential confounding impacts of habitat variance on anuran community composition along the pollution gradient. Anuran species richness was, however, significantly associated with the type of land use (urban, industrial or rural) surrounding study sites, as well as the distance that study sites were located from the Melbourne CBD. Species richness was highest in rural areas, which were located further away from the city, and these sites generally had lower overall levels of metal pollution. These findings indicate that activities undertaken in urban and industrial areas have been the primary source of heavy-metal pollution throughout the Merri Creek corridor.

In addition to showing a negative association between heavy-metal pollution and species richness, our data also indicate that heavy-metal pollution was negatively associated with the distribution patterns of individual species. In total, six species were found throughout the Merri Creek corridor, and three species had distributions that were significantly negatively correlated with the total level of metal pollution. The species (*L. raniformis, C. parinsignifera* and *L. dumerilli*) whose distributions were not correlated with metal pollution were only present in a small number of sites (4-9 sites), so the power to detect an effect of metal pollution was limited. The species (*C. signifera*, *L. tasmaniensis* and *L ewingi*) whose distributions were negatively correlated with metal pollution are all common Victorian species that are known to inhabit highly disturbed and degraded habitats (Hamer and McDonnell 2010; Lane and Burgin 2008). However, our data indicate that these species may differ in their sensitivity to heavy-metal pollution. *Crinia signifera* and *L. tasmaniensis* were recorded in the highest number of sites (60% and 40% respectively), and both species were present in all but the most extremely polluted sites, suggesting they have moderate to high metal tolerance. In contrast, *L. ewingi* only occurred in a moderate number of sites, and was absent from many of the more polluted sites, suggesting this species may have limited metal tolerance. To confirm these inferences, it will be necessary to conduct experimental tests that evaluate differences between species in the lethal (toxic) and sub-lethal physiological and behavioural effects of metal exposure (Clements 1991; Hopkins and Rowe 2010). Importantly, these studies should aim to examine the effects of metals in combination, and over chronic time periods (weeks to months), so as to simulate natural scenarios (Hopkins and Rowe 2010).

Interestingly, all species, with the exception of *C. signifera* and *L. dumerilii***,** were absent from relatively unpolluted sites. This was a surprising result because previous landscape-scale studies of anuran species assemblages have generally reported maximum species diversity in relatively pristine sites (Bishop *et al.* 1999; Ferraro and Burgin 1993; Hazell 2003; Hecnar and M'Closkey 1996). However, our finding is comparable to a recent study by Lane and Burgin (2008) that evaluated frog species richness in urban and non-urban sites throughout the Blue Mountains near Sydney, Australia. In that study, six frog species were present in degraded urban sites, but only a single species (*C. signifera*) was present in relatively pristine sites (Lane and Burgin 2008). The proposed explanation for this pattern was that chytrid fungus, a highly infectious disease linked to mass frog mortality, might be unable to tolerate chemical contaminants, including heavy metals, present in urban wetlands. This postulate is in line with an earlier experimental study by Parris and Baud (2004) who reported that exposure to copper significantly reduced the detrimental effects of chytridiomycosis on larval growth rates in the North American gray treefrog *Hyla chrysoscelis* (Parris and Baud 2004).

Although metal pollution is a pervasive problem in Australia, and past studies have shown negative effects on the composition of freshwater invertebrate communities (Wright and Burgin 2009), our study is the first to provide evidence for a negative association between metal pollution and anuran species richness. Critically, these findings add to a small but growing body of evidence that metal pollution can significantly alter global amphibian community structure. Throughout the northern hemisphere, the ecological impacts of metals have been considered for several decades and there is now some emerging evidence that anuran species richness has been negatively impacted by agricultural sources of copper in southern Spain (García-Muñoz *et al.* 2010a; García-Muñoz *et al.* 2010b) and industrial sources of copper, cadmium, nickel, aluminium, and zinc in Sudbury Canada (Glooschenko *et al.* 1992) and cadmium, chromium and lead in Wisconsin USA (Karasov *et al.* 2005). By providing evidence that metal pollution correlates negatively with anuran species richness in the southern hemisphere, our study adds further weight to the long held notion that heavy metal pollution resulting from anthropogenic activities is an important factor contributing to amphibian decline.

Globally, amphibians are not the only freshwater taxon in which negative associations between heavy-metal pollution and species richness have been found. Over the past two decades similar associations have been reported in algae (Medley and Clements 1998), macro-invertebrates (Hickey and Clements 1998; Wright and Burgin 2009) and fish (Clements 1991; Greig *et al.* 2010). This broad taxonomic association between heavy metal pollution and species decline underscores the urgent need for continued research focussed on understanding the threat of heavy metals to global freshwater biodiversity.

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Figure legends

Fig. 1. Map illustrating the location of 35 wetland study sites along the Merri-Creek corridor in Victoria, Australia. Solid triangles represent wetlands with low to moderate metal pollution, solid circles represent sites with moderate to high metal pollution, and open squares represent sites with high to severe metal pollution. Sites were located in three distinct land-use zones (urban, industrial and rural) demarcated by horizontal lines and double sided arrows.

Fig. 2. Relationships between anuran species richness and wetland-sediment concentrations (mg/Kg dry weight) of (a) copper, (b) nickel, (c) lead, (d) zinc, (e) cadmium, and (f) mercury at 35 study sites along the Merri Creek corridor.

Fig. 3. Relationships between anuran species richness and wetland-water levels of (a) orthophosphate (ppm), and (b) electrical conductivity (μS/cm) at 35 study sites along the Merri Creek corridor.