

1 **Stormwater wetlands can function as ecological traps for urban frogs**

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15 Running head: Frogs caught in ecological traps

16

17 **ABSTRACT**

18

19 Around cities, natural wetlands are rapidly being destroyed and replaced with  
20 constructed wetlands created to capture stormwater. Although the intended purpose of  
21 these wetlands is to manage urban stormwater, they are inhabited by wildlife that  
22 might be exposed to contaminants. These effects will be exacerbated if animals are  
23 unable to differentiate between stormwater treatment wetlands of varying quality, that  
24 is, some function as ‘ecological traps’ (i.e. habitats that animals prefer despite fitness  
25 being lower than in other habitats). To examine if urban stormwater wetlands can be  
26 ecological traps for frogs, we tested if survival, metamorphosis-related measures and  
27 predator avoidance behaviours of frogs differed among stormwater wetlands with  
28 different contaminant levels in a mesocosm study, and paired this with a natural  
29 oviposition experiment to assess breeding-site preferences. We provide the first  
30 empirical evidence that these wetlands can function as ecological traps for frogs.  
31 Tadpoles had lower survival and were less responsive to predator olfactory cues when  
32 raised in more polluted stormwater wetlands, but also reached metamorphosis earlier  
33 and at a larger size. A greater size at metamorphosis was likely a result of the  
34 combined effects of increased food availability due to eutrophication, and reduced  
35 competition due to higher mortality rates. Breeding adults laid comparable numbers of  
36 eggs across wetlands with high and low contaminant levels, indicating no avoidance  
37 of the former. Since stormwater treatment wetlands are often the only available  
38 aquatic habitat in urban landscapes we need to better understand how they function to  
39 guide management decisions that mitigate their potential ecological costs. This may  
40 include improving wetland quality so that fitness is no longer compromised,  
41 preventing colonisation by animals, altering the cues animals use when selecting

42 habitats, pretreating contaminated water prior to release, providing off-line wetlands  
43 nearby or simply not constructing stormwater treatment wetlands in sensitive areas.  
44 Our study confirms the potential for urban stormwater treatment wetlands to function  
45 as ecological traps and highlights the need for greater awareness of their prevalence  
46 and impact at landscape scales.

47

48 **Keywords:** *amphibians, constructed wetland, HIREC, metapopulation, predator*  
49 *avoidance, urbanisation*

50 **INTRODUCTION**

51 Urbanisation is changing the biological, chemical and physical characteristics of  
52 natural aquatic ecosystems (Walsh et al. 2005). For example, in Oregon, USA, land-  
53 use changes from underdeveloped and agricultural to urban and residential led to  
54 almost all the palustrine emergent/open water wetlands being graded as either fair  
55 (46%) or poor (43%) quality (Kentula et al. 2004). Within urban areas, stormwater  
56 treatment wetlands are being created to provide ecosystem services such as flood  
57 control, and water filtration and purification (Hammer 1989). These habitats are  
58 becoming ubiquitous throughout urban and suburban landscapes, modifying both the  
59 distribution and nature of wetlands (Kentula et al. 2004). Often resembling natural  
60 wetlands, stormwater treatment wetlands attract animals (Tixier et al. 2011, Hassall  
61 and Anderson 2015), and thus may provide valuable aquatic habitat in urban  
62 landscapes where most natural waterbodies have been lost or heavily degraded.

63 One of the primary functions of stormwater treatment wetlands, however, is to  
64 capture and treat various contaminants (e.g. nutrients, sediments, heavy metals,  
65 pesticides) prior to release into receiving waters (Hammer 1989). Animals that inhabit  
66 them may thus suffer a range of deleterious effects, such as lower survival or physical  
67 abnormalities (Sparling et al. 2004, Snodgrass et al. 2008, Gallagher et al. 2014).  
68 Contaminants common in stormwater wetlands can also cause maladaptive  
69 behaviours to develop *via* olfactory disruption (Tierney et al. 2010). For example,  
70 Cuban tree frog tadpoles do not avoid predator cues and are hyperactive following  
71 exposure to the herbicide atrazine (Ehrsam et al. 2016). These behavioural changes  
72 can indirectly reduce fitness by increasing the probability of predation (Broomhall  
73 2004).

74 The impact of highly polluted stormwater treatment wetlands will be

75 exacerbated if animals do not recognise them as poor-quality habitats, and they  
76 function as ‘ecological traps’. An ecological trap occurs when there is differential  
77 fitness between available habitats, and animals show a preference (severe trap) or an  
78 equal-preference (equal-preference trap) for the habitat within which fitness is lower  
79 (Robertson and Hutto 2006). Ecological traps are defined by impacts to individuals,  
80 not by demographic effects (Robertson et al. 2013) and so the identification of  
81 ecological traps requires information about how habitats impact the fitness (i.e.  
82 survival, reproduction) and habitat preferences of individual animals.

83         Although the potential for stormwater wetlands to function as ecological traps  
84 has been discussed previously (Brand and Snodgrass 2010, Gallagher et al. 2014,  
85 Hale et al. 2015a, Sievers et al. *in press*), we currently lack empirical tests to confirm  
86 this. Animals may be attracted to stormwater wetlands by cues such as native  
87 vegetation but subsequently are exposed to contaminants that reduce fitness.  
88 Distinguishing when stormwater wetlands are functioning as ecological traps is  
89 important as these wetlands are often the only available aquatic habitat within urban  
90 areas. If traps are present, they will draw animals from higher- to lower-quality  
91 stormwater wetlands, potentially reducing the likelihood of both local and regional  
92 population persistence (Donovan and Thompson 2001, Hale et al. 2015b).  
93 Understanding how traps form and their consequences to animals has thus become an  
94 emerging management challenge. Although traps have been identified and studied  
95 broadly in terrestrial systems (Hale and Swearer 2016), there are few examples from  
96 aquatic environments, despite the significant environmental changes occurring in  
97 these habitats worldwide (Vörösmarty et al. 2010, Halpern et al. 2015).

98         For taxa that lack post-hatching parental care, the selection of oviposition sites  
99 is a critical decision that directly affects offspring fitness, and ultimately, population

100 dynamics (Reich and Downes 2004). In particular, strong selective forces should be  
101 acting on the oviposition behaviour where larvae are unable to leave unsuitable sites  
102 (Rudolf and Rodel 2005, Resetarits and Silberbush 2016). Oviposition-site selection  
103 in amphibians can be influenced by a range of cues, such as the presence of predators  
104 (Resetarits and Wilbur 1989) and pesticides (Vonesh and Kraus 2009). Amphibians  
105 can thus respond adaptively to stimuli by preferring unpolluted or predator-free  
106 conditions. However, as stormwater wetlands are often the only available breeding  
107 habitat within urban landscapes, oviposition-site selection is likely to involve choices  
108 between wetlands that all are degraded/polluted/contaminated to varying degrees,  
109 complicating the decision-making process.

110         Given that approximately 40% of amphibian species face the threat of  
111 extinction (Monastersky 2014), there is an urgent need to understand the  
112 consequences to amphibians of inhabiting urban stormwater treatment wetlands. In  
113 this study, our overarching goal was to test if stormwater wetlands can function as  
114 ecological traps. While ecological trap studies often compare sites that are  
115 hypothesised to be traps with less disturbed (e.g. reference) sites, we examined  
116 responses to replicate pairs of stormwater wetlands of differing quality, which better  
117 reflect the reality of urban landscapes. We conducted a mesocosm experiment to  
118 quantify the fitness and behaviour of tadpoles, coupled with field-choice trials to  
119 examine the oviposition preferences of breeding frogs. We hypothesized that: (1.)  
120 tadpole survival and size/mass at metamorphosis would be lower within more  
121 contaminated wetlands, and (2.) their ability to respond adaptively to predator cues  
122 would be reduced. If urban stormwater treatment wetlands are ecological traps, we  
123 predicted that females would not avoid contaminated wetlands where offspring fitness  
124 was reduced. We discuss our results in the context of managing stormwater wetlands

125 that are ecological traps to reduce the potential that they compromise the persistence  
126 of aquatic animals in urban landscapes. .

127

128

## 129 **METHODS**

130

### 131 *Study species*

132 Marsh frogs (Genus *Limnodynastes*, Fitzinger, 1843) are ground-dwelling frogs native  
133 to Australia, New Guinea and the Torres Strait Islands. We focused on the spotted  
134 marsh frog *Limnodynastes tasmaniensis* (Günther, 1858) and the striped marsh frog  
135 *Limnodynastes peronii* (Duméril and Bibron, 1841), two species common in south-  
136 eastern Australia that are known to occupy and breed in urban wetlands (Hamer and  
137 Parris 2011, Ficken and Byrne 2013). Nocturnal call surveys confirmed the presence  
138 of both species at all study sites.

139

### 140 *Study sites*

141 As of 2015, nearly 500 wetlands had been constructed in Melbourne, with more than  
142 80% of those built in the last two decades alone (Hale et al. 2015a). To examine frog  
143 responses to stormwater wetland quality, we selected six stormwater wetlands that  
144 differed in the level of contamination within the Greater Melbourne Region,  
145 Australia. These wetlands were grouped in three pairs, with one that we hypothesized  
146 to be of high quality (hereafter HQ) and another we hypothesized to be of low quality  
147 (hereafter LQ), based on sediment and water quality data recently collected by the  
148 Centre for Aquatic Pollution Identification and Management. In particular LQ and HQ  
149 status was based on the presence and concentrations of contaminants (heavy metals

150 and pesticides) that we predicted could have an effect on amphibian fitness (Egea -  
151 Serrano et al. 2012).

152

### 153 *Mesocosm design*

154 We established 48 circular mesocosms (150L, 875 mm diameter x 310 mm deep) at  
155 The University of Melbourne, Burnley Campus (37°49'47.17" S, 145°01'28.64" E),  
156 with eight randomly assigned replicates of each source wetland. Each mesocosm was  
157 filled with 120 L of 100 µm-filtered water, 2 L of 64 µm-filtered sediment, and 4 L of  
158 unfiltered, autoclaved sediment from the respective source wetland in September  
159 2016, and covered with 50% shade-cloth to keep out predators. Filtered sediment was  
160 added to allow the establishment of natural benthic communities, and unfiltered,  
161 autoclaved sediment to provide benthic structure; both methods also removed  
162 predatory invertebrates (Pettigrove and Hoffmann 2005). We collected zooplankton  
163 from an on-campus wetland using a 50 µm plankton net, with samples diluted and  
164 added to each mesocosm in 0.2 L aliquots after screening to exclude predators.  
165 Mesocosms were allowed to settle and mature for 4 weeks. We conducted fortnightly  
166 25 L water changes using water from the respective source wetland to help maintain  
167 water quality conditions. Evaporated water was replaced with aged tap water or  
168 natural rainfall so that depth was kept consistent.

169 We collected ten spotted marsh frog egg masses from an off-line wetland  
170 (37°37'08.06" S, 145°00'10.67" E) created for the endangered growling grass frog  
171 *Litoria raniformis* (Keferstein, 1867). Each egg mass was hatched in an individual 2L  
172 beaker in the laboratory, and we transferred two-day-old tadpoles into the mesocosms.  
173 Each mesocosm received ten tadpoles on the 16<sup>th</sup> October 2016, one from each of the  
174 ten egg masses to remove any confounding genetic/parental effects.

175

176 ***Fitness responses***

177 Tadpoles were removed from mesocosms as they began to metamorphose and placed  
178 in individual 0.5 L plastic containers until complete tail resorption. We then recorded  
179 days to, length at, and mass at metamorphosis, and the incidence of any physical  
180 abnormalities (e.g. missing or malformed limbs, scoliosis). Survival was calculated as  
181 the number of tadpoles reaching metamorphosis relative to the number of tadpoles  
182 released into each mesocosm (after accounting for those removed for the predator  
183 avoidance trials; see below).

184

185 ***Periphyton, water and sediment analysis***

186 After the final tadpole metamorphosed (80 days), we collected periphyton, water and  
187 sediment samples from each mesocosm. To quantify food availability, we removed all  
188 periphyton from a 5 x 10 cm section, 5 cm below the high-water level of each  
189 mesocosm with a razor blade. Samples were oven dried at 60°C for 24 h, weighed,  
190 inserted into a blast furnace at 475°C for 18h, and re-weighed to calculate ash-free  
191 dry-weight (AFDW). We measured ammonia, nitrite, nitrate, phosphate and total  
192 alkalinity using an eXact® Eco-Check photometer (Industrial Test Systems, Inc,  
193 USA), and pH and conductivity using a WP-81 meter (TPS, Brisbane, Australia). We  
194 air-dried and filtered sediments to 1 mm, and metal concentrations were quantified by  
195 TrACEES at The University of Melbourne (Supplementary Appendix 1;  
196 [www.chemicalanalysis.unimelb.edu.au](http://www.chemicalanalysis.unimelb.edu.au)).

197

198 ***Predator avoidance trials***

199 We harvested odours from dragonfly larvae – a ubiquitous and voracious tadpole  
200 predator commonly used in predator detection and avoidance experiments (Carlson  
201 and Langkilde 2013) – and conducted predator avoidance trails in a choice tank split  
202 into three zones: near predator, a middle zone and an away from predator zone (see  
203 Supplementary Appendix 2 for detailed experimental design). Trials were run with  
204 tadpoles from two source wetland pairs, as insufficient tadpoles were available from  
205 the third due to high mortality. For the first pair, we used 16 tadpoles per wetland, and  
206 for the second we used 12 tadpoles per wetland. For each source wetland, we  
207 conducted 12 replicates of each of four treatments: control (i.e. aged tap water) vs  
208 control, visual cues vs control, olfactory cues vs control, and visual and olfactory cues  
209 combined vs control. Therefore, we tested tadpoles from the first pair three times and  
210 tadpoles from the second pair four times, with no single tadpole experiencing the  
211 same treatment type more than once.

212 We placed a tadpole in the centre of a choice tank and provided cues at the  
213 beginning of the 2-min acclimation period. We recorded each 5-min trial using a  
214 GoPro Hero 3+, and calculated the proportion of time spent in the zone away from the  
215 predator cues relative to the time spent in the zone with the cues, based on recording  
216 the position of tadpoles every 30 seconds.

217

### 218 *Oviposition site preference*

219 We monitored oviposition preference concurrently with mesocosm experiments so  
220 that water and sediment quality would be largely comparable. We deployed three  
221 pairs of 100 L pre-formed, rock-style fibreglass ponds around each of the six source  
222 wetlands used in the mesocosm experiment. Each pond-pair simulated each wetland-  
223 pair used previously by reciprocally adding sediment and water from source wetlands.

224 This provided breeding frogs with a choice between two source wetland conditions  
225 with differing contaminant loads. We deployed pond-pairs approximately 10–40 m  
226 from the wetland edge, with the two ponds in a pair consistently deployed 50 cm apart  
227 in the same orientation. Pond-pairs were separated from each other by at least 50 m.  
228 Similar to the mesocosm experiments, we conducted fortnightly water changes.

229         Within each pond, we added a potted, semi-aquatic plant abundant at the  
230 source wetlands to encourage oviposition (foliage approximately 30 cm above water  
231 level; *Carex* sp.). We inspected ponds for egg masses at least weekly over the  
232 spring/summer breeding period. We removed and photographed egg masses, and  
233 emptied and refilled any pond containing eggs with sediment and water from the  
234 respective source wetland to avoid confounding future oviposition decisions.

235

## 236 ***Statistical analysis***

### 237 *Mesocosm experiment*

238 We analysed survival, mass and length at metamorphosis, time to metamorphosis,  
239 total periphyton dry weight, ash-free fry weight of periphyton (AFDW), water quality,  
240 and the concentration of heavy metals in sediments using linear models. For each  
241 model, we fitted treatment (HQ or LQ) as a fixed effect and location (wetland pair) as  
242 a blocking factor. Data were log-transformed for all variables except survival in order  
243 to meet the assumptions of normality and homogeneity of variances.

244

### 245 *Predator-avoidance trials*

246 To examine responses to predator cues, we used generalised linear mixed effects  
247 negative binomial models with treatment and cue (control, visual, olfactory and visual  
248 + olfactory) fitted as fixed effects, location as a blocking factor, and tadpole ID and

249 site (source wetland) fitted as random effects. Overdispersion was examined prior to  
250 calculation of model estimates and CIs. We constructed separate models for each  
251 treatment (i.e. HQ or LQ), and suppressed the intercepts so that model estimates and  
252 95% CIs could be extracted. Avoidance or attraction was then deemed significant  
253 when 95% CIs did not overlap 0.5 (i.e. no avoidance or attraction). In addition, since  
254 copper is a well-known olfactory disruptor in aquatic taxa (Tierney et al. 2010), we  
255 examined if the proportion of time spent away from predators was related to copper  
256 sediment concentration within mesocosms using linear regression, with treatment and  
257 copper concentration (log-transformed) fitted as fixed effects, and location as a  
258 blocking factor.

259

#### 260 *Oviposition-site preference experiment*

261 We assessed oviposition-site preference using Chi-squared tests of the conditions (HQ  
262 or LQ ponds) eggs were laid into and the conditions (HQ or LQ source wetland)  
263 ovipositing frogs came from as factors. The number of eggs per egg mass was  
264 analysed using a linear model with treatment fitted as a fixed effect and location as a  
265 blocking factor.

266 We assessed normality and homogeneity of variances before all analyses using  
267 Q-Q and residual plots, respectively. We performed analyses on R 3.2.2 (R  
268 Development Core Team 2015), using the lmerTest package (Kuznetsova et al. 2015)  
269 to fit mixed effects models.

270

271

## 272 **RESULTS**

273

274 ***Fitness responses***

275 All measured parameters were significantly different between HQ and LQ treatments  
276 (Table 1). Based on model estimates, tadpoles raised in HQ treatments exhibited on  
277 average 42.0% higher survival, took 5.9 days longer to reach metamorphosis, and  
278 were 0.29 g lighter and 2.5 mm shorter than those raised in LQ treatments (Fig. 1). No  
279 physical abnormalities were observed in any of the tadpoles.

280

281 ***Periphyton, water and sediment analysis***

282 Periphyton was approximately three times more abundant in LQ mesocosms (Table 1;  
283 Fig. 1). Water from LQ mesocosms also had higher pH, conductivity, total alkalinity,  
284 and phosphate levels (Table 2; Fig. 2; Supplementary Fig. 1). Although ammonia,  
285 nitrite and nitrate were often detected in LQ treatments, levels were below the  
286 detection limit for most HQ treatments (Supplementary Fig. 1). LQ mesocosms also  
287 had significantly higher concentrations of nine of the analysed metals (Fig. 2;  
288 Supplementary Table 2; Supplementary Fig. 1).

289

290 ***Predator avoidance trials***

291 Overdispersion was not present in the full generalized linear mixed model ( $\chi^2_{143} =$   
292 99.2,  $p = 0.99$ ). Control trials with no stimuli indicated no inherent biases in terms of  
293 preference for one side of the tank (Fig. 3). Tadpoles from different wetlands  
294 exhibited variable responses to olfactory cues from predators, but none responded to  
295 visual cues (Fig. 3). Tadpoles from HQ treatments significantly avoided predator  
296 odours, but those from LQ conditions showed a weak attraction to cues (Fig. 3).  
297 When visual and olfactory cues were provided simultaneously, all tadpoles responded  
298 by avoiding the stimuli (Fig. 3).

299           There was a significant, negative correlation between the proportion of time  
300 tadpoles spent away from predator olfactory cues and the copper concentration of  
301 mesocosm sediments ( $F_{1, 29} = 4.22$ ,  $p = 0.049$ ; Fig. 4). Tadpoles from the mesocosms  
302 with the lowest copper concentration (~16 mg/kg) spent more than 85% of the  
303 observations away from predators, while those raised in the highest concentration  
304 (~33 mg/kg) spent less than 20% of the observations away from predators (Fig. 4).

305

### 306 *Oviposition site preference*

307 In total, 23 egg masses were laid into both high- and low-quality ponds, with no effect  
308 of the quality of the site they were laid at, or the conditions they were laid into ( $\chi^2_1 = 0$ ,  
309  $p = 1$ ; Fig. 5). The number of eggs per egg mass was also similar for masses laid into  
310 different treatment conditions ( $F_{1, 42} = 0.51$ ,  $p = 0.47$ ; Fig. 5).

311

312

## 313 **DISCUSSION**

314 We provide the first empirical evidence that urban stormwater treatment wetlands can  
315 be equal-preference ecological traps for frogs. Tadpoles had lower survival and were  
316 less responsive to predator cues when raised in more polluted conditions, but breeding  
317 adults laid comparable numbers of eggs at sites regardless of habitat quality.

318           Amphibians are often considered sensitive to habitat modification and  
319 contamination (Blaustein et al. 1994, Sievers et al. *in press*), and survival differed  
320 most for the two source wetland pairs with the greatest differences in environmental  
321 conditions (see Supplementary Fig. 1, 2). Heavy metals (Lefcort et al. 1998),  
322 pesticides (Egea-Serrano et al. 2012), hydrocarbons (Jelaso et al. 2002) and nitrogen  
323 (Marco et al. 1999) can all reduce amphibian survival, and a combination of

324 contaminants can have even greater impacts (Relyea 2009). The concentration of  
325 metals within the mesocosms closely matched concentrations from initial wetland  
326 sampling (see Marshall et al. 2016, Sharley et al. 2017), so pesticide and hydrocarbon  
327 levels were also likely to have been higher within LQ treatments, further contributing  
328 to reduced survival. In addition, we would likely have observed even stronger fitness  
329 reductions if we also examined the survival of eggs and embryos, given these early  
330 life history stages can be very susceptible to contaminants (Brand et al. 2010).

331         Although survival was significantly reduced in LQ treatments, we also  
332 observed a reduction in the time taken to reach metamorphosis and an increase in the  
333 size of metamorphs. This may be a consequence of higher food availability, due to  
334 increased phosphates and nitrogen within more contaminated conditions, which  
335 enhances periphyton growth (Smith 1982). Additionally, greater food availability and  
336 enhanced development may be due to reduced competition because of density-  
337 dependent mortality effects. Alternatively, developing faster may allow animals to  
338 more quickly escape polluted conditions (Boyle et al. 2016). Although  
339 metamorphosis-related measures can be influenced by hydroperiod, predation and  
340 contamination (Edge et al. 2016), we found in a recent meta-analysis that survival and  
341 reproductive success differed more frequently between altered and reference wetlands  
342 than these measures, which were largely similar between wetland types (Sievers et al.  
343 *in press*). Indeed, many studies have reported conflicting findings on the effects of  
344 pollution on metamorphosis-related measures (Egea-Serrano et al. 2012). For example,  
345 in Ontario, Canada, tadpoles exposed to copper suffered reduced survival rates but  
346 increased growth rates (Leduc et al. 2016), while in Rhineland-Palatine, Germany,  
347 tadpoles exposed to cropland and the associated agrichemicals were smaller at, and  
348 took longer to reach, metamorphosis than tadpoles from natural wetlands (Wagner et

349 al. 2014). Therefore, in isolation, these measures may provide less reliable  
350 information on individual fitness and habitat quality.

351 Behaviour also differed between treatments. Tadpoles raised in more  
352 contaminated conditions failed to avoid olfactory stimuli from predators, which will  
353 likely further reduce survival rates within low-quality wetlands. The attraction  
354 towards predator olfactory cues (particularly by tadpoles raised in the most  
355 contaminated conditions) mirrors similar responses observed in Cuban tree frog  
356 tadpoles after exposure to the pesticide atrazine (Ehram et al. 2016). We also found  
357 that tadpoles exposed to higher copper concentrations within sediments were less  
358 likely to avoid predator odours. Copper is a known olfactory inhibitor in aquatic  
359 animals (Tierney et al. 2010), and environmentally relevant levels of copper in the  
360 water can reduce the capacity of tadpoles to detect predators, ultimately leading to  
361 increased predation rates (Hayden et al. 2015).

362 Importantly, stormwater wetlands typically contain a mixture of contaminants,  
363 often at individual concentrations below those deemed biologically relevant (Allinson  
364 et al. 2017). Therefore, although frogs can discriminate and avoid unfavourable  
365 conditions (e.g. Vonesh and Buck 2007), their ability to do so may be reduced when  
366 they are exposed to complex mixtures of contaminants. As a consequence, females  
367 may lay eggs in sites that are highly polluted in multiple ways, rather than them being  
368 redirected to higher quality sites elsewhere, with implications for offspring fitness,  
369 and potentially population persistence.

370 It is critical that habitat preference is studied using an appropriate design to  
371 reflect the natural behaviour of the focal species. We offered breeding frogs a  
372 simultaneous choice between high- and low-quality conditions, an approach that is  
373 appropriate for urban stormwater treatment wetlands that are often made up of

374 multiple, connected, sections, constructed in a deliberate sequence to maximise  
375 capture and treatment of contaminants (Lawrence and Breen 1998). As a consequence,  
376 within a single stormwater wetland network, breeding amphibians may be able to  
377 choose between areas of differing contaminant concentrations, with particular sections  
378 (e.g. sediment settlement ponds) more likely to act as ecological traps. Furthermore,  
379 although we tested habitat selection with two conditions provided simultaneously, it is  
380 possible that some amphibians exhibit sequential habitat selection, where multiple  
381 habitats may be assessed in turn until one meeting a certain threshold for acceptance  
382 is encountered (Stamps et al. 2005). In addition, a recent study found that more than  
383 20% of frogs within a breeding population bred at more than one wetland (Lannoo et  
384 al. 2017), suggesting some that amphibians will be required to make multiple choices  
385 amongst breeding sites. While research into ecological traps is still in its infancy, the  
386 discovery and apparent prevalence of traps is starting to increase along with  
387 appreciation of the significant problem they pose for wildlife (Hale and Swearer 2016,  
388 Robertson and Chalfoun 2016). Regardless of how dispersing or breeding frogs are  
389 selecting sites, our results suggest that contaminated urban stormwater treatment  
390 wetlands are capable of reducing juvenile fitness, have the capacity to attract breeding  
391 frogs, and ultimately threaten population viability because of their capacity to  
392 function as ecological traps. Given the global vulnerability of amphibians and the  
393 increasing predominance of, and reliance by wildlife on, stormwater treatment  
394 wetlands in cities around the world, understanding the ecological risk these artificial  
395 systems pose is critical to successful wildlife management.

396

397 ***Management implications***

398 When will the presence of low-quality stormwater wetlands become detrimental to  
399 amphibian populations, and how can we manage this risk? Even low-quality wetlands  
400 may be beneficial in some instances (Hale et al. 2015b). For example, if urban areas  
401 are built in landscapes previously devoid of natural wetlands, the construction of  
402 stormwater wetlands provides frogs with aquatic habitat that was not there  
403 previously. Even if the quality of these stormwater wetlands is relatively low, the  
404 local frog (meta)population may still increase in size because of this new habitat.  
405 However, if these wetlands are attracting and drawing in dispersing and breeding  
406 individuals away from high-quality habitats, the presence of these ecological traps can  
407 have serious consequences for metapopulations (Hale et al. 2015b). A logical next  
408 step would be to quantify both the net effect of urban development on the availability  
409 of stormwater and natural wetlands within the landscape, and the effect of these  
410 changes on local and regional (meta)populations. From this, the full impact of having  
411 stormwater wetlands functioning as ecological traps within urban landscapes can be  
412 evaluated at a scale more appropriate to managers.

413         When the construction of a stormwater wetland is likely to lead to the creation  
414 of an ecological trap, we need to consider how best to mitigate the risks it poses to  
415 animals. This could involve managers: (1) conserving and maintaining high-quality  
416 wetlands in the landscape; (2) restricting access by wildlife; (3) determining the cues  
417 used by animals when selecting wetlands and removing these cues, or adding them to  
418 high-quality sites; (4) finding alternative stormwater treatment solutions in sensitive  
419 areas; (5) strategically positioning stormwater wetlands within urban landscapes to  
420 limit their connectivity to other aquatic habitats; or (6) constructing high-quality, off-  
421 line wetlands that do not receive large volumes of stormwater near stormwater  
422 wetlands.

423           The active management of high-quality wetlands is an obvious management  
424 action to ensure at least some viable habitat is available for aquatic and semi-aquatic  
425 animals. For example, the dredging of accumulated sediment and organic debris to  
426 physically remove contaminants can improve habitat quality for wildlife, leading to  
427 greater amphibian richness and abundance (Stevens et al. 2002). Instigating a regular  
428 maintenance regime, however, has its limitations due to unknown pollutant  
429 accumulation rates, intermittent pollution events, and the substantial costs associated  
430 with intensive management regimes (Weiss et al. 2007).

431           Alternatively, stormwater wetlands can be designed or modified to exclude  
432 animals from potential traps. For example, designing wetland banks/edges with steep  
433 slopes may make sites less attractive than those with the gently sloping, vegetated  
434 banks that suit most amphibian species. However, steep banks/edges may also result  
435 in increased mortality of species whose metamorphs cannot escape the water and  
436 drown (Parris 2006), and this design likely reduces the overall capacity to vegetate  
437 wetlands. Other options to prevent animals accessing highly-polluted sites include  
438 displaying predator mimics, utilising sonic deterrents, or erecting exclusion fences  
439 (Martin 1979, Bomford and O'Brien 1990, Letnic et al. 2015).

440           It may also be possible to use our understanding of animal behaviour (Greggor  
441 et al. 2014) to discourage settlement. For example, removing fringing vegetation from  
442 wetlands is a broad-scale approach to eliminate a probable cue used by amphibians  
443 when selecting oviposition sites (Egan and Paton 2004). However, given the  
444 important role vegetation plays in reducing contaminants and improving the aesthetics  
445 of urban stormwater treatment wetlands, this method may only be suitable for the  
446 protection of specific species in need of conservation, with vegetation removal limited  
447 to certain host plant species. This strategy requires detailed knowledge of species'

448 behaviour, habitat preferences and selection cues, and thus remains largely  
449 unexplored (although see Robertson 2012, Greggor et al. 2014). Indeed, relatively  
450 little is known about the specific habitat-selection cues amphibians use when  
451 choosing breeding sites and this remains an important and interesting area for future  
452 research.

453         Employing proactive strategies to pre-treat stormwater prior to release such as  
454 hybrid filtration methods have obvious broader benefits in that it likely benefits the  
455 entire wetland ecosystem. Methods such as deep sump catch basins, settling devices,  
456 vegetated filter strips and sand filtration would reduce overall inputs to wetlands (e.g.  
457 Johir et al. 2010), and could improve the survival of animals exposed to stormwater  
458 (Spromberg et al. 2016). For example, high mortality adult coho salmon suffer when  
459 exposed to unfiltered stormwater can be prevented when this water is pre-treated by  
460 soil infiltration (an inexpensive, green infrastructure technology; Spromberg et al.  
461 2016).

462         However, despite this diversity of potential management options, combining  
463 the promotion of wildlife with pollution management (i.e. multi-objective  
464 management; Benyamine et al. 2004) may still be detrimental to animal populations.  
465 In addition, active monitoring in, and proactive management of, a large number of  
466 stormwater wetlands may be largely unrealistic in most circumstances. The strategic  
467 placement of stormwater wetlands within the landscape, or creating off-line wetlands  
468 near stormwater wetlands may enhance and promote urban wildlife. Theoretical  
469 models have shown that metapopulations will be most compromised when ecological  
470 traps represent a large proportion of available habitats, are highly attractive, and  
471 severely reduce individual fitness (Hale et al. 2015b). Therefore, creating stormwater  
472 wetlands predicted to receive considerable contamination beyond the dispersal

473 distance of most animals will help prevent, or at least reduce, colonisation, and  
474 ultimately lower the risk that emigrants will settle into ecological traps. Alternatively,  
475 creating off-line wetlands disconnected from the urban stormwater network provides  
476 animals with habitat that is likely to be higher quality than even the least polluted  
477 stormwater wetlands. Given our current findings, research is first needed to determine  
478 whether animals are capable of making good decisions in this circumstance (i.e.  
479 choosing “unpolluted” off-line *versus* “polluted” on-line wetlands), and, thus, that the  
480 nearby on-line stormwater wetland is not functioning as an ecological trap.

481 Ecological traps are an emerging global ecological and conservation issue  
482 (Robertson and Chalfoun 2016). Identifying the potential for habitats to function as  
483 ecological traps – as we have done here – is important for minimising the harm and  
484 realising the ecological potential of human-altered habitats. Our results highlight the  
485 potential for some stormwater wetlands to impact individuals, with the assertion that  
486 this may have demographic implications. We need to better understand how a wider  
487 range of stormwater wetlands perform as habitat, particularly in terms of these  
488 broader landscape-level consequences if some stormwater wetlands are functioning as  
489 ecological traps. Future empirical studies should document how other animals  
490 respond when these habitats vary in quality, and what fitness costs are incurred,  
491 preferably using individual-level metrics (e.g. survival, growth) in addition to the  
492 more commonly used community- or population-level metrics which only indicate  
493 that animals are present at a site (Sievers et al. *in press*). In general, when traps exist,  
494 we recommend identifying the factors responsible for reduced fitness, determining the  
495 specific cues animals rely on when selecting habitats, and assessing the potential to  
496 manipulate these cues to eliminate the trap (Hale et al. 2015a). Ultimately, given the  
497 growth in stormwater treatment wetlands in cities around the world, minimising the

498 ecological costs these artificial habitats can impose will be critical for sustaining  
499 aquatic animal populations in urban environments.

500

501

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511

512

## 513 **DATA ACCESSIBILITY**

514 Data and metadata will be made publicly available on Figshare.

515

516

517 **LITERATURE CITED**

- 518 Allinson, M., P. Zhang, A. Bui, J. H. Myers, V. Pettigrove, G. Rose, S. A. Salzman, R.  
519 Walters, and G. Allinson. 2017. Herbicides and trace metals in urban waters in  
520 Melbourne, Australia (2011–12): concentrations and potential impact.  
521 *Environmental Science and Pollution Research* **24**:7274-7284.
- 522 Benyamine, M., M. Backstrom, and P. Sanden. 2004. Multi-objective environmental  
523 management in constructed wetlands. *Environmental Monitoring and Assessment*  
524 **90**:171-185.
- 525 Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994. Amphibian declines: judging  
526 stability, persistence, and susceptibility of populations to local and global  
527 extinctions. *Conservation Biology* **8**:60-71.
- 528 Bomford, M., and P. H. O'Brien. 1990. Sonic deterrents in animal damage control: a  
529 review of device tests and effectiveness. *Wildlife Society Bulletin (1973-2006)*  
530 **18**:411-422.
- 531 Boyle, R. L., M. N. Hoak, V. J. Pettigrove, A. A. Hoffmann, and S. M. Long. 2016.  
532 Comparing the impacts of sediment-bound bifenthrin on aquatic  
533 macroinvertebrates in laboratory bioassays and field microcosms. *Ecotoxicology*  
534 *and Environmental Safety* **133**:489-500.
- 535 Brand, A. B., and J. W. Snodgrass. 2010. Value of Artificial Habitats for Amphibian  
536 Reproduction in Altered Landscapes. *Conservation Biology* **24**:295-301.
- 537 Brand, A. B., J. W. Snodgrass, M. T. Gallagher, R. E. Casey, and R. Van Meter. 2010.  
538 Lethal and Sublethal Effects of Embryonic and Larval Exposure of *Hyla*  
539 *versicolor* to Stormwater Pond Sediments. *Archives of Environmental*  
540 *Contamination and Toxicology* **58**:325-331.
- 541 Broomhall, S. D. 2004. Egg temperature modifies predator avoidance and the effects of  
542 the insecticide endosulfan on tadpoles of an Australian frog. *Journal of Applied*  
543 *Ecology* **41**:105-113.
- 544 Carlson, B. E., and T. Langkilde. 2013. A common marking technique affects tadpole  
545 behavior and risk of predation. *Ethology* **119**:167-177.
- 546 Donovan, T. M., and F. R. Thompson. 2001. Modelling the ecological trap hypothesis: a  
547 habitat and demographic analysis for migrant songbirds. *Ecological Applications*  
548 **11**:871-882.
- 549 Edge, C. B., J. E. Houlahan, D. A. Jackson, and M. J. Fortin. 2016. The response of  
550 amphibian larvae to environmental change is both consistent and variable. *Oikos*.
- 551 Egan, R. S., and P. W. Paton. 2004. Within-pond parameters affecting oviposition by  
552 wood frogs and spotted salamanders. *Wetlands* **24**:1-13.
- 553 Egea-Serrano, A., R. A. Relyea, M. Tejedo, and M. Torralva. 2012. Understanding of the  
554 impact of chemicals on amphibians: a meta-analytic review. *Ecology and*  
555 *evolution* **2**:1382-1397.
- 556 Ehrsam, M., S. A. Knutie, and J. R. Rohr. 2016. The herbicide atrazine induces  
557 hyperactivity and compromises tadpole detection of predator chemical cues.  
558 *Environmental Toxicology and Chemistry* **35**:2239-2244.
- 559 Ficken, K., and P. G. Byrne. 2013. Heavy metal pollution negatively correlates with  
560 anuran species richness and distribution in south-eastern Australia. *Austral*  
561 *Ecology* **38**:523-533.

- 562 Gallagher, M. T., J. W. Snodgrass, A. B. Brand, R. E. Casey, S. M. Lev, and R. J. Van  
563 Meter. 2014. The role of pollutant accumulation in determining the use of  
564 stormwater ponds by amphibians. *Wetlands Ecology and Management* **22**:551-  
565 564.
- 566 Greggor, A. L., N. S. Clayton, B. Phalan, and A. Thornton. 2014. Comparative cognition  
567 for conservationists. *Trends in Ecology & Evolution* **29**:489-495.
- 568 Hale, R., R. Coleman, V. Pettigrove, and S. E. Swearer. 2015a. Review: Identifying,  
569 preventing and mitigating ecological traps to improve the management of urban  
570 aquatic ecosystems. *Journal of Applied Ecology* **52**:928-939.
- 571 Hale, R., and S. E. Swearer. 2016. Ecological traps: current evidence and future  
572 directions. *Proceedings of the Royal Society of London B: Biological Sciences*  
573 **283**:20152647.
- 574 Hale, R., E. A. Treml, and S. E. Swearer. 2015b. Evaluating the metapopulation  
575 consequences of ecological traps. *Proceedings of the Royal Society of London B:*  
576 *Biological Sciences* **282**:20142930.
- 577 Halpern, B. S., M. Frazier, J. Potapenko, K. S. Casey, K. Koenig, C. Longo, J. S.  
578 Lowndes, R. C. Rockwood, E. R. Selig, and K. A. Selkoe. 2015. Spatial and  
579 temporal changes in cumulative human impacts on the world's ocean. *Nature*  
580 *communications* **6**.
- 581 Hamer, A. J., and K. M. Parris. 2011. Local and landscape determinants of amphibian  
582 communities in urban ponds. *Ecological Applications* **21**:378-390.
- 583 Hammer, D. A. 1989. *Constructed wetlands for wastewater treatment: municipal,*  
584 *industrial and agricultural.* CRC Press.
- 585 Hanlon, S. M., and R. Relyea. 2013. Sublethal effects of pesticides on predator-prey  
586 interactions in amphibians. *Copeia* **2013**:691-698.
- 587 Hassall, C., and S. Anderson. 2015. Stormwater ponds can contain comparable  
588 biodiversity to unmanaged wetlands in urban areas. *Hydrobiologia* **745**:137-149.
- 589 Hayden, M. T., M. K. Reeves, M. Holyoak, M. Perdue, A. L. King, and S. C. Tobin.  
590 2015. Thrice as easy to catch! Copper and temperature modulate predator-prey  
591 interactions in larval dragonflies and anurans. *Ecosphere* **6**:1-17.
- 592 Jelaso, A. M., E. Lehigh-Shirey, A. Predenkiewicz, J. Means, and C. F. Ide. 2002.  
593 Aroclor 1254 alters morphology, survival, and gene expression in *Xenopus laevis*  
594 tadpoles. *Environmental and Molecular Mutagenesis* **40**:24-35.
- 595 Jahir, M., S. Vigneswaran, and J. Kandasamy. 2010. Hybrid filtration method for pre-  
596 treatment of stormwater. *Water Science and Technology* **62**:2937-2943.
- 597 Kentula, M. E., S. E. Gwin, and S. M. Pierson. 2004. Tracking changes in wetlands with  
598 urbanization: Sixteen years of experience in Portland, Oregon, USA. *Wetlands*  
599 **24**:734-743.
- 600 Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2015. Package 'lmerTest'. R  
601 package version **2**.
- 602 Lannoo, M. J., R. M. Stiles, M. A. Sisson, J. W. Swan, V. C. Terrell, and K. E. Robinson.  
603 2017. Patch Dynamics Inform Management Decisions in a Threatened Frog  
604 Species. *Copeia* **105**:53-63.
- 605 Lawrence, I., and P. F. Breen. 1998. *Design guidelines: Stormwater pollution control*  
606 *ponds and wetlands.* Cooperative Research Centre for Freshwater Ecology.

607 Leduc, J., P. Echaubard, V. Trudeau, and D. Lesbarrères. 2016. Copper and nickel effects  
608 on survival and growth of northern leopard frog (*Lithobates pipiens*) tadpoles in  
609 field-collected smelting effluent water. *Environmental Toxicology and Chemistry*  
610 **35**:687-694.

611 Lefcort, H., R. Meguire, L. Wilson, and W. Ettinger. 1998. Heavy metals alter the  
612 survival, growth, metamorphosis, and antipredatory behavior of Columbia spotted  
613 frog (*Rana luteiventris*) tadpoles. *Archives of Environmental Contamination and*  
614 *Toxicology* **35**:447-456.

615 Letnic, M., J. K. Webb, T. S. Jessop, and T. Dempster. 2015. Restricting access to  
616 invasion hubs enables sustained control of an invasive vertebrate. *Journal of*  
617 *Applied Ecology* **52**:341-347.

618 Marco, A., C. Quilchano, and A. R. Blaustein. 1999. Sensitivity to nitrate and nitrite in  
619 pond-breeding amphibians from the Pacific northwest, USA. *Environmental*  
620 *Toxicology and Chemistry* **18**:2836-2839.

621 Marshall, S., D. Sharley, K. Jeppe, S. Sharp, G. Rose, and V. Pettigrove. 2016.  
622 Potentially toxic concentrations of synthetic pyrethroids associated with low  
623 density residential land use. *Frontiers in Environmental Science* **4**:75.

624 Martin, L. R. 1979. Effective use of sound to repel birds from industrial waste ponds.

625 Monastersky, R. 2014. Biodiversity: Life--a status report. *nature* **516**:158-161.

626 Parris, K. M. 2006. Urban amphibian assemblages as metacommunities. *Journal of*  
627 *Animal Ecology* **75**:757-764.

628 Pettigrove, V., and A. Hoffmann. 2005. A field-based microcosm method to assess the  
629 effects of polluted urban stream sediments on aquatic macroinvertebrates.  
630 *Environmental Toxicology and Chemistry* **24**:170-180.

631 R Development Core Team. 2015. R: A language and environment for statistical  
632 computing. R Foundation for Statistical Computing, Vienna, Austria.

633 Reich, P., and B. J. Downes. 2004. Relating larval distributions to patterns of oviposition:  
634 evidence from lotic hydrobiosid caddisflies. *Freshwater Biology* **49**:1423-1436.

635 Relyea, R. A. 2009. A cocktail of contaminants: how mixtures of pesticides at low  
636 concentrations affect aquatic communities. *Oecologia* **159**:363-376.

637 Resetarits, W. J., and A. Silberbush. 2016. Local contagion and regional compression:  
638 habitat selection drives spatially explicit, multiscale dynamics of colonisation in  
639 experimental metacommunities. *Ecology Letters* **19**:191-200.

640 Resetarits, W. J., and H. M. Wilbur. 1989. Choice of oviposition site by *Hyla*  
641 *chrysoscelis*: role of predators and competitors. *Ecology* **70**:220-228.

642 Robertson, B. A. 2012. Investigating Targets of Avian Habitat Management to Eliminate  
643 an Ecological Trap. *Avian Conservation and Ecology* **7**:2.

644 Robertson, B. A., and A. D. Chalfoun. 2016. Evolutionary traps as keys to understanding  
645 behavioral maladaptation. *Current Opinion in Behavioral Sciences* **12**:12-17.

646 Robertson, B. A., and R. L. Hutto. 2006. A framework for understanding ecological traps  
647 and an evaluation of existing evidence. *Ecology* **87**:1075-1085.

648 Robertson, B. A., J. S. Rehage, and A. Sih. 2013. Ecological novelty and the emergence  
649 of evolutionary traps. *Trends in Ecology and Evolution* **28**:552-560.

650 Rudolf, V. H. W., and M. O. Rodel. 2005. Oviposition site selection in a complex and  
651 variable environment: the role of habitat quality and conspecific cues. *Oecologia*  
652 **142**:316-325.

653 Sharley, D. J., S. M. Sharp, S. Marshall, K. Jeppe, and V. J. Pettigrove. 2017. Linking  
654 urban land use to pollutants in constructed wetlands: Implications for stormwater  
655 and urban planning. *Landscape and Urban Planning* **162**:80-91.

656 Sievers, M., R. Hale, K. M. Parris, and S. E. Swearer. *in press*. Impacts of human-  
657 induced environmental change in wetlands on aquatic animals. *Biological*  
658 *Reviews*.

659 Smith, V. H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes:  
660 an empirical and theoretical analysis. *Limnology and Oceanography* **27**:1101-  
661 1111.

662 Snodgrass, J. W., R. E. Casey, D. Joseph, and J. A. Simon. 2008. Microcosm  
663 investigations of stormwater pond sediment toxicity to embryonic and larval  
664 amphibians: Variation in sensitivity among species. *Environmental Pollution*  
665 **154**:291-297.

666 Sparling, D. W., J. D. Eisemann, and W. Kuenzel. 2004. Contaminant exposure and  
667 effects in red-winged blackbirds inhabiting stormwater retention ponds.  
668 *Environmental Management* **33**:719-729.

669 Spromberg, J. A., D. H. Baldwin, S. E. Damm, J. K. McIntyre, M. Huff, C. A. Sloan, B.  
670 F. Anulacion, J. W. Davis, and N. L. Scholz. 2016. Coho salmon spawner  
671 mortality in western US urban watersheds: bioinfiltration prevents lethal storm  
672 water impacts. *Journal of Applied Ecology* **53**:398-407.

673 Stamps, J. A., V. Krishnan, and M. L. Reid. 2005. Search costs and habitat selection by  
674 dispersers. *Ecology* **86**:510-518.

675 Stevens, C. E., A. W. Diamond, and T. S. Gabor. 2002. Anuran call surveys on small  
676 wetlands in Prince Edward Island, Canada restored by dredging of sediments.  
677 *Wetlands* **22**:90-99.

678 Tierney, K. B., D. H. Baldwin, T. J. Hara, P. S. Ross, N. L. Scholz, and C. J. Kennedy.  
679 2010. Olfactory toxicity in fishes. *Aquatic toxicology* **96**:2-26.

680 Tixier, G. T., M. Lafont, L. Grapentine, Q. Rochfort, and J. Marsalek. 2011. Ecological  
681 risk assessment of urban stormwater ponds: literature review and proposal of a  
682 new conceptual approach providing ecological quality goals and the associated  
683 bioassessment tools. *Ecological Indicators* **11**:1497-1506.

684 Vonesh, J. R., and J. C. Buck. 2007. Pesticide alters oviposition site selection in gray  
685 treefrogs. *Oecologia* **154**:219-226.

686 Vonesh, J. R., and J. M. Kraus. 2009. Pesticide alters habitat selection and aquatic  
687 community composition. *Oecologia* **160**:379-385.

688 Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green,  
689 S. Glidden, S. E. Bunn, C. A. Sullivan, and C. Reidy Liermann. 2010. Global  
690 threats to human water security and river biodiversity. *nature* **467**:555.

691 Wagner, N., W. Zueghart, V. Mingo, and S. Loetters. 2014. Are deformation rates of  
692 anuran developmental stages suitable indicators for environmental pollution?  
693 Possibilities and limitations. *Ecological Indicators* **45**:394-401.

694 Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P.  
695 Morgan. 2005. The urban stream syndrome: current knowledge and the search for  
696 a cure. *Journal of the North American Benthological Society* **24**:706-723.

697 Ward, A. J. W., and T. Mehner. 2010. Multimodal mixed messages: the use of multiple  
698 cues allows greater accuracy in social recognition and predator detection

699 decisions in the mosquitofish, *Gambusia holbrooki*. Behavioral Ecology **21**:1315-  
700 1320.  
701 Weiss, P. T., J. S. Gulliver, and A. J. Erickson. 2007. Cost and pollutant removal of  
702 storm-water treatment practices. Journal of Water Resources Planning and  
703 Management **133**:218-229.  
704  
  
705

706 **Tables**

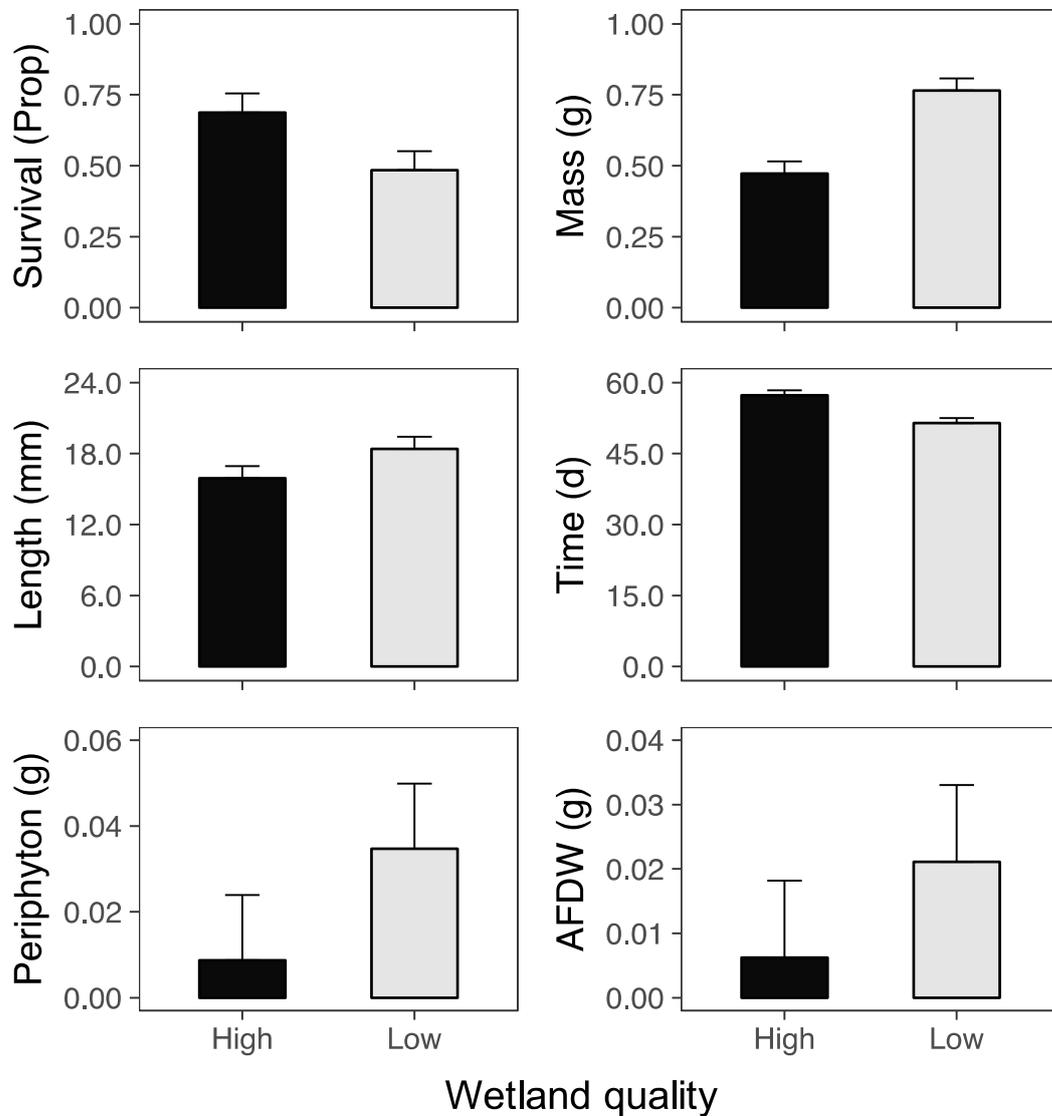
707 Table 1. Output from ANOVA for the mesocosm experiment with treatment ('high' or  
 708 'low' quality conditions) and location (wetland pair) fitted as fixed effects. Significant p-  
 709 values are in boldface. Asterisks indicate measures that were log-transformed to meet the  
 710 assumptions of normality and homogeneity of variance.

711

<b>Measure</b>	<b>Factor</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
Survival	Treatment	1	0.180	5.10	<b>0.030</b>
	Location	2	0.087	2.40	0.104
	Treat×Loc	2	0.085	2.30	0.111
	Residuals	42	0.036		
*Mass at metamorphosis	Treatment	1	0.034	12.61	<b>0.001</b>
	Location	2	0.006	2.24	0.120
	Treat×Loc	2	0.006	2.09	0.137
	Residuals	40	0.003		
*Length at metamorphosis	Treatment	1	0.022	14.20	<b>&lt;0.001</b>
	Location	2	0.006	3.97	<b>0.027</b>
	Treat×Loc	2	0.004	2.53	0.093
	Residuals	40	0.002		
*Time to metamorphosis	Treatment	1	0.029	13.45	<b>&lt;0.001</b>
	Location	2	0.007	3.18	0.052
	Treat×Loc	2	0.005	2.43	0.101
	Residuals	40	0.002		
*Periphyton	Treatment	1	0.0034	9.85	<b>0.003</b>
	Location	2	0.0005	1.35	0.270
	Treat×Loc	2	0.0001	0.40	0.673
	Residuals	42	0.0003		
*Ash-free dry-weight	Treatment	1	0.0022	10.30	<b>0.003</b>
	Location	2	0.0003	1.50	0.235
	Treat×Loc	2	0.0002	0.84	0.440
	Residuals	42	0.0002		

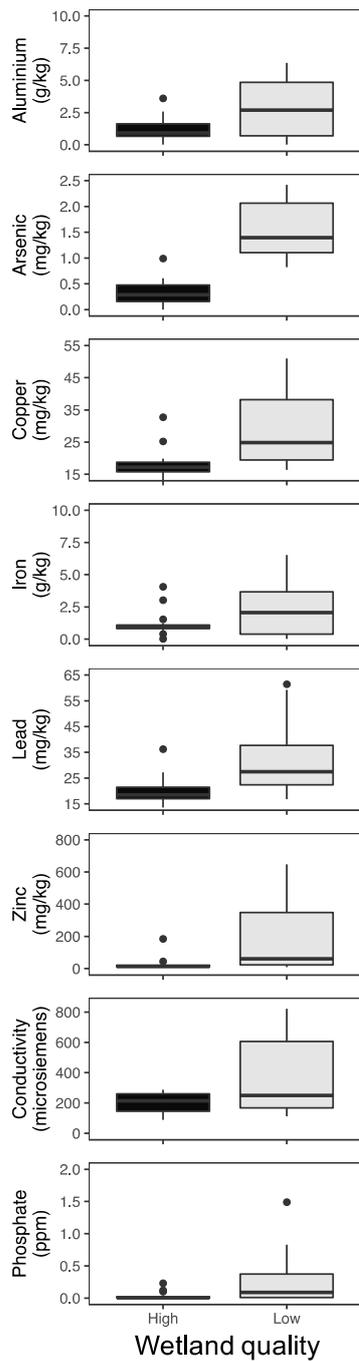
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713 **Figures**



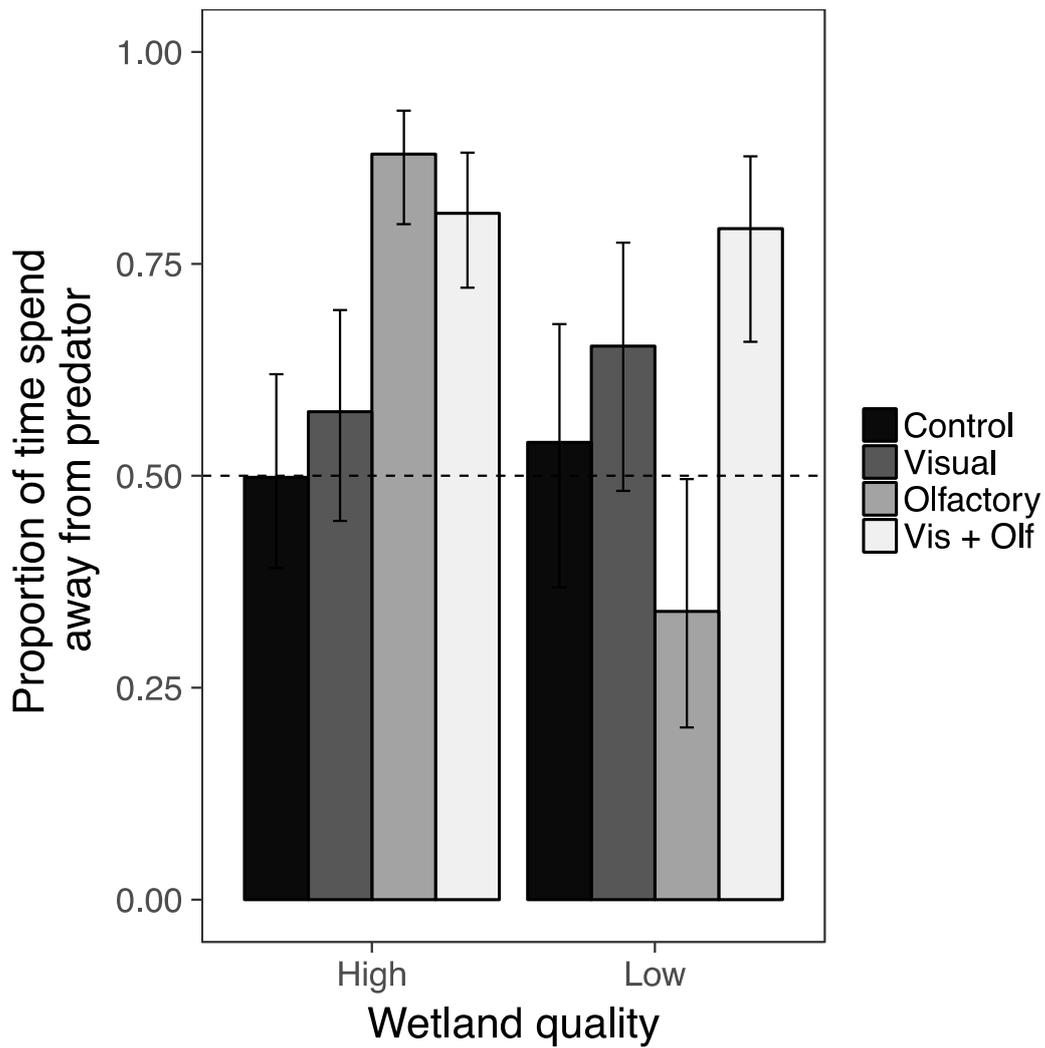
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715 Figure 1. Model estimates (+ SE) for tadpole fitness (survival to, mass at, length at, and  
716 time to metamorphosis) and food availability (periphyton dry weight and periphyton ash-  
717 free dry weight; AFDW – g/dm<sup>2</sup>) based on linear model estimates with treatment (‘high’  
718 or ‘low’ quality conditions) and location (wetland pair) fitted as fixed effects.



719

720 Figure 2. A subset of the sediment heavy metal concentrations (mg/kg), and the  
 721 conductivity (microsiemens;  $\mu\text{S}$ ) and phosphate ( $\text{PO}_4$ ; ppm) concentrations within  
 722 mesocosm water. N = 24 per treatment. Note different x-axis units for some metals. For  
 723 visualisations of all measured sediment and water variables see Supplementary Figure 2.

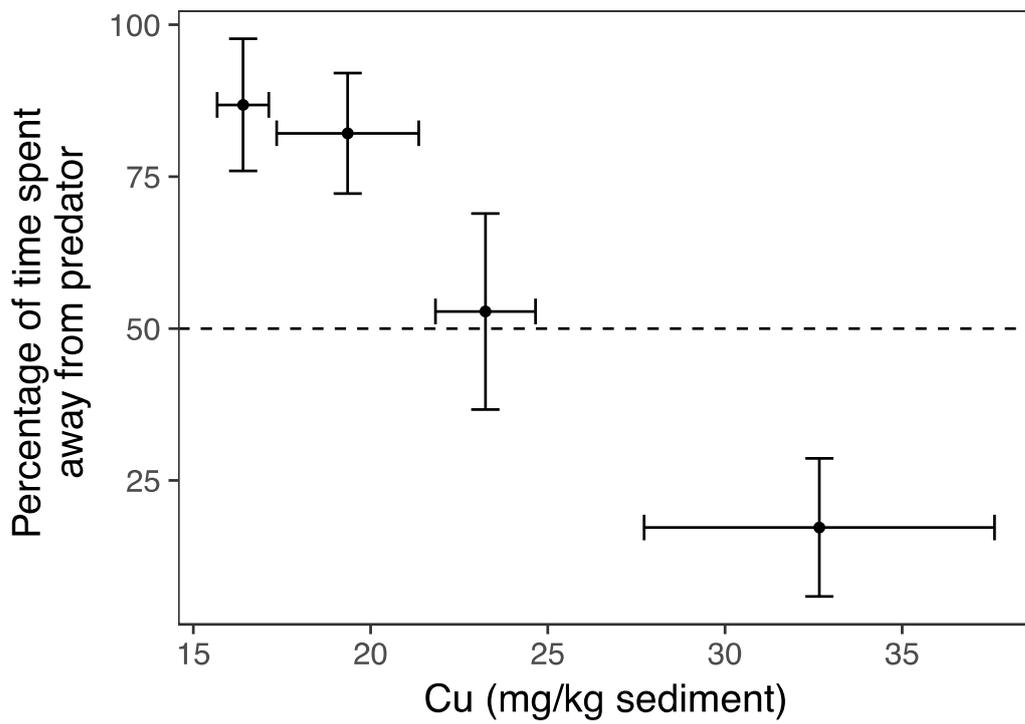


724

725 Figure 3. Median (+95% CI) model estimates from generalized linear mixed effects

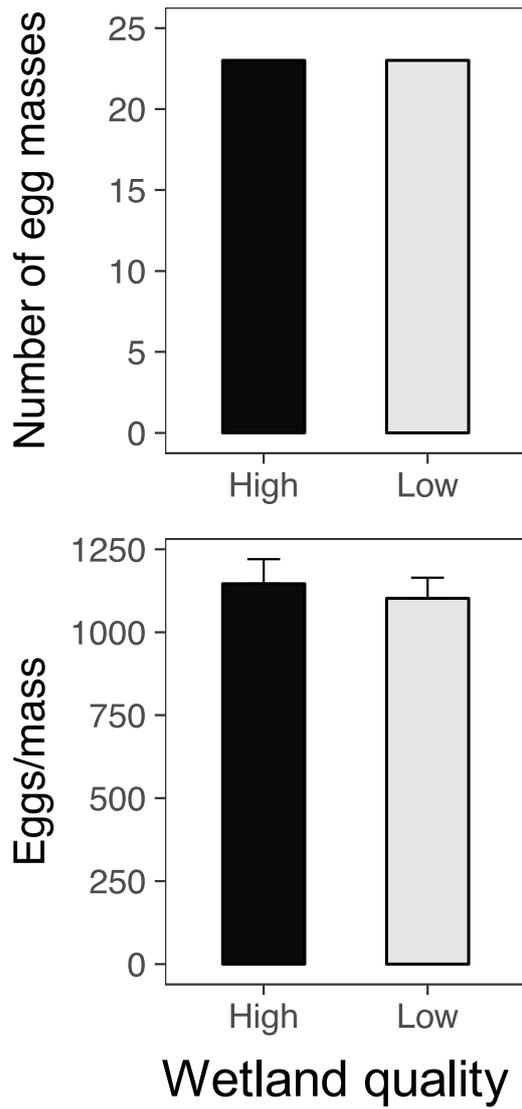
726 model for the proportion of time spent in the ‘away from predator’ zone relative to the

727 ‘near predator zone’ in the choice tank.



728

729 Figure 4. Relationship between copper concentration (mg/kg) within mesocosm  
 730 sediments and predator odour avoidance behaviour of tadpoles raised in conditions  
 731 simulating Lynbrook, Chandler, Yarrabing and Ringwood wetland conditions. Error bars  
 732 are standard errors.



733

734 Figure 5. Oviposition site selection: the number of egg masses and the number of eggs

735 per egg mass (+SE) laid into high- and low-quality conditions.

736

737 **SUPPLEMENTARY INFORMATION**

738 ***Supplementary Appendix 1 - Sediment digestion and ICP-OES analyses***

739 All reagents were of analytical reagent grade and were used as received. Deionised  
740 water was used for the preparation of all solutions. Solutions of hydrochloric and  
741 nitric acids were prepared by dilutions of the corresponding concentrated reagents.  
742 Reverse *aqua regia* was made by mixing concentrated nitric and hydrochloric acids in  
743 a ratio of 3:1.

744         Oven dried (50°C) sediment samples (0.5 g) were digested with 5 ml of  
745 reverse aqua regia solutions. Samples were weighed into 75 ml volumetric  
746 borosilicate digest tubes which were placed into a heating block digester. Each  
747 sediment sample was mixed with the digestion solution and left for  $16 \pm 1$  h  
748 (overnight) at room temperature (pre-digestion step). Then the suspension was heated  
749 at 110 °C for 5 h (digestion step). This temperature was chosen as the average  
750 digestion temperature of frequently used soil digestion methods. After the digestion,  
751 the samples were cooled down to room temperature and made up to 75 ml. Digest  
752 samples were then filtered and dispensed into sampling vials for elemental analyses  
753 using a Perkin Elmer Optima 8300 ICP-OES equipped with a charge coupled solid  
754 state detector and an auto sampler. Elemental concentrations were derived using linear  
755 calibration of the operating software was Syngistix. The analysis was performed  
756 under the following conditions: RF power at 1300 W, plasma flow at 15.0 L/min,  
757 auxiliary flow at 0.2 L/min, and nebulizer gas flow at 0.8 L/min. The spectrometer  
758 was set to normal resolution with a normal purge gas flow and read delay time of 30 s.  
759 Five replicate readings were made for each analytical sample. The sample flow rate  
760 was 1.50 ml/min with a flush time of five seconds between samples. The wash  
761 parameters were set as wash rate 1.5 ml/min and wash time 30 s.

762 ***Supplementary Appendix 2 - Predator avoidance trials***

763 We obtained locally collected dragonfly larvae (Suborder: Epiprocta) – a  
764 ubiquitous and voracious tadpole predator commonly used in predator detection and  
765 avoidance experiments (e.g. Carlson and Langkilde 2013, Hanlon and Relyea 2013).  
766 To harvest predator odours, we kept 36 dragonfly larvae in 4 L of aged tap water for  
767 18 h, which was diluted to 20 L, resulting in a concentration of 1 larva per 0.56 L of  
768 odour water (similar to Carlson and Langkilde 2013, Ehram et al. 2016). All cue  
769 water was made fresh and used within 12 h.

770 We ran predator avoidance trials with tadpoles from Pairs 1 and 3 only, as  
771 insufficient tadpoles were available from the other pair due to high mortality. We  
772 collected tadpoles from mesocosms using tadpole traps made from inverted 600 mL  
773 water bottles with lettuce inside. For the Lynbrook-Chandler trials, we collected two  
774 tadpoles from each mesocosm (16 tadpoles per source wetland; Length:  $50.4 \pm 1.9$  mm;  
775 mean  $\pm$  SE). For the Yarrabing-Ringwood trials, tadpoles were not available from two  
776 mesocosms from each source wetland, so we collected 12 tadpoles per source wetland  
777 (Length:  $45.9 \pm 1.6$  mm). Tadpoles were held individually in 0.5 L plastic containers  
778 with source water in the laboratory at  $\sim 25^\circ\text{C}$  and under a 12L:12D light cycle before  
779 trials.

780 For each wetland, we conducted 12 replicates of each of four treatments:  
781 control (i.e. aged tap water) vs control, visual cues vs control, olfactory cues vs control,  
782 and visual and olfactory cues combined vs control. Given there were 32 tadpoles for  
783 the Chandler-Lynbrook trials and 24 for the Ringwood-Yarrabing, we tested the  
784 former tadpoles three times, whilst we tested the latter four times. No single tadpole  
785 experienced the same treatment type more than once.

786 We conducted each trial within a glass choice tank (50 x 14 x 15 cm; L x W x  
787 H), with side glass tanks (30 x 14 x 15 cm) to provide visual cues, and two containers  
788 above to provide olfactory cues (Ward and Mehner 2010). We created sections within  
789 the choice tank to create three 'zones' (two key zones at either end, 11 cm in length,  
790 and a middle zone, 28 cm in length). We provided visual cues by holding ten  
791 dragonfly larvae in a side tank, with the larvae pushed up to the side nearest the  
792 choice tank using a piece of plastic (reducing the functional tank length from 30cm to  
793 10cm; Ward and Mehner 2010). We also added a similar piece of plastic to the side  
794 tank on the other side of the choice tank. We provided olfactory cues by dripping in  
795 water from containers held above each side of the choice tank at a flow rate of ~50  
796 ml/min (Ward and Mehner 2010). We switched the side that the predator cues were  
797 applied to the choice tank every 12 trials. Each trial lasted a total of 7 min; a 2 min  
798 acclimation period and a 5 min examination period. Preliminary tests with dye  
799 revealed this was sufficient for some cue to reach the middle of the choice tank  
800 without too much mixing of cues.

801 We placed a single tadpole in the centre of the choice tank within a clear,  
802 perforated cylinder (9 cm diameter) and provided cues at the beginning of the  
803 acclimation period. We then lifted the cylinder using a string and pulley so that  
804 tadpoles did not see the observer. We recorded each 5-min trial using a GoPro Hero  
805 3+, and calculated the proportion of time spent in the zone away from the predator  
806 cues, based on recording the position of tadpoles every 30 seconds and excluding the  
807 observations when tadpoles were in the middle zone. Human error led to a portion of  
808 the choice tank not being visible in some videos, so these replicates were excluded  
809 from analyses.

810 Supplementary Table 1. Initial sediment quality data used to select and dichotomise  
811 the wetland pairs into high and low quality (HQ and LQ, respectively). All data  
812 collected by the Centre for Aquatic Pollution and Integrated Management  
813 (www.capim.com).

	Pair 1		Pair 2		Pair 3	
	HQ Lynbrook Estate Wetland	LQ Chandler Rd	HQ Woodlands Lake	LQ Cheltenham Rd	HQ Yarrabing Wetland	LQ Ringwood L
<b>Heavy metals (mg kg-1 dry weight)</b>						
Aluminium	21100	16200	23800	20000	7480	
Antimony	2.5*	5	2.5*	2.5*	17	
Arsenic	16	39	17	37	2.5	
Barium	90	430	130	150	40	
Beryllium	0.5*	0.5*	1	1	0.5*	
Boron	25*	25*	25*	25*	25*	
Cadmium	0.5*	2	0.5*	2	0.5*	
Chromium	45	73	43	48	12	
Cobalt	10	19	10	13	2	
Copper	72	299	45	113	14	
Iron	25400	29300	26300	28000	6360	
Lead	29	176	24	283	13	
Manganese	109	187	91	136	79	
Mercury	0.1	0.4	0.1	0.4	0.05*	
Molybdenum	1*	13	8	2	1*	
Nickel	34	159	27	36	4	
Selenium	2.5*	2.5*	2.5*	2.5*	2.5*	
Silver	1*	1*	1*	1*	1*	
Strontium	24	38	46	37	8	
Thallium	2.5*	2.5*	2.5*	2.5*	2.5*	
Tin	2.5	48	2.5	11	2.5	
Titanium	180	430	40	370	40	
Vanadium	49	47	80	58	16	
Zinc	606	3790	299	2390	138	
<b>Pesticides (ppm)</b>						
Bifenthrin	136	68.4	2	37.2	2.5	
Diethyltoluamide	4.8	2.5	17.6	1*	20	
Diuron	165	20	6	22	3	
Fenamiphos	69.6	10	20	10	5	
Permethrin	49.2	209	10	39.4	5	
Prometryn	5	2.5	5	5	2.5	
Pyrimethanil	1	7.6	4	1	1	

Triclosan	69.6	155	20	80.8	10
Trifloxystrobin	2.5	2.5	5	2.5	20
<b><i>Other</i></b>					
Moisture Content	4.3	3	8	4.6	2.8
Total Organic Carbon	4.22	4.29	4.19	4.25	6.14
TPH	1940	21810	500	5420	1690

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Note: Asterisk indicates lowest detection limit

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816 Supplementary Table 2. Output from ANOVA for the mesocosm experiment with  
817 treatment ('high' or 'low' quality conditions) fitted as a fixed effect and location  
818 (wetland pair) fitted as a blocking factor. Significant p-values are bold-face at alpha =  
819 0.05. Asterisks indicate measures that were log-transformed to meet the assumptions  
820 of normality and homogeneity of variance. Mercury was rarely detected (8 of 48) so  
821 was not analysed.

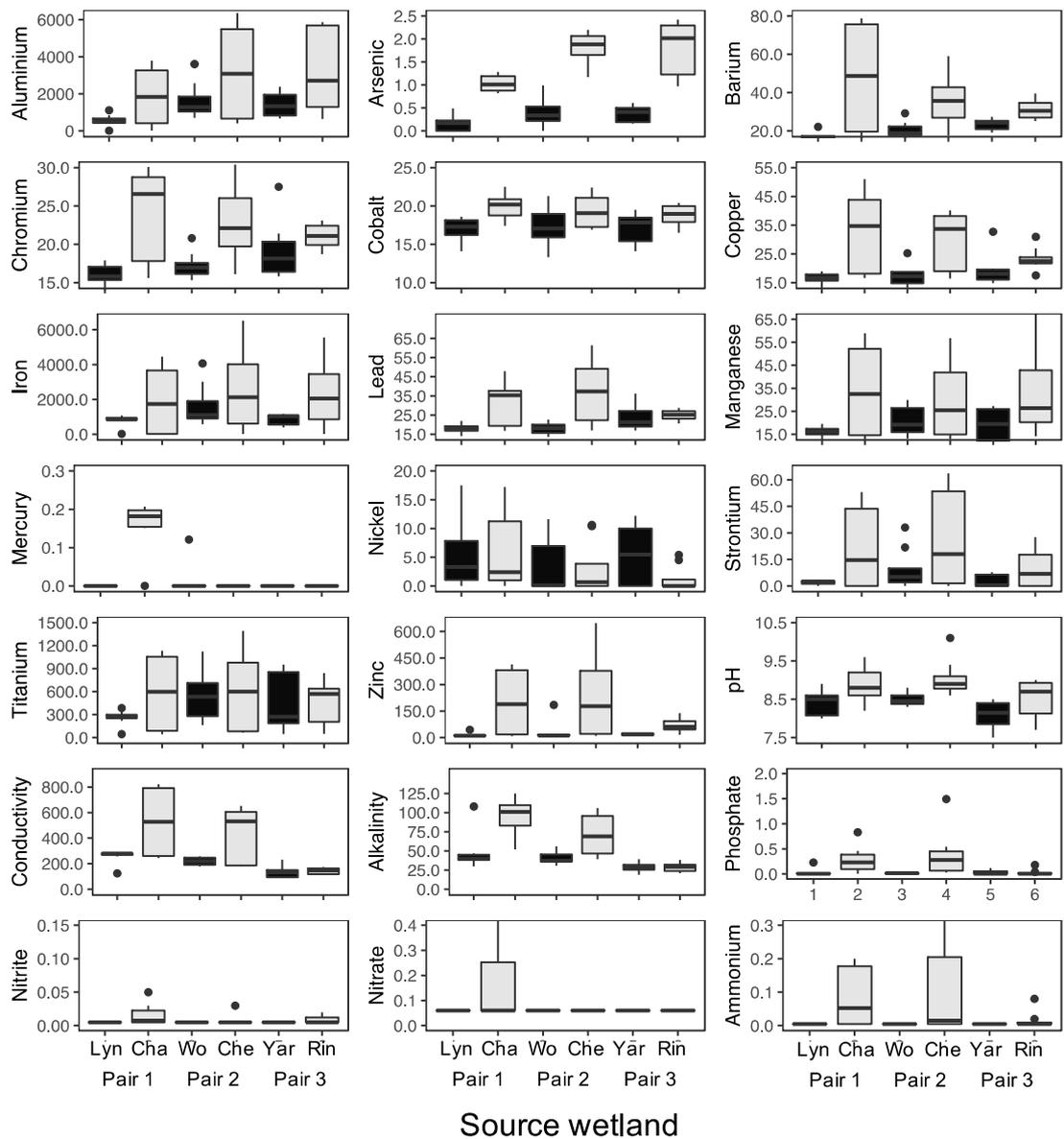
	<b>Factor</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
<i>pH</i>	<b>Treatment</b>	<b>1</b>	<b>2.95</b>	<b>18.22</b>	<b>&lt;0.001</b>
	<b>Pair</b>	<b>2</b>	<b>0.95</b>	<b>5.87</b>	<b>0.006</b>
	Residual	44	0.16		
<i>Conductivity</i>	<b>Treatment</b>	<b>1</b>	<b>329528</b>	<b>13.03</b>	<b>&lt;0.001</b>
	<b>Pair</b>	<b>2</b>	<b>280483</b>	<b>11.09</b>	<b>&lt;0.001</b>
	Residual	44	25296		
<i>Alkalinity</i>	<b>Treatment</b>	<b>1</b>	<b>7967</b>	<b>17.57</b>	<b>&lt;0.001</b>
	<b>Pair</b>	<b>2</b>	<b>7480</b>	<b>16.49</b>	<b>&lt;0.001</b>
	Residual	44	454		
<i>PO4*</i>	<b>Treatment</b>	<b>1</b>	<b>0.06</b>	<b>11.63</b>	<b>0.001</b>
	Pair	2	0.01	2.64	0.082
	Residual	44	0.00		
<i>Al</i>	<b>Treatment</b>	<b>1</b>	<b>28729338</b>	<b>11.53</b>	<b>0.001</b>
	Pair	2	6972271	2.80	0.072
	Residual	44	2491767		
<i>As</i>	<b>Treatment</b>	<b>1</b>	<b>18.60</b>	<b>146.52</b>	<b>&lt;0.001</b>
	<b>Pair</b>	<b>2</b>	<b>1.29</b>	<b>10.18</b>	<b>&lt;0.001</b>
	Residual	44	0.13		
<i>Ba*</i>	<b>Treatment</b>	<b>1</b>	<b>3.56</b>	<b>24.57</b>	<b>&lt;0.001</b>
	Pair	2	0.01	0.10	0.906
	Residual	44	0.14		
<i>Cr</i>	<b>Treatment</b>	<b>1</b>	<b>329.18</b>	<b>20.57</b>	<b>&lt;0.001</b>
	Pair	2	0.46	0.03	0.972
	Residual	44	16.00		
<i>Co</i>	<b>Treatment</b>	<b>1</b>	<b>62.34</b>	<b>15.64</b>	<b>&lt;0.001</b>
	Pair	2	0.88	0.22	0.802
	Residual	44	3.99		
<i>Cu</i>	<b>Treatment</b>	<b>1</b>	<b>1399.68</b>	<b>21.21</b>	<b>&lt;0.001</b>
	Pair	2	43.37	0.66	0.523
	Residual	44	66.00		
<i>Fe*</i>	Treatment	1	0.10	0.04	0.852
	Pair	2	4.31	1.58	0.217

<i>Pb</i>	Residual	44	2.72		
	<b>Treatment</b>	<b>1</b>	<b>1564.08</b>	<b>16.59</b>	<b>&lt;0.001</b>
	Pair	2	57.11	0.61	0.550
<i>Mn*</i>	Residual	44	94.28		
	<b>Treatment</b>	<b>1</b>	<b>2.16</b>	<b>8.51</b>	<b>0.006</b>
	Pair	2	0.02	0.07	0.930
<i>Ni</i>	Residual	44	0.25		
	Treatment	1	26.11	0.99	0.326
	Pair	2	25.43	0.96	0.391
<i>Sr*</i>	Residual	44	26.49		
	Treatment	1	1.33	3.55	0.066
	Pair	2	0.53	1.41	0.255
<i>Ti</i>	Residual	44	0.37		
	Treatment	1	215821	1.46	0.233
	Pair	2	92976	0.63	0.538
<i>Zn*</i>	Residual	44	147728		
	<b>Treatment</b>	<b>1</b>	<b>27.10</b>	<b>21.16</b>	<b>&lt;0.001</b>
	Pair	2	0.19	0.15	0.862
	Residual	44	1.28		

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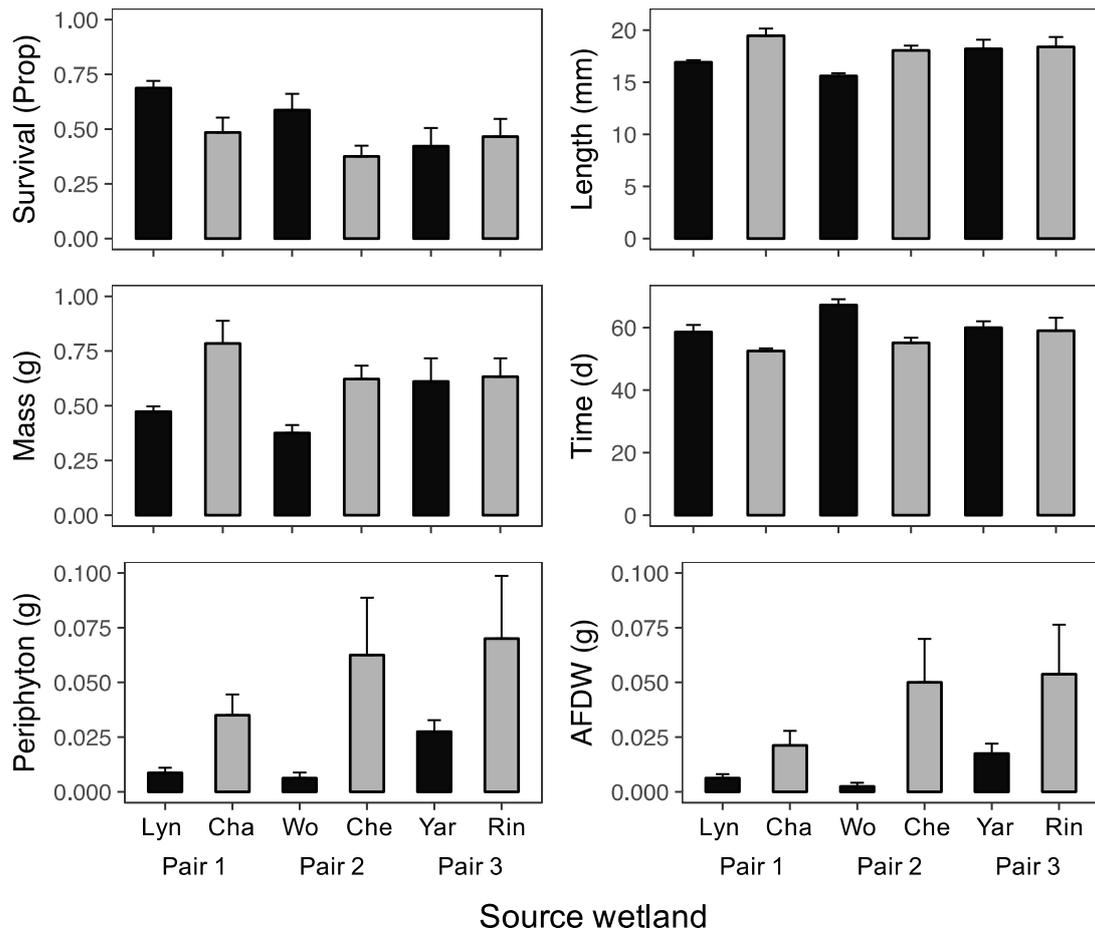
824

825 Supplementary Figure 1. Sediment heavy metal concentrations (mg/kg), pH,  
 826 conductivity (microsiemens;  $\mu\text{S}$ ), alkalinity (ppm), phosphate ( $\text{PO}_4$ ), nitrite (ppm),  
 827 nitrate (ppm) and ammonium (ppm). Dark bars indicate high-quality conditions and  
 828 light grey bars indicate low-quality conditions. N = 8 per treatment. Lyn: Lynbrook;  
 829 Cha: Chandler; Wo: Woodlands; Che: Cheltenham; Yar: Yarrabing; Rin: Ringwood.  
 830 Pair 1: Lynbrook (HQ;  $38^\circ 03' 20.84''$  S,  $145^\circ 15' 03.89''$  E) and Chandler (LQ;  
 831  $38^\circ 00' 00.56''$  S,  $145^\circ 10' 46.19''$  E); Pair 2: Woodlands (HQ;  $38^\circ 00' 11.49''$  S,  
 832  $145^\circ 07' 18.82''$  E) and Cheltenham (LQ;  $37^\circ 59' 27.68''$  S,  $145^\circ 09' 12.58''$  E), and; Pair

833 3: Yarrabing (HQ; 37°50'19.19" S, 145°13'12.39" E) and Ringwood (LQ;

834 37°48'48.58" S, 145°14'19.10" E).

835



836

837 Supplementary Figure 2. Mean (+SE) tadpole fitness (survival to, mass at, length at,  
 838 and time to, metamorphosis) and food availability (periphyton dry weight and  
 839 periphyton ash-free dry weight; AFDW –  $\text{g}/\text{dm}^2$ ) for each source wetland. Dark bars  
 840 indicate high-quality conditions and light grey bars indicate low-quality treatments.  
 841 Lyn: Lynbrook (HQ); Cha: Chandler (LQ); Wo: Woodlands (HQ); Che: Cheltenham  
 842 (LQ); Yar: Yarrabing (HQ); Rin: Ringwood (LQ). Pair 1: Lynbrook (HQ;  
 843  $38^{\circ}03'20.84''$  S,  $145^{\circ}15'03.89''$  E) and Chandler (LQ;  $38^{\circ}00'00.56''$  S,  $145^{\circ}10'46.19''$   
 844 E); Pair 2: Woodlands (HQ;  $38^{\circ}00'11.49''$  S,  $145^{\circ}07'18.82''$  E) and Cheltenham (LQ;  
 845  $37^{\circ}59'27.68''$  S,  $145^{\circ}09'12.58''$  E), and; Pair 3: Yarrabing (HQ;  $37^{\circ}50'19.19''$  S,  
 846  $145^{\circ}13'12.39''$  E) and Ringwood (LQ;  $37^{\circ}48'48.58''$  S,  $145^{\circ}14'19.10''$  E).