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3 *Can road stormwater ponds be successfully exploited by the European green frog (Pelophylax sp.)?*

4

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12

13 Abstract

14 Stormwater ponds ("SWPs") are artificial roadside structures, conceived to retain and clean
15 polluted runoff water, before its release to the environment. SWPs were not designed as
16 suitable habitats for wildlife. However, SWPs in Europe are frequently used as breeding sites
17 by amphibians, especially by the European green frog (*Pelophylax sp.*). Yet, the potential long-
18 term effects of pollutants within SWPs on the populations of this species are unknown. In this
19 study, we investigated the suitability of SWPs as a habitat for European green frogs living in
20 Alsace, NE France, a region with a highly modified landscape that mainly consists of croplands
21 with few natural wetlands remaining. We compared various biological indicators of individual
22 health in 709 green frogs belonging to different life history stages, living in 9 semi-natural
23 ponds (SNPs) and in 25 SWPs. There were no differences between these two pond types for
24 most of the indicators investigated. However, juveniles had a lower body condition (-124%) in
25 SWPs, while adults were 8.3% larger and 41.4% heavier in SWPs. Based on these results, we
26 suggest that SWPs are not a suitable habitat for the development of European green frogs
27 (i.e. juveniles), but may serve as a substitute habitat for adults in a highly modified landscape
28 with few natural wetlands remaining. SWPs may especially act as relay sites, allowing frogs to
29 colonize relict wetlands.

30 **Keywords:** amphibians; fitness; ecological source; ecological trap; road ecology

31

32 1. Introduction

33 During the 20th century, wetland areas have decreased globally by at least 64%,
34 making them the most threatened habitats (Davidson, 2014; Hassall & Anderson 2015).
35 Wetlands are destructed directly through urbanization and agricultural practices (Fischer &
36 Lindenmayer 2007; Van der Ree et al. 2015; Metcalfe et al. 2018) but also indirectly through
37 chemical pollution of water and soil (*e.g.* agriculture & road traffic; Mander & Mitsch 2009;
38 Van der Ree et al. 2015; Maxwell et al. 2016). For instance, polycyclic aromatic hydrocarbons
39 (PAHs) from domestic heating emissions and heavy metals from road traffic contaminate
40 wetlands (Finlayson-Pitts & Pitts 1997; Alam et al. 2018). Nowadays, urban planning uses
41 infrastructures such as stormwater ponds (SWPs, also referred to as retention ponds) to
42 reduce the pollution impacts from road water runoff to the environment (EPA 2009). In fact,
43 SWPs are designed to collect, regulate, store and clean up runoff water, before its release to
44 the environment (EPA 2009). Although the water in SWPs is polluted, especially by PAHs,
45 heavy metals and salt (Gallagher et al. 2014; Snodgrass et al. 2017), some studies have shown
46 that SWPs are frequently colonized by fauna and that amphibians in particular inhabit them
47 (Scher et al. 2004; Brand & Snodgrass 2010; Le Viol et al. 2012; Hassall & Anderson 2015;
48 Jumeau et al. 2020). Apart from studies indicating a low tadpole survival in SWPs (Dorchin &
49 Shanas 2010; Sievers et al. 2018), overall, little is known about the suitability of these sites for
50 amphibian populations.

51 All amphibians share a common bimodal life cycle, consisting of seasonally alternating
52 terrestrial and aquatic phases. This complex life cycle exposes amphibians to different
53 environmental stressors throughout the year. After the winter, they move from their
54 terrestrial wintering area to their aquatic breeding site, causing mass migrations that often
55 lead to massive roadkill (Cushman 2006; Joly 2019). However, roadkill is certainly not the only
56 threat these amphibians face. Especially in developed and industrialized regions, amphibians
57 encounter numerous threats. One of the biggest contributor to amphibian declines is human
58 land use that leads to habitat destruction and the isolation of populations (Gibbons et al.
59 2000; Stuart et al. 2004; Catenazzi 2015). Amphibians are also impacted by the introduction
60 of invasive species that compete with local species (Gibbon et al. 2000; Falaschi et al. 2020)
61 and that might lead to disease proliferation (Gibbons et al. 2000; Stuart et al. 2004; Duffus &
62 Cunningham 2010; Kik et al. 2011). Emerging viral and fungal pathogens affect amphibians
63 worldwide (Price et al. 2014; Catenazzi 2015). Moreover, amphibians, like most animal and

64 plant species, are strongly impacted by global warming (Gibbon et al. 2000; Falaschi et al.
65 2020) and pollution (Salice 2012; Dirzo et al. 2014; Catenazzi 2015). Given their permeable
66 skin, amphibians are especially vulnerable to pollutants present in the water (Wilbur 1980;
67 Miaud et al. 2000; Quaranta et al. 2009). Elevated concentrations of PAHs and heavy metals
68 have been reported to directly and indirectly impact amphibians across various temporal
69 scales. For instance, aforementioned pollutants have been shown to cause disorders during
70 embryonic development (Bryer et al. 2006; Brand et al. 2010), body malformations that result
71 in a greater fluctuating asymmetry (Guo et al. 2017) and, ultimately, reduce survival (Dorchin
72 & Shanas 2010; Gallagher et al. 2014). Thus, the SWPs, containing polluted water, may
73 negatively affect amphibian reproduction, which is of great concern for one of the most
74 threatened vertebrate taxa in the world (Stuart et al. 2004).

75 Amphibian reproduction and population trends can be affected by factors other than
76 pollution. For example, aquatic vegetation (*e.g.* macrophytes and algae) can have a positive
77 effect by providing ideal breeding sites and shelter for amphibians, and by supplying
78 additional food for their larvae. Predation by fish and interspecific competition may have
79 negative effects on the entire amphibian community (Hamer & Parris 2011; Martins et al.
80 2013; Oertli & Parris 2019). Additionally, different abiotic characteristics of the ponds that
81 amphibians use, regardless if they are semi-natural or artificial, may affect their suitability for
82 amphibians (Sievers et al. 2019). For example, the presence of gently sloping areas (<30°)
83 seems to be most favourable for amphibians (Lesbarrères & Lodé 2002). In artificial ponds
84 (*e.g.* SWPs), the presence of specific structures, such as oil removers, found on the SWP
85 banks, where they skim the surface water and remove oil and other organic matter, have
86 recently been implicated to create death traps for amphibians (Jumeau, personal
87 communication). Finally, beside the pond itself, the surrounding landscape is of great
88 importance for amphibians, not only during their movement to/from the breeding pond, but
89 also after reproduction, since most amphibians overwinter on land. For instance, amphibian
90 movements can be disrupted in highly modified landscapes that are mainly composed of
91 croplands and roads. Moreover, species requirements differ, so that the quality rating of the
92 landscape surrounding the breeding sites depends on the ecological niche and habitat use of
93 the species in question (Indermaur et al 2009). A number of important functions have to be
94 provided by the landscape surrounding the breeding site. Firstly, the provision of refuge areas

95 to avoid predators but also for thermoregulation (microclimate; Schwarzkopf & Alford 1996;
96 Seebacher & Alford 2002). A sufficient prey availability is essential for survival and, especially,
97 successful breeding (Waelti & Reyer 2007), particularly if the access to alternative ponds,
98 located in more favourable habitats, is reduced because of environmental degradation and
99 fragmentation (Cushman 2006). In such altered landscapes, natural and semi-natural ponds
100 have almost disappeared, increasing isolation of the few breeding sites and, thus, decreasing
101 amphibian population viability (Joly 2019; Cayuela et al., 2020).

102 **Research objectives**

103 The current investigation is part of a long-term study on the ecological role and
104 importance of stormwater ponds for amphibian conservation. Here, we investigated the
105 suitability of SWPs as a habitat for amphibians. Whereas previous studies mainly focused on
106 population and community level characteristics (*e.g.* species richness; Le Viol et al. 2012;
107 Jumeau et al. 2020), we used a qualitative approach by assessing individual health criteria
108 (Pulliam 1988; Runge et al. 2006; Furrer & Pasinelli 2016). In our study, we compared fitness
109 indices of European green frogs (*Pelophylax sp.*) living in SWPs with fitness indices of frogs
110 living in semi-natural ponds (SNPs), the latter being the control group. Fitness was assessed
111 using two commonly used indicators of fitness: (1) the body condition index (BCI), which
112 refers to the size-corrected body mass of an animal and has been used as a proxy of individual
113 energy reserves and fitness in many species (*e.g.* amphibians, reptiles, birds and mammals;
114 Green 2001; Schulte-Hostedde et al. 2005) and (2) the fluctuating asymmetry index (FA),
115 which reflects minor deviations from a perfect bilateral symmetry of animals (Palmer &
116 Strobeck 1986; Leung et al. 2000). FA is used as an indicator of developmental stability (Guo
117 et al. 2017; see also Breno et al. 2013 for mammals), past exposure to stressors, and fitness in
118 amphibians (Costa & Nomura 2016; Guo et al. 2017; Zhelev et al. 2019). Taking into account
119 previous studies concerning SWPs (Snodgrass et al. 2008; Dorchin & Shanas. 2010; Sievers
120 2017), we predicted that individuals found in SWPs should have a lower health index (*i.e.*
121 body condition) than those found in SNPs. We then investigated the environmental
122 parameters associated with individual SWPs (*e.g.* biotic/pollutant/conception/landscape; Tab.
123 1) and linked them with the indicators of individual frog fitness. We predicted that
124 environmental parameters of individual SWPs would affect the fitness of green frogs.

125 **2. Materials and Methods**

126

127 2.1. Study area and sites

128 The study was carried out during the summer of 2019 in the agricultural plain of Bas-Rhin,
129 Alsace, NE of France (as described in Jumeau, 2020). This area consists of an extensive
130 agricultural landscape, where 87 stormwater ponds (SWPs) and 41 semi-natural ponds (SNPs)
131 have been identified and geolocated using data from the French National Geographic
132 Institute (<https://www.geoportail.gouv.fr>). All SWPs were artificial ponds, surrounded by
133 fences to exclude large fauna and in 80% of the cases also small fauna (e.g. rodents), while all
134 SNPs had no fences. However, most of the small fauna fences were in poor condition,
135 enabling small fauna to enter SWPs. Only ponds with safe access, relatively shallow depth and
136 a sparse surrounding vegetation (enabling field prospection by foot) were considered in the
137 present study.

138 Hence, 25 SWPs and 9 SNPs were included in the current study (**Figure 1**). Among the 25
139 SWPs, 5 were composed of two basins (one for water-infiltration and one for water-
140 retention). In the case of dual ponds, we only captured individuals from the retention basin,
141 which contains water throughout the year. Furthermore, 9 out of 25 SWPs contained a
142 specific structure to skim oil from the road water run off (oil remover). It consists of a
143 concrete drainage, positioned at a ~45° angle, covered with a metal grid that skims the water
144 surface, once it has reached the lower rim of the structure. However, amphibians may be
145 carried inside this structure (depending on the nature of the grid) with the water flow and
146 become trapped. Potential effects of these structures on frog biometrics/body condition were
147 investigated.

148 2.2. Study animals

149 We used the European green frog as our model species, which is the most abundant species
150 encountered in the SWPs of the study area (Jumeau *et al.*, 2020). In Alsace, all species of the
151 *Pelophylax* complex are locally classified as ‘Least Concern’ on the Alsatian Red List
152 (*Pelophylax kl. esculentus*, *Pelophylax ridibundus*), with the exception of *Pelophylax lessonae*,
153 classified as ‘Near Threatened’ (BUFO 2014).

154 From June to August 2019, green frogs were collected by hand during nocturnal patrols of the
155 above-mentioned 25 SWPs and 9 SNPs. All visits to SWPs and SNPs were conducted at
156 random and mean visiting date (11/07/2019) did not differ between pond type. Individual

157 frogs were located by listening for singing males and approached using headlights, to allow
158 hand capture with telescopic nylon nets. Captures were simultaneously conducted by two to
159 three persons, each person having a bucket to store the individuals until the time of body
160 measurements. To minimize the impact of our study on amphibians, a maximum of 30
161 individuals were captured per site and each site was sampled only once. This protocol limited
162 the disturbance but also avoided the potential recapture of individuals.

163 All individuals captured in a given water pond were placed in a plastic bag and weighed. The
164 sex of adult frogs was determined by the presence of nuptial pads and vocal sacs in adult
165 males (Duellman & Trueb 1994). For the immatures (i.e. <25g), we distinguished two groups
166 according to size: sub-adults (body length ≥ 34 mm) and juveniles (body length <34mm, see
167 Results). We did not determine the exact species, to avoid identification mistakes between
168 species of the *Pelophylax* complex, which are hard to identify in the field (Patrelle et al. 2011).
169 Body mass (BM) was measured with a spring balance (± 0.3 g, Pesola[®], Schindellegi,
170 Switzerland) for individuals ≥ 25 g (thereafter referred to as adults *i.e.* sexually mature;
171 Kyriakopoulou-Sklavounou & Loumbourdis, 1990) and with a precision scale (± 0.01 g, Aroma-
172 Zone[®], France) for juveniles and sub-adults (< 25g; *i.e.* sexually immature). Body length
173 measurements were conducted using an electronic calliper (0-150 ± 0.03 mm, Tesa
174 technology[®], Renens, Switzerland). We measured body length from the snout to the tip of the
175 ischium (thereafter referred to as SVL for Snout-Vent Length) and the length of the left and
176 right forearms and tibias. After the measurements, individuals were kept shortly in a second
177 bucket, before being released at the exact location of their capture.

178 To prevent the spreading of pathogens such as *Batrachochytrium sp.*, we used single-use
179 gloves for all handlings, that were replaced between sites. Boots, nets and buckets were
180 disinfected with a Virkon[®] solution between sites (Miaud 2014). All handling procedures were
181 authorized by the “Arrêté Préfectoral n° 2019-DREAL-EBP-0030”.

182 2.3. Fitness indicators

183 We used two indices of individual fitness. (1) A body condition index (BCI) was calculated
184 following Green (2001), using the individual residuals of the linear regressions between
185 Ln(BM) and Ln(SVL) for each group (adult females, adult males, sub-adults and juveniles).
186 Fluctuating asymmetry (FA) was calculated for the length of forearms and tibias, according to

187 Palmer and Strobeck (1986): $= \frac{abs(C_l - C_r)}{mean(C_l, C_r)}$, where “abs” is the absolute difference between C_l
188 and C_r , and C_l and C_r are the length of the left and right forearms or tibias, respectively. In
189 addition to these two indices, we also calculated the sex-ratio (SR) for each site as the number
190 of males divided by the total number of males and females collected.

191 2.4. Environmental parameters of stormwater ponds

192 We used 23 environmental parameters, based on the landscape surrounding the pond (*i.e.*
193 pond conception, biotic conditions and pollution levels; Tab.1), to investigate potential
194 relationships with fitness indicators. Unfortunately, because of the lack of data concerning
195 pollution levels, such analysis was not possible for the SNPs. For the SWPs pollutants, we only
196 retained concentrations of dissolved nitrogen, chlorine and copper in our characterization,
197 since they were the only pollution parameters that had been consistently recorded in most of
198 the SWPs (14 ponds for nitrogen and copper, 15 ponds for chlorine; Jumeau et al. 2020).
199 Consequently, environmental parameters were investigated in 17 out of 25 SWPs. The
200 surrounding landscape of each of the 17 SWPs was characterised in terms of landscape
201 elements based on the “Coopération pour l’Information Géographique en Alsace” database
202 (with a precision of 1:10 000, CIGAL 2018). The presence and surface area of crop fields,
203 forests, grasslands, and wetlands, were calculated within a 500 m radius (hereafter referred
204 to as ‘nearby environment’) and within a 5000 m radius (referred to as ‘distant environment’)
205 around each SWP (Smith & Green 2005), using geographical information system software
206 (QGIS v3.10, Development Team, 2020). For the wetland elements, we distinguished
207 important wetlands from ordinary wetlands based on the presence of an exceptional
208 biodiversity in important wetlands (Agence de l’Eau Rhin-Meuse, DREAL 2016). Due to strong
209 statistical dependencies between parameters of the nearby environment, only the
210 presence/absence of each element was used for these parameters. Some landscape variables
211 such as moorlands and orchards were not included in our analysis because they were not
212 present within the 5000 m radius for many SWPs.

213 2.5. Statistical analyses

214 Sexual dimorphism in body length (SVL) and body mass (BM) in each of our 34 study sites
215 (SWPs and SNPs) was tested using a Wilcoxon test. An analysis of variance (ANOVA), based on
216 Linear Mixed Models (LMM) with site as random factor, was used for comparing SVL, BM, BCI

217 and the two FA indices (forearms and tibias) between SWPs and SNPs for each individual
218 group (males/females/sub-adults/juveniles). To obtain a Gaussian distribution, a BoxCox
219 transformation was applied to FA indices. The relationships between SWP environmental
220 parameters and SVL, BM, BCI and FA indices were tested in males only, using Linear Model
221 (LM) based ANOVAs (n=17 SWPs), since males were the only group that was present in
222 sufficiently large numbers (n≥6) at all ponds. Each environmental parameter for the SWPs was
223 standardized before analysis. Collinear environmental parameters were identified and
224 removed using Spearman tests. All statistical analyses were conducted using R (v3.5.3,
225 RStudio v1.2.1335, R Core Team, 2020) and all figures were plotted with GraphPad Prism
226 8.2.1 (San Diego, California, USA). Each model was selected by a BIC bidirectional elimination
227 procedure. Results are shown as mean±SEM with a statistical significance threshold of 5% (*
228 for p< 0.05, ** for p< 0.01 and *** for p<0.001). The GLM estimates are indicated as “β”.

229 3. Results

230 A total of 709 individuals was captured in 25 SWPs (N=509 individuals) and 9 SNPs
231 (N=200 individuals). A rank-frequency histogram of individual body length ([see suppl. figure 1](#))
232 and body mass showed a clear separation within our immatures (*i.e.* <25g), allowing us to
233 distinguish two groups of immatures: sub-adults (body length ≥34mm) and juveniles (body
234 length <34mm). The sampled population was composed of 393 males, 127 females, 112 sub-
235 adults, and 77 juveniles. On average, 20.3±1.5 individuals were captured per site (range for
236 males: 0 to 30; females: 0 to 14; sub-adults: 0 to 13; juveniles: 0 to 21). Since vocalizing males
237 were easier to locate, the sex ratio of captured individuals was biased towards males.
238 However, this sampling bias was identical for both pond types.

239 3.1. Body length, body condition and fluctuating asymmetry

240 There was a significant sexual dimorphism for body length and body mass of adult frogs in
241 both SWPs and SNPs. Females were, on average, about 10% longer and 40% heavier than
242 males (body length: SWPs: W=4750, p<0.01 and SNPs: W=5778, p<0.01; body mass: SWPs:
243 W=4463.5, p<0.01 and SNPs: W=5778, p<0.01; **Tab. 2, Fig. 2A & 2B**).

244 Adult individuals from SWPs were significantly larger (+6.6% for the males $\chi^2=5.76$, p<0.05,
245 +12% for females $\chi^2=3.98$, p>0.05) and heavier (+27.5% for the males $\chi^2=6.04$, p<0.05, +40.8%
246 for females $\chi^2=5.18$, p<0.05) than individuals from SNPs (**Tab. 3, Fig. 2A & B**). Conversely,

247 juveniles were smaller in the SWPs when compared with the SNPs (-10.4%, $\chi^2=5.12$, $p<0.05$;
248 **Tab. 3**), while body mass did not differ between pond types (**Tab. 3**). Lastly, the size and body
249 mass of the sub-adult frogs did not differ between pond types ($p>0.05$ **Tab. 3**).

250 Because of this sexual dimorphism in body length and body mass, the body condition index
251 (BCI) was calculated for each sex and group, separately. Our analysis showed a significantly
252 lower BCI in juveniles from SWPs, when compared with individuals from SNPs (-124% $\chi^2=5.97$,
253 $p=0.01$; **Tab. 3**). By contrast, there were no significant differences in BCI for the other groups
254 (males, females, sub-adults) between SWPs and SNPs ($p>0.05$). In addition, there were no
255 significant differences in fluctuating asymmetry between individuals from SNPs and SWPs for
256 any of the investigated groups ($p>0.05$; **Tab. 2**).

257

258 3.2. Relationship between environmental parameters of SWPs and male green frog biometrics

259 Despite the high variability for the concentration of the different pollutants analysed at the
260 SWPs (coefficient of variability >40%), we found no significant relationship between any of the
261 pollutants at SWPs included in the analysis (dissolved nitrogen, chlorine, copper) and the
262 biometric variables recorded in adult male green frogs ($p>0.05$). Model analysis showed that
263 adult male biometrics were correlated with landscape parameters, whereby larger frogs were
264 found in SWPs surrounded by a greater proportion of cropland and forested areas within a
265 5000m radius ($\beta=4.92$, $p<0.05$; $\beta=4.12$, $p<0.05$). By contrast, smaller frogs were found in
266 SWPs surrounded by a greater proportion of important wetlands on the same spatial scale
267 ($\beta=-2.93$, $p<0.05$). Adult male body condition index (BCI) at the SWP was negatively correlated
268 with the surface area of grassland fields ($\beta=1.2^{E-2}$, $p<0.01$) within a 5000m radius. The FA
269 based on forearm-length was negatively correlated with the surface area of grassland and
270 important wetlands within a 5000m radius (**Tab. 4**). By contrast, the FA based on Tibia-length
271 was not related to any of the characteristics of the SWPs tested in this study, as the BIC
272 selection did not retain any relevant variables (*i.e.* FA tibias \sim 1; **Tab. 4**). Finally, male BCI was
273 lower (-286%) in SWPs equipped with an oil remover, when compared with SWPs without
274 such a device ($F=12.8$, $d=1$, $p<0.01$; **Tab. 4**).

275

276 4. Discussion

277 Stormwater ponds are designed to remove pollutants from road runoff waters and the
278 latest designs often include additional elements to achieve this, such as oil removers. As such,
279 SWPs are likely more polluted than the surrounding areas. Accordingly, our initial hypothesis
280 was that SWPs are not a suitable habitat for wildlife, including the European green frog. Our
281 results show that adult green frogs captured at SWPs were larger and heavier, but not in a
282 better body condition, than those captured at SNPs. Juveniles, captured at SWPs, were
283 smaller and their body condition was lower than in juveniles captured at SNPs. Hence, our
284 results only partially support our original hypothesis.

285 We caught significantly more males than females at both pond types. Such an unbalanced sex
286 ratio during capture has been reported previously for green frogs (Loman & Madsen 2010;
287 Green 2013) and also for other amphibian species (Wells 1977; Loman & Madsen 2010;
288 Grayson et al. 2012; Green 2013). A possible explanation might be that male amphibians
289 actively search for females, while females typically hide after spawning (Wells 1977).
290 Accordingly, males are more conspicuous (also given their vocal behaviour) and more
291 susceptible to capture, when compared with females. Apparently, the situation at SWPs does
292 not differ in that respect from natural ponds.

293

294 4.1. Body size

295 Body size is a strong driver of reproduction, leading to sexual dimorphism in many taxa
296 (Masello & Quillfeldt 2003; Baird et al. 2007; Whitman 2008). This is the case in amphibians,
297 where reproduction is explosive, with females laying hundreds, if not thousands of eggs in
298 one time. Consequently, most amphibians show sexual dimorphism, with females being larger
299 than males, as is the case for green frogs (Shine 1979). Our results on adult body size show
300 that female European green frogs were on average 8.3% larger and 41.4% heavier than males,
301 which is consistent with previously reported values (Shine 1979). Females were larger and
302 heavier in SWPs than in SNPs, leading to an even more pronounced female-biased
303 dimorphism in SWPs. While this might seem counterintuitive, a larger size at the SWPs might
304 be related to a greater food availability for individuals. Sievers et al. (2018) found that the
305 tadpoles of frogs inhabiting more polluted stormwater wetlands survived less well but also
306 reached metamorphosis earlier and at a larger size than at less polluted sites. They attributed

307 the larger tadpole size to a greater food availability for surviving individuals, due to the
308 combined effects of a higher mortality (density-dependent effects; Berven & Gill 1983;
309 Semlitsch et al. 1988) and eutrophication. Presumably, individuals that are larger during
310 metamorphosis will become larger adults, as has been found for a number of salamander and
311 frog species (Berven & Gill 1983; Semlitsch et al. 1988). However, the larger body size of adult
312 frogs captured at the SWPs, despite their similar body condition with adults from the SNPs,
313 might have also been caused by a difference in age. Hence, the larger individuals captured at
314 the SWPs may have been older than the smaller individuals captured at the SNPs. The
315 skeletochronology approach could be used to test this hypothesis (Guarino et al. 1995). Older
316 populations at the SWPs may result from a potentially lower mortality at such artificial sites,
317 due to a reduced predation pressure, either because of negative effects of road density on
318 predator numbers and activity (i.e. 'predation release hypothesis'; Downing et al. 2015) or
319 because of predator exclusion via fences. The latter may limit the entrance of some
320 amphibian predators, such as mustelids (Parris 2006; Otero et al. 2018) or fish that might be
321 introduced by other species. Similarly, fences might limit frogs to disperse from the SWPs,
322 resulting in a longer residence time, which might lead to older populations at SWPs, when
323 compared with SNPs. Behavioural studies are needed to assess the capabilities of green frogs
324 to pass fences surrounding SWPs.

325 Last but not least, the differences in body size we found between green frogs captured at
326 SNPs and at SWPs may also be related to a potential species difference. The European green
327 frog complex (the *Pelophylax* complex) includes different species (*Pelophylax kl. esculentus*,
328 *Pelophylax ridibundus*, and *Pelophylax lessonae*), which are hard to differentiate in the field
329 (Patrelle et al., 2011). For instance, *Pelophylax lessonae* can be morphologically smaller than
330 the two other species and is more frequently found in (semi-)natural environments than the
331 other two species (Pagano et al. 2001). Accordingly, frogs captured in SNPs in our study may
332 have been specimens of the smaller *Pelophylax lessonae*. Unfortunately, we could not
333 conduct any genetic or further morphological analyses, which would have allowed a precise
334 determination.

335

336 4.2. Body condition and fluctuating asymmetry

337 Despite the above-mentioned differences in body size and body mass between green frogs
338 captured at SWPs and at SNPs, we found no differences in body condition or in fluctuating
339 asymmetry between green frogs (adult males/females and sub-adults) captured at both pond
340 types. Yet, juveniles captured at SWPs had a lower BCI and were also smaller than those
341 captured at SNPs. A smaller body size and a lower body condition in juveniles of the SWPs
342 suggest that they underwent an earlier metamorphosis and/or were smaller at
343 metamorphosis. An earlier metamorphosis of frogs at SWPs is supported by results from
344 Snodgrass et al. (2008) and Dorchin & Shanas (2010), that concern *Anaxyrus americanus* and
345 *Bufoes viridis*, respectively.

346 If we neglect the possibility that frogs collected at both pond types belonged to different
347 species of the green frog complex (see above) and apart from potential differences in
348 population age, the similar BCI observed in sub-adults and adults at SWPs and SNPs might also
349 be explained by the following scenarios: (1) Juveniles that encountered potentially lower
350 trophic conditions at SWPs and that survived to become sub-adults and adults were able to
351 compensate for this. (2) Alternatively, while trophic conditions between pond types differed
352 for juveniles, this was not the case for adults and sub-adults.

353 Body condition (BCI) is the most commonly used proxy of individual energetic reserves and
354 fitness (Green 2001; Schulte-Hostedde et al. 2005). The lower BCI of juveniles that
355 developed at the SWPs (embryonic development), and the similarity of the BCI observed in
356 adult and sub-adult individuals caught at the SWPs and the SNPs, suggests that the artificial
357 nature of SWPs did not affect the trophic conditions experienced after metamorphosis, but
358 rather affected embryonic development. Previous studies have found a relationship between
359 pollutant levels (*e.g.* heavy metals and pesticides) and body mass in amphibians, where a
360 higher pollutant level is associated with a lower body mass (Egea-Serrano et al. 2012) and,
361 consequently, lead to a reduced BCI. In our study, pollutant levels were not measured for all
362 SWPs sites and not at all for the SNPs sites. However, our assumption was that pollutant
363 levels should be lower at the SNPs than at the SWPs. Despite this, we found no relationship
364 between pollutant levels and biometric measurements (BCI, FA). This could mean that any
365 potential effects of pollutants investigated in this study on adult and sub-adult European
366 green frogs might have been similar in both pond types. Given the relatively low pollutant
367 level and/or the specific combination of pollutants, it is possible that they had no measurable

368 effect on the biometrics of collected frogs. Alternatively, it is also possible that individuals that
369 were exposed to a greater (pollutant) stress level and, therefore, might have shown a lower
370 BCI and a higher AF index, did not survive and were, consequently, not captured. However,
371 during our study, we did not find any dead individuals. The pollutant issue is further
372 complicated by species differences concerning the sensitivity to pollution (Katz 1975) and
373 further studies are urgently needed to address this issue.

374 Fluctuating asymmetry (FA) has been proposed as a good indicator of past stressful periods
375 and of individual fitness in amphibians (Costa & Nomura 2016; Guo et al. 2017; Zhelev et al.
376 2019). FA has, thus, been used to compare environmental quality of different habitats used
377 by amphibians (e.g. Guillot et al. 2016; Sievers 2017). Guillot et al. (2016) compared the FA in
378 common toads (*Bufo bufo*) that were captured in farmland areas and in forested areas, while
379 Sievers (2017) investigated FA in the common eastern froglet (*Crinia signifera*), living in
380 different wetland habitats. Sievers (2017) reported significant differences in FAs (forearms
381 and tibias) between individuals captured in habitats of contrasting quality, whereas Guillot et
382 al. (2016) only reported differences using forearm FA (**Fig. 3**). In our study, FA calculated from
383 forearms was higher than the FA calculated from tibias. However, we did not find differences
384 between individuals captured at SWPs and at SNPs. Differences in FA between forearms and
385 tibias may be explained by the difficulty to accurately measure forearm length in amphibians,
386 given its considerably shorter length, when compared with the length of the tibia (e.g. in
387 juveniles, forearm versus tibia length is on average 5.5 ± 0.07 mm and 12.9 ± 0.3 mm,
388 respectively). In addition, interpretation of FA also depends on the threshold value used to
389 assign environmental quality for amphibians. Accordingly, we strongly encourage colleagues
390 to carefully consider FAs based on forearm length, because measuring the forearms of living
391 amphibians can lead to inaccurate measurements (Alford et al. 1999). Considering the limits
392 of using forearm FA, the measurements must be replicated to estimate a measurement error
393 (Alford et al. 1999; Sievers 2017). In addition, when a measurement concerns a short
394 structure (e.g. forearm), the measurement error of the instrument (e.g. electronic calliper)
395 must also be taken into account. Therefore, the use of a calliper to measure the forearms to
396 obtain the FA is risky. A better approach might be to take an X-ray of the animal, to allow an a
397 posteriori measurement on a computer (Breno et al. 2013).

398 The FA investigation of our study could not demonstrate a difference in developmental
399 conditions for European green frogs at the two pond types investigated (**Fig. 3**). However, our
400 investigation concerning the BCI, clearly indicates such a difference (**Tab. 3**). There is a wide
401 spectrum of tools available to assess individual health in amphibians. For example,
402 corticosterone levels in the blood have been used to investigate potential stress effects
403 associated with habitat fragmentation and temperature increase in common toads (Janin et
404 al. 2011; Brischoux et al. 2018). Hematological parameters, such as leucocyte count and

405 haemoglobin concentration have also been used for health assessment in the context of
406 pollution effects on amphibians (Zhelev et al. 2018). While such parameters provide a clear
407 answer when assessing individual health status, blood sampling requires skills, training and
408 specific ethical authorisations. By contrast, biometric measurements are relatively easy to
409 obtain.

410 4.3. Linking stormwater pond design and surrounding landscape with adult male 411 biometrics

412 When investigating the relationship between various environmental parameters and
413 adult frog biometrics, our model did not retain any pollutant variables. Hence, with respect to
414 the pollutants included in our analysis and the considered sampling period, we found no
415 negative impact of SWP water on adult green frog biometrics. The most likely explanation is
416 that all pollutant concentrations (Cu=8.1±1.02 µg/l, N=0.05±0.01 mg/l, Cl=9.7±1.8 mg/l) were
417 well below reported sub-lethal concentrations for amphibians (Cu=31.6 mg/l, N=20 mg/l,
418 Cl=1721.4 mg/l; Jumeau et al. 2020). Hence, with respect to the pollutants investigated in our
419 analysis, this confirms that SWPs and SNPs are not highly contrasted environments for
420 European green frogs.

421 By contrast, we found a strong correlation between adult male green frog biometrics
422 and landscape parameters. Frogs were larger in SWPs surrounded by a greater proportion of
423 cropland and forests within a 5000 m radius. When SWPs were surrounded by a greater
424 proportion of important wetlands, frogs were smaller. A size increase might be explained by
425 the absence of alternative habitats such as wetlands, as suggested by the negative
426 relationship between the size of individuals (SVL) and surrounding wetland surface area. The
427 mechanism behind this might be that in the absence of sufficiently large wetlands, older
428 individuals might stay at SWPs, rather than abandoning them and moving to nearby wetlands.
429 A further mechanism that could be verified experimentally might be the increasing input of
430 agricultural fertilizers into the environment (N,P,K), which are known to favour the
431 proliferation of aquatic vegetation (Taziki et al. 2015). Accordingly, eutrophication at SWPs
432 surrounded by croplands might increase food availability to green frog larval stages. The
433 quantity of proteins ingested (both vegetable and animal sources) has been shown to affect
434 the growth rate and survival of tadpoles in several amphibian species (Martinez et al. 1993,
435 1996; Martins et al. 2013) and possibly also affects adult size. While, the effect of food

436 availability on tadpole body-size has been shown, it would be interesting to use a semi-
437 controlled approach (e.g. mesocosm), to investigate this effect throughout the different life
438 history stages of individuals. Such an investigation might also be possible in the field, using
439 capture-mark-recapture methods (Miaud et al. 1999).

440 Apart from the link between the surrounding landscape of SWPs and male green frog
441 biometrics, we found that individuals caught at SWPs that were equipped with an oil remover
442 had a body condition ~3 times lower than frogs caught at SWPs lacking such a device. We
443 believe that in SWPs equipped with an oil remover some frogs are washed through the metal
444 grid during an overflow and become trapped inside the concrete drainage when the water
445 level falls below the rim of the skimmer. Hence, trapped frogs might only be able to escape
446 during the subsequent overflow, which might occur within a few days, weeks or months
447 (Jumeau, personal communication). These stochastic events obviously depend on the
448 weather and the season. Unable to feed when trapped inside the concrete drainage, the
449 effect on frog body condition is obvious.

450 Unfortunately, for the SNPs, a statistical analysis investigating the potential effects of the
451 surrounding landscape on frog biometrics was not possible because of a too small number of
452 animals in each category.

453

454 Are SWPs a suitable habitat for the European green frog?

455 In our study, the results obtained from juveniles are in line with previous studies. A number of
456 studies argued that SWPs are poor quality habitats for amphibian development (i.e.
457 embryonic development), leading to small size and a poor body condition in this group
458 (Bishop et al. 2000; Brand & Snodgrass 2010; Dorchin & Shanas 2010; Sievers 2017).
459 However, our results obtained from sub-adults and adults contrast with results from the
460 juveniles. The lack of a difference between pond types with respect to body condition and
461 fluctuating asymmetry in adult and sub-adult frogs does not support our original hypothesis
462 of SWPs being an unsuitable habitat for these groups, nor would they appear to be less
463 favourable than SNPs.

464 A number of factors might have contributed to the differences we found between juvenile
465 and adult/sub-adult green frogs. First, the hatching place of sub-adult and adult green frogs

466 caught during the study is uncertain so that animals might have moved more recently to the
467 SWPs. Most green frogs spend nearly all their adult life in the vicinity of the pond where they
468 hatched (Peter, 2001), for example at a neighbouring pond, except if pond access has been
469 altered (e.g. landscape fragmentation), as is the case for most of the SWPs in our study.
470 Hence, we do not know if the adult/sub-adult green frogs caught at the SWPs originated from
471 there. By contrast, for the juveniles it is certain that they spent their entire developmental
472 period at the capture site because in some individuals the tail had not been fully resorbed.
473 This emphasizes the need to investigate the suitability of SWPs for green frogs, by comparing,
474 *in situ*, growth and survival rates of tadpoles from SWPs and SNPs.

475 Second, our sampling might have coincided with a non-representative year, with less
476 pollution from the roads in the water of the SWPs than typical for the study area of Bas-Rhin.
477 According to the European Environmental Agency, an upgrading of combustion engines and
478 an improvement in anti-pollution technology has occurred during the last 20 years (EEA
479 2020). However, at the same time, agricultural pollution has continued to increase (Zabel et
480 al. 2019). These developments were confirmed in a recent study conducted between 2015
481 and 2016 on 82 SWPs in the Bas-Rhin area (Jumeau et al. 2020). That study measured more
482 than 20 different pollutants and found that their concentrations in these SWPs were below
483 established sub-lethal thresholds for amphibians (Jumeau et al. 2020). In this context, the
484 protocols used in most studies investigating the effects of pollutants in a habitat on
485 amphibians do not reflect the actual conditions of a site *in situ* but are mostly conducted
486 within the laboratory (Bishop et al. 2000; Brand & Snodgrass 2010; Dorchin & Shanas. 2010).
487 Studying the condition of individuals (e.g. BCI reflecting fitness) at a particular site, should
488 better reflect the effects of the concerned environment on amphibians. Indeed, SWPs are not
489 only structures that retain road pollutants. Instead, a complex set of factors, existing at these
490 SWPs and their synergistic or opposing interactions can lead to positive or negative effects on
491 the individuals that live at these sites.

492 Our study focused on the European green frog, a taxonomic complex that is ubiquitous and
493 presumably relatively tolerant with respect to pollution (Zhelev et al. 2015). However, green
494 frogs were not the only amphibian species present at our study SWPs but European green
495 toads (*Bufo viridis*) could also be found (Jumeau et al. 2020). The reason for the over-
496 representation of these two species at the SWPs is likely linked to the attractiveness of these

497 sites for these species (*e.g.* nearest site to the wintering habitat; presence of first singing
498 males), rather than to the intrinsic ecological quality of SWPs in a disturbed landscape.
499 Because amphibian species may respond differently to a given stressor (Katz 1975, 1981;
500 Otero et al. 2018), a way to solve and prioritise the question of the intrinsic quality of the
501 SWPs for amphibians requires further consideration, especially because it may also concern
502 the most endangered amphibian species in the area, the European green toad. Finally, we
503 suggest that future studies should address questions concerning the health status of
504 amphibians at SWPs more directly and investigate whether individuals at these sites
505 (presumably more polluted and eutrophic) are more susceptible to disease and parasite load.
506 Internal (*e.g.* trematodes; Johnson et al. 2007) and external parasites (*e.g.* leeches; Schalk et
507 al. 2009) might affect the health of numerous amphibian species at SWPs.

508

509 To conclude, in a landscape highly fragmented by roads and agricultural fields, where
510 natural wetlands have almost vanished, stormwater ponds (SWPs) could be considered as
511 suitable substitute habitats for adult and sub-adult European green frogs. However, potential
512 physical traps, such as oil removers, have to be modified, to prevent trapping and, potentially,
513 killing amphibians. By contrast, our results for juveniles, known to have hatched at the SWPs
514 investigated, suggest that SWPs are less suitable for the early development of green frogs. It
515 is therefore most likely, that the adult and sub-adult green frogs captured at the SWPs
516 originated from different, more favorable sites. Since a healthy and sufficiently large juvenile
517 population is crucial for population survival, the use of SWPs as habitat will likely not support
518 a viable green frog population. The major function of SWPs for green frogs might be to act as
519 relay sites, allowing frogs to recolonize relict wetlands. The development of specific
520 management measures could also help to improve the quality of SWPs for amphibians. For
521 example, the regular drainage of such ponds during the non-breeding season and the removal
522 of polluted sediment might be an important measure in this context. While further research is
523 clearly needed, a conservation strategy that will preserve existing wetlands and restore
524 former wetlands will be most beneficial to many amphibian species.

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528

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535

536 **Conflicts of interest/Competing interests**

537 None of the authors of this manuscript has any potentially competing interests.

538

539 **Ethics approval**

540 All experimental protocols for this study complied with national legislation and were
541 approved by the appropriate authorities: “Arrêté Préfectoral n° 2019-DREAL-EBP-
542 0030”.

543

544 **Consent to participate**

545 All authors participated in the writing of this manuscript.

546 **Consent for publication**

547 All authors have approved the submission to ‘Urban Ecosystems’.

548

549 **Availability of data and material**

550 The datasets generated and/or analysed during the current study are available from
551 the corresponding author upon request.

552

553 **Code availability**

554 The codes used with R software during the current study are available from the
555 corresponding author upon request.

556

557 **Author contributions**

558 All authors approve this version of the manuscript for publication. They agree to be
559 accountable for the aspects of the work that they conducted, and will ensure that any
560 questions related to the accuracy or integrity of any part of their work are
561 appropriately investigated and resolved. AC, JJ, & YH conceived the study and
562 designed the methods; AC & ND collected the data; AC & JJ analysed the data; AC
563 drafted the manuscript with contributions from JJ, ND, ME, JYG & YH.

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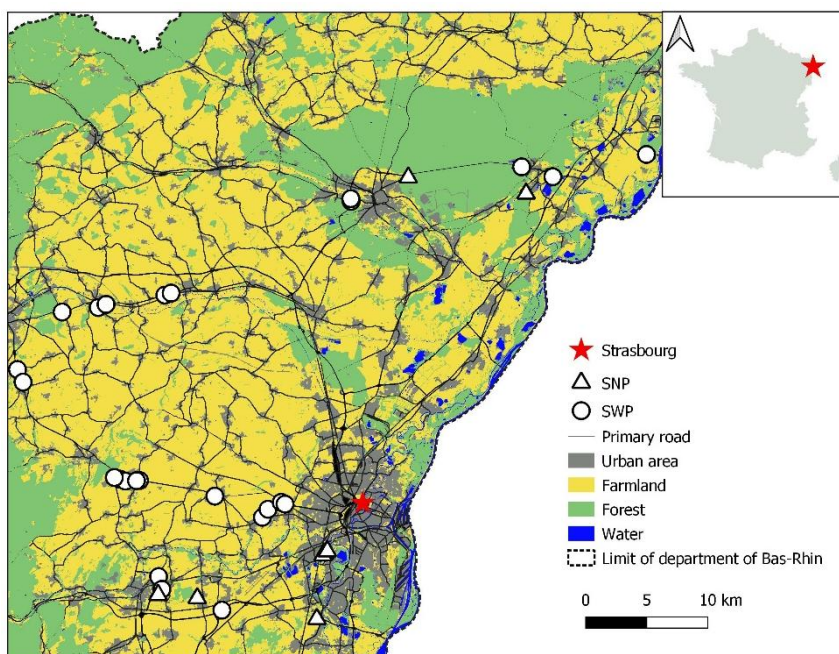
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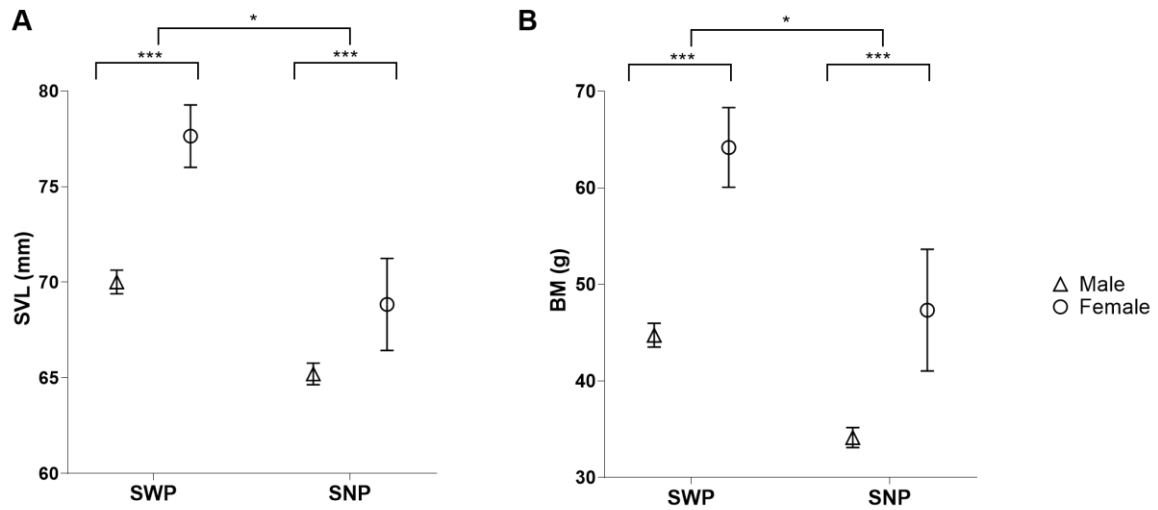
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 820 **Figures**



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 823 **Figure 1: Study area and pond locations.**

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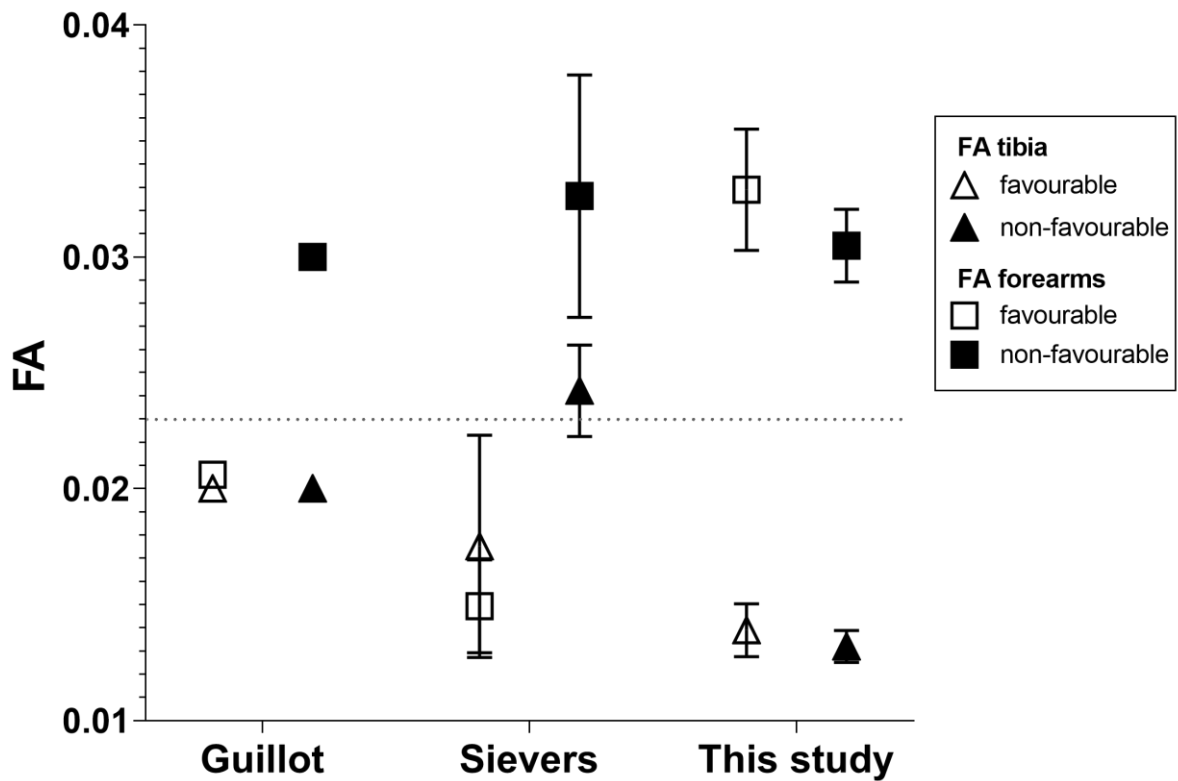


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826 Figure 2: Comparison (means±SEM) of body length (Snout Vent Length, SVL; A) and body mass (BM; B) between adult
 827 male (triangles) and adult female (circles) European green frogs (*Pelophylax sp.*) captured in SWPs ($N_{\sigma}=250$; $N_{\phi}=58$) and
 828 SNPs ($N_{\sigma}=107$; $N_{\phi}=27$). Females were larger and heavier than males in both pond types (ANOVA, *** $P<0.001$).
 829 Individuals caught at SWPs were larger and heavier than individuals caught in SNPs (Wilcoxon test, * $P<0.05$).

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833 Figure 3: Fluctuating asymmetry (FA) based on forearm (squares) and tibia (triangles) measurements in amphibian species
 834 inhabiting contrasting environments (favourable in white and non-favourable in black) in agricultural areas (common toad,
 835 Guillot et al. 2016), wetlands (common eastern froglet, Sievers 2017) and road landscapes (European green frog, this study).
 836 The horizontal dashed line indicates a hypothetical threshold of FA common for all 3 studies and emphasizes the difficulties
 837 associated with using FA indices in interspecific studies.
 838

839 **Tables**

840

841 **Tab. 1: Environmental parameters of SWPs used to test for potential effects on fitness indicators of male European green**
 842 **frogs (*Pelophylax sp.*)**

Conception of SWPs	Water pollutants	Immediate landscape (500 m radius)	Distant landscape (5000 m radius)	Biotic elements
Age (year)	Nitrogen (mg/l)	Presence of crop field	Surface area of crop fields (m ²)	Green frog abundance
Presence of oil remover	Chlorine (mg/l)	Presence of forest	Surface area of forests (m ²)	Presence of algae
Nearest road distance (m)	Copper (mg/l)	Presence of hedges	Surface area of grassland (m ²)	Presence of aerial vegetation
Presence of slope <30°		Presence of a road	Surface area of highway network (m ²)	Amphibian species richness
Volume (m ³)		Presence of wetland	Surface of wetland (m ²) Surface area of notable wetland (m ²)	

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846 Tab. 2: Biometric measurements (means±SEM) for four different groups of European green frogs, captured in SWPs and SNPs

		Males	Females	Sub-adults	Juveniles
SWPs (n=25)	SVL (mm)	70.0±0.6	77.6±1.6	50.2±0.7	25.1±0.4
	BM (g)	44.7±1.2	64.1±4.2	15.1±0.7	1.9±0.1
	BCI ^(E-3)	1.3±2.5	8.7±5.1	4.2±4.5	-9.2±5.0
	AF Tibias ^(E-3)	13±0.7	12±1.3	13±1.2	21±2.2
	AF Forearms ^(E-3)	31±1.6	32±2.9	34±3.6	53±4.9
SNPs (n=9)	SVL (mm)	65.2±0.6	68.8±2.4	50.6±0.8	27.9±1.1
	BM (g)	34.1±1.0	47.3±6.3	15.9±0.8	2.4±0.3
	BCI ^(E-3)	-0.8±2.8	-7.3±4.9	-7.5±5.9	38±10
	AF Tibias ^(E-3)	14±1.1	13±1.8	12±1.5	16±3.5
	AF Forearms ^(E-3)	33±2.6	34±4.9	34±3.9	33±4.7

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849 Tab. 3: Statistical comparisons for biometric measurements of European green frogs (*Pelophylax sp.*) captured in SWPs versus
850 SNPs

Measure	Males (n=357)			Females (n=85)			Sub-adults (n=112)			Juveniles (n=77)		
	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
SVL	5.76	1	0.016	3.98	1	0.046	0.15	1	0.69	5.12	1	0.02
BM	6.04	1	0.014	5.18	1	0.022	0.43	1	0.51	3.04	1	0.08
BCI	0.05	1	0.81	1.75	1	0.18	0.94	1	0.33	5.97	1	0.01
FA Tibias	0.25	1	0.61	0.11	1	0.73	0.69	1	0.41	0.74	1	0.39
FA forearms	0.37	1	0.54	0.18	1	0.66	4E-4	1	0.98	1.86	1	0.17

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Results of ANOVA on LMM with site as a random factor. Significant differences (p<0.05) between SWPs and SNPs are indicated in bold.

863 Tab. 4: Statistical outputs from Linear Model Analysis testing for relationships between male frog biometric variables and
 864 environmental parameters at SWPs

LM				
Dependant variable	Parameter	Estimate (β)	SE	P
SVL	<i>(intercept)</i>	73.98	1.55	***
	Surface crops 5000 m	4.92	2.01	*
	Surface forest 5000 m	4.12	1.72	*
	Surface notable wetlands 5000 m	-2.93	0.97	*
BCI	<i>(intercept)</i>	8.1E-03	4.1E-03	ns
	Presence of an oil remover	-2.3E-02	3.2E-03	**
	Surface grassland 5000 m	-1.2E-02	6.3E-03	**
FA forearms	<i>(intercept)</i>	2.9E-02	1.3E-03	***
	Surface grassland 5000 m	-3.6E-03	1.4E-03	*
	Surface notable wetlands 5000 m	-4.5E-03	1.4E-03	**
FA tibias~1				

ns = not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

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