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2

3 **Title:** Cost-effective spatial sampling designs for field surveys of species distribution

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24 **Abstract**

25 High-quality biodiversity inventories are key tools to develop effective conservation strategies, but  
26 financial resources devoted to systematic species inventories are usually limited. Different sampling  
27 strategies have been proposed to efficiently allocate such limited resources (i.e. accessibility-based,  
28 stratified random and grid samplings), but their effectiveness may depend on the aim of the survey. Our  
29 aim was to assess which approach can provide the best trade-off between sampling costs and accuracy  
30 in estimating both single species distribution and regional species set composition. We generated  
31 simulated species distribution data to compare costs and performances of the three sampling methods in  
32 assessing species distribution. When we aim at measuring species range (i.e. area of occupancy or  
33 extent of occurrence), or obtaining baseline ecological data for conservation assessments (i.e. niche  
34 breadth), grid sampling usually provided the best trade-off between performances and costs at both the  
35 species and regional levels. Otherwise, the stratified random sampling outperformed the other methods  
36 when we are interested in assessing the relative rarity (i.e. species frequency) of the species across the  
37 study area. Low quality distribution data can lead to heavily biased conclusions on biodiversity trends  
38 or impacts of environmental changes; our findings highlight that selecting the right sampling strategy is  
39 essential to obtain reliable estimates of both single species distribution and regional species set  
40 composition.

41

42 **Keywords:** field survey design, grid sampling, stratified random sampling, accessibility-based sampling,  
43 species frequencies, area of occupancy, extent of occurrence, niche breadth

44

## 45 **Introduction**

46 Species inventories are a key tool to obtain baseline data on the distribution of organisms and to  
47 develop effective conservation strategies (Barthlott & Winiger 1998). Systematic field surveys can  
48 enhance our knowledge of species occurrences and relative frequencies, which are essential to detect  
49 and track changes in biodiversity patterns (e.g. modifications in species richness or community  
50 composition following climate change, urbanization or agricultural intensification), to identify species  
51 or areas of high conservation priority, and to develop successful management measures (Austin &  
52 Heyligers 1989; Neldner et al. 1995; Hortal & Lobo 2005). Although survey campaigns are widely  
53 acknowledged as a primary tool in conservation planning and management, human and financial  
54 resources devoted to biodiversity survey and monitoring are limited. As a consequence, one of the main  
55 issues for conservationists and managers remains how to allocate limited resources to carry out the best  
56 conservation outcomes (McCarthy et al. 2012; Ficetola et al. 2018).

57         Surveying costs, in terms of time and/or funds, can be reduced by selecting sampling sites that  
58 are more easily accessible, usually close to roads (“accessibility-based” sampling) (Greenwood, 1996;  
59 Jobe & White 2009). However, site accessibility is seldom uniform across a region. For instance, road  
60 distribution is related to multiple factors, such as the physical properties of the landscape (e.g.  
61 elevation, orography, presence of barriers), and the distribution of human activities (e.g. presence of  
62 urban, agricultural or industrial areas) (Nelson 2008; Uchida & Nelson 2010). Therefore, easily  
63 accessible sites are often associated with anthropogenic stresses that are likely to affect species  
64 distribution. Many plant and animal species show limited frequency and / or activity nearby roads (e.g.  
65 edge effect) because of lower habitat quality and increased mortality (Forman & Alexander 1998;  
66 Trombulak & Frissell 2000; Fahrig & Rytwinski 2009). As a consequence, even if appealing from a  
67 cost perspective, accessibility-based samplings may provide spatially and/or ecologically biased data

68 (Kadmon et al. 2004). It is thus fundamental that these aspects are carefully accounted for before any  
69 inference is made about patterns and potential drivers of biodiversity.

70         Given the spatial bias of many species distribution datasets (Ficetola et al. 2013; Yang et al.  
71 2014), several methods have been proposed to optimize and standardize efforts in collecting  
72 biodiversity information across a given area. Stratified (habitat-specific) random and grid sampling are  
73 among the most popular methods (Smith et al. 2017). However, outputs, spatial bias and costs may be  
74 very different among these methods, and their effectiveness mostly depends on the aims of the study.  
75 Stratified random sampling could return spatially unbiased information about species distribution and  
76 frequency across the study area by sampling all the potential suitable habitats (Yoccoz et al. 2001;  
77 Smith et al. 2017) but, due to logistic constraints, its application may be limited to surveying a reduced  
78 number of taxa in relatively small study areas (Guisan & Zimmermann 2000). This method seems  
79 particularly appropriate for investigating the distribution of rare or endangered species with well-  
80 known ecological constraints, as it requires some *a-priori* knowledge of the requirements of target  
81 species (e.g. inhabited vegetation types, elevational range); consequently, setting up a multi-habitat and  
82 multi-species (i.e. assemblage level) stratified sampling over large study areas can be technically  
83 complex and expensive (Guisan & Zimmermann 2000). Grid sampling (systematic survey *sensu*  
84 Wessels et al. 1998) could be more appropriate if the aim is to collect data on distribution patterns on a  
85 large set of species (e.g. assemblages) within a study area. In this case, a uniform sampling of the study  
86 area would be desirable. This approach could provide spatially unbiased estimates of species  
87 distribution, which are helpful to map biodiversity patterns within the study area; however it could be  
88 excessively expensive, and may not always lead to reliable estimates of species frequencies (Overton &  
89 Lehmann 2003). Even if statistically representative, both of these approaches may nevertheless under-  
90 represent or even lack species living in extremely rare habitats, for which ad-hoc strategies of site  
91 selection could be advisable (Økland 2007; Rolaček et al. 2007).

92           The choice of the sampling method is a crucial and challenging task that requires awareness  
93 about the strengths and weaknesses associated with each sampling approach. The relative performances  
94 and costs of different approaches may be assessed by comparing data collected with different protocols  
95 in the same area (Kadmon et al. 2004; Mccarthy et al. 2012). However, no method provides a perfect  
96 knowledge of true species distribution, thus hampering the estimation of the absolute biases. The  
97 analysis of simulated data on species distribution provides several advantages, such as the perfect  
98 knowledge of species occupancy and frequency, and community composition across the study area;  
99 this, in turn, allows the quantification of the sampling bias in relation to the real pattern (i.e. the  
100 “truth”), and the comparison of the biases of estimators based on different sampling methods (Hirzel &  
101 Guisan 2002; Zurell et al. 2010; Smith et al. 2017).

102           Here we used simulated species distribution data to compare costs (in terms of time needed to  
103 reach and survey the sites; i.e. total time) and performances of three different sampling methods  
104 (accessibility-based, stratified random and grid samplings) in assessing both single species distribution  
105 and species set composition across the study area. Stratified random and grid are rigorous sampling  
106 strategies, which can allow unbiased estimation of the parameters of interest (Smith et al. 2017). On the  
107 contrary, accessibility-based sampling often has high bias, but such data are frequent in occasional  
108 inventories, thus it is important to assess their relative performance. We considered three landscapes  
109 configurations, differing for their accessibility (i.e. road densities) and also assessed the robustness of  
110 our results to the issues of imperfect detection (MacKenzie et al. 2006; Kery & Royle 2016) and edge  
111 effect (Palomino & Carrascal 2007; Semlitsch et al. 2007), given their pervasive effects on species  
112 distribution data and on the reliability of survey results. Water dependent organisms were selected as it  
113 is easy to identify relationships between the distribution of presence sites (i.e. waterbodies) and  
114 accessibility, but results can be applied to many organisms that can be sampled in sites where  
115 appropriate resources (habitats) are. The aim of our study was to provide guidelines for researchers as

116 well as for non-profit organization and government agencies dealing with biodiversity survey and  
117 monitoring. This will allow optimizing sampling design depending on both the survey aim and  
118 available resources, thus maximizing the reliability of the gathered data in term of species distribution.

119

120

## 121 **Methods**

### 122 *Simulated species and landscape*

123 Our simulation approach mimicked surveys aiming at detecting water-dependent organisms (e.g.  
124 amphibians, water birds, insects, or any kind of aquatic taxon). Artificial distribution data were  
125 generated for 15 hypothetical aquatic species differing in their habitat preferences, response to  
126 elevational gradients, and occupancy probabilities. For habitat preferences, we considered three species  
127 typologies: specialists for lentic habitats (e.g. ponds or small lakes), specialist of lotic habitats (e.g.  
128 streams), generalist (present in both typologies; Table 1). For elevation, each species showed an  
129 optimal elevation, and we assumed a Gaussian response to the altitudinal gradients (i.e. each species  
130 responded to the elevational variation with a symmetrical and decreasing occurrence probability around  
131 an optimum value, following a Gaussian probability curve). Species differed in optimum value (mean)  
132 and amplitude of their responses (standard deviation, sd) (see Table 1). Although variables other than  
133 elevation (e.g., water depth) also affect the distribution of aquatic species, and elevation may not be the  
134 key environmental driver of distribution *per se*, elevation is directly or indirectly linked to major  
135 variables (e.g. temperature, solar radiation, oxygen pressure, hydroperiod and wind), that can deeply  
136 influence organisms occurrence and frequency and overall biodiversity patterns (Guisan &  
137 Zimmermann 2000; Körner 2007; Graham et al. 2014). Furthermore, orography strongly determines  
138 the distribution of roads. To obtain realistic species distributions, occupancy probability was set to 0.5  
139 (6 species) or 0.25 (9 species): only a randomly selected portion of suitable sites was thus considered

140 effectively populated. Consequently, for each species, realized occupancy was higher around the  
141 optimum value (mean) and decreased following a Gaussian probability curve. Potential biotic  
142 interactions among simulated species were not considered. See Electronic Supplementary Material 1  
143 (ESM1) for an example of the scripts used to generate species distribution data.

144 To obtain simulations mimicking the complexity of real landscapes, simulated data were  
145 generated on a true area of  $40 \times 40$  km placed at the foothills of the Eastern Italian Alps (upperleft  
146 corner:  $x = 714,000$  m,  $y = 5,114,000$ ; Map projection: UTM zone 32N), characterized by an  
147 elevational range of more than 2,000 m. Patterns of spatial aggregation of lentic waters and paths of  
148 both roads and lotic waters are mainly determined by local orography, geomorphological and  
149 lithological features. Selecting a true area allowed us obtaining a realistic distribution of both sampling  
150 sites and road network without compromising the generality of results (Hirzel et al. 2001; Meynard &  
151 Quinn 2007).

152

### 153 *Environmental variables*

154 For the study area, elevation data were obtained from the Shuttle radar topographic mission (SRTM;  
155 original resolution = 3 arc-seconds; downloaded on 20<sup>th</sup> April 2010), reprojected to UTM 32N  
156 (resolution = 92.66 m) and slightly rescaled to vary between 0 and 2,252 m a.s.l. (Figure 1a). The  
157 complete road network was obtained from the database DBPrior10K (downloaded on 15<sup>th</sup> January 2016  
158 from <http://www.centrointerregionale-gis.it/DBPrior/DBPrior.asp>). Single roads, both main and  
159 secondary roads (branches), were manually reclassified to three different classes (class 1 to class 3;  
160 Figure 1b). In our simulations we explored three scenarios of accessibility (low, medium and high road  
161 densities). In the low accessibility scenario we only considered class 1 roads (main roads); class 1 + 2  
162 roads (main roads and their first branches) were considered in the medium accessibility scenario, and  
163 for the high accessibility scenario we considered all roads as exploitable during the survey.

164            Sampling sites included both lentic and lotic sites. Lotic sites were obtained by simplifying the  
165 hydrographic network available on the Italian National Geoportal website (downloaded on 7<sup>th</sup>  
166 November 2015 from [http://wms.pcn.minambiente.it/ogc?map=/ms\\_ogc/wfs/Aste\\_fluviali.map](http://wms.pcn.minambiente.it/ogc?map=/ms_ogc/wfs/Aste_fluviali.map) via the  
167 Web Feature Service in Quantum GIS 2.2). For each stream we set a sampling site every 1,500 m with  
168 a minimum of 2 sampling sites per stream, obtaining a total of 719 lotic sites (Figure 1c). Lentic sites  
169 were detected from the toponym layer (downloaded on 13<sup>th</sup> November 2015 from  
170 [http://wms.pcn.minambiente.it/ogc?map=/ms\\_ogc/wfs/Toponimi\\_2011.map](http://wms.pcn.minambiente.it/ogc?map=/ms_ogc/wfs/Toponimi_2011.map) via the Web Feature  
171 Service in Quantum GIS 2.2), by selecting sites representing water-related typologies (118 points).  
172 Available maps certainly underestimate lentic sites, given that small ponds are often undetected by  
173 aerial photos (Ficetola et al. 2015). To approximate a 2:1 ratio between lotic and lentic sampling sites  
174 and retain at the same time the spatial aggregation pattern typical of lentic habitats, we randomly  
175 generated 225 additional lentic points within a buffer of 2,000 m from the extant ones (total lentic sites  
176 = 343; Figure 1c). This led to a total of 1,062 sampling sites (719 lotic + 343 lentic sites). For each  
177 potential sampling site, travelling costs (in term of time) were calculated using the *gdistance* R package  
178 (van Etten 2015) and applying the Tobler's Hiking Function. This function provides a rough estimate  
179 for the maximum speed of off-path hiking given the slope of the terrain (Tobler 1993). Once obtained  
180 the inter-cell speed (m/s), the correction (ratio) for the inter-centroid distance converts the speeds in  
181 reciprocal of times (1/s): simply summing the reciprocal of these reciprocals ( $\Sigma 1/(1/s)$ ) allow us to  
182 obtain the total travelling time. For each of the three accessibility scenarios, costs were estimated  
183 between each sampling site and the closest road. Despite in the real world it is not always feasible to  
184 gain access to the whole set of sampling sites, here we considered all sites potentially accessible and  
185 differing only in the travelling cost to be spent in reaching them.

186

187 ***Survey design***



188 We evaluated three survey strategies (grid, stratified random and accessibility-based samplings) under  
189 three scenarios of accessibility (low, medium and high). In 999 simulations, we generated the  
190 distribution of artificial species; simulated species sets were then sampled according to the three  
191 different methods (see Supplementary Figure 1b-d in ESM2 for an example of site selection). To  
192 simplify comparisons, we employed the same sampling effort (i.e. same number of sampling sites) in  
193 the three sampling methods. Grid sampling was performed by building grids of different cell size and  
194 selecting, whenever present, one lotic and one lentic site within each cell of the grid. To account for  
195 scale dependent effects, analyses were run using cell sizes of 10, 6.67, 5, 4, 3.33 and 2.5 km  
196 (corresponding to 32, 69, 118, 167, 235 and 373 sampling sites). We applied the same sampling effort  
197 to the three methods, thus the same number of sampling sites ( $n$ ) used in the grid approach was  
198 subsequently sampled with the stratified random and accessibility-based methods. For the stratified  
199 random sampling we considered just one ecologically informative stratum, i.e. the availability of water  
200 resources (both streams and ponds) across the whole study area. Sampling was then performed by  
201 randomly selecting from the whole dataset of water resources  $n$  sampling sites. Only for the  
202 accessibility-based sampling, we selected the  $n$  sampling sites with the lowest travelling costs;  
203 consequently, the total cost is the same for all the replicates with the same  $n$  within the same  
204 accessibility scenario. Travelling cost estimation and sampling selection were repeated for each of three  
205 accessibility scenarios. For purpose of comparison, two additional values of  $n$  (600 and 750 sites) were  
206 further sampled with the accessibility-based sampling only. A total of 60 combinations were thus  
207 analysed for each of the 999 simulated species sets: 3 sampling methods  $\times$  6 sampling efforts  $\times$  3  
208 accessibility scenarios, plus two additional sampling efforts (i.e. 600 and 750 sites)  $\times$  3 accessibility  
209 scenarios for the accessibility-based sampling only.

210 We performed two additional simulation runs to assess the impact of edge effect and imperfect  
211 detection on our conclusions. To assess the consequences of edge effect, sites within 90 m from roads

212 were considered unsuitable for the target species (average travel time: about 110 s from the nearest  
213 road), all other parameters being constant. Furthermore, in standard analyses, we assumed just one  
214 survey per site and perfect detection of all the present species. However, detection probability is almost  
215 always below one, and multiple surveys are needed to obtain robust estimates of species distribution  
216 (MacKenzie et al. 2006; Petitot et al. 2014). We therefore repeated simulations assuming that species  
217 have imperfect detection; the detection probability of each species was randomly drawn from the  
218 interval [0.1,0.7]. Each site was surveyed in three distinct sampling occasions, while all the other  
219 parameters remained consistent with the other simulations.

220

### 221 *Assessing the efficiency of survey methods*

222 The performance of each survey method (grid, stratified random and accessibility-based methods) was  
223 evaluated by its ability to assess species distribution at a given survey cost. At the regional level, two  
224 measures of species distribution were used, reflecting different survey aims: area of occupancy and  
225 species frequency across the landscape. Area of occupancy is a measure of the spatial distribution of  
226 species, while frequency across the landscape is the proportion of sites with species presence. These  
227 two metrics are not necessarily correlated and allow to describe and represent different forms of rarity  
228 (Rabinowitz 1981). For instance, a species can occupy a very large number of sites within a small area  
229 (e.g. small range species that are locally abundant), or can occupy very large ranges with just a few  
230 populations (sparse populations over broad ranges). For each cell size used during the grid sampling  
231 (i.e. 10, 6.67, 5, 4, 3.33 and 2.5 km), area of occupancy was calculated as the total number of cells in  
232 which a given species was present (true occupancy) or collected (sampled occupancy) standardized by  
233 total number of cells; this approach is similar to the one used during IUCN species assessment. Species  
234 frequency across the study area was calculated as the total number of sites in which the species was  
235 present (true frequency) or collected (sampled frequency), standardized by the total number of sites or

236 the number of surveyed sites, respectively. At the regional level, bias was calculated as the overall  
237 Renkonen (Percentage) dissimilarity (Renkonen 1938) between standardized sampled (i.e. sampled  
238 occupancy or frequency) and true (i.e. true occupancy or frequency) species sets. Renkonen  
239 dissimilarity corresponds to Bray-Curtis dissimilarity when this is calculated on relative rather than  
240 absolute abundances, and solves the problem of density invariance highlighted for this latter index (Jost  
241 et al. 2011). At the species level, we measured performance also for two additional parameters: niche  
242 breadth and extent of occurrence. For each species, niche breadth was calculated as the altitudinal  
243 range experienced by the species, while extent of occurrence as the area contained within the minimum  
244 convex polygon enclosing all sites occupied by the species.

245 For each estimator of sampling performances, we calculated relative bias as (true value -  
246 estimated value) / true value. Consequently, bias values can range between -1 and 1 when dealing with  
247 species frequency across the landscape, and between 0 and 1 in all the other cases (i.e., area of  
248 occupancy, extent of occurrence and niche breadth). We report species-level measures of bias for a  
249 subset of species representing the whole range of simulated species: the commonest (Species 1), the  
250 rarest (Species 9) and one species with an intermediate frequency (Species 10).

251 In biodiversity surveys, the time required by operators to complete sampling is a major  
252 determinant of total survey cost. We used two metrics to measure the sampling cost of each survey  
253 scheme: cumulative travel time, and number of surveyed sites. Cumulative travel time was the sum of  
254 the time needed to reach all the  $n$  sites, as the time to reach survey sites constitute a major part of the  
255 working time of operators. Furthermore, we considered the total number of surveyed sites, as sampling  
256 more sites requires a larger effort. The number of surveyed sites ranged between 32 and 373 (up to 750  
257 for the accessibility-based sampling only). These measures were calculated for each of the three  
258 different accessibility scenarios (from low to high road density). We finally calculated the total survey  
259 time as (number of surveyed sites  $\times$  site sampling time) + cumulative travel times, by assuming an

260 average sampling time of 20 minutes per site, which is a typical survey effort for the national  
261 monitoring of amphibians and reptiles in Italy (Stoch & Genovesi 2016). Times other than off-path  
262 hiking (e.g., driving time from a “base”) and costs for materials (e.g., sampling equipment or fuel) were  
263 not considered for the calculation of costs as they strongly depend on the positioning of the base and  
264 the sampling methodology, respectively.

265 Analyses were performed using the R programming environment (R 3.2.5; R Development  
266 Core Team 2016) and associated packages (Goslee & Urban 2007; Bivand & Rundel 2014; Bivand et  
267 al. 2015; Hijmans 2015; van Etten 2015). Data sets and R scripts used to run the analyses are available  
268 as supplementary material (ESM1).

269

270

## 271 **Results**

272 Analyses of relationships between sampling costs and bias showed that an increase in total survey time  
273 was always associated with a decrease in sampling bias (Figs. 2-4). However the different monitoring  
274 strategies showed substantial differences in bias for all the measures of species distribution used, i.e.  
275 area of occupancy (Figs. 2a-c and 3a-c), frequency (Figs. 2d-f and 3d-f), extent of occurrence (Fig. 4a-  
276 c) or niche breadth (Fig. 4d-f), and across the accessibility scenario considered.

277

### 278 ***Regional level analysis: species area of occupancy***

279 When we considered the reliability of estimates of area of occupancy across the whole species set and  
280 study area, the accessibility-based sampling always showed smaller total and travel times than the other  
281 methods (Fig. 2a-c and Supplementary Figure 2a-c in ESM2, respectively). The relative performances  
282 (biases) of the three methods considerably varied depending on the accessibility scenario (Fig. 2a-c).  
283 Grid sampling consistently provided the best estimates across all the accessibility scenarios, although

284 accessibility-based samplings slightly outperformed the others when the greatest number of sites was  
285 sampled (750 sites). Accessibility-based and random sampling showed similar performance in the high  
286 and medium accessibility scenarios (Fig. 2a-b), while random sampling generally showed lower bias  
287 than the accessibility approach in the low accessibility one (Fig. 2c). See Supplementary Figure 2 in  
288 ESM2 for an estimation of sampling cost, separately showing cumulative travel times and number of  
289 sampled sites and Supplementary Figures 5a-c in ESM2 for the consequences that edge effect has on  
290 sampling bias.

291

### 292 ***Regional level analysis: species frequency in the landscape***

293 When we considered the reliability of species frequency estimates across the whole species set and  
294 study area, the relative performances of each method were consistent across the three accessibility  
295 scenarios (Fig. 2d-f, Supplementary Figure 3 in ESM2). Stratified random sampling returned the most  
296 accurate estimation of the species set at the regional level (Fig. 2d-f), while the accessibility-based  
297 sampling provided the worst estimates, irrespective of the landscape accessibility and the measures of  
298 cost used. See Supplementary Figure 3 in ESM2 for an estimation of sampling cost, showing separately  
299 cumulative travel times and number of sampled sites and Supplementary Figures 5d-f in ESM2 for the  
300 consequences that edge effect has on sampling bias.

301

### 302 ***Species level analysis***

303 The performances of the three sampling methods in describing area of occupancy, frequency, extent of  
304 occurrence and niche breadth of single species revealed patterns partially similar to the ones from the  
305 regional level analyses (Figs. 3 and 4). Here we focus on the results of the high accessibility scenario,  
306 but conclusions for the other scenarios were similar (Supplementary Fig. 4 in ESM2). For all the  
307 species, sampling bias ranged more widely with respect to the regional level analyses. Considering the

308 bias in estimating the area of occupancy (Fig. 3a-c), the accessibility-based method showed the best  
309 performances for common species only (Fig. 3a), whereas grid sampling outperformed accessibility-  
310 based sampling for rare species (Fig. 3c). For the estimation of species frequencies across the landscape  
311 (Fig. 3d-f), the results are consistent with the patterns observed at the regional level: the stratified  
312 random sampling provides bias values very close to zero for all the species and thus clearly  
313 outperformed the other methodologies. For the estimation of the extent of occurrence (Fig. 4a-c), the  
314 accessibility-based sampling slightly outperformed the other methods for species with high and  
315 intermediate frequencies (Fig. 4a-b), while grid sampling showed the lowest bias when dealing with  
316 rare species (Fig. 4c). Lastly, considering the bias in estimating niche breadth (Fig. 4d-f) all the  
317 methods provide a similar performance for species with high and intermediate frequency (Fig. 4d-e),  
318 while grid sampling returned the best estimates for rare species (Fig. 4f).

319

### 320 *Imperfect detection*

321 At the regional level, the overall performances of the three sampling methods were consistent with  
322 previous results, when imperfect detection was included in simulations (Supplementary Figure 6 in  
323 ESM2). Grid and stratified random samplings returned the best estimates of area of occupancy (Fig.  
324 S6a-c) and species frequency (Fig. S6d-f), respectively, but incomplete detection and multiple  
325 sampling occasions increased both the uncertainties in estimating the species set at the regional level,  
326 and the sampling costs.

327

### 328 **Discussion**

329 Efficient and reliable biodiversity surveys are necessary to obtain distribution data, but substantial  
330 resources are required to obtain robust estimates of species range and frequency. At a given sampling

331 cost, different approaches show strong heterogeneity in performance, and our results help to select the  
332 optimal sampling strategy depending on both the aims of the survey and the landscape accessibility.

333         When the main aim is obtaining measures of geographic range of species, baseline data for  
334 conservation assessments (IUCN 2001; Tracewski et al. 2016), or overall biodiversity patterns across  
335 the landscape, grid -based sampling provides a good trade-off between sampling bias and costs at both  
336 the regional and single species levels (Figs. 2a-c, 3a-c, 4). Accessibility-based sampling effectively  
337 estimated the area of occupancy of commonest species, but suffers multiple drawbacks. First, species  
338 distributions can be accessibility-biased (e.g. lower abundance nearby roads, a classical case of edge  
339 effect) (Palomino & Carrascal 2007; Semlitsch et al. 2007), and under these circumstances selecting  
340 sites on the basis of accessibility would provide biased results (discussed below). Furthermore, grid  
341 sampling considerably outperforms the accessibility-based one in estimating areas of occupancy level  
342 (Fig. 2) and the distribution of rare species (Fig. 3c, Fig. 4c and Fig. 4f). Grid sampling allows a  
343 homogeneous spatial distribution of sampling sites across the whole study area, thus providing more  
344 balanced estimates of single species relative distribution and maximising spatial coverage, which is  
345 essential for the assessment of species ranges. The grid approach we used can be particularly effective,  
346 as it may be seen as a grid-based stratified sampling: in fact, within each cell, two different typologies  
347 of sites (i.e. one lentic plus one lotic habitat) were randomly selected, allowing to take into account  
348 ecological variation and thus improving the overall quality of the estimates.

349         Conversely, if the main aim of the survey is to collect reliable data on species frequency across  
350 the landscape, the stratified random sampling outperformed the other methods in describing both  
351 regional patterns and single species frequencies (Fig. 2d-f, Fig. 3d-f). This can be due to its ability to  
352 gather data proportionally to the resource typology and spatial availability, allowing a more reliable  
353 estimation of species frequency within the study area. The excellent performance of random sampling  
354 in estimating species frequency at both the regional and the single species level was independent of

355 landscape accessibility and the measures of cost used (Fig. 2d-f, Fig. 3d-f, Supplementary Figures 3 &  
356 4d).

357 Occasional samplings are often biased by accessibility. As occasional sampling is a main source  
358 of biodiversity distribution data, accessibility-based sampling is perhaps the most frequent strategy for  
359 the collection of distribution data, even though this is only seldom explicitly stated. For instance,  
360 citizen science provides a huge amount of data over large temporal and spatial scales but it is prone to  
361 spatial biases from infrastructure and human population density (Geldmann et al. 2016) because roads,  
362 cities, and other physical features determine accessibility for observers. This bias may be reduced using  
363 effective protocol development and volunteer training (Flesch & Belt 2017), still it remains pervasive  
364 in biodiversity datasets. In principle, selecting sampling sites on the basis of accessibility greatly  
365 reduces sampling time, and thus allows visiting a larger number of sites. For instance, in this study the  
366 travel time needed to visit the 373 most accessible sites (53 h) was about seven times lower than the  
367 time required to visit the same number of sites selected using the alternatives schemes (355 and 362 h  
368 for grid and stratified random sampling, respectively), in the intermediate accessibility landscape  
369 (Supplementary Fig. 2b). Unfortunately, surveying such a large number of sites does not improve the  
370 quality of results, confirming the existing concerns on road-biased sampling. Accessibility-based  
371 sampling is sometimes thought to represent the most cost-effective solution to sample an area (Albert et  
372 al. 2010), but its effectiveness strongly depends on the density of the road network: in fact, sampling  
373 sites close to roads reduces costs only within highly accessible landscapes or for common species (Fig.  
374 2, Fig. 3a and Fig. 4a and d), and only if road distribution is not heavily biased by spatial and  
375 ecological features (e.g. landscape composition or orography). Given that such biases are widespread,  
376 and given that the usefulness of the accessibility-based sampling is restricted to specific conditions, if  
377 possible other sampling strategies should be preferred in most of programmes.



378 In addition, roads often have negative effects such as direct killing by vehicles, disturbance,  
379 barrier effects and pollution (Forman & Alexander 1998; Rytwinski & Fahrig 2015). Consequently,  
380 occupancy is generally reduced in sites nearby roads (edge effect) (Palomino & Carrascal 2007;  
381 Semlitsch et al. 2007), posing additional issues to the accessibility-based sampling. If we assume that  
382 sites within 90 m from roads are unsuitable for the target species, accessibility-based sampling  
383 becomes even less reliable (Supplementary Fig. 5 in ESM2). When we estimate area of occupancy and  
384 species frequencies accounting for edge effect, the performances of the accessibility-based survey were  
385 far from being reliable. In practice, edge effects determine the highest observed bias values  
386 (Supplementary Fig. 5), and completely erases any potential advantage of accessibility-based sampling.  
387 Nevertheless, the interactions between roads, species occurrence, accessibility, and performance of  
388 surveys can be complex, and there are cases in which performing sampling along roads do not provide  
389 biased estimates of species distribution (Mccarthy et al. 2012).

390 In the real world, imperfect detection of species is pervasive, further increasing the complexity  
391 of planning biodiversity surveys (MacKenzie et al. 2006; Petitot et al. 2014). If detection is imperfect,  
392 multiple visits must be performed to each site, thus increasing the overall cost and the uncertainties of  
393 species distribution estimates. Nevertheless, after taking into account imperfect detection we obtained  
394 the same overall pattern, with grid sampling providing the best assessment of species range, and  
395 stratified sampling providing the best assessments of species frequencies (Supplementary Figure 6 in  
396 ESM2). This is probably due to the fact that detection probability was not different among sites with  
397 different accessibility, and the number of surveys per site was adequate to obtain reliable estimates of  
398 species occupancy. The situation could be more challenging when detection probability of species is  
399 not spatially random (Gu & Swihart 2004). For instance, species detection might be lower for rare  
400 species (Tanadini & Schmidt 2011) or nearby roads: in this case we expect that non-random imperfect  
401 detection would further increase the bias of accessibility-based sampling.

402 Our simulations were developed assuming aquatic species as the target of the survey and testing  
403 the effectiveness of three alternative sampling strategies. Small wetlands and streams often are discrete  
404 habitats, thus an *a-priori* selection of sites with a stratified sampling can be easily performed using  
405 geographic information systems, if information on wetland distribution is available. The selection of  
406 sampling sites may be more complex for terrestrial or marine organisms, whose habitats are often  
407 represented as polygon-like features (Smith et al. 2017). For these organisms there is the additional  
408 question of the appropriate position of the sampling site, within the polygon extent. The definition of  
409 the appropriate sampling site (e.g. point count, transect or trapping station) is strongly dependent on the  
410 study taxon and on the research aims, and is beyond the scope of the present study. Still, the increasing  
411 availability of informative strata (e.g. habitat typology, altitude, and microclimate data layers) can  
412 allow integrating multiple information sources, in order to optimize the sampling strategy even in the  
413 most complex situations. Therefore, grid and stratified random sampling can also be used for the  
414 selection of sampling sites for terrestrial organisms, once the potential sampling sites have been  
415 defined. At the same time, alternative sampling strategies such as the generalized random-tessellation  
416 stratified (GRTS; Stevens & Olsen, 2004) and the gradient directed transects (grandsects; Gillison &  
417 Brewer, 1985; Wessels et al., 1998) could be just as reliable as those tested here to optimize and  
418 standardize efforts in collecting biodiversity information across a given area. All of these objective  
419 approaches to site selection have the advantage to strongly limit subjective choices driven by  
420 environmental attractiveness or accessibility (Soberón et al. 2000; Parnell et al. 2003; Moerman &  
421 Estabrook 2006; Romo et al. 2006).

422 There is not a single sampling approach suitable for all the circumstances and, when setting up  
423 a survey or monitoring programme, the optimal sampling strategy should be defined on the basis of the  
424 landscape structure and the aims of the programme (Yoccoz et al. 2001). If the aim is to collect  
425 unbiased data on the spatial distribution of the species (e.g. for a distribution atlas) and to use them to

426 assess biodiversity patterns, a grid sampling, eventually associated with a stratified selection of sites  
427 within each cell, is the more appropriate and cost-effective method. Conversely, the stratified random  
428 sampling returns the best trade-off between data reliability and sampling cost, when the focus is on  
429 species frequencies (e.g. assessing species rarity). Monitoring programmes must be repeated in time, to  
430 discover potential biodiversity changes, assess the consequences of environmental modifications, and  
431 test whether populations are declining or increasing (Nichols & Williams 2006; Wintle et al. 2010;  
432 Ficetola et al. 2018). However, low quality distribution data can lead to heavily biased conclusions  
433 when we test species or biodiversity trends, and impacts of environmental changes (Yoccoz et al.  
434 2001). Selecting an optimal and objective approach to survey or monitoring is important to optimize  
435 the results, but is also the key to obtain reliable assessments of the long-term trajectories of species and  
436 ecosystems, and thus to best inform conservation and management.

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598

599

600 **Author Contributions**

601 SM, AR and FL conceived the idea; SM and GFF designed the methodology; SM, GFF and FL

602 analysed the data; FL and SM led the writing of the manuscript. All authors critically contributed to the

603 drafts and gave final approval for publication.

604 **Figure captions**

605

606 **Figure 1** (b/w in print)

607 Study area: Digital elevation model (a). Road network with classified roads: class 1 only = low  
608 accessibility scenario; class 1 + 2 = medium accessibility scenario; all classes = high accessibility  
609 scenario (b). Sampling stations (N = 1062) showing separately the 719 lotic sites (along streams) and  
610 the 343 lentic sites. Green triangles = lentic sites; blue circles = lotic sites (c).

611

612 **Figure 2** (b/w in print)

613 Regional level: relationships between total sampling costs and bias for the three sampling methods  
614 (grid, random and accessibility-based samplings) at three different accessibility scenarios (high,  
615 medium and low road densities). Grid sampling was performed using cell sizes of 10, 6.67, 5, 4, 3.33  
616 and 2.5 km (corresponding to 32, 69, 118, 167, 235 and 373 sampling sites). Total sampling cost was  
617 measured as total time: (number of surveyed sites × site sampling time) + cumulative travel times. Bias  
618 was calculated as Renkonen (Percentage) dissimilarity between true and sampled species sets based on  
619 area of occupancy (Fig. 2a-c) and species frequency (Fig. 2d-f). Bars represent the 0.025 and 0.975  
620 quantiles: vertical bars refer to distribution of the bias, whereas horizontal bars refer to total sampling  
621 times. Blue circles = grid sampling; green squares = random sampling; black diamonds = accessibility-  
622 based sampling; grey diamonds = accessibility-based sampling, 600 and 750 sampling sites.

623

624 **Figure 3** (b/w in print)

625 Species level: relationships between total sampling costs and bias for the three sampling methods (grid,  
626 random and accessibility-based samplings) using the high accessibility scenario. Grid sampling was  
627 performed using cell sizes of 10, 6.67, 5, 4, 3.33 and 2.5 km (corresponding to 32, 69, 118, 167, 235

628 and 373 sampling sites). Total sampling cost was measured as total time (see Fig. 2). Three species  
629 were reported: the commonest (Species 1), the rarest (Species 9) and one species with an intermediate  
630 frequency (Species 10). Estimates of single species distribution were based on area of occupancy (Fig.  
631 3a-c) and species frequency (Fig. 3d-f). Relative bias was calculated as  $(\text{true value} - \text{estimated value}) /$   
632 true value. Bars represent the 0.025 and 0.975 quantiles: vertical bars refer to distribution of the bias,  
633 whereas horizontal bars to total sampling time. Blue circles = grid sampling; green squares = random  
634 sampling; black diamonds = accessibility-based sampling; grey diamonds = accessibility-based  
635 sampling, 600 and 750 sampling sites.

636

637 **Figure 4** (b/w in print)

638 Species level: relationships between total sampling costs and bias for the three sampling methods (grid,  
639 random and accessibility-based samplings) using the high accessibility scenario. Grid sampling was  
640 performed using cell sizes of 10, 6.67, 5, 4, 3.33 and 2.5 km (corresponding to 32, 69, 118, 167, 235  
641 and 373 sampling sites). Total sampling cost was measured as total time (see Fig. 2). Three species  
642 were reported: the commonest (Species 1; Fig. 4a and d), the rarest (Species 9; Fig. 4c and f) and one  
643 species with an intermediate frequency (Species 10; Fig. 4b and e). Estimates of single species  
644 distribution were based on extent of occurrence (Fig. 4a-c) and niche breadth (Fig. 4d-f). Relative bias  
645 was calculated as  $(\text{true value} - \text{estimated value}) / \text{true value}$ . Bars represent the 0.025 and 0.975  
646 quantiles: vertical bars refer to distribution of the bias, whereas horizontal bars to total sampling time.  
647 Blue circles = grid sampling; green squares = random sampling; black diamonds = accessibility-based  
648 sampling; grey diamonds = accessibility-based sampling, 600 and 750 sampling sites.

649 **Tables**

650 **Table 1:** Ecological preferences and occupancy probability of the 15 artificial species. We assumed  
651 Gaussian responses of the species to elevational gradients: each species was characterized by its  
652 optimal value (mean) and amplitude of the response (sd - standard deviation). Three species typologies  
653 were considered, according to their habitat preferences: specialists for lentic habitats (e.g., ponds),  
654 specialists for lotic habitats (e.g., streams) and generalists. Finally, two occupancy probabilities (0.5  
655 and 0.25) were used to control the relative rarity of the species within suitable habitats.

656

Species	Elevation range (m a.s.l.)		Habitat typology	Occupancy
	mean	sd		
Species 1	250	125	lotic	0.5
Species 2	900	200	lotic	0.5
Species 3	1900	300	lotic	0.5
Species 4	600	300	lotic	0.25
Species 5	1150	325	lotic	0.25
Species 6	1600	300	lotic	0.25
Species 7	250	125	lentic	0.5
Species 8	900	200	lentic	0.5
Species 9	1900	300	lentic	0.5
Species 10	600	300	lentic	0.25
Species 11	1150	325	lentic	0.25
Species 12	1600	300	lentic	0.25
Species 13	500	250	lentic + lotic	0.25
Species 14	1000	250	lentic + lotic	0.25
Species 15	1500	250	lentic + lotic	0.25

657

658

659 **Electronic Supplementary Materials**

660 Supplementary Data: ESM1 - Data sets and R scripts used to run the analyses

661 Supplementary Figures: 1-6 (ESM2)