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Comparative success of two sampling techniques for high-altitude Alpine grassland reptiles under different temporal designs

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Abstract. Monitoring of wildlife populations is essential for their conservation and requires a carefully chosen methodology. We compared survey effectiveness of reptiles using coverboards and visual encounter surveys in two study sites in the Italian Alps with similar habitats and reptile communities. The two sites shared similar methodologies, cover boards and visual encounter surveys (VES), except for the temporal approach, with one employing a long-lasting monitoring scheme and the other operating on a much shorter time-frame. Coverboards were placed two years before the beginning of the monitoring in the first site, while they were installed only for ten days and then removed each year in the second site. Similarly, VES were spread across the whole reptile activity season (May-September) in the first site, while conducted over nine consecutive days in the second site. Although the observation rate of any species was mainly associated with its relative abundance, reptiles preferred long-established coverboards and all three species present (*Zootoca vivipara*, *Anguis veronensis* and *Vipera berus*) were found underneath them. Only *Zootoca vivipara* used recently installed ones. On the other hand, short-term daily visual encounter surveys led to a much higher observation rate of *Z. vivipara* than those spread over the entire season. Our results suggest that coverboards may provide a valuable monitoring tool for reptiles when projects are conducted over long periods. Conversely, when only short-term assessments are possible, no real difference exists between the two methods and observation rate is more influenced by the species abundance than by the chosen method.

Keywords: Alps, *Anguis veronensis*, coverboards, monitoring, *Vipera berus*, visual encounter surveys, visual searches, *Zootoca vivipara*.

Introduction

Reptiles and amphibians are amongst the most threatened taxa worldwide (Baillie et al., 2010; Alroy, 2015), with species that are locally adapted to extreme environmental conditions, peripheral and relict populations, and endemics being particularly prone to extinction (McNeely, 1990; Araújo, Thuiller and Pearson, 2006; Parmesan, 2006; Henle et al., 2008). Conservation of these species is thus a priority

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that requires effective and efficient monitoring methods.

Long-term monitoring is constrained by resources and time availability (Del Vecchio et al., 2019), and successful survey methods should seek to minimize the trade-off between imperfect detection and financial costs, and permit the most efficient completion of study aims (Field, Tyre and Possingham, 2005). Traditionally, herpetological monitoring relies on three main methods: visual encounter surveys (VES; Doan, 2003), active trapping systems, such as funnel and pitfall traps (Jenkins, McGarigal and Gamble, 2003; Willson and Gibbons, 2010), and arrays of coverboards or similar artificial refuges providing temporary shelters to the animals (Willson and Gibbons, 2010). All these techniques have pros and cons and the application of any method must be carefully chosen based on the habitat characteristics (Ryan et al., 2002), season (Todd et al., 2007), species ecology (Greenberg, Neary and Harris, 1994; Todd et al., 2007; Ribeiro-Júnior, Gardner and Ávila-Pires, 2008) as well as the availability of labourforce and time (Hutchens and DePerno, 2009).

Although a vast literature is available on herpetological monitoring, several aspects remain contradictory. For instance, the material used for coverboards is known to influence their attractiveness (Halliday and Blouin-Demers, 2015; Fish, 2016), but it remains unclear whether or not the duration of their deployment has an effect on their efficacy. While some studies suggest that the longer their deployment, the higher the capture rate (Grant et al., 1992; Bonin and Bachand, 1997), others suggest no or negligible effects of deployment time (Houze and Chandler, 2002; Carlson and Szuch, 2007; Michael et al., 2019). Similarly, the efficiency of sampling methods is known to depend on habitat, yet the research focus is often biased toward certain habitats over others (Ribeiro-Júnior, Gardner and Ávila-Pires, 2008). For instance, boreal and alpine regions are extremely underrepresented, especially in regards of ecological studies on reptiles and amphibians (Corn, 2005; Slough and Mennell, 2006; Slatyer, Umbers and Arnold, 2021). Nevertheless, the particular environmental conditions combined with the short activity periods of ectothermic species characterizing alpine landscapes might influence the efficiency of certain methods working well in other environments.

In this study, we provide an assessment of methodologies suitable for reptiles in mountain areas by using data collected within two different study sites, with the aim to compare the probability of observing reptiles using different methods in alpine grasslands. In the two sites, we employed similar methodologies (coverboards and visual encounter surveys) but different temporal designs. Specifically, in the first site we deployed coverboards on the ground for several months before inspecting them and organized surveys at regular intervals over the whole activity season of reptiles. For the second site, we restricted the monitoring over short, consecutive time windows, both in regards of the coverboards' placement and of the visual surveys. More specifically, our objective was to compare the efficacy of the two designs: the former simulating a long-lasting monitoring plan, the latter a short-term one.

Materials and methods

Study sites

We conducted the study in two protected areas of north-eastern Italy in Trentino-Alto Adige Region: Stelvio National Park (SNP: 46°27′N, 10°30′E) and Paneveggio-Pale di San Martino Nature Park (PPSMNP: 46°18′N, 11°45′E), located in the Rhaetian Alps and in the Dolomites, respectively.

The two sites are situated in a relative short geographic distance and host similar reptile communities (Caldonazzi, Pedrini and Zanghellini, 2002). Both sites share similar landscapes and environments and, in order to further minimize environmental differences between sites, we restricted the data collection between 1850 and 2150 m a.s.l. in open habitats (pastures, other grasslands and rocky areas), dominated by grass and shrub vegetation, alternated to coniferous forests of Norway spruce (*Picea abies*) and European larch (*Larix decidua*). In total, seven and 12 surveyed locations (i.e., plots) have been selected in SNP and PPSMNP, respectively.

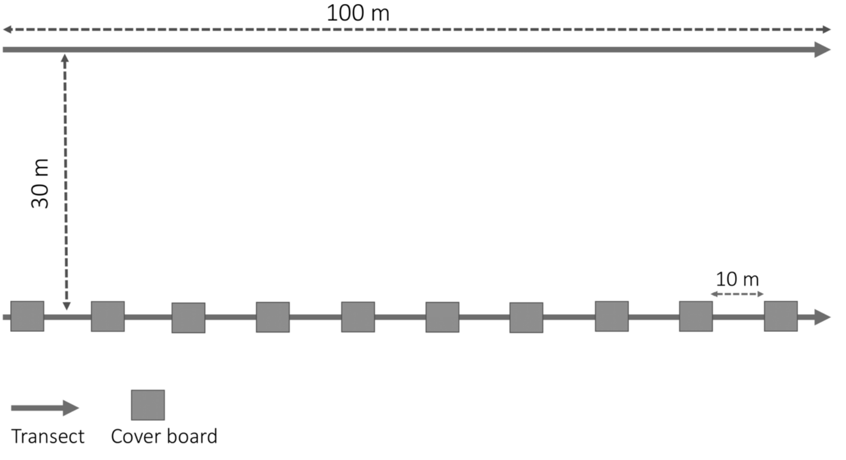

Figure 1. Schematic representation of the plots' setup in Paneveggio-Pale di San Martino Nature Park.

Survey design

In both sites, we employed coverboards and visual encounter surveys (VES). Although sharing similar methods, the two sites had different temporal sampling designs.

In SNP, coverboards were placed in September 2016 and were never removed. Each plot included four or five corrugated bitumen coverboards (70 × 90 cm, ≃1 kg) and one straight transect (150-470 m). A single observer (AB) sampled each plot during reptile activity hours at least ten times every year between May and September in 2018 and 2019.

In PPSMNP, a different observer (MC) surveyed the plots. Each plot consisted of two parallel 100-m transects and ten bitumen coverboards (40 × 50 cm, ≃0.5 kg) distributed every 10-m along one of them (fig. 1). Each plot was sampled for nine consecutive days after an initial day for placing the coverboards on the ground, and another day to let them settle. After the nine days of survey, the coverboards were removed and moved to another plot. Surveys were conducted between June and August of 2018 and 2019.

While walking the transects, the observer recorded the total number of animals encountered, differentiating between those found under coverboards or during VES. Following Doan (2003), we did not adopt a time-constrained search but rather the observer walked at a standard pace while visually searching the entire transect taking as much time as needed to check the coverboards and carefully examine each area thoroughly.

Data analysis

To assess the effects of the methodological design on the counts of the species, we used a generalized linear mixed modeling approach (GLMM; McCullagh and Nelder, 1989; Baayen, 2008; Bolker et al., 2009). Because at this stage our main intent was to highlight differences between designs rather than between methods, we did not compare results between coverboards and VES, but only within each method. Due to the abundance of zero data (i.e., no individuals observed under coverboards or during VES) and the limited variability in the number of observed individuals, we first converted our response variable into a binary variable (observed/not observed), which allowed us to adopt a binomial conditional distribution, thus to model the species observation rate.

To evaluate the effect of coverboards' running time and survey frequency on the observation rate of any given species, we used the number of days since the first placement of the coverboard ("running time") as test predictor for the coverboard data, and a categorical variable describing both the observer and the survey frequency adopted for the VES data ("survey type"). We also included the daily mean temperature retrieved from the closest weather stations (stations T0065, T0076, T0380 in SNP, and T0103 in PPSMNP; available on: www.meteotrentino.it), the Julian date, and the year (table 1). However, after testing for collinearity between variables, we observed that the daily mean temperatures were strongly correlated with the survey type ($W = 13\,304$, $p < 0.001$ for VES; $r(378) = 0.22$, $p < 0.001$ for coverboards); hence we discarded the temperature data from the model selection. Furthermore, because differences in abundance between plots may influence detection probability, we included the total yearly number of observations gained from both techniques (N) standardized as catch per unit effort (VES $= N/$(transect length $*$ number of visits) $*$ 100; coverboards $= N/$(number of boards $*$ number of visits) $*$ 100) for each species in each plot as a proxy for abundance (i.e., "relative abundance"). However, for *Vipera berus* and *Anguis veronensis* this value was always strongly correlated with the covariate specifying the survey design. For this reason, we tested two models separately for both species: one including the total abundance as covariate, and the other with the survey design. Finally, to account for other environmental differences, we included the plot itself as a random effect while differences in survey effort were accounted for by including the number of coverboards and the transect length as log-transformed offsets, assuming a clog-log link function (Dunn and Smyth, 2018). We performed our analyses in R 4.0.3 (R Development Core Team, 2020) using the packages *glmmTMB* (Brooks et al., 2022).

Table 1. List of predictors used in the global model comparing observation rate between the two study sites.

Species	Method	Predictors
Zootoca vivipara	VES	Survey type + Abundance + Year + Julian + 1|Plot + offset (Length)
	Coverboard	Running time + Abundance + Year + Julian + 1|Plot + offset (Boards)
Anguis veronensis	VES	NA
	Coverboard (1)	Running time + Year + Julian + 1|Plot + offset (Boards)
	Coverboard (2)	Abundance + Year + Julian + 1|Plot + offset (Boards)
Vipera berus	VES (1)	Survey type + Year + Julian + 1|Plot + offset (Length)
	VES (2)	Abundance + Year + Julian + 1|Plot + offset (Length)
	Coverboard	NA

To find the optimal fixed structure of the model, we fitted all possible term combinations using the dredge function implemented in the package *MuMIn* (Barton, 2017). We then performed a model comparison using Akaike's information criterion adjusted for small samples (AICc). To obtain parameter estimates, we recalculated model weights for models within ΔAICc $\leqslant 2$ and averaged parameter values (Grueber et al., 2011). Full models were significantly different from the null model unless otherwise stated. Residual diagnostics have been inspected using quantile residuals with the package DHARMa (Hartig, 2022).

A second analysis was conducted to test for differences in the observation rate of any given species between coverboards and VES. Due to the issue of standardizing the survey effort between the two techniques, in this analysis we used only data collected in PPSMNP, where the number of boards and the transect length was kept constant for all plots and years and therefore the effort was assumed as constant. Parameters' choice was analogue to that of the first analysis except that a predictor defining the method (VES or Coverboard) replaced those specifying survey type or coverboards' placement time.

Results

In both study sites we recorded two species of reptiles, namely *Zootoca vivipara* and *Vipera berus*, while *Anguis veronensis* was only seen in SNP. The total number of encountered individuals differed remarkably between the two sites as well as between the two methods. Overall, coverboards with a long running time captured all of the three reptile species, while those with a short running time used in PPSMNP attracted only *Z. vivipara*. Contrariwise, VES in PPSMNP resulted in a higher number of individuals for both *Z. vivipara* and *V. berus*, whereas *A. veronensis* was almost never encountered through VES in either of the two study sites.

In the model selection regarding *Z. vivipara* observation through VES, the species observation rate was mainly driven by the survey type (table 2) and was higher in PPSMNP, where surveys were conducted consecutively for nine days. In addition, the relative abundance of the species and the Julian date were also included in the best model and appeared to have the same importance as the survey design (table 3). On the other hand, the model selection for *Z. vivipara* beneath coverboards resulted in several models that were equally supported (table 2). Although only the relative abundance was positively significant, the coverboards' running time was the second most important predictor (table 3).

Zootoca vivipara was also the only species for which we could test between VES and coverboard surveys in PPSMNP. Of the parameters tested only relative abundance of the species was significant (table 4), which had a positive effect on the observation rate.

For *A. veronensis* and *V. berus*, the data was more unevenly distributed between the two study sites. Overall, *A. veronensis* was only detected in SNP, where more individuals were found sheltering beneath coverboards than observed in VES; in PPSMNP we never observed this species with either of the two methods, hence we could only perform the model selection for coverboards. While no parameters resulted to be significant in the model including the coverboards' running time, in the one including the species relative abundance this was the only significant parameter

Table 2. Model selection for the observation rate of three reptile species surveyed in two study sites. Only models within ΔAICc ⩽ 2 are shown. Variables in bold are statistically significant. All model selections include the plot as random effect and the transect length/number of coverboards as offsets.

Species	Method	Model	AICc	ΔAICc	Weight
Zootoca vivipara	VES	**Survey type** + **Julian** + **Abundance**	344.8	0.00	0.63
		Survey type + **Julian** + **Abundance** + Year	345.9	1.14	0.36
	Coverboard	Running time + **Abundance**	286.8	0.00	0.40
		Abundance	287.9	1.19	0.22
		Running time + **Abundance** + Year	288.0	1.22	0.21
		Running time + **Abundance** + Julian	288.7	1.91	0.15
Anguis veronensis	VES	NA	NA	NA	NA
	Coverboard (1)	Year + Running time + Julian	122.3	0.00	0.19
		Running time + Julian	122.4	0.12	0.18
		1	122.8	0.56	0.14
		Running time	123.2	0.92	0.12
		Year	123.2	0.96	0.12
		Year + Julian	123.3	1.08	0.11
		Julian	123.5	1.21	0.10
	Coverboard (2)	**Abundance**	118.2	0.00	0.46
		Abundance + Julian	118.8	0.65	0.33
		Abundance + Year	119.9	1.74	0.19
Vipera berus	VES (1)	**Year**	141.5	0.00	0.54
		Year + Survey type	143.2	1.65	0.23
		Year + Julian	143.3	1.76	0.22
	VES (2)	**Abundance** + **Year**	125.6	0.00	0.72
		Abundance + **Year** + Julian	127.6	1.93	0.27
	Coverboard	NA	NA	NA	NA

Table 3. Estimates, standard errors, significance and importance of all predictors in the averaged model comparing observation rate for the three focal species between the two study sites.

Species	Method	Predictor	Estimate	SE	*p*-value	Importance
Zootoca vivipara	VES	Year	0.087	0.191	0.648	0.36
		Survey type	−1.436	0.294	1.2e-06*	1.00
		Julian	−0.475	0.148	0.001*	1.00
		Abundance	0.228	0.106	0.031*	1.00
	Coverboard	Year	−0.058	0.175	0.740	0.22
		Running time	0.188	0.153	0.220	0.78
		Julian	0.008	0.062	0.889	0.16
		Abundance	0.775	0.105	<2.e-16*	1.00
Anguis veronensis	VES	NA	NA	NA	NA	NA
	Coverboard (1)	Year	−2.075	2913.9	0.999	0.43
		Running time	3.024	3499.7	0.999	0.51
		Julian	−0.326	223.11	0.999	0.61
	Coverboard (2)	Year	0.050	0.231	0.826	0.20
		Julian	−0.065	0.133	0.622	0.34
		Abundance	0.601	0.279	0.031*	1.00
Vipera berus	VES (1)	Year	1.869	0.666	0.005*	1.00
		Survey type	−0.228	0.843	0.786	0.24
		Julian	−0.036	0.163	0.821	0.22
	VES (2)	Year	1.807	0.729	0.013*	1.00
		Julian	−0.099	0.281	0.725	0.28
		Abundance	1.520	0.306	8.0e-07*	1.00
	Coverboard	NA	NA	NA	NA	NA

Table 4. Estimates, standard errors, significance and importance of all predictors in the averaged model comparing observation rate for *Zootoca vivipara* between methods in Paneveggio-Pale di San Martino Nature Park.

Predictor	Estimate	SE	*p*-value	Importance
Year	−0.004	0.073	0.946	0.12
Method	0.130	0.202	0.518	0.44
Temperature	0.009	0.053	0.855	0.25
Abundance	0.562	0.080	<2.e-16 *	1.00

and the model resulted having a lower AICc (table 4). There were not enough observations for *A. veronensis* with VES to fit a robust comparison between the two study sites for this technique ($N = 3$ in SNP and $N = 0$ in PPSMNP).

On the other hand, *V. berus* was observed only during VES and never using coverboards in PPSMNP, while it was observed with both methods in SNP. The year of survey appeared in all the best selected models for VES (table 2). However, the species relative abundance was equally important in the model including this parameter (table 3). Similar to *A. veronensis*, there were insufficient observations for *V. berus* under coverboards ($N = 8$ in SNP and $N = 0$ in PPSMNP) and we did not perform a model selection.

Discussion

While many studies have compared the relative effectiveness of different methods in sampling reptile species (Sung, Karraker and Hau, 2011; Michael et al., 2012; Bartman et al., 2016), very few have explored how different designs of these methods can affect the observation rate. For instance, there is a general agreement that visual encounter surveys work better with diurnal heliotherms (Michael et al., 2012), while coverboard arrays are best suited for cryptic species that are difficult to detect (Bartman et al., 2016; Fish, 2016). On the other hand, how the temporal distribution of VESs or the time of placement of coverboards lead to different results is less clear.

With this study, we provide the first methodological assessment for reptiles in alpine areas (but see Menegon, 2007). Overall, we found that the three focal species responded differently to each of the methods and to the designs we adopted. Specifically, *Zootoca vivipara* and *Vipera berus* were observed with both techniques, although, especially in the case of *V. berus*, with strong differences in their numbers, possibly as a consequence of the sedentary habits of this species (Neumeyer, 1987), which allowed the same specimens to be observed during all sampling days. On the other hand, *Anguis veronensis* was almost exclusively observed while sheltering under coverboards, which is in line with the results of several studies highlighting the low-mobility and shy behaviour of the sister taxon *A. fragilis* (Fish, 2016; Schmidt et al., 2017).

The confounding effect between methodology and site imposes caution when interpreting the results since possible differences between populations might appear as different response to the two designs. For instance, the number of individuals found beneath coverboards in SNP but not in PPSMNP would suggest that both *V. berus* and *A. veronensis* require more time than *Z. vivipara* to start using the boards as shelters, either because of extreme low mobility or mistrust toward newer objects. However, the different observation rate within Stelvio National Park indicates this to be a more likely consequence of spatial differences in the species abundance and, especially in the case of *A. veronensis*, it is possible that the species in Paneveggio-Pale di San Martino Nature Park has too low densities to be detected. In fact, although it has been suggested that coverboards placed for longer work better (Grant et al., 1992; Bonin and Bachand, 1997) and most studies often place these objects months or even years prior to the beginning of surveys (Adams, West and Kalmbach, 1999; Houze and Chandler, 2002; Grasser and Smith, 2014), Fish (2016) found slowworms already a week after the first placement and Sato et al. (2013) monitored alpine skinks in Tasmania with just a 4-days survey period. Interestingly, while most studies have used plywood coverboards for snakes (Halliday and Blouin-Demers, 2015; Bartman et al., 2016), we herein proved that corrugated bitumen roofing material can work as well.

Finally, we should acknowledge that coverboards used in Stelvio National Park were bigger and heavier than those in Paneveggio-Pale di San Martino Nature Park. However, Stumpel and van der Werf (2012) observed all study species under small boards and, at least in the case of the slowworm, being the species often associated with moist and damp substrate (Badziukiewicz, 2021), the dry environment created by larger boards (Hesed, 2012) should actually make them less suitable than smaller ones. We do not consider coverboard size being relevant for *Zootoca vivipara* either, since it was found in both sites. In fact, for species of comparable size, the dimension of the coverboards has been proven as uninfluential (Carfioli et al., 2000), and the observation rate of *Z. vivipara* remains a reflection of its abundance in the area, although the positive relationship with the age of coverboards suggests a preference for older shelters.

The higher observation rate of daily-repeated VES compared to that of surveys spread over the entire season is more difficult to interpret. While it has been proven that daily visited coverboards have a significantly lower capture rate than those checked weekly, probably as a consequence of the disturbance of sheltering animals (Marsh and Goicochea, 2003; Hesed, 2012), whether this happens also with active searches is unknown. Our results seem to reject this hypothesis.

With respect to environmental characteristics, on average the two sites did not differ in vegetation density and rock cover. Although our results might reflect a true difference in the abundances of the surveyed populations, future research is needed to understand if difference between the two sites do exist. While in fact this could be the cause of the higher number of counted *V. berus* in Paneveggio-Pale di San Martino Nature Park, it might not be the case in *Zootoca vivipara*, for which the survey design and the relative abundance were equally supported in the model selection. Naturally, since the abundance measured by us is a product of the real population size and detectability given the different observers and survey designs, it remains untested whether the apparent differences between temporal survey design would hold if parallel sampling with the two designs was conducted by the same observer in the same sites.

The only detected environmental difference was in temperature regimes, which was on average lower in PPSMNP than in SNP. Therefore, our results may be a consequence of the thermal preference of the species and it is possible that animals were observed more frequently in the former since they sheltered more frequently during the warmest days.

Alternatively, these results may reflect an observer bias. Differences among observers in ability to detect individuals has been long acknowledged as a potential source of error in ecological monitoring programs (Kepler and Scott, 1981). These differences can be the results of several factors, such as observer experience, fatigue and even taxon of interest or environmental characteristics (Anderson et al., 2015; Lardner et al., 2019). In the present study, the confounding effects of observer, site and study design make the effects of different variables difficult to disentangle. Although both observers employed in these sites were trained ecologists of similar age and with previous experience in herpetological samplings, the VES designs likely influenced the detectability of the species in regard of the learning process of the observer. On the one hand, it is possible that the daily surveys conducted in Paneveggio-Pale di San Martino Nature Park reduced the independence of each consecutive visit since the observer gained more and more confidence with the plots and "learned" the location of individuals within as the days passed (compare Thompson and Mapstone, 1997; Wintle et al., 2005). On the other hand, because observer skills increase with time and it has been observed that they already improve from the first to the second day (Garel et al., 2005), it is possible that the occasionality of survey frequency in Stelvio National Park suffers from a constant first-time observer bias (Kendall, Peterjohn and Sauer, 1996; Jiguet, 2009).

Although the confounding effect between site and methodology makes it impossible to disentangle the relative effect of each one of the two, we regard our results as valuable despite the weakness. While we cannot exclude that the different observation rates are a consequence of differences in the reptile populations of the two Parks, the overall environmental similarities suggest that these results may reflect different methodological designs. On the understanding that all pros and cons of the methods are secondary to the abundance of the species, if time allows, coverboards may offer a more comprehensive approach. This technique captured all three species while VESs almost entirely missed *Anguis veronensis*, and it could be too much dependent on the observer's experience. On the other hand, however, most environmental-impact assessments operate on much shorter time-frames. In this case, installing coverboard arrays might not be convenient or worth the effort and VESs can offer a good compromise, especially if the target species are not particularly elusive or rare and the observer is kept consistent between surveys. However, new emerging techniques, such as the use of wildlife detection dogs (Grimm-Seyfarth, Harms and Berger, 2021) or the analysis of environmental DNA (Bohmann et al., 2014), might overcome these limits and new studies should test their feasibility, efficacy and cost/labour convenience with different species and across different environments.

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