Monitoring of saproxylic beetles in Croatia: following the path of the stag beetle

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Abstract

As a member of the European Union, Croatia is obliged to report on the conservation status of 220 animal non-bird species listed in the annexes of the Habitats Directive (92/43/EEC), for which purpose a monitoring system is being established. Concerning saproxylic beetles, seven species present in its territory have to be monitored: Lucanus cervus, Cerambyx cerdo, Morimus funereus, Rhysodes sulcatus, Cucujus cinnaberinus, Rosalia alpina and Osmoderma eremita complex. Out of these species, a monitoring programme has only been established for Lucanus cervus, which partially includes participation of non-experts. In 2015 and 2016, a public campaign was organised in order to collect observations of Lucanus cervus and two other saproxylic beetles that are easily recognisable by the public: Morimus funereus and Rosalia alpina. Data gathered through this campaign serve as an addition to the mapping activities and monitoring of the species’ range. So far, more than 650 citizen observations have been collected, providing data on species presence in 216 10×10 km² grid cells intended for reporting on the species’ range. Besides the public campaign, since 2014, public institutions for managing nature protected values have been involved in population monitoring for which they received education through several workshops. Altogether, 21 sites have been included in the monitoring of the stag beetle so far. Data collected for Lucanus cervus on standard transects, by tree and ground pitfall traps and tree trunk surveys at night will be discussed. To the present time, eight public institutions have been involved in stag beetle population monitoring and the number has been continuously increasing.

Keywords
citizen science data (CSD), Lucanus cervus, Habitats Directive, habitat suitability model, monitoring, reporting, Morimus funereus, Rosalia alpina, species distribution range, observation on transects
Introduction

As the most recent member of the European Union, the Republic of Croatia, joining in 2013, has an obligation, according to Article 17 of the Habitats Directive (92/43/EEC) and Article 12 of the Birds Directive (2009/147/EC), to report on the conservation status of the species and habitats listed in the annexes of the directives by 2019. The conservation status assessment has to be undertaken for seven saproxylic beetles, *Lucanus cervus*, *Cerambyx cerdo*, *Morimus funereus*, *Rhysodes sulcatus*, *Cucujus cinnaberinus*, *Rosalia alpina* and *Osmoderma eremita* complex which are present in its territory and listed in the annexes of the Habitats Directive.

Of the seven saproxylic beetles, to date a monitoring programme at the national level has only been developed for the stag beetle (Šerić Jelaska 2013). It was compiled during the pre-accession period, through the European Union’s Instrument for Pre-Accession Assistance (IPA) Programme “Natura 2000 Management and Monitoring – NATURA MANOMN” (EuropeAid/129747/D/SER/HR) in 2012, following the experiences of other EU countries (Harvey et al. 2011a, b, Alvarez and Alvarez 1995 etc.), especially of neighbouring Slovenia and Italy (Campanaro et al. 2012, Vrezec et al. 2012).

However, Croatia has its own specificities concerning geographical position, geology, forest management practice, as well as socio-economic and political history. The country spreads across three biogeographical regions: Mediterranean, Continental and Alpine and the stag beetle has existed in all three of them (Harvey et al. 2011a, Šerić Jelaska 2013). Forests and woodlands cover around 40% of the land area (Oikon Ltd 2004) and the majority is represented by natural forests. Virgin forests without logging activities are spread over 1400 ha comprising 4.4% of the total forested area. Most of the forests are national property, managed by the state owned company, Croatian Forests Ltd. Two management methods have dominated: selective logging for mixed aged stands in beech-fir mixed forests and regular logging in other forests types (Matić 2009).

Before mapping and monitoring activities connected to the Habitats Directive, no other systematic surveillance for the stag beetle has been conducted in Croatia (Harvey et al. 2011a). Historic data on species presence in Croatia, taken from museum collections in the country and abroad (Croatian Natural History Museums in Zagreb, Split and Rijeka, Hungarian Natural History Museum, Royal Belgian Institute of Natural Sciences, Faculty of Science and Faculty of Forestry at the University of Zagreb), relevant literature (e.g. Depoli 1926–1940, Koča 1900, 1905, Novak 1952, 1970) and also some latest observations made by biologists and foresters, were summarised and published in a paper by Harvey et al. (2011a). These data represented the baseline for formulating the national monitoring programme for the stag beetle.

In the European Union, several non-invasive monitoring methods have been developed and tested over the last 20 years such as trapping of adults (with or without lures), counting living adults during transect walks (flying or on the ground), surveys of tree trunks, mapping adults by citizens, counting road kill individuals and predation remains etc. (e.g. Alvarez and Alvarez 1995, Campanaro et al. 2012 and 2016, Chiari et al. 2014, Harvey et al. 2011a, b, Mason et al. 2015, Vrezec et al. 2012). Most of these methods
are suitable for non-experts, relatively inexpensive and non-invasive, but still have some weaknesses when it comes to application and standardisation (Campanaro et al. 2016, Harvey et al. 2011b, Mason et al. 2015, Zapponi et al. 2017). Different practices within EU countries and lack of standardised protocols may impede the conservation actions of this species and also many other threatened species at the European level (Campanaro et al. 2016).

Due to a lack of experts, vis-à-vis the need for a large input when the Habitats Directive requirements are applied, it was decided to gradually include non-experts as far as possible in order to have financially feasible and scientifically relevant long-term surveillance of the stag beetle across three biogeographical regions. For the first time, the results have been presented on a systematic survey by non-experts from public institutions for management of nature protected values (national parks, nature parks, county institutions etc.) in monitoring stag beetle populations following the national monitoring programme; and unsystematic data gathering by citizens as a part of the surveillance that provided additional sources of data for mapping and monitoring of the range of the stag beetles. In addition, data gathering by citizens were organised for Morimus funereus and Rosalia alpina, that are among largest European saproxylic coleopterans and easily recognisable. After four years of surveillance, the efficiency of the stag beetle collecting methods and the potential of non-experts were discussed, as well as the problems which were faced during monitoring and mapping activities.

**Material and methods**

**Methodology according to the national monitoring programme (Šerić Jelaska, 2013)**

According to the Croatian national monitoring programme for the stag beetle, monitoring of the conservation status consists of (i) monitoring of population size and structure on plots, utilising three data sampling methods, counts on evening transects, survey of tree trunks and tree pitfall traps with lures; and of (ii) systematic range mapping, accompanied by non-systematic data gathering on the species distribution. The methodology was suggested as described below.

Monitoring on transects should be conducted along forest roads or walking paths inside the forest or at the forest edge during favourable weather conditions (e.g. without rain) in June/July period when it is the seasonal peak of adults’ activity (Harvey et al. 2011a, Vrezec 2008, Vrezec et al. 2009). Transects should be undertaken in the evenings (between ca. 8 pm and 9 pm) by one person slowly and continuously walking along a transect 100 to 1000 m long, counting flying adults and those on the ground. Evening transects should be applied in 10 successive days in two repetitions, between the middle of June and the middle of July. The methodology was adjusted according to Vrezec et al. (2009, 2012).

The night survey of one to ten tree trunks should be conducted during warm and dry evenings, immediately after the evening transect. Tree trunks should be surveyed in
ten successive days in two repetitions, preferably one in the second half of June and one in first half of July. This method was suggested by Vrezec et al. (2012) for monitoring stag beetle populations based on aggregation of stag beetles on live standing tree trunks where they were present mainly for feeding.

The tree pitfall traps should be made of plastic bottles (2 litres) cut off to two thirds of their height, placing the upper part inside the lower part and thus making a plastic pot with a funnel. The opening should be broad enough for the stag beetle to fall into the trap. Three to ten traps should be placed per site, attached to a trunk of live standing tree, at 1.5–2 m of tree height. The traps should be filled with bait, e.g. fruit, rum with sugar or grated fresh ginger root (Harvey et al. 2011b). As with previous methods, traps should be checked every day in 10 successive days in two repetitions, preferably one in June and one in July.

The efficacy of these three sampling methods, proposed by the monitoring programme, was tested in the Continental region at two locations, Mt. Medvednica and Maksimir Park in Zagreb town, during June/July in 2013 and in the Mediterranean region, at Mt. Učka and Krk Island locations, in 2014, where the presence of the stag beetle had already been confirmed.

Involvement of non-experts in monitoring of conservation status

As mentioned above, due to the lack of experts, it was concluded that involvement of non-experts in monitoring the conservation status was required to fulfil the reporting obligations. As a part of the nature protection sector is already directly present in the field, employees of the public institutions (PIs) for management of nature protected values were recognised as prized field researchers who could facilitate the monitoring activities. In order to stimulate and educate these potential associates, in summer 2015, the Croatian Agency for the Environment and Nature Protection (CAEN), within the EU Natura 2000 Integration Project - NIP (IBRD 8021-HR), organised two workshops for the implementation of the monitoring of stag beetle population. The workshops consisted of theoretical and practical parts. During the theoretical part, participants were educated on saproxylic beetle ecological functions, threat status and conservation measures, stag beetle biology and finally on monitoring methodology. The practical part consisted of field visits to stag beetle habitats where all three methods were demonstrated and tested by the participants themselves. In total, 29 employees from 17 PIs participated at the workshops.

Besides population monitoring, all PIs were encouraged every season to record stag beetle observations in order to facilitate species mapping and range monitoring. For the same purpose, in spring 2015 and spring 2016, CAEN initiated a broad citizen science campaign for collecting observations of stag beetle and other two larger and easily recognisable species *Morimus funereus* and *Rosalia alpina*. The campaign was announced via the CAEN website, websites and mailing lists of several biological civil society organisations, most popular social networks and media. As well as data on observation locality and date, photographic evidence was requested for species iden-
Citizen involvement was encouraged by rewarding every hundredth record and most attractive species photograph with the set of red books of wild species of Croatia. All reported observations were published and credited on the Bioportal-geoportal of the Croatian Nature Protection Information system (CAEN 2017).

Implementation of the monitoring programme

As a result of previous actions aimed at encouraging PIs to participate in monitoring and mapping activities, eight PIs joined the monitoring activities within the next two years and conducted the monitoring on an additional six locations in the Continental biogeographical region.

At each location, one to two sites were selected (Table 1). Study sites were placed in natural and urban forests and forest edges, within protected areas and outside. The principal tree species at each location are listed in Table 1. Study sites were scattered from eastern to western Croatia, including lowland areas, mountains and one island.

Altogether, 21 transects at lengths between 100–700 m were set along forest roads or walking paths inside forests or at forest edges. The survey was conducted in the evenings between 7 pm and 9 pm by one person spending 30 minutes of slow continuous walking along the transect and counting flying adults and those on the ground. Evening transects were applied in successive 9 to 20 days when weather conditions were favourable. The relative abundance in Table 2 represented the number of beetles counted on a transect per 100 metres per day at each location.

In addition, 4 to 12 traps were employed at each site and each trap was attached to the trunk of a live tree. The traps were filled with three different baits: fruit (peach and banana mixture), rum with sugar (only the first year, Vrezec et al. 2012), red wine-with vanilla flavoured sugar and grated fresh ginger root (Harvey et al. 2011b). Traps were set for a period of 10 to 20 days. During the field study, traps were checked every to every second day prior to counting on transects.

Although it was not suggested by the national programme, ground pitfall traps were also used at three sites (Table 2). The ground pitfall traps were formed by 0.5 litre plastic pots, buried in the soil, with an opening at the soil surface level. They were filled with the same bait as tree traps. At each sampling site, traps were set randomly and approximately 10 metres apart. They were exposed for 19 and 20 days. The relative abundance was calculated as average number of individuals caught per one trap per night at each location (one trap night is the catch of one trap in one night).

The survey of tree trunks was carried out between 8 p.m. and 9.30 p.m. in warm and dry nights. Ten to twenty trunks were randomly surveyed per site per day using an electric torch.

The relative abundance was calculated as the average number of individuals observed per surveyed trunk per day.

Monitoring of adult beetles was undertaken from 2013 to 2016 in period between 15 June and 19 July.
Table 1. Characteristics of study areas, PIs that were included and the year when monitoring was undertaken.

<table>
<thead>
<tr>
<th>Biogeographical region</th>
<th>Location</th>
<th>Public Institution</th>
<th>No. of sites</th>
<th>Year</th>
<th>Habitat type</th>
<th>Main tree species at location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continental</td>
<td>Mt. Medvednica</td>
<td>Medvednica Nature Park</td>
<td>1</td>
<td>2016</td>
<td>Natural forest</td>
<td>Quercus petraea, Fagus sylvatica, Robinia pseudoacacia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2015</td>
<td>Natural forest</td>
<td>Quercus petraea, Fagus sylvatica, Robinia pseudoacacia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2*</td>
<td>2013</td>
<td>Natural forest</td>
<td>Quercus petraea, Robinia pseudoacacia, Fagus sylvatica, Picea abies</td>
</tr>
<tr>
<td></td>
<td>Zagreb</td>
<td>Maksimir Park</td>
<td>2</td>
<td>2013</td>
<td>Urban forest</td>
<td>Quercus petraea, Q. robur, Q. cerris</td>
</tr>
<tr>
<td></td>
<td>Nedelišće</td>
<td>Medimurje Nature</td>
<td>1</td>
<td>2016</td>
<td>Forest edge</td>
<td>Quercus sp., Carpinus betula</td>
</tr>
<tr>
<td></td>
<td>Radoboj</td>
<td>Krapina-Zagorje County</td>
<td>1</td>
<td>2016</td>
<td>Quercus petraea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mt. Žumberak</td>
<td>Zumberak-Samoborskogorje</td>
<td>1</td>
<td>2013</td>
<td>Natural forest</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slavonski Brod, Mladavodica</td>
<td>Natura Slavonica, Brod-Posavina County</td>
<td>1</td>
<td>2016</td>
<td>Forest edge</td>
<td>Fagus sylvatica, Quercus petraea, Carpinus betula, Corylus avellana, Salix alba, Populus sp., Prunus sp., Fraxinus sp.</td>
</tr>
<tr>
<td></td>
<td>Spačva</td>
<td>Vukovar-Srijem County</td>
<td>2</td>
<td>2015</td>
<td>Natural forest</td>
<td>Quercus robur, Carpinus betula</td>
</tr>
<tr>
<td></td>
<td>Spačva</td>
<td>Vukovar-Srijem County</td>
<td>2</td>
<td>2014</td>
<td>Natural forest</td>
<td>Quercus robur, Carpinus betula</td>
</tr>
<tr>
<td></td>
<td>Mt. Papuk</td>
<td>Papuk Nature Park</td>
<td>2</td>
<td>2015</td>
<td>Natural forest</td>
<td>Quercus petraea, Fagus sylvatica, Prunus sp.</td>
</tr>
<tr>
<td></td>
<td>Mt. Učka*</td>
<td>Faculty of Science</td>
<td>2</td>
<td>2014</td>
<td>Forest edge</td>
<td>Quercus pubescens, Ostrya carpinifolia, Carpinus orientalis</td>
</tr>
<tr>
<td></td>
<td>Krk Island*</td>
<td>Faculty of Science</td>
<td>2</td>
<td>2014</td>
<td>Natural forest</td>
<td>Quercus ilex, Q. pubescens, Carpinus orientalis</td>
</tr>
</tbody>
</table>

*Sites that were used for testing methods proposed by the monitoring programme (Šerić Jelaska 2013)

Data analyses

For comparisons of sampling data and methods efficiency, non-parametric statistical tests, $\chi^2$ and Kruskal-Wallis were used. In addition, cumulative average number of individual species recorded per day at each transect was calculated to reveal how many consecutive days were needed before the average number of observed specimens per day stabilised on the transect (i.e. reduction of variation).

Spatial analyses of the results were made in ArcGIS 10.1. (ESRI 2012).

To estimate the efficiency of the citizen science campaign with respect to spatial coverage of data on the stag beetle, habitat suitability maps have been produced for the stag beetle using Maxent (Phillips et al. 2006). All data collected were geo-referenced with assigned values of their spatial uncertainty. In this analysis, only those data with spatial precision being equal to circle with 2000 metres radius, or smaller, have been
Table 2. The results of simultaneous observations of stag beetles during 2013-2016 using three methods at 21 sites (and ground pitfall traps as an additional method at two locations in Spačva and Slavonski Brod area) in Croatia. Number of observations/individuals (No. obs. and No. ind.) and relative abundance for used methods at each location representing sampling effort are presented.

<table>
<thead>
<tr>
<th>Location/Year</th>
<th>No. transects/sites (length, m)</th>
<th>Transect evenings</th>
<th>Transect No. tree traps per site</th>
<th>No. ground traps per site</th>
<th>Traps exposure days</th>
<th>Evening transects No. obs</th>
<th>Evening transects No. obs/100 m * day</th>
<th>Evening transects No. ind</th>
<th>Evening transects No. ind/one trap*day</th>
<th>Tree traps No. ind</th>
<th>Tree traps No. ind/one trap*day</th>
<th>Ground traps No. ind</th>
<th>Ground traps No. ind/one trap*day</th>
<th>Night survey of trunks No. ind</th>
<th>Night survey of trunks No.ind/night*site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt. Medvednica/2016</td>
<td>1 (700 m)</td>
<td>9</td>
<td>10</td>
<td>/</td>
<td>19</td>
<td>13</td>
<td>0.21</td>
<td>7</td>
<td>0.037</td>
<td>0</td>
<td>/</td>
<td>0</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mt. Medvednica/2015</td>
<td>1 (700 m)</td>
<td>9</td>
<td>10</td>
<td>/</td>
<td>17</td>
<td>19</td>
<td>0.30</td>
<td>54</td>
<td>0.318</td>
<td>0</td>
<td>/</td>
<td>0</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mt. Medvednica/2013</td>
<td>2 (100 m; 100 m)</td>
<td>20</td>
<td>10</td>
<td>/</td>
<td>20</td>
<td>160</td>
<td>2.00</td>
<td>14</td>
<td>0.018</td>
<td>0</td>
<td>/</td>
<td>4</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zagreb, Maksimir Park/2013</td>
<td>2 (100 m; 100 m)</td>
<td>12</td>
<td>10</td>
<td>/</td>
<td>20</td>
<td>122</td>
<td>1.53</td>
<td>2</td>
<td>0.003</td>
<td>0</td>
<td>/</td>
<td>4</td>
<td>0.050</td>
<td></td>
<td></td>
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<tr>
<td>Međimurje, Nedelišće/2016</td>
<td>1 (350 m)</td>
<td>20</td>
<td>10</td>
<td>/</td>
<td>20</td>
<td>683</td>
<td>9.76</td>
<td>140</td>
<td>0.700</td>
<td>0</td>
<td>/</td>
<td>15</td>
<td>0.750</td>
<td></td>
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<tr>
<td>Zagorje, Radoboj/2016</td>
<td>1 (340 m)</td>
<td>14</td>
<td>12</td>
<td>/</td>
<td>16</td>
<td>270</td>
<td>5.67</td>
<td>6</td>
<td>0.031</td>
<td>0</td>
<td>/</td>
<td>3</td>
<td>0.214</td>
<td></td>
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<tr>
<td>Mt. Žumberak/2016</td>
<td>2 (390 m; 900 m)</td>
<td>11</td>
<td>4</td>
<td>/</td>
<td>17</td>
<td>39</td>
<td>0.14</td>
<td>0</td>
<td>0.000</td>
<td>0</td>
<td>/</td>
<td>25</td>
<td>0.568</td>
<td></td>
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<tr>
<td>Slavonski Brod, Mladavodica/2016</td>
<td>1 (100 m)</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>20</td>
<td>43</td>
<td>3.07</td>
<td>14</td>
<td>0.058</td>
<td>7</td>
<td>0.029</td>
<td>3</td>
<td>0.214</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spačva (Vukovar-Srijem County)/2015</td>
<td>2 (200 m; 200 m)</td>
<td>19</td>
<td>12</td>
<td>12</td>
<td>19</td>
<td>137</td>
<td>1.14</td>
<td>19</td>
<td>0.021</td>
<td>0</td>
<td>0.000</td>
<td>20</td>
<td>0.333</td>
<td></td>
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</tr>
<tr>
<td>Spačva (Vukovar-Srijem County)/2014</td>
<td>2 (200 m; 200 m)</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>20</td>
<td>50</td>
<td>0.39</td>
<td>1</td>
<td>0.001</td>
<td>2</td>
<td>0.002</td>
<td>3</td>
<td>0.047</td>
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<tr>
<td>Mt. Papuk/2015</td>
<td>2 (100 m; 100 m)</td>
<td>15</td>
<td>12</td>
<td>/</td>
<td>10</td>
<td>21</td>
<td>0.53</td>
<td>11</td>
<td>0.023</td>
<td>0</td>
<td>/</td>
<td>4</td>
<td>0.100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mt. Učka/2014</td>
<td>2 (200 m; 200 m)</td>
<td>17</td>
<td>12</td>
<td>/</td>
<td>22</td>
<td>119</td>
<td>0.88</td>
<td>12</td>
<td>0.011</td>
<td>0</td>
<td>/</td>
<td>7</td>
<td>0.103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krk Island/2014</td>
<td>2 (200 m; 200 m)</td>
<td>10</td>
<td>12</td>
<td>/</td>
<td>15</td>
<td>187</td>
<td>1.56</td>
<td>16</td>
<td>0.022</td>
<td>0</td>
<td>/</td>
<td>26</td>
<td>0.433</td>
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</table>
used, resulting in 449 input data, out of which as many as 392 had their precision below 1000 metres radius. As descriptors (i.e. environmental variables), two independent sets have been used, one consisting of anthropogenic-related variables (distance from settlements, distance from roads and interpolated human population density), the second representing climatic and topographical conditions (seasonal amount of precipitation and mean temperature, slope and aspect). Due to the circular nature of aspect values (e.g. 1˚ and 359˚ representing an almost identical aspect), the sine and cosine of aspect values were used, transforming them into two variables ranging from -1 to 1, representing an inclination from north (cosine) and from east (sine) as in, for example Guisan et al. (1999) and Jelaska et al. (2003). The resulting Maxent maps, with their logistic outputs (i.e. values ranging from 0 to 1) were reclassified to binary (0-non-suitable and 1-suitable habitat) grids using two threshold values. In the first step, a conservative 0.5 value was used as a threshold based on which every pixel of Maxent grid is classified into non-suitable (0) or suitable (1) habitat. Given the fact that the stag beetle is a species of special concern, in the second step a less strict rule was used and a threshold value that contained 80% of input data was selected. The latter resulted in using 0.336 as a threshold for the Maxent model developed using human population oriented variables and 0.414 for the Maxent model based on climatic and topographic variables. Thereafter, the above described two pairs of reclassified Maxent models were spatially overlapped, (one based on 0.5 threshold value and one with customised threshold values that included 80% of input data) and percentages of overlapping and unique areas of suitable habitats for the stag beetle were calculated.

Results

Population monitoring

Overall, there were 2282 records of stag beetle adults after monitoring activities on 21 sites within ten locations, sampled during June/July from 2013 to 2016. Of the 21 sites, four were located within the Mediterranean biogeographical region and the rest in Continental Croatia. There was no systematic surveillance conducted in the Alpine region during this period. Such differences in the number of sites in each region were the consequence of the lower response from the public institutions of Alpine and Mediterranean regions to the workshops and monitoring activities.

Amongst three methods applied on all sites, the evening transect was the most efficient in detecting the species presence (Figure 1) with 1863 observations making 81.6% of the total record. Tree traps had 296 records (13% of the total record) but the lowest detection efficiency (at 16 of 21 sites making 76.2%, Figure 1) and the night survey of tree trunks had 114 records (5%) with detection efficiency at 18 sites. Kruskal-Wallis confirmed significant differences between those three methods (H=19.9, p<0.05). Only eight records (0.04 % of total records) using ground pitfall traps, applied at three locations, were obtained (Table 2).
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Figure 1. Stag beetle detection efficiency using three sampling methods presented as a proportion of sites with recorded individuals (N sites = 21). The differences were not significant ($\chi^2 = 0.3658$, $p<0.05$).

Furthermore, all tested methods were sex biased, with the detection of significantly more males than females ($\chi^2 = 13.0384$, $p$-value =0.004554, $p<0.05$). The lowest proportion of males, 80.7%, for the survey of trunks, 81.7% at counts on evening transects, 88.9% in ground pitfall traps and 90.2% in tree pitfall traps was found.

Twenty days of evening transects, in two sequences of 10 days each as suggested by the monitoring programme, were achieved at five sites and observations at the other 16 sites were undertaken within 9 to 17 days. During that period, in eastern continental Croatia,
in Spačva T1 2015, it can be clearly seen that, after 11 days, the average number stabilised at ca. 3.5 observed individuals on the transect and at the Mlade Vodice 2016 transect, the average number of individuals at ca. 3.5 was reached after 10 days of transect walk (Figure 2a). In northern continental Croatia (Figures 2c and d), the average number of observed individuals on most of the transects stabilised between 11 and 14 days and in the Mediterranean region, the peak of activity was caught at Krk T2 2014 transect, where the number of observations stabilised at ca. 2.5 individuals between 11 and 14 days (Figure 2b).

Citizen science campaign

Altogether 640 observations of the three saproxylic beetle species were reported to the CEAN by the public during the citizen science campaign, the majority being of stag
beetle (555 observations, 87%), then 45 observation of *Morimus funereus* (7%) and 40 of *Rosalia alpina* (6%).

Observations were reported from all three biogeographical regions in Croatia, with the majority from the Continental biogeographical region (Figure 3 and Figure 4).

In total, citizen observations confirmed the presence of these three species in 216 10×10 km² grid cells, intended for reporting on species’ range, out of which the presence of the stag beetle was confirmed in 204 grid cells (Figure 5). Comparing the distribution data of the stag beetle in Croatia given by Harvey et al. (2011a) and the data
collected through this citizen science campaign, the stag beetle presence is recorded in 100 new 10×10 km² grid cells. Areas where stag beetle presence was left unconfirmed, when comparing to data from Harvey et al. (2011a), are mostly in the Mediterranean biogeographical region and could be attributed to the lower response from citizens.
Analyses of habitat suitability maps derived by Maxent using two sets of descriptors are shown in Figure 6 where obvious differences can be seen in the spatial arrangement of suitable habitats depending on whether model was derived based on human population oriented variables or those representing climatic and relief conditions. The percentages of suitable habitats for the stag beetle identified by the Maxent model based on human population oriented variables, based on variables describing climatic and relief conditions and those where both models were congruent, are shown in Figure 7.

Discussion

Stag beetle population monitoring

Of the three methods proposed by the Croatian stag beetle monitoring programme (Šerić Jelaska 2013), the standard transect walk proved to be the most efficient, with the stag beetle recorded at all sites in each year and with the highest number of stag beetle observations. All three methods were biased towards males, but the night survey of trunks yielded the lowest proportion of males. The results on detection efficiency are in line with data in Vrezec et al. (2012) using the same methods. These very first data, collected from 2013 to 2016, during methodology testing activities and by involving PIs in population monitoring, indicated that evening transects could be a trustworthy method, as this is also suggested by the latest results on the application of this method at a European scale (Campanaro et al. 2016) and is easy to conduct by non-experts. Of the 17 PIs who attended the workshops, only eight of them were involved in the mapping and monitoring activities. There is still room therefore for progress in encouraging PIs to join, especially PIs from the Mediterranean region since they have not been involved so far. The evening transect, although not technically demanding, may still appear unattractive to employees of PIs as it may demand time after working hours. As the duration of transects varied from 9 to 20 evenings a year, it may be expected that the number of days on transects will be shorter in practice than the number proposed by the programme (twenty days). As suggested by Campanaro et al. (2016), the three weeks survey, including the period between the middle of June and the first week of July, has a high probability of detection of stag beetles. The same June/July period was included within the conducted monitoring activities in Croatia, resulting in high number of observations at all sites, but the proposed three to five weeks period could be hard to achieve by PI employees. However, the five-week period (Campanaro et al. 2016) should be proposed within the national programme to monitor the distribution range when species absence within the empty grid in certain areas needs to be confirmed. For population monitoring on transects, according to the data presented in Figure 2, it seems that minimum number of nights on transects should not be less than 11 and there is no need to exceed 20 days within the favourable June/July period as proposed by the monitoring programme (Šerić Jelaska 2013). Even in the Mediterranean region where the monitoring was started earlier than in other areas (Table 2), the peak of activity was reached and the number of observations were stabilised on Krk.
T2 transect after 11 days (Figure 2b), while on Krk T1, it seems that the trend started to stabilise a few days later, with a slightly higher number of observations. On Mt. Učka, monitoring of stag beetles’ activity had obviously started too early, as can best be seen from the first eight days without any individual observed on transect Učka T2, while, afterwards, the number increases continuously. In northern continental Croatia (Figure 2d), the average number of observed individuals at two transects (Žumberak T1 and T2) was very low and it is surmised that the research probably missed the seasonal peak of their activity.

The lower detection efficiency of tree pitfall traps and trunk surveys and lower number of recorded individual species might raise the question of justification for their application. Trunk surveys proved to be more sensitive to females than the other two methods and pitfall traps in comparison with evening transects and trunk surveys can be set and checked during the working hours of PIs. Therefore, the pitfall trap method with appropriate bait can still be utilised when night surveys are not an option.

**Citizen science campaign**

Collecting observations of the saproxylic beetle species through a citizen science campaign in Croatia proved that involvement of the public in mapping of these charismatic and easily recognisable species can make a considerable contribution to the knowledge of their distribution. The same conclusion is given by Zapponi et al. (2017) in their study which analysed the distribution data, amongst others on the same three species, collected by the citizen science programme organised in Italy. The number of citizen observations of *Lucanus cervus*, *Morimus funereus* and *Rosalia alpina* collected during the campaign in Italy is very similar (607) to the number of observations collected during the campaign in Croatia, but with a higher share of *Morimus funereus* (39%) and *Rosalia alpina* (11%) in the total number of observations. As for this study, Zapponi et al. (2017) found that photographic documentation accompanying the observation data is sufficient to validate species identification and thus should be an obligatory part of the observation report.

Spatial analyses of data collected through citizen science campaign (Figure 6) confirmed that those data could be spatially biased towards settlements and roads, leaving a significant portion of unexplored habitats for the stag beetle, hence without data on the status of species of interest in those areas. Even though only citizen science collected data were used (thus closer to settlements and roads) for developing the Maxent model based on variables describing climatic and relief conditions, significant areas were designated uniquely by this model as suitable for the stag beetle (Figure 7, 28% and 51%). As these areas are further from settlements, the citizen science campaign to ensure data in those areas cannot be expected in the future. Hence additional efforts for monitoring of the stag beetle should be focused in those areas, mostly by experts and PIs. Usage of two threshold values for classification into binary suitable/non suitable areas for stag beetles resulted in differences in absolute areas, as well as those in percentages of unique and shared contributions (Figure 7) of used environmental vari-
ables (e.g. Humans vs. TopoClim) to predict suitable habitats. Although it is out of the scope of this paper, the importance of threshold selection has been discussed by a number of authors (e.g. Liu et al. 2005; Phillips et al. 2006; Li and Guo 2013; Liu et al. 2016) hence it should be mentioned here and treated with due attention in the future.

Conclusions

Based on the four years’ experience in establishing a monitoring system for saproxylic beetles, it is concluded that, with appropriate education, non-experts can be successfully involved in population monitoring of these species, thus compensating for the lack of experts as well as reducing financial costs for the monitoring system. Although citizen science programmes, with accompanying media campaigns, can also considerably contribute to the mapping activities of easily recognisable and charismatic species, they cannot substitute targeted mapping projects due to the observation bias to urban areas.

Author contributions

LSJ and LK developed the initial concept of the paper. LSJ, LK and SDJ wrote the manuscript together. LSJ was involved in population monitoring activities and data analyses; LK was involved in contacting PIs, providing citizen science observations and data analyses and SDJ performed the spatial analyses of presented data and contributed to the initial concept of this paper.

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