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Detection of stag beetle oviposition sites by combining telemetry and emergence traps

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Abstract

The European stag beetle, *Lucanus cervus*, is a flagship species for biodiversity conservation of old-growth forests and is protected under the Habitats Directive. Although it has been the focus of active research in the last two decades, many aspects of its ecology and habitat requirements for the larvae remain poorly known, particularly to what extent certain factors limit larval development. The objectives of this preliminary work were: (1) to explore the feasibility of a non-invasive method for detecting oviposition sites; (2) to attempt the characterisation of above-ground ecological factors recorded in the oviposition sites and (3) to quantify the number of traps and operators needed for obtaining a number of beetles suitable for statistical analysis. In 2014, twelve females were followed by means of radio-telemetry to detect potential oviposition sites in a relict broadleaf forest of northern Italy. In 2015, emergence traps were set in nine sites selected from the 21 sites where females were recorded digging deeply in the soil near to dead wood during the previous year. Traps were checked during the 2015 and 2016 flight seasons. Overall, 15 stag beetles were detected (8 males and 7 females) from five emergence trap sites which were therefore regarded as real oviposition sites. All oviposition sites were characterised in terms of typology of dead wood, tree species, canopy openness, trunk diameter, dead wood volume, decomposition stage (five classes) and wood hardness (four classes). All the detected emergence sites belonged to the genus *Quercus,* two being from the allochthonous *Q. rubra,* but no preferences for a dead wood species nor for a typology were shown and a broad variation was apparent for all the considered variables. The mean values of canopy openness, diameter, dead wood volume, decay status and wood hardness were 2.54%, 51cm, 4.92m^3, 3 and 3.4 respectively. These data suggested an important heterogeneity in the oviposition sites selection. Although this method (telemetry + emergence traps) provided substantial aid to finding newly emerged beetles, it required a large amount of fieldwork effort, both in terms of time and man-hours. The advantage of the method is its low degree of invasion while its drawback is the amount of effort needed. Calculations were made to assess the minimum number of operators and traps needed to gather a number of data suitable for statistical analysis. It was found that two full time operators should be able to detect about 50 potential oviposition sites in one flight season, 28 of which were expected to be real oviposition sites.

Keywords

emergence traps, radio-telemetry, saproxylic insects, dead wood, oviposition sites

Introduction

Detecting the breeding sites for a protected species is of great importance for its conservation and monitoring, as the knowledge of these crucial spots is needed to optimise management and surveillance.

The European stag beetle, *Lucanus cervus* (Linnaeus, 1758) (Coleoptera: Lucanidae), is a flagship species for conservation of forest ecosystems, particularly for the saproxylic community (Pratt 2000, Thomaes et al. 2008, Carpaneto et al. 2015, Campanaro 2016, Bardiani et al. 2017b). The stag beetle is protected at the European level and listed in the Annex II of the Habitats Directive. Its larvae develop underground for three to five years, often up to 1m deep, feeding on decaying wood of stumps, logs or roots of a wide range of broadleaf trees and shrubs (Franciscolo 1997, Percy et al. 2000, Harvey et al. 2011a). A key problem for conservation of the stag beetle is that larvae are hard to detect because they live in the subterranean/saproxylic interface between soil and deadwood where they spend the most part of their life cycle. Sampling methods aimed at directly searching for larvae through excavation under log and tree roots are probably too invasive as they could harm the larva and alter the ecological conditions (Harvey et al. 2011b, Chiari et al. 2014b). For these reasons, stag beetles are usually monitored only during their adult phase (Sprecher-Uebersax and Durrer 2001, Sprecher 2003, Campanaro et al. 2011a, b, 2016, Fremlin and Hendriks 2011, Harvey et al. 2011b, Chiari et al. 2014a, Bardiani et al. 2017a, b).

Three methods have been developed for detecting larvae in a monitoring context: Rink and Sinsch (2008) used radio-telemetry to track females up to oviposition sites in a suburban context of Germany, while Harvey et al. (2011b) developed both a chemical method for detecting compounds produced by the larvae and an acoustic method for recording their stridulations in a suburban context in England. A standardised method for detecting sites of larval development and capturing newly emerged beetles could be crucial for conservation and studies on habitat requirements and dispersal ability of the target species (Rink and Sinsch 2007,Tini et al. in press a). In fact, previous telemetric studies showed that the longest distances covered by a stag beetle were recorded from freshly emerged individuals and highlighted the importance of the first period of adult life for dispersal (Rink and Sinsch 2007, Tini et al. in press a). Furthermore, the characterisation of the larval development sites is a key aspect for their conservation, as this type of habitat is likely to be the most critical and limiting factor for the species development. Another important limiting factor is its modest reproduction rate (for an insect) that ranges from 15 to 36 eggs (24 on average) for a single clutch (Sprecher 2003, Harvey et al. 2011a).

In this preliminary study, for the first time radio-telemetry was used in combination with emergence traps set to detect and describe the oviposition sites. The specific objectives were: (1) to explore the feasibility of a mildly invasive method for detecting oviposition sites in forest habitats, assuming that they were also potential development sites for the larvae; (2) to verify whether the method could be used to characterise the oviposition sites in terms of above-ground ecological factors to be used as a proxy for underground conditions of wood and (3) to quantify the average number of individuals captured by emergence traps in order to assess the minimum quantity of traps and operators needed for obtaining the amount of data suitable for statistical analysis.

Materials and methods

Study area

The study area "Bosco della Fontana" is located in northern Italy (Marmirolo, province of Mantua, Region Lombardy) (45°12'N 10°44'E, altitude: 24–26m a.s.l.). This area is one of the last remains (233ha) of the lowland broadleaf forests in the Po valley, an intensively cultivated area. The forest has been a Biogenetic Nature Reserve since 1977, included in the Nature 2000 network as Special Protected Areas (SPAs IT20B0011) since 1998 and a Site of Community Importance (SCI IT20B0011) since 2004. This State Reserve, formerly managed by the national forestry service (CFS), is currently managed by the Ufficio Territoriale Carabinieri per la Biodiversità di Verona [= Territorial Office of Carabinieri for Biodiversity of Verona]. Since 2007, it has also been part of the Italian Long Term Ecological Research Network (LTER-Italy). Around 85% of the Reserve is covered by broadleaf forests, the remaining part consisting of grassland and a small wetland. The deciduous forest of the study area is dominated by *Quercus cerris* L., *Q. robur* L., *Carpinus betulus* L. and *Fraxinus ornus* L., with *Alnus glutinosa* (L.) and *Fraxinus oxycarpa* Vahl along the main watercourses (Mason 2004). Silvicultural practices officially stopped in 1994 (Mason 2004) and forest management was aimed at increasing deadwood for restoring the natural character of the ecosystem. Actions included favouring the ageing of native trees and converting the alien species (*Q. rubra* L., *Juglan snigra* L., *Platanus* spp.) which have been inappropriately introduced in the past, to coarse woody debris (CWD) (Cavalli and Mason 2003). The increase in deadwood, in this way, would benefit saproxylic insects and the whole forest community. Currently, the study area hosts many large saproxylic organisms, such as *Lucanus cervus, Morimus asper* (Sulzer, 1776) and *Cerambyx cerdo* Linnaeus, 1758 (Mason et al. 2015).

Data collection

In 2014, from 29th May to 10th July, twelve females were captured, radio-tagged and released for detecting potential oviposition sites. In 2015, before the emergence of the adults (early May), these sites were covered with anti-aphid plastic nets (hereafter: emergence traps) to capture newly emerged individuals and hence demonstrating the role of those dead wood spots as larval development sites. After studying emergence activities during these two years, the traps were removed at the end of the study.

The females for radio-tracking were captured mostly at sunset, by hand while they were crawling on the ground or with a hand net while they were flying. The hand net had a circular frame (50cm diameter) and a telescopic handle (up to 199cm). Each captured adult was weighed, marked ventrally with a permanent marker and with a numbered sticker on the right elytron, then equipped with a battery-powered radio transmitter (LB-2X / 0.31g; Holohil Systems Ltd., Carp, Ontario, Canada) (Figure 1). Transmitters were attached to the pronotum using a cyanoacrylate glue (LOCTITE, Super Attack flex Gel; Henkel, Düsseldorf, Germany), as previously undertaken for *O. eremita* by Chiari et al. (2013). The nominal lifetime of transmitters was 21 days with a lifespan range of 13-22 days. The antenna of the transmitters was reduced to 5cm and adjusted above the elytral suture to limit disturbance to beetle behaviour (Rink and Sinsch 2007). According to Boiteau and Colpitts (2001), electronic tags should weigh no more than 33% of the beetle's weight in order not to hamper flight; in this study, the transmitter weight was less than 18% of the initial body mass (weight of females ranged between 1.8–4.1g), thus the transmitter load was well below the threshold. Transmitter signals were detected by means of a hand-held antenna (Yagi three elements; Wildlife Materials Inc., Murphysboro, IL, USA) connected to a radio receiver (NEW TRX-1000S W; Wildlife Materials Inc.).

The radio-tagged beetles were released the next evening, at the same location from where they had been captured and at a time when there was no rainfall and the temperature was mild. The 'homing technique' (White and Garrott 1990) was used to detect the location of each beetle (Tini et al. in press a, b). To obtain information on beetle activities during the whole 24 hours of the day, one homing procedure each hour for each individual was performed, within modules of four hours per day moved forward by four hours every day (six shifts per week). In this way, the twenty-four hours of the day were covered in six days. The homing procedure always started at the last known location of the individual and lasted, in most cases, for a few minutes, but sometimes it could also occur over several hours. To avoid interference with the behaviour of tagged individuals, they were approached no closer than 0.5m. Although visual detection was not possible when individuals were underground, in dead wood or in the canopy, the

Figure 1. A female of *Lucanus cervus* marked with a numbered sticker on the right elytron and equipped with a battery-powered radio transmitter (LB-2X / 0.31g; Holohil Systems Ltd., Carp, Ontario, Canada).

location was recorded. A potential oviposition site (hence, a potential larval development site) was assumed to be found when the radio-signal of a female was heard for at least 3 successive days underground (within 2m of suitable dead wood including tree, stump and snag or directly under logs). As the radio-signal became progressively less clear, suggesting an increasing depth day by day, the detection probability of an oviposition site was very high. If the female remained at one place for three days, but not near to dead wood, the place was not considered as a potential oviposition site and at least 5 days passed before a hole was dug for checking (in all these cases, a dead female or a radio-tag detached from the beetle was found). Coordinates of each location were identified using a Garmin GPS (Garmin Ltd., Olathe, KS, USA) (MAP 60 CSX).

In the first half of May 2015, emergence traps were set in nine potential oviposition sites based on data obtained during the previous year. Different typologies of dead wood spots were considered as potential oviposition sites: standing dead trees (SDT), lying dead trees (LDT), logs (portions of a trunk or a large branch), stumps, snags, uprooted LDTs, uprooted stumps and roots. A snag was defined as a standing dead tree without branches, with height >130cm and diameter at breast height (DBH) >10 cm; if branches were present, the snag was considered as an SDT; if the snag was less than 130cm in height, it was considered as a stump. The DBH of SDT, LDT and snags was measured.

Emergence traps were made with anti-aphid plastic nets wrapping a large dead wood spot and fixed with nails to the ground and/or to the deadwood (Figures 2 a and b). The margins of the net were carefully kept attached to the bark or the wood surface to avoid the escape of the beetles from the trap. Each trap was checked twice a week from 15th May until 15th July 2015 and from 3rd June until 4th July 2016.The control of each trap lasted about thirty minutes. When adults of *L. cervus* were found, these were captured by opening the net. Each captured beetle was weighed, measured and marked with a numbered sticker on the right elytron and with a permanent marker ventrally. As it was not known what the best time was for net checking, the nets were checked in the evening up to early night (16:00–22:00h) when stag beetles were mostly active in the study area (Campanaro et al. 2016, Bardiani et al. 2017b, Tini et al. in press b), using a headlamp.

For each potential oviposition site, at the end of the 2014 flight season, the following environmental variables were recorded (Table 1): typology of dead wood, tree species, diameter, percentage of canopy openness, dead wood volume, decay status and wood hardness. As the stag beetle larvae exist underground in the interface between soil and dead wood, it was hard to assess the variables which affect their development and it was possible only with excavation (e.g. Rink and Sinsch 2008). Therefore, it was assumed that some variables measured above-ground, such as decay status and wood hardness, may be good proxies for similar subterranean environmental conditions. The percentage of canopy openness was calculated by means of a Gap Light Analyser (GLA version 2.0), a digital image processing software that allows the estimation of gap light transmission indices from true-colour hemispherical (fisheye) photographs (Frazer et al. 1999).The photographic lens was a Fisheye converter FC-E8 0.21×. Photographs were taken under a uniformly overcast sky or close to sunset; these sky conditions provided a perfect diffuse sky, thus avoiding the interference of direct sunlight which can cause errors up to 50% (Nobis and Hunziker 2005). Dead wood volume was calculated by approximating the shape of trunks to a cylinder and the shape of uprooted stumps to two cylinders. Height of trees and length of fallen trunks was measured by means of a dendrometer Vertex III (produced by Haglöf Sweden); this instrument uses ultrasonic signals to determine heights and distances. Decay status was recorded for the

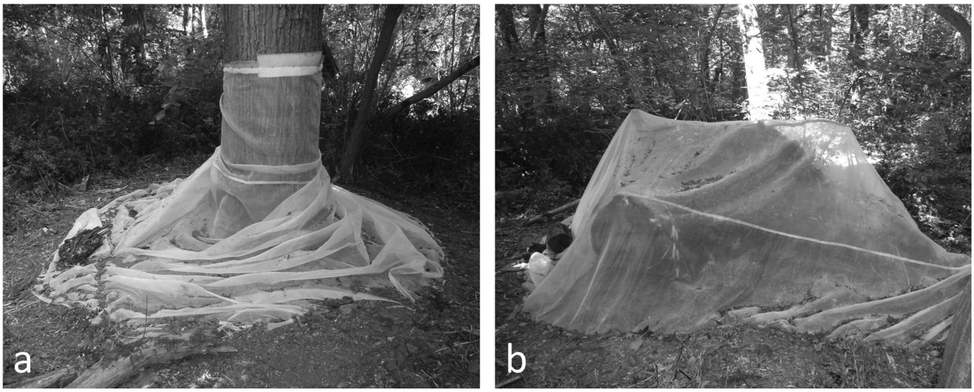

Figure 2. Emergence traps placed on larval development sites (DS) detected in 2014 by means of radio-telemetry. **A** Emergence trap on *Quercus* sp. SDT (DS01) **B** Emergence trap on *Quercus rubra* Uprooted stump (DS10).

above-ground part of the wood piece, according to Hunter and Malcolm (1990), using a score of five classes: (1) no evidence of decay; (2) solid wood, less than 10% changed structure due to decomposition, the wood being solid at its surface and attacked only to a very small extent by wood decomposing organisms, bark being intact or lost only in part, twigs (diameter < 3cm) being absent, unaltered colour of wood; (3) slightly decayed, 10–25% of the wood having a changed structure due to decomposition, twigs being absent, bark being present only in trace amounts, colour of wood having faded; (4) decomposed wood, 26–75% of the wood being soft to very soft, bark and twigs absent, wood colour being light, from faded brown to yellow and (5) very decomposed wood, 76–100% of the wood being soft, bark and twigs being absent, wood colour fading to light yellow or grey. The wood hardness of each dead wood spot was assigned to four classes (from 1 to 4, in order of decreasing hardness) upon the degree of penetration of a knife blade (Opinel n°8, as in Redolfi De Zan et al. 2014): (1) the knife blade penetrates less than 1cm; (2) the knife blade penetrates about 1cm; (3) the knife blade penetrates more than 1cm; (4) the wood is highly soft throughout its entire thickness, the knife blade penetrates completely into the wood.

Number of traps and operators assessment

To estimate the minimum number of operators and traps needed to obtain an amount of data suitable for statistical analysis, the following values were calculated. The emergence site detection ratio was calculated by dividing the number of emergence sites detected by the number of traps used. To estimate the number of emergence sites which can be expected to yield emergence data, the number of set traps was multiplied by the emergence site detection ratio. The minimum, maximum and mean numbers of capture expected in one season were also estimated.

Table 1. Number of beetles captured by emergence traps and dead wood variables. ID number of females radio-tagged in 2014 (eight on the whole) which remained at least three days in one dead wood spot, therefore considered as a potential oviposition site and a potential larval development site (DS); number of emerging males and females captured by emergence traps in 2015 and 2016 in each DS N° and environmental variables. SDT: standing dead tree; LDT: lying dead tree; Ø: diameter (for snags, SDT, LDT is considered as DBH); DW: dead wood.

Digging Female	DS N°	Emerging Males	Emerging Females	Date of emergence	Typology	Species	Canopy openness (%)	Ø (cm)	DW volume (m^3)	Decay status	Wood hardness
F061	DS01	6	1	22/06/15	SDT	*Quercus* sp.	3.06	72.5	12.97	3	4
F061	DS02	0	0		LDT	*Q.robur*	4.83	50	8.39	2	3
F061	DS03				LDT	*Carpinus betulus*	0.78	33	1.03	3	3
F038	DS04	2		27/06/15 04/07/15	Log	*Quercus* sp.	2.94	47	0.90	4	3
F060	DS05		2	9 and 27/06/16	Uprooted LDT	*Q. rubra*	1.28	36.5	1.93	3	4
F060	DS06				Roots	*C. betulus*	4.48	8	0.01	1	1
F075	DS07				Stump	*Quercus* sp.	2.75	68	0.32	5	4
F038	DS08				Snag	*C. betulus*	1.02	52	0.91	4	4
F075	DS09	0	0		Stump	*Q. rubra*	0.70	65	0.40	5	4
F075	DS10	1	2	27/05/15 (1M) 1/07/15(1F)	Uprooted stump	*Q. rubra*	1.28	52	4.40	3	4
				9/06/16 (1F)							
F075	DS11				SDT	*Q. rubra*	1.86	55	4.03	3	4
F075	DS12				Uprooted LDT	unidentified	1.60	80	4.49	5	4
F072	DS13				SDT	*Prunus avium*	8.61	60	4.23	2	1
F072	DS14				SDT	*C.betulus*	4.24	22	0.53	1	1
F020	DS15		1	27/06/16	Uprooted LDT	*Quercus* sp.	4.14	47	4.98	2	2
F091	DS16				Log	*Quercus* sp.	5.36	32	4.5	2	2
F091	DS17				Log	*Fraxinus ornus*	11.38	16	8.14	3	2
F091	DS18				Stump	unidentified	5.28	32	0.06	2	1
F013	DS19	0	0		Stump	unidentified	3.76	45	0.08	4	3
F013	DS20	0	0		Uprooted LDT	*C. betulus*	12.29	31	2.48	1	1
F013	DS21				LDT	*C. betulus*	2.50	29	0.77	5	4

0 means that no beetle was found in that emergence trap.

As this work was a preliminary study, data on which the estimates are based were few, thus the results of the number of traps and operators assessment have very broad confidence intervals and a solid statistical approach to evaluate the dead wood productivity cannot be performed.

Results

Capture data

At the end of the reproductive season for 2014, the 12 radio-tagged females allowed the detection of 21 dead wood spots as potential oviposition sites and these were covered by emergence traps. In the first half of May 2015, due to logistic constraints, only nine of these spots were chosen, based on their accessibility and feasibility of being covered by emergence traps without damaging the surrounding vegetation. In 2015 (from 27th May to 27th June) and 2016 (from 9th to 27th June), 11 (9 males and 2 females) and 4 individuals (all females) were respectively captured by the emergence traps.

During the radio-telemetry study carried out in 2014 (Tini et al. in press a, b), eight females were recorded digging deeply at the periphery of at least one dead wood spot and to spend at least three days underground. It was assumed that these dead wood spots were potential oviposition sites and hence potential larval development sites (Tini et al. in press a) (Table 1). In 2015, 11 individuals were found inside three of the nine emergence traps in five checking days and, in 2016, four individuals were captured inside three traps in two checking days. Overall, five emergence sites were detected.

Characterisation of the oviposition sites

Approximately half (10/21) of the dead wood spots, identified as potential oviposition sites, could be assigned to the genus *Quercus* with certainty: five *Quercus* sp., four *Q. rubra* and one *Q. robur* (Table 1). The other half were: six *Carpinus betulus*, one *Prunus avium* and one *Fraxinus ornus*. For three sites, it was not possible to identify the genus due to the advanced decaying. The typologies of the five sites that were found to be suitable for larval development were: two uprooted LDT, one uprooted stump, one SDT and one group of logs (Table 1). All these five sites belonged to the genus *Quercus*: three were probably *Q. robur* (uncertainty due to advanced rotting stage) and the other two belonged to the allochthonous *Q. rubra*. Canopy openness of these five sites was 2.54% (SD = ±1.24) on average. The mean diameter value was 51cm (SD = ±13.28). Dead wood volume was 4.92m^3 (SD = ±4.49) on average and the mean decay status was 3 (SD = ±0.71). Wood hardness values averaged 3.4 (SD = ±0.89).

The highest number of beetles (7) was captured within the trap DS01 wrapping an SDT of *Quercus* sp. (Figure 2a; Table 1). This emergence trap contained the largest amount of dead wood and, although the decay status was not extremely advanced (score value: 3), the wood was the least hard (score value: 4). The trunk appeared to be full of wood mould, at least below breast height. All the emergences recorded in this trap were detected during the same check. Only one site (DS10) was found to be suitable for larval development in both checking seasons.

Number of traps and operators assessment

In 2015, 29 stag beetles (20 males and 9 females) were radio-tracked by two operators, thus it was calculated that 30 females can be tracked by the same number of operators during the same period. As the radio-tracking of 12 females led to the detection of 21 potential oviposition sites, it was calculated that, with 30 females, it would be possible to detect about 50 potential oviposition sites (30 * 21 / 12 = 52.5). As five emergence sites were detected by mean of nine traps, an emergence site detection ratio of 0.56 (5 / 9 = 0.56) was calculated. Thus, by setting 50 traps, 28 sites are expected to yield emerging adults (50 * 0.56 = 28). It was calculated that 50 traps would be required for the capture of at least 28 emerging adults. For the highest number of captures, the same value as observed in the present work (seven adults) was used (28 * 7 = 196). The mean number of captures obtained by the emergence sites was three, thus for a total of 50 traps a mean number of captures of 84 stag beetles is expected (28 * 3 = 84). Considering about 30 minutes for checking one trap, one operator should be able to control about 25 traps twice a week, working about 4 hours per day. Therefore 2 operators should be able to check about 50 traps twice a week.

Discussion

This study is only a pioneering approach for the combined use of telemetry and emergence traps (wrapping nets), with the aim of detecting the oviposition sites of the stag beetle. Moreover, it was also a preliminary investigation on the characteristics of dead wood spots suitable for oviposition. Emergence traps have previously been used to capture freshly emerged stag beetles by Rink and Sinsch (Rink 2006), but at spots where adults had already been seen emerging in previous occasions, a quite easily detectable event in urban environments. In this case, the study was developed in a forest with a large amount of dead wood where it was hard to detect the effective emergence sites. As the number of observations was low and cannot be analysed in statistical terms, some preliminary considerations were given on the data obtained, valid for launching a number of working hypotheses. Obviously, it cannot be concluded that the dead wood spots that yielded no captures were not occupied by the larvae of *L. cervus*.

According to these results, *L. cervus* showed a broad heterogeneity in the selection of potential oviposition sites, in agreement with previous literature (Percy et al. 2000, Smith 2003, Rink and Sinsch 2008, Harvey et al. 2011a). In fact, these consisted of a diverse typology of dead wood spots with a high range of values for all variables considered. However, *Quercus* was the only tree genus where larvae developed successfully. About half of the trees with positive results were *Q. rubra*, showing that exotic oaks are also potentially suitable for this beetle. *Q. rubra* was found to be suitable for larval development in the major part of the cases where traps were placed (2/3: 67%) probably because this alien species, destined for a progressive eradication in the study area, was transformed into dead wood and used by the management authorities for increasing saproxylic biodiversity (Cavalli and Mason 2003). Dead wood of *Q. rubra* is now very abundant and has the right age and stage of decomposition for larval development. The stag beetle does not seem to have a preference either for this species with respect to native oaks, nor for other deciduous trees, although in a previous study developed in England, oak, apple, ash and cherry were found to be more commonly used, likely because they were commonly available (Percy et al. 2000). Smith (2003) reported 27 species as suspected or confirmed oviposition sites, supporting the idea that stag beetles will utilise a range of tree species as breeding sites. A clear preference for a species was not even shown in the study of Rink and Sinsch (2008), where the suitable trees for larval development were oak, sessile oak, white willow, silver birch, cherry and plum trees. Harvey et al. (2011a) reported that, in Britain, larvae have been recorded from 60 different hosts and that, although oaks were dominant, they formed only 9%–19% of records. In the study developed by Rink and Sinsch (2008), almost all the breeding sites were exposed to sunlight, while, in this study, they were located in rather shaded places with a mean value of canopy openness of 2.54%. Probably, such differences in sun exposure are linked to the different climatic conditions. In fact, average temperatures in Germany are generally lower than in northern Italy and sun exposure may be favourable or dangerous, depending on the local climate. Furthermore, in the study by Rink and Sinsch (2008), dead wood diameters ranged between 24cm and 79cm, similar to the values of this study (36.5cm to 72.5cm). Both studies supported the idea that the stag beetles showed a large variation in the oviposition sites selection. In parallel, the stag beetle was not associated to any typology of the selected oviposition sites, although roots were present in most dead wood spots, except for logs. In fact, oviposition sites, in the proximity of roots, were also observed by previous authors (e.g. Percy et al. 2000, Fremlin 2009). These data suggest that oviposition sites of the stag beetles are distributed over several microhabitat types with a wide range of values for each parameter considered. Probably, management actions focused on increasing the heterogeneity and dead wood in a forest, with such uprooting or cutting of alien species trees, without removing the wood, potentially leading to favourable conditions for stag beetle reproduction.

This method could be used for improving the knowledge of dead wood requirements for larval development and for studying the first part of the adult lifespan when individuals are more active, at least concerning their dispersal movements (Rink and Sinsch 2007, Tini et al. in press a). Although the proposed method required a large amount of fieldwork, in terms of time and man-hours and yielded a low number of individuals, it was probably less invasive and more feasible than other methods proposed. Moreover, it may yield a larger amount of data if conducted by several operators collaborating in net setting and checking. It was calculated that, in this study area, two full time personnel were able to radio-track up to 30 females in two consecutive months of the local reproductive period (i.e. June-July). Such a fieldwork could lead to the detection of more than 50 potential oviposition sites, a number suitable for a statistical analysis focused on characterising the above-ground ecological parameters, as a proxy for subterranean environmental conditions. In the successive year, a second phase of fieldwork, also conducted by two operators, could record the emergence of about 80 adult stag beetles i.e. a number suitable for the evaluation of each oviposition site. The limited amount of data presented here was mainly related to the fact that it was not possible to work on a full time basis for this research.

In any case, the monitoring of a high number of oviposition sites, investigated by emergence traps, cannot last for more than five years because the nets hinder the females laying their eggs and the number of emergent individuals will become zero in the fifth year after the trap setting. This hindrance to egg-laying, due to the presence of nets over the oviposition site, may lead to an important impact on the reproduction of *L. cervus* in areas where suitable dead wood is scarce and localised. In this study area, where there is plenty of dead wood, such an impact is probably less important and the application of this method during a long-term study, could also be useful for investigating how long the dead wood is suitable for larval development.

An unsolved issue concerns the selection of the best checking time during the day, a problem which can be addressed with this working protocol, based on controls twice a week. In fact, even with more controls per day at different time slots, this problem is linked to beetle detectability under the net mesh that may vary consistently throughout the day in relation to species behaviour and to the visual acuity of the checking operator. Many studies revealed that the peak of stag beetle activity, at least in northern Italy, is in late afternoon to almost one hour after sunset (Chiari et al. 2014a, Campanaro et al. 2016, Tini et al. in press b). Probably, in the late morning and at noon, beetles are not easily detectable because high temperature values make them stand still in the shadow, under fallen leaves or in cavities where they are not visible to the checking operator, while from late evening to sunset, they are more prone to move and become easier to detect, although human sight is less efficient after sunset and needs the use of artificial light.

Conclusions

The data available with this method, if applied to a long term study, could be of great importance for the conservation of *L. cervus*, as they could give information on the effects of different wood decaying stages on stag beetle larval development. The combined use of radio-telemetry and emergence traps is a useful method for finding oviposition sites and for detecting emerging individuals. Little evidence is available on the length of the larval development of the stag beetle from a single oviposition site and location. In fact, the duration of the life cycle may vary between three to five years dependent on several factors such as quality of food and climatic conditions. A long term monitoring of the emergence of stag beetles from a single site can help to calculate the duration of its suitability as a larval development site. Such knowledge could be very helpful in order to build artificial oviposition sites in a protected area, thus allowing the managing authorities to plan the dates for cutting logs or uprooting trees and could predict how long these would be suitable for larval development thus ensuring a continuing availability of essential resources.

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