**Title**

A cost-efficient and accurate pheromone-baited monitoring protocol for *Elater ferrugineus*, a biodiversity indicator of hollow trees.

**Authors**

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**Journal**

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**Abstract**

Introduction: To evaluate the loss of biodiversity, monitoring of priority species has been proposed. As these species need to include different taxonomical and functional groups, there is a need for cost-efficient and accurate monitoring protocols for saproxylic indicator species. *Elater ferrugineus* is known as an indicator of hollow trees and the associated biodiversity and can be monitored easily with the use of pheromones.

Aims/Methods: We evaluated the effect of the trap type (funnel and bottle trap) and the required sampling effort to detect *E. ferrugineus* or to monitor its trend with a power of 80%.

Results: The effort needed for species detection is 7 and 13 days for funnel and bottle trap respectively. While for trend monitoring 27 and 63 days are needed respectively. Trap type was not significant for species detection while for trend monitoring, funnel traps performed significantly better.

Discussion: The sampling effort is high compared to earlier studies performed on this species, which is likely related to the low habitat quality of the study areas. The local variation found within sites was low reflecting the mobility of the beetle. We conclude that distribution surveys and trend monitoring of this species is feasible with a citizen science approach even in areas with low habitat quality using commercially produced pheromones in respectively bottle and funnel traps.

Implications for insect conservation: For distribution surveys, we propose to use 4 bottle traps during 3 days in each site. For trend monitoring, we propose to use 1 funnel trap during 27 days or multiple traps in a shorter period.

**Key-words**

7-methyloctyl-(Z)-4-decenoate, statistical power, species conservation, rusty click beetle, hollow pollarded trees

**Introduction**

Monitoring biodiversity is essential for nature conservation documenting its current loss (Lindenmayer and Likens 2010; Reynolds et al. 2011; Jones et al. 2015). As monitoring the entire scope of biodiversity is impossible, lists with priority species have been proposed to focus monitoring and conservation (e.g. Stanton et al. 2016). The reasoning is that a list comprised of species from different habitats, taxonomical and functional groups should enable an overall evaluation of the biodiversity trend. Therefore, species selected for this purpose should not be biased towards more familiar or noticeable species (Clark and May 2002; Regan et al. 2008; Franklin et al. 2011). However, underrepresentation of invertebrates has often been argued (Cardoso et al. 2011; D'Amen et al. 2013; Leather 2013). Furthermore, insects included mainly represent noticeable species of open habitats like butterflies and dragonflies (e.g. Larsson and Svensson 2011; Gerlach et al. 2013) while saproxylic beetles and fungi are underrepresented in national sets of priority species (Thomaes and Vandekerkhove 2017). The difficulty to sample and determine saproxylic beetles, their great taxonomic diversity as well as their elusive lifestyle has led to a lack of knowledge about their distribution and main threats (Horak and Pavlicek 2013; Kadej et al. 2015; Larsson 2016). Consequently, there is a strong need for innovative cost-efficient and accurate monitoring techniques and protocols for saproxylic indicator species.

The use of pheromone lures are a promising technique to allow accurate and reliable monitoring of many insect species (Kadej et al. 2015; Larsson 2016), giving better understanding on their distribution (e.g. Kadej et al. 2015; Harvey et al. 2017; Rukavina et al. 2018) and threats (Andersson et al. 2014; Ray et al. 2014). Pheromone lures have a long history in pest management but the use in conservation biology is still rather limited (Larsson 2016). Nevertheless, this technique has many advantages: a low detectability threshold, species specificity avoiding major sorting and determining problems of random trap systems, a higher detection probability resulting in better estimates of population size, long-range attraction and large scale deployment possibilities (Larsson 2016). Main disadvantageous encountered are the difficulty to develop pheromone lures, the need for highly specialised and costly laboratories to identify and reproduce pheromones, a limited commercial interest for species of conservation interest compared to pest species and finally, pheromone trapping could potentially harm the target species by disturbing its pheromone communication (Larsson 2016). When using known pheromone lures for national or regional wide monitoring schemes (Musa et al. 2013; Andersson et al. 2014; Harvey et al. 2017), the main challenge might be to reduce costs, to ensure the pheromone supply for the coming years and to simplify the monitoring protocol in order to be able to use a citizen science approach.

For the conservation policy of Flanders (Northern Belgium), a list of 57 priority species, breeding birds not included, was compiled (Westra et al. 2019). *Elater ferrugineus* L., 1758 was included as an indicator of hollow tree communities (Thomaes et al. 2016). This species was selected because of (I) its fairly wide distribution in Flanders, (II) its use as an indicator species for hollows and associated fauna and (III) the potential to use pheromone monitoring (Thomaes et al. 2016). The pheromones have been identified (Svensson et al. 2012, Tolasch et al. 2007) and applied in different studies revealing its potential for monitoring. Formerly, the species was often considered to be quite rare as little data was available (e.g. Kadej et al. 2015). For Flanders, only 8 records are known between 1950 and 2011, until the use of pheromones revealed a much wider and more common distribution. Due to this fairly wide distribution in Flanders, its monitoring could represent a regional indicator of its habitat. Furthermore, *E. ferrugineus* is known as a habitat indicator associated with the local density of tree hollows (Musa et al. 2013; Oleksa et al. 2015) as well as an indicator of species rich communities in these hollows (Andersson et al. 2014; Oleksa et al. 2015). An exploratory study in Flanders (Thomaes et al. 2015a) revealed a similar relation with habitat availability and species diversity for this region. In contrast to *Osmoderma eremita* (Scopoli, 1763), the presence of *E. ferrugineus* is indicative on a larger spatial scale making it a better indicator for broad landscape patterns and its pheromone monitoring is more efficient (Musa et al. 2013; Andersson et al. 2014). As *O. eremita* is critically endangered in Flanders (lacking records since 2008, Thomaes et al. 2015b), it could not be included in the list of priority species for monitoring.

Previous pheromone studies on *E. ferrugineus* have mainly been using funnel traps (Svensson et al. 2004, 2012; 2008; Larsson and Svensson 2009, 2011; Musa et al. 2013) while others have used window traps (Svensson and Larsson 2008; Andersson et al. 2014; Zauli et al. 2014; Kadej et al. 2015; Rukavina et al. 2018) which are both expensive to purchase or built and costly to send to numerous volunteers. Some studies have used small self-build cross vane window traps (Tolasch et al. 2007; Oleksa et al. 2015; Harvey et al. 2017) which are still labour intensive to make and need to be send around. However, the effect of the trap type on the performance of monitoring *E. ferrugineus* has not yet been evaluated. Bottle traps are cheaper and homemade alternatives can be used in a citizen science approach excluding the costs to send a trap (Steininger et al. 2015). Bottle traps have been widely used as baited traps with success in numerous studies on other species (e.g. Mazon et al. 2013; Steininger et al. 2015; Klingeman et al. 2017; Miller et al. 2018). However, studies on the pheromone trapping of Bark and Ambrosia beetles reveal non conclusive trap effects (Mazon et al. 2013; Steininger et al. 2015; Klingeman et al. 2017; Miller et al. 2018). Furthermore, population size and study aim of these studies differ strongly so conclusions cannot be generalised to the monitoring of rare species. Consequently, it is unclear whether a simple bottle trap would perform as successfully as a funnel traps to monitor the distribution or population trend of *E. ferrugineus.*

The goal of this study is to test possibilities of monitoring *E. ferrugineus* in a cost-efficient and accurate way based on pheromone-baited monitoring. Musa et al. (2013) and Harvey et al. (2017) and already showed that it is possible to obtain the national distribution of the species by the use of pheromone baited traps in a citizen science approach. Our goal is to study options to further reduce the costs and simplify the method in order to use pheromone baited trap in a citizen science monitoring context. Moreover, we intended to compare 16 unit funnel traps with homemade bottle traps and analyse the monitoring effort needed to ensure presence detection or abundance trend monitoring.

**Marterial & methods**

The study species, *E. ferrugineus*, is a large click beetle (Elateridae) with a broad distribution in Europe. The larvae of this species live for several years in wood mould of hollow trees of various tree species (e.g. Svensson et al. 2004; Tolasch et al. 2007). The larvae are considered facultative carnivorous hunting on larvae, mainly of larger scarabaeid beetles (Schaffrath 2003; Svensson et al. 2004; Tolasch et al. 2007). Also the adults exhibit a hidden life style due to the short phenology and high flights. Their habitat includes wood pastures, parks, old forests, orchards and sites with pollarded trees. Due to the strong loss of these kinds of habitats, the species is listed as Near Threatened on the IUCN European Red List (Nieto and Alexander 2010).

The study was performed in Flanders (Northern Belgium) where 6 study sites were selected for which the presence of *E. ferrugineus* was recently ascertained (Table 1, Figure 1). The different sites represent varying habitat types where the species is found. At each site 4 trapping locations (named from A to D) were selected which are about 100 m away from each other (along a 400 m transect or in a 100 by 100 m square). This distance ensured independent trapping effects (Svensson et al. 2012). Each location had a pheromone baited trap. At start, location A and C had a funnel trap (Lindgren 16 units funnel trap, Phero Tech Inc, Canada with 20 cm top and 10 cm funnel bottom) and B and D had a bottle trap (1 L PET bottle with top inverted as funnel with approximately 9.5 cm top and 2.5 cm funnel bottom, Figure 2). The traps were baited with 7-methyloctyl-(Z)-4-decenoate in rubber septa (Pherobank, The Netherlands) which were placed in the trapping bottle.

In general, traps were checked every 2 to 3 days, rarely less frequently with a maximum of 11 days. Traps were shifted one location about weekly so that funnel and bottle traps were used at each location. Traps were followed up in 2019 and 2020 from early July till early August. In some sites traps were followed up till end of August or early September but only data gathered up to 2 days after the last beetle was captured (end of the season) were used for the data analysis. Individuals were marked at two sites in 2019 but no recaptures were registered.

Table 1: Study sites in Flanders including habitat description, coordinates and years of monitoring for each site.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study site | Town | Coordinates | Years | Habitat description |
| Bos t’Ename | Oudenaarde | 50.85°N 3.65°E | 2019-2020 | Poplar, Ash and Alder dominated nature reserve (105 ha) |
| Geraardsbergen | Geraardsbergen | 50.76°N 3.88°E | 2019-2020 | Garden with old orchard at the border of the town, surrounded by agriculture and forests |
| Helix | Geraardsbergen | 50.78°N 3.93°E | 2019-2020 | Agricultural area with pollarded willows |
| Meerdaalwoud | Bierbeek | 50.80°N 4.70°E | 2019-2020 | Oak dominated coppice with standard reserve (32 ha) in a 1350 ha oak forest |
| Neigembos | Ninove | 50.81°N  4.06°E | 2020 | Garden with old orchard and edge of Beech dominated forest reserve (72 ha) |
| Voeren | Voeren | 50.76°N 5.83°E | 2019 | Garden in an agricultural area with pollarded trees |



**Fig. 1** Map of Flanders with observations of *Elater ferrugineus* (between 2010-2021, filled 5\*5 km UTM squares) and study sites (asterisks with first two letters of the location). See Table 1 for names of the study sites. Inset map shows Flanders within Northwest Europe.

**Fig. 2** Lindgren 16 units funnel trap (left) and 1 L PET bottle trap (right).

We used a generalised linear mixed model for the number of trapped individuals (*Y*) assuming a Poisson distribution with log link. The linear predictor η includes three parts: I) the log number of days trapped (log(d)) as offset, II) the density in bottle traps in 2019 (β0), the difference in density between bottle and funnel traps (βt) and the difference in density between 2019 and 2020 (βy) as fixed terms and III) a random intercept for the site (bs), a random intercept for the location, nested in site (bl) and a second order random walk along the day of the season (bd). We fit the model in R 4.0.2 (R Core Team 2021) using the INLA package (Rue et al. 2009; Rue et al. 2017) which is a framework for Bayesian inference using Integrated Nested Laplacian Approximation. The fixed effects β0, βt and βy get uninformative Gaussian priors N(0,1000) and random effects bs, bl and bd get a penalised complexity prior for the precision: P(σs>0.6)=0.01, P(σl>0.6)=0.01 and P(σd>0.6)=0.01 (Sørbye and Rue 2016).

In order to analyse the effort needed to monitor a certain decline, a statistical power analysis would be needed (e.g. Thomaes et al. 2017). As we only have two years of data, the year-to-year variance in the studied area is currently unknown. Alternatively, we calculated the time (effort) needed to observe at least 5 individuals as an initial proxy for abundance estimates which could be used in abundance trend monitoring.

In a second and third model we use survival analysis to determine the time (t) needed to detect the first (species detection) or the fifth individual (trend monitoring) in a trap. f(t) is the density of finding the first or fifth individual based on a Weibull distribution with shape parameter α and scale parameter λ. The scale parameter λ is linked to the linear predictor η through a log link. The linear predictor η includes two parts: I) the density in bottle traps in 2019 (β0), the difference in density between bottle traps and funnel traps (βt) and the difference in density between 2019 and 2020 (βy) as fixed terms and II) a random intercept for the site (bs) and a random intercept for the location, nested in site (bl). The fixed effects β0, βt and βy get uninformative Gaussian priors N(0,1000). The random effects bs, bl and bd get a penalised complexity prior for the precision: P(σs>10)=0.5 and P(σl>10)=0.5. We use INLA’s default penalised complexity prior for the shape parameter α. As the traps were not monitored on a daily base, we define the event either as the time interval in which we found the fifth individual or as a right censored time interval in case we never found the fifth individual during a trap rotation.

**Results**

In total, 666 trapping events were used for the data analysis and between 0 (n=473) and 10 (n=1) beetles were trapped at a single event with an average of 0.6 beetles. Beetles were already active the 10th of July 2019 and 27th of June 2020 when the experiment started and last individuals were found 25th of August in 2019 and 20th of August in 2020. Summoning the number of beetles trapped by a single trap on a single location (trap rotation) results in 136 cumulative trapping events (10 site-season combinations\*4 locations\*2-4 trap rotations). The cumulative number of beetles trapped ranges from 0 (n=52) to 32 (n=1).

The model that explains the number of beetles trapped shows a significant effect of trap type (Table 2). Funnel traps were 35% more effective compared to bottle traps (credible interval 9-65%). A clear seasonal pattern is present (Figure 3) clarifying that number of beetles trapped peaked in the second half of July, but declined quickly in the second half of August.

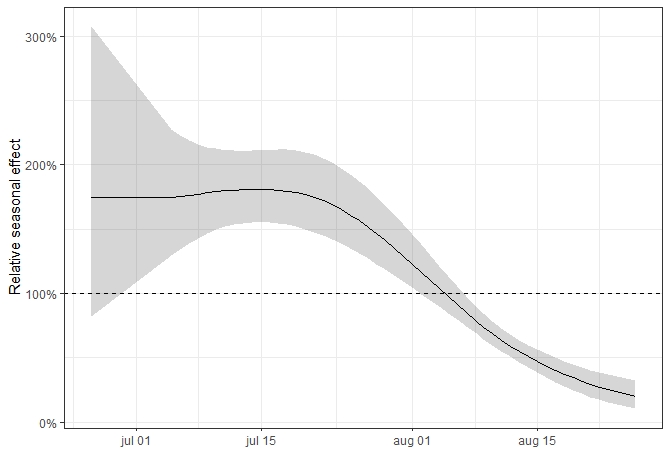
Table 2: Model parameter estimates of the fixed effects in log scale for the model explaining the number of beetles trapped giving the mean, standard deviation (s.d.) and the 95% credibility interval (quantile 2.5 and 97.5%).

mean s.d. quantile 2.5% quantile 97.5%

Intercept -1.871 0.280 -2.429 -1.321

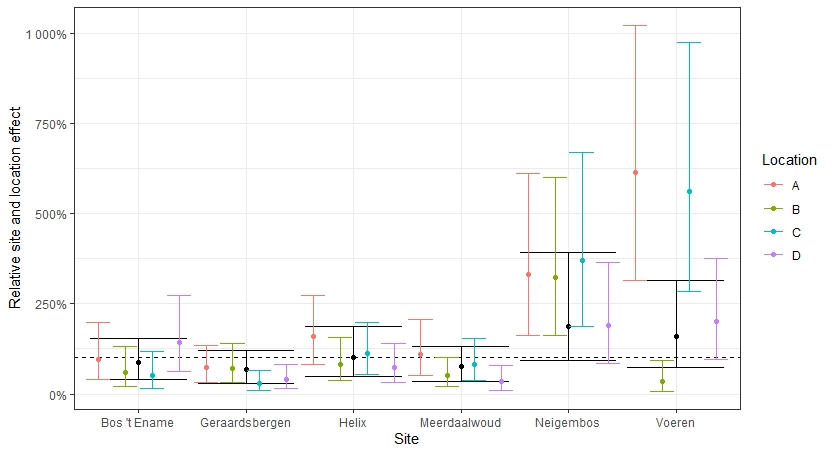
Trap (funnel) 0.291 0.107 0.083 0.501

Year (2020) -0.231 0.154 -0.535 -0.231



**Fig. 3** Relative seasonal effect from the INLA model explaining the number of beetles captured giving the mean as a full line and credibility interval as grey scale

There are no significant differences among sites, but Neigembos and Voeren have slightly higher amounts of beetle captured (Figure 4). Within Voeren, locations A and C perform significantly better than location B. At other sites, there are no significant location effect.



**Fig. 4** Relative effect of site (black) and location (colours) as random variables in the INLA model explaining the number of beetles captured giving the mean as a dot and credibility interval as whiskers

The survival model shows that the chance to capture a first beetle (species detection) faster with a funnel trap is 92% but credibility interval is not discriminative (credibility interval for hazard rate includes 1, Table 3). To have 80% chance of capturing at least one beetle (20% type II error), the trapping effort is 7 vs. 13 days, for funnel and bottle trap respectively (95% credibility intervals are 3-19 and 5-33 days, respectively). When aiming to capture at least 5 individuals (trend monitoring), there is a 99% chance to be faster with a funnel trap compared to a bottle trap (discriminative, Table 3) and to reduce the type II error below 20%, the trapping effort is 27 vs. 63 days, for funnel and bottle trap respectively (95% credibility intervals are 13-67 and 28-182 days, respectively).

Table 3: Hazard rate (expressed as chance of success with funnel trap divided by chance of success with bottle trap) for the INLA survival models to capture at least one beetle or at least five beetles giving the mean, standard deviation (s.d.) and the 95% credibility interval (quantile 2.5 and 97.5%).

Hazard rate funnel/bottle

mean s.d. quantile 2.5% quantile 97.5%

1 beetle 1.962 0.867 0.806 4.151

5 beetles 2.573 1.099 1.210 5.425

**Discussion**

Our results show that a 16 unit funnel trap performs slightly better compared to a homemade bottle trap for capturing *E. ferrugineus*. This was significant for the number of beetles trapped but not for the time needed to capture one beetle (species detection). However, when aiming to capture at least 5 individuals (as a proxy for abundance estimate for trend monitoring), funnel traps performed significantly better. Effort needed for species detection was 7 and 13 days for respectively funnel and bottle trap. For trend monitoring the effort resulted in 27 and 63 days respectively. The activity peaked in the second half of July.

The finding that a large funnel trap performs better than a simple bottle trap might seem intuitive. However, contrasting effects had been found in some (but not all) studies on Bark and Ambrosia beetles (Scolytinae) comparing the same trap types (Steininger et al. 2015; Miller et al. 2018). As the funnel trap is not significantly better for species detection, we suggest to use bottle traps for large scale distribution surveys. Especially when a citizen science approach is used and shipping costs need to be taken into account, a bottle trap is preferred to a funnel trap. To monitor the abundance trend, funnel traps seem to be better as they reduce the effort to 43%. As a scheme for trend monitoring generally consists of a limited number of fixed locations, the overall shipping costs involved are less of an issue.

The effort needed to detect the species (13 days, 80% power) is much higher compared to efforts mentioned in previous papers. Andersson et al. (2014) detected the species on all studied sites after 6 to 9 days of monitoring. Kadej et al. (2015) even reached 60% accuracy after 1 day of monitoring. In contrast, Musa et al. (2013) mention the possibility that populations can be so small that a full flight season of monitoring is needed to detect them or that a population is not detected as adults are not produced every year. The differences between these studies are likely related to local differences in population size which can be expected to depend on habitat availability and connectivity and historic landscape continuity (see further).

In our experiment, the amount of beetles trapped in general did not differ among locations from a single site at distances of 100 m. Only for one location at one site, a lower number of beetles was trapped. This is in line with the finding of Harvey et al. (2017) who did not obtain positive records from any 10 km square where previous negative records had been obtained. Also Tolasch et al. (2007) already concluded that one or two traps would be sufficient to study the presence in a large area. Mapping the distribution of *E. ferrugineus* in Flanders (2018-2021) yielded 5 false negatives on 44 trapping events (11%) using pheromone baited bottle traps with at least 13 days of trapping which is in line with the 80% power.

No recaptures were found at the two sites were beetles were marked. Also Harvey et al. (2017) found little recaptures while Svensson et al. (2012) had a recapture rate of more than 60%, Zauli et al. (2014) of more than 50% and Andersson et al. (2014) 30%. Intermediate results were found by Svensson and Larsson (2008) and Larsson and Svensson (2009) (4%) and Musa et al. (2013) (16%). A first explanation is the trap effort, Svensson et al. (2012) used 12 and 16 traps per study site and Zauli et al. (2014) used 27 traps per site. However, Andersson et al. (2014) used only one trap per site similar to other studies (Musa et al. 2013; Harvey et al. 2017) but with contrasting recapture rates. Secondly, high recaptures relate to sites with high but local habitat availability which likely result in lower dispersal distances of males in search of females and consequently higher recapture rates. The habitat availability (hollow trees) in the Flemish study sites is more scattered throughout the landscape and consequently males need to disperse further in search of females. We therefore can conclude that in a regional monitoring set-up, where traps are placed at different sites rather than at a single site, a mark-recapture approach is only successful at sites with high and local habitat availability.

The high effort needed as well as this low recapture rate might reveal overall poor site conditions in Flanders compared to many of the previous studies. Previous studies were mainly done in wood pastures with many old hollow oaks (Larsson and Svensson 2011; Svensson et al. 2012; Musa et al. 2013; Andersson et al. 2014), a high-quality habitat that has disappeared in Flanders centuries ago (Tack et al. 1993; Verhulst 1995). Other studies include old growth forests and avenues with locally large numbers of veteran trees (Zauli et al. 2014; Oleksa et al. 2015; Rukavina et al. 2018). The main habitat in the study sites in Flanders are pollarded willows and old orchard trees that may be present in low densities throughout the landscape but are also rare in the rest of Flanders. Furthermore, these hollows are much smaller and form a less stable habitat due to the relatively young age of the trees and the higher decomposition rate, as compared to veteran oaks. Furthermore, these hollows are dependent on the maintenance of the pollarding management. These habitats also have a low historical continuity compared to habitat in wood pastures or old-growth forest of previous studies, due to intensive management in the past decades and even centuries (Tack et al. 1993; Verhulst 1995). Finally, scarabaeid larvae are often stated as the main food source (Schaffrath 2003; Svensson et al. 2004; Tolasch et al. 2007), but they seem to be scarce or lacking in Flanders. Most of the potential species have been listed as extinct (*Gnorimus variabilis* (L, 1758) and *Protaetia* marmorata (Fabricius, 1792)) or strongly threatened (*O. eremita* and *Gnorimus nobilis* (L, 1758)) in Flanders, with the exception of *Cetonia aurata* (L, 1758) which is recolonizing Flanders since 1990 (Thomaes et al. 2015b). *E. ferrugineus* has been found both in areas where *C. aurata* has been rediscovered as well as in areas where itwas still lacking. However other food sources might also be of importance. It is likely that the larvae prey on many other invertebrates and earthworms. Furthermore, Tolasch et al. (2007) mention that the larvae can also be reared on wood mould alone.

Our study demonstrates that in sites with low population sizes of *E. ferrugineus*, pheromone monitoring of this species is still possible. The species is a good indicator of the availability of hollow trees as well as the associated communities of hollow depending species and this along the entire gradient from optimal to quite poor habitat conditions (Musa et al. 2013; Andersson et al. 2014; Oleksa et al. 2015; Thomaes et al. 2015a). Furthermore, the monitoring results from this species reflect large scale landscape quality and connectivity up to 4.5 km (Musa et al. 2013; Andersson et al. 2014). Therefore, we believe that pheromone-baited monitoring is promising for the further conservation of hollow tree associated biodiversity. It could provide information on the potential for landscape restoration concerning biodiversity associated with hollow trees. It could also point out where conservation efforts are best allocated in order to restore and reconnect habitat fragments.

The use of commercially produced pheromones as in our study can further reduce the costs and ensure a steady supply needed for monitoring. Another advantage is that these pheromones are produced in rubber septa which are easier to use as they do not need any further handling by the volunteer compared to pheromones in microcentrifuge tubes used in all previous studies. This can simplify the protocol which is very important when including a citizen science approach.

Regarding the species’ phenology, our results are very similar to many other studies (Musa et al. 2013; Andersson et al. 2014; Zauli et al. 2014; Harvey et al. 2017). We found that the species was already active the 27th of June and peaked in the second half of July while the activity declined in the second half of August. There seems little difference in activity across Europe and monitoring protocols don’t need to be adjusted for that. We propose that trend monitoring should be conducted in July to ensure comparable phenology.

In the current analysis, we used a preliminary proxy of 5 specimens per site to allow certain abundance estimates. This will likely assure trend evaluation if about 30 sites are monitored. To make a better estimate of the effort needed for trend monitoring, a statistical power analyses would be needed. However, data from a preliminary monitoring is needed to perform this statistical power analyses (Lang et al. 2016; Thomaes et al. 2017) and such data is currently not available for Flanders. Starting up a monitoring of 30 sites will allow some first insights in the trend and allow a power analysis in the future.

We conclude that it is possible to develop a cost-efficient and accurate pheromone-baited monitoring protocol for *E. ferrugineus*. We propose to use the layout as represented in Table 4 for species distribution surveys and trend monitoring studies.

Table 4: Proposed layout for species distribution surveys and trend monitoring studies on *E. ferrugineus*.

|  |  |  |  |
| --- | --- | --- | --- |
| Survey type | Trap type | Effort (No. traps x days) | Set up |
| distribution | bottle | 4 x 3 | every 6j (cf. Harvey et al. 2017) |
| population monitoring | funnel | 1 x 27 (2 x 14 or 3 x 9) | 30 sites in 3y cycle (10 sites /year) |

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Consent for publication: All authors approved the manuscript for publication

**Authors’ contributions**

Arno Thomaes: Conceptualization, methodology, helped with formal analysis and investigation, writing - original draft preparation

Thierry Onkelinckx: Formal analysis and investigation and wrote part of the article

Toon Westra: Helped with formal analysis and investigation and wrote part of the article

Sam Van de Poel, Luc De Keersmaeker, Hannes Ledegen & Axel Neukermans: helped with the methodology

All authors reviewed and edited the manuscript

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