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High-accuracy sampling of saproxylic diversity indicators at regional scales with pheromones: the case of *Elater ferrugineus* (Coleoptera, Elateridae)

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¹ MCL, PM & KOB conceived and designed the study. KA & NJ collected most of the data. KA, PM & MCL analysed the data. FA, EH, JB, MCL & IW contributed reagents/materials/analysis tools. KA, PM & MCL wrote the paper with assistance from all other coauthors.

Abstract

The rare beetle *Elater ferrugineus* was sampled at 47 sites in the county of Östergötland, Sweden by means of pheromone-baited traps to assess its value as an indicator species for hollow oak stands rich in rare saproxylic beetle species. In addition, *Osmoderma eremita* was also sampled with pheromone baits. These data were then compared against species survey data collected at the same sites by pitfall and window traps. Both species co-occur with many Red Listed saproxylic beetles, with *E. ferrugineus* being a somewhat better indicator for the rarest species. The conservation value of a site (measured as Red List points or number of Red Listed species) increased with the number of specimens of *E. ferrugineus* and *O. eremita* caught. Accuracy of sampling by means of pheromone trapping turned out to be radically different for the two model species. *E. ferrugineus* traps put out during July obtained full accuracy after only 6 days, whereas *O. eremita* traps needed to be out from early July to mid-August in order to obtain full accuracy with one trap per site. By using *E. ferrugineus*, or preferably both species, as indicator species, accuracy would increase and costs decrease for saproxylic biodiversity sampling, monitoring and identification of hotspots.

Keywords: Beetles, conservation, indicators, monitoring , pheromone, saproxylic

Highlights

- Saproxylic environments are difficult and expensive to sample
- Traditional data on saproxylic species have low accuracy
- Using pheromones, rare species can now be sampled with high accuracy
- We show two examples of indicator species that can be sampled with pheromones
- This enables new conservation strategies for this high biodiversity environment

1 Introduction

Identifying, protecting and monitoring key areas or habitat types that support a high number of rare or threatened species is essential in conservation (Myers et al. 2000, Henle et al. 2013). However, complete surveys are expensive, time-consuming and often prevented by the lack of taxonomic experts. Therefore, indicator species are often used instead of complete surveys to locate sites with high species richness and/or conservation value (Simberloff 1998, Duelli & Obrist 2003, Fleishman & Murphy 2009). Many of the indicator species used are plants or invertebrates, the latter being used principally in marine and aquatic environments and recently also in terrestrial environments. The most notable terrestrial indicators are butterflies and wild bees, particularly for open environments like grasslands (Rosenberg et al. 1986, Sparrow et al. 1994, Nilsson et al. 1995, McGeoch 1998, Bazelet & Samways 2011, 2012, Bommarco et al. 2012, Gerlach et al. 2013). Despite the fact that conspicuous day flying species such as these are often sampled several times per year, there is still a substantial risk of species being under-reported in broad surveys (e.g. Wikström et al. 2009, Jonason et al. 2010, Quinto et al. 2013). Hence we risk generating expensive data of low accuracy and precision.

Saproxylic insects and other invertebrates constitute a significant overall proportion of threatened biodiversity and a major component of the biodiversity of old-growth forest habitats (Speight 1989, Grove 2002, Toivanen & Kotiaho 2007). As the dynamics of old-growth forest habitats are slow, conservation of old-growth forest biodiversity requires a multi-layered strategy. In the short term, this includes identification and preservation of key habitats that still harbor a diverse range of species, whereas long-term measures might focus on regeneration of future old-growth habitat in order to expand or integrate isolated habitat fragments into a larger landscape framework (e.g. Margules & Pressey 2000, Lindenmayer et al. 2006). In order to achieve both the short- and long-term aims, reliable indicators are needed. In this context, saproxylic fauna provide a significant challenge as they are notoriously elusive, difficult to sample, and comprise great taxonomic diversity, requiring an unusually high level of taxonomic expertise to identify completely (e.g. Horak & Pavlicek 2013). As yet, there seem to be only a few cases of useful indicator species, e.g. *Osmoderma eremita* (Ranius 2002, Jansson et al. 2009).

At present, there is a paucity of tools for the multiple parallel tasks required for effective conservation of the saproxylic fauna: urgent identification of the most valuable saproxylic biodiversity hotspots, and subsequent monitoring of the processes involved in long-term preservation efforts. In the last decade, trapping systems based on pheromones and other attractive semiochemicals (information chemicals) have offered a potential solution for efficient sampling and monitoring of insect biodiversity. Pheromone systems of a limited number of red-listed species have been studied specifically for conservation purposes (Larsson et al. 2003, Tolasch et al. 2007, Harvey et al. 2010, Millar et al. 2010) and semiochemicals have been employed to study insect distribution, population and dispersal dynamics, and effects of landscape processes (Gandhi et al. 2009, Larsson & Svensson 2009, 2011, Svensson et al. 2011, 2012, Musa et al. 2013). Pheromone-based trapping systems for insects have been used for decades in efficient monitoring of a wide range of pest species in both agriculture and forestry (Johnson et al. 2006, Witzgall et al. 2010). Hence, we might very well assume pheromone monitoring to be appropriate also for conservation purposes (Larsson et al.

2009), especially for saproxylic insects, which are dominated by beetle families known or expected to rely extensively on pheromone communication (Francke & Dettner 2005). Pheromone-based trapping has the potential to entirely reverse the present situation for sampling and monitoring of saproxylic biodiversity, by opening up the possibility of sampling insect groups previously disregarded as indicators because of costly and cumbersome sampling procedures. In this manner, the conservation value of sites and the status of cryptic and/or difficult-to-sample species of high conservation value, could be assessed and monitored much more efficiently.

Pheromone monitoring systems are generally species-specific, although cross-attraction exists, e.g., among saproxylic species (Hanks and Millar 2012). This selectivity would be advantageous for a focus on defined indicator species, but naturally limits the range of species that could be covered. Ultimately, the usefulness of pheromone-based indicator systems for saproxylic biodiversity would be determined by the aggregate information obtained from selected indicator species chosen to represent characteristic habitats and dynamic landscape processes. In the present study, we demonstrate how pheromone-trapping of two saproxylic beetle species, whose pheromone systems have recently been characterized, could provide extensive information about the conservation value of sites for the insect fauna associated with hollow trees.

The first pheromone identified for sampling of a rare and threatened insect species was the male-produced sex pheromone of *Osmoderma eremita* (Larsson et al. 2003), a beetle confined to hollow trees and an indicator of saproxylic biodiversity (Ranius 2002, Jansson et al. 2009). Pheromone-baited traps have been used in the field to estimate population sizes and dispersal dynamics of this rare species, showing that populations may be significantly larger than previously suggested based on unbaited pitfall traps (Larsson & Svensson 2009, 2011).

The rare Rusty red click beetle *Elatер ferrugineus* is commonly associated with *O. eremita* but known from far fewer sites in Sweden (Svensson et al. 2004). Nilsson & Baranowski (1994) found that many click beetle species, including *E. ferrugineus*, live exclusively at sites with long hollow-tree continuity, but also assumed that these beetles would not be useful as indicators since the chance of detecting them is low. However, recent studies have shown that *E. ferrugineus* can be monitored by traps baited with the *O. eremita* sex pheromone (Svensson et al. 2004), and even more efficiently with its own highly attractive, female-produced sex pheromone (Tolasch et al. 2007, Svensson et al. 2012), thus radically expanding its potential as an indicator species. Population studies with pheromone-baited traps have suggested that population sizes of *O. eremita* and *E. ferrugineus* may sometimes be comparable, but that the latter exhibits considerably higher population fluctuations (Larsson & Svensson 2009, 2011). *E. ferrugineus* also depends on hollow tree resources at larger spatial scales than *O. eremita* (Ranius et al. 2011, Bergman et al. 2012, Musa et al. 2013). Consequently, *E. ferrugineus* could be a very sensitive and cost-effective indicator of the biological effects of landscape fragmentation, especially given the novel potential for sampling with a highly attractive and species-specific pheromone.

The main aim of this study was to study the co-occurrence between *E. ferrugineus* and other saproxylic insects, and to evaluate the usefulness of pheromone-trapped *E. ferrugineus* as an indicator species for hollow tree stands with a rich saproxylic insect

fauna. The indicator potential of *E. ferrugineus* was studied by means of pheromone trapping at sites previously sampled for saproxylic insect diversity in 1994-2010 (see e.g. Jansson 2009, Jansson et al. 2009). Since many of the beetle species associated with hollow trees are assumed to have low dispersal rates, they are believed to depend on the continuity of hollow trees within a relatively close range (~2 km proposed by Nilsson & Baranowski 1994, Jansson 2006), and many are classified as threatened (Gärdenfors 2010).

The experiment examined to what extent pheromone trap catch of *E. ferrugineus* is a good predictor for the presence of individual rare and threatened species, as well as for sites rich in rare species. Species Red Listed by the IUCN in 2010 were used to test the hypothesis that *E. ferrugineus* is more abundant at sites with high richness of Red Listed saproxylic beetle species associated with hollow oak stands. The indicator potential of *E. ferrugineus* was compared with that of *O. eremita*, which has already been shown to have high indicator potential for saproxylic beetles when using conventional trapping or search methods (Ranius 2002, Jansson et al. 2009). Methods used in this project were matched against the data on *E. ferrugineus* and *O. eremita* sampled by Jansson (2009). In this way we have determined both the accuracy and usefulness of *E. ferrugineus* as an indicator species for hollow oak stands with high conservation value, and the efficiency of the two pheromones in detecting the species compared to other methods.

2 Material & methods

2.1 Background of trapping methodology and datasets used in this study

The study was conducted in the province of Östergötland, where sites with high density of old and/or hollow oaks were selected. These sites generally have high species richness (Nilsson et al. 1995, Økland et al. 1996) but have suffered from severe decline and fragmentation over the last 200 years in Sweden, mainly due to the change in ownership of the oaks and shifts in farming and forestry practices (Eliasson & Nilsson 2002). The 47 sites used were all pasture woodlands with varying canopy cover, and they were selected because they were known or believed to harbour a species-rich saproxylic fauna. Several of the sites are nature reserves. At the sites, *Quercus robur* was the dominant tree but at some sites there were also old or dead trees from other species. The sites are located in a broader landscape dominated by coniferous forests and arable fields. The number of large oaks per site varied (range from 0.1 to 6 large oaks ha⁻¹ when considering a circle with radius 327 m around the traps) and this has implications for the occurrence of individual species (Bergman et al. 2012, Musa et al. 2013).

2.1.1 Pitfall- and window-trapping

The saproxylic insect survey 1994-2010 was carried out with trapping inside individual trees with pitfall traps inside hollows and window traps (Ranius & Jansson 2002) hung near the trunk near a hollow (one of each in every individual oak sampled; Jansson 2009). The data used here are derived from the sum of four oaks per site, combining data from both trap types. This sampling intensity is likely to trap approximately 40% of the species present at a site (Jansson 2009). We considered only saproxylic beetle

species associated with hollow deciduous trees (hence ignored the many other insect species trapped).

2.1.2 Sites for pheromone trap placement

There was one trap per site in 2011, the target position being the centroid of the four trees sampled in 1994-2010 (the final location depended on availability of a suitable tree for suspending the trap). We decided on a minimum distance of 500 m between traps, in order to avoid potential conflicts between traps (the sex pheromone of *E. ferrugineus* potentially being effective at relatively large distances; Larsson et al. *unpubl.*). This led to the selection of 47 sites, unevenly distributed over the study area (10,000 km²).

2.2 Pheromone-targeted species

2.2.1 *Elater ferrugineus* Linnaeus, 1758 (Elateridae)

The red click beetle *Elater ferrugineus* (synonym: *Ludius ferrugineus*) is Sweden's second largest click beetle (imago size about 17-24 mm), and the largest click beetle to be found in hollow trees. It is found in deciduous hollow trees such as oak (*Quercus* spp.) and beech (*Fagus sylvatica*) (Palm 1954, 1959, Nilsson & Baranowski 1994). The life cycle of *E. ferrugineus* is between 4-6 years, probably depending on food availability (Palm 1954, 1959). Larvae of *E. ferrugineus* are generalist predators, but seem to prefer large prey species such as larvae of *O. eremita*, but given the chance, it will also be cannibalistic (Nilsson & Baranowski 1994, Palm 1954, 1959). Its position at a higher trophic level may cause it to be more sensitive to habitat fragmentation than many other saproxylic species that consume decaying wood (Holt et al. 1999, Davies et al. 2000, Holt 2002). *E. ferrugineus* was, until 1993, only known from a few scattered sites in Sweden, and is often thought to be an under-sampled species in surveys (Nilsson & Baranowski 1994, Jansson 2009).

2.2.2 *Osmoderma eremita* Scopoli, 1763 (Cetoniidae)

The hermit beetle *Osmoderma eremita* (imago size 25-35 mm) is a saproxylic beetle associated with hollow trees, most often oaks. In Sweden it has only been found in deciduous hollow trees, where the larvae feed on fungal mycelia and chew the trunk from the inside, thereby enlarging the hollow (Ranius & Nilsson 1997, Ranius 2002, Ranius et al. 2005). The normal life cycle of *O. eremita* in Sweden is about 3 years; imagos emerge in late June-early July and the season is usually over before the end of August (Ranius & Nilsson 1997). *O. eremita* is known from 270 sites in Sweden but has been recorded in only 130 of these after 1990, despite extensive surveys. It is therefore likely that the rate of extinction from sites is higher than the rate of colonization (Ranius et al. 2005). *Osmoderma eremita* is protected by law in many European countries and is classified as Near Threatened according to the Red List of 2010 in Sweden (Anonymous 1992, Gärdenfors 2010). Several studies have shown that the presence of *O. eremita* indicates hollow oak stands with high conservation value (Ranius 2002, Jansson et al. 2009).

2.3 Pheromone trapping

Pheromone trapping can be a highly species-specific method, depending on the pheromone used and species aimed for. The male-produced sex pheromone for *O. eremita* has been shown to attract mostly females of *O. eremita*, but to a lesser extent also conspecific males, as well as females of *E. ferrugineus*. The female-produced sex

pheromone for *E. ferrugineus* attracts only males but in much higher numbers than the sex pheromone of *O. eremita* (Larsson et al. 2003, Svensson et al. 2004, Svensson et al. 2012).

All field trapping in the present study was performed with cross-vein funnel traps (Svensson & Larsson 2008). Each trap was baited with both the sex pheromone for *E. ferrugineus* (7-methyloctyl (Z)-4-decenoate; Tolasch et al. 2007, Svensson et al. 2012) and *O. eremita* ((R)-(+)- γ -decalactone; Larsson et al. 2003). The sex pheromone of *E. ferrugineus* (>98% purity) was synthesized according to Tolasch et al. (2007) and Svensson et al. (2012). Lures for *E. ferrugineus* were made from 200 μ L PCR tubes loaded with 2 μ L aliquots of neat compound, and pierced with an insect pin size 3 just below the lid to release the pheromone (Tolasch et al. 2007). The sex pheromone of *O. eremita* was obtained as a racemic mixture from Sigma-Aldrich, as the racemate can be used instead of the pure (R)-enantiomer (Svensson & Larsson 2008). Lures for *O. eremita* were made from 4 mL glass vials loaded with 600 μ L of neat pheromone, with a cotton dental roll (Celluron, Paul Hartmann S.A., France) inserted as a wick. Only one trap per site (47 in total) was used for simultaneous capture of both species, with each trap containing two different baits; one for each of the two target species. The presence of two baits together has no negative influence on the capture of either species (Larsson, Burman unpublished data). Each trap was placed at approximately 2.5 m height from the ground on a branch facing north from the stem, in order to reduce direct sun exposure of the trap.

At a few sites, with known large populations of *E. ferrugineus*, some monitoring traps were put up in mid-June, to enable coordinated trap activation with the start of the flight period of the species. Traps were emptied every third day during the first three weeks, a frequency that guaranteed almost no mortality (except late in the season) nor any escapes among trapped specimens. This trapping interval was pursued for the rest of the season for traps that had caught beetles during the first three weeks. Traps that had not initially caught beetles were thereafter checked less frequently.

Beetles were marked with a queen bee marking pen (Uni paint marker PX-21, Mitsubishi Pencil co., LTD) on the elytra with a unique combination of marks for each individual. Beetles caught were released in the field after marking. The release procedure for *E. ferrugineus* was to take the beetles approximately ten meters away from the trap and throw them up into the air, forcing them into flight. In contrast, specimens of *O. eremita* were put on a tree at least 15 m from the trap (as they are less prone to fly than *E. ferrugineus*).

2.4 Trapping periods used in statistical analyses

It was of particular interest to assess for how long traps should be active to produce reliable results. Therefore, we used two trapping periods in the statistical analyses, each extending over 6 days, in the beginning of July (Period A; 5-12 July) and end of July (Period B; 23-30 July). Only new individuals caught within the respective interval were used (hence, re-captures within the same period were ignored). For the statistical analyses on these data, one site was excluded for Period A, and the whole season, since its trap was not activated until mid-July (because permission was given late by landowner).

2.5 Red List points for individual sites

Red List score 2010 was calculated according to a conservation priority index (CSPI; Jansson et. al. 2009), attributing a number to each site based on the Red List classification of its species. Each species recorded was given a score, and these were summed per site: *Not Listed* and *Least Concern* 0 p; *Near Threatened* 1 p; *Vulnerable* 3 p; *Endangered* 5 p.

2.6 Odds ratios and statistical analyses

Odds ratios (OR) are related to the probability of finding species x at a site with species y (*Elater* or *Osmoderma*).

$$OR=(a/b) / (c/d)$$

Where: a= x and y co-occur
b= x present but y absent
c= x absent but y present
d= both x and y absent

If OR is significant and >1 it means that species x is more likely to occur when species y is present. If OR approximates 1, it means that the distribution is random, and if OR <1 it means that species x is less likely to occur when species y is present. Odds ratios are commonly used in other disciplines, most notably medicine, but more rarely so in environmental sciences and ecology (Rita & Komonen 2008, Morris & Gardner 1988, Bland & Altman 2000), despite being a statistically sound way of analysing proportions.

Odds ratios were calculated in Comprehensive meta-analysis V2™ (Englewood, New Jersey, U.S.A.). This program calculates the exact confidence intervals and was used for all OR related calculations in this paper. It was also used to calculate the weighted average (random model) for species grouped according to the Red List categories; *Not Listed* (NL), *Least Concern* (LC), *Near Threatened* (NT) and *Vulnerable* and *Endangered* combined (VU+EN).

2.7 Generalized linear model analysis (GLZ)

We used GLZ to find the best models to explain (i) species richness, (ii) number of Red Listed species, (iii) CSPI, (iv) number of NT species and (v) number of EN/VU species. The best model was selected based on Akaike information criterion (AIC; Burnham & Anderson 2004). The explanatory variables were the catch results for *E. ferrugineus* and *O. eremita*. GLZ analysis was performed with normal distribution and log-link. The catch data had been log(x+1) transformed in order to lower the variance that affected the residuals. We used Statistica 10 for all GLZ analyses (StatSoft Inc. 2011).

2.8 Assessing accuracy

Overall accuracy, i.e. to what extent trapping results reflect the underlying pattern (Allouche et al. 2006), was calculated as:

$$Accuracy = (a+b) / N$$

Where: a=number of sites correctly detected as sites with species x present
b=number of sites correctly detected as sites with species x absent
N=total number of sites sampled

The main purpose of using accuracy was to assess the length of trapping needed to reach agreement with the whole-season dataset (no other data than the pheromone trapping performed were considered here).

Cumulative accuracy-graphs, using different hypothetical start dates for trapping based on a data set for the whole season, can show when, and for how long, a trap should be active in order to get high accuracy. Three-day intervals were used for traps that caught beetles, but on a few occasions the interval was up to five days, in which case trapped beetles (if any) were noted as captured on the last day. A few sites, that were presumed to lack both pheromone-trapped species, resulted in *O. eremita* captures after longer periods without being emptied (maximum 17 days).

3 Results

In total 985 catches of 693 individuals of *E. ferrugineus* and 99 catches of 95 individuals of *O. eremita* were recorded. The maximum number of catches for one site, i.e. one trap, was 143 catches of 91 individuals of *E. ferrugineus* at Runstorp (Table 1). There were more catches of *E. ferrugineus* in early July (period A: 246 individuals; 18/18 active traps catching), than in late July (period B: 138 individuals; 19/19 traps catching). For *O. eremita* corresponding catches were 17 individuals for period A (12/27 traps catching) and 22 for period B (14/28 traps catching).

The probability of re-capturing *E. ferrugineus* (29.6%) was higher than re-capturing *O. eremita* (4.0%; Table 1). Since the pheromone for *O. eremita* works as a kairomone and also attracts females of *E. ferrugineus*, beetles caught in some of the traps were sexed. In these traps, 3.8% of the individuals were female (7 out of 182).

Pheromone traps caught *E. ferrugineus* at 19 sites, while window/pitfall traps had indicated its presence at only five of these (Table 1). In contrast, pheromone traps caught *O. eremita* at 28 sites, and window/pitfall traps had indicated its presence on 26 of these (Table 1). In addition, window/pitfall traps had previously caught *O. eremita* at an additional five sites where no *O. eremita* was caught in the present study.

3.1 Temporal catch pattern

Catches of *E. ferrugineus* were skewed towards the early part of the field season, with the highest number of new individuals being captured during the first week (Figure 1). A few monitoring traps ensured that the true beginning of the flight season had not been missed. Catches remained relatively high during most of the field season, with most traps catching new individuals until the first week of August, after which only a few stray individuals appeared (Figure 1). In contrast, *O. eremita* catches exhibited no distinct peaks over the season, other than perhaps a brief initial series of captures and a subsequent skew in the catch towards the latter half of the season. The catch pattern of *O. eremita* suggested that the flight season of this species was somewhat longer than that of *E. ferrugineus*. A suggested dip in the catches of both species after the first 10 days coincided with a brief spell of unusually cold and rainy weather (Anonymous 2011).

3.2 Generalized Linear models (GLZ)

Correlation between the abundance of *E. ferrugineus* and *O. eremita*, used as predictors in the GLZ, was surprisingly low. The *r*-values for period A, B and for the whole season was -0.43 ($n=23$), -0.11 ($n=27$) and -0.095 ($n=33$), respectively. The indication of negative correlations, albeit not significant, is likely explained by the differences in peak flight activities by the two species.

In the GLZ analyses, the best model included abundance of *E. ferrugineus* alone in four cases (CSPI; VU+EN; total species number in Period B; VU+EN in Period A). In all other cases, the best model included both *E. ferrugineus* and *O. eremita*. (Table 2).

3.3 Odds ratios

The weighted average of odds ratios for *E. ferrugineus* ($OR_{E. ferrugineus} = 1.53$; $p < 0.0001$) and *O. eremita* ($OR_{O. eremita} = 1.74$; $p < 0.0001$) indicated that the odds of finding any random species was higher if either *E. ferrugineus* or *O. eremita* was present, compared when they were absent (Figure 2). When considering the classification according to the Red List of 2010, *E. ferrugineus* had a stronger connection to the most rare species (VU/EN) in this study (weighted average odds ratio of finding a VU/EN species at a site with *E. ferrugineus* compared to a site without *E. ferrugineus* was 5.48; $p < 0.0001$). The corresponding odds ratio for *O. eremita* was not significantly different from 1. The corresponding odds ratios for NT species were large for both *E. ferrugineus* and *O. eremita* ($OR_{E. fer.} = 2.16$; $OR_{O. ere.} = 2.68$; p in both cases < 0.0001). Beetles from the LC category also had OR significantly larger than 1 ($OR_{E. fer.} = 1.53$, $p = 0.047$ and $OR_{O. ere.} = 1.92$, $p < 0.0001$).

Thirteen species occurred significantly more often on sites with *E. ferrugineus* than without (Table 3), and one – *Anthrenus scrophulariae* (Dermestidae) – occurred less frequently when *E. ferrugineus* was present ($p = 0.022$). Of the 13 species, two are considered as near threatened (NT) and two as vulnerable (VU) according to the Red List (Gärdenfors 2010) (Table 3).

Nine species occurred significantly more often at sites with *O. eremita*, (Figure 2), and of these three are listed as near threatened (NT) according to the Red List of 2010 (Table 4).

3.4 Accuracy

The accuracy for catching *E. ferrugineus* and *O. eremita* varied over the season (Figure 3). Traps that were set out from the beginning of July until late July reached 100 % accuracy within 6-9 days for *E. ferrugineus*. In contrast, traps for detection of *O. eremita* needed to be out from the beginning of July until early August in order to reach 100 %, whereas traps that were set out after 14th of July never obtained 100 % accuracy for *O. eremita*.

4 Discussion

This study showed that pheromone-based monitoring of selected indicator species has great potential to alleviate some of the caveats that plague conservation of saproxylic biodiversity. Inexpensive surveys of selected indicator species could be used to pinpoint biodiversity hotspots quickly and efficiently over large geographic areas with

small effort. Furthermore, the same systems would greatly facilitate subsequent monitoring schemes to follow the future spatiotemporal dynamics of these species in relation to different landscape management regimes. Thus, they are tools that offer novel approaches to biodiversity monitoring with unprecedented accuracy, and allowing extensive geographic coverage. For this approach to be valid, it is essential that indicator species provide extensive information, and that their relationships to specific landscape processes related to extinction are well characterized. Our study demonstrates the validity of these assumptions for *E. ferrugineus* and *O. eremita*, and our model species serve as illustrative examples of what could be expected when generalizing from the present study to a broader context.

4.1 Reducing uncertainty regarding distribution

The primary advantage of pheromone-based monitoring systems is that they could, with a small sampling effort, virtually eliminate uncertainty about the true distribution of the target species caused by false zeros (e.g. Tyre et al. 2003, Martin et al. 2005, Dorazio et al. 2011). Our two model species occupy different ends of the scale for monitoring efficiency with long-range pheromones, where the monitoring system for *E. ferrugineus* is known to attract a much higher fraction of the population than that for *O. eremita* (Svensson et al. 2012). In our study *O. eremita* was less abundant in the traps than *E. ferrugineus*, and the necessary trapping effort was quite different between the two species. When using trapping methods more adapted to field surveys (shorter periods rather than the whole season), full accuracy was reached for *E. ferrugineus* within 6-9 days after activation, as long as the traps would be activated in July. For *O. eremita* the accuracy drastically decreased the later the activation date and could only reach 100 %, relative to the maximum number of detected sites in this study, if the traps were placed early in July and left out until mid-August. The recapture rates, which were considerably higher for *E. ferrugineus* than *O. eremita*, indicate that differences in trap catch reflect fundamental differences in sampling range and capture probability between the *O. eremita* pheromone trapping system and that of *E. ferrugineus* (Östrand & Anderbrant 2003). These differences could be due to several factors, including innate responsiveness to the respective pheromone, differences in attraction radius of the two types of pheromone traps, and that *O. eremita* appears to be more sedentary than *E. ferrugineus*, with only a minor fraction of the population leaving their natal trees (Ranius & Hedin 2001, Larsson & Svensson 2009, 2011).

The high levels of attraction of males to the female-produced sex pheromone of *E. ferrugineus* seem comparable to classical sex pheromones of moths and other insect groups (see also Tolasch et al. 2007). Its monitoring efficiency was extraordinary compared to other methods employed in the present study, with confirmed presence at 19 sites including all five sites previously identified by means of pitfall trapping and window traps. It is thus difficult to provide independent validation of the monitoring efficiency of the *E. ferrugineus* pheromone based on currently available data from a single season, other than noting that it appears very high. Judging from the general characteristics displayed by the pheromone system in this and previous studies, we expect that the detection probability of local populations would be near 100% when deployed during a full season, even at very low population density. Individual males have been recaptured multiple times for up to three weeks at the same site (Svensson et al. 2011), making it unlikely for even few males to completely evade capture for a whole season. When comparing *E. ferrugineus* pheromone data with records from

window/pitfall traps in earlier years, and additional information records, it was apparent that *E. ferrugineus* has been severely under-sampled in studies of saproxylic species, as previously proposed by Nilsson & Baranowski (1994) and Jansson (2009).

Although the efficiency of the *O. eremita* pheromone system for monitoring is probably near the low end of what could be achieved with long-range pheromones, the surveys by means of single pheromone-baited funnel traps nevertheless provided reasonably good detection of this species (see also Svensson et al. 2004). Comparisons with previous surveys of *O. eremita* (Jansson 2009, Jansson et al. 2009, Jansson *unpublished data*) demonstrated that a single pheromone trap was sufficient to indicate its presence at most localities. The overall agreement between pheromone traps and pitfall/window traps was relatively high, indicating that both systems have good detection rates and between them present a near complete picture of the true distribution of *O. eremita*. This implies that *O. eremita* is generally over-sampled relative to other species when using pitfall and window traps, where the pitfall traps placed inside the hollow trees usually have a high probability of catching *O. eremita*, likely due to its sedentary ecology (Ranius 2001, Ranius & Nilsson 1997; but see Chiari 2011, Chiari et al. 2013). In the present study, single pheromone traps were placed according to a fixed centroid position at each site, but the accuracy of the system could presumably be improved further, with negligible added effort, by placing multiple traps per site using and close to large hollow trees.

4.2 Potential for pheromone-based trapped indicator species

Extensive pheromone trapping more or less confirmed the previously known distribution for *O. eremita*, and for the first time provided accurate information about the true distribution of *E. ferrugineus*, over a large number of saproxylic biodiversity hotspots. This novel information could be compared with a large inventory of saproxylic insects, that is more or less unique in its systematic approach and geographic and taxonomic scope, to assess their value as indicators for conservation purposes. Our analysis demonstrated that both model species were good indicators of the conservation value of individual sites. *O. eremita* has previously been shown to be an indicator for species richness of saproxylic beetles (Ranius 2002, Jansson et al. 2009). The present study is the first to include both species, showing that *Elater ferrugineus* sampled by pheromone traps was a somewhat better indicator for sites with high conservation value (and considerably better considering the smaller trapping effort). *E. ferrugineus* was shown to be more abundant at sites where many other rare saproxylic beetle species were found, and statistical models showed that the rarest saproxylic beetle species (EN/VU) were more frequently observed at sites occupied by *E. ferrugineus*. Both the odds ratios and GLZ models indicated that the rarest categories of beetle species appear more frequently where *E. ferrugineus* was present, but they were not significantly more common where *O. eremita* was present. This implies that sites where *E. ferrugineus* is found contain more rare species than those with *O. eremita*. However, it is important to note that a high number of species occur more often with both *E. ferrugineus* and *O. eremita*, most easily seen in the odds ratios (Figure 2).

Our analysis not only demonstrated the potential for a limited set of indicator species to identify and prioritize among biodiversity hotspots, but the underlying patterns emerging from the analysis also showed that these indicators, and many other red-listed species from the saproxylic community, responded in a predictable hierarchical manner

to landscape processes affecting the extinction of threatened species. Other large-scale analyses have also demonstrated similar patterns (Ranius 2002, Jansson 2009, Hill et al. 2011), strongly suggesting that saproxylic species form functional clusters and extinction hierarchies based on their sensitivity to different habitat and landscape factors. In the present study, the occupancy patterns of the two beetles formed a perfectly nested hierarchy, where *E. ferrugineus* was in no place present where *O. eremita* was absent (when considering the combined window, pitfall and pheromone trap results). Occupancy relationships between the two species generally conform to a similar pattern, with both species found in similar habitats, but with *E. ferrugineus* occupying a more exclusive subset of habitat patches (Svensson et al. 2004, Tolasch et al. 2007, Ranius et al. 2011, Bergman et al. 2012, Musa et al. 2013, *unpublished data*). Note that there are also exceptions where *E. ferrugineus* is found without *O. eremita*, including Great Britain (where only *E. ferrugineus* is present). We suggest that the persistent hierarchical relationship between the two species reflects fundamental differences in how they are affected by landscape processes leading to extinctions. *O. eremita* is able to sustain viable populations for extended time periods in very small patches of suitable trees, and its present distribution thus primarily reflects historical patterns of continuity of hollow trees. In contrast, *E. ferrugineus* exhibits greater overall fluctuations in population density (Larsson & Svensson 2011) and appears to be more affected by diminishing habitat resources and fragmentation than many other saproxylic species (Ranius et al. 2010, 2011, Bergman et al. 2012, Musa et al. 2013). Its presence may thus be a sensitive indicator for sufficient habitat quality and connectivity required for the long-term persistence of many vulnerable species. *O. eremita* and *E. ferrugineus* thus seem to be excellently matched tools for understanding short-term and long-term conservation priorities of saproxylic species at the landscape level.

4.3 Increased accuracy when sampling saproxylic beetles

The example set by the present study could open up for the possibility of using limited assemblies of saproxylic indicator species, extensively validated based on knowledge about their biology and co-occurrence with other valuable species. At present, uncertainty regarding the true distribution of individual species, and regarding our ability to monitor them efficiently, makes the establishment and implementation of such indicator sets impractical. With few exceptions, obtaining sufficiently informative data about individual saproxylic species, beyond educated guesses, would require additional large systematic sampling and monitoring efforts. This is exemplified in the present study by *E. ferrugineus*, which was not detected by pitfall and window traps at most of the sites subsequently identified by pheromone monitoring (although other methods such as wood mould sampling might have proven somewhat more efficient in this case; see Ranius & Jansson 2002). Furthermore, an indicator could not be used as an efficient proxy for large-scale surveys of saproxylic species unless it could be monitored with greater efficiency than is possible for most species today. Developing efficient pheromone-based monitoring systems for potential indicators would thus aid in both their validation and subsequent implementation.

In order to be able to rank individual sites according to their value for conservation (cf. Jansson et al. 2009) using the results from pheromone trapping, more indicator species would be desirable. Many potential indicators are already highlighted by statistical investigations and informed suggestions by experienced entomologists. Although pheromones for further potential indicator species are currently lacking, many of these

species likely use sex or aggregation pheromones; the list could therefore be extended further. By using multiple traps baited with different pheromones, it would be possible to provide more fine-grained ranking of the sites according to the presence of more species besides *E. ferrugineus* and *O. eremita*, which are specifically associated with the most valuable sites represented by hollow deciduous trees. Many additional species could provide information about other types of saproxylic habitats at different successional stages. It should be obvious that a certain indicator species is only useful within its geographical distribution. However, it is also known that an indicator identified within one geographic area will become less efficient as one moves away, simply because of shifts in species richness and composition (Jansson et al. 2009). For example, in Sweden there is an increase in the richness of saproxylic assemblages when moving from west to east. It is therefore important to have the geographical constraints of a specific indicator species in mind. Routine use of pheromone-baited traps in conservation would considerably increase accuracy while simultaneously reducing the costs of surveying large areas in order to identify objects of interest for conservation measures. If the time assigned to field work is limited, this method would constitute a very good tool for decision makers, especially since only one trap per site in one week may be enough to obtain satisfying results, as shown in the present case.

4.4 Conclusions

This study shows that both *Elater ferrugineus* and *Osmoderma eremita*, when using pheromone baited trapping, are highly useful as indicator species for rare species associated with deciduous hollow tree areas in Sweden. Many of the rarest species were present more often together with either *E. ferrugineus* or *O. eremita*, with the former having a more tight connection with the most vulnerable species according to the Red List of 2010 (Gärdenfors 2010). *E. ferrugineus* could also generate reliable data with fewer traps, being active for shorter time periods, than *O. eremita*.

We believe that pheromone-baited monitoring traps bring much promise for conservation, both for surveying and monitoring targeted species, and by using pheromone-trapped species as indicators for other aspects of biodiversity. It also opens up the possibility of sampling on larger spatial scales with high temporal resolution, which might assist in a more process oriented study of rare species. Apart from yielding highly accurate data, the costs are much lower than with conventional methods. Also, conservation planning might gain momentum by better knowledge of cryptic parts of biodiversity. Furthermore, the feasibility of trapping and marking large number of individuals opens up an opportunity for autecological studies of rare species, by estimates of population size, dispersal distances and other relevant parameters.

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Table legends

Table 1. Presence (P) of *Elater ferrugineus* and *Osmoderma eremita* on site sampled by window & pitfall (WP) traps, and by pheromone (Ph) traps (individuals caught/number of catches). “-“ denotes not detected.

	<i>Elater ferrugineus</i>		<i>Osmoderma eremita</i>	
	WP	Ph	WP	Ph
Adelsnäs	-	15/25	P	-
Bjärka äng	P	52/59	P	5/5
Borensberg	-	-	-	-
Borg	-	-	P	4/4
Brokind	P	51/61	P	2/2
Bråborg	-	-	P	5/5
Bäck	-	-	-	1/2
Börsjö	-	-	-	-
Disevid	-	-	-	-
Djursö	P	8/9	P	4/4
Ekön	-	-	P	9/9
Engelholm	-	18/19	P	7/7
Fornhagen	-	94/141	P	6/6
Garnison	-	-	P	2/2
Gunnarsbo	-	16/23	P	-
Hamra	-	-	P	1/1
Harsbo	-	-	P	3/3
Hjorthänget	-	34/42	P	1/1
Hägerstad	-	-	P	4/4
Händelö	-	-	P	1/1
Labbenäs	-	12/13	P	1/1
Lagnebrunna	-	-	-	-
Långvassudde ^a	-	5/6	P	1/1
Malmölan	-	-	P	1/1
Mauritzberg	-	-	-	-
Norrby sjön	-	-	-	-
Norsholm	-	19/23	P	-
Orräng	-	24/44	P	-
Prästbola	-	-	-	-
Ravnäs	-	-	P	5/5
Ruda	-	-	-	-
Runstorp	-	91/143	P	1/2
Räckeskog	-	40/62	P	3/3
Skaggebo	-	43/62	-	1/1
Skärblacka	-	20/40	P	1/1
Solberga	-	-	P	2/2
St.Löpgöl	-	-	-	-
Stegeborg	P	26/29	P	13/15
Sund	-	-	P	2/2
Sundsbro	P	62/86	P	8/8
Sundsnäs	-	-	-	-

Svaltorp	-	-	-	-
Såtra-Humpen	-	66/98	P	-
Tisenhult	-	-	-	-
Tolefors	-	-	-	-
V Eneby	-	-	P	1/1
Östantor	-	-	-	-

^atrapping started 13 days after the other sites.

Table 2. GLZ (normal distribution log-link) results, continuous predictors in columns. Period A and B refers to early vs late July. Model 1: *E. ferrugineus* abundance only; model 2: *O. eremita* abundance only (tested but never selected as the best of the 3 models); model 3: both *E. ferrugineus* abundance and *O. eremita* abundance in model. CSPI (conservation priority index) was calculated based on the Red List classification of species present at a site.

Time period considered	Type of model selected	Mode 1						
		p-value	Intercpt	±CI _{95%}	Elat er	±CI _{95%}	Osmode rma	±CI _{95%}
NUMBER OF SPECIES RECORDED AT A SITE								
Period A	3	0.047	3.69	0.08	5	0.10	0.28	0.34
Period B	1	0.017	3.69	0.08	0.15	0.14		
Whole season	3	0.002	3.64	0.09	9	0.08	0.22	0.17
NUMBER OF RED-LISTED SPECIES RECORDED AT A SITE								
Period A	3	0.000	4	1.81	0.18	0.27	0.19	0.83
Period B	3	0.000	48	1.82	0.17	0.39	0.24	0.52
Whole season	3	<0.00	01	1.67	0.18	0.20	0.12	0.26
NUMBER OF SPECIES CLASSIFIED AS NEAR-THREATENED AT A SITE								
Period A	3	0.001	5	1.74	0.16	0.21	0.18	0.54
Period B	3	0.002	7	1.74	0.16	0.29	0.23	0.50
Whole season	3	<0.00	01	1.62	0.17	0.14	0.12	0.26
NUMBER OF SPECIES CLASSIFIED AS VULNERABLE OR ENDANGERED AT A SITE								
Period A	1	0.002	8	-0.69	0.58	0.65	0.51	
Period B	1	0.000	39	-0.71	0.62	1.03	0.65	
Whole season	3	<0.00	01	-0.99	0.66	0.64	0.40	1.24
CSPI OF A SITE								
Period A	3	0.000	64	1.96	0.23	0.34	0.23	0.69

Period B	1	0.000 18	1.98	0.22	0.51	0.29		
Whole season	3	<0.00 01	1.8	0.23	0.28	0.16	0.61	0.32

Table 3. Odds ratio (OR) for species that had significantly higher probability of being

Species	OR	CI _{95%}	p-value	Red list category
<i>Globicornis rufitarsis</i>	19.6	4.24-90.15	0.00014	NT
<i>Gastrallus immarginatus</i>	13.3	3.03-58.62	0.00061	LC
<i>Cryptophagus micaceus</i>	39.0	2.15-708.8	0.0013	LC
<i>Tenebrio opacus</i>	24.3	2.72-217.1	0.0043	VU
<i>Osmoderma eremita</i>	20.8	2.43-177.6	0.0056	NT
<i>Trox scaber</i>	7.4	1.43-38.08	0.017	-
<i>Orchesia micans</i>	7.6	1.37-42.09	0.021	-
<i>Mycetochara linearis</i>	4.3	1.21-15.44	0.024	-
<i>Hapalaraea pymaea</i>	27.4	1.44-523.5	0.028	LC
<i>Hedobia imperialis</i>	4.1	1.1-15.52	0.035	-
<i>Conopalpus testaceus</i>	4.4	1.08-17.63	0.039	LC
<i>Corticeus fasciatus</i>	21.6	1.12-418.6	0.042	VU
<i>Elater ferrugineus</i> ^a	21.6	1.12-418.6	0.042	VU

found at a site with *E. ferrugineus* present.

^a OR calculated based on window & pitfall results vs pheromone results.

Table 4. Odds ratio (OR) for species that had a significantly higher probability of being found at a site with *O. eremita* present.

Species	OR	CI _{95%}	p-value	Red list category
<i>Osmoderma eremita</i> ^b	36.4	6.24-212.4	<0.0001	NT
<i>Scraptia fuscata</i>	63.0	3.36-1180	0.0056	LC
<i>Procræus tibialis</i>	6.3	1.68-23.88	0.0065	NT
<i>Cryptophagus micaceus</i>	6.7	1.66-26.76	0.0075	LC
<i>Mycetophagus piceus</i>	7.6	1.37-42.09	0.021	LC
<i>Globicornis rufitarsis</i>	4.3	1.21-15.44	0.024	NT
<i>Protaetia marmorata</i>	4.3	1.21-15.44	0.024	LC
<i>Gastrallus immarginatus</i>	3.9	1.13-13.45	0.031	LC
<i>Calambus bipustulatus</i>	4.6	1.10-19.50	0.037	NT

^b OR calculated based on window & pitfall results vs pheromone results.

Figure legends

Figure 1. The temporal pattern of catches of new individuals per trap (dots, right y-axis) of (A) *E. ferrugineus* and (B) *O. eremita*. Solid lines denote average catches per day (left y-axis), dashed line is the mean daily temperature at Malmslätt meteorological station (obtained from SMHI.se) (right y-axis, in the lower graph (B) the temperature is divided by 10). Note the differences in catch number and lack of distinct peaks for *O. eremita*. Note also that the individual points showing the highest catches in the two species all come from different sites in each species, suggesting that individual sites may exhibit peak densities at somewhat different times of the field season.

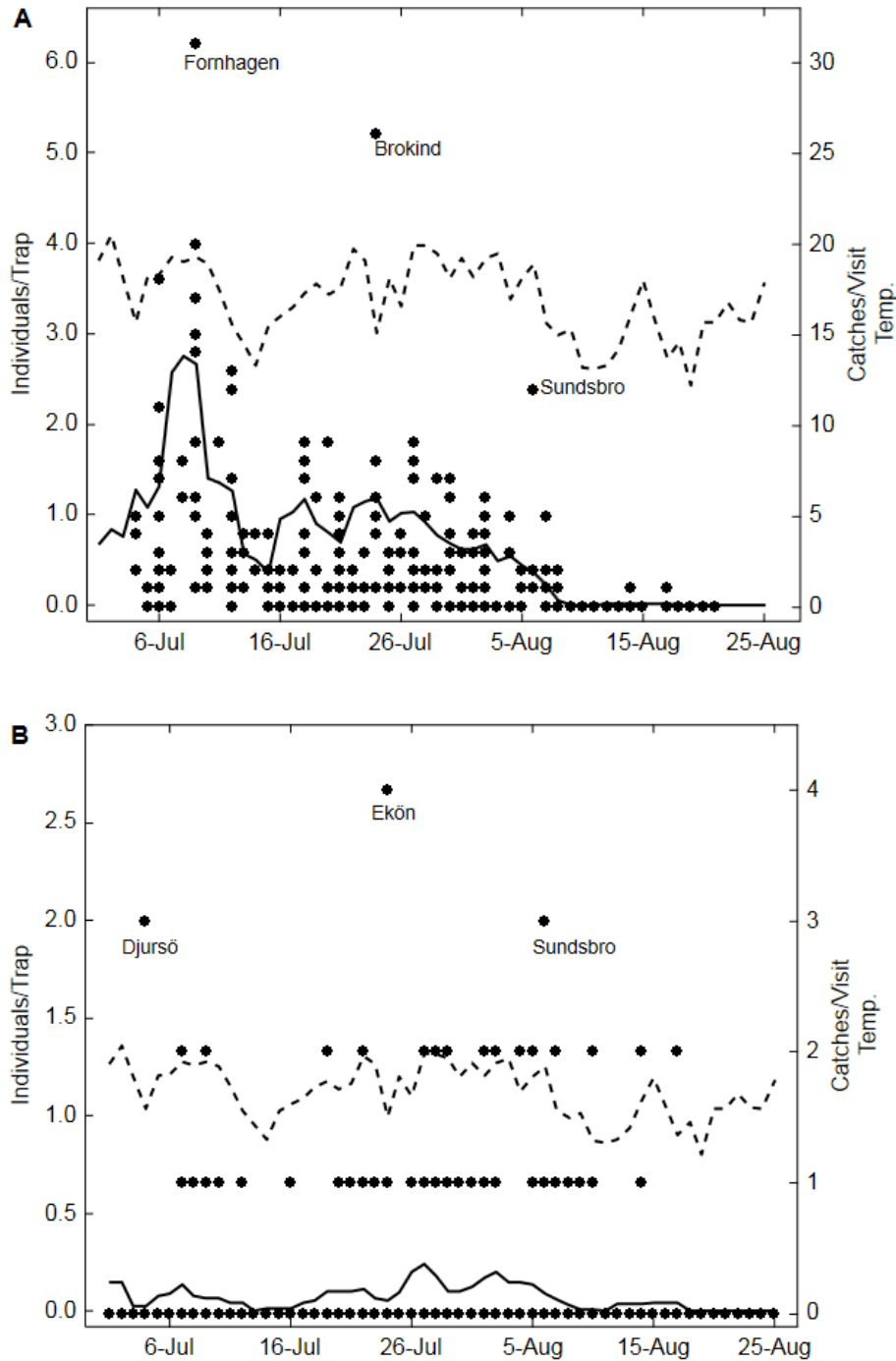


Figure 2. Graphs of log odds ratio on presence/absence data of *E. ferrugineus* (left) and *O. eremita* (right) from pheromone traps vs. window & pitfall traps. Circles denote the odds ratio; filled circles indicate significant odds ratio, and bars 95% confidence intervals. 13 species occurred significantly (5% level) more often when *E. ferrugineus* was present and one significantly more often when *E. ferrugineus* was absent. Eight species occurred more often when *O. eremita* was present.

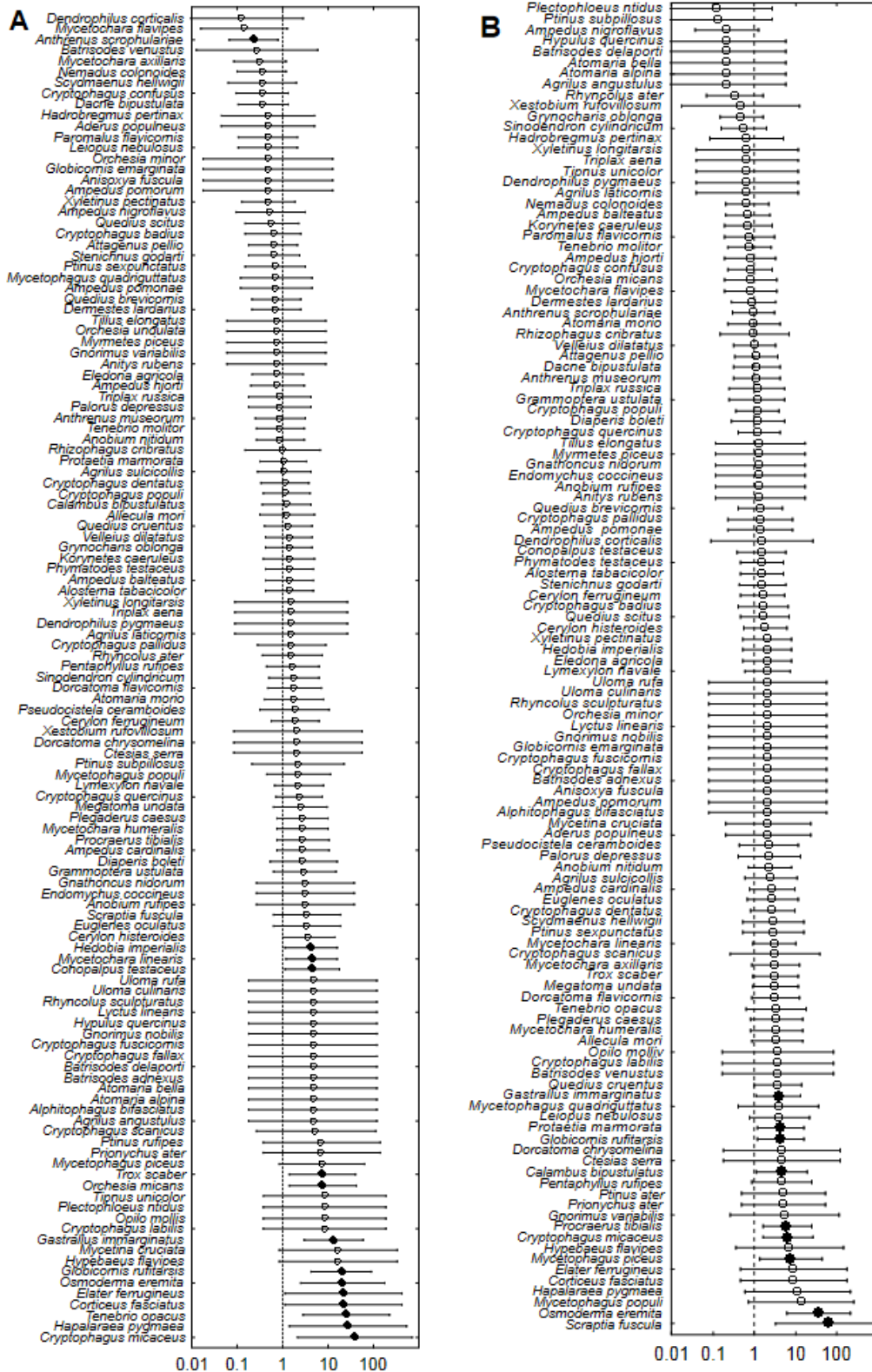


Figure 3. Accuracy graphs showing the overall agreement with the complete dataset of 2011: total number of sites with presence of *E. ferrugineus* (A) and *O. eremita* (B), based on pheromone trapping at different times during the field season. Bold line denotes how the accuracy at each single visit (corresponding to three days of trapping) changed over the field season. The dotted lines around the bold line denote 95% CI. Thinner lines emerging from the bold line show the cumulative change in accuracy over time if trapping would have been started at that date and continued until end of the season. For example: if the trap is up on the 26th of July it will take only two visits before *E. ferrugineus* has reached an accuracy of 100%, whereas the accuracy for *O. eremita* will increase from 55% to about 80% accuracy over the next five visits and thereafter level out, as no catches were noted at new sites.

