A pheromone-based toolbox of longhorn beetles (Cerambycidae) for monitoring biodiversity in ephemeral deadwood substrates of oak

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Cover: A flight-intercept trap with attached pheromone lures, two species of longhorn beetles *Plagionotus detritus* and *Pyrrhidium sanguineum*, and a recently collapsed oak with large quantities of ephemeral fresh, wood substrates, suitable to the larvae of the various species that were studied in this work.
(photos: M. Molander)

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Abstract

Forest biodiversity is exhibiting a worldwide decline in response to environmental changes that result in the rapid loss, degradation, and fragmentation of essential forest habitats. Saproxylic insects, especially beetles, are an important part of forest biodiversity by contributing to deadwood decomposition, and serving as important components of food webs. Many saproxylic beetles display negative population trends, and are listed on national and European Red Lists of threatened species. Despite their great importance, present knowledge on the ecology and conservation requirements of these beetles is limited, in part due to the absence of efficient tools to sample populations of many species. Recently, pheromone-based methods have been proposed as a novel tool to study saproxylic insects. Unfortunately, thus far, pheromones have only been identified for a small number of species of interest to conservation.

In this work I identified the aggregation-sex pheromones of longhorn beetles dependent on fresh, recently dead, wood substrates of oak in Sweden, and examined the usefulness of the pheromone-based trapping approach for detecting local populations, and studying the species' ecology. The pheromone-chemistry of eight species was considered, with a total of seven identified pheromone compounds (hydroxyketones, alcohols, and one ketone). The pheromones were used for systematic, large-scale monitoring studies in southern Sweden. The results served to significantly change the perception of several species' distribution and abundance. Further, local beetle abundance (trap captures), was best correlated with habitat at relatively large spatial scales, indicating that future detailed analyses of the species' ecology need to consider large spatial scales. Effects of oak forest management and habitat structure were examined in a three-year monitoring study. Generally, beetle abundance did not differ between ordinary oak production stands and two types of set-aside habitats for biodiversity. Most species also preferred more open, sun-exposed oak habitats. In addition, the beetles displayed short-term positive responses to logging in oak production stands, when fresh oak substrates were retained on site. The work clearly demonstrated the advantages of using pheromones to study these species and also offered early insights into the complicated, and highly dynamic, ecology of the species.

Keywords: Saproxylic species, Red List, indicators, GC-MS, hydrocarbon, forest management, forest biofuel, spatial scale, nature management, conservation.

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Feromonbaserad övervakning av långhorningsskalbaggar (Cerambycidae) som representanter för biodiversitet i kortvariga dödvedssubstrat av ek

Abstract

Den globala skogsbiodiversiteten minskar tillföljd av miljöförändringar i skogen som resulterar i förlust, försämring och fragmentering av nödvändiga livsmiljöer. Vedlevande (saproxyliska) insekter, i synnerhet skalbaggar, är en viktig del av skogens biodiversitet där de fungerar som nedbrytare av död ved och födokälla för andra arter. Många vedlevande skalbaggar uppvisar negativa populationstrender och finns upptagna på nationella och europeiska rödlistor över hotade arter. Trots deras stora betydelse är kunskapen om saproxyliska skalbaggars ekologi och bevarandebiologi liten, delvis som ett resultat av att det saknas effektiva redskap för att kunna studera många sällsynta arter som lever undanskymt, eller på annat sätt är svåra att kvantifiera. Feromonbaserad övervakning har nyligen föreslagits som en ny metod för att kunna studera saproxyliska arter som tidigare varit svåra att undersöka, men feromoner har hittills endast identifierats för ett litet antal arter av betydelse för bevaradebiologiskt arbete.

I detta arbete identifierade jag sexual-aggregationsferomoner för långhorningsskalbaggar som lever i tunna, nyligen döda vedsubstrat av ek i Sverige, samt undersökte vilken potential feromonerna har för att användas som ett verktyg för att studera arternas förekomst och ekologi. Åtta arter studerades vilket resulterade i identifieringen av totalt sju feromonämnen (hydroxyketoner, alkoholer och en keton). Feromonerna användes för storskaliga, systematiska studier av arternas utredning i sydligaste Sverige. Resultaten var förvånande då flera arters utbredning och abundans visade sig vara större än tidigare känt. De lokala fångsterna användes för att undersöka arternas relation till sitt habitat över olika spatiala skalor. Jämförelserna indikerade att arterna samspelar med sitt habitat över stora skalor och framtida mer detaljerade analyser kommer sannolikt att behöva kvantifiera miljöfaktorer över stora områden. Effekter av skogliga skötselåtgärder i ekskog och ekhabitatstyp undersöktes i en treårig studie. Arternas abundans skiljde sig generellt inte mellan produktionsskog och två typer av avsatta områden för biodiversitet. Flertalet arter föredrog också soliga miljöer och svarade positivt på gallring i ekskog när färska vedsubstrat sparades på plats. Arbetet visade på feromonernas höga potential att användas som verktyg för att studera dessa arter och gav värdefulla inblickar i deras komplicerade ekologi.

Keywords: Saproxylisk art, rödlista, indikatorart, GC-MS, kolväte, biobränsle, skogsskötsel, spatial skala, naturskötsel, bevarandebiologi.

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Dedication

To my father, who raised me, and showed me the bee hives.

‘‘Gör vad ditt hjärta lyster.
Till det du har lust, har du lycka.’’

Carolus Linnæus

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Molander MA***, Winde IB, Burman J, Nyabuga NF, Lindblom TUT, Hanks LM, Millar JG, Larsson MC. Common cerambycid pheromone components as attractants for longhorn beetles (Cerambycidae) breeding in ephemeral oak substrates in Northern Europe. *Journal of Chemical Ecology*, accepted, minor revisions.

II **Molander MA***, Larsson MC (2018). Identification of the aggregation-sex pheromone of the cerambycid beetle *Phymatodes pusillus* ssp. *pusillus* and evidence of a synergistic effect from a heterospecific pheromone component. *Journal of Chemical Ecology*, 44 (11), pp. 987-998.

III **Molander MA***, Helgesson J, Winde IB, Millar JG, Larsson MC (2019) The male-produced aggregation-sex pheromone of the cerambycid beetle *Plagionotus detritus* ssp. *detritus*. *Journal of Chemical Ecology*, 45 (1), pp. 28-36.

IV **Molander MA***, Eriksson B, Winde IB, Zou Y, Millar JG, Larsson MC (2019) The aggregation-sex pheromones of the cerambycid beetles *Anaglyptus mysticus* and *Xylotrechus antilope* ssp. *antilope*: New model species for insect conservation through pheromone-based monitoring. *Chemoecology*, (in press).

V Imrei Z*, **Molander MA**, Winde IB, Lohonyai Z, Csonka ÉB, Fail J, Hanks LM, Zou Y, Millar JG, Tóth M, Larsson MC (2019) Identification of the aggregation-sex pheromone of *Plagionotus arcuatus* ssp. *arcuatus* (Coleoptera: Cerambycidae) from two geographically separated European populations. *The Science of Nature,* (in press).

VI **Molander MA***, Backström L, Ponsonby C, Brunet J, Millar JG, Larsson MC. Interspecies associations and response to habitat at different spatial scales in four longhorn beetles breeding in ephemeral oak wood substrates. Manuscript.

VII **Molander MA***, Eriksson B, Vestlund M, Zou Y, Millar JG, Larsson MC. Pheromone-based monitoring in oak forests reveal no effect of set-aside conservation sites, but short-term effects of logging on habitat use by saproxylic longhorn beetles (Cerambycidae) with fast population dynamics. Manuscript.

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The contribution of Mikael Molander to the papers included in this thesis was as follows:

I Developed and performed the field work in 2017 with MCL, performed part of the laboratory work and analyzed the field data, wrote the manuscript with MCL, and the assistance of the other co-authors.

II Planned the work with MCL, performed the laboratory and field work, analyzed the field and laboratory data. Wrote the manuscript with the assistance of MCL.

III Participated in developing the work with IBW and MCL, performed part of the field work, analyzed the field data, and wrote the manuscript with the assistance of JGM and MCL.

IV Planned the work with IBW and MCL, performed the main part of the laboratory and field work. Compiled the field data and analyzed the data with BE. Wrote the manuscript with the assistance of all co-authors.

V Planned the work with IBW and MCL, performed the main part of the laboratory and field work (in Sweden) and compiled the data. Wrote the paper with ZI, JGM and LMH.

VI Developed the questions and design with LB and MCL. Assisted during the field and laboratory work, compiled and analyzed the data. Wrote the manuscript with assistance of the co-authors.

VII Developed the questions and design with MCL, planned and performed or supervised the field and laboratory work. Assisted BE in compiling and analyzing the data. Wrote the manuscript with assistance of the co-authors.

Abbreviations

EAD	Electroantennographic detection
FID	Flame ionization detector
GC	Gas chromatography
GIS	Geographic information system
MS	Mass spectrometry
WKH	Woodland key habitat

1 Introduction

1.1 Background

Forest ecosystems worldwide are under steadily growing pressure to deliver increasing amounts of resources to human societies. For millennia, forests have delivered crucial ecosystem services, but this is now under severe threat due to the widespread loss of biodiversity in forests. The current situation is due to the extensive changes which human use has inflicted on forest ecosystems and wooded pastures during particularly the 19th and 20th century (e.g. Bengtsson et al. 2000; Paillet 2010; Halme 2013). These changes have resulted in widespread loss, redisposition and fragmentation of essential forest habitats and resources for biodiversity (Bouget et al. 2012). Many forest species are highly specialized, with restricted capacity to adapt to quick changes, which also means that they are more susceptible to be adversely affected by environmental changes (Groove 2002). Thus, increasing conservation efforts and more basic knowledge on how to preserve biodiversity is urgently needed.

Forests dominate the land cover in Sweden and forestry has been a key industrial sector for centuries, which today is highly efficient. Only small remnants of undisturbed, relatively natural forest (Figure 1) remain in the country (Dahlberg and Stokland 2004). Early conservations efforts in Sweden primarily aimed to protect forest habitats and their affiliated biodiversity by setting aside smaller areas to serve as protected reservoirs for biodiversity (e.g. nature reserves). However, during later decades of the 20th century and early 21st century, it has become increasingly clear that active efforts to conserve forest biodiversity are also needed outside of protected areas, within production forests (Angelstam et al. 2003). This has given rise to for instance the implementation of PEFC™ and FSC® forest certification systems, which require that production forests are managed with a higher degree of attention to biodiversity. Despite the overall increasing conservation efforts, national trends for forest biodiversity continue to be negative in Sweden (Larsson 2011; ArtDatabanken 2015; Andersson et al. 2019). Part of the problem is that the environmental requirements of many groups of organisms are unknown, or poorly understood. Optimal, cost-efficient, integration of goals for production of forest resources and preservation of biodiversity is not possible without accurate knowledge on what ecological characteristics and nature management that are necessary, and most beneficial, for different groups of taxa. One issue explaining why the requirements of biodiversity are poorly known is that the sheer number of organisms living in forests is exceedingly high, and that studying many of them is time-consuming and difficult for practical reasons.

Saproxylic insects, particularly beetles, are one of the most species rich groups of forest taxa, and have received significant attention from biologists and conservation researchers. However, many of these species are elusive and difficult to sample quantitatively, or even come into contact with. Recently, pheromones (attractive odors) have been proposed as a tool to study such saproxylic insects (Larsson et al. 2009). Initial studies have shown promising results, but few insect species of interest to conservation have identified pheromones (Larsson 2016).

Figure 1. Undisturbed, comparatively natural, deciduous forests are rich in biodiversity, but have almost disappeared in Sweden. Current forests are designed to maximise production of forest resources and differ from natural forests in a number of ways, perhaps most strikingly in terms of the amount of dead wood present, which is considerably lower in production forests. Hornsö Ecopark 2015 (photo. M. Molander).

1.2 Deadwood substrates and biodiversity

Dead wood and old trees are key environmental features for forest and woodland biodiversity due to the many different ecological niches that occur on these biological structures (Lassauce 2011; Ulyshen 2018). The occurrence of these structures differs between natural forests and modern production forests in a number of ways. Modern production forests have far lower volumes of dead wood than natural forests (Siitonen 2001; Stokland et al. 2012), denser tree coverage, and overall younger trees of similar age that lack most of the micro habitats which are commonly associated with old trees (De Jong and Almstedt 2005; Hjältén et al. 2012). Particularly, the lack coarse dead wood in production forests has long been highlighted as a problem (De Jong and Almstedt 2005), but is problematic to resolve as coarse wood is also the most economically important resource for forestry. However, lately, the harvesting of other wood substrates, primarily twigs and thin branches (Figure 2) for production of forest biofuel has also emerged as a new potential threat to saproxylic biodiversity (Jonsell 2008; Hedin et. al. 2008; Hiron et al. 2017). These substrates were previously left in the forest after thinning or logging, providing significant amounts of substrates for many species.

Various different factors affect how, and which saproxylic taxa, that utilize a particular type of dead wood. Species of tree, moisture content, position of the wood (standing or lying down), stage of decay, and substrate type (roots, twigs, or trunks etc.), are some examples (Dahlgren and Stokland 2004). Different types of wood substrates also exhibit different spatiotemporal dynamics, which has driven the evolution of different life-history strategies among the species that utilize them. Certain deadwood substrates such as coarse, decaying logs and tree hollows can take hundreds of years to form, but are comparatively stable once formed and can function as suitable substrates for many decades (Ranius et al. 2009). Other types of substrates such as thin, recently dead twigs and branches can be generated rather quickly, but typically only function as suitable substrates for short periods of time (a few years). Species associated with substrates that are suitable for long periods of time often exhibit slow spatiotemporal population dynamics, and respond slowly to environmental changes, while species that utilize substrates with fast dynamics are forced to also display rapid population dynamics.

Saproxylic beetles are species of Coleoptera which at some point during their lifecycle are dependent directly, or indirectly, on living or dead wood and its derivatives (Speight 1989). Derivatives are substrates that have formed from trees and dead wood, such as fruit bodies of bracket fungi, sap runs, and tree hollows. By this definition, about 1 200 beetle species in Sweden are to be regarded as saproxylic species (Dahlgren and Stokland 2004; Sörensson 2012).

Beetles account for about one third of all saproxylic insects in Sweden (Sörensson 2012). Some of the most well-known groups with predominantly saproxylic species are the jewel beetles (Buprestidae), click beetles (Elateridae), false darkling beetles (Melandryidae) bark beetles (Curculionidae: Scolytinae), and the longhorn beetles (Cerambycidae). Saproxylic beetles play an important role in forest ecosystems due to their function as decomposers of wood (Grove 2002; Buse et al. 2008; Ulyshen 2018; García-Lópes et al. 2016). Larvae and the adult beetles are also important sources of food for other arthropod predators as well as higher taxa such as woodpeckers (Picidae) (Bell et al. 2015). Diminished areas of distribution and abundance are well documented for many saproxylic beetles in Sweden through the work of particularly amateur entomologists (e.g. Nilsson & Baranowski 1994; Nilsson et al. 2002; Jeppson et al. 2010; Lindhe et al. 2010).

Figure 2. Wood substrates of thin dimensions are now commonly harvested for production of biofuel, but are frequently stored in the forest to dry. Many saproxylic insects oviposit on the substrates, but the material is usually shredded before the insects emerge, resulting in a complete loss of the reproductive output (photo. M. Molander).

1.3 Oak and its fauna of saproxylic beetles

Among the trees in Sweden, oak (*Quercus robur* and *Q. petraea*) is the tree species with the most species rich fauna of saproxylic beetles (Palm 1959; Dahlberg and Stokland 2004; Jansson 2009), and many of the species are monophagous on oak (Jonsell et al. 1998; Jansson 2009). Oak does not only support the highest total species richness, but also the highest number of red listed species in Sweden (Jonsell et al. 1997). Particularly the long life-span, and large size, of oaks give rise to a multitude of different ecological niches that can be exploited by different species. At the same time, oak is an important species of tree also in modern forestry that can yield high-value timber (Löf et al. 2016).

The distribution and abundance of particularly old, veteran oaks has changed considerably over the last few hundred years in Sweden, primarily due to altered management methods throughout the landscape (Niklasson and Nilsson 2005). After 1830, the number of old oaks in Sweden decreased rapidly (Eliasson and Nilsson 2002), and remaining concentrations of trees suffered negative effects particularly when the management that was keeping the habitats open seized (Nilsson 1997; Jansson 2009). The combination of old oaks being both rare, and supporting the highest biodiversity, has resulted in research largely concentrating on the conservation requirements of saproxylic beetles dependent on old, large-diameter veteran oaks (Figure 3), usually situated in pastures and meadows of the agricultural landscape (e.g. Ranius 2000 Hedin 2003; Jansson 2009). Much fever studies have focused on oak in typical forest habitats and saproxylic species less dependent on veteran trees. However, Franc (2007) performed important work in oak-dominated forest habitats and saproxylic beetles in the Swedish boreo-nemoral zone.

Figure 3. Oak habitats with veteran trees have received much attention from conservation biologists (**left**). However, other oak habitats can also important, such as oak shrub forests on dry, rocky ground cover (**right**), which tend to generate significant quantities of thin deadwood substrates, particularly during years of drought (photos. M. Molander).

1.4 Saproxylic longhorn beetles

Longhorn beetles, or cerambycids (Cerambycidae), is a diverse, species-rich family of primarily saproxylic beetles with worldwide distribution and about 35 000 described species (Nearns 2013; Švácha and Lawrence 2014). A few species are problematic pest species, and potentially invasive pest species, with great economic importance, such as the old house borer (*Hylotrupes bajulus*) and the Asian long-horned beetle (*Anoplophora glabripennis*) (Reddy 2007; Faccoli and Gatto 2015), but the vast majority of species are of little direct concern to human activities as they only utilize already dead or dying wood substrates for their larval development. Instead, these species serve as important components of forest ecosystems, where they contribute to the decomposition of dead wood, create microhabitats for other organisms, and are vital components of forest food webs (Hogstad and Stenberg 1997; Evans et al. 2007; Buse et al. 2008). Different species occupy a wide variety of ecological niches. For instance, larvae of most species in the Cerambycinae subfamily are associated with fresh wood substrates and colonize these immediately after their demise (Monné et al. 2017), while species of the Lepturinae subfamily, are dependent on substrates that have progressed to later decay stages. Certain species develop in partially living trees, in the zone between living and dead plant tissues, and some species seem to prefer sun-exposed conditions, others shady habitats. The position of the wood (downed logs or vertical high stumps) is also an important aspect (Kariyanna et al. 2017). Different species have often developed specific wood-diameter preferences, some species feed on the trunk parts, others on branches, and further species utilize the thin twigs or even the roots (Ehnström and Axelsson 2002). Many species are oligophagous, others are polyphagous in terms of host tree choice (Linsley 1959). In Sweden, oak is the host tree that has the highest total number of cerambycid species, red listed species, as well as species included in national action plans for threatened species (Ehnström and Holmer 2007; Molander unpubl. summaries). Adult beetles may or may not feed on a variety of food sources such as nectar, pollen, tree sap, or foliage.

Similar to other saproxylic beetles, longhorn beetles have suffered declines in their distribution and abundance, in response to the recent extensive environmental changes in forests (McCorquodale et al. 2007; Jeppsson et al. 2010; Cálix et al. 2018). In Sweden, 108 saproxylic species are, or have been present, but a handful is now considered regionally extinct. A high proportion (42%) of the resident species is red listed on the national Red List (ArtDatabanken 2015), and 17 species are included in national action plans for threatened species, issued by the Swedish Environmental Protection Agency. Many longhorn beetles are also listed on European Red Lists (Cálix et al. 2018;

García et al. 2018). Some highly threatened and iconic species such as the great capricorn beetle (*Cerambyx cerdo*) and the alpine longhorn (*Rosalia alpina*) have become flagship species for conservation of saproxylic insects in Europe. Despite the popularity of longhorn beetles as study animals, much is still unknown concerning the distribution and ecology of most non-pest species.

1.5 Pheromones of longhorn beetles

Insects rely heavily on chemical signals (odors) and olfactory systems to locate suitable habitats, food resources, and to communicate with conspecifics (Renou 2014). The chemical compounds that mediate communication between individuals over short to long distances are small, volatile, organic compounds are called pheromones. Pheromones are released into the ambient environment by one individual of a certain species and trigger a behavioural, or physiological change, in a receiving individual of the same species (Karlson and Luscher 1959). Pheromones are separated into several different groups with the most common types being alarm, sex, and aggregation pheromones (Norin 2007; Nandagopal et al. 2008). Sex pheromones typically attract individuals of the opposite sex, and are important for mate-finding and sexual selection. Aggregation pheromones can have different functions, such as coordinating defence or attack. Some male-produced pheromones attract both sexes and have commonly been referred to as a separate category called aggregation-sex pheromones (Cardé 2014). Pheromones often function in combination with other types of volatile compounds that act between different species (allelochemicals). Volatile compounds emitted by host plants often function as attractants for many insects, and may synergize response to sex pheromones (Reddy and Guerrero 2004).

Next to bark beetles (Scolytinae), longhorn beetles have recently emerged as a comparatively well-studied group of saproxylic beetles in terms of pheromone chemistry, and particularly long-range sex pheromones have been shown to play important roles in the mating systems of cerambycids (Millar et al. 2009; Millar and Hanks 2017). The first cerambycid pheromone was reported in 1984 (Sakai et al. 1984), but recent reviews now recognize more than 100 species of longhorn beetles with identified pheromone compounds or other attractants (Hanks and Millar 2016; Millar and Hanks 2017). Species of the subfamilies Cerambycinae, Spondylinae and Laminae are known to use male-produced aggregation-sex pheromones that attract both sexes, while Prioninae and Lepturinae use ‘‘traditional’’ female-produced sex pheromones that exclusively attract males. In male beetles, long-range sex pheromones appear to most commonly be emitted from pores on the prothorax (Iwabuchi

1986; Ray et al. 2006; Hoshino et al. 2015). Females release the pheromones from a gland on the ovipositor (Barbour et al. 2006; Hanks & Millar 2016).

The first studies of species of the Cerambycinae subfamily, which is the subfamily that has received the most attention, indicated that hydroxyketone and 2,3-alkanediol type pheromones were highly conserved across species and genera. However, recent studies have shown that the diversity of compounds might actually be high in this subfamily as alcohols, terpenoids, a pyrrole structure, and other classes of compounds have been discovered more recently (Hanks & Millar 2016). Species of the subfamily Spondylidinae use compounds of the geranylacetone class, which are also found among Lamiinae species (the alcohol fuscumol, and the acetate ester fuscumol acetate). Besides geranylacetone, Lamiinae species also use hydroxyethers and related compounds. Monochamol (2-undecyloxy-1-ethanol) is a characteristic compound of the genus *Monochamus* (Fierke et al. 2012).

Female sex pheromones have only been reported for a few lepturine and prionine species. Among lepturines, *Ortholeptura valida* uses *cis*-vaccenyl acetate (Ray et al. 2011), and members of the *Desmocerus* genus use *R*-desmolactone ((4*R*,9Z)-hexadec-9-en-4-olide) (Ray et al. 2014). A couple of different classes of compounds are known to occur within the Prioninae subfamily, members of the *Prionus* genus use prionic acid ((3*R*,5*S*)-3,5-dimethyldodecanoic acid), while *Tragosoma* species use different enantiomers of 2,3-hexanediol (Barbour et al. 2011; Ray et al. 2012).

1.6 Pheromones for insect conservation

The benefits of exploiting pheromones to detect and monitor a wide variety of pest insect species have long been recognized (Baker and Heath 2005; Witzgall et al. 2010), but pheromones have rarely been used for monitoring and studies of rare species of conservation concern until recently (Larsson et al. 2009; Tolasch et al. 2013; Oleander et al. 2015; Larsson 2016; Leal 2017). The first rare saproxylic insect to have its pheromone identified for the explicit purpose of using it as a tool to study the species' conservation requirements was the hermit beetle (*Osmoderma eremita*) (Larsson et al. 2003). Later, the pheromone of the rare click beetle *Elater ferrugineus* was identified (Tolasch et al. 2007). Both species develop exclusively in the cavities of old, hollow trees, particularly oaks. Insect traps with lures consisting of synthetic copies of the pheromone compounds of these species have proven highly efficient to document the distribution of the species occur. The quantitative trap captures also function as estimates of local abundance that can be analyzed in relation to various environmental factors. Such analyses can provide detailed information on how to design and direct nature management actions that help preserve the species and their habitats (e.g. Musa et al. 2013; Oleksa et al. 2015).

Up till now, ecological studies utilizing pheromone-based trapping of rare and threatened insects have largely concentrated on *O. eremita* and *E. ferrugineus* (Larsson 2016), likely because the identification of pheromones is a significant obstacle that requires considerable effort to overcome. However, the extensive use of long-range aggregation-sex pheromones among longhorn beetles, paired with their relatively well-known general biology in a broad sense (see e.g. Ehnström and Holmer 2007; Klausnitzer et al. 2016), could make this group advantageous for obtaining other saproxylic model species for pheromone-based monitoring. So far, conclusively identified pheromones have only been reported for a few European cerambycids (see Millar and Hanks 2017). The two Prioninae species *Tragosoma depsarium* and *Prionus coriarius* (Figure 4), whose larvae feed in coarse pine logs and various decaying tree roots respectively, can be sampled efficiently with prionic acid and 2,3-hexanediols (see above) (Larsson et al. unpublished studies). Recently, the aggregation-sex pheromone of *Rosalia alpina*, a species included in the EU Habitat's Directive (Council of Europe 1992), was also reported (Žunič Kosi et al. 2017), but this species is regionally extinct in Scandinavia (Lindhe et al. 2010). Much in the same way as pheromones have proved highly advantageous to study sedentary, elusive saproxylic beetles living in hollow trees, pheromones could potentially also be useful for studying longhorn beetles that exhibit rapid population changes and high spatiotemporal variation, particularly species dependent on fresh, ephemeral deadwood substrates, provided their pheromones are first identified.

Figure 4. The prionid cerambycids *T. depsarium* (**left**) and *P. coriarius* (**right**) are two species of only a few non-pest longhorn beetles that can be studied with pheromone-based trapping. (photos. Marcus Vestlund, M. Molander).

2 Thesis aims

The overall aim of the present work was to examine to what extent aggregation-sex pheromones of longhorn beetles can be used to study species dependent on ephemeral, fresh oak wood substrates in southern Sweden. Field studies of this group of beetles are few, and previous research directed at saproxylic beetles in oak-dominated habitats has resulted in limited quantitative data for these species (cf. Franc 2007). Thus, could pheromone-based trapping be a useful tool to overcome the substantial knowledge gaps concerning the conservation status and ecological requirements of cerambycids dependent on fresh oak wood substrates?

The work was organized in two principal segments of basic and applied research, respectively. In the first part, the aim was to conduct basic research in the laboratory and field to identify pheromones and other attractants of multiple species of oak-associated longhorn beetles, with different substrate niches (wood-diameter preference) and population status (common and red listed species, respectively). In the second part, the aim was to exploit the identified pheromones in an applied context for quantitative field-based studies. The purpose of the field studies were to provide initial examples of how the pheromone-based trapping system can be utilized as a practical tool to obtain new insights into the species' ecology and conservation requirements, by means of pheromone-based monitoring of the spatiotemporal occurrence of populations in relation to various environmental factors.

The specific objectives were to:

i) Identify the aggregation-sex pheromones of multiple species of ephemeral oak longhorn beetles, to assemble a toolbox (portfolio) of species that can be efficiently monitored with pheromone-based trapping (papers I-V).

ii) Explore the effectiveness of pheromone-based monitoring to detect and quantify local populations of the study species, and document the geographic distribution of the species at small and large spatial scales (papers VI, VII).

iii) Perform ecological field studies to quantify interspecific relationships, relevant spatial scales where the species interact with their habitat, and examine the effects of different nature management regimes and oak habitat structure (papers VI, VII).

3 Methods

3.1 Study species

The pheromone chemistry of eight species of longhorn beetles was studied in this work (Figure 5, Tables 1 and 2). Largely the same procedure and methods were used for all species. For seven species, the work included collection of volatile compounds and subsequent field bioassays. For one species (*Phymatodes testaceus*), no collections of volatiles were performed, but attraction to the previously identified pheromone, and a heterospecific pheromone component, was studied in field bioassays. All species belong to the subfamily Cerambycinae (Danilevsky 2018). The three *Phymatodes* species and *Pyrrhidium sanguineum* belong to the Callidiini tribe, while the *Plagionotus* species and *Xylotrechus antilope* belong to Clytini tribe. *Anaglyptus mysticus* is a member of the Anaglyptini tribe (Danilevsky 2018).

Table 1. *The species whose pheromone chemistry was studied, their body length (mm), wood diameter (cm) preference, and adult phenology in south-eastern Sweden.*

Species	Body length[1]	Substrate ø[2]	Phenology[3]
Anaglyptus mysticus (L.)	6-10	>3.0	Mid May - late June
Plagionotus arcuatus arcuatus (L.)	8-20	>5.0	Mid May - mid June
Plagionotus detritus detritus (L.)	10-19	>20.0	Late May - late June
Phymatodes alni alni (L.)	4-7	1.0 - 2.5	Mid May - mid June
Phymatodes testaceus (L.)	6-18	>5.0	Early June - mid July
Phymatodes pusillus pusillus (F.)	5-10	2.0 - 6.0	Late April - early June
Pyrrhidium sanguineum (L.)	6-15	>5.0	Late April - early June
Xylotrechus antilope antilope (S.)	7-14	2.0 - 6.0	Early June - mid July

1. After Ehnström and Holmer (2007).
2. Author’s own observations and various literature sources.
3. According to the Swedish Species Observation System (all records of adults 1990-2015).

Figure 5. The study species (top-left to bottom-right); *P. sanguineum*, *P. arcuatus*, *P. detritus*, *P. testaceus*, *P. pusillus*, *X. antilope*, *P. alni*, *A. mysticus* (photos: David Andersson (*P. alni*, *A. mysticus*) and M. Molander)

In Sweden, oak (*Quercus robur* and *Q. petraea*) is the primary host tree of seven species, while *Anaglyptus mysticus* is rather polyphagous on deciduous trees, but is particularly common in hazel (Ehnström and Axelsson 2002). Six of the oak-species are only rarely (or never) found in other host trees than oak, but *P. testaceus* also utilizes other deciduous trees to a greater extent, although oak remains the primary host also for this species (Ehnström and Holmer 2007). All species are dependent on fresh, recently dead substrates and colonize these immediately after their demise. The substrates are normally only suitable for oviposition by one generation of beetles, and all species usually require one to two years for their development, but *A. mysticus* requires two to three years (Ehnström and Axelsson 2002; author's personal observations). Although all seven oak-species utilize the same general type of oak substrates, different species utilize different, but partially overlapping, niches on the same type of substrate (Table 1) (Ehnström and Axelsson 2002; author pers. obs.). The larvae feed primarily in the nutrient rich phloem, cambium and secondary xylem, but pupate in the primary xylem (Ehnström and Axelsson 2002). The species are only occasionally observed visiting flowers (except *A. mysticus*), and adults are typically observed on their host substrate (Ehnström and Axelsson 2002; Ehnström and Holmer 2007).

Table 2. *Status of the study species in southern Sweden and their national Red List category. Two species are included in national action plans for threatened species, issued by the Swedish Environmental Protection Agency.*

Species	Distribution[1]	Status[1]	Red List[2]	Action plan
A. mysticus	Widespread	Relativ. rare, local	NT	
P. arcuatus	Widespread	Common	LC	
P. detritus	Stockholm-region	Rare, nearly extinct	EN	X[3]
P. alni	Widespread	Uncommon, local	LC	
P. pusillus	Small area in SE Swe.	Rare, local	VU	X[4]
P. testaceus	Widespread	Common	LC	
P. sanguineum	Widespread	Relativ. rare, local	NT	
X. antilope	Small area in SE Swe.	Relativ. rare, local	NT	

1. Mainly after Ehnström and Axelsson (2002) and Ehnström and Holmer (2007).
2. After ArtDatabanken (2015).
3. See Ehnström (2005).
4. See Franc (2013).

3.2 Collection of experimental insects

To obtain adult beetles of the study species, recently dead wood substrates of primarily oak were collected in south-eastern Sweden (Blekinge, Småland, and

Öland provinces). For this purpose, heaps of forest biofuel material (woody debris from logging or thinning management, with relatively small diameters such as twigs and branches) proved useful (Figure 6). The large quantities of fresh, dead wood, usually stored in the forests at sun-exposed positions, are highly attractive to the study species, and female beetles may oviposit great numbers of eggs on these substrates. Thus, relatively small volumes of wood from biofuel heaps can provide hundreds of emerging individuals. The wood substrates were brought back from the field to the laboratory and transferred to boxes inside a greenhouse. The first beetles would start to emerge after about a week and the different species would then hatch sequentially in about the same order as their activity periods start during the season. The beetles were removed from the boxes as they hatched, separated by sex, and kept in smaller containers with fresh oak substrates (to potentially stimulate pheromone release), and paper tissues saturated with a solution of honey and water for nourishment. Large numbers of individuals emerged of several of the study species, and only subsets were used for collection of pheromones.

Individuals of the majority of study species were obtained from heaps of forest biofuel, but *Anaglyptus mysticus* was reared from wood substrates of hazel that were not logging residues. Another exception was *Plagionotus detritus*, a species which is almost extinct in southern Sweden. For this species, animals breed in captivity were used, within the framework of a national action plan for the recovery of this threatened species' (Ehnström 2005).

Figure 6. Large, sun-exposed stack of wood residues from logging activities (mainly twigs and branches of oak). Substrates containing great numbers of individuals of several of the study species were collected from this stack. Shortly after, all insects were killed when the material was shredded for production of forest biofuel. Barnebo 2015 (photo: M. Molander).

3.3 Collection of pheromone compounds

Volatile compounds, emitted from the beetles, were collected using the headspace sampling technique. Typically, the containers with males and females separated were placed in a climate chamber the afternoon before headspace sampling was to commence. The following morning, male and female beetles were placed in groups of about five to eight individuals of each sex in two separate glass bottles, and a third empty bottle was used as a blank control (Figure 7). Each bottle had two top openings. The bottles were connected with Teflon® tubing to an air pump, which would pull ambient air through the bottles. Upon exiting the glass bottles, the air would pass through beds of adsorbent material (Porpak™ Q) in Teflon® tubing. The volatile compounds that were emitted from the beetles would be captured on the adsorbent material and could thereafter be eluted using a solvent (hexane). A set of the same collectors, or a set of granulated active charcoal collectors, were used at the inlet of the glass bottles to filter the ambient air coming into the bottles. Collection of the pheromones generally took place for about four to six hours between 10 AM and 4 PM, as most species appear to have their maximum activity at this time of day under natural conditions (author's pers. obs.). Multiple separate collections were made per species with partially different sets of individuals. The headspace sampling was performed in a climate chamber, but not the same chamber as where the beetles had been held during the previous night.

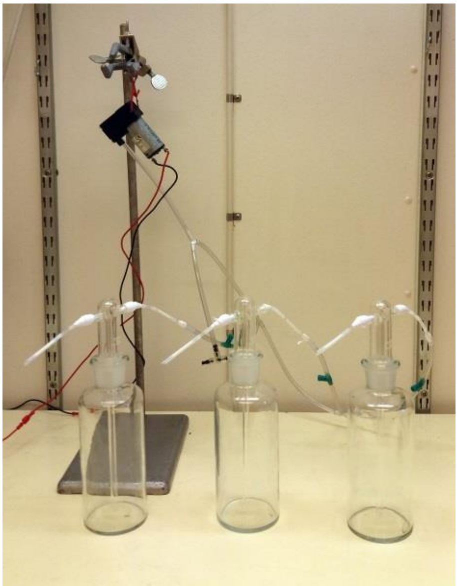


Figure 7. Equipment for head-space collections. Male and female beetles were held in separate glass bottles with an empty bottle used as a control. Collectors consisted of Porapak™ Q columns. Porapak™ collectors were also used to purify the air going into the system. A single air pump was used to draw ambient air through the vessels.

3.4 Analyses of extracts of volatiles

3.4.1 Gas chromatography – mass spectrometry

Extracts of volatiles were typically first analysed with two GC-MS systems, equipped with a nonpolar HP5 column and a polar DB-WAX column respectively, at the Alnarp Campus. To recognize which compounds that could constitute pheromone compounds or components of pheromone blends, the chromatograms of males, females and the blank control were compared visually with the Agilent ChemStation software, using the overlay function. Compounds that were specific to the sample of males would appear as elevated concentrations of ions (peaks) that were not present in the corresponding sample of females, or the blank control.

For tentative identification of the compounds that were consistently unique to the extracts of males, online mass spectral databases, and/or standards of compounds that were previously known to constitute pheromones of longhorn beetles were used. The identities were then confirmed by GC-MS analyses of synthetic standards and comparisons of the retention time and mass spectra of the standards to those of the beetle-produced compounds. The chirality of the suggested pheromone-compounds was examined by collaborators at University of California Davies with a Cyclodex B GC column.

3.4.2 Gas chromatography – electroantennographic detection

Electrophysiological studies were performed with a few of the study species using a coupled GC-EAD apparatus to examine the response of beetle antennae to the extracts of volatiles from males and screen for compounds that were likely to be behaviourally active. The entire head, or an antenna, of the beetle were excised and mounted between two glass micro capillaries containing Beadle-Ephrussi Ringer solution, with recording and reference electrodes of silver protruding into the solution (Figure 8). The recording electrode was connected to a pre-amplifier probe (EAG combi probe), further connected to a DC amplifier interface box (IDAC 2). The extract was injected onto a GC fitted with an HP5 column. At the GC effluent, the output was split 1:1 between a flame ionization detector (FID) and a transfer line (tracking the GC temperature program) ending in a glass tube with the antenna positioned immediately in front of the end of the tube. The neural pulses from the antenna, and the FID data, were translated into digital signals for visualisation. Response of the antenna to eluting compounds would be presented by sudden spikes in the EAD that coincided with FID peaks, which would indicate

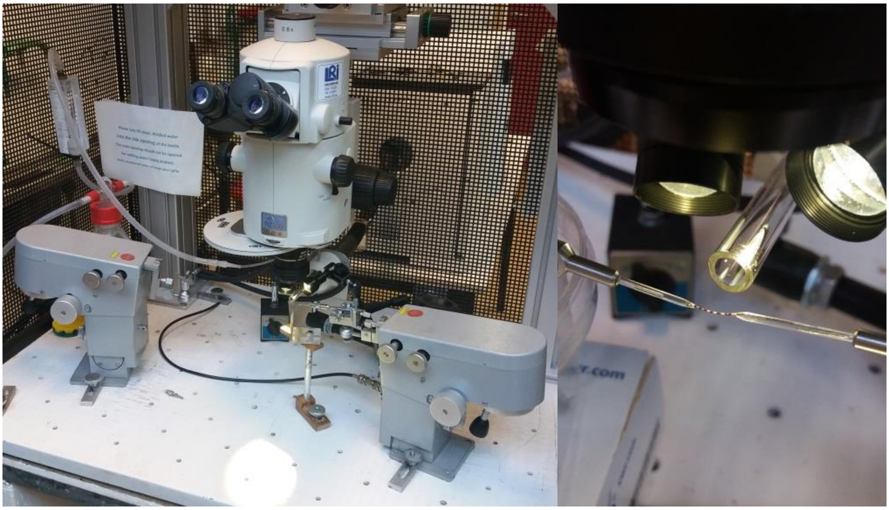


Figure 8. **Left**; setup used for GC-EAD analyses in a Faraday cage with GC and transfer line visible behind the microscope. **Right**; A mounted antenna of a longhorn beetle between micro capillaries in front of the transfer line's terminal glass tube (photos; M. Molander).

physiologically active compounds possibly attractive to the species.

3.5 Bioassays of pheromone-components

Prospective pheromone compounds, identified from GC-MS (and sometimes GC-EAD), were tested directly in field bioassays from 2015 to 2017 in order to determine if the beetles displayed significant attraction to the synthetic copies. The compounds were either bought from commercial companies, or synthesized by collaborators at the University of California Davis.

3.5.1 Trapping areas for bioassays

The majority of bioassays were performed within Hornsö Ecopark (located 40 km north-west of Kalmar) in south-eastern Sweden. The large (9 200 hectares), mostly forested area, is advantageous for this type of work for several reasons. First, oak is noticeably abundant and several of the study species appear more common within the Ecopark compared to other areas (Nilsson and Huggert 2001; author's pers. obs.). Secondly, the area is sparsely populated, has a single land owner (state-owned forestry company), and replicates can be positioned at undisturbed, sunny sites with adequate distance in between the sites.

However, for two species, *P. detritus* and *A. mysticus*, we used different areas for the bioassays respectively. The bioassay for *P. detritus* took place at Norra Djurgården in Stockholm City, as the Stockholm region is the only area in Sweden where the species is known to maintain a population (Lindhe et al. 2010). The bioassay for *A. mysticus* was performed in the Mittland forest on the island of Öland in the Baltic Sea, where the species is comparatively common (Lindhe et al. 2010; Swedish Species Observation System 2018).

Figure 9. Example of a trap replicate from a bioassay of compounds attractive to *P. pusillus* in Hornsö Ecopark 2016 (photo: M. Molander).

3.5.2 Flight-intercept traps

The same type of trap was used in all bioassays. The trap was a relatively small, custom-built, cross-vane flight-intercept trap, with a total vertical area of 0.2 sqm (Figure 9 and 10). Spatial and temporal replication was used to increase the number of observations. The compounds were tested separately and in blends of varying ratios. Each replicate would include one trap with each pheromone lure (treatment) and a control trap (solvent only). Isopropanol (2-propanol) was used as the solvent for all treatments. In most cases the lures were exchanged with about three week intervals, and the traps were also emptied and their positions interchanged within the replicate to create a new configuration and reduce any possible systematic errors emanating from the specific position of the trap within the replicate.

In all studies, the pheromone dispensers consisted of Grippie® zip-lock bags. The pheromones would be loaded into the dispensers at the field sites. Killing traps were used in the bioassays, except for *Plagionotus detritus*. A catch and release version of the trap was used for *P. detritus*, the traps were emptied late every afternoon and the beetles released. Emptying the killing traps could be performed quickly by filtering the propylene glycol (preservative in the trap jar) with the trapped insects through a tea filter which was saved in a plastic bag and brought back to the laboratory (Figure 10).

Figure 10. Schematic drawing of a flight-intercept trap (catch-release version), and the author servicing traps (drawing; M. Molander, photos: Bengt Lundberg, Björn Eriksson).

All longhorn beetles (except *P. detritus*) were identified at the laboratory with the key by Ehnström and Holmer (2007). Males and females were separated either based on external characters (colour differences, or the relative length of the antennae compared to body length). After examination, the material was stored in 70% ethanol at Alnarp Campus (for the time being).

3.6 Application of pheromones for monitoring studies

To examine the usefulness, and feasibility, of utilizing the pheromone-based trapping approach for large-scale general surveys to detect and quantify local populations of the ephemeral oak longhorn beetles, three separate trapping studies in the field were performed, each including a considerable number of individual trapping sites. One study comprised a dense network of sites in a comparatively small area, while two studies utilized much larger study areas with a lower density of trapping sites. Killing traps and the same type of flight-intercept trap (see above) was used in all monitoring studies.

3.6.1 Hornsö Ecopark, Småland province, 2014

To study the occurrence of the species at a finer scale, a subset of the species were monitored at 30 sites in a comparatively small sampling area (7 × 8 km) within the boreo-nemoral zone (average inter-site distance of ~1 km) (Figure 11). The sites were mainly chosen based on the trapping sites used in a non-related study the previous year, utilizing passive flight-intercept traps to survey saproxylic beetles. At each trapping site, three traps with a blend of common pheromone components (racemates of 3-hydroxy-2-hexanone and 2-methyl-1-butanol) were placed in a triangle to survey primarily *P. sanguineum*, *P. alni* and *P. testaceus*, but also *P. pusillus*. The traps were deployed throughout the activity period of all species, and serviced with three-week intervals. Limited information on the occurrence of the species within the area was available through previous general surveys of saproxylic beetles and collections by amateur entomologists (compiled in Nilsson and Huggert 2001).

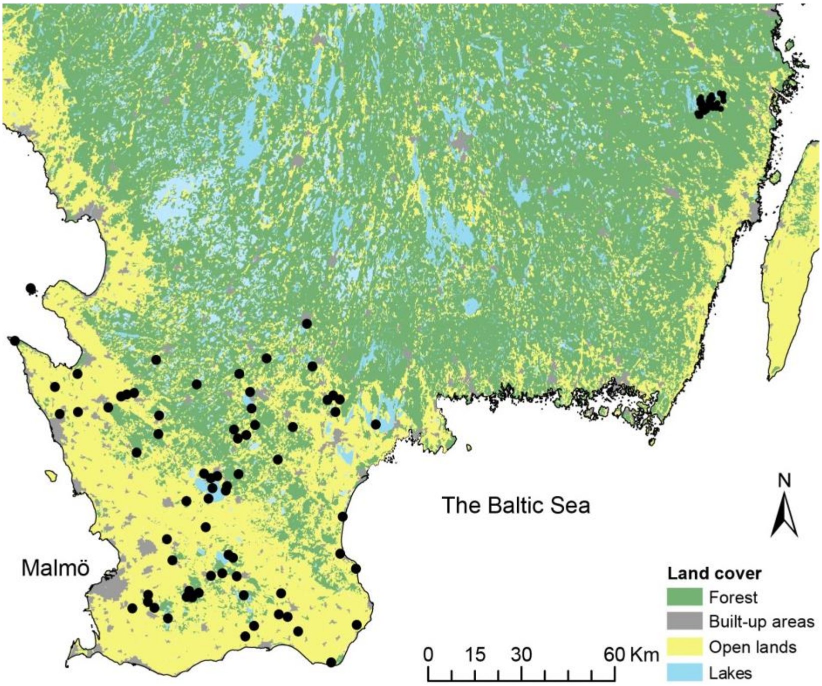


Figure 11. The trapping sites in southern Sweden used for monitoring of *P. sanguineum*, *P. alni*, *P. testaceus* and *P. pusillus* in 2014 and 2015. The area to the northeast (Hornsö Ecopark) was surveyed with a high density of trapping sites in 2014, and the study area in Skåne province (the remaining sites) was surveyed in 2015. Map: Vägkartan, vector © Lantmäteriet, Gävle, Sweden.

3.6.2 Skåne province 2015

Skåne province, situated in the nemoral forest zone at the southernmost tip of Sweden, is one of the areas in Scandinavia with the most available information on saproxylic insects, due to numerous recent targeted surveys by various authorities and researchers, and the large number of active amateur entomologists. Knowledge on the distribution and population status of saproxylic deciduous beetles is considered comparatively comprehensive and up-to-date, at least when compared to most other areas in the country (e.g. Lindhe et al. 2010; Sörensson 2012). The pheromone-based study in 2015 included 70 sites (Figure 11) that were monitored with three traps per site utilizing a blend of common pheromone components (racemates of 3-hydroxy-2-hexanone and 2-methyl-1-butanol) to obtain local, site-specific estimates of the presence and abundance of the three species *P. sanguineum*, *P. alni* and *P. testaceus*. The sites were chosen rather arbitrarily to represent a wide number of different habitats with varying degree of oak presence in the surroundings, but included most sites with recent records of *P. sanguineum* and *P. alni* (1980 to 2014). The trapping period covered the full activity periods of *P. alni* and *P. testaceus*, and the majority of the activity period of *P. sanguineum*. The observations from the study were compared qualitatively with the previously reported records of the species from the province.

3.6.3 Småland and Blekinge provinces 2016-2018

This study stretched over three years with sites distributed at large spatial scale in two provinces (Figure 12). A total of 62 sites were monitored in 2016 and 2017, and 29 sites in 2018. The sites were the same each year and belonged to five different categories of specifically selected oak-dominated forest and woodland habitat types (see below under section 3.7.3). With nine traps per site, it was possible to monitor all six, primarily oak-dependent, species from the toolbox of species with identified pheromones: *P. sanguineum*, *P. alni*, *P. testaceus*, *P. pusillus*, *X. antilope* and *P. arcuatus* (*P. detritus* is essentially not present in the study area, but was reintroduced to a site in 2017). Each year, either the entire activity period, or a substantial part of the activity period, of each species was covered by systematic trapping that commenced in late April and was terminated in late July. The traps were for the most part hung from bars of reinforcement steel that were forced into the ground, but were hung from tree branches at sites with grazing animals. The results were compared qualitatively to the previously known distribution areas of the species in this part of the country.

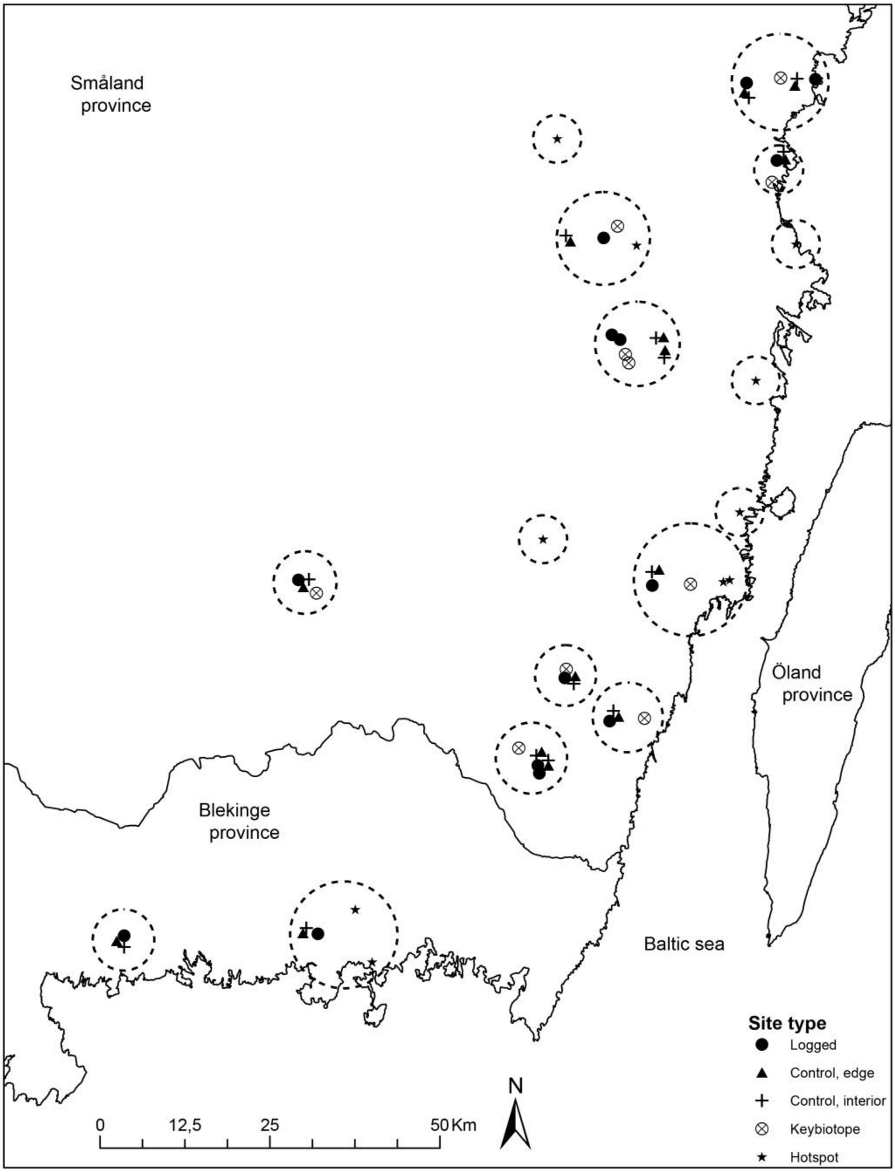


Figure 12. Trapping sites in south-eastern Sweden used for monitoring in 2016 to 2018 and their site/oak habitat type. Recently logged oak-dominated production stands and the edge of matching control sites consisting of oak production forests without recent logging activities were surveyed all three years, while the interior of control stands, woodland key biotopes and oak conservation hotspots were surveyed for two years (2016-2017). Hatched circles show the grouping of the sites into spatial clusters used for analyses. Map: GSD-Översiktskartan, vector © Lantmäteriet, Gävle, Sweden.

3.7 Interspecific correlations, associations with oak forest cover and effects of oak habitat type

3.7.1 Interspecific correlations

Given the high dependency of the study species on the same species of host tree (oak), and the same main type of fresh wood substrates, local species presence and abundance could theoretically show strong interspecific correlations in terms of local presence-absence and abundance patterns based on common patterns of substrate availability. Previously, a high degree of interspecific correlation in local abundance was observed in the two species *O. eremita* and *E. ferrugineus* when pheromone-based trap catches were examined (Larsson and Svensson 2011). To explore the presence of such correlations could be beneficial to select indicator, or umbrella species, that have similar ecology and habitat management requirements (cf. Ranius 2002; Andersson et al. 2014; Foit et al. 2016). Selecting such species from the toolbox of species with identified pheromones could result in a more efficient monitoring approach by decreasing the number of species that need to be monitored. Qualitative nestedness among the species was examined as presence and absence patterns of the species at individual sites, and quantitative relationships were studied by correlations of quantitative trap catches (local abundance) of the different species. Correlations were examined in the two datasets from the monitoring efforts in 2014 and 2015.

3.7.2 Forest cover, spatial scales and beetle abundance

Understanding at what spatial scales species interact with their habitat is important in order to design appropriate conservation measures and nature management strategies (e.g. Lindenmayer 2000; Jackson and Fahrig 2012). However, difficulties associated with accurate sampling of many taxa, as well as obtaining data on relevant habitat variables over large geographic extents are often a major obstacle to study species' response to habitat at different scales. Data from pheromone-based sampling have previously been used to identify relevant spatial scales for the hollow-tree dependent beetle *E. ferrugineus* (Musa et al. 2013), and local abundance of saproxylic longhorn beetles has been correlated to general forest cover (Holland et al. 2004).

In Sweden, detailed forestry maps of the volume of different tree species exist for the whole country, including a map of oak tree volume. The oak map is a raster sheet that shows an estimate of the quantity of standing, living oak wood per pixel. Each pixel is equivalent to 25 x 25 m ground surface. The map can be visualized in a GIS-program and the oak volume at different spatial scales (buffer zones with different radii) extracted (Figure 13). Local abundance of the study species, as measured in the pheromone-based field studies of 2014 and 2015, was correlated to the oak volume at different spatial scales to examine at what spatial scales the study species showed the strongest association with their habitat.

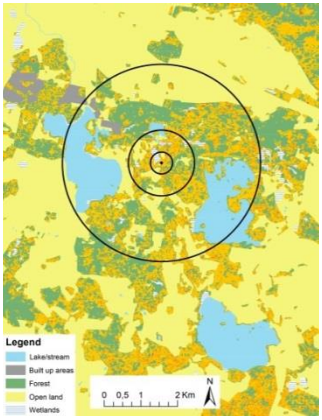


Figure 13. Illustration of a trapping site (black dot), with buffer zones (spatial scales) at 300 m, 900 m and 2 700 m radii. The forest map of oak volume is shown as the raster in yellow, orange and red colours depending on the oak volume present (the darker the higher volume). Map: Vägkartan, vector © Lantmäteriet, Gävle, Sweden.

3.7.3 Oak habitat structure and management

The trap sites that were monitored in 2016 to 2018 were selected to represent five different types of oak-dominated forests and woodland habitats (Figure 14). Beetle abundance was surveyed with the pheromone-based trapping system in three different habitat types at stands of oak production forests and in two types of set-aside oak forest and woodland habitats intended to benefit biodiversity. Fourteen recently logged oak production stands, with comparatively large quantities of fresh oak wood substrates from the previous winter, were compared to fourteen similar oak control production stands, but which had not recently been managed, and thus lacked significant quantities of fresh wood substrates. The control stands were sampled at the sunny edge, which was more similar to conditions at the recently logged stands, and at the interior, shady centre of the same production stand, respectively. Abundance at the recently logged stands was compared to the edge of the control stands over a period of three years to examine the effect that logging and retention of fresh wood substrates had on local beetle populations over time as the wood substrates aged.

Further, to study potential differences in beetle abundance between production stands and set-aside oak habitats, trapping results from the edge of the control stands was compared to the beetle abundance at the edge of so called Woodland Key Habitats (WKH, small, set-aside habitats) and oak conservation hotspots (often formally protected, set-aside habitats, renowned for their high number of threatened saproxylic beetles associated with oak). The WKH were structurally similar to ordinary oak control production stands, while the hotspots consisted of semi-open woodlands with large-diameter veteran oaks. Finally beetle abundance at the interior of the oak production stands was compared with the edge of production stands, as well as the two set-aside habitats, to examine particularly the effect of the different sun-exposure between the edge and interior of the production stand.

Figure 14. The five surveyed oak habitat types during the monitoring study of 2016-2018. **Top-left**; recently logged oak production stand with fresh branches and twigs. **Top-right**; south-facing edge of control production stand. **Center-left**; interior of control production stand. **Center-right**; edge of oak-dominated woodland key habitat site. **Bottom-left**; typical oak conservation hotspot. **Bottom-right;** Fresh oak substrates are colonized immediately by the study species and are quickly depleted. The substrates in the picture were fresh in spring 2016, but little over a year later (July 2017) the bark is falling off, and most beetles of the study species have already emerged from the wood (photos: M. Molander).

4 Results and discussion

4.1 Identified pheromone components

Headspace collections and bioassays demonstrated the presence and significant attraction of the eight study species to male-produced aggregation-sex pheromones under field conditions. In total, seven different attractive compounds were identified; four hydroxyketones, two alcohols, and one ketone (Table 3, Figure 15). The eight species represent a large share of the total number of longhorn beetles that are characteristic to fresh, dead oak wood in Scandinavia. Likely pheromone compounds were also identified for *Ropalopus femoratus* (L.) and *Cerambyx scopolii* ssp. *scopolii* (F.), but bioassays could not be performed (or finished) within the scope of this thesis. The alkanediones 2,3-hexanedione and 2,3-octanedione, and hydroxyalkan analogs of the ketols, such as 2-hydroxy-3-hexanone and 2-hydroxy-3-decanone, were also frequently observed in extracts of male beetles, but these compounds were not studied further as they are likely to be artefacts by thermal rearrangement during GC analysis (see paper 5; Sakai et al. 1984; Schröder et al. 1994; Hanks and Millar 2016). Occasionally, various trace compounds were observed in the extracts of males at average ratios of about one percent or less of the main component. These were deemed unlikely to be important for attraction, and were not studied further.

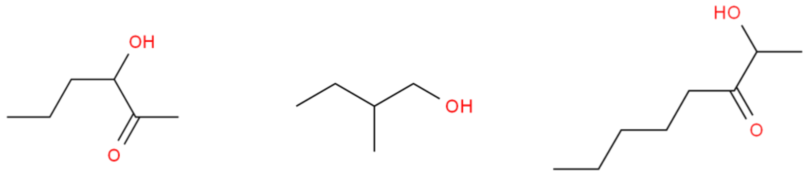
The pheromones of most species proved to consist of two-component blends, but *X. antilope* and *P. testaceus* (see Hanks et al. 2019) produce, and were significantly attracted to, single pheromone compounds. For species producing more than one compound, single components were not significantly attractive, or elicited only weak attraction. Typically, synergism in attraction was obtained when using blends, resulting in significantly higher attraction compared to controls and single compounds. *Plagionotus arcuatus* was unusual among the study species (and cerambycids in general, see Millar and Hanks 2017) as it produced relatively large quantities of three hydroxyketones. The ternary blend captured the highest absolute number of beetles in the large dataset from the bioassay in Hungary, but was not statistically significantly different from the two-component blend. However, as 3-hydroxy-2-octanone was available in sufficient quantities, the ternary blend was used for the large motoring study in paper 7. Trap captures of males and females of each species were in most cases approximately equal in the bioassays.

Table 3. *Summary of the aggregation-sex pheromone components (or attractants) identified in this work and used by the different study species.*

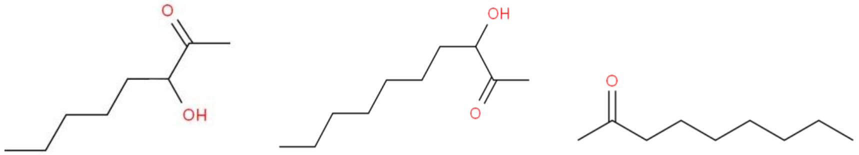
Compound Species	(*R*)-3-Hydroxy-2-hexanone	(*R*)-2-Methyl-1-butanol	(*S*)-2-Hydroxy-3-octanone	(*R*)-3-Hydroxy-2-octanone	(*R*)-3-Hydroxy-2-decanone	2-Nonanone	1-Hexanol
A. mysticus	X					X	
P. arcuatus	X			X	X		
P. detritus	X		X				
P. alni	X	X					
P. pusillus		X[1]					X
P. testaceus[2]	X	X					
P. sanguineum	X	X					
X. antilope			X				

1. The specific enantiomer produced by this species was not identified.
2. Attraction was determined through bioassays; the pheromone of the species has previously been identified as (*R*)-2-methyl-1-butanol alone (Hanks et al. 2019).

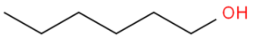
The two pheromone compounds 3-hydroxy-2-hexanone and 2-methyl-1-butanol were shared by multiple study species, and traps with these compounds can simultaneously monitor three species efficiently (and to some extent also *P. pusillus*). Other compounds such as 3-hydroxy-2-decanone and 1-hexanol were more species-specific. Racemic solutions of the chiral compounds elicited significant attraction, indicating that the enantiomer which was not produced by the beetles, was not a strong inhibitor or deterrent, which has also been observed among North American species (Hanks and Millar 2016). Cross-attraction to heterospecific compounds was also observed by *P. pusillus* and *X. antilope* (papers II and IV). However, the species-specific pheromones were clearly more attractive than the heterospecific components, and trapping with the specific pheromones, rather than other attractants, can be important for detection and quantification of rare species (paper IV; Svensson et al. 2012). However, in *P. testaceus* attraction appeared essentially equal to both the species-specific pheromone (2-methyl-1-butanol alone; see Hanks et al. 2019) and to the heterospecific pheromone component 3-hydroxy-2-hexanone (paper I).



3-hydroxy-2-hexanone 2-methyl-1-butanol 2-hydroxy-3-octanone



3-hydroxy-2-octanone 3-hydroxy-2-decanone 2-nonanone



1-hexanol

Figure 15. Skeletal formulas of the seven identified pheromone-components from the eight cerambycid study species (see Table 3).

4.1.1 Pheromones of *P. sanguineum*, *P. alni*, and *P. testaceus* (paper I)

Previous work had established (*R*)-3-hydroxy-2-hexanone as the major pheromone-component of *P. sanguineum* though headspace collections and wind tunnel assays, in combination with minor quantities of (2*S*,3*R*)-2,3-hexanediol and (2*R*,3*R*)-2,3-hexanediol (Schröder et al. 1994; Schröder 1996; Fettkoetter et al. 2001). *Phymatodes testaceus* has recently been studied in North America, where the species has been introduced, and the aggregation-sex pheromone identified as (*R*)-2-methyl-1-butanol alone through headspace collections and field bioassays (Hanks et al. 2019). The pheromone chemistry of *P. alni* had not previously been studied.

The headspace collections from *P. sanguineum* and *P. alni* included two main compounds, (*R*)-2-methyl-1-butanol and (*R*)-3-hydroxy-2-hexanone (and minor quantities of 2,3-hexanedione), that were specific to the extracts of

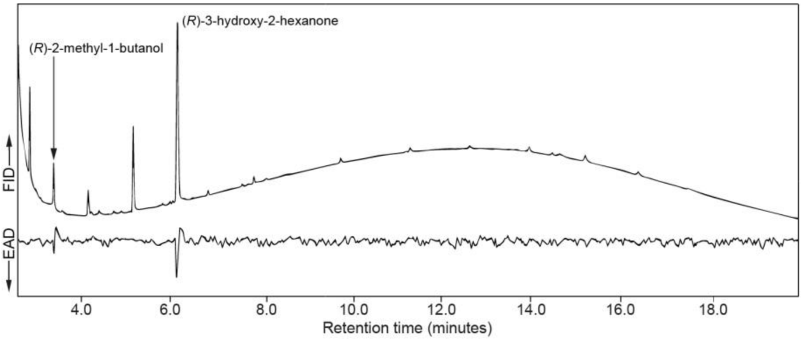


Figure 16. Chromatography-electroantennogram detection analysis of headspace volatiles from males of *P. sanguineum* with an antenna of a male beetle of the same species. The antennae repeatedly responded to (*R*)-2-methyl-1-butanol and (*R*)-3-hydroxy-2-hexanone.

volatiles from male beetles. Ratios ranged from 1-15:100 (*P. sanguineum*) and 70-110:100 (*P. alni*). GC-EAD studies with *P. sanguineum* demonstrated that (*R*)-2-methyl-1-butanol and (*R*)-3-hydroxy-2-hexanone elicited consistent antennal response (Figure 16), but not 2,3-hexanedione. The 2,3-hexanediols were not observed in the extracts of *P. sanguineum*. Only a few individuals of *P. testaceus* hatched, which were overlooked in the emergence boxes, possibly due to their crepuscular/nocturnal habits. Thus, no headspace collections were made from this species.

In the bioassays, single component treatments were generally unattractive to *P. sanguineum* and *P. alni*, but various blends elicited significant attraction (Figure 17). As in previous wind tunnel experiments (Schröder 1996), 3-hydroxy-2-hexanone alone elicited significant attraction in one of the bioassays, but most blends captured significantly more beetles than single

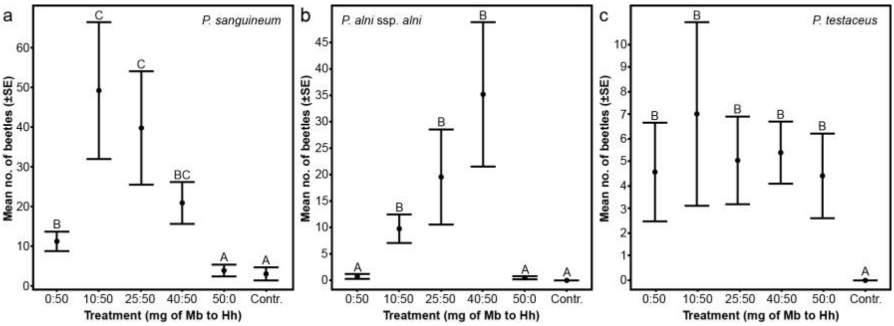


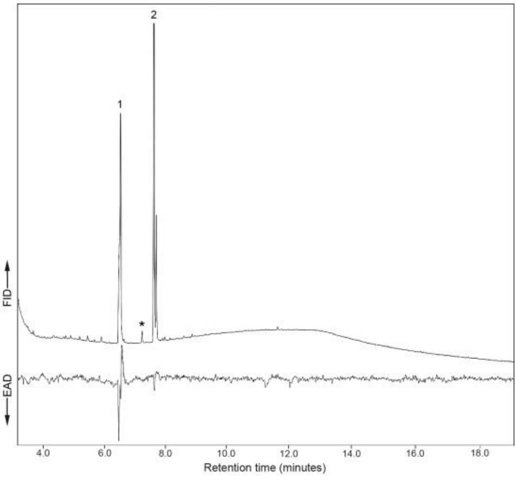
Figure 17. Mean number of captured beetles of three cerambycid species per trap and collection date for treatments with different ratios of the racemates of 2-methyl-1-butanol and 3-hydroxy-2-hexanone (bioassay 2017). Treatments that do not share a common letter are significantly different (adjusted $P<0.05$).

compounds. Interestingly, *P. sanguineum* and *P. alni* produced the same two compounds, but extract-ratios, as well as absolute captures in the 2017 bioassay, indicated that the species produced different ratios of the compounds, and responded optimally to ratios that were similar to the ratios found in the extracts, although the differences between blends were in most cases not statistically significant. Significant attraction of *P. testaceus* to (*R*)-2-methyl-1-butanol alone was also confirmed, but the heterospecific pheromone component 3-hydroxy-2-hexanone alone, and all blends of 2-methyl-1-butanol and 3-hydroxy-2-hexanone also elicited significant attraction, which was similar across treatments. Based on the observations, all three species can be monitored efficiently with the same lure treatment. For this purpose, a 50:100 blend of racemic 2-methyl-1-butanol to racemic 3-hydroxy-2-hexanone, is a suitable tool.

4.1.2 Pheromone of *P. pusillus* (paper II)

This species had previously been studied by Schröder (1996) who identified 1-hexanol as a major compound specific to the extracts of males, and tentative pheromone of the species, in addition to trace quantities of 1-butanol and 1-octanol, but no behavioral assays were reported.

In the monitoring study of 2014 (paper VI), when the blend of the racemates of 2-methyl-1-butanol and 3-hydroxy-2-hexanone was used for cerambycid surveys at 30 sites in Ecopark Hornsö (Småland province), limited numbers of *P. pusillus* were also captured, which indicated attraction to the heterospecific blend. When headspace collections from *P. pusillus* were performed in winter 2015, analyses showed that these contained 1-hexanol as a major component and minor quantities of 2-methyl-1-butanol (Figur 18). Trace



Figur 18. Chromatography-electroantennogram detection analysis of headspace volatiles from males of *P. pusillus* with an antenna of a conspecific female. The antennae repeatedly responded to (*R*)-2-methyl-1-butanol (1) and 1-hexanol (2). The asterisk denotes 2-methyl-1-pentanol.

quantities of 2-methyl-1-pentanol and 1-octanol were also observed. In GC-EAD recordings, antennal response by male and female *P. pusillus* was observed to 1-hexanol and 2-methyl-1-butanol, but not to 2-methyl-1-pentanol or 1-octanol.

The bioassay with 1-hexanol and racemic 2-methyl-1-butanol showed that both components were inactive when applied single, but blends with different proportions of 2-methyl-1-butanol were significantly attractive (Figure 19). Likewise, the heterospecific blend of the racemates of 2-methyl-1-butanol and 3-hydroxy-2-hexanone was significantly attractive, and the addition of the heterospecific component 3-hydroxy-2-hexanone synergized response to 2-methyl-1-butanol as further field tests in 2017 of the ketol as a single compound showed that also this compound was inactive as a single component.

Surprisingly, male and female *P. pusillus* displayed a significantly different response to the heterospecific blend, contra the species-specific pheromone. Both sexes were attracted in about equal numbers to the heterospecific pheromone, but females showed a significantly higher attraction to the species' own pheromone compared to males. The latter difference was in contrast to the other bioassays where males and females were captured in roughly equal numbers with the species-specific pheromones, and the pattern was repeated in the much larger landscape study of 2016. The heterospecific pheromone may function as a general substrate cue, which is equally adaptive to respond to for both males and females. By comparison, it might be more adaptive for females to respond to the species own male-produced pheromone, than for other males.

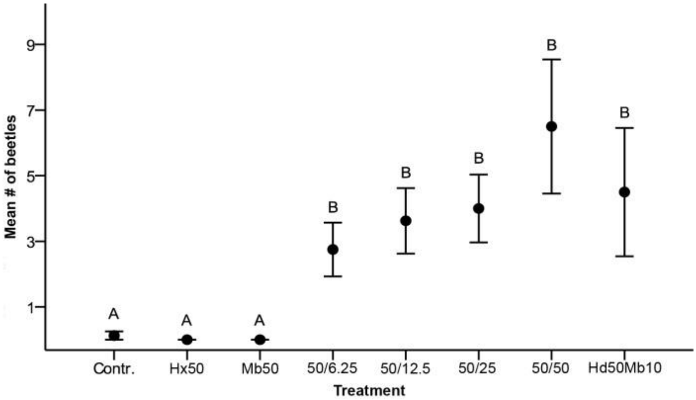


Figure 19. Mean number (±SE) of male and female *P. pusillus* captured per trap and collection date (n=8 samples) with different lure treatments of 1-hexanol (Hx), 2-methyl-1-butanol (Mb), and 3-hydroxy-2-hexanone (Hd). Four treatments tested blends of 1-hexanol (50 mg per lure) and a variable quantity of 2-methyl-1-butanol. Means with different letters are significantly different (adjusted $P < 0.05$).

Males of the species may benefit the most from emitting their own pheromone to attract females, or use host volatiles and the heterospecific blend to search for females, rather than responding to the pheromone source emitted by conspecific males.

The specific aggregation-sex pheromone of *P. pusillus* should be the preferred tool for monitoring the species. In the large monitoring study from 2016 to 2018, when each site had separate sets of traps with the species-specific pheromone and the heterospecific blend, the species' own pheromone repeatedly captured higher total numbers of beetles than the heterospecific blend (e.g. totals of 178 versus 30 individuals in 2016).

4.1.3 Pheromone of *P. detritus* (paper III)

Male beetles, of this essentially unstudied species, from the Swedish captive breeding program produced large quantities of (*R*)-3-hydroxy-2-hexanone and (*S*)-2-hydroxy-3-octanone at an average ratio of 20:100 (main component: (*R*)-3-hydroxy-2-hexanone). Minor quantities of the alkanediones 2,3-hexanedione and 2,3-octanedione were also observed in the majority of samples. Individual compounds were not attractive to the species in the catch and release bioassay (Figure 20 and 21), but significant attraction was achieved when the compounds were applied in a 20:100 blend mimicking the average proportions found in the extracts of volatiles. *Plagionotus detritus*, is the first species of longhorn beetles shown to utilize a combination of two hydroxyketones with different chain length in which the positions of the hydroxyl and carbonyl groups are interchanged between the two compounds.

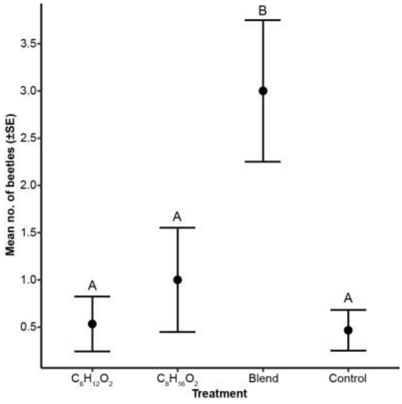


Figure 20. Mean number of *P. detritus* captured per trap and collection day with different lure treatments of the racemates of 3-hydroxy-2-hexanone and 2-hydroxy-3-octanone as single components and in a 20:100 blend (main component: 3-hydroxy-2-hexanone). Means with different letters are significantly different (adjusted $P < 0.05$).

Figure 21. Replicate from the bioassay targeting *P. detritus* (Stora Skuggan, Stockholm, 2015), and *P. detritus* individuals in a trap jar.

4.1.4 Pheromones of *A. mysticus* and *X. antilope* (paper IV)

Extracts of volatiles from male *Anaglyptus mysticus* contained a number of compounds that were not present in the corresponding extracts from female beetles (Figure 22). However, the two compounds (*R*)-3-hydroxy-2-hexanone and 2-nonanone dominated the samples. Extracts indicated that 2-nonanone was the minor component, with an average ratio of 70:100 to the (*R*)-3-hydroxy-2-hexanone. In the bioassay, both compounds were inactive when applied single, but significant attraction was achieved when the compounds were applied in blends with higher ratios of 2-nonanone (100:100 and 160:100) to that of the hydroxyketone’s (*R*)-enantiomer (Figure 23).

Only two compounds were consistently preset, and specific, to extracts of males of *Xylotrechus antilope*; (*S*)-2-hydroxy-3-octanone and minor quantities of 2,3-octanedione (Figure 24). In two separate bioassays, lures of both racemic and pure (*S*)-2-hydroxy-3-octanone were significantly attractive to *X. antilope*.

Significant cross-attraction of *X. antilope* to the pheromone of *P. arcuatus* (see paper IV) was also observed. However, the species-specific pheromone was significantly more attractive, e.g. capturing 7.4 times more beetles than the heterospecific blend during the second year of the three-year monitoring study (paper VII). The heterospecific blend also failed to detect *X. antilope* at 26% of the sites where the species-specific pheromone trapped the species in 2017.

Anaglyptus mysticus is the first species from the Palearctic to be shown to use a combination of (*R*)-3-hydroxy-2-hexanone and 2-nonanone as an aggregation-sex pheromone, but a species in North America utilizes the same two compounds (Mitchell et al. 2013). Schröder (1996) reported (*S*)-2-hydroxy-3-octanone as the major male-specific compound of *X. antilope*, in addition to minor quantities of the enantiomers of 3-hydroxy-2-octanone and traces of the diastereomers of 2,3-octanediol, but no behavioral assays were reported. 3-Hydroxy-2-octanone was likely an artefact due to thermal rearrangement of 2-hydroxy-3-octanone (cf. Sakai et al. 1984), but the significance of the 2,3-octanediols is uncertain as these compounds were not observed, or tested in the work presented here.

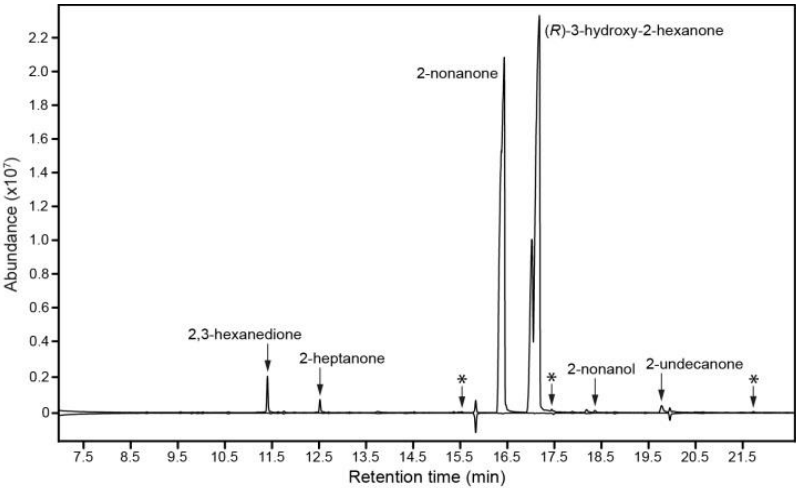


Figure 22. Total ion chromatograms (DB-WAX column) of headspace volatiles collected from adult male (top trace) and female. *A. mysticus* (inverted trace). Asterisks denote trace compounds that were not identified. (*R*)-3-Hydroxy-2-hexanone was in part rearranged into 2-hydroxy-3-hexanone, forming a partially separate peak in front of (*R*)-3-hydroxy-2-hexanone.

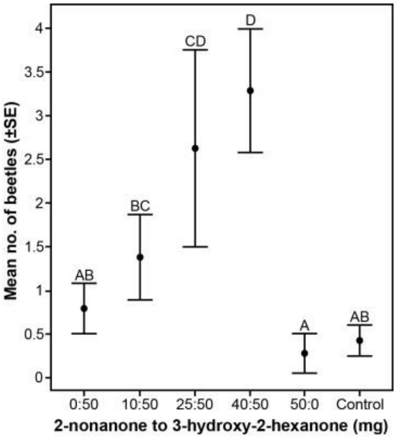


Figure 23. Mean numbers of *A. mysticus* captured per trap and collection date with different quantities of 2-nonanone and racemic 3-hydroxy-2-hexanone (n = 14 samples). Mean values that do not share a common letter are significantly different ($P < 0.05$).

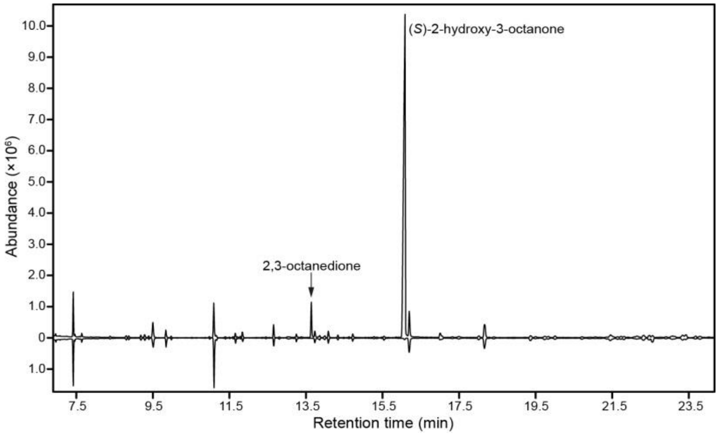


Figure 24. Total ion chromatograms (DB-WAX column) of headspace volatiles collected from adult male (top trace) and female *X. antilope* (bottom, inverted trace).

4.1.5 Pheromone of *P. arcuatus* (paper V)

As in previous work by Schröder (1996), extracts of volatiles from male beetles of this species contained a number of male-specific compounds, but three were present in large quantities and dominated the samples; (*R*)-3-hydroxy-2-hexanone, (*R*)-3-hydroxy-2-decanone and (*R*)-3-hydroxy-2-octanone (Figure 25). GC-MS analyses with high and low injector temperatures also indicated that several compounds such as 2-hydroxy-3-decanone and 2-hydroxy-3-octanone formed through thermal rearrangement of the main components (Figure 26). Eluting the collectors with hexane and diethyl ether gave essentially the same average proportions with a ratio of roughly 7:2:1 (hexanone:decanone:octanone), while dichloromethane gave strikingly different average ratios close to 1:1:1. According to Schröder (1996), (*R*)-3-hydroxy-2-decanone and 2-hydroxy-3-decanone were the two main components, and (*R*)-3-hydroxy-2-hexanone and (*R*)-3-hydroxy-2-octanone were present in minor quantities.

In parallel bioassays in Sweden and Hungary, racemates of the compounds were tested as single compounds, two-component blends, and as a full ternary blend, with ratios similar to those observed in the extracts of volatiles. Results were similar across the two countries, both 3-hydroxy-2-hexanone and 3-hydroxy-2-decanone were necessary to include in the lures in order to achieve significant attraction (Figure 27). Single compounds and blends not including both these compounds were not different from controls. The ternary blend including 3-hydroxy-2-octanone did not capture significantly more beetles than the two-component blend, but the relatively limited bioassays may have been insufficient to detect a difference between these two blends, and further studies should be undertaken to determine the importance of 3-hydroxy-2-octanone.

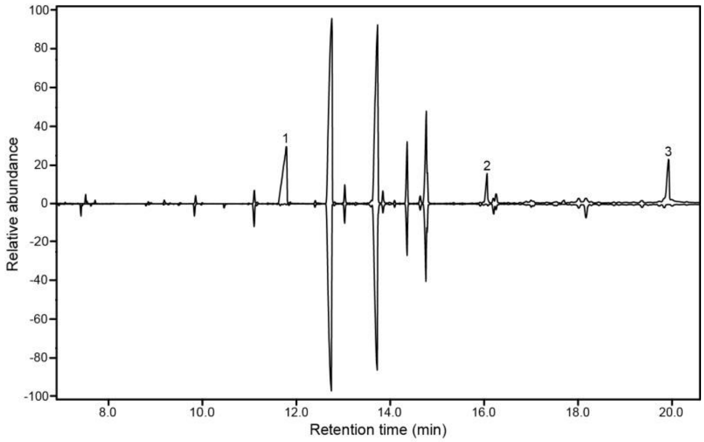


Figure 25. Total ion chromatograms (HP-5ms column) of headspace volatiles collected from adult male (top trace) and female (inverted trace) *P. arcuatus*. Three compounds, (*R*)-3-hydroxy-2-hexanone (1), (*R*)-3-hydroxy-2-octanone (2), and (*R*)-3-hydroxy-2-decanone (3), were male-specific and consistently present in relatively large quantities.

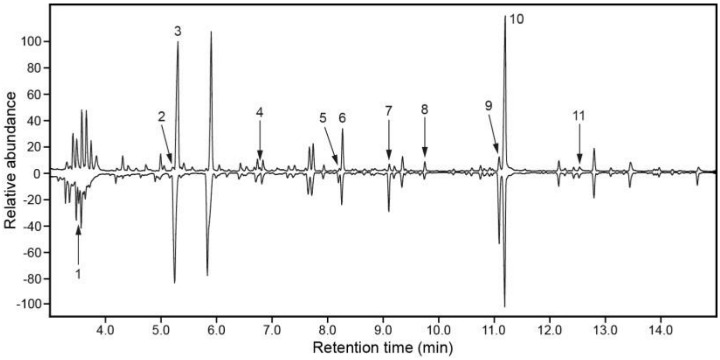


Figure 26. Total ion chromatograms of a hexane extract of headspace volatiles from male *P. arcuatus* collected on Porapak™ Q and analyzed on a DB-17 GC column in splitless mode, showing the effects of thermal isomerization and degradation of the hydroxyketones. Top trace was run with an injector temperature of 125 °C, and the bottom, inverted trace with an injector temperature of 250 °C. Compound identities (italic, confirmed; normal font, tentative): (1) *2,3-hexanedione*, (2) *2-hydroxyhexan-3-one*, (3) *3-hydroxyhexan-2-one*, (4) 3-hydroxyheptan-2-one, (5) *2-hydroxyoctan-3-one*, (6) *3-hydroxyoctan-2-one*, (7) *2,3-decanedione*, (8) 3-hydroxynonan-2-one, (9) *2-hydroxydecan-3-one*, (10) *3-hydroxydecan-2-one*, and (11) 3-hydroxyundecan-2-one

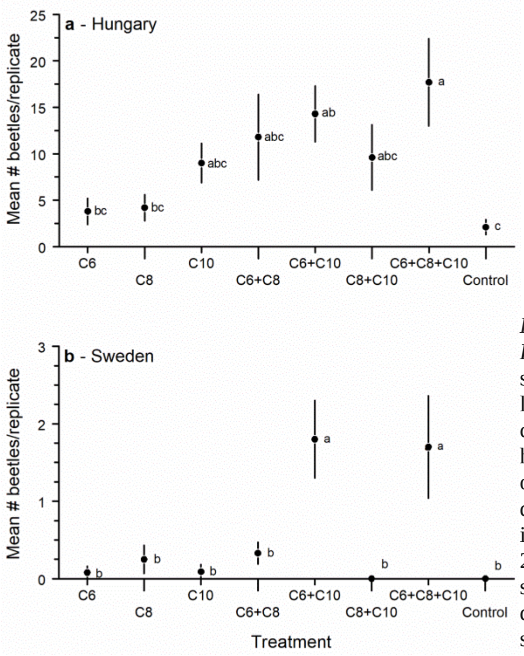


Figure 27. Mean numbers (± SE) of *P. arcuatus* captured per trap and sampling interval in **a** Hungary with lures of different ratios and combinations of 3-hydroxy-2-hexanone (C6), 3-hydroxy-2-octanone (C8), and 3-hydroxy-2-decanone (C10) (all racemates) in isopropanol. **b** Sweden (datasets from 2015 and 2016 combined) with the same lures as in panel a. Means that do not share a common letter are significantly different ($P < 0.05$).

4.2 Application of pheromones for monitoring ephemeral longhorn beetles

The pheromones showed promising qualitative results already during bioassays. For instance, the total number (178) of individuals of *P. pusillus* that were captured in the bioassay, was five times as many as the total number individuals that had been hand-collected by entomologists during a period of 60 years (Lindhe et al. 2010). The three subsequent large monitoring studies clearly demonstrated the effectiveness of using pheromone-based trapping to detect the presence of these ephemeral species. The surveys worked to change the general perception of the distribution and abundance of several species, and how they utilize the landscape. In the literature, red listed species such as *P. sanguineum* and *X. antilope* are described as fairly rare, with local occurrence, but pheromone-based surveys over a mere four-year period revealed that these species have a more or less continuous presence in southwest and south-east Sweden respectively. *Phymatodes alni* was also shown to have a more continuous distribution, except in the southern part of Skåne province where the species appears to be rather local. These three species, and *P. pusillus*, were detected at a great number of sites without previous records and the pheromone-based monitoring also served to extend the current known distribution of several species (particularly *P. pusillus* in Småland province, and *P sanguineum* and *P. alni* in Skåne province). The primary reason why the species have been overlooked is likely that they are highly difficult to observe unless fresh substrates are accessible at ground level and can be inspected during favorable weather conditions, and to some extent also that a couple of species (*P. sanguineum* and *P. pusillus*) are active early in the season, when few entomologists perform field work. Most species were also considerably more numerous in terms of sheer number of individuals present at individual sites, and in the landscape as a whole, compared to what has been observed in pheromone-based surveys of *O. eremita* and *E. ferrugineus* that develop in tree cavities in the same study areas (Forsmark 2012; Larsson et al. unpublished).

The pheromone-based trapping also delivered unprecedented quantitative data that could be used for the first comparisons of habitat utilization and effects of management among the studied species. Previous, large surveys with passive flight-intercept traps at many sites in oak-dominated forest habitats in southeastern Sweden had generated low total captures of these ephemeral species (cf. Franc 2007), leaving little opportunity for meaningful comparisons at the species level. The first quantitative examinations of interspecific associations in terms of correlations of local abundance of the different species revealed none or surprisingly weak degrees of association (section 4.2.3), and correlations between local beetle abundance and oak habitat at different spatial scales indicate that it is necessary to consider rather large spatial scales when examining how the species interact with their environment (section 4.2.4).

The present work did not include applied studies utilizing the pheromone of the endangered species *P. detritus*. The fact that the species is almost extinct in Sweden at present limits the usefulness of the pheromone (Lindhe et al. 2010). However, reintroductions using beetles breed in captivity are ongoing, and the pheromone could provide a cost-efficient, noninvasive tool to systematically monitor reintroductions, in addition to being used for studies of the surviving population in the Stockholm-area.

4.2.1 Hornsö Ecopark 2014 and Skåne province 2015 (paper VI)

The first larger survey to test the trapping efficiency of the blend of common pheromones components (2-methyl-1-butanol and 3-hydroxy-2-hexanone) in 2014 revealed that all four species that can be sampled with this blend had a continuous distribution in the comparatively small study area within Hornsö Ecopark (Figure 28). Virtually all species were captured at all individual trapping sites. Particularly *P. pusillus* and *P. alni* seemed to have been overlooked in many subareas during an extensive survey with passive flight-intercept (window) traps and manual collecting methods (Nilsson and Huggert

2001). The total mean catch of about 60 individuals per trap and site of *P. sanguineum* and *P. alni* respectively was highly surprising given that such numbers of beetles have never before been encountered in surveys using passive flight-intercept traps or hand-collecting (Table 4). Typically single, or a few, individuals are recorded in such surveys. Average captures of *P. pusillus* were much lower by comparison, as the pheromone blend used for the survey is a heterospecific attractant with lower trapping efficiency for this species (paper II). Meaningful quantitative comparisons may be difficult when this blend is used to trap *P. pusillus*. Captures also showed that the average abundance of the species could differ considerably between sites, despite sites being located only one to two kilometer from each other.

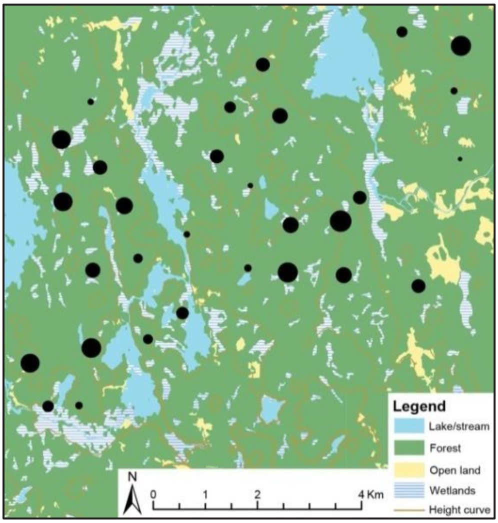


Figure 28. Results from pheromone-based trapping of *P. alni* in Hornsö Ecopark 2014. Filled, black circles denote sites where the species was present. The size of the circle denotes the total average number of captured beetles per trap relative to the catch from the other sites. Map: Vägkartan, vector© Lantmäteriet, Gävle, Sweden.

Table 4. *Results from monitoring studies in 2014 and 2015. The total number of captured individuals only includes the tap catches from the sites that were used for quantitative analyses. Mean catch per trap is the total number of beetles divided by three traps per analysed site.*

Study	Species	Sites with detection	Sites with no detection	Sites in quant. analyses	Total catch	Mean catch/trap
Hornsö Ecopark 2014	*P. sanguineum*	30	0	30	5289	58.8
	P. alni	30	0	30	5511	61.2
	P. testaceus	30	0	30	922	10.2
	P. pusillus	29	1	30	189	2.1
Skåne province 2015	*P. sanguineum*	66	4	57	2574	15.1
	P. alni[1]	44	25	67	589	2.9
	P. testaceus	67	3	68	955	4.7
	P. pusillus	0	70	NA	0	0

1. One site excluded due to excessive trap malfunction.

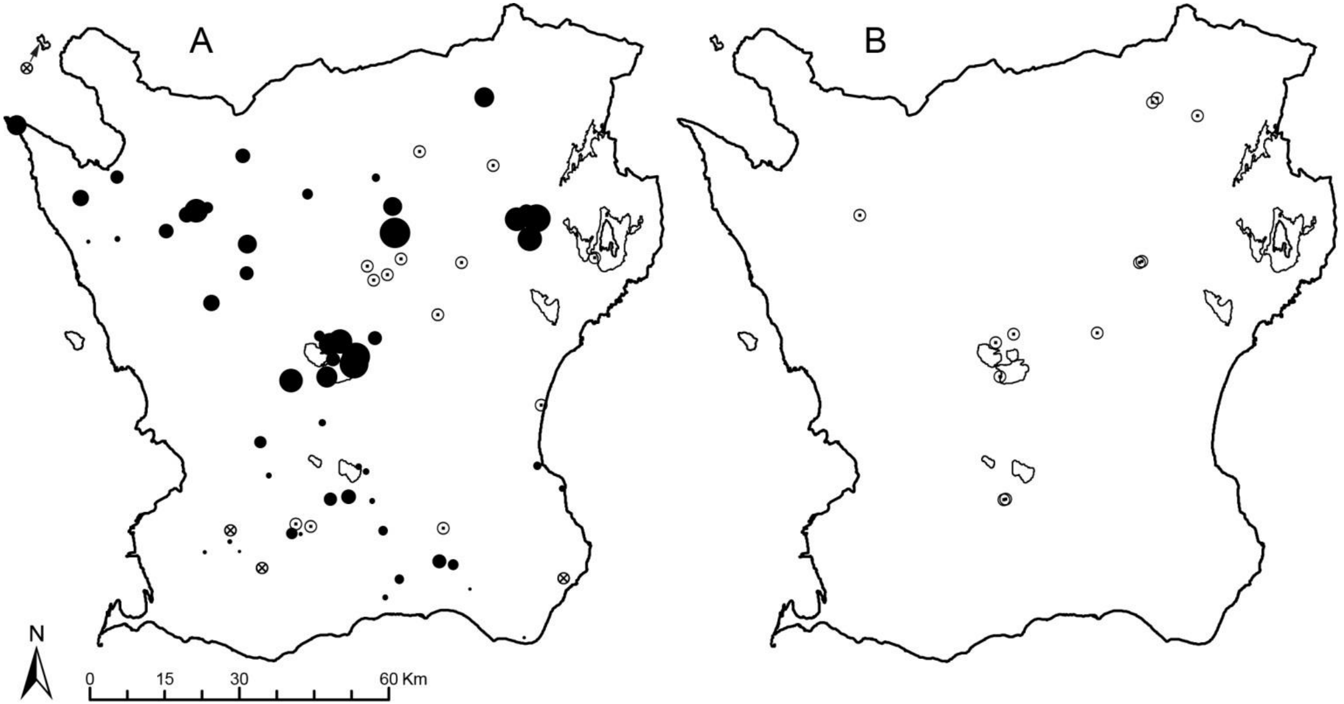


Figure 29. (**A**) Results from pheromone-based trapping of *P. sanguineum* in Skåne province (2015). Filled, black circles denote sites where the species was present. The size of the circle represents the relative total number of captured beetles per trap in relation to the catches from the other sites. Circles with a cross denote sites where the species was not captured. Circles with a small black dot in the center denote sites where the species was present, but from which trap captures were not used for quantitative analyses (due to late deployment of traps). (**B**) All older, publicly reported observations of the species from 1980 to 2014 (Swedish Species Information System 2019a). A circle with black dot in the center denotes the position of an old observation. Map: GSD-Översiktskartan, vector © Lantmäteriet, Gävle, Sweden

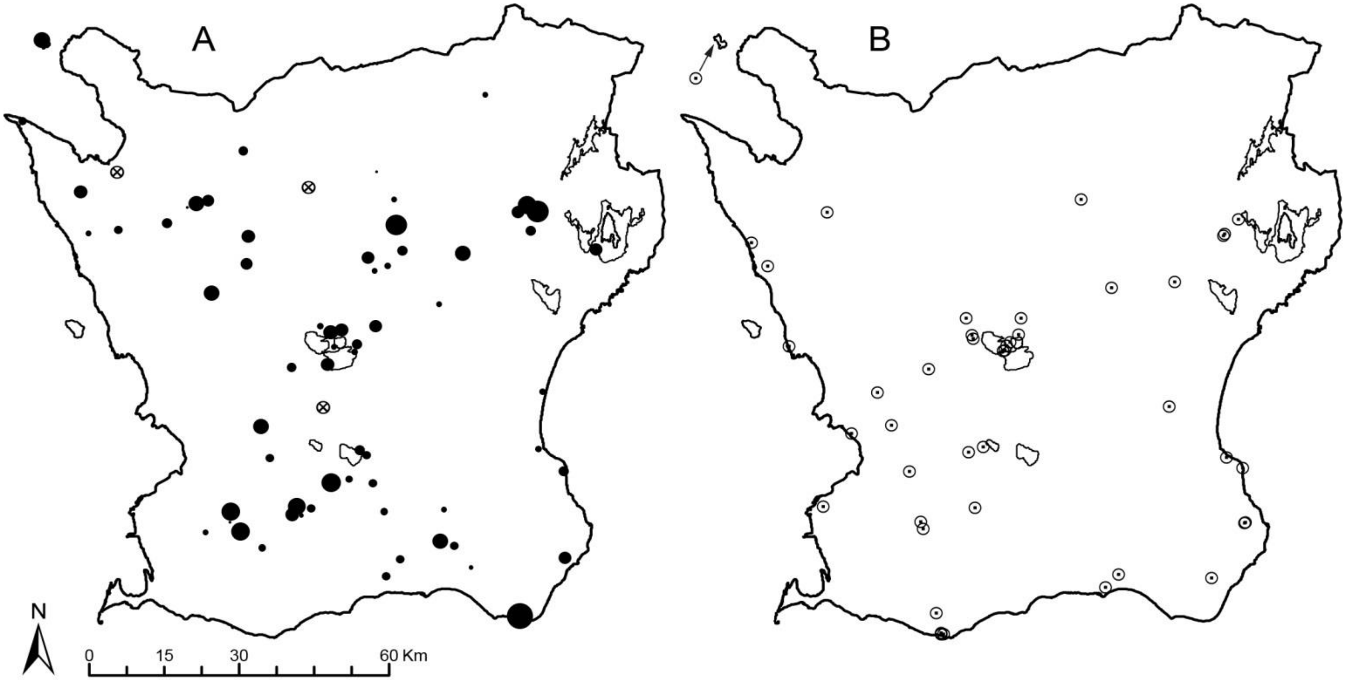


Figure 30. (**A**) Results from pheromone-based trapping of *P. testaceus* in Skåne province (2015). Filled, black circles denote sites where the species was present. The size of the circle represents the relative total number of captured beetles per trap in relation to the catches from the other sites. Circles with a cross denote sites where the species was not captured. (**B**) All older, publicly reported observations of the species from 1980 to 2014 (Swedish Species Information System 2019b). A circle with black dot in the center denotes the position of an old observation. Map: GSD-Översiktskartan, vector © Lantmäteriet, Gävle, Sweden.

Results from the trapping study in Skåne province (2015) with the same pheromone blend showed remarkable results. Past records of *P. sanguineum* indicated that this species was present primarily in the central and northeast part of the province (Figure 29), and that *P. alni* was almost exclusively present in the central part. Based on this, it was expected that the two species would not be present at a high proportion of the surveyed sites. However, in contrast to expectations, the pheromone-based study showed that the species were present at high proportions of the surveyed sites. The survey also showed that previous records gave a highly inaccurate picture of the species true distribution. *Pyrrhidium sanguineum* is present at many sites in the southwest, southeast and northwest of the province, where nearly no recent records were known. Similarly, *Phymatodes alni* was found at a number of sites in particularly the northeast and northwest part of the province, where no recent records were reported, but the species was also missing at quite a high proportion of the sites in the southwest and southeast parts of the province. Interestingly, the results from the pheromone-based trapping of the common species *P. testaceus* were basically identical to the distribution of recent records within the province (Figure 30).

In both study areas, one single year of pheromone-based surveys significantly changed the perception of the occurrence of the species, despite decades of efforts by entomologists, and targeted surveys of saproxylic beetles.

4.2.2 Småland and Blekinge provinces 2016-2018 (paper VII)

This study was the first to use the full toolbox of species with identified pheromones that are associated with oak, and it was also the first study to examine temporal variation of populations. Patterns of presence and absence of the different species at the studied sites tended to be stable from year to year, but local abundance exhibited rather extensive variation from year to year. Much like results from Skåne province, the trapping showed that the species were not local, but in fact occurred at virtually all studied sites within their area of distribution (Figure 31), and the great numbers of individuals present at individual sites were difficult to comprehend. Even the rare species *P. pusillus* was present at all sampled sites located within its previously known distribution, and the study showed that the species also maintains a current presence further north than previously known (Lindhe et al. 2010), although only single individuals were trapped at some of those sites. The small area of distribution of this species is difficult to explain, but clearly indicates that other factors than the availability of suitable substrates influence the distribution of this species, as well as *X. antilope*.

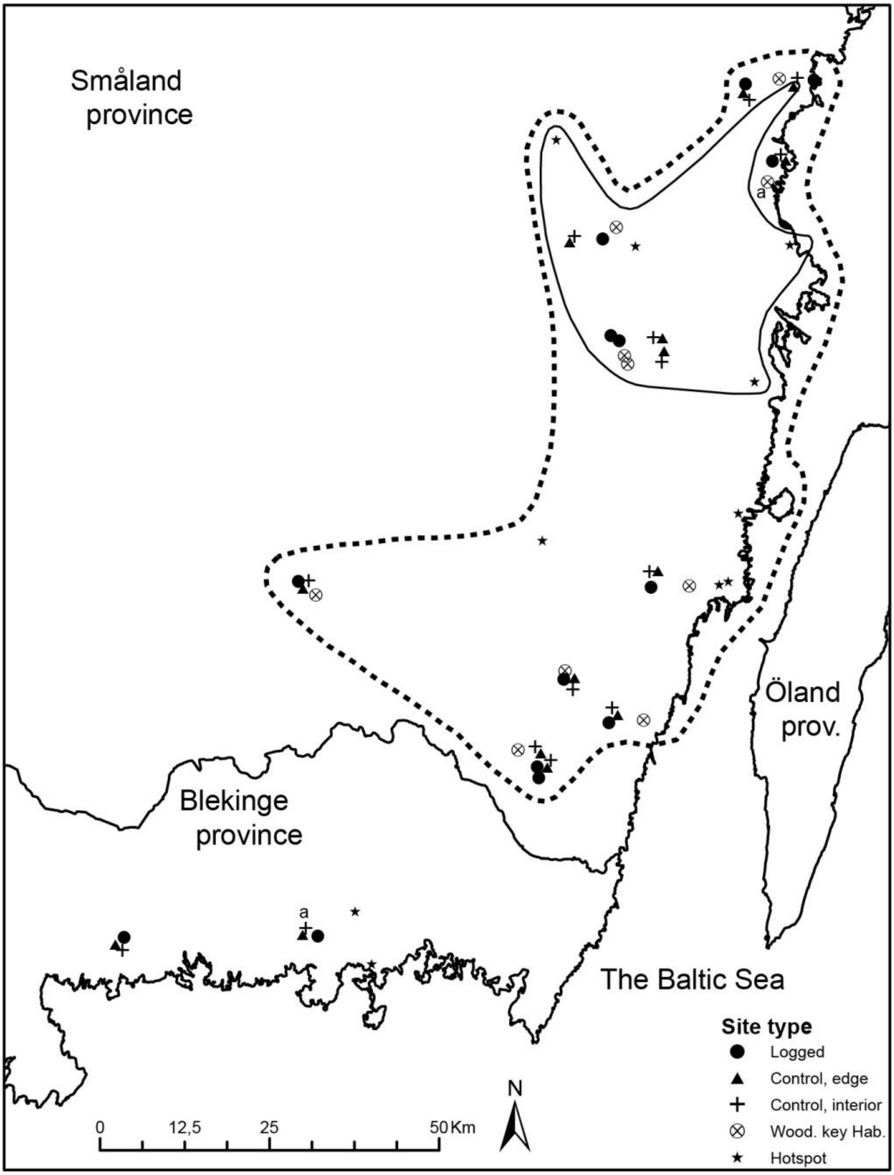


Figure 31. Map showing the distribution of the six studied species 2016-2018 in south-eastern Sweden. Four species, *P. sanguineum*, *P. alni*, *P. testaceus*, and *P. arcuatus* were captured at least one year at all surveyed sites (with the exception of two sites, denoted by a, where *P. arcuatus* was not captured). *Xylotrechus antilope* (hatched line) and *P. pusillus* (solid line) occupied smaller areas, but the two species were captured at all sites within their particular distribution, during at least one year of the study. Due to the large scale, the location of certain sites is shown with a minor offset (<2.0 km) to enhance visibility. Map: GSD-Översiktskartan, vector © Lantmäteriet, Gävle, Sweden.

There was a general tendency for average captures of most species to increase substantially (regardless of oak habitat type) from particularly 2016 to 2017, while results were more mixed from 2017 to 2018. The pattern was especially prominent among trap captures from the edge of the ‘‘stable’’ control sites, where no succession of fresh wood substrates took place. Possibly, this increase was caused by the unusually cold spring of 2015, which exhibited below average temperatures in southern Sweden during May, and a near complete absence of warmer periods (SMHI 2015). Four of the species have their peak activity, or substantial parts of their activity, in this month. Low beetle activity in 2015 could have resulted in low reproductive output with fewer beetles emerging particularly in 2016. The more favorable weather in 2016 may have allowed for a better reproductive output with populations recovering through higher emergence in 2017 and 2018. These types of large scale population changes would be impossible to reveal without the systematic pheromone-based monitoring method, and further studies over time of these species could yield valuable insights into their ecology.

4.2.3 Interspecific associations (paper VI)

Correlations of local species abundance using the datasets from 2014 and 2015 resulted in none, or only weak degrees of monotonic association (Table 5). This was surprising as the species share many similarities in terms of substrate and choice of host tree, and the pheromone-based monitoring should have served to collect systematic data with high precision for particularly the three species utilizing the blend of common pheromone components (*P. sanguineum*, *P. alni* and, *P. testaceus*). Preliminary examinations of species’ correlations using the 2016-2018 dataset were also made, but gave similar results with essentially no, or only weak, correlations among most species. Fine differences in the species’ specific autecology, such as preferred substrate diameter, and perhaps dispersal capacity, paired with strong effects of stochastic processes may explain the lack of strong interspecific correlations in abundance.

In Skåne province, local presence of *P. alni* was almost certain to be associated with occurrence of the two other species, and presence of *P. alni* could to some extent be used as a qualitative indicator of important areas for this set of species, particularly in the southern part of the province. In southeastern Sweden, local presence of *P. pusillus* is also associated with near certain occurrence of the other five study species, but only within the small area of distribution of *P. pusillus*. Outside of that area, presence of any study species is likely to be associated with occurrence of all the other species.

To summarize, it is therefore at present difficult to point to any specific suitable indicator species that should be selected for monitoring from a quantitative or qualitative perspective among the surveyed species. Studies are also needed to examine to what extent the ephemeral longhorn beetles may function as indicators of the presence and abundance of other saproxylic beetles or saproxylic insects in general.

Table 5. *Rank correlations of average total trap catch per site of each species with Kendall's Tau-b correlation for two datasets from Hornsö Ecopark and Skåne province. Significant correlations in* ***bold****.*

Study	Species couplet	Tau-b	*Z*	*P*
Kalmar 2014	*P. sanguineum - P. alni*	0.23	1.77	0.077
	P. sanguineum - P. testaceus	<0.01	0.02	0.986
	P. sanguineum - P. pusillus	0.12	0.89	0.374
	P. alni - P. testaceus	0.02	0.16	0.872
	P. alni - P. pusillus	**0.34**	2.53	**0.011**
	P. testaceus - P. pusillus	0.10	0.74	0.460
Skåne 2015	*P. sanguineum - P. alni*	**0.32**	2.66	**0.008**
	P. sanguineum - P. testaceus	**0.26**	2.59	**0.010**
	P. alni - P. testaceus	0.06	0.54	0.596

4.2.4 What is a relevant spatial scale for interactions between habitat and ephemeral oak longhorn beetles? (paper VI)

In the dataset from 2015, local abundance of *P. sanguineum* and *P. alni* showed an increasing degree of association with habitat (measured as a proxy of total standing oak wood volume in the landscape) when the spatial scale increased (Table 6). The species responded most strongly to habitat at the largest examined scale with a radius of 2.7 km. In contrast, *P. testaceus* showed the highest (but low) degree of association at 900 m, and was essentially not associated with oak volume at the two larger scales. *Phymatodes testaceus* is less dependent on oak compared to the two other species, and the forest map of oak volume may thus be less functional as a habitat predictor for this species.

In the 2014 dataset, non-significant and generally low degrees of associations between abundance and oak wood volume were found for all species at the examined radii of 100 m and 300 m (Table 6). Associations also had a tendency to be negative. The negative associations may appear counterintuitive, but could in fact be informative. Rocky areas in Hornsö

Ecopark are common and are often dominated by large shrub forests of oak (Anonymous 2008; Nilsson and Huggert 2001). These forests tend to continuously produce significant amounts of dead wood of thin dimensions (Figure 3), that likely constitute suitable substrates particularly to *P. alni* and *P. pusillus*. Thus, the shrub forests may be an important habitat type for the beetles, despite low total volumes of standing oak wood. Especially the somewhat higher negative coefficient already at a 100 m radius for the smallest species *P. alni*, that develop in twigs with diameters of about two cm, could be a result of a strong association with local shrub forests of oak, situated in close proximity to the traps. Interactions between saproxylic oak beetles and their habitat at a relatively small scale, as well as at a much larger scale (dataset from 2015), have been observed before (cf. Musa et al. 2013).

Table 6. *Results from Spearman's rank correlations of average total trap catch per site (abundance) and total volumes of standing oak wood (m^3) in circular buffer zones with different radius (scale, meters) around the trapping sites. Radii of 900 and 2 700 were not tested for the dataset from Kalmar County 2014, due to violation of spatial independence of the zones.*

Scale (m) / Study		100		300		900		2 700	
	Species	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
Skåne 2015	*P. sanguineum*	0.09	0.496	0.20	0.133	0.20	0.140	0.32	0.092
	P. alni	0.01	0.991	0.21	0.115	0.23	0.077	0.34	**0.031**
	P. testaceus	0.08	0.571	0.13	0.319	-0.09	0.501	-0.01	0.961
Kalmar 2014	*P. sanguineum*	0.05	0.783	-0.15	0.444				
	P. alni	-0.31	0.102	-0.21	0.274				
	P. testaceus	0.12	0.546	0.26	0.168				
	P. pusillus	-0.07	0.709	-0.12	0.536				

Overall, the limited examination here indicates that the national kNN oak volume forest map has some potential to be used as a proxy for habitat availability for these species, and as a basis for detailed analyses of relevant spatial scales and the species' landscape ecology. Such analyses should consider relatively large spatial scales as indicated by the 2015-dataset, and it is possible that examination of even larger scales could have resulted in even higher degrees of association. However, the observations from the 2014 dataset also suggest that the association between beetle abundance and the volume of oak can be modified by oak habitat type and its structure, and points to the necessity of including other environmental variables in analyses. In a similar study by Holland et al. (2005) nearly all cerambycid species (12 tested) showed the greatest degree of association with forest cover at a scale of 1.5 km or less (up to 2 km were tested), but all studied species belonged to the subfamilies Lepturinae or Laminae, and these species are usually associated with less ephemeral wood substrates than the species examined here. Species associated with older wood substrates are often more sedentary, which could explain their interaction with habitat at smaller spatial scales.

4.2.5 Effects of oak habitat management and habitat structure (p. VII)

Four species, including the three red-listed species, showed no differences when comparing beetle abundance at the edge of oak production stands (without recent logging activities) and the two types of set-aside oak habitats (woodland key habitats and oak conservation hotpots) (Figure 32). Further, the abundance of *P. arcuatus* was significantly higher at the edge of the production stands, compared to the WKH, but did not differ from the abundance at the hotspots. One species (*P. testaceus*) exhibited significantly higher abundance at the two set-aside habitats. This suggests that the studied set-aside oak-dominated habitats, with comparatively passive management (i.e. practically no management, or grazing alone to maintain overall habitat structure), did not provide any substantial positive effects for the studied species, and that most species utilized set-aside habitats at about the same extent as ordinary oak production stands managed for high yields of quality timber. This is a striking difference compared to what has been observed in many studies of other saproxylic beetles (e.g. Franc et al. 2007; Sverdrup-Thygeson et al. 2014; Hämäläinen et al. 2018), and particularly the species that depend on oaks with tree hollows or large-diameter substrates. The latter species are often missing in production stands and rely almost exclusively on set-aside, typically protected, woodland and forest habitats that are not actively managed for production of forest resources. The survey of fresh wood substrates at the sites in 2016 indicated that substrate quantities were low, but similar, across the three different oak habitats (see supplement paper VII), and this was likely the main reason as to why beetle abundance of most species did not differ.

The recently logged oak production stands, where the active forest management had generated higher quantities of suitable wood substrates, were a sharp contrast to the largely unmanaged oak habitats discussed above. The comparison of beetle abundance at the logged stands and the edge of the matching production stands (without recent management) revealed species-specific, and varying, responses over time (Figure 33). However, most species tended to be significantly more abundant at the recently logged stands compared to the controls. The fresh wood at the logged sites likely recruited

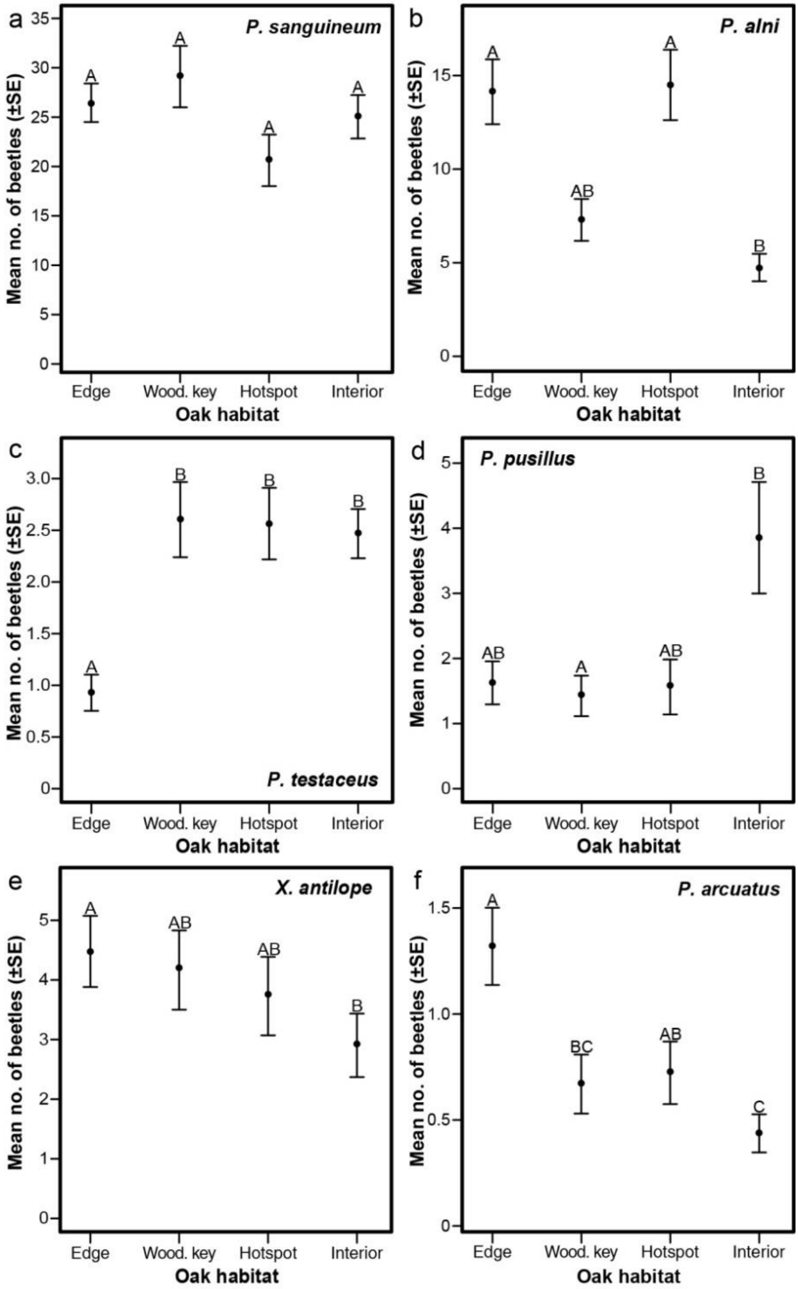


Figure 32. Mean number of beetles per pheromone trap and trapping interval of six species of longhorn beetles captured in four different types of oak habitats; the edge of production stands, woodland key habitats, hotspots and the interior of production stands. Two years (2016-2017) of repeated sampling are pooled. Habitats that do not share a letter are significantly different ($P < 0.05$), according to pairwise least squares means analysis following GLMMs.

individuals from the surrounding landscape that oviposited on the substrates in 2016. The colonization phase was replaced by new generations of beetles that emerged from the substrates in the following two years (2017-2018). The abundance of four species also showed a tendency to become more even at the logged and control stands over time, indicating that the positive effect of the fresh substrates quickly diminished, but *P. alni* and *X. antilope* displayed a different pattern over time. Similar positive, but short-lived, responses have been observed for conifer-associated saproxylic beetles when fresh, deadwood substrates of conifers were created in boreal forests (Komonen et al. 2014). Previous assessments of the whole saproxylic beetle community following partial cutting in oak-dominated WKH and nature reserve forests in southern Sweden by Franc and Götmark (2008) have shown mixed effects. Many species increased in abundance after partial cutting, but red listed species richness did not change, and the latter group of species may in fact have been disfavored by the cutting (Franc and Götmark 2008).

It should be noted that the results from the study of the short-term effects of logging, and the comparison of species' abundance at oak production stands and set-aside habitats, were obtained from a landscape which is rich in oak and results from monitoring showed that most species maintained rather continuous distributions in the area. Local habitat utilization by species can vary depending on the structure and habitat availability in the surrounding landscape (e.g. Gibb et al. 2005). Thus, in a landscape with lower, and more fragmented, occurrence of oak (such as the southern part of Skåne province), the positive effects of logging could be proportionally greater, and the role of set-aside habitats may differ.

The comparison of beetle abundance at the sunny edge and the shady interior of oak production stands (controls) also revealed species-specific preferences in four species, and no preference in two species (*P. sanguineum*, and *P. pusillus*). Three of the four species were significantly more abundant at the edge of the control stands, and one species was significantly more abundant at the shady interior (*P. testaceus*). The mixed preferences are similar to what was observed in another study utilizing pheromones for sampling saproxylic cerambycids in North America (Allison et al. 2019), but the result is also similar to previous qualitative observations in Sweden that most of the study species prefer more open, sun-exposed habitats (e.g. Palm 1959). A study of sun-exposure and the entire saproxylic beetle community on fresh oak high-stumps have presented similar results (Lindhe et al. 2005), and early-successional saproxylic insects in general are favored by sun-exposed habitats, while late-successional species prefer shaded environments (Jonsell et al. 1998). The result suggests that oak habitats with relatively open structure are

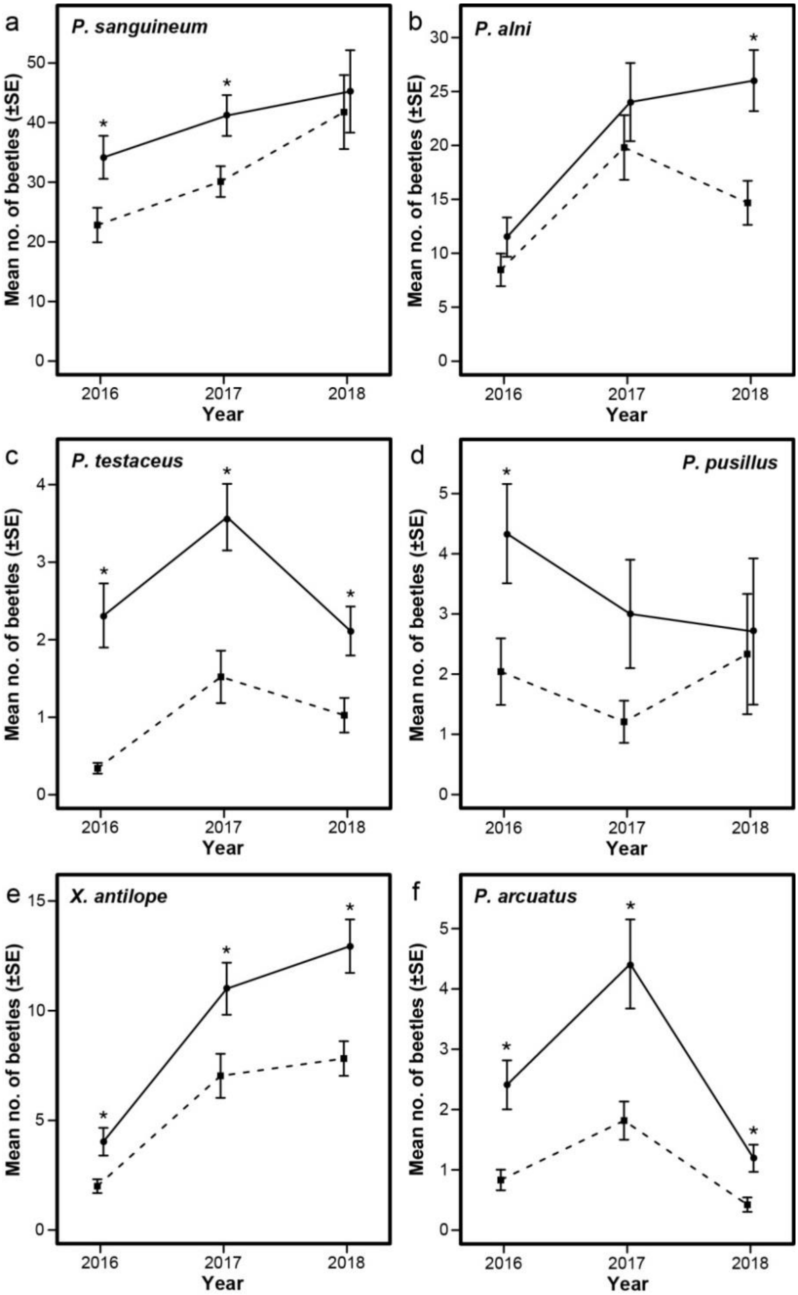


Figure 33. Mean number of beetles of six species of longhorn beetles captured per pheromone trap and trapping interval at recently logged oak production stands (solid line), and matching control stands without recent logging activities (hatched line). The stands were sampled for three consecutive years. Years with an asterisk indicate a significant difference ($P < 0.05$) between the logged stand and the control stand, according to pairwise least squares means analysis following GLMMs

more beneficial for the guild of ephemeral longhorn beetles, than dense, closed-canopy habitats, and that nature management that opens up, or maintain, relatively open oak habitats will favor the majority of species. Interestingly, the two species which showed no difference in abundance are also the two species that are active earliest in the season, with peak-activity at the same time as the oak leaves begin to unfold, which likely compromised the comparison.

To summarize, the observations indicate that three of the different oak habitats have similar ability to support ephemeral oak longhorn beetles, but the recently logged sites are more favorable particularly due to the presence of comparatively large quantities of fresh substrates. Shady oak habitats are less attractive to several species. The preference of three species for sun-exposed oak habitat were likely also a contributing factor to the positive response that was observed to the recent logging and thinning activities in production stands. Both the structural change from relatively dense, shady oak forest to a more open, sunlit habitat, combined with the substantial increase of fresh wood substrates, was positive in a short-term perspective. The effect of a more open canopy-cover after the logging event will also last longer than the effect of the fresh substrates. In light of these results, ephemeral oak longhorn beetles appear to be an example of a group of species that can easily benefit from active management of oak forests for production purposes, provided that forest management and biodiversity preservation becomes more integrative (cf. Löf et al. 2016; Heikkala et al. 2016; Roth et al. 2019), and wood substrates are left on site. On the whole, this may provide a more positive (and cost-efficient) effect for these species compared to setting aside habitats without any particular active management. However, it should be stressed that many other saproxylic beetles associated with oak, such as species dependent on tree cavities, are difficult to maintain in non-set-aside areas in a way that is sustainable from economic perspectives, and the set-aside areas are crucial to the survival of these species at present. The quantity of substrates generated from logging or thinning that need to be retained on site for the study species I this work is a key issue and further studies of this complex question are urgently needed to achieve optimal cost-benefit tradeoffs. It is also likely that ephemeral longhorn beetles with their rapid temporal and spatial population fluctuations exhibit source-sink dynamics. Sites with fresh logging residues could serve as important source habitats that export beetles to the surrounding landscape. If so, complete and long-term removal of all wood substrates at logged sites may have significant negative effects for populations over time. Pheromone-based monitoring may prove to be a useful tool to also target these and many other questions associated with ephemeral longhorn beetles.

5 Conclusions and future perspectives

The first part of this work resulted in identifications of the aggregation-sex pheromones of three species of longhorn beetles whose pheromone chemistry had not previously been studied. Revisiting the pheromone chemistry, paired with behavioral assays, for five other species either confirmed previous identifications, or resulted in important differences, particularly for *P. pusillus* and *P. sanguineum*. In fact, the two latter species proved to produce blends of partially different components compared to what had been reported previously (Schröder 1996). The studies revealed that the pheromones consisted of single compounds or simple blends of two to three compounds. Altogether seven different main compounds could be recognized; four hydroxyketones, two alcohols and one ketone. Several species shared the same compounds and racemic solutions were adequate to elicit significant attraction, even though the beetles produced one specific enantiomer. For species that produced blends, single compounds were usually unattractive, but a synergy occurred when the compounds were applied in a blend, and significant attraction could be reached. The ratio of the components also appeared important for attraction, which is analogous to many other insects (e.g. Löfstedt and Herrebout 1988). Significant cross-attraction to the pheromones of heterospecifics was observed by a couple of species (*P. pusillus* and *X. antilope*). This was confusing during early work stages, but later comparisons showed that the species' own pheromones were more attractive, and should be the preferred tool to obtain a high trapping sensitivity.

Several factors observed in this work combine to make the pheromones suitable for large-scale monitoring studies of the cerambycid community on ephemeral oak substrates; (1) the compounds and blends are relatively non-complex from a chemical point of view, (2) beetle attraction to racemic solutions is significant, (3) most compounds are commercially available, or can be synthesized in large quantities at reasonable cost, and (4) several species share the same compounds. It may be possible to monitor all species with a single multi-component pheromone blend, which would serve to make the monitoring more effective. The number of traps needed per site to monitor all species is a key determinant of how many sites that can be sampled due to time- and cost-constraints. If the same traps can be used to capture all species, it would be possible to either monitor more sites, or increase the number of traps per site to achieve greater precision of the estimates of local beetle abundance (if necessary). However, the blends need to first be studied for antagonistic effects as the compounds of one species can occasionally inhibit responses of other species (Millar and Hanks 2017; Hanks et. al. 2019). A handful of other cerambycid species occur in Sweden, which should be studied in terms of their pheromone chemistry as well, in order for the toolbox to include all relevant species dependent on fresh, ephemeral deadwood substrates of oak. However, the work here has significantly expanded the available knowledge on the use of pheromones by European species of the Cerambycinae subfamily, which had received little recent attention compared to North American species of this subfamily (see Millar and Hanks 2017).

In the second part of the work, large-scale pheromone-based surveys served to significantly change the basic perception of which parts of the landscape in southern Sweden that several species utilize, over the course of just a few seasons of field work. Species such as *P. sanguineum* and *P. alni* had been perceived as occurring locally, but the pheromone-based monitoring demonstrated that these species in fact maintain a much more continuous presence in the landscape, and that the sheer numbers of beetles present is much higher than what could be inferred from previous studies (Franc 2007). The surveys also presented an example how the pheromone-based approach largely corroborated the distribution of one rare species, *P. pusillus*, which due to unknown factors is limited to a small geographic area. Overall, the pheromone-based trapping approach is considerably more efficient and reliable for detection and quantification of the species than other survey methods, partly due to that passive flight-intercept trap can rarely be positioned at relevant micro-habitats (fresh wood) where the species gather, and that many potentially suitable wood substrates are impossible to inspect as they occur above ground level. The three-year monitoring study also revealed substantial temporal variation in total beetle abundance at landscape level and that individual survey years may be poor indicators of the status and development of populations, pointing to the necessity of obtaining structured time series to properly evaluate population trends during for instance Red List assessments (Fox et al. 2018). Such variation, driven by environmental factors acting at large scales, would be exceedingly difficult to quantify with any other approach than systematic pheromone-based trapping.

The surveys also showed that local abundance of the different study species exhibited none, or low degrees of interspecific association. Thus, it appears difficult to select indicator species from the toolbox of species that can represent the whole community of cerambycids on fresh, ephemeral oak wood substrates. An important task for the future will be to examine to what extent the species may function as indicators or umbrella species for other saproxylic insect biodiversity dependent on various types of oak substrates.

The first applied ecological studies utilizing the local pheromone-based trap captures as estimates of abundance showed promising results and opens up several avenues for future work. The quantitative estimates of local abundance of the species were successfully used to study effects of oak forest management and habitat structure, and species' response to their habitat at different spatial scales. In contrast to many other studies of saproxylic beetles associated with oak, the studies here showed that most study species utilized oak habitats designated for production of forest resources at about the same extent as set-aside habitats specifically intended to support biodiversity. Examination of the response of the study species to a common active management practice (logging or thinning events) in oak production stands showed that this benefitted the species, and appeared to be chiefly dependent on the generation and retention of the fresh wood substrates that are pivotal for their reproduction. Similar to many other studies of saproxylic oak beetles, most species also preferred relatively open, sunlit conditions to closed, shady conditions, and nature management that results in habitats with a less dense structure should be most favorable for this group of species. These findings are important as they indicate that the most effective strategy to preserve the species studied here, in terms of cost to benefit tradeoffs, is to make adjustments of oak forest management within habitats that are currently used for production of high-quality oak timber, rather than setting aside habitats as protected, but usually unmanaged, areas. For this strategy to be successful, it will be essential to retain certain quantities of fresh deadwood substrates of low dimensions after forest management actions.

However, the quantitative applied studies and qualitative observations also point to that this community of beetles, in similarity to their substrates, occur as a spatially and temporally highly dynamic 'cloud'' of individuals in the landscape, and that particular sites serve opportunistically as source patches, exporting individuals to the surrounding landscape, while other sites serve as sink habitats (cf. Hedin et al. 2008). These conditions, the stochastic nature of events that benefit (or impair) the species, and the fact that the species seem to interact with their habitat at large spatial scales pose significant challenges for constructing more detailed models of their habitat requirements. Further, direct quantification in the field of relevant environmental factors for these species is difficult, and already existing, up-to-date datasets with large spatial coverage (such as the kNN oak forest wood volume map), and high resolution, are few. To speculate, the availability of suitable wood substrates may be the single most important factor at local scale, whereas at larger scales the proportion of oak in the landscape, complexity of the landscape (heterogeneity, measured as amount of forest edges etc.), and climate may prove to be essential factors. Future studies will have to determine what role pheromone-based data can have for extensive, detailed analyses of the species' ecology, but the pheromone-based trapping stands out as the best way to begin to disentangle the problems and sort out the important signals from background noise. As exemplified by the study of beetle response to logging, the sensitivity of the pheromone-based monitoring system is clearly high enough to capture some of the important factors and processes.

Lastly, the work presented here confirms previous conclusions that pheromone-based monitoring of saproxylic insects is useful, and in many situations highly advantageous. These inferences have up till now been derived primarily from studies of species dependent on veteran oaks and tree-cavities (e.g. Burman 2016; Harvey et al. 2017; Larsson 2016). The portfolio of ephemeral cerambycid species that was established in this work will form an important addition to the small, but growing, number of rare and threatened species that can be monitored with pheromones, and has made it possible to study a completely new community of species with ecology and conservation requirements that are different from those of the species dependent on veteran oaks. The present toolbox should be relevant for applications primarily in Northern Europe (but *P. detritus* and *P. pusillus* are also rare and threatened in parts of Central and Western Europe). However, if the pheromone chemistry of other species, with similar substrate requirements, could be studied in different parts of Europe, it would be possible to assemble a larger toolbox and initiate integrated pan-European monitoring schemes (cf. Henry et al. 2008). Among many other applications, this could aid in the process to evaluate to what extent conventions for preserving biodiversity in the EU are fulfilled (European Environment Agency 2012). Given the species' dependency on fresh substrates, these could also serve as models in an early warning system to efficiently detect changes in fresh deadwood availability across Europe at early stages, before these changes affect guilds of species that depend on late-successional wood decay stages. Such monitoring systems are needed in face of the rapid and accelerating biotic and abiotic changes that are currently transforming European forest and woodland ecosystems.

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Populärvetenskaplig sammanfattning

Den biologiska mångfalden i världens skogar minskar alarmerande snabbt. Den huvudsakliga faktorn bakom tillbakagången är människans allt intensivare nyttjande av skogen för produktion av olika råvaror såsom sågvirke och bränsle, vilka är av stor ekonomisk betydelse i många länder, inklusive Sverige. Med nuvarande brukningsmetoder leder detta utnyttjande tyvärr också till att en stor mängd livsmiljöer som olika arter är helt beroende av för sin överlevnad minskar, försämras och fragmenteras (delas upp i mindre områden som arter har svårt att överleva i eller hitta till). När livsmiljöerna minskar, minskar också de arter som är beroende av dem eftersom arterna har mycket små möjligheter att hinna utveckla evolutionära anpassningar som hjälper dem överleva i nya miljöer när förändringarna sker så snabbt som för närvarande. Framöver kan även effekterna från den accelererande klimatförändringen komma att påverka många arter och livsmiljöer negativt. Omkring ett femtiotal skogslevande arter som tidigare varit bofasta i landet har försvunnit helt från Sverige och betraktas som nationellt utdöda. Förlusten av biologisk mångfald är oroande eftersom den kan orsaka störningar i ekosystemet som gör att de ekosystemtjänster som det mänskliga samhället fortfarande är beroende av upphör att fungera, med enorma ekonomiska kostnader som följd. Från ett ekonomiskt perspektiv är det därför viktigt att nyttja skogarna på ett sätt som är både kortsiktigt ekonomiskt, såväl som långsiktigt biologiskt, hållbart för att kommande generationer inte ska belastas med stora miljöproblem som kan visa sig mycket dyra och näst intill omöjliga att lösa.

Skogar hyser en enorm biologisk mångfald som fördelar sig på ett stort antal olika grupper av djur och växter. Bland dessa märks särskilt en stor mångfald av olika vedlevande, eller saproxyliska, insekter och i synnerhet vedlevande skalbaggar som omfattar omkring 1 200 arter i Sverige (eller cirka en sjundedel av alla vedlevande arter i landet). Vedlevande skalbaggar är på ett eller annat sätt beroende av död ved eller gamla träd under någon del av sin livscykel. Oftast utvecklar sig deras larver inuti död ved som de förtär som föda och därmed bidrar till att bryta ned veden och möjliggöra cirkulationen av näringsämnen i skogen. Larverna kan leva flera år i veden medan de vuxna skalbaggarna oftast lever under några veckor på sommarhalvåret. I stort sett alla typer och delar av döda träd utnyttjas av olika vedskalbaggars larver, allt från grova stamdelar och bark, till tunna kvistar och skott samt rötter. Olika arter är ofta specialiserade och lever endast i en viss typ av ved eller på en enda trädart. Vedskalbaggar är också en viktig födoresurs för andra arter, särskilt hackspettar. Många vedskabaggar har minskat kraftigt i Sverige och i vissa fall helt försvunnit från landet, alternativt försvunnit från stora delar av sina forna utbredningsområden. En hög andel av arterna har tagits upp på den nationella rödlistan över hotade arter som riskerar att försvinna från landet inom ett kortare till medellångt tidsperspektiv. Insatser har gjorts speciellt under senare årtionden för att på olika sätt vända den negativa trenden för vedskalbaggar, och skogsbiodiversitet i allmänhet, men tyvärr pekar nuvarande bedömningar på att dessa ansträngningar sammantaget inte varit tillräckliga för att vända, eller stabilisera, den negativa trenden. I samma takt som positiva förändringar genomförts har nya skötselmetoder för att maximera produktiviteten också utvecklats, vilket fått motsatt effekt. Exempelvis är tillvaratagandet av tunna kvistar och grenar (så kallat GROT) för flisning till biobränsle en ny företeelse som sannolikt påverkar många arter negativt.

För närvarande försöker samhället lösa problemen kring ekonomiskt hållbar produktion i skogen, och samtidigt bibehållen biodiversitet, genom en kombination av olika åtgärder. En del områden sätts helt av från produktion som skyddade områden för biologisk mångfald (exempelvis naturreservat). I produktionsskogen tas olika grader av hänsyn till de specifika miljöer och strukturer visas är viktiga. Exempelvis lämnas en del död ved kvar vid skogliga skötselåtgärder såsom gallring eller slutavverkning. Dessa åtgärder tycks hittills dock inte vara tillräckliga och en del av problemet kan vara att kunskapen om vad olika typer av biologisk mångfald kräver för sin överlevnad saknas eller är otillräcklig. Detta beror i sin tur på att arters krav ofta är komplexa, samtidigt som antalet arter är mycket stort, men också delvis på att det saknas praktiska verktyg för att kunna studera många arter som är sällsynta, lever undanskymt, eller vistas på platser som är svåra att inventera praktiskt. Bland vedskalbaggar finns många sådana arter som av olika anledningar är svåra att komma i kontakt med. På senare tid har sexuella lockdofter, så kallade feromoner, i insektsfällor lanserats som ett effektivt verktyg för att kunna studera arter som annars nästan aldrig observeras med andra inventeringsmetoder. Doftämnena lockar till sig individer av specifika arter som fångas i fällorna. Arternas närvaro och antal på en plats kan ge information om hur gynnsam en naturmiljö är, eller hur pass positiv effekten av en viss typ av miljöskötsel är. Hittills har främst skalbaggar som lever tillbakadraget inuti ihåliga träd studerats med feromoner, framförallt därför att feromoner bara identifierats från ett litet antal naturvårdsintressanta arter.

I det här arbetet identifierade jag feromoner för långhorningsskalbaggar, vars larver lever i färska nyligen döda, tunna vedtyper av ek, främst kvistar och grenar eller tunna stamdelar. Dessa arter utgör en annan grupp av vedskalbaggar och lever inte undanskymt, men arterna har varit svåra att studera tidigare eftersom andra typer av insektsfällor har svårt att påvisa och uppskatta deras förekomst på ett tillförlitligt sätt. Antagligen lever arter ofta i vedsubstrat som sitter uppe i träden där de är svåra att komma i kontakt med. Med hjälp av doftuppsamlingar från långhorningarna, instrument för kemiska analyser och fångst med insektsfällor i naturen kunde jag identifiera sju ämnen som utgör attraktiva lockdofter för åtta olika arter av långhorningar. Ämnena är så kallade kolväten som huvudsakligen består av kolkedjor och väteatomer. Efter att jag identifierat ämnena använde jag dessa för omfattande fältstudier på många platser i sydligaste Sverige för att undersöka hur pass effektiv den feromon-baserade inventeringen är för att påvisa lokala förekomster av studiearterna. Resultaten visade att feromonerna var mycket fördelaktiga för att påvisa lokala förekomster av långhorningarna och undersökningarna kunde visa att flera arter hade en större utbredning och rikare förekomst än vad som tidigare varit känt, antagligen därför att andra inventeringsmetodiker lätt förbiser dem. Jag studerade också huruvida de lokala antalen fångade individer av olika arter samvarierade, men fann att dessa uppvisade liten tendens till samvarians. Detta indikerar att man behöver inventera samtliga arter, snarare än enskilda representativa indikatorarter, om man vill skaffa sig information om deras kvantitativa förekomst på lokal nivå. Vidare visade mina studier att antalet fångade skalbaggar på lokal nivå (åtminstone för ett par arter) tycktes kunna ha ett samband med volymen stående ekved som var starkast när relativt stora områden studerades. Detta pekar mot att arterna samspelar med sin livsmiljö över betydande ytor och att man behöver analysera naturmiljön över stora områden om man ska få en djupare förståelse för vilka miljöfaktorer som är viktiga för arterna, samt att skötselåtgärder för dessa långhorningar troligen behöver appliceras storskaligt i landskapet, snarare än lokalt.

I en treårig studie använde jag feromonfångst för att jämföra hur mängden individer av de olika arterna fördelade sig på olika typer av ekdominerade trädmiljöer. Antalet individer av de flesta arterna var detsamma i produktionsbestånd av ek som i nyckelbiotoper och i så kallade värdekärnor för eklevande insekter (oftast naturreservat eller Natura 2000-områden). Båda de senare naturtyperna är avsatta från skoglig produktion för att gynna biologisk mångfald, men positiva effekter föreföll saknas för flertalet av de studerade långhorningarna. Vidare föredrog de flesta arterna ljusöppna ekmiljöer framför skuggiga miljöer. Jag studerade också nyligen gallrade eller delvis slutavverkade ekbestånd och jämförde dessa med liknande kontrollbestånd där inga skogliga åtgärder utförts sedan en tid tillbaka. Jämförelsen visade att de flesta arterna åtminstone kortsiktigt gynnades av gallring och huggning under åren som följde direkt efter åtgärden i beståndet, eftersom antalet skalbaggar av de flesta arterna generellt var högre i de behandlade bestånden jämfört med i kontrollbestånden, även om olika arters svar på skogsåtgärden varierade. Den viktigaste faktorn som förklarar arternas högre antal i de nyligen huggna ekbestånden var av allt att döma att färska vedsubstrat av ek (grenar och tunna stamdelar) lämnats i samtliga studerade bestånd efter de skogliga åtgärderna, vilka skalbaggarna kunde reproducera sig i, och som gav synbart mer lämplig ved för arterna än i de obehandlade kontrollbestånden. Dessutom blev de behandlade bestånden mer ljusöppna, vilket sannolikt bidrog till den positiva effekten.

Sammantaget visade sig långhorningarnas feromoner vara ett ypperligt verktyg för att studera dessa arters utbredning och antal, och tycks även kunna utnyttjas för omfattande studier av arternas ekologi. Ur skogskötselsynpunkt förefaller det som att just dessa arter lätt, och kanske rentav bäst, kan bevaras i produktionslandskapet snarare än i skyddade områden, men det förutsätter att färska vedsubstrat lämnas i produktionsskogen i samband med skötselåtgärder, och att inte alla substrat tas om hand som biobränsle. Det ska också poängteras att andra grupper av vedlevande arter, med andra miljökrav, har svårt att överleva i produktionsskogen och därför behövs både en bättre anpassad skötsel av produktionsbeståden, såväl som skyddade områden, om den biologiska mångfalden i skogen ska kunna säkras. Framtida studier får utvisa hur mycket vedsubstrat som bör lämnas och kanske kan feromon-baserade studier av långhorningarna spela en roll även vid sådana studier. Det är också möjligt att feromonerna skulle kunna användas för årlig, systematisk och kostnadseffektiv övervakning av dessa arter för att utvärdera hur deras populationer utvecklar sig över tid både i Sverige och internationellt. Liknande nationella övervakningssystem finns för exempelvis fåglar och dagfjärilar. Övervakning av vedlevande långhorningar, eller vedlevande insekter i stort, skulle kunna vara ett värdefullt komplement till existerande miljöövervakningssystem.

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