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„The status of a Mediterranean forest reserve (Pineta san Vitale, Ravenna) prone to degradation: A case study on moths“

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„The status of a Mediterranean forest reserve (Pineta san Vitale, Ravenna) prone to degradation: A case study on moths“

Cover photo: Pictures of Pineta san Vitale in 2011 (left, grey) and 2017 (right, colored) exemplarily showing the changes in vegetation due to succession. The Cream-spot Tiger moth (*Arctia villica*) indicates the analysis of moth communities in the course of the time. © Mirko Wölfling.

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1. Abstract

The biodiversity crisis is in full swing and countless species of insects are affected. Their functions in ecosystems and for us humans are irreplaceable. Therefore, scientists collect data to quantify the extent of the crisis and to find solutions. The acquisition of new data and their comparison with historical samples is fundamental for such analyses.

In my thesis, I took a look in the past and reconstruct, by means of a rare long-term record, how moth communities have changed in the isolated forest reserve Pineta san Vitale (NE Italy, Ravenna). Historical collections from museums and own empirical data spanning 15 years of sampling were used to create a time series over 80 years.

Therefore, I searched for solutions how to analyze the combination of historical and empirical data as reliable as possible. The first step was, to analyze the settling behavior of 1426 moth individuals at a manual light trap to check for a possible sampling bias depending on the type of the trap. I distinguished between two response types: 1) animals that sit and stay motionless on the trap immediately after arrival and 2) animals that continue to fly actively around the trap. This difference in behavior does not lead to bias during manual collection, since all individuals are recorded. In the case of automatic traps, however, some moths settle down immediately and therefore do not get into the collection container. This results in an underrepresentation of certain taxa, e.g. small species in the Nolidae, Eupitheciini, and Lithosiini. I also found that these behavioral differences are contingent with ambient temperature and the effect increases by combining temperature and wingspan. As a result, when analyzing moth samples automated traps may produce a biased result.

In order to analyze possible causes of long-term changes in the species richness of macro-moths in Pineta san Vitale, I was interested in the role of succession. I assumed, based on historical records, that after the area had received status as protected reserve, vegetation succession has led to the loss of open habitats and the recovery of forest. I therefore expected that open habitat specialists among moths have vanished over time while simultaneously forest bound species have increased. I also wanted to check whether succession may override the manifold anthropogenic influences that act on the reserve from its surroundings. Accordingly, I checked whether the moth assemblage in the reserve has undergone a directional shift, for example that larger or specialized moths have experienced a higher risk of extinction in this isolated area.

I compared historical (1933–1976: 107 species; 1977–1996: 157 species) and own empirical data (1997–2002: 174 species; 2011 + 2012: 187 species). As it is usually unknown how historic collections have been assembled, there might be a bias towards representation of species that are attractive for collectors. For my own work therefore, I had to consider these potential sources of error, for example by only using incidence data, as individual numbers recorded per species are influenced by the trap type or the sampling habits of former collectors.

The proportion of habitat generalists among all moths recorded increased from 20 to 33% over the past 80 years. In contrast, forest as well as open habitat species decreased by 10 percentage points. If one does not consider the proportions but rather the absolute numbers of species, apparent gains were greater than losses for forest species and habitat generalists. Overall, 18 species that prefer open habitats and 10 species that prefer reed habitats have disappeared. The Pineta san Vitale reserve has indeed undergone massive changes in its

vegetation structure due to succession. This can be attributed to the abandonment of extensive forest use. I conclude from my results that habitat generalists were better able than habitat specialists to cross landscapes altered by humans and to re-colonize this isolated habitat fragment. I also conclude, that the succession caused establishment of a near natural forest structure rises the number of species in the course of the decades. This succession effect might be large enough to override possible effects of anthropogenic impacts like increased soil salinity or the pesticide use in nearby agricultural land.

Records of 300 moth species from Pineta san Vitale showed that local extinction risk was indeed related to size of the animals and their degree of ecological specialization. Specialization was classified by 1) larval food affiliations, adult habitat preferences and the position of the northern distribution limit in Europe, and 2) by analysis of the functional dispersion of species assemblages (FDis) over time on the basis of 12 species traits. Locally lost species (mean wingspan: 36.9 mm) were on average larger than persisting (33.2 mm) or previously unrecorded species (30.7 mm). By using rough categories to classify specialization, I could not confirm any connection with the local risk of extinction. Using a multivariate measure of FDis, on the other hand, there were significant differences according to the species status. Hence, simple classification systems may not be sensitive enough for analyzing changes in the specialization of the moth community. In contrast, FDis was more informative, since this analysis method reflects the multitude of ecological niche dimensions. After the end of extensive anthropogenic forest use in Pineta san Vitale, the composition of the moths seems to have shifted in the direction of forest-affine species, which led to a decrease in FDis values (not to be confused with the decline in the proportions of forest species). Multivariate analyses also confirmed a general change in species composition over the past 80 years.

2. Zusammenfassung

Die Biodiversitätskrise ist in vollem Gange und unzählige Insektenarten sind betroffen. Ihre Funktionen in Ökosystemen und für uns Menschen sind unersetzlich. Daher sammeln Wissenschaftler Daten, um das Ausmaß der Krise zu quantifizieren und Lösungen zu finden. Die Erfassung neuer Daten und deren Vergleich mit historischen Stichproben ist für solche Analysen von grundlegender Bedeutung.

In meiner Dissertation habe ich einen Blick in die Vergangenheit geworfen und anhand seltener Langzeitdaten rekonstruiert, wie sich die Artenzusammensetzung von Nachtfaltern im isolierten Waldreservat Pineta san Vitale (Nordostitalien, Ravenna) verändert hat. Aus historischen Museumssammlungen und eigenen, empirischen Daten aus 15 Jahren wurde eine Zeitreihe von insgesamt über 80 Jahren erstellt.

Dann habe ich nach Lösungen gesucht, um die Kombination von historischen und empirischen Daten so zuverlässig wie möglich zu analysieren. Der erste Schritt bestand darin, das Anflugverhalten von 1426 Nachtfaltern an einer künstlichen Lichtquelle zu beobachten. Dabei habe Ich zwischen zwei Verhaltensweisen unterschieden: 1) Tiere, die sich unmittelbar nach der Ankunft bei der Lichtquelle setzen und dort sitzen bleiben, und 2) Tiere, die weiterhin aktiv um die Lichtquelle fliegen. Dieser Unterschied im Verhalten führt bei einer manuellen Erfassung zu keinem Fehler, da alle Individuen berücksichtigt werden. Bei automatischen Fallen hingegen gelangen einige Nachtfalter vermutlich nicht in den Auffangbehälter, wenn sie sich sofort setzen. Dies führt zu einer Unterrepräsentation bestimmter Taxa, z.B. sind kleine Arten aus den Gruppen Nolidae, Eupitheciini und Lithosiini in Fallenfängen oft gering vertreten. Ich habe auch herausgefunden, dass dies von der Umgebungstemperatur abhängt. Bei kühleren Temperaturen tendieren auch mittelgroße Nachtfalter dazu, sofort nach Ankunft am Licht sitzen zu bleiben. Infolgedessen können Stichproben aus Automatikfallen bei der Analyse von Nachtfaltergemeinschaften zu einem verzerrten Ergebnis führen.

Um mögliche Ursachen für langfristige Veränderungen des Artenreichtums von Nachtfaltern in Pineta san Vitale zu analysieren, habe ich mich dafür interessiert, welche Rolle die Sukzession der Vegetation im Lebensraum dabei spielt. Aufgrund historischer Aufzeichnungen bin ich davon ausgegangen, dass die Vegetationsfolge nach der Unterschutzstellung des Gebietes zum Verlust offener Lebensräume zu Gunsten des Waldes geführt hat. Ich habe daher erwartet, dass unter den Nachtfaltern Spezialisten für offene Lebensräume im Laufe der Zeit verschwunden sind, während gleichzeitig waldgebundene Arten zugenommen haben sollten. Es stellte sich auch die Frage, ob die Sukzession die vielfältigen, möglichen anthropogenen Einflüsse, die auf das Reservat aus seiner Umgebung einwirken, kompensieren kann. Dem entsprechend habe ich geprüft, ob es eine gerichtete Veränderung in der Artenzusammensetzung der Nachtfalter im Reservat gegeben hat, zum Beispiel, dass größere oder spezialisierte Arten in diesem isolierten Gebiet ein höheres Aussterberisiko haben.

Für diese Analyse habe ich historische (1933–1976: 107 Arten; 1977–1996: 157 Arten) und eigene empirische Daten (1997–2002: 174 Arten; 2011 + 2012: 187 Arten) verglichen. Leider ist es nicht bekannt, wie historische Sammlungen zusammengestellt wurden. Individuen von Arten, welche für Sammler attraktiv erschienen, wurden möglicherweise häufiger mitgenommen und sind folglich überrepräsentiert. Für meine Arbeit musste ich daher diese potenziellen Fehlerquellen berücksichtigen, indem ich beispielsweise nur Inzidenzdaten verwendet habe, da die Individuenzahlen in den Belegserien vom Fallentyp oder den Sammelgewohnheiten früherer Entomologen abhängen.

Dabei habe ich herausgefunden, dass der Anteil der Habitat-Generalisten an allen registrierten Nachtfaltern in über 80 Jahren von 20 auf 33% gestiegen ist. Im Gegensatz dazu nahmen sowohl Wald- als auch Offenlandarten um 10 Prozentpunkte ab. Wenn man nicht die Anteile, sondern die absolute Anzahl der Arten betrachtet, waren für Waldarten und Habitat-Generalisten die Gewinne größer als die Verluste. Insgesamt sind 18 Arten verschwunden, die offene Lebensräume bevorzugen und 10 Arten, die Schilflebensräume bevorzugen.

Das Reservat Pineta san Vitale hat aufgrund der Sukzession massive Veränderungen in seiner Vegetationsstruktur erfahren. Dies ist auf die Aufgabe der extensiven Waldnutzung zurückzuführen. Ich schließe aus meinen Ergebnissen, dass Habitat-Generalisten besser als Habitat-Spezialisten in der Lage sind, von Menschen veränderte Landschaften zu durchqueren, um ein isoliertes Habitat-Fragment wie Pineta san Vitale neu zu besiedeln. Ich komme auch zu dem Schluss, dass die durch Sukzession verursachte Bildung einer naturnahen Waldstruktur die Anzahl der Nachtfalter-Arten im Laufe der Jahrzehnte erhöht hat. Dieser Effekt könnte groß genug sein, um mögliche Auswirkungen anthropogener Einflüsse, wie einen erhöhten Salzgehalt des Bodens oder den Einsatz von Pestiziden, in den nahe gelegenen landwirtschaftlichen Flächen, zu kompensieren.

Meine Auswertungen zeigen, dass das lokale Aussterberisiko tatsächlich mit der Größe der Tiere und ihrem Grad an ökologischer Spezialisierung zusammenhängt. Die Spezialisierung wurde einmal grob klassifiziert nach 1) Larvenfutterzugehörigkeiten, Präferenzen für Lebensräume der adulten Tiere und der nördlichen Verbreitungsgrenze in Europa und 2) durch Analyse der funktionellen Dispersion (Streuung) innerhalb von Artengruppen (FDis) über die Zeit, auf der Basis einer feiner aufgelösten Betrachtung von 12 Arteigenschaften („Traits“). Lokal verloren gegangene Arten (mittlere Flügelspannweite: 36,9 mm) waren im Durchschnitt größer als persistente (33,2 mm) oder zuvor nicht erfasste Arten (30,7 mm). Durch die Verwendung grober Kategorien zur Klassifizierung der Spezialisierung konnte ich keinen Zusammenhang zum lokalen Aussterberisiko aufdecken. Hingegen bei der Verwendung des multivariaten FDis-Maßes gab es signifikante Unterschiede. Daher sind einfache Klassifizierungssysteme möglicherweise nicht empfindlich genug, um subtile Änderungen im Spezialisierungsgrad der Nachtfaltergemeinschaft abzubilden. Im Gegensatz dazu war FDis informativer, da diese Analysemethode eine Vielzahl ökologischer Nischendimensionen widerspiegelt. Nach dem Ende der extensiven anthropogenen Waldnutzung in Pineta san Vitale scheint sich die Zusammensetzung der Nachtfalter in Richtung waldaffiner Arten verschoben zu haben, was zu einem Rückgang der FDis-Werte führte (nicht zu verwechseln mit dem Rückgang der Anteile von Waldarten). Multivariate Analysen bestätigten auch eine allgemeine Veränderung der Artenzusammensetzung in den letzten 80 Jahren.

3. List of manuscripts with statement of personal contribution

Chapter 6: How differences in the settling behavior of moths (Lepidoptera) may contribute to sampling bias when using automated light traps (2016) *European Journal of Entomology*. 113: 502-506. DOI: 10.14411/eje.2016.066

M. Wölfling, M. Becker, B. Uhl, A. Traub & K. Fiedler

Personal contribution:

- Procurement of the international long-term scientific relationship with the Comune di Ravenna and application for the sampling permit
- Planning of the sampling design
- Procurement, management and implementation of the field work
- Species identification, partially confirmed by genital dissections
- Data preparation and statistical analysis (supervision by K. Fiedler)
- Compilation of the literature (supervision by K. Fiedler)
- Preparation of figures (supervision by K. Fiedler)
- Drafting, co-writing and co-editing of the manuscript (supervision by K. Fiedler)

Chapter 7: Multi-decadal surveys in a Mediterranean forest reserve – do succession and isolation drive moth species richness? (2019) *Nature Conservation*. 35: 25-40. DOI: 10.3897/natureconservation.35.32934

M. Wölfling, B. Uhl & K. Fiedler

Personal contribution:

- Procurement of the long-term scientific relationship with the Comune di Ravenna and application for the sampling permit
- Planning of the sampling design
- Search for historic PsV moth collections
- Inquiry for access to the historic collections and data digitization
- Procurement, management and implementation of the field work
- Species identification, if necessary, confirmed by genital dissection
- Data preparation and statistical analysis (supervision by K. Fiedler)
- Compilation of literature (supervision by K. Fiedler)
- Preparation and co-editing of figures (supervision by K. Fiedler)
- Drafting, co-writing and co-editing of the manuscript (supervision by K. Fiedler)

<u>Chapter 8:</u> Ecological drift and directional community change in an isolated Mediterranean forest reserve – larger moth species under higher threat. (2020) *Journal of Insect Science*. 20(5), 7. DOI: 10.1093/jisesa/ieaa097

M. Wölfling, B. Uhl & K. Fiedler

Personal contribution:

- Procurement of the long-term scientific relationship with the Comune di Ravenna and application for the sampling permit
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- Compilation of the literature (supervision of K. Fiedler)
- Preparation and editing of figures (supervision of K. Fiedler)
- Drafting, co-writing and co-editing of the manuscript (supervision of K. Fiedler)

4. Selection of already released publications

- 📚 Wölfling, M., Fiedler, K., Uhl, B. (2020). Ecological drift and directional community change in an isolated Mediterranean forest reserve – larger moth species under higher threat. *Journal of Insect Science*, 20(5),7, 1-10.
  https://doi.org/10.1093/jisesa/ieaa097

- Uhl, B., Wölfling, M., Fiedler, K. (in press). Local, forest stand and landscape-scale correlates of plant communities in isolated coastal forest reserves. *Plant Biosystems*.
  https://doi.org/10.1080/11263504.2020.1762776

- Uhl, B., Wölfling, M., Fiedler, K. (2020). Understanding small-scale insect diversity patterns inside two nature reserves: the role of local and landscape factors. *Biodiversity and Conservation*, 29(7), 2399-2418.
  https://doi.org/10.1007/s10531-020-01981-z

- 📚 Wölfling, M., Uhl, B., Fiedler, K. (2019). Multi-decadal surveys in a Mediterranean forest reserve – do succession and isolation drive moth species richness? *Nature Conservation*, 35, 25-40.
  https://doi.org/10.3897/natureconservation.35.32934

- Wölfling, M. & Nässig, W. (2017). *Cymbalophora pudica* (Esper, [1785]) in Switzerland (Ticino) — former occurrence apparently confirmed by rediscovered historic specimen in private collection (Lepidoptera: Erebidae, Arctiinae). *Nachrichten des Entomologischen Vereins Apollo*, 38, 129-130.

- 📚 Wölfling, M., Becker, M. C., Uhl, B., Traub, A., Fiedler, K. (2016). How differences in the settling behavior of moths (Lepidoptera) may contribute to sampling bias when using automated light traps. *European Journal of Entomology*, 113, 502-506.
  https://doi.org/10.14411/eje.2016.066

- Uhl, B., Wölfling, M., Fiala, B., Fiedler, K. (2016). Micro-moth communities mirror environmental stress gradients within a Mediterranean nature reserve. *Basic and Applied Ecology*, 17(3), 273-281.
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- Uhl, B. & Wölfling, M. (2015). Anthropogenic influences on the condition of *Pinus pinea* L. and *Quercus robur* L. in Pineta san Vitale (Ravenna, Italy). *Journal of Mediterranean Ecology*, 13, 5-12.

- Kohnle, A. & Wölfling, M. (2015). Mapping of the wing scale types of *Pieris brassicae* (Linnaeus, 1758) and *Pieris rapae* (Linnaeus, 1758) (Lepidoptera: Pieridae). *Nachrichten des Entomologischen Vereins Apollo*, 36, 153-160.

📚 = The book symbol indicates publications which are part of this thesis.

- Naseem, M., Wölfling, M., Dandekar, T. (2014). Cytokinins for immunity beyond growth, galls and green islands. *Trends in Plant Science*, 19(8), 481-484. https://doi.org/10.1016/j.tplants.2014.04.001

- Uhl, B. & Wölfling, M. (2013). New record of the invasive pest species *Cydalima perspectalis* (Walker, 1859) in Emilia Romagna (Italy) (Lepidoptera: Crambidae, Spilomelinae). *Nachrichten des Entomologischer Verein Apollo*, 34, 91-93.

- Kohnle, A. & Wölfling, M. (2012). Number of wing scales in normal sized exemplars and dwarf forms of *Inachis io* Linnaeus, 1758 and *Argynnis paphia* Linnaeus, 1758 (Lepidoptera: Nymphalidae). *Entomologische Zeitschrift*, 122(6), 277-281.

- Rostás, M. & Wölfling, M. (2009). Caterpillar footprints as host location kairomones for *Cotesia marginiventris*: persistence and chemical nature. *Journal of Chemical Ecology*, 35(1), 20-27. https://doi.org/10.1007/s10886-009-9590-z

- Wölfling, M. & Rostás, M. (2009). Parasitoids use chemical footprints to track down caterpillars. *Communicative and Integrative Biology*, 2(4), 353-355. https://doi.org/10.4161/cib.2.4.8612

5. Introduction

Long term analyses by using museum and private collections

Worldwide over three billion biological objects are preserved and stored in natural history museums (Yeates et al. 2016). Suarez & Tsutsui wrote 2004: “These biological collections make innumerable contributions to science and society in areas as divergent as homeland security, public health and safety, monitoring of environmental change, and traditional taxonomy and systematics”. Especially, the scientific value of such collections for ecology and conservation is high (Kharouba et al. 2018, Meineke et al. 2019, Tarli et al. 2018). For example, vouchers are now commonly used with the help of cutting-edge sequencing technology as treasury of DNA data (Yeates et al. 2016). Also, the analysis of changing species distributions over time, in the course of climate change, requires that geo-referenced specimens can be connected with climatic data (Pyke & Ehrlich 2010).

To top of the described biological objects, half a billion preserved insects (Short et al. 2018), are waiting to be used in science, posing a possibility to find answers to major ecological problems of the Anthropocene (Cadotte et al. 2017). Approaches of forecasting climate change are urgently needed and insect collections pose a possibility for this (Kharouba et al. 2018), since many species are showing range shifts caused by climate warming (Chen et al. 2011, Kellermann & Heerwaarden 2019). But, also massive warnings to humanity because of global insect decline (Cardoso et al. 2020), sometimes even termed ‘Ecological Armageddon’ (Leather 2017), with accompanying ecological effects can be estimated by using museum collections (Meineke et al. 2019, Montgomery et al. 2020). This kind of applied entomology is furthermore relevant to agriculture and forestry when vouchers of insects, that are held in museums, give insight in population dynamics and evolution of pest species (Meineke & Davies 2019, Summerville & Crist 2008). Finally analyzing changes in species composition in environments under intense anthropogenic influence, in near natural habitats and in ecological reserves is a powerful tool for conservation planning and management (Habel et al. 2016, 2019a, 2019b).

Next to museums also some private insect collections were already processed in prominent works providing basic knowledge of taxonomy and phenology (de Freina & Witt 1987, Bertaccini et al. 2008). Unfortunately, such sources have mostly to nourish from old collections since private insect sampling has become ever more difficult because of legal regulations and prohibitions in the past (Klausnitzer & Segerer 2018). Kim & Byrne (2006) collated limiting factors for biodiversity science also related to vanishing private collecting activity: “1) the lack of data concerning site-specific species compositions 2) decreasing number of taxonomists 3) insufficient feasibility for inventory and assessment of biodiversity as well as 4) missing taxonomic resources and its related service structure for biodiversity science.” Citizen science projects are a possibility to counteract these problems. In many cases, participants can report online where they have found which species (e.g. www.gbif.org, www.schmetterlinge-d.de). Therefore, such projects offer the opportunity to generate large data sets without collecting the individuals. However, citizen science data are not collected by trained taxonomists, so it is not clear if all individuals are always identified correctly. Pictures of the registered specimens can solve this problem in some cases, when the species does not belong to complexes that need to be dissected (Vantieghem et al. 2016). Furthermore, citizen science data cannot replace the value of museal collections for other analyzes investigating changes over time in physiology (e.g. body size or weight) or DNA barcoding. In conclusion, citizen science can contribute to long-term recordings, but cannot replace the classic museum collections completely. Specially in the age of biodiversity crisis (Montgomery et al. 2020) analyses over decades are needed to detect trends in and reasons for change (Macgregor et al. 2019, Seibold et al. 2019). But also shifts in community composition and the degree of species turnover (Mori et al. 2018), which may arise through species presence/absence or by fluctuations in abundances (Kinsella et al. 2020), need to be analyzed to avoid massive damage to ecosystems and consequently harmful effects to humanity. As an example, assuming that all pollinators are lost, the financial costs of missing ecosystem services were already in 2009 rated to amount to 172 billion US$ (Gallai et al. 2009). Instead the global costs of invasive insects are at least 70 billion US$ per year, and associated healthcare costs over 6.9 billion US$ (Bradshaw et al. 2016). In such a scenario of missing pollination 87% of all flowering plant species (Ollerton et al. 2011) and thus 35% of all food crops (Klein et al. 2007) would also be affected. The loss of pollinators would have a massive impact on countless other ecosystem services linked to pollination of flowering plants and, in terms of primary agricultural production, would result in a global annual loss of today estimated US$ 235-577 billion (FAO 2020a). Not to mention the social consequences for the nutrition of the world population, where 820 million people already do not have enough to eat (FAO 2020b).

Why choosing moths for analyses?

Most important for ecological analyses is the proper choice of target organisms, which also implies the availability of the required data. As a species-rich and abundant group in practically all terrestrial and freshwater ecosystems, insects are therefore often in the focus of ecological science (Wagner 2019). Actually, about 1 million insect species have been named (Stork 2018). But which group of insects is most suitable for data sampling? The number of taxonomically named species reveals that there are four hyper-diverse insect orders: Coleoptera 387.000, Lepidoptera 157.000, Diptera 155.000 and Hymenoptera 117.000 (Stork 2018). Unfortunately, Diptera and Hymenoptera are not easy to determine and in many cases taxonomic literature is not available. In addition, there are mostly no uniform sampling methods for these groups, whereas moths can be collected easily and in a standardized manner by using light traps. Furthermore, Lepidoptera have important properties for ecological analyses, as details about their species traits and ecology in Europe are sufficiently known (Ebert 1994-2003, Leraut 2006-2019, Nowinszky 2003).

Regarding valuable long-term studies, the selection of the proper organism group is finally shaped by the availability of collection specimens or record data. Nobody can sample insects over a century because human lifespan is limited. Consequently, museum collections are the only possibility to assemble datasets over longer timespans. Hence, we have to take into account the availability of vouchers in museum collections. Classically, mainly Lepidoptera and Coleoptera were collected in the past by dedicated amateurs (today termed ‘citizen scientists’) (Arnett et al. 2019), offering numerous datasets that persist to the present day. Using target groups with short time responses on disturbance – such as moths – has the advantage that the effect of certain environmental factors may be rapidly seen within a data set (Rákosy & Schmitt 2011). Consequently, moths are in many cases suitable for analyses of anthropogenic impacts and natural changes in an ecosystem in long-term analyses, provided that the pertinent data are available.

Mediterranean Ecosystems

It is undisputed that Mediterranean-type ecosystems are hotspots of biodiversity (Blondel et al. 2010). Such ecosystems can be found in various parts of the world, viz. California, central Chile, the western Cape Region in South Africa, southwest and south Australia and around the Mediterranean basin (IUCN 2020). In the latter region we can find a wide variety of habitats, many of which are protected, following the FFH guidelines. Examples of known types are Mediterranean salt marshes (Juncetalia maritimi), Laurel forest (Laurisilva) and also dune forests of *Pinus pinea* and *Pinus pinaster*. After millennia of intense land-use, the remaining primary vegetation of the Mediterranean basin today covers only about 110.000 km^2 (i.e., just 4.7 % of its original extent), including 42.123 km^2 of protected areas. In the Mediterranean region, about 25.000 plant species occur of which 13.000 are endemic (4.3% of all plant species worldwide) (Myers et al. 2000). However, Myers et al. omitted invertebrates (estimated 95% of all species) from their study because these are still largely undocumented (Myers et al. 2000). The high level of biological diversity is due to the richness of landscape and ecosystem structures that has developed since the emergence of the Mediterranean basin (Blondel et al. 2010).

This variety of Mediterranean ecosystems and immense biodiversity are threatened by human impacts, especially in the densely populated coastal areas (Romano & Zullo 2014). Land-use change (Falcucci et al. 2007), urbanization (García-Nieto et al. 2018) and tourism (Davenport & Davenport 2006) are the main drivers. During the last decades surface sealing increased whereas extensive land-use decreased at the coastline (Falcucci et al. 2007). As a consequence, many typical Mediterranean plants and animals became threatened (Falcucci et al. 2007). Moreover, the loss of ecosystem services for agriculture and forestry is a rising problem as a result of intense urbanization (García-Nieto et al. 2018). In addition, there are 330 million tourists per year (as of: 2016) spending their holidays in the Mediterranean region, leading to massive pollution (Tovar-Sánches et al. 2019) or the transformation of coastal habitats by ruining the last natural dunes (Sytnik & Stecchi 2015). Therefore, there is an intense conflict potential between conservation efforts and anthropogenic land-use in Mediterranean coastal zones. Therefore, research on human impacts on the few remaining natural areas required, to ameliorate conservation management of the last remains of natural habitats in the Mediterranean biodiversity hotspot.

My focal area: Characteristics and history of Pineta san Vitale

Today, there are about 3.4 million ha land protected by national and regional parks (Federparchi 2020) in Italy. This contrasts with a population of about 60.5 million people (worldometer 2019) and an immense loss of land for urbanization of about 14.000 ha per year (Romano et al. 2017). Particularly alarming are the gha-numbers (gha = global hectare: a measure of the ecological footprint with regard to production and consumption) of the Emilia Romagna (Malucelli et al. 2014), which show that the former “granary of Italy” is no longer sufficient to sustain the consumption of the population. Consequently, there is a high risk that valuable protected areas, which are essential for the basic supply of ecosystem services, will come under increasing anthropogenic pressure even when conservation areas are fixed by legislations (Romano et al. 2017). One of the reasons for this problem is that surrounding areas also become urbanized (Romano et al. 2017). Concerning urbanization, the Po Valley is one of the main critical regions where over 20.5 million people (35% of the Italian population) are living on 1/6 of the whole area of Italy (Romano et al. 2017). Extremely alarming are consequently the 33 ha/day that have been transformed over the last 50 years, including even ecosystems that are necessary e.g. for flood-protection (Romano et al. 2017). The absolute hotspot for this land transformation is the 1km wide coastline, where the urbanized area has more than doubled from the year 1950 to 2000 (Romano et al. 2017). This resulted in ongoing subsidence (Corbau et al. 2019) coupled with massive soil salinization as one of the major threats to agriculture as well as to reserve areas (Antonellini et al. 2008). So, the immense anthropogenic pressure on the one hand and the conservation goals associated with the formation of the Parco Regionale del Delta del Po on the other hand collide on the coast of the Emilia Romagna. This contrast is particularly evident when cities with big industrial areas and surrounded by intensively cultivated farmland are situated close to nature reserves. Ravenna is the largest coastal city in the Emilia Romagna and has a spacious industry (Airoldi et al. 2016). The closest nature reserve of the Parco Regionale del Delta del Po is the forest reserve Pineta san Vitale (hereafter in all chapters PsV) (Fig. 5.1), which is so close that the southern edge of the reserve is almost seamlessly connected to the area built-up with industrial plants (Lucialli et al. 2007). Additionally, the PsV reserve is furthermore isolated from other remaining reserves by areas under intensive agriculture (Benini & Pezzi 2011).

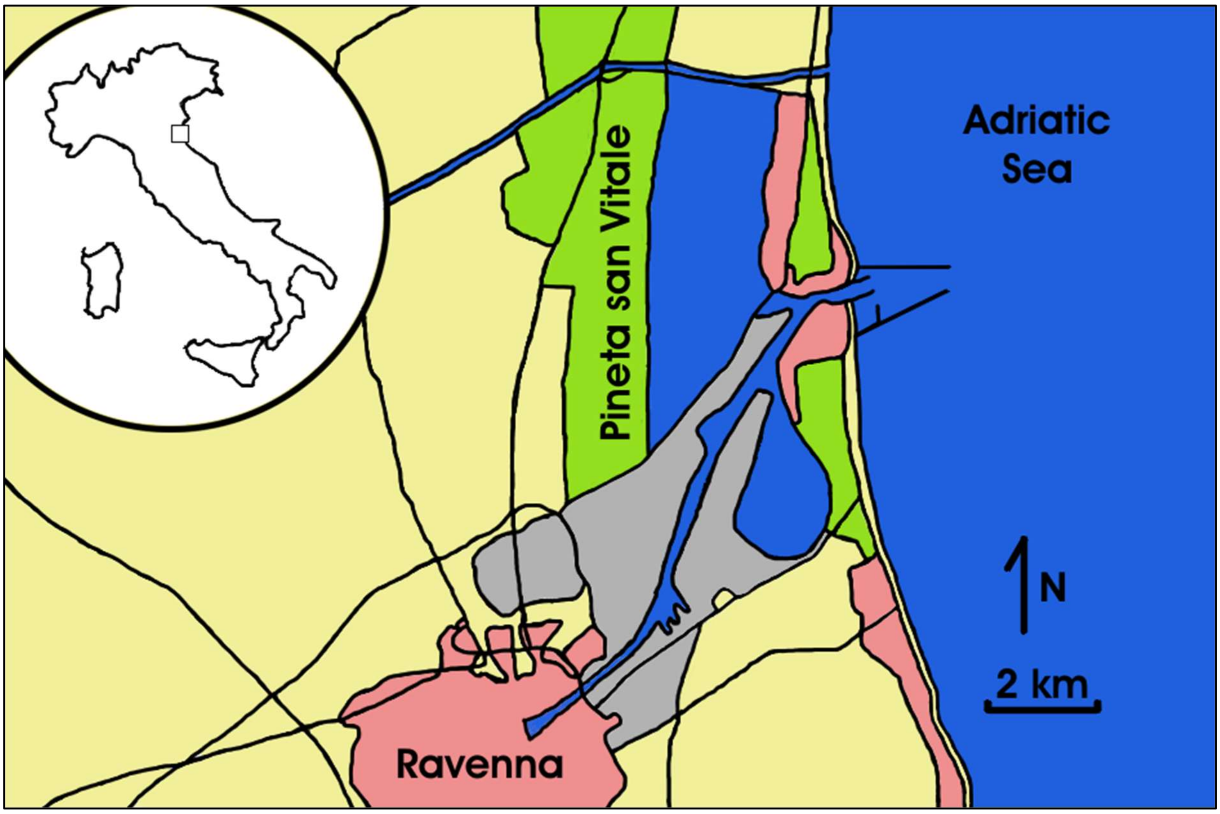
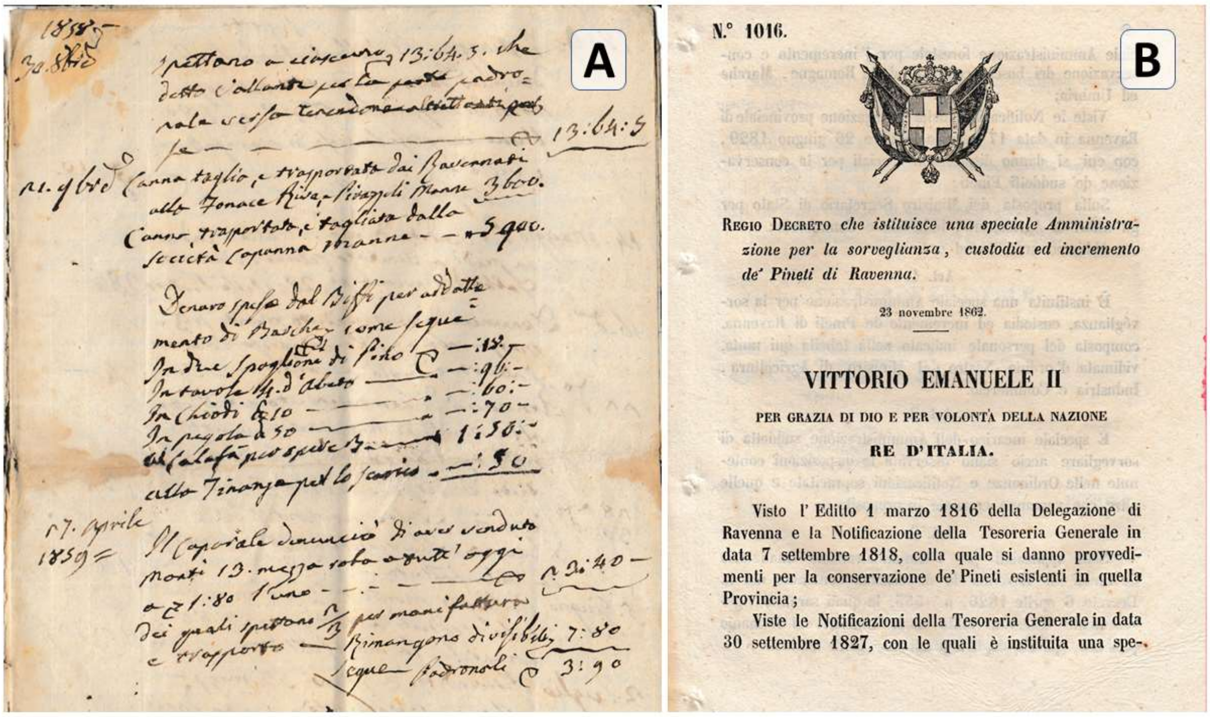


Figure 5.1: Overview map of the study area. The square on the black and white outline of Italy shows the enlarged map area. Red = urban area, grey = industrial area, green = near-natural area, yellow = agricultural land, blue = open water bodies and sea.

In order to understand today's situation of PsV, its ecological developments and changes in the recent past, one has to start with its origin and early landscape development. PsV is a Mediterranean coastal forest that exists since the 12th century, grown on paleodunes (Andreatta et al. 2010). The enormous economic value of the forests was recognized very early on and people used the pinewoods extensively for cattle grazing as well as for reed and herb harvest (Fig. 5.2 A). Since 1496, Doges and Popes have drawn up numerous statutes in order to maintain PsV permanently, prevent overexploitation of the flora and regulate permanent use (Rava 1897). In addition to the economic value, socio-ecological contents were recognized as early as in the 18th century. Already the first book about the Ravenna pinewoods (Ginanni 1774) contained detailed descriptions of the entire area with its numerous animals, including the first mentioned Lepidoptera species. Thus, it was possible for me to identify 31 butterfly and moth species (Appendix S1) after 240 years on the basis of historic literature. Since Ginanni published weak engravings and text descriptions of the species, I could assign some of the described species only with a degree of uncertainty to taxa that are valid today.



1858
30. 8bre
spettano a ciascuno 13:64.3. che
detto l'allante per la parte padro=
nale verso [illegible]
fa ——— 13:64:5

11. 9bre Canna taglio, e trasportata dai Ravennati
alla Fornace Riva, Pirazzoli Manne 3600.
Canne trasportate, e tagliata dalla
Società Capanna Manne —— 5900.

Denaro speso dal Biffi per addatta=
mento di Barche come segue
In due Spoglioni di Pino C —:12.—
In tavole 14. d'abeto —— —:96:—
In Chiodi &10 —— —:60:—
In pegola a 50 —— —:70—
Al calafà per spese B[illegible] —— 1:50:—
allo Tinanza per lo scarico —:50

17. Aprile
1859 = Il Caporale denunciò di aver venduto
monti 13. mezza rota a tutt' oggi —— C 23:40 —
a C 1:80 l'uno
dei quali spettano 1/3 per manifattura
e trasporto — Rimangono divisibili 7:80
segue padronali C 3:90

N.° 1016.

Regio Decreto *che istituisce una speciale Amministrazione per la sorveglianza, custodia ed incremento de' Pineti di Ravenna.*

23 novembre 1862.

VITTORIO EMANUELE II

PER GRAZIA DI DIO E PER VOLONTÀ DELLA NAZIONE

RE D'ITALIA.

Visto l' Editto 1 marzo 1816 della Delegazione di Ravenna e la Notificazione della Tesoreria Generale in data 7 settembre 1818, colla quale si danno provvedimenti per la conservazione de' Pineti esistenti in quella Provincia;

Viste le Notificazioni della Tesoreria Generale in data 30 settembre 1827, con le quali è instituita una spe-

Figure 5.2: A = Manuscript from 1858 documenting the extensive mode of use of the Ravenna pinewoods. B = Royal Decree of 1862 by Vittorio Emanuele II deploying the first pinewood attendant. Both documents are stored in Coll. Mirko Wölfling (Niederwerrn, Germany). Photos: Mirko Wölfling.

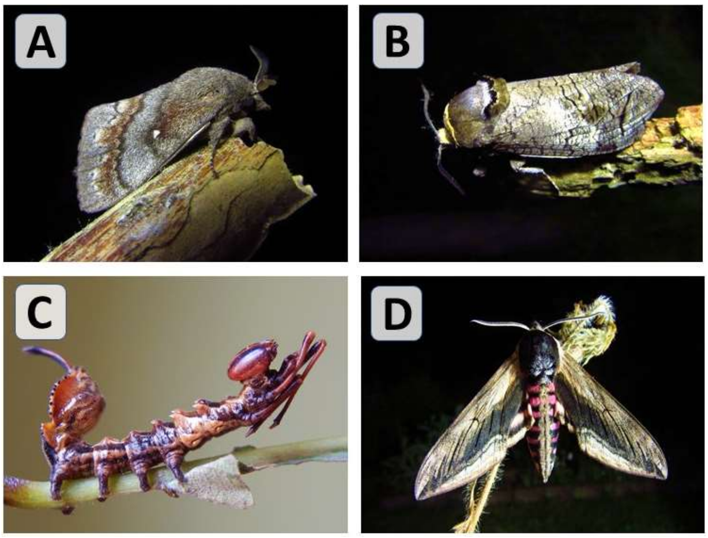


Figure 5.3: Moth species from PsV that were registered 246 years ago: A = *Dendrolimus pini*, B = *Cossus cossus*, C = *Stauropus fagi*, D = *Sphinx ligustri*. A-C were again registered by own empiric data. Photos: Britta Uhl.

Unfortunately, there is also no indication of how Ginanni created this list of species or the aspects according to which he described species in his work and which he possibly did not name. Specimens from this time have also not been preserved, which makes reliable evidence even more difficult. In addition, there is a span of over 100 years between Ginanni's records and the oldest preserved museum vouchers the stem from a collector named Callegari. It therefore made no sense to include Ginanni's rudimentary data in my analyses. However, they should be given a brief look here, as they are part of the story of PsV as first records ever of Lepidoptera. So, for example, these few ancient records prove that *Colocasia coryli* was present in PsV in 1774. No later evidence for the existence of this noctuid moth, that is widespread a common throughout most of Europe, was found in the historical collections or records, or in my own data. To give another example, *Sphinx ligustri* (Fig. 5.3 D) was recorded by Ginanni and was later also found in old museum collections. However, I have not been able to detect this species in PsV during the entire time of my own samplings, starting in 1997 and continuing to the present day. Other moth species instead, traced in Ginanni's descriptions, were found among old museum vouchers as well as in my own records, e.g. *Dendrolimus pini* (Fig. 5.3 A), *Cossus cossus* (Fig. 5.3 B) and *Stauropus fagi* (Fig. 5.3 C).

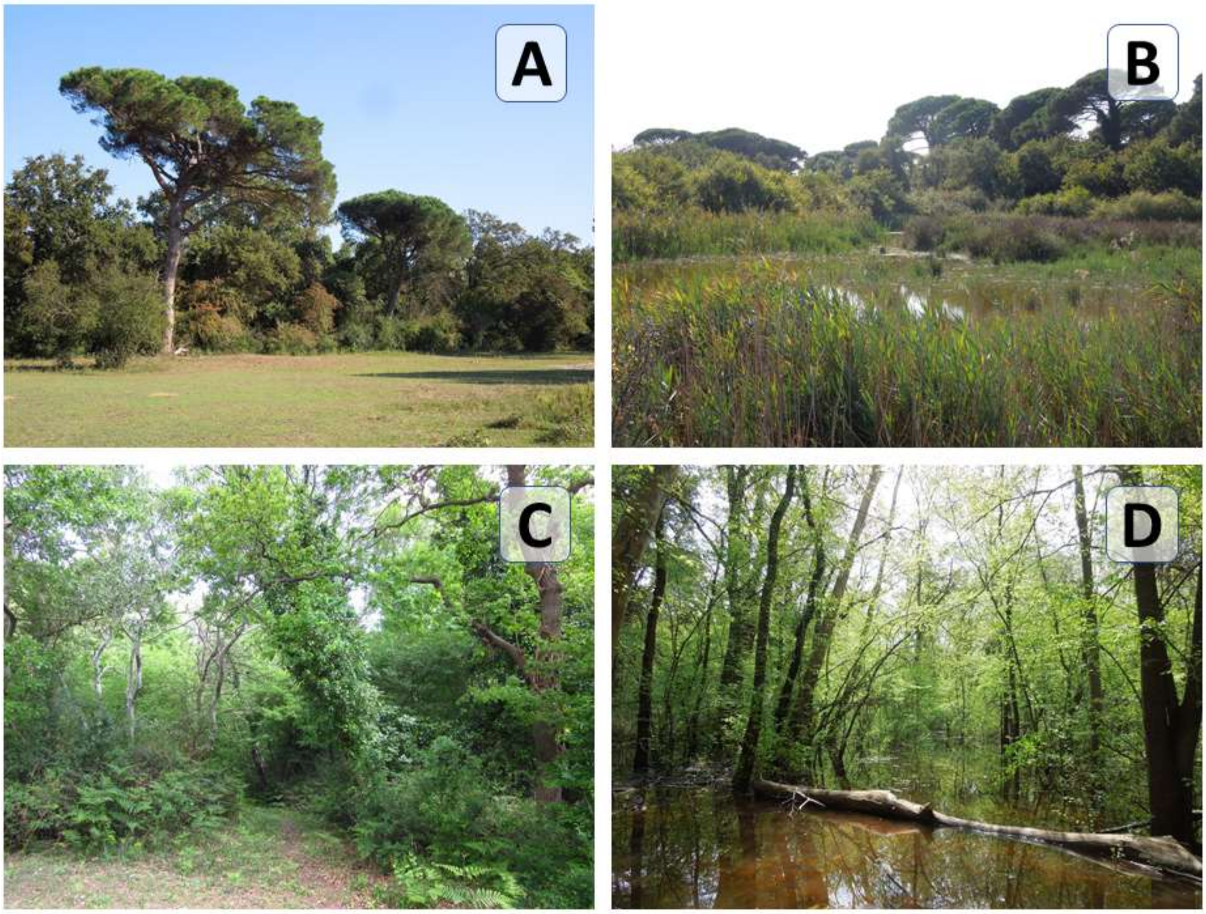


Figure 5.4: The major vegetation types of PsV. A = sandy pioneer floor, B = reed and open water, C = downy oak forest, D = riparian forest. Photos: Mirko Wölfling.

From March 1st, 1816 onwards, PsV was finally better protected by King Vittorio Emanuele II, and with a subsequent decree in 1862, the first pinewood attendant was deployed (Fig. 5.2 B). The Comune di Ravenna was finally able to buy PsV from the government and the church in 1873 (4500 ha), but lost many pines and other vegetation due to the harsh winter of 1879/80 and a fire that followed shortly afterwards (Rava 1897). Since 1988, PsV is legally protected as Part of the Parco Regionale del Delta del Po (Assemblea Legislativa Regione

Emilia-Romagna 1988) affiliated to the EU-Life programme (European Union 2010) and subject to the Natura 2000 agreement (Montanari 2010). It is furthermore an UNESCO biosphere reserve (UNESCO 2015) and has been declared by the Ramsar Convention as "Wetland of international importance" (Ramsar Convention Secretariat 2013). However, since being placed under protection, succession has also been a driving factor for the development of the vegetation and habitats in the park (Wölfling et al. 2019) and is increasingly covering the formerly open area with a forest-like character. The major vegetation types in the reserve (Fig. 5.4) include actually pine and downy oak forests, sandy pioneer floors as well as reed and water bodies (Geoportale 2016).

Rationale of this study

Condensing the past and present situation, PsV is an area that has developed over centuries under multifactorial anthropogenic and natural influences to the unique remnant of near-natural area it presents today. Ultimately, I chose PsV as the target of my study not only because of its aforementioned characteristics but also because former moth collectors were already active in this area and sampled a lot of data.

For a data series of over 80 years it is essential to use samples from historical material. Such vouchers were found in two local museums (in Venice and Bagnacavallo) and in a private collection (Forli). Unfortunately, in most cases the sampling of the historic material was not done in a standardized manner and often it is not known which type of light-trap was used or why former collectors kept (or discarded) certain individuals. The manifold developments of light sources and trap systems over the past 100-150 years makes it a challenge to compare old collection data with own recent ones. For example, it can be assumed that automated traps and manual collection at artificial light sources produce different results. This aspect had to be clarified first in order to know whether abundance-based analyzes are generally possible. The first part of the dissertation is therefore:

> *Determination of the sampling error based on the flight-to-light behavior of moths in regard to body size, taxonomic status and ambient temperature.*

Long term ecological studies (e.g. from standardized monitoring efforts) are the basis for solving the long-cherished dream of mankind to be able to predict what happens in nature. One possibility for this is offered by experiments in which e.g. a "ground zero" is established by clearing (Abella et al. 2018) and later this area is left to natural succession. The subsequent repopulation processes are recorded and evaluated. From the results, deductions can be derived to predict processes in other, similar areas. This becomes particularly complicated when not only the succession of plants is to be considered, but especially their effects on higher trophic levels such as herbivores. In this case the assumed order of predictability is: species richness, species functions, and species composition (Abella et al. 2018). Differentiated knowledge about precisely these aspects is rare but of enormous importance when it comes to implementing or improving nature conservation management. The analysis and interpretation of such successional processes at higher trophic levels is rendered even much more difficult if near-natural areas are under ongoing but changing anthropogenic influences. As a consequence, till today there is a lack of knowledge about how far succession towards “wilderness” in conservation areas can compensate for anthropogenic impacts that act from the surroundings of a reserve.

In PsV we have got exactly this situation, but fortunately a documented history of this area and its surrounding landscape, as well as evaluable historic private and museum collections of moths plus own empiric data spanning 15 years. Together these data result in a nearly unique record spanning about 80 years. Consequently, this rare situation offers the unique possibility to analyze long term changes in moth assemblages, leading to the second part of the dissertation:

Cause analysis for long term changes in the PsV moth species richness with special consideration, how succession shapes the composition of the moth community.

With this database, I attempted to address what happened in the last 80 years to the moths in PsV, including information about the sensitivity of individual species to factors such as isolation or anthropogenic disturbances. Certain properties of the species or groups of species can be decisive in determining whether they suffer from an increased risk of local extinction. It is therefore important to take a close look at physiological and functional traits. There are already studies that suggest that larger species in isolated habitats also have a higher risk of extinction (Nolte et al., 2019). Concerning specialized species, we have a similar situation (Mangels et al. 2017). It was also observed that biotic homogenization often takes place and specialists are replaced by generalists (Tanjona et al. 2018, Bagchi et al., 2018). It was therefore tempting to ask whether the sum of the influences in combination with the physiological and functional properties of the Heterocera would result in an all-encompassing shift in species composition. These considerations led to the third part of this dissertation:

Clarification of the question whether there was in the past 80 years a directional shift in the moth community of PsV and therefore, whether large or specialized moths in particular have a higher risk of extinction.

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6. How differences in the settling behavior of moths (Lepidoptera) may contribute to sampling bias when using automated light traps

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Abstract

Quantitative community-wide moth surveys frequently employ flight-interception traps equipped with UV-light emitting sources as attractants. It has long been known that moth species differ in their responsiveness to light traps. We studied how the settling behavior of moths at a light trap may further contribute to sampling bias. We observed the behavior of 1426 moths at a light tower. Moths were classified as either, settling and remaining still after arrival, or continually moving on the gauze for extended periods of time. Moths that did not move after settling may not end up in the sampling container of the light trap and therefore are under-represented in automated trap samples relative to their true proportions in the community. Our analyses revealed highly significant behavioral differences between moths that differed in body size. Small moths were more likely to remain stationary after settling. As a corollary, representatives of three taxa, which in Europe are predominantly small species (Nolidae, Geometridae: Eupitheciini, Erebidae: Lithosiini), usually settled down immediately, whereas most other moths remained active on or flying around the trap for some time. Moth behavior was also modulated by ambient temperature. At high temperatures, they were less likely to settle down immediately, but this behavioral difference was most strongly apparent among medium-sized moths. These results indicate the likely extent of the sampling bias when analyzing and interpreting automated light-trap samples. Furthermore, to control for temperature modulated sampling bias temperature should always be recorded when sampling moths using flight-interception traps.

Key words

Lepidoptera, moths, biodiversity assessment, sampling method, light-trapping, sampling bias

Introduction

Flight-interception traps using UV light as an attractant are the most widely applied method for assessing the diversity of nocturnal moths (Southwood et al. 2003, Lamarre et al. 2012, Jonason et al. 2014, Merckx & Slade 2014). Like all survey methods light-trap samples do not perfectly mirror the true compositions of animal communities (Southwood et al. 2003, Merckx & Slade 2014). Light intensity, spectral composition of the emitted light (Cowan & Gries 2009, van Langevelde et al. 2011, Somers-Yeates et al. 2013), light pollution from nearby alternative illuminations, trap design (Intachat & Woiwod 1999, Muirhead-Thompson 2012, Bates et al. 2013) and moonlight all modulate the attraction of moths to light traps (Davies et al. 2012, 2013, Gaston et al. 2013). Other factors that influence the likelihood of moths being captured by light traps include their wing shape or flight times (Beck & Linsenmair 2006, Beck et al. 2011, Fuentes-Montemayor et al. 2012, Lintott et al. 2014). Nevertheless, using a standardized design of light-trap is a convenient way of characterizing moth communities along ecological gradients.

Comparing catches obtained with automated light traps and hand-sampling revealed that some moth groups, especially small species of Geometridae, tend to be strongly under-sampled (Axmacher & Fiedler 2004, Merckx & Slade 2014). However, the reasons for this under-representation of small moths remained obscure. So, there are possibly other factors, influencing the effectiveness of automated light traps. Temperature can modulate activity of nocturnal moths (Hrdy et al. 1996, Pinault et al. 2012) and therefore could also lead to differences in flight behavior. Another potential, but under-explored source of the variation in capture probability is the difference in behavior of moths after arrival at a light trap. Bates et al. (2013) report that "observations of moth behavior at traps have shown that it is not just the proportion of moths captured by a trap but also the proportion of moths retained by a trap, that combine to influence trap capture efficiency". For example, catches of *Noctua pronuba* differed significantly in their numbers depending on the type of trap used by Bates et al. (2013).

Flight-interception light traps usually consist of a sheet or cylinder of a transparent material (such as acrylic glass). Moths that collide with this obstacle may fall through the funnel into the container at the bottom of the trap. The probability of moths falling through the funnel is increased when moths are highly active in flying around the light trap, which results in them colliding many times with the obstacle. Other moths, in contrast, immediately settle on these surfaces or elsewhere on the trap and do not fall into the collecting device. In this study we recorded moth settling behavior at a light tower in order to determine whether the species differed in behavior depending on their body size and taxonomic affiliation.

Our goals were to:

1. Assess whether certain moth groups (defined by body size or phylogenetic relationships) have a higher likelihood of settling down directly upon arrival at the light source, as compared to other groups; and

2. Establish if this behavioral response of moths is contingent on ambient air temperature. Given that small moths tend to be under-represented in samples obtained using automated light traps, the expectation was that these moths might differ from large moths in their settling behavior after arrival at a light source.

Material and Methods

Observations were recorded at seven locations on 13 nights in PsV, Parco regionale del Delta del Po (Ravenna, Italy). We sampled hygrophil forest, pine forest, downy oak forest, wetland with reed vegetation and dry and open grassland habitats. Observations were recorded from twilight (depending on the season, which on average was 9:00 PM) till 12:00 PM. Observations were terminated when hardly any moths arrived at the light trap due to low temperatures (i.e. when the temperature dropped to about 15°C in June to October 2013). As the light source, an Osram 500 W HWL lamp powered by a Honda EM 500 gasoline generator was used. The light tower consisted of a gauze cuboid, 1.80 m high with a top edge length of 0.40 m (Fig.

6.1). Temperature was measured directly at the light tower using a digital thermometer (Febi Bilstein 37476 Sensor). Mean night temperatures during observations ranged from 14.5°C to 28.5°C. Depending on their behavior immediately upon arrival, moths were classified either as "settled" (if they remained at their initial landing place for longer than 30 s) or "restless" (if moths behaved otherwise). Temperature was recorded at the time of a moth's arrival. Since this was not always possible, for a couple of records there are no temperatures. To avoid pseudoreplication all moths were then caught and kept for later determination. For practical reasons, we only considered so-called "large" moths belonging to the families Cossidae and Limacodidae and to the "Macro-heterocera" sensu Regier et al. (2013). Moths were divided into three size classes based on their wing span (big: > 40 mm, mean = 51 mm, standard error = 8.54, n = 131; medium: 30–39 mm, mean = 34 mm, standard error = 2.08, n = 765; small: < 30 mm, mean = 23 mm, standard error = 2.41, n = 317). Inspection of the frequency distribution of our data revealed that this classification yielded a rather even partitioning. Data on wing span were obtained from http://ukmoths.org.uk/ (last visited 11.10.2015) and by direct measurements of specimens when data was not available. Moths were also classified according to their systematic affiliation. Six families were explicitly considered (Geometridae, Nolidae, Noctuidae, Erebidae, Notodontidae, Lasiocampidae) plus two tribes (Eupitheciini within the Geometridae; Lithosiini within the Erebidae). Three other moth families (Cossidae, Limacodidae, Drepanidae) were too poorly represented in our data to warrant representation as distinct taxa in our statistical model, but were included in the analyses of size classes. Sphingidae did not appear at our light traps and hence were not included in our analysis. We analyzed our data using the logistic regressions in the package Statistica 7.1 (StatSoft Inc.). In this analysis only those species for which more than eight individuals were observed were included. Furthermore, Generalized Linear Models (GLM) with binomial error structure and logit link function were used.

Figure 6.1: The light trap used in this study.

Results

The regression analysis revealed that the settling behavior of moths of the three body size classes differed. Small moths were far more likely to settle down immediately upon arrival than medium or large moths (Wald's χ^2 2df = 62.20, $p < 0.0001$; Fig. 6.2). The probability of settling down immediately decreased with increase in temperature (regression coefficient in

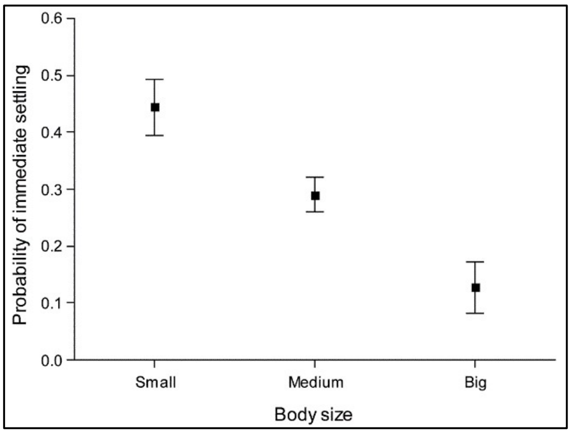

Figure 6.2: Probability of small, medium and big moths settling down immediately after arrival at a light source. Given are the means ± 95% confidence intervals. The small moths were more likely than the medium and big moths to settle down immediately after arrival at the light source.

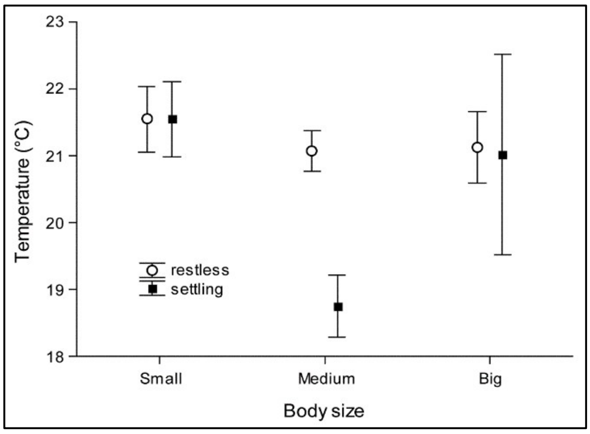

Figure 6.3: Mean temperatures ± 95% confidence intervals at which moths in the three body size classes settled down immediately after arrival at the light source (solid squares) or remained active (empty circles). Medium-sized moths mostly tended to settle at low and remained active at high temperatures, which differs from the behavior recorded for the small and large moths.

logistic model: $b = –0.0882$, $t_{1399df} = 5.612$, $p < 0.0001$), but this effect was largely restricted to medium-sized moths (Fig. 6.3). For this size category, the temperature effect was much stronger (regression coefficient in logistic model: $b = –0.1608$; $t_{821df} = 7.525$; $p < 0.0001$). Settling behavior was also strongly contingent on ambient temperature, but in a more complex way.

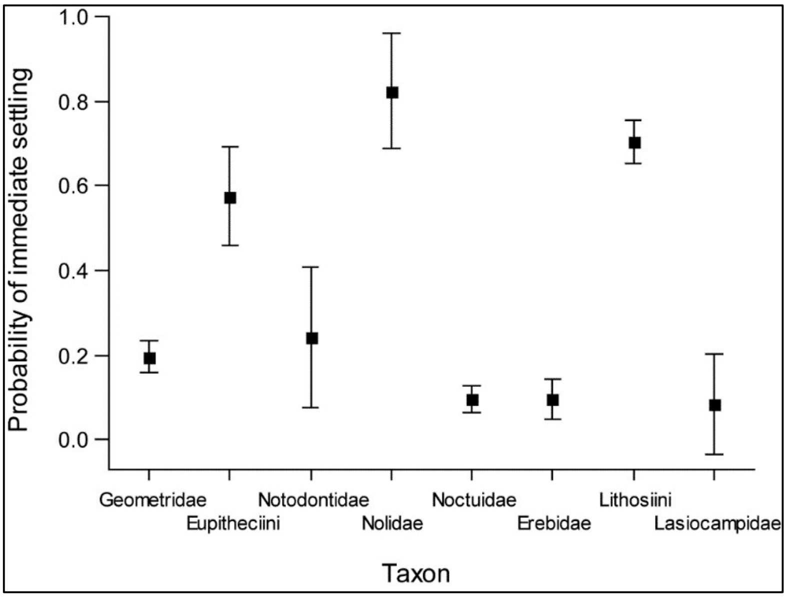

Figure 6.4: Probability of moths belonging to eight taxa settling down immediately on arrival at a light source. Given are the means ± 95% confidence intervals. Species of Eupitheciini, Lithosiini and Nolidae were highly significantly more likely to settle down than those of the other groups (Wald's χ^2 7df = 433.57, $p < 0.0001$).

GLM revealed that settling behavior was significantly correlated with body size (df = 2, Wald's $\chi^2 = 17.591$, $p = 0.0002$) and temperature (df = 1, Wald's $\chi^2 = 5.284$, $p = 0.0215$). This effect was even more obvious when body size and temperature were combined (df = 2, $p < 0.0001$). In addition, we observed highly significant differences in settling behavior of the different moth taxa (Fig. 6.4; $p < 0.001$). Species of Eupitheciini, Lithosiini and Nolidae were more likely (60–80%) to settle down immediately after arrival at the light source. In all the other groups caught in sufficient numbers, 80% or more of the moths continued flying or crawling around after arriving at the trap. Of the Cossidae 6 of 19 moths (31.6%) settled upon arrival, of the Drepanidae 0 of 7 and of the Limacodidae 0 of 5 moths.

Discussion

The settling behavior of moths at light traps was strongly associated with their wing span, with small moths more likely to remain stationary after settling. This behavior reflects the sampling bias towards large moths that is reported in two earlier studies (Intachat & Woiwod 1999, Bates 2013). We suggest that this big difference in behavior is one of the main causes of the under-representation of small moths in the catches of automated light-traps. Our findings also indicate that moth behavior after arrival at a trap may be modulated by ambient temperature. While in general ectothermic moths are obviously more active at high temperatures (Van Dyck 2012), especially in medium-sized moths the probability of remaining active for some time was much higher at high air temperatures. This also makes it more likely that medium-sized moths will be caught by automated flight-interception traps at high temperatures, whereas at low temperatures the same moths are more likely to immediately settle down when coming into contact with a light trap and thus escape being collected. If this is a general phenomenon it indicates that the efficiency of automated light traps will be constrained at low temperatures (Summerville 2013, Jonason et al. 2014) by some kind of size-temperature interaction effect. Generally, large moths flew around the light source for longer and were less likely to settle down immediately than small moths. This may be explained by their greater ability to store heat even at low temperatures, since moths with a large thorax have a physiological advantage in being able to retain for longer more of the heat, they produce through muscle activity, whereas convective cooling acts more rapidly in small insects (Heinrich 2013). Since we collected all the moths that settled and remained stationary on the gauze it is unknown whether these moths would have remained inactive throughout the night or would have become active again. If moths resume activity sometime after arrival at the light trap, this could again increase their likelihood of their being caught. Furthermore, the material the light trap is made of might influence the moths settling behavior. It might be easier, especially for large heavy moths, to settle down on gauze than on acrylic glass. To test this, further studies are needed. In this study, small moths did not differ in their behavior at different temperatures. Both, settling and restless individuals were recorded at 21 to 22°C. However, most of these moths were caught in early summer, when night temperatures often reach 21 or 22°C. So, most of these moths are likely to have been sampled at these temperatures and this result is an effect of sampling mainly on warm nights. To better understand the settling behavior of small moths, further data needs to be collected for cold nights. Light source and intensity can affect the species of moths sampled (Cowan & Gries 2009, van Langevelde et al. 2011, Somers-Yeates et al. 2013). In addition, the comparison of the results of studies using manual sampling and automated light traps also reveal irregularities in the number of species and specimens sampled. For example, in our study, Eupitheciini made up 13.6% of all Geometridae species and 14.4% of all Geometridae specimens caught. Unpublished data of manually light-trapped moths sampled in the botanical garden of Bayreuth also indicate that Eupithecia made up 8.0% of all Geometridae species caught (and 4.1% of the Geometridae individuals). However, in a study in the Swiss Alps using automated traps (Beck et al. 2010), the genus Eupithecia accounted for 2.3% of the species of Geometridae, but these made up only 0.4% of the geometrids caught. In samples collected by Truxa & Fiedler (2012), Eupitheciini made up 8.6% of the 140 automatically light trapped species of Geometridae, but accounted for only 2.1% of all the specimens of that family. Comparing the percentage of

Eupitheciini in all four studies, manual light trapping recorded more specimens of Eupitheciini (although the percentage of Eupithecia species recorded was more or less the same). So, based on our behavioral data reported above, the contribution to the community in terms of Eupitheciini seems underrecorded by automated light traps. Based on our results and in line with earlier direct comparisons of automated versus hand sampling at light traps we conclude that automated traps may inaccurately quantitatively characterize assemblages of small lepidopterans (Bates et al. 2013). In automatically sampled data we therefore expect an under-representation of families with small species like Tortricidae, Gelechiidae or Pyralidae, which are abundant and species-rich in many habitats. For example, in a comparison of moth assemblages in different types of flood-plain forests in Central Europe based on catches by automated light-traps (Truxa & Fiedler 2012), the predominantly small Pyraloidea accounted for 17.2% of the species recorded, but made up only 8.1% of the total catch of > 32,000 individuals. As a consequence, functionally important moth guilds predominantly composed of small species might be under-represented, such as species with endophagous larvae (like many Eupitheciini, but also micro-moths like Tortricidae or Pyraloidea) or detritivorous species (e.g. the genus Idaea). Our observations of moth behavior at a light trap also indicate that lichen moths (Erebidae: Lithosiini) might sometimes be under-represented in automatic samples. This could be important when numbers of lichenophagous species caught are used as indicators of ecosystem status (Thorn et al. 2015). However, in the study of Truxa & Fiedler (2012) no such under-representation was obvious, as lichen moths made up 20.5% of the Erebidae species caught, but accounted for 84.0% of the individuals, mainly due to the massive representation of one species (*Pelosia muscerda*) in the trap samples. These examples illustrate that differences in moth settling behavior at light traps associated with their size and/or phylogenetic position, may account for the deviations between capture rates in surveys and their abundance in their respective habitats, but that these relationships do not allow for simple generalizations. Moreover, it should be stressed that this does not devalue light trap samples as sources of information on moth biodiversity or community ecology (Merckx et al. 2012 a, 2012b, Truxa & Fiedler 2016). As long as the same light sources and types of traps are used in studies carried out along ecological gradients, there is little reason to assume that sampling bias will result in seriously distorted ecological patterns. Nevertheless, in future studies the fact that small moths and other taxa might be undersampled, especially when sampling different habitats, should be considered. However, in view of our observations it would be desirable to further elucidate the relationships between sampling results and behavior. More studies on the individual flight behavior of a wide range of different groups of moths that simultaneously address the effect of temperature, light conditions (moonlight, spectral characteristics of light sources) and body size of nocturnal moths are needed.

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7. Multi-decadal surveys in a Mediterranean forest reserve – do succession and isolation drive moth species richness?

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Abstract

Isolated fragments of semi-natural habitats are often embedded in a landscape matrix that is hostile to organisms of conservation concern. Such habitat islands are prone to changes in their biota over time. For insects, few studies on long-term trends in species richness within conservation areas are available, mainly due to the lack of historical data. We here use moths in the coastal pine wood reserve PsV (Ravenna, NE Italy) to assess how local fauna has changed over the last 85 years. This reserve has experienced massive changes in vegetation structure due to secondary succession. We compared historical collections (1933–1976: 107 species; and 1977–1996: 157 species) with our own samples (1997–2002: 174 species; and 2011+2012: 187 species). Over the last 85 years, the proportion of habitat generalists in relation to all recorded moth species increased from 20 to 33%. The fractions of woodland and open habitat species concomitantly decreased by 10 percentage points, respectively. Amongst woodland and habitat generalist species, gains outnumbered losses. In contrast, 18 species of open habitats and 10 reed species were lost over the decades. We attribute these changes to vegetation succession and to the isolation of the reserve. Generalist species are presumably better able to pass through anthropogenically exploited landscapes and colonize isolated habitat fragments than habitat specialists.

Keywords

Anthropogenic influence, biodiversity, conservation biology, generalists, habitat specialists, Italy, Lepidoptera, long-term changes, moth diversity

Introduction

Mediterranean coastal pine forests are vulnerable and rare ecosystems. They provide habitat for many species, but have experienced massive contractions over centuries and are nowadays strongly at risk through anthropogenic land-use intensification (Gasparella et al. 2017). Only few coastal pine forests have persisted on the Italian Peninsula. Some of them have been awarded high legal conservation status (Gasparella et al. 2017), but the current status of their biodiversity is often imperfectly known (Scarascia-Mugnozza et al. 2000). In general, isolated nature reserves, embedded in a landscape matrix dominated by intense human land-use, run the risk of losing over time those organisms for which they had been established (Mora & Sale 2011). Even for large-sized conservation areas, such detrimental trajectories have been documented from a range of biomes (Gauthier et al. 2015, Hautier et al. 2015, Uhl et al. 2016). Some species may persist for decades in conservation areas, but eventually get lost over time, be it due to ecosystem degradation or just attributable to stochasticity. This notion has led to the concept of an extinction debt and, only in the long term, can it be evaluated whether organisms are really safe in the reserves that have been set aside for their conservation (Carroll et al. 2004, Halley et al. 2016). Especially in areas where anthropogenic influence has been severe over decades, long term studies are of great interest because they can mirror changes in an ecosystem best and shed light on the steady erosion of biodiversity (Habel et al. 2016).

To understand long-term changes of the insect fauna in an isolated nature reserve, we investigated moth communities in PsV. Nowadays protected as a Natura 2000 site (Montanari 2010) and listed in the Convention of Ramsar (Ramsar Convention Secretariat 2013) as "wetland of international importance", the coastal pine forest PsV, since the year 1988, forms part of the Parco Regionale del Delta del Po which is also covered under the EU life programme (http://ec.europa.eu). Therefore, it is of high interest which long term changes might have taken place in this reserve.

Originally PsV is believed to have been covered by dune vegetation that later changed to a humid forest. Around the 12th century, Italian stone pine trees (*Pinus pinea*) were planted for wood and pine nut production. Timber extraction and commercial pine nut harvest were abandoned in 1988 when the Parco Regionale del Delta del Po was established (Enrica Burioli, pers. communication). In general, Italian coastal areas with wooded dune habitats show strong signs of vegetation succession during the past decades. In particular, the grassland fractions in these areas have prominently declined since the 1960s (Prisco et al. 2016). Comparisons of old photographs with the current vegetation status also indicate that, in PsV, shrub and tree cover has massively increased at the expense of grassland that historically provided a habitat for numerous non-forest species (Fig. 7.1).

Apart from succession, there are multiple external sources of environmental stress acting on the nature reserve. These include the neighboring industrial harbor of Ravenna (Lucialli et al. 2007) as well as a surrounding landscape dominated by intense agriculture (Benini & Pezzi 2011). The heavy use of pesticides in Ravenna's agriculture is also well documented (Paris et al. 2016). Therefore, pesticide drift must be considered to possibly affect organisms inside the reserve. Furthermore, the whole region is subject to subsidence induced soil salinization, raising salt concentrations in soil water up to 22 g/l in PsV due to continual groundwater pumping and offshore gas production (Antonellini et al. 2008). Finally, climate change is also evident in the region of Ravenna. Surface solar radiation in northern Italy decreased from 1959 until the mid-1980s, followed by an increase later on (Manara et al. 2016). Mean annual temperatures increased between 1961 and 2010 (Antolini et al. 2015), accompanied by substantial changes in precipitation levels. As a consequence, the overall aridity in the region has increased, particularly near the coastline (Appiotti et al. 2013). Against this background, it is expected that − apart from mere stochastic effects on local colonization and extinction promoted through isolation (Haddad et al. 2015) − natural succession, air pollution, soil salinization, pesticide drift and climate change may have triggered long-term directional ecosystem changes in PsV.

We here combine multi-annual data from our own observations with a rare set of historical records to assess the transformation of the insect fauna in a Mediterranean coastal pine forest over the last 85 years. Regional lepidopterists visited PsV over many decades to conduct light-trapping, thereby collating faunal data for the area. We consider macromoths as suitable focal organisms for this type of study since they occur in high numbers, are rich in species and have short generation times (usually one year or less), rendering quick responses to environmental change visible.

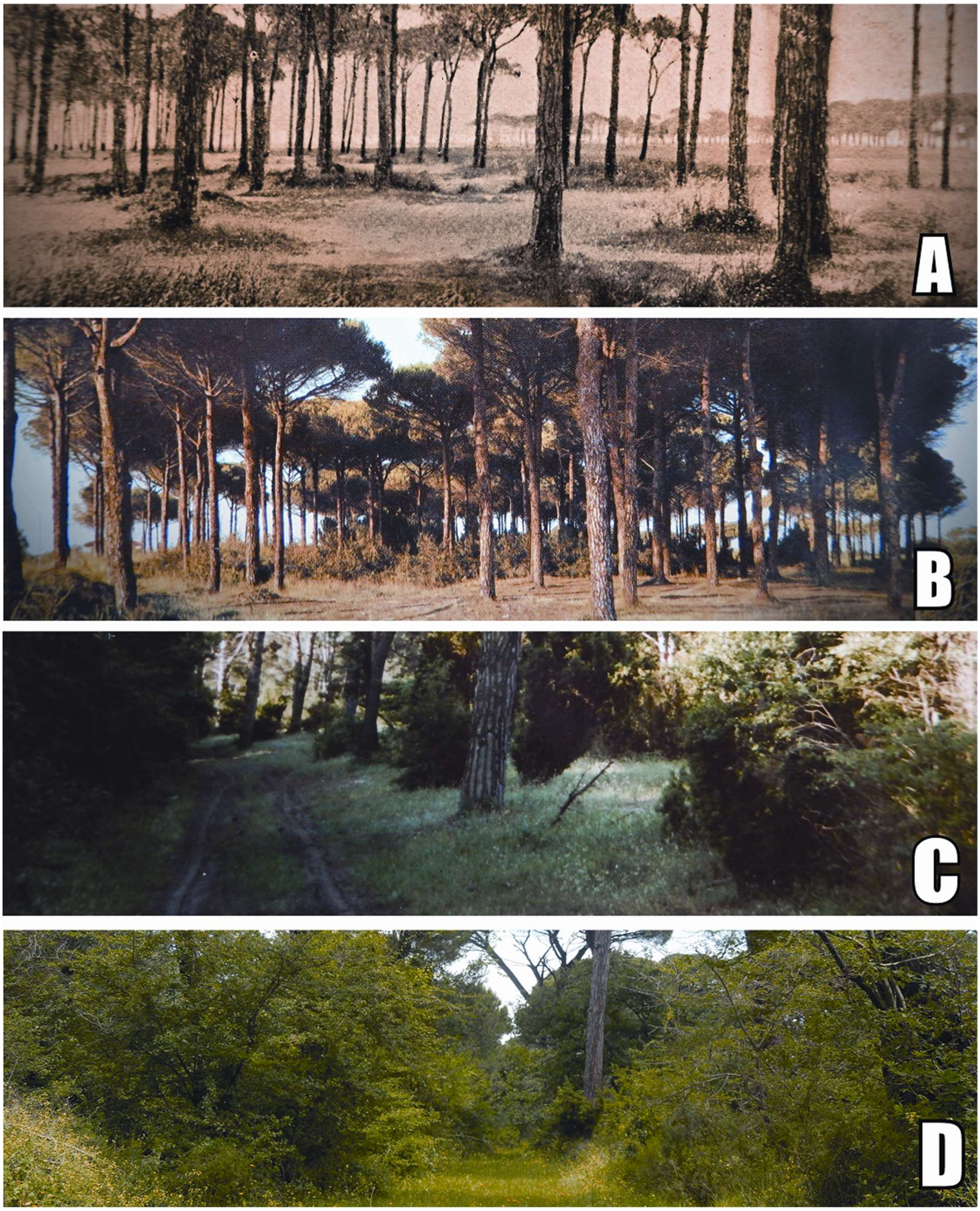

Figure 7.1: Succession of vegetation in PsV. A = 1910, B = 1970, C = 1996, D = 2017.

Moreover, moth species span a wide range of trophic affiliations, especially during their larval stages. Many species are quite specialized to particular host plants or habitat structures, which renders them susceptible to anthropogenic habitat changes (Fox 2013). With these data, we test the following hypotheses:

1. Species richness has overall decreased because of manifold anthropogenic influences and stochastic extinctions;
2. Species numbers of forest-bound moths have increased and non-forest moths decreased, due to the succession towards more forest cover within the reserve.

Material and Methods

Historical moth data

To analyze multi-decadal changes in moth assemblages, data spanning a period of about 80 years were collated (Tab. 7.1). Two historical collections of moth specimens, accompanied by reliable data on sampling localities and sampling dates, were traced in regional natural history museums in Italy, viz. the Museo di Storia Naturale di Venezia (vouchers from 1933–1968) and the Museo Civico delle Cappuccine, Bagnacavallo (vouchers collected between 1966–1976). Further data were made available by a private collector, providing samples from the years 1977–1996 (E. Bertaccini, pers. communication). All moths, reliably labelled to have been collected in PsV, were considered for analysis. Identifications were cross-checked and corrected according to up-to-date taxonomy, whenever required. These historical voucher collections were qualitative in the sense that we have no information as to the reasons why collectors decided to keep or discard observed specimens. For sure, no large voucher series of common species were assembled at these earlier times. We consider it likely that early collectors always kept vouchers of species that appeared to be ‘new’ to them for the site, whereas they may have ignored common species after their first observations.

Extant moth data

Data on the extant moth assemblages in PsV (expansion from north 44°31'39.15"N, 12°14'19.82"E to south 44°27'48.09"N, 12°13'43.67"E and west 44°29'51.96"N, 12°13'22.79"E to east 44°29'50.50"N, 12°14'15.56"E) were sampled by means of lighttrapping in two time periods between 1997 and 2012, but in different manners. From 1997 to 2002, moths were attracted to one single light trap and manually sampled, mainly in early summer and early autumn, at seven locations within different types of vegetation (viz. downy oak forest, hygrophilous forest and, occasionally, reed or remaining open habitats). In the years 2011 and 2012, we more systematically collected moths in spring, early summer, high summer and autumn. This was done in four different habitat types prevalent in PsV (viz. reed, hygrophilous forest, downy oak forest and open habitats) to cover the moth community of the entire reserve as completely as possible. In 2011, automated light-traps were run at 20 sites rather equally distributed within PsV, which allowed sampling multiple habitats simultaneously. In 2012, the light trap employed from 1997 to 2002 was used again at nine locations. Moths were manually collected at this trap, but due to the high demand of manpower, this could be realized only at a smaller number of sites (see Tab. 1 for further details). All vouchers, sampled since 1997, are stored in the private collection of Mirko Wölfling (Niederwerrn, Germany).

Table 7.1: Overview of the moth collections from PsV, available for evaluation. Only those moth species which qualified for a comparative analysis are considered in this tabulation (see Methods section).

Collection	**Period**	**Number of recorded species**	**Number of trap locations**	**Type of trap and lamps**
Callegari + Martinasco combined	1933–1976	107	Unknown	Unknown
Bertaccini	1977–1996	157	Unknown	Unknown
Wölfling Early extant data	1997–2002	174	7	500 W HWL, manual
Wölfling Recent extant data	2011–2012	187	20 (2011) 9 (2012)	2011: Sylvania 15 W BL + 15 W white BL, automated 2012: 500 W HWL, manual

Data management and analysis

Our primary target group were species of the monophyletic clade Macroheterocera sensu Regier et al. (2017), augmented by a handful of larger-sized representatives of Cossidae and Limacodidae that have traditionally been treated as ‘macro-moths’ by earlier European lepidopterists. Since our extant data were exclusively derived through light-trapping, we removed all strictly diurnally active Macroheterocera from the historical data to improve comparability. We also omitted species from the historical records that are on the wing only during the cold seasons, since we have no recent data for these parts of the year. Finally, we took out from all time periods species that only show up in NE Italy as sporadic or seasonal long-distance migrants, but which are not able to build up persistent populations there. In a couple of cases, cryptic species diversity has been uncovered amongst moths represented in our data in recent years, while in the historical collections, these were still treated as just one species each. We then adopted the older (more inclusive) taxonomic species delineations for our analyses, since it was not possible to re-examine all historical records by means of anatomical or DNA-sequence based methods.

For analysis, we partitioned our data into four time horizons. The first time horizon covered collections from 1933–1976 (collections Callegari and Martinasco), the second one refers to the period 1977–1996 (collection Bertaccini). The third time horizon was represented by our own samples from 1997–2002 and the fourth group by our own samples collected in 2011 and

2012. For sample-based species richness estimation, each of the four time horizons had to be subdivided into sampling units. Since we have no information about the details of moth sampling in the old collections, we instead used individual calendar years as proxy for sampling units. This way, the moth data of the first time horizon were allocated to 33 subsamples and those of the second time horizon to 20 subsamples. For the time horizon from 1997–2002, we instead used sampling nights as units. The same was done for the year 2012. In 2011, when automated light traps were used, we decided to choose sampled habitats per season rather than sampling nights. As the four automated light traps sampled four different habitats in one sampling night, choosing sampling nights as a unit would mean pooling data from different vegetation types. With manual light trapping, as it was performed in all the other years of our own sampling, just one vegetation type per night could be sampled. To adjust automated to manual samples by number of sampling units, choosing sampled habitats rather than sampling nights therefore seemed to be the most logical approach. As a consequence, we came up with 30 subsamples for the 1997–2002 timespan and 41 subsamples for the most recent timespan 2011/12.

For comparisons of moth species richness between the four temporal layers, we then analyzed species accumulation by incidence data using the programme iNEXT online (Chao et al. 2016). We further partitioned observed moth species into inhabitants of wooded habitats, species of open habitats, reed habitats and habitat generalist species, respectively. In three of these subsets, we again checked for temporal changes in species richness by means of species accumulation analysis across the four temporal layers, as described above. Reed species were too few to allow for a meaningful analysis through species accumulation statistics.

Finally, we used the information of species incidence counts to calculate the proportions of these for classes of habitat affiliations across the four time horizons. Using χ2 -tests, we checked for significant differences in the representation of species per category of habitat use over the four time horizons. With these data, we also created pie charts to visualize the relationships between the different habitat users and how these might have changed proportionally over time.

Information about habitat affiliations of moth species was compiled from Ebert (1994–2003), Hausmann & Viidalepp (2012), Redondo et al. (2009), Rákosy (1996) and from various internet sources (www.lepiforum.de; www.pyrgus.de; www. euroleps.ch). The resulting classification of moth species into the four groups of habitat use can be found in Suppl. material (Appendix S2).

Results

In total, we assembled records of 403 macro-moth species for PsV. From these species, 103 species recorded in older collections had to be deleted from the analyses presented below, as they are either on the wing during the cold seasons only, show exclusively diurnal flight activity or only reach the area as sporadic long-distance migrants, leaving exactly 300 species of macro-moths for the present analyses. With the above adjustments, historical records could be traced for 219 macro-moth species that were observed in PsV during the 20th century. The two sets of historical collections comprised 107 (1933–1976: Callegari + Martinasco) and 157 species, respectively (1977–1996: Bertaccini). Our own samples covered in total 174 species from 1997–2002 and 187 species in the years 2011+2012 (237 species in total since 1997). Altogether, 63 of the 219 species covered by historical records (28.8%) have never been observed again by us in PsV since 1997. We consider these below as 'lost' species. On the other hand, our data comprise records of 81 species that were not represented in the earlier collections ('gained' species). These gross figures indicate a substantial turnover in moth species composition over time, but they need to be controlled for sampling intensity prior to interpretation.

An incidence-based comparison of older time layers with the more recent datasets clearly shows a substantial increase in total macro-moth species richness after correcting for sampling intensity (Fig. 7.2). When extrapolated to a standardized number of 40 sample units, an estimated plus of about 67 species has occurred. In particular, 119 macromoth species (± 13) were estimated for the oldest data, 193 species (± 21) for the time period from 1977–1996, 190 species (± 14) for the years 1997–2002 and 186 species (± 10) for the newest data. This corresponds to an increase by 56.3–59.7% in total moth species richness over the course of the entire time span, whereas almost no change is apparent if only the more recent data from the Bertaccini collection are taken as the basis. Concerning the three classes of moth species according to their habitat use (Fig. 7.2), our comparisons at a standardized number of 40 samples revealed the following results. In total, 75 generalist species were observed over the last 80 years. From the oldest to the most recent time horizon, habitat generalists showed a plus of 28 species (1933–1976: 24 ± 6, 1977–1996: 56 ± 12, 1997–2002: 51 ± 4, 2011+2012: 52 ± 7), viz. an increase by 112.5–116.7%. Altogether, 123 woodland moth species were represented in the records. Woodland moths showed an estimated plus of 26 species over the full timespan (1933–1976: 54 ± 10, 1977–1996: 70 ± 14, 1997–2002: 76 ± 9, 2011+2012: 80 ± 5), which means an increase by 40.7–48.1%. Overall, 73 open habitat species have thus far ever been recorded from PsV. With an estimated plus of 6 species, which refers to an 18.8–46.9% increase, open habitat users had the lowest increase in species numbers (1933–1976: 32 ± 7, 1977–1996: 40 ± 9, 1997–2002: 47 ± 10, 2011+2012: 38 ± 5). Observed species counts and the respective estimates for a standardized number of 40 sample units of macro-moths at the four time horizons in PsV, including segregation into classes of their habitat use, are listed in Tab. 7.2.

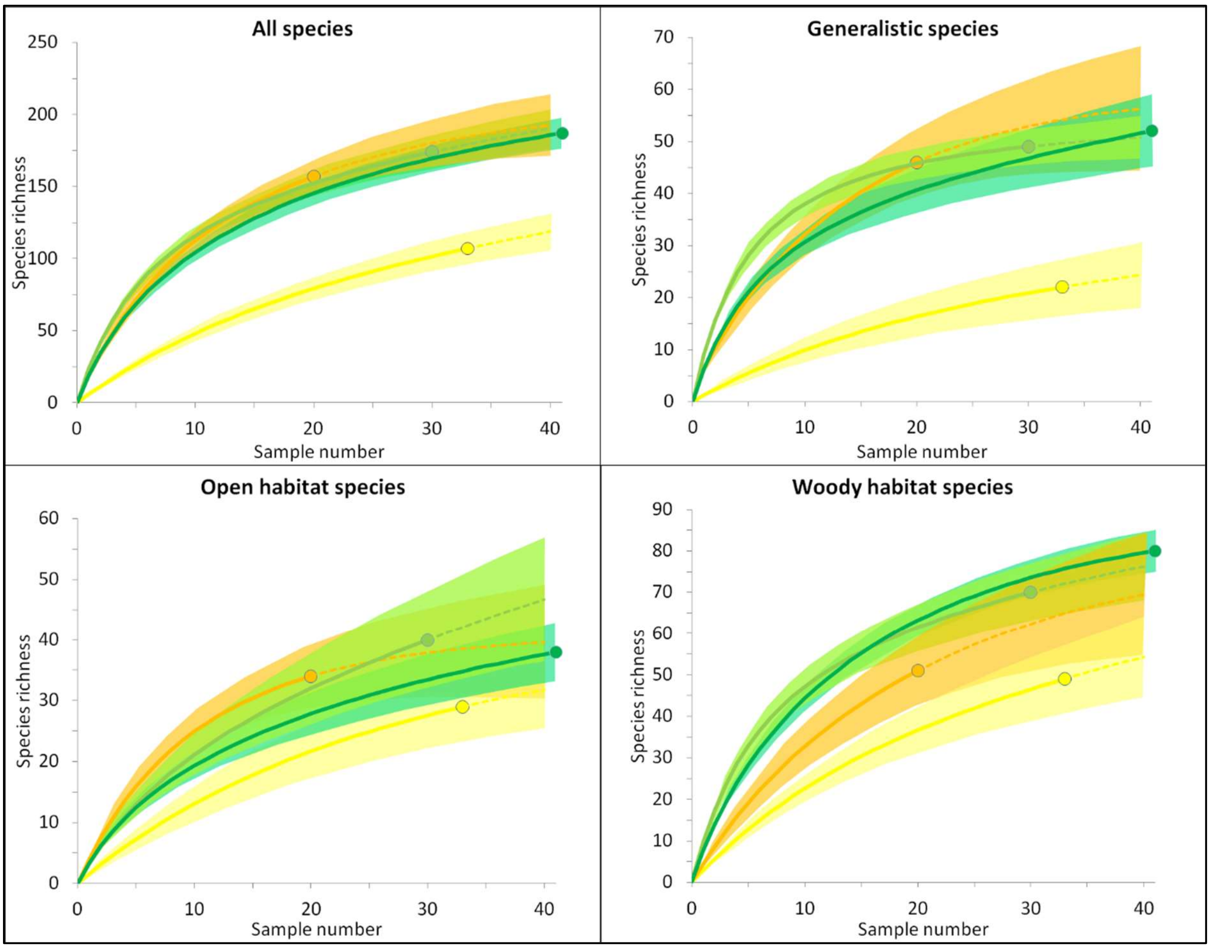

Figure 7.2: Species richness accumulation of macro-moths in PsV according to their habitat use, across four time horizons, as a function of the number of sampling units calculated in iNEXT. Shaded areas: 95% confidence limits. Yellow = 1933–1976, orange = 1977–1996, light green = 1997–2002, dark green = 2011/2012. Filled circles indicate observed species numbers at the respective number of available sampling units.

The contribution of moth species associated with individual habitat types (Fig. 7.3) revealed an increase in the proportion of generalist species, from 20.5% in the earliest samples to 32.3–37.4% around the year 2000 and later on. In contrast, the proportion of woodland species slightly decreased from 48.2% to 40.5%. Similarly, moth species of open habitats decreased in relative prevalence from 26.7% down to 13.6–17.5% of observed species. Reed species contributed only a minor fraction of 4.6–9.7% of the observed species richness per time horizon, except for the decades spanned by the Bertaccini collection, when almost one quarter of the observed macro-moth species were reed dwellers. However, these differences of species numbers in the different types of habitat affiliations and time periods were just not significant ($\chi2_{9df}$=16.73, p=0.055). A comparison of the number of species which disappeared during the last 80 years with those that were newly recorded since the mid-1990s revealed a substantial turnover in all four classes of moths according to their habitat use (Tab. 7.3). Amongst woodland species and habitat generalists, gains were almost twice as large as losses. In contrast, moth species of open or reed habitats were disproportionally prone to losses. These differences were statistically significant ($\chi2_{6df}$=15.78, p=0.015).

Table 7.2: Species counts and species richness estimates of macro-moths in PsV, segregated according to temporal layers and habitat use.

Habitat use	Old data	1970ies	1997-2002	2011 & 2012
All species observed	107	157	174	187
Estimated species total	119	193	190	186
Generalist species observed	22	46	49	52
Generalist species estimated	24	56	51	52
Woodland species observed	49	51	71	81
Woodland species estimated	54	70	76	80
Open habitat species observed	29	34	40	38
Open habitat species estimated	32	40	47	38
Reed species observed	7	26	14	16
Reed species estimated	-	-	-	-

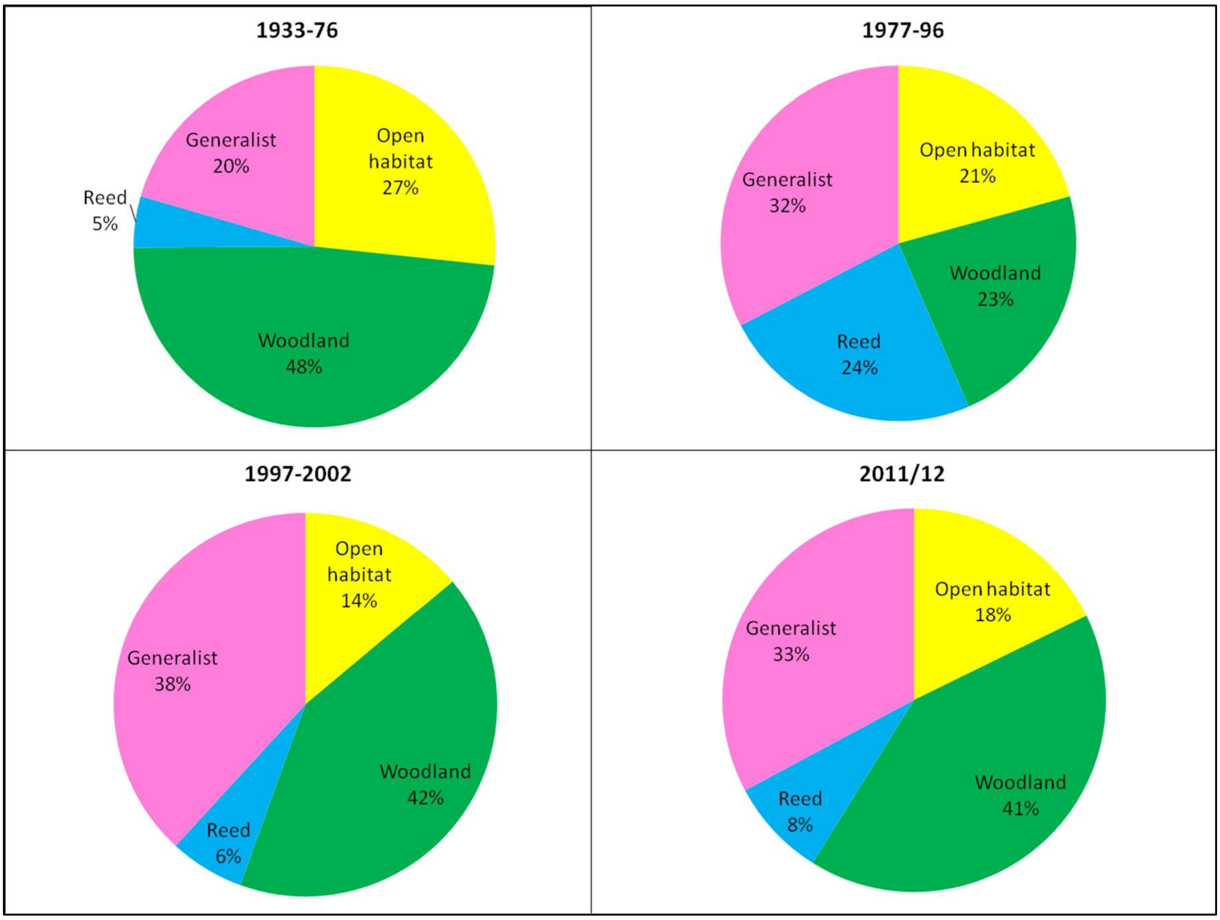

Figure 7.3: Proportions of macro-moth species in four classes according to habitat use, in the four time horizons.

Table 7.3: Numbers of moth species no longer observed after 1995 in the reserve Pineta san Vitale ('lost'), only observed after 1997 ('gained') and present in historical as well as recent surveys ('persistent'), according to their major habitat affiliations.

Habitat use	**Lost**	**Persistent**	**Gained**
Open habitats	18	35	20
Woodland	25	57	41
Generalist	10	46	19
Reed	10	18	1

Discussion

Our study revealed that (1) contrary to expectation, total species richness of macromoths did not decline obviously over the past 85 years; yet (2) indeed a substantial species turnover has occurred, favoring generalist and, to some extent, woodland species, while macro-moths of dry open grassland became far less prevalent than before and also reed species suffered from losses. The first observation is surprising, given the numerous stressors that act heavily on the isolated nature reserve PsV from its immediate surroundings. Observations in German nature reserves, embedded in landscapes of intensive agriculture, indicated that insect biomass has undergone severe reductions in the last decades (Hallmann et al. 2017). The same trend should be expected for PsV.

Apart from pressures exerted by the surrounding land-use, a severe extinction debt in isolated nature reserves such as PsV should be expected from demographic and environmental stochasticity alone (Bommarco et al. 2014). Hence, one might have anticipated a strong erosion of species richness over time (Halley et al. 2016). On the contrary, overall species richness of macro-moths appears to have increased over time, either when considered at a standardized sampling intensity or using the raw species counts. This richness pattern was also largely consistent across all groups of moths according to their habitat affiliations, though this apparent increase was strongest when all moths or only generalist species were considered. We attribute this apparent, unexpected increase in moth species richness to two complementary reasons, viz. secondary succession and sampling intensity. After the definitive abandonment of land-use following the implementation of the current conservation status of PsV, succession has changed the vegetation of the area towards a more complex suite of woodland habitats, at the expense of dry open grassland (Fig. 1). Similar vegetation developments have also been observed elsewhere in northern Italy (Prisco et al. 2016). An increase in species richness should therefore be expected, since the number of niches available in an area usually increases with succession (Hilmers et al. 2018). Indeed, species accumulation analysis suggests that the number of woodland species steadily increased in PsV from the 1930s to the end of the 20th century, but has subsequently remained on the same high level over the past 20 years.

In this context, the species thriving in open and often xeric habitats are also informative. Richness of this group of species has increased the least and even decreased in the most recent collections, although our own quantitative light-trap samples were much larger and thus more comprehensive than earlier records available from PsV. Specifically, our own collections from the years 1997–2002 comprised 1655 moth individuals and those from 2011 and 2012 even 3192 individuals, as opposed to the Bertaccini collection (1459 specimens) and the oldest data (454 specimens). Hence, despite a higher likelihood of detecting open habitat species in these much larger samples, their contribution was low in our data. This well matches the fact that open xeric habitats have shrunk considerably in PsV over the last decades. Moth species of reed habitats contributed only a minor fraction to the moth fauna of PsV, even though this particular nature reserve is part of a wetland national park of international relevance (Montanari 2010, Ramsar Convention Secretariat 2013). This habitat-specialist group of insects, like open habitat species, appears to have experienced disproportional biodiversity losses in recent decades. Despite the much larger size of our own moth samples, only one single additional reed species could be detected, whereas 10 moth species of reed habitats, present in old collections, have never been observed since 1997. However, our sampling efforts were not specifically targeted to surveying wetland species, so this might also be an effect of preferred sample locations of old collectors, for which we do not have concrete information. In contrast, generalist species have increased in absolute species numbers, as well as in their relative contribution to the local fauna. This might indicate that generalists are better able to colonize isolated semi-natural areas than some specialized groups (Slade et al. 2013).

Apart from the overall increase in woodland, open and generalist species richness, which might be due to succession and increased sampling effort, the change in the proportions of the groups over time indicates that PsV, as an isolated nature reserve, might today favor the colonization by generalist species and therefore fail in conserving specialized species (Rossetti et al. 2017). In fact, the proportion of generalists compared to the whole community increased most. These trends are in line with the notion that increasing human pressure on habitats favors generalists over specialists, thereby contributing to biotic homogenization (Mangels et al. 2017). Apart from true species turnover, the apparent increase in species richness may partially be due to the way in which historical collections have been assembled. We do not know which kind of light trap was used by early lepidopterists, but in the 1930s, collectors did not have access to lamps powered by electricity with substantial light emission in the near-UV range. They instead often used petrol lanterns with lower efficiency in attracting nocturnal insects. Moreover, lepidopterists with a keen interest in faunistic research tended to be biased towards keeping records preferentially of the ‘more interesting’ species, i.e. those that are regionally rare or otherwise charismatic. In hindsight, it is impossible to safely tell which species, lacking in old collections, are ‘false negatives’ (i.e. species that were present, but went unnoticed or no vouchers were kept). However, a number of conspicuous species like *Hemithea aestivaria*, *Opisthograptis luteolata* and *Lacanobia w-latinum*, which collectors of the old data would surely have taken, only appeared in the new data. In contrast, small and ‘uncharismatic’ species like *Idaea straminata* and *Deltote pygarga* were sampled by old collectors. In order to compensate for differences in sampling effort, we allocated the old data into two time horizons yielding subsets of roughly similar size. We consider comparing two ‘historical’ periods of low effort sampling with six years of medium and two years of high sampling effort, suitable to facilitate comparisons. We acknowledge that analysis of data from non-standardized sampling by extrapolation is always prone to critique. However, even by comparing the raw data, old collections comprised fewer species than the newer ones (about 80 species). We therefore conclude that the lower richness of the older collections is not only due to sampling effects, but indeed reflects the appearance of new species in the reserve over time.

Even though our results might indicate that preservation of moth biodiversity works quite well within the reserve PsV, many moth species have apparently completely disappeared. We never observed 63 (out of 219) species recorded at least once between 1933 and 1996 during our own light-trapping campaigns. These lost species include conspicuous species (e.g. *Calophasia lunula*, *Plusia festucae*, *Diachrysia chryson* and *Sphinx ligustri*) that are very unlikely to have gone undetected by chance in the period between 1997 and 2012. Lost species also comprise a few species of high conservation concern (e.g. *Calyptra thalictri* and the very rare and localized wetland geometrid *Chariaspilates formosaria*). Even though one can never be entirely sure whether 'lost' species are really locally extinct or whether 'gained' species had not existed earlier in PsV, yet escaped discovery, our analyses show that species turnover in the reserve was non-random. Overall, these considerations indicate that (a) a substantial extinction debt still remains a risk for the fauna of PsV: more local species extinctions are to be expected, just as the losses that have occurred in earlier decades; and that (b) the process of biotic homogenization (Newbold et al. 2018) is likely to proceed here as well. Generalist species already play a larger role in faunal composition than was the case with the historical data. Similarly, moth communities across many regions in Europe tend to become ever more homogeneous, with generalist ubiquitous species replacing specialists (Mangels et al. 2017; Franzén & Betzholtz 2012).

Our analyses indicate that, apart from an apparent increase in recorded species numbers, this area of high legal conservation status is indeed threatened by further erosion of its biodiversity, mainly due to the risk of a strong extinction debt, as well as by landscapelevel constraints on recolonization once species have locally gone missing. In the long run, even though the vegetation in PsV may continue to converge to a more 'natural' structure, the insect fauna in this highly isolated area might be prone to further homogenization. Therefore, active conservation management is most desirable, for example with focus on wetland or open habitat fractions remaining as niches for specialist organisms, in order to safeguard the function of PsV in the context of preserving biodiversity.

Conclusions

To understand changes in insect diversity, there is a strong need for long term analyses. Yet, long-term data from standardized monitoring are largely lacking. Historical collections not only provide an opportunity to gain an insight into community change, but also pose challenges, such as selective or variable sampling effort and gaps in time series. We tried here to extract valuable information on the long term development of biota in an isolated nature reserve by analyzing such old collections. In contrast to our expectations, species richness increased although isolation effects, increased salinity and pesticide use in nearby agricultural areas might have affected the reserve. Therefore, succession might even override these negative effects and conceal possible influences on moth species richness. Open habitat specialists have been lost to a disproportionate extent, whereas generalist and woodland species have increased. These trends reflect both the succession inside the forest reserve, as well as constraints on species dispersal in fragmented landscapes. From a conservation perspective, enhancing connectivity between such reserves is of the highest importance for protecting specialized and rare species.

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8. Ecological drift and directional community change in an isolated Mediterranean forest reserve – larger moth species under higher threat

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Abstract

Long-term data are important to understand the changes in ecological communities over time but are quite rare for insects. We analyzed such changes using historic museum collections. For our study area, an isolated forest reserve in North-East Italy, data from the past 80 yr were available. We used records of 300 moth species to analyze whether extinction risk was linked to their body size or to their degree of ecological specialization. Specialization was scored 1) by classifying larval food affiliations, habitat preferences, and the northern distributional limit and 2) by analyzing functional dispersion (FDis) within species assemblages over time. Our results show that locally extinct species (mean wingspan: 37.0 mm) were larger than persistent (33.2 mm) or previously unrecorded ones (30.7 mm), leading to a smaller mean wingspan of the moth community over time. Some ecological filters appear to have selected against bigger species. By using coarse specialization categories, we did not observe any relationship with local extinction risk. However, FDis, calculated across 12 species traits, significantly decreased over time. We conclude that simple classification systems might fail in reflecting changes in community-wide specialization. Multivariate approaches such as FDis may provide deeper insight, as they reflect a variety of ecological niche dimensions. With the abandonment of extensive land use practices, natural succession seems to have shifted the moth community toward a preponderance of forest-affiliated species, leading to decreased FDis values. Multivariate analyses of species composition also confirmed that the moth community has significantly changed during the last 80 yr.

Key words: extinction risk, ecological specialization, functional dispersion, body size, long-term data

Introduction

Ecological communities are dynamic networks that may respond to all kinds of environmental constraints (Vellend 2016). During the last decades, multiple anthropogenic stressors dramatically affected remaining near-natural systems, such as nature reserves, finally culminating in the definition of a new geological epoch: the Anthropocene (Laurance 2019). Landscapes are modified on large scale by anthropogenic land use, including agricultural intensification and urbanization (Plieninger et al. 2016). Besides the loss of suitable habitat area (Thompson et al. 2017), reduced connectivity between patches alters species immigration and genetic flux between isolated habitats (Habel & Schmitt 2018). Consequently, remnant patches of near-natural habitat areas are nowadays often isolated from one another and embedded in landscapes under intense human pressure.

The large-scale changes of terrestrial habitats have recently been shown to threaten regional insect diversity even inside conservation areas. Over the last 20 yr, scientists found a drastic decline in terrestrial insect biomass and gamma diversity (Seibold et al. 2019). Insects are the most species-rich group of terrestrial Eucaryota. They form an important part of manifold trophic interactions and ecosystem processes such as pollination, herbivory, and pest control (Noriega et al. 2018). Moreover, insects serve as a food resource for animals at higher trophic levels such as birds and bats (Maas et al. 2016). The landscape wide loss of insect species raises attention on how conservation management might be ameliorated to stop further biodiversity erosion and to maintain ecosystem function. Rösch et al. (2013) showed that insect species richness declined with increasing habitat isolation and especially with the reduced size of the remnant habitats. They observed highest extinction rates and lowest probabilities of recolonization in small, isolated fragments. Maintaining or restoring habitat connectivity therefore is an important goal in biodiversity conservation (Correa Ayram et al. 2016), especially as large remnants of near-natural habitats are particularly important for the maintenance of disturbance-sensitive and threatened taxa (Melo de Melo et al. 2019).

Besides the general observation of insect diversity decline within fragmented habitat patches, we lack in many cases information about species' individual sensitivities to isolation and disturbance. Are there some species groups that face—due to their physiological or functional characteristics—a greater risk of local extinction inside isolated nature reserves? For vertebrates, numerous studies have shown that body size is an important factor shaping extinction risk (Ripple et al. 2017, Smith et al. 2018). Large-sized animal species are dependent on the primary productivity of their habitat, which plays an important role to nourish the individuals. Without sufficient food resources, no viable population can persist. Consequently, with decreasing habitat size, a lower carrying capacity for larger consumers is expected especially when additional stressors might reduce food quality. Indeed, also for insects, there is increasing evidence that larger species are under elevated extinction risk in isolated habitats (e.g., Koh et al. 2004, Nolte et al. 2019).

Looking at species functional traits, there is evidence for specialized species vanishing more strongly due to land use intensification (Mangels et al. 2017) and urbanization (Concepción et al. 2015). The phenomenon of specialized species being replaced by a few generalist ones is described as biotic homogenization of communities (Knop 2016). Fragmentation can also favor functional homogenization and the replacement of specialized species by generalist ones (Bagchi et al. 2018, Ramiadantsoa et al. 2018). Thus, Keinath et al. (2017) suggested that conservationists should pay particular attention to specialized species whenever anthropogenic disturbances could further fragment remaining habitats. However, there is no uniform method to measure the degree of specialization. For animals, a common approach is to classify species according to their feeding habits or habitat preferences. Either only one of these characteristics is considered (Mangels et al. 2017) or various specialization values covering different aspects of the ecological niche requirements of organisms are aggregated into one synthetic specialization score (Eskildsen et al. 2015). Additionally, different methods to quantify functional diversity have become popular at the community level. On the one hand, community weighted means can give insight into conditions favoring shifts with regard to single factors (Neff et al. 2019). On the other hand, functional dispersion (FDis) represents the abundance-based dispersion of species in trait space (Laliberté & Legendre 2010). As functional specialization is defined as the relative distance of a species from the centroid (Bellwood et al. 2006, Laliberté & Legendre 2010), FDis can be interpreted as mean functional specialization at the community level. To calculate FDis, a variety of ecologically relevant species traits needs to be assembled and subjected to multivariate analysis.

In this study, we evaluate if body size or functional specialization of insect species are linked to a higher risk of extinction in an isolated conservation area. To achieve that goal, we analyzed historic data (1933–1996) and recent samples (1997–2012) from the coastal pinewood reserve Pineta san Vitale (hereafter termed PsV) in North-East Italy, comprising a time span of about 80 yr. When no long-term monitoring data are available, museum collections can serve as important clues for community composition in the past. PsV, as most conservation areas in Europe, has become ever more disconnected from other remaining near-natural habitats over the past century through urbanization and the spread of intense agriculture (Andreatta 2010). So, the distances of PsV to other larger, near natural areas are as follows: 32 km to Bosco Mesola (North), 40 km to the forests in the Apennines (west), and 10 km to Pineta di Classe (south). There is mainly agricultural land located between PsV and the other mentioned regions (Uhl et al. 2020).

Additionally, different potential pollution sources developed in the vicinity of the conservation area, like the industrial harbor of Ravenna (Airoldi et al. 2016), influencing the air (Lucialli et al. 2007) and water quality (Guerra et al. 2014) in the direct surroundings of the forest reserve. Finally, the vegetation structure inside the conservation area has changed due to the abandonment of former extensive management practices (wood and reed production, pine-nut harvest, cattle grazing) and the progress of natural forest succession (Wölfling et al. 2019). While developing toward a more natural forest habitat structure, early successional stages like open areas largely vanished. However, recent conservation management in the reserve tries to maintain different habitat structures, such as grassland and reed, by keeping horses inside the reserve area. Here, we explore multi-decadal changes in moth communities of PsV and investigate whether long-term isolation and habitat change had an effect on body size and functional specialization of the moth community. We address the following specific hypotheses:

1. Larger moth species have experienced a higher risk of local extinction in PsV, as the isolated area might fail to provide sufficient food resources and habitat quality. Subsequently, the mean wingspan of the whole community should have decreased over time.

2. Specialized species were more likely to go extinct, as they are more sensitive to environmental changes. The isolated forest remnant might be unsuitable to maintain specialist species and functional homogenization might have occurred inside the nature reserve. Therefore, the mean degree of specialization should have decreased over time.

3. Extinction and colonization events have contributed to directional shifts in the moth community composition in PsV over the last 80 yr. Taking into account the natural succession that has transformed the vegetation of PsV, a loss of open habitat species is expected.

Materials and Methods

The forest reserve PsV (expansion of 950 ha from north 44°31′39.15″N, 12°14′19.82″E to south 44°27′48.09″N, 12°13′43.67″E and west 44°29′51.96″N, 12°13′22.79″E to east 44°29′50.50″N, 12°14′15.56″E) is located in the Emilia Romagna (Italy) close to the city of Ravenna. The area of PsV developed from the tenth century onwards through sedimentation forming dunes. The pine woods, which were planted afterward for firewood and pine-nut production in the 10th and 11th centuries, were also used extensively for cattle grazing keeping the understory open and therefore forming a very open forest structure (Malfitano 2002, Andreatta 2010). Extensive land use was then abandoned in 1988, when PsV became a part of the Parco Regionale del Delta del Po (Consorzio Del Parco Regionale Del Delta Del Po 2004) and therefore was protected as UNESCO biosphere reserve (UNESCO 2015). Since then, succession formed more forest like habitats with a diverse understory in PsV (Wölfling et al. 2019). Today, PsV consists mainly of downy oak forest and hygrophilous forest, but also smaller patches of pine stands, reed, and open habitats. PsV is protected under several levels of legislation based on Natura 2000 (Montanari 2010) and the Convention of Ramsar (Ramsar Convention Secretariat 2013). For the present paper, three historical moth collections were analyzed:

- Museo di Storia Naturale di Venezia (vouchers of 93 species from 1933 to 1968)
- Museo Civico delle Cappuccine, Bagnacavallo (vouchers of 41 species from 1966 to 1980)
- Private collection of Edgardo Bertaccini (vouchers of 157 species from 1977 to 1996)

Additionally, we sampled in PsV between 1997 and 2012. For each year, the samples should represent the reserve-wide species assemblage. Therefore, we always tried to sample the different habitats of PsV for each year and subsequently pooled the data into one species list for each year. Seven locations were sampled between 1997 and 2002 with a 500 W HWL manual light trap, mainly in early summer and early autumn in downy oak forest, riparian forest, reed, and open habitats. In 2011, we used weaker light tubes (15 W BL + 15 W white BL) in automated light traps at 20 locations to sample all mentioned vegetation types simultaneously in spring, early summer, high summer, and autumn to get the most complete dataset of the whole PsV moth community. The technical characteristics of the automated traps are different from that of the manual trap and can therefore cause a sampling bias (Axmacher and Fiedler 2004) that is discussed later. In 2012, again the 500 W HWL manual light trap was used like in the years 1997–2002 at nine different locations. In total, 237 species were sampled from 1997 to 2012. All vouchers from the own sampling are stored in the private collection of Mirko Wölfling (Niederwerrn, Germany).

Over a span of 80 yr, 300 moth species (Macroheterocera sensu Regier et al. 2013 plus Cossidae and Hepialidae) were considered for the subsequent analyses after phenological cleaning of all data. In particular, all early- and late-flying species as well as long-distance migrants and strictly diurnal species were removed, since these were not represented in our own light-trap samples. For the analysis, only incidence data were used, as we were not able to count abundances out of the historic collections. Further details about data sources and management were described in Wölfling et al. (2019).

For the first hypothesis, the typical wingspan (averages) of each species was extracted from a database (http://ukmoths.org.uk) or, if not available there, specimens were measured from our collections. The log-transformed wingspan served as a measure of body size. For analyzing the hypothesis on overall specialization, we created a toolbox (Tab. 8.1) that considered three ecologically important dimensions for classifying all species according to their degree of specialization. The niche dimensions considered and their scoring are listed in Tab.8.1.

Table 8.1: Register for scoring the specialization levels of each moth species.

Dimension of Resource	**Classification factors**	**Classification value**
Specialization in larval food selection	The species' phagism-type is:	
	Monophagous within one plant genus	→ 1
	Oligophagous within one plant family	→ 2
	Polyphagous: > 1 plant family	→ 3
	Highly polyphagous: > 5 plant families	→ 4
Northern limit of distribution in Europe	The northern distribution limit of the species is:	
	43°-46° N (range limit: south of the Alps)	→ 1
	47°-50° N (range limit: German highlands)	→ 2
	51°-54° N (range limit: North or Baltic sea coast)	→ 3
	55°-71° N (range limit: further north than class 3)	→ 4
Specialization in habitat choice	The species occurs in:	
	1-2 habitat types (e.g. xeric grasslands, coppice forests, pine forests etc.)	→ 1
	3-4 habitat types	→ 2
	5 or more habitat types or common-/ non special habitat types (e.g. forest, deciduous mixed forest, edge of the woods, woody habitats, meadows etc.)	→ 3
	Numerous and / or anthropogenic influenced habitats (gardens, parks, urban areas, common meadows.	→ 4

We chose descriptors pertaining to larval resource requirements, to the climatic niche, and the habitat use since these are essential aspects for defining how specialized a species is. Data on larval host selection were extracted from Ebert (1994–2003) and from the websites www.euroleps.ch and www. pyrgus.de. The northern limit of the distribution of each species in Europe was taken from www.gbif.org. Specialization in habitat use (including their preference for open habitats) was extracted from Ebert (1994–2003) and the two databases www.euroleps.ch and www.pyrgus.de. We scored each niche dimension on a rank scale from 1 (most specialized) to 4 (least specialized). Aggregating over these three dimensions, the degree of total specialization of each species is therefore the sum of the scores along all three dimensions. Accordingly, highly specialized species may attain a minimum value of 3 (3 × score 1) and highly generalist species may reach a maximum value of 12 (3 × score 4). A complete species list with all scorings regarding the different dimensions of specialization is provided in Suppl. material (Appendix S3).

Furthermore, we compiled a trait matrix containing information on 12 species traits of functional relevance, viz. wingspan, presence or absence of proboscis, larval food source (detritivore, lichen feeder, and 15 plant families), salt tolerance of larval food plants, use of ruderal food plants, growth form of larval host plants (e.g., woody or herbaceous), degree of larval food specialization (from monophagous to highly polyphagous), habitat type (forest, open habitats, shrub-land, or reed), phenology of adult activity period (spring, early summer or summer), voltinism, hibernating stage, and latitudinal extent of European distributional range. From these trait data, we calculated a Gower dissimilarity matrix, which was—together with the moth incidence data—used for calculating FDis as an index for community-wide specialization using the ‘dbFD’ function in the ‘FD’ package in R (Laliberté and Legendre 2010). Comparing these two different methods for classifying moth specialization, we wanted to analyze which index is more useful.

To analyze local extinction risk, we partitioned all moth species under consideration into three groups: species observed only before 1997 (named ‘lost’ hereafter), species recorded only after 1997 (named ‘previously unrecorded’), and species occurring before and after 1997 (named ‘persistent’). The year 1997 was chosen for separation because this was the year when our own sampling started. Body size was used as the response variable in a linear mixed-effect model with taxonomic family included as a random factor and species status as the fixed factor, using the R package ‘nlme’ (Pinheiro et al. 2019). Food specialization, northern limit of distribution, habitat specialization, and total specialization were used as response variables in separate generalized linear models with Poisson-type error distribution, again modeling species status as fixed and moth family as random factors.

Additionally, generalized additive models (hereafter GAM) were built to look for changes in body size and specialization over the observed time series. For the historic collection data, we had to pool some years since there were usually too few individuals present as vouchers in the collections per year. The historic museum collection data were split into four partitions, two for the Callegari/Martinasco collection (1933–1949 summed up as ‘1940s’ and 1950–1976 summed up as ‘1960s’) and two more for the Bertaccini collection (1977–1984 summed up as ‘1970s’ and 1985–1996 summed up as ‘1980s’). Our own samples, which comprised far more vouchers, were split up by year. The calculation of the GAM was done using the ‘mgcv’ package (Wood 2011). The log-transformed mean wingspan of all observed moth species per year was used as the response variable. Furthermore, the annual degree of specialization at the community level was calculated as mean value of the specialization scores of all observed moth species per year. Similarly, annual FDis was calculated using the moth incidence data from each time period. The proportion of open habitat species (logit transformed) served as the response variable in GAM to analyze whether open habitat users may have decreased during the last decades due to succession.

Finally, to check whether changes in species composition in PsV over time were related to shifts in wingspan, ecological specialization, or preference for open habitats, we used non-metric multidimensional scaling (NMDS) and a permutation test (999 permutations). We first prepared our own sample data by separating them according to single sampling years, yielding eight data points. Museum collections data were as above split into the four partitions '1940s', '1960s', '1970s', and '1980s'. From the resulting species × time layer incidence matrix, a triangular similarity matrix was calculated using the Sørensen similarity measure. As potential explanatory factors, we included community mean values of wingspan (log transformed), mean total specialization score, and the proportion of open habitat species (logit transformed) per each time unit. For visualization of possible temporal trends, we created an ordisurf diagram using the package 'vegan' (Oksanen et al. 2018). Ordisurf is a function performed on the NMDS ordination, based on GAM (Oksanen 2007).

Results

In PsV, we counted 63 lost, 156 persistent, and 81 previously unrecorded moth species, summing up to a total of 300 species (Appendix S3). As predicted, locally lost species were on average larger than previously unrecorded moth species (p = 0.002, df = 287; Fig. 8.1, Tab. 8.2). The GAM also revealed substantial changes across the entire community in wingspan over time (Fig. 8.2 A, Tab. 8.2). Only one species, *Nola cristatula* Hübner, 1793 (Lepidoptera: Nolidae), reached the highest possible degree of specialization according to our classification system. Most moth species matched a specialization score of 10 or even higher, indicating a high proportion of generalist species in our data set (Fig. 8.3).

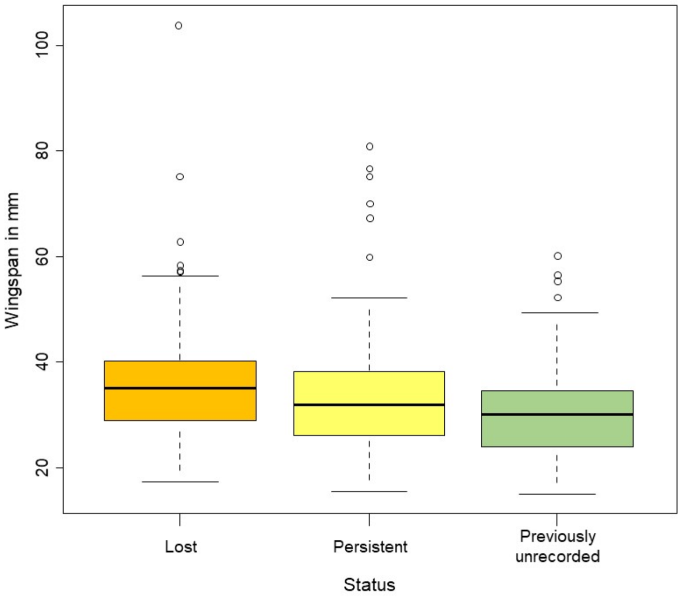

Figure 8.1: Wingspan of moth species from Pineta san Vitale (PsV) partitioned into three categories according to their occurrence status. 'Lost' (orange) represents moth species that were only found pre-1997 (n = 63). 'Persistent' (yellow) denotes moths occurring pre-1997 as well as afterward (n = 156). 'Previously unrecorded' (green) refers to species only observed in 1997 or later (n = 81). 'Previously unrecorded' and 'Lost' species differed significantly in body size (p = 0.002). Bar in the middle represent median, box limits are third and first quartiles, and whiskers describe data points within 1.5 times of the interquartile range.

Looking at the three groups of lost, persistent, and previously unrecorded species, the specialization scores showed no significant differences. This was true for all three dimensions separately—larval food, habitat, and northern distribution limit—as well as for total specialization (Tab. 8.2). Likewise, the GAM showed no consistent decline in total specialization or proportion of open habitat species over time (Figs. 8.2 B and C). Contrary to these results, FDis declined significantly over the last 80 yr (R^2 = 0.60, p = 0.002, Fig. 8.2 D). The unconstrained ordination plot reveals substantial changes in the species composition of moth assemblages over time (Fig. 8.4). According to a permutation test, variation in mean wingspan (R^2 = 0.23, p = 0.001), mean total specialization (R^2 = 0.19, p = 0.001), and the proportion of open habitat species (R^2 = 0.14, p = 0.004) were all significantly associated with variation in species composition. However, moth samples partitioned into 15 time periods did not indicate a simple directional shift. Rather, older (museum) samples were ordinated in the periphery in reduced ordination space, with the most recent (quantitative light trap) samples in the center.

Discussion

Even though they usually provide only snapshots of biota encountered at earlier times, museum collections may give important insight into past insect communities and therefore are valuable for the reconstruction of long-term community shifts. We were able to analyze changes in the PsV moth communities over the last 80 yr, where a clear shift in species composition has occurred. Larger species were more likely to go extinct locally, leading to a reduced community-wide mean wingspan over time. For ecological specialization, our results were contingent on the analytical method used. Although a coarse classification failed to reveal any differences in the degree of specialization between extinct and previously unrecorded species, a multivariate approach such as FDis well captured a significant decrease in specialization over the last 80 yr. Altogether the moth community changed significantly in PsV. These shifts in the moth assemblage seem to have been more intense in the first half of the 20th century because these oldest moth samples showed the largest differences to the recent ones, for example in the ordination analysis.

Table 8.2: Results of generalized mixed-effect models for the three groups of ‘persistent’, ‘lost’, and ‘previously unrecorded’ moth species with regard to mean wingspan and ecological specialization score.

	t-value/z-value	p-value	Regression coefficient β	Marginal R^2	Conditional R^2
1. Hypothesis Larger species have higher extinction risk					
Mean wingspan	**3.15**	**0.002**	**0.43**	**0.01**	**0.60**
2. Hypothesis Specialized species have higher extinction risk					
Total specialization	-1.22	0.22	-0.07	0.01	0.01
Food specialization	-0.26	0.79	-0.03	<0.01	<0.01
Habitat specialization	-0.76	0.45	-0.08	<0.01	<0.01
Northern distribution limit	-1.02	0.31	-0.09	<0.01	<0.01
Generalized additive models (observed change over 15 time periods)				Adjusted R^2	
Mean wingspan	**-3.43**	**0.006**	**-0.73**	**0.5**	
Total specialization	0.02	0.98	0.01	-0.1	
Proportion of open habitat species	-1.90	0.09	-0.52	0.19	
Functional dispersion	**-4.16**	**0.002**	**-0.80**	**0.60**	

A higher rate of local losses among larger species was expected because similar developments have been observed with other insects in isolated conservation areas worldwide (Coulthard et al. 2019, Nolte et al. 2019). However, because our historic data were taken from museum vouchers rather than from standardized sampling or monitoring programs, one might ask to what extent apparent body size patterns might be shaped by the unknown whereabouts around the earlier samples. For example, citizenscientists as collectors might have favored larger species or have overlooked small species. Dealing with historic data, there is always the problem that we have no information about how exactly the moths were sampled. We also do not know why a collector has taken, or discarded, particular species. For most historical natural history collections, personal interest in certain taxa or aiming to increase the completeness of the collection might have been decisive for the selection of the voucher individuals.

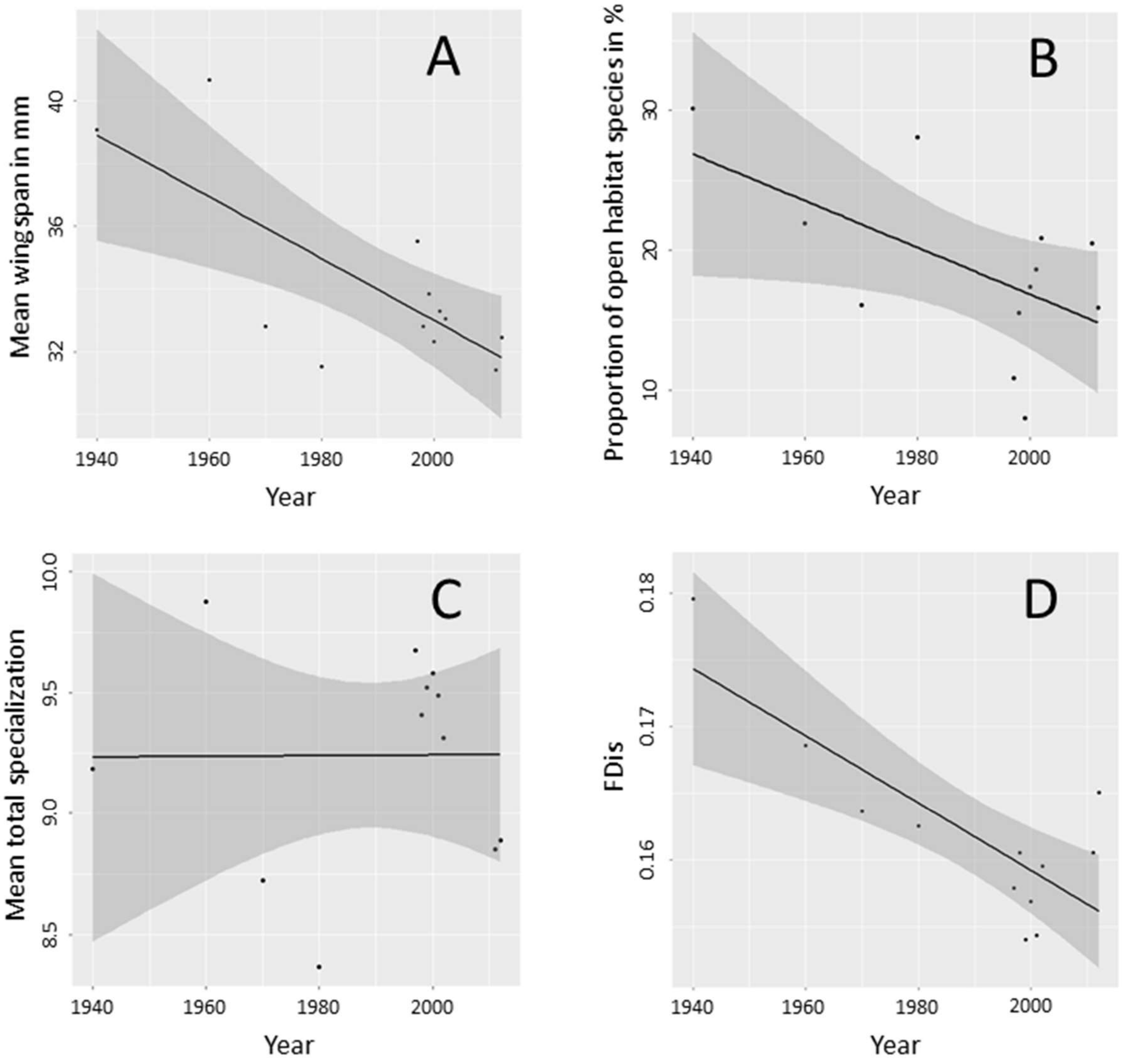

Figure 8.2: Plots showing (A) mean wingspan in millimeters, (B) proportion of open habitat species in percentage, (C) mean degree of total specialization, and (D) functional dispersion (FDis) in the moth community over time. Moth data from PsV were partitioned into 15 time layers for analysis. The dark line indicates the generalized additive model (GAM) function and its confidence intervals (shaded area). Statistical details of the GAMs can be looked up in Tab. 2.

Therefore, museum collections are obviously prone to sampling bias (Graham et al. 2004). So, proper adjustment of vouchers to be considered and a critical questioning of the results are crucial when historic collections are used for comparisons with more standardized surveys. In our case, however, such a sampling bias can mostly be refuted as many small and inconspicuous moth species were preserved in the evaluated historical collections (Wölfling et al. 2019). Furthermore, there are some obvious big moth species that have definitely disappeared from the area and have not been found again during our own intense sampling activities. For example, *Sphinx ligustri* Linnaeus, 1758 (Lepidoptera: Sphingidae), *Minucia lunaris* Denis & Schiffermüller, 1775 (Lepidoptera: Erebidae), *Catocala puerpera* Giorna, 1791 (Lepidoptera: Erebidae), or *Catocala elocata* Esper, 1787 (Lepidoptera: Erebidae) never showed up again after 1997, even though these moth species are easily recorded by light-trapping and are not considered as endangered in southern Europe. Hence, the fact that we never recorded any of these moths between 1997 and 2012 (and actually not beyond: B. Uhl & M. Wölfling, unpublished results) gives strong evidence that these large-sized species have really been lost in PsV.

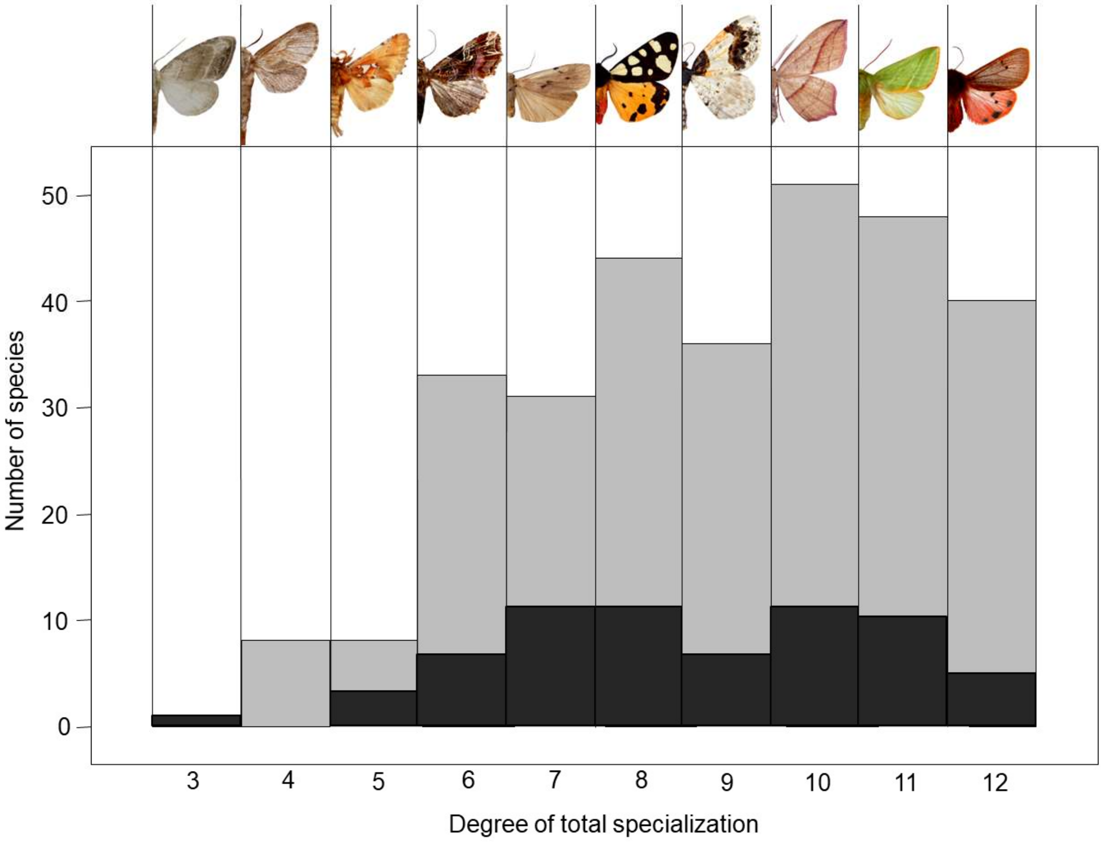

Figure 8.3: Number of species in regard to their total specialization score (gray bars). Black bars indicate the number of lost species. For each degree of specialization one representative species was selected viz. (from left): *Nola cristatula* Hübner 1783 (Lepidoptera: Nolidae), *Dyspessa ulula* Borkhausen 1790 (Lepidoptera: Cossidae), *Spatalia argentina* Schiffermüller 1775 (Lepidoptera: Notodontidae), *Callopistria juventina* Stoll 1782 (Lepidoptera: Noctuidae), *Arctia villica* Linnaeus 1758 (Lepidoptera: Erebidae), *Ligdia adustata* Schiffermüller 1775 (Lepidoptera: Geometridae), *Timandra comae* Schmidt 1931 (Lepidoptera: Geometridae), *Pseudoips prasinana* Linnaeus 1758 (Lepidoptera: Nolidae), *Phragmatobia fuliginosa* Linnaeuas 1758 (Lepidoptera: Erebidae).

During the observed time span, PsV did not suffer from any significant loss of area, nor did the host plants of these lost species disappear. We therefore assume that some other aspects of habitat quality or indirect effects of anthropogenic actions on the landscape scale might have been the determining factors that have led to the disappearance of these species. Looking at the landscape scale, light pollution seems to have become more intense in recent years, caused by the development of the industrial harbor of Ravenna. Although Merckx et al. (2018) found that increasing urbanization favored bigger moth species, we observed an apparent selection against larger species. We can only hypothesize that this might be an effect of bigger species reacting differently to nearby light pollution sources. Mark and recapture experiments showed that moth families comprising large species, such as Sphingidae and Erebidae, tend to be attracted by light from wider distances than other families, e.g., Noctuidae or Geometridae (Merckx & Slade 2014). So, these families might be more affected by nearby light pollution around the reserve than others. However, the complex effects of light pollution on moths are only poorly understood to date (Owens et al. 2020), and so, the effect of light pollution on moth functional and physiological characteristics needs to be investigated in future analyses.

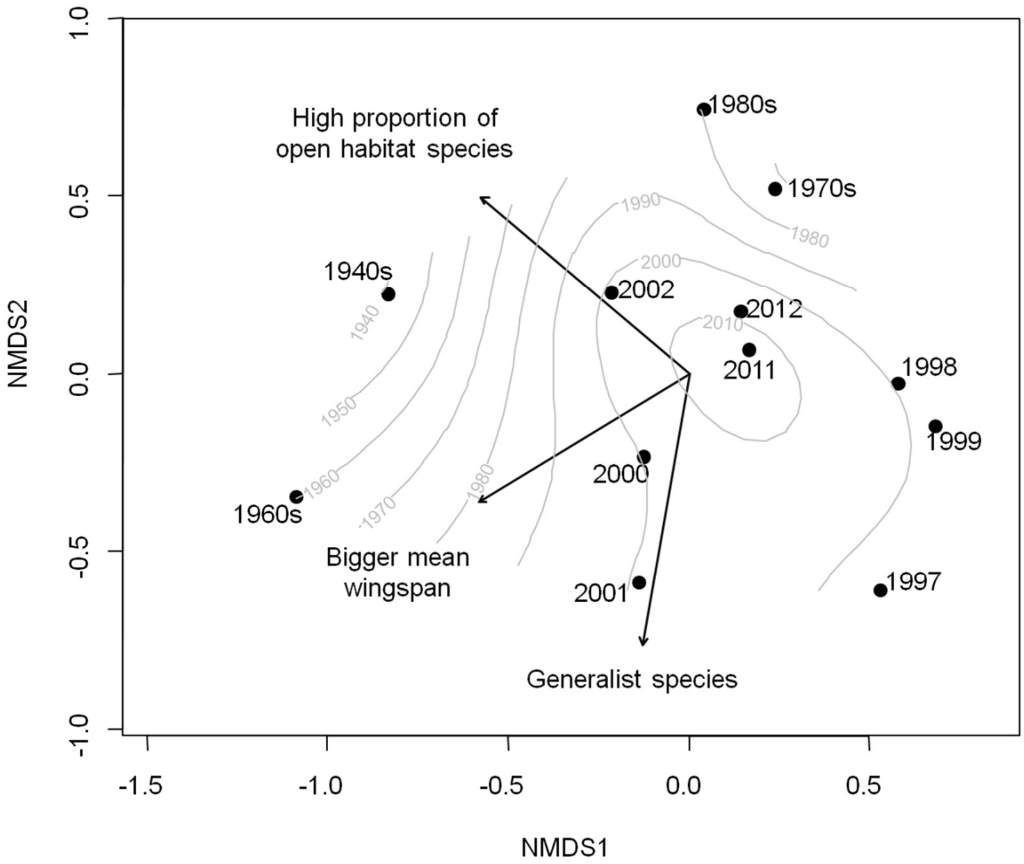

Figure 8.4: Non-metric multidimensional scaling (NMDS) ordination based on moth species lists from 15 time layers sampled in PsV. Proportion of open habitat species, mean wingspan, and total degree of specialization were superimposed as descriptors of the moth communities. The sampling years are projected on the NMDS through an ordisurf function based on a generalized additive model (GAM). Stress: 0.13 (non-metric fit $R^2 = 0.98$, linear fit $R^2 = 0.91$).

Besides the industrial development, agricultural intensification in the surroundings of PsV and accompanied nutrient influx has contributed to homogenization of the plant community in the park (Uhl et al. 2020). This may have filtered out some moth species with peculiar nutrient demands. Besides, elevated nutrient levels can alter food plant quality and directly loop back on the fitness of some moth species (Kurze et al. 2018). Finally, subsidence induced soil salinity

(Antonellini & Mollema 2010) also might reduce food plant quality (Okon 2019). Elevated salt content in soil may influence the composition and structure of the mycorrhiza and can lead to the decline of mature trees (Montecchio et al. 2004). Massive damage on trees has already been observed all over PsV (Uhl & Wölfling 2015). As a bottom-up effect, salinity stress is known to influence the development and population parameters of insects (Quais et al. 2019). Whatever mechanistic pathway may have been involved, we therefore assume that larger species may have suffered more from the adverse effects of environmental stress, rendering them more prone to local extinctions.

For the degree in specialization, our results were strongly dependent on the method used to define specialization. Using only three coarse dimensions of ecological specialization (breadth of larval food niche, habitat preference, and northern distribution limit) yielded no clear patterns. In contrast, the result based on multivariate FDis was clearly significant. The small number of classification factors obviously resulted in a classification with too poor resolution. Furthermore, completely different ecological niches are rated with the same total score. For example, one only counts the numbers of used habitats or host plants, but does not distinguish between different types. Accordingly, the value of such simplified approaches is low, especially if alternative measures based on broader trait information is available. Fortunately, for European Lepidoptera, species– trait information is more complete than for most other insect orders (Ebert 1994–2003).

For calculating FDis, the communities' mean dispersion from the functional trait space centroid, a species–trait matrix consisting of multiple different ecological traits is used (Fig. 8.5 A). Assuming that 'specialization' is the distance of each species from this centroid, a decline in FDis might indicate either a generally lower specialization of species in the community (Fig. 8.5 B) or a shift of the moth community from multiple different niches to be occupied to one prevalent niche type (Fig. 8.5 C). We know that in PsV the vegetation structure has changed during the last 80 yr due to natural succession (Wölfling et al. 2019). Following this process favoring more near-natural forest areas to develop, it is plausible that the moth community today is predominantly composed of forest-associated species. Functional dispersion of the community therefore would have shifted from species spread all over the trait space to species concentrated in the direction of forest dwellers (Fig. 8.5 C). As we found no increase in strictly forest-bound species (Wölfling et al. 2019), we conclude that moderately generalistic species that depend on woody structures were responsible for the observed shift in FDis. In fact, the proportion of generalist habitat users has increased during the last 80 yr. With the proportion of open habitat species only slightly tending to decrease over time (Fig. 8.2 B, Tab. 8.2), we furthermore conclude that within open habitats, there might have been a shift from strict open habitat users to those also tolerating or even preferring some bushy vegetation structures. Such a more subtle shift would have gone undetected with a coarse classification system. We therefore conclude that the observed change in community-wide FDis cannot be associated with one single habitat type or niche type that has vanished, but rather with multiple small changes in the occupied fraction of moth trait space.

Looking at the community composition over the last 80 yr, there was a significant change. Compositional differences between older samples were higher than among the newer ones, resulting in some kind of nested arrangement, with the earliest samples in the outer regions of the ordination plot, clearly distant from each other, and the newer own collections closer together in the center (Fig. 8.4). For the analysis of species composition, the use of historic collections and different trap types has its limits. The collectors of the historic data likely did not sample in a standardized manner, but more likely have taken only individuals of interest. Vouchers of common species are more likely kept by entomologists only when a collection is started, while later on citizen-scientists may have focused on species missing in their collections. As a result, common species may not have been registered every year.

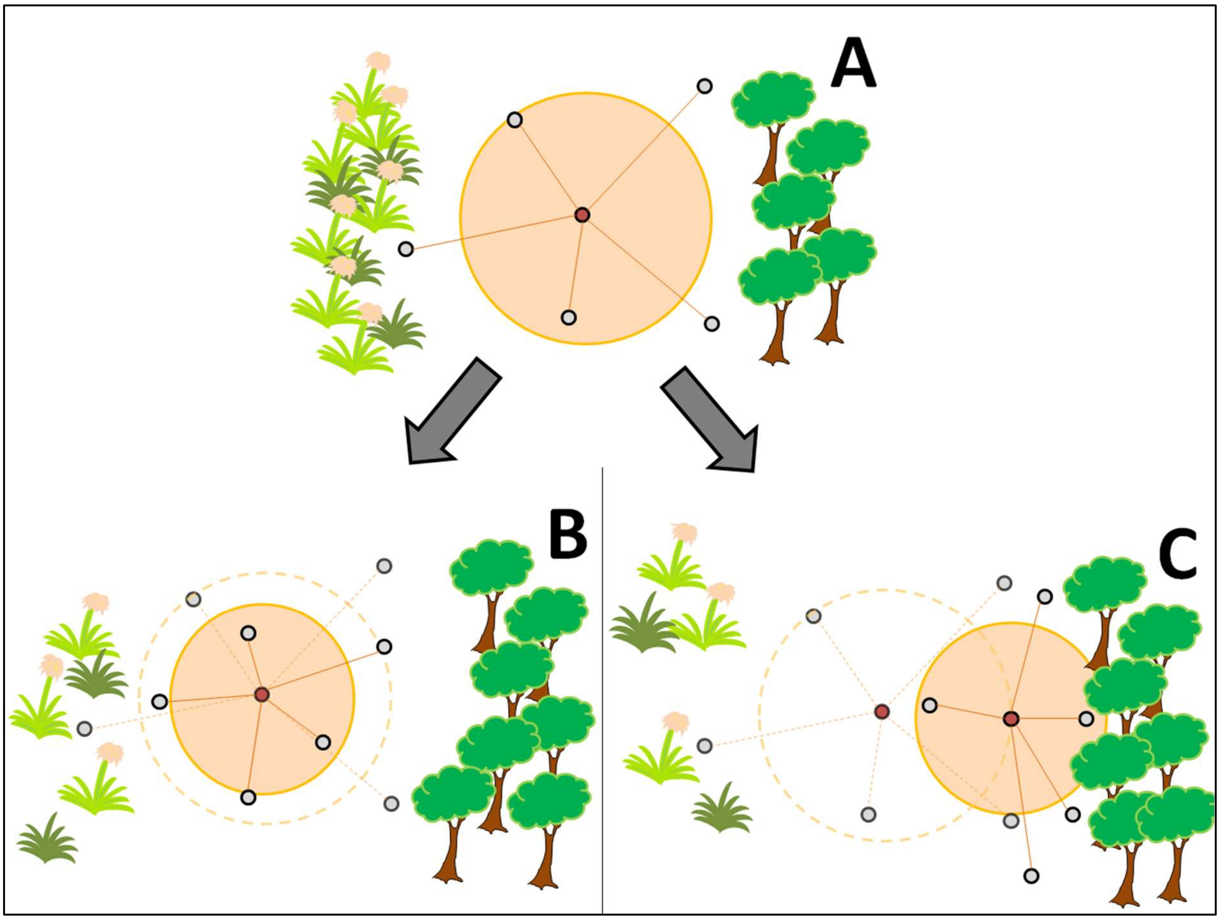

Figure 8.5: Change of functional dispersion over time in a conservation area where due to abandonment of land use and natural forest succession the open grassland biota gradually declines. Gray points represent species in functional trait space. The red point indicates the trait space center. The distance between each species and the trait space centroid is shown as a line. The orange circle symbolizes the mean functional dispersion FDis (mean distance across all species to the centroid). By simplifying multivariate trait space into two-dimensions, we indicate open habitat species to the left of the picture (grass symbols), and species of woody habitat on the right (tree symbols). (A) Situation about 80 yr ago with historic extensive land use. (B) First scenario: Species in general got 'less specialized'. The functional centroid keeps its position, but individual species distances are on average smaller compared to the past (dashed lines). Consequently, mean functional dispersion decreases (compared with the ancient dispersion, shown as dashed circle). (C) Second scenario: Occupied functional trait space shifts toward more forest-affiliated species. Species might be also specialized, but predominantly concentrate in one habitat type. The dispersion of species toward open habitats therefore decreases, the trait space centroid shifts toward forest species, leading to smaller mean distances of species to the new trait space center.

Using different light-trap types might have had an effect on the composition of the assemblages of attracted moths. We do not know which trap types were used by early collectors in Italy. Additionally, also the more recent samples are based on two different trap types (a manual light trap from 1997 to 2002 and in 2012; automated light traps in 2011). Different trap types are known to influence the composition of moth catches (Axmacher & Fiedler 2004). However, a large part of this influence is due to changes in relative abundances of individual species, and not so much in the presence or absence of species (Axmacher & Fiedler 2004). As we only used incidence data in our present analysis, and pooled all samples to one species list per year, we tried to minimize these sampling effects as best as possible. Therefore, we suggest that the observed drastic alterations in species composition can also be attributed to the substantial environmental changes that have taken place in the reserve. When cattle were grazing under the pine trees, shrubs were regularly removed to facilitate pine-nut harvest, and fallen branches were collected as firewood. So, in these times, PsV still offered rather large tracts of open habitat due to frequent disturbances. More recently, the community seems to have become more stable, as community composition differences were smaller among the newer data sets. The recent forest-associated moth community of PsV can be seen as a consequence of secondary succession that started since the whole area received legal protection status in 1988. Also, in other studies, moth communities of locations in mature forest systems were more similar than in secondary, younger forest types (Axmacher & Fiedler 2004).

This community similarity in recent times also suggests that the use of different types of traps (manual vs automated, each with different light sources) obviously did not have a major impact on the overall results of our study. Pooling the data by year therefore seems a useful procedure to make the incidence data from different trap types comparable. Three key processes drive community assembly, viz. dispersal limitation, ecological filtering, and ecological drift (Sydenham et al. 2017). There is general evidence that larger moth species disperse more easily (Ockinger et al. 2010, Kuussaari et al. 2014). But for most of the species, there is a lack of knowledge concerning their dispersal capacity. So, it is quite difficult to make any assumptions about this important point driving community assembly. However, PsV—although being isolated from other forest areas—is still connected with other near-natural areas like wetlands and open habitats in the North. Furthermore, there are conservation efforts, trying to better connect PsV with other reserves (Estreguil et al. 2013). Streets are often edged by bigger trees, hedgerows, and field edges with various grass and herb species, and individual trees can often be found in agricultural areas around PsV. Such small microhabitats may serve as important stepping stones, facilitating dispersion between natural areas (Slade et al. 2013). As such, dispersal limitation should not be the major threat to the PsV moth communities, as is also indicated by the rather large number of previously unrecorded species over time.

With regard to ecological filters, we conclude that the abandonment of extensive use of forest commodities after the 1970s and subsequent succession were the major drivers of the observed moth community shift. This led to a reduction in the proportion of open habitat species and a shift toward more generalist forest species. However, the loss of open areas was recognized in time, so that conservation efforts in PsV were attempted to keep the structurally rich habitats. Therefore, horses were released as ecosystem engineers, keeping open areas free from bushes and counteracting natural succession. Concerning ecological drift, it is often overlooked that local populations may go extinct due to stochastic reasons even in the absence of environmental stress, such that changes in community composition can be mistaken for indicating an environmental trend (Sgardeli et al. 2016). Therefore, some random extinctions may have occurred. For example, also small and rather generalistic species such as *Eupithecia absinthiata* Clerck, 1759 (Lepidoptera: Geometridae) and *Tephronia sepiaria* Hufnagel, 1767 (Lepidoptera: Geometridae) appear to have disappeared. Due to our intensive sampling, we are confident that *E. absinthiata* was likely not overlooked. *Tephronia sepiaria* has disappeared although other lichen feeders are still found in numbers in PsV. Simultaneously, large species such as *Mormo maura* Linnaeus, 1758 (Lepidoptera: Noctuidae) and *Deilephila porcellus* Linnaeus, 1758 (Lepidoptera: Sphingidae) were previously unrecorded. The latter two species would definitely have been documented by earlier collectors, had they ever encountered them.

In summary, the moth communities in PsV currently seem mostly to be shaped by ecological filters combined with random ecological drift. With the protection status conferred to the area in the late 1980s, most constraints driven by former extensive forest use were suspended. For the preservation of open habitat structures in PsV, nowadays horses are held within the reserve. By doing so, the diversity of different habitat structures is maintained. Additionally, with a relatively large area of about 900 ha, PsV seems to be able to preserve also a range of specialized insect species (Slade et al. 2013).

Supplementary Data

Supplementary data are available at Journal of Insect Science online and in the Appendix S3.

Acknowledgments

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9. Synopsis

“Nature is essential for human existence” is one of the key findings published by the IPBES in the Global Assessment Report on Biodiversity and Ecosystem Services (IPBES 2019). Species diversity, genetic diversity and habitat diversity were analyzed by 450 scientists over three years. In its core result the analyses predict that up to one million species are going to face extinction in the next decades inferring dramatic consequences for humanity (IPBES 2019). It is therefore of the utmost importance to understand the underlying processes driving biodiversity loss.

The most species rich group among eukariotic animals is insects. Their disappearance on the large-scale is in line with the predictions of the IPBES. The losses of insect species are rated up to 40% for the next decades and may be up to eight times faster than in vertebrates (Sánchez-Bayo & Wyckhuys 2019). This loss might lead to imbalanced herbivore-parasitoid interactions (Tscharntke et al. 2005, Geiger et al. 2010), favoring the drastic increase of certain agricultural pest species, but also the spread of insect vectors threatening human healthcare (Sánchez-Bayo & Wyckhuys 2019). The subsequent estimated costs of 70 billion US$ per year, caused by invasive insects are least concern (Bradshaw et al. 2016) in comparison to missing pollination services in agriculture, already estimated to a deficit of 235-577 billion US$, with accompanying devastating consequences for the nutrition of 820 million people who already have too little to eat (FAO 2020 a, 2020 b).

As insect decline is a temporal process over decades to centuries, analysis of long-term data offers the great opportunity to better understand ecological processes, extinction events and how species assemblages change over time. However, standardized long-term data series for insects are rare and are lacking for most regions in the world. Leather (2018) found clear words about this knowledge-gap, when he wrote: “we desperately need funding for more long-term studies, particularly of invertebrates and plants. Unfortunately, this may however, be a case of locking the stable door, after the horse has bolted. We also need to find instances where the data already exist but have not yet been analyzed”. Against this background, I explored in my thesis the potential of small historic museum collections to get an insight into long-term insect community change by using moths as target organisms. Furthermore, I investigated potential pitfalls rendering comparisons between samples or studies problematic.

Investigating historic collections is sometimes a bit like playing lottery: If collection boxes with moth specimens are the tickets, we can hope for the jackpot to find a scientific fine box. But to pull the drain is disproportionally higher, because we usually cannot tell exactly how the moths had been sampled. Accordingly, we have to assume that the sampling across different collections was not standardized. As a consequence, it is difficult to say whether a moth species that occurred in ancient times, but is missing in the box, just reflects preferences of ancient collectors or random sampling error. It is also likely that different light trap types and other sampling devices were used over time. All these circumstances may influence the outcome, posing also a problem for meta-analyses. Looking at light-trap sampling, it is known that different light sources can affect the species composition of attracted moths (Axmacher & Fiedler 2004, Jonason et al. 2014). Additionally, the trap type - manually or automated trap - can affect apparent species composition (Chapter 6). Moths are not all the same – they behave differently when flying to light and they have different manners when they are trying to sit down on a trap. Some species directly fly to the trap and sit down immediately, while others keep circling around the trap and collide multiple times with the trap surface, until they may eventually end up in the sampling container. Therefore, my first hypothesis was dealing with the determination of the possible sampling error based on the flight-to-light behavior of moths in regard to body size, taxonomic status and ambient temperature (Chapter 6).

The results show that in particular, small species remained seated after their arrival and would therefore have been less likely to collide with a baffle plate of an automatic trap and thus landed in its collecting container. This particularly affects taxonomic groups, which in Central Europe mainly contain small species amongst the Macroheterocera, such as the Nolidae, Eupitheciini and Lithosiini. Species in these taxa sat down 60-80% faster and stayed seated. Consequently, my findings underline the urgent need to take a closer look at the sampling bias in terms of the trap type in connection with animal behavior. Automated trap types have on the one hand multiple advantages when moths need to be sampled. On the other hand, however, they seem unpractical when certain taxa are considered.

Besides this taxonomic bias, ambient temperature played a significant role, with mostly medium-sized moths (30-39mm) becoming more active with increasing temperature, rendering them more likely to be sampled than at cooler conditions. So, temperature can have major effects on the sampled species composition, as in warmer nights or locations, medium-sized moths are more likely to get caught. My findings suggest, that not only more individuals are caught in warmer nights, but also the composition might change, as certain groups of moths are more likely to fly to the trap. To avoid such a bias, each study site should always be sampled multiple times. The resulting pooled species composition might counteract a strong influence of variable weather conditions in individual sampling nights on the outcome.

Half a billion insects have been scientifically sampled worldwide over the decades with light-traps and other methods (Short et al. 2018). Of course, my findings do not mean that this immense mass of museum material is unusable for analysis now. But it is an indication to be careful with the data. An evaluation of historical material using individual numbers as proxy for abundances is only useful if it was meticulously documented how the samples were taken. Irrespective of all caveats surrounding the use of old collection data, my findings offer an example for the use of data extracted from museal collections as I managed to do both: Using historic collections and combining the data with an own empiric long term study (Chapter 7). My test area was the isolated coastal forest reserve Pineta san Vitale (Italy, Ravenna) (hereafter PsV) where my own data records were collated from 1997 to 2012, in combination with three historic collections of moths that I found (in museums and private). Together, these data span over more than 80 years. PsV has one additional important property that made it interesting to study: due to the protected status of this area, former extensive land-use was abandoned and in the course of the resulting succession vegetation has changed profoundly over the past decades (Fig. 9.1).

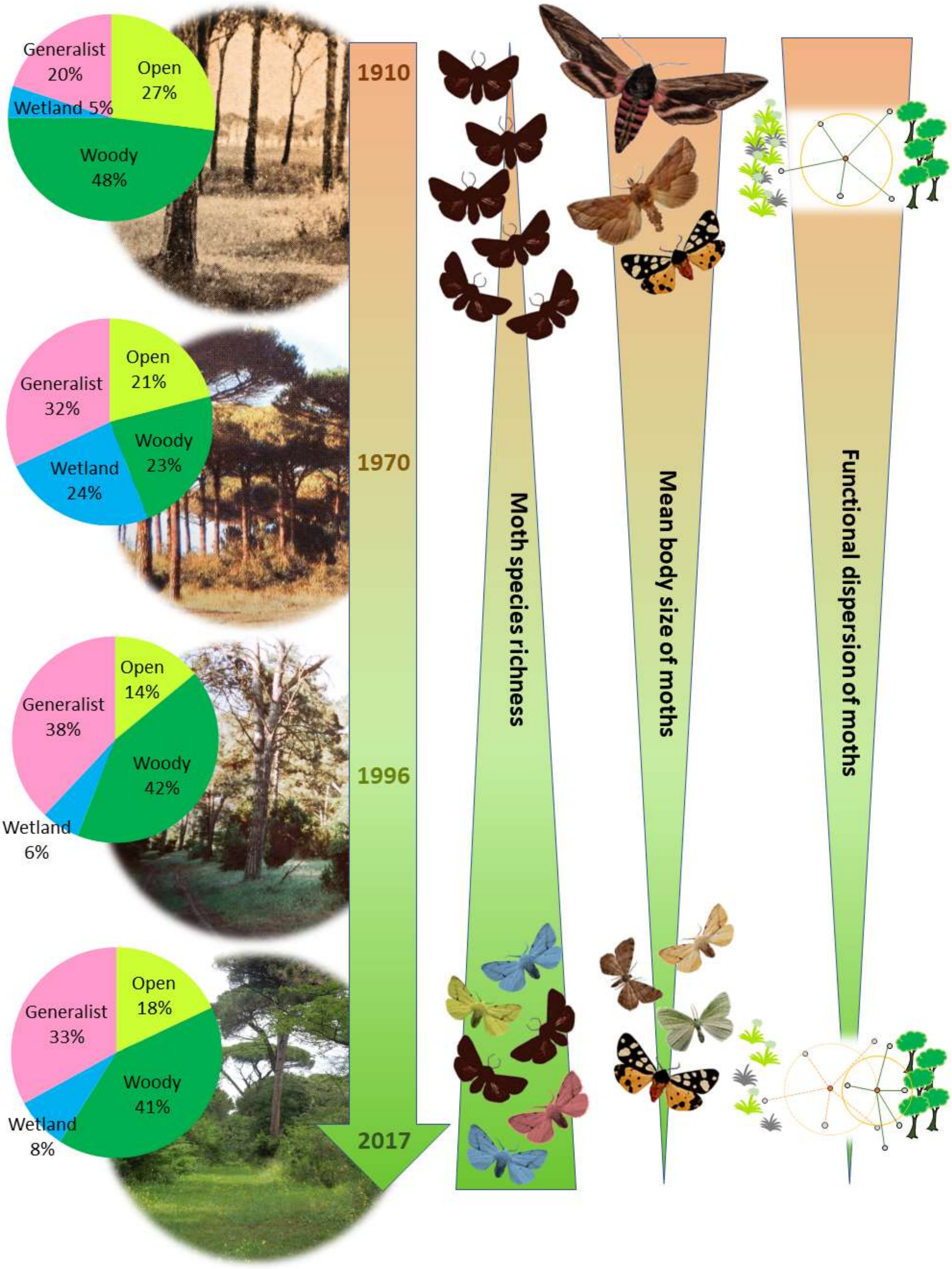

Figure 9.1: Overview of the results of my thesis showing the environmental changes in Pineta san Vitale in the course of the last eight decades. The pie charts illustrate the moth species composition, split up after their habitat use. The round pictures exemplarily show the vegetation that could be found in the course of the time (years are given within the arrow).

On the other hand, as a ‘forest island’ embedded into a highly transformed cultivated landscape PsV has to withstand the manifold stressors on the landscape scale, such as pollution from neighboring industrial plants (Lucialli et al. 2007), pesticide input from the surrounding intense agriculture (Benini & Pezzi 2011, Paris et al. 2016) as well as subsidence induced soil salinization (Antonellini et al. 2008). With PsV as focal area, I therefore had the unique opportunity to answer the fundamental question how succession shapes the composition of the moth community when land-use inside a conservation area has ceased, but at the same time this reserve is exposed to manifold external stressors at the landscape scale. Can a conservation area under these circumstances still fulfill the preservation of near-natural biodiversity, which is one goal of setting aside land for conservation purposes (Young et al. 2005)? Or, is the insect fauna of such an area prone to degradation?

My results have shown that over the decades, the number of observed moth species has increased in PsV (Chapter 7, Fig. 7.2, Fig. 9.1). In more recent samples species richness was enhanced by about 67 additional species (estimator based on incidence data). This increase was in particular high among forest and generalist moths, where the number of species nearly doubled over time (Chapter 7). The number of open habitat species, in contrast, nearly stayed the same over the last 80 years. Natural succession has favored the development of a near-natural forest structure, since the abandonment of extensive land use in PsV in the 1980s. Previously open grassland habitats in consequence disappeared in favor of forest habitats. Subsequently the composition of the moth community has also shifted accordingly in the course of the decades, favoring the establishment of more forest and generalist species. This change in habitat use is especially visible, when I compared the proportions of species groups using one or another habitat type over the years. The proportion of habitat generalists has increased (from 20 to 33%), while the proportion of open habitat species has dropped by 9 percentage points. The proportion of forest species has also increased during the last 80 years, but compared to habitat generalists, this increase was less pronounced (Chapter 7, Fig. 7.3, Fig. 9.1). In conclusion, habitat generalists seem to more easily reach an isolated area by passing the anthropogenically influenced landscape matrix around the reserve. For forest specialist species, in contrast, open agricultural areas can be insurmountable barriers, what makes forest species less likely to migrate between isolated forest areas. This is finally in line with the results from Summerville & Crist (2004), corroborating that the number of forest affiliated species goes down when the patch-size and the proportion of forest in the landscape decreases. This way, my findings can also be used in nature conservation management. In this context, habitat connecting elements and stepping stones are already known as basic tools for the improvement of species dispersal (Slade et al. 2013). But with regard to insect biodiversity loss, my results represent a renewed appeal to attach particular importance to a permanent and future improved network of biotopes in our anthropogenic characterized landscape.

Without further analyses, one might conclude that this is a consistently positive development and that possible anthropogenic influences on this isolated forest reserve are simply over-compensated by natural secondary succession. However, it is not that simple. In order to provide a first outlook, I inspected the developments over the past 20 years, based on my own field observations (Fig. 9.2). This first glance reveals that the total number of species, after controlling for sample-size effects through rarefaction, has decreased during this period. Insect decline might be a phenomenon that especially accelerated over the past 20 years (Hallmann et al. 2017, Seibold et al. 2019). To check whether this is just a fluctuation, I continued to collect moth data in PsV after 2012 almost annually until today, but these data still await comprehensive analysis. So, for future evaluations, I can not only tell whether PsV has passed through a climax community, but may also link my insect samples to historic weather records, revealing possible links to climate change. In addition, my own data are abundance based and can therefore also be evaluated concerning individual numbers over a span of 23 years.

Today, however, nature conservation management has to do more than improve habitat connectivity. One of the most important elements is to determine the risk of extinction of certain groups or individual species to initiate targeted countermeasures. The results of the third part of this dissertation (Chapter 8) give new insights into the risk of extinction of moth species in isolated, near-natural reserves. In birds and mammals, the body size of the species as well as their degree of specialization play a decisive role in modulating their extinction risk (Ripple et al. 2017, Smith et al. 2018). However, publications in regard to the extinction risk of insects in isolated, near-natural reserves are still mostly missing. For this purpose, a mere species list is not sufficient as data source to solve this problem and properties of each individual species have to be checked carefully. For such an instructive analysis I compiled all properties of each of the 300 moth species of Pineta san Vitale (e.g. wingspan as a proxy for body size) and determined the total degree of ecological specialization (Chapter 8).

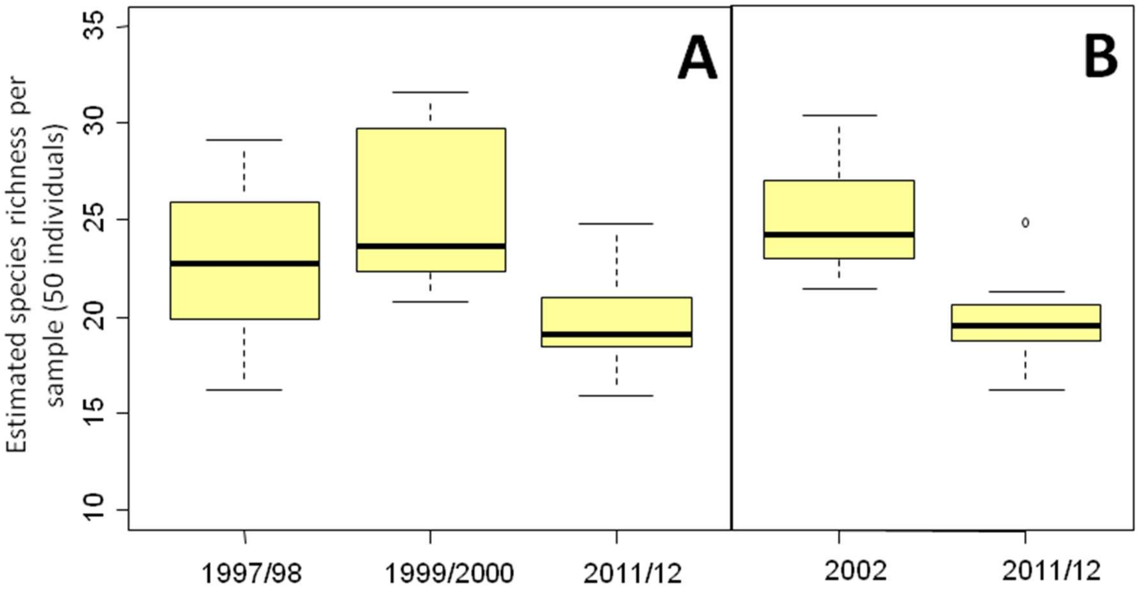

Figure 9.2: Estimated species richness (50 individuals per sample) of Pineta san Vitale. A) shows early summer moth species richness in 1997/98, 1999/2000 and 2011/12 samples while (B) illustrates summer moth species richness in 2002 and 2011/12 samples.

In my analyses, it seems that body size plays a major role concerning the extinction risk of moths in the isolated forest reserve PsV. In connection with the insect decline, it is an important finding that in isolated, semi-natural habitats mainly large species of moths are affected. Previously unrecorded or persistent species were on average smaller than lost species (mean wingspan: 30.7 mm and 33.2 mm vs. 37.0 mm) (Fig. 8.1, Fig. 9.1).

This contrasts with the findings of Merckx et al. (2018), who found moth wingspan to increase along an urbanization gradient. The correlation between body size and extinction risk is therefore quite difficult to evaluate. Large species might have some advantages in urban areas (Merckx et al. 2018) and seem also to be more predisposed for the recolonization of isolated areas (Slade et al. 2013). However, a larger size might also come with the risk of being more likely to get caught by predators (Tammaru et al. 2018). Finally, to date there is no explanation for the increased extinction risk of large species in PsV. Extinction can be driven by multiple different factors, and body size can be an advantage or disadvantage, influencing also species' persistence. Here, future investigations are needed to more precisely evaluate the correlation between environmental gradients and body size in insects.

With regard to the degree of specialization, I was unable to identify any groups with a higher risk of extinction by using rough classification of species. By means of a multivariate analysis (FDis over 12 species characteristics) to analyze the functional dispersion within species groups, however, I detected significant differences in functional specialization. I attribute this to the fact that more ecological niche dimensions are taken into account by using a metric based on multiple graded traits. Mere categorizations and specialization ranks can obscure changes in species habitat affiliations or might simply be too rough to resolve ecological shifts. Only multivariate analyses like the FDis can show what is actually going on as they consider more niche dimensions and e.g. distinguish against different habitat types. FDis of macro-moths in PsV decreased during the last 80 years, indicating that either the whole community has become more specialized, or the community has shifted towards one habitat type, diminishing the ancient wider dispersion of functional niche space. Most likely, the latter scenario took place, as I found that the PsV moth community changed towards forest affiliated species in the course of the time. So, multivariate analyses are more appropriate for such analyses and showed in the case of PsV that a decrease in FDis can indicate some kind of niche homogenization, by shifting towards an overall more forest-affine insect community (Fig. 8.5, Fig. 9.1).

Overall, my results show that PsV was in the last 80 years not prone to degradation because succession increased the total number of moth species. I can state that good nature conservation management by the Comune di Ravenna has ensured that PsV has been preserved as an important element in the network of the Po Delta National Park despite diverse anthropogenic influences. A good hybrid was found between succession and the small-scale habitat diversity. However, first abundance-based results from my recent data (1997-2012) indicate decreasing species richness over the last 20 years (Fig. 9.2). The continuation of actually 23 years of own data, as well as the associated abundance-based evaluation, are therefore essential in order to identify negative trends at an early stage. Combined with weather data it will be possible to evaluate the effect of climate change on PsV since it is known, that climate change can undermine conservation efforts (Araújo et al. 2011). Suitable habitats within conservation areas might vanish because of changing climatic conditions, and so distribution areas of species do also shift (Stuhldreher & Fartmann 2018). Studies have already shown that, due to global warming, some species that are tied to cooler and more humid locations will locally become extinct or migrate northwards (Forsman et al. 2016). Calculations forecast that the Mediterranean zone will also extend northwards in the next 30 years and 157.000 km^2 (half the size of Italy) will be replaced in favor of arid zones in the Mediterranean basin (Barredo et al. 2018). Adapting conservation efforts also to the challenges posed by climate change will afford landscape-scale actions for better connectivity between conservation areas. By doing so, species will be able to migrate more easily between reserves, what is especially important when their distribution areas shift due to climatic and habitat changes.

Only counting species numbers is finally not sufficient to get an insight about what is driving insect decline. The analysis of community composition and functional characteristics here provides much more opportunities to understand diversity changes over time. For an application-related analysis, in terms of community shifts, the current situation should therefore always be determined as detailed as possible, ideally using multivariate methods.

Historic collections can serve as important excerpts in order to gain insight into past processes in a protected area (Kim & Bryne 2006). Although historical collection material harbors many pitfalls, I showed that historic data can be useful, in combination with modern data, beyond the mere fact to describe who has been there at an earlier point in time.
Besides the analysis of museum collections, we urgently need to mobilize more biodiversity data sources like private collections and citizen science projects (Leather 2018) to collate long term data and get a better insight in the "Ecological Armageddon". As diverse as the solution approaches are to get the urgently needed long-term data, it is also difficult to create it. As Kim and Bryne (2006) wrote: "Systematic collections, the material basis of biodiversity information, have been neglected and abandoned, particularly at institutions of higher learning". Without a re-orientation towards the education of trained taxonomists and the analysis of site-specific biodiversity trends, we cannot fight biodiversity decline.

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11. Appendix

Appendix S1: Lepidoptera species from the Ravenna Pinewoods from 1774.

Actebia praecox
Aglais urticae
Allophyes oxyacanthae
Catocala nupta
Ceramica pisi
Cerura vinula
Colocasia coryli
Cossus cossus
Crambus spec.
Deilephila elpenor
Dendrolimus pini
Euclidia glyphica
Favonius quercus
Gonepteryx rhamni
Hemaris tityus
Hyphoraia spec.
Lithosia quadra
Maniola jurtina
Operophtera spec.
Phlogophora meticulosa
Plebejus cf. idas
Pseudotelphusa tessella
Pyrgus malvae
Retinia cf. resinella
Rhyacionia spec.
Smerinthus ocellata
Sphinx ligustri
Stauropus fagi
Thaumetopoea pityocampa
Thyria jacobaeae
Zygaena cf filipendulae

Appendix S2: Species of Pineta san Vitale split into different types of habitat use.

Woody	**Open**	**Wetland**	**Generalist**
Abrostola triplasia	*Abrostola asclepiadis*	*Archanara dissoluta*	*Aedia leucomelas*
Acronicta aceris	*Acronicta auricoma*	*Archanara neurica*	*Allophyes oxyacanthae*
Agrochola circellaris	*Acronicta rumicis*	*Chariaspilates formosaria*	*Angerona prunaria*
Amphipyra pyramidea	*Agrotis bigramma*	*Chilodes maritima*	*Arctia villica*
Apeira syringaria	*Agrotis puta*	*Coenobia rufa*	*Caradrina morpheus*
Apoda limacodes	*Anarta stigmosa*	*Denticucullus pygmina*	*Charanyca ferruginea*
Arctornis l-nigrum	*Anarta trifolii*	*Diachrysia chryson*	*Chloroclystis v-ata*
Ascotis selenaria	*Apamea monoglypha*	*Globia algae*	*Conisania luteago*
Atethmia centrago	*Aplasta ononaria*	*Globia sparganii*	*Deltote pygarga*
Axylia putris	*Aplocera plagiata*	*Helotropha leucostigma*	*Diachrysia chrysitis Komplex*
Cabera exanthemata	*Athetis gluteosa*	*Lacanobia blenna*	*Dypterygia scabriuscula*
Calliteara pudibunda	*Calophasia lunula*	*Laelia coenosa*	*Egira conspicillaris*
Callopistria juventina	*Calyptra thalictri*	*Lenisa geminipuncta*	*Eilema complana*
Catarhoe rubidata	*Caradrina kadenii*	*Leucania obsoleta*	*Eilema depressa*
Catephia alchymista	*Charanyca trigrammica*	*Macrochilo cribrumalis*	*Eilema lurideola*
Catocala elocata	*Chiasmia aestimaria*	*Mythimna congrua*	*Eilema sororcula*
Catocala nupta	*Chlorissa viridata*	*Mythimna pudorina*	*Elaphria venustula*
Catocala promissa	*Chrysodeixis chalcites*	*Mythimna riparia*	*Epirrhoe alternata*
Catocala puerpera	*Cilix glaucata*	*Mythimna straminea*	*Eucarta amethystina*
Cerura erminea	*Deilephila porcellus*	*Nonagria typhae*	*Eucarta virgo*
Cerura vinula	*Diaphora mendica*	*Orthonama vittata*	*Eupithecia pusillata*
Clostera anastomosis	*Dyspessa ulula*	*Pelosia obtusa*	*Euplexia lucipara*
Clostera curtula	*Eilema pygmaeola*	*Phragmataecia castaneae*	*Habrosyne pyritoides*
Clostera pigra	*Episema glaucina*	*Plusia festucae*	*Hemistola chrysoprasaria*
Colobochyla salicalis	*Eublemma purpurina*	*Rhizedra lutosa*	*Hemithea aestivaria*
Colostygia pectinataria	*Eupithecia absinthiata*	*Scopula caricaria*	*Herminia tarsipennalis*
Comibaena bajularia	*Eupithecia centaureata*	*Senta flammea*	*Herminia tenuialis*
Conistra vaccinii	*Eupithecia ericeata*	*Simyra albovenosa*	*Hoplodrina blanda*
Cosmia affinis	*Eupithecia ultimaria*	*Thumatha senex*	*Horisme vitalbata*
Cosmia pyralina	*Eupithecia virgaureata*		*Idaea aversata*
Cosmia trapezina	*Euxoa segnilis*		*Idaea degeneraria*

Cosmorhoe ocellata
Cossus cossus
Costaconvexa polygrammata
Craniophora ligustri
Crocallis elinguaria
Cryphia algae
Cryphia ochsi
Cyclophora punctaria
Cyclophora puppillaria
Deilephila elpenor
Dendrolimus pini
Drymonia dodonaea
Drymonia querna
Earias clorana
Earias vernana
Eilema caniola
Ennomos erosaria
Ennomos quercinaria
Epione repandaria
Eupithecia abbreviata
Eupithecia dodoneata
Euproctis chrysorrhoea
Euproctis similis
Furcula bifida
Furcula furcula
Gastropacha quercifolia
Harpyia milhauseri
Herminia grisealis
Herminia tarsicrinalis
Heterogenea asella
Horisme radicaria
Hypena proboscidalis
Hypomecis punctinalis
Idaea seriata
Idia calvaria
Ipimorpha retusa
Ipimorpha subtusa
Laothoe populi
Laspeyria flexula
Leucoma salicis
Ligdia adustata
Lithosia quadra
Lobophora halterata
Lomaspilis marginata
Lomographa bimaculata
Macaria liturata
Macaria notata
Malacosoma neustria
Meganola togatulalis
Melanthia procellata
Miltochrista miniata
Minucia lunaris
Moma alpium
Mormo maura
Mythimna turca
Noctua janthina / janthe
Nola aerugula
Notodonta tritophus
Notodonta ziczac

Euxoa temera
Gymnoscelis rufifasciata
Hadena bicruris
Hadena perplexa
Hecatera bicolorata
Hecatera dysodea
Hoplodrina ambigua
Hyphantria cunea
Idaea filicata
Idaea pallidata
Idaea rusticata
Lacanobia suasa
Lemonia taraxaci
Leucania zeae
Luperina dumerilii
Lygephila pastinum
Macdunnoughia confusa
Macrothylacia rubi
Malacosoma castrensis
Mamestra brassicae
Mesapamea secalis
Mesoligia furuncula
Metachrostis velox
Minoa murinata
Mythimna pallens
Mythimna sicula
Noctua orbona
Nola subchlamydula
Nyctobrya muralis
Oligia latruncula
Parahypopta caestrum
Pelurga comitata
Perizoma flavofasciata
Photedes morrisii
Phyllophila obliterata
Pyrrhia umbra
Scopula emutaria
Scopula rubiginata
Sesamia cretica
Thalera fimbrialis
Thetidia smaragdaria
Tholera decimalis

Idaea dimidiata
Idaea muricata
Idaea rubraria
Idaea straminata
Idaea subsericeata
Lacanobia oleracea
Lacanobia w-latinum
Lygephila craccae
Lymantria dispar
Macaria alternata
Meganola albula
Menophra abruptaria
Menophra japygiaria
Mythimna ferrago
Noctua fimbriata
Noctua interjecta
Nola chlamitulalis
Ochropleura plecta
Opisthograptis luteolata
Orgyia antiqua
Pasiphila chloerata
Peribatodes rhomboidaria
Phaiogramma etruscaria
Phragmatobia fuliginosa
Polyphaenis sericata
Pseudeustrotia candidula
Pseudoterpna pruinata
Rivula sericealis
Schrankia costaestrigalis
Scopula imitaria
Scopula nigropunctata
Scotopteryx cf mucronata
Scotopteryx luridata
Sideridis rivularis
Spilosoma lubricipeda
Spilosoma lutea
Synopsia sociaria
Timandra comae
Trachea atriplicis
Trichiura crataegi
Trigonophora flammea
Xanthorhoe fluctuata
Xanthorhoe vidanoi
Xestia xanthographa

Ocneria rubea
Odonestis pruni
Orgyia recens
Parascotia fuliginaria
Pechipogo plumigeralis
Pelosia muscerda
Peridea anceps
Petrophora chlorosata
Phalera bucephala
Pheosia tremula
Philereme transversata
Philereme vetulata
Pseudoips prasinana
Pterostoma palpina
Smerinthus ocellata
Spatalia argentina
Sphinx ligustri
Spilosoma urticae
Stauropus fagi
Stegania trimaculata
Subacronicta megacephala
Tephronia sepiaria
Tethea ocularis
Thaumetopoea pityocampa
Thaumetopoea processionea
Thyatira batis
Watsonalla binaria
Watsonalla cultraria
Xanthia gilvago
Xanthia ocellaris
Xanthorhoe ferrugata
Zanclognatha lunalis
Zeuzera pyrina

Appendix S3: Table of the 300 moth species from Pineta san Vitale (Italy). Listed is their occurrence status (lost, persistent, or previously unrecorded), their classification with regard to specialization (total specialization, larval food specialization, northern distribution range limit, and habitat specialization), their wingspan (in mm), and their taxonomic affiliation at family level.

		Classification of the degree of specialization					
Species	**Status**	**Total**	**Food**	**North**	**Habitat**	**Wingspan**	**Family**
Abrostola asclepiadis	Previously unrecorded	6	1	4	1	33.5	Noctuidae
Abrostola triplasia	Persistent	8	1	4	3	29.9	Noctuidae
Acronicta aceris	Lost	11	3	4	4	39.7	Noctuidae
Acronicta auricoma	Lost	12	4	4	4	38.9	Noctuidae
Acronicta rumicis	Persistent	12	4	4	4	32.4	Noctuidae
Aedia leucomelas	Persistent	4	1	2	1	33.9	Noctuidae
Agrochola circellaris	Previously unrecorded	9	2	4	3	35.4	Noctuidae
Agrotis bigramma	Persistent	10	3	4	3	43.8	Noctuidae
Agrotis puta	Previously unrecorded	8	3	4	1	31.0	Noctuidae
Allophyes oxyacanthae	Persistent	10	2	4	4	39.7	Noctuidae
Amphipyra pyramidea	Persistent	12	4	4	4	45.6	Noctuidae
Anarta stigmosa	Persistent	5	2	2	1	30.5	Noctuidae
Anarta trifolii	Persistent	12	4	4	4	32.4	Noctuidae
Angerona prunaria	Persistent	12	4	4	4	39.7	Geometridae
Apamea monoglypha	Lost	10	2	4	4	49.8	Noctuidae

Apeira syringaria	Persistent	11	3	4	4	40.0	Geometridae
Aplasta ononaria	Lost	8	1	4	3	28.4	Geometridae
Aplocera plagiata	Lost	8	1	4	3	39.9	Geometridae
Apoda limacodes	Previously unrecorded	11	4	4	3	25.9	Limacodidae
Archanara dissoluta	Persistent	6	1	4	1	29.9	Noctuidae
Archanara neurica	Persistent	6	1	4	1	27.5	Noctuidae
Arctia villica	Persistent	8	3	3	2	52.0	Erebidae
Arctornis l-nigrum	Lost	10	3	4	3	39.7	Erebidae
Ascotis selenaria	Lost	8	4	2	2	40.3	Geometridae
Atethmia centrago	Previously unrecorded	7	1	4	2	33.9	Noctuidae
Athetis gluteosa	Previously unrecorded	9	3	4	2	28.4	Noctuidae
Axylia putris	Persistent	10	3	4	3	29.4	Noctuidae
Cabera exanthemata	Previously unrecorded	11	3	4	4	32.4	Geometridae
Calliteara pudibunda	Previously unrecorded	12	4	4	4	49.0	Erebidae
Callopistria juventina	Persistent	6	1	4	1	35.9	Noctuidae
Calophasia lunula	Lost	8	2	4	2	28.8	Noctuidae
Calyptra thalictri	Lost	6	1	4	1	45.5	Erebidae
Caradrina kadenii	Previously unrecorded	8	3	3	2	30.0	Noctuidae
Caradrina morpheus	Lost	11	3	4	4	34.9	Noctuidae
Catarhoe rubidata	Persistent	9	1	4	4	28.4	Geometridae
Catephia alchymista	Persistent	4	1	2	1	44.9	Erebidae
Catocala elocata	Lost	8	2	2	4	74.8	Erebidae
Catocala nupta	Persistent	10	2	4	4	69.8	Erebidae
Catocala promissa	Lost	6	1	4	1	62.5	Erebidae
Catocala puerpera	Lost	6	2	2	2	58.0	Erebidae
Cerura erminea	Persistent	8	2	4	2	59.6	Notodontidae
Cerura vinula	Lost	9	2	4	3	56.1	Notodontidae
Charanyca ferruginea	Previously unrecorded	12	4	4	4	35.8	Noctuidae
Charanyca trigrammica	Lost	11	3	4	4	37.4	Noctuidae
Chariaspilates formosaria	Lost	7	3	3	1	38.4	Geometridae
Chiasmia aestimaria	Persistent	4	1	1	2	22.9	Geometridae
Chilodes maritima	Persistent	7	1	4	2	32.3	Noctuidae
Chlorissa viridata	Persistent	10	3	4	3	25.5	Geometridae
Chloroclystis v-ata	Previously unrecorded	12	4	4	4	16.3	Geometridae
Chrysodeixis chalcites	Persistent	12	4	4	4	38.1	Noctuidae
Cilix glaucata	Previously unrecorded	10	2	4	4	19.9	Drepanidae
Clostera anastomosis	Persistent	8	1	4	3	31.9	Notodontidae
Clostera curtula	Persistent	8	2	4	2	30.7	Notodontidae
Clostera pigra	Lost	9	2	4	3	24.4	Notodontidae
Coenobia rufa	Lost	6	1	4	1	23.5	Noctuidae
Colobochyla salicalis	Previously unrecorded	7	1	4	2	27.9	Erebidae
Colostygia pectinataria	Persistent	10	3	4	3	24.4	Geometridae
Comibaena bajularia	Previously unrecorded	8	1	4	3	24.9	Geometridae
Conisania luteago	Persistent	6	1	4	1	38.0	Noctuidae
Conistra vaccinii	Previously unrecorded	12	4	4	4	31.8	Noctuidae
Cosmia affinis	Persistent	10	3	4	3	31.3	Noctuidae
Cosmia pyralina	Lost	11	3	4	4	31.4	Noctuidae
Cosmia trapezina	Persistent	12	4	4	4	28.7	Noctuidae
Cosmorhoe ocellata	Previously unrecorded	9	1	4	4	22.4	Geometridae
Cossus cossus	Persistent	11	3	4	4	80.8	Cossidae
Costaconvexa polygrammata	Persistent	8	1	4	3	26.0	Geometridae
Craniophora ligustri	Previously unrecorded	11	3	4	4	32.4	Noctuidae
Crocallis elinguaria	Persistent	11	4	4	3	36.2	Geometridae
Cryphia algae	Persistent	9	1	4	4	26.8	Noctuidae
Cryphia ochsi	Previously unrecorded	7	4	1	2	21.0	Noctuidae
Cyclophora punctaria	Previously unrecorded	8	1	4	3	26.5	Geometridae
Cyclophora puppillaria	Previously unrecorded	9	4	3	2	31.8	Geometridae
Deilephila elpenor	Persistent	12	4	4	4	52.0	Sphingidae
Deilephila porcellus	Previously unrecorded	9	1	4	4	42.4	Sphingidae

Deltote pygarga	Persistent	10	3	4	3	21.0	Noctuidae
Dendrolimus pini	Previously unrecorded	7	2	4	1	56.1	Lasiocampidae
Denticucullus pygmina	Lost	9	2	4	3	25.8	Noctuidae
Diachrysia chrysitis Komplex	Persistent	10	3	4	3	31.3	Noctuidae
Diachrysia chryson	Lost	9	3	3	3	48.7	Noctuidae
Diaphora mendica	Previously unrecorded	12	4	4	4	32.6	Erebidae
Drymonia dodonaea	Previously unrecorded	7	1	4	2	35.4	Notodontidae
Drymonia querna	Lost	7	2	3	2	39.9	Notodontidae
Dypterygia scabriuscula	Previously unrecorded	10	3	4	3	34.4	Noctuidae
Dyspessa ulula	Persistent	4	1	2	1	22.2	Cossidae
Earias clorana	Previously unrecorded	8	1	4	3	17.9	Nolidae
Earias vernana	Persistent	8	1	4	3	19.4	Nolidae
Egira conspicillaris	Previously unrecorded	6	1	3	2	38.9	Noctuidae
Eilema caniola	Persistent	8	1	3	4	31.3	Erebidae
Eilema complana	Persistent	8	1	4	3	34.0	Erebidae
Eilema depressa	Persistent	7	1	4	2	31.8	Erebidae
Eilema lurideola	Previously unrecorded	9	1	4	4	31.3	Erebidae
Eilema pygmaeola	Persistent	7	1	4	2	25.9	Erebidae
Eilema sororcula	Persistent	8	1	4	3	28.5	Erebidae
Elaphria venustula	Persistent	11	3	4	4	20.9	Noctuidae
Ennomos erosaria	Lost	11	3	4	4	32.4	Geometridae
Ennomos quercinaria	Previously unrecorded	12	4	4	4	45.8	Geometridae
Epione repandaria	Persistent	11	3	4	4	27.4	Geometridae
Epirrhoe alternata	Persistent	9	1	4	4	22.4	Geometridae
Episema glaucina	Previously unrecorded	4	1	2	1	33.3	Noctuidae
Eublemma purpurina	Previously unrecorded	7	2	4	1	25.0	Erebidae
Eucarta amethystina	Persistent	6	2	2	2	34.0	Noctuidae
Eucarta virgo	Persistent	8	2	4	2	29.5	Noctuidae
Eupithecia abbreviata	Lost	7	1	4	2	20.5	Geometridae
Eupithecia absinthiata	Lost	12	4	4	4	20.0	Geometridae
Eupithecia centaureata	Persistent	11	4	4	3	17.9	Geometridae
Eupithecia dodoneata	Persistent	7	1	4	2	20.5	Geometridae
Eupithecia ericeata	Persistent	6	3	2	1	18.9	Geometridae
Eupithecia pusillata	Previously unrecorded	7	1	4	2	18.9	Geometridae
Eupithecia ultimaria	Previously unrecorded	5	1	3	1	14.9	Geometridae
Eupithecia virgaureata	Previously unrecorded	11	4	4	3	19.8	Geometridae
Euplexia lucipara	Persistent	11	4	4	3	29.4	Noctuidae
Euproctis chrysorrhoea	Previously unrecorded	12	4	4	4	38.9	Erebidae
Euproctis similis	Lost	12	4	4	4	31.3	Erebidae
Euxoa segnilis	Lost	7	4	2	1	32.5	Noctuidae
Euxoa temera	Lost	5	2	2	1	38.0	Noctuidae
Furcula bifida	Persistent	9	2	4	3	39.7	Notodontidae
Furcula furcula	Persistent	10	3	4	3	30.7	Notodontidae
Gastropacha quercifolia	Persistent	10	3	4	3	67.1	Lasiocampidae
Globia algae	Lost	7	2	4	1	38.0	Noctuidae
Globia sparganii	Lost	6	1	4	1	35.8	Noctuidae
Gymnoscelis rufifasciata	Persistent	12	4	4	4	16.9	Geometridae
Habrosyne pyritoides	Persistent	8	1	4	3	37.4	Drepanidae
Hadena bicruris	Lost	10	2	4	4	34.6	Noctuidae
Hadena perplexa	Persistent	8	1	4	3	31.2	Noctuidae
Harpyia milhauseri	Previously unrecorded	7	2	4	1	54.8	Notodontidae
Hecatera bicolorata	Lost	10	2	4	4	31.3	Noctuidae
Hecatera dysodea	Lost	10	2	4	4	33.0	Noctuidae
Helotropha leucostigma	Persistent	8	3	4	1	40.4	Noctuidae
Hemistola chrysoprasaria	Persistent	9	1	4	4	29.9	Geometridae
Hemithea aestivaria	Previously unrecorded	12	4	4	4	25.5	Geometridae
Herminia grisealis	Persistent	10	3	4	3	25.9	Erebidae
Herminia tarsicrinalis	Persistent	11	3	4	4	29.9	Erebidae
Herminia tarsipennalis	Persistent	10	3	4	3	32.4	Erebidae

Herminia tenuialis	Lost	8	4	2	2	22.5	Erebidae
Heterogenea asella	Persistent	10	3	4	3	17.3	Limacodidae
Hoplodrina ambigua	Persistent	9	1	4	4	33.0	Noctuidae
Hoplodrina blanda	Lost	11	3	4	4	32.9	Noctuidae
Horisme radicaria	Previously unrecorded	7	1	3	3	26.3	Geometridae
Horisme vitalbata	Persistent	8	1	4	3	32.4	Geometridae
Hypena proboscidalis	Previously unrecorded	8	1	4	3	30.8	Erebidae
Hyphantria cunea	Previously unrecorded	11	4	3	4	31.3	Erebidae
Hypomecis punctinalis	Persistent	12	4	4	4	50.3	Geometridae
Idaea aversata	Persistent	12	4	4	4	26.3	Geometridae
Idaea degeneraria	Persistent	11	3	4	4	28.4	Geometridae
Idaea dimidiata	Persistent	11	3	4	4	15.3	Geometridae
Idaea filicata	Previously unrecorded	5	3	1	1	15.3	Geometridae
Idaea muricata	Persistent	11	4	4	3	19.0	Geometridae
Idaea pallidata	Previously unrecorded	9	3	4	2	18.4	Geometridae
Idaea rubraria	Previously unrecorded	9	3	3	3	23.0	Geometridae
Idaea rusticata	Previously unrecorded	10	4	4	2	20.0	Geometridae
Idaea seriata	Persistent	12	4	4	4	20.0	Geometridae
Idaea straminata	Persistent	11	3	4	4	30.4	Geometridae
Idaea subsericeata	Persistent	10	4	4	2	23.5	Geometridae
Idia calvaria	Lost	9	3	2	4	29.0	Erebidae
Ipimorpha retusa	Previously unrecorded	10	2	4	4	28.8	Noctuidae
Ipimorpha subtusa	Previously unrecorded	10	2	4	4	28.5	Noctuidae
Lacanobia blenna	Persistent	6	2	3	1	38.0	Noctuidae
Lacanobia oleracea	Persistent	12	4	4	4	34.4	Noctuidae
Lacanobia suasa	Persistent	12	4	4	4	34.4	Noctuidae
Lacanobia w-latinum	Previously unrecorded	11	3	4	4	39.4	Noctuidae
Laelia coenosa	Persistent	8	3	4	1	48.0	Erebidae
Laothoe populi	Persistent	10	2	4	4	76.5	Sphingidae
Laspeyria flexula	Previously unrecorded	9	1	4	4	24.9	Erebidae
Lemonia taraxaci	Lost	5	2	2	1	44.0	Brahmaeidae
Lenisa geminipuncta	Previously unrecorded	6	1	4	1	29.4	Noctuidae
Leucania obsoleta	Persistent	6	1	4	1	38.0	Noctuidae
Leucania zeae	Persistent	7	2	1	4	35.9	Noctuidae
Leucoma salicis	Lost	10	2	4	4	43.0	Erebidae
Ligdia adustata	Persistent	9	1	4	4	22.4	Geometridae
Lithosia quadra	Previously unrecorded	9	1	4	4	43.9	Erebidae
Lobophora halterata	Previously unrecorded	10	2	4	4	22.4	Geometridae
Lomaspilis marginata	Previously unrecorded	10	3	4	3	33.8	Geometridae
Lomographa bimaculata	Previously unrecorded	11	3	4	4	23.9	Geometridae
Luperina dumerilii	Persistent	6	2	3	1	33.0	Noctuidae
Lygephila craccae	Persistent	8	2	4	2	42.9	Erebidae
Lygephila pastinum	Persistent	9	2	4	3	39.4	Erebidae
Lymantria dispar	Persistent	12	4	4	4	42.0	Erebidae
Macaria alternata	Persistent	11	4	4	3	24.4	Geometridae
Macaria liturata	Previously unrecorded	8	2	4	2	24.4	Geometridae
Macaria notata	Lost	11	3	4	4	29.9	Geometridae
Macdunnoughia confusa	Persistent	12	4	4	4	34.9	Noctuidae
Macrochilo cribrumalis	Persistent	8	2	4	2	28.5	Erebidae
Macrothylacia rubi	Persistent	11	4	4	3	51.0	Lasiocampidae
Malacosoma castrensis	Persistent	10	4	4	2	35.7	Lasiocampidae
Malacosoma neustria	Lost	11	3	4	4	29.6	Lasiocampidae
Mamestra brassicae	Persistent	12	4	4	4	40.8	Noctuidae
Meganola albula	Persistent	10	3	4	3	20.8	Nolidae
Meganola togatulalis	Persistent	5	1	3	1	20.8	Nolidae
Melanthia procellata	Persistent	9	1	4	4	29.4	Geometridae
Menophra abruptaria	Persistent	11	3	4	4	38.9	Geometridae
Menophra japygiaria	Lost	7	4	1	2	28.5	Geometridae
Mesapamea secalis	Persistent	10	2	4	4	30.5	Noctuidae

Mesoligia furuncula	Persistent	9	2	4	3	24.8	Noctuidae
Metachrostis velox	Persistent	4	2	1	1	17.0	Erebidae
Miltochrista miniata	Persistent	8	1	4	3	24.9	Erebidae
Minoa murinata	Persistent	7	1	3	3	15.9	Geometridae
Minucia lunaris	Lost	6	1	4	1	56.9	Erebidae
Moma alpium	Persistent	11	3	4	4	32.4	Noctuidae
Mormo maura	Previously unrecorded	9	3	4	2	59.8	Noctuidae
Mythimna congrua	Persistent	6	2	3	1	33.4	Noctuidae
Mythimna ferrago	Previously unrecorded	10	2	4	4	37.4	Noctuidae
Mythimna pallens	Persistent	10	2	4	4	32.4	Noctuidae
Mythimna pudorina	Lost	8	3	4	1	36.5	Noctuidae
Mythimna riparia	Persistent	7	3	2	2	32.4	Noctuidae
Mythimna sicula	Persistent	6	2	3	1	28.8	Noctuidae
Mythimna straminea	Persistent	8	3	4	1	35.8	Noctuidae
Mythimna turca	Persistent	8	3	4	1	40.8	Noctuidae
Noctua fimbriata	Persistent	12	4	4	4	49.8	Noctuidae
Noctua interjecta	Persistent	11	4	4	3	33.4	Noctuidae
Noctua janthina / janthe	Persistent	12	4	4	4	34.6	Noctuidae
Noctua orbona	Lost	12	4	4	4	41.4	Noctuidae
Nola aerugula	Persistent	8	3	4	1	17.3	Nolidae
Nola chlamitulalis	Lost	3	1	1	1	18.0	Nolidae
Nola subchlamydula	Persistent	5	2	2	1	17.0	Nolidae
Nonagria typhae	Lost	7	2	4	1	47.4	Noctuidae
Notodonta tritophus	Persistent	9	1	4	4	49.8	Notodontidae
Notodonta ziczac	Previously unrecorded	11	3	4	4	42.4	Notodontidae
Nyctobrya muralis	Previously unrecorded	9	1	4	4	30.3	Noctuidae
Ochropleura plecta	Previously unrecorded	12	4	4	4	27.4	Noctuidae
Ocneria rubea	Lost	7	4	2	1	28.3	Erebidae
Odonestis pruni	Persistent	11	4	4	3	49.6	Lasiocampidae
Oligia latruncula	Previously unrecorded	10	2	4	4	25.5	Noctuidae
Opisthograptis luteolata	Persistent	11	3	4	4	34.4	Geometridae
Orgyia antiqua	Previously unrecorded	12	4	4	4	27.4	Erebidae
Orgyia recens	Persistent	10	3	4	3	37.4	Erebidae
Orthonama vittata	Persistent	11	3	4	4	24.5	Geometridae
Parahypopta caestrum	Previously unrecorded	6	1	3	2	29.6	Cossidae
Parascotia fuliginaria	Previously unrecorded	10	2	4	4	22.5	Erebidae
Pasiphila chloerata	Previously unrecorded	8	1	4	3	18.0	Geometridae
Pechipogo plumigeralis	Previously unrecorded	8	3	3	2	23.9	Erebidae
Pelosia muscerda	Persistent	7	1	4	2	25.9	Erebidae
Pelosia obtusa	Persistent	6	1	4	1	25.0	Erebidae
Pelurga comitata	Lost	10	2	4	4	27.4	Geometridae
Peribatodes rhomboidaria	Persistent	12	4	4	4	33.8	Geometridae
Peridea anceps	Lost	9	1	4	4	57.0	Notodontidae
Perizoma flavofasciata	Previously unrecorded	10	2	4	4	28.8	Geometridae
Petrophora chlorosata	Persistent	7	1	4	2	33.9	Geometridae
Phaiogramma etruscaria	Persistent	6	3	1	2	17.9	Geometridae
Phalera bucephala	Persistent	12	4	4	4	48.1	Notodontidae
Pheosia tremula	Persistent	10	2	4	4	49.8	Notodontidae
Philereme transversata	Previously unrecorded	10	3	4	3	32.8	Geometridae
Philereme vetulata	Persistent	11	3	4	4	26.8	Geometridae
Photedes morrisii	Persistent	6	1	4	1	29.7	Noctuidae
Phragmataecia castaneae	Persistent	6	1	4	1	36.7	Cossidae
Phragmatobia fuliginosa	Persistent	12	4	4	4	32.4	Erebidae
Phyllophila obliterata	Persistent	4	1	2	1	22.5	Noctuidae
Plusia festucae	Lost	8	2	4	2	39.6	Noctuidae
Polyphaenis sericata	Previously unrecorded	6	3	2	1	42.0	Noctuidae
Pseudeustrotia candidula	Persistent	11	3	4	4	22.0	Noctuidae
Pseudoips prasinana	Persistent	11	4	4	3	32.4	Nolidae
Pseudoterpna pruinata	Lost	8	2	4	2	32.4	Geometridae

Pterostoma palpina	Persistent	11	3	4	4	43.9	Notodontidae
Pyrrhia umbra	Persistent	12	4	4	4	30.7	Noctuidae
Rhizedra lutosa	Persistent	6	1	4	1	45.8	Noctuidae
Rivula sericealis	Persistent	10	2	4	4	19.9	Erebidae
Schrankia costaestrigalis	Persistent	9	3	4	2	18.8	Erebidae
Scopula caricaria	Lost	8	2	4	2	21.5	Geometridae
Scopula emutaria	Persistent	8	3	4	1	24.5	Geometridae
Scopula imitaria	Previously unrecorded	9	3	4	2	27.5	Geometridae
Scopula nigropunctata	Persistent	12	4	4	4	31.4	Geometridae
Scopula rubiginata	Lost	10	4	4	2	17.3	Geometridae
Scotopteryx cf mucronata	Lost	8	2	4	2	33.8	Geometridae
Scotopteryx luridata	Lost	7	2	4	1	34.9	Geometridae
Senta flammea	Persistent	6	1	4	1	35.8	Noctuidae
Sesamia cretica	Persistent	7	2	1	4	31.4	Noctuidae
Sideridis rivularis	Persistent	10	2	4	4	28.5	Noctuidae
Simyra albovenosa	Persistent	9	4	4	1	35.8	Noctuidae
Smerinthus ocellata	Persistent	11	3	4	4	74.8	Sphingidae
Spatalia argentina	Lost	5	1	2	2	35.4	Notodontidae
Sphinx ligustri	Lost	11	3	4	4	103.9	Sphingidae
Spilosoma lubricipeda	Persistent	12	4	4	4	40.4	Erebidae
Spilosoma lutea	Previously unrecorded	12	4	4	4	33.5	Erebidae
Spilosoma urticae	Lost	10	4	4	2	41.8	Erebidae
Stauropus fagi	Previously unrecorded	10	4	4	2	52.0	Notodontidae
Stegania trimaculata	Persistent	6	1	3	2	27.0	Geometridae
Subacronicta megacephala	Persistent	11	3	4	4	42.0	Noctuidae
Synopsia sociaria	Lost	6	3	2	1	35.1	Geometridae
Tephronia sepiaria	Lost	7	2	2	3	20.0	Geometridae
Tethea ocularis	Persistent	9	1	4	4	34.9	Drepanidae
Thalera fimbrialis	Persistent	11	4	4	3	27.4	Geometridae
Thaumetopoea pityocampa	Persistent	4	2	1	1	38.2	Notodontidae
Thaumetopoea processionea	Lost	9	1	4	4	29.6	Notodontidae
Thetidia smaragdaria	Previously unrecorded	10	3	4	3	35.0	Geometridae
Tholera decimalis	Lost	10	2	4	4	38.0	Noctuidae
Thumatha senex	Persistent	6	1	4	1	17.3	Erebidae
Thyatira batis	Persistent	8	1	4	3	34.9	Drepanidae
Timandra comae	Persistent	10	2	4	4	25.4	Geometridae
Trachea atriplicis	Persistent	11	3	4	4	40.0	Noctuidae
Trichiura crataegi	Persistent	9	3	4	2	27.4	Lasiocampidae
Trigonophora flammea	Previously unrecorded	9	4	3	2	47.8	Noctuidae
Watsonalla binaria	Previously unrecorded	6	1	4	1	23.2	Drepanidae
Watsonalla cultraria	Previously unrecorded	7	2	4	1	23.7	Drepanidae
Xanthia gilvago	Previously unrecorded	11	3	4	4	34.9	Noctuidae
Xanthia ocellaris	Previously unrecorded	6	1	4	1	33.9	Noctuidae
Xanthorhoe ferrugata	Previously unrecorded	10	3	4	3	19.9	Geometridae
Xanthorhoe fluctuata	Previously unrecorded	11	3	4	4	21.2	Geometridae
Xanthorhoe vidanoi	Lost	7	3	1	3	19.9	Geometridae
Xestia xanthographa	Previously unrecorded	11	4	4	3	33.5	Noctuidae
Zanclognatha lunalis	Previously unrecorded	10	3	4	3	33.9	Erebidae
Zeuzera pyrina	Persistent	12	4	4	4	45.8	Cossidae