

**Doctoral School in Biology**  
**Section: Biodiversity and Ecosystem Analysis**  
**XXVIII Cycle A.A. 2014/2015**

**DISTRIBUTION PATTERNS AND POPULATION ANALYSIS  
OF THREATENED LONGHORN BEETLES IN FOREST  
HABITATS OF CENTRAL ITALY**

**Sarah Rossi de Gasperis**



**Tutor Prof. Giuseppe M. Carpaneto**



DOCTORAL SCHOOL IN BIOLOGY

Section: Biodiversity and Ecosystem Analysis

XXVIII Cycle

**DISTRIBUTION PATTERNS AND POPULATION  
ANALYSIS OF THREATENED LONGHORN BEETLES  
IN FOREST HABITATS OF CENTRAL ITALY**

Distribuzione e analisi di popolazione di coleotteri cerambicidi  
minacciati in habitat forestali dell'Italia centrale

**Sarah Rossi de Gasperis**

A.A. 2014/2015

Tutor: Prof. Giuseppe M. Carpaneto

PhD School Coordinator: Prof. Marco A. Bologna



Rossi de Gasperis S. 2015. Distribution patterns and population analysis of threatened longhorn beetles in forest habitats of Central Italy

Sarah Rossi de Gasperis

Department of Science, Roma Tre University, 446 Viale Marconi,  
00146 Rome, Italy.

E-mail: sarah.rossidegasperis@uniroma3.it, sarahrodega@gmail.com

Front cover: *Rosalia alpina* with the marking number made during the capture-mark-recapture study. Picture by Lara Redolfi De Zan

Thesis defense on the 15th February 2016 in front of the following jury:

Prof. Ferdinando Boero,

Prof. Giovanna Abbate,

Prof. Romolo Fochetti.

## CONTENTS

|  |       |
|--|-------|
| ABSTRACT   | 7p.   |
| PREFACE  | 8p.   |
| CHAPTER 1 Introduction   | 9p.   |
| 1.1 Saproxyllic beetles  | 9p.   |
| 1.2 Target species of the project: <i>Morimus asper/funereus</i><br>and <i>Rosalia alpina</i>  | 10p.  |
| 1.2.1 <i>Morimus asper/funereus</i>  | 10p.  |
| 1.2.2 <i>Rosalia alpina</i>  | 12p.  |
| 1.3 Population study with Capture-Mark-Recapture method  | 15p.  |
| 1.4 Threats and forest management  | 16p.  |
| 1.5 Overview of the thesis and aims  | 17p.  |
| 1.6 References   | 18p.  |
| CHAPTER 2 Overwintering ability and habitat preference<br>of the threatened longhorn beetle <i>Morimus asper / funereus</i> :<br>a two years mark-recapture study with implications for<br>conservation and forest management. | 23p.  |
| CHAPTER 3 Sexual dimorphism and intra-specific<br>allometric relationships between secondary sexual character<br>and body size in <i>Morimus asper/funereus</i> .  | 51p.  |
| CHAPTER 4 Computer-aided Photographic Identification of<br><i>Rosalia alpina</i> (Coleoptera: Cerambycidae): first application<br>in a mark-recapture study.   | 76p.  |
| CHAPTER 5 Conclusions with implications for conservation<br>and forest management  | 109p. |
| 5.1 Summary of the results   | 109p. |
| 5.1.1 Results of chapter 2   | 109p. |
| 5.1.2 Results of chapter 3   | 110p. |
| 5.1.3 Results of chapter 4   | 111p. |
| 5.2 Suggestion for monitoring and preserving <i>M. asper/funereus</i><br>and <i>Rosalia alpina</i>   | 112p. |
| 5.3 References   | 113p. |

|                      |       |
|----------------------|-------|
| ACKNOWLEDGEMENTS     | 117p. |
| LIST OF PUBLICATIONS | 118p. |

## ABSTRACT

*Morimus asper* and *Rosalia alpina* are two saproxylophagous longhorn beetles associated to mature broadleaf forests. Both are threatened by local fragmentation and micro-habitat loss due to dead wood removal by forest management practice. Our two years mark-recapture study, revealed new information on population dynamics and habitat preference of *M. asper* and *R. alpina* in different beech forests of central Italy. Dispersal was recorded in few individuals for both species. The sampling method performed, allowed to get population size estimates for the two species. Both species showed preference for old huge trees in a medium decay class. *M. asper* proved to be sensitive to temperature effect and it resulted to prefer lying dead trees, coarse woody debris and high canopy closure in the surroundings. Sun-exposed huge trees resulted to be preferred plants for *R. alpina* and for oviposition of *M. asper*. Probably, for *M. asper* the standing dying trees, still owning a part of tree foliage, provide a good compromise between larval development in sun-exposed parts and adult activity in shady parts. “Key trees” for population viability of the two species were identified in the study areas. Forest management practices should promote the maintenance of old and huge trees as “stepping stones” to preserve the habitat connectivity and favour genetic flow in both the species.

Overwintering ability was observed for *M. asper* adults, both males and females, with a very long lifespan (282-409 days). Sexual dimorphism in *M. asper* was detected in several morphological traits, and the scaling relationship between antennal length and body size divided the male population into two groups, smaller and larger males, which showed different mating frequencies.

Regarding *R. alpina*, the computer-aided photographic identification resulted a reliable and less invasive capture-mark-recapture method, and we provided suggestion to standardize and facilitate the procedure.

## PREFACE

This research produced three major papers submitted to peer-reviewed journals. In the common introduction (chapter 1) were briefly addressed the issues related to target species and the overall aims of the project. Each one of the following chapters corresponds to one paper, in particular:

CHAPTER 2: Rossi de Gasperis S., Passacantilli C., Redolfi De Zan L., Carpaneto G.M. Overwintering ability and habitat preference of the threatened longhorn beetle *Morimus asper* / *funereus*: a two years mark-recapture study with implications for conservation and forest management. Submitted to: Biological Conservation.

CHAPTER 3: Rossi de Gasperis S., Redolfi De Zan L., Romiti F., Hardersen S., Carpaneto G.M. Sexual dimorphism and intra-specific allometric relationships between secondary sexual character and body size in *Morimus asper/funereus*. Submitted to: Journal of Morphology.

CHAPTER 4: Rossi de Gasperis S., Carpaneto G.M., Nigro G., Antonini G., Chiari S., Cini A., Mancini E., Mason. F., Mosconi F., Redolfi de Zan L., Roversi P.F., Sabbatini G., Solano E., Campanaro A. Computer-aided Photographic Identification of *Rosalia alpina* (Coleoptera: Cerambycidae): first application in a mark-recapture study. Submitted to: Insect Conservation and Diversity.

In the final section (chapter 5), the results of these papers were utilized for drawing conclusions on the analyses conducted at population level for target species and their habitat relationships.



### Introduction

#### 1.1 Saproxylic insects

Saproxylic insects are all species dependent on dead wood or on other saproxylic organisms, at least in some part of their life cycle (Speight 1989; Alexander 2008; Stokland et al. 2012).

Based on the degree of association with dead wood material, saproxylic organisms can be either *obligate* or *facultative* species (Stokland et al. 2012). The *obligate* saproxylic species are strictly related to dead wood which represents their unique trophic resource (directly feeding on wood or indirectly as a consumer of other saproxylic species) or essential material for their life cycle (e.g. breeding site, laying site, matter for larval development). On the contrary, the *facultative* saproxylic species represent all those that regularly use dead wood but do not depend completely upon it, using alternative resources as well (e.g. many predatory insects that hunt larvae in decaying wood but that can also search for prey in other substrates like forest floor).

Many longhorn beetles (Coleoptera Cerambycidae) belong to the functional group of saproxylic insects, and play an important role in the decomposition of dead wood in forest ecosystems.

Several species of longhorn beetles feed almost exclusively on the cambium of dying trees (Ehnström and Axelsson 2002). Among them, there are xylophagous species that feed on the inner part of wood, like sapwood or heartwood. These species typically colonize trees recently dead, when sap and nutrients-rich cytoplasm in the cells are still present in the inner bark.

The ecology of many Cerambycidae is closely related to old-growth forests with many mature trees and great dead wood amount. Larvae of longhorn beetles dig tunnels in trunks and adults emerge from trunk releasing typical exit holes. These insects represent the main trophic resource many parasitoid insects and for many birds, such as woodpeckers, which control xylophagous larvae and contribute to tree preservation (Pechacek and Kristin, 2004; Stokland 2012).

## 1.2 Target species of the project: *Morimus asper/funereus* and *Rosalia alpina*

### 1.2.1 *Morimus asper/funereus*

*Morimus asper* (Sulzer 1776) and *M. funereus* (Mulsant 1862), are two flightless saproxylophagous longhorn beetle (Coleoptera Cerambycidae) mainly associated to deciduous mature forests.

Since 1980s, the taxonomical classification of these two closely related species and their western Palearctic allies (Fig. 1), such as *M. ganglbaueri* (Reitter 1894), *M. orientalis* (Reitter 1894) and *M. verecundus* (Falderman 1836), is debated owing to the high similarity in their morphological traits (Danilevsky, 2013; Koren, 2010; Sama, 2011, 1988; Stanić et al. 1986). Furthermore, a recent molecular study supports the high genetic relatedness among *M. funereus*, *M. asper* and *M. ganglbaueri* (Solano et al. 2013) at subspecies level. For this reason, we used the expression *asper/funereus*.

Therefore, because of nomenclature rules, *M. asper* (sensu lato) should be listed in the Annex II of the European Habitats Directive 92/43EEC instead of *funereus* (currently listed) which could be ranked as subspecies of the former, together with *ganglbaueri* as synonym.

The distribution range of *Morimus asper* (sensu lato) extends in Southern Europe (Sama, 2004). In Italy, populations of *M. asper* occur in all the country, including the major islands (Fig. 2).

The larval development of *Morimus asper/funereus* can vary from three-five years in natural conditions (Campanaro et al., 2011) to one year or less in captivity (Dojnov et al. 2012; Romero-Samper and Bahillo 1993); pupation occurs in spring and the adult's active period is reported from April to August. Adult size and exit holes diameter varies greatly, but the shape of holes is typically more or less circular (Fig 3).

Little is known about the biology and ecology of this species, in particular, few studies were conducted in field (Vrezec et al. 2010, 2012; Polak 2010, Chiari et al. 2013). Moreover, no study investigated the sexual dimorphism and size variability of morphological traits potentially involved into eco-ethological aspects. On the whole, the lack of information on taxonomy, distribution, ecology and ethology of these beetles makes difficult to plan conservation actions for them.

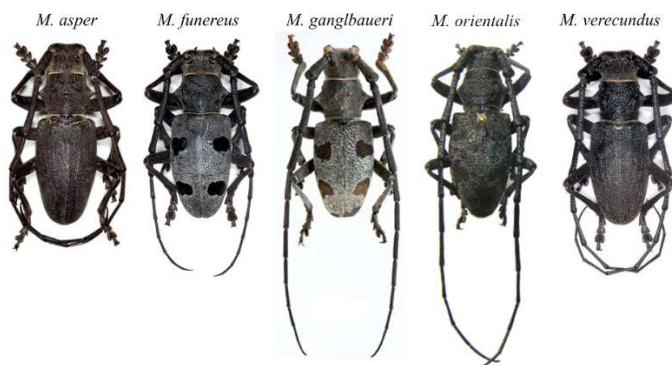


Figure 1. The five taxa of the genus *Morimus* occurring in south Europe (all males) (Images available on: [www.cerambyx.uochb.cz](http://www.cerambyx.uochb.cz); [www.coleo\\_net.de](http://www.coleo_net.de); [www.hmyz.photo.cz](http://www.hmyz.photo.cz); <http://projects.biodiversity.be/openuprbins/about>).

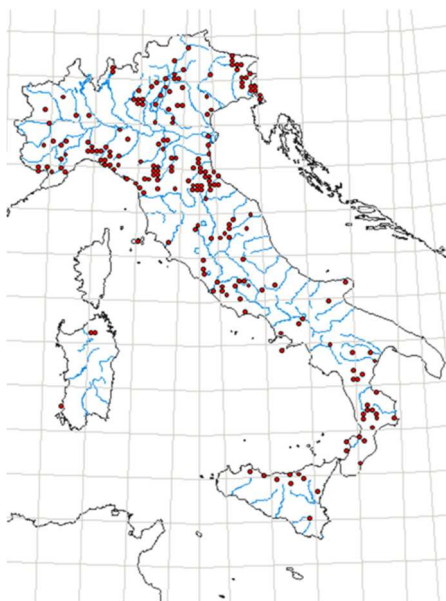


Figure 2 Distribution pattern of *M. asper* in Italy, presence records (red dots) (Sama 2004, Stoch 2005).



Figure 3. A male of *Morimus asper* on a trunk of *Fagus sylvatica*. Two typical *Morimus* adult exit holes are visible in the picture (by Sarah Rossi de Gasperis)

### 1.2.2 *Rosalia alpina*

The longhorn beetle *Rosalia alpina* (Linnaeus, 1758) is considered a flagship species, due to its distinctive colorful elytral pattern (Duelli & Wermelinger 2005) (Fig. 4). The larval development lasts 3-4 years, pupation occur in spring and adult phenology ranges from July to September (Duelli and Wermelinger 2005, Trizzino et al. 2013). The adult size ranges between 14-40 mm and exit holes show a typical elliptic shape about 10 mm long (Fig. 5) (Duelli and Wermelinger 2005; Russo et al. 2013).

*R. alpina* is listed in the Annexes II e IV of the European Habitats Directive 92/43EEC. The distribution range extends from central-southern Europe to Caucasic Region (Sama 1988, 2002). In Italy, the species occurs in all regions, excluding Sardinia (Sama 2005), showing a scattered distribution with large gaps within its areal (Bosso et al. 2013) (Fig. 6). *R. alpina* is mainly associated to ancient deciduous forests dominated by *Fagus sylvatica* (Lachat et al. 2013) and for this reason is mainly located in the Alpine and Apennine mountain ranges. The species was also found associated with other host trees (e.g. mountain elm, sycamore maple, European ash) (Michalcewicz et al. 2011; Michalcewicz and Ciach 2012; Michalcewicz et al. 2013).

A population study recently conducted in Czech Republic (Drag et al. 2011), where the species showed a higher abundance respect to Italian populations, highlighted the short adult life span (4-7 days) and a high dispersal ability (around 1,6 km) of *R. alpina*.

The sampling methods suggested for monitoring *R. alpina* are based on visual encounter surveys (Campanaro et al. 2011; Vrezec et al. 2012, Trizzino et al. 2013), but a standard protocol for population study is still missing. Few field studies were conducted in Italy concerning micro-habitat preference and distribution range (Russo et al. 2013; Bosso et al 2013); therefore, more information on population ecology are needed for monitoring protocol and conservation plans.



Figura 4. A male of *Rosalia alpina* on a trunk of *Fagus sylvatica* (by Sarah Rossi de Gasperis).



Figure 5. Typical elliptic adults exit holes of *R. alpina* on a trunk of *Fagus sylvatica* (by Sarah Rossi de Gasperis).



Figure 6. Distribution pattern of *Rosalia alpina* in Italy, presence records (black dots) (Bosso et al. 2013).

### 1.3 Population study with Capture-Mark-Recapture method

Population studies performed during the current research were based on Capture-Mark-Recapture method (CMR). This method involved: a) the capture of the specimens, b) the marking procedure, which assigned an individual code of recognition, and c) the release of the marked specimen in the place of finding. Repeated surveys were performed to get a size estimate of the sampled population.

Population size is the first objective of the CMR method, but other population parameters like survival ability and catchability can be estimated with different models in data analysis (Amstrup 2005). In relation to the aim of the research, several population models can be applied to obtain the population parameter estimates (Cooch and White 2015). The most common models are: closed-population models and open-population models.

Some assumptions are common to all closed-population models (Chao and Huggins 2005):

- 1) population remains constant over the study period (e.g. there is no immigration or emigration), although known removal (e.g. deaths on capture) are allowed (the closure assumption);
- 2) animals do not lose their marks or tags;
- 3) all marks or tags are correctly recorded;
- 4) animals act independently.

However, in some cases some assumptions may be violated, therefore models are continuously improved (e.g. Bhurnam 1972, Otis et al. 1978; White et al. 1982).

The open-population model more common is the Jolly-Seber model (Jolly 1965; Seber 1965) which need the following assumptions (Pollok and Alpizar-Jara 2005):

- 1) every animal in the population at a given sample time  $j$  as an equal chance ( $p_j$ ) of being captured in that sample (equal catchability);
- 2) every marked animal alive in the population at a given sample time  $j$  as an equal chance of survival ( $\phi_j$ ) until the next sampling occasion (implicitly, this assumption applies to all animals, marked and unmarked, in order to estimate the survival of all animals in the population;
- 3) marked animals do not lose their mark and marks are not overlooked;
- 4) sampling period are short (i.e. effectively instantaneous);
- 5) all emigration from the population is permanent.

Also in this case, open-population models have been improved (e.g. Lebreton et al. 1992, Pollock 2001).

#### **1.4 Threats and forest management**

Like for all the saproxylic organisms, the major threats affecting the two target species are represented by habitat loss and some forest management practices (Davies et al. 2008). Agricultural exploitation has been recognized as the main reason of forest loss in the past but more recently an increasing proportion of deforestation has been caused by industrial factors (Butler and Laurence 2008). In particular, industrial timber exploitation implies road expansion, which generally leads to further deforestation increasing the fragmentation process (Stockland et al. 2012). Moreover, human-induced fires contribute to forest loss (Achard et al. 2006). Fragmentation of woodlands represents the main limit to the dispersal ability of the species, and connectivity gaps will increase the isolation among their populations (Siitonen and Saaristo 2000; Bosso et al. 2013) with consequences on population viability. In particular, saproxylic species with a low dispersal ability suffer more the fragmentation effects (Jonsson and Nordlander 2006).

At lower scale, habitat loss is seriously affected by the local removal of the preferred habitats of saproxylic species (Martikainen et al. 2000), mainly represented by fallen trees and tree logs. Dead wood resulting from natural disturbances tend to be harvested, to ensure human security or for aesthetic reason; moreover, this kind of forest management is performed under a point of view of “not wasting resource”. These are the main factors which explain why low dead wood amount is less present in managed forests than in natural forests (Kirby et al. 1998, Brin et al. 2008).



## 1.5 Overview of the thesis and aims

Few population studies were conducted on *Morimus asper* and *Rosalia alpina* in European countries (Vrezec et al. 2010; Polak 2010, Drag et al. 2011). In Italy, only one population study was performed on *Morimus asper*, showing the high attraction power of fresh tree logs for the species (Chiari et al. 2013) and only one study provided information on the micro-habitat preference of *Rosalia alpina* by using the adult exit holes as indicator of habitat quality (Russo et al. 2013). Currently, no population study has been conducted for more than one year in natural conditions, avoiding the use of attractive features (e.g. tree logs) to evaluate population dynamics and habitat preference of *M. asper*. Moreover, different aspects of the common sampling design used for *R. alpina* need to be improved.

The overall scope of this research project is to increase the ecological knowledge on *M. asper* and *R. alpina* in beech forests of central Italy. Indeed, this study provided specific sampling methods to gather qualitative and quantitative population data in order to plan monitoring programs and forest management practices, which ensure the conservation of these taxa.

The following aims will be pursued:

- 1) Estimating population size of the target species in selected forest areas of central Italy, where previous data have proved their occurrence.
- 2) Setting up a sampling methodology for *Rosalia alpina* through a new approach based on photographic marking.
- 3) Correlating the abundance of the target species to environmental parameters.
- 4) Increasing the knowledge of the poorly studied species *Morimus asper*, in particular exploring the morphological variability to evaluate if morphological traits may affect its behavioral ecology.

## 1.6 References

- Achard F., Mollicone D., Stibig H.-J., Aksenov D., Laestadius L., Li Z., Potapov P., Yaroshenko A. 2006. Areas of rapid-forest cover change in Boreal Eurasia. *Forest Ecology and Management*, 237: 322-334.
- Alexander K.N.A. 2008. Tree biology and saproxylic Coleoptera: issues of definitions and conservation language. *Rev. Écol. (Terre Vie)*, vol. 63, 2008.
- Amstrup S.C., McDonald T.L., Manly B.F.J. 2005. Handbook of capture-recapture analysis. Princeton University Press, Princeton.
- Bosso L., Rebelo H., Garonna A.P. and Russo D. 2013 Modelling geographic distribution and detecting conservation gaps in Italy for the threatened beetle *Rosalia alpina*. *Journal for Nature Conservation* 21: 72– 80.
- Brin A., Meredieu C., Piou D., Brustel H., Jactel H. 2008. Change in quantitative patterns of deadwood in maritime pine plantations over time. *Forest Ecology and Management*, 256: 913-921.
- Burnham K.P. 1972. Estimation of population size in multiple capture-recapture studies when capture probabilities vary among animals. Thesis, Oregon State University, Corvallis.
- Butler R.A., Laurence F.L. 2008. New strategies for conserving tropical forests. *Trends in Ecology and Evolution*, 23: 469-472.
- Campanaro A., Bardiani M., Spada L., Carnevali L., Montalto F., Antonini G., Mason F., Audisio P., 2011. Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxylica/ Guidelines for monitoring and conservation of saproxylic insects. Quaderni Conservazione Habitat, 6. Cierre Grafica, Verona.
- Chao A. and Huggins R.M., 2005. Classical Closed-population Capture-Recapture Models. In: Handbook of capture-recapture analysis. (eds) Amstrup, S.C., McDonald, T.L., Manly, B.F.J. 2005. Princeton University Press, Princeton, 22-36 p.
- Chiari S., Bardiani M., Zauli A., Hardersen S., Mason F., Spada L., Campanaro A. 2013. Monitoring of the saproxylic beetle *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) with freshly cut log piles. *J. Insect Conserv.* 17: 1255-65. DOI:10.1007/s10841-013-9606-4.
- Cooch E G., White G.C. 2015. Program MARK: a gentle introduction (14<sup>th</sup> edition). Available online in PDF format at: <http://www.phidot.org/software/mark/docs/book/>.

- Davies Z.G., Tyler C., Stewart G.B., Pullin A.S. 2008. Are current management recommendations for saproxylic invertebrates effective? A systematic review. *Biodivers. Conserv.* 17:209-234.
- Danilevsky M.L. 2013. A check-list of Longicorn Beetles (Coleoptera, Cerambycoidea) of Europe. 41 pp.  
<http://www.cerambycidae.net/europe.pdf>.
- Dojnov B., Vujčić Z., Božić N., Margetić A., Vujčić Z.M., Nenadović V., Ivanović J. 2012. Adaptations to captive breeding of the longhorn beetle *Morimus funereus* (Coleoptera: Cerambycidae); application on amylase study. *J. Insect Conserv.* 16, 239–247.
- Drag L., Hauck D., Pokluda P., Zimmermann K., Cizek L. 2011. Demography and dispersal ability of a threatened saproxylic beetle: A mark-recapture study of the rosalia longicorn (*Rosalia alpina*). *PLoS One*, 6(6), e21345.  
<http://dx.doi.org/10.1371/journal.pone.0021345>
- Duelli P., Wermelinger B. 2005. Der Alpenbock (*Rosalia alpina*). Ein seltener Bockkafer als Flaggschiff Art. *Merkblatt für die Praxis*, 39, 1–8.
- Ehnström B., Axelsson R. 2002. Insektsnag i bark och ved. ArtDatabanken, SLU, Uppsala.
- Jolly, G.M. 1965. Explicit estimates from capture-recapture data with both death and immigration: stochastic model. *Biometrika*, 52: 225-2862.
- Jonsson M., Nordlander G. 2006. Insects colonization of fruiting bodies of the wood-decaying fungus *Fomitopsis pinicola* at different distances from an old growth forest. *Biodiversity and Conservation*, 15: 295-309.
- Kirby K.J., Reid C.M., Thomas R.C., Goldsmith F.B. 1998. Preliminary estimates of fallen deadwood and standing dead trees in managed and unmanaged forests. *Journal of Applied Ecology*, 35: 148-155.
- Koren T., 2010. Longhorn beetles (Coleoptera: Cerambycidae) in Central Istria. *Entomol. Croat.* 14, 77-84.
- Lachat T., Ecker K., Duelli P., Wermelinger B. 2013. Population trends of *Rosalia alpina* (L.) in Switzerland: a lasting turnaround? *J Insect Conserv.* DOI 10.1007/s10841-013-9549-9.
- Lebreton J.D., Bhurnam K.P., Clobert J., Anderson D.R. 1992. Modelling survival and testing biological hypotheses using marked animals: a unfield approach with case studies. *Ecological Monographs* 62:67-118.
- Pollok K.H., Alpizar-Jara R. 2005 Classical Open-population Capture-Recapture Models. In: *Handbook of capture-recapture analysis.* (eds)

- Amstrup, S.C., McDonald, T.L., Manly, B.F.J. 2005. Princeton University Press, Princeton, 36-55p.
- Martikainen P., Siitonen J., Punttila P., Kaila L., Rauh J. 2000. Species richness of Coleoptera in mature managed and old-growth boreal forests in southern Finland. *Biol. Conserv.* 94: 199–209.
- Michalcewicz J., Ciach M., Bodziarczyk J. 2011. The unknown natural habitat of *Rosalia alpina* (L.) (Coleoptera: Cerambycidae) and its trophic association with the mountain elm *Ulmus glabra* in Poland—a change of habitat and host plant. *Pol J Entomol*, 80:23–31.
- Michalcewicz J., Ciach M. 2012. *Rosalia longicorn Rosalia alpina* (L.) (Coleoptera: Cerambycidae) uses roadside European ash trees *Fraxinus excelsior* L. – an unexpected habitat of an endangered species. *Polish Journal of Entomology*, 81, 49–56.
- Michalcewicz J., Bodziarczyk J., Ciach M. 2013. Development of the *rosalia longicorn Rosalia alpina* (L.) (Coleoptera: Cerambycidae) in the sycamore maple *Acer pseudoplatanus* L.– the first report from Poland. *Polish Journal of Entomology*, 82, 19–24.
- Otis D.L.K., Bhurnam K.P., White G.C., Anderson D.R. 1978. Statistical inference from capture data on closed animal populations. *Wildlife Monographs* 62:1-135.
- Polak S. 2010. Phenology and mating behavior of *Morimus funereus* (Coleoptera, Cerambycidae), in: Abstracts of the 6th European symposium and workshop on the conservation of saproxylic beetles (June 15-17, 2010, Ljubljana), p.19.
- Pollock K.H. 2001. George Seber: a statistical ecology pioneer and scientist par excellence. *Journal of Agricultural, Biological, and Environmental Statistics*, 6:152-157.
- Pechacek P., Kristin A. 2004. Comparative diets of adult and young three-toed woodpeckers in a European alpine forest community. *J. Wildl. Manage.* 68, 683-693.
- Redolfi De Zan L., Bellotti F., D’Amato D., Carpaneto G.M. 2014. Saproxylic beetles in three relict beech forests of central Italy: analysis of environmental parameters and implications for forest management. *For. Ecol. Manag.* 328: 229-244. DOI: 10.1016/j.foreco.2014.05.040
- Romero-Samper J., Bahillo P. 1993. Algunas observaciones sobre la distribución y biología de *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) en la Península Ibérica. *Boln. Asoc. Esp. Ent.* 17, 103-122.
- Russo D., Cistrone L., Garonna A. 2011. Habitat selection by the highly endangered long-horned beetle *Rosalia alpina* in Southern Europe: A

- multiple spatial scale assessment. *Journal of Insect Conservation*, 15, 685–693.
- Sama G. 1988. Coleoptera, Cerambycidae. *Catalogo Topografico e Sinonimico. Fauna d'Italia. XXXVI*. Ed. Calderini, Bologna.
- Sama G. 2002. Atlas of the Cerambycidae of Europe and Mediterranean Area: Northern, Western, Central and Eastern Europe. British Isles and Continental Europe from France (excl. Corsica) to Scandinavia and Urals. Vit Kabourek, Kabourek, Zlín.
- Sama, G. 2004. Fauna Europaea: Cerambycidae. In: Audisio P. (ed.), *Fauna Europaea: Coleoptera 2, Beetles. Fauna Europaea version 1.0*. Available at <http://www.faunaeur.org> (accessed September 2015).
- Sama G. 2005. Coleoptera Cerambycidae, pp. 217–219 + CD-ROM. In: Ruffo S. & Stoch F. (eds.), *Checklist e distribuzione della fauna italiana. Memorie del Museo civico di Storia naturale di Verona, II serie, Sezione Scienze della Vita*, 16.
- Sama G., Rapuzzi P. 2011. Una nuova Checklist dei Cerambycidae d'Italia (Insecta Coleoptera Cerambycidae). *Quad. Studi e Notizie di Storia Nat. Romagna* 32, 121-164.
- Seber G. A. F. 1965. A note on the multiple recapture census. *Biometrika*, 49: 249-259.
- Siitonen J., Saaristo L. 2000. Habitat requirements and conservation of *Phyto colwensis*, a beetle species of old-growth boreal forest. *Biological Conservation*, 94:211-220.
- Solano E., Mancini E., Ciucci P., Mason F., Audisio P., Antonini G., 2013. The EU protected taxon *Morimus funereus* Mulsant, 1862 (Coleoptera: Cerambycidae) and its western Palaearctic allies: Systematics and conservation outcomes. *Conserv. Genet.* 14, 683–694. DOI:10.1007/s10592-013-0461-3.
- Speight M.C.D. 1989. Saproxylic invertebrates and their conservation. *Nature and Environment Series 46*, Council of Europe, Strasbourg.
- Stanić V., Ivanović J., Janković-Hladni M., Nenadović V., Marović R. 1986. Feeding habits, behavior, oviposition and longevity of the adult cerambycid beetle *Morimus asper funereus* Muls. (Col., Cerambycidae) under laboratory condition. *Acta Ent. Jugosl.* 21, 87-94.
- Stoch F. 2000-2005. CK map for Windows Version 5.1. Ministero dell'Ambiente e della Tutela del Territorio, Direzione per la Protezione della Natura. <http://checkmap.faunaitalia.it>
- Stokland J.N. 2012. The Saproxylic food web. In: Stokland J.N., Siitonen J., Jonsson B.G. (eds.), *Biodiversity in dead wood*. Cambridge University Press, New York. pp. 29-54.

- Trizzino M., Audisio P., Bisi F., Bottacci A., Campanaro A., Carpaneto G.M., Chiari S., Hardersen S., Mason F., Nardi G., Preatoni D.G., Vigna Taglianti A., Zauli A., Zilli A., Cerretti P. 2013. Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat, 7. CFS-CNBFVR, Centro Nazionale Biodiversità Forestale. Cierre Grafica, Sommacampagna, Verona.
- Vrezec A., Ambrožič Š., Kapla A. 2010. Biology and ecology of flightless cerambycid *Morimus funereus* (Mulsant, 1862) as a background for monitoring application: laboratory and large-scale field study, in: Abstracts of the 6th European symposium and workshop on the conservation of saproxylic beetles (June 15-17, 2010, Ljubljana), p.20.
- Vrezec A., Ambrožič Š., Kapla A. 2012. An overview of sampling methods tests for monitoring schemes of saproxylic beetles in the scope of Natura 2000 in Slovenia. In: Maja J. (ed.), Saproxylic beetles in Europe: monitoring, biology and conservation. Studia Forestalia Slovenica 137, 73–90.
- White G.C., Anderson D.R., Bhurnam K.P., Otis D.L. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos Natinal Lab, LA-8787-NERP, Los Alamos, NM.

## CHAPTER 2

### **Overwintering ability and habitat preference of the threatened longhorn beetle *Morimus asper* / *funereus*: a two years mark-recapture study with implications for conservation and forest management**

Sarah Rossi de Gasperis<sup>1</sup>, Chiara Passacantilli<sup>1</sup>, Lara Redolfi De Zan <sup>2-3</sup>,  
Giuseppe Maria Carpaneto<sup>1</sup>.

<sup>1</sup> Dipartimento di Scienze, Università Roma Tre, Viale Guglielmo Marconi 446, 00146 Roma, Italy.

<sup>2</sup> CREA - ABP Consiglio per la Ricerca in Agricoltura e Analisi dell'Economia Agraria, Via di Lanciola 12/a, Cascine del Riccio, 50125, Firenze (FI), Italy.

<sup>3</sup> Corpo Forestale dello Stato, Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale 'Bosco Fontana', Strada Mantova 29, 46045 Marmirolo (MN), Italy.

Corresponding author: Sarah Rossi de Gasperis, sarahrodega@gmail.com, mobile phone +39 3291631385

Dipartimento di Scienze, Università Roma Tre, Viale Guglielmo Marconi 446, 00146 Roma, Italy.

## Abstract

*Morimus asper* is a saproxylophagous longhorn beetle associated to mature forests. Its flightless condition makes difficult colonizing new trees in fragmented areas. Our two years mark-recapture study, revealed new information about the survival ability, population dynamics and habitat preference of *M. asper* in three beech forests of central Italy. Daily surveys were performed from May to July in 2013 and 2014, inspecting suitable trees for hosting the species. A low percentage of overwintered adults was observed for both sexes, with a very long lifespan: 282-409 days. Most recaptures were made on the same tree; dispersal (20-451 m) was recorded only in thirteen individuals. Adult phenology showed two peaks of activity (middle of May and middle of June). Population estimates varied significantly among sites and years. Mean daily temperature affected recapture probability and total captures (optimal range of 15-26°C). The species showed preference for huge lying trees, branches on the ground, low percentage of debarked surface and high canopy closure. *M. asper* showed a high survival probability, but the few movements observed, due to its sedentary nature combined with the flightless condition, represent the ecological limit of the species, highly threatened by the local fragmentation. Furthermore, almost total captures were made in the same tree, giving to this a role of “key tree” for population viability. The species will benefit by the maintenance of microhabitat conditions, including many lying trees, abundant coarse woody debris and dense canopy ensuring fresh temperature in the understory, which should be preserved during forest management techniques.

**Key words:** POPAN; Coleoptera; Cerambycidae; saproxylic; population ecology, GLMMs



## 1. Introduction

*Morimus asper* (Sulzer 1776) is a flightless saproxylophagous longhorn beetle (Coleoptera Cerambycidae) associated to deciduous mature forests. The genetic diversity and the status of conservation of this taxon and its western Palearctic allies, *M. funereus* (Mulsant 1862), *M. ganglbaueri* (Reitter 1894), *M. orientalis* (Reitter 1894) and *M. verecundus* (Falderman 1836), are still debated. All these taxa apparently differ from *M. asper* for dorsal microsculpture and color (from dark to light and bluish grey, with more or less visible black spots on elytra) (Dajoz, 1976). Overall, they have a large distribution range from the Iberian Peninsula to northwestern-Iran, through Italy, the Balkans and Anatolia (Sama, 2004). Since 1980s, Sama and other authors started to consider *M. funereus* as a subspecies of *M. asper* and *M. ganglbaueri* as a synonym or another subspecies of the former (Danilevsky, 2013; web site of Fauna Europaea, 2012; Koren, 2010; Sama, 2011, 1988; Stanić et al., 1986). A recent molecular study (Solano et al., 2013) supports the high genetic relatedness of these three taxa and the hypothesis that they could be merged into the same species, bearing the name of *M. asper* (the first one to be described). These preliminary results lead to revise the status of conservation of the whole group because only *M. funereus* is currently protected by the European Habitats Directive 92/43EEC (HD) (Annex II), and this status should be shared by *M. asper* and its closely related taxa.

In the light of these new taxonomic evidences, the distribution range of *Morimus asper* (sensu lato) extends in Southern Europe, from northern Spain to the Balkan region, through France and Italy (Sama, 2004). In Italy, populations of *M. asper* occur in all the country, including the major islands.

This large longhorn beetle belongs to the functional group of saproxylic insects, i.e. all species dependent on dead wood or on other saproxylic organisms at least in some part of their life cycle (Speight, 1989; Stokland et al., 2012). Like all the saproxylic organisms, it plays an important role in the decomposition of dead wood in forest ecosystems. In particular, longhorn beetle larvae feed on wood and dig tunnels in old trees, improving development of fungi and other parasites. These beetles represent an important trophic resource for many birds, such as woodpeckers, which control xylophagous larvae and contribute to tree preservation (Virkkala, 2006).

Few biological studies were focused on the genus *Morimus*, apart from some physiological investigations on eastern populations (those protected

by HD under the name '*funereus*'). These studies mainly concerned with the larval feeding metabolism and the adult antennal neuronal transmission (Božić et al., 2008; Dojnov et al., 2012; Spasic, 2015). To our knowledge, the only field studies were conducted in Slovenia (Vrezec et al., 2010, 2012; Polak, 2010) and in N Italy (Chiari et al., 2013). The guidelines for monitoring this protected species were recently discussed (Campanaro et al., 2011, Vrezec et al., 2012; Trizzino et al., 2013, Mason et al., 2015).

The larval development of *Morimus asper* / *funereus* can vary from three - five years in natural conditions (Campanaro et al., 2011) to one year or less in captivity (Dojnov et al., 2012; Romero-Samper and Bahillo, 1993); pupation occurs in spring and the adult's active period is reported from April to August. Studies on the life cycle of *M. asper* in laboratory condition (lab.) date back to the 40s (Pavan, 1947, 1948; Ghidini and Pavan, 1937). Stanić et al. (1986) observed the adult overwintering ability of *M. asper* in lab., but the adult lifespan of this species in natural conditions is still unknown. Overwintering ability is uncommon in adults beetles, but several examples have been recognized among Cerambycidae, Chrysomelidae, Coccinellidae and Curculionidae (Bazzocchi et al., 2004; Greenberg et al., 2007; Koutroumpa et al., 2008; Sileshi and Kenis, 2001), showing that diapause is performed by adults (mainly females) to overcome the low temperatures of winter season and to readily breed in early spring.

*M. asper* is poorly studied and more information about its habitat preference is needed to plan conservation action. Until now, no population study has been conducted on *M. asper* for more than one year using capture-mark-recapture method (CMR), to evaluate the life span of adults in its natural habitat, and to evaluate the habitat preference without artificial attractive features (like freshly cut tree logs or traps) as our study did.

We provided more information about the survival ability of *M. asper* in three beech forests of central Italy, assessing how the local environmental conditions can affect the abundance of the species.

In particular, we aimed to the following objectives:

- i) to detect the overwintering ability for both sexes in natural conditions;
- ii) to estimate population size of the target species using open population models on CMR data, and provide more information on adult phenology and dispersal ability;
- iii) to evaluate the effects of weather factors on the recapture probability, to give indications for surveying the species;
- iii) to assess the habitat preference of *Morimus asper* to provide information useful for planning conservation, with ad hoc forest management suggestions, to preserve this priority species.

## 2. Material and methods

### 2.1 Study area

The present study has been conducted in three fragments (study sites) of beech woodlands in the Lazio region (central Italy): Allumiere (500-600 m a.s.l.), Mount Fogliano (600-960 m a.s.l.) and Mount Cimino (950-1,053 m a.s.l.) (Fig.1). All the three forest fragments are located in Sites of Community Importance (SCI) assigned by the HD: “Boschi Mesofili di Allumiere” (SCI IT 6030003), “Monte Fogliano e Monte Venere” (SCI IT 6010023) and “Monte Cimino (versante nord)” (SCI IT 6010022).

Allumiere beech forest (60 ha), located at 20 km from the Tyrrhenian coast, belongs to the low-elevation beech forests (400–800 m a.s.l.), typically located on fertile (e.g. volcanic) soils, generally near lakes or at short distance from the sea coast. This old growth forest is dominated by *Fagus sylvatica* associated to *Acer pseudoplatanus*, *Carpinus betulus*, *Castanea sativa*, *Hedera helix*, *Ilex aquifolium*, *Quercus cerris*, *Ruscus aculeatus* and *Spartium junceum*.

M. Fogliano beech forest (318 ha) showed a vegetation cover similar to the former site, becoming monospecific mature forest of *F. sylvatica* on the top with scattered *Q. cerris* trees, and *Narcissus poeticus* and *Lilium bulbiferum* in the understory.

M. Cimino beech forest (60 ha) is covered by a pure old growth forest of *F. sylvatica* associated to *Acer opalus*, *A. pseudoplatanus*, *C. betulus*, with understory species like *Campanula trachelium*, *Corydalis cava* and *Galanthus nivalis* (Alessandrini et al., 2008); in this forest many trees have diameter larger than one meter with a height of 30-35 meters.

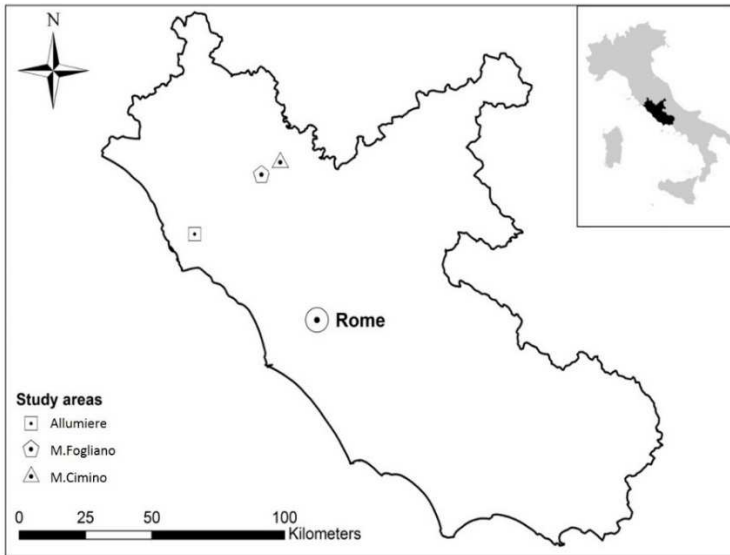


Figure 1. Study area. The map shows the three selected study sites of *M. asper* sampling.

## 2.2 Sampling of *Morimus asper*

In 40 ha of each study site, we selected thirty trees (Vrezec et al. 2012) suitable for hosting *Morimus asper* (i.e. trees entirely or partially dead with a great diameter and presence of cerambycid exit holes).

Visual Encounter Survey (VES) was performed, in order to gather data on the activity of *M. asper*, getting real information on the habitat preference of the species and observing the effects of environmental changes on population dynamics. The same observer made all the surveys.

We inspected selected trees during 29 April - 30 July in 2013 and 2014 and each study site was visited every five days, excluding rainy days.

The few field studies conducted on *Morimus* revealed that these beetles are active at both dusk and daytime (Romero-Samper and Bahillo, 1993; Vrezec et al., 2012). For this study, not being possible to carry out nocturnal inspections, we restricted our surveys to the interval between 9:30 am and

5:00 pm, until the last tree was surveyed; the duration of the survey varied in relation to the number of captures and study site features (e.g. distance among selected trees on field). Tree sampling order has been inverted on every subsequent survey to inspect each tree at different daytime. Adults were captured by hand or with an insect net. Following the CMR method, an individual code was assigned to recognize specimens, by marking them with non-toxic permanent colored spots (CFG Paint Marker). Colored spots were applied on the ventral surface (Suppl. Mat. Figure A1), avoiding an increase in predation rate (Campanaro et al., 2011). At first capture, we recorded the sex and, in addition, we measured individual variables (e.g. elytra length, antennal length etc.) useful to recognize adults in case of partial loss of marks between years. During the survey, the mating events and the individuals in mate were recorded. Finally, for each survey, we recorded three weather parameters every hour: temperature (°C), relative humidity (%) and wind speed (m/s) by using the Weather Meter Kestrel 4000NV.

### 2.3 Environmental variables sampling

The selected ninety trees were mapped using a Global Positioning System (GPS) receiver (Garmin 60CSX). The seventeen environmental variables measured for each tree (cf. Ranius and Jansson, 2000; Redolfi De Zan et al., 2014) are summarized in Table I. Two of these, are strictly related to the target species: cerambycid exit holes (CH) and the occurrence of *M. asper* laying pits (MLP), i.e. peculiar shallow pits dug by females for laying eggs (Sama, 1988) (Fig.2).

Table I. Description of the seventeen environmental variables measured for each selected tree.

| Environmental variable   | Type of variable            | Measuring method   |
|--|-----------------------------|--|
| DNC= Distance from the Nearest Colonized tree  | continuous (m)              | Garmin MapSource software  |
| DBH = Tree Diameter at Breast Height   | continuous (cm)             | Tree girth measured at 1.3 m height  |
| TH = Tree Height   | continuous (m)              | Smart phone application Measure Height 1.4 (Android Play Store) for standing trees, meter for lying trees  |
| CH = Mean number of Cerambycid Holes   | continuous (count of holes) | We made 4 pictures of tree trunk 2-meters height from the ground for standing trees (one for each cardinal direction) and 3 pictures of tree trunk 2-meters length for lying trees (the forth side was ground lying).<br>We counted the cerambycid holes of each picture to get the mean number per tree (CH). Percentages of MS, LS, IS, DBS and WAS were obtained by analyzing picture with Image J 1.48 V software. |
| MS = Mean Bark Surface covered by Moss   | continuous (%)              |  |
| LS = Mean Bark Surface covered by Lichens  | continuous (%)              |  |
| IS = Mean Bark Surface covered by Ivy  | continuous (%)              |  |
| DBS = Mean Debarked Surface  | continuous (%)              |  |
| WAS = Mean Bark Surface with Woodpecker Activity (e.g. surface with numerous holes done by woodpeckers foraging activity). | continuous (%)              |  |
| CC = Mean Canopy Closure   | continuous (%)              |  |
| TS = Tree Status   | categorical                 | Three category: A = Standing living tree, B = Standing dead tree, C = Lying dead tree  |
| ST= Stump  | binary                      | Tree code: presence 1 – absence 0  |
| MLP = shallow Laying Pits made by <i>Morimus asper</i>   | binary                      |  |
| MMB = Major and Minor Branches on the ground   | binary                      |  |
| CA = Cavity  | binary                      |  |
| WN = Woodpecker nest   | binary                      |  |
| FF = <i>Fomes fomentarius</i>  | binary                      |  |



Figure 2. *Morimus asper* shallow lying pits. Red arrows show a female of *M. asper* laying inside the shallow pits she only just dug, patrolled by the male (left picture), and shallow lying pits located on a fallen beech tree (right picture) (pictures made by S. Rossi de Gasperis).

### 3. Theory/calculation

#### 3.1 Population analysis

The observed lifespan for overwintering adults was calculated as the number of days between the first capture in 2013 and last capture in 2014 (Suppl. Mat. Table A1). Dispersal was reported as the linear distance covered between two consecutive captures (Suppl. Mat. Table A2). Sex rate was analyzed in marked and recaptured individuals by Kruskal-Wallis test. The total number of marked individuals was compared among sites in both years with Tukey's Honestly Significant Difference test (THSD).

CMR data were analyzed with Mark 8.0 software (Cooch and White 2015), under the assumptions of the open population Jolly-Seber model with the POPAN parametrization (White and Burnham, 1999) to estimate the population size  $N$  and three primary parameters:  $\phi_i$ - apparent survival probability,  $p_i$ - recapture probability, and  $pent_i$ - the probability of entering the population (combining birth and immigration). Models were built with parameters  $\phi$  and  $p$  constant in time (.) or time-dependent (t). We assumed the probability of entering the population always time-dependent:  $pent(t)$ . The goodness of fit of the started model  $\{\phi(t) p(t) pent(t)\}$  was tested with the Release GOF TEST 2 + TEST 3 (best fitting for  $\hat{c} = 1$ )

(implemented in Mark package). Parameters estimates are reported for the best population model selected by AIC (Akaike Information Criterion theory approach) (Suppl. Mat. Table A3) and daily population size estimates  $N\text{-hat}$  of 2013 and 2014 were compared with One-way Anova test.

The correlation between captures dataset 2013-2014 (mark events + recaptures) and weather parameters (mean value per survey) was assessed by using the Spearman's  $r_s$  correlation coefficient. Since only temperature (T) showed significant correlation with captures (see results), it was used like *time varying covariate* to build constrained linear models (CLM) (Lebreton et al., 1992, Amstrup et al., 2005) evaluating its influence on the recapture probability  $p$  (Suppl. Mat. Table A4). A non-linear fit model analysis, supported by AIC and partial F test ("anova" function in R), was used to investigate the relationship between T and captures. Finally, THSD test was performed to compare T among study sites.

### 3.2 Habitat preference analysis

Tree colonization between the two years was graphed and described. A Spearman's  $r_s$  correlation coefficient, focusing on the most important *Morimus* variables (MLP and CH), was used to check the correlation with the other environmental variables.

Generalized Linear Mixed effects Models (GLMMs) (family = *poisson*, link = *log*, "glmer" function in lme4 R-package) were built to light out the habitat preference of the species. We used the number of marked individuals as the response variable, and the environmental variables as the explanatory variables (fixed effect). On the started model with full environmental variables, we applied three random effects on the intercept (separately or in combination): i) Site (3 levels), ii) Season, represented by the 35 surveys of May-July (19 in 2013 and 16 in 2014) grouped in 15 days per month (6 levels), and iii) Tree(each tree investigated, 90 levels). We chose these three random effects to take into account unknown variation of the number of marked individuals due to site features, phenology, and intrinsic features of trees (e.g. wood decay class). The Tree effect, allowed us also to maintain in to the dataset, the environmental variables changed in 2014 for some trees (e.g. TS, MMB, CC etc.) due to natural events (e.g. tree fell) or human activity (e.g. dead wood removal) evaluating their effect on the response variable. The AIC selection, supported by Likelihood Ratio test (LRT), was used to select the best random effect model, and the final



best model resulted by backward and AIC selection. The collinearity among variables was explored by computing the Variance Inflation Factor (VIF); all the variables with VIF values below 4.0 were considered without a serious collinearity and retained for building the GLMMs models.

All the data analyses were performed using R3.0.3 software (R Development Core Team 2010), CMR data were normalized for parametric test (see 3.1) using logarithmic function; the alpha set for all the analyses was 0.05.

## 4. Results

### 4.1 Population analysis

CMR data of both years, recorded in Allumiere (AL), M. Cimino (MC) and M. Fogliano (MF), are showed in Table II. Of the 28 overwintering adults, the mean life span observed was of  $355 \pm 34$  days (282-409, i.e. 9-13 months), with no difference observed among sites: AL  $332 \pm 43$  (282-396), MC  $359 \pm 31$  (288-409), and MF  $377 \pm 9$  (370-383) (Suppl. Mat. Table A1). The female with the longest observed life span (362 days) was found in mating in 2013 but recaptured recently dead in 2014. Mating events in both years were recorded only for three of the 26 males, and one of the them showed the longest observed life span (409 days).

Two main peaks of adult abundance, middle of May and the middle of June, were observed in 2013 (Fig.3a-b). No difference was observed in sex phenology but the observed sex rates and sex recapture rates were in favor of males (Table II). The total number of marked individuals varied significantly among study sites only in 2013 resulting significantly lower in MF respect to AL (THSD: diff. = - 0.92, p adj. = 0.002) and MC (THSD: diff. = - 1.38, p adj. =  $6.1e^{-06}$ ).

On the 177 recaptured males, 64% were recaptured once, 24% twice, 7% thrice and 3% and 2% four and five times, respectively; while of the 40 recaptured females, 48% were recaptured once and 52% twice. Dispersal was observed only in thirteen adults and the distance covered varied between 20 and 451 meters. The maximum distance covered by a male (451 m) and by a female (404 m) was observed in MC (Suppl. Mat. Table A2).

The fully time dependent model resulted in general with good c-hat (0.55-0.7) except for MF 2013 (c-hat = 0.10), consequently, parameter estimated for this model resulted biased. The best POPAN population model selected by AIC (Suppl. Mat. Table A3) showed:  $\phi$  constant in time in each study

site for both years, with high mean values among sites (0.74 - 0.98), *p* constant or in function of time (values ranging from 0.07 to 0.47) and *pent* values ranging from 0.06 to 0.73. Daily population size estimates ( $N\text{-hat}$ ) varied significantly between years in each study site (AL:  $F = 17.5$ ,  $Df = 1$ ,  $p = 0.0002$ ; MC:  $F = 30.22$ ,  $Df = 1$ ,  $p = 4.25e^{-06}$ ; MF:  $F = 48.29$ ,  $Df = 1$ ,  $p = 1.02e^{-07}$ ) (Fig. 3 c and d).

Temperature (mean value per survey) resulted the only weather parameter correlated to captures (Spearman's Coeff. = 0.43,  $p = 0.001$ , Fig. 4). CLM models showed recapture probability *p* depending on temperature (T) or on both temperature and time (t + T) (Suppl. Mat. Table A4). Non-linear fit models between temperature and captures reduced the choice of best fitting models between linear function ( $r^2 = 0.27$ ,  $p < 0.001$ ,  $AIC = 261.7$ ) and quadratic function ( $r^2 = 0.33$ ,  $p < 0.001$ ,  $AIC = 254.5$ ), with the latter selected as best model by AIC (Delta AIC = 7.2) and partial F test ( $F_{1,101} = 9.30$ ,  $p = 0.003$ ). During the sampling period, temperature varied among sites showing higher value in AL ( $T = 22.12^\circ\text{C} \pm 3.76$ ) respect to MF ( $T = 19.24^\circ\text{C} \pm 4.43$ , THSD: diff. = 4.20,  $p \text{ adj.} = 0.01$ ) and MC ( $T = 18.88^\circ\text{C} \pm 3.92$ , THSD: diff. = 4.71,  $p \text{ adj.} = 0.003$ ).

Table II. CMR results of two sampling years (2013 and 2014) for each study site. Table shows number of Marked, number of Recaptured and total Recaptures of both sexes; recaptured rate (Recapt. rate) calculated as the Number of recaptured / Number of marked; number of overwintering adults (OW) and overwintering recaptured rate (OW Rate) calculated as the number of marked in 2013 recaptured in 2014 / the number of marked in 2013 (see text 3.1), and total number of mating events (ME) recorded in each study site. Kruskal-Wallis test performed for Marked sex rate and sex total recaptures rate (Recaptures  $\text{♂} / \text{♀}$ ) in each population, (\*) significant results.

| Study Sites         | Marked<br>(♂/♀) | Recaptured<br>(♂/♀) | Recaptures<br>(♂/♀) | Recapt.<br>rate | OW        | OW<br>rate      | ME | K-W test<br>sex rate | K-W test<br>sex recapt.<br>rate |
|---------------------|-----------------|---------------------|---------------------|-----------------|-----------|-----------------|----|----------------------|---------------------------------|
| Allumiere 2013      | 150<br>(96/54)  | 46<br>(35/11)       | 69<br>(56/13)       | 30.7%           | 6♂        | 7.3 %           | 38 | H = 3.9<br>p = 0.05* | H = 8.9<br>p < 0.01*            |
| Allumiere 2014      | 80<br>(60/20)   | 22<br>(20/2)        | 34<br>(32/2)        | 27.5%           |           |                 | 9  | H = 7.7<br>p = 0.01* | H = 16.6<br>p < 0.01*           |
| M. Cimino<br>2013   | 257<br>(168/89) | 75<br>(61/14)       | 107<br>(89/18)      | 29.2%           | 18♂<br>2♀ | 10.7 %<br>2.1 % | 54 | H = 4.5<br>p = 0.03* | H = 12.6<br>p < 0.01*           |
| M. Cimino<br>2014   | 92<br>(67/25)   | 36<br>(30/6)        | 58<br>(50/8)        | 39.1%           |           |                 | 19 | H = 4.9<br>p = 0.03* | H = 7.2<br>p < 0.01*            |
| M. Fogliano<br>2013 | 51<br>(42/9)    | 10<br>(9/1)         | 12<br>(11/1)        | 19.6%           | 2♂        | 4.8 %           | 3  | H = 8.1<br>p < 0.01* | H = 8.1<br>p < 0.01*            |
| M. Fogliano<br>2014 | 97<br>(62/35)   | 28<br>(22/6)        | 41<br>(33/8)        | 28.9%           |           |                 | 17 | H = 2.3<br>p = 0.12  | H = 3.3<br>p = 0.05*            |

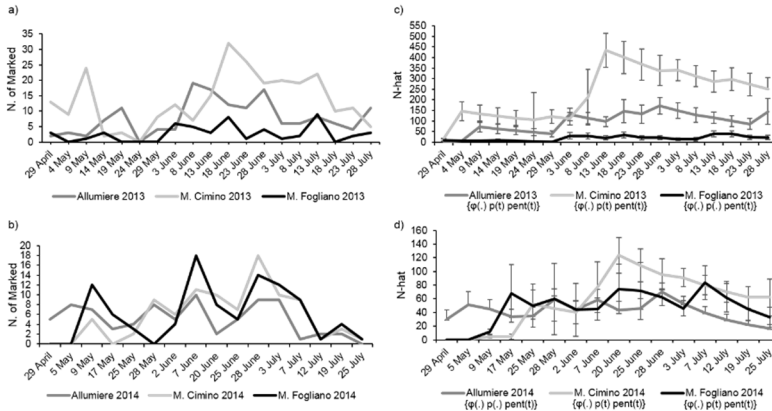


Figure 3. Adult Phenology and Daily Population size estimates of *Morimus asper*. Adult phenology is represented by the number of Marked individuals in 2013 (a) and 2014 (b) in each study site; the daily population size  $\hat{N}$  estimated of the best AIC selected model for each study site (Suppl. Mat Table A3) in 2013 (c) and 2014 (d) with  $\phi$  = apparent survival probability,  $p$  = recapture probability,  $pent$  = probability of entering the population, that can be constant in time (.) or time dependent (t).

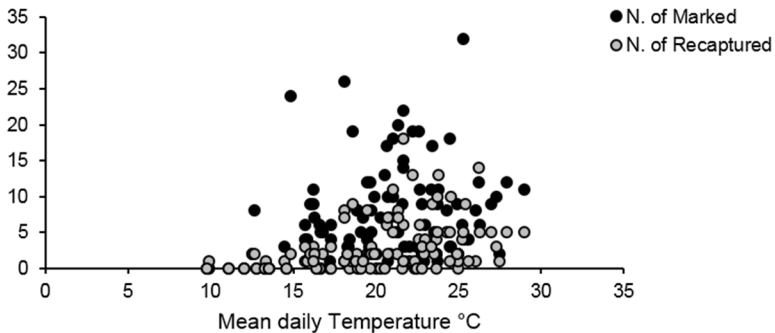


Figure 4. Temperature effect on *Morimus asper* daily captures. Number of captures (mark events and recaptures) of three study sites in 2013 and 2014 in function of the mean daily survey temperature (°C).

## 4.2 Habitat preference analysis

A different percentage of colonized trees was observed among study sites: 77% in AL, 50% in MC and 53% in MF, with a very different number of captures between years in several trees (Fig. 5a-d). In each study site, trees with the highest number of captures in both years showed DBH greater than 100 cm (AL7 DBH = 178 cm, MF28 DBH = 114 cm and MC9 DBH = 150 cm), a TH of 24-31 meters, low percentage of DBS and occurrence of MMB. On these same trees we recorded the highest number of total mating events (AL7 = 17, MF28 = 8, MC9 = 40). Mating events decreased strongly in MC24 tree (17 mating events in 2013 and only one in 2014).

The Spearman's correlation showed many significant values: MLP showed a positive correlation with CH (coeff. = 0.35,  $p < 0.001$ ), WN (coeff. = 0.30,  $p = 0.002$ ) and WAS (coeff. = 0.21,  $p = 0.04$ ); on the contrary, a negative correlation resulted with CC (coeff. = -0.22,  $p = 0.03$ ). CH showed other positive correlations with WAS (coeff. = 0.49,  $p < 0.001$ ), DBS (coeff. = 0.34,  $p < 0.001$ ) and DBH (coeff. = 0.22,  $p = 0.03$ ), while resulted negatively correlated to LS (coeff. = -0.43,  $p < 0.001$ ) and CC (coeff. = -0.28,  $p = 0.004$ ).

Results of GLMMs analyses (Table III a-b), showed M4 (full variables with Tree + Season as random effect) as best random effect model and significantly different from the model M3 (full variables with Tree random effect) (LRT: Chisq. = 112.58, df. = 1,  $p < 2.2e^{-16}$ ). The AIC top ranked model form backward selection resulted M4a (deviance = 2519.6, df. resid = 3240,  $r^2 = 0.55$ ), highlighting six explanatory variables correlated with the number of marked individuals: MS and DBS (negative correlation), TS (C category), DBH, MMB and CC (positive correlation).

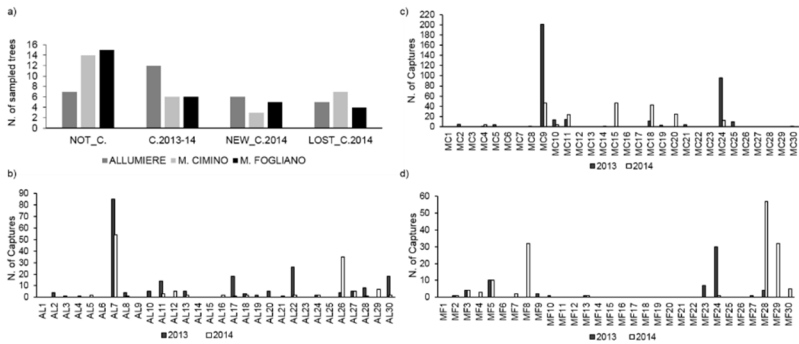


Figure 5. Tree Colonization dynamics by *Morimus asper*. The sampled beech trees in the three study sites in 2013 and 2014 a): Not\_C. = number of not colonized trees in both years, C.2013-2014 = number of colonized trees in both years, New\_C.2014 = number of trees not colonized in 2013 but colonized in 2014, Lost\_C.2014 = number of trees colonized in 2013 but not colonized in 2014. Comparisons of total captures (Marked + recaptured) individuals between 2013 and 2014 for each sampled tree b) Allumiere (AL), c) M. Cimino (MC) and d) M. Fogliano (MF).

Table III. Generalized Linear Mixed effects Poisson Models results. a) Random effects selection and final best model obtained by backward selection for *Morinus asper* (number of marked individuals). For each model are reported the AIC value and Delta AIC for selecting the best model, the value of variance (Var.) and standard deviation (S.D) of each random effect and the two  $r^2$  values of the model (considering the fixed effects and the random effect). Total number of marked individuals = 707, total number of observations = 3150 (90 trees x 35 surveys), random effects levels: Site = 3, Season = 6, Tree = 90 (see 2.3). b) Estimates and standard error (S.E.) of the six explanatory variables of the best model; significant codes: 0 ‘\*\*\*’, 0.001 ‘\*\*’, 0.01 ‘\*’, 0.05 ‘.’. TS = Tree Status (three categories A, B and C) (see Table I).

| a)  |  |           |           |  |            |             |
|---|--|-----------|-----------|--|------------|-------------|
| Model   | Function   | AIC       | Delta AIC | Random effect<br>(Var. $\pm$ S.D.)               | fix. $r^2$ | rand. $r^2$ |
| M4a   | marked ~<br>TS+DBH+MMB+CC+MS+DBS+<br>(1 TREE)+(1 Season) | 2539.6    | 0         | Tree: 3.98 $\pm$ 1.99<br>Season: 0.20 $\pm$ 0.45 | 0.32       | 0.54        |
| M4  | marked ~ Full variables+<br>(1 TREE)+(1 Season)          | 2553.3    | 13.7      | Tree: 3.71 $\pm$ 1.92<br>Season: 0.20 $\pm$ 0.45 | 0.36       | 0.55        |
| M3  | marked ~ Full variables +<br>(1 TREE)                    | 2663.9    | 124.3     | Tree: 3.56 $\pm$ 1.89                            | 0.34       | 0.47        |
| M2  | marked ~ Full variables + (1 Season)                     | 2929.3    | 389.7     | Season: 0.19 $\pm$ 0.43                          | 0.19       | 0.19        |
| M1  | marked ~ Full variables +<br>(1 Site)                    | 3039.4    | 499.8     | Site: 0 $\pm$ 0                                  | 0.21       | 0.21        |
| b)  |  |           |           |  |            |             |
| Best model M4a: marked ~ TS+DBH+MMB+CC+MS+DBS+(1 TREE)+(1 Season) |  |           |           |  |            |             |
| Fixed effects   | Parameter  | Estimates | S.E.      | z value  | pr(> z )   |             |
|   | Intercept  | -10.561   | 1.399     | -7.548   | ***        |             |
|   | TSB = Standing dead tree                                 | 0.416     | 0.609     | 0.682  | p = 0.495  |             |
|   | TSC = Lying dead tree                                    | 3.129     | 0.463     | 6.760  | ***        |             |
|   | DBH = Tree Diameter                                      | 0.060     | 0.007     | 8.284  | ***        |             |
|   | MMB = Major and Minor branches on the ground             | 1.447     | 0.227     | 6.382  | ***        |             |
|   | CC = Canopy Closure                                      | 0.035     | 0.014     | 2.459  | *          |             |
|   | MS = Mean Bark Surface covered by Moss                   | -0.041    | 0.012     | -3.460   | ***        |             |
|   | DBS = Mean Debarked Surface                              | -0.089    | 0.022     | -4.103   | ***        |             |

## 5. Discussion

A low percentage of overwintering adult of *M. asper* under natural conditions (e.g. predatory pressure, environmental and human disturbance) was observed for both sexes and some males were found in mating also in 2014. The long adult lifespan that we observed, more than 400 days, was never evidenced before this study. Stanić et al. (1986) reported mean

lifespan of 334 (98-481) days, for *M. asper* adults collected in the wild and reared in captivity; this value is slightly less than our results for wild specimens, 355 (282-409) days. However, the real lifespan is still unknown and could be longer. Other cerambycid beetles showed diapause in lab condition with a life span of a hundred of days (Keena, 2002; Koutroumpa et al., 2008). *M. asper* is able to hibernate probably inside refugees under bark or in soil where it can survive the low temperatures of winter, like observed for the longhorn beetles (Baur et al., 2005).

The total adult phenology fell within the sampling period and showed two peaks of activity (middle of May and middle of June) with no difference between sexes, but the sex ratio was unbalanced in favor of males. Probably females tend to be more elusive than males, affecting also the sex recapture rate, as observed in other studies (Drag et al. 2011, Tikkamäki and Komonen 2011, Chiari et al. 2013). On the other hand, males can be more visible because of their searching for females and fighting activities, affecting the recapture rate.

The dispersal ability of *M. asper* is for sure influenced by its flightless condition; however, the high recapture rate on the same tree can be also a consequence of sedentary behaviours like observed in other cerambycid beetles of the Lamiini tribe (Hanks 1999). Males were recaptured also four-five times at the same tree, this could be related also to their strong territoriality (Romero-Samper and Bahillo, 1993). However, for the few movements recorded, a high distance covered (around 500 meters) was observed, showing the high potential dispersal ability of *M. asper* respect to other flightless longhorn beetles, like *Dorcadion fuliginator* (Linnaeus, 1758) (Baur et al., 2005). Except two shifts, adults moved to the tree with the highest number of captures recorded. According to Hanks (1999), it is unlikely that Lamiine beetles are attracted by long-range sex pheromones, and one may assume they wander until reach a tree with a good chance of mate.

The differences in population abundance observed between years can be explained by the natural fluctuations of adult emergencies due to the long larval development period of the species (Samways, 2005).

The survival probability of the species resulted obviously high and constant in time, while the recapture probability was generally temperature dependent, suggesting an ideal range of mean daily temperature of 15-26°C for survey. This lead to avoid too hot or cold days even if peak of activity occurs, preferring survey time interval fitting with the optimal temperature range (e.g. early morning or evening in hot sites). Two studies on *Osmoderma eremita* (Scopoli, 1763) (Scarabaeidae) and *Elater ferrugineus*

(Linnaeus, 1758) (Elateridae) revealed a different influence of temperature on adult activity: positive effect was observed on the flight activity in Sweden (Larsson and Svensson, 2011); while a temperature threshold limiting the adult activity was recorded in Mediterranean region (Zauli et al., 2014).

In each study site, most of captures and mating events of both years were recorded on one huge tree (DBH from 113 to 177 cm); this can allow us to define this tree like a “key tree” for the population. This result also suggest a strong aggregative behavior in *M. asper* (Romero-Samper and Bahillo, 1993).

Cerambycid holes and *Morimus* shallow laying pits resulted positively correlated to some selected woodpecker variables, supporting the importance of the cerambycid larvae as trophic resource of these specialized forest key stone birds (Pechacek and Kristin, 2004). Furthermore, these two *Morimus* features showed a negative correlation with canopy closure. This preliminary result could mean that the species prefer trees with sun exposed parts for laying and consequently for larval development, like observed for the longhorn beetle *Cerambyx cerdo* (Linnaeus, 1758) (Albert et al., 2012). Adults were significantly more abundant on lying dead trees, meaning the preference for the lying position of tree (i.e. dead or recently dead trees). Maybe, after the fall, the suitable standing trees (live or dead) release attractive compounds, i.e. kairomones (Allison et al. 2004) from bark split and broken branches due to the impact with soil, or maybe microorganisms growing in the fallen tree modified kairomones (Bouget et al., 2009) increasing the tree attraction power for beetles which recognize that trees like potential larval host plant (Hanks 1999).

Great diameter and the presence of major and minor branches on the ground, in the surrounding of the fallen or standing tree, affected positively the species. Therefore, huge mature trees and deadwood on the ground showed again an important role in the habitat preference of saproxylic beetles (Cocciufa et al., 2014; Ranius and Jansson, 2000; Redolfi de Zan et al., 2014).

Canopy closure showed positive effect on the adults' abundance. For sure, captures were positively affected by lying trees, generally with low local canopy, but a high canopy cover in the surrounding probably preserves the preferred range of daily temperature.

The negative effect of debarked surface on adult abundance could mean that the species prefer the early or medium stages of decaying wood when the bark still covers the largest part of the tree. Probably, like observed in *Cerambyx cerdo* (Buse et al. 2007), a tree with higher decay stage could



have already been overexploited and not represent more a good substrate for egg laying and larval developing. In fact, the complex interactions between cerambycid larvae and the decaying process could bring the wood toward an advanced decay class, useful for other saproxylic species (Buse et al., 2008).

At last, the species seems to prefer trees not covered by moss, maybe this could indicate again a preference for an early decay stage or a dry wood microclimate, in particular for the laying pits location chosen by females.

On the bases of the habitat preference results, we may explain how in a same tree was observed a strong difference in number of captures between years (Fig.5). The natural fluctuations of populations previously mentioned can be responsible of the adult abundance variation observed between years for trees with unchanged environmental conditions, like M. Cimino “key tree” (MC9) and other trees (AL 26, MC15 and MF24). Although this intrinsic biological feature combined with natural events like tree fell, increasing the amount of coarse woody debris, could explain the higher number of captures observed in 2014 for M. Fogliano “key tree” (MF28) and in other trees (MF8, MF29, MC20). The same combined effect maybe affected negatively the abundance in Allumiere “key tree” (AL7); being Allumiere a hot study site, the lower canopy closure due to tree fell may have caused higher local temperature decreasing the daily occurrence of *M. asper*. In fact, canopy closure and its effect on microclimate can influence the saproxylic beetle distribution in forest (core and edge areas) (Vodka and Cizec 2013). Finally, human disturbance (i.e. removal of branches on the ground by local people) could explain the capture decline in 2014 in Allumiere’s trees (AL17, AL22 and AL30). Strong human impact due to forest management, by removing all branches and almost all the whole trunk, affected the huge lying tree MC24 in M. Cimino, a prime candidate to become a “key tree”. These changes, in combination with clear-cut in the surrounding forest edge, which reduced the canopy cover (therefore increasing local temperature), affected seriously the occurrence of the species.

## **6. Conclusions with implication for conservation and forest management**

The sampling protocol performed in our study allowed to gather qualitative and quantitative data on a poorly studied species. *M. asper* showed an unexpected longevity in natural conditions, therefore a very high survival

probability. Some overwintered males were observed in fighting still showing good conditions, and some males were found in mating in both years, meaning at least two reproductive seasons. However, even if the species could have advantages by its long lifespan, the sedentary nature combined with the flightless condition, represents the ecological limit of *M. asper* for colonizing new trees, making the species seriously threatened by woodland fragmentation. The local fragmentation could have also an effect on temperature, which can increase in clear-cut areas, limiting the adult daily activity and having effect on survey results. We suggest to survey *M. asper* taking into account the optimal temperature range of the local site, like observed in our study, and to use a capture-mark-recapture method to have real evidence of new adult generations. Retaining lying trees and maintaining coarse woody debris, such as major and minor branches on the ground, could be the correct strategy to preserve *M. asper*. A special attention must be given to “key trees” that must not be removed because they ensure population viability, acting like a “source” for facilitating colonization. Our results suggest that key trees are preferred spots for mating and egg-laying, representing a need for a *Morimus* population; therefore, the population viability would be seriously affected by the removal of these trees. Furthermore, key trees should be used as important indicators of the growth population trend in a monitoring protocol. Finally, the mitigation of the forest management impact, not only in the interior part of a forest but also in the forest edge, trying to preserve their original microhabitat conditions (e.g. canopy closure and temperature), will improve the dispersal ability and colonization success of the species.

## Acknowledgements

We are grateful to Dr. Tommaso Jucker, for his suggestions in data analysis. We thank our colleagues, Federico Romiti, Massimiliano Tini and Agnese Zauli, for their support. Special thanks goes to Maria Piscitelli, Simone Viglietta, Roberto Passacantilli and Guido Prola, for their great help on field during the sampling period. Finally, we thank the park rangers of “Lago di Vico” Regional Natural Reserve and Antonio Pasquini, chief of the management authority of the Allumiere forest, for their facilitations in the study areas.

## References

- Albert, J., Platek, M., Cizek, L., 2012. Vertical stratification and microhabitat selection by the Great Capricorn Beetle (*Cerambyx cerdo*) (Coleoptera: Cerambycidae) in open-grown, veteran oaks. *Eur. J. Entomol.* 109, 553–559.
- Alessandrini, A., Blasi, S., Di Filippo, A., Piovesan, G., Ziaco, E., 2008. Alla scoperta di una foresta vetusta, la Faggeta di Soriano nel Cimino- il Sentiero Natura. Edizioni Sette Città, Viterbo.
- Allison, J. D., Borden, J. H., Seybold, S. J. 2004. A review of the chemical ecology of the Cerambycidae (Coleoptera). *Chemoecology* 14:123–150.
- Amstrup, S.C., McDonald, T.L., Manly, B.F.J., 2005. Handbook of capture-recapture analysis. Princeton University Press, Princeton.
- Baur, B., Corey, A., Minoretti, N., Zschokke, S., 2005. Dispersal of the endangered flightless beetle *Dorcadion fuliginator* (Coleoptera: Cerambycidae) in spatially realistic landscapes. *Biol. Conserv.* 124, 49–61. DOI:10.1016/j.biocon.2004.12.011.
- Bazzocchi, G.G., Lanzoni, A., Accinelli, G., Burgio, G., 2004. Overwintering, phenology and fecundity of *Harmonia axyridis* in comparison with native coccinellid species in Italy. *Bio. Control.* 49, 245–260.
- Bouget, C., Brustel, H., Brin, A., Valladares, L., 2009. Evaluation of window flight traps for effectiveness at monitoring dead wood-associated beetles: the effect of ethanol lure under contrasting environmental conditions. *Agric. Forest Entomol.* 11, 143–152. DOI:10.1111/j.1461-9563.2008.00400.x.
- Buse, J., Schröder, B., Assmann, T., 2007. Modelling habitat and spatial distribution of an endangered longhorn beetle – A case study for saproxylic insect conservation. *Biol. Conserv.* 137: 372–381. DOI:10.1016/j.biocon.2007.02.025.
- Buse, J., Ranius, T., Assmann, B., 2008. An endangered longhorn beetle associated with old oaks and its possible role as an ecosystem engineer. *Conserv. Biol.* 22, 329–337. DOI:10.1111/j.1523-1739.2007.00880.x
- Bozić, N., Ivanović, J., Nenadović V., Bergström J., Larsson, T., Vujčić, Z., 2008. Purification and properties of major midgut leucyl aminopeptidase of *Morimus funereus* (Coleoptera, Cerambycidae) larvae. *Comp. Biochem. Phys. B-Biochem. & Mol. Bio.* 149, 454–462.

- Campanaro, A., Bardiani, M., Spada, L., Carnevali, L., Montalto, F., Antonini, G., Mason, F., Audisio, P., 2011. Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxylca/ Guidelines for monitoring and conservation of saproxylc insects. Quaderni Conservazione Habitat, 6. Cierre Grafica, Verona.
- Chiari, S., Bardiani, M., Zauli, A., Hardersen, S., Mason, F., Spada, L., Campanaro, A., 2013. Monitoring of the saproxylc beetle *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) with freshly cut log piles. J. Insect Conserv. 17: 1255-65. DOI:10.1007/s10841-013-9606-4.
- Cocciufa, C., Gerth, W., Luiselli, L., Redolfi De Zan, L., Cerretti, P., Carpaneto, G.M., 2014. Survey of saproxylc beetle assemblages at different forest plots in central Italy. Bull. Insectology 67: 295-306.
- Cooch E G. & G.C. White 2015. Program MARK: a gentle introduction (14<sup>th</sup> edition). Available online in PDF format at: <http://www.phidot.org/software/mark/docs/book/>
- Dajoz, R., 1976. Etude morphologique et biometrique des *Morimus* (Col., Cerambycidae) de la faune europeenne. Entomologiste 32, 212-231.
- Danilevsky, M.L., 2013. A check-list of Longicorn Beetles (Coleoptera, Cerambycoidea) of Europe. 41 pp. <http://www.cerambycidae.net/europe.pdf>.
- Drag, L., Hauck, D., Pokluda, P., Zimmermann, K., Cizek, L., 2011. Demography and Dispersal Ability of a Threatened Saproxylc Beetle: A Mark-Recapture Study of the Rosalia Longicorn (*Rosalia alpina*). PLoS ONE 6, e21345. DOI:10.1371/journal.pone.0021345.
- Dojnov, B., Vujčić, Z., Božić, N., Margetić, A., Vujčić, Z.M., Nenadović, V., Ivanović, J., 2012. Adaptations to captive breeding of the longhorn beetle *Morimus funereus* (Coleoptera: Cerambycidae); application on amylase study. J. Insect Conserv. 16, 239–247.
- Fauna Europaea, 2012. Fauna Europaea version 2.5. Web Service <http://www.faunaeur.org>, (accessed August 3<sup>rd</sup> 2015).
- Ghidini, G.M., Pavan, M., 1937. Appunti sulla larva di *Morimus asper* Sulz. Boll. Zoologia, VIII, 91-100.
- Greenberg, S.M., Jones, G.D., Eischen, F., Coleman, R.J., Adamczyk, J.J., Liu, T.X., Setamou, M., 2007. Survival of boll weevil (Coleoptera: Curculionidae) adults after feeding on pollens from various sources. Insect Sci. 14, 503-510. DOI:10.1111/j.1744-7917.2007.00179.x.
- Hanks, L.M., 1999. Influence of the larval host plant on reproductive strategies of cerambycid beetles. Annu. Rev. Entomol. 44, 483–505.

- Keena, M.A., 2002. *Anoplophora glabripennis* (Coleoptera: Cerambycidae) Fecundity and Longevity Under Laboratory Conditions: Comparison of Populations from New York and Illinois on *Acer saccharum*. *Popul. Ecol.*, 31:490-498.
- Koren, T., 2010. Longhorn beetles (Coleoptera: Cerambycidae) in Central Istria. *Entomol. Croat.* 14, 77-84.
- Koutroumpa, F.A., Vincent, B., Roux-Morabito, G., Martin, C., Lieutier, F., 2008. Fecundity and larval development of *Monochamus galloprovincialis* (Coleoptera Cerambycidae) in experimental breeding. *Ann. For. Sci.* 65, 1. DOI:10.1051/forest: 2008056.
- Larsson, M.C, Svensson, G.P., 2011. Monitoring spatiotemporal variation in abundance and dispersal by a pheromone-kairomone system in the threatened saproxylic beetles *Osmoderma eremita* and *Elatер ferrugineus*. *J. Insect Conserv.* 15, 891–902. DOI:10.1007/s10841-011-9388-5.
- Lebreton, J., Burnham, K.P., Clobert, J., Anderson, D.R., 1992. Modelling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecol. Monogr.* 62, 67–118.
- Mason, F., Roversi, P.F., Audisio, P., Bologna, M.A., Carpaneto, G.M., Antonini, G., Mancini, E., Sabbatini Peverieri, G., Mosconi, F., Solano, E., Maurizi, E., Maura, M., Chiari, S., Sabatelli, S., Bardiani, M., Toni, I., Redolfi De Zan, L., Rossi de Gasperis, S., Tini, M., Cini, A., Zauli, A., Nigro, G., Bottacci, A., Hardersen, S., Campanaro, A., 2015. Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). *Fragmenta Entomol.* 47, 51-52.
- Pavan, M., 1947. Significato anatomico e funzionale delle varie porzioni dell'apparato copulatore in *Morimus asper* Sulz. e *Lamia textor* L. (Coleoptera Cerambycidae). *Bull. Zool. Agraria Bachicolt.* XIV, 1-27.
- Pavan, M., 1948. Sulla durata del ciclo biológico di *Morimus asper* Sulz. (Col. Cerambycidae). *Ital. J. Zool.* 15, 33-36.
- Pechacek, P., Kristin, A., 2004. Comparative diets of adult and young three-toed woodpeckers in a European alpine forest community. *J. Wildlife Manage.* 68, 683-693.
- Polak, S., 2010. Phenology and mating behavior of *Morimus funereus* (Coleoptera, Cerambycidae), in: Abstracts of the 6th European symposium and workshop on the conservation of saproxylic beetles (June 15-17, 2010, Ljubljana), p.19.

- R Development Core Team 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Romero-Samper, J., Bahillo, P., 1993. Algunas observaciones sobre la distribución y biología de *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) en la Península Ibérica. Boln. Asoc. Esp. Ent. 17, 103-122.
- Ranius, T., Jansson, N., 2000. The influence of forest regrowth, original canopy cover and tree size on saproxylic beetles associated with old oaks. Biol. Conserv. 95, 85-94.
- Redolfi De Zan, L., Bellotti, F., D'Amato, D., Carpaneto, G.M., 2014. Saproxylic beetles in three relict beech forests of central Italy: analysis of environmental parameters and implications for forest management. For. Ecol. Manag. 328: 229-244. DOI:10.1016/j.foreco.2014.05.040
- Sama, G., 1988. Coleoptera, Cerambycidae. Catalogo Topografico e Sinonimico. Fauna d'Italia. XXXVI. Ed. Calderini, Bologna.
- Sama, G., 2004. Fauna Europaea: Cerambycidae. In: Audisio P. (ed.), Fauna Europaea: Coleoptera 2, Beetles. Fauna Europaea version 1.0. Available at <http://www.faunaeur.org> (accessed August 3<sup>rd</sup> 2015).
- Sama, G., Rapuzzi, P., 2011. Una nuova Checklist dei Cerambycidae d'Italia (Insecta Coleoptera Cerambycidae). Quad. Studi e Notizie di Storia Nat. Romagna 32, 121-164.
- Samways, M.J., 2005. Insect Diversity Conservation. Cambridge University Press, Cambridge, UK.
- Sileshi, G., Kenis, M., 2001. Survival, longevity and fecundity of overwintering *Mesoplatys ochroptera* Stål (Coleoptera: Chrysomelidae) defoliating *Sesbania sesban* (Leguminosae) and implications for its management in southern Africa. Agric. Forest Entomol. 3, 175-181.
- Solano, E., Mancini, E., Ciucci, P., Mason, F., Audisio, P., Antonini, G., 2013. The EU protected taxon *Morimus funereus* Mulsant, 1862 (Coleoptera: Cerambycidae) and its western Palaearctic allies: Systematics and conservation outcomes. Conserv. Genet. 14, 683–694. DOI:10.1007/s10592-013-0461-3.
- Spasic, S., Kestic, S., Stojadinovic, G., Petkovic, B., Todorovic, D., 2015. Effects of the static and ELF magnetic fields on the neuronal population activity in *Morimus funereus* (Coleoptera, Cerambycidae) antennal lobe revealed by wavelet analysis. Comp. Biochem. Phys. A-Mol. Int. Phys. 181, 27-35.
- Speight, M.C.D., 1989. Saproxylic invertebrates and their conservation. Council of Europe, Publications and Documents Division, Strasbourg.

- Stanić, V., Ivanović, J., Janković-Hladni, M., Nenadović, V., Marović, R. 1986. Feeding habits, behavior, oviposition and longevity of the adult cerambycid beetle *Morimus asper funereus* Muls. (Col., Cerambycidae) under laboratory condition. Acta Ent. Jugosl. 21, 87-94.
- Stokland, J.N., Siitonen, J., Jonsson, B.G., 2012. Biodiversity in Dead Wood. Cambridge University Press, Cambridge, UK.
- Tikkamäki, T., Komonen, A. 2011. Estimating population characteristics of two saproxylic beetles: a mark-recapture approach. J. Insect Conserv. 15, 401–408. DOI:10.1007/s10841-010-9313-3.
- Trizzino, M., Audisio, P., Bisi, F., Bottacci, A., Campanaro, A., Carpaneto, G.M., Chiari, S., Hardersen, S., Mason, F., Nardi, G., Preatoni, D.G., Vigna Taglianti, A., Zauli, A., Zilli, A., Cerretti, P., 2013. Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat, 7. CFS-CNBFVR, Centro Nazionale Biodiversità Forestale. Cierre Grafica, Sommacampagna, Verona.
- Virkkala, R., 2006. Why study woodpeckers? The significance of woodpeckers in forest ecosystems. Ann. Zool. Fenn. 43, 82-85.
- Vodka, Š., Cizec, L., 2013. The effects of edge-interior and understorey-canopy gradients on the distribution of saproxylic beetles in a temperate lowland forest. For. Ecol. Manag. 304, 33–41. DOI:10.1016/j.foreco.2013.04.007
- Vrezec, A., Ambrožič, Š., Kapla, A., 2010. Biology and ecology of flightless cerambycid *Morimus funereus* (Mulsant, 1862) as a background for monitoring application: laboratory and large-scale field study, in: Abstracts of the 6th European symposium and workshop on the conservation of saproxylic beetles (June 15-17, 2010, Ljubljana), p.20.
- Vrezec, A., Ambrožič, Š., Kapla, A., 2012. An overview of sampling methods tests for monitoring schemes of saproxylic beetles in the scope of Natura 2000 in Slovenia. In: Maja J. (ed.), Saproxylic beetles in Europe: monitoring, biology and conservation. Studia Forestalia Slovenica 137, 73–90.
- White, G.C., Burnham, K.P., 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46, 120–139.
- Zauli, A., Chiari, S., Hedenström, E., Svensson, G.P., Carpaneto, G.M., 2014. Using odour traps for population monitoring and dispersal analysis of the threatened saproxylic beetles *Osmoderma eremita* and *Elater ferrugineus* in central Italy. J. Insect Conserv. 18, 801–813. DOI:10.1007/s10841-014-9687-8.

**Supplementary Materials**

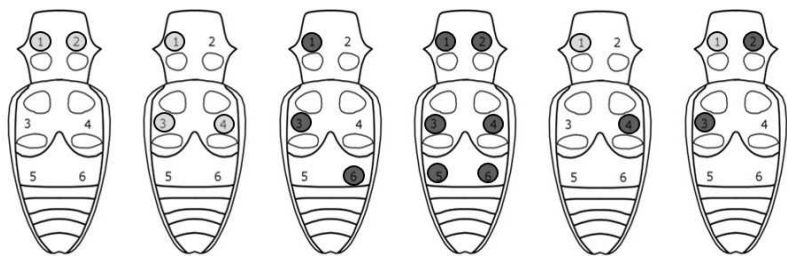


Figure A1. The marking method used for *Morimus asper*: Each specimen was marked by a unique combination of different positioned and colored spots on ventral sclerites of pronotum and abdomen of the individual, as suggested in Campanaro et al. (2011). Some examples are given in the figure where numbers represent color spots position and empty spaces represent the insertion of the insect's legs.



Table A1. Distances covered by individuals in each study site. ID Adult shows the individual code and sex (M = male and F = female), Capture date before movement and capture date after movement indicates the two consecutive captures during which the movement (Distance covered) was recorded.

| ID    |      |             |                          | Capture         |                  |
|-------|------|-------------|--------------------------|-----------------|------------------|
| Adult | Year | Study site  | Capture Date before mov. | Date after mov. | Distance covered |
| M 123 | 2013 | Allumiere   | 13 July                  | 23 July         | 50 m             |
| M 30  | 2014 | Allumiere   | 27 May                   | 26 June         | 28 m             |
| M 61  | 2014 | Allumiere   | 30 June                  | 4 July          | 47 m             |
| M 26  | 2013 | M. Cimino   | 11 May                   | 5 July          | 451 m            |
| M 51  | 2013 | M. Cimino   | 21 May                   | 30 July         | 49 m             |
| M 71  | 2013 | M. Cimino   | 5 June                   | 25 June         | 110 m            |
| M 97  | 2013 | M. Cimino   | 20 June                  | 25 July         | 20 m             |
| M 152 | 2013 | M. Cimino   | 30 June                  | 15 July         | 49 m             |
| M 174 | 2013 | M. Cimino   | 5 July                   | 10 July         | 20 m             |
| F 76  | 2014 | M. Cimino   | 3 July                   | 19 July         | 404 m            |
| F 42  | 2014 | M. Fogliano | 7 June                   | 3 July          | 338 m            |
| M 67  | 2014 | M. Fogliano | 28 June                  | 3 July          | 220 m            |
| M 68  | 2014 | M. Fogliano | 28 June                  | 3 July          | 79 m             |

Table A2. Observed lifespan of the overwintering adults of *Morimus asper* in each study site. For each adult is reported: the individual code (Id) bearing sex (M = male, F = female) and progressive number of marking procedure; date of first capture in 2013 and date of the last capture in 2014; total number of recaptures (N. Recapt.) in 2013 and in 2014; number of mating events (ME) in 2013 and 2014 and observed days of lifespan calculated as the number of days between the first capture in 2013 and last capture in 2014.

| Id.                | FC.<br>2013 | LC<br>2014 | N. R.<br>2013 | N. R.<br>2014 | ME<br>2013 | ME<br>2014 | Lifespan<br>(days) |
|--------------------|-------------|------------|---------------|---------------|------------|------------|--------------------|
| <b>Allumiere</b>   |             |            |               |               |            |            |                    |
| M31                | 3 June      | 4 July     | 3             | 5             | 1          | 0          | 396                |
| M123               | 13 July     | 19 May     | 1             | 1             | 1          | 0          | 310                |
| M141               | 28 July     | 6 May      | 0             | 1             | 0          | 0          | 282                |
| M122               | 13 July     | 6 May      | 0             | 0             | 0          | 0          | 297                |
| M49                | 8 June      | 27 May     | 0             | 2             | 0          | 0          | 353                |
| M43                | 8 June      | 27 May     | 3             | 0             | 2          | 0          | 353                |
| <b>M. Cimino</b>   |             |            |               |               |            |            |                    |
| M98                | 20 June     | 9 May      | 0             | 0             | 0          | 0          | 323                |
| M71                | 5 June      | 19 July    | 2             | 3             | 1          | 1          | 409                |
| M78                | 10 June     | 9 May      | 0             | 0             | 0          | 0          | 333                |
| M195               | 10 July     | 19 July    | 1             | 6             | 0          | 0          | 374                |
| M243               | 25 July     | 9 May      | 0             | 0             | 0          | 0          | 288                |
| M171               | 30 June     | 28 May     | 2             | 1             | 0          | 0          | 332                |
| M148               | 25 June     | 9 May      | 3             | 0             | 0          | 0          | 318                |
| M194               | 10 July     | 25 July    | 0             | 5             | 0          | 1          | 380                |
| M174               | 5 July      | 28 May     | 1             | 0             | 1          | 0          | 327                |
| M180               | 5 July      | 2 June     | 0             | 0             | 0          | 0          | 358                |
| M100               | 20 June     | 3 July     | 1             | 2             | 1          | 1          | 378                |
| M245               | 25 July     | 12 July    | 0             | 4             | 1          | 1          | 352                |
| M95                | 20 June     | 25 July    | 0             | 4             | 0          | 1          | 400                |
| M57                | 31 May      | 7 June     | 1             | 0             | 0          | 0          | 372                |
| M184               | 5 July      | 25 July    | 0             | 2             | 0          | 0          | 385                |
| M153               | 25 June     | 19 July    | 2             | 4             | 0          | 0          | 389                |
| M64                | 5 June      | 20 June    | 1             | 0             | 1          | 0          | 380                |
| M232               | 15 July     | 19 July    | 0             | 1             | 0          | 0          | 369                |
| F244               | 25 July     | 12 July    | 0             | 0             | 0          | 0          | 352                |
| F222               | 15 July     | 12 July    | 1             | 0             | 2          | 0          | 362                |
| <b>M. Fogliano</b> |             |            |               |               |            |            |                    |
| M42                | 14 July     | 19 July    | 0             | 4             | 1          | 0          | 370                |
| M23                | 19 June     | 7 July     | 1             | 4             | 0          | 2          | 383                |

Table A3. Best POPAN Jolly-Seber population models selected by AICc of two sampling years for each study site. For each model are reported the AICc value and AICc Weights, mean and standard deviation of estimates for primary parameters:  $\phi$  = apparent survival probability,  $p$  = recapture probability,  $pent$  = probability of entering the population and  $N$  = population size. For parameters depending on time ( $t$ ) we reported the lower - upper mean value at 95% C.I. of the reliable values given in Mark 8.0.

| Sites   | Best Model                 | AICc  | AICc W | $\phi$          | $p$             | $pent$        | $N$          |
|---------|----------------------------|-------|--------|-----------------|-----------------|---------------|--------------|
| AL 2013 | $\{\phi(.) p(t) pent(t)\}$ | 608.5 | 0.95   | $0.86 \pm 0.03$ | $0.07 - 0.27$   | $0.07 - 0.42$ | $378 \pm 46$ |
| AL 2014 | $\{\phi(.) p(.) pent(t)\}$ | 329.1 | 1      | $0.75 \pm 0.04$ | $0.17 \pm 0.03$ | $0.06 - 0.39$ | $182 \pm 27$ |
| MC 2013 | $\{\phi(.) p(t) pent(t)\}$ | 934.8 | 0.81   | $0.92 \pm 0.02$ | $0.08 - 0.19$   | $0.06 - 0.66$ | $587 \pm 53$ |
| MC 2014 | $\{\phi(.) p(t) pent(t)\}$ | 440.8 | 0.95   | $0.88 \pm 0.04$ | $0.07 - 0.32$   | $0.06 - 0.73$ | $164 \pm 29$ |
| MF 2013 | $\{\phi(.) p(.) pent(t)\}$ | 178.1 | 0.99   | $0.58 \pm 0.08$ | $0.20 \pm 0.07$ | $0.06 - 0.33$ | $142 \pm 38$ |
| MF 2014 | $\{\phi(.) p(t) pent(t)\}$ | 349.1 | 0.98   | $0.74 \pm 0.05$ | $0.07 - 0.47$   | $0.06 - 0.59$ | $223 \pm 42$ |

Table A4. Constrained Linear Model (CLM) evaluating Temperature effect on recapture probability. Results of CLM showed two best population models selected by AICc in 2013 and 2014 in each study site with recapture probability ( $p$ ) dependent on temperature ( $T$ ). For each model are reported the AICc scores, model likelihood (M. Likelihood) and the number of parameters estimated (Num. Par) given in Mark 8.0.

| Site    | Model                        | AICc   | Delta AICc | AICc Weights | ML   | Num. Par |
|---------|------------------------------|--------|------------|--------------|------|----------|
| AL 2013 | $\{\phi(.) p(t+T) pent(t)\}$ | 605.92 | 0          | 0.78         | 1    | 26       |
| AL 2013 | $\{\phi(.) p(t) pent(t)\}$   | 608.51 | 2.59       | 0.22         | 0.27 | 27       |
| AL 2014 | $\{\phi(.) p(T) pent(t)\}$   | 326.55 | 0          | 0.79         | 1    | 10       |
| AL 2014 | $\{\phi(.) p(.) pent(t)\}$   | 329.14 | 2.59       | 0.22         | 0.27 | 10       |
| MC 2013 | $\{\phi(.) p(t) pent(t)\}$   | 934.87 | 0          | 0.65         | 1    | 25       |
| MC 2013 | $\{\phi(.) p(t+T) pent(t)\}$ | 937.19 | 2.33       | 0.20         | 0.31 | 26       |
| MC 2014 | $\{\phi(.) p(T) pent(t)\}$   | 436.17 | 0          | 0.83         | 1    | 7        |
| MC 2014 | $\{\phi(.) p(t) pent(t)\}$   | 440.80 | 4.64       | 0.08         | 0.10 | 19       |
| MF 2013 | $\{\phi(.) p(.) pent(t)\}$   | 178.14 | 0          | 0.83         | 1    | 12       |
| MF 2013 | $\{\phi(.) p(T) pent(t)\}$   | 181.31 | 3.17       | 0.17         | 0.21 | 13       |
| MF 2014 | $\{\phi(.) p(T) pent(t)\}$   | 346.43 | 0          | 0.65         | 1    | 10       |
| MF 2014 | $\{\phi(.) p(t) pent(t)\}$   | 349.06 | 2.63       | 0.17         | 0.27 | 21       |

## CHAPTER 3

### **Sexual dimorphism and intra-specific allometric relationships between secondary sexual character and body size in *Morimus asper/funereus* (Coleoptera: Cerambycidae)**

Sarah Rossi de Gasperis<sup>1</sup>, Lara Redolfi De Zan<sup>2-3</sup>, Federico Romiti<sup>1</sup>, Sönke Hardersen<sup>3</sup>, Giuseppe Maria Carpaneto<sup>1</sup>

<sup>1</sup> Dipartimento di Scienze, Università Roma Tre, Viale Guglielmo Marconi 446, 00146 Roma, Italy.

<sup>2</sup> CREA - ABP Consiglio per la Ricerca in Agricoltura e Analisi dell'Economia Agraria, Via di Lanciola 12/a, Cascine del Riccio, 50125, Firenze (FI), Italy.

<sup>3</sup> Corpo Forestale dello Stato, Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale 'Bosco Fontana', Strada Mantova 29, 46045 Marmirolo (MN), Italy.

**Short title:** *Morimus asper*: sexual dimorphism and allometry

**Corresponding author:** Sarah Rossi de Gasperis, Dipartimento di Scienze, Università Roma Tre, Viale Guglielmo Marconi 446, 00146 Roma, Italy. E-mail: sarahrodega@gmail.com, mobile phone +39 3291631385

## Abstract

The longhorn beetle *M. asper* exhibits the typical sexual dimorphism of cerambycid beetles: males have longer antennae than females. The antennal length seems to vary greatly among males of the same population. This study aimed to explore the sexual dimorphism in *M. asper* and the scaling relationship between antennal length and body size in both sexes in order to assess the allometric pattern of the traits studied and their potential effects on individual eco-ethological features. This study revealed sexual dimorphism also in other morphological traits: males showed a longer pronotum and females larger and longer elytra, suggesting that in males more resources are allocated in developing the anterior body traits, whereas in females more resources are invested to the development of post-prothoracic body parts, which contain the reproductive apparatus. In males, the allometric relationship between antennal length and elytra length resulted to be best described by a segmented regression, which identified a switch point dividing the population in two different groups. Males with elytra length below the switch point showed a positive allometry, while in larger males, the scaling relationship between the two morphological traits tends to isometry. In females, a sigmoidal model better fitted the data, and described an allometric pattern similar to that of males, with a decreasing slope only for the largest females. Morphometric traits are correlated to behavior and ecology and we found that the largest males, above the switch point, were more frequently observed in mating when compared to those smaller than the switch point.

**Key words:** antennae; segmented regression; longhorn beetle; body size

## Introduction

*Morimus asper* (Sulzer 1776) is a flightless saproxylic longhorn beetle, mainly associated with mature hardwood forests. The taxonomic and conservation status of this species complex is still object of discussion. A recent study (Solano et al. 2013) highlighted a genetic variability among the Euro-Anatolian populations of the genus *Morimus* which cannot be easily assigned to the currently accepted five species, and may actually represent a single, genetically and morphologically variable species (*M. asper*). Therefore, due to the high genetic relatedness between *M. asper* and the protected taxon *M. funereus* (Mulsant 1862), which is included in the Annex II of European Habitat Directive 92/43 CEE (HD), the conservation status of *M. asper* sensu lato should be revised.

In males of *M. asper* the antennae are at least two times longer than the body length, while in females they slightly exceed the length of the body (Sama 1988). This sexual dimorphism is present in almost all longhorn beetles where the antennae are longer in males than females (Hanks et al. 1996 a,b; Saikia et al. 2012). The fact that male *M. asper* have antenna of exaggerated length suggests that the evolution of this trait is driven by sexual selection and promoted by intrasexual competition, or mating preferences in the opposite sex (Darwin 1874; Linsley 1959; Johnstone 1995). The extreme development of such structures in the male may be very costly for the bearer (Møller and Zamora Muñoz 1997). The scaling relationship between body size and secondary sexual structures (e.g. antennal length) can vary intraspecifically, from small, relatively normally proportioned, to very large individuals with grossly enlarged structures (Emlen and Nijhout 2000).

Allometry which explores the relationship between the size variations of an organ in respect to the body size in adult individuals within a species (intraspecific) or among related species (interspecific) are usually defined as *static allometry* (Huxley 1932; Gould 1966; Levinton 1988; Emlen and Nijhout 2000; Knell et al. 2004). A *positive allometry* occurs when the slope of the scaling relationship (i.e., allometric coefficient) between the studied trait and body size is greater than 1 (Green 1992), as is typical for sexually selected traits.

Commonly, the study of allometry is conducted by searching for the model which best fits the scaling relationship of the two variables (generally log transformed). Often, the allometric growth trajectory is described by a simple linear relationship, however in many holometabolous insects, the best model deviates from simple linearity and follows a curvilinear and/or

discontinuous model, defining a non-linear or discontinuous allometric relationships (Knell 2009; Hardersen et al. 2011; Painting and Holwell 2013). The growth of the imaginal disks, from which appendages develop, like head structures and a portion of the thorax, undergo different speeds of growth, competing for the same resources, mainly during prepupal and pupal stage, in an essentially closed system (Williams 1980). These brief and asynchronous developments of imaginal structures in respect to imaginal body size may result in non-linear or discontinuous allometries (Nijhout and Wheeler 1996; Knell 2009).

In discontinuous allometries data points can be divided into more than one distinct group, i.e. morph, identifying a switch point (usually a threshold in body size) at which individuals switch from one morph to another (Knell 2009). Different approaches have been used to investigate this kind of allometric relationships between secondary sexual characters and body size (Eberhard and Gutierrez 1991; Kotiaho and Tomkins 2001; Cook and Bean 2006; Knell 2009).

When two (or more) morphs occur within a sex, the slopes of their scaling relationships indicate how the growth of the secondary sexual traits changes in respect to body size. When the slope becomes steeper with increasing body size, larger individuals bear secondary sexual characters proportionally larger than smaller individuals (Knell et al. 2004), but in some cases, the opposite situation may be observed (Eberhard and Gutierrez 1991; Hanley 2001). The development of the individuals in each morph seems to depend on both genotype and environmental conditions (e.g. resource availability) (Eberhard and Gutierrez 1991; Nijhout and Wheeler 1996; Hardersen et al. 2011).

The phenotypical expression of different morphs within a sex, generally in males, has been observed in different beetle families like Lucanidae, Scarabaeidae, Cerambycidae (Zeh and Zeh 1992; Emlen and Nijhout 2000; Knell et al. 2004; Kawano 2006) with some examples of male trimorphism (Rowland and Emlen 2009; Iguchi 2013).

Eco-ethological aspects like fighting, mating and survival ability may differ between morphs (Goldsmith 1996; Moczek and Emlen 2000; Hanley 2001; Forslund 2003; Romiti et al. 2015).

Like in other longhorn beetles (Hanks et al. 1996b), *M. asper* males fight for females: rivals use their anterior body like a battering ram in order to reverse the opponent with the aid of pronotal spines, pushing the opponent and biting mainly the appendages (pers. obs.). Winning males benefit from more mating opportunities (Hughes and Hughes 1982) and increase their fitness.

As stated above, cerambycids are well known for their sexual dimorphism (e.g. Zeh and Zeh 1992; Saikia et al. 2012) and the allometry body size vs. antennal length has been investigated for some Cerambycidae (e.g. Hanks et al. 1996a; Wang and Zeng 2004). However, to our knowledge these allometries have not been explored applying non-linear or discontinuous models. The allometry of *M. asper* has not been studied and the correlation between the size of morphological traits and ecological aspects is also unknown, as generally *M. asper* has been poorly studied and little is known about its ecology in the natural context (i.e. natural habitat conditions of the species like woodlands) (Sama 1988; Romero Samper and Baillo 1993).

In this study, we aimed: i) to investigate sexual dimorphism of *M. asper* in biometric variables other than antennae; ii) to investigate the allometric relationship between antennal length and elytra length in both sexes; iii) to evaluate if morphological traits (e.g. antennal length and elytra length) may affect adult ecology (e.g. mating, movements and overwintering ability).

## Material and methods

### Study area

We collected biometric data on adult individuals of three populations of *M. asper* from three study sites located in the Lazio region (central Italy): Allumiere, Mount Fogliano and Mount Cimino. All three study areas are Sites of Community Importance (SCI) and Special Protection Zones (SPZ) defined by the Habitats Directive 92/43/CEE and the Bird Directive 79/49/CEE.

Allumiere (UTM zone 32 T, WGS84, 740236 4671138; 500-600 m a.s.l.) is located 20 km from the Mediterranean sea and is a beech forest consisting of *Fagus sylvatica* associated to *Ilex aquifolium*. M. Fogliano (UTM zone 33 T, WGS84, 263969 4690438; 800-950 m a.s.l.) and M. Cimino (UTM zone 33 T, WGS84, 269751 4698603; 950-1053 m a.s.l.) are located in the inner part of Lazio region, both are beech forests, the first one with some *Q. cerris* interspersed.

### Sampling of *Morimus asper*

Thirty trees, evaluated *a priori* as suitable breeding habitats for the target species (e.g. living or dead trees with emergence holes of longhorn beetles),



were selected in each site. Trees were visually inspected approximately every five days, avoiding rainy days, in 2013 and 2014, when adults were active (May - July). In order to recognize each individual, adults were marked by using a unique combination of permanent colored spots (i.e. identification code) on the ventral sclerites of the pronotum and abdomen (Campanaro et al. 2011) with a non-toxic CFG Paint Marker. At first capture, each individual was measured, sexed and marked. The individual identification code of both partners, observed in mating, was recorded. Eight morphological traits (Fig. 1) were measured for individuals of both sexes. After marking and measuring, each individual was released at the same tree where it had been found. Four morphological traits were measured during the 2013 survey; four extra measurements were added in 2014. The same operator (S. Rossi de Gasperis) made all measurements with a digital caliper (precision 0.05 mm) as described in Table I.

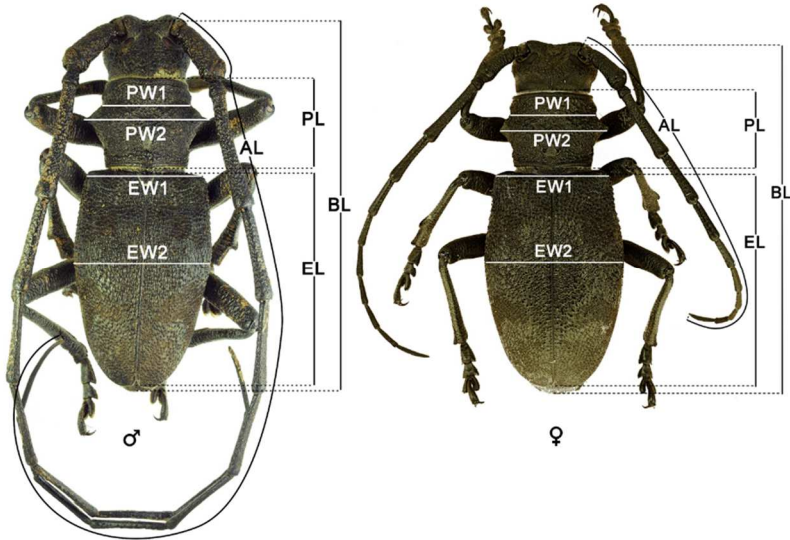


Figure 1 Morphological traits measured on males and females of *Morimus asper*. All abbreviations were reported in Table I. (pictures by Federico Romiti).

Table I. List of measurements collected on *Morimus asper* males and females for the allometric analysis. (\*) Extra measurements added in 2014.

| Measurement | Description  |
|-------------|--|
| BL*         | Body Length: distance from the front of the head to pygidium. This was measured when adults assumed the complete closed posture. |
| PL*         | Pronotum Length: length of the vertical midline of the pronotum.   |
| EL          | Elytra Length: distance from the base of the scutellum to the apex of the elytra, along the suture line.                         |
| AL          | Antennal Length: distance from the insertion of the scape to the last antennal segment.  |
| PW1         | Pronotum Width 1: distance between the insertions of the spines.   |
| PW2         | Pronotum Width 2: distance between the apexes of the spines.   |
| EW1*        | Elytra Width 1: distance measured at the base of the elytra.   |
| EW2*        | Elytra Width 2: maximum width of elytra.   |

## Data analysis

### Sexual dimorphism and allometric analysis

Sexual dimorphism was investigated by testing significant differences for all morphometric traits of the total dataset with the One-way Anova test (Table II).

Allometric relationships were investigated in both sexes using the biometric variables measured in both years: i.e. the four variables: PW1, PW2, EL and AL.

One Way Anova test was used to highlight differences in EL among populations.

Redundancy analysis (RDA) with forward selection (“forward.sel” function in R “packfor” package) was computed on the total dataset for each sex, to

highlight the correlation between AL (response variable) and PW1, PW2 and EL (explanatory variables) and to select the best correlated variable by a permutation test (n. perm. 999), on the basis of highest adjusted  $r^2$  explained, (Borcard et al., 2011). The best explanatory variable was then used in the allometric analysis.

Before proceeding with the allometric analyses, we tested for both sexes if EL data of the two years were significantly different (One Way Anova) in the three populations and among them.

Allometric analyses for males and females were conducted separately for each of the three populations and subsequently for the pooled dataset. Following the suggestions of Knell (2009), the scaling relationship of AL and EL was checked for discontinuity. As no clear discontinuity was found the allometry was investigated by fitting four models: linear regression, segmented regression (“segmented” function in R “segmented” package), three parameters Gaussian and sigmoidal model (“nls” function in R). The Akaike Information Criterion (AIC) was used to select the best fitting model and the difference between the two top models was tested with a partial F-test (“anova” function in R).

## Effects of morphological traits on behavioral ecology

To evaluate if body size affected the observed mating frequency (i.e. number of observed matings) a One-way Anova was performed between EL of individuals observed mating and EL of individuals found unmated, for both sexes of each population.

The estimated switch point (SP) of the segmented regression (see Results) was used to discriminate males in two groups, those smaller than the switch point (g1) and those larger than the switch point (g2). The ratio of observed males for both groups (i.e. mated males g1/ total males of g1 and mated males g2/ total males g2) was calculated for each population, to compare their observed mating frequencies with a Kruskal-Wallis test.

The male/female EL ratio was calculated for all observed matings, in order to investigate the size ratio (and its range). The number of males belonging to g1 and g2 observed within the male/female EL ratio, was reported to make a comparison.

To investigate if the size of the exaggerated male character, AL, was correlated to dispersal, two scatterplots were created: AL vs. maximum observed distance travelled and AL vs. mean daily distance recorded. Dispersal was recorded as the distance covered between two consecutive

captures. The mean daily distance was calculated by dividing the maximum distance observed by the number of days separating the two sightings, which define the maximum distance.

Finally, for each population, the mean EL of the males, which were re-sighted after the wintering period, was compared with the mean EL of all males. This analysis was not carried out for females, as only two females were re-sighted after winter.

RDA and allometric analysis were conducted in R 3.0.3 (R Development Core Team, 2010), One-Way Anova test and K-Wallis test were performed with Past 3 (Hammer et al. 2001); the  $\alpha$  set for all the analysis was 0.05.

## Results

### Sexual dimorphism and allometric analysis

During the sampling period 2013-2014 we gathered biometric data from a total of 461 males and 223 females (Table II).

In males the mean AL/BL rate was near 2:1 (Mean  $\pm$  S.D. of AL/BL =  $2.09 \pm 0.22$ ), while AL resulted slightly longer than EL in females (Mean  $\pm$  S.D. of AL/BL =  $1.16 \pm 0.09$ ). Males had a longer pronotum ( $F = 18.03$ , d.f. = 1,  $p < 0.0001$ ) and longer antennae ( $F = 1005$ , d.f. = 1,  $p < 0.0001$ ) than females; moreover females had longer elytra than males ( $F = 53.33$ , d.f. = 1,  $p < 0.0001$ ) and the maximum elytra width (EW2) was larger in females compared to males ( $F = 23.53$ , d.f. = 1,  $p < 0.0001$ ). The female EW1/ EW2 rate resulted significantly lower than that of males ( $F = 247.8$ , d.f. = 1,  $p < 0.0001$ ).

RDA between AL and explanatory variables (PW1, PW2, EL) showed highly significant results in males:  $r^2 = 0.928$ ,  $\text{adj.}r^2 = 0.927$ ,  $F = 2021.3$ , Perm. = 999,  $p < 0.001$ ; EL resulted to be the variable with the highest adjusted  $r^2$  ( $\text{adj.}r^2 = 0.91$ ,  $F = 4997.18$ ,  $p < 0.001$ ). We obtained similar results for females:  $r^2 = 0.893$ ,  $\text{adj.}r^2 = 0.892$ ,  $F = 612.86$ , Perm. = 999,  $p < 0.001$ , and again EL was the best explanatory variable ( $\text{adj.}r^2 = 0.86$ ,  $F = 1435.5$ ,  $p < 0.001$ ).

No significant difference was found comparing the EL between the two years for both sexes in each population (Fig. 2), therefore we used the pooled dataset 2013-2014 for males and for females of each population for further analysis (Table III). Comparing the elytra length among populations, Allumiere males resulted significantly larger than those from M. Fogliano ( $F = 12.47$ , d.f. = 1,  $p < 0.01$ ) and from M. Cimino ( $F = 7.24$ , d.f. = 1,  $p <$

0.01). In females the results were similar but only the difference between Allumiere and M. Cimino was significant ( $F = 5.92$ , d.f. = 1,  $p < 0.05$ ).

The best fitting model for males and females of the three populations and for the three populations pooled data are shown in Table IV.

The segmented model (switch point EL = 17.99 mm) in Allumiere males (Fig. 3 a) showed no significant difference from Gaussian and sigmoidal models, therefore they were considered equally valid. In contrast, the segmented model of M. Fogliano (switch point EL = 16.28 mm) was the best model, on the basis of AIC score, and showed a significant difference from the Gaussian model ( $F_{1,99} = 9.36$ ,  $p = 0.003$ ) (Fig. 3b). The same result was obtained for M. Cimino (switch point EL = 16.94 mm) (segmented vs Gaussian model:  $F_{1,213} = 6.18$ ,  $p = 0.013$ ). Also when pooling all data for males, the segmented model fitted the data best (switch point EL = 16.78 mm) (segmented vs Gaussian model:  $F_{1,455} = 9.53$ ,  $p = 0.002$ ) (Fig. 3 c,d).

The segmented model, divided males into two groups in relation to the estimated switch points: g1 (smaller then switch point) and g2 (larger then switch point). The numbers of males assigned to the respective groups resulted: g1 = 99 and g2 = 42 in Allumiere, g1 = 143 and g2 = 76 in M. Cimino, g1 = 67 and g2 = 34 in M. Fogliano.

For the females, AIC values were very similar for the different models and no differences were found from pair ways comparisons for the models of Allumiere, M. Fogliano and M. Cimino. Considering the pooled dataset for females, the sigmoidal model was significantly different from the linear model ( $F_{1,215} = 4.29$ ,  $p = 0.04$ ) but not significant difference was found with the Gaussian model. We plotted the best fitting model (i.e. sigmoidal model) only for the pooled dataset for females. (Fig. 3 e-h).

Table II. List of the morphological traits measured on males and females of *Morimus asper* and summary of their mean  $\pm$  standard deviation (SD). For each morphological traits are reported the total number of marked adult ( $N_{tot}$ ) and the total number of males ( $N_m$ ) and females ( $N_f$ ).

| Morphological trait | $N_{tot}$ | $N_m$ | $N_f$ | Mean ( $\pm$ SD) males | Mean ( $\pm$ SD) females |
|---------------------|-----------|-------|-------|------------------------|--------------------------|
| BL                  | 258       | 184   | 78    | 27.51 ( $\pm$ 4.32)    | 27.38 ( $\pm$ 3.37)      |
| PL                  | 258       | 184   | 78    | 6.48 ( $\pm$ 1.03)     | 5.93 ( $\pm$ 0.79)       |
| EL                  | 684       | 461   | 223   | 16.02 ( $\pm$ 2.35)    | 17.42 ( $\pm$ 2.27)      |
| AL                  | 684       | 461   | 223   | 57.65 ( $\pm$ 13.46)   | 31.62 ( $\pm$ 4.55)      |
| PW1                 | 684       | 461   | 223   | 7.30 ( $\pm$ 1.22)     | 7.21 ( $\pm$ 0.99)       |
| PW2                 | 684       | 461   | 223   | 9.17 ( $\pm$ 1.53)     | 9.06 ( $\pm$ 1.25)       |
| EW1                 | 258       | 184   | 78    | 8.84 ( $\pm$ 1.40)     | 8.83 ( $\pm$ 1.21)       |
| EW2                 | 258       | 184   | 78    | 9.66 ( $\pm$ 1.35)     | 10.52 ( $\pm$ 1.32)      |
| EW1/EW2             | 258       | 184   | 78    | 0.91 ( $\pm$ 0.03)     | 0.84 ( $\pm$ 0.04)       |

Figure 2. Mean and standard deviation of elytra length (EL) for males and females in both years for each population.

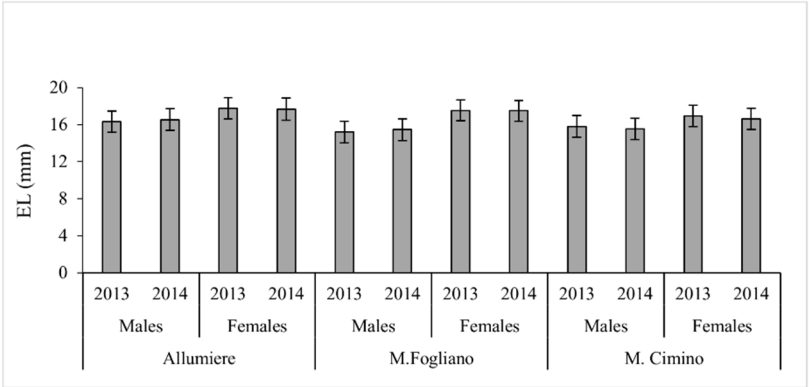


Table III. Summary of the morphological traits, with their mean  $\pm$  standard deviation (SD), used in the allometric analyses of males (a) and females (b) of each population (N = total number of individuals).

| a)                  |           |                      |             |                      |           |                      |
|---------------------|-----------|----------------------|-------------|----------------------|-----------|----------------------|
| Morphological trait | Allumiere |                      | M. Fogliano |                      | M. Cimino |                      |
|                     | N         | Mean ( $\pm$ SD)     | N           | Mean ( $\pm$ SD)     | N         | Mean ( $\pm$ SD)     |
| BL                  | 57        | 28.72 ( $\pm$ 4.05)  | 61          | 26.86 ( $\pm$ 4.91)  | 66        | 27.12 ( $\pm$ 4.67)  |
| PL                  | 57        | 6.79 ( $\pm$ 0.97)   | 61          | 6.35 ( $\pm$ 1.17)   | 66        | 6.36 ( $\pm$ 1.13)   |
| EL                  | 141       | 16.55 ( $\pm$ 2.27)  | 101         | 15.53 ( $\pm$ 2.36)  | 219       | 15.90 ( $\pm$ 2.34)  |
| AL                  | 141       | 60.43 ( $\pm$ 12.62) | 101         | 55.23 ( $\pm$ 14.01) | 219       | 56.92 ( $\pm$ 13.48) |
| PW1                 | 141       | 7.48 ( $\pm$ 1.15)   | 101         | 7.04 ( $\pm$ 1.26)   | 219       | 7.29 ( $\pm$ 1.22)   |
| PW2                 | 141       | 9.35 ( $\pm$ 1.46)   | 101         | 8.84 ( $\pm$ 1.52)   | 219       | 9.19 ( $\pm$ 1.54)   |
| EW1                 | 57        | 9.09 ( $\pm$ 1.39)   | 61          | 8.67 ( $\pm$ 1.59)   | 66        | 8.80 ( $\pm$ 1.56)   |
| EW2                 | 57        | 9.98 ( $\pm$ 1.33)   | 61          | 9.54 ( $\pm$ 1.59)   | 66        | 9.51 ( $\pm$ 1.54)   |
| b)                  |           |                      |             |                      |           |                      |
| Morphological trait | Allumiere |                      | M. Fogliano |                      | M. Cimino |                      |
|                     | N         | Mean ( $\pm$ SD)     | N           | Mean ( $\pm$ SD)     | N         | Mean ( $\pm$ SD)     |
| BL                  | 19        | 27.11 ( $\pm$ 4.47)  | 34          | 27.90 ( $\pm$ 2.42)  | 25        | 26.86 ( $\pm$ 3.55)  |
| PL                  | 19        | 6.06 ( $\pm$ 1.06)   | 34          | 6.02 ( $\pm$ 0.57)   | 25        | 5.71 ( $\pm$ 0.78)   |
| EL                  | 72        | 17.91 ( $\pm$ 2.48)  | 44          | 17.58 ( $\pm$ 1.71)  | 109       | 16.81 ( $\pm$ 2.26)  |
| AL                  | 73        | 32.06 ( $\pm$ 4.87)  | 44          | 31.65 ( $\pm$ 3.46)  | 109       | 30.5 ( $\pm$ 4.64)   |
| PW1                 | 73        | 7.25 ( $\pm$ 1.12)   | 44          | 7.37 ( $\pm$ 0.71)   | 109       | 7.13 ( $\pm$ 1.01)   |
| PW2                 | 73        | 9.18 ( $\pm$ 1.39)   | 44          | 9.07 ( $\pm$ 0.90)   | 109       | 8.91 ( $\pm$ 1.26)   |
| EW1                 | 19        | 8.69 ( $\pm$ 1.72)   | 34          | 8.99 ( $\pm$ 0.90)   | 25        | 8.71 ( $\pm$ 1.13)   |
| EW2                 | 19        | 10.36 ( $\pm$ 1.84)  | 34          | 10.70 ( $\pm$ 0.91)  | 25        | 10.40 ( $\pm$ 1.35)  |

Table IV. AIC results and  $r^2$  values (reliable for linear models) of the four model used to investigate the allometric relationship between antennal length and elytra length in both sexes for each population and for the total dataset.

| Males       |          |           |       | Females     |         |           |       |
|-------------|----------|-----------|-------|-------------|---------|-----------|-------|
| Allumiere   |          |           |       | Allumiere   |         |           |       |
| Models      | AIC      | Delta AIC | $r^2$ | Models      | AIC     | Delta AIC | $r^2$ |
| Gaussian    | -390.69  | 0.00      |       | Linear      | -194.39 | 0.00      | 0.82  |
| Sigmoidal   | -389.19  | 1.50      |       | Gaussian    | -192.46 | 1.93      |       |
| Segmented   | -389.15  | 1.54      | 0.90  | Sigmoidal   | -192.45 | 1.94      |       |
| Linear      | -369.26  | 21.43     | 0.89  | Segmented   | -190.75 | 3.64      | 0.82  |
| M. Fogliano |          |           |       | M. Fogliano |         |           |       |
| Models      | AIC      | Delta AIC | $r^2$ | Models      | AIC     | Delta AIC | $r^2$ |
| Segmented   | -229.48  | 0.00      | 0.91  | Gaussian    | -153.39 | 0.00      |       |
| Gaussian    | -226.89  | 2.59      |       | Sigmoidal   | -153.24 | 0.15      |       |
| Sigmoidal   | -226.52  | 2.96      |       | Linear      | -152.69 | 0.70      | 0.84  |
| Linear      | -219.67  | 9.81      | 0.90  | Segmented   | -152.56 | 0.83      | 0.85  |
| M. Cimino   |          |           |       | M. Cimino   |         |           |       |
| Models      | AIC      | Delta AIC | $r^2$ | Models      | AIC     | Delta AIC | $r^2$ |
| Segmented   | -547.96  | 0.00      | 0.91  | Segmented   | -316.54 | 0.00      | 0.87  |
| Gaussian    | -543.75  | 4.21      |       | Linear      | -316.07 | 0.47      | 0.86  |
| Sigmoidal   | -541.13  | 6.83      |       | Sigmoidal   | -315.10 | 1.44      |       |
| Linear      | -521.13  | 26.83     | 0.90  | Gaussian    | -315.01 | 1.53      |       |
| Pooled data |          |           |       | Pooled data |         |           |       |
| Models      | AIC      | Delta AIC | $r^2$ | Models      | AIC     | Delta AIC | $r^2$ |
| Segmented   | -1174.12 | 0.00      | 0.91  | Sigmoidal   | -653.86 | 0.00      |       |
| Gaussian    | -1166.59 | 7.53      |       | Gaussian    | -653.73 | 0.13      |       |
| Sigmoidal   | -1161.56 | 12.56     |       | Segmented   | -653.52 | 0.84      | 0.85  |
| Linear      | -1105.68 | 68.44     | 0.90  | Linear      | -651.54 | 2.32      | 0.84  |



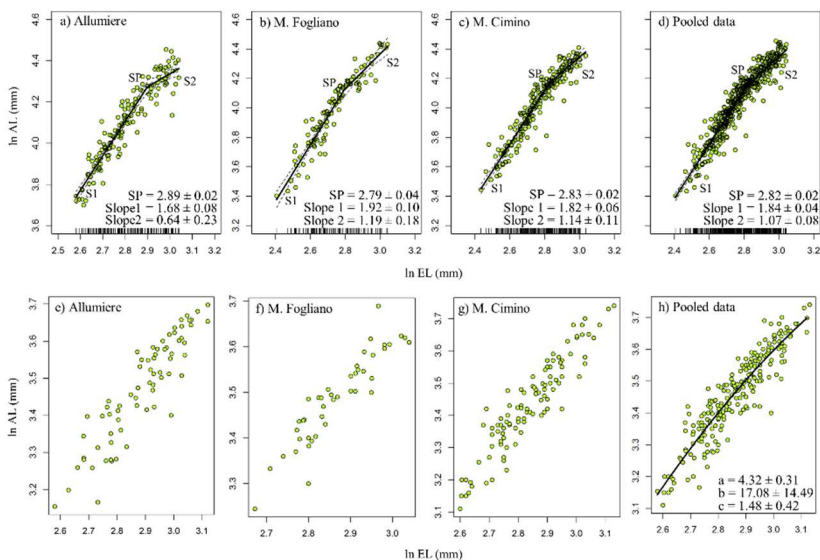


Figure 3. Scatterplots of the scaling relationship between the natural logarithm (ln) of antennal length (AL) and elytra length (EL), for males (a-d) and females (e-h) of *M. asper*, for the three populations (Allumiere:  $N_m = 141$ ,  $N_f = 70$ ; M. Fogliano:  $N_m = 101$ ,  $N_f = 43$ ; M. Cimino:  $N_m = 219$ ,  $N_f = 104$ ) and the pooled dataset (Pooled data:  $N_m = 416$ ,  $N_f = 217$ ). Estimated parameters are shown in figures: the two slopes of the segmented model, slope1 and slope 2 (S1 and S2), and switch point (SP) values (Mean and S.D.) and three parameters (a, b, and c) of the sigmoidal model in pooled female data. Solid lines represent the best model; dashed lines represent the 95% C.I. for the segmented model. The scaling range of the two axes of Allumiere males (a) differs from the others to better visualize the growth pattern.

## Effects of morphological traits on behavioural ecology

During the two years of sampling, 140 mating events were recorded: 47 in Allumiere, 20 in M. Fogliano and 73 in M. Cimino.

In M. Fogliano, the elytra length of mating males (Mean  $\pm$  S.D. = 17.05 mm  $\pm$  2.09) resulted significantly longer than in males non mating (Mean  $\pm$  S.D.

= 15.23 mm  $\pm$  2.31) ( $F = 9.06$ , d.f. = 1,  $p < 0.01$ ). The same result was found for M. Cimino (Mean  $\pm$  S.D. EL mating = 16.73 mm  $\pm$  2.25, Mean  $\pm$  S.D. EL non mating = 16.55 mm  $\pm$  2.29,  $F = 12.69$ , d.f. = 1,  $p < 0.001$ ). Also for Allumiere mating males had on average longer elytra, but the difference was not significant (Mean  $\pm$  S.D. EL mating = 17.04 mm  $\pm$  2.71, Mean  $\pm$  S.D. EL non mating = 16.37 mm  $\pm$  2.07,  $F = 2.56$ , d.f. = 1,  $p = 0.11$ ). In all females datasets no significant differences were found between EL of mating and non-mating individuals.

The individuals belonging to g2 (larger than switch point) where more frequently found in mating than individuals of g1 (Tab. V) (K-W test:  $H = 3.86$ ,  $p = 0.049$ ).

On the total of 140 observed mating males, 70 males belonged to g1 and 70 to g2. The mean and standard deviation of male/female EL ratio, calculated for the individuals observed mating, resulted  $0.97 \pm 0.16$ , the majority of matings observed were in the range of 0.8-1.1 (Fig. 4).

Dispersal was recorded for eleven males and only one dispersal event was recorded for each individual. The male M4 (AL = 64.01 mm) covered the longest maximum distance (451m) in 26 days. The male M10 (AL = 47.01 mm) covered the longest mean daily distance (44m), with a maximum distance of 220 m in 5 days (Fig.5).

Overall, 26 males were recorded after having overwintered: six males in Allumiere (mean EL  $\pm$  S.D.: 17.41  $\pm$  2.91 mm), eighteen males in M. Cimino (mean EL  $\pm$  S.D.: 16.32  $\pm$  2.02 mm) and two males in M. Fogliano (mean EL  $\pm$  S.D.: 16.71  $\pm$  2.36 mm); they showed a mean EL slightly exceeding the mean EL of the relative populations (Tab. III). Due to the low number of overwintered males, no statistic test was performed.

Table V. Number of males belonging to g1 and g2 of each population and their relative mating frequencies. N. mated g1 = number of males of g1 found in mating, N. mated g2 = number of males of g2 found in mating, Mating fr. g1 = relative mating frequencies of males of g1 calculated as the N. mated g1 / N. g1, Mating fr. g2 = relative mating frequencies of males of g2 calculated as the N. mated g2 / N. g2

| Males       | N. g1 | N. mated g1 | Mating fr. g1 (%) | N. g2 | N. Mated g2 | Mating fr g2 (%) |
|-------------|-------|-------------|-------------------|-------|-------------|------------------|
| Allumiere   | 99    | 27          | 27.27             | 42    | 20          | 47.62            |
| M. Fogliano | 67    | 5           | 7.46              | 34    | 15          | 44.1             |
| M. Cimino   | 143   | 38          | 26.57             | 76    | 35          | 46.05            |

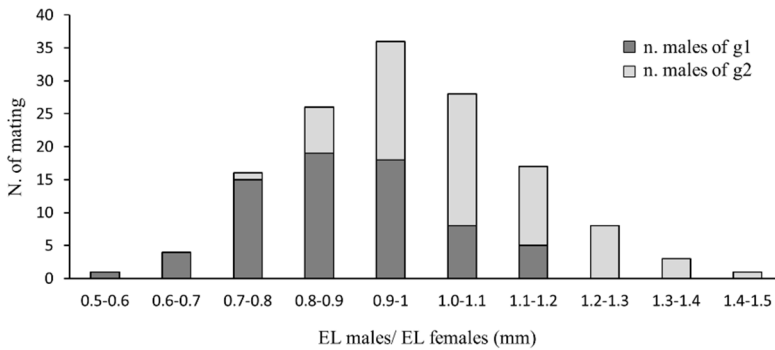


Figure 4. Number of mating events observed within each male/female EL ratio categories. In each category was reported the number of males belonging to g1 (below the switch point) and g2 (above the switch point) observed in mating.

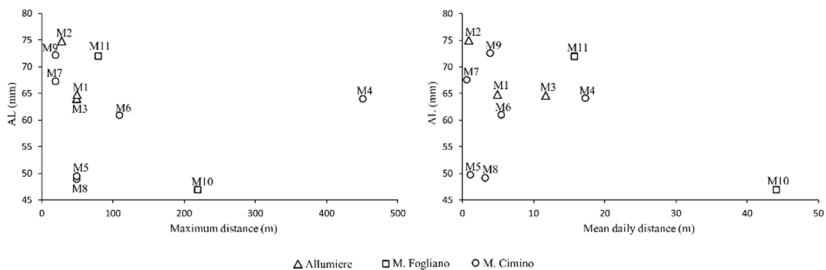


Figure 5. Maximum distance (left side) and mean daily distance (right side) in relation to the antennal length (AL) of the eleven shifted males (M1-M11). The maximum distance was recorded between two consecutive captures of the individual and the mean daily distance was obtained by dividing the maximum distance for the number of days between two consecutive captures.

## Discussion

Previous studies on longhorn beetles highlighted the existence of sexual dimorphism in different morphological traits (e.g. foreleg, head, mandibles, scape and antennal segments) (Zeh and Zeh 1992; Kawano 2006; Saikia et al. 2012). In the present study, sexual dimorphism was confirmed in *M. asper* for various morphological parameters: males had longer antennae than females; the antennal length was two times longer than total body length in males and slightly longer than body length in females like observed in other studies (e.g. Linsley 1961; Cherepanov 1988; Hanks 1996 a,b). The antennal length of males turned out as the biometric trait with the highest variability. Males had a longer pronotum than females, while the maximum width of elytra and the elytra length were larger in females. This sexual dimorphism suggests that more resources are used for developing the anterior body traits in males (e.g. antennae and pronotum), while in females more resources are invested into the development of the post-prothoracic body parts, which contain the reproductive apparatus.

Males from Allumiere were larger than those from the other two populations, while for females this difference was significant only for the comparison of Allumiere and M. Cimino. Two main hypotheses may explain this difference in body size: (a) genetic variability among populations, probably due to isolation; (b) differences in habitat factors of the three study sites. The second hypothesis is supported by the fact that the Allumiere beech forest is located at a lower altitude, closer to sea and subjected to higher average temperatures in respect to the other two study sites. Previous studies on insect populations (Plaistow et al. 2005; Hardersen et al. 2011; Painting et al. 2014) claimed that seasonal constraints, limiting the feeding resources, can affect adult size. Geographic variation in environmental parameters, as well as seasonal changes, can produce size differences in populations of a species (Kaiser et al. 2007; Entling et al. 2010). Differences in environmental parameters among sites may affect the adult size directly, by altering the activity period of the larvae and, indirectly, affecting the availability of feeding resources (Fattorini et al. 2013; Painting et al. 2014).

The allometric relationships between the antennal length and the elytra length in males of *M. asper* did not follow a simple linear model; two of the three populations exhibited a significant change in the slope described by the segmented model. For the males from Allumiere, all three non-linear models showed non-distinguishable explanatory power for the AL vs EL scaling relationship, including the segmented model. The estimated switch

points varied among the populations and the population from Allumiere had the highest value of the switch point; this is probably due to the fact that this population presents the largest males (see above). Pooling males of the three populations, again the segmented model was the best one. All these results led us to divide the male population into two groups with two different allometric trajectories.

The positive allometry of g1 males was very steep (1.68-1.92), this indicates that males of this group allocate a disproportionate amount of resources into antennae than larger males (g2). However, for males larger than the switch point (g2) the antennae growth trajectory changes and its allometric coefficient is close to 1. For these males the advantage of developing ever longer antennae is lower, probably because of practical constraints. To our knowledge, only a few other studies reported non-linear allometries in insects due to a relative reduction in trait size for the largest males (Pomfret and Knell 2006; Painting and Holwell 2013). The various authors seem to agree that the changes in the allometric slope may reflect changes in the fitness benefits associated with increases in trait size and body size change as an animal gets larger. Painting and Holwell (2013) suggest a limitation in resource allocation or a diminishing requirement for large males to invest increasingly into an exaggerated structure (here rostrum).

We do not consider the two groups as two different morphs because our data did not show a clear bimodal distribution (Knell 2009).

The competition for resources by the rapidly growing body parts in the pupa seems to be the more reasonable hypothesis to explain growth trend like that observed in larger males of *M. asper* (Huxley 1932; Nijhout and Wheeler 1996; Knell et al. 2004). In particular, for those beetles with more exaggerated characters, the fast growing of secondary sexual traits may locally deplete the resources necessary for their body development (Knell et al. 2004). Furthermore, the decreasing slope for larger males probably deals with genetic variability, hormonal interaction (juvenile JH and ecdysone) and feeding resource availability for the development not only of the exaggerated structures but also of their trade-off supporting traits (Eberhard 1982; Emlen and Nijhout 2001; Emlen and Allen 2004; Nijhout 1994; Painting and Holwell 2013). In fact, resource allocation trade-offs during larval development affects the final size of adult structures (Moczek and Nijhout 2004).

Considering that males observed in mating were generally larger than unmated males and that the mated/unmated ratio was higher for larger males (group above the switch point) in all three populations, we may infer that larger males win more contests and mate more often, as also observed

for *Monochamus scutellatus* (Hughes and Hughes 1982) and as predicted by theory (Darwin 1871). Little is known about the reproductive behavior of *M. asper* (Romero-Samper and Bahillo 1993) and there are no information about sexual pheromones and intrasexual communication, which in insects involves mainly receptors located on the antennae (Cardè and Bell 1995). According to Hanks (1999), it seems that Lamiini beetles do not own long-range sex pheromones; therefore we may suppose that longer antennae in *M. asper* may increase the surface for sexual pheromone reception giving to larger males more chance to detect females. In our study, we may hypothesize that in larger males more resources are allocated in trade-off supporting traits, i.e. to enlarge and to strengthen the body to bear such long antennae, increasing their success in combat.

However, the male/female EL ratio, for mating pairs was close to 1, with the majority of matings in the range between 0.8 and 1.1. This means that male and females tended to mate with a partner of more or less the same size, evidence for a non-random mating pattern (Hanks et al. 1996a).

Smaller males were observed outnumbering the larger one in each population; this fact may be responsible of an equal mating possibility even if smaller males lose contests for mating. Probably small males are favored by natural selection due to other factors, such as predation by birds and mammals, because they are more hardly detected, however, this hypothesis should be tested by further studies.

For females, no single model best fitted the allometry data for the antennae of the three populations and one reason could be the paucity of data points. However, pooling all data, the scaling relationship followed a sigmoidal model and the data are described by the curve above the inflection point. This growth trend means that at first antenna increasing disproportionally with overall size (elytra length) and that the AL/ EL ratio decreases as females become ever larger, a result that is similar to that obtained for males.

Observing the recorded dispersal, eight of the eleven males showed antennae longer than 60 mm and one of them covered the longest distance; however, a male with relatively short antennae (47 mm) covered the longest distance in less time. Regarding effects of exaggerated structures on movements, Goyens et al. (2015) observed that larger males of the stag beetle *Cyclommatus metallifer* (Lucanidae) with exaggerated mandibles adjusted their locomotor due to their heavy head appendages.

Within each population, the mean elytra length of the overwintered males was slightly longer than the average of the entire population, and this suggests that larger individuals have a higher overwintering probability.

Unfortunately, the data on overwintering ability and dispersal were too few to allow statistical tests and no firm conclusions can be drawn.

## Conclusions

Sexual dimorphism in *Morimus asper* was observed not only in the antennal length but also in width and length of elytra. In general, a non-linear allometric relationship was found between antennal length and body size in males and females showing a positive allometry for smaller individuals and an allometric relationship tending to isometry for the largest ones. This change in growth rate is stronger in males, where a switch point determines a threshold in body size above which males exhibit antennae which scale more or less isometrically. Morphometric traits do affect ecological features, in particular larger males were observed mating more frequently and had a higher probability to survive the winter.

## Acknowledgements

The authors are grateful to Roberto Sacchi for his technical advices on statistical analysis, and to Chiara Passacantilli for her field support.

## References

- Cardè RT and Bell WJ. 1995. Chemical ecology in insects II. Chapman and Hall, London. 433p.
- Borcard D, Gillet F, Legendre P. 2011. Numerical Ecology With R. New York: Springer. 306 p.
- Campanaro A, Bardiani M, Spada L, Carnevali L, Montalto F, Antonini G, Mason F, Audisio P. 2011. Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxylca/ Guidelines for monitoring and conservation of saproxylc insects. Quaderni Conservazione Habitat, 6. Cierre Grafica, Verona. 8p.
- Cherepanov AI. 1988. Cerambycidae of Northern Asia, Vol. 1, Amerind, New Delhi. 300p.
- Cook JM and Bean D. 2006. Cryptic male dimorphism and fighting in a fig wasp. Anim Behav 71: 1095 – 1101.

- Darwin CR. 1874. The descent of man and selection in relation to sex. Hurst, New York. 705p.
- Eberhard WG. 1982. Beetle horn dimorphism, making the best of a bad lot. *The American Naturalist* 119: 420-426.
- Eberhard WG. and Gutierrez EE. 1991. Male dimorphisms in beetles and earwigs and the question of developmental constraints. *Evolution* 45: 18–28.
- Emlen DJ. 2001. Costs and the Diversification of Exaggerated Animal Structures. *Science* 291:1534-1536.
- Emlen, DJ and Allen CE. 2004. Genotype to phenotype: physiological control of trait size and scaling in insects. *Integr Comp Biol* 43: 617–634.
- Emlen DJ and Nijhout HF. 2000. The Development and Evolution of Exaggerated Morphologies in Insect. *Annu Rev Entomol* 45:661-780.
- Emlen DJ and Nijhout HF. 2001. Hormonal control of male horn length dimorphism in the horned beetle *Onthophagus Taurus* (Coleoptera: Scarabaeidae): a second critical period of sensitivity to juvenile hormone. *J Insect Physiol* 47: 1045–1054.
- Entling W, Schmidt-Entling MH, Bacher S, Brandl R, Nentwig W, 2010. Body size e climate relationships of European spiders. *J. Biogeogr.* 37: 477-485.
- Fattorini S, Lo Monaco R, Di Giulio A, Ulrich W. 2013. Latitudinal trends in body length distributions of European darkling beetles (Tenebrionidae). *Acta Oecol* 53: 88-94.
- Forslund P. 2003. An experimental investigation into status-dependent male dimorphism in the European earwig, *Forficula auricularia*. *Anim Behav* 65: 309–316.
- Goldsmith SK, Stewart Z, Adams S, Trimble A. 1996. Body Size, Male Aggression, and Male Mating Success in the Cottonwood Borer, *Plectrodera scalator* (Coleoptera: Cerambycidae). *J Insect Behav* 9: 719-727.
- Gould SJ. 1966. Allometry and size in ontogeny and phylogeny. *Biol. Rev* 41: 587-640.
- Goyens J, Dirckx J, Aerts P. 2015. Costly sexual dimorphism in *Cyclommatus metallifer* stag beetles. *Funct Ecol* 29: 35–43.
- Green A. J. 1992 Positive allometry is likely with mate choice, competitive display and other functions. *Anim Behav* 43: 170–172.
- Hammer O, Harper D, Ryan P. 2001. PAST: Paleontological statistics software package for education and data analysis. *Paleontol Electron* 4: 1-9.



- Hanks LM, Millar JG, Paine TD. 1996 a. Body Size Influences Mating Success of the Eucalyptus Longhorned Borer (Coleoptera: Cerambycidae). *J Insect Behav* 9: 369-382.
- Hanks LM, Millar JG, Paine TD. 1996 b. Mating Behavior of the Eucalyptus Longhorned Borer (Coleoptera: Cerambycidae) and the Adaptive Significance of Long "Horns". *J Insect Behav* 9: 383-393.
- Hanks LM. 1999. Influence of the larval host plant on reproductive strategies of cerambycid beetles. *Annu. Rev. Entomol.* 44: 483–505.
- Hanley RS. 2001 Mandibular allometry and male dimorphism in a group of obligately mycophagous beetles (Insecta: Coleoptera: Staphylinidae: Oxyporinae). *Biol. J. Linn. Soc.* 72: 451–459.
- Hardersen S, Macagno ALM, Sacchi R, Toni I. 2011. Seasonal constraints on the mandible allometry of *Lucanus cervus* (Coleoptera: Lucanidae). *Eur J Entomol* 108: 461–468.
- Hughes AL, Hughes MK. 1982. Male Size, Mating Success, and Breeding Habitat Partitioning in the Whitespotted Sawyer *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae). *Oecologia (Berl)* 55:258-263.
- Huxley JS. 1932. Problems of Relative Growth. Methuen, London. 276p.
- Iguchi Y. 2013. Male mandible trimorphism in the stag beetle *Dorcus rectus* (Coleoptera: Lucanidae). *Eur. J. Entomol.* 110: 159–163, 2013.
- Johnstone RA. 1995. Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biol Rev* 70: 1-65.
- Kawano K. 2006. Sexual dimorphism and the making of oversized male character in beetles (Coleoptera). *Ann Entom Soc Am* 99:327-341
- Kaiser A, Klok CJ, Socha J, Lee W, Quinlan MC, Harrison JF. 2007. Increase in tracheal investment with beetle size supports hypothesis of oxygen limitation on insect gigantism. *Proc Natl Acad Sci U. S. A.* 104: 13198-13203.
- Kotiaho JS, Tomkins JL. 2001. The discrimination of alternative male morphologies. *Behav Ecol* 12: 553 – 557.
- Knell RJ. 2009. On the analysis of non-linear allometries. *Ecol Entomol* 34: 1-11.
- Knell RJ, Pomfret JC, Tomkins JL. 2004. The limits of elaboration: curved allometries reveal the constraints on mandible size in stag beetles. *Proc R Soc Lond B* 271: 523–528.
- Levinton J. 1988. Genetics, Paleontology and Macroevolution. Cambridge: Cambridge University Press. 637p.
- Linsley EG. 1959. Ecology of Cerambycidae. *A Rev Entomol* 4: 99–138.

- Linsley EG. 1961. The Cerambycidae of North America. I. Introduction. Univ Calif Publ Entomol 18: 1-135.
- Moczek AP, Emlen DJ. 2000. Male horn dimorphism in the scarab beetle *Onthophagus Taurus*: do alternative reproductive favour alternative phenotypes? Anim Behav 59:459-466.
- Moczek AP, Nijhout HF. 2004. Trade-offs during the development of primary and secondary sexual traits in a horned beetle. Am Nat 163:184-191.
- Møller A.P. and Zamora Muñoz C. 1997. Antennal asymmetry and sexual selection in a cerambycid beetle. Anim Behav 54: 1509–1515.
- Nijhout HF. 1994. Insect hormones. University Press, Princeton. 267p.
- Nijhout HF, Wheeler DE. 1996. Growth models of complex allometries in holometabolous insects. Am Nat 148: 40-56.
- Painting C., Holwell G. 2013. Exaggerated Trait Allometry, Compensation and Trade-Offs in the New Zeland Giraffe Weevil (*Lasiorhynchus barbicornis*). PlosOne 8: e82467.
- Painting CJ, Buckley TR, and Holwell GI. 2014. Weapon allometry varies with latitude in the New Zealand giraffe weevil. J Evol Biol 27: 2864-2870.
- Plaistow S.J., Tsuchida K., Tsubaki Y. and Setsuda K. 2005. The effect of a seasonal time constraint on development time, body size, condition, and morph determination in the horned beetle *Allomyrina dichotoma* L. (Coleoptera: Scarabaeidae). Ecol Entomol 30: 692–699.
- Pomfret J.C. and Knell R.J. 2006. Sexual selection and horn allometry in the dung beetle *Euoniticellus intermedius*. Anim Behav 71: 567–576.
- R Development Core Team 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Romero-Samper J, Bahillo P. 1993. Algunas observaciones sobre la distribución y biología de *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) en la Península Ibérica. Boln Asoc Esp Ent 17: 103-122.
- Romiti F, Tini M, Redolfi De Zan L, Chiari S, Zauli A, Carpaneto GM. 2015. Exaggerated allometric structures in relation to demographic and ecological parameters in *Lucanus cervus* (Coleoptera: Lucanidae). J Morphol 276: 1193-1204.
- Rowland JM, Emlen DJ. 2009. Two thresholds, three male forms result in facultative male trimorphism in beetles. Science 323:773–776.
- Saikia K, Thakur NSA, Ao A, Gautam S. 2012. Sexual Dimorphism in *Pseudonemorphus versteegi* (Ritsema) (Coleoptera: Cerambycidae), Citrus Trunk Borer. Fla Entomol 95: 625-629.

- Sama G. 1988. Coleoptera, Cerambycidae. Catalogo Topografico e Sinonimico. Fauna d'Italia. XXXVI. Ed. Calderini, Bologna. 216p.
- Solano E., Mancini E., Ciucci P., Mason F., Audisio P., Antonini G. 2013. The EU protected taxon *Morimus funereus* Mulsant, 1862 (Coleoptera: Cerambycidae) and its western Palaearctic allies: Systematics and conservation outcomes. *Conserv Genet* 14: 683–694.
- Wang Q., Zeng W. 2004. Sexual selection and male aggression of *Nadezhdiella cantori* (Hope) (Coleoptera: Cerambycidae: Cerambycinae) in relation to body size. *Environ Entomol* 33: 657-661.
- Williams CM. 1980. Growth in insects. In: *Insect biology in the future*, Locke M. and Smith D.S. (eds.), Academic Press, New York. p 369-382,
- Zeh DW, Zeh JA. 1992. Sexual Selection and Sexual Dimorphism in the Harlequin Beetle *Acrocinus longimanus*. *BIOTROPICA* 24: 86-96.

## CHAPTER 4

### **Computer-aided Photographic Identification of *Rosalia alpina* (Coleoptera: Cerambycidae): first application in a mark-recapture study**

Sarah Rossi de Gasperis<sup>1</sup>, Giuseppe M. Carpaneto<sup>1</sup>, Giulio Nigro<sup>2</sup>, Gloria Antonini<sup>3</sup>, Stefano Chiari<sup>4,5</sup>, Alessandro Cini<sup>4,5,6</sup>, Emiliano Mancini<sup>1</sup>, Franco Mason<sup>7</sup>, Fabio Mosconi<sup>3,4</sup>, Lara Redolfi De Zan<sup>4,5</sup>, Pio Federico Roversi<sup>4</sup>, Giuseppino Sabbatini Peverieri<sup>4</sup>, Emanuela Solano<sup>3,4</sup>, Alessandro Campanaro<sup>4,5</sup>

<sup>1</sup> Dipartimento di Scienze, Università Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy.

<sup>2</sup> Facoltà di Scienze Matematiche, Fisiche e Naturali- Università di Pisa, Via Lungarno Pacinotti 43; 56126 Pisa, Italy

<sup>3</sup> Dipartimento di Biologia e Biotecnologie Charles Darwin, Università La Sapienza, Via A. Borelli 50, 00161 Roma, Italy

<sup>4</sup> Consiglio per la ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per l'Agrobiologia e la Pedologia (CREA-ABP) - Via di Lanciola 12/a, 50125 Cascine del Riccio Firenze, Italy

<sup>5</sup> CFS Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale "Bosco Fontana" di Verona - Strada Mantova 29, 46045 Marmirolo Mantova, Italy.

<sup>6</sup> Current address: Dipartimento di Biologia, Università di Firenze, Via Madonna del Piano, 6 - 50019 Sesto Fiorentino Firenze, Italy

<sup>7</sup> Corpo Forestale dello Stato, Centro Nazionale Biodiversità Forestale, Laboratorio Nazionale Tassonomia Invertebrati "Lanabit" - Via Carlo Ederle 16/a & Via Tomaso da Vico 1 - I-37100 Verona, Italy.

**Corresponding author:** Sarah Rossi de Gasperis, sarahrodega@gmail.com

## Abstract

Quantitative population studies are strongly needed in order to assess conservation status of protected species, and are often carried out by Capture-Mark-Recapture methods (CMR). Such a methodology often uses artificial marking procedures, which may stress and/or injure individuals. Therefore, the exploitation of natural marking (e.g. spots or other individual morphological traits) may be a viable alternative to avoid invasive methods. In our two-year study, we tested the reliability of the photographic identification method on elytra spots of *Rosalia alpina*. Moreover, we provided more information on population ecology and habitat preference of the species in order to give suggestions for monitoring programmes. Data were collected in two National Parks of central Italy during two years: 2014-2015. Suitable trees for hosting the species were visually inspected from 10:00 am to 4:00 pm from 15<sup>th</sup> July to 30<sup>th</sup> August (seven surveys) in 2014. In 2015 we reduced the number of inspected trees and increased the number of sampling surveys in order to get better estimates of population size, survival and recapture probability of the species. Computer-aided photographic identification resulted in a reliable method for *R. alpina* within a CMR population study. Our results suggested that to optimize beetle image acquisition in the field, the use of a small box for single specimen containment during acquisition (e.g. a wide mouth square sample bottle) allows getting more clearly comparable images to facilitate the I<sup>3</sup>SC screening process. Furthermore, our results also showed that using only the elytral central spot contours made the tracing contour process less time-consuming. In our analysis, the I<sup>3</sup>SC output scores, obtained from the images comparison, were used to identify a threshold value for the identification of new individuals or recaptures, facilitating the final identification made by operators. In 2015, the adjusted sampling design resulted more efficient allowing to get population parameters estimates. The species was positively associated to decaying huge trees, in a medium decay class, with low canopy closure; these trees hosted also the protected longhorn beetle *Morimus asper/funereus*. Among the selected trees, “key trees” for population viability were identified. Forest management should promote the maintenance of old and huge trees interspersed within opened areas and preserve, in particular, old trees distributed around “key trees” to increase dispersal and colonization by the species.

**Keywords:** Natural marking, Saproxylic insects, longhorn beetles, protected species, Habitats Directive, Population size, I<sup>3</sup>SC software, GLMMs

## 1. Introduction

The longhorn beetle *Rosalia alpina* (Linnaeus, 1758) is a flagship species among insects, having a charismatic attractive power for people due to its distinctive colorful elytral pattern (Duelli & Wermelinger 2005). *R. alpina* is an obligate saproxylophagous beetle, and then belongs to saproxylic beetles, i.e. dependent, during some part of their life cycle, upon the dead or dying wood of decaying trees or upon wood-inhabiting fungi, or upon the presence of other saproxylics (Speight 1989; Alexander 2008; Stokland et al. 2012).

In terms of insect conservation, this beetle is ranked as a priority species and listed in the Annexes II e IV of the European Habitats Directive 92/43EEC (hereafter HD). The distribution range of *R. alpina* extends from central-southern Europe to northern Turkey and Caucasian region (Sama 1988; 2002); its current distribution is highly fragmented with few isolated populations in most of central Europe (Sama 2002, Binner and Bussler 2006, Cizek et al. 2009; Jurc et al. 2008). In Italy, the historical distribution of the species includes all administrative regions, excluding Sardinia (Sama 2005; Campanaro et al. 2011). *R. alpina* is mainly associated to ancient deciduous forests dominated by *Fagus sylvatica* (Lachat et al. 2013) and for this reason it is mainly located in Alpine and Apennine mountains. Across its distribution range, it has been also recorded from forests of lowland and hilly areas, where the species was found associated to different larval-host trees (e.g. mountain elm, European ash, sycamore maple) (Sama 1988; Ciach et al 2007; Cizek et al. 2009; Michalciewicz et al. 2011; Michalciewicz and Ciach 2012; Michalciewicz et al. 2013). As all the obligate saproxylophagous beetles, this species requires deadwood for its biological cycle, in particular dry sun-exposed deadwood (Russo et al. 2011). The larval development lasts 3-4 years, pupation occur in spring and adult phenology ranges from July to September (Duelli and Wermelinger 2005, Trizzino et al. 2013). Due the short adult life span of *R. alpina* and its specific microhabitat (Drag et al. 2011; Russo et al. 2011), it is difficult to choose an appropriate sampling method to obtain data on population ecology and to provide a monitoring program (Bosso et al 2013).

Monitoring protocols for saproxylic beetles listed in the HD have been recently discussed (Campanaro et al. 2011; Vrezec et al. 2012, Trizzino et al. 2013). The most common sampling method for monitoring *R. alpina* is the visual encounter survey (VES), which requires an high sampling effort (i.e. long transects, an high number of sessions). In general, the use of traps is more rapid in terms of person/hours needed on field, and odor-traps

baited with the specific sexual pheromone may be the best sampling method for endangered species with low detectability. In this regard, successful examples obtained for *Osmoderma eremita* (Scopoli, 1773) have been discussed (Larsson et al. 2003; Svensson et al. 2009; Chiari et al. 2013b; Zauli et al. 2014). A recent study (Ray et al. 2009) tested the attractive power of the aggregation pheromone produced by males of the American species *Rosalia funebris* (Motschulsky, 1845), but to our knowledge, until now, no pheromone has been identified and synthesized for the European species. Another limit to the sampling method, in particular for endangered species, is the need of a non-invasive sampling technique. Population studies, which aim to gather information on survival ability, demography and dynamics, are generally carried out by a Capture-Mark-Recapture method (CMR) which allows to recognize each individual and gather quantitative data (Amstrup et al. 2005). One of the assumptions of CMR is that the marker must be permanent for all the sampling period. The artificial marking procedure usually involves capturing and handling, which can produce stress and/or lead to injury individuals, and can lead to violate the other assumption of CMR about the equal recapture probability of individuals. Therefore, natural marking (e.g. spots and other individual morphological traits) can be a viable alternative (Bradfield 2004). Some successful examples of photo-identification by using the individual coloured pattern occur in mammals, reptiles and amphibians (Wilson et al. 1999; Bradfield 2004; Gamble et al. 2008; Schofield et al. 2008; Hoque et al. 2011; Lahiri et al. 2011; Knox et al. 2012; Rydell and Russo 2015). In this regard, it was recently demonstrated that the colored elytral pattern of *R. alpina* can be used as natural marking for photographic identification by visual assessment (Pagola Carte 2011). However, photographic approach showed some limits, in particular, it may be complex and time-consuming by processing large databases. In this case, the computer-aided matching of photographs was a successful solution for individual identification of other animals (Kelly 2001, Speed et al. 2007, Hiby et al. 2009, Sacchi et al. 2010). The photo-identification of *R. alpina* by using specific software to facilitate the identification procedure was recently applied mainly on museum collections samples (Caci et al. 2013). However, the reliability of computer-aided photographic identification and its feasibility in the field (i.e. on natural populations) has never been tested before.

The present study was performed within the framework of the Project LIFE11 NAT/IT/000252 MIPP "Monitoring of Insects with Public Participation" (Mason et al. 2015), which aims at testing different monitoring protocols for saproxylic beetles listed in HD.

We assessed the reliability of computer-aided photographic identification method for *R. alpina* within a two years CMR study and we improved the survey sampling design to achieve a quick and efficient method for monitoring the species. In particular we aimed to: i) test the reliability of computer-aided photo identification to be used as CMR method providing a standard field protocol; ii) to define an efficient sampling method for *R. alpina* to obtain population parameters estimates; iii) to get information on habitat preference in order to give suggestion for the conservation of the species.

## 2. Material and Methods

### 2.1 Study area

The study was conducted in two National Parks and Sites of Community Importance (SCI) of Central Italy: (i) Parco Nazionale Foreste Casentinesi, Monte Falterona and Campigna (PNFC), in the Northern Apennines; (ii) Parco Nazionale Abruzzo, Lazio and Molise (PNALM) in the Central Apennines (Fig. 1).

Two study sites, Poggio Ghiaccione, and Solforosa, both at mean altitude of 700 m a.s.l., were selected in the PNFC, within the “Foresta di Campigna, Foresta La Lama, Monte Falco” (SCI: IT4080001). The forests of these two study sites are mostly closed, mainly consisting of silver fir (*Abies alba*) and beech (*Fagus sylvatica*), with minor presence of other deciduous species (e.g. *Acer pseudoplatanus*, *Fraxinus excelsior*, *Carpinus betulus*).

The other three study sites were selected in the PNALM within the “Parco Nazionale d’Abruzzo” (SCI: IT7110205). Two study sites, Difesa di Pescasseroli (1.348 m a.s.l.) and Val Fondillo (1.217 m a.s.l) are characterized by pure beech forests with wide clearings including old-growth trees; the third study site, Zio Mas (1.694 m a.s.l) is characterized by fragmented beech woodlands interspersed among open mountain grasslands.



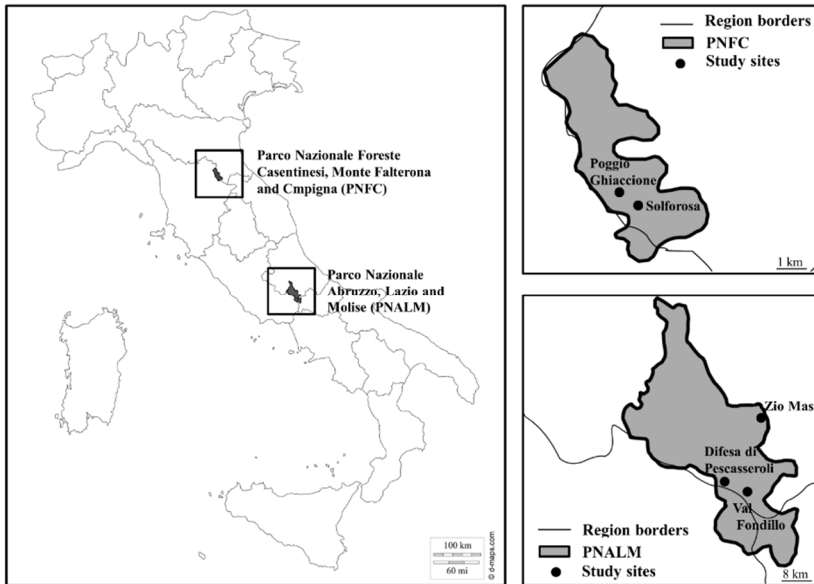


Figure 1. Study sites selected for surveying *Rosalia alpina* within two National Parks of Central Italy

## 2.2. Fieldwork and data collection

In 2014, an explorative sampling was performed in the two study sites of PNFC and in the three study sites of PNALM. In 2015, the same sampling design of 2014 was conducted in the two study sites of PNFC, while an adjusted sampling design was performed only in the two study sites of PNALM because the highest species abundance was recorded there in 2014, i.e. Difesa di Pescasseroli and Val Fondillo.

### 2.2.1 *Rosalia alpina* sampling design 2014

We selected 136 suitable trees for hosting *R. alpina* (e.g. standing dead or moribund trees and fallen big trunks, without part or the entire bark, with

presence of emerging holes of longhorn beetles): in Soflorosa (28 trees), Poggio Ghiccione (28), Val Fondillo (28); Difesa di Pescasseroli (30) and Zio Mas (30).

Trees were visually inspected during the warmest hours, from 10:00 am to 4:00 pm, every seven days (avoiding rainy and windy days) for a total of 7 surveys for each study site. The survey's duration varied in relation to number of captures and intrinsic field features. Surveys were performed from 15<sup>th</sup> July to 30<sup>th</sup> August, i.e. during the adult flight period. In order to inspect each tree at different daytime, we inverted the tree sampling order on every subsequent survey.

Data were collected by following CMR method: adults were captured directly by hand or with an insect net and then released in the same spot after the marking procedure. At first capture, adults were sexed and marked by using the two types of marking:

- 1) Artificial marking, by writing a progressive number of capture on the elytra with a non-toxic black permanent color (Stabilo S OHPen universal) (cf. Drag et al. 2011). The number was reported on both elytra to reduce at minimum the possibility of loss of data (for example in cases of ink abrasion or by recognizing dead individuals with single elytral remain).

- 2) Natural marking, by photographing the adult dorsal surface to acquire images of the elytral spots in order to recognize each specimen. During image acquisition, according with Caci et al. (2013), the angle under which images must be taken should not exceed 30°. For this reason, and in order to standardize pictures of our database, each individual was placed on the bottom of a 1000 ml High Density Polyethylene (HDPE) wide mouth square sample bottle. Pictures were taken by putting the camera lens (Canon® PowerShot D20) directly on the mouth of the bottle, so that the framing angle was maintained as much as possible perpendicular to the specimen. In most cases, within the bottle, individuals usually remained quiet, so that the image acquisition was relatively easy; pictures were made for each adult when it was completely still and, such as possible, placed in the centre of the bottle. Four pictures were collected for each individual in order to choose the best one during image analysis.

During the surveys, when a previously marked beetle was intercepted (i.e. recaptured), the number of the individual was annotated and its elytral pattern images were acquired. The same procedure was made for marked remains of dead individuals.

### 2.2.2 Collection of environmental variables in 2014

The selected trees were mapped using a Global Positioning System (GPS) receiver (Garmin 60CSX) and for each tree, we measured nine environmental variables (cf. Ranius and Jansson, 2000; Redolfi De Zan et al., 2014).

Garmin MAP Source software was used to measure the distance of the nearest colonized tree (DNC); tree diameter (DBH) was calculated by measuring the tree girth at 1.30 m from the ground; tree status (TS) was assessed by coding each tree with one of the three categories: dead, decaying and living tree. Tree height (TH) was measured by using the Smart phone application Measure Height 1.4 (Android Play Store); canopy closure (CC) was measured by visual assessment in an area of 5-10 m of radius around each tree. Presence-absence data were recorded for cavities (CA), woodpecker activity (WA) (i.e. foraging holes made by woodpeckers) and for the typical shallow laying pits of *Morimus asper/funereus* (MLP) (Sama 1988, Romero-Samper and Bahillo, 1993), which is another saproxylic longhorn beetle listed in Annex II of HD. Finally, the wood decay class (WDC) was measured according to Hunter (1990), based upon the degree of penetration of a knife blade (Opinel n°8), attributing each tree to one of the five decay classes. WDC is classified in five categories: I) recently dead, no evidence of decay, intact bark; II) solid wood, less than 10% of decaying wood, knife blade penetrates less than 1 cm, intact bark; III) 10% to 25% of decaying wood, knife blade penetrates up to 1 cm, and soft wood in surface with some bark slippage; IV) more than 26% decaying wood, knife blade penetrates more than 1 cm, and soft wood to the touch also in depth, bark missing; V) 76% to 100% decaying wood, highly soft wood throughout its entire thickness.

### 2.2.3 *R. alpina* adjusted sampling design 2015

In 2015, taking into account results obtained in 2014 (see section 3.1), only the natural marking was used; each adult was sexed and photographed at every capture. In addition, remains of dead individuals collected at the base of inspected trees were photographed with the same method.

The sampling design was modified in order to get more information on survival and recapture probability of the species by following the suggestions of MacKenzie and Royle (2005). The number of surveyed trees was reduced to 15, maintaining those trees where the species was found in

2014. If the species had been found in few trees, we maintained also the more suitable trees for the species among the original selected ones, in order to achieve a total of 15 trees. Reducing the number of trees allowed us to reduce the sampling effort, of each survey. We increased the number of surveys to 14 in each study site, two surveys per week: four consecutive sampling days, alternating two sampling days between the selected study sites (i.e. Difesa di Pescasseroli and Val Fondillo), followed by three days of rest. Finally, we reduced the sampling period to 21<sup>st</sup> July - 28<sup>th</sup> August, on the base of the local phenology observed in the previous year. As in 2014, we inverted the tree sampling order on every subsequent survey.

## 2.3 Data analysis

### 2.3.1 The I<sup>3</sup>SC software

Photographic analysis was conducted by using the Interactive Individual Identification System version “Contour” 3.0 (hereafter I<sup>3</sup>SC) (Van Tienhoven et al. 2007), available on <http://www.reijns.com/i3s/index.html>. I<sup>3</sup>SC was already applied to *R. alpina* identification by Caci et al. (2013). Briefly, I<sup>3</sup>SC allows to trace the contour (i.e. fingerprint) on the elytral black spots of *R. alpina* image, using a semiautomatic tracing algorithm. The screening process is made by matching the contour of the unknown individual under examination with the contours of all the images included in the reference library (i.e. all photo records of known individuals). The match output gives a ranked photo list where each rank shows a score value of similarity between the two contours matched; therefore, photo ranked at first place shows the contour most similar to the one of the unknown individual under examination. The perfect match between two fingerprints will correspond to a zero score in the first rank position. However, the final identification, between the unknown image and those ranking highest in the database (i.e. ranks 1-3), is always left to the operator (Caci et al. 2013). For further information about the use of I<sup>3</sup>SC we refer to den Hartog and Reijns (2011).

### 2.3.2 Photographic analysis

Dataset of 2014, containing artificial marking data and photo-records data, was used to validate the photographic identification method performed by I<sup>3</sup>SC, i.e. evaluating whether the software made errors in the identification

of recaptured individuals. Only one of the four pictures of each individual was used, obtaining a total 148 pictures, which built up the database for analysis. This led to the condition that recaptured individuals had at least two images in the dataset: the image of first capture (i.e. mark event) and the images of the recapture events.

The validation procedure was performed in the three following steps:

1) The elytral spot contours were traced for each image and all the 148 images were included in the reference library.

2) Images were matched one by one against the reference library. In this way, we obtained two comparisons for images of the recaptured individuals: the first one match between the image of the first capture (mark event) against the reference library in which is included the image of recapture events, and the second one match between the image of recapture event against the reference library in which is included the image of the first capture (mark event). Therefore, we obtained three results for recaptured individuals: i) correct identification, i.e. the correct images ranked in the three top positions of the output score rank list (Caci et al. 2013); ii) erroneous identification, i.e. no correct identification occurs in both matches, and iii) discrepancy, i.e. erroneous identification occurs in one of the two matches.

3) We recorded errors and discrepancies for the identification of recaptured individuals by comparing the I<sup>3</sup>SC results with the artificial marking of the specimens.

In order to find out a less time-consuming contour tracing method, the validation procedure was separately performed using contours of different spots area of the elytra: the total elytral spot contours (TSC) and a contour for each pair of elytral spots: anterior spot contours (ASC), central spot contours (CSC) and posterior spot contours (PSC) (Fig.2).

One-way Anova test was performed to compare the discrimination power, i.e. Delta score, of the best two tracing methods. The Delta score is defined as the difference between the score of the correct recapture identification ranked at first place (or at most 2<sup>nd</sup> and 3<sup>rd</sup>) and the subsequent ranked score identified as a different individual.

The time spent in the contour-tracing operation, made by a trained operator, was recorded by an analogic chronometer for the two best contours, for a random sample of images (n = 20) in order to compare the two best methods (Kruskall-Wallis test).

The best contour was used for the photographic analysis of the dataset of 2015.

In 2015, 222 individuals were photographed. In this case, we had no information about recaptures in the dataset because no artificial marking was used. Therefore, we proceeded by including in the starting reference library only the images of individuals photographed in 2015 during the first survey among the study sites (i.e. 33 images). Then the new images collected during the subsequent surveys were progressively included in the library as new individuals or recaptured individuals.

One-way Anova test was used to compare, in each year and between years, the first ranked score obtained for the identification of new individuals and recaptures, and to compare Delta score of recaptures identifications between years.

Finally, we graphed match frequencies and Delta scores in order to identify a possible threshold value of scores facilitating the operator in the final identification of new individuals and recaptured.

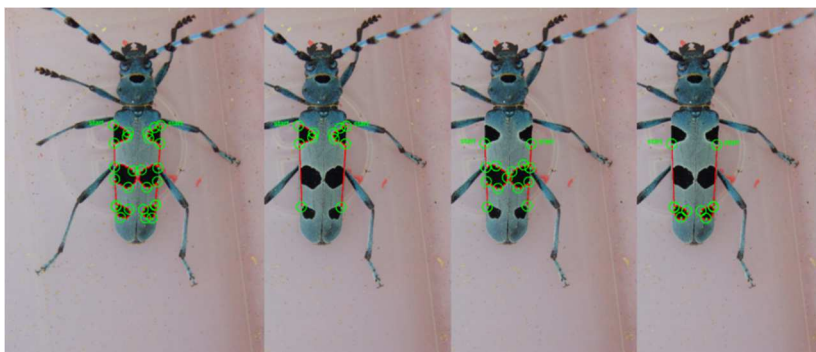


Figure 2. Comparison of four spot contours of *Rosalia alpina*. Figure shows the four different contours from left to right: total spot contours (TSC), anterior spot contours (ASC), central spot contours (CSC) and posterior spot contours (PSC).

### 2.3.3 Population analysis of 2014 and 2015

Population analyses were performed on 2014 and 2015 datasets of Difesa di Pescasseroli and Val Fondillo, in order to get comparisons between data gathered with the two different sampling designs in each study site

Sex ratio of marked individuals was analyzed by Kruskal-Wallis test for each study site.

Total number of marked individuals per tree and adult shifts in 2014 and 2015 were obtained. Shifts were recorded as the linear distance between the spots of two consecutive captures.

Population size was estimated by using two different models:

1) POPAN parametrization of the Jolly-Seber open population model (White and Burnham, 1999) implemented in Mark software (Cooch and White 2015) was performed using CMR data. This model allows to estimate the population size  $N$  and three primary parameters:  $\phi_i$ - apparent survival probability,  $p_i$ - recapture probability, and  $pent_i$ - the probability of entering the population (combining birth and immigration). Models were built with parameters  $\phi$  and  $p$  constant in time (.) or time-dependent (t). We assumed the probability of entering the population always time-dependent:  $pent(t)$ .  $N$ -hat (daily population size estimates) were graphed for the best POPAN model to visualize and compare phenology and species abundance in 2014 and 2015.

2) Royle Repeated Count (RRC) model (Royle 2004) was performed to get an estimate of the population size by recording the abundance (i.e. number of sighted individuals per tree) of the species over multiple surveys, by using PRESENCE 8.3 software (Hines & MacKenzie 2004). The coefficient of variation (CV) of the models was calculated as the standard error (SE) of the population size estimate ( $N$ ) divided by the number of individuals estimated ( $N$ ), it indicates the precision of the estimate. For POPAN models as well as RRC models the Akaike Information Criterion (AIC) model selection procedure was used to select the best model.

#### 2.3.4 Habitat preference analysis

Due to the paucity of data gathered in PNFC, habitat preference analysis was performed by using the 2014 dataset of the three study sites of PNALM: Difesa di Pescasseroli, Val Fondillo and Zio Mas. In these study sites, all trees were standing trees, and no tree showed class V of WDC.

Principal component analysis (PCA) was performed on the environmental variables to visualize the similarity among the three study sites and a MANOVA test was computed to find which variable was significantly different among the three study sites.

In order to investigate the habitat preference of adult beetles, we used Generalized Linear Mixed effects Models (GLMMs) (family = *poisson*, link

= *log*, “*glmer*” function in lme4 R-package) between marked individuals (response variables) and the 9 environmental variables (explanatory variables, fixed effect). To take into account an unknown variation in the number of marked individuals, due to site features and adult phenology, we applied two random effects on the intercept (separately or in combination): Site (3 levels) and Survey (7 levels). The AIC and a Likelihood Ratio Test (LRT), were used to select the best random effect model; then, backward selection and AIC were used to obtain the best final model. The Variance Inflation Factor (VIF) was computed to highlight collinearity among the explanatory variables; all the variables with VIF values below 4.0 were considered without a serious collinearity and retained for building the GLMMs models.

The habitat preference analyses and parametric and non-parametric tests, overall data analysis, were performed using R3.0.3 software (R Development Core Team 2010). Data were normalized (when required) for parametric test using logarithmic function; the alpha set for all the analyses was 0.05.

### **3. Results**

#### **3.1 Photographic analysis**

The 2014 dataset included photo records of 148 individuals gathered from the study areas: 133 marked ( $n = 13$  from PNFC and  $n = 120$  from PNALM) and 15 recaptured (all from PNALM), of which 12 living individuals and 3 dead individuals (remains).

In Table 1 are shown the results of the validation procedure obtained by comparing the artificial marking with the identification results of I<sup>3</sup>SC analysis of recaptured individual images. Among the four types of contours used for this analysis, TSC and CSC identified correctly all the 12 images of recaptured living individuals, and both types of contours ranked the correct image at first place of the output list. In both methods, the same erroneous identifications occurred for the 3 images of marked remains of dead individuals.

The mean and standard deviation of Delta score for recaptured identification resulted:  $2315.8 \pm 1948.4$  for CSC and  $3791.13 \pm 2551.8$  for TSC. Both methods discriminated in the same way ( $F = 3.16$ , d.f. = 1,  $p = 0.09$ ).

The total time spent for tracing the spot contours of 20 images was 24.13 minutes for TSC and 15.27 minutes for CSC. The mean and standard



deviation of the time used for the contour-tracing procedure on a single image was  $48.7 \pm 6.3$  sec for TSC and  $24.5 \pm 2.9$  sec for CSC. Comparing the two methods, CSC turned out to be the less time-consuming contour tracing method ( $H = 29.27$ ,  $p < 0.001$ ).

The 2015 dataset included 222 images of which 35 photo records from PNFC (34 individual and 1 remains of dead individual) and 187 photo records from PNALM (180 individuals and 7 remains of dead individuals). The starting reference library only included 33 individuals, those photographed in the first survey among the study sites ( $n = 15$  from PNFC and  $n = 18$  from PNALM).

The I<sup>3</sup>SC identification procedure by using the CSC less time-consuming contour tracing method, gave back the following results for new individuals (NI) and recaptures (R): 32 NI and 3 R in PNFC, 135 NI and 45 R in PNALM. Of the 48 R, 91.7% ( $n = 44$ ) was ranked at first score, 6.2% ( $n = 3$ ) was ranked at second score, and 2.1% ( $n = 1$ ) was ranked at third score. Only one error for a single NI, which was recognized as a recapture, was found for an image collected in PNFC. All images of remains of dead individuals ( $n = 8$ ) under examination were identified as NI.

On the total identifications, the first ranked scores of NI showed higher values than that of R in 2015, while no difference resulted in 2014 (Table 2). Between years, NI and R first ranked scores of 2014 resulted higher than 2015, while no difference resulted for Delta score for R identification.

In 2015, the highest number of matches, which identified NI were recorded within the range 7,000 - 10,000 of first ranked scores, highlighting 8,000 as a good threshold to identify a new specimen from that value onward (Fig. 3). Match frequencies of R resulted partially overlapped with that of NI, therefore it is difficult to identify a clear threshold for the identification of recaptured individuals by using only the first score value. Of the 48 recaptures, 32 showed a Delta score  $> 1,000$ ; this Delta score value was used as good threshold to be confidence in R identification in 2015. The remaining 16 recaptures of 2015 with delta score  $< 1,000$ , which can indicate uncertainty, were recorded mostly for R with first ranked scores ranging between 5,500 and 7,500 (Fig. 3).

Table 1. Results of the 15 recaptured individuals, obtained from the validation procedure of I<sup>3</sup>SC using the four different elytral spot contours. Total spot contours (TSC), anterior spot contours (ASC), central spot contours (CSC) and posterior spot contours (PSC).

| Spot contours type | Correct identification rate | Erroneous identification rate | Discrepancy rate |
|--------------------|-----------------------------|-------------------------------|------------------|
| TSC                | 12/15                       | 3/15                          | 0/15             |
| CSC                | 12/15                       | 3/15                          | 0/15             |
| ASC                | 4/15                        | 3/15                          | 8/15             |
| PSC                | 1/15                        | 12/15                         | 2/15             |

Table 2 Comparisons of Mean and SD of first ranked score for individuals identified as new (NI) and individuals identified as recaptured (R), and Delta score between first and second ranked scores of recaptured identifications, for dataset of 2014 and 2015.

|                                      | NI                   | R                     | Delta score         |
|--------------------------------------|----------------------|-----------------------|---------------------|
| Year                                 | Mean $\pm$ SD        | Mean $\pm$ SD         | Mean $\pm$ SD       |
| 2014                                 | 11734.1 $\pm$ 4058.9 | 10583.5 $\pm$ 2131.33 | 2315.8 $\pm$ 1948.4 |
| 2015                                 | 8687.8 $\pm$ 2327.4  | 6048.8 $\pm$ 1495.9   | 1832.4 $\pm$ 1240.3 |
| One-way Anova test results           |                      |                       |                     |
| NI 2014 vs R 2014                    | F = 1.60             | d.f. = 1              | p = 0.21            |
| NI 2015 vs R 2015                    | F = 38.37            | d.f. = 1              | p < 0.001*          |
| NI 2014 vs NI 2015                   | F = 42.1             | d.f. = 1              | p < 0.001*          |
| R. 2014 vs R 2015                    | F = 101              | d.f. = 1              | p < 0.001*          |
| Delta score 2014 vs Delta score 2015 | F = 1.29             | d.f. = 1              | p = 0.26            |

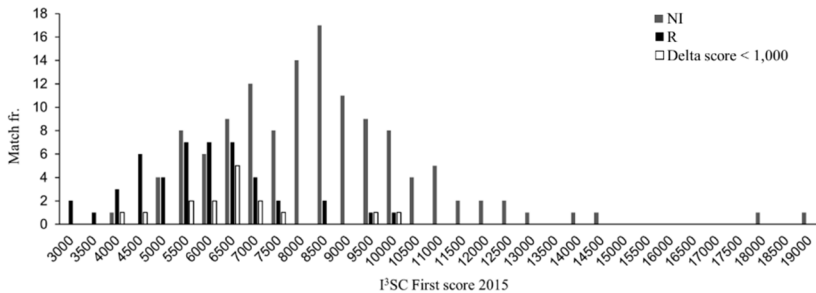


Figure 3. In figure are shown the number of matches for new individuals (NI) and recaptures (R) respect to their first ranked score value given in I³SC for the dataset of 2015. Uncertainty in R. identification is represented by Delta score < 1000. The scores of NI over 19,000 (range 20,000-29,000, only 1 match) were excluded from the graph for better visualizing the frequencies.

### 3.2 Population analysis

Sex ratio of marked individuals was in general unbalanced in favour of males (Table 3), with a significant difference observed in 2015 for Val Fondillo (VF) ( $H = 4.42$ ,  $p = 0.03$ ) and an almost significant difference for Difesa di Pescasseroli (DP) ( $H = 3.43$ ,  $p = 0.06$ ).

In each study site, the same tree had the highest number of marked individuals in both years (Fig. 4). The percentage values of recaptured individuals were: 7.8 % in DP and 3.7 % in VF in 2014; 25.3% in DP and 18.8 % in VF in 2015. In 2014, no female was recaptured while 7 males were recaptured only once. In 2015, two females were recaptured only once, 20 males were recaptured once (15 in DP and 5 in VF) and 10 males more than once (5 in DP and 3 in VF were recaptured twice, 1 in DP trice and 1 in VF four times).

In 2015, two shifts were recorded in VF: one male was recaptured at 165 m from the first capture spot, after 18 days; one female was recaptured at 371 m after 2 days. Four shifts were recorded in DP: 68 m (one female, after two days); 226 m (one male, two days); two shifts of 750 m each one (one male in three days between the same two trees, i.e. 1.5 km in 6 days).

The two models used to estimate population size showed similar values in both years for DP dataset (Table 4). The best POPAN model selected by AIC for VF 2014 resulted the fully time dependent model  $\varphi(t)p(t)pent(t)$ , which did not give reliable estimates due to lack of fit of the capture history. Therefore, in order to allow comparisons between population size estimates, we reported for VF 2014 the only reliable value ( $N = 55 \pm 10$ ) obtained from the most parsimonious model  $\varphi(.)p(.)pent(t)$  (delta AIC < 7). Adult phenology resulted anticipated in 2015 respect to 2014, and the daily population size estimates varied between years, in particular in VF (Fig. 5). Survival probability showed the highest value in 2015 (Table 5) and the observed longest life span was of 20 days for two individuals (8-28 August, 2-22 August) in VF 2015.

Table 3. CMR data of 2014 and 2015 recorded in the two study sites of the Abruzzo region: Difesa di Pescasseroli (DP) and Val Fondillo (VF).

| Study site | Captures( $\mathcal{B}/\mathcal{Q}$ ) | Marked( $\mathcal{B}/\mathcal{Q}$ ) | Recaptured( $\mathcal{B}/\mathcal{Q}$ ) | Recaptures( $\mathcal{B}/\mathcal{Q}$ ) |
|------------|---------------------------------------|-------------------------------------|---|---|
| DP 2014    | 83 (61/22)                            | 77 (55/22)                          | 6 (6/0)                                 | 6 (6/0)                                 |
| DP 2015    | 116 (90/26)                           | 87 (62/25)                          | 22 (21/1)                               | 29 (28/1)                               |
| VF 2014    | 28 (22/6)                             | 27 (21/6)                           | 1 (1/0)                                 | 1 (1/0)                                 |
| VF 2015    | 64 (49/15)                            | 48 (34/14)                          | 10 (9/1)                                | 16 (15/1)                               |

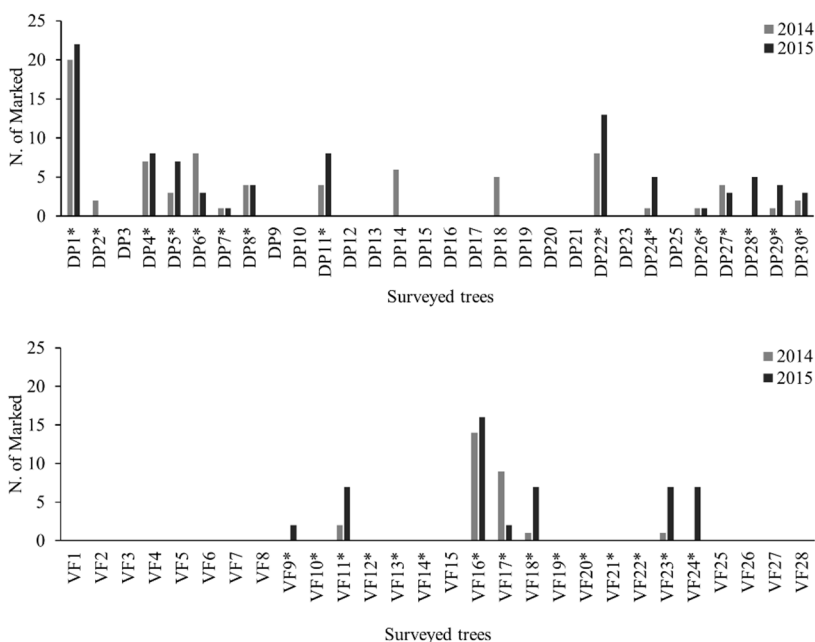


Figure 4. Number of marked individuals found in each tree during 2014 and 2015 at Difesa di Pescasseroli (DP) and Val Fondillo (VF). Trees with '\*' were surveyed in both the years.

Table 4. Population size estimates (N) and standard error (SE) obtained by using CMR data (POPAN model) and abundance data (RRC model) of the two study sites: Difesa di Pescasseroli (DP) and Val Fondillo (VF); for each estimate is reported the coefficient of variation (CV). (\*) The only reliable population size estimate among the POPAN models is given by  $\phi(.)p(.)pent(t)$  for VF 2014 (see 3.2).

| Study site | Model | N   | SE | CV (%) |
|------------|-------|-----|----|--------|
| DP 2014    | POPAN | 127 | 37 | 29.1   |
|            | RRC   | 115 | 29 | 24.9   |
| DP 2015    | POPAN | 141 | 18 | 12.7   |
|            | RRC   | 114 | 31 | 27.6   |
| VF 2014*   | POPAN | 55  | 10 | 18.2   |
|            | RRC   | 19  | 5  | 26.7   |
| VF2015     | POPAN | 110 | 24 | 21.8   |
|            | RRC   | 31  | 7  | 24.3   |

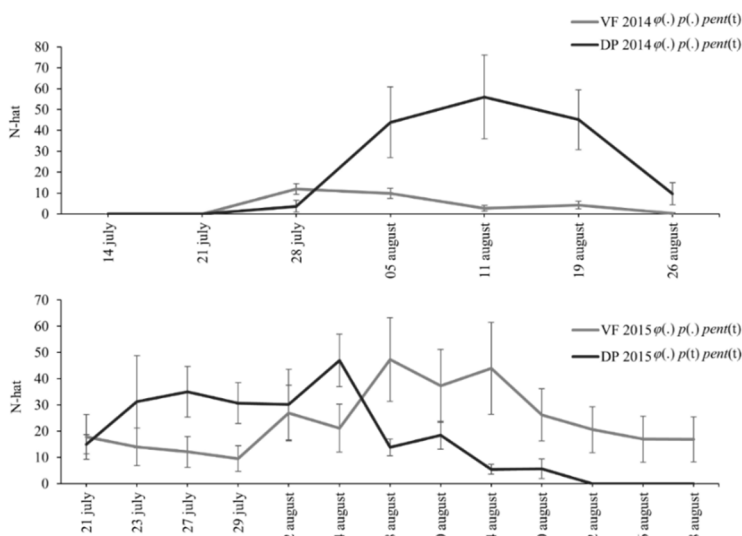


Figure 5. Phenology and abundance of marked individuals of 2014 and 2015 in Val Fondillo (VF) and Difesa di Pescasseroli (DP). The graphic shows the estimated daily population size ( $N\text{-hat}$ ) of the best POPAN model selected by AIC (excepted for VF 2014 model, which was not placed at first AIC score, but it was the only model which estimated population size and  $N\text{-hat}$ , see 3.2). Thirteen surveys are reported in 2015 instead of fourteen, because one missed due to bad weather conditions in both study sites.

Table 5. Primary parameter estimates  $\varphi$  (survival probability)  $p$  (recapture probability)  $pent$  (probability of entering the population) of best POPAN population model in the two study sites, Difesa di Pescasseroli (DP) and Val Fondillo (VF). For each model are reported the AICc values, AIC weight, Maximum Likelihood and Number of Parameter estimated. For parameters time dependent (t), it was reported the mean and SE of the reliable estimates given in Mark program.

| Study site | Model                   | AICc   | AICc W | ML   | N. Par | $\varphi \pm S.E.$ | $p \pm S.E.$    | $pent \pm S.E.$ |
|------------|-------------------------|--------|--------|------|--------|--------------------|-----------------|-----------------|
| DP 2014    | $\varphi(.)p(.)pent(t)$ | 99.28  | 0.88   | 1    | 6      | $0.21 \pm 0.06$    | $0.54 \pm 0.18$ | $0.32 \pm 0.06$ |
| DP 2015    | $\varphi(.)p(t)pent(t)$ | 228.9  | 0.76   | 1    | 16     | $0.74 \pm 0.03$    | $0.48 \pm 0.18$ | $0.13 \pm 0.07$ |
| VF 2014*   | $\varphi(.)p(.)pent(t)$ | 36.29  | 0.03   | 0.04 | 5      | $0.06 \pm 0.04$    | -               | $0.18 \pm 0.07$ |
| VF 2015    | $\varphi(.)p(.)pent(t)$ | 182.92 | 0.99   | 1    | 10     | $0.88 \pm 0.03$    | $0.21 \pm 0.07$ | $0.23 \pm 0.10$ |

(\*) The only reliable parameter estimates among the POPAN models for VF 2014 (see 3.2).

### 3.3 Habitat preference analysis

PCA analyses showed a strong dissimilarity in environmental variables between Zio Mas (ZM) and Difesa di Pescasseroli (DP), while Val Fondillo (VF) resulted in an overlapping between them (Fig. 6). Of the nine principal components axes, PC1:PC7 explained the 94% of cumulative proportion of variance. Six variables differed significantly among sites: DBH ( $r^2 = 0.59$ ,  $p = 0.001$ ), WDC ( $r^2 = 0.27$ ,  $p = 0.001$ ), WA ( $r^2 = 0.21$ ,  $p = 0.001$ ), MLP ( $r^2 = 0.18$ ,  $p = 0.001$ ), DNC ( $r^2 = 0.13$ ,  $p = 0.01$ ) and TH ( $r^2 = 0.07$ ,  $p = 0.04$ ). In particular, ZM showed: a lower number of trees involved by WA and MLP and trees with smaller diameter (Mean and SD: 40 cm  $\pm$  13) respect to DP (Mean and SD: 118 cm  $\pm$  23) and VF (Mean and SD: 72 cm  $\pm$  38). A larger number of trees of middle-advanced decay status was observed in DP (WDC class I: 0 class II: 3 class III: 16 class IV: 9) respect to ZM (WDC class I: 1 class II: 15 class III: 12 class IV: 0) and VF (WDC class I: 1 class II: 11 class III: 16 class IV: 0). Colonized trees were closer each other and uniformly distributed in DP (Mean SD: 112 m  $\pm$  78), respect to the grouped distribution ( $SD \geq \text{Mean}$ ) in VF (Mean SD: 226 m  $\pm$  217) and ZM (Mean SD: 360m  $\pm$  389).

Testing the two random effects on the starting model with full variables (nine environmental variables), the best model selected by AIC resulted M3 (Site + Survey as random effect) which was significantly different from the model M2 (Survey random effect) (LRT: Chisq. = 97.83, df. = 1,  $p < 0.0001$ ) (Table 6a). The final best model obtained by backward and AIC selection resulted M3a (deviance = 445.3, df. resid = 605,  $r^2 = 0.65$ ) with seven significant explanatory variables: tree status, tree diameter, tree height, wood decay class, canopy closure, presence of cavities and presence of *Morimus* laying pits (Table 6b).

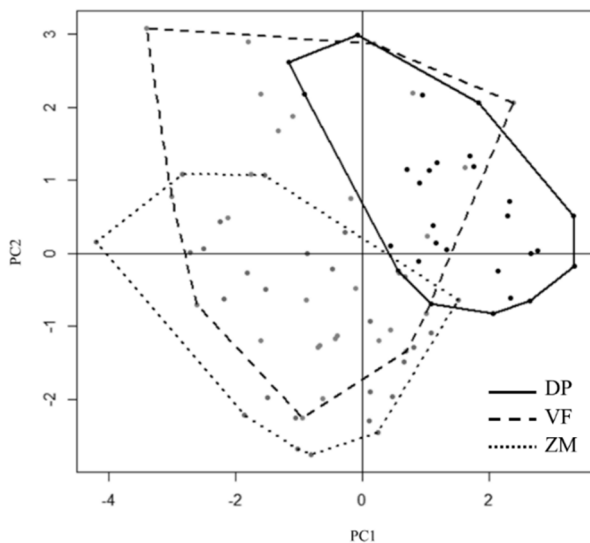


Figure 6. Ordination plot obtained by PCA analysis on the environmental variables collected for the selected trees of the three study sites in the Abruzzo region: Difesa di Pescasseroli (DP), Val Fondillo (VF) and Zio Mas (ZM).



Table 6. Generalized Linear Mixed effects Poisson Models results. a) Random effects selection and backward selection of the best model (with all significant explanatory environmental variables) for *Rosalia alpina* (number of marked individuals). For each model are reported the AIC value and Delta AIC for selecting the best model, the value of variance (Var.) and standard deviation (SD) of each random effect and the two  $r^2$  values of the model (considering the fixed effects and the random effect). Total number of marked individuals = 117, total number of observations = 616 (88 trees x 7 surveys), random effects levels: Site = 3, Survey = 7. b) Parameters estimates and standard error (S.E.) of the seven explanatory variables of the best model; significant codes: 0 ‘\*\*\*’, 0.001 ‘\*\*’, 0.01 ‘\*’, 0.05 ‘.’. Environmental variables abbreviations: TS = Tree Status (three categories living, decaying and dead tree), DBH = Tree Diameter, WDC = Wood Decay Class, TH = Tree Height, CC = Canopy Closure, CA = Cavity, MLP = *Morimus* laying pits (see Table I).

| a)   |   |           |           |  |            |             |
|--|---|-----------|-----------|--|------------|-------------|
| Model  | Function  | AIC       | Delta AIC | Random effect<br>(Var. + SD)             | fix. $r^2$ | rand. $r^2$ |
| M3a  | marked ~ TS + DBH + WDC + TH + CC +<br>CA + MLP + (1 Survey) + (1 Site) | 467.3     | 0         | Survey: 4.83 ± 2.2<br>Site: 2.03 ± 1.43  | 0.40       | 0.65        |
| M3   | marked ~ Full variables+ (1 Site)+(1 Survey)                            | 468.4     | 1.1       | Survey: 4.91 ± 2.22<br>Site: 2.35 ± 1.53 | 0.40       | 0.66        |
| M2   | marked ~ Full variables + (1 Survey)                                    | 487.8     | 20.1      | Survey: 4.18 ± 2.05                      | 0.30       | 0.50        |
| M1   | marked ~ Full variables + (1 Site)                                      | 564.3     | 97.0      | Site: 1.89 ± 1.38                        | 0.30       | 0.30        |
| b)   |   |           |           |  |            |             |
| Best model M3a: marked ~ TS + DBH + WDC + TH + TC + CA + MLP + (1 Survey) + (1 Site) |   |           |           |  |            |             |
| Fixed effects  | Parameter   | Estimates | SE        | z value                                  | pr(> z )   |             |
|  | Intercept   | -12.661   | 1.873     | -6.759                                   | ***        |             |
|  | TS decaying   | 2.257     | 0.371     | 6.078                                    | ***        |             |
|  | TS living   | -0.427    | 0.399     | -1.069                                   | p = 0.3    |             |
|  | DBH   | 0.015     | 0.004     | 4.374                                    | ***        |             |
|  | WDC   | 2.236     | 0.352     | 6.349                                    | ***        |             |
|  | TH  | 0.166     | 0.032     | 5.145                                    | ***        |             |
|  | CC  | -0.035    | 0.006     | -6.045                                   | ***        |             |
|  | CA  | -0.836    | 0.232     | -3.603                                   | ***        |             |
|  | MLP   | 0.933     | 0.269     | 3.460                                    | ***        |             |

#### 4. Discussion

Population studies of animal species should be carried out carefully if they involves artificial marking (Hagler & Jackson, 2001); this is particularly true for *Rosalia alpina*, its being a species protected under the European HD and having slight elytra and delicate body, this requiring special care during the handling. Therefore, some studies (Campanaro et al. 2011; Trizzino et al. 2013) have suggested using natural marking in population studies, based on individual identification, for monitoring the species.

Our study demonstrated that the computer-aided photographic identification method, by using images of natural marking of *R. alpina*, is a reliable method for a CMR study.

In our sampling protocol, we used a wide mouth bottle to facilitate the image acquisition of the elytral pattern and to speed up the fieldwork during the CMR procedure, responding to the criterion of an instantaneous marking (Amstrup et al. 2005). The photographic framing constraints given by the square bottle, allowed us to obtain standardized and comparable images, giving the best conditions for the analysis performed with I<sup>3</sup>SC software. In fact, the I<sup>3</sup>SC performance resulted with the 100% of living recaptured individuals correctly identified considering the first three top positions in the output list, and the 91.7% if we consider only the first rank position. Caci et al. (2013) obtained similar results using a dataset mostly made by images of museum specimens: 94.8% when considered the three top positions, and 92.1% if considered only the top rank. However, the identification procedure resulted limited when the I<sup>3</sup>SC examined images of dead individual remains, probably because these assumed a different shape due to degradation process.

The approach followed in this study, by comparing the contours of different elytral spots, suggested that using either only the central spots or the total number of spots we gather images which allow the same level of correct identification among individuals. Indeed, the variation that allows the correct discrimination is mainly concentrated in the central elytral spots. Sub-setting an area of the natural marking is a technique used in other studies, in particular when the analysis are performed by following the ROI approach, i.e. by selecting the region of interest of the image (Hoque et al. 2011; Lahiri et al. 2011). Both contours, central and total spots, have returned the same identification results, but using only the central spot contours made the tracing contour process less time-consuming, reducing the image elaboration to half the time. In few cases, the central spot contours of two different specimens resulted very similar, therefore, the operator must focus the attention on the anterior or posterior spots in the final recognition. When individuals exhibited one elytron damaged or covered by the wing, only two specimens in our dataset, it was possible to perform the match by using the contour of only one elytron (Caci et. al. 2013). In our case, during the identification process, it was possible to find a threshold of certainty to identify new individuals or recaptures by looking at the first score value (or at least the second or third score) in combination with the discriminant value (i.e. Delta score). Even if the final decision must be always carried out by the operator, the screening process is facilitated by

I<sup>3</sup>SC software, otherwise it would be time-consuming and hard to perform manually (Kelly 2001, Speed et al. 2007; Sacchi et al. 2010). Although the photo identification method used in our study does not avoid the need of capture, the procedure that we performed was quick and probably less invasive than other marking methods. Indeed, photographic identification method limited the manipulation of individuals respect to the artificial marking procedures, like observed in other studies (Bradfield 2004; Gamble et al. 2008; Hoque et al. 2011, Knox et al. 2012).

The two different models used to estimate population size resulted almost equivalent in Difesa di Pescasseroli, while showed very different estimates for Val Fondillo, in both years. Probably, the Royle Repeated Count model works better when the species abundance is distributed more uniformly among the surveyed trees during the sampling period, like in Difesa di Pescasseroli, otherwise it could underestimate the population size due to violations of prior parameters assumptions of the model (Royle 2004). On the other hand, also the POPAN model did not give reliable population parameter estimates for Val Fondillo in 2014, due to the low amount of data collected.

Sampling designs and data analysis to get information on population parameters and species ecology were largely discussed (Williams et al. 2002; Burnham and Anderson 2002; MacKenzie et al. 2004; Thompson 2004; Amstrup et al. 2005; Sutherland W.J. 2006). In this study, adjusting the sampling design by inspecting the sub-set of trees selected in 2014, and by increasing the number of surveys, allowed us to get population estimates in the same sites where it was performed in 2015, acquiring more information on survival probability and dispersal. In our case, the sampling effort was reduced in some aspects (lower number of trees to inspect and hours per survey) but increased in others (a higher number of surveys); however, this approach is generally suggested for species with low detectability (MacKenzie and Royle 2005).

Sex ratio and sex recapture rate observed in both years led us to suggest a higher detectability of males, probably due to their greater activity, like observed in other beetles (Tikkamäki and Komonen 2011, Chiari et al. 2013a). Probably, females are more elusive, but previous data showed that they move from a tree to another to find sites for laying eggs (Drag et al 2011); in this regard, the only two females recaptured in our study were both found in a different tree from the one of their first capture. Dispersal was observed for few individuals, and the longest distance was recorded for a male, 750 m, which was slightly higher of that one observed by Drag et al. (2011). In both years, the higher percentage of marked individuals was

recorded for the same tree; similar aggregative behaviour was observed in other longhorn beetles (Hanks et al. 1999). The trees in which was found the highest number of marked individuals may represent a “key tree” for population viability, and could give information on the population trend during a monitoring program. In the study sites, where the distribution of suitable trees was scattered, dispersion could have been limited and have suffered of the gap effect given by large areas where no suitable habitats occur (Bosso et al. 2013). This distribution pattern may result in isolation between populations and consequently it may increase the local extinction risk (Hanski and Gaggiotti 2004, Laschat et al. 2013). A low number of data was collected in the PNFC, which resulted to be a less suitable area respect to PNALM, where the species showed different patterns of abundance in the three study sites. Decaying huge trees, in the III-IV stages of decay class, mainly located in opened areas (i.e. with a low canopy closure), represented the preferred habitat of the species, like observed in previous studies (Duelli and Wermelinger 2005, Russo et al. 2011). The sun-exposed location of trees may limit the humid microclimate typical of the trunk cavities, explaining their negative association with the species. Trees colonized by *R. alpina* represented also a good host plant for *M. asper/funereus*. These two species may be probably considered as umbrella species for the saproxylic community, like another longhorn beetle *Cerambyx cerdo* (Buse et al., 2008). Therefore, old huge trees confirmed their important role as resource for saproxylic beetles (Ranius and Jansson, 2000; Lindhe et al. 2005, Redolfi de Zan et al., 2014) and in our study the co-occurrence of two species currently protected by the Habitats Directive 92/43EEC, highlighted the need to preserve these kind of trees.

## 5. Conclusions with implication for monitoring plans

The computer-aided photographic identification method resulted to be a reliable, less invasive method for population studies on *Rosalia alpina*. Nevertheless, to facilitate the identification procedure with I<sup>3</sup>SC software, images must be taken following some advices. We suggest to standardize pictures limiting the framing angle variations by photographing the specimens within a polyethylene wide mouth bottle or a similar tool, facilitating the procedure on field. Furthermore, using only the elytral central spot contours in the I<sup>3</sup>SC software, made all the identification procedure less time-consuming.

After a first explorative year, an adjusted sampling design by balancing data acquisition and sampling effort may result in a quick and more efficient sampling protocol, always in relation to the aim of the research. This approach can represent a good strategy, in particular when the target species shows a reduced abundance and scattered distribution. Finally, trees identified as “key trees” for the species must not be removed, because they ensure the population viability and give information on phenology and growth population trend during a monitoring program. The forest management should promote the maintenance of old and huge trees interspersed within opened areas and, in particular, should preserve old trees distributed around “key trees” to increase dispersal and colonization by the species.

## **Acknowledgements**

The present work was developed within the EU project LIFE11 NAT/IT/000252, with the contribution of the LIFE financial instrument of the European Union. The authors thank all the MIPP staff and the Insitutions involved: Corpo Forestale dello Stato, Consiglio per la ricerca in Agricoltura e l'Analisi dell'Economia Agraria - Centro di Ricerca per l'Agrobiologia e la Pedologia, Università degli studi di Roma La Sapienza, Università degli studi di Roma Tre, Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Regione Lombardia. Moreover, the authors are grateful to the local offices of the Corpo Forestale dello Stato that administrate the study sites, i.e. the UTB Castel di Sangro (Tiziana Altea, Federica Desprini, Lucia Eusepi, Mario Romano) and the UTB Pratovecchio (Giovanni Quilghini, Silvia Bertinelli, Sandro Marsella, Matteo Padula, Barbara Rossi, Antonio Zoccola), and also the staff of the Abruzzo National Park (Cinzia Sulli, Paola Tollis).

We are also grateful to all the field assistants which voluntarily help during the surveys: Sara Amendolia, Marco Boscaro, Giulia Caruso, Anna Cuccurullo, Patrizia Giangregorio, Andrea Mancinelli, Marco Molfini, Alessandro Morelli, Margerita Norbiato, Giulia Albani Rocchetti, Randi Rollins, Rosaria Santoro, Ventura Talamo, Melissa Yslas, Ilaria Zappitelli and Cooperativa In Quiete.

## **References**

- Alexander K.N.A., 2008. Tree biology and saproxylic Coleoptera: issues of definitions and conservation language. *Rev. Écol. (Terre Vie)*, vol. 63, 2008.
- Amstrup, S.C., McDonald, T.L., Manly, B.F.J., 2005. Handbook of capture-recapture analysis. Princeton University Press, Princeton.
- Binner V. and Bussler H. 2006. Erfassung und Bewertung Von Alpenbock-Vorkommen. *Naturschutz und Landschaftsplanung* 38: 378-382.
- Bosso L., Rebelo H., Garonna A.P. and Russo D. 2013 Modelling geographic distribution and detecting conservation gaps in Italy for the threatened beetle *Rosalia alpina*. *Journal for Nature Conservation* 21: 72– 80.
- Bradfield, K.S. 2004. Photographic identification of individual Archey's frog *Leiopelma archeyi*, from natural markings. Department of Conservation, PO Box 10-420, Wellington, New Zealand.
- Burnham K. and Anderson D., 2002. Model Selection and Multi-Model Inference (2nd Edition). Springer-Verlag.
- Buse, J., Ranius, T., Assmann, B., 2008. An endangered longhorn beetle associated with old oaks and its possible role as an ecosystem engineer. *Conserv. Biol.* 22, 329–337. DOI:10.1111/j.1523-1739.2007.00880.x
- Caci G., Biscaccianti A.B., Cistrone L., Bosso L., Garonna A.P., Russo D. 2013. Spotting the right spot: computer-aided individual identification of the threatened cerambycid beetle *Rosalia alpina*. *Journal of Insect Conservation*, DOI 10.1007/s10841-013-9561-0.
- Campanaro, A., Bardiani, M., Spada, L., Carnevali, L., Montalto, F., Antonini, G., Mason, F., Audisio, P., 2011. Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxylica/ Guidelines for monitoring and conservation of saproxylic insects. *Quaderni Conservazione Habitat*, 6. Cierre Grafica, Verona.
- Chiari, S., Bardiani, M., Zauli, A., Hardersen, S., Mason, F., Spada, L., Campanaro, A., 2013a. Monitoring of the saproxylic beetle *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) with freshly cut log piles. *J. Insect Conserv.* 17: 1255-65. DOI:10.1007/s10841-013-9606-4.
- Chiari S., Zauli A., Mazziotta A., Luiselli L., Audisio P., Carpaneto G.M. 2013b. Surveying an endangered saproxylic beetle, *Osmoderma eremita*, in Mediterranean woodlands: a comparison between different capture methods. *Journal of Insect Conservation*, 17 (1): 171-181.

- Ciach M., Michalcewicz J., Fluda M. 2007. The first report on development of *Rosalia alpina* (Linnaeus, 1758)(Coleoptera: Cerambycidae) in wood of *Ulmus* L. in Poland. Polish Journal of Entomology, 76: 101–105.
- Cizek, L., Schlaghamerský, J., Bořucký, J., Hauck, D. and Helešic J. 2009. Range expansion of an endangered beetle: Alpine Longhorn *Rosalia alpina* (Coleoptera: Cerambycidae) spreads to the lowlands of Central Europe. Entomologica Fennica, 20, 200–206.
- Cooch E. G. & G.C. White 2015. Program MARK: a gentle introduction (14th Edition). Available online in PDF format at: <http://www.phidot.org/software/mark/docs/book/>
- den Hartog J. & Reijns R. 2011. I3S Contour Manual: Interactive Individual Identification System. Available free on [www.reijns.com/i3s](http://www.reijns.com/i3s)
- Drag, L., Hauck, D., Pokluda, P., Zimmermann, K., & Cizek, L. (2011). Demography and dispersal ability of a threatened saproxylic beetle: A mark-recapture study of the rosalia longicorn (*Rosalia alpina*). PLoS One, 6(6), e21345. <http://dx.doi.org/10.1371/journal.pone.0021345>
- Duelli, P., & Wermelinger, B. (2005). Der Alpenbock (*Rosalia alpina*). Ein seltener Bockkafer als Flaggschiff Art. Merkblatt für die Praxis, 39, 1–8.
- Gamble L., Ravela S. and McGarigal K. 2008. Multi-scale features for identifying individuals in large biological databases: an application of pattern recognition technology to the marbled salamander *Ambystoma opacum*. Journal of Applied Ecology, 45:170–180.
- Hagler, J.R. & Jackson, C. G. (2001). Methods for marking insects: current techniques and future prospects. Annual Review of Entomology 46, 511–543.
- Hanks L.M., 1999. Influence of the larval host plant on reproductive strategies of cerambycid beetles. Annu. Rev. Entomol. 44: 483–505.
- Hanski I. and Gaggiotti O. 2004. Ecology, genetics and evolution of metapopulations. Elsevier Academic Press, San Diego.
- Hiby L., Lovell P., Patil N., Samba Kumar N., Gopalaswamy A.M., Ullas Karanth K. 2009. A tiger cannot change its stripes: using a three-dimensional model to match images of living tigers and tiger skins. Biol Lett 5:383–386
- Hines JE, MacKenzie DI (2004) PRESENCE, version 8.2. Available at <http://www.mbr-pwrc.usgs.gov/software.html>

- Hoque S., Azhar M.A.H.B. and Deravi F. 2011. ZOOMETRICS – Biometric Identification of Wildlife Using Natural Body Marks. *International Journal of Bio-Science and Bio-Technology*, 3: 45-53
- Hunter Jr., M.L., 1990. *Wildlife, Forests, and Forestry. Principles of Managing Forests for Biological Diversity*. Prentice Hall, Englewood Cliffs, NJ.
- Jurc, M., Ogris, N., Pavlin, R., & Borkovic, D. (2008). Forest as a habitat of saproxylic beetles on Natura 2000 sites in Slovenia. *Revue d'Ecologie*, 63, 53–66.
- Kelly M.J. 2001. Computer-aided photograph matching in studies using individual identification: An example from Serengeti cheetahs. *J Mammal* 82:440–449.
- Knox C.D., Cree A. and Seddon P.J. 2012. Accurate identification of individual geckos (*Naultinus gemmeus*) through dorsal pattern differentiation. *New Zealand Journal of Ecology*, Vol. 37 N.1
- Lachat T., Ecker K., Duelli P. and Wermelinger B., 2013. Population trends of *Rosalia alpina* (L.) in Switzerland: a lasting turnaround? *J Insect Conserv.* DOI 10.1007/s10841-013-9549-9
- Lahiri M., Tantipathananandh C., Warungu R., Rubenstein D.I. and Berger-Wolf T.Y. 2011. Biometric Animal Databases from Field Photographs: Identification of Individual Zebra in the Wild. *Proceedings of the 1st ACM International Conference on Multimedia Retrieval* New York, NY, USA. ISBN: 978-1-4503-0336-1 doi>10.1145/1991996.1992002.
- Larsson M.J., Hedin J., Svensson G.P., Tolasch T., Francke W. 2003. The characteristic odour of *Osmoderma eremita* (Coleoptera: Scarabaeidae) identified as a male–released pheromone. *Journal of Chemical Ecology*, 29: 575–587.
- Lindhe A., Lindelöw A., Asenblad N. 2005. Saproxylic beetles in standing dead wood – Density in relation to substrate sun-exposure and diameter. *Biodiversity & Conservation* 14: 3033–3053.
- MacKenzie, D.I., Royle, J.A., Brown, J.A. & Nichols, J.D. 2004. Occupancy estimation and modeling for rare and elusive populations. *Sampling Rare or Elusive Species* (ed. W.L. Thompson), pp. 149–172. Island Press, Washington, DC.
- MacKenzie D.I. and Royle J.A. 2005. METHODOLOGICAL INSIGHTS Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology*, 42: 1105-1114.



- Mason, F., Roversi, P.F., Audisio, P., Bologna, M.A., Carpaneto, G.M., Antonini, G., Mancini, E., Sabbatini Peverieri, G., Mosconi, F., Solano, E., Maurizi, E., Maura, M., Chiari, S., Sabatelli, S., Bardiani, M., Toni, I., Redolfi De Zan, L., Rossi de Gasperis, S., Tini, M., Cini, A., Zauli, A., Nigro, G., Bottacci, A., Hardersen, S., Campanaro, A., 2015. Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). *Fragmenta entomologica*, 47, 51-52.
- Michalciewicz J., Ciach M., Bodziarczyk J. 2011. The unknown natural habitat of *Rosalia alpina* (L.) (Coleoptera: Cerambycidae) and its trophic association with the mountain elm *Ulmus glabra* in Poland—a change of habitat and host plant. *Pol J Entomol*, 80:23–31.
- Michalciewicz, J., & Ciach, M. (2012). *Rosalia longicorn* *Rosalia alpina* (L.) (Coleoptera: Cerambycidae) uses roadside European ash trees *Fraxinus excelsior* L. – an unexpected habitat of an endangered species. *Polish Journal of Entomology*, 81, 49–56.
- Michalciewicz J., Bodziarczyk J. and Ciach M. 2013. Development of the *rosalia longicorn* *Rosalia alpina* (L.) (Coleoptera: Cerambycidae) in the sycamore maple *Acer pseudoplatanus* L.— the first report from Poland. *Polish Journal of Entomology*, 82, 19–24.
- Nieto, A., & Alexander, K. N. A. (2010). European red list of saproxylic beetles. Luxembourg: Publications Office of the European Union.
- Pagola Carte S. 2011. Seguimiento de la poblaciòn de *Rosalia alpina* en el hayedo de trasmochos de Oieleku (LIC de Aiako Harria) (Acciòn E.7 del Proyecto Life + Maneyo y Conservaciòn de los habitats de *Osmoderma eremita*, *Rosalia alpina* y otros saproxílicos de interés comunitario en Gipuzkoa“). Dissertation, Donostia-SanSebastiàn, 57 pp.
- Ranius T. and Jansson N. 2000. The influence of forest regrowth, original canopy cover and tree size on saproxylic beetles associated with old oaks. *Biological Conservation* 95: 85–94.
- Ray A.M., Millar J.G., McElfresh S., Swift I.P., Barbour J.D. and Hanks L.M. 2009. Male-Produced Aggregation Pheromone of the Cerambycid Beetle *Rosalia funebris* *J Chem Ecol*, 35: 96–103. DOI 10.1007/s10886-008-9576-2.
- R Development Core Team 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

- Redolfi De Zan, L., Bellotti, F., D'Amato, D., Carpaneto, G.M., 2014. Saproxyllic beetles in three relict beech forests of central Italy: analysis of environmental parameters and implications for forest management. *For. Ecol. Manage.* 328: 229-244. DOI:10.1016/j.foreco.2014.05.040
- Romero-Samper, J., Bahillo, P., 1993. Algunas observaciones sobre la distribución y biología de *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) en la Península Ibérica. *Boln. Asoc. Esp. Ent.* 17, 103-122.
- Royale J.A., 2004. - N-mixture models for estimating population size from spatially replicated counts. *Biometrics* 60:108-115.
- Russo, D., Cistrone, L., & Garonna, A. 2011. Habitat selection by the highly endangered long-horned beetle *Rosalia alpina* in Southern Europe: A multiple spatial scale assessment. *Journal of Insect Conservation*, 15, 685–693.
- Rydell J. and Russo D. 2015. Photography as a low-impact method to survey bats. *Mammalian Biology*, 80: 182 – 184.
- Sacchi R., Scali S., Pellitteri-Rosa D., Pupin F., Gentili A., Tettamanti S., Caviglioli L., Racina L., Maiocchi V., Galeotti P. and Fasola M. 2010. Photographic identification in reptiles: a matter of scales. *Amphibia-Reptilia* 31: 489-502
- Sama, G., 1988. Coleoptera, Cerambycidae. *Catálogo Topográfico e Sinonímico. Fauna d'Italia. XXXVI. Ed. Calderini, Bologna.*
- Sama, G. 2002. Atlas of the Cerambycidae of Europe and Mediterranean Area: Northern, Western, Central and Eastern Europe. British Isles and Continental Europe from France (excl. Corsica) to Scandinavia and Urals. *Vit Kabourek, Kabourek, Zlín.*
- Sama, G., 2004. Fauna Europaea: Cerambycidae. In: Audisio P. (ed.), *Fauna Europaea: Coleoptera 2, Beetles. Fauna Europaea version 1.0.* Available at <http://www.faunaeur.org> (accessed September 2015).
- Sama G., 2005. Coleoptera Cerambycidae, pp. 217–219 + CD-ROM. In: Ruffo S. & Stoch F. (eds.), *Checklist e distribuzione della fauna italiana. Memorie del Museo civico di Storia naturale di Verona, II serie, Sezione Scienze della Vita*, 16.
- Schofield G., Katselidis K.A., Dimopoulos P., Pantis J.D. 2008. Investigating the viability of photoidentification as an objective tool to study endangered sea turtle populations. *J Exp Mar Biol Ecol* 360:103–108

- Speed CW, Meekan MG, Bradshaw CJA (2007) Spot the match— wildlife photoidentification using information theory. *Front Zool* 4:2. doi:10.1186/1742-9994-4-2
- Speight M.C.D., 1989. Saproxylic invertebrates and their conservation. Council of Europe, Publications and Documents Division, Strasbourg.
- Stokland J. N., Siitonen J., Jonsson B. G. 2012. Biodiversity in Dead Wood. Cambridge University Press, Cambridge, UK.
- Sutherland W.J. 2006. Ecological census techniques: a handbook. Cambridge University Press, Cambridge, United Kingdom.
- Svensson GP, Oleksa A, Gawronski R, Lassance JM, Larsson MC 2009. Enantiomeric conservation of the male-produced sex pheromone facilitates monitoring of threatened European hermit beetles (*Osmoderma* spp.). *Entomol Exp Appl* 133:276–282.
- Tikkamäki, T., Komonen, A. 2011. Estimating population characteristics of two saproxylic beetles: a mark-recapture approach. *J. Insect Conserv.* 15, 401–408. DOI:10.1007/s10841-010-9313-3.
- Thompson W.L. 2004. Sampling Rare or Elusive Species: Concepts, Designs, and Techniques for Estimating Population Parameters, Island Press, Washington, D.C., USA.
- Trizzino, M., Audisio, P., Bisi, F., Bottacci, A., Campanaro, A., Carpaneto, G.M., Chiari, S., Hardersen, S., Mason, F., Nardi, G., Preatoni, D.G., Vigna Taglianti, A., Zauli, A., Zilli, A., Cerretti, P., 2013. Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat, 7. CFS-CNBFVR, Centro Nazionale Biodiversità Forestale. Cierre Grafica, Sommacampagna, Verona.
- Van Tienhoven AM, den Hartog JE, Reijns RA, Peddemors VM (2007) A computer-aided program for pattern-matching natural marks on the spotted ragged tooth shark *Carcharias Taurus* (Rafinesque, 1810). *J Appl Ecol* 44:273–280
- Vrezec, A., Ambrožič, Š., Kapla, A., 2012. An overview of sampling methods tests for monitoring schemes of saproxylic beetles in the scope of Natura 2000 in Slovenia. In: Maja J. (ed.), Saproxylic beetles in Europe: monitoring, biology and conservation. *Studia Forestalia Slovenica* 137, 73–90.
- White, G.C., Burnham, K.P., 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study* 46, 120–139.
- Williams K., Nichols J. and Conroy M., 2002. Analysis and Management of Animal Populations. Academic Press.

- Wilson B., Hammond P.S., Thompson P.M. 1999. Estimating size and assessing trends in a coastal bottlenose dolphin population. *Ecol Appl* 9:288–300
- Zauli, A., Chiari, S., Hedenström, E., Svensson, G.P., Carpaneto, G.M., 2014. Using odour traps for population monitoring and dispersal analysis of the threatened saproxylic beetles *Osmoderma eremita* and *Elatér ferrugineus* in central Italy. *J. Insect Conserv.* 18, 801–813. DOI:10.1007/s10841-014-9687-8.

### Conclusions with implications for conservation and forest management

#### 5.1 Summary of the results

5.1.1 Results of chapter 2: Overwintering ability and habitat preference of the threatened longhorn beetle *Morimus asper* / *funereus*: a two years mark-recapture study with implications for conservation and forest management.

The long adult lifespan in natural conditions that we observed, more than 400 days, was never evidenced before this study. *M. asper* is able to hibernate, probably inside refuges under bark or within tree hollows, where it can survive the low temperatures of winter (Baur et al., 2005). The survey protocol performed (inspecting suitable trees for the species in several surveys) allowed us to collect many data about *M. asper*. The adult phenology showed two peaks of activity (middle of May and middle of June) with no difference between sexes. Behavioral aspects (females are more elusive and males more visible) probably affected the sex rate and sex recapture rate in favor of males (Drag et al. 2011, Tikkamäki and Komonen 2011, Chiari et al. 2013). The dispersal ability of *M. asper* is for sure influenced by its flightless condition; however, the high recapture rate on the same tree can also be a consequence of sedentary behavior (Hanks 1999). The differences in population abundance observed between two years can be explained by the natural fluctuations of adult emergencies due to the long larval development period of the species (Samways, 2005) in combination with natural (e.g. tree fell that releases attractive compounds) or anthropic events (e.g. dead wood removal which reduces resource availability). The recapture probability was generally temperature dependent, suggesting an ideal range of mean daily temperature of 15-26°C for survey. In the three study sites investigated, most of captures and mating events of both years were recorded on a single huge tree; this can allow us to define this tree like a “key tree” for the population. Exit holes and shallow laying pits resulted positively correlated to some woodpecker variables, confirming the importance of the larvae as trophic resources for birds (Pechacek and Kristin, 2004). Furthermore, these two *Morimus* features showed a negative correlation with canopy closure, which could mean that the species prefer trees with sun exposed parts for laying eggs and consequently for larval development (Albert et al., 2012). The species showed preference for the lying position of tree (i.e. dead or recently dead

trees) with minor and major branches on the ground, like observed for other saproxylic beetles (Cocciufa et al., 2014; Ranius and Jansson, 2000; Redolfi de Zan et al., 2014). Canopy closure showed a positive effect on adult abundance, because probably high canopy cover in the surrounding of lying trees preserves the preferred range of daily temperature. Furthermore, the species prefer the early and medium stages of decaying wood. Probably, like observed in *Cerambyx cerdo* (Buse et al. 2007), a tree at higher decay stage could have already been overexploited and not represent more a good substrate for egg laying and larval developing.

### 5.1.2 Results of chapter 3: Sexual dimorphism and intra-specific allometric relationships between secondary sexual character and body size in *Morimus asper/funereus*.

The sexual dimorphism assessed in our study, suggests that in males, a great investments of resource is directed in development of anterior body traits (e.g. antennal and pronotum length), whereas, in females, more resources are invested for developing wider and longer posterior body traits, which contain reproductive apparatus. The allometric relationships between antennal length and elytra length in males exhibits a significant change in the slope described by the segmented model, with a switch point dividing the male population in two groups. From the switch point elytra value onward (i.e. larger males), the growth rate between the two morphological traits is lower than that of males with elytra length below the switch point (i.e. smaller males) (Goldsmith 1985; Pomfret and Knell 2006). Due to some overlaps in the independent variable, it is difficult to identify two morphologies (Knell 2009). However, smaller males showed a positive allometry while larger males growth trend tends to isometry (Emlen and Nijhout 2000; Pomfret and Knell 2006). The growth trend observed in larger males seems to be related to competition for resources among rapidly growing body parts in the pupa (Huxley 1932; Nijhout and Wheeler 1996; Knell et al. 2004). Furthermore, feeding resource availability are used for the development of trade-off supporting traits (Eberhard 1982; Emlen and Nijhout 2000; Emlen and Allen 2004; Nijhout 1994; Painting and Holwell 2013). Probably, in larger males, more resources are allocated to enlarge and to strengthen the body to bear such long antennae. Males observed in mating were generally larger than unmated males, and larger males mated more frequently. We may suppose that they won more contests for get females (Hughes and Hughes 1982). The fact that the smaller males

outnumbered the larger ones may give them an equal mating possibility even if they lose contests. Probably small males are favored by natural selection due to other factors, such as predation by birds and mammals, because they are more hardly detected, even if this hypothesis should be tested by further studies. Our observations suggest also a possible influence of body size on dispersal and overwintering ability, but they need further investigations.

#### 5.1.3 Results of chapter 4: Computer-aided Photographic Identification of *Rosalia alpina* (Coleoptera: Cerambycidae): first application in a mark-recapture study

Our study demonstrated that the computer-aided photographic identification method, by using images of natural marking of *R. alpina*, is a reliable method for a CMR study. However, if identification will be performed by using I<sup>3</sup>SC software, images have to be of good resolution to make possible the spots contour tracing process and they need to be as much as possible standardized to each other. In our case we get comparable images by photographic adults within a wide mouth square bottle. Using the central spot contours made the tracing contour process less time-consuming and facilitated the identification. When individuals exhibited one elytron damaged or covered by the wing, we successfully matched images by using the contour of only one elytron (Caci et al. 2013). During the identification process, it was possible to find a score threshold value of certainty to identify new individuals or recaptures. Even if the final decision must be always carried out by the operator, the screening process has been facilitated by I<sup>3</sup>SC software, otherwise it would be time-consuming and hard to perform manually (Sacchi et al. 2010; Caci et al. 2013). The computer-aided photographic identification method was quick and less invasive, because it limited the manipulation of individuals (Bradfield 2004; Gamble et al. 2008; Hoque et al. 2011; Knox et al. 2012). The Royle Repeated Count model resulted to be a good model when applied to Difesa di Pescasseroli data, where the species abundance was distributed more uniformly among the surveyed trees during the sampling period (Royle 2004), otherwise the model underestimated the population size. In this study, adjusting the sampling design by inspecting the sub-set of trees

selected in 2014, and by increasing the number of surveys, allowed us to get population estimates in the same sites where it was performed in 2015, acquiring more information on survival probability and dispersal. In our case, the sampling effort was reduced in some aspects (lower number of trees to inspect and hours per survey) but increased in others (a higher number of surveys); however, this approach is generally suggested for species with low detectability (MacKenzie and Royle 2005). In both years, the higher percentage of marked individuals was recorded for the same tree, therefore defined as “Key tree”; similar aggregative behaviours were observed in other cerambycid beetles (Hanks et al. 1999). Dispersion could have been limited and have suffered of the gap effect given by large areas where no suitable habitats occur (Bosso et al. 2013). Dying huge trees, in a medium decay class, therefore with some bark missing, mainly located in opened areas (i.e. with a low canopy closure) represented the preferred habitat of the species, like observed in other studies (Duelli and Wermelinger 2005; Russo et al. 2011). Trees colonized by *R. alpina* represented also a good host plant for *M. asper/funereus*.

## **5.2 Suggestion for monitoring and preserving *M. asper/funereus* and *Rosalia alpina***

*M. asper* showed an unexpected longevity in natural conditions, and overwintered males were observed in mating in two consecutive years, providing the evidence of at least two reproductive seasons for these individuals. However, even if the species could have benefits by its long lifespan in terms of reproductive success, the sedentary nature combined with the flightless condition, represents the ecological limits of *M. asper* for colonizing new trees, making the species seriously threatened by woodland fragmentation. We suggest to plan the surveys of *M. asper* taking into account the optimal temperature range for adult activity. Therefore, surveys should be conducted at early morning or late evening for hot study sites, and warmest hours of the day for cold study sites.

The computer-aided photographic identification method resulted to be a reliable and less invasive method for population study of *Rosalia alpina*. Nevertheless, to facilitate the identification procedure with I<sup>3</sup>SC software, images must be taken following some precautions. In particular, we suggest to standardize pictures limiting the framing angle variations by



photographing the specimens within a polyethylene wide mouth square bottle or a similar tool, facilitating the procedure on the field.

In relation to the aim of the research, an adjusted sampling design by balancing data acquisition and sampling effort may result in a quick and more efficient sampling protocol. This approach can represent a good strategy in particular when the target species shows a reduced abundance and scattered distribution.

A special attention must be given to “key trees” which have been identified for both species. These trees must not be removed because they ensure population viability, acting like a “source” for facilitating colonization. Our results suggest that key trees are preferred spots for mating and egg-laying, in particular for *M. asper* because they represent a need for its populations; therefore, the population viability would be seriously affected by the removal of these trees, to avoid that they become a “sink”. Furthermore, key trees should be used as important indicators of the growth population trend in a monitoring protocol.

Forest management should promote the maintenance of old and huge trees, lying dead trees and coarse woody debris for both species, ensuring a resource availability and connectivity within woodlands and between wood fragments. In particular, within open areas, decaying old trees should be retained around “key trees” to increase their colonization by dispersing individuals of *R. alpina*. Furthermore, the mitigation of the forest management impact, not only in the interior part of a forest but also in the forest edge, trying to preserve the original microhabitat conditions (e.g. canopy closure and temperature), will improve the dispersal ability and colonization success of *M. asper*.

### 5.3 References

- Albert, J., Platek, M., Cizek, L., 2012. Vertical stratification and microhabitat selection by the Great Capricorn Beetle (*Cerambyx cerdo*) (Coleoptera: Cerambycidae) in open-grown, veteran oaks. Eur. J. Entomol. 109, 553–559.
- Baur, B., Corey, A., Minoretti, N., Zschokke, S., 2005. Dispersal of the endangered flightless beetle *Dorcadion fuliginator* (Coleoptera: Cerambycidae) in spatially realistic landscapes. Biol. Conserv. 124, 49–61. DOI:10.1016/j.biocon.2004.12.011.

- Bosso L., Rebelo H., Garonna A.P. and Russo D. 2013 Modelling geographic distribution and detecting conservation gaps in Italy for the threatened beetle *Rosalia alpina*. *Journal for Nature Conservation* 21: 72– 80.
- Bradfield, K.S. 2004. Photographic identification of individual Archey's frog *Leiopelma archeyi*, from natural markings. Department of Conservation, PO Box 10-420, Wellington, New Zealand.
- Buse, J., Schröder, B., Assmann, T., 2007. Modelling habitat and spatial distribution of an endangered longhorn beetle – A case study for saproxylic insect conservation. *Biol. Conserv.* 137: 372-381. DOI:10.1016/j.biocon.2007.02.025.
- Chiari S., Bardiani M., Zauli A., Hardersen S., Mason F., Spada L., Campanaro A. 2013. Monitoring of the saproxylic beetle *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) with freshly cut log piles. *J. Insect Conserv.* 17: 1255-65. DOI:10.1007/s10841-013-9606-4.
- Cocciufa, C., Gerth, W., Luiselli, L., Redolfi De Zan, L., Cerretti, P., Carpaneto, G.M., 2014. Survey of saproxylic beetle assemblages at different forest plots in central Italy. *Bull. Insectology* 67: 295-306.
- Caci G., Biscaccianti A.B., Cistrone L., Bosso L., Garonna A.P., Russo D. 2013. Spotting the right spot: computer-aided individual identification of the threatened cerambycid beetle *Rosalia alpina*. *Journal of Insect Conservation*, DOI 10.1007/s10841-013-9561-0.
- Drag L., Hauck D., Pokluda P., Zimmermann K., Cizek L. 2011. Demography and dispersal ability of a threatened saproxylic beetle: A mark-recapture study of the *rosalia longicorn* (*Rosalia alpina*). *PLoS One*, 6(6), e21345. Duelli and Wermelinger 2005
- Eberhard WG. 1982. Beetle horn dimorphism, making the best of a bad lot. *The American Naturalist* 119: 420-426.
- Emlen, DJ and Allen CE. 2004. Genotype to phenotype: physiological control of trait size and scaling in insects. *Integr Comp Biol* 43: 617– 634.
- Emlen DJ and Nijhout HF. 2000. The Development and Evolution of Exaggerated Morphologies in Insect. *Annu Rev Entomol* 45:661-780.
- Gamble L., Ravela S. and McGarigal K. 2008. Multi-scale features for identifying individuals in large biological databases: an application of pattern recognition technology to the marbled salamander *Ambystoma opacum*. *Journal of Applied Ecology*, 45:170–180.
- Goldsmith SK, Stewart Z, Adams S, Trimble A. 1996. Body Size, Male Aggression, and Male Mating Success in the Cottonwood Borer,

- Plectrodera scalator* (Coleoptera: Cerambycidae). *J Insect Behav* 9: 719-727.
- Hanks, L.M., 1999. Influence of the larval host plant on reproductive strategies of cerambycid beetles. *Annu. Rev. Entomol.* 44, 483–505.
- Hoque S., Azhar M.A.H.B. and Deravi F. 2011. ZOOMETRICS – Biometric Identification of Wildlife Using Natural Body Marks. *International Journal of Bio-Science and Bio-Technology*, 3: 45-53
- Hughes AL, Hughes MK. 1982. Male Size, Mating Success, and Breeding Habitat Partitioning in the Whitespotted Sawyer *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae). *Oecologia* (Berl) 55:258-263.
- Huxley JS. 1932. Problems of Relative Growth. Methuen, London. 276p.
- Knell RJ. 2009. On the analysis of non-linear allometries. *Ecol Entomol* 34: 1-11.
- Knell RJ, Pomfret JC, Tomkins JL. 2004. The limits of elaboration: curved allometries reveal the constraints on mandible size in stag beetles. *Proc R Soc Lond B* 271: 523–528.
- Knox C.D., Cree A. and Seddon P.J. 2012. Accurate identification of individual geckos (*Naultinus gemmeus*) through dorsal pattern differentiation. *New Zealand Journal of Ecology*, Vol. 37 N.1
- MacKenzie D.I. and Royle J.A. 2005. METHODOLOGICAL INSIGHTS Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology*, 42: 1105-1114.
- Nijhout HF. 1994. Insect hormones. University Press, Princeton. 267p.
- Nijhout HF, Wheeler DE. 1996. Growth models of complex allometries in holometabolous insects. *Am Nat* 148: 40-56.
- Painting C., Holwell G. 2013. Exaggerated Trait Allometry, Compensation and Trade-Offs in the New Zeland Giraffe Weevil (*Lasiornhynchus barbicornis*). *PlosOne* 8: e82467.
- Pechacek, P., Kristin, A., 2004. Comparative diets of adult and young three-toed woodpeckers in a European alpine forest community. *J. Wildlife Manage.* 68, 683-693.
- Pomfret J.C. and Knell R.J. 2006. Sexual selection and horn allometry in the dung beetle *Euoniticellus intermedius*. *Anim Behav* 71: 567–576.
- Ranius, T., Jansson, N., 2000. The influence of forest regrowth, original canopy cover and tree size on saproxylic beetles associated with old oaks. *Biol. Conserv.* 95, 85-94.
- Redolfi De Zan L., Bellotti F., D’Amato D., Carpaneto G.M. 2014. Saproxylic beetles in three relict beech forests of central Italy: analysis

- of environmental parameters and implications for forest management. *For. Ecol. Manag.* 328: 229-244. DOI: 10.1016/j.foreco.2014.05.040
- Royle J.A., 2004. - N-mixture models for estimating population size from spatially replicated counts. *Biometrics* 60:108-115.
- Russo D., Cistrone L., Garonna A. 2011. Habitat selection by the highly endangered long-horned beetle *Rosalia alpina* in Southern Europe: A multiple spatial scale assessment. *Journal of Insect Conservation*, 15, 685–693.
- Sacchi R., Scali S., Pellitteri-Rosa D., Pupin F., Gentili A., Tettamanti S., Caviglioli L., Racina L., Maiocchi V., Galeotti P. and Fasola M. 2010. Photographic identification in reptiles: a matter of scales. *Amphibia-Reptilia* 31: 489-502
- Samways, M.J., 2005. *Insect Diversity Conservation*. Cambridge University Press, Cambridge, UK.
- Tikkamäki, T., Komonen, A. 2011. Estimating population characteristics of two saproxylic beetles: a mark-recapture approach. *J. Insect Conserv.* 15, 401–408. DOI:10.1007/s10841-010-9313-3.

## ACKNOWLEDGEMENTS

My heartfelt thanks go to my tutor Giuseppe M. Carpaneto, who represented my reference point from the beginning to the end of the PhD; he always guided me and encouraged me during these three years. Thank you Giuseppe for your great and irreplaceable support.

I wish to thank all my colleagues and friends of Lab. 5.8 for their cooperation and their help: Lorenzo Mentil, Lara Redolfi De Zan, Federico Romiti, Massimiliano Tini and Agnese Zauli.

Thank you Lorenzo for your enthusiasm and support.

Thank you Massimiliano for your help and support, we postponed a work, but we will do it.

Thank you Federico for your great help and for having worked with me, I have learned much from our collaboration.

Thank you Agnese for all your suggestions and for the constant help, you have been always present when I needed.

A special thanks for you Lara, thank you for your strong support in every circumstances, you are always ready to assist me during the fieldwork (in good and very bad weather conditions!), during the data analysis and the writing of the manuscripts; but most of all, thank you for being such a great friend!

I would like to thank Chiara Passacantilli for her help on field and for sharing with me “The *Morimus* adventures”! It has been a pleasure for me to help you with your thesis, I have found a new friend and, I am sure, also a future colleague.

I want to thank all the members of PhD school in particular the PhD school coordinator Prof. Marco A. Bologna for being always available and ready to help.

Thanks to all the people involved in the MIPP Project for the facilitations and help, in particular Alessandro Campanaro, Stefano Chiari, Sönke Hardersen, Emiliano Mancini, Michela Maura and Emanuela Maurizi. Special thanks go to Emiliano for his constant support.

Finally, I thank God for giving me a wonderful family and an extraordinary husband and I thank them for their love, their constant presence and their great patience, but most of all for believing in me always.

## LIST OF PUBLICATIONS

- Rossi de Gasperis S.**, Redolfi De Zan L., Battisti C., Reichegger I., Carpaneto G.M. (2015). Distribution and abundance of hole-nesting birds in Mediterranean forests: impact of past management patterns on habitat preference. *Ornis fennica*, in publication (manuscript, accepted 10/12/2015).
- Mason, F., Roversi, P.F., Audisio, P., Bologna, M.A., Carpaneto, G.M., Antonini, G., Mancini, E., Sabbatini Peverieri, G., Mosconi, F., Solano, E., Maurizi, E., Maura, M., Chiari, S., Sabatelli, S., Bardiani, M., Toni, I., Redolfi De Zan, L., **Rossi de Gasperis, S.**, Tini, M., Cini, A., Zauli, A., Nigro, G., Bottacci, A., Hardersen, S., Campanaro, A., 2015. Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). *Fragmenta Entomologica* 47, 51-52.
- Romiti F., **Rossi de Gasperis S.**, Redolfi De Zan L., Carpaneto G.M. 2015. Intraspecific allometry in two saproxylic species *Lucanus cervus* (Lucanidae) and *Morimus asper* (Cerambycidae): effects on demographic and ecological parameters, in: Proceedings of the 76<sup>th</sup> National Conference of the Unione Zoologica Italiana. Quaderni del Centro Studi Alpino-IV. ISBN-978-88-903595-4-5.
- Rossi de Gasperis S.**, Carpaneto G.M. 2015. Population analysis on *Morimus asper* in beech forests of central Italy: preliminary results, in: Papers and abstract of the 8<sup>th</sup> Symposium on the Conservation of Saproxylic Beetles 13-15 June 2014. Journal of the Swiss Entomological Society 88 (1-2): 52.
- Submitted:
- Rossi de Gasperis S.**, Passacantilli C., Redolfi De Zan L., Carpaneto G.M. Overwintering ability and habitat preference of the threatened longhorn beetle *Morimus asper* / *funereus*: a two years mark-recapture study with implications for conservation and forest management. *Biological Conservation*.
- Rossi de Gasperis S.**, Redolfi De Zan L., Romiti F., Hardersen S., Carpaneto G.M. Sexual dimorphism and intra-specific allometric relationships between secondary sexual character and body size in *Morimus asper/funereus*. *Journal of Morphology*.
- Rossi de Gasperis S.**, Carpaneto G.M., Nigro G., Antonini G., Chiari S., Cini A., Mancini E., Mason. F., Mosconi F., Redolfi de Zan L., Roversi P.F., Sabbatini G., Solano E., Campanaro A. Computer-aided Photographic Identification of *Rosalia alpina* (Coleoptera:

Cerambycidae): first application in a mark-recapture study. *Insect Conservation and Diversity*.

Redolfi De Zan L., **S. Rossi de Gasperis S.**, Fiore L., Battisti C., Carpaneto G.M. The importance of dead wood for hole-nesting birds: a two years study in three beech forests of Central Italy. *Israel Journal of Ecology & Evolution* (in revision).

### **Conferences**

76<sup>th</sup> National Conference of the Unione Zoologica Italiana, Viterbo, Italy, 15-18 September 2015. Poster presentation: Intraspecific allometry in two saproxylic species *Lucanus cervus* (Lucanidae) and *Morimus asper* (Cerambycidae): effects on demographic and ecological parameters.

8<sup>th</sup> Symposium and Workshop on the Conservation of Saproxylic Beetles Basel – Switzerland, 13-15 June 2014. Oral presentation: Population analysis of *Morimus asper* in beech forests of Central Italy: preliminary results.

