Functional diversity, trophic interaction, development, and metabolism of ground beetles (Coleoptera: Carabidae) in perennial Mediterranean agroecosystems

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FUNCTIONAL DIVERSITY, TROPHIC INTERACTION, DEVELOPMENT, AND METABOLISM OF GROUND BEETLES (COLEOPTERA: CARABIDAE) IN PERENNIAL MEDITERRANEAN AGROECOSYSTEMS

DOCTORAL THESIS

First mentor: Lucija Šerić Jelaska, PhD

Second mentor: Assoc. prof. Tomislav Kos

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PRIRODOSLOVNO-MATEMATIČKI FAKULTET
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Lara Ivanković Tatalović

FUNKCIONALNA RAZNOLIKOST, TROFIČKI ODNOSI, RAZVOJ I METABOLIZAM TRČAKA (COLEOPTERA: CARABIDAE) VIŠEGODIŠNJIH MEDITERANSKIH AGROEKOSUSTAVA

DOKTORSKI RAD
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SUPERVISORS

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Lucija Šerić Jelaska, PhD, is a research associate employed at the Faculty of Science of the University of Zagreb, where she received her doctorate on the ecology of insects in forest ecosystems. As a post-doctoral student, she was granted with the fellowship (HRZZ), to work at the University of Cardiff, in Great Britain, where she investigated the feeding relationships of insects using modern technologies and methods of molecular biology. She is currently the project leader of the MEDITERATRI project (HRZZ), EntoCryoArk (HAZU) and the development project of the saproxylic NATURA 2000 beetle monitoring program (OPKK). At the Faculty of Science, she participates in the courses "Zoology", "Introduction to Forensic Biology", “Basics of Molecular Ecology" and teaches "Forensic Entomology" at the Faculty of Veterinary Medicine in Zagreb. She is the co-author of the university textbook "Entomology - the science of insects". She actively participated in the work of the Croatian EntomologicalSociety and its journal Entomologia Croatica and was the secretary of the Croatian Ecological Society. She participated in numerous international scientific meetings through presentations, but also as a member of organizational and scientific committees.

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Tomislav Kos, Assistant Professor, works at the University of Zadar, where he holds six subjects: Plant nutrition, Viticulture and winemaking, Plant protection, Integrated plant protection, General fruit growing. He graduated at the Faculty of Agriculture, University of Zagreb, in 2004. He worked at the Department of Agricultural Zoology, where he defended his doctoral thesis in 2011. His scientific interest is focused on field pest control systems. In addition to entomology, he also deals with phytopharmacy, researching the effectiveness of insecticides in the laboratory and in the field experiments, and consulting in phytomedicine. He is an authorized lecturer of the Ministry of Agriculture for the implementation of the sustainable action plan of pesticide use. He was a coordinator of the PESCAR project, which include methods of proper monitoring of certain diseases and pests as well as methods of controlled and targeted protection against them.

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Funkcionalna raznolikost, trofički odnosi, razvoj i metabolizam trčaka (Coleoptera: Carabidae) višegodišnjih mediteranskih agroekosustava

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U sklopu disertacije provedena su istraživanja utjecaja različitih tipova gospodarenja koja se provode u mediteranskim maslinicima i vinogradima na zajednicu, populacije i jedinke trčaka (Insecta, Coleoptera, Carabidae). Većina istraživanja bila je usmjerena na grabežljive vrste trčaka koje svojom ishranom suzbijaju nametnike na biljkama i time doprinose održivosti poljoprivrednih ekosustava. U istraživanju su korištene metode koje se primjenjuju u morfološkim istraživanjima, molekularnoj biologiji (PCR dijagnstika) i toksikologiji (izlaganje aktivnim tvarima, HPLC, GC-MS) kako bi usporedili udjele različitih funkcionalnih grupa trčaka ovisno o načinu poljoprivrednog gospodarenja i utjecaju gospodarenja na njihov razvoj, ishranu i metabolizam, ali i kako bi provjerili šire li se insekticidi ishranom kroz različite trofičke interakcije unutar hranidbenih mreža tla. Analizom objavljenih podataka vezanih za mediteransko područje i onih prikupljenih na terenu na području Zadarske županije dokazali smo da gospodarenje utječe na sastav zajednice te da negospodarena staništa imaju značajno viši udio mesojednih vrsta trčaka. Negospodarena staništa su također sadržavala viši udio velikih vrsta koje ne lete i vezana su uglavno za stabilnije stanišne uvjete. Vrijednosti fluktuacijske asimetrije izmjerene na populacijama dviju vrsta ukazale su da su maslinici povoljniji za razvoj trčaka od vinograda, dok su negospodarena staništa bila povoljnija u odnosu na maslinike i vinograde. Od dvije testirane vrste, *Pterostichus melas* se pokazao prikladnijom vrstom za analizu fluktuacijske asimetrije kao pokazatelja promjena u okolišu koje negativno utječu na populacije, s obzirom da su se u toj vrsti iskazale statistički značajne razlike u razini fluktuacijske asimetrije između populacija, dok kod vrste *Poecilus koyi* razlika među populacijama nije bilo. Nadalje, akutno trovanje tiametoksanom, insekticidom iz skupine neonikotinoida, izazvalo je niz subletalnih reakcija kod jedinki trčaka. Jedinke izložene višim koncentracijama konzumirale su značajno manje količine hrane, a motorika im je bila poremećena. Nadalje, promjene u koncentraciji pojedinih metabolita u tretiranih jedinki ukazuju da je slijedom trovanja došlo do oksidativnog stresa u tkivu trčaka. Međutim, mjerenjem aktivnosti superoksid dismutaze, enzima koji sudjeluje u obrani od oksidativnog stresa, nisu dobiveneznačajne razlike između grupa ovisno o koncentraciji. U ovom istraživanju prvi put je laboratorijski dokazan prijenos insekticida iz supstrata (tlo) na odabrani plijen (gujavice), te potom s plijena na grabežljive trčke putem ishrane. Molekularnim metodama smo potvrdili da se trčci s istraživanih ploha hrane gujavicama. Ovim istraživanjem je potvrđeno da: (i) staništa na kojima se ne gospodari pogoduju grabežljivim vrstama i njihovom normalnom razvoju i predstavljaju stabilniji okoliš za istraživanu skupinu na što ukazuje veća brojnost vrsta koje ne lete; (ii) insekticidi u tkivo tračaka mogu doći i neizravnim doticajem s aktivnim tvarima hraneći se kontaminiranim plijenom; (iii) više koncentracije insekticida thiametoksama izazivaju poremećaje u hranjenju, kretanju i metabolizmu trčaka, što narušava njihovu grabežljivu aktivnost koja u poljoprivrednim ekosustavima doprinosi biološkoj zaštiti. Ovi rezultati pridonose razvoju načina gospodarenja koji podržavaju zajednicu grabežljivih trčaka, te time smanjiti gubitak bioraznolikosti u mediteranskoj regiji.

(110 stranica, 30 slika, 216 literaturnih navoda, jezik izvornika engleski)

Ključne riječi: Biološka zaštita, carabidae, fluktuacijska asimetrija, funkcionalne grupe, maslinici, insekticidi, poljoprivredni ekosustavi, vinogradi.

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Functional diversity, trophic interaction, development, and metabolism of ground beetles (Coleoptera: Carabidae) in perennial Mediterranean agroecosystems

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The goals of this dissertation were to measure impact of different types of management that are carried out in Mediterranean olive groves and vineyards on the community, populations and individuals of carabid beetles, who provide valuable ecosystem services to agriculture. A special emphasis was placed on predatory species of carabid beetles, which can control harmful pests on plants with their predatory activity. Research methods included morphological analyses, molecular biology (PCR diagnostics), and toxicology (intoxication with active ingredients, HPLC, GC-MS) were used in order to compare the proportions of different functional groups of carabids depending on the type of agronomic management, the impact of management on their development, predatory activity, and metabolism, but also to check whether insecticides spread through soil foodwebs via trophic interactions. By combining the collected data from Zadar County and data published from the other Mediterranean areas, we proved that management affects the composition of the community and that unmanaged habitats have a significantly higher proportion of carnivorous carabid species. Unmanaged habitats also contained a higher proportion of large flightless species. The method of fluctuating asymmetry, performed on two species, indicated that olive orchards are less adverse habitats for the development of carabid beetles than vineyards, however, unmanaged habitats are more suitable compared to both vineyards and olive orchards. Of the two tested species, *Pterostichus melas* proved to be more suitable species for fluctuating assymetry as a sign of negative enviromental impact on populations, given that it showed statistically significant differences in the level of fluctuating asymmetry between populations, while in the species *Poecilus koyi* there were no differences between populations. Furthermore, acute poisoning with thiamethoxam, an insecticide from the group of neonicotinoids, caused a series of sub-lethal effects in carabids. Individuals exposed to higher concentrations consumed significantly less food and exhibited obvious locomotory impairments, such as slow, irregular gait, intense cleaning of antenae, and inability to return to their feet when turned over on their backs. Furthermore, changes in the concentration of individual metabolites in the treated individuals indicate that following the intoxication, oxidative stress occurred in the tissues of the carabids. However, measurement of the activity of superoxide dismutase, an enzyme involved in the defense against oxidative stress, did not show any significant differences between the groups depending on the concentration. In this research, for the first time, the transfer of insecticides from the soil to selected prey (earthworm), and then from the prey to predatory species through feeding, was demonstrated in the laboratory. Using molecular methods, we confirmed that carabids from the research sites feed on earthworms. This research confirmed that: (i) unmanaged, stable habitats favor a higher proportion of predatory species and their normal development; (ii) insecticides can enter carabid beetle's tissue indirectly, by feeding on the contaminated prey; (iii) higher concentrations of the insecticide thiamethoxam cause disturbances in the feeding, movement, and metabolism of carabids, disrupting their predatory activity and thus reducing ecosystem services that might be provided by carabids, which in agricultural ecosystems contributes to the biocontrol of plant pests. These results may help to develop more sustainable management practices that support the community of predators and reduce the loss of biodiversity in the Mediterranean region.

(110 pages, 30 figures, 216 references, original in English)

Keywords: Agroesystems, biological control, carabid beetles, fluctuating assymetry, functional groups, insecticides, olive orchards, vineyards

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TABLE OF CONTENT

1. INTRODUCTION

The cultivation of olive trees (*Olea europaea* L.) and grapevines (*Vitis vinifera* L.) in the Mediterranean region dates back to ancient times, and both plants continue to serve as essential economic commodities as well as an important cultural role in the nations that are part of the Mediterranean basin today (Kavvadias and Koubouris 2019). These cultivations are in danger from a number of pest species, which forces farmers to apply a variety of pesticides to minimize the damage (Picchi et al. 2017). Farmers have switched from conventional management (CM) practices to ecological (EPM) or integrated (IPM) pest management to increase the control of insect and invertebrate pests and to reduce the damaging impact of pesticides on non-target organisms. The foundation of both EPM and IPM is a variety of control strategies, including biological control, host-plant resistance breeding, and cultural measures. Although synthetic chemical pesticides (SCPs) are not utilized at all in EPM, IPM and CM both continue to use a variety of SCP that target various organisms (Tshernyshev 1995, Thomas 1999). However, the usage of substances permitted in EPM can still harm native or non-pest arthropod communities (Iannotta et al. 2007, Scalercio et al. 2009). According to Myers et al. (2000), the Mediterranean basin is a hotspot for biodiversity worldwide and is consequently vulnerable to climate change. The Mediterranean basin's poorly studied biodiversity may be at even higher risk of extinction as a result of climate change, intensive agriculture, and the use of SCP. One of more frequently used insecticides in the world are neonicotinoid insecticides (Sparks 2013), and their use has an impact on non-target vertebrate and invertebrate species in agroecosystem communities (Whitehorn et al. 2012, Hallmann et al. 2014, Douglas et al. 2015). They have been discovered to spread to surface waters and harm the community of macroinvertebrates (Van Dijk et al. 2013). Farmers are encouraged by current agro-environmental initiatives in Europe to employ non-chemical technologies as well as low pesticide input control strategies. According to EU Directive 2009/128/EC, low chemical input principles and criteria are now required as of January 1, 2014. However, these beneficial legalizations may be revoked. For instance, the EU approved emergency authorizations for thiamethoxam-containing products in 15 European countries in 2020, including Croatia, even though it was banned in 2018, necessitating more research on the substance's impact on natural predators.

Due to their feeding on numerous plant and insect pests, carabid beetles (Coleoptera: Carabidae) are one of the most significant groups of beneficial arthropods in agroecosystem food webs (Kromp 1999, Giglio et al. 2011, Sommaggio et al. 2018). They frequently dominate numerically among soil-active arthropods (Lövei and Sunderland 1996), and they are able to consume enough food each day to equal their body mass (Thiele 1977). They have been identified as olive fruit fly (*Bactrocera oleae* Gmelin), 1790 predators in several investigations, both in the laboratory (Dinis et al. 2016, Albertini et al. 2018) and in the field (Orsini et al. 2007, Pizzolotto et al. 2018). For the production of grapevines, maintaining the native predatory insect fauna is beneficial (Nicholls et al. 2008).

This doctoral research was conducted as part of the MEDITERATRI project (Croatian Science Foundation (HRZZ) UIP-2017-05-1046) which aims to explore the potential transfer of pesticides to general predators through trophic interactions and to connect their application with the predator survival and diversity of non-target invertebrates in the field. The project takes place in the Mediterranean area of Croatia where biodiversity and antropogenic impact on invertebrate fauna, especialy within agroecosystems, are understudied. Some of the prior faunistic reseach in the area include the extensive collection of Dalmatian beetle fauna by Petar Novak (1879-1968) in the first half of 20th Century. Among the others notable entomologist from that period in Dalmatia were Eduard Karaman (1849-1923), Wolgang Bath (1882-1932), and Joseph Müller (1880-1964). After the first half of 20th century, entomological research in North Dalmatia has been mostly neglected (Durbešić 2011). The goal of my research was to meassure impact of agricultural activity on the carabid beetles by finding the answers to the following research objectives:

1.1. Research aims and hypothesis

Research aims are the following:

The general aim of this dissertation is to study the functional traits, morphometry, and physiology of carabid beetles from olive orchards and vineyards with EPM and IPM systems, as well as impact of selected pesticides on the activity of predatory carabids, including the spread of these pesticides via trophic interactions. This aim is achieved by finding the answers to specific goals:

1. To analyse the distribution of the functional diversity of carabid beetles by calculating the share of ecological and morphological characteristics in their assemblages between agricultural sites and unmanaged habitats, and by identifying predatory beetle's prey
2. To measure FA among the carabid beetle communities that have developed under the different management systems and unmanaged habitat in order to compare the developmental stress that theese managements have on carabid beetle's populations
3. To examine the impact of direct pesticide exposure *in vivo* on the physiology of predatory carabid beetles: at the level of entire organism (feeding, locomotion, metabolism) and at the cellular level (oxidative stress)

4. To test if there is a transmission of selected pesticides in carabid beetles via their feeding on earthworms that had been exposed to pesticide, and how it affects oxidative stress in carabid beetles

By evaluating published research so far and field-observations the following hypothesizes are defined:

1. Distribution of functional traits differs in carabid beetle assemblages depending on management types
2. The level of fluctuating asymmetry (FA) will be higher in populations from agricultural sites compared to the populations from unmanaged sites
3. Exposure to pesticides will impact the feeding rate, locomotion, metabolites, and oxidative stress levels in carabid beetles
4. Pesticides capable of spreading through the food web will impact the levels of oxidative stress in carabids that feed on contaminated earthworms, the prey that they consume in the filed

2. LITERATURE REVIEW

2.1. Biology and ecology of carabid beetles

Carabid beetles (Carabidae) are a family of insects (Insecta) from the order Coleoptera, and the suborder Adephaga. With over 40,000 described species, they are one of the most numerous families of beetles, making up one of the groups richest in species on earth (Lövei and Sunderland 1996; Lorenz 2005). The suborder Adephaga consists primarily of aquatic insects such as the diving beetles (Dytiscidae), whirligig beetles (Gyrinidae), crawling water beetles (Haliplidae), and water scavenger beetles (Hygrobiidae) families, and the carabid beetles are almost the only terrestrial member of this group, along with their subfamily tiger beetles (Cicindelinae) (Thiele 1977). They evolved in the early Tertiary in the tropics from where they spread to higher latitudes, and today they are a cosmopolitan group (Erwin and Adis 1982). Carabid beetles inhabit a wide range of habitats, from wet and coastal biotopes, to dry steppes and deserts, and prefer leaf litter as a microhabitat and hide under rocks (Hurka 1996). Moisture, however, is a limiting environmental factor for carabids (Lövei and Sunderland 1996), so in desert areas they occur only in oases or by some other water source (Erwin 1985). The suborder Adephaga, including the carabid beetles, are a group of monophyletic origin and despite the large number of species, the carabids have little variation in morphological structure (Thiele 1977). The thighs of the hind legs are connected to the hind sternite, the head is in an exile position with filamentous antennae that always have 11 antennomers, and the legs are without exception adapted for running. The body is firmly sclerotized in most species, with a dorsal side of a dark color that often has a blue, green, or copper sheen, and the body extensions and ventral side may be black, brown, red, and yellow. The prothorax is precluded by a solid neck shield (pronotum). Beneath the tightly sclerotized forewings (elitrae) is a posterior pair of wings that may be well developed (macropteric forms), partially or completely reduced (brahipteric and apteric forms), and in some species both forms of hind wings (dimorphic species) occur (Brandmayr 1991). The body size range is 2-60 mm (Homburg et al. 2014). The surface contains the tactile organs of the set (Thiele 1977, Trautner and Geigenmüller 1987, Hurka 1996) (Figure 1).

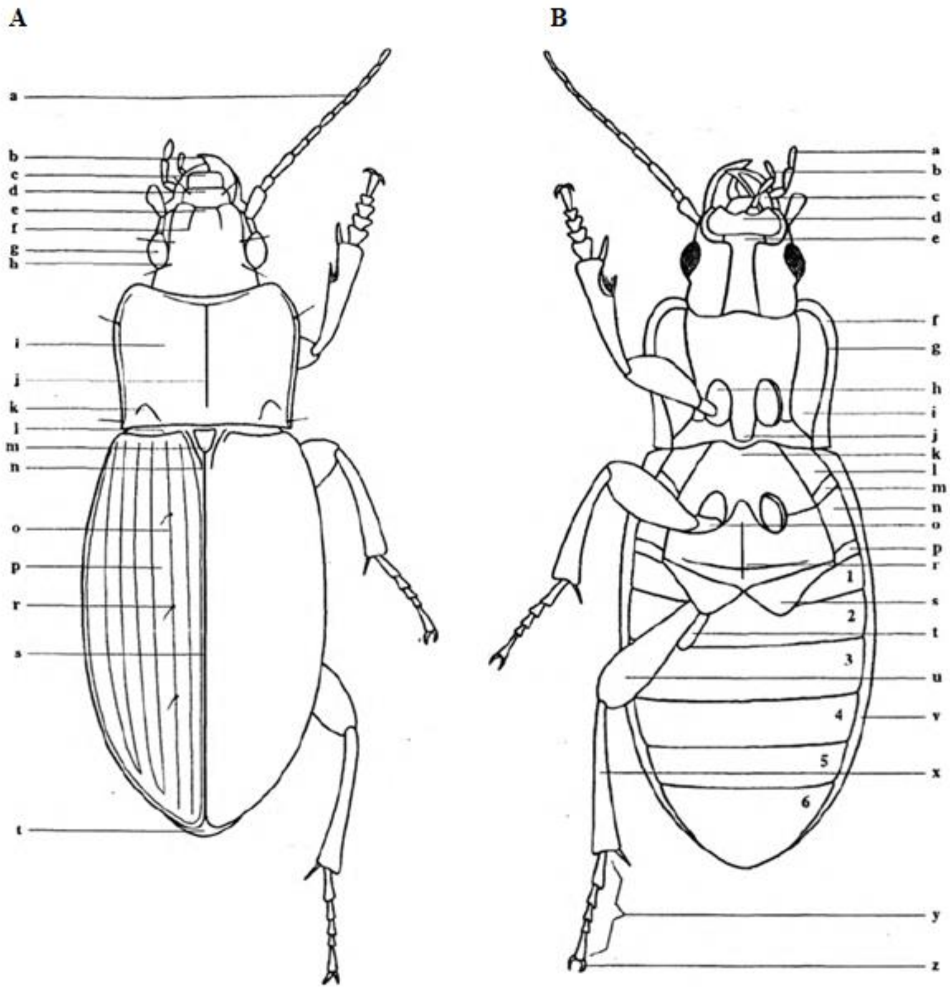

Figure 1. (A) The morphology of an adult carabid beetle, dorsal side (a - antenna; b - mandible; c - labrum; d - clypeus; e - frons; f - frontal furrov; g - eye; h – setiferous puncture at inner edge of eye; i - pronotum; j – median line of pronotum; k – basal impression (fovea) of pronotum; l – basal margination of elytrae; m - scutellum; n – scutellar stria; o – elytral stria 3; p – elytral interval 3; r – dorsal setiferous puncture; s- elytral suture; t - pygidium).

(B) The morphology of an adult carabid beetle, ventral side (a – maxillary palpus; b – labial palpus; c - maxilla; d - mentum; e - gula; f – epipleuron of pronotum; g – notopleural suture; h – front coxa; i – episternum of prothorax; j - prosternal projection; k mesothorax; l – episternum of mesothorax; m – epimeron of mesothorax; n - episternum of mesothorax; o – middle trochanter; p - epimeron of metathorax; r - methatorax; s – hind coxa; t – hind trochanter; u – hind femur; v – epipleuron of elytron; x – hind tibia; y – hind tarsus; z - claw; 1-6 – visible abdominal segments). Taken from Hurka (1996).

Carabid beetles, with their uniform body shape, are adapted to the life of a predator on the ground, which is why they are also known as ground beetles. Most of the pronounced morphological variations are associated with a specialized type of diet (Thiele 1977). Most predatory species are generalists, and specialized diets are more often conditioned by the environment than by genetics. An example is the coastal species *Eurynebria complanata*, which feeds almost exclusively on *Talitrus saltator* shrimp in its natural habitat, but has demonstrated a wider range of prey in the laboratory. In contrast, some species of the genus *Cychrus* feed exclusively on snails, and to adapt to such a diet they have a narrowed head and pronotum and elongated jaws that allow them to penetrate deep into the snail's shell (Trautner and Geigenmüller 1987). The genus *Procerus* developed a large broad-headed body for breaking snail shells (Thiele 1977).

The majority of carabid beetles from temperate zones are unovoltine, having one generation per year (Hurka 1996). Depending on the breeding period, carabid beetles can be *larval hibernators* or *adult hibernators*, as proposed by Lindroth (1949). Breeding period is influenced by latitude, which is why in norther hemisphere most species breed in spring and summer, adults of new generation emerge in summer or early autumn, and hibernate over winter (Hurka 1996, Jambrošić Vladić 2020). This allows them to reach a necessary level of body development prior to winter. In the case of larval hibernators, adults mate in autumn, and new generation hibernates as larvae and emerge in early spring. In some species of genera *Abax*, *Molops*, *Ditomus* and *Pterostichus* a parental care has been observed (Brandmayr et al. 1994, Hurka 1996).

Most carabid beetles are nocturnal animals, especially in regions with a hot climate (Kromp 1999). Factors such as: changes in temperature (Jones 1979), humidity, light intensity (Thiele 1977), period of prey activity (Alderweireld and Dessender 1990) and dormancy periods, either due to high temperatures (eg. *Rhytidognathus* sp.), (Castro et al. 2014) or low temperatures (Desender 1982), affect daily and annual rhythm (entomophenology) of this family. This allows survival, harmonizes life expectancy with food stocks, and helps in competition avoidance, etc. (Danks 1987, Delinger 2002, Kotze et al. 2011).

Functional traits (feeding, wing development) and body size are often-used characteristics of carabid beetles in ecological studies (e.g. Šustek 1987, Ribera et al. 2001, Šerić Jelaska et al. 2007). Body size affects the ability to live in a certain type of environment, migration, and in the case of predatory species, it sets the size range of their prey (Šustek 1987, Hatteland 2010). It is possible to expect changes in body size structure of a carabid beetle assemblage under the pressure of anthropogenic factors, such as land management (Šerić Jelaska and Durbešić 2009, Ivanković Tatalović et al. 2020). Habitat age can affect body size and life-history traits (Šerić Jelaska et al. 2011), with large carabid species and species that overwinter as larvae were dominant in older forest habitats. Blake et al. (1994) noted that if a particular assemblage of species is typical of a particular habitat type, then similar patterns of body size distributions will appear between similar sites. Furthermore, different species of carabid beetles have different dispersal capabilities, with some species having well-developed hind wings (macropterous), others being flightless with hind wings reduced (brachypterous), and some species may develop both types of hind wings (dimorphic) (Brandmayr 1991). Small carabid species are more often macropterous than the large-sized species (e.g. *Carabus*) (Blake et al. 1994, Ribera et al. 2001). Šerić Jelaska and Durbešić (2009) found that more macropterous species were present in isolated habitat and more brachypterous in continuous forest area, especially medium-sized brachypterous beetles, and that higher proportions of small-sized carabids were present in isolated area and large species were more common in continuous forest areas, implying that the power of dispersal is an important factor that can be used in analyses of isolation effects on carabid assemblages, both at small and large scales. Landscape stability can serve as an ecological filter of carabid beetles in relation to their dispersal and phenology (Duflot et al. 2014), which includes the impact that agricultural land use and management may have on the ecological composition of carabid beetle assemblages (Cole et al. 2002).

2.2. The role of Carabid beetles in agroecosystems

Carabid beetles are among the most important and numerically dominant group of beneficial arthropods in the agroecosystem food webs as they can act as a biocontrol against many pests and weeds (Kromp 1999; Saska et al. 2008, Giglio et al. 2011, Sommaggio et al. 2018). They are voracious feeders, capable of consuming close to their body mass of food daily. Agroecosystems are noted to harbor rich carabid fauna despite intensive anthropological impact on such habitats (Holland and Luff 2000), and in Europe, Thiele (1977) lists 26 species that can be found in at least one-third investigated arable habitats stretching from England over Central Europe towards Belorussia. Among those species the following were most common: *Pterostichus melanarius* (Illiger, 1798), *Poecilus cupreus* (Linnaeus, 1758), *Harpalus rufipes* (DeGeer, 1774), *Harpalus affinis* C.R. Sahlberg, 1827, *Anchomenus dorsalis* (Pontoppidan, 1763), *Agonum muelleri* (Herbst, 1784), *Bembidion lampros* (Herbst, 1784), and *Trechus quadristriatus* (Schrank, 1781), as was later confirmed by other studies (Luff 1987, Lövei and Sárospataki 1990). In Croatia, Lemić et al. (2017) collected 26 carabid beetle species in arable land in Podravina region. More than 30 years ago, Štrbac (1983) identified 31 species in the same area. Even though most species found in agroecosystems are classified as eurytopic, and of no special interest in the terms of rarity, arable habitats can harbour 'interesting' carabid species, for example *Harpalus zabroides* Dejean, 1829*, Amara littorea* C.G. Thomson, *1857, Amara municipalis* (Duftschmid, 1812), which are on the German Red list (Schnitter 1994).

It has been demonstrated that carabid beetles feed on agricultural pests, however, most of this data is based on laboratory feeding trials, rather than field observation (Kromp 1999). Lindroth (1992) noted that feeding on animals is not the same as predation and that many carabids attack already weakened or injured prey, making them more of a scavenger. However, he considers genera *Agonum, Bembidion* (partly), *Calathus, Calosoma, Carabus, Cychrus, Dyschirius, Elaphrus, Notiophilus* and *Pterostichus* (partly) to be true predators, of which six (*Agonum, Bembidion, Calathus, Calosoma, Carabus, and Pterostichus*) can be found on Thiele's (1977) list of common species in arable lands. Furthermore, nowadays methods used in molecular biology, such as polymerase chain reaction (PCR) and DNA metabarcoding of carabid guts, confirmed their potential in pest control against slugs and dipterans (Šerić Jelaska et al. 2014, Kamenova et al. 2018). Frei et al. (2019) demonstrated high levels of carabid weed seed consumption in cereal fields across Central Europe using molecular analysis. Symondson et al. (2002) concluded that generalist predators significantly reduce prey numbers in field experiments.

Among their prey are aphids, which remains have been detected in the guts of *Poecilus cupreus, P. lepidus* (Leske, 1785), *Pterostichus macer* (Marsham, 1802), *Calathus fuscipes* (Goeze, 1777)*, Harpalus rufipes, Anchomenus dorsalis, Agonum muelleri,* and *Nebria brevicollis* (Fabricius, 1792) (Luf 1974, Hajek et al. 2007). The negative correlation between aphid densities and those of carabids, especially *Anchomenus dorsalis* (Kromp 1999).

Carabids also feed on eggs, larvae and pupae of dipterans. Many laboratory and controlled field experiments have been conducted to study the impact of carabid predation on dipteran populations. Floate et al. (1990) identified 14 carabid species that feed on wheat midge in the field. In greenhouse trials, *Amara familiaris* (Duftschmid, 1812) and *Abax parallelopipedus* (Piller and Mitterpacher, 1783) reduced larvae of cabbage root fly (*Delia radicum* (Linnaeus, 1758)) by 50% (Finch and Elliott 1994). Even though carabid predation on dipteran eggs is reduced due to the female flies burying their eggs in the soil, *Bembidion quadrimaculatum* (Linnaeus, 1760) consumed up to 25 fly eggs per day in the laboratory and reduced onion maggot numbers in field cages by up to 57% (Grafius and Warner 1990). Olive fruit fly (*B. oleae*) is another intensely studied dipteran species. It is one of the most serious pests on olive trees (Malheiro et al. 2015), and if left unchecked, it can cause up to 90% damage in commercial plantations by causing the early fall of afflicted fruits, devouring the pulp, and lowering the quality of olive oil (Pereira et al. 2004, Ordano et al. 2015). Olive fruit flies lay their eggs within olive fruits where larvae feed for the first two instars. In mid-autumn, the third instar falls to the ground, where it pupates in the top 3 cm of soil, making it available to active predators and parasitoids on the ground (Neuenschwander et al. 1983, Dimou et al. 2003, Orsini et al. 2007). Carabid beetles, according to Gonçalves and Pereira (2012), are a strong biocontrol of *B. oleae* since they are active in olive orchards during the autumn and winter (Lasinio and Zapparoli 1993). Dinis et al. (2016) conducted feeding preference assays in which they proved that carabids *Calathus granatensis* and *Pterostichus globosus* can be important in reducing the densities of *B. oleae* in olive groves. Several field studies (Orsini et al. 2007; Pizzolotto et al. 2018) have listed Carabid beetles as *B. oleae* predators.

Slugs inflict some of the most extensive damage to agricultural and horticultural crops in temperate zones (Asteraki 1993). They are present on the fruit opening its skin, which contaminates the final product and exposes it to fungal infestation. The damage they do in field under organic management has to be solved by biological control measures, including carabids as naturally occurring predators. Kromp (1999) listed carabid beetles as effective agents in slug biocontrol, feeding on slug eggs and juveniles (Scaccini 2020). Slug control by *Abax parallelepipedus* and *Pterostichus madidus* was shown to be equally effective as methio-carb application (Asteraki 1993). Species of genera *Carabus*, *Cychrus*, and *Licinus* are heliciphagous, capable of feeding on large snails and slugs, and can overcome their mucus defense (Bursztyka et al. 2013).

Moths (Insecta, Lepidoptera) are another group capable of causing significant damage in agriculture. Moths like *Prays oleae* and *Zeuzera pyrina* can cause substantial damages within olive plantations (Ramos et al. 1998) while grapevine moths *Lobesia botrana* and *Eupoecilia* (syn. *Clysia*) *ambiguella* are frequent pest in vineyards. Carabid beetles have been detected as lepidopteran feeders (Fuller 1988, Šerić Jelaska et al. 2014, Candan et al. 2021).

2.3. Management types within the agroecosystems

Current agro-environmental initiatives in Europe for biodiversity conservation urge farmers to use minimal pesticide input management approaches, including non-chemical methods. Since January 1, 2014, the European Union (EU) has made low chemical input principles and guidelines mandatory (EU Directive 2009/128/EC). Agriculture's significance in providing habitat for birds, arthropods, and other organisms, as well as contributing to conservation aims, is now recognized in countries where rural landscapes prevail. Increasing the effectiveness of natural enemies by changing the environment or the pest control methods in use allows for biological control (Eilenberg et al. 2001). Natural mortality is an important part of pest management in both agriculture and forest environments because it keeps insect populations under control. The natural environment may supply food, refuge, and alternative prey to biocontrol agents such as carabid beetles within a patchy territorial texture containing crops, weeds, and forests, according to the ecological landscape concept (Goodell 2009, Ortega and Pascual 2014). There are several management types in agroecosystems:

Conventional management (CM) is significantly more focused on pest control using pesticides as the main method to control outbreaks. In several situations, it responded only after pests had been discovered, with minimal emphasis on prevention and no system-wide perspective. There are several problems with this approach. Firstly, due to the genetic variations, pest species can develop the resistance to the pesticides. Due to the genetic selection, surviving specimens will dominate the reproduction leading to the population resistant to the pesticides. On the other hand, population of natural pest enemies will diminish in numbers, resulting in even bigger increase in pest abundance. Pesticide residues may be left in the environment because pesticides are difficult to confine to the target. These can harm wildlife and humans directly through occupational exposure or indirectly through bio-magnification and accumulation of pesticide levels in the food chain. Residues, when coupled with other environmental elements, generate a magnified effect greater than the individual compound effects put together, is also a problem brought on by synergism, when mixed compounds cause a magnified effect greater than the individual compound effects added together. Nowadays, more farmers are switching from conventional pest management (CM) to organic (EPM) or integrated pest management (IPM).

Integrated pest management (IPM) is "the coordinated use of pest and environmental information with available pest control methods to prevent unacceptable levels of pest damage by the most economical means and with the least possible hazard to people, property, and the environment" as defined by the United States Environmental Protection Agency (2007). According to the European commission for Food Safety, IPM is one of the tools for low-pesticide-input pest management, and must be implemented by all professional users. IPM entails a thorough examination of all available plant protection methods as well as the integration of appropriate measures that discourage the growth of harmful organism populations, limit the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified, and reduce or eliminate risks to human health and the environment. IPM emphasizes the growing of a healthy crop with as little damage to agro-ecosystems as possible, while also encouraging natural pest control mechanisms. IPM has the following principles as listed on the official European union site (ec.europa.eu): Plant protection methods include crop rotation, use of adequate cultivation techniques, use of resistant or tolerant cultivars and standard/certified seed and planting material, balancing fertilization, regular cleansing of machinery and equipment to prevent the spreading of harmful organisms, and "protection and enhancement of important beneficial organisms, e.g. by adequate plant protection measures or the utilization of ecological infrastructures inside and outside production sites." Appropriate procedures and equipment must be used to monitor harmful species, such as observation in the field and consultation with the experts. The professional user must decide whether and when to apply plant protection measures based on the monitoring results, and before treatments, the region, specific areas, crops, and climatic circumstances must all be considered. Sustainable biological, physical and other non-chemical methods must be preferred to chemical methods, and if pesticides must be applied, it should be in minimal effective doses, with the least side effects on human health, non-target organisms and the environment.

Many features of ecological pest management (EPM) are used in integrated pest management (IPM), but EPM is based on maintaining and supporting the agro-natural ecosystem's stability and suppressing insect outbreaks early on when the pest population density is still low. Certain combinations of diverse crops and lands with natural plant complexes can produce agro-ecosystem stability and high biodiversity. If naturally beneficial insects are unable to control the pest population density, pest enemies that have been artificially raised should be used (Tshernyshev 1995). Unlike CM and IPM, EPM does not use synthetic chemical pesticides (SCP) targeting different organisms (Thomas 1999), although it may still use natural compounds, like copper oxides, Azadirachtin, or Rotenone etc. (Ianotta et al. 2007).

2.3.1. Pesticides - types, history of use and research on them

European Commission defines a pesticide as "something that prevents, destroys, or controls a harmful organism ('pest') or disease, or protects plants or plant products during production, storage and transport". This includes herbicides, fungicides, insecticides, acaricides, nematicides, molluscicides, growth regulators, repellents, rodenticides and biocides. Pesticides used to protect crops or some other plants cultivated and used by humans are called plant protection products. With the introduction of chemicals like 2,4-D and DDT during World War II, modern synthetic pesticides became widely used. Because the potential applications of these insecticides were endless and the risks were modest, pest control began to follow a specific pattern (New Jersey Department of Environmental Protection). Many farmland vertebrate and invertebrate species have had population decreases due to increased use of pesticides and chemical fertilizers, as well as increased mechanization, earlier planting and harvesting, and simplification and loss of traditional crop cycles (Freemark and Kirk 2001). As of June 2022, there are 453 approved active substances, safeners and synergists used in EU. The European Commission provided the first report on the Farm to Fork strategy's progress in lowering the use and risk of chemical pesticides for the European Union on May 31, 2021. In comparison to the 2015-2017 baseline period, there was an 8% reduction in the usage and danger of chemical pesticides in the European Union in 2018, and a further 5% reduction in 2019 (ec.europa.eu). Neonicotinoid insecticides are the most frequently used insecticides in the world (Sparks, 2013), and their use has an impact on agroecosystem communities, including non-target invertebrate and vertebrate species (Whitehorn et al. 2012, Hallmann et al. 2014, Douglas et al. 2015). They've been discovered to spread to surface waterways, wreaking havoc on the macroinvertebrate population (Van Dijk et al. 2013). Neonicotinoids are nicotinic acetylcholine receptor agonists that bind to nAChRs in insects' central nervous systems. They are pesticides that have a systemic effect. Systemic pesticides, unlike contact pesticides, are taken up by the plant and distributed throughout the plant, rather than remaining on the surface of the treated sections of the plant (e.g. leaves), and can eventually end up even in nectar and pollen. This makes them dangerous to bees by negatively impacting their nutritional and energetic homeostasis, both of which are important factors in maintaining colony health (Cook 2019).

Pesticides' effects on carabid beetles have been extensively researched, due to their role in biocontrol and the ease with which they can be sampled in pesticide field trials (Holland and Luff 2000). The direct toxicity of pesticides, particularly insecticides, has been studied in laboratory, semi-field, and field trials (Mauchline et al. 2004, Goulson 2013, Plavšin et al. 2015, Tooming et al. 2017). Topical (Vickerman and Sunderland 1977) and surface (Hassan et al. 1991) pesticide exposure has been shown to be fatal to carabids. Sublethal effects of pesticides on carabid behavior include changes in locomotory behavior and foraging behavior (Jensen et al. 1997). The overall effect of insecticides on carabid beetles depend on many biotic and abiotic factors as outlined in Figure 2.

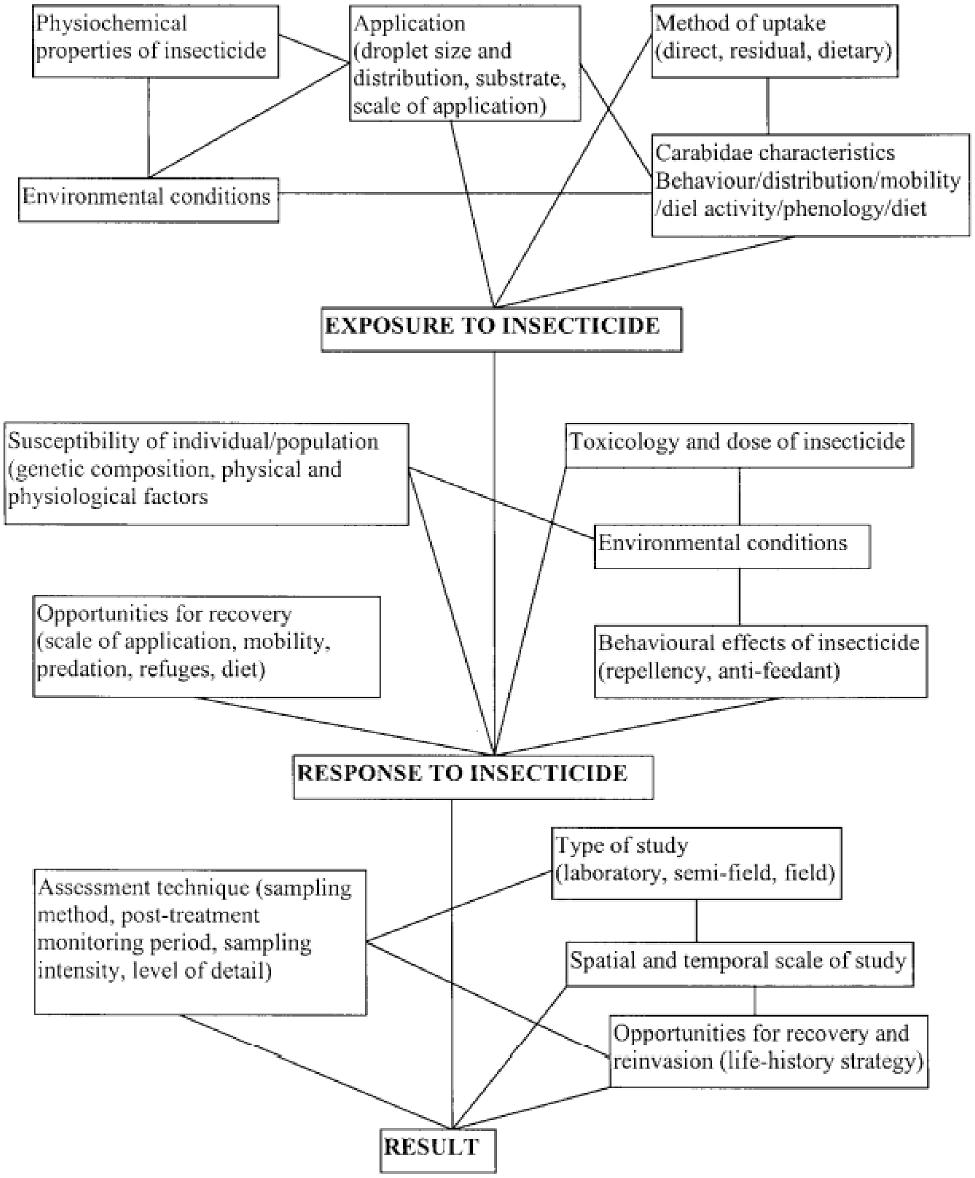

Figure 2. Interactions between pesticides and Carabidae, taken from Holland and Luff (2000).

It has been demonstrated that certain compounds can bioaccumulate in invertebrates and thus spread via trophic interactions (Šerić Jelaska et al. 2007, 2014, Douglas et al. 2015, Pisa et al. 2015). It should be noted that insecticides are not the only pesticides that can impact carabid beetles. Herbicides decrease the abundance and diversity of plant species, which directly affects carabid beetle's food and coverage, as well as it changes soil moisture, thus making habitat more welcoming to xerophylic species (Holland and Luff 2000).

2.4. Measures of enviromental stress

2.4.1. Fluctuating assymetry as an indicator of environmental stress

Geometric morphometrics (GMM) is a type of morphometric analysis used to quantify and visualize morphological variations which allows for better interpretation of morphological data (Alibert et al. 2001). While traditional morphometry uses linear distances, GMM is based on *landmark* coordinates which represent the shape of researched organism (Mitteroecker et al. 2013). In current morphometrics, Procrustes-based geometric morphometrics is the most often used methodology (Adams et al. 2013). The first step in using Procrustes-based GMM is to represent each specimen using the relative positions of morphological markers (landmarks) that can be precisely located and build a one-to-one correlation between all specimens in the analysis (Figure 12). This can be done on both live and preserved specimens.

The geometric information about a configuration of landmarks, save for its size, position, and orientation, is thus defined as shape (Dryden and Mardia 1998). Procrustes superimposition, which removes variation in size, position, and orientation from data on landmark coordinates and is at the heart of geometric morphometric, is used to retrieve the shape information (Figure 3). The superimposed landmarks' coordinates can be employed in multivariate statistical studies to answer a variety of biological problems (e.g. Klingenberg 2010).

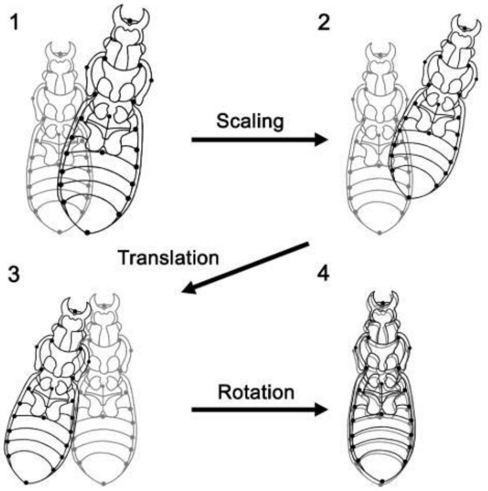

Figure 3. Schematic presentation of Procrustes superimposition, that includes scaling to the same size, translating to the same location of the centroids, and rotating to the best fit of the corresponding landmarks. This way, components of variations other than shape (size and position) are eliminated. Taken from Benitez (2013).

Fluctuating asymmetry (FA) assesses random tiny deviations from bilateral symmetry and is utilized in the assessment of varying levels of stress, environmental or genetic, that influenced organisms during their development. Developmental instability (DI) or developmental noise (DN) is the tendency of a development system to produce morphological changes in response to random disturbances (Polak 2003). The FA has been frequently used to assess DI levels in populations subjected to a variety of environmental stressors, including temperature, nutrition, radiation, chemical compounds, population density, noise, parasitism, light, predation risk, moisture conditions, and habitat structure (Huk and Kühne 1999, Alibert et al. 2001, Hoffmann and Woods 2003). Asymmetry of carabid beetle populations has been employed in several studies to assess the effects of urbanization, intense forestry practices, and agricultural activities (Labrie et al. 2003, Weller and Ganzhorn 2004, Henrquez et al. 2009, Elek et al. 2014, Benitez et al. 2018). There are variations in the approaches taken by different authors. Elek et al. (2014) showed that carabid beetles common along a Danish urbanization gradient do not appear to indicate differences in habitat quality by their level of FA, and Labrie et al. (2003) found no differences in FA levels between sites with different agricultural practices. On the other hand, Wealler and Ganzhorn (2004) demonstrated that in species that are vulnerable to urbanization, FA increases in close proximity to urban areas. Additionally, eurytopic species have reduced amounts of FA because they are less susceptible to environmental changes, according to Weller and Ganzhorn (2004). This led to the conclusion that environmental disturbances, ranging from changes in urbanization to the use of pesticides in agriculture, oftentimes do not result in changes in FA that allow for the differentiation between different levels of disturbances, such as higher levels of pesticides. As a feature that has been overlooked in many studies, the difference in FA levels between the sexes must also be highlighted (Hardersen 2000, Ribeiro et al. 2006, Elek et al. 2014, Lemić et al. 2020).

2.4.2. Oxidative stress and metabolomics as indicators of intoxication

Sublethal effects of environmental contaminants on insects can be expressed in metabolism and at a cellular level in the form of oxidative stress. Insects have unique systems for eliminating reactive oxygen species (ROS), making them well-adapted to the environmental changes (Kobayashi et al. 2019). Oxidative stress is defined as an imbalance between an organism's generation and removal of ROS, which can cause damage to biomolecules required for normal function of the organism (Rahman et al. 2006). Gaining of one electron by reduction of oxygen molecules (O_2) forming superoxide O_2^-, the anionic form of O_2, is what creates ROS. Catalytic actions of oxidative enzymes, such as nicotinamide adenine dinucleotide (NADH)/reduced NADPH oxidase (NOX) and cytochrome oxidase, generate superoxide which is toxic to the organism (Navar et al. 2020). The efficacy of several insecticides has been proven to be based on the oxidative imbalance that they produce in insects (Kolawole and Kolawole 2014). Superoxide dismutases (SODs) are enzymes that detoxify superoxide anions (O_2^-) by converting (dismutating) them into two less harmful species: oxygen (O_2) and hydrogen peroxide (H_2O_2), in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron (Nimse and Pal 2015) (Figure 13). The dismutation reaction is an important antioxidant mechanism that occurs in practically all cells in response to O_2 exposure to defend against its toxicity (Plavšin et al. 2015). However, various pollutants, including pesticides, can diminish such antioxidant response, and thus disturb the health of the organism.

The role of oxidative stress has been studied in shaping variation in survival, ageing rates, reproduction, growth, and immunity (Cram 2022). Zhang et al. (2006) proved that environmental cadmium (Cd) induces oxidative damage in insects by altering antioxidant defense enzyme systems. Their results demonstrated that Cd induced stress caused a significant increase in superoxide (SOD) dismutase and catalase (CAT) activities. By measuring SOD activity, Aslanturk et al. (2011) demonstrated that organophosphate methidathion induces oxidative stress in the gypsy moth, *Lymantria dispar* L., a serious defoliator of deciduous trees. The adverse effects of another insecticide, 2,2-Dicholorovinyl dimethyl phosphate, on the oxidative stress in insect pest was demonstrated by Kolawole et al (2014), in whose research synthetic insecticide enchanced SOD activity. The elevated antioxidant response in insect can even lead to pesticide tolerance (Müller et al. 2008). The impact of agricultural management on oxidative stress in non-target invertebrates needs to be further investigated. However, some studies have already proved that both synthetic and organic pesticides cause significant oxidative stress in non-targetr species, including the biocontrol agents (Palma-Onetto et al. 2021).

Diverse environmental factors have different effects on how organisms respond, including how they develop, reproduce, and how they regulate their basic metabolism. Assuming that exposure to a contaminant will disrupt energy allocation in an organism, physiological energetics can explain the effect and method of action of many toxicants (Bagheri et al. 2010). Toxicants can affect an animal's energetics in at least two separate ways: by altering the animal's locomotor activity and by having a direct impact on its metabolic rate as a result of rising detoxifying costs or damage from a toxicant. As a result, it is challenging to forecast a final outcome of a toxicant (Bednarska et al. 2010). Nowadays, many ecotoxicological studies measure alterations in the metabolite profiles to evaluate the effect of environmental disturbance. Bagheri et al. (2010) found that juvenoid pyriproxyfen significantly affected total lipid and carbohydrate amount in juveniles of

Brachynema germarii (Kolenati, 1846) , which may disrupt oviposition in adults. Carabid beetle *Pterostichus oblongopunctatus* (Fabricius, 1787) , when exposed to high nickel (Ni) levels, had increased respiration rates (Bednarska et al. 2010). Non-target analysis for metabolome profiling was used to evaluate the effect of bioaccumulation of emerging contaminants on caddisfly larvae by Previšić et al. (2020). They discovered that exposure to contaminants caused fine metabolic changes in studied insects. Finaly, metabolomics analysis has been used to study impact of neonicotinoids on insects, particulary on pollinators such as honey bees. By using liquid chromatography–mass spectrometry (LC-MS)-based combined with gas chromatography–mass spectrometry (GC-MS)-based metabolomics approaches, Shi et al. (2018) profiled the metabolic changes that occur in the head of honey bee after subchronic exposure to 2 mg/L thiacloprid over 3 days. The results showed that there were 115 metabolites significantly affected in thiacloprid-treated bees compared to control, and these changes are associated with oxidative stress, detoxification, and hindered brain activity.

Often-used analytical method in metabolomics profiling is GC-MS, which combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample (Sparkman et al. 2011). It consists of gas chromatograph and the mass spectrometer. The capillary column used in the gas chromatograph has molecule-separation characteristics that rely on the phase (5% phenyl polysiloxane, for example) and column dimensions (length, diameter, film thickness). As the sample moves along the column's length, the separation of the molecules will be aided by differences in the chemical characteristics of the various molecules in the mixture and their relative affinities for the stationary phase. The retention time of the molecules permits the mass spectrometer downstream to catch, ionize, accelerate, deflect, and detect the ionized molecules individually because the molecules are kept by the column and then elute (come off) from the column at distinct times (Sparkman et al. 2011).

2.4.3. Dignostic PCR in trophic analyses

Carabid and rove beetles, spiders, centipedes, and other terrestrial predatory arthropods as well as parasitic invertebrates like wasps and nemathodes are important in cotroling prey, which makes it important to characterize their diet. First pioneering work was done in 19th century by Forbes (1883), and ever since than researchers implemented various methods to detect the prey consumed by invertebrates. Predatory carabids as dominant and ground active group of species have been of a special interst to the reasercehre studying trophic ecology. Direct observation and gut content analysis under microscope were used at first, but these methods proved to be very limited. Many carabid species are nocturnal hunters, not to mention that plenty of prey species are soft-bodied, making their remains inside the beetle’s gut unrecognisible. Recently molecular gut content analysis where polymerise chain reaction (PCR) is applied is being used instead of these older techniques. It is possible to detect the prey's target sequences from the semi-digested remains in the predator's gut using PCR, the most used molecular approach for gut content analyses (Harper et al. 2005, González-Chang et al. 2016).

PCR is an enzyme-driven technique for amplifying small DNA segments *in vitro*. The method is based on knowing at least partial sequences of the target DNA beforehand as to construct oligonucleotide primers that hybridize specifically to the target sequences. Amplified DNA can than be tested using electroforesis and scored on stained agarose gel, where samples positive to certain prey can be told according to the amplicon of particular size (number of DNA base pairs) that appeared on the gel. As with most techniques, there are several limitations with PCR. Because there is a relatively small detection window, predation can be underestimated if decay rates of stomach contents are high. Variables such as predator identity, prey species, sex, and temperature, which may be unknown at the time of testing, influence decay rates and detection window size (Hagler and Naranjo 1997, Greenstone et al. 2014), making it difficult to determine predation rates. Scavenging and secondary predation (predation by another predator after the target prey has been devoured) cannot be distinguished from primary predation and may exaggerate trophic connection estimations.

Carabid beetles show potential in biocontrol against pests such as lepidopterans and dipterans, and when pest species are not available, alternative prey, for example earthworms, sustain the carabid beetle assemblages (King et al. 2010). Methods used in molecular biology have been utilized to investigate complicated trophic interactions in a range of ecosystems, including agroecosystems, together with prey abundance data, natural enemy-pest population dynamics, and/or behavioral trials (Šerić Jelaska et al. 2014 a, b, 2016, Athey et al. 2016, Albertini et al. 2018). Most studies were screening the predators for detecting single prey species of particular interest, like agricultural pests (Šerić Jelaska et al. 2014, Kamenova et al. 2018). However, some studies show potential of PCR diagnostic by anaying multiple prey within single predatory individual (King 2010) or by screening the entire predatory community for multiple prey species that was done for the first time in forest ecosystems, as a predecessor method to metagenomics (Šerić Jelaska et al. 2014 a, b).

3. RESEARCH SITES

Croatia is located in the Southeastern Europe, on the eastern shore of the Adriatic Sea. The country's relief is quite diverse, and the Dinaric Alps and neighboring Adriatic coast are one of the world's biodiversity hotspots (Myers et al. 2000). Croatia is divided into three biogeographic regions in Europe: continental, Alpine, and Mediterranean (European Environmental Agency 2016).

The first part of sampling for this research was conducted within two vineyards, two olive orchards (one of each under IPM and one under EPM), and adjacent Mediterranean scrubland serving as control site located in Zadar County, within the Mediterranean part of the county (Table 1, Figure 9). Zadar County is situated in the heart of Croatian Adriatic coast. The total area is 7,486.91 km^2, with the land area being 3,641.91 km^2. It geographically covers the northern Dalmatian coast, the Ravni kotari and Bukovice coasts, Pozrmanje, and a portion of southern Lika. Ravni kotari is defined by its location in relation to the sea, the rocky inland, and southern boundary of Vrana Lake. This geological-geomorphological structure produces a considerable amount of arable land, making it the most notable area of the Croatian coast in terms of agricultural land size (Franin 2016). Warm, dry summers and mild, rainy winters characterize the climate. Evergreen shrubs and trees make typical vegetation, especially olive trees, so it is sometimes called "climate of olives" (Kraljev et al. 2005). The highest temperatures are in July and August, and the lowest in January. Precipitation is highest in late autumn and lowest in July (Bralić and Faričić 2010).

Cultivation of olive trees (*O. europaea*) and grapevines (*V. vinifera*) in the Mediterranean dates back to antiquity, and they continue to play an important economic and cultural role in the Mediterranean basin today (Loumou and Giourga 2003, Froidevaux et al. 2017, Kavvadias and Koubouris 2019). Olive groves are stable ecosystems, partly due to the rich beneficial arthropod fauna (Cirio 1997), while several studies emphasized the importance of functional arthropod biodiversity in vineyards as well (e.g. Franin et al. 2016, Froidevaux et al. 2017).

Olive orchard with EPM

Olive orchard under EPM management is located near Poličnik, 9 km from sea (Figure 4). It was transformed from a maquis to an olive grove 12 years ago. We considered that the time span (>10 years) was sufficient to allow invertebrate fauna to develop communities and display stability in species composition, distribution, and abundance due to the small-medium field size, absence of crop rotation, and stability of the olive orchards (Cirio 1997, Kromp 1999). Autochthonous olive *orkula* is planted here as it is most resilient to suboptimal pedological and climate factors (arid, rocky terrain, dry climate), and harvested by hand. Only the compounds allowed under EPM are used, in this case copper, which is regularly controlled by authorized monitoring station AgriBioCert.

Figure 4. Olive orchard with EPM. Photo: MEDITERATRI team.

Olive orchard with IPM

This research site is located near Škabrnja, and is surrounded by coppice as well as other nearby orchards, including apples and cherries. The soil within the orchards is covered by plants which are regularly mowed (Figure 5). It has altitude of 104 m, and surface area of 0.85 ha.

Figure 5. Olive orchard with IPM. Photo: MEDITERATRI team.

Vineyard with EPM

Vineyard under EPM is located near Nadin, at the altitude of 70 m. It has 15 ha in area, covered with tilled soil with weeds (Figure 6). Copper and sulfur-based agents are used as fungicides, and authorized monitoring station AgriBioCert controls that principles of ecological production are being met. The production started in 2007, making it the first vineyard with ecological production in Croatia. Nadin is also known as Croatia's most environmentally friendly town, with 90 percent of its 600 acres of vineyards being cultivated under the observation of ecological stations. Maraština, Chardonnay, Merlot, Syrah, Cabarnet sauvignon, Greanage, Plavina and Crljenak (Zinfandel) are cultivated vine varieties.

Figure 6. Vineyard with EPM. Photo: MEDITERATRI team.

Vineyard with IPM

This site is located near the settlement of Islam Greek and belongs to the agricultural good Baštica owned by the University of Zadar (Figure 7). It has area of 6 ha located within the agricultural complex, where the majority of the plantations are perennial, such as other vineyards and fruit orchards. Vegetable farming is also conducted nearby. The vineyard is covered by grass that is controlled by mowing and herbicides. Maraština, Merlot, Cabarnet sauvignon, and Plavina are cultivated vine varieties.

Figure 7. Vineyard with IPM. Photo: MEDITERATRI team.

<u>Control site (C)</u>

It is an unmanaged site that had never been an arable land, situated near olive orchard with EPM. It has typical Mediterranean habitat with rocky soil, maqius and garrigue formed by region’s natural vegetation (Figure 8).

Figure 8. Control site. Photo: MEDITERATRI team.

Table 1. Research sites and their characteristics.

Type of study site	Location	Site (Mark)	Pest management type	Vegetation	Mulching	Ploughing	Altitude
Olive groves	Poličnik, Zadar County	OLOM1	Ecological	Rocky soil with little plant coverage	Yes	No	81
	Škabrnja, Zadar County	OLIPM1	Integrated	Grass covarage, surrounded by coppice	Yes	No	104
Vineyards	Nadin, Zadar County	VYOM1	Ecological	Tilled soil with weeds	No	Yes	70
	Baštica, Zadar County	VYIPM1	Integrated	Tilled soil with weeds	Yes	Yes	105
Control	Suhovare, Zadar County	C	Unmanaged	Maquis and *garrigue*	No	No	95

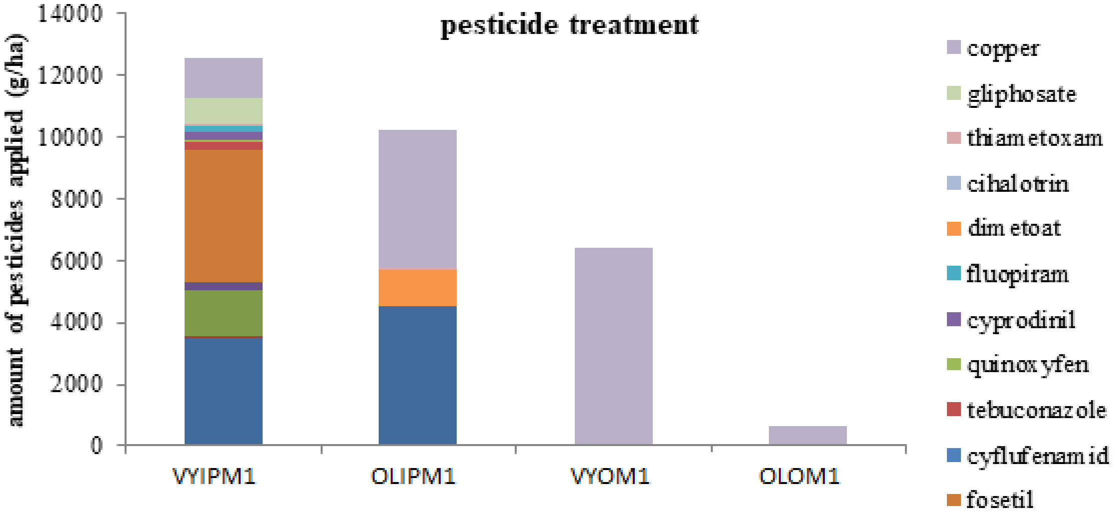

Figure 9. The amount of pesticides added at each study site during 2018 shown as grams of active substances applied per hectare. Copper was used either as Copper (I) oxide or copper oxychloride ($H_6C_{12}Cu_4O_6$). Sites abbreviations: vineyard with EPM (VYOM1) and one with IPM (VYIPM1), olive grove with EPM (OLOM1) and one with IPM (OLIPM1). Taken and adapted from Ivanković Tatalović et al. 2020.

The second part of sampling, where live samples for ecotoxicological tests were collected, was conducted in deciduous forests in Zagreb County, Velika Buna (Pokupsko municipality) (Figure 10), in the area far from agricultural sites and pesticide use. This site was chosen as it has never been under the agricultural management, and due its proximity to Zagreb, which made beetle sampling and rearing simpler. Hornbeam, oak, chestnut, and beech are typical vegetation, and soil is covered with leaf litter. The area lays between the Sava and Kupa valleys, before their junction. From 2000 to 2020, the average total precipitation in the winter (December, January, and February) was 196 mm, while the average total precipitation in the spring/summer (May, June, and July) was 223 mm per Croatian Meteorological and Hydrological Service (meteo.hr). These samples were used for *in vivo* studies on thiamethoxam impact on carabid beetles. Contol groups were scanned to confirm that there are no pesticides in the enviroment.

Figure 10. Temperate oak and hornbeam forest ecosystem in Pokupsko municipality where carabid beetle samples for *in vivo* toxicity experiments were collected. Photo: Ivanković Tatalović L.

4. MATERIALS AND METHODS

4.1. Sampling

Carabid beetles used in this study were caught by hand and using pitfall traps, which is the most widely used sampling technique for carabid beetles (Kromp 1999). Sampling by hand included searching for beetles under tree trumps, stones, and moss, for the molecular analyses and experiment where live beetles were needed. The trap (usually a plastic container) is dug in the ground, so that soil reaches the opening (Figure 11). It should be covered from the rain in a way that still allows the beetles to reach the opening and fall in. Pitfall traps can be filled with preservative or attractant, depending on the purpose of sampling. Number of traps and the duration of their exposure make the sampling effort. This is the passive sampling method, so the number of caught specimens depends on several factors, including species activity, trap size and shape, vegetation cover, and weather. Since the catchment depend on these factors, comparative studies between the sites can be difficult (Sunderland et al. 1995). Furthermore, there is a danger of predation inside the trap (Stapp 1997). Despite these shortcomings this is still the most reliable and often used sampling method for carabid beetles (Spence and Niemela 1994).

Figure 11. A pitfall trap covered by piece of wood. Photo: Ivanković Tatalović L.

4.1.1. Carabid beetle sampling in Croatian agroecosystems - preserved specimens

In order to record the complete vegetation season, carabid beetles were sampled in 2018 from April to July and from September to November. As previous years suggested, draughts and high air temperatures occurred in August (meteo.hr), so August was left out of the sample period. Furthermore, due to aestivation in August, carabid beetle activity decreased (Thiele 1977). Plastic pitfall traps with an 8 cm diameter and

300 mL volumen were used to gather the specimens. There were 12 traps per site positioned at the plantation, and the distance between traps was approximately 10 m. All pitfall traps were placed under olive trees or under grapevine stumps. They were positioned at least 20 m from the field margins, to avoid changes in population and community structure that occure at the boundary of two habitats, called edge effects (Porensky and Young 2013). For the purpose of statistical analysis, the capture for each species at each location for the whole sampling period was standardised as activity density (AD) [1] because the number of traps and their exposure length varied between sites and sample periods.

$$[1]\ AD = \frac{N\ (sampled\ specimens)}{\sum n[traps \times days]} \times 1000$$

where n is the number of sampling periods, N is the number of sampled specimens, traps is the number of open pitfall traps, and days refers to the number of days that traps were active.

For the preservation of trapped arthropods water salt solution was used. Traps were emptied every two-three weeks and material was transferred in 80% ethanol and analysed in the laboratory. In total, seven sampling events occurred across all sites, four in spring/summer season and three in autumn. Carabid beetles were isolated and identified to species using taxonomic keys by Trautner and Geigenmüller (1987), Freude et al. (2004), and Hurka (1996), and following the nomenclature of Fauna Europaea (Vigna-Taglianti 2013).

4.1.2. Carabid beetle sampling in Croatian agroecosystems - live specimens

For the purposes of scaning carabid beetle gut content for selected prey, carabid beetles were sampled from April to November in 2018 and 2019, with August being excluded from sampling period due to the draught (meteo.hr) and carabid beetle estivation in that month (Thiele 1977). Plastic containers (volume of 300 mL) with vinegar serving as a lure were placed beneath the olive trees and vines overnight, and checked for the live beetles in the morning. Each site had 12 traps positioned parallel within the plantation, with a space between traps of about 10 meters. All of the pitfall traps were placed beneath olive trees or grapevine stumps. To avoid edge effects, they were placed at least 20 meters apart from the field's edges. Carabid beetles that shared a container with a dead prey were discarded. Handpicking was used as a sampling method alongside the pitfall traps. Samples were immediately preserved in absolute ethanol to preserve their gut content eDNA. All the collected samples were sorted in the laboratory where predatory species were identified for scanning gut content DNA for specific prey.

4.1.3. Carabid beetle sampling and rearing in deciduous forests of Zagreb County

In order to guarantee that the experimental beetles had not previously been exposed to long- or short-term pesticide exposure, beetles were caught by hand and pitfall traps in late March and during May 2020 in the edge of deciduous forests in Zagreb County, in the area far from agricultural sites and pesticide use. *Abax ovalis* (Duftschmid, 1812) and *Abax parallelus* (Duftschmid, 1812) , frequent predatory carabid beetles in this habitat, were selected as a model organism for the feeding trials. In the ground, 300 mL plastic containers were utilized as pitfall traps, with vinegar acting as a bait. *A. parallelus* and *A. ovalis* specimens were moved to a laboratory for rearing every two days until an adequate number of the specimens had been collected.

Before the start of the experiment, they were kept in plastic containers with 2 cm of humid soil (Mauchline et al. 2004, Douglas et al. 2015), in chamber with 12h light, 4h dimmed light, and 8h dark photoperiod and 20°C (±2°C) temperature. They were fed with moistened dog food (Kunkel et al. 2001; Mauchline et al. 2004, Douglas et al. 2015). Water mist was applied to the containers to provide humidity and drinking fluids (Young 2008). Pieces of wood and moss were offered as shelter (Hatteland et al. 2010).

4.2. Analysis of carabid beetle assemblages and distribution of functional traits in olive orchards and vineyards

To assess the assemblage and the carabid beetles traits composition, in addition to study sites in Zadar county described in section 3, published studies with following criteria were selected: (i) conducted in vineyards and olive orchards within the Mediterranean basin; (ii) having samples collected by pitfall traps since the pitfall traps are most widely used sampling technique for carabid beetles (Kromp 1999); and (iii) containing the list of sampled carabid beetles identified to the species level.

To determine whether the distribution of features among carabid beetle assemblages can be connected to researched agricultural practices, three ecological and morphological traits were selected. In order to characterize carabid beetle assemblages in agricultural fields, it is typical to employ life traits such as feeding preferences, body size, and wing development (Cole et al. 2002, Pizzolotto et al. 2018). The following few attributes were used to further break down each trait: (i) feeding preferences (carnivores, omnivores, and herbivores); (ii) average body size of the species (very small (5 mm), small (5-10 mm), medium (10-15 mm), and large (>15 mm); and (iii) wing development (macropterous, dimorphic, brachypterous). Information on the selected traits was acquired from the recent literature and the online database Carabids.org (Homburg et al. 2014).

4.3. Morphological analysis – geometric morphometry (GMM) and fluctuating asymmetry (FA)

Specimens of *P. koyi* were photographed using an Epson Perfection V600 Photo scanner while specimens of *P. melas* were photographed using a digital Nikon D60 camera with Sigma macroobjective. For all *P. koyi* and *P. melas* specimens, the dorsal portion of the body was imprinted in clay to maintain the specimen's horizontal level while the ventral portion of the body was photographed. A total of 16 landmarks on the ventral part of the body was used for *P. koyi* and *P. melas* (Figure 12). The tpsDig 2.31 software was used to digitize proposed landmarks (Rohlf 2018). Landmarks were digitized three times in the case of *P. melas* and twice in the case of *P. koyi* in order to assess the significance of fluctuating asymmetry.

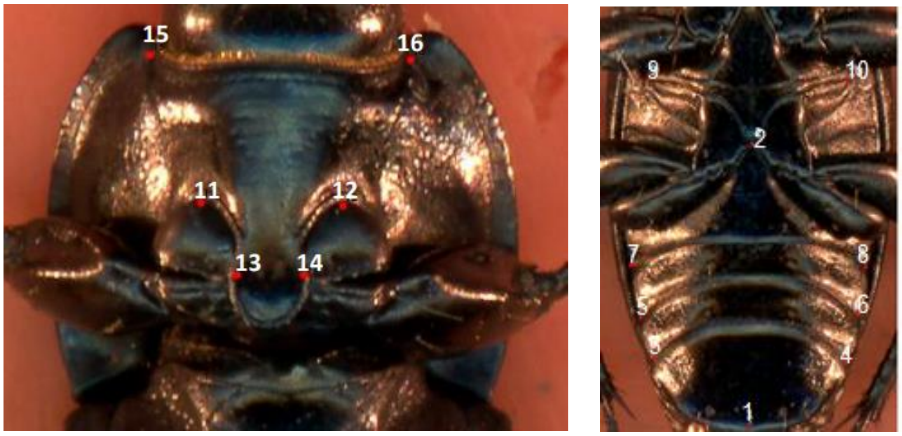

Figure 12. Selected landmarks on the ventral side of the pronotum (left) and abdomen (right) of the *P. koyi* specimen. (1) pygidium; (2) middle point of the metastern; (3,5,7) left lateral vertex of the abdominal segment; (4,6,8) right lateral vertex of the abdominal segment; (9,10) metathorax; (11,12,13,14) coxa of the first legs; (15) left vertex of the pronotal epimere; (16) right vertex of the pronotal epimere. Photo: MEDITERATRI team.

4.4. Ecotoxicological tests

In order to ensure that every *A. parallelus* individual enters the treatment in the same state, food was provided ad libidum for 24 hours four days before to the treatment. Following that time, the food was taken away, and the beetles were starved for the following 72 hours. Every individual had their weight and sex recorded for the day of the treatment. Beetles were exposed to thiamethoxam (Thiamethoxam, 100 MGM, Sigma-Aldrich) by conventional dipping method, thus ensuring that every beetle was treated equally and all body parts were exposed to the pesticide. First, stock solution (100mg Ai/L) was prepared and then diluted with distilled water (dH2O) to the following concentrations: 3.9 mg/L (C1), 9.1 mg/L (C2), 20.0 mg/L (C3), and 40.0 mg/L (C4). Control group (C0) was treated with the dH_2O. The starting concentration

(C1) was based on the highest total body concentration of pesticides which was measured in carabids collected in the vineyards under IPM (Šerić Jelaska et al. unpublished data), and on preliminary experiments. Fifteen beetles per concentration were submerged in the 15 mL of the solution for 15 seconds, then returned to the clean plastic container.

4.4.1. Feeding trial and assesing locomotor activity post treatment

Each *A. parallelus* individual received two fresh blowfly larvae (Calliphoridae) two hours after treatment and was allowed to eat them for twelve hours. To make feeding easier, the larvae were notched. Before giving food to the beetles and again immediately after it was taken out of the container after 12 hours, weights of larvae and later larvae remains were recorded. In the container's 100% humidity was maintained with wet filter paper, preventing any weight loss due to evaporation.

The locomotor ability of each beetle was tested by turning it on its back and observing the reaction for one minute. They were then classified in the four categories: (1) Normal - normal walk, energetic and fast movement of legs, 10 sec or less to turn themselves over after being placed on their backs; (2) slightly intoxicated - beetles are still reacting to a stimulus, but their movements are slower and less coordinated. They may demonstrate excessive twitching and cleaning of antennae; (3) intoxicated - extremely uncoordinated movement, walking with legs fully extended, unable to return on their legs when put on their backs; (4) dead or moribund - completely paralyzed or had only minute nerve twitches. Those beetles whose behaviour fell between these categories were given the average value of two categories. Beetle's condition was observed 4, 12, 24, and 48 hours after the treatment. After 48 hours specimens were frozen at -80°C for the metabolomics profiling.

4.4.2. Measuring metabolites using gas chromatography–mass spectrometry

The method outlined by Pan et al. (2010) was used to extract metabolites. In an essence, methanol was utilized for the first extraction, and chloroform, methanol, and water (1:2:1, v/v/v) were employed for the second extraction. After centrifugation at 12000 x g and 4°C, combined supernatants were put into analytical vials and dried by evaporation. Methoximation was performed using 80 µL of methoxyamine hydrochloride (15 mg/mL) (Sigma-Aldrich, St. Louis, USA) in pyridine (BDH PROLABO, UK). The resultant mixture was incubated for 16 h at room temperature. Derivatization was performed using 80 µL of N-methyl-N-trimethylsilytrifluoroacetamide (MSTFA) with 1% of Trimethylsilyl chloride (TMCS) (Sigma-Aldrich, St. Louis, USA) at 70°C for 1h. The metabolic profiling analysis was performed on a

Shimadzu single quadrupole GCMS-QP2010 gas chromatograph-mass spectrometer (Shimadzu, Kyoto, Japan). Metabolites were separated on a 30 m × 0.25 mm × 0.25 μm BPX-5 capillary column (SGE, Austin, TX, USA). The injector temperature was 250°C and high-purity helium was used as the carrier gas. The GC temperature was programmed with initial temperature at 60°C for 2 minutes, then increased to 330°C at 15°C/minute and maintained for 10 minutes. Ion source temperature was 200°C and interface temperature was 280°C. The selected mass range was set to 45-600 m/z. The identification of low molecular weight metabolites was carried out using a commercially available GC-MS Metabolite Mass Spectral Database (NIST and Wiley). The metabolites concentrations are expressed as total ion current (TIC) for each individual beetle.

4.4.3. Oxidative stress and superoxide dismutase (SOD)

There are many assay kits in the market designed to test the activity of SODs in the blood and/or tissue of the organisms. Superoxide Dismutase Activity Assay Kit (Colorimetric) by Abcam used in this study uses the tetrazolium salt WST-1, which when reduced with superoxide anion forms a water-soluble formazan dye. The rate of WST-1 decrease is proportional to the activity of SOD in inhibiting xanthine oxidase (XO). SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen, lowering WST-1 levels (Figure 13). This inhibitory activity of SOD is measured by colorimetric technique at OD 450nm.

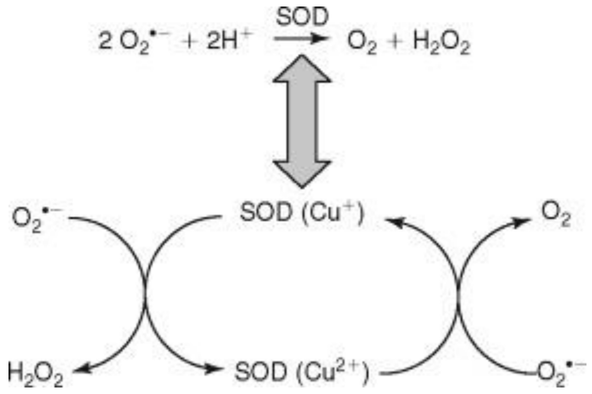

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Figure 13. Illustration of the simplified SOD mechanism in the cell: biological process of $O_2^{\bullet-}$ dismutation into O_2 and H_2O_2 via a cyclic oxidation-reduction electron transfer mechanism. Taken from Zhang et al. (2011).

The beetles were fed ad libidum and then starved for 24h so they all enter the experiment in the same condition. They were treated with thiamethoxam (0, 3.9, 9.1, and 20.0 mg/L) as described in section

4.4.1. after which they were allowed to rest for 30 minutes. Then, they were killed by quick freezing in a liquid nitrogen and preserved at -80°C. The SOD activity in beetle tissue was measured using Superoxide Dismutase Activity Assay Kit (Colorimetric) by Abcam per producer's instructions. SOD activity was calculated as the inhibition activity of xanthine oxidase (XO) by SOD.

4.4.4. *Transmission of insecticides via trophic interactions and post-feeding oxidative stress levels in carabid beetles*

A. ovalis specimens collected in the deciduous forest in Zagreb County were kept as described in section 4.1.3. Earthworms of the genus *Eisenia,* weighting from 0.6 to 1.2 grams, were obtained from fishing store to serve as a prey to the carabids in the following feeding trial. 2.5 kg of store-bought black soil was dried at 70◦C and cooled to the room temperature. The soil was distributed in five glass jars, each containing 0.5 kg. After cooling, four jars were spiked with insecticides solutions, two with thiamethoxam, and two with dimethoate. The soil in the first jar was mixed with 15 ml of thiamethoxam stock solution (100mh Ai/L) which equals 3 mg Ai/kg. The soil in the second jar was mixed with 40 ml of thiamethoxam stock solution (100mh Ai/L) which equals 8 mg Ai/kg. The soil in the third jar was mixed with 5 ml of dimethoate stock solution (100mg Ai/L) which equals 1 mg Ai/kg. The soil in the fourth jar was mixed with 50 ml of dimethoate stock solution (100mg Ai/L) which equals 10 mg Ai/kg. Stock solution is mixed with distilled water to make 125 ml solution that is used to rehydrate the soil, so that moisture level is 25% (Martin 1986), and achieve final concentrations of 3 and 8 mg Ai/kg for thiamethoxam, that is, 1 and 10 mg Ai/kg for dimethoate. Earthworms are added to the soil (3 animals per 0,5 kg of soil) and left to accumulate pesticides for seven days. Jars were enclosed to retain moisture and toxic vapor and were kept in the dark (Martin 1986). After seven days earthworms were taken from the soil and left 24h to empty their guts of all the soil (Svendsen and Week 1997). One or two earthworms out of each group were frozen for the detection of pesticides in their tissue. Earthworms were cut with the scalpel and offered to the beetles to feed *ad libidum.* Beetles who were not observed to feed were removed from the experiment. The rest of them were separated into two groups. Beetles in the first group were left to rest for three hours and then killed by quick freezing in liquid nitrogen. These beetles were used to measure SOD activity in their tissue. Beetles in the other group were left to empty their guts and then were frozen and sent alongside earthworm samples to EUROFINS CROATIAKONTROLA d.o.o. where pesticide concentrations were measured by liquid chromatography - mass spectrometry (LCMS / MS) to confirm the transfer of pesticides from prey to the predators. The sample of store-bought soil that was used in the experiment (>20g) was also sent, to discard the possibility pesticide contamination prior to the start of the experiment. The list of samples sent for analysis is in the Table 2.

Table 2. The list of carabid beetle, earthworm, and soil samples sent for the liquid chromatography - mass spectrometry (LCMS / MS) to measure concentration of insecticides in the sample.

Specimen ID	**Taxon**	**Insecticide treatment (mg/kg)**	**N (individuals)**	**Sample mass (g)**	**Insecticide for detection**
1	Lumbricidae	3	1	1.0416	Thiamethoxam and clothianidin
2	Lumbricidae	8	2	1.2889	Thiamethoxam and clothianidin
3	Lumbricidae	1	2	1.3556	Dimethoatee
4	Lumbricidae	10	2	1.2195	Dimethoatee
5	Carabidae	3	4	0.9744	Thiamethoxam and clothianidin
6	Carabidae	8	5	1.1146	Thiamethoxam and clothianidin
7	Carabidae	1	5	1.2845	Dimethoate
8	Carabidae	10	5	1.1494	Dimethoate
9	Soil	/	/	23.3200	Any pesticide

The measurement of SOD activity in the carabid beetle tissue was performed as described in section 4.4.3.

4.5. Gut content analyses using Polymerase chain reaction (PCR)

Carabid beetle foregut was extracted from the animal by gently pulling the beetle apart at the joint between elytra and pronotum. Foregut was then separated from the thorax using curved forceps and transferred to an Eppendorf tube for the further DNA extraction. Microscope was used for the smaller individuals and forceps bleeched, washed, and flame-sterilized after each individual to avoid cross contamination. DNA was extracted from the foregut of predatory carabid beetles using DNeasy Blood & Tissue Kit (Qiagen) per manufacturer's instructions and kept at -80°C. To screen for potential DNA carry-over contamination during extraction, each set of samples included negative controls with no animal tissue added. DNA from *B. oleae*, and earthworm and moth species was also extracted to serve as positive controls during PCR. The primers used to screen carabid gut content for these three prey groups are listed and described in Table 3.

Table 3. List of primers used to screen carabid beetle gut content for specific and non-specific prey.

Taxon	Primer name	Primer sequence	Amplicon size (bp)	Reference
Earthworms general	185F: 14233R	TGTGTACTGCCGTCGTAAGCA AAGAGCGACGGGCGATGTGT	225–236	Harper et al. 2005
Moths general	LM-14259-F LM-14423-R	TCTGCATCTTGATCTGAT TTTGGCGGTATTTTAGTTCAT	165	Sutherland 2000
B. oleae	p_Cox1D F p_Cox1D R	CAGTAGTACTAACAGCCCTAC TTGGTAAAGGATTGGGTCTCC	136	Panni and Pizzolotto 2018
Terrestrial arthropods general	BF3 BR2	CCHGAYATRGCHTTYCCHCG TCDGGRTGNCCRAARAAYCA	458	Elbrecht and Leese 2017

Following procedure was used: For earthworm general primers, each PCR (6µL) contained the following: 1.76µL of dH2O (QIAGEN), 3µL of DreamTaq ThermoFisher Scientific polymerase, 0.12µL of each primer, and 1µL of template DNA. PCRs were carried out in BioRad thermal cycler using the following conditions: initial heat activation (95°C, 5 min), denaturation (95°C, 30 s), annealing (65°C, 30 s), extension (72°C, 60 s), final extension (72°C, 10 min). Denaturation, annealing, and extension were repeated for 40 cycles.

For moths general primers, each PCR (6µL) contained the following: 1.76µL of dH2O (QIAGEN), 3µL of DreamTaq ThermoFisher Scientific polymerase, 0.12µL of each primer, and 1µL of template DNA. PCRs were carried out in BioRad thermal cycler using the following conditions: initial heat activation (95°C, 3 min), denaturation (95°C, 30 s), annealing (50.2°C, 30 s), extension (72°C, 60 s), final extension (72°C, 10 min). Denaturation, annealing, and extension were repeated for 40 cycles.

For *B. olea* primers, each PCR (6µL) contained the following: 1.76µL of dH2O (QIAGEN), 3µL of DreamTaq ThermoFisher Scientific polymerase, 0.12µL of each primer, and 1µL of template DNA. PCRs were carried out in BioRad thermal cycler using the following conditions: initial heat activation (95°C, 3 min), denaturation (95°C, 30 s), annealing (55.5°C, 30 s), extension (72°C, 60 s), final extension (72°C, 10 min). Denaturation, annealing, and extension were repeated for 40 cycles.

Using universal invertebrate primers for a 458-bp fragment of the mitochondrial cytochrome oxidase I (COI) gene (Table 1), extractions were examined by PCR to determine whether DNA was present after extraction and to prevent false negatives (Šerić Jelaska et al. 2014 a, b, 2016, Panni and Pizzolotto 2018). Each PCR (6µL) contained the following: 1.3µL of dH2O (QIAGEN), 2.5µL of QIAGEN Multiplex PCR Master Mix, 0.2µL of each primer (BF3 and BR2), and 1µL of template DNA. PCRs were carried out in BioRad thermal cycler using the following conditions: initial heat activation (95°C, 15 min), denaturation (94°C, 30 s), annealing (46°C, 90 s), extension (72°C, 90 s), final extension (72°C, 10 min). Denaturation, annealing, and extension were repeated for 35 cycles.

4.6. Statistical analysis

In the analysis of carabid beetle assemblages and functional traits within Mediteranean agroecosystems, the Shapiro-Wilks test was employed to determine whether the data were normal. To determine if there was a significant difference in the proportion of traits between unmanaged sites, sites under EPM, and sites under IPM, as well as to evaluate the difference in the functional diversity between the management types, a nonparametric Kruskal-Wallis test was conducted. For the purpose of visualizing trait composition in relation to agricultural practice, redundancy analysis (RDA) was carried out. To include many aspects of functional composition and variety in carabid beetle assemblages, a Rao coefficient was developed as explained in Lepš et al. (2006). In order to compare the results, it was first calculated for every site using presence-absence data and then just for Croatian sites using abundance data. All analyses were performed using STATISTICA 13 (Statistica Inc. TIBCO Software), PAST 4.03 (Hammer et al. 2001), and Microsoft Excel 2010.

Statistical analyses of shape variations were carried out in MorphoJ (Klingenberg, 2011) and STATISTICA 13 (Statistica Inc. TIBCO Software). The Cartesian coordinates of each landmark were used for further analyses (Rohlf and Slice 1990) after Procrusted Superimposition was done in MorphoJ. To analyse measurement error Procrustes ANOVA was performed on combined datasets of repeated landmark digitization for both *P. koyi* and *P. melas*. To evaluate size differences between specimens from different sites and sexes, analysis of variance (ANOVA) and post hoc unequal honest significant difference (HSD) was performed in STATISTICA 13. Procrustes ANOVA was performed, with sites and sex as extra effects. Better visualization between groups was achieved using canonical variate analysis (CVA). After a modification of the axes from the principal component analyses, using the maximum variance of selected groups, to assess the differences across the study sites and in order to statistically quantify the Mahalanobis and Procrustes differences between sites, a pairwise comparison after a 10.000 permutation was performed. All the analyses were performed using symmetric components of the shape variation.

In ecotoxicology experiments, the mass of consumed food 12 hours after the treatment was divided with the mass of the beetle, to standardize the numbers. A Shapiro-Wilks test was used to test the normality of the data ($p < 0.05$), after which a nonparametric Kruskal-Wallis test was performed to analyse differences in the mass of consumed dipteran larvae between the different treatments and sexes. The same test was performed to check the difference in metabolomics and SOD activity in tissue between the groups. We used PCA to visualise the data on metabolites relevant in the detoxification process and anti-oxidative defence. The changes in metabolites were calculated as a deviation of treated groups from the control group.

5. RESULTS

5.1. *Carabid beetle assemblages and distribution of functional traits in olive orchards and vineyards*

5.1.1. Meta-analysis of life traits in carabid beetles assemblages

Sites selected from the published studies, three olive orchards in Italy and two in Greece, and one vineyard in Italy, were under similar agricultural practice as sites in Zadar County (Table 1) with mainly regular mowing activities. Data on mechanical soil preparations and mowing were not available for all locations, hence they were not used as co-variables in the subsequent analyses. The research areas' typical Mediterranean natural habitats are represented by unmanaged control sites in Croatia, Italy, and Greece. The analyses included information on the species makeup of carabids from a total of thirteen sites (Table 4). Furthermore, selected agricultural sites were separated into two categories: those under the EPM and those under the IPM (Table 4). Proportion of the traits described was calculated for each site based on presence-absence data for the carabid beetle species since data on abundance were not available in most of the published studies.

Table 4. Study sites with applied pest managements, ground vegetation, soil mechanical treatments where the data was available, and study references (Abbreviations: IPM - Integrated Pest Management, EPM - Ecological Pest Management, N/A - not available). Taken and adapted from Šerić Jelaska et al. 2022.

Type of study site	Location	Site (Mark)	Pest management type	Ground vegetation and weed procession	Mulching	Ploughing	Reference
Olive orchards	Poličnik, Zadar County, Croatia	OLOM1	EPM	Rocky soil with little plant coverage and regular mowing	Yes	No	Our study
	Škabrnja, Zadar County, Croatia	OLIPM1	IPM	Grass coverage, surrounded by coppice, regular mowing	Yes	No	Our study
	Tuscany, Central Western Italy	OLIPM2, OLIPM3, OLIPM4	IPM	Regular weed mowing	No	No	Albertini et al. (2017)
	Fthiotida, Central Greece	OLOM2, OLOM3	EPM	N/A	No	No	Chapman (2014)

Table 4. Continuation.

Vineyards	**Nadin, Zadar County, Croatia**	**VYOM1**	**EPM**	**Tilled soil with weeds**	**No**	**Yes**	**Our study**
	Baštica, Zadar County, Croatia	VYIPM1	IPM	Tilled soil with weeds	Yes	Yes	Our study
	Val d'Agri, Basilicata, Italy	VYOM2	EPM	N/A	N/A	N/A	Letardi et al. (2015)
Control	Suhovare, Zadar County, Croatia	Con1	Unmanaged	Maquis and garrigue with *Juniperus*, *Pinus* and *Quercus* species	No	No	Our study
	Tuscany, Central Western Italy	Con2	Unmanaged	Coniferous and *Quercus* species, poor understory vegetation	No	No	Albertini et al. (2017)
	Island of Kos, Greece	Con3	Unmanaged	Arid habitats with pine and cedar forests	No	No	Assing (2017)

There were 113 carabid beetles species noted within thirteen selected agricultural sites across the Mediterranean basin, 81 in the olive orchards and 54 in the vineyards, and 24 species on unmanaged sites. Kruskal-Wallis test on different feeding preferences, body size, and wing development groups of carabid beetles from seven olive orchards, three vineyards, and three unmanaged sites, showed that there is a significant difference (Table 5) in the proportion of carnivores based on the management type (unmanaged sites, EPM, IPM) (Figure 14a).

Table 5. Results of Kruskal-Wallis ANOVA by ranks on the proportions of traits and Rao index values. Management type was the independent (grouping) variable: three (Unmanaged, EPM, IPM) for the proportion of the traits; two (unmanaged, managed) for the Rao index. *p* values lower than 0.05 are bolded. Taken from Šerić Jelaska et al. 2022.

		N (sites)	df	KW test	*p*
Proportion of the traits	Carnivores	13	2	6.593	**0.037**
	Herbivores	13	2	5.406	0.067
	Omnivores	13	2	5.507	0.064
	Macropterous	13	2	3.288	0.193
	Brachypterous	13	2	4.478	0.106
	Dimorphic	13	2	1.307	0.52
	Very small	13	2	1.939	0.379
	Small	13	2	3.473	0.176
	Medium	13	2	1.554	0.459
	Large	13	2	2.142	0.342
Functional diversity	Feeding preferences	13	1	4.828	**0.028**
	Wing development	13	1	0.714	0.398
	Body size	13	1	4.828	**0.028**

Multiple Comparisons *p* values (2-tailed) showed that unmanaged sites have significantly higher proportion of carnivorous species than sites with EPM ($p = 0.0183$), but not than sites with IPM ($p = 0.2025$). There were no significant differences in different size and dispersal ability in the carabid beetle assemblage caused by management types. However, sites with IPM had a higher percentage of small and macropterous individuals when compared to those with EPM and unmanaged sites (Figure 14b, c). Variations in carabid beetle assemblages between different managements, according to their feeding preferences, wing development, and body size are presented on the F1 × F2 ordination plot of RDA. The first three axes explained 22.75%, 8.93%, and 0.86% of variance for olive orchards, vineyards, and unmanaged sites, explained 32.54% of the variance (Figure 15). The first axis separates unmanaged sites and OLOM1 from other managed sites, with carnivorous, large, and brachypterous species inclining towards unmanaged sites, confirming the results of Kruskal-Wallis test. Rao index (calculated on presence-absence data) showed the lowest values for all three traits on unmanaged sites compared to the agricultural sites (Figure 16). When the two management types were pooled together (unmanaged sites, agricultural sites), the Kruskal-Wallis test revealed that functional diversities for feeding preferences are significantly lower on the unmanaged sites (Table 5). On the other hand, when agricultural sites were separated by the management type (EPM and IPM), there was no statistically significant difference in Rao index values between the management types (unmanaged sites, sites with EPM, and sites with IPM).

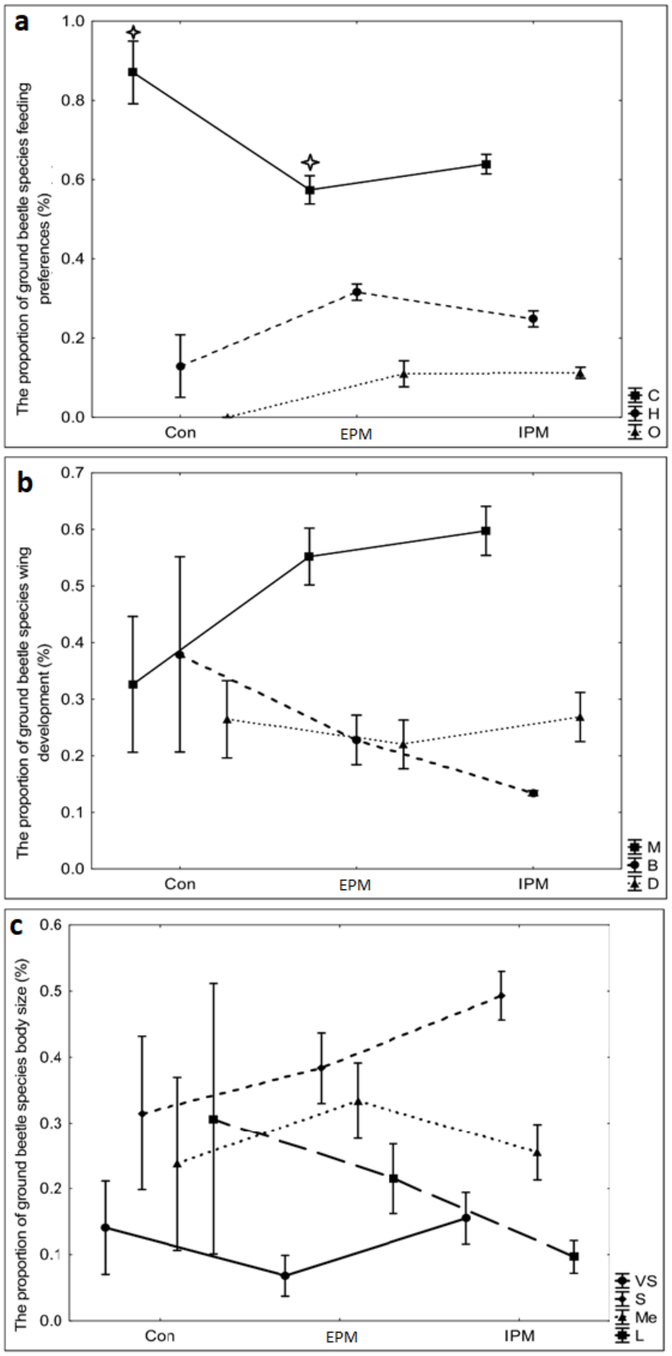

Figure 14. The average proportion (%) of: (a) - carnivorous (C), herbivorous (H), and omnivorous (O); (b) - macropterous (M), brachypterous (B), and dimorphic (D); and (c) - very small (Vs), small(S), medium (Me), and large (L) carabid beetle species depending on agricultural management. (Con. – unmanaged habitats; EPM – sites with ecological pest management; IPM – sites with integrated pest management). Vertical bars denote +/− standard errors. Star denotes the statistical significance in the values. Taken and adapted from Šerić Jelaska et al. 2022.

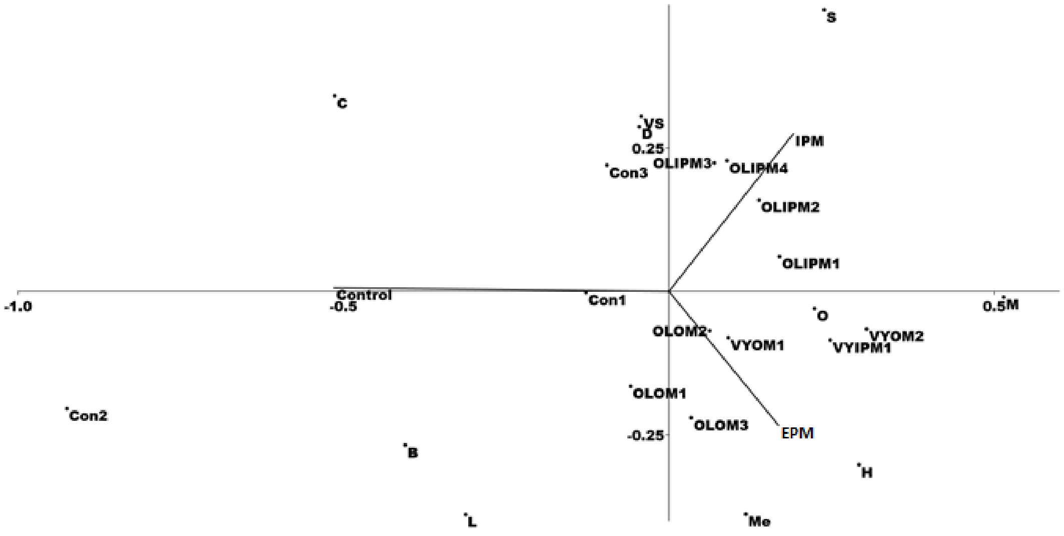

Figure 15. Redundancy analysis (RDA) biplot of axes 1 and 2 of RDA for the proportion of carabid beetle traits in olive orchards and vineyards (C – carnivores; H – herbivores; O – omnivores; M – macropterous; B – brachypterous; D – dimorphic; VS – very small; S – small; Me – medium; L – large). For site abbreviations see Table 4. The environmental variables (management types) analyzed are indicated as vectors. Taken and adapted from Šerić Jelaska et al. 2022.

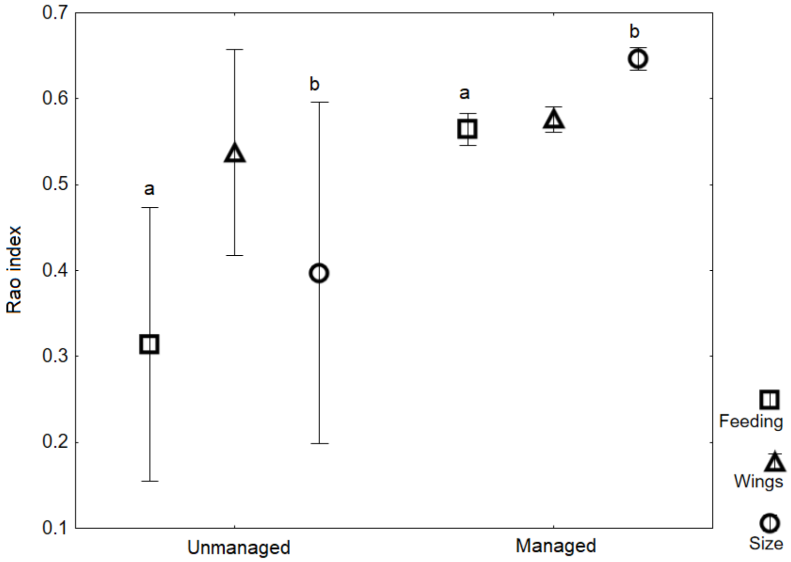

Figure 16. Mean Plot of Rao index values grouped by management (unmanaged, managed) according to the traits. Vertical bars denote +/− standard errors. Taken from Šerić Jelaska et al. 2022.

5.1.2. Carabid beetle assemblages and traits analyses at study sites in Zadar County

In the study period, a total of 4344 individuals belonging to 65 species and 26 genera of family Carabidae was recorded (Table 7). Carnivorous carabid beetles individuals were the dominant feeding group at each site (Figure 17a). This was particularly expressed at OLIPM1 and VYOM1, where their proportion was 96.6% and 96.4% respectively (contrary to proportion of carnivorious species which was 68.8% and 58.6% for these sites). As for the dispersion ability, macropterous individuals were the most numerous at every site with the exception of Con1, where the brachypterous individuals dominated (Figure 17b). The majority of the sampled carabid beetles were larger than 5 mm. Very small individuals (<5 mm) made only 0.5% of the total catch, and were most numerous at VYIPM1, where their proportion was 3.1%. Medium-sized individuals dominated at every site but Con1 and OLIPM1, where the large-sized and small-sized carabid beetles were the dominant groups respectively (Figure 16c). Rao index values were highest for category *size* and lowest for category *diet* on each site except for VYIPM1, where diet had the highest Rao index value (Figure 17a, b, c). Diet also had the lowest value in meta-analysis when only the presence-absence data was used.

Table 6. List of carabid beetle species in alphabetical order, their traits, and AD (Activity density) values (calculated as a number of captured individuals divided with multiplication of number of traps and number of days that traps were exposed) are given for each species on each site in Zadar County, Croatia. C – carnivores; O – omnivores; H – herbivores; B – brachypterous; D – dimorphic; M – macropterous. (Con1 – unmanaged site; OLOM1 – olive orchard with EPM; OLIPM1 – olive orchards with IPM; VYOM1 – vineyard with EPM; VYIPM1 – vineyard with IPM.

Species	**Diet**	**Wing development**	**Body size**	**Sites**				
				Con1	OLOM1	OLIPM1	VYOM1	VYIPM1
***Abax carinatus* (Duftschmid, 1812)**	C	B	Large	0	0	0	0	5.8×10^{-4}
***Acinopus megacephalus* (P. Rossi, 1794)**	H	B	Medium	7.5×10^{-1}	5.9×10^{-1}	0	0	0
***Acinopus picipes* (Olivier, 1795)**	H	B	Medium	7.5×10^{-1}	5.9×10^{-1}	0	0	0
***Acupalpus interstitialis* Reitter, 1884**	O	M	Very small	0	0	0	6.1×10^{-1}	0
***Acupalpus meridianus* (Linne, 1761)**	O	M	Very small	0	0	0	0	1.2
***Agonum duftschmidti* J. Schmidt, 1994**	C	D	Small	0	0	0	6.1×10^{-1}	0
***Amara aenea* (De Geer, 1774)**	O	M	Small	0	0	5.7	6.1	8.7

Table 6. Continuation.

Amara anthobia **A. Villa & G.B. Villa, 1833**	**H**	**M**	**Small**	**0**	**5.9×10^{-1}**	**1.7**	**1.8**	**1.2**
Amara dalmatina **Dejean, 1828**	H	M	Small	0	1.8	0	0	0
Amara equestris **(Duftschmid, 1812)**	H	M	Medium	0	5.9×10^{-1}	0	0	0
Amara eurynota **(Panzer, 1796)**	H	M	Medium	0	0	0	4.9	0
Anchomenus dorsalis **(Pontoppidan, 1763)**	C	M	Small	0	0	8.9×10	0	5.8×10^{-1}
Anisodactylus binotatus(Fabricius, 1787)	H	M	Medium	0	0	5.7×10^{-1}	0	0
Anisodactylus signatus **(Panzer, 1796)**	O	M	Medium	0	0	5.7×10^{-1}	0	0
Brachinus brevicollis **Motschulsky, 1844**	C	M	Small	0	0	5.7×10^{-1}	0	0
Brachinus crepitans **(Linne, 1758)**	C	M	Small	0	5.9×10^{-1}	5.7	1.2×10^{2}	5.8×10^{-1}
Brachinus elegans **Chaudoir, 1842**	C	M	Small	0	0	1.1	0	0
Brachinus explodens **Duftschmid, 1812**	C	M	Small	0	4.7	3.8×10	0.14	4.7
Brachinus immaculicornis **Dejean, 1826**	C	M	Small	0	0	5.7×10^{-1}	0	0
Brachinus psophia **Audinet-Serville, 1821**	C	M	Small	0	0	1.1	0	0
Brachinus sclopeta **(Fabricius, 1792)**	C	M	Small	0	1.8	0.13	4.4×10	0
Calathus cinctus **(C.R. Sahlberg, 1827)**	C	D	Medium	0	0	3.4	6.1×10^{-1}	0
Calathus fuscipes **(Goeze, 1777)**	C	D	Medium	7.5×10^{-1}	1.2	6.8×10	0.11	5.8×10^{-1}
Calathus luctuosus **(Latreille, 1804)**	C	B	Medium	0	5.9×10^{-1}	0	0	0
Calathus melanocephalus **(Linne, 1758)**	C	D	Small	1.5	1.8	1.4×10	1.9×10	0
Calathus mollis **(Marsham, 1802)**	C	D	Small	0	0	2.3	3.0	0
Callistus lunatus **(Fabricius, 1775)**	C	M	Small	0	0	0	0	5.8×10^{-1}
Carabus caelatus dalmatinus **Duftschmid, 1812**	C	B	Large	0	0	0	0	0
Carabus cancellatus **Illiger, 1798**	C	B	Large	0	0	5.7×10^{-1}	1.2	0
Carabus coriaceus **Linne, 1758**	C	B	Large	3.0×10	2.4×10	2.7×10	4.9	4.7
Carabus hortensis **Linnaeus, 1758**	C	B	Large	0	1.2	0	6.1×10^{-1}	0
Carabus ullrichi **Germar, 1824**	C	B	Large	0	0	5.7×10^{-1}	2.4	5.8×10^{-1}
Chlaenius decipiens (L.Dufour, 1820)	C	M	Medium	0	0	1.7	4.3	5.8×10^{-1}
Chlaenius nigricornis (Fabricius, 1787)	C	M	Medium	0	0	0	6.1×10^{-1}	0

Table 6. Continuation.

***Harpalus affinis* (Schrank, 1781)**	H	**M**	**Medium**	**0**	**0**	**0**	**0**	**7.0**
***Harpalus albanicus* Reitter, 1900**	H	M	Small	0	0	0	1.8×10	0
***Harpalus dimidiatus* (P. Rossi, 1790)**	H	M	Medium	0	0	0	0	1.2×10^{2}
***Harpalus cupreus* Dejean, 1829**	H	M	Medium	0	0	1.0×10	6.1	5.8×10^{-1}
***Harpalus distinguendus* (Duftschmid, 1812)**	H	M	Medium	0	0	0	0	2.3
***Harpalus picipennis* (Duftschmid, 1812)**	H	D	Small	0	0	0	6.1×10^{-1}	0
***Harpalus saxicola* Dejean, 1829**	H	M	Medium	0	1.2	0	0	0
***Leistus imitator* Breit, 1914**	C	B	Small	7.5×10^{-1}	4.1	0	6.1	0
***Leistus fulvibarbis* Dejean, 1826**	C	D	Small	7.5×10^{-1}	5.9×10^{-1}	2.3	0	5.8×10^{-1}
***Leistus spinibarbis* (Fabricius, 1775)**	C	M	Small	0	0	2.3	0	1.8
***Licinus silphoides* (P. Rossi, 1790)**	C	M	Medium	0	1.6×10	0	0	0
***Microlestes minutulus* (Goeze, 1777)**	C	M	Very small	0	0	0	0	1.0×10
***Notiophilus germinyi* Fauvel in Grenier, 1863**	C	D	Small	1.5	0	0	0	0
***Ocys berytensis (*Netolitzky, 1917)**		M	Very small	0	0	0	0	5.8×10^{-1}
***Olisthopus fuscatus* Dejean, 1828**	C	M	Small	7.5×10^{-1}	0	0	0	0
***Ophonus azureus* (Fabricius, 1775)**	H	D	Small	0	0	0	3.7	0
***Ophonus puncticeps* Stephens, 1828**	H	M	Small	0	5.9×10^{-1}	5.7×10^{-1}	0	0
***Ophonus rufibarbis* (Fabricius, 1792)**	H	M	Small	0	0	5.7×10^{-1}	1.2	4.7
***Ophonus sabulicola* (Panzer, 1796)**	H	M	Medium	0	0	5.7×10^{-1}	0	0
***Ophonus stictus* Stephens, 1828**	H	M	Medium	0	0	0	6.1×10^{-1}	0
***Ophonus subquadratus* (Dejean, 1829)**	H	M	Small	0	0	3.4	0	0
***Parophonus dejeani* (Csiki, 1932)**	H	M	Small	0	0	5.7×10^{-1}	0	0
***Parophonus maculicornis* (Duftschmid, 1812)**	H	M	Small	0	0	0	0	2.3
***Parophonus mendax* (P. Rossi, 1790)**	H	M	Small	0	0	0	0	2.3
Poecilus koyi (Germar, 1824)	C	M	Medium	6.0	6.8×10^{-2}	1.8×10^{2}	4.8×10^{2}	7.7×10

Poecilus cupreus (Dejean, 1828)	O	M	Medium	0	0	0	1.2	1.8
Pseudoophonus rufipes DeGeer, 1774	O	D	Medium	0	0	0	8.5	4.9×10

Table 6. Continuation.

***Pterostichus cylindricus* (Herbst, 1784)**	C	B	**Large**	**0**	**1.1**	**0**	**0**	**0**
***Pterostichus melanarius* (Illiger, 1798)**	C	D	Medium	0	5.9×10^{-1}	3.1×10	8.9×10	2.6×10
***Pterostichus melas* (Creutzer, 1799)**	C	B	Large	0	5.9×10^{-1}	9.1×10	0.19	7.6×10
***Trechus quadristriatus* (Schrank, 1781)**	C	D	Very small	0	0	5.7×10^{-1}	0	5.8×10^{-1}
***Zabrus tenebrioides* (Goeze, 1777)**	H	M	Medium	4.5	4.7	5.7×10^{-1}	0	1.8

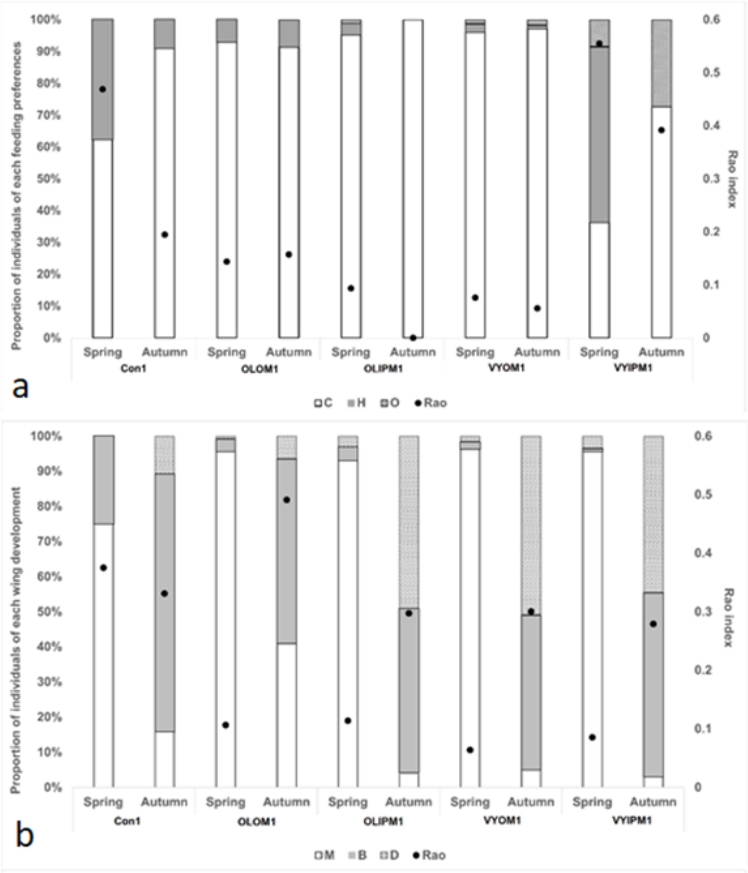

Figure 17. The proportion of individuals and RAO index according to the traits (feeding preferences (a), wing development (b), and body size (c)) they shared at each site within Zadar County, through the

spring/early summer and autumn seasons. (C – carnivores; H – herbivores; O – omnivores; M – macropterous; B – brachypterous; D – dimorphic; VS – very small; S – small; Me – medium; L – large). For site abbreviations, see Table 4. Taken from Šerić Jelaska et al. 2022.

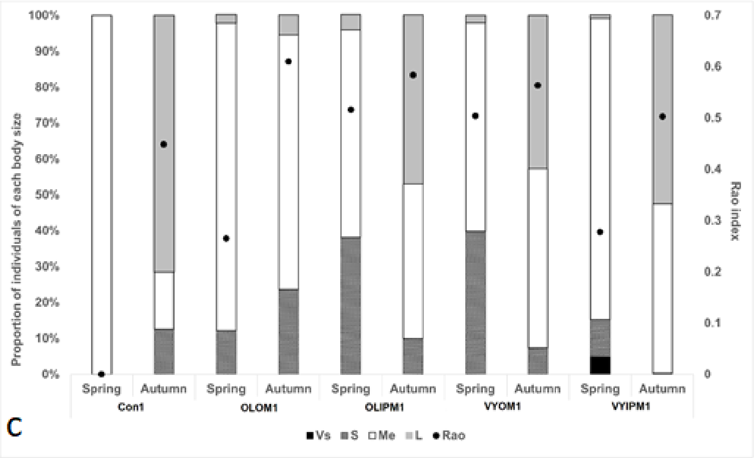

Figure 17. Continuation.

5.2. The impact of agricultural practices on fluctuating asymmetry

Two widespread species, *Poecilus koyi* (Germar, 1824) and *Pterostichus melas* (Creutzer, 1799), were present on nearly all of the sites in a significant number of individuals, hence these species were selected for further analysis and fluctuating assymetry measurements out of the entire sample of carabid beetles (Table 7). Even with extra collection efforts, there were not enough samples of *P. melas* from OLOM1 and *P. koyi* from ontrol site to include this population in further analysis. Samples from two different control sites were combined to create the *P. melas* control sample. Males and females were separated, based on sex combs on the first pair of legs, or in a case that feature was insufficient or absent, external genital parts were examined.

Table 7. Number of individuals used in the analyses of each species per site. Site abbreviations: vineyard with EPM (VYOM1) and one with IPM (VYIPM1), olive grove with EPM (OLOM1) and one with IPM (OLIPM1), and control sites (C). Taken and adapted from Ivanković Tatalović et al. 2020.

Type of study site	Site (Mark)	Pest management type	Number of specimens for *Poecilus koyi*	Number of specimen for *Pterostichus melas*

			♀	♂	♀	♂
Olive groves	OLOM1	EPM	27	53	0	0
	OLIPM1	IPM	26	53	50	50
Vineyards	VYOM1	EPM	25	74	50	50
	VYIPM1	IPM	22	64	50	50
Control	C	unmanaged	0	0	20	9

The Procrustes ANOVA applied on repeated measures of individuals in order to assess the measurement error (ME) showed that ME was negligible (MSindiv>ME, MSind*side>ME) between sets of measurements for both *P. melas* and *P. koyi* (Table 8). The same analysis found significant variances in *P. melas* populations and sexes for size and shape ($p<0.0001$), but only for shape in *P. koyi*. Females of *P. melas* were larger than males on every site (Figure 18). Post hoc test shows a significant difference in size between females from VYIPM1 and control ($p=0.00216$) and females from VYOM1 and control ($p=0.00427$) in case of *P. melas*. *P. koyi* showed no differences between males and females from different sites, or between two sexes within same site.

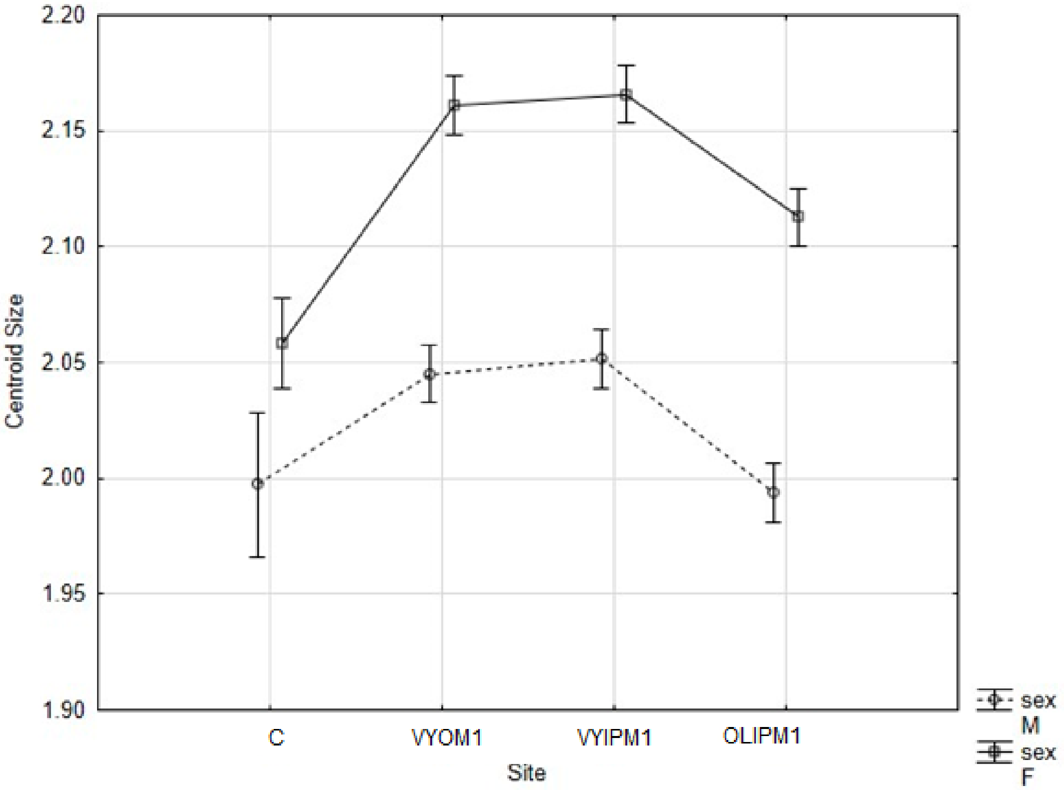

Figure 18. Average centroid size of male (M) and female (F) *P. melas* in four study sites. Vertical bars denote +/− standard errors. For site abbreviations, see Table 2. Taken and adapted from Ivanković Tatalović et al. 2020.

Table 8. Procrustes ANOVA performed for centroid size and shape of *P. melas* (325 individuals) and *P. koyi* (344 individuals). Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). Taken from Ivanković Tatalović et al. 2020.

	Effect	**SS**	**MS**	**df**	**F**	**P (param.)**
Pterostichus melas	**Centroid size**					
	Individual	10.438	0.0377	277	35.14	<.0001
	Error 1	0.5855	0.001	546		
	Shape					
	Individual	0.6295	0.0002	3878	5.39	<.0001
	Side	0.0045	0.0003	14	10.71	<.0001
	Ind * Side	0.1168	3.0113×10^{-5}	3878	28.63	<.0001
	Error 1	0.0161	1.0518×10^{-4}	15288		
Poecilus koyi	**Centroid size**					
	Individual	5.8027	0.0167	348	409.65	<.0001
	Error 1	0.0137	4.1×10^{-5}	336		
	Shape					
	Individual	0.2256	4.6311×10^{-5}	4872	3.25	<.0001
	Side	8.8546×10^{-3}	6.3247×10^{-4}	14	44.43	<.0001
	Ind * Side	0.0694	1.4237×10^{-5}	4872	11.69	<.0001
	Error 1	0.0115	1.2175×10^{-5}	9408		

A canonical variate analysis (CVA) applied to the symmetrical components of shape variation in *P. melas* demonstrated a significant difference in Mahalanobis distance (p<0.0001) between populations from studied sites, except for males from the control sites that did not differ from other groups. Furthermore, there is a significant Mahalanobis distance between sexes within the same population (p<0.0001) (Table 9, Figures 19, 20). Results of the CVA analysis with Procrustes distances on shape variations within *P. melas* populations were similar to those with Mahalanobis distance except for the control populations that did not differ at p <.0001 from other populations, Furthermore, CVA showed a significant Procrustes distance between males and females from same populations except for the control populations (<.0001) (Table 10).

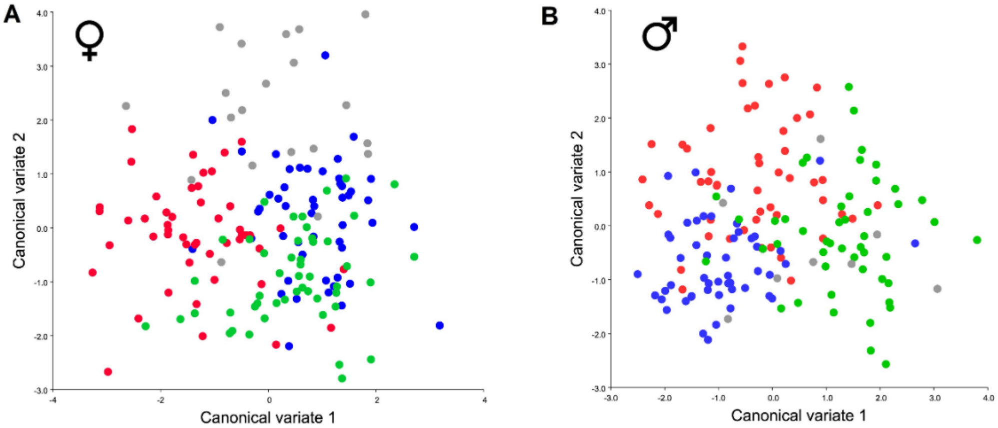

Figure 19. A CVA applied to symmetrical components of shape for *P. melas*. Female (A) and male (B) individuals are shown separately (♂ males, ♀ females). Individuals from VYIPM1 site are presented by blue dots, those from VYOM1 by red dots, OLIPM1 by green and C by grey dots. For site abbreviations, see Table 2. Taken from Ivanković Tatalović et al. 2020.

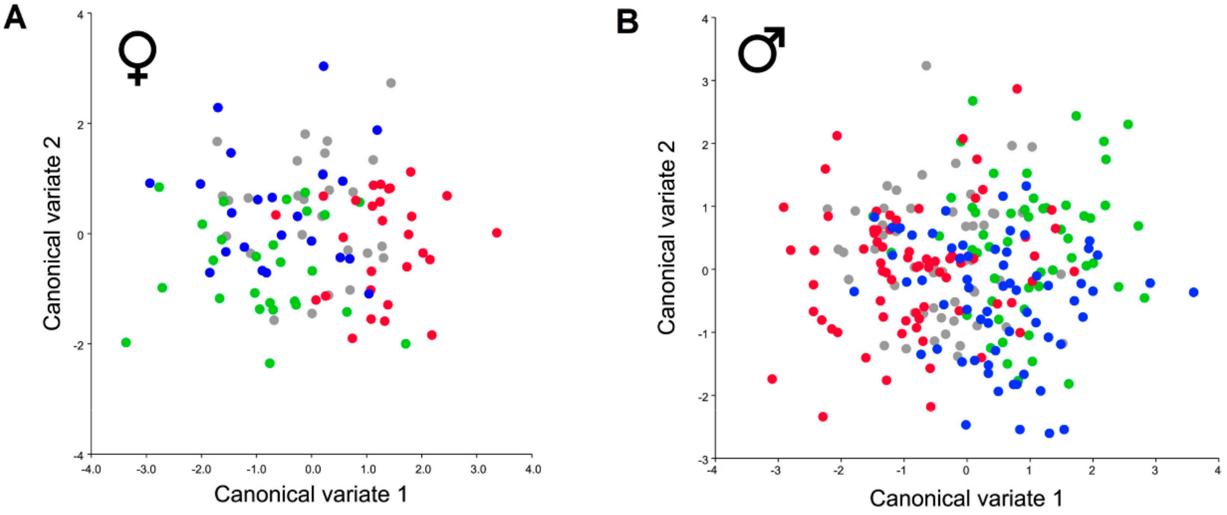

Figure 20. A CVA applied to symmetrical components of shape for *P. koyi*. Female (A) and male (B) individuals are shown separately (♂ males, ♀ females). Individuals from VYIPM1 site are presented by

blue dots, those from VYOM1 by red dots, OLIPM1 by green and C by grey dots. For site abbreviations, see Table 2. Taken from Ivanković Tatalović et al. 2020.

Table 9. Results after t-testing pairwise distances reported as Mahalanobis distance and p-values after 10000 permutations runs. For site abbreviations, see Table 2. Taken and adaptedfrom Ivanković Tatalović et al. 2020.

Species	Site & sex Distance P value	C ♂ Mahalanobis P value	OLIPM1 ♂ Mahalanobis P value	VYOM1 ♂ Mahalanobis P value	VYIPM1 ♂ Mahalanobis P value	C ♀ Mahalanobis P value	OLIPM1 ♀ Mahalanobis P value	VYOM1 ♀ Mahalanobis P value
Pterostichus melas	OLIPM1 ♂	1.7771 0.0677						
	VYOM1 ♂	2.3878 0.0057	2.0741 <.0001					
	VYIPM1 ♂	2.2988 0.0007	2.3702 <.0001	1.6511 <.0001				
	C ♀	3.8665 <.0001	4.7129 <.0001	4.1761 <.0001	4.1955 <.0001			
	OLIPM1 ♀	2.781 <.0001	2.9191 <.0001	2.922 <.0001	2.5668 <.0001	3.0542 <.0001		
	VYOM1 ♀	3.2551 <.0001	3.6275 <.0001	2.7623 <.0001	2.8527 <.0001	2.6604 <.0001	2.1433 <.0001	
	VYIPM1 ♀	3.4221 <.0001	3.9055 <.0001	3.4544 <.0001	2.7985 <.0001	2.5228 <.0001	1.5866 <.0001	1.9401 <.0001

Species	Site & sex Distance P value	OLOM1 ♀ Mahalanobis P value	OLIPM1 ♀ Mahalanobis P value	VYOM1 ♀ Mahalanobis P value	VYIPM1 ♀ Mahalanobis P value	EO ♂ Mahalanobis P value	OLIPM1 ♂ Mahalanobis P value	VYOM1 ♂ Mahalanobis P value
Poecilus koyi	OLIPM1 ♀	1.1751 0.206						
	VYOM1 ♀	1.4212 0.0041	1.8508 <.0001					
	VYIPM1 ♀	0.9435 0.6635	1.1502 0.3692	1.7277 0.0002				
	OLOM1 ♂	2.9347 <.0001	3.2679 <.0001	3.3421 <.0001	2.9529 <.0001			
	OLIPM1 ♂	3.3318 <.0001	3.3071 <.0001	3.8005 <.0001	3.2384 <.0001	1.6127 <.0001		

	VYOM1 ♂	3.0853 <.0001	3.3635 <.0001	3.1367 <.0001	3.0515 <.0001	0.9469 0.0123	1.8619 <.0001	
	VYIPM1 ♂	2.9559 <.0001	3.0142 <.0001	3.4758 <.0001	2.7716 <.0001	1.2945 0.0001	1.0131 0.0128	1.4896 <.0001

Table 10. Results after t-testing pairwise distances reported as Procrustres distance and p-values after 10000 permutations runs. For site abbreviations, see Table 2. Taken and adapted from Ivanković Tatalović et al. 2020.

Species	Site & sex Distance P value	C ♂ Procrustes P value	OLIPM1♂ Procrustes P value	VYOM1 ♂ Procrustes P value	VYIPM1 ♂ Procrustes P value	C ♀ Procrustes P value	OLIPM1♀ Procrustes P value	VYOM1 ♀ Procrustes P value
Pterostichus melas	OLIPM1♂	0.0088 0.4891						
	VYOM1 ♂	0.0152 0.0572	0.0189 <.0001					
	VYIPM1 ♂	0.0187 0.001	0.0247 <.0001	0.0152 <.0001				
	C ♀	0.0275 0.0002	0.0344 <.0001	0.0234 <.0001	0.0243 <.0001			
	OLIPM1♀	0.0161 0.0143	0.0219 <.0001	0.0164 <.0001	0.0147 <.0001	0.0176 0.0001		
	VYOM1 ♀	0.0237 0.0003	0.0294 <.0001	0.0181 <.0001	0.0227 <.0001	0.0113 0.0228	0.0177 <.0001	
	VYIPM1 ♀	0.025 0.0003	0.032 <.0001	0.0217 <.0001	0.016 <.0001	0.0123 0.0063	0.0116 0.0001	0.0161 <.0001

Species	Site & sex Distance P value	OLOM1 ♀ Procrustes P value	OLIPM1♀ Procrustes P value	VYOM1 ♀ Procrustes P value	VYIPM1 ♀ Procrustes P value	OLOM1 ♂ Procrustes P value	OLIPM1♂ Procrustes P value	VYOM1 ♂ Procrustes P value
Poecilus koyi	OLIPM1♀	0.0071 0.0672						
	VYOM1 ♀	0.006 0.1352	0.01 0.0036					
	VYIPM1 ♀	0.0055 0.2602	0.0039 0.6577	0.0091 0.0169				
	OLOM1 ♂	0.0109 0.0001	0.0155 <.0001	0.0107 0.0002	0.014 <.0001			
	OLIPM1♂	0.0133 <.0001	0.0163 <.0001	0.0136 <.0001	0.0162 <.0001	0.0079 0.0005		

VYOM1 ♂	0.0125 <.0001	0.0171 <.0001	0.0108 <.0001	0.0156 <.0001	0.0034 0.3014	0.0096 <.0001	
VYIPM1 ♂	0.0094 0.0014	0.0134 0.0001	0.0108 0.0002	0.0123 0.0001	0.0045 0.1098	0.0061 0.0057	0.007 0.0021

Fluctuating asymmetry levels (mean squares value of ind*side interaction, corrected for error variance) differed greatly in between studied species. In *P. melas*, populations from all three agricultural sites had higher FA levels than the control population, with FA level of populations from ecological vineyard (VYOM1) being the highest. Population from olive groves had lowest FA levels than those from vineyards, but higher than individuals from the unmanaged sites. There was no significant difference in fluctuating asymmetry between males and females within the same site. In *P. koyi*, on the other hand, males were more asymmetrical at every site, but there were no significant differences between four sites, although sites with ecologically based pest management had slightly higher levels of FA in both vineyards and olive orchards (Figure 21). Regression of the Mahalanobis and Procrustes FA with individual shape was performed in order to see the magnitude in FA values, showing olive orchards as indeed less influential in FA than vineyards (Figure 22).

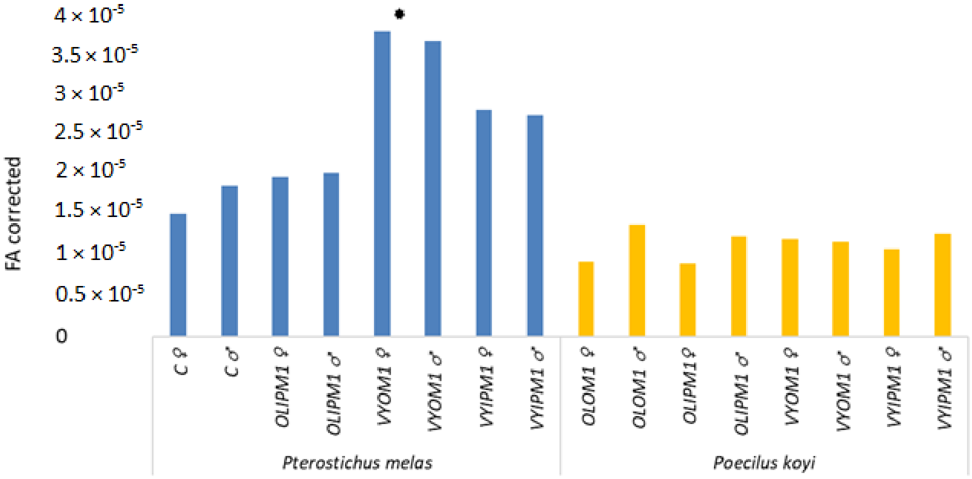

Figure 21. Histogram of the intensity of fluctuating asymmetry (FA) in different populations and sexes of *P. melas* and *P. koyi*. Blue bars show values obtained from populations of *P. melas*, and yellow bars depict populations of *P. koyi*. Mean squares of ind*side interaction corrected for error variance were used as a corrected measure of FA. (Star denotes *P. melas* populations significantly different in Procrustes FA scores

from others, ANOVA, $p<0.05$). For site abbreviations, see Table 2. Taken and adapted from Ivanković Tatalović et al. 2020.

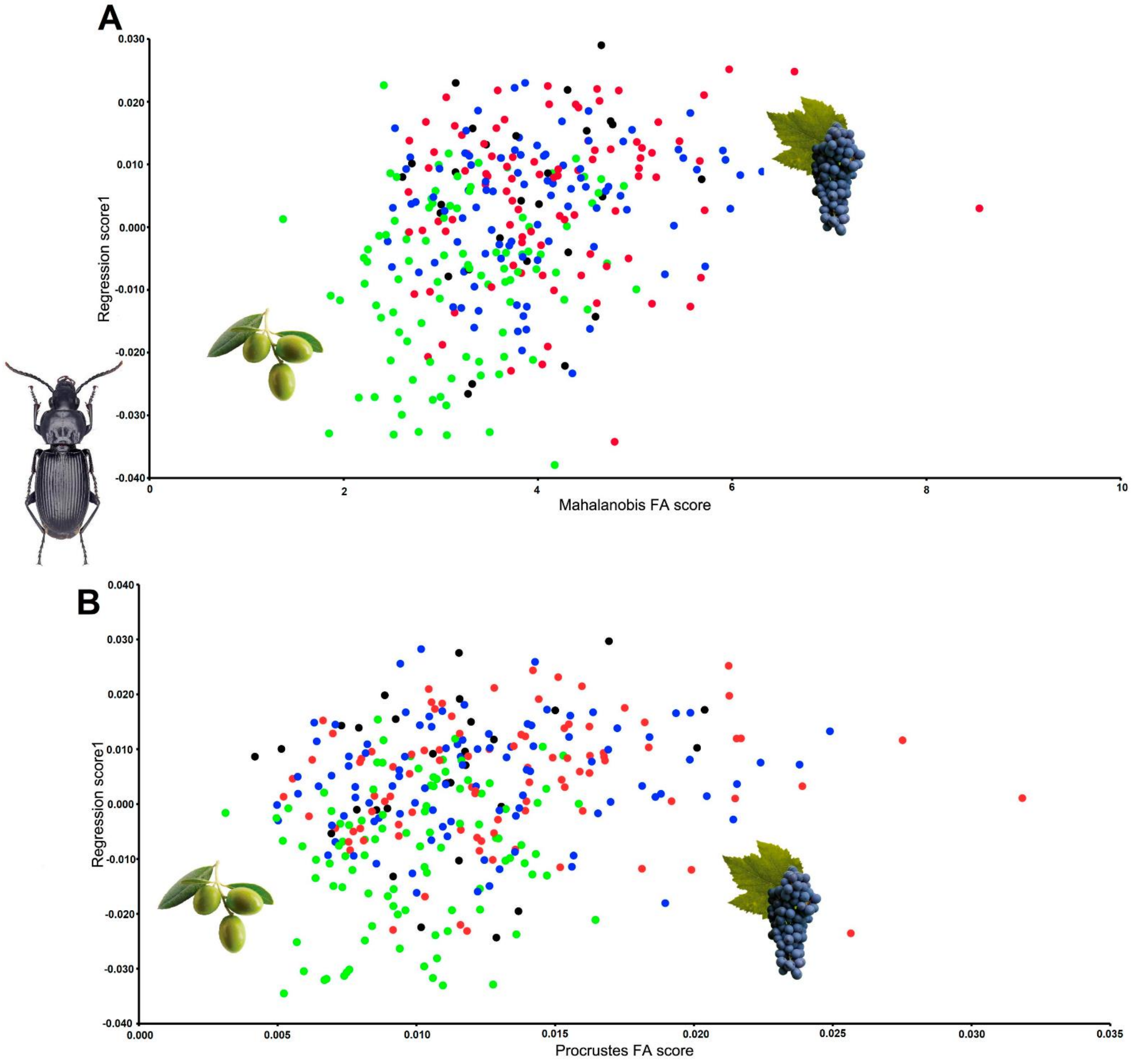

Figure 22. Regression of the Mahalanobis (A) and Procrustes (B) FAs for the shapes of *P. melas* individual species from four sites (OLIPM1 = green, C = black, VYIPM1 = blue, and VYOM1 = red). For site abbreviations, see Table 2. Taken and adapted from Ivanković Tatalović et al. 2020.

5.3. *Behavioural and metabolic reactions of common predatory carabid beetle to thiamethoxam intoxication*

5.3.1. *Feeding trial*

The Kruskal-Wallis tests (H (4, 71) = 42.1531, *p*<0.0001) showed significant differences for the mass of the consumed food per body mass of *A. parallelus* based on the thiamethoxam concentration used in the treatment. The food consumption decreased with the increasing concentrations of thiamethoxam in the treatment (Figure 23). Multiple Comparisons *p* values (2-tailed) revealed that group C3 statistically differed from groups C1 (*p* = 0.0154) and C2 (*p* = 0.0491), and group C4 was statistically different from groups C0 (*p* = 0.000016), C1 (*p* < 0.001), and C2 (*p* < 0.001). The mean food consumption per body mass was higher in females, but not significantly different compared to male beetles (H (1, 67) = 0.1615, *p* = 0.6878). The mean food consumption per body mass within each treatment was higher in females, but not significantly different compared to male beetles (H (1, 67) = 0.1615, *p* = 0.6878).

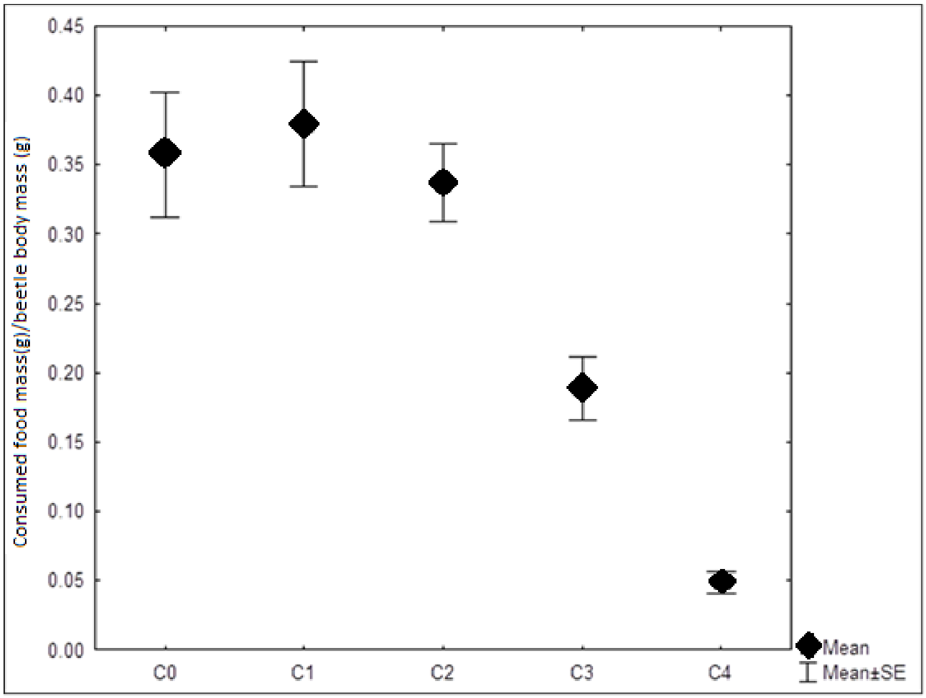

Figure 23. Mean of consumed food (g) per beetle body mass (g) for each treatment with thiamethoxam. C0 – group treated with 0 mg Ai/L, C1 - group treated with 3.9 mg Ai/L, C2 - group treated with 9.1 mg Ai/L,

C3 - group treated with 20 mg Ai/L, C4 - group treated with 40 mg Ai/L. Vertical bars denote standard errors. Submited to *Ecotoxicology* journal.

5.3.2. Locomotor activity and mortality following thiamethoxam exposure

The signs of intoxication were visible only in groups exposed to higher concentrations of thiamethoxam (C3 and C4), with shortest time period for signs to appear was 12 hours (Figure 23). All beetles in C3 survived 48-hour long period after treatment, while one individual was found dead in C4 (Figure 24). Overall, 20% of C3 beetles showed signs of intoxication at one point during the observation period, while for C4 beetles that share was 46.7%. In the period of 48h after the treatment there was one dead individual, belonging to the C4 group.

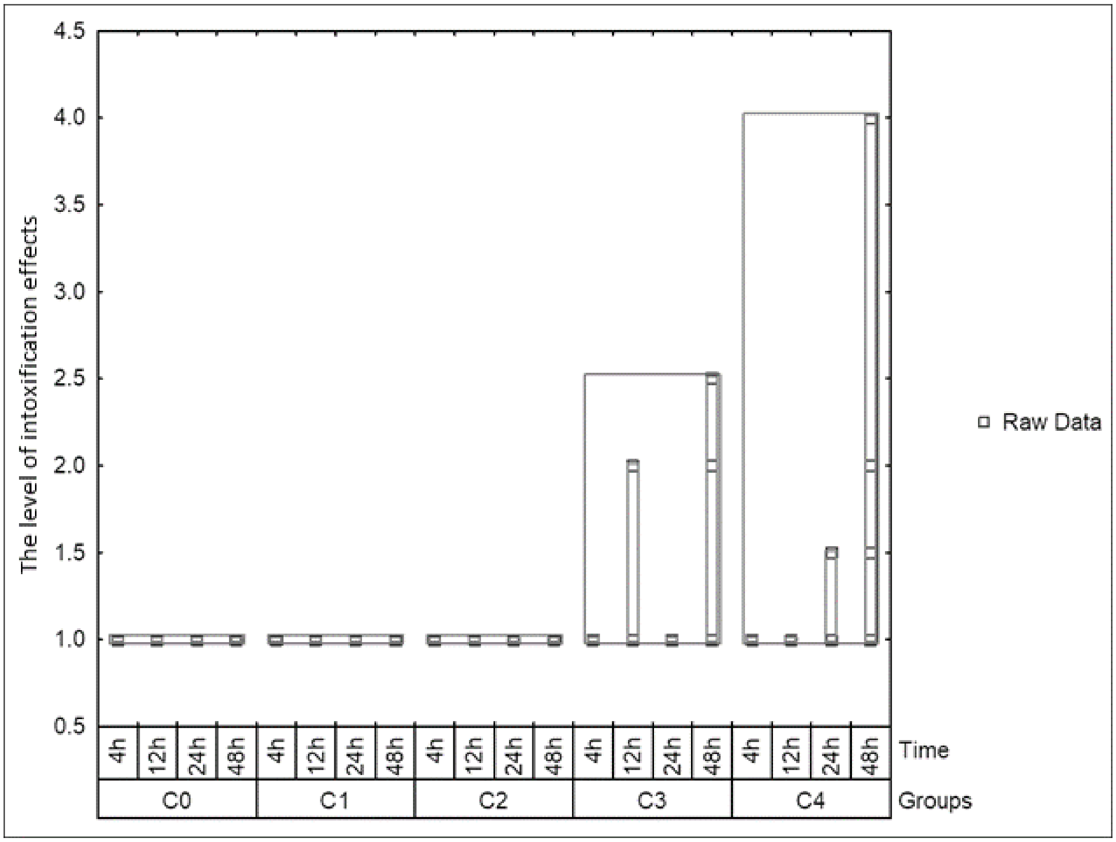

Figure 24. The number of beetles in each group expressing the signs of intoxication in their locomotor activity at some point during the 48h period after the end of the treatment. Submited to *Ecotoxicology* journal.

5.3.3. Untargeted metabolomics profiling

Kruskal-Wallis test revealed that there were statistically significant differences in concentrations of metabolites between treated groups (C3 and C4) and the control group (Table 11).

Table 11. Metabolites of *A. paralellus* after exposure to higher conentartions of thiamethoxam by dipping method (Kruskal-Wallis with multiple comparison *p* values (two-tailed)). Column *Separated treatments* depicts results when C3 and C4 were evaluated separately and each group was compared to C0, while column *Merged treatments* depicts results with C3 and C4 evaluated as one group (treated) and compared to C0 (non-treated). Statistically significant values are bolded. Submited to *Ecotoxicology* journal.

	Separated treatments				Merged treatments		
Metabolite	H	df	*p* (C3)	*p* (C4)	H	df	*p* (C3&C4)
Aminoisobutryc acid	7.0403	2	**0.0393**	1	1.8933	1	0.1688
Cholesterole	7.2221	2	**0.0222**	0.5121	5.0402	1	**0.0247**
d-Glucose	11.508	2	**0.0021**	0.5918	6.4248	1	**0.0113**
Glutamine	15.397	2	0.0661	**0.0003**	14.182	1	**0.0001**
2-hydroxyglutaric acid	11.227	2	**0.0195**	1	0.8863	1	0.3465
L-isoleucine	7.4396	2	**0.0371**	1	1.6993	1	0.1923
L-ornithine	9.6318	2	**0.0076**	0.1451	7.9773	1	**0.0047**
L-proline	8.9987	2	**0.0378**	1	0.8863	1	0.3465
Pyroglutamic acid	8.8623	2	**0.0183**	1	2.0979	1	0.1475
Succinate	13.253	2	**0.0008**	0.3773	7.9773	1	**0.0047**
Uric acid	5.9851	2	**0.0434**	0.9414	3.5455	1	0.0597

An increase of total ion current (TIC) was detected in L-isoleucine, succinate, pyroglutamic acid, d-Glucose, uric acid, and cholesterol, while glutamine and aminoisobutryc acid showed a decrease in TIC in both groups. Succinate showed the largest difference, whose concentration was 439% higher in the C3 group and 47% higher in the C4 group (Figure 25) than in the control group. Opposite changes between the groups were noted for several metabolites. L-proline and L-ornithine decreased in C3 but increased in C4 (overall decrease in treated groups), and the opposite occurred in 2-hydroxyglutaric acid concentration (Figure 25). Avarage TIC value for each metabolite and group is depicted in Table 12.

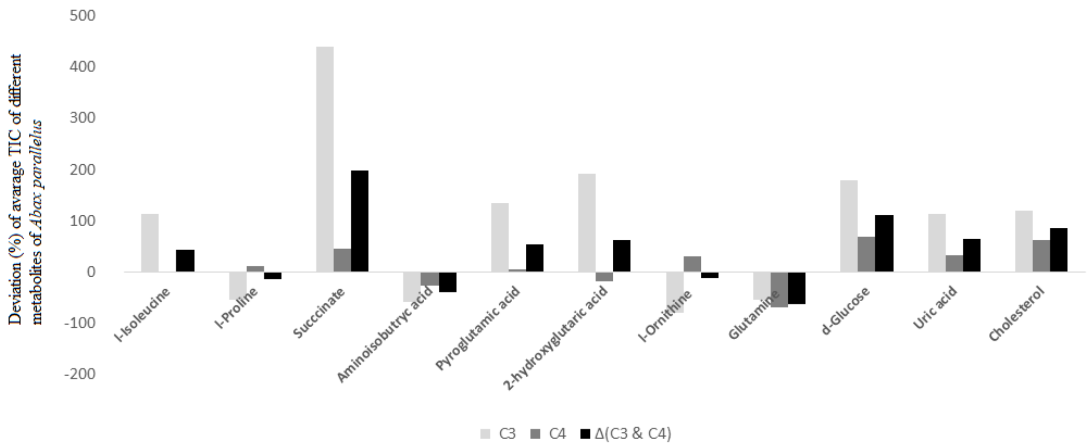

Figure 25. Deviation (%) of average TIC of different metabolites of *Abax parallelus* in C3 and C4 (individual group and combined) from C0. Only metabolites with significant deviations are depicted (after Kruskal-Wallis with multiple comparison *p* values (two-tailed) in Table 11). Submited to *Ecotoxicology* journal.

Table 12. The average values of TIC of each scanned metabolite of *Abax parallelus* individuals post thiamethoxam treatment (C3 – group treated with 20 mg/L, C4 – group treated with 40mg/L), and their deviation from the average TIC of the control group (C0).

Metabolites	**TIC**				**Deviation from C0**		
	ΔC0	ΔC3	ΔC4	ΔC3&C4	C3	C4	C3&C4
Lactic acid	0.59	1.15	0.65	0.84	96%	11%	44%
L-alanine	9.55	10.17	9.79	9.94	7%	2%	4%
L-valine	2.40	2.77	2.32	2.49	15%	-3%	4%
Glycerol	7.23	6.19	8.04	7.33	-14%	11%	1%
Phosphoric acid	13.46	12.48	15.67	14.45	-7%	16%	7%
L-isoleucine	2.30	4.88	2.32	3.30	113%	1%	44%
L-proline	24.62	11.58	27.72	21.51	-53%	13%	-13%
L-glycine	4.35	5.34	4.16	4.61	23%	-4%	6%
Succcinate	0.74	4.01	1.09	2.21	439%	47%	198%
L-serine	0.86	1.06	1.02	1.04	24%	19%	21%
L-threonine	0.96	1.67	0.88	1.18	74%	-8%	24%
L-methionine	0.53	0.56	0.48	0.51	5%	-10%	-4%
Aminoisobutyric acid	0.59	0.24	0.44	0.37	-59%	-25%	-38%
Pyroglutamic acid	1.38	3.27	1.45	2.15	136%	5%	55%
2-hydroxyglutaric acid	0.20	0.59	0.17	0.33	192%	-17%	63%
L-ornithine	0.24	0.05	0.31	0.21	-79%	31%	-12%

Table 12. Continuation.

Glutamine	**6.61**	**3.06**	**2.08**	**2.45**	**-54%**	**-69%**	**-63%**
L-phenylalanine	0.58	1.70	0.54	0.99	191%	-8%	69%
L-asparagine	1.10	0.91	1.12	1.04	-18%	1%	-6%
L-glutamine	1.37	0.65	0.77	0.73	-52%	-44%	-47%
L-lysine	0.12	0.04	0.08	0.06	-70%	-32%	-47%
Citric acid	2.57	1.11	1.58	1.40	-57%	-38%	-45%
d-Glucose	2.03	4.98	3.05	3.79	180%	69%	112%
L-histidine	0.50	0.11	0.22	0.18	-79%	-55%	-64%
L-tyrosine	3.81	1.19	1.36	1.30	-69%	-64%	-66%
Palmitoleic acid	1.37	1.19	0.95	1.04	-13%	-30%	-24%
Palmitic acid	2.00	5.28	2.26	3.42	164%	13%	71%
Inositol	0.91	0.52	0.78	0.68	-43%	-14%	-25%
Uric acid	1.11	2.37	1.49	1.83	113%	34%	64%
Tryptophan	0.18	0.06	0.11	0.09	-65%	-39%	-49%
Oleic acid	2.14	3.10	2.15	2.52	45%	0%	17%
Stearic	0.77	1.39	0.67	0.95	81%	-12%	23%
Uridine	0.31	0.28	0.39	0.35	-11%	26%	12%
Cholesterol	0.50	1.11	0.82	0.93	121%	64%	86%

These results were confirmed with PCA analysis, where the first two axes explained 91.95% of variance. First axis (68.33%) separated C0 individuals from groups C3 and C4, while the second axis (23.61%) separated C3 and C4 (Figure 26).

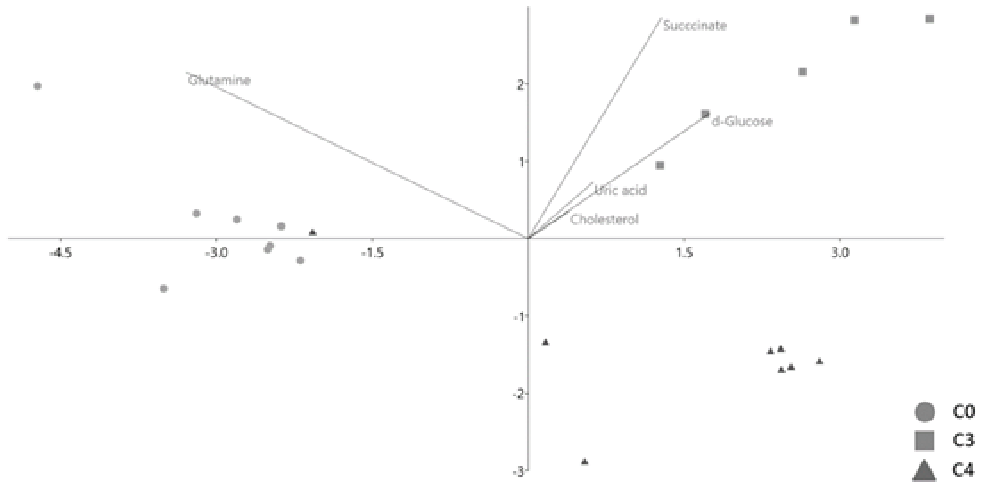

Figure 26. PCA score plots of thiamethoxam treated *A. parallelus* individuals (C3 - squires, C4 - triangles), and control group (C0 - dots). In the score plot, each data point represents one beetle sample. Submited to *Ecotoxicology* journal.

5.3.4. Superoxide Dismutase activity after thiamethoxam exposure

Kruskal-wallis test (H (3, N = 51) = 3.601957) revealed that there is no significant difference (p = 0.3078) in SOD activity between groups C0, C1, C2, and C3. SOD activity was higher in C0 groups (Figure 27).

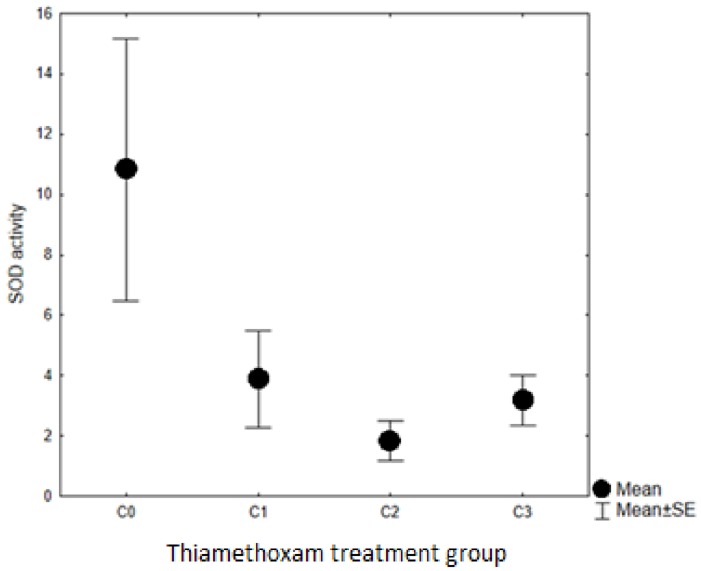

Figure 27. SOD activity in *Abax parallelus* tissue depending on thiamethoxam treatment, calculated as the inhibition activity of xanthine oxidase (XO) by SOD. Vertical bars denote standard errors. Submited to *Ecotoxicology* journal.

5.4. PCR gel diagnostics of predatory carabid beetle gut content on the selected prey

Eleven carabid beetles were positive for the earthworms (2.37%), and 4 for lepidopterans (1.56%). None of the scanned beetles was positive for the *B. oleae* (Table 13).

Table 13. The results of PCR gel diagnostics of carabid gut content for selected pray from five sites in Zadar County. For site abreviations, see Table 2.

Taxon	**Primer name**	**Number of Carabids scanned**	**Percentage (%) of carabids positive**	**Positive samples**	**Sites**
Earthworms	185F: 14233R	464	2.37	*Poecilus koyi* (x4) *Pterostichus melas* (x3) *Carabus coriaceus* (x1) *Calathus fuscipes* (x3)	OLIPM1(x8) OLOM1 (x1) VYOM1 (x2)
Moths	LM-14259-F LM-14423-R	256	1.56	*Poecilus koyi* (x3) *Carabus coriaceus* (x1)	OLIPM1 (x4)
B. oleae	p_Cox1D F p_Cox1D R	151	0	/	/

5.5. Transmisson of selected pesticides via trophic interactions and its impact oxidative stress in carabid beetles

Trend line in SOD activity depicts its slight rise in the beetles fed by the earthworms contaminated by thiamethoxam (Figure 28), and decline in the beetles fed by the earthworms contaminated by dimethoate (Figure 29). However, very high number of inhibition (>100%) imply a procedure error.

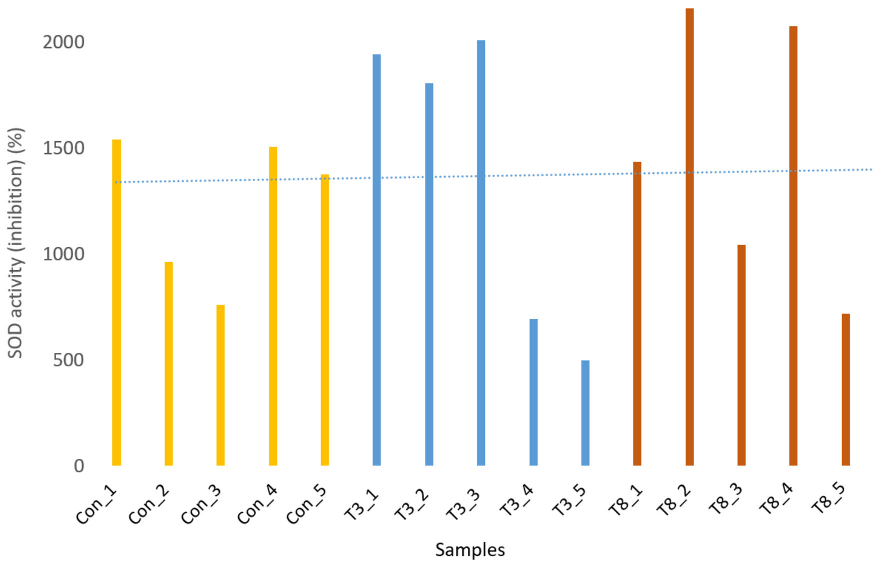

Figure 28. The SOD activity (% of inhibition) in the carabid beetle tissue. Each column is one beetle sample belonging to the control group (yellow bars, Con_1-5) or groups fed with earthworms from soil that had 3 mg/kg (blue bars, T3_1-5) or 8 mg/kg (red bars, T8_1-5) of thiamethoxam. Trend line is depicted by dotted blue line.

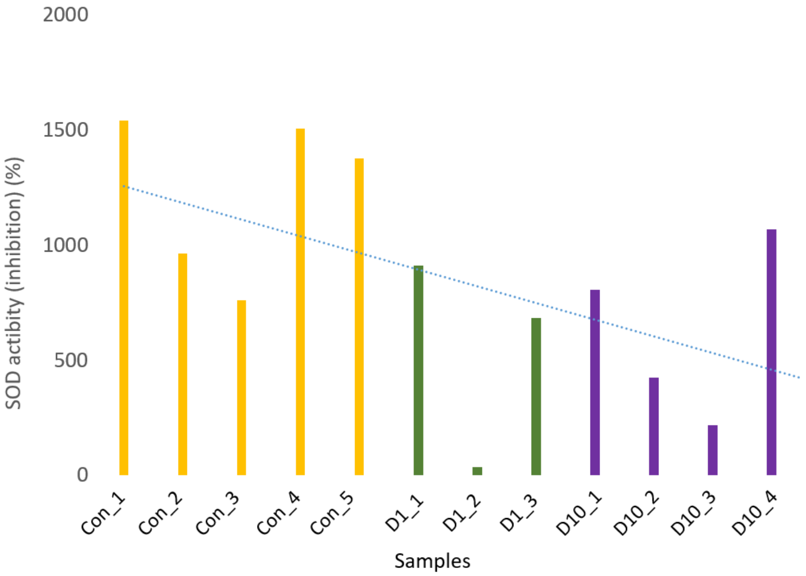

Figure 29. The SOD activity (% of inhibition) in the carabid beetle tissue. Each column is one beetle sample belonging to the control group (yellow bars, Con_1-5) or groups fed with earthworms from soil that had 1 mg/kg (green bars, D1_1-3) or 10 mg/kg (purple bars, D10_1-4) of dimethoate. Trend line is depicted by blue line.

Both thiamethoxam and its byproduct clothianidin were detected in all the earthworms kept in the soil contaminated with this insecticide. Concentration of thiamethoxam in the soil did not affect the concentration in the tissue (Figure 30). Every carabid beetle sample consisting of beetles that fed on these earthworms also had both clothianidin and thiamethoxam detected in the tissue. On the other hand, dimethoate was detected only in the earthworm tissue in the case of the higher concentration (Figure 30). No pesticide was detected in the store-bought soil sample which was used in the experiment.

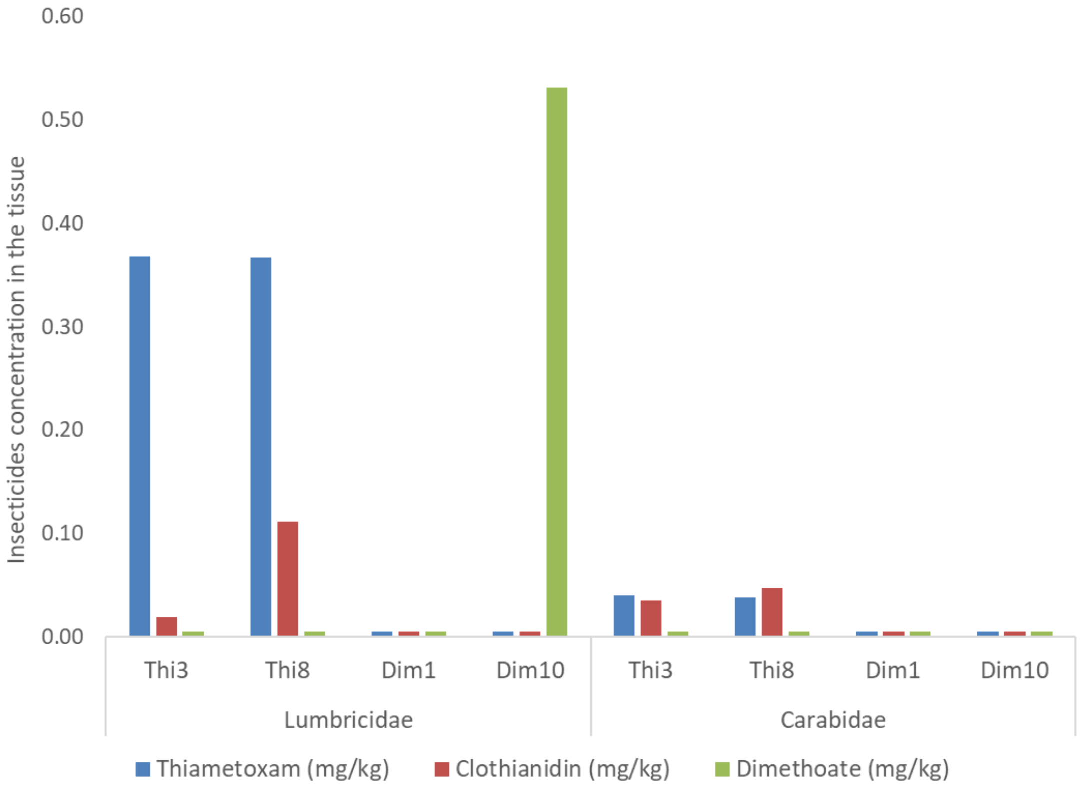

Figure 30. The concentrations of thiamethoxam, its chemical product clothianidin, and dimethoate in the tissue of carabid beetles and earthworms. Thi3 - earthworms from soil in which 3 mg/kg of thiamethoxam was added and carabids that fed on them; Thi8 - earthworms from soil in which 8 mg/kg of thiamethoxam was added and carabids that fed on them; Dim1 - earthworms from soil in which 1 mg/kg of dimethoate was added and carabids that fed on them; Dim10 - earthworms from soil in which 10 mg/kg of dimethoate was added and carabids that fed on them.

6. DISCUSSION

6.1. The impact of management practices in perennial Mediterranean agroecosystems on the functional diversity, development, and metabolism of carabid beetles

The aim of this dissertation was to investigate the impact of agricultural management practices, including the use of synthetic pesticides on the carabid beetles, from the community level, to the population and individuals. For the first time a meta-analysis on the functional diversity and life traits of carabid beetles within Mediterranean agroecosystems has been conducted, revealing the differences between the communities from unmanaged and agricultural land. Furthermore, the effect that land management has on the development of common predatory carabid species was researched using GMM, with the environmental stress that beetles suffer from in the olive orchards and vineyards being measured with FA. At the individual level, the sublethal behavioral and metabolic effects in carabid beetles following acute neonicotinoid exposure were noted. Lastly, carabids gut content was scanned for earthworms, lepidopterans and olive fruit fly, and the role of trophic interactions in pesticide spread was investigated, as well as the oxidative stress it causes to the predators.

The results confirmed the array of negative consequences that agricultural management has on carabid beetles. Although vineyards and olive orchards within Mediterranean basin harbored rich carabid assemblages, they had lower share of predatory species that are beneficial in pest control. It was confirmed that level in fluctuating asymmetry is higher in managed land compared to unmanaged habitats. Thiamethoxam causes decreased feeding, uncoordinated locomotion, and changed levels of key metabolites in treated beetles, but it did not cause increased levels of SOD. Carabid beetles from research sites in Zadar County were confirmed to feed on earthworms and lepidopterans, and laboratory feeding trials showed that insecticide residues can spread from earthworms to carabids via trophic interactions.

6.1.1. The comparison of ecological and morphological traits within carabid beetle communities from olive orchards, vineyards, and unmanaged habitats within the Mediterranean basin

The composition of carabid beetles and the distribution of their traits across agroecosystems are influenced by a complex web of edaphic, physical, ecological, and human-made factors, only some of which were looked at in this work (Eyre et al. 2013, Albertini et al. 2017). Carabid beetle assemblages from unmanaged sites were different from assemblages in the agroecosystems based on the distribution of the studied traits. The managed sites had a higher percentage of macropterous and small to medium-sized species compared to the unmanaged sites, which had a considerably higher number of carnivorous species. Studies conducted in agroecosystems other than the Mediterranean observed the same pattern. For instance, Cole et al. (2002) found that the type of agricultural techniques utilized on Scottish farmlands have the greatest impact on large predators belonging to the genus *Carabus*. Carnivores are more sensitive to landscape changes than omnivores and herbivores, hence it is projected that habitats with more anthropogenic disturbances will have a smaller proportion of purely predatory carabid beetles (Purtauf et al. 2005, Gobbi and Fontaneto 2008, Šerić Jelaska and Durbešić 2009). On the other hand, managed habitats had the largest relative abundance of herbivores. In comparison to the combined data from both management types, functional diversity (presence-absence based) for feeding preferences and size was much lower at unmanaged locations. However, the functional diversity between the EPM and IPM management types studied in this study did not show any significant differences. Elek et al. (2021) and Šerić Jelaska et al. (2011) likewise noticed the limited functional diversity in control plots and unmanaged woods for forest carabid beetle populations, where larger, flightless carnivorous species predominated. These results imply that the big carnivorous species have developed assemblages in stable environments. IPM and EPM programs promote non-invasive management techniques that maintain the effectiveness of beneficial arthropods in pest control (Albertini et al. 2017, Picchi et al. 2017).

When only Croatian sites were considered, herbivore activity peaked in the spring on Con1, OLIPM1, and VYIPM1, however in VYOM1 and OLOM1 they are similarly active in all seasons. The only research site where brachypterous and big species predominated, particularly in the fall, was the unmanaged site. Longer-lasting larval stages in large species and fall breeders are more susceptible to soil disturbances (Gobbi and Fontaneto 2008, Šerić Jelaska et al. 2011). The unmanaged site offers a more stable ecosystem than the other research sites since it is devoid of practices like tillage and pesticide use that may otherwise have a negative impact on carabid beetles (Holland and Luff 2000). The proportion of brachypterous species (Brandmayr 1991, Hof et al. 2012) and brachypterous individuals in dimorphic species (Lövei and Sunderland 1996) may increase when habitat persistence increases. According to Blake et al. (1994), disturbed environments sustain a fauna of smaller-sized carabid beetles on average, and carabid beetle body size falls as management intensity increases. These results corroborate what we saw at the Croatian research sites, where OLIPM1, a frequently mulched site, had the largest number of small individuals. The distribution of body size among the different management types revealed in the meta-analysis we conducted further support this finding.

To sum up, our prediction that EPM and unmanaged sites will maintain an assembly with more large, flightless carnivores than IPM sites was only partially proven. Compared to IPM sites, both unmanaged and EPM sites exhibited larger proportions of big and brachypterous species, while compared to agricultural sites, unmanaged sites had a significantly higher proportion of carnivorous species. These findings were further supported by functional diversity, which was significantly higher at agricultural sites but not between EPM and IPM, and RDA, which distinguished between managed and unmanaged sites. The carabid beetle assemblage in the study was maintained by more substantial, flightless carnivores in unmanaged, natural settings. Nevertheless, it seems that the proportion of carabid beetle features is significantly impacted by differences between organic and integrated pest management at study locations. Predatory carabid beetle populations of various sizes that were active all year around were prevalent in Croatian agricultural sites. They can control a variety of prey using biocontrol (Kromp 1999). In order to support agricultural practices that maintain ecosystem services and to ensure a better understanding of insect biodiversity in Mediterranean ecosystems, it is crucial to comprehend the distribution of carabid beetle functional traits between various agricultural practices and unmanaged sites.

6.1.2. The impact of agricultural management on carabid beetle populations as discerned using shape analysis

FA as a method of assessing environmental stress has few advantages compared to other methods: it is relatively non-expensive and simple to use, it can be performed on both alive and dead specimens, the norm (bilateral symmetry) is known prior to the start of the experiment, it is a permanent feature of the organism, and it is applicable on the large array of stress factors and organisms. However, method is not without the flaws. The source of stress may not always cause FA, even when the increase in the mortality is noted (Labrie et al. 2003). The complexity and interchangeability of stress factors, and organism's potential adaptation to them can make the method not applicable or results difficult to interpret. Furthermore, even when increased FA is present, the deviation from perfect symmetry could be very small, making it easy to enter the human error or get the false negative results in statistical analysis (Hendickx et al. 2003).

Increased FA scores in *P. melas* populations from vineyards compared to populations from olive groves may suggest that olive grove management strategies may be less harmful to carabid predators. Higher FA values were found in both species from sites with ecological pest control practices, defying our expectations that IPM would increase levels of asymmetry within populations because a wider spectrum of pesticides would be used (Hardersen 2000, Abaga et al. 2011, Coda et al. 2016). However, for *P. koyi*, these differences were not as noticeable as in *P. melas*, which appeared to be the less robust species. These results, however, are consistent with those of other studies (Labrie et al. 2003, Henríquez et al. 2015), which found that FA levels did not vary between populations of various carabid beetle species in continuous forest and fragmented habitats in a plantation region. The fact that this study site experiences the most frequent mechanical tillage of the soil may account for the higher FA score for *P. melas* from vineyard under EPM. As a component of the soil fauna, carabids are susceptible to mechanical disturbances of the soil, particularly when they are developing. According to Benítez et al. (2018), communities in annual arable lands had higher FA scores and were more unstable than those in perennial agroecosystems. The fact that this group has the largest density at the designated study location also lends weight to these findings. Higher population densities encourage intraspecific competition, which lowers individual food availability and raises FA (Hunt et al. 2000, Henríquez et al. 2015). Additionally, Nattero et al. (2019) shown that levels of asymmetry that were reduced by pesticide treatment were a result of the greater mortality rates of less acclimated animals, who were frequently those with higher degrees of asymmetry. This implyes that anthropogenic influences (such as the use of pesticides and tillage) operate as selecting pressures that favor more symmetrical individuals and may explain why FA values are higher at sites employing EPM than those utilizing IPM. Individuals in control populations from pristine ecosystems, on the other hand, develop without anthropogenic forces interfering with their developmental stability and are, as a result, the least asymmetric. A variable mortality hypothesis was put up as an explanation for this phenomenon by Floate and Fox (2000). More investigation is required to establish FA as a marker of the negative effects of pesticides on predatory organisms, keeping in mind that field settings are considerably more varied than laboratory conditions. When compared to the control, studies on two distinct populations of *P. melas* in Croatian agricultural sites did not find any appreciable differences in FA values (Benítez et al. 2018), while studies on populations of the wolf spider *Pirata piraticus* did not find any associations between FA and heavy metal pollution (Nunes et al. 2015). In this study, the method was not completely reliable as it gave inconsistent results depending on the species used and were somewhat inconclusive about the connection of FA level and environmental stress, as was the case in previously mentioned researches (Nunes et al. 2015, Benitez et al. 2018). Furthermore, because *P. koyi* was one of the dominant species at our research sites, it may be less vulnerable to environmental stress (Weller et al. 2014), which could possibly account for the lack of variation in FA values between populations. However, due to the dubious reliability of FA as a method of stress-evaluation, both in this research and previous studies, this is only speculative. *P. melas* was able to adapt to the conditions in Mediterranean regions maintained for agricultural purposes, such as olive orchards, despite being a predator from natural forest habitats (Pizzolotto et al. 2018). This modification is thought to have benefited from the movement of species from nearby wild settings to agricultural fields. Additionally, *P. melas* may have been more vulnerable to asymmetry changes because of farming activities in regions where this transition was more challenging. Furthermore, *P. koyi* may be better adapted to various types of agricultural fields, where it frequently ranks among the most numerous species, explaining the difference in asymmetry levels between the two species.

Previous studies (Hardersen 2000, Ribeiro et al. 2007, Elek et al. 2014) found that there were differences in FA between the sexes, with males of the examined species typically being less asymmetric than females. In our investigation, males of *P. koyi* were more asymmetrical at all sites save vineyard under EPM, whereas no sex-related variations were found in *P. melas* except for the control group, where the males were slightly more asymmetrical. The lowest FA score was observed for the *P. melas* control group, and since the FA in *P. koyi* was significantly lower than that in *P. melas*, differences in FA between sexes may be disregarded in this instance. This slight difference in FA between the sexes within the control group may result from the collection of *P. melas* from two distinct pristine sites.

To conclude, the control population of *P. melas* had the lowest FA intensity, as was to be predicted, which supports the use of FA as a technique to evaluate specific environmental stresses brought on by agricultural methods. The findings do show that various species do not react to FA in the same way, though. The populations of *P. melas* from olive groves demonstrated a lower FA than populations from vineyards, and the FA values were closer to the control group from the pristine environment. In contrast, the populations of *P. koyi* showed no differences in FA between populations from different agricultural locations. Therefore, we propose *P. melas* as a test animal for next research employing FA on environmental stress. Furthermore, FA noted that, independent of pest management practices, olive groves may be a less harmful environment for predatory carabids than vineyards (IPM or EPM). Future research can use the FA of *P. melas* as a technique to identify more natural agriculture approaches.

However, in the case of *P. melas*, both males and females had the smallest average body size at the control sites, and largest in two vineyards. Venn (2007) noted that undisturbed habitats harbor smaller individuals of the same species compared to the populations from disturbed and intermediate habitas. This observation further confirms that vineyards are more adverse to carabids than olive orchards, but that unmanaged habitats are preferable to agroecosystems.

6.1.3. The impact of acute thiamethoxam exposure on carabid beetle behavior and physiology

Shortly after thiamethoxam administration, the CFCR diminished significantly in the C3 and C4 treatment groups, but not in the lower concentrations of C1 or C2. All of the beetles did, however, feed, showing that thiamethoxam did not impair their ability to recognize and eat the food. Additionally, within the first 14 hours following the treatment, feeding was decreased. This behavioral change following thiamethoxam intoxication was noted by Tooming et al. (2017) in predatory carabid *Platynus assimilis*, and by Yao et al. (2015) in the biological agent *Serangium japonicum*. According to Knopp and Uhnavá (2014), proper feeding is a crucial component of survival, growth, and fecundity. Therefore, toxic stress that results in decreased feeding may decrease the quantity of beneficial predatory insects in arable land, such as carabid species from the tribe Pterostichinae, including the genera *Abax*, *Poecilus*, and *Pterostichus* (Kromp 1999).

Beetles were moving with evident signs of intoxication between 12 and 48 hours after treatment, but only one of them was dead at that time. According to Goulson (2013), the neurotoxin thiamethoxam binds to nicotinic acetylcholine receptors (nAChRs) in insects' central nervous systems, resulting in loss of balance and orientation, paralysis, and death (Jensen et al. 1997, Desneux et al. 2007, Moser and Obrycki 2009). In our investigation, beetles showed negative locomotor effects at dosages of 20 and 40 mg/L after 12 hours. According to Tooming et al. (2017), greater dosages of thiamethoxam given orally cause carabids to become hyperactive right away, and the day after the treatment, all beetles, regardless of dose, are in a state of hypoactivity. Other neurotoxic pesticides, such as pyrethroids and organophosphates, produced similar effects in insect predators (Prasifka et al. 2008). The neurotoxic sublethal consequences of thiamethoxam in our investigation included poor walking, difficulty to turn on the legs after being thrown over, and excessive grooming. Despite the fact that our experiment had a low mortality rate, it is important to remember that it was carried out in a controlled setting. Predatory carabid beetles are active hunters, thus the restricted movement seen in this study and comparable ones can easily hamper their ability to both catch prey and avoid predators. Lower carabid activity density in fields treated with neonicotinoid seeds may be due to hypoactivity brought on by neonicotinoid poisoning (Douglas et al. 2015).

Following the treatment, succinate and d-glucose levels experienced the largest shift (a 198% and 122% increase respectively). Reactive oxygen species are created and eliminated as a result of succinate. The overproduction or under-consumption of succinate is necessary for mitochondrial leakage. Accumulation of succinate is associated with SDH mutations, hypoxia, or energy imbalance (Tretter et al. 2016, Pekny et al. 2018). Given that neonicotinoids are known to cause it, this may represent an increase in oxidative stress following acute exposure to thiamethoxam (Yan et al. 2021). Furthermore, elevated glutamine metabolism also results in the accumulation of succinate, and in the carabid beetles treated with the higher concentrations of thiamethoxam, we found lower glutamine concentrations. When glutamine contributes its amide nitrogen to the cytosol for the creation of nucleotides and hexosamines, glutamate is created. Through the synthesis of glutathione (GSH), cytosolic glutamate is essential for preserving redox equilibrium and shielding cells from oxidative damage (Yu 2008, Zhang et al. 2017). An explanation for this metabolic demand for glutamate and the ensuing breakdown of glutamine in treated carabid beetles may be an oxidative stress response. Important elements of the nutritional and metabolic physiology of honey bees were affected by neonicotinoids clothianidin and imidacloprid, and high-dose imidacloprid exposure caused bees to have a slowed metabolic rate (Derecka et al. 2013, Cook 2019). Both the decline in food consumption and the higher glucose concentrations found in this study could be attributed to the lowered metabolic rate. Following organophosphate treatment, the enzyme succinate dehydrogenase, which catalyzes the oxidation of succinate into fumarate in the Krebs cycle, was significantly reduced in silkworms, suggesting a decrease in respiration rate at the tissue level due to toxicity brought on by these insecticides (Nath 2002). Uric acid is another metabolite whose levels rose in the treated beetles. In the prior research, protein loss in tissues after pesticide exposure was reported (Srinivas 1986, Jeschke et al. 2016). By maintaining the amount of free amino acids in hemolymph and addressing osmoregulatory issues caused by pesticide intoxication, this may offer intermediates for the Krebs cycle (Srinivas 1986). According to Jeschke et al. (2016), the amino acids produced by protein breakdown were predominantly deaminated, resulting in ammonia that was detoxified by being changed into uric acid. Furthermore, study on termites (Tasaki et al. 2017) showed that uric acid plays a beneficial function in resistance to oxidative stress. Termite survival under highly oxidative conditions was significantly improved by uric acid accumulation as well as externally supplied uric acid. Lastly, Etebari et al. (2007) reported a correlation between elevated glucose levels in the haemolymph and lower protein levels in silkworm larvae following pesticide (pyriproxyfen) administration. Because it was noted that trehalase activity was increased in the midgut of silkworms treated with pesticides, it was hypothesized that this might be because trehalase activity is boosted in silkworm haemolymph. The predominant sterol present in the majority of insects is cholesterol, whose amounts in tissue also increased after treatment (Behmer and Nes 2003). According to Etebari et al. (2007), changes in cholesterol were comparable to changes in uric acid and glucose, but they demonstrated a considerable rise in treatment effects after 120 hours.

Following administration of thiamethoxam, the there was no statistically significant variations in SOD activity in beetle tissue, however, the trend indicated slightly more activity in the control group compared to the three treated groups. The neurotoxic pesticides pirimiphos-methyl and deltamethrin, which have an inhibitory impact on SOD molecules, have been shown by Plavšin et al. (2015) to dramatically reduce the total antioxidative capacity in the beetle *Tribolium castaneum* (Herbst 1797) (Coleoptera, Tenebrionidae). This resulted from the pesticide's interference with the hormone adipokinetic hormone (AKH), which is involved in the defense mechanisms used by insects to combat oxidative stress. Chlorpyrifos, trifluralin, and chlorothalonil were tested to see how they affected the activities of SOD and glutathione S-transferase (GST) in *Daphnia magna* Straus, 1820 (Diplostraca, Daphniidae), and Song et al. (2017) discovered that both activities were induced at low concentrations but inhibited at high concentrations. It is possible that while thiamethoxam did cause oxidative stress in the beetles, it also supressed that their antioxidative reaction (SOD activity), which would account for the decreased SOD activity in the treated beetles. The substantial intragroup variability might also suggest that factors other than acute thiamethoxam exposure, such as age, health, and/or environmental circumstances, had a greater influence on SOD activity before the capture (Simone-Finstrom et al. 2016).

To sum up, changes in CFCR and locomotor skills may be related to a decline in the predatory and survival ability of the carabid *A. parallelus*. Thiamethoxam intoxication seems to promote insect protein catabolism while decreasing insect carbohydrate catabolism. The potential inhibitory effect of thiamethoxam or the sensitivity of beetles to other inner and/or external stress factors could be the cause of the mild SOD activity in the treated groups. Thiamethoxam does produce oxidative damage in the beetle, according to changes in glutamine, succinate, and uric acid, but another biomarker besides SOD may be more telling. Every impact that has been seen is concentration-dependent.

6.1.4. PCR gel diagnostics of predatory carabid beetle gut content on the selected prey

In field, carabid beetles act as agents in biocontrol against various plant parasites, and during periods when pest species are not available, other fauna sustains the beetle community. Earthworms and lepidopterans are frequent food choice for carabid beetles (King et al 2010, Šerić Jelaska et al. 2014, 2016), which was confirmed for Mediterranean agroecosystems using DNA metabarcoding (MEDITERATRI project, unpublish results). In this research, beetles were positive for earthworms and lepidopterans, however, the share of positive beetles was lower compared to forest ecosystems (Šerić Jelaska et al. 2014, 2016). Lepidopterans in olive orchards and vineyards include some plant parasite, such as olive moth (*Prays oleae* Bernard, 1788), which was confirmed at research sites (Anđelić Dmitrović et al. 2022, PESCAR project unpublished data), leopard moth (*Zeuzera pyrina*, (Linnaeus, 1761)), or Europaean grapevine moth

(*Lobesia botrana*, Denis and Schiffermüller, 1775), and carabid beetles are considered to be one of the agents of biocontrol against these pest species. Earthworms are not pests, but they sustain the carabid beetle communities in the periods when pest prey is low in abundance or absent (Symondson et al. 2000).

Carabid beetles were observed to feed on *B. oleae*, both in the field (Orsini et al. 2007, Gonçalves and Pereira 2012, Pizzolotto et al. 2018) and in the laboratory (Dinis et al. 2016, Albertini et al. 2018). However, in this study no individual was positive for *B. oleae*, which can be explained by pests' low abundance on the research sites that year (PESCAR project, unpublished data).

6.1.5. Bioaccumulation of insecticides via trophic interactions and its impact on carabid beetles

After seven days in soil spiked with thiamethoxam, all earthworm samples (consisting of one, or two pooled animals), had traces of thiamethoxam detected in them. None of the earthworms was found dead. The highest detected concentration was 0.368 mg/kg of tissue. Interestingly, this concentration was found in samples from both groups (3 mg/kg and 8 mg/kg), implying that there might be a threshold of how much insecticide it can accumulate in the body. However, the earthworms that were kept in soil with higher concentration of thiamethoxam had more clothianidin detected in the tissue, which is a degradation product of thiamethoxam, making the total amount of insecticides in the tissue higher. Earthworms' and other invertebrates' propensity to accumulate pesticides in their tissue has been recorded before (Mosleh et al. 2002) and on our research sites neonicotinoids and other pesticides were detected in both predator and prey (Šerić Jelaska et al. unpublished data). Given that carabids can come in contact with neonicotinoid residues in many ways, not just via trophic interactions, that pathway was tested in this experiment in the controlled environment. All carabid samples also had both clothianidin and thiamethoxam detected in them. Ritchie et al. (2019) also tested the effects of thiamethoxam contaminated soil on earthworms, and noted that both thiamethoxam and clothianidin have negative effect on the reproduction, but not on the survival, of earthworm *Eisenia andrei*. Dimethoate was detected in only one sample, earthworms that were kept in the soil with concentration of 10 mg/kg. It is not clear why it was not detected in beetles that fed on these earthworms. In field study (Šerić Jelaska et al. unpublished data) dimethoate was detected in just one pooled sample of carabid beetles from the olive orchard under IPM.

The SOD activity measurements yielded abnormally high inhibition rates, implying some failure in the process. Due to the limited number of live beetle specimens the measurements were not repeated. In spite of these difficulties, the results are presented here as they may point to some trends and propose what can be evaluated in the further studies. In the case of thiamethoxam, trend line shows negligible differences in

SOD activity among the groups, while in the case of dimethoate, the highest SOD activity was in control group. These results correspond to the observations section 6.1.3., where SOD assay kit did not detect any significant differences in the enzymes' activity among the groups based on the treatment, but the trend showed that the SOD inhibition rate decreases as the concentration of thiamethoxam in the treatment increases. The questionable results of this experiment make it difficult to draw any conclusion, except that Assay kits, while certainly useful in many experiments, should be used with care and may not be best choice for carabid beetles. Regardless of these results, it was proven that by eating contaminated prey, carabid beetles intake neonicotinoid insecticide in their tissue, and many previous researches, leave no doubt that this has hazardous effect on them. This should be taken in account when planning agricultural management strategies.

6.2. The importance of entomological research within arable land

In the last 10,000 y the human population has grown for 7.7 billion people (Wagner et al. 2021) and as a result, most of the arable land today has been transformed into agricultural sites (Raven and Wagner 2021). That, coupled with deforestation, pollution, rising CO_2 levels, draught, fires and other unfavorable anthropogenic effects have led to what some scientist believe is the sixth global mass extinction (Ceballos et al 2017, Curtis et al. 2018, Stokstad 2018). How this reflects in insect populations is still poorly understood. While there are several published works describing negative trends in insect biomass, abundance, and species richness (e.g. Hallman et al. 2018, Forister et al. 2019, Seibold et al. 2019), other studies seem to contradict these alarming reports (e.g. Willig et al. 2019, Schowalter et al. 2021). In 2020, three meta-analyses on this topic were conducted, two of which reported decline of terrestrial insects abundance (van Klink et al. 2020, Pilotto et al. 2020), and one where no such trend was noted (Crossley et al. 2020). Whichever findings turn out to be closer to the true abundance shifts in insect populations, there is no doubt that insects as the group provide numerous and invaluable ecological services, both on the arable lands and outside of it. This research focused on only the small portion of it, connected to one beetle family and its role in pest control within specific Mediterranean agroecosystems. Human activity in the form of agricultural management was shown to impact carabid beetles, from the community to the cellular level. Over the years, there have been myriad of studies on human-insect interactions - services they provide and our effect on them. These efforts were not in vain. As discussed earlier in this dissertation, EU banned three neonicotinoids harmful to the pollinators, and pesticide use has been in constant decline in EU since 2015 (European Commission, 15 June 2022). Individual countries, such as Germany and Sweden, have their own national projects with the aim to protect insect populations (Vogel 2019, Government Office of Sweden 2020). The Mediteratri project (UIP-05-2017), within which this dissertation was made, is funded by Croatian Science Foundation that is a part of a Science Europe Member Organization. Conventional agricultural management is being abandoned for the more nature-friendly EPM and IPM practices (Vasileiadis et al. 2015). In the United States millions of dollars are annually allocated by the Agriculture Improvement Act of 2018 for pollinator research and conservation around the country, and in January 2020, the Environmental Protection Agency released an interim decision on the use of neonicotinoids in the United States aimed at protecting pollinators (Environmental Protection Agency 2020). Insects are becoming more and more the focus of community (citizen) science and education projects that aim to survey, conserve, and promote understanding of their significance as pollinators, prey, nutrient recyclers, and focal organisms in science and technology as well as in literature, art, and other facets of culture (Wagner et al. 2021). However, just as these positive legalizations have been proposed and put into effect, they can be retracted. For example, in 2020 EU granted emergency authorizations for products containing thiamethoxam in 15 European countries including Croatia, making further studies of its effect on natural predators necessary. Furthermore, climate change in combination with already existing anthropogenic stressors poses novel challenges (Halsch et al. 2021). As a result, extended monitoring and research of insect communities and their response to human-made stressors continues to be necessary. MEDITERATRI project continued biodiversity assesment in understudied Mediterranean area of Croatia, after faunistic research from 20th Century by Novak, Karaman, and Müller, trying to emphasise the importance of Mediterranean biodiversity and ecosystem sevices provided by non target fauna within agriculutural settings. It represents the first such broad-scoped research in Croatian Mediterranean agroecosystems. Within the project, including also the results presented in this doctoral thesis, modern techniques, such as metagenomics and gass chromatography with tandem mass spectrometry (GC-MS) have been utilized in order to discern the spread of pesticides via trophic interactions in the agroecosystems under different management types. My doctoral research measured the impact of these practices on important part of ground fauna, carabid beetles.

6.3. Guidelines for further studies

Due to the complexity of anthropogenic impact on carabid communities, morphology, physiology, and behavior, integrative approach which uses different methods should be implemented. In both studies on fluctuating asymmetry and functional diversity, samples from control differed from the samples from agricultural sites regarding the variables researched in those studies, but there was less difference between agricultural sites under different management. Research on fluctuating asymmetry level among the populations suggests that vineyards might be more stressful for carabids compared to the olive orchards, even when vineyards is under EPM, but why is that so is less clear. Furthermore, we demonstrated that not all carabid species are equally suitable for GMM, showing the need to research further into which carabid species are good model organisms for GMM and FA as indicators of environmental stress.

Meta-analysis of carabid communities within Mediterranean agroecosystem revealed differences in share of carnivorous species depending on the management. However, the analysis was done using only presence-absence data, due to the mismatch of data among the used studies. Since the abundance of species in the field is an important factor in the species' effectiveness in biocontrol, it would be beneficial to conduct the similar analysis using abundance data if possible, and see if it reveals differences in community composition between EPM and IPM systems, which were not so evident in this study.

Changes in metabolites implicated oxidative stress in carabid beetle cells following thiamethoxam application. However, SOD turned out to be poor choice in detecting it by enzymatic tests. Further studies using some other antioxidative enzyme are recommended. Alternatively, methods such as measuring respiratory metabolism (Žagar et al. 2018). Secondly, beetle locomotion was recorded by observing them shortly four times over the period of two days. This leaves a chance for some locomotion or other behavioral issue to remain unnoticed. In further studies, I propose using technologically more advanced system with camera recorder and software, for example EthoVision XT Version 9 software (Noldus Information Technology, Wageningen, The Netherlands) (Tooming et al. 2017). Lastly, ecotoxicological tests revealed effects of acute exposure to thiamethoxam in laboratory by emerging them in dipping method. While it is relatively simple method useful in observation of damage neonicotinoids may cause, it does not reflect what beetles can experience in the field. Further studies should research how longer exposure to low concentration residues of pesticides affect the parameters described in section 5.3.

The experiments on insecticide spreading via trophic interaction offered several new study questions. For further research, I propose using different prey groups for carabid beetles, such as slugs or, as demonstrated by PCR gel diagnostic, lepidopterans, to discover which prey group is most responsible for prey to predator bioaccumulation of pesticides. Different pesticides should also be tested, as this experiment showed differences in accumulation of dimethoate and thiamethoxam, at least when earthworms and carabid beetles are concerned. Lastly, as these studies were conducted in laboratory, I propose that long-term impact of pesticides via trophic interactions in the field be investigated.

7. CONCLUSIONS

1. Fluctuating asymmetry analysed within carabids populations proved to have a potential in measuring developmental instability induced by agricultural practice. FA singled out olive groves as a potentially less adverse habitat to predatory carabids when compared to vineyards, regardless of the pest management (IPM or EPM) and unmanaged habitats are less adverse compared to agroecosystems. However, different species of carabid beetles do not have the same response in respect to fluctuating asymmetry and most appropriate species should be determined prior to FA analyses. Thus, FA is not very reliable method, and should be used in combination with methods that are more straightforward, with results interpreted carefully.
2. Carabid assemblages differ between managed and unmanaged sites regarding life traits distribution and this may impact the level of ecosystem service they provide in terms of pest control. Unmanaged sites supported a higher proportion of carnivorous carabid species compared to agricultural sites (vineyards and olive orchards) within Mediterranean region.
3. Acute thiamethoxam intoxication causes reduced feeding and locomotion difficulties in predatory carabid species which could lead to their lower survival rate in nature, and a loss may reduce the level of ecosystem service they provide in terms of pest control.
4. Changes in metabolism imply that thiamethoxam causes oxidative damage in the carabid beetles, but some other biomarker than SOD may be more indicative.
5. Lepidopterans and earthworms were found as carabid prey within Mediterranean agroecosystems confirming that as generalist predators, carabids predate on both herbivorous insects as potential pests and non pest invertebrates like earthworms that can sustain their populations when pest populations are low.
6. Laboratory feeding tests showed that carabid beetles can accumulate insecticide residues within their tissue and confirmed transmission by feeding on prey which dwelled in contaminated soil. Besides direct impact of insecticides on non-target fauna, they can further affedt predators via transmission through trophic interaction and spread futher through ecosystems

8. LITERATURE

Abaga NOZ, Alibert P, Dousset S, Savadogo PW, Savadogo M, Sedogo M (2011) Insecticide residues in cotton soils of Burkina Faso and effects of insecticides on fluctuating asymmetry in honey bees (*Apis mellifera* Linnaeus). Chemosphere, 83: 585-592

Adams D, Rohlf FJ, Slice D (2013) A field comes of age: geometric morphometrics in the 21st century.

Agriculture Improvement Act of 2018 (Farm Bill), https://www.govtrack.us/congress/bills/115/hr2. Accessed 6 November 2020 (see also https://www.ers.usda.gov/agriculture-improvement-act-of-2018-highlights-and-implications/).

Albertini A, Pizzolotto R, Petacchi R (2017) Carabid patterns in olive orchards and woody semi-natural habitats: first implications for conservation biological control against *Bactrocera oleae*. BioControl 62 (1): 71-83

Albertini A, Marchi S, Ratti C, Burgio G, Petacchi R, Magagnoli S (2018) *Bactrocera oleae* pupae predation by *Ocypus olens* detected by molecular gut content analysis. BioControl 63(2): 227-239.

Alderweireldt M, Desender K (1990) Variation of carabid diel activity patterns in pastures and cultivated fields. In: Stork NE, (ed.) The Role of Ground Beetles in Ecological and Environmental Studies. Intercept, Andover, 335–338.

Alibert P, Moureau B, Dommergues JL, David B (2001) Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. Zoologica Scripta 30: 299e311.

Anđelić Dmitrović B, Jelić M, Rota E, Šerić Jelaska L (2022) DNA Barcoding of Invertebrates Inhabiting Olive Orchards and Vineyards Accelerates Understudied Mediterranean Biodiversity Assessment. Diversity, 14(3): 182.

Aslanturk A, Kalender S, Uzunhisarcikli M, Kalender Y (2011) Effects of methidathion on antioxidant enzyme activities and malondialdehyde level in midgut tissues of *Lymantria dispar* (Lepidoptera) larvae. Journal of the Entomological Research Society, 13(3): 27-38.

Assing V (2017) On the Staphylinidae of the Greek island Kos, with an appendix on Carabidae and additional records from other islands (Insecta: Coleoptera). Linzer Biologische Beiträge 49(1): 191-205

Asteraki EJ (1993) The potential of carabid beetles to control slugs in grass/clover swards. Entomophaga 38: 193–198.

Athey KJ, Dreyer J, Kowles KA, Penn HJ, Sitvarin MI, Harwood JD (2016) Spring forward: molecular detection of early season predation in agroecosystems. Food Webs, *9*: 25-31.

Bagheri F, Talebi K, Hosseininaveh V (2010) Cellular energy allocation of pistachio green stink bug, *Brachynema germari* Kol.(Hemiptera: Pentatomidae) in relation to juvenoid pyriproxyfen. African Journal of Biotechnology, 9(35): 5746-5753

Bednarska AJ, Gerhardt A, Laskowski R (2010) Locomotor activity and respiration rate of the ground beetle, *Pterostichus oblongopunctatus* (Coleoptera: Carabidae), exposed to elevated nickel concentration at different temperatures: novel application of Multispecies Freshwater Biomonitor®. Ecotoxicology, 19(5): 864-871.

Behmer ST, Nes WD (2003) Insect sterol nutrition and physiology: a global overview. In: Simpson SJ (ed.), Advances In Insect Physiology, 1-72, Elsevier Ltd, Kidlington

Benítez HA (2013) Assessment of patterns of fluctuating asymmetry and sexual dimorphism in carabid body shape. Neotropical Entomology, 42(2): 164-9.

Benítez HA, Lemić D, Püschel TA, Gašparić HV, Kos T, Barić B, Bažok R, Živković IP (2018) Fluctuating asymmetry indicates levels of disturbance between agricultural productions: An example in Croatian population of *Pterostichus melas melas* (Coleptera: Carabidae). Zoologischer Anzeiger, 276:42-9.

Blake S, Foster GN, Eyre MD, Luff ML (1994) Effects of habitat type and grassland management practices on the body size distribution of carabid beetles. Pedobiologia 38(6): 502-512

Brandmayr P (1991) The reduction of metathoracic alae and dispersal power of carabid beetles along the evolutionary pathway into the mountains. In: Lanzavechia, Valvassory R (eds.) Form and function in zoology, Selected Symphosia and Monographs, Mucchi, Modena, 363-378.

Brandmayr P, Pizzolotto R, Brandmayr TZ (1994) Oral communications: Population biology and nesting of *Carterus calydonius* Rossi (Coleoptera, Carabidae, Ditomini).

Bralić A, Faričić J (2010) Višnja maraska: bogatstvo Zadra i zadarske regije. Sveučilište u Zadru, Zadar.

Bursztyka P, Saffray D, Lafont-Lecuelle C, Brin A, Pageat P (2013) Chemical compounds related to the predation risk posed by malacophagous ground beetles alter self-maintenance behavior of naive slugs (*Deroceras reticulatum*). PLoS One. 8(11): e79361.

Candan S, Özyurt Koçakoğlu N, Serttaş A (2021) Histoanatomy of Malpighian tubules and the digestive tract of adult of biocontrol agent *Calosoma sycophanta* L. (Coleoptera: Carabidae). International Journal of Tropical Insect Science, 41(2): 1373-1386.

Castro AV, Porrini DP, Cicchino AC (2014) Annual activity density of groundbeetles (Coleoptera: Carabidae) of a *Celtis ehrenbergiana* (Rosales: Celtidaceae) forest of Buenos Aires province, Argentina. Journal of Insect Science *14*(1): 252.

Ceballos G, Ehrlich PR, Dirzo R (2017) Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proceedings of the national academy of sciences, 114(30): E6089-E6096.

Chapman MAN (2014) The influence of landscape heterogeneity-ground beetles (Coleoptera: Carabidae) in Fthiotida, central Greece. Biodiversity Data Journal (2).

Cirio U (1997) Agrochemicals and environmental impact in olive farming. Olivae, 65: 32-39.

Coda J, Gomez D, Martínez JJ, Steinmann A, Priotto J (2016) The use of fluctuating asymmetry as a measure of farming practice effects in rodents: A species-specific response. Ecological Indicators, 70: 269-275.

Cole LJ, McCracken DI, Dennis P, Downie IS, Griffin AL, Foster GN, Murphy KJ, Waterhouse T (2002) Relationships between agricultural management and ecological groups of ground beetles (Coleoptera: Carabidae) on Scottish farmland. Agriculture, Ecosystems & Environment 93(1-3): 323-336.

Cook SC (2019) Compound and dose-dependent effects of two neonicotinoid pesticides on honey bee (*Apis mellifera*) metabolic physiology. Insects, 10(1):18.

Cram DL (2022) Oxidative stress and cognition in ecology. Journal of Zoology, Early view.

Crossley MS, Meier AR, Baldwin EM, Berry LL, Crenshaw LC, Hartman GL, Lagos-Kutz D, Nichols DH, Patel K, Varriano S, Snyder WE (2020) No net insect abundance and diversity declines across US Long Term Ecological Research sites. Nature Ecology & Evolution, 4(10): 1368-1376.

Curtis PG, Slay CM, Harris NL, Tyukavina A, Hansen MC (2018) Classifying drivers of global forest loss. Science. 361(6407):1108-11.

Danks HV (1987) Insect dormancy: an ecological perspective. Biological Survey of Canada (Terrestrial Arthropods), National Museum of Natural Sciences, Ottawa, Canada.

Delinger DL (2002) Regulation of diapause. Annual Review of Entomology 47: 93–122.

Derecka K, Blythe MJ, Malla S, Genereux DP, Guffanti A, Pavan P, Moles A, Snart C, Ryder T, Ortori CA, Barrett DA (2013) Transient exposure to low levels of insecticide affects metabolic networks of honeybee larvae. PLoS One. 8(7):e68191.

Desender K (1982) Ecological and faunal studies on Coleoptera in agricultural land. II. Hibernation of Carabidae in agro–ecosystems. Pedobiologia 23: 295–303.

Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. Annual Reviews in Entomology 52:81-106.

Dimou I, Koutsikopoulos C, Economopoulos AP, Lykakis J (2003) Depth of pupation of the wild olive fruit fly, *Bactrocera (Dacus) oleae* (Gmel.) (Dipt., Tephritidae), as affected by soil abiotic factors. Journal of applied entomology 127(1): 12-17.

Dinis AM, Pereira JA, Benhadi-Marín J, Santos SA (2016) Feeding preferences and functional responses of *Calathus granatensis* and *Pterostichus globosus* (Coleoptera: Carabidae) on pupae of *Bactrocera oleae* (Diptera: Tephritidae). Bulletin of entomological research 106 (6):701-709.

Douglas MR, Rohr JR, Tooker JF (2015) Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. Journal of Applied Ecology 52(1): 250-260.

Dryden IL, Mardia KV (1998) Statistical shape analysis. Wiley, Chichester.

Durbešić P (2011) Croatian entomofauna–Looking Back from the Present and Future Plans. Entomologia croatica, 15(1-4): 17-01.

Eilenberg J, Hajek A, Lomer C (2001) Suggestions for unifying the terminology in biological control. BioControl (4): 387-400.

Elbrecht V, Leese F (2017) Validation and development of COI metabarcoding primers for freshwater macroinvertebrate bioassessment. Frontiers in Environmental Science, 5: 1– 11.

Elek Z, Lövei G L, Bátki M (2014) No increase in fluctuating asymmetry in ground beetles (Carabidae) as urbanisation progresses. Community Ecology 15: 131–138.

Environmental Protection Agency, Proposed Interim Decision on Neonicotinoids, (2020). https://www.epa.gov/pollinator-protection/epa-actions-protectpollinators. Accessed 6 November 2020.

Erwin TL (1985) The taxon pulse: a general pattern of lineage radiation and extinction among carabid beetles. In: Ball GE (ed.) Taxonomy, phylogeny and zoogeography of beetles and ants. Dordrecht, Junk Publishers, 437–472.

Erwin TL, Adis J (1982) Amazonian inundation forests: Their role as short– term refuges and generators of species richness and taxon pulses. In: Prance G (ed.) Biological Diversification in the trphics. Proceedings of the Fifth International Symposium for the Association for Tropical Biology, NY USA, Columbia University Press, 479–484.

Etebari K, Bizhannia AR, Sorati R, Matindoost L (2007) Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. Pesticide Biochemistry and Physiology, 88(1): 14-19.

Eyre MD, Luff ML, Leifert C (2013) Crop, field boundary, productivity and disturbance influences on ground beetles (Coleoptera, Carabidae) in the agroecosystem. Agriculture, Ecosystems & Environment. 15: 165:60-7.

Finch S, Elliott MS (1994) Predation of cabbage root fly eggs and larvae by carabid ground beetles—fact or fantasy? IOBC/WPRS Bulletin 17(8): 109–114.

Floate KD, Doane JF, Gillot G (1990) Carabid predators of the wheat midge (Diptera: Cecidomyiidae) in Saskatchewan. Environtal Entomology 19(5): 1503–1511.

Floate KD, Fox AS (2000) Flies under stress: A test of fluctuating asymmetry as a biomonitor of environmental quality. Ecological Applications, 10: 1541-1550.

Freude H, Harde KW, Lohse GA, Klausnitzer B (2004) Die Käfer Mitteleuropas 2. Springer, Berlin.

https://food.ec.europa.eu/plants/pesticides/sustainable-use-pesticides/integrated-pest-management-ipm_en. Acessed om 30th July 2022.

Forbes SA (1883) The food relations of the Carabidae and Coccinellidae. The Canadian Entomologist. 1883 (1):13-4.

Forister ML, Pelton EM, Black SH (2019) Declines in insect abundance and diversity: We know enough to act now. Conservation Science and Practice. (8):e80.

Franin K, Kuštera G, Šišeta F (2016) Fauna of ground-dwelling arthropods in vineyards of Zadar County (Croatia). Poljoprivreda, 22(2): 50-56.

Freemark KE, Kirk DA (2001) Birds on organic and conventional farms in Ontario: partitioning effects of habitat and practices on species composition and abundance. Biological Conservation, 101(3): 337-350.

Frei B, Guenay Y, Bohan DA, Traugott M, Wallinger C ()2019 Molecular analysis indicates high levels of carabid weed seed consumption in cereal fields across Central Europe. Journal of Pest Science 92(3): 935-42.

Froidevaux JS, Louboutin B, Jones G (2017) Does organic farming enhance biodiversity in Mediterranean vineyards? A case study with bats and arachnids. Agriculture, ecosystems & environment, 249: 112-122.

Fuller BW (1988) Predation by *Calleida decora* (F.) (Coleoptera: Carabidae) on velvet bean caterpillar (Lepidoptera: Noctuidae) in soybean. Journal of Economical Entomology 81: 127–129.

Giglio A, Giulianini PG, Zetto T, Talarico F (2011) Effects of the pesticide dimethoate on a non-target generalist carabid, *Pterostichus melas italicus* (Dejean, 1828) (Coleoptera: Carabidae). Italian Journal of Zoology 78(4): 471-477.

Gobbi M, Fontaneto D (2008) Biodiversity of ground beetles (Coleoptera: Carabidae) in different habitats of the Italian Po lowland. Agriculture Ecosystems & Environment, 127 (3-4): 273-276

Gonçalves MF, Pereira JA (2012) Abundance and diversity of soil arthropods in the olive grove ecosystem. Journal of Insect Science, 12(20): 1-14.

González-Chang M, Wratten SD, Lefort MC, Boyer S (2016) Food webs and biological control: a review of molecular tools used to reveal trophic interactions in agricultural systems. Food Webs, 9: 4–11.

Goodell PB (2009) Fifty years of the integrated control concept: the role of landscape ecology in IPM in San Joaquin valley cotton. Pest Management Science: formerly Pesticide Science, 65(12): 1293-7.

Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology, 50(4): 977-987.

Government Office of Sweden, SEK 70 million to benefit pollinating insects. (2020). https://www.government.se/press-releases/2019/09/sek-70-million-tobenefit-pollinating-insects.

Grafius E, Warner FW (1989) Predation by *Bembidion quadrimaculatum* (Coleoptera: Carabidae) on *Delia antiqua* (Diptera: Anthomyiidae). Environmental entomology, 18(6): 1056-9.

Greenstone MH, Payton ME, Weber DC, Simmons AM (2014) The detectability half-life in arthropod predator-prey research: what it is, why we need it, how to measure it, and how to use it. Molrcular Ecology, 23: 3799-3813.

Hagler JR, Naranjo SE (1997) Measuring the sensitivity of an indirect predator gut content ELISA: Detectability of prey remains in relation to predator species, temperature, time, and meal size. Biological Control, 9: 112-119.

Hajek AE, Hannam JJ, Nielsen C, Bell AJ, Liebherr J K (2007). Distribution and abundance of Carabidae (Coleoptera) associated with soybean aphid (Hemiptera: Aphididae) populations in central New York. Annals of the Entomological Society of America, 100(6): 876-886.

Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Müller A, Sumser H, Hörren T, Goulson D (2018) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PloS one, 12(10):e0185809.

Halsch CA, Shapiro AM, Fordyce JA, Nice CC, Thorne JH, Waetjen DP, Forister ML (2021) Insects and recent climate change. Proceedings of the national academy of sciences, 118(2):e2002543117.

Hardersen S (2000) The role of behavioural ecology of damselflies in the use of fluctuating asymmetry as a bioindicator of water pollution. Ecological Entomology, 25: 45-53

Harper GL, King RA, Dodd CS, Harwood JD, Glen DM, Bruford MW, Symondson WO (2005) Rapid screening of invertebrate predators for multiple prey DNA targets. Molecular ecology, 14(3): 819-27.

Hassan SA, Bigler F, Bogenschütz H, Boller E, Brun J, Calis JN, Chiverton P, Coremans-Pelseneer J, Duso C, Lewis GB, Mansour F (1991) Results of the fifth joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". Entomophaga, 36(1): 55-67.

Hatteland BA, Grutle K, Mong CE, Skartveit J, Symondson WOC, Solhøy T (2010) Predation by beetles (Carabidae, Staphylinidae) on eggs and juveniles of the Iberian slug *Arion lusitanicus* in the laboratory. Bulletin of Entomological Research, 100(5): 559-567.

Hendrickx F, Maelfait JP, Lens L (2003) Relationship between fluctuating asymmetry and fitness within and between stressed and unstressed populations of the wolf spider Pirata piraticus. Journal of Evolutionary Biology, 16: 1270-1279.

Henríquez P, Donoso DS, Grez AA (2009) Population density, sex ratio, body size and fluctuating asymmetry of *Ceroglossus chilensis* (Carabidae) in the fragmented Maulino forest and surrounding pine plantations. Acta Oecologica, 35: 811–818.

Hof C, Brändle M, Dehling DM, Munguía M, Brandl R, Araújo MB, Rahbek C (2012) Habitat stability affects dispersal and the ability to track climate change. Biology Letters, 8(4): 639-643.

Hoffmann AA, Woods R. (2003) Associating environmental stress with developmental stability: problems and patterns. In: Polak M (Ed.), Developmental Instability: Causes and Consequences. Oxford University Press, Oxford, 387e401.

Holland JM, Luff ML (2000) The effects of agricultural practices on Carabidae in temperate agroecosystems. Integrated pest management reviews, 5(2): 109-29.

Homburg K, Homburg N, Schäfer F, Schuldt A, Assmann T (2014) Carabids.org: a dynamic online database of ground beetle species traits (Coleoptera, Carabidae). Insect Conservation and Diversity 7:195-205.

Huk T, Kühne B (1999) Substrate selection by *Carabus clatratus* (Coleoptera, Carabidae) and its consequences for offspring development. Oecologia, 121(3): 348-354.

Hunt J, Allen GR (2000) Larval density and developmental instability in the acoustically orienting parasitoid *Homotrixa alleni*. Acta ethologica, 2(2): 129-132.

Hurka K (1996) Carabidae of the Czech and Slovak republics. Print-centrum, Zlín, Czech Republic.

Iannotta N, Belfiore T, Brandmaya P, Noce ME, Scalercio S (2007) Evaluation of the impact on entomocoenosis of active agents allowed in organic olive farming against *Bactrocera oleae* (Gmelin, 1790). Journal of Environmental Science and Health, 42(7): 783-788.

Ivanković Tatalović L, Anđelić B, Jelić M, Kos T, A. Benítez H, Šerić Jelaska L (2020) Fluctuating asymmetry as a method of assessing environmental stress in two predatory carabid species within Mediterranean agroecosystems. Symmetry, 12(11):1890.

Jambrošić Vladić Ž (2020) Ekologija i biogeografija odabranih endemskih epigejskih vrsta trčaka (Coleoptera: Carabidae) dinarskog krša (Doctoral dissertation, University of Zagreb. Faculty of Science. Department of Biology).

Jensen CS, Garsdal L, Baatrup E (1977) Acetylcholinesterase inhibition and altered locomotor behavior in the carabid beetle *Pterostichus cupreus*. A linkage between biomarkers at two levels of biological complexity. Environmental Toxicology and Chemistry: An International Journal, (8): 1727-32.

Jeschke V, Gershenzon J, Vassão DG (2016) A mode of action of glucosinolate-derived isothiocyanates: detoxification depletes glutathione and cysteine levels with ramifications on protein metabolism in Spodoptera littoralis. Insect Biochem. Molecular Biology, 71:37-48.

Jones MG (1979) The abundance and reproductive activity of common carabidae in a winter wheat crop. Ecological Entomology 4: 31–43.

Kamenova S, Bretagnolle V, Plantegenest M, Canard E (2018) DNA metabarcoding diet analysis reveals dynamic feeding behaviour and biological control potential of carabid farmland communities. BioRxiv, 332312.

Kavvadias V, Koubouris G (2019) Sustainable soil management practices in olive groves. In: Panpatte DG, Jhala YK (eds) Soil fertility management for sustainable development, pp. 167-188 Springer, Singapore.

King RA, Vaughan IP, Bell JR, Bohan DA, Symondson WO (2010) Prey choice by carabid beetles feeding on an earthworm community analysed using species-and lineage-specific PCR primers. Molecular Ecology, 19(8): 1721-32.

Klingenberg CP, Debat V, Roff DA (2010) Quantitative genetics of shape in cricket wings: developmental integration in a functional structure. Evolution, 64.

Kobayashi Y, Nojima Y, Sakamoto T, Iwabuchi K, Nakazato T, Bono H, Toyoda A, Fujiyama A, Kanost MR, Tabunoki H (2019) Comparative analysis of seven types of superoxide dismutases for their ability to respond to oxidative stress in *Bombyx mori*. Scientific reports, 9(1): 1-2.

Kolawole AO, Kolawole AN (2014) Insecticides and bio-insecticides modulate the glutathione-related antioxidant defense system of Cowpea storage Bruchid (*Callosobruchus maculatus*). International Journal of Insect Science 6: IJIS-S18029.

Kotze JD, Brandmayr P, Casale A, Dauffy-Richard E, Dekoninck W, Koivula MJ, Lövei GL, Mossakowski D, Noordijk J, Paarmann W, Pizzolotto R, Saska P, Schwerk A, Serrano J, Szyszko J, Taboada A, Turin H, Venn S, Vermeulen R, Zetto T (2011) Forty years of carabid research in Europe-from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. ZooKeys, 100: 55–148.

Kraljev D, Gajić – Čapka M, Zaninović K (2005) U okrilju sunca i mora: klimatska monografija Zadra. Hrvatsko meteorološko društvo, Zagreb.

Kromp B (1999) Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agriculture, Ecosystems and Environment, 74: 187– 228.

Kunkel BA, Held DW, Potter DA (2001) Lethal and sublethal effects of bendiocarb, halofenozide, and imidacloprid on *Harpalus pennsylvanicus* (Coleoptera: Carabidae) following different modes of exposure in turfgrass. Journal of Economic Entomology, 94(1): 60-67.

Labrie G, Prince C, Bergeron JM (2003) Abundance and developmental stability of Pterostichus melanarius (Coleoptera: Carabidae) in organic and integrated pest management orchards of Quebec, Canada. Environmental Entomology, 32(1): 123-32.

Lasinio PJ, Zapparoli M (1993) First data on the soil arthropod community in an olive grove in central Italy. In: Coleman DC, Foissner W, Paoletti MG (eds.) Soil biota, nutrient cycling, and farming systems 113-121.

Lemić D, Benítez HA, Bjeliš M, Órdenes-Claveria R, Ninčević P, Mikac KM, Živković IP (2020) Agroecological effect and sexual shape dimorphism in medfly *Ceratitis capitata* (Diptera: Tephritidae) an example in Croatian populations. Zoologischer Anzeiger, 288: 118-24.

Lemić D, Čačija M, Gašparić HV, Drmić Z, Bažok R, Živković IP (2017) The ground beetle (Coleoptera: Carabidae) community in an intensively managed agricultural landscape. Applied Ecology and Environmental Research, 15: 661-74.

Lepš J, de Bello F, Lavorel S, Berman S (2006) Quantifying and interpreting functional diversity of natural communities: practical considerations matter. Preslia 78: 481–501.

Letardi A, Arnone, S, Cristofaro M, Nobili P (2015) Species composition of carabid communities (Coleoptera Carabidae) in apple orchards and vineyards in Val d'Agri (Basilicata, Italy). Biodiversity Journal 6(1): 11-16.

Lindroth CH (1949) Die Fennoskundischen Carabiden, Part 3, Algemeiner Teil. Stockholm: Broderna Lagerstrom Boktrychare, 911.

Lindroth CH (1992) Ground beetles (Carabidae) of Fennoscandia: a zoogeographic study: Part 1. Specific knowledge regarding the species. Smithsonian Institution Libraries and National Science Foundation, Washington, D.C.

Lorenz W (2005) Systematic list of extant ground beetles of the world (Insecta Coleoptera "Geadephaga": Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae). 2nd edn. Titzing, Germany.

Loumou A, Giourga C (2003) Olive groves: ``The life and identity of the Mediterranean''. Agriculture and Human Values 20(1): 87-95.

Lövei GL, Sárospataki MI (1990) Carabid beetles in agricultural fields in Eastern Europe. In: Stork NE (Ed.) The Role of Ground Beetles in Ecological and Environmental Studies, Intercept, Andover 87–93.

Lövei GL, Sunderland KD (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual review of entomology 41 (1):231-256.

Luff ML (1974) Adult and larval feeding habits of *Pterostichus madidus* (F.) (Coleoptera: Carabidae). Journal of Natural History 8: 403–409.

Luff ML (1987) Biology of polyphagous ground beetles in agriculture. Agricultural Zoology Reviews 2: 237-78.

Malheiro R, Casal S, Baptista P, Pereira JA (2015) A review of *Bactrocera oleae* (Rossi) impact in olive products: from the tree to the table. Trends in Food Science & Technology 44 (2): 226-242.

Martin NA (1986) Toxicity of pesticides to Allobophora caliginosa (Oligochaeta: Lumbricidae). New Zealand journal of agricultural research, 29(4): 699-706.

Mauchline AL, Osborne JL, Powell W (2004) Feeding responses of carabid beetles to dimethoate-contaminated prey. Agricultural and Forest Entomology, 6(2):99-104.

https://meteo.hr/index_en.php. Accessed on 30th July 2022

Mitteroecker P, Gunz P, Windhager S, Schaefer K (2013) A brief review of shape, form, and allometry in geometric morphometrics, with applications to human facial morphology. Hystrix, the Italian Journal of Mammalogy, 24(1): 59-66.

Moser SE, Obrycki JJ (2009) Non-target effects of neonicotinoid seed treatments; mortality of coccinellid larvae related to zoophytophagy. Biological Control, 51: 487–492.

Mosleh YY, Paris-Palacios S, Couderchet M, Vernet G (2002) Biological effects of two insecticides on earthworms (*Lumbricus terrestris* L.) under laboratory conditions. Mededelingen (Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen), 67(2):59-68.

Müller P, Chouaibou M, Pignatelli P, Etang J, Walker ED, Donnelly MJ, Simard F, Ranson H (2008) Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in Anopheles arabiensis sampled from an area of cotton fields in Northern Cameroon. Molecular ecology, (4): 1145-55.

Myers N, Mittermeierf RA, Mittermeierf CG, da Fonsecat GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature, 403: 853-858.

Nath BS (2002) Shifts in glycogen metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticides toxicity. Pesticide Biochemistry and Physiology, 74(2): 73-84.

Nattero J, Piccinali RV, Gaspe MS, Gürtler RE (2019) Fluctuating asymmetry and exposure to pyrethroid insecticides in *Triatoma infestans* populations in northeastern Argentina. Infection, Genetics and Evolution, 74: 103925.

Navar LG, Maddox D., Munger KA (2020).The renal circulations and glomerular filtration. Brenner and rector's the kidney. Amsterdam: Elsevier, 80-114.

Neuenschwander P, Bigler F, Delucchi V, Michelakis S (1983) Natural enemies of preimaginal stages of *Dacus oleae* Gmel. (Dipt., Tephritidae) in Western Crete. I. Bionomics and phonologies. Bollettino del Laboratorio di Entomologia Agraria'Filippo Silvestri 40: 3-32.

Nicholls CI, Altieri MA, Ponti L (2008) Enhancing plant diversity for improved insect pest management in northern California organic vineyards. Acta horticulturae, 785: 263-278.

Nimse SB, Pal D (2015) Free radicals, natural antioxidants, and their reaction mechanisms. RSC advances, 5(35): 27986-28006.

Nunes LA, de Araújo ED, Marchini LC (2015) Fluctuating asymmetry in *Apis mellifera* (Hymenoptera: Apidae) as bioindicator of anthropogenic environments. Revista de Biología Tropical, 63: 673-682.

Ordano M, Engelhard I, Rempoulakis P, Nemny-Lavy E, Blum M, Yasin S, Lensky IM, Papadopoulos NT, Nestel D (2015) Olive fruit fly (*Bactrocera oleae*) population dynamics in the Eastern Mediterranean: influence of exogenous uncertainty on a monophagous frugivorous insect. PloS ONE 10 (5).

Orsini MM, Daane KM, Sime KR, Nelson EH (2007) Mortality of olive fruit fly pupae in California. Biocontrol Science and Technology 17(8): 797-807.

Ortega M, Pascual S (2014) Spatio-temporal analysis of the relationship between landscape structure and the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae). Agricultural and Forest Entomology, 16(1): 14-23.

Palma-Onetto V, Oliva D, González-Teuber M (2021) Lethal and oxidative stress side effects of organic and synthetic pesticides on the insect scale predator *Rhyzobius lophanthae*. Entomologia Generalis, (4): 345-55.

Pan L, Qiu Y, Chen T, Lin J, Chi Y, Su M, Zhao A, Jia W (2010) An optimized procedure for metabonomic analysis of rat liver tissue using gas chromatography/time-of-flight mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis, 52(4):589-96.

Panni S, Pizzolotto R (2018) Fast molecular assay to detect the rate of decay of *Bactrocera oleae* (Diptera: Tephritidae) DNA in *Pterostichus melas* (Coleoptera: Carabidae) gut contents. Applied entomology and zoology, 53(3): 425-31.

Pekny JE, Smith PB, Marden JH (2018) Enzyme polymorphism, oxygen and injury: a lipidomic analysis of flight-induced oxidative damage in a succinate dehydrogenase d (Sdhd)-polymorphic insect. Journal of Experimental Biology, 221(6): jeb171009.

Pereira JA, Alves R, Casal S, Oliveira MBPP (2004) Effect of olive fruit fly infestation on the quality of olive oil from cultivars Cobrançosa, Madural and Verdeal Transmontana. Italian Journal of Food Science, 16: 355-365.

Picchi MS, Marchi S, Albertini A, Petacchi R (2017) Organic management of olive orchards increases the predation rate of overwintering pupae of *Bactrocera oleae* (Diptera: Tephritidae). Biological Control, 108: 9-15.

Pilotto F, Kühn I, Adrian R, Alber R, Alignier A, Andrews C, Bäck J, Barbaro L, Beaumont D, Beenaerts N, Benham S (2020) Meta-analysis of multidecadal biodiversity trends in Europe. Nature communications, 11(1):1-1.

Pisa LW, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs CA, Goulson D, Kreutzweiser DP, Krupke C, Liess M, McField M, Morrissey CA (2015) Effects of neonicotinoids and fipronil on non-target invertebrates. Environmental Science and Pollution Research, 22(1): 68-102.

Pizzolotto R, Mazzei A, Bonacci T, Scalercio S, Iannotta N, Brandmayr P (2018) Ground beetles in Mediterranean olive agroecosystems: Their significance and functional role as bioindicators (Coleoptera, Carabidae). PloS ONE, 13(3): e0194551

Plavšin I, Stašková T, Šerý M, Smýkal V, Hackenberger BK, Kodrík D (2015) Hormonal enhancement of insecticide efficacy in *Tribolium castaneum*: oxidative stress and metabolic aspects. Comparative Biochemistry & Physiology, 170: 19-27.

Previšić A, Rožman M, Mor JR, Acuña V, Serra-Compte A, Petrović M, Sabater S (2020) Aquatic macroinvertebrates under stress: Bioaccumulation of emerging contaminants and metabolomics implications. Science of The Total Environment, 20:704:135333.

Polak M (2003) Developmental Instability: Causes and Consequences. Oxford University Press, Oxford, United Kingdom

Porensky LM, Young TP (2013) Edge-effect interactions in fragmented and patchy landscapes. Conservation Biology, 27(3): 509-519.

Purtauf T, Dauber J, Wolters V (2005) The response of carabids to landscape simplification differs between trophic groups. Oecologia, 142(3): 458-464

Rahman I, Biswas SK, Kode A (2006) Oxidant and antioxidant balance in the airways and airway diseases. European Journal of Pharmacology, 533: 222-239.

Ramos P, Ramos JM, Jones OT (1989) The influence of asynchrony between olive moth (*Prays oleae* Bern.) adult emergence and olive fruit phenology in determining subsequent fruit infestation. InInternational Symposium on Olive Growing, 286: 391-394.

Raven PH, Wagner DL (2021) Agricultural intensification and climate change are rapidly decreasing insect biodiversity. Proceedings of the National Academy of Sciences. 118(2):e2002548117.

Ribera I, Dolédec S, Downie IS, Foster GN (2001) Effect of land disturbance and stress on species traits of ground beetle assemblages. Ecology, 82(4): 1112-29.

Ribeiro B, Guedes RNC, Corrêa AS, Santos, CT (2007) Fluctuating asymmetry in insecticide-resistant and insecticide-susceptible strains of the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). Archives of environmental contamination and toxicology, 53: 77-83

Ritchie EE, Maisonneuve F, Scroggins RP, Princz JI (2019) Lethal and sublethal toxicity of thiamethoxam and clothianidin commercial formulations to soil invertebrates in a natural soil. Environmental toxicology and chemistry, 38(10): 2111-20.

Rohlf FJ (2018) tpsDig Version 2.31; Digitize Landmarks and Outlines: Stony Brook, NY, USA.

Saska P, Van der Werf W, De Vries E, Westerman PR (2008) Spatial and temporal patterns of carabid activity-density in cereals do not explain levels of predation on weed seeds. Bulletin of Entomological Research 98(2): 169-81.

Scaccini D, Panini M, Chiesa O, Nicoli Aldini R, Tabaglio V, Mazzoni E (2020) Slug monitoring and impacts on the ground beetle community in the frame of sustainable pest control in conventional and conservation agroecosystems. Insects 11(6): 380

Scalercio S, Belfiore T, Noce ME, Vizzarri V, Iannotta N (2009) The impact of compounds allowed in organic farming on the above-ground arthropods of the olive ecosystem. Bulletin of Insectology 62(2): 137-141

Schnitter PH (1994) The development of carabid communities from uncultivated fields and meadows in the first years of a succession. In: Desender K, Dufréne M, Loreau M, Luff ML, Maelfait JP (Eds.) Carabid Beetles: Ecology and Evolution, Kluwer Academic Publishers, Dordrecht/Boston/London, 361–366.

Schowalter TD, Pandey M, Presley SJ, Willig MR, Zimmerman JK (2021) Arthropods are not declining but are responsive to disturbance in the Luquillo Experimental Forest, Puerto Rico. Proceedings of the National Academy of Sciences. 118(2):e2002556117.

Seibold S, Gossner MM, Simons NK, Blüthgen N, Müller J, Ambarlı D, Ammer C, Bauhus J, Fischer M, Habel JC, Linsenmair KE (2019) Arthropod decline in grasslands and forests is associated with landscape-level drivers. Nature. 574(7780):671-4.

Shi T, Burton S, Wang Y, Xu S, Zhang W, Yu L (2018) Metabolomic analysis of honey bee, *Apis mellifera* L. response to thiacloprid. Pesticide biochemistry and physiology, 152: 17-23.

Simone-Finstrom M, Li-Byarlay H, Huang MH, Strand MK, Rueppell O, Tarpy DR (2016) Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. Scientific Reports, 6(1): 1-10.

Sommaggio D, Peretti E, Burgio G (2018) The effect of cover plants management on soil invertebrate fauna in vineyard in Northern Italy. BioControl 63(6): 795-806

Song Y, Chen M, Zhou J (2017) Effects of three pesticides on superoxide dismutase and glutathione-S-transferase activities and reproduction of *Daphnia magna*. Archives of Environmental Protection, 43:80-86

Sparkman OD, Penton Z, Kitson FG (2011) Gas chromatography and mass spectrometry: a practical guide. Academic press, Elsevier, Oxford.

Sparks TC (2013) Insecticide discovery: an evaluation and analysis. Pesticide Biochemistry and Physiology 107: 8–17.

Spence JR, Niemelä JK (1994) Sampling carabid assemblages with pitfall traps: the madness and the method. The Canadian Entomologist, 126(3): 881-894.

Srinivas P (1986) Studies on metabolic stress in silk moth, *Bombyx mori* (L.) induced by selective insecticides. Kakatiya University, Warrangal, India

Stapp P (1997) Small mammal predation on darkling beetles (Coleoptera: Tenebrionidae) in pitfall traps. The Southwestern Naturalist, 43: 352–355.

Stokstad E (2018) New global study reveals the ‘staggering’loss of forests caused by industrial agriculture. Science. 13:10.

Sunderland KD, De Snoo GR, Dinter A, Hance T, Helenius J, Jepson P, Kromp B, Lys J-A, Samu F, Sotherton NW, Toft S, Ulber B (1995) Density estimation for invertebrate predators in agroecosystems. In: Toft S, Riedel W (Eds.) Arthropod Natural Enemies in Arable Land I. Density, Spatial Heterogeneity and Dispersal, Acta Jutlandica 70(2): 133–162.

Sutherland RM (2000) Molecular Analysis of Avian Diets. PhD Thesis, University of Oxford, United Kingdom

Symondson WOC, Sunderland KD, Greenstone MH (2002) Can generalist predators be effective biocontrol agents? Annual Review of Entomology 47: 561–594.

Svendsen C, Weeks JM (1997) Relevance and Applicability of a Simple Earthworm Biomarker of Copper Exposure. I. Links to Ecological Effects in a Laboratory Study withEisenia andrei. Ecotoxicology and Environmental Safety, 36(1): 72-79.

Šerić Jelaska L, Blanuša M, Durbešić P, Jelaska SD (2007) Heavy metal concentrations in ground beetles, leaf litter, and soil of a forest ecosystem. Ecotoxicology and Environmental Safety, 66(1): 74-81.

Šerić Jelaska L, Durbešić P (2009) Comparison of the body size and wing form of carabid species (Coleoptera: Carabidae) between isolated and continuous forest habitats. Annales de la Société entomologique de France 45(3): 327-338

Šerić Jelaska L, Dumbović V, Kučinić M (2011) Carabid beetle diversity and mean individual biomass in beech forests of various ages. ZooKeys, (100), p.393.

Šerić Jelaska L, Jurasović J, Brown DS, Vaughan IP, Symondson WOC (2014a) Molecular field analysis of trophic relationships in soil-dwelling invertebrates to identify mercury, lead and cadmium transmission through forest ecosystems. Molecular ecology 23(15): 3755-66.

Šerić Jelaska L, Franjević D, Jelaska SD, Symondson WOC (2014b) Prey detection in carabid beetles (Coleoptera: Carabidae) in woodland ecosystems by PCR analysis of gut contents. European Journal of Entomology, 111(5): 631-638.

Šeric Jelaska L, Symondson WOC (2016) Predation on epigeic, endogeic and anecic earthworms by carabids active in spring and autumn. Periodicum biologorum, 118(3): 281-289.

Šerić Jelaska L, Ivanković Tatalović L, Kostanjšek F, Kos T (2022) Ground beetle assemblages and distribution of functional traits in olive orchards and vineyards depend on the agricultural management practice. BioControl. 3: 1-12.

Štrbac P (1983) Fauna, bionomija i morfološko-taksonomske karakteristike klisnjaka i trčuljaka (Col.; Elateridae; Carabidae) u agroekološkim uslovima Slavonije i Baranje. Doctoral thesis, University of Osijek, Osijek, Croatia.

Šustek Z (1987) Changes in body size structure of carabid communities (Coleoptera, Carabidae) along an urbanisation gradient. BIOLOGIA (BRATISL.), 42(2): 145-56.

United States Environmental Protection Agency. 2007. Pesticides and food: What "Integrated Pest Management" means [Online]. Available at https://www.epa.gov/safepestcontrol/integrated-pest-management-ipm-principles (verified 15 July 2022).

Tasaki E, Sakurai H, Nitao M, Matsuura K, Iuchi Y (2017) Uric acid, an important antioxidant contributing to survival in termites. PLoS One, 12(6): e0179426.

Thiele HU (1977) Carabid beetles in their environments. Springer, Berlin.

Thomas MB (1999) Ecological approaches and the development of “truly integrated” pest management. Proceedings of the National Academy of Sciences 96(11): 5944-5951.

Tooming, E., Merivee, E., Must, A., Merivee, M.I., Sibul, I., Nurme, K. and Williams, I.H. 2017. Behavioural effects of the neonicotinoid insecticide thiamethoxam on the predatory insect *Platynus assimilis*. Ecotoxicology, 26(7), 902-913.

Trautner J, Geigenmüller K (1987) Tiger beetles, ground beetles. Illustrated key to the Cicindelidae and Carabidae of Europe. TRIOPS Verlag, Stuttgart, Germany.

Tretter L, Patocs A, Chinopoulos C (2016) Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. Biochimica et Biophysica Acta 1857(8): 1086-1101.

Tshernyshev, W. B. (1995). Ecological pest management (EPM): general approaches. Journal of applied entomology 119(1-5): 379-381.

Van Dijk TC, Van Staalduinen MA, Van der Sluijs JP (2013) Macro-invertebrate decline in surface water polluted with imidacloprid. PloS One. 8(5), e62374. https://doi.org/10.1371/journal.pone.0062374

van Klink R, Bowler DE, Gongalsky KB, Swengel AB, Gentile A, Chase JM (2020) Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. Science. 368(6489):417-20.

Vasileiadis VP, Otto S, Van Dijk W, Urek G, Leskovšek R, Verschwele A, Furlan L, Sattin M (2015) On-farm evaluation of integrated weed management tools for maize production in three different agro-environments in Europe: Agronomic efficacy, herbicide use reduction, and economic sustainability. European Journal of Agronomy. 63:71-8.

Vickerman GP, Sunderland KD (1977) Some effects of dimethoate on arthropods in winter wheat. Journal of Applied Ecology, 1: 767-77.

Vigna-Taglianti A (2013) Fauna Europea: Carabidae. Fauna Europaea version 2.6. URL: http://www. faunaeur.org.

Vogel G (2019) 100 million German insect protection plan will protect habitats, restrict weed killers, and boost research. Science.

Wagner DL, Grames EM, Forister ML, Berenbaum MR, Stopak D (2021) Insect decline in the Anthropocene: Death by a thousand cuts. Proceedings of the National Academy of Sciences. 12, 118(2):e2023989118.

Weller B, Ganzhorn JU (2004) Carabid beetle community composition, body size, and fluctuating asymmetry along an urban-rural gradient. Basic and Applied Ecology, 5: 193-201.

Whitehorn PR, O'connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science, 336(6079): 351-352.

Willig MR, Woolbright L, Presley SJ, Schowalter TD, Waide RB, Heartsill Scalley T, Zimmerman JK, González G, Lugo AE (2019) Populations are not declining and food webs are not collapsing at the Luquillo Experimental Forest. Proceedings of the National Academy of Sciences. 116(25):12143-4.

Yao FL, Zheng Y, Zhao JW, Desneux N, He YX, Weng QY (2015) Lethal and sublethal effects of thiamethoxam on the whitefly predator *Serangium japonicum* (Coleoptera: Coccinellidae) through different exposure routes. Chemosphere 128: 49-55.

Yang S, Rothman RE (2004) PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. The Lancet. Infectious diseases, 4(6): 337–348.

Young OP (2008) Body weight and survival of *Calosoma sayi* (Coleoptera: Carabidae) during laboratory feeding regimes. Annals of the Entomological Society of America, 101(1): 104-112.

Yu SJ (2008) Detoxification Mechanisms in Insects. In: Capinera JL (ed) Encyclopedia of Entomology. Springer, Dordrecht.

Zhang X, Ju H, Wang J (2011) Electrochemical sensors, biosensors and their biomedical applications. Academic Press, Massachusetts, United States.

Zhang Y, Sun G, Yang M, Wu H, Zhang J, Song S, Ma E, Guo Y (2011) Chronic accumulation of cadmium and its effects on antioxidant enzymes and malondialdehyde in *Oxya chinensis* (Orthoptera: Acridoidea). Ecotoxicology and Environmental Safety, (5): 1355-62.

Žagar A, Carretero MA, Marguč D, Simčič T, Vrezec A (2018) A metabolic syndrome in terrestrial ectotherms with different elevational and distribution patterns. Ecography, (41): 1728-1739.

9. CURICULUM VITAE

Lara Ivanković Tatalović was born on 2nd April 1993 in Split, Croatian. There, she was enrolled in primary school Ravne Njive, and after moving to Zagreb in 2005 she completed her primary education in Primary school Davorin Trstenjak. She went to The Fifth Gymnasium in Zagreb where she graduated in 2012, and that same year she entered the Faculty of Science, University of Zagreb, division: Biology. In January 2016, she successfully completed the Course in Winter Ecology at S. N. Skadovsky Zvenigorod Biological Station of Lomonosov Moscow University. In 2017, she traveled to Ireland for three months as part of Erasmus+ program, and stayed at University College Cork as part of the research group led by Professor Simon Harris. Her final thesis, entitled "Phenology patterns and ecology of family Dixidae (Insecta, Diptera) on a longitudinal gradient of Plitvice lakes", was done under the tutorship of Associate Professor Marija Ivković, and she graduated in 2018. She was a participant at 11th Symposium for European Freshwater Sciences in Zagreb. From 2018 to 2019 she worked as Curator in the Croatian Natural History Museum, during which she participated as professional associate in the creation of exhibition and catalogue entitled Superorganism (Authors: Iva Mihoci PhD and Martina Podnar Lešić, PhDIn 2019 she enrolled postgraduate study of Biology at Faculty of Science, Department of Biology, as part of project "Human Resources development – young scientists carrier development (DOK-01-2018)". That same year she is employed as assistant on MEDITERATRI project (Croatian Science Foundation (HRZZ) UIP-2017-05-1046) where she currently works.

Within postgraduate study and MEDITERATRI project activities she participated in several national conferences including: 57th Croatian and 17th International Symposium on Agriculture (19/96/2022-24/06/2022, Vodice, Croatia - Oral presentation), 6th PhD student Symposium (23/04/2022-24/04/2022, Zagreb, Croatia - Poster), 4th PhD student Symposium (28/02/2020, Zagreb, Croatia - Oral presentation), 55th Croatian & 15th International Symposium on Agriculture (16/02/2020 – 21/02/2020 – Vodice, Croatia - Oral presentation), 57th Croatian & 17th International Symposium on Agriculture (19/06/2022 – 24/06/2022 – Vodice, Croatia - Oral presentation), and 6th PhD student Symposium (23/04/2022 - 24/04/2022, Zagreb, Croatia - Poster). As a project associate she participated in following workshops: ACS on Campus (16/03/2022, Zagreb, Croatia); Korištenje računalnog klastera Isabella", University in Zagreb, University Computing Centre (23/06/2021 – 25/06/2021, Zagreb, Croatia); „Priprema podataka u R-u (S771)", University in Zagreb, University Computing Centre (19/04/2021 – 22/04/2021 - Zagreb, Croatia); „Molecular Phylogeny and Evolution", institute of Molecular and Organismic Evolution, University in Mainz (22/09/2020 – 24/09/2020 – Mainz, Germany); "Upoznavanje sa sintaksom jezika R i njegova primjena u osnovnoj statističkoj i grafičkoj analizi podataka", University in Zagreb, University

Computing Centre (02/03/2020 – 05/03/2020 Zagreb, Croatia); "Inkscape ili vektorska grafika za svakoga (Inkscape 0.91), University in Zagreb, University Computing Centre (30/01/2020 – Zagreb, Croatia); The Arachnology course, Masaryk University Centre in Telč (13/01/2017 – 17/01/2020 – Telč, Czech Republic).

10. LIST OF PUBLICATIONS

Research papers published in journals

1. Ivanković L, Ivković M, Stanković I (2019) Perennial phenology patterns and ecological traits of Dixidae (Insecta, Diptera) in lotic habitats of a barrage lake system. Limnologica. 76:11-8.
2. Ivković M, Ivanković L (2019) The genus *Dixa* (Diptera, Dixidae) in Croatian lotic habitats, with a checklist of species and relationships with the fauna of neighbouring countries. ZooKeys. 867:45.
3. Ivanković Tatalović L, Anđelić B, Jelić M, Kos T, Benítez AH, Šerić Jelaska L (2020) Fluctuating Asymmetry as a Method of Assessing Environmental Stress in Two Predatory Carabid Species within Mediterranean Agroecosystems. Symmetry, 12(11): 1890. (https://www.mdpi.com/2073-8994/12/11/1890)
4. Šerić Jelaska L, Ivanković Tatalović L, Kostanjšek F, Kos T (2022) Ground beetle assemblages and distribution of functional traits in olive orchards and vineyards depending on their management system. Biocontrol, 67:275-286. https://doi.org/10.1007/s10526-022-10133-x
5. Ivanković Tatalović L, Mašek M, Šerić Jelaska L (2022) Dietary, locomotory, and metabolic reactions of *Abax parallelus* (Coleoptera, Carabidae) to acute thiamethoxam intoxication. Ecotoxicology (accepted by the editor, in revision)

Abstracts bublished in books of abstracts

1. Šerić Jelaska L, Kos T, Jelić M, Anđelić B, Ivanković L (2020) MEDITERATRI projekt: priča o utjecaju pesticida na predatorske člankonošce. 64. Seminar biljne zaštite- zbornik sažetaka, Bažok, Renata - Zagreb : Hrvatsko društvo biljne zaštite, 2020, 66-67 (https://www.bib.irb.hr/1050330; https://hdbz.hr/wp-content/uploads/2019/12/64-Seminar-biljne-zastite-Program.pdf)
2. Šerić Jelaska L, Jelić M, Ivanković L, Anđelić B, Gajski D, Kos T (2020) Raznolikost beskralježnjaka u ekološkom i integriranom upravljanju maslinicima i vinogradima. Zbornik skupa Održivi razvoj poljoprivrede i turizma u kontekstu klimatskih promjena. Krapac, Marin Goreta Ban, Smiljana (ur.).Poreč: Institut za poljoprivredu i turizam, str. 54-55 (http://145g.iptpo.hr/wp-content/uploads/2020/11/ZBORNIK-SKUPA-Odr%C5%BEivi-razvoj-poljoprivrede-i-turizma.pdf)
3. Anđelić B, Jelić M, Ivanković L, Kos T, Šerić Jelaska L (2020) Metabarkodiranje za potrebe molekularnih trofičkih analiza. SIMPOZIJ STUDENATA DOKTORSKIH STUDIJA PMF-a: Knjiga sažetaka, Rončević S, Barišić D (ur.) Zagreb: Prirodoslovno-matematički fakultet,

Sveučilište u Zagrebu, str. 84-84 (http://www.pmf.unizg.hr/_download/repository/Knjiga_sazetaka_SSDS_PMF_2020.pdf)

4. Ivanković L, Anđelić B, Jelić M, Kos T, Šerić Jelaska L (2020) Raznolikost i ekologija faune beskralješnjaka u agroekosustavima s ekološkim i integriranim tipom upravljanja. SIMPOZIJ STUDENATA DOKTORSKIH STUDIJA PMF-a: Knjiga sažetaka / Knjiga sažetaka / Rončević S, Barišić D (ur.). Zagreb: Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu, str. 34-34 (http://www.pmf.unizg.hr/_download/repository/Knjiga_sazetaka_SSDS_PMF_2020.pdf)
5. Ivanković L, Anđelić B, Gajski D, Jelić M, Kos T, Šerić Jelaska L (2020) Diversity and ecology of non-target invertebrate fauna in Integrated and Ecologically based Pest Managements in agricultural sites within MEDITERATRI project. 55th Croatian & 15th International Symposium on Agriculture- Knjiga sažetaka, Mioč, B., Širić, I. - Zagreb: Sveučilište u Zagrebu, Agronomski fakultet, 2020, 62-62 (http://sa.agr.hr/pdf/2020/sa2020_abstracts.pdf)
6. Ivanković Tatalović, L., Šerić Jelaska, L. (2021): The impact of thiamethoxam exposure on feeding and locomotor activity of Abax parallelus (Coleoptera: Carabidae), in Proceedings of the 1st International Electronic Conference on Entomology, 1–15 July 2021, MDPI: Basel, Switzerland, doi:10.3390/IECE-10541 (https://sciforum.net/paper/view/10541)
7. Lucija Šerić Jelaska, Barbara Anđelić Dmitrović, Lara Ivanković Tatalović, Tomislav Kos (2021) *Drosophila suzukii* RECORDED IN MEDITERRANEAN OLIVE ORCHARDS IN CROATIA. 4th Croatian Symposium on Invasive Species – Knjiga sažetaka, Zagreb, Hrvatsko ekološko društvo, 29-30. 11. 2021, str. 78. http://www.ekolosko-drustvo.hr/4CSIS-Programme.pdf
8. Ivanković Tatalović L, Mašek M, Šerić Jelaska L (2022) Utjecaj izlaganja tiametoksamom na hranjenje, kretanje i metabolizam vrste *Abax parallelus* (Coleoptera:Carabidae). SIMPOZIJ STUDENATA DOKTORSKIH STUDIJA PMF-a: Knjiga sažetaka / Schneider P (ur.). Zagreb: Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu, str. 201
9. Šerić Jelaska L, Ivanković Tatalović L, Kostanjšek F, Kos T (2022) Carabid assemblages and functional traits distribution in olive orchards and vineyards within the Mediterranean basin. 20th European Carabidologist Meeting - Book of Abstracts, Sklodowski J and Sienkiewicz P (eds.). Warszawa, Poland. pp. 25
10. Ivanković Tatalović L, Mašek T, Šerić Jelaska L (2022) The impact of neonicotionoid on behaviour and metabolomics of carabid beetles and its spreading by trophic interactions. 20th European

Carabidologist Meeting - Book of Abstracts, Sklodowski J and Sienkiewicz P (eds.). Warszawa, Poland. pp. 14

11. Šerić Jelaska L, Ivanković Tatalović L, Kostanjšek F, Kos T (2022) Carabid beetle assemblages and functional traits distribution in olive orchards and vineyards within Mediterranean basin. In: 57th Croatian and 17th International Symposium on Agriculture.

Data published in databases

1. Šerić Jelaska L, Anđelić Dmitrović B, Ivanković Tatalović L, Rešetnik I (2022). Meditera3. University of Zagreb Faculty of Science. Occurrence dataset https://doi.org/10.15468/5jkd4t