16TH MEETING OF THE EUROPEAN ASSOCIATION OF FORENSIC ENTOMOLOGY

BORDEAUX June 6th-7th, 2019

Pôle juridique et judiciaire
35, Pey Berland square

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16th Meeting of the European Association of Forensic Entomology

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University of Bordeaux, France

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PROGRAMME
June 6, 2019

	Legal and Judicial dpt building (room 1K / 1st floor)
8.30-12.30	*Registration* (entry hall)
9.30-10.00	Welcome Speech (room 1K / 1st floor) Jean-Bernard Huchet (organizer), Anne Delagne (Head of PACEA laboratory) and Luc Bourguignon (EAFE President)
10.00	Oral Communications Section I — Chairman: Jens Amendt
10.00-10.15	Amoret Whitaker, Eleanor Chessun-Lawrence **Effect of prior concealment of cadavers on blow fly oviposition**
10.15-10.30	Denise Gemmellaro, George Hamilton, Mariela Domínguez, Lauren Weidner **An analysis of blow fly (Diptera: Calliphoridae) species richness and distribution along elevation gradients in Sicily (Italy) and Ecuador**
10.30-10.45	Amely M. Bauer, Jeffery K. Tomberlin **Effects of photoperiod on the development of forensically important blow fly *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae)**
10.45-11.00	Fabiola Tuccia, Giorgia Giordani, Stefano Vanin ***Physiphora alceae* (Preyssler, 1791) (Diptera: Ulidiidae) found on a cadaver in Northern Italy: case, morphology and phylogeny**
11.00-11.30	**Coffee break**
11.30	Oral Communications Section II — Chairman: Luc Bourguignon
11.30-11.45	Stefano Vanin **Entomology or not Entomology: that is the question**
11.45-12.00	Cindy Aubernon, Valery Hedouin, Damien Charabidzé **Developmental strategies in Calliphoridae larvae**
12.00-12.15	Calil Makhoul **Advances of Forensic Entomology research in Lebanon**
12.15-12.30	Krystal Hans , Trevor Stamper **Purdue University's Forensic Entomology area of concentration: Preparing a future generation of forensic entomologists with a unique program in insect biology**
12.30-14.00	**Lunch Time**
14.00-14.30	Poster session (room RE, ground floor)
14.30	Oral Communications Section III — Chairman: Amoret Whitaker
14.30-14.45	Martin Hall **The relationship between research and casework in forensic entomology: how do we encourage the next generation of caseworkers from research?**

PROGRAMME
June 6, 2019

Legal and Judicial dpt building (room 1K / 1st floor)	
14.45-15.00	Jennifer Pechal, M. Eric Benbow **Insect-microbiome interactions, importance, and applications for Forensic Entomology**
15.00-15.15	Lavinia Iancu, Georgiana Necula-Petrareanu, Cristina Purcarea **Necrophagous Insect Microbiome – qualitative and quantitative insights**
15.15-15.30	Elvan Hortac, Ali Açikgöz, Abdullah Zübeyir Ceylan, Erhan Büken, Meriem Taleb, Halide Nihal Açikgöz **Microbiome and environmental conditions: the first study from Turkey**
15.30-15.45	Martin Novák, Katarzyna Frątczak-Łagiewska, Anna Mądra-Bielewicz, Szymon Matuszewski **Eye-background contrast as a quantitative indicator for pupal age**
15.45-16.15	**Coffee break** 
16.15	Oral Communications Section IV Chairman: Denise Gemmellaro
16-15-16.30	Joanna Gruszka, Szymon Matuszewski **Development of *Necrodes littoralis* L. (Coleoptera: Silphidae) at five constant temperatures – preliminary data**
16.30-16.45	Kyu Jin Youm, Sang Eon Shin, Ju Hee We, Ha Jin Kim, Tae Mo Kang, Da Som Lee, Kwang Soo Ko, Seong Hwan Park **Morphological key features of five Calliphoridae species common in Korea : with emphases on the comparison between unique oriental and universal sister species (family Calliphoridae)**
16.45-17.00	Trevor Stamper **iFly: forensic entomology mobile field app**
17.00-17.15	**Conclusions of the first day**
20.00	**SOCIAL DINNER ("K Baroque", 1 quai des Chartrons)** **Tram B: " *CAPC-Musée d'Art Contemporain*" Stop**

PROGRAMME
June 7, 2019

Legal and Judicial dpt building (room 1K / 1st floor)	
9.00-12.30	*Registration* (entry hall)
9.30	Oral Communications Section V — Chairman: Katherine Brown
9.30-9.45	Larissa Komo, Damien Charabidzé, Valery Hedouin **Time to move: using larval migration for minimum Postmortem interval estimation in Forensic Entomology**
9.45-10.00	Gaetan Moreau, Lena Lutz, Jens Amendt **Necrophagous insect activity modulates the thermal microhabitat of concealed carcasses**
10.15-10.30	M. Eric Benbow, Breanna Wydra, Brianna Timmons, Katelyn Smiles, Nicholas Babcock, Joseph Receveur, Sierra Kaszubinski, Courtney Weatherbee **Aquatic and terrestrial invertebrate colonization of vertebrate remains in a pond: an experimental test for a cold case death investigation**
10.30-10.45	Chong Chin Heo, Razuin Rahimi, Azmiera Zamri **Unusual Diptera colonization on Human corpses in Malaysia: Implications and opportunities in Forensic entomological services**
10.45-11.00	Marise Heyns, Devin Finaughty, Kyle Kulenkampff **Species composition of forensically important blow flies (Diptera: Calliphoridae) in the Western Cape of South Africa**
11.00-11.30	**Coffee break**
11.30	Oral Communications Section VI — Chairman: Daniel Martín-Vega
11.30-11.45	Hannah Moore **Cuticular Hydrocarbons from empty puparial cases for identification of species and geographical location**
11.45-12.00	Jennifer Pradelli, Lorenzo Franceschetti, Fabiola Tuccia, Cristina Cattaneo, Stefano Vanin **Geographical distribution of forensically important flies in Milan, Northern Italy: morphological identification and molecular comparison of different species**
12.00-12.15	Katharina Köchling, Lena Lutz, Jens Amendt **Effect of inbreeding on development of the forensically important blow fly species, *Calliphora vicina***
12.15-12.30	Meriem Taleb, Halide Nihal Açikgöz **Molecular identification of *Stearibia nigriceps* Meigen and *Piophila casei* Linnaeus (Diptera: Piophilidae) from Algeria with description of the first african record of *Stearibia nigriceps***
12.30-14.00	**Lunch Time**
14.00-14.30	Poster session (room RE, ground floor)

PROGRAMME
June 7, 2019

Legal and Judicial dpt building (room 1K / 1st floor)	
14.30	Oral Communications Section VII — Chairman: Stefano Vanin
14.30-14-45	Emma Harvey, Katherine Brown **Accelerated flies? The effects of petrol and lighter fluid on piglet decomposition and blowfly succession & development**
14.45-15.00	Lena Lutz, Jens Amendt **Insect breeding in Forensic Laboratories - A Survey**
15.00-15.15	Falko Drijfhout **Metabolites as internal age markers in *Calliphora vicina* and *Lucilia sericata***
15.15-15.30	Meghan Beutler, Andrew Hart, Martin Hall **The use of wing fray and sex ratios to determine the origin of flies at an indoor crime scene**
15.30-15-45	Alexandra Bauer, Jeffery Tomberlin **Impact of diet moisture on the larval development of the forensically important blow fly *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae)**
15.45-16.15	**Coffee break** 
16.15	Oral Communications Section VIII — Chairman: Damien Charabidzé
16.15-16.30	Yanko Kolev, Dobrinka Radoinova **The use of Diptera in the determination of the time since death in Bulgaria**
16.30-16.45	Judit Altarriba Fatsini, Marta I. Saloña Bordas **Muscidae (Diptera) associated to a pig carcass (*Sus scrofa*) in Leioa (Bizkaia, Spain)**
16.45-17.00	**Conclusions of the second day**
17.00-18.00	**XVIth EAFE Annual General Meeting**

Oral presentations

Effect of prior concealment of cadavers on blow fly oviposition

Amoret Whitaker [1,@], Eleanor Chessun-Lawrence [1]

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Concealment of a cadaver prior to final deposition may occur, especially in cases of death which are not premeditated. Concealment may be in environments which are mainly inaccessible to blow flies, such as a basement or garage, vehicle, sealed container or tight wrappings. Decomposition of the cadaver will commence, but oviposition by blow flies may be prevented. The succession of insects on a body is well-documented, such that different species of insect are known to be attracted to a decomposing cadaver at different stages of decomposition. However, there is currently little published data on whether partially decomposed remains will still be attractive to blow flies, or for how long. If there is a delay in oviposition, this will affect the estimation of minimum post-mortem interval (minPMI), so any prior concealment, if known, should be taken into account. A study was undertaken to investigate whether prior concealment had an effect of oviposition, with the exposure of fresh pigs heads, alongside some which had been concealed for a period of one, two or three weeks, prior to exposure. Preliminary results suggest that a longer period of concealment results in a longer delay in oviposition, which should be taken into account when estimating minPMI.

An Analysis of Blow Fly (Diptera: Calliphoridae) species richness and distribution along elevation gradients in Sicily (Italy) and Ecuador

Denise Gemmellaro [1,@], George Hamilton [1], Mariela Domínguez [2], Lauren Weidner [2]

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A survey of blow flies (Diptera: Calliphoridae) was conducted in Sicily, Italy and Ecuador across four different altitudinal levels. The elevations ranged from 20m to 1552m in Sicily, and 561m to 3336m in Ecuador. Species richness, relative abundance and diversity were calculated, as well as the ratio of females to males and community assemblage. Twelve species were collected in Sicily and 17 species were collected in Ecuador. In Sicily, the most abundant blow fly species was *Lucilia sericata* (Meigen) accounting for 68.50% of the total capture. While in Ecuador, *Consomyiops verena* (Walker) accounted for 51.67% of all species captured. In Sicily, significant differences were only observed in the relative abundance of *L. sericata* across elevation gradients. In Ecuador, significant differences were observed in the relative abundance of *Calliphora nigribasis* (Macquart), *Chrysomya albiceps* (Weidemann), *C. verena*, *Hemilucilia semidiaphana* (Rondani), *Lucilia ibis* (Shannon), *Lucilia purpurescens* (Walker), and *Paralucilia* sp. across elevation gradients. These data can help build a checklist of blow fly species in these two regions and can be instrumental in forensic investigations

Effects of photoperiod on the development of forensically important blow fly *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae)

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The estimation of the time of colonization (TOC) is among the most important practical applications of forensic entomology. As it is often based on laboratory studies that document arthropod development, precise data for forensically important species, such as blow flies (Diptera: Calliphoridae), are therefore essential for accuracy in the estimate of the TOC. Blow fly development (e.g., instar duration, weight or length over time) is a quantitative trait, and thus depends on genetic and environmental factors, such as temperature, humidity, and photoperiod. Developmental data are generated under controlled environmental conditions, and as insects are poikilothermic, effect of temperature is studied best. In calliphorids, studies showed photoperiod can play a role in development. However, there has been little research to date and the data suggest that various species are affected differently. The hairy maggot blow fly, *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), is a fly of great medical and legal importance and is often encountered on vertebrate remains in temperate and tropic regions throughout the world, including North and Central America, Asia and Australia. Analyzing how photoperiod affects the development of this species will add to the knowledge of its effect in forensically important blow flies and will therefore help limit error in TOC estimations.
In this study, the effects of photoperiod on the larval development time, larval length and weight over time, adult emergence and weight are examined under three different light regimes of 12, 16 and 24 h of light measured at 28.0 ±1.0°C. It is hypothesized that the duration of light plays a distinct role in post-embryonic development.

Physiphora alceae (Preyssler, 1791) (Diptera: Ulidiidae) found on a cadaver in Northern Italy: case, morphology and phylogeny

Fabiola Tuccia [1,2,@] , Giorgia Giordani [2,1], Stefano Vanin [2,1]

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The correct identification of species is of paramount importance in forensic entomology if one wants to have a reliable interpretation of the criminal event. At the same time, the scientific community is well aware of the huge gap existing between the morphological resources available for the adults and for the immatures of Diptera, especially for poorly researched species that only recently have started raising interest. This is the case of the family Ulidiidae which, in contrast to the common colonizers of cadavers (Calliphoridae, Sarcophagidae, Muscidae, Stratiomyidae and Phoridae), is rarely reported in human cases despite the larvae of some species are known as feeders on plant and animal decomposing matter. In this study, *Physiphora alceae* (Preyssler, 1791) (Diptera: Ulidiidae) is reported for the first time within the insect assemblage colonizing a human cadaver in Northern Italy. The immature stages of this species are described in detail using the information provided by the research program of the FLEA lab, with the final goal to reduce the risk of misidentification and provide useful information to forensic entomology investigations. In addition, a phylogenetic analysis, based on the molecular analysis of the species, was performed to validate the morphological result. This approach provided also the possibility to review the phylogeny of the family.

Entomology or not Entomology: that is the question

Stefano Vanin [1, 2, @]

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Forensic Entomology deals with the use of insects and other arthropods in medico-legal investigations. If we consider as well urban and stored products entomology, forensic entomology deals with the application of knowledge about insects to legal investigations. Knowledge about insects is the domain of the discipline called entomology, so forensic entomology is a branch of entomology. If it is true, a forensic entomologist has to have a deep knowledge not only of the techniques but, as well, of the rules typical of the discipline updated to the specific time in which he or she lives. Unfortunately, in the last years of reading papers or chapters of books or - worst - the comments of some reviewers, it seems that some fundamental aspects of entomology have been forgotten or are unknown. Classification, determination, identification are used as synonyms when they are not synonyms. "Be consistent in using or not using brackets in the name of the species descriptor (authority)" is one of the most recurrent "blasphemies" in some reviewer's comments. Are we losing our entomologists? Are we doing our proper job as authors, reviewers, editors, lecturers, experts or are we underestimating the rules of our discipline? Words are important, they are not only sounds, but they contain information. The aims of this presentation are to report a series of errors that are commonly found in the forensic literature and raise awareness, especially among students, of the importance of taxonomy and systematics in entomology and more specifically in forensic entomology.

Developmental strategies in Calliphoridae larvae

Cindy Aubernon [1,@], Valery Hedouin [1], Damien Charabidzé [1]

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Calliphoridae larvae growing on cadavers experience strong selection pressures during their development. This extreme, competitive and constraining environment has favored the emergence of efficient developmental strategies, based on mechanisms such as thermal regulation but also sociality. Our findings indicate the existence of individual and collective behavioral development strategies allowing larvae to develop efficiently under harsh conditions.
In this context, larvae are subject to behavioral trade-offs: forming large aggregations to obtain collective benefits, avoiding overcrowding, locating places with sufficient food or staying on warm body areas are not always compatible goals. Our studies on the behavior of Calliphoridae larvae highlight that gregariousness is intrinsically linked to a balance between these costs and benefits on larval development. Under field conditions, growth is a never-ending trade-off.
Currently, minimum Post-Mortem Interval calculations use developmental data obtained under laboratory conditions omitting to take account of larval behavior. This talk will describe, based on detailed experimental studies, how behavioral trade-offs and sociality affect the development of Calliphoridae larvae, highlighting the importance of an awareness of the maggot's point of view.

Advances of Forensic Entomology Research in Lebanon

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Forensic entomology has been regarded as one of the most useful fields in forensic sciences especially for the estimation of the postmortem interval. Even if, on a worldwide scale, forensic entomology has tremendously evolved, in Lebanon, the first steady steps have only been taken in the last five years. Accordingly, studies focused not only on the main factors influencing the development of necrophagous insects, but also on the use of molecular-based techniques in order to assist the taxonomic identification of the species (Shayya *et al*, 2018), adding to the diversity of insects and their succession (Makhoul, 2014). Moreover, the use of cytochrome oxidase genes has been employed in the taxonomic identification of species (Shayya *et al*, 2018). Furthermore, pioneer Lebanese researches have been conducted on species compositions and development of necrophagous insects recovered from dead bodies (Shayya, S. et al., 2016) as well as on the colonization animal models decomposing in various conditions. These have been carried on in several locations including North Lebanon (Shayya *et al.*, 2016), Beirut (Makhoul, 2014), and Mount-Lebanon (Shayya, 2018). Nevertheless, data from the other regions in Lebanon are currently lacking. Since Lebanon is the smallest country on the Levantine coast (10,457 km2) and has a variety of ecological conditions throughout the country, it is important to have data on the influence of geographic location on species composition and development of necrophagous insects. In addition, studies comparing Lebanon with surrounding countries have not been reported so far. In 2015, forensic entomology only became officially implied in the Lebanese forensic investigations and it was a bedrock for the development of further research. All pertinent research conducted in this field in Lebanon are described. However, more research must be conducted to provide more inclusive information of information pertaining to insects of forensic importance, to enhance our database and to understand thoroughly the intrinsic and extrinsic factors that influence entomofaunal succession.

Purdue University's Forensic Entomology area of concentration: Preparing a future generation of forensic entomologists with a unique program in insect biology

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In spring 2019, the Department of Entomology at Purdue University announced the approval of the Bachelor of Science in Insect Biology with a Forensic Entomology area of concentration. This unique area of concentration is the first program to explicitly focus on forensic entomology. Set to begin fall 2019, this track within the Insect Biology degree incorporates general entomology courses, forensic science courses and forensic entomology courses that provide students with a broad background in both molecular biology and ecology of forensically relevant insects. This area of concentration takes a 4-fields approach, incorporating urban, stored-product, medical/veterinary, and medicolegal forensic entomology, and requires students to complete a capstone research project in one of these areas. This forensic entomology area of concentration offers the necessary coursework for the American Board of Forensic Entomology technician certification, and meets the forensic science standards of the Forensic Science Education Programs Accreditation Commission (FEPAC). This program is suitable for students who are interested in pursuing careers in forensic entomology, crime scene investigation, forensic analysis, or wish to enter graduate programs in these areas.

The relationship between research and casework in forensic entomology: how do we encourage the next generation of caseworkers from research?

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Research is a vital component of all forensic sciences and is often stimulated by casework, which identifies lacunae in our knowledge. In such a niche area of forensic science as entomology there should be a close and mutually beneficial relationship between research and casework: to some extent there is a continuum between the two and many forensic entomologists are involved in both to a greater or lesser degree.
However, research and casework involve quite differing challenges, from the replicated, highly controlled, sometimes esoteric aspects of research to the very individual, sometimes chaotic and disruptive, but highly applied aspects of casework. Ideally casework will include the full involvement of a forensic entomologist, who will collect the insect and climate evidence at the scene and produce a robust witness statement based on a full analysis of this data. Unfortunately, it can also include situations where samples, if collected at all, are poorly preserved, not representative of the full cadaver fauna available and presented to the entomologist months or years after the event, without local temperature data.
While research is recognised though publications and their citation indices, casework and its associated expert witness statements often receive no credit in an academic environment. How then can researchers be encouraged to undertake casework? This presentation will examine this important question from a UK perspective, considering how challenges from cuts to budgets for forensic science and investigation might impact on our ability to recruit the next generation of forensic entomology caseworkers, hopefully stimulating discussion of how we can create an environment that values the contribution of both research and casework.

Insect-microbiome interactions, importance, and applications for Forensic entomology

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Forensic entomology is the study of arthropods used during litigation. However, insects are not the only organisms found in the environment, but rather they are a host for the millions of microbes residing in or on the insect. It is becoming more recognized that microbial-host interactions are ubiquitous in nature. This scientific principle has been made possible through the improvements and increased technological capacity (e.g., targeted amplicon sequencing, whole shotgun metagenomics, transcriptomics, proteomics, etc.) to explore microbiomes. Stable, transient, commensal or pathogenic members of the insect microbiota have been shown to influence host behavior, mate selection, defense mechanisms, vector competence, and tolerance to perturbations. Yet, thorough exploration of microbiome interactions, importance, and applications for forensically relevant arthropods are still in the early stages. Here, we review the current literature related to ten groups of forensically relevant arthropods [Calliphoridae, Sarcophagidae, Muscidae, Piophilidae, Phoridae, Silphidae, Staphylinidae, Cleridae, Dermestidae, and "Others" (e.g., Siphonaptera, Acari, Blattodea, Cimicidae)] to identify the body of microbiome work associated with each taxon. We then discuss the potential for using knowledge of these insect-microbiome interactions for interpreting evidence during death investigation. Unsurprisingly, Calliphoridae had the most microbiome data published (29.5%) followed by Sarcophagidae (17.5%) and Muscidae (14%). Blow flies (Calliphoridae) are most well known for their use in forensic science applications to estimate the time since death. There is evidence of cross-domain associations of microbiota, necrophagous flies and decomposing bodies that suggests insects may be dispersing and inoculating naïve resources with microbes acquired at a decomposing resource. Knowing how often this potentially happens could have forensic applications. The resulting frameworks, protocols, models, and theories published are informative to improving knowledge of insect-microbiome interactions after death.

Necrophagous insect microbiome – qualitative and quantitative insights

Lavinia Iancu [1,@], Georgiana Necula-Petrareanu [1], Cristina Purcarea [1]

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Forensic entomology is a well-established science, based on the development stages and succession of necrophagous insects, for postmortem interval estimation. While the insect-microbe interactions are well investigated, scarce information exists on the bacterial communities associated with the necrophagous insect species, having forensic implications. Several studies focused on the horizontally and trans-generationally metagenomic assessment of bacteria from Calliphoridae species (Sigh et al. 2014), and on the investigation of the bacterial communities from different development stages of Stratiomyidae species (Zheng et al. 2013).
Considering the need of data on the bacterial communities' diversity and dynamics within the necrophagous insect species and throughout their life stages and the insect-bacterial transfer to the colonizing tissue, the current paper presents qualitative and quantitative data on bacteria associated with different necrophagous insect species. The first study presents the insect microbial community profile, assessing the diversity and dynamics of the bacterial communities via 16S rRNA Next Generation Illumina MiSeq, from adult and larval specimens of Staphylinidae, Muscidae and Phoridae collected from decomposed rat carcasses buried 40 cm deep in soil (June 2016). A following study was aimed at the investigation of insect-specific bacterial species quantitative transfer throughout *Lucilia illustris* Meigen, 1826 (Diptera: Calliphoridae) life stages and to the colonizing tissues via qPCR assays (June 2017).
Both studies provide insights into the microbiome associated with both necrophagous insect adult and larval stages and data on bacterial quantitative transfer to the colonizing tissues, showing an ascendant trend link between the bacterial insect-specific identified from larval life stages and from the colonized tissues. Moreover, Proteobacteria and Firmicutes were identified as dominant phyla, while *Ignatzshineria*, *Proteus* and *Clostridium* were identified as common genera regardless of the necrophagous insect family.

References

Singh, B. et al. Appl. Microbiol. Biotechnol. 99, 869–883 (2015).
Zheng, L. et al. J. Med. Entomol. 50, 647–658 (2013).

Microbiome and environmental conditions: the first study from Turkey

Elvan Hortac [1], Ali Açikgöz [2], Abdullah Zübeyir Ceylan [3], Erhan Büken [4], Meriem Taleb [5], Halide Nihal Açikgöz [6,@]

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Microbial fingerprint is the most important element for forensic entomology. It allows estimation of the time of death, to determine the location of lost graves, to establish the relationship between the victims and to prove strong connections between items. Every sample obtained from the scene may not be used as evidence because of degradation due to decomposition. Conversely, necrobiome products develop with decomposition.
This study was conducted between 23.05.2018 and 07.07.2018. Samples were collected on the animal model *Sus scrofa domestica* right before slaughter and were also collected after the placement of the dead pig and on the first, second, 14th and 28th day from body orifices such as the mouth, the ears, the nose, and the anus, and from the skin.
After the identification, the pre-slaughter samples revealed that the pig was healthy with presence of a normal bacterial flora and the absence of pathogenic strains. The first transmigration after death was presented by *Staphylococcus aureus*, followed by other enteric bacteria mainly *Escherichia coli*, *Staphylococcus* sp. and *Neisseria* sp. dominance declined and was replaced by *Bacillus*, *Pseudomonas* and *Micrococcus*species. As the decomposition progressed, other anaerobes started to appear. In spite of the increase of species diversity in the flora, it was observed that the number of aerobic bacteria decreased due to acid products and the anaerobic environment produced by metabolism. Although the facultative anaerobic bacteria decreased with the dominance of the anaerobes, it was found to be intense in every stage of decomposition.
In addition, the presence of *Pseudomonas aeruginosa* and pseudomonas-like bacteria observed intensively during the early stages of decomposition was explained by the presence of moisture in the body as a result of its proximity to water sources and frequent rainfall during the period of exposure of the carcass.

Eye-background contrast as a quantitative indicator for pupal age

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Age determination of immature insects sampled at a death scene is frequently used to estimate post-mortem interval (PMI). These estimates are usually based on the age of larvae, due to their abundance on cadavers, ease of sampling and availability of quantitative age indicators (e.g. length). A considerable portion of development takes place in the pupal stage. Pupae are, however, less frequently used to estimate PMI owing, among other things, to the lack of reliable and easy to use quantitative indicators for their age. Most methods developed for pupal Diptera and Coleoptera have been based on combinations of qualitative morphological traits. We have investigated quantitative changes of eye pigmentation in pupae of *Necrodes littoralis* (Linnaeus, 1758) (Coleoptera: Silphidae) and their usefulness for pupal age estimation. Color changes in the eye begin from the onset of the pupal stage, turning from white through pink and violet into black in a mature pupa. We quantified these changes as a contrast in intensity between the eye and a grey standard. Pupae (reared in the laboratory at 17, 20 and 23°C) were killed (boiling water; 5 min) at intervals of 5-10% of the stage duration and after cooling were preserved in 75% ethanol. Eyes were pictured in lateral view under standard light conditions using a light stereomicroscope (Leica M165C). Standard grey photo plates were pictured in the same conditions. Average intensity of pixels in eyes and plates was quantified using a histogram function of Adobe Photoshop. Eye-background contrast changed linearly with an almost perfect fit of the regression model. Currently we are validating the model to determine error rates for the pupal age estimation using the eye-background contrast. The method is reliable and simple and may further increase the accuracy of PMI estimates based on pupae. Moreover, the technique seems universal for all forensically useful beetles and flies.

Development of *Necrodes littoralis* L. (Coleoptera: Silphidae) at five constant temperatures – preliminary data

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Necrodes littoralis (Coleoptera: Silphidae) is forensically important Palearctic carrion beetle. It may be useful for postmortem interval estimation, as its immature stages are frequently found on human cadavers. Unfortunately, there is no forensically useful developmental model for this species. Here, we present preliminary results of an ongoing study, covering developmental data from five constant temperatures: 14°C, 17°C, 18°C, 19°C and 20°C.
Adult beetles to start and maintain the colony were collected from pig carcasses. Eggs were placed in incubators and checked for the presence of the first instar larvae. Then, 400 first instar larvae were transferred to eight containers (50L1 per container) for each of the predefined temperatures. Larvae were fed with pork *ad libitum* and had constant access to cotton wool soaked with water. Six randomly chosen larvae from each of the four 'measured' containers were measured *in-vivo* at intervals representing no more than 10% of the stage duration. Larvae from the other four containers were not measured. Insects in all containers were monitored for developmental landmarks. Postfeeding larvae were transferred into smaller containers filled with soil to allow for a pupal chamber formation. Length and weight of adult beetles were measured at emergence in parallel with determination of sex.
The time to emergence of *N. littoralis* was 89,11±3,33 days for 14°C, 63,07±2,43 for 17°C, 48,42±3,28 for 18°C, 44,44±2,44 for 19°C and 44,54±1,79 for 20°C. There were differences in the duration of development between measured and unmeasured larvae, they were however inconsistent between the temperatures. Males had a longer development than females and were smaller. Higher mortality was recorded at 14°C and 17°C. Moreover, preliminary thermal summation models and growth curves will be presented.

Morphological key features of five Calliphoridae species common in Korea: with emphases on the comparison between unique oriental and universal sister species

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In the practice of forensic entomology, species identification is very important to estimate minimum post mortem interval (minPMI). Unlike morphological identification keys for adult flies, there was no study on morphological identification keys for maggots of forensically important flies collected in Korea. In this study, we compared morphological appearances of five Calliphoridae species, i.e., *Calliphora vicina*, *Calliphora lata*, *Chrysomya megacephala*, *Chrysomya pinguis*, and *Lucilia sericata*, commonly observed in Korea. In particular, we focused on comparative features between unique oriental and their sister-universal species, i.e., *C. lata* versus *C. vicina* and *Ch. pinguis* versus *Ch. megacephala*. In addition, *L. sericata*, the dominant species in Korea, was compared with foreign conspecifics in the previously reported papers by Velasquez et al. (2010) and Szpila (2010). Maggot samples were obtained from autopsy cases in Seoul and its suburb area from 2015 to 2019. To confirm the species of the maggots, DNA barcoding for cytochrome C oxidase subunit I (COI) gene was performed. As a result, differently from *C. vicina*, *C. lata* did not have closed peritremes in their posterior spiracles, but otherwise, no difference was found. No significant difference could be observed between *Ch. megacephala* and *Ch. pinguis*. In addition, the morphology of Korean *L. sericata* maggots were consistent with that of the previously published features. To our best knowledge, this study is the first to describe the morphological features of *C. lata* and *Ch. pinguis* maggots. However, for two closely related sister species without any differential larval morphology, i.e., *Ch. pinguis* and *Ch. megacephala*, more studies are needed to find possible key clues for species identification.

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iFly: forensic entomology mobile field app

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Most entomologists construct their own "scene data sheets" to record what they consider to be necessary data for a decomposition scene. iFly is a mobile application for recording data from death scenes, with a focus on forensic entomological specimen collection and Total Body Score (TBS) estimation. iFly was created to provide a flexible digital platform for recording this same data from the scene, and for sharing data between users in a standard format. Cases can be organized around any number of single or mixed model assemblages using the default models in iFly. Each model can then be tracked through any number of sampling events. Scene data collection layouts can be formatted as a template to conform to standard operating procedures, allowing for many users to record the same data. iFly allows for the first ever routine field data exchange between carrion ecology or vertebrate decomposition researchers. Individual screens can be exported as .csv files, or the entire case can be exported for peer-to-peer transfer of cases on iFly enabled devices.

Time to move: using larval migration for minimum postmortem interval estimation in Forensic Entomology

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Several studies have already shown that biotic parameters such as food type or larval density influence the development of necrophagous larvae. Although it is known that divergent development rates may reduce or even falsify the precision of postmortem interval (PMI) estimations, it is difficult to avoid such bias. Indeed, the biotic parameters existing from the outset at a crime scene, and thus the growing conditions of the larvae, are largely unknown. Therefore, the present research has been searching for the development event on which the influence of biotic parameters has the least effect. The migration of larvae (i.e., the beginning of the postfeeding stage) and the eclosion of flies were on the shortlist, since both are precisely determinable and easy to observe. Comparing published data from studies testing different growth conditions, migration was the development event with the least variability. As an example, focusing on developmental studies with *L. sericata* at 25 °C, the possible period of oviposition extended by 72h when calculating back from the time of eclosion, but only by 48h (i.e., ± 24h) from the time of migration. Therefore we suggest using the migration time instead of the eclosion for minPMI estimations and to always include postfeeding data in future development studies.

Necrophagous insect activity modulates the thermal microhabitat of concealed carcasses

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Both necrophagous insect activity on a cadaver and body putrefaction generate heat. However, these two processes act inconsistently during the decomposition process and are likely to differ if the cadaver is concealed due to the absence of convective heat transfer from air flow. In this field study, we have examined the biotic and abiotic factors influencing the thermal microhabitat of carcasses stored within containers. The containers used in the study (i.e., suitcases, trashcans and drums) presented different levels of constraints to the colonization of necrophagous insects. The thermal microhabitat of the different containers was reconstructed during the first 100 days postmortem using a meteorological weather station, button-type thermo data loggers and generalized mixed additive modeling. Results showed that inside containers that allowed insects to access the carcass (i.e., suitcases and trash cans), average temperature was 5 to 6°C higher in early decomposition than later in the process. Although insect colonization was impossible in sealed containers (i.e., drums), their average temperature was also 1°C higher in early decomposition than later in the process, an effect we attributed to carcass cooling and putrefaction. These results indicate that the heat radiating from carcasses increases when carcasses can be accessed by insects and show that the absence of convective heat transfer from air flow can result in a substantial increase of ambient temperature under the influence of insects. While maggot mass effects have been documented in the past, we show here that insect activity generates enough heat to alter the entire thermal microhabitat of concealed carcasses, and not only the area where maggot masses are located. This study also highlights the challenges of forensic meteorology and the limitations and issues of current methods used in forensic sciences to model in situ temperatures.

Aquatic and terrestrial invertebrate colonization of vertebrate remains in a pond: an experimental test for a cold case death investigation

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Compared to terrestrial carcass decomposition knowledge there is much less understood from aquatic habitats, especially with direct relevance to a death investigation. The objective of this study was to determine if, and to what extent, aquatic and terrestrial invertebrates would colonize submerged/floating vertebrate carcasses during summer months to compare with circumstances of a human body (referred to as the Aquatic Case [AC]) recovered from a small lake after 21 days missing.
The study was conducted from 26 July – 16 August 2018 (similar time of year of the AC) in a remote pond. Five swine carcasses (each ~ 45 kg) were euthanized and immediately transported to the study location where they were dressed in clothing to represent the apparel of AC body and placed into the pond. The carcasses were evaluated for aquatic and terrestrial invertebrates at daily or every three day intervals.
There was not a significant difference in air temperatures at the experimental pond compared to those recorded during the AC 21 days missing. There was adult terrestrial blow fly presence (Diptera: Calliphoridae) on 29 July and obvious blow fly egg and larval colonization on 30 July on all carcasses. Larvae and adults of *Cochliomia macellaria* and *Phormia regina* and numerous taxa of aquatic invertebrates were collected from all three carcasses on most dates. When comparing the experimental results to the lack of any insect evidence reported in the AC autopsy and death investigation reports, the findings suggested that the AC body had a low probability of being submerged for 21 days; these findings matched autopsy photos and medical examiner notes indicating only moderate decomposition. While the experimental results suggested extensive colonization of a carcass by both terrestrial and aquatic invertebrates it is possible that the AC body had sunk and remained neutrally buoyant for 20 days.

Unusual Diptera colonization on Human corpses in Malaysia: Implications and opportunities in forensic entomological services

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In Malaysia, there are at least 24 species of forensically important Diptera that have been reported from human corpses, with *Chrysomya megacephala* being the primary coloniser followed by other species of calliphorids, sarcophagids and muscids. Forensic entomology cases examined at the Faculty of Medicine, Universiti Teknologi MARA revealed a few uncommon dipteran species colonising human corpses. We report one case of colonisation by *Eristalinus arvorum* (Diptera: Syrphidae) within a human skull, which is the first documentation of this species as forensically important. Another case reported third instars of *Piophila casei* (Diptera: Piophilidae) from a human skeleton where numerous empty pupae of *Chrysomya nigripes* (Diptera: Calliphoridae) were attached to the skull and scapula surfaces. As *C. nigripes* was not the earliest coloniser based on the known insect succession, PMI was expected to be much earlier than what we have estimated. Another indoor case revealed a co-infestation by *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and *C. megacephala*. As the indoor environment is biotically and abiotically different from the outdoors, insect succession sequences might be altered and consequently, it may be difficult to decide which candidate species should be selected in the estimation of mPMI, in addition to the uncertain duration of delay in fly arrival. Nevertheless, the unique biology and behaviours of these species could provide indications to the case pertaining to mPMI, stages of decomposition, and the ecoregion of the scene. However, gaps in knowledge and limitations from these cases should be addressed to improve future applications and forensic entomological services. Furthermore, research opportunities and innovations in forensic entomology are also highlighted in this presentation.

Species composition of forensically important blow flies (Diptera: Calliphoridae) in the Western Cape of South Africa

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Forensic entomological research was initiated at the University of Cape Town in the Western Cape of South Africa in 2012. Investigating insect succession, along with the developmental study of carrion insects, constitutes some of the most fundamental and important work of forensic entomology. Our aim is therefore to identify the local blow fly species that are active in land-based environments and to determine baseline development rates for these species, which will aid in more accurate PMI (Post Mortem Interval) determinations.

Blow flies of Chrysomya spp., Calliphora spp. and Lucilia spp. have been identified. Rare species and previously unknown species have also been identified and will be reported on in this presentation.

Current research includes the investigation of species abundance in terms of habitat or seasonal associations. Field observations of baited traps and pig carcasses provided data for occurrence and activity patterns of these necrophagous insects. This data in conjunction with case reports contributed to an up to date list of species in this region.

It is the intention of the research group to expand the collection and identification of site-specific species to other national regions as well as to neighbouring countries, and the first results from Botswana will be included in the presentation.

Cuticular Hydrocarbons from empty puparial cases for identification of species and geographical location

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Research in social insects has shown that hydrocarbons found on the cuticle are species-specific. This has also been proven in studies of flies and is a promising tool for identifying forensically important fly species in Forensic Entomology.

There has been a recent increase in research analysing the cuticular hydrocarbons of forensically important blowflies for identification as well as ageing, presenting promising results for this chemical analysis technique. This presentation will primarily concentrate on the profiles of empty puparial cases from 7 forensically important blowflies and a flesh fly species. The species examined were *Chrysomya albiceps, Lucilia caesar, Lucilia sericata, Lucilia silvarum, Protophormia terraenovae, Phormia regina, Calliphora vicina and Sarcophaga caerulescens.*

The aim was to examine their profiles for identification purposes but also investigate geographical differences by comparing profiles of the same species from different regions. The cuticular hydrocarbons were extracted with hexane and analysed using Gas Chromatography-Mass Spectrometry (GC-MS), which is a standard technique for cuticular hydrocarbon analysis.
Our results show that there were distinguishing chemical differences within the cuticular hydrocarbon profiles allowing for identification to be established. There were also differences shown in the profiles of *Calliphora vicina* which were sampled from Germany, Spain and Norway, indicating that geographical locations can be determined from this chemical analysis technique and therefore the environment does have an impact on the chemical profiles. These results show great potential because the empty puparial cases are often the only form of entomological evidence left at older crime scenes. They are fragile and often only found in fragments which makes morphological identification challenging. The results of this study also provides us with proof of concept to further develop this technique for geographical mapping of insects.

Geographical distribution of forensically important flies in Milan, Northern Italy: morphological identification and molecular comparison of different species

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A combined morphological and molecular approach is commonly applied for entomological species identification. Proper species identification and up-to-date locality information are essential in criminal investigations.
In this study, immature stages of Diptera were collected from crimes scenes and during autopsies of 40 cases investigated at the Institute of Legal Medicine, University of Milan (Italy) in 2017 and 2018. The geographical distribution of the cases involved urban and suburban habitats in and around the city of Milan.
The specimens were first morphologically identified, then total DNA was isolated and five different molecular targets, three mitochondrial (COI, ND5, CYT-B) and two nuclear (EF-1a, ITS2), were tested. Results from the two approaches were compared.
The most common species were morphologically identified as *Lucilia sericata*, *Lucilia ampullacea*, *Lucilia caesar, Calliphora vicina*, *Calliphora vomitoria*, *Chrysomya albiceps* (Calliphoridae), *Sarcophaga argyrostoma* and *Sarcophaga crassipalpis* (Sarcophagidae). Specimens within the Phoridae family were not identified at the species level.
The discriminatory power of the molecular targets can be summarized as follows: the 658bp COI barcoding fragment was the most reliable taxonomic marker for identification; good results were obtained by ND5, EF-1a and ITS2 analyses; the CYT-B showed high percentage of misidentification. These differences are likely due to the different evolutionary rate of the analyzed DNA region; however issues related to the reliability of the published DNA sequences cannot be excluded. This study corroborates previous observations on the choice of the molecular targets aimed to confirm the morphological identification and enlightens the need to improve the accuracy of the databases to strengthen the use of the combined approach for entomological analysis.
Results also showed an unchanged geographical distribution of forensically important flies over the past two decades and can be used to establish a national database of forensically important species as essential tool for entomological reports in forensic contexts.

Effect of inbreeding on development of the forensically important blow fly species, *Calliphora vicina*

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Forensic entomology is focusing on PMImin estimations by age calculation of juvenile blow flies. This is made possible by reference data obtained from laboratory populations of the species in question. Such colonies, however, can easily suffer from inbreeding due to the lack of genetic regeneration. If inbreeding were to impact development and growth in laboratory environments, this could bias reference data applied in forensic case work. We therefore analysed the effect of inbreeding on the development from first instar to imago of *Calliphora vicina* at three different temperatures; 10°C, 20°C and 30°C. The inbred colony had been established in the laboratory for years and was only refreshed by its own offspring, while the outbred colony had been through just two generations. Three repetitions were done for each temperature, with five boxes, each containing 40 first instar larvae, respectively. Five larvae (one per box) were sampled, killed and measured every 24 (10°C), 8 (20°C) or 6 (30°C) hours. After reaching post-feeding, the boxes were controlled for pupariation and fly eclosion every 24 hours. We hypothesised that inbreeding depression is greatest at the more extreme temperatures, 10°C and 30°C, resulting in slower development.
The results show a slightly faster growth of the outbred flies during the third larval stages at all three temperatures, with most distinctive differences at 10°C and 30°C. However, after the end of the feeding phase this discrepancy was adjusted by the inbred flies when reaching the post-feeding stage. At 10°C, the outbred flies required even more time for pupariation and needed approximately two days longer until eclosion.
In general, this study confirms inbreeding depression for laboratory colonies of the blow fly *C. vicina*. It highlights the importance of the regular renewal and addition of new flies to prevent this as a source of error in PMImin estimations.

Molecular identification of *Stearibia nigriceps* Meigen and *Piophila casei* Linnaeus (Diptera: Piophilidae) from Algeria with description of the first african record of *Stearibia nigriceps*

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Stearibia (=Piophila) nigriceps Meigen occurs in the Palearctic Region from Europe and Far East Russia, in the Nearctic Region, in the Neotropical Region and in the Oriental Region. Recently, only *Piophila casei* Linnaeus and *P. megastigmata* McAlpine have been reported from Algeria. Despite the fact that Piophilidae species are regularly recorded in succession surveys and forensic cases, there is a lack of information regarding this group. In this study, we report the first occurrence of *Stearibia nigriceps* from Africa collected in Algeria. In addition, our results provide DNA sequences of *P. cas*ei and *S. nigriceps* that may serve as reference data for future identification.

Specimens were collected on rabbit carcasses during spring in a semi-urban habitat, 188 m above sea level. After morphological identification and confirmation of the species identification, DNA was extracted from the whole individual. A region of the cytochrome c oxidase I gene marker of 473 bp was amplified and DNA was sequenced. The obtained sequences were deposited in GenBank databases.

All the specimens were correctly identified using the nBLAST tool from the GenBank database. The sequences obtained were aligned corresponding to positions 1589–2983 of the reference sequence of *Drosophila yakuba* Burla (GenBank accession number NC_001322). The phylogenetic tree included not only the Algerian specimens but also sequences from specimens worldwide acquired from GenBank. *P. casei* and *P. nigriceps* individuals from Algeria were found to be separated from the specimens of *P. casei* and *P. nigriceps* originating from the other countries with a 100% high bootstrap value. Haplotypes of *P. nigriceps* acquired from GenBank showed no geographic partitioning. The result was a mixed pattern of specimens originating from Europe and USA.

Our results contribute to update the knowledge of the distribution of the Piophilidae family. They also contribute to build up online DNA sequence databases.

Accelerated flies? The effects of petrol and lighter fluid on piglet decomposition and blowfly succession & development

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Although 693 fire related fatalities were recorded in Great Britain between 2015 and 2017 and ignitable liquids are commonly used in criminal cases, there is still limited research and conflicting data on the effects of accelerants and burning on cadaver decomposition and associated entomological evidence. This research explored the effects of petrol and lighter fluid on blowfly larval development, insect succession and cadaver decomposition.

Calliphora vicina larvae were reared under controlled conditions, on pig's liver soaked in lighter fluid and their lengths were measured over the course of their development. In addition, six repeated control samples fed on unadulterated liver enabled production of comparative growth charts to document size differences. This experiment concluded that the presence of lighter fluid on the liver produced smaller larvae and prevented the reduction in larval size when nearing pupariation.

Between 1st and 12th June 2018, three piglet carcasses underwent decomposition; one was soaked with 100ml of lighter fluid, a second was soaked in 100ml of petrol and a third was left without the influence of a chemical. Observations were recorded, and arthropods were collected frequently over the course of this experiment. Although neither accelerant influenced the pattern of insect succession, the presence of lighter fluid attracted a larger quantity of insect activity. Although it was unclear if initial insect oviposition was affected, it was found that both lighter fluid and petrol did delay the decomposition process.

This research-derived data provides a valuable preliminary dataset and indicates the need for exploring variables in isolation to fully untangle complex environmental and anthropogenic effects, such as fire, on taphonomic and entomological processes.

Insect breeding in Forensic Laboratories - A Survey

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Developmental studies on forensically important insects are crucial for any minPMI estimation and thus form, as reference data, an important basis for casework in forensic entomology. As a result, rearing of forensically important Diptera and Coleoptera in controlled environments is a routine operation for many forensic laboratories around the world. To get an overview of the institutes that rear insects, the various techniques to do so and the different insect species bred, we designed an online survey on the insect breeding in forensic laboratories. The survey was shared via the European Association of Forensic Entomology (EAFE) and accessible via a Website. Overall, 30 institutes from 17 countries participated, 27 of which breeding insects. In addition to museums, Institutes for Forensic Science and Criminal Investigation departments, the majority of the laboratories are based at Universities. 16 species of blow flies (Diptera: Calliphoridae), 6 species of houseflies (Diptera: Muscidae), 6 species of flesh flies (Diptera: Sarcophagidae), 2 species of latrine flies (Diptera: Fanniidae) and 7 Coleoptera species are breed permanently or occasionally in the institutes. The blow fly *Calliphora vicina* is the species most frequently bred (in 12 institutes). Most populations of species originate from field samplings but also from human bodies or commercial suppliers. The methods applied, like e.g. food supply for the adults, larval diet, pupation substrate, or number of specimens in a cage varied enormous between the different laboratories and will be outlined. We see a need of guidelines and standards for breeding forensically important insect species especially when using them for developmental studies. The practicability and necessity of a "universal breeding protocol" is discussed.

Metabolites as internal age markers in *Calliphora vicina* and *Lucilia sericata*

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Determining the age of entomological evidence collected at the crime scene is crucial in all post-mortem interval estimations and several methods already exist to calculate the age of larvae collected at the crime scene. These include larval length, larval instar and ADH calculations as well as more recently developed methods such as using cuticular hydrocarbons and DNA techniques. In this preliminary study the focus is on internal small molecules (metabolites) and how these can be used as age markers in two blowfly species of forensic importance.

Larval extracts from *C. vicina* and *L. sericata* were obtained by daily extractions (n = 10) using an aqueous solvent (methanol/water, 1:1). 500 µL was added for samples < 50 mg and 1 mL solvent for samples > 50 mg. The larvae (varying from 40 to 2 larvae per extract) were cut into small pieces with scissors and then homogenised using glass beads. Samples were sonicated for 15 minutes and centrifuged for 15 minutes. The supernatant was removed and retained as the aqueous extract. A quality control (QC) sample was made by combining 40µL from each of the larval extracts. These extracts, including the QC's, were analysed in a random sequence on an Agilent LC-Q-TOF-MS with ESI on a C18 column, at a flowrate of 0.3 ml/min using gradient elution.

Analysis of the data was performed using XCMS on-line to extract relevant features ('accurate mass' and 'retention time' combinations) from each sample followed by multivariate analysis on the obtained dataset. Results from this preliminary study indicate that using the metabolites in the aqueous extracts, larvae from different days can be distinguished in the PCA plot. Several biomarkers were highlighted as either increasing or decreasing with larval age. In the presentation the use of this novel method will be discussed, alongside together with the challenges of using this method.

The use of wing fray and sex ratios to determine the origin of flies at an indoor crime scene

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When adult flies are collected at indoor crime scenes, the question of their origin arises, i.e., whether they originated from the outdoors, coming in to oviposit on the body, or if they developed through the larval and pupal stages from eggs laid on the body. This is particularly important if no empty puparia were collected, potentially because they were overlooked, with a consequent impact on the accuracy of the minimum post-mortem interval estimation. This study used two methods to determine if flies sampled in various experimental conditions and at an actual crime scene were wild flies attracted to the body or had developed on the body, i.e., through the assessment of: (1) wing fray and; (2) sex ratio. A template was created to measure the amount of wing fray damage quantitatively in a simple, rapid and efficient manner. The experiments demonstrated that by combining the two methods it is possible to establish the origin of a population of adult flies at a crime scene.

Impact of diet moisture on the larval development of the Forensically important blow fly *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae)

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Cochliomyia macellaria (Fabricius) (Diptera: Calliphoridae) is a carrion-breeding species of veterinary, medical, and forensic importance. As it is very abundant in the Nearctic and Neotropical regions and is one of the most common colonizers of vertebrate remains in the southern United States, it is of great evidential value in estimating the minimum time of colonization (TOC) of remains related to forensic investigations. So far, studies have investigated the effects of several biotic and abiotic factors on *C. macellaria* (e.g., temperature, tissue type, and photoperiod). However, no data on the specific impact of food source moisture on the larval development of this species are known to have been published.
In this study, the effects of diet moisture on larval development time, larval length, and weight over time, as well as adult emergence and weight were investigated. Eggs (< 1 h old) were placed on substrates prepared from freeze-dried bovine liver, with moisture content being either 0, 33, 50, or 71 % at 25.6 °C, 77 % RH, and 14L:10D. Frozen-thawed liver was used as a control. No larvae hatched from eggs placed on substrates with 0 % moisture. Larvae were able to develop on 33 % moisture for up to 201 h, though none reached pupal stage. Larvae fed diets at 71 % moisture developed faster, grew larger, and gained more weight than those reared on diets at 50 % moisture. Adult emergence and weight were similar across treatments, indicating immature traits were affected the most.
Due to the impact of diet moisture on the development of *C. macellaria*, the water content found in colonized tissues of vertebrate remains should be considered when estimating the TOC based on this species. This recommendation applies especially to regions where (e.g., due to weather conditions) (partial) mummification of remains is known to occur.

The use of Diptera in the determination of the time since death in Bulgaria

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The use of flies in forensic entomology in postmortem interval estimations is hindered by lack of information of local species in Bulgaria. Basic distribution data for the most likely indicator species of insects are required. It is apparent that the seasonality and species assemblage vary in different geographical areas. For accurate PMI estimations using flies, the most important information is the species identity of the immature flies found upon a corpse and their temperature-biased developmental times, specific for the local population.
Seasonal carrion decomposition studies were conducted in the suburban area of Gabrovo, Bulgaria. In 2007 the first field experiments started, using piglet corpses for successional studies on insect colonisation. Domestic pig carcasses (N=4) were placed in the study site in spring, summer, autumn-winter and a second summer experiment (2008). Carcasses were monitored during the decay process to document the temporal variation in decay patterns and the composition of the associated carrion fly communities, together with the temperatures during their development from immature stages to adult flies. The insects were collected and identified. Forensically important species of blow flies (*Calliphora vicina, Calliphora vomitoria, Lucilia sericata, Lucilia caesar* and *Chrysomya albiceps*) were determined for the geographical region and their temperature-dependent times of development were calculated. A summary of their specific ecological data and seasonal patterns for the region was made from the experiments and real medico-legal cases. Field protocols for the collection and processing of entomological evidence from the crimescene were made, according to the EAFE standards.
This work was the first replicated decomposition study in Bulgaria and contributes to a development of a national database in forensic entomology. The first cases involving the use of insects were solved and the corresponding experimental data results gives hope to the future of the discipline and its statute at national level.

Muscidae (Diptera) Associated to a Pig Carcass (*Sus scrofa*) in Leioa (Bizkaia, Spain)

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This study reports adults of muscid flies (Diptera, Muscidae) associated with a domestic pig carcass (*Sus scrofa domestica*) placed in an oak forest in Leioa (Spain), in the Bizkaia campus of the University of the Basque Country. Samples were collected daily during the summer over 60 days. A total of 11 genera and 16 species of this family have been identified. Adult activity and diversity observed suggest the need to carry out studies of distribution, development and phenology of this family, as it can provide useful information to police research, i.e. post-mortem interval estimation or scene manipulation. Four species are recorded for the first time in the autonomous community of the Basque Country (*Neomyia cornicina, Polietes domitor, P. meridionalis* and *Pyrellia vivida*) and nine more species are recorded for the first time in Bizkaia (*Eudasyphora cyanella, Graphomya maculata, Musca domestica, Muscina levida, M. prolapsa, Myospila meditabunda, Phaonia subventa, P. tuguriorum* and *P. valida*).

Poster presentations

Study of the decomposition of boar corpses during two seasons, winter and spring in the Zeralda Game Reserve (Algeria)

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This study aims to identify necrophagous insects from northern Algeria in two seasons : winter and spring. The work was done in Algeria at the hunting reserve of Zéralda. Two trapping techniques have been used from December 2017 until May 2018 on four corpses of boars. During the bioecological study, necrophagous insects were captured, and the results showed that the insect fauna of cadavers evolved in successive waves. Three orders of insects dominated during the experiment. These were the Diptera, Coleoptera and Hymenoptera. The inventory made using the pot trap included 4348 individuals belonging to 6 classes, 22 orders, 78 families and 158 species. The class of Insecta was represented by 3635 individuals. The Diptera dominated with 2445 individuals and then the Coleoptera with 998 individuals. Hymenoptera were registered with only 172 individuals. The other orders were poorly represented. The inventory made using colored plates revealed 17484 individuals belonging to 5 classes, 15 orders, 110 families and 251 species. The class of Insecta was represented by 16327 individuals that included 8 orders of Diptera and 12571 individuals for a set of 90 species (A.R.% = 71.90%). The Coleoptera occupied the second place with 2336 individuals for a total of 86 species (AR% = 13.36%), followed by 27 species of Hymenoptera represented by 1235 individuals (AR% = 7.06 %). The observed necrophagous Diptera belong to several families, the most important being the Calliphorida: *Calliphora vicina* and *Chrysomya albiceps*. In Coleoptera, the important families were Staphylinidae, Nitidulidae, Dermestidae, and Histeridae. In Hymenoptera, two families were the most abundant, the Vespidae and Braconidae.

The succession of buried pig (*Sus scrofa domesticus* Linnaeus, 1758) carcass in Turkey

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One of the most commonly used methods to hide a victim's body is to bury it. However, it is usually overlooked that insects can also reach buried bodies. In homicide and poaching cases buried bodies can be investigated with forensic entomological techniques. Indeed, besides the medicolegal methods, most of the valuable information to determine the post-mortem interval is obtained by forensic entomology studies.

Our literature survey regarding observations on predators and carrion insects visiting buried corpses.revealed that internationally there were a few studies published, but none in Turkey. The aim of our study was to determine the succession of insects on a buried carcass.

A carcass of *Sus scrofa domesticus* was buried under 1.5 m of soil in an open field in Ankara, Turkey (994 m, 39°53''19.7268''N and 32° 39'5.5944'' E) between 30 June and 01 August 2018 and observed using night vision cameras. The ambient temperature was 16.5 ºC ± 2.4ºC and the temperature at the meteorological station was19.5ºC ± 2.9ºC. Mature pupae and adult insects were collected. Third instar larvae were recovered and reared at ambient temperatures in the field, using chicken liver. Morphological identification of the adult insects was conducted in the laboratory. In total, 1402 insects were collected. The following species were identified: *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) 11%, *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) 10%, *Chyrosomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) 6%, *Lucilia silvarum* (Meigen, 1826) (Diptera: Calliphoridae) 4%, *Saprinus sp.* (Motschulsky, 1849) (Coleoptera: Histeridae) 29%, *Creophilus maxillosus* (Linnaeus, 1758) (Coleoptera: Staphylinidae) 21%, *Necrobia rufipes* (Fabricius, 1781) (Coleoptera: Cleridae) 11%, *Dermestes frischii* (Kugelann,1792) (Coleoptera: Dermestidae) 8%, *Saprinus sp.*was the numerically dominant taxon in our study.This manuscript presents this work as the first such study conducted in Turkey.

Lucilia sericata vs *Chrysomya megacephala* (Diptera, Calliphoridae): first comparison of the preimaginal development of two synanthropic flies in SW Europe

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Lucilia sericata (Meigen, 1826) is one of the most common forensic indicators in SW Europe. The species *Chrysomya megacephala* (Fabricius, 1794) plays a similar role in their original area of distribution, but its potential in the Mediterranean region should not be ignored. Knowledge of the potential interrelationships of both species is required as an important tool for application in forensic investigations. Under laboratory conditions (25 ± 2.49°C, 48.29 ± 9.95% humidity and 12 h daylength), two different breeding media were used, ie.: liver and minced pork, for rearing both species. The instar, the length of the larva and the weight of the puparia were measured daily. The data obtained were used to construct growth curves of larvae and pupae. These results were compared with previous data of both species at constant temperatures (18, 23 and 28°C; RH 60%, 14 h daylength). Larval and pupal periods, Accumulated Day Degrees (ADD) and isomorphic diagrams were also constructed for both species. Results showed differences between the development times in the two media. For *Ch. megacephala*, the larval period was shorter in minced meat, and reduced by one-day with respect to liver. In the case of *L. sericata,* larval periods were similar in both substrates, but larvae reared in minced pork were lighter. Regarding the grow curves, the results obtained in laboratory conditions followed similar trends to those obtained in constant temperatures. The total time required to reach the adult stage decreased with increased temperature. The weight of puparia decreased as time passes, until its stabilization prior to the emergence of the adult, in both species, and the time to pupariation varied in both larval media. The implications of these results for forensic entomology are discussed in this study.

Instability in growth data and accumulated degree hours for *Chrysomya megacephala* (Fabricius, 1794) (Diptera, Calliphoridae) reared on a diet of liver

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With the exception of second instar and adult tibia length and weight, all stages of growth were significantly greater (larger, wider and heavier $p < 0.01$) when *Chrysomya megacephala* (Fabricius, 1794) was reared on heart, compared with when it was reared on liver. Little significant difference was observed when reared on fresh or aged heart, except that second instar larvae were significantly longer and wider on 3 day aged heart compared to fresh heart.
Conversely, mixed results were achieved from *Chrysomya megacephala* reared on fresh or aged liver. Second instar larvae grew longest and widest on 3 day aged liver and longer but narrower on 5 day aged liver compared to fresh liver. Third instar larvae grew longest on 5 day aged liver and shortest on 3 day aged liver longer; while being widest on fresh liver and narrowest on 3 day aged liver. Pupae were longest when fed 5 day aged liver. There was no significant difference in larval or pupal weight as liver aged.
The chi-square analysis of ADH for *Chrysomya megacephala* laboratory reared at 20.4°C indicated that the association between tissue and age of tissue is real ($p<0.01$) and hence the difference observed between the relative ADH values are real.
The main impact of the results demonstrate that the type of tissue diet (in this case heart or liver) and the age of that tissue (in terms of progression of decomposition) have an effect on the measurements of specimens retrieved and the calculated number of days (or ADH) since death. We observed a time difference of only half a day when larvae were reared on heart tissue, but of one day and eight hours using liver as diet. The effect on growth comparisons and crime scene analysis on *alibi* confirmation could be significant.

The potential effect of type and age of food on developmental growth and the estimation of minimum *post mortem* interval in *Lucilia cuprina* (Wiedemann, 1830) (Diptera, Calliphoridae)

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Growth rates of blowfly larvae encountered at crime scenes are frequently assessed against laboratory cultured growth trials, commonly using homogenised liver as a dietary base. We observed variability of growth in different batches of reared *Lucilia cuprina* (Wiedemann, 1830) (Diptera, Calliphoridae) under laboratory conditions at 20.4°C using liver. We subsequently set out to trial age-effects on the presumption that decomposing liver was inconsistent as a growth medium using fresh and aged bovine heart and liver.

With the exception of second instar and adult weight, all stages of growth were significantly greater (larger, wider and heavier $p < 0.01$) when *Lucilia cuprina* was reared on heart, compared with when reared on liver. Results indicated that *Lucilia cuprina* reared on fresh or aged liver were stunted as the liver aged, perhaps indicating diminishing nutrient return or toxic effects of decomposing protein on growth. Chi-square analysis of accumulated degree hour (ADH) indicated that the association between tissue type and age is real ($p<0.01$) and hence the difference observed between the relative ADH values are real: increasing ADH as decomposition of heart progressed and decreasing ADH as liver aged.

This has potential implications when laboratory-generated growth models using different food sources are used to calculate post mortem intervals (PMI). The main impact of the results demonstrate that the type of tissue encountered by larvae as they grow, and the age of that tissue (in terms of progression of decomposition) have an effect on the measurements of specimens retrieved and the estimated post mortem interval. We observed a time difference of one day and seven hours when larvae grew on heart tissue and one day and fourteen hours when larvae grew on liver tissue. The effect during crime scene analysis on *alibi* confirmation could be significant.

Repulsive effect of decomposed beef liver on blowfly larvae

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Blowflies are among the first insect colonizers of carrion: when larvae start feeding, the bacteria-driven decomposition process is just beginning and many tissues are still fresh. However, bacteria abundance can vary according to tissue location, with higher decomposition rate where bacteria are more abundant (e.g. guts). Larvae could then be exposed to tissues of different decomposition levels during development, leading them to choose the one to feed on. This choice can be of critical importance as decomposed food is known to impact larval development. Moreover, while feeding, larvae aggregate into groups composed of thousands of individuals which likely influence larval choices. The goal of this study was to investigate the behavioral response of *Lucilia sericata* larvae to variations in food decomposition level. In a binary choice test with 2 spots of minced beef liver decomposed from 0 (fresh spot) to 7 days (rotten spot), the distribution of 40 larvae was photographed every 2 minutes for 48 hours and analyzed with ImageJ software. The experiment was repeated by tripling the number of larvae and the spot area. During the first 20 hours, the 40 larvae groups chose the fresh spot, showing a repulsive effect of decomposed food, a result consistent with the negative effect of this food on larval development. However, this choice was not observed when the spot area was increased, but occured again with groups of 120 larvae. These findings suggest that the repulsive effect of decomposition is weak at the individual level but amplified by aggregation behavior, becoming significant when group size is large enough to offset individual variation. From a forensic point of view, this study highlights the importance in considering larvae behavior when estimating minimum post-mortem intervals.

The effectiveness of hand-held FLIR cameras in the search for the buried bodies of missing persons

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The rise in missing persons cases is a current cause of concern for many police forces across the world. Despite this there is currently a lack of research surrounding effective methods of search in locating these missing persons, particularly when the missing persons are believed to be deceased. This study expands on previous work conducted by Demarais (2014) and Amendt et al. (2017) which looked at the use of FLIR cameras to detect bodies of missing persons.

The experiment used four piglet cadavers; three were buried in soil at different depths within plastic containers and one was placed on the surface of the soil in another container. A container full of soil only was used as a control. The containers were then placed in a greenhouse to prevent scavenging and photographed using a E60BX FLIR camera for a period of a month during summer 2018.

The findings from the experiment showed a that a distinct heat signature was detected from the cadaver on the surface due to heat produced by larval mass aggregations, however it was inconclusive as to whether the FLIR camera could specifically identify the buried cadavers due to the heat from the greenhouse greatly raising soil and container temperatures. Despite this, the soil disturbance caused by the burial and decomposition of the cadavers was detectable using the FLIR camera.

The results here show promise for use in low-level, targeted missing persons searches, particularly when access to aerial vehicles is not possible, however larger-scale feasibility studies in a variety of outdoor scenarios are required for suitability as a go-to tool.

Rearing of *Musca domestica* (L.) on different larval substrate: effect on development

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Different food can influence rate of larvae growth, and consequently a PMI estimate. For this reason, studies were conducted to evaluate the larval development on different substrates (tissue of mammals, fish or artificial diets.)
Musca domestica (L.) is a common fly that can be found associated in forensic case, and used to determine the minimum PMI. In our study, three different diets were tested to investigate their suitability and adequacy for larval growth. *Musca domestica* was reared under controlled condition RH = 70 ±5 %, photoperiod L:D = 16:8, and temperature = 25°C. Samples of 200 eggs were put in a plastic box (10 x 6 cm) onto 100 g of substrate: Diet 1 (D1) was a Gainesville diet (Alfalfa meal 20%, Wheat bran 50%, Corn meal 30%), Diet 2 (D2) was a modified Gainesville diet (Alfalfa meal 50%, Wheat bran 35%, Dry food 15%), and Diet 3 (D3) was pork meat. Development time, larval length and pupal weight were measured every 24h (30 insects per day, 710 larvae measured, and 310 pupae weighted in total).
The three diets were evaluated using a Growth Index and Index of Adequacy, which incorporates larval growth, development times, and adult emergence. Growth curves of larvae among diets were compared according to Radhakrishna (1958). The pupal weight among diets were analyzed using one-way ANOVA. $P<0.05$ was chosen for the significance of tests.
The larvae developed significantly faster ($p<0.05$) on D3 with a larval development period of only 6 days (D1 and D2 reach pupal stage in 11 days); total development time from egg to adult was 18 days for D1 and D2, and 13 days for D3. Pupal weight did not differ between D1 and D2, while pupae on D3 were significantly heavier ($p<0.01$).
The Growth Index and Index of Adequacy was for D3 7.5 and 0.28, respectively; for D1 3.3 and 0.06, respectively; and for D2 3.9 and 0.08, respectively. The data confirm that D3 was much more adapt to insect growth in confined environment.
The use of different larval substrates results in high variability in development of the insects, which impacts the subsequent interpretation of data during the investigation. Hopefully, this research will inspire similar studies to produce best practice for larval rearing of Diptera involved in forensic cases.

Reference

Radhakrishna R.C., 1958. Some statistical methods for comparison of growth curves. – Biometrics 14: 1-17.

The insect fauna found on a corpse concealed in a soft shell suitcase and estimation of the time of death

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Criminals have been known to dispose of bodies inside zipped suitcases in an attempt to conceal murder. In such circumstances the species of insect and the succession patterns could be different from exposed carcasses due to colonization being hindered or delayed. Here we report a case in which a un-named female corpse was found in a soft shell suitcase in an area of bushes in Guangdong, China, on 13 May, 2018. The suitcase was unbroken and sealed well by zippers. The victim was a young woman, naked and curled up in the suitcase. The insect evidence we collected included (1) puparia and empty puparium of *Chrysomya nigripes* (Aubertin) and *Fannia canicularis* (Linnaeus), (2) larvae, puparia and empty puparium of *Megaselia scalaris* (Loew), (3) larvae and puparia of *Hermetia illucens* (Linnaeus). According to these insect evidences, we estimated the minPMI was 55 days. Bhadra et al.'s research (2014) showed that larvae of *Calliphora vomitoria* (Linnaeus) and *C. vicina* (Robineau-Desvoidy) could colonize carcasses concealed in suitcases sealed by zip fasteners. Although, the size of the zipper (7mm) in this case was larger than Bhadra's (4mm), we did not find flies such as *Chrysomya megacephala* (Fabricius), *C. rufifacies* (Macquart) or *Lucilia sericata* (Meigen) which usually colonize fresh carcasses. So, we deduced that the victim was killed in winter. If the death was in late winter, close to the spring, the body would still be fresh after a short cold snap of winter and be able to attract those flies that usually colonize fresh stage carcasses. Hence, we estimated that the victim died at the beginning of winter, that is early in January of that year. After the crime was solved, the murderer confessed that he killed the victim on 5 January, which is consistent with our estimation.

Chrysomya albiceps predation on *Lucilia sericata* larvae under experimental conditions and real cases: implications for Forensic Entomology

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Larval development of *Chrysomya albiceps* (Wiedemann, 1819) and *Lucilia sericata* (Meigen, 1826) is a valuable tool to estimate *post-mortem* interval and also provide indications of the death scene location. *Chrysomya albiceps* is a facultative predator on the other dipteran larvae during the third larval instar, and its negative influence on the abundance of other blowflies is an important factor to consider. Frequently, both species are found during autopsies in SE Spain, so precise knowledge of larval coexistence data is essential for accuracy in the evaluation of the *post-mortem* interval. The aim of this study was to investigate the effect of the coexistence between *Ch. albiceps* and *L. sericata* in laboratory conditions and real cases. To study the coexistence under laboratory conditions, colonies of both species were established at University of Alicante (SE, Spain). To analyse the intra- and interspecific competition, four larval densities were used (50, 100, 150 and 300 specimens) under controlled abiotic conditions. Larvae in first instar were introduced in plastic pots with the same amount of pig liver (15 g). To compare these data with real coexistence in natural conditions, annual activity of both species (captured on baited traps) were also analysed. Finally, several forensic cases were studying from autopsies at the Institute of Legal Medicine of Alicante. Results indicate that mortality of immatures increased for both species, according the density levels; moreover, predation of *Ch. albiceps* on *L. sericata* was evident. Preimaginal developmental time and adult wing size were analysed and compared on intraspecific and interspecific treatments; both variables were affected by competition in all cases. In the field, annual activity and sex ratio of adults were analysed, confirmed their coexistence during spring-summer. The coexistence of both species colonising human corpses is frequent in the studied area.

Comparative wing geometric morphometrics as a tool for identification of Calliphoridae (Diptera) of forensic importance in the Iberian Peninsula

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The Calliphoridae, as members of the sarcosaprophagous insect community, play an important role as forensic estimators of *post-mortem* interval (PMI). However, in some cases, the insects collected in forensic cases comprise shattered and fragmented specimens, thus identification could be complicated if a taxonomic specialist it is not available. A tool that allows species to be identified easily and efficiently is essential. In the Iberian Peninsula the blowflies with a higher medico-legal importance are: *Calliphora vicina, Calliphora vomitoria, Chrysomya albiceps, Chrysomya megacephala, Lucilia caesar* and *Lucilia sericata*. Morphometrics analysis of the wings (shape and size) of these species is proposed as a tool to improve the accuracy of taxonomical identification, especially of fragmented specimens. A total of 600 individuals were used, 50 of each gender of the six species. To identify these species, seventeen *landmarks* were selected per wing, and centroid size and shape by geometric morphometrics, and size by classical morphometrics were obtained and compared. Results showed differences in the shape of all species, as well as between sexes within each species. Wing size by traditional (morphology of veins) and geometric morphometrics, showed significant differences in species, except between *C. vicina-L.caesar-Ch.megacephala*. Sexual dimorphism of *Lucilia* and *Calliphora* species was detected when size with both methods was used, with females being larger than males. However, there were no such between-sex differences for both *Chrysomya* species. These results indicate that geometric morphometrics, especially shape, is an easy-to-use tool to discriminate common blowflies of forensic importance.

A dead newborn baby: entomological traces as surprising evidence at the court

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In this criminal case, forensic entomology was used to calculate the time of a murder of a newborn baby, where the body was found in two plastic bags by a tractor driver in a field during spring field preparation. The expert's report provided the calculation and establishment that the first oviposition was 20 to 32 days before the finding. The entomological traces also showed that the newborn was originally enclosed in the plastic bags and/or buried in a shallow grave. Finally, the entomological traces pointed to the fact that at the latest 8 days before the finding, the original placement of the newborn was changed, which meant that the plastic bags with the newborn were pulled out from the shallow grave and/or the plastic bags were torn (both probably by an agricultural machine). All these facts were important evidence at the court that allowed for the accurate documentation of the situation between the disposal of the newborn's body in the field and finding the corpse. At the end of the expert's testimony the court used the entomological conclusions in a surprisingly different way than that expected by forensic experts. The mother claimed she had put the still living newborn baby on the field in open bags, but the entomological traces showed that the plastic bags were bound from the beginning, so the baby had no chance of surviving, even if he was alive. The court decided that entomological evidence confirmed the mother's will to murder her child and used the finding as an aggravating circumstance in determining the sentence. Finally, the mother was sent to 15 years in prison.

Dermestidae and Cleridae in forensic cases in the Czech Republic

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Since 2010, there have been records of families of Dermestidea in 21 forensic cases and Cleridae in 26 forensic cases. Although Dermestidae is represented by synanthropic species and are often known as pests in homes, warehouses, collections, etc., they can also be found on corpses in makeshift shelters of homeless people or on dead bodies in outdoor environments. On the other hand, species of the family Cleridae are common on outdoor corpses, but can be also be found on indoor bodies. In 9 cases both families were reported. The first representatives of the families Cleridae and Dermestidae commonly appear in the first days to weeks of colonization of a corpse. As the post mortem interval increases their importance and number of individuals also increases. Nevertheless, their representation on the corpse is slightly different. Dermestidae can represent the dominant group of insects on the corpse after one to three months, being found as larvae, pupae, and adults all over the whole corpse ; however, Cleridae are found with accompanying groups of other insects because they are unable to dominate the whole corpse due to the interspecies competition. *Dermestes frischii* was found to be the most common representative on human corpses in the Czech Republic, but in recent years, the increased presence of *Dermestes haemorrhoidalis* has been reported in Prague, the capital of the Czech Republic. Other representatives of the family Dermestidae that can have been recorded on human corpses or animal carcasses are *Dermestes lardarius*, *D. undulatus*, *D. murinus*, *D. laniarius*, and *Attagenus smirnovi*. From the family Cleridae, all three species of the Czech Republic can be found on corpses : *Necrobia rufipes*, *N. violacea*, and *N. ruficollis*. *Necrobia rufipes* is the most common and *N. ruficollis* is the rarest and has only been detected on human corpses in two cases.

Pediculosis as a crucial proof of the time of cruelty to an infirm mother

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In the case of neglect and abuse of an infirm mother, knowledge of lice was used to determine the degree of fault. This criminal case began with the death of the elderly woman from natural causes in November 2010. Her son called a rescue service which found the dead woman in appalling sanitary conditions. The medical staff decided to call the Police of the Czech Republic for suspicion of neglect and cruelty of the woman. On the basis of a medico judicial report, it was decided that the woman had suffered in unsanitary conditions for 3 to 4 weeks. The son was subsequently sentenced to three years in prison and his common-law wife was sentenced to two years in prison. However, the prosecutor claimed that the woman suffered a much longer period. Consequently, the Court of Appeal returned the case for reconsideration. It was in February 2013 that the Institute of Criminalistics in Prague was asked to process an expert opinion in forensic entomology. Since the dead woman was already cremated, only photographs from the crime scene, photo documentation from her autopsy, autopsy protocols, and a lock of her hair were available. Based on the presented evidence, it was possible to establish that the victim suffered from pediculosis at least 4 to 5 months before her death. During the first 1 to 2 months the condition was likely a normal pediculosis, where the lice on her head may not have been noticed; however, at least 3 months before her death the lice were so intense that they covered her whole body. On the basis of this entomological evidence the court increased the initial sentences of the convicts: the son was sentenced to 6 years in prison and his common-law wife to 5 years.

Use of geometric morphometrics for the identification of Piophilidae (Diptera) species of forensic importance

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The Piophilidae (Diptera) are a relatively small family comprising around 80 species of mainly sarcosaprophagous habits. Piophilids can represent major pests for the food industry, but can also be used as forensic indicators in forensic investigations. In forensic investigations, a reliable identification of the collected material is an essential prerequisite. However, the identification of piophilids can sometimes be difficult, e.g. due to diagnostic morphological characters that are not always easily observable. Molecular identification may also be sometimes problematic, for example, if the sample is not properly preserved. In this study, we used geometric morphometrics as a method for the identification of 12 piophilid species of forensic relevance. Fourteen landmarks of each right wing were used to explore differences not only between species, but also between sexes and different geographic populations. In addition, we explored landmark-based differences between morphs in the dimorphic species *Prochyliza nigrimana* (Meigen, 1826). The results gave percentages of correct identification of 83.3–100%, including the unequivocal discrimination of individuals of the species *Piophila casei* (L.) and *Piophila megastigmata* McAlpine, which have frequently been misidentified. The results at the level of sex and population origin gave different percentages of success depending on the species, ranging from 36.84–80% and 14.29–75%, respectively. Discrimination of *P. nigrimana* morphs was relatively successful, with a percentage of cases correctly identified of 80%. We suggest the utility of geometric morphological analysis of wing landmarks as an additional method for the identification of Piophilidae species.

Gravidity prevalence in a wild population of *Calliphora vicina* and the frequency of precocious eggs

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The prevalence of gravid females in a given area is one major factor influencing the oviposition probability by blow flies and therefore the start of their development on a dead body. Ageing the juvenile and developing specimens resulting from oviposition leads to the estimation of a PMImin (minimum post-mortem interval), and it is often stated that the "oldest specimens" should be used for this estimation. A factor that is mentioned in this context is the presence of precocious egg in the genital tract of some species (e.g., *Calliphora vicina, C. terraenovae, C. vomitoria*) of Calliphoridae. Despite the fact that several authors mention precocious egg development as potential error source for PMImin estimates, and some even take it into account in their development studies, just two studies were performed to examine the occurrence and relevance of precocious eggs both in laboratory and field populations of blow flies. We surveyed the occurrence and seasonal distribution of gravid females with precocious eggs in a wild population of *C. vicina* in Frankfurt am Main in 2017. During 152 sampling occasions, 5.126 female specimens of *C. vicina* were sampled of which 44 % were gravid; 54 % of all gravid females had a precocious egg/1st instar larvae in their genital tract. This number varied depending on the season and showed the highest proportion in Spring with almost 64% of all gravid females. Our results show that the phenomena of precocious eggs in wild populations of blow flies can occur more frequently than mentioned in forensic literature (8,82 % to 62,0 %). The answer to the question whether this is a problem in forensic entomology is directly linked to the way in which a sample (e.g., 200-300 maggot mass) is considered in an entomological report. Is only the largest maggot of relevance?

Protocol for rearing of *Necrodes littoralis* L. (Coleoptera: Silphidae)

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Necrodes littoralis (Linnaeus, 1758) is recognized as one of the forensically most important beetle species among Palaearctic carrion entomofauna. Intensive studies are currently underway to explore its development. Accordingly, protocols for its rearing are needed to enable efficient use of specimens from casework and to make results of basic studies more reproducible. We present protocol currently used in our laboratory.
Adult insects are kept in plastic containers on damp and peaty flower growing substrate. Insects are fed with pieces of pork and are supplied with wet cotton. Each colony is covered with aluminum foil to prevent desiccation of meat and soil substrate. Colonies are kept under the fume hood at room temperature (20-23°C) and humidity (30-50%). To induce oviposition fresh soil substrate and fresh meat are necessary. After 3-5 days females start to oviposit. Eggs are laid in batches (30-40 eggs) into the soil, near container walls. Larvae are fed with pieces of pork. We suggest to keep larvae in groups, where their feeding and development is much more effective. If possible use groups of 30-50 larvae, however groups of 10 larvae were also efficiently reared. If individual larvae need to be reared (for casework), we suggest slightly decayed meat, necessarily covered with aluminum foil. Post-feeding larvae should be kept in humid soil substrate and transferred into plastic Petri dishes (90 mm; 1-5 larvae/dish) after they start burying themselves. Petri dishes should be watered during the early post-feeding stage by immersing in water for several seconds. Larvae form pupal chambers where pupae emerge. Tenerals stay in the chambers for 1-3 days, afterwards they dig themselves out. Intra-specific killing occurs very infrequently. Mite infestation needs to be controlled, as it may decrease survival of beetles.

The influence of burning on blow fly (Diptera: Calliphoridae) attraction to meat substrates

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Crime scenes involving burnt victims are among the most difficult to investigate (Geberth, 1990). When part of a criminal act, fire is commonly used to eliminate evidence from the body and the crime scene (Ubelaker, 2008; Gruenthal et al., 2012).
Several studies have observed that usually burning does not deter insect colonisation, and an accurate minPMI estimation can still be made. In the event of a fire, two effects can be observed on the cadaver: a change in odour which can alter the attraction of insects to the body (Avila and Goff, 1998), and physical changes like dehydration and reduction of the protein content. When estimating the minPMI, it is crucial to consider how these changes alter the colonisation of blow flies (Whitaker, 2017).
To improve the communication between forensic specialists, the Crow-Glassman Scale (CGS) was devised, which describes five different levels of destruction of the body caused by fire.
The aim of this study is to determine how burning at different CGS levels influences the attraction of blow flies to meat substrates, and therefore the oviposition. The most common species of blow flies in the United Kingdom were considered for this experiment: *Calliphora vomitoria* and *Calliphora vicina*.
To simulate the texture of human skin, pork belly was used for this experiment. Small portions were burned at different CGS levels in a controlled environment. These samples were then placed in insect cages with gravid female specimens coming from the laboratory colonies. In the following 24 hours, attraction to the meat substrate and oviposition were noted.
Previous studies have suggested that CGS levels 2-3 are more likely to be colonised than CGS levels 1-4-5. Results investigate how different levels of burning affect the attraction of blow flies to meat substrates and determine under what conditions these substrates become unsuitable for oviposition.

Larval instar determination of the forensically important species *Dermestes frischii* (Coleoptera: Dermestidae)

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Dermestid beetles (Coleoptera: Dermestidae) are frequently found in cadavers in the advanced stages of decomposition, feeding on dry tissue remains. Because of this, dermestids are potentially useful indicators in forensic investigations concerning skeletonized and mummified human remains. In those cases, a minimum post-mortem interval (minPMI) may be estimated on the basis of crime scene temperature records and available development data for the collected dermestid species and developmental stage. Determining the larval instar is an essential prerequisite in the application of those reference developmental data for minPMI estimations. The aim of this ongoing study is to develop a method for the accurate determination of the larval instar of *Dermestes frischii* (Kugelann), one of the most frequent dermestids on carrion, combining both quantitative and qualitative markers. For qualitative markers, potential age-diagnostic characters, such as the presence of urogomphi or the number of setae, are currently being studied. For quantitative markers, six features from the cephalic structures were measured to develop classifiers using linear discriminant analyses. However, only three of those six measurements were finally selected to develop the classification model, due to their greater classificatory power. Validation with the larvae (N = 120) used to fit the resulting model gave a percentage of cases correctly classified of 96.67%, whereas validation with independent test larvae (N = 42) classified 100% of cases correctly. Moreover, the classification functions developed for *D. frischii* were also tested with sets of larvae of known instar of *Dermestes maculatus*(Brahm) (N = 54) and *Dermestes undulatus* (De Geer) (N = 39), to see if the classifiers could be used at a genus level. In those cases, the percentage of cases correctly classified was 59.26% for *D. maculatus* and 23.08% for *D. undulatus*, suggesting that it would be necessary to develop specific classifiers for each species.

Developmental times of the forensically important carrion beetle *Thanatophilus sinuatus* (Silphidae: Silphinae) at three constant temperatures

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Developmental timing of insect immature stages are of great potential for forensic entomology investigations in Europe. *Thanatophilus* (Silphidae: Silphinae) species are relevant for estimating a post mortem interval (PMI), an interval often used as evidence for medico legal investigations. *Thanatophilus sinuatus* is a common carrion beetle found during early stages of on vertebrate carrion and human corpse decomposition across Europe. Despite the benefits, no information is currently available for *Th. sinuatus* development rate at different temperatures; as a result, thermal summation models for PMI calculatations cannot be used. Thermal summation models, or accumulated degree-days (ADD) methods, are used as solid evidence to estimate PMI. This study provides premilinary information regarding the developmental times of *Thanatophilus sinuatus* at three constant temperatures (18°C, 20°C, 21°C). Immature stages of insects for the study were obtained from laboratory rearing under controlled conditions (16:8 h of light/dark period) in climate chambers. Based on the data of temperature and development length a linear regression model was used to calculate the *Lower Temperature-Threshold* (LTT) and the *Accumulated Degrees-Days* (ADD). As expected, egg to adult development times decreased based on the rise of the temperature. Results for each of the four developmental stages of *Th.sinuatus* from ovipositions to adult were: LTT(Egg): 17 °C ADD(Egg): 12 DD; LTT(Larvae1): 17.4 °C ADD(Larvae1): 6.0 DD; LTT(Larvae2): 18.0 °C ADD(Larvae2): 5.9 DD; LTT(Larvae3): 16.9 °C, ADD(Larvae3): 43.3 DD; LTT(Pupae): 16 °C, ADD(Pupae): 46.4 DD. As mentioned before, results are preliminary and will be improved over the completion of the study. Results of this study provide vital data for the use of *Th. sinuatus* for PMI estimation in criminal investigations.

A preliminary investigation of faunal colonisation of remains in open water

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Insect and invertebrate colonisation of remains in aquatic environments is a current topic of interest in forensic entomological research. Here, three approaches for investigating invertebrate colonisation on animal remains in open water were tested, with a view to providing the first steps towards a robust methodology for conducting this type of research as a stepping stone to a larger scale study. Method 1 used a modified crayfish pot to enclose a piglet carcass and incorporated a GoPro camera to monitor decomposition and marine fauna feeding behaviour using timelapse photography. Method 2 made use of a whelk pot to house the carcass and improve the rate of trapping feeding fauna. Finally, method 3 took a combined approach using a lobster pot to both house the piglet carcass and trap feeding fauna, as well as using timelapse photography to monitor decomposition and feeding behaviour. Of these, method 3 was the most successful for monitoring decomposition and colonisation for forensic purposes as it was possible to collect must more data using this method than with the other two.

The sarcosaprophagous entomofauna as geographical and environmental indicators

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Insects can detect the presence of a cadaver at a great distance, colonizing it rapidly and being the first to exploit this resource. Their distribution and abundance depend on the geographic location, time of year, environmental characteristics and the habitat in which the corpse is located. Hence, there is a need to undertake studies of the colonization and succession of such fauna on animal carcass models in different natural and urban environments to determine the differences between the communities of each locality. For this reason, a study in a wild mountainous area of the province of Murcia (SE, Spain) was carried out in different seasons to provide information on the entomosarcosaprophagous fauna composition and activity in such environmental conditions, thereby broadening our knowledge of the forensic entomology community associated with cadaver decomposition in semiarid regions.
The study was conducted in Sierra Espuña Regional Park, a protected area (Site of Community Importance ES0000173) in the centre of the province of Murcia (Murcia Region) (37°49'50"N 1°29'56"W). The area is located at about 400 meters above sea level, facing South, and characterized by an undergrowth dominated by *Rosmarinus* sp. and *Thymus* sp., and an arboreal stratum of *Pinus* sp.
Samples were collected with a 60 x 70 x 70 cm modified Schoenly trap baited with a 5 Kg piglet (*Sus scrofa* Linnaeus, 1758). In total, 52,567 individuals were collected, with *Chrysomya albiceps, Calliphora vicina* and *C. vomitoria* being the most valuable species as seasonal indicators. Moreover, these species, together with *Dermestes frischii, Camponotus sylvaticus* and those of the Chalcidoidea family, defined a clear pattern of colonization that varied according to the season. *Saprinus detersus* and *Formica subrufa* may, additionally, be of potential value as habitat indicators of the studied area.

The Impact of Shallow Burials on the Soil Meso-fauna (Acari)

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The fauna of a buried cadaver is associated with reduced activity of forensic arthropods such as Diptera and Coleoptera that can otherwise easily colonise exposed cadavers. Consequently, Post mortem interval (PMI) investigations of buried bodies based on entomological data can be challenging. Leakage of nutrient-rich cadaver fluids contributes to the alteration of the micro-environment of the soil which directly affects the Meso-fauna; particularly the mites (Acari). Much like carrion insects, mites will colonise a cadaver in a successive pattern; specific species are attracted to different stages of decay. Along with the regular soil mite fauna, phoretic mites may be amongst the first arthropods to arrive at a corpse along with smaller carrion flies and beetles that are able to access buried bodies through crevices in the soil. A significant difference in the abundance and species richness of mites in the grave soil of buried pig cadavers (*Sus scrofa)* throughout the decay process and bare soil of the same depth was found ($P < 0.05$). The most abundant and diverse mites in the grave soil were Mesostigmata mites (49%); of which soil and phoretic Parasitidae and Macrochelidae mites were the most abundant and diverse families. Successional patterns of mite taxa was observed in relation to the decay stages; fresh stages were associated with common soil dwelling species of Oribatida, Astigmata and Prostigmata, which disappeared during the onset of bloated stages after which Mesostigmata mites reached peak abundance during active and advanced decay and dry stages, after which the soil fauna started to stabilise again. This novel study demonstrates that mites may be useful as forensic markers of decay stages in PMI estimations of buried cadavers as the analysis of mite taxa composition and the visitation patterns of species in correspondence to decay stages may allow for more accurate estimations of the PMI.

Cuticular Hydrocarbons as a Tool for Determining the Age of *Chrysomya rufifacies* (Diptera: Calliphoridae) larvae

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Calliphoridae are one of the most important insect groups encountered as evidence collected from a crime scene. Age determination of the immatures of these necrophagous flies is an important step towards estimating time of colonization and inferring a minimum postmortem interval in most instances. To scrutinize the potential use of cuticular hydrocarbon for age estimation, hydrocarbons from 1st, 2nd, 3rd and post feeding larvae of *Chrysomya rufifacies* were extracted and analyzed by using gas-chromatography coupled to mass spectrometry (GC-MS) and principal component analysis (PCA). The intent of this study was to identify and quantify hydrocarbon profiles associated throughout the larval stage of the blow fly, *Chrysomya rufifacies*. A total of 23 compounds were identified with most of them being alkanes (65%) with carbon chain length of 9 to 33 carbons, alkenes (18%) and methyl branched alkanes (17%). For 1st instars, nonane was the most abundant (17% of the total hydrocarbon content). With a percentage of 11% and 10%, tricosane and pentacosane, respectively, were the notable hydrocarbons in second instars. For post-feeding larvae, heptacosane, hentriacontane and tritriacontane were present with relative abundances of 16%, 18% and 15%, respectively; whereas for the same compounds these were 15%, 17% and 13% in feeding 3rd instars. The amount of alkanes, alkenes, and methyl branched alkanes in first instars ranged from 0.12 - 2.09 ng, 0.56 - 0.14 ng and 0.41 - 0.48 ng, respectively; while, in post-feeding stage, they ranged from 1.87 - 83.06 ng, 9.81 -15.07 ng, and 6.28 - 18.39 ng. On average there was a shift from low molecular hydrocarbons to high molecular hydrocarbons as larvae aged. These results proved the efficiency of using these hydrocarbons to determine the age of insects and hence will aid in post mortem interval estimations.

Niche partitioning of necrophagous flies using contour plots

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Tolerance limits of necrophagous flies for environmental factors not only provides an understanding of niche partitioning, but can also be used as a basis for seasonal appearance. The authors placed 104 fly traps without illuminators in four habitats every two weeks for 5 years (2014-2018), and simultaneously measured environmental factors using a data logger. In addition, contour graphs were plotted for necrophagous species with a relative frequency of 13% or more.

As a result, 4,448 individuals were identified from 23 species. *Lucilia sericata* was in competition with *L. illustris*. *Lucilia caesar* showed an ecological optimal range similar to *L. illustris*, but fitness was higher than L. *illustris* around 23 °C / 47% RH. The ecological optimal range of *Triceratopyga calliphoroides, Calliphora vicina*, and *C. nigribarbis* (= *C. lata*) were around 18-19 °C / 53% RH, 22 °C / 53% RH, and 18 °C / 47% RH, respectively. *Sarcophaga similis* showed the same ecological and physiological optimal range around 31 °C / 33% RH. *Sarcophaga peregrina*had bimodal ecological optimal ranges were approximately 23 °C / 55% RH and 31 °C / 60% RH due to environmental constraints. *Chrysomyia pinguis* was a specialist in a narrow range between 26-30 °C / 57% RH. In addition, there was no significant correlation between collected individual numbers and light intensity (P value > 0.05).

These results provide the basis for the appearance of necrophagous flies in unrecorded microenvironments where the body is located. In particular, because there is no larval growth data for *C. pinguis* yet, we can refer to narrow ecological range observed in this species to rear the maggots for larval growth experiments.

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Competition between larvae of *Chrysomya megacephala* and *Lucilia sericata* (Diptera: Calliphoridae)

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Chrysomya megacephala (Fabricius, 1794) and *Lucilia sericata* (Meigen, 1826) are two forensically important flies that coexist in southern Europe. While the European green blowfly, *L. sericata* is found in most areas of the world, the oriental latrine fly, *Ch. megacephala,* was recently recorded in Europe. Both species can be found on carrion, but little is known about the interactions between them. The aim of this study was to evaluate the effect of inter- and intraspecific competition on development and survival of both species. Laboratory experiments were performed on mixed and pure cultures with first-instar larvae at different densities (50, 100, 200, 500 ind/pot) and the same quantity of pig liver (15 g). In mixed and pure cultures, survival and development of *L. sericata* was greater and longer than *Ch. megacephala*. For *Ch. megacephala* at the lowest density, the mortality was greater in the pure cultures than in the mixed cultures. But in mixed cultures, mortality of *Ch. megacephala* increased (50%) at high densities. Both species developed fastest when the rearing density was increased but there were no differences between the two cultures. For *Ch. megacephala,* the effects of interspecific competition with *L. sericata* on survival were greater than the effects of intraspecific competition in a pure culture. For *L. sericata,* the effects of inter – and intraspecific competition were low, suggesting a competitive advantage by *L. sericata* over *Ch. megacephala*.

A case study estimating the time period that parts of a dismembered corpse spent floating in a river

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Due to the relatively few indicators of time since death for corpses in aquatic ecosystems and limited research on this topic, it is difficult to estimate the time of death of bodies found in water, let alone individual body parts. We present a case in which entomological evidence was used to estimate the period of time spent floating in a river by dismembered body parts. On March 10, 2019, a portion of a corpse was found in a river in Zhongshan City, Guangdong Province. We were invited to assist in estimating the time of death. Insects collected from the body parts included puparia of *Parasarcophaga similis* (Meade, 1876), an empty puparium of *Lucilia sericata* (Meigen, 1826), 3rd instar and post-feeding larvae of *Chrysomya megacephala* (Fabricius, 1794), a puparium of *Fannia canicularis* (Linnaeus, 1758), a cockroach ootheca and an aquatic arthropod (species unknown). According to the puparium of *L. sericata* and the larvae of *C. megacephala*, we estimated that the body parts had floated on the water surface for at least 20 days and 11.3 days, respectively. According to the intra-puparial morphological characteristics of *P. similis*, the body parts appeared at the water surface at least 20.1 days before recovery. Therefore, the period over which the body parts floated was at least 20.1 d. After the crime was solved, the murderer admitted that the victim was killed on January 29, and that he had dismembered the body into 11 parts over the next two days,before throwing them into the river on January 31. Hence, the post-mortem submersion interval was equal to or shorter than 17.9 d. More indicators need to be researched to help estimate the post-mortem interval of bodies in aquatic environments. In this case, we obtained a first estimation through determining the intra-puparial morphological characteristics, thereby proving the practical value of studies of intra-puparial morphological changes.

Analysis of blow fly (Diptera: Calliphoridae) colonization of human remains in Indiana, USA

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Over the past several decades, an increased understanding of the utility of insects for legal investigations has led to substantial growth in the field of forensic entomology. Although great strides have been made in the field, work pertaining to the colonization of human remains is still limited. Here we present we present the forensically important blow fly species (Diptera: Calliphoridae) that were found colonizing human remains in Indiana, USA, along with other forensically important insects that were collected from the remains. A total of 25 cases with insect infestations were analyzed. Cases ranged from June 2016 through September of 2018. Along with the species composition of the larvae that were colonizing the remains, variables including weather (temperature, humidity, precipitation), time of year (season and month), and relevant scene information (i.e. indoor vs. outdoor scene) were also included. In the cases presented here, *Phormia regina* (Meigen), *Lucilia sericata* (Meigen), and *Cochliomya macellaria* (Fabricus) were the predominant colonizing blow fly species.

Preliminary assessment of an odourless rearing medium for laboratory culture of blowflies (Diptera, Calliphoridae)

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Experimental studies supporting forensic investigation and the rearing of blowflies from crime scene investigation are reliant on easy to handle growth media on which to feed larvae. Many investigations make use of homogenised liver and various other meat extracts and products, to provide the essential protein and nutrition requirement for regular growth and development. Nutritional intake differs with varying tissue type and tissue age, or with rearing medium. In addition, we observed variation in development as result of feeding larvae on liver, and encountered adverse responses in a mixed-laboratory to the malodorous release of volatile organic compounds (VOC), especially if ammonia levels are uncontrolled.
To facilitate development of blowfly cultures for experimentation purposes in a multi-user laboratory environment, we set about to develop an odourless rearing medium. We sought to find a medium that provided regular growth and development and which can be substantially demonstrated to be consistent with growth of larvae on a human body.
We settled on an agar based diet amended in various ways to optimise blowfly growth, which we have called the *standard agar-yeast-l-tryptone* diet (StAYT). Rearing tests were undertaken to demonstrate that this diet was similar if not better than homogenised pork liver, minced pork or diced pork. We then benchmarked against samples from legal cases with known times of death. For *C. vomitoria* (Linnaeus, 1758), Fisher's exact measure of significance showed no significant difference ($F_{0.05, 23} = 0.22$) between StAYT reared third instar larvae.

Bomb-proof insects: Is decomposition affected by the chemical effects of a terrorist chlorine bomb attack?

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With the current threat level for terrorism at severe and important figures within the community recognising the potential threat of chemical attacks, it is important to understand what impact this could have on the taphonomic and entomological aspect of investigations. There is a distinct lack of research surrounding insect behaviour and decomposition rates when chemicals have been involved in a death, and how this could in turn impacts post-mortem interval (PMI) estimation. Therefore, this research has been conducted to ascertain whether the use of the chemical chlorine would influence insect succession and the rate of decomposition.
Three pig carcasses were decomposed in a remote woodland; one as a control, one exposed to a low dose of chlorine, and one exposed to a high dose of chlorine. Over a period of three weeks, decomposition was monitored and entomological samples collected.
Data indicated no distinct differences between the decomposition of the control and low dose chlorine piglets, therefore cadavers not located within a close distance to a chlorine bomb would not be adversely affected. In contrast, the high dose chlorine carcass attracted different and unpredictable insect arrival early on within the study, however the decomposition rate was unaffected by the chemical influence. Cadavers located within close proximity to a chlorine bomb or gas attack would therefore be expected to decompose at a similar rate, but the entomological succession patterns and markers used for PMI estimation would be different.