EAFE 2014
Lille, France

9th to 11th April 2014

11th Meeting of the European Association for Forensic Entomology

Abstract Book

Univ. Lille 2 – Pôle Recherche, Lille, France

Lille Forensic Taphonomy Unit
Univ. Lille 2

EAFE
European Association for Forensic Entomology

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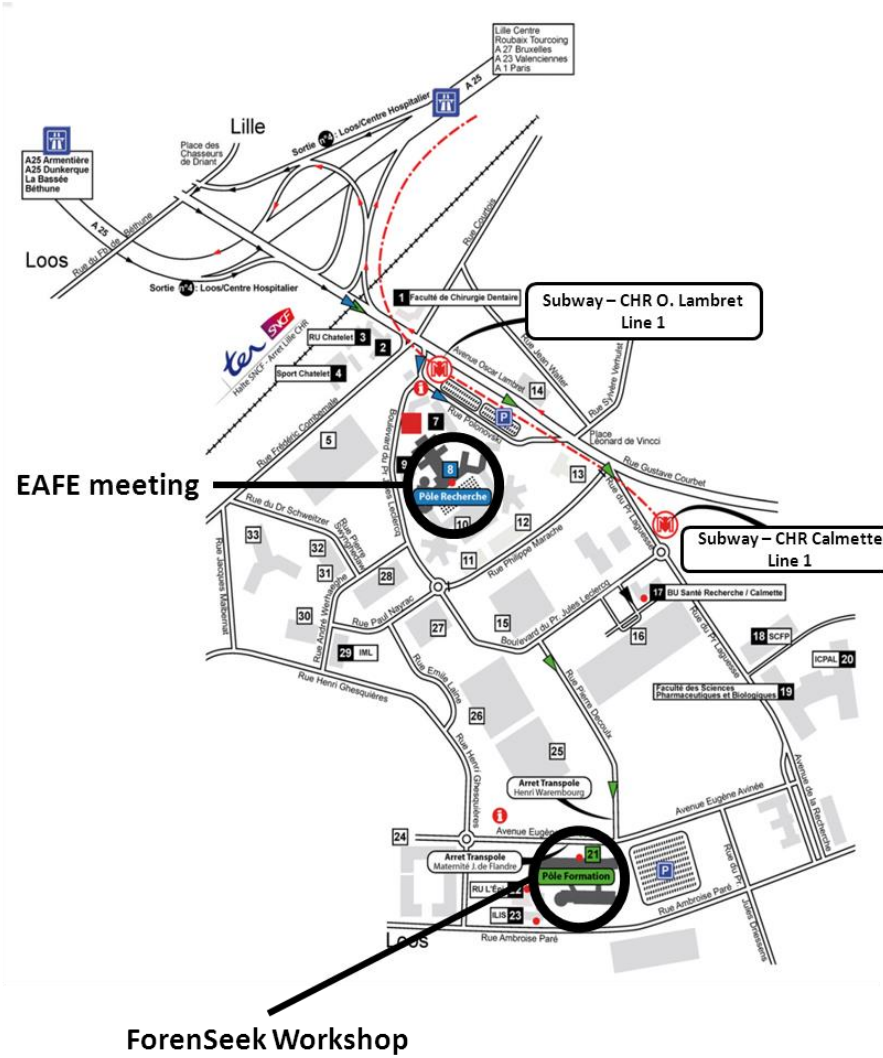
Lille Forensic Taphonomy Unit
Rue A. Verhaeghe 59000 Lille
eafe2014.sciencesconf.org

Lille Forensic Taphonomy Unit
Univ. Lille 2

EAFE
European Association for Forensic Entomology

EAFE | 2014

UNIVERSITY LILLE 2 - MAP



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A LETTER FROM L. BOURGUIGNON

EAFE PRESIDENT

It is with great pleasure that invite you to discover the abstract book of the 11th meeting of the European Association for Forensic Entomology.
In the following pages you will read and discover the results of a year of research and intensive studies made in forensic entomology by various teams and individuals throughout the world, with emphasis on Europe.
The wide range of topics proposed is an illustration of the dynamism and the curiosity of forensic entomologists, and their tireless efforts to bring the best of science to support justice.
Publishing research is of course part of the Scientific Method, but it is also a way to create networks. I hope the information you will find here encourages you to maintain existing contacts and develop new opportunities.

Luc Bourguignon

C'est avec un grand plaisir que je vous invite à parcourir le catalogue de la 11ème réunion de l'Association Européenne pour l'Entomologie Forensique.
Dans les pages qui suivent, vous pourrez lire et découvrir les résultats d'un an de recherches et d'études effectuées par des équipes du monde entier, avec une emphase particulière sur les travaux effectués en Europe.
Le large panel de sujets proposés est une illustration du dynamisme et de la curiosité des entomologistes forensiques, et de leurs efforts incessants pour venir en aide à la Justice avec le meilleur de la Science.
Rendre ses recherches publiques est bien entendu partie intégrante de la Méthode Scientifique, mais c'est aussi un moyen de créer des réseaux. J'espère que les informations que vous trouverez ici vous encourageront à maintenir les contacts existants et en développer de nouveaux.

ENGLISH/FRENCH DICTIONNARY

Termes de base/ To start
Yes = Oui
No = Non
Thank you = Merci
You're welcome = de rien, je vous en prie
Please = S'il vous plaît
Excuse me = pardon, excusez-moi
Hello = Bonjour
Goodbye = Au revoir
See you soon = à bientôt
Good morning = Bonjour
Good afternoon = Bon après-midi
Good evening = Bonsoir
Good night = Bonne nuit
I don't understand = Je ne comprends pas
How do you say X in French? Comment dit-on X en Français?
What is your name? = Comment vous appelez-vous? Quel est votre nom? =
Nice to meet you = Enchanté (de faire votre connaissance)
How are you? = Ça va?
Cheers! = À votre santé! À la vôtre!

Acheter/Shopping
How much does this cost? = Quel est le prix? Combien ça coûte?
What is this? = Qu'est-ce que c'est?
I'll buy it = Je le prends
I would like to buy ... = Je voudrais acheter
Do you have ... = Avez-vous ...
Do you accept credit cards? = Acceptez-vous les cartes de crédit?
Open = ouvert
Closed = fermé
Postcard = la carte postale
Stamps = des timbres

Se déplacer/ Transports
Where is ...? = Où est ...?
How much is the fare? = Quel est le prix
Ticket = Billet
One ticket to ... = Un billet pour ...
Where are you going? = Où est-ce que vous allez?
Where do you live? = Où habitez-vous?
Train = le train
Bus = un autobus
Subway, Underground = le métro
Airport = un aéroport
Train station = la gare
Bus station = la gare routière
Departure = le départ
Arrival = une arrivée
Car rental agency = Agence de location de voitures
Parking = le stationnement, le parking
Hotel = un Hôtel
Room = une chambre
Reservation = la réservation
Are there any vacancies for tonight? = Avez-vous des chambres disponibles pour ce soir?
No vacancies = Complet
Passport = le passeport

Orientation
Left = à gauche
Right = à droite
Straight = Tout droit
Far = Loin
Near = près, proche
Map = Carte
Tourist Information = office de tourisme
Where is the bathroom? Where is the toilet? = Où sont les toilettes?
Post office = La Poste
Museum = le musée
Bank = la banque
Police station = le commissariat
Hospital = l'hôpital
Pharmacy, Chemists = la pharmacie
Store, Shop = le magasin
Restaurant = le restaurant
Street = la rue
Square = la place

Heure/Time
What time is it? = Quelle heure est-il?

PLENARY SESSION

Archaeoentomology
Friday, Apr. 11 – 9:00am-10:00am

Jean-Bernard Huchet[1,2,3]

[1]UMR 7209 du CNRS, Archéozoologie, Archéobotanique
Sociétés, Pratiques et Environnements, MNHN, Paris; [2]UMR 7205 du CNRS, Institut de Systématique, Evolution, Biodiversité (ISyEB), MNHN, Paris; [3]Université de Bordeaux, PACEA - UMR 5199, laboratoire d'Anthropologie (A3P), Pessac.

Jean-Bernard Huchet works as archaeoentomologist (the study of insect remains recovered in archaeological contexts) at the National Museum of Natural History (Paris). In 1996, from the study of the entomofauna found in a 10th century sarcophagus attributed to Guillaume Taillefer, the Count of Toulouse, he laid the groundwork for a new discipline: "l'Archéoentomologie funéraire" (Funerary Archaeoentomology), which can be defined as the application of Forensic entomology to the study of graves and human remains recovered within archaeological contexts.

The EAFE meeting will be a good opportunity for him to present several "old cases" dealing with past funerary practices evidenced by the study of relevant, well-preserved insect remains as well as some amazing cases of human bone modifications perpetrated by several families of hexapods formerly misinterpreted as palaeopathologies.

EAFE MEMBERS MEETING

Friday, Apr. 11, 4:00pm – 5:00pm - Amphi

Interlaboratory projects 2013-2014:
Preliminary results and future developments

The EAFE organized 2 interlaboratory projects during the season 2013-2014. They both were launched mid-2013.

The **Project 1** was a collaborative exercise. 3 samples of adult insects were provided to the participants. The aim was to identify their species (Family, genus, species and gender), so that a total of 12 answers were expected.

33 individual or laboratories answered. Results were spread like this:

- 27 respondents gave the correct answer to each of the 12 questions,
- 2 respondents gave 11 correct answers
- 2 respondents gave 9 correct answers, but did not specify the gender,
- 1 respondent gave correct answers but apparently intertwined the 3 samples,
- 1 respondent gave 10 incorrect answers.

Collaborative exercises of the kind will be organized on a regular basis in the upcoming years.

The **Project 2** consisted in making a monthly trapping throughout Europe, according to a common calendar. So far 20 laboratories or individuals showed interest to participate in this project.

The results will be collected, and discussed between the participants, as well as the future outcomes and improvements of both projects.

BEST ORAL COMMUNICATION &

BEST POSTER COMMUNICATION PRIZES

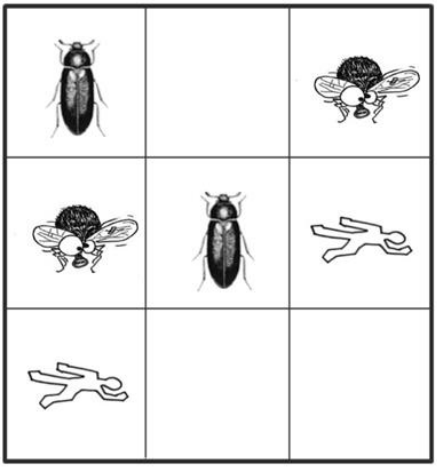
A Prize will be awarded for the best oral communication presented during the meeting. Another price will be awarded for the best poster communication.

Winners will be selected by the scientific committee.

Good luck!

The organizing committee

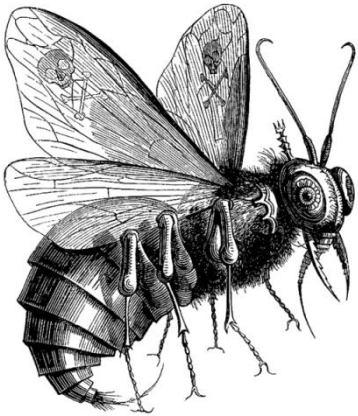
PLANNING



COMPLETE THE GRID TO HAVE EACH PICTURE ONLY ONCE PER LINE AND COLUMN

Wednesday, Apr. 9

5:30pm – 6:30pm	**Registration |** Hall of the conference building
6:00pm – 8:00pm	**Welcome Reception |** Eureka Room



Thursday, Apr. 10

8:30am – 14:00pm	**Registration |** Hall
9:00am – 9:30am	**Welcome Speech – Pr. Didier Gosset |** Amphi

Session 1	***Chairman:* Stefano Vanin (IT)**
9:35am – 9:50am	**E. Garrett |** Effect of novel psychoactive substance 'Benzofury' and legal replacement 5-EAPB on weight, length and cuticular hydrocarbons for ageing larvae
9:55am – 10:10am	**K. Szpila |** Flesh flies (Diptera: Sarcophagidae) colonizing pig carcasses in Central Europe
10:15am – 10:30am	**C. Aubernon |** Some like *eat* hot? Experimental study of Blowflies larvae thermal preferences
10:30am – 11:00am	**Coffee Break |** Hall
Session 2	***Chairwoman:* Paola Magni (IT)**
11:10am – 11:25am	**L. Bourguignon |** One more species in the forensic entomologist's toolbox: developmental data of *Cynomya mortuorum* (L., 1761)
11:30am – 11:45am	**M. Hall |** Factors affecting accessibility to Blowflies of bodies disposed in suitcases
11:50am – 12:05am	**K. Brown |** Optical Coherence Tomography: Age estimation of Calliphora vicina pupae in vivo?
12:10am – 12:25am	**I. Dadour |** The pre-colonization period: What do we really know?
12:30am – 1:30pm	**Lunch |** Atrium

Session 3	***Chairman:*** **Luc Bourguignon (Be)**
1:40pm – 1:55pm	**S. Matuszewski** **|** Best practice in the pre-appearance interval studies
2:00pm – 2:15pm	**M.F. Caneparo** **|** Forensic entomology in Brazil: challenges and achievements
2:20pm – 2:35pm	**C. Von Hoermann** **|** How does a piglet cadaver smell for the burying beetle *Nicrophorus vespilloides* (Coleoptera: Silphidae)?
2:40pm – 2:55pm	**S. Bortolini** **|** Molecular identification of 20 years old samples collected from a crime scene
3:00pm – 3:30pm	**Coffee Break |** Hall
Session 4	***Chairman:*** **Bryan Turner (UK)**
3:40pm – 3:55pm	**P. Magni** **|** Effect of cooling on different densities of larvae of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae)
4:00pm – 4:15pm	**L. Manelli** **|** Body colonization and degradation in a freshwater system: the role of the crayfish *Procambarus clarkia*
4:20pm – 4:35pm	**J. Boulay** **|** Aggregation of Diptera larvae on homogenous & patchy environment
5:00pm – 6:00pm	**ForenSeek Workshop - |** Computer Room Meeting Point in the Hall of the conference building

Friday, Apr. 11

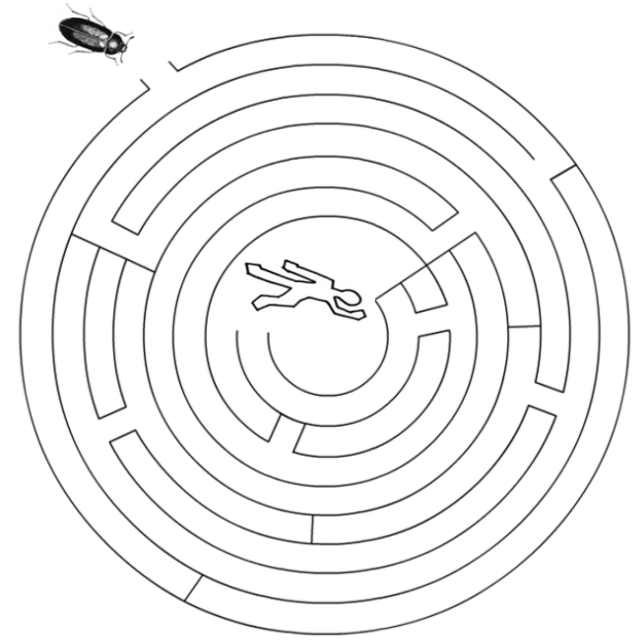
8:30am – 14:00pm	**Registration |** Hall
9:00am – 10:00am	**Archeo-entomology – Jean-Bernard Huchet (FR) |** Amphi

Session 5	***Chairwoman:*** **Katherine Brown (UK)**
10:05am – 10:20am	**B. Morris |** Olfactory responses of larval *Chrysomya rufifacies*
10:25am – 10:40am	**K. Barnes |** Nocturnal oviposition behaviour of forensically-important Diptera in Central England
10:30am – 11:00am	**Coffee Break |** Hall
11:10am – 11:25am	**L. Dourel |** Forensic entomology in expertise context: the weight of identified uncertainties
11:30am – 11:45am	**E.M. Benbow |** An ecoregion perspective of Calliphoridae microbiome diversity
11:50am – 12:05am	**J. Wells |** Calculating probabilities for a succession-based PMI estimate
12:10am – 1:30pm	**Lunch |** Atrium

Session 6	***Chairman:*** **Jens Amendt (DE)**
1:40pm – 2:15pm	**S. Vanin |** Teaching Forensic Entomology from the college to the PhD: experience from the University of Huddersfield (UK) Teaching Forensic Entomology in Italy: the GIEF experience
2:20pm – 2:35pm	**G. Suwannapong |** Effect of constant temperatures on developmental rate of important forensically Blowfly of Thailand, *Chrysomya rufifacies* (Macquart)

2:45pm – 3:10pm	**Coffee Break |** Hall
3:20pm – 3:35pm	**B.K. Zajac |** MACE (Massive Analysis of cDNA-Ends) - Highly sensitive digital gene expression profiling of *Calliphora vicina* (Calliphoridae) pupae
3:40pm – 3:55pm	**D. Gemmellaro |** Forensic entomology's first steps in Sicily: lava fields and caves
4:00pm – 5:00pm	**Members Meeting |** Amphi
6:00pm – 7:00pm	**Social Event - Lille City Tour** Meeting Point - Place General De Gaulle
7:30pm – 11:30pm	**Closing Gala |** Omnia Restaurant (9 rue Esquermoise)

ORAL PRESENTATIONS



Effect of novel psychoactive substance 'Benzofury' and legal replacement 5-EAPB on weight, length and cuticular hydrocarbons for ageing larvae

Garrett Emma [1], Falko Drijfhout [1]

[1] Keele University,
School of Physical and Geographical Sciences, Keele, Staffordshire, ST5 5BG

Forensic entomology relies on the correct identification and aging of insect samples. It is well documented that drugs can affect the growth of larvae leading to incorrect PMI estimations. Previous research has shown the influences of illegal drugs such as cocaine and amphetamines but the area of Novel Psychoactive Substances is currently un-documented, but forensically important due to a growing number of deaths and substances increasingly worrying government officials, enough to implement the illegalisation of some.
The research focus is on discovering the effect of such substances on larval length and wet weight alongside the effect on the hydrocarbons found on the larval cuticle.
Prior research has shown encouraging data for aging developing larvae using Cuticular hydrocarbon analysis and it is possible that hydrocarbon profiles may not be affected by drugs and toxins and therefore may prove an invaluable method for accurately aging larvae affected by such chemicals.

Data was collected after feeding forensically important *C. vicina* with Novel Psychoactive substance 'Benzofury' and 5-EAPB, in the hope of showing length and weight changes, effect on ovipostion and also indicating the chemical profile changes and to discover if this is affected by the Novel Psychoactive Substances. The active compound found in Benzofury is 6-APB and it is thought to be a stimulant based on MDA (3,4-methylenedioxyamphetamine). In addition to 6- APB, there are other components present in Benzofury, such as caffeine. 5- EAPB (1-(benzofuran-5-yl)-N-ethylpropan-2-amine) is thought to be the legal replacement now the previously named substance has been illegalised.

The hydrocarbon extracts were analysed using Gas Chromatography-Mass Spectrometry (GC- MS) and the results were further analysed using Principal Component Analysis. Larval growth was also monitored by measuring length on a daily basis, weight twice daily and rearing the drugged colony through to adults and providing oviposition material to determine any effect the chemicals may have.
Data showed differences between the control and drugged samples in terms of growth and this will be presented alongside the cuticular hydrocarbon profiles and the consequences for PMI estimations.

Flesh flies (Diptera: Sarcophagidae) colonizing pig carcasses in Central Europe

Szpila Krzysztof[1], Bajerlein Daria[2], Konwerski Szymon[3], Szafalowicz Michal[4], Madra Anna[4,3], Matuszewski Szymon[4]

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[2] Department of Animal Taxonomy and Ecology, Adam Mickiewicz University Umultowska 89 61-614 Poznan, Poland
[3] Natural History Collections, Adam Mickiewicz University Umultowska 89 61-614 Poznan, Poland
[4] Laboratory of Criminalistics, Adam Mickiewicz University sw. Marcin 90 61-809 Poznan, Poland

In Europe, large vertebrate carrion, including human bodies, attracts about 30 species of Sarcophagidae, mostly from genus Sarcophaga. However, only some of them breed in this kind of substrate. Diversity and abundance of carrion flesh flies increase toward south, with the highest values in Mediterranean region. Unfortunately results on larval and adult Sarcophagidae from forensic carrion studies are virtually absent. The actual impact of this taxon on decomposition of large carrion has not been reliably evaluated, so far.

As a result of several pig carrion studies in Poland large body of data on adult and larval Sarcophagidae was collected. We defined: 1) assemblages of adult flesh flies visiting pig carrion in various habitats, 2) species of flesh flies which actually realise development in pig carrion, 3) temporal distribution of flesh fly larvae during decomposition.
Fifteen species of flesh flies were observed to visit pig carcasses, with much higher diversity and abundance in grasslands than in forests. Larvae of five species were collected, with *S. caerulescens* and *S. similis* regularly breeding in carcasses. First instar larvae of flesh flies were recorded on carrion earlier or concurrently with first instars of blowflies. Postfeeding larvae of *S. caerulescens* and *S. similis* were usually observed before the appearance of the fully grown blow fly larvae.
These results contest the view expressed in old FE manuals that flesh flies are later colonizers than blow flies.

Some like *eat* hot? Experimental study of Blowflies larvae thermal preferences

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[1] Unité de Taphonomie Médico-Légale (UTML)
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It is well known that Blowflies larvae (Diptera Calliphoridae) development time is mainly controlled by local temperature. Accordingly, it has been experimentally demonstrated that each species has an optimal development temperature, characterized by a short development time and a high survival rate. Furthermore, in recent study, it has been showed that larvae move inside masses to select preferential thermic areas. Then, temperature felt by larvae during their development on a corpse may be behaviorally selected. If so, use of ambient temperature or maximum maggot-mass temperature to estimate development time of larvae on a corpse would be meaningless.

In a first experiment we designed the ThermoGrad, a 80cm long thermal gradient setup, to determine specific preferential temperatures. The temperature gradient was set with linear increase from 15°C to 45°C. This experiment was performed on 3 different species: Lucilia sericata (N=10), Calliphora vomitoria (N=10) and Calliphora vicina (N=10). 80 young third instars were homogeneously placed on the ThermoGrad with mixed beef liver. The location of larvae was observed after 20h in the dark. Larvae of each species formed masses always located at the same temperature. This selected temperature was 34°C for L. sericata, while C. vomitoria and C. vicina selected lower temperatures (respectively 29°C and 19°C). These values are in agreement with the optimal development temperature noticed in the literature for these species.

The second experiment was performed on L. sericata larvae only (80 young third instars, N=10). One extremity of a small ThermoGrad (40cm long) was heated at 25°C while the opposite was heated at 35°C. We observed in these conditions that L. sericata masses were not always located on optimal temperature areas.
These results will be discussed from both a fundamental (gregariousness) and applied (development time calculation) point of view.

One more species in the forensic entomologist's toolbox: developmental data of *Cynomya mortuorum* (L., 1761)

Bourguignon Luc [1], Braet Yves, Vanpoucke Sofie, Drome Valerie, Hubrecht Francoise

[1] National Institute for Criminalistics and Criminology (INCC-NICC)
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Cynomya mortuorum (L. 1761) (Diptera : Calliphoridae) is a species of forensic interest, mainly present in the paleartic region, but its rarity makes it a species for which little is known. Its practical usage is therefore limited at the moment. We found it to be a very trustworthy and predictable species.
Its thermal constant and developmental time were studied under constant and variable regimes of five daily average temperatures commonly encountered in Belgium (14, 16, 18, 20 and 22°C) respectively.

During the constant regime, temperature followed no day/night variations. During the variable regime, temperature was higher during the day than during the night. The photoperiod in all incubators was set at 16:8 (day:night). All rearings were monitored with Testo T175-T1 data loggers (temperature) and Testo Saveris H2D (temperature and relative humidity).
The entire protocol was repeated up to 10-15 times for each temperature tested.

Under the constant temperature regime, egg-to-adult development time ranged from 29.67 days (± 2.38) at 14.45°C, to 16.82 days (± 0.40) at 22.11°C. The temperature threshold was calculated at 4.06°C and the Accumulated Degree-days (ADD) needed to complete total development was 305.75 (± 15.67) DD.
Under variable temperature conditions, the egg-to-adult development time ranged from 32 days (± 1.77) at 13.99°C to 17.05 days (± 0.67) at 22.25°C. The temperature threshold was calculated at 4.55°C and the Accumulated Degree-days (ADD) needed to complete total life cycle was 300.87 (± 14.71) DD.

The difference between the observed ADD for the two regimes of temperatures was not significant at (t test, p=0.066). An article discussing our observations and findings is being submitted to a forensic journal.

Factors affecting accessibility to blowflies of bodies disposed in suitcases

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content.met.police.uk
[3] Natural History Museum, London (NHM) Department of Life Sciences
Cromwell Road, London, SW7 5BD, UK www.nhm.ac.uk

Criminals have been known to dispose of bodies in zipped suitcases in an attempt to conceal murder. In order to investigate the forensic implications of this mode of disposal on calculating time of death, it is necessary to study the accessibility of bodies in suitcases to blowflies (Diptera: Calliphoridae) and the possibility of oviposition and infestation under these circumstances.

An experimental apparatus was designed that incorporated different zips (toothed and coil) of various gauges (4-6 mm) above a chicken liver bait. Gravid *Calliphora vomitoria* and *C. vicina* females were attracted to and oviposited on and through these zips, both under laboratory and field conditions. Egg laying was significantly more frequent and with greater numbers of eggs when zips were in contact with the bait than when they were placed approximately 6 cm above the bait. In the absence of bait, adult females could be stimulated to lay eggs on moistened zips, although the presence of blood accelerated egg laying compared to water alone. No eggs were laid on dry zips in the absence of bait. Of the first instar larvae tested, 89% were able to colonise the bait below the zips by passing through gaps between the teeth.
Preliminary field studies using suitcases baited with a pig's head indicated that there was a delay of 1-3 days in oviposition when compared to laboratory conditions. This information has practical value in explaining the presence of larvae on enclosed bodies in suitcases and, with further studies, will help forensic entomologists estimate a more accurate minimum time since death.

Optical Coherence Tomography: age estimation of *Calliphora vicina* pupae in vivo?

Brown Katherine[1], Harvey Michelle[2]

[1] School of Biological Sciences, University of Portsmouth
King Henry Building, King Henry I St, Portsmouth, Hampshire PO1 2DY
[2] School of Life and Environmental Sciences, Deakin University Waurn Ponds, Victoria, 3217

Much of the morphological analysis of blowfly pupae for developmental studies and age estimation is conducted on preserved individuals, using stereomicroscopy on whole organisms or compound microscopy on histological sections. Preservation and subsequent dissection or sectioning are destructive methods of age estimation. These current methods can limit or even inhibit additional analyses, such as gene expression and rearing to the adult stage. The advantages of in vivo observation of morphological development are therefore clear. This study demonstrates the potential application of optical coherence tomography (OCT) to age and PMI estimation of *Calliphora vicina* pupae.

Four- and seven-day old pupae were sampled from a laboratory-reared colony. Individuals were observed i) in vivo, in their puparia and ii) after being killed by submersion in near-boiling water and their puparia removed. Pupae were scanned using a bench-top OCT microscope from Michelson Diagnostics, which employs a multibeam system to increase resolution over other OCT technologies, to <7.5 µM. 2D and 3D rendered images were created and are shown, demonstrating the depth and resolution of images achievable with the current systems and the suitability of the method for age estimation.

The pre-colonisation period: What do we really know?

Dadour Ian[1], Cook David[1], Voss Sasha[1], Hung Wei-Feng[1], Magni Paola[1]

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Forensic entomology is the study of insects within a legal framework. The outstanding variable in determining an accurate post mortem interval (PMI) is the time that adult flies first lay live larvae or eggs onto a body.

The predicted order of insects, typically blowflies and flesh flies that are attracted to a decomposing corpse or cadaver is pivotal to determining the PMI in forensic entomology. The predicted order is divided into primary, secondary and tertiary insects. This correlates with the process of decomposition and how it changes over time. Blowflies generally arrive after death and recent research has shown that *Calliphora dubia*, *Calliphora varifrons* and sarcophagid adults lay live larvae onto guinea pig carcasses within 1 hour of exposure. These flies are typically the primary visitors to a corpse. Very little is known about ovoviviparous blowflies, which are invariably the first blowflies to visit a corpse in south western Australia and are often the critical species in a PMI determination.

New trials exposed 30 guinea pig carcasses throughout the day (0600-2000hrs) during spring and summer in bushland on each of 5 successive days. Replicate carcasses were set up randomly along a kilometre of bushland track and a carcass was removed every 0.5, 1.0, 2.0, 4.0, 8.0hrs throughout the day blowflies layed onto carcasses within 30 minutes (33% of carcasses), and on average on all carcasses within 1.5hrs of death. Eight different oviparous (*e.g. Lucilia sericata, Chrysomya rufifacies*, *Chrysomya varipes* and *Australophyra rostrata*), and the oviviviparious fly species above deposited either eggs or live larvae onto the carcasses within 6hrs.

This study highlights the previously unknown rapidity with which blowfly species are able to commence laying onto carcasses and from a large number of fly species, some of which are considered in the literature to be late colonisers of carcasses. Two repeat trials of exposure of carcasses again over 5 successive days in summer revealed 5 similar species of blowflies laid onto carcasses. This research has many ramifications for the post mortem interval estimation especially if all flies previously designated secondary and tertiary are in fact primary visitors to a corpse.

Best practice in the pre-appearance interval studies

Matuszewski Szymon[1], Madra Anna[1,2]

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An interval preceding appearance of adult or larval stage on a corpse (called the pre-appearance interval or PAI) is strongly temperature-dependent in many forensically important insects. Due to the strength of these associations, it was proposed to estimate PAI simply from temperature. This kind of estimation requires species-specific temperature models for PAI and case-specific temperature data.
The simplest way for PAI estimation involves prediction of PAI for a given temperature by using the model which regress PAI against temperature. The quality of temperature models for PAI certainly depends on many factors, among which the most important pertain to the protocols for PAI studies. Here we analyze effects of sampling frequency and techniques, the kind of temperature data, as well as the size and the kind of a sample in PAI studies on the quality of temperature models for PAI. Models differing according to the above factors were calculated by using PAI data from 30 pig carcasses decomposing in various temperature conditions. Carcasses were separated in time (six placements from April to August) and space (open and forest habitats). Insects were sampled with pitfall traps and manually. On-site, ground level temperatures were logged. Performance of the models was tested with another body of PAI data.
It was found that low frequency of insect sampling distinctly deteriorates temperature models for PAI. The effect of sampling techniques is clearly smaller, although single technique models were in all aspects worse as compared to mixed technique models. Temperature data from local weather station gave models of poor quality. Retrospective correction of weather station temperatures clearly improved the models. Most importantly, however, current study demonstrates that sample size in PAI studies may be substantially reduced, with no model deterioration. Samples consisting of 11-14 carcasses gave models of high quality, as long as the whole range of relevant temperatures was examined and carcasses were uniformly distributed across that range. Based on the current results the following guidelines for PAI studies are proposed:

1. Collect insects at least on a daily basis.
2. Sample insects with manual techniques and pitfall traps.
3. Collect on-site, twenty-four-hour temperature data. Use weather station data only after its retrospective correction.
4. Get PAI data from the whole range of relevant temperatures. Use more placements with fewer carcasses, distributed evenly over habitats differing in temperature (e.g. 7-8 placements x 2 carcasses, one in a dense forest, the other in an open habitat).
5. Reduce summer placements and multiply spring or autumn placements.

Forensic entomology in Brazil: Challenges and achievements

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Researches about species of flies and beetles that breed on carcasses have a long tradition in Brazil. Despite this, Universities and Police only cooperate to analyze entomological traces in a small percentage of cases. In addition, the utility of entomological evidence in criminal cases is not fully recognized by crime scene investigators and prosecutors.

The recent legal recognition of forensic entomology (FE) as a subarea of Forensic Biology; the absence of a required degree in Natural Sciences for crime scene investigators (CSIs) employment and the lack of forensic entomology classes in the great majority of police training courses are some of the aspects that have delayed the use of insects in legal investigations in Brazil. However, in the past few years, this scenario has been changing due to local partnerships between researchers and Police Departments.

One example is the Group of Forensic Entomology of Universidade Federal do Paraná (Southern Brazil) that in the last three years received entomological data from 26 cases to analyze, providing the estimative of death when possible. In about 35% of the cases it was impossible to estimate the post mortem interval (PMI). The main reasons for this high number involve the lack of data from the death scene, mainly temperature and humidity. In addition, most CSIs sampled only a few specimens, which could impair the rearing in laboratory. Also, Brazil has a megadiverse necrophagous fauna, differing from USA and Europe, which turns the research and application of FE more difficult due to the lack of biological data for many species. Regardless of all those issues, it was possible to estimate PMI in most cases, thus encouraging CSIs to collaborate, showing them the importance of FE in their routine. Unfortunately, researchers can't access testimonial evidence and trial results which slows down the spread of the academic knowledge.

To make easier and legitimate FE in Brazil an ongoing standardization project for forensic sciences will propose protocols for all procedures, from collecting evidence up until trial, an important step for the progress of this science.

How does a piglet cadaver smell for the burying beetle *Nicrophorus vespilloides* (Coleoptera: Silphidae)?

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In the necrophagous burying beetle *Nicrophorus vespilloides* (Coleoptera: Silphidae) cadaver preference appears to depend on cadaver size and maturity of the beetles. In a previous study I showed that newly emerged females prefer later stages of decomposition of large cadavers. Therefore, *N. vespilloides* females with immature ovaries, retrieved by forensic entomologists from large cadavers such as human corpses, can and should be regarded as potential indicators of prolonged PMIs. Until now, only a few workgroups have studied the postmortem VOC profiles emanating from human remains, pigs as human analogues and other vertebrate cadavers. Even less work has been done regarding the verification of bioactivity of specific cadaveric VOCs for insects despite our possibility of exploiting their fascinating olfactory performance as a prerequisite for refining the PMI determination in legal investigations. For accurate and more precise PMI estimation in real case work it is essential to consider the whole time frame from cadaveric VOC detection by insects till final insect colonization. Therefore, my aim was to determine specific chemical compounds involved in the discrimination of cadaveric odour bouquets by the burying beetle. Applying chemoecological methods I have been able to identify 13 electrophysiologically active compounds for burying beetles in the headspace of piglet cadavers during the whole decomposition period. By means of these compounds newly emerged females are potentially able to discriminate between all five decomposition stages of large cadavers: The bouquet of the fresh decomposition stage was mainly characterized by high relative amounts of trimethylpyrazine. High relative amounts of dimethyl trisulfide represented the bloated, post-bloating and advanced decay stages. The advanced decay and the dry remains stage were characterized by high relative amounts of phenol. This alcohol mainly contributed to the chemical separation of the fresh decomposition stage important for the reproduction of mature burying beetles, from the advanced decay stage that is nutritionally more attractive for newly emerged beetles. Phenol might therefore function as a key substance for newly emerged female burying beetles to locate suitable cadavers for maturation feeding. In summer field trapping experiments with the synthetic blend of a piglet cadaver in advanced decay, I trapped no N. vespilloides. However I caught high numbers of the necrophagous silphid beetle *Oiceoptoma thoracica*. For forensic entomological evaluations of seasonality during crime solving, it is important to know that my trapping results indicate seasonality in *O. thoracica* from the end of June till the end of September and not exclusively during spring as reported in other pig decomposition studies. Further trapping experiments will be needed to test whether seasonal constraints are responsible for successful attraction of burying beetles to synthetic scent in the field.

Molecular identification of 20 years old samples collected from a crime scene

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On March 2010, 17 years after her disappearance, the body of a 16 years old woman was found under the roof of a church in Potenza (Southern Italy). Entomological evidence and analysis on the insect feeding activity on the hair were the key elements in order to estimate the season of death and post mortem mutilation of the body (hair fetishism).
The insect fauna collected on the body was composed by several puparia and adult fragments belonging to Diptera (Calliphoridae, Sarcophagidae, Muscidae, Fanniidae and Phoridae), Coleoptera (Dermestidae, Cleridae, Histeridae, Anobiidae) and Hymenoptera (Pteromalidae, Vespidae).

The insect species identification of the samples collected from the crime scene, at the time of the body discovery, was performed using a morphological approach. In 2013 a project for the DNA extraction and the molecular identification of the puparia samples was undertaken in order to investigate the possibility of a molecular identification of old samples using standard molecular methods.
Despite the large amount of DNA extracted from intact and crushed puparia no positive results were obtained using the standard primers for the COI region (LCO1490 and HCO2198, ~709pb), the same results were obtained using specific primers designed for shorter regions (153-491bp). In contrast a positive result was obtained from a complete pupal cage, containing a uneclosed specimen. A 373bp region was amplified and sequenced. The BLAST comparison, gave an identity score of 99% with *Chrysomya albiceps*, confirming the morphological identification. This result show the possibility to extract and sequencing DNA from entomological evidence for species identification 20 years after the body colonisation, despite no positive results having been previously obtained working on the puparia. The longest sequences (694bp) obtained from these samples belong to environmental contamination or, more probable, from microorganisms potentially developed on the decomposed body, like Himatismenida (Amoebozoa), Oomycota and Nematoda.

Further investigations, with different extraction methods and different primers, are in progress as well in order to define «a posteriori» the Microbiome developed on the body.

Effect of cooling on different densities of larvae of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae)

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Following a homicide the regulation of forensic practice in many countries prevents the pathologist performing an immediate autopsy. Preceding each autopsy the corpse and the insects associated with it are stored in a cool room in a mortuary (4°C) to slow down the decomposition process as well as the development of most of the necrophagous insects associated with the corpse. In late stages of decay, fly immatures may be present in masses. The aim of this research was to investigate the growth and development of different larval masses of the blowfly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) when stored in a cold environment (4°C).
The experiments were divided into a number of trials comprising different storage conditions (continuous exposure or cyclic exposure) for immature stages (L2 and L3) and included four different densities of larvae (100, 500, 1000, 5000 larvae) each placed on 3kg of liver. *L. sericata* adults were first established in colony and allowed to complete one generation prior to each experiment.

Results show that if the larval mass has a density between 1000 and 5000 larvae and have already reached the third instar, then there is no influence on the development time of larvae. Therefore, the identification of the size of the larval mass as well as the instar of the larvae present are crucial data for any subsequent determination of a correct Post Mortem Interval.

Body colonization and degradation in a freshwater system: the role of the crayfish *Procambarus clarkii*

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The study of decay in freshwater systems is important for the estimation of the mPMI, the PMSI and the manner of death. In previous experiments, the importance of *Procambarus clarkii* in the consumption of dead bodies in freshwater was assessed. The present study aimed at characterizing the role of *P. clarkii* in the decomposition of carcasses by studying the population dynamics in relation to the decomposition stage and the wounds caused by the crayfish's feeding activity.

The study was conducted in Nonantola, Northern Italy, and consisted in two experiments, one in July, and one in August-September. Five pig carcasses enclosed in lobster pots were placed inside a freshwater dew pond. The number of P.clarkii detected on each carcass was recorded following a fixed sampling protocol, together with the type and outline of the wounds caused by the crayfish's feeding activity, and meteorological data.

Results show that the Crustaceans attacked the carcasses a few hours after their positioning, starting to damage the external epidermal layer with lacerations up to 4cm, generally ascribable to round shape wounds. The initial day corresponded with the first peak in *P. clarkii* population with up to 100 specimens detected in each lobster pot. In the following days, the crayfishes continued their activity, increasing the percentage of damaged skin, until the full laceration of the dermal layer. The second peak in crayfish's population occurred at end of the floating decay stage, with the carcass lying on the pond's bottom. From that moment, the feeding activity continued in the internal part of the carcass, until the flesh was completely consumed.

This study shows that the feeding activity of *P.clarkii* is crucial in the dismemberment of bodies in freshwater, and the importance in the forensic field is on two main points. First, by detecting differences in the colonization in relation to the position in the water column, these results are useful for the estimation of the mPMI interval; second, it emerged that the typical shape of the wounds made by the Crustaceans could be confused for sharp force injuries which could lead to a wrong report on the causes of death.

Aggregation of Diptera larvae on homogenous and patchy environment

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Blowfly (Diptera Calliphoridae) larvae are usually found on vertebrates cadavers in large masses of hundreds to thousands of individuals. One of the most impressive consequences of these aggregations is the elevation of temperature inside the aggregate. This local increase of temperature can be an efficient mechanism to reduce development time on corpse and thus increase larval fitness. However, the aggregation mechanisms of necrophagous larvae have been poorly studied.

In a first experiment, forty larvae of *Lucilia sericata* were placed in a dish with a homogeneous diet (pig blood & agar-agar) for 30min, 1h, 3h, 5h or 24h. Results indicate that aggregation took place quickly and was reinforced with time. This experiment demonstrated for the first time under controlled conditions the active aggregation of *L. sericata* larvae. To highlight the inter-attraction mechanism between larvae, we performed binary-choice experiments. Forty larvae were randomly placed in a circular arena (with agar-agar) with 2 identical food-patches (beef liver & water) and video-tracked. These experiments were performed on 2 Calliphoridae species: *L. sericata* and *Calliphora vomitoria*.

For the 2 species, a choice did occur: larvae aggregates on a single food-patch (winner spot). Aggregation on the winner spot took place quickly (<30min) and was stable in time. Same results were observed with the 2 species and with heterospecific groups (20 *L. sericata* + 20 *C. vomitoria*). However, an important number of individuals were observed outside food-patches (15 larvae in average).

This observation suggests a behavioral cycle during foraging. Indeed, monitoring of one individual over time showed that larvae did not stayed in aggregate but moved out and came back. In other words, the larvae explore their environment all around the aggregate but returned in contact with congeners. This result, together with previous tests, strongly suggests existence of an aggregation signal used by larvae to locate conspecifics.

Results of these experiments bring better comprehension of the aggregation behaviour of Diptera larvae which impacting on the development of individuals and so the estimation of post-mortem interval.

Olfactory responses of larval *Chrysomya rufifacies* to each other: the formation of larval feeding aggregation on dead bodies

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The physiology and behaviour of blowflies across their life stages have received considerable attention in relation to forensic entomology and yet we know relatively little about the behaviour of individual maggots in their food supply of carrion or cadaver.
Usually, a short time after egg hatching or larval deposition, larvae aggregate and feed *en masse* as they consume decaying soft tissue. How such aggregations form has not been precisely determined, although thigmotaxis is often cited as what helps maintain the aggregation.

Based on the premise that olfaction is the primary sense used by insects to detect and locate various resources, this study explores the behaviour of laboratory-reared *Chrysomya rufifacies* third stage larvae when provided with odour choices in a four-arm airflow olfactometer. Individual larvae were introduced into a chamber which presented odour choices comprised of (a) 100 conspecific maggots of the same age in a vial with no meat; (b) beef heart which had not been occupied by larvae but which was of the same quality and age as that in which the 100 maggots had been reared; and (c) a control of distilled water. Directional responses over time were recorded.

The clear cut choice of the larvae amongst the odour possibilities provided substantial statistical power to the premise that individual larvae move towards aggregations of other larvae based on odour. A possible explanation of such behaviour is the adaptive benefit conferred by subsequent cooperative feeding.

Nocturnal oviposition behaviour of forensically- important Diptera in Central England

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When determining a minimum post mortem interval (mPMI) from insect evidence, occurrence of diurnal oviposition is commonly assumed. To date, there is variable information relating to nocturnal oviposition and very few studies can be applied to UK casework.
This study assessed the occurrence of nocturnal oviposition in Central England for the first time, at 25 time points from 2011 to 2013. Fresh lamb's liver (100g) was placed in bottle traps at a height of 1.5m in an urban location during control (diurnal) and nocturnal periods. Bottle traps were collected at the end of each 5 hour experiment and the presence or absence of eggs recorded. No attempt was made to rear eggs to adulthood. Temperature and humidity were recorded throughout each experimental period, rainfall was monitored as either no rainfall (0), light rainfall (1) or heavy rainfall (2) and the phase of the moon was recorded to indicate potential light levels during nocturnal experiments. A wireless camera was used to observe and record blow fly activity around the bait.

No nocturnal activity or oviposition was observed during the course of the study even during periods when environmental conditions were conducive to blow fly activity and oviposition in diurnal data sets. For diurnal data, backward stepwise linear regression indicated no significant interaction effects, and temperature was the only significant single effect for both activity and oviposition occurring. However, the spread amongst the levels within rainfall and moon phase was very limited and therefore the data were unlikely to provide the necessary contrast to detect significant effects due to these variables. Activity and oviposition occurred at warmer temperatures, above 14°C and 17°C respectively. However, these temperatures should not be treated as cut-off values as there was a paucity of data points at temperatures just below these.
There was also marked overlap in temperature ranges where oviposition occurred and didn't occur, suggesting the influence of other factors. These may not have been detected here due to the extent of variation in environmental conditions relative to the number of data points.

These findings indicate that nocturnal oviposition does not occur in Central England and highlight the importance of the need for further study to improve the accuracy of mPMI calculations by enabling practitioners to assess more accurately when colonization could have taken place.

Forensic entomology in expertise context: the weight of identified uncertainties

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In a suspicious death, investigators or magistrates could request the forensic entomologist to provide the minimum post-mortem interval. The forensic entomologist leads his expertise regarding his scientific knowledge but also with the constraints and realism of the judiciary system. That means he is a justice agent first with the mission to help to the demonstration of the truth in a general context.
Thus, beside reducing the potential inaccuracies of the different methods used in expertise in the spirit of a quality assurance process, he has to make sure of the good understanding of his conclusion. Through 20 years of practice, the different feedbacks led by the department of forensic entomology have permitted to reorganise the FE report and modified the conclusion to simplicity and transparency with the limits of the method clearly communicate. But, justice tends to wait more and more accurate results with a statistic or probabilistic approach.

In order to achieve a useful and mastered result in an investigative context, and not only in a classification perspective, any result has to take into account the weight of identified uncertainties. These uncertainties occur in the different kind of causes that could lead to any observation: sampling conditions and local aspects do provide some examples.
A common bias in establishing forensic evidence is also to consider the collection of observed characteristics without any assessment of their possible mutual dependence. Furthermore, it could be possible to infer on the relationship of presence and absence of different species, not only on entomology reasoning, but helped with statistical assessment on a big data set taken from real cases.

An ecoregion perspective of Calliphoridae microbiome diversity

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Blow flies (Diptera: Calliphoridae) are forensically important insects commonly used as evidence collected for estimating the postmortem interval (PMI). While blow fly diversity has been recorded throughout many locations of the world, there is a need to better understand community variation within and across ecoregions in any given country. Additionally, recent studies have demonstrated the potential to use microbial community succession in assisting with PMI estimates. Given that blow flies directly affect corpse decomposition, they may be important biological mediators of microbial succession and thereby changing these communities through three primary pathways: 1) directly competing with microbes for the resource through feeding activity; 2) introducing antimicrobial compounds to the resource; 3) transporting exogenous microbial species to the resource in a way that shifts community composition and succession; or 4) some combination of these activities.

In an effort to assess how blow fly communities varied with fly species-specific microbiome communities, we conducted a survey of blow flies and their associated internal microbiome communities in several ecoregions of the USA. In June 2013, blow flies were collected from nine sites within six ecoregions of the eastern USA (Central Appalachians, Ridge and Valley, Northern Piedmont, Piedmont, Blue Ridge Mountains, and Southwestern Appalachians), and in August 2013 from seven locations representing two ecoregions near Juneau, Alaska (Pacific Coastal Mountains and Coastal Western Hemlock-Sitka Spruce Forest).

The most abundant blow fly taxa from the eastern USA were *Phormia regina*, *Lucilia spp*., *Calliphora spp*. and *Cynomya spp.*, representing 90.9%, 7.4% and 1.7% of Calliphoridae, respectively, while *C. terraenovae* was most abundant in Alaska. The internal microbiome communities were variable among ecoregions at the phyletic level. In the eastern USA, *P. regina* microbiomes were dominated by Bacteriodetes while Firmicutes and Proteobacteria dominated *C. terraenovae microbiomes* in Alaska.

These results confirm that ecoregion dictates local blow fly species diversity and abundance, while providing the first descriptions of the Calliphoridae internal microbiome community and how these communities vary with species across ecoregions. As molecular sequencing technologies continue to improve, postmortem microbiome communities and their interactions with necrophagous flies may become more important considerations in criminal investigations.

Calculating probabilities for a succession-based PMI estimate

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No published carrion arthropod succession study included sufficient replication to calculate a confidence interval about a PMI estimate based on occurrence data.
We exposed 53 pig carcasses (16±2.5 Kg), near the likely minimum needed for such statistical analysis using two taxa, at a site in Indiana, USA, over three consecutive summer seasons. Insects and Collembola were sampled daily from each carcass for a total of 14 days, by which time each was skeletonized. A life stage of a given species was judged to be potentially useful for succession-based PMI estimation if it was 1) non-reoccurring (not one that has two or more separate periods of presence on a corpse), and 2) found in a sufficiently large proportion of carcasses to support a PMI 90% confidence interval. For this data set that proportion threshold is 45/53.

Daily occurrence was recorded for 266 hexapod species. 17 of these were observed on $\geq$ 45 of the carcasses. At least one gap in occurrence was recorded for every common species. Given that this included dipteran larvae, it is clear that a gap can represent a false negative, and this highlights the fact that "occurrence" is in part a function of collection method. Creophilus maxillosus (Staphylinidae) adults, Phormia regina larvae and Lucilia illustris (Calliphoridae) larvae displayed exceptional forensic utility in that they were observed on every pig and had occurrence gaps of only one day on a single carcass. Inverse prediction of 95% confidence intervals about the duration of insect succession, which we term the succession interval (SI), was more precise when based on two species rather than one species.

Teaching Forensic Entomology from the college to the PhD: experience from the University of Huddersfield (UK)

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Crime Scene Investigation portrayed in television programs has a big impact on the public especially in young people. The fascinating world of crime fighting creates dreams and job expectations in new generations. At the same time the increase request of qualified professionals in the forensic context obliges Universities and other High Educational Institutions to create courses in different forensic disciplines. In addition Forensic Sciences lectures are used to advertise University degrees at the College level.

University of Huddersfield count more than 24000 in different school. Forensic courses are mainly developed in the School of Applied Sciences. To date the School is offering students a BSc and a MSc in Forensic and Analytical Sciences and a new MSc in Forensic Sciences with 4 specialisms (DNA/Body fluids, Forensic Toxicology, Forensic Entomology and Forensic Anthropology).

Forensic Entomology is taught at different levels during the BSc student progression. A general introduction to Forensic Entomology is conducted during the 1st year Forensic Sciences practicals. A further detailed course is run with a 36 hours workshop during the final year. During this workshop students learn about insect collection (indoor and outdoor cases), preparation and identification as well as mPMI estimation using the meteorological data. In addition, the interpretation of the entomological evidence is discussed during the final year. The specific module «Evidence Interpretation» is built on real cases from the specific literature or on the lecturers professional experience.

A campus with different environments, where real outdoors scenarios are mimicked, a crime scene house and a further crime scene room with internal CCTV allow the lecturers to create a realistic teaching environment.

From 2011 Forensic Entomology is taught also at doctoral level with projects on species biology, behaviour characterisation and molecular species identification.
The structure of the modules and teaching strategies will be presented.

Teaching Forensic Entomology in Italy: the GIEF experience

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In Italy, to date does not exist a certification for forensic scientists, and only the different professional bodies, like for example Biologists, Geologists and Medical Doctors, can guarantee a certain professionalism of their affiliates. Unfortunately the belonging to a professional body is not require to work as a forensic expert, with the consequence that people without or with very poor preparation are working on forensic cases. In addition, at the moment only few Master of Forensic Sciences are offered by the Italian Universities and Forensic Entomology is taught in only a few of them. Furthermore Crime Scene Investigation portrayed in television programs has a big impact on the public especially in young people. The fascinating world of crime fighting creates dreams and job expectations in new generations.

In order to offer a solid background in Forensic Entomology, the Italian Association for Forensic Entomology (GIEF) has organised in the last 6 years 4 courses for a total of more than 120 participants. Courses have been done in collaboration with the University of Pisa, Bari and Modena-Reggio Emilia. Participants were mainly Forensic Pathologists, MDs specializing in Legal Medicine and Entomologists working for National Institutions.
The duration of the courses was of 3-4 full days, with a large practical section. This section was run using pig and rabbit carcases to explain the collection, preparation, and identification methodologies following the EAFE standards and guidelines.
Theoretical sections of law, insect biology, insect ecology, systematics, microscopy, temperature reconstruction, molecular biology and thanatology have been offered by the member of the GIEF board and by external national and international experts. In addition a theoretical and practical section on the stored product insects is presented in order to give a complete overview of the discipline.

The feedback from the participants, collected using a questionnaire, about topics, teaching strategy, contents, organisation, praticals and teaching material has been very positive (average >85%) and courses of II level will be run from this year, in order to stimulate the team work and the collaboration between the different specialists working on a casa.
The structures of the course and some teaching strategies will be presented.

Effect of constant temperatures on developmental rate of important forensically blowfly of Thailand, *Chrysomya rufafacies* (Macquart)

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In this study, we investigated the developmental rates of the forensically important blowflies of Thailand, *Chrysomya rufifacies* at five different constant temperatures regimes, 27, 30, 33, 36, 39 °C.
The results showed that developmental time from egg to adult under all regimes ranged from 171.41 to 206.33 h. No adult emergence at 39 °C was found. The sizes of the largest larvae rearing at five different temperatures were statistically different ($F=731.00$, $df=8$ $P<0.0001$). The base threshold temperature was generated using a linear model calculated from different five temperatures, it was 9.43 °C.

MACE (Massive Analysis of cDNA-Ends) - Highly sensitive digital gene expression profiling of *Calliphora vicina* (Calliphoridae) pupae

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Frequently, new methodological approaches in forensic sciences open up advanced perspectives that allow cases to be investigated more differentiated and faster. Such a novel approach is presented by MACE (Massive Analysis of cDNA Ends). Only cDNA Ends of transcripts are sequenced which leads to increased coverage. Each cDNA molecule is represented by one highly specific tag, originating from a 100-500 bp region of the 3' end of a transcript. This achieves ultra-deep analyses to include the rare transcripts at about a 20 times lower sequencing depth as RNAseq. A typical transcriptome consists mainly of a few transcript species in high copy number, which can make up 40-80% of all transcripts, and many transcripts in very low copy number. MACE captures these low-level transcripts.

In order to break new ground in the field of age determination of forensic relevant blowflies, we analyzed the transcriptome of *Calliphora vicina* pupae at 15 different development stages. Determining a post mortem interval using the weight or lentgh of blow fly larvae for the age estimation is well established. But to date there are only a handful studies dealing with the age estimation of blow fly pupae. From this study, we obtain new insights on the gene activity during metamorphosis. Genetic markers for the molecular age estimation of pupae can extend the period for a successful post-mortem interval determination. We got 15 libraries with 3-8 million reads per library. 53538 different transcripts were identified, 7548 were annotated to known insect genes. As *Calliphora vicina* is a non-model organism a de novo transcriptome assembly was performed.

The results help us to identify and understand the function of mRNAs included in blowfly metamorphosis which will assist in molecular age determination of blowfly pupae. The data provides us with insights on gene activity in general and the relationship between different genes. It facilitates the choice and increases the success of gene expression assays. Based on this study gene expression assays of new molecular markers for age estimation are being designed and tested.

Forensic entomology's first steps in Sicily: lava fields and caves

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Sicily is a major Mediterranean island and one of the largest regions of Italy; it contains a variety of ecosystems, ranging from rocky and sandy coasts to volcanic formations and grassy plains and valleys. Also numerous are the volcanic and karst caves scattered throughout the island. Unfortunately Sicily is also one of the most violent Italian regions in terms of organized crime and related violent acts.
These characteristics have stimulated our interest in offering a deeper knowledge of the insects of forensic importance and an additional investigative tool to Sicilian law enforcement agencies. We also want to explore the potential insect activity on decaying matter in dark subterraneous environments.

We started our study in the area surrounding Mt. Etna, the tallest volcano in Europe (over 3000 m). The first study site was set up around the town of Bronte, north-west of Mt. Etna. We placed four carcasses (2 pigs and 2 chickens) on a field of ancient lava, facing west, from December (winter) until skeletonization. We visited our site twice daily and sampled at least once daily.

Two of the carcasses were predated, making it impossible to collect pupae from the field; we were able to collect pupae from the remaining two. The predominant species observed was *Calliphora vicina*, which was the first to colonize the carcasses; less abundant but still present were *Lucilia sp*. We then placed two pig carcasses and one liver trap at different depths within a cave in proximity of our original site. No activity was observed for 20 days; then, *C. vicina* was observed on one of the carcasses, laying eggs in complete darkness at a temperature of 6.4° C. Development under these conditions was delayed and mortality rate was high.
This preliminary trial made it possible to better understand the local requirements and permits for forensic scientific trials in Sicily. It also allowed us to improve our experimental design and helped map new sites and caves where we are expanding our research to collect new data on the distribution of forensically important insects in Sicily and to further explore their activity in caves.

ABSTRACTS FOR POSTER PRESENTATIONS

FORENSIC
LARVAE
ADD
ENTOMOLOGY
FLY
LILLE
EAFE
PMI
LUCILIA
DEVELOPMENT
COURT
INSECT
CRIME
NECROBIA
PAI
TEMPERATURE
TAPHONOMY
CSI

Preliminary observation of ADD value of six landmarks within the pupal stage of the Forensically Important fly *Lucilia sericata* (Diptera, Calliphoridae)

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The pupal stage needs about 50% of the total immature development time of a blow fly species. Establishing the age of this big window of time is difficult and needs alive specimens which are reared up to the adult stage or expensive molecular techniques. However, estimating the age of a pupae can be a crucial task for determining the minimum postmortem interval.

This study reports on a series of laboratory experiments that explore the effects of constant temperature on the development of the species *Lucilia sericata* with particular reference to Accumulated Degree Days (ADD) estimation and the observation of six landmarks to fix different pupal phases. The constant temperatures were 20, 22, 24, 26, and 28°C (± 1°C). Lower developmental threshold temperature (12,5°C) for the development were extrapolated from the linear regression of the developmental rates on each temperature. Following the removal of pupal case, five pupae every 24h were examined with stereo-microscope using 15x magnification, and six landmarks are showed as described below.

Cryptocephalic pupa [S1]: the head is still invaginated. Phanerocephalic pupa [S2]: complete eversion of the head. Pharate adult [S3]: visible segmentation in the thorax, legs and abdomen. Yellow eyed [S4]: beginning of eye pigmentation and ocelli are visible. Tanned chaetae [S5]: all the bristles, both macro- and microchaetae, are fully tanned, except a few at the distal margin of the leg and abdominal segments, which are still brownish. Tanned legs [S6]: all the bristles, legs and wings are fully tanned. Emergence [S7].
For each of the landmarks, the ADD value was calculated for every rearing temperature involved. Data processing showed that the mean ADD value at 20°C was 67.5for S1, 75 for S2, 85.5 for S3, 112,5 for S4, 127,5 for S5, 142,5 for S6 and 157,5 for S7.

In this study the development times from oviposition to adult eclosion were different from those reported in other studies. This discrepancy could be attributed to a variation in the geographic adaptation (intrinsic factors) or to the experimental method (extrinsic factors). Therefore it would be necessary to create a suitable protocol for the observation of pupal characters.

Beetles (Coleoptera) recovered from forensic entomological studies conducted in lowland and montane forested areas in Malaysia

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Beetles (Order: Coleoptera) visiting the monkey carcasses in forensic entomological studies in lowland and montane forested areas, and their succession on different decomposition stages were studied.

A total of 4 replicates were used in each study sites. A total of 24 Coleoptera species belonging to 12 families was recorded on monkey carcasses placed outdoor and indoor. More species of beetles were found in indoor than in outdoor. The species of beetles collected from carcasses increased from the fresh stage to the remains stage in studies conducted in outdoor and indoor in both study sites, indicating that beetles preferred to colonize late decomposition stages of monkey carcasses.

A total of 15 species of beetles were visited the carcasses placed in a montane forested area and 13 species in the a lowland forested area. Staphylinids were dominant on carcasses placed in a montane forested area; while Hybosorids were dominant on carcasses placed in a lowland forested area. Our studies revealed that beetles found on carcasses strictly are specific in certain geographical regions, indicating that they can serve as an evidence in forensic cases occurring in similar ecological habitats. However, geographical data with beetles must be used with caution when a moving of the cadaver is suspected.

A preliminary report on ants (Hymenoptera: Formicidae) recovered from forensic entomological studies conducted in different ecological habitats in Malaysia

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This study reported ant species that were recovered from monkey carcasses in three different ecological habitats in Malaysia. The study was conducted in forested area (Gombak, Selangor), coastal area (Tanjong Sepat, Selangor) and highland area (Bukit Cincin, Pahang), respectively.

Monkey carcasses were used as a model for human decomposition in this study since they are phylogenetically related to human. A total of 4 replicates were used in each study sites. The monkey carcasses were placed about 100m apart each other. Ants attracted to the carcasses were collected using forceps. Ants were observed to prey on eggs, larvae, pupae and newly emerged flies.

A total of 11 species of ants were collected. However, only 7 species were able to identify to species level. This study found that ant species could be found at all stages of decomposition, indicating that ants were not a significant indicator for faunal succession. However, different species of ants were obtained from monkey carcasses placed in different ecological habitats. *Cardiocondyla sp.* was only found on carcasses placed in the coastal area; while *Pheidole longipes*, *Hypoponera sp*. and *Pachycondyla sp*. were solely found on carcasses placed in the highland area. On the other hand, *Pheidologeton diversus* and *Paratrechina longicornis* were found in several ecological habitats. Some ants are common in a same region; geographical data with species must be used with caution.

These data suggests that specific ant species can act as geographic indicators for different ecological habitats in forensic entomology cases in Malaysia.

The first report of *Telomerina flavipes* (Meigen, 1830) (Diptera, Sphaeroceridae) in a forensic case, with redescription of its pupa

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The contribution of Forensic Entomology to forensic practice has been widely documented and in some cases has provided interesting results on the fauna related to corpses. Perhaps the most interesting application to forensic practice deals with the estimation of the postmortem interval (PMI) on the basis of entomological evidence recovered from the corpse or the forensic scene. Such estimation can be done taking into account the larval development of the species breeding in the corpse as well as the succession model of the sarcosaprophagous fauna. For these purposes, the insects of most forensic importance are those that breed in the corpse because they afford an estimation of the time of death when the developmental time of their preimaginal stages is known. Thus, one of the primary goals of forensic entomology studies is to identify those species associated with corpses and, for this goal, actual forensic cases provide excellent substrates for these studies.

This contribution aims to describe an actual forensic case from the Southeastern Iberian Peninsula in which the entomological specimens recovered were used to determine the time of death. These included numerous fly puparia which were identified as *Telomerina flavipes* (Meigen, 1830) (Diptera, Sphaeroceridae). This is the first time this species has been identified from a human corpse, and this is the first record from a forensic case. In addition, its puparia had not previously been well characterized. Thus, the pupa is newly described and illustrated for the first time. A redescription of the cephalopharyngeal skeleton is also presented, providing images and details that can be useful for identification purposes.

An experimental approach to charred remains and entomological evidence identification

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Despite the great importance of entomology in the determination of time of death, there is very little data in the literature concerning its application to the frequently encountered scenario of burnt bodies. In addition, a commonly asked question in court is whether it is possible to determine if a body has been burnt before or after decomposition. In order to answer this question two experiments were performed using larvae, pupae and puparia of *Calliphora vomitoria* (exp 1,2) and puparia of *Megaselia scalaris* and *Lucilia sericata* (exp 2).

In the first experiment larvae, pupae and puparia were placed onto a piece of pork flesh, wrapped in different fabrics (cotton, denim, polyester) and heated under anoxic conditions (nitrogen) for a period of 1 hour at 200, 500 and 700 °C. All the experiments were performed in triplicate and results indicated that the fabric wrappings had a statistically significant impact on the loss of water compared to the control ($p=0.000$) whereas no differences were observed between the different types of fabric ($p>0.470$). The presence of larvae could be detected at 200°C but not at higher temperatures whereas the presence of pupae and puparia was detectable until 700°C under the anoxic conditions used.

In the second experiment a hot-stage microscope with a digital imaging system was used to record pictures and reflected light intensity profiles of puparia heated at 10 °C min-1 to 700 °C under static air. Under these conditions, the pictures (recorded at 25 °C intervals) showed that the puparia are completely destroyed between 475 and 550 °C. No significant differences were detected between species ($p=0.070$). Interestingly, the shape of the posterior region and the spiracles were not affected by the heating until immediately prior to complete combustion allowing potential identification of the samples.

Further experiments are planned in order to study the burning process under aerobic conditions. However, the results to date can already be considered as a useful tool when investigating burned remains.

A preliminary study of insect succession and decomposition patterns on hung pig carcasses (*Sus scrofa domestica*) in Nan province, Thailand

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Forensic entomology is the study of insects and other arthropods associated with dead bodies in order to estimate the mPMI or obtain other important information related with the death. Diptera, especially blow flies, are the most abundant insects that can be found on dead corpses.

This research aims to establish the base line of Forensic Entomology in Thailand by identifying the key species and the succession order on dead bodies. A preliminary comparison between the entomofauna collected from suspended pig carcasses (*Sus scrofa domestica*: weight approximately 22 kg.) in mixed deciduous forest and suburban area were performed in summer 2011, at Nan province, Thailand. The air temperature and relative humidity in the vicinity of the carcasses, the internal carcass temperature and the rate of carcass decomposition were compared with the insect succession for each site.

Over 40 taxa were collected and identified, with flies predominating. The diversity of carrion insects was higher in the mixed deciduous forest than the suburban area. *Chrysomya megacephala* and *Achoetandrus rufifacies* were the dominant fly species in both cases. The first species arriving at the suspended carcasses were C. megacephala and *A. rufifacies*. The first flies colonizing the carcass in mixed deciduous forest where different to the species from the suburban area. *Chrysomya bezziana*, *Chrysomya pinguis*, *Chrysomya chani* and *Hypopygiopsis infumata* were found only in the mixed deciduous forest.The decomposition of the pig carcasses was divided into five discernible stages. Decomposition of suspended carcass in the sub urban area was slower than in the mixed deciduous forest during stage 1 to stage 3 of the decomposition process.

Development of an interactive Puparium identification key for common forensically important fly species of Belgium

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Insects collected on the corpse at different immature stages are used to calculate post-mortem interval (PMI). For any given species, the pupae is the last immature stage, therefore the first to be used to determine PMI when found at a crime scene. Empty or damaged puparia are sometimes the only entomological traces to be collected on a scene after the body has been removed by human intervention or scavenger activity.

Therefore in some circumstances it may be useful to know what species are present though the only traces are puparia.

In this explorative work 7 species present in the center of Europe were studied (*Calliphora vicina, C. vomitoria, Cynomya mortuorum, Lucilia sericata, Phormia regina, Protophormia terraenovae and Sarcophaga tibialis*). Puparia were obtained from laboratory rearing and minimally prepared for observation under stereomicroscope and tabletop Scanning Electron Microscopy (SEM). Only external morphological criteria were used, and those few criteria were found sufficient to create an effective interactive key usable even with empty puparia. The key was developed with Lucid3.3 (*www.lucidcentral.com*).

Necrophilous beetle assemblages in shrub habitat of western Poland

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Type of habitat is one of the most important factors influencing insect community composition, structure and succession on carcasses. Forest and open habitats belong to the best studied in this regard. Their distinct nature and contrasting abiotic and biotic conditions cause substantial differences in both decomposition and insect succession. Shrub habitats have not been the object of such studies. They are characterized by a significant shading and the lack of stratification, and thus large daily temperature variations. In forensic context shrubs are common habitats where human corpses are being found.

The main aim of the study was to characterize necrophilous beetle assemblages occurring on pig carcasses in shrubs during spring, summer and autumn. The second important goal was to compare necrophilous beetle assemblages in shrubs against open (one typical and two transitional) and forest habitats (one typical and two transitional).

A total of 38 domestic pig carcasses were placed in various habitat types starting from early spring until early fall: 12 in shrubs, 13 in open areas and 13 in forest habitats. Examinations took place every day until the end of active decay and less frequently afterwards. Beetles were collected manually and using pitfall traps.

In shrubs a total of 94 species and over 19 000 individuals were identified. Nine families were represented: Staphylinidae (42 species), Histeridae (14 species), Silphidae (12 species), Leiodidae: Cholevinae (12 species), Nitidulidae (5 species), Dermestidae (3 species), Cleridae (3 species), Geotrupidae (2 species) and Trogidae (1 species). The most numerous were Silphidae and Histeridae. Larvae numbered over 15 000 specimens and belonged to six families. The highest species richness was observed in spring and decreased through summer until autumn. Some species showed a clear seasonality: spring, spring-summer or autumn. There were no species related to summer.

Some species showed a clear preference for shrub, open or forest habitats. In this regard shrubs can be placed between open and forest areas, containing species from both. Interestingly shrubs differed from open and forest transitional habitats.

Microbes and maggots on a human corpse: towards a better understanding (step 1)

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Microbes are considered as major contributors in the decomposition of a human cadaver. It is known that insects interact with microorganisms and that particular interaction is being used in medicine for a long time now. In a forensic perspective, microbes (especially bacteria) and insects interactions aren't that detailed in the literature. Better knowledge in this area would be necessary to appreciate the potential implications in the forensic entomology post mortem interval evaluation. Our study aims to better understand the interactions between bacteria and Calliphoridae maggots.

This research requires an initial identification of bacteria of interest during the corpse decomposition, to be able to confront them later to larvae in controlled conditions. In that purpose, we selected corpses with known and short post mortem intervals and took samples of blood in the subclavian area. After culture on blood agar under aerobic and anaerobic conditions, bacterial isolation and identification was done by phenotypic and biochemical methods, completed by mass spectroscopy identification concerning the anaerobic bacteria. In six samples, we managed to isolate 24 bacterial strains. 19 of them are known to be part of the gut microbiota, even though our samples were done at a certain distance from the intestines. These first results prove that bacteria originated from the gut are present, viable and spreading in the early stage of decomposition.

As a second step, we intend to confront sterile maggots with those bacteria in monospecific- inoculated Petri dishes. A chronological observation of bacteria and larvae growth will be done, both qualitatively and quantitatively, These experiments are still in process.

These findings will allow a more accurately appreciation of the human corpse microbiota when colonized by insects, and could be taken into account when interpreting the post mortem interval, which is a crucial stake in the forensic investigation.

Population dynamics of *P. clarkii* as a function of the body submersion/floating stage

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Procambarus clarkii (Girard, 1852) is a freshwater crayfish, native of Southern USA, which spread in Europe after its introduction for aquaculture. Little information is available on the decomposition of bodies in freshwater systems, yet the study of body transformations in this environment is important for the estimation of the minPMI and the alterations of the body that may affect the estimation of the cause of death. In previous experiments, the importance of P. clarkii in the consumption of dead bodies in freshwater systems was assessed.

A new series of experiments was set out in order to analyze the population dynamics of *P. clarkii* in relation to the position of the body in the water column, and, therefore, with the decomposition stage of the carcass. The experiments were performed in Nonantola (MO), Northern Italy (both during summer, one in July and the other in August). In each experiment, five pig carcasses (*Sus scrofa*, L) enclosed in lobster pots were placed inside an artificial freshwater dew pond (67m x 15m, with a maximum depth of 1,5m). The number of *P. clarkii* detected on each carcass was recorded following an established sampling protocol, together with water level and meteorological data.

Results of both experiments show two main crayfish population peaks on the carcasses: the first peak occurs immediately after the deposition of the body inside the pond and the second one occurs 7-10 days after the placement, at the end of the floating stage. This peculiarity is probably connected with a difficulty for the crayfish to reach the carcasses during the floating stage, because of their poor swimming ability. Both peaks occurred in the submersion stage, with the carcass lying on the pond bottom, where it was easily reachable by crayfish.

This study is important in the forensic field related to freshwater systems, because the feeding activity of P. clarkii is crucial in the dismemberment of the body in water. These experiments showed differences in the colonization of the body in relation to its position in the water column. This may be useful for the estimation of the minPMI interval on bodies found in freshwater ponds.

The life cycle of *Dermestes frischii* (Kugelann, 1792) and *Dermestes undulatus* (Brahm, 1790) under regulated conditions

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To improve our data on the lifecycle of *Dermestes frischii*, we observed it at different rearing conditions. This is one of the most abundant species among the family Dermestidae in Northern Italy whose developmental time is not fully known yet. The family Dermestidae is not predatory but interested in dehydrated carcasses as a source of food for adult and immature specimens and it is a valuable indicator of the PMI. Although *D. undulatus* is a Holarctic species, it is rarer in Northern Italy compared to *D. frischii*. In this work we focused our interest on the comparison of the lifecycle of *D. frischii* and *D. undulatus* reared at the same controlled conditions.

In the past we obtained data on the lifecycle of *D. frischii* at 28°C and at 75% RH; now we chose a temperature of 23°C and 75% RH to have a different set of data and to assess development at a more commonly occurring temperature in our region.
Several specimens of *D. frischii* and *D. undulatus*, collected on a dehydrated pig carcass were reared in an incubator and were fed the carcass that had attracted them. The duration of the lifecycle was measured in each single stage and in total; mortality was also considered in each stage and overall.

In the case of *D. frischii*, eggs, larvae, pupae and adults were sampled, fixed and measured and all the results are compared to the ones previously obtained.
The several colonies of *D. undulatus* showed an heterogeneous evolution: the number of the larval stages went from 4 to 6 and such colonies comprehended a lower number of specimens compared to *D. frischii.* The developmental cycle of this species seems to be more influenced by environmental conditions than *D. frischii's*: ovoposition is less regular and the number of the eggs is lower; moreover, the eggs were significantly fragile and hatched with more difficulty. This is why we have not obtained the morphometric data of this species yet.
Nevertheless, the duration of the immature stages and the rate of mortality, which is quite high at this temperature, are comparable to *D. frischii*.

Megaselia scalaris burial behaviour

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Body colonisation patterns may vary due to intrinsic and environmental factors, for example, has the cadaver been buried or is it located above ground?
Colonisation in buried remains depend on the slower decomposition rate of buried bodies, reduced dispersion of the decomposition odours but as well the reduced accessibility to the body.

Phoridae are commonly found amongst the entomofauna of exhumed crypts, coffins and graves. The phorid Megaselia scalaris has been reported as being able to borrow up to 2 m. Limited information is available about the kind of soil this fly is able to dig through to reach a cadaver. In order to answer this question, two different kinds of «soil» were investigated: sand and sandy loam garden soil.

One litre glass cylinders were used to determine depth of soil at 125, 250, 500 and 1000 ml. Ten male and female adult M. scalaris flies were added to the cylinder, sealed and left for one month at room temperature. In addition, a similar experiment with 30 larvae was undertaken. At the end of the experiments the contents of the cylinders were laid out in 50 ml intervals, the developmental stages recorded and counted. All the experiments have been in triplicate.

The maximum number of pupae was collected from the experiments (performed with adults); 125 ml of soil had 171±67 whereas from the 250 and 500 soil experiments 134±165 and 29±50 pupae were collected respectively. No pupae were detected in the 1000 ml soil as well as in all the sand experiments. All the adults placed at the beginning of the experiment were located in the soil columns.
The majority of the pupae were found close to the bait, only a few were found close to the surface. Our experiments have demonstrated that both *M. scalaris* adults and larvae were able to successfully burrow through the sandy loam garden soil (till 500 ml) and oviposit/develop at three depths however neither the adults of larvae were able to burrow through the sand and all specimens deceased on top of the soil with no burial progress made.

Species-specific temperature thresholds for oviposition in forensically important Blowflies (Diptera: Calliphoridae)

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A number of factors affect oviposition behaviour in blowflies with research indicating that temperature is the most significant. Although temperature thresholds for activity and development have been investigated, there is a lack of literature on temperature thresholds for oviposition, and importantly this extends to forensically important blowflies. Here, a pilot study was conducted under controlled conditions in the laboratory in order to develop a method for determining temperature thresholds for oviposition. Temperatures tested were on a scale between 17.5 and 35° C.

Eggs were laid by *Calliphora vomitoria* between 25 and 35° C, with no eggs being laid at temperatures below 25° C. At 35°C, fewer eggs were laid than at the lower temperatures, suggesting that the temperature was approaching a threshold at which oviposition would no longer occur. Results indicate that temperature had a significant effect on oviposition. However, further work is necessary to precisely determine thresholds for oviposition.

Therefore, following this pilot study, a further experiment has been designed to determine lower temperature thresholds for oviposition in three species of forensically important blow fly (Calliphora vomitoria, Calliphora vicina and Lucilia sericata, Diptera: Calliphoridae). Replicates will be established for each species at temperatures ranging from 5 to 40° C, and the number of eggs laid will be recorded. Providing lower temperature thresholds for oviposition in the three named species of blowfly will yield critical information for forensic entomologists in the UK, enabling more reliable determination of the minimum time since death

Postmortem injuries caused by the freshwater crayfish *Procambarus clarkii* (Girard, 1852)

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The feeding activity of animals can affect the decomposition of human cadavers because of tissue laceration and consumption. Nonetheless postmortem animals' activity may cause considerable damage to the body, producing perimortem wounds' modification that could lead to an incorrect assessment of the PMI or a wrong cause of death. Moreover, evaluation of aquatic arthropods activity can be useful in estimating the PMSI (Post Mortem Submersion Interval).

Procambarus clarkii (Girard, 1852) is a freshwater crayfish native of south-eastern USA, present in Europe with invasive populations, that are found also in Northern Italy. Previous experiments assessed the influence of the crayfish in the decomposition of animal carcasses in a freshwater system. The aim of this study was to investigate the role of *P. clarkii* as a carrion-feeder, studying the type and outline of the wounds caused by the crayfish's feeding activity on the carcasses.

Two experiments were performed in Nonantola (MO), Northern Italy (both during summer, one in July and one in August). Five pig carcasses (*Sus scrofa*) enclosed in lobster pots were placed inside an artificial freshwater dew pond. For each carcass the number of feeding *P. clarkii*, together with the type and size of lesion was carefully recorded following a fixed sampling protocol, together with water level and meteorological data.

Results show that in both the experiments, the Crustaceans attacked the carcasses a few hours after their positioning, starting to damage the external epidermal layer with lacerations up to 4cm, generally ascribable to round shape wounds. In the following days, the crayfishes continued their activity, increasing the percentage of damaged skin until the full laceration of the dermal layer. From that moment, the feeding activity continued mostly in the internal part of the carcass, with the attack of the internal organs, until the flesh was completely eaten and only bones remained.

The study of post mortem injuries could be very important in real cases of dead bodies recovered in freshwater systems, because the typical shape of the wounds caused by the Crustaceans could be confused for sharp force injuries and lead to a wrong report on the causes of death.

Ubiquity™: Uniform Base InQUIry utiliTY

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Knowledge of the Taxonomy, biology, ecology, behavior and placement of arthropods found on a crime scene provide useful data about when, where and how, a crime was committed. By now these samples must be considered a physical evidence just as blood stains, fingerprints, hairs, fibers, or any other biological material, the collection and delivery of which is summarized in Amendt et al (2007).Unfortunately, barely «real world» follows ideal guidelines: something in the protocol should miss, accidentally or by several diverse reasons.

IT tools can actually help by suggesting the operator to follow appropriate steps and proper techniques during survey, data and evidence collection.

Here we present a software based on UBIQUITY™ (that is the acronym of "*Uniform Base InQUIry utiliTY*") the underlying idea of a rational approach to field data acquisition and quasi-certified storage.
The software originates from the collaboration among the winners of "NO-BLE Ideas Project", a competition aimed to promote the innovation in goods and services production, DiSSPA-UNIBA Aldo Moro and GIEF (Gruppo Italiano Entomologia Forense).

The «Crime UBIQUITY™ » is shaped to run on an Apple iPad™, and can drive operators during survey from the approach to the crime scene to a first arthropods identification for their appropriate collection and storage.

Crime UBIQUITY™ helps the operator during the whole survey providing a checklist of equipment for the survey, allowing data recording by voice clips to store descriptions associate with photo, movies, alphanumeric data, GPS coordinates and more. The data can be stored both in the tablet and online in real-time into a Cloud repository, if a proper connection is available. The software can help in data management by exporting a report draft from the tablet, once the fieldwork is out.

The effect of larder beetle (Coleoptera: Dermestidae) long-term feeding in low temperature conditions

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In April 12, 2013 in the basement of a tenement house located in the very center of Łódź - the third largest city in Poland - there was found a corpse of an unidentified man. The body was fully clothed, lying on a pile of debris and boards. The head was separated from the rest of the body. During autopsy, it turned out that the corpse is partly mummified, partially converted into adipocere. In clothing, in the body cavities and under the skin there were numerous remains of insects, and very large amounts of fibrous material resembling coir.

Entomological analysis revealed the presence of very large number of exuviae and singular beetle larvae of the genus Dermestes. Several imagines found represented two species - *Dermestes lardarius* and *D. haemorrhoidalis* (quite rare in Poland). In addition, there were found about 20 empty and damaged puparia of Diptera, probably of the genus Lucilia.

Fibrous material consisted exclusively of faeces of larder beetle larvae. Individual fibers reached considerable length of more than 5 cm, which may indicate an uninterrupted, long-term feeding, without much movement from place to place.
A significant part of the beetle faeces has been consumed and used for the construction of cocoons by the larvae of Tineidae. There were no live specimens, only empty pupal cuticles and several dead specimens of *Niditinea fuscella*, species usually encountered in the nests of birds, feeding on their feathers and droppings.

The cellars of this type of houses served for years as storage rooms for coal intended for fuel, now they are mostly abandoned. On the basis of long-term study conducted in Łódź it can be stated, that conditions prevailing in such places throughout the year are fairly constant, when compared to the open space, with temperatures exceeding 10°C only during the hottest summer months and almost never falling below zero. Such conditions are favorable to substantial elongation of larval period of beetles and their reduced mobility, which can lead to the effects observed.

Temperature data have been gathered thanks to National Science Center grant N N304 286640.

Life cycle and larval morphology of *Euspilotus azureus* (Coleoptera: Histeridae): First record of the species on corpses

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Histeridae is one of the most sampled beetles on carcasses in Brazil and *Euspilotus azureus* (Sahlberg) is the most collected histerid on carcasses in South America. This species is found in all decay stages. It is usually used to estimate post mortem interval (PMI) through succession, although some authors consider that its immature development time is underrated for PMI estimative. Despite its forensic interest, little is known about *E. azureus* biology and immature morphology.

The aim of this work is to describe the life cycle and larvae of the species, and also report the first record of the species in a corpse. For this purpose, a rearing protocol was developed and an experiment was conducted in Universidade Federal do Paraná (Brazil) where 280 individuals were monitored individually from egg to adult under controlled conditions (Temperature: 25°C; Photophase: 12hrs; Relative Humidity: 65%). Immature specimens (N=20) were fixed on Kahle-Dietrich solution for morphological studies.

Biological results show that *E. azureus* presents four ontogenetic stages: egg, larvae (two instars), pupae and adult. The total life cycle was 41.4±2 days. The lifetime of first larval instar (L1) was 5.5±0.7 days and second larval instar (L2) was 13.2±2 days. The life cycle data corroborate those observed for *Phelister haemorrhous* Marseul, however, *P. haemorrhous* larval development was shorter.
Regarding the morphological data, L1 presents total length of 6±1mm; body cream-coloured, largely membranous with distinct segments; prognate head with strong sclerotization; coronal suture present on the basal sixth; antennae 3-segmented with the second antennomere about half the length of the first; pronotal plate about 2 times wider than mesonotum in lateral view; membranous abdomen 9-segmented with dorsal, ventral and lateral setae; one pair of spiracles per segment (1-8) and 2-segmented urogomphus. The L2 differs from L1 by its body length (12±2mm); more distinctly segmented; reduced coronal suture and pronotal plate about 1.5 times wider than mesonotum.

In conclusion, the life cycle results help comprehend E. azureus biology and its importance on forensic entomology. Morphological data will help identifying the species when collected on carcasses or corpses. In addition, it is worth mentioning these results are unprecedented for Histeridae in Brazil.

Beetles to estimate mPMI? Case reports utilizing beetles in Brazil

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Coleoptera are commonly used to estimate post mortem interval (PMI) through entomological succession, mostly due to lack of knowledge about the biology of necrophagous beetles. Some authors even suggest that beetles are underrated when estimating the time of death. Thus, a compilation of three cases is presented for the state of Paraná (Brazil), where Coleoptera larvae were used to perform the minimum PMI (mPMI) estimative.

Entomological samples were collected on corpses by crime scene investigators from the Instituto de Criminalística do Paraná and sent to the «Group of Forensic Entomology» from Universidade Federal do Paraná (GFE- UFPR). The estimative was based on the development of immatures of *Oxelytrum sp.* (Silphidae) previously studied by our group.
On *Oxelytrum* studies, individuals were monitored from egg to adult on six different temperatures (not published data) and it has been used to mPMI estimative.

On the first case, a corpse of an adult man was found in a rural area, on October 18th, 2011, the mean temperature was 23.7°C and mPMI was approximately 9 days. The second case, a male corpse was found near a dense forest on December 22nd, 2011, the mean temperature was 24.1° C and the mPMI was approximately 9 days. On the third case, the corpse of an adult man was found on Curitiba suburbs on February 12th, 2013, the mean temperature was 20.2°C and the estimated mPMI was around 18 days.

This set of case reports highlights beetles as a functional group for estimating time of death. In European case reports, Coleoptera have been collected and rarely used for this kind of estimative. Considering the potential importance of some necrophagous beetles for the PMI estimative, GFE-UFPR is performing a series of studies to elucidate the biological parameters and development time of those species. It is also noteworthy that GFE-UFPR is the only group known to use beetle larvae to estimate mPMI in Brazil.

The Mummy and I: how to preserve a friendship

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On October 2013, in a small city in the North of Italy an old woman (R.) died after a long illness. The woman was known to have a long friendship with her daughter-in-law's mother (G.), insomuch as the women lived together. Furthermore, G. was known to be a sage, able to predict the future and to bless people. Even though people did not see her for many years, they continued to bring offerings to obtain her blessings.

When R. died, G. unexpectedly failed to attend the funeral of her best friend. Worried about the well-being of G. her relatives together with law enforcement forcibly entered into her apartment. The corpse of G. was found in a room of the apartment, seated on an armchair, completely mummified and covered with a linen sheet. Further investigations confirmed that balms had been used to preserve the corpse and to protect it from the colonization of insects.

Despite this, on the mummified corpse *Dermestes frischii* Kugelann (Coleoptera: Dermestidae) larva exuviae, frass and artifacts were found. Dermestid beetles are unpredictable colonisers as they have been reported arriving at a corpse between 24h to 3-6 months after death, depending on the environmental situation. Dermestid frass has been found between 1 month and 10 years after death. In the present case the date the death of G. was estimated to be between 16 and 18 years before discovery.

Dermestes murinus (Coleoptera: Dermestidae) colonization of hanging pig carcasses in Central Europe

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Larder beetles (Dermestidae) are mostly scavengers feeding on dry skin, hair and bones. Many observations on Dermestidae from Europe and other continents have shown that they are associated with advanced decomposition and remains stage. From the other hand in warmer regions larder beetles can be present on carcasses few days after death. In such cases their presence on carcasses was observed during bloating or even on fresh carcasses. Such observations have not been made for decomposing bodies in temperate climate of Central Europe.

In 2012 and 2013 a field experiment on insect succession and carrion decomposition of hanging carcasses was carried out in hornbeam-oak forest in western Poland. The experiment was conducted in three seasons: spring, summer and autumn. In each period of research four domestic pig carcasses weighing approximately 27.5 kg were used. Two carcasses have been hung on branches of trees and carrion of the other two were placed on the ground as a control sample. The examination of carcasses took place every day until the beginning of advanced decay.

During this study *Dermestes murinus* was observed frequently but only during spring and only on hanging carcasses. In 2012 D. murinus appeared on hanging carcasses as a first beetle individual before the beginning of active decay (5th day of decomposition). In 2013 spring appearance of *D. murinus* was observed later (11th and 19th day of decomposition) but it was after a long and cold winter and in the result colonization of insects was delayed and started in the 10th day of decomposition. Difference in *Dermestes murinus* appearance on two types of carcasses could be a result of various humidity. Hanging carcasses were exposed to the wind and consequently were drier than carcasses which were on the ground. Hanging carcasses did not have a contact with ground which was frozen after winter and it also could have an impact on these differences.

Results of this study could be very useful for estimating PMI in case of hanging bodies and can prevent misleading use of Dermestidae beetles in forensic entomology.

Instar determination in forensically useful beetles *Necrodes littoralis* (Silphidae) and *Creophilus maxillosus* (Staphylinidae)

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In order to estimate postmortem interval from immature insects, it is necessary to accurately determine which instars are present in a corpse sample. Unfortunately, most forensically useful beetles lack morphological features specific for particular instars. Consequently, the only way to distinguish larval instars of beetles is to measure some of their morphological features. The wide range of prevalence, intensive reproduction on the remains and PAI (preappearance interval) being dependent on the temperature, make both, *Necrodes littoralis* and *Creophilus maxillosus*, extremely important species in assessing the time of death in the Palaearctic Region. The main aim of the research was to find the features useful for instar determination of *N. littoralis* and *C. maxillosus* and to create easy to use classifier of larval stages.

Six features were measured during the research: the distance between dorsal stemmata, the width of the pronotum, the length of the body, the width of the mesonotum, the width of the 8th abdominal tergit, and the length of the 1st segment of urogomphus. To create a classifier, a linear discriminant analysis (LDA) was used. Using the measurements of two morphological features with the best discriminant power, simple classifier was created. The main part of validation of the classifiers was performed by fully sclerotized larvae. To get the view of the performance of classifiers with difficult specimens, we additionally tested them with larvae just after ecdysis. All features were incorporated into discriminant functions in the case of both species. Classification functions were solved for training larvae and perfect results were achieved.

Perfect results were also attained for test larvae of both species in main part of validation and for N. littoralis in the additional validation. In the case of *C. maxillosus* two larvae of third instar just after ecdysis were misclassified as a second instar. Simple classifiers performed similarly well with fully sclerotized larvae, but in the case of larvae just after ecdysis they revealed higher misclassification rate than complete classifiers.
Consequently, it is recommended for casework to use simple classifiers for classification of fully sclerotized larvae and complete classifiers for classification of larvae just after ecdysis.

Molecular identification of forensically important cheese skippers (Diptera: Piophilidae) using COI and EF1?

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Species-specific identification of flies plays an important role in forensic entomology and is obligatory for an accurate post-mortem interval calculation. Many important Diptera and Coleoptera taxa of the cadaver community can already be identified by common barcoding approaches, i.e. by sequencing a 648 base-pair region in the mitochondrial cytochrome c oxidase 1 gene. Nevertheless there is still a lack of reference for barcodes of species that can be especially found later during decomposition on a cadaver (up to several weeks or even months post mortem).

Flies of the family Piophilidae are a good example for this gap of knowledge. Until the recent past a jumping larva on a cadaver would be mostly identified as *Piophila casei*, the most popular skipping fly in literature. However, *P. casei* is not the only piophilid occurring on carrion, and for example, the species *P. megastigmata* could be easily mistaken for it. Due to the fact that a sufficient key of immature stages is still missing and the larvae of many piophilid species even remain unknown there is the need for additional tools to support the sound identification of forensically relevant Piophilidae. Therefore, various adult piophilid specimens were collected at 11 locations in four European countries: Germany (n = 3 stands), Poland (n = 1), Spain (n = 5) and Portugal (n =2).

Beside the mentioned barcoding region we analyzed additionally a 400bp long region of the nuclear elongation factor 1 alpha (EF1a) and established the molecular ID of 10 piophilid species. Moreover the study presents the molecular phylogeny of the examined taxa.

An investigation that examines the development rate of blowfly pupae in a range of substrates

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Very little research is currently conducted that examines the pupal stage of development. This is surprising as particularly in blowflies 50% of the species development is spent as a pupa. Here we investigated the effect of temperature on pupal development of two common blowfly species (Diptera: Calliphoridae) associated with cadaver succession, *Lucilia sericata* (Meigen) and *Calliphora vomitoria* (Robineau-Desvoidy) were chosen for the study Potential interaction effects were included by utilising five forensically relevant substrates; sawdust, sand, soil gravel and a control of no substrate categorised as air. A controlled laboratory temperature (24.5°C); with a 16:8 light:dark regime in a walk in incubator was established as a baseline treatment. Equivalent experiments were also conducted in an outdoor suburban setting in the north west of England and conducted between June and August 2007. For each experiment, a 15 cm plastic beaker was filled to a depth of eight cm with substrate, and this was placed into a square plastic container filled with the same substrate. Five pupae were placed on the surface of the substrate in each beaker and then covered by a further three cm of the same substrate.

The plastic containers were then placed outdoors (natural temperature regime) or in the incubator (steady temperature regime) until the flies emerged. The maximum and minimum temperature and humidity of each day was recorded in the area where the containers located. A Microsoft webcam and USB low-level lamps were attached to a laptop computer located above the experiments. A Microsoft Timer Shot program was setup to capture an image every five minutes, enabling the accurate documentation of the date and time of the emergence of each fly. Due to time constraints the constant temperature regime was only carried out using L. sericata with no effect on development rate ($F_{8,80} = 0.05$, p ? 1.00). However in a natural temperature regime there were significant substrate effects in both species. The development was significantly different in each substrate ($F_{4,60} = 91$, p Substrate does make a difference to time of pupal development in fluctuating temperatures. Time constraints and sample size were 2 of the main problems with this study. Future studies should focus on determining the thermal conductivity of common substrates and the link, if any with this development stage. All the substrates used in this investigation were dry but research into thermal conductivity suggests that as the moisture content of a substrate increases so does the thermal conductivity.

Entomotoxicology to determinate drugs substances and the determinations of PMI: real cases

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Entomotoxicology deals with toxicological analyses of carrion-feeding insects in order to identify drugs and toxins present in tissues from dead bodies. It also investigates the effects of drugs on insects development and lifecycle in order to avoid a wrong mPMI. The main application of this methodology is on advanced decomposed bodies where the substrates routinely used for toxicological tests may be subject to degradation or contamination.

Two cases studied at the Institute of Forensic Medicine of University of Pisa between August 2009 and May 2012 are presented. Case 1 concerned a 36years old woman found dead in her home and seen alive 5 days before. Case2 involved a 32years old man found in an abandoned warehouse. Insects found in the first case were Calliphoridae (*Lucilia sericata, Chrysomya albiceps*) and Sarcophagidae (*Sarcophaga sp*). In the second case insects found were Calliphoridae (*Lucilia sericata*), Fannidae (*Fannia scalaris*), Muscidae (*Hydrotaea capensis, Muscina stabulans*). They were sampled; some of them were stored in ethanol, after hot water fixation for identification and age estimation, whereas others (*Lucilia Sericata*) were frozen at -20°C for toxicological analysis. Larvae (500 mg), after repeated washing to remove any external contamination, were placed in a tube containing ceramic beads. Three ml of phosphate (pH 5.2) and the deuterated internal standards of the substances to be searched were added. We proceeded to complete homogenization with a homogenizer model Precellys (Bertin, France). After centrifugation, the supernatant was recovered and a solid phase extraction(SPE) was performed. After derivatization with PFPA/ PFPOH samples were analysed with gas chromatography/mass spectrometry(GC-MS).

Larvae from the first case indicated the presence of benzoylecgonine and cocaine from the second the presence of benzoylecgonine, cocaine and morphine. Both results are in agreement with the data obtained from cadaveric tissues. Entomotoxicology is a useful to identify drugs and toxins present in death body tissues, but unfortunately is a scarcely known discipline among forensic pathologists and often toxicologists. At the moment we are creating a new research group on this topic at the University of Pisa in order to develop this discipline and analyse in detail the most common drugs reported from our region.

Effect of body mass and clothing on carrion entomofauna

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Body mass and clothing are factors of high forensic importance. Both factors, and in particular carcass mass probably have large effect on the composition and structure of carrion insect assemblages. Unfortunately, these effects were not studied in a forensically-relevant range of body masses. Here simultaneous effects of carcass mass and clothing are analysed.

Complete factorial block design was used with four levels of carcass mass (small carcasses: 5-15 kg, medium carcasses: 15.1-30 kg, medium/large carcasses: 35-50 kg, large carcasses: 55-70 kg) and two levels of carcass clothing (clothed and unclothed). Pig carcasses (N=24) were split into three blocks (the day of carcass exposition: 17 May, 16 July and 27 August 2012). Insect samples were taken on a daily basis until about the 20th day after the exposure, and less frequently afterwards. Sampling was performed by pitfall traps (2 per carcass) and manual collection.

Necrophilous species from families Calliphoridae, Sarcophagidae, Muscidae and Piophilidae (Diptera), as well as Silphidae, Histeridae, Dermestidae, Cleridae and Nitidulidae (Coleoptera) were identified and analysed. Generally, effect of carcass mass was much larger than effect of carcass clothing. As for the adult insects, larger carcasses attracted them more abundantly and these differences were particularly evident in the case of late-arriving species. As for the immature insects, some late- colonizing species were not present on small corpses at all and were less frequent on medium corpses, as for example *Necrodes littoralis* (Coleoptera: Silphidae), *Necrobia spp*. (Coleoptera: Cleridae), *Omosita colon* (Coleoptera: Nitidulidae) or *Stearibia nigriceps* (Diptera: Piophilidae). Moreover, in majority of species residencies distinctly increased with carcass mass, whereas the preappearance interval revealed no relation to both factors. Summarizing carrion insect assemblages are more complex, abundant and long-lasting on larger carcasses as compared to smaller carcasses.

Effect of carcass mass and clothing on pattern and rate of decomposition

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Body mass and clothing are factors of high forensic importance. In casework corpses differ according to mass and kind or extent of clothing. Unfortunately, effects of these factors on decomposition are unclear. Here simultaneous effects of carcass mass and clothing on gross processes in decomposition, carcass mass loss and overall rate of decomposition are analysed.
The experiment followed a complete factorial block design with four levels of carcass mass (small carcasses: 5-15 kg, medium carcasses: 15.1-30 kg, medium/large carcasses: 35-50 kg, large carcasses: 55-70 kg) and two levels of carcass clothing (clothed and unclothed). Pig carcasses (N=24, 6 in each category of mass, half clothed and half unclothed) were grouped into three, separated in time blocks.

Generally, carcass mass revealed significant and large effects in almost all analyses, whereas carcass clothing had only minor influence on advanced decay. Carcass mass differently affected particular gross processes in decomposition. Larger carcasses revealed more efficient putrefaction, and less efficient active decay. The average rate of active decay showed significant, logarithmic increase with an increase in carcass mass, however only if it was driven solely by blowflies. In case of multi-guild active decay (larval blowflies followed by larval *Necrodes littoralis* (Coleoptera: Silphidae)), which was regularly recorded in medium/ large and large carcasses, the average rate showed only slight and insignificant increase with an increase in mass.
These results demonstrate that lower efficiency of active decay in larger carcasses results from the multi-guild and competition-related pattern of this process. Interestingly, pattern of mass loss in large and medium/large carcasses was not sigmoidal, but rather exponential. The overall rate of decomposition was strongly, but not linearly, related to carcass mass. In a low mass range it increased with an increase in mass, then at about 30 kg a distinct decrease was present, and again at about 50 kg decomposition rate began to increase. Until about 100 accumulated degree-days larger carcasses gained higher Total Body Scores than smaller carcasses, afterwards the pattern was reversed. In conclusion, current results demonstrate that cadaver mass is a factor of key importance for decomposition, and as such it should be taken into account by decomposition-related methods for PMI estimation.

Estimating post-mortem interval from Total Body Score, correcting for body mass

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One of the most promising approaches to post-mortem interval (PMI) estimation from decomposition uses Total Body Score (TBS) system and regress it against accumulated temperature. It was however demonstrated that this approach gives inaccurate estimates for some geographical locations. Recent results indicate that low accuracy of TBS system may result from large effects of corpse mass on the rate at which TBSs increases during decomposition.

Here we analyse what can be done to accommodate corpse mass effects while estimating PMI. For that purpose multiple regression model was tested with carcass mass and TBS as predictor variables and accumulated degree-days (ADD) as response variable. The model was calculated by using results of the complete factorial block experiment with four levels of carcass mass studied (small carcasses: 5-15 kg, medium carcasses: 15.1-30 kg, medium/large carcasses: 35-50 kg, large carcasses: 55-70 kg). Pig carcasses (N=24) were grouped into three, separated in time blocks. TBSs were determined on a daily basis until about 20th day post-mortem, and less frequently afterwards. The system of Megyesi *et al.* [2005] was used after modification to take pig carcasses into account. The model performance was tested with external body of data originating from another large experiment with pig carcasses. The initial results are promising and at present the main part of analyses is under way.

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In the 1930's, the application of forensic entomology in Hungary was based on the observations of an expert in forensic medicine called Dr. Dénes Schrancz. According to expert advice in the 1970's made by Ferenc Mihályi, a museologist of the Hungarian Natural History Museum, acquitment was delivered in a murder case, which has been mentioned as the captain's case in numerous comprehensive articles. Forensic entomology has fallen into oblivion or rather has been disused in our country since then. From the beginning of the 21st century, the Hungarian Police in the Hungarian Institute for Forensic Sciences and developed appropriate protocols for the reuse of forensic entomology with the application of their latest knowledge and modern devices available.

Identification of Forensically and Medically Important Blowfly: Calliphora vicina (Rob-Desvoidy) in TURKEY

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Blowfly species, of great importance in forensic sciences, form colonies on human and animal corpses. They belong to Calliphoridae family and the most important feature of these species is that they are the first to arrive at corpses. They lay their larvae on corpses and play an important role in decomposition. Many species of blowflies appear globally while some of their species live in certain regions. A Calliphoridae species, *Calliphora vicina* is frequently encountered in Turkey just as they widely appear in the rest of the world.

The aim of this study was to summarize features of *Calliphora vicina* in Turkey. This is an experimental study. The specimens were obtained through traps hung on trees at certain distances and morphological features of the species in third instar and adult stages were evaluated by using different keys to identify *Calliphora vicina*. A summarize key was created to identify *Calliphora vicina* in Turkey.

There was not a significant difference in morphological features of *Calliphora vicina* in Turkey and in Europe.

Insects from Pre-Colombian Peruvian mummies: a funerary archaeoentomological approach

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Funerary Archeoentomology was used for the first time by Jean-Bernard Huchet in 1996. This author defines "l'Archeoentomologie funeraire" as the use of the information provided by the insect fauna associated with archaeological human remains in order to define the peri (around) and post mortem events or the funerary practices.

The museum of Human Anatomy (University of Pisa) hosts a large collection of artifacts and human remains belonging to the Chimù-Chancay population. This material, partially analysed in the 18th century, is now under a more concentrated study. The preliminary observations of the samples collected from different kind of materials (cotton, leaves, plant fibers, etc) present between the fabric layers that formed a sort of cocoon in which the body was wrapped (the “fardo”) revealed a very complex entomofauna. Insects and other arthropods found in the fardos can be grouped in five major groups: 1) insects related with body decomposition, 2) insects associated with the offerings, 3) ectoparasites, 4) organisms associated with the environment where the bodies were stored and 5) contamination occurring in previous years in the museum store
In the first group, remains of insects belonging to Diptera (Sphaeroceridae undetermined) and Coleoptera (*Dermestes maculatus, Mezium americanum*), in the second group, Coleoptera belonging to the family Tenebrionidae (*Gnatocerus cornutus* and undetermined species) and Anobiidae associated with corn and cereals. The ectoparasites found in two of the five analysed « fardos» belong to a species of flea (Aphaniptera, Pulex irritans), whereas some nits, louse's eggs (Phthiraptera, *Pediculus humanus capitis*), were collected from hairs. Several fragments of spiders and pseudoscorpions were found on the analyzed materials. Pseudoscorpions are arthropods related with the soil and often with hypogean cavities. Their presence suggests that the fardos stored at the Museum of Pisa had been buried or stored in soil holes before their discovery. This observation is confirmed by other “fardo” findings (Huchet et al., 2013).

Insect feeding on museum samples, especially belonging to the Dermestidae and Lepismatidae (Thysanura) families have been found among the collected material. These findings require a rethinking of the storage and preservation strategy in place at Pisa museum in order to avoid the destruction of such an important collection.

Preliminary study on the relationship between the size of the body and the arrival of insects

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Many works on the decomposition of corpses say that the chronology for the arrival of insects on a decomposing body does not necessarily follow the pattern of eight wave squads. Furthermore, it is well accepted intervention Coleoptera, very soon after the decomposition. Therefore, they are the second group of insects to colonize a corpse, just after the Diptera.

In Algeria, works addressing forensic entomology are very recent and fragmentary and mainly Diptera. As for carrion beetles, no study has been conducted. It is in this perspective that our investigations were made on two dead animals, an adult dog and a puppy.

The study was conducted in the spring in an open space which level, the two cadavers are installed in separate cages, specially designed to welcome and protect them from predators. Just hours after their deposit, the two bodies were already visited by insects begin who to lay. After the capture of insects and their identification, the results reveal specimens belonging to two major orders, flies and beetles that best represent the necrophagus fauna. Our daily sampling has allowed us to collect a total of 797 specimens on the cadaver of dog adult, 58% of which belong to the order Diptera and 39% of those beetles. On the cadaver of the puppy, we could identify 165 specimens of which 26% belong to the order Diptera and Coleoptera 74%.

At the end of our investigation, analysis results reveal an abundance of necrophagus insects on the large substrate decomposition. It is represented mainly by Diptera compared to the small body which is visited especially by beetles.

Development and survival of immature *Muscina prolapsa* (Diptera: Muscidae): Effects of interspecific competition

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The genus Muscina (Diptera: Muscidae) is an important part of the necrophagous community and some of its members are able to colonize buried remains even in a depth of up to 40 cm. Despite its common occurrence, knowledge about development is still poor. We studied the immature development of *Muscina prolapsa* under three constant temperature regimes (17 °C, 20 °C, 25 °C) in the laboratory and under fluctuating temperatures in a field setting (average: 23,03 °C). Since the occurrence of the forensically important blow fly *Calliphora vicina* cannot be ruled out in shallow burials, we also investigated possible interspecific competition and its influence on the development and survival of *M. prolapsa*. Three predator-prey-ratios were reared at constant 20 °C (Muscina: Calliphora, 10:30, 20:20 and 30:10).

In the pure cultures of *M. prolapsa*, development time ranged from 16,7 days at 25 °C to 29.7 days at 17 °C. The lower development thresholds by use of linear regression were calculated for larvae (4.65 °C) and pupae (9.35 °C). Larval growth was plotted against accumulated degree hours (ADH). After a mean of 2668 ADH larval growth ceased. Larvae showed no migratory phase and pupate on the food substrate, without reducing size like it is known from blow fly larvae. Interspecific competition with *C. vicina* led to a prolonged development time of pupation up to 1.5 days for *M. prolapsa*. Larval mortality of *M. prolapsa* decreased with decreasing number of *C. vicina* larvae (10:30, 25 % to 30:10, 9.95 %). The larval mortality of the pure *M. prolapsa* culture revealed a rate of 31.25 %, the total mortality rate was 48.75 %.

The development time of *C. vicina* was also influenced by the presence of *M. prolapsa*. Larvae of predator-prey-ratios 30:10 and 20:20 started their post-feeding-stage significant earlier (up to
1.9 days) compared to a pure *C. vicina* culture. At a ratio of 30:30 and 20:20 larvae pupate up to 1.2 day earlier and the adult flies need up to 1.9 days less for finishing the pupal period than the specimens of the pure *C. vicina* cultures.

Development of a GC-MS method for the detection of α and β Endosulfan in *Calliphora vomitoria* (L.) (Diptera: Calliphoridae)

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Forensic entomology is accepted as an essential tool in many homicide cases. Entomotoxicology is a branch of forensic entomology focused on detecting drugs or other toxic substances in decomposing tissues from insects. The literature mostly reports the extraction of drugs and chemicals (morphine, cocaine, opiates, benzodiazepines, paracetamol, mercury, etc.) from blowfly larvae. However, a small number of studies are focused on the entomotoxicological analyses of chemical compounds associated with poisoning wildlife and pets.Organochlorine pesticides such as Endosulfan are the most common substances used to prepare poisoned baits (1223 cases between 2005 to 2009, data from Centro Regionale Antidoping of Torino, Italy). In a few cases Endosulfan has been involved in human fatalities. Due to its toxicity in 2012 the Stockholm Convention Persistent Organic Pollutants (POPs) dismissed the production and the use of Endosulfan by 2018. In this study larvae *of Calliphora vomitoria* L. were reared on substrates (beef liver) spiked with three different amounts of α and β Endosulfan: 10ppm, 25ppm, 50ppm. Another liver was used as control. These levels of Endosulfan were calculated based on concentrations found in livers of both humans and animals which died as a result of Endosulfan poisoning.
100mg of *C. vomitoria* larvae, pupae and spent pupae in each experimental group were processed using QuEChERS method and analyzed using gas chromatography coupled to mass spectrometry (GC-MS) in order to determine the presence of Endosulfan in the chitinic array. Furthermore, 30 individuals of each experimental group at these different stages were sacrificed to determine their developmental stage. This study demonstrated that it was possible to extract Endosulfan from larvae reared on beef liver spiked with 25ppm and 50ppm Endosulfan (with α Endosulfan concentration generally higher) but not with 10 ppm. Developmental times for *C. vomitoria* reared on 10ppm and 25ppm were not significantly different to the control, whereas individuals reared on 25ppm showed a significantly different developmental time from the control. *C. vomitoria* reared on 50ppm developed much slower and never reached the pupal instar. As larvae their development was significantly slower than the control.

Detection of Gunshot Residues (GSR) in blowfly larvae using Scanning Electron Microscopy equipped with Energy Dispersive X-ray microanalysis (SEM-EDX) and Inductively Coupled Plasma Mass Spectrometry (ICP- MS)

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In addition to estimating the postmortem interval (PMI) insects that feed on carcasses may also represent a reliable specimen for toxicological analyses (entomotoxicology). This is especially so in the absence of tissues and fluids normally taken for such purposes. There are many studies involving the extraction of drugs and chemicals from blowfly larvae (Diptera: Calliphoridae) associated with a corpse, but only a few papers have focused on the analyses of gunshot residues (GSR, generally lead (Pb), barium (Ba) and antimony (Sb)) detection in blowfly larvae.

Mostly the detection of GSR elements in blowfly larvae has been conducted using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The focus of these studies was on the detection of Pb, Ba and Sb in larvae as pure chemical elements.

The aim of this study was to determine if there are any chemical and/or morphological changes of Pb, Ba and Sb following the uptake of GSR by larvae of *Calliphora vomitoria* (L.) (Diptera: Calliphoridae).

Five pieces of swine meat (500g and 16x16x7cm each) were shot using a Beretta 98f gun. One piece was not shot (control). The ammunitions used were 9x21mm Winchester with Pb contained in the primer and 9x21mm Fiocchi Leadless no Pb in the primer, and two shooting distances of 2 and 40cm. 200 *C. vomitoria* eggs were placed on each piece of meat as close as possible to the shot hole and allowed to develop until the third instar.

500mg of larvae were analyzed by ICP-MS while a total amount of 50 stubs each one prepared with the stomach contain of a single larva of each experimental groups were analyzed using two different SEMs (Leica StereoScan 420 and Zeiss EVO 50XVP with variable pressure) both coupled with Energy Dispersive X-ray microanalysis (SEM-EDX). The gloves and the hands of the gunman were also analyzed.

This study demonstrated the presence of actual GSR particles which was obtained by using variable pressure SEM. The EDX analyses showed that GRS particles were in the stomach content of those larvae feeding on the meat shot at close range (2 cm).

Comparative larval morphology of nine flesh fly species (Sarcophagidae) of forensic importance in southern Brazil

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Necrophagous species of flies provide useful data to estimate the post-mortem interval in forensic cases because their immatures are the most common entomological evidence collected in death investigations. However, identification of muscoid Diptera larvae is considered difficult because only few references present detailed morphological descriptions, which hampers the identification process. A detailed description, with a comparative approach to find characters with inter-specific variation, is fundamental to a proper use and assessment of morphological diversity. For this reason, we present detailed descriptions comparing nine species of Sarcophagidae found in southern Brazil.

Gravid females were collected in the states of Paraná and Santa Catarina and brought to the laboratory. After larviposition, maggots were reared until the emergence of males to confirm the identification. A colony was established to obtain enough larvae for complete documentation. Third instar larvae were killed, fixed and mounted for optical and scanning electron microscopy analysis using standard procedures. Larval documentation was produced for *Oxysarcodexia paulistanensis* (Mattos), *O. riograndensis* (Lopes), *Microcerella halli* (Engel), Peckia (Euboettcheria) florencioi (Mattos), Peckia (E.) australis (Fabricius), Peckia (Sarcodexia) lambens (Wiedemann), Peckia (Pattonella) intermutans (Robineau-Desvoidy), Peckia (P.) resona (Lopes) and Sarcophaga (Bercaea) africa (Wiedemann).

Larvae of these species are mainly separated by the shape of their mouthhooks; development of apodema on basal part of mouthhooks; shape of intermediate sclerite; width of vertical plate; presence/absence of hair-like spines or warts on the body surface; distribution of spines on thoracic and abdominal segments and shape of anal papillae. These characters are useful to separate the third instar larvae of all species except *O. paulistanensis* and *O. riograndensis*, which differ only in size and behavior. The morphological data will enable us to produce a pictorial key to identify flesh fly larvae from entomological evidence and thus avoid time-consuming rearing. In megadiverse countries like Brazil, keys for forensic purposes should be regional and contain only necrophagous species with forensic importance confirmed by larviposition on large carrion.

Indoor arthropod succession in an urban environment in central Spain

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Arthropod succession studies are required to determine the sequence and patterns of insect colonization on a corpse and to properly estimate the minimum postmortem interval. The composition and the succession pattern of the insect fauna associated with carcasses vary among habitats and seasons, but it has been noted that «while the majority of cases with insect evidence occur indoors, nearly all insect succession studies take place outside» (in Amendt et al., 2010: Current concepts in forensic entomology, p. 353-368).

The aim of this ongoing project is to study the arthropod succession and carrion decomposition on an indoor scenario in an urban habitat of central Spain. Three 20-25 kg pig carcasses were placed at three separated rooms, with three respective, equally orientated open windows, inside a building in Alcalá de Henares city center at the beginning of each season, from summer 2013 to spring 2014. In total, 12 pig carcasses were used. The carcasses were examined twice per day during the first week and subsequently once per day, recording new egg-layings and the hatching of larvae, and the subsequent insect succession pattern.

The current poster shows the preliminary results from summer, autumn and winter trials, with a checklist of the insect species recorded on the carcasses. During both summer and autumn, the Calliphoridae species *Calliphora vicina* Robineau-Desvoidy and *Lucilia sericata* (Meigen) were the pioneer colonizators of carcasses, although the active decay was mainly driven by larvae of *Chrysomya albiceps* (Wiedemann), which was also the first species completing its life cycle. Sarcophagidae, Muscidae and Fanniidae were also recorded during the first days of decomposition, and their larvae also occurred during the active decay. Piophilidae species appeared later in decomposition and their puparia were only collected during the last days of the experiment. During winter, *C. vicina* was virtually the only species feeding on carcasses during the active decay. Among Coleoptera, species from families Cleridae, Dermestidae, Histeridae, Silphidae and Staphylinidae were also collected on the carcasses. In general, the species composition was not highly diverse, but the interest of the occurrence of some species is briefly discussed.

Register and systematization of the insect fauna associated with corpses in Chile.

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In Chile, as in several countries of South America, in recent years Forensic Entomology has developed in both its aspect: experimental or basic science and police investigations. In the context of FONDEF Project D09I1035, the Investigations Police of Chile (PDI) has collected insects associated with the decomposition process from corpses, in order to create a systematized database about diversity, phenology and conditions these species were found, something never done in Chile before.

Between May 29/2011 and February 19/2014, entomological evidence was taken from 60 sites where bodies were discovered in 8 regions of the country, from Arica (18° 28'S.L.) to Lanco (39° 26' S.L.). There have been 25 species of necrobiotic arthropods grouped into 14 families and 6 orders. It was possible to associate the presence of these species with the rural / urban and indoor / outdoor conditions, in addition to the seasonal environments in which they were found. Since this type of registration is open, it can't be considered definitive, since there are many variables that can be considered in each finding (latitude, type of natural or anthropic environments, seasons, specific conditions of the body, etc).

Oxelytrum lineatocolle (Coleoptera: Silphidae) in corpses of south central Chile (*)

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The presence of larvae and adults of *O. lineatocolle* (Coleoptera: Silphidae) have been reported for the first time in the mid valley of Central-south-central Chile, on corpses exposed to outside weather conditions.
This silphids were manualy colected by trained PDI officers from corpses in the finding sites, stored in ethanol-acetic solution and sent to FE Lab in Temuco.

Adults were identifed with the key of the genus Oxelytrum in South America (Oliva, 2012) and we assumed that the larvae and adults found in all bodies are of the same species (Oliva, 2004), because immature stages of this species haven't been described yet.
Due to the special conditions in which these corpses were found (outdoor, shaded, wet, close to native vegetation, and semi-immersed at different times, during their disappearance period), the presence of diptera was limited; therefore, the development degree of the immature stages of this silphid (necrophagus sensu stricto) could be used as an indicator to determine the PMI. However, comparing the cases, there are differences in the number of adults and larvae that were found in corpses with similar time of disappearance, as well as a greater average length of larvae in one case compared to another with a shorter disappearance period.

These apparent inconsistencies could be explained by the delay in the colonization of this silphid due to the specific adverse conditions of the corpses (hanged, immersed due to rainfall accumulation, etc), therefore it is important to consider these variables, when it comes to calculating the PMI through the immature stages of this silphid.

(*) This work was sent to the Forensic Science International Journal and in this moment is in revision

Necrophilous and opportunistic fauna as PMI and seasonal indicator. Case of the Staphylinidae (Coleoptera) in Southeastern Spain*

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Necrophilous fauna is composed of a quite great variety of insect groups predating on necrophagous species or parasitizing them. Opportunistic species use the carrion as an extension of their own habitat or an additional food and shelter resource.

Staphylinidae is one of the most diverse Coleoptera families, with 45724 species described worldwide. This group comprises species with a variety of feeding habits, but most of them are predators. Particularly, staphylinid species have been considered the commonest predators found on cadavers, thus belonging to the necrophilous component of the community. They are usually attracted to carrion to feed on maggots and the larvae of other insects. Since many staphylinid species feed on fungi, a usual resource in a decomposing cadaver, they also belong to the opportunistic component. Among the predacious species, some are specialists, while others have generalist diets. These categories of sarcosaprophagous arthropods can provide some valuable environmental data that may contribute to the estimation of time since death and other interesting factors for forensic purposes. In fact, there are a few references of this family related to human corpses.

A study on sarcosaprophagous community was conducted at the top of a mountain system in Murcia province (SE Spain) using a Schoenly trap as collecting device. Samplings were taken in all seasons covering about eight week periods. Forty-three staphylinid species were recorded. *Bisnius sparsus* characterized the spring and *Aleochara bipustulata* the summer. The family was much less abundant during fall and winter. Thus, these species can act as seasonal indicators. Despite isolated specimens may be present at the bloated stage, these two species showed a pattern of appearance clearly related to more advanced decomposition stages thus allowing an estimation of the PMI.

At the light of our results compared with those of other authors we can affirm that the specific composition of the Staphylinid community can also be used as an environmental indicator.

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Occurrence of necrophagous Diptera in a wild environment of South-eastern Spain*

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Necrophagous fauna is usually the most important component of the sarcosaprophagous community and most useful for forensic purposes. Among it, Diptera are usually the most abundant group, arriving to the corpse first after death and providing the best tools to estimate PMI. Since the fauna is affected by several factors, such as seasonality and environment, it becomes necessary to study this fauna in all environments as possible in order to avoid potential miscalculations when estimating PMI. Sarcosaprophagous community is usually unknown from wild environments, at least in the Iberian Peninsula, while periurban ones are quite well known in certain areas.

Thus, a study was conducted in a site near the summit of Sierra Espuña, a Natural Park of the Murcia Region (South eastern Iberian Peninsula) reaching 1560 m altitude, using a Schoenly type trap baited with a 5 kg piglet. Seasonal samplings were made covering a whole year period. Collections were made daily for the first two weeks and on alternate days during the following 8-9 weeks.

Forty one Diptera families were collected in the whole period. Families Calliphoridae, Fanniidae, Muscidae, Phoridae, Piophilidae, Sarcophagidae and Sphaeroceridae were considered the most representative from a forensic point of view according to literature. Differences in dipteran families/species composition associated with season and decomposition stages were determined applying Dominance and Frequency of occurrence indexes proposed by Oliveira & Vasconcelos (2010).

Calliphoridae resulted as dominant and very frequent in all seasons. Muscidae was also dominant and very frequent in summer and Sphaeroceridae dominated in spring and was very frequent in spring and summer. The other families were not as dominant although could be very frequent in qualitative terms.

Among Calliphoridae, *Calliphora vicina* was the dominant species in all seasons. *C. vomitoria* dominated in spring and winter, and *Chrysomya albiceps* in summer and fall. Among Muscidae, *Musca domestica* dominated in summer. No other dipteran species showed dominance at all despite the family dominance.

Reference: Oliveira, T.C. & Vasconcelos, S.D., 2010. Forensic Science International, 198: 97-102.

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Forensic entomology in French criminal cases: snapshot and trend

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Created in 1992, the department of forensic Fauna Flora Forensic (ex entomology) of the Forensic Science Institute of the French Gendarmerie has provided more than 1350 expertises. From these cases, some information appears concerning geographical, environmental parameters and some different trends regarding the species identified. Thus, this accurate reflexion in France shows the main cases come from rural spot; concern a majority of the corpses discovered outside a building or a shelter. 6 states of decomposition have been drawn up with a majority of putrefied and highly putrefied. At last, although most of the corpses are found in a location without physical constraint to lead an estimation of the post mortem interval, some of them need caution regarding their situation: buried, packaged, floated or hanged.

Considering Dipterans, 97% of the cases treated have Calliphoridae identified. Among us, twenty species have been identified more than 5 times with 6 species encountered more than 100 times: *Calliphora vicina*, *Calliphora vomitoria*, *Lucilia sericata*, *Lucilia caesar*, *Chrysomya albiceps* and *Phormia regina*. More precisely, *C. vicina* can be found in rural spot but *C. vomitoria* is more infrequent in urban spot. But, the association of these two species is more frequent in rural than in urban area. For the species with a higher thermal threshold, the presence of *C. albiceps* and *P. regina* alone is quite similar but their association is very rare. Regarding the last 17 years, *P. regina* tend to increase its presence to the detriment to *C. albiceps*.

In the estimation of the post mortem interval, many parameters can affect the accuracy of the result. The association of the species is one of them and its study could be integrated in a widely model of FE analysis linking ecological data and statistical approach.

Pupal age estimation in *Megaselia scalaris*: from microscopy to sincrotrone

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The temporal resolution of the pupal stage is severely limited because no changes in the external morphology of the pupal case occur during the metamorphosis. Several methods have been used to study the development of pupae in more detail. Traditional methods focus on morphology by describing distinct external cuticular markers during development after removal of the puparial case. Advances in this approach have been achieved using SEM, X-ray computed microtomography (μCT) and gene expression.
Little information is available about metamorphosis in Phoridae, and recently an article was published about *Megaselia scalaris*.

For this species, laboratory-based μCT imaging can provide useful information only on the external morphology of the pupa and indication of the development of electron dense structures, but due to its small size and low contrast between the different soft tissues only little information can be extracted about internal features. In order to bypass this problem synchrotron-based μCT with a monochromatic radiation and phase-contrast imaging are required. Observations using this approach have been done at the Elettra - Sincrotrone Trieste. The obtained images have been compared with optical microscopy observations performed on pupae after the pupal cage removal in order to define characters useful for the age estimation of the pupae. Pupae at their 0, 15, 35, 50, 70 85 and 100% development were analysed for both sexes. First appearance of antennae and legs was observed at the 15% of the development, but the strongest change is evident between 50% and 70% of development when eyes and thoracic setae (dorsal and ventral) are well distinguishable, legs are darkened, wings are visible as well as postocellar setae and ocelli. Internal organs like the reproductive apparatus appear as very electron dense structures since the beginning of the pupariation. These structures appear in different positions in the two sexes, more distal in the males.

As observed by other authors the fact that X-ray technologies are more and more common in different institutions in the near future they will be a useful tool for the pupal age estimation. Only a little sample preparation is required as well as a short recording time (1-3h).

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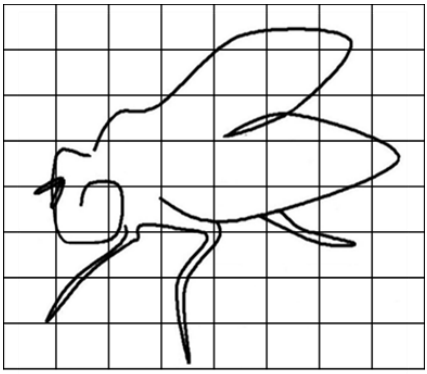

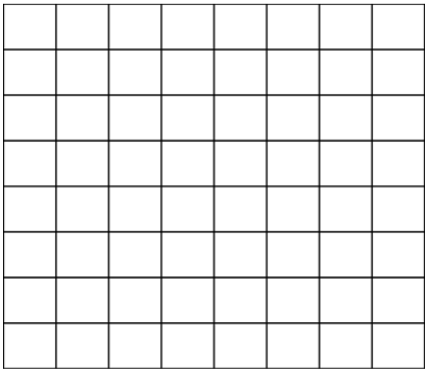

REPRODUCE THE EAFE LOGO USING THE GRID

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