



12th Meeting of the European Association for Forensic Entomology

# 2015

6th-9th May 2015 University of Huddersfield, Huddersfield, UK

12<sup>th</sup> Meeting of the European Association for Forensic Entomology

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12<sup>th</sup> Meeting of the European Association for Forensic Entomology

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## Programme

	7 <sup>th</sup> May 2015
8.30-12.30	Registration
9.15-9.30	Welcome Speech
9.30	Invited Speaker
9.30-10.30	M.J.R. Hall
	Forensic entomology, a consilience – past, present and future
10.35	Oral Communications Section I chairman : S. Vanin
10.35-10.50	J. Haynes, K. Chapman
	Yorkshire and the Humber Police
10.50-11.05	S. Wagenknecht, F. Reckel, C-P. Schuch, H. Sprenger L. Weidlich, J.
	Amendt
	Can you see the heat? Helicopter thermal imaging for detecting insect
	infested human remains
11.05-11.30	Coffee break
11.30	Oral Communications Section II chairman: F. Reckel
11.30-11.45	D. Charabidze, A. Depeme, <u>C. Aubernon</u> , C. Devigne, V. Hedouin
	Do flies truly lay their eggs in wounds? From a crimlinal case to laboratory
11.45.10.00	experiments
11.45-12.00	L. Bourguignon, Y. Braet, S. Vanpoucke, V. Drome, F. Hubrecht
	Influence of overcrowding during rearing on the developmental duration
12.00.12.15	and the size of adults of five diptera of forensic importance
12.00-12.15	<b>L. Zheng</b> , <b>I. L. Crippen</b> , <b>A. Gordy and J.K. Tomberin</b>
	development of Lucilia seriesta (Diptore: Callinhoridae)
12 15 12 30	H Ody M Bulling and K Barnes
12.13-12.30	Species-specific temperature thresholds for ovinosition in two species of
	forensically important blow flies (Diptera:Callinhoridae)
	Torensieury important blow mes (Diptera.eamphoridae)
12 30-14 00	Lunch Time and Poster Section
12.30 17.00	

14.00	Oral Communications Section III chairman: L. Bourguignon
14.00-14.15	A.F. Spindola, P. J. Thyssen, L. Zheng, J.K. Tomberlin
	Attraction and oviposition of Lucilia eximia (Diptera: Calliphoridae) to
	resources colonized with the predator, Chrysomya albiceps
14.15-14.30	M.A. Perotti, S. Lambiase
	Timing neglect using a severe parasitosis
14.30-14.45	J. Tschui, E. Obrecht, C. Schyma, C. Jackowski, J. Amendt
	A fly up to its neck in water: A case report
14.45-15.00	<u>S. Matuszewski</u> , A. Mądra, K. Frątczak, A. Grzywacz
	Patterns of multiple colonization of large pig cadavers in a long-term study
	of carrion insects
15.00-15.15	A.E. Perez, N.H. Haskel, <u>J.D. Wells</u>
	Commonly used intercarcass distances appear to be sufficient to ensure
	independence of carrion insect succession pattern
15.15-15.45	Coffee break
15.45	Oral Communications Section IV chairman: K. Brown
15.45-16.00	J. Amendt, M. Baqué, J.B. Butcher, <u>C.R. Day</u>
	Progress towards the automated analysis of <i>Calliphora vicina</i> larval
	morphology and temperature data for age estimation
16.00-16.15	D. Martín-Vega, T.J. Simonsen, M.J.R. Hall
	Looking into the puparium: estimating the age of blowfly pupae with micro-
1 < 1 = 1 < 20	computed tomography
16.15-16.30	<u>S. Bortolini</u> , L. Maistrello, S. Vanin
16 20 16 45	Molecular identification and phylogeny of Diptera of forensic importance
16.30-16.45	<b><u>B.K. Zajac</u></b> , J. Amendt, R. Horres, M.A. Verhoff, R. Zehner
	Molecular age estimation of <i>Calliphora vicina</i> pupae (Diptera:
	Calliphoridae) – Identification of genetic Markers using Massive Analysis
16 45 17 00	of cDNA Ends (Mace)
16.45-17.00	A.M. Seuri; F.H. Galai; A.A. Ibranim, A.A. Elnemawy
	Comparative evaluation studies of RAPD-PCR marker system and
	(DMI)
17.00-17.15	Conclusion of the first day
17.00-17.15	
17.30	Student workshop
17.30-18.30	M. Pala
	How to do a Presentation
18.45	Real Ale Trail (Huddersfield) [social event]
18.45	

	8 <sup>th</sup> May 2015
8.30-12.30	Registration
9.30	Oral Communications Section V chairman: J. Amendt
9.30-9.45	J.A. Cammack, S.N. Thornton, J.K. Tomberlin Implications of nutrition and larval feeding activity on the microbial community function in an artificial insect diet: potential use in forensic entomology
9.45-10.00	<b><u>E. Crooks</u></b> , <b>M. Bulling</b> , <b>K. Barnes</b> Impact of bacteria on the development of forensically important blow-flies
10.00-10.15	J.L. Pechal, M. E. Benbow Influence of resource pulses on cadaver networks: insects, microbiomes, and their potential use in forensic entomology
10.15-10.30	<u>M. Hani</u> , C. Frost, C. Toone, H.R. Braig, M. Perotti The collection of micro-arthropod trace evidence from human clothing
10.30-10.45	N.A. Che Kamaruzaman, Y. Velásquez, A. Lindström, A. G. Medina, H.R. Braig, P. Mašán, M.A. Perotti <i>Macrocheles</i> species as markers of stages of decomposition: three case reports from Europe
10.45-11.15	Coffee break
11.17	
11.15	Ural Communications Section VI chairman: M.J.R. Hall
11.15-11.50	<b><u>J.F. Farren</u></b> , A.E. Whitington, M.F. Zalucki Carrion café –using insects to determine post mortem intervals in Queensland, Australia
11.30-11.45	<u>N. Feddern</u> , C. Schyma, C. Jackowski, J. Amendt, J. Tschui Spatio-temporal distribution of blowflies (Diptera:Calliphoridae) of potential forensic interest in the area of Bern, Switzerland
11.45-12.00	<b><u>R.J. Stock</u></b> , <b>N.H. Haskell</b> The distribution of forensically relevant insect species across North America
12.00-12.15	S. Shayya, C. Bassil, C. Makhoul, M. Al-Zayed, A. Nel, D. Azar First succession study of a cadaver entomofauna in Lebanon
12.15-12.30	S.E. Shin, J. H. Park, K.S. Ko, S.H. Park A review of forensic entomology in Korea
12.30-14.00	Lunch Time and Poster Section

14.00	Oral Communications Section VII chairman: T. Pasquerault
14.00-14.15	A.M. Faris, H.H.Wang, A.M. Tarone, W.E. Grant
	An ecological modeling approach for quantifying uncertainty associated
	with insect age estimates
14-15-14.30	<b><u>D. Charabidze</u></b> , B.Vincent, T. Pasquerault, V. Hedouin
	The biology and ecology of <i>Necrodes littoralis</i> , a species of forensic interest
	in Europe
14.30-14.45	M.D. Gemmellaro, G. Hamilton, L.M. Weidner, C. Sollami
	A new joint Italian/American entomology laboratory – implications for
	research, training and case work in Sicily
14.45-15.00	J. Amendt, M.A. Perotti, M.I. Saloña-Bordas, M.L. Goff, H.R. Braig
	The dark side of open access: predatory journals in forensics
15.00-15.15	<u>S. Vanin</u>
	Forensics or not Forensics: that is the question
15 15 15 20	
15.15-15.30	Conclusions of the second day
15 00 16 00	
15.30-16.00	Coffee break
16.00	
16.00	EAFE meeting
16.00-17.00	EAFE meeting
17.00	
17.00	Student workshop
17.00-18.00	A. Laws, K. Brown
	How to write a grant
20.00	Social dinner

	9 <sup>th</sup> May 2015
9.30-12.30	Workshop: Tricky identification

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# ORAL COMMUNICATIONS AND POSTER PRESENTATIONS

Invited speaker

#### FORENSIC ENTOMOLOGY, A CONSILIENCE - PAST, PRESENT AND FUTURE

#### M.J.R. Hall

#### Department of Life Sciences, Natural History Museum, London, UK m.hall@nhm.ac.uk

Forensic entomology is a true consilience, linking together principles from many disciplines to form a comprehensive theory. That consilience, or convergence of evidence, takes place at many levels within forensic entomology, where evidence drawn from independent methods of measurement leads to the same answer. Examples of this include verification of a species identification by morphological and molecular methods, or of a minimum post-mortem interval by developmental rate, hydrocarbon, gene expression and successional analyses. A consilience makes the presentation of evidence in court more robust than it would be if a single method was used. Although the roots of forensic entomology can be traced back to thirteen century China and some significant publications appeared in Europe in the midnineteenth to early twentieth centuries, forensic entomology as a scientific discipline only really started to flourish in the 1980's, stimulated in particular by the landmark publication of Ken Smith in 1986, A Manual of Forensic Entomology. This book drew together a wide and diverse literature making it accessible to a much wider readership than before. Since then there has been an exponential growth in the scientific output on forensic entomology, with no real signs of a slowdown, especially as more and more areas of general science are brought to bear on forensic applications. It is probably true to say that developments in the science of forensic entomology are running ahead of their actual application to help investigate crime, but time and the rigorous testing of techniques in court will allow for a catch-up and a winnowing of those techniques that will be adopted from those that will be discarded. Advances in forensic entomology are being made at a time when there are substantial changes in the requirements for forensic science testimony in the courts of many countries in an effort to raise quality standards. There is now an expectation that standard operating procedures are followed in collecting and processing insect evidence and that conclusions are reached following the application of validated, peer-reviewed methods. While this is a challenging time for forensic entomology it is also an exciting time, with great potential for increasing the future use of insect evidence in criminal investigations at a global level, taking account of the accelerating input from pure and applied studies and from statistically robust interpretations.

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#### CAN YOU SEE THE HEAT? HELICOPTER THERMAL IMAGING FOR DETECTING INSECT INFESTED HUMAN REMAINS

S.Wagenknecht<sup>1</sup>, F. Reckel<sup>2</sup>, C-P. Schuch<sup>3</sup>, H. Sprenger<sup>3</sup>, L.Weidlich<sup>4</sup>, J. Amendt<sup>1</sup>

<sup>1</sup>Institute of Forensic Medicine, Goethe-University, Frankfurt/Main, Germany; <sup>2</sup>Bavarian State Criminal Police Office, Munich, Germany; <sup>3</sup>University of Applied Science of North Rhine-Westphalia, Gelsenkirchen, Germany <sup>4</sup> Flying Squadron, State Office of Central Police Services, Düsseldorf, Germany amendt@em.uni-frankfurt.de

Thermal imaging (TI), also known as infrared imaging) shows the thermal patterns emitted from objects, without requiring visual light. There has been little research into the efficiency of TI in the detection of cadavers and their remains. As temperature profiles of animal cadavers are usually recorded during insect succession studies there is awareness that insect infestation temperature can exceed ambient air temperatures by up to 20°C, depending e.g. on the season and the body mass of the cadaver. However, intensity and duration of these heat signatures and their potential use as a detector source for locating human remains especially in the later stages of search and recovery missions by TI has been virtually unstudied. This was highlighted by a case 2010 in Germany when a 10-year old boy was kidnapped and killed. The investigators were concerned about the operation of a TI-system and its appropriate timing, when it took almost 2 weeks before the first (unsuccessful) search.

We examined the decomposition, heat signature and insect infestation of two pig cadavers (70 & 90 kg), which were placed in an open field in the vicinity of Duisburg/Germany. The experiment took place from 20.05. – 10.06.2014. Ambient, cadaver and maggot mass temperatures were recorded daily. Immature insects were sampled regularly and identified. Heat signatures of the cadavers and the environment were measured by means of a TI-system (Model FLIR (Forward Looking InfraRed) Star Safire HD) during 8 flights with a police helicopter (Model BK117 B2) in an altitude between 900 – 1500 ft.

Insect fauna was dominated by Diptera (Calliphoridae: *Calliphora vicina, C.vomitoria, Lucilia sericata, Phormia regina, Protophormia terraenovae*) and beetles, especially the silphid *Necrodes littoralis*. Ambient temperature profile during the three weeks was between 10°C and 30°C. Body temperature (surface and core) of the decomposing cadaver was never below the daily ambient temperature, and the maggot masses dominated the temperature profile of both cadavers. TI was successful in detecting thermal emissions from all insect larval masses and differentiating the remains from the surrounding environment until 20 days after placing the cadavers in the field, especially when ambient temperatures got low e.g. during night.

This is to our knowledge the first published experiment to prove a possible window of time for detecting human remains via TI.

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#### DO FLIES TRULY LAY THEIR EGGS IN WOUNDS? FROM A CRIMINAL CASE TO LABORATORY EXPERIMENTS

D. Charabidze<sup>1</sup>, A. Depeme<sup>1</sup>, <u>C. Aubernon<sup>1</sup></u>, C. Devigne<sup>1, 2</sup>, V. Hedouin<sup>1</sup>

<sup>1</sup>UnivLille, Forensic Taphonomy Unit, Lille, France; <sup>2</sup>Laboratoire Ecologie & Biodiversité, UCLILLE, FLST, Lille, France cindy.aubernon@unite-taphonomie.fr

It is frequently reported in forensic entomology manuals that blowflies lay their eggs inside wounds. This assertion has recently called our attention. In 2009, the naked body of a young woman was discovered in a corn field a few hours after her disappearance. Several wounds caused by a sharp object were present, and her hair was soaked with blood. An autopsy concluded that the victim was first throttled but did not die of asphyxiation and was subsequently stabbed in the head following an attempted rape. The only forensic entomology evidence was the unhatched fly eggs that were discovered in the eyes and nostrils of the victim, which were bred and identified as belonging to *Lucilia sericata*. According to the literature, gravid females are supposed to lay eggs in orifices and wounds. Thus, the complete lack of eggs on the bloody wounds in the skull would appear to suggest that the victim had not already been stabbed when the flies deposited their eggs on the corpse. However, this scenario was inconsistent with subsequent investigations, and it was lastly proven that the victim was stabbed before being deposited in the field where she was discovered.

Following this case, a study was designed so to investigate the preferential egg-laying sites of *L. sericata*. In a first experiment, rat cadavers were placed during 5 hours into insectariums with 10 males and 30 gravid females. Eggs were then removed using a head louse comb, separated with hot water and numerically counted. We did not observe a single egg inside the wound in any of the replicates.

In a second experiment, we observed the effect of eggs immersion (body fluids often exudes from wounds) on the survival rate of larvae, using water and blood at high and low volumes.

Results show that fluids strongly decreased the survival rate of the larvae and impact their development.

These data, obtained under controlled conditions using rat cadavers, does not support the assertion that *L. sericata* females lay their eggs in wounds. This result should be kept in mind when trying to interpret forensic entomology evidences.

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#### INFLUENCE OF OVERCROWDING DURING REARING ON THE DEVELOPMENTAL DURATION AND THE SIZE OF ADULTS OF FIVE DIPTERA OF FORENSIC IMPORTANCE

L. Bourguignon, Y. Braet, S. Vanpoucke, V. Drome, F. Hubrecht

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To estimate the minimum PMI, the Accumulated Degree Day method requires rearing the larvae sampled on a body, taking into account the emergence of adult Diptera, and using specific ADD values established in optimal conditions (food *ad libitum*, optimum temperature range, no competition, etc.).

In casework, it is not possible to control for the level of comfort that the larvae encountered before the body was found, and it is usually implicitly considered that the food resources were probably always present in sufficient quantity.

However, it has been shown that several factors, such as overcrowding or scarce food resource, could affect the normal development cycle of diptera, leading to adult individuals, exhibiting sub-optimal morphological characteristics (eg.: size and weight) or shortened development times.

Five species of diptera of forensic importance (*Calliphora vicina* Robineau-Desvoidy, 1830; *Cynomya mortuorum* (L., 1761); *Lucilia sericata* (Meigen, 1826); *Protophormia terraenovae* Robineau-Desvoidy, 1830 and *Sarcophaga tibialis* Macquart, 1851 were reared in less-than-optimal conditions (limited amount of food available), and the dry mass of the adults were compared with the mass of adults reared in comfortable conditions, where food was always available in excess.

The mass of those two groups appear to be so significantly different that it allows us to propose, for each species, a mass threshold below which the size of the imagos reflects a development in stressful conditions (Figg. 1 and 2).



**Figures 1 and 2**: the dashed line represents the distribution of the mass of adults (dry) when food supply is limited. The continuous line represents the distribution of the mass of adults (dry) receiving an unlimited amount of food. In those two examples (*P. terraenovae* and *L. sericata*), an obvious separation in the mass ranges of the two groups is visible.

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#### EVALUATION OF STERILIZED DIET FORMULATIONS AND LARVAL DENSITY EFFECTS ON THE DEVELOPMENT OF *LUCILIA SERICATA* (DIPTERA: CALLIPHORIDAE)

L. Zheng<sup>1</sup>, T.L. Crippen<sup>2</sup>, A. Gordy<sup>1</sup>, J.K. Tomberlin<sup>1</sup>

<sup>1</sup>Department of Entomology, Texas A&M University; <sup>2</sup>USDA-ARS zhengle@tamu.edu

Lucilia sericata (Diptera: Calliphoridae) larvae have wide applications and importance in medical entomology as principal organisms for maggot debridement therapy, as well as in forensic entomology (e.g., minimum postmortem interval, neglect) as they are often associated with human remains. This research evaluated the larval development of L. sericata at different densities (1:0.5, 1:1, 1:2 larvae/g diet) on six sterilized diets and fresh beef liver (a commonly used rearing diet), which served as the control. The sterilized diets were decomposed liver, powdered liver, powdered fishmeal, blood-based, milk-based, and an artificially constructed diet. Sterilization of diets were autoclaved at 121°C for 20min. All the liver base diets were beef liver, and decomposed liver diet composed with 5 days old aerobic decomposed liver at 25°C. End point performance measurements included the following lifehistory traits: larval survivorship (proportion to pupation), pupal weight, proportion to eclosion, and adult longevity. Data were analyzed in R with generalized linear regression using a random effect model. This research provides a baseline measurement for density and dietary effects, and determined threshold values for future L. sericata development experiments. Results revealed that larval survivorship increased with larval density and that the life-history traits of larvae reared on decomposed liver (P = 0.21) and blood-based diets (P = 0.48) correlated more consistently with the control diet than did the traits of other diets. From a forensic entomology perspective, these results serve as a primer for future research exploring the influence of nutrition and exogenous and endogenous microbes on the development of this forensically important species.

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#### SPECIES-SPECIFIC TEMPERATURE THRESHOLDS FOR OVIPOSITION IN TWO SPECIES OF FORENSICALLY IMPORTANT BLOW FLIES (DIPTERA:CALLIPHORIDAE)

#### H. Ody, M. Bulling, K. Barnes

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Forensic entomologists rely on the colonisation times and development rates of specific blow fly species to estimate the minimum Post Mortem Interval (mPMI). A number of factors are known to affect blow fly behaviour and oviposition, with temperature being the most significant. However, temperature thresholds for oviposition in forensically important blow flies are not well understood. In this study, temperature thresholds for oviposition in *Lucilia sericata* and *Calliphora vomitoria* were established within a laboratory environment.

Experiments were run under controlled conditions in the laboratory with at least three replicates of each environmental configuration for each species. 40 adult flies (20 male and 20 female) were introduced into a plastic cage (30cmx30cmx30cm) containing 55g of pig liver, sawdust to cover the floor and a water pot. Cages were placed in an insect growth chamber for 24 hours at constant temperatures ranging from 10 to  $40^{\circ}$ C (intervals of 5°C in most cases with intervals of 2 or 1°C being used to narrow down the threshold temperature), and a constant relative humidity of 55% and constant light. Any resulting eggs were collected and reared to the puparial stage at which they were counted.

Temperature was found to have a significant effect on oviposition occurrence (p<0.001). Lower temperature thresholds of 12°C and 16°C were established for *C. vomitoria* and *L. sericata* respectively. Both species laid a large number of eggs at the highest temperature tested (40°C), but few of these survived through to the puparial stage. The highest rate of survival to pupa in *C. vomitoria* occurred at 25°C. Survival was low overall for *L. sericata*, with the highest rate of survival occurring at 15°C. A positive correlation was observed between the number of eggs laid and the number of pupae reared for *C. vomitoria* (correlation coefficient of 0.7522971) but a negative correlation existed for *L. sericata* (correlation coefficient of -0.3382404). Preliminary data (using a partial data set (n=4)) indicated that humidity did not have a significant effect on *L. sericata* oviposition occurrence with (p=<0.084).

These results suggest that different oviposition temperature thresholds may exist for different blow fly species. Such effects are likely to alter the calculation of mPMI. More precise knowledge of temperature thresholds in specific blow fly species is therefore needed for improved accuracy of insect based mPMI estimations.

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#### ATTRACTION AND OVIPOSITION OF *LUCILIA EXIMIA* (DIPTERA: CALLIPHORIDAE) TO RESOURCES COLONIZED WITH THE PREDATOR, *CHRYSOMYA ALBICEPS* (DIPTERA: CALLIPHORIDAE)

<u>A.F. Spindola<sup>1</sup></u>, P.J. Thyssen<sup>1</sup>, L. Zheng<sup>2</sup>, J.K. Tomberlin<sup>2</sup>

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Since the introduction of the invasive and predatory blowfly species, *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae), to Brazil, native blow fly populations, such as those of *Lucilia eximia* (Wiedemann) (Diptera: Calliphoridae), have been declining. Knowledge about the impact of the invasive species on the native blow fly fauna of Brazil is critical for forensic entomology as it could alter their patterns of development and succession on human remains.

This study was carried out with laboratory-based colonies of these two species at the Department of Microbiology and Parasitology, Federal University of Pelotas (UFPel), Rio Grande do Sul state, Brazil. The goal was to determine if the level of attraction and oviposition to a decomposing resource (minced beef) by *L. eximia* adults, based on sex and ovarian status, was impacted by the presence of immature *C. albiceps*. This work was carried out utilizing a dual-choice assay. Their preference between a resource not colonized (control) or pre-colonized (treatment) with eggs, different instars, or densities of *C. albiceps* was determined. Data were analyzed using PROC logistic (SAS Institute Inc, Cary, NC, USA) with significance set at P < 0.05.

*Lucilia eximia* attraction was influenced by the presence/absence of different life stages of *C*. *albiceps*. Data obtained showed that gender and physiological state significantly influence adult responses to  $2^{nd}$  instars (P = 0.0178) and  $3^{rd}$  instars (P = 0.0053) but not to eggs (P = 0.3546). The estimated probability values indicate nongravid females responded more when exposed to treatments eggs or  $3^{rd}$  instars, than males and gravid females. Gravid females were significantly repelled from the  $2^{nd}$  instar treatment. *Lucilia eximia* attraction to  $2^{nd}$  instar *C*. *albiceps* at different densities was marginally significantly (P = 0.0508). *Lucilia eximia* oviposition was negatively influenced by the presence of *C*. *albiceps* eggs or larvae, with flies oviposition preference between the treatments, gravid females were more attracted to (28.4%) and oviposited more (55.7%) on the egg treatment than on  $2^{nd}$  instar treatment (attraction = 23.8%; oviposition = 44.3%).

These results demonstrate that the presence of immature *C. albiceps* on a food resource significantly influenced the behaviour of the native blow fly *L. eximia*. These responses are possibly due to *C. albiceps* being an effective predator on all instars of native species. The attraction and oviposition of these native species could be governed by volatiles emitted by bacteria present on the external surface of eggs and salivary glands of *C. albiceps* larvae (Ma et al. 2012) and not by pheromones (Brodie et al. 2014). The present data may explain the response of *L. eximia* to a resource previous attended by a predator such *C. albiceps*. Such information could have implications with regards to using succession patterns to estimate a minimum postmortem of human remains as the succession trajectory may have shifted since the introduction of *C. albiceps* into Brazil.

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#### TIMING NEGLECT USING A SEVERE PARASITOSIS

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The associations between arthropods and human skin are manifold. Parasitic insects, bloodsucking, stinging or biting arthropods and even those secreting or spraying irritant substances can produce visible lesions on our skin. Some parasites, like blood-sucking lice, might reach so severe and prolonged infestations that the skin itself changes permanently.

*Pediculus humanus capitis* or the well-known human head louse is a blood-sucking insect that completes its entire life cycle just on the hairy human scalp. It is a species-specific ectoparasite that, at the very moment, is causing epidemic outbreaks within populations of school children from around the world. Interestingly, head lice as well as their siblings, body lice (*Pediculus humanus humanus*), are not known to infest human adults unless under special circumstances of homelessness, indigence, poverty, bad sanitation or neglect. The latest studies on the origin of body lice indicate that they have originated multiple times from head lice. Therefore, if there is a case of extreme head louse infestation, and it is left unattended for years, head lice might spread onto the body, being able to breed in the clothes of the person.

The most comprehensive reports on acute lice infestations and the consequence on the human host were written at the time of and just after World War I with its trench warfare. While head lice were more or less under control by shaving, body lice thrived, with very bad consequences for the poor soldiers already facing very deprived conditions. Body lice are also vectors of fevers, hence, the disease of the time, trench fever (caused by *Bartonella quintana*).

In most cases of homelessness, indigence, etc., extreme infestations of head lice can be a useful proxy for timing neglect; however, this has never being shown before. By analysing a case of severe head louse infestation of an elderly lady from North Italy, who was badly cared for, we were able to estimate the latest onset of a continuous head lice infestation, and therefore put a clock on her neglect which took place circa 3 years before the time of discovery.

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#### A FLY UP TO ITS NECK IN WATER: A CASE REPORT

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In August 2013 a naked, putrefied male corpse was found lying in a dry part of a river bed in a rural area at the foothill of the Swiss Alps (river Ärgera, canton Fribourg). The Ärgera is a wild prealpine mountain river known for its largely unpredictable changes of stream gauge. The body was covered by driftwood and in an advanced stage of decomposition. The skull was largely skeletonized and heavily traumatized. The body was colonized by a large amount of fly larvae. Initially, the case circumstances and the manner of death were completely unclear and the local police force asked for an estimation of the post mortem interval by the means of forensic entomology. Insect larvae, air, body and water temperature data were collected on site. After rearing of the larvae and conduction of fly taxonomy the species Lucilia caesar (13 specimens), Lucilia illustris (15 specimens), Lucilia sericata (3 specimens) and Chrysomya albiceps (1 specimen) were documented. The minimum postmortem interval based on the entomological evidence was estimated as being about 6 days. Police investigation revealed that the deceased was last seen alive 10 days before his body was discovered. This apparent contradiction could eventually be explained by further investigations, which eventually lead to the conclusion, that the deceased probably drowned a couple of kilometres upstream, was washed to the site where he was found by a flood caused by a wild summer storm and accessed by the flies after the water level had dropped.

This case confirmed that the minimum post mortem interval estimated by forensic entomology does not always correspond with the actual time of death, but interpretation of additional data, in the present case meteorological and hydrological aspects, should be taken into account for useful interpretation when dealing with bodies near water.

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#### PATTERNS OF MULTIPLE COLONIZATION OF LARGE PIG CADAVERS IN A LONG-TERM STUDY OF CARRION INSECTS

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Long-term changes of carrion entomofauna are poorly understood. No single carrion study lasted longer than one year. We studied entomofauna of large pig carcasses in the second and the third year postmortem. Ten carcasses were exposed in xerothermic grasslands of Western Poland in spring, early and late summer of 2012. Entomofauna was monitored until September of 2014. 24 larval taxa were recorded in the second year, and four in the third year after the exposure. Several taxa, as for example Calliphora vomitoria, Hydrotaea ignava, Lucilia caesar, L. sericata, Necrodes littoralis, Phormia regina or Sarcophaga caerulescens colonized carcasses only once and in the year of carcass exposure. Recolonizations were always recorded after the winter, in the second or the third year after the exposure, with no such case within a single year. Only necrophagous taxa breeding in long-lasting carrion parts as well as predators feeding on arthropods present in these parts were found to recolonize. Patterns of multiple colonization depended on the time of carcass exposure in the first year. Some species (e.g. Creophilus maxillosus or Stearibia nigriceps) revealed one colonization on carcasses exposed in spring, whereas carcasses exposed in the early or the late summer were frequently recolonized by these insects in the second year. Another pattern was found in the case of Nitidula sp. and Omosita sp. These taxa recolonized regularly on spring carcasses, whereas on the early or the late summer carcasses they revealed only one colonization, but in the second year of the study. Moreover, several taxa colonized some carcasses for the first time in the second year of the study, e.g. Dermestes frischii or Omosita sp. Such delays were particularly frequent on the late summer carcasses. There was however no such taxon, which colonized all carcasses not until the second year of the study. Tineidae and Omosita sp. colonized carcasses just in the second year with high regularity, however on some carcasses they were found also in the first year. These results indicate that only larger cadavers may be recolonized and by just some carrion insects. Moreover, the multiple colonization depends on the time of a year when cadaver is exposed (and resultant amount of biomass available for insects after overwintering) and on the pattern of a taxon year-round activity. Implications for post-mortem interval (PMI) estimation in long PMI cases are discussed.

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#### COMMONLY USED INTERCARCASS DISTANCES APPEAR TO BE SUFFICIENT TO ENSURE INDEPENDENCE OF CARRION INSECT SUCCESSION PATTERN

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Insect succession on carrion can be used to estimate the postmortem interval if the victim died where she or he was found and if there is a predictive model suitable for conditions at the scene. Carrion insect succession models describe the insect species and life stages present on a corpse over time, and this pattern is likely to be affected by many environmental factors requiring experimental investigation. It is difficult to do this with human corpses because there are few locations authorized for their outdoor exposure and because replicate corpses are in short supply. Instead succession researchers have most often used the domestic pig as a model for human postmortem decay. It is also often difficult to find field sites to expose a large decomposing carcass. Therefore an investigator will probably want to optimize both travel time between carcasses and the number of carcasses for a given area by using the smallest possible intercarcass distance. Presumably, though, there is a trade off between optimizing time and space and maintaining the statistical independence of replicate carcasses. Carrion insect field researchers have typically placed experimental corpses < 50 m apart. Direct observations indicate that this intercarcass distance probably prevents cross contamination by crawling larvae, but seems insufficient to prevent highly mobile adult insects from detecting or visiting more than one carcass. Therefore the succession patterns of carcasses too closely spaced might not be independent, violating an assumption of common statistical analyses.

We looked for evidence of nonindependence in 32 domestic pig carcasses (range of intercarcass distance 30 to >300 m) exposed during two consecutive summers as part of a larger succession experiment (Perez et al. 2014. Forensic Sci Int 241:91-95). Analyses included calculating relationships between: (1) average distance to another carcass and aspects of the succession patterns of forensically important species and (2) intercarcass distance was investigated through semivariogram analyses of aspects of the succession interval as well as community similarity indices. Pairwise similarity of time to first occurrence of a species was the only variable that displayed a consistent, in this case negative, relationship to intercarcass distance, although not statistically significant for any single analysis. Semivariogram analysis suggested that under the conditions used for our experiment commonly employed intercarcass distances are satisfactory to ensure independence of carcasses.

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# PROGRESS TOWARDS THE AUTOMATED ANALYSIS OF *CALLIPHORA VICINA* LARVAL MORPHOLOGY AND TEMPERATURE DATA FOR AGE ESTIMATION

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The work reported here is an interdisciplinary collaboration between forensic entomology and the machine learning computer sciences.

One of the main environmental factors that are known to influence the rate at which blow fly larvae develop is the ambient temperature during the principal stages of their development. In this work several cohorts of *Calliphora vicina* larvae were reared for up to 5 to 10 days in controlled environmental conditions at three separate ambient temperatures: 15, 20 and 25°C. Amongst the information recorded for each cohort of larvae were measurements of body length, weight and larval stage at up to 5 hour intervals: with the frequency of measurement reducing as the rate of development slowed.

Supervised and unsupervised neural networks involving Self-Organising Maps (SOMs) and Multi-Layer Perceptrons (MLPs) have been used to analyse this simple morphological and environmental data to see if reliable determinations of the post mortem interval (PMI) can be made.

Early indications are that up to 90% accuracy to around +/- 1 PMI day can be obtained from the MLP neural networks. In addition, the SOM neural networks (whose main strength is to deliver two-dimensional topologically related clusters derived from higher dimensional input data) have developed clusters which support the MLPs' classifications but also augment those findings with information about the nature of the ambiguities surrounding those specimens that were misclassified.

An important benefit that arises from using the machine learning techniques we are reporting here is the reduced effort required in the form of expert data analysis. These techniques also hold the promise of giving more systematic operational results.

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#### LOOKING INTO THE PUPARIUM: ESTIMATING THE AGE OF BLOWFLY PUPAE WITH MICRO-COMPUTED TOMOGRAPHY

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With the pupal stage lasting more than 60% of the total immature development period, determining the age of blowfly pupae is a crucial task in forensic investigations. However, whereas most forensic entomology research has focussed on the larval stages, the temporal resolution of the pupal stage is still very limited, and reliable methods for estimating the age of blowfly pupae are thus strongly needed.

A recent study (Richards et al., 2012) explored the use of micro-computed tomography (micro-CT) for describing and visualising the changes occurring during metamorphosis inside the puparium, with promising results. Here we present the first preliminary results from an ongoing project aimed at developing a novel and reliable method for aging blowfly pupae for forensic purposes using micro-CT scanning, and thereby providing a greater temporal resolution than that currently available.

Five pupae of the blowfly *Calliphora vicina* Robineau-Desvoidy reared at a constant temperature of 24  $^{0}$  C were collected at every 10% developmental interval, killed in hot water, stained in 0.5 M iodine and scanned in a Nikkon Metrology HMX ST 225 system (exposure: 500 ms; voltage: 110 kV; current: 100  $\mu$ A). The procedure was replicated three times. Slice stacks in the three principal planes (cross, horizontal and sagittal) were reconstructed for each specimen, enabling the determination of age-related morphological changes in key structures. Moreover, virtual three-dimensional reconstructions allowed for volume measurement of key structures such as the alimentary canal and the indirect flight muscles, potentially yielding a quantitative measure of development. The advantages and disadvantages of this non-destructive method for age determination, and the next steps for developing it will be discussed.

Richards CS, Simonsen TJ, Abel RL, Hall MJR, Schwyn DA, Wicklein M. 2012. Virtual forensic entomology: Improving estimates of minimum post-mortem interval with 3D micro-computed tomography. *Forensic Science International* 220: 251–264.

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#### MOLECULAR IDENTIFICATION AND PHYLOGENY OF DIPTERA OF FORENSIC IMPORTANCE

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The identification of species plays an important role in several science fields, from research to applied topics. The identification of the species can be performed by two different approaches: the classic morphological identification, and the more recent molecular approach, based on sequencing and comparison of specific mitochondrial and nuclear nucleotide regions. Both approaches have advantages and disadvantages. The classical morphological identification method is not always possible especially if the sample is composed by insect fragments or the insect is in an immature stage of development, due to the lack of complete identification keys for immature stages of the majority of the species. On the other hand, the recent molecular identification method is limited by the lack of information in the sequence databases.

The aim of this work is to evaluate the ability of molecular and statistical methods for the identification to species level and for the phylogenetic grouping of samples of Diptera of forensic interest. The specimens analysed were collected between 2011 and 2014 in Italy (Emilia Romagna and Veneto) and England (West Yorkshire). Eighty four specimens were identified using a morphological approach, and subsequently processed to perform a molecular identification. The molecular identification involved four different genes; two mitochondrial genes, COI and ND5, and two nuclear genes, EF-1 $\alpha$  and PER. These four genes have already been used for the species identification in several research papers. The sequences were obtained by direct sequencing and the identification was performed using BLAST and GenBank. The phylogenetic trees of the species under study were built with the Maximum Likelihood (ML) method.

Within the eighty four specimens analysed, 61.90% were collected in Italy, 34.52% in England, and three samples were from Belgium. All the analysed specimens belong to fourteen different species. Among them *Calliphora vicina* and *Lucilia sericata* were the most abundant species with 29.76% and 27.38% respectively. The analysis of the data from the identification with COI, ND5, EF-1 $\alpha$ , and PER genes highlights a match between morphological and molecular identification in 87.5%, 72.5%, 77.1%, and 67.9% of the specimens, respectively. A higher resolution in the identification of the species is obtained with the analysis of sequences in a phylogenetic tree and with the analysis of "supergenes". The results obtained with the phylogenetic approach presented a pattern of grouping similar to the data present in literature, giving a correct identification of the specimens under analysis. A better identification of sibling species was obtained with the analysis of supergenes.

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#### MOLECULAR AGE ESTIMATION OF CALLIPHORA VICINA PUPAE (DIPTERA: CALLIPHORIDAE) – IDENTIFICATION OF GENETIC MARKERS USING MASSIVE ANALYSIS OF CDNA ENDS (MACE)

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Determining the age of juvenile blow flies providing evidence for the minimum post mortem interval is one of the key tasks of forensic entomology. While the age determination of blow fly larvae is well established using morphological parameters, current studies focus on developing methods to determine the age of blow fly pupae. The pupal stage, which lasts about half the total juvenile development time, still features scope for new applications in forensic entomology and casework. Among the eligible methods gene expression analyzes are predominant. Gene expression is a biological process in which the information of a gene is used to synthesize a functional gene product. Depending on the need of a certain gene product, the expression of the corresponding gene can be turned on or off. This process affects and interacts with the development of organisms. Due to this context, it might be feasible to use the correlation between gene expression and development for age predictions. It has been demonstrated in several studies that the variance in expression of several genes is often too high to assign a certain expression level to a distinct developmental stage or age. To overcome this problem we identified new transcripts which show a sharp age dependent expression course during immature development by analyzing NGS generated transcriptome data (Zajac et al., 2015). We used Massive Analysis of cDNA Ends (MACE) for high resolution gene expression analysis of 15 different development stages of Calliphora vicina pupae reared at constant 17°C. In total, 53,539 distinct transcripts have been detected. By analyzing the MACE data we have filtered for transcripts showing a significant increase of gene expression at a certain development stage during metamorphosis.

We identified more than 100 putative pupal age related transcripts showing an unambiguous increase in gene expression for a particular pupal development stage. For each of the 15 analyzed development days genes of interest have been identified. In the current study, we designed gene expression assays for each day of pupal development. Each assay has been tested concerning its specificity and efficiency. For the development of an age determination kit of *C. vicina* pupae qPCR gene expression analyzes are being performed. First promising genetic markers could be identified for testing in qPCR.

Zajac, B.K., Amendt, J., Horres, R., Verhoff, M.A. and Zehner, R. (2015), "De novo transcriptome analysis and highly sensitive digital gene expression profiling of *Calliphora vicina* (Diptera: Calliphoridae) pupae using MACE (Massive Analysis of cDNA Ends)", *Forensic science international. Genetics* 15: 137–146.

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#### COMPARATIVE EVALUATION STUDIES OF RAPD-PCR MARKER SYSTEM AND TRADITIONAL MORPHOMETRIC METHOD IN THE ESTIMATION OF POSTMORTEM INTERVAL (PMI)

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Experiments were designed to use third larval instar of *Sarcophaga carnaria* as an indicator to estimate PMI by traditional morphometric method (larval length, width and weight) and RAPD-PCR marker system. Statistical analysis of traditional methods clarified that traditional morphometric method was unsatisfactory and insufficient to be used for age determination, independently. Data analysis of randomly amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR) technique demonstrated the suitability of these molecular markers to differentiate to some extent between the ages of 3<sup>rd</sup> instar larvae of *Sarcophaga*. We concluded that RAPD markers are very useful in determining PMI in comparison to traditional method. Further research is needed on other primers to provide a greater resolution of the affinities among following 3<sup>rd</sup> instar larvae of *Sarcophaga*.

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#### IMPLICATIONS OF NUTRITION AND LARVAL FEEDING ACTIVITY ON THE MICROBIAL COMMUNITY FUNCTION IN AN ARTIFICIAL INSECT DIET: POTENTIAL USE IN FORENSIC ENTOMOLOGY

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Microorganisms, particularly bacteria, play an important role in the decomposition of organic matter such as carrion. Microbial metabolic community function, with regards to nutrient quality and the decomposition of organic matter, is heavily linked to insect activity. If arthropod access to a decomposing resource is delayed or inhibited due to factors such as weather, concealment, or post mortem movement of the remains, the succession and function of the associated epinecrotic microbial community is altered and the rate of nutrient recycling inhibited. In this study, larvae of the black soldier fly, Hermetia illucens (L.), (Diptera: Stratiomyidae), were reared on one of three artificial diets that differed only in proteincarbohydrate ratios, at three moisture levels. BioLog Ecoplates<sup>™</sup> (Biolog Inc., Hayward, California, USA), which quantify carbon source utilization by bacteria, were used to determine the changes in microbial metabolic community function with respect to differences in protein-carbohydrate ratios and moisture content of the artificial diets, and feeding activity of the black soldier fly larvae. Previously published information indicates that the microbial metabolic community functional profile changes over time as a resource changes in quality (e.g., throughout decomposition), and as a result, could be useful in forensic entomology for estimating the minimum postmortem interval. However, the potential effects of the highlyvariable nutritional and moisture content of the decomposing resource itself has not been considered. Such information could provide insight into understanding the mechanisms regulating arthropod colonization and consumption efficiency of decomposing organic matter, and the subsequent recycling of nutrients back into the environment.

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#### IMPACT OF BACTERIA ON THE DEVELOPMENT OF FORENSICALLY IMPORTANT BLOW-FLIES

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Forensic entomology uses colonisation times and development rates of specific blow fly species to estimate the minimum Post Mortem Interval (mPMI). The presence or absence of bacteria on a corpse can potentially affect the development and survival of blow fly larvae. Therefore an understanding of how bacterial communities affect the development of blow flies is important for improving the accuracy of mPMI calculations. In this study, the effect of two common bacteria (Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*) on the growth rate and survival of three forensically important blow fly species (*Lucilia sericata, Calliphora vicina* and *Calliphora vomitoria*) was investigated.

Sterile larvae were raised in a controlled environment (16:8h day: night cycle, 23°C day and 21°C night and at 35% RH) on four artificial diets prepared with 100  $\mu$ l of 10<sup>5</sup> CFU bacterial solutions as follows: 1) *E. coli* 2) *S. aureus* 3) a 50:50 *E. coli*: *S. aureus* mix and 4) a sterile bacteria-free control diet. Daily measurements (length, width and weight) were taken from first instar larvae through to the emergence of adult flies. Survival rates were also recorded. Experiments were repeated a minimum of six times for each blow fly species

The bacteria were not essential for the development of any of the blow-fly species. Growth rates however were dependent on both bacterial diet and blow fly species. Adult fly weights varied by a maximum of 19% between diets in *C. vicina* and *C. vomitoria*, with *S. aureus* diets leading to the lowest adult fly weights.

In summary, the bacteria used in this study had a significant effect on larval development in three European blow flies of forensic importance. These results indicate the potential for the bacteria that larvae are exposed to while developing on a corpse to alter developmental rates and emergence times, and to distort mPMI estimates. This is particularly important for practitioners utilising blow fly weight, length or width to age larvae. Extending this work to cover other bacterial species and combinations would help elucidate further the extent to which bacterial communities on a corpse may influence blow fly development and therefore affect the accuracy of mPMI estimates.

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#### INFLUENCE OF RESOURCE PULSES ON CADAVER NETWORKS: INSECTS, MICROBIOMES, AND THEIR POTENTIAL USE IN FORENSIC ENTOMOLOGY

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Resource subsidies, such as vertebrate carrion or cadavers, are recognized to cross ecosystem boundaries and influence the structure and function of micro and macroorganisms associated with the decomposing remains – creating cadaver networks. Cadaver networks consist of groups of interconnected and interacting organisms, such as bacteria, insects, and vertebrate scavengers, that are associated with decomposing organic material. Blow flies (Diptera: Calliphoridae) are typically the first insects to arrive at human remains and carrion, and predictable succession patterns and larval development on the resource can provide information to assist a forensic entomologist with estimates of the minimum post-mortem interval (PMI<sub>min</sub>). More refined estimates of period of insect activity on human remains may also result from studying the microbial community dynamics thus leading to greater precision in estimating the time of death of the individual. However, few studies have evaluated the internal bacterial communities of insects associated with carrion. Given that blow flies directly affect vertebrate decomposition they may be important biological mediators of microbial succession. The objective of this study was to assess the internal microbiome of blow flies (larvae and adults) and decomposing vertebrate carcasses.

adults associated with decomposing post-spawning salmon carcasses Blow flv (Oncorhynchus keta) were surveyed in Juneau, AK, USA. Carcasses naturally deposited on the stream bank were characterized and assessed by dipteran larvae mass activity; those carcasses with third instar masses were sampled for matched larvae collections and adults attracted to the carcasses. The most abundant blow fly species attracted to and colonizing vertebrate carcasses were Calliphora terraenovae and Phormia regina. The internal microbiome communities, as characterized by Illumina MiSeq, demonstrated Firmicutes and Proteobacteria were predominate phyla in adult C. terraenovae while the adult P. regina microbiomes were dominated by Bacteriodetes. Initial results describing the third instar internal microbiomes of C. terraenovae revealed that larval microbiomes were substantially different than those of the adult blow flies, and from those of the carcass. Additionally, C. terraenovae adults had an increase in unique OTUs (5,400) when compared to C. terraenovae larvae (1,515) developing on salmon carcasses, which suggests possible important salmon carcass effects on the insect microbiome.

Our data demonstrate unique shifts in the microbial community of the insects and resources found within a cadaver network. As molecular sequencing technologies continue to improve, the identification of microbiome communities and their interactions within necrophagous flies insects may be useful to improve  $PMI_{min}$  estimations made by forensic practitioners, and potentially increase the use of insect microbiomes at crime scenes.

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#### THE COLLECTION OF MICRO-ARTHROPOD TRACE EVIDENCE FROM HUMAN CLOTHING

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Microarthropods are among the unseen faunal diversity in nearly all human habitats. Despite their dominance, their importance is seldom appreciated in forensics and opportunities for using them as trace evidence in routine forensic investigations have been ignored.

Mites or the Acari are found in almost every conceivable environment. This could be on plants, animals, stored food, furniture, dust, and on humans and their clothing. Many insects and mites have developed intimate associations with humans and human habitation. Their occurrence on humans is of importance in providing evidence in forensic investigations.

Some of the mite species that are found on and in human clothing feed off skin flakes and dander. They also breed in human clothing or any fabrics that are associated with humans or are available in human habitation. Notwithstanding their prominence and occurrence, they have been omitted from forensic investigations, due to the absence of sampling protocols that help forensic scientists and investigators to collect this kind of trace evidence.

We have developed an easy and cost-effective approach for the extraction and screening of microarthropods from fabrics. This method will facilitate and, moreover, quicken the collection and sampling of microarthropod specimens from human clothes in routine forensic analysis.

Different types of commercially available forensic lifters and tapes were tested. The cosmopolitan mite *Tyrophagus putrescentiae* (Astigmata: Acaridae) was used as a model species; it can be found in human clothing as well as on sofas, beddings, floors, carpets, upholstery curtains and window stills. We analyzed three main points: i) the most suitable lifter or tape in terms of quality and quantity of the materials lifted; ii) quality of visualization of mite structure and specimens after extraction; and iii) the number of mites lifted after exposure to treatment.

In this experiment we took advantage of manipulating a number of factors and treatments to speed adhesion of living invertebrates to lifters and fingerprint tapes. The development of microarthropods-screening protocols, like this one, might facilitate their inclusion as trace evidence in routine forensic examination.

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#### MACROCHELES SPECIES AS MARKERS OF STAGES OF DECOMPOSITION: THREE CASE REPORTS FROM EUROPE

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*Macrocheles* is the largest genus in the Macrochelidae family (Acari: Mesostigmata); a family of numerous predator and phoretic mite species. Adult virgin females of *Macrocheles* are commonly carried by insects, particularly beetles and muscoid flies, and in most cases this insect-mite association is species-specific. Phoresy allows the unmated females to rapidly colonise ephemeral environments such as decomposing organic mater. Macrochelidae mites also belong to a haplodiploid clade of the Gamasina; the moment they arrive at a carcass or corpse, they immediately reproduce parthenogenetically and produce sons. At optimal environmental conditions these sons will develop into adults in about 3 days and will mate with the females available. Therefore, in a matter of a few days, the sex ratio of the population will change, and this information can be used as a proxy of the time since the fly carrier arrived. This is especially valuable for estimations of time of death based on insect activity.

Macrochelids predate on other micro-arthropods including fly eggs and other detritivorous mites co-occurring in the carcass. *Macrocheles* species are often used as bioindicators, and in forensic analyses depending on the species as markers of early or, later stages of decay. Members of the Macrochelidae have already been reported from animal carcasses but currently only two records exist of their presence on human corpses. This work discusses the occurrence of three *Macrocheles* species associated with early and advanced decomposition, each sampled from one of three human corpses found in two geographically distant European countries, Spain and Sweden. In each of the three cases, the species reported served not only as an indicator of time but also as a marker of the environment. This information can increase the value in trace evidence analysis.

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#### FROM DEATH TO DUST – USING INSECTS TO DETERMINE POST MORTEM INTERVALS IN QUEENSLAND, AUSTRALIA.

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Forensic entomology and necrophagous insect succession have been extensively studied in southern Australia, however long-term data describing insect taxa associated with decomposition are scarce in northern and sub-tropical Australia. A succession study of the invertebrates associated with carrion in south-east Queensland was conducted over two years to investigate seasonal and annual variation. Colonisation, successional patterns, species diversity, relative abundance of sarcosaprophagous insects, and their potential as forensic indicators were investigated. Data were collected from 64 pig carcases (*Sus scrofa* Linnaeus) decomposing in open grassland and timbered peri-urban habitats at a field site on the western Darling Downs.

*Caloglyphus berlesei* (Acari) outnumbered all other necrophagous invertebrates, however the Calliphoridae and Sarcophagidae (Diptera) are the most useful forensic indicators in terms of specimens observed, collected and reared from the carcases. The primary colonisers of the carcases varied seasonally, and seven calliphorid (blow fly) and four sarcophagid (flesh fly) species were identified as forensically significant for the region. The most important calliphorids and sarcophagids from a forensic viewpoint were *Calliphora augur, Calliphora stygia, Chrysomya megacephala, Sarcophaga impatiens, Sarcophaga aurifrons, Sarcophaga froggatti* and *Sarcophaga praedatrix* because they indicated clear seasonal preferences and bred in the pig carrion. The suites of species occurring as primary and secondary invaders in Queensland are quite different to those recorded in southern and Western Australia. The implication is that succession data generated elsewhere in Australia could not be accurately used for post mortem interval estimations in Queensland.

Comparative data were also collected on an opportunistic basis from road-kill, farmed livestock carcases in the region, and from human remains in the Queensland Health Forensic and Scientific Services mortuary in Brisbane. Results indicate similar succession patterns and dominant species over a range of vertebrate remains in south-east Queensland.

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#### SPATIO-TEMPORAL DISTRIBUTION OF BLOWFLIES (DIPTERA: CALLIPHORIDAE) OF POTENTIAL FORENSIC INTEREST IN THE AREA OF BERN, SWITZERLAND

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Blowflies play an important role for the PMI estimation because they belong to the first colonizers of a cadaver. However, the distribution and therefore the expected species composition depends on geographical region, habitats and season. The knowledge about the spatio-temporal distribution of Calliphoridae is still rare. Here we present the first results of a monitoring of blowflies in the area of Bern, Switzerland.

Starting April 2014, the monitoring lasted 9 months and covered 6 different locations ranging from rural to urban habitats. Bottle traps baited with a combination of beef liver and dimethyl trisulfide (1:10 in MeOH) were used for trapping the flies. Water with liquid soap was used as the trapping liquid. The traps were placed approximately 1 m above ground and were covered with small plastic plates serving as rain shelters. The traps were emptied weekly or every two weeks in the winter sampling period, the containing flies stored in ethanol and the bait refilled. Flies were then morphologically identified.

In total, 5581 blowflies belonging to 13 species were captured, including one finding of the invasive species *Chrysomya albiceps*. The most abundant species was *Lucilia sericata* (3648 specimens), followed by *Calliphora vicina* (684 specimens) and *Protophormia terraenovae* (584 specimens). Most species showed a habitat preference. *Lucilia sericata* e.g. preferred explicitly the location in the city center and was only represented by a few specimens in the forest. *Calliphora vicina* occurred in all habitats but was most abundant in the forest, followed by the suburban habitats. *Lucilia illustris* on the other hand was a generally less abundant species but showed a more or less even distribution over all habitats. All documented species were most abundant in the summer months with a peak in July and August. In the winter month only *Calliphora vicina* and *Calliphora vomitoria* could be documented. Over all, *Calliphora vicina* was the species with the most even distribution over the seasons.

Systematic studies about flies of forensic interest in Switzerland are still scarce. Up to now they have mainly focused on the western part of the country while there are none in central Switzerland. Our preliminary results are in line with common knowledge about the distribution of Calliphoridae in urban regions in Central Europe. However, studies should be extended to the local challenges of the variegated habitats of Switzerland with its urban, suburban, rural, pre-alpine and alpine regions and extreme seasonal variations in temperature.

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# FIRST STUDY OF THE SUCCESSION OF INSECTS ON A CADAVER IN LEBANON

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Forensic entomology studies insects in relation to court cases. It is probably the only accurate method of estimating the minimum Post Mortem Interval (PMI<sub>min</sub>) when more than two-three days after death have passed. In order to investigate the colonization and pattern of decomposition of a pig, an entomological based experiment was carried out on an open field in Bedghan (Aley district, Mount Lebanon Governorate, Lebanon). Stages of decomposition and the insects that marked them were investigated. Adult flies were the first to arrive to the corpse in the fresh stage and their larvae, especially those of *Chrysomya albiceps* (Wiedemann, 1819), dominated the active phase of decay. Throughout the decay process, different fly and beetle species were collected as they fed on the carcass until the stage of dried remains. Three Calliphoridae species were recorded; Chrysomya albiceps, Lucilia sericata (Meigen, 1826) and Calliphora vomitoria (Linnaeus, 1758). Three Sarcophagidae species were identified for the first time in the fauna of Lebanon; Sarcophaga protuberans Pandellé, 1896, Sarcophaga africa (Wiedemann, 1824) and Sarcophaga carnaria (Linnaeus, 1758). Three other Sarcophagidae subgenera belonging to the genus Sarcophaga Meigen, 1826 were recognized; Liopygia Enderlein, 1928, Parasarcophaga Johnston and Tiegs, 1921 and Liosarcophaga Enderlein, 1928. Among beetles, Staphilinidae, Dinothenarus pubescens (De Geer, 1774), Creophilus maxillosus (Linnaeus, 1758) and Histeridae Saprinus maculatus (Rossi, 1792), Saprinus caerulescens (Hoffmann, 1803) were identified for the first time for the Lebanese entomofauna. Dermestes frischii Kugelann, 1792 among dermestids and Necrobia rufipes (De Geer, 1775) among clerids were observed especially during the post decay stage. Within Diptera, the thermophilous predator Chrysomya albiceps was the dominant species and within Coleoptera, Creophilus maxillosus, which feeds on Diptera larvae, was the major species. This is the first record of insect succession on a cadaver in Lebanon; future work will validate the results.

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#### A REVIEW OF FORENSIC ENTOMOLOGY IN KOREA

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Forensic entomology is a branch of forensic science. Forensic entomologists use information about insect lifecycles to help interpret evidence in a legal context. For the purpose of presenting current conditions and future fields of forensic entomology in Korea, 39 articles during the last 20 years were carefully reviewed. Subjects of the articles were insects attracted to carrier (18, 46.1 %). DNA based identification of necrophagous fly species (11, 28.2 %), reviews (4, 10.3 %), and practical application to postmortem interval estimation or case reports (3, 7.7 %). Most articles focused on basic research rather than a practical approach. Moreover, no articles recorded insect fauna attracted to human cadavers or larval developmental data of forensically important insect species in Korea. Because research on DNA-based identification mainly focused on flies, other taxonomic groups, particularly beetles, should be investigated in the future. To enhance the applicability of forensic entomology to the scene, larval developmental studies for major species are also required. Despite these limited studies to date, the change in medicolegal environments due to the tragic sinking of Korean Ferry has stimulated a growing interest in forensic entomology in Korea. In conclusion, although forensic entomology in Korea is still at an early stage, more practical research and attention from the medicolegal and police personnel will encourage its growth.

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#### THE DISTRIBUTION OF FORENSICALLY RELEVANT INSECT SPECIES ACROSS NORTH AMERICA

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When a vertebrate carcass is left to decompose naturally, it normally becomes infested with insect fauna. The most common use of forensic entomology is to estimate the minimum Post-Mortem Interval ( $PMI_{min}$ ), or the most likely time of death using the growth stage of these insects. They can also be used to tell if the cadaver has been moved since death. Some insect species are widespread and are found throughout North America and the rest of the world, whilst others are more narrowly distributed and found in particular regions. Those found only in certain areas can determine if the body is located where the killing occurred or if this scene is further afield. If insects typically found in other areas are found in a crime-scene, it is indicative that the body has been re-located, and primary colonisation at another location has occurred, before any colonisation at the site of discovery.

The Forensic Entomology Investigative Service (FEI) has conducted analysis or consulted on more than nine-hundred cases across North America and has collected over two-hundred and sixty different taxa of invertebrates, across forty-four states of the USA. Included in the database are all the species found within the crime-scenes which have been analysed. However, there are some species which are incidental insects, and on the body by mere coincidence, whilst there are others which are associated with the consumption of dead bodies, but not currently used in forensic entomology analysis. These species are still included, but cannot tell us very much. By analysing the relevant insects and which cases they were collected in, and in which state, we can show the distribution of the species across the United States.

Many of the insects collected in these cases are not found in Europe and are therefore not overly relevant, but it is hoped that this study can demonstrate the benefit of establishing such databases outside of the USA and that the information found can be used in future cases.

As the FEI analyse future cases, the information will be added to this database, with the aim to identify other areas where specific insects can be found. Additionally, collaboration with other Forensic Entomologists, with their casework in North America and around the globe would be beneficial and will increase the data points in this study, therefore identifying the distribution of many more forensically relevant insects and increasing the relevance and reliability of the database.

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#### AN ECOLOGICAL MODELING APPROACH FOR QUANTIFYING UNCERTAINTY ASSOCIATED WITH INSECT AGE ESTIMATES

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Estimates of insect age can be informative in death investigations, and when certain assumptions are met, can be useful for estimating the postmortem interval (PMI). PMI estimates are based on species-specific development data that are temperature-dependent, can be highly variable, and therefore prone to error. Currently, the precision of PMI estimates is unknown, as error can arise due to different sources of variation such as temperature fluctuations, phenotypic variation due to non-temporal related environmental variation or genetic variation, and predation. Ecological models are an abstract, mathematical representation of an ecological system that can make predictions about the dynamics of the real system. Legal and professional pressures have been placed on forensic disciplines to develop a more scientific approach to forensic sciences. One area within forensic entomology in need of restructuring is the approach to understanding error associated with PMI estimates. To quantify the uncertainty associated with PMI estimates, we developed an ecological model that simulates the colonization of vertebrate remains by a blow fly species, to represent the uncertainty associated with local temperature variability, and to refine PMI estimates at local sites. The model is based on a Texas-specific development data set for a primary colonizer of carrion in the southern US, Cochliomyia macellaria (Fabricius) (Diptera: Calliphoridae). After a PMI estimate is calculated for each individual, the model calculates the maximum, minimum, and mean PMI, as well as the range and standard deviation, for the entire sample collected from the remains in Texas investigations. Development rates for all stages were slower and more variable during colder months, and faster and less variable during warmer months. These differences in development rates resulted in longer, more variable PMI estimates during colder months and shorter, less variable PMI estimates during warmer months. Our results suggest that PMI estimates would result in variation of approximately one to eighteen days in colder months and less than four days in warmer months. This study suggests that for this species in Texas, forensic entomologists should be more confident in PMI estimates for warmer months than colder months. The model outlined here is a cost-effective approach to evaluate potential error and can be used to discover likely sources of error for any given prediction algorithm that can guide future research directions regarding estimates of insect age. Such discoveries can lead to new research questions and help guide systematic efforts to better understand error.
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#### THE BIOLOGY AND ECOLOGY OF NECRODES LITTORALIS, A SPECIES OF FORENSIC INTEREST IN EUROPE

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Necrodes littoralis (Coleoptera Silphidae) (Linnaeus, 1758), also known as the "shore sexton beetle," is a common burying beetle that visits and breeds on large vertebrate cadavers. Compared to 1028 French forensic entomology cases analyzed between 1990 and 2013, N. littoralis was observed, on average, in 1 case out of 8. Most of these cases occurred during spring and summer (73.5%) and 90% of the cases were located outdoors. In outside locations, most corpses were located in woodlands, bushes and fields. The decomposition stage of the corpse varied, with more than 50% in the advanced decomposition stage, 36% in the early decomposition stage and less than 10% in the fresh, mummified or skeletonized stages. Regarding other necrophagous species sampled with N. littoralis, Calliphorids flies were found in 94% of the cases and Fanniidae/Muscidae in 65% of the cases. Chrysomya albiceps, a heliotropic species mostly located in the Mediterranean area, was present in 34% of the cases (only 20% in the whole dataset). The most common coleopteran species were Necrobia spp. (Coleoptera: Cleridae) and *Creophilus maxillosus* (Coleoptera: Staphylinidae); these beetles were observed in 27% of the cases. The over-representation of these species is likely due to similar requirements regarding the climate and decomposition stage. As N. littoralis is frequently observed and tends to become more common, we conclude that the developmental data for this species would be a precious tool for forensic entomologists in Europe.

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### A NEW JOINT ITALIAN/AMERICAN ENTOMOLOGY LABORATORY – IMPLICATIONS FOR RESEARCH, TRAINING AND CASE WORK IN SICILY

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Forensic entomology continues to gain attention throughout Italy; specifically, in the last year, a great deal of effort has been invested towards the growth of this discipline in Sicily. Following the forensic entomological research conducted around Mt. Etna and inside its caves, and after several educational events organized during the last months, public administration, law enforcement agencies and local institutions have become more sensitive towards this field and have decided to offer the concrete support that the discipline needs to become established and continue to grow in Sicily. As a result of this work, a new Entomology Laboratory has been created and inaugurated in Caltanissetta (Sicily, Italy). The laboratory is the result of the collaboration among the University Consortium of Caltanissetta, CEFPAS (an institute specialized in biomedical training) and Rutgers University (USA). The laboratory is located inside the CEFPAS complex and is already furnished with all the necessary equipment to be operative. Denise Gemmellaro and the inloco representative, Claudia Sollami, direct the laboratory. This lab will offer training for university students, police forces and public employees (ASP and ASL personnel, people working in the judiciary system and professionals). The laboratory will also be actively involved in research projects supervised by Rutgers University and the Consortium. While the main focus of the lab will be forensic entomology, our intention is to expand to other areas of entomology, particularly veterinary entomology, medical entomology and biological control. The lab will seek and develop collaborations with other experts in the various areas of entomology with the aim of becoming an entomology hub for southern Italy.

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#### THE DARK SIDE OF OPEN ACCESS: PREDATORY JOURNALS IN FORENSICS

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The quality of journals has been driven by two parties: on one hand the consumers (individual subscribers and libraries) who were only prepared to pay for journals when they recognised at least a minimal level of quality and integrity, and on the other hand, by learned societies that try to uphold similar standards. This all changed with the appearance of Open Access journals, where the force to sell to a demanding consumer was replaced by the willingness of the authors to pay for the publication. In some countries this made research articles legally a piece of advertisement.

The desire to publish and the often insurmountable obstacles of traditional journals were so strong that many enterprises sprung up that were only interested in making profit without any effective mechanisms of quality control, the so called predatory publishers which only interest is to predate on authors. This phenomenon is most prevalent in the medical sciences because of the sheer numbers of potential authors and because of the financial resources of the authors in this field.

However, forensic sciences have not escaped this trend. There are now predatory journals in the forensic sciences as well and forensic entomology is affected too on a global scale, either because entomologists started publishing in predatory journals or have even joined the editorial board of such journals. This is a critical trend. The aim of this presentation is to warn against the temptations of predatory journals and start a discussion whether it is the responsibility of an organisation like EAFE to react with e.g. an own recommendation or guideline.

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#### FORENSICS OR NOT FORENSICS: THAT IS THE QUESTION

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Forensic biology is defined as the application of Biology to legal investigation and it covers a wide spectrum of disciplines from genetics to zoology, of organisms from algae to vertebrates and of cases from homicide to protect organisms' trade.

More specifically Forensic Entomology deals with the use of insects and other arthropods in medico legal investigations. If we consider as well urban and store products entomology, forensic entomology deals with the application of the knowledge about insects to legal investigations.

In all these definitions, and in others reported in the wide literature about this discipline there are three common words that make the difference between Forensic entomology and the other branches of Entomology: the magic words are "application", "legal" and "investigations/cases".

We can apply our knowledge, analyses, studies to a legal investigation and we are doing Forensic Entomology. In contrast the study of the colonization of a pig by insects and/or other arthropods can be considered a work of classic ecology. The results obtained from this kind of study may have forensic application if someone will you them in a forensic context.

The creation of a database with sequences that can be used to identify species of forensic importance is not a forensic work is part of molecular taxonomy. In addition a forensic species doesn't exist, but as previously mentioned we can have species of forensic importance: species that if presents in a forensic case can be used in order to provide useful information to answer at the 6Ws questions.

In addition the use of insects in the reconstructions of events occurred more than 100 years ago is not related with Forensic but with historical or archaeological cases.

Different definitions can be applied to other disciplines like for example forensic archaeology where the word refers to a specific approach (methodologies and techniques) but we have to be trapeze artists to explain the meaning of forensic palaeontology.

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#### MEGASELIA SCALARIS (DIPTERA: PHORIDAE) ACTIVITY IN LONG AND SHORT PHOTOPERIODS

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Circadian clocks have evolved to synchronize physiology, metabolism, and behaviour to the 24-hour geophysical cycles of the Earth. The understanding of the circadian clock mechanism is a crucial element of forensic entomology because it is able to control routines such as locomotor activities, location of food sources, feeding, mating, ovipositing, and emergence times. Colonization of carrion and human cadavers by insects allows for the minimum Post Mortem Interval (mPMI) to be estimated.

The prevailing opinion is that flies that are the first colonizers of a cadaver are not active during the night-time and therefore do not oviposit during this time. Determining the prevalence – if any – of nocturnal activity in forensically important flies, is fundamental for an accurate estimation of the mPMI.

Previous studies demonstrated that the scuttle fly *Megaselia scalaris* (Diptera: Phoridae) has nocturnal activity during the night and in dark conditions under 12:12 LD photoperiod in controlled condition.

In this paper we present the effects of longer 16:8 and shorter 8:16 photoperiods on the activity of this fly. In addition we demonstrated that after being entrained in long and short photoperiods, flies recover a 24hr cycle if maintained in dark conditions, confirming the role of the circadian clock in the activity of this fly.

As in previous work, these experiments were also performed using Trikinetics technology, commonly used in *Drosophila* studies, which allows for factual data rather than observational data as reported in many articles. The *M. scalaris* rhythms were monitored at 20°C.

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#### A PRELIMINARY SURVEY OF THE NECROPHAGOUS INSECTS OF ALGERIA: SPECIES DIVERSITY AND MOLECULAR TAXONOMY

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After death, a corpse becomes a habitat and food source for various species of insects including Diptera and Coleoptera. In Algeria the works done in this discipline do not result in publications. The present work brings new information on the succession of necrophagous insects during the decomposition of 3 boar carcasses weighing about 40 kg. The study was performed in Zéralda (Algeria) during autumn 2014. The boars were hunted, killed and placed in metal cages for monitoring. The study area has a sub-humid climate with warm winter. The insect sampling was started the day after the sacrifice of the animals and continued for two months at 2 times a week. Two sampling techniques were used in this study: pitfall traps and yellow plates. The sampling focused on adult insects.

Calliphoridae dominated with the species *Chrysomya albiceps*. The Cleridae *Necrobia rufipes* and the Histeridae *Saprinus semistriatus* were the most abundant beetles. 2 taxa, belonging to the family Muscidae, were recorded for the first time in Algeria: *Synthesiomyia nudiseta*, *Hydrotaea ignava*. Also the flesh fly *Sarcophaga argyrostoma* was not recorded officially in Algeria.

As there are no specific keys for the identification of flies of forensic importance in North Africa, we establish a molecular database for 11 fly species, belonging to four families of forensic importance, Calliphoridae, Muscidae, Fanniidae and Sarcophagidae.

These data are preliminary and may bring new knowledge on the succession of necrophagous insects and their development within the ecosystem carrion in northern Algeria.

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#### THE FALSE CHRYSOMYA MARGINALIS - PITFALLS OF LARVAL IDENTIFICATION ON BASIS OF MORPHOLOGICAL STRUCTURES

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In June 2014, an unknown male corpse was discovered in an apartment in the city of Frankfurt, Germany. The stage of decomposition was advanced with a massive larval infestation by blow flies. During the autopsy we sampled larvae to determine the minimum post-mortem interval.

Morphological identifications of 243 third instar larvae were performed using the "Key for identification of European and Mediterranean blowflies (Diptera, Calliphoridae) of forensic importance - third instars" (Szpila, 2010) and revealed altogether seven species: *Lucilia sericata, L. illustris/caesar, Calliphora vicina, Calliphora vomitoria, Phormia regina, Protophormia terraenovae,* and eventually *Chrysomya marginalis.* 

The latter species was represented by 7 specimens. According to the current stage of knowledge, *Ch. marginalis* does not occur in Central Europe. Morphological comparisons with reference specimens of *Ch. marginalis* and the similar *C. vomitoria* still supported or at least did not exclude an ambiguous identification. The oral sclerite was rather shorter than sclerotized in whole length. The posterior spiracles were close to each other with an incomplete peritreme. It was not possible to use the shape of thoracic spines as they are similar in both species: being large, with blunt tips and arranged separately. For molecular identification DNA-Barcoding of the cytochrome oxidase subunit-I (COI) was performed and resulted in all specimens being identified as *C. vomitoria*.

A possible explanation for the ambiguous identification might be that the "*Ch. marginalis*" larvae in question were young third instars and important structures like the oral sclerite and peritreme of posterior spiracle were not fully sclerotized. Young third instar larvae of *C. vomitoria* without a well pigmented oral sclerite and an incomplete peritreme may be also misidentified as larvae of *Ch. megacephala*. This highlights the benefits of an additional use of molecular methods for identification in cases, where some characters might be doubtful.

Szpila, K. (2010). Key for the Identification of Third Instars of European Blowflies (Diptera: Calliphoridae) of Forensic Importance. In J. Amendt, C. P. Campobasso, M. L. Goff, & M. Grassberger (Eds.), *Current Concepts in Forensic Entomology* (pp. 43–56).

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#### **BODY REGION AND TISSUE SPECIFIC INFESTATION PATTERNS OF FORENSICALLY IMPORTANT DIPTERA – MUCH ADO ABOUT NOTHING?**

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In the recent past several authors have noted the part of a corpse from which larvae are collected due to different growth rates on different body tissues. Kaneshrajah & Turner (2004) for example showed that *Calliphora vicina* larvae grow faster on lung than on liver. Clark et al. (2006) observed the same for *Lucilia sericata* larvae feeding on lung and heart. Ireland & Turner (2006) showed that fly eclosion of *Calliphora vomitoria* is delayed by at least two days when fed on brain.

From May to November 2014, we sampled larvae from 17 corpses during autopsy, assigning the samplings to 15 different body regions and tissues: eyes, brain, outer head/neck, oral cavity, throat, outer thorax, muscle, abdominal cavity, liver, heart, lung, gut, anal-genital-region, arms and legs. The presumed post-mortem interval ranged between three and thirty days. Half of the specimens of each single body region were reared on ground meat (pork/beef) until adults emerged. The flies were identified and the date of eclosion recorded. The remaining half of larvae was killed with hot water and stored in 96% ethanol. Identification and length measurement was performed immediately. Altogether 1637 specimens were reared to the adult stage and around the same number of larvae were identified and measured.

53 % to 71 % of the bodies were infested by maggots in the brain (n= 10), throat (n= 10), lung (n= 12), legs (n= 9), the outer head/neck (n= 10), the oral cavity (n= 10) and the outer thorax (n= 12). Organs like liver, heart and gut revealed a colonization of just up to 12 %. We identified nine species belonging to the families of the blow flies, flesh flies and true flies. *L. sericata* occurred on 76 % of the bodies (n= 13).

No species-specific preferences for a certain body region or tissue were observed. Focusing on the most abundant species, *L. sericata*, distribution of the juvenile stages on single body regions was heterogeneous and didn't show a certain age specific pattern.

The recommendation of sampling and sorting larvae according to their place of feeding seems to be an academic one - the ecosystem cadaver isn't that easy to handle. Assigning larvae found in the cavity of a badly decomposed cadaver to a certain body region or organ is just one dilemma. Moreover, as larvae are highly mobile it seems to be impossible to reconstruct their nutrition history. Last but not least a different stage of development of the same species on different organs or body regions doesn't necessarily indicates a tissue specific artifact of development: it simply can mirror different times of colonization as e.g. the natural orifices of a body are preferred oviposition sites

Clark, K., Evans, L. & Wall, R., 2006. Growth rates of the blowfly, Lucilia sericata, on different body tissues. *Forensic Science International*, 156(2-3), pp.145–9.

Ireland, S. & Turner, B., 2006. The effects of larval crowding and food type on the size and development of the blowfly, *Calliphora vomitoria. Forensic Science International*, 159(2-3), pp.175–81.

Kaneshrajah, G. & Turner, B., 2004. *Calliphora vicina* larvae grow at different rates on different body tissues. *International Journal of Legal Medicine*, 118(4), pp.242–4.

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#### EFFECTS OF AMITRIPTYLINE ON THE DEVELOPMENT OF *MEGASELIA* SCALARIS (DIPTERA: PHORIDAE)

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In Forensic Entomology the estimation of the age of the insects is used for the estimation of the minimum post mortem interval (mPMI). Insect development rate is mainly temperature dependent despite of other parameters can affect the developments. Several studies demonstrated that drugs and other chemicals can affect the growth of larvae, feeding on the dead body, leading to incorrect mPMI estimations. Amitriptyline is a commonly used antidepressant in case of major depressive disorder. It is a tricyclic molecule absorbed in the gastrointestinal tract and metabolized into the liver. This molecule shows a high toxicity in cases of overdose.Studies on the effect of Amitriptyline on insect development and accumulation/excretion have been performed in the '90 on Parasarcophaga ruficornis (Diptera: Sarcophagidae) and on *Calliphora vicina* (Diptera: Calliphoridae) whereas no data are available for other taxa. The results of these studies demonstrated the non effect of the molecule on the growth rate. In the same years Amitriptilyne and derivates have been isolated from empty puparia of Megaselia scalaris (Diptera: Phoridae) and from skin and fecal material of Dermestes maculatus (Coleoptera: Dermestidae) collected from a mummified body in New England. The aim of this study was to investigate the effect of Amitriptyline, often found on cadavers, on the development of Megaselia scalaris, a common species in indoor cases both in Europe and in the USA. This species is very important for mPMI estimation in indoor cases, as observed by the authors and reported in the specific literature.

Larvae of *M. scalaris* were reared on pork liver with four different concentrations of amitriptyline (0=control, 120, 240, 800 ng/g). One hundred twenty larvae per each concentration were killed in hot water after 48 and 72 hours from the experiment beginning (eggs) and measured using a M60 Leica microscope equipped with a camera and LAS software with an automatic calibration of the measurements. Pupa and wing measurements were also collected and analysed. Statistic tests (one way and factorial Anova, Tukey posthoc) were performed using IBM Statistical v22 software, using 0.05 as significant level.

Statistically significant differences were observed in the larval size of the 4 treatments after 48 and 72hrs ( $F_{3,476}$ =62.59 p=0.000;  $F_{3,476}$ =13.66 p=0.000 respectively) The same result was obtained for the pupa length ( $F_{3,476}$ =12.42 p=0.000).

The wing size, used in order to detect differences in size in the adults, shows statistically significant differences (p=0.000) with the control being smaller when compared to the specimens fed on food with different antidepressant concentrations.

Durations of the immature stage (larval and puparial stages) despite of the size differences were not statistically different form the control at all the tested concentrations.

In conclusion this experiment demonstrated that on *M. scalaris* Amitriptyline has an effect on the larval size but not on the total immature developmental time, so the mPMI estimation can be affected if base on the larval size and not on the complete development.

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# COASTAL INSECTS: DECOMPOSITION AND SUCCESSION IN PORTSMOUTH, UK

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Throughout the world, there are a variety of studies highlighting local species distribution and insect succession. Only six UK-based studies have been published to date, all of which are at least six years old. Compounding this, there are indications that climate change is affecting species distributions (Picard, 2013). Our aim was to produce preliminary decomposition and succession studies for future reference and monitoring the effects of climate change.

Two studies were conducted in Portsmouth, UK. The first study in October 2012 monitored decomposition of a whole pigs leg (*Sus scrofa*) within a vehicle, for one month. High levels of activity from only *Calliphora vicina* were observed despite the cool and damp autumnal weather, at an average temperature of 11.1°C. Oviposition occurred on day five. The mean larval development temperature was 12.3°C, with the increase attributed to the formation of a larval mass from days eleven to twenty-two. Interestingly, the longest larvae exceeded that of the dataset provided by Donovan et al. (2006), making age estimation using this dataset troublesome.

The second study was conducted in August 2013 on whole rabbit cadavers (*Lepus curpaeums*) both exposed to a garden environment and within a car, for two weeks. Decomposition was accelerated; in addition to *C. vicina*, various *Lucilia sericata*, *Lucilia caesar* and *Lucilia illustris* adults and immature stages were collected. The exposed rabbit decomposed to the dry stage within one week, at an average temperature of ~18°C. Colonisation occurred on day four. Within the car, colonisation occurred on day seven, the day after the rabbit bloated. The dry stage was reached by day twelve.

Direct comparison between the studies showed seasonal variation and discrepancies in the estimated period of colonisation from visual inspection and larval measurement.

Donovan, S. E., Hall, M. J. R., Turner, B. D., & Moncrieff, C. B. (2006). Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures. *Medical and Veterinary Entomology*, 20(1), 106–14.

Picard, C. J. (2013). First Record of *Chrysomya megacephala* Fabricius. (Diptera: Calliphoridae) in Indiana, U.S.A. *Proceedings of the Entomological Society of Washington*, *115*(3), 265–267.

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#### SSAP-BARCODING: ESTABLISHING A SEQUENTIAL SHORT AMPLICON PCR FOR ANALYSIS OF DEGRADED SAMPLES

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The sound identification of fly species collected from a corpse is essential for determining the age of the specimens and calculating the minimum postmortem interval. However, it remains challenging to distinguish several forensically important fly species based on morphological criteria. On-site killing or preservation by the crime scene technician makes rearing to the adult stage impossible. Therefore, DNA-based methods for species identification are applied. Hebert et al. (2003) suggest using a part of the *coI* gene for standardized molecular identification of all animals due to its high degree of nucleotide variation. For amplification and sequencing of this 648 bp region, primers established by Folmer et al. (1994) are commonly utilized. However, in case of degraded DNA (may e.g. occur during decomposition or inadequate killing/preservation) barcoding may fail due to the length of the folmer fragment. Therefore, we established a system of sequential primers, each generating amplicons of 100 - 170 bp.

In this study we present the first approach to establish amplification- and sequencing-primers for the *coI* barcoding region of several forensically relevant flies that offer molecular identification of species in case of degraded DNA.

We designed 9 primer pairs amplifying the mentioned barcoding region as overlapping fragments. They were established according to an alignment of nearly 50 forensically important fly species belonging to the families Calliphoridae, Muscidae, Piophilidae, Phoridae and Sarcophagidae. Due to high sequence polymorphism the use of wobble bases are necessary.

In order to verify the designed primers for amplification efficiency, DNA originating from the blow flies *Calliphora vicina*, *Calliphora vomitoria* and *Lucilia sericata* was used. All amplicons generated, exhibited the expected molecular weight. Currently, we are validating these primers for sequencing.

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#### A COMPARISON OF DIFFERENT STORAGE AND MEASURING METHODS ON LARVAL LENGTH VALUES OF THE BLOW FLIES *LUCILIA SERICATA* AND *CALLIPHORA VICINA*

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Sampling, killing and storing methods are very important in forensic entomology (FE) because they can influence survival and growth rates of living samples as well as the result of the morphometric examination of the dead specimens. There are several manuals about best practice in FE, leading to a certain amount of heterogeneity regarding methods of sampling and storing insect evidence. Interestingly, the scientific background for these recommendations and manuals is quite fragile as only a few studies have examined the effects of different killing and storage methods.

We examined the influence of different killing and storing methods on two forensically important blow flies, Lucilia sericata and Calliphora vicina. For the latter species we additionally were evaluating three different length measurement methods. Newly hatched Lucilia sericata larvae were supplied with ground beef ad libitum in an incubator at 20°C. For three days, larvae were sampled from the source of food every 24h and divided in two equal subsamples. The first subsample was killed by hot but not boiling water (HW) and stored in 75%-ethanol. Half of these were stored at room temperature and the other in a fridge at +6°C. The second subsample was killed by boiled up 75%ethanol and left in this killing solution (HE). Again half of them were stored at room temperature and the other in a fridge at  $+6^{\circ}$ C. Lengths of all larvae were measured immediately after killing and every 24h until day 4 and once more after 7 days. No significant changes in the lengths of the stored larvae were seen except for 2 treatments in the HW group stored under room temperature in which 24 h old larvae showed a significant decrease in length after 4 days of storage time, and 72 h old larvae revealed a slight significant increase in length after one week of storage. There were no differences which would lead to different age estimations of L. sericata, as the observed variance in length seems to be a negligible natural variation. In a similar second experiment, newly hatched *Calliphora vicina* larvae were supplied with ground beef ad libitum in an incubator at 23°C. For three days, larvae were sampled from the source of food every 24h and divided in two equal subsamples. Both subsamples were killed by hot but not boiling water. Subsample I was stored at room temperature in 70%-ethanol, subsample II was stored at room temperature in 96%-ethanol. Lengths of all larvae were measured immediately after killing and every 24h until day 7 and then every week for one more month. There was an increase in length over time, but significant only for the subsamples I (stored in 70%-ethanol), especially in the younger specimens (day 1 and day 2.). The observed differences could lead to a wrong estimation of age for those larvae which were stored in 70%-ethanol, but not for those which were stored in 96%-ethanol.

The results of both experiments suggest that it is possible to kill and store fly larvae directly in (not hot)  $\geq$ 75%-ethanol. This simplifies the sampling and storing of fly evidence.

We also compared the influence of three different measuring methods for estimating the length of  $L_1$ -  $L_3$  *C. vicina* larvae by using a) a ruler with a 0,1 mm scaling, b) a geometrical micrometer and c) a computer-aided stereomicroscope. No significant differences were detected, supporting the view, that a simple tool like a geometrical micrometer can produce reliable results.

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# DECOMPOSITION OF CONCEALED PORCINE REMAINS IN THE NORTH CAROLINA PIEDMONT

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The decomposition and subsequent colonization of pig carrion (Sus scrofa (L.)) placed in concealed and open environments, by necrophagous insects, was studied during the spring, summer, and fall from 2010 - 2012, in Raleigh, North Carolina, USA. Remains were concealed in simulated attics in one of three manners, ranging from minimal (no additional concealment aside from the attic) to well concealed (remains were placed inside a plastic trash bag and plastic storage bin). Concealment in the attics had a significant impact on the insect community colonizing the remains. Although blow flies (Diptera: Calliphoridae) and other fly species colonized remains located indoors, the beetles Necrobia rufipes (DeGeer) (Coleoptera: Cleridae) and Dermestes maculatus (DeGeer) (Coleoptera: Dermestidae) were the only species indicative of remains located indoors. In contrast, numerous fly species, beetle species, and an ant species (Hymenoptera: Formicidae: Prenolepis), were species indicative of remains located outdoors. Season also significantly affected the insect species, particularly the blow flies (Diptera: Calliphoridae), that colonized decomposing porcine remains. Lucilia illustris (Meigen) was indicative of the spring, Cochliomyia macellaria (F.) and Chrysomya rufifacies (Macquart) indicative of the summer, and Calliphora vicina (Robineau-Desvoidy) and *Calliphora vomitoria* (L.) indicative of the fall. Additionally, across all seasons, concealment delayed colonization by 35-768 hours, depending on the degree of concealment. These differences between the insect communities across seasons and concealment treatments, and the effects of concealment on colonization indicate that such information is important and should be considered when analyzing evidence for criminal investigations.

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# LITERATURE REVIEW OF SOCIAL WASPS (HYMENOPTERA, VESPIDAE, POLISTINAE) ASSOCIATED WITH VERTEBRATE CARRION IN BRAZIL

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Some species of social wasps, especially members of the epiponine genera *Agelaia* Lepeletier and *Angiopolybia* Araujo, utilize vertebrate carrion to feed their larvae. Foragers from these genera display aggressive behavior toward adults of carrion-related flies and prey on their immatures, thus reducing their numbers and retarding the decomposition process. Given that literature on necrophagic behavior in social wasps is still scattered, we reviewed the literature for inventories of these insects collected from vertebrate carcasses in Brazil. This review was based on a bibliographical survey that included only journal articles, excluding abstracts and papers presented at congresses or conferences. The articles that we found reported the following taxa: *Polybia (Myrapetra) fastidiosuscula* de Saussure, *Polybia (Trichothorax) ignobilis* (Haliday), *Polybia (Trichothorax) sericea* (Olivier), *Polybia (Myrapetra) paulista* Ihering, *Angiopolybia pallens* (Lepeletier), *Angiopolybia paraensis* (Spinola), *Apoica* (*Apoica) gelida* Van der Vecht, *Agelaia cajennensis* (Fabricius), *Agelaia fulvofasciata* (Degeer), *Agelaia angulata* (Fabricius), *Agelaia pallipes* (Olivier), *Agelaia vicina* (de Saussure) and *Agelaia multipicta* (Haliday).

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#### REVIEWING THE HOUSE DUST MITE FAUNA: ITS VALUE AS INDOOR TRACE EVIDENCE

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Most environments are characterised by a huge diversity of microscopic, eukaryotic organisms, ranging from single cell algae (diatoms) and testate amebae to animals like nematodes and mites. Their small size facilitates exchange between objects, people, and the environment. House dust mites (HDMs) are arthropods belonging to several clades of the Acari. What makes the house as an environment very special is that a new house doesn't come with mites. House dust mites are imported into the house by humans, which makes every house slightly different. This also means that these dust mites are very much associated with humans. The house itself offers a wide variety of microenvironments colonised by an equal variety of mite species. An almost ideal situation for employing house dust mites in forensic trace analyses. The fact that a few house dust mite species are also responsible for human allergies has led to an overwhelming body of medically oriented literature on the subject; a forensic investigator will struggle to find the mites in the haystack.

To make the house dust mite literature more accessible for forensic purposes, a comprehensive review has been started to chart the biodiversity of mite species inside houses to specific environments like living room, kitchen, bath room, bed room, and microenvironments like dado rail, windowsill, curtain, foot-end of the bed, pillow and so on. Peculiarities of biogeographic regions, urban and rural houses, and living standards are taken into account. The bigger picture of the mite diversity is visualised with heat maps for biogeographic regions and graphical representations of the interior of houses, while the forensic detail is accommodated in species-specific descriptions of the biology.

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#### FROM DEATH TO DUST – USING INSECTS TO DETERMINE POST MORTEM INTERVALS IN QUEENSLAND, AUSTRALIA.

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Forensic entomology and necrophagous insect succession have been extensively studied in southern Australia, however long-term data describing insect taxa associated with decomposition are scarce in northern and sub-tropical Australia. A succession study of the invertebrates associated with carrion in south-east Queensland was conducted over two years to investigate seasonal and annual variation. Colonisation, successional patterns, species diversity, relative abundance of sarcosaprophagous insects, and their potential as forensic indicators were investigated. Data were collected from 64 pig carcases (*Sus scrofa* Linnaeus) decomposing in open grassland and timbered peri-urban habitats at a field site on the western Darling Downs.

*Caloglyphus berlesei* (Acari) outnumbered all other necrophagous invertebrates, however the Calliphoridae and Sarcophagidae (Diptera) are the most useful forensic indicators in terms of specimens observed, collected and reared from the carcases. The primary colonisers of the carcases varied seasonally, and seven calliphorid (blow fly) and four sarcophagid (flesh fly) species were identified as forensically significant for the region. The most important calliphorids and sarcophagids from a forensic viewpoint were *Calliphora augur, C. stygia, Chrysomya megacephala, Sarcophaga impatiens, S. aurifrons, S. froggatti* and *S. praedatrix* because they indicated clear seasonal preferences and bred in the pig carrion. The suites of species occurring as primary and secondary invaders in Queensland are quite different to those recorded in southern and Western Australia. The implication is that succession data generated elsewhere in Australia could not be accurately used for post mortem interval estimations in Queensland.

Comparative data were also collected on an opportunistic basis from road-kill, farmed livestock carcases in the region, and from human remains in the Queensland Health Forensic and Scientific Services mortuary in Brisbane. Results indicate similar succession patterns and dominant species over a range of vertebrate remains in south-east Queensland.

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#### A REVIEW OF NECROPHAGOUS INSECTS COLONISING HUMAN REMAINS IN SOUTH-EAST QUEENSLAND, AUSTRALIA.

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A review of insects collected from decomposing human remains in south-east Queensland yielded 32 species in three orders (Diptera, Coleoptera, Hymenoptera) and 11 families (Calliphoridae, Sarcophagidae, Muscidae, Phoridae, Sepsidae, Chironomidae, Dermestidae, Cleridae, Histeridae, Staphylinidae, Encyrtidae). There were 15 cases where remains were located indoors and five cases where remains were outdoors, in both terrestrial and aquatic environments. Coleoptera were strongly associated with outdoors remains, while dipteran species composition was similar in both indoor and outdoor habitats. Some Diptera were only associated with indoors remains, while others were similarly restricted to remains recovered outdoors. Hymenopteran parasitoids were active in both habitats.

The predominance of Calliphoridae (*Chrysomya rufifacies, Ch. megacephala, Ch. saffranea,* and *Ch. nigripes*), which were present in 15 of the 20 cases, reflects its close association with human and other vertebrate remains, justifying the ongoing use of blowflies in forensic investigations. Sarcophagidae species were collected from 9 of the 20 mortuary cases, and in three of these cases, were the only larvae present, indicating a potential for *Sarcophaga crassipalpis* and *S. impatiens* to behave as primary invaders. They appeared to behave as secondary invaders in other cases where much more developmentally advanced calliphorid larvae were present.

Comparative collections were made from other vertebrate remains, including road-kill and farmed animals throughout south-east Queensland and northern New South Wales during the same period. Similar succession patterns and dominant species were observed over a range of vertebrate remains in south-east Queensland.

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#### NEW DECOMPOSITION STAGES TO DESCRIBE CARCASSES DECOMPOSING IN THE PARTIAL OR COMPLETE ABSENCE OF INSECTS

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Decomposition is a complex process that is highly influenced by many interrelated variables. It has been determined that when temperature is accounted for, insects play the most significant role in the rate of the process. The use of decomposition stages helps investigators better characterize the state of remains. The most commonly used stages in the field are those which were published by Payne in 1965. A set of six stages were established to describe carcasses decomposing in the presence of insects, and a set of five stages to describe carcasses decomposing in the absence of insects. This study proposes new stages of decomposition to describe scenarios involving the partial exclusion of insect. It also proposes additional stages for the complete exclusion of insects which better describe observations made in a temperate zone, such as the Great Lakes region.

The current study was conducted during the summer months of three consecutive years (2011-2013) in Southern Ontario, Canada. It investigated the decomposition processes of pig (*Sus scrofa domestica*) carcasses exposed to insects, and those which were partially or completely excluded. The insect-exposed carcasses acted as controls and were described using Payne's six stages (fresh, bloat, active decay, advanced decay, dry, and remains). However, the other groups of carcasses exhibited different decomposition patterns, necessitating the creation of new, descriptive stages.

Carcasses partially excluded from insects were characterised by: fresh, bloat, localized tissue removal, dry decomposition, and desiccation. One carcass from each of the 2012 and 2013 studies were never accessed by carrion, causing them to decompose differently from the partially excluded carcasses, and they did so at a much slower rate. Four stages were recognized for these carcasses: fresh, bloat, deflation, and dry decomposition.

Under specific environmental conditions, such as partial or complete exclusion of insects, carcasses decompose differently than the more commonly observed carcasses actively accessed by carrion. It is hoped that the characterisation of more suitable decomposition stages will aid investigators and researchers to more accurately describe the remains. Additional studies should be conducted in different environments to compare these observational stages.

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#### EFFECT OF BROMADIOLONE POISON ON DIPTERA COLONIZATION IN BODY RATS (*RATTUS NORVERGICUS*) PLACED IN SEMI-CONFINED SPACES

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Some species of Diptera will lay their eggs on corpses are lying on the surface of the ground, but in buried corpses the physical barrier of soil and some factors, as temperature, shadow or oxygen, restrict their access or reduce their activity. However, some Diptera species are able to locate bodies below ground. Sealing a body within an airtight container also reduces its rate of decay because of the reduced oxygen level and the inability of invertebrates to gain access to it. There are few experimental data on how the colonization happens in bodies with few or nothing reachability.

In urban ambient the most common dead animal are birds and rats. In the last years the control of rat pest with use of poisons has increment to the detriment to the typical traps. Certain poisons, as the bromadiolone, have an effect in medium long term, rodent killing after a continuous intake of such poisons. Poison causes the dead, but animal body is hidden in burrows or other places, causing unpleasant odours and the presence of Diptera. Many of the manufacturers of these poisons ensure rapid dehydration of the body and the absence of Diptera as a result.

The objective of this study was to analyze the entomofauna attracted to cadavers confined and to know the effect of bromadiolone on the presence of detritivorous Diptera species. Dead rats with bromadiolone,  $CO_2$  and clamping device, were introduced in sealed boxes buried at 30 cm with two tubes of 50 cm on each side that reached the surface and allowed the access to the dead body. After 10 days all specimens present in dead bodies were breed in lab at controlled condition. Number of specimens and mortality rate in preimaginal stages were obtained in base to type of dead.

Preliminary results showed that Diptera of Calliphoridae, Sarcophagidae, Muscidae, Phoridae and Piophilidae families were the most abundant. The most common blowflies were *Lucilia sericata* and *Calliphora vicina* joint to *Chrysomya albiceps*, which appears exceptionally. The bromadiolone caused a high level of dead pupae and low abundance of Phoridae and Piophilidae adults.

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#### GROWING CURVES IN THE BLACK SOLDIER FLY, HERMETIA ILLUCENS (DIPTERA: STRATIOMYIDAE)

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Recently *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae) has been found in human corpses in Europe, so this species can be considered as a forensic indicator to estimate the post-mortem interval (PMI). But this species is known by its capacity to develop in a wide variety to substrates, as wastes, excrement, etc. Determine the accumulated degree-days are a key factor on applied research with this species, not only in forensic entomology but also for mass production as animal feed.

The aim of this study was known the life cycle of this species in two different larvae medium (meat pig and hen feed) and at three constant temperatures (25, 30 and 35°C). Larvae were measured and weighed every day during the time of experiment. When they became in pupae, these were individualized and weighed until adult emergence. The variation in development time, size of larva and adult, and weight of pupa were recorded in the two different diets at the three temperatures indicated above. Moreover, the minimum development time in each stage, the minimum development threshold temperature ( $t_0$ ), the accumulated degree-days and isomorphic diagrams were obtained.

The results indicate a decrease in the duration of larval stage with temperature increases. However, pupae reared at maximum temperature needed more time to complete their development. The number of required degree-days for total development in *H. illucens* varied with the temperature but also with the quality of larval medium. Larvae fed in hen feed needed fewer degree-days than developed in meat of pork. Regarding the temperature, the number of degree-days to complete the development at 30°C is greater than at 25 or 35°C. Finally, size, weight and duration in each stages of development were compared in base to temperature and larvae rearing substrates.

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#### SYNTHESIOMYIA NUDISETA (DIPTERA, MUSCIDAE): AN ACTIVE PREDATOR?

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Intraguild predation (IGP) is a combination of competition and predation, which is the killing and eating within potential competitors. When dipteran larvae feed on corpses, intraspecific and interspecific competition phenomena take place on them. *Synthesiomyia nudiseta* (Van der Wulp, 1883) is a species with forensic importance in tropical, subtropical, and recently south of template regions (Velasquez et al., 2013). The mature larva of this species is facultative predator, but not many scientific papers of *S. nudiseta* larvae predation has been published until now. However, *Chrysomya albiceps* (Wiedemann, 1819) is a facultative predator on other necrophagous larvae during part of its larval stage, and its negative influence on abundance of blowflies is frequently cited.

The aim of this study was to investigate the role of *S. nudiseta* with other necrophagous species. Analyse of intra- and interspecific competition was carried on. The species used for this study were the most common Calliphoridae in corpses in southwestern of Europe (*Ch. albiceps, Calliphora vicina, Lucilia sericata*). To study the predation behaviour of *S. nudiseta*, four densities were used (50, 100, 150 and 300 larvae). Each larvae group in first instar were placed in a plastic cup with the same amount of pig liver (15g).

This study allowed us to evaluate factors, such as competition and initial density, affect in the survival rate, development and adults size in blowflies and *S. nudiseta*. In general results show that when density increased mortality increased, size decreased and development decreased too. Mortality rates at interspecific competition condition were higher than in intraspecific competition in all studied species, *Ch. albiceps* even died in competition with *S. nudiseta* at 25°C but not at 23°C. In all species, the larval period was more influenced than pupation period by the initial larval density. In the case of *Ch. albiceps* the larval period was double in the mixed cultures with *S. nudiseta* than in the pure cultures. The implications of *S. nudiseta* presence in the sarcosaprophagous community dynamics will be discussed.

Velásquez, Y., Ivorra, T., Grzywacz, A., Martínez-Sánchez, A., Magaña, C., García-Rojo, A. and Rojo, S. 2013. Larval morphology, development and forensic importance of *Synthesiomyia nudiseta* (Diptera: Muscidae) in Europe: a rare species or just overlooked? *Bulletin of Entomological Research*, 103: 98-110.

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#### A NEW TOOL TO FORENSIC ENTOMOLOGICAL INVESTIGATIONS IN SPAIN

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Frequently, in Spain available forensic data in the literature is incomplete or invalid in a large number of cases, which often prevents or limits their usefulness in legal proceedings. However, in recent years it has significantly increased the number of studies and forensic cases that provide information to correct these shortcomings. Unfortunately, these data are often scattered in multidisciplinary scientific literature or sometimes its dissemination is limited.

Due to the difficulty that exists in our country for the implementation of time of death based on entomological evidence in professional field (police and medical - legal), the main objective of this work is to obtain a software tool for managing entomological evidence. This application allows to calculate a correct time of death by estimating the Post Mortem Interval (PMI) and the factors that influence its calculation, because it would use the data on the necrophagus fauna in Spain and the Iberian peninsula but also all accessible data which can be incorporated from other areas of the world, extracted from scientific articles and unpublished experiments, obtaining a very complete database.

For the development of this tool, we have discarded using proprietary or payment technologies, opting for the use of open source technologies (*mysql, php, apache, javascript*). Given all this, we have decided to develop a web application that is easily accessible by different types of users. Forensic indicators used in the project are species of the families: Calliphoridae, Sarcophagidae and Muscidae, fundamentally, because these allow us to estimate the precise time of the colonization of the body. As a result, this new tool and its functionality are presented. This instrument will permit having a mechanism for managing individual projects that a researcher can perform; besides, the knowledge generated is shared with the scientific community, expanding that knowledge in a collaborative manner.

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#### DEVELOPMENT OF THREE BLOW FLIES (DIPTERA: CALLIPHORIDAE) IN THE SOUTH-WESTERN EUROPE

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The study of biology and development of necrophagous insects is necessary to achieve a proper application of forensic entomology, especially when some species from different geographic origin have different behavior. In order to increase the knowledge of the forensic entomofauna from southern Europe we studied the life cycle of three species that commonly appear in decomposing matter in the Iberian peninsula: *Calliphora vicina* Robineau-Desvoidy, 1830, *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia sericata* (Meigen, 1826). We evaluated developmental time and growth curves in the laboratory under three constant temperatures (18, 23 and 28°C) with 60-70% RH and a photoperiod of 14: 10 (L:D). The temperature had a significant effect on the maximum average size of the larvae, both in length (F = 74.57, df=8, P = <0.001) and weight (F = 185.26, df=8, P = <0.001). In *C. vicina*, there was no significant difference in the maximum length, but larvae reared at 23°C were the heaviest. Larvae of *Ch megacephala* maintained at 23°C were longer than those reared at 23°C and 28°C, and larvae kept at 18°C had the lowest weight. In *L. sericata*, larvae reared at 23°C were significantly bigger in both length and weight, than those maintained at 18°C.

The complete development in *C. vicina* ranged from  $27.4 \pm 0.5$  days at 18°C to  $15.2 \pm 0.9$  days at 28°C. In *Ch. megacephala*, the time of development varied between  $26.7 \pm 0.5$  days at 18°C and  $9.0 \pm 0.0$  days at 28°C. The total time required for *L. sericata* to complete development ranged from  $31.1 \pm 1.0$  days at 18°C to  $13.6 \pm 0.5$  days at 28°C.

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#### DETERMINATION OF EMBRYONIC MORPHOLOGICAL LANDMARKS FOR AGING CALLIPHORA VICINA EGGS

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Although larvae and pupae are the most frequent developmental stages collected by the time a cadaver is discovered, blowfly eggs can sometimes be the only entomological evidence in a forensic case, especially in cooler weather when hatching might take several days; hence a method for estimating their age is strongly needed. Numerous studies on larval development of forensically important blowflies have been published and different approaches for age estimation of blowfly pupae are currently being developed. However, developmental data on blowfly eggs are limited virtually just to records of the time of larval hatching. The aim of the current study is to describe the morphological changes occurring during the embryogenesis of the blowfly *Calliphora vicina* Robineau-Desvoidy and their timing, in order to determine those which could be used for egg age estimation.

Eggs of C. vicina were obtained from a laboratory colony, collected with a fine brush from the oviposition medium (fresh pig liver) within 30 minutes of oviposition, placed on moistened tissue paper on a Petri dish, and then into an incubator under a constant temperature of 25°C. Ten eggs were randomly collected at each of the 11 10% time intervals (from 0-100%), dechorionated in a 1:1 solution of sodium hypochlorite, then rinsed and placed on a cavity slide in Hoyer's medium. The dechorionated living embryos were photographed with transmitted light under a binocular microscope during the 30-minute period after collection. The whole procedure was replicated three times using a different incubator each time. Moreover, to test the killing and preservation method, ten eggs of each age were killed with hot water, transferred to 80% ethanol and stored at 4°C, while another ten eggs of eeach age were placed directly in 80% ethanol and also stored at 4<sup>o</sup>C. One week after storage, the eggs were dechorionated and visualised following the aforementioned method. Fourteen morphological landmarks easily-visualised under a binocular microscope were identified in living embryos of C. vicina, allowing for their age estimation with a resolution of 10-20%. The observed age intervals are compared to the embryonic stages described for *Drosophila melanogaster* Meigen at 25 <sup>o</sup>C, which are used as reference data in many developmental studies. Hot water killing and fixation is recommended prior to preservation of egg samples in 80% ethanol. The resolution of landmarks in preserved material is discussed in comparison to that observed in living embryos.

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#### DIPTERANS ASSOCIATED WITH A DECOMPOSING ANIMAL CARCASS IN RIYADH, SAUDI ARABIA

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This study aimed to provide the first checklist of forensically-important dipteran species in Riyadh, Saudi Arabia, a region exposed to high rates of dryness. Using a decomposing rabbit as experimental model. Adult flies were collected during the five stages of decomposition (fresh, bloated, decay, advanced decay and dry). It allowed quantitative and qualitative comparisons to be made in three habitats (desert, urban and agriculture). There was a significant diversity in fly species between the different habitats. Ten families of necrophagous flies were collected in the three habitats; the most important families to breed on carrions were Calliphoridae such as *Chrysomya albiceps, Chrysomya putoria* and *Lucilia sericata*, Muscidae such as *Atherigona orientalis, Musca domestica, Hydrotaea capensis* and Sarcophagidae such as *Ravinia pernix, Sarcophaga babiyari, Sarcophaga hirtipes* which can used as forensic indicators to estimate the postmortem interval (PMI). The most important species in respect to abundance and frequency was *Chrysomya albiceps*.

#### MOLECULAR BIOLOGIST, PLEASE, DO NOT DESTROY THE EVIDENCE!

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In Forensic Entomology the estimation of the age of the insects is used for the estimation of the minimum post mortem interval (mPMI). Insect development rate is mainly temperature dependent and species specific, so correct species identification is fundamental for any other consideration.

The identification of the species can be performed by two different approaches: the classic morphological identification, and the more recent molecular approach, based on sequencing and comparison of specific mitochondrial and nuclear nucleotide regions.

Molecular identification is considered a destructive technique and, depending on the legal system, it requires the authorization of the authority in charge of the case, especially when only few larvae are available.

In this paper we demonstrate that a molecular identification can be performed several times on the same larva without affecting the anatomical characters used for morphological identification.

The suggested technique allow the preservation of the larval esoskeleton and of the not used tissue in the same vial under ethanol as preservative solution in order to be able to repeat both the molecular and morphological analyses and reducing the risk of loss of the evidence.

The technique has been tested using larvae of *Calliphora vomitoria* and *Lucilia sericata* (Diptera: Calliphoridae) and *Megaselia scalaris* (Diptera: Phoridae).

In the first two species, of large size, the minimum amount of DNA useful for identification was obtained from a fragment corresponding to 1/128 for *C. vomitoria* and 1/64 for *L. sericata* of the whole LIII larva. The minimum amount of *M. scalaris* larva, few millimetres in size, was 1/8.

This paper underlines the need of a competent specialist/entomologist when insects are collected from a crime scene in contrast to the deleterious idea that DNA is the solution to all the problems and that non specialists can identify, in all the cases, species through the molecular approach.

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#### EFFECT OF SALT WATER AND ELEVATION OF THE CORPSE ON THE RATE OF DECOMPOSITION AND SUBSEQUENT INSECT SUCCESSION.

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The post-mortem decomposition and corresponding insect succession is a continuous process. This commences from the moment of death until the final stage of decomposition. Many studies have been carried out looking at these processes, and what factors may have an effect upon them. The effect of salinity levels in water and elevation have on a corpse is very rarely touched upon at all. With the field of forensic entomology growing in popularity with law enforcement agencies all the time, there is a demand for more investigative studies to be undertaken. Essential data can be gathered from studies that aids in the successful conclusion of investigations of many types of crime. This study is designed to investigate the effects that elevation of the corpse and salinity level have on decomposition rates, whilst comparing insect biodiversity and population levels of insects on the individual corpses. To my knowledge, no research has been carried out in this particular area of the field in the UK. Three pig corpses were left to decompose in the open air and therefore exposed to the insect population. One corpse was elevated, using a wooden gallows and homemade port ledge, limiting its contact with the ground. A second corpse was submerged and soaked in a saltwater solution and left to dry (touch dry) for 6 hours before being placed on the ground; whilst a third corpse was used as a control. Insects were sampled using different techniques (netting, hand collection using tweezers, Tullgren funnel, pit fall traps) and pictures were taken, to monitor decomposition rate, for a 43-day period during the months of March and April, in Leicestershire, England.

The results indicate that insect succession and the rate of decomposition are both affected respectively, with regards to salinity and elevation level.

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#### SAPRINUS SEMISTRIATUS- A LAZY LITTLE FELLOW?

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The estimation of the minimum post-mortem interval using entomological evidence is a common practice. Four different ecological groups of insects can be classified on a corpse: necrophagous species that feed on the dead organic matter directly, predators and parasites of insects and other arthropods, omnivorous and adventive species. The occurrence, age, abundance and succession of certain insect species during the different stages of a decomposing body can be of forensic importance. In a field experiment near Neuchâtel, Switzerland we have studied the presence of Coleoptera on decomposing pig cadavers (Sus scrofa) over time. Five pig cadavers were placed directly on the ground and five pig cadavers were hung one metre above ground. Two pitfall traps were placed next to the cadavers: one ventrally and one dorsally. The beetles were collected from the pitfall traps on a daily basis for 26 days after the cadavers were placed and less frequently until day 32.The decomposition stages of each cadaver were monitored and recorded at the same time. Most Coleoptera families showed a higher abundance on the cadavers lying directly on the ground than on the hanging cadavers. The family Histeridae belonged to the three most abundant families and among the Histeridae adult Saprinus semistriatus was the most frequent and abundant species. Histeridae are typical carrion and dung predators and feed mainly on Diptera larvae. In this study we focused on the following questions: Does the abundance of Saprinus semistriatus differ between hanging and ground pigs? Can we relate the presence of Saprinus semistriatus to a certain decomposition stage? Our results show that Saprinus semistriatus is found in a greater abundance on the ground pig cadavers in comparison to the hanging pig cadavers with significant differences between the two treatments. The majority of individuals appeared in the active decay stage with only a few present in the bloated stage. The abundances of Saprinus semistriatus in the ground pig treatments differed significantly between the bloated and the active decay stage and between the active and the advanced decay stage. This was not the case in the hanging pig treatment. We suggest that Saprinus *semistriatus* can be used to mark the onset of the active decay stage preferably when cadavers and their associated abundance of Diptera larvae are easily accessible as is the case in cadavers decomposing on the ground.

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## BURIED REMAINS IN A MEDITERRANEAN ENVIRONMENT: A MULTIDISCIPLINARY APPROACH FOR A 3 YEARS RESEARCH

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Burying and burning are the two most largely methods used in Southern Italy by organize crime in order to conceal/destroy the bodies. The most typical reports of buried remains in this region arise from confessions which are inaccurate. In such conditions, the search carried out by conventional methods is extremely difficult, time-consuming and rarely successful. In these cases, the use of ground penetrating radar GPR, now widely used, is extremely beneficial. In order to investigate the effect of time on body localization and recovery a project has been started in June 2011 an finished in July 2014. Data from this three years research carried out in a hilling area in Calabria region, where pigs were buried at 5 different deepness (0.9, 1.4, 1.7, 2 and 3.2 m) are reported.

GPR analysis was performed with a IDS-RIS 2K georadar. Recovery of the remains was performed with the help of an earth-mover and soil was sieved (2cm - 300  $\mu$ m) in order to collect insect remains associated with decomposition. GPR surveys were performed at time 0, after 1 year and at the end of the experiment (after 3 years) in order to assess variations in the radar signal levels. A final excavation and recovery of the carrions was performed in order to define: 1) the decomposition levels (skeletonization, presence of tissues, saponification) and 2) the carrion colonization by saprophagous insects (presence/absence; species). GPR data indicate that signals of abnormalities under the soil indicating, in our case the presence of the carrions, were present in all the collection times. The majority of the pigs were found in a complete skeletonized state, except pigs at 2 and 3.2 m where abundant adipocere was present. In these pigs internal organs were still visible and the stomach content analyzable. Fly puparia of a single species (*Ophyra capensis*, Diptera, Muscidae) were found abundant among the bones and the soil covering the pig at 0.9 m, whereas in the pig at 1.4 m a few puparia of Heleomyizidae were collected and only one puparium of the same family was collected among the bones of the pig at 1.7 m.

In conclusion our experiments underlying that in a Mediterranean environment deepness plays a fundamental role both in body decomposition and in insect colonization It is worth mentioning that GPR was able to clearly identify underground variation index of the presence of , in our case, a carcass although in skeleton state.

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#### MOLECULAR AGE ESTIMATION OF CALLIPHORA VICINA PUPAE (DIPTERA: CALLIPHORIDAE) – IDENTIFICATION OF GENETIC MARKERS USING MASSIVE ANALYSIS OF CDNA ENDS (MACE)

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Determining the age of juvenile blow flies providing evidence for the minimum post mortem interval is one of the key tasks of forensic entomology. While the age determination of blow fly larvae is well established using morphological parameters, current studies focus on developing methods to determine the age of blow fly pupae. The pupal stage, which lasts about half the total juvenile development time, still features scope for new applications in forensic entomology and casework. Among the eligible methods gene expression analyzes are predominant. Gene expression is a biological process in which the information of a gene is used to synthesize a functional gene product. Depending on the need of a certain gene product, the expression of the corresponding gene can be turned on or off. This process affects and interacts with the development of organisms. Due to this context, it might be feasible to use the correlation between gene expression and development for age predictions. It has been demonstrated in several studies that the variance in expression of several genes is often too high to assign a certain expression level to a distinct developmental stage or age. To overcome this problem we identified new transcripts which show a sharp age dependent expression course during immature development by analyzing NGS generated transcriptome data (Zajac et al., 2015). We used Massive Analysis of cDNA Ends (MACE) for high resolution gene expression analysis of 15 different development stages of Calliphora vicina pupae reared at constant 17°C. In total, 53,539 distinct transcripts have been detected. By analyzing the MACE data we have filtered for transcripts showing a significant increase of gene expression at a certain development stage during metamorphosis.

We identified more than 100 putative pupal age related transcripts showing an unambiguous increase in gene expression for a particular pupal development stage. For each of the 15 analyzed development days genes of interest have been identified. In the current study, we designed gene expression assays for each day of pupal development. Each assay has been tested concerning its specificity and efficiency. For the development of an age determination kit of *C. vicina* pupae qPCR gene expression analyzes are being performed. First promising genetic markers could be identified for testing in qPCR.

Zajac, B.K., Amendt, J., Horres, R., Verhoff, M.A. and Zehner, R. (2015), "De novo transcriptome analysis and highly sensitive digital gene expression profiling of *Calliphora vicina* (Diptera: Calliphoridae) pupae using MACE (Massive Analysis of cDNA Ends)". *Forensic Science International. Genetics* 15: 137–146.

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## EFFECT OF FUR ON THE MICROBIAL AND ENTOMOLOGICAL COMMUNITIES ON RABBIT CARCASSES

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Different factors, both intrinsic and extrinsic, have been reported affecting the decomposition of a carrion/body. These factors mainly interact with the speeds of the biological and chemical reaction happening after the death. The biological reactions are mainly due to the activity of microorganism and insects. Pigs (*Sus scrofa domesticus*) have been used as a model for human studies and the results obtained have been applied to other mammals without considering the effect that fur can have on the decomposition process and on the insect or microbial colonization. In order to investigate this point, rabbits (*Oryctolagus cuniculus*) with fur and without were used in two sets of experiments in Huddersfield in 2014 (summer) and in 2015 (spring).

Entomological data for the first experiment revealed the presence of Diptera Calliphoridae (*Lucilia sericata*, *Calliphora vicina*, *Protophormia terranovae*), Muscidae (*Ophyra* sp); Sphaeroceridae (*Coproica* spp.), and Piophilidae (gen. sp.), Hymenoptera Pteromalidae (*Nasonia vitripennis*) and Coleoptera Cleridae (*Necrobia rufipes*). Differences in colonization time were observed only in spring: animals without fur were colonized two days before animals with fur. No significant differences were observed in summer experiment.

The microbial community was investigated using BIOLOG EcoPlate<sup>TM</sup> and by pyrosequencing (data under analysis). The functional diversity of the bacterial community on all carcasses showed a big variability dependent on the stages of decomposition and the sampling region (skin, mouth, soil-carrion interface). The content of water seems to play the most important role in the bacterial community growth, whereas the presence or absence of fur does not seem to affect the functional diversity. At the beginning of the sampling the bacterial community is very high in the mouth area, whereas the community in the interface soil-carrion is negligible. This community increased its diversity during the decomposition process through to the end of the experiment (4 months). The community on the exposed skin is a function of the drying process with a belt shape: limited diversity at the beginning and at the end of the decomposition process and a maximum during the active decomposition. Fur seems to partially affect the bacterial community only on the exposed surface. These observations will be confirmed and better explained when the pyro-sequencing data is available.

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#### FORENSIC ENTOMOLOGY IN CENTRAL AND NORTHERN ITALY, A GENERAL OVERVIEW

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It seems to be a habit of forensic entomologists to complain about the lack of data and the scarcity and poverty of information about the distribution, phenology, biology and ecology of forensically important species. In several papers, we repeat that "more data at regional scale are necessary", "further investigations in the phenology and distribution of the collected species are necessary in order to …". This lack of data could be due not only to a "real lack of cases" but also the lack of synthesis of the cases that are already collected.

In order to provide useful information on cases in which insects were collected or used for forensics in Northern Italy, we presented a first "state of the art" paper in 2010. Now, a few years later, we present an update on this topic with additional cases.

The data summarized here, concerns more than 260 cases that occurred in the period of 1998-2014 in Central (10%) and Northern Italy (90%), and more particularly in the regions of Marche, Abruzzo, Tuscany, Lombardy, Piedmont, Valle d'Aosta, Emilia Romagna and Veneto. The majority of the dead subjects were male (65.1%), aged from 22 to 89, Their bodies were found indoors (63.9%), and the main cause of death being cardiac arrest. The majority of the bodies were in an active/advanced decay when found.

The dead found indoors were young people often involved in drug-abuse. Moreover several bodies were found along river or canal shores, representing a key component of the North Italian landscape.Flies belonging to Calliphoridae, Sarcophagidae, Muscidae and Fanniidae were the most abundant taxa collected, whereas Coleoptera were available only in a few cases, mainly when an entomologist was involved in the entomological evidence sampling. Larvae were present in more than 80% of the cases, whereas eggs and pupae/puparia in about 20% of cases.Nine species of Calliphoridae (*Phormia regina, Protophormia terranovae, Lucilia sericata, L. caesar, L. illustris, Chrysomya albiceps, Calliphora vomitoria, C. vicina, C. loewi*) were collected. The specimens of Muscidae collected belonged to species of the genus/genera *Ophira/Hydrotaea* (*O./H. dentipes, O./H. capensis*) *Musca* and *Muscina* and the Fanniidae to the genus *Fannia (Fannia scalaris, Fannia cannicularis*, etc). The Flesh flies were identified only at the genus level (*Sarcophaga*).

Beetles (Dermestidae, Cleridae, Tenebrionidae, Nitidulidae, Silphidae, Histeridae, Staphylinidae) were present mainly in the samples carried out by an entomologist and they result underestimated.

This second summary is important not only as "state of the art" but also because it may help to orientate the further investigations on carrion-breeding insects in a useful way for the real cases in which forensic entomology will be used to define the time since death and to obtain other important information (e.g.: body transfer, drugs, etc).

12<sup>th</sup> Meeting of the European Association for Forensic Entomology

#### BODY COLONIZATION AND DEGRADATION IN A FRESHWATER SYSTEM, THE ROLE OF THE CRAYFISH *PROCAMBARUS CLARKII* (GIRARD, 1852) L. Manelli<sup>1,2</sup>, S.Vanin<sup>1,3</sup>, L. Maistrello<sup>1,2</sup>

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Little information is available on the decomposition of bodies in freshwater systems, yet the study of decay in this type of environment is important for the estimation of the minimum Post Mortem Interval (mPMI), the Post Mortem Submersion Interval (PMSI) and the manner of death. In a previous experiment, the importance of *Procambarus clarkii* (Girard, 1852) in the consumption of dead bodies in freshwater ponds in Northern Italy was assessed. This crayfish, native of Southern USA, recently spread in Europe after its introduction for aquaculture. The present study aimed at characterizing the role of P.clarkii in the decomposition of carcasses in freshwater systems by studying the population dynamics in relation to the decomposition stage, the period of the year and the position of the body in the column of water, and the wounds caused by the crayfish feeding activity. The study was conducted in Nonantola (MO), Northern Italy, and it consisted of four experiments, the first in July, the second in August-September, the third from November to June, the fourth in June-July. Five pig carcasses (Sus scrofa, L) enclosed in lobster pots were placed in different positions inside an artificial 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 feeding activity on the carcasses, the water level and meteorological data. Results show that in the non-winter experiments, the crustaceans attacked the carcasses a few hours after their positioning, starting to damage the external epidermal layer with lacerations up to 20-30mm, generally ascribable to round shape wounds. The day of deposition of the body inside the pond corresponded with the first peak in *P.clarkii* population with up to 100 specimens detected in each lobster pot. In the following days, the crayfish continued their activity, increasing the percentage of damaged skin until the full laceration of the dermal layer. The second peak in crayfish population occurred after the end of the floating decay stage, with the carcass lying again on the bottom of the pond; this peak could be explained with the low swimming ability of the crayfish. From that moment, the feeding activity continued mostly in the internal part of the carcass, with the attack to the internal organs, until the flesh was completely consumed by the crustaceans and only bones remained. The situation was different in the winter experiment: crayfish colonised the carcasses from the first day after pigs positioning in water, but the decomposition carried on slowly. The crayfish feeding activity was reduced because of the low water temperatures, and the skeletisation occurred only after seven months. At the end of all the experiments, saponified tissues remained together with the bones, and the crayfish did not show any interest in these body parts. 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. On one side, by detecting differences in the colonization of the body in relation to the season and the position in the water column, these results are useful for the estimation of the mPMI interval of bodies found in freshwater systems. On the other side, 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.

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#### AUTOMATED IDENTIFICATION OF ADULT BLOWFLIES FROM WING IMAGES

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The Old World screwworm fly (OWSF), Chrysomya bezziana (Diptera: Calliphoridae), is an important agent of traumatic myiasis and, as such, a major health and welfare problem for humans and animals. It can also have forensic value as an indicator of neglect. Morphological and molecular studies have demonstrated the existence of two distinct lineages of the species, one African the other Asian. Within these lineages there is considerable mitochondrial DNA diversity linked to the area of geographical origin. We have explored the potential for a morphometric identification of these OWSF lineages by a wing landmark analysis. Using a bootstrapped log likelihood ratio test of the output of a Procrustes PCA of wing landmark data, a highly significant difference was shown in wing morphometry between African and Asian OWSF forms, supporting previous molecular and morphological studies (Hall et al., 2014). Wing morphometry is, therefore, known to be capable of providing a reliable index of intraspecific population identification. However, wing landmark analysis can be time consuming and is subject to errors caused by operator subjectivity. In order to further develop this research track we have compared and contrasted results obtained from the landmarkbased study with those from a novel, direct morphometric analysis of digital images of wing morphology. In the latter analysis, standard digital images of wings were transformed into an 8-bit monochrome format, subjected to automatic brightness level adjustment and downsampled to a 20 x 40 pixel matrix, output as a column vector of ASCII pixel brightness values. The data matrix of values was subjected to principal components analysis (PCA) and canonical variate analysis (CVA), as were the landmark data. Geographic lineage and genderbased comparison analysis by morphometric landmarks and processed images returned results that were fully comparable to one another. The fact that no attempt was made to improve the quality of the processed images makes these results remarkable and encouraging because, even under extremely low-resolution conditions, sufficient information was retained by the processed images to return results as useful and statistically significant as the analysis of laboriously placed morphometric landmarks. Our results suggest that a fully-automated, quick and easy-to-use identification system for OWSF is a realistic possibility as is the potential expansion for identification of other Chrysomya species and other blowfly genera of forensic value.

Hall, M.J.R., MacLeod, N. and Wardhana, A.H. (2014). Use of wing morphometrics to identify populations of the Old World screwworm fly, Chrysomya bezziana (Diptera: Calliphoridae): a preliminary study of the utility of museum specimens. Acta Tropica, 138S, S49-S55.

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