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Universidad del País Vasco

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ENTOMOFAUNA ASSOCIATED WITH DOMESTIC PIG (*SUS SCROFA*) DECOMPOSITION IN AN ATLANTIC ENVIRONMENT (AIAKO HARRIA, BASQUE COUNTRY, SPAIN)

TESIS DOCTORAL

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In the end we will conserve only what we love,
we will love only what we understand,
we will understand only what we are taught.

Baba Dioum

Llevo horas delante de un papel en blanco, sin saber por donde empezar, y eso que ya estamos en el final. Llega más tarde de lo que jamás hubiera imaginado, y como todo lo viejo, hay que terminarlo para dejar sitio a lo nuevo.

Esta tesis siempre ha sido mi responsabilidad, una carga que ha sido tan liviana como pesada. Desde las largas caminatas por Aiako Harria esquivando vacas con cara de mala leche, hasta las interminables horas de números y ordenadores. Sin embargo, en todo este camino ha habido personas que también han sido partícipes del proceso, ya sea como fuertes postes a los que amarrarse o barcos que van y vienen.

En una dedicatoria los padres y hermanos no pueden faltar, y en esta con más motivo. Siempre han estado ahí, animándome a conseguir mis metas sin perder por ello mis valores en el camino. Acompañandome en mis caminatas, haciendo fotos, aguntando mis rabietas y llevándome al cine o de compras compulsivas… Y eso sí, llenando siempre el tupper, que el estrés da mucha hambre. Si este trabajo ve un final, desde luego es gracias a ellos.

Algo más intermitente por su viaje a America Latina, pero siempre prestando una mano amiga (incluso como psicólogo improvisado), ese es Alberto Castro. Siempre me acordaré de la primera vez que lo vi allá por tiempos de Cidemco estaba aplaudiendo a los mosquitos (literalmente, aplaudía para cazar algún aventurero que conseguía salir de su jaula). Con él llegué a ver la luz al final del túnel de la Bioestadística Aplicada, aunque sigue siendo muy pequeñita, que le vamos a hacer.

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Una mención especial es para Aranzadi y toda su gente. Desde el primer día me acogieron y me dejaron usar el Departamento de Entomología como cuartel general. Es genial contar con todos ellos, tanto para conseguir bibliografía como té de importación de Sri Lanka, o ya sea solo para

divagar en las comidas intentando arreglar este maltrecho mundo que tenemos.

Gracias también a mi gurú del buen karma, Jaime, que como siempre dice, lo hemos hecho lo mejor que hemos sabido hacer. Y también quiero darle las gracias a Esther, que aunque la conozco desde hace poquito, ha sido un fuerte apoyo en la etapa final.

Y volviendo a los inicios de ésta mi andadura por lo forense, quiero recordar a todas esas personas que van pasando por la vida dejando su huella, aquellas a las que siempre les viene bien un café a cualquier hora o una tarde de tomar el sol en un lugar secreto para desconectar. Javi, Dani, Nerea, Montse, Maite…

A todos, gracias.

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That which is cut in pieces,
or engraved/segmented.

Entomos, from the Greek

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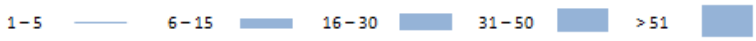
overlap between them is due to their asynchrony in carcasses. Abundance of each taxon follows this key: 

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Abstract

To a good approximation,
all species are insects.

Robert May, quoted in Richard Dawkins, The Selfish Gene

Resumen

El Análisis Forense es un mundo multidisciplinar que engloba un gran número de especialidades tan diversas como lo puede ser la naturaleza de las pruebas que han de estudiarse para resolver un caso. Entre ellas, podemos encontrar especialidades tales como Antropología, Medicina, Toxicología o incluso el Arte. También se incluye en este ámbito la Entomología Forense, una rama del Análisis Forense que aplica el conocimiento de los insectos (y otros artrópodos en general) para asistir en la resolución de determinados casos legales (contaminación de alimentos, tráfico de especies, muerte de personas, etc.). A día de hoy, se desconoce si existe un método que permita determinar con precisión el momento exacto de una muerte. En el caso de la Entomología Forense, se hace una estimación del periodo de actividad de los artrópodos (PAI) en el cuerpo (GOFF, 2010). Esta medida se aproxima al intervalo post-mortem (IPM) dado que, en condiciones, óptimas, la llegada de los colonizadores sucede minutos después del fallecimiento. El cálculo puede basarse bien en la tasa de desarrollo de las especies involucradas o en la sucesión de estas sobre el cuerpo (GENNARD, 2012). Para la correcta aplicación de los métodos de estimación del IPM basados en la fauna cadavérica, es fundamental identificar correctamente las especies asociadas a los procesos cadavéricos, cuyos patrones de sucesión resultan de gran importancia a la hora de responder preguntas tales como *"¿Cuándo ocurrió la muerte?"* (SCHOENLY *et al.*, 1992). Teniendo en cuenta que la comunidad de artrópodos asociada a los procesos de reducción cadavérica varía en gran medida según el área biogeográfica, la estación del año o el tipo de hábitat entre otros (MATUSZEWSKI *et al.*, 2008), dichos patrones de sucesión son específicos para cada localidad, con el problema que esto supone para regiones que carecen de este tipo de conocimiento entomológico. En el caso de la Península Ibérica, se han realizado numerosos estudios en torno a la Entomología Forense. Sin embargo, la situación geográfica y variada orografía de la Península hacen que ésta presente una notable diversidad climática que, a su vez, dificulta la extrapolación de resultados de un área a otra. Esto conlleva la necesidad de analizar cada zona climática de forma individual, con el reto que supone iniciar

el estudio en un área poco estudiada y con un clima tan particular como lo es la Cornisa Cantábrica (CHAZARRA *et al.*, 2011).

El asesoramiento técnico para esclarecer el momento en que se da una muerte debería incluir, por tanto, datos sobre las condiciones ambientales de una determinada zona y las especies de insectos existentes en la misma (CAMPOBASSO *et al.*, 2001). De esta forma, se pretende que el presente trabajo sirva como referencia para futuros estudios y casos forenses en la Comunidad Autónoma del País Vasco (C.A.P.V.) y otras regiones biogeoclimáticas similares.

El presente estudio se centra principalmente en la aplicación de la Entomología como herramienta para datar la muerte de un individuo, para lo cual se busca una correcta identificación de la fauna de interés forense en un área de clima Atlántico, prestando especial atención a los procesos de sucesión y desarrollo de dicha fauna y los factores que pueden influir sobre ellos, tales como la insolación, temperatura o la lluvia. Del mismo modo, se busca identificar posibles especies bioindicadoras de las fases del proceso de reducción cadavérica que ayuden futuras aplicaciones del conocimiento que de aquí se derive. Para ello, se han depositado 10 cuerpos de cerdo doméstico (5 durante el verano de 2009 y 5 durante el verano de 2010) en un entorno reforestado, sobre los que se han estudiado los procesos de reducción cadavérica y la entomofauna asociada a ellos mediante capturas manuales (con manga entomológica, pinzas, pincel u otros medios). Las capturas se realizaban de forma diaria durante las primeras semanas, y en días alternos a medida que disminuía la actividad de los artrópodos. Se tomaba nota del aspecto físico de los cuerpos, las condiciones climatológicas de la zona, y cualquier otro dato considerado relevante. A fin de ordenar el proceso de reducción cadavérica, y también para facilitar al lector la comparación de este trabajo con otros previos, este se presenta aquí dividido en las 5 etapas bien delimitadas propuestas por (PAYNE, 1965).

En el presente estudio se ha recolectado una fauna asociada muy diversa y variable. Hay 16 órdenes representados en 7837 individuos recolectados, siendo los más abundantes los insectos (suponen más del 97% del total) y muy

especialmente los dípteros y coleópteros (los dos juntos acumulan el 94.28% del total). También se aportan numerosas nuevas citas de estos dos órdenes: 50 especies, incluyendo incluso dos nuevas especies para la Ciencia: *Crossopalpus* sp. n. (nr. *nigritellus* and *aeneus*) y *Drapetis* sp. n. (group *exilis*) (Diptera: Hybotidae). Esto denota el poco conocimiento que se tiene de la fauna cadavérica de nuestra región, de la que aún quedan numerosas zonas por explorar.

La descomposición de un cuerpo inicia un proceso dinámico de colonización, desarrollo y sucesión, en el que los artrópodos compiten entre sí y con otros organismos por los recursos que el cadáver ofrece (CARTER *et al.*, 2007). El periodo fresco es el momento de mayor dominancia y menor diversidad, debido principalmente al bajo número de especies que actúan como colonizadoras del cadáver (principalmente moscardas). Por el contrario, el estado seco ha sido el más diverso. Para estos cálculos de diversidad es habitual utilizar el índice de Margalef. Sin embargo, este está fuertemente influenciado por el esfuerzo de muestreo, por lo que se recomienda contrastar con el cálculo del índice de Fisher, menos influenciado, para obtener un cálculo de diversidad más objetivo en este tipo de experimentos.

De forma adicional, se ha realizado un análisis de la diversidad β, en el cual se ha encontrado que el cambio de periodo fresco a hinchado ocurre con mínimas variaciones en la identidad de las familias capturadas, si bien se dan mayores cambios en la abundancia de cada una de ellas (las especies dominantes en el estado fresco se vuelven menos importantes en número con la llegada de nuevas especies al inicio de la fase hinchada). Este reemplazo de familias es mayor en las últimas etapas del proceso, a pesar de que en este punto no son importantes los cambios de abundancia de cada una.

Todo este proceso de colonización, desarrollo y recambio de especies está fuertemente influenciado por las condiciones climáticas, siendo de especial importancia la temperatura. Es de sobra conocido que cuanto más favorable sea el clima, mayor número de insectos estarán presentes en el proceso, teniendo a su vez una consecuencia directa en la duración de dicho proceso y de cada una de sus fases. Incluso en algunos casos, parte de esas fases

pueden desaparecer o no llegar a ser distinguidas por el investigador, tal como sucedió durante el presente estudio en dos de los cerdos, al no llegar a apreciarse un aspecto hinchado en dichos cadáveres. Este hecho ha sido previamente explicado por otros autores como consecuencia de la alta actividad de los insectos. Sin embargo, este no es el caso dado que no se encontró una población significativamente alta en dichos cerdos en ese momento. Si además tenemos en cuenta el hecho de que la temperatura ambiente en los puntos donde se encontraban dichos cadáveres era ligeramente superior al resto, la fase hinchada sería más notoria pero de menor duración, de modo que es probable que la frecuencia de muestreo (diaria) haya dificultado la observación de esta fase.

El clima templado característico del área de estudio favoreció la rápida colonización de los cuerpos de los cerdos, que empezó segundos después de depositar los cadáveres. Sin embargo, en 2009 las abundantes lluvias que se dieron en el area de estudio durante los primeros días del experimento dificultaron la llegada de los insectos necrófagos y, por tanto, el inicio de la sucesión del resto de artrópodos asociados. Este hecho pone de manifiesto la importancia de recabar información no solo de la temperatura, sino también de otros factores climatológicos que puedan afectar (insolación, lluvia, viento fuerte…). Por norma general, esta información se obtiene de la Estación Meteorológica más cercana. Pero incluso en aquellos casos en los que hubiera una muy cerca del lugar a estudiar/investigar, las condiciones de los distintos microhábitats pueden diferir considerablemente debido a diferencias en la cobertura vegetal, insolación, drenaje, pendiente u otros factores no biológicos ajenos a la labor de investigación, tal como mal funcionamiento o rotura de la estación, haciendo que ambos, estación y lugar, sean incomparables. De hecho, se han encontrado notorias diferencias incluso entre los distintos cadáveres de cerdo utilizados, los cuales se separaban entre sí a una distancia aproximada de 10 metros.

Para resolver este problema de incertidumbre, se ha realizado un análisis sobre cuán apropiadas resultan las tres Estaciones Meteorológicas más cercanas a la zona de muestreo. Los resultados apoyan la práctica habitual de escoger la más cercana, si bien cabe decir que se encontraron diferencias

estadísticamente significativas en todos los casos. Pero, ¿qué pasaría en el caso de tener dos estaciones a idéntica distancia pero en ambientes diferentes? ¿O en caso de mal funcionamiento temporal? El cálculo del *tamaño del efecto* (COHEN, 1988) de las diferencias entre los registros de temperatura de la estación y el lugar de muestreo (o de descubrimiento de un cadáver en un caso real) ha resultado ser una buena técnica para evaluar el significado e importancia biológica de dichas diferencias, así como el error que suponen en la estimación del IPM, aportando una herramienta de análisis al técnico forense que le permita escoger la Estación con mayor objetividad. En el presente estudio, la estación más cercana al lugar de estudio no tuvo registros durante varias semanas, con lo que se analizaron la segunda y tercera estación en distancia, y se obtuvieron resultados contradictorios según se utilizaran registros horarios o temperaturas medias diarias. La aplicación del tamaño del efecto puso de manifiesto que no es necesariamente la más próxima la mejor, sino aquella con un ambiente similar. Así, la segunda en distancia se encuentra en un valle, mientras que la tercera está situada en un ambiente de montaña; por tanto, el tamaño del efecto era menor en esta última en comparación con la segunda.

Volviendo a la sucesión de artrópodos y centrándonos en los adultos, se ha registrado y analizado el periodo en que cada taxón se encontró presente durante el proceso de descomposición. Estos siguen una secuencia predecible, si bien la comunidad de artrópodos no forma grupos específicos y cerrados según la fase de descomposición en que se encuentra un cuerpo. Lo que se deduce de los datos aquí obtenidos están en línea con el pensamiento *gleasoniano* del proceso como un continuo (ver MOURA *et al.*, 2007). Es decir, el inicio de cada fase no depende solo de los factores ambientales y de la entomofauna, ya que hay muchos factores que afectan al proceso, aunque no haya habido medios para poder evaluarlos (temperatura de los tejidos, humedad, propiedades del suelo, etc.).

Sin embargo, el hecho de que no hay una clara separación entre fases de descomposición no entra en conflicto con la idea de que ciertas especies aparecen asociadas a un determinado periodo de tiempo dentro del proceso (MOURA *et al.*, 2005). Nuestros resultados indican que en dicho proceso pueden

observarse diferentes etapas atendiendo a la comunidad de artrópodos presente: una etapa inicial de colonización, con la llegada de especies como *Lucilia caesar* y *Musca autumnalis*; una etapa intermedia de recambio continuo de especies; y una etapa final caracterizada por especies de aparición tardía, tales como *Nemopoda nitidula*, *Parapiophila vulgaris*, *Spelobia luteilabris* y *Spelobia clunipes*.

No obstante, la sucesión de artrópodos es, en términos generales, similar a estudios previos, aunque se han encontrado ciertas diferencias en cuanto al papel que tienen ciertos taxones de gran interés forense. La más notable es la escasa presencia de la familia Sarcophagidae, o el carácter depredador observado en algunos geotrúpidos. También se han dado diferencias a nivel de especie. A modo de ejemplo, *Lucilia caesar* ha sido la especie más abundante en la colonización inicial de los cuerpos de los cerdos, tal como ha sido recogido en estudios previos realizados en verano en otros países centroeuropeos; Polonia (MATUSZEWSKI *et al.*, 2008), Alemania (ANTON *et al.*, 2011; REIBE & MADEA, 2010) o Portugal (PRADO E CASTRO *et al.*, 2012), aunque este último en primavera. En cambio, *Lucilia sericata* fue la especie más abundante en estudios previos realizados al sur de la Cornisa Cantábrica, tanto en la zona continental (CASTILLO MIRALBÉS, 2002; GARCÍA-ROJO, 2004) como en la costa mediterránea (MARTÍNEZ – SÁNCHEZ *et al.*, 2000a; VANIN *et al.*, 2008). Los datos que aquí se aportan confirman las diferencias entre las diferentes regiones biogeográficas de la Peninsula Ibérica, siendo la C.A.P.V. más similar a otros estudios llevados a cabo en países centroeuropeos que tienen a su vez unas condiciones climatológicas parecidas durante el verano y una vegetación similar (ANTON *et al.*, 2011; GRASSBERGER & REITER, 2004; MATUSZEWSKI *et al.*, 2008, 2010b).

Este tipo de experimentos generan grandes inventarios de especies (CASTILLO MIRALBÉS, 2002; MATUSZEWSKI *et al.*, 2010c). Por tanto, se hace necesaria la correcta identificación de especies características o bioindicadoras, a fin de facilitar tanto la investigación científica como el trabajo de técnicos forenses. En este sentido, el análisis de los patrones de permanencia de las especies propuesto por MATUSZEWSKI *et al.* (2010b) permite la selección de taxones de mayor utilidad desde el punto de vista forense para una determinada región, a

pesar de que tiene cierta limitación cuando el número de réplicas es reducido. Con este análisis, se seleccionaron 17 taxones que debieran conformar el modelo de sucesión de referencia para futuros estudios en la zona norte peninsular, siendo *Hydrotea similis, Coproica hirticula, Anoplotrupes stercorosus* y *Necrodes littoralis* las especies de mayor interés.

Los datos de desarrollo de los estadios larvarios de las especies colonizadoras son de gran utilidad para la estimación del IPM, sobre todo cuando se complementan con patrones de sucesión de adultos (TABOR, 2009). De hecho, en los primeros momentos tras la muerte de un individuo, el desarrollo larvario de los dípteros necrófagos colonizadores es una de las principales fuentes de información (AMENDT *et al.*, 2007). Los datos que aquí se presentan son un breve resumen del desarrollo de las especies más abundantes halladas en relación a los cadáveres. En la mayoría de los casos, puede observarse un solapamiento de dos o más generaciones de larvas, lo que puede conllevar a no recoger los ejemplares de mayor tamaño que darían un IPM infraestimado. Esto recuerda la importancia que tiene el seguir un buen procedimiento de recogida de muestras antes, durante y después del levantamiento de un cadáver (BYRD *et al.*, 2010).

MATUSZEWSKI *et al.* (2010b) destacan la utilidad de las fases larvarias dada su estrecha asociación con el cadáver, permitiendo demostrar casos en los que se haya movido un cuerpo, por ejemplo. Por ello, se ha realizado una descripción de la sucesión de los estadios larvarios de las especies que visitan un cuerpo en descomposición. Se ha confirmado que estos siguen un patrón muy similar al de los adultos, siendo los califóridos los primeros en desarrollarse formando grandes masas larvarias que afectaron en gran medida a la temperatura ambiente (se detectó una diferencia máxima de 25.5ºC). Cuando las larvas de Calliphoridae y Sarcophagidae migran fuera de los restos en descomposición para pupar en el suelo circundante, adquieren protagonismo las larvas de las familias Muscidae y Fanniidae. En la etapa final, las familias más abundantes han sido Piophilidae y Sepsidae.

Por otra parte, no se ha medido objetivamente el efecto que tiene la práctica habitual de recoger en los muestreos (ya sea en campo o en laboratorio) los

ejemplares de mayor tamaño. El efecto del investigador sobre este tipo de estudios ha sido analizado con respecto a los adultos (JONG & HOBACK, 2006) y la temperatura y pérdida de peso del cadáver (JONG *et al.*, 2011). Por su parte, WELLS *et al.* (2015) analizaron en el laboratorio el efecto de coger las larvas de mayor tamaño o el conjunto total de larvas de mayor edad en modelos de desarrollo. Ante esto, será conveniente realizar nuevos estudios de características similares con la población larvaria.

A modo de conclusión, destacar que nos encontramos ante los primeros resultados de fauna cadavérica realizados en un extremo de la cornisa cantábrica y que se necesita continuar con la labor investigadora en diferentes áreas de nuestra geografía, estaciones del año o hábitats, a fin de poder tener una sólida base de información que pueda ser aplicada en futuros estudios forenses. Este es un punto crucial, sobre todo teniendo en cuenta las grandes diferencias encontradas en las distintas regiones de la Península Ibérica (ARNALDOS *et al.*, 2004; CASTILLO MIRALBÉS, 2002; MARTÍN-VEGA, 2011; PRADO E CASTRO *et al.*, 2012), y las similitudes con otras regiones centroeuropeas (KOČÁREK, 2003; MATUSZEWSKI *et al.*, 2008). Por último, en esta tesis se aplican una serie de herramientas matemáticas de escaso uso en la práctica forense habitual (por ejemplo, estimar el tamaño del efecto de Cohen (COHEN, 1988) o los patrones de permanencia de especies de Matuszewski *et al.* (2010b) que serían de gran utilidad para los técnicos forenses y que ayudarían también a mejorar el proceso de estimación del IPM. Por ello se recomienda su inclusión en los protocolos de actuación de Entomología Forense.

Palabras Clave: Entomología Forense, País Vasco, sucesión, artrópodos.

Abstract

Forensic Analysis is a multidisciplinary field that includes a huge number of specialties as diverse as the nature of the evidence that must be studied in legal cases. Among forensic practitioners, scientists from Anthropology, Medicine, Toxicology or even professionals of Arts can contribute to its development. It also includes Forensic Entomology, which applies information derived from the presence of arthropods to assist in the resolution of many types of cases (food contamination, wildlife traffic, death bodies, etc.). This work mainly focuses on the application of Entomology as a tool for dating the moment of death of an individual. With this aim, decomposition studies were conducted in Aiako Harria Natural Park (Errenteria, Gipuzkoa), during the summer months of 2009 and 2010, and using as non-human model piglet (*Sus scrofa*) carcasses of 8-12 kilograms.

A checklist of 18 orders and 255 species is included, with 21 taxa/families already pendant of a more detailed revision. The vast majority of specimens identified (94%) correspond to flies (Diptera) and beetles (Coleoptera), orders in which a huge amount of new records has been found (50 species, including 2 new species for Science (*Crossopalpus* sp. n. (nr. *nigritellus* and *aeneus*) and *Drapetis* sp. n. (group *exilis*) (Diptera: Hybotidae)). Their presence follows a predictable sequence, being fresh stage the moment when a higher degree of dominance and less diversity were found, and the dry stage the most diverse one. That expected sequence presents important differences when comparing with previous studies from the Iberian Peninsula, both at species level and attending to the role of some forensically important taxa. By contrast, results obtained in this research are found to be quite similar to those performed in central Europe regions with a similar climate.

From the large inventory of species found, a residency pattern analysis was performed with adult presence/absence data. 17 taxa were found that should be included on a baseline succession model for the northern maritime facade of the Iberian Peninsula, being just 4 species ascribed as indicators for its usefulness in Forensic Entomology through the analysis of residency patterns:

Hydrotaea similis, Coproica hirticula, Anoplotrupes stercorosus and *Necrodes littoralis*.

Regarding weather data, an analysis was made about the degree of appropriateness of the nearest National Weather Stations in distance to the study area. Results supported that choosing the nearest one was the best option in this research, but recommended to include the calculation of Cohen' Effect Size to gain objectivity, as it allows easier inference of the significance of the differences between the Weather Station and the site of discovery of a corpse.

Another source of uncertainty in PMI calculation is the lack of development models for most of the species involved on carcass reduction, in special models performed outdoors, under a regime of fluctuating temperature. Data presented here will serve as a reference for future research on this regard, as briefly summarize the development of the most abundant species associated to pig carcasses and reared under field conditions. Additionally, larval succession pattern is also included, and follows the general trend observed for adult succession. Calliphoridae and Sarcophagidae species were the first developing on carcass tissue, followed by Muscidae and Fanniidae. The last stages of decomposition were dominated by Piophilidae and Sepsidae immature stages.

This work contributes to enhance the knowledge of the carrion fauna of the Basque Country. It also applies some analytical methods with scarce use in routinely forensic practice (i.e., Cohen's effect size or residency patterns), although they can aid the forensic technician to estimate the PMI on an easiest and objective way. However, the recommendation is to keep studying other regions, seasons, habitats, etc. in order to have an acceptable baseline of carcass decomposition and its related fauna.

Keywords: Forensic Entomology, Basque Country, succession, arthropods.

INTRODUCTION

The farther backward you can look,
The farther forward you will see.

Winston Churchill

You know my methods. Apply them […]. I never guess. It is a shocking habit – destructive to the logical faculty […]. I never make exceptions. An exception disproves the rule […]. Eliminate all other factors, and the one which remains must be the truth.

One should always look for a possible alternative, and provided against it. It is the first rule of criminal investigation.

Sherlock Holmes
(*The sing of the four* and *The adventure of Black Peter*,
by Sir Arthur Conan Doyle;
Image from the 22nd Insect Fear Film Festival of the University of Illinois, USA)

◈ The game is afoot, forenseic science in criminal investigations

Born in 1887 by the hand of Sir Arthur Conan Doyle, Sherlock Holmes became famous in the late nineteenth century for his logical reasoning, his skilful use of observation and his use of forensic science skills to solve difficult cases. But even being a fictional character, Mr Holmes has all the characteristic features that could be found in a real forensic investigator, as the character is inspired on the surgeon and forensic detective Joseph Bell.

For both, real or fictional, there is only one aim: corroborating that each evidence is what is supposed or alleged to be. To achieve this goal, the investigator will use Forensic Sciences, which involve the application of a broad spectrum of sciences to answer questions of interest to a legal system (civil actions or crimes).

The word forensic comes from the Latin *"forensis"*, which meaning "of or before the forum" is related to the way in which the ancient Roman civilization solved criminal cases: both the person accused of a crime and the person who accused had to present their point of view of the story before a group of public individuals in the forum, so as that individuals could determine the outcome of the case.

Nowadays, Forensic Science has a high number of branches or subdivisions that are as diverse as the nature of the evidence that must be studied in each case. So this multidisciplinary field involves scientists from Medicine or Anthropology, as well as from Toxicology, Crystallography or even Arts.

Forensic sciences includes also the study of insects which, due to its ability to occupy almost all conceivable habitats worldwide, often act as living witness to crime or to the cause of litigation (*cf.* BYRD & CASTNER, 2009a). In other words, forensic entomology is the branch of forensic science in which information about insects is used to draw conclusions when investigating legal cases relating to both humans and wildlife, although on occasion the term may be expanded to include other arthropods (GENNARD, 2012).

This broad definition includes entomologists who are able to testify about subjects pertaining to the proper use of chemicals or other pesticides, control of fly populations in animal waste, damage to structures due to insects, or food contamination. These cases are usually adjudicated in civil courts (urban and stored products entomology). A more specific concept of forensic entomology, which focuses this research, is the use of insects during a crime investigation or other legal matters where the forensic entomologist will culminate his or her investigation with a report or testimony in court (medicolegal entomology) (HALL, 1990; HASKELL, 2006).

◈ A BRIEF REVIEW OF FORENSIC ENTOMOLOGY' DEVELOPMENT

Forensic entomology is a branch developed due to the dedicated work of a relative low number of scientists as compared to other medical and paramedical fields. It has been developed as a continuum, and there have been very few, if any, radical discoveries (GUPTA & SETIA, 2004).

Figure 2: Necklace from Ancient Egypt (from http://egyptopia.com)

However, it is not a new discipline. Travelling back

in time we are reminded of the association between corpses and arthropods, traceable almost to the dawn of recorded history (GREENBERG & KUNICH, 2002). One of the first references of this association brings the reader to the 10-year siege of Troy, when Achilles worries over the dead body of his dear friend, Patroclus, "*that flies will settle upon the son of Menoetius and breed worms about his wounds, so that his body, now he is dead, will be disfigured and the flesh will rot*". His mother Thetis answers: "*I shall endeavour to drive from him the swarming and fierce things, those flies, which feed upon the bodies of men who have perished...*" (Homer, *The Illiad*, Book 19. Translated by Samuel Butler (http://classics.mit.edu/Homer/iliad.19.xix.html) (GREENBERG & KUNICH, 2002).

In our time familiarity may breed contempt but among the ancients the fly appears to have bred a kind of reverence, even was often deified. In the Chaldean, Philistine, and Phoenician pantheon flies were deified as Baalzebub (Lord of Flies); the Greek god Apollo, as pasture god, has the function of fly-chaser; and in ancient Egypt, they symbolized impudence, persistence and courage, and may have even assisted in the embalming process (*cf.* GREENBERG & KUNICH, 2002).

Notwithstanding, the Chinese were the first to report the use the flies and other insects as a part of their analysis tools in a crime scene investigation, and instances of their use are recorded as early as the mid-tenth century. Such was the importance of insects that in 1235, a training manual on investigating death, *Washing Away of Wrongs*, was written by Sung Tz'u recording the resolution of a stabbing near a rice field. It explains how the landing of a number of blowflies on a particular sickle caused its owner to confess to murdering a fellow farm worker with that sickle (BENECKE, 2001; GENNARD, 2012).

During the Middle Ages (until the 15th century), sculptors, painters and poets accurately depicts the insect mediated pattern of body mass reduction. The most remarkable artworks could be "Dance of the Death", "Skeleton in the Tumba" or the poem "Une Charogne" (written in 19th century) (*cf.* BENECKE, 2001).

...
Et le ciel regardait la carcasse superbe
Comme une fleur s'épanouir.
La puanteur était si forte, que sur l'herbe
Vous crûtes vous évanouir.

Les mouches bourdonnaient sur ce ventre putride,
D'où sortaient de noirs bataillons
De larves, qui coulaient comme un épais liquide
Le long de ces vivants haillons.
...

Une Charogne- Fleurs du mal de Charles Baudelaire

Figure 3: A fragment from the poem *Une Charogne* (C. Baudelaire, 1857).

Having seen the role of entomology in the medieval times, there are barely two notable experiments between the 15th and 19th centuries: the rebuttal of the theory of spontaneous generation by Redy (1668), proving that that seemingly spontaneous generation of maggots on meat was indeed the larvae of flies that hatched from eggs previously laid; and the work by Linnaeus (1775) in developing a system of classification (BYRD & CASTNER, 2009a; GENNARD, 2012; GUPTA & SETIA, 2004).

The credit for the first modern forensic entomology case goes to French doctor Bergeret (GUPTA & SETIA, 2004). As well as Orfila (another famous French doctor) before him, Bergeret noted the presence of insects on dead bodies, and pointed out that Orfila failed to apply their development to medicolegal matters, as he did to estimate the post mortem interval of a baby's mummified body in 1850 (GENNARD, 2012; GREENBERG & KUNICH, 2002; GUPTA & SETIA, 2004). Bergeret did not focus on forensic entomology in his report to the court but used entomology as one forensic tool among others (BENECKE, 2001).

Pierre Mégnin can be regarded as the first person who undertook a scientific research on forensic entomology. More than 15 years of experience at the Paris morgue allowed him to relate eight stages of decomposition to the succession of insects colonizing the body after death. He published his observations and conclusions in *Faune des Tombeaux* (1887) and *La Faune des Cadavres: Application de l'Entomologie à la Médecine Légale* (1894). In the latter book, Mégnin described the different groups or squads of arthropods (mainly insects

and mites) that colonize corpses in predictable and accurate successional waves. He was also the first person to develop a method to calculate the age of a dead body by the insects present (BENECKE, 2001; GENNARD, 2012; GREENBERG & KUNICH, 2002; GUPTA & SETIA, 2004).

These stages of decomposition were subsequently shown to be dependent upon environmental conditions among others. As his contemporaries caution, *the chief danger to be feared by Mégnin's imitators is that they might tend to apply rules to countries and climates where they where inapplicable* (GREENBERG & KUNICH, 2002).

Figure 4: Front page from Megnin's book *La faune des cadavres*.

Mégnin established the basis of forensic entomology as a science, but the seed lay dormant until after the mid-twentieth century as the number of specialists, the occasions when they were required and the communication between them and experts on legal medicine decreased considerably (GREENBERG & KUNICH, 2002; MAGAÑA, 2001).

In the turn of the 20th century, France and Germany enjoyed a general increase in the interest in zoological studies and several books were published, such as *Thierleben* (Life of Animals, by Alfred Brehm) and *Souvenirs entomologiques* (Souvenirs of Insect Life, by Jean Henri Fabre). The general interest was also focused on pest control and new methods for treating wounds using maggots, a treatment actually known as maggot therapy (BENECKE, 2001; GRASSBERGER & FLEISCHMANN, 2002; MAGAÑA, 2001).

Nevertheless, there are few works concerning forensic entomology during the first decades of the 20th century. Among them, it is remarkable the research performed by Stefan von Horoskiewicz and Eduard Ritter von Niezabitowski (Krakau University), in which they demonstrated that human corpses share the same fauna with animal corpses, both vertebrate and invertebrate (BENECKE, 2001). This was a very important achievement, as human corpses are extremely difficult to obtain legally and researches usually employ nonhuman

carcasses in order to include replication and repeated sampling in a succession study (WELLS & LAMOTTE, 2010).

It was in 1978 when Marcel Leclercq published *Entomologie et Médecine Légale. Datation de la mort.*, and later in 1986 with the publication of *A manual of forensic entomology* by K.G.V. Smith, the interest for this science was again on the rise. These scientists, together with Pekka Nuorteva among others, became pioneers in modern forensic entomology and introduced it into the habitual routine of forensic laboratories of many countries (LECLERCQ, 1978; MAGAÑA, 2001; SMITH, 1986).

From this moment, Forensic Entomology experienced an exponential growth, being a high number of researches performed and numberless the cases in which entomologists have contributed (MAGAÑA, 2001).

◈ Forensic Entomology in Spain

The Iberian Peninsula is an area with a set of particular characteristics due to the environmental influences of different biogeographic areas, which make it the area in whole Europe with the major entomological diversity. Indeed, it is where the highest number of new animal species is found every year in the continent, being the majority of these new species arthropods. This may be due to scientific backwardness that exists with respect to other European regions.

From an historical point of view, the first author that used arthropods with forensic purpose was Mariano de la Paz Graells (1886). In his article, he resumed the most important findings of Mégnin, including the real case in which Mégnin himself assisted the police, and used these ideas as an aid for the Spanish police in the resolution of a case of murder (ARNALDOS *et al.*, 2006; CASTILLO MIRALBÉS, 2002; GONZÁLEZ PEÑA, 1997).

It is also worth to mention here the not-very-well-known article entitled *Los insectos y la putrefacción de los cadáveres*, by Teodoro Ríos (1902), in which the cadaveric fauna known at that moment can be found, as well as the sequence of appearance of the different groups, even though the author did not

believe in “Mégnin’s squads” as well and closely defined groups (GONZÁLEZ PEÑA, 1997).

Apart from some punctual studies (LECHA-MARZO, 1924; PIGA PASCUAL, 1928) which strive to warn about the hazards of extrapolating Mégnin’s conclusions to different areas, there is little more in our background until the publications of BÁGUENA (1952), DOMÍNGUEZ MARTÍNEZ & GÓMEZ FERNÁNDEZ (1957). These, while still have references to Mégnin, did also include their own observations and reports of preliminary studies about the life cycle of some forensically important species.

In 1975, on the occasion of the first centenary of the Spanish Royal Society of Natural History, PÉREZ DE PETINTO published an article which included a review of the artistic representations of insects in association with death, a description of several practical cases, and the life cycle of dipterans, in addition to pointing out the importance of coleopterans in the decomposition of corpses (CASTILLO MIRALBÉS, 2002).

From this moment onwards, the number of experimental works and, therefore, the publications, grew up considerably as can be seen in Figure 5.

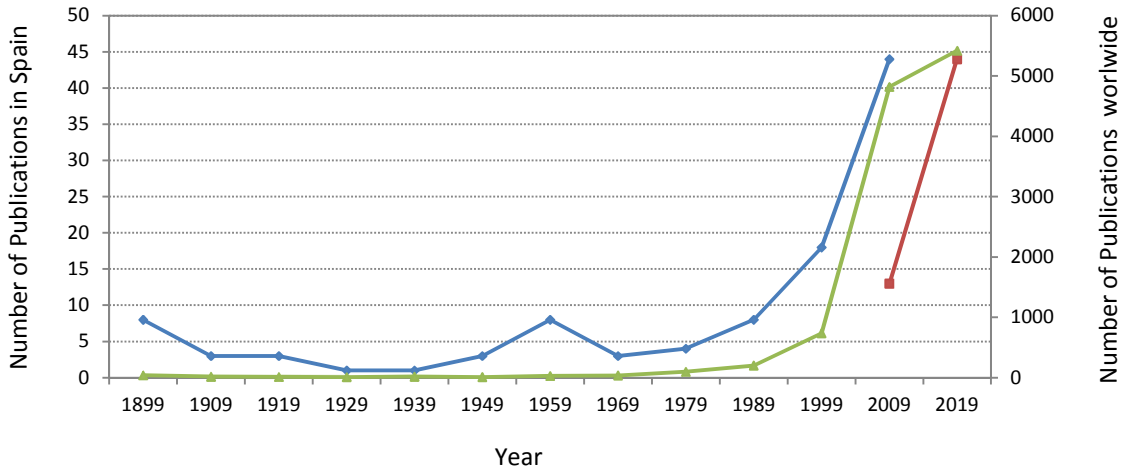


Figure 5: Number of publications about Forensic Entomology in Spain through the last century (data from GÓMEZ-GÓMEZ, 2007; blue line), actualized with data from Web of Kownledge (WOK; the search was made on 24th August 2015; red line); and number of publications about Forensic Entomology worldwide (search with Google Scholar on 22nd August 2015; green line).

Since the 90's, two main lines of research has been developed within Forensic Entomology:

1) The study of the biology of main arthropod species involved in the decomposition process (life cycle, ecology, distribution...), especially early colonizers (blowflies, fleshflies).
2) The study of succession patterns in different conditions (shade *vs.* sun, different season, location...).

Based on these lines, several works and active research groups deserve remarking. Among them, the first research performed by Castillo Miralbés, which included a detailed study of entomological succession in Alto Aragón, was the reference work about sarcosaprophagous fauna in our country (CASTILLO MIRALBÉS, 2002). In Madrid, Ana García Rojo (GARCÍA-ROJO, 2004; GARCÍA-ROJO & HONORATO, 2006; GARCÍA-ROJO *et al.*, 2009) works for the police in a Forensic Entomology laboratory as well and assists in real cases. The anthropologist Concha Magaña made a review of the status of Forensic Entomology in Spain (MAGAÑA, 2001) and sample collection guidelines for forensic entomological cases (MAGAÑA LOARTE & PRIETO CARRERO, 2009), assisting in real cases in the Police Sciences and University Research Institute (IUICP, University of Alcalá, Madrid; https://iuicp.uah.es/pagina-principal.html). Finally, in the southeastern region, it needs to be remarked the research and formative work of Mª Dolores García, María Isabel Arnaldos and Juan José Presa in the University of Murcia, updating knowledge of the composition and biology of the carrion-related fauna of the Mediterranean region (including regions of (ARNALDOS *et al.*, 2004) and Portugal (PRADO E CASTRO *et al.*, 2011a). And also for this Mediterranean region of the peninsula, Santos Rojo and Ana Isabel Martínez Sanchez, from the University of Alicante, have consolidated different lines of investigation based on forensically important species: taxonomy (VELÁSQUEZ *et al.*, 2010), succession studies (MARTÍNEZ SANCHEZ *et al.*, 2000a and 2000b) and applied Entomology (BARROSO *et al.*, 2014) among others.

This list of Spanish teams ends with the consolidated group in the Basque Country (Northern Spain), where this research has been developed. Leaded by

the Molecular Biologist Mª Angeles M. De Pancorbo and the Entomologist Marta I. Saloña-Bordas, this group contributes to the maturity of Forensic Entomology in our region. They started the research focusing in one of the most forensically important family (Diptera: Calliphoridae) (SALOÑA BORDAS *et al.*, 2009). Nowadays, several lines of investigation have been developed: blowfly genomics (GILARRIORTUA *et al.*, 2013), distribution and life cycle (DÍAZ MARTÍN *et al.*, 2014; ZABALA *et al.*, 2014); pest control (VALBUENA LACARRA & SALOÑA BORDAS, 2010; DÍAZ MARTÍN *et al.*, 2012); and succession of arthropods in carrion (DÍAZ MARTÍN & SALOÑA BORDAS, 2015; CARLES-TOLRÁ *et al.*, 2012). This thesis is focused on the last area of research, by conducting a field survey of carrion-related fauna associated with pig carcass decomposition in Aiako Harria Natural Park (Gipuzkoa, Spain).

◈ CURRENT CONCEPTS

The time elapsed since death, also called post-mortem interval (PMI) is a matter of crucial importance in legal Medecine, allowing to establish a possible guilt or to identify a missing person for instance. During the first 72 hours after death, a good method to estimate PMI is the external observation of the body, which includes factors such as body temperature, cadaveric lividity, stiffness, signs of dehydration, external injuries, and signs of activity of animal invasion (scavengers or arthropods) (ABALONE & MICHALZUCK, 2010; GENNARD, 2012).

After these first 72 hours, medical information loses precision. Therefore, other areas of expertise can provide useful details in order to estimate the time of death. In this case, forensic entomology is used to approximate the post mortem interval, based upon either aging the insects or studying the succession of arthropods present on the body (GENNARD, 2012) and the soil (BORNEMISSZA, 1957; BRAIG & PEROTTI, 2009; SALOÑA *et al.*, 2010).

◈ THE DECOMPOSITION PROCESS

Keeping in mind that decomposition is a continuous process, which begins at the time of death and ends when the body has been reduced to a skeleton, it has been divided into a series of stages, ranging from one to as many as nine depending on the author and the geographic region (GOFF, 2010).

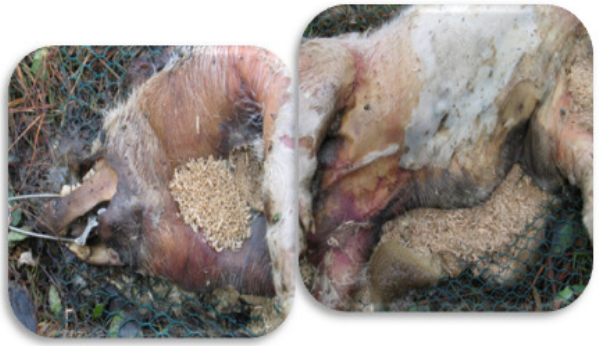
Decay stages are a useful mean of summarizing decomposition patterns, while some authors believe that they are in close agreement with changes in arthropod community composition (SCHOENLY & REID, 1987). In any case, in this research, unless otherwise stated, we will refer to the five stages as originally described in PAYNE (1965) and ANDERSON & VANLAERHOVEN (1996): Fresh, Bloated, Active Decay, Advanced Decay, and Dry – Remains (Table 1).

It is important to remark that every corpse is a dynamic ecosystem, with its particular biotope and biocoenosis, both in continuous change and clearly differentiated from the surrounding environment. Thus, different organisms will occur over time on the body, modifying it. So that when a species can no longer feed on the body due to the condition in which this is, a new species arrives and modifies the body for the next (MAGAÑA, 2007). A higher degree of complexity can be reached when taking into account the competition relationships between species, not only for sharing the same niche but also for predation, species displacement or parasitism.

“Fresh” stage decomposition is associated with the cessation of the heart and the depletion of internal oxygen. Autolysis and fly colonization can begin within minutes and soil microbes are observed to positively respond to cadaver presence within 24 hours. The lack of oxygen inhibits aerobic metabolism and creates an ideal environment for anaerobic microorganisms of the gastrointestinal tract and the respiratory system, which transform carbohydrates, lipids and proteins into organic acids (propionic and butyric acids) and gases (hydrogen sulphide, sulphur dioxide, carbon dioxide, methane, ammonia, hydrogen and carbon dioxide); a further retail can be found in CARTER, 2007 and GENNARD, 2012.

Table 1: Summary of changes during a decomposition process and the most important groups of arthropods associated with them (BRAIG & PEROTTI, 2009; GENNARD, 2012; GUNN, 2009; MÉGNIN, 1896; PAYNE & KING, 1969).

STAGE OF DECOMPOSITION	CHARACTERISTICS	ARTHROPODS		PHOTOGRAPHY
Fresh	Body starts to cool. Autolysis begins. Gas production by bacterial anaerobic metabolism. Hypostasis and *rigor mortis* may be observed.	Diptera	Calliphoridae Sarcophagidae	
		Coleoptera	Silphidae	
		Acari	Mesostigmata Oribatida Prostigmata	
Bloated	Discoloration of skin surface. Body swells from accumulation of gasses. Fluid expelled from orifices.	Diptera	Calliphoridae Sarcophagidae Muscidae	
		Coleoptera	Silphidae Staphylinidae Histeridae	
		Acari	Mesostigmata Prostigmata Ixodida	

Active Decay	Rapid decay owing to intense microbial and invertebrate activity. Body deflates as decomposition gasses escape. Progressive loss of skin. Soft tissues visibly decaying.	Diptera: Fanniidae, Piophilidae, Phoridae, Sepsidae, Sphaeroceridae, Sciaridae Coleoptera: Silphidae, Staphylinidae, Cleridae Acari: Mesostigmata, Astigmata, Prostigmata, Ixodida	
Advanced Decay	Decay owing to invertebrate and microbial activity starts to slow down. Body starts to dry out.	Diptera: Piophilidae, Sepsidae, Sphaeroceridae, Phoridae Coleoptera: Staphylinidae, Histeridae, Leiodidae, Nitidulidae Lepidoptera: Various species Acari: Mesostigmata, Astigmata, Oribatida, Prostigmata, Ixodida	

Dry – Remains	Skin and soft tissues lost. Decay proceeds more slowly. Skeleton may become disarticulated through environmental and biological processes.	Coleoptera Dermestidae Nitidulidae Trogidae Lepidoptera Tineidae Acari Mesostigmata Astigmata Oribatida Prostigmata Ixodida	

This results in a definite change in the physical nature and/or appearance of the body (GENNARD, 2012): colour change, odour and bloating of the cadaver. This process is also known as putrefaction and leads to the onset of “Bloated” stage. At this point, internal pressure from gas accumulation forces purge fluids to escape from cadaveric orifices and flow into the soil, creating discrete “islands of fertility” in the soil in association with plants. Putrefactive bloating and maggot feeding activity causes ruptures in the skin, allowing oxygen back into the corpse and exposing more surface area for the development of maggots and aerobic bacterial activity (CARTER, 2007).

This designates the beginning of “the Active Decay stage”, characterized by a rapid mass loss and the production of several compounds such as fatty acids, skatole, putrescine and cadaverine (GENNARD, 2012). During this phase, previous decomposition islands merge and create a single cadaver decomposition island (CDI), an area of increased soil carbon, nutrients and pH (CARTER, 2007).

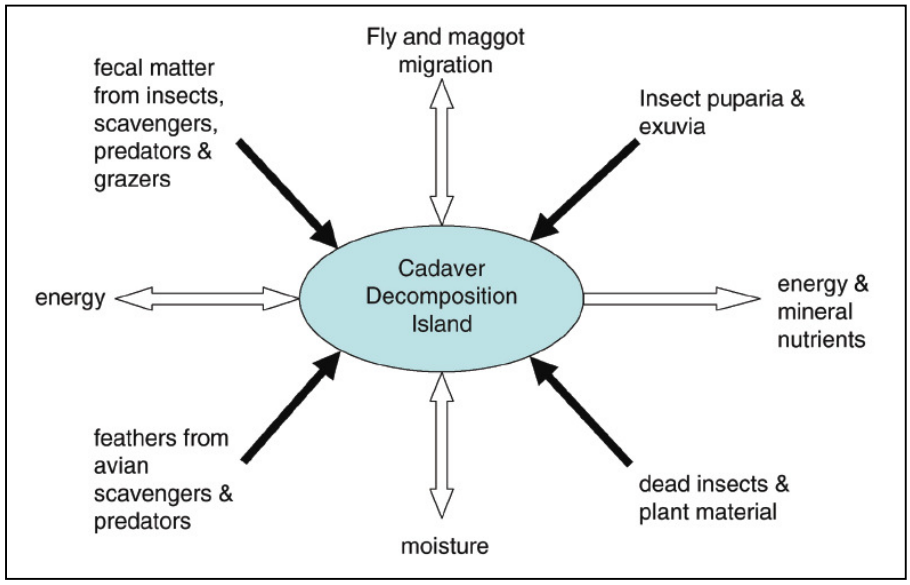


Figure 6: Diagram of the cadaver decomposition island (CDI) as a highly concentrated hub of energy and nutrient flow (CARTER, 2007).

Active Decay finishes when fly larvae migrate from the cadaver to pupate, a phenomenon that represents the beginning of “Advanced Decay”. This stage is associated with the death of underlying and nearby vegetation, which might

happen due to nitrogen toxicity, excretion of antibiotics by fly larvae and/or some unknown factor (CARTER, 2007).

The transition from “Advanced Decay stage” to “Dry stage” and to “Remains” is very difficult to identify (CARTER, 2007). Once all soft tissues are removed, skeletal material is further broken down by microbial activity and environment conditions, being finally reduced to mineral components (GENNARD, 2012; GUNN, 2009).

◈ THE ROLE OF ARTHROPODS

In order to understand the process of decomposition and the stages involved, it helps to have some knowledge of the organisms that will be involved in it (GOFF, 2010). There are four primary groups or taxa involved:

- Bacteria, they are associated with both external and internal aspects of the human body, begin to digest the body from inside out (GOFF, 2010).

- Fungi, which appear on the surface of animal and human cadavers. Forensic scientists and other researchers have yet paid little attention to them, and therefore, little is known on their role on the decomposition process (ISHII *et al.*, 2006).

- Insects, mainly, although other arthropods are involved too, are responsible for the major reduction of the body in terrestrial environments and in absence of scavengers.

- Vertebrate scavengers. They are predators from different vertebrate families that occasionally or predominantly feed on corpses, and their activity can rapidly deplete food in a corpse. They can profoundly impact the decomposition process of a dead body (GOFF, 2010). Additionally, it is worth mention those other animals that feed on insects, as although they do not feed directly on carcasses, they can also modify the ongoing of the decomposition process.

Even when there is a wide range of organisms that can exploit decomposing remains, not all allow an accurate estimation of PMI. Scavengers can accelerate the decomposition process by dismemberment or opening wounds, but do not provide enough information to estimate PMI without other tools. Otherwise, forensic microbiology is a promising field, but is still evolving. METCALF *et al.* (2013) found that microbes may provide a novel method for estimating PMI, but still at laboratory level. Arthropods are often the first to arrive and the predominant group, and provide the majority of the information aiding forensic investigations. Therefore, this is the group where this research will focus.

To the casual observer, it would appear that all arthropods inhabiting animal carcasses share the same ecological resource. However, each species have its own ecological niche, and it develops on a carcass because of its unique characteristics that give it some adaptive advantage over all other species (BYRD & CASTNER, 2009b). Therefore, one of the first steps is to identify the different types of relationships between a decomposing body and arthropods, which will have different value to the forensic investigation (GOFF, 2010).

There are mainly five basic relationships:

◇ *Necrophagous species*

These taxa feed only on decomposing tissues. This group includes Diptera (especially Calliphoridae and Sarcophagidae) and Coleoptera (Silphidae and Dermestidae) (CAMPOBASSO *et al.*, 2001). There are also mite species specialise on feeding on dry skin, as well as on bacteria, algae and fungie that develop on carcass surfaces (BRAIG & PEROTTI, 2009).

Figure 7: *Lucilia caesar* (Diptera: Calliphoridae) in the mouth of a domestic pig (*Sus scrofa*) (left), and two specimens of *Thanatophilus sinuatus* (Coleoptera: Silphidae) below ground (right).

◇ *Predators and Parasites of Necrophagous species*

They belong to the second most important category. It includes many beetles (Coleoptera: Silphidae, Staphylinidae, Histeridae) and parasitic wasps (Hymenoptera) (CAMPOBASSO *et al.*, 2001; GOFF, 2010).

Some fly species (Calliphoridae, Muscidae, Stratiomyidae) are also included in this category. This is because their larvae, although being necrophagous at first instance, can become predators on other larvae during their later states of development (e.g. *Chrysomya* spp. and *Hydrotaea aenescens*) (CAMPOBASSO *et al.*, 2001; GOFF, 2010).

Finally, several families of mites (Macrochelidae, Parasitidae, Parholaspididae, Uropodidae) were described by BRAIG & PEROTTI (2009) as predators of other mites, insects, and nematodes found on carcasses.

Figure 8: On the left, a predator (Coleoptera; Staphylinidae) of dipteran larvae and a parasitoid (Hymenoptera) of fly larvae and puparia (on the right).

◇ *Omnivorous species*

Taxa such as wasps, ants (Hymenoptera) and some beetles (e.g. Coleoptera: Staphylinidae, Histeridae) can feed both on decomposing remains and associated arthropods (CAMPOBASSO *et al.*, 2001).

Figure 9: On the left, it can be seen a group of ants taking blowflies' eggs away. On the right, there are two specimens of Histeridae.

◇ *Adventive species*

This category includes those taxa that simply use the corpse as an extension of their normal habitat, as is the case for springtails (Collembola), spiders, ticks, centipedes, millipedes and wood lice (GOFF, 2010).

They come from the surrounding vegetation or from the subsoil and use the corpse as a hiding refuge, or visit it from time to time. Occasionally, they can become predators of necrophilous species (CAMPOBASSO *et al.*, 2001).

A minor group of mite species will end up at carcasses as adventice species, mainly through foresy on necrophagous or necrophilous insects (BRAIG & PEROTTI, 2009).

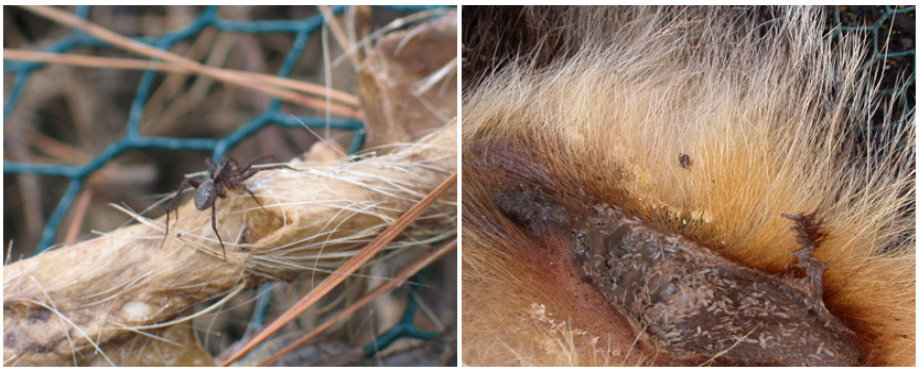


Figure 10: A spider (Araneae) on the left, and a little tick (Acari: Ixodidae) on the right.

◇ *Accidental species*

This is a category often ignored, even by entomologists, and not always recognized but may still be of significance, especially on cases of post mortem movement of a body (GOFF, 2010). It refers to those species that have no real relationship to the corpse but still found on it because they may have fallen or landed on the body (GOFF, 2010).

To summarize the relationships between these groups and the site of decaying remains, it has been made a diagram:

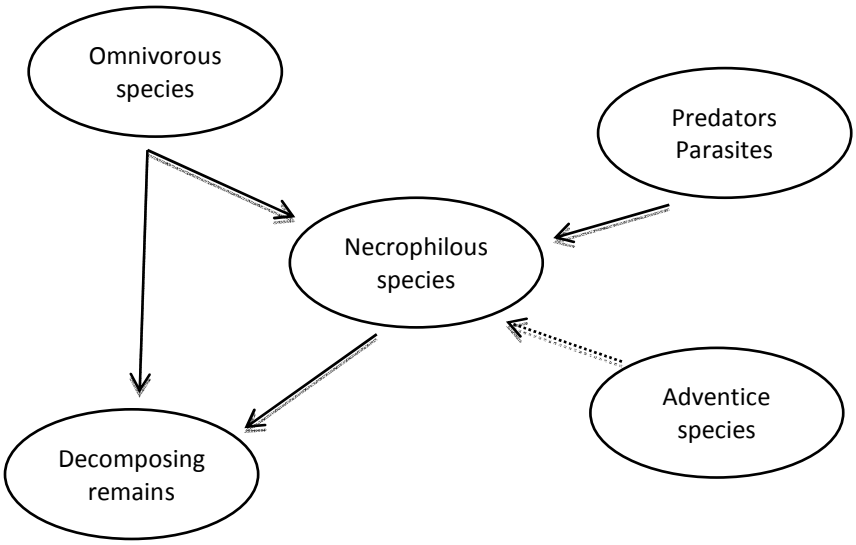


Figure 11: Relationships between each ecological role and decomposing remains (*cf.* SMITH, 1986).

Each group of arthropods will be attracted to decomposing remains at different stages of decomposition, as changes within the remains and abundance of

insect species present in them result in the availability of different resources (Table 1). This predictable order in which insect group is attracted to and observed on remains can be used in forensic investigations to estimate the post mortem interval (PMI) or time since death (ANDERSON & VANLAERHOVEN, 1996). This method is most valuable in the later stages of decomposition, when the earliest colonizers are no longer present (TABOR *et al.*, 2004) and the medical methods (body temperature, stiffness, etc) are useless.

In cases where decease is recent, the PMI can be estimated by analyzing the degree of development of early-arrival species that colonize the corpse. This approach enables investigators to establish a minimum PMI, because flies generally do not deposit their eggs on a living individual (TABOR *et al.*, 2004).

However, insect succession and development on a corpse may be influenced by many factors, including geographical region, exposure, season and habitat.

◈ FACTORS AFFECTING DECOMPOSITION

The rate of post mortem decay can be affected by variables of different nature concerning the corpse itself (intrinsic factors) and the external environment (extrinsic factors) (CAMPOBASSO *et al.*, 2001).

In accordance with CAMPOBASSO *et al.*, 2001, worth mentioning among the intrinsic factors are:

- Age: putrefaction is slower in foetuses and newborns
- Constitution: body size and weight affect both the development and dissemination of bacteria and the attraction of insects to the body.
- Cause of death: each type presents its own set of unique circumstances that affect the many factors during the decomposition process.
- Integrity of the corpse: wounds or cuts in the skin are an easy way in for external bacteria and Diptera.

Among the extrinsic factors, the most important is certainly ambient temperature, followed by ventilation and air humidity. Unfavourable temperature and humidity conditions can easily destroy eggs, affect the rate of maggot

development and can inhibit adult flies' activity (CAMPOBASSO *et al.*, 2001). Other factors to keep in mind are rainfall and wind, which serve as temporary barriers inhibiting insect flight (GOFF, 2010).

The degree of sunlight also affects both macro and microfauna. Vertebrate scavengers are found less frequently in sunny habitats (ANDERSON, 2009), whereas it has been observed that cloudy days affect insect access (mainly Diptera: Sarcophagidae) to human remains (CAMPOBASSO *et al.*, 2001).

Under certain conditions (normally wet, anaerobic situations), bodies may undergo saponification and develop waxy substances called adipocere which will delay the process for several months. On the other hand, in extremely dry conditions, the normal process of decaying is halted, causing mummification (*cf.* GOFF, 2010).

Access of the body to insects is the next variable in importance, as there can be several barriers (physical or chemical) that may affect the decomposition rate. Clothing of the body, burial or submersion prevent, though differently, direct access to decomposing tissues, as well as in those corpses found in closed spaces (buildings, vehicles...) (ANDERSON, 2009; CAMPOBASSO *et al.*, 2001; GOFF, 2010).

Besides, several chemical compounds are known to accelerate or delay the decomposition. For example, embalming is an ancestral practice to preserve bodies for centuries as the chemicals used on it repeal insects, as well as the presence of pesticides on the body or surroundings (GOFF, 2010). Other chemicals that can be present on a body, such as drugs, can affect the rate of development of the maggots, delaying (e.g. ethanol) or accelerating it (e.g. paracetamol) (GROSSELIN *et al.*, 2011; O'BRIEN & TURNER, 2004; TABOR *et al.*, 2005; VERMA & PAUL, 2013).

◈ MAIN APPLICATION FIELDS

Knowledge of the distribution, biology and behaviour of insects found in a crime scene can assist many types of forensic investigation by providing information

on when, where, and how, under certain conditions, a crime was committed or a person died (AMENDT *et al.*, 2007). Thus, forensic entomologist is often required to aid in the answering to the following tasks:

- Estimation of the PMI through the study of sarcosaprophagous fauna found in decomposing remains. It is used for this task larval development (early stages of decomposition) or the successional data (later stages) (GARCÍA-ROJO *et al.*, 2009; ARNALDOS *et al.*, 2005).

- Determine the period of the year (summer, autumn, winter or spring) in which the crime was committed, as species show a particular frequency and seasonality through the year (ARNALDOS *et al.*, 2005).

- Determine possible movements of a corpse by analysing the arthropod community, as each one is characteristic of a particular region (ARNALDOS *et al.*, 2005; BONACCI *et al.*, 2009).

- Neglect and abuse. Several cases of myiasis (maggot infestation of human tissues) have been observed on disregarded persons, mainly children and elderly (BENECKE *et al.*, 2004; BENECKE & LESSIG, 2001).

- Intoxication and drug consumption. This kind of substances can be detected on maggots and their puparia, even when this is no longer possible on human remains (O'BRIEN & TURNER, 2004; TABOR *et al.*, 2005).

- Wildlife traffick, as the biogeographical distribution is such a characteristic that allow the specialist to determine the origin of an individual or remain with the insects found on it (PETNEY, 1997).

- Food quality. To prevent economic losses, since the presence of insects in food can contaminate or devaluate the product (BEGUM *et al.*, 2007; JESS *et al.*, 2007).

General Objectives

If you don´t know where you´re going,

you will probably end up somewhere else.

Laurence J. Peter

◈ OBJECTIVES

Current work focuses mainly on the use of Entomology as a tool for estimating the post-mortem interval when a corpse has found. The correct identification of the fauna of forensic interest of northern Spain still remains as a research gap. Previous works in nearby areas with Atlantic climate has delved into the biology and development of several necrophagous families (BAHILLO DE LA PUEBLA *et al.*, 2004; MONEO & SALOÑA, 2009; SALOÑA BORDAS *et al.*, 2009). However, to our knowledge this is the first attempt to study and describe the whole process of carcass reduction by arthropods in a forest area of the Atlantic region of northern Spain. The identification of the species involved, as well as the description of their ecological roles, is a key point for the development of Forensic Entomology in our region. Moreover, from the extensive list of organisms that have been collected through this research, it will be identified the most important species of the process, in order to check their usefulness to design a succession model for our region.

Additionally, one of the tools of Forensic Entomology to estimate a post-mortem interval is the development on carcass tissues of pre-imaginal stages of those forensically important species. Complementarily, and as well as with adult specimens, a description of their succession patterns is needed for this region, due to the lack of previous references.

Finally, it is known that decomposition process and the species of arthropods involved are greatly influenced by temperature among other factors, so a detailed description of climatic conditions of the area is needed. In a real case, this information is of crucial importance when estimating PMI using entomological evidence. Hence, best practices in Forensic Entomology (AMENDT *et al.*, 2007) states that, in that cases, temperatures of the discovery site must be compared with the nearest Weather Station by means of a correlation analysis, in order to reconstruct climatic events before discovering the corpse. However, to our knowledge, the appropriateness of this standard has not been analysed in depth and therefore, this work will also focus on this protocol.

As a summary, a list of the general objectives of this thesis work is presented:

- Study the process of pig carcasses decomposition in Gipuzkoa during summer months, describing the arthropod community associated to it.
- Define the main climatic characteristics (temperature, rainfall, humidity) of the study area, and compare them with records from nearby Weather Stations in order to check its appropriateness as reference data source in possible forensic research in nearby locations.
- Determine the arthropod community dynamics and its ecological succession stages during the carcasses decomposition.
- Identify possible forensic bioindicators.
- Describe the larvae development pattern of relevant Diptera species under field conditions and the appropriateness of an extrapolation from laboratory development studies in PMI estimations.
- Create a reference database that gather all the information collected in this study, in order to be a reference for forensic investigations in Gipuzkoa and similar regions.

Site description and General Methodology

The method of scientific investigation
is nothing but the expression of the necessary mode
of working of the human mind.

Thomas Henry Huxley

◈ STUDY SITE DESCRIPTION

This research has been conducted in the Natural Park of "Aiako Harria", Site of Community Importance (SCI): ES2120016, which is located in the north-eastern corner of Gipuzkoa (Basque Country, Spain) (CANALES *et al.*, 2003).

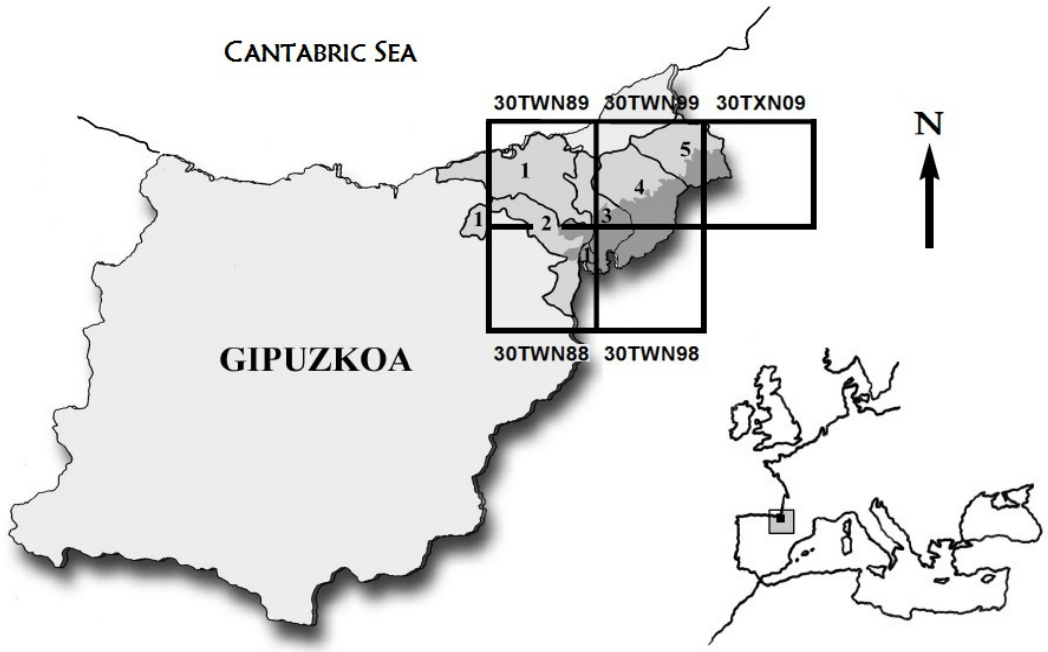


Figure 12: Location of "Aiako Harria" Natural Park (dark grey) inside the province of Gipuzkoa and Europe. Limits of the municipalities are pointed out in the map (1: San Sebastián; 2: Hernani; 3: Errenteria; 4: Oiartzun; 5: Irún), so as the UTM squares (10 x 10 km) of the mentioned area (*cf.* PAGOLA CARTE *et al.*, 2005).

It has a total surface of 6913 hectares and 105,5 km of perimeter. Its rough orography and intricate relief are composed of a succession of narrow valleys and hillsides of steep slopes and gorges with numerous streams. The maximum altitude corresponds to "Aiako Harria", with 834 meters, and the minimum to lower Bidasoa basin (6 meters), although most of the territory (71% approximately) is between 200 and 500 meters (CARLES TOLRÁ *et al.*, 2003; LIZAUR SUKIA *et al.*, 1996).

Regarding climate, this area is part of the Eurosiberian region, which means a humid temperate climate without dry season. However, as a consequence of its particular location near the coast, as well as the situation of Gipuzkoa at the far end of the bay of Biscay, makes this region to have a lower average

temperature in comparison with nearby regions, and the highest records of rainfall in the Basque Country (more than 2000 l/m^2 in many points) (BIURRUN *et al.*, 2011; CARLES TOLRÁ *et al.*, 2003; SALOÑA BORDAS *et al.*, 2009).

A special characteristic of the landscape of the Park is its geological peculiarity. Most of the land dates back to the Paleozoic, being the oldest materials reported from the whole Basque Country. It also constitutes the western end of the Pyrenean axial zone (CANALES *et al.*, 2003; LIZAUR SUKIA *et al.*, 1996).

The scenario of "Aiako Harria" Natural Park is predominantly forest, with vast areas of both native deciduous forest (beechwoods, oakwoods, and alder groves) and tree cultures (*Pinus sp.*). There are also some intermittent open areas for grazing and disperse country farms (caseríos) (LIZAUR SUKIA *et al.*, 1996).

One of the most remarkable enclaves is the "Urdaburu-Añarbe" forest, which is a mixed forest of oaks (*Quecus robur*) and European beeches (*Fagus silvatica*) situated quite close to the "Añarbe" reservoir. It is in this area where the field work of this project was carried out, specifically in a part of the mountain which previously served as an area for the recuperation of the wild boar in the Park (UTM coordinates: 30TWN91458860).

Nowadays, it is an unused area for more than 10 years, with scarce tree cover (mainly isolated pine trees) and high density of shrubbery, brambles and ferns (VENTURA *et al.*, 2012). The area is delimited by a wire enclosure and it presents two abandoned wood shelter (Figure 13). One of them is an old drinking trough covered by a rudimentary roof, and the other one is a little construction for storage.

◈ Animal model and Sample plots

The experiment was carried out using carcasses of domestic piglets (*Sus scrofa* Linnaeus, 1758) as animal models. As it has been pointed out by several authors (CATTS & GOFF, 1992; GOFF, 1993; SCHOENLY *et al.*, 2006), it is the most adequate species for Forensic Entomology research with comparative

purposes, as its results can be extrapolated to human beings (CASTILLO MIRALBÉS, 2002). Both species, human and pig, show similar shape and physiological pattern of decomposition, and share other characteristics such as diet, internal anatomy, fat distribution, size of chest cavity and lack of heavy fur (BYRD & CASTNER, 2001; CAMPOBASSO *et al.*, 2001; CATTS, 1992; SCHOENLY *et al.*, 2006).

The appropriate weight suggested by CATTS & GOFF (1992) is of about 23 kg, which is in accordance with results obtained by SPICKA *et al.* (2011). However, HEWADIKARAN & GOFF (1991) stated that there are no size-related differences observed between carcasses of different weight with respect to composition of the arthropod fauna and/or patterns of succession. Therefore, piglets of about 8-12 kg were decided to use mainly for practical reasons.

Most of the studies carried out with the aim of detecting differences between seasons of the year have not included replicate samplings, neither in time (doing just one experiment per season) nor with the number of animals (one carcass per season/treatment) (CENTENO *et al.*, 2002; JOY *et al.*, 2006; PRADO E CASTRO *et al.*, 2011b, among others), or locations. Nevertheless, environmental conditions can change not only in space (geographically), but also in time (from one year to another). On the other hand, the lack of replication for each season/treatment can generate problems of confounding, which means that differences due to experimental treatments cannot be separated from other factors that might be causing the observed differences (QUINN & KEOUGH, 2002). Hence, this study was developed during summer and autumn months of both 2009 and 2010, placing 5 carcasses per year in the research area:

- 2009: from 6th August to 29th October.
- 2010: from 27th July to 22nd October.

As space availability and logistic considerations constraint carcass distance, a compromise solution between independence, desired randomization and effort was applied, (QUINN & KEOUGH, 2002). Postfeeding larval dispersal was also taken into account. It ranges from a few centimetres to up to 8.1 meters (GREENBERG, 1990). Additionally, GOMES *et al.* (2002) established a distance of 6 meters from a carcass to search for pupae at a crime scene. Therefore, the

design was a partially radial distribution of them, with a minimum distance of 10 meters between one carcass and the next one (Figure 13).

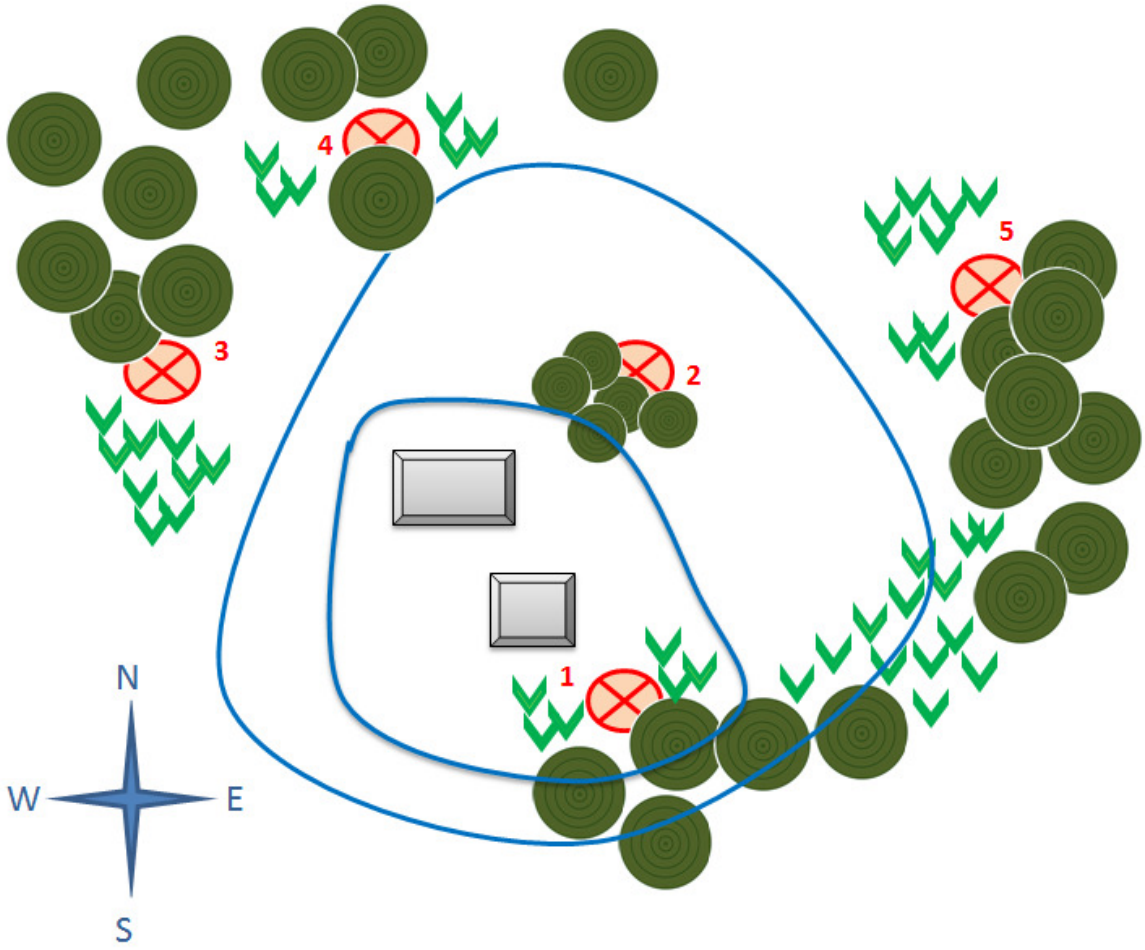


Figure 13: Schematic representation of the sampling area, with detail of carcasses position (numbered from 1 to 5, as it will be referred in results). Grey square: abandoned wood shelter; Blue lines: slope changes (from null on the centre to high on the limits); Green circles: pine trees; Green ticks: shrubbery, brambles and ferns; Red circles: carcasses.

Sun exposure of each carcass was measured the 21st June 2011, as it was the longest day of the year. The measure was made by placing an A3 paper (297 x 420 mm) on each carcass emplacement, and drawing on the paper the shadowed areas. Then, it was calculated the percentage of the shadowed area of that paper. This procedure was repeated at three different hours throughout the day (10:00, 14:30, and 18:00). Results are summarized on Table 2.

Table 2: Sun exposure of pig carcasses. Results are expressed as percentages.

	10:00		14:30		18:00	
	SUN	**SHADOW**	**SUN**	**SHADOW**	**SUN**	**SHADOW**
C1	0	100	8,3	91,7	18,2	81,8
C2	32,8	67,2	67,8	32,2	100	0
C3	30,48	69,52	42,31	57,69	54,14	45,86
C4	26,75	73,25	12,53	87,47	54,67	45,33
C5	100	0	33,04	66,96	15,6	84,4

◈ Equipment, Tools and Preservatives

Insects, scavengers and bacteria compete for cadaveric resources. Insects can consume a cadaver before a scavenger has utilized it, and microorganisms can release repellent toxins to prevent competitors. When insects are less active, scavenger success can approach (CARTER, 2007).

The study is focused on the knowledge of the arthropod fauna related to the carcasses. Therefore, each carcass was placed inside a weld-wire cage of 1145 x 845 x 975 mm to reduce the effects of vertebrate scavengers (*cf.* SCHOENLY *et al.*, 2006). Although this reduce the possibility of what HURLBERT (1984) described as *demonic intrusion* (non-malevolent entities which alter the perfection of your experimental design), the traditional recreational function of the park and livestock grazing might have added some extra 'noise' to the data.

Piglets were obtained in a local farm and killed by a stab in the carotid in the local slaughterhouse, following welfare aspects and approved ethical protocols (Spanish directives R.D. 54/1995 and R.D. 731/2007). According to GOFF (2010), carcasses were double bagged and transported to the site immediately after decease.

To facilitate the sampling procedure, each selected plot was cleaned of organic debris and a wire mesh was placed on it before depositing the carcasses.

Once cage, wire-mesh and carcass were placed, two i-Button thermometers were deployed: one in the mouth of the pig (to record the temperature in the carcass, through the mouth for being the most accessible part of it without causing an additional penetrative trauma) and the other one in the cage (to

record the temperature of each microhabitat). All i-buttons were protected from the environment in vials which allow air entry, tested in the laboratory and programmed for register temperature every hour.

At the same time, a datalogger was placed on a tree to record the ambient temperature every hour, and a second one was placed inside one of the abandoned shelters, to check the existence of any difference due to exposure to wind, rain and sun. In addition, rainfall was recorded with a rain gauge (Figure 14).

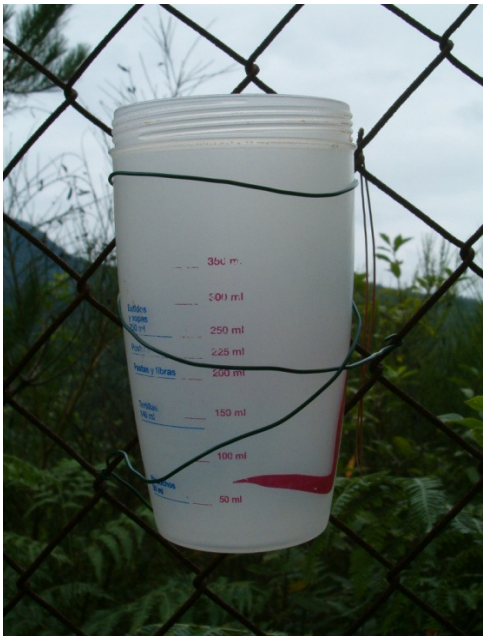


Figure 14: Rain gauge.

Following the European Association of Forensic Entomology (EAFE) standards and guidelines (AMENDT *et al.*, 2007), weather data from the three nearest meteorological stations were requested in order to assess possible biases incurred when using them for estimating the *postmortem* interval based on environmental temperature. In situ sampling was conducted using the following equipment:

- Tool box.
- Camera for picture documentation.
- Notebooks for technical notes as hour, temperature, description of the corpse, etc.

- Dark graphite pencil and pen with waterproof.
- Labels.
- Forceps.
- Fine paintbrush for collecting eggs, small insects and mites.
- Vials and storage boxes of different sizes.
- Thermometer for measuring the body and ambient temperature, as well as larval mass temperature.
- Handheld insect capture net for catching flying insects.
- Ethanol for killing and storing specimens (except maggots, which should be boiled in hot water for 1 minute before storing in ethanol).
- Plastic glasses for immature insects to be reared.

And finally, all collection of samples was done wearing protective clothing to avoid any contamination of the scene (AMENDT *et al.*, 2007) as well as to protect the investigator against hematophagous such as ticks, horseflies and others. This included wearing overalls, gloves and boots.

◈ PROCEDURE

The collection of the samples was based on a daily visit during the first stages of the decomposition (2009: days 1 to 13; 2010: days 1 to 9). Thereafter, sampling days were more spaced in time, allowing for days with no sampling (2009: days 14 to 85; 2010: days 10 to 88). The date of death and placement of pig carcasses was designate as day 1 (D1).

The sampling procedure for each carcass and day followed CASTILLO MIRALBÉS (2002) and GOFF (2010) procedure, and consisted in:

1. First of all, observations of the physical condition of each carcass were recorded. Several photographs were taken and a brief description was written down to have a diary of changes.
2. With the aid of a temperature measuring probe, temperatures on and around the pig were annotated, including ambient temperature, soil, interface, mouth, anus, larval masses and pig's surface.

3. At the same time, the rain gauge was checked in each visit, taking note of the volume of water on it and emptying it for next lecture.
4. Representative samples of immature arthropods were taken from the different parts of the animal. A group of ten small and 10 big maggots was collected in order to have almost all the sizes available. Other groups were only taken if larvae with different external morphology were present. All larvae were preserved in ethanol 80% at the sampling site, and boiled in hot water for one minute at the arrival to the laboratory keeping the sample back into the alcohol.
5. When available, larvae of the last growing periods (LIII, LIII migrating and pupae) were isolated in plastic containers with soil as substrate for pupation, rearing them to adults. These cultures were kept on the shelter close to pig 1 (Figure 13), with a datalogger attached to the roof for recording temperature and humidity there.
6. A representative sample of adult arthropods present was intended to be captured, from both areas, above and below the corpse, using a paintbrush, forceps or insect net.
7. The whole procedure (1-6) was repeated on every pig every sampling day.
8. Bones and any other remains were removed once the experiment finished.

Further specific information of methods applied in laboratory, and statistical treatments are explained in detail in corresponding chapters.

Arthropod of forensic interest associated to pig carcasses in Aiako Harria Natural Park (Basque Country, northern Spain)

Discovery consists of looking at the same things as everyone else and thinking something different.

Albert Szent Györgi

◈ INTRODUCTION

Arthropods, scavengers and microbes compete for cadaveric resources on a dynamic process of colonization, development and succession patterns (CARTER *et al.*, 2007). Natural inhabitants of the soil disappear (BORNEMISSZA, 1957; SALOÑA *et al.*, 2010), and this new ephemeral ecosystem provided by the dead corpse becomes dominated by cadaveric organisms.

Arthropods are the main responsible of corpse consumption, reducing it to skeleton in few days under adequate conditions. Necrophagous species feed on the corpse, and live or breed in/on it, depending on their biological preferences, and on the state of body decomposition. Other arthropods predate and parasite the previous ones. This produces a faunal succession which varies through season and environment conditions, and which can be useful for the estimation of time since death (BENECKE, 1998, 2005) and other circumstances associated to a decease.

The use of these succession patterns is necessarily based on an adequate knowledge of the local diversity, as species vary widely through regions (ARNALDOS *et al.*, 2005; SHARANOWSKI *et al.*, 2008). This sort of information is often generated in forensics through field experiments based on carrion decomposition in which nonhuman carcasses are usually employed, due to legal impediments on using human corpses as models (PRADO E CASTRO *et al.*, 2012; WELLS & LAMOTTE. 2010). Without this baseline knowledge of the relevant fauna associated to a corpse, the forensic entomologist needs to draw upon previous research and use that one (or a set of them) developed in areas of similar characteristics, although the accuracy of post-mortem interval estimation could be diminished as a consequence of it (ARNALDOS *et al.*, 2006; SHARANOWSKI, 2008).

In the Iberian Peninsula, detailed studies in this field are scarce, and most papers contribute data only for certain groups (ARNALDOS *et al.*, 2004). Besides, they have been mostly performed in areas south of the Atlantic bioregion of the Cantabric Shelf, leaving this area unexplored (PRADO E CASTRO *et al.*, 2012; ARNALDOS *et al.*, 2004; CASTILLO MIRALBÉS, 2002; GARCÍA ROJO, 2004; ROMERO PALANCO *et al.*, 2006). This lack of information is more remarkable since the

predominant climate types of our peninsula differ considerably, and the Atlantic climate of the whole coastal strip of the Cantabric Shelf and part of the Atlantic Facade discourages any extrapolation of data obtained in the Spanish continental plateau (Alto Aragón and Madrid) and Mediterranean regions (Alicante, Murcia, Cádiz and Lisbon) without assuming uncertainty on post-mortem calculations.

It is also of interest to check the effect of changes of the environment on the community. Therefore, it is necessary to have previous information about the biologic diversity of the community (species richness or α diversity) and the rate of change or replacement in species composition between different communities (β diversity) (KOLEFF, 2005; MORENO, 2001).

Therefore, this study aims to establish baseline knowledge of cadaveric fauna reporting an extensive inventory of carrion-associated arthropods, as well as to provide information about their successional patterns to create a database of reference for the area and close nearby with similar biogeoclimatic characteristics.

◈ MATERIAL AND METHODS

9 IDENTIFICATION

Arthropods constitute the most important group in terms of abundance and diversity of terrestrial fauna, and their classification is under continuous revision (SCHENK & MCMASTERS, 1956). Therefore, multidisciplinary teams are needed for an accurate identification of all the species involved in carcass reduction.

Following experts' instructions and the last review published by the Spanish Association of Entomology (BARRIENTOS coord., 2004), collected arthropods were divided in groups, according their class, order or family when it was possible. For this Chapter, only adults were taken into account.

More specific keys were used for genus and species level, with advice, guidance and supervision of experts when required. As consequence of the particular complexity associated with the correct identification of some groups,

they were directly sent to specialists for identification. This is the case of Hymenoptera, Hemiptera, Chelicerata and several families of Diptera and Coleoptera (see the list below), which were included for ease the comparison with previous works.

For the most abundant groups, which are also the better studied ones, the following taxonomic keys were used:

- COLEOPTERA: BAHILLO DE LA PUEBLA (2009, unpublished data); BÁGUENA, 1960; CHARRIER, 2002; MARTÍN PIERA & LÓPEZ COLÓN, 2000; PRIETO PILOÑA & PÉREZ VALCÁRCEL, 2002; YÉLAMOS, 2002.
- DIPTERA (adults and maggots): SZPILA (2012); SZPILA (2010) ; SZPILA *et al.* (2015); SZPILA & GRZYWACZ (2010, unpublished data); GONZÁLEZ MORA, 1989; PERIS & GONZÁLEZ MORA, 1991; ROGNES (1980, 1991, 1998); ROZKOŠNÝ *et al.*, 1997.

For remain taxa, either for limited information available or due to the complexity of identifying them correctly, the following specialists are aiding for the identification process:

- COLEOPTERA
    - Carabidae: Jesús Lencina
    - Histeridae: Tomás Yélamos
    - Leiodidae: Javier Fresneda
    - Nitidulidae: Pablo Bahillo
    - Staphylinidae: Raimundo Outerelo
    - Trogidae: Pablo Bahillo, Jesús Romero Samper
- DIPTERA
    - Agromyzidae: Miloš Černý
    - Chironomidae: Oscar Soriano
    - Chloropidae: Emilia P. Nartshuk
    - Ephydridae: Tadeusz Zatwarnicki
    - Fanniidae: Andrzej Grzywacz
    - Muscidae: Krzysztof Szpila, Andrzej Grzywacz
    - Phoridae: Henry Disney

    - Psychodidae: Rüdiger Wagner
    - Sarcophagidae: Dolores González Mora
    - Scatopsidae: Jean Paul Haenni
    - Sciaridae: Kai Heller
    - Tachinidae: Hans Peter Tschorsnig
    - Acartophthalmidae, Carnidae, Heleomyzidae, Milichiidae, Opomyzidae, Piophilidae, Sepsidae, Sphaeroceridae, Syrphidae, Tabanidae, Ulidiidae: Miguel Carles Tolrá
    - Ceratopogonidae, Dolichopodidae, Empididae, Hybotidae: Daniel Ventura
- HYMENOPTERA:
    - Formicidae: María Dolores Martínez Ibañez
    - Parasitoids: José Francisco Gómez Sánchez
- HEMIPTERA: Xanti Pagola
- ARACHNIDA: Alberto Castro
- ACARI: Alejandra Perotti

All these material is kept in ethanol in the reference collection of the Forensic Entomology research group of the Basque University (UPV/EHU, Leioa, Biscay, Spain).

9 ANALYSIS OF DIVERSITY

In the absence of information at species level on several taxa, all calculations have been made at family level with the statistical software PAST (HAMMER *et al.*, 2001) (Table 4).

Margalef index, which estimates the rate at which species are added when expanding the sample, was used to evaluate the diversity. It is easy to implement and widely applied in similar studies allowing comparability of results. Notwithstanding, it can be strongly influenced by sampling effort; therefore Fisher index was also applied to allow for a safer comparison among samples (MORENO, 2001; MAGURRAN, 2004).

Simpson's index, which is strongly influenced by the most dominant species, was applied, to improve our understanding of the community diversity, and to quantify the representativeness of each species.

On the other hand, decomposition process can be understood as a temporal gradient (TABOR, 2009). Therefore, the rate of species replacement through time of decomposition was evaluated (Figure 17). With this aim, it was calculated the turnover rate, which gives a percentage of similarity between successive time periods (*cf.* MAGURRAN, 2004):

$$t = \frac{a + b}{N_A + N_B}$$

where *a* and *b* are the number of species present only in sample A and B respectively, and N_A and N_B are the total number of species in each sample.

The Bray-Curtis dissimilarity index (1-D) is also reported, in order to account not only for the presence or absence of species, but also their abundance (MORENO, 2001). Both indexes, the turnover rate and the Bray-Curtis dissimilarity, show complementarily if the community composition changes through time and, if so, how it does.

◈ RESULTS

A total of 7837 adult specimens were collected during the research, being 3317 specimens in 2009 and 4520 in 2010. They ascribed up to 255 known species while 21 taxa (several families and orders) still remain unidentified and pendant of a more detailed revision.

C1 carcasses reported fewer individuals (a total of 829 adults that represented the 10.61% of the arthropod collection of the study). C2 and C3 carcasses, with an average of 1071 and 1083 specimens respectively, were the carcasses with the major number of specimens collected. C4 carcasses represented the 19.39% of the study collection, and C5 carcasses contribute with 621 and 548 specimens.

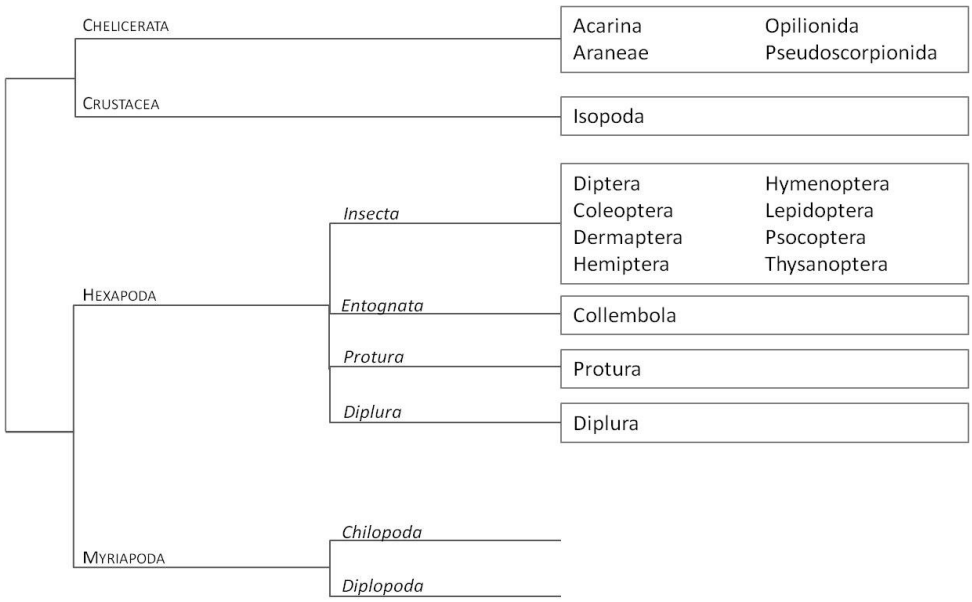


Figure 15: Main taxa collected during the whole experiment (from left to right: subphylum, class, order). Note that the length of lines does not imply any evolutive distance between groups.

There were 18 taxa represented (Figure 15), being the most important those of the class Insecta (Figure 16). Diptera was the predominant order (82%), with 30 families and more than 177 species collected. They were followed by beetles (Coleoptera, 12%), with 13 families and more than 64 species; and Hymenoptera (3%) already unidentified. Relative abundance of the dominant families is shown in Figure 16.

Table 3 reports the presence of the different taxa (up to 253 species from 12 families, 10 orders and 2 classes) associated to each stage of decomposition, as well as the total number of adults recorded during the whole experiment. New records are included, detailing the region from which the taxon is first reported.

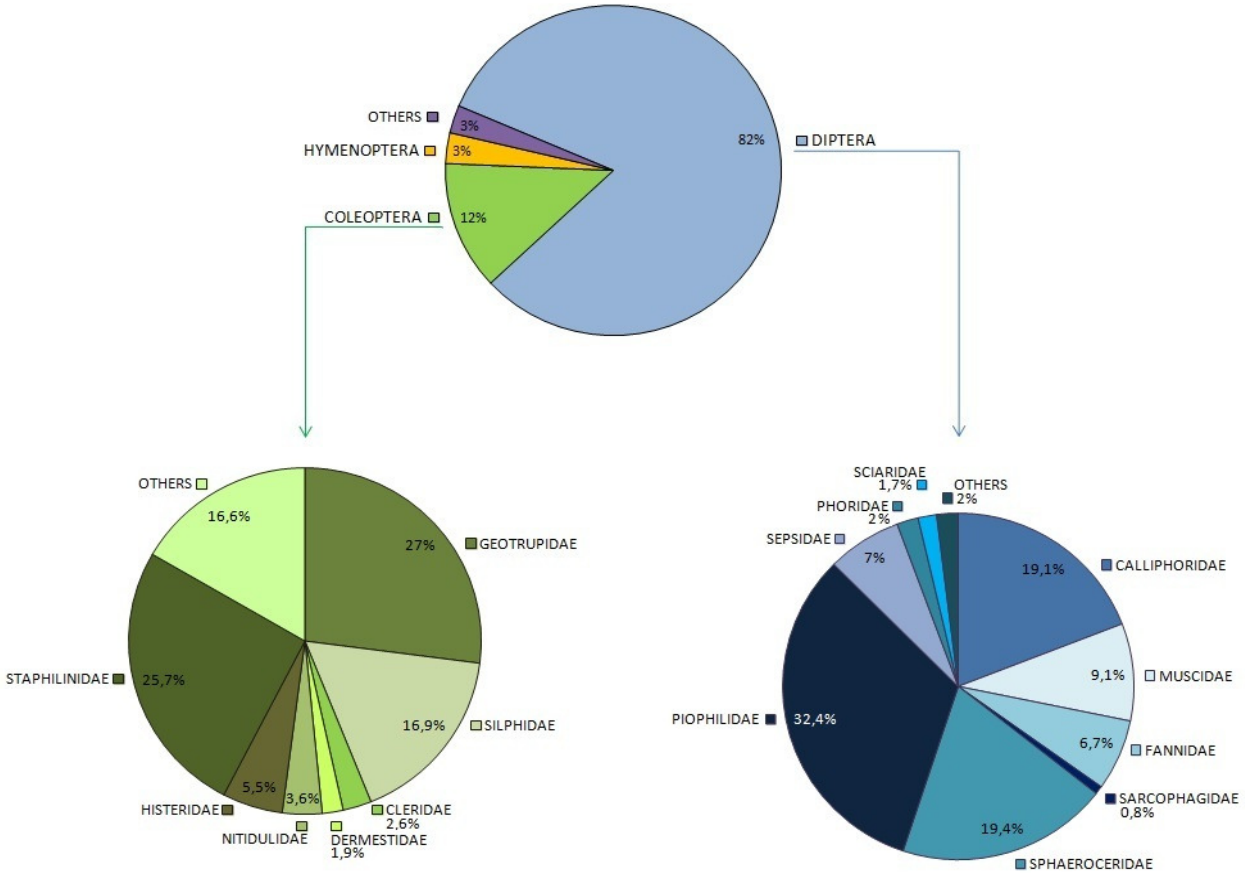


Figure 16: Relative abundance of main insect orders, detailing main families of Diptera and Coleoptera collected.

Table 3: Presence of arthropods, with the total sample size collected throughout the study period. New record of species, genera or family are specified with a superindex (G: Gipuzkoa; BC: Basque Country; S: Spain; SP: Spanish Peninsular territory; IP: Iberian Peninsula); names in bold refer to new species for Science. n.c.: not quantified.

		STAGE OF DECOMPOSITION					
ORDER / FAMILY	**SPECIES**	**F**	**B**	**AcD**	**AdD**	**D**	**NUMBER**
DIPTERA							
ACARTHOPHTHALMIDAE^{G}	*Acarthophthalmus^{G} bicolor^{BC}*			x	x		5
AGROMYZIDAE	*Liriomyza pusilla*				x		1
ANTHOMYIDAE	Unidentified species		x		x	x	9
CALLIPHORIDAE	*Bellardia* sp.				x	x	13
	Calliphora vicina	x	x	x	x	x	23
	Calliphora vomitoria	x	x	x	x	x	67
	Chrysomya albiceps	x	x	x	x	x	154
	Lucilia ampullacea	x	x		x	x	27
	Lucilia caesar	x	x	x	x	x	926
	Lucilia illustris	x	x		x		5
	Lucilia sericata				x		1
	Pollenia rudis					x	1
	Rhiniinae (un. sp.)		x		x		5
CARNIDAE^{BC}	*Meoneura^{BC} neottiophila^{BC}*		x	x	x	x	18
	Meoneura sp.				x		1
CECIDOMYIIDAE	Unidentified species					x	7
CERATOPOGONIDAE	*Culicoides (Avaritia) scoticus*	x					1
	Culicoides (Culicoides) impunctatus					x	1
	Culicoides (Silvaticulicoides) pallidicornis		x				1
	Forcipomyia (Euprojoannisia) titillans					x	1
	Forcipomyia (Forcipomyia) ciliata			x			1
CHIRONOMIDAE	Subfam Orthocladiinae					x	2
CHLOROPIDAE	*Oscinella frit*			x			1
	Siphunculina aenaea^{S}					x	1
	Siphunculina quinquangula^{S}				x	x	5
	Tricimba cincta				x	x	2
DOLICHOPODIDAE	*Chrysotus gramineus*				x		1
EPHYDRIDAE	*Hydrellia* sp.			x			1
FANNIIDAE	*Fannia canicularis*		x	x	x	x	17
	Fannia fuscula		x	x	x	x	17
	Fannia manicata	x	x	x	x	x	43
	Fannia scalaris					x	3
	Fannia sp.	x	x	x	x	x	352
HELEOMYZIDAE	*Suillia affinis*					x	2
	Suillia variegata				x		1
HYBOTIDAE	***Crossopalpus* sp. n. (nr. *nigritellus* and *aeneus*)**					x	1
	Crossopalpus humilis^{IP}				x	x	13
	Drapetis* sp. n. *(group exilis)			x		x	2
MILICHIIDAE	*Leptometopa^{BC} latipes^{BC}*				x		1

MUSCIDAE	*Azelia* sp.	x	x	x	x	x	84
	Dasyphora albofasciata		x			x	2
	Eudasyphora sp.	x		x	x	x	9
	Graphomya sp.		x	x	x	x	9
	Gymnodia sp.		x	x	x		6
	Hebecnema sp.				x	x	3
	Hydrotaea aenescens			x	x		28
	Hydrotaea armipes			x	x	x	15
	Hydrotaea capensis		x	x	x		3
	Hydrotaea dentipes	x	x	x	x	x	64
	Hydrotaea ignava		x	x	x	x	53
	Hydrotaea pilipes			x	x	x	22
	Hydrotaea similis	x	x	x	x		157
	Hydrotaea sp.	x			x	x	12
	Morellia sp.	x				x	2
	Musca autumnalis	x	x	x	x	x	58
	Musca domestica				x	x	4
	Muscina levida			x	x	x	14
	Muscina pascuorum			x		x	4
	Muscina prolapsa		x	x	x	x	8
	Mydaea sp.	x			x	x	5
	Myospila sp.					x	1
	Phaonia sp.	x		x		x	10
	Stomoxys calcitrans	x	x		x	x	5
	Other Muscidae species		x	x		x	5
OPOMYZIDAE	*Geomyza tripunctata*					x	1
PHORIDAE	*Conicera floricola*					x	2
	Conicera tibialis				x		2
	Diplonevra florescens			x			3
	Megaselia albicaudata					x	1
	Megaselia brevicostalis	x		x	x	x	71
	Megaselia citrinella[S]				x		1
	Megaselia elongata			x			1
	Megaselia giraudii	x					1
	Megaselia meconicera[S]					x	1
	Megaselia tama[S]				x	x	4
	Megaselia tarsalis			x	x	x	4
	Megaselia verna					x	1
	Megaselia sp near angusta	x			x	x	5
	Megaselia sp A	x			x	x	3
	Megaselia sp B					x	1
	Megaselia sp C		x				1
	Metopina perpusilla			x	x		2
	Pseudacteon formicarum[S]				x	x	5
	Pseudacteon lundbecki				x		2
PIOPHILIDAE	*Liopiophila varipes*	x	x	x	x	x	441

	Parapiophila[BC] *vulgaris*[BC]			x	x	x	48
	Piophila[BC] *casei*[BC]				x		1
	Piophila megastigmata[BC]			x	x		13
	Prochyliza[BC] *nigrimana*[BC]		x	x	x		6
	Protopiophila latipes		x	x	x	x	118
	Stearibia[G] *nigriceps*[G]	x	x	x	x	x	1447
PSYCHODIDAE	*Psychoda albipennis*					x	4
	Psychoda minuta					x	1
	Psychoda cf. s*urcoufi*					x	3
	Psychoda sp.					x	1
	Tinearia alternata					x	1
SARCOPHAGIDAE	*Ravinia pernix*				x	x	5
	Sarcophaga (Bellieriomima) subulata					x	2
	Sarcophaga (Helicophagella) noverca					x	1
	Sarcophaga (Parasarcophaga) aratrix				x	x	2
	Sarcophaga (Sarcophaga) pyrenaica				x	x	3
	Sarcophaga albiceps	x		x	x	x	16
	Sarcophaga incisilobata					x	1
	Sarcophaga sp.		x		x	x	20
SCATOPSIDAE	*Coboldia fuscipes*					x	3
	Thripomorpha cf. coxendix					x	1
SCIARIDAE	*Bradysia angustipennis*					x	1
	Bradysia hilaris					x	1
	Bradysia subrufescens					x	1
	Bradysia sp.					x	1
	Cratyna sp.					x	1
	Hyperlasion wasmanni				x	x	9
	Lycoriella cellaris					x	2
	Phytosciara (Dolichosciara) flavipes					x	1
	Phytosciara sp.					x	1
	Pnyxia scabiei					x	1
	Scatopsciara atomaria				x	x	2
	Scatopsciara multispina			x	x	x	86
	Other Sciaridae species			x			1
SEPSIDAE	*Meroplius*[BC] *fukuharai*[IP]			x	x	x	16
	Meroplius minutus[BC]		x	x	x	x	295
	Nemopoda nitidula	x		x	x	x	45
	Nemopoda speiseri[IP]			x	x	x	6
	Sepsis fulgens			x	x	x	27
	Sepsis luteipes[IP]	x		x	x	x	17
	Sepsis punctum	x		x	x	x	28
	Other Sepsidae species				x	x	5
SIMULIDAE	Unidentified species					x	7
SPHAEROCERIDAE	*Alloborborus*[IP] *pallifrons*[IP]		x				1
	Bifronsina bifrons				x	x	3
	Borborillus[G] *vitripennis*[G]	x			x		3

	Chaetopodella scutellaris	x	x	x	x	x	347
	Coproica ferruginata				x	x	7
	Coproica hirticula[BC]		x	x	x	x	260
	Coproica lugubris[BC]				x	x	16
	Coproica pusio		x	x	x	x	41
	Coproica rohaceki			x	x	x	18
	Coproica vagans[G]	x	x	x	x	x	35
	Elachisoma[BC] *aterrimum*[BC]				x	x	15
	Elachisoma bajzae[BC]			x	x	x	10
	Elachisoma pilosum[BC]					x	1
	Gonioneura[BC] *spinipennis*[BC]			x	x	x	7
	Ischiolepta denticulata[E]	x				x	2
	Ischiolepta vaporariorum[BC]			x	x		2
	Leptocera caenosa		x	x	x	x	43
	Minilimosina[G] *alloneura*[BC]			x		x	5
	Minilimosina fungicola[BC]				x	x	5
	Minilimosina parvula[BC]	x		x	x	x	22
	Norrbomia costalis	x					1
	Opalimosina[BC] *calcarifera*[BC]				x	x	22
	Opalimosina collini[BC]					x	2
	Opalimosina czernyi[BC]					x	1
	Opalimosina liliputana[BC]			x	x	x	76
	Opalimosina mirabilis[BC]				x	x	9
	Opalimosina simplex[BC]		x	x	x	x	24
	Paralimosina fucata	x	x		x	x	16
	Phthitia empirica[IP]				x		3
	Spelobia baezi[BC]			x	x	x	19
	Spelobia cambrica[IP]				x	x	7
	Spelobia clunipes	x		x	x	x	37
	Spelobia luteilabris	x	x	x	x	x	69
	Spelobia nana[BC]				x	x	4
	Spelobia palmata	x					2
	Sphaerocera curvipes	x			x	x	5
	Telomerina flavipes			x	x	x	12
	Telomerina levifrons[SP]			x	x	x	39
	Terrilimosina schmitzi				x		2
	Trachyopella[BC] *kuntzei*[IP]				x	x	11
	Trachyopella lineafrons[BC]		x	x	x	x	38
	Other Sphaeroceridae species				x		1
SYRPHIDAE	*Episyrphus balteatus*			x	x	x	10
	Eristalis similis					x	1
	Meliscaeva cinctella					x	1
	Syritta pipiens				x		1
TABANIDAE	*Dasyrhamphis atra*	x					1
	Tabanus bromius	x					1
TACHINIDAE	*Peribaea tibialis*					x	1

	Voria rurales					x	1
	Winthemia cf. quadripustulata					x	7
ULIDIIDAE	*Euxesta pechumani*				x		2
COLEOPTERA							
APHODIDAE	Unidentified species				x		1
CARABIDAE	*Steropus (Steropus) madidus*	x		x			3
CLERIDAE	*Necrobia ruficollis*					x	1
	Necrobia rufipes			x	x	x	10
	Necrobia violácea				x	x	15
CURCULIONIDAE	Unidentified species	x	x		x		6
DERMESTIDAE	*Dermestes frischii*				x	x	6
	Dermestes mustelinus				x	x	3
	Dermestes undulatus				x	x	10
GEOTRUPIDAE	*Anoplotrupes stercorosus*	x	x	x	x	x	124
	Sericotrupes niger					x	1
	Thyphaeus thypoeus					x	1
	Trypocopris pyrenaeus	x	x	x	x	x	142
HISTERIDAE	*Carcinops pumilio*				x	x	7
	Hister unicolor				x	x	3
	Margarinotus (Ptomister) brunneus	x	x		x	x	24
	Saprinus (Saprinus) planiusculus			x			3
	Saprinus (Saprinus) semistriatus	x	x	x	x		6
	Saprinus (Saprinus) subnitescens		x	x	x		15
LEIODIDAE	*Sciodrepoides fumatus*				x	x	18
	Sciodrepoides watsoni				x		1
NITIDULIDAE	*Nitidulidae* sp.					x	8
	Omosita colon					x	12
	Omosita depressa				x	x	14
	Omosita discoidea					x	2
OEDEMERIDAE	Unidentified species			x			1
SILPHIDAE	*Necrodes littoralis*	x	x	x	x		141
	Nicrophorus humator			x			1
	Nicrophorus vespilloides	x	x	x	x		24
	Oeceoptoma thoracica				x		1
	Tanatophilus rugosus		x				1
	Tanatophilus sinuatus			x			2
STAPHYLINIDAE	*Acrotona pygmaea*				x	x	10
	Acrulia inflata					x	1
	Aleochara (s.str.) curtula		x	x	x	x	22
	Aleochara (s.str.) stichai					x	1
	Anotylus complanatus	x			x	x	8
	Anotylus sculpturatus		x				1
	Atheta (Microdota) amicula	x				x	3
	Atheta (Microdota) indubia					x	1
	Atheta (Microdota) mortuorum				x	x	7
	Atheta (s.str.) aquatica					x	6

	Atheta (s.str.) fungicola				x	x	3
	Autalia impressa					x	2
	Autalia puncticollis					x	1
	Bisnius fimetarius		x	x	x	x	38
	Creophilus maxillosus		x	x	x	x	46
	Dimetrota nigripes					x	5
	Drusilla canaliculata			x		x	2
	Gabrius exiguus		x				1
	Gyrohypnus (s.str.) fracticornis				x	x	3
	Megarthrus denticollis					x	1
	Myrmecopora (Iliusa) fugax					x	1
	Ontholestes tesellatus			x		x	2
	Oxytelus (Epomotylus) sculptus	x	x	x		x	9
	Philonthus cochleatus	x	x	x	x	x	12
	Philonthus coprophilus				x		1
	Philonthus nitidus					x	1
	Philonthus politus			x	x	x	11
	Philonthus succicola	x	x		x	x	12
	Philonthus varians				x	x	4
	Platysthetus arenarius		x		x	x	27
	Proteinus brachypterus					x	3
	Rugilus (s.str.) orbiculatus					x	1
	Tachinus flabolimbatus					x	2
	Other Staphylinidae species					x	9
TROGIDAE	*Trox scaber*[BC]				x	x	3
OTHER families	Unidentified species	x	x	x	x	x	133
HYMENOPTERA							
FORMICIDAE	Unidentified species	x	x	x	x	x	136
UNIDENTIFIED	Unidentified species	x	x	x	x	x	100
EXOPTERYGOTA							
PSOCOPTERA						x	6
THYSANOPTERA						x	2
HEMIPTERA							
CICADELLIDAE	Unidentified species	x		x		x	4
CIXIIDAE	Unidentified species					x	1
DELPHACIDAE	Unidentified species	x		x	x	x	6
ISSIDAE	*Issus coleoptratus*					x	3
ANTHOCORIDAE	*Xylocoris (Proxylocoris) galactinus*					x	5
CERATOCOMBIDAE[BC]	*Ceratocombus*[BC] *(Ceratocombus) coleoptratus*[BC]				x	x	4
COREIDAE	*Coreus marginatus marginatus*				x	x	3
LYGAEIDAE	*Scolopostethus affinis*					x	1
PENTATOMIDAE	*Piezodorus lituratus*		x				1
APHIDIDAE	Unidentified species				x	x	3
OTHER HEXAPODA							
LEPIDOPTERA		x					1

DERMAPTERA				x		x	3
COLLEMBOLA		x			x	x	67
PROTURA				x		x	6
DIPLURA						x	1
CHELICERATA							
ACARI	Unidentified species	x	x	x	x	x	n.c.
ARANEAE							
LINYPHIIDAE	*Diplocephalus latifrons*					x	3
	Erigone dentipalpis					x	1
	Tiso vagans					x	2
	Centromerita concinna					x	1
	Centromerus sylvaticus					x	4
	Diplostyla concolor					x	3
	Tenuiphantes sp.					x	2
	Tenuiphantes flavipes					x	6
	Tenuiphantes tenuis				x		1
LYCOSIDAE	*Pardosa sp.*				x	x	4
	Pardosa lugubris					x	1
	Trochosa terricola	x				x	5
SALTICIDAE	*Heliophanus sp.*					x	1
THERIDIIDAE	*Episinus truncatus*				x		1
OPILIONIDA						x	10
PSEUDOSCORPIONIDA						x	4
OTHER TAXA							
MYRIAPODA	DIPLOPODA					x	1
	CHILOPODA					x	20
ISOPODA						x	23

Values of diversity indexes are detailed in Table 4. Advanced Decay stage reported the higher number of specimens collected, but it was during the Dry stage when the highest diversity was found, as estimated from Margalef and Fisher indexes. Regarding Simpson index, it had lowest values during the Fresh stage, indicating that one or few taxa dominated the carcass environment.

Table 4: Diversity indexes associated with the five decomposition stages.

	STAGE OF DECOMPOSITION				
	Fresh	**Bloated**	**Active Decay**	**Advanced Decay**	**Dry**
Number of adults	755	412	1120	3250	2300
Number of Families	24	20	30	37	47
Margalef index	3,471	3,154	4,132	4,452	5,943
Fisher index (alpha)	4,724	4,391	5,674	5,854	8,362
Simpson index (1-D)	0,6145	0,8162	0,8626	0,7643	0,9035

Dissimilarity was highest during the first and last moments of decomposition (Figure 17A), remaining lower between those moments. On the other hand, species replacement expressed by turnover rate indicated a very low change on species during Fresh and Bloated stages (Figure 17B), suggesting that in these stages the most important changes were in the abundance of the present species. These findings agree with results summarised on Table 4. Fresh stage was dominated by adults of two species (*Lucilia caesar and Musca autumnalis*) which became less important in number with the arrival of new species during the ongoing of bloated stage, resulting in a peak of dissimilarity at the Active Decay stage (see Figure 17A). After this first moment, species turnover (Figure 17B) kept more or less constant.

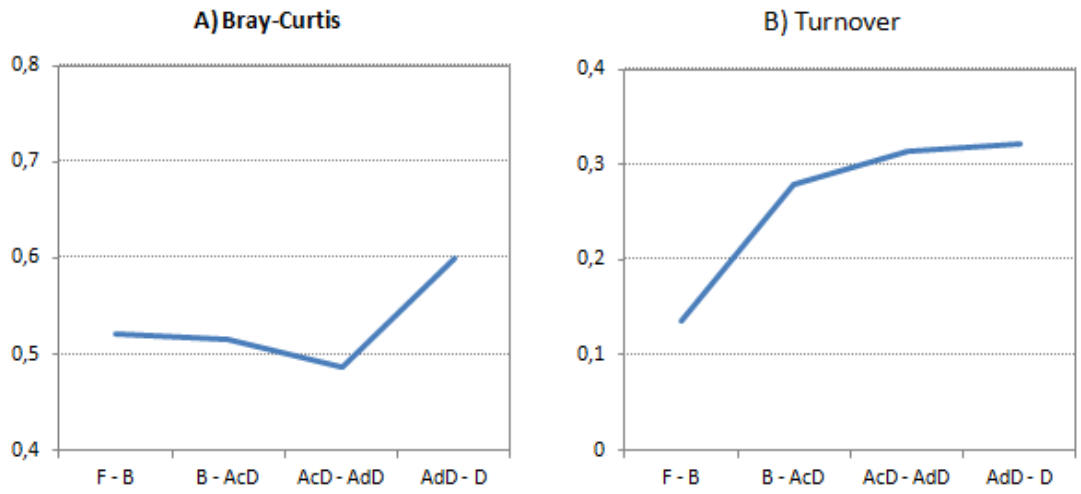


Figure 17: A) Bray-Curtis dissimilarity index shows differences between stages attending the abundance of the families represented on each. Zero values indicate that the two stages have the same composition, whereas 1 means they do not share any family. B) Turnover of families attending their presence/absence on stages. The lower it is the more similar the stages are.

◈ Discussion

The current study presents considerable information about arthropods of forensic interest. Each stage of decomposition is characterized by a particular group of organisms, each of which occupies a particular niche (Payne, 1965). However, in summer the process occurred so fast as temperate temperature and high humidity (an average of 16.7ºC and 86.9% relative humidity) favour

bacterial growing and larval development (ANTON *et al.*, 2011; HORENSTEIN *et al.*, 2012). This leads the dry stage to be the longest one, and therefore, the general trend for specimens was to be captured then as was also observed by ARNALDOS *et al.* (2004). Therefore, a gradual change in biological diversity (α diversity) was observed.

Insecta was found to be the most abundant class, being more than 97% of the total 7837 adult specimens collected (for further detail on their succession, see Chapter 6). This result is much greater than the 70-86% proposed by other authors (HORENSTEIN *et al.*, 2012; PAYNE, 1965; TANTAWI *et al.*, 1996), which could be related to different factors as for instance the kind of methodology employed. In fact, unlike this study, based on manual capture methods, HORENSTEIN *et al.* (2005, 2012) used a Schoenly trap, which allowed the continuous recollection of arthropods and, therefore, the collection of a great number of non-insect arthropods. Aditionally, PAYNE (1965) emphasized the need for animal model uniformity, which could be not as easy as it might expect. For instance, HORENSTEIN *et al.* (2005) used chicken carcasses; TANTAWI *et al.* (1996) used rabbits; PAYNE (1965) used small piglets of 1-1.4kg; HORENSTEIN *et al.* (2012) used piglets of about 8kg and current study used piglets of 8-12kg.

It is also important to remark that mites, which were previously reported as another dominant group (CASTILLO MIRALBÉS, 2002; HORENSTEIN *et al.*, 2012), remain already under revision and are just mentioned in this section, as there is evidence they provide forensically important information (PEROTTI, 2009; RASMY, 2011), and therefore are conserved for future research.

Among Insecta, the most abundant families belong to the orders Diptera and Coleoptera, both added up to 94.28% of the total necrophagous fauna found on carcasses, and are considered the most reliable forensic indicators usually found (BYRD & CASTNER, 2009b; GENNARD, 2007, 2012; PAYNE, 1965).

The amount of new records and new species collected (a total of 1105 adult specimens (14.1%) from 49 species, see Table 3) denotes the low level of knowledge of the carrion fauna in the area and gives us an idea of the necessity of increasing the effort dedicated to faunistic studies in this region. The finding of new records in this kind of studies is not unusual. For instance, PAYNE (1965)

reported 4 new species for science; CASTILLO MIRALBÉS (2002), found a new report for Spain (*Fannia leucosticta*: Fanniidae) and a new species for Science (*Pholioxenus castilloi*: Histeridae); and a field work in Coimbra reported also 25 species of Diptera and 5 species of Coleoptera new for continental Portugal (PRADO E CASTRO & GARCÍA, 2009 and 2010; PRADO E CASTRO *et al.*, 2009, 2010a, 2010b). To give a taste of the importance and diversity of new records here obtained, it is noticeable that just among Diptera (82% of the whole specimens collected, and one of the most studied necrophagous order) there were:

a) 2 new species for Science: *Crossopalpus* sp. n. (nr. *nigritellus* and *aeneus*) and *Drapetis* sp. n. (group *exilis*) (Diptera: Hybotidae).
b) 1 genus and 8 new species for the Iberian Peninsula.
c) 7 new species for Spain.
d) 1 new species for peninsular Spain.
e) 1 family, 11 genus and 28 species new for the Basque Country.
f) 1 family, 4 genus and 3 species new for Gipuzkoa.

For Coleoptera, a first record was reported for the species *Trox scaber* (Coleoptera: Trogidae) in the Basque Country Autonomous Region (C.A.P.V.). It is a small beetle associated with advanced stages of decomposition, due to its breeding habits over keratin-based remains. Nevertheless, to our knowledge this is the first research on Forensic Entomology to record this species feeding on carcass remains (DÍAZ MARTÍN & SALOÑA BORDAS, 2012).

Another first record for the Basque Country was *Ceratocombus coleoptratus*, a species of the family Ceratocombidae (Hemiptera: Heteroptera). Most species of this family are generalist predators of other small arthropods, but their role on cadaveric environments may be adventive or opportunist (PAGOLA-CARTÉ & DÍAZ, 2012).

In this study, carrion fauna has been found to be very diverse and quite variable, with up to 255 species listed. This is in line with previous studies performed in the Iberian Peninsula; for instance, CASTILLO MIRALBÉS (2002) reported 273 species in Alto Aragón; ARNALDOS *et al.* (2004) included a total of 208 species in southern Spain; and PRADO E CASTRO *et al.* (2012, 2013)

identified 71 species of dipterans and 82 species of coleopterans in Lisbon (Portugal).

Regarding succession process, general trends described on previous studies (CATTS & GOFF, 1992; PAYNE, 1965) have been confirmed. Fresh and Bloated stages are characterized by groups of arthropods that depend predominantly on the carrion as their direct source of food. Blowflies arrive within seconds to the corpse, and maggots take an important role in the carcass reduction (see Chapter 6 and 8 for further details). Hence, lowest values of Margalef index are recorded in Fresh and Bloated staged, and the community shows a small degree of dominance (Table 4).

Active and Advanced Decay stages represented an inflexion point between Fresh and Bloated stages and Dry stage. In the later one, the dominance became less influent and the diversity raised up. Additionally, the greatest value on Margalef index occurred on the late decay stage, concurring with the highest value of Simpson index. This means that very few of the species present during the first stages of decomposition remained until dry stage, as was previously reported by PAYNE (1965).

Decomposition stages were also analysed as a continuous process of changes in the community composition (β diversity, Figure 17), rather than as independent units, and similar conclusions have been obtained in comparison with previous studies (*cf.* ARNALDOS *et al.*, 2004). Changes from fresh to bloated stage occurred with minimal variations in the identity of the families collected, but with major changes in the abundance of each family. There were also noticeable changes in dominance, which was higher during the Fresh as compared to the Bloated stage. By contrast, family replacement was higher in latter stages, although the abundance of each one did not vary significantly (and so the dominance, which was much lower on the late stages).

Previous studies also included diversity indexes (ARNALDOS *et al.*, 2004; HORENSTEIN *et al.*, 2005; PRADO E CASTRO *et al.*, 2011a), although the use of different methodologies and/or sampling procedures prevents through comparison. Nevertheless, our results are in accordance with these studies, tending diversity to be higher in late stages of decomposition. Highest similarity

was reported by PRADO E CASTRO *et al.* (2011a), probably for being this research conducted in an area with Atlantic influence on climate. However, this study included only data of adults of Diptera community, so differences may be higher if all the community had been included (as for instance in ARNALDOS *et al.* (2004) or HORENSTEIN *et al.* (2005)).

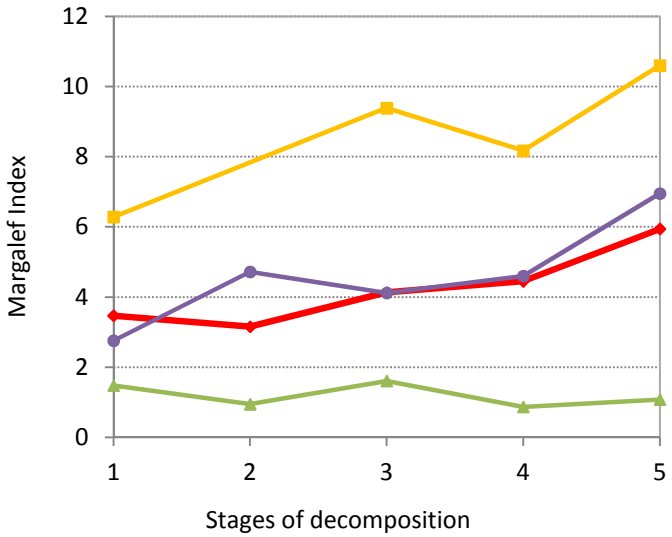


Figure 18: Margalef index values for current study (red line and diamonds) and those performed by ARNALDOS *et al.* (2004) (yellow line and square points), HORENSTEIN *et al.* (2005) (green line and triangles) and PRADO E CASTRO *et al.* (2011a) (purple line and round points).

Is it safe enough to choose the closest Weather Station as reference in Forensic Research?

An analysis of the effect of the distance to the crime scene in the final estimations of the post-mortem interval

... it is the care we bestow on apparently trifling, unattractive and very troublesome minutiae which determines the result.

Theobald Smith

◈ INTRODUCTION

Medico-criminal entomology focuses mainly on providing evidence of the amount of time that a corpse has been exposed to insects, which helps to estimate the post-mortem interval (PMI) (VILLET *et al.*, 2010). However, the decomposition of a carcass is a complex process driven primarily by biochemical reactions, bacterial activity, and necrophagous activity (CARTER *et al.*, 2007; MICHAUD & MOREAU, 2011). These factors are in turn dependent on environmental conditions, among which ambient temperature is known to be determinant (CAMPOBASSO *et al.*, 2001; MICHAUD & MOREAU, 2011). This thermal dependence, along with the knowledge of insect succession patterns and lifecycles, has allowed forensic entomologists to develop quantitative methods to estimate PMI by means of insect age estimated through the calculation of accumulated degree-days (ADD) (ARCHER, 2004; MEGYESI *et al.*, 2005).

Entomologist's estimation is based on a series of generally valid assumptions, which are susceptible to variance and can therefore skew the accuracy of the estimated PMI (CATTS, 1992). One of these sources of error is the effect of heat and humidity, as unfavourable temperature and humidity conditions can easily destroy eggs or lead them to a state of quiescence called diapause. On the other hand, higher temperatures favour egg hatching and accelerate maturation of larvae (CAMPOBASSO *et al.*, 2001).

Typically, in order to achieve an accurate PMI estimation, hourly temperature is recorded with data loggers placed for several days at a corpse discovery site and compared with the temperature recorded usually from the nearest weather station over the same interval (AMENDT *et al.*, 2007). In absence of a perfect relation, a partial remedy of interpolation between weather station and discovery site is usually applied (CATTS & HASKELL, 1991). This is normally done by means of a regression analysis, whose results are used to reconstruct temperature conditions and other key weather events of the discovery site in the days/weeks prior to discovery and removal of the corpse (ARCHER, 2004; SCALA & WALLACE, 2010).

Therefore, the nearest weather station is, implicitly, assumed to record the most similar data to the crime scene. However, even with a weather station close by, microhabitat conditions may differ considerably due to differences in vegetation cover, air drainage, slope exposure or any other factor that make both, site and station, incomparable (CATTS, 1992). Surprisingly, the appropriateness of using the nearest weather station remains largely untested as well as the impact it might have in PMI estimation (DABBS, 2010; CATTS, 1992). To the best of our knowledge, there are only two studies dealing with this matter. Archer (2004) measured the effect of differences between body discovery site and length of time after discovery of a corpse, and found that temperature records from the local weather station did not adequately correlate with the field data. Furthermore, DABBS (2010) used an anthropological framework to examine variations in PMI estimation arising from temperature data sets of different weather stations, demonstrating that the nearest weather station is not always the best model being inappropriate a blind use of its temperature records.

Therefore, in this chapter, effects of using the nearest weather station on PMI estimation will be tested, as well as how the distance between the weather station and the corpse discovery site correlates with estimation error.

◈ METHODS

Main data set was collected from 6th August 2009 to 29th October 2009, during the first year of the study of corpse-associated fauna.

Ambient temperature and humidity data from the study area were collected using two data logger (Escort RH iLog). One of them (referred to as DA hereafter) was placed in the middle of study area, on a tree near carcass number 2 (see Figure 13), at two meters above ground level. The second data-logger (DC, hereafter) was placed at 10 meters from DA in a more protected environment, taking advantage of the presence of an abandoned shelter where maggots were reared until adult emergence. Both dataloggers were programmed to register hourly ambient temperature. Daily average was used for posterior analyses, in order to apply the Single Sine Method for degree-day

calculation as described by REITER & GRASSBERGER (2002) (see also the formula proposed by MICHAUD & MOREAU (2011), described below). In situ values were compared with records from the three nearest weather stations (Jaizkibel (J), Oiartzun (O) and Añarbe (A)), obtained through the Basque Agency of Meteorology (EUSKALMET) (Figure 19).

Jaizkibel weather station (545 meters above sea level, UTM 30T 592554 4799722) is located on the top of Jaizkibel mountain, at a distance of 11,18 km from the sampling point. This mountain is included on the network NATURA 2000, and is considered the first coastal mountain of the northwest of Pyrenees. Its landscape has been strongly modified through human activities as forestry, grazing and recreational activities (LIZAUR SUKIA *et al.*, 1996).

Oiartzun weather station (53 meters above sea level, UTM 30T 590556 4795682) is placed at a distance of 7,14 km from the study site, on the banks of the Oiartzun river in the town of the same name. Surrounding areas include suburbial neighbourhoods, patched with small green areas and parks, and an industrial area.

Añarbe weather station (184 meters above sea level, UTM 30T 593537 4786631) is the nearest one to the research area (2,87 km). It is located close to the Añarbe reservoir, inside the “Aiako Harria” Natural Park. Due to the low human pressure, this area constitutes one of the most remarkable examples of beech and oak forest in the region (LIZAUR SUKIA *et al.*, 1996). For unknown reasons, data from this station from 22nd October 2009 to 10th December 2009 are not available. Therefore, data used for the comparison consisted on records from the above mentioned stations and the two dataloggers from 6th August 2009 to 22nd October 2009.

Figure 19: Aerial view of the eastern corner of Gipuzkoa, with the location of the study area (white rhombus) and the three nearest weather stations considered (white pins) (modified from Google Earth).

For all the stations, hourly temperature records were gathered from EUSKALMET database (http://www.euskalmet.euskadi.eus/), and daily average temperature was calculated following the same methodology than for the study site (see REITER & GRASSBERGER, 2002; MICHAUD & MOREAU, 2011).

To test differences between weather stations and datalogger records, we used the Paired Hotelling's test (Statistical software PAST, HAMMER *et al.*, 2001). This multivariate test is a particular case of Multiple Analysis of Variance (MANOVA) for paired designs, and tests against the null hypothesis of no differences between pairs of data. It looks for significant differences at least in one of the pairs (HAMMER *et al.*, 2001). Therefore, data were organized in pairs (DC-O; DC-J; DC-A; DC-DA; DA-O; DA-J; DA-A). In case of positive results, differences within each pair of data were specifically analysed with aid of the t-test for paired data, using the SPSS Statistics 20 package (IBM CORP. RELEASED, 2011). In cases of statistical significance in the tests, the effect size value (EF) was also calculated (COHEN, 1988). The EF gives an idea of the relevance and practical importance of the observed differences, allowing easier inference of their biological sense (MARTÍNEZ-GONZÁLEZ *et al.*, 2006). Three degrees or

categories of EF have been used: small (EF=0.2), medium (EF=0.5), and large (EF=0.8).

Finally, the accumulated error in PMI estimation incurred when choosing either weather station was estimated. For that purpose, standard procedures proposed by AMENDT *et al.* (2007) were applied.

In our case, the most abundant species reared in the field was *Chrysomya albiceps* (Wiedemann, 1819) (see Chapter 7). Cultures were kept inside the abandoned shelter near carcass number 1 (see Figure 13, Chapter 2), where the DC datalogger was placed. Hence, records from this datalogger and own field experimental data for *Chrysomya albiceps* development were used as reference values to measure the degree of the estimation error.

Therefore, a comparison between DC and DA temperature records and between DC and the three weather stations was made to obtain the equation relating them and the coefficient of their correlation. For this calculation, hourly temperatures were used instead of average day temperature, in order to obtain a more accurate relationship and to account not only for maxima and minima, but also to evaluate the effect of hourly variation among stations. Hence, r^2 values may differ from those obtained in the t-test (paired) analyses. Once the equations had been calculated, DA and the three weather stations temperature records were corrected to approximate their values to the temperatures recorded in the study area. However, both data sets, corrected temperature records and original ones, are presented to evaluate its effect on PMI estimation.

PMI was then estimated by means of ADD calculation, for being the most common method used (RICHARDS *et al.*, 2008), and following formula from MICHAUD & MOREAU (2011):

$$ADD_{thd} = [\{T_{min} + T_{max}\}/2] - thd$$

being $[\{T_{min} + T_{max}\}/2]$ is the average daily temperature, and *thd* is the threshold value (minimum temperature at which the development of a specific organism can be completed). *Ch. albiceps* was selected as reference species

(GRASSBERGER *et al.*, 2003; RICHARDS *et al.*, 2008), and we used 10.2ºC as lower development threshold (*cf.* MARCHENKO, 2001).

Negative values of degree-days were considered as zero. The effect of larval mass in carcass temperature was disregarded because, although it is well reported in forensic literature and recently efforts have been made to understand its origin and characterization (CHARABIDZE *et al.*, 2011), field conditions are rarely predictable (CATTS, 1992; REITER & GRASSBERGER, 2002) and there are no indications on how to include its effect.

◈ RESULTS

During the study period 2034 hourly temperature records were collected with each datalogger, as well as from each of the three weather stations (Oiartzun, Jaizkibel, Añarbe). Basic features of temperature recordings are summarised in Table 5.

Table 5: Descriptive summary of temperatures recorded at each station. DA: open-air datalogger; DC: sheltered datalogger. Temperature data are expressed in ºC.

	Oiartzun	Jaizkibel	Añarbe	DA	DC
Mean	19,9571	15,8955	16,8353	17,1032	17,5885
Standard Deviation	4,52278	4,02846	3,95410	3,90743	4,12557
Variance	20,456	16,229	15,635	15,268	17,020
Asymmetry	-,426	-,113	-,808	-,499	-,487
Typ. error of asymmetry	,272	,272	,272	,272	,272
Kurtosis	,547	,696	1,350	,873	,884
Typ. error of kurtosis	,538	,538	,538	,538	,538
Minimum value	7,10	4,30	4,70	5,20	5,10
Maximum value	30,85	25,25	25,40	25,95	26,85

Examination of the whole data with Paired Hotelling's test revealed significant differences ($p<0,001$). Therefore, each pair of data set was analysed using the t-test. Results of the t-tests, as well as the correlation values and effect size index for each pair are summarized in Table 6.

Table 6: Results from t-test (paired) application with its significance value, effect size and correlation degree (r^2). O: Oiartzun; J: Jaizkibel; A: Añarbe; DA: open-air datalogger; DC:

sheltered datalogger. Effect Size categories: small (EF=0.2), medium (EF=0.5), Large (EF=0.8), after COHEN (1988).

	Mean difference	95% confidence interval for difference		t	Sig. (bilateral)	Effect Size		Correlation r^2
		Inferior	Superior			Value	Category	
DC – DA	0,48526	0,28710	0,68341	4,876	<0,001	0,1176	Small	0,978
DC – O	-2,36859	-2,83846	-1,89872	-10,038	<0,001	-0,574	Large	0,888
DC – J	1,69295	1,36697	2,01893	10,341	<0,001	0,41	Medium	0,937
DC – A	0,75321	0,46599	1,04042	5,222	<0,001	0,1826	Small	0,951
DA – O	-2,85385	-3,27000	-2,43769	-13,655	<0,001	-0,73	Large	0,914
DA – J	1,20769	0,92888	1,48651	8,625	<0,001	0,309	Medium	0,952
DA – A	0,26795	0,03981	0,49608	2,339	0,022	0,0686	Small	0,967

Significant differences were found in all the pairs, even between dataloggers. However, temperatures recorded by both dataloggers were very similar despite different degree of shelter. In fact, although there were significant differences, the effect size was very small (0.1176) and they showed a high degree of correlation (97.8%). Interestingly, the smallest effect size was found for DA and the nearest weather station (Añarbe), even more similar than the DA and DC pair. Comparison with Jaizkibel also resulted in a high degree of correlation, but the effect size was greater. Finally, Oiartzun presented the lowest degree of correlation and the highest effect size, despite being closer to the study area than Jaizkibel.

As mentioned earlier, the sheltered datalogger (DC) was used as reference, so a regression was made with DC records and those from meteorological stations (Figure 20). With the following correlation equations, records from DA and weather stations were adjusted to study area temperatures, and ADD was then calculated.

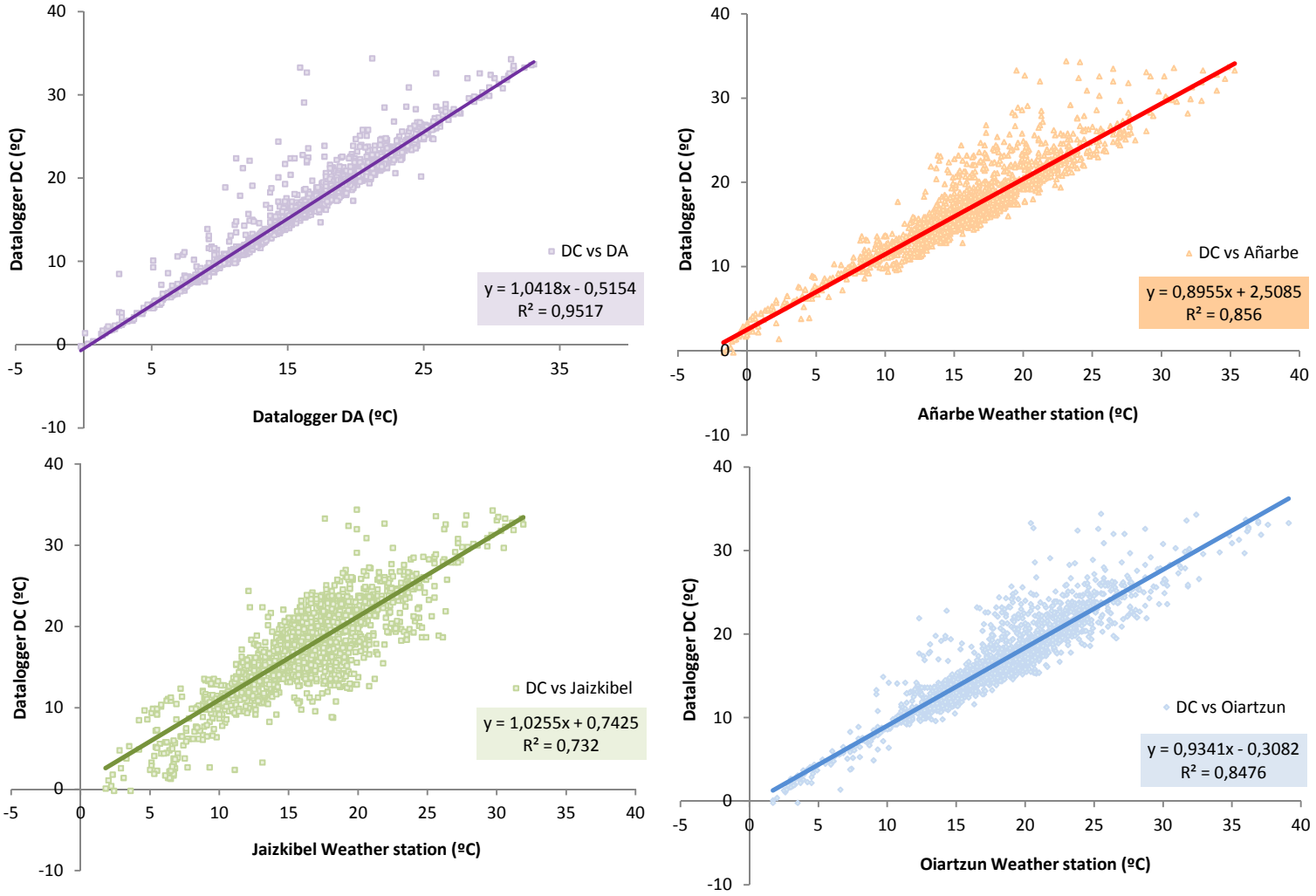


Figure 20: Correlation graphic between sheltered datalogger (DC) and different weather stations (Oiartzun, Jaizkibel, Añarbe); and between DC and the open-air datalogger (DA). Formula and r^2 values for each correlation are detailed.

To evaluate differences on PMI estimations, ADD values of *Chrysomya albiceps* larvae reared in the shelter were used. They completed their development after an average of 18 days at an average of 19.6ºC ± 4.2 ºC (see Chapter 7), which corresponds to 185.2 ADD according to DC temperature records. This value of 185.2 ADD was regarded as reference value and used to estimate larval age based on records from the three weather stations and DA datalogger. In order to assess the effectiveness of this temperature correction, the estimation was also done using uncorrected data.

In general, corrected temperatures yielded more accurate results (Table 7), whereas the use of uncorrected temperature data resulted in a bias of at least one day of infra/over estimation, depending on site temperatures being higher/lower than weather station records. Once again, the best accuracy was obtained using data from Añarbe, the nearest station. Oiartzun led to the worst time estimation even when temperature records were corrected, with a difference of up to 5 days to the reference emergence data; and Jaizkibel incurred in an error of 2 days.

Table 7: Fly emergence time estimation, with DC calculation as reference (185.2 ADD and 18 days for emergence of *Chrysomya albiceps* adults). Uncorrected temperature data correspond to estimations done using the original data from each station, whereas corrected temperature data refers to the estimation done after fitting the weather station data to the site temperatures (which are DC records for this hypothetical simulation).

	UNCORRECTED TEMPERATURES		CORRECTED TEMPERATURES	
WEATHER DATA SET	MINIMUM	MAXIMUM	MINIMUM	MAXIMUM
DA Datalogger	19d 20h 42’	20d 15h 44’	18d 4h 40’	19d 4h 34’
Añarbe	19d 10h 38’	20d 6h 58’	17d 20h 9’	18d 19h 58’
Jaizkibel	24d 6h 52’	25d 4h 54’	20d 8h 3’	21d 10h 12’
Oiartzun	11d 15h 32’	12d 18h 40’	13d 5h 59’	14d 4h 28’

.

◈ DISCUSSION

Comparison of daily average temperatures of research area with the three closest weather stations selected has shown significant differences,

independently of the distance between site and station. Absolute differences between means range from 0.27ºC to 2.85ºC. A possible explanation may lay on factors that can produce modifications on microhabitat conditions, such as topography, cover, air drainage, etc (CATTS, 1992), whose analyses may be of interest on future works.

An accurate record of environmental conditions during the post-mortem period is a key element to estimate the age of corpse-associated maggots (CATTS & GOFF, 1992) and, therefore, a minimum PMI. Weather stations usually provide detailed records of ambient conditions, but rarely one can be found nearby the place where a corpse has been found. At first sight, results presented here are in accordance with the hypothesis that the farther the station is, the greater the error is committed when estimating a PMI based on the ADDs accumulated by the oldest insect collected on the carcass

According to correlation values of daily mean temperatures recorded (Table 6), the least error has been incurred using data from the nearest weather station, Añarbe. The use of hourly records (Figure 20) yielded similar results, reinforcing the validity of the nearest station. Notwithstanding, correlation values obtained with hourly records were somewhat diminished when comparing with daily records. This is logical as just one data per day were used in the daily approach, whereas 24 data per day were used in the hourly approach in order to get the most accurate correlation for PMI calculation. As REITER & GRASSBERGER (2002) indicated, most methods are adequate considering the accuracy of weather instruments used and the difficult inherent to the calculation of degree-days for daily temperature fluctuations than occur in nature. However, if hourly temperature data are available, then degree-hours can be calculated.

These results support the standard procedures for Forensic Entomology (AMENDT *et al.*, 2007), suggesting to use data from the nearest station. However, there may be times in which the closest station may not reflect similar conditions than the crime scene or is not available due to malfunction, breakage or any other causes (as it happened when recording temperatures for this experiment), or plainly the nearest station could be a considerably far-away

one. Therefore, different weather stations need to be analysed, and it is recommendable to consider the margin of error when doing the final estimation. In this scenario, opposing results were obtained for Oiartzun and Jaizkibel stations that advised caution. Using hourly temperatures (Figure 20), the best station second to Añarbe, was Oiartzun, as could be expected from distance to study site. However, using daily average temperatures, Oiartzun showed the lowest correlation value whereas the performance of Jaizkibel improved markedly.

Inspection of the effect sizes showed that the importance of deviation in results obtained from the Oiartzun data set was greater than that obtained for the Jaizkibel data set, despite being the second one farther from the study site. Therefore, this latter station would resemble more the site temperature, and the error assumed on following estimations would also be lower than that for Oiartzun. This result could be due to the fact that Jaizkibel has a similar environment (mountain habitat and similar altitude), whereas Oiartzun station is located at a lower zone surrounded by urban areas which can increase the average temperature.

Regarding ADD calculation, results support previous findings, as calculations made using data from Añarbe are the closest to those made using temperatures recorded at the experimental site. Again, Oiartzun, despite being closer to the study site than Jaizkibel, performed worse (Figure 20). This is probably due to environmental characteristics of the station locations. As ARCHER (2004) reported, a greater error may result from using uncorrected temperatures (Table 7).

As confirmed by results, thermal differences between the discovery site and nearby weather stations are likely to be variable (ARCHER, 2004) and may affect PMI estimation. Differences can be due to different geographic characteristics of the area (altitude, sun exposure, etc.), vegetation of the surroundings or other characteristics. Additionally, microhabitat conditions of each site and carcass may differ also in time (trees or grass can be cut down, buildings constructed, etc), producing additional variation in ambient temperatures that cannot be explained by simple correlation (ARCHER, 2004). That is why we strongly

recommend the inclusion of effect size, together with regression procedures, for choosing the best weather station on each case. Including the effect of the maggot mass temperature in the calculation might improve estimations, but this relationship remains poorly understood and difficult to implement (CHARABIDZE *et al.*, 2011; GENNARD, 2007).

Although much progress has been made on detecting and describing the effect of confounding variables that may impact on the accuracy of the estimations (CATTS & GOFF, 1992; VILLET *et al.*, 2010), in concordance with ARCHER (2004), further work of this nature would aid Forensic Entomology in gaining objectivity and judicial acceptance, and allowing implementation of quality control.

The analysis of the Effect Size of differences regarding temperature records was found to be a useful tool for objectively choosing a Weather Station, regardless of distance to the crime scene. It would be also interesting to go deeply into the study of microhabitat conditions of Weather Stations and their inference on PMI estimations, as well as increasing the number of Stations compared.

Carrion-Related Arthropod Succession

The timing of death, like the ending of a story,
gives a changed meaning to what preceded it.

Mary Catherine Bateson

◈ Introduction

Basic descriptive knowledge of the ecological processes and its regional characteristics is useful to understand variations and design any experimental research (Matuszewski *et al.*, 2010a). In the case of cadaveric fauna, these patterns are of outmost importance to assess information collected in forensic research. Since Megnin's first description of succession waves, forensic entomologists have aimed to create proper models of arthropod succession in cadavers worldwide (Castillo Miralbés, 2002; García Rojo, 2004; Grassberger & Frank, 2004; Payne, 1965; Prado e Castro *et al.*, 2012; Richards & Goff, 1997; among others), often generating large inventories of taxa (Matuszewski *et al.*, 2010b). Within those inventories, a researcher decides which taxa would be interesting and useful to create a ground model for forensic purposes, as many species frequenting carcasses may not contribute to the main objective of the research and can be considered of no forensic interest (*cf.* Matuszewski *et al.*, 2010c). That is why the identification and use of entomological indicators in a succession-based PMI estimation promises to be a reliable technique in different periods of decomposition (Schoenly *et al.*, 1992). However, the use of a particular "baseline fauna" is usually restricted to the area in which it was collected, as species composition and its phenology vary among biogeographical regions (Arnaldos *et al.*, 2006; Prado e Castro *et al.*, 2012).

It is remarkable that we still lack an adequate model of succession that could be considered as reliable for the PMI estimation for most of the biogeographical areas of Europe and its typical habitats (Matuszewski *et al.*, 2008). Furthermore, in the Iberian Peninsula, scarce works have been done to specifically create a model of arthropod succession in cadavers. Works conducted hitherto have been focused on areas of the peninsula with Continental or Mediterranean influence (Arnaldos *et al.*, 2004; Castillo Miralbés, 2002; García Rojo, 2004; Prado e Castro *et al.*, 2012), while the northern maritime facade lacks the necessary background to create a useful model for its particular orography and climatic conditions.

Succession models have been typically associated to stages of decomposition attending to physical and chemical characteristics of the decaying matter, being afterwards used to describe the arthropod community present at each (SCHOENLY & REID, 1987). However, this has raised much dispute, since the distinction of the ending of each stage and the beginning of the next one can be somewhat open to subjectivity. According to MOURA *et al.* (2005), the recognition of resource utilization patterns and the association of some species with specific decomposition stages could be the reasons why several researchers consider the process as discrete. However, a discrete approach ignores the continuous nature of decomposition as well as the fact that each carcass usually represents a mosaic of features of different stages (MATUSZEWSKI *et al.*, 2010a).

It is, therefore, the aim of this research to 1) analyse the discrete or continuum nature of the process according to the arthropod community, 2) identify the taxa to be included in the succession model, while keeping in mind the forensic usefulness of the species involved to identify possible forensic bioindicators, and, 3) contribute to increasing the knowledge of carrion-related community composition and successional patterns in a forest of northern Spain.

◈ MATERIALS AND METHODS

This chapter will focus only on adults of the species collected. As a brief reminder, the collection of samples took place during the summer months of 2009 and 2010 (from 6th August 2009 to 29th October 2009; and from 27th July 2010 to 22nd October 2010), in outdoor unused premises in the Añarbe forest (Aiako Harria Natural Park) closed to public. The study site was a small glade in a forested area, with high density of shrubbery, brambles and ferns. Climate characteristics are these of the Eurosiberian region, with mild temperatures (an average of 12.5ºC in the last 15 years) and abundant rainfall (an average of 2061.7 mm in the last 15 years) (data from Añarbe Weather Station, obtained on the website (http://www4.gipuzkoa.net/).

For further information about study area, carcass emplacement and sampling procedures, see the detailed description provided on “Site Description and General Methodology” (Chapter 3).

All the statistical procedures were run with the statistical software PAST (HAMMER *et al.*, 2001), and significance was set at α= 5%.

CLIMATIC DATA

Following the standards and guidelines proposed by AMENDT *et al.* (2007), microclimatic conditions were summarily characterized. In order to do so, daily temperature data were collected with a datalogger Escort RH iLog placed on a tree in the centre of study area, and daily rainfall data was recorded with a rain gauge (Figure 14; Chapter 3). In the same way, both temperature and rainfall data recorded by the nearest weather station (Añarbe; 2.873 meters to the study area) were also considered. Data were obtained from the EUSKAL METEOROLOGIA AGENTZIA – EUSKALMET (http://www.euskalmet.euskadi.eus/) (See also Chapter 5 for information about the appropriateness of this station for using it for forensic purposes in this area).

DECOMPOSITION PROCESS

It is a common practice in carrion studies to combine data from replicate carcasses into a single succession diagram, producing a cumulative portrait of carrion-arthropod succession for a given location and time of year (SCHOENLY, 1992). In spite of that, each carcass and its decomposition and successional patterns were considered separately, in order to avoid confounding problems in the interpretation, and taking into account the potential variability among carcasses even within a single site and/or season, as for instance substantial variations in biotic and abiotic conditions, as well as in the duration of decomposition processes within the same season.

- STAGES OF DECOMPOSITION

Every carcass' sampling period was divided into the five stages of decomposition usually considered (fresh, bloated, active decay, advanced decay and dry). As previous experiments reported that the head usually decomposed faster than the trunk (ANTON *et al.*, 2011), only characteristics of the trunk were taken into account to determine the phase of decomposition of each carcass and each day (being the first day of experiment (D1) when pigs were euthanized and deployed on the study area).

The main idea behind the use of clear-cut stages of decomposition is to facilitate the description of the process. However, as stated before, the discrete or continuum nature of the process is a controversial matter and has also been analysed. In order to do that, we hypothesized that in the case of a discrete process more similar species composition within a particular phase of decomposition than between decomposition stages could be expected (MOURA *et al.*, 2005; QUINN & KEOUGH, 2002). Therefore, in order to test if sampling days of the same decomposition stage appeared together in the cluster due to their arthropod community, a cluster analysis using unweighted pair-group average method (UPGMA) was performed using days of sampling as objects and species or higher taxa as variables. Complementarily, arthropods could appear in the carcass as a result of the development of new niches, so a second cluster was constructed using species as objects to test for the structured presence of guilds on carcasses. Clustering method starts creating a matrix of pairwise similarities or dissimilarities between the objects of the occurrence matrix (QUINN & KEOUGH, 2002), and represents them on a cluster diagram or dendrogram. The Morisita–Horn similarity index was selected for its broad use and suitability to the data, as it is not influenced by species richness and sample size (*cf.* MAGURRAN, 2004). To reduce error attributable to particular conditions of each carcass succession pattern, taxa that appeared either accidentally or occasionally in one or few carcasses were discarded from these analyses. Therefore, taxa that presented significant differences between carcasses in the study area (see the column Carcass location on Table 9) were discarded from ulterior analyses.

- CARRION – RELATED COMMUNITY

A good succession model for forensic purposes might discard those species whose presence in carrion is unpredictable. Hence, effects of time and location (by means of year of experiment and location of carcasses respectively) were firstly analysed. For this purpose, an occurrence matrix was constructed with those taxa minimally abundant. The minimal abundance was arbitrarily established at 30 individuals; teneral adults and preimaginal instars were not included in the analysis. The null hypothesis (H_0) considered factors not to have an influence on the arthropod community, and was tested using the χ^2 test. As sample size was very large and there were too many categories, Monte Carlo permutation test was run to obtain a confidence interval to the p-value of the χ^2 test, by using 10.000 randomizations as it is recommended for biological studies, especially when dealing with unequal sample sizes and/or small sample sizes for some groups and multiple, nested categories (MANLY 2006). This is the most common form of categorical data analysis, especially with counts or frequencies of units (QUINN & KEOUGH, 2002; SCHOENLY, 1992). The analyses were run with SPSS 20.0.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.).

In the same way, species with a very long presence period should be also discarded. Therefore, an additional χ^2 with Monte Carlo simulation was run to test the H_0 of that species are equally distributed during the whole process. Species that have shown significant differences for carcass location were excluded from this analysis. Species that have shown significant temporal variation among years on previous χ^2 were included, although they migth be taken into account with caution.

To ease the identification of representative species that could be useful in forensic investigations in our region, the percentage of each species per stage of decomposition and year of study were obtained.

Finally, a succession pattern was made for each carcass considering only species detected over 30 individuals. Less abundant species were included together as “Other species” in each arthropod family. Additionally, to facilitate comparison of this study with those from other areas, other forensically

important taxa such as Sarcophagidae, Cleridae and Dermestidae were also considered although they were less abundant, due to its close association to carcass environment in previous works worldwide (ROMERA *et al.*, 2003; SCHROEDER *et al.*, 2002). Each of these cases will be further explained later.

9 FORENSIC BIOINDICATORS

The potential usefulness of every species as forensic indicators was determined by analyzing their features of residency as described in MATUSZEWSKI *et al.* (2010b), which refers to the period of time that a taxon stays in a corpse, taking into account if it is continuous or have breaks (days without presence before appearing again on the carcass). According to their procedures, species/taxa with less than ten individuals per carcass were discarded from the analyses, so that patterns of residency were analysed only for 13 species (Table 11). Length of the presence period (LPP), number of breaks in the presence period, and length of the longest unbroken period were determined for each of them. The significance of the relationship between the appearance time of a given taxon and the onset of bloating was also evaluated by means of Pearson product-moment correlation.

MATUSZEWSKI *et al.* (2010b) stated that, from these parameters, the most important in determining the forensic usefulness of a taxon are the length of presence period and regularity of appearance in a particular moment of decomposition. At the light of both features, they suggested the use of the average relative length of the presence period (ARLPP) of a given species as an indicator of usefulness. The ARLPP is expressed as the percentage of days of the sampling interval in which a given species or taxon was found. After it, MATUSZEWSKI *et al.* (2010b) proposed 5 categories to summarise the results:

- Taxa of no usefulness: ARLPP>80 or no significant relationship between appearance time of a taxon and onset of a given decompositional period.
- Taxa of low usefulness: ARLPP (60-80) or significant, weak (r <0.5), positive relationship between appearance time of a taxon and onset of a given decompositional period.

- Taxa of moderate usefulness: ARLPP (40-60) or significant, moderate (0.5<r>0.8), positive relationship between appearance time of a taxon and onset of a given decompositional period.
- Taxa of high usefulness: ARLPP ≤40 or significant, strong (r ≥0.8), positive relationship between appearance time of a taxon and onset of a given decompositional period.
- Taxa of unknown usefulness: those for which it was impossible to analyse patterns of residency or significance of relationship between appearance time of a taxon and onset of a given decompositional period.

◈ RESULTS

9 CLIMATIC DATA

Ombrothermic conditions of the research area are detailed in Figure 21. Both years showed the typical temperature fluctuation pattern of an Atlantic region, with an average of 16.7ºC and 17.1ºC respectively in 2009 and 2010 for summer months (August to October). It is worth noting that 2009 was rainier than 2010, even to double the rate of precipitation on certain dates. This is an important fact, as rainy conditions during the first days of the experiment in 2009 directly affected the access to the body and, therefore, delayed most of the decay processes (Figure 21; Table 8).

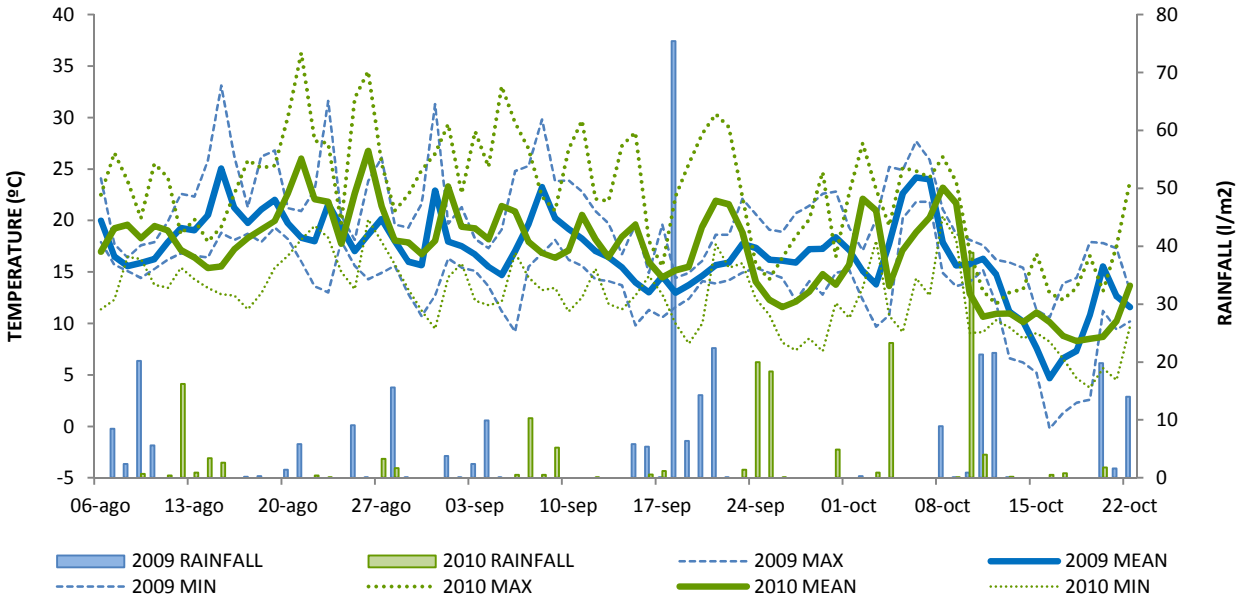


Figure 21: Daily temperature recorded by the datalogger placed in the sampling area and rainfall data from the Añarbe Weather Station. Each year is represented by one colour: 2009: blue lines; 2010: green lines. Rainfall is represented in l/m^2 by bar diagram, whereas temperature, in Celsius degrees, is represented by lines (solid line for average temperature, discontinuous thin line for minimum temperature, and discontinuous bigger line for maximum temperature).

9 DECOMPOSITION PROCESS

▪ STAGES OF DECOMPOSITION

Duration of decomposition stages was very similar in all carcasses, being worth noting the absence of bloating appearance in carcasses C4 and C5 during the trial of 2010, and the long duration of advanced decay in C3 in both summers. Table 8 summarizes their main characteristics and duration in carcasses from both years; 2009 and 2010.

Table 8: Decomposition stages: definition based on GENNARD, 2012; PAYNE, 1965; and duration (in days). White lines correspond to the experiment of 2009, and grey ones to 2010. C1 to C5 are the code names of carcasses. (n.a.: not appreciated).

Stage	Definition	Days post-mortem				
		C1	C2	C3	C4	C5
Fresh (F)	The body preserves its typical appearance.	1-3	1-3	1-3	1-3	1-2
		1-2	1-3	1-4	1-4	1-4
Bloated	Inflation of the abdomen.	4-5	4	4-5	4-6	3-5

(B)		3-4	4-5	5	n.a.	n.a.
Active Decay (AcD)	Body deflation and strong odour. Skin detachment. Active feeding of dipterans' larval masses.	6-7	5-9	6-8	7-9	6-8
		5-7	6-7	6-7	5-8	5-6
Advanced Decay (AdD)	Migration of dipterans' larval masses. Remains of the body are skin, cartilage and bones with some remnants of flesh.	8	10-13	9-32	10-18	9-13
		8-9	8-11	8-29	9-18	7-13
Dry (D)	There are only hair, dry skin and bones.	9-85	15-85	36-85	21-85	15-85
		11-88	13-85	34-88	21-88	15-88

When testing for the continuity of the different stages, cluster analysis showed that it was not possible to establish any clear boundary between them, and sampling days assigned to each stage were not uniformly grouped together (Figure 22).

Examining Figure 22, there was no consistence for the discrete stage approach, as sampling days were not ordered chronologically or grouped in separated branches of the cluster. From bottom to top, the last branches created small clusters with just few days in each one. These groups of days were assigned in the field to dry stage, which leads to the conclusion that a high degree of variability occurs in the community present at this stage. In the fifth subdivision of the clusters, there was a group of sampling days from active and advanced decay stages. The final group of branches contained three different classes of sampling days: one for early stages (fresh and bloated), another for mid-late stages (active and advanced decay), and a third one for remaining dry stage's days.

Therefore, sampling days of early stages (fresh and bloated) were grouped together, as well as those of active and advanced decay, which were divided in two groups. Dry stage is the exception, being distributed along all the groups formed in the cluster.

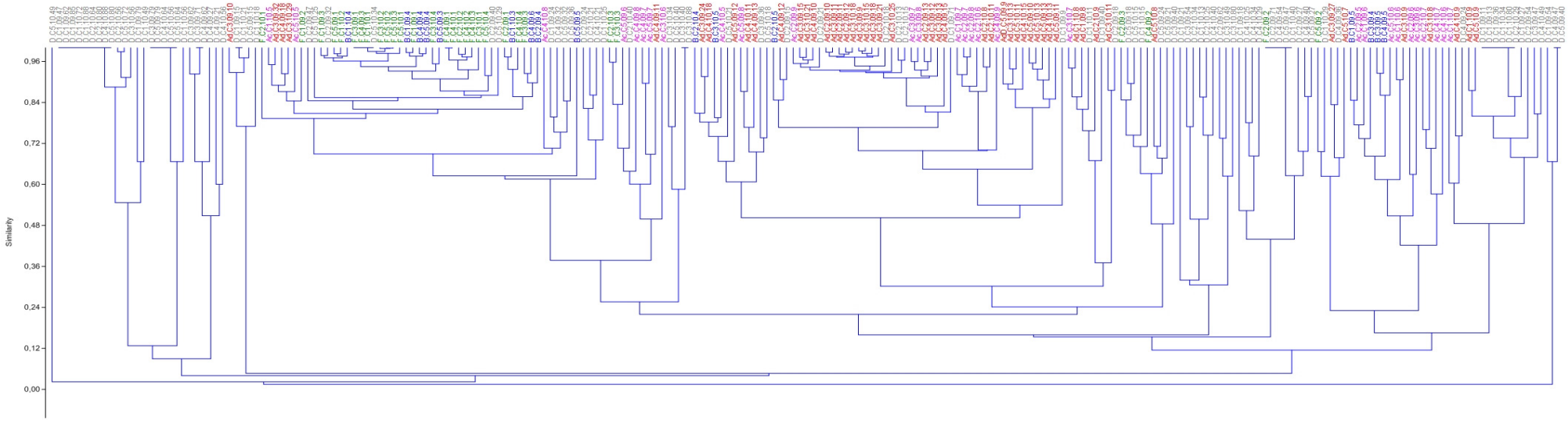


Figure 22: Cluster analysis showing the distance (similarities) between sampling days. Each colour corresponds to one stage of decomposition (Fresh: green; Bloated: blue; Active Decay: pink; Advanced Decay: red; Dry: grey).

When examining the cluster of similarities between species (Figure 23), several groups can be recognized attending to the niche they occupy. Using the average percentage of both years of study, *Lucilia caesar* and *Musca autumnalis* were found to be more abundant on fresh stage; they were also grouped together in cluster analysis (Figure 23, Table 10). Another group was made up with species that were more likely to appear at the end of the decomposition process: *Nemopoda nitidula*, *Parapiophila vulgaris*, *Spelobia luteilabris* and *Spelobia clunipes*. The remaining species were organised in various groups, in an apparently random pattern.

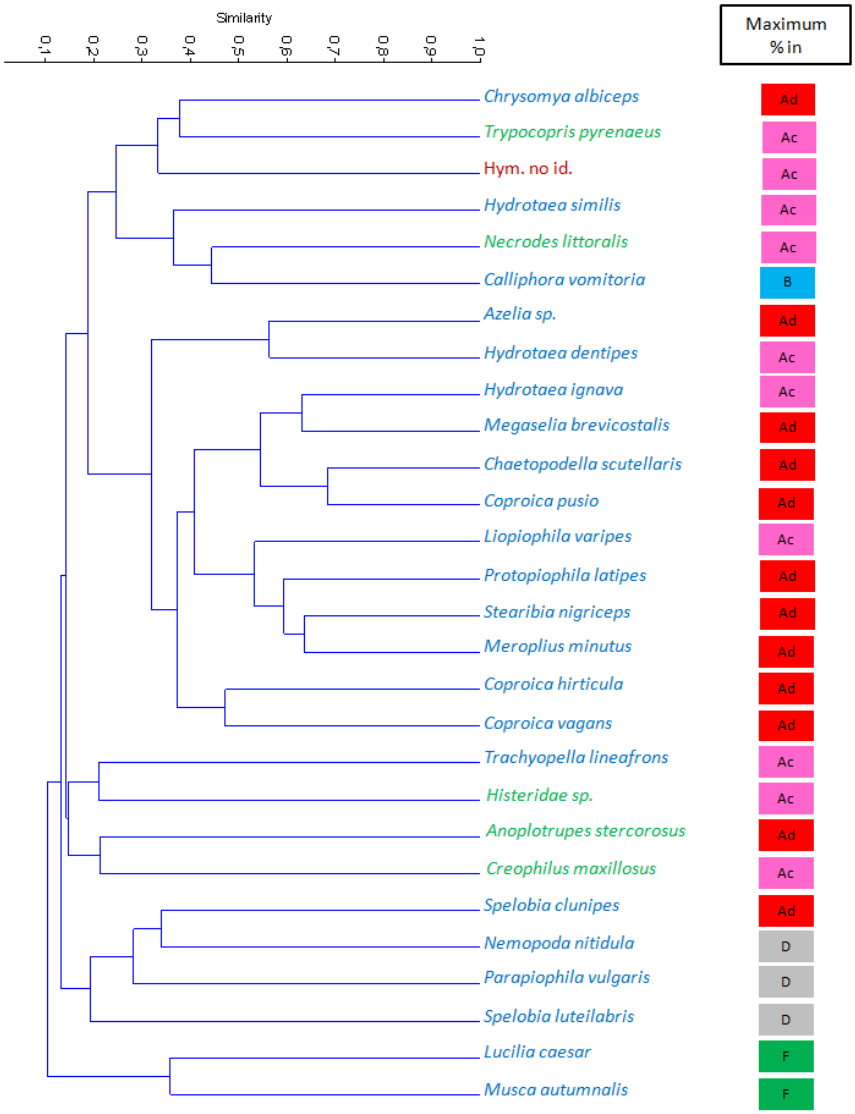


Figure 23: Cluster analysis of similarities between species. Colour of the species' name indicates the order (Diptera: blue; Coleoptera: green; Hymenoptera: brown). The stage of decomposition when their adults reach the maximum average percentage is also included (Fresh: F, green; Bloated: B, blue; Active Decay: Ac, pink; Advanced Decay: Ad, red; Dry: D, grey).

- CARRION – RELATED ARTHROPODS

A detailed checklist of the species collected, as well as the description of the identification procedure, can be found on Chapter 4.

In a first step, to decide which species could be a potential forensic indicator and should be included in the model, it was analysed the effect that time and location had on them. The χ^2 test (Table 9) detected 8 species not equally distributed among carcasses: *Fannia sp., Fannia manicata, Leptocera caenosa, Opalimosina liliputana, Telomerina levifrons, Scatopsciara multispina, Bisnius fimetarius* and non identified Formicidae. 11 species showed differences in temporal distribution: *Calliphora vomitoria, Musca autumnalis, Fannia sp., Fannia manicata, Coproica hirticula, Leptocera caenosa, Opalimosina liliputana, Scatopsciara multispina, Necrodes littoralis,* and non identified Histeridae and Formicidae. Of these, 6 were significantly affected by both the time and location: *Fannia sp., Fannia manicata, Leptocera caenosa, Opalimosina liliputana, Scatopsciara multispina* and non identified Formicidae.

Table 9: χ^2 test for location ("Pig" column) and time ("Year" column). It has shown the p value of Monte Carlo simulation after 10.000 randomizations, and the 95% Confidence Interval. Significant results are highlighted. Statistical differences indicate that abundance of a given taxon was significantly different among pigs or years. ($p<0.05$).

		PIG			YEAR		
		p	Lower Limit	Upper Limit	p	Lower Limit	Upper Limit
CALLIPHORIDAE	***Calliphora vomitoria***	0,104	0,098	0,11	<0,001	<0,001	0,001
	Lucilia caesar	0,281	0,272	0,29	0,095	0,089	0,101
	Chrysomya albiceps	0,08	0,074	0,085	0,149	0,142	0,156
MUSCIDAE	***Azelia* sp.**	0,075	0,07	0,08	0,174	0,166	0,181
	Hydrotaea dentipes	0,168	0,161	0,175	0,123	0,116	0,129
	Hydrotaea ignava	0,189	0,181	0,196	0,052	0,048	0,056
	Hydrotaea similis	0,334	0,325	0,343	0,704	0,695	0,713
	Musca autumnalis	0,121	0,115	0,128	0,024	0,021	0,027
FANNIIDAE	***Fannia* sp.**	0,04	0,036	0,043	<0,001	<0,001	<0,001
	Fannia manicata	0,039	0,035	0,043	0,026	0,023	0,03
SPHAEROCERIDAE	***Chaetopodella scutellaris***	0,158	0,151	0,165	0,08	0,074	0,085
	Coproica hirticula	0,058	0,053	0,062	0,041	0,037	0,045
	Coproica pusio	0,391	0,381	0,4	0,159	0,152	0,166
	Coproica vagans	0,181	0,173	0,188	0,179	0,171	0,186
	Leptocera caenosa	0,004	0,003	0,005	0,001	0,001	0,002

	Opalimosina liliputana	0,008	0,006	0,01	<0,001	<0,001	<0,001
	Spelobia clunipes	0,964	0,96	0,967	0,187	0,179	0,194
	Spelobia luteilabris	0,374	0,364	0,383	0,085	0,08	0,091
	Telomerina levifrons	0,026	0,023	0,029	0,059	0,055	0,064
	Trachyopella lineafrons	0,066	0,061	0,071	0,415	0,405	0,424
PIOPHILIDAE	***Liopiophila varipes***	0,7	0,691	0,709	0,268	0,259	0,277
	Parapiophila vulgaris	0,331	0,322	0,341	0,888	0,882	0,894
	Protopiophila latipes	0,065	0,06	0,07	0,077	0,071	0,082
	Stearibia nigriceps	0,295	0,286	0,304	0,439	0,429	0,448
SEPSIDAE	***Meroplius minutus***	0,633	0,624	0,643	0,347	0,337	0,356
	Nemopoda nitidula	0,168	0,16	0,175	0,19	0,183	0,198
PHORIDAE	***Megaselia brevicostalis***	0,656	0,646	0,665	0,402	0,393	0,412
SCIARIDAE	***Scatopsciara multispina***	0,009	0,008	0,011	<0,001	<0,001	<0,001
GEOTRUPIDAE	***Anoplotrupes stercorosus***	0,059	0,054	0,063	0,088	0,082	0,094
	Trypocopris pyrenaeus	0,683	0,674	0,692	0,548	0,539	0,558
SILPHIDAE	***Necrodes littoralis***	0,204	0,196	0,211	0,022	0,019	0,025
HISTERIDAE	**Unidentified Histeridae**	0,081	0,076	0,087	0,04	0,036	0,044
STAPHYLINIDAE	***Bisnius fimetarius***	0,003	0,002	0,004	0,392	0,382	0,401
	Creophilus maxillosus	0,605	0,595	0,614	0,666	0,656	0,675
HYMENOPTERA	**Unidentified wasps**	0,994	0,992	0,996	0,149	0,142	0,156
	Unidentified Formicidae	0,004	0,003	0,005	<0,001	<0,001	<0,001

As explained before, species not equally distributed among carcasses were excluded from following analyses due to their unsuitability for creating a standard baseline pattern of carrion-related arthropod succession. Additionally, species with temporal variations were considered on subsequent analyses, although they require a special caution and have been evaluated individually.

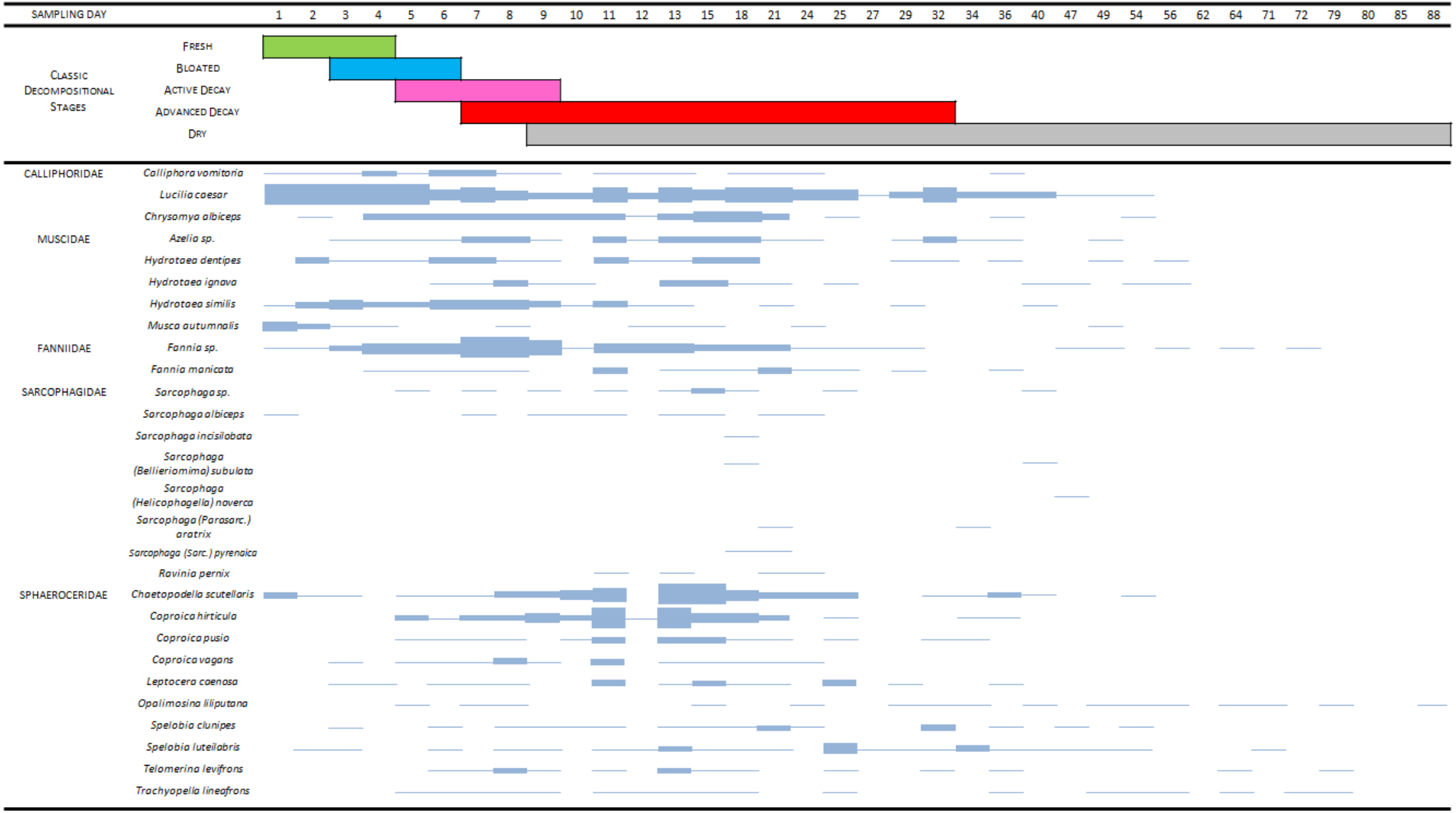
With this in mind, the 28 species that met the criteria to be considered as potential forensic indicator were tested with a second χ^2 test in order to detect those with a very long presence period. Eleven out of that 28 species analysed were found to be equally distributed among the different stages of decomposition (Table 10), being less useful as potential succession indicators due to the negative correlation of the length of presence period and the accuracy of PMI estimation (MATUSZEWSKI *et al.*, 2010b). Remaining 17 species had shorter presence periods or they showed peaks of abundance in a particular stage, giving a more precise PMI approach.

Table 10: χ^2 test for temporal distribution of species and their percentage among stages of decomposition (namely F: Fresh; B: Bloated; Ac: Active Decay; Ad: Advanced Decay; and D: Dry). It has shown the p value of Monte Carlo simulation after 10.000 randomizations, and the 95% Confidence Interval. Significant results are highlighted, indicating those species whose adults were more abundant in one or more stages of decomposition and, therefore, are potential forensic indicators. ($p<0.05$).

		MONTE CARLO			Percentage (%)					
		p	Lower Limit	Upper Limit	Year	F	B	Ac	Ad	D
CALLIPHORIDAE	***Calliphora vomitoria***	<0,001	<0,001	<0,001	**2009**	0,36	1,55	1,44	0,41	0,02
					2010	0,24	0	0,08	0,17	0,02
	Lucilia caesar	<0,001	<0,001	<0,001	**2009**	4,93	5,18	3,00	2,93	1,42
					2010	20,12	8,60	4,08	3,88	1,05
	Chrysomya albiceps	0,015	0,013	0,018	**2009**	0,00	0,36	2,13	1,85	0,37
					2010	0,35	1,20	0,08	0,75	0,24
MUSCIDAE	***Azelia* sp.**	0,373	0,363	0,382	**2009**	0,00	0,09	0,75	0,22	0,18
					2010	0,12	0,00	0,31	1,33	0,29
	Hydrotaea dentipes	0,011	0,009	0,012	**2009**	0,00	0,18	0,50	0,19	0,07
					2010	0,47	0,40	0,62	0,79	0,14
	Hydrotaea ignava	0,005	0,003	0,006	**2009**	0,00	0,00	0,63	0,30	0,12
					2010	0,00	0,00	0,38	0,71	0,10
	Hydrotaea similis	<0,001	<0,001	<0,001	**2009**	0,43	0,73	3,13	0,48	0,04
					2010	2,65	1,40	1,08	0,42	0,03
	Musca autumnalis	0,01	0,008	0,012	**2009**	0,14	0,09	0,13	0,22	0
					2010	2,47	0,20	0	0	0,07
SPHAEROCERIDAE	***Chaetopodella scutellaris***	0,493	0,483	0,503	**2009**	0,36	0	0,69	1,74	0,68
					2010	0,53	0,80	0,08	7,00	1,09
	Coproica hirticula	0,003	0,002	0,004	**2009**	0	0	0,69	1,19	0,35
					2010	0	0,60	0,92	6,21	0,57
	Coproica pusio	0,097	0,092	0,103	**2009**	0	0	0,06	0,37	0,05
					2010	0	0,20	0,08	0,58	0,19
	Coproica vagans	0,268	0,26	0,277	**2009**	0,07	0,00	0,13	0,11	0,02
					2010	0	0,20	0,15	0,75	0,12
	Spelobia clunipes	0,447	0,437	0,457	**2009**	0,07	0	0,13	0,26	0,30
					2010	0	0	0,08	0,25	0,05
	Spelobia luteilabris	0,875	0,868	0,881	**2009**	0,14	0,09	0,19	0,11	0,25
					2010	0	0	0,08	0,33	0,64
	Trachyopella lineafrons	0,121	0,115	0,127	**2009**	0	0	0,19	0,26	0,12
					2010	0	0	0,31	0,13	0,24
PIOPHILIDAE	***Liopiophila varipes***	<0,001	<0,001	<0,001	**2009**	0,07	0,36	5,56	2,56	0,18
					2010	0,59	3,00	4,15	6,21	0,69
	Parapiophila vulgaris	0,393	0,384	0,403	**2009**	0	0	0,06	0,11	0,33
					2010	0	0	0	0,21	0,34
	Protopiophila latipes	0,001	<0,001	0,001	**2009**	0	0	0,44	1,37	0,25
					2010	0	0	0,08	1,63	0,34

	Stearibia nigriceps	<0,001	<0,001	<0,001	**2009**	0	0	9,63	20,19	0,54
					2010	0,06	5,00	2,08	20,63	2,91
SEPSIDAE	***Meroplius minutus***	0,014	0,012	0,016	**2009**	0	0	2,13	2,11	0,47
					2010	0	0	0,46	2,96	1,72
	Nemopoda nitidula	0,472	0,462	0,481	**2009**	0,07	0	0	0,22	0,40
					2010	0	0	0,08	0,21	0,16
PHORIDAE	***Megaselia brevicostalis***	0,013	0,011	0,015	**2009**	0	0	0,13	0,78	0,16
					2010	0,12	0	0,08	1,42	0,12
GEOTRUPIDAE	***Anoplotrupes stercorosus***	0,001	<0,001	0,001	**2009**	0,07	0,27	0,31	0,56	0,42
					2010	0,24	1,40	1,46	1,33	0,24
	Trypocopris pyrenaeus	<0,001	<0,001	<0,001	**2009**	0,07	0	1,44	1,19	0,33
					2010	0,59	0,80	1,38	1,13	0,14
SILPHIDAE	***Necrodes littoralis***	<0,001	<0,001	<0,001	**2009**	0,14	2,64	3,75	0,07	0
					2010	0	0,60	2,54	0,50	0
HISTERIDAE	**Unidentified Histeridae**	0,257	0,249	0,266	**2009**	0	0	0,63	0,15	0,09
					2010	0,12	0,40	0,62	0,38	0,26
STAPHYLINIDAE	***Creophilus maxillosus***	<0,001	<0,001	<0,001	**2009**	0	0,09	0,50	0,15	0,02
					2010	0	0,40	1,46	0,42	0,02
HYMENOPTERA	**Unidentified wasps**	0,296	0,287	0,304	**2009**	0,36	0,45	0,81	0,44	0,19
					2010	0,12	0,60	0,23	0,46	0,60

At the light of results, these 17 species, out of 36 evaluated, are potentially the best ones to construct a representative and meaningful succession pattern which will constitute the baseline model for this region. However, we decided to include also all the species with a minimum representativity in terms of numbers and other forensically important groups, as this allows comparison with previous models developed in other regions (Figure 24). These include the families Fanniidae, because they were found to be good complementary indicator species (ABALLAY *et al.*, 2012); Sarcophagidae, as they have been found to be important in summer in nearby areas (ARNALDOS *et al.*, 2004; CASTILLO MIRALBÉS, 2002; MARTÍNEZ SANCHEZ *et al.*, 2000b; ROMERA *et al.*, 2003; PRADO E CASTRO *et al.*, 2012); and Sciaridae, which are frequently associated to carcasses (ANDERSON & VANLAERHOVEN, 1996; HORENSTEIN *et al.*, 2012; PRADO E CASTRO *et al.*, 2012), although the later ones usually are not analysed in great detail due to their typical occurrence in low numbers.


SAMPLING DAY
1 2 3 4 5 6 7 8 9 10 11 12 13 15 18 21 24 25 27 29 32 34 36 40 47 49 54 56 62 64 71 72 79 80 85 88
CLASSIC DECOMPOSITIONAL STAGES
FRESH
BLOATED
ACTIVE DECAY
ADVANCED DECAY
DRY
CALLIPHORIDAE
Calliphora vomitoria
Lucilia caesar
Chrysomya albiceps
MUSCIDAE
Azelia sp.
Hydrotaea dentipes
Hydrotaea ignava
Hydrotaea similis
Musca autumnalis
FANNIIDAE
Fannia sp.
Fannia manicata
SARCOPHAGIDAE
Sarcophaga sp.
Sarcophaga albiceps
Sarcophaga incisilobata
Sarcophaga (Bellieriomima) subulata
Sarcophaga (Helicophagella) noverca
Sarcophaga (Parasarc.) aratrix
Sarcophaga (Sarc.) pyrenaica
Ravinia pernix
SPHAEROCERIDAE
Chaetopodella scutellaris
Coproica hirticula
Coproica pusio
Coproica vagans
Leptocera caenosa
Opalimosina liliputana
Spelobia clunipes
Spelobia luteilabris
Telomerina levifrons
Trachyopella lineafrons

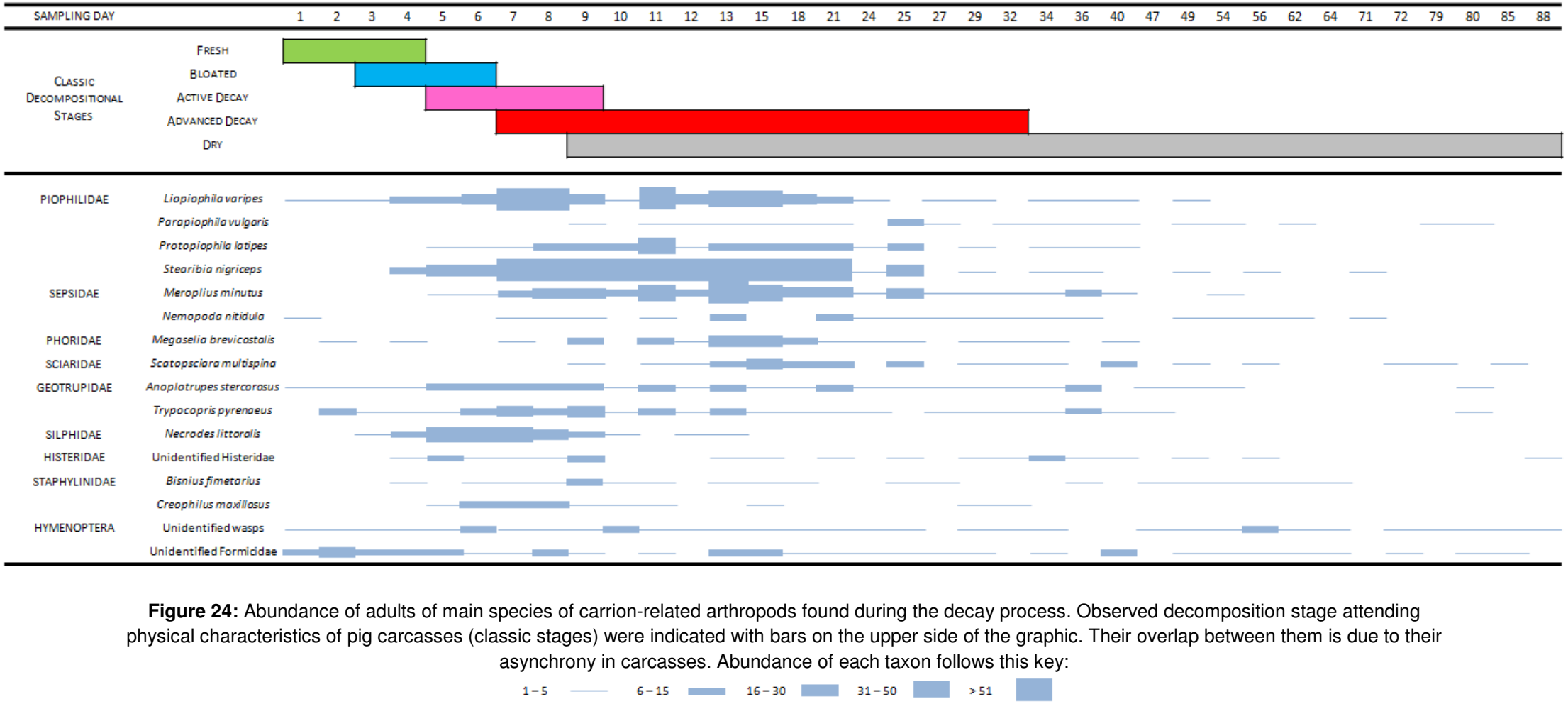


Figure 24: Abundance of adults of main species of carrion-related arthropods found during the decay process. Observed decomposition stage attending physical characteristics of pig carcasses (classic stages) were indicated with bars on the upper side of the graphic. Their overlap between them is due to their asynchrony in carcasses. Abundance of each taxon follows this key:

All the species included in the succession pattern followed a predictable sequence in the colonization of the carcass (see also Appendix I). Amongst Diptera, Calliphoridae was one of the first families to arrive, being *Lucilia caesar* its most abundant species. It was typically accompanied by Muscidae, especially *Musca autumnalis* and *Hydrotaea* species.

As the process proceeded, these families became numerically less important, while the number of species and individuals of the families Piophilidae, Sphaeroceridae and Sepsidae increased. *Stearibia nigriceps* was the most abundant piophilid (69.77% of the piophilids; see Chapter 4), followed by *Liophiophila varipes* (21.26%) and *Protopiophila latipes* (5.69%). Sphaeroceridae had a great diversity (41 species), and both *Chaetopodella scutellaris* and *Coproica hirticula* were the most abundant, with a total of 347 and 260 individuals captured respectively. Finally, *Meroplius minutus* was the most abundant species of the family Sepsidae (67.2%).

First arrivals among coleopterans were specimens of the families Geotrupidae, Silphidae and Staphylinidae. They correspond to almost 70% of the beetles associated to the carcasses. It is remarkable the species *Necrodes littoralis*, the most abundant silphid, which is typically characterized for its preference for large carcasses (WATSON & CARLTON, 2005). From Staphylinidae, which was well represented with 33 species listed, *Creophilus maxillosus* and *Bisnius fimetarius* were found to be the most abundant ones, representing respectively the 17.9% and 14.8% of whole rove beetles.

Hymenoptera were never collected in abundance, and it hardly represented a 3% of the total of arthropods associated to the carcasses. Collected specimens belong to two trophic groups (ants and parasitoid wasps), which were collected through all days of experiment. Ants were more likely to be captured on early stages, as they prey on eggs and first instar maggots, whereas parasitoid wasps appeared later, parasitizing different instars of insects. Common wasps were also observed preying on flies.

9 Forensic Bioindicators

13 out of 36 taxa evaluated met the prerequisites of abundance established for the analysis of residency patterns (i.e more than 10 imagoes per carcass). Most of them showed an average relative length of presence period (ARLPP) of between 22 and 46%. Only *Necrodes littoralis* was below this range, with 6.3%; and *Anoplotupes stercorosus* exceeded it with 53.4%.

Regarding the reoccurrence character, most taxa have an average of 2.2 breaks evenly distributed in their presence period. Once again, *Necrodes littoralis* was below this range, as it had a presence period almost without breaks (the average number of breaks (ANBPP) was bellow 1, and the length of the longest unbroken period (ARLLUP) was above 89%), whereas *Anoplotrupes stercorosus* was the opposite, with the highest ANBPP value and the lowest ARLLUP (3.8 and 17.7 respectively).

On the other hand, *Necrodes littoralis* had a strong, negative relationship between appearance time and the onset of bloating, while *Coproica hirticula* showed a strong, positive relationship with it. Other strong, positive relationships were found between the onset of Advanced Decay stage and the species *Hydrotaea similis* and *Anoplotrupes stercorosus*. No other significant relationship was found among the other taxa analysed, probably due to the low number of carcasses included in the design.

Table 11: Residency patterns (ARLPP: Average Relative Length of Presence Period; ANBPP: Average Number of Breaks in the Presence Period; ARLLUP: Average Relative Length of the Longest Unbroken Period) and relationship between appearance time of adults of the different taxa analysed and onset of bloating (n: number of carcasses which met the prerequisites of abundance; r: degree of correlation; p: significance of the correlation). Significant results are highlighted.

		Residency patterns				Pearson-correlation coefficient					
		ARLPP	ANBPP	ARLLUP	N	Onset of Bloating		Onset of Active Decay		Onset of Advanced Decay	
						r	p	r	p	r	p
CALLIPHORIDAE	***Lucilia caesar***	45,8	2,7	41,9	10	-	-	-	-	-	-
	Chrysomya albiceps	27,4	1,7	43,1	6	0,78	0,066	0,53	0,276	0	1
MUSCIDAE	***Hydrotaea similis***	25,3	2,0	29,8	6	0,43	0,399	-0,11	0,834	0,86	0,027
FANNIIDAE	***Fannia* sp.**	39,1	2,6	24,7	8	0,56	0,149	0,54	0,168	-0,34	0,409
SPHAEROCERIDAE	***Chaetopodella scutellaris***	36,7	3,0	28,9	6	0,49	0,324	0,58	0,223	-0,14	0,789
	Coproica hirticula	22,2	1,8	38,3	6	0,87	0,026	0,26	0,621	0,25	0,086
PIOPHILIDAE	***Liopiophila varipes***	32,6	2,3	33,9	10	0,12	0,732	-0,16	0,666	-0,08	0,821
	Protopiophila latipes	22,8	1,8	37,4	6	0	1	0,39	0,44	0,64	0,176
	Stearibia nigriceps	32,3	1,6	46,3	9	0,14	0,722	0,52	0,153	0,51	0,157
SEPSIDAE	***Meroplius minutus***	31,5	1,8	53,1	9	0,33	0,379	0,56	0,119	0,-0,05	0,905
GEOTRUPIDAE	***Anoplotrupes stercorosus***	53,4	3,8	17,7	5	0	1	0	1	0,97	0,005
	Trypocopris pyrenaeus	39,9	2,8	27,1	9	0,24	0,542	-0,15	0,707	0,10	0,802
SILPHIDAE	***Necrodes littoralis***	6,3	0,3	89,7	7	-0,83	0,021	-0,06	0,894	-0,41	0,366

When classifying these taxa into different categories attending to their usefulness in succession investigations, only 3 out of the 5 categories described by MATUSZEWSKI *et al.* (2010b) were observed (Table 12). Most of the taxa analysed were found to be useless, as no significant relationship between arrival time and onset of any stage of decomposition was found for them. Only 4 species (*Hydrotaea similis, Coproica hirticula, Anoplotrupes stercorosus* and *Necrodes littoralis*) were found to be potential PMI indicators of a specific period of decomposition. It was not possible to calculate the relationship between arrival time and the onset of bloating for *Lucilia caesar*, as the sampling was made on daily basis and, therefore, precluded from this analysis those species that appear shortly after death (MATUSZEWSKI *et al.*, 2010b). Hence, the usefulness of residency patterns of *Lucilia caesar* adults remains unknown.

Table 12: Classification of the minimally abundant species attending to their residency patterns (see MATUSZEWSKI *et al.* (2010b) for more details on calculations).

Adults of no usefulness	Adults of high usefulness	Adults of unknown usefulness
Chrysomya albiceps *Fannia* sp. *Chaetopodella scutellaris* *Liopiophila varipes* *Protopiophila latipes* *Stearibia nigriceps* *Meroplius minutus* *Trypocopris pyrenaeus*	*Hydrotaea similis* *Coproica hirticula* *Anoplotrupes stercorosus* *Necrodes littoralis*	*Lucilia caesar*

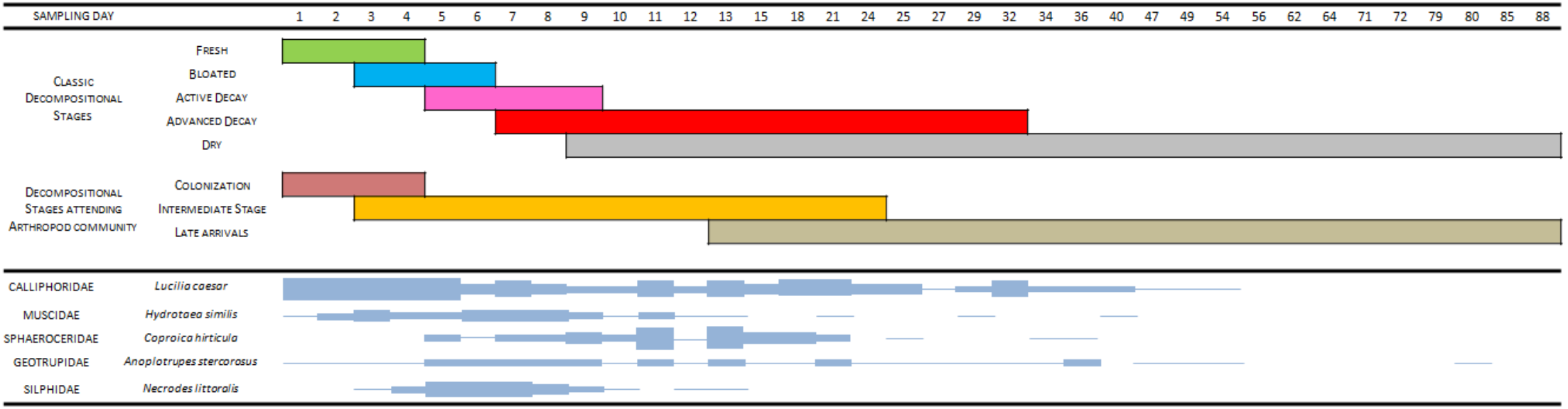


Figure 25: Chronograph summarising the results of the decomposition process. Classical decomposition stages are detailed for comparison with decomposition stages discriminated in results attending to the arthropod community. Abundance and presence periods of the adults of the species of high or unknown usefulness according residency features (ARLLP) are also included. Bars' overlapping is due to asynchrony between carcasses in the classical approach, and to the absence of discrete boundaries in the process observed in this study.

◈ Discussion

9 Climatic Effect

Forensic Entomology is based on the arrival timetable of the different species of sarcosaprophagous insects to the carcass and the sequence and time of development of immatures of different taxa (Matuszewski *et al.*, 2010c; Nuorteva, 1977). These processes are influenced by a variety of environmental factors such as temperature, humidity, precipitation and insolation (Lopes de Carvalho & Linhares, 2001). Hence, the assessment of death chronology must correlate both environmental conditions and insect species (Campobasso *et al.*, 2001).

The temperate weather of the area of study favoured the rapid colonization of the carcass in summer, which could start within few seconds after deploying the piglets. However, the abundant rainfall recorded in 2009 from the second day of experiment (31.33 l/m^2 in 5 days; see Figure 21) might have hampered the survival and development of the first generation of maggots, and made difficult the arrival of new adults. For example, just 2 adults of *Lucilia caesar* were captured that second day at all carcasses, being 47 the previous day (see Appendix I). These worst weather conditions might be one of the reasons of finding an average of one-day delay on the onset of every stage of decomposition in 2009 in comparison with the experiment of 2010 (Table 8). Another reason could be related to the placement of the carcasses in the same study area both years, as local populations of carrion arthropods might be increased in 2010 as a consequence on 2009 trial. Nevertheless, this fact highlights the importance of the evaluation of weather conditions during forensic investigations, as in the majority of cases an absence of a specific taxa may be weather dependant and not the end of its presence period (Matuszewski *et al.*, 2010b). Furthermore, as it is done routinely, weather conditions around the estimated date of decease needs to be inspected to decide whether the PMI estimation should be corrected or its coefficient interval expanded if rainy or adverse weather conditions had occurred.

9 DECOMPOSITION PROCESS

▪ STAGES OF DECOMPOSITION. DISCRETE OR CONTINUUM PROCESS?

Pattern of decomposition observed in the present study followed the general trend described in previous research (ANTON *et al.*, 2011; ARNALDOS *et al.*, 2004; CASTILLO MIRALBÉS, 2002; GARCÍA ROJO, 2004; GRASSBERGER & FRANK, 2004; MATUSZEWSKI *et al.*, 2010a). Notwithstanding, two unusual observations should be highlighted. Firstly, in two cases (carcasses C4 and C5 in 2010), no bloating was observed. The absence of the bloated stage had been previously reported by MATUSZEWSKI *et al.* (2010a), and authors explained it as a consequence of low temperatures during the research. However, this explanation cannot be extrapolated to this research, because it was performed in summer and autumn, and there was no cold episode recorded during the first weeks of the research (Figure 21). In our case, the absence of evident bloating could be related to the temperature of the tissues of the carcasses (KORSHUNOV *et al.*, 2003), as it affects the microbial activity and internal gas production. Additionally, it should be stressed out that the duration of this stage is also dependent on the number of larvae infesting the carcass rather than on ambient temperature, and in consequence is affected by differences in breeding biology of blowflies (KOČÁREK, 2003; TANTAWI *et al.*, 1996). Regarding carcasses C4 and C5 in 2010, oviposition was lower than in other replicates of the same year (see Figure 26), and the air temperature was higher. Under these conditions, internal gas production might have been higher and the bloating more notorious and shortened, difficulting its observation with the sampling methodology of daily basis employed.

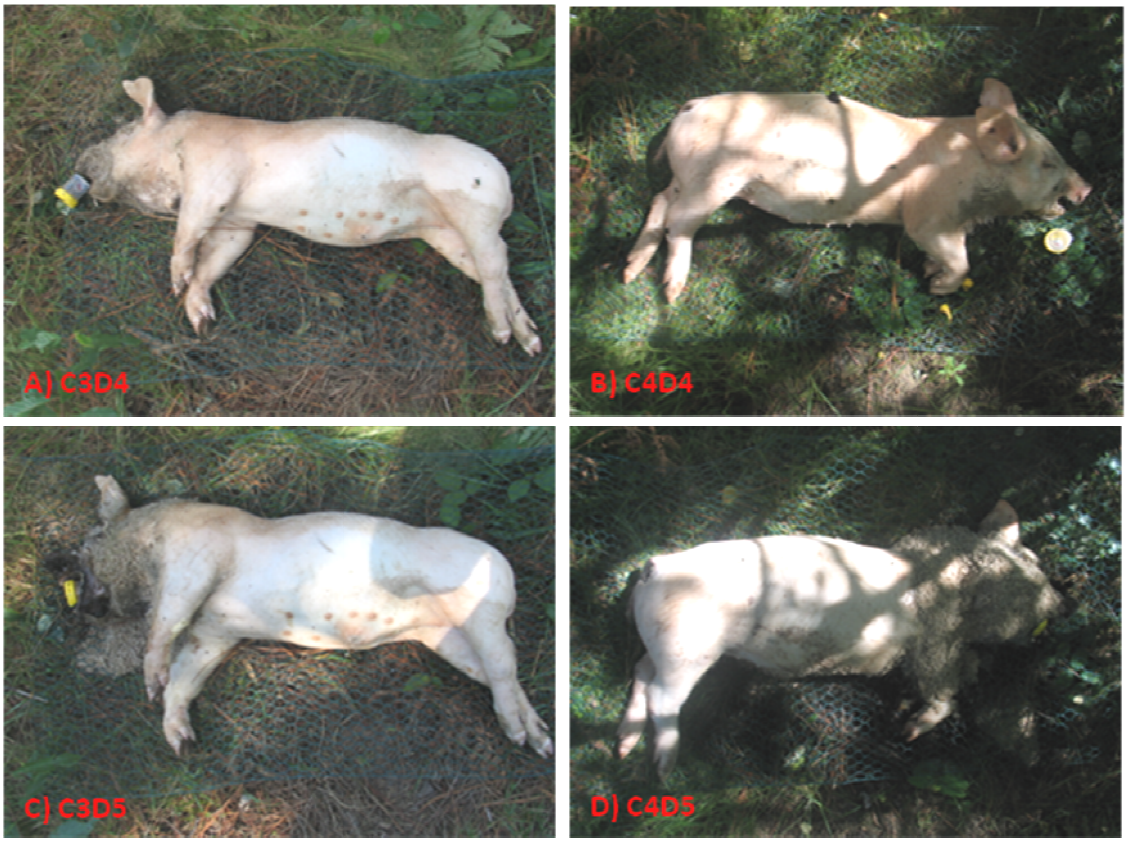


Figure 26: At the left, carcass C3 on the 4th (A, last day of fresh stage) and 5th (C, the only day on bloating) day of sampling in 2010. At the right, carcass C4 on the 4th (C, last day of fresh stage) and 5th (D, onset of Active Decay) day of sampling in 2010. Note that inside the mouth of piglets, there is an i-button protected into a plastic vial to record the internal temperature of the carcass (see also Chapter 7).

Secondly, advanced decay on both C3 carcasses (2009 and 2010 samples) was prolonged in time in comparison with the process in other carcasses. A possible explanation may lay in the fact that, in both years, those carcasses accumulated important amounts of decomposition fluids under the skin remains and bones, possibly favoured by the high soil moisture appreciated in the area. The large quantity of wet, viscous material in the soil may prolong the period of arthropod activity in C3 carcasses and, therefore, the duration of advanced decay stage. This highlights the importance of considering soil properties and texture in the future when recording data for the estimation of the PMI (TUMER *et al.*, 2013).

Despite exceptions as those above described, decay stages were easily recognized trough observed physical changes in carcasses, and were used to

describe both the decomposition process and the succession of carrion-related fauna (SCHOENLY & REID, 1987). However, while there could be five recognisable stages of decomposition, there were not clearly defined boundaries between decomposition stages attending carrion fauna, making subjective the decision of stage change (LEBLANC & LOGAN, 2010; SCHOENLY & REID, 1987). As in previous works (MOURA *et al.*, 2005; SCHOENLY & REID, 1987), results of our analyses showed that similarities between decomposition periods (in days, as sampling was made on a daily basis) support the continuum concept of the process (Figure 22), and that stage boundaries were not defined by abrupt changes in the composition of the arthropod community.

Nevertheless, this does not conflict with the fact that some species can be associated with a certain period of time of the decomposition process (MOURA *et al.*, 2005), although our results indicate that these periods do not necessarily correspond with the classical stages of decomposition. In other words, our results define only three succession periods attending the arthropod community (see Figures 22-25): an initial period characterised by first arrival imagoes (colonization stage), such as *Lucilia caesar* and *Musca autumnalis* (see Figures 23 and 24); a second "middle" period characterised by a continuous replacing of species and niche overlapping; and a dry stage characterised by late arrivals, such as *Nemopoda nitidula*, *Parapiophila vulgaris*, *Spelobia luteilabris* and *Spelobia clunipes*. But, as well as with classic decompositional stages, these arthropod-community-based periods did not present discrete boundaries, as a gradual change in the community was observed.

- CARRION – RELATED ARTHROPODS

Arthropod succession was in line with the relatively predictable sequence described in previous carrion studies (CASTILLO MIRALBÉS, 2002; GARCÍA ROJO, 2004; GENNARD, 2012; GRASSBERGER & FRANK, 2004; PAYNE, 1965; PRADO E CASTRO *et al.*, 2012; RICHARDS & GOFF, 1997; SCHOENLY, 1992; among others). Under adequate environment conditions, as it is summer outdoors in a temperate region, there is a rapid invasion by flies, which lay eggs preferably on natural orifices and wounds. Large numbers of the greenbottle fly *Lucilia caesar*

were observed to arrive within minutes to all carcasses both years, accompanied in some cases by the face fly *Musca autumnalis*, ants (Formicidae) and other less abundant species. The main reason for ants being present during the first days of decomposition seems to be the predation of insects' eggs and first instar larvae, and may have an important effect on carcass reduction (CASTILLO MIRALBÉS, 2002; MARTÍNEZ *et al.*, 2002). In fact, in some carcasses the wound of the neck was rapidly infested by ants, with the result of no fly ovipositing there and the delay of soft tissues removal in that area with respect to other carcasses where no ants were detected. Additionally, although it was not observed here, ants can also show a necrophagous behaviour, causing post-mortem injures over the skin surface or modifications on pre-existing skin lesions (CAMPOBASSO *et al.*, 2009). These artefacts require a considerable experience from forensic technicians, as they can be misidentified as ante-mortem injuries leading to wrong PMI estimations (CAMPOBASSO *et al.*, 2009; MARTÍNEZ *et al.*, 2002; RAMÓN & DONOSO, 2015).

The fact that *Lucilia caesar* was the most abundant species is not in concordance with previous studies conducted in the Iberian Peninsula, where this species was of minor importance in summer. For instance, *Chrysomya albiceps* was the most abundant species on summer studies performed by PRADO E CASTRO *et al.* (2011a) in Portugal, being *Lucilia caesar* the most abundant in spring (PRADO E CASTRO *et al.*, 2012). Moreover, *Lucilia sericata* has been reported in previous studies as the most abundant species in most of the studied regions of Spain (CASTILLO MIRALBÉS, 2002; GARCÍA-ROJO, 2004; MARTÍNEZ – SÁNCHEZ *et al.*, 2000a). By contrast, *Lucilia caesar* was the most abundant summer species in other countries, such as Poland (MATUSZEWSKI *et al.*, 2008) or Germany (ANTON *et al.*, 2011; REIBE & MADEA, 2010). All these findings strengthen the need of performing this kind of studies in the Atlantic area of the Iberian Peninsula, as previous research confirm the dissimilarities between this and other biogeographic areas of the Peninsula (ARNALDOS *et al.*, 2006; CASTILLO MIRALBÉS, 2002), and the closer similarities with central Europe dynamics (SALOÑA BORDAS *et al.*, 2009; ZABALA *et al.*, 2014).

Muscidae was the second important family during the first stages of decomposition. Although *Musca autumnalis* was collected at the beginning of

the decomposition process, it did not really play an important role in the process, as it just bred on decomposition fluids, without having been observed either oviposition or immature stages collected. By contrast, *Hydrotaea* species appeared to be important in the breakdown of carcasses (see also Chapter 7). Species of this genus are commonly referred as later visitors of carrion remains (CASTILLO MIRALBÉS, 2002; MATUSZEWSKI *et al.*, 2008). However, muscids occurred from the first day onwards, breeding and laying eggs, which is in concordance with previous research done under similar conditions (ANTON *et al.*, 2011; PRADO E CASTRO *et al.*, 2012).

Another interesting difference with previous studies performed in the Iberian Peninsula, was the scanty abundance of flesh flies (Diptera: Sarcophagidae). They were very low in number and widely spread throughout all the process. The minor role of flesh flies has been previously reported by MATUSZEWSKI *et al.* (2008), and CASTILLO MIRALBÉS (2002) found them only during the first days of decomposition. This contrasts with the high number and diversity found in several studies developed in southern areas of the Iberian Peninsula (ARNALDOS *et al.*, 2004; PRADO E CASTRO *et al.*, 2012), and provides further evidence of the greater similarities between our region and central Europe (KOČÁREK, 2003; MATUSZEWSKI *et al.*, 2008) than with nearest areas, probably due to biogeographic and climatic differences between the northern maritime facade and more southern areas.

The remains and the huge number of fly adults and larvae attracted also a considerable number of beetles, mainly Geotrupidae, Silphidae and Staphylinidae. Interestingly, these families are barely reported on the summer trial of CASTILLO MIRALBÉS (2002), highlighting again the differences between carrion-related arthropod community of different bioclimatic areas. Geotrupidae species have been previously reported as regular visitors of carcasses (KOČÁREK, 2003; MATUSZEWSKI *et al.*, 2008), and were classified as coprophagous species which especially appeared during the onset of active decay (PAYNE, 1965), when contents of the stomach and gut are exposed (VEIGA & LOBO, 1986). It was therefore surprising to observe them preying on maggots. This necrophagous behaviour was previously reported (PÉREZ-LÓPEZ & HERNÁNDEZ-RUIZ, 1995), but had not been observed in cadaveric

environments until now. This unclearness about its role in decomposition process asks for further studies, as its trophic level is rather supposed than confirmed (KOČÁREK, 2003). On the other hand, Silphidae species belong to both necrophagous and predator categories (KOČÁREK, 2003). Its most abundant species, *Necrodes littoralis*, was found to be of high usefulness from a forensic point of view (Table 12) as stressed out by MATUSZEWSKI *et al.* (2010b), being absent in summer and barely present in the autumn experiment of CASTILLO MIRALBÉS (2002). On its behalf, Staphylinidae species are commonly referred as predators of other insects (FERNÁNDEZ *et al.*, 2010; HORENSTEIN *et al.*, 2012; PRADO E CASTRO *et al.*, 2011a). Only *Creophilus maxillosus* has often been reported on previous studies performed in the Iberian Peninsula (GARCÍA ROJO, 2004; ROMERO PALANCO *et al.*, 2006; PRADO E CASTRO *et al.*, 2010a), being also the most abundant rove beetle in the present study. The remaining Staphylinidae species vary with the location of the study (BAZ *et al.*, 2014; CASTILLO MIRALBÉS, 2002; FERNÁNDEZ *et al.*, 2010).

Within the roles of Hymenoptera, most of the collected species were parasitoids of third instar larvae and pupae of different insects, mainly Calliphoridae (HORENSTEIN & SALVO, 2012; GRASSBERGER & FRANK, 2003). However, common wasps were also observed preying on adult flies; they captured and killed the flies, leaving the head on the ground near the corpses.

With the onset of active decay, other flies that were absent or scarce during fresh and bloated stage started to acquire more importance in terms of abundance, the most abundant of which were Piophilidae, Sepsidae and Sphaeroceridae. Piophilidae and Sepsidae were confirmed to complete their life cycle on carcasses (see Chapter 7). Moreover, Sepsidae and Sphaeroceridae are also a common part of the insect fauna that inhabit decomposing organic matter, including carrion and excrements (BYRD & CASTNER, 2009b). These three families, Piophilidae, Sepsidae and Sphaeroceridae, have been frequently related to advanced stages of decomposition (CASTILLO MIRALBÉS, 2002; MARTÍNEZ *et al.*, 2007; PAYNE, 1965), and to a lesser extent to early stages (ANDERSON & VANLAERHOVEN, 1996; PRADO E CASTRO *et al.*, 2012). However, these families are frequently reported without being identified to species level,

probably due to technical difficulties for a specific identification, and if more information could be obtained from species specific identification is yet to see.

Piophilidae could provide valuable information at advanced stages of decomposition in some cases, although adults can be occasionally collected during the first days (ANDERSON & VANLAERHOVEN, 1996; MARTÍN-VEGA, 2011; PRADO E CASTRO *et al.*, 2012; VITTA *et al.*, 2007) as happened in this research. Frequently, biological information about this family is limited to *Phiophila casei*, due to its forensic, medical and economical interest (MARTÍN-VEGA, 2011), or just for being the only species of this family present in many popular field guides as pointed out by CARLÉS-TOLRÁ (2006) for the species *Sarcophaga carnaria*. However, *Phiophila casei* has been the least abundant species from the 8 piophilids listed in this research, being the most abundant *Stearibia nigriceps*, which has been found to be more frequent than the former, and has been previously reported from a forensic case in our region (SALOÑA *et al.*, 2010). These results are in line with those obtained by PRADO E CASTRO & GARCÍA (2010) and PRADO E CASTRO *et al.* (2012), although in continental areas of the Iberian Peninsula during summer *Stearibia nigriceps* was absent (CASTILLO MIRALBÉS, 2002) or scarce (MARTÍN-VEGA, 2011).

Sepsidae is another group of tiny flies with periods of activity similar to Piophilidae (PRADO E CASTRO *et al.*, 2012). *Meroplius minutus* was the most abundant one amongst this family, which is in accordance with findings reported by TABOR *et al.* (2004) in Southwest Virginia; and differing from previous results of the Iberian Peninsula, which collected *Sepsis fulgens* (CASTILLO MIRALBÉS, 2002; MARTÍN-VEGA, 2011) or *Nemopoda nitidula* (PRADO E CASTRO *et al.*, 2012) as the only species of this family.

Chaetopodella scutellaris and *Coproica hirticula* were found to be the most abundant of the 41 species of lesser dung flies (Sphaeroceridae). This is in accordance with BARTÁK & ROHÁČEK (2011), who collected these species with meat bait traps in Czech Republic. CASTILLO MIRALBÉS (2002) also found *Coproica hirticula* in his forensic research developed in Alto Aragón (Spain).

When soft tissues begin to be scarce, there is a gradual decline in species abundance and an increase of species richness (see Chapter 4). Trough

advanced decomposition stage, new carrion-frequenting arthropods such as Phoridae, Sciaridae, Histeridae, Dermestidae, Cleridae or Nitidulidae, arrived to the carcasses. But interestingly, only on carcasses C3 they were minimally relevant in number, probably due to the large quantity of wet, viscous material in the soil among other factors not account for.

- CONSIDERATIONS TO CREATE A BASELINE MODEL

In order to create a baseline model by using the entire carrion insect community, some knowledge is needed about site-specific arthropods and succession patterns (AMENDT *et al.*, 2007).

Firstly, succession patterns are usually described following the classic decompositional stages proposed by PAYNE (1965), which does not necessarily match the description of the process attending insect community. Clustering analyses were useful to define the arthropod-community-based periods through adults' diversity, finding just three succession periods on this summer trial on this area: colonization, intermediate period, and late arrivals.

Secondly, it is important to decide which taxa must be included in an adult succession model, as most of them are of no usefulness due to their low abundance, unpredictability or very long presence period (MATUSZEWSKI *et al.*, 2010c).

The χ^2 test allowed for a quick identification of those species whose presence during the reduction of a carcass is not constant due to either low abundance or unpredictability. Thereby, adults of those species which have shown significant differences between carcasses of the experiment should not be taken into account to create the baseline pattern. From the 8 species included in this group, it is somewhat surprising to find species of the genus *Fannia*, as they are typically related to decomposition processes (BENECKE & LESSIG, 2001; VELÁSQUEZ *et al.*, 2013) and were considered to have a moderate usefulness for forensic purposes in previous works (ABALLAY *et al.*, 2012; MATUSZEWSKI *et al.*, 2010b). Hence, this species with significant differences between carcasses can

provide useful information in some cases, but might be absent under special conditions, so each particular case needs to be analysed with caution.

On the other hand, another note of caution is needed with those species showing significant variations between both years of study, as there may be several factors influencing beneath. MATUSZEWSKI *et al.* (2010c) found that appearance time of carrion taxa differed across years due to differences in temperature, but pointed out that it is more important the lack of annual differences in the composition of carrion fauna. Therefore, it is recommended to evaluate each of these taxa carefully to determine if they should be included or not.

An additional χ^2 test attending the temporal distribution of species among the process itself determined those species whose adult presence period is too long and, hence, have scarce forensic usefulness for PMI estimation in our region. Most Sphaeroceridae species and some other taxa fell into this group, as well as parasitoid wasps. Concerning the latter, it should be stressed out that they are already considered as a group pendant of revision, and that a more specific analysis is already needed in this way as other authors have found some important forensic indicators among the species of this group (DISNEY & MUNK, 2004; GRASSBERGER & FRANK, 2003).

9 FORENSIC BIOINDICATORS

In view of the above mentioned results, a proper identification of characteristic or indicator species is necessary to accurate the PMI estimation based on adult succession models (DUFRENE & LEGENDRE, 1997; MATUSZEWSKI *et al.*, 2010b).

MATUSZEWSKI *et al.* (2010b) developed a method to determine the forensic usefulness of carrion insects, which includes the evaluation of the reoccurrence of each taxon and the number of breaks in its presence period, the length of that presence period, and the relationship between the taxon and a particular state of the carcass. With those parameters taken into account, just four species have been identified as having high usefulness for this region and habitat: *Hydrotaea similis, Coproica hirticula, Anoplotrupes stercorosus* and

Necrodes littoralis. Notwithstanding, these are preliminary results obtained from a low number of carcasses, so a note of caution is required.

The presence of *Anoplotrupes stercorosus* in the list of high usefulness taxa is noteworthy, taking into account that, to our knowledge, this is the first work reporting a predator behavior of this beetle in cadaveric environments. This finding supports what KOČÁREK (2003) stated about the trophic level of some species involved, which are rather supposed than confirmed.

Finally, as MATUSZEWSKI *et al.* (2010b) reported, the sampling methodology employed was not suitable for the analysis of those species that appeared shortly after death, which is probably why *Lucilia caesar* adults were classified as a taxon of unknown usefulness in this study (Table 5), despite being a species frequently used and of importance in other studies (BENECKE, 1998; GRASSBERGER & FRANK, 2004; REIBE & MADEA, 2010; VANIN *et al.*, 2008). It is worth mentioning that Calliphoridae is one of the most important families associated with decaying matter (see for instance MONEO & SALOÑA, 2009; SALOÑA BORDAS *et al.*, 2009; ZABALA *et al.*, 2014, for data from areas nearby the study area), and also one of the most important flies from a forensically point of view. They are described as first arrivals to carcasses in numerous works, and adults remain there while food is available (CASTILLO MIRALBÉS, 2002). Nevertheless, the value of the species collected in this research should be especially analysed based on their preimaginal instars.

Larval Succession on Carcasses

And though she be but little,

she is fierce.

William Shakespeare

◈ Introduction

Larval development of arthropods is a key matter on Forensic Entomology, as it allows the estimation of the minimum time since a decease occurred (the minimum post-mortem interval, commonly referred as minimum PMI) (Amendt *et al.*, 2007; von Zuben *et al.*, 1998). This is usually done by aging the largest individual through measuring its length, since it is usually considered the oldest one on a carcass (Amendt *et al.*, 2007; Arnaldos *et al.*, 2005; Grassberger & Reiter, 2002; Reiter & Grassberger, 2002) and, therefore, its age is assumed to manifest the PMI (Catts & Goff, 1992).

The age of larvae found on a corpse can be described using their length, width or body weight (Villet *et al.*, 2010), although the last one is the least used. These corporal measures are then transformed to development time with the aid of a set of graphical, mathematical and/or physical models inferred from laboratory experiments (Grassberger & Reiter, 2001; Villet *et al.*, 2010; von Zuben *et al.*, 1998). Being poikilotherm animals, their growth is dependent on ambient temperature (Tabor, 2009). However, air temperature is not necessarily representative of insect' body temperature (Villet *et al.*, 2010), as carcass tissues may act as a protective layer against sudden changes in environment temperature, and also due to the gregarious behaviour of larvae which form aggregations called maggot mass (Campobasso *et al.*, 2011; Charabidze *et al.*, 2011; Slone & Gruner, 2007; Wells & LaMotte, 2010). This gregarious behaviour is well reported in forensic literature for increasing local temperature (Amendt *et al.*, 2007; Grassberger & Frank, 2004; Slone & Gruner, 2007). Additionally, larvae on maggot mass suffer thermal stress and competition and to minimize them, they migrate inside out the mass as a process of thermoregulation. Maggot mass temperature, size, and its location on a carcass are usually registered to minimize PMI estimation bias, but all factors that are influencing beneath are very difficult to account for (Villet *et al.*, 2010).

Villet *et al.* (2010) proposed to use the development of species with thermal niches on the corpse that could be more similar to ambient conditions, either to base the PMI estimation on this kind of species or to make a cross-validate-

estimation. This approach requires baseline data of development of the different species that can be found in cadaveric environments, many of which are still without being studied thoroughly. These gaps of knowledge force to assume some errors when extrapolating data from different species, even when they belong to the same family.

Additionally, conditions at a crime scene often do not resemble those of any laboratory experiment, and size measurements of wild populations are almost non-existent (WELLS & LAMOTTE, 2010). Most field work surrounding insect succession has only been focused on adult specimens (CENTENO *et al.*, 2002; GARCÍA-ROJO, 2004) that have been reported to provide a poor PMI estimation (AMENDT *et al.*, 2007; see also Chapter 6), although usually some information about presence of larvae is also reported (ANTON *et al.*, 2011; GRASSBERGER & FRANK, 2004). Some studies also include information on the length of larvae of one or two forensically important species found on their field work. CASTILLO MIRALBÉS (2002) reported the maximum size found for *Lucilia sp.* and *Chrysomya albiceps* on pig carcasses from a succession research in Alto Aragón (Spain). JOY *et al.* (2006) recognized the necessity of field studies to gain a better insight of fly development under variable climatic conditions, and provided detailed information of third instar larvae measurements of *Phaenicia* (=*Lucilia*) *coeruleiviridis* and *Phormia regina* concurring on pig carcasses, but provided no data on first and second instars. Finally, CALDERÓN-ARGUEDAS *et al.* (2005) studied the larval succession on rabbits focusing mainly on Muscidae family, but provided no information regarding their development.

To the best of our knowledge, this report represents the first attempt to describe larval succession and their development on a field study conducted on the northern maritime facade. Additionally, information about temperature and maggot mass effect is also included due to their influence on PMI estimation.

◈ Materials and Methods

Information about study area, carcass emplacement and sampling procedures can be found on “Site Description and General Methodology” (Chapter 3).

As it was explained on previous chapters (Chapter 3, 5 and 6), ambient temperature was recorded hourly with a datalogger placed on a tree in the middle of study area. However, each carcass placement showed different degree of vegetation cover and sun exposure (see Figure 13 and Table 2 on Chapter 3). Hence, the ambient temperature of each carcass microhabitat was continuously registered with an i-Button thermometer attached to the wire cage that covered them. Additionally, a second i-Button thermometer was placed inside the mouth of the pig to record the temperature at the carcass, in order to see if arthropods' activity has any impact on ambient temperature. Latter i-buttons were kept on head area through all the experiment, in order to minimize disturbances effects due to investigator inference. All i-buttons were protected from the environment in vials which allow air entry, and were programmed for registering the temperature every hour. As maggot mass is usually formed during the onset of Active Decay stage, only temperatures of the first month of the experiment are shown.

During sampling, immature instars of colonizing arthropods were daily collected from different areas of the carcass and its surrounding, including the head (mouth, ears, and eyes), neck wound, anus and soil. When carcasses lost their form, a single area of collection, called remains, was considered. In order to ensure representativeness of the samples, and to account for most sizes present, ten small and ten big larvae were collected from each carcass area whenever available. Larvae were killed in alcohol 80% at the sampling site. Once in the laboratory, they were boiled in hot water for one minute, returned to alcohol 80% and kept properly labelled (AMENDT *et al.*, 2007).

When available, third instar maggots (L3), migrating larvae and/or pupae were isolated in plastic containers with soil as substrate for rearing them into adult stage. These cultures were kept *in situ* in the field on a shelter close to pig carcass number 1 (see Figure 13), with a data logger inside the shelter to record temperatures in there.

In the laboratory, preserved larvae were separated into families following a taxonomic key by SMITH (1986). Specimens of Calliphoridae, Muscidae, Fannidae and Sarcophagidae families were further identified to species level,

with the aid of taxonomic keys developed SMITH (1986), SZPILA (2010), SZPILA (2012), SZPILA *et al.* (2015) and SZPILA & GRZYWACZ (2010, unpublished data). For other families as Piophilidae or Sepsidae, data are detailed at family level, due to technical difficulties for a specific identification of their larvae.

Once identified to the most detailed level (either family or species), each specimen was classified according to its developmental stage: egg (E), first instar (L1), second instar (L2), third instar (L3), pupa or teneral adult. This latter category refers to those adult specimens which emerged from cultures. Finally, length of all instars was measured in the laboratory to the nearest 0.1 mm using a stereomicroscope Nikon SMZ 1500. The average length of each species, carcass area and day of collection was calculated attending the developmental stage. Only data for the most abundant species (those with more than 100 individuals) are included in this report.

◈ RESULTS

9 MICROCLIMATIC CONDITIONS

Table 13 shows the average temperature conditions in each carcass emplacement, as well as the internal temperature of the carcasses. Several i-button failed and did not register data, which could be related to heavy rains that continuously soaked the i-buttons despite being protected inside a vial. Alternatively, another cause of malfunction could have been the erratic activity of small larvae during Bloated stage, as they were found repeatedly inside the protective vial of some i-buttons.

Similar ambient conditions were found between carcasses in 2009. However, greater differences were found within 2010, being remarkable the lower temperature recorded in C2 and the higher temperature of C3.

Table 13: Average temperature (± standard deviation) of carcass temperature and the ambient temperature of their surrounding recorded with i-buttons. Average temperature of the study area recorded with DA datalogger (see Chapter 6) was also included. Only data for the first month after decease were taken in consideration.

	2009		2010	
Average Temperature	18,62		16,94	
	Ambient Temperature	**Carcass Temperature**	**Ambient Temperature**	**Carcass Temperature**
C1	-	-	17,79 ± 4,3	-
C2	18,95 ± 4,29	21,04 ± 3,98	15,29 ±5,07	-
C3	19,67 ± 4,98	-	19,20 ±6,11	21,16 ± 5,47
C4	18,85 ± 3,86	-	18,11 ±4,27	20,12 ± 4,28
C5	19,35 ± 4,72	-	18,56 ±5,35	22,03 ± 5,49

Figures 27 to 30 detail temperature variations during the first month of the study for both carcass temperature and their surrounding ambient temperature. Only data for carcass C2 in 2009 and carcasses C3, C4, and C5 in 2010 are displayed, due to malfunction of some i-buttons.

Attending to 2010 carcasses, differences have been appreciated on ambient temperature of carcasses emplacement, being daily temperature of C3 higher in comparison with C4 and C5. These differences were more noticeable during the first week of exposure, when maximum records on ambient temperature of C4 during the day was near 25ºC, C5 reached 30ºC, and C3 even exceeded 40ºC in some hours. Notwithstanding, temperature at night hours was more similar in all carcasses.

Regarding carcass temperature, high differences were found in comparison with ambient temperature of carcass surrounding recorded with the i- button during the first days of exposure in all the cases presented, even becoming more than the double than ambient temperature. For instance, in C3-2010 (Figure 28), a difference of 25.5ºC was found during the night of 5th day of exposure, when ambient temperature record was 17.5ºC and carcass temperature reached 43ºC. After an average time lapse of ten days, carcass and ambient temperature were more similar each other in all carcasses (see figures 27 to 30).

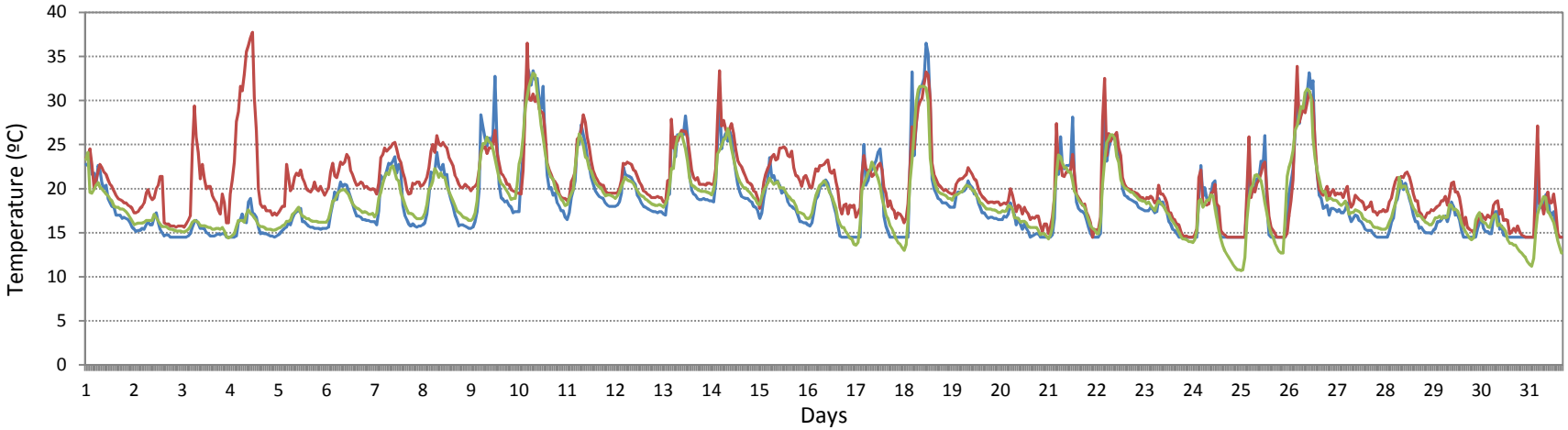


Figure 27: Ambient temperature of study area (green line), carcass surroundings (blue line) and carcass temperature (red line) of C2, 6th August to 5th September 2009.

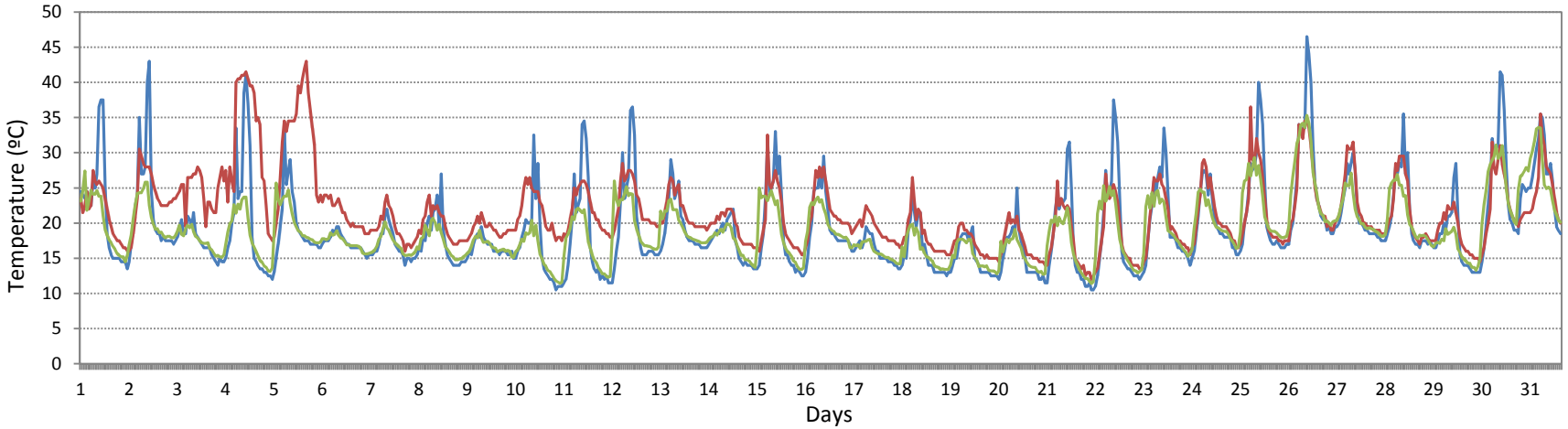


Figure 28: Ambient temperature of study area (green line), carcass surroundings (blue line) and carcass temperature (red line) of C3, 27th July to 26th August 2010.

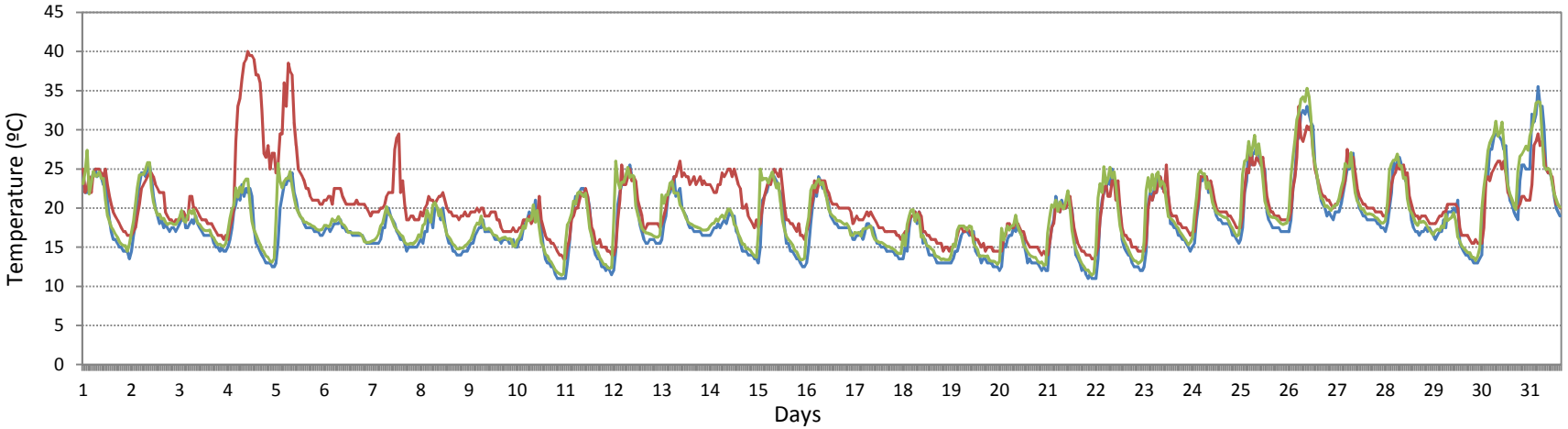


Figure 29: Ambient temperature of study area (green line), carcass surroundings (blue line) and carcass temperature (red line) of C4, 27th July to 26th August 2010.

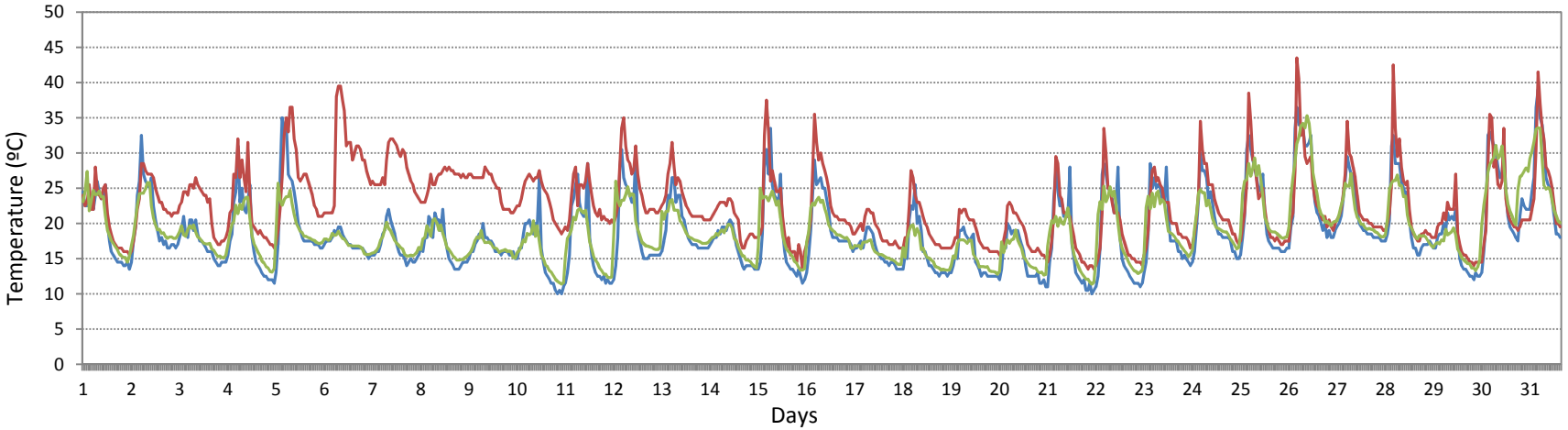


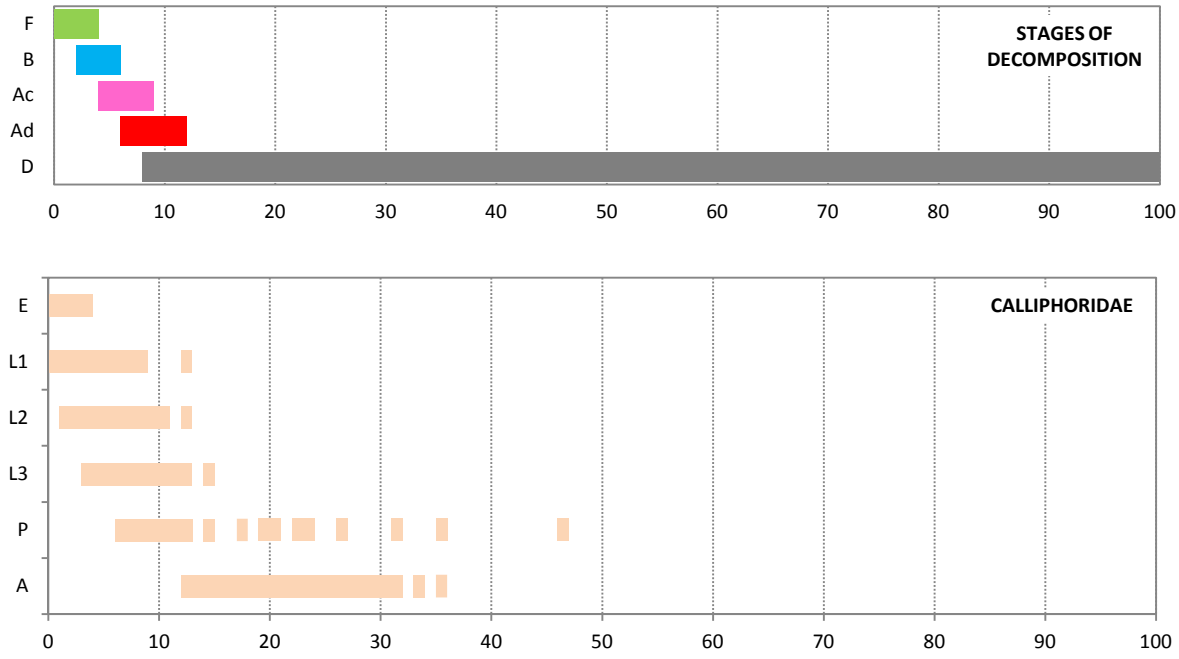
Figure 30: Ambient temperature of study area (green line), carcass surroundings (blue line) and carcass temperature (red line) of C5, 27th July to 26th August 2010.

9 LARVAL SUCCESSION

The succession sequence observed for immature instars of different Diptera families is summarized in Figure 31. Calliphoridae were the first colonizers of carcasses, and its presence on carcasses was coincident with the days when differences between ambient and carcasses temperature were highest. Fleshflies (Sarcophagidae) also appeared as colonizers from the 2nd day onwards, although their presence was very scarce.

The second most important family in terms of abundance was Muscidae. They appeared at the end of fresh stage and remained almost until the end of the study (D5 to D88). Similarly, Fanniidae were collected from D8 onwards, coincident with the onset of Active Decay stage, and practically until the end of the experiment. Specimens of both families were detected and collected mainly as third instar (L3).

Piophilidae and Sepsidae larvae started to be appreciated when blowfly maggots began to migrate out of remains. Piophilidae and Sepsidae larvae were collected on carcasses until D72 and D54 respectively, being Piophilidae the most abundant representative on latter stages of decomposition.



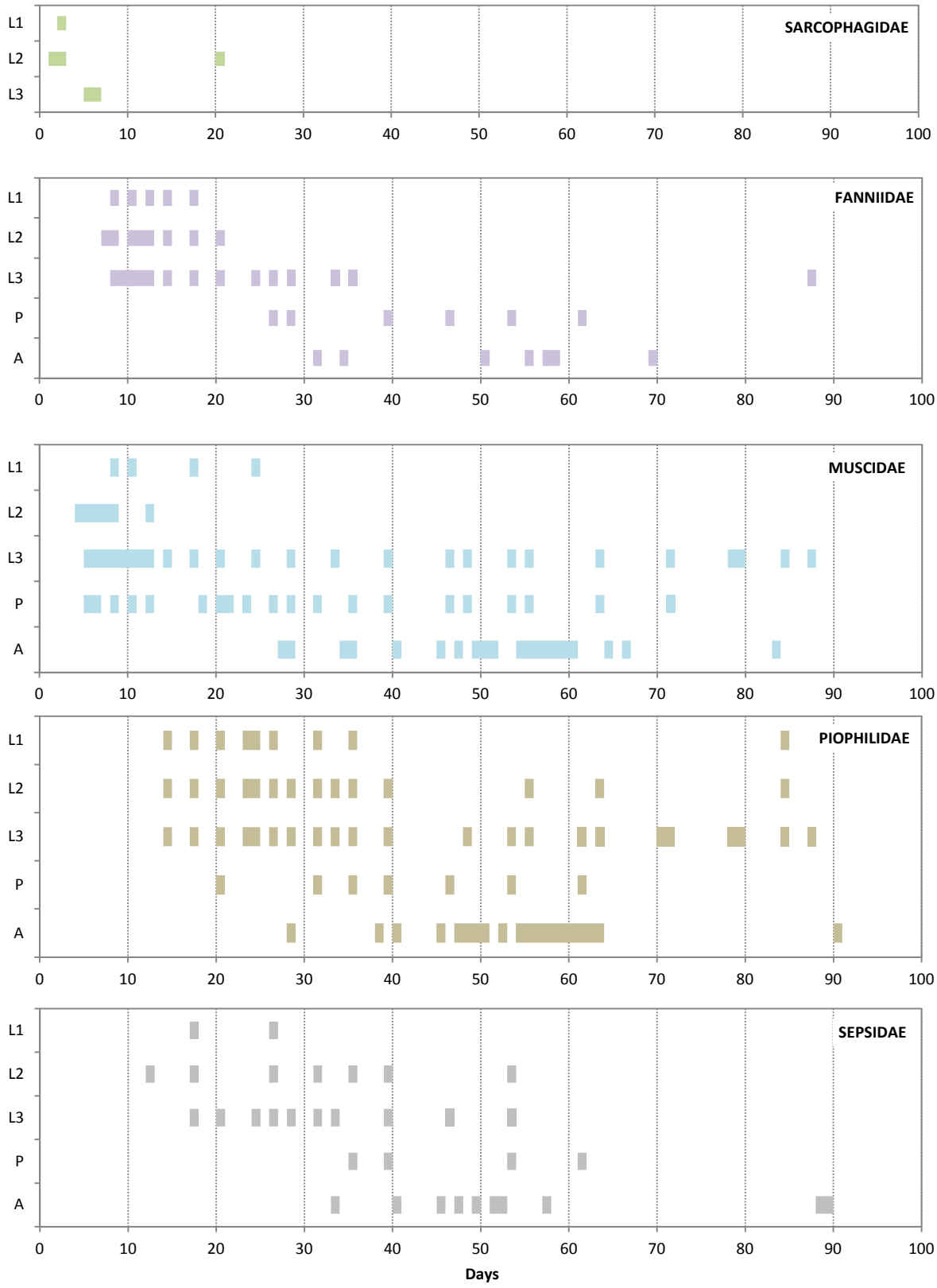


Figure 31: Presence of each developmental stage of the different Diptera families found on carcasses (E: egg; L1: first instar larva; L2: second instar larva; L3: third instar larva; P: pupa; A: teneral adult). Duration of classic decomposition stages is also included.

9 Larval Development

A total of 325 eggs, 5864 larvae, 377 pupae and 389 teneral adults of several Diptera families were collected and measured to the nearest 0.1mm. Blowfly maggots played the major role in carcass reduction, being almost 67.8% of the total amount of specimens collected in this study. It was well represented by six species, being *Lucilia caesar* and *Chrysomya albiceps* the most abundant species, with a total of 907 and 362 larvae collected from carcasses. They were also the most abundant species reared at cultures, with 44 and 205 adults obtained respectively. Other species among Calliphoridae were *Calliphora vomitoria*, *Lucilia ampullacea* and *Lucilia illustris*, all with high presence as larvae at carcasses, but not in cultures. *Lucilia sericata* was also present on carcasses, but its presence was punctual.

Muscidae family was also well represented with 8 species, 734 larvae at carcasses and 36 adults at cultures. *Hydrotaea ignava* and *Hydrotaea similis* were the most abundant among this family, being the 69.11% of all muscids. *Hydrotaea dentipes* and *Hydrotaea pilipes* were also present, but in less number. It is remarkable the presence of a single individual of *Musca autumnalis* reared at cultures, although no more larvae were collected from carcasses. *Fannia manicata* is the only species of the family Fanniidae with a good representation at all stages of development (except eggs). *Fannia scalaris* had only a punctual presence with two L3 larvae.

Piophilidae was another family collected in abundance, with a total of 884 larvae of different instar and 67 teneral adults emerged from cultures. Sepsidae were not very abundant either on carcasses or cultures, but have been included on further analysis due to the well representation of every developmental stage. Finally, the Sarcophagidae family was barely present on this study, but it was also included due to its forensic importance.

Table 14: Number of specimens of each stage of development collected for each species. Shaded lines indicate scarce data available and analyses not conducted. Data recorded as "Calliphoridae, Muscidae, Piophilidae and Sepsidae" refers to those specimens that cannot be identified to species level. E: egg; L1: first instar larva; L2: second instar larva; L3: third instar larva; P: pupa; A: teneral adult.

	E	L1	L2	L3	P	A	Total
CALLIPHORIDAE	325	1090	1046	12	15		2488
Calliphora vomitoria			23	131	3	3	160
Chysomya albiceps			90	272	140	205	707
Lucilia ampullacea			4	75	4	6	89
Lucilia caesar		2	45	920	29	44	1040
Lucilia illustris			26	123		5	154
Lucilia sericata				4			4
Fannia manicata		12	45	275	8	9	349
Fannia scalaris				2			2
Fannia sp.		5	9	2	27		43
MUSCIDAE					39		39
Musca autumnalis					1	1	2
Hydrotaea sp		6	48	12			66
Hydrotaea aenescens				1			1
Hydrotaea capensis				25	1	1	27
Hydrotaea dentipes			1	61	2	2	66
Hydrotaea ignava				239	23	23	285
Hydrotaea pilipes		1		43	8	8	60
Hydrotaea similis			1	296	1	1	299
Sarcophaga sp.		2	9				11
Sarcophaga similis				2			2
PIOPHILIDAE		35	187	593	69	67	884
SEPSIDAE		4	7	78	7	14	96
Total	**325**	**1157**	**1541**	**3166**	**377**	**308**	**6874**

Tables 25 to 35 (Appendix II) show the average length of each stage of development of each sampling day of the most abundant species. Furthermore, Figures 32 to 42 represented these data graphically, including also information about the area of carcasses were samples were collected.

In many species, overlap of two or more generations was observed. For instance, *Chrysomya albiceps* L2 were found on soil and wound at the same time as L3 and pupae were collected. The same happened with *Fannia manicata*, Piophilidae and Sepsidae, as L1, L2 and L3 specimens were collected simultaneously on the first days of their first detection. This overlapping is more notorious on *Hydrotea* species, as L3 larvae were found

until the end of the study, when pupae and teneral adult were obtained on cultures several weeks before.

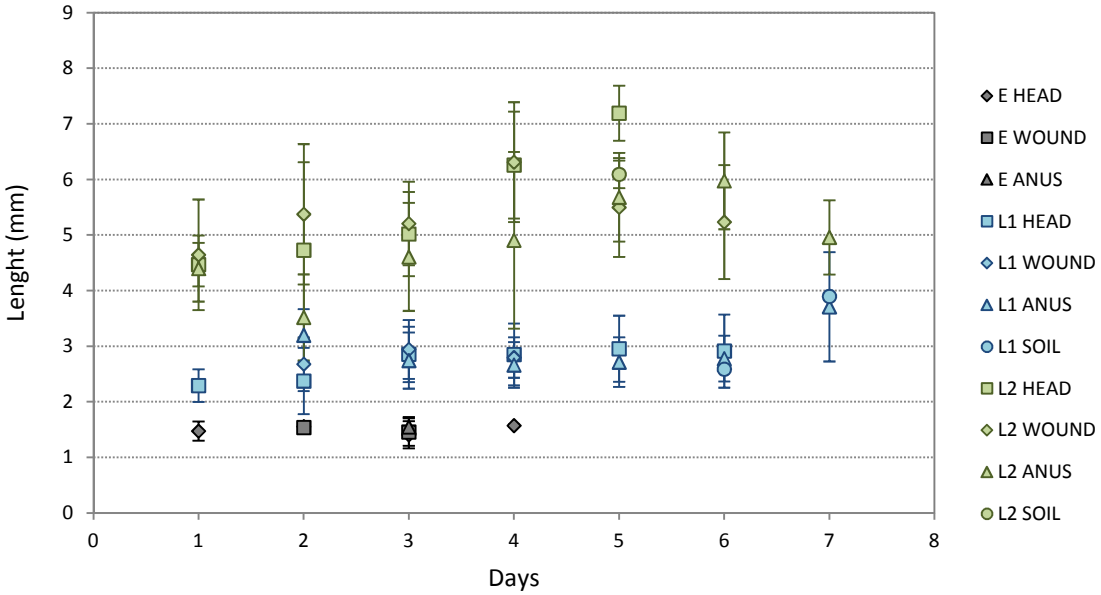


Figure 32: Unidentified maggots. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: first instar (L1) in blue, second instar (L2) in green, and third instar (L3) in red.

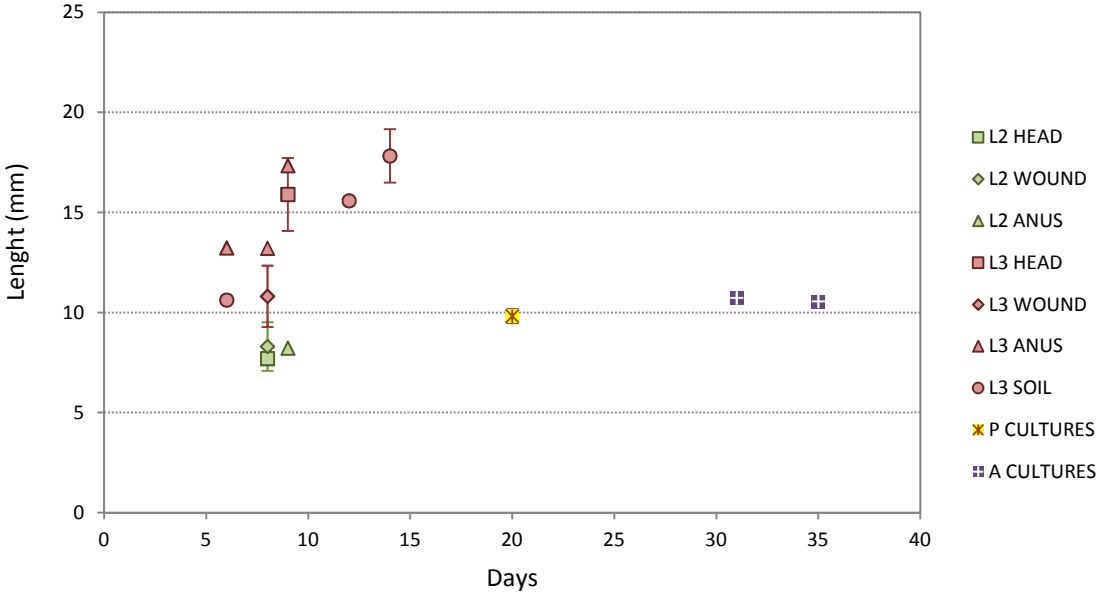


Figure 33: *Calliphora vomitoria*. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, pupae (P) in yellow, and teneral adult (A) in purple.

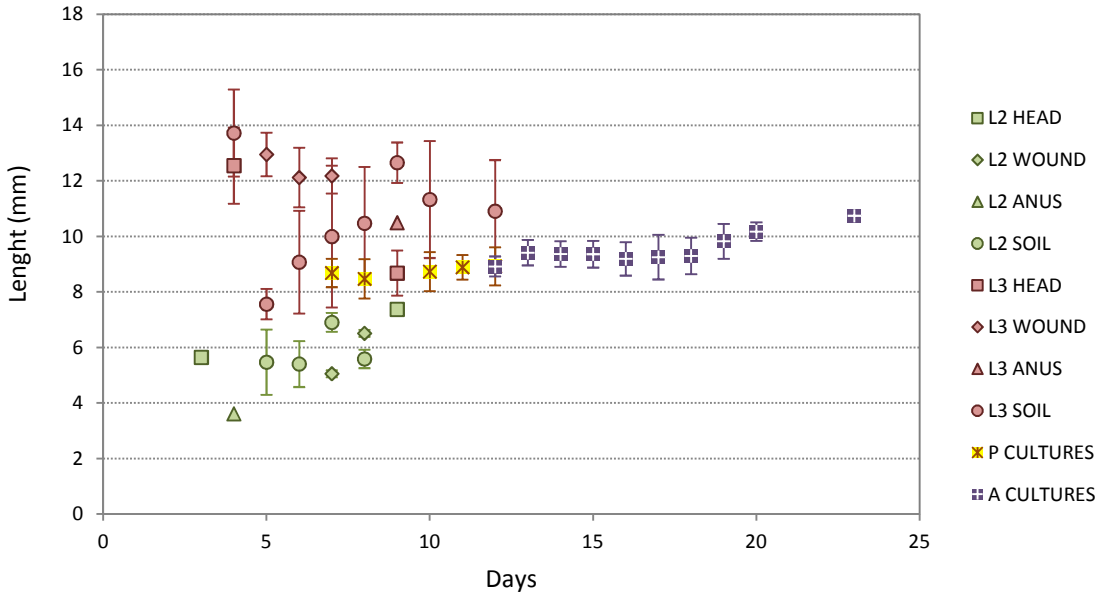


Figure 34: *Chrysomya albiceps*. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, pupae (P) in yellow, and teneral adult (A) in purple.

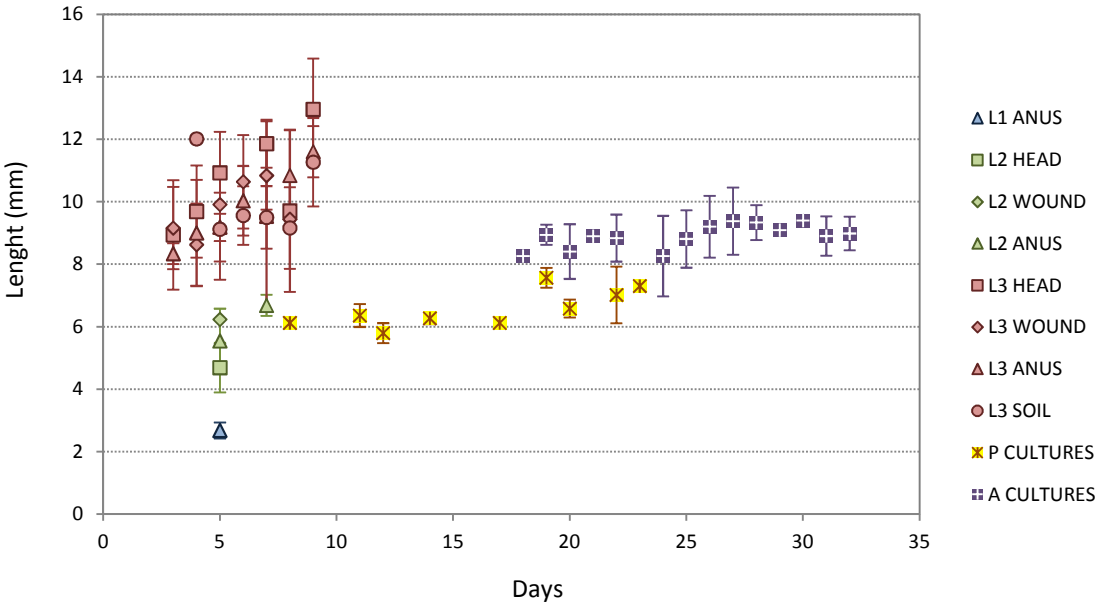


Figure 35: *Lucilia caesar*. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, pupae (P) in yellow, and teneral adult (A) in purple.

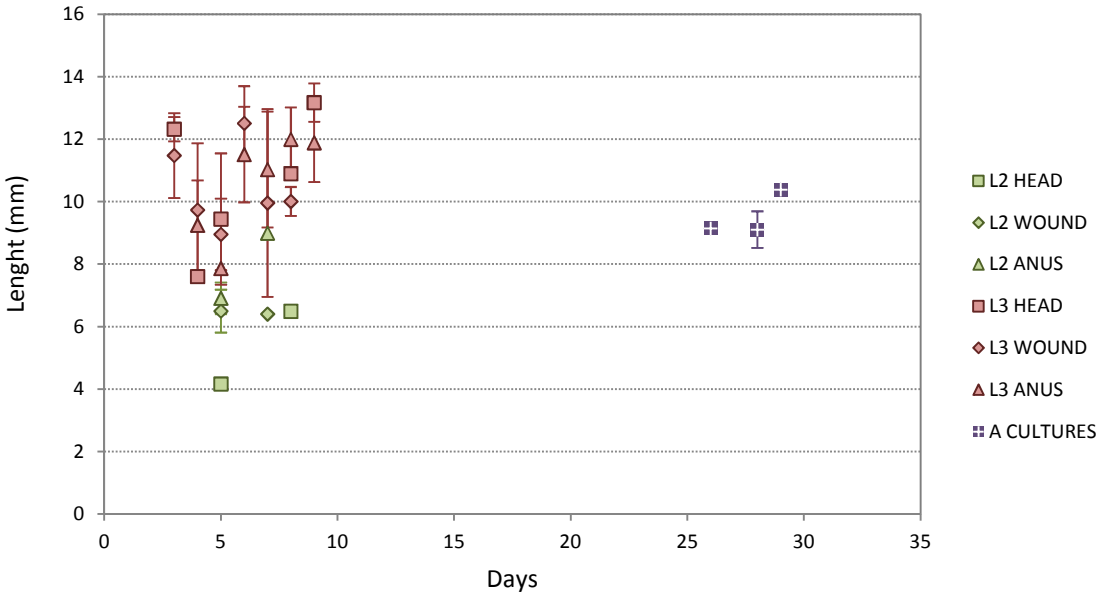


Figure 36: *Lucilia illustris*. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, and teneral adult (A) in purple.

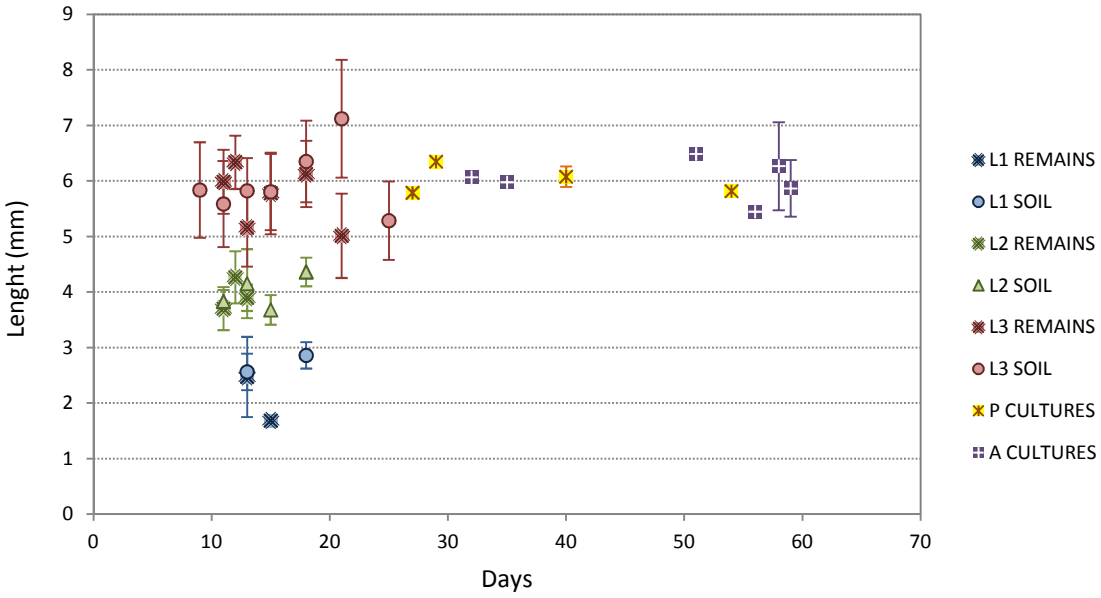


Figure 37: *Fannia manicata*. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, pupae (P) in yellow, and teneral adult (A) in purple.

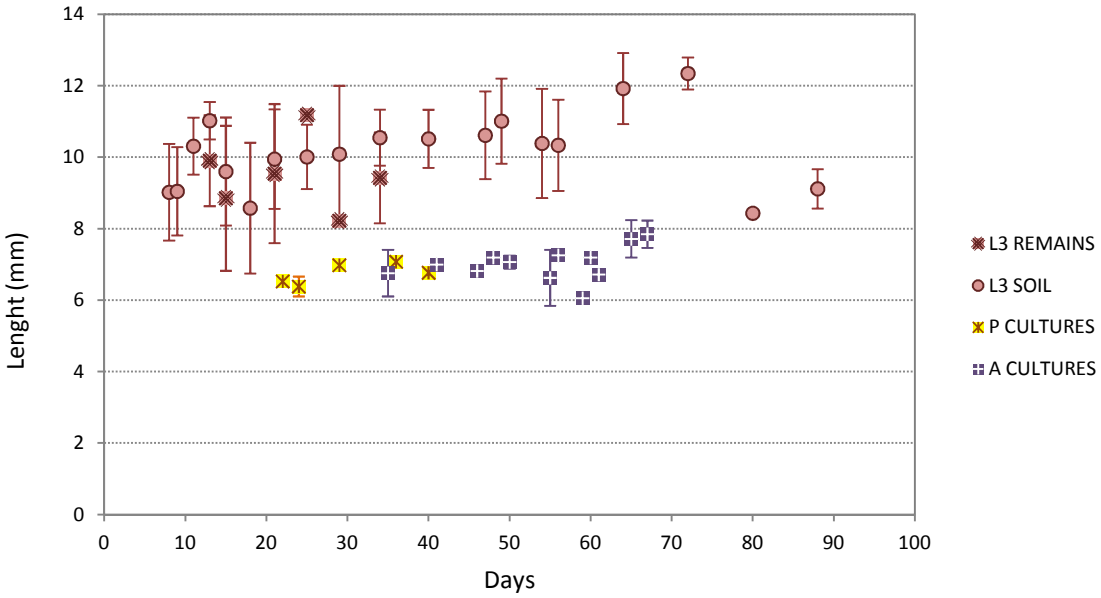


Figure 38: *Hydrotaea ignava*. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, pupae (P) in yellow, and teneral adult (A) in purple.

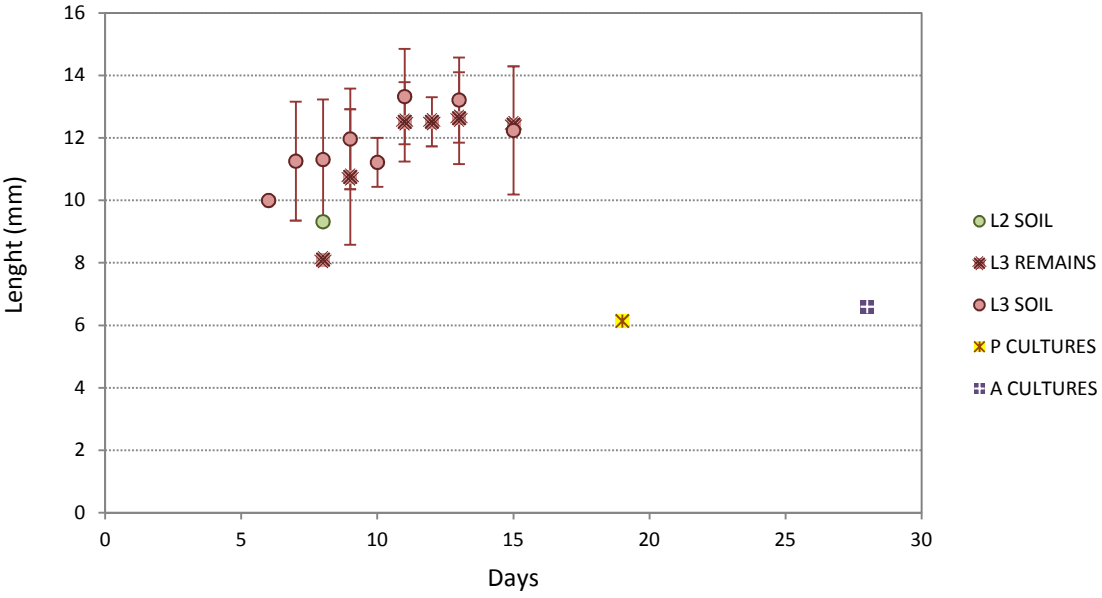


Figure 39: *Hydrotaea similis*. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, pupae (P) in yellow, and teneral adult (A) in purple.

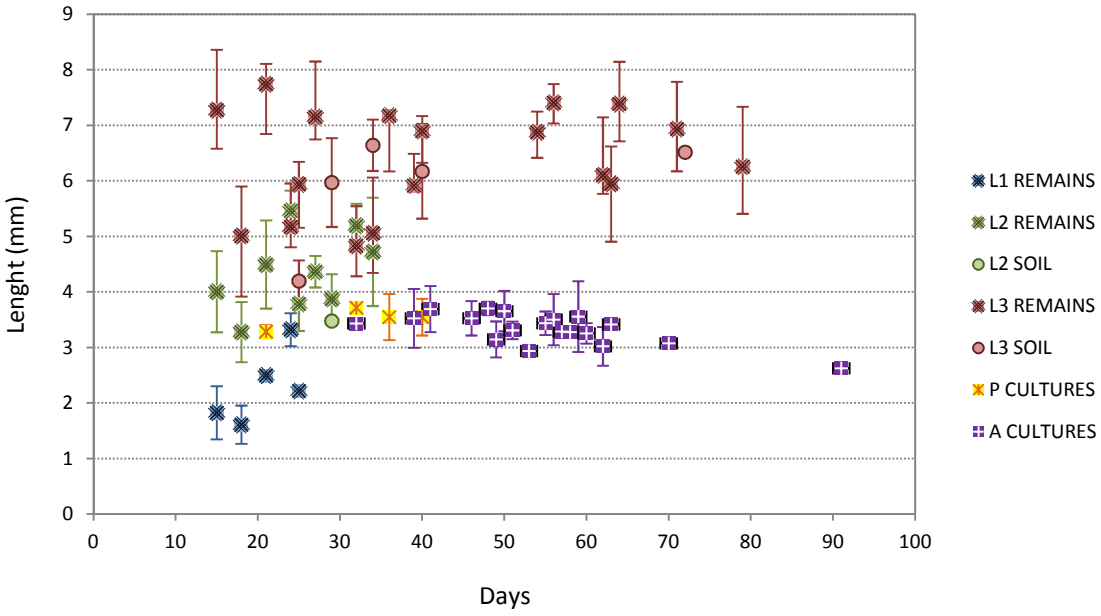


Figure 40: Piophilidae. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, pupae (P) in yellow, and teneral adult (A) in purple.

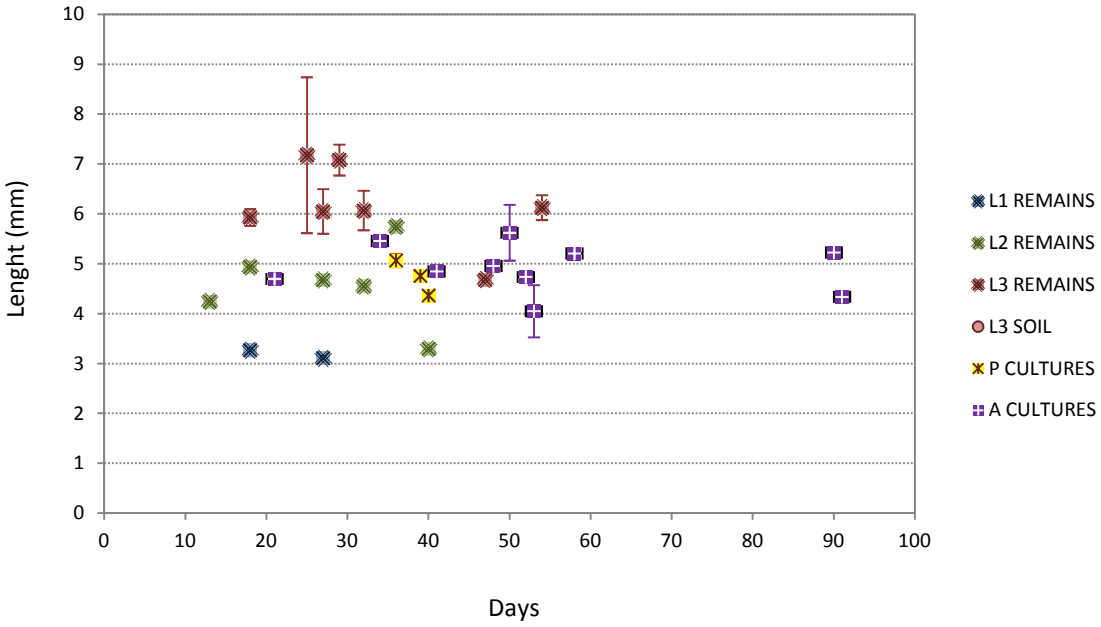


Figure 41: Sepsidae. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: second instar (L2) in green, third instar (L3) in red, pupae (P) in yellow, and teneral adult (A) in purple.

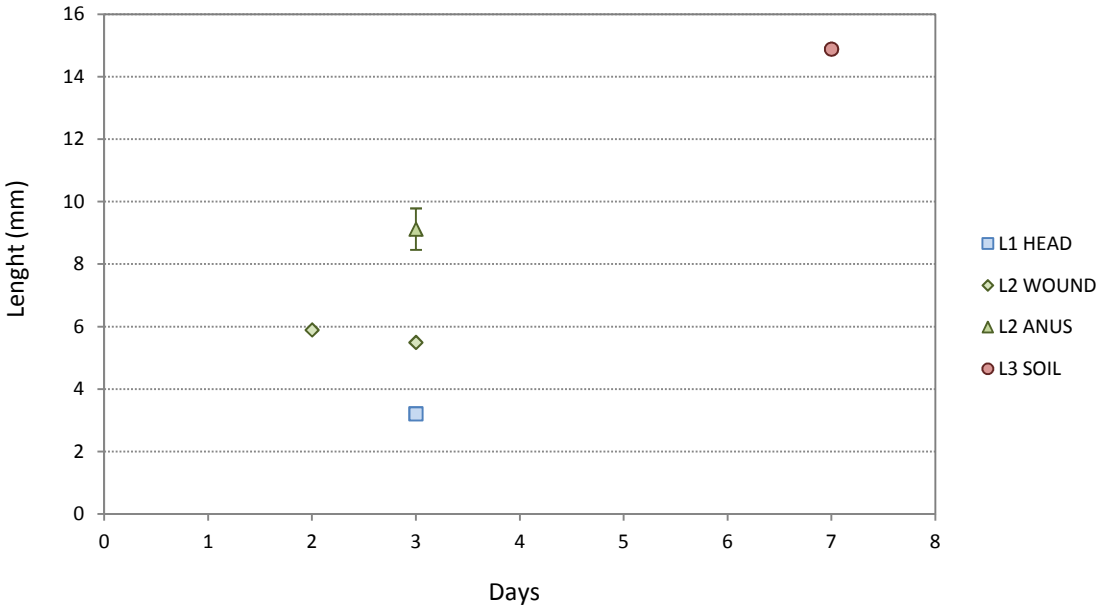


Figure 42: Sarcophagidae. Average length of larvae (± standard deviation) from different days and zones. Larval instar is also specified: first instar (L1) in blue, second instar (L2) in green, and third instar (L3) in red.

◈ Discussion

9 Microclimatic Conditions

Ambient temperature of carcass placements recorded with the i-buttons was found to be closely resembled to the average temperature of the study area recorded with DC datalogger (see Table 13 and Chapter 5). Carcass C2 and C3 were particularly different in 2010, with a difference of 4ºC between them and 1-3ºC with respect to other carcasses. These differences can profoundly affect fly larvae development. For instance, Niederegger *et al.* (2010) obtained adults of *Lucilia sericata* after an average of 120 hours at a constant temperature of 29ºC, but the same species were unable to pupate at 13ºC and even failed to hatch when trying to rear them at laboratory under fluctuating temperature between 5 and 29ºC (average of 17ºC). Extrapolating Niederegger *et al.* (2010) results to field conditions, where larvae suffer from competition, predation and parasitism, implicates that the minimum difference on temperature can imply an advantage for one species that can imply the displacement or no development of other species.

In agreement with previous studies, microenvironmental temperature was influenced by heat emissions of larval aggregations or larval masses. It is worth noting that carcass temperature was recorded only on the head area, and therefore gives information on maggot masses formed in that area especially during the first days of carcass exposure. Nevertheless, the effect is notorious in all the carcasses (see Figures 27 to 30). The temperature on carcass occasionally raised 25.5ºC above surrounding ambient temperature. This huge increment on carcass temperature was previously reported by GRASSBERGER & FRANK (2004), who found an increment of 25ºC, and was coincident with the period of activity of blowfly species on carcasses (see also Figure 31). Larval masses also showed small differences on their average temperature values between carcasses, but these differences could have been a consequence of the influence of several factors, such as quantity of larvae forming the mass, environmental temperature and other factors influencing beneath (CHARABIDZE *et al.*, 2011).

9 LARVAL SUCCESSION AND DEVELOPMENT

Larval succession on carcasses was in line of adult succession pattern (see Chapter 6), and with previous studies performed in similar biogeographic areas (for instance, MATUSZEWSKI *et al.*, 2008; PRADO E CASTRO *et al.*, 2012).

Blowfly maggots were the most abundant breeders on carcasses, as previously reported by several authors (CASTILLO MIRALBÉS, 2002; GRASSBERGER & FRANK, 2004; MATUSZEWSKI *et al.*, 2008; PRADO E CASTRO *et al.*, 2012). The rapid colonization by blowflies, which took place within minutes after exposure and was favoured by the temperate ambient temperature, allowed the collection of eggs and small larvae since the first day of sampling. The collection and identification of these specimens is a crucial step on legal investigations. However, identify those specimens to species level could be a very difficult task, as it is necessary a specific training and the use of a scanning electron microscope (SZPILA *et al.*, 2008; VILLET & AMENDT, 2011) or DNA-based identification tools (HARVEY *et al.*, 2008) that were not available for this research.

The first maggots that could be identified to species level were *Lucilia caesar* and *Chrysomya albiceps*, which were also the most abundant ones in carcasses and also in cultures. Both species were also found as dominant on the summer trial performed in Lisbon area by PRADO E CASTRO *et al.* (2012). Although third instar larvae of *Chrysomya albiceps* were collected with one day of delay with respect to *Lucilia* species, it was the first species to emerge as adult in cultures. This is related to the competitive and predator behaviour of *Chrysomya* species, which can clear a corpse from all earlier colonizers (MONEO & SALOÑA, 2009; GRASSBERGER & FRANK, 2004). *Lucilia illustris* and *Calliphora vomitoria* were also abundant on carcasses, but had a more discrete occurrence on cultures, as few adults were obtained.

Similar to MATUSZEWSKI *et al.* (2008) results, Sarcophagidae did not play an important role on cadaveric reduction, as there were barely four days in which larvae were collected. However, this shortly appearance could be of special importance on real cases, giving them the role of early forensic indicator, so it is important to focus on larvae residency patterns on future research to complement the information of adult residency pattern (see Chapter 6).

Another similarity with that study and other of nearby regions (CASTILLO MIRALBÉS, 2002; GARCÍA ROJO, 2004; GRASSBERGER & FRANK, 2004; PRADO E CASTRO *et al.*, 2012) was the role of Muscidae species: they were detected later than Calliphoridae species. *Hydrotaea similis* and *Hydrotaea ignava* were the most abundant species. It is worth noting that the latter species, *Hydrotaea ignava*, showed marked overlap of several generations of larvae present on carcasses, as L3 larvae were still found on carcass surroundings several weeks after the rearing of adults in cultures (Figure 38). This overlap can have an impact on PMI estimations, as if adults or pupae of the first generation are not collected (as for instance in cases of carcass movement), it may end with an underestimation of PMI. This reminds also the importance of collection procedures for entomological samples in a legal investigation, in order to have a representative sample of the faunal population present on the remains without getting unnoticed any valuable envidence (BYRD *et al.*, 2010). On this regard, BYRD *et al.* (2010) describe a detailed proper collection procedure, which includes a surface collection from the body at the scene, collect from under the

body and its surrounding once the remains are removed, and sampling from within the tissues of the body during the autopsy.

An interesting difference with previous results was the abundance of immature specimens of Fanniidae species. In our case, *Fannia manicata* was the most abundant species of the family, being very similar in number to both *Hydrotaea similis* and *Hydrotaea ignava*. However, their presence seems to be scarce on studies performed on summer by PRADO E CASTRO *et al.* (2012), who found a similar number of Fanniidae adults than on this study, but only reported collection of eggs from bloated to advanced decay. This difference may be a consequence of different environment used for the study and the preference of some species of Fanniidae for urban environments (CARVALHO, 1997): PRADO E CASTRO *et al.* (2012) research was performed at a small woodland park inside an urban perimeter whereas present report was run inside a Natural Park. Moreover, the experimental design was also different, as PRADO E CASTRO *et al.* (2012) used a modified Schoenly trap that keep carcasses in a more protected environment, whereas in current research carcass were directly exposed and arthropods handly collected. However, as numbers on the abundance of instars were not detailed by PRADO E CASTRO *et al.* (2012), this explanation should remain under revision until more information is obtained on this regard.

Once blowfly larvae had left the carcasses, as a consequence of their migratory behavior, members of the Piophilidae family started to acquire importance and became the dominant family of advanced stages of decomposition (D11-D91). Their larvae breed mainly on high-protein resources, as for instance decomposing fungi and plant matter or carrion of vertebrate animals (PRADO E CASTRO & GARCÍA, 2010), and were easily distinguishable due to the skipping behavior (MARTÍN-VEGA, 2011), being frequently observed jumping above carcass remains.

Regarding Sepsidae family, although it was not a very abundant group, their presence on latter stages of decomposition (D13-D90) was more or less constant. It is interesting the complete absence of them on summer trial of PRADO E CASTRO *et al.* (2012).

9 Implications in Forensic Entomology

Larval succession was found more useful than adult succession in some cases due to the close association between larvae and carcasses, such as in cases of carcass movement (Matuszewski *et al.*, 2010b). Results gained in this study constitute the first attempt to describe both larvae development and their succession on field conditions in the northern maritime facade.

The general pattern of larval succession was in line with results obtained with adult specimens (Chapter 6). Calliphoridae species were the most important ones in the reduction of carcasses, arriving within minutes of exposure and generating dense populations of maggots that consumed the carcasses in a few days. Maggot mass had a great impact on carcass temperature, which contributed to accelerate larval development within a carcass. More studies are needed to implement the knowledge about how larval mass affect PMI estimation, in special during the early post-mortem period, as age of blowfly maggots is a key point on early PMI estimation (Amendt *et al.*, 2007).

Muscidae were well represented with seven species, and Fanniidae were also collected during the early post-mortem period, as soon as Calliphoridae species started to migrate out of carcasses to pupariate. The late post-mortem period was characterized by larvae of Piophilidae and Sepsidae.

It is worth noting that for most of these taxa, forensically useful models are lacking even for well-studied taxa of carrion related arthropods (Matuszewski *et al.*, 2010b). Therefore, more development studies should be conducted with other species of importance in carcass reduction in order to improve PMI estimations. In this way, the Laboratory of Entomology of the Institute of Forensic and Social Medicine has recently developed Forenseek, a software to facilitate forensic entomology sample analysis, including also a database of development data of forensically important species (https://www.forenseek.org/beta/). In addition, this thesis includes novel information about development of some most abundant Dipteran species on carcasses under variable/natural conditions.

Another gap in forensic research is that development models under field conditions are, to the best of our knowledge, almost non-existent. NIEDEREGGER *et al.* (2010) performed a laboratory trial rearing different species of forensically important flies under fluctuating temperatures, and found that results did not correspond to the development under the resulting mean constant temperature. Much work is needed on this regard, and results presented here aim to contribute to the baseline database for larvae development under field conditions in our region and other with similar characteristics.

Finally, the effect of investigator disturbance, regularly taking the largest individuals from the carcass (or bait in the case of laboratory experiments) has not been evaluated on this research. Previous studies analysed this kind of effect on the collection of adult samples (JONG & HOBACK, 2006) or carcass mass loss and temperature (JONG *et al.*, 2011). In addition, WELLS *et al.* (2015) analysed the effect of subsampling the largest larvae or entire age cohorts on development predictive models under laboratory conditions. None has been conducted up today on field conditions. Therefore, future research should be focused on the sampling methodology employed in development experiments, being them performed under laboratory or field conditions.

General Discussion

One worthwhile task carried to a successful conclusion is worth half-a-hundred half-finished tasks.

Malcom S. Forbes

9 Arthropod of Forensic Interest (Chapter 4)

It is generally accepted that there is nowadays no reliable analytical tool to determine the exact period of time elapsed since death. For a correct application of the methods of estimating PMI, it is essential a correct identification of the carrion-related fauna, which successional patterns are of outmost importance to answer questions such as *"When did the death take place?"* (Schoenly *et al.*, 1992). What is usually done in Forensic Entomology is an estimation of the period of arthropod activity on a body (Goff, 2010). This approximate measure of a post-mortem interval (PMI) can be based on either the development rate of the species involved or their succession on the body (Gennard, 2012).

As carrion-related arthropod community varies considerably according to biogeographic areas, season of the year or type of habitat among others (Matuszewski *et al.*, 2008), those patterns are locality-specific, which is a problem for many regions without this knowledge. The assessment of death chronology must indeed correlate both environmental conditions and insect species (Campobasso *et al.*, 2001). In this line, with this study we establish solid grounds to develop a sound model to serve as a reference for future forensic research in the Basque Country and similar biogeoclimatic areas.

Carrion fauna has been found to be very diverse and quite variable. Just among adults, there have been 16 orders identified represented in 7837 specimens, being the most important those of the class Insecta (more than 97% of the total), especially Diptera and Coleoptera (both make up about 94% of the total fauna collected). There is also an important amount of new records among these two classes (50 species, including 2 new species for Science (*Crossopalpus* sp. n. (nr. *nigritellus* and *aeneus*) and *Drapetis* sp. n. (group *exilis*) (Diptera: Hybotidae)). This denotes the low level of knowledge of the carrion fauna in our region previous to this research.

In order to summarize decomposition patterns and to allow readers to compare with similar research, the whole process was divided into the five *clear-cut* stages of decomposition proposed by Payne (1965), although the dinamic of carrion fauna did not support this approach.

Fresh stage was the period when a higher degree of dominance and less diversity were found, as very few species act as colonizers (mainly blowflies). Dry stage was, by contrast, the most diverse one, which could be expected as it was the longest stage of decomposition (the probability of collecting more species raised up with sampling time), and dominance of first colonizers disappeared, allowing other species to arrive to the carcass and to occupy the new niches that had been generated through the decomposition process. It should be noticed that, although Margalef index was included, it is strongly influenced by sampling effort, so Fisher index, less influenced, is a more objective calculation of diversity in this kind of research.

Additionally, β diversity analysis determined that changes from fresh to bloated stage occurred with minimal variations regarding the identity of the families collected, but with major changes in the abundance of each family (dominant species became less important in total abundance of individuals with the arrival of new species during the ongoing of bloated stage). This family replacement rate was higher in latter stages, although the abundance of each one did not vary significantly.

9 Is it Safe to Choose the Closest Weather Station (Chapter 5)

The temperate climate of the study area favoured the rapid colonization of the carcass in summer, although the abundant rainfall in the area during the first experiment year hampered the arrival of necrophagous insects and, therefore, the beginning of carrion-related arthropod succession. This fact reinforces the necessity of recovering information not only of temperature records in the area, but also other factors that could have influenced on the process, such as rainfall or strong wind among others. Traditionally, this kind of information is taken from the nearest Weather Station. But even if one of them is close by, extrapolation can incur in errors due to differences in vegetation cover, air drainage, slope exposure or many other factors, including technical problems such as malfunction or breakage.

To solve this matter, an analysis was made about the degree of appropriateness of the three nearest National Weather Stations in distance to the study area. Results supported that choosing the nearest one was the best option in this case, although in all cases statistical significant differences were found. Nevertheless, what would happen in case of having two Weather Stations at equal distance? Or in case of breakage? The calculation of the Effect Size of differences between the Weather Station and the body discovery site temperature records was found to be a reliable technique to evaluate the significance of those differences and the error assumed in PMI estimation, aiding the technician to choose more objectively. For instance, during this study, nearest Weather Station to the study area had no records for several weeks for unknown reasons, so the second and third Weather Stations in distance were also analysed, giving conflicting results depending on the kind of data used for comparison (hourly or daily average records). The application of the Effect Size showed that is not necessarily the nearest one the best option, but the nearest one in a similar environment: the second Weather Station in distance is placed inside a valley and performed worst Effect Size than the third one, which is placed in a mountain environment, more similar to study area.

9 Arthropod Succession (Chapter 6)

The process of colonization, development and species replacement is strongly influenced by climate, being the temperature of outmost importance. It is known that the more suitable the weather is the more number of arthropods is involved, having a consequence on the duration of the whole process and each stage of decomposition. In some cases, some stages may completely disappear or may be not noticeable, as happened with bloated stage in 2 out of ten of the carcasses. Several authors had previously explained it as a consequence of the high activity of insects, but this is not the case to which we refer. On those 2 carcasses with no observed bloating, oviposition was lower and air temperature higher in comparison with other carcasses, probably making bloating more notorious and shortened, so probably the methodology employed, based on daily sampling, difficulted its observation.

Getting back to adult succession on carcasses, the period in which each taxon was present during the decomposition of piglet carcasses were found to follow a predictable sequence, although they did not form closed groups attending to the stage of decomposition in which each carcass was. What is derived from data here presented was in line with the *Gleason's continuum view* of community structure. In other words, the onset of each stage does not depend only on ambient temperature and the arthropod community itself, being another factors not considered in this research probably influencing beneath (temperature of tissues, wet, soil properties, insolation, slope, etc.).

However, the fact that discrete boundaries were not found did not conflict with the fact that some species were associated with a certain period of time of the decomposition process (MOURA *et al.*, 2005). Our results defined three succession waves through decomposition attending the arthropod community: an initial period of colonization, with the arrival of species such as *Lucilia caesar* and *Musca autumnalis*; an intermediate period characterised by a continuous replacing of species and niche overlapping; and a last period characterised by late arrivals, such as *Nemopoda nitidula*, *Parapiophila vulgaris*, *Spelobia luteilabris* and *Spelobia clunipes*.

Apart from that, succession of adult arthropods was in line with previous studies, although some important differences were found attending to the role of some forensically important taxa. The most noticeable was the practical absence of Sarcophagidae in the area, and the predator behaviour observed in Geotrupidae. Important differences were also found at species level. As an example, *Lucilia caesar* was the most abundant species colonizing the carcasses, as well as it has been reported from central Europe countries; Poland (MATUSZEWSKI *et al.*, 2008); Germany (ANTON *et al.*, 2011; REIBE & MADEA, 2010); or Portugal in spring (PRADO E CASTRO *et al.*, 2012). By contrast, *Lucilia sericata* has been previously reported as the most abundant species in most of the studied regions of Spain (CASTILLO MIRALBÉS, 2002; GARCÍA-ROJO, 2004; MARTÍNEZ – SÁNCHEZ *et al.*, 2000a) and other mediterranean areas (VANIN *et al.*, 2008). All these findings confirm the dissimilarities between northern maritime facade and other biogeographic areas of the Iberian Peninsula, being

the results of this research more similar to other works performed in central European regions with more similar climate.

This kind of experiments results on large inventories of taxa (MATUSZEWSKI *et al.*, 2010c). Therefore, a proper identification of characteristic or indicator species is urged to improve the efficiency of future forensic investigations based on entomological evidence. In this way, residency pattern analysis proposed by MATUSZEWSKI *et al.* (2010b) allowed the selection of the forensically most useful adults for each region, although it has some limitations when there are a low number of replicate samples. These analyses found that 17 taxa can be included in the baseline succession model for the northern maritime facade of the Iberian Peninsula, being *Hydrotaea similis, Coproica hirticula, Anoplotrupes stercorosus* and *Necrodes littoralis*, the most useful adults for future succession research in Forensic Entomology in our region.

9 LARVAE SUCCESSION (CHAPTER 7)

Development data of larvae of colonizer species can be very useful on PMI estimations, especially when used in conjunction with patterns of arthropod succession (TABOR, 2009). In fact, early after death, maggot development is one of the main sources of information (AMENDT *et al.*, 2007).

Data presented here briefly summarize the development pattern of the most abundant species associated to pig carcasses and reared under field conditions. In many cases, they showed marked overlap of several generations of larvae present on carcasses, which may end with an underestimation of PMI if oldest individuals are not collected. This reminds the importance of following a proper collection procedure before, during and after removing a carcass (BYRD *et al.*, 2010).

Results regarding larval succession are in agreement with patterns observed in adult succession and also with previous studies of regions with similar climate (MATUSZEWSKI *et al.*, 2008; PRADO E CASTRO *et al.*, 2012). Calliphoridae species were the first to arrive and most abundant ones, forming large larval masses

that increased ambient temperature up to 25.5ºC. When blowfly maggots left the carcasses to pupariate in soil, Muscidae and Fanniidae species acquired importance on carcass reduction. The late post-mortem period was dominated by Piophilidae and Sepsidae, as well as it was found during adult succession.

It was found a gap on standard sampling methodology employed, as the effect of investigator disturbance, regularly taking the largest individuals from the carcass (or bait in the case of laboratory experiments) was not analyzed. It should be a core objective for future research.

9 Final considerations

Forensic Entomology is a relatively new field that is still developing in many parts of the world. The marked differences found in this study with regard to those from nearby regions underline the need to investigate other areas, seasons and/or habitats, in order to have barely acceptable baseline studies of carcass decomposition in this region. This is a crucial point, as results here reported differ considerably from those reported previously from nearby regions of the Iberian Peninsula (Castillo Miralbés, 2002; Martín-Vega, 2011; Prado e Castro *et al.*, 2012), being more similar to central Europe areas (Kočárek, 2003; Matuszewski *et al.*, 2008). Additionally, a set of mathematical and ecological tools (i.e., Cohen's effect size or residency patterns) not often applied in forensic entomology would aid forensic technicians to better estimate the PMI attending entomological evidence. Their inclusion in common practice protocols is recommended.

Additionally, knowledge of development rates for most of the species involved on carcass reduction are still lacking, being models performed under field conditions almost non-existent. This also increases the uncertainty of PMI calculations and reinforces the need of further studies on this regard. In this line, results presented here could serve as reference data for future research, as they include not only the presence/absence of species on their immature stages, but also their development patterns under field conditions.

Finally, Forensic Entomology, as a field that focuses on the most diverse and abundant phylum of Animal Kingdom, needs the collaboration of researchers and technicians from different areas of expertise. This multidisciplinary approach is the key point for the development of Forensic Entomology.

CONCLUSIONS

Quickly jump to conclusions
rarely leads to happy landings.

S. Siporin

- This study reports 255 species of carrion related arthropods, from which 97% are insects.

- The important amount of new records (50 species, including 2 new for Science) denotes the low level of knowledge of the carrion fauna in the area previous to this study.

- Findings on arthropod succession confirm the dissimilarities between northern maritime facade and other biogeographic areas of the Iberian Peninsula, being our region more similar to regions with more similar weather in central Europe.

- Analysis of the appropriateness of National Weather Stations suggests that other factors than distance to study area might play an important role on PMI estimation, which could be improved considering similar environment (vegetation, altitude...) into/between the nearest stations.

- The calculation of the Effect Size of differences between the Weather Station and the body discovery site temperature records seems to be a reliable technique to evaluate the significance of differences between them.

- Few species colonize carcasses just after decease, reporting lower values of diversity index and higher values of dominance index. This situation turned to the opposite with the ongoing of the process and the arrival of new species that cohabit with the colonizers whenever food is available (this contributes to a higher diversity and lower dominance on latter stages).

- Regarding the decay process, although classical stages were almost always easily differentiated attending the physical condition of carcasses, adults of arthropod community did not form close groups, so no discrete boundaries were clearly appreciated between the ending of a specific stage of decomposition and the onset of the next one.

- Attending the arthropod community, only three different succession periods could be defined: colonization, intermediate period, and dry.

- Residency pattern analysis was used to determine the usefulness of the species involved on the process. Four possible forensic indicators as adults for PMI estimation in our region were identified: *Hydrotaea similis* and *Necrodes littoralis*, which appeared related to Active decay; and *Coproica hirticula* and *Anoplotrupes stercorosus*, more related to Advanced decay.

- Larval succession was in line with adult appearance on carcasses. The first immature specimens belonged to the family Calliphoridae and, to a lesser extent, Sarcophagidae. On a second stage, Muscidae and Fanniidae were the main responsibles of carcass reduction and finally, the later stages were dominated by Piophilidae and Sepsidae.

- Results presented here report data of succession and development of most abundant species of this trial. They can be used as reference for future studies on this regard, but further studies are still needed on several matters. On one hand, it is important to keep investigating other seasons and/or habitats to have scarcely acceptable baseline knowledge of carcass decomposition in our region. On the other hand, this report needs to be updated with new laboratory experiments focused on larval development with availability of different substrates, interspecific competition and/or under variable environmental conditions to create forensically useful models of larval development.

Conclusiones

- Se presenta una relación de 255 especies de artrópodos encontradas asociadas a restos cadavéricos, de los cuales el 97% son insectos.

- Se recopila una cantidad importante de nuevas citas (50 especies, incluyendo 2 nuevas para la Ciencia), lo que denota el bajo nivel de conocimiento que se tiene de la fauna cadavérica en la cornisa Cantábrica.

- El modelo de sucesión de artrópodos observado confirma las diferencias entre la Cornisa Cantábrica y otras regiones de la península Ibérica, siendo nuestra región más similar a otras áreas centroeuropeas con clima de tipo eurosiberiano.

- El análisis de cuán apropiados son los datos de las estaciones meteorológicas más cercanas al área de estudio sugiere que hay más factores aparte de la distancia que pueden ser importantes en las estimaciones del IPM. Este análisis podría mejorar considerando, además de la distancia, que la estación esté en un ambiente similar al del area de estudio en relación a cobertura vegetal, altitud, etc.

- El cálculo del "tamaño del efecto" (Effect Size) de las diferencias entre los registros de temperatura del lugar donde se descubre un cuerpo y la estación meteorológica más cercana promete ser una técnica útil a la hora de evaluar el significado de las diferencias encontradas entre ambos puntos.

- Son pocas las especies que colonizan los cadáveres, tal como se refleja en el bajo valor de los índices de diversidad y el alto valor de los índices de dominancia. La situación varía a medida que avanza el proceso, con la llegada de nuevas especies que cohabitan con las primeras colonizadoras, siempre que haya alimento disponible. Esto es, hay mayor diversidad y menor dominancia en las últimas etapas de reducción cadavérica.

- En relación al proceso de descomposición cabe decir que, a pesar de ser reconocidas de forma relativamente sencilla las fases clásicas de

descomposición atendiendo al estado físico del cadáver, no se han encontrado cambios abruptos entre el final de una fase y el inicio de la siguiente atendiendo a la comunidad de artrópodos.

- Atendiendo a los adultos de la comunidad de artrópodos, solo se han diferenciado tres fases de sucesión: colonización, una etapa intermedia, y una última etapa de restos esqueléticos.

- Se ha utilizado el análisis de los patrones de residencia de los adultos en los cadáveres para determinar la utilidad de los mismos como indicadores forenses. En el presente caso, se han identificado cuatro indicadores para nuestra región: *Hydrotaea similis* y *Necrodes littoralis*, los cuales aparecen relacionados con la fase de descomposición activa; y *Coproica hirticula* y *Anoplotrupes stercorosus*, más relacionados con la fase de descomposición avanzada.

- La sucesión larvaria sigue el mismo patrón que la de los adultos. Los primeros especímenes recogidos pertenecen a la familia Calliphoridae y, en menor abundancia, Sarcophagidae. En una segunda fase, las familias Muscidae y Fanniidae fueron las principales responsables de la reducción cadavérica. Finalmente, las últimas etapas estavieron dominadas por las familias Piophilidae y Sepsidae.

- Se reconoce la necesidad de continuar con esta investigación desde diferentes puntos de vista. Por un lado, es importante seguir estudiando otras estaciones del año y/o hábitats, de forma que pueda llegarse a establecer una base de datos para nuestra región. Por otro lado, este trabajo debe completarse con nuevos estudios de desarrollo larvario en laboratorio que contemplen aspectos como disponibilidad de diferentes substratos, competición interespecífica y/o condiciones ambientales variables, de forma que se obtengan modelos de desarrollo útiles para las ciencias forenses.

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The less we read,
the more harmful it is what we read.

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APPENDIXES

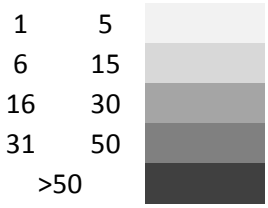
A good book
has no ending.

R. D. Cumming

Appendix I

In order to make the reading of Chapter 6 lighter, and also for easing the comprehension of it, succession tables were separated in this appendix.

Each table represents the sampling of each pig carcass, which goes from C1 to C5 in both years of the study: 2009 and 2010. They include the most important species, and their abundance along the sampling period is indicated both in total number of specimens and with a colour key as indicated:



Additionally, the stage of decomposition was also included:

- F: fresh
- B: bloated
- AcD: active decomposition
- AdD: advanced decomposition
- D: dry.

Table 15: Succession associated to pig carcass C1 in summer of 2009.

C1	STAGE	F	F	F	B	B	AcD	AcD	AdD	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
2009	DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	15	18	21	24	27	32	36	40	47	54	62	71	79	85	TOTAL	
CALLIPHORIDAE	*Calliphora vomitoria*	1			2		1																						4	
	Lucilia caesar	7	2	7	9	1	3	4	3	1						1	2	2	1										43	
	Chrysomya albiceps														5														5	
	Other Calliph. species	2		1														2											5	
MUSCIDAE	*Azelia sp.*							3								1		1											5	
	Hydrotaea dentipes						2	3																					5	
	Hydrotaea ignava						1	2	1															1					5	
	Hydrotaea similis	1			1	1	2	1	2																				8	
	Musca autumnalis				1				1																				2	
	Other Muscidae species	1		1						1								1			1								5	
FANNIIDAE	*Fannia* sp.							2	4																				6	
	Other Fanniidae species						1										1	1											3	
SARCOPHAGIDAE	*Sarcophaga sp.*							1								1													2	
SPHAEROCERIDAE	*Chaetopodella scutellaris*							2								1													3	
	Coproica hirticula									1																			1	
	Coproica vagans							1									1												2	
	Leptocera caenosa																1												1	
	Opalimosina liliputana								1																				1	
	Spelobia clunipes			1						1							5							1					8	
	Spelobia luteilabris						1									1	1												3	
	Telomerina levifrons								1																		1		2	
	Trachyopella lineafrons						1	1	1	1																	1		5	

	Other Sphaeroc. species			1			2	3	1	1							8						1					17
PIOPHILIDAE	*Liopiophila varipes*						3	10	9													1						23
	Parapiophila vulgaris									1						1	2										1		5
	Protopiophila latipes						1		2	1							1												5
	Stearibia nigriceps							14	4	2																1			21
SEPSIDAE	*Meroplius minutus*						1		2								1												4
	Nemopoda nitidula																2												2
	Other Sepsidae species	1							1								1												3
PHORIDAE	*Megaselia brevicostalis*									1								1											2
	Other Phoridae species									1							1												2
SCIARIDAE	*Scatopsciara multispina*																										1		1
	Other Sciaridae species																											1	1
Other Diptera	Other Diptera species							1	1								2												4
GEOTRUPIDAE	*Anoplotrupes stercorosus*		1			1		1	1			1		3					2		5								15
	Trypocopris pyrenaeus						1		1	1																			3
	Other Geotrup. species																	1											1
SILPHIDAE	*Necrodes littoralis*					3	8	5																					16
	Other Silphidae species					2																							2
STAPHYLINIDAE	*Bisnius fimetarius*											2							1										3
	Creophilus maxillosus							1	2																				3
	Other Staph. species			1			1			1				2					3	1	1	1	3	2	1				17
Other Coleoptera	Other Coleoptera species						4			1		2						1	1		2		1	14		1	4	6	37
HYMENOPTERA	Parasit. Hym.		1	1		1												1					1		2		1	1	9
	FORMICIDAE		1					1										1	1					1	1			1	7

Table 16: Succession associated to pig carcass C2 in summer of 2009.

C2	STAGE	F	F	F	B	AcD	AcD	AcD	AcD	AcD	AdD	AdD	AdD	AdD	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
2009	DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	15	18	21	24	27	32	36	40	47	54	62	71	79	85	TOTAL
CALLIPHORIDAE	*Calliphora vomitoria*				2	1	4	3	3	2			2	3															20
	Lucilia caesar	8		1	7	1		4	1	8	4	2	1	2	5	1	3	2	1	9		4	5						69
	Chrysomya albiceps						3	3	2	1	9	8	1	1	3		1												32
	Other Calliph. species										1		1	2				1		2	1	2							10
MUSCIDAE	*Azelia sp.*					1		4	2				3	1			1												12
	Hydrotaea dentipes									1			2		1														4
	Hydrotaea ignava								1	1	2													1					5
	Hydrotaea similis	4			2		1	5	5	3	1	2	2	1			1												27
	Musca autumnalis												1																1
	Other Muscidae species	1		4		1	4	1	2	1	3	2	9		3		3			1			3	1					39
FANNIIDAE	*Fannia sp.*				1		2	15	24	21	3	2	16	1		1			1				1						88
	Fannia manicata				1			1																					2
	Other Fanniidae species												4																4
SARCOPHAGIDAE	*Sarcophaga sp.*													1		4						1							6
	Ravinia pernix																2												2
SPHAEROCERIDAE	*Chaetopodella scutellaris*			3					1	1					1	1													7
	Coproica hirticula							3	1	3		1	1	1		13													23
	Coproica pusio											2		1		1	1												5
	Leptocera caenosa											1																	1
	Opalimosina liliputana																									1	1		2
	Spelobia clunipes								1		1									1			2						5
	Spelobia luteilabris		2						1				1						2				1			1			8
	Telomerina levifrons									2										1									3

	Trachyopella lineafrons														2	1														3
	Other Sphaeroc. species			6				1	1						2	1	1			1	1			1			1		1	17
PIOPHILIDAE	*Liopiophila varipes*				3		6	14	13	8		9	22	3	1	4			1											84
	Parapiophila vulgaris									1		1	2						1						2					7
	Protopiophila latipes							1			1	9	1			3	2													17
	Stearibia nigriceps						2	19	15	36	55	84	137	20	8		12	1												389
	Other Piophilidae species						1		1			1	1																	4
SEPSIDAE	*Meroplius minutus*							2	4	8	3	13	13	1	2	4	3	2	1											56
	Nemopoda nitidula																								1					1
	Other Sepsidae species							1	4	3	1	1	2	1		1		1						2						17
PHORIDAE	*Megaselia brevicostalis*									1		1	2	4	1		1													10
	Other Phoridae species	1										1																		2
SCIARIDAE	*Scatopsciara multispina*																												1	1
	Other Sciaridae species																									1	2			3
Other Diptera	Other Diptera species				2			2	1	2		2					1										1	3		14
GEOTRUPIDAE	*Anoplotrupes stercorosus*									1			1		1		1								2					6
	Trypocopris pyrenaeus						1	1		2	2	2		2	1	1			1	1		2	1							17
SILPHIDAE	*Necrodes littoralis*				1	3	6	6	4	2	1		1																	24
HISTERIDAE	Histeridae													2						1										3
STAPHYLINIDAE	*Bisnius fimetarius*																								2	1				3
	Creophilus maxillosus									1					1															2
	Other Staph. species		1	1					1	1	1							1		1				2			2	5	2	18
Other Coleoptera	Other Coleoptera species				1			1		1	2				1									1	3	2	10	5	1	28
HYMENOPTERA	Parasit. Hym.	1		1		1	1	1		1	5																			11
	FORMICIDAE	1	4		2														1			1								9

Table 17: Succession associated to pig carcass C3 in summer of 2009.

C3	STAGE	F	F	F	B	B	AcD	AcD	AcD	AdD	AdD	AdD	AdD	AdD	AdD	AdD	AdD	AdD	AdD	AdD	D	D	D	D	D	D	D	D	
2009	DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	15	18	21	24	27	32	36	40	47	54	62	71	79	85	TOTAL
CALLIPHORIDAE	*Calliphora vomitoria*			3	5		1						1			2		1											13
	Lucilia caesar	10		7	6	2	3	4	1	1		2	1	2	4	2	1	8		10	7	1		2					74
	Chrysomya albiceps				2		7	4	5	3	3	3	2	4	1	4	1				1			1					41
	Other Calliph. species				1			1							1			1			1								5
MUSCIDAE	*Azelia sp.*														1														1
	Hydrotaea aenescens						1	3	3	1		1		1															10
	Hydrotaea dentipes						1									1				1	1								4
	Hydrotaea ignava								1	1					1	1													4
	Hydrotaea similis			1		1		1		1																			4
	Musca autumnalis								1						1			2											4
	Other Muscidae species							1	1		1		1								1	1							6
FANNIIDAE	*Fannia sp.*	1						4	3	1					1		2	1											13
	Fannia manicata																	2			1								3
	Fannia canicularis					1									1			1	1										4
SARCOPHAGIDAE	*Sarcophaga sp.*							1		3		1			1		2	1											9
	Ravinia pernix											1		1				1											3
SPHAEROCERIDAE	*Chaetopodella scutellaris*							1								1		2			5	1		1					11
	Coproica hirticula								1	2		1	1			2	2				1								10
	Coproica pusio							1				1		1															3
	Coproica vagans																2	1											3
	Leptocera caenosa								2																				2
	Opalimosina liliputana															1								1					2
	Spelobia clunipes											1						1		2	1								5

	Spelobia luteilabris																						1							1
	Trachyopella lineafrons									1					1									1			1			4
	Other Sphaeroc. species	1	1									1								2	2	1	2	1						11
PIOPHILIDAE	*Liopiophila varipes*						1	5	9	5			2		3	2	2	3			1									33
	Parapiophila vulgaris																				5			2	1					8
	Protopiophila latipes								2	3		2	1				2	2			3	1								16
	Stearibia nigriceps							18	22	40		26	9	12	18	25	19	3			3									195
	Piophila megastigmata							1		2		1																		4
SEPSIDAE	*Meroplius minutus*							1	2	2		2			2			3			5	1		1						19
	Other Sepsidae species	1						3	2	2			1	1			1	7		1	2			2	3	1				27
PHORIDAE	*Megaselia brevicostalis*									4			1	2	2	2		1				1								13
	Other Phoridae species		1				2	1		2											4									10
SCIARIDAE	Other Sciaridae species													1							1									2
Other Diptera	Other Diptera species						1		1			3		1							1				1	4		1		13
GEOTRUPIDAE	*Anoplotrupes stercorosus*					1		2						1				2		3	2		1							12
	Trypocopris pyrenaeus						3	2	1	4		3	1	1		1	2		2		2	1								23
SILPHIDAE	*Necrodes littoralis*			2	6	15	5	1	1																					30
	Nicrophorus humator										2					1	2		1											6
HISTERIDAE	Histeridae							1													4									5
STAPHYLINIDAE	*Bisnius fimetarius*							2		1														1						4
	Creophilus maxillosus						1	2	3											1										7
	Other Staph. species		2					1					1	1						1	1		2	2	2	5			18
Other Coleoptera	Other Coleoptera species									2	1	1								1	5	4	2	2	1	5	1	4		29
HYMENOPTERA	Parasit. Hym.				1	2	4		1	1	1			1		1		1						1						14
	FORMICIDAE	2	1	2																				1						6

Table 18: Succession associated to pig carcass C4 in summer of 2009.

C4	STAGE	F	F	F	B	B	B	AcD	AcD	AcD	AdD	AdD	AdD	AdD	AdD	AdD	D	D	D	D	D	D	D	D	D	D	D	D	
2009	DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	15	18	21	24	27	32	36	40	47	54	62	71	79	85	TOTAL
CALLIPHORIDAE	*Calliphora vomitoria*				2		2	4	2	1				1															12
	Lucilia caesar	8		5	7		8	6	1		4	3	4	3		4				8		1							62
	Chrysomya albiceps						2	1	3	1	1	1	2	1		1													13
	Other Calliph. species											1	2		1	1				1	1								7
MUSCIDAE	*Azelia sp.*																			4									4
	Hydrotaea dentipes					1									1					1									3
	Hydrotaea ignava								1					1									1						3
	Hydrotaea similis						2	7	7	3		2					1												22
	Musca autumnalis	2							1					1															4
	Other Muscidae species							2		2	2	1	2	1		1	2	2			1								16
FANNIIDAE	*Fannia sp.*							9	7	2	2		3	1			1												25
	Fannia canicularis				1												1												2
SARCOPHAGIDAE	*Sarcophaga sp.*										2					1													3
SPHAEROCERIDAE	*Chaetopodella scutellaris*		1								1			1							1								4
	Coproica hirticula									1	3																		4
	Coproica pusio										1	1																	2
	Coproica vagans									1																			1
	Opalimosina liliputana																			2							1		3
	Spelobia clunipes									1				1						2									4
	Spelobia luteilabris									1					1					1		2		1					6
	Telomerina levifrons																			1									1
	Trachyopella lineafrons									1			1																2
	Other Sphaeroc. species	1		1						1										3	1								7

PIOPHILIDAE	*Liopiophila varipes*				1			4	1	3	4	1							1													15
	Parapiophila vulgaris																				3											3
	Protopiophila latipes										5	1	2																			8
	Stearibia nigriceps							2	3	12	26		5	4	3	1	1															57
	Other Piophilidae species													1																		1
SEPSIDAE	*Meroplius minutus*								2	3	1		1	3							1	1										12
	Other Sepsidae species									5	1						2		1	3	1				3							16
PHORIDAE	*Megaselia brevicostalis*									1		1								1												3
	Other Phoridae species					1								1																		2
SCIARIDAE	*Scatopsciara multispina*									1			1							2												4
	Bradysia subrufescens																1															1
Other Diptera	Other Diptera species									1			1							1									1		1	1	6
GEOTRUPIDAE	*Anoplotrupes stercorosus*					1		1		1	1					2	1	1		1												9
	Trypocopris pyrenaeus						2	1	2					2	1	1		1	1	1	3	1										16
	Other Geotrup. species																							1								1
SILPHIDAE	*Necrodes littoralis*				3	1	5	1	3																							13
	Nicrophorus vespilloides				1							1		2																		4
HISTERIDAE	Histeridae									8				1																		9
STAPHYLINIDAE	*Bisnius fimetarius*									1	1											1										3
	Other Staph. species	1	1		2		1				1	1	1	1			1			1		1	1	1							14
Other Coleoptera	Other Coleoptera species					2			1						1							1	3	1	1		1				11
HYMENOPTERA	Parasit. Hym.					1			1			1					1			1											5
	FORMICIDAE																	1														1

Table 19: Succession associated to pig carcass C5 in summer of 2009.

C5	STAGE	F	F	B	B	B	AcD	AcD	AcD	AdD	AdD	AdD	AdD	AdD	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
2009	DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	15	18	21	24	27	32	36	40	47	54	62	71	79	85	TOTAL
CALLIPHORIDAE	*Calliphora vomitoria*	1			2	2	1						1								1								8
	Lucilia caesar	14		7	8	2	4	6	2	2	7	1	2	6		4	4	4	1	4	8								86
	Chrysomya albiceps						1	1	2	2	1	1			9		1												18
	Other Calliph. species	2		1	1					2		1			1		1												9
MUSCIDAE	*Azelia sp.*				1		1		1				1							2	1								7
	Hydrotaea dentipes			1					1							1													3
	Hydrotaea ignava						1		2					1		2	1					1								8
	Hydrotaea similis			1			8	5	2	1	1																		18
	Other Muscidae species	1			1		2	2	2	7	1	1		1			2	1		2	3		2						28
FANNIIDAE	*Fannia sp.*						1	3		2						1													7
	Fannia manicata							1									2												3
	Other Fanniidae species								1	1						1													3
SARCOPHAGIDAE	*Sarcophaga sp.*	1								1	1	1											1						5
SPHAEROCERIDAE	*Chaetopodella scutellaris*	1						1	5	9	17	5		11		12	5	5		1	5								77
	Coproica hirticula							1	1	13	3		2			2	3												25
	Coproica pusio										1	1		1						1									4
	Leptocera caenosa																				1								1
	Opalimosina liliputana													1			1	1		1						1			5
	Spelobia clunipes													1			1			1	2								5
	Spelobia luteilabris			1										1					1		1		1						5
	Telomerina levifrons								1					3						1	1								6
	Trachyopella lineafrons											1									1						1		3
	Other Sphaeroc. species				1		1	1		4	1	2	1	5		1	6	1		9	3		3						39

PIOPHILIDAE	*Liopiophila varipes*	1					1	6	5	3	1						1														18
	Protopiophila latipes								3	3	2			1	1	1	1														12
	Stearibia nigriceps						1	1	9	23	13	3	2	13		2	1														68
	Other Piophilidae species								1					1																	2
SEPSIDAE	*Meroplius minutus*							1	10	4	4			3	1	2	1				1										27
	Nemopoda nitidula	1										1		3			3	3													11
	Sepsis fulgens						2		1					1			1	1		4	1										11
	Other Sepsidae species						1			2				1		1	1	1	1		2										10
PHORIDAE	*Megaselia brevicostalis*											1				1	2														4
	Metopina perpusilla							1			1																				2
SCIARIDAE	Other Sciaridae species						1														1								1		3
Other Diptera	Other Diptera species			1						1				1											2	1					6
GEOTRUPIDAE	*Anoplotrupes stercorosus*											1	1	1			2				1										6
	Trypocopris pyrenaeus		1				1	3	3	3	2	1		1							1										16
SILPHIDAE	*Necrodes littoralis*						2	5	3																						10
	Nicrophorus vespilloides				1		1		1																						3
HISTERIDAE	Histeridae						1							1																	2
STAPHYLINIDAE	*Bisnius fimetarius*									3															1						4
	Creophilus maxillosus					1					1																				2
	Other Staph. species				2								1				1		1						1	3					9
Other Coleoptera	Other Coleoptera species									1					2					3				2	2			1		11
HYMENOPTERA	Parasit. Hym.		1				1	1	1		1						2														7
	FORMICIDAE																						1								1

Table 20: Succession associated to pig carcass C1 in summer of 2010.

C1	STAGE	F	F	B	B	AcD	AcD	AcD	AdD	AdD	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
2010	DAY	1	2	3	4	5	6	7	8	9	11	13	15	18	21	25	29	34	40	49	56	64	72	80	88	TOTAL
CALLIPHORIDAE	*Calliphora vomitoria*		1																							1
	Lucilia caesar	33	10	7	14	13	1	2	3		2					2										87
	Chrysomya albiceps												1	4	3											8
	Other Calliph. species		4						2			1				6	1						1			15
MUSCIDAE	*Azelia sp.*								2		2	3		1												8
	Hydrotaea dentipes		1		1			2				1														5
	Hydrotaea ignava						1														1					2
	Hydrotaea similis			3		1																				4
	Other Muscidae species	1							2		1		1		1		1				1					8
FANNIIDAE	*Fannia sp.*			1		6	1	1	8	1		1														19
	Fannia manicata					1	1	1	2		1															6
	Other Fanniidae species							1								1										2
SPHAEROCERIDAE	*Chaetopodella scutellaris*								1			1	3													5
	Coproica hirticula								2		1															3
	Coproica pusio												1													1
	Leptocera caenosa			1	1						2	1	3	2	1	2										13
	Opalimosina liliputana								3			2		1	2	3	1									12
	Spelobia luteilabris													2		1	1		1							5
	Telomerina levifrons								1		2	2														5
	Trachyopella lineafrons								1											2	1					4
	Other Sphaeroc. species								7		2	4	2	3	1	14	1									34
PIOPHILIDAE	*Liopiophila varipes*				1	1			14		5	6								2						29
	Parapiophila vulgaris											1														1

	Stearibia nigriceps								2			2																4
SEPSIDAE	*Meroplius minutus*								2	1		17	1	1		1												23
	Nemopoda nitidula									1		1																2
	Other Sepsidae species												1															1
PHORIDAE	*Megaselia brevicostalis*												1			1	1											3
	Other Phoridae species									1								1		2	1							5
SCIARIDAE	*Scatopsciara multispina*												4	2	1	1	2											10
	Other Sciaridae species																	2									2	4
Other Diptera	Other Diptera species	1		1				1		1		1		1	2	2	1	2	1	3	2							19
GEOTRUPIDAE	*Anoplotrupes stercorosus*			1	2	4	4	3	2	3	2		1				1	2						1			26
	Trypocopris pyrenaeus		1	1				2	1	1	1						3										10
SILPHIDAE	*Necrodes littoralis*					1	4																					5
	Nicrophorus vespilloides				1																							1
HISTERIDAE	Histeridae					2																						2
STAPHYLINIDAE	*Bisnius fimetarius*				1		1	1	1	2	2	1	1	1		1						1						13
	Creophilus maxillosus						1		1								1											3
	Other Staph. species		1		1	3	3			3			3		1	2	2	1	1									21
Other Coleoptera	Other Coleoptera species							4	2	1	2	2	1	3	2	4	2	2			1	1		1			28
HYMENOPTERA	Parasit. Hym.				1						1					1	1			2			1		2		9
	FORMICIDAE	1	4	1			1	2							1									1			11

Table 21: Succession associated to pig carcass C2 in summer of 2010.

C2	STAGE	F	F	F	B	B	AcD	AcD	AdD	AdD	AdD	D	D	D	D	D	D	D	D	D	D	D	D	D	
2010	DAY	1	2	3	4	5	6	7	8	9	11	13	15	18	21	25	29	34	40	49	56	64	72	80	88	TOTAL
CALLIPHORIDAE	*Calliphora vicina*	1										1			1											3
	Calliphora vomitoria	1						1																		2
	Lucilia caesar	12	24	10	9	7			2		1	1	4	4	3	2		1								80
	Chrysomya albiceps		1		1	4							1	3												10
MUSCIDAE	*Azelia sp.*										2			5												7
	Hydrotaea dentipes		3				1				2			3												9
	Hydrotaea ignava											2														2
	Hydrotaea similis			12	1	2	1												1							17
	Musca autumnalis	6	6		1							3														16
	Other Muscidae species						2	1	3	1	4			2			1	2		1						17
FANNIIDAE	*Fannia sp.*		2	3	7	4	5	6	10		4	1		3									2			47
	Fannia manicata										6			2												8
	Other Fanniidae species				1						1			1												3
SARCOPHAGIDAE	*Sarcophaga sp.*					1						1	4			1			4							11
SPHAEROCERIDAE	*Chaetopodella scutellaris*	4							2		5	2	12		1											26
	Coproica hirticula								5	1	18	20	7													51
	Coproica pusio								1		1	2	3													7
	Coproica vagans								7			2	1													10
	Leptocera caenosa										4	1														5
	Opalimosina liliputana								1		1	2	1			6				1		1				13
	Spelobia clunipes										2				1											3
	Spelobia luteilabris						1				2	4														7
	Telomerina levifrons								4			1	1													6

	Trachyopella lineafrons											1				1					1							3
	Other Sphaeroc. species		1						2		7	8	4	1	5	2	1											31
PIOPHILIDAE	*Liopiophila varipes*	1	3	1	5	7	11	2	18	2	35	6	9	2														102
	Parapiophila vulgaris											2	2			3		1										8
	Protopiophila latipes								3		3	5	3			3												17
	Stearibia nigriceps				5	15	9		18		39	63	63	23														235
	Other Piophilidae species								1		1																	2
SEPSIDAE	*Meroplius minutus*						1		1		5	17	14	1	3	11		1										54
	Other Sepsidae species	1							2				3															6
PHORIDAE	*Megaselia brevicostalis*										2		1															3
	Other Phoridae species			1				1											1									3
SCIARIDAE	*Scatopsciara multispina*										1		7	1	9	3	1		3	1								26
	Other Sciaridae species																1										1	2
Other Diptera	Other Diptera species				1						3	1	2		1			2		1	1		1					13
GEOTRUPIDAE	*Anoplotrupes stercorosus*	1				3			1		1						1											7
	Trypocopris pyrenaeus		1	1	2		1	4	2	1	1																	13
SILPHIDAE	*Necrodes littoralis*					3	3	3																				9
	Nicrophorus humator							1																				1
HISTERIDAE	Histeridae					1		1	1						1													4
STAPHYLINIDAE	*Creophilus maxillosus*					2	3	3	1																			9
	Other Staph. species						1	3	1		1	5	1	2	2	1							1	1	1			20
Other Coleoptera	Other Coleoptera species				1				2			5		2	1	2	2	1	3				1		1			21
HYMENOPTERA	Prasit. Hym.			1	2						1							1			3	1		2			11
	FORMICIDAE				4	5	1	1		1		1	3	2					1	1	1						21

Table 22: Succession associated to pig carcass C3 in summer of 2010.

C3	STAGE	F	F	F	F	B	AcD	AcD	AdD	AdD	AdD	AdD	AdD	AdD	AdD	AdD	AdD	D	D	D	D	D	D	D	D	
2010	DAY	1	2	3	4	5	6	7	8	9	11	13	15	18	21	25	29	34	40	49	56	64	72	80	88	TOTAL
CALLIPHORIDAE	*Calliphora vomitoria*				1							1			1											3
	Lucilia caesar	14	47	5	16	6	2	1			9	4	1	6	11	2	7	6								137
	Chrysomya albiceps				2	1						2	6	5	1	2										19
	Other Calliph. species		2	1		2	1	2			3		3				1						1			16
MUSCIDAE	*Azelia sp.*			1				2		1	1	4		4												13
	Hydrotaea dentipes					1	1	2				2		3							1					10
	Hydrotaea ignava						2	1				4	6	1												14
	Hydrotaea similis		3	10	3	1	4	6			1	1			1											30
	Musca autumnalis	9																								9
	Other Muscidae species					1	1	1			3			1	3			1	1							12
FANNIIDAE	*Fannia sp.*		1	9	1	1	6	8	4		9	4	4	6	1		1				2	1	1			59
	Fannia manicata										2	1		2	2											7
	Fannia canicularis							2			2			2			1									7
SARCOPHAGIDAE	*Sarcophaga sp.*											1	1	1	1			1								5
SPHAEROCERIDAE	*Chaetopodella scutellaris*					4	1		1		19	58	4	5	3			1								96
	Coproica hirticula					3	3	6			69	24	6	3	4			1								119
	Coproica pusio					1	1				3	4	3					1								13
	Coproica vagans					1		1			3	3	1	1												10
	Leptocera caenosa										4	1	3	1	1											10
	Opalimosina liliputana										3		1	3	2			1								10
	Spelobia clunipes											1		1												2
	Spelobia luteilabris										1	1		2	1			2								7
	Telomerina levifrons							2			2	2	1													7

	Trachyopella lineafrons											1	1							1								3
	Other Sphaeroc. species						1	2			7	8	9	1	2	2	1	1				1			2			37
PIOPHILIDAE	*Liopiophila varipes*		2		2	2	3	31			14	9	9	7	4		1	1										85
	Parapiophila vulgaris										1		1					1		5				1				9
	Protopiophila latipes										10	1	2	4		3		4										24
	Stearibia nigriceps				1	5	3	5			29	58	85	104	37	16	2	1		3	2							351
	Other Piophilidae species						1				2		2															5
SEPSIDAE	*Meroplius minutus*							2			3	7	6	3	2	2	1											26
	Other Sepsidae species							1				1	1				1	3	1	1								9
PHORIDAE	*Megaselia brevicostalis*		1					1			1	12	9	5														29
	Other Phoridae species										3	1	1	1		1					1							8
SCIARIDAE	*Scatopsciara multispina*											1			1	2												4
	Other Sciaridae species																1	1	1		1							4
Other Diptera	Other Diptera species				1						2	1	1		4	2		3	4	3								21
GEOTRUPIDAE	*Anoplotrupes stercorosus*					1	1		1	3	1				2	1	2	1										13
	Trypocopris pyrenaeus					1	3	2	2	1	3	1	1		1		1								1			17
SILPHIDAE	*Necrodes littoralis*						2	3	3	3		3																14
	Other Silphidae species					1	1	2				1																5
CLERIDAE	*Necrobia sp.*							1					1	1			3	3	2	1								12
DERMESTIDAE	*Dermestes sp.*															1	6	3		1								11
HISTERIDAE	Histeridae				1	1	1						2			4	1	8	2	1							1	22
STAPHYLINIDAE	*Bisnius fimetarius*						2																					2
	Creophilus maxillosus						2	1	6		1																	10
	Other Staph. species			1									1		2	1	1	1						1	6	1		15
Other Coleoptera	Other Coleoptera species			1		1									2	1	1		1	1		4	1	2	1			16
HYMENOPTERA	Parasit. Hym.						3				1	2	1		1		1				7							16
	FORMICIDAE	4	6	2	3	3					1	2	1	1				1	1									25

Table 23: Succession associated to pig carcass C4 in summer of 2010.

C4	STAGE	F	F	F	F	AcD	AcD	AcD	AcD	AdD	AdD	AdD	AdD	AdD	D	D	D	D	D	D	D	D	D	D	D	
2010	DAY	1	2	3	4	5	6	7	8	9	11	13	15	18	21	25	29	34	40	49	56	64	72	80	88	TOTAL
CALLIPHORIDAE	*Calliphora vomitoria*				1						1			1												3
	Lucilia caesar	41	42	8	11	11	1	4	7		7	9	8	11	10	8	2			1						181
	Chrysomya albiceps		1			1							2			1										5
	Other Calliph. species								1			1					1	1								4
MUSCIDAE	*Azelia sp.*				1				2	2	2	6	4	1	2		1	1		1						23
	Hydrotaea dentipes		4				1		1	1	3		4	1			1									16
	Hydrotaea ignava								1			2	3			3										9
	Hydrotaea similis		5	1	7	1		1			3	1					1									20
	Musca autumnalis	11	7	3																						21
	Other Muscidae species					1			2		3	2	3	2		2	1		1	1						18
FANNIIDAE	*Fannia sp.*			1	11	4	1	14	8	6	12	4	4		1	4				2	1	1				74
	Fannia manicata							1			3	1	4		1	1										11
	Other Fanniidae species							1	1			1		1		1	1			1						7
SARCOPHAGIDAE	*Sarcophaga sp.*										1		2													3
SPHAEROCERIDAE	*Chaetopodella scutellaris*	1								1	1	19	12	5	4	10		2								55
	Coproica hirticula					3					2	6	3			3										17
	Coproica pusio											2				1										3
	Coproica vagans						1				2				1											4
	Leptocera caenosa						1	1					1		1	5	1									10
	Opalimosina liliputana					3		1			2		1		3	2	2	1	1	2	1				1	20
	Spelobia clunipes						1						1													2
	Spelobia luteilabris												1		3	15	2	4		2						27
	Telomerina levifrons						1				2	2	1	1		1						1				9

	Trachyopella lineafrons					2	2															2				6
	Other Sphaeroc. species					1					5	2	2	2	3	11	1	3	3	2						35
PIOPHILIDAE	*Liopiophila varipes*				1		1	4		2	7	7	13	4	2		1										42
	Parapiophila vulgaris											1	1	1		2			1								6
	Protopiophila latipes					1					3	2	3	1													10
	Stearibia nigriceps					5	1		1		10	18	38	7		2											82
	Other Piophilidae species					1		1																			2
SEPSIDAE	*Meroplius minutus*										11	7	8	5	6	13	4	2	1								57
	Other Sepsidae species					1							1			1	2	1			1						7
PHORIDAE	*Megaselia brevicostalis*				1							1	2	1			1	1									7
	Other Phoridae species					1						3		1			1										6
SCIARIDAE	*Scatopsciara multispina*										1	4	1	1	1	1	2		9	1	4		2				27
Other Diptera	Other Diptera species						1	1			1	1					3		1		3	2					13
GEOTRUPIDAE	*Anoplotrupes stercorosus*				3	2	3		1	2	3	3		2						5							24
	Trypocopris pyrenaeus		4	1			1	1	3	2	1		1					1									15
SILPHIDAE	*Necrodes littoralis*					2	2	4	4																		12
	Nicrophorus vespilloides			1					1	1																	3
HISTERIDAE	Histeridae					2	2					1									1						6
STAPHYLINIDAE	*Bisnius fimetarius*															2				1							3
	Creophilus maxillosus					1	3	5		1																	10
	Other Staph. species					1	3	5		1				2	1	4	1	2	1	2	7		1	2	1		34
Other Coleoptera	Other Coleoptera species	1							1	1	2	1			1	1	5	2	4		2		1	1	1		24
HYMENOPTERA	Parasit. Hym.										1	1			1		1	2		2	1				1		10
	FORMICIDAE	1							10		4	3	3	1	1	4	2		4		1	1	1				36

Table 24: Succession associated to pig carcass C5 in summer of 2010.

C5	STAGE	F	F	F	F	AcD	AcD	AdD	AdD	AdD	AdD	AdD	D	D	D	D	D	D	D	D	D	D	D	D	
2010	DAY	1	2	3	4	5	6	7	8	9	11	13	15	18	21	25	29	34	40	49	56	64	72	80	88	TOTAL
CALLIPHORIDAE	*Calliphora vomitoria*														1											1
	Lucilia caesar	28	24	9	8	11			2		5	5		3	4	2	3	2	1							107
	Chrysomya albiceps				2									1												3
	Lucilia ampullacea			1																						1
MUSCIDAE	*Azelia sp.*							1			2		1													4
	Hydrotaea dentipes								1		2		1							1						5
	Hydrotaea ignava								1																	1
	Hydrotaea similis		2	1	1			1	2																	7
	Musca autumnalis																			1						1
	Other Muscidae species		1			2					1	1			1					1	1					8
FANNIIDAE	*Fannia sp.*				1	1		2	2	1	3	1			1	1					1					14
	Fannia manicata													1	1		1									3
	Fannia fuscula								2																	2
SARCOPHAGIDAE	*Sarcophaga sp.*																		1							1
SPHAEROCERIDAE	*Chaetopodella scutellaris*	4							5		15	12	24	3												63
	Coproica hirticula										4	2			1											7
	Coproica pusio												2		1											3
	Coproica vagans										1		3													4
	Opalimosina liliputana										2	1	2	2							1					8
	Spelobia clunipes										1			1	1											3
	Trachyopella lineafrons												1	1							1	1	1			5
	Other Sphaeroc. species		1							1	2	3	17	1	2		1				2	1				31
PIOPHILIDAE	*Liopiophila varipes*					1					3		2	2	1		1									10

	Parapiophila vulgaris															1												1
	Protopiophila latipes										3	1	3	1			1											9
	Stearibia nigriceps					3					23	9	8	1	1													45
SEPSIDAE	*Meroplius minutus*					3						7	2	4	1													17
	Nemopoda nitidula								1		1	2			2													6
	Other Sepsidae species	1							4		3	1	1	4					1									15
PHORIDAE	*Megaselia brevicostalis*											1		1														2
SCIARIDAE	*Scatopsciara multispina*										3	2	4	2					1			1						13
	Other Sciaridae species						1						1							2								4
Other Diptera	Other Diptera species											1	6	1	2	2		4		3	1							20
GEOTRUPIDAE	*Anoplotrupes stercorosus*					1			3	2																		6
	Trypocopris pyrenaeus				2		1	4	1	1	1		1		1													12
SILPHIDAE	*Necrodes littoralis*					3	2	3																				8
	Nicrophorus vespilloides				1		1		2																			4
HISTERIDAE	Histeridae				1															1								2
STAPHYLINIDAE	*Bisnius fimetarius*													1		1						1						3
	Other Staph. species				1						1	2	6	3		1				1	1	1	2	1	3			23
Other Coleoptera	Other Coleoptera species			2				1				1	1		2		1	1		1			1	1	7			19
HYMENOPTERA	Parasit. Hym.		1								1	1	1	1								2	1				8
	FORMICIDAE	2	4	2								2	1	1	2		1	1	1		1			1				19

Appendix II

In order to make the reading of Chapter 7 lighter, and also for easing the comprehension of it, development data for the species included on that Chapter are detailed here.

Each table represents the average length of each stage of development of each sampling day of the most abundant species.

Table 25: Unidentified maggots. Average length of larvae (± standard deviation) from different days.

	Unidentified Maggots		
DAY	**E**	**L1**	**L2**
1	1,48 ± 0,17	2,30 ± 0,3	
2	1,55 ± 0,09	2,56 ± 0,64	4,58 ± 0,82
3	1,45 ± 0,24	2,85 ± 0,52	4,76 ± 1,51
4	1,58 ± 0,02	2,76 ± 0,46	5,01 ± 0,83
5		2,75 ± 0,47	5,79 ± 1,41
6		2,83 ± 0,42	5,84 ± 1,01
7		3,50 ± 0,67	5,91 ± 0,96
8		3,32	5,01 ± 0,73
9		2,86	
11			7,46 ± 1,74
13		6,01 ± 0,75	

Table 26: *Calliphora vomitoria*. Average length of larvae (± standard deviation) from different days.

	Calliphora vomitoria			
DAY	**L2**	**L3**	**P**	**A**
6		11,89		
7		11,93 ± 1,84		
8	6,54 ± 0,69	12,84 ± 1,02		
9	7,83 ± 1,33	11,07 ± 3,68		
10	7,88 ± 0,37	14,23 ± 2,97		
11	6,61	13,68 ± 2,19	7,23	
12		16,39 ± 1,58		
13	8,63	15,80 ± 2,00		
15		16,81 ± 2,08		

21			9,83 ± 0,35	
32				10,56
36				10,75 ± 0,16

Table 27: *Chrysomya albiceps*. Average length of larvae (± standard deviation) from different days.

	Chrysomya albiceps			
DAY	**L2**	**L3**	**P**	**A**
4	5,65			
5	3,62	13,14 ± 1,53		
6	5,74 ± 0,94	10,61 ± 3,65		
7	5,95 ± 1,5	9,56 ± 1,92		
8	5,61 ± 1,3	10,87 ± 2,35	8,69 ± 0,51	
9	8,68 ± 3,95	10,53 ± 1,96	8,48 ± 0,71	
10	7,38	11,16 ± 1,94		
11		11,64 ± 1,96	8,74 ± 0,7	
12		12,18 ± 4,8	8,89 ± 0,45	
13		11,19 ± 1,45	8,93 ± 0,69	8,93 ± 0,36
14				9,42 ± 0,46
15			8,58 ± 0,73	9,38 ± 0,46
16				9,37 ± 0,48
17				9,20 ± 0,6
18			9,07 ± 0,83	9,26 ± 0,8
19				9,31 ± 0,66
20				9,83 ± 0,63
21			9,09	10,18 ± 0,33
24			8,70 ± 0,34	10,75
32			9,26 ± 0,52	
36			8,64	
47			9,48 ± 0,05	

Table 28: *Lucilia caesar*. Average length of larvae (± standard deviation) from different days.

	Lucilia caesar				
DAY	**L1**	**L2**	**L3**	**P**	**A**
4			9,01 ± 1,5		
5			9,29 ± 1,47		
6	2,69 ± 0,25	5,89 ± 1,06	9,97 ± 1,47		
7			9,98 ± 1,25		
8		6,71 ± 1,29	9,88 ± 1,63		
9		6,92	10,29 ± 1,63	6,13	
10		8,41	11,32 ± 1,39		

11		10,73 ± 1,36		
12		11,85 ± 0,81	6,37 ± 0,37	
13		10,95 ± 0,75	5,81 ± 0,32	
15			6,28 ± 0,19	
18			6,13	
19				8,27
20			7,58 ± 0,32	8,95 ± 0,32
21			6,59 ± 0,29	8,41 ± 0,88
22				8,90 ± 0,11
23			7,02 ± 0,91	8,84 ± 0,75
24			7,31	
25				8,27 ± 1,29
26				8,82 ± 0,92
27			6,70	9,21 ± 0,99
28				9,39 ± 1,08
29				9,34 ± 0,56
30				9,10
31				9,41
32				8,91 ± 0,63
34				8,99 ± 0,53
36				10,11

Table 29: *Lucilia illustris*. Average length of larvae (± standard deviation) from different days.

	Lucilia illustris		
DAY	**L2**	**L3**	**A**
4		11,82 ± 1,08	
5		9,43 ± 1,88	
6	6,71 ± 0,83	8,98 ± 1,28	
7	9,58	11,42 ± 1,41	
8	7,71 ± 1,83	10,90 ± 1,66	
9	6,50	10,60 ± 1,39	
10	7,02 ± 2,36	12,24 ± 1,24	
27			9,15
29			9,11 ± 0,59
30			10,39

Table 30: *Fannia manicata*. Average length of larvae (± standard deviation) from different days.

Fannia manicata

DAY	L1	L2	L3	P	A
9		3,97	6,09 ± 0,77		
10			5,93 ± 0,6		
11		3,72 ± 0,36	5,51 ± 0,67		
12		4,27 ± 0,47	5,97 ± 0,48		
13	2,51 ± 0,55	4,00 ± 0,42	5,70 ± 0,66		
15	1,69 ± 0,1	3,26 ± 0,56	5,55 ± 0,78		
18	2,86 ± 0,24	4,37 ± 0,26	6,01 ± 0,76		
21			5,60 ± 1,27		
25			5,36 ± 0,72		
27			5,78	5,79	
29			5,17 ± 0,56	6,35	
32					6,07
34			6,58 ± 0,33		
35					5,98
36			5,99 ± 0,29		
40				6,08 ± 0,18	
51					6,49
54				5,82	
56					5,45
58					6,27 ± 0,79
59					5,87 ± 0,51
70					5,85

Table 31: *Hydrotaea ignava*. Average length of larvae (± standard deviation) from different days.

Hydrotaea ignava

DAY	L3	P	A
8	8,44 ± 1,35		
9	9,12 ± 1,23		
11	12,35 ± 0,8		
13	11,10 ± 1,35		
15	10,19 ± 1,57		
18	10,39 ± 1,83		
21	10,84 ± 1,5		
22		6,53	
24		6,39 ± 0,28	
25	10,64 ± 0,89		
29	10,42 ± 1,93	6,98	

34	10,14 ± 1,08			
35				7,03 ± 0,65
36		7,08		
40	10,09 ± 0,81	6,77 ± 0,06		
41				7,72
46				7,85
47	10,01 ± 1,23	6,55 ± 0,47		
48				6,72
49	9,95 ± 1,19	6,78 ± 0,26		
50				7,20 ± 0,18
54	8,58 ± 1,53			
55				7,26 ± 0,78
56	9,60 ± 1,28			6,06
59				7,20
60				7,07
61				6,63
64	11,03 ± 1,00	6,66 ± 0,13		
65				7,00 ± 0,53
67				6,82 ± 0,38
72	10,31 ± 0,45	6,59		
80	9,05			
84				6,76
88	9,02 ± 0,53			

Table 32: *Hydrotaea similis*. Average length of larvae (± standard deviation) from different days.

	Hydrotaea similis			
DAY	**L2**	**L3**	**P**	**A**
6		9,87 ± 2,06		
7		11,26 ± 1,9		
8	9,32	11,23 ± 1,96		
9		11,64 ± 1,84		
10		8,97 ± 2,22		
11		12,87 ± 1,43		
12		12,52 ± 0,79		
13		13,03 ± 1,4		
15		12,25 ± 1,97		
19			6,14	
28				6,60

Table 33: Piophilidae. Average length of larvae (± standard deviation) from different days.

PIOPHILIDAE				
DAY	**L1**	**L2**	**L3**	**P**
15	1,83 ± 0,48	4,01 ± 0,73	6,18 ± 0,69	
18	1,61 ± 0,34	3,28 ± 0,54	6,04 ± 1,09	
21	2,50	4,50 ± 0,79	5,94 ± 0,89	3,29 ± 0,12
24	3,32 ± 0,30	5,46 ± 0,36	6,94 ± 0,37	
25	2,22 ± 0,01	3,79 ± 0,49	6,30 ± 0,74	
27	3,68	4,37 ± 0,28	7,39 ± 0,41	
29		3,86 ± 0,45	6,01 ± 0,95	
32	3,38 ± 0,22	5,20 ± 0,39	7,41 ± 0,55	3,72
34		4,73 ± 0,98	6,27 ± 0,69	
36	4,04	5,36 ± 0,7	6,90 ± 1,01	3,55 ± 0,42
40		4,38 ± 0,64	6,74 ± 0,69	3,55 ± 0,33
47				3,44 ± 0,39
49			5,92 ± 0,27	
54			7,18 ± 0,46	3,56 ± 0,37
56		3,74 ± 0,08	5,06 ± 0,37	
62			7,15 ± 0,34	3,73
64		4,32	4,84 ± 1,04	
71			5,94 ± 0,67	
72			4,20	
79			7,74 ± 0,76	
80			5,18 ± 0,85	
85	2,84	3,44 ± 0,48	7,28 ± 1,08	
88			5,01 ± 0,79	

Table 34: Sepsidae. Average length of larvae (± standard deviation) from different days.

SEPSIDAE				
DAY	**L1**	**L2**	**L3**	**P**
13		4,25		
18	3,27 ± 0,12	4,94	6,13 ± 0,17	
21			5,98 ± 0,93	
25			5,19 ± 0,7	
27	3,11	4,68	6,07 ± 0,45	
29			6,96 ± 0,62	
32		4,55	6,05 ± 0,4	
34			5,56 ± 0,97	
36		5,75		4,37 ± 0,13
40		3,30	5,20 ± 0,38	5,26

47			7,18	
54		3,14	5,93 ± 0,25	4,76
62				5,07

Table 35: Sarcophagidae. Average length of larvae (± standard deviation) from different days.

	SARCOPHAGIDAE		
DAY	**L1**	**L2**	**L3**
2		5,90	
3	3,22 ± 0,08	8,61 ± 1,5	
6			15,14
7			14,89
21		9,04	

Appendix III

A relation of the articles published up to date from this thesis.

Title	**Arthropods of forensic interest in the Basque Country (Northern Spain).**
Authors	B. Díaz & M. Saloña
Journal	*Ciencia Forense*, 12: 211-232
Abstract	A checklist of carrion-related arthropods collected in association to pig carcasses in Aiako Harria Natural Park (Basque Country, northern Spain) is presented. Leaving aside the subfilum Miriapoda, there are 16 orders represented in 7837 specimens, being the most important those of the class Insecta (7667 specimens; 98%). The moment in which they were present during the decomposition of a piglet carcass is detailed, and an analysis of the diversity of each decomposition stage was performed. Fresh stage is the moment when a higher degree of dominance and less diversity were found, being the dry stage the most diverse one. It should be stressed out the huge amount of new records: 2 new species for Science (*Crossopalpus* sp. n. (nr. *nigritellus* and *aeneus*) and *Drapetis* sp. n. (group *exilis*) (Diptera: Hybotidae)); 1 genus (*Alloborborus*) and 8 species new for the Iberian Peninsula (*Crossopalpus humilis, Meroplius fukuharai, Nemopoda speiseri, Sepsis luteipes, Alloborborus pallifrons, Phthitia empirica, Spelobia cambrica, Trachyopella kuntzei*); 7 new species for Spain (*Siphunculina aenaea, Siphunculina quinquangula, Megaselia citrinella, Megaselia meconicera, Megaselia tama, Pseudacteon formicarium, Ischiolepta denticulata*); 1 new species for the peninsular Spain (*Telomerina levifrons*); 2 family, 12 genus and 29 species new for the Basque Country; and 1 family, 4 genus and 3 species new for Guipúzcoa. This study may serve as a reference for future forensic studies in the Basque Country and other similar biogeoclimatic areas.
Keywords	Forensic Entomology, arthropods, diversity, pig carcass, Basque Country

Title	***Ixodes ricinus* (Ixodidae), an occasional phoront on necrophagous and coprophagous beetles in Europe**
Authors	M. Saloña, P. Bahillo, B. Díaz, J. Sumner & M.A. Perotti
Journal	*Experimental and Applied Acarology*, Vol 65: 243-248 (2015)

Abstract | For ticks, phoretic behaviour using insects associated with vertebrates might offer an alternative strategy to host-seeking. Here we report for the first time the presence of immature stages of the most widespread tick species in Western Europe, Ixodes ricinus (Acari: Ixodidae), on three beetle species belonging to families Silphidae and Geotrupidae (Coleoptera). Specimens were collected while performing fieldwork surveys on insect diversity during the peak of tick's questing behaviour, in July and August of 2009 and 2010. The collections took place in two Natural Parks, the Aiako Harria, Guipúzcoa in Northern Spain and Wellington Country Park, Berkshire, in England. The silphid beetle Nicrophorus vespilloides and the geotrupid Trypocopris pyrenaeus were collected from pig-carcasses and both carried nymphs of I. ricinus; the geotrupid Anoplotrupes stercorosus was carrying a tick larva while feeding on red deer dung. These findings revealed an unnoticed but common relation of ticks not only with decomposed animals but also with insect scavengers. We discuss the rationale of this phenomenon

Keywords | Tick, Ixodes ricinus, Silphidae, Geotrupidae, Phoretic, Phoresy, Carcass

Title | **Primer registro de la familia Ceratocombidae Fieber, 1860 (Hemiptera: Heteroptera) en la Comunidad Autónoma Vasca.**

Authors | S. Pagola-Carté & B. Díaz

Journal | *Heteropterus Revista Entomológica*, Vol. 13 (1): 59-63 (2013)

Abstract | *Ceratocombus coleoptratus* (Zetterstedt, 1819) has been collected on pig carcasses in the Aiako Harria Natural Park (Gipuzkoa), being the first record of the family Ceratocombidae from the Basque Autonomous Community. Some comments on ecology are given and the short list of Iberian records of the infraorder Dipsocoromorpha (Hemiptera: Heteroptera) is updated.

Keywords | Ceratocombidae, *Ceratocombus coleoptratus*, Basque Autonomous Community, Iberian Peninsula

Title | **New Chloropidae from the Basque Country (northern Spain). Additions to the Chloropidae (Diptera) fauna from the northern Spain, with new records.**

Authors | E. Petrovna Nartshuk, B. Díaz & M. Saloña

Journal | *Boletín de la Asociación Española de Entomología*, Vol. 37 (3-4): 173-179 (2013)

Abstract | For the first time, the finding of three genera and four species of the family Chloropidae associated to cadaveric environment has been reported. Two out of the four species listed, Siphunculina aenea (Macquart, 1835) and Siphunculina quinquangula (Loew, 1873), are also reported for the f irst time from the Iberian Peninsula.

Keywords | Forensic Entomology, Diptera, Chloropidae, sarcosaprophagous fauna,

Iberian Peninsula, Basque Country, Spain

Title | **Algunos dípteros necrófilos capturados sobre cadáveres de cerdos en el País Vasco (España) (Insecta: Diptera: Brachycera).**

Authors | M. Carles-Tolrá, B. Díaz & M. Saloña

Journal | *Heteropterus Revista Entomológica*, Vol. 12 (2): 213-222 (2012)

Abstract | A list of 68 species of necrophilous flies captured on pig carcasses during a research of Forensic Entomology is reported from the Natural Park Aiako Harria (Guipúzcoa, Basque Country, Spain). Results obtained here have significantly increased the knowledge of necrophilous Diptera in the Iberian Peninsula, especially those from the Basque Country. We report, for the first time: a) 1 genus (Sphaeroceridae: *Alloborborus* Duda, 1923) and 7 species (Sepsidae: *Meroplius fukuharai* (Iwasa), *Nemopoda speiseri* (Duda) and *Sepsis luteipes* Melander & Spuler; Sphaeroceridae: *Alloborborus pallifrons* (Fallén), *Phthitia empirica* (Hutton), *Spelobia cambrica* (Richards) and *Trachyopella kuntzei* (Duda)) for the Iberian Peninsula; b) 1 species (Sphaeroceridae: *Ischiolepta denticulata* (Meigen)) for Spain; and c) 1 species (Sphaeroceridae: *Telomerina levifrons* (Spuler)) for peninsular Spain.

Keywords | Insecta, Diptera, Iberian Peninsula, Basque Country, faunistics

Title | ***Crossopalpus humilis* (Frey, 1913) en la Península Ibérica y la relación de la familia Hybotidae con cadáveres de vertebrados (Diptera: Empidoidea: Hybotidae).**

Authors | D. Ventura, B. Díaz & M. Saloña

Journal | *Boletín de la Sociedad Entomológica Aragonesa*, Vol. 50: 527-532 (2012)

Abstract | *Crossopalpus humilis* (Frey, 1913) in the Iberian Peninsula and the relation of the family Hybotidae to vertebrate carcasses (Diptera, Empidoidea).
Crossopalpus humilis (Frey, 1913) is recorded for the first time in the Iberian Peninsula, found on pig carcasses during a forensic entomology research carried out in Aiako Harria Natural Park, Gipuzkoa province (Basque Country). The presence of species of the family Hybotidae on vertebrate carcasses is revised.

Keywords | Diptera, Hybotidae, *Crossopalpus humilis*, first record, Iberian Peninsula, forensic entomology

Title | **Primera cita y nuevos datos sobre los hábitos necrófagos de *Trox scaber* (Linnaeus, 1767) (Coleoptera: Trogidae) en la Comunidad Autónoma del País Vasco (C.A.P.V.).**

Authors | B. Díaz & M. Saloña

Journal | *Boletín de la Asociación Española de Entomología*, Vol. 36 (1-2): 53-59 (2012)

Abstract | First record of the species *Trox scaber* (Linnaeus, 1767) is reported from the Basque Country (Errenteria, Guipúzcoa) with new details related to the biology. It should be considered a species of forensic interest, associated with advanced stages of decomposition and other animal remains. Despite being widely distributed through the northern and central areas of the Iberian Peninsula, the collection of three specimens in the Natural Park of Aiako Harria provides new data that confirms its presence in the Basque Country autonomous region.

Keywords | Cadaveric fauna, Scarabeoidea, Trogidae, *Trox scaber*, Basque Country