



Universidade Federal de Minas Gerais

Instituto de Ciências Biológicas



Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre

**SYSTEMATIC REVIEW OF FORENSIC ENTOMOLOGY AND A META-ANALYSIS  
OF THE EFFECT OF THE PRESENCE OF CHEMICALS IN CARCASSES**

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Belo Horizonte – 2019

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Dissertação apresentada ao Programa de Pós-graduação em Ecologia, Conservação e Manejo da Vida Silvestre, do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito para obtenção do título de Mestre.

Orientador: Prof. Dr. Ricardo Ribeiro de Castro Solar

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## ABSTRACT

Despite being considered a relatively recent science, it is already possible to find a vast literature on Forensic Entomology, and an extensive quantity of informations about it. With it, arises a need to gather and synthesize these informations, in order to organize and integrate the knowledge. It is in this scenario that systematic reviews and meta-analyzes are inserted, once they are extremely essential tools in perform this synthesis. Therefore, we reviewed the current global state of Forensic Entomology and we performed a meta-analysis of the effect of the presence of chemical substances on insect parameters of the Forensic Entomofauna. We pulled data from 309 articles for the systematic review, and among those, 24 articles composed the meta-analysis. Articles about this science have been found in 33 countries, suggesting that Forensic Entomology needs to develop even more, to consolidate its global status. Studies of survey, insect successional and that focus on the Diptera order were the most abundant. Overall, the presence of chemical substances in carcasses had positive effect on insect development time, insect colonization, insect length and insect width, and had negative effect on insect survival and insect performance. The effects of the presence of chemical substances vary according to the type of substance and the type of the carcass containing the substance, due to its individual characteristics.

Key-words: Review- Meta-analysis- Entomotoxicology- Forensic- Insect- Drugs- Carcass

## RESUMO

Apesar de ser considerada uma ciência relativamente recente, já é possível encontrar uma vasta literatura sobre Entomologia Forense, e uma extensa quantidade de informações sobre ela. Com isso, surge a necessidade de reunir e sintetizar essas informações, a fim de organizar e integrar o conhecimento. É nesse cenário que revisões sistemáticas e meta-análises são inseridas, uma vez que são ferramentas extremamente essenciais para a realização dessa síntese. Assim, revisamos o atual estado global da Entomologia Forense e realizamos uma meta-análise do efeito da presença de substâncias químicas nos parâmetros da entomofauna forense. Puxamos dados de 309 artigos para a revisão sistemática e, desses, 24 artigos compuseram a metanálise. Artigos sobre essa

ciência foram encontrados em 33 países, sugerindo que a Entomologia Forense precisa se desenvolver ainda mais para consolidar seu status global. Estudos de levantamento, sucessão de insetos e foco na ordem Diptera foram os mais abundantes. No geral, a presença de substâncias químicas nas carcaças teve efeito positivo no tempo de desenvolvimento dos insetos, na colonização dos insetos, no comprimento dos insetos e na largura dos insetos, e teve efeito negativo na sobrevivência dos insetos e no desempenho dos insetos. Os efeitos da presença de substâncias químicas variam de acordo com o tipo de substância e o tipo de carcaça que contém a substância, devido às suas características individuais.

Palavras-chave: Revisão - Meta-análise - Entomotoxicologia - Forense - Insetos - Drogas-Carcaça

## INTRODUCTION

Insects have a great importance in the environment as well as in society. Besides playing a role in the ecological and epidemiological contexts, they are also extremely valuable in the field of forensic sciences. In this last case, the so-called Forensic Entomology is the science that associates the study of insect development, behavior and ecology with criminal procedures, which the main goal is to discover significant information about an investigation (Linhares & Thyssen, 2007).

One of the subdivisions of Forensic Entomology is the Medical-Legal Entomology, which deals with the use of insects found associated with criminal scenes, especially those against life (Caneparo, Corráša, Mise, & Almeida, 2012). In this branch, insects have several applications as they are the first organisms to arrive in a corpse. Thus, they participate during most of the decomposition process, contribute to the determination of the *post-mortem* interval (i.e., the interval between the moment of the death and the discovery of the corpse), provide parameters of the circumstances of death, help the determination of the geographical origin of death and verify body movement. They also permit the identification of the victim even in the

absence of the body and the detection of negligence and maltreatment of humans and animals. Finally, they allow the discovery of the presence of chemical substances in the corpse (Linhares & Thyssen, 2007).

The entomological fauna used in such functions is composed of a variety of species and orders that coexist and eventually compete with each other for the carcass, an ephemeral food resource (Barton et al., 2013). Although many orders are found in the corpses, it is outstanding the presence of Diptera, Coleoptera, Hymenoptera and Lepidoptera. It is also noticeable that there is a process of succession among them, since the decomposition process offers ideal conditions for the development of each order in a certain stage (Hobson, 1932; Keh, 1985).

Although Forensic Entomology is considered to be a relatively recent science (Amendt, Krettek & Zehner, 2004), it is possible to find an extensive literature about this subject that evaluated the process of succession that occurs in a carcass and several other factors and variables related to the response of the entomological communities during the decomposition process of a carcass. Climatic conditions, location of the corpse, body size, presence of toxic substances, clothes, injuries, burial, and entomofauna behavior, among others, are some of the variables already studied and published. Despite the great number of studies, there is an evident need to gather and synthesize this knowledge, providing organization, systematization and integration of this existing information (Denis, 2010). It is also necessary to determine secure ways to summarize independent studies produced in a given research area (Wolf, 1986), to provide reliable information that will facilitate a better understanding of the subject by the public as well as function as a tool for decision making.

In this scenario, meta-analysis and systematic reviews are extremely useful and suitable tools to synthesize the available results and information, analyze general trends and evaluate factors that can cause heterogeneity on certain responses (Borgestein et al., 2009; Koricheva, Gurevitch & Mengersen, 2013). The objective of such tools is concentrating the results of several studies in a single piece of research and determining the state of the field of knowledge about a certain research problem (Figueiredo, Paranhos, Silva, Rocha, & Alves, 2014). Therefore, using such tools, in this study we aimed to perform a systematic review about the current global state of Forensic Entomology, and a meta-analysis of the effect of the presence of chemical substances on insect parameters of the Forensic Entomofauna. Each type of substance presents a certain

individual unique effect over the different parameters of the insect life, that may lead to incorrect *post-mortem* interval, and many studies show different effects for the same substance tested. With this in mind, we performed our meta-analysis to show the general effects of chemical substances, evaluating these different results from many studies and generating a value that translate the global effects of these substances. We also evaluate the effect of mediators not evaluated before.

With the review, our goals are to understand the main trends of research, such as country, the main type of carcasses used, and the questions made by forensic scientists, and to summarize a great amount of data available in forensic entomology and therefore generated a robust database about and an overview of this science around the world. In the meta-analysis, our hypothesis is that the presence of chemical substances in carcasses will influence the insect parameters of the entomofauna, since these factors can alter the phases of decomposition, colonization and succession of the species in the cadaver, the time of decomposition and the determination of the *post-mortem* interval.



## METHODS

### Database construction

We performed the data collection searching for articles about Forensic Entomology in the ISI Web of Science and Scielo databases. We searched for the following terms in English: “Forens\* Entomolog\*”, including titles, abstracts and keywords, and we found 1156 articles in Web of Science platform and 144 in Scielo, totalizing 1300 articles. We also used the following terms, in order to be more specific: “Forens\* Entomolog\* Effects” (returning 167 articles by Web of Science, two articles by Scielo) and “Forens\* Entomolog\* Factor\*” (87 articles by Web of Science, one article by Scielo) to make sure we comprehended all the possible combinations about the subject. All duplicates were excluded. Articles were searched from June to December 2017. Since not all articles presented useful data for the review and the meta-analysis, they had to meet the following criteria to be selected:

#### *Review*

- (1) Studies should be articles;
- (2) Articles should focus on Forensic Entomology;
- (3) Articles should be written in English, Portuguese or Spanish;
- (4) Articles should be published in scientific journals, considered to be the journals that have an editorial board;
- (5) We should be able to retrieve some information such as number of authors, year of publication, objective of the study, journal in which the article was published, type of study (e.g.: case-study, quantitative, qualitative), insect order, family and species studied, habitat or location where the study was performed, country, language, carcass type and the variables studied;

#### *Meta-analysis*

- (1) Studies should be articles;
- (2) Articles should focus on Forensic Entomology;

- (3) Articles should be written in English;
- (4) Articles should be published in scientific journals, considered to be the journals that have an editorial board;
- (5) Articles should have reported data for the number of observations or replicas, measures of variance (standard deviation, standard error or confidence interval) and other information considered to be important for the realization of this meta-analysis;
- (6) The quantitative information should have been reported for both control and experimental groups;

All researches and articles that did not fit one of these criteria were excluded. In the review, when the articles reported data for more than three insect orders or families, it was labeled as reporting data for “Various”.

To perform the meta-analysis, we used data of sample size, standard deviation of the mean and the mean of both control and experimental groups. Measurements of variance reported, other than standard deviation of the mean (standard error or interval of confidence), were converted to it using MetaWin Statistical Calculator (Rosenberg *et al.*, 2000). When the qualitative data were available on figures, they were obtained by using the program ImageJ, after calibration of each picture.

We conducted a meta-analysis to evaluate the effect that the presence of the chemical substances ethanol, caustic soda, hydrochloric acid, insecticide, perfume, nordiazepam, flunitrazepam, p-Hydroxymethamphetamine, methamphetamine, morphine, ketamine, malathion, nandrolone, methylphenidate hydrochloride, phenobarbital, buscopan, bleach, mosquito repellent, unleaded gasoline, cocaine, ciprofloxacin, ampicillin, pentane, tramadol, sodium hypochlorite, isopropyl alcohol, povodine iodine, hydrogen peroxide, gas and citronella have over the forensic entomofauna. Based on the information provided by the authors, we calculated the effect of chemical substances according to the moderators carcass type (pig, beef, kangaroo, kangaroo mixed with lamb meat, liver, rabbit, artificial diet, rat and chicken) and substance type (described above). We evaluated the effects over the following parameters: abundance, species

richness, development time, colonization, length, weight, mortality, width, emergence interval, survival, sex ratio, and performance of the insects.

#### Data analysis (Meta-analysis)

To homogenize the parameter to be analyzed, we categorized them according to their similarities in length (included: coastal length, tibial length, puparial length and size), development time (included: time required to maggots to reach pupal stage, duration of development of pupae from maggots, time from egg hatching to completion of stage, developmental duration of the stage, duration of pupal stage, duration of larval stage, mean development time, duration of the inoculation of the maggots until abandonment, duration of the maggot stage, time to total development and pupation period), weight (included: body mass and growth rates of larval body weights), mortality (included: mortality rate, larval mortality at the beginning of the pupal stage, pupae mortality at the end of the pupal stage and unmerged adults), survival (included: remainder and survival rate), and performance (included: walking speed, total track length and upwind length). All insects included in this meta-analysis are holometabolous. To perform the meta-analysis we excluded the substances and carcasses types that had less than two independent comparisons.

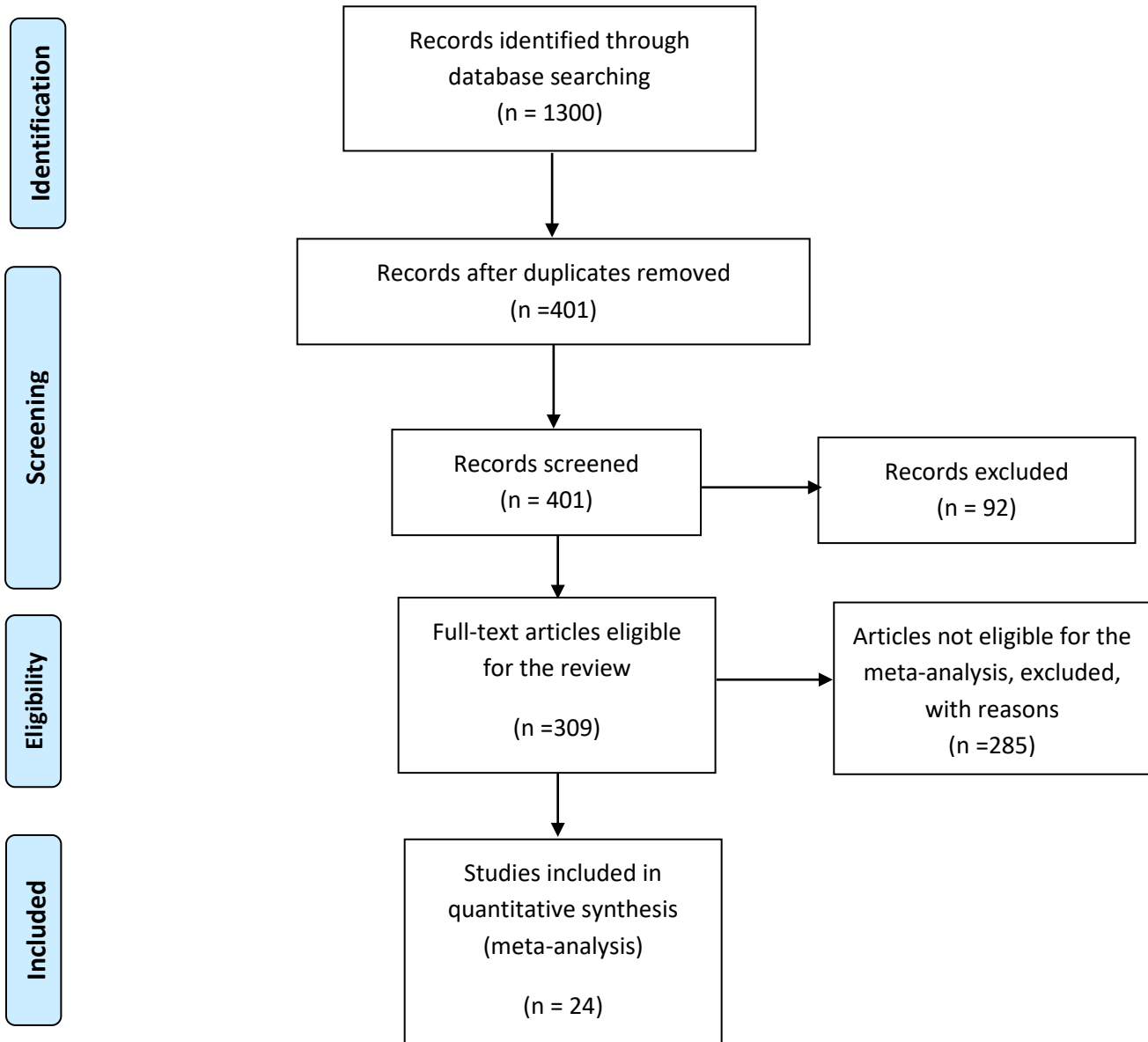
We used MetaWin 2.1.3.4. (Rosenberg et al. 2000) to conduct the meta-analysis for each effect and the overall level of effect (Hedge's  $d$ ). This software calculated the size of the effect of the presence of chemical substances for each study here included, based on the means of both control and experimental groups and their standard deviation. Fail-safe numbers (Rosenthal's method) were also calculated in order to generate the number of studies that would need to be produced or added to the meta-analysis to change its significance (Vasconcelos, Maravalhas, & Cornelissen, 2017). The software also performed the analyses of heterogeneity (Q statistic), which indicates if the categorical groups were homogeneous between them (QB). We excluded variables with only two independent comparisons from the analyses.

First, we performed the analyses using a random-effects meta-analysis model to see the general effect that the presence of chemical substances has over the entomofauna, then we performed a meta-regression model to test the effects of the moderators carcass type and type of substance. Following Vasconcelos *et al.* (2017), we considered the effect sizes significant if the confidence intervals did not overlap with zero.

## RESULTS

### Systematic review

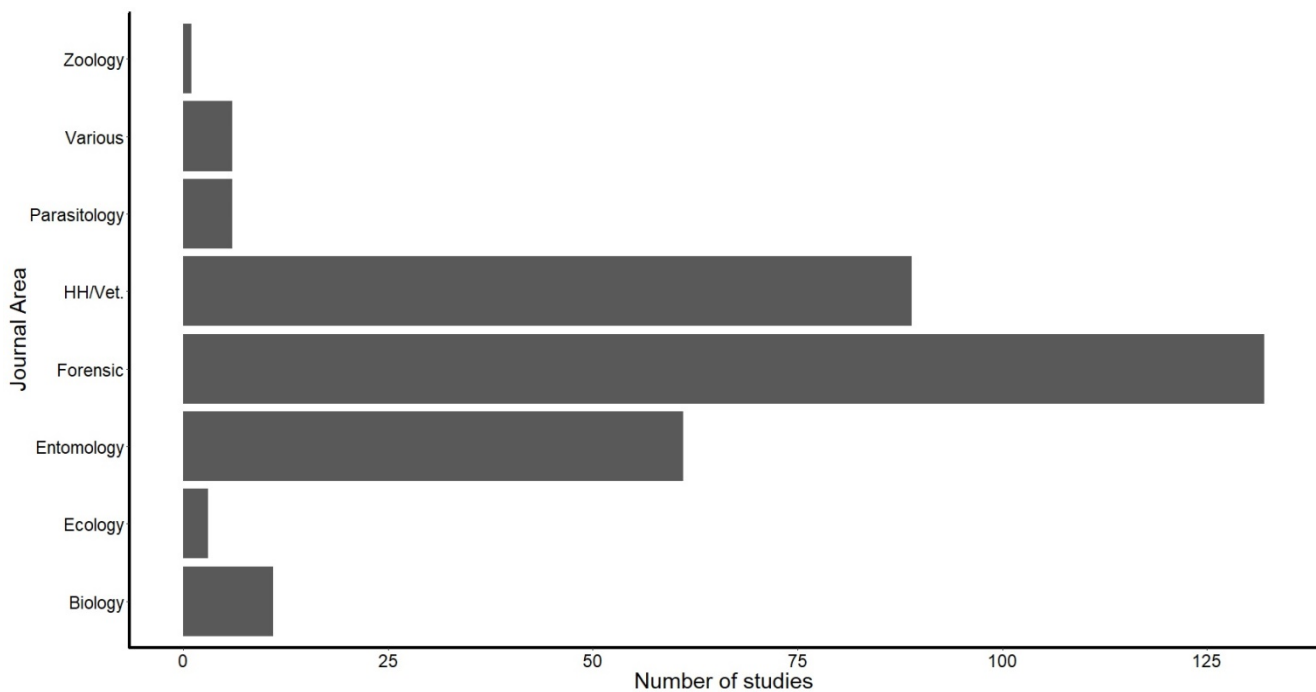
With our more general search, we found 1300 articles in total. We downloaded all articles that, in the first moment, had any relation with Forensic Entomology detected, and 401 articles were selected to the next phase of screening. In that phase, we excluded 92 studies after a deep analytical reading, eliminating those that focused on another area of the forensic sciences or that did not focus on insects specifically, dissertations and texts that were not published in Scientific Journals. Thus, 309 articles remained and composed this systematic review. For the meta-analysis, we included a filter, in which we removed another 13 articles that were not written in English. From this universe of 296 studies, all articles that studied the effect of chemical substances over the entomofauna, contained the values of the mean standard error or standard deviation and the number of observations, and that were published until 2017 were elected to be part of the meta-analysis (24 articles) (Fig. 1).



Source: PRISMA 2009 Flow Diagram

**Figure 1-** Number of articles analyzed and excluded in each screening phase. The *n* stands for the number of articles.

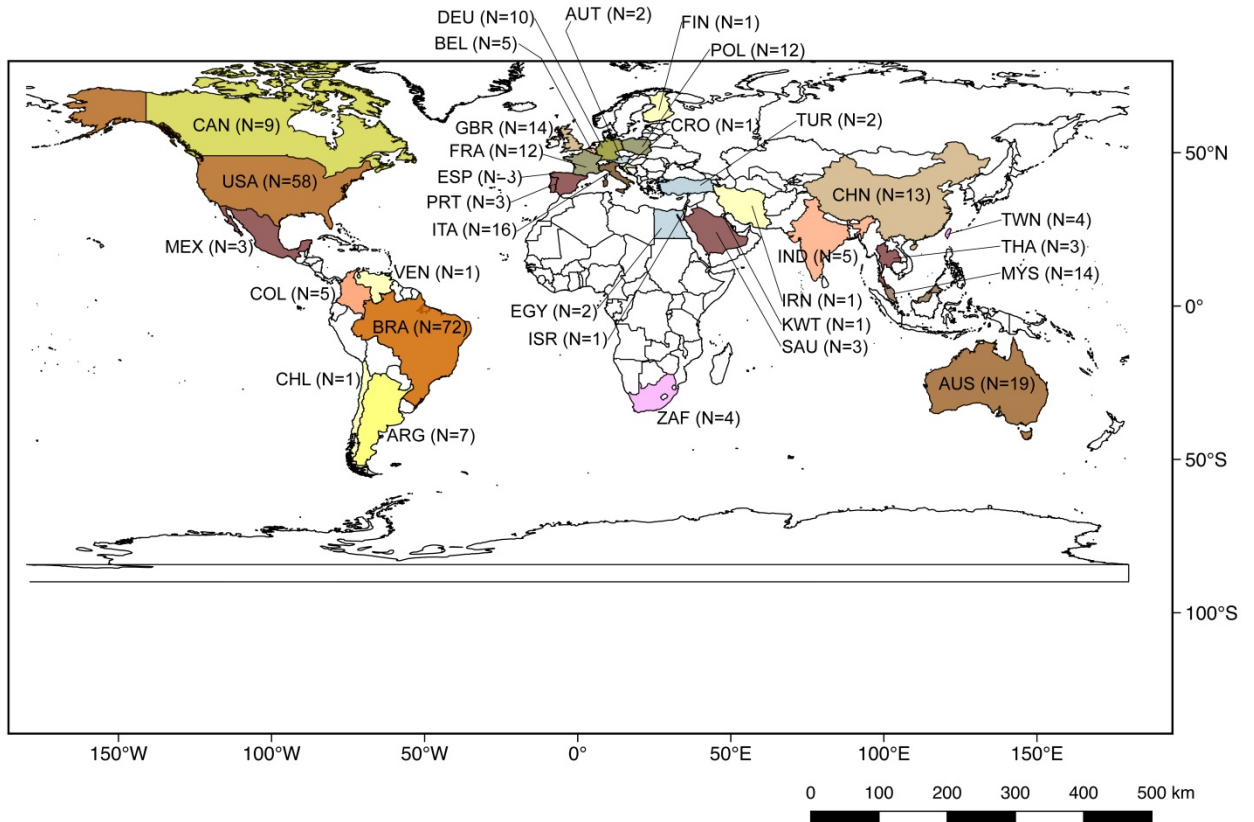
From the 309 articles used in this review, 132 articles, the majority (42.72%), were published in dedicated Forensic Journals. The other areas include fields of Human Health and Veterinary (89 articles or 28.80%), Entomology (61 articles or 19.74%), Biology (11 articles or 3.56%), Parasitology (6 articles or 1.94%), Ecology (3 articles), Zoology (1 article) and journals with more than one specific area (6 articles) (Fig. 2).



**Figure 2** - Histogram of the number of publications per knowledge areas. HH/Vet. = Human Health and Veterinary Journals.

Studies about Forensic Entomology were conducted in the Americas, Europe, Asia, Africa and Oceania, comprising 33 countries. Brazil has the biggest number of studies, 72 articles (23.3%), followed by United States (58 articles or 18.77%), Australia (19 articles or

6.15%), Italy (16 or 5.18%), Malaysia (14 articles or 4.53%), England and China, both with 13 articles, representing 4.21%, France and Poland (12 articles or 3.9%) and Germany (10 articles or 3.24%). The remaining countries contributed to this review with less than 10 articles (Fig. 3). The mean number of authors per study is four, with 11 authors being the greater number found and one author, the minimum. The great majority, 95.8% or 296 articles, was written in English. Nine articles (2.9%) were written in Portuguese and four articles (1.3%) in Spanish.



**Figure 3-** Map of the world with the location of the studies. N stands for the number of studies made per country. ARG= Argentina, AUS= Australia, AUT= Austria, BEL= Belgium, BRA= Brazil, CAN= Canada, CHL= Chile, CHN= China, COL= Colombia, CRO= Croatia, EGY= Egypt, FIN= Finland, FRA= France, GBR= Great Britain, IND= India, IRN= Iran, ISR= Israel, ITA= Italy, KWT= Kuwait, MYS= Malaysia, MEX= Mexico, POL= Poland, PRT= Portugal, SAU= Saudi Arabia, ZAF= South Africa, ESP= Spain, TWN= Taiwan, THA= Thailand, TUR= Turkey, USA= United States of America, VEN= Venezuela.

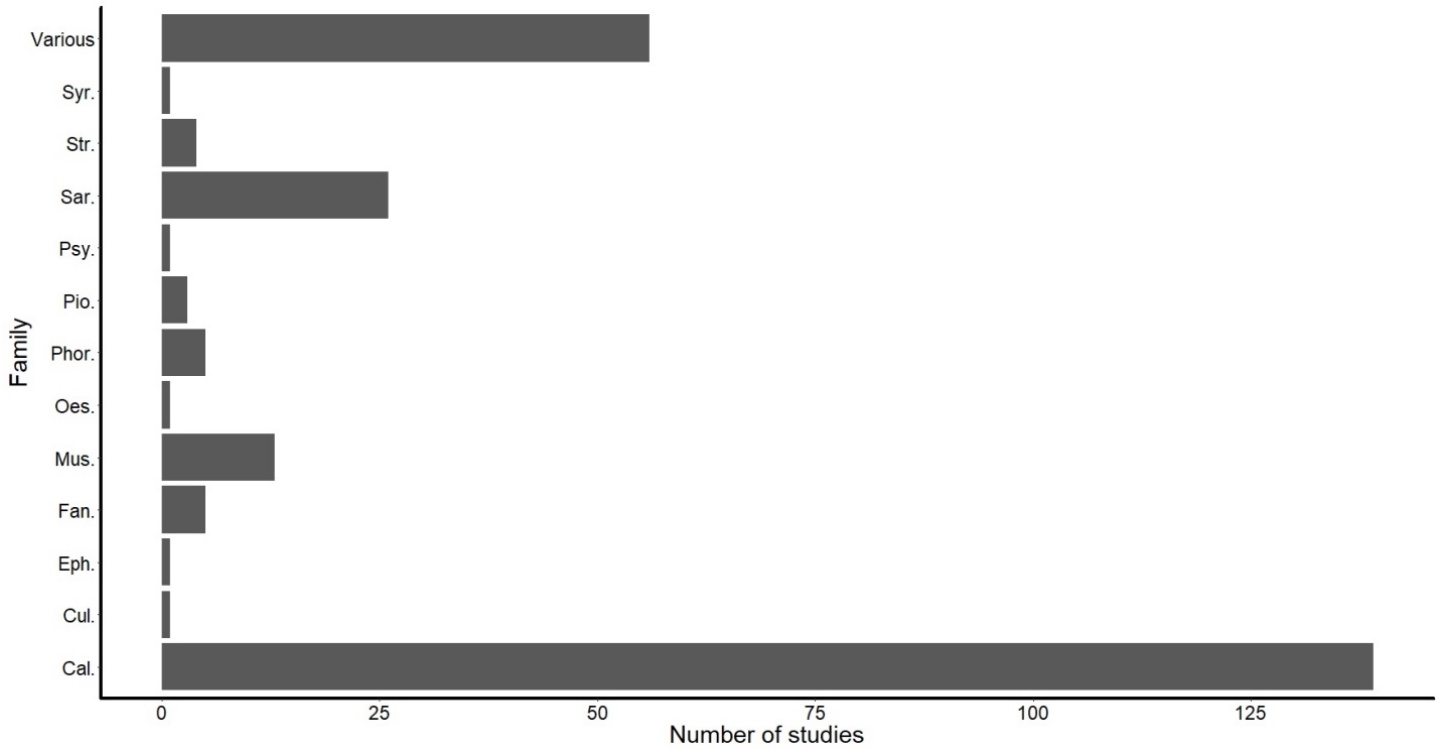
In total, eight insect orders were reported: Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera and Phthiraptera. From all the articles, 74.17%, or 223

articles, reported data or performed experiments with only one insect order, being Diptera the more often studied order (183 articles or 59.22%). Twenty-nine articles (9.34%) studied only the Coleoptera, nine articles (2.91%) studied Hymenoptera, two articles (0.65%) studied Lepidoptera and only one article was about each of the Phthiraptera, Orthoptera, Heteroptera and Blattodea (Table 1).

Studies that focused on two different orders represented 10.68%, or 33 articles. Twenty seven of these articles focused on Coleoptera and Diptera, three articles on Diptera and Hymenoptera, and one article on Diptera and Lepidoptera, Orthoptera and Hymenoptera and Diptera and Heteroptera, each. Only 2.27% (7) of the articles focused in three orders. Among those, six articles reported data of Coleoptera, Diptera and Hymenoptera, and one article reported data of Coleoptera, Diptera and Lepidoptera. From the remaining articles, 9.4% (29) report data of “Various” orders, and 17 articles (5.5%) don’t focus on any order, but talk about Forensic Entomology in general.

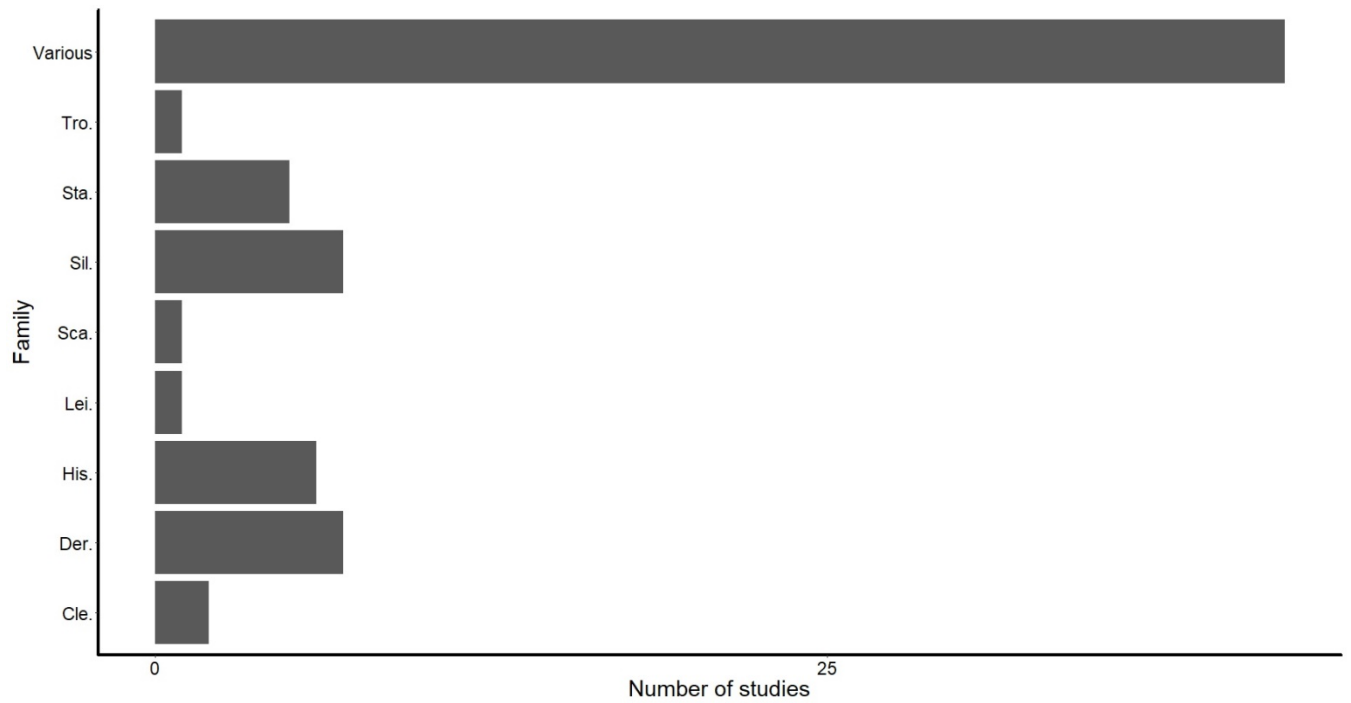
Specifically for the Dipterans, this order appeared in 222 articles (71.85%). Among those, 143 articles report data for only one Diptera family, with dominance for the family Calliphoridae, appearing 139 times, followed by the family Sarcophagidae, with 26 studies. The word “Various”, when referring to Diptera order appeared 56 times (Fig. 4).





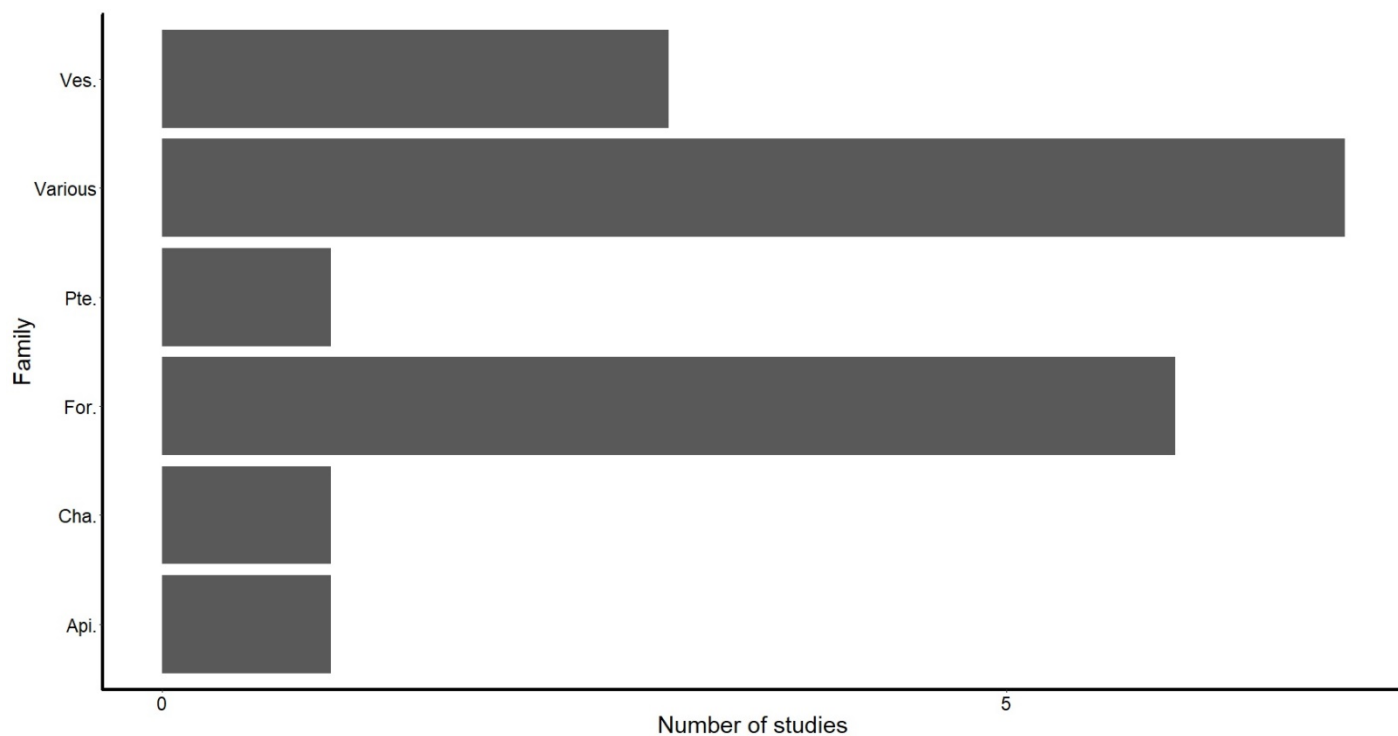
**Figure 4-** Histogram of the number of publications per Diptera family. Syr.= Syrphidae, Str.= Stratiomyidae, Sar.= Sarcophagidae, Psy.= Psychodidae, Pio.= Piophilidae, Phor.= Phoridae, Oes.= Oestridae, Mus.=Muscidae, Fan.= Fanniidae, Eph.= Ephydriidae, Cul.= Culicidae, Cal.= Calliphoridae.

The Coleoptera Order was found in 63 articles. Among those, the families Silphidae and Dermestidae were the most represented, appearing seven times each. Histeridae family came in second place, appearing six times. The word “Various”, when referring to Coleoptera order appeared 42 times (Fig. 5).



**Figure 5-** Histogram of the number of publications per Coleoptera family. Tro. = Trogidae, Sta= Staphylinidae, Sil.= Silphidae, Sca.= Scarabaeidae, Lei.= Leiodidae, His.= Histeridae, Der.= Dermestidae, Cle.= Cleridae.

The third most represented order was the Hymenoptera, appearing in 19 articles. The Formicidae family appeared more times, six in total. Vespidae family came in second, being found three times. Apidae, Pteromalidae and Chalcididae appeared one time each. Seven articles reported data for more than three families (Fig. 6).



**Figure 6-** Histogram of the number of publications per Hymenoptera family. Ves.= Vespidae, Pte.= Pteromalidae, For.= Formicidae, Cha.= Chalcididae, Api.= Apidae.

**Table 1-** Insect groups in the articles.

<b>ORDER</b>	<b>FAMILY</b>	<b>SPECIES</b>
Blattodea	Termitidae	-
Coleoptera	Cleridae	<i>Necrobia ruficollis</i> <i>Necrobia rufipes</i>
	Dermestidae	<i>Dermestes ater</i> <i>Dermestes maculatus</i>
	Histeridae	<i>Margarinotus brunneus</i> <i>Margarinotus marginatus</i> <i>Margarinotus ruficornis</i> <i>Margarinotus striola succicola</i> <i>Margarinotus ventralis</i> <i>Onthophilus striatus</i>

		<i>Operclipygus hospes</i>
		<i>Saprinus lugens</i>
		<i>Saprinus planiusculus</i>
		<i>Saprinus</i> sp.
	Leiodidae	<i>Catoposchema tasmaniae</i>
	Scarabaeidae	<i>Coprophanæus lancifer</i>
	Silphidae	<i>Necrodes littoralis</i>
		<i>Necrophila americana</i>
		<i>Nicrophorus vespilloides</i>
		<i>Nicrophorus vespilloides</i>
		<i>Oiceoptoma thoracicum</i>
		<i>Oxelytrum discicolle</i>
		<i>Thanatophilus micans</i>
		<i>Thanatophilus sinuatus</i>
	Staphylinidae	<i>Creophilus maxillosus</i>
	Trogidae	<i>Omorgus</i> sp.
Diptera	Calliphoridae	<i>Calliphora augur</i>
		<i>Calliphora croceipalpis</i>
		<i>Calliphora dubia</i>
		<i>Calliphora hilli</i>
		<i>Calliphora stygia</i>
		<i>Calliphora varifrons</i>
		<i>Calliphora vicina</i>
		<i>Calliphora vomitoria</i>
		<i>Chloroprocta idioidea</i>
		<i>Chrysomya albiceps</i>
		<i>Chrysomya chloropyga</i>
		<i>Chrysomya marginalis</i>
		<i>Chrysomya megacephala</i>
		<i>Chrysomya nigripes</i>
		<i>Chrysomya pinguis</i>
		<i>Chrysomya putoria</i>
		<i>Chrysomya rufifacies</i>
		<i>Chrysomya saffrana</i>
		<i>Chrysomya varipes</i>
		<i>Cochliomyia hominivorax</i>
		<i>Cochliomyia macellaria</i>
		<i>Cynomya cadaverina</i>

	<i>Hemilucilia segmentaria</i>
	<i>Hemilucilia semidiaphana</i>
	<i>Hemipyrellia ligurriens</i>
	<i>Lucilia caesar</i>
	<i>Lucilia cuprina</i>
	<i>Lucilia eximia</i>
	<i>Lucilia illustris</i>
	<i>Lucilia sericata</i>
	<i>Paralucilia fulvinota</i>
	<i>Paralucilia paraensis</i>
	<i>Phaenecia coeruleiviridis</i>
	<i>Phormia regina</i>
	<i>Protophormia terraenovae</i>
	<i>Sarconesia chlorogaster</i>
Culicidae	-
Ephydriidae	-
Fanniidae	<i>Fannia manicata</i> <i>Fannia pusio</i>
Muscidae	<i>Australophyra rostrata</i> <i>Hydrotaea dentipes</i> <i>Hydrotaea rostrata</i> <i>Musca domestica</i> <i>Muscina stabulans</i> <i>Ophyra aenescens</i> <i>Ophyra albuquerquei</i> <i>Ophyra capensis</i> <i>Synthesiomyia nudiseta</i>
Oestroidea	-
Phoridae	<i>Megaselia scalaris</i>
Piophilidae	<i>Parapiophila vulgaris</i> <i>Parasarcophaga ruficomis</i> <i>Piophila casei</i> <i>Stearibia nigriceps</i>
Psychodidae	-
Sarcophagidae	<i>Helicobia pilifera</i> <i>Microcerella erythropyga</i> <i>Microcerella halli</i> <i>Oxysarcodexia fringidea</i>

		<i>Oxysarcodexia riograndensis</i>
		<i>Peckia (Pattonella) resona</i>
		<i>Peckia (Peckia) pexata</i>
		<i>Ravinia belforti</i>
		<i>Sarcophaga (Lyopygia)</i>
		<i>crassipalpis</i>
		<i>Sarcophaga albiceps</i>
		<i>Sarcophaga argyrostoma</i>
		<i>Sarcophaga bullata</i>
		<i>Sarcophaga haemorrhoidalis</i>
		<i>Sarcophaga hirtipes</i>
		<i>Sarcophaga peregrina</i>
		<i>Sarcophaga</i> sp.
	Stratiomyidae	<i>Hermetia illucens</i>
	Syrphidae	<i>Ornidia obesa</i>
Heteroptera	Alydidae	<i>Alydus eurinus</i>
Hymenoptera	Apidae	-
	Formicidae	<i>Cephalotes clypeatus</i>
		<i>Crematogaster scutellaris</i>
		<i>Solenopsis invicta</i>
	Pteromalidae	<i>Nasonia vitripennis</i>
	Vespidae	<i>Agelaia fulvofasciata</i>
Lepidoptera	-	-
Orthoptera	Tettigoniidae	<i>Pediocetes haldemani</i>
Phthiraptera	Pediculidae	<i>Pediculus humanus capitis</i>
		<i>Pediculus humanus humanus</i>

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Among the carcasses or tissues/diet used in all the studies, pig was the most used animal, appearing in 120 articles (38.8%). Beef came in second, appearing in 43 studies (14%). Human meat or tissue came in third, being found in 40 studies (13%) (Table 2). Forty articles used a combination of more than one type of carcass, tissue or diet.

**Table 2-** Relation of carcass/diet or tissue type and number of Forensic Entomology articles (N).

<b>Carcass/Tissue/Diet</b>	<b>N</b>
Pig	120
Beef	43
NA	42
Human	40
Rabbit	26
Chicken	14
Rat	13
Artificial diet	9
Fish	6
Liver	5
Sheep	4
Minced meat	3
Black Bear	2
Goat	2
Grain-based diet	2
Kangaroo	2
White-tailed deer	2
American alligator	1
Apple	1
Canine muscle	1
Courgette	1
domestic guinea pig	1
Equine tissue	1
hedgehogs	1
Honey	1
<i>Lucilia illustris</i>	
pupae	1
Monkey	1
Peanut butter	1
Pet	1
Raccoon	1
Shrimp paste	1

Snake	1
Squid	1
Sugar	1

From a total of 172 articles analyzed that had only one theme, 27% (84 articles) aimed at observing the effects of external drivers on the entomofauna. Another 20 (6.5%) were case-studies (e.g. following a police investigation) and 19 (6.2%) were reviews. Also, 10 articles (3.24%) studied the type of diet, tissue and bait, in total. Finally, 10 articles only intended to perform an inventory of the entomofauna found in the study (Table 3).

**Table 3-** Relation of the subject studied and number of articles (N).

<b>Type of Study</b>	<b>N</b>
Activity	3
Behavior	3
Case-Study	20
Development of models	3
DNA Analysis	1
Effect	84
Improvements in the area	4
Morphology	3
Occurrence	6
Review	19
Residues analysis	1
Survey	10
Diet type	3
Bait type	2
Tissue type	5
Others	5

One hundred and thirty-seven articles (44.34%) focused in studying more than one subject in the Forensic Entomology Field. Eighteen articles (5.8%) studied the effect of a determined factor, the patterns of succession and performed a survey, all together. Sixteen (5.18%) articles studied the patterns of succession, the seasonality and also performed a survey. Twelve (3.88%) articles studied the patterns of succession and performed a survey (Table 4).



**Table 4-** Relation of the subject studied and number of articles (N).

<b>Type of Study</b>	<b>N</b>
Case-Study/DNA Analysis	1
Case-Study/Effect	3
Case-Study/Occurrence	3
Effect/Behavior	14
Effect/Carcass type	1
Effect/Development of models	1
Effect/Diet type	2
Effect/Morphology	1
Effect/Occurrence	2
Effect/Survey	3
Effect/Tissue type	3
Survey/Habitat	5
Survey/Seasonality	6
Survey/Succession	12
Activity/Occurrence/Type of Bait	1
Case-Study/Occurrence/Behavior	1
Review/DNA Analysis	2
Review/Improvements in the area	1
Review/Succession	1
Review/Survey	2
Survey/Bait type	2
Effect/Seasonality/Habitat	1
Effect/Survey/Seasonality	1
Effect/Survey/Succession	18
Effect/Survey/Succession/Habitat	3
Effect/Survey/Succession/Seasonality	4
Review/Survey/Habitat	1
Review/Survey/Succession	1
Succession/Seasonality/Occurrence	1
Survey/Habitat/Bait type	2
Survey/Habitat/Seasonality	3
Survey/Habitat/Time variation	2
Survey/Seasonality/Carcass type	1
Survey/Seasonality/Time variation	1
Survey/Succession/Habitat	6
Survey/Succession/Time variation	2
Survey/Succession/Seasonality	16
Survey/Habitat/Bait type/Annual variation	1
Survey/Habitat/Bait type/Seasonality	1
Survey/Succession/Seasonality/Habitat	5

Articles that tried to verify the influence of a factor over the entomofauna, alone or associated with another type of study, were in great number, resulting in 141 in total (45.6%). Among those, 44 articles studied the effects of temperature only, being temperature the most studied factor. The second most studied effect was the effects of the night over the oviposition behavior, which was almost five times less studied. Seven articles focused on studying the effects of burial and burned versus unburned carcasses each (Table 5).

**Table 5-** Relation of the factor studied and number of articles (N).

<b>Effect of</b>	<b>N</b>
Temperatura	44
Night	9
Burial	7
Burned, Unburned	7
Sun, Shade	5
Indoor, Outdoor	4
Morphine	3
Fresh, Frozen	2
Hanging, Not hanging	2
Light, Dark	2
Malathion	2
Stage of Decomposition	2
Temperature, Light Intensity	2
Wrapping	2
3,4-Methylenedioxymethamphetamine	1
Abiotic environmental factors, Light intensity	1
Altitude	1
Ant presence	1
Antibiotic Ampicillin	1
Antibiotic Ciprofloxacin	1
Antibiotics Ampicillin, Cefazolin, Ceftizoxime, Clindamycin, Gentamicin, Mezlocillin, Vancomycin	1
Barbiturates (thiopentone, phenobarbitone, amylobarbitone, barbitone, brallobarbitone) Analgesics (Aspirin, sodium salicylate, paracetamol, sodium aminohippurate, amphetamine sulfate)	1
Buscopan	1
Carcass mass, Clothing	1
	22

Chemical Thiamethoxam	1
Climate, Chemicals (Insecticide, Gasoline, Mosquito repellent, Caustic soda, Perfume, Bleach)	1
Clothing	1
Clothing, Sun, Shade	1
Cocaine	1
Competitor	1
Concealed, Exposed	1
Dermestes maculatus in wounds	1
Drugs (opiates,cocaine, henobarbital,Levopromazine,Amitriptyline,Nortriptyline,Tioridazine,Clomipramine)	1
Egg immersion, Wetness, Artificially wounded areas, Short-haired areas	1
Ethanol	1
Flunitrazepam	1
Household Products (Bleach, Repellent, Perfume, Caustic Soda, Insecticide, Gasoline, Hydrochloric Acid)	1
Household Products (Soda, Repellent, Insecticide, Gas, HCl), Sun, Shade	1
Insecticide	1
Investigator Disturbance	1
Isopropanol	1
Ketamine	1
Ketamine, Temperatura	1
Length of Time After Death	1
Length, Temperature	1
Light, Bait Height	1
<i>Lucilia sericata</i>	1
Malathion, Rain, Sun, Shade	1
Malignant tumor	1
Methamphetamine, p-Hydroxymethamphetamine	1
Methylphenidate hydrochloride, Phenobarbital and their association	1
Nandrolone Decanoate	1
Nordiazepam	1
Paracetamol	1
Photoperiod, Depth of burrowing	1
Refrigeration, Frozen-Thawed	1
Sheltered, Unsheltered	1

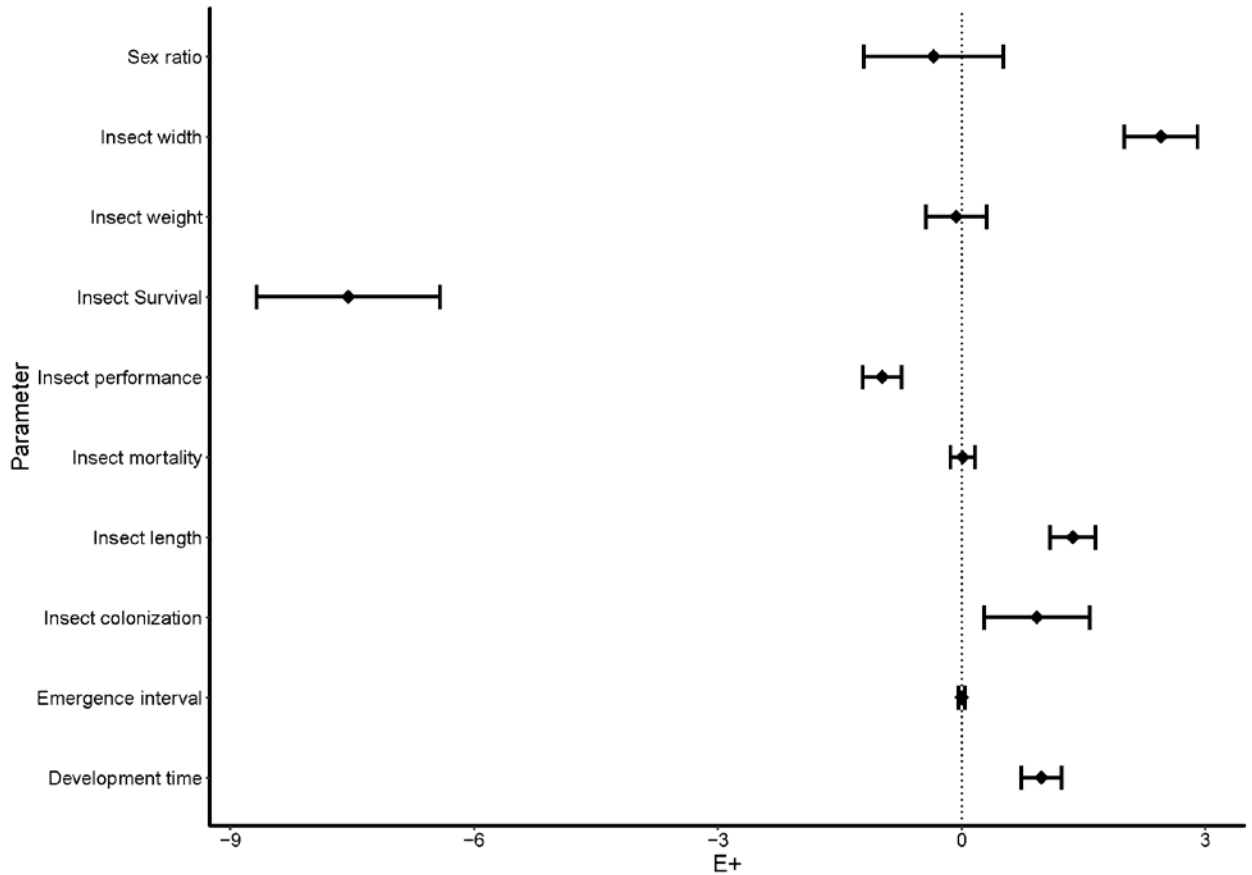
Steroids, Burial	1
Temperature, Humidity, Light Intensity	1
Tramadol	1
Type of death	1
Wound cleansing treatments	1

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## Meta-analysis

From a total of 309, 24 studies met our criteria and were selected to be part of this meta-analysis. These 24 studies generated 999 comparisons of the effects of the presence of substances on different insect parameters.

Overall, in the random-effects meta-analysis model, the presence of chemical substances accelerated insect development time by 98.04% ( $E_{++} = 0.9804$ ,  $CI = 0.7351$  to  $1.2256$ ,  $df = 113$ ), and increased insect colonization by 92.42% ( $E_{++} = 0.9242$ ,  $CI = 0.2726$  to  $1.5757$ ,  $df = 6$ ), insect length by 136.65% ( $E_{++} = 1.3665$ ,  $CI = 1.0860$  to  $1.6469$ ,  $df = 85$ ) and insect width by 245.16% ( $E_{++} = 2.4516$ ,  $CI = 2.0040$  to  $2.8993$ ,  $df = 23$ ). On the contrary, the presence of chemical substances decreased insect survival by 754.95% ( $E_{++} = -7.5495$ ,  $CI = -8.6750$  to  $-6.4240$ ,  $df = 57$ ) and insect performance by 97.81% ( $E_{++} = -0.9781$ ,  $CI = -1.2193$  to  $-0.7369$ ,  $df = 14$ ), but had no visible effect on insect weight ( $E_{++} = -0.0684$ ,  $CI = -0.4426$  to  $0.3069$ ,  $df = 116$ ), insect mortality ( $E_{++} = 0.0114$ ,  $CI = -0.1372$  to  $0.1601$ ,  $df = 13$ ), emergence interval ( $E_{++} = 0$ ,  $CI = -0.0391$  to  $0.0391$ ,  $df = 35$ ) and sex ratio ( $E_{++} = -0.3465$ ,  $CI = -1.2042$  to  $0.5113$ ,  $df = 3$ ) (Fig. 7).



**Figure 7-** General effects of the presence of chemical substances on insect development time, emergence interval, colonization, length, mortality, performance, survival, weight, width and sex ration. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

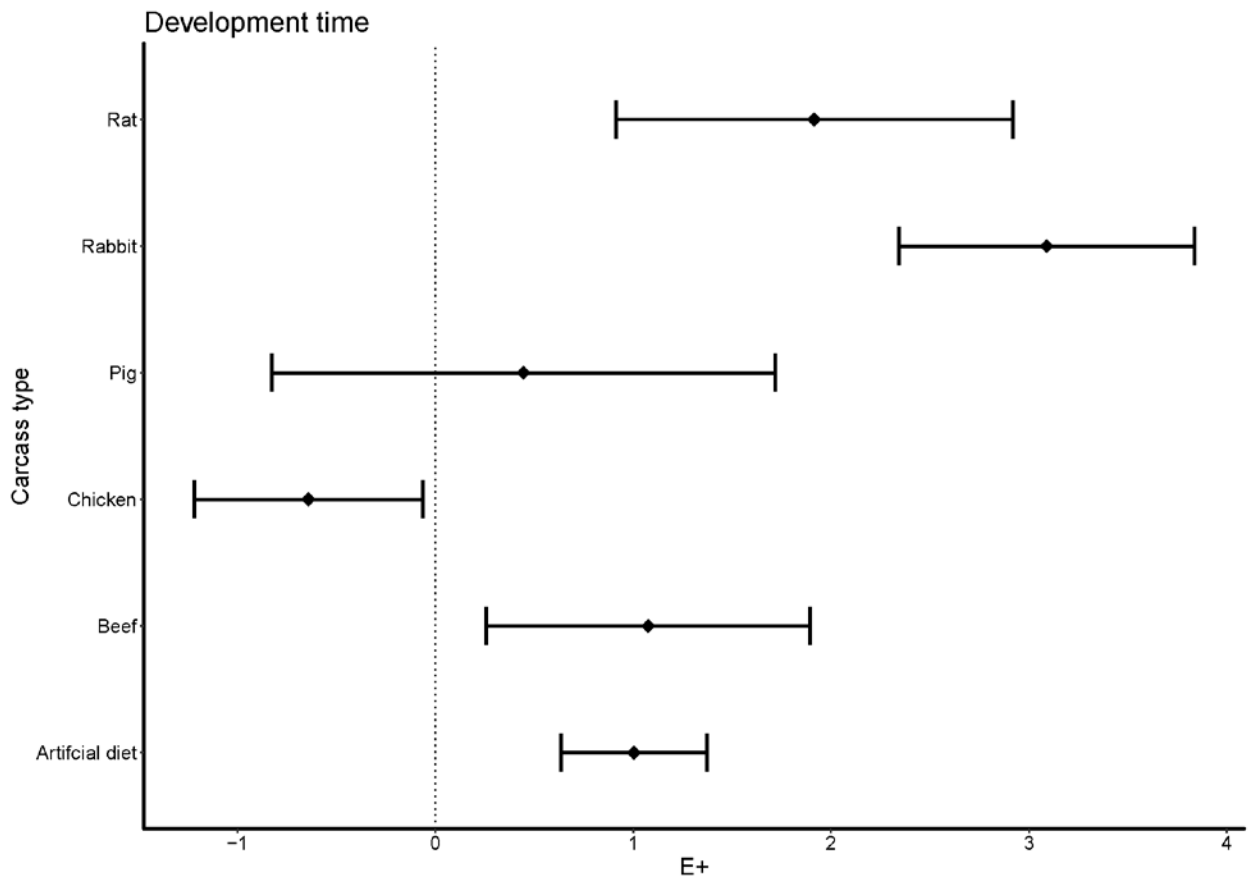
Fail-safe numbers for effects of insect survival (8106.6 studies), insect length (5255.5 studies), development time (3285.7 studies), insect performance (408.3 studies), insect colonization (22.2 studies) and insect width (2602.9 studies) were large compared to the number of independent comparisons included in the meta-analysis (58, 86, 114, 15, 7 and 24 comparisons, respectively), indicating the strength of our results in these parameters. Insect weight, insect mortality, insect emergence interval and insect sex ratio all had fail-safe numbers equal to zero studies.

We also tested the effects of the moderators carcass type and chemical substance, using the meta-regression model. Carcass type influenced the development time, length, weight, width and mortality of insects, as described below. All the parameters analyzed showed a statistically

significant difference among all the carcasses types (development time:  $Q_B = 74.1884$ ,  $p < 0.01$ ; length:  $Q_B = 237.7359$ ,  $p < 0.01$ ; weight:  $Q_B = 161.0255$ ,  $p < 0.01$ ,  $p < 0.01$ ; width:  $Q_B = 161.2579$ ,  $p < 0.01$  and mortality:  $Q_B = 9.7737$ ,  $p = 0.0075$ ).

### Development time

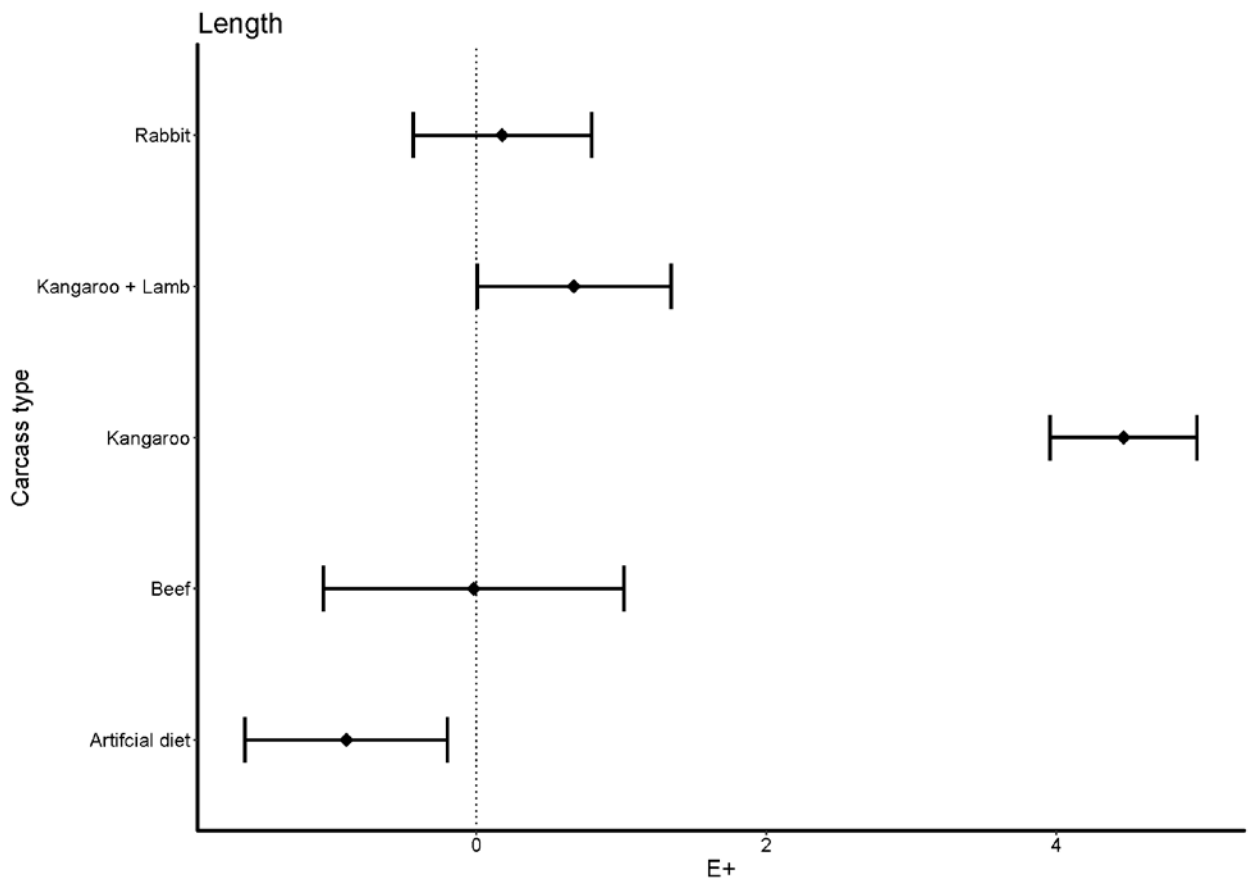
The development time of insects is accelerated in rabbits by 308.8% ( $E_{++} = 3.0898$ ,  $CI = 2.3438$  to  $3.8357$ ,  $df = 19$ ), rats by 191.49% ( $E_{++} = 1.9149$ ,  $CI = 0.9116$  to  $2.9182$ ,  $df = 10$ ), artificial diet by 100.29% ( $E_{++} = 1.0029$ ,  $CI = 0.6341$  to  $1.3717$ ,  $df = 39$ ) and beef by 107.54% ( $E_{++} = 1.0754$ ,  $CI = 0.2580$  to  $1.8927$ ,  $df = 11$ ). In chicken, the insect development time slowed by 64.21% ( $E_{++} = -0.6421$ ,  $CI = -1.2202$  to  $-0.0640$ ,  $df = 23$ ) (Fig. 8). Pig did not have a significant effect over insect development. It was not possible to evaluate the effect of liver, kangaroo and kangaroo mixed with lamb meat, because there were not enough comparisons.



**Figure 8-** Effects of the presence of chemical substances on insect development time according to carcass type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

### Length

Kangaroo and kangaroo mixed with lamb meat had a positive effect over insect length, increasing it by 446.43% ( $E_{++} = 4.4643$ ,  $CI = 3.9596$  to  $4.9689$ ,  $df = 35$ ), and by 67.40% ( $E_{++} = 0.6740$ ,  $CI = 0.0060$  to  $1.3421$ ,  $df = 11$ ), respectively. Artificial diet had a negative effect, decreasing the length of insects by 89.60% ( $E_{++} = -0.8960$ ,  $CI = -1.5943$  to  $-0.1977$ ,  $df = 12$ ). Rabbit and beef did not have a significant effect over insect length (Fig. 9). It was not possible to evaluate the effect of liver, pig, rat and chicken, because there were not enough comparisons.

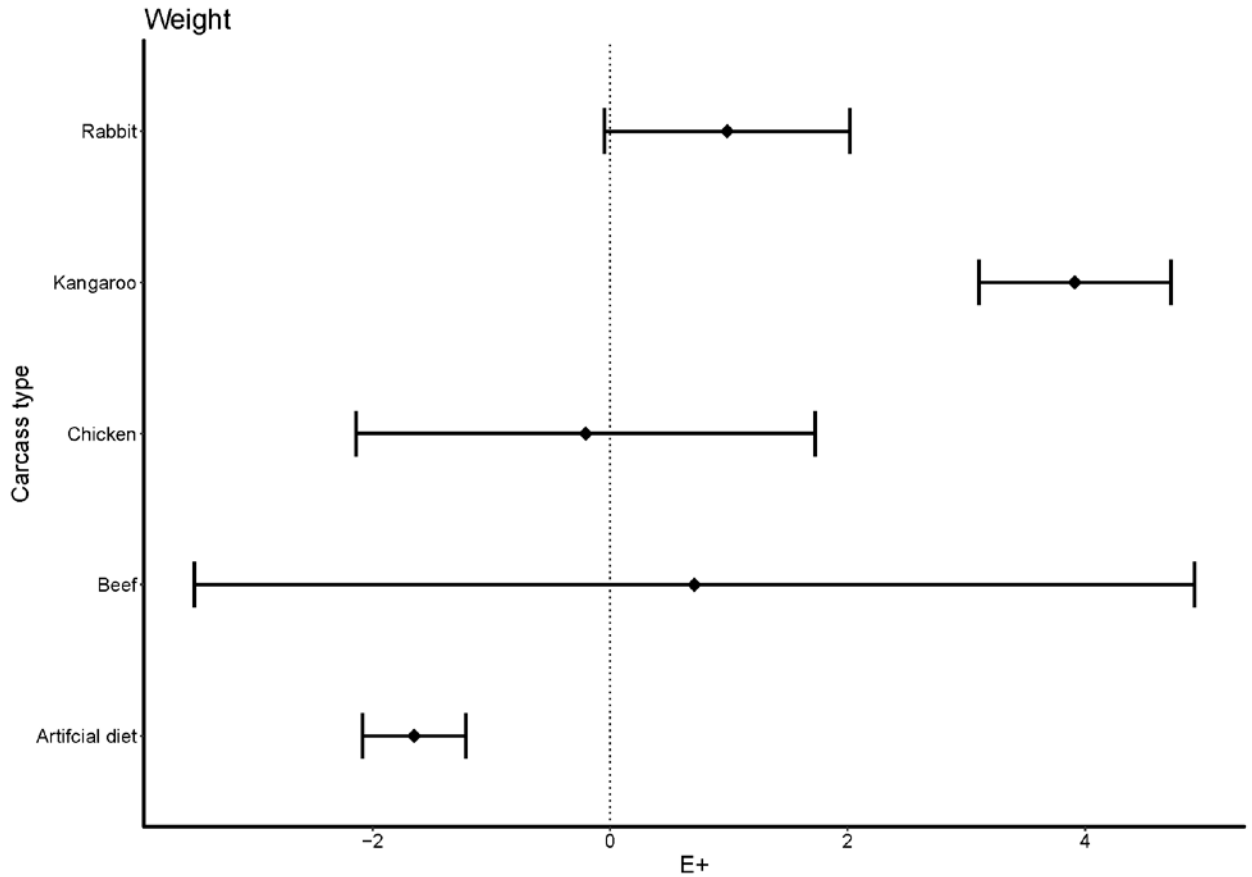


**Figure 9-** Effects of the presence of chemical substances on insect length according to carcass type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

### Weight

Kangaroo had a positive effect over insect weight, increasing it by 391.47% ( $E_{++} = 3.9147$ ,  $CI = 3.1083$  to  $4.7212$ ,  $df = 26$ ). Artificial diet decreased it by 165.11% ( $E_{++} = -1.6511$ ,

CI=-2.0870 to -1.2152, df= 66), respectively. Rabbit, beef and chicken did not have a significant effect over insect weight (Fig. 10). It was not possible to evaluate the effect of liver, pig, rat, kangaroo mixed with lamb meat because there were not enough comparisons.

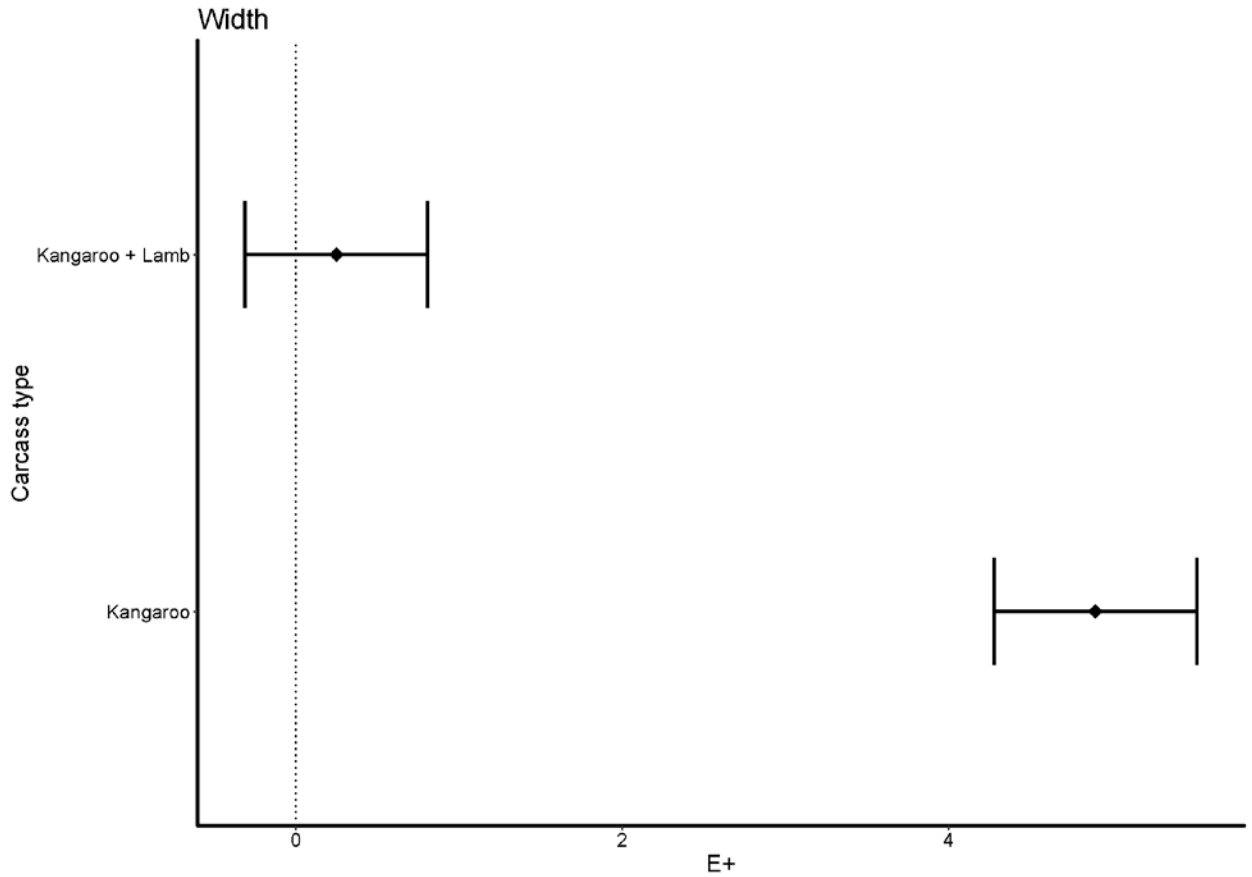


**Figure 10-** Effects of the presence of chemical substances on insect weight according to carcass type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

### Width

Only kangaroo had a positive effect over insect width, increasing it by 489.90% ( $E_{++} = 4.8990$ , CI= 4.2777 to 5.5203, df= 17). Kangaroo mixed with lamb meat did not have a significant effect over insect width (Fig. 11). It was not possible to evaluate the effect of liver, pig, rabbit, rat, beef, artificial diet and chicken, because there were not enough comparisons.

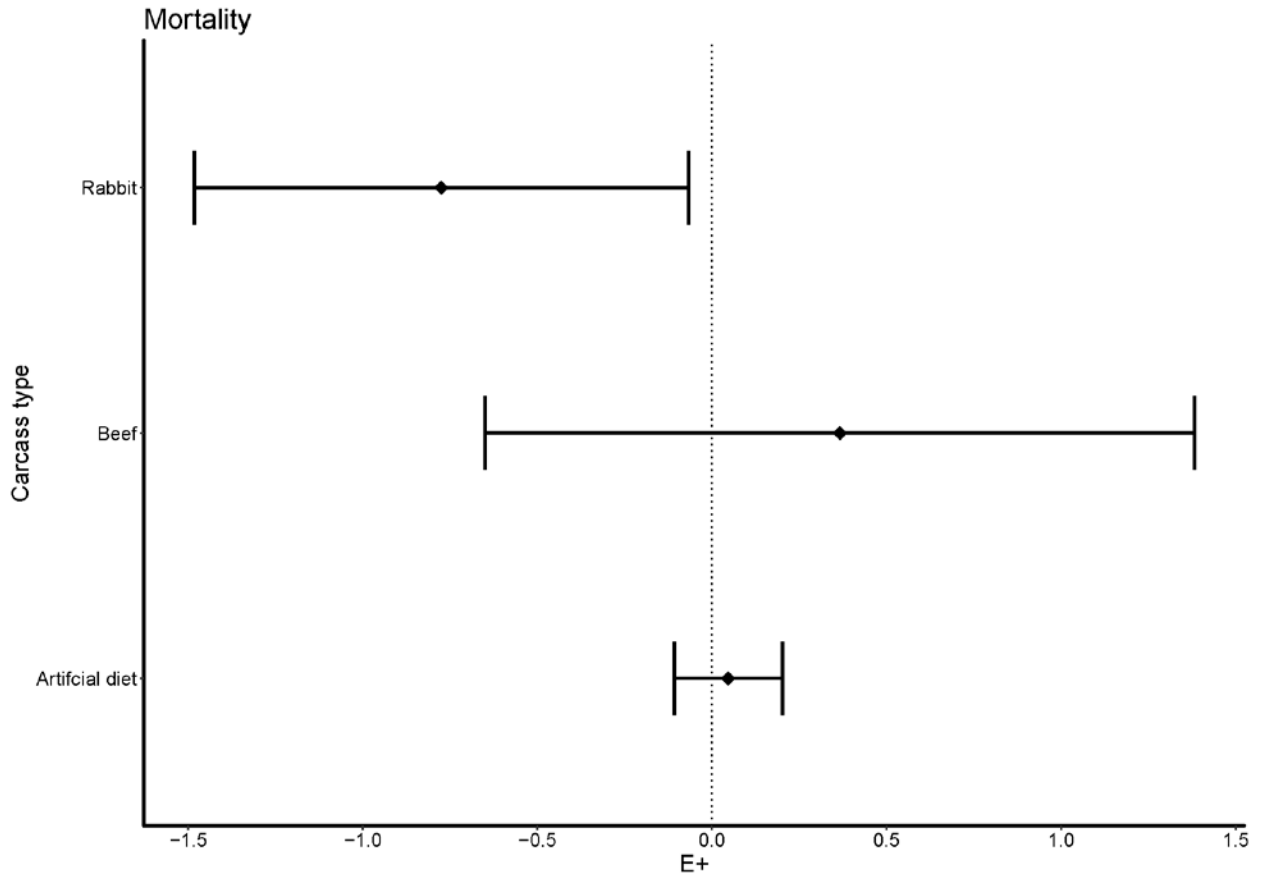




**Figure 11-** Effects of the presence of chemical substances on insect width according to carcass type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

### *Mortality*

Rabbit had a negative effect on insect mortality, decreasing it by 77.50% ( $E_{++} = -0.7750$ ,  $CI = -1.4832$  to  $-0.0669$ ,  $df = 5$ ). Neither beef nor artificial diet had a significant effect over insect mortality (Fig. 12). It was not possible to evaluate the effect of liver, pig, rabbit, rat, kangaroo, kangaroo mixed with lamb meat and chicken, because there were not enough comparisons.



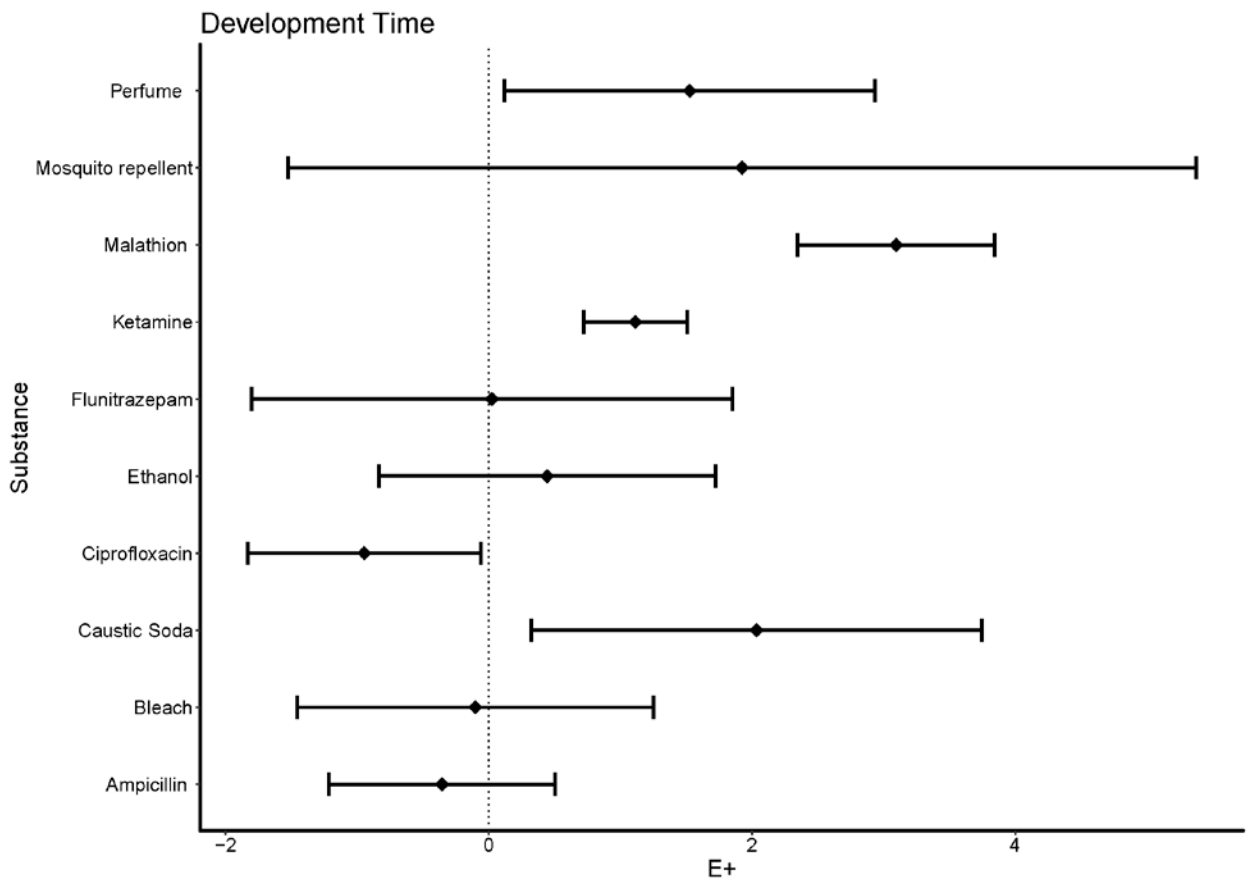
**Figure 12-** Effects of the presence of chemical substances on insect mortality according to carcass type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

The different types of substances had an effect over the development time, length, weight, width, mortality, emergence interval and survival of insects, as described below. Only emergence interval of insects was not significantly different from zero for the different types of substances ( $Q_B = 0$ ,  $p=1$ ). All the other parameters showed a statistically significant difference among all the substances types (development time:  $Q_B = 83.1392$ ,  $p < 0.01$ ; length:  $Q_B = 230.3087$ ,  $p < 0.01$ ; weight:  $Q_B = 199.6982$ ,  $p < 0.01$ ; width:  $Q_B = 166.2894$ ,  $p < 0.01$ , mortality:  $Q_B = 8.6327$ ,  $p = 0.00330$ , and survival:  $Q_B = 495.8186$ ,  $p < 0.01$ ).

#### *Development time*

The development time of insects is accelerated in the presence of caustic soda by 203.58% ( $E_{++} = 2.0358$ ,  $CI = 0.3219$  to  $3.7498$ ,  $df = 4$ ), perfume by 152.73% ( $E_{++} = 1.5273$ ,  $CI =$

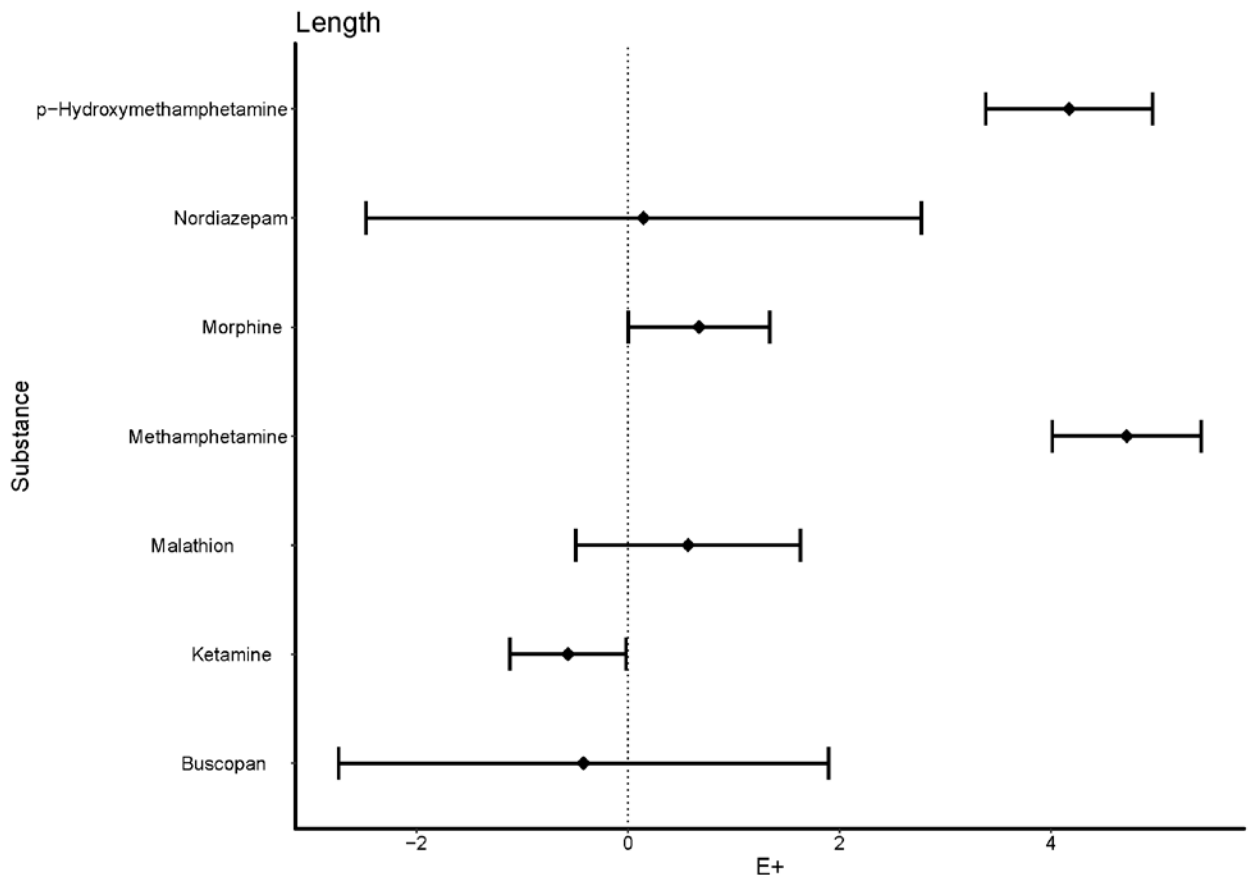
0.1182 to 2.9364, df= 5), ketamine by 111.44% ( $E_{++} = 1.1144$ , CI= 0.7213 to 1.5075, df= 35) and malathion by 309.61% ( $E_{++} = 3.0961$ , CI= 2.3470 to 3.8453, df= 19). Ciprofloxacin slowed insect development time by 94.67% ( $E_{++} = -0.9467$ , CI= -1.8330 to -0.0604, df= 11). Ethanol, Flunitrazepam, bleach, mosquito repellent and ampicillin did not affect significantly the development time (Fig. 13). It was not possible to evaluate the effect of hydrochloric acid, citronella, insecticide, gas, Nordiazepam, p-Hydroxymethamphetamine, methamphetamine, morphine, nandrolone, methylphenidate hydrochloride, phenobarbital, Buscopan, sodium hypochlorite, isopropyl alcohol, povodine iodine, hydrogen peroxide, unleaded gasoline, cocaine, pentane and Tramadol because there were not enough comparisons.



**Figure 13-** Effects of the presence of chemical substances on insect development time according to substance type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

## Length

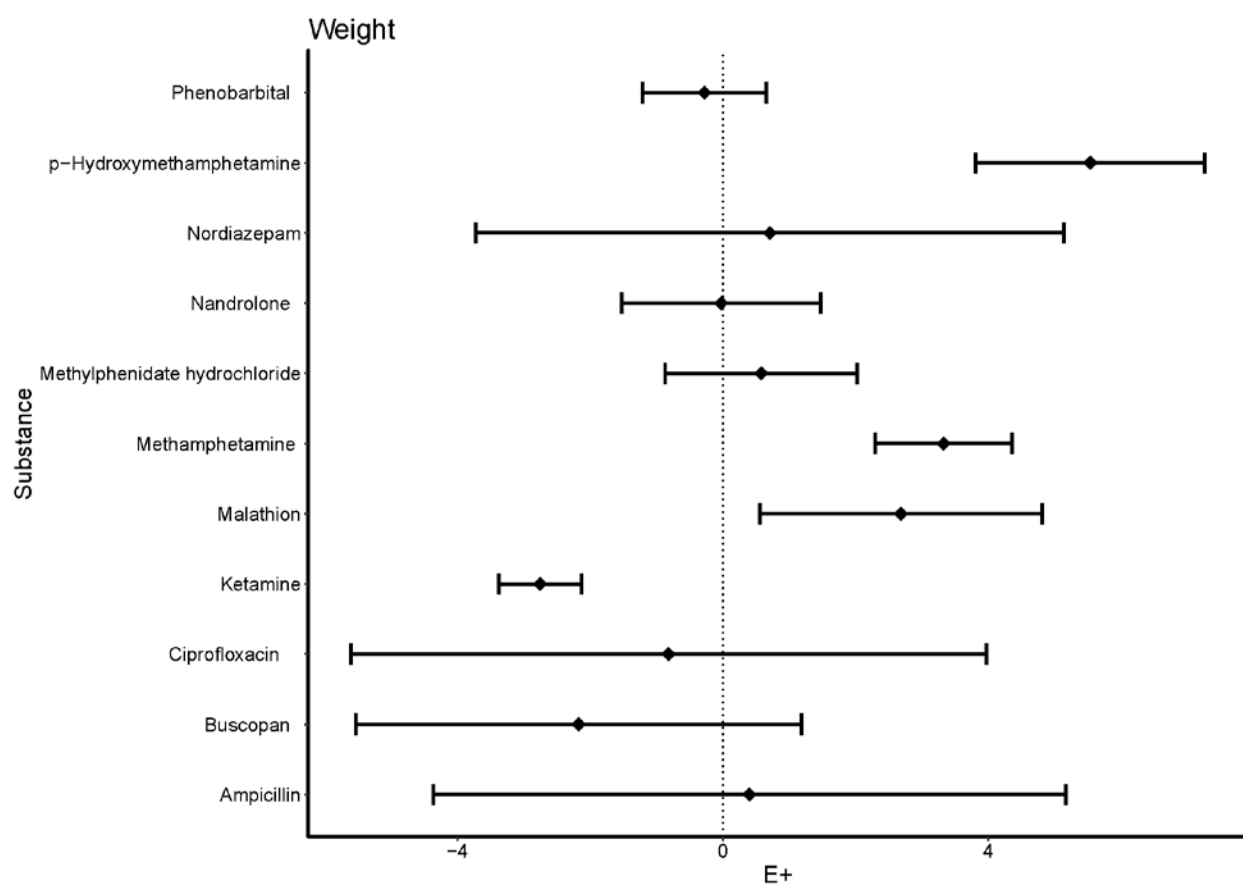
The length of insects increased in the presence of p-Hydroxymethamphetamine by 417.17% ( $E_{++} = 4.1717$ , CI= 3.3836 to 4.9598,  $df= 14$ ), methamphetamine by 471.49% ( $E_{++} = 5.1272$ , CI= 4.0099 to 5.4200,  $df= 20$ ) and morphine by 67.40% ( $E_{++} = 0.6740$ , CI= 0.0052 to 1.3429,  $df= 11$ ). Ketamine decreased insect length by 56.79% ( $E_{++} = -0.5679$ , CI= -1.1166 to -0.0193,  $df= 16$ ). Nordiazepam, malathion and Buscopan did not affect significantly the length (Fig. 14). It was not possible to evaluate the effect of hydrochloric acid, citronella, ethanol, insecticide, gas, Flunitrazepam, caustic soda, bleach, mosquito repellent, ampicillin, nandrolone, methylphenidate hydrochloride, phenobarbital, sodium hypochlorite, isopropyl alcohol, povidine iodine, hydrogen peroxide, unleaded gasoline, cocaine, ciprofloxacin, perfume, pentane and Tramadol because there were not enough comparisons.



**Figure 14-** Effects of the presence of chemical substances on insect length according to substance type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

## Weight

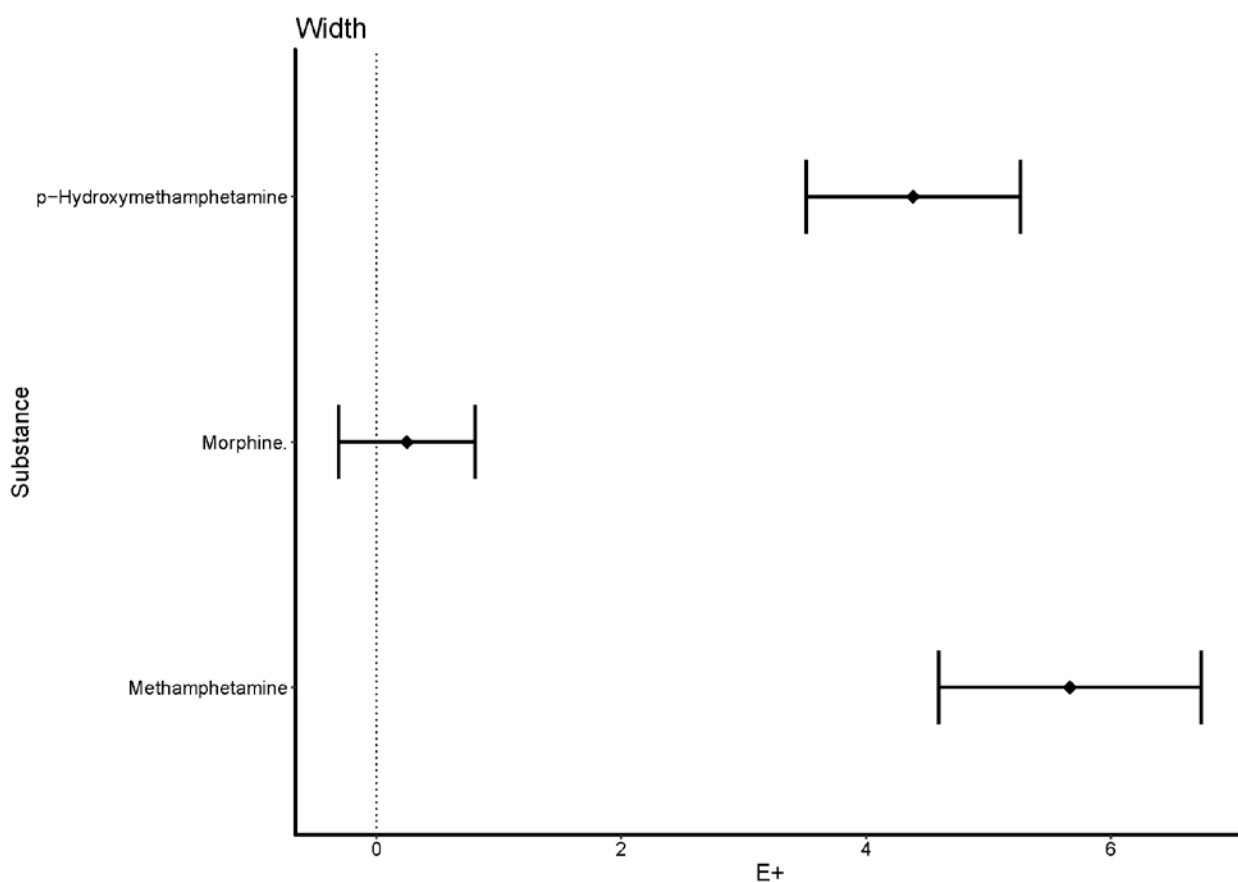
The weight of insects increased in the presence of p-Hydroxymethamphetamine by 553.71% ( $E_{++} = 5.5371$ , CI= 3.8140 to 7.2603,  $df= 8$ ), methamphetamine by 332.68% ( $E_{++} = 3.3268$ , CI= 2.2993 to 4.3544,  $df= 17$ ) and malathion by 268.36% ( $E_{++} = 2.6836$ , CI= 0.5606 to 4.8065,  $df= 5$ ). Ketamine decreased insect weight by 275.28% ( $E_{++} = -2.7528$ , CI= -3.3733 to -2.1323,  $df= 34$ ). Nordiazepam, nandrolone, methylphenidate hydrochloride, Buscopan, ciprofloxacin, ampicillin and phenobarbital did not affect significantly the weight (Fig. 15). It was not possible to evaluate the effect of hydrochloric acid, citronella, ethanol, insecticide, gas, morphine, Flunitrazepam, caustic soda, bleach, mosquito repellent, sodium hypochlorite, isopropyl alcohol, povodine iodine, hydrogen peroxide, unleaded gasoline, cocaine, perfume, pentane and Tramadol because there were not enough comparisons.



**Figure 15-** Effects of the presence of chemical substances on insect weight according to substance type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

### Width

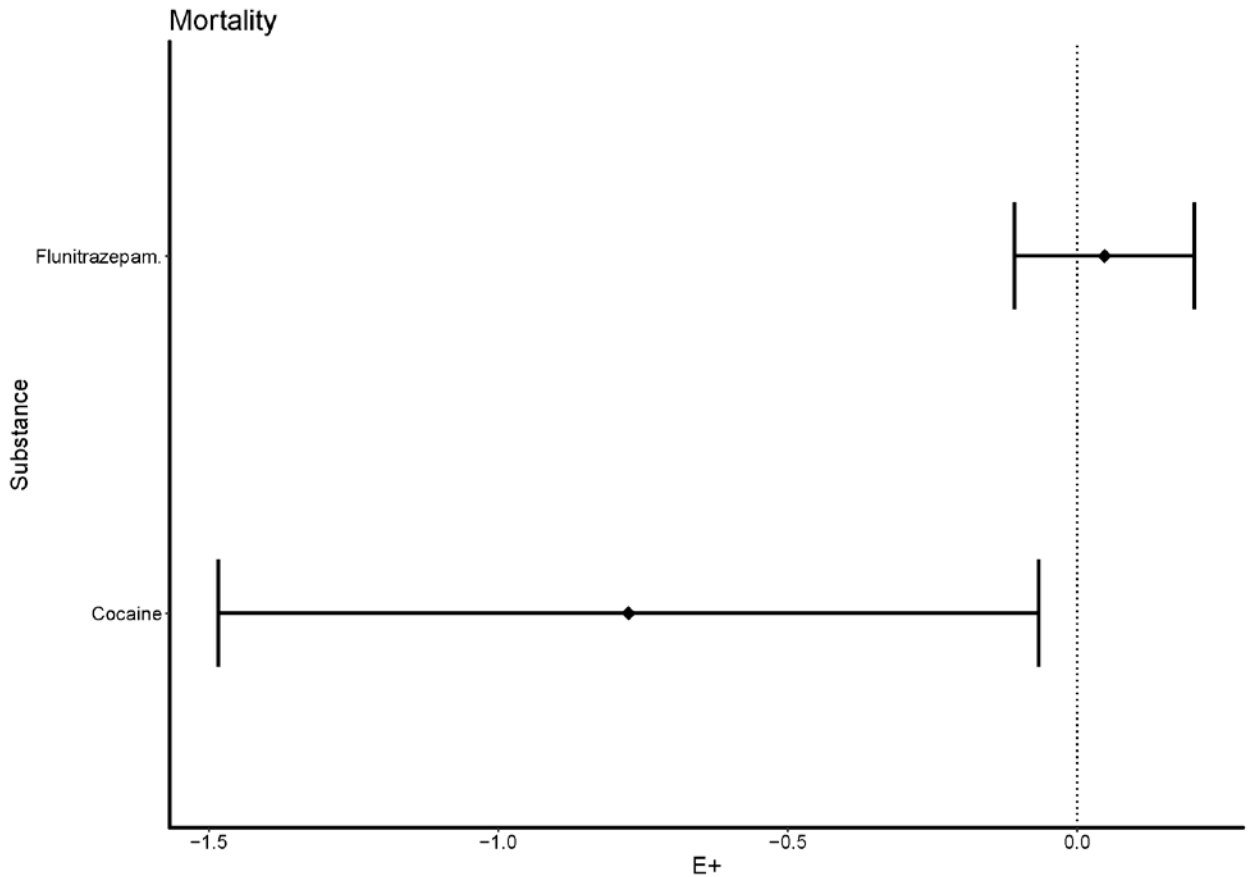
The width of insects increased in the presence of p-Hydroxymethamphetamine by 438.32% ( $E_{++} = 4.3832$ , CI= 3.5083 to 5.2580,  $df= 8$ ) and methamphetamine by 566.32% ( $E_{++} = 5.6632$ , CI= 4.5897 to 6.7366,  $df= 8$ ). Morphine did not affect significantly the width (Fig. 16). It was not possible to evaluate the effect of hydrochloric acid, citronella, malathion, ethanol, insecticide, gas, Flunitrazepam, caustic soda, bleach, mosquito repellent, ampicillin, Buscopan, sodium hypochlorite, isopropyl alcohol, povodine iodine, hydrogen peroxide, unleaded gasoline, cocaine, ciprofloxacin, perfume, pentane, Tramadol, Nordiazepam, ketamine, nandrolone, methylphenidate hydrochloride and phenobarbital because there were not enough comparisons.



**Figure 16-** Effects of the presence of chemical substances on insect width according to substance type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

### Mortality

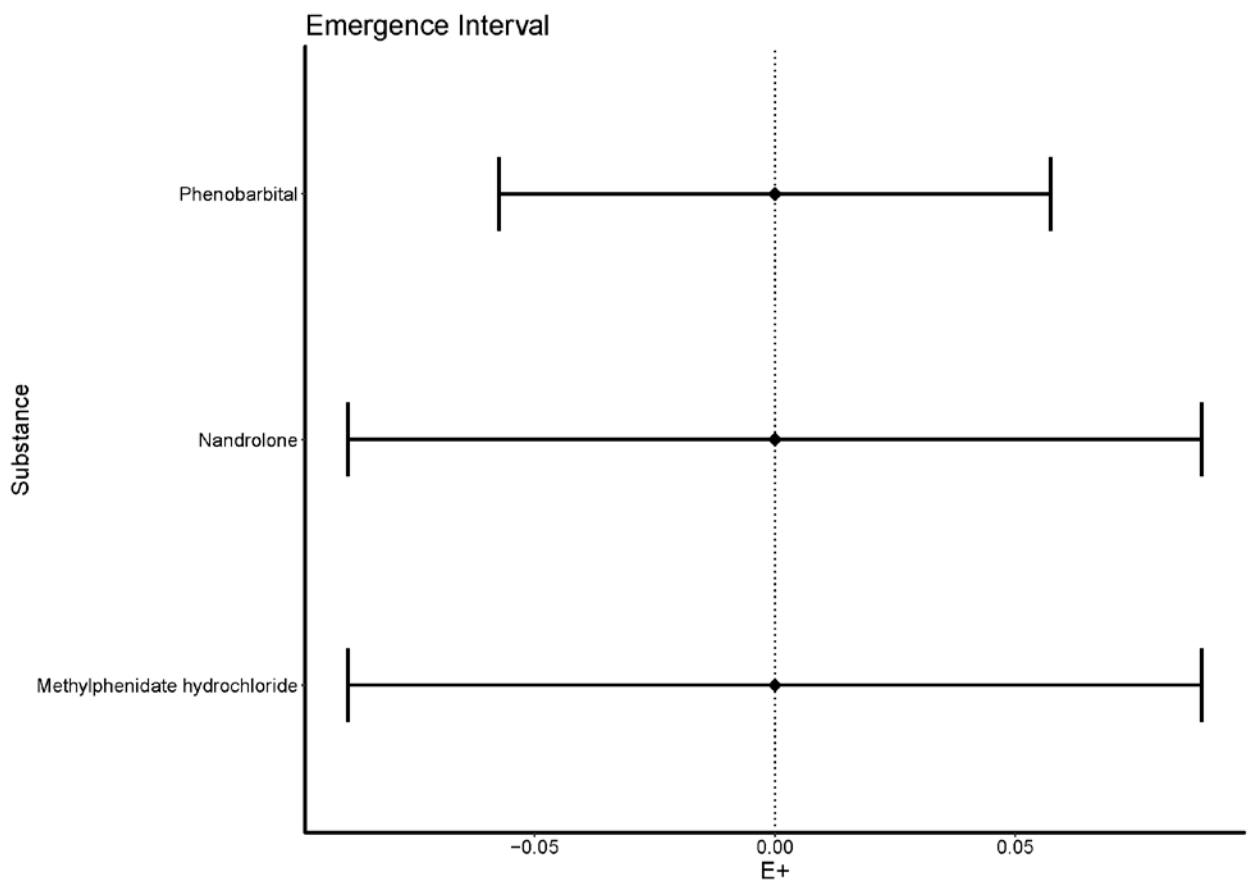
The mortality of insects decreased in the presence of Cocaine by 77.50% ( $E_{++} = -0.7750$ ,  $CI = -1.4832$  to  $-0.0669$ ,  $df = 5$ ). Flunitrazepam did not affect significantly the mortality of insects (Fig. 17). It was not possible to evaluate the effect of hydrochloric acid, citronella, malathion, methamphetamine, ethanol, insecticide, gas, caustic soda, bleach, mosquito repellent, ampicillin, Buscopan, sodium hypochlorite, isopropyl alcohol, povodine iodine, morphine, hydrogen peroxide, unleaded gasoline, ciprofloxacin, perfume, pentane, Tramadol, p-Hydroxymethamphetamine, Nordiazepam, ketamine, nandrolone, methylphenidate hydrochloride and phenobarbital because there were not enough comparisons.



**Figure 17-** Effects of the presence of chemical substances on insect mortality according to substance type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

### Emergence interval

The emergence interval of insects did not alter in the presence of Nandrolone, methylphenidate hydrochloride and phenobarbital (Fig. 18). It was not possible to evaluate the effect of hydrochloric acid, citronella, malathion, methamphetamine, ethanol, insecticide, gas, caustic soda, bleach, mosquito repellent, ampicillin, Buscopan, sodium hypochlorite, isopropyl alcohol, povodine iodine, Flunitrazepam, cocaine, morphine, hydrogen peroxide, unleaded gasoline, ciprofloxacin, perfume, pentane, Tramadol, p-Hydroxymethamphetamine, Nordiazepam and ketamine, because there were not enough comparisons.

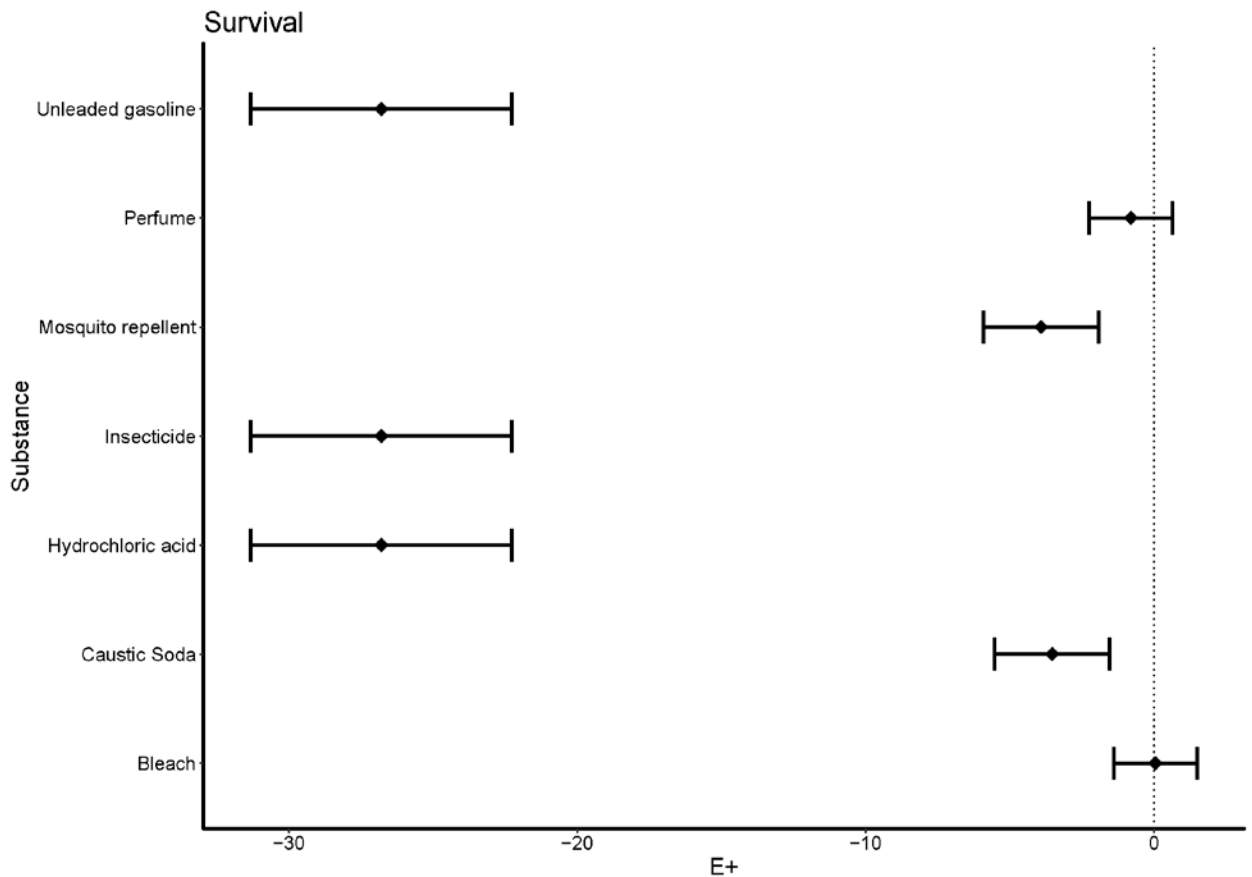


**Figure 18-** Effects of the presence of chemical substances on insect emergence interval according to substance type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.



## Survival

The survival of insects was decreased in the presence of caustic soda by 352.85% ( $E_{++} = -3.5285$ ,  $CI = -5.5137$  to  $-1.5433$ ,  $df = 7$ ), hydrochloric acid, unleaded gasoline and insecticide, each by 267.90% ( $E_{++} = -26.7900$ ,  $CI = -31.3217$  to  $-22.2583$ ,  $df = 7$ ) and mosquito repellent by 390.99% ( $E_{++} = -3.9099$ ,  $CI = -5.9045$  to  $-1.9153$ ,  $df = 7$ ). Perfume and bleach did not alter the survival of insects (Fig. 19). It was not possible to evaluate the effect of, citronella, malathion, methamphetamine, ethanol, phenobarbital, gas, ampicillin, Buscopan, sodium hypochlorite, isopropyl alcohol, povidone iodine, Flunitrazepam, cocaine, morphine, hydrogen peroxide, nandrolone, ciprofloxacin, methylphenidate hydrochloride, pentane, Tramadol, p-Hydroxymethamphetamine, Nordiazepam and ketamine, because there were not enough comparisons.



**Figure 19-** Effects of the presence of chemical substances on insect survival according to substance type. The cumulative effect is reported for each effect with its 95% confidence intervals and effects are significant when confidence intervals do not overlap with zero.

## Assessment of publication bias

Fail-safe numbers for effects of the presence of chemical substances according to the type of carcasses were high for insect length (5579.7 studies), development time (3340.5 studies) and insect width (2916.7 studies), indicating the strength of our results. Insect weight and insect mortality all had fail-safe numbers equal to zero studies, demonstrating some weakness in these results.

When the type of substances were the moderator, fail-safe numbers for effects of the presence of chemical substances were high for insect survival (10799.8 studies), insect length (5631.5 studies), insect width (2921.4 studies) and development time (2799.9 studies), also indicating the robustness of our results. Insect mortality had safe-fail number equal to 0.1 studies, and insect weight and insect emergence interval had fail-safe numbers equal to zero studies, indicating that these results are weak.

## DISCUSSION

### Review

Regarding the journals that published Forensic Entomology articles, it is clear that this science is related to different fields of study. The oldest article here included was published in the human health domain, in 1985, and, since then, Forensic Entomology has been studied with focus in many other areas. However, with the advance of the Forensic Sciences, journals specialized in this field were created around the sixties, and, now, hold the majority of the articles published. Although the first article here included dates from 1985, the science of Forensic Entomology has been reported since the 13<sup>th</sup> century (Benecke, 2001). There are records of older texts about this science, for instance the study of Freire, published in 1914, but it was not possible to locate them in the databases used,

The Forensic Entomology area has been studied in all continents, except in Antarctica, and in 33 countries, but only two of them hold a significant amount of articles (Brazil 23.3% and United States 18.77%). All other countries have contributed smaller numbers; this evidence concludes that we have more room to develop in this area. Brazil had the greater number of articles about Forensic Entomology in this review and this may be because this country has a consolidate tradition in studying the orders Diptera and Coleoptera, which has contributed to the advancement of this science in the country (Santana & Boas, 2012). Almost 96% of the articles were written in English, which, although expected, confirms the international character of this science.

Most researchers preferred to study only one insect order. It may be due to the fact that usually people specialize in one type of group or because it is easier to focus in only one taxon. The most studied order was the Diptera, since flies are considered to be the most essential insect in forensic entomology (Jens Amendt, Krettek, & Zehner, 2004). Along with the Dipterans, the Coleopterans, the second most studied order, are of great forensic importance. Both taxa are largely used in the estimation of the *post-mortem* interval, since they compose the first groups to colonize the carcass, which is a source of food and a substrate for reproduction (J. Amendt, Richards, Campobasso, Zehner, & Hall, 2011). The other orders have fewer studies in the area

and this can be due to the fact that orders, such as Lepidoptera and Hymenoptera, can parasitize or predate necrophagous species or they can use the carcass as an alternative resource (Campobasso *et al.*, 2001)

Among the Dipterans, the Calliphoridae family was by far the most represented. This can be explained for its ubiquitous distribution and its great number of species (approximately 1000 species), which make it easy to find, study and compare results of this family worldwide (Jens Amendt *et al.*, 2004). Also, the species of this family are one of the pioneers in colonizing a carcass and are considered to be the most precise tools for the *post-mortem* interval calculation (Alves, Santos, Farias, & Creão-Duarte, 2014). *Chrysomya megacephala* appeared in more studies. This species occurs in all continents and has a distinguished facility to encounter carrion, which may explain its great number in studies (Badenhorst & Villet, 2018).

Sarcophagidae family was the second most studied Dipiteran family and, alongside with the Calliphoridae family, it is one of the most common families of dipterans found on carrion (Martín-Vega & Baz, 2013). Although it was the second most studied order, the number of studies of the Sarcophagidae family differs largely of the Calliphoridae family (five times smaller than the number of Calliphoridae studies). According to Dias *et al.* (2015), the small number of studies about this family is perhaps due to the difficulty in identifying the species of this family, especially in the larval stage. The Muscidae family is also considered to be important in Forensic Entomology, but studies about this family in the forensic field are yet incipient.

For Coleopterans, Dermestidae and Silphidae are the most studied families. According to Kulshrestha (2001), these two families are among the most important families of Coleopterans in Forensic Sciences. Still according to this author, *Dermestes maculatus* (Dermestidae), also known as skin beetle, is one of the most significant specie in this field. The Silphidae family, also known as carrion beetle, can be used by investigator to obtain information about the death, such as time since death, but knowledge about carrion beetle is not abundant (Dekeirsschieter, Verheggen, Haubruge, & Brostaux, 2011). Despite being one of the main orders in Forensic Entomology, there are considerably fewer studies about Coleoptera, when compared to Dipterans, and consequently, the information about Coleopterans families in this field is limited. It is solid information that beetles are found in advanced stages of decomposition, and due to

that, studies about these insect are not that abundant, once fewer studies report data about the entomofauna of later stages of putrefaction (Kulshrestha & Satpathy, 2001).

Very few studies reported data for Hymenoptera order, being Formicidae the most represented family. Ants are normally classified as omnivorous, feeding on the carrion or on the entomofauna occurring in it (Chen et al., 2014). In the process of decomposition, ants may disrupt the colonization of other necrophagous insects, as they can predate them, thus, affecting the decomposition and successional process (Gomes et al., 2007). Therefore, in many studies, researchers try to eliminate these insects using traps to avoid their arrival to the carrion.

Regarding carcass type, as expected, the majority of studies used pig as the animal model. Swine carcasses are preferred because they approximate of the human's in their insect community and rate of decomposition (Rodríguez Olivares, Quijas, Cupul Magaña, & Navarrete Heredia, 2015). Beef meat or tissue came in second, and it is usually used in bait form. The use of baits represents a low cost and a gain of time, aside from permitting higher numbers of replication allowing a high number of replicates (Goh et al., 2013). Most studies that used human as models were case-studies and had the association of the Police Department included, once there are many ethical and legal issues that involve the use of human bodies or parts in research. The association of carcass or diet and tissue type in a study was mainly conducted to compare the efficiency of each.

Most of the studies focused on performing an inventory and study the successional pattern of the entomofauna in the region where the study was conducted, which is very important for the advances of this field. The main use of Forensic Entomology is to determine the *post-mortem interval* (PMI) and to do so, it is crucial to know the species biology, anatomy, distribution, ecology and behavior (Sharma, 2015). Also, the attempt to understand the effect of a determined factor over the entomofauna was the main goal in many studies. The understanding of the factors that can influence the colonization of carrion by insects, the development of immature stages or the rate of decomposition of the body is also fundamental for the correct estimation of the PMI, in addition to provide information about the circumstances and cause of death, *post-mortem* injuries on the body, use of drugs or other substances (Linhares and Thyssen 2007). However, generally, there was only one study about the effect of a factor over determined

specie, therefore the amount of information available is limited, which compromises the precision reliability of the conclusions one can make.

## Meta-analysis

Although the effects of the presence of determined chemical substance on insect parameters had been revealed in previously individual studies, our meta-analysis demonstrates the generalization of those effects and how each insect parameter is affected. For instance, Tabor *et al.*, in 2005, indicated that the ingestion of alcohol by pigs before death delayed the development time of maggots. However, Monthei demonstrated in his doctoral dissertation, in 2009, that ethanol in pig tissue does not increase the time of duration of larval stage unless its concentration is higher than 0.10% w/v. In general, we demonstrate a strong effect of the presence of chemical substances, accelerating insect development time, and increasing insect colonization, insect length and insect width. In 1986, Goff *et al.* demonstrated that lethal doses of cocaine in decomposing rabbit tissue considerably accelerated the growth rate of maggots. Also, according to Catts and Goff (1992), related researches showed accelerated maggot growth in the presence of heroin and a positive relation between the quantity of morphine present in tissues and the duration of pupariation. Carvalho *et al.* (2001) investigated the effect of diazepam on flies using decomposing rabbit tissue, and observed that in the presence of this drug, the larvae that fed on the liver containing the drug developed more rapidly than the control larvae. The authors also demonstrated that the flies in contact with diazepam presented faster growth rates when compared to the controls and weighed almost two times more than the others. The emergence time of these insects also took longer than the control group. Different studies demonstrate similar effects in the presence of methamphetamine, amitriptyline and cocaine. According to Bourel *et al.* (1999), in a study performed with rabbits, the effects of morphine in larvae may be dependent of the dosage of this substance. In this study, the larvae of *Lucilia sericata* reared in the rabbit with the greatest concentration of morphine developed slower. In 2001, the same author studied the effects of morphine hydrochloride solutions in minced beef, and found almost no concentration of the substance in pupae of the same species, indicating the capacity of these larvae to eliminate great quantity of morphine during the post-feeding stage.

When the death occurs, the autolysis of the body begins and its rate is increased in higher temperatures, and so is the decomposition rate. Illicit and prescribed drugs, such as the ring-derivate amphetamines, cocaine and drugs that have an effect similar to the drug atropine may be the cause of high body temperatures at the moment of death (Zhou & Byard, 2011), which may cause an accelerated body decomposition and attract more insect. This might be the reason of the increase in insect colonization when in presence of some chemical substances that we found in this study.

On the other hand, we showed a significant decrease in insect survival and insect performance. Trivia *et al.* (2018) performed a study to evaluate the effect of chemotherapeutic drugs present in beef, on the necrophagous fly *Chrysomya megacephala* and found that the drug methotrexate reduces the survival rate of this specie, but has no significant effect in the sex ratio. Many chemical substances, such as insecticides, act over the nervous system of insects, altering numerous behavior aspects (Haynes, 1988). This might be the main reason of the decrease of insect performance, which may directly affect insect survival.

We also revealed that not all carcasses types cause the same effects on these insects' parameters, as significant differences were observed amongst groups. Kaneshrajah *et al.* (2004) says that the type of tissue used as substrate may alter the effects of different drugs in the growth of some blowfly larvae. In a dissertation, written by Derek Reed Monthei in 2009, the author attests that the substrate in which the larvae are reared can influence its development time, among other parameters. In a study performed in 2003, Green *et al.* found that pupae of *Phormia regina* weighted more when reared in artificial diet with 53% protein and its development time was shorter compared to the pupae reared in lamb's liver or in meridic diets that varied in protein and carbohydrate quantity. The same effect of artificial diet, regarding development time, was found in our results. The study also revealed that the weight of pupae increased as protein in a diet increased, but the level of carbohydrate maintained constant. It showed that in greater quantities of protein, the larvae presented a decrease in development time. The author concluded that if the larvae of this species are reared in a media with insufficient quantity of protein, it will develop in smaller adults. Also, Day *et al.* (2006) demonstrated that larvae that consumed more fat spend less energy on metabolism than if they have fed on substrates purely proteinaceous, directing more energy into growth. Cosgrove *et al.* (2005) attested that red meats (e.g.: beef,

lamb and pork) have higher quantity of fat when compared to white meat (e.g.: chicken and turkey). In addition, in the same study, Cosgrove noticed that the intake of protein by humans was significantly highest and the intake of carbohydrate was significantly lowest in high consumers of red meat, indicating that this type of meat has greater quantity of proteins. This may be the reason of the positive effects of beef, rat and kangaroo meat, and the negative effect of chicken carcass in insect development.

Kangaroo carcass had a positive effect in insect length, weight and width. This may be due to its higher percentages of polyunsaturated fatty acids than beef meat (Sinclair, Slattery, & Deaa, 1982). Insects use this type of lipid in development, locomotors activities, and, when associated with other components, fatty acids compose cellular and subcellular biomembranes, aiding in the structure of cells and tissues (Stanley-Samuels *et al.*, 1988). Also, it is possible that, because of the increase in insect length, the weight increases directly, and the width grows with this last one. Regarding to rabbit carcass, although classified as white meat, it is a lean meat of high protein value, in contrast to red meat and chicken (Nistor *et al.*, 2013), which may explain its positive effect in insect development and its decrease in insect mortality.



## CONCLUSION

In conclusion, in our systematic review we noticed that some countries developed more studies about Forensic Entomology than others, such as Brazil and United States, which demonstrates that this science has much more room to develop itself. We also perceived that the most studied order was the Diptera, followed by the Coleoptera, and that the most performed studies were surveys of the community of insects, studies of ecological succession that occurs in the carcass and studies that aimed to evaluate the effects of a factor on the community of insects. Regarding this last type of studies, they are still very limited.

In our meta-analysis, we demonstrated that in general the presence of chemical substances positively affects insects' development time, colonization, size and width. On the contrary, it affects negatively insects' survival and performance. These effects vary according to the type of chemical substances and the type of carcasses.

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## ATTACHMENT

Article	Re	Me
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		alysis
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