How lasting are the effects of pesticides on earwigs? A study based on energy metabolism, body weight and morphometry in two generations of Forficula auricularia from apple orchards

Adrien Le Navenant, Corinne Brouchoud, Yvan Capowiez, Magali Rault, Séverine Suchail

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Abstract: Widespread use of pesticides to control crop pests is still the dominant system in apple orchards. Therefore, to avoid adverse side effects, there is a growing interest in promoting alternative methods such as natural enemies. The European earwig Forficula auricularia L. (Dermaptera: Forficuidae) is an effective predator in apple orchards. Pesticide pressure has been shown to divert energy resources which could have a negative impact on life history traits. In this study we assessed (i) whether variations in pesticide exposure could differentially impact energy reserves, body weight and morphometric parameters of F. auricularia, and (ii) whether these effects last or not in the next generation reared under optimal conditions. Individuals from the first generation were collected in late October from organic, IPM and conventional orchards. The next generation was obtained under a rearing program, in the absence of pesticide exposure. Earwigs collected from conventional orchards exhibited lower values for all morphometric parameters compared to those collected in organic orchards. However, a relaxed period without pesticide exposure (in autumn) appears to have allowed the females to recover their energy reserves to ensure reproduction and maternal care. Glycogen contents are the reserves that were more easily restored. However, due to the rearing conditions (food ad libitum), all the earwigs from the second generation exhibited higher body weights and energy reserves than their parents. They finally reached the same level in these parameters regardless of the protection system from which their parents were collected. Sex-specific responses appeared to depend on earwig life cycle.

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Professor Jay Gan
Co-Editor-in-Chief (Science of the Total Environment)
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Object: manuscript submission

Avignon, August 10, 2020

Dear Professor Jay Gan,

Please find enclosed a manuscript entitled “**How lasting are the effects of pesticides on earwigs? A study based on energy metabolism, body weight and morphometry in two generations of *Forficula auricularia* from apple orchards”** for submission to Science of the Total Environment in the topic “Ecotoxicology and risk assessment”.

Apple orchards are highly productive crops where the use of pesticides is still the dominant system, and we focus our investigations on alternative methods such as biological control. The earwigs *Forficula auricularia* is a widespread generalist predator in pome fruit orchards, involved in the regulation of pests. Earwigs were collected in orchards conducted under different management strategies (organic, IPM and conventional). The objective of this manuscript was to assess whether different pesticide use could differentially impact energetic reserves, body weight and morphometric parameters, and how long last these effects on earwig’s lifespan and on the next generation. This work is of particular importance since it allows us to better understand the vulnerability of earwig and their progeny towards agricultural practices.

Our results indicated that a relaxed period without pesticide applications (in autumn) allows the females to recover their energetic reserves to face the reproduction and the maternal care. Glycogen contents are the reserves to be more easily restored. Moreover, we highlighted time-specific and sex-specific differential responses depending on both the pesticide application period and the life cycle of earwigs.

We believe and hope that this kind of original study may be of interest for the readers of your journal.

Best regards

Magali RAULT

*Title Page

How lasting are the effects of pesticides on earwigs? A study based on energy metabolism, body weight and morphometry in two generations of *Forficula auricularia* from apple orchards

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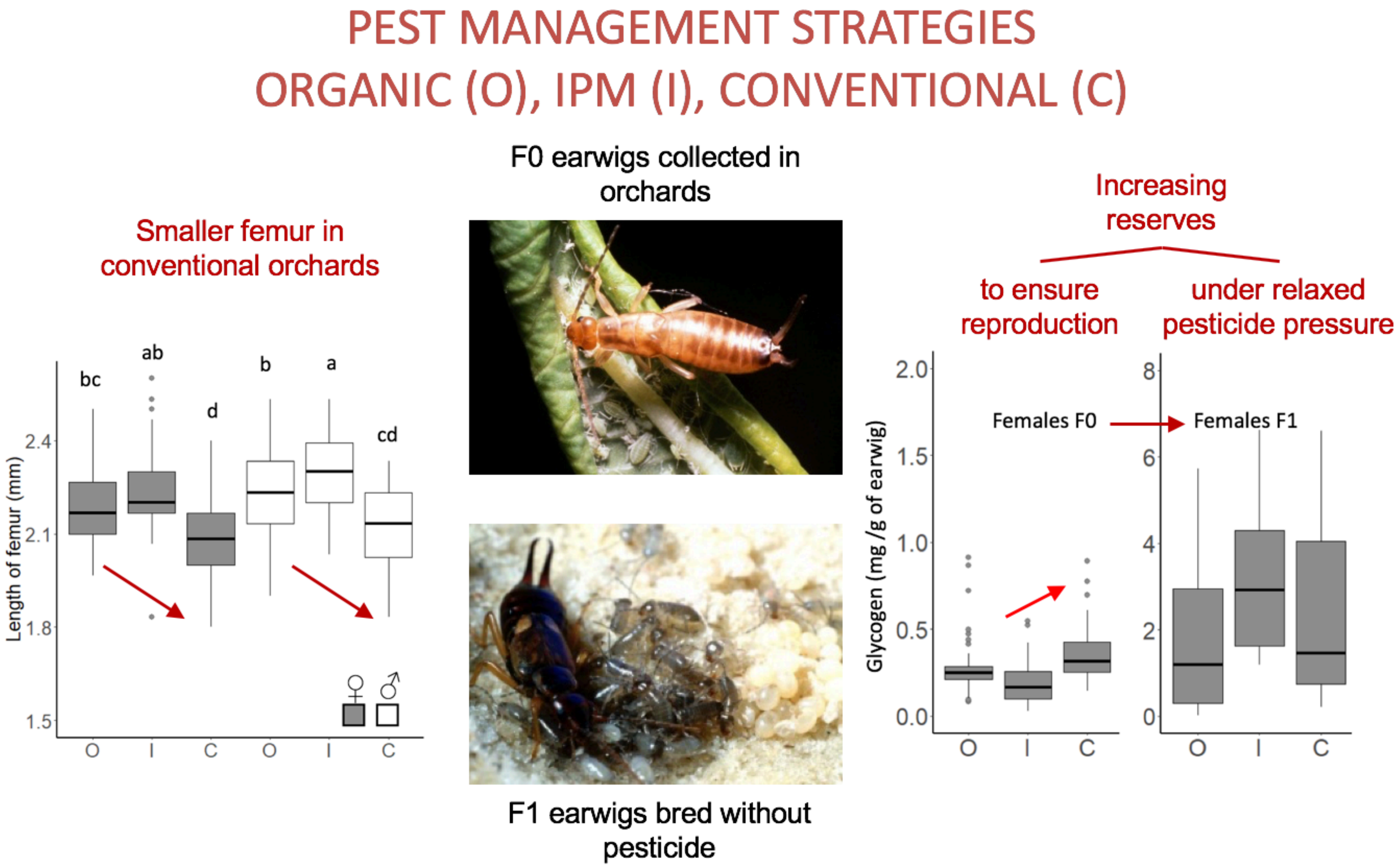
*Graphical Abstract



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

HIGHLIGHTS

- The long-lasting effects of pesticide treatments in orchards on earwigs are unknown
- Morphometric traits are the lowest in earwigs sampled in conventional orchards
- Females appeared to fair better under pesticide pressure to ensure reproduction
- Management strategy did not impact earwig growth according to Dyar's rules
- Under relaxed pesticide pressure during development energy reserves increased

How lasting are the effects of pesticides on earwigs? A study based on energy metabolism, body weight and morphometry in two generations of *Forficula auricularia* from apple orchards

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ABSTRACT

Widespread use of pesticides to control crop pests is still the dominant system in apple orchards. Therefore, to avoid adverse side effects, there is a growing interest in promoting alternative methods such as natural enemies. The European earwig *Forficula auricularia* L. (Dermaptera: Forficuidae) is an effective predator in apple orchards. Pesticide pressure has been shown to divert energy resources which could have a negative impact on life history traits. In this study we assessed (i) whether variations in pesticide exposure could differentially impact energy reserves, body weight and morphometric parameters of *F. auricularia*, and (ii) whether these effects last or not in the next generation reared under optimal conditions. Individuals from the first generation were collected in late October from organic, IPM and conventional orchards. The next generation was obtained under a rearing program, in the absence of pesticide exposure. Earwigs collected from conventional orchards exhibited lower values for all morphometric parameters compared to those collected in organic orchards. However, a relaxed period without pesticide exposure (in autumn) appears to have allowed the females to recover their energy reserves to ensure reproduction and maternal care. Glycogen contents are the reserves that were more easily restored. However, due to the rearing conditions (food ad libitum), all the earwigs from the second generation exhibited higher body weights and energy reserves than their parents. They finally reached the same level in these parameters regardless of the protection system from which their parents were collected. Sex-specific responses appeared to depend on earwig life cycle.

KEYWORDS: European earwigs; energy reserves, biometry, pesticides, pest management strategy

1. INTRODUCTION

Agricultural production needs to increase to meet the needs of a growing world population. Doing so in ways that do not compromise environmental integrity and public health is still a great challenge (Gorbach, 2001; Tilman et al., 2002). However, intensive production systems and widespread use of pesticides to control crop pests still dominate, with adverse side effects on the environment (Köhler and Triebskorn, 2013). Pesticides have an impact at all levels of biological organization from neurotoxicity, immunotoxicity, metabolism to behavior, which could induce cascade events at higher levels (Köhler and Triebskorn, 2013). Such practices reduce biodiversity, affect ecosystem functioning and the production of goods and services by non-target organisms (Mace et al., 2012).

This is particularly the case in apple orchards which are highly productive crops with, an average of 30 pesticide applications per year in Provence, South-Eastern France (Sauphanor et al., 2009; Mazzia et al., 2015). Among these treatments, organophosphorus (OP), neonicotinoid and synthetic pyrethroid compounds are the most used insecticides in Integrated Pest Management (IPM) strategies. Today, there is general agreement among the public and growers, that crop protection should rely less on the use of synthetic pesticides and more on agro-ecology principles (Marliac et al., 2015; Jones et al., 2016). Among these principles, biological control through the utilization and enhancement of natural enemies is of prime importance (Beers et al., 2016). In order to improve the delivery of ecosystem services, we need to identify the effects of factors that could explain the variability in the response of arthropod communities involved in such services. Among those factors, pesticides, beside their lethal effect, induce sublethal physiological or behavioral effects, which may cause long-term effects on the population dynamics of natural enemies, and consequently on pest regulation (Biondi et al., 2013).

The European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae), is a natural enemy in apple orchards. They are effective generalist predators involved in the regulation of pests such as aphids, leaf rollers and psyllids (Dib et al., 2010; Dib et al., 2011; Orpet et al., 2019). The abundance and diversity of earwigs could be influenced either by practices such as tillage and hedge quality (Sharley et al., 2008; Moerkens et al., 2012) or by pesticides use (French-Constant and Vickerman, 1985; Malagnoux et al., 2015a). Moreover, their ease of capture and identification makes them an interesting potential bioindicator of the possible detrimental effects of orchard management. Exposure to sublethal concentrations of pesticides may affect *F. auricularia* behavior. Diflubenzuron has been shown to be highly toxic to *F. auricularia*, affecting its weight, mobility and predatory activity (Sauphanor et al., 1993). More recently, Malagnoux et al. (2015b) reported that Spinosad (used in organic orchards), acetamiprid (neonicotinoid) and chlorpyrifos-ethyl (organophosphorus) have significant negative effects, on the predatory behavior of adult *F. auricularia* earwigs.

Beside direct impacts, long term pesticide exposure is responsible for inducing tolerance to OP insecticides in *F. auricularia* (Le Navenant et al., 2019). Insecticide tolerance and/or resistance can be associated with fitness costs (Rivero et al., 2011), and this explains why susceptible genotypes increase in frequency when insecticide pressure is relaxed (Foster et al., 2017). Resistance to OPs insecticides was observed to lead to fitness costs in the dipteran *Culex pipiens* (Culicidae), with an impact on predation activity (Berticat et al., 2004), mating (Berticat et al., 2002), or fecundity and development (Berticat et al., 2008) in resistant strains. The diversion of the energy flow is the consequence of trade-offs, resulting in the reallocation of energetic resources from primary functions (growth, reproduction) to maintain secondary functions (overproduction of detoxifying enzymes). Such a reallocation of energy reserves induces a negative impact on life history traits (Ribeiro et al., 2001; Da Silva et al., 2004). In insects, life history traits have been shown to be linked to various environmental changes such as habitat loss (Öckinger et al., 2010), climate change (Musolin, 2007) and agricultural management strategies (Kliot and Ghanim, 2012).

Glycogen and the lipid triacylglycerol are the predominant energy reserves in animal cells. Both are stored in the fat bodies distributed throughout the body of the insect with triglycerides representing more than 50% of their dry weight (Arrese and Soulages, 2010). Because glycogen can be quickly hydrolyzed to glucose in the hemolymph of insects, its content may vary depending on physiological status and/or environmental conditions (Anand and Lorenz, 2008; Chowanski et al., 2015). Triacylglycerol also provides energy during diapause for organism development and to sustain flight activity (Van der Horst, 2003). Proteins produce energy through catabolism and are essential in tissue building, repair and protection. All of these reserves are used for growth, maintenance and reproduction (Arrese and Soulages, 2010).

Recently, we reported a severe depletion in energy reserves associated with lower weights and lower values for morphometric parameters in the earwig *F. auricularia* sampled in IPM orchards compared to those sampled in organic apple orchards (Suchail et al., 2018). However, further points remain to be addressed to accurately assess the impact of pesticide pressure on *F. auricularia*. Firstly, IPM management covers a wide range of practices (Barzman et al., 2015; Caffi et al., 2017; Dumont et al., 2020) and little is known concerning the physiological effect in either the earwigs subjected to such a gradient of practices or in their progeny. Do all the IPM management approaches used in apple orchards have effects that impair energy reserves and morphometric sizes? Secondly, following energy reserve depletion, are the earwigs able to rapidly restore their own energy reserves once the pesticide treatment period is over (e.g. in autumn)? And would these impacts have a visible effect on the next generation? In this study, we aimed to assess the potential recovery of energetic reserves, modification of body weight and morphometric parameters, in two succeeding generations of earwigs with sex distinction. The organisms from the first generation were collected in late October in organic and IPM orchards with different levels of pesticide use. The next generation of these earwigs was obtained under a laboratory-rearing program, in the absence of pesticide exposure.

2. MATERIALS AND METHODS

2.1. Chemical compounds

Sodium chloride, Tris-HCL, trichloroacetic acid, ethanol, oyster glycogen, glucose, sodium acetate, amyloglucosidase from *Aspergillus niger*, phosphovanilline, sulfuric acid and bovine serum albumin were purchased from Sigma-Aldrich® (Saint Quentin-Fallavier, France). The glucose GOD-PAP kit was purchased from BioLabo SAS® (Maizy, France)

2.2.Characterization of crop management strategies in the apple orchards

The study was carried out in the South-East of France, where 24% of French apple orchards are concentrated (Agreste, 2019). To describe the diversity of protection management strategies within the orchards, the following characteristics were identified and recorded: pesticide usage (including microbial and classical insecticides) and the use of alternative strategies (presence of nets, sexual confusion). According to these characteristics, nine apple orchards were chosen in a 20 km circular region around Avignon (France) and were separated into three groups: organic, IPM and conventional orchards, with three orchards per group. Organic orchards were group one, where only “natural” pesticides are authorized. Those orchards were managed following organic production guidelines where no synthetic pesticides or fertilizers were used. The main pesticides used in organic apple production are copper and sulphur (fungicides), mineral oils as well as neem against aphids and Spinosad and microbial insecticides (granulovirus and *Bacillus* *thuringiensis*) against codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae). The second group included three orchards classified as IPM where in addition to the use of sexual confusion, chemical insecticides are applied sparingly according to damage thresholds; the third group was composed of three orchards considered as conventional exhibiting higher rates of insecticide applications than regular IPM. In those orchards sexual confusion against codling moths was not used.

For each orchard, treatment calendars were recorded and analyzed to compute the overall treatment frequency index (TFI) as the total number of pesticide treatments per hectare with commercial products, weighted by the ratio of the dose used to the recommended dose (Jørgensen, 1999). However, since organic farming uses different kinds of pesticides, the TFI was determined for insecticides depending on their composition, microbial and organic insecticides were pooled together and compared to synthetic ones.

$$\mathrm{TFI} = \sum_{i=1}^{n} \frac{\mathrm{ADi}}{\mathrm{HD}}$$

where *n* is the total number of insecticides applied in one year in an orchard, AD_i is the amount of each insecticide applied and HD is the recommended amount per hectare.

2.3.Earwig sampling and rearing

Male and female *F. auricularia* adults were sampled in October 2015 in the nine orchards using cardboard traps placed on apple tree trunks. Since the *F. auricularia* species is univoltine (Dib et al., 2017), adults were of roughly the same age. Organisms were either directly stored at -20°C until biochemical and morphometric measurements were carried out (20 for each sex), or reared in an outdoor laboratory, under shelter and submitted to natural photoperiod, temperature and humidity, with no pesticide exposure. Twenty females per orchard were used for earwig breeding. All the individuals were fed *ad libitum*, regardless of the orchards group from which they were collected. Nymphs at the two last life-stages before reaching adulthood (N3 and N4), and adults (male and female) of the new generation (October 2016) were randomly chosen and withdrawn from the rearing. Ten earwigs per stage (N3, N4, female and male) were thus stored at -20°C for morphometric and reserve contents analysis.

2.4.Morphometric measurements

Frozen earwig specimen were slightly thawed to avoid any change in morphometric parameters, and dried. Then, they were weighed individually using a XT0120A Precisa® electronic precision balance with an accuracy of 0.01 mg. All morphometric measurements were carried out using an eyepiece micrometer on a Nikon® binocular microscope. Measurements were first recorded as a unit of the micrometer scale to the nearest 1 mm and were converted into millimeters (± 0.001 mm). Each earwig was positioned carefully to avoid bias in measurement and to achieve the same plan of view. A single person carried out all measurements to avoid user bias. For adults (males and females) and nymphs, we measured the maximum width of the prothorax, inter-eye and telson and the length of the left femur of the third pair (Suchail et al., 2018). The growth of earwig nymphs was assessed through the increase in body weight, femur length and prothorax width by calculating the ratio between two successive life-stages (N4/N3 or adult/N4). Only males were used to calculate the ratio between adult life-stage and N4 to avoid errors due to the possible presence of premature eggs in the females.

Adults from apple orchards and from breeding were termed F0 or F1 earwigs, respectively.

2.5.Crude extract preparation and biochemical measurements

After the morphometric assessment, the whole earwig body was homogenized on ice in 10% (w/v) low-salt buffer containing 10 mM Tris-HCL (pH 7.3) and 10 mM NaCl and centrifuged for

10 min at 3000 x g according to Suchail et al. (2018). The supernatant was used for further glycogen, lipid and protein measurements.

To determine glycogen content, the method based on enzymatic hydrolysis of glycogen by amyloglucosidase (EC 3.2.1.3) was used (Parrou and Francois, 1997). Solution trichloroacetic 4% acid was added for deproteinization to 250 µL, 70 µL and 30 µL of crude extract (v/v) for adults, N4 and N3, respectively. The solution was centrifuged at 3000 x g for 1 min at 4 °C. Two volumes of 95% ethanol were added to precipitate glycogen that was present in the supernatant. Glycogen was finally pelleted by centrifugation at 5000 x g for 5 min at 4 °C. Ethanol was removed and the pellet was dried for 2 h at room temperature. Once dried, the glycogen pellet was incubated for 2 h at 60 °C in 500 µL (adults) or 100 µL (nymphs) 0.2 M sodium acetate pH 5.2 containing 7 IU (International Units) of amyloglucosidase. After incubation, the solution was cooled on ice and the amount of glucose generated from glycogen was determined in each sample using the Glucose GOD-PAP method adapted to a 96-well microplate format. The Solution mixture containing 250 µL of Glucose GOD-POD and 25 µL of sample, was left to stand for 20 min at room temperature before glucose was detected by measuring the absorbance at 505 nm. The amount of glucose was calculated from a standard curve (A505 = f[glucose]) containing pure glucose as a standard treated with the same conditions. Because the final values were included, the amount of glycogen was corrected for the glucose content in samples that were not incubated with amyloglucosidase.

Total lipids were determined according to Frings et al. (1972) adapted to a 96-well microplate format (Suchail et al., 2018). Concentrated sulfuric acid (298 µL) was added to either 2 µL of sample or 2 µL of standard solution (olive oil). The solutions were homogenized and placed in water at 98 °C for 10 min and cooled on ice for 5 min. A 700 µL of phospho-vanillin reagent was added to each extract or lipid standard. After homogenization and incubation at 37 °C for 15 min, samples were cooled on ice and the absorbance was recorder at 540 nm.

The protein content was determined by the Lowry method modified according to Markwell et al. (1978), with bovine serum albumin as a standard.

The energy reserve values were directly derived from a standard curve prepared with known concentrations of glycogen, lipid or serum albumin. All assays were conducted in triplicate and the above components were express as milligrams per gram of earwig.

2.6. Statistical analysis.

All data analyses were carried out using Software; R version 3.4.3 and RStudio version 1.1.423 (R Core Development Team, Vienna, Austria). The significance of all results was tested using permutation tests due to non-respect of normality and homoscedasticity. We used the ‘lmPerm’ package (Wheeler and Torchiano, 2016) and the function aovp combined with the function pairwise.perm.t.test from RVAideMemoir package. “Fdr” adjusted p-values were used due to the risk of multiple comparisons. ANOVA were used to test the effects of sex and management strategy (organic, IPM and conventional).

3. RESULTS

3.1. Pesticide applications

Only organic and microbial insecticides were applied in organic orchards with a TFI of about 6.6 compared to IPM (2) and conventional (1) orchards. Both IPM and conventional orchards used synthetic insecticides with an average TFI of 4.5 and 7.6, respectively. However, considering the total TFI, no significant differences were observed between management strategies (Figure 1).

3.2. Forficula auricularia body weight

No significant differences in the weight of adult males (p-value=0.965) or females (p-value=0.692) were observed among management strategies. However, regardless of the protection strategy factor, the earwig generation obtained after one year in laboratory conditions (F1) exhibited significant higher weights than the earwigs sampled in apple orchards (F0) (p-value<0.001; Figure 2). Whatever the orchard management or the generation of earwigs, females were significantly heavier (p-values<0.05) (49.9±1.7 mg (for F0) and 61.8±2.1 mg (F1)) than males (46.7±1.7 mg (F0) and 53.4±1.5 mg (F1)).

3.3. Morphometric traits and growth

Among the morphometric parameters measured, telson width was the only one that was significantly different between males and females (p-value<0,0001), either for earwigs sampled in orchards (F0) or for reared earwigs (F1). In both cases, males exhibited larger telson than females at about 37 % and 20 % for F0 and F1 adult's earwigs, respectively (Table 1). Orchards characteristics did not appear to affect telson width.

Femur length (and the width of prothorax, data not shown) was significantly different depending on the orchards groups from which earwigs were collected with lower values in conventional orchards (p-value<0.0001; Figure 3). No significant differences in these parameters were observed between the sexes in earwigs that were field sampled (p-values equivalent to 0.888, 0.058 and 0.995 for organic, IPM and conventional orchards, respectively, for femur length as an example). However, F0 earwigs had significant longer femurs (p-value<0,05) when collected from organic (2.206±0.135 mm) or IPM orchards (2.270±0.138 mm), compared to earwigs caught in conventional orchards (2.104±0.130 mm) (Figure 3). In the progeny earwigs (F1), we observed a homogenization in the femur length and prothorax width data for almost all the adult earwigs. The exception was the femur length of F1 females whose parents were previously sampled in conventional orchards, with these exhibiting a significantly smaller sized femur (p-value<0.0001, Table 2).

The management practices did not have an impact on earwig growth (Table 2). The mean ratio calculated between two successive life-stages for body weight was 2.02±0.76 between N3 and N4, and 2.62±0.40 between N4 and adults (Table 2). It is interesting to note that for all the earwigs, regardless of their origin, the growth of femur length and prothorax width obeyed Dyar's law with an average growth ratio of 1.43±0.07 and 1.33±0.09 from N3 to N4 respectively or 1.23±0.06 and 1.10±0.06 from N4 to adults, respectively.

3.4. Energy reserves

Energy reserves in the earwigs were found to depend on sex and management strategies. For the F0 earwigs, a higher lipid content was measured in females collected from organic orchards (p-values<0.0001). F0 females (IPM and conventional groups) and F0 male earwigs (all studied groups), contained the same amount of lipids (Figure 4A). Concerning glycogen contents, we observed a significant higher glycogen content for F0 females collected in conventional compared to organic and IPM orchards (p-value<0.05) 4C). Overall, males exhibited higher glycogen contents than females, with the highest significant value in those from organic orchards (p<0.0001) and the lowest in those from conventional orchards, which was also similar to females from conventional orchards (p-value=0.987; Figure 4C). No significant differences were observed for protein content (data not shown) with values close to 58 mg. g^{-1} of earwig, for both F0 and F1. A slight, but non-significant, reduction in protein content was measured in males sampled in orchards compared to those reared in the lab (p-value=0.053; data not shown).

Except for protein content, it is noteworthy that energy reserves in all the earwigs reared under our breeding program (generation F1) were higher than in their parents. Glycogen was approximately four times higher and lipids twice as high in F1 than F0 (4B, 4D). In addition, both glycogen and lipid contents reached similar levels in all F1regardless of F0 provenance.

4. DISCUSSION

In this study we aimed to highlight the potential benefit of an interruption in synthetic insecticide exposure on *F. auricularia* earwig parents as well as their progeny. Physiological, behavioral or morphological trade-offs occur in insects allowing them to adapt to environmental changes due to agricultural practices (pesticide usage and habitat loss). Such strategies are developed to minimize negative environmental effects but they are costly to the organism in terms of metabolic resources and energy demand (Calow, 1991). Because an organism's energy resources are generally limited, agricultural management practices can lead to additional metabolic costs and modify energy reserves and/or morphometric parameters (Congdon et al., 2001; Yasmin and D'Souza, 2010).

Firstly, our results showed that there were no significant differences in weight for earwigs sampled from the orchards under different management practices in late October. The main difference observed for fresh weight was sex determined with females always being heavier than males, either for F0 or later for F1 earwigs. Our previous study reported contrasting results for measurements on earwigs sampled in the same orchards in July. In that latter study, earwigs from organic orchards were the heaviest and females were lighter than males (Suchail et al., 2018). There are several explanations as to why time-specific and sex-specific differential responses could be observed. Firstly, earwigs are omnivores and can feed on several types of plant (leaves, flowers) and animal matter. Insecticides treatments stop at the end of August in apple orchards (Sauphanor et al., 2009), thus from then the earwigs could return to their normal foraging behavior. Increased food availability and consumption could have contributed to body weight increases in earwigs from the conventional and IPM orchards which compensated for differences between management strategies. Secondly, the earwig life cycle and the sampling period (July in Suchail et al. (2018) versus October in the present study) could account for sex-specific differential responses. According to Albouy and Caussanel, (1990) and Sauphanor et al., (1993), female earwigs start their oogenesis in late summer/early autumn and most were pregnant in October. A visual check during dissection confirmed that most of the females from both generations investigated (F0 and F1) were full of pre-mature eggs in October. Females also cease feeding between egg laying and egg hatching (Kölliker, 2007; Meunier et al., 2012) and they continuously groom their eggs to prevent the growth of pathogenic fungi, a behavior crucial to reproductive success (Boos et al., 2014; Diehl and Meunier, 2018). Over the course of winter, we can assume that females need to accumulate resources to sustain themselves and ensure their maternal care. In contrast, males live for a shorter time than females with a lifespan of eight compared to 18 months, respectively (Ratz et al., 2016). They invest most of their energy into mating and die in late winter or early spring (Lamb and Wellington, 1975; Albouy and Caussanel, 1990; Dib et al., 2017). This differential life-history trait could account for the sex-specific responses we observed in earwigs' body weight as well as energy reserves (see below).

Interestingly, we observed that earwigs from IPM orchards displayed characteristics close to those of earwigs collected in organic orchards (regarding morphometric traits, and glycogen content), with the exception of lipid content in female earwigs. Earwigs sampled in conventional orchards had shorter femurs suggesting that a high historic level of insecticide exposure is needed to influence body shape of these organisms. These findings are consistent with a decrease in body size and elytron length seen for the carabidae, *Calathus fuscipes,* sampled in potato fields treated with the larvicide lambda-cyhalothrin (Giglio et al., 2017) and with previous analyses on earwigs

(Suchail et al., 2018). However, other studies failed to highlight changes in morphological size under insecticide exposure, as for *C. pipiens* mosquitoes (Taskin et al., 2016) as an example. Moreover, laboratory exposure to insecticides did not induce any morphometric variations in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Marcus and Fiumera, 2016). These latter results, together with ours, point out the absence of a relationship between single insecticide exposure and insect morphometric traits. They also revealed that even if pesticides are mostly sprayed during periods when insects are developing, high levels of repeated pesticide exposure (such as those found in conventional orchards) are needed to shape morphological traits in adults.

It is noteworthy that we did not observe a legacy effect on second-generation earwigs since values for morphometric traits measured in F1 earwigs did not differ according to the insecticide exposure history of their parents. The absence of pesticide exposure and the unlimiting food provided during earwig breeding allowed them to reach about the same body size. These findings are in agreement with previous studies of the effect of exposure to Spinosad at the adult stage on the body size of offspring, in the yellow dung fly *Scathophaga stercoraria* L. (Diptera: Scathophagidae) (Mahdjoub et al., 2020). Another interesting point is a weak but significant decrease in telson width for F1 males compared to F0 males. This could be explained by captivity which provided a less hostile environment compared to the realistic environment conditions in orchards. The telson shape is generally related to forceps shape (Albouy and Caussanel, 1990). It is well known that male forceps are used as weapons in male contests for accessing to females, which also choose males based of their forceps length (Rantala et al., 2007). Telson structure plays thus an important role in impact-resistance during contest, and such multifunctional structures of sexual ornaments can limit the development of other morphological traits (Elmen, 2001). We overall questioned whether the observed effects on the offspring are due to captivity and/or rearing conditions rather than the exposure history of their parents. Previous results pointed out that the development of macrolabic males (large earwigs with longer forceps) was not fully related to a favorable diet or rearing density (Tomkins, 1999). To disentangle which parameters are having the most impact on morphometric traits further experiments are needed with varying rearing conditions, diets, pesticide exposure and safety in captivity.

Energy reserves give an insight into rapid physiological responses. When *F. auricularia* were collected in July, both glycogen and lipid contents were lower in the earwigs from IPM apple orchards than from organic orchards (Suchail et al., 2018). According to the treatment calendars, July is the peak of pesticide usage as they are applied from late March to late August (Sauphanor et al., 2009; Marliac et al., 2015). At this time, earwigs have to cope with pesticide exposure to survive. As a result, *F. auricularia* collected in conventional orchards, were shown to exhibit tolerance to chlorpyrifos-ethyl with higher constitutive enzymes activities involved in detoxication mechanisms such as Glutathione-S-transferases and carboxylesterases compared to earwigs from organic orchards (Le Navenant et al., 2019). These latter results highlight the ability of earwigs to develop physiological and biochemical modifications to adapt to high levels of pesticide exposure, which could be costly for the organisms.

The first interesting point observed in F0 earwigs collected in late October (i.e. two months after the last pesticide application) lies in the sex-specific energetic contents, which could be attributed to differential lifespans depending on sex, as specified above. The absence of significant differences in males either for lipid or glycogen contents contrasted with the lower lipid content observed in females sampled in conventional and IPM orchards. Females also contained more glycogen in conventional compared to other orchards. If we assume that females are able to quickly accumulate glycogen reserves to prepare their clutch (up to October), it appears that they could not make up the gap in lipid content. However, when a surplus of carbohydrates is available, pyruvate generated through glycolysis could be used to produce acetyl coenzyme A (acetyl-CoA), a central intermediate for lipid metabolism (Wakil, 1961). Then, because lipid content was very low in females, we cannot exclude the possibility that lipids were burned at a faster rate than at which they were produced, resulting in stable lipid loss. In honey bees, mechanisms of stable lipid loss are known to depend either on behavioral maturation and novel regulatory processes (Ament et al., 2011) or on *Nosema ceranae* infection (Li et al., 2018). However, little is known about the molecular mechanisms involved in rapid body mass changes in insects. Establishing whether our observations were sued to stable lipid loss or an absence of lipogenesis will need further analysis. For example, in the parasitic wasp, the incorporation of radioactively marked glucose into lipids showed that lipogenesis did not occur at a sufficient level to increase lipid reserves and compensate for lipid use (Giron and Casas, 2003).

To conclude, our results demonstrate that energy reserves, body weight and morphological parameters can be used to detect the effects of environmental stress in the European earwig, *F. auricularia*. They suggest that a relaxed period without pesticide exposure allows females to recover energy reserves to ensure reproduction and maternal care from egg-laying to the emergence of juveniles. Females appear to quickly adapt their energetic flow, from maintenance/tolerance to reproduction. We confirmed previous results concerning lower values for some morphometric traits of F0 earwigs collected in highly treated orchards compared to those collected in organic orchards. However, due to the rearing conditions (food given ad libitum), all the F1 earwigs exhibited higher body weights and energy reserve contents than their parents. In addition, all parameters in the F1 earwigs finally reached the same levels regardless of the historic provenance of their parents. In the future, metabolic pathways and specific enzymes involved in lipogenesis and glycogenesis, should be investigated to better understand the physiological and biochemical responses induced by pest management strategies on *Forficula* species and other important natural enemies. These findings open new research paths to explore the impact of management strategies on fecundity, maternal care and the success of egg hatching and nymph survival. They also pointed out the high variability of practices within IPM strategy and thus when possible TFI should be provided if we want to take this variability into account.

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TABLES

Table 1: Telson width (mm) (mean ± standard deviation) depending on sex, generation (parents sampled from orchards (F0) and their progeny resulting from laboratory rearing (F1) and management strategy in apple orchards. Significant differences between generation and sex are indicated by different letters (p-value<0.0001).

		Organic	IPM	Conventional	Mean
F0	Male	2.743 ± 0.261[a]	2.787 ± 0.218 [a]	2.749 ± 0.229 [a]	2.762 ± 0.236 [a]
	Female	2.003 ± 0.130 [c]	2.046 ± 0.129 [c]	1.991 ± 0.112 [c]	2.018 ± 0.126 [c]
F1	Male	2.337 ± 0.149 [b]	2.434 ± 0.208 [b]	2.475 ± 0.162 [b]	2.421 ± 0.127 [b]
	Female	2.042 ± 0.093 [c]	2.028 ± 0.120 [c]	1.962 ± 0.156 [c]	2.013 ± 0.184 [c]

Table 2: Femur length, prothorax width and ratio calculated between two successive instars for body weight, femur length and prothorax width in the European earwigs, *Forficula auricularia* resulted from laboratory rearing (F1). The ratio adult/N4 was calculated using the weight and size of the adult males to avoid errors due to the presence of premature eggs in the females. No significant differences were observed in adults depending on sex and/or management strategy, excepted for femur length (*** stands for p-value< 0.0001).

Management strategy		Organic	IPM	Conventional	Mean ± sd
Body weight	N4/N3	1.81 ± 0.64	2.21 ± 0.83	1.96 ± 0.69	2.02 ± 0.76
	F1 Adult/N4	3.08 ± 0.47	2.65 ± 0.44	2.23 ± 0.30	2.62 ± 0.40
Femur length	N4/N3	1.44 ± 0.08	1.45 ± 0.07	1.39 ± 0.06	1.43 ± 0.07
	F1 Adult/N4	1.22 ± 0.07	1.23 ± 0.06	1.23 ± 0.03	1.23 ± 0.06
	F1 Male (mm)	2.115 ± 0.117	2.165 ± 0.104	2.134 ± 0.059	2.142 ± 0.098
	F1 Female (mm)	2.152 ± 0.155	2.170 ± 0.090	1.993 ± 0.140***	-
Prothorax width	N4/N3	1.32 ± 0.10	1.35 ± 0.10	1.32 ± 0.07	1.33 ± 0,09
	F1 Adult/N4	1.12 ± 0.06	1.11 ± 0.06	1.08 ± 0.06	1.10 ± 0,06
	F1 Adult (mm)	1.736 ± 0.094	1.768 ± 0.088	1.741 ± 0.095	1.751 ± 0.092

FIGURE LEGENDS

Figure 1: Treatment Frequency Index. Mean ± standard deviation of the Treatment Frequency Index (TFI) for the synthetic and organic insecticides applied during the year 2015 in the studied orchards, according to their management strategy.

Figure 2: *F. auricularia* body weight (g) depending on sex and generation (parents sampled from orchards (F0) and their progeny resulting from laboratory rearing (F1). Tukey box plots indicate the median, the 25th and 75th percentiles (box edges), the range (whiskers) and outliers (black dots). Significant differences are indicated by different letters (p-value<0.001).

Figure 3: Femur length of adult European earwigs (F0) sampled from orchards of different management strategies (O: Organic. I: IPM and C: Conventional). Tukey box plots indicate the median, the 25th and 75th percentiles (box edges), the range (whiskers) and outliers (black dots). Significant differences are indicated by different letters (p-value < 0.05).

Figure 4: Energy reserves for F0 earwigs collected in orchards (A) Lipid; (C) Glycogen; and for F1 earwigs reared in a breeding program for 1-year (B) Lipid; (D) Glycogen. Tukey box plots indicate the median, the 25th and 75th percentiles (box edges), the range (whiskers) and outliers (black dots). Different letters indicate significant differences (p-value < 0.05).

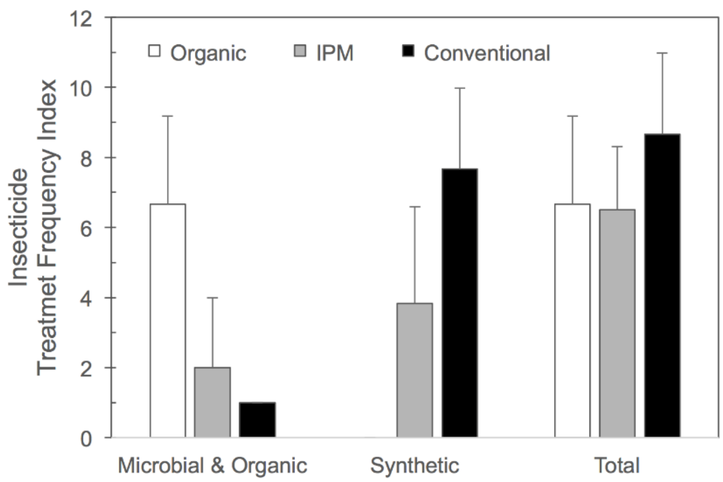
Organic
IPM
Conventional
Insecticide
Treatmet Frequency Index
0
2
4
6
8
10
12
Microbial & Organic
Synthetic
Total

Figure 1

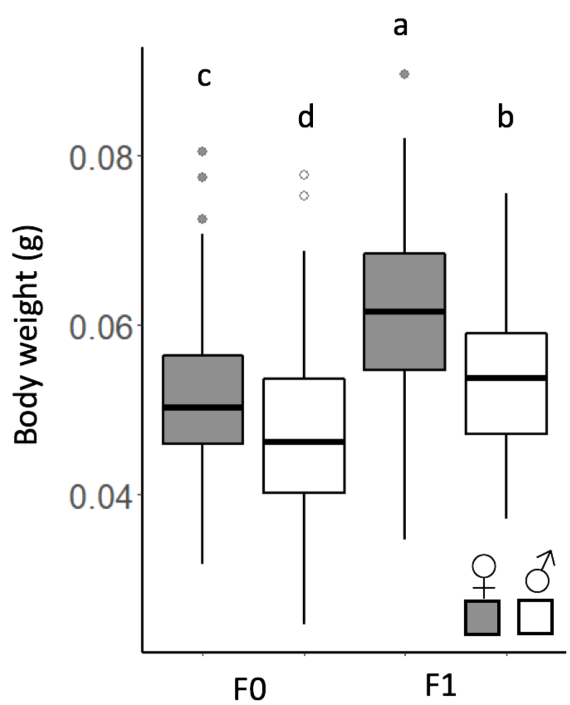

Figure 2

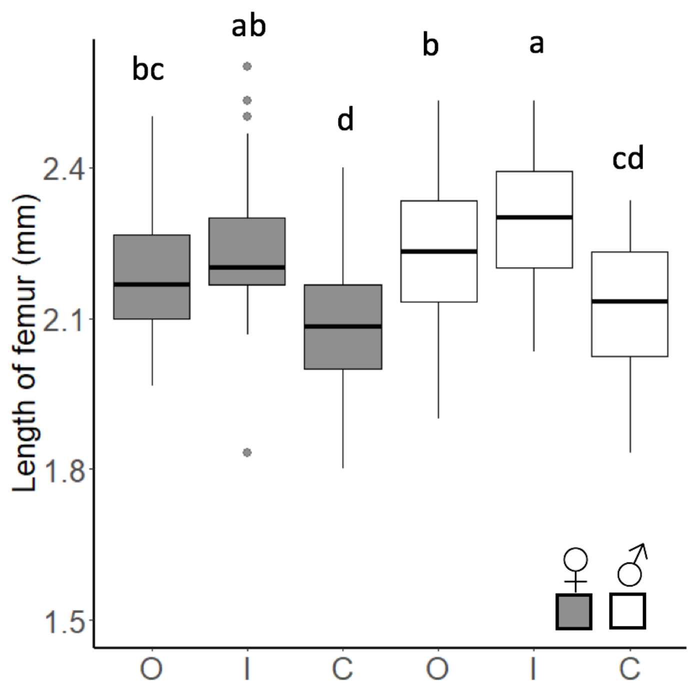

Figure 3

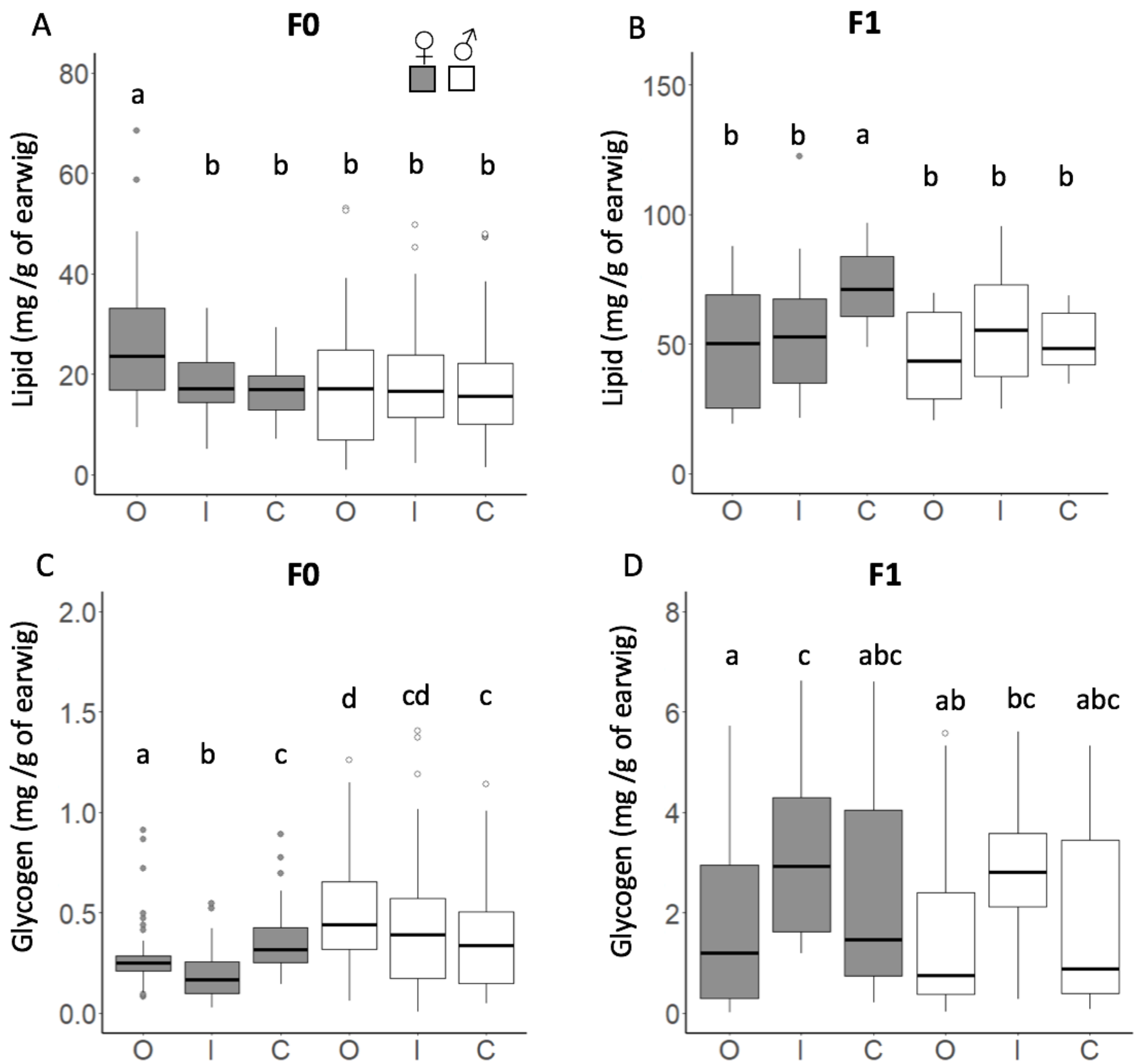
A
F0
♀ ♂
Lipid (mg /g of earwig)
80
60
40
20
0
a
b b b b b
O I C O I C
B
F1
Lipid (mg /g of earwig)
150
100
50
0
b b a
b b b
O I C O I C
C
F0
Glycogen (mg /g of earwig)
2.0
1.5
1.0
0.5
0.0
d cd c
a b c
O I C O I C
D
F1
Glycogen (mg /g of earwig)
8
6
4
2
0
a c abc
ab bc abc
O I C O I C

Figure 4

***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: