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## An exploratory study of energy reserves and biometry as potential tools for assessing the effects of pest management strategies on the earwig, *Forficula auricularia* L

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### ► To cite this version:

Séverine Suchail, Adrien Le Navenant, Yvan Capowiez, Alain Thiéry, Magali Rault. An exploratory study of energy reserves and biometry as potential tools for assessing the effects of pest management strategies on the earwig, *Forficula auricularia* L. *Environmental Science and Pollution Research*, 2018, 25 (23), pp.22766-22774. 10.1007/s11356-018-2371-x . hal-02047731

**HAL Id: hal-02047731**

**<https://univ-avignon.hal.science/hal-02047731>**

Submitted on 25 Feb 2019

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1 **An exploratory study of energy reserves and biometry as potential tools for assessing the effects**  
2 **of pest management strategies on the earwig, *Forficula auricularia* L.**

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32 **Abstract**

33

34 Apple orchards are heavily treated crops and some sprayed insecticides are recognized to have toxic  
35 effects on non-target arthropods. Earwigs are important natural enemies in pip-fruit orchards and  
36 contribute to the biological control of aphids. In addition, due to their ease of capture and  
37 identification, they are an interesting potential bioindicator of the possible detrimental effects of  
38 different orchard management strategies. In this study, we measured the energy reserves and some  
39 morphological traits of *F. auricularia* L. sampled in apple orchards under management strategies  
40 (organic versus Integrated Pest Management (IPM)). We observed a significant decrease in mass (22  
41 to 27%), inter-eye width (3%) and prothorax width (2 to 5%) in earwigs from IPM compared to  
42 organic orchards. Energy body reserves also confirmed these results with a significant decrease of  
43 48% in glycogen and 25 to 42% in lipid content in earwigs from IPM compared to organic orchards.  
44 However, the protein content was approximately 70% higher in earwigs from IPM than in organic  
45 orchards. Earwigs sampled in IPM orchards, may adapt to minimize to the adverse toxic effects of  
46 pesticide treatments using a large number of strategies, which are reflected in changes to their energy  
47 reserves. These strategies could in turn influence the population dynamics of natural enemies and  
48 impair their role in the biological control of pests in apple orchards.

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51 **Keywords:** earwig, energy reserves, glycogen, lipid, protein, biometry, pest management strategy

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55 **Highlights**

- 56
- Female and male from organic orchards have higher weight
  - Inter-eye and prothorax widths are lower in IPM orchards
  - Lipid and glycogen contents decrease in earwigs coming from IPM orchards
  - Protein increase in female and male from IPM versus organic orchards
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- 58
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## 61 **1. Introduction**

62 The United Nations Food and Agricultural Organization reported that the world's population will  
63 reach to nearly 10 billion by 2050. This will lead to an increase in world food production in order to  
64 keep pace with the demand of this growth (Gill et al. 2014). Agricultural chemicals can enhance crop  
65 productivity. However, pesticides can also have negative effects on the environment, ecosystem and  
66 biodiversity. Pesticides can have an impact on non-target and beneficial species through direct or  
67 indirect routes, such as water contamination and runoff, pesticide residues, and by consuming food  
68 that has been sprayed (Martinou et al. 2014). The destruction of these beneficial arthropods can  
69 exacerbate pest problems as they play an important role in regulating pest population levels. In France,  
70 apple orchards are heavily treated with, on average, more than 30 pesticide applications per year  
71 (Sauphanor et al. 2009; Mazzia et al. 2015). Two pest management strategies are generally identified  
72 in commercial apple orchards: Integrated Pest Management (IPM) and organic farming. The European  
73 earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae) is a common, sub-social and omnivorous  
74 insect in agro-ecosystems worldwide. These arthropods are commonly found in apple orchards. Adults  
75 and nymphs are nocturnal and hide during the day under shelters such as bark or in holes in the  
76 ground. They can be found in high abundance in artificial shelters and traps (Sauphanor 1992;  
77 Fountain and Harris 2015). In apple orchards, earwigs are a key biological control agent against  
78 numerous insect pests such as aphids, psyllids and spider mites (Dib et al. 2010; Debras et al. 2007;  
79 Romeu-Dalmau 2012). Their ease of capture and identification make them an interesting potential  
80 bioindicator of the intensity of pesticide use in orchards (Dib et al. 2010).

81 Earwigs are sensitive to different management strategies in IPM apple orchards where several  
82 pesticides can be applied, e.g., organophosphates (Ffrench-Constant and Vickerman 1985; Malagnoux  
83 et al. 2015a), pyrethroids (Badjii 2004) or neonicotinoids (Shaw and Wallis 2010; Malagnoux et al.  
84 2015b). Other agricultural practices such as tillage and the presence and quality of hedges can also  
85 influence earwig abundance (Sharley et al. 2008; Moerkens et al. 2012) and the presence and quality  
86 of hedges (Debras et al. 2007). Management strategies in apple orchards also influence diversity in the  
87 earwig community, with *F. auricularia* being less sensitive to insecticides than *F. pubescens*  
88 (Malagnoux et al. 2015b). Studies concerning *F. auricularia* focused on its ecological role in orchards  
89 (Kölliker and Vancassel 2007; Malagnoux et al. 2015a) or the direct impact of commonly used  
90 pesticides on its populations (Ffrench-Constant and Vickerman 1985; Campos et al. 2011; Malagnoux  
91 et al. 2015a; Fountain and Harris 2015). However, there is, to our knowledge, little available data on  
92 the negative physiological effects of pesticides on energy reserves in these earwigs.

93 Glycogen and the lipid, triacylglycerol, are the predominant energy reserves stored in the fat bodies  
94 distributed throughout the body of the insect (Arrese and Soulage 2010). This compartment plays an  
95 essential role in energy storage and has important functions throughout the insect's life cycle including  
96 growth, timing of metamorphosis and egg development. In addition, the fat body also synthesizes most  
97 of the hemolymph proteins and circulating metabolites. Glycogen can be rapidly hydrolyzed to

98 glucose in the haemolymph of insects to be used as a glycolytic fuel (Steele 1982). The glycogen  
99 content may vary depending on physiological state (Anand and Lorenz 2008) or environmental  
100 conditions (Chowanski et al. 2015).

101 Triacylglycerol is formed by fatty acids that can be used for energy production through  $\beta$ -oxidation.  
102 Lipids are always the main component of the fat body which, in insect, represents more than 50% of  
103 their dry weight (Ziegler 1991). Lipids provide energy during diapause (Hahn and Denlinger 2007),  
104 for embryo development (Ziegler and Van Antwerpen 2006) and sustained flight activity (Van der  
105 Horst 2003).

106 Proteins can also supply energy through catabolism and are essential in tissue building and repair. For  
107 this reason, it is also relevant to study the variability of insect protein content under different  
108 conditions.

109 Living organisms use reserves to maintain their bodies, growth, development, and reproduction  
110 (Arrese and Soulage 2010). Under stressful conditions, there is an increased expenditure associated  
111 with detoxification which can have a negative impact on life-history traits in the life cycle. After  
112 exposure to pesticides, it has been shown that energy reserves decreased in several insects (Ribeiro et  
113 al. 2001; Nath 2003; Da Silva 2004; Rharrabe et al. 2008).

114 A low level of energy reserves could potentially influence the population dynamics of earwigs and  
115 decrease their role in pest management in apple orchards. Markaw (1995) and Nestel et al. (2016)  
116 showed that ecological stressors can affect developmental instability, which leads to reduced fitness.  
117 Morphological parameters e.g. mass or insect size can be used to detect the effects of environmental  
118 stress on an organism.

119 The aim of our study was to assess the effects of two orchard management strategies, organic and IPM  
120 practices, on earwig (*F. auricularia*) weight, energy reserves (glycogen, lipid and protein contents)  
121 and two morphometric parameters (inter-eye and prothorax widths).

122

## 123 **2. Materials and methods**

### 124 2.1. Chemical compounds

125 Sodium chloride, Tris-HCl, trichloroacetic acid, ethanol, Oyster glycogen, glucose, sodium acetate,  
126 amyloglucosidase from *Aspergillus Niger*, phosphovanillin, sulfuric acid and bovin serum albumin  
127 were purchased from Sigma-Aldrich® (Saint Quentin-Fallavier, France) while glucose RTU™ was  
128 purchased from bioMérieux SA® (Geneva, Suisse).

### 129 2.2. Earwigs

130 Male and female adults of *F. auricularia* L. earwigs were caught in July 2015 using cardboard traps  
131 placed on apple tree branches in five orchards located in Noves, near Avignon (south-eastern France).  
132 The landscape around these orchards is similar and characterized by the presence of a dense network  
133 of windbreak hedgerows, which protects orchards against the prevailing northern winds. Since this

134 species is univoltine, these adults were of roughly the same age. The five orchards had two  
135 management strategies: three were under IPM management and thus applied synthetic plant protection  
136 products and two were under organic farming and used a restricted range of products (such as  
137 carbovirusine, spinosad, copper, sulfur...). Twenty male and 20 female earwigs were collected from  
138 each management system (i.e. 40 and 60 earwigs per sex for organic and IPM strategies, respectively),  
139 frozen and stored at  $-20\text{ }^{\circ}\text{C}$  until biochemical and morphometry measurements were carried out.

### 140 2.3. Morphometric measurements

141 Frozen earwigs were slightly thawed to avoid change to morphometric measurements. All  
142 morphometric measurements of earwigs were carried out using an eyepiece micrometer on a Nikon<sup>®</sup>  
143 binocular microscope. Measurements were first recorded as units of the ocular micrometer scale to the  
144 nearest  $1\mu\text{m}$  and were then converted into mm ( $\pm 0.1\text{ mm}$ ). Each earwig was positioned carefully to  
145 avoid bias in measuring and to achieve the same plane of view. A single person carried out all the  
146 measurements to avoid user bias. We measured the maximum prothorax and inter-eye (i.e., distance  
147 between the eyes) widths. The inter-eye width was measured in a straight line between the two eyes  
148 and the prothorax width between two reference points (Fig.1).

### 149 2.4. Scanning electron microscopy

150 We used scanning electron microscope photography (Philips XL 30 ESEM with detector SE) to obtain  
151 images (e.g. Figure 1) of dried specimens coated with a gold layer of  $10^{-12}\text{ m}$ . The equipment was set  
152 to operate at 20KV acceleration voltage, at 35 for the magnification and at 28.9 mm for the working  
153 distance.

### 154 2.5. Crude extract preparation

155 After morphometric measurements, earwigs (male and female) were weighed individually using a  
156 Precisa<sup>®</sup> XT120A electronic precision balance with a readability of 0.1 mg. The whole body of the  
157 earwig was homogenized on ice in 10% (w/v) low-salt buffer containing 10 mM Tris-HCl (pH 7.3)  
158 and 10 mM NaCl and centrifugated for 10 min at 3000 g. This supernatant was the crude extract on  
159 which further glycogen, lipid or protein measurements were carried out.

### 160 2.6. Biochemical measurements

#### 161 *Determination of glycogen content*

162 The method, based on enzymatic hydrolysis of glycogen by amyloglucosidase (EC 3.2.1.3), was used  
163 according to Parrou and François (1997). Solution trichloroacetic 4% acid was added to 250  $\mu\text{l}$  of  
164 crude extract (v/v) for deproteinization and the solution was centrifuged at 3000 g for 1 min at  $4\text{ }^{\circ}\text{C}$ .  
165 After centrifugation, two volumes of 95% ethanol were added to precipitate the glycogen, which was  
166 present in the supernatant. Glycogen was finally pelleted by centrifugation at 5000 g for 5 min at  $4\text{ }^{\circ}\text{C}$ .  
167 Ethanol was removed and the pellet was dried at room temperature. The dried pellet was incubated for

168 2 h at 60 °C in 500 µL of 0.2 M sodium acetate, pH 5.2, containing 7 UI of amyloglucosidase. After  
169 incubation, the solution was cooled on ice for 5 min and the amount of glucose generated from  
170 glycogen was determined using the Glucose RTU™ method adapting to 96-well microplate format.  
171 The reaction mixture (275 µL final volume), containing 250 µL Glucose RTU™ and 25 µL glucose  
172 produced above, was left to stand for 20 min at room temperature, before glucose detection by  
173 measuring the absorbance at 505 nm. The amount of glucose was calculated from a standard curve ( $A$   
174  $505 = f[\text{glucose}]$ ) containing pure glucose as a standard treated within the same conditions. Because  
175 the final values included the amount of glycogen was corrected for the glucose content in samples that  
176 were not incubated with amyloglucosidase.

#### 177 *Determination of lipid content*

178 Total lipids were determined using a sulphophosphovanillin reaction according to the method of  
179 Frings et al. (1972) adapted to a 96-well microplate format. 294 µL concentrated sulfuric acid was  
180 added either to 6 µL of earwig crude extract or to 6 µL of a standard solution (olive oil). The solutions  
181 were homogenized and placed in boiling water for 10 min and cooled on ice for 5 min. A 700 µL of  
182 the phosphovanillin reagent was added to each extract or lipid standard, homogenized and incubated at  
183 37 °C in a water bath for 15 min. After the samples were allowed to cool for 5 min the absorbance was  
184 recorded at 540 nm.

#### 185 *Determination of protein content*

186 Total protein content was determined by the Lowry method modified according to Markwell et al.  
187 (1978), with bovine serum albumin as the standard.

188 The energy reserve values were derived directly from a standard curve prepared with known  
189 concentrations of bovine serum albumin, glycogen and lipid. All assays were run in triplicate.

190 All the above components were expressed as milligrams per g of earwig.

#### 191 2.7. Statistical analysis

192 For all the parameters (energy reserves and morphometric measurements), the effects of orchard  
193 management (organic vs IPM) and earwig sex were included in a mixed model with management and  
194 sex as fixed factors and apple orchard as a random factor. The assumptions of normality and  
195 homogeneity of variance were first tested, and log-transformation was applied when required (weight  
196 and morphometric measurements). We compared all the means for both factors using the 'lsmeans'  
197 package. The effects of the factor were thus assessed with the general model but sometimes also on  
198 simplified models (one factor only) depending on the significance of the interaction between the  
199 factors. All computations were carried out using R (version 2.15.3).

200

## 201 **3. Results**

### 202 3.1 Weight

203 Both earwig sex and orchard management had a significant effect on earwig weight ( $p < 0.001$ ) and no  
204 significant interaction was observed between the two factors ( $p = 0.37$ ). Regardless of orchard  
205 management strategy, male earwigs were significantly heavier than females. The weight of both sexes  
206 of earwigs sampled in organic orchards was also higher than those sampled in IPM orchards (Fig. 2).

### 207 3.2 Morphometric parameters

#### 208 *Prothorax and inter-eye widths*

209 Similar observations to above were obtained with the morphometric analysis of the earwigs. For both,  
210 prothorax and inter-eye widths measurements, sex did not have a significant effect, and the interaction  
211 between sex and management strategy was also not significant. Only management strategy had a  
212 significant effect with significantly smaller prothorax ( $p = 0.032$ ) and inter-eye widths ( $p = 0.026$ ) for  
213 earwigs sampled in IPM orchards compared to those sampled in organic orchards (Fig.3). The  
214 decrease in inter-eye width was about 3% and between 2 to 5% for the prothorax width.

### 215 3.3 Biochemical analysis

#### 216 *Glycogen*

217 The interaction between sex and management strategy was highly significant ( $p < 0.001$ ) for the  
218 glycogen content. For both management practices ( $p < 0.001$ ) sex had a significant effect with females  
219 having higher glycogen content than males. Management practices had no overall effect but when only  
220 females were considered, higher glycogen contents were observed in earwigs from organic compared  
221 to IPM orchards. No significant difference was observed for males explaining the significant  
222 interaction.

#### 223 *Lipids*

224 Sex and the interaction between sex and management strategy had no significant effect on the earwig  
225 lipid contents. However, management strategy had a significant effect ( $p = 0.008$ ) with lower lipid  
226 content in earwigs sampled in IPM orchards than those sampled in organic orchards ( $23.49 \text{ mg} \pm 10.57$   
227 vs  $32.10 \text{ mg} \pm 10.39$  and  $24.23 \text{ mg} \pm 11.12$  vs  $29.49 \text{ mg} \pm 10.57$  respectively for females and males  
228 (Fig. 4 B)). On average, earwigs sampled in IPM orchards had 25 to 42 % less triacylglycerol than  
229 those under organic management.

#### 230 *Proteins*

231 Sex and the interaction between sex and management strategy had no significant effect on earwig  
232 protein contents. However, management strategy had a significant effect ( $p = 0.016$ ) with higher body  
233 protein content in earwigs from IPM orchards than organic orchards (Fig. 4 C). Earwigs in IPM  
234 orchards had 70% more protein than those under organic management for both female and male.



235

#### 236 **4. Discussion and conclusion**

237 It is well recognized that agricultural management practices including the use of pesticides affects  
238 non-target species and leads to a decline in biodiversity in agroecosystems (Mac Laughlin and Mineau  
239 1995; Kromp 1999; Nash et al. 2008). The hazard of pesticide usage depends on the application rate,  
240 intrinsic toxicity and the level of uptake, persistence, volatilization, metabolization and bioavailability  
241 of the active ingredient (Sanchez-Bayo 2009). The exposure time is also a critical variable in risk  
242 assessments of agrochemicals. Organisms may develop a number of strategies to minimize the  
243 negative toxic effects induced by chemical stress. All responses to resist toxicants are costly for the  
244 organism in terms of metabolic resources and energy demand (Calow 1991). Because the energy  
245 resources of organisms are limited and used for growth, reproduction and basal metabolism, the  
246 additional metabolic costs due to pesticide management results in a reallocation of energy resources,  
247 and can increase energy consumption leading to reduced energy reserves (Congdon et al. 2001;  
248 Yasmin and Souza 2010). Resource allocation and life history strategies are inevitably interconnected  
249 for all organisms because of the competition and the limitation of available energy reserves either in  
250 the environment or within the individual itself.

251 Our results confirm that pest management strategy can result in changes to energy reserves in earwigs.  
252 Our measurements of energy reserves indicated a lower lipid and glycogen contents in earwig bodies  
253 collected from IPM compared to organic orchards. The major differences between the organic and  
254 IPM strategies were lower total Treatment Frequency Index (TFI: total number of pesticide treatment  
255 weighted by the ratio of the dose used to the recommended dose (Jørgensen 1999), and agricultural  
256 practices such as tillage, total weed removal or the presence and quality of hedges in the organic  
257 orchard. In organic strategy, pest control is based on a greater use of mineral fungicides (copper and  
258 sulphur) and natural insecticides (granulovirus, mineral oils and spinosad). In contrast, non-organic  
259 orchards mainly used synthetic pesticides which are known to be more efficient against pests. Beside  
260 the fact that the total TFI, was slightly but significantly lower in organic orchards (22.2), than in IPM  
261 orchards (26.6) it has been previously shown that the earwig community of *F. auricularia* was lower  
262 in IPM orchards compared to organic orchards and was correlated to a higher insecticide use (4.9 in  
263 organic vs 9.2 in IPM) (Malagnoux et al. 2015b). By their toxicity, insecticides increase lethality  
264 and/or affect the behavior of non-target organisms. For example, high sensitivity of *F. auricularia* to  
265 many insecticides was observed after exposure to organophosphates (Ffrench-Constant and Vickerman  
266 1985; Malagnoux et al. 2015a), pyrethrinoids (Badji et al. 2004) and neonicotinoids (Shaw and Wallis  
267 2010; Malagnoux et al. 2015b). Moreover, insecticide exposure induced disturbed behavior in  
268 predation activity of earwigs (Malagnoux et al. 2015a), cast production of earthworms (Jouni et al.  
269 2018), feeding rate of the white shrimp (Garcia de La Parra et al. 2006) or locomotion of zebrafish  
270 (Tilton et al. 2011). Because the process of food detection involves complex nervous activity that can

271 be disrupted by neurotoxic pesticides (Desneux et al. 2007), impairments on behavior modify the  
272 feeding ability of the organisms and leads to a decrease in their energy reserves. Then, despite the  
273 possible loss in prey availability induced by higher TFI in IPM orchards (Sauphanor and Stäubli 1994;  
274 Altieri 1999; Pekar 1999; Dib et al. 2016), we assumed that the main factor responsible for depletion  
275 in energy reserves lies to a direct effect of insecticides on the organisms rather than on environmental  
276 modification (i.e. food availability). Our assumption was supported by several studies that have been  
277 conducted in laboratory experiments without food limitation. These studies have shown that exposure  
278 to organophosphorus insecticides, either severely disturbed glycogen metabolism in the Lepidoptera  
279 *Bombyx mori* (Nath 2003), or induced a significant depression of glycogen, lipid, and protein contents  
280 in the terrestrial isopod *Porcellio dilatatus* (Ribeiro et al. 2001).

281 Glycogen is a major glucose reserve and can be rapidly mobilized whereas lipids and proteins are  
282 considered as long-term reserves (Arrese and Soulage 2010). In our study, the protein values were  
283 higher in male and female earwigs sampled from the orchard under IPM. Our results are in agreement  
284 with those of Jeon et al. (2013) who observed an increase in protein of *Daphnia magna* after carbaryl  
285 exposure, associated to a decrease in lipid and carbohydrate contents, or with the results of Staempfli  
286 et al. (2007) in the soil arthropod, *Folsomia candida* after dinoseb exposure. Reallocation of energy  
287 reserves to maintain homeostasis under pesticide exposure mainly focuses on protein induction to  
288 improve defense mechanisms, and they often involve overproduction of detoxification enzymes  
289 (Calow 1991). According to those results and ours, it is unlikely that the increase in protein levels in  
290 IPM practices may offset the reduction in glycogen and lipid but, on the contrary, the consumption of  
291 glycogen and lipid content allow protein synthesis, which is highly cost effective. According to the  
292 thermodynamics of each nutrient (17.5 kJ/g glycogen, 24 kJ/g protein, and 39.5 kJ/g lipids) (Gnaiger  
293 1983), total energy reserves are higher in term of global kJ in IPM orchards by 19% and 28% for male  
294 and female earwigs, respectively. However, this amount of energy is not available fast enough. When  
295 the organisms become unable to cope with stress or pollutants, the energy budget results of imbalance  
296 between the rate of synthesis and rate of degradation. Then, after the use of glycogen and lipids,  
297 proteins constitute the last energy reserve to change or be used upon stress exposure (Ferreira et al.  
298 2015). In this context, studies have reported that a decrease of protein content is associated to an  
299 increase in mortality. It was the case observed by Staempfli et al. (2007) where a biphasic  
300 concentration-response linked to the phenomenon of hormesis was observed, showing that after  
301 having produced an effort to increase growth and reproduction, lethality increases. Rharrabe et al.  
302 (2008) demonstrated that for lepidoptera *Plodia interpunctella* Hübner, the insecticide azadirachtin  
303 caused a severe reduction in protein levels and led larval weight loss, developmental delay followed  
304 by high lethality. These authors postulated that azadirachtin exerts its effect on insects by modifying  
305 the protein synthesis capacity of the fat body as also observed for *S. litura* (Huang et al. 2004).  
306 Moreover, Ribeiro et al. (2001) observed a decrease in protein content in parathion-intoxicated isopods

307 along with an increase in mortality, indicating that physiological adaptability to compensate pesticide  
308 stress requires high-energy but ineffective demand associated with.

309 In addition to agricultural practices, other factor can have an impact on rates of energy consumption,  
310 e.g. age and environmental conditions including temperature or dehydrating state. Anurag and Lorenz  
311 (2008) reported that the cricket, *Gryllus bimaculatus*, fat body energy reserves were age-dependent.  
312 Climatic conditions also change abundance of energy reserves (Chowanski et al. 2015). *F. auricularia*  
313 is univoltine, and in south of France, earwigs reach the adult stage in early summer. It is noteworthy  
314 that because all the organisms analyzed were caught in July, they were of roughly the same age.  
315 Moreover this study was carried out on a same town and thus a reduced geographic area (less than 10  
316 km radius), climatic conditions and landscape type are very similar. In this region, all orchards are  
317 surrounded by hedges due to the prevailing wind. Moreover, earwigs are nocturnal, inactive and  
318 hidden during the day inside the cardboard traps. When they are captured, we can assumed they are all  
319 in the same diet stage with an almost empty gut that will limit differences in energy reserves. Thus, in  
320 the studied apple orchards, the main factor governing energy budget in *F. auricularia* was assumed to  
321 be management strategy, and suggested that insecticide use, quantitatively and qualitatively, is able to  
322 decrease *F. auricularia* energy reserves. Other pollutant molecules can also lead to changes in energy  
323 reserves under natural conditions. Indeed, studies of chronically polluted with metals effects on the  
324 beetle *Pterostichus oblongopunctus*, showed a significant decrease in energy reserves (Bednarska et al.  
325 2013). Givaudan et al. (2016) showed higher lipid and glycogen contents in earthworms collected in  
326 abandoned and organic orchards compared to IPM orchards. Thus, the depletion of such biochemical  
327 constituents can be due to the major mobilization of these nutrients in response to the toxic effect of  
328 the pesticides.

329 Biochemical parameters appear to be good biomarkers for monitoring environmental management  
330 strategies but they need to be complemented with morphological parameters to confirm the impact of  
331 orchard management practices. Weight and morphometric traits in our earwigs were lower in IPM than  
332 in organic orchards. Earwigs from IPM orchards have to make trade-offs between maintenance,  
333 growth, storage and resistance to agrochemicals so as to maximize their fitness. The observed  
334 reduction in their prothorax and inter-eye widths is likely to be the consequence of these trade-offs  
335 induced by pesticides. A lot of studies in other insects showed that pesticides can lead to  
336 morphometric variations. Marcus and Fiumera (2016) showed that exposure to atrazine, the one of the  
337 most widely used herbicides significantly affected male and female adult body size of *Drosophila*  
338 *melanogaster*. In *Pterostichus melasitalicus*, a carabidae, morphometric analyses also showed that  
339 dimethoate application caused a reduction in body or elytra lengths in females (Giglio et al. 2011).  
340 Giglio et al. (2017) showed in another carabidae, *Calathus fuscipes*, that morphological parameters  
341 e.g. body and pronotum width decreased after an exposure to the larvicide lambda-cyhalothrin.  
342 Moreover, studies of the effects of an inorganic insecticide (boric acid) against *Blatella germanica*  
343 showed a reduction in the size of basal oocytes, which affected its reproduction (Habes et al. 2013).

344 All these studies suggest that agrochemical molecules can have an impact on the morphometric  
345 parameters of exposed organisms.

346 Our results underline the possible modifications in biochemical and morphometric parameters in  
347 earwigs due to orchard management strategies. Body energy reserves and prothorax and inter-eye  
348 widths in earwigs may be interesting biomarkers to characterize changes induced by agricultural  
349 farming both with pesticides and for other practices in orchards. This study shows more widely that *F.*  
350 *auricularia*, along with some other characteristics (ease of capture, identification, univoltine species)  
351 is an interesting potential bioindicator of the orchard management practices, especially in pip-fruit  
352 orchards, where it is considered an active natural enemy of aphids. In order to characterize the specific  
353 effects of pest management strategy on earwigs (direct and indirect effects), future studies should also  
354 be performed in abandoned orchards to assess the consequence of a free-pesticide environment. Other  
355 morphometric parameters could additionally be investigated to further determine if agricultural  
356 strategies influence other earwig life-history traits and further weakens these non-target species. In  
357 conclusion, this present study highlights the need to develop an integrated approach to assess both the  
358 physiological and biochemical modifications induced by pest management strategies on natural  
359 enemies.

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364 **Acknowledgments**

365 We thank Alain Tonetto (Pratim, Aix Marseille University) for assistance in taking the electronic  
366 microscopy photos. We are grateful to the Rovaltain Foundation for its financial support of the  
367 I-ResPect project.

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369 **References**

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371 Altieri MA (1999) The ecological role of biodiversity in agroecosystems. *Agric Ecosyst Environ*  
372 74:19-31.

373 Anand A and Lorenz MW (2008) Age-dependent changes of fat body stores and the regulation of fat  
374 body lipid synthesis and mobilisation by adipokinetic hormone in the last larval instar of the cricket,  
375 *Gryllus bimaculatus*. *J Insect physiol* 54:1404-1412.

376 Arrese EL and Soulage JL (2010) Insect faty body: energy metabolism and regulation. *Ann Rev*  
377 *Entomol* 55 :207-225.

378 Badji CA, Guedes RNC, Silva AA, Araujo RA (2004) Impact of deltamethrin on arthropods in maize  
379 under IPM and no-tillage cultivation. *Crop Prot.* 23 : 1031–1039.

380 Bednarska AJ, Stachowicz I, Kurianska L (2013) Energy reserves and accumulation of metals in the  
381 ground beetle *Pterostichus oblongopunctatus* from two metal-polluted gradients. *Environ Sci Pollut*  
382 *Res* 20 :390-398.

383 Beenackers AMT, Vanderhorst DJ, Vanmarrewijk WJA (1984) Insect flight metabolism. *Insect*  
384 *Biochem* 14:243–60.

385 Calow P (1991) Physiological costs of combating chemical toxicants: ecological implications. *Comp*  
386 *Biochem Physiol* 100(1/2) :3-6.

387 Campos MR, Picanço MC, Martins JC, Tomaz AC, Guedes RNC (2011) Insecticide selectivity and  
388 behavioral response of the earwig *Doru luteipes*. *Crop Prot* 30 : 1535–1540.

389 Chowanski S, Lubawy J, Spochacz M, Paluch E, Smykalla G, Rosinski G, Slocinska M (2015) Cold  
390 induced changes in lipid, protein and carbohydrate levels in the tropical insect *Gromphadorhina*  
391 *coquereliana*. *Comp Biochem and Physiol Part A* 183 : 57-63.

392 Congdon JD, Dunham AE, Hopkins WA, Rowe CL (2001) Resource allocation-based life histories : a  
393 conceptual basis for studies of ecological toxicology. *Environ Toxicol Chem* 20(8) :1698-1703.

394 Debras JF, Dussaud A, Rieux R, Dutoit T (2007) Prospective research on the "source" role of hedges  
395 in integrated fruit production. The case of earwigs: *Forficula auricularia* L. et *Forficula pubescens* L.  
396 *Gené. C.R. Biol.* 330, 664–673. (in French)

397 Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial  
398 arthropods. *Annu Rev Entomol* 52:81-106.

399 Dib H, Simon S, Sauphanor B, Capowiez Y (2010) The role of natural enemies on the population  
400 dynamics of the rosy apple aphid, *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) in organic  
401 apple orchards in south-eastern France. *Biol. Control* 55 : 97–109.

402 Dib H, Sauphanor B, Capowiez Y (2016) Effect of management strategies on arthropod communities  
403 in the colonies of rosy apple aphid, *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) in south-  
404 eastern France. *Agric Ecosyst Environ* 216 : 203-206.

405 Ferreira NGC, Morgado R, Santos MJG, Soares AMVM, Loureiro S (2015) Biomarkers and energy  
406 reserves in the isopod *Porcellionides pruinosus*: The effects of long-term exposure to dimethoate. *Sci*  
407 *Total Environ* 502 :91-102.

408 Ffrench-Constant RH, Vickerman GP (1985) Soil contact toxicity of insecticides to the European  
409 earwig *Forficula auricularia* (Dermaptera). Entomophaga 30 : 271–278.

410 Frings CS, Fendley TW, Dunn RT, Queen CA (1972) Improved determination of total serum lipids by  
411 the sulfo-phospho-vanillin reaction. Clin Chem 18(7): 673-674.

412 Fountain MT, Harris AL (2015) Non-target consequences of insecticides used in apple orchards on  
413 *Forficula auricularia* L. (Dermaptera: Forficulidae). Biological Control 91: 27-33.

414 García-de la Parra LM, Bautista-Covarrubias JC, Rivera-de la Rosa N, Betancourt-Lozano M,  
415 Guilhermino L (2006) Effects of methamidophos on acetylcholinesterase activity, behavior, and  
416 feeding rate of the white shrimp (*Litopenaeus vannamei*). Ecotox. Environ. Safe. 65: 372–380.

417 Giglio A, Cavaliere F, Giuliani PG, Mazzei A, Talarica F, Vommaro ML, Brandmayr P (2017)  
418 Impact of agrochemicals on non-target species : *Calthus fuscipes* Goeze 1777 (Coleoptera : Carabidae)  
419 as model. Ecotox Environ Safety 142 :522-529.

420 Giglio A, Giuliani PG, Zetto T, Talarica F (2011) Effects of the pesticide dimethoate on a nontarget  
421 generalist carabid, *Pterostichus melas italicus* (Dejean, 1828) (Coleoptera: Carabidae). Italian J  
422 Zoology 78(4) :471-477.

423 Gill HK and Garg H. (2014) Pesticides : Environmental impacts and management strategies.  
424 Pesticides – Toxic Aspects. pp 187-230.

425 Givaudan N, Suchail S, Rault M, Mouneyrac C, Capowiez Y (2016) Impact of orchard management  
426 strategies on earthworm (*Allobophora chlorotica*) energy reserves. Soil Biol Chem 100:252-254.

427 Gnaiger E. (1983) Calculation of energetic and biochemical equivalents of respiratory oxygen  
428 consumption. In: Gnaiger E, Forstner H (eds) Polarographic oxygen sensors. Aquatic and  
429 physiological applications. Springer, Berlin, pp 337–345.

430 Habes D, Messiad R, Gousmia S, Grib L (2013) Effects of an inorganic insecticide (boric acid) against  
431 *Blattella germanica*: Morphometric measurements and biochemical composition of ovaries. Afr J  
432 Biotech 12(18) :2492-2497.

433 Hahn DA, Denlinger DL. (2007) Meeting the energetic demands of insect diapause: nutrient storage  
434 and utilization. J. Insect Physiol. 53:760–73

435 Huang Z, Shi P, Dai J, Du J (2004) Protein metabolism in *Spodoptera litura* (F.) is influenced by the  
436 botanical insecticide azadirachtin. Pest Biochem Physiol 80: 85–93.

437 Jeon J, Kretschmann A, Escher BI, Hollender J (2013) Characterization of acetylcholinesterase  
438 inhibition and energy allocation in *Daphnia magna* exposed to carbaryl. Ecotox Environ Safety  
439 98 :28-35.

440 Jørgensen LN (1999). Denmark's action plans for pesticides: status and role of research. Nordisk  
441 Jordbrugsforskning 81: 201-202.

442 Kölliker M and Vancassel M (2007) Maternal attendance and the maintenance of family groups in  
443 common earwigs (*Forficula auricularia*): a field experiment. Ecological Entomology 32 :24-27.

444 Kromp B (1999) Carabid beetles in sustainable agriculture: A review on pest control efficacy,  
445 cultivation impacts and enhancement. Agric Ecosyst Environ 74:187–228.

446 Malagnoux L, Capowiez Y, Rault M (2015a) Impact of pesticide exposure on the predation activity of  
447 the European earwig, *Forficula auricularia*. Environmental Science and Pollution Research 22:  
448 14116-14126

449 Malagnoux L, Marliac G, Simon S, Rault M, Capowiez Y (2015b) Management strategies in apple  
450 orchards influences earwig community. Chemosphere 124 : 156–162.

451 Marcus SR, Fiumera AC (2016) Atrazine exposure affects longevity, development time and body size  
452 in *Drosophila melanogaster*. J Insect Physiol 91-92:18-25.

453 Markwell MAK, Hass SM, Bieber LL, Tolbert NE (1978) A modification of the Lowry procedure to

454 simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* 87 : 206–210.

455 Martinou AF, Seraphides N, Stavrinides MC (2014) Lethal and behavioral effects of pesticides on the  
456 insect predator *Macrolophus pygmaeus*. *Chemosphere* 96 : 167-173.

457 Mazzia C, Pasquet A, Caro G, Thénard J, Cornic J-F, Hedde M, Capowiez Y (2015) The impacts of  
458 management strategies in apple orchards on the structural and functional diversity of epigeal spiders.  
459 *Ecotoxicology* 24: 616-625.

460 Mclaughlin, Mineau P (1995) The impact of agricultural practices on biodiversity. *Agric Ecosyst*  
461 *Environ* 55 :201-2012.

462 Moerkens R, Leirs H, Peusens G, Belien T, Gobin B (2012) Natural and human causes of earwig  
463 mortality during winter: temperature, parasitoids and soil tillage. *J. Appl. Entomol.* 136 : 490–500.

464 Nash MA, Thomson LJ, Hoffmann AA (2008) Effect of remnant vegetation, pesticides, and farm  
465 management on abundance of the beneficial predator *Notonomus gravis* (Chaudoir) (Coleoptera:  
466 Carabidae). *Biol Control* 46: 83–93.

467 Nath BS (2003) Shifts in glycogen metabolism in hemolymph and fat body of the silkworm, *Bombyx*  
468 *mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticides toxicity. *Pest Biochem*  
469 *Physiol* 74 :73-84.

470 Nestel ND, Papadopoulos NT, Pascacio-Villafán C, Righini N, Altuzar-Molina AR, Aluja M (2016)  
471 Resource allocation and compensation during development in holometabolus insects. *J Insect Physiol*  
472 95:78-88.

473 Parrou JL and François J (1997) A simplified procedure for a rapid and reliable assay of both glycogen  
474 and trehalose in whole yeast cells. *Anal Biochem* 248:186-188.

475 Pekar S (1999) Effect of IPM practices and IPM spraying on spider population dynamics in an apple  
476 orchard. *Agric Ecosyst Environ* 26 : 155–166.

477 Rharrabe K, Amri H, Bouayad N, Sayah F (2008) Effects of azadirachtin on post-embryonic  
478 development, energy reserves and a-amylase activity of *Plodia interpunctella* Hübner (Lepidoptera:  
479 Pyralidae). *J Stored Prod Res* 44 :290-294.

480 Ribeiro S, Sousa JP, Nogueira AJA, Soares AMVM (2001) Effect of Endosulfan and Parathion on  
481 Energy Reserves and Physiological Parameters of the Terrestrial Isopod *Porcellio dilatatus*. *Ecotox*  
482 *Environ Safety* 49 :131-138.

483 Romeu-Dalmau C, Pinol J, Espadaler X (2012) Friend or foe? The role of earwigs in a Mediterranean  
484 organic citrus orchard. *Biol Control* 63 : 143–149.

485 Sánchez-Bayo F (2009) From simple toxicological models to prediction of toxic effects in time.  
486 *Ecotoxicology* 18:343 –354.

487 Sauphanor B, Stäubli A (1994) Evaluation au champ des effets secondaires des pesticides sur  
488 *Forficula auricularia* et *Anthocoris nemoralis*: validation des résultats de laboratoire. In: Vogt, H.  
489 (Ed.), OILB–SROP Section Régionale Ouest Paléarctique, Pesticides and beneficial organisms, vol.  
490 17. Bulletin OILB/SROP, pp. 83–88.

491 Sauphanor B, Dirwimmer C, Boutin S, Chaussabel AL, Dupont N, Fauriel J, Gallia V, Lambert N,  
492 Navarro E, Parisi L, Plenet D, Ricaud V, Sagnes JL, Sauvaitre D, Simon S, Speich P, Zavagli F (2009)  
493 Comparative analysis of different systems in fruit tree farming. In: INRA (Ed.), *Ecophyto R&D:*  
494 *Towards crop management systems that save crop protection products*, Expert report, Volume IV,  
495 National Institute of Agronomic Research.

496 Sauphanor B. (1992). An aggregation pheromone in the European earwig *Forficula auricularia* L.  
497 *Entomol Exp Applic* 62 (3): 285–291.

498 Sharley DJ, Hoffmann AA, Thomson LJ (2008) The effects of soil tillage on beneficial invertebrates  
499 within the vineyard. *Agric. For. Entomol.* 10 : 233–243.

500 Shaw PW, Wallis DR (2010) Susceptibility of the European earwig, *Forficula auricularia*, to  
501 insecticides residues on apple leaves. *New Zeland Plant Prot.* 63 : 55–59.

502 Staempfi C, Tarradellas J, Becker-van Slooten K (2007) Effects of dinoseb on energy reserves in the  
503 soil arthropod *Folsomia candida*. *Ecotox Environ Safety* 68 :263-271.

504 Steele JE (1982) Glycogen phosphorylase in insects. *Insect Biochem.* 12 (2) :131-147.

505 Tilton FA, Bammler TK, Gallagher EP (2011) Swimming impairment and acetylcholinesterase  
506 inhibition in zebrafish exposed to copper or chlorpyrifos separately, or as mixtures. *Comp. Biochem.*  
507 *Phys. C.* 153: 9–16.

508 Yasmin S and Souza D (2010) Effects of pesticides on the growth and reproduction of earthworm: A  
509 review *Applied and Environmental Soil Science* 1-9. doi:10.1155/2010/678360.

510 Ziegler R (1991) Changes in lipid and carbohydrate metabolism during starvation in adult *Manduca*  
511 *sexta*. *J Comp Physiol Part B* 161 :125-131.

512 Ziegler R, Van Antwerpen R. (2006). Lipid uptake by insect oocytes. *Insect Biochem. Mol. Biol.*  
513 36:264–72.

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517 **Figure captions**

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519 Figure 1. Scanning electron microscopy dorsal view of head and prothorax of a male earwig showing  
520 the measurement method applied to selected body parts: the inter-eye (A) and the prothorax (B).

521 Figure 2. Mean weight (+SE) of the female and male adults of earwigs depending on the management  
522 strategy in apple orchards. (n=40 for male and n=40 for females for each strategy).

523 Figure 3. Measurements of morphometric parameters: prothorax and inter-eye widths (means + SE)  
524 according to the management strategy in apple orchards. For each morphometric parameter, bars with  
525 the same letters are not significantly different from each other (n=40 for males and females for each  
526 strategy).

527 Figure 4. Energy reserves content of the female and male of earwigs for each management strategy in  
528 apple orchards. A: Glycogen, B: lipid and C: protein. Data represent the means (+SE). Bars with the  
529 same letters are not significantly different from each other (n=40 for males and females for each  
530 strategy).

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