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1 **Stress response in terrestrial isopods: a comparative study on glycaemia**

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14 **Abstract**

15 Stressors are omnipresent in the environment and trigger specific physiological and/or
16 behavioural responses that remain relatively less known in invertebrates. Among the
17 parameters used to quantify the impact of stress on physiology, the circulating level of
18 glucose is commonly used in aquatic crustacean species. Terrestrial isopods are small
19 crustaceans commonly used as bioindicators of soil quality; however, their response to
20 environmental stressors remains poorly documented. In laboratory conditions, we
21 investigated how different stressors influence the physiological response of two terrestrial
22 isopod species. Thus, we exposed *Armadillidium vulgare* and *Porcellio dilatatus* to a series
23 of both acute (physical) and chronic (temperature, social isolation, and exposure to herbicide)
24 stressors that mimic potentially stressful situations encountered in their environment. Our
25 results revealed that, for both species, glycaemia is not impacted by thermic shock (1 h at
26 4°C), mechanical disturbances (1 minute of shaking or upside-down motion) or social
27 isolation (4 weeks) at the population level. Nevertheless, we observed a significantly higher
28 sensitivity in male *P. dilatatus* exposed to upside-down movements and in males of both
29 species exposed to social isolation. Acute (96 hours) and chronic (21 days) exposure to
30 glyphosate (1.8-2.5 kg.ha⁻¹) did not impact glycaemia levels in *A. vulgare*. However, at both
31 tested doses, individuals exposed to glyphosate for 25 days and subjected to an additional
32 stressor (haemolymph sampling at 96 hours) had significantly higher glycaemia in
33 comparison to their control. Survival was also impacted by the joint application of chronic
34 glyphosate exposure and intermediate haemolymph sampling. We conclude that glucose is a
35 potentially good indicator to study the stress response of terrestrial isopods. Additional
36 studies for this parameter, combined with other physiological indicators, are needed to better
37 characterize this response.

38 **Keywords:** bioindicator, glyphosate, acute stress, social isolation, *Armadillidium vulgare*,
39 *Porcellio dilatatus*

40

41 **1. Introduction**

42 In ecology, the ability of an organism or a population to respond to a given change can be
43 used as a biological indicator, also called a bioindicator, of the quality of an environment
44 (Whitfield and Elliott, 2002). For instance, bioindicator species can be used to measure and
45 evaluate the impact of potentially harmful chemicals, such as heavy metals or pesticides, on
46 the environment (Bargańska et al., 2016; Hopkin et al., 1993). Changes can be measured at
47 different levels, such as specific diversity and abundance (Santorufó et al., 2012), or at the
48 individual level with records of mortality and fecundity (Jones and Hopkin, 1996),
49 physiology (Folguera et al., 2011) or behaviour (Zidar et al., 2005) to evaluate the direct
50 impact of environmental changes. To date, several vertebrate and invertebrate species have
51 been identified as reliable bioindicators of their natural habitat (Bargańska et al., 2016;
52 Carravieri et al., 2013; Gerlach et al., 2013; Paoletti and Hassall, 1999; Park, 2015; Pearce
53 and Venier, 2005; Souty-Grosset et al., 2005). Among invertebrate bioindicator species,
54 terrestrial isopods have the potential to be used as indicators in both moist areas and
55 grasslands (Gerlach et al., 2013; Souty-Grosset et al., 2005). Terrestrial isopods are important
56 decomposers of leaf litter, and their integrity may be rapidly threatened when a disturbance
57 occurs in the soil (Dallinger et al., 1992; Souty-Grosset et al., 2005).

58 When an organism is threatened, its resources are mobilized and redirected from functions
59 such as growth or reproduction to face the challenges and preserve its integrity (Moberg and
60 Mench, 2000). This response is known as the stress response. Invertebrates are frequently
61 exposed to a number of stressors, including repeated predation attempts (Hawlena et al.,
62 2011), adverse environmental conditions (Chown and Nicolson, 2004) and parasitism (Chang
63 et al., 2005). As stressors are omnipresent in the environment, the stress response is essential
64 for the survival of an individual and relatively well preserved during the course of evolution
65 (Nesse et al., 2016). Reactions towards stress are highly variable not only among different
66 species but also within individuals of the same species (Koehn and Bayne, 1989). As the
67 stress response is initially adaptive and protective, prolonged exposure to a given stressor or a
68 combination of stressors can further evolve into maladaptive responses and be detrimental for
69 an organism or a community (Moberg and Mench, 2000). Stress can cause severe damage to
70 an organism with a negative impact on its survival and fitness and potential consequences at
71 the population level (Hoffmann and Hercus, 2000). As an example, the accumulation of
72 various stressors resulted in the recent worldwide increase in colony failure rates, or ‘colony
73 collapse disorder’, in honeybee (Even et al., 2012).

74 The stress response has been extensively studied in vertebrates and is the result of
75 physiological changes in response to stressful stimuli (Kassahn et al., 2009). The stress
76 response can be measured at three levels. The primary stress response is the release of stress
77 hormones, such as corticosteroids and catecholamines, in the bloodstream (Tsalafouta et al.,
78 2014). The main role of these compounds is to restore homeostasis following exposure to
79 stressors. The secondary stress response acts at the cellular level by mobilizing and
80 reallocating energy and increasing cardiac output and oxygen uptake and transfer. In
81 mammals, for example, cortisol can facilitate the increase in the production and availability
82 of metabolites such as glucose. Gluconeogenesis is promoted in the liver, while glucose use
83 by the cells is limited to increase blood glucose availability to meet the needs of the organism
84 in response to stressful stimuli (Desborough, 2000). The tertiary response affects the entire
85 organism and can inhibit functions such as growth, reproduction, immune response and the
86 ability to face additional stressors (Moberg and Mench, 2000; Sapolsky et al., 2000; Tsigos
87 and Chrousos, 2002).

88 Although invertebrates are susceptible to stressors, the stress response in invertebrates has
89 been studied to a lesser extent than it has in vertebrates. Therefore, fewer cellular and
90 physiological indicators of stress in invertebrates are known. At the cellular level, the
91 increased production or activation of antioxidant proteins and heat shock proteins is seen in
92 response to a stressor, which is similar to the vertebrate response (Feder and Hofmann, 1999).
93 Relatively few invertebrate species have been studied regarding the stress response. Among
94 them, we find some model species for biological processes, such as *Caenorhabditis elegans*
95 or *Drosophila melanogaster* (Feder and Hofmann, 1999), or economically and ecologically
96 important species, such as the honeybee *Apis mellifera* (Even et al., 2012) and aquatic
97 crustacean species, such as the American lobster *Hommarus gammarus* (Chang et al., 2005)
98 or the white leg shrimp *Litopenaeus vannamei* (Chang et al., 2009). In aquatic crustacean
99 species, it has been shown that exposure to harmful physical or chemical stimuli resulted in
100 an elevation of glucose haemolymph (Lorenzon, 2005; Lorenzon et al., 1997). These
101 physiological changes were shown to be under the control of the crustacean hyperglycaemic
102 hormone (CHH) (Chang et al., 1998; Chang et al., 2005; Lorenzon, 2005; Lorenzon et al.,
103 1997). This hormone is secreted from the sinus gland (Fanjul-Moles, 2006) in response to
104 stressful stimuli in crustaceans (Chang et al., 2005). Moreover, this hormone has been
105 described as the crustacean equivalent of cortisol and corticosterone (Elwood et al., 2009).

106 Terrestrial isopods have been used in tests of the toxicity of soil, and the parameters of
107 evaluation were mainly the abundance of individuals (Faulkner and Lochmiller, 2000),
108 reproduction rate (Niemeyer et al., 2009) and survival (Stanek et al., 2006). *Porcellio scaber*
109 were studied most often regarding the metal bioaccumulation process (da Silva Souza and
110 Fontanetti, 2011). In this study, we chose to investigate the impact of several potential
111 stressors on the glucose levels of circulating haemolymph in two terrestrial isopod species,
112 *Armadillidium vulgare* (Latreille, 1804) and *Porcellio dilatatus* (Brandt, 1833). These species
113 belong to two different ecomorphological groups, with different defence strategies: *A.*
114 *vulgare* is a roller and *P. dilatatus* is a clinger (Schmalfuss, 1984). In their habitat, these
115 organisms can be exposed to various stressors that can be biotic or abiotic and acute or
116 chronic. The main stressors encountered can be separated into three categories: 1) biological
117 stressors (predators, parasites, fungi, bacteria and viruses); 2) chemical stressors (pesticides,
118 fungicides, and herbicides), which can cause damage to non-target species; and 3)
119 environmental stressors (agricultural practices, (micro)climate change, resource abundance
120 fluctuation, social interactions, humidity, etc.). In our laboratory, we investigated the impact
121 of five different stimuli that mimicked the whole range of potentially stressful situations
122 encountered in the natural habitat or cultivated areas of these species (Swan et al., 2011;
123 Szlavec et al., 2018). The main objective of the present study was to assess the importance
124 of three extrinsic factors (temperature, shaking, and falling), one intrinsic factor (gender) and
125 social privation on the glycaemia level.

126 The approach of using more subtle endpoints, such as physiological and behavioural changes,
127 to assess the toxicity of potentially harmful substances has become more popular and has
128 been shown to be more accurate than the traditional survival, growth and reproduction
129 responses (Drobne, 1997). In line with this approach, we use glycaemia as a physiological
130 endpoint to measure the potential stress responses in two terrestrial isopod species.

131 **2. Material and methods**

132 **2.1 Animals**

133 The terrestrial isopods *A. vulgare* and *P. dilatatus* were derived from two natural populations
134 collected in France (Nice, Alpes Maritimes, 1967, for *A. vulgare* and Rom, Deux-Sèvres,
135 1988, for *P. dilatatus*). The animals were maintained in standard laboratory conditions (in
136 boxes of moistened compost, at 20°C, and with the natural photoperiod of Poitiers, France

137 46°40'N) and fed *ad libitum* fresh organic carrots and dried leaves of linden (*Tilia* sp.). For
138 Experiments 1 and 2, we selected one-year-old adult *A. vulgare* and *P. dilatatus* of similar
139 size (around 60 mg and 150 mg respectively). For Experiment 3, we selected a mixture of
140 virgin 1- to 3-year-old *A. vulgare* individuals (60-150 mg). All animals used were in
141 intermoult (di-ecdysis or beginning the pre-ecdysis stages C/D₀) (for growth cycle details, see
142 Beauché and Richard, 2013 and Zidar et al., 1998). The period of each experiment was
143 chosen to match the moult cycle of each species (at least 26 days for both species; Beauché
144 and Richard, 2013; Zidar et al., 1998) in order to avoid moulting during the course of
145 experiment. None of the tested individuals were moulting during the course of the
146 experiment. Gravid females were excluded from the experimental pool. The experimental
147 procedures and animal manipulations did not require an ethics statement. The stimuli used in
148 these experiments were carefully selected to elicit a subtle response without causing any
149 unnecessary distress.

150 **2.2 Experimental procedure**

151 All procedures were carried out in laboratory conditions during daylight hours between 10:00
152 and 16:00 in the spring and summer of 2016. We chose five different stimuli to be applied to
153 the isopods: “cold”, “shake”, “upside-down” (Experiment 1), social isolation (Experiment 2)
154 and chronic exposure to glyphosate (Experiment 3). The stimuli were chosen as they could
155 mimic threats that the animals encounter in natural situations.

156 *2.2.1 Experiment 1: biological stressor*

157 Animals were randomly subjected to one of the three following acute stimuli: exposure to
158 cold temperature, shaking or an upside-down movement. The cold stimulus was chosen as
159 ectothermic animals, including terrestrial isopods, are sensitive to severe temperature changes
160 as their body temperature is mostly dependent on the temperature of their environment
161 (Dahlhoff and Rank, 2007). The shaking stimulus was used to mimic the impact of modern
162 agricultural practices, such loud noises and vibration disturbances caused by heavy
163 machinery (Schlacher and Thompson, 2007; Soane and van Ouwerkerk, 1994). The upside-
164 down stimulus aimed to mimic the impact of predator attacks. In nature, terrestrial isopods
165 can be targeted by numerous predators which could catch, move and drop them (Tuf et al.,
166 2015).

167 For each species, 20 undisturbed individuals (10 males and 10 females) were randomly
168 picked from the holding tanks and served as control groups. The ‘cold’ stimulus consisted of
169 placing two individuals in one box (Ø 8 cm, H 5 cm) kept at 4°C for one hour. The light
170 condition was kept constant during this period (refrigerator with a glass door), and the box
171 was kept moist using water-dipped tissues in the bottom). The “shake” stimulus consisted of
172 shaking the animals individually in a box (Ø 8 cm, H 5 cm) attached to an IKA KS 130 Basic
173 2D shaker (IKA, Staufen, Germany) for one minute at 560 rpm. The “upside-down” stimulus
174 consisted of placing the animals in individual boxes (Ø 9.5 cm, H 5 cm) and overturning the
175 box repeatedly by performing ample movements (180°) from right to left for one minute.
176 This stimulus was carried out by the same experimenter at a constant speed (approximately
177 70 movements). Stimuli were applied randomly to different individuals.

178 *2.2.2 Experiment 2: social isolation*

179 We investigated the impact of social isolation, which could be considered a chronic stressor
180 for the studied species. Terrestrial isopods are known to be gregarious organisms. Living in
181 groups has been demonstrated to offer several advantages, such as the prevention of
182 desiccation, the potential protection from predators and better access to mates and secondary
183 food resources (Broly et al., 2013). For this stimulus, 36 animals per species (18 males and 18
184 females) were placed in individual boxes (Ø 8 cm, H 5 cm) and kept isolated for a period of 4
185 weeks. The boxes were visually isolated from each other by cardboard sheets. In parallel, 120
186 individuals were sexed and kept in groups of 6 (20 boxes, Ø 11 cm, H 5 cm; 10 boxes for
187 males and 10 boxes for females) and served as the controls. On the sampling day, the
188 analyses of both the isolated and grouped individuals were performed simultaneously. In
189 total, 20 animals (10 males, 10 females, one individual per box) were sampled.

190 *2.2.3 Experiment 3: chemical stressor*

191 The animals were chronically exposed to the glyphosate-based herbicide Roundup®
192 (Monsanto, USA). Glyphosate-based herbicides are among the most widely used herbicides
193 for weed control and have become one of the most commonly used agrochemicals worldwide
194 (Shang et al., 2011). Despite studies of the impact of glyphosate on several non-target species
195 (Balbuena et al., 2015; Giesy et al., 2000), the sublethal effect remains poorly studied.
196 Terrestrial isopods have been proposed as good candidates to study the impact of pollutants
197 in terrestrial environments (Drobne, 1997).

198

199 Animals (*A. vulgare*) were randomly exposed to one of three doses of glyphosate (0, 1.8 and
200 2.5 kg.ha⁻¹) for up to 25 days. These doses are related to the mg of glyphosate present in the
201 glyphosate-formulated herbicide Roundup®. In this study, we did not aim to observe high
202 mortality but rather to test the impact of the doses recommended by the manufacturer and
203 approved by the French Ministry of Agriculture and Food at the time of the experiment for
204 use against easy-to-control (1.8 kg.ha⁻¹) and difficult-to-control (2.5 kg.ha⁻¹) weeds. Animals
205 were subsequently placed in plastic boxes (Ø 10 cm, 5 cm H) containing 70 (±0.5) g of
206 moistened compost. Each box contained 5 animals of the same sex and same age class
207 (N=40/treatment). The animals were acclimated in the boxes for four days, during which they
208 received their usual food. After this acclimatization period, the uneaten food was removed.
209 The glyphosate used in our study was in the form of isopropylamine salt (170 g.l⁻¹) as
210 specified in the Roundup® formulation. Animals were exposed to the desired dose of
211 glyphosate (Roundup®) through the food and the soil. For each box, 5-6 pieces of thinly
212 sliced carrot and the equivalent of two linden leaves were soaked with a mixture of 7 ml of
213 water and 0 µl, 8.3 µl or 11.5 µl of Roundup® for 15 minutes prior to being placed in the
214 appropriate box. The remaining solution was subsequently pipetted homogeneously into the
215 box, where it was placed on the soil, not directly on the animals. This process was repeated
216 weekly until the completion of the experiment (25 days maximum). Mortality was recorded
217 during these processes, and dead individuals were removed from the experimental boxes. One
218 group was sampled after 96 hours (acute exposure), and one group was sampled after 21 days
219 (chronic exposure). In addition, after the puncture, the animals from the group sampled after
220 96 hours were kept moist in recovery boxes with water-dipped tissues at the bottom for 24
221 hours. Surviving individuals were placed back into their original experimental boxes and
222 sampled after 21 additional days (25 days in total). This was done to investigate the effect of
223 an extra stressor (haemolymph sampling, mimicking a wound) in addition to the chronic
224 exposure to glyphosate.

225 Immediately after each stimulus, circulating haemolymph was sampled by making a puncture
226 in the intersegmental membrane between the thorax and the abdomen. The respective control
227 groups from each experiment were also sampled for haemolymph at the same time. In total,
228 5-10 µl of haemolymph per individual was collected using a micropipette and stored at -20°C
229 until further analyses. After the puncture, the animals were kept moist in recovery boxes
230 using water-dipped tissues on the bottom for 24 hours. Surviving individuals (>50%) were

231 placed back into their original housing boxes with moistened compost litter and were not
232 further used for another study.

233 **2.3 Glucose determination**

234 Haemolymph glucose was measured with a commercially available enzymatic test kit
235 (Glucose HK, Sigma-Aldrich, St. Louis, USA), with protocols adapted to a 96-well
236 microplate (Schram et al., 2010). Samples are first diluted in distilled water (1:6); then, 10 µl
237 of the diluted sample or standard (5.55 mmol.l⁻¹ glucose) are mixed with 200 µl of the reagent
238 provided with the kit and incubated for 15 minutes at 20°C. Absorbance was read within 30
239 minutes at 340 nm (Berthold LB941 Multimode reader, Berthold Technologies, Zug,
240 Switzerland).

241 **2.4 Data analyses and statistics**

242 Data are summarized by box and whisker plots that present first, second (median), and third
243 quartiles as horizontal lines in the box and upper and lower values as “whiskers” extending
244 from the boxes; In some instances, not all samples collected were analysed because of
245 insufficient haemolymph quantity. For Experiment 1 (the “cold” and “shake” stimuli) and
246 Experiment 3, there were no gender differences, and, therefore, the data were pooled.
247 However, for the “upside-down” stimulus (Experiment 1) and Experiment 2, the data were
248 split between males and females. The data were not normally distributed; therefore, the non-
249 parametric Mann-Whitney test was used to assess statistical significance for all the
250 experiments. We used the Chi-square test to compare the survival of individuals in
251 Experiment 3.

252 **3. Results**

253 **3.1 Experiment 1: biological stressor**

254 There were no differences in the glucose haemolymph concentration regardless of the species
255 or the type of stimulus tested in comparison with the control condition when the data were
256 pooled (Fig 1A and 1B) (Mann-Whitney test; for *P. dilatatus* and *A. vulgare*, respectively:
257 “cold”: W=59, p=0.32; W=151, p=0.27; “shake”: W=93.5, p=0.41; W=141, p=0.17; “upside-
258 down”: W=100, p=0.16; W=180.5, p=0.79). For *A. vulgare*, no gender-specific differences
259 were found (Mann-Whitney test, p> 0.05 for all stimuli, Fig 1B and 2B). Similarly, *P.*
260 *dilatatus* subjected to the cold and shake stimuli did not display gender differences regarding

261 their glucose haemolymph (Fig 1A). However, male *P. dilatatus* had significantly
262 haemolymph glucose levels compared to those of their female counterparts for the upside-
263 down stimulus only (Mann-Whitney test, $W=20$, $p=0.03$, Fig 2A). These levels were
264 significantly higher than those of the male control group (Mann-Whitney test, $W=13$, $p=0.02$,
265 Fig 2A).

266 **3.2 Experiment 2: social isolation**

267 There were no differences in the glucose haemolymph concentration for either species in
268 comparison with their respective controls when the data were pooled (Mann-Whitney test;
269 $W=106.5$, $p=0.20$ for *P. dilatatus* and $W=157$, $p=0.08$ for *A. vulgare*). However, we observed
270 a gender difference for both species: male *P. dilatatus* and *A. vulgare* subjected to social
271 isolation for a month had significantly higher haemolymph glucose levels compared to those
272 of their respective control groups (Mann-Whitney test, $W=56$, $p=0.04$ for *P. dilatatus*,; and
273 $W=14.5$, $p<0.01$ for *A. vulgare*, Fig 3A and 3B). No differences in glucose haemolymph were
274 found between females subjected to social isolations and females kept in group for either
275 species (Mann-Whitney test, $W=98$, $p=0.96$ for *P. dilatatus* and $W=53$, $p=0.47$ for *A.*
276 *vulgare*).

277 **3.3 Experiment 3: chemical stressor**

278 There was no effect on the glucose haemolymph concentration when *A. vulgare* individuals
279 were exposed to either dose of glyphosate for 96 hours (Mann-Whitney test, $W=506.5$,
280 $p=0.47$ for the $1.8 \text{ kg}\cdot\text{ha}^{-1}$ group and $W=475$, $p=0.21$ for the $2.5 \text{ kg}\cdot\text{ha}^{-1}$ group, Fig 4A) or 21
281 days (Mann-Whitney test, $W=353$, $p=0.45$ and $W=295.5$, $p=0.18$, Fig 4B). However,
282 individuals who were sampled after 25 days of exposure to either concentration and received
283 another stressor (i.e., a haemolymph puncture after 96 hours) had significantly higher glucose
284 haemolymph than that of the control group (Mann-Whitney test, $W=57$, $p=0.01$ for the 1.8
285 $\text{kg}\cdot\text{ha}^{-1}$ group and $W=64.5$, $p=0.05$ for the $2.5 \text{ kg}\cdot\text{ha}^{-1}$ group, Fig 4C). Survival tended to
286 decrease naturally with time in all groups (non-significant; $X^2=0.13$, $\text{ddl}=2$; $X^2=0.33$, $\text{ddl}=2$;
287 and $X^2=0.58$, $\text{ddl}=2$ for the control group and the 1.8 and $2.5 \text{ kg}\cdot\text{ha}^{-1}$ glyphosate groups,
288 respectively) (Fig 5). Survival was only negatively affected by the combination of chronic
289 exposure to glyphosate and the application of an extra stressor (Fig 5). No differences in
290 survival were observed between individuals from the control group samples after 96 hours
291 and those from the group exposed to glyphosate for 96 hours + 21 days ($X^2=3.63$, $\text{ddl}=1$),

292 while survival decreased in both the 1.8 and 2.5 kg.ha⁻¹ glyphosate groups ($X^2=6.23$, ddl=1
293 and $X^2=7.41$, ddl=1, respectively). There were no differences in survival among all groups
294 after 96 hours and 21 days of exposure ($X^2=0.58$, ddl=1; $X^2=0.01$, ddl=1; and $X^2=0.053$,
295 ddl=1 for the control group and the 1.8 and 2.5 kg.ha⁻¹ glyphosate groups, respectively) (Fig
296 5).

297 **4. Discussion**

298 The elevation of circulating glucose levels is a common response to stressors and is found
299 throughout the animal kingdom, including crustaceans. Crustaceans have been widely used
300 for stress risk assessment and are used as bioindicators or biomonitors, especially in various
301 aquatic systems (Rinderhagen et al., 2000). Among commercial aquatic crustaceans species,
302 stress exposure, such as emersion, transport and handling, is common and causes hypoxia and
303 variation in physiology (Lorenzon, 2005). For example, the decapods, *Liocarcinus depurator*
304 and *Munida rugosa* showed a higher concentration of glucose when subjected to trawling in
305 comparison to creel-caught crustaceans (Bergmann et al., 2001). Another study revealed that
306 the CHH hormone concentration increased in the haemolymph in less than 1 hour after acute
307 stress (handling) in lobsters, which is slower than the appearance of biogenic amines (Chang
308 et al., 2005). The production and action of these hormones leads to mobilization of energy
309 reserves, such as glucose. In the current study, we used two terrestrial isopod species to test
310 the glucose level variation in response to different stressors.

311 We found that stress induced in *A. vulgare* and *P. dilatatus* adults by the cold and shake
312 stimuli had no significant impact on the haemolymph glucose levels, which were collected
313 right after the application of the stimuli to test the short-term impact of our stressors.

314 When exposed to the upside-down acute stressor, we observed that males of *P. dilatatus* had
315 significantly higher glucose levels than those of their controls, while there was no effect on
316 male *A. vulgare*. These two species have different defence strategies. *A. vulgare* can roll and
317 may isolate themselves from the exterior (Schmalfuss, 1984). However, *P. dilatatus*
318 corresponds to the clinger ecomorphological group (Schmalfuss, 1984) and is highly
319 responsive to stimuli, but the duration of response (tonic immobility) is relatively short, with
320 no difference between sex (Quadros et al., 2012).

321 In the second experiment, we observed a sex difference for both species when individuals
322 were isolated for one month. The glucose levels of male are apparently affected, while those

323 of females are not. These results indicate that male isopods, regardless of their defence
324 strategies, are more sensitive to non-physical and chronic stress, such as social isolation, than
325 their female counterpart. Here, we showed that different types of stress have different impacts
326 between species, but we also observed differences in sensitivity between females and males.
327 Differences in sensitivity between sexes exist and have been previously observed in several
328 invertebrates and in both aquatic and terrestrial systems (Sornom et al., 2010) and reference
329 therein (McClellan-Green et al., 2007; Wilczek et al., 2008; Zeng et al., 2011). For example,
330 under oxidative stress conditions, male flies ingested and excreted less food than females
331 (Zeng et al., 2011). In two species of spider, cellular reactions against stress caused by metal
332 pollutants seemed to be sex-dependant (Wilczek et al., 2008). In the terrestrial isopod *P.*
333 *scaber*, a close relative of *P. dilatatus*, an exposure to dimethoate for 48 hours impacted the
334 locomotor behaviour (increased hyperactivity and suppressed turning rate) of males only
335 (Bayley, 1995).

336 In the third experiment, we evaluated the effect of two recommended concentrations of
337 glyphosate used in agriculture (1.8 and 2.5 kg.ha⁻¹). Glyphosate ingestion might affect the
338 learning and retrieval of memory for the recognition of food (Balbuena et al., 2015), and
339 chronic exposure to traces of glyphosate reduces the responsiveness to sucrose in honeybee
340 (Herbert et al., 2014). Glyphosate is also known to reduce growth in the earthworm (Springett
341 and Gray, 1992) and affect reproduction and development in the freshwater snail (Tate et al.,
342 1997). In our experiment, we observed no significant impact on *A. vulgare* glycaemia.
343 However, individuals who were chronically exposed to glyphosate and an additional stressor
344 (intermediate haemolymph sampling) had altered glucose levels in comparison with
345 individuals that only received the additional stressor without glyphosate. No individual
346 moulted between the two sampling sessions. Stressors are omnipresent in the environment
347 and can potentially interact with each other and affect the capacity of an organism to cope
348 with a specific stressor (Sornom et al., 2010). While assessing the sublethal impact of a
349 contaminant on an organism, it is important to take into account that the potential of an
350 organism to cope with stressors and survive is determined by the ability of all its biological
351 systems to work in concert (Hebel et al., 1997). For instance, here, we observed no direct
352 impact of exposure to the recommended doses of glyphosate, but when animals were exposed
353 to an additional stressor (haemolymph puncture) that can be compared to a minor wound, we
354 observed that its combination with glyphosate exposure resulted in stress.

355 In conclusion, changes in glycemia can be used to assess stress in terrestrial isopods. Males
356 of both species seemed to be more sensitive than female to stressors such as upside-down
357 movements or social isolation. In our study, we have focussed on the very short term impact
358 of single stressor. The effect combined stressors and the impact of glyphosate exposure on
359 the middle and longer term require further investigations.

360

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- 525

526 **Figure legend**

527 **Fig 1.** Haemolymph glucose concentrations (whisker plot) in *Porcellio dilatatus* (A) and
528 *Armadillidium vulgare* (B) in the control situation and following different stressful stimuli:
529 one hour at 4°C (“cold”) and one minute of constant shaking at 560 rpm (“shake”) (females
530 and males pooled). NS: not significant (Mann-Whitney test).

531 **Fig 2.** Haemolymph glucose concentrations (whisker plot) in *Porcellio dilatatus* (A) and
532 *Armadillidium vulgare* (B) in the control situation and one minute of repeated manual
533 overturns in a box (“upside-down”). NS: not significant, *: p<0.05 (Mann-Whitney test).

534 **Fig 3.** Haemolymph glucose concentrations (whisker plot) in *Porcellio dilatatus* (A) and
535 *Armadillidium vulgare* (B) kept in groups of 6 individuals (“control”) or kept isolated
536 (“social isolation”) for a period of 4 weeks. NS: not significant, *: p<0.05, **: p<0.01 (Mann-
537 Whitney test).

538 **Fig 4.** Haemolymph glucose concentrations (whisker plot) in *Armadillidium vulgare* exposed
539 to 0 (control), 1.8 and 2.5 kg.ha⁻¹ of glyphosate for 96 hours (A), 21 days (B) and 25 days
540 with an extra stressor (haemolymph sampling) after 96 hours (C) NS: not significant, *:
541 p<0.05, Mann-Whitney test).

542 **Fig 5.** Survival (%) of *Armadillidium vulgare* exposed to 0 (control), 1.8 and 2.5 kg.ha⁻¹ of
543 glyphosate for 96 hours (T=96 H), 21 days (T=21 D) and 25 days with an extra stressor
544 (T=96 H + 21 D) after 96 hours. *: p<0.05 Chi-square test.

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