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## Effect of static magnetic field on terrestrial isopods (Isopoda: Oniscidea)

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### ABSTRACT

We assessed the effect of exposure of three terrestrial isopods, *Armadillidium vulgare* (Latreille, 1804), *A. granulatum* (Brandt, 1833), and *Porcellio laevis* Latreille, 1804, to a static magnetic field (SMF) (200 mT). Exposure altered mortality, reproduction, and behavior (volvation). Mortality rate increased significantly ( $P < 0.05$ ) in the three species as compared to non-exposed individuals. Volvation changed from euspheric to mesospheric in *A. vulgare*, and from mesospheric to the absence of volvation in *A. granulatum* ( $P < 0.01$ ). The size of the male copulatory organ and the female marsupium of *P. laevis* were significantly changed ( $P < 0.001$ ) after exposure to SMF and maintained until reaching maturity.

**Key Words:** behavior, mortality, reproduction, volvation, woodlice

### INTRODUCTION

The development of new technologies have resulted in a potential exposure to high intensities of static magnetic fields (SMF) in humans and other animals. Sources of SMF in residential and work environments include small permanent magnets in magnet clips and magnetic attachments (bags, buttons, magnetic necklaces and bracelets, magnetic belts, magnetic toys), which generate local static fields exceeding 0.5 mT (ICNIRP, 2009). Previous reported effects of SMF dealt mainly with different responses among living organisms to different SMF intensity ranges: low intensity in the microtesla ( $\mu\text{T}$ ) range, including a geomagnetic field (25–65  $\mu\text{T}$ ) (Finlay *et al.*, 2010); moderate intensity in the millitesla (mT) range; and high intensity in the tesla (T) range, including ultrahigh SMF intensity ( $> 5$  T) (Okano, 2008). The highest non-occupational exposure occurs in patients undergoing a diagnostic examination by magnetic resonance (MR). Medical procedures using magnetic resonance imaging (MRI) are becoming more commonplace. In MRI, magnetic flux intensities typically range between 0.15 and 3 T and the exposure is usually limited to less than 1 h (ICNIRP, 2009).

The effects of magnetic fields on biological systems have been demonstrated at various levels. Epidemiological studies suggest that repeated exposure to magnetic fields in residential or work environments could increase the risk of various types of cancers in humans (Poole & Trichopoulos, 1991; Coogan *et al.*, 1996). Such prolonged exposure induces a modulatory action of apoptosis in animals (Thun-Battersby *et al.*, 1999; Chater *et al.*, 2005). SMF disrupts the menstrual cycle of rats (Levin & Ernst, 1997), causing changes in the aging rate (Kholodov, 1971; Bellossi, 1986) and inducing morphological and histochemical changes in the

microstructure of the central nervous system (Abdullakhodzhayeva & Razykov, 1986). 1T SMF is lethal in young mice while 420 mT caused the death of embryos and the destruction of the adult uterus (Kholodov, 1971).

A weak static magnetic field can also affect invertebrates (Dubrov, 1978; Martin *et al.*, 1989). Cockroaches exposed to static electric fields (SEF) showed significant changes in locomotion by covering less distance, walking slowly, and turning more often (Jackson *et al.*, 2011). The freshwater crab *Barytelphus acunicularis* (Westwood in Sykes, 1836) showed a maximum aggregation and aggressiveness when exposed to low frequency electromagnetic fields (LF-EMF). The change in the behavior of crabs was similar to that reported for serotonin neurotransmitters on the same species (Rosaria & Martin, 2010). Similarly, the direction of movement of the crayfish *Faxonius limosus* (Rafinesque, 1817) was significantly affected by a static magnetic field applied at the entrance of hideouts of ceramic pipes placed in a natural area (Tanski *et al.*, 2005). Magnetic pollution could affect reproduction and impair embryonic development (Pan, 1996; Levin & Ernst, 1997). Gastrulation was interrupted after exposure to 8.35 T magnetic fields (MF) in frogs (Neurath, 1968). A constant and rotating magnetic field (25 mT) treatment induced a significant increase in the hatching percentage of *Artemia* cysts and the development of parthenogenetic eggs of the cladoceran *Daphnia magna* Straus, 1820 showed accelerated rates of embryonic development after EMF exposure (Shckorbatov *et al.*, 2010). Females of *D. magna* that developed from exposed eggs to EMF (435–500 Hz) showed an accelerated release from the internal egg membrane and decrease in the number of viable offspring in their first brood (Krylov, 2010).

Woodlice, the common terrestrial isopod, has an important role in the trophic chain and subsequently in the equilibrium of the forest ecosystem. Some woodlice species are nevertheless characteristic of grasslands. These species may be useful as easily identified bioindicators of undisturbed, semi-natural conditions (Souty-Grosset *et al.*, 2005). Woodlice are involved in leaf litter decomposition (Abd El-Wakeil, 2015). Decomposition of isopod feces in a long-term experiment was lower than litter decomposition, which may support stabilization of organic matter in soil (Spaldonová & Frouz, 2014). Changes in the behavior of these isopods that could result from possible exposure to magnetic fields could have repercussions on the respective ecosystems. Terrestrial isopods are suitable for experimental work because they are small, abundant, breed easily (Medini *et al.*, 2000; Hamaied & Charfi-Cheikhrouha, 2004), and their presence in domestic environments passively expose them to magnetic fields.

We present the results of a study designed to assess the effects of SMF on three species of terrestrial isopods collected in northern Tunisia. The investigation focused on the mortality, volvation, and reproduction after exposure.

## MATERIALS AND METHODS

### Test species and sampling

Specimens of the three woodlice, *Armadillidium vulgare* (Latreille, 1804), *A. granulatum* (Brandt, 1833), and *Porcellio laevis* Latreille, 1804, were collected from yards at the University of Bizerte, northern Tunisia (37°52.0251 N, 9°52.4428 E) (Supplementary material Fig. S1). The habitat is covered by plants, is in the shade, moist, and rich in decaying matter and litter. Woodlice were reared in boxes under controlled conditions (temperature  $24 \pm 2$  °C, humidity > 80%, and photoperiod 16h light:8h dark). Individuals were fed with plants and/or decomposing litter from the sampling site. Individuals were divided into four groups, two controls and two under experimental exposure to SMF, and were used to study mortality and volvation in *A. vulgare* and *A. granulatum*. Nine groups

of *P. laevis* were bred, two for examining mortality (one control, one exposure to SMF) and seven for the study of reproduction: three control groups (males with females, males only, and females only) and four exposed groups (males exposed with females, females exposed with males, exposed males only and exposed females only).

Dead individuals were counted after each weekly exposure to study mortality resulting from exposure to SMF. Volution in *A. vulgare* was tested by touching the specimens. Three states of this behavior were considered where volvation persisted, changed (euspheric to mesospheric), or disappeared.

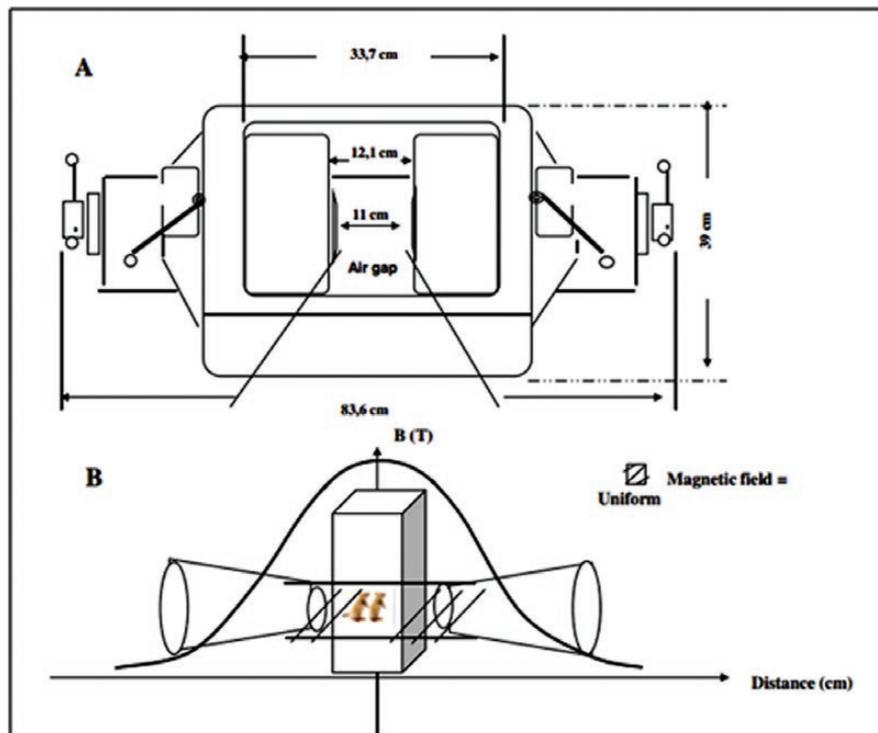
For the reproduction of *P. laevis*, the marsupial size:body size ratio, was calculated for each individual of the tested batches and compared with controls. The genitalia:body size ratio for the males was also determined. Body length and the size of the male copulatory organ and the female marsupium were measured under a stereomicroscope equipped with a micrometer. Thirty specimens were used in each experiment (six replicates with five specimens per replicate).

### Exposure design

We used an electromagnet (Model EM4-HVA, Lake Shore Cryotronics, Westerville, OH, USA) and a magnetic power supply (Model 647, Lake Shore Cryotronics) (Supplementary material Fig. S2) with an air gap of 11 cm (Fig. 1A). Water-cooled coils provide an excellent field for stability and uniformity when a high power is required to achieve the maximum field capability for the electromagnet. An accurate pole alignment was obtained by the construction of the air gap adjustment mechanism (Abdelmelek *et al.*, 2006).

### Static magnetic field exposure

The intensity of the SMF was measured and standardized at 200 mT over the total floor area of the glass bottom ( $10 \times 5 \times 5$  cm) using a digital tesla-meter (model 13610.93, Phywe, Göttingen,



**Figure 1.** Design of the electromagnet (Model EM4-HVA) used in the experiment (A) and magnetic field propagation (B) (from Ghodbane *et al.*, 2015).

Germany) with an axial hall probe determining both the static and electromagnetic fields at a frequency range of DC-5 kHz and a magnetic field intensity range of 10  $\mu$ T-2.5 T (Hassan & Abdelkawi, 2010). The bottom was placed in the gap between the two bobbins of the Lake Shore System (Abdelmelek et al., 2006). Woodlice were placed at the center of the uniform field area. Individuals were exposed to SMF 1h daily for 5 d. The bottom contained five individuals for each exposure (Fig. 1B). The control woodlice were placed in the same conditions without applying SMF.

#### Statistical analysis

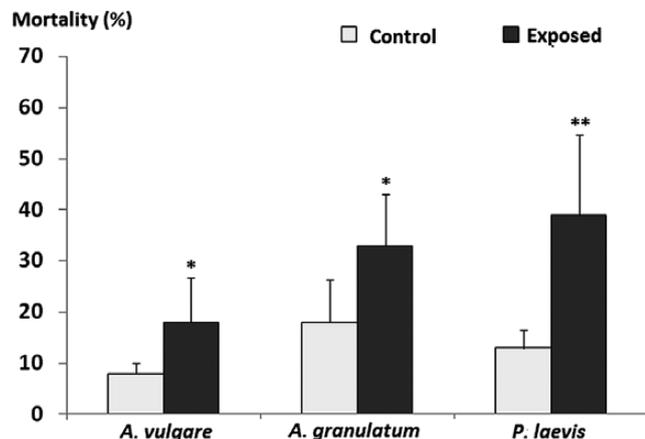
All data were expressed as the mean  $\pm$  standard deviation. The data were first tested in order to assess normality (Kolmogorov-Smirnov test) and equality of variance (Bartlett test). One-way ANOVA was used to test for overall differences in the considered parameters (mortality, volvation, and reproduction) between the controls and the exposed samples, and the Newman-Keuls multiple-comparison test was used in pairwise comparisons of exposed samples and controls. Difference among treatments was considered at a significance level of  $P < 0.05$ . All statistical analyses were performed with Statistica 6.0 software for Windows.

## RESULTS

After 5 d of exposure to SMF (1 h daily), a significant mortality rate was recorded for all three species of woodlice: 18% for *P. laevis* ( $P < 0.01$ ), 33% for *A. vulgare* ( $P < 0.05$ ), and 39% for *A. granulatum* ( $P < 0.05$ ) (Fig. 2).

Exposure to SMF caused a significant change in the shape of volvation, from originally euspheric to mesospheric in *A. vulgare* (from mean number of individuals 0.83 in controls to 6.66 after exposure), and even the disappearance of this phenomenon in several individuals (Fig. 3); whereas in *A. granulatum* the originally mesospheric volvation significantly disappeared ( $P < 0.01$ ) after exposure to SMF (Fig. 4), from a mean of 0.83 individual in controls to 15.16 after exposure.

The presence of females of *P. laevis* had no significant effect on the size of the male copulatory organ. In contrast, the presence of the male had a highly significant ( $P < 0.01$ ) effect on the development of the size of the female marsupium. Exposure to SMF induced a significant decrease in the ratio (copulatory organ length:total body length) for both sexes ( $P < 0.001$ ) as well as males alone ( $P < 0.001$ ) (Fig. 5). A significant regression was obtained for the size of the marsupium in females with males ( $P < 0.05$ ) (mean

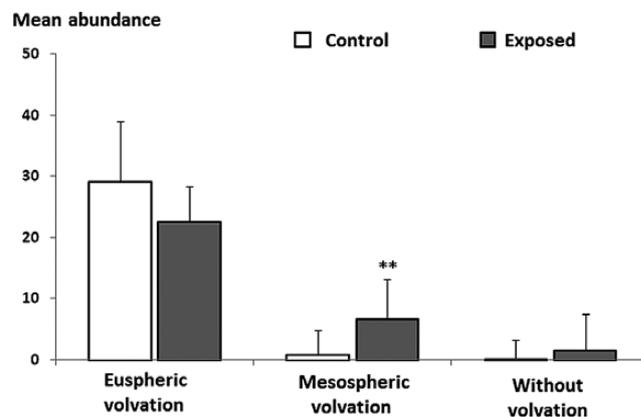


**Figure 2.** Effect of static magnetic field (200 mT) on the mortality of the woodlice *Armadillidium vulgare*, *A. granulatum*, and *Porcellio laevis*; \* significant difference compared to control at  $P < 0.05$ ; \*\* at  $P < 0.01$ .

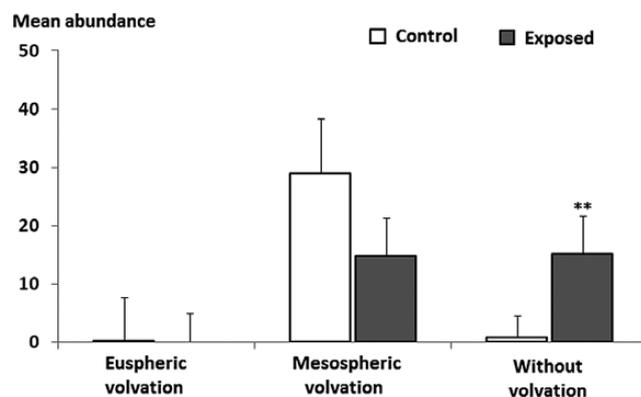
ratio of 0.376 in the controls versus 0.326 in exposed individuals) and a significant regression in females only ( $P < 0.001$ ) (mean ratio of 0.370 in the controls versus 0.331 in exposed individuals) (Fig. 6).

## DISCUSSION

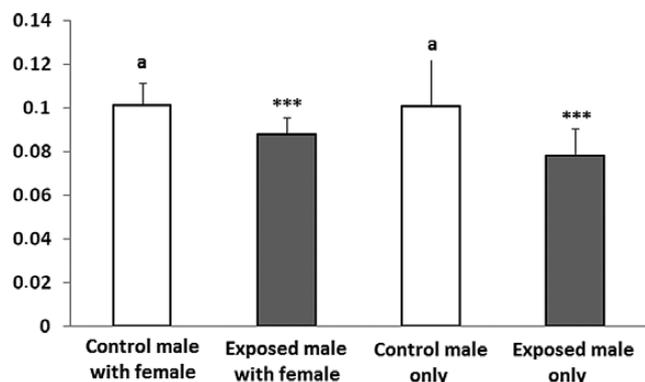
After five-day exposure to SMF (1 h daily), mortality increased significantly in the two species of woodlice *Armadillidium* ( $P < 0.05$ ) and *P. laevis* ( $P < 0.01$ ). The high mortality in the three species was observed in various generations (different ages, both sexes). This mortality could be explained by the reduction in the frequency of hatching, which was delayed. A similar effect was observed in *Heliothis virescens* (Fabricius, 1777) (Insecta, Lepidoptera) after exposure to high SMF (7 T) (Pan, 1996). Maximal, significant mortality was recorded after seven days of exposure to AMF (alternating magnetic field) of 500 Hz in *Daphnia magna* (Krylov, 2008). The mortality of *Drosophila* eggs treated for 48 h with SMF (4.5 mT) was lower in controls than the exposed ones, and the viability of its adults decreased by 35% (Ramirez et al., 1983). Mortality was significantly increased in the adult instars of the aphid *Sitobion avenae* (Fabricius, 1775) under a treatment of 0.065 T for 60 min (He et al., 2012). Furthermore, the increase in mortality after exposure to a mean SMF (10 mT-0.1 T) in two species of sea urchins was attributed to the alteration of the time of the first cell division and the appearance of embryonic anomalies due to the increase of the exogastrulation frequency (Pan, 1996). The increase of the death rate after the nocturnal exposure of



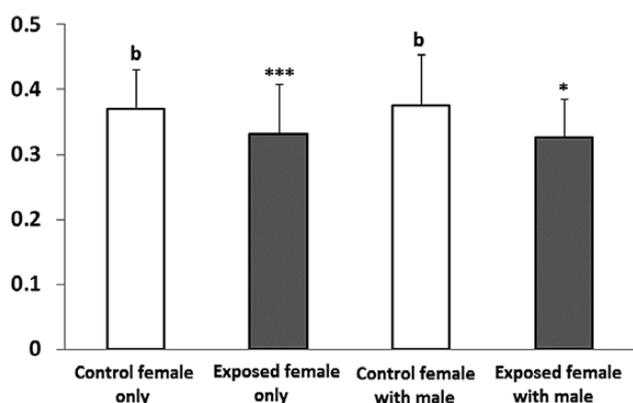
**Figure 3.** Effect of static magnetic field (200 mT) on the volvation of *Armadillidium vulgare*; \*\* highly significant difference compared to control at  $P < 0.01$ .



**Figure 4.** Effect of static magnetic field (200 mT) on the volvation of *Armadillidium granulatum*; \*\* highly significant difference compared to control at  $P < 0.01$ .



**Figure 5.** Effect of static magnetic field (200 mT) on the male copulatory organ:body length ratio in *Porcellio laevis*; a, no significant difference between control males with and without females; \*\*\* highly significant difference compared to control at  $P < 0.001$ .



**Figure 6.** Effect of static magnetic field (200 mT) on the female marsupium:body length of *Porcellio laevis*; b, highly significant difference between control females with and without males at  $P < 0.001$ ; \* significant difference compared to control at  $P < 0.05$ , \*\*\* at  $P < 0.001$ .

the snail *Cepaea nemoralis* (Linnaeus, 1758) to SMF was attributed to the disruption of the endogenous opioid system, which in turn disturbs the modulation of homeostatic mechanisms and increases the effects of stress, thus altering immune responses (Kavaliers & Ossenkopp, 1991, 1992).

The volvation of isopods can be simple (mesospheric) or perfect (euspheric) (Vandel, 1948) with several intermediate forms. Volution is a passive defense strategy against predators. The mechanical requirements of the coil are the same for all volvational isopods, but if the result is the same, the structures involved are often different (Vandel, 1960, 1962). We found that volvation changed from euspheric to mesospheric in *A. vulgare*, and from mesospheric to no volvation in *A. granulatum* ( $P < 0.01$ ). SMF can affect either the articulation mechanism between different segments of the body, or the neural mechanisms that control the involved muscles. The excitatory post-synaptic potential (EPSP) produced via electrical and chemical synapses in the lateral giant neuron were enhanced after 30 min of SMF exposure (8.08 mT) in the crayfish *Faxonius limosus*. Such exposure increased the efficacy of synaptic transmission in the tail-flip escape circuit (Yeh *et al.*, 2008).

Most isopods reproduce seasonally. Such as the case of *Armadillidium pelagicum* Arcangeli, 1957, which reproduces from March/April to the end of August, followed by sexual inactivity from September to February/March (Hamaied & Charfi-Cheikhrouha, 2004). In the iteroparous *P. laevis*, reproduction extends over nine months from February to October, followed by a three-month inactivity (November to January) (Medini *et al.*, 2000).

The presence of males with ovigerous females of *P. laevis* had a significant effect on female reproduction but not in males. The marsupium was found to be more developed in females that had bred with males than those that did not bred (Figs. 5, 6). In *P. variabilis* Lucas, 1849, the stimulation of female reproduction does not require repetitive coupling with males, and the spermatophore acquired by the female will be used, in part, for several generations (Medini *et al.*, 2000). The effect of the presence of a male on the reproduction of females occurs at other levels where the male acts on the female by stimulating the second phase of vitellogenesis. Couple formation in the woodlouse *Hemilepistus reaumurii* (Milne Edwards, 1840) stimulated reproduction (Lefebvre & Caubet, 1998).

Our research showed that the exposure of *P. laevis* to SMF had induced a significant decrease in the size of male copulatory organ ( $P < 0.001$ ) and in the marsupium size of females ( $P < 0.05$ ). In contrast, the exposure of the mussel *Mytilus edulis* Linnaeus, 1758 to SMF (3.7 mT) for several weeks, did not influence gonadal or condition indices (Bochert & Zettler, 2004). Exposure to SMF (7 T) in the lepidopteran *Heliothis virescens* (Fabricius, 1777) greatly delayed egg hatching ( $21.7 \pm 0.9$  h) and reduced hatching rates ( $58 \pm 8\%$ ), which affects the number of larvae (Pan, 1996).

Similarly, a chronic exposure to AMF (500 Hz) in *Daphnia magna* increased the number of vital offspring while decreasing the size of the offspring (Krylov, 2008). Exposures of *D. magna* to an electric low frequency magnetic field (ELF MF) for several generations showed to result a decrease in the size and biomass of the animals, beside a change in the generalized variance of morphometric parameters for viable offspring and neonates for the first five generations (Krylov & Osipova, 2013).

Overall, the reduction in female marsupial size in *P. laevis* could be probably explained by the decrease in the number of eggs and/or juveniles as a result of the increase in the frequency of exogastrulation or juvenile mortality. Such results have been validated by Wan *et al.* (2014), who had demonstrated that the exposure of two homopterans, *Laodelphax striatellus* (Fallén, 1826) and *Nilaparvata lugens* (Stål, 1854), to a near zero magnetic field (NZMF) decreased adult weights, female fecundity, and delays in the development of eggs and nymphs. The transcription rates of vitellogenin in newly moulted females and in the number of eggs per female were also significantly reduced in both species (Wan *et al.*, 2014).

## SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Figure. Sampling sites.

S2 Figure. Electromagnet used to generate the static magnetic field in the experiments.

## ACKNOWLEDGMENTS

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