The Company of Biologists

RESEARCH ARTICLE

The magnetic orientation of the Antarctic amphipod *Gondogeneia antarctica* is cancelled by very weak radiofrequency fields

K. Tomanova and M. Vacha*

ABSTRACT

Studies on weak man-made radiofrequency (RF) electromagnetic fields affecting animal magnetoreception aim for a better understanding of the reception mechanism and also point to a new phenomenon having possible consequences in ecology and environmental protection. RF impacts on magnetic compasses have recently been demonstrated in migratory birds and other vertebrates. We set out to investigate the effect of RF on the magnetic orientation of the Antarctic krill species *Gondogeneia antarctica*, a small marine crustacean widespread along the Antarctic littoral line. Here, we show that upon release, *G. antarctica* (held under laboratory conditions) escaped in the magnetically seaward direction along the magnetic sea–land axis (*y*-axis) of the home beach. However, the animals were disoriented after being exposed to RF. Orientation was lost not only in an RF field with a magnetic flux density of 20 nT, as expected according to the literature, but even under the 2 nT originally intended as a control. Our results extend recent findings of the extraordinary sensitivity of animal magnetoreception to weak RF fields in marine invertebrates.

KEY WORDS: Amphipoda, Magnetoreception, Radical pair mechanism, Larmor frequency, Narrow-band magnetic field

INTRODUCTION

Along with ongoing research into animal orientation according to the magnetic field of the earth (MFE), a remarkable phenomenon has appeared suggesting that orientation could be disturbed or biased by very weak electromagnetic radiofrequency (RF) waves. For decades, the biological effect of such electromagnetic anthropogenic smog, mostly associated with urban areas, has been overlooked, particularly in view of its low flux density and unknown mechanism of biophysical action. However, a growing number of studies have indicated a loss of animal magnetoreception after exposure to RF emission. Although the behavioural phenomenon of magnetoreception loss or modification under weak RF has recently been documented by a number of laboratories (Malkemper et al., 2015; Landler et al., 2015; Engels et al., 2014; Kavokin et al., 2014; Ritz et al., 2009), its clear biophysical interpretation is not easy to provide (Lee et al., 2014; Engels et al., 2014). Notably, RF impacts animals even under intensities of its magnetic component a thousand times weaker than the natural MFE (approximately 50 µT). Such sensitivity was reported by Engels et al. (2014), whose migratory robins lost orientation under the anthropogenic wide-spectrum electromagnetic noise with a magnetic flux density of less than 10 nT associated with their university campus as an omnipresent background. Bird

magnetosensitivity and correct orientation was restored only in an electromagnetically shielded chamber. The disruptive effect of RF on the magnetic orientation of animals therefore represents the only known case of biological sensitivity to RF electromagnetic fields three orders below thermal effects and exposure doses recommended for human health protection (International Commission on Non-Ionizing Radiation Protection, 2009).

Migratory birds have been repeatedly shown to lose magnetic orientation under weak RF (Ritz et al., 2004; Thalau et al., 2005; Wiltschko et al., 2007, 2015; Engels et al., 2014; Kavokin et al., 2014). The magnetoreception of insects and some mammals has also been shown to be sensitive to RF bias (Vacha et al., 2009; Malkemper et al., 2015, respectively), while mole-rats (Thalau et al., 2006) did not react to an RF field. The opportunity to investigate amphipod magnetic orientation in an Antarctic ecosystem devoid of electromagnetic noise sources led us to two main questions: (1) is there any disruptive effect of RF fields on the magnetosensitivity of marine isopods; and if so, (2) what is the RF field flux density threshold?

Tiny marine crustaceans living in littoral zones all over the world are the most abundant part of the zooplankton. Antarctic krill – the general name for small crustaceans in the south polar zone – is the main food source for fish, penguins, seals and whales. Reliable spatial orientation in a rapidly changing microenvironment is crucial for crustaceans living along the littoral line. The sea–land axis (*y*-axis; discussed below) plays the key role in orientation in rapidly changing flood and drain streams. The MFE provides a number of marine and freshwater animals living on the edge of land and water, such as crustaceans [Ugolini and Ciofini, 2016; terHorst, 2012; Ugolini and Pezzani, 1995; S. C. Rothsey, The effect of the earth's magnetic field and other orientation cues on direction seeking in four crustaceans (three intertidal – Amphipoda and Isopoda, one freshwater – Decapoda), University of New England, 2006], frog tadpoles (Diego-Rasilla et al., 2013) and newts (Deutschlander et al., 2000), with a stable orientation cue helping them to find and keep a shoreward or waterward *y*-axis bearing.

The goal of our work at the Czech scientific polar station (Johann Gregor Mendel Station, James Ross Island, Antarctica) in the spring of 2013 was to: (1) verify the spontaneous magnetoreception-based *y*-axis orientation of *G. antarctica*, (2) determine whether spontaneous orientation is influenced by RF, and, if so, (3) compare the flux density threshold of Larmor frequency (LF; see Discussion) with thresholds of different frequencies. The last of these goals was not achieved because of the limited duration of the polar season. However, animal sensitivity to extremely weak narrow-band RF levels was found.

MATERIALS AND METHODS
Animals
For testing, we used adult individuals of *Gondogeneia antarctica* (Chevreux 1906) (Calliopioidea superfamily, Amphipoda), the

Department of Animal Physiology and Immunology, Faculty of Science, Masaryk University, Brno, Kamenice 735/5, Brno 625 00, Czech Republic.

*Author for correspondence (vacha@sci.muni.cz)

> **List of abbreviations**
> CCW   counterclockwise
> CW   clockwise
> LF   Larmor frequency
> MFE   magnetic field of the earth
> RF   radiofrequency
> RP   radical pair

most abundant amphipod of the open seawater along the coast of Antarctica. Animals were caught with an aquarist net in the shallow water on the beach of Cape Lachman (the northern part of James Ross Island, Antarctica; 63°79′S, 57°80′W). The land–sea shoreline axis (*y*-axis) of this location creates an angle of between 30 and 80 deg with the north–south magnetic axis (magnetic azimuth of the beach). The spontaneous escape orientation was, for this reason, expected within this span (Fig. S1).

Animals were transported in PET bottles to the Johann Gregor Mendel station (transport time was no longer than 2 h; Fig. S1) and transferred to a covered tank with airflow and seawater from the collection site. All individuals regardless of sex were tested in a darkened room (Fig. S2) after up to 5 days in captivity.

Testing device, and magnetic and RF field settings
Initially, animals were tested with the generator of an RF field (BK precision 4085) switched off under the natural MFE and under a field with the horizontal component rotated 60 deg clockwise (CW) and 60 deg counterclockwise (CCW). The testing device consisted of a single Helmholtz coil to rotate the local MFE (48 cm diameter, 2×25 wraps), a glass bowl (25 cm in diameter) containing approximately 2 cm of seawater, a white cloth tunnel (45 cm high) around the bowl to shield visual orientation cues and distractions, a tripod with a source of white light (40 W light bulb) and a camera above the cloth shielding (Fig. S2). The horizontal magnetic field vector generated by the coil enabled us to change the local magnetic vector so that the experimental magnetic north rotated by 60 deg CW, 60 deg CCW and 180 deg could be set without changing the total intensity or inclination. The natural vector in the testing location had an inclination of 53 deg and a total flux density of 35 µT (measured by an HMR 2300 magnetometer, Honeywell). The Helmholtz coil was supplied by an electric power supply 2 m away (RMG 1501, MC POWER).

The 60 deg CW and 60 deg CCW rotated angles were chosen because: (1) to ensure that the total flux density is constant, the single Helmholtz coil made it possible to set only four conditions: natural MFE, 60 deg CW and 60 deg CCW rotated angles, plus reversed vector 180 deg; and (2) if the reversed vector 180 deg was used as a reference to natural MFE, no behavioural change would appear if the orientation turned out to be bimodal (axial). Because unimodal magnetic orientation had been found in the first set of experiments, the MFE reversed by 180 deg was used in the second set of experiments.

A second set of tests was performed with running generator of RF field under the natural MFE and under a field rotated by 180 deg. An antenna consisting of a single coaxial cable loop (diameter of loop 83 cm) with the 2 cm shielding cover removed in the centre was placed permanently to test the effect of RF. The loop was mounted horizontally around the Helmholtz coil, forming an angle of 37 deg between the vertical axis of the loop and the local geomagnetic vector. The glass bowl with the test animals was placed in the centre of the loop approximately 3 m from the RF generator.

No background RF fields were measurable within the range 0.9–30 MHz in the laboratory (electric and magnetic components were measured separately in the centre of the bowl by an EHP200A Electric and Magnetic Field Analyser, Narda; magnetic field sensitivity: 0.1 nT; electric field sensitivity: 0.02 V m^{-1}). To determine any possible changes of the RF signal running through the seawater (conductive), the RF was set to 20 nT, 1 MHz. Then, we submerged the sensor under the water so that it was surrounded with a 3 cm water layer and we measured RF in: (1) deionized water, (2) water containing 34.5 ppt sea salt (ocean salinity) and (3) double salinity water (69 ppt). There were no measurable differences among these conditions, showing that damping effects of water were irrelevant in our test.

When the disrupting effect of RF was tested, a 20 nT field flux density was applied as an experimental treatment (electric field intensity of 0.14 V m^{-1}), as this value exceeds threshold levels for LF found previously in insects (Vacha et al., 2009) and birds (Wiltschko et al., 2015). A magnetic flux density of 2 nT was used at first as a control (the electric field intensity was immeasurable). Why was 2 nT used in particular? We applied measures according to Kirschvink et al. (2010) so that the RF generator was operating at the same power level during all experiments. Experiments with the RF generator feeding the antenna and a gap in the shielding cover of the coaxial cable shortened by a switcher were meant as a control set-up. Under such wiring, the antenna should have emitted irrelevant control RF intensities. However, it turned out that even with a gap in the antenna, a shortened RF field of 2 nT was still present and behavioural reactions appeared. Therefore, to exclude the effect of RF completely, a third set of tests – final control experiment – was performed without a running RF generator.

Variants of experiments (switching 20 nT/2 nT RF field) were altered blind and randomly by an operator not informed of the experimental protocol.

The light conditions in the glass bowl were homogeneous from 2.0 W m^{-2} in the centre of the bowl to 1.7 W m^{-2} along the edge of the bowl (the intensity of light was measured by an SKE 510 energy sensor; Skye Instruments, Llandrindod Wells, UK).

Test procedure and evaluation
When testing spontaneous orientation, each animal was taken gently from a storage bottle containing approximately 10 animals and placed on the inner wall of a small glass funnel (diameter 3 cm) with a fine brush. The funnel was then dipped into the seawater in the centre of the glass bowl so that its wide part with the animal was submerged. The cloth tunnel was drawn down to cover the bowl and the animal was left for 1 min in the dark to acclimatise. The light was then switched on and at the same time the glass funnel was lifted by a rope and the animal was released into the entire space of the bowl water. The animal's motion was recorded by a camera connected to a permanently running PC. All video records are available online at https://is.muni.cz/www/1376/62242471/Tomanova_Vacha_Videos.zip. The escape direction of each animal was later scored as the angle between the magnetic north–south axis and the line between the centre of the bowl and the point at which the animal touched the edge first by means of Screen Protractor software (Iconico.com). Only animals that reached the edge of the bowl within 1 min were evaluated. The individuals were released separately; each individual was tested only once. Animals were tested from 25 January to 11 February 2013 between 09:00 and 20:00 h. The initial preliminary evaluations necessary for timing subsequent steps of the plan schedule were performed at the Johann Gregor Mendel base; final scoring was performed fully

blind by a non-participating person after our return to the Czech Republic. The animals' escape directions were evaluated by standard circular statistics: the Rayleigh test and the Mardia–Watson–Wheeler test of homogeneity (Oriana 4.02, Kovach Computing Services, Anglesey, UK).

RESULTS

At first, we tested whether there is any spontaneous orientation of the Antarctic amphipod *G. antarctica* and whether the main bearing of escape movement from the centre of a round dish correlates with the sea–land direction (*y*-axis) of the beach. This direction was hypothesised according to data from the literature (Ugolini and Pezzani, 1995; Rothsey, 2006) as a crucial orientation bearing helping marine crustaceans to avoid becoming stranded in basins of shallow water during the draining phase. Under the natural MFE, the animals displayed significant spontaneous orientation with magnetic azimuth μ=47 deg (*n*=81, *r*=0.239, *P*=0.010; Fig. 1A).

The hypothesis that animals use exclusively magnetic cues was tested by rotation of the horizontal magnetic field vector by 60 deg CW and 60 deg CCW. After rotation, the average escape orientation of *G. antarctica* was rotated accordingly, both in the 60 deg CW shift (μ=134 deg, *n*=43, *r*=0.243, *P*=0.078; Fig. 1B) and in the 60 deg CCW shift (μ=332 deg, *n*=61, *r*=0.217, *P*=0.056; Fig. 1C). Both reactions are close to the threshold for statistical significance. However, if all samples from the three described rotations of magnetic axis (0 deg, 60 deg CW and 60 deg CCW, plus 180 deg; see below) are pooled together and plotted with respect to magnetic north, a very clear and significant escape direction magnetically seaward appeared (μ=61 deg, *n*=221, *r*=0.237, *P*<0.001; Fig. 1E). The average escape bearing in the laboratory tests therefore matches the magnetic *y*-axis towards the sea of the Cape Lachman beach very well (magnetic azimuth 55±25 deg; Fig. S1). In addition, 95% confidence intervals in all magnetic directions overlap with seaward

direction spans (Fig. 1). As a control of possible non-magnetic orientation bias from the laboratory, identical samples were pooled topographically, giving no significant orientation (μ=15 deg, *n*=221, *r*=0.105, *P*=0.087; Fig. 1F). A test of homogeneity (Mardia–Watson–Wheeler test) confirmed differences between both pooled groups (*n*=221, *W*=7.065, *P*=0.029; Table 1).

Based on these results, we concluded that *G. antarctica* uses magnetic cues for escape orientation, which is not just axial (along both ends of the *y*-axis without polarity differentiation), as the animals also determine the polarity of the magnetic *y*-axis (unimodal reaction). Because we were limited by the climatic conditions of the short Antarctic summer and the continuous sea ice cover of the littoral areas, we could not collect animals from sites with a differently oriented *y*-axis. Nevertheless, the magnetic orientation of littoral amphipods along the *y*-axes from variously oriented beaches has already been reported by Rothsey (2006) and Ugolini and Pezzani (1995), and testing the *y*-axis phenomenon was not our primary goal.

Sensitivity to weak RF was tested following the verification of magnetosensitivity in the primary sets of experiments. For James Ross Island, the LF (see Discussion) is 969 kHz, for which reason we tested this frequency first. Two flux densities were tested: 20 nT (see Materials and methods) and 2 nT. The loss of magnetic orientation during exposure to LF 969 kHz, 20 nT, was hypothesised, whereas exposure to a 2 nT field was expected to result in normal magnetic orientation along the magnetic *y*-axis. Our first expectation was confirmed: the animals were disoriented when tested under 969 kHz and 20 nT RF in non-rotated MFE (μ=353 deg, *n*=72, *r*=0.141, *P*=0.238; Fig. 2A). Nevertheless, the animals' escape directions were scattered randomly even under 969 kHz and 2 nT (μ=207 deg, *n*=70, *r*=0.061, *P*=0.769; Fig. 2B), conditions meant as a positive control. Persistent loss of magnetosensitivity even in a very weak 2 nT RF field was surprising and led us to change the RF frequency so that it was

Table 1. Overview of statistical evaluations

Condition	*n*	Mean vector μ (deg)	95% confidence interval for μ (deg)	Vector length **r**	Rayleigh *P*	Mardia–Watson–Wheeler *P*
Natural magnetic field, no RF	81	47	10.7–83.4	0.239	0.010	
Rotated magnetic field (60 deg CW), no RF	43	134	85.5–183.5	0.243	0.078	
Rotated magnetic field (60 deg CCW), no RF	61	332	285.4–17.9	0.217	0.056	
Rotated magnetic field (180 deg), no RF	36	297	253.9–341.0	0.297	0.041	
Summation to magnetic north, no RF	221	61	38.9–83.3	0.237	≤0.001	0.029
Summation to topographic north, no RF	221	15		0.105	0.087	
Natural magnetic field, 969 kHz, 20 nT	72	353		0.141	0.238	
Natural magnetic field, 969 kHz, 2 nT	70	207		0.061	0.769	0.009
Natural magnetic field, 10 MHz, 2 nT	51	182		0.121	0.473	
Rotated magnetic field (180 deg), 10 MHz, 2 nT	68	251		0.129	0.324	
Summation to magnetic north, 2 nT	189	139		0.099	0.632	
Summation to topographic north, all RFs	261	256		0.046	0.571	

Conditions correspond with those given in Figs 1 and 2. Note the significant difference between summations to magnetic north differing only by 2 nT radiofrequency (RF) field application. The grey area highlights experiments with RF field applications.
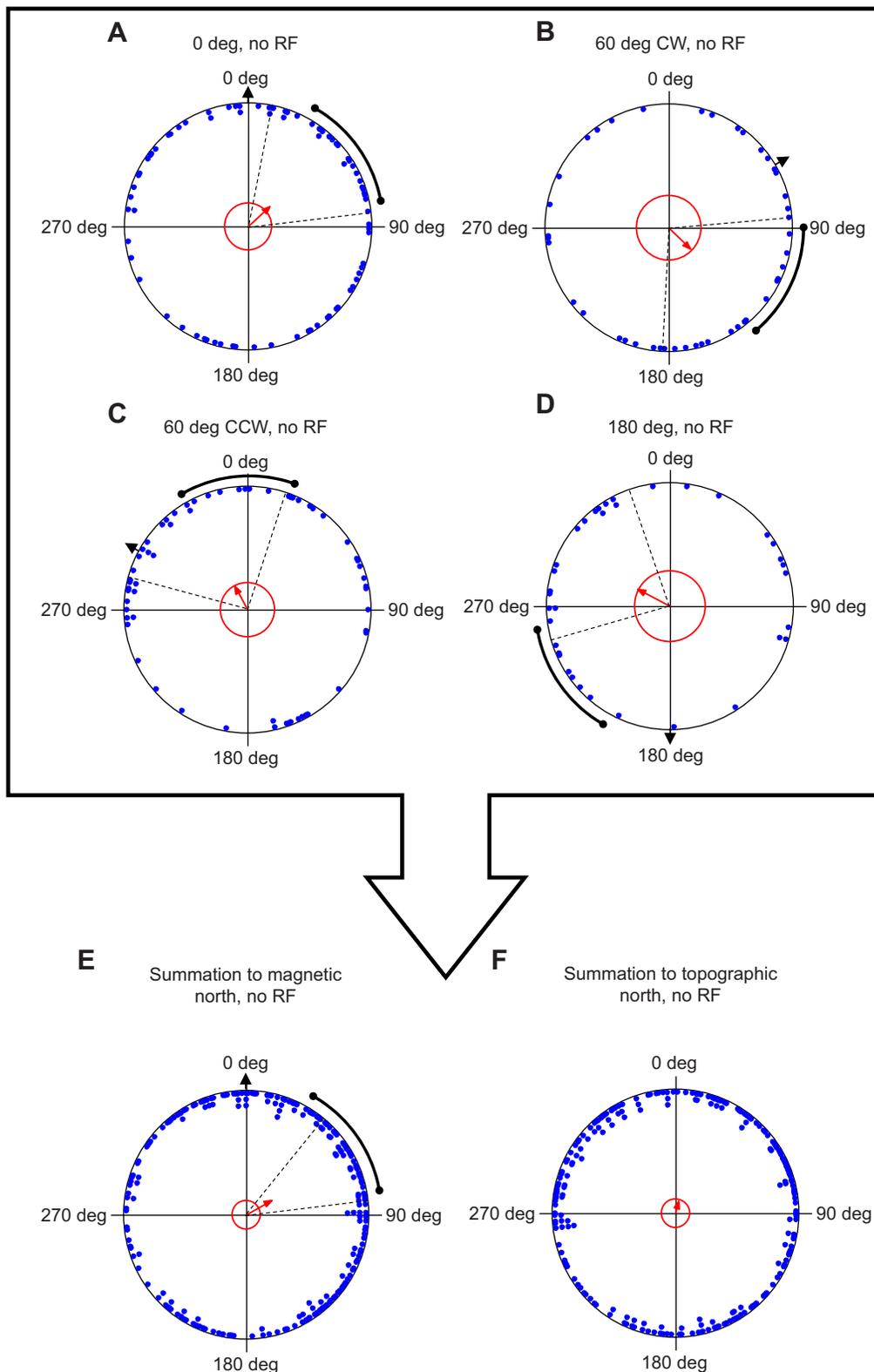
Fig. 1. Spontaneous orientation of *Gondogeneia antarctica* under different positions of geomagnetic north with no radiofrequency interference.
(A) Orientation in natural magnetic field (μ=47 deg, n=81, r=0.239, P=0.010), (B) magnetic north rotated 60 deg CW (μ=134 deg, n=43, r=0.243, P=0.078), (C) magnetic north rotated 60 deg CCW (μ=332 deg, n=61, r=0.217, P=0.056), (D) magnetic north rotated 180 deg (μ=297 deg, n=36, r=0.330, P=0.019), (E) summation to magnetic north (μ=61 deg, n=221, r=0.237, $P\leq0.001$) and (F) summation to topographic north (μ=15 deg, n=221, r=0.105, P=0.087). In the diagrams, each point represents the escape direction of one individual. The inner red circles mark the 5% significance border of the Rayleigh test. The lengths of the arrows are proportional to the mean vector **r**. The arrows exceeding the circles indicate significant directional orientation. The dashed lines within the major circle indicate 95% confidence intervals. The black arrow outside the circle depicts the magnetic north position. The black line outside the circle marks span of seaward directions.

different from LF: if high sensitivity to the disturbing effect of RF is caused by specific resonance effects, then the change to a frequency different from LF should restore orientation under a weak 2 nT RF (Ritz et al., 2009). Nevertheless, the orientation of *G. antarctica* was random even under an RF 10-fold higher than LF, 10 MHz and a weak flux density of 2 nT (μ=182 deg, n=51, r=0.121, P=0.473; Fig. 2C). Correct orientation was also not observed in experiments with an RF of 10 MHz, 2 nT in non-rotated natural MFE (Fig. 2C) or in experiments with the same RF settings but with MFE reversed by 180 deg (μ=250 deg, n=68, r=0.129, P=0.324; Fig. 2D). However, spontaneous orientation of *G. antarctica* was restored in the subsequent final control, in 180 deg reversed MFE with the RF generator turned off – as it was in the first set of experiments (μ=297 deg, n=36, r=0.330, P=0.019; Fig. 1D). The disrupting effect of a mere 2 nT RF, regardless of frequency, was seen when all three tests under 2 nT RF were pooled together and plotted with respect to magnetic north: no orientation appeared (μ=139 deg, n=189, r=0.099, P=0.632; Fig. 2E). A test of homogeneity (Mardia–Watson–Wheeler test) confirmed a significant difference between groups pooled to magnetic north and tested with and without 2 nT RF (n=221/189, W=9.352, P=0.009; Table 1). As in the first set of experiments, as a control of possible non-magnetic orientation bias from the laboratory, all four RF samples were pooled topographically, giving no significant orientation (μ=256 deg, n=261, r=0.046, P=0.571; Fig. 2F).

DISCUSSION

Taken as a whole, our results show that the Antarctic amphipod *G. antarctica* uses a magnetic compass for orientation. If all the samples from the four different rotations of magnetic axis (Fig. 1A–D) are plotted with respect to magnetic north, a clear escape in the seaward direction appears (Fig. 1E). This escape bearing corresponded with the magnetic position of the beach from which the animals were collected (Fig. S1). Correct orientation in the sea–land axis (*y*-axis) has been reported as being crucial for small animals living in the littoral zone. Supratidal crustaceans dwelling in the seagrass wrack accumulated on beaches above the water line prefer to escape in the shoreward direction (Rothsey, 2006; terHorst, 2012; Ugolini and Pezzani, 1995), but this motivation may be reversed by environmental factors; for example, beach hoppers (*Orchestia* sp.) move towards the shore in wet environments and towards the water in dry environments (terHorst, 2012; Rothsey, 2006).

Our animals, which occupy open seawater, were expected to prefer an escape direction towards the sea, and this hypothesis of seaward movement motivation was confirmed. As to the biological meaning of seaward escape, we had assumed that animals trapped at low tide in shallow pools on rocky beaches without being able to find their way back to the open ocean were exposed to a high risk of predation, overheating or desiccation. Despite a rather limited swimming efficiency in water currents, movement toward the sea could be a universal escape reaction of the animal in danger. As to accessible orientation cues, amphipods occupying sandy beaches are known to orient themselves along the *y*-axis with the aid of visual markers, as well as a complex of environmental stimuli dependent on time, slope, magnetism (Arendse, 1978), humidity, etc. (reviewed in terHorst, 2012; Scapini, 2006). However, orientation according to visual or other clues seems to be rather difficult in Antarctic waters because of strong winds and rough seas. Magnetosensitivity could make orientation in this turbid environment much more reliable, and MFE is one of the very few stable guideposts for small aquatic animals.

The phenomenon of RF interfering with the magnetic compass orientation of animals is remarkable not only for its possible impact on organisms living and migrating near systems emitting RF, but also for its diagnostic evidence in the search for the biophysical basis of the magnetoreception mechanism (Henbest et al., 2004). Indeed, this was the primary goal of the first experiments with RF and magnetic orientation (Ritz et al., 2004). Despite 60 years of research, the principles of magnetoreception – the unique ability to use omnipresent MFE clues for orientation and/or navigation – are still not fully understood. There are two plausible mechanisms of terrestrial magnetoreception. In salmon and other vertebrates such as subterranean mole-rats (Thalau et al., 2006) and bats (Holland et al., 2008), magnetoreception is probably based on the presence of magnetic particles of iron oxides in their tissues (Eder et al., 2012, but see also Edelman et al., 2015). Other animals, including invertebrates, seem to possess a different system for magnetic field detection: according to radical-pair (RP) theory, MFE affects the dynamic of biochemical reactions of molecules forming transient RPs. Cryptochromes found in the retina of birds (reviewed in Liedvogel and Mouritsen, 2010) and *Drosophila* (Mazzotta et al., 2013) have been suggested to create RP with the cofactor Flavin and are the most widely accepted candidates for the molecules sensitive to MFE direction (e.g. reviewed in Dodson et al., 2013). A combination of both mechanisms is expected in birds (e.g. Wiltschko and Wiltschko, 2007). The principle of the disruptive effect of RF on magnetosensitivity was originally suggested as a selective diagnostic tool separating RF-sensitive RP photochemical processes from RF-insensitive iron particle mechanotransduction processes (Ritz et al., 2004). However, the current situation is complicated by the fact that the disrupting effect of extremely weak RF on the RP reaction to a static MFE some 3000 times stronger seems to be problematic even within the RP paradigm, and is not generally accepted by biophysicists (Hogben et al., 2009). Similarly, the thermal or other reactions of iron particles are not fully clear, especially in broadband RFs (Engels et al., 2014).

There are, however, specific resonance effects bound to the interaction between RF and unpaired electrons of an RP. If RF shows a peak of its effect at the LF, such a finding would be much more compatible with an RP mechanism than reception mediated by iron oxide particles (Ritz et al., 2004). In theory, spinning electron (s) of the RP interact with an external magnetic field in a frequency-dependent manner that corresponds to the LF (Ritz et al., 2009). Provided that one or both radicals are free of hyperfine magnetic interactions with nuclei and the earth's field vector is the only relevant local field, the LF falls into frequencies of radio waves close to 1 MHz (Ritz, 2011). If the RF of this frequency is applied in a non-parallel way to the earth's field, it causes resonance effects on spinning electrons interfering with their singlet–triplet interconversion, and possibly hindering sensitivity to the direction of the MFE (Ritz et al., 2009). The condition of no hyperfine interaction points to radical species involving oxygen as likely magnetoreceptors sensitive to LF, though some other key prerequisites are considered to be questionable (Hogben et al., 2009; Mouritsen and Hore, 2012; Lee et al., 2014).

What is the behavioural evidence for LF-mediated effects? While experiments on birds (Ritz et al., 2009) and insects (Vacha et al., 2009) have shown the dominant impact of LF in line with RP-specific resonance bias, no exceptional effects of LF on magnetoreception were reported by Engels et al. (2014) on the European robin under broadband RFs or by Malkemper et al. (2015) on wood mice. Such heterogeneity may still be understandable because of the limited number of studies and the
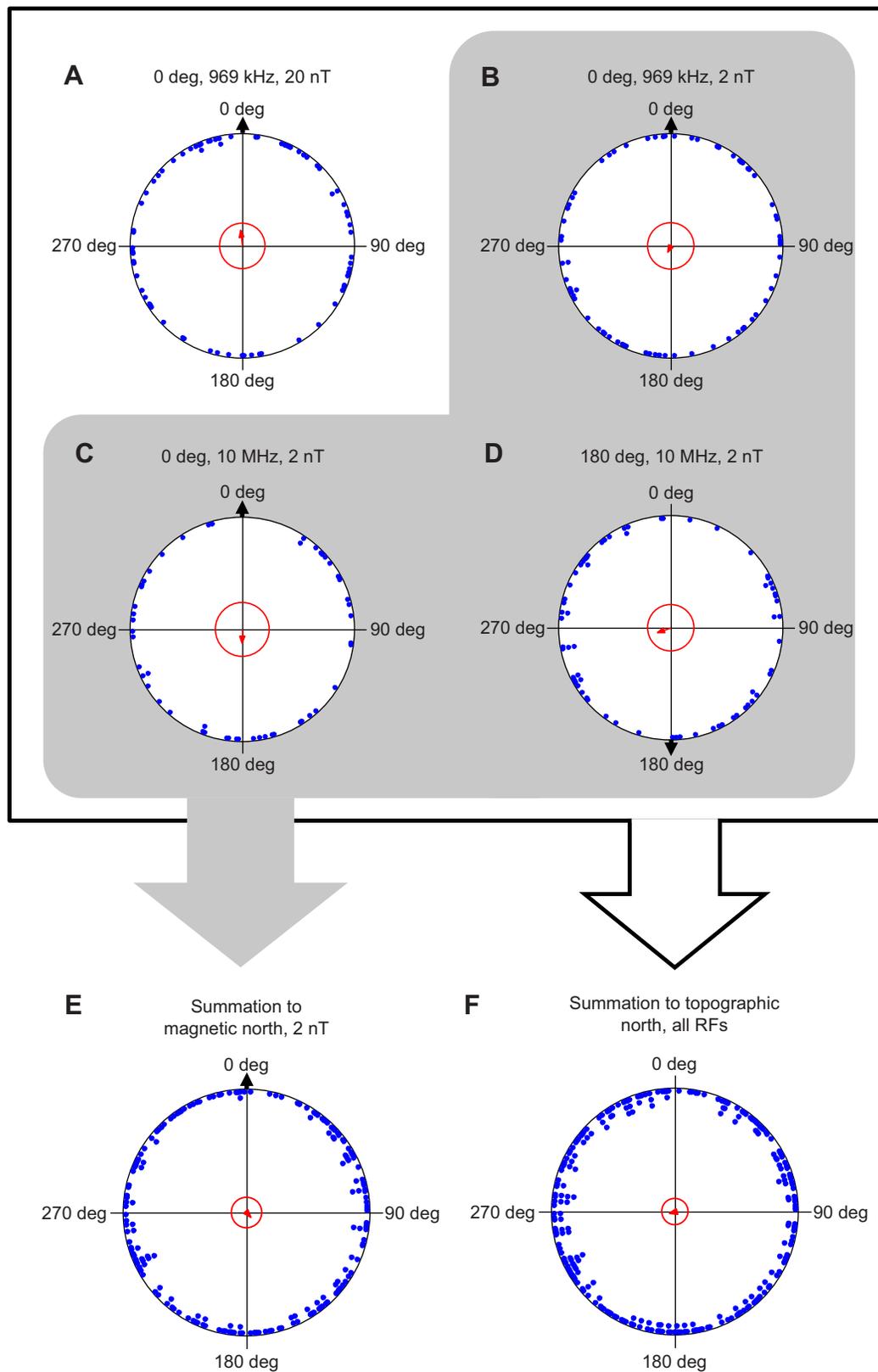
Fig. 2. Spontaneous orientation of *G. antarctica* under different positions of geomagnetic north and radiofrequency (RF) field interference.
(A) Orientation in natural magnetic field, RF 969 kHz, 20 nT (μ=353 deg, n=72, r=0.141, P=0.238), (B) natural magnetic field, RF 969 kHz, 2 nT (μ=207 deg, n=70, r=0.061, P=0.769), (C) natural magnetic field, RF 10 MHz, 2 nT (μ=182 deg, n=51, r=0.121, P=0.473), (D) magnetic north rotated 180 deg, RF 10 MHz, 2 nT (μ=250 deg, n=68, r=0.129, P=0.324), (E) summation to magnetic north, RF 2 nT only (μ=139 deg, n=189, r=0.049, P=0.632) and (F) summation to topographic north, all RFs (μ=256 deg, n=261, r=0.046, P=0.571). The grey area covers experiments with 2 nT RF regardless of frequency. Symbols and descriptions as for Fig. 1.

variety of protocols from multidisciplinary fields combining demanding biological and physical approaches. Deciphering the problem of interactions of weak RF fields and living organisms is, therefore, an important task of frontier research on the boundary between biology and physics.

The results of our experiments suggest the remarkable sensitivity of the magnetic orientation of the Antarctic amphipod *G. antarctica* to RF. Spontaneous escape bearings became random not only after exposure to an RF of 20 nT, 969 kHz (LF) (Fig. 2A), but also under RF 2 nT both at 969 kHz (Fig. 2B) and at a frequency 10 times higher (10 MHz; Fig. 2C,D). Even 2 nT RF, regardless of frequency, was able to disrupt orientation (Fig. 2E). Orientation was lost whenever the set consisting of a signal generator connected to an antenna loop generated an RF. However, biasing factors other than RF potentially caused by feeding the set such as vibrations or ultrasounds are not likely. Measurement of ultrasounds produced by running the generator–antenna system (bat detector Pettersson D980) at a distance of 2 m from the generator gave no signal. To check the possibility that loss of orientation was due to vibrations spread from running the RF generator, we performed additional vibration measurements under conditions resembling as much as possible those at Johann Gregor Mendel station. Measurements by a specialised detector (Seismic Accelerometer, Wilcoxon Research, Model 731A, Wilcoxon, Power unit Amplifier Model P-31, Audacity 1.3 software) showed that differences between generator on/off states were very apparent when the probe was on the table in the direct proximity of the generator (Fig. S3A), as well as under the table on the chipboard floor (Fig. S3B). However, the record curves did not differ when the probe and generator were separated by a distance of 1 m or more (Fig. S3C–E), making the likelihood of vibrations from the RF generator reaching the 3 m distant testing device highly unlikely.

In addition, the animals showed no orientation towards or away from any cue in the laboratory with the exception of the magnetic field (Fig. 2F), which provides evidence of no directional bias. RFs remain, therefore, the most likely factor explaining the loss of magnetic orientation.

Our results confirm the extraordinary sensitivity of animal magnetoreception to electromagnetic fields from a radio wave spectrum of tens of nanoTesla flux densities previously reported in research into the animal magnetic compass (Landler et al., 2015; Engels et al., 2014; Ritz et al., 2009). Our work extends recent findings on vertebrate compass orientation for marine invertebrates living abundantly in polar seas. Should sensitivity to man-made RF be widespread across the animal kingdom, it may point to a new phenomenon with possible important ecological consequences. Animal directional magnetoreception of MFE seems to be the only known biological phenomenon responsive to electromagnetic fields of nanoTesla flux densities and a megahertz spectrum. A magnetic compass need not, however, be the only biological process receptive to extremely weak oscillating fields, as the report of nociception in mice by Prato et al. (2013) shows. Such findings affirm, *inter alia*, the importance of studying animal magnetoreception and perhaps other selected physiological events in environments isolated from man-made electromagnetic noise, whether in shielded laboratories or far from sources of anthropogenic noise.

There is currently no generally accepted biophysical model covering biologically relevant processes underlying such extreme sensitivity to RF, and the nature of the interference remains unresolved (Lee et al., 2014; Engels et al., 2014; Wiltschko et al., 2015). Our present work cannot contribute to the discussion on the validity of the LF resonance effect on RP magnetoreception

because we did not reach a flux density threshold for any frequency. Magnetoreception was lost under all intensities and frequencies of RF used. Similarly, to discuss the possible impacts of the electrical and magnetic component of RF separately, we would have had to screen out or to produce only one or the other component. Unfortunately, such experiments could not be carried out within the limited time frame of the Antarctic season and the limited space of the polar base facility. We believe, however, that our report of extraordinary sensitivity to RF may inspire practical replications as well as new theoretical biophysical and biological insights.

Data availability
All video records are available online at https://is.muni.cz/www/1376/62242471/Tomanova_Vacha_Videos.zip

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.132878/-/DC1

References
Arendse, M. C. (1978). Magnetic field detection is distinct from light detection in the invertebrates *Tenebrio* and *Talitrus*. *Nature* **274**, 358-362.
Deutschlander, M. E., Phillips, J. B. and Borland, S. C. (2000). Magnetic compass orientation in the eastern red-spotted newt, *Notophthalmus viridescens*: rapid acquisition of the shoreward axis. *Copeia* **2000**, 413-419.
Diego-Rasilla, F. J., Luengo, R. M. and Phillips, J. B. (2013). Use of a light-dependent magnetic compass for y-axis orientation in European common frog (*Rana temporaria*) tadpoles. *J. Comp. Physiol. A* **199**, 619-628.
Dodson, C. A., Hore, P. J. and Wallace, M. I. (2013). A radical sense of direction: signalling and mechanism in cryptochrome magnetoreception. *Trends Biochem. Sci.* **38**, 435-446.
Edelman, N. B., Fritz, T., Nimpf, S., Pichler, P., Lauwers, M., Hickman, R. W., Papadaki-Anastasopoulou, A., Ushakova, L., Heuser, T., Resch, G. P., Saunders, M., Shaw, J. A. and Keays, D. A. (2015). No evidence for intracellular magnetite in putative vertebrate magnetoreceptors identified by magnetic screening. *Proc. Natl. Acad. Sci. USA* **112**, 262-267.
Eder, S. H. K., Cadiou, H., Muhamad, A., McNaughton, P. A., Kirschvink, J. L. and Winklhofer, M. (2012). Magnetic characterization of isolated candidate vertebrate magnetoreceptor cells. *Proc. Natl. Acad. Sci. USA* **109**, 12022-12027.
Engels, S., Schneider, N.-L., Lefeldt, N., Hein, C. M., Zapka, M., Michalik, A., Elbers, D., Kittel, A., Hore, P. J. and Mouritsen, H. (2014). Anthropogenic electromagnetic noise disrupts magnetic compass orientation in a migratory bird. *Nature* **509**, 353-356.
Henbest, K. B., Kukura, P., Rodgers, C. T., Hore, P. J. and Timmel, C. R. (2004). Radio frequency magnetic field effects on a radical recombination reaction: a diagnostic test for the radical pair mechanism. *J. Am. Chem. Soc.* **126**, 8102-8103.
Hogben, H. J., Efimova, O., Wagner-Rundell, N., Timmel, C. R. and Hore, P. J. (2009). Possible involvement of superoxide and dioxygen with cryptochrome in avian magnetoreception: origin of Zeeman resonances observed by in vivo EPR spectroscopy. *Chem. Phys. Lett.* **480**, 118-122.
Holland, R. A., Kirschvink, J. L., Doak, T. G. and Wikelski, M. (2008). Bats use magnetite to detect the earth's magnetic field. *PLoS ONE* **3**, e1676.

International Commission on Non-Ionizing Radiation Protection (2009). ICNIRP statement on the 'Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz)'. *Health Phys.* **97**, 257-258.

Kavokin, K., Chernetsov, N., Pakhomov, A., Bojarinova, J., Kobylkov, D. and Namozov, B. (2014). Magnetic orientation of garden warblers (*Sylvia borin*) under 1.4 MHz radiofrequency magnetic field. *J. R. Soc. Interface* **11**, 20140451.

Kirschvink, J. L., Winklhofer, M. and Walker, M. M. (2010). Biophysics of magnetic orientation: strengthening the interface between theory and experimental design. *J. R. Soc. Interface* **7** Suppl. 2, S179-S191.

Landler, L., Painter, M. S., Youmans, P. W., Hopkins, W. A. and Phillips, J. B. (2015). Spontaneous magnetic alignment by yearling snapping turtles: rapid association of radio frequency dependent pattern of magnetic input with novel surroundings. *PLoS ONE* **10**, e0124728.

Lee, A. A., Lau, J. C. S., Hogben, H. J., Biskup, T., Kattnig, D. R. and Hore, P. J. (2014). Alternative radical pairs for cryptochrome-based magnetoreception. *J. R. Soc. Interface* **11**, 20131063.

Liedvogel, M. and Mouritsen, H. (2010). Cryptochromes – a potential magnetoreceptor: what do we know and what do we want to know? *J. R. Soc. Interface* **7**, S147-S162.

Malkemper, E. P., Eder, S. H. K., Begall, S., Phillips, J. B., Winklhofer, M., Hart, V. and Burda, H. (2015). Magnetoreception in the wood mouse (*Apodemus sylvaticus*): influence of weak frequency-modulated radio frequency fields. *Sci. Rep.* **4**, 9917.

Mazzotta, G., Rossi, A., Leonardi, E., Mason, M., Bertolucci, C., Caccin, L., Spolaore, B., Martin, A. J. M., Schlichting, M., Grebler, R. et al. (2013). Fly cryptochrome and the visual system. *Proc. Natl. Acad. Sci. USA* **110**, 6163-6168.

Mouritsen, H. and Hore, P. J. (2012). The magnetic retina: light-dependent and trigeminal magnetoreception in migratory birds. *Curr. Opin. Neurobiol.* **22**, 343-352.

Prato, F. S., Desjardins-Holmes, D., Keenliside, L. D., DeMoor, J. M., Robertson, J. A. and Thomas, A. W. (2013). Magnetoreception in laboratory mice: sensitivity to extremely low-frequency fields exceeds 33 nT at 30 Hz. *J. R. Soc. Interface* **10**, 20121046.

Ritz, T. (2011). Quantum effects in biology: bird navigation. *Procedia Chem.* **3**, 262-275.

Ritz, T., Thalau, P., Phillips, J. B., Wiltschko, R. and Wiltschko, W. (2004). Resonance effects indicate a radical-pair mechanism for avian magnetic compass. *Nature* **429**, 177-180.

Ritz, T., Wiltschko, R., Hore, P. J., Rodgers, C. T., Stapput, K., Thalau, P., Timmel, C. R. and Wiltschko, W. (2009). Magnetic compass of birds is based on a molecule with optimal directional sensitivity. *Biophys J.* **96**, 3451-3457.

Rothsey, S. C. (2006). The Effect of the Earth's Magnetic Field and Other Orientation Cues on Direction Seeking in Four Crustaceans: three Intertidal - Amphipoda and Isopoda, One Freshwater – Decapoda, *University of New England theses*, University of New England, n. of pages: 256.

Scapini, F. (2006). Keynote papers on sandhopper orientation and navigation. *Mar. Freshw. Behav. Physiol.* **39**, 73-85.

terHorst, C. P. (2012). Context-dependent orientation cues in a supratidal amphipod. *Mar. Freshw. Behav. Physiol.* **45**, 45-50.

Thalau, P., Ritz, T., Stapput, K., Wiltschko, R. and Wiltschko, W. (2005). Magnetic compass orientation of migratory birds in the presence of a 1.315 MHz oscillating field. *Naturwissenschaften* **92**, 86-90.

Thalau, P., Ritz, T., Burda, H., Wegner, R. E. and Wiltschko, R. (2006). The magnetic compass mechanisms of birds and rodents are based on different physical principles. *J. R. Soc. Interface* **3**, 583-587.

Ugolini, A. and Ciofini, S. (2016). Landscape vision and zonal orientation in the equatorial sandhopper *Talorchestia martensii*. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **202**, 1-6.

Ugolini, A. and Pezzani, A. (1995). Magnetic compass and learning of the Y-axis (sea-land) direction in the marine isopod *Idotea baltica basteri*. *Anim. Behav.* **50**, 295-300.

Vacha, M., Puzova, T. and Kvicalova, M. (2009). Radio frequency magnetic fields disrupt magnetoreception in American cockroach. *J. Exp. Biol.* **212**, 3473-3477.

Wiltschko, W. and Wiltschko, R. (2007). Magnetoreception in birds: two receptors for two different tasks. *J. Ornithol.* **148**, 61-76.

Wiltschko, W., Freire, R., Munro, U., Ritz, T., Rogers, L., Thalau, P. and Wiltschko, R. (2007). The magnetic compass of domestic chickens, *Gallus gallus*. *J. Exp. Biol.* **210**, 2300-2310.

Wiltschko, R., Thalau, P., Gehring, D., Niessner, C., Ritz, T. and Wiltschko, W. (2015). Magnetoreception in birds: the effect of radio-frequency fields. *J. R. Soc. Interface* **12**, 20141103.