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Insights into the behavioural responses of juvenile thornback ray *Raja clavata* to alternating and direct current magnetic fields

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Abstract :

As part of energy transition, marine renewable energy devices (MRED) are currently expanding in developed countries inducing the deployment of dense networks of submarine power cables. Concern has thus raised about the cable magnetic emissions (direct or alternating current) because of potential interference with the sensorial environment of magneto-sensitive species, such as sharks and rays. This study sought to assess the short-term behavioural responses of juvenile thornback rays (*Raja clavata*) (n = 15) to direct and alternating (50 Hz) uniform 450-µT artificial magnetic fields using 1 h focal-sampling design based on a detailed ethogram. Careful control of magnetic fields' temporal and spatial scales was obtained in laboratory conditions through a custom-made Helmholtz coil device. Overall, qualitative or quantitative behavioural responses of juvenile rays did not significantly vary between control *vs*. exposed individuals over the morning period. Nonetheless, rays under direct current magnetic field increased their activity over the midday period. Synchronisation patterns were also observed for individuals receiving alternating current exposure (chronologic and qualitative similarities) coupled with a high inter-individual variance. Further studies should consider larger batches of juveniles to address the effect of long-term exposure and explore the sensitivity range of rays with dose-response designs.

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1 Introduction

Recent technological improvements and renewable energy goals are driving the growth of energy infrastructures in the ocean. Such infrastructures are essential to the offshore energy sector (oil, gas, wind, wave, and tidal energy) and global communication, and include platforms, submarine pipelines, and moorings, as well as power and telecommunication cables (Meißner *et al.*, 2006). In many countries, ocean energy exploitation is an attractive alternative to fossil fuels, which are major sources of atmospheric CO_2 (IEA, 2015). Therefore, an increasing number of offshore renewable energy devices (MREDs) is being planned in coastal areas (IRENA, 2019; Soares-Ramos *et al.*, 2020). Within this context, submarine power cables (SPCs) are extensively deployed to bring ashore the electricity produced by offshore wind farms and tidal/wave turbines, as well as to establish electrical interconnections between countries (Ardelean and Minnebo, 2015; Rte, 2019).

One of the major concerns related to the operation of SPCs is the introduction of artificial magnetic (expressed in teslas, T) and electric fields (expressed in volts per meter, V/m) into the marine environment (Otremba *et al.*, 2019; Taormina *et al.*, 2018). Such artificial sources may mask or alter the natural magnetic field (i.e. the Earth's magnetic field, also referred to as the geomagnetic field) and electric cues, thereby impacting ecological processes in magneto- and electro-sensitive species (e.g. teleost fish, mammals, crustaceans, molluscs, elasmobranchs; reviewed in Fischer and Slater, 2010). Therefore, vital functions and behaviours such as spawning or feeding migrations, homing, predation efficiency, and detection of sexual mates could be disrupted (Klimley *et al.*, 2016; Normandeau Associates, Inc. *et al.*, 2011; Ohman *et al.*, 2007).

Although modern cable insulation fully shields the electric fields, the magnetic fields radiate to the outside, and their potential interactions with marine life largely depend on the characteristics of SPCs (CSA Ocean Sciences Inc. and Exponent, 2019). The geomagnetic field is a direct current (DC) field, meaning that its polarity is constant over time and varies from 20 to 70 µT. Cables carrying DC also produce a constant magnetic field and can thus modify the ambient geomagnetic field *(Otremba et al.*, 2019). Cables can also transfer alternating current (AC) that changes direction 50 or 60 times per second

(50-60 Hz) and produce independent magnetic fields outside the cable (Kavet *et al.*, 2016). According to the law of electromagnetic induction, a moving object (e.g. a swimming organism or ocean current) in a conductive environment (e.g. seawater) with a DC or AC magnetic field can induce weak DC or AC electric fields, respectively (CSA Ocean Sciences Inc. and Exponent, 2019; Worzyk, 2009). The magnetic field strength (i.e. magnetic induction) depends on the number, twisting, and inter-conductor distance, but also on the power, voltage, and current intensity (Meißner *et al.*, 2006; Worzyk, 2009). Specifically, this field strength is linearly proportional to the current intensity and decreases with distance from the cable (Otremba *et al*., 2019). Based on simplified formulas that do not integrate the magnetic field compensation effect, the potential emission range of cables can be estimated. For example, at a similar current intensity (1000 A), AC or DC single-phase cables (15 cm diameter), three-phase AC cables, and bipolar DC cables (27 cm diameter, 12 cm space between conductors) that are buried at a 0.3-3 m depth (Albert *et al.*, 2020; Meißner *et al.*, 2006; Taormina *et al.*, 2018), produce magnetic fields of 670-70, 180-3,and 170-3 µT at the seabed, respectively [personal calculations based on simplified formulas from Salinas *et al.*, 2009; detailed in the Supplementary figure (1)].

Nevertheless, the rise in SPC number has not been accompanied by a commensurate research effort to evaluate the potential impacts of these infrastructures, even for species identified as vulnerable such as elasmobranchs (Dulvy *et al.*, 2014; Myers *et al.*, 2007). Globally, sharks and rays are under heavy fishing pressure, both directly and as by-catch, and 25% of their populations are currently in decline (Janse *et al.*, 2017; Molina and Cooke, 2012). These animals use an extremely sensitive electrosensory organ (the ampullae of Lorenzini) (Kalmijn, 1982; Tricas and Sisneros, 2004) with detection thresholds ranging between 20 $nV \cdot cm^{-1}$ and 100×10^3 $nV \cdot cm^{-1}$ to localise prey, predators, and conspecifics through low-frequency bioelectric field emissions (0–15 Hz). Fish, including elasmobranchs, also detect subtle variations in magnetic field components (i.e. polarity, inclination angle, and total intensity), which are assumed to assist large-scale and homeward orientation (Bonfil *et al.*, 2005; Kalmijn, 1978; Weng *et al.*, 2007). For example, the European eel (*Anguilla anguilla*), displayed orientation shifts in response to very subtle variations in the ambient field (e.g. a 2.4 µT intensity

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increase and a 2° inclination decrease) (Naisbett-Jones *et al.*, 2017). In addition to this orientation sense based on polarity (Kalmijn, 1978; Newton and Kajiura, 2020a), recent findings demonstrate that elasmobranchs possess a magnetic map sense (Keller *et al.*, 2021; Newton and Kajiura, 2020b). Current knowledge suggests that marine species may be responsive to extremely subtle magnetic field variations that are associated with power cables and occur within a limited spatial scale away from the cable sheath (Hutchison et al, 2021). However, elasmobranchs are also known to be repulsed by or attracted to deterrent devices associated with strong artificial magnetic fields and electrical voltages (O'Connell *et al.*, 2011). Nevertheless, given that the magnetic field strengths produced by submarine power cables can be far superior to those of the geomagnetic and AC type fields, movement ecology may not be the only aspect of fish behaviour altered by these infrastructures.

Currently, three empirical mechanisms have been proposed as the main drivers of magnetic orientation in fish: induction-based electroreception, magnetite-based magnetoreception, and radical pair mechanism. The first hypothesis states that when a fish is swimming, its electroreceptors detect self-generated bioelectric fields caused by its motion through the geomagnetic field (Nimpf et al, 2019). Constant heading is then achieved by detecting voltage variations relative to this bioelectric field background (Kalmijn, 1982, 1978; Molteno and Kennedy, 2009; Montgomery and Bodznick, 1999; Paulin, 1995). However, recent behavioural evidence supports a direct reception mechanism through magnetoreceptive cells containing ferromagnetite crystals (Fe_3O_4) that orient to the Earth's field (Anderson *et al.*, 2017; Hodson, 2000). Finally, the third hypothesis proposes that external magnetic fields are detected via the product of radical pair chemical reactions located within the visual system (Hore and Mouritsen, 2016).

Even though SPCs emit within the range of elasmobranch magnetosensitivity, the current knowledge on their potential interactions is mostly theoretical and there is not enough concrete evidence to conduct proper environmental impact assessments (Boehlert and Gill, 2010; Gill *et al.*, 2014; Normandeau Associates, INC. *et al.*, 2011). In the case of SPCs, some authors have hypothesised that repulsive effects could disrupt migration routes within the range of the cables' magnetic and electrical

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influence, whereas attraction would stimulate superfluous foraging behaviour, leading to poor success and unnecessary energy expenditure (Normandeau Associates, Inc. *et al.*, 2011; Wilson et al., 2010). In a field mesocosm study, Gill *et al*. (2009) tracked the individual movements of thornback rays (*Raja clavata*), spiny dogfish (*Squalus acanthias*), and small-spotted catsharks (*Scyliorhinus canicula*) in the presence of energised and non-energised SPCs. Rays increased their rate of movement during cable activation. Catsharks were found closer to the cable when it was switched on and their rate of movement increased once it was switched off. However, these responses were highly variable among individuals and were not repeated across different trials with the same individuals. In a similar experiment, the exploratory and foraging activity of little skate (*Leucoraja erinacea*) increased in the presence of a buried high voltage DC energised cable versus a cable-free enclosure (Hutchison *et al.*, 2020). Moreover, in laboratory studies, Orr (2016) demonstrated that the exploratory behaviour of New Zealand carpet sharks (*Cephaloscyllium isabellum*) increased around a DC magnetic field but remained unchanged under AC magnetic field exposure.

Variations in both the design and settlement of SPCs make it highly difficult to define a realistic range of magnetic field emissions (Albert *et al*., 2020). Field and natural experiments offer realistic scenarios with intensity gradients that are crucial in environmental risk assessments. However, such results are often not comparable due to environmental variables that cannot be controlled but only monitored and accounted for (Gill *et al.*, 2009; Hutchison *et al.*, 2020). These limitations can be partly solved with controlled environment approaches that allow testing constant intensities of an independent magnetic field. Despite not being the most realistic scenario, exposure to a uniform field intensity enables researchers to draw causal conclusions regarding the detection abilities and responses of a given species (Spicer, 2014).

Our laboratory-based study sought to investigate the individual behavioural responses of the thornback ray *Raja clavata* to a sudden change in the magnetic field environment in terms of both intensity (i.e. ten times higher than the ambient magnetic field) and field type (DC and AC). This bottom-dwelling species is widely distributed in the Eastern Atlantic and is both a target and bycatch of

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commercial fishing along European coasts. As a solitary benthic predator forager (Marandel, 2018; Nottage and Perkins, 1983), the thornback ray uses its highly specialised and sensitive electrosensory system to find prey and displays evidence of magnetosensitivity (Kalmijn, 1971; Tricas, 2001). Moreover, *R. clavata* spends most of its time buried in fine sediment (often at a 10–60 m water depth) and exhibits onshore/offshore seasonal migrations (Hunter *et al.*, 2006; Marandel, 2018; Maxwell *et al.*, 2009). Therefore, *R. clavata* is very likely to encounter and detect magnetic emissions produced by cables buried in the superficial sediment layers. As an initial approach to characterise the short-term (1 h exposure) behavioural responses of rays to magnetic fields, we designed and constructed an experimental device to produce constant-intensity (450 µT) AC or DC magnetic fields. Such intensity occurs at the water-sediment interface above a 0.4 m buried AC or DC single-phase cable (15 cm diameter) carrying 1000 A (calculations based on Salinas *et al.*, 2009) and is thus relevant for bottom-dwelling species. Behaviour analysis is one of the most integrative and sensitive tools to assess the effect of environmental stressors on organisms (Weis, 2014). Captive-born juveniles were studied both to gain preliminary insights into the magneto-sensitivity of young stages towards artificial electric field sources and to avoid the transfer-related stress of wild specimens (Dehart, 2004). Based on the available literature, we hypothesised that exposure to a magnetic field would cause behavioural alterations in captive *R. clavata* in the form of quantitative (i.e. hypo- or hyper-activity) and qualitative (e.g. distinct behavioural sequences) changes or both.

2 Materials and Methods

2.1 Animal maintenance and experimental facilities

Fifteen *R. clavata* juveniles (full siblings, aged 13 to 16 months over the study course) were provided by the Oceanopolis Center of Scientific Culture. All individuals were kept in one 600-L recirculating stock tank (length × width × height: 1 × 1 × 0.6 m) filled with fine yellow sand and supplied with natural aerated seawater at seasonal ambient temperature. Rays were maintained by the Oceanopolis staff and fed daily with a variety of frozen food (squid, mussels, shrimp, or fish). Throughout

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the study period, rays ranged in length from 12.2 cm to 20.1 cm (average size 17.0 cm ± 2.3 cm) and weighed on average 106.4 g ± 37.5 g. All data are reported as mean ± standard deviation (SD).

The whole experiment was carried out in a private and thermally regulated experimental room (19–22 °C) also located in the Oceanopolis Center of Scientific Culture. The room was equipped with 3 acclimation glass tanks (size: 60 x 50 x 40 cm; water depth: 30 cm) aligned along a wall [see Supplementary figure (2)]. The water temperature of all acclimation and experimental tanks was maintained at 13.8 ± 1.4 °C using a water chiller. This temperature was similar to that recorded in the rearing stock tank. Both the acclimation and experimental tanks were supplied with aerated seawater pumped from the bay of Brest at an 84 $L \cdot h^{-1}$ flow rate. All tanks were connected to a semi-closed water recirculating system with a 45% hourly renewal (fresh seawater supply). The water outflow of the tanks was UV-irradiated and then filtered with a mechanical polyethylene filter followed by Biogrog biological filtration before recirculating. Each tank contained a 3 cm layer of yellow sand and an air pump to maintain a > 90% oxygen saturation. Oxygen levels were monitored before each behavioural test to ensure that behavioural impairments were not caused by hypoxic conditions. The water was maintained at a pH of 7.9 and a 33.2 PSU salinity. Other parameters were kept under threshold values (NH_4^+ < 0.1 mg/L; NH_3 <0.01 mg/L; NO_2 <0.05 mg/L; NO_3 <10 mg/L). The room was kept on a 10:14 light:dark cycle.

2.2 Magnetic field exposure system and experimental tank

The artificial magnetic fields were produced by two square Helmholtz coils (1.5 × 1.5 m) (Figure 1), each composed of 200 vertically stacked turns of copper wire (2.5 mm^2 diameter; 1.2 km total length) inside a hermetically sealed Plexiglas hollow frame. The coils were spaced one meter apart, parallel to one another, and were laid on a Plexiglas support. The experimental glass tank was located between both coils. In DC conditions, the Helmholtz coils were supplied with an electric current generated by a DC power supply (30V–16A), which provided a uniform 450-µT static field (447.2 ± 6.3 µT) within the experimental tank. In AC conditions (50 Hz), the 450-µT homogeneous zone was similar but the coils

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were powered by connecting the sector current to a portable isolation transformer that was controlled with a rheostat.

Our exposure system was based on the same physical principle as those of other research teams (Jakubowska *et al.*, 2019; Scott *et al.*, 2018; Taormina *et al.*, 2020; Woodruff *et al.*, 2013) but is mobile and designed for regular use across various experimental conditions. Moreover, all the electrical parameters (voltage, electric intensity, on/off switching, and coil temperature) are monitored, recorded, and programmed from a purpose-built software developed by MAPPEM Geophysics (http://www.mappem-geophysics.com/). The magnetic induction is measured in real time and maintained at a fixed value through an automatic control loop that continuously adjusts the electric intensity. Prior to the trials, the Helmholtz coils were supplied by a 7.7 A electrical current (20 V) and a 3D map of the magnetic field measurements was constructed (MATLAB software 3D plotting function, MathWorks, Natick, Massachusetts; Figure 1) using a magnetometer (Mag690 Three-axis, Bartington Instruments) according to a 10 × 10 cm grid. When the coils were turned off, the geomagnetic field inside the experimental tank was measured at 47 µT. Additional measurements indicated that the acclimation tanks were not influenced by the exposure system (see the Supplementary figure (1)).

2.3 Experimental procedure

The different stages of the experiment are illustrated in Figure 2. Because behavioural trials were conducted at the individual level, each ray was acclimated alone for 3 days in one of the 3 acclimation tanks and fed daily. Then, the ray was transferred to the experimental tank 18 hours before the behavioural trials. As appetite loss is commonly used as a stress indicator, only animals that had eaten on the previous 2 consecutive days were transferred to the experimental tank using a hand net (Charbeneau, 2004).

In a pilot study conducted one year prior under similar conditions, ray activity varied hourly and was higher during midday. As time dependency was also observed in the responses of elasmobranchs to magnetic fields in Gill *et al.* (2009), two time periods were included in the present study. Therefore,

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the behaviour of each ray was recorded during two distinct periods on the same day for each trial: morning (9:00-10:00 am) and midday (1:00-2:00 pm). Over each period, the rays experienced one of the following magnetic treatments: (1) the ambient magnetic field of 47 µT with the coil turned off as the control (CT), (2) a uniform DC magnetic field of 450 µT (DC), (3) a uniform 50 Hz AC magnetic field of 450 µT (AC). Based on the treatment type and exposure period, trials were categorised into 5 groups (Groups A-E) that are further described in Figure 2. First, 15 rays were randomly assigned to three groups (Groups A-C) of 5 individuals each to receive the appropriate treatments over a 20-day period. After these trials, the first 10 rays tested were returned to their initial stock tank for a minimum of 10 days up to 20 days (the latter corresponding to the first individual tested) before a second experimental session. The rays were then randomly divided into Groups D and E. This experimental design was selected due to the reduced size of the experimental population. Nevertheless, based on current literature and evidence of short-term memory in elasmobranchs (e.g. 12 h to 3 weeks with reinforcement experiments), a 10-day period was considered sufficient to minimise the post-exposure effects or repeated measure effects (Kimber *et al.*, 2013; O'Connell *et al.*, 2011).

Individual behaviour was recorded using a GoPro HERO5 Black camera located 40 cm above the experimental tank (4K resolution, large screen, 30 frames per second). The camera did not modify the magnetic intensity recorded inside the experimental tank. The magnetic field values inside the experimental tank were monitored and recorded at a 0.1 Hz frequency during the experiment. As a precaution to avoid stressing the rays, video recording was remotely launched with the GoPro mobile application 15 minutes before the actual start of the behavioural test. During video recording, the air pump and water inflow were turned off to improve visibility and to avoid behavioural bias such as ray attraction. After each trial, the tested individual was weighed and transferred to a post-exposure tank. To limit stress, ray size (i.e., pectoral fin span) was measured from calibrated pictures using the open-source Kinovea software (v. 0.9.1).

2.4 Behavioural and statistical analysis

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A total of 12 *R. clavata* juveniles were first observed individually for 5 hours each to establish a detailed ethogram (Table 1). Of the fifteen behavioural variables evaluated herein, six were taken from the work of Greenway et al. (2016) whereas the others were new contributions. In the present study, video analysis was performed using the BORIS software (Behavioural Observation Research Interactive Software v. 7.9.7) and consisted of measuring the time spent in each of the variables (from 0 to 3600 s) for the compilation of time budgets (i.e. focal sampling). Using principal component analysis (PCA), variables were pooled into higher behavioural categories to facilitate interpretation. Prior to analysis, the matrix sampling adequacy was verified using a Bartlett sphericity test and no rotation factor was applied. Principal components were extracted following the parallel analysis method, as suggested by Glorfeld (1995).

To model the proportion of time spent in the different behavioural categories (i.e. time budget) for each magnetic treatment, generalised additive models for location, scale, and shape (GAMLSS) with zero or one inflated beta regression were established (Rigby and Stasinopoulos, 2005). Regarding the experimental design, morning and midday data were modelled separately. Because the same individuals were studied across both periods, CT midday treatments occurring after a DC or AC morning exposure were specified as post-DC and post-AC, in case the magnetic treatment would lead to prolonged or delayed behavioural adjustments. To check for potential differences, the CT treatment was compared with the post-treatments (i.e. post-DC and post-AC) and the treatments (i.e. AC and DC), respectively. Accordingly, the explanatory variable "treatment" was categorical with three levels for both the morning period (i.e. CT, AC, or DC) and the midday period performed with two distinct tests (i.e. CT, AC and DC or CT, post-AC and post-DC).

The beta distribution is often used to describe the proportion of data within the interval (0, 1) and is described by two parameters: μ (location parameter, i.e. distribution mean) and σ (precision/scale parameter) (Ospina and Ferrari, 2010). In the present study, the occurrence of each behavioural event (response variable) was defined either by a high proportion of 1 (e.g. immobile 100 % of the time) or else by a high proportion of 0 (e.g. 0 % of vertical or horizontal activity). To allow observations on the

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intervals (0, 1] and [0, 1), data were thus modelled using a two-distribution mixture of beta distribution on (0, 1) and Bernoulli distribution for the probability mass at 0 or 1 (v: shape parameter) (Ospina and Ferrari, 2010). Candidate models were selected using the generalised Akaike information criterion (GAIC) (Akaike, 1973). The maximum likelihood estimation (i.e. likelihood ratio test, LRT) approach was performed to test the contribution of the 'treatment' variable to the data structure (Stasinopoulos *et al*., 2015). Finally, assumptions were checked by plotting residuals versus fitted values and covariate values.

In addition to time budget comparisons, a descriptive analysis using hierarchical clustering was applied to the raw data (i.e. containing all the 15 behavioural variables) to emphasise potential qualitative and quantitative similarities among individual behavioural sequences. To achieve this, all ray behavioural sequences were compared using a distance matrix with Euclidian distance and ward linkage methods according to the experimental periods. Additionally, the individual chronologies of behavioural events were compared. All statistical analyses were performed at a significance level of 5% using R version 4.0.2 (RStudio Team, 2019) with the 'FactoMineR' (Lê et al, 2008), 'paran', and 'psych' packages (Revelle, 2021) for PCA and the 'gamlss' package (Rigby and Stasinopoulos, 2005) for zero/one inflated beta regressions.

3 Results

Data were collected from 15 rays: control: n = 5; DC: n = 10; and AC: n = 10 individuals (re-tested after a 10-day period). The individual mass (mean ± SD) of the rays in the control group was 106.6 ± 21.0 g, 106.3 ± 44.6 g for DC, and 110 ± 34.3 g for AC.

3.1 Reduction and structure of the variables with PCA

Following PCA analysis, the 15 behavioural variables evaluated herein (see Table 1) were condensed into two principal components (PC1 and PC2) explaining 72.9% of the variance contained in the dataset (Figure 3). PC1 explained 54.5 % of the total variability and was described by two groups of opposing variables: six variables (i.e. "rising", "burying", "crab steering", "rotating", "crawling", and

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“stepping”) with positive values and one variable “cryptic horizontal resting” with a negative value. PC2 explained 18.3% of the total variability and was mainly described by five variables with positive contribution (i.e. “snout glass”, “vertical swimming”, “surface breaking”, “swimming”, and “digging”) and two variables with negative contribution (i.e. “searching”, “obvious horizontal resting”). The “vertical resting” variable was poorly represented by PC1 and PC2 and was thus assigned to a third axis.

Based on PCA output and intra-variable correlation coefficients, the variables were clustered in three main categories: “immobility”, “horizontal activity”, and “vertical activity”. “Immobility” referred to “cryptic horizontal resting” behaviour that was negatively correlated with all variables. “Horizontal activity”, described by both PC1 and PC2, was linked to bottom-related active behaviours (i.e. “rising”, “burying”, “crab steering”, “rotating”, “crawling”, “stepping”, “searching”, and “obvious horizontal resting”). Finally, “vertical activity”, described by PC2, referred to behaviours in which the animals were attracted towards the edge and top of the aquarium (Levin *et al.,* 2007; Maximino *et al.*, 2010) (i.e. “snout glass”, “vertical swimming”, “surface breaking”, “swimming”, “digging” and “vertical resting”).

3.2 Time budget comparison

Across both periods and all magnetic conditions, the activity of the rays was overwhelmingly dominated by the “immobility” behavioural category, which occurred 79% to 100% of the experimental time, whereas “horizontal activity” and “vertical activity” occurred far less frequently (0%–16% or 0%–11% of the total experimental time), albeit at comparable average proportions (Figure 4). All model coefficients are available in the Supplementary figures (3) and (4).

3.2.1. Morning period

During the morning period, magnetic treatment (i.e. CT 47 µT, AC 450 µT, or DC 450 µT) did not affect the proportion of time spent in the “immobility” (*LRT*= 4.9, *p-value* = 0.087), “vertical activity” (*LRT* = 3.8, *p-value* = 0.149), or “horizontal activity’” (*LRT* = 3.0, *p-value* = 0.223) categories (Table 2; Figure 4a). Based on visual inspection, model residuals were homogeneous and normally distributed.

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3.2.2. Midday period

During the midday period, the magnetic treatments had a significant effect on the time spent in "immobility" (LRT =15.7, *p-value* = 0.0004), with a significant decrease associated with the DC treatment (t value= -4.897, *p-value* = 0.0006) [details in supplementary material (4)]. However, the treatments had no significant effects neither on the "vertical activity" (LRT = 2.0, *p-value* = 0.4), nor the "horizontal activity" (LRT = 4.0, *p-value* = 0.1) categories (Table 2; Figure 4b). Finally, the CT treatment did not significantly differ from the AC and DC post-treatments in neither of the behavioural categories (Table 2). The model residuals were homogeneous and normally distributed.

3.3 Descriptive analysis of behavioural sequences

Individual hierarchical clustering was summarised in dendrograms for the morning and midday periods (Figure 5A). During the morning period, rays were clustered into 5 major groups. The largest group was C1, which gathered all individuals (n = 18/25 rays) that exhibited "immobility" (i.e. "cryptic horizontal resting") at least 96% of the time. Among them, 9 rays received CT treatment, 5 received DC treatment, and 3 were exposed to AC treatment. Other clusters were grouped based on the proportion of time spent active, as opposed to "immobility". However, no obvious patterns were observed neither in the type of behaviour nor in their chronology (cf. Supplementary figure (5)).

During the midday period, individuals were grouped into 5 clusters. Once again, motionless rays (i.e. exhibiting "immobility" at least 99 % of the time) constituted the largest cluster C1 (n = 18/25) and included 3 rays from the AC treatment, 4 from the post-AC treatment, 3 from the post-DC treatment, 3 from the DC treatment, and 5 from CT treatment. A second cluster (C2) gathered two rays that were both exposed to AC treatment. Their behavioural sequences exhibited strong temporal, qualitative, and quantitative similarities (Figure 5B). First, their latencies of first movement were extremely close (7 min 5s and 6 min 18 s, respectively) after the magnetic field was turned on and both rays remained active for similar periods (102.83 and 115.56 s). Additionally, these two rays performed similar behaviours (5/7

behaviours in common) belonging to the "horizontal activity" category with very similar time budgets. No behavioural patterns were found in the three remaining clusters.

4 Discussion

Despite the increase in the number of subsea cables and the resulting rise in magnetic field emissions in coastal waters, their potential interactions with electro- and magneto-sensitive species remain largely uncharacterised. Therefore, our study sought to investigate the short-term behavioural responses of juvenile thornback rays *(R. clavata)* to 450 µT magnetic fields of both alternating (AC) and direct current (DC). Over the past decade, both laboratory and field studies investigating the effects of DC magnetic fields on elasmobranchs identified an increase in exploratory behaviours towards areas of magnetic emission. More specifically, the investigatory responses of New Zealand carpet sharks (*Cephaloscyllium isabellum*) increased during the first minutes immediately after DC cable activation (maximum magnetic field of 3.96 mT) (Orr, 2016). Such responses were attributed to an attraction/exploration of the magnetic field source (i.e. the cable). Similarly, Hutchison *et al.* (2020) demonstrated that the exploratory behaviour of little skates (*Leucoraja erinacea*), which was estimated based on the travelled distance, the number of large turns, the time spent in the zone of highest emissions, and the proximity to the seabed, were higher in the vicinity of an HVDC cable (maximum magnetic field anomaly of 14 µT). In the present study, results showed an increase of active behaviours for the rays that had received the 450 µT DC treatment, during the midday period. However, the horizontal and vertical activities occurred over the same proportion for the DC treatment. Though consistent with current literature, such result should be interpreted with care due to the total inactivity of the control group during the midday period, which may not account for the natural inter-individual variability. Accordingly, since no difference was found during the morning period across the treatments, such results could arise from a sample size bias or to the natural variation of the rays behavioural responses throughout the day.

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The present work is innovative in several experimental aspects. First, this laboratory-based approach recreates magnetic fields in a controlled environment. Moreover, the experiments were conducted on the juvenile stages of *R. clavata* exposed to constant-intensity magnetic fields. To the best of our knowledge, this is the first time that an elasmobranch species is exposed to such conditions without an intensity gradient or an identifiable source. Finally, the behavioural analysis was based on an exhaustive ethogram and focused on both qualitative and quantitative aspects. Nevertheless, many factors could have contributed to the discrepancies observed herein (i.e. lack of statistical significance) relative to other studies. One first aspect to consider relates to the experimental population and sample size. The present study was conducted on a small number of fish within a similar sample size to that of other studies (e.g. Greenway *et al.*, 2016; n=14) that were able to show significant behavioural differences. Such density is highly reasonable for laboratory studies considering the husbandry work and facilities required for animal maintenance. Furthermore, Hutchison *et al.* (2020) reported significant behavioural changes among a larger batch of 39 skates. Therefore, research on elasmobranchs requires an experimental design with sufficient statistical power to detect magnetic effects and overcome low sample size, subtle effects, and/or inter-individual variance. Additionally, the present work was conducted with a consistent experimental population both in terms of life stage, traits, and genetic background, whereas populations from other studies were more heterogeneous.

In the present work, we observed a high inter-individual variance of responses, especially when considering moving (i.e. exhibiting "horizontal and vertical activities") and non-moving individuals (i.e. exhibiting "immobility"). Gill *et al.* (2009) also observed species-dependent and individual-specific responses in adult *R. clavata* and *S. canicula* towards magnetic fields. Immobility is a common behaviour in the *Raja* genus, which is comprised of sit-and-ambush predators that spend a large proportion of time buried in the sediment to capture prey (Greenway *et al.*, 2016; Wearmouth *et al.*, 2014). Such dichotomy between individuals might result from the existence of two behavioural strategies in response to new environmental cues. In natural conditions, populations experience fluctuating environmental contexts that drive variable selective pressures on individual behaviour (Nannini *et al.*,

2012). Previous studies have demonstrated that a given population might display numerous behavioural phenotypes because they guarantee a variety of adaptive solutions to different environmental challenges (Mittlebach *et al.*, 2014; Wolf *et al*., 2012). Therefore, it is not uncommon for some individuals to remain relatively inactive and alert in novel conditions, whereas others are more active and display more exploratory behaviours (Sneddon, 2003; Wilson and Godin, 2009; Wilson *et al.*, 1993). In juvenile flatfishes and rays, camouflage through burial and cryptic colouration coupled with reduced activity is known to be a first-line mechanism against predation risk (Gibson *et al.*, 2014; Snow *et al*., 1996). Elasmobranchs usually rely on their electrosensory system to detect bioelectric stimuli from potential predators (Kempster *et al.*, 2012). During the embryonic stage, predator-mimicking electric fields trigger a "freeze behaviour" during which individuals stop ventilating through tail undulation or gill movements in thornback ray (Ball *et al.,* 2016), clearnose skates (*Raja eglanteria*) (Sisneros *et al.*, 1998), catsharks (*Schyliorhnius canicula*) (Peters and Evers, 1985), and banded bamboo sharks (*Chiloscyllium punctatum*) (Kempster *et al.*, 2013). Close to an operating cable, Hutchison *et al.* (2020) measured AC-induced electric fields within the detection range of electro-sensitive species (<1–100 µV cm−1, 1–100 Hz) and close to those causing freeze responses in elasmobranch embryos (6.1–10.5 V/m; 5x10-5 to 9.5x10-4 V/m; 9.0x10-5 V/m in Ball *et al.*, 2016; Kempster *et al*., 2013; Sisneros *et al.*, 1998). As highlighted by Nyqvist *et al.* (2020), electric fields co-occur with magnetic fields and most laboratory studies investigating the effects of the latter could not discriminate which one was causing the behavioural changes. Accordingly, given that the Helmholtz coils should have emitted electric fields within the tank, some individuals may have detected them and could have expressed an anti-predator strategy commonly associated with bioelectric signals. However, this hypothesis remains speculative as it is not supported by the electric fields measured in the exposure system.

Based on the descriptive analysis, strong qualitative and quantitative similarities were observed between two rays, both of which were exposed to a 450 µT AC magnetic field during the midday period. Particularly, the rays shared analogous behavioural chronologies, with very close chronological steps such as the total duration of the moving phase, the starting and ending times, and comparable time

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budgets. Furthermore, both rays performed the same type of behaviours exclusive to the "horizontal activity" category. Such similarities did not occur in any other rays and were specific to the AC treatment (see Supplementary figure (5)). Because the rays were acclimated and tested individually at a 3-day interval with very minor and uncontrollable variations in their experimental environment, such behavioural similarities are unlikely to be due to experimental bias. These results are inconsistent with those of Orr (2016), who did not observe any attraction or avoidance responses upon exposure to a 50-Hz AC magnetic field (maximum value of 1.43 mT) in the New Zealand carpet shark (*C. isabellum*). Nevertheless, Gill *et al.* (2009) reported that AC cable energisation (maximum magnetic field anomaly of 8 µT and electric field of 2.2 µV/m) caused occasional attraction towards the exposed zone in the catshark (*S. canicula*), as well as an increase in the rate of movement in the thornback ray (*R. clavata*). The tested values varied greatly between studies conducted at 8 µT and up to 1430 µT, which could explain the seemingly opposite responses observed in elasmobranchs exposed to magnetic/electric fields. In natural conditions, elasmobranchs should not often experience the extreme value selected by Orr (2016) which matches a situation of close proximity to the cable armour (Albert *et al.*, 2020; Otremba *et al.*, 2019), as cables are often buried or protected by thick structures (e.g. rock mattresses up to 2 m thick). Moreover, elasmobranch magneto-sensitivity is species-specific (Gill *et al*., 2009) and potentially limited by sensitivity thresholds, making inter-studies comparisons difficult. Elasmobranchs might exhibit distinct responses when experiencing magnetic strengths that are likely to occur in natural conditions (i.e. bioelectric fields) compared to very high values out of their familiar range.

As previously mentioned, this study is the first to evaluate the responses of elasmobranchs to a constant magnetic field value in controlled conditions. Field studies offer realistic ecological contexts in which the individuals are subject to the inherent environmental variations of their natural habitat. However, it is quite impossible to fully characterise the subtle variations in the abiotic (e.g. boundary layer flows, sediment texture, and microtopography) and biotic factors (e.g. prey, predators) that characterise a given environment, and therefore generalising field study results is questionable (Spicer *et al*., 2014). The experimental approach adopted herein sought to draw causal relationships and to

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isolate the effects of magnetic fields from all possible extraneous variables encountered in the field. Due to spatial scale constraints related both to the size of the tested rays and to the exposure system, we chose to test the responses to a constant intensity magnetic field rather than a gradient to standardise the exposure in the whole tank and to avoid complex interactions between spatial, temporal, and individual parameters.

In the field, the electrosensory system of elasmobranchs detects local electric field fluctuations induced by prey, predators, and conspecifics and allows their spatial localisation (Whitehead and Collin, 2004). Additionally, during migratory periods, elasmobranchs are assumed to orient to geomagnetic cues and anomalies based on a compass or a magnetic map sense (Klimley, 1993; Meyer *et al.*, 2005). Such a sense is based on an extreme sensitivity to low magnetic field gradients (Mouritsen, 2018; Nyqvist *et al.*, 2020). Accordingly, Klimley *et al*. (2021) recently suggested that studies on the impacts of magnetic fields on marine organisms should primarily focus on their effects on movement patterns, as this would facilitate reaching population-level conclusions. In the present study, we instead characterised a larger range of basic behavioural responses to various magnetic signals (field type and intensity) for two major reasons: (1) the values of cable-induced magnetic emissions are very high compared to the small-scale changes in the geomagnetic field, and therefore would not likely be perceived by fish as orientation cues; (2) magneto-sensitive species are also totally naïve to AC fields and thus should not use them as orientation cues.

Furthermore, the juvenile rays studied herein originated from a single spawning event, which guaranteed that they were all at a similar development stage and experienced similar ambient magnetic field conditions throughout their life. This sample homogeneity would have been highly difficult to obtain and standardise using wild subjects. *R. clavata* exhibits polyandrous behaviour (i.e., multiple paternities), thereby increasing the genetic diversity within the same litter. Therefore, potential bias due to genetic similarities was not considered a major issue in the present study. Captive breeding allows for a careful control of the experimental population with a traced origin and ensures their naivety towards cable-induced magnetic fields. Nevertheless, captivity may also lead to behavioural

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adjustments through the domestication process even after only one generation (Pasquet, 2018), thus potentially changing the sensory abilities of a species (Putman *et al.*, 2014). Given that the behavioural changes between wild and captive fishes are more quantitative than qualitative (Pasquet, 2018), we cannot rule out the possibility that wild subjects might exhibit different responses. Moreover, the present study evaluated individual responses to magnetic fields; however, animal density is known to strengthen foraging activity and stimulate movement as a result of intra-specific competition and imitation (Ward *et al.*, 2006). For example, Greenway *et al.* (2016) demonstrated that high densities of *R. clavata* individuals reared in captivity correlated with higher activity and more stress-associated behaviours. Further experiments are thus required to test behavioural responses at the population level.

In conclusion, our experiments highlighted the challenges of identifying behavioural responses to magnetic signals in a species that naturally displays long periods of inactivity and high inter-individual variability. Further long-term studies are thus required to assess the interaction between magnetic fields and daily, tidal, or seasonal rhythms, as well as how these could potentially contribute to the habituation process. Moreover, behavioural experiments should be coupled with physiological approaches to better understand the biological processes triggered by magnetic signals. In coastal areas, species may soon encounter an increasing number of power cables (Hutchison *et al.*, 2020) and the cumulative effects of constant exposure to varying magnetic field intensities should be promptly addressed with dose-response experiments, as these might affect the adaptive capacities of aquatic species.

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Ethical statements

The Oceanopolis center was accredited for the use of animals in scientific purposes. Oceanopolis staff had required qualifications and validation from the veterinarian of the regional ethics committee was obtained. A proper ethics committee was not required due to the supervision of the aquarium staff (during ray transfer and manipulation) and because experiment were exclusively observational.

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Figure 1

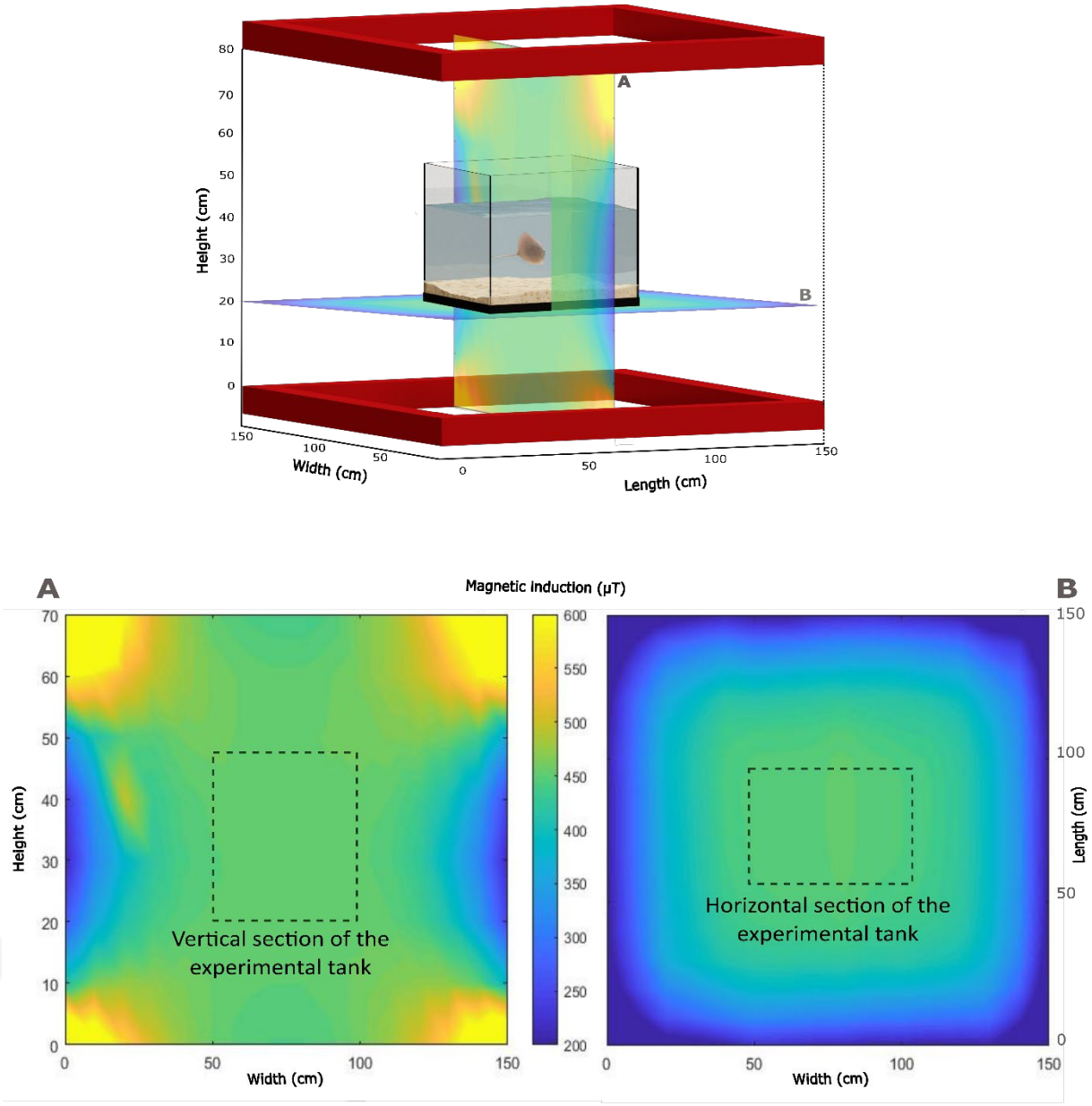

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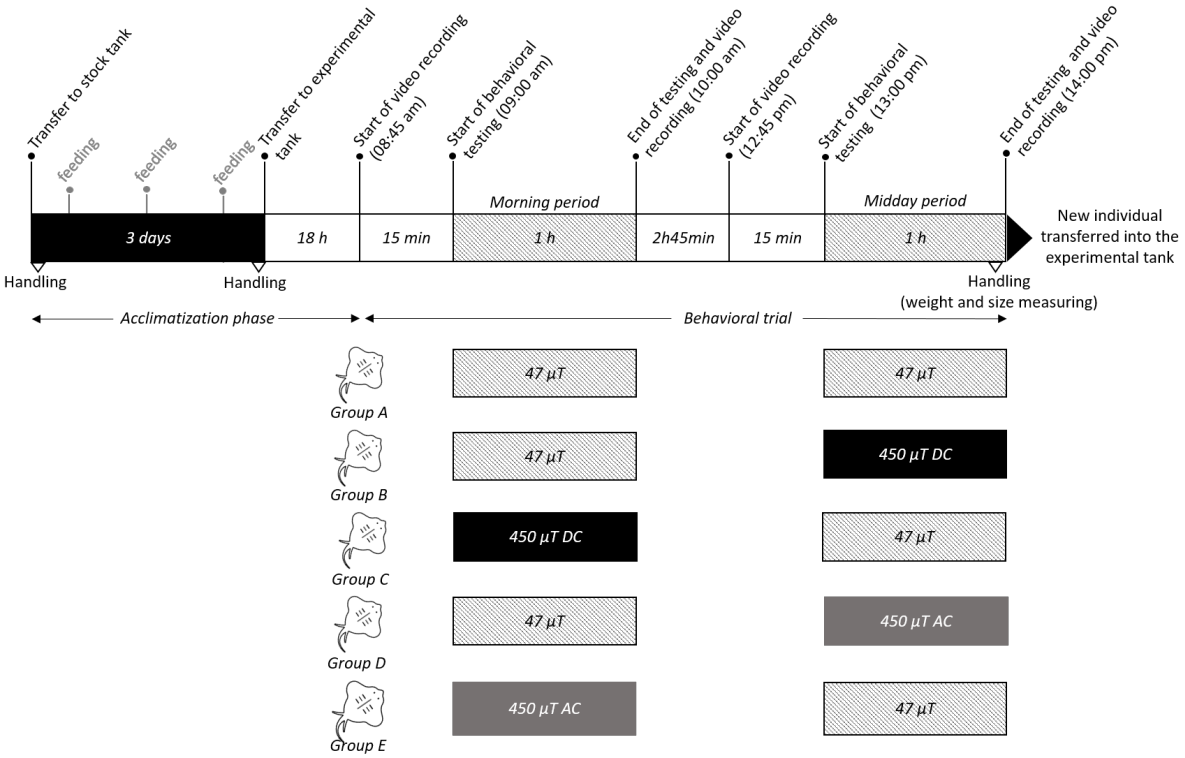
Figure 2



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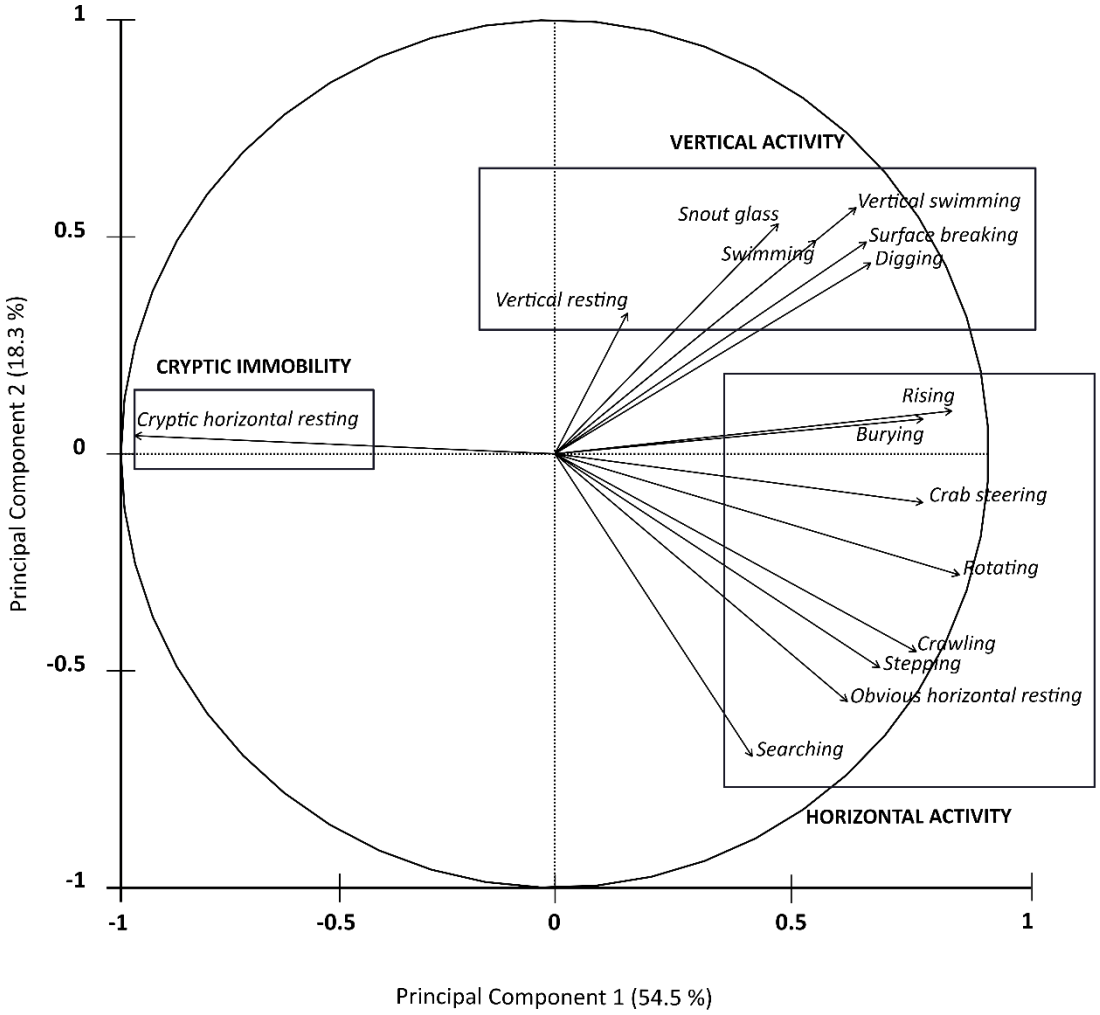

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Figure 4

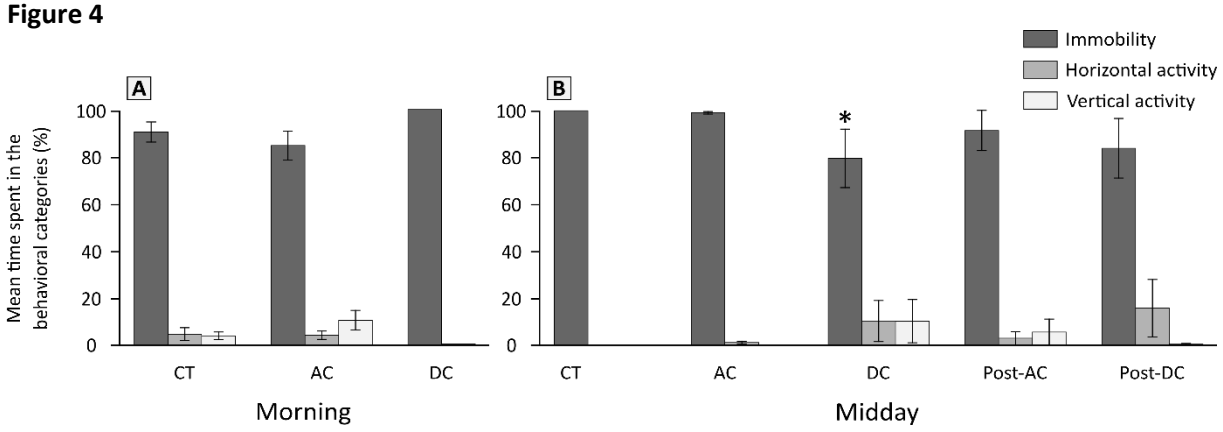

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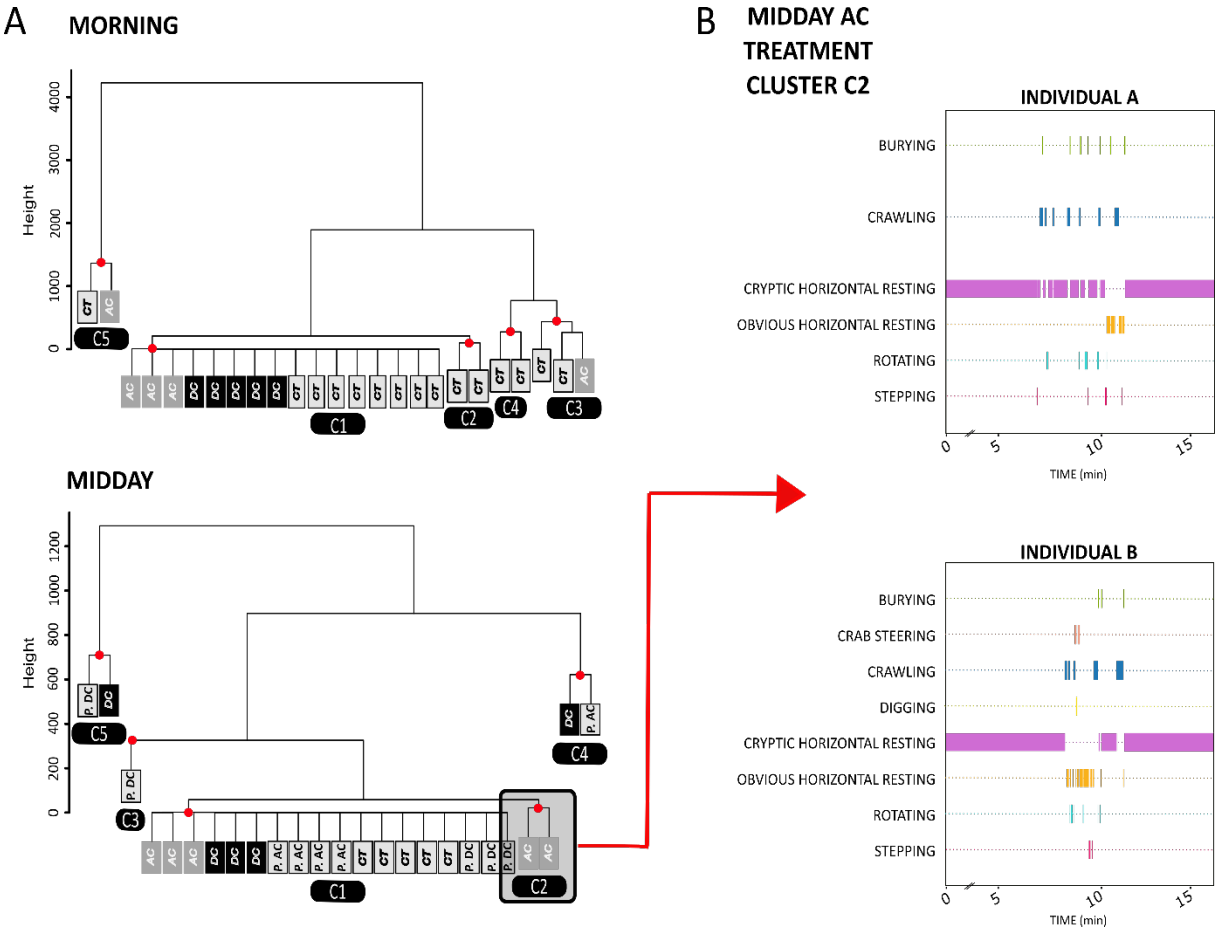
Figure 5. (A) (B)



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FIGURES CAPTIONS

Figure 1: Distribution of the magnetic field induction (expressed in µT) between the two Helmholtz coils and within the experimental tank. The highest field strengths are generated at coils location, along the height axis at 0 cm and 70 cm (coloured orange and yellow). (A) and (B) refer to either vertical or horizontal measurement section, respectively. The experimental tank is represented in dashed line across both sections according to its spatial location, showing the magnetic field values experienced by the ray.

Figure 2: Experimental timeline illustrating the sequence of events and behavioral tests for each ray *Raja clavata*. The acclimation phase was made in a stock tank (black box) and the test phase (grey and white boxes) in the experimental tank. Five batches of animals were used (A, B, C, D, and E) corresponding to either control 47 µT (A) or exposure to a continuous 450 µT magnetic field during midday (B) or morning (C), or to an alternating 450 µT magnetic field during midday (D) or morning (E).

Figure 3: Principal component analysis (PCA) of the behavioural variables (see Table 1 for definition) observed in the thornback ray (*Raja clavata*) across experimental treatments. The two principal components (PC 1 and PC 2) explained 72.9 % of the variance. The behavioural variables are grouped into 3 higher categories: "vertical activity", "horizontal activity" and "immobility". The PCA was obtained from a correlation matrix.

Figure 4: *Raja clavata* time budgets (in %) spent in the 3 behavioural categories across morning and midday periods, in relation to magnetic treatment. Over morning period, control rays were exposed to ambient magnetic field (47 µT) and gathered individuals from group A, B and D (n=15). DC treatment (C) rays received a 450-µT constant magnetic field (n=5), and AC treatment (E) a 450-µT alternative magnetic field (n=5). Over midday period, rays were exposed to control treatment CT (A; n=5), DC treatment (B; n=5), AC treatment (D; n=5), post-DC treatment (C; n=5) and post-AC treatment (E; n=5). Statistical difference resulting from the comparison with the CT treatment are shown by an asterisk '*'.

Figure 5: (A) Dendrogram of hierarchical cluster analysis for morning and midday periods. Each box matches an individual (*Raja clavata*) and is coloured according to either CT (control), AC (450 µT alternative magnetic field) or DC (450 µT direct magnetic field). Based on their similarity, individuals are gathered in clusters C1 to C5, highlighted by a red dot. (B) Behavioural chronology of the two individuals A and B gathered in cluster 2 (C2) over midday treatment. Both individuals experienced AC treatment.

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Table 2 ……..p.9

Legend*:** Likelihood ratio tests for morning and midday period, comparing *Raja clavata* time budgets as a function of magnetic treatment. '' Indicates p-values under the 5 % threshold.

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Table 1. *Raja clavata* ethogram of the behaviours observed over both the pilot and experimental studies; this completes the work of Greenway et al., 2016.

CATEGORY	BEHAVIORAL VARIABLES	DEFINITION
Horizontal activity	Crawling	Moving on the ventral face using the pelvic fins one after the other to push sand to the back
	Obvious horizontal resting	Remaining still on the floor but conspicuous with little or no sand covering the body. This behavior is usually part of a moving sequence.
	Rotating	Changing orientation (snout as the reference point)
	Searching	Remaining at the same spot but moving slightly from side to side and digging the sand
	Stepping	Short forward movement
	Rising	Initiating an upward movement along the glass and then returning to the sand
	Burying	Oscillating pelvic fins to move sand onto the body and dig a burrow.
	Crab steering	Touching the tank wall with the snout and moving towards the left or right side, shaking body
Vertical activity	Digging	Agitating fins, undulating on the sand
	Snout glass	Swimming with the snout touching the tank wall
	Surface breaking	Swimming with the snout above the water's surface
	Swimming	Moving through the water column without touching any surface (tank wall, sand)
	Vertical swimming	Swimming vertically touching the tank wall with the ventral face
	Vertical resting	Remaining still with ventral face touching the tank wall
Immobility	Cryptic horizontal resting	Remaining still on the floor covered with a thick layer of sand

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Table 2. Likelihood ratio tests. '***' Indicates p-value under the 5 % threshold.

Effect	Model fitting criteria (AIC)	Likelihood ratio tests		
		χ^2	df	Significance (p-value)
Morning period				
CT vs. DC and AC				
		IMMOBILITY		
Intercept	18.774			
Treatment	19.657	4.883	2	0.087
		VERTICAL ACTIVITY		
Intercept	17.927			
Treatment	17.741	3.814	2	0.149
		HORIZONTAL ACTIVITY		
Intercept	4.547			
Treatment	3.549	3.002	2	0.223
Midday period				
CT vs. DC and AC				
		IMMOBILITY		
Intercept	-5.1314	15.741	2	0.0004***
Treatment	6.6099			
		VERTICAL ACTIVITY		
Intercept	13.854			
Treatment	11.856	2.002	2	0.368
		HORIZONTAL ACTIVITY		
Intercept	1.8290			
Treatment	1.8718	4.043	2	0.133
CT vs. post-DC and post-AC				
		IMMOBILITY		
Intercept	12.947			
Treatment	11.893	2.947	2	0.229
		VERTICAL ACTIVITY		
Intercept	13.526			
Treatment	11.852	4.200	2	1
		HORIZONTAL ACTIVITY		
Intercept	9.828			
Treatment	10.153	4.326	2	0.115

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