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BRIEF COMMUNICATION

Behavioral responses of European sea bass, *Dicentrarchus labrax,* fingerlings under the influence of an electromagnetic field

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Abstract
The advent of wind farming as a renewable energy source necessitates assessment of the effects of electromagnetic fields (EMF) on fish, especially when done in conjunction with aquaculture. In this study, we investigated the effects of an EMF on the behavior of European sea bass fingerlings. A T-maze apparatus was used to record the behavior of the fish with and without the presence of a static EMF of 10 mT and subsequently, several behavioral metrics were analyzed using specialized software. Our results suggest that E. sea bass can perceive magnetic fields. Compared to the control group, fish exposed to the EMF showed reduced exploratory behavior with longer periods of immobility, traveled a shorter total distance, and exhibited freezing behavior at an increased frequency. In addition, fish showed a preference for the geomagnetic south. The findings offer valuable contributions to our understanding of EMF effects on fish, which in turn may be useful in shaping the future of fish farming in conjunction with energy production.

Keywords *Dicentrarchus labrax* · T-maze · Magnetoreception · Electromagnetic field · Behavior

Introduction

Food security mandates the increase of food production from the sea, mainly in the form of aquaculture (Bjørndal et al. 2024), with offshore aquaculture in particular gaining recent interest (Michler-Cieluch et al. 2009; Nassar et al. 2020). Meanwhile, the energy crisis necessitates the increase of energy supply. Wind energy already exceeds one fifth of the global renewable energy production, and its expansion in high energy offshore environments has flourished in recent years (Golestani et al. 2021). Thus, it appears that

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an efficient use of offshore space would be the combination of several compatible activities such as fish farming and energy production in what is known as offshore multi-purpose platforms (MPP) (Abhinav et al. 2020). However, the activities developed on multi-purpose offshore platforms pose risks to both wild and farmed fish, with wind turbines and the presence of extensive networks of submarine cables producing potentially stressful stimuli for fish, such as, among others, electromagnetic fields (Ohman et al. 2007; Svendsen et al. 2022).

Studies on various fish (salmonids, tilapia, yellowfin tuna, zebrafish) have shown that they utilize the local magnetic field for orientation and respond experimentally to artificial stimuli (Formicki et al. 2015; Hutchison et al. 2020; Ohman et al. 2007; Putman et al. 2020; Svendsen et al. 2022). However, little is known about possible effects of the magnetic field when it comes to aquaculture fish. Due to the obvious link between migration and magnetoreception, concerns are raised for possible EMF effects on physiology or behavior. Historically, most behavioral studies with EMFs have focused on elasmobranchs and migratory species such as salmonids, where the effects on their orientation ability have been extensively documented (Anderson et al. 2017; Formicki et al. 2019). Specifically, it has been shown that these species perceive changes in orientation along the north–south axis and align with it. However, this ability is also reported for non-migratory species such as carp, where a preference for body alignment with the magnetic field lines of the earth's magnetic field has been observed (Hart et al. 2012). Another study on zebrafish (Ward et al. 2014) showed that the effect of a strong static magnetic field (4.7–11.7 T) results in disruption of orientation and locomotor behavior. However, even today, the way in which different fish species detect and/or orient themselves to the magnetic field remains enigmatic (Naisbett-Jones and Lohmann 2022; Schneider et al. 2023).

With the motive of assessing the feasibility of fish farming along with marine energy production, in this short communication we aimed to investigate potential effects of EMFs on the behavior of European sea bass (*Dicentrarchus labrax*); one of the most important species in the Mediterranean aquaculture. To examine whether the species exhibits magnetoreception and to what extent it is malleable to external stimuli, we subjected E. sea bass fingerlings to an artificial EMF and analyzed several behavioral metrics under a controlled behavioral-testing setup (T-maze).

Materials and methods

Husbandry

The behavioral trial was performed in the spring of 2021 using E. sea bass fingerlings with a mean weight of 3 g. They were obtained from the intensive hatchery of the Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research. The fish were placed in a 500 dm^3 cylindrical holding tank equipped with a biological and mechanical filter, fed manually three times a day, and were kept under a 12L:12D photoperiod regime at 23 °C. The fish were left to acclimate to those conditions for 10 days prior to any behavioral testing. The trial was approved by the Ethics Committee of the IMBBC (Ref Number 114/2023) and was conducted in certified laboratories (EL91-BIOexp-04) in accordance with legal regulations (EU Directive, 2010/63).

The experimental setup

The experimental protocol entailed the use of a Helmholtz coil (YP Magnetic Technology Development Co., Ltd, model number HLY30-100) to generate a homogeneous static EMF in combination with a T-maze (Fig. 1a), which is a widespread experimental setup for conducting behavioral tests (Braida et al. 2014; Echevarria et al. 2016; Vignet et al. 2013). The T-maze (homemade construction, UoC) was made of plexiglas and was peripherally covered with opaque material to cut off visual stimuli to the subjects inside. It comprised two arms and a start zone, which could be isolated from the rest of the device by a screen made of the same material. The T-maze was placed inside the Helmholtz coil, with the arms aligned along the north–south axis of the Earth's magnetic field and the orientation of the EMF being horizontal to the T-maze. The magnetic induction of the EMF was 10 mT DC, which, despite being of higher intensity than that of marine power cables (order of μT, Hutchison et al. 2020), was chosen in order to elicit effects as is typically done in behavioral and physiological tests on marine animals (EMF intensity of 0.1–36 mT over 35 studies, Albert et al. 2020). An IP camera (Basler AG) was installed 50 cm above the T-maze and was used to record videos that were subsequently analyzed using specialized software.

The T-maze protocol

Recordings were made individually on fish in the absence of EMF (control group), and on fish while the Helmholtz coil was in operation, which were designated as the EMF group. The intensity of the EMF was 10 mT and its orientation was offset by 180° relative to the earth's magnetic field. Following established protocols in zebrafish (Avdesh et al. 2012; Colwill et al. 2005), acclimation of fish to the T-maze was performed prior to the start of recordings. For each video recording, a single fish was placed in the start zone of the maze, with the separator closed, approximately 2–3 h after its morning meal. After 2 min, the separator was removed, and the fish was allowed to freely explore the maze while the process was being recorded continuously. Recordings were made at 25 frames per second and the duration was 20 min. Between recordings, the maze was flushed with fresh water and

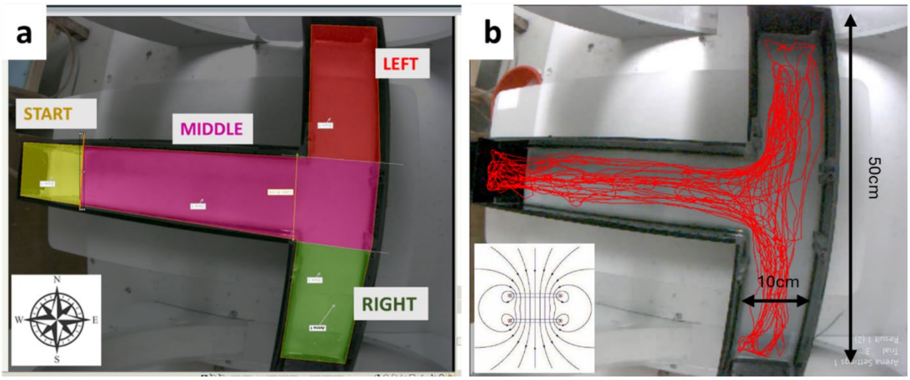

Fig. 1 Use of Ethovision software to study behavior in European sea bass (*Dicentrarchus labrax*) fingerlings: **a** the four characterized T-maze activity zones and their orientation relative to earth's polarity and **b** an example of the movement of a single individual (red line), as detected and recorded during a 20-min video. The arrows on the bottom left corner indicate the orientation of the EMF and the direction of the magnetic field lines

replenished with new seawater from the reservoir. For each treatment, 25 individuals were used, one for each recording, resulting in an equal number of measurements (25 individual biological replicates). During the process, some fish exhibited freezing behavior which was defined as instances of fish remaining in the maze starting area for at least the first 10 min of the recording. In such cases, the presence of freezing behavior was noted manually by the experimenter upon an initial screening of the video; the recording was discarded, and a new fish was used as a replacement.

Behavioral analysis

The recorded videos were analyzed with specialized software (Ethovision, Noldus Information Technology BV). Specifically, the maze was divided into distinct zones, as shown in Fig. 1a, which corresponded to the starting position of the fish, the intermediate zone, and the two arms. Then, using the software, the position of the fish was determined for each frame of the video (Fig. 1b) allowing the automatic calculation of several metrics of swimming behavior. These metrics included the distance traveled (total distance in mm covered during the 20-min video), the latency period (the time from the start of the recording until the fish first reached either of the T-maze arms), the average velocity (average speed during the recording, mm s^{-1}), the maximum acceleration (maximum value of accelerated swimming, mm s^{-2}), meandering (deg min^{-1}), and time (s) spent in each of the maze zones. Regarding meandering, it is a measure of the tortuosity of a trajectory, and it was measured here as the change in moving direction of the central body point, relative to the distance moved. Treatment means and SD for the above metrics are reported. Treatment effects were analyzed in SPSS software (version 22) via two-tailed *t*-test for the parameter "distance" at $P < 0.05$ level of significance after confirming the assumptions of normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). The non-parametric Mann–Whitney *U* test was applied for "latency," "velocity," "meandering," and "max acceleration," since those assumptions were not met.

Results

The first behavioral metric analyzed was the freezing behavior. This behavior occurred in only one fish in the control group, accounting for 4% of the counts, while it was markedly more frequent in the EMF group with 4 fish (16%). Examples of this behavior are given in Fig. 2.

There was also a significant difference in the latency period, which in this case was the time from the start of recording to the arrival of the fish on either of the arms of the maze. On average, this time was six times higher for the group exposed to the EMF, suggesting that fish in the control group commenced exploratory behavior faster than those exposed to EMF. The total distance traveled by fish was also significantly lower for the EMF group. Moreover, the trajectories of the animals differed in their tortuosity, with the parameter meander being significantly higher for the EMF fish (Table 1). On the contrary, the presence of EMF did not affect the average swimming velocity. However, the maximum acceleration values recorded in the videos were affected, with the EMF group exhibiting higher values.

Finally, the distribution of the time spent by the fish in the different zones of the maze was analyzed and is shown in the graphs of Fig. 3 for the different groups. Regarding the

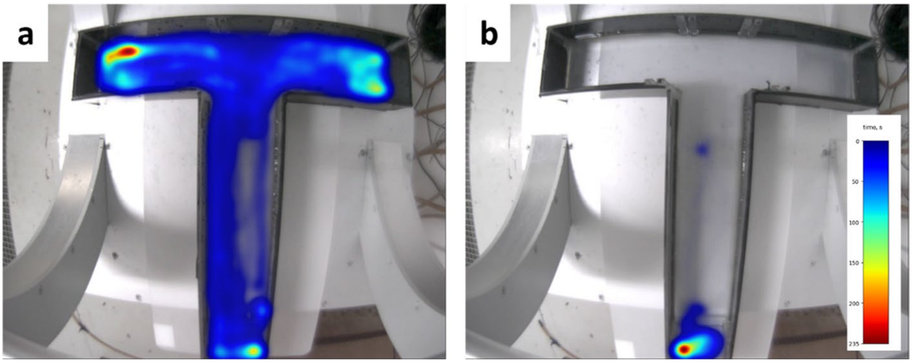

Fig. 2 Activity distribution heatmap (time, s) of a single European sea bass (*Dicentrarchus labrax*) fingerling displaying typical exploratory behavior (**a**) and freezing behavior (**b**) during a 20-min recording in a T-maze

Table 1 Behavioral metrics of the European sea bass (*Dicentrarchus labrax*) fingerlings in the absence (control) and presence (EMF) of a 10-mT static electromagnetic field as recorded in a T-maze apparatus and analyzed with specialized software (Ethovision, Noldus Information Technology BV). Mean values ± SD are provided

Behavioral metric	Control	EMF	statistics
Latency period (s)	46.9 ± 56.1	305.6 ± 130.8	$U= 8, n_1 = n_2 = 25, P< 0.001$
Distance (cm)	24.6 ± 11.1 10^3	18.1 ± 5.8 10^3	$t - value= 2.58, df = 48, P= 0.006$
Meander (deg min^{-1})	6.1 ± 5.1	13.7 ± 8.1	$U= 127, n_1 = n_2 = 25, P< 0.001$
Velocity (mm s^{-1})	16.8 ± 11.7	21.1 ± 13.4	$U= 127, n_1 = n_2 = 25, P= 0.293$
Max acceleration (mm s^{-2})	18.2 ± 16.6 10^3	75.2 ± 43.2 10^3	$U= 49, n_1 = n_2 = 25, P< 0.001$

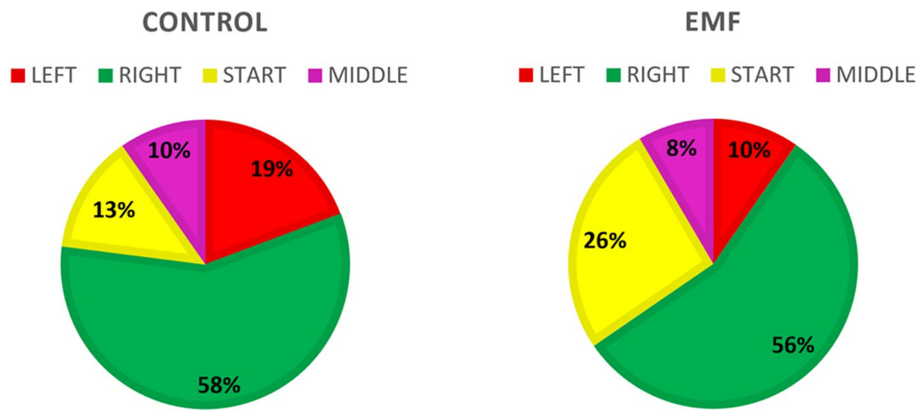

Fig. 3 Percentage distribution of time spent by European sea bass (*Dicentrarchus labrax*) fingerlings in the absence (control) and presence (EMF) of a 10 mT static electromagnetic field in the different zones of a T-maze

control group, the fish showed a preference for the right arm of the maze, oriented toward the geomagnetic south, where they spent 58% of their total time. Time allocated to the left, middle, and initial zones of the maze was, respectively, 19%, 10%, and 13% of the time. The preference for the geomagnetic south was also strong in the EMF group, despite the reversal of EMF polarity (56%). However, in this group, time spent in the initial zone doubled (26%), which came at the expense of time spent in the left arm (10%), while the proportion of time fish spent in the middle zone was unaffected.

Discussion

In this trial, we applied a T-maze protocol to analyze behavioral metrics of E. sea bass fingerlings in the presence and absence of an EMF. An important initial finding was the increase of immobility under the EMF. Freezing behavior is expected to have minimal occurrence under control conditions, but an increase in the presence of additional stressors. In our trials, only one fish in the control group exhibited this behavior; the fish generally started exploring the apparatus soon after the start of recording (low latency), traveled a long total distance, and the time they spent at the start zone reflected a small percentage (13%) of their total travel time. This is similar to the observations of Benhaïm (2012) who also recorded a low percentage (15%) of time spent at the start zone of the T-maze in E. sea bass fingerlings.

Conversely, when exposed to the EMF, immobility generally increased with the fish delaying exploration of the maze as well as decreasing the total distance traveled. The occurrence of freezing behavior increased substantially. Latency also increased with the fish requiring an average period of 5 min to reach either arm of the maze since the start of the recording. In addition, the time spent at the start zone was higher for the EMF group, accounting for 26% of the total time. These patterns suggest that E. sea bass exhibited some sensitivity to EMF and in fact experienced the presence of the EMF as stressful stimuli, as has been the case in experiments with zebrafish where a strong magnetic field resulted in the disruption of locomotor behavior (Ward et al. 2014). Interestingly, Tański et al. (2011) studied the behavioral effects of EMF on E. sea bass but did not report a decrease in activity or any correlation of EMF with the direction of movement. However, considering that the intensity of the EMF on that trial was much lower (0.2 mT opposed to 10 mT used here), it is likely that low EMF intensities are not capable of eliciting a stress response or any other potential disruption of magnetoreceptive orientation or sensory perception, which, however, exceeds the scope of this paper. Moreover, we observed changes in the mode of swimming. The maximum acceleration and meandering were significantly higher for the EMF group, indicating high tortuosity in the movement; a sign of indecisiveness. Finally, based on the proportion of time spent on the various zones of the T-maze, the control and EMF fish showed a preference for the right arm, which coincided with the geomagnetic south. Despite the intensity of the geomagnetic field (25–65 μT) being low (Finlay et al. 2010), it seems that the EMF fish were able to maintain the preference, albeit with higher difficulty (increased latency, lower distance traveled).

This information is highly relevant for aquaculture, specifically when it comes to the designation of farming sites in conjunction with offshore energy production. If EMF can cause these locomotory disruptions in E. sea bass fingerlings, it is likely that the feeding behavior (among other potential physiological impairments), including the detection and capture of feed, will also be impaired, thus, reducing growth performance and fish welfare. Overall, this work shows clear signs of EMF effects on E. sea bass behavior constituting an early step in understanding the magnetoreceptive sensitivity of E. sea bass to a strong EMF. While further experimental work mainly in the direction of behavioral conditioning as well as on the cellular mechanisms underlying magnetic perception is needed, the present work offers a considerable contribution to our understanding of EMF effects on fish, which in turn may be useful in shaping the future of fish farming in conjunction with energy production.

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Author contribution Conceptualization (Papandroulakis Nikos), Formal Analysis (Stavrakidis-Zachou Orestis), Data Acquisition (Stavrakidis-Zachou Orestis, Paraskevi Sidera), Methodology (Stavrakidis-Zachou Orestis, Papandroulakis Nikos), Visualization (Stavrakidis-Zachou Orestis), Funding acquisition (Papandroulakis Nikos), Writing – original draft (Stavrakidis-Zachou Orestis), Writing – review & editing (Stavrakidis-Zachou Orestis, Paraskevi Sidera, Papandroulakis Nikos). All authors have read and agreed to the published version of the manuscript.

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Data availability The experimental data supporting the findings of this study are available within the paper while additional details may be provided from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The trial was approved by the Ethics Committee of the IMBBC (Ref Number 114/2023) and was conducted in certified laboratories (EL91-BIOexp-04) in accordance with legal regulations (EU Directive, 2010/63).

Competing interests The authors declare no competing interests.

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