



Do magnetic fields related to submarine power cables affect the functioning of a common bivalve?

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ABSTRACT

The aim of the study was to determine the effect of static magnetic field (SMF) and electromagnetic field (EMF), of values usually recorded near submarine cables, on the bioenergetics, oxidative stress, and neurotoxicity in the cockle *Cerastoderma glaucum*. Bivalves maintained a positive energy balance, but the filtration rate and energy available for individual production were significantly lower in SMF-exposed animals compared to the control treatment. No changes in the respiration were noted but ammonia excretion rate was significantly lower after exposure to EMF. Changes in the activities of antioxidant enzymes and the lipid peroxidation were not observed however, exposure to both fields resulted in increased protein carbonylation. After exposure to EMF a significant inhibition of acetylcholinesterase activity was observed. As the present study for the first time revealed the oxidative damage and neurotoxicity in marine invertebrate after exposure to artificial magnetic fields, the need for further research is highlighted.

1. Introduction

The demand for sustainable energy sources has resulted in the rapid development of the offshore renewable energy infrastructure. Offshore wind farms have become the most popular source of marine renewable energy and the sector is expected to grow by at least 25% by the year 2030 (DeCastro et al., 2019). The optimal areas for building the offshore wind farms are coastal shallow waters which are typically characterised with high biodiversity (Otremba and Andrulewicz, 2014; Kulkarni and Edwards, 2021). One of the main environmental concerns related to the operation phase of offshore wind farms and other marine renewable energy devices (MREDS) is the introduction of artificial magnetic fields generated by submarine cables into the environment (Petersen and Malm, 2006). Cables present in marine habitats are not only connected with MREDS but also provide electrical interconnections between the distant regions or countries (Chatzivasilieiadis et al., 2013). In 2015 the total length of all submarine cables (including telecommunication cables) has been estimated to be 10^6 km, of which almost 8000 km were high voltage direct current (HVDC) submarine power cables (Ardelean

and Minnebo, 2015) mostly used for transfer energy over long distances (Soares-Ramos et al., 2020). Direct current (DC) cables placed on the sea bottom generate the static magnetic field (SMF) thus modify the natural geomagnetic field. Cables carrying alternating current (AC) which produce low-frequency electromagnetic field (EMF; alternating magnetic fields) are currently most commonly used in offshore wind farms (Soares-Ramos et al., 2020). The intensity of magnetic field (measured as the magnetic induction expressed in teslas (T)) depends on the distance from the cable cores, the relative position of the cables, and is linearly dependent on the intensity of the flowing current (Otremba et al., 2019). Maximum value of magnetic induction which can be noted in the close vicinity of the cable vary between few and 8000 μ T (Cada et al., 2011; Taormina et al., 2018; Albert et al., 2020), and depends on a number of factors such as the voltage applied, conductor diameter, layers of insulation and internal cable arrangement (Meißner et al., 2006; Hutchison et al., 2021).

As many marine organisms, especially migrating fish are able to perceive the natural geomagnetic field and use it for orientation, navigation and homing (Kirschvink et al., 2001), the presence of artificial

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magnetic fields in the environment may interfere with this ability (Klimley et al., 2021). The electromagnetic energy is also regarded as a type of environmental pollution (GESAMP, 1991; Redlarski et al., 2015). Both static magnetic and electromagnetic fields may be harmful for living organisms (Zhadin, 2001; Ghodbane et al., 2013; Otremba et al., 2019). However, according to existing data based on the theoretical considerations and research conducted at the cellular level and on model organisms, low frequency EMF is considered to be particularly hazardous for biological structure, which is also reflected by the international evaluations of risks to human health (Suzuki et al., 2006; EC, 2013). The European Commission Marine Strategy Framework Directive requires the member states to take the necessary measures to ensure that the introduction of energy, including electromagnetic fields, is at levels that do not adversely affect the marine environment (EC MSFD, 2008). Despite this fact, the research related to the potential effects of magnetic fields generated by submarine cables, on marine fauna is scanty, and therefore relevant environmental safety regulations concerning the introduction of these physical fields to the marine environment are missing. Benthic fauna that lives near the cables may be especially exposed to magnetic fields generated by submarine cables both buried in the sediment and laid on the seafloor. It holds true especially for the sessile organisms with limited capabilities to avoid of or escape from unfavourable conditions. As the ability to perceive magnetic fields is documented in some crustacean species (Arendse and Kruyswijk, 1981; Lohmann, 1985), the number of studies related to the effects of magnetic fields and submarine cables on crustaceans has been increasing in recent years, focusing mainly on the potential alterations of behaviour in crabs and lobsters (Love et al., 2017; Ernst and Lohmann, 2018; Scott et al., 2018, 2021; Hutchison et al., 2020; Taormina et al., 2020). However, much less attention has been paid to other important groups of benthic fauna, including bivalves. In addition, the results obtained so far are not conclusive. No significant effects of SMF were documented on the survival, condition and gonad index of blue mussel *Mytilus edulis* (3.7 mT; Bochert and Zettler, 2004), and on the behaviour of freshwater clam *Elimia clavaeformis* (up to 36.4 mT; Cada et al., 2011), whereas significant changes were observed in hydration and amine nitrogen in blue mussel (5.8–80 mT; Aristarkhov et al., 1988). The negative effect of EMF, in turn, was manifested in cellular stress responses in blue mussels (0.3–0.6 mT; Ottaviani et al., 2002; Malagoli et al., 2003, 2004) and in significantly elevated genotoxicity endpoints in Baltic clam *Macoma balthica* (1 mT; Stankeviciūtė et al., 2019).

The aim of the present study was to find out if exposure to static magnetic field and electromagnetic field (50 Hz), of values typically recorded in the close vicinity of submarine cables, affect the physiological processes and bioenergetics, and induce oxidative stress and neurotoxicity in the cockle *Cerastoderma glaucum*. *C. glaucum* is a common bivalve of shallow environments of the North-East Atlantic Ocean and European seas, including the Baltic Sea. Sites inhabited by this opportunistic filter feeder, which lives near the sediment surface, are characterised by high bioturbation potential (Rueda and Smaal, 2002; Gogina et al., 2017).

We hypothesize that both magnetic fields affect the basic physiological processes of *C. glaucum* and consequently the amount of energy for growth and reproduction, but that more pronounced effect is observed after exposure to potentially more hazardous EMF. Therefore, to assess the potential effects on the overall fitness we examined the rates of food consumption, oxygen consumption, ammonia excretion and energy balance (scope for growth) which constitute a complex bioassay of environmental stress (Naylor et al., 1989; Widdows et al., 2002). One of the main mechanisms that magnetic fields interact with biological systems is the stimulation of free radicals' activity, increasing of their concentration and prolonging of their life time, which consequently lead to oxidative stress (Ghodbane et al., 2013; Lai, 2019). There is also evidence that activity of acetylcholinesterase (AChE), a key enzyme for the functioning of nervous system, usually declines in response to magnetic fields (Stegemann et al., 1993; Ravera et al., 2010).

However, the parameters related to oxidative stress and the AChE activity have never been investigated in marine animals exposed to magnetic fields. Nevertheless, it is assumed that the exposure to both magnetic fields will result in significant changes in parameters related to the antioxidant defence and/or oxidative damage as well as in the activity of whole-body AChE.

2. Materials and methods

2.1. The experimental setup

Two experiments were performed, one on the effects of SMF and the other on the effects of EMF. The experimental set-up consisted of a SMF or EMF generator (depending on the experiment), two identical experimental (located in SMF/EMF) and reference (located in natural geomagnetic field ~0.05 mT) aquaria (V = 25 L), connected by flow-through system to a conditioning tank (V = 900 L) equipped with a cooling system (Titan 4000, Aqua Medic, Bissendorf, Germany). The magnetic field generators were designed and constructed at Gdynia Maritime University. The generators were previously described by Fey et al. (2019) and Jakubowska et al. (2021). Briefly, the generators consist of Helmholtz coils, which were powered by laboratory switching mode power supplies HCS-3602 (Manson, China) or variable auto-transformer RAVISTAT 15 P-1 (Ravi Electricals Pvt. Ltd, India), for SMF and EMF respectively. The generators produce spatially almost uniform (throughout the entire volume of the experimental aquaria) SMF or EMF with any value of the magnetic induction controlled by the current in the coils, up to 15 mT. A teslameter (13610–93, PHYWE, Gottingen, Germany) was used to monitor and adjust the values of the magnetic induction of both SMF and EMF. The Helmholtz coils in both generators were cooled by circulating water from the cooling unit (Titan 2000; Aqua Medic; Bissendorf, Germany).

An algebraic model [1] based on the Biot-Savart law that relates the induction value to the distance from an infinitely thin line conductor through which the electric current flows, was used to determine the distribution of magnetic field induction and thus to estimate the experimental value.

$$B = \frac{\mu_r \mu_0}{2\pi} I a^{-1} \quad [1]$$

where: a – distance from the wire; B – magnetic induction (T); μ_0 – permeability of a vacuum = $12.56637 \cdot 10^{-7} \text{ V s A}^{-1} \text{ m}^{-1}$; μ_r – relative permeability of water (0.999992); I – current carried by the wire (A).

Chosen experimental value of magnetic induction (6.4 mT) was calculated as the highest values potentially perceived in the close proximity to the cable surface (diameter of the cable core 4.4 cm and the outer cable diameter 10 cm; Cross-linked polyethylene (XLPE) Submarine Cable Systems, 2010) characterized by electrical current 1500 A. The value was calculated using the finite element method (i.e. taking into account the current flow in the entire core, not only in its center; more detailed description of calculation is presented in the Supplementary Material).

2.2. Animal collection and maintenance

Animals for experiments were collected from the Puck Bay (inner Gulf of Gdańsk, southern Baltic Sea). For SMF experiment *C. glaucum* individuals were obtained from the sandy site located at Kuźnica at a ~100 cm water depth in June 2020. Bivalves for EMF experiment were collected in outer Puck Bay from 9 m depth (Long: 18.5538381 Lat: 54.6279667) during research cruise using the bottom dredge, in October 2020. During each animal collection the sediment was also gathered and sieved on site over a 1 mm mesh. After transportation to the laboratory bivalves were kept in natural sediment and seawater (T = 16 °C, S = 7) for one week before the transferring to the experimental setup.

2.3. General experimental design

Since two independent experiments were carried out - on the effects of SMF and EMF, all the steps described below were taken in an analogous way for both. Bivalves were placed in 1.7 L containers filled with 1 L of filtered (GF/F filter, Whatman 47 mm, 0.7 μm) artificial seawater (commercial sea salt, Sea Salt, Aquaforest, Poland; $T = 16\text{ }^{\circ}\text{C}$, $S = 7$) and ashed ($450\text{ }^{\circ}\text{C}$, 12 h) fine sediment (97% of size fraction in a range 0.125–0.250 mm). Ten (SMF) or eleven (EMF) individuals were put in each container. The water in each container was aerated with air stones and an aquarium air pump (Pro Silent a400, JBL, Germany). Three containers with bivalves were placed in experimental aquarium (situated in SMF/EMF) and three in the control one. Bivalves were fed twice a day with planktonic green algae *Monoraphidium contortum* (BA05) isolated from the coastal zone of the Gulf of Gdańsk, and maintained as unialgal cultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk. *M. contortum* with high lipid content was chosen as feed for bivalves (Bogen et al., 2013). After 3 days of acclimation the SMF/EMF generator was switched on and bivalves were exposed to appropriate magnetic field of 6.4 mT for 8 days. Survival was checked daily and dead individuals were immediately removed. The behaviour of bivalves assessed as the number of individuals on the sediment surface was monitored at the same time every day. The water temperature was monitored constantly by Hobo loggers UA-001-08 (Onset, USA). The water in each container was exchanged every three days. For the determination of physiological processes and SFG, 18 individuals per treatment (shell length 12.84 ± 0.59 in SMF experiment; 14.45 ± 0.98 in EMF experiment) were used. Remaining bivalves, which survived to the end of the experiment, were frozen in liquid nitrogen and then transferred to $-80\text{ }^{\circ}\text{C}$. Those individuals were used for the analyses of whole-body oxidative stress biomarkers and AChE activity. After experiments the length, width and height of shell ($\pm 0.01\text{ mm}$) as well as total wet weight of each bivalve ($\pm 0.01\text{ mg}$) were determined. In case of bivalves used for scope for growth also the dry weight (48 h at $60\text{ }^{\circ}\text{C}$) of tissue and shell were determined. The detailed morphometric parameters of bivalves used in the experiments are shown in Table S1.

2.4. Scope for growth

2.4.1. Food consumption rate

The food consumption rate of *C. glaucum* was calculated based on the determination of the clearance rate. Clearance rate was estimated by measuring the removal of suspended particles from the water in a static stirred system. Containers (six per SMF/EMF treatment, six per relevant control and one control container without bivalves per experiment) were filled with 1.2 L of filtered (GF/F filter, Whatman 47 mm, 0.7 μm) artificial seawater ($T = 16\text{ }^{\circ}\text{C}$, $S = 7$) and tightly sealed with lids with built-in electrical stirrers. Three bivalves of a similar size were placed at the bottom of each container. Containers were put in the tank ($V = 900\text{ L}$) which served as a water bath with the constant temperature. The following steps were performed according to method proposed by Sobral and Widdows (1997a, b) with slight modifications: After 30 min (sufficient time for bivalves to resume pumping activity) the known volume of *M. contortum* culture was added to each container to reach the initial algal concentration $2000\text{ cells mL}^{-1}$. After 5 min (a period for the algal cells to be thoroughly mixed), a 2 mL sample was collected from the centre of each container. Samples were collected from each container every 20 min for 100 min (i.e. on five occasions). The cell concentrations were counted using a BD Accuri C6 Plus flow cytometer (BD Biosciences, San Jose, USA) according to the method described by Śliwińska-Wilczewska et al. (2018). Cell concentrations used in calculations were the means of 3 counts. Clearance rate was calculated using equation [2] given by Coughlan (1969):

$$\text{CR} = V (\ln C_1 - \ln C_2) / t \quad [2]$$

where CR is the clearance rate (litres L h^{-1}), V - volume of water used (L), C_1 and C_2 - cell concentrations between two sampling times, and t - time increment (h).

CR was calculated for each of 5 time increments and the averaged value was used in further calculations.

The dry weight of food was determined by filtrating particular volumes of algae culture of known cell concentration through pre-weighted GF/F filters and weighting of dried (48 h at $60\text{ }^{\circ}\text{C}$) material. The dry weight of the 10^6 cells amounted to $0.73 \pm 0.04\text{ mg}$ ($N = 6$) in the culture medium used in SMF experiment and $1.23 \pm 0.19\text{ mg}$ ($N = 7$) in the culture used in EMF experiment. The energy value of food was determined by burning the material (dried microalgae) and measuring heat produced using bomb microcalorimeter MK-100 (modified Philipson type described by Prus, 1975) following Normant et al. (2002). The energy value of *M. contortum* cells from the culture amounted to 20.55 ± 0.29 ($N = 10$) joules per milligram of dry weight ($\text{J mg}^{-1}\text{ DW}$). The food consumption rate of *C. glaucum* was calculated from clearance rates (CR) multiplied by the concentration of food (mg L^{-1} ; Widdows, 1978) and expressed in milligrams of dry weight per gram of DW of tissue per day ($\text{mg g}^{-1}\text{ DW d}^{-1}$), and then converted into energy equivalents - joules per gram of DW of tissue per day ($\text{J g}^{-1}\text{ DW d}^{-1}$) by multiplying by the energy value of food.

2.4.2. Food absorption efficiency

Faeces were collected daily from each container with bivalves through 8 days of SMF/EMF exposure. Faeces were collected using pipette and were rinsed with deionised water in order to remove potentially attached sediment particles. Then they were put onto pre-ashed (12 h at $450\text{ }^{\circ}\text{C}$) GF/F glass filters, dried (48 h at $60\text{ }^{\circ}\text{C}$) and, after determination of their dry weight, ashed again to determine the content of organic matter (Gnaiger and Bitterlich, 1984). The organic content of faeces ranged from 22.82 ± 4.05 (control EMF) to 30.68 ± 0.12 (control SMF). The formation of pseudofaeces was not observed. The organic matter content of food, calculated from the weight loss on ashing filters with algae culture in a muffle furnace, reached $97.50 \pm 2.52\%$ ($N = 6$) in SMF experiment and $95.79 \pm 5.73\%$ ($N = 7$) in EMF one. The food absorption efficiency (AE), which represents the efficiency of absorption of organic matter from ingested food, was calculated using formula [3] given by Conover (1966):

$$\text{AE} = (F - E) \cdot [(1 - E) \cdot F]^{-1} \cdot 100 \quad [3]$$

where F is the ratio of ash-free dry weight to total dry weight in the food, and E is the ratio of ash-free dry weight to total dry weight in the faeces.

2.4.3. Oxygen consumption rate

The oxygen consumption rate was determined after 8 days of SMF/EMF exposure in closely sealed respirometric glass chambers ($\sim 125\text{ mL}$, $9 \times 4 \times 4\text{ cm}$ external dimensions) using the UnderWater Meter (Unisense, Denmark) equipped with oxygen needle microsensors (OX-500). Measurement of oxygen consumption of each individual was performed in two replicates. Each bivalve was gently transferred to a respirometric chamber filled with aerated artificial seawater ($T = 16\text{ }^{\circ}\text{C}$, $S = 7$). The chambers were placed into exposure and reference aquaria used as the water baths. Three identical respiration chambers but without bivalves served as controls. The oxygen concentration in each chamber was measured at the beginning (initial oxygen concentrations) and after 1.5–2 h of incubation (final oxygen concentrations). The oxygen concentration in the chambers did not fall below 7.8 mg L^{-1} . The resting respiration rate ($\text{mg O}_2\text{ g}^{-1}\text{ DW d}^{-1}$) was calculated from the difference between the initial and final oxygen concentrations in the respiration chambers, taking into account the controls, according to Sobral and Widdows (1997a). The amount of energy ($\text{J g}^{-1}\text{ DW d}^{-1}$) expended on metabolic processes was calculated using the oxycaloric equivalent ($1\text{ }\mu\text{mol O}_2 = 0.450\text{ J}$) given by Gnaiger (1983).

2.4.4. Ammonia excretion rate

The rate of ammonia excretion was determined based on Koroleff (1976) indophenol method. The concentrations of ammonia were measured in water collected from respirometric chambers (three replicates per chamber, including controls) after the measurements of respiration rate (Hulathduwa et al., 2007; Jakubowska et al., 2019). The ammonia excretion rate was calculated ($\mu\text{mol NH}_4^+ \text{g}^{-1} \text{DW d}^{-1}$), and then converted into energetic units ($\text{J g}^{-1} \text{DW d}^{-1}$) using the ammonia energy equivalent ($\mu\text{mol NH}_4^+ = 0.348 \text{ J}$; Elliott and Davison, 1975).

2.4.5. Energy available for production

The quantity of energy assimilated from food (A) was calculated by multiplying the amount of energy consumed as food (C) by the absorption efficiency (AE). In order to calculate the amount of energy available for individual production (scope for growth), the rates of physiological processes, expressed in energy units were put to the energy budget equation [4] (Winberg, 1956):

$$P = A - U - R \quad [4]$$

where P is energy available for production, A - energy assimilated from food, U - energy excreted as ammonium and R - energy expended on metabolic processes.

2.5. Determination of biomarkers of oxidative stress and neurotoxicity

The samples (1 individual per sample) were homogenized with a Teflon-pestle homogenizer in ice-cold buffer containing 50 mM Tris-H₂SO₄, pH 7.6 with 0.1 mM EDTA, 2 mM DTT and 0.2% TritonX-100. The homogenates were centrifuged at 14 000 RCF for 30 min at 4 °C. The supernatants were transferred into fresh tubes and used for analysis. Protein was determined by the Lowry method (1951) with modification of Peterson (1977). Enzyme activities of glutathione S-transferase, catalase and superoxide dismutase were measured spectrophotometrically, using UV-VIS Spectrophotometer (Beckman Coulter), according to methods described in Supplementary Material S1A. Total glutathione content (tGSH) and Total Antioxidant Capacity (TAC) were measured using a spectrophotometer, Synergy 2 Multi-Mode Reader (BioTek) and assay methods were included in Supplementary Material S1B. Lipid peroxidation product (malondialdehyde - MDA) and protein peroxidation products (carbonyl groups - CBO) were also determined spectrophotometrically using Synergy 2 Multi-Mode Reader (BioTek). A description of the method for the determination of these products is provided in Supplementary Material.

The whole-body acetylcholinesterase (AChE; EC 3.1.1.7) activity in *C. glaucum* proposed for assessment of pollution by anticholinesterase agents in estuarine areas (Scaps et al., 1996, 1997; Scaps and Borot, 2000) was measured with Acetylcholinesterase Activity Assay Kit (MAK119, Sigma-Aldrich). This assay is an optimized version of the Ellman method in which thiocholine, produced by AChE, reacts with 5, 5'-dithiobis (2-nitrobenzoic acid) to form a colourimetric product, proportional to the AChE activity present. The absorbance was followed for 10 min at 412 nm in Synergy 2 Multi-Mode Reader (BioTek). Total AChE activity was expressed in $\text{nmol min}^{-1} \text{mg}^{-1}$ of total protein concentration.

2.6. Statistical analyses

The normal distribution of the data was verified by the Shapiro-Wilk test. Parametric *t*-test (for data characterised by a normal distribution) or Mann-Whitney *U* test (for non-parametric data) at a confidence level of $\alpha = 95\%$ were used to test the significance of the differences between rates of physiological processes and the activities/concentrations of biomarkers of oxidative stress and neurotoxicity between organisms exposed to SMF/EMF and relevant controls. The significance of differences in the proportion of individuals buried in the sediment were

compared among the treatments using the difference between two proportions test. All analyses were performed using STATISTICA software (10.0 Software, Inc. PA, USA).

3. Results

Averaged ($N = 3$) survival of *C. glaucum* after 8 days of exposure to SMF amounted to 80%. The same survival rate was observed in the control group. After exposure to EMF, the survival reached 97% and 94% in the relevant control.

In SMF experiment the percentage of bivalves present on the sediment surface averaged for 8 days, ranged between the replicates ($N = 3$) from 7.5 to 22.1.0%, whereas in the control treatment it varied between 8.1 and 15.8% (Fig. S1A). During EMF exposure, the percentage on bivalves present on the sediment surface varied from 19.45 to 26.5% (23.4–39.0% in control treatment; Fig. S1B). The rest of the bivalves were completely or partially buried in the sediment. The differences in number of bivalves buried in the sediment as well as present on the sediment surface were negligible (difference between two proportions test, $P > 0.05$) between the individuals exposed to magnetic fields and relevant controls.

The food consumption rate of *C. glaucum* exposed to SMF amounted to $353.54 \pm 147.29 \text{ mg g}^{-1} \text{DW d}^{-1}$ and was significantly lower (Mann-Whitney *U* test, $U = 43$, $N1 = 17$, $N2 = 18$, $P = 0.003$) than in adequate control treatment ($602.67 \pm 217.18 \text{ mg g}^{-1} \text{DW d}^{-1}$).

In EMF-exposed bivalves the food consumption rate ($454.65 \pm 208.93 \text{ mg g}^{-1} \text{DW d}^{-1}$) was not significantly different (Mann-Whitney *U* test, $U = 128$, $N1 = 18$, $N2 = 18$, $P = 0.289$) from the control ($578.77 \pm 326.71 \text{ mg g}^{-1} \text{DW d}^{-1}$).

Neither the exposure to SMF nor EMF affected the respiration rate of *C. glaucum* (Mann-Whitney *U* test, $U = 136$, $N1 = 17$, $N2 = 18$, $P = 0.586$; *t*-test, $t = 1.556$, $N1 = 18$, $N2 = 18$, $P = 0.128$; respectively) which amounted to $107.62 \pm 52.83 \text{ mg O}_2 \text{ g}^{-1} \text{DW d}^{-1}$ in SMF ($94.14 \pm 44.31 \text{ mg O}_2 \text{ g}^{-1} \text{DW d}^{-1}$ in relevant control) and $51.74 \pm 11.86 \text{ mg O}_2 \text{ g}^{-1} \text{DW d}^{-1}$ in SMF ($58.90 \pm 15.51 \text{ mg O}_2 \text{ g}^{-1} \text{DW d}^{-1}$ in adequate control).

The ammonia excretion rate in bivalves was not affected by the exposure to SMF (*t*-test, $t = 0.489$, $N1 = 17$, $N2 = 18$, $P = 0.489$). The rate of this process amounted to $384.48 \pm 107.79 \mu\text{mol NH}_4^+ \text{g}^{-1} \text{DW d}^{-1}$ in SMF and $421.01 \pm 191.63 \mu\text{mol NH}_4^+ \text{g}^{-1} \text{DW d}^{-1}$ in relevant control. In EMF-exposed bivalves the excretion rate ($105.58 \pm 31.96 \mu\text{mol NH}_4^+ \text{g}^{-1} \text{DW d}^{-1}$) was significantly lower (*t*-test, $t = 2.153$, $N1 = 18$, $N2 = 18$, $P = 0.038$) than in the control treatment ($135.44 \pm 49.44 \mu\text{mol NH}_4^+ \text{g}^{-1} \text{DW d}^{-1}$).

The amount of energy available for production (scope for growth) was positive in bivalves exposed to both SMF and EMF as well as in adequate control treatments since the quantity of energy assimilated from the food was higher than the energy expenditure (Fig. 1, Table 1). The differences in the amount of energy for production between SMF-exposed bivalves and individuals from relevant control were significant (Mann-Whitney *U* test, $U = 34$, $N1 = 17$, $N2 = 18$, $P = 0.001$), whereas no significant changes in relation to control treatment were recorded in *C. glaucum* exposed to EMF (Mann-Whitney *U* test, $U = 130$, $N1 = 18$, $N2 = 18$, $P = 0.319$). The rates of physiological processes expressed in energy units and bioenergetic parameters are presented in Table 1.

Increased CBO in relation to relevant controls was recorded in *C. glaucum* after exposure to both magnetic fields (*t*-test, $t = -3.301$, $N1 = 8$, $N2 = 6$, $P = 0.006$; Mann-Whitney *U* test, $U = 10.000$, $N1 = 13$, $N2 = 10$, $P = 0.001$, respectively for SMF and EMF; Figs. 2F and 3F). No significant changes were observed in indicator of lipid peroxidation (MDA) and in whole-body biomarkers of antioxidant activity (GST, GSH, SOD and CAT) after exposure to both SMF and EMF (*t*-test, Mann-Whitney *U* test, $P > 0.05$; Figs. 2 and 3).

Exposure to electromagnetic field caused significant decrease in the activity of whole-body AChE in *C. glaucum* (*t*-test, $t = 2.115$, $N1 = 13$,

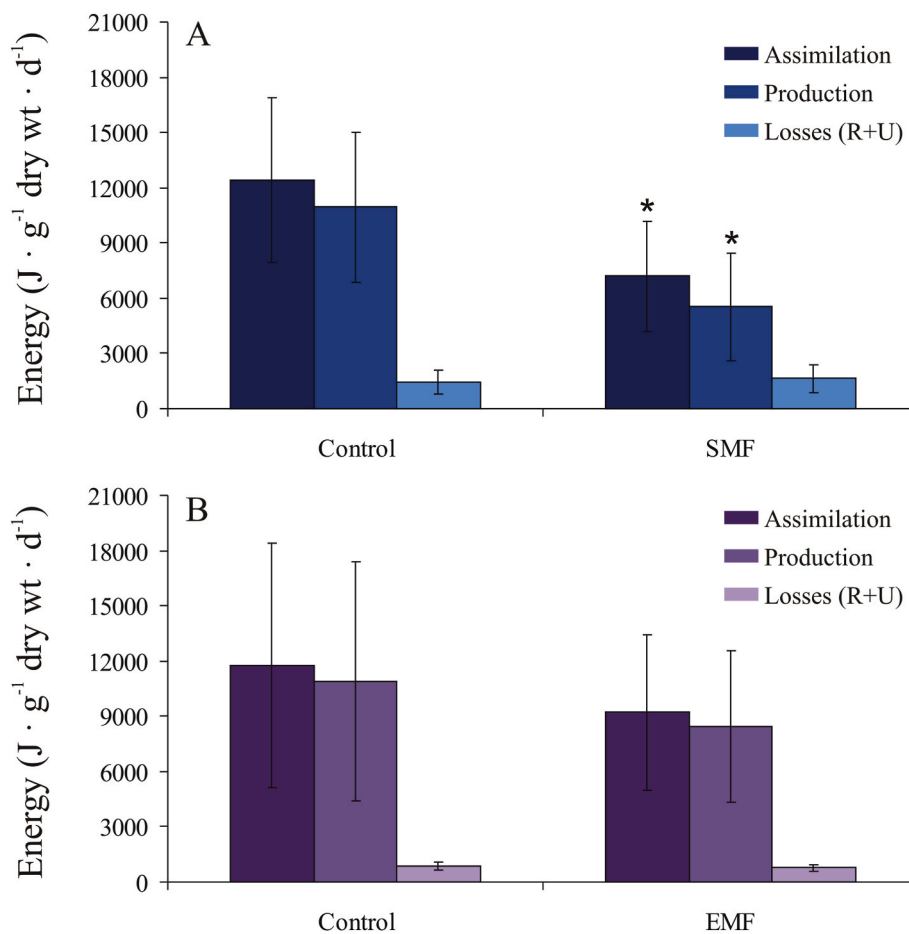


Fig. 1. Rates of energy losses (R + U) and energy available for production (P) in relation to energy assimilated (A) from food of *C. glaucum* after 8-day exposure to SMF (A) and EMF (B) of 6.4 mT in relation to relevant controls (mean \pm SD; $N = 17-18$). Asterisks indicate significant ($P < 0.05$) changes in relation to adequate control treatments.

Table 1

Bioenergetic parameters ($J g^{-1} DW d^{-1}$), AE coefficient (%) and K_2 coefficient of *C. glaucum* after 8-day exposure to SMF (A) and EMF (B) of 6.4 mT in relation to relevant controls. Asterisks indicate significant changes in relation to adequate control treatments.

	SMF experiment		EMF experiment	
	Control	SMF	Control	EMF
Energy allocation				
Ingested (C)	12573.07 \pm 4539.90	7265.18 \pm 3026.83*	11893.72 \pm 6713.94	9343.01 \pm 4293.43
Assimilated (A)	12430.71 \pm 4488.76	7193.29 \pm 2995.48*	11752.67 \pm 6632.71	9218.23 \pm 4225.07
Respired (R)	1323.87 \pm 623.13	1513.34 \pm 742.84	828.29 \pm 218.12	727.56 \pm 166.81
Excreted (U)	146.51 \pm 66.69	133.8 \pm 37.51	47.13 \pm 17.20	36.74 \pm 11.12*
Production (P)	10960.33 \pm 4083.92	5546.15 \pm 2927.70*	10877.24 \pm 6501.90	8453.92 \pm 4122.10
AE (%)	98.87 \pm 0.1	99.02 \pm 0.7	98.82 \pm 0.8	98.69 \pm 0.29
K_2 (P/A)	87.95 \pm 4.36	74.43 \pm 11.27	90.95 \pm 3.97	90.69 \pm 4.11

$N_2 = 10$, $P = 0.046$; Fig. 4B), whereas no such effect was recorded in bivalves exposed to static magnetic field (t -test, $t = 0.407$, $N_1 = 8$, $N_2 = 6$, $P = 0.691$, Fig. 4A).

4. Discussion

C. glaucum maintained positive energy balance after exposure to both static magnetic and electromagnetic fields. The positive and high value of SFG is similar to the results of our previous study on the polychaete *Hediste diversicolor*, in which it amounted to 85% after 8-day exposure to both 1 mT EMF and to control conditions (Jakubowska et al., 2019). However, in the present study, the amount of energy for production was high in bivalves exposed to SMF, but significantly lower than in the relevant control (K_2 (P/A) = 74 and 88%, respectively). It was connected with significantly decreased filtration rates in SMF-exposed animals compared to the control treatment. Filtration rate, and pumping activity in general, are sensitive endpoints for evaluating the biological effects of various stressors in marine bivalves and are usually diminished in unfavourable conditions (Akberali and Trueman, 1985; Loayza-Muro and Elías-Letts, 2007). Thus, decreased food intake or its assimilation are often connected with the lower SFG (Naylor et al., 1989; Sobral and Widdows, 1997a). It should be underlined that algal monoculture used as food in the present study was characterised by high energy value and organic matter content and was provided in high concentrations, thus the amount of assimilated energy was very high. In the natural environment the potential food for suspension feeding bivalves may be composed of different macroalgae species of lower energetic value and poorer digestibility. In addition, food availability may be limited. Therefore, if exposure to SMF reduces the filtration rate independently on food concentration and its energetic value, it may have severe effect on the overall energy budget of the bivalve. Consequently, it may affect the local phytoplankton biomass. In EMF-treated bivalves both the

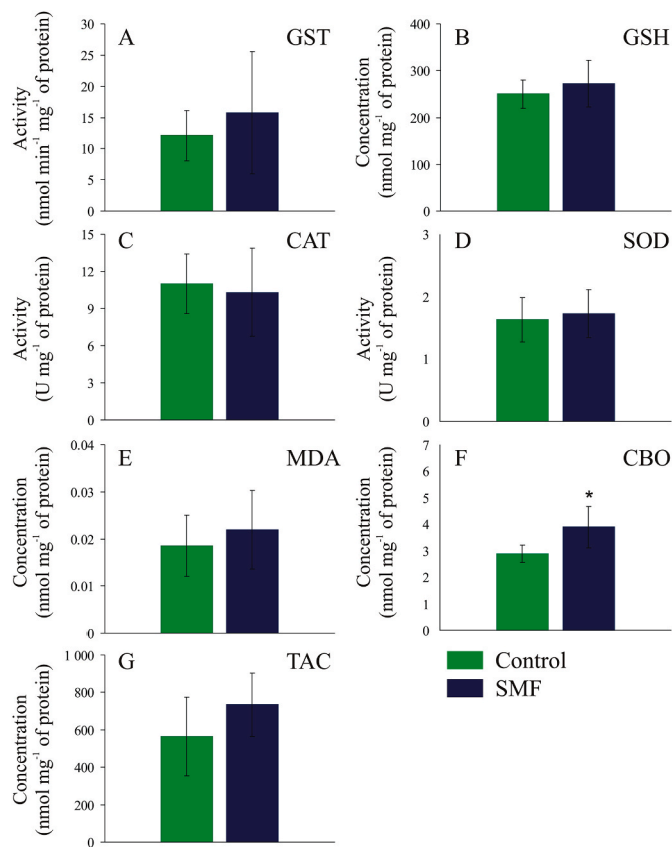


Fig. 2. Biomarkers of oxidative stress: glutathione S-transferase (A), glutathione (B), catalase (C), superoxide dismutase (D), malondialdehyde (lipid peroxidation) (E), protein carbonyl content (CBO) (F), and total antioxidant capacity (G) in *C. glaucum* exposed to SMF of 6.4 mT for 8 days (mean \pm SD; $N = 6-8$). Statistically significant ($P < 0.05$) differences from the control treatment are indicated by asterisks.

filtration rates as well as SFG were not different from the control conditions. The interpretation of obtained results is difficult due to lack of comparable data on the filtration rates of bivalves exposed to artificial magnetic fields. Similarly, food consumption rate in *H. diversicolor* was not significantly affected by exposure to EMF (Jakubowska et al., 2019).

Although coping with environmental stressors is often reflected in increased metabolic rates, the oxygen consumption rate and thus the energy expended on metabolic processes was not affected by the exposure to SMF or EMF. The lack of significant effect of magnetic fields on the respiration rate of *C. glaucum* is in line with previous reports for marine invertebrates such as *H. diversicolor* exposed to 1 mT EMF (Jakubowska et al., 2019) and shrimps *Palaemon squilla* and *Crangon crangon* exposed to SMF and EMF of 3.2 mT (Bochert and Zettler, 2006).

The ammonia excretion rate of *C. glaucum* exposed to SMF was not significantly different from the control conditions, whereas exposure to EMF resulted in its significant (~21%) decrease. Similar effect was observed in *H. diversicolor* exposed to EMF, in which excretion rate was 27% lower compared to the control treatment. As there is a lack of other comparable findings related to ammonia excretion rates in response to magnetic fields, it is difficult to explain the mechanism behind these responses. However, it might be assumed that they are somehow related to the effect of EMF on the permeability of biological membranes (Rosen, 1996) as changes in ammonia excretion and in overall nitrogen metabolism are connected to the osmoregulation and cell volume regulation processes in euryhaline invertebrates (Emerson, 1969; Gosling, 2003). The amount of energy lost in the conversion of nitrogen compounds is very low, therefore changes in excretion rates are not crucial from the bioenergetical point of view. Nevertheless, the effect of

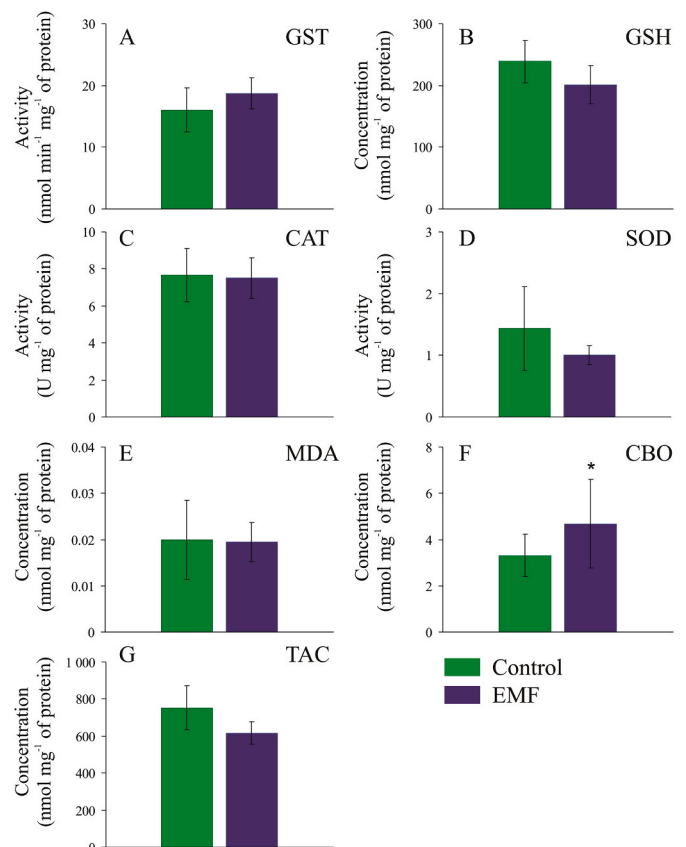


Fig. 3. Biomarkers of oxidative stress: glutathione S-transferase (A), glutathione (B), catalase (C), superoxide dismutase (D), malondialdehyde (lipid peroxidation) (E), protein carbonyl content (CBO) (F), and total antioxidant capacity (G) in *C. glaucum* exposed to EMF of 6.4 mT for 8 days (mean \pm SD; $N = 10-13$). Statistically significant ($P < 0.05$) differences from the control treatment are indicated by asterisks.

EMF on excretion of marine invertebrates definitely require further research.

The activities of enzymes and non-enzymatic mechanisms responsible for the direct interaction with free radicals and protection of cells against the oxidative stress were not significantly suppressed after exposure to SMF and EMF. Despite that, it might be assumed that these mechanisms were insufficient to mitigate ROS generation, which resulted in protein damage after exposure to both magnetic fields. Static and extremely low frequency (ELF) electromagnetic fields accelerate the Fenton reaction - a metal-catalysed conversion of hydrogen peroxide to highly reactive hydroxyl free radicals, therefore increase the activity and concentration of free radicals as well as prolong their life (Ghodbane et al., 2013; Lai, 2019). The lack of changes in the first-line oxidative defence system i.e., activities of SOD and CAT despite observed oxidative protein damage, is not surprising. The presence of free radicals might be associated with both elevation and decline of these enzymes' levels, and those two processes might occur simultaneously with no noticeable change in their activity (DiGulio et al., 1989). Additionally, magnetic fields may also directly influence the activity of ROS-scavenging enzymes. As concluded by Pandir and Sahingoz (2014) the metal ions (Zn^{2+} and Cu^{2+}) contained in SODs enzymes may be influenced by external magnetic fields due to electron distribution in these metals.

Protein carbonylation is the most harmful and irreversible effect of oxidative stress. It is a kind of protein oxidation related to the incorporation of the new functional groups into their structure, which may result in biomolecule malfunctions, increased toxicity and finally lead to cell death (Suzuki et al., 2010; Fedorova et al., 2014). Interestingly,

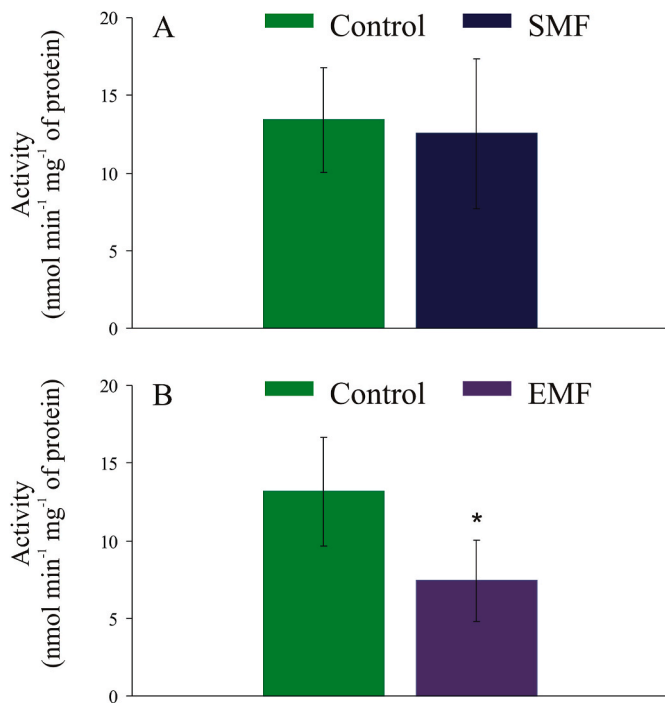


Fig. 4. AChE activity in *C. glaucum* exposed to SMF (A) and EMF (B) of 6.4 mT for 8 days (mean \pm SD; $N = 6-13$). Statistically significant ($P < 0.05$) differences from the control treatment are indicated by asterisks.

increased levels of malondialdehyde (MDA) were not observed in the present study, therefore it might be concluded that protein carbonylation in bivalves is more sensitive to magnetic fields than lipid peroxidation. The fact that similar effects related to oxidative stress were observed after exposure to both SMF and EMF is not surprising, as according to Brocklehurst and McLauchlan (1996) the lifetime of free radicals is much shorter than the period of low frequency EMF, thus free radicals experience EMF as a static field. Strong oxidative stress connected with the suppressed activity of antioxidant enzymes and lipid peroxidation was observed in land invertebrate - flour moth *Ephesia kuehniella* after 72-h exposure to 1.4 T SMF (Pandir and Sahingoz, 2014). Nonetheless, observed effects related to oxidative stress, (i.e., increased/decreased activities of antioxidant enzymes, increased/unchanged MDA concentration) in cells, tissues and organs of land mammals vary depending on the exposure time, magnetic field intensity and type of cells/tissues (Goraca et al., 2010 and references therein; Ghodbane et al., 2013 and references therein).

Inhibition of AChE was noted in various marine invertebrates after exposure to different factors, mainly to chemical pollutants (Scaps et al., 1997; Lionetto et al., 2003; Deidda et al., 2021 and references therein). Whole-body AChE level in some invertebrate species is even proposed for the assessment of pollution in marine and estuarine areas (Scaps et al., 1996; Deidda et al., 2021). Nevertheless, present study indicated for the first time that exposure to electromagnetic field results in the significant decrease of AChE activity in marine bivalves. The inhibition of this enzyme has been previously observed after exposure to ELF EMF of 0.7–2.5 mT in mammalian synaptosomal membranes (Morelli et al., 2005; Ravera et al., 2010) or in AChE enzyme *in vitro* (Fathi and Farahzadi, 2012). According to the latter authors, the observed effect might be related to structural changes in the secondary and tertiary structure of the enzyme, which consequently can affect AChE interaction with the substrate (acetylcholine iodide), and its activity. On the other hand, Afrasiabi et al. (2014) observed increased activity of synaptosomal AChE after exposure to EMF of 0.3–0.6 mT, whereas higher intensity (1.2–1.7 mT) with the same frequency (50–90 Hz) resulted in its decrease.

Interestingly, in bivalves exposed to SMF, AChE levels remained unchanged in relation to the relevant control. Contrary to our results, inhibition of AChE activity after exposure to SMF (1.4 T) was observed in murine bone cells (Stegemann et al., 1993), whereas subjection to 1 mT SMF resulted in increased AChE activity in rat brain (Dinčić et al., 2018). It has been concluded that, at least at the cellular and molecular level, both increasing and decreasing of AChE activity may be observed, depending on the field frequency, intensity and orientation and time of exposure, but explanation of the exact mechanism behind this phenomenon is complicated and yet unknown (Afrasiabi et al., 2014; Dinčić et al., 2018). In the context of abovementioned findings, it would be interesting to test wide range of environmentally relevant values of magnetic fields on AChE activity in marine bivalves in the future studies, to determine eventual thresholds.

AChE inhibition or inactivation which results in acetylcholine accumulation in synapses is usually connected with the alteration of neurotransmission responsible for neuromuscular and behavioural reactions (Deidda et al., 2021). The changes in the behaviour are also the first observable responses of an organism exposed to environmental stress and in case of clams it is related to the burrowing behaviour and burrowing depth (Compton et al., 2016). The behavioural assay which was performed in the present study consisted on the observation of the bivalves' presence or absence on the sediment surface in regular time intervals. No clear pattern in behaviour of *C. glaucum* was observed. Most of the bivalves remained entirely or partially burrowed in the sediment throughout experiments. In the SMF experiment less bivalves were observed on the surface but this observation applies to SMF-treated animals as well as to control ones. It might be related to the smaller sizes of bivalves used in SMF experiment than in EMF as relative burial depth of larger individuals is generally shallower than smaller ones in many bivalve species (Kondo, 1987). In the present study SMF and EMF did not significantly affect the behaviour related to the bivalves' emergence from the sediment. It should be kept in mind, however, that the behavioural tests were restricted to the observations of the presence/absence of bivalves on the sediment surface and that the variability in this parameter was almost as high between the replicates within each treatment as between the treatments. Based on the literature it may be concluded that *C. glaucum* does not exhibit strong behavioural reactions during environmental stress. For example, Griffiths and Richardson (2006) did not observe any behavioural response in *C. glaucum* after exposure to chemical cues emitted from shore crabs, whereas other bivalve - *Macoma balthica* responded by doubling their burial depth to avoid predation. On the other hand, some behavioural alterations were observed in *C. glaucum* subjected to microplastics (Urban-Malinga et al., 2021). Concerning artificial magnetic fields, so far it has been only documented that 48-h long exposure to SMF gradient between 0.19 and 36 mT had no effect on the spatial distribution of freshwater molluscs - clam *Corbicula fluminea* and snail *Elimia claviformis* (Cada et al., 2011).

The present study demonstrated that *C. glaucum* maintained a positive energy balance after exposure to both SMF and EMF as the amount of energy available for individual production (scope for growth) was very high, i.e., the energy intake and assimilation was high compared to the expenditure. However, the filtration rate was significantly lower in SMF-exposed animals compared to the control ones, which resulted in less amount of energy for production. The impair of feeding activity in bivalves is a stress response analogous to the avoidance behaviour. Interestingly, no such situation was observed in EMF-exposed bivalves. The ammonia excretion rate was significantly lower in EMF-exposed bivalves comparing to the adequate control, what, together with similar findings for Baltic polychaete (Jakubowska et al., 2019) allow us to presume that EMF may affect the osmoregulation process or membrane permeability in euryhaline invertebrates, but this issue requires further investigations. No influence was noted on either activities of antioxidant enzymes and non-enzymatic antioxidants or in levels of malondialdehyde (indicator of protein peroxidation), but 8 days of

exposure to both magnetic fields resulted in increased protein carbonylation, which is an equivalent of irreversible protein damage. After exposure to EMF, but not to SMF, a significant inhibition of AChE activity was observed. This neurotoxic effect observed in EMF-treated individuals was not however accompanied by the changes in bivalves' behaviour expressed as the frequency of emergence from the sediment.

It should be emphasized that present study investigated the effect of magnetic fields on physiology and endpoints of oxidative stress and neurotoxicity in marine invertebrates for the first time and it is one of the first research concerning effect of magnetic fields on bivalves at all. Therefore, we decided to apply potentially highest value of magnetic induction which can be recorded in the close vicinity of submarine cables. Although the magnetic field emitted from particular cable can be easily calculated, it is difficult to determine to what values of the magnetic field marine organisms are or will be most often exposed in the near future. The formula based on the Biot-Savart law, which main elements are the current carried by the cable core and the distance from the core, is commonly used to estimate the field induction from an electric cable. However, information concerning the value of the electric current conducted through the core of the submarine cables is scattered and varied. The same situation concerns the information relating to the geometry of the cross-section of the cables i.e., the diameter of cable core and the outer diameter which vary greatly among the cables and depends on whether it is the main cable or the return cable (which has weaker insulation and thus higher induction on its surface). Additionally, according to the rising number of MREDS, increasing demands for higher capacity cables is also expected (Hutchison et al., 2021). On the other hand, marine organisms may be exposed to wide range of the magnetic induction values, which are not only dependent on the current intensity of the cable. The intensity of magnetic induction decreases with the increasing distance from the cable wire and 6 m from the HVDC cable it is close to the natural geomagnetic field (Bochert and Zettler, 2004). Moreover, cables are often buried under 0.3–2 m sediment layer (Albert et al., 2020), whereas cables laid directly on the seabed may be covered by rocks, steel plates or cable anchoring of 15–30 cm (Taormina et al., 2018). Therefore, in future studies it would be reasonable and necessary to investigate variety of lower values of SMF/EMF. Applying of multiple SMF/EMF strengths in experimental research is also necessary to assess the value of magnetic induction that results in minimal negative effects (Scott et al., 2021). It is especially important due to lack of any recommendations concerning the technology used for underwater energy transmission and the potential implementation of available mitigation measures. It would be also interesting to examine if synergistic effects between artificial magnetic fields and other anthropogenic or environmental factors exist.

CRedit authorship contribution statement

Magdalena Jakubowska-Lehrmann: Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Marcin Białowas:** Investigation, Writing – review & editing. **Zbigniew Otremba:** Methodology, Writing – original draft, Writing – review & editing. **Anna Hallmann:** Investigation, Writing – original draft, Writing – review & editing. **Sylwia Sliwińska-Wilczewska:** Investigation, Visualization. **Barbara Urban-Malinga:** Conceptualization, Investigation, Writing – original draft, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2022.105700>.

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