

Master's Thesis
Year 2022-2023
Master Sciences de la Mer

The effect of electromagnetic
field on the development of
early life stages of Atlantic cod
(*Gadus morhua*)

Gauthier LORILLARD



Produced under the supervision of Dr.
Caroline DURIF and Dr. Prescilla PERRICHON

Institute of Marine Research
Austevoll Research Station, Norway

A qui de droit,

L'administration du Master Sciences de la Mer de l'Université Sorbonne Université, est l'unique dépositaire des manuscrits servant à valider les Thèses de Master. Nous ne diffusons des copies que sur demande écrite, après avoir sollicité l'accord de l'auteur et du ou des responsables du stage qui a conduit à l'écriture de la Thèse. En apposant la mention ci-dessous dans la première page du manuscrit (page de couverture intérieure), nous nous engageons à ne pas diffuser le manuscrit et à transmettre toute demande à l'auteur et au responsable de stage qui la traitera indépendamment.

Alain NIERGA

Gestionnaire de la formation

**SORBONNE UNIVERSITE
FACULTE SCIENCES et INGENIERIE
MASTER SCIENCES DE LA MER**
Tour 46 - 46/00 - Etage 1 - BC 6
4 place JUSSIEU
75252 PARIS Cedex 05

Avertissement/Disclaimer

Cette Thèse de Master a été réalisée de manière indépendante, pour remplir une partie des conditions d'obtention du titre de Master Sciences de la Mer de l'Université Sorbonne Université. Aucune autre assistance ou source que celles mentionnées n'ont été utilisées. Aucune donnée ne pourra être prise de ce manuscrit sans l'approbation de l'auteur et du responsable de stage.

This Master Thesis has been independently composed for the partial fulfillment of the title of Master of Science in Marine Sciences at University «Sorbonne Université ». No other than the indicated aid and sources have been used. It has not been presented to any other examination board. No data can be taken out of this manuscript without prior approval of the author and the thesis-promoter.

Table of contents

Acknowledgement	
Figures and tables list	
Symbols list	
Abbreviations list	
1. Introduction	1
1.1. EMF surveys.....	1
1.2. Electromagnetic senses.....	1
1.3. Effects of EMF and MF	2
1.4. Atlantic cod.....	2
1.5. Reproduction of Atlantic cod	3
1.6. Objective and hypothesis.....	5
2. Materials and methods	6
2.1. Broodstock	6
2.2. EMF exposure.....	7
2.3. Experimental procedure.....	8
2.3.1. EMF exposure of fertilized eggs	8
2.3.2. EMF exposure of adult male cod.....	9
2.4. Biological analyses.....	9
2.4.1. Sperm motility.....	9
2.4.2. Morphology and cardiac functions	11
2.5. Statistical analyses.....	11
3. Results	12
3.1. Early stages exposure	12
3.1.1. Morphology.....	12
3.1.2. Heart rate and ventricular functions.....	13
3.2. Male exposure and offspring assessment.....	14
3.2.1. Sperm motility of exposed parents (males).....	14
3.2.2. Fertilization.....	16
3.2.3. Offspring morphology	16
3.2.4. Heart rate and ventricular functions.....	16
4. Discussion	18
4.1. EMF effects on developing cod larvae.....	18
4.2. EMF effects on male cod and on their offspring	20
5. Conclusion and perspectives	22
References	

Acknowledgement

I would like to thank Caroline DURIF and Prescilla PERRICHON for their hospitality, their guidance, and their kindness throughout this thesis. This master's thesis would have been really different without you two. Thank you for all, I learned a lot thanks to you and in other word: tusen takk!

I would like to thank the Ecosystem Acoustic research group, its group leader Rolf KORNELIUSSEN, as well as the Reproduction and Development biology Research group and its group leader Anna WARGELIUS.

I would like to thank the Institute of Marine Research and more specially the Austevoll Research station, its department manager Kjetil STENSLAND and all the people who works there, researchers and technicians.

I also would like to thank Glenn SANDTORV for his help on the fishing, Stig Øve UTSKOT for the help on the experiment and his supervision during the maintenance and the monitoring of the batches and Anders THORSEN for his time and equipment.

Special thanks to Alessandro CRESCI, Maud ALIX, Rosa ESCOBAR LUZ, Marina MIHALJEVIC, Gabriel HAARVEI, Shelby CLARKE, Christos SIAPAZIS and Jasmine TALEVI for their help, emotional support, laugh and joy during the 5 months of this thesis.

Then, I would like to thank my family for their support, even if we were far away.

And last but not least, I would like to thank Lucie for her everyday support and her patience.

Figures and tables list

Number of the figures/tables	Short caption
Figure 1	Different stages of Atlantic cod embryonic development
Figure 2	Map of the fishing area
Figure 3	Incubator unit
Figure 4	Sperm motility parameters schematic
Figure 5	Cardiac functions of Atlantic cod larvae after a direct exposure to three levels of electromagnetic field
Figure 6	Sperm motility parameters of male Atlantic cod exposed to one level of electromagnetic field
Figure 7	Cardiac functions of Atlantic cod larvae after an indirect exposure to three levels of electromagnetic field
Figure 8	Stroke volume and cardiac output of Atlantic cod larvae after an indirect exposure to three levels of electromagnetic field
Table I	Electromagnetic field measurement in the Electromagnetic Survey Simulator tank
Table II	Total malformation rate of the larvae exposed to three levels electromagnetic field

Symbols list

Symbol	Description	Unit (S.I.)
μT	Microtesla	Tesla
mT	Millitesla	Tesla
m	Meter	Meter
$^{\circ}\text{C}$	Degree Celsius	Celsius
kg	Kilogram	Gram
t	Ton	Ton
mm	Millimeter	Meter
L	Liter	Liter
μm	Micrometer	Meter
$\%$	Percent	
mL	Milliliter	Liter
mg	Milligram	Gram
min	Minutes	Second
μL	Microliter	Liter
$\mu\text{m}\cdot\text{s}^{-1}$	Micrometer per second	Meter per second
$\text{Beat}\cdot\text{min}^{-1}$	Beats per minute	Beat per minute
s	Second	Second
$< \text{ or } >$	Superior or inferior	
nL	Nanoliter	Liter
$\text{nL}\cdot\text{min}^{-1}$	Nanoliter per minute	Liter per second
Hz	Hertz	Hertz
GHz	Giga hertz	Hertz

Abbreviations list

Abbreviation	Description
EMF	Electromagnetic field
MF	Magnetic field
CSEM	Controlled source electromagnetic
mpf	Minutes post fertilization
hpf	Hours post fertilization
Dpf/DPF	Days post fertilization
DD	Degree days
EMSS	Electromagnetic Survey Simulator
dph	Days post hatching
BPM	Beats per minute
min	Minimum
max	Maximum
KW	Kruskal-Wallis
C	Control
L	Low
M	Medium
H	High
VSL	Velocity Straight Line
VCL	Velocity Curvilinear Line
VAP	Velocity Average Path
F2	Fish two
F3	Fish three
F4	Fish four

1. Introduction

An electromagnetic field (EMF) is created when electric charges are in movement. They have different applications, such as domestic uses (microwaves, televisions, radios) or industrial uses (generators, sensors, or electric vehicles). In the marine environment, the dominant magnetic field (MF) is generated by the Earth's mantle field (25 to 65 μT). EMFs can be used to carry out electromagnetic surveys (Nyqvist et al., 2020).

1.1. EMF surveys

Such EMFs activities, specifically Controlled Source Electromagnetic (CSEM) are used for hydrocarbon exploration. CSEM gives information about the nature of the fluids in the sea bottom. If applied correctly, CSEM technology can be a real advantage in hydrocarbon exploration (Constable, 2010; Constable and Srnka, 2007; Hesthammer et al., 2010b). CSEM technology can also be used in geological survey, to create models, which can give information about the crust and cooling processes that occur in it (Young and Cox, 1981). CSEM technology is increasingly used, with a 56% success rate (Hesthammer et al., 2010a), as an alternative to seismic technology. There is a real conservation challenge around hydrocarbon exploration, and specifically exploration in areas that display important marine biodiversity, because those activities can overlap over habitats and/or spawning season of several marine species. But effects of anthropogenic EMF on marine ecosystems are poorly documented (Kark et al., 2015).

1.2. Electromagnetic senses

Electromagnetic senses in marine life have evolved in many species with a variety of sensory systems. Magnetoreception is the capacity of an animal to detect and use a magnetic field (generally the Earth's geomagnetic field). They mostly use it as a compass to orient, combined or not with other environmental cues, such as stars or sun light. The geomagnetic field is a common cue in the marine environment, used by many long distance migratory species (Putman, 2018). Magnetite based magnetoreception, radical-pair mechanisms and electric field mediated magnetic orientation are the three main mechanisms used to explain magnetoreception for animals. But these mechanisms, even if they have been identified, are not fully understood. Interfering with animal senses can have an impact on their development, even more if the exposure occurs at the early life stages.

1.3. Effects of EMF and MF

Experimental studies have demonstrated that anthropogenic MF have different effects on the following fish species: haddock (*Melanogrammus aeglefinus*, Linnaeus) (Cresci et al., 2022a), roach (*Rutilus rutilus*, Linnaeus) (Chebotareva et al., 2009), sea trout (*Salmo trutta* m. *trutta*, Linnaeus) (Brysiewicz and Formicki, 2019), guppy (*Lebistes reticulatus*, Peters) (Brewer, 1979), little skate (*Leucoraja erinacea*, Mitchill) (Hutchison et al., 2020) and lumpfish (*Cyclopterus lumpus*, Linnaeus) (Durif et al., 2023). Some have not been impacted at all, such as lesser sandeel larvae (*Ammodytes marinus*, Raitt) (Cresci et al., 2022b), European lobster (*Homarus Gammarus*, Linnaeus) (Taormina et al., 2020). Among these effects, MF can have negative impacts by disorienting and reducing the swimming performance of haddock larvae (Cresci et al., 2022a). MF and EMF enhanced the absorption rate of the yolk-sac (Fey et al., 2019). This can lead to severe problems for larval development, reducing survival of the larvae in natural conditions. Because they develop faster, larvae grow smaller and are easier to catch by predators. Constant MF exposure during the development of embryos can slow down the embryogenesis of the sea trout (*Salmo trutta* m. *trutta*, Linnaeus) and the rainbow trout (*Oncorhynchus mykiss*, Walbaum) (Formicki et al., 2021), leading to longer development time and reduced survival. Impact of MF on melanophores of European whitefish (*Coregonus lavaretus*, Linnaeus) and vendace (*Coregonus albula*, Linnaeus) was also studied during the first steps of embryogenesis. The study showed that long-term exposure can delay eye and body pigmentation when the larvae were exposed to an increasing MF (from 1 mT to 5 mT) (Brysiewicz et al., 2017). On the other hand, MF can also have a positive impact on the sexual performance of fish, by improving sperm motility and fertilization rate of trout (*Salmo trutta*, Linnaeus) (Formicki et al., 2015) or of the Danube huchen (*Hucho hucho*, Linnaeus) (Formicki et al., 2013). Even though MF and EMF are two different sources, and because we have a lot of variability between the species' responses, it is important to test the potential impacts on cod.

1.4. Atlantic cod

The Atlantic cod (*Gadus morhua*, Linnaeus) is a Teleost fish belonging to the Gadidae family. Cod are found in the North Atlantic Ocean, from Canada to Norway. They live in nearshore areas, between the surface and near the sea floor, with a preference for depths between 40 and 130 m, with a maximum of 400 m. Their habitats can be characterized as

rocky slopes and ledges. It is a cold water species (between 0 and 20°C) (Drinkwater, 2005) and some ecotypes are migratory (Lough, 2004). They have a distinguishing barbel under the jaw tip and display some variation in their colorations, depending on their habitats. Therefore, they all have in common small color spots and a pale lateral line on their body. Concerning their feeding habits, they have a non-exclusive diet, and seize any opportunity to eat. Their nutrition varies throughout their life (Lough, 2004).

Cod represents the second most economically important fish in Europe. Fish landings represented 0.9 million kg in 2019 (Nardi et al., 2021). This number has declined since 1988, when the landings were over 34 million kg. In Norway, cod farming started in the late 1880s, with a production focused of cod juveniles. The maximum production occurred in 2010 with 21,000 t, but it collapsed one year later, in 2011 and until 2014, because of disease, escapees, problems with growth and eggbound mortality. A new interest for cod farming appeared between 2019 and 2020, with new methods, such as live holding or capture-based aquaculture (Nardi et al., 2021).

It is assumed that the Atlantic cod is a metapopulation, i.e. genetically different subpopulations (Kent Smedbol and Wroblewski, 2002). Depending on the stock, the characteristics of the fish are different, which means the migratory behavior can be different between stocks, as the spawning period can be postponed. Based on the stock from which they come from, some cod can be seasonal spawners, and some of them lay eggs during spring (from March to May) (Brandner and Working Group on Cod and Climate Change, 2005). The eggs are released in several batches, with a cycle between 48 to 72 hours depending on temperature (Kjesbu, 1989; Kjesbu et al., 2023).

1.5. Reproduction of Atlantic cod

During reproduction, the females enter the male territory. Through courtship, the fish swim together near the surface and fertilization occurs through a ventral mount, with the male under the female. The release of eggs and sperm is done simultaneously by the male and the female. Other males may follow the mating couple and fertilize the eggs if the female takes several minutes to release her batch. In this way, the egg batch can be fertilized multiple times (Zemeckis et al., 2014). Certain anthropogenic activities can overlap with spawning and disturb the fish. Sperm cells are activated by contact with sea water and after which they start

to swim. Eggs harden when in contact with sea water (Lonning et al., 1984), making fertilization impossible after hardening. Therefore, a synchronized release of gametes is necessary for successful spawning. Eggs and sperm quality vary during the spawning season, as does the quantity of eggs per batch.

Eggs are transparent, planktonic and float in the water column. They have a diameter between 1.2 and 1.7 mm, with a unsculptured chorion (Lough, 2004). Spawning occurs where ocean circulation will allow favorable food conditions for the larvae (Stanley et al., 2013). Embryonic development is temperature dependent, and hatching occurs between 8 to 60 days post fertilization (dpf) in varying temperatures (Lough, 2004). Embryonic development can be described in six steps at $7 \pm 0.2^\circ\text{C}$ (Hall et al., 2004): (1) the zygote period (300-335 minutes post fertilization (mpf)), which occurs just after fertilization and corresponds to the fusion of the gametes, (2) the cleavage period (335 mpf – 22 hours post fertilization (hpf)) corresponds to cell division, from the one-cell stage to (3) the formation of the blastula (22-56 hpf). After (4) the formation of the gastrula (56-113 hpf) comes (5) the segmentation period (113-256 hpf), during which the organogenesis takes place. Hatching occurs after this and so begins (6) the larval period (>256 hpf) (Figure 1; (Skjærven et al., 2011).

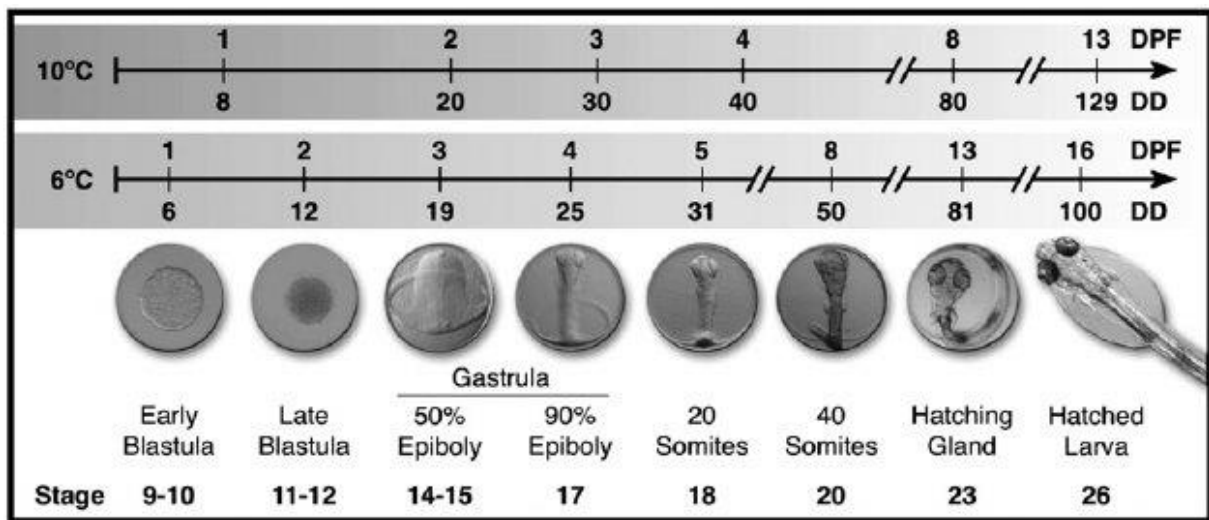


Figure 1. Different steps of Atlantic cod (*Gadus morhua*) embryonic development depending on the temperature (from Skjærven et al, 2011). DD: degree days; DPF: days post-fertilization.

During their development, cod larvae draw their energy from the yolk-sac, which contains a high concentration of lipids (Finn et al., 1995), even if no oil globule is visible. Once it is depleted, the larvae present a functional jaw and can find preys (such as copepods) to feed and survive. The larvae are found between the surface down to 75 m depths. Their survival relies on the presence of prey during a short time window after hatching, usually between 5 and 7 days.

1.6. Objective and hypothesis

This study aims to study the effects of CSEM surveys on Atlantic cod. Specifically, this study investigated (i) the effect of direct EMF exposure on the morphology of early developing cod larvae and their development, and (ii) the effect that EMF may have on male gametes and the consequences of that indirect exposure on its offspring.

Eventually, this study contributes to improve knowledge about Atlantic cod and its early life stages, and how these can be affected by anthropogenic effects. To do this, fertilized eggs were monitored after a direct exposition to different EMF levels, which were equivalent to those generated during CSEM surveys. In a second experiment, adult male cod were exposed to EMF before spawning and their offspring were subsequently monitored.

2. Materials and methods

2.1. Broodstock

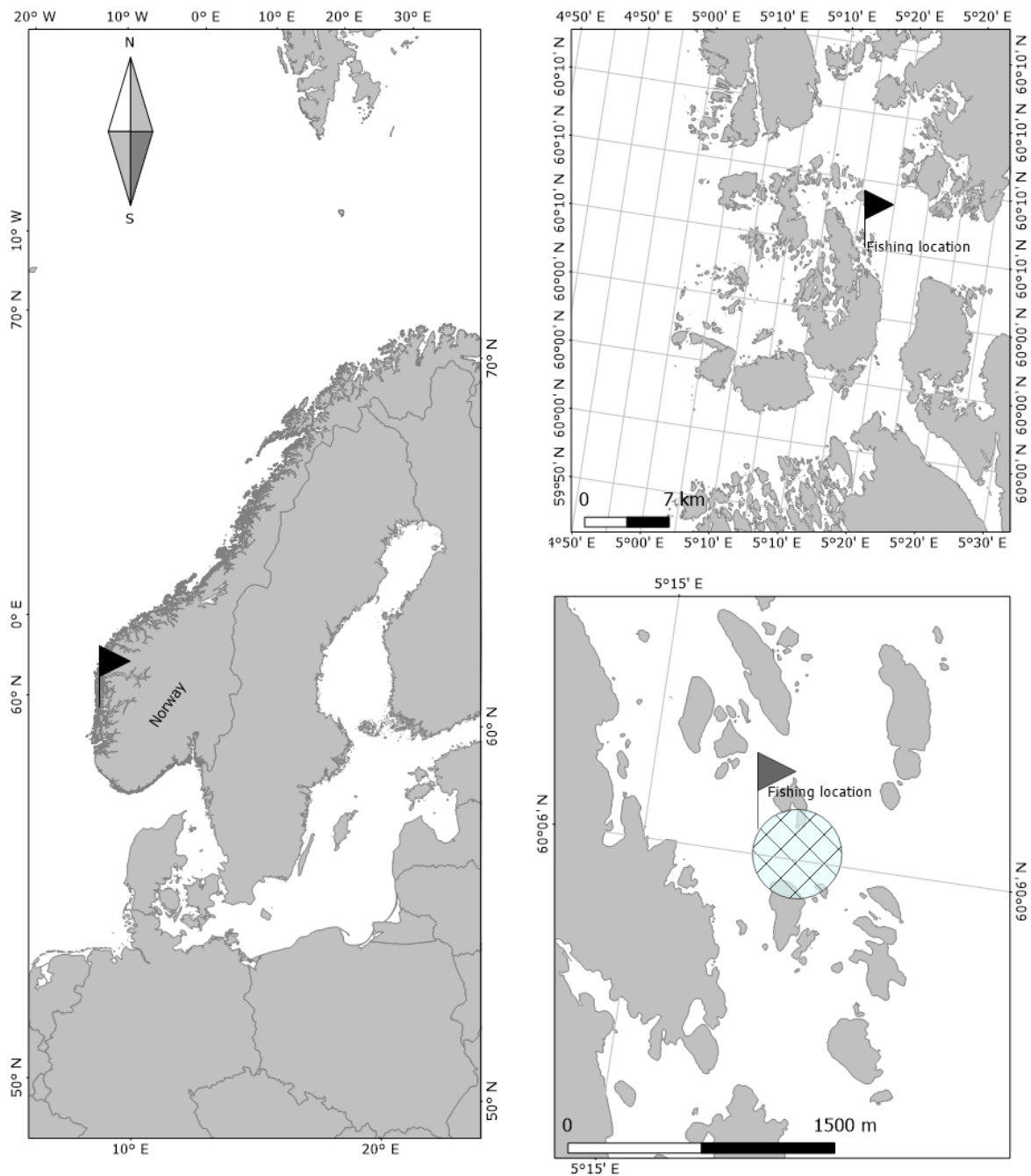


Figure 2. Map of the Atlantic cod (*Gadus morhua*) fishing area used in the electromagnetic field experiment. The circle depicts the fishing area (approximately 200-meter radius).

Wild Atlantic cod were captured in January and February 2023, by using 3 fyke nets (65 mm mesh-size, Høge Toggegarn). The nets were set in approximately the same area throughout the fishing period (Figure 2). After capture, the cod were maintained in an aquaculture cage until we transferred them to 2 7000 L-circular land-based tanks before the EMF experiments.

2.2. EMF exposure

An Electromagnetic Survey Simulator tank (EMSS tank) was used to create the EMF (Durif et al., 2022). An electric wire forms a coil around the tank (1.60 m in diameter). The homogenous electrical field inside the tank is generated using a hardware switch and a resistor box, connected to a power supply unit. The electrical field is dispersed homogeneously using electrodes which cover the wall surface. The magnetic and the electric fields are perpendicular to each other.

A signal generator was used to generate 3 different field levels to simulate CSEM survey conditions: level 1 (low), level 2 (medium), and level 3 (high). All the measurements were taken inside the EMSS tank (Table I).

*Table I. Measurement of the three electromagnetic levels inside the EMSS tank, used to expose eggs and adult Atlantic cod (*Gadus morhua*).*

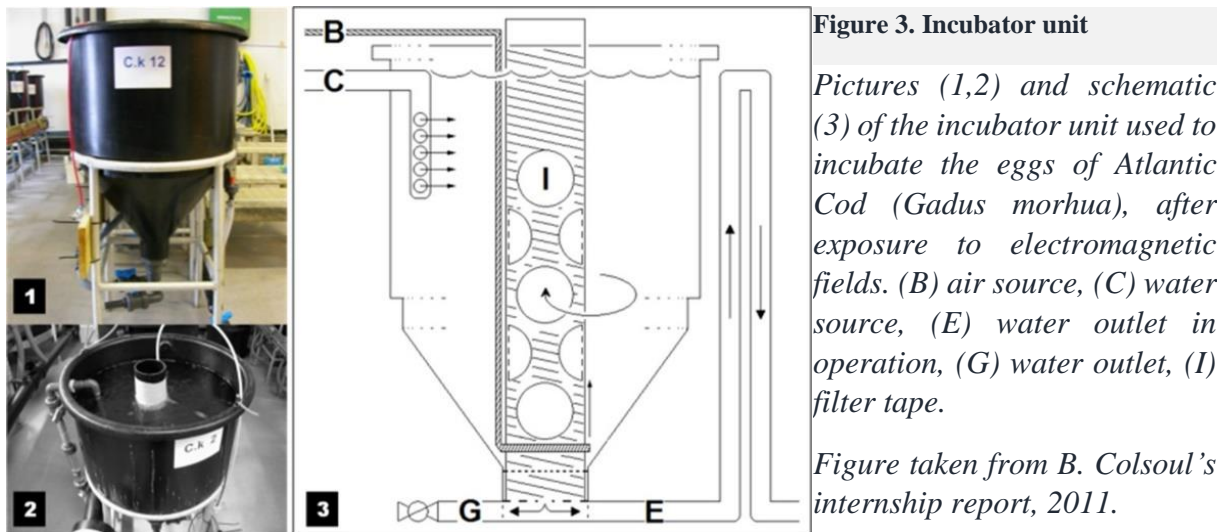
Field level, estimated				True field levels (9.6 degrees C water temperature)			
Field	H [A/m]	Coil I [A]	Distance source (approx) [m]	H [A/m]	Coil I [A]	Voltage PSU [V]	PSU Voltage generating estimated field [V]
Low field Level 1	0.1	0.0063	1000	0.1	0.0094	9.4	9.4
Strong field Level 2	10	0.63	100	8.9	0.83	9.4	10.6
Nearfield Level 3	40	2.53	30	26.9	2.50	9.4	14.0
Field	E [mV/m]	Electrode I [mA]	Distance source (approx) [m]	E [mV/m]	Electrode I [mA]	Voltage PSU [V]	PSU Voltage generating estimated field [V]
Low field Level 1	0.1	0.28	1000	0.14	NM	5.2	NA
Strong field Level 2	16	44	100	16	51	6.56	6.56
Nearfield Level 3	64	180	30	64	192	6.95	6.95

2.3. Experimental procedure

The saltwater used during the experiments was pumped from the sea at a depth of 60 m. The temperature was recorded before and during all the experiments with a temperature probe and was measured between 6 and 8°C.

2.3.1 EMF exposure of fertilized eggs

After each spawning event that occurred naturally in the broodstock tank, the eggs were collected by leading overflow from the tank to a 100-L container lined with a 500- μ m bag capturing the eggs. Once incubated, eggs were monitored daily; the fertilization rate (%) was calculated for every egg batch for one month (from 26/02/2023 to 26/03/2023) by the same operator, dead eggs were removed, and their volume was measured to estimate the mortality rate (%). Eggs were kept until they hatch for the first experiment, then killed using an overdose of MS222 (Tricaine mesylate). After the experiment we killed the eggs just after. 70 L incubators (Figure 3) were then cleaned and prepared for a new batch of eggs.



During the first experiment, we collected four times 1100 mL of the same incubator water, containing the eggs at 2dpf/19.25 degree-days (DD), which correspond to a late blastula stage. We exposed each fraction to the 3 EMF levels (low = level 1, medium = level 2, high = level 3, Table I). For the control treatment, the eggs were manipulated in the same manner as the exposed eggs, but the EMF was turned off. Each group of eggs was placed in a net in the center of the EMSS tank and the EMF was activated. After a 15-minute exposure, the eggs were carefully transferred to different plastic bottles, which were transported within

the hour to a climate-controlled room in a cool box to maintain the temperature of the eggs during the transfer (less than 10 minutes). 144 eggs, at the same developmental stage (late blastula), were then selected and individually placed in Nunclon™ Surface Nunc plates. These plates were kept until the larvae hatched in a climate-controlled room at a constant water temperature, between 7.8 and 8°C, and a 12/12 day-night light cycle. The light source was a desk lamp with a standard light bulb lamp with constant intensity. Every plate received the same light intensity, by carefully moving them regularly. In order to replicate the findings, this experiment was carried out a second time using a different batch of cod eggs, but at the same developmental stage as the first one.

2.3.2 EMF exposure of adult male cod

For the second experiment, we exposed 5 males to the high field strength (EMF level 3). After anesthetizing the fish with MS222 (20 mg/L), we gently stripped it to collect between 10 and 25 mL of sperm, used as '*control*' sperm sample. The fish was then transferred to the EMSS tank and was exposed to EMF for 15 min. After the EMF exposure, the male was anesthetized and stripped a second time, to collect a second '*exposed*' sperm sample. The procedure was repeated with five fish. After observation of the collected '*control*' sperm, samples from two individuals displayed low quality and were discarded. Therefore, only three fish were retained. Sperm samples were transported in a climate-controlled room and analyzed within 3 hours after the collection, at room temperature (8°C).

Three females from the broodstock tank were also anesthetized and gently stripped to collect their respective egg batches just after the EMF exposure of the males. One egg batch contained feces and was discarded. We mixed the two remaining egg batches to confound any female-variable effect on the results. A total of 240 mL of egg volume was collected and divided into six buckets (40 mL in each bucket), where fertilization took place. The buckets were kept for 45 min in the outside laboratory at a temperature of 8°C. Each 40 mL fertilized fractions were then maintained in 6 separate 70 L egg incubators.

2.4. Biological analyses

2.4.1 Sperm motility

The sperm motility of male cod (non-exposed and exposed, see section 2.3.2) was measured using a microscope (LEICA DM1000) coupled with a camera (Teledyne Lumenera

Infinity 5). Between 10 to 25 mL of sperm was collected on three fish by hand-stripping, before and after the EMF exposure, then analyzed within 3 hours after the sampling.

The sperm was used pure, without any dilution. 2.5 μL of this sperm was dropped in a plastic petri dish, then the sperm was quickly activated with 2 mL of sea water. Next, 2.5 μL of the solution was distributed on a 20- μm count Leja slide (HOUM, Norway) already setup under the microscope for video recording. Two videos of 1-min spermatozoids motility were recorded for each fish.

The videos were then processed using ImageJ (Schneider et al., 2012) to transform it into a 61 frames sequence, which started 30 seconds after the activation of the sperm cells. Contrast and luminosity were modified to make the cells more recognizable by the tracking software, using a threshold between 125 and 255. Next, we used the motility analyzer of the Open CASA plugin (Alquézar-Baeta et al., 2019) to assess the motility of each sperm cell from the different treatments.

We extracted 3 parameters from this plugin, the Straight Line Velocity (VSL), which is the average velocity of a sperm head along the straight line between its first and last detected positions; the Curvilinear Line Velocity (VCL), which is the average velocity measured over the actual point-to-point track followed by the cell and the Average Path Velocity (VAP), which measures the sperm head along its spatial average trajectory (Sloter et al., 2006) (Figure 4). These three parameters are expressed in $\mu\text{m}\cdot\text{s}^{-1}$.

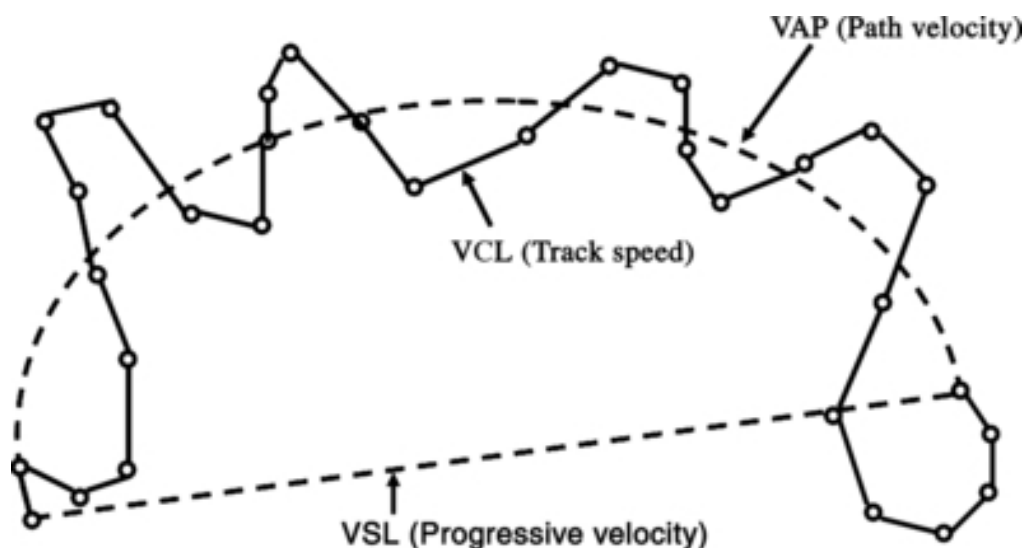


Figure 4. Sperm motility parameters, Velocity Straight Line (VSL), Velocity Curvilinear Line (VCL) and Velocity Average Path (VAP), used to characterize the sperm cells motility of Atlantic cod (*Gadus morhua*), after an electromagnetic exposure. From Kathiravan et al., 2011.

2.4.2. Morphology and cardiac functions

For the pictures and video recording of early life stages of the larvae, we used a Moticam 1080 camera (Motic®, Richmond, BC, Canada) mounted on a binocular microscope (Olympus SZX10). The larvae were individually placed in a methylcellulose gel (2% methylcellulose, 98% seawater), in a left lateral view to take a photo of the whole larva and assess the standard length (in mm) of the body, with a x0.8 zoom. A zoom of x3.2 was used to take pictures of the head and heart areas. Each larvae's heart was recorded once for 20 seconds with a zoom of x6.3. A x2 lens (Olympus DFPL 2X/-4) was used for all the pictures and videos. To maintain a stable temperature (8°C) setting, a thermally regulated microscope stage (Brook industries, Lake Villa, IL) was used. For the first experiment, we used a total of 240 larvae at a 6 dph/135 DD. For the second, we use a total of 40 larvae per fish (120 in total), at the same development stage as the first experiment. Only the DD was slightly different (136 DD).

We studied the malformation rate of the larvae by characterizing different categories of malformation: craniofacial malformations (jaw, eye and head proportion), yolk sac edema, spinal curvature, heart malformation, formation of the digestive system and development delay (Aranguren-Abadía et al., 2022).

Heart rate (beat.min⁻¹/BPM) was determined by manually counting the number of ventricular contractions in each 20 s video. The videos were then transformed into stacks of images with Prism Video Converter Software (NCH software), and the parts of the video exhibiting complete contraction and relaxation of the heart were selected. The ventricle area, at the end of the diastole and at the end of the systole for a same contraction/relaxation event, was measured using ImageJ. The ventricle measurement protocol was repeated three times for each larva. Stroke volumes were determined from the outlined ventricular perimeter and calculated using the conventional prolate spheroid volume (Perrichon et al., 2017). The stroke volume (nL) was calculated as the difference between diastolic and systolic ventricular volumes. Cardiac output (nL/min) was calculated as the product of stroke volume and heart rate (Perrichon et al., 2017).

2.5. Statistical analyses

The data were analyzed using Kruskal-Wallis tests, Chi-2 tests, two-way ANOVA with rank transformation of the data, and Post hoc tests to assess differences between our

variables Assumptions of normality and homogeneity, were tested by visualizing Q-Q plots and histograms of the residuals, residual-fit plots and residual lag plots. All data analysis was carried out with RStudio (R Core Team, 2021).

3. Results

3.1. *Early stages exposure*

Both egg batches used for the early life stages exposure exhibited a fertilization rate of $95\pm 13.2\%$ and $85\pm 13.2\%$ respectively for the first and second replicates. These two rates represented two of the highest rates observed during the whole spawning period (min = $46.1\pm 13.2\%$, max = $96\pm 13.2\%$, total assessed spawning= 12).

3.1.1. *Morphology*

There was no significant difference in standard length of the hatched larvae (min = 3.13 mm, max = 4.88 mm) after an early exposure to different EMF treatments (KW, p-value = 0.5118).

We also assessed the total malformation rate of the larvae for the four treatments and found that the highest malformation rate occurred in the low (level 1) treatment and the lowest malformation rate occurred in the high (level 3) treatment (Table II). No significant differences were found between them (Chi-2, p-value = 0.2133).

Table II. Total malformation rate for the Atlantic cod (Gadus morhua) larvae exposed to the three EMF levels (Low = level1, Medium = level 2 and High = level 3).

	Control	Low	Medium	High
Total malformed larvae (%)	3.33 ± 4.7	8.33 ± 2.3	5 ± 2.3	1.6 ± 2.3

3.1.2. Heart rate and ventricular functions

We observed no significant difference in heart rate (KW, p-value = 0.706), stroke volume (KW, p-value = 0.7522), or cardiac output (KW, p-value = 0.7722) after eggs were exposed to EMF (Figure 5).

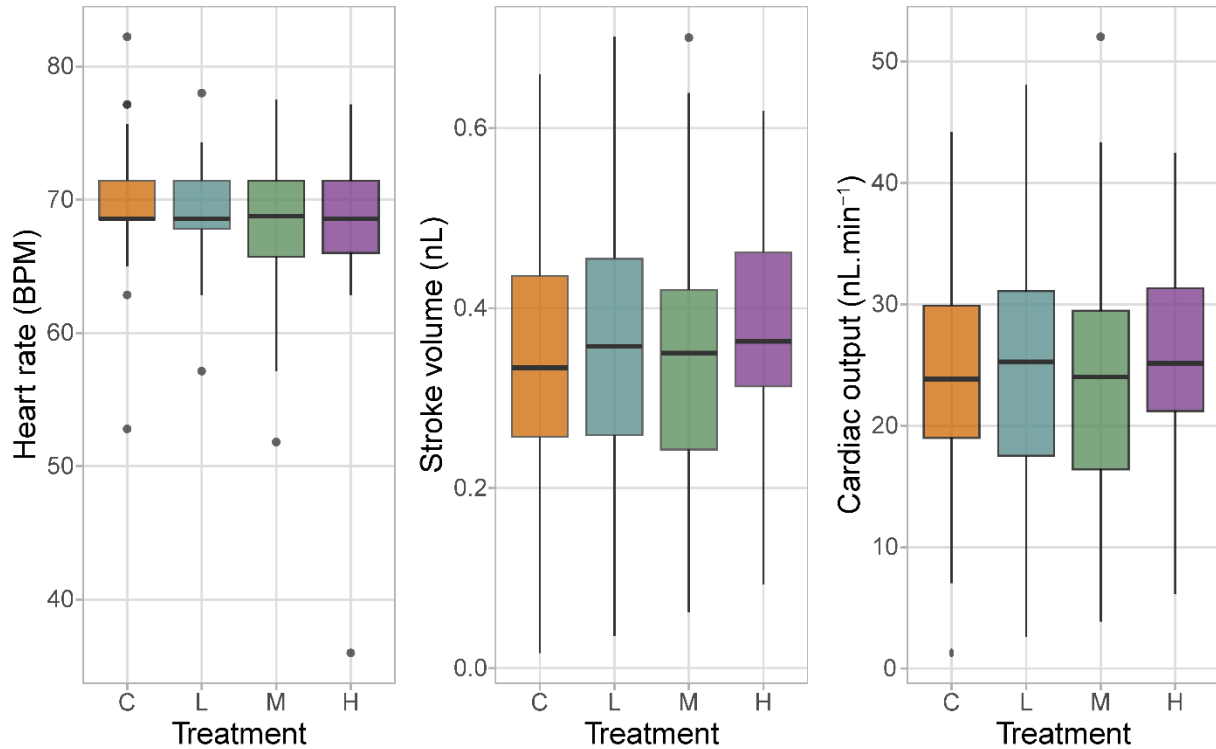


Figure 5. Cardiac functions of Atlantic cod (*Gadus morhua*) after a direct exposure to three level of electromagnetic fields (Low (L), level 1; Medium (M), level 2 and High (H)), level 3). For controls (C), the EMF was turned off. The middle line represents the median, while the bars represent the minimum and the maximum of our data. $N = 30$ larvae per treatment.

3.2. Male exposure and offspring assessment

3.2.1. Sperm motility of exposed parents (males)

VSL (Velocity Straight Line)

We observed a statistically significant difference in the VSL of the sperm cells between the control and the EMF exposed groups (two-way ANOVA, p-value = 0.0006). Sperm cells from the exposed groups were faster than the control ones for F2 and F3 (two -way ANOVA, p-value < 0.0001 for both) while the sperm cells were slower for F4 (two -way ANOVA, p-value < 0.0001) (Figure 6).

VCL (Velocity Curve Line)

There was also a significant difference in the VCL of the sperm cells between the control and the EMF exposed groups (two -way ANOVA, p-value = 4.49e-13). Only F2 displayed a significant difference between the control and exposed groups (two -way ANOVA, p-value < 0.0001). Like the results for the VSL, we found that the exposed group is faster than the control group (Figure 6).

VAP (Velocity Average Path)

Finally, we found a significant difference in VAP between the control and the EMF exposed groups (2-way ANOVA, p-value = 4.111e-08). Sperm cells from F2 and F3 were faster after EMF exposure compared to control samples (two -way ANOVA, p-value <0.0001). Sperm cells from F4 displayed slower speed (two -way ANOVA, p-value < 0.0001) (Figure 6).

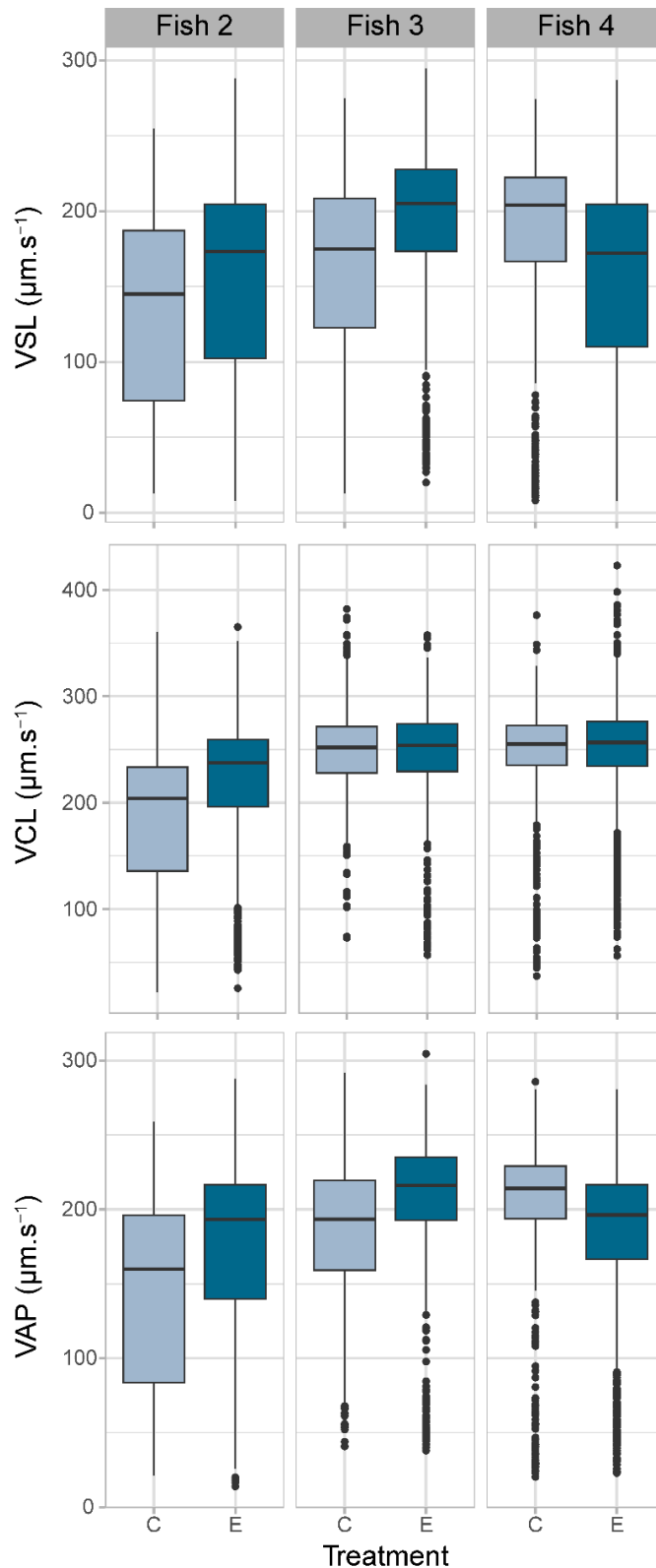


Figure 6. Sperm motility parameters (Velocity Straight Line (VSL), Velocity Curvilinear Line (VCL), Velocity Average Path (VAP)) of our three exposed Atlantic cod (*Gadus morhua*). They were exposed to one level of electromagnetic field (Exposed (E)), level 3). For controls (C), the EMF was turned off. The middle line represents the median, while the bars represent the minimum and the maximum of our data. $N = 20$ larvae per treatment per fish.

3.2.2. Fertilization

Fertilization rates after exposure of sperm to EMF was equal to $71.3 \pm 10.7\%$ for the control sample and to $82.6 \pm 10.2\%$ for the exposed sample and we found no significant differences between the two groups (Chi-2, p-value = 0.1573). We calculated a mortality rate by incubator equal to $43 \pm 6.3\%$ for the control group and $35 \pm 4.3\%$ for the exposed group.

3.2.3. Offspring morphology

There was no difference in the standard length of larvae (min = 3.92 mm, max = 5.09 mm) resulting from the control and EMF exposed males (KW, p-value = 0.5118). Malformation rate was $1.6 \pm 3\%$ for the control group and $11.6 \pm 5.7\%$ for the exposed group and no significant difference was found between the two groups (Chi-2, p-value = 0.1573).

3.2.4. Heart rate and ventricular functions

There was no significant difference in the heart rate of the offspring (KW, p-value = 0.7426). Nevertheless, differences in stroke volume and cardiac output of larvae resulting from the control and EMF exposed males were significant (KW respectively, p-value = 0.06592 and p-value = 0.07242, Figure 7).

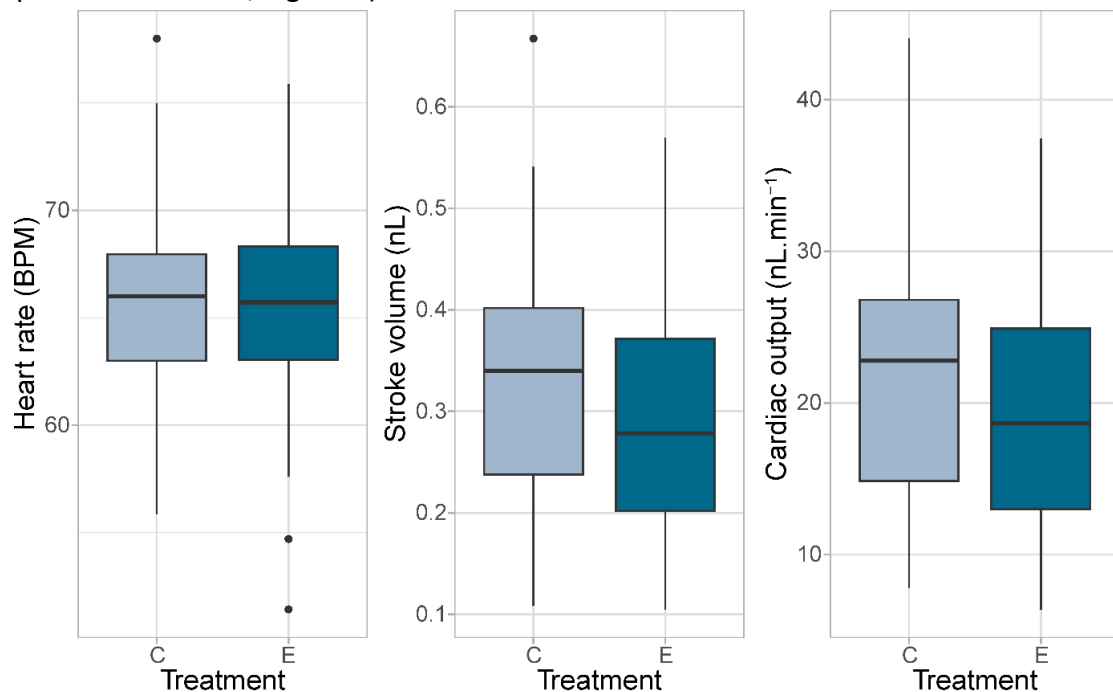


Figure 7. Cardiac functions of our three exposed Atlantic cod (*Gadus morhua*). There were exposed to one level of electromagnetic fields (Exposed (E)), level 3). For controls (C), the EMF was turned off. The middle line represents the median, while the bars represent the minimum and the maximum of our data. N = 20 larvae per treatment per fish.

However, results between fish were opposite for the stroke volume and the cardiac output (Figure 8). Two fish (F2 and F4) presented lower values for the exposed group compared to the control group, while F3 displayed the opposite (higher values in the exposed group).

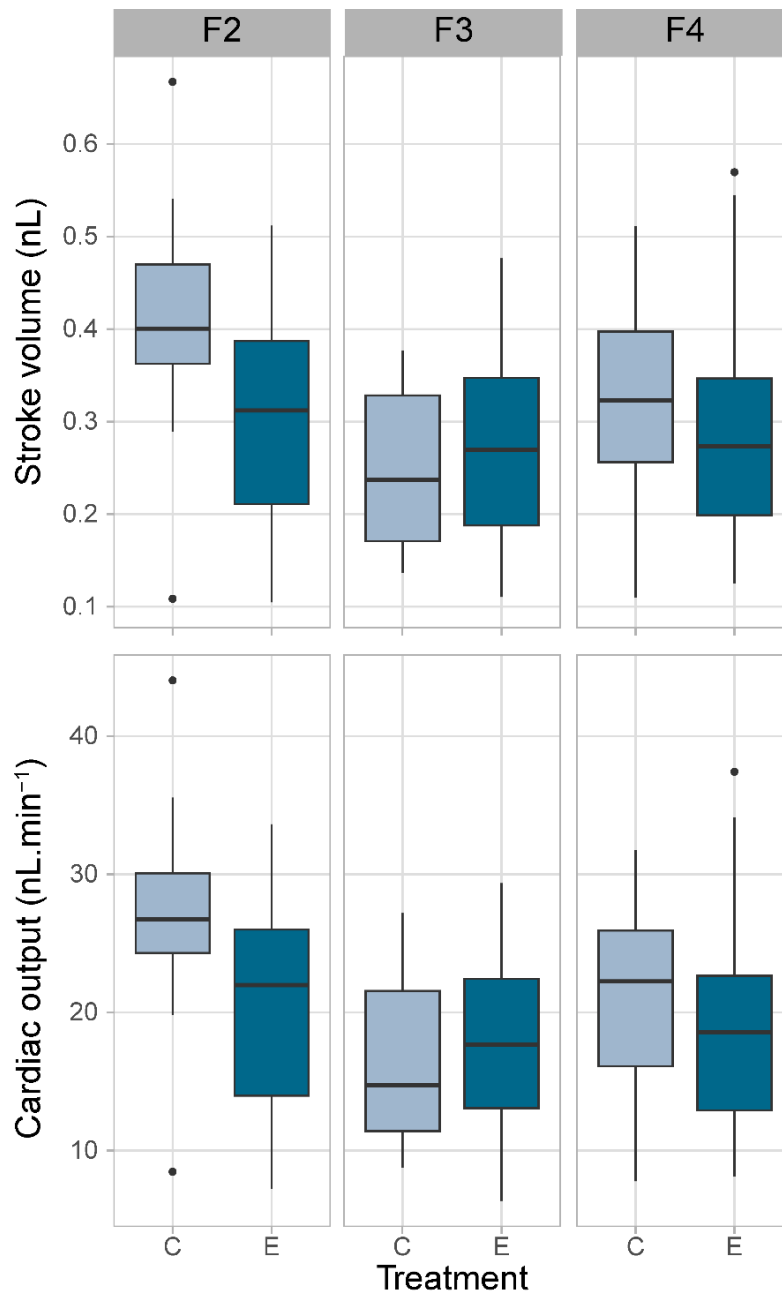


Figure 8. Stroke volume and cardiac output of three exposed Atlantic cod (*Gadus morhua*). They were exposed to one level of electromagnetic fields (Exposed (E)), level 3). For controls (C), the EMF was turned off. The middle line represents the median, while the bars represent the minimum and the maximum of our data. $N = 20$ larvae per treatment per fish

4. Discussion

Through this study, we aimed to assess the effects of EMF on Atlantic cod. Specifically, we focused on two aspects: (i) the impact of EMF on the morphology of early developing cod larvae, and (ii) the effects of EMF on male cod and the subsequent consequences on their offspring.

Two experiments were performed in this study. The first experiment involved exposing eggs to three different EMF levels. The results showed that early exposure of EMF had no significant effect on the morphology of Atlantic cod larvae. In the second experiment mature male cod were exposed to the highest EMF level and their sperm was used to fertilize a set of eggs. We found an increase in sperm motility after EMF exposure in two out of three fish. The high individual variability did not allow us to give a definite conclusion about the effect of EMF on sperm motility. However, this increase, at least in two fish, did not affect subsequent development and morphology of the offspring.

4.1. EMF effects on developing cod larvae

Fertilization rates observed in our production setting were within a similar range as those reported in the wild (26 to 91%, Lanes et al., 2012). Since our male and female fish were kept together in the same tank, it is assumed that the proximity between the fish and the absence of external parameters (i.e., no strong currents, variations of temperature, etc.) that could potentially reduce the fertilization have contributed to an improvement in fertilization rates. The mortality rate was unfortunately not followed during the development of the eggs in the nunc plates, but previous similar experiments on haddock showed no effects of EMF on mortality (Guillebon 2022).

The size and morphology of the cod larvae were not affected by the EMF exposure when they were exposed at the late blastula stage. An EMF exposure (500 Hz, 150 μ T, for 27 to 108 hours and at a temperature between 21 and 16°C) of roach eggs during the embryogenesis resulted in a reduced body length (Chebotareva et al., 2009). However, in a similar experiment conducted on haddock exposed to the same EMF as in our study, their results showed no effect, as for the malformation rate (Guillebon, 2022). Indeed, they found a rate between 13.8 and 31%, when we found a rate between 1.6 \pm 2.3% and 8.33 \pm 2.3%, and both were no significant, which induce that EMF exposure as no effect on the malformation rate.

EMF exposure during late blastula stage did not have any significant effect on the frequency and contractility of the heart of developing larvae. We observed the same results for the stroke volume and unsurprisingly also for cardiac output, since it is calculated using the stroke volume and the heart rate. However, tachycardia events have been observed in haddock larvae after being exposed to EMF (same intensity as ours) during the embryogenesis, and precisely during medium and high exposure (Guillebon, 2022). These effects were confirmed after a long-term high exposure (1 hour) and resulted in a heart rate 1.2 times higher compared to the control. Heart rate has been assessed on different species after an exposure to a static MF or an EMF, i.e., sea trout, common carp (*Cyprinus carpio*, Linnaeus), northern pike (*Esox Lucius*, Linnaeus) and European whitefish. The exposure of static MF (51-70mT) on the common carp led to an increase in the heart rate in the first minute of experiment, but it came back to a normal level after 15 min (Formicki et al., 2021). The exposure of northern pike to static MF (10 mT) causes similar effects as those observed in common carp (Formicki et al., 2021). However, when sea trout was exposed to a static MF of 4 mT, a temporary increase in the frequency of heart contractions was observed during the first 10 min. The heart frequency then returned to normal after 30 min (Formicki et al., 2021). The European whitefish exposed to static MF (4mT) presented a slowing down of the heart rate. However, after an initial decrease, the heart rate increased by the 10th min and then returned to normal levels (slightly higher than the control) (Formicki et al., 2021). It seems that an exposure to MF has primarily short-term effects on the heart rate of different fish species. The effect reported were reversible, which seems to corroborate our results, where we found no effect of EMF on this parameter. It is important to acknowledge the lack of literature on this subject, making the comparison within the same conditions harder. The heart is a primary vital organ, so any impact on its morphology or function may influence the other functions. The energy cost necessary to a good cardiac functioning may reduce the energy necessary for the other secondary biological functions such as swimming, feeding, predator escaping and in longer term, can reduce the survival capacity of the organism.

4.2. EMF effects on male cod and on their offspring

Regarding sperm motility, we observed a significant difference between groups in the three parameters (VSL, VCL and VAP). For two individual, sperm cells were faster in the exposed group compared to the control group. Overall, the variability between the fish had a higher impact than the EMF, or at least the variability between the fish made it harder to conclude about the effect of the EMF exposure. The number of individual fish should have been higher to be able to draw more solid conclusions about a possible effect of EMF on sperm motility. Nevertheless, even if the sperm motility is faster, it may not be crucial for a better fertilization in Atlantic cod (Trippel and Neilson, 1992). However, in an MF exposure (1,5 and 10 mT) on trout induced a higher sperm motility, which led to a higher fertilization rate (Formicki et al., 2015).

Even if we assessed some differences in sperm motility, the fertilization rate we calculated for the two groups (respectively $75\pm 11.5\%$ and $83\pm 10.2\%$) was in the same range as the other rates assessed during the spawning period of our fish (min = $46.1\pm 13.2\%$ and max = 96 ± 13), no significant difference were found, meaning that EMF exposure as no effect on the fertilization rate. This confirmed that in our experiment, a higher sperm motility did not have an impact on the egg batch quality, neither an improvement nor a degradation of the quality.

About the mortality rate, it was found a higher rate for the control group than for the exposed group (respectively $43.3\pm 6.3\%$ and $35\pm 4.3\%$). Fey et al. (2019) found no statistically significant differences in the mortality of rainbow trout larvae after EMF exposure (0.9%) compared to control larvae (0.5%), as we found in our experiment.

No significant difference was observed on the size of the offspring larvae indicating that indirect exposure to EMF had no impact on their growth. However, reduced body length have been noted on the roach exposed to EMF (500 Hz, $150\mu\text{T}$, for 27 to 108 hours and at a temperature between 21 and 16°C) (Chebotareva et al., 2009).

Furthermore, the heart rate was not significantly different. The contractility of the ventricular chamber showed a tendency to be diminished, but it was not statistically different. This suggests that there may have been a decrease in the force of contraction or the ability of the ventricular chamber to pump effectively. The variability between the three fish was

substantial and higher than the variability due to the EMF treatment. Working with live fish implies a higher individual variability but is more representative of natural conditions.

It is also crucial to consider that there is an important lack of knowledge on the transgenerational effect of EMF exposure. Experiments have been conducted on different models (e.g. *Daphnia magna* and human being) and resulted in no adverse effect of extremely low frequency MF on a neuronal disease of the offspring from exposed parents (De Roos et al., 2001). On the other hand, an exposure of radio frequency EMF (900 GHz) on *D. magna* showed a reduction in fertility and in the body size of their offspring (Sarapultseva et al., 2023). No existing literature are currently focusing on the transgenerational influence of EMF on the offspring in fish, making the discussion of our findings limited.

5. Conclusion and perspectives

We found no significant effect of EMF exposure on the development of cod embryos. However, our sample size in the parental effect experiment was too low to draw any definitive conclusion. Similar experiments were run using haddock and the same experimental set up (Guillebon, 2022). It appears that cod is less sensitive than haddock to EMF exposure.

It is also important to consider that EMF can be emitted by different sources. They can be emitted by High-Voltage Direct Current (HVDC) power cables, which are used for transporting electricity produced by offshore wind farms (Öhman et al., 2007), but also to supply current to islands, marines platforms and observatories. As the Atlantic cod larvae may live near the seafloor, it can lead to an exposition to EMF. But they must be really near the HVDC cable to be affected. The EMF conditions used in our experiment were low compared to the ones used in the literature but were environmentally realistic in a CSEM context and corresponded to three different distances between the EMF source and the fish.

In conclusion, the objectives of this study were to extend our knowledge on how Atlantic cod could be impacted by CSEM, which are increasingly used nowadays. Hydrocarbon exploration is an important sector for the marine environment and can have a huge impact on marine life, by overlapping important areas (e.g., spawning or feeding areas) for the marine life. EMF can be an alternative to seismic surveys, which can have an impact on ecosystems. Our findings will help making informed decisions about the use of EMF and ensure the preservation of marine ecosystems.

References

- Alqu zar-Baeta, C., Gimeno-Martos, S., Miguel-Jim nez, S., Santolaria, P., Y niz, J., Palac n, I., Casao, A., Cebri n-P rez, J. ., Mui no-Blanco, T., P rez-P , R., 2019. OpenCASA: A new open-source and scalable tool for sperm quality analysis. *PLOS Comput. Biol.* 15, e1006691. <https://doi.org/10.1371/journal.pcbi.1006691>
- Aranguren-Abad a, L., Yadetie, F., Donald, C.E., S rhus, E., Myklatun, L.E., Zhang, X., Lie, K.K., Perrichon, P., Nakken, C.L., Durif, C., Shema, S., Browman, H.I., Skiftesvik, A.B., Goks yr, A., Meier, S., Karlsen, O.A., 2022. Photo-enhanced toxicity of crude oil on early developmental stages of Atlantic cod (*Gadus morhua*). *Sci. Total Environ.* 807, 150697. <https://doi.org/10.1016/j.scitotenv.2021.150697>
- Brandner, K., Working Group on Cod and Climate Change (Eds.), 2005. Spawning and life history information for North Atlantic cod stocks, ICES cooperative research report. Internat. Council for the Exploration of the Sea, Copenhagen.
- Brewer, H.B., 1979. Some preliminary studies of the effects of a static magnetic field on the life cycle of the *Lebistes reticulatus* (guppy). *Biophys. J.* 28, 305–314. [https://doi.org/10.1016/S0006-3495\(79\)85178-4](https://doi.org/10.1016/S0006-3495(79)85178-4)
- Brysiewicz, A., Formicki, K., 2019. The effect of static magnetic field on melanophores in the sea trout (*Salmo trutta m. trutta* Linnaeus, 1758) embryos and larvae. *Ital. J. Anim. Sci.* 18, 1431–1437. <https://doi.org/10.1080/1828051X.2019.1680319>
- Brysiewicz, A., Formicki, K., Ta ski, A., Wesolowski, P., 2017. Magnetic field effect on melanophores of the European whitefish *Coregonus lavaretus* (Linnaeus, 1758) and vendace *Coregonus albula* (Linnaeus, 1758) (Salmonidae) during early embryogenesis. *Eur. Zool. J.* 84, 49–60. <https://doi.org/10.1080/11250003.2016.1272644>
- Chebotareva, Yu.V., Izyumov, Yu.G., Krylov, V.V., 2009. The effect of an alternating electromagnetic field upon early development in roach (*Rutilus rutilus*: Cyprinidae, cypriniformes). *J. Ichthyol.* 49, 409–415. <https://doi.org/10.1134/S0032945209050075>
- Constable, S., 2010. Ten years of marine CSEM for hydrocarbon exploration. *GEOPHYSICS* 75, 75A67–75A81. <https://doi.org/10.1190/1.3483451>
- Constable, S., Srnka, L.J., 2007. An introduction to marine controlled-source electromagnetic methods for hydrocarbon exploration. *GEOPHYSICS* 72, WA3–WA12. <https://doi.org/10.1190/1.2432483>
- Cresci, A., Durif, C.M.F., Larsen, T., Bjelland, R., Skiftesvik, A.B., Browman, H.I., 2022a. Magnetic fields produced by subsea high-voltage direct current cables reduce swimming activity of haddock larvae (*Melanogrammus aeglefinus*). *PNAS Nexus* 1, pgac175. <https://doi.org/10.1093/pnasnexus/pgac175>
- Cresci, A., Perrichon, P., Durif, C.M.F., S rhus, E., Johnsen, E., Bjelland, R., Larsen, T., Skiftesvik, A.B., Browman, H.I., 2022b. Magnetic fields generated by the DC cables of offshore wind farms have no effect on spatial distribution or swimming behavior of lesser sandeel larvae (*Ammodytes marinus*). *Mar. Environ. Res.* 176, 105609. <https://doi.org/10.1016/j.marenvres.2022.105609>
- De Roos, A.J., Teschke, K., Savitz, D.A., Poole, C., Grufferman, S., Pollock, B.H., Olshan, A.F., 2001. Parental Occupational Exposures to Electromagnetic Fields and Radiation and the Incidence of Neuroblastoma in Offspring. *Epidemiology* 12, 508–517.
- Drinkwater, K., 2005. Drinkwater, K. F. The response of Atlantic cod (*Gadus morhua*) to future climate change. *ICES Journal of Marine Science. Ices J. Mar. Sci. - ICES J MAR SCI* 62, 1327–1337. <https://doi.org/10.1016/j.icesjms.2005.05.015>
- Durif, C., Zhang, G., Cresci, A., Berit, A., Browman, H., Doks, L., 2022. EFFECTS OF THE ELECTROMAGNETIC FIELD USED IN HYDROCARBON SURVEYS ON MARINE ORGANISMS.
- Durif, C.M.F., Nyqvist, D., Taormina, B., Shema, S.D., Skiftesvik, A.B., Freytet, F., Browman, H.I., 2023. Magnetic fields generated by submarine power cables have a negligible effect on the

- swimming behavior of Atlantic lumpfish (*Cyclopterus lumpus*) juveniles. *PeerJ* 11, e14745. <https://doi.org/10.7717/peerj.14745>
- Fey, D.P., Jakubowska, M., Greszkiewicz, M., Andrulowicz, E., Otremba, Z., Urban-Malinga, B., 2019. Are magnetic and electromagnetic fields of anthropogenic origin potential threats to early life stages of fish? *Aquat. Toxicol.* 209, 150–158. <https://doi.org/10.1016/j.aquatox.2019.01.023>
- Finn, R.N., Henderson, J.R., Fyhn, H.J., 1995. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Lipid metabolism and enthalpy balance. *Mar. Biol.* 124, 371–379.
- Formicki, K., Korzelecka-Orkisz, A., Tański, A., 2021. The Effect of an Anthropogenic Magnetic Field on the Early Developmental Stages of Fishes—A Review. *Int. J. Mol. Sci.* 22, 1210. <https://doi.org/10.3390/ijms22031210>
- Formicki, K., Szulc, J., Korzelecka-Orkisz, A., Tański, A., Kurzydowski, J.K., Grzonka, J., Kwiatkowski, P., 2015. The effect of a magnetic field on trout (*Salmo trutta* Linnaeus, 1758) sperm motility parameters and fertilisation rate. *J. Appl. Ichthyol.* 31, 136–146. <https://doi.org/10.1111/jai.12737>
- Formicki, K., Szulc, J., Tański, A., Korzelecka-Orkisz, A., Witkowski, A., Kwiatkowski, P., 2013. The effect of static magnetic field on Danube huchen, *Hucho hucho* (L.) sperm motility parameters. *Arch. Pol. Fish.* 21. <https://doi.org/10.2478/aopf-2013-0016>
- Guillebon, C., 2022. Effects of electromagnetic field on Atlantic haddock (*Melanogrammus aeglefinus*) early life stages.
- Hall, T.E., Smith, P., Johnston, I.A., 2004. Stages of embryonic development in the Atlantic cod *Gadus morhua*. *J. Morphol.* 259, 255–270. <https://doi.org/10.1002/jmor.10222>
- Hesthammer, J., Stefatos, A., Boulaenko, M., Fanavoll, S., Danielsen, J., 2010a. CSEM performance in light of well results. *Lead. Edge* 29, 34–41. <https://doi.org/10.1190/1.3284051>
- Hesthammer, J., Stefatos, A., Boulaenko, M., Vereshagin, A., Gelting, P., Wedberg, T., Maxwell, G., 2010b. CSEM technology as a value driver for hydrocarbon exploration. *Mar. Pet. Geol.* 27, 1872–1884. <https://doi.org/10.1016/j.marpetgeo.2010.08.001>
- Hutchison, Z.L., Gill, A.B., Sigray, P., He, H., King, J.W., 2020. Anthropogenic electromagnetic fields (EMF) influence the behaviour of bottom-dwelling marine species. *Sci. Rep.* 10, 4219. <https://doi.org/10.1038/s41598-020-60793-x>
- Kark, S., Brokovich, E., Mazor, T., Levin, N., 2015. Emerging conservation challenges and prospects in an era of offshore hydrocarbon exploration and exploitation: Challenges of Marine Oil and Gas. *Conserv. Biol.* 29, 1573–1585. <https://doi.org/10.1111/cobi.12562>
- Kathiravan, P., Kalatharan, J., Karthikeya, G., Rengarajan, K., Kadirvel, G., 2011. Objective Sperm Motion Analysis to Assess Dairy Bull Fertility Using Computer-Aided System – A Review. *Reprod. Domest. Anim.* 46, 165–172. <https://doi.org/10.1111/j.1439-0531.2010.01603.x>
- Kent Smedbol, R., Wroblewski, J.S., 2002. Metapopulation theory and northern cod population structure: interdependency of subpopulations in recovery of a groundfish population. *Fish. Res.* 55, 161–174. [https://doi.org/10.1016/S0165-7836\(01\)00289-2](https://doi.org/10.1016/S0165-7836(01)00289-2)
- Kjesbu, O.S., 1989. The spawning activity of cod, *Gadus morhua* L. *J. Fish Biol.* 34, 195–206. <https://doi.org/10.1111/j.1095-8649.1989.tb03302.x>
- Kjesbu, O.S., Alix, M., Sandø, A.B., Strand, E., Wright, P.J., Johns, D.G., Thorsen, A., Marshall, C.T., Bakkeplass, K.G., Vikebø, F.B., Skuggedal Myksvoll, M., Ottersen, G., Allan, B.J.M., Fosshem, M., Stiansen, J.E., Huse, G., Sundby, S., 2023. Latitudinally distinct stocks of Atlantic cod face fundamentally different biophysical challenges under on-going climate change. *Fish Fish.* 24, 297–320. <https://doi.org/10.1111/faf.12728>
- Lanes, C.F.C., Bizuayehu, T.T., Bolla, S., Martins, C., de Oliveira Fernandes, J.M., Bianchini, A., Kiron, V., Babiak, I., 2012. Biochemical composition and performance of Atlantic cod (*Gadus morhua* L.) eggs and larvae obtained from farmed and wild broodstocks. *Aquaculture* 324–325, 267–275. <https://doi.org/10.1016/j.aquaculture.2011.10.036>

- Lonning, S., Kjorsvik, E., Davenport, J., 1984. The hardening process of the egg chorion of the cod, *Gadus morhua* L., and lump sucker, *Cyclopterus lumpus* L. *J. Fish Biol.* 24, 505–522. <https://doi.org/10.1111/j.1095-8649.1984.tb04821.x>
- Lough, R.G., 2004. Atlantic Cod, *Gadus morhua*, Life History and Habitat Characteristics (Second edition). NOAA Tech. Memo. NMFS-NE 190.
- Nardi, G., Prickett, R., van der Meeren, T., Boyce, D., Moir, J., 2021. Atlantic cod aquaculture: Boom, bust, and rebirth? *J. World Aquac. Soc.* 52, 672–690. <https://doi.org/10.1111/jwas.12811>
- Nyqvist, D., Durif, C., Johnsen, M.G., De Jong, K., Forland, T.N., Sivle, L.D., 2020. Electric and magnetic senses in marine animals, and potential behavioral effects of electromagnetic surveys. *Mar. Environ. Res.* 155, 104888. <https://doi.org/10.1016/j.marenvres.2020.104888>
- Öhman, M.C., Sigra, P., Westerberg, H., 2007. Offshore Windmills and the Effects of Electromagnetic Fields on Fish. *AMBIO J. Hum. Environ.* 36, 630–633. [https://doi.org/10.1579/0044-7447\(2007\)36\[630:OWATEO\]2.0.CO;2](https://doi.org/10.1579/0044-7447(2007)36[630:OWATEO]2.0.CO;2)
- Perrichon, P., Grosell, M., Burggren, W.W., 2017. Heart Performance Determination by Visualization in Larval Fishes: Influence of Alternative Models for Heart Shape and Volume. *Front. Physiol.* 8, 464. <https://doi.org/10.3389/fphys.2017.00464>
- Putman, N., 2018. Marine migrations. *Curr. Biol.* 28, R972–R976. <https://doi.org/10.1016/j.cub.2018.07.036>
- Sarapultseva, E.I., Uskalova, D.V., Ustenko, K.V., Tikhonov, V.N., Ivanov, I.A., Tikhonov, A.V., 2023. Transgenerational changes in *Daphnia magna* under radio frequency radiation in the juvenile and puberty period. *Int. J. Radiat. Biol.* 99, 551–560. <https://doi.org/10.1080/09553002.2022.2087928>
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. <https://doi.org/10.1038/nmeth.2089>
- Skjærven, K.H., Olsvik, P.A., Finn, R.N., Holen, E., Hamre, K., 2011. Ontogenetic expression of maternal and zygotic genes in Atlantic cod embryos under ambient and thermally stressed conditions. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 159, 196–205. <https://doi.org/10.1016/j.cbpa.2011.02.026>
- Sloter, E., Schmid, T.E., Marchetti, F., Eskenazi, B., Nath, J., Wyrobek, A.J., 2006. Quantitative effects of male age on sperm motion. *Hum. Reprod.* 21, 2868–2875. <https://doi.org/10.1093/humrep/del250>
- Stanley, R.R.E., deYoung, B., Snelgrove, P.V.R., Gregory, R.S., 2013. Factors Regulating Early Life History Dispersal of Atlantic Cod (*Gadus morhua*) from Coastal Newfoundland. *PLoS ONE* 8, e75889. <https://doi.org/10.1371/journal.pone.0075889>
- Taormina, B., Di Poi, C., Agnalt, A.-L., Carlier, A., Desroy, N., Escobar-Lux, R.H., D’eu, J.-F., Freytet, F., Durif, C.M.F., 2020. Impact of magnetic fields generated by AC/DC submarine power cables on the behavior of juvenile European lobster (*Homarus gammarus*). *Aquat. Toxicol.* 220, 105401. <https://doi.org/10.1016/j.aquatox.2019.105401>
- Trippel, E.A., Neilson, J.D., 1992. Fertility and Sperm Quality of Virgin and Repeat-Spawning Atlantic Cod (*Gadus morhua*) and Associated Hatching Success. *Can. J. Fish. Aquat. Sci.* 49, 2118–2127. <https://doi.org/10.1139/f92-235>
- Young, P.D., Cox, C.S., 1981. Electromagnetic active source sounding near the East Pacific Rise. *Geophys. Res. Lett.* 8, 1043–1046. <https://doi.org/10.1029/GL008i010p01043>
- Zemeckis, D.R., Dean, M.J., Cadrin, S.X., 2014. Spawning Dynamics and Associated Management Implications for Atlantic Cod. *North Am. J. Fish. Manag.* 34, 424–442. <https://doi.org/10.1080/02755947.2014.882456>

The effect of electromagnetic field on the development of early life stages of Atlantic cod (*Gadus morhua*)

Abstract: Controlled source electromagnetic (CSEM) surveys are used for hydrocarbon exploration in the marine environment. Because those activities can overlap habitats and spawning period of Atlantic cod (*Gadus morhua*), it is needed to study the impact of CSEM on this species. CSEM survey use electromagnetic field (EMF) to explore the seafloor by dragging an antenna above it. Eggs of Atlantic cod are planktonic and developing in the water column are likely to be exposed to EMF. We tested three different levels of EMF, which represented three different distances from the EMF source. We assessed the direct exposure of living eggs, then followed their development, but also tested the indirect effect of an EMF exposure by exposing male cod, focusing on their sperm motility and followed the development of their offspring. We found no effect of the EMF on the size, the morphology nor the cardiac functions on the two experiments. Nonetheless, we observed a higher sperm motility for the exposed group than for the control group in the second experiment. However, our sample size for the transgenerational effect experiment, a high variability between the fish and the lack of the literature on this subject made the discussion of our experiment limited.

Keywords: CSEM – Morphology –Atlantic Cod – Offspring – Cardiac functions

L'effet des champ électromagnétiques sur le développement des premiers stades de vie de la morue d'Atlantique (*Gadus morhua*).

Résumé : Les études électromagnétique à source contrôlée (CSEM) sont utilisées pour la recherche d'hydrocarbures dans l'environnement marin. Ces activités peuvent chevaucher les habitats ou les périodes de pontes de la morue d'Atlantique (*Gadus morhua*). Il est donc nécessaire d'étudier les impacts de ces études électromagnétiques sur cette espèce. Ces études emploient des champs électromagnétiques (CEM) pour sonder le plancher océanique par dragage d'une antenne au-dessus de celui-ci. Les œufs de morue sont planctoniques et se développent dans la colonne d'eau. Ils sont susceptibles d'être exposés aux CEM. Nous avons donc testé trois différents niveaux de CEM, qui représentent trois distances différentes à la source. Nous avons étudié une exposition direct d'œufs vivants, puis avons suivi leur développement. Nous avons également testé l'effet indirecte des CEM en exposant des mâles, étudiant leur sperme et en suivant le développement de leur descendance. Nous n'avons pas trouvé d'effets significatifs des CEM sur la taille, la morphologie et les fonctions cardiaques durant nos deux expériences. Nonobstant, une augmentation de la vitesse des spermatozoïdes après exposition a été observé pendant la deuxième expérience. Cependant, la taille de notre échantillon pour l'expérience sur l'effet transgénérationnel, une haute variabilité entre les poissons et un manque de littérature sur ce sujet, ont rendu limitée la discussion de nos expériences.

Mots-clefs : CSEM– Morphologie – Morue d'Atlantique – Descendant – Fonctions cardiaques