The effects of weak electromagnetic field (EMF) exposure on developing *Xenopus laevis* tadpoles

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Abstract

The effect of electromagnetic fields (EMFs) on biological systems continues to be explored in the scientific literature and is expanded upon in the present study by using *Xenopus laevis* tadpoles as a model for vertebrate physiology and behaviour . Previous research has shown that weak EMF exposure causes delays in tadpole metamorphosis. The current study assesses the influence of EMFs on tadpole social behaviour by quantifying the strong natural gathering instinct of the animals. EMFs that have an impact on tadpole behaviour are hypothesized to further show quantifiable differences in tadpole neuroanatomy. Results from the present study do not show a significant interaction between field exposure and tadpole growth or social behaviour. Strong limitations are discussed with respect to the data collected. How the data from this experiment coincides with the current literature on biological-EMF interaction is rationalized. Finally, aspects of the present study that can be utilized to guide future research are identified.

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The Effects of Weak Electromagnetic Field (EMF) Exposure on Developing *Xenopus laevis* Tadpoles

Severini et al. (2010) proved that exposure to weak EMFs delay tadpole development. Although their observations of growth were important, it provided limited insight into the targets and mechanisms of EMF-biological interactions. This present study contributes to the literature by expanding previous tadpole EMF exposure research to include a behavioural component. Quantifying behavioural as well as physiological measures (that are responding to EMF exposure) can shed deeper insight into what neuronal or biomolecular substrates are being targeted by EMF exposure. This is because changes in behaviour may be indicative of changes in neurophysiology.

The Effects of EMF Exposure on Biological Systems

According to Maxwell's equations, a charged particle moving at a constant velocity generates a static (constant) electric and magnetic field; while a charged particle that is accelerating in speed generates an oscillating electric and magnetic field (Cifra, Fields, & Farhadi, 2010). This is the foundation for how electromagnetic fields (EMFs) are generated. As Levin (2003) and many other researchers have pointed out, the intensity of the field can be as important as the specific pattern of the field with regards to having effects on biological systems. Developing biological systems have been shown to be particularly responsive to low intensity EMF exposure. Early research by Delgado, Leal, Moneagudo, and Gracia (1982), demonstrated that chicken embryos exposed to a weak EMF show increased teratogenic effects and delays in development. Zusman, Yaffe, Pinus, and Ornoy (1990) also demonstrated developmental abnormalities in rat embryos as a result of weak EMF exposure. Milder developmental delays have been shown in sea urchin eggs (Zimmerman, Zimmerman, Winters, & Camerone, 1990) and in *Xenopus laevis* tadpoles (Severini et al., 2010).

Reports of biology being affected by EMF exposure are highly varied due to the wide range of parameters of the field that can be manipulated. This includes the frequency of the EMF, its distance

from the target, the duration of exposure, continuous versus pulsing application of the field, and

whether the field is static or dynamic in nature (Cifra et al., 2010). All of the characteristics of an EMF

contribute to the varying biological response. EMF exposure at the cellular level is known to induce a

range of biological effects including changes to cellular proliferation (Lisi et al., 2006), suppressed

apoptosis/programmed cell death (Tian et al., 2002), changes in protein phosphorylation (Sun et al.,

2001), and increased enzymatic activity (Kula, Sobczak, and Kuska, 2000).

Whether EMFs have an inherent or purposeful function in affecting biological systems is a

continuously evolving topic in the scientific literature. Examples of EMFs being used as a medium for

cellular communication include the coupling of germination rates in cherry pollen grains (Budagovskii,

Turovtseva, & Budagovskii, 2001), coupling of growth rate in yeast cells (Musumeci, Brizhik, & Ho, 1999),

and coordinated proliferation of osteoblasts (Zhang & Zhang, 2007). EMF communication has also been

suggested as the mechanism for synchronous processes, such the coordination of microtubule

formation, which is a key component of cellular differentiation (Pokorny, Hasek, Jelinek, Saroch, &

Palan, 2001). Calculations for the applied force of a magnetic field on a cell membrane have been shown

to be substantial enough to decrease the adhesion between cells; a weakening that may in turn result in

metastatic growth (Pokorny, 2009).

Mechanism for EMF Interactions with Biological Systems

There are a number of theories explaining how EMFs interact with biological systems. Early

ideas on the topic suggested that enzymatic systems within cell membranes convert electric field energy

into chemical energy (Tsong, 1992). This theory is known as the electroconformational coupling (ECC)

theory. In this theory, the characteristics of the field must match certain properties of the biological

system in order to affect the activation barrier for the reaction. As previously mentioned, the

information within the pattern of the EMF is more relevant to the biological effect than the intensity of

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the field (Levin, 2003). Tsong's theories have been substantiated experimentally by showing that there

are optimal frequencies and amplitudes of EMFs that are able to induce activity of the Na+/K+ pump and

stimulate ATP hydrolysis (Lui, Astumian, & Tsong, 1990). Tsong's theories are further substantiated by

work that shows that branching chain reactions (such as those found in enzymatic reactions) are

necessary components of magnetoception that depend on the amplification of a small amount of energy

(delivered by EMF exposure) to have a biological effect (Voeikov & Beloussov, 2007).

Resonant interactions are another possible mechanism for how EMFs interact with biological

systems. Any cellular structure is a potential target for these interactions since any of these structures or

their substructures can oscillate at their resonance frequency or "eigenfrequency" when stimulated with

energy (Cifra et al., 2010). These cellular structures are also the source of cellular EMF generation

because most proteins in a cell are polar (contain electric charges) and will generate an EMF when they

vibrate (Cifra et al., 2010). The frequency of the vibration of these electrically polar proteins will be

equal to the frequency of the EMF that is generated. For an external EMF to be able to affect a biological

system, Adair (2003) has argued that the energy associated with the external EMF must be enough to

overcome the energy generated by the cell's EMF, or the thermal noise of the biological system. This

notion suggests that the intensity of an EMF is particularly germane to observing biological effects.

However, principles of stochastic resonance propose a mechanism by which relatively weak EMFs can

have major effects when the noise in the system compliments the resonance properties of the system

alongside the applied EMF (Hanggi, 2002).

Another potential mechanism for how weak EMFs provoke biological responses is that clusters

of water molecules oscillate at the frequency of weak externally applied EMFs, and this subsequently

amplifies the signal to the point that it can overcome the internal resistance of a biological system

(Sinitsyn et al., 2000). Quantum electrodynamics predicts that special properties of water give it unique

properties at interfaces compared to water in bulk (Giudice, Spinetti, & Tedeschi, 2009). An interface

that causes the separation of bulk water into a different phase can include a cell membrane or polar

molecules. The properties of cytosolic water surrounding these structures would include having an

increased viscosity, lower thermal motion (energy) of molecules, and a strong separation of charge. It

has been further reported that the microvolumes of cytosolic water that surround a molecule may not

diminish the EMF signal as much as a signal traveling through bulk water (Preparata, 1995). The

excitation of proteins in the cellular space could lead to a change in the tertiary or quaternary structure

of macromolecules since the application of a weak EMF has an effect on weak intra-molecular forces

that orient and maintain the structure of the protein (Blank, 2008).

Specific effects on calcium flux have been explored in the scientific literature and are suggested

to be the origin of cascading EMF effects that result in large scale changes to biological systems. Zhadin

(1996) suggested that magnetic fields increase the kinetic energy of Ca2+, which would subsequently

cause changes in the electrical-chemical balance of the cellular system. Ding, Nakahara, Tian, Guo, and

Miyakoshi, (2001) further substantiated this notion in their experiment by demonstrating that a Ca2+

influx into MCF-7 cells can be induced by EMF exposure and also can be used to prevent cellular

apoptosis. Calcium as a candidate for EMF reception has also been suggested by Grundler and Kaiser

(1992), who propose that intracellular calcium oscillations lock onto the oscillations of electromagnetic

fields. The redistribution of calcium and other ions in cells by EMFs can induce weak currents that

subsequently affect the functioning of the cell and surrounding tissue (Funk, Monsees, & Ozkucur,

2009). Fanelli et al. (1999) have also shown that magnetic fields modulate the flux of Ca2+ across cell

membranes. In their study, several different chemical agents were used to induce cellular apoptosis

(programmed cell death) while cells were exposed to a static magnetic field. The fields prevented

cellular apoptosis by enhancing the Ca2+ ionic influx from the extracellular space.

The cellular substructure known as a centriole has also been considered as an antennae for

detecting EMFs (Albrecht-Buehler, 2010). This notion is based on the fact that the centrioles orient

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themselves perpendicular to the cellular chromatin and this conformation allows them to localize the

source of EMFs. Other structures implicated in EMF detection are natural magnetic nanoparticles which

have been described by Psfai and Dunin-Borkowski (2009). These particles have been found in a wide

range of species from bacteria to humans and suggest a selective advantage for EMF detection (Binhi &

Rubin, 2007).

Magnetic field interactions with melatonin have also been explored in conjunction with

behavioural changes in planaria (Mulligan, Gang, Parker, & Persinger, 2012). This is especially relevant to

research exposing EMFs to tadpoles, since melatonin has an antagonistic effect on thyroid hormone

(Wright et al., 2000) and thyroid hormone is the principle hormone for inducing metamorphosis in

amphibians (Severini et al., 2010). This idea may explain why developmental delays are triggered in

tadpoles exposed to EMFs (Severini et al., 2010).

Significance of EMF Pattern

As previously mentioned, the pattern of magnetic field is directly relevant to the biological

consequence. There are a massive combination of frequencies and intensities available to choose from

and this interplay is further complicated by how the field is administered (pulsing or constant exposure),

the developmental stage of the biological system, and the type of biological system itself (Cifra et al.,

2010). Even the environment the experiment is conducted in can vary the results. Geomagnetic storm

events have been shown to cause a wide array of biological dysfunctions including disturbed circadian

rhythms (across a variety of species and organic structures), loss of attention and increased anxiety in

humans, disturbances of cellular adhesion and aggregation in heterokaryons, and more (Volpe, 2003).

Even a temperature change as little as 0.2 °C can cause biological effects that can confound the results

of experiments trying to observe the physiological targets of EMF exposure (Morrissey, 2008).

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To reinforce a consistency in the scientific literature, a similarly weak EMF as used in Severini et al. (2010) will be used once again in this experiment to try and replicate the developmental delays observed in the original experiment. In addition to this field, an LTP pattern of EMF exposure will be applied to the groups of tadpoles rather than the sinusoidal wave pattern used in Severini et al. (2010). The LTP pattern has been previously shown to cause behavioural abnormalities in rats (Mach & Persinger, 2009). Whether the effects from this pattern extend to other vertebrate models was explored in this research.

McCaig, Rajnicek, Song, and Zhao (2005) proposed that static electric fields may produce physiological consequences, such as the redistribution of ions in cellular space. He further suggests that internally generated "quasi-static" (or slowly varying magnetic fields) may even have a purposeful role in directing the distribution of charges for the cell to perform physiological functions. Because of the difficulty in maintaining a static electric field, only the effects of dynamic fields will be explored. There have been numerous examples of dynamic fields affecting biological systems (Volpe, 2003). In addition to this, the pattern of a dynamic field has a greater chance of engaging with macromolecules through resonance phenomena (Cifra et al., 2010).

Justification for Tadpole Use

This study is based on previous research that has observed morphological changes in tadpoles (Xenopus laevis) in response to EMF exposure (Severini et al., 2010). Xenopus laevis tadpoles are in a constant state of development, making them highly susceptible to the effects of magnetic field exposure (Severini et al., 2010). Lesser animals reach the point of sexual maturity too quickly or are too primitive to fully explore the behavioral effects of magnetic field exposure. Tadpoles are widely cited for their susceptibility to magnetic field exposure and for their ease of use in research.

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Tadpole Neuroanatomy and Histology

The following information on tadpole neuroanatomy has been compiled based on research by

Roberts, Le, and Soffe, (2010) that explored how neurons generate behaviour in developing tadpoles.

The tadpole central nervous system (CNS) can be divided into the forebrain, midbrain, hindbrain, and

spinal cord. Eight types of neurons are present in the nervous system which includes two types of

sensory neurons, one type of motor neuron, and five types of interneurons. Only a few thousand

neurons in the entire nervous system are responsible for conducting behaviours for flexion, swimming,

sense of direction and orientation, accelerating/decelerating, struggling, social behaviour, proprioceptive

sensations, detecting light, detecting water currents, and detecting noxious stimuli. Major nervous

system structures in the tadpole include the trigeminal ganglia, which innervates and provide a sense of

proprioception to the entire body surface (in conjunction with the spinal cord). Sensory neurons

innervating the skin are highly excitable and trigger explosive all-or-none neuronal activity and motor

reflexes. Like many aquatic species, the tadpole has a lateral line that allows it to detect water currents.

A pineal gland allows tadpoles to detect light and establish circadian rhythms. All of the aforementioned

neuro-anatomical structures are potential candidates for histological analysis.

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The Present Study

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The purpose of this study was to assess the influence of EMFs on tadpole (*Xenopus laevis)* social

behaviour by quantifying the strong natural gathering instinct of the animal. EMFs that have an impact

on tadpole behaviour were hypothesized to further show quantifiable differences in tadpole

neuroanatomy. Tadpoles were selected as a model for vertebrate physiology for both their well

substantiated responsiveness to EMF exposure, and to expand the pool of animal models used in this

line of research. Previous research has shown that weak EMF exposure causes delays in tadpole

metamorphosis. Observing physiological changes in conjunction with behavioural changes in tadpoles

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exposed to weak EMFs will help us elucidate the mechanism of EMF interactions, as well as help us to

make inferences on the neurological targets of EMF interaction. This is because changes in behaviour

are often reflective of changes in neurophysiology.

The morphological data gathered from this experiment was predicted to be similar to the

morphological data collected in a study previously conducted by Severini et al. (2010). The data from the

previous study suggested that there would be readily apparent delays in morphological development.

The behavioural paradigm was hypothesized to show marked differences between EMF exposed v.

control groups as was apparent in rats exposed to LTP patterned weak EMFs in the previously

mentioned Mach and Persinger (2009) study.

Materials

The experiment utilized *Xenopus laevis* tadpoles to observe the behavioral and physiological

changes that occurred after exposure to a weak electromagnetic field. This specie is widely cited for its

ease of care in laboratory experiments. An LTP patterned electro-magnetic field of intensity 3.4-6.9 mG

was generated using a solenoid chamber that could contain all field conditions. The solenoid chamber

consists of a large metal cage wrapped in copper wire that is connected to a computer controlling the

intensity and magnitude of the field. The field was applied to 6 groups of 15 tadpoles (15 tadpoles per

tank). Three groups were exposed to an LTP patterned EMF of intensity 6.4-6.9 mG by positioning their

tanks toward the perimeter of the solenoid chamber (high intensity condition). Another three groups

were exposed to an LTP patterned EMF of intensity 3.4-4.0 mG by positioning them towards the center

of the solenoid chamber. A gaussmeter was used to discern the areas with differences in intensites

within the solenoid chamber. EMF intensities were measured daily by a gaussmeter to ensure that

tadpoles were immersed in an EMF throughout the duration of the experiment. EMF exposure has

previously been found to have sub-lethal effects on tadpole development such as delaying

morphogenesis into a frog (Severini et al., 2010).

The *Xenopus laevis* stage series of development (Nieuwkoop, 1994) was used to track the

morphological development of tadpoles for 6 days. Tadpoles reached approximately stage 48 of

development. The generation of *X. laevis* tadpoles was purchased for use from Carolina Biological Supply

Company. Tadpoles were fed with *X. laevis* tadpole food that was purchased from the same tadpole

supplier. Tadpoles were housed in plastic aquariums about the size of a large shoebox. Plastic pipettes

were used to handle tadpoles, and a piece of grid paper was placed beneath the transparent aquariums

to be used as a scale for measurements in photos. A 13 megapixel cell phone camera was used to

capture images and ImageJ software was used to analyze the pictures.

Procedure

Upon arrival, the tadpoles were separated into 12 equal groups (15 tadpoles per group) and

placed in their respective housing with their group. Behavioural and morphological paradigms were

measured by taking a picture of a group tank on top of a piece of grid paper. Each plastic aquarium was

placed in a solenoid chamber. Each solenoid chamber continuously applied a magnetic field that was

accurately controlled through specialized computer software. The intensity of the magnetic field

depended on the group (Groups A2-F2 = control groups; Groups A1, B1, and F1 = High intensity; Groups

C1, D1, E1 = Low intensity).

The experiment proceeded as follows: After the tadpoles arrived, they were separated into 12

equal groups (15 tadpoles per group). Each group was housed in a separate tank. For the duration of the

experiment, each group was exposed to the variable which was either a high intensity field (6.4-6.9 mG),

a low intensity field (3.4-4.0 mG), or no field (control). The background EMF activity for all conditions

was 0.5-0.6 mG. Behavioural tests and observations were made at the same time every day for 6 days. A

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picture of the tank on top of a piece of grid paper (0.5cm squares) was taken at this time and ImageJ

software was used to analyze the distance each tadpole was to the nearest tadpole (measure of social

behaviour) and also was used to measure the length of each tadpole. Water was changed daily and

tadpoles were fed once every three days.

Results

Analyses were conducted with IBM SPSS Statistics 21 for Windows. The tables below show the

descriptive statistics for tadpole length and social behaviour. No post hoc analysis is substantiated.

Table 1

One Way Descriptive Statistics for Overall Average Tadpole Length

Variable	Min (cm)	Max (cm)	Mean (cm)	SD	SE	N
No Field	0.65	0.78	0.699	0.053	0.022	6
Low Field	0.65	0.70	0.665	0.029	0.0169	3
High Field	0.69	0.79	0.734	0.054	0.031	3

Note. SD: standard deviation; SE: standard error; Low Field = 3.4-3.9 mG; High Field = 6.4-6.9 mG.($p > .05$)
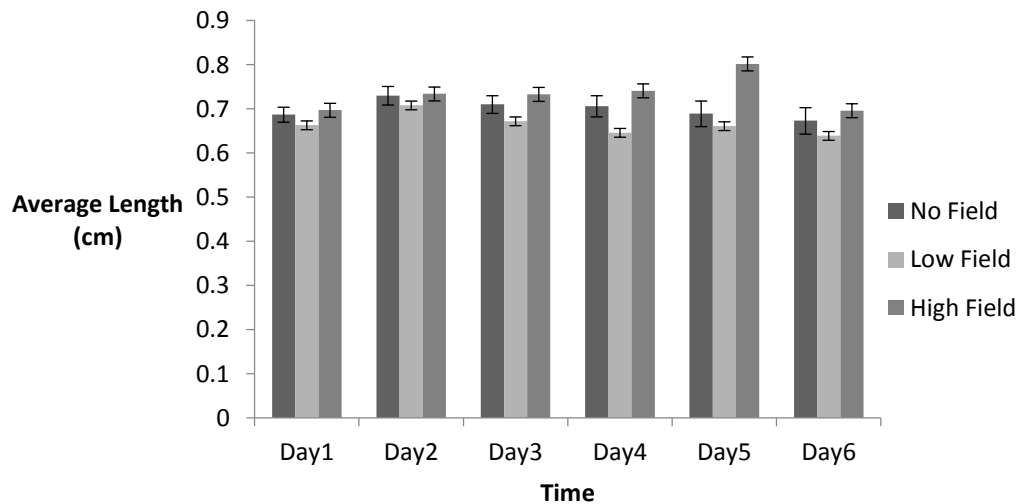
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Table 2

One Way Descriptive Statistics for Overall Average Tadpole Social Behaviour

Variable	Min (cm)	Max (cm)	Mean (cm)	SD	SE	N
No Field	1.31	2.27	1.83	0.354	0.144	6
Low Field	1.35	1.70	1.52	0.178	0.103	3
High Field	1.08	1.89	1.57	0.431	0.249	3

Note. SD: standard deviation; SE: standard error; Low Field = 3.4-3.9 mG; High Field = 6.4-6.9 mG (p>.05)

$F(10,45) = 1.642$, p > .05

Figure 1. Average tadpole length between fields by day. Measurements were taken by analyzing tank photos using ImageJ software. There are no statistically significant relationships in the data (p >.05).

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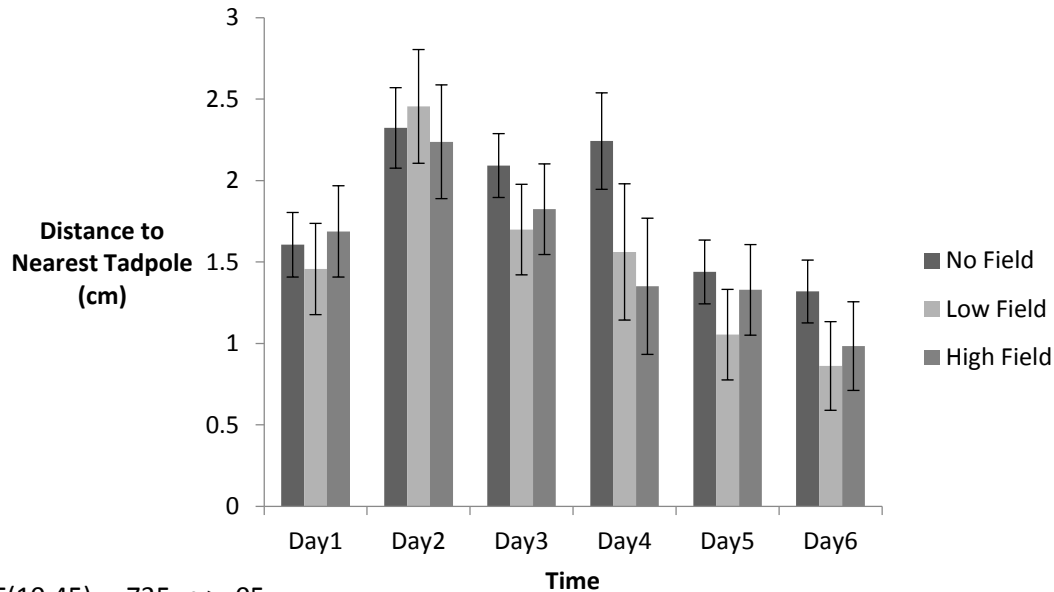

F(10,45) = .735, p > .05

Figure 2. Average social behaviour of tadpoles between fields by day. Measurements were taken by analyzing photos of each group using ImageJ software. There are no statistically significant relationships in the data (p >.05).

The effect of field on tadpole length was not significant, $F(10,45) = 1.642$, $p > .05$. The effect of field on social behaviour was also not significant, $F(10,45) = .735$, $p > .05$. Multiple regression analysis was used to discern whether or not a high intensity field had an effect on relative scores of tadpole length on day 5. The results of the regression indicated that the predictors explain variance in tadpole relative length on day 5 ($R^2=.43$, $F(1,11)= 13.505$, $p <.05$), see *Figure 1*. The analysis shows that there is a significant effect on day 5 where high intensity field exposure predicts longer tadpole lengths ($\beta=.758$, $p< .05$). However, no one predictor in this analysis could account for a significant portion of the variability. Field's (2009) recommends that there be at least 10 cases of data for each predictor in this type of regression analysis. In the current study, the total 'n' for the experiment is n= 12, insufficient to generate reliable predictors (needs at least an 'n' of 120). It is important to collect more data before we can obtain a more reliable regression model. In addition to this, external variables which are not factored into the regression model could have affected the outcome of dependent variables. For these

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reasons, the data collected in this experiment is insufficient to determine whether EMFs had an influence on tadpole growth or social behaviour.

Averages from 6 control groups were used and averages from 3 groups for each condition were used (low intensity and high intensity fields). Taking individual tadpole measurements would have significantly increased the power of this experiment. This is because individual tadpole measurements would result in 15 samples per group (12 groups) or 180 total samples (n = 180). Although individual measurements provide more data, it would require individual storage of each tadpole and subsequently interfere with the observation of tadpole aggregation behaviour (the quantification of social behaviour).

Discussion

Although the experiment generated limited data, a response to EMF exposure in tadpoles is well substantiated in the scientific literature (Severini et al., 2010; Grimaldi et al., 2000, Grimaldi et al., 2004). Thus, this discussion will attempt to rationalize why the EMF exposure in this research did not provoke a biological response.

As previously mentioned, for an external EMF to be able to affect a biological system, Adair (2003) has argued that the energy associated with the external EMF must be enough to overcome the energy generated by the cell's EMF, or the thermal noise of the biological system. In this experiment the weak intensity of the magnetic field may have indeed prevented the observation of a marked EMF interaction. The strength of the EMF used in the current study ranged from 3.4 to 6.9 mG, which is considered a generally weak intensity (Severini et al., 2010). It is possible that the energy associated with the application of this EMF was not enough to overcome the noise of the tadpole biological system. EMF patterns in conjunction with their intensity are both important factors that can influence the biological response (Cifra et al., 2010).

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In the ECC theory, the characteristics of the field must match certain properties of the biological system in order to affect the activation barrier for the reaction (Tsong, 1992). However, if there was excess chlorine in the water (discussed in limitations), it could have interfered with the enzymatic systems capable of amplifying energy delivered by the EMF exposure. In the same way that chlorine could have affected enzymatic systems, so too could it have affected other biomolecular pathways.

It is difficult to discuss how these results fit into the current scientific literature due to their weak statistical and methodological efficacy. Therefore, a critical examination of the limitations encountered in this experiment is necessary to define future directions for research.

Limitations

Unfortunatley, tadpoles in this experiment did not reach the desired stage of maturity. The *Xenopus laevis* stage series of development (Nieuwkoop, 1994) was used to track the morphological development of tadpoles for 6 days. Tadpoles reached approximately stage 48 of development (6 days of growth) out an intended 59 stages (approxiamatley 45 days of development). Numerous factors could have contributed to poor tadpole survivability in the experiment.

One possible factor that affected tadpole survivability was excess chlorine in the water. The method of dechlorination used in this experiment was to allow the chlorine to evaporate out of tanks of tap water over a period of 48 hours (Lens et al., 2002). Although this is a well known method of dechlorination, the rate of diffusion of chlorine out of the water can be affected by storage conditions (Lens et al., 2002). The poor ventilation in the experimental room could have slowed the rate of diffusion of chlorine out of the water and subsequently affected tadpole health. Future experiments should implore the use of techniques for detecting aqueous chlorine concentrations. Conducting future experiments in well ventilated rooms or using automatic de-chlorinators in the aquariums can help control these limiting factors in future experiments.

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Another possible factor affecting tadpole survivability had to do with the loss of a portion of the mucosal membrane that surrounds the tadpoles. When tadpoles were separated into their respective groups, a portion of their mucosal membrane was lost during the transfer. It is possible that this disturbance had an effect on their survivability since the mucosal membrane is a source of nutrients to the tadpoles during early development.

Disease may have also influenced tadpole survivability. The tadpoles used in this experiment were all laboratory reared and genetically similar. Genetic similarity makes a group or specie especially susceptible to disease (Jirtle & Skinner, 2007). Even stressful life events such as the shipping and handling process, potential exposure to the harsh Sudbury climate, and being transferred from the shipping containers into the aquariums all could have been stressful events that had an effect on survivability.

In the end it could have been a combination of many of the aforementioned factors that resulted in poor survivability. Because these variables could not be controlled in the experiment, the data collected cannot say with statistical efficacy whether the EMF had an effect.

Implications

Although this experiment produced limited data, it is not without its implications. One important implication for this research is that it contributes to the diversification of animal models used in science. A report from the European Union (European Commission, 2013) states that almost 4/5ths of all research animals used by members states of the European Union are either rats or mice. It is important to diversify the animal model so that the information we learn from these animals (that we apply to understanding nature) isn't understood only in the narrow context of lab rats and lab mice. Factors that may be influencing research associated with laboratory animals include genetic similarity due to inbreeding, rearing animals in sterilized environments, overfeeding animals, and animals being under stimulated. Factors like these divorce the organism from its natural state, and subsequently

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narrows the conclusions we can draw from them (how well the sample applies to natural populations). Using multiple animal models will provide deeper insights into how different experimental effects scale among higher and lower species. This would provide a deeper understanding of how an experiment on an animal can provide insight into human research/development.

Another important implication for this research is that it was the only experiment in the animal care research center (Laurentian University) that utilized aquatic organisms at the time (2014-2015). The drafting of a successfully passed animal care protocol in this experiment allows it to serve as a ledger by which future experiments can model their protocols after. This will allow scientists at Laurentian University interested in working with aquatic or amphibian animals to move forward faster with their research. This experiment further developed the body of literature regarding ethical considerations for animals at Laurentian University.

Future Directions

Future directions for this line of research will address the severe limitations encountered in this experiment. This includes monitoring/reducing the chlorine concentration of tank water and increasing the amount of groups used in the experiment. Once the limitations are sufficiently addressed, the following behavioural paradigms can be used in conjunction with observing social behaviour to further quantify tadpole behaviour:

Startle Habituation: A tadpole is placed in a culture dish individually. The tadpole will remain untouched in the dish for two minutes to overcome handling effects. After this period, the side of the dish is tapped to initiate a startle (jerk-like movement in a random direction). Each tap will be separated by approximately 10 seconds. The number of taps before a startle response is no longer observed will be recorded up to a maximum of 30 trials (5 minutes). This test will quantify changes in sensory gating mechanisms.

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Avoidance Test: A tadpole is placed in a culture dish individually. The tadpole will remain untouched in the dish for two minutes to overcome handling effects. A black object will be placed under the dish as far as possible from the tadpole. A measure will be taken on how close this object can get to the tadpole before it escapes (rapid movement away from object). This is also a test for sensory gating mechanisms.

These two behavioural paradigms are expected to show differences between field exposure groups, such that tadpoles in the field exposure groups are predicted to show a higher number of trials before startle habituation occurs, they may show larger average distances in the avoidance test, and show increased distances between tadpoles (abnormally) in the social behaviour paradigm.

It is difficult to predict what neuroanatomical structures will be affected by the EMF exposure since there has been no previous examination of the tadpole nervous system following EMF exposure in the scientific literature. For histological purposes, careful attention should be paid to reflexive pathways since startle habituation and avoidance test behavioural paradigms strongly reflect sensory-motor gating mechanisms. In addition to this, the thyroid gland and pineal gland can be dissected out and concentrations of thyroid hormone (in thyroid) and melatonin (in pineal) can be considered. The thyroid and the pineal gland are significant since the release of thyroid hormone is heavily implicated in the process of metamorphosis (Severini et al., 2010) and the antagonistic effect of melatonin on thyroid hormone may be responsible for delaying metamorphosis in tadpoles. Based on this, it is predicted that a histological analysis of the tadpole nervous system following EMF exposure may show lower concentrations of thyroid hormone (due to delay in morphogenesis) in the thyroid, higher concentrations of melatonin in the pineal organ, and differences in the anatomy of the field exposure group's central nervous system (CNS).

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At the end of future experiments, tadpoles can be euthanized by adding buffered tricaine methanesulfonate (MS 222) to the tadpole water bath. Up to 1 hour of immersion in this water bath at concentrations of 5-10 g/L may be necessary to achieve anaesthetic overdose. The use of this compound is necessary for this type of study because it creates minimal histological artifacts. At this point, the gross anatomy of the tadpoles can be assessed before deciding what histological staining procedure is most beneficial in observing neuroanatomical structures. All animal used in any subsequent experiments will adhere to the recommendations of the Canadian Council on Animal Care, under the requirements of the Animals for Research Act, as well as to Laurentian University's Animal Care Policies and Guidelines (and other applicable procedures).

Conclusion

To fully realize the effects of external EMF exposure on biological systems, in depth analysis of the neuroanatomical consequences of EMF exposure must be pursued. Changes in tadpole neuroanatomy as a result of this exposure may provide insights into how EMFs can influence other biological and vertebrate models. As the nature of these interactions is extremely complex, it is important to thoroughly analyze all changes and aspects of an organism that are vulnerable to EMF exposure, from the macroscopic behavioural level, to the microscopic cellular level. In this experiment, severe limitations prevented the observation of a marked EMF interaction with the tadpole biological system. Utilizing more tadpole participants and imploring the startle habituation and avoidance test paradigms will provide deeper insights into the mechanism of EMF interaction. Neurological targets of EMF interactions can be inferred through the observation of changes in tadpole behavioural paradigms.

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