Received: 28 January 2022 | Revised: 22 April 2022 | Accepted: 23 May 2022

DOI: 10.1002/wer.10747

RESEARCH ARTICLE

water ENVIRONMENT RESEARCH

No evidence for collateral effects of electromagnetic fields used to increase dissolved oxygen levels on the behavior and physiology of freshwater fishes

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Funding information
Natural Sciences and Engineering Research Council of Canada, Grant/Award Number: NSERC Alliance Grant no. ALLRP 561435 - 20; Carleton University Animal Care Committee, Grant/Award Number: # 112698

Abstract

Hypoxia in surface waters driven by warming climate and other anthropogenic stressors is a major conservation concern, and technological solutions for water quality remediation are sorely needed. One potential solution involves the use of low-intensity electromagnetic fields (EMFs) to increase dissolved oxygen levels, but potential collateral effects of the EMFs on aquatic animals have not been formally evaluated. We examined the effects of EMF exposure on wild-caught, captive sunfish (*Lepomis* spp.) over 8-day and 3-day exposures, with and without aeration in mesocosms and stock tanks (respectively). We also quantified ambient fish abundance in close proximity to EMF devices deployed in Opinicon Lake (ON). We found no significant differences in a suite of blood-based stress physiology biomarkers, behaviors, and putative aerobic capacities between EMF and control conditions over 8 days. Aerated mesocosms equipped with activated EMFs consistently had higher oxygen levels in the water than aerated controls. There were no differences in mortality during 3-day oxygen depletion trials under EMF or control conditions, and we detected no differences in fish abundance when the devices were activated in the lake. Our findings suggest that deploying EMF devices in field settings is not likely to exert negative effects on exposed fish populations.

Practitioner Points

- Low-cost, low-energy technological solutions to remediate aquatic hypoxia are sorely needed
- Electromagnetic fields (EMFs) can increase oxygen flux across air/water interfaces and increase dissolved oxygen levels
- We found no evidence of negative effects of EMFs on fish physiology or behavior and our results support their use in alleviating hypoxic conditions

KEYWORDS

aquatic remediation, avoidance, behavior, centrarchid, hypoxia, oxygen depletion, stress response, swim endurance

INTRODUCTION

Freshwater biodiversity is in crisis (Harrison et al., 2018), and many of the freshwater ecosystem services that we depend on are threatened. Freshwater ecosystems are subject to several key long-standing threats including, pollution, flow modification, and other forms of habitat degradation (Dudgeon et al., 2006). These issues are further exacerbated by climate change and interactions between cumulative stressors (Reid et al., 2019). Global estimates reveal that freshwater populations have declined on average by 83% over the last four decades (WWF, 2018), and given the extent to which humans around the globe depend on freshwater ecosystems for food, potable water, and livelihoods (Dodds et al., 2013; Postel & Carpenter, 1997), this is alarming. The problems facing freshwater ecosystems are real and pervasive—what we need are solutions (Tickner et al., 2020). A common characteristic for many degraded freshwater ecosystems is decreased levels of biologically vital dissolved oxygen (DO) and hypoxic or anoxic conditions (Diaz, 2001). Low levels of DO in freshwater systems can lead to changes in ecosystem structure and function that result in loss of biodiversity (Steckbauer et al., 2011), and low oxygen conditions following harmful algal blooms (Watson et al., 2016) can cause fish kills (Farrell & Richards, 2009) that result in socio-economic and health consequences for humans (Diaz & Rosenberg, 2011).

Given the manifold negative effects of hypoxic and anoxic condition on freshwater ecosystems, much effort has been devoted to initiatives that reduce the input of pollutants associated with low DO levels. For example, agricultural land use practices have been modified (Kleinman et al., 2015) and sewage treatment facilities have been upgraded (X. Zhang et al., 2019) to reduce the input of excess nutrients that drive eutrophication and algal blooms and produce low oxygen conditions. However, in instances where hypoxic or anoxic conditions are already common and where efforts to reduce nutrient inputs have failed, there are relatively few tools available to mitigate hypoxia in freshwater systems aside from directly increasing DO levels (Kerr et al., 2013). Increasing DO in freshwater ecosystems is not easy (Boys et al., 2021) and often involves mechanical approaches such as bubblers and aerators that tend to have very localized effects (Baldwin et al., 2021; Whitworth et al., 2013). In regulated lotic systems, it is possible to manipulate flows (Watts et al., 2018) but that is not applicable to unregulated rivers or lentic systems. Chemical approaches to increasing DO levels are limited to small, closed systems and thus far have shown to be largely ineffective (e.g., Dunalska et al., 2015). During the last decade, there have been developments that use magnetic or electric (i.e., electromagnetic) fields (EMFs) to increase DO concentrations in freshwater systems and they are showing promising outcomes (e.g., Hassan & Rahman, 2016; Yap et al., 2021; Yin et al., 2011). However, given that the use of such technology involves altering electrical charges in freshwater ecosystems, there are concerns regarding their potential collateral effects on aquatic organisms that need to be evaluated before full-scale field applications in natural waterbodies can be approved.

The effects of EMFs on aquatic animals have been well studied for decades, mostly in the context of submarine electrical cables (Albert et al., 2020) or marine renewable energy infrastructure (Copping et al., 2020; Kulkarni & Edwards, 2021). The majority of this work has occurred in marine environments and has revealed evidence for localized effects on the behavior of fishes (e.g., Gill et al., 2012; Gill & Taylor, 2001; Normandeau Associates et al., 2011), invertebrates (Scott et al., 2021), and marine mammals (Teilmann & Carstensen, 2012) when they are in close proximity (within several meters) to cables or infrastructure (reviewed in Albert et al., 2020; Kulkarni & Edwards, 2021; Woodruff et al., 2012). However, several other studies have failed to identify negative impacts of EMFs on the behavior of marine animals (e.g., Love et al., 2015) and most documented behavioral alterations tend to be rather small (Öhman et al., 2007). Similar work in freshwater systems is less common but has also revealed minor impacts on behavior for some fish species when in close proximity to cables (Bevelhimer et al., 2013; Cada et al., 2011). Physiological outcomes have also been studied, mostly in lab environments, with some evidence of histopathological (Samiee & Samiee, 2017) and endocrine (Lerchl et al., 1998) effects on freshwater fishes, although there are also examples where no effects were observed (Bochert & Zettler, 2004). However, the context of an animal being in direct proximity to infrastructure that is incidentally emitting EMFs may be different than when low-intensity EMFs are intentionally emitted as a means of increasing DO levels as these are intended to produce constant, consistent output. There has been very little research on that topic aside from some recent studies that have focused on testing whether such devices can be used to improve growth performance and physiological status of fish in aquaculture (Ahmed & Abd El-Hamed, 2020; Charmi et al., 2020; Hassan et al., 2018; Irhayyim et al., 2020). No studies have yet evaluated electromagnetic water treatment technology on freshwater fish in the context of understanding the potential biological risk of such devices when used in real-world field applications.

Low-intensity EMFs have shown promise for improving water quality and hypoxia remediation. DO levels have been successfully increased in even highly polluted sewage lagoons in southwestern Ontario via increased surficial air-to-water flux of oxygen when these EMFs are present (Laursen et al., 2021b). There are many places where such technology is needed, but deployments cannot proceed without understanding any biological impacts beyond improving DO levels, which will generally benefit aquatic life. The goal of this study is to assess any potential effects of low-intensity EMFs on freshwater fishes. To achieve this goal, we had three objectives that involved both lab and field experiments and endpoints spanning a range of biological responses (physiological state, behavior, and survival). First, we held wild-caught adult pumpkinseed (*Lepomis gibbosus*) in outdoor replicate mesocosms for 8-day trials with active or inactive EMF devices and sampled subsets of the fish at 2-day intervals for blood stress and behavioral parameters (Experiment 1). Second, we held adult bluegill (*Lepomis macrochirus*) in replicate stock tanks with active or inactive EMF devices, plus control tanks without bluegill, to track water oxygen levels over time while allowing biological oxygen consumption and monitored the tanks regularly for mortalities (Experiment 2). Third, we deployed active and inactive EMF devices equipped with waterproof video cameras at different sites in a small lake for 1-h periods and analyzed the videos to estimate ambient fish abundance as an indicator of attractive versus repellent localized effects of EMFs (Experiment 3).

METHODS AND MATERIALS

EMF device

The device (EMF-1000, EM Fluids Inc., Ottawa, ON, Canada: https://emfluids.com/) is a portable, free-floating, solar-powered, buoyant hull approximately the size of a shoebox containing a battery, a solar panel, a Bluetooth antenna, and a GPS antenna connected to a control chip. The hull floats on the surface of the water and a cylindrical EMF transducer arm extends down ~20 cm into the water column (Figure 1). The device is operated via proprietary companion application for Android OS that allows the device to be activated and deactivated, as well as device status notifications and automated prompts to upload device log files for transmission back to the manufacturer via email. Internal and commercial field testing of these devices indicates that each may have an effective range radius of over 200 m (Laursen et al., 2021a; 2021b; D.T. Fung, personal communication).

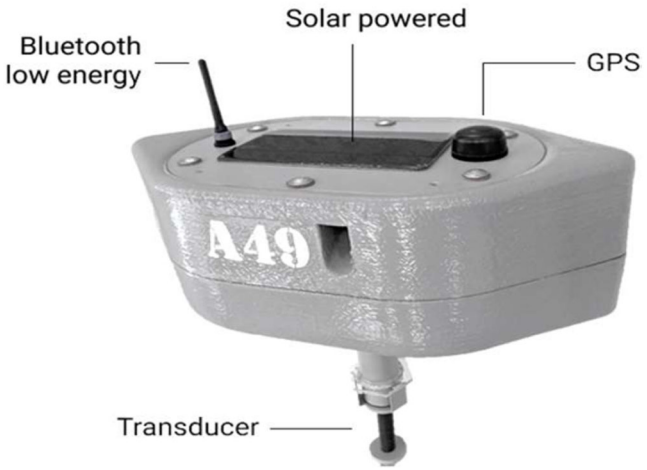

FIGURE 1 The electromagnetic field (EMF)-1000 device (EM Fluids Inc., https://emfluids.com/) with submerged EMF transducer arm below the buoyant hull

Study animals

This study was conducted at the Queen's University Biological Station (QUBS; www.qubs.ca; Elgin, ON, Canada) between July and September 2021 using wild-caught, postspawning sunfish (Experiment 1: pumpkinseed *Lepomis gibbosus* and Experiment 2: bluegill *L. macrochirus*) from Opinicon Lake. Test fish were collected via angling from a boat away from the QUBS property using size 8 baitholder hooks with pieces of commercially obtained earthworm (*Lumbricus* sp.). Only fish that were captured rapidly (<30 s from hooking to landing) and hooked shallowly in the jaw were retained, and all fish with deep hooking involving the tongue, gills, or esophagus, or who were showing any signs of bleeding upon capture, were immediately released back into the lake. All retained fish were held in 20-L aerated coolers filled with lakewater for <1 h following capture and then transferred to 500-L holding tanks supplied with flow-through lakewater on the QUBS boat dock for <24 h prior to experimentation. Several studies on physiological outcomes of angling-induced stress responses in centrarchid fishes have demonstrated that 24 h is sufficient for recovery to baseline, prestressor levels, and minimizing holding times reduces long-term fitness effects (reviewed in Kieffer & Cooke, 2009).

Experiment 1: Prolonged EMF exposures in aerated mesocosms

Four circular replicate mesocosms (gray fiberglass, 3.5-m diameter, 1.4-m depth) were filled with unfiltered water from Opinicon Lake to depths of 0.5 m (~5000 L). Each

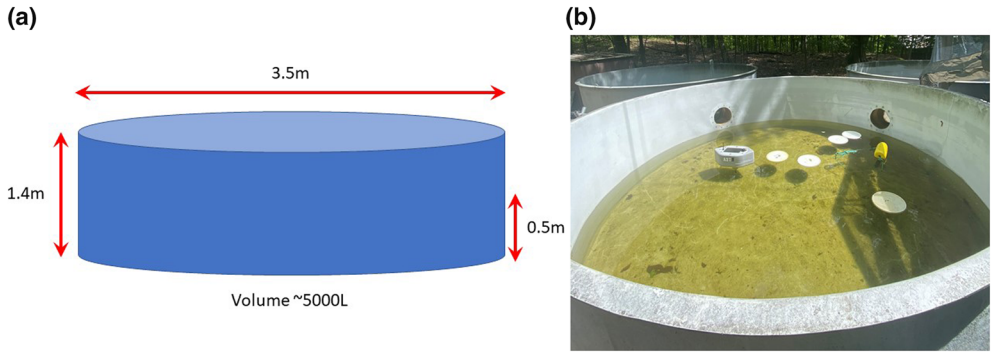

FIGURE 2 Dimensions (a) of the four replicate mesocosms (b) used to hold pumpkinseed (*Lepomis gibbosus*; $N = 60$ each) for 8-day electromagnetic field (EMF) exposures with aeration in Experiment 1. The EMF device is floating at upper left, and the dissolved oxygen logger is suspended from the yellow buoy (upper right). White circles are bucket lids added to provide shelter

mesocosm was equipped with one EMF-1000 device and an aquaMeasure DO (Innovasea Systems Inc.) DO logger that recorded oxygen saturation (%) at 2-h intervals throughout each 8-day experimental round (Figure 2). Each mesocosm was aerated via a 15-cm airstone and standard aquarium airline tubing to maintain similar baseline oxygen levels throughout the experiment to isolate the effects of EMF exposure from any water quality effects of the devices and to offset biological oxygen consumption. Each mesocosm was supplied with five white plastic bucket lids to serve as shelter for the fish. Half of the EMF devices were activated 1 h before fish were introduced to serve as treatment replicates, and the other half were left off to serve as control replicates. Each mesocosm was stocked with $N = 60$ pumpkinseed on Day 0, and the fish were held without additional food for 8 days. Every second day (i.e., Days 2, 4, 6, and 8), a subset of fish were removed from the mesocosms ($N = 10$ from each mesocosm, $N = 40$ per sampling day) for physiological ($N = 6$ per mesocosm, $N = 24$ per sampling day) and behavioral ($N = 4$ per mesocosm, $N = 16$ per sampling day) assays.

Physiological sampling consisted of extracting 0.3-ml blood samples within 3 min of removal from the mesocosms (Lawrence et al., 2018) via heparinized syringe (25 gauge needle). Point-of-care devices (Lactate Plus Lactate Meter, Nova Biomedical, Waltham, MA; Accu-Chek Glucose Meter, Roche Diagnostics, Laval, QC) that have been validated for use in fish (Stoot et al., 2014) were used to quantify blood lactate (mmol/L) and blood glucose (mmol/L). Hematocrit (% red blood cells) was determined by using a hematocrit centrifuge. All three measures have been positively associated with elevated physiological stress levels including oxygen deficits (Donaldson et al., 2014; Sopinka et al., 2016; Suski et al., 2007).

The behavioral assay consisted of two parts. First, two fish from each mesocosm were placed into one of two glass aquaria (60 cm × 30 cm × 30 cm, length × width × height) filled with 40 L of lake water and allowed to acclimate for 1 h following established protocols (Brown et al., 2009; Lloren et al., 2019). A digital video camera (Apexcam, 1080p, 60fps) mounted in front of the aquaria on a tripod was then activated, and the fish were recorded for 20 min. From the videos, at 30-s intervals, we recorded shoaling index (SI) where values ranged from 0 (the fish were not within 1 body length of each other) to 1 (the fish were within 1 body length of each other) and area use (AU) where values ranged from 2 (both fish were in the bottom half of the aquarium) to 4 (both fish were in the upper half of the aquarium). These endpoints are commonly used to evaluate behavioral differences between fish in different experimental groups (Brown et al., 2009; Lloren et al., 2019). In addition, after the behavioral observations, focal fish were transferred individually to a circular arena (1.2-m diameter) filled to a depth of 10 cm with unfiltered lake water and then chased via hand at consistent speed and intensity by one experimenter (CJB) until a swimming quit point was reached (Elvidge & Cooke, 2020; Roche et al., 2013) as a measure of aerobic capacity (Kieffer, 2000; Kieffer et al., 2002), endurance (Hammer, 1995), and/or motivational state (Elvidge & Cooke, 2020). At the end of each sampling, focal fish were measured (total length and mass) and released directly back into the lake, as were all remaining fish in the mesocosms at the end of each 8-day exposure. In total, we performed three full rounds ($N = 4$ mesocosms each with $N = 60$ fish) plus an initial half-round ($N = 2$ mesocosms) as proof-of-concept and to assess fish mortality in the mesocosms (none observed) for a total of $N = 840$ fish in $N = 7$ replicates per treatment ($N = 420$ each

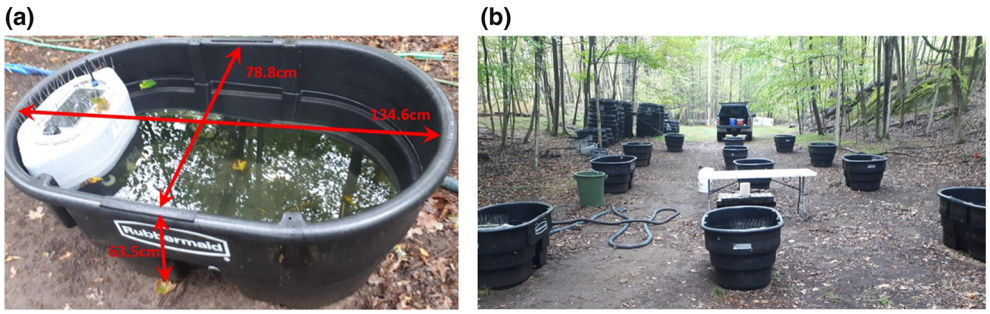

FIGURE 3 Dimensions (a) of the 12 commercial stock tanks (b) used to hold bluegill (*Lepomis macrochirus*, $N = 8$ each) for 3-day oxygen depletion trials in Experiment 2

in the treatment and control groups) with approximately $N = 336$ assayed for blood physiology ($N = 168$ in each treatment group) and $N = 224$ assayed for behavior and aerobic capacity ($N = 112$ in each treatment group). Experimental rounds began on July 9, July 18, August 6, and September 2 (2021).

Experiment 2: Short-term EMF exposures with oxygen depletion

Commercial stock tanks (Rubbermaid; 378 L; $N = 12$; Figure 3) were filled with ~280 L of unfiltered lake water to track DO levels over 3-day (72 h) depletion trials. DO (mg/L), oxygen saturation (%), and temperature were recorded at 4–6 h continuously throughout the trials using an OxyGuard Handy Polaris DO meter (OxyGuard International), and mortalities were removed and recorded as needed at each sampling time. We subsequently compared the oxygen concentration and saturation measurements via linear correlation to support our saturation measures in Experiment 1. As the beginning of this experiment overlapped with the final round of Experiment 1, the number of EMF devices was initially limited to six (the other four being used in the mesocosms). Consequently, the number of tanks and EMF devices increased after the first experimental round. Round 1 used nine tanks, with six of them stocked with $N = 10$ bluegill each and half assigned to each of the EMF treatment and control groups ($N = 60$ bluegill total). The other three tanks did not receive fish and served as indicators of background oxygen levels in the tanks. Rounds 2 and 3 each used the full set of 12 tanks, with eight tanks stocked with bluegill ($N = 80$ fish per round) and four left unstocked. Of the four unstocked tanks, all served as background oxygen level controls in Round 2, while two were equipped with an EMF device in Round 3 with one device activated and one left off. At the end of each 3-day trial, all fish were removed, measured (total length and mass), and released back into Opinicon Lake. Fish masses from each tank were then used to calculate metabolic oxygen consumption (MO) between sampling intervals (ratio of concentration changes to fish mass per unit time). In total, there were $N = 11$ replicates each of the treatment and control bluegill trials, $N = 9$ of the background oxygen level trials, and $N = 1$ each of the EMF treatment and control background oxygen level trials. Experimental rounds were started on September 7, September 12, and September 18 (2021).

Experiment 3: Lake deployments and ambient fish abundances

The EMF devices were equipped with waterproof cameras (Apexcam; Experiment 1) attached to the submerged transponder arm so the cameras would rest below the surface of the water. The units were then tethered to cinder block anchors and yellow marker buoys with nylon nautical rope and deployed for 1-h observation periods at different points (1- to 3-m depth) separated by at least 300 m around the shores of Opinicon Lake. From the videos, we extracted still frames at 30-s intervals, counted the number of fish visible in those frames, and identified them to at least the Family level or genus or species whenever possible (as per Glassman et al., 2022) to determine if the EMFs influenced space use by wild, free-swimming fishes in their natural habitat. We recorded a total of $N = 51$ videos consisting of $N = 25$ treatment (EMF device activated) and $N = 26$ control (EMF device off) trials conducted at midday to maximize light availability and underwater viewing range. Field deployment videos were recorded during July and August 2021.

Statistical analyses

Response data from Experiments 1 and 2 were analyzed as linear mixed-effects models (LMEs) with mesocosm (Experiment 1) or tank (Experiment 2) nested within

experimental round in a hierarchical error term, EMF treatment as a fixed effects term, and either testing day (Experiment 1) or elapsed time (Experiment 2) as additional factors in factorial designs using the "*lme4*" (Bates et al., 2015) and "*lmerTest*" (Kuznetsova et al., 2017) packages with Type II sums of squares. Behavioral measures from the aquarium observations were analyzed as above but without mesocosm as a nested error term. Mortalities in Experiment 2 were treated as binary data and analyzed between treatments in a generalized linear mixed model (GLMM) with binomial error distribution in the "*lme4*" and "*car*" packages (J. Fox & Weisberg, 2011), and fish sizes in both experiments were compared between treatments in one-way ANOVAs against treatment.

Fish counts from the lake deployments in Experiment 3 were analyzed as total values per trial and mean counts per frame at 30-s intervals in two-way factorial ANOVAs with treatment and genus as fixed-effects terms, and genus occurrence in each trial was scored as a binary variable and analyzed in a factorial GLM with treatment and genus as fixed-effects terms against a binomial distribution. Analyses were conducted in R version 4.1.0 (R Core Team, 2021), and figures were generated with "*ggplot2*" (Wickham, 2016), "*cowplot*" (Wilke, 2019), and the "*wesanderson*" (Ram & Wickham, 2018) packages.

RESULTS

Experiment 1: Prolonged EMF exposures in aerated mesocosms

Oxygen saturation (%) was significantly greater in the aerated EMF treatment mesocosms than in the aerated controls (LME: $F_{1,9.2} = 13.74$, $P = 0.0047$) throughout the 8-day trials, with similar patterns of change over time between the replicates (Figure 4a) and a significant effect of mesocosm as a random term (LME: likelihood-ratio χ^2_1=213.1, $P < 0.0001$). Oxygen saturation did not vary significantly between experimental rounds (LME: $P > 0.05$).

Pumpkinseed (107- to 241-mm total length, mean 176.2 mm) did not differ in size between treatment and control groups (ANOVA: total length: $F_{1,603}$=0.304, $P = 0.58$; mass: $F_{1,603} = 3.45$, $P = 0.064$) and did not differ in their physiological responses between experimental treatments (Table 1), although there were some significant effects of exposure day, the treatment × day interaction, and the random error terms. Blood glucose was significantly influenced by the treatment × day interaction (Table 1), but this did not result in a clear pattern in the data (Figure 5a). Blood lactate did not differ between treatments or days (Figure 5b) but did vary significantly between experimental rounds (Table 1).

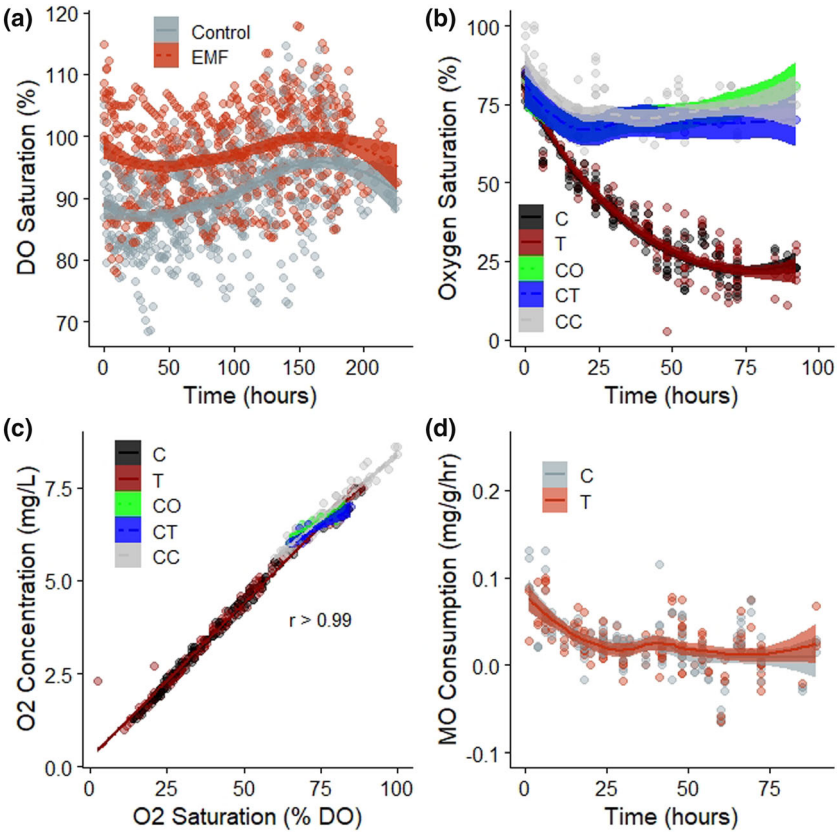

FIGURE 4 (a) Mean (± SE) oxygen saturation levels measured at 2-h intervals in four replicate aerated mesocosms stocked with $N = 60$ pumpkinseed (*Lepomis gibbosus*) and equipped with activated (orange) or unactivated (gray) electromagnetic field (EMF) devices, plus one round with two mesocosms only in Experiment 1. (b) Oxygen depletion curves for bluegill (*L. macrochirus*) in stock tanks ($N = 8$ each) over 3-day (72 h) unaerated trials, (c) oxygen concentration readings plotted against oxygen saturation (C: bluegill, control treatment; T: bluegill, EMF treatment; CO: no bluegill, EMF off; CT: no bluegill, EMF on; CC: no bluegill, no EMF device), and (d) mass-specific metabolic oxygen consumption of bluegill (C: control; T: EMF) during Experiment 2

TABLE 1 Linear mixed-effects models (LMEs) of blood parameters and time to exhaustion in pumpkinseed (*Lepomis gibbosus*) held in replicate aerated mesocosms for 8-day trials under electromagnetic field (EMF)-exposed or control treatment conditions

Response	Factor	*df*	*F*	*P*
Blood glucose (mmol/L)	Treatment	1, 349	0.14	0.71
	Day	3, 349	0.15	0.93
	Treatment:Day	3, 349	3.70	0.012
	Error term	*df*	*LRT* χ^2	*P*
	Tank:Round	1	0	1
	Round	1	0	1
Blood lactate (mmol/L)	Treatment	1, 9.1	0.59	0.46
	Day	3, 337.7	1.98	0.12
	Treatment:Day	3, 337.8	2.19	0.088
	Error term	*df*	*LRT* χ^2	*P*
	Tank:Round	1	0.12	0.73
	Round	1	7.15	0.0075
Hematocrit (%)	Treatment	1, 345.9	0.036	0.85
	Day	3, 345.9	5.68	0.00084
	Treatment:Day	3, 345.9	0.22	0.88
	Error term	*df*	*LRT* χ^2	*P*
	Tank:Round	1	0	1
	Round	1	14.85	0.00012
Time to exhaustion (s)	Treatment	1, 236.9	0.051	0.82
	Day	3, 237	21.32	< 0.0001
	Treatment:Day	3, 236.9	0.62	0.60
	Error term	*df*	*LRT* χ^2	*P*
	Tank:Round	1	0	1
	Round	1	24.30	< 0.0001
Area use index (AU)	Treatment	1, 86.1	2.86	0.094
	Day	3, 86.5	1.29	0.28
	Treatment:Day	3, 86.1	0.82	0.49
	Error term	*df*	*LRT* χ^2	*P*
	Round	1	1.59	0.21
Shoaling index (SI)	Treatment	1, 85.9	0.33	0.57
	Day	3, 86.9	1.79	0.16
	Treatment:Day	3, 85.9	0.71	0.55
	Error term	*df*	*LRT* χ^2	*P*
	Round	1	0.041	0.84

Hematocrit percentages differed significantly between exposure days (Table 1) with progressive increases in both the treatment and control groups (Figure 5c). Hematocrit also varied significantly between experimental rounds (Table 1). Time to exhaustion during forced swim events differed significantly between exposure days (Table 1), with progressive decreases at each sampling period for both the treatment and control groups (Figure 5d) and significant variation between experimental rounds (Table 1).

In the aquarium assays, area use was not significantly influenced by any of the explanatory model terms (Table 1) although pumpkinseed in the control treatment demonstrated a trend towards occupying the upper part of the water column compared with fish in the EMF treatment (Figure 6a). SI was similarly unrelated to the model terms (Table 1) despite pumpkinseed in the EMF treatment demonstrating more cohesive shoaling behavior on days 4, 6, and 8 compared with controls (Figure 6b).

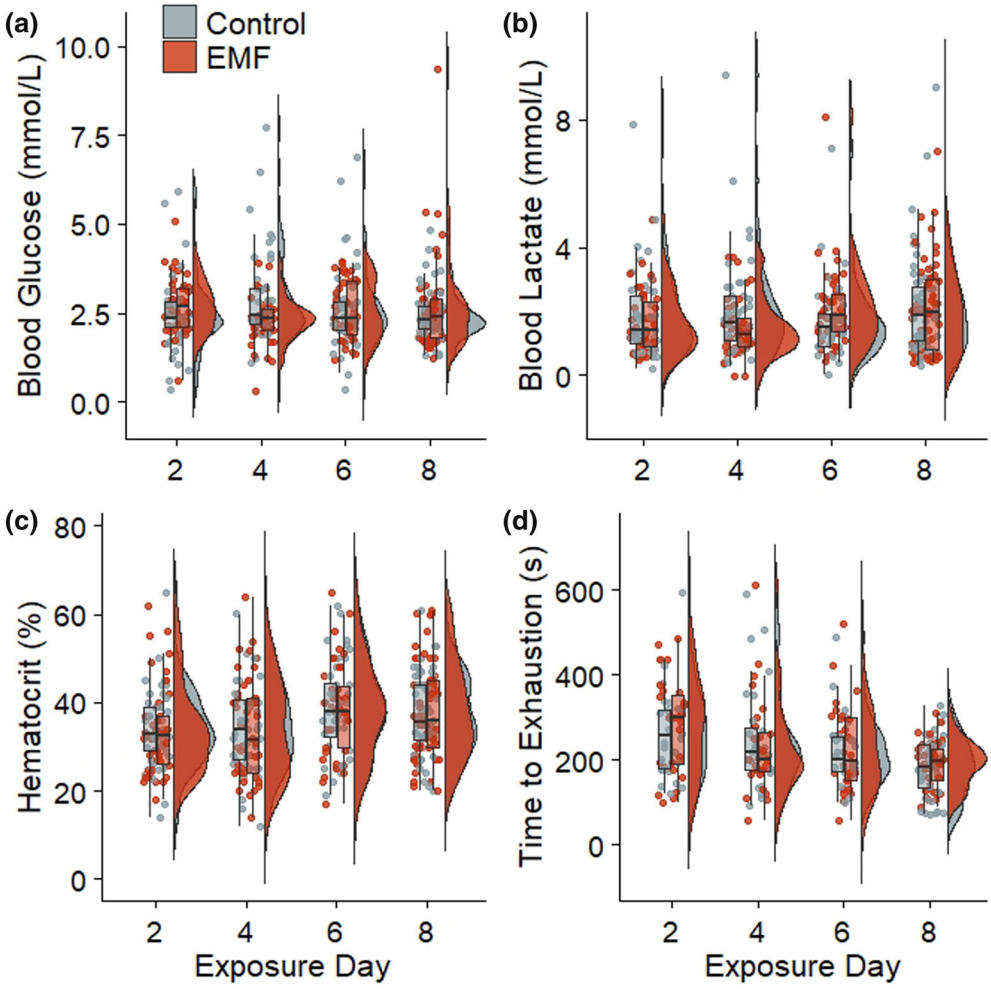

FIGURE 5 Blood (a) glucose and (b) lactate concentrations (both mmol/L), (c) blood hematocrit (%), and (d) time to exhaustion (s) during forced chases in pumpkinseed (*Lepomis gibbosus*) held for 8-day electromagnetic field (EMF) exposure trials in replicate aerated mesocosms in Experiment 1

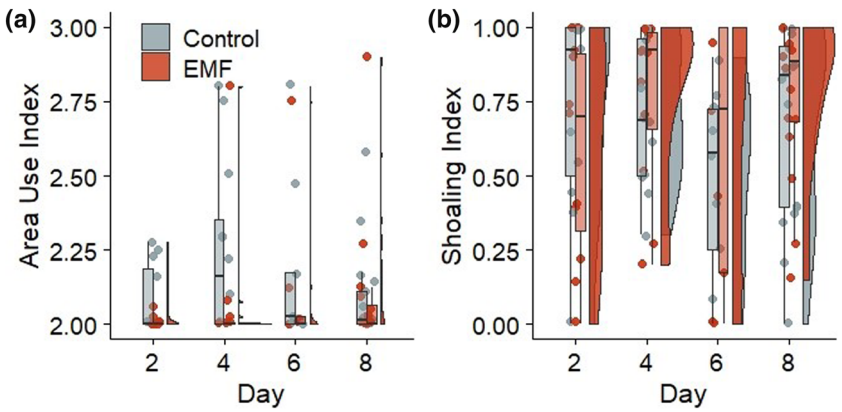

FIGURE 6 Behavioral patterns in (a) area use (2 = both fish in the lower half of the tank, 4 = both fish in the upper half of the tank) and (b) shoaling index (0 = fish greater than 1 body length apart, 1 = fish less than one body length apart) of pumpkinseed (*Lepomis gibbosus*) held in replicate aerated mesocosms for 8-day electromagnetic field (EMF) exposure trials in Experiment 1

Experiment 2: Short-term EMF exposures with oxygen depletion

Unaerated stock tanks housing bluegill (136- to 217-mm total length, mean 180.8 mm) demonstrated lower water oxygen saturation levels than tanks without bluegill (Figure 3b) and oxygen levels decreased over time (Table 2) consistent with biological consumption over the 3-day holding periods. In the stock tanks without bluegill, there was no apparent effect of the EMF device on oxygen levels compared with stock tanks without the EMF devices (Figure 4b). As the oxygen loggers used in Experiment 1 record only oxygen saturation but the probe used in Experiment 2 measured both oxygen saturation and concentration, we compared the values via linear correlation (Figure 4b) and found that the saturation-concentration values were reliably interchangeable (Figure 4c; Pearson's $r_{478} = 0.99$, $P < 0.0001$),

validating our decision to include an evaluation of oxygen consumption by the focal fish. MO did not differ between EMF treatment and control groups but did change significantly between sampling times (Table 2), with a trend towards decreasing MO (Figure 4d) concurrent with decreasing ambient oxygen levels in the stock tanks (Figure 4b). Mortality was not influenced by treatment (GLMM, Wald's $\chi^2_{1,167} = 2.64$, $P = 0.104$; three mortalities in the control treatment vs. nine mortalities in the EMF treatment or 5.5% overall), and qualitative examination of deceased fish identified fungal infections possibly resulting from capture and handling as the likely causes of death.

Experiment 3: Lake deployments and ambient fish abundances

The number of fish recorded in each frame at 30-s intervals in 1-h videos (Figure 7a) and the total number of fish recorded in each video (Figure 7b) did not differ between EMF and control treatments (ANOVA, both $F_{1,49} = 0.55$, $P = 0.46$) nor did the number of frames in which fish were visible (ANOVA, $F_{1,49} = 3.83$, $P = 0.056$). Species diversity was limited to pumpkinseed and bluegill (*Lepomis* spp.), black crappie (*Pomoxis nigromaculatus*), largemouth bass (*Micropterus salmoides*), yellow perch (*Perca flavescens*), and unidentified cyprinid minnows. Of these, largemouth bass could be classified as adults or juveniles based on visual size assessments. Occurrence of fishes in the video frames was not influenced by EMF treatment (GLM, activated versus controls: $F_{1,343} = 2.02$, $P = 0.16$), but species were not detected equally between frames (GLM, $F_{1,343} = 24.53$, $P < 0.0001$). Centrarchid sunfish (*Lepomis* spp.) occurred more frequently when the EMF devices were activated than when they were not (27 vs. 15.9 mean total observations per video), as were yellow perch and minnows (0.04 vs. 0 mean per video). Black crappie followed an opposite pattern (0.04 during controls vs. 0 during treatments), and largemouth bass were overall equally common between treatments (17.03 control vs. 17.8 EMF). By age class, juvenile bass were more abundant during treatment trials than controls (17.4 vs. 15.7), while adults were more common during

TABLE 2 Linear mixed-effects models (LMEs) of oxygen concentrations in unaerated stock tanks and metabolic oxygen consumption by bluegill (*Lepomis macrochirus*) held for 3-day trials under electromagnetic field (EMF)-exposed or control treatment conditions

Response	Factor	*df*	*F*	*P*
Dissolved oxygen concentration (DO: mg/L)	Treatment	1, 119.5	0.65	0.42
	Time	1, 325.9	1602.8	>0.0001
	Treatment:Time	1, 325.9	0.23	0.63
	Error term	*df*	*LRT* χ^2	*P*
	Tank:Round	1	0.04	0.84
	Round	1	0	1
Metabolic oxygen consumption (MO: mg/g/h)	Treatment	1, 288	0.053	0.82
	Time	1, 288	11.31	0.00087
	Treatment:Time	1, 288	0.002	0.97
	Error term	*df*	*LRT* χ^2	*P*
	Tank:Round	1	0	1
	Round	1	0	1

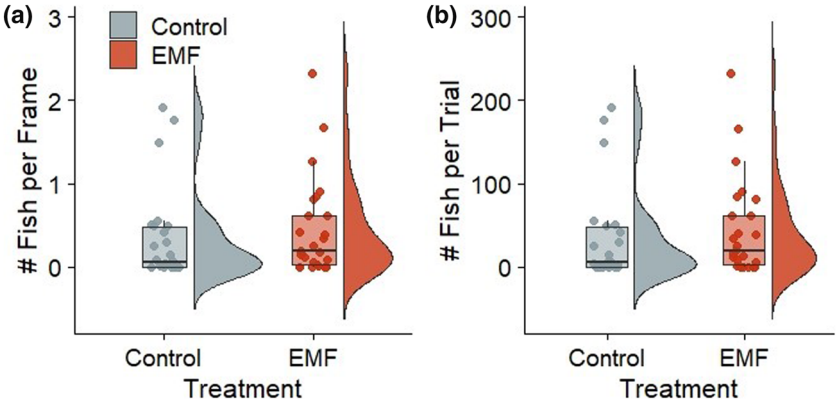

FIGURE 7 (a) Number of fish (all species) detected in video frames at 30-s intervals and (b) total number of fish (all species) observed during 1hr videos of open-water lake deployments of the electromagnetic field (EMF) device in Experiment 3

control trials (1.3 vs. 0.4). Overall, more fish were observed independent of species during EMF treatment trials than during control trials (44.8 vs. 33.04; Figure 6).

DISCUSSION

With increased interest in using electromagnetic devices to increase DO in fresh surface waters as a means of restoring aquatic ecosystems, there is precautionary need to ensure that the EMFs emitted do not have negative effects on aquatic organisms. The balance of evidence we collected based on physiological and behavioral responses during and after 8-day exposures with aeration, mortalities during 3-day exposures with oxygen depletion, and local fish abundance during lake deployment trials strongly indicates that the EMFs produced by the devices tested here (i.e., the EM Fluids Inc. EMF-1000 devices) do not exert any negative effects on the freshwater fish species included in this study over these timescales. Such an outcome is not entirely unexpected given that previous research involving fish and exposure to EMFs has tended to identify effects, if any, that are relatively small and limited to instances where organisms are in very close proximity to the source (e.g., within several meters of a submarine cable: Albert et al., 2020; Kulkarni & Edwards, 2021; Woodruff et al., 2012). In the three experiments conducted here, we studied fish that were also within several meters (Experiments 1 and 3) or within 1 m (Experiment 2) of the device, and even within those proximities to the transducer where EMF intensity would be greatest, we failed to detect significant effects relative to appropriate controls. In field deployments, these devices have an effective area that can cover hundreds of square meters (Laursen et al., 2021a, 2021b; D.T. Fung, personal communication). If fish were distributed randomly through space, then most fish in the effective area would be at much greater distances from the device than were studied here.

For the first objective, we did not observe any evidence that there were differences in physiological status or putative aerobic capacity assessed via a forced swimming challenge between control fish and those exposed to the EMFs. We used a number of different biomarkers that involve different physiological pathways and biological systems (e.g., metabolic and cardiorespiratory function, locomotion) to derive that conclusion. Blood lactate is an indicator of metabolic disturbance associated with anaerobic exercise or hypoxia, while glucose can indicate nutritional status and is also a secondary indicator of a glucocorticoid stress response (Barton et al., 2002; Sopinka et al., 2016). Hematocrit is a measure of red blood cell volume within the blood which is indicative of oxygen carrying capacity (Fánge, 1992), while the swimming challenge we used is indicative of aerobic capacity (Kieffer, 2000) and motivational state (Elvidge & Cooke, 2020). Forced swimming duration also serves as an indicator of overall organismal performance and fitness (Hammer, 1995). All of those biomarkers are commonly used as robust indicators of stress in fish under different scenarios (Sopinka et al., 2016). We did observe some temporal variability in physiological measures with hematocrit increasing and time to exhaustion decreasing over time, as well as a significant interaction between time and treatment on blood glucose levels, but these patterns were not consistent with apparent negative effects of EMF exposure compared with control conditions. Importantly, these observations cannot be adequately dissociated from the effects of captivity as hematocrit increased in both treatment groups nor from random temporal variability between successive experimental rounds as the pattern of the interaction between treatment and time was equivocal. Further, these observed differences are unlikely to manifest under fully natural conditions in free-swimming fishes exposed to EMFs due to their unconstrained ability to behaviorally mitigate physiological stress that tends to be expressed at greater levels in captive animals (Archard & Braithwaite, 2010). The significant effect of mesocosm as a random term that we observed may be due to a confound with treatment because although treatments were alternated between mesocosms in successive experimental rounds, the odd number of rounds (three) resulted in each of the four mesocosms receiving one treatment twice and the other treatment once. However, central to our primary objective, no consistent or statistically significant treatment-associated physiological effects were detected.

The same conclusions were evident for our behavioral assays where we failed to find any compelling evidence that the EMF device altered behavior in a biologically meaningful way. The behavioral endpoints (space use, shoaling) have been used to assess fish responses to different chemical stimuli including biological cues (Jones & Hara, 1985) and pollutants (Armstrong et al., 2019). While we observed some variation between trials, the patterns were not consistent over the sampling periods and EMF versus control effect sizes were small and statistically nonsignificant. Time to exhaustion in forced chases varied significantly during Experiment 1 with a decreasing stepwise pattern between successive sampling days but no effect of EMF treatment compared with controls. Collectively, the physiological and behavioral patterns observed in Experiment 1 suggest that the effects of captivity over time, including food limitation, had stronger effects on the responses of captive fish than the experimental EMF treatment.

To address the second objective, we held adult bluegill in replicate tanks with active or inactive EMF devices, plus control tanks without bluegill. MO did not differ between EMF treatment and control groups suggesting that the EMFs did not yield a metabolic cost that could be plausibly attributed to elevated stress levels (Barton & Schreck, 1987). Indeed, that finding aligns well with the observations in Experiment 1 where we did not find evidence for differences in physiological measures typical of stress responses in fish (Sopinka et al., 2016). We also monitored the tanks at 6-h intervals for mortalities, which were not significantly influenced by treatment with three mortalities observed in the control treatment versus nine mortalities in the EMF treatment. Our sample size was large ($N = 220$) such that these levels of mortality were low (5.5% overall) and likely related to the inherent challenges of retaining wild, unfed fish in captivity at relatively high densities compared with Experiment 1 (Fox & Flowers, 1990).

Video recordings taken during field deployments did not capture any differences in localized fish abundance when the EMF devices were activated or left off. Opinicon Lake has an abundance of fish, and as such, the recordings commonly contained images of fish. However, such data are also inherently variable (e.g., Glassman et al., 2022) with sampling instances in which no fish were observed. The statistical analysis revealed some variation in fish presence and diversity between recordings at different sites but none that was clearly linked to device operation and EMF transmission. Similar to our findings, research on EMFs associated with submarine cables in shallow waters of Lake Ontario found no influence of the cables on fish presence as assessed using various sampling methods (Dunlop et al., 2016). In our study, the devices were not operated prior to monitoring so that localized changes in DO related to EMFs would not influence ambient fish abundance. That is, our goal was to assess the effects of the EMFs on the fish rather than the effects of the EMFs on DO levels and subsequent effects on fish distribution and space use.

We found equivocal support for the effectiveness of EMFs produced by the devices tested at increasing the DO levels in the mesocosms and stock tanks. In Experiment 1, mesocosms with activated EMF devices consistently had higher DO levels than control mesocosms even though both groups were aerated and we did not detect signals of biological oxygen consumption over time. Conversely, in the unaerated 3-day exposure stock tanks (Experiment 2), there were clear patterns of biological oxygen consumption over time and no apparent effect of EMFs on DO levels in stock tanks containing fish. Further, we detected no differences in DO levels between fish-free stock tanks with activated or unactivated EMF devices, suggesting that under still-water conditions (i.e., unaerated and with minimal surface disruption) and the limited interfacial air/water surface area of the tanks, any oxygen-increasing effects of EMFs are incapable of either offsetting biological oxygen consumption or of raising DO levels above ambient conditions. The DO trends towards higher levels in the treatment mesocosms, however, strongly suggest that EMFs may increase oxygen saturation rates in the presence of large air/water interfacial areas provided by the air bubbles. These patterns are consistent with internal testing done by EM Fluids Inc. demonstrating higher oxygen levels in bubble-aerated versus unaerated tanks when EMFs are present (D.T. Fung, personal communication).

Hypoxia and anoxia are major issues in freshwater ecosystems around the globe (Nürnberg, 2004) directly linked to human activity (Jenny et al., 2016). Predicted climate change patterns are likely to make hypoxia and anoxia more prevalent and extreme (Rabalais et al., 2010). As such, there is dire need to develop effective means of mitigating hypoxia and restoring degraded aquatic systems. We conducted lab and field experiments using a variety of biological endpoints and found no evidence that the low-intensity EMFs emitted from the devices tested had any negative effects on fish in the contexts studied here. Because we only included fish in close proximity to the devices, we tested the most extreme EMF exposure levels such that our findings are both robust and risk averse, and this research should provide the necessary basis to ameliorate collateral damage concerns over deploying such technology where it may be helpful in increasing DO levels in the field. Here we focused on freshwater fish and ecosystems, but hypoxia is also an issue in estuaries and coastal marine environments (J. Zhang et al., 2013) and there is no apparent reason to believe that our conclusions here would not also apply to marine teleost fishes. Technological solutions to address persistent water quality problems such as hypoxia are potentially valuable tools, and our results here should provide regulators with confidence to deploy EMF devices to enhance water quality without exerting any collateral effects from the use of low-intensity EMFs.

ACKNOWLEDGEMENTS

All work was approved by the Carleton University Animal Care Committee (# 112698) and funded by the Natural Sciences and Engineering Research Council of Canada (NSERC Alliance Grant no. ALLRP 561435 - 20). We thank M. Thapar, A.L. Jeanson, D. Jeanson, J.C. Hendricks, B. Etherington, T. Lepine, M. Dusevic,

and A.J. Zolderdo for valuable assistance with fish collection, project logistics, and helpful comments. While EM Fluids Inc. has a direct interest in results supporting the use of the proprietary device tested here, all data were collected and analyzed independently by the academic team from Carleton University (CKE, CJB, JCV, and SJC), and we declare no conflicts of interest in our results.

AUTHOR CONTRIBUTIONS

Chris Elvidge: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization. **Christian Bihun:** Data curation; investigation; methodology. **Colin Davis:** Methodology; resources. **Saad Ulhaq:** Resources. **David Fung:** Conceptualization; funding acquisition; project administration; resources. **Jesse Vermaire:** Conceptualization; funding acquisition; validation. **Steven Cooke:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision.

DATA AVAILABILITY STATEMENT

All data collected during this study and supporting R code are freely available online via Open Science Framework (URL: https://osf.io/vbsh4/; doi: 10.17605/OSF.IO/VBSH4).

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How to cite this article: Elvidge, C. K., Bihun, C. J., Davis, C., Ulhaq, S., Fung, D. T., Vermaire, J. C., & Cooke, S. J. (2022). No evidence for collateral effects of electromagnetic fields used to increase dissolved oxygen levels on the behavior and physiology of freshwater fishes. *Water Environment Research*, *94*(6), e10747. https://doi.org/10.1002/wer.10747