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Effects of Electromagnetic Fields on Fish and Invertebrates

Task 2.1.3: Effects on Aquatic Organisms Fiscal Year 2012 Progress Report

Environmental Effects of Marine and Hydrokinetic Energy

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May 2013



Pacific Northwest
NATIONAL LABORATORY

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Richland, Washington 99352

Summary

Energy generated by the world's oceans and rivers offers the potential to make substantial contributions to the domestic and global renewable energy supply. However, the marine and hydrokinetic (MHK) energy industry faces challenges related to siting, permitting, construction, and operation of pilot- and commercial-scale facilities. One of the challenges is to understand the potential effects to marine organisms from electromagnetic fields, which are produced as a by-product of transmitting power from offshore to onshore locations through underwater transmission cables.

This report documents the progress of the third year of research (fiscal year 2012) to investigate environmental issues associated with marine and hydrokinetic energy (MHK) generation. This work was conducted by Pacific Northwest National Laboratory (PNNL) for the U.S. Department of Energy's (DOE's) Office of Energy Efficiency and Renewable Energy (EERE) Wind and Water Technologies Office. The report addresses the effects of electromagnetic fields (EMFs) on selected marine species where significant knowledge gaps exist. The species studied this fiscal year included one fish and two crustacean species: the Atlantic halibut (*Hippoglossus hippoglossus*), Dungeness crab (*Metacarcinus magister*), and American lobster (*Homarus americanus*).

These species were chosen based on their conservation and management status, and ecological and economic importance in the United States. A behavioral approach was used as a means of providing a sensitive yet rapid screening level assessment. Experimental trials were conducted in a controlled laboratory setting where EMF exposures and responses were actively monitored and quantified. A maximum EMF strength between 1.0 - 1.2 mT direct current (DC) was used for all tests to provide consistency and comparability among species. The EMF intensity was selected as an upper bound of an anthropogenic source that might be encountered based on reviewed literature. Behavioral observations were recorded in a test tank where the maximum EMF strength occurred at the tank center, decreasing to approximately 0.2 mT at each end and compared to identically configured control tanks with uniform background EMF intensities (~0.05 mT). Experimental trials were conducted for 24 hours (lobster), 72 and 96 hours (crab), and 72 hours (halibut). Attributes examined included the location (i.e., where time was spent with respect to the EMF source) and changes in activity levels (e.g., swimming, walking, resting, buried, sheltered, and burrowed). The overall approach was designed to provide preliminary information regarding key marine organisms responses to anthropogenic sources of EMF that will inform the siting and permitting process for MHK energy projects.

Based on the initial laboratory screening studies, the weight of evidence to date for the three tested species showed relatively few behavioral responses that would indicate explicit avoidance or attraction to an approximate 1.1 mT DC EMF intensity. However, for each species there were statistically significant differences related to the use of space and/or activity level within the experimental tanks. Further study is needed to clarify whether these results are related to the directional flow of water current in the tanks, a response to a change in EMF vector orientation relative to background, or some other tank effect. There was a large amount of variability observed between individuals, trials, and seasons; hence the results need to be considered from this perspective. As benthic species, test animals spent a majority of their time in an inactive state, independent of treatment; halibut were motionless 88% of the time; crab were buried/resting 80-91% of time; and lobster were sheltered or burrowed 76% of time. Given these largely inactive baselines, subtle changes in behavior were difficult to detect.

Recommendations for future research include: testing of other life stages (e.g. juveniles) that are likely to have different sensitivities and responses to EMFs; inclusion of other species of regulatory concern in future testing that may be sensitive to EMF (e.g. green sturgeon, elasmobranchs); implementation of tests using a variety of EMF configurations, including an AC source for comparison to the DC EMF results; and implementation of an experimental design in the laboratory to specifically address test animals responses to changes in EMF vector orientation. Finally, larger scale studies using activated cables in the field are needed in order to realistically assess EMF effects on navigation and migration patterns of priority species, including distribution of populations.

Acknowledgments

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Acronyms and Abbreviations

°C	degree(s) Celsius
μT	microtesla
uV	microvolt
AC	alternating current
B-field	magnetic field
BOEMRE	Bureau of Ocean Energy Management, Regulation and Enforcement
CI	confidence interval
cm	centimeter(s)
d	day(s)
DC	direct current
DO	dissolved oxygen
DOE	U.S. Department of Energy
EERE	DOE Office of Energy Efficiency and Renewable Energy
E-field	electric field
EMF	electromagnetic field
FY	fiscal year
g	gram(s)
hr	hour(s)
iE-field	induced electrical field
km ²	square kilometer(s)
L	liter(s)
L/min	liter(s) per minute
lux	lumen per square meter (unit of illumination)
m	meter(s)
mg	milligram(s)
mg/L	milligram(s) per liter
MHK	marine and hydrokinetic
min	minute(s)
mT	millitesla
MSL	Marine Sciences Laboratory
nT	nannotesla
PNNL	Pacific Northwest National Laboratory
psu	practical salinity unit
R&D	research and development
S.D.	standard deviation
wk	week(s)

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1.0 Introduction

This report documents the progress of the third year (fiscal year 2012) of research conducted by Pacific Northwest National Laboratory (PNNL) for the U.S. Department of Energy's (DOE's) Office of Energy Efficiency and Renewable Energy (EERE) Wind and Water Power Technologies Office to investigate environmental issues associated with marine and hydrokinetic (MHK) energy generation. Specifically, this progress report addresses the effects of electromagnetic fields (EMFs) on selected marine species where knowledge gaps currently exist. The species investigated this past year included Atlantic halibut (*Hippoglossus hippoglossus*; Order Pleuronectiformes), Dungeness crab (*Metacarcinus magister*; Order Decapoda), and American lobster (*Homarus americanus*, Order Decapoda).

1.1 MHK Research and Development Background

The emerging MHK energy industry seeks to harness energy generated by the world's oceans and rivers to contribute to the domestic and global renewable energy supply. In doing so, its project developers face challenges related to siting, permitting, construction, and operation of pilot- and commercial-scale facilities, as well as the need to develop robust technologies, secure financing, and gain public acceptance. In addition, in many cases little is known about the potential effects of MHK energy generation on the aquatic environment from either a small number of devices or a large-scale commercial array. Nor are the potential effects that may occur after years or decades of operation understood. This lack of knowledge affects the solvency of the industry, the actions of regulatory agencies, the opinions and concerns of stakeholder groups, and the willingness of developers and investors to commit to developing and investing in MHK energy-generation projects.

To address the complexity of environmental issues associated with MHK energy generation, PNNL developed a program of research and development (R&D) that draws on the knowledge of the industry, regulators, and stakeholders and builds on investments made by the EERE Wind and Water Power Technologies Office. The PNNL R&D program—together with complementary efforts of other national laboratories, national marine renewable energy centers, universities, and industry—supports DOE's market acceleration activities through focused R&D related to environmental effects and siting issues. Thus far, PNNL research activities have focused on the following four major areas:

- **Categorizing and evaluating the effects of environmental stressors.** A knowledge management system called *Tethys* was developed to facilitate the creation, annotation, and exchange of information about the environmental effects of MHK technologies. *Tethys* now exists as an interactive web site that enables regulatory agencies, industry, research institutions, and stakeholder groups to assess relevant information and disseminate results. *Tethys* has also supported an Environmental Risk Evaluation System developed by PNNL to categorize and assess relative risks associated with MHK technologies.
- **Effects on physical systems.** Computational numerical modeling systems were created and used to understand the effects of energy removal on water bodies from the short- and long-term operation of MHK devices and arrays.
- **Effects on aquatic organisms.** Testing protocols and laboratory exposure experiments were developed to evaluate the potential for adverse effects from operation of MHK devices in the aquatic environment. Studies included laboratory assessments of the potential effects from EMFs or noise

associated with MHK installations and assessment of the potential risk of the physical interaction of aquatic organisms with devices.

- **Permitting and planning.** Stakeholder communication and outreach activities were structured to ensure research products and results are effectively communicated and disseminated. This task includes activities to promote consideration of renewable ocean energy in national and local Coastal and Marine Spatial Planning activities.

1.2 Purpose and Scope

Anthropogenic sources of EMF are produced by underwater power transmission cables that transmit electrical power through a network of inter-array cables offshore and bring power onshore via an export cable. Knowledge gaps exist with respect to the effects of these EMF sources on marine organisms. During the past 3 yr, PNNL has examined the effects of EMF exposure on a variety of fish and invertebrates in the laboratory. These efforts were conducted as part of Task 2.1.3 – Effects on Aquatic Organisms, Subtask 2.1.3.1 – Electromagnetic Fields, under the EERE Water Power Program.

During the first year of Subtask 2.1.3.1 – Electromagnetic Fields (fiscal year 2010 [FY10]), PNNL conducted a literature survey, created an EMF exposure system, and developed testing protocols that could measure morphological and behavioral endpoints for species of interest. The literature survey revealed that acute effects were not likely from EMF exposures; therefore, further protocol development focused on development of sub-lethal measurement endpoints that would have importance at community and population level scales. The second year (FY11) of activities and reporting focused on conducting preliminary laboratory experiments to assess the sub-lethal effects that reflected change in developmental stages and physiological responses and initial studies of behavioral responses in a variety of aquatic organisms, including coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), Atlantic halibut, California halibut (*Paralichthys californicus*), and Dungeness crab.

The goal of the experimental work conducted during FY12 was to conduct laboratory screening level assessments of behavioral responses to EMFs by selected marine organisms considered to be high priority by regulators and stakeholders, and for which scientific data are currently lacking. Hence further work was conducted with Atlantic halibut and Dungeness crab. Preliminary investigations with small adult American lobster were also conducted. These species were selected because of their conservation and management status and their ecological and economic importance in the United States.

1.3 Report Contents and Organization

This report documents the FY12 research with selected species, in particular examining behavioral responses of Atlantic halibut, Dungeness crab, and American lobster to an EMF source. The ensuing sections begin with an overview of the methods and general approach used during the past year's studies (Section 2.0). Sections 3.0, 4.0, and 5.0 document the specific experimental methods, results, and discussion of EMF effects for Atlantic halibut, Dungeness crab, and American lobster, respectively. Section 6.0 summarizes the results and conclusions of the study. Section 7.0 lists the literature cited in this report.

2.0 Background and Approach

Energy generated by the world's oceans and rivers offers the potential to make substantial contributions to the domestic and global renewable energy supply. However, the MHK energy industry faces a number of challenges in developing and operating pilot- and commercial-scale facilities, including the need to understand the potential effects of EMF on marine organisms, a by-product of transmitting power from offshore to onshore locations through underwater transmission cables. Although underwater power transmission cables have existed for decades, potential new sources of offshore renewable energy facilities will greatly increase the extent and magnitude of these cables in the United States (Normandeau et al. 2011).

EMFs in the marine environment arise from both natural and anthropogenic sources. The coupling between the electric and magnetic fields is very weak at frequencies associated with power cables, hence these two fields are treated separately. A primary source of a natural magnetic field is the Earth's geomagnetic field that is derived primarily from heat convection currents within the Earth's core. This geomagnetic field has both intensity (25-65 microtesla [μT]) and directional components (www.ngdc.noaa.gov/geomag/faqsgeom.shtml); the latter deriving from both the north-south orientation of the magnetic poles as well as the inclination of the magnetic field vectors that vary from north to south (Figure 1). Additional sources of geomagnetism derive from differential occurrence of ferromagnetic minerals in the earth's crust that produce local distortions of the main field, with spatial variability over a few km of up to 10% ($2 \mu\text{T}$) of the larger background field.

These two aspects of the earth's magnetic environment thus provide a potential source of information for a diverse suite of animals including terrestrial (e.g. Wiltschko, 1993; Kirschvink 1997) and marine species (e.g. Lohmann et al. 1995; Normandeau et al. 2011) for orientation or navigation over small and large spatial scales. For example, recent studies have demonstrated that the ocean migratory pathways of sockeye salmon (*Oncorhynchus nerka*) around Vancouver Island, Canada are influenced by decadal changes in the earth's geomagnetic field (Putman et al. 2013).

The mechanism or mechanisms by which animals can exploit these fields is incompletely understood. Some species may sense magnetic fields directly through biogenic magnetite crystals that reorient as the animal moves to maintain alignment with geomagnetic field lines (e.g., Kirschvink et al. 2001). Magnetite-based magnetoreceptive cells have been found in the olfactory epithelium of trout, for example, indicating salmonids may be able to directly sense magnetic fields (Eder et al. 2012). Alternatively, the movement of seawater through magnetic fields (e.g. via current or tidal flow) induces localized electric fields that, although small (0.05-0.5 $\mu\text{V}/\text{cm}$), may be detectable by certain species, e.g., elasmobranchs (e.g., Kalmijn 1982). Species using such electromagnetic induction may not be able to detect geomagnetic field vector inclination, but would be sensitive to variations in geomagnetic field strength (e.g., Walker et al. 2003).

In the marine environment, evidence of sensing, responding to, or orienting to natural magnetic field cues has been shown for elasmobranchs (Kalmijn 1982; Meyer et al. 2005), bony fishes including tuna, salmon, trout and eels (Walker et al. 1984; Mann et al. 1988; Diebel et al. 2000; Moore and Riley 2009), sea turtles (Lohmann et al. 2001, 1999; Lohmann and Lohmann 1998, 1996, 1994), marine mammals (Klinowska 1985; Kirschvink et al. 1986; Walker et al. 1992), and invertebrates including molluscs and

arthropods (Lohman and Willows 1987; Ugolini and Pezzani 1995; Ugolini 2006; Boles and Lohmann 2003).

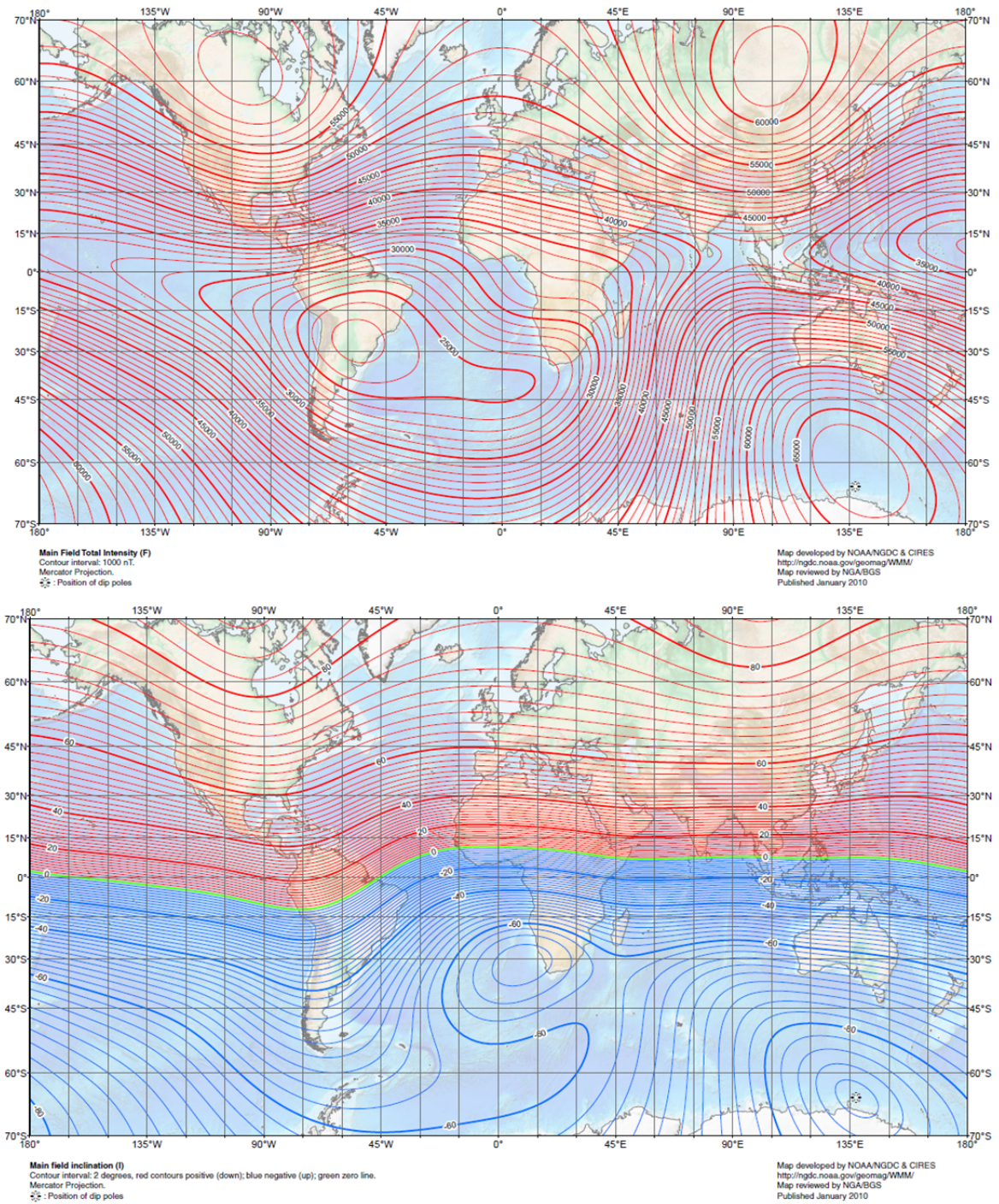


Figure 1. Main geomagnetic field intensity in 1000 nT contour interval (upper) and main field inclination in 2 degree contour intervals (lower), in 2010 (Maus et al. 2010).

Evidence of electro-reception in marine organisms is well documented for elasmobranchs (Yano et al. 2000; Lowe et al. 1994; McGowan and Kajiura 2009; Gill et al. 2009), and has been demonstrated in other fishes such as lampreys and sturgeons (Muraveiko 1984; Bodznick and Northcutt 1981; Collin and Whitehead 2004; Bullock 2005) and decapod crustaceans (Patulla and Macmillan 2007). Electro-reception is used primarily to detect very low-frequency bioelectric fields emitted by prey, locate mates, and for orientation (Boehlert and Gill 2010).

EMFs emitted from underwater power transmission cables consist of the electric field (E-field), an induced magnetic field (B-field), and induced electrical field (iE-field) created by movement of water or an organism through the magnetic field (Normandeau 2011; Cada et al. 2011). Conductive sheathing can shield cables from direct emission of the E-field. However, it is currently not feasible from a design and cost perspective to completely contain the B-field, hence both B-field and iE-fields are emitted from alternating current (AC) and direct current (DC) underwater power cables. Magnetic and iE fields would be experienced as a gradient relative to the distance from the cable, either vertically or horizontally (Normandeau 2011). These EMFs represent not only a distortion to the background field intensity, but a change in vector field orientation (i.e. directionality) as well. The EMF lines consist of curved vectors surrounding the power transmission cable, with the vectors directed upward on the left side of the cable (facing away from the power source) and downward on the right side of the cable. Depending upon the intensity of the source current, these distortions could produce local anomalies in the EMF vector fields. Thus, the potential for EMF effects from cables will vary depending upon the specifics of cabling and energy generation on a project- and site-specific basis (Normandeau et al. 2011).

Recent literature reviews and syntheses of effects of EMF on marine organisms (Normandeau et al. 2011; Fisher and Slater 2010; Gill et al. 2012; Cada et al. 2011) have shown that species vary in sensitivity and response. In addition, there is a great deal of uncertainty regarding the strength of EMF that may be expected from tidal, wave, and offshore wind ocean energy deployments. A project in European waters encountered field strengths that ranged from 0.01 μT at one site (Ardtow) to up to 0.6 μT at another site (Burbo) (Gill et al. 2009). Modeled EMF strengths from electrical cables of MHK systems have predicted a greater range of possible strengths at the sediment-water interface depending on cable configurations and depth of cable burial. Estimated EMF strengths for 10 projects with AC cables buried at 1 m depth were 8 μT at the sediment surface compared to 78 μT for 8 projects using DC cables buried at the same depth (Normandeau et al. 2011), while magnetic flux estimated from amperage and cable diameter information ranged from 0.4 to 8 mT at the cable surface, depending on a variety of factors including cable diameter, conductors and type of insulation (Cada et al. 2011). The necessity of specialized equipment to conduct *in-situ* measurements and inherent difficulties arising with underwater research projects in general, has also limited field measurements.

In order to address some of the basic knowledge gaps, the research conducted this past fiscal year focused on three marine species that have not been investigated with respect to natural EMF sensitivities or the effects of anthropogenic EMF sources. A behavioral approach was used as a means of providing a sensitive yet rapid screening level assessment. A controlled laboratory setting was used where EMF exposures and behavioral responses could be accurately quantified. A maximum EMF strength of 1.1 to 1.2 mT DC was used for all tests to provide consistency and comparability among tested species. This EMF level was selected as a possible upper bound that might be encountered based on reviewed literature. The overall approach was designed to provide preliminary information about important benthic marine species responses to anthropogenic sources of EMFs that could be used to inform the siting and permitting process for MHK energy projects.

3.0 Atlantic Halibut

Atlantic halibut are a demersal marine fish, found primarily in the northern portions of the eastern and western Atlantic Ocean. Adults can range in size from 80 to 220 cm in length, and are considered the largest flatfish in the world (Brodziak and Col 2006; Cargnelli et al. 1999). They feed primarily on other fishes (cod, haddock, herring, capelin). Younger halibut also feed on a variety of crustaceans and other benthic species including crab, lobsters, and worms (Cargnelli et al. 1999; Nickerson 1978). Atlantic halibut are an important commercial and recreational resource in the northern and mid-Atlantic regions of the United States and are farmed as an aquaculture species in several northern latitude countries. They are listed as a Species of Concern by the U.S. National Marine Fisheries Service, based on overfishing in certain parts of the world.

The developmental stages of Atlantic halibut have been well characterized; their classic stages of early development have been defined based primarily on eye migration and pigmentation (Gisbert et al. 2002; Sæle et al. 2004). Proper eye migration is closely linked to larval survivorship, thus making the halibut an ideal model organism for test exposures. During FY11, larval stages of Atlantic halibut were tested to determine the effects of EMFs on their development through several early life stages (Woodruff et al. 2012). The results of these experiments indicated a possible effect on growth and development, but the results were not conclusive. To augment the developmental work conducted with larval halibut the behavioral responses of juvenile halibut (approximately 1 yr old) exposed to an artificial EMF source were examined in FY12.

The goal of this experimental work was to determine if an anthropogenic EMF source above background levels would elicit changes in the observed behaviors of Atlantic halibut, a bottom-dwelling teleost of that occurs in areas of planned or existing MHK sites. Reviews of published literature studies by Gill et al (2005; 2012) and Normandeau et al. (2011) indicate a general lack of available scientific literature for bottom-dwelling teleosts; however, several species have shown an electro- and/or magneto-sensitive response to natural or anthropogenic EMFs. The type of response varies and appears to be species specific. To date there have been no reported studies of the effects of EMF on Atlantic halibut or the species' response to EMF.

The specific objectives of the laboratory experiments were to 1) determine if an avoidance or attraction response was elicited by halibut in the presence of an anthropogenic EMF source, measured by the spatial distribution of the halibut in relation to the EMF source, and 2) determine if there were changes in the level of activity that would indicate the animal's behavior patterns were being affected by the EMF source.

3.1 Methods

This section describes methods related to the laboratory EMF exposure system (3.1.1), halibut care and holding (3.1.2), the experimental design (3.1.3), and data analysis (3.1.4).

3.1.1 EMF Exposure System

The configuration for all experiments conducted during FY12 used a single electrical coil (described in greater detail by Woodruff et al. [2012]) centered around each of two 1700-L rectangular tanks (3.3 m long \times 0.75 m wide \times 0.69 m deep) that allowed some freedom of movement for organisms placed in the tank. Both tanks were oriented approximately orthogonal to the Earth's magnetic poles. A coil in one tank (hereafter termed the Exposure Tank) was energized to produce a 1.23-mT DC EMF in the center of the exposure tank, decaying to \sim 0.27 mT at either end of the tank. In the Control Tank, the coil was not energized, but otherwise provided an identical physical configuration, with a background level of \sim 0.05-mT EMF. The magnetic flux density was measured using an Alpha Lab Model VGM gauss meter. For the halibut and crab exposures, only one coil could be energized, hence the same tank was energized for each trial. However for the lobster exposures either coil could be energized and the exposure and control were randomly assigned to each tank.

3.1.2 Halibut Care and Holding

Juvenile Atlantic halibut were reared from the larval stage at PNNL's Marine Sciences Laboratory (MSL). The larval stage fish were cultivated and hatched offsite by Scotian Halibut Limited in Nova Scotia, Canada, and shipped to the MSL at 22 d after first feed. The larval halibut were used during FY11 to assess whether EMFs would affect the developmental transformation between the larval and juvenile phases (Woodruff et al. 2012). After the completion of these experiments, the control (unexposed) fish were returned to holding tanks and maintained on pellet food ad libitum until behavioral experiments were conducted with this cohort, as described below. At the time of experimentation, fish were approximately 1 yr post-hatch, averaged 29 cm (\pm 1.5 S.D.) in fork length, and weighed 267 g (\pm 52 S.D.) (Figure 2).

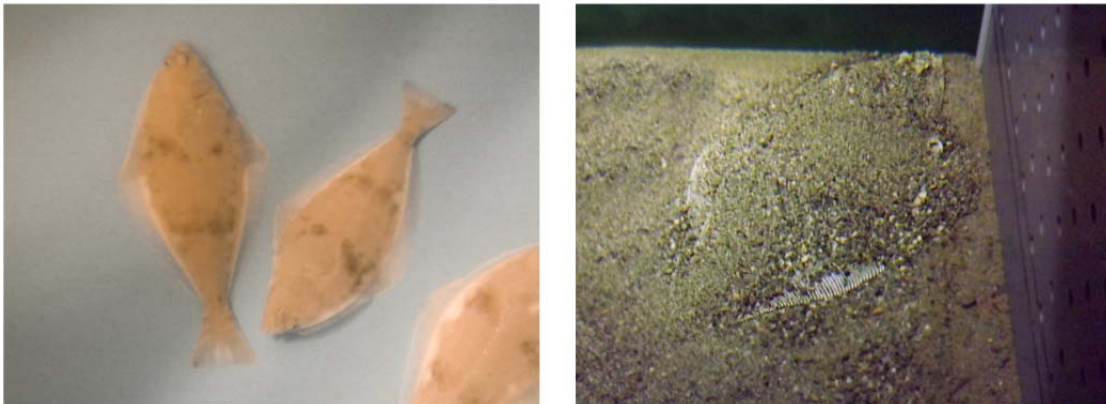


Figure 2. Atlantic Halibut 1 yr post-hatch at time of testing in a) a holding tank at MSL (left photo), and b) an experimental tank camouflaged by sand (right photo).

3.1.3 Experimental Design

The bottom of each experimental tank contained \sim 15 cm of clean sand, providing the halibut with an opportunity to camouflage/bury themselves at will. Filtered flow-through seawater was delivered to each tank at a mean rate of 19.1 (\pm 0.9 S.D.) L/min at one end of the tank (upstream), exiting at the downstream end of the tank. Water quality was maintained at 8.6 ± 0.4 °C, 30.9 ± 0.5 psu, 8.6 ± 1.4

mg/L dissolved oxygen (DO), and pH 7.56 ± 0.17 . Baffles at either end of the tank provided a uniform longitudinal flow from one end of the tank to the other. The effective surface area available for halibut movement was 2.475 m^2 . Subdued daylight spectrum lighting ($16 \pm 3.5 \text{ lux}$) synchronized to civil sunrise and sunset was provided during daylight hours, and infrared illumination was provided at night.

Prior to the initiation of each experimental trial, water quality was monitored and the electromagnetic coil was energized in the Exposure Tank. Halibut were then moved by net from the holding tank and placed in the center of the control and exposure tanks (one fish per tank). Six trials using two fish per trial (one in each tank) were conducted. Each experimental trial was initiated during daylight hours and consisted of a 72-hr continuous exposure. The fish were not fed during the experiments. A video-capture system described in the FY11 progress report (Woodruff et al. 2012) was used to record behaviors in both tanks for the duration of the experiment.

Behavioral observations can be recorded as either “states” or “events” (Altmann 1974). States are considered to have appreciable duration (e.g. resting), whereas events occur over a much shorter duration or instantaneously (e.g. camouflaging by Atlantic halibut, repositioning). A majority of the behavioral patterns observed in the three species studied were considered as a behavioral state, largely because of the benthic and/or sedentary nature of the organisms. For example, the Atlantic halibut uses a “sit-and-wait” foraging strategy (Nilsson et al. 2010), meaning considerable amounts of time were spent lying motionless on the bottom of the tank.

3.1.4 Data Analysis

Behavioral observations were recorded every 15 min using instantaneous sampling (Altmann 1974) where the observer recorded the location and “event” or “state” at a preselected frequency. This is used primarily in studies where the objective is to estimate the proportion of time an individual devotes to various activities. The behavioral observations for Atlantic halibut were recorded as a motionless state (i.e. resting/buried) or an active state with specific events noted (i.e. swimming, drifting, camouflaging, repositioning along the bottom without a forward swimming movement). To discount any initial effects of introducing fish into tanks, the initial 15-min intervals of each trial were not included in the analyzed data sets.

The location and behavioral patterns of the halibut were recorded and analyzed with respect to five approximately equal-sized zones within each tank (Figure 3, top). Zone 3 corresponded to the highest experimental EMF strength in the Exposure Tank ($\sim 1.0 \text{ mT}$); Zones 2 and 4 averaged ~ 0.77 and 0.53 mT in the Experimental Tank, respectively; and Zones 1 and 5 in the Experimental Tank averaged 0.3 and 0.23 mT , respectively. The Control Tank received background levels of EMF present at the MSL ($\sim 0.05 \text{ mT}$), and it was delineated into similar zones for analytical comparison (Figure 3, bottom).

Statistical analyses were formulated to test the following null hypotheses:

- H_{01} : *The proportion of time Atlantic halibut spend within each zone is independent of EMF treatment – reflecting temporal use of space.*
- H_{02} : *The number of times Atlantic halibut settled within each zone is independent of EMF treatment – reflecting choices of settling places.*

- H_03 : The frequency with which Atlantic halibut change between buried/resting and active behaviors is independent of the presence of EMF treatment.

To evaluate H_01 a nonparametric Kruskal-Wallis test was used to compare the median proportion of time spent in a given zone between treatments. For each trial ($n=6$), the average proportion of time spent in each zone shown in Figure 3 was calculated as the average number of minutes the halibut spent in a zone each time it was observed in that zone divided by the total amount of time for which observations were made. To evaluate H_02 a nonparametric Kruskal-Wallis test was used to compare the median number of times halibut were observed settling in a zone between treatments. The number of times the halibut were observed within a zone was standardized between trials by dividing by the total amount of time for which observations were made during a given trial multiplied by 1000.

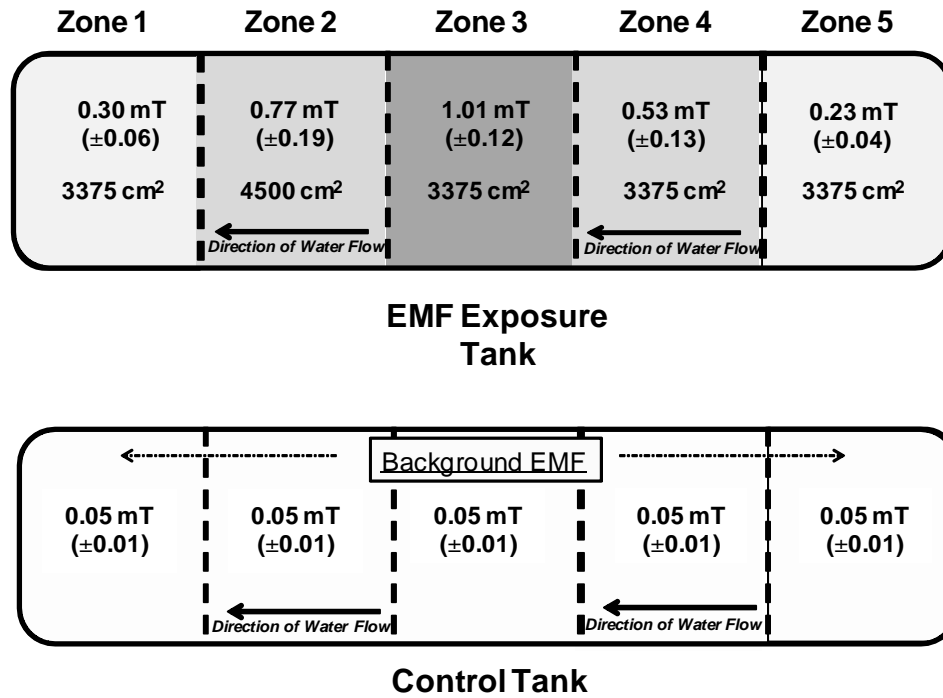


Figure 3. EMF intensities (mean \pm S.D.) in the Exposure Tank (top) and Control Tank (bottom) for 5 delineated zones. This configuration was used for Atlantic halibut and Dungeness crab. Shading indicates EMF intensity ranges (*High* – Zone 3, *Mid* – Zones 2 and 4, *Low* – Zones 1 and 5).

For each hypothesis tested the data was analyzed sequentially based on the zone delineations described in Figure 3, hereafter referred to as five-zone, three-zone, and two-zone. The first analysis used five zones delineated by water flow direction and EMF strength: upstream – low EMF, upstream-moderate EMF, high EMF, downstream -moderate EMF, downstream – low EMF. The use of space within the tank is a function of the combined effects of water flow, EMF strength and electromagnetic vector orientation, and any nuances specific to the environment in and around the Control and Exposure tanks. It was not possible to separate these effects without combining zones. Hence, a second pair of analyses was conducted to determine if field strength alone was determining spatial distribution. This analysis combined the five zones into three (low, moderate, and high) such that the upstream and

downstream EMF low field strengths (Zones 1 and 5) and upstream and downstream EMF moderate field strengths (Zones 2 and 4) were combined. A third analysis was conducted with only two zones, inside the high zone (Zone 3) and outside the high zone (Zones 1, 2, 4, and 5 combined). As the number of zones is decreased, the number of effects examined is reduced. Thus, the third analysis addresses attraction to or avoidance of the highest EMF field. For all analyses, observations of location were excluded from the analyzed data sets if a fish was actively swimming during the last minute of the 15-min observation interval.

Differences in the change in activity (H_{03}) within each zone between treatments were evaluated with a nonparametric Kruskal-Wallis test. A change of activity was defined as a change in behavior between a motionless state (resting/buried) and an active state (swimming, drifting, camouflaging, repositioning), or between active behaviors. Observations with less than 15 min of defined behavior(s) (i.e., behavior could not be definitively determined or confirmed) were excluded from the statistical analysis. For each trial, a change in activity was analyzed as a proportion based on the average from each time period spent in a single zone, calculated as the number of changes of state divided by the number of minutes spent within that zone. A three-sequence series of analyses of behavior was performed in the same manner as the zone location analyses above (i.e., five-zone, three-zone, and two-zone). The average over zones of the proportion of changes in activity was calculated for both night and day and for each tidal stage (ebb and flood). For each day/night time period and tidal stage, the nonparametric Kruskal-Wallis test was used to compare the median proportions of active behavior between treatments (control and EMF). A Mann-Whitney test was used to compare the median proportion of active observations between each day/night time period and each tidal stage.

3.2 Results

Study results are presented in terms of observed location (Hypotheses H_{01} and H_{02} - Section 3.2.1) and behavioral changes (Hypothesis H_{03} - Section 3.2.2) as they relate to the EMF source.

3.2.1 Location

During the six trials, a total of 3,281 observations of location were made at 15-min intervals and 46,095 observations of behavior at 1-min intervals. The median proportion of time (15-min intervals) halibut spent in the upstream-low zone was significantly greater in the Control Tank than in the Exposure Tank (H_{01}) (Kruskal-Wallis; $p = 0.028$) when the data were analyzed by five zones. However, for the four other zones, the median proportion of time spent in each zone was not significantly different between treatments ($p > 0.15$) (Figure 4a). The median number of times that halibut settled in each zone, normalized by total time observed, was not significantly different between the Control and Exposure tanks (H_{02}) (Kruskal-Wallis; $p > 0.12$).

When the data were combined for the three-zone analysis, the effects of EMF strength were examined. The median proportion of time spent was not significantly different between treatments (Kruskal-Wallis; $p > 0.15$) (Figure 4b). The median number of times that halibut were observed in each zone, normalized by total time observed, was also not significantly different between the Control and EMF Exposure tanks ($p > 0.52$). Thus, it appears that the EMF strength alone does not affect the use of space. The two-zone analysis (attraction to or avoidance of the high EMF strength) did not demonstrate a significant difference for either the median proportion of time spent or the median number of times that a

halibut is observed in a zone ($p > 0.52$) (Figure **Error! Reference source not found.4c**). Recognizing the small sample size ($n=6$), the combined analytical results suggest that EMF strength alone does not affect the spatial distribution of Atlantic halibut, however statistically significant differences between the Control and Exposure tanks in the upstream-low zone could be related to other effects (e.g. orientation to upstream current flow, electromagnetic vector orientation, other tank effects).

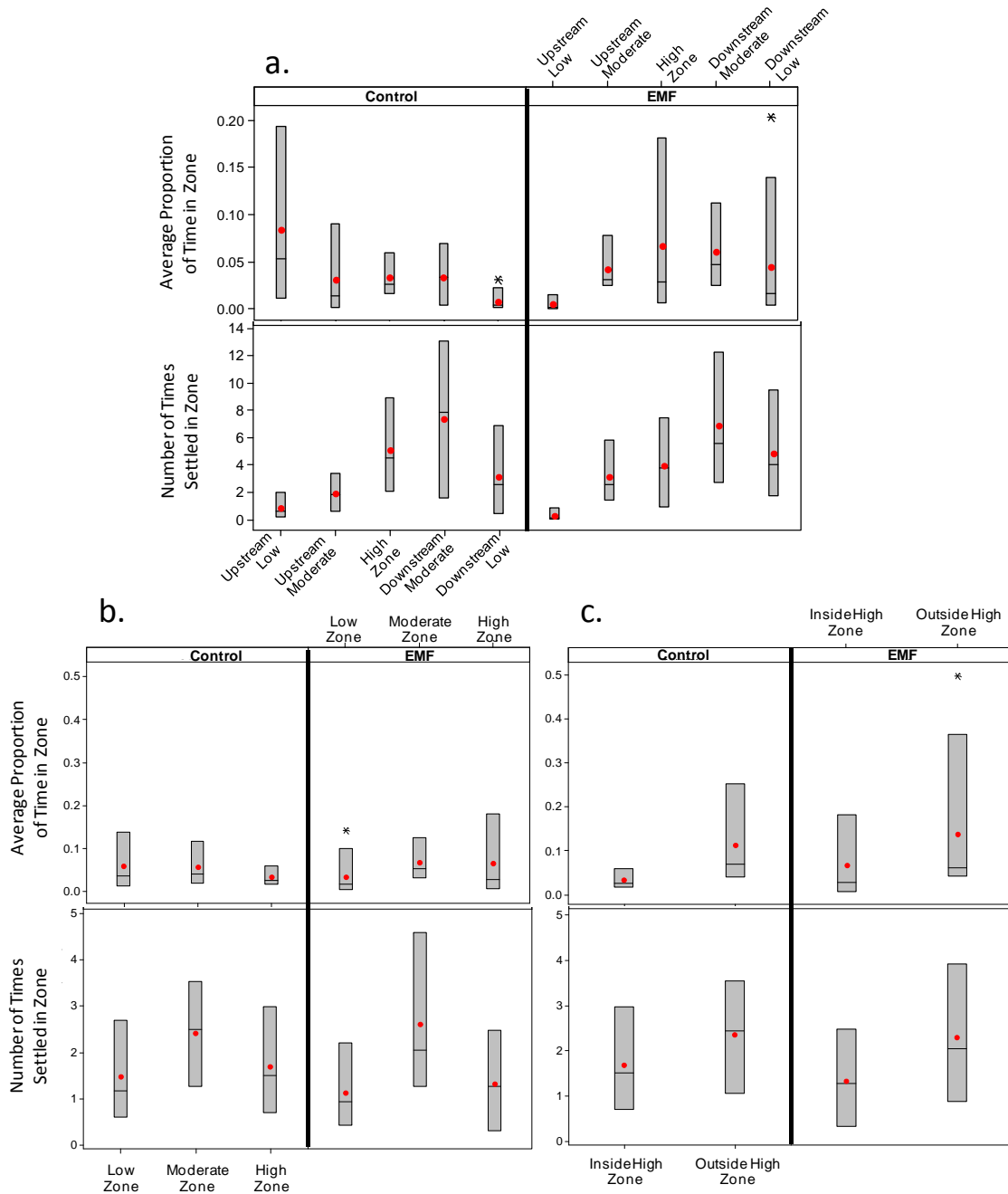


Figure 4. Box plot of Atlantic halibut showing the 95% confidence interval (CI) at about the median ($n=7$) of the average proportion of time spent in a zone (top two cells of plot) and the number of times fish settled in each zone normalized to the total time observed (bottom two cells of each plot) for a) five zones, b) three zones, and c) two zones.

3.2.2 Behavioral Changes/Activities

The halibut spent most of the time lying motionless on the bottom of the Control and Exposure tanks, frequently camouflaged with sand (Figure 2). For this reason, we chose to record observations at a greater frequency (once a minute) to have a better chance of capturing changes in activity. Overall, only 12% of the observations with a zone designation had a recorded change in activity (5,421 out of 45,180 observations). The median proportion of changes in activity was not significantly different between the Control and Exposure tanks during either the ebb or flood tide periods (Kruskal-Wallis; $p > 0.52$) or during either the day or night (Kruskal-Wallis; $p > 0.75$). Combining data from the two tanks, the median proportion of changes in activity was not significantly different between the ebb and flood tide periods (Mann-Whitney; $p = 0.21$); however, fish were significantly more active during the night (Mann-Whitney; $p < 0.001$). Hence, further analysis was conducted separately for the night and on the combined (day and night) proportion of observations with a change in activity.

The median proportional number of changes in activity within each of the five zones was not significantly different between the control and EMF treatments during the night (Kruskal-Wallis; $p > 0.064$) or during the combined night and day ($p > 0.053$) (Figure 5). For the three-zone analysis, the effect of treatment was also not significant during the night ($p > 0.423$) or during the combined night and day ($p > 0.522$). Likewise, the two-zone analysis showed the effect of treatment was not significant during the night ($p > 0.423$) or during the night and day combined ($p > 0.522$).

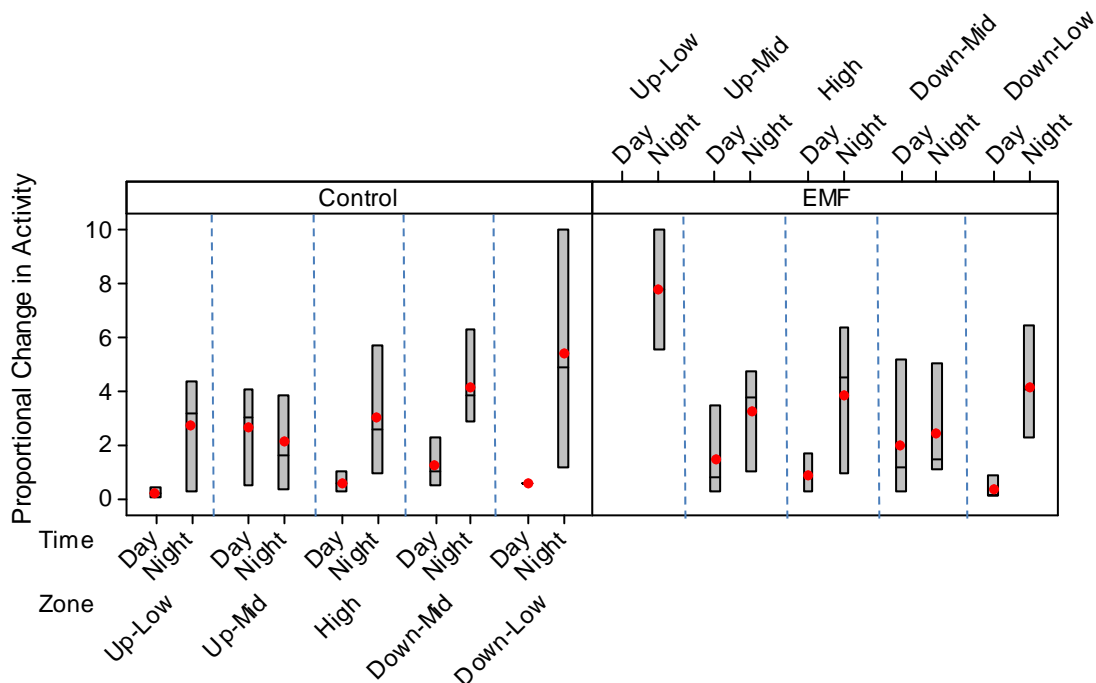


Figure 5. Box plot of Atlantic halibut showing the 95% CI at about the median number of changes in activity divided by the number of observations for the five zones and two time periods in the Control and EMF Exposure tanks (n=6 trials).

3.2.3 Summary

Atlantic halibut exhibited a single significant response to the EMF exposure in terms of spatial distribution (less time spent in the upstream low zone; $p = 0.03$). Halibut did not exhibit a significant change in behavioral activity as measured by the frequency of change in behavior as a result of EMF exposure. Although not quite reaching the level of statistical significance, the frequency of change in behavior for the EMF-exposed fish during the night and day-night combined was greater in the upstream low zone ($p = 0.06$ and $p = .05$ respectively). This observation is consistent with the observation regarding spatial distribution in the upstream-low zone. The observed differences between Control and Exposure in this zone could be due to orientation related to the upstream current, responses to changes in the vector field orientation produced by the EMF coil, or other tank effects.

The activity level of the halibut was higher during the night, but overall their activity level was very low. This could be partially explained by their foraging habits. Atlantic halibut are considered to be a “sit and wait” predator (Nilsson et al. 2010); they remain undetected until prey is within a lunging range. In these experiments, prey availability was intentionally excluded as part of the study design.

4.0 Dungeness Crab

Dungeness crabs are recreationally and commercially important benthic marine crustaceans, inhabiting nearshore waters along the North Pacific between Alaska and California (Pauley et al. 1986). They are found in estuaries and nearshore regions as well as subtidal waters up to 100 m deep. They occur most commonly in sand or muddy-sand substrate, often partially buried in sand. Dungeness crab reproduce in coastal nearshore areas; larval and juvenile stages move onshore and settle in shallow coastal bays, tidal flats, and estuaries. As adults they move offshore into deeper waters and forage as opportunistic predators on fish, shrimp, mollusks, and crustaceans.

Although a few physiological and behavioral studies have been conducted that examine the sensitivity of marine crustaceans to EMFs, there had been no studies of Dungeness crab prior to the FY11 progress report (Woodruff et al. 2012). The lack of study of Dungeness crab was noted by Normandeau et al. (2011) and Fisher and Slater (2010) in EMF literature reviews of marine species. The Bureau of Ocean Energy Management, Regulation and Enforcement (BOEMRE) includes Dungeness crab on a priority listing of invertebrate species in U.S. waters that are either likely to sense EMFs or for which there is little or no information documenting their sensitivity, but are considered important because of their conservation or management status. Dungeness crabs represent an actively managed fishery on the West Coast (PSMFC 2012), which adds to their importance as a study organism. Based on behavioral studies of other decapods, the most likely effect of EMFs on this species would be a disruption of orientation or navigation, possibly leading to altered onshore/offshore migration patterns of adults. Other life stages (e.g., larval and juvenile) could be exposed to EMFs from power cables as well, based on their potential proximity to the cables in nearshore and coastal waters.

During FY11, the sensitivity of Dungeness crabs to EMF was evaluated by examining their antennular flicking rate when crabs were exposed to an elevated EMF source, and a food source in the presence of EMF. There was no significant response to the EMF source for either test when compared to control responses. An initial experiment was also conducted in FY11 (Trial 1) to examine the spatial location of crabs through time with respect to the EMF, and whether their activity pattern over 3 d of exposure was modified. A potential acclimation to EMF was suggested in the FY11 results based on a change in activity level, therefore a second experiment was conducted during FY12 (Trial 2) adding an additional day of exposure (4 d total).

The specific objectives of the laboratory experiments were to 1) determine if an avoidance or attraction response was elicited in the crab in the presence of an anthropogenic EMF source, measured by the spatial distribution of the crab in relation to the EMF source, and 2) determine if changes in a crab's level of activity that would indicate the animal's behavior patterns were being affected by the presence of an anthropogenic EMF source.

4.1 Methods

This section describes methods related to the laboratory EMF exposure system (4.1.1), crab care and holding (4.1.2), experimental design (4.1.3), and data analysis (4.1.4).

4.1.1 EMF Exposure System

The EMF exposure system for Dungeness crab used the same electromagnetic coil and Exposure Tank design as that used for the Atlantic halibut exposures (Section 3.1.1 above) and the behavioral experiments conducted with Dungeness crab during FY11 (Woodruff et al. 2012). The energized coil produced a mean flux density of 1.01 ± 0.12 S.D. mT DC EMF in the center of the Exposure Tank, decaying approximately one order of magnitude at either end of the tank.

4.1.2 Dungeness Crab Care and Holding

Locally trapped adult male Dungeness crabs were used for all behavioral test exposures. Crabs were held at the MSL in outdoor tanks containing ~20 cm of clean sand and unfiltered flow-through seawater from Sequim Bay. Crabs were held for 1 to 3 wk until being tested and were provided an ad libitum diet of native bivalves or fish. When testing occurred, crabs were moved to the indoor experimental system and food was withheld for the duration of testing. At the time of testing, the crabs' mean weight was 743 ± 62 S.D. g.

4.1.3 Experimental Design

The laboratory experiments conducted with Dungeness crabs during FY12 (Trial 2) used an experimental design similar to that used in FY11 (Trial 1); a fourth day of observation was added during Trial 2. The bottom of each Exposure Tank contained ~15 cm of clean sand, providing a crab an opportunity to bury itself at will. Each tank received filtered flow-through seawater at a mean rate of $\sim 20.0 \pm 0.2$ S.D. L/min. Water quality was maintained at ambient conditions of $10.8 \pm 0.3^\circ\text{C}$, 31.1 ± 0.4 psu, 6.3 ± 0.2 mg/L DO, and pH 7.2 ± 0.4 . Baffles at either end of the tank provided a uniform longitudinal flow from one end of the tank to the other. Subdued daylight spectrum lighting (16 ± 3.5 lux) synchronized to civil sunrise and sunset was provided during daylight hours, and infrared illumination was provided at night.

For Trial 2, 10 adult male Dungeness crabs, tagged with unique identifiers on their carapaces, were placed in the Control and Exposure tanks (five crabs per tank). Crabs were partitioned individually at the downstream end of each tank with a polyvinyl chloride (PVC) enclosure and allowed to acclimate for 30 min. Previous experience with this species suggested a 30-min acclimation time was sufficient to mitigate for non-treatment behavioral responses. At the initiation of the experiment, the PVC enclosures were removed and the crabs' locations and behaviors were video recorded for 96 hr. Additional live observations were made every 30 min during the day, noting the location and behavior of each crab. Trained observers post-processed the video data and recorded observations of crab location and behavioral responses at 15-min intervals. All data were checked for quality assurance using a second observer. Trial 1 (FY11) was conducted in the same manner (i.e., number of crab, acclimation period, observation intervals), but it was conducted for 72 hr.

4.1.4 Data Analysis

The video data were analyzed statistically for Trial 2 using the five zone locations shown in Figure 3, described in Section 3.1.4. Trial 1 had zone location data for only three EMF field strength zones (low, moderate, and high). For both trials, the behavioral state of each crab was recorded at the end of every

15-min interval as either buried, resting on the surface of the sediment (Figure 6), or engaged in an active behavior such as walking or digging. For the initial Trial 1 and Trial 2 studies, each tank contained five crabs and every crab was viewed as responding to a landscape, hence each was treated as an independent replicate for this screening level assessment and location and associated behaviors were recorded for each.



Figure 6. Example of Dungeness crab categorized as buried in the Exposure Tank.

Statistical analyses were formulated to test the following null hypotheses:

- H_{01} : *The average amount of time Dungeness crab spend within each zone is independent of the presence of EMF treatment – reflecting temporal use of space.*
- H_{02} : *The number of times Dungeness crab visit a zone is independent of the presence of EMF treatment – reflecting choices of settling places.*
- H_{03} : *The frequency with which Dungeness crab change between buried/resting and active behaviors is independent of the presence of EMF treatment – reflecting level of agitation.*

Exposure tanks were not randomized between replicates (trials), thus, for each defined zone (Figure 3 and Section 3.1.4), a nonparametric Kruskal-Wallis test was used to compare the median response between the Control and Exposure tanks. For Trial 2, the sequential analysis of data by five-zone, three-zone, and two-zones was conducted, similar to the Atlantic halibut analysis. As before, the sequential reduction of the number of zones decreases the total number of observations. The confounding environmental and experimental conditions (including the tank effect) that affect the choice of location are also reduced.

The dependent variable for the H_{01} analyses (i.e., the average number of 15-min intervals each crab spent within a zone), was similar to the dependent variable for the halibut. However, because each crab's position could be located for all observations, it was not necessary to normalize the data by the total time observed, as was done for the halibut. The dependent variable for the H_{02} analyses was the number of times a crab visited a zone. A nonparametric Kruskal-Wallis test was used to compare the median length of time spent within a given zone and the median number of visits to that zone (low, moderate, high) between trials for a given treatment. When the median results between trials were not significantly different, a Mann-Whitney test was used to compare treatments with the trials combined.

The frequency of an activity change (H_03) was analyzed using observations collected within the first minute of every 15-min interval for 96 hr. A change of activity was defined as a change between buried/resting and any other active behavior (digging, walking, and climbing). Crabs resting on the surface or buried were considered equivalent for this analysis. Crabs were buried or resting for a majority of the time (91%) during Trial 2. For the activity analysis, the combined low (1 and 5), moderate (2 and 4) and high (3) zones were evaluated. The time of day and tidal stage were examined as the average number of changes in activity, calculated for both night and day time periods and for each tidal stage (ebb and flood). A nonparametric Kruskal-Wallis test was used to compare the median proportions of changes in activity between each time period (day and night) and each tidal stage (ebb and flood) for the control and EMF-exposed data separately. Data from Trial 1 were included in the analysis to increase the level of replication. A Kruskal-Wallis test was used to examine the effect of treatment for each of three zones. The analyses were conducted using all days combined and treating each 24-hr period separately. For the latter, a Kruskal-Wallis test was used to compare Control versus Exposure tanks combining the data for all zones.

4.2 Results

The crabs used for the experimental trial conducted in FY11 (Trial 1) were active more frequently (e.g., walking, digging, climbing) than crabs tested in FY12 (Trial 2). During Trial 1, approximately 20% of the crab observations taken every 15 min were recorded as active (570 out of 2890 total observations), whereas only 9% of the observations were recorded as active during Trial 2 (346 out of 3840 observations). However, for both trials, crabs spent a majority of the time buried, based on the frequency of observations and proportion of behaviors (active, buried, resting) shown in **Error! Reference source not found.** for each zone (low, mid, high).

4.2.1 Location

When the data were analyzed by five zones (Figure 7a), the median time (average number of intervals spent within a zone) spent within the upstream low zone, the high zone, and the downstream moderate zone was not significantly different between the crabs in the Control and Exposure tanks during Trial 2 (Kruskal-Wallis; $p > 0.60$; Figure 7a, top graphs). However, the median time spent within the upstream moderate zone and the downstream low zone was significantly different between the Control and Exposure tanks (Kruskal-Wallis; $p < 0.03$; Figure 7a). The median number of visits to the upstream low, upstream moderate, high, and downstream low zones was not significantly different between the control and exposed crabs (Kruskal-Wallis; $p > 0.24$; Figure 7a, bottom graphs). However, the median number of crab visits to the downstream moderate zone was significantly greater in the Control Tank than in the Exposure Tank (Kruskal-Wallis; $p = 0.01$, Figure 7a, bottom graphs). When the data were combined into three zones (low, moderate, and high), the median time spent and the number of visits to each zone were not significantly different between Control and Exposure tanks (Kruskal-Wallis; $p > 0.24$; Figure 7b). Likewise, when the data were combined into two zones (inside and outside the high zone), the median time spent and the number of visits to each zone were not significantly different between the control and exposed treatments (Kruskal-Wallis; $p > 0.24$; Figure 7c).

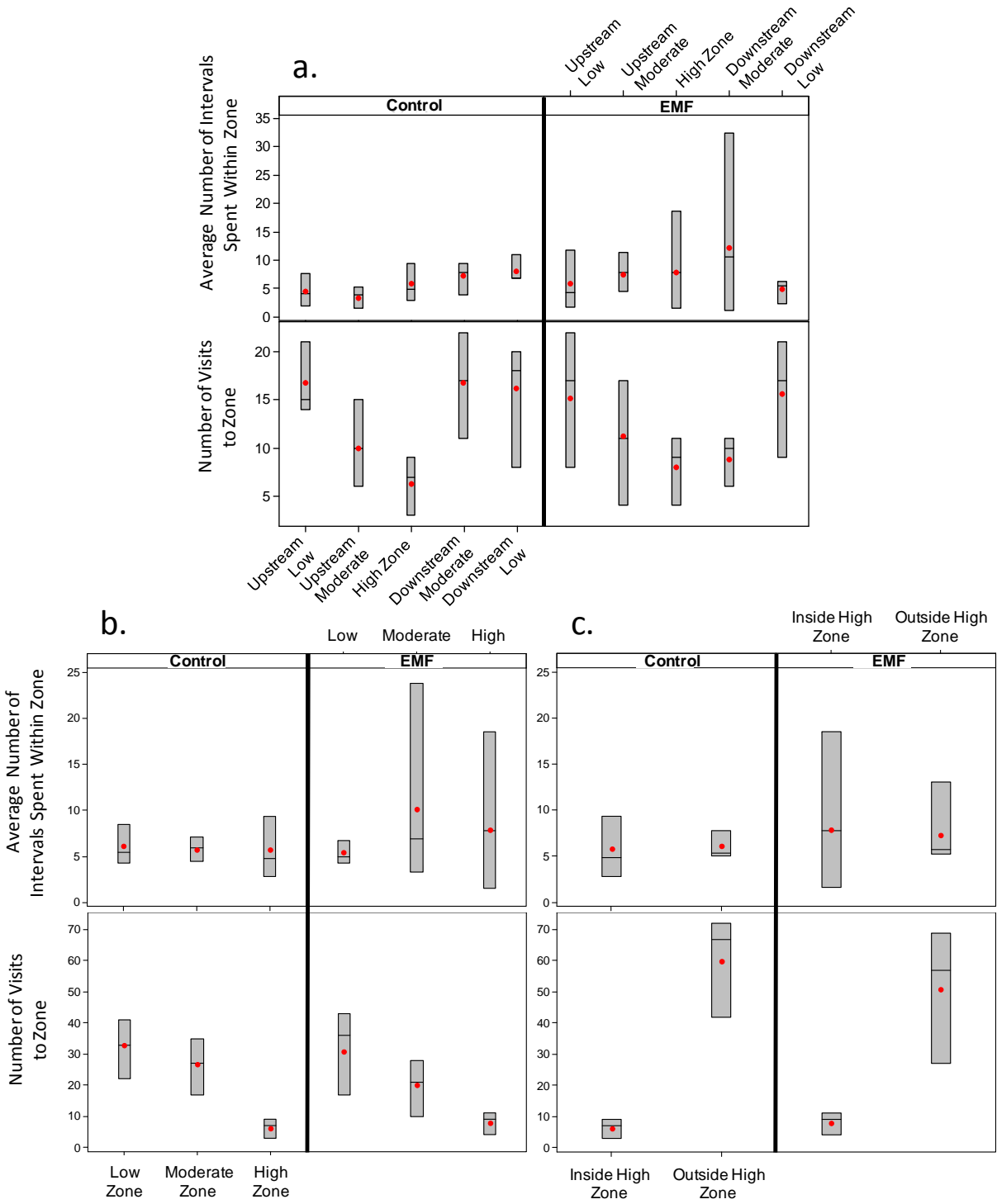


Figure 7. Dungeness crab Trial 2 showing 95% CI at about the median ($n=5$) of the average time spent in a zone (top 2 cells of each plot) and the average number of intervals observed in a zone (bottom 2 cells of each plot) for data compiled into a) five zones, b) three zones, and c) two zones.

The use of space within the low, moderate, and high zone locations was compared between Trial 1 and Trial 2 (Figure 8). The median number of 15-min intervals spent within the low, moderate, or high zones was not significantly different between trials for the control and EMF-exposed crabs (Kruskal-Wallis; $p > 0.117$ and $p > 0.076$, respectively; Figure 8 top graphs). The median number of visits to the low and moderate zones were significantly greater for the control crabs in Trial 2 (Kruskal-Wallis; $p = 0.021$ and $p = 0.05$, respectively; Figure 8 bottom graphs), but not for the high zone ($p = 0.17$), nor for the EMF exposed crab in any zone ($p > 0.465$). When both trials are combined, the median number of 15-min intervals control crabs spent within the low zone was significantly greater than that for the EMF-exposed crabs ($p = 0.02$; Figure 9); however, there was no significant difference between treatments in the moderate or high zones ($p > 0.91$).

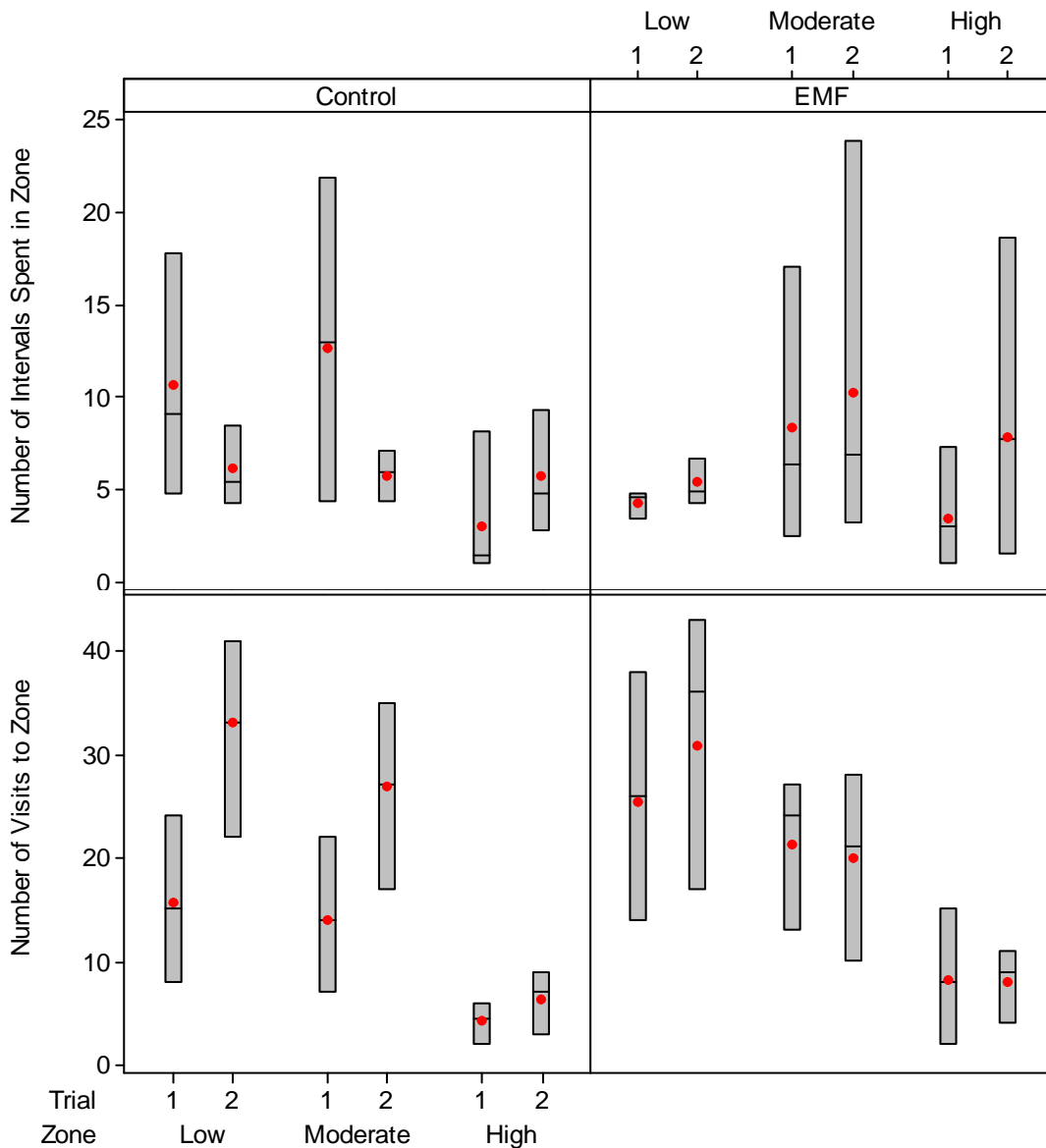


Figure 8. Dungeness crab Trial 1 and Trials 2 compared as 95% CI about the median time spent within a zone (top 2 cells) and the number of visits to a zone (bottom 2 cells) for the control ($n=5$ crabs) and EMF ($n=5$) exposures.

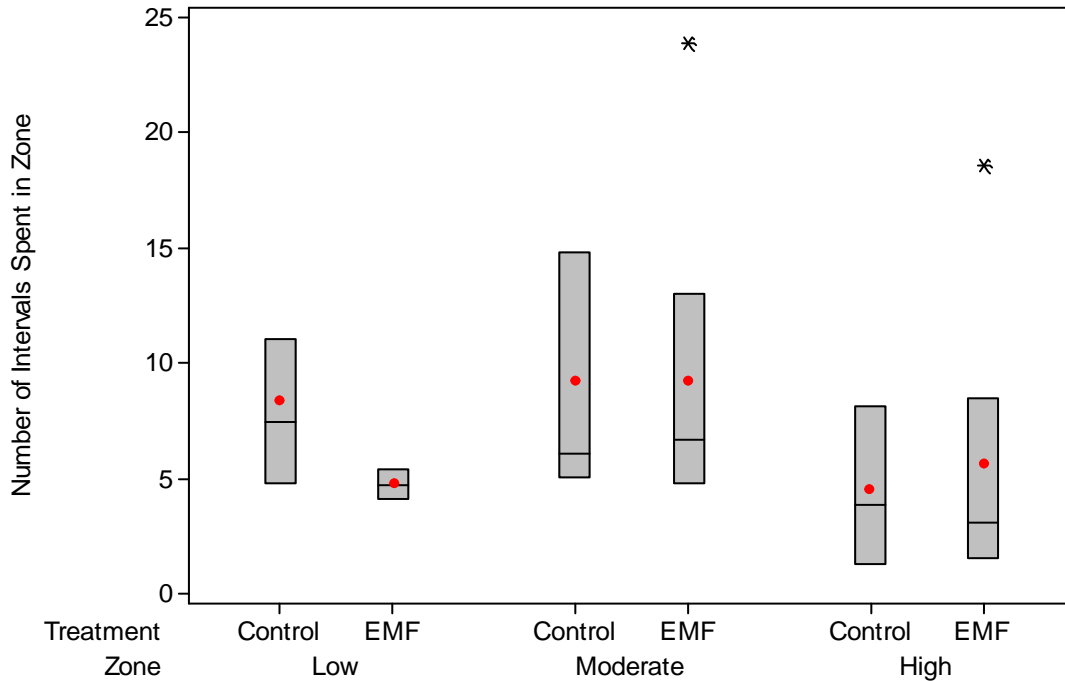


Figure 9. Dungeness crab Trial 1 and 2 combined, showing the 95% CI about the median time spent within the low, moderate, and high zones for the control (n=10) and EMF (n=10) exposures.

4.2.2 Behavioral Changes/Activities

The frequency of behavioral changes of the crab were evaluated by examining the number of observed changes between behavioral responses (i.e., between buried/resting and active behavior) within a given zone divided by the number of observations of behavior within that zone. Differences between the ebb and flood tide stage were examined initially using the combined Control Tank data from Trials 1 and 2, and combined Exposure Tank data. The median frequency of changes in activity was not significantly different between the ebb and flood tide stages for the control data (Kruskal-Wallis; $p = 0.10$) or the exposure data (Kruskal-Wallis; $p = 0.55$); therefore, tidal stage was not considered further with respect to the frequency of behavior changes. Differences between the nighttime and daytime were examined as well.

For the combined Control Tank data from both trials and all zones, the median activity change frequency was significantly greater during the night than during the day (Kruskal-Wallis; $p = 0.002$). However, for the combined Exposure Tank data, the median frequency of changes in activity was not statistically significantly different between night and day (Kruskal-Wallis; $p = 0.06$). Because Trial 2 had so few observations of active behaviors during the day (9 active observations out of 820 possible), statistical analyses of the frequency of changes in behavior were conducted on both the nighttime only data and on the combined day and nighttime data.

The data were also evaluated by examining the activity levels (frequency of changes in behavioral states between buried/resting and active) between the crabs in the Control and Exposure tanks on a daily basis for the duration of the experiments. During Trial 1, there was no statistically significant difference

in the frequency of behavior changes between the Control and Exposure tanks (Kruskal-Wallis; Day 1 $p = 0.09$; Day 2 $p = 0.06$; and Day 3 $p = 0.47$; Figure 10).

During Trial 1 (FY11 progress report, Woodruff et al. 2012) the number of times crabs changed behavior during the 72-hr exposure was greater in the Exposure Tank than in the Control Tank (Figure 10), although it did not quite reach statistical significance (Kruskal-Wallis; $p = 0.08$). When the data were partitioned into 24-hr periods and evaluated separately, Day 1 and Day 2 crabs showed significantly more changes in the Exposure Tank than in the Control Tank (Kruskal-Wallis, Day 1 $p < 0.05$, Day 2 $p < 0.05$), but by Day 3, the number of changes was similar between tanks (Kruskal-Wallis, $p = 0.35$; Figure 10).

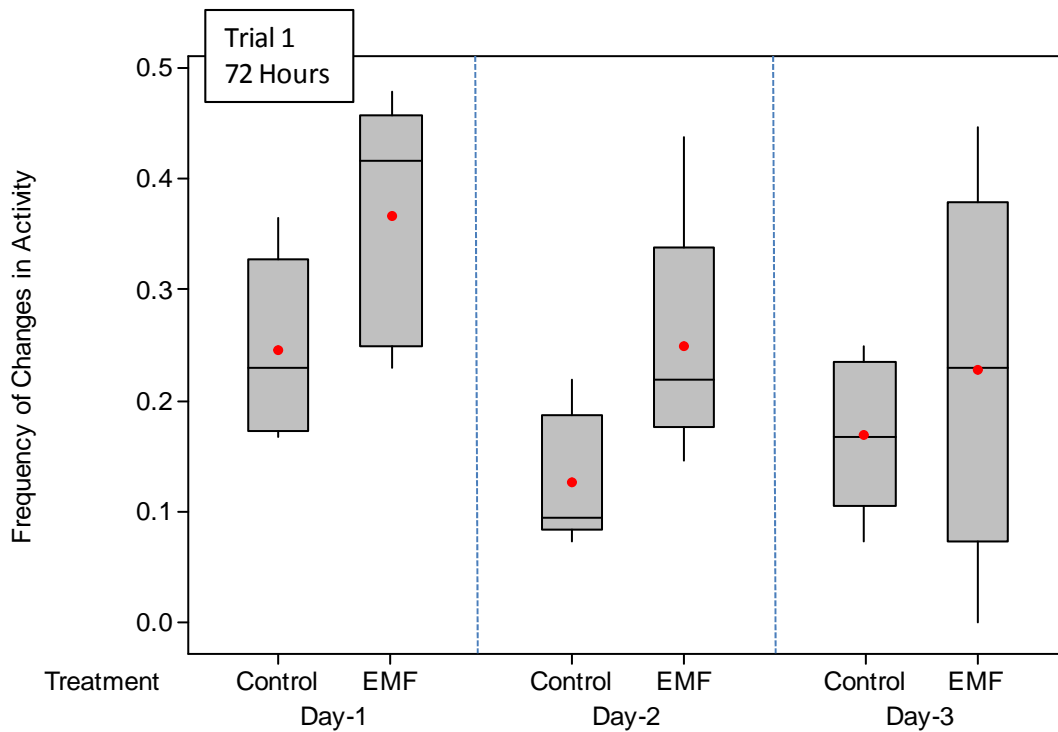


Figure 10. Boxplot of Trial 1 frequency of changes in crab activity between behavioral responses (buried/resting and active) for the Control and Exposure tanks evaluated for each day of the experiment. Boxplot whiskers represent the boundary used to define extreme values.

For Trial 2 during the night, the frequency of changes in behavioral states (buried/resting and active) was not significantly different between treatments in any of the five zones (Kruskal-Wallis; $p > 0.17$), three zones ($p > 0.34$), or two zones ($p > 0.46$). However, when the nighttime and daytime observations are combined, the behavior change frequency was significantly greater for the EMF-exposed crabs than for the Control crabs in the upstream low zone ($p = 0.01$). The Control crabs had a significantly greater behavior change frequency in the downstream moderate zone ($p = 0.05$) than those in the EMF-exposed tank. The treatment was not significantly different in the remaining three zones and the combined set of three and two zones ($p > 0.12$).

During this 4-d trial, there was no significant difference between treatments in the frequency of changes in behavior either in the night or overall (Kruskal-Wallis, $p > 0.60$). Further, when the data were

partitioned by day, there was no statistically significant difference between Control and Exposure tanks on any day (Kruskal-Wallis; Day 1 $p = 0.83$, Day 2 $p = 0.75$, Day 3 $p = 0.14$, and Day 4 $p = 0.30$; Figure 11).

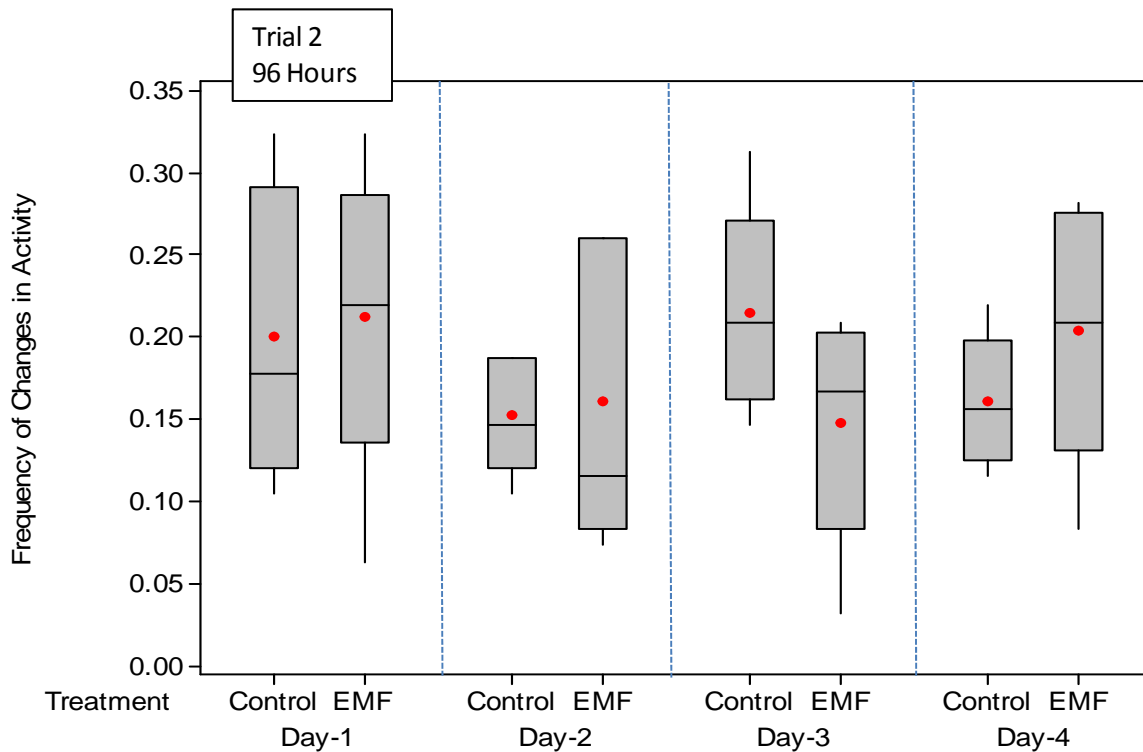


Figure 11. Boxplot of Trial 2 frequency of changes in crab activity between behavioral responses (buried/resting and active) for the Control and Exposure tanks evaluated for each day of the experiment. Boxplot whiskers represent the boundary used to define extreme values.

4.2.3 Summary

There were no statistically significant responses due to EMF strength alone with respect to spatial distribution or activity levels of Dungeness crabs. However, there were several significant responses when the data was examined with respect to the combined effects of EMF strength and vector orientation, water flow direction and other tank effects ($p < 0.05$ for Trial 2 flow-specific zone data). Although suggestive, these results are confounded by the fact that crab exhibited relatively large differences in behavior among days and between the two trial periods. In general, the crabs spent more time buried in areas closer to the ends of the tanks than in the center of the tanks. This general pattern of burial and location has also been noted in holding tanks of similar size and shape at MSL prior to the experimental trials. Activity levels also showed mixed results. During Trial 1, the first 2 d showed marginally higher levels of activity in the EMF Exposure Tank relative to the Control Tank ($p < 0.10$); however, this pattern was not repeated during Trial 2.

5.0 American Lobster

The American lobster is widely distributed over the continental shelf of the western North Atlantic ocean, ranging from Labrador to North Carolina (ASFMC 2012; Cobb and Castro 2006). They are considered one of the most valuable commercial fisheries along the U.S. Atlantic coast, particularly in New England waters (ASFMC 2012; Steneck and Wilson 2001). Lobster fishery efforts and landings have expanded since the early 1950s; most of the landings occur in state waters (i.e., within 3 miles from shore) (ASFMC 2012). American lobsters are found primarily in shallow sub-tidal regions to depths of 50 m or more, but can be found to depths of 700 m along the continental slope (Cobb and Castro 2006). Although lobsters generally remain in a home range of about 5 to 10 km², large mature lobsters in offshore areas make seasonal migrations inshore to reproduce. Lobsters, particularly juveniles and adolescents, prefer to remain sheltered, using crevices in rocks and boulders or burrowing in sediment when necessary. Lobsters become more active at night as they forage for food, and are opportunistic feeders of fish, crabs, clams, mussels, and sea urchins (Cobb and Castro 2006; Karnofsky et al 1989).

Although the sensitivity to EMFs has been examined for a few species of marine crustaceans (Normandeau et al. 2011), there have been no previous studies conducted with the American lobster. The most notable work has been carried out with the Caribbean spiny lobster (*Panulirus argus*), where it was determined through a number of laboratory and field studies that the spiny lobster can sense the Earth's magnetic field and uses this information for long-range navigation and homing (Lohmann 1984, 1985; Boles and Lohmann 2003; Cain et al. 2005; Lohmann et al. 2007). BOEMRE has included the American lobster on the priority listing of invertebrate species for EMF in U.S. waters (Normandeau et al. 2011), primarily because of the lack of information documenting its sensitivity to EMFs, as well as its importance from an ecological and management perspective. One of the concerns for this species related to EMFs is the possible disruption of navigation, potentially leading to an altered offshore/onshore migration pattern.

During FY12, experiments were conducted with small adult American lobsters to examine their behavioral responses to an anthropogenic EMF source in the laboratory. The specific objectives were to 1) determine if the spatial distribution of American lobster is affected by the presence of anthropogenic EMF, and 2) determine if changes in activities (e.g., sheltering, burrowing, exploring) that might indicate behavioral disturbance are affected by an EMF source.

5.1 Methods

This section describes methods related to the laboratory lobster care and holding (5.1.1), EMF exposure system (5.1.2), experimental design (5.1.3), and data analysis (5.1.4).

5.1.1 American Lobster Care and Holding

Four shipments of small adult American lobsters (male and female) were shipped live from a locally sourced supplier in Boothbay Harbor, Maine, and held at the MSL in indoor tanks with flow-through filtered seawater. The 75-L holding tanks each housed between 2 and 4 lobsters (Figure 12a). All lobsters arrived with their chelae immobilized with elastic bands to minimize aggressive interactions, and they remained banded until testing. They were held between 3 and 25 d before testing and were fed

minced squid (*Loligo opalescens*) ad libitum until testing occurred, at which point food was withheld. Their mean weight was 492 ± 29 S.D. g and cephalothorax length was 11.0 ± 0.4 cm.



Figure 12. American lobster experimental system including a) communal holding tanks prior to testing (left photo), and b) test tank with the PVC shelter in the center of tank (right photo).

5.1.2 EMF Exposure System

The EMF exposure system for American lobster used the same electromagnetic coil and tank design described above for the halibut and crab trials (e.g., Section 3.2.1, EMF Exposure System), however the system was modified to allow either coil to be energized, producing a similar exposure for each tank (Tanks A and B) when it was energized. A maximum field strength of approximately 1.1 mT DC was produced at the center of each tank with a uniformly decaying field on either side of the center (Figure 13). The zone delineations were reduced to three (i.e., two low zones and one high zone) with an approximate boundary at 0.5 mT DC (Figure 13). The mean exposure for each zone is shown in **Error! Reference source not found.** Control tanks had a mean field strength of 0.05 mT.

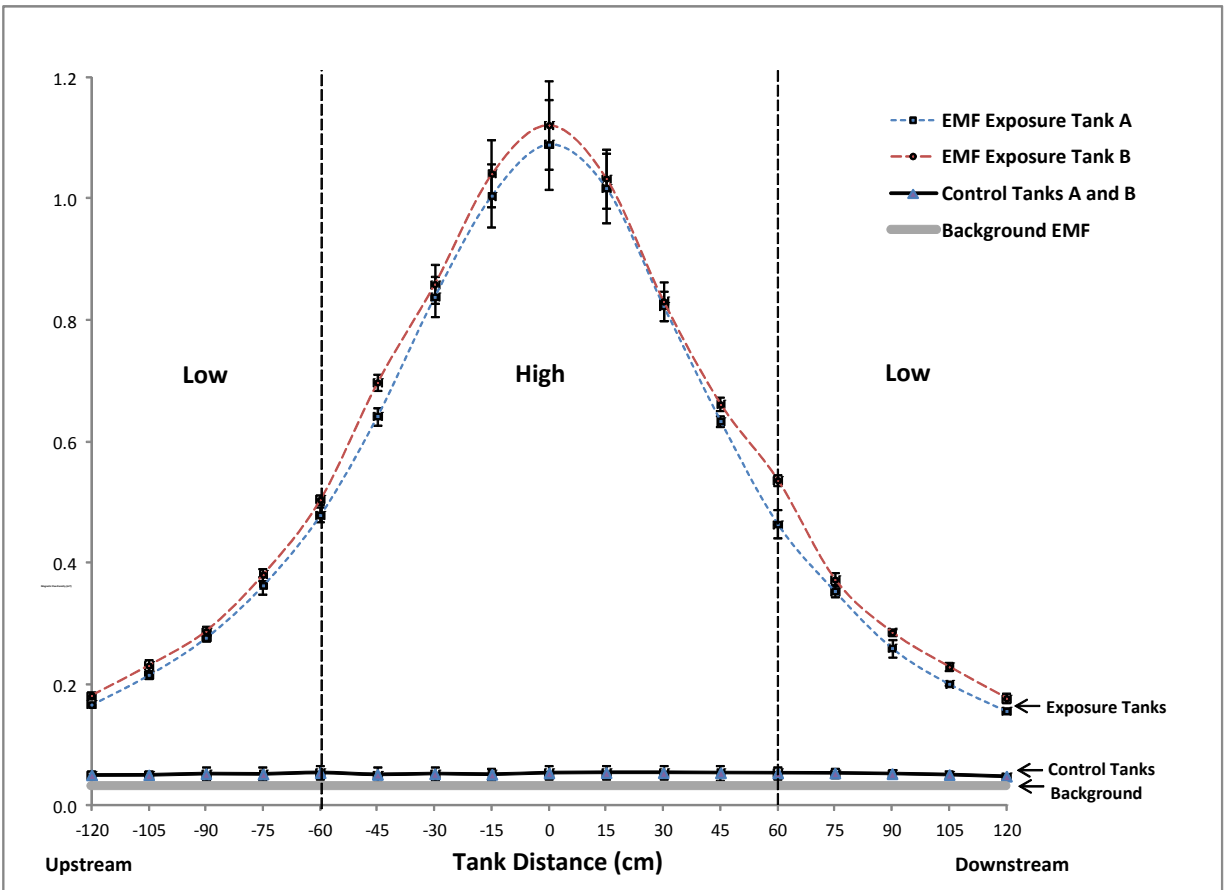


Figure 13. Magnetic flux density (mean \pm S.D.) in the Exposure tanks (Tank A and B) and the Control tanks used for American Lobster experiments.

Table 1 Magnetic flux density (mT DC) (mean \pm S.D.) of lobster Tanks A and B used as either an Exposure or Control tank.

EMF Treatment and Zone Location	Tank A (mT DC)	Tank B (mT DC)
Low EMF Exposure – upstream zone	0.29 \pm 0.11	0.32 \pm 0.13
High EMF Exposure – center zone	0.86 \pm 0.18	0.89 \pm 0.17
Low EMF Exposure – downstream zone	0.30 \pm 0.11	0.32 \pm 0.12
High EMF Peak – at center of tank	1.09 \pm 0.07	1.12 \pm 0.07
Control Treatment – all zones	0.05 \pm 0.003	0.05 \pm 0.003

5.1.3 Experimental Design

The experimental tanks previously described for the halibut and crab trials were used. The bottom of each tank contained \sim 15 cm of clean sand and filtered flow-through seawater was provided at a rate of 6.9 ± 0.4 S.D. L/min. Ambient water quality conditions throughout the experimental trials were 13.7 ± 0.8 $^{\circ}$ C, 28.2 ± 3.6 psu, 8.5 ± 0.7 mg L $^{-1}$ DO, and pH 7.9 ± 0.1 . A longitudinal flow of water was maintained across the tank; subdued daylight spectrum lighting (16 ± 3.5 lux) synchronized to civil sunrise and sunset

was provided during daylight hours and infrared illumination was provided at night. One lobster shelter was placed perpendicular to the flow of water in the center of each tank (Figure 12b). The shelters were composed of PVC and measured $38 \times 20 \times 10$ cm.

For each trial, one lobster was placed in the Control and Exposure tank on top of the PVC shelter with a PVC cover positioned over the lobster and shelter and allowed to acclimate for 30 min before the experiment was started. The choice of which tank would be energized for the EMF exposure was determined through random selection before each trial was initiated. However, the EMF vector orientation in Tank A with respect to the direction of water current flow was opposite that of Tank B requiring the data from each tank to be analyzed separately. Shelters were placed only in the center of the tanks in the high zone, which may have served as an incentive for lobsters to be in that zone. At the beginning of each test, the PVC covers were removed and video recording was initiated for 24 hr. Each trial was started at approximately the same time during the daylight hours in the morning. Live observations were made throughout the day as reference notes for later post-processing of the video. The video data were post-processed by trained observers, and quality assurance checked by a second observer prior to analysis of the data.

5.1.4 Data Analysis

Statistical analyses were formulated to test the following null hypotheses:

- H_{01} : *The proportion of time American lobsters spend within each zone is independent of the presence of EMF treatment.*
- H_{02} : *The number of times American lobsters are observed in each zone is independent of the presence of EMF treatment.*
- H_{03} : *The frequency with which American lobster change behaviors is independent of the presence of EMF treatment.*

Differences in location (H_{01} and H_{02}) were evaluated for each tank and zone separately using a Kruskal-Wallis test of equal treatment medians. Zones were analyzed in a sequential fashion: first by using three zones representing EMF strength, EMF vector orientation, and water flow direction. This analysis allowed for the assessment of confounding environmental and experimental conditions in the tanks. The analysis using only two zones (low combined and high) allowed for an assessment of avoidance/attraction to the highest EMF field or shelter with environmental effects minimized. The dependent variable in the H_{01} analysis was the average number of 15-min intervals in which the lobster was found within a given zone. The dependent variable in the H_{02} analysis was the number of times a lobster visited a given zone.

To evaluate H_{03} , the behavior of each lobster was recorded at the end of every 15-min interval; behavioral states recorded included *sheltered* (located inside the PVC shelter – Figure 14a), *burrowed* (partially buried in sand outside the shelter – Figure 14b), *resting* (motionless on the sand but not burrowed), or *active* (a suite of behaviors that included walking, digging, climbing). Variables were compared between Control and Exposure tanks and included the number of times lobsters crossed the high zone within each 30-min interval (i.e., moving from one end of the tank to the opposite end within two consecutive 15-min intervals), the number of times a lobster changed zones between 15-min

intervals, and the number of times a change in activity occurred. A change in activity was defined as a change in behavior between any of the behavioral states or events in successive observation periods.

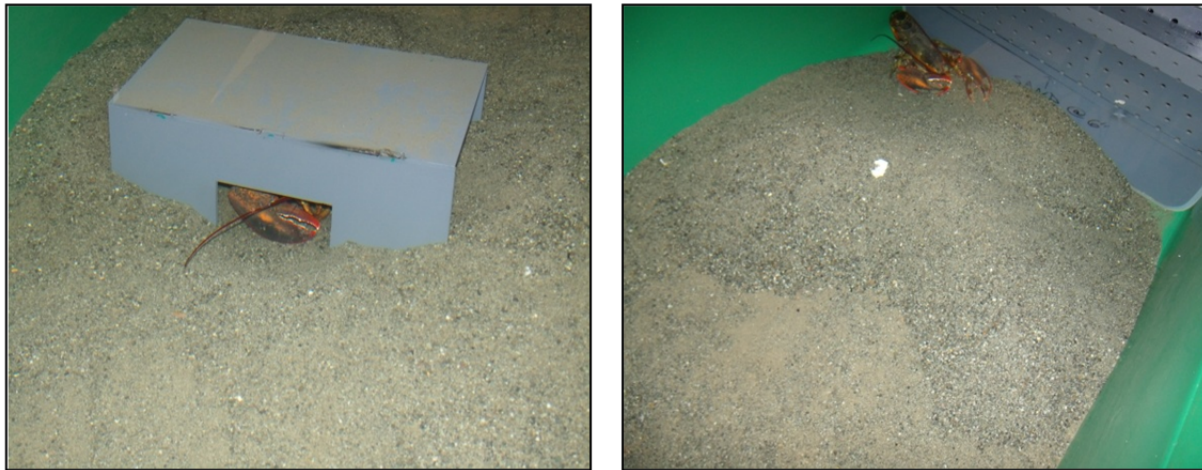


Figure 14. American lobster behaviors observed in experimental trials including a) sheltered in the PVC shelter (left photo), and b) burrowed in sand (right photo).

Data from the Control tanks (n=15 lobsters) were evaluated to assess the level of influence of each environmental/experimental factor including gender, time of day (day/night), tidal stage (ebb/flood), lobster shipment (i.e., batch #), and tank replicate (A or B). A Kruskal-Wallis test was used to test the null hypothesis that the median number of occurrences of each variable was not different for each environmental/experimental factor.

5.2 Results

Analysis of the environmental/experimental factors indicated there was no statistically significant difference ($p > 0.05$, 8 males, 7 females) for any environmental condition (i.e., time of day, tidal stage, and tank replicate) of the three behavioral activities examined (crossing high zone, changing zones, changing activity). In addition, when genders were combined, there was no statistically significant difference in behavioral activities for tidal stage, lobster shipment, or tank replicate (Tank A or Tank B). There were statistically significant differences were observed between night and day for one of the behavioral activities: there was a statistically significant difference for lobsters changing zones (Kruskal-Wallis; $p = 0.04$), but not for crossing the high zone (Kruskal-Wallis; $p = 0.09$), or between day and night (Kruskal-Wallis; $p = 0.14$). Further statistical analyses of behavioral activities were separated by day and night for comparison of EMF-exposed and control lobsters.

5.2.1 Use of Space

During the experimental trials, lobsters spent a majority of their time either burrowed (36% of time) or sheltered (40% of time), and only 23% of their time exploring, regardless of whether they were in a Control Tank or an Exposure Tank. Because the PVC shelter was placed in the center of the tank (high EMF zone), sheltering could only occur in a single zone; however, lobsters that burrowed chose to do so only in the low zones of the Control and Exposure tanks, rather than in the high zone.

For three zones, the time spent within the downstream low and the high zone was not significantly different between treatments for Tank A (Kruskal-Wallis; $p > 0.2$, Figure 15). However, in Tank A, the EMF-exposed lobster spent significantly more time in the upstream low zone than the control lobster (Kruskal Wallis; $p = 0.04$). This result was not observed in Tank B, for which the treatments were not significantly different (Kruskal-Wallis; $p = 0.518$). In Tank A, the number of times a lobster visited a zone was not significantly different between treatments for any zone (Kruskal-Wallis; $p > 0.32$). For Tank B, the number of times a zone was visited was not statistically significantly different between the control and EMF treatments for the downstream low and high zones ($p > 0.35$), or the upstream low zone ($p = 0.06$).

When the upstream and downstream low zones were combined into one low zone, the time spent inside or outside the high zone was not significantly different between treatments for either tank (Kruskal-Wallis; $p > 0.20$). The number of visits to each zone was also not significantly different between treatments for either tank ($p > 0.23$).

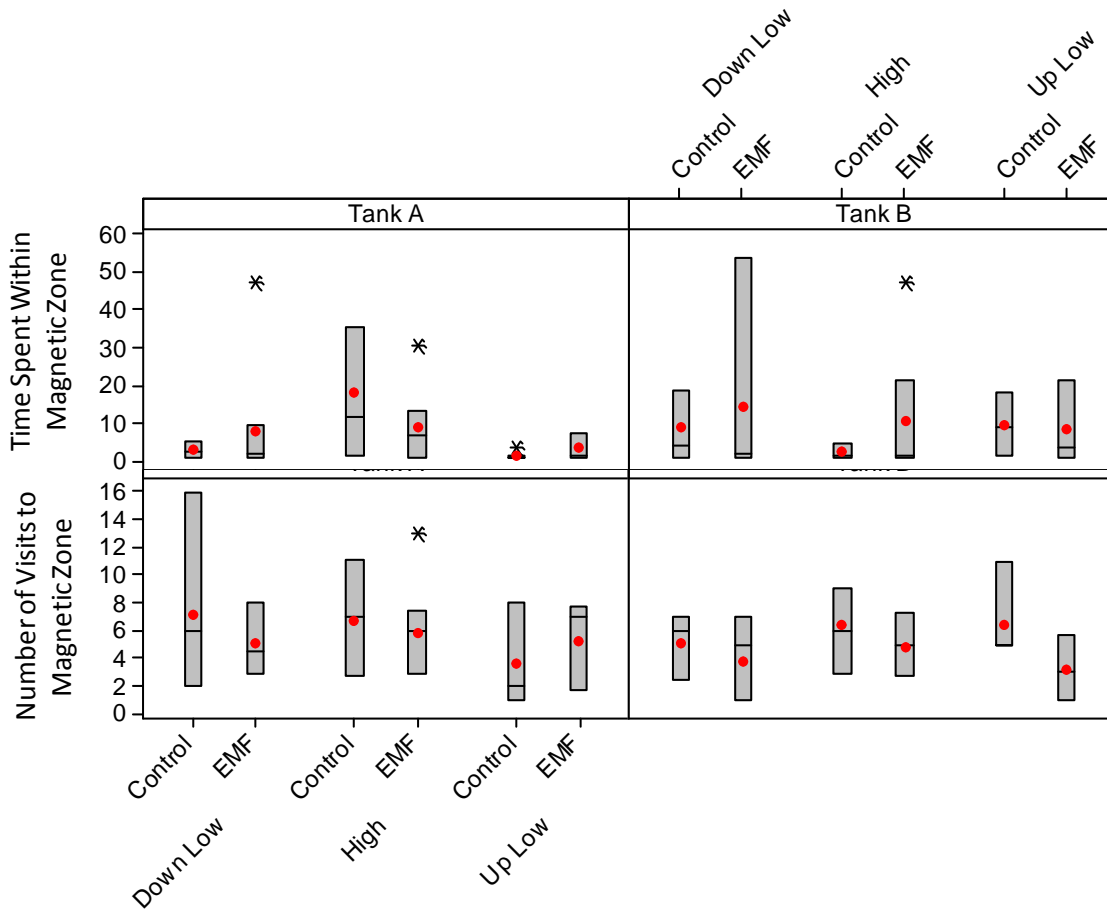


Figure 15. American lobster trials (n=15) for Tanks A and B showing the mean (red dot), median (black bar) and 95% CI of the median time spent within each zone (top) and the number of visits to each zone (bottom) for each treatment.

5.2.2 Behavioral Changes/Activities

Lobster responses related to active behaviors and sheltering were examined with respect to differences between the Control and Exposure tanks. In all cases, there were no statistically significant differences between the treatment conditions. The number of times lobsters changed activity was not significantly different during the day (Kruskal-Wallis; $p = 0.66$) or the night (Kruskal-Wallis; $p = 0.82$) (Figure 16), nor was the number of times lobsters changed zones during the day (Kruskal-Wallis; $p = 0.39$) or the night (Kruskal-Wallis; $p = 0.98$) (Figure 17).

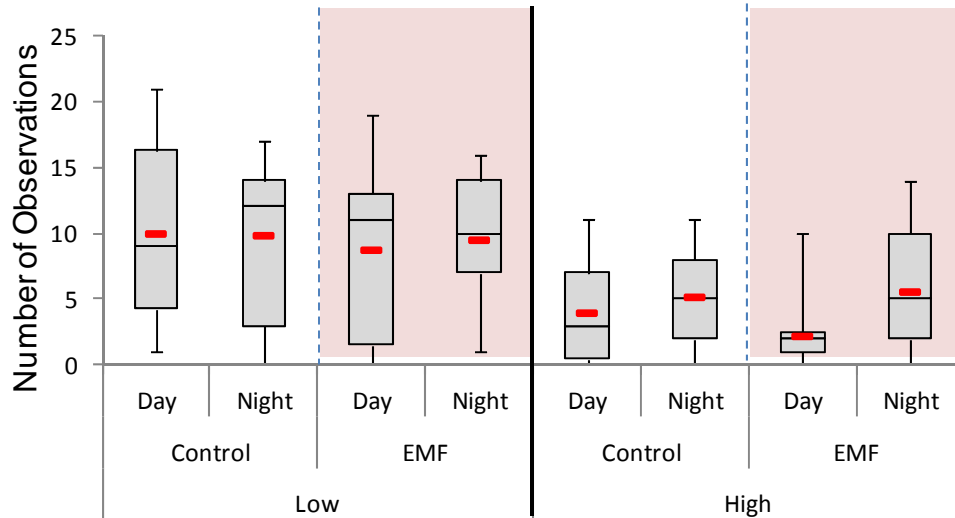


Figure 16. Boxplot of the number of observations where lobsters changed activity. Boxplot whiskers indicate the maximum and minimum number of responses.

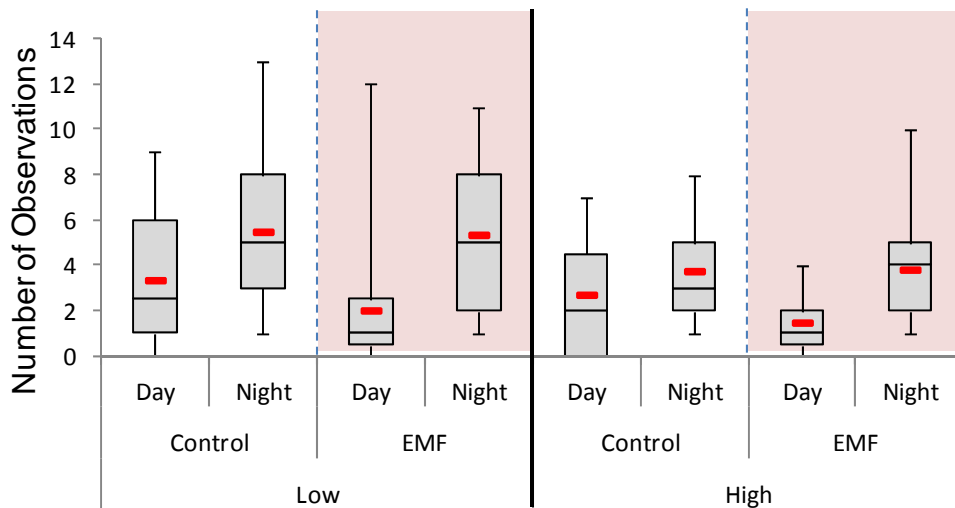


Figure 17. Boxplot of the number of observations where lobsters changed zones (low to high; high to low; or low to low at the other end of the tank). Whiskers indicate the maximum and minimum response.

An added behavioral measure was evaluated to provide insight into the willingness of a lobster to cross an area of higher EMF intensity, thus serving as a potential proxy for lobsters to cross an area of higher EMF strength in a natural environment where an underwater power cable might be present. This behavioral measure was calculated as the number of times a lobster completely crossed the high zone from one low zone to the other within one-half hour. It was possible to evaluate this behavior because the lobsters overall activity level was greater than that of either crabs or halibut. For this, the median number of times lobsters crossed the high zone was not significantly different between the Control and Exposure tanks during the day (Kruskal-Wallis; $p=1.00$) or the night (Kruskal-Wallis; $p= 0.78$) (Figure 18). The individual variability was very high for this activity.

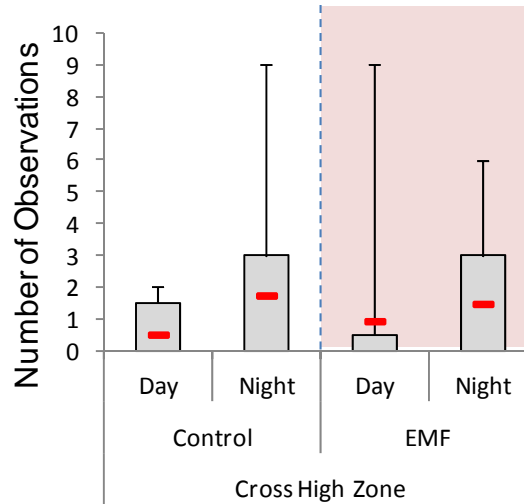


Figure 18. Boxplot of the number of observations where lobsters crossed from one low zone to the other; moving through the high zone (tank center). Boxplot whiskers indicate the maximum and minimum response.

5.2.3 Summary

Individual lobster exhibited a high level of variability in both space use and behavior within the experimental tanks independent of the EMF treatment. Within the 15 trials conducted, the lobsters did not exhibit a statistically significant response to the EMF, indicated by their use of space or a change in activity. They spent a majority of time either in the shelters (40%) or burrowed (a form of sheltering, 36%), and considerably less time (24%) out in the open (e.g., walking, digging, climbing, resting). A greater level of activity was noted during the nighttime compared to daytime hours for both control and EMF-exposed lobsters.

6.0 Discussion and Conclusions

The increasingly documented use of geomagnetic fields and weak electric fields by at least some marine taxa for a range of functions (e.g., prey detection, detecting conspecifics, orientation, navigation, migration) has prompted questions about the potential effects that anthropogenic EMF emissions might have in this environment. The proliferation of electric cabling in the marine environment that would result from MHK development heightens the importance of understanding the nature and significance of biological responses to EMF.

There is limited literature available regarding the responses of marine organisms to EMFs generated by undersea cables. The approach used by PNNL in FY12 was to acquire data from ecologically, recreationally, or commercially important species that had not been evaluated with respect to responses or effects from anthropogenic sources of EMFs. Controlled laboratory studies were conducted that focused on the organisms spatial distribution and level of observable behavioral activities when exposed to an EMF source. The three species selected for study (Atlantic halibut, Dungeness crab, and American lobster) were chosen for the following reasons:

- Species are listed as a priority species for study by BOEMRE (American lobster, Dungeness crab)
- Populations are of concern due to their management, exploitation, or conservation status (American lobster, Dungeness crab, Atlantic halibut)
- There were no reported studies regarding EMF responses (American lobster, Dungeness crab, Atlantic halibut)
- The species occupies benthic or demersal zones that would increase the likelihood of exposure to cables on or buried in the seabed (American lobster, Dungeness crab, Atlantic halibut)
- Species is migratory and therefore movements could be disturbed by EMF from a submarine cable (Dungeness crab, American lobster)

These studies should be considered preliminary screening level assessments; further investigation is warranted to confirm findings and evaluate other life stages.

6.1 Weight of Evidence

All of these species spent a majority of the time they were under experimental observation in an inactive state, independent of treatment; halibut were motionless 88% of the time; crab were buried/resting 80-91% of time; and lobster were sheltered or burrowed 76% of time. Given these largely inactive baselines, subtle changes in behavior are difficult to detect. However, the weight of evidence to date shows relatively few behavioral responses that would indicate an explicit avoidance of, or attraction to an ~ 1.1 mT EMF intensity based on the spatial distribution and activity levels of the three species tested (**Error! Reference source not found.**, p values of Significance Relative to EMF Strength, and Significance Relative to Avoidance/Attraction). The data do suggest possible behavioral differences between the Control and Exposure treatments occurring in specific areas (zones) of the tank (Table 2, p values of Significance Relative to EMF Strength, Vector Orientation, Water Current Flow, and Tank Effects). These observations were not consistently produced and were associated with a great deal of variability among individuals, and between seasons for Dungeness crab (i.e. significant difference

between FY11 trials and FY12 trials of time EMF Exposed crab spent in the low zone; $p=0.021$). Hence, additional study with further replication and a modified experimental design is needed to explain the statistically significant differences between treatments in specific tank zones that could be related to the directional flow of water in the tank, a response to a change in EMF vector orientation relative to background, or some other effect.

Table 2. Summary of statistical results for EMF exposure experiments with marine fish and crustaceans for FY12. P-values are from the Kruskal-Wallis nonparametric test between the control and exposed median response. P-values highlighted in yellow (<0.05), highlighted in tan (<0.10). The results for the high EMF zone are not repeated.

Experiment	Measurement Endpoint	Significance Relative to EMF Strength, EMF Vector Orientation, Water Current Flow Direction, other Tank Effects					Significance Relative to EMF Strength		Significance Relative to Avoidance/Attraction
		Upstream Low	Upstream Moderate	High	Downstream Moderate	Downstream Low	Low	Moderate	Outside High Zone
Atlantic halibut (<i>Hippoglossus hippoglossus</i>) N = 6 replicates 72-hr trials Exposure = 1.01 mT DC 1 halibut per Exposure and Control tank	Time spent within zone	Control > EMF p=0.028	p=0.150	p=1.000	p=0.150	p=0.173	p=0.150	p=0.522	p=0.873
	Number of times settled in zone	p=0.121	p=0.628	p=0.520	p=0.873	p=0.520	p=0.629	p=0.936	p=0.749
	Number of activity changes during the night	EMF > Control p=0.064	p=0.201	p=0.423	p=0.144	p=0.402	p=0.873	p=0.631	p=0.631
	Number of activity changes total	EMF > Control p=0.053	p=1.000	p=0.522	p=0.262	Control > EMF p=0.082	p=0.749	p=0.522	p=0.522
Dungeness crab (<i>Metacarcinus magister</i>) N = 1 replicate 96-hr trial FY12 only Exposure = 1.01 mT DC 5 crabs per each Exposure and Control tank	Time spent within zone	p=0.602	EMF > Control p=0.028	p=0.917	p=0.602	Control > EMF p=0.009	p=0.754	p=0.347	p=0.602
	Number of times visited zone	p=0.834	p=0.600	p=0.239	Control > EMF p=0.012	p=1.000	p=0.834	p=0.249	p=0.347
	Number of activity changes during the night	p=0.0.754	p=0.465	p=0.754	p=0.602	p=0.173	p=0.347	p=0.754	p=0.465
	Number of activity changes total	p=0.754	Control > EMF p=0.047	p=0.754	p=0.602	EMF > Control p=0.009	p=0.117	p=0.465	p=0.917

Table 2. (cont'd)

Experiment	EMF Strength, EMF Vector Orientation, Water Current Direction, other Tanks Effects/Measurement Endpoint	Tank A		Tank B			Tank A	Tank B	
		Downstream Low	High	Upstream Low	Downstream Low	High	Upstream Low	Outside High	Outside High
American lobster (<i>Homarus americanus</i>) N = 7 to 8 replicates 24 hr trials Exposure = 1.1 mT DC 1 lobster per each Exposure and Control tank	Time spent within zone	p=0.712	p=0.203	EMF > Control p=0.040	p=0.935	p=0.409	p=0.518	p=0.1153	p=0.524
	Number of times visited zone	p=0.606	p=0.600	p=0.326	p=0.458	p=0.350	Control ≥ EMF p=0.060	p=0.642	p=0.237
	Time of Day	Day			Night			Total	
	Number of activity changes through time	p = 0.662			p = 0.819			p = 0.506	
	Movement across the high EMF zone	p = 1.00			p = 0.783			p = 0.946	
Movement between zones	p = 0.397			p = 0.983			p = 0.603		

6.4

By examining the proportion of observations when individual test subjects were found closest to the EMF source (high zone, center of tank) for all species, it is possible to get an overall sense of whether higher intensity EMFs serve as an attractant or deterrent on the basis of relative field strength alone. Figure 19 shows the proportion of observations in the high zone for individuals as well as the mean response for each control and exposure group. The expected value of observations based on the amount of surface area in each zone is also shown (blue line). The mean response is remarkably similar between the control and exposure groups, particularly given the large individual variability among test organisms. The variability for lobsters is particularly notable and reflects the fact that most lobsters established a preferred location early in the experimental test that was maintained throughout the exposure period. This is shown by the clustering of a large percentage of observations in the high zone (which contained the PVC shelter) (Figure 19).

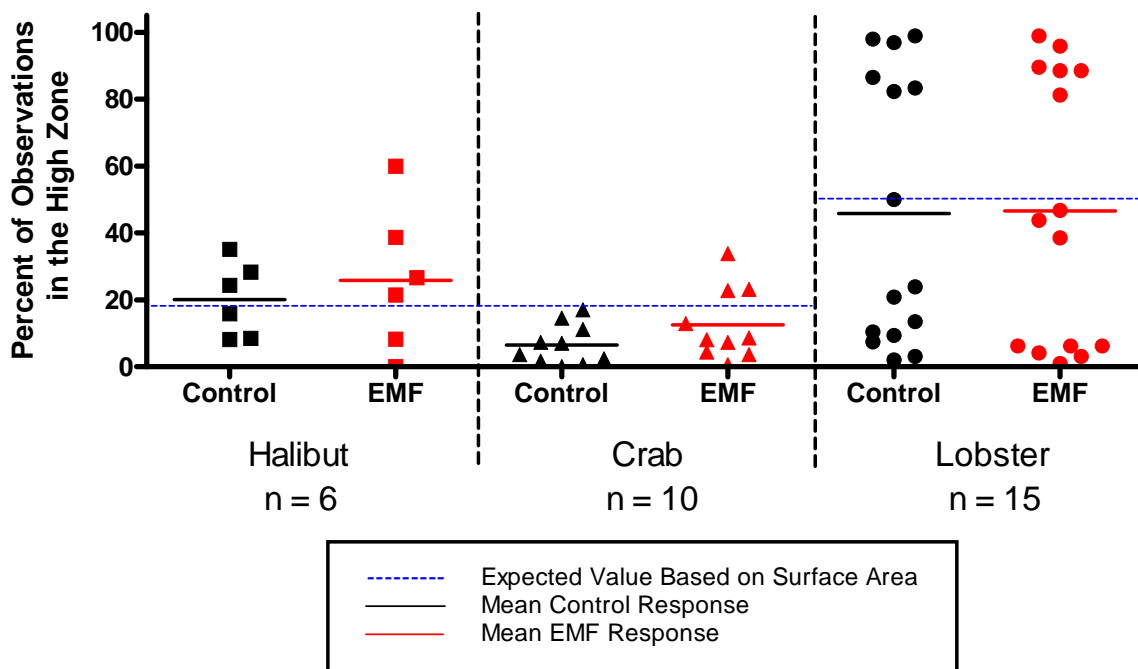


Figure 19. Observed and mean (line) proportion of all observations recorded by species in the high zone for Atlantic halibut (n=6), Dungeness crab (n=10), and American lobster (n=15).

6.2 Relevance to Field Deployments

The current studies are useful for developing hypotheses and directed experiments regarding potential effects of EMF arising from electrical cables in a field situation. For example, the distortion of geomagnetic fields caused by EMF from cables may alter species distribution and behavior in ways that are subtler than simple avoidance/attraction. The screening level studies described here were not designed to address questions related to EMF vector anomalies that may be generated by subsurface cables, although our statistical analyses raise the possibility that these localized anomalies may affect at least some species. Additionally, the laboratory studies have demonstrated that the presence of other environmental attributes (e.g. shelters in the case of American lobster) may influence distribution to a greater extent than that

induced by the EMF. However, extrapolation from a controlled laboratory situation to the field without further knowledge of the context is not advisable. The use of shelters, social behavior, orientation/migration and foraging habits are extremely important aspects of juvenile and adult life stages of the American lobster. While controlled laboratory studies are extremely important for understanding specific behavioral responses, the context of the field is required to understand the behavioral ecology of the animal and effects of EMF in a broader sense, especially with respect to questions of barriers to migration.

In addition to the behavioral/motivational aspects of a field versus laboratory situation, consideration should be given to the nature of the EMF source in the field compared to the laboratory. In the laboratory, the source used for these experiments was a coil surrounding the center of the tank. This configuration produced a vector orientation and orientation anomalies that are dissimilar to those that would be encountered in the field with a cable, either buried or on the sediment surface. In a field situation, the vector anomalies produced from a cable, while less complex, would be more extensive. Given that the overall approach of the laboratory investigation was to provide screening level information, these findings can be used to refine and inform future laboratory and field studies.

6.3 Extrapolation of Results to Population and Cumulative Impacts

Results from these screening-level studies do not provide sufficient information to assess the significance of effects of EMF at a population level. Likewise, extrapolating from a single exposure to multiple exposures (cumulative impact) is also speculative (Normandeau et al. 2011). It is not currently known how a subsea infrastructure of cables (i.e., multiple cables over short distances) will affect organisms in the area (Gill et al. 2012). These studies have demonstrated that EMF intensity alone is not a primary factor in determining the distribution of these test species in experimental tanks; however, we cannot confirm whether EMF sources would have effects on local orientation /navigation or longer-distance migrations where geomagnetic fields (Figure 1) and naturally-occurring local anomalies are likely to be used. For organisms that migrate seasonally along a coastline, exposures could result in navigational miscues (assuming these are not corrected by the use of other senses) or interference with feeding (Normandeau et al. 2011). For onshore-offshore migrants, the migratory endpoint and orientation of the cables (e.g., cable orientation parallel or perpendicular to species direction of travel) could determine the length of exposure, cumulative exposures, and potential miscues or redirection of a migratory route. It has recently been established, for example, that natural shifts in the local geomagnetic fields can redirect migrating sockeye salmon into using completely different routes of return to spawning rivers (Putnam et al. 2013). For sedentary species and those that occupy separate habitats during different life stages, the potential for cumulative impacts may be the greatest, particularly for those species whose early life stages might be exposed for great lengths of time. As benthic and demersal species, the organisms we investigated occupy distinct habitats during part or all of their life stages. In addition, Dungeness crab and American lobster are both seasonal onshore/offshore migrants. Hence the potential for cumulative impacts at multiple life stages is possible.

6.4 Recommendations

The laboratory studies have provided an initial assessment of behavioral responses by several marine species to artificially induced EMFs. Based on the findings to date, the following recommendations are provided for future research:

- The screening level assessments were conducted using DC EMF. It is expected that these species and other species may respond differently to EMF produced from AC cables (Normandeau et al. 2011). Additional controlled laboratory investigations are suggested utilizing an AC EMF source to provide comparative responses for key species. Sources should emulate realistic submarine cable characteristics to the extent possible.
- These experiments were conducted with adult and young adult life stages of each species tested. For many species, juveniles are considered to be more vulnerable. Hence additional study of responses and effects of EMF on larval and juvenile life stages of the American lobster and Dungeness crab, in particular, is warranted.
- Additional studies that specifically examine responses to EMF vector orientation anomalies produced by cables are warranted. Laboratory study designs that minimize confounding factors would then provide the basis for specifically directed field studies.

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