U.S. DEPARTMENT OF ENERGY

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Effects of Electromagnetic Fields on Fish and Invertebrates

Task 2.1.3: Effects on Aquatic Organisms – Fiscal Year 2011 Progress Report

Environmental Effects of Marine and Hydrokinetic Energy

D. Woodruff
I. Schultz
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V. Cullinan

September 2011

Pacific Northwest
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Pacific Northwest National Laboratory
Richland, Washington 99352

Abstract

In the FY11 progress report, we provide a description of the methods and results for experiments conducted in FY10/FY11 to evaluate the potential for adverse effects of electromagnetic field (EMF) exposure on aquatic organisms. The work described supports Task 2.1.3: Effects on Aquatic Organisms, Subtask 2.1.3.1: Electromagnetic Fields. EMF experiments with fish and invertebrates included short- and longer-term exposures of juvenile coho salmon (Oncorhynchus kisutch), Atlantic (*Hippoglossus hippoglossus*) and California halibut (*Paralicthys californicus*), rainbow trout (*Oncorhynchus mykiss*), and Dungeness crab (*Metacarcinus magister*). These species are ecologically, commercially, and recreationally important, and have the potential to encounter an MHK device or transmission cable during part or all of their life cycle. Acute effects such as mortality were not expected to occur from EMF exposures based on earlier studies, hence our test endpoints focused on changes in test organism behavior (detection of EMF, interference with feeding behavior, avoidance, attraction), organism development (growth and survival from egg or larvae to juvenile), and exposure markers indicative of physiological responses such as stress. EMF strengths during the tests ranged from 0.1 to 3 mT, representing upper bounding conditions, as available evidence suggests actual EMF strengths associated with devices and cables could potentially be much lower.

Results from the past two years of experimentation indicate there is little evidence to suggest major detrimental effects to the species tested under high EMF and extended exposure conditions. Although there were several developmental, physiological, and behavioral responses to a high EMF exposure, most were not statistically significant. Further analysis of these data and follow-on experiments with several other species in FY12 will allow a reasonable assessment of potential individual, community and population affects from MHK devices or cables on aquatic organisms. During FY12, the PNNL project team will complete behavioral testing with an additional invertebrate species, the American lobster *Homarus americanus*, and conduct behavioral testing of an elasmobranch specie (e.g. shark, skate, ray) known to use the earth's magnetic field for navigation and/or prey detection, to expand the findings of EMF responses by species representative of those important to stakeholders.

Project Overview

Energy generated from the world's oceans and rivers offers the potential to make substantial contributions to the domestic and global renewable energy supply. The U.S. Department of Energy (DOE) Office of Energy Efficiency and Renewable Energy (EERE) Wind and Water Power Program supports the emerging marine and hydrokinetic (MHK) energy industry. As major players in an emerging industry, MHK project developers face challenges with 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 many cases, little is known about the potential effects of MHK energy generation on the aquatic environment from a small number of devices or a large-scale commercial array. Nor do we understand potential effects that may occur after years or decades of operation. This lack of knowledge affects the solvency of the industry, the actions of regulatory agencies, the opinions and concerns of stakeholder groups, and the commitment of energy project developers and investors.

To unravel and address the complexity of environmental issues associated with MHK energy, Pacific Northwest National Laboratory (PNNL) is developing a program of research and development that draws on the knowledge of the industry, regulators, and stakeholders and builds on investments made by the EERE Wind and Water Power Program. The PNNL program of research and development—together with complementary efforts of other national laboratories, national marine renewable energy centers, universities, and industry—supports DOE's market acceleration activities through focused research and development on environmental effects and siting issues.

Research areas addressed include

- **Categorizing and evaluating effects of stressors** – Information on the environmental risks from MHK devices, including data obtained from in situ testing and laboratory experiments (see other tasks below) will be compiled in a knowledge management system known as *Tethys* to facilitate the creation, annotation, and exchange of information on environmental effects of MHK technologies. *Tethys* will support the Environmental Risk Evaluation System (ERES) that can be used by developers, regulators, and other stakeholders to assess relative risks associated with MHK technologies, site characteristics, waterbody characteristics, and receptors (i.e., habitat, marine mammals, and fish). Development of *Tethys* and the ERES will require focused input from various stakeholders to ensure accuracy and alignment with other needs.
- **Effects on physical systems** – Computational numerical modeling will be used to understand the effects of energy removal on water bodies from the short- and long-term operation of MHK devices and arrays. Initially, PNNL's three-dimensional coastal circulation and transport model of Puget Sound will be adapted to test and optimize simulated tidal technologies that resemble those currently in proposal, laboratory trial, or pilot study test stages. This task includes assessing changes to the physical environment (currents, waves, sediments, and water quality) and the potential effects of these changes on the aquatic food webs) resulting from operation of MHK devices at both pilot- and commercial-scale in river and ocean settings.
- **Effects on aquatic organisms** – Testing protocols and laboratory exposure experiments will be developed and implemented to evaluate the potential for adverse effects from operation of MHK devices in the aquatic environment. Initial studies will focus on electromagnetic field effects, noise

associated with construction and operation of MHK devices, and assessment of the potential risk of physical interaction of aquatic organisms with devices. A variety of fish species and invertebrates will be used as test animals, chosen due to their proximity to and potential susceptibility to MHK devices.

- **Permitting and planning** – Structured stakeholder communication and outreach activities will provide critical information to the project team to support execution of other project tasks. Input from MHK technology and project developers, regulators and natural resource management agencies, environmental groups, and other stakeholder groups will be used to develop the user interface of *Tethys*, populate the database, define the risk attributes of the ERES, and communicate results of numerical modeling and laboratory studies of exposure of test animals to MHK stressors. This task will also include activities to promote consideration of renewable ocean energy in national and local Coastal and Marine Spatial Planning activities.

The team for Activity 2.0 - MHK Environmental Impacts & Siting – is made up of staff, faculty, and students from

- Pacific Northwest National Laboratory
  - Marine Sciences Laboratory (Sequim and Seattle, Washington)
  - Risk and Decision Sciences (Richland, Washington)
  - Knowledge Systems (Richland, Washington)
- Oak Ridge National Laboratory (Oak Ridge, Tennessee)
- Sandia National Laboratories (Albuquerque, New Mexico; Carlsbad, California)
- Oregon State University, Northwest National Marine Renewable Energy Center (Newport, Oregon)
- University of Washington, Northwest National Marine Renewable Energy Center (Seattle, Washington)
- Pacific Energy Ventures (Portland, Oregon).

Acronyms and Abbreviations

ANOVA	analysis of variance
cm	centimeter(s)
dpf	days post fertilization
dpff	days post first feed
DOE	U.S. Department of Energy
EERE	DOE Office of Energy Efficiency and Renewable Energy
EMF	electromagnetic field
ERES	Environmental Risk Evaluation System
FD	food extract
ft	foot, feet
gal	gallon(s)
GLM	general linear model
hr	hour(s)
in.	inch(es)
L	Liter(s)
µm	micrometer(s)
MHK	marine and hydrokinetic
mL	milliliter(s)
mT	millitesla
MSL	Marine Sciences Laboratory
ng	nanogram(s)
NOAA	National Oceanic and Atmospheric Administration
NWFSC	Northwest Fisheries Science Center
pg/mL	picograms per milliliter
psu	practical salinity units
PNNL	Pacific Northwest National Laboratory
SW	seawater

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1.0 Introduction

As part of Task 2.1.3, Effects on Aquatic Organisms, Subtask 2.1.3.1 is focused on evaluating the potential effects of electromagnetic field (EMF) exposure on fish and invertebrates. This report documents the preliminary experimental work conducted in FY 2011 to evaluate those effects.

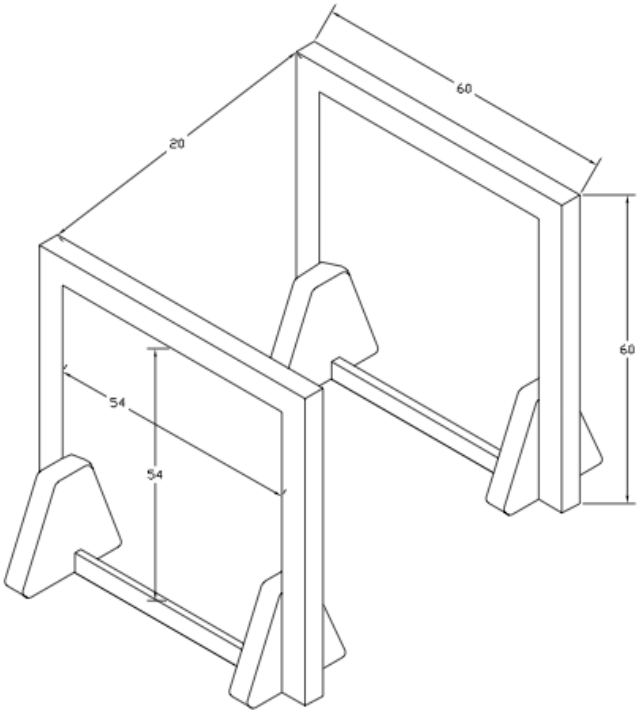
Section 2 of this report provides a description of the Helmholtz coil system that was used to generate the electromagnetic fields used in the experiments; Sections 3 and 4 provide the approach, assumptions, methods, and results for fish and invertebrate experiments, respectively. Chapter 5 provides a discussion of the overall results and planned activities to complete the aquatic assessments in Year 3 (FY 2012) of the project. Literature cited is presented in Section 6.

2.0 Helmholtz Coil Exposure System Description

During the initial stages of the project, the Pacific Northwest National Laboratory (PNNL) team reviewed relevant scientific literature to assess the existing state of knowledge concerning potential EMF effects on aquatic species. While limited, this information suggested that effects were possible at magnetic field strengths ranging from approximately 0.1 to 5 mT. Peer-reviewed literature and technical reports also demonstrated that in many cases, there was a high degree of uncertainty related to the exposure of test organisms because most experimental systems used employed small rare-earth or electromagnets that resulted in a nonuniform exposure field. To reduce the effect of this potential confounding factor, PNNL used internal funding to purchase a specialized Helmholtz coil system that has been used to support biological testing. What follows is a description of the Helmholz coil system and examples of coil configurations used during the project to evaluate potential EMF effects on marine, estuarine, and freshwater species.

2.1 Coil System and EMF Mapping

The Helmholtz coil system used to support biological effects testing was purchased from Walker LDJ Scientific, Lake Orion, Michigan, in early 2010. This device consists of two square frames measuring 60 in. (1.5 m) on each side with an external 750-W power supply capable of operating in both AC and DC mode (Figure 2.1).



CUSTOMER	PACIFIC NW NAT'L LAB
QUOTE	
S/O	
CODE	BATTPNNL
ICS X	20"
ICS Y	N/A
ICS Z	N/A
X SQ	60"
Y SQ	N/A
Z SQ	N/A
WEIGHT	250 lbs/coil
COOLING	AIR
POWER	750 W MAX 13-1/2A / 60V DC
FIELD Oe	30 Oe DC
UNIF. %	<20%
UNIF. VOL.	2'D x 1'L Cylinder
NOTE: ALL DIMENSIONS ARE APPROXIMATE.	

Figure 2.1. Helmholtz Coil Dimensions and Specifications

Initial EMF experiments focusing on organism survival, growth, and behavior were conducted in dual-coil configuration, with the two coils side by side (Figure 2.1). Prior to use in testing, the EMF field

generated by the Helmholtz coils was mapped to determine if PNNL's requested specifications were met and to quantify the EMF test organisms would experience between the coils. The coils framed an area that was 24 in. wide × 60 in. long × 40 in. high. A grid was established between the coils at 4-in. intervals vertically and horizontally, and EMF strength was determined using a Holaday HI-3550 magnetic field monitor. A three-dimensional image depicting field strength (Figure 2.2) was generated using the MATLAB software 3D plotting function (The MathWorks Inc., Natick, Massachusetts). The highest field strength generated was along the *y*-axis at 4 in. and 20 in. where the coils are positioned; the average field strength was 3.2 ± 0.13 mT, which is 64 times stronger than the Earth's magnetic field. The majority of the testing area is between 2.5 and 3.5 mT, which provided a uniform-field-testing area within a cube 24 in. to a side and met our stated requirements for exposure effects testing.

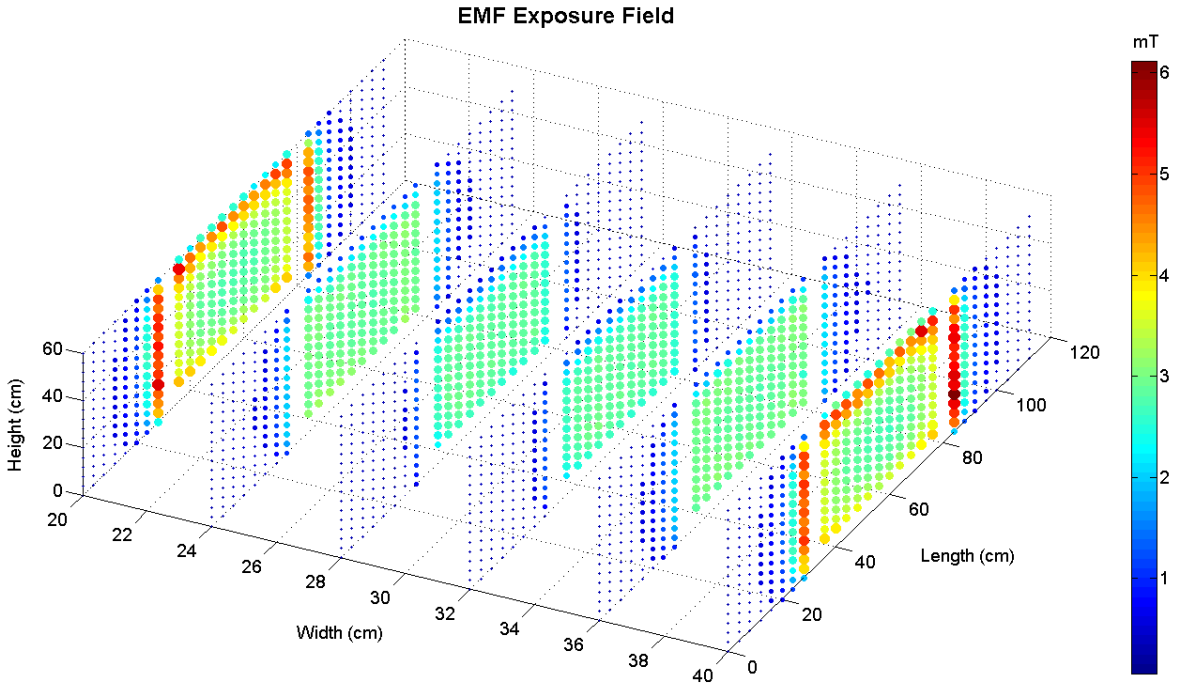


Figure 2.2. EMF Generated as milliTesla (mT) in Dual-Coil Configuration

To support avoidance/attraction testing with Dungeness crab this past year, the Helmholtz coils were separated to create a decaying magnetic field. As described below, this configuration allowed one energized coil to be used as the positive test system, and the second unenergized coil was used as the negative (control) system. EMF mapping prior to testing showed a maximum field strength at the center of the energized coil of ~1 mT, which decayed to nearly background within ~1 m (Figure 2.3)

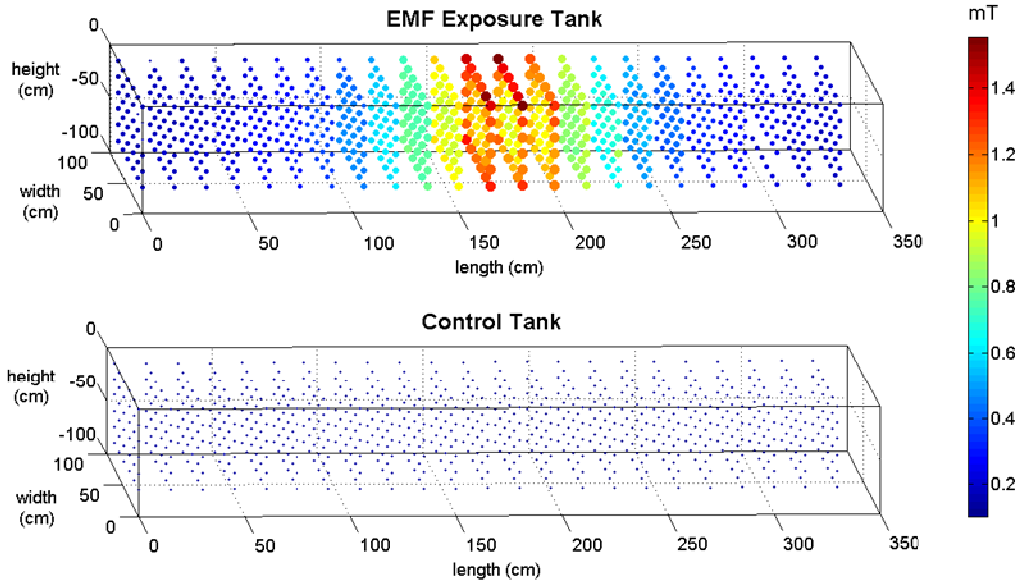


Figure 2.3. EMF Generated as milliTesla (mT) in Single-Coil Configuration

2.2 Example Test Systems

2.2.1 Dual-Coil Configurations

As described above, initial EMF experiments were conducted in dual-coil mode to ensure a uniform magnetic field within the experimental enclosures. In this configuration, test containers holding test organisms were positioned between the coils on an elevated platform to ensure all organisms received the same EMF exposure. Examples of dual-coil configurations used for fish and invertebrate testing are provided in Figures 2.4 through 2.7. Because ancillary stimuli not directly associated with the experimental treatments (i.e., sound, personnel movement, extraneous lighting) could compromise behavioral testing results, a temporary wall was constructed around the Helmholtz system to isolate it from the rest of the laboratory, and individual test chambers were isolated using black plastic or physical barriers, depending on the experimental design. In addition, all behavioral tests were video recorded to permit detailed analyses after the experiments were competed and to provide visual documentation of test results (Figure 2.8).

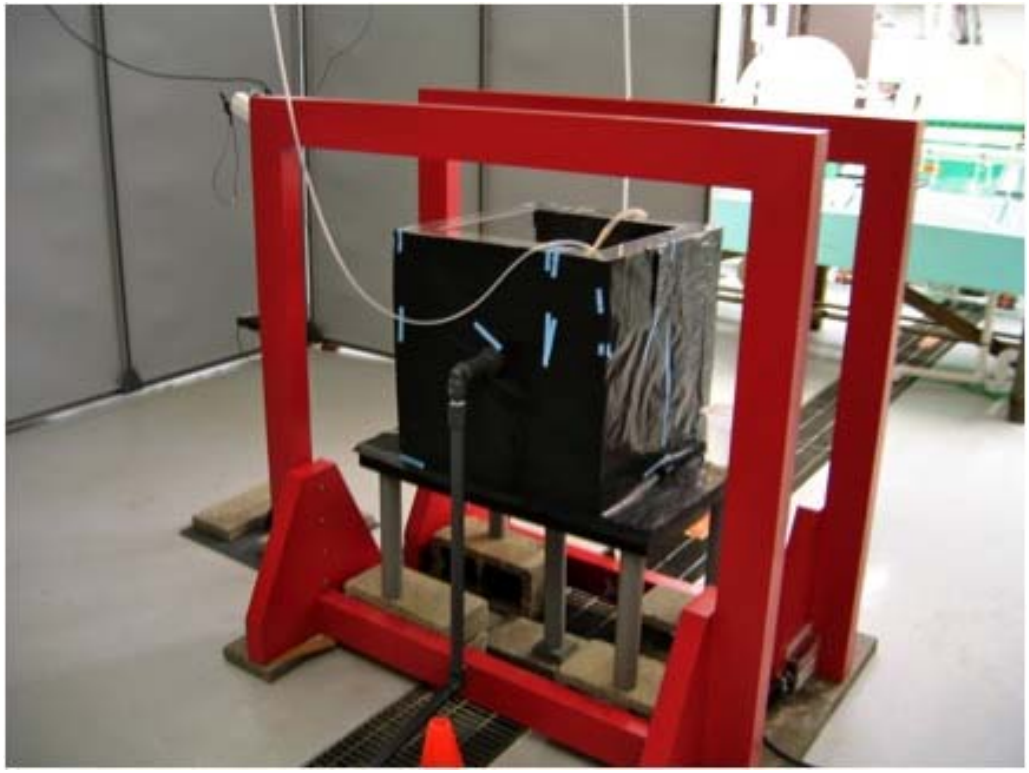
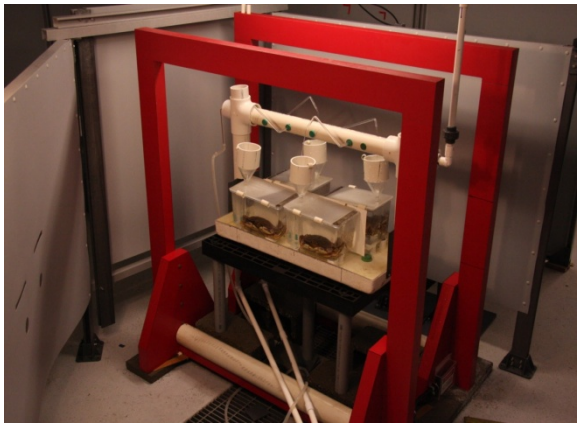


Figure 2.4. Experimental System for Coho Salmon and Atlantic Halibut

Figure 2.5. Experimental System for California Halibut

Figure 2.6. Experimental System for Rainbow Trout



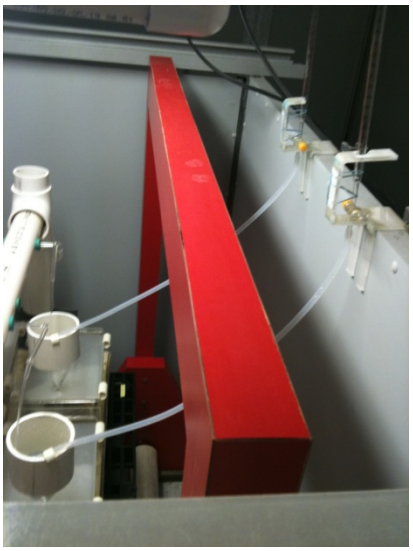


Figure 2.7. Experimental System for Dungeness Crab Antennular Flicking (left) and Modification of Buret Delivery System for Food Detection (right)

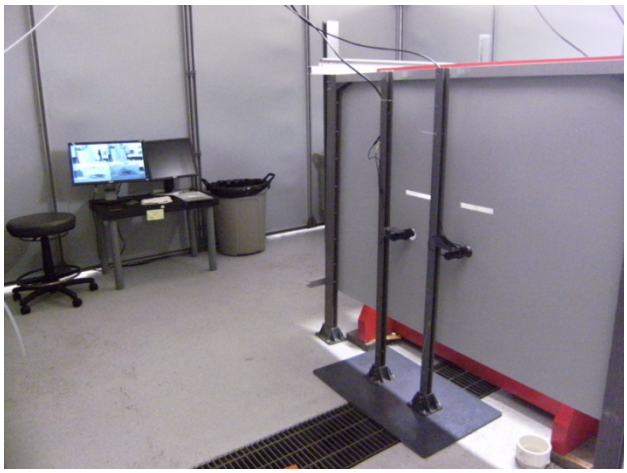


Figure 2.8. Recording System and Visual Blinds Placed Around Helmholtz Coil and Experimental System Shown in Figure 2.7

2.2.2 Single-Coil Configurations

As described above, the design of the Helmholtz coil system allowed the coils to be separated for use in Dungeness crab avoidance/attraction experiments. In this configuration, one energized coil was positioned at the midpoint of a rectangular environmental tank to serve as the positive test system. A short distance away, but outside the EMF field effects, a second non-energized coil was positioned over an identical tank to provide a control (negative) exposure (Figure 2.9). As described above, a video-capture system was used for both tanks to record test organism behavior.

Figure 2.9. Control and EMF Exposure Tanks for Dungeness Crab Avoidance/Attraction Experiments

3.0 Fish Experiments

3.1 Introduction

This subtask is investigating the effects of EMF on marine fishes. Prior to the initiation of experiments, a literature search was performed to review previous studies of EMF exposure to fishes. This exercise indicated relatively little has been published on the impact of EMF on fishes. However, three studies stood out based on their findings and more rigorous experimental design. Skauli et al. (2000) observed a delay in zebrafish (*Danio rerio*) embryogenesis after exposure to a 1-mT EMF (AC current 50 Hz). The EMF was generated using Helmholtz coils of a design similar to that described in Section 2 (although smaller in scale). This finding is in general agreement with those observed using other non-fish vertebrate models that suggest EMF exposure can alter the timing of developmental processes (Juutilainen 2005). Lerchl et al. (1998) reported that brook trout (*Salvelinus fontinalis*) exposed for 45 min to a 0.04-mT EMF (pulsing DC current, 800 ms off – 200 ms on) significantly increased the nighttime melatonin levels in both plasma and the pineal gland. This study also used Helmholtz coils for EMF generation. This finding is interesting as it is in general agreement with the "melatonin hypothesis" (Reiter 1995) that EMF can alter melatonin secretion, but it differs in that typically EMF exposure reduces melatonin levels (Stevens and Davis 1996). Gill et al. (2009) observed that a weak EMF (0.008 mT) produced by a simulated underwater power cable significantly altered swimming patterns of catsharks (*Scyliorhinus canicula*) held in large estuarine enclosures. Although these are three independent fish studies, they collectively suggest the potential for EMF to influence select developmental, physiological, and behavioral processes in sensitive fishes. With so little known about potential EMF effects of MHK devices, our experimental approach was then designed to both expand and confirm the findings of previous studies using fish species representative of those important to MHK stakeholders.

3.1.1 Testing Goals and Objectives

Testing goals and objectives were as follows:

- Determine the potential for adverse developmental effects related to EMF exposure to a variety of salmonid and flatfish species. The goal is to provide information on the potential for EMF exposure to affect growth and development or to invoke behavioral changes that could affect species spatial or temporal distribution, predator-prey relationships, or food-web dynamics.
- Develop a rapid assessment of the effects of EMF on a range of organisms in the laboratory and recommend organisms for in depth dose-response experiments.

3.1.2 Species Selection

Fish species selection was based on availability, established methods for laboratory culturing, and relevance for the endpoints to be measured. EMF impacts on fish development processes are being studied at three different life history stages: embryogenesis and larval – juvenile and parr – smolt transformations. For the embryogenesis studies, rainbow trout were selected based on the principal investigator's past experience (Schultz et al. 2003, 2008; Brown et al. 2007). For the larval – juvenile studies, flatfish metamorphosis was chosen as the model system. We selected Atlantic halibut (*Hippoglossus hippoglossus*) and California halibut (*Paralicthys californicus*) as the test species because

both are cultured commercially, are found in areas of planned or existing MHK sites, and both have well-characterized developmental staging and, therefore, are good model organisms to use for these experiments (Gisbert et al. 2002; Saele et al. 2004). The parr – smolt transformation and initial behavioral and physiological studies used coho salmon (*Oncorhynchus kisutch*).

3.1.3 Rationale for Experimental Designs

Many fishes, such as rainbow trout (*O. mykiss*) and coho salmon, possess magnetic-field sensing magnetite (Fe_3O_4) crystals that are concentrated in the snout or anterior head region (Wiltschko and Wiltschko 2005; Hellinger and Hoffmann 2009). Highest concentration of these crystals is typically associated with the olfactory lamellae and trigeminal nerve, which provides neuronal inputs to the hippocampus and other higher brain regions. This implies a connection between magnetic field perception, olfaction, and memory or imprinting of a stimulus (e.g., predator odor). Demersal fishes such as flatfish have a complex life history involving a symmetrical free-swimming larval stage and an asymmetrical post-larval, juvenile – adult stage. Demersal fishes are anticipated to receive higher EMF exposures in the field due to their benthic life style. Physiological or hormonal markers of exposure were tested in coho salmon and focused on plasma cortisol and melatonin levels. Cortisol is an established stress response marker and known to participate in the parr– smolt transformation (Bjornsson et al. 2011). Similarly, melatonin is well characterized in coho salmon and also involved in smoltification of salmonids (Gern et al. 1984).

3.2 Coho Salmon Alarm-Response

During FY 2010 and FY 2011, experiments were conducted to determine if exposure to EMF affected the ability of coho salmon to detect alarm odorants that are used to detect the presence of predators. Follow-on experiments were conducted in early FY 2011, as described below. Inhibition of predator detection following exposure to EMF could significantly affect survivability of both juvenile and adult salmon, resulting in potential effects at community and population levels if the likelihood of exposure were high and comprised a significant number of individuals.

3.2.1 Methods

For behavioral studies, juvenile coho salmon (approximately 15 cm fork length) were exposed to EMF and then stimulated with a predatory alarm substance to determine if their predator avoidance behavior had been affected by EMF exposure. This response has been used by the National Oceanic and Atmospheric Administration (NOAA) Fisheries and others (Stone et al. 1994; Brown and Smith 1997; Scholz et al. 2000; Tierney et al. 2006) to determine if exposure to chemicals from stormwater or other sources affects the predator-response ability of these fish. For EMF exposures, each experiment was conducted using one to five hatchery-reared juvenile salmon that had been acclimated in the exposure cubes for 7 days. Following acclimation, the fish were exposed to a static 3-mT field for 1–14 days. A predatory alarm substance consisting of 10 mL of homogenized coho salmon skin was added to the tank through a remote syringe apparatus to simulate a predator, and fish response was monitored by the video cameras.

3.2.2 Results

Under non-stressed conditions, coho salmon were expected to swim throughout the water column. When the alarm substance was added, the fish were expected to become motionless until the alarm substance was diluted by clean water inflow and cleared from the cube (Brown and Smith 1997). Four experiments were conducted in which the salmon were exposed to EMF for varying lengths of time and then introduced to the predatory alarm substance. During the first two experiments, the alarm substance was introduced during the daytime hours when the lights were on. Throughout the exposure, the fish remained situated primarily at the bottom of the cube and moved very little within the water column prior to and after the alarm substance was introduced. This response indicated the fish were displaying the stereotypical avoidance behaviors constantly throughout the experiment.

Through video monitoring of the non-EMF exposed control tank under non-stressed conditions, fish were observed to be more active at night when the lights were off. For this reason, the original protocol was altered to add the alarm substance 30 min after the lights went off instead of during the day. The experiments in the dark conditions provided variable results.

During the third trial, fish were exposed to EMF 5 days prior to the addition of the predator stimulus. The skin extract was added to the tanks 30 min after dark, and fish were monitored for the behavior. Before the extract was added, fish were observed swimming in the water column at various depths within the cube. Immediately after the extract was added, the fish displayed noticeable defensive behavior—schooling at the bottom of the cube, becoming increasingly motionless, and aligning themselves into the flow of water. This behavior lasted 30–60 min following stimulus addition; the fish returned to the full water column once the alarm substance was cleared from the cube. For this trial, the observed behavioral response suggested that exposure to EMF did not inhibit the alarm response in coho salmon.

During the fourth trial, fish were exposed to 1 day of EMF and the stimulus was added 30 min after dark. In the video footage, the fish appeared to be displaying an alarm response both prior to and after the addition of the skin extract. It is possible the variability in behavior between trials three and four could be due to human disturbances prior to the addition of the alarm substance, despite precautions taken to reduce non-treatment responses through the use of physical barriers and limiting fish view through exposure containers. Because the fish respond to light cues, adding the alarm substance in the dark with low-level auxiliary lighting may have created shadows that were interpreted as predator movement by the test fish.

A subsequent experiment used juvenile coho of smaller size (approximately 7 cm fork length) to assess whether better acclimation to the exposure system would occur. However, results similar to those found using the larger fish were obtained. At this point, further coho salmon behavioral testing was stopped, as it did not appear tractable under the restrictions imposed by the exposure system.

3.2 Coho Salmon Exposure Markers

Measurement of exposure markers was pursued using coho salmon as a means to detect underlying physiological changes that may precede more overt responses at the whole organism level. Exposure marker testing was done using short-term EMF exposures that allowed testing of a variety of exposure levels and currents (AC and DC).

3.2.1 Methods

Fish. Juvenile coho salmon were obtained from NOAA-NWFSC (Seattle, Washington). These fish were initially transported to the Marine Sciences Laboratory (MSL; Sequim, Washington) in freshwater. After 3 months of acclimation at MSL, these fish were then smolted by gradually raising the salinity 5–7 ppt per week, until full-strength seawater was obtained. After 2 months of seawater acclimation, the fish were then used in subsequent EMF experiments. At this time, the fish varied in weight between 150 and 250 grams.

Exposure Protocol and Cortisol and Melatonin Measurements. All EMF exposures lasted 80 hr. For each EMF exposure, four fish were placed in the acrylic experimental cubes (2 ft × 2 ft × 2 ft) filled with 40–50 L of filtered Sequim Bay seawater supplied via a flow-through system. Temperatures were maintained between 9°C–10°C, and water flow rates ranged between 2–4 L/min. One cube was situated in the center of the Helmholtz coils, and one cube was set up 20 ft away, which was confirmed to be out of range of the EMF generated by the Helmholtz coils. A concurrent control group was always sampled for each treatment. The fish were then exposed to measured EMF levels of 0.1 and 3 mT using DC electrical current. These exposure levels were also repeated using a pulsed EMF generated every 2 sec (on/off cycle). A fifth EMF exposure was performed using a field intensity of 0.13 mT generated using AC electrical current operated at 40 Hz. The EMF exposure concluded at 12:00 a.m. (0000 hr). Fish were euthanized by tricaine methanesulfonate (MS-222) overdose (250 mg/L), and a blood sample was obtained from the caudal vein and the plasma separated by centrifugation (3,000 × *g* for 5 min). All fish sampling was done in complete darkness. Fish handlers used headlamps fitted with red filters to prevent stimulation of the pineal gland. Both cortisol and melatonin were measured in plasma using commercially available ELISA kits (Neogen Inc.).

3.2.2 Results

Cortisol. Plasma cortisol levels were observed to vary substantially between the treatment groups. Mean values in the EMF treatments ranged from 235–1510 µg/L and varied from 382–1520 µg/L in the control groups. Visual inspection of the results did not identify any trends in the results, and the cortisol levels appeared to randomly vary among individuals. One-way analysis of variance (ANOVA) showed no significant differences between EMF treatments and the control groups ($p > 0.1$ for all treatments).

Melatonin. Mean plasma melatonin levels exhibited greater consistency among the control groups, varying between 33 and 49 ng/L. In the EMF treatment groups, mean values were typically lower than those of control fish and varied between 1.5 and 34 ng/L. A graphical summary of the results is shown in Figure 3.1 (note that melatonin analysis in the 3-mT constant on group has yet to be completed). Inspection of the results suggested a dose-response trend in that the largest difference in mean values was observed in the 3-mT on/off EMF exposure while relatively similar values were observed for the 0.1-mT EMF exposure. However, the 0.13-mT EMF (AC current) appeared to produce a change intermediate in scope. It is worth noting that statistical analysis (ANVOA) did not identify any significant differences between treatment groups. The lack of statistical significance appears to be due to relatively high inter-individual differences in melatonin levels among control fish. Despite the lack of statistical significance, the results are consistent with those obtained from other vertebrate model systems suggesting EMF exposure suppresses nighttime melatonin production by the pineal gland (Steven and Davis 1996).

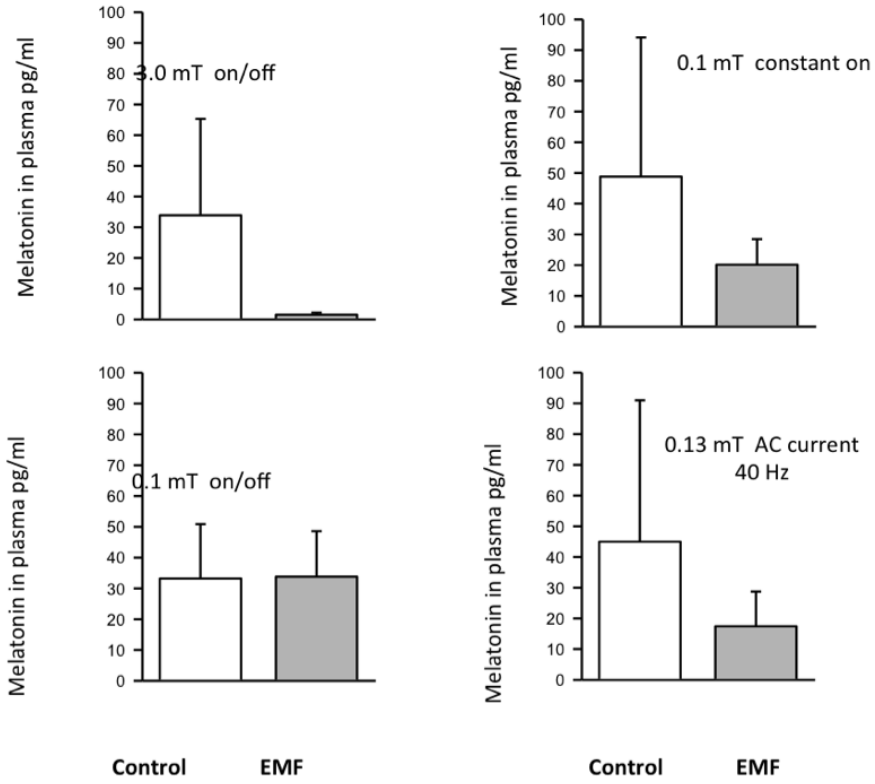


Figure 3.1. Plasma Melatonin Levels in Coho Salmon Exposed for 80 hr to Various EMF Levels. Values are mean ±SD (n = 4).

3.3 Rainbow Trout Egg Development

This experiment investigated the effects of EMF exposure on embryogenesis in rainbow trout, a species also under investigation by Oak Ridge National Laboratory. Two groups of fertilized trout eggs were exposed to a constant 3-mT EMF field ranging from 10 to 17 days, then examined to assess developmental progress. Concurrent control exposures without EMF were also conducted. Interference with normal egg development could result in death, delayed development, or greater susceptibility to predation, leading to both community and population-level effects if the potential for exposure were high and involved a large number of individuals.

3.3.1 Methods

Egg Fertilization. Approximately 24 hr prior to the arrival of the rainbow trout eggs from a commercial fish hatchery (TroutLodge, Inc., Lacey, Washington), one individual male rainbow trout (of the OSU x Arlee strain) was stripped of semen. The semen was calculated to have an average sperm count of 1.793 million/µL. Eggs were fertilized at a ratio of 4.4×10^5 sperm cells/egg in 50-ml fertilization buffer (60 mM NaHCO3 and 50 mM TRIS). Twenty-five eggs were set aside as an unfertilized control.

Apparatus and Experimental Design. Directly after fertilization, the eggs were dispensed evenly into 15 identical replicate cups. The 15 replicate cups were then separated into three groups and placed in fertilization trays. The trays were exposed to consistent temperature, constant flow rate, and light regime. Equal treatment was given to all fertilized eggs at this stage. Three days post fertilization (dpf), the eggs were moved into two 20-gal aquaria. Five replicates were chosen at random and placed in one aquarium, centered between Helmholtz coils, which were supplied with a direct current that resulted in a 3-mT EMF equally distributed throughout a 60cm/side cube. This group served as the early EMF-exposed treatment. The remaining replicates (including the unfertilized eggs) were moved to an identical aquarium away from the EMF source to serve as the unexposed control and also to provide individuals for late stage EMF exposure treatments.

The aquaria were set-up with constant freshwater flow-through. The Helmholtz coils were turned on at approximately 3 dpf. The eggs starting the study in the Helmholtz coils were referred to as the early EMF exposure group and were exposed to an EMF for 17 continuous days until 20 dpf. A second group, consisting of five replicates, entered the coils at 10 dpf. This treatment was referred to as the late EMF-exposed group, and the replicates were exposed to the EMF for the remainder of the 17-day exposure (10 days total). A control group of unexposed eggs remained farther than 180 cm outside the coils in a comparable experimental setup throughout the duration of the EMF exposure. This exposure schedule was influenced by a similar study involving zebrafish by Skauli et al (2000). Daily temperature recordings and frequent flow rate readings were taken in both aquaria so that conditions remained within 0.1°C of one another. At 20 dpf, each replica cup was split into two groups. One-half was collected and fixed in Stockard's solution for observation directly following termination of EMF exposure. The other half was allowed to develop until hatching in the absence of the EMF. Twice a day, eggs were observed for hatched fry, which were then removed, documented, and fixed for observation.

Post Mortem and Developmental Progress Scoring. The 20-dpf eggs and the hatched fry were scored on different developmental parameters. Eggs received one point for a positive observation in the following parameters: fertilization, growth and development of head and spinal cord, development of a distinct bi-hemispherical brain, large dark pigmented eyes, and a combined spinal cord to head length of greater than half the circumference of the egg itself. Eggs with mutations such as scoliosis, eye malformations, and improper brain development were docked one point. The fry were scored based on developmental parameters including the following: complete hatching, complete separation of caudal fin from the egg yolk, opening of the mouth, straightness of the spine, and length. The scores ranged from zero to five. Zero was assigned to either unfertilized eggs or non-hatched embryos for each observation. Five was assigned to either fully developed embryos or hatched fry with no spine deformities. The unfertilized eggs were counted, removed, and discarded. Egg mortalities were removed upon visible signs of death and were tracked by date and exposure group. The mortalities were fixed in Stockard's solution for later observation.

Statistical Analysis. For the rainbow trout experiments, a one-way ANOVA using the ANOVA calculator from http://faculty.vassar.edu/lowry/anova1u.html was performed on each of the parameters analyzed to determine significance. Differences were considered significant at $p < 0.05$.

3.3.2 Results

Fertilization Success. Fertilization success was used as the endpoint to evaluate how EMF exposure affected overall larval development. Fertilization success was expressed as the percentage of eggs that underwent any embryonic growth (determined at 20 dpf) relative to the total eggs that were fertilized. The unexposed group, the early EMF-exposed group, and the late EMF-exposed groups had fertility successes of 59.8%, 65.4%, and 65.4%, respectively. The variation between the five replicates in each of the three treatment groups was not statistically significant ($p > 0.05$).

Developmental Progress to 20 dpf. To track developmental progress before hatching, half of the eggs in each replicate were removed at 20 dpf and scored for their developmental progress. The average developmental scores for the unexposed control, early exposed, and late exposed groups were 4.67 (0.43), 4.86 (0.28), and 4.34 (0.74), respectively, and were not statistically different ($p > 0.05$). The average percentage of embryos receiving a score of five was 75.64% (6.75) for the control group, 86.78% (6.3) for the early EMF-exposed group, and 44.7% (14.6) for the late EMF-exposed group. The early exposed group had a significantly higher percentage of embryos scoring a five compared to the control and late-exposed group ($p < 0.05$; Figure 3.2). The percentage of embryos scoring a five in the late EMF-exposed group was significantly lower than those of the control and early exposed group ($p < 0.05$; Figure 3.2). These data suggest that rate of development could be affected by EMF exposure at different periods of embryogenesis. Given that eggs were removed from EMF exposure at 20 dpf, it is uncertain if this pattern would have continued throughout the remainder of embryogenesis.

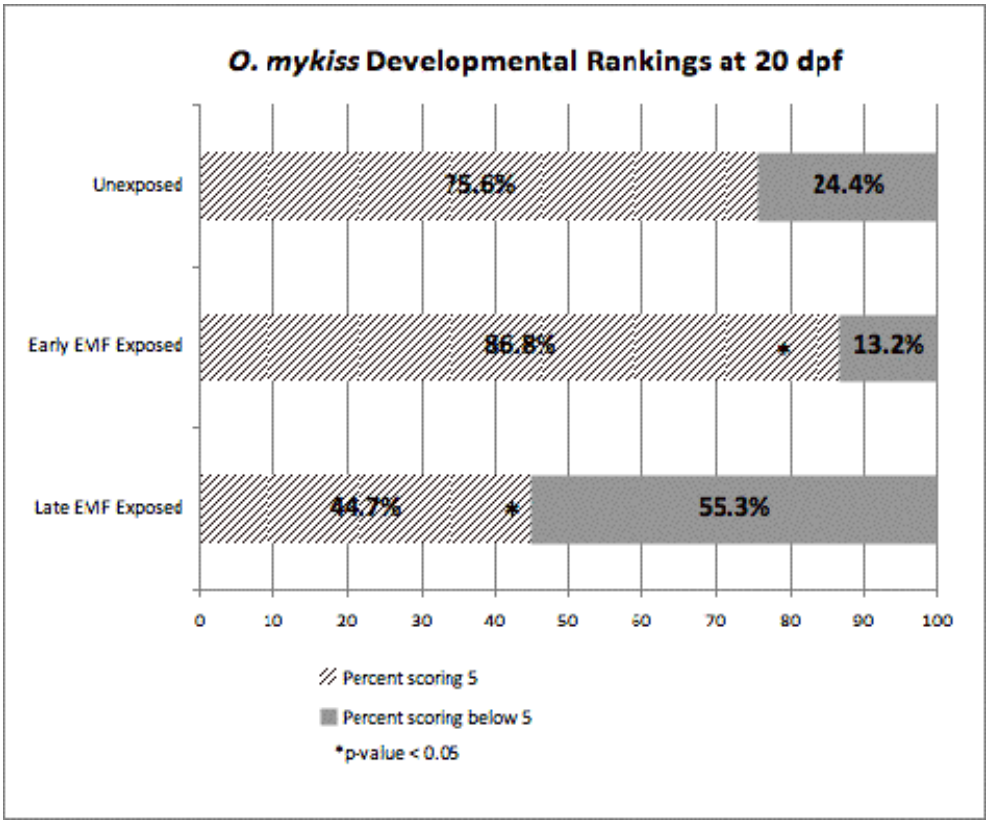


Figure 3.2. Rainbow Trout Developmental Rankings at 20 dpf

Developmental Progress After Hatching. Examination of trout development after hatching provided an indication of whether EMF exposure affected the ability of the fry to emerge from the egg. Any noticeable differences may have been the result of an increase in mutations within an experimental group or delays in embryogenesis. The hatching success of the fertilized eggs was 85.0% for the unexposed group, 79.6% for the early EMF-exposed group, and 91.7% for the late EMF-exposed group. There was no significant difference between the three groups when examining fry mutation rates ($p > 0.05$). The average developmental score of the fry for the unexposed group and the early and late EMF-exposed group were 4.01 (0.18), 4.22 (0.266), and 3.989 (0.219), respectively. The percentage of fry receiving a score of five was 35.5% (13.8) for the control group, 43.66% (18.28) for the early EMF-exposed group, and 36.9% (12.1) for the late EMF-exposed group. There were no significant differences among the groups ($p > 0.05$; Figure 3.3).

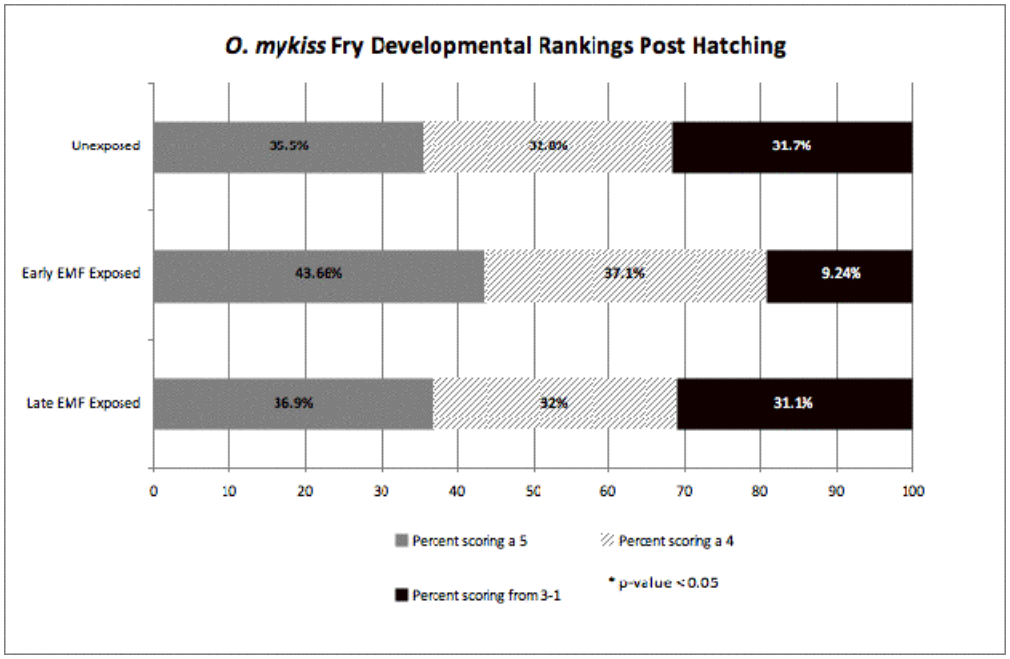


Figure 3.3. Post-Hatch Developmental Rankings for Rainbow Trout

Hatching Rates. Hatching rates across all experimental groups were also examined. The date, time, and number of total fry hatchings per day were recorded for all the treatments (Figure 3.4). The average dpf until hatching for each treatment was as follows: 30.7 dpf for the unexposed group, 30.9 dpf for the early EMF-exposed group, and 41 dpf for the late EMF-exposed group. These differences were not significant, suggesting that short-term EMF exposure did not overtly affect embryogenesis through hatching ($p > 0.05$; Figure 3.4).

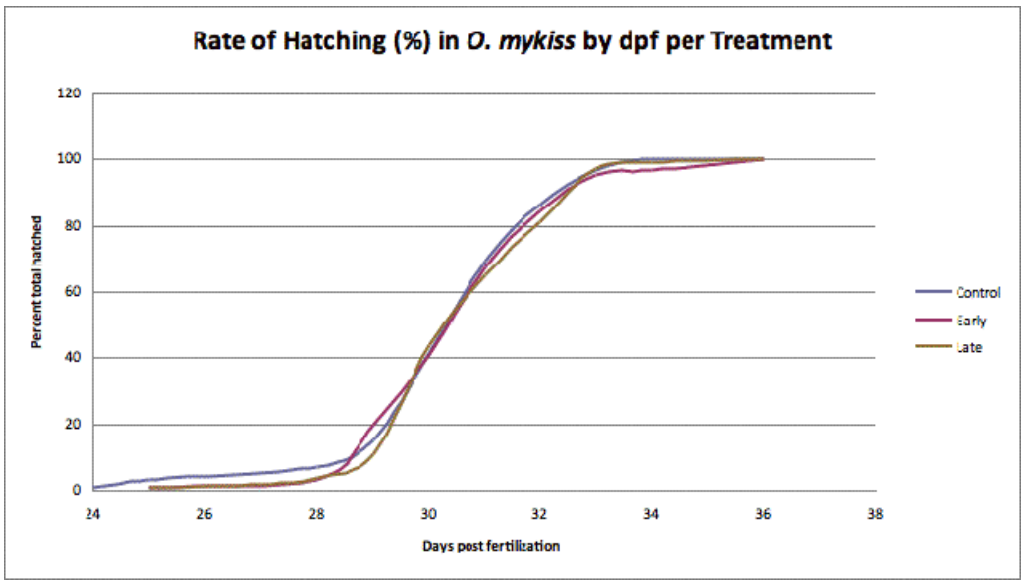


Figure 3.4. Hatching Rate for Rainbow Trout

3.4 Atlantic Halibut Development and Survival

Atlantic halibut are an important commercial and recreational resource in the North and Mid-Atlantic regions and are under consideration as a species of interest for net pen and fish farming in Nova Scotia. Larval halibut are found in the water column during early development; they become associated with benthic environments at the completion of eye migration. Because the developmental stages of this species have been well characterized (Gisbert et al. 2002; Saele et al. 2004), this species is a good model organism for use in EMF testing. Given their position in the water-column, both larval and adult halibut receive an exposure from MHK devices and cables. If significant developmental effects occur (e.g., delayed or incomplete eye migration or reduced growth), community and population-level effects could occur. To determine if EMF exposure can affect marine fish larval – juvenile transformations, larval Atlantic halibut were exposed to a constant 3-mT EMF field during two distinct time periods before metamorphosis to the juvenile stage. This transition is an important stage in halibut development because population recruitment is based on transitioning between larval forms with eyes on both sides of the body to juvenile forms with eyes on one side of the body (Saele et al. 2004).

Fish were exposed to a 3-mT EMF in two separate experiments: 1) for 32 days starting at 27 days post first feed (dpff) and 2) for 7 days starting at 59 dpff. In the first experiment, fish were harvested after the 32-day exposure and examined for the following parameters: fish size, developmental progress as indicated by pigmentation, eye migration stage, and overall subjective developmental state, and mortality rate. In the second experiment, the larvae were transferred to holding tanks after the 7-day EMF exposure and, then cultured (without further EMF exposure) until 90 dpff, at which point most of the control fish had appeared to have completed metamorphosis and were then assessed for the same metrics as in the first experiment.

3.4.1 Methods

Arrival, Care, and Holding. The Atlantic halibut larvae were cultivated and hatched offsite by Scotian Halibut Limited in Halifax, Nova Scotia, Canada. Approximately 1,000 Atlantic halibut larvae aged 22 days dpff, arrived with an approximate mortality rate of 40%. The fish were fed SELCO-enriched second instar brine shrimp twice a day for the duration of the study. Upon arrival, the halibut were initially held in large cylindrical 300-L tanks with constant filtered seawater flow-through. Tanks were greened with gray porcelain potter's clay following feedings to decrease fish stress. Dead fish and detritus were siphoned from the tanks three times a week, and mortality rates were documented.

Apparatus and Experimental Design. For experiment 1, 150–24 dpff larvae were placed in two experimental cubes sized 60 cm/side that were filled with 40–50 L of filtered seawater resupplied via a flow-through system. Temperatures were maintained between 9°C–10°C, and water flow rates ranged between 1–2 L/min. The flow rates were adjusted to alter temperature if the cube temperatures differed by more than 0.2°C from each other. One cube was situated directly in the center of the same Helmholtz coils used for the rainbow trout egg exposure, and one cube was set up as the early established control. Mortalities were counted and removed 2 days after transfer to the cubes. To replace the fish lost, approximately 80 fish were transferred to both the experimental and the control cubes. After 72 hr acclimatization (relative to the initial transfer of 150 larvae), the Helmholtz coil were turned on to produce a 3-mT EMF. At this time, we also established a second control cube (late control) containing 100 larvae at a similar age (27 dpff). After 32 days of EMF exposure, each fish was euthanized and analyzed for the following parameters: degree of eye migration, pigmentation patterning, myotome height, standard length (head to fork), and overall developmental stage.

In experiment 2, 22–24 larvae aged 59 dpff were placed in the EMF and control exposure cubes and exposed to a 3-mT EMF until 63 dpff. At this point, the larvae were transferred to fiberglass culturing tanks until 90 dpff, when they were similarly evaluated as in experiment 1.

Staging of Larvae. In examining developmental progress (staging) of the fish, numerical rankings were given for pigmentation, eye migration, and overall developmental stage. Observed eye migration rankings were scaled from zero to five. Zero was defined as having symmetrical eyes or no visible signs of migration. One was defined as partial migration where half of the migrating eye was visible when lying flat. Five was defined as fully migrated.

Statistical Analysis. Because of the lack of tank replication, confidence intervals were used to compare groups rather than ANOVA. Confidence intervals of 95% and 99% were applied to compare the distributions of individual data sets. Confidence intervals were determined using Microsoft Excel.

3.4.2 Results

Fish Size. Overall fish size was evaluated by measuring myotome height, standard length, and the ratio of the two measurements to determine if EMF affected larval size. There is a comparable relationship between age and size that can represent developmental progress (Saele et al. 2004).

In experiment 1, the average myotome height for the early-established control and the EMF-exposed treatments were 3.77 mm (±0.74) and 3.73 mm (±0.73), respectively. The average standard length for the early-established control and the EMF-exposed treatments were 17.5 mm (±1.4) and 16.7 mm (±1.4),

respectively. The ratio of myotome length to standard length for the early established control and the EMF-exposed treatments were 0.22 (±0.03) and 0.21 (±0.03), respectively. The differences between the control tank and EMF-exposed tank with respect to all observed parameters lie within a 99% confidence interval of one another and thus are not considered to be different. Larval developmental stages were also subjectively scored based on representative fish diagrams in Saele et al. (2004). Fish stages ranged from 1–9 based on cranial ossification, which corresponds highly with eye migration. Stage 9 larvae are defined as having fully completed eye migration. The developmental stage for the early-established control group was 7.26 (±0.88) and 7.19 (±0.74) for the EMF-exposed group (Figure 3.5). The differences observed between the control tank and the EMF-exposed tank lie within a 99% confidence interval of one another and are not considered different.

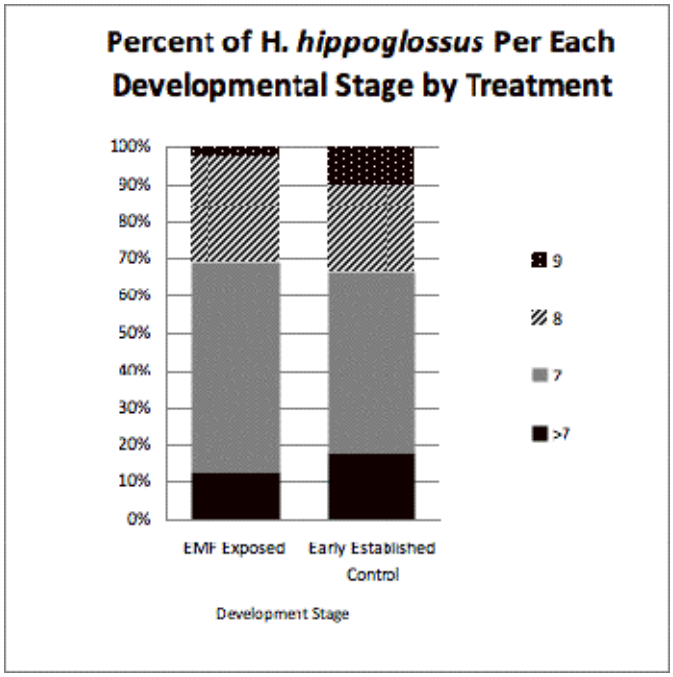


Figure 3.5. Percentage of Atlantic Halibut for Each Developmental Stage by Treatment

Observations on Early and Late Established Control Comparison in Experiment 1. The important difference between the early and late controls is the acclimation time in the exposure cubes prior to the start of the experiment. Two control cubes were established to provide some replication for assessing tank level variation in larvae growth and development. In order to draw conclusions from a comparison of the EMF-exposed fish with one or both of the control tanks, there must not be variation between the two controls. Different dpff of movement and starting population density must be considered when comparing the early- and late-established controls. The developmental pigmentation changes were constant across all but one of the 104 fish observed from all three experimental cubes. However, average eye migration stage, average myotome height, average standard length, and the percentage of fish in each developmental stage were different between the early- and late-established controls. The average myotome lengths for the early- and the late-established controls were 3.77 mm (±0.742) and 4.35 mm (±0.675), respectively. The overall fish lengths in the early- and late-established controls were 17.51 mm (±1.36) and 18.37 mm (±1.41), respectively. Due to variation between the controls, no comparison was made between the late-established control and the EMF-exposed tank.

Mortality Rate. The average mortality rate in each cube was calculated by counting and removing dead fish three times a week. Based on the slope of best fit for the population numbers per tank an average death rate per day was calculated to be 2.98 %/day for the early established control, 2.7 %/day for the late established control, and 3.03 %/day for the EMF-exposed tank, (Figure 3.6). At the time of termination only 21.5% of the early-established cube, 26 % of the late established control cube, and 23.1 % of the EMF-exposed cube, were still alive. For each treatment exposure a two parameter exponential decay model ($Y=(100-Plateau)*e^{-K*Days} + Plateau$) was fit to the percentage of the initial population remaining over the number of days elapsed ($R^2 > 0.95$ for both treatments). The two curves were significantly different (Regression, p= 0.01). The *Plateau* parameters were not significantly different (p = 0.52) and was estimated to be 7.68 (95% Confidence Interval 0 to 18.7). The mortality rate, however, was significantly greater (p = 0.003) in the control (K = 0.087; 95% Confidence Interval 0.065 to 0.110) than in the EMF exposed group (K = 0.070; 95% Confidence Interval 0.064 to 0.075).

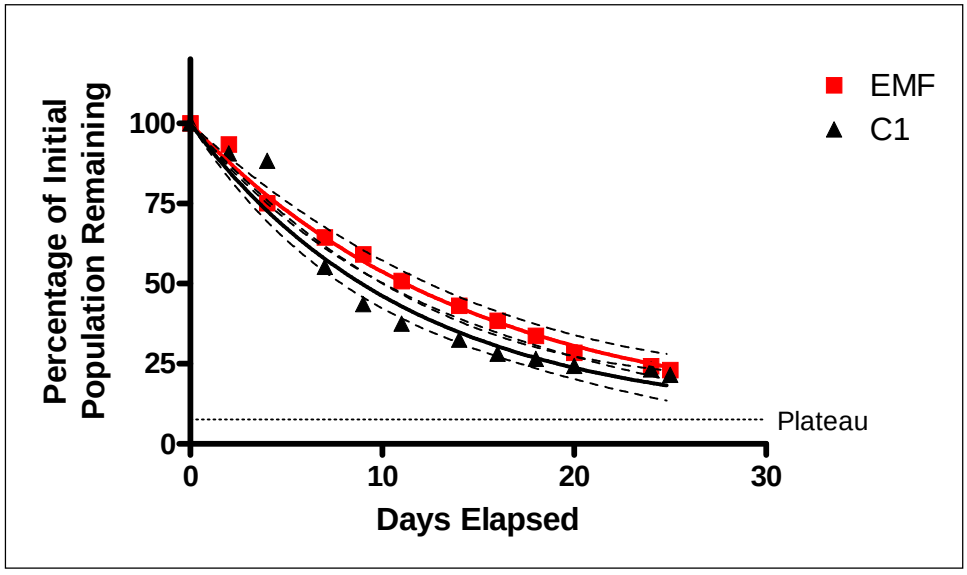


Figure 3.6. Mortality of Atlantic Halibut (*H.hippoglossus*) in Each Experimental Cube

In experiment (2), the average myotome height for the control and the EMF-exposed treatments were 7.12 mm (0.57) and 5.74mm (0.47), respectively. The average standard length for the early established control and the EMF-exposed treatments were 24.5mm (1.5) and 20.5mm (1.3), respectively. Subjective comparison of larval staging found that the majority of EMF exposed larvae were in larval stage 8 compared to the control fish where the majority had completed metamorphosis (Figure 3.7). The differences between the control tank and EMF-exposed tank with respect to these parameters did not lie within a 99% confidence interval and thus are considered to be different. In this experiment, overall mortality was low with greater than 90% survival of the control and EMF fish.

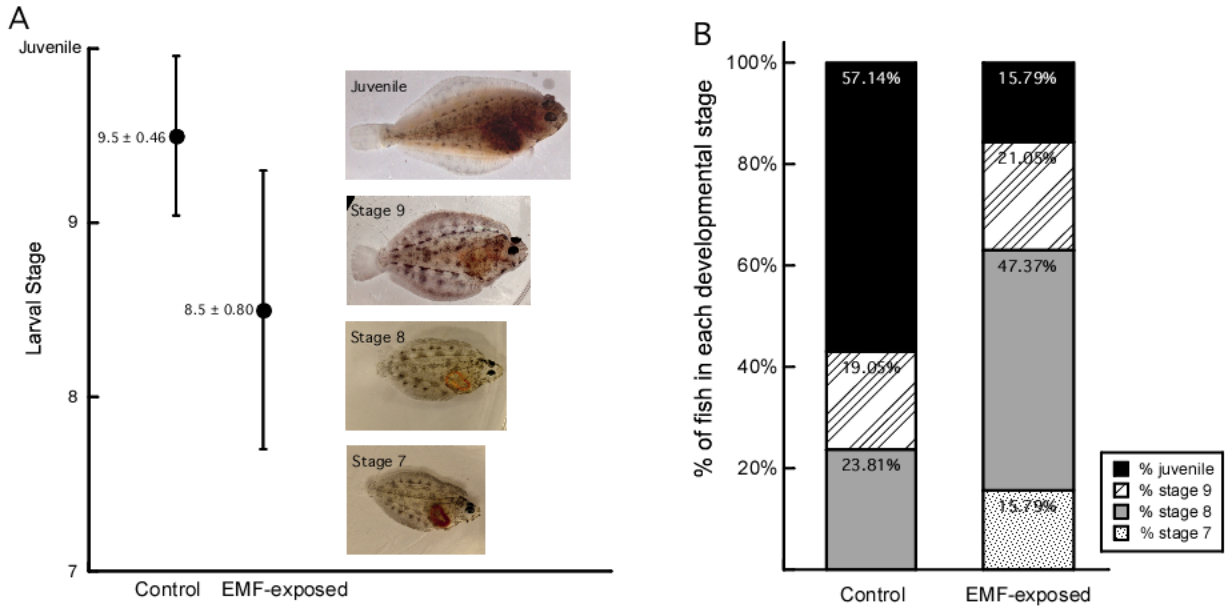


Figure 3.7. Staging of Halibut larvae at 90 dpff. Larvae received a 7-day EMF exposure between 59–63 dpff. A greater number of EMF exposed larvae have not completed metamorphosis as compared to control. Mean plus 99% confidence interval (n = 20 – 22).

3.5 California Halibut Growth and Development

California halibut were also used as a model flatfish to evaluate the effect of EMF exposure on the larval – juvenile transformation. California halibut were received from The Cultured Abalone, Ltd. (Santa Barbara, California) at the Marine Sciences Laboratory within 72 hr of hatching. Through preliminary culturing efforts, we developed a protocol that maximized fish survivability and permitted testing during the time period when metamorphosis is occurring. Using this new protocol, 48-hr post-hatch larvae were cultured to 32 days post-hatch, when most larvae were in the flexion – postflexion developmental stage and close to the initiation of eye migration. At this point, the larvae were used in experiments described below.

3.5.1 Methods

California halibut were contained in 2.5-gal aquaria nested in large water baths (Figure 2.3.). Larval staging was examined prior to the start of exposure, and larvae were distributed across treatment groups based on size and developmental stage; each group received equal numbers of each stage totaling 18–20 fish per tank. Two replicates of control non-exposed tanks and two replicates of EMF-exposed tanks were used. The EMF was set to a measured value of 3 mT (constant on). Fish were held in their respective treatment tanks until all fish in the control non-treated tank appeared to have undergone eye migration, which occurred 12 days after the exposure began. After the experiment ended, each fish was measured for standard length, eye migration stage, pigmentation pattern, and developmental stage.

3.5.2 Results

Survival of the larvae during the exposure was 63%–74% for the control group and 42%–47% for the EMF exposed groups. Indices of metamorphosis such as standard length and eye migration were similar between all treatment groups (Figure 3.8), as was the level of pigmentation in the larvae.

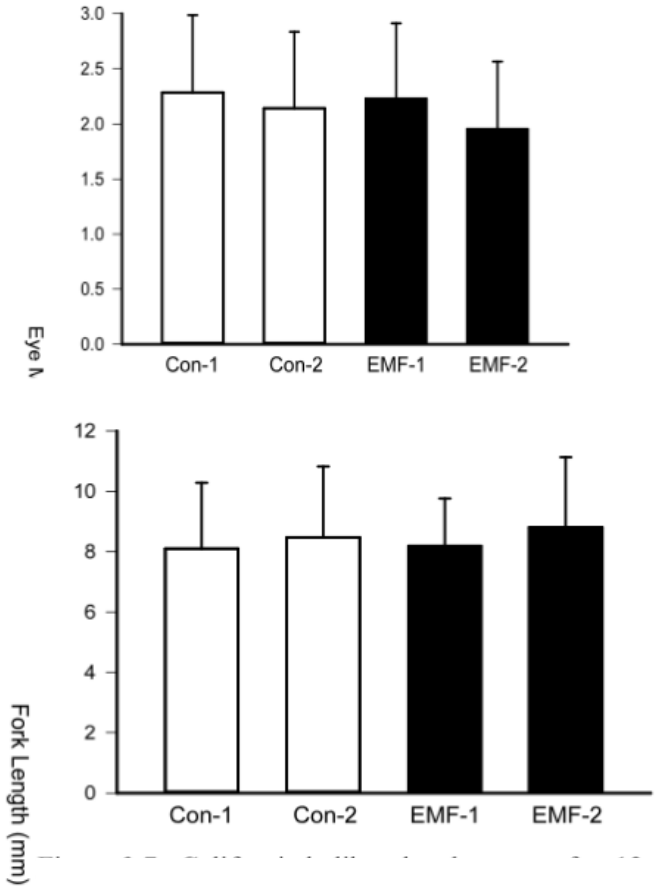


Figure 3.8. California Halibut Development After 12 Days at 3-mT Exposure (Top: Eye Migration; bottom: Fork Length. Values are mean ±SD (n = 114).)

3.5.3 Summary of FY 2010 and FY 2011 Fish Experiments

During FY 2010 and FY 2011, a variety of experiments were conducted with ecologically, commercially, and recreationally important fish species to determine if exposure to EMF from MHK devices and cables could result in adverse outcomes that could influence individuals, communities or populations. The results of these studies are presented in Table 3.1. Behavioral experiments with coho salmon were inconclusive, due primarily to non-treatment influences that masked a potential behavioral response to EMF. There was, however, no compelling evidence that exposure to EMF affected the ability of this species to detect predators. Exposure marker experiments showed no evidence of stress while exposed to EMF, but some evidence was seen of reduced melatonin levels after exposure to EMF that was not statistically significant. Exposure of fertilized trout eggs to 3-mT EMF for extended periods appeared to influence egg development but was also not statistically significant. Experiments with Atlantic halibut

suggested exposure to 3-mT EMF reduced both growth and development, but neither endpoint was statistically different from control; changes to growth and development of California halibut were not observed.

Although EMF exposures approaching 3 mT have appeared in peer-reviewed literature, this field strength is not expected to occur near devices or from cables associated with MHK devices and represents an upper bounding limit scenario. Given the lack of statistically significant behavior, growth, or exposure marker responses in the species tested in FY 2010 and FY 2011, there is no reason to believe that EMFs associated with MHK devices or cables will result in adverse impacts at individual, community, or population levels for the species evaluated in this study.

Table 3.1. Summary of EMF Experiments with Fish from FY 2010 and FY 2011

Experiment	Species Tested	Testing Endpoint	Result
Coho salmon alarm response	Coho salmon *Oncorhynchus kisutch*	Decreased swimming	Inconclusive results
Coho salmon exposure marker	Coho salmon *Oncorhynchus kisutch*	Melatonin/cortisol	3 mT and 0.13 mT (AC) ↓ melatonin levels
Trout egg development	Rainbow trout *Oncorhynchus mykiss*	Survival and development	EMF caused developmental delay at 20 dpf
Atlantic halibut effects	*Hippoglossus hippoglossus*	Growth	EMF ↓ during late exposures.
		Development	EMF ↓ during late exposures.
California halibut effects	*Paralichthys californicus*	Growth	No effect
		Development	No effect

3.6 FY 2012 Activities

FY 2012 activities will include exposure assessments of a representative elasmobranch (e.g. shark, skate or ray). We are currently assessing the options with respect to relevance, availability, ease of handling, and stage of life and organism size. In addition the Helmholtz coil will be reconfigured to accommodate the swimming nature and size of the specie selected. Past fish exposures will be used to guide the experimental design as well as available literature.

4.0 Invertebrate Experiments

4.1 Introduction

Dungeness crab (*Metacarcinus magister*) are an ecologically, commercially, and recreationally important resource in temperate coastal ecosystems ranging between Alaska and California. Because there is anecdotal evidence that some species of crustaceans may be sensitive to EMF, a series of experiments was conducted to 1) evaluate crab sensitivity to EMF, 2) assess the influence of EMF on the ability to detect food, and 3) evaluate whether the presence of EMF from electrical cables created avoidance or attraction behavior. Antennular flicking rate was used as an endpoint for EMF detection and food detection experiments; crab position and behavior (burying or visible on sediment surface) in relation to EMF was used to support avoidance/attraction exposures. Tests were conducted using the Helmholtz coil system described in Section 2; sensitivity and food detection experiments were conducted at approximately 3 mT between the coils; to support avoidance/attraction experiments, the coils were separated as configured to create a decaying field emanating from the center at approximately 1 mT of a rectangular enclosure. For all experiments, a combination of visual observations and video recordings was used to document endpoints of interest.

4.1.1 Testing Goals and Objectives

The testing goals for Dungeness crab for FY 2011 were focused on behavioral endpoints that could be measured in the laboratory and would provide a range of assessment criteria:

- *Phase 1 – Detection of EMF* – Determine the ability of Dungeness crab to detect a relatively high dose of EMF using their antennules.
- *Phase 2 – Detection of a food odor in the presence of EMF* – Determine the ability of Dungeness crab to detect the presence of a food odor (clam extract) after a moderate exposure (~20 hr) to EMF.
- *Phase 3 – Avoidance/attraction to EMF* – Develop a protocol and assess the behavioral response of crab when presented with choices of habitat with a spatially-decaying EMF field.

4.1.2 Test Organism and Holding Facilities

Locally trapped adult male Dungeness crabs (*M. carcinus*) were used for all 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. They were held for 1 to 3 weeks until tested, and crabs 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.

4.2 EMF Detection of Acute Exposure

The initial phase of experimentation with Dungeness crab was conducted in fall 2010 to determine in a general sense whether a crab's response to an acute increase in EMF exposure was overtly obvious, subtle, or nonexistent. The results of these experiments, using the antennular flicking rate and other behavioral movements as a proxy for detection, were then used to design follow-on tests.

4.2.1 Experimental Design

For the Phase 1 experiments—detection of EMF—the exposure system shown in Figure 2.7 was used. The test system contained four plastic chambers (30 x 20 x 20 cm.) fitted with an opaque plexiglass cover clamped to it. A funnel and inlet manifold delivered approximately 1 L/min of 35-µ filtered flow-through seawater from a dripper arm to the bottom of each chamber. A photoperiod synchronized to civil sunrise and sunset provided approximately 500 lux of daylight spectrum lighting.

Four crabs were moved from outdoor holding tanks to the testing system and allowed to acclimate overnight before testing. Seawater flow rates were adjusted, and partitions placed completely around the testing apparatus to reduce visual disturbance to the crab, prior to and during testing. Small openings in the partition allowed for camera placement and video recording as well as visual observation (Figure 2.8). Each day of testing occurred entirely within an incoming or outgoing tidal cycle during daylight hours.

For each test, the initial position and posture of each crab was noted (e.g., anterior or posterior placement, resting, standing, antennules active or retracted). To initiate testing, the video recorder was turned on to record four cameras simultaneously, capturing the crab's body posture, movement, and antennular flicking rate of one antennule (Figure 4.1). In addition, a trained observer recorded the flicking rate of one crab for the duration of the test as a quality assurance measure. The crab's behavior was recorded for 5 min (i.e., EMF off), the Helmholtz coil was then turned on, generating 3 mT EMF, and the recording continued for an additional 5 min (i.e., EMF on).

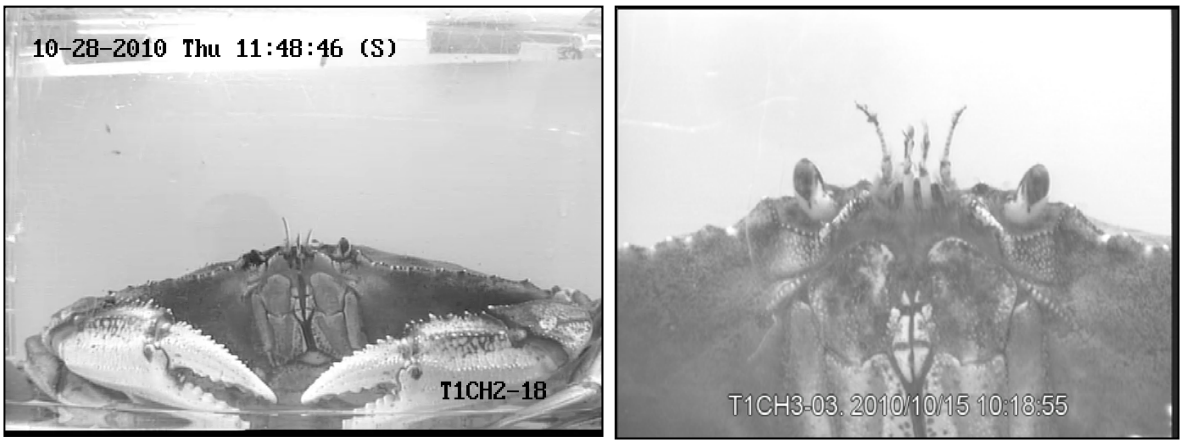


Figure 4.1. Video Images of a Dungeness Crab in the EMF Exposure Chamber in a Resting Position (left) and a Close-Up of the Antennules Centered Between the Antenna and Eyestalk (right)

The video data for each crab was post-processed, and antennular flicking rate per minute was measured for the 5 min prior to and 5 min during the EMF exposure. A one-sample *t*-test was used to test the null hypothesis that the difference between the average pre- and average post-exposure antennular flicking rate was equal to zero. Additional behavioral observations were also evaluated to assess the response to an acute exposure to EMF.

4.2.2 Results

Observations of 34 crabs were evaluated by post-processing the video recordings prior to and during an acute EMF exposure. Five 1-min intervals were evaluated before EMF exposure (i.e., minute 1 through 5) and five 1-min intervals during exposure to EMF (i.e., minute 6 through 10). Eleven crabs were removed from this analysis because some minute intervals were missing (e.g., antennules retracted, not visible to camera).

The range of pre-EMF exposure flicking rate averaged between 5.6 to 40 flicks/min, which was consistent with the flicking rate of a resting Dungeness crab found in Pearson et al. (1979; 5 to 47 flicks/min). The mean pre-exposure flicking rate was 17 ± 3.6 flicks/min. The average flicking rate during EMF exposure was 15.2 ± 2.7 flicks/min. Figure 4.2 shows the mean antennular flicking rate per minute pre-exposure (EMF off) and during exposure (EMF off). Although a slight decrease was noted between the pre-exposure flicking rate of 17 and the during-exposure rate of 15.2/min, a non-parametric comparison of the difference between pre- and during-exposure rate was not statistically significant (one-sample *t*-test; $n = 23$; $p = 0.21$).

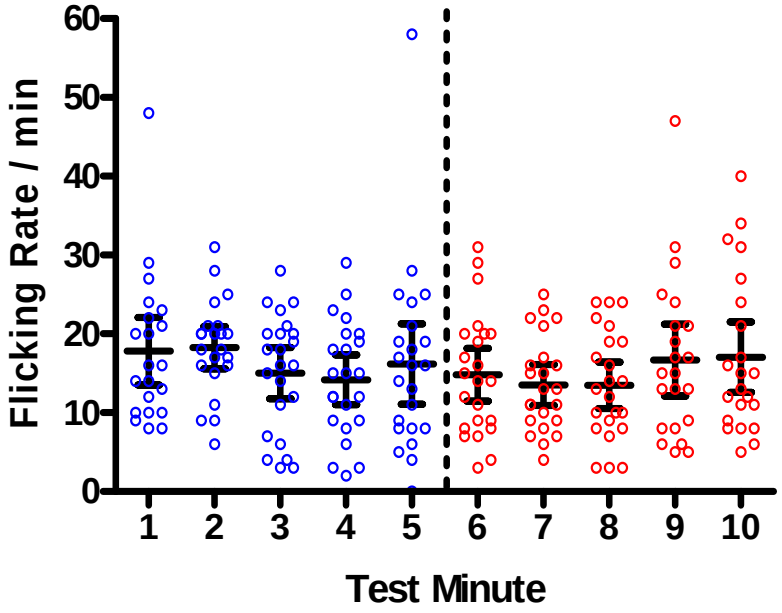


Figure 4.2. Mean ± 95% CI of Dungeness Crab Antennular Flicking Rate ($n = 23$) for 5 min Prior to EMF Exposure and 5 min During 3-mT EMF Exposure

Video recordings were evaluated to determine if other behavior changes occurred between the pre- and acute EMF exposure trials. These included the posture in the test chamber prior to testing (i.e., resting, sleeping with eyestalks retracted in sockets, standing, and climbing), the posture during EMF exposure, and other movements associated with the antennae, eyestalks, mouthparts, dactyls, and chelae. Although these overt behaviors and movements occurred on occasion, there was no obvious or explicit pattern of change in behavior between the pre- and post-EMF exposure.

4.3 Food Detection During EMF Exposure

Phase 2 of experimentation evaluated the ability of Dungeness crab to recognize a food odor source after an approximate 20-hr exposure to a 3-mT EMF source. Crabs were exposed to filtered seawater (SW) as a stimulant and/or a food extract (FD) derived from clams. These experiments were conducted as two separate trials:

- ***Trial 1*** – 20-hr exposure to 3-mT EMF or control (no exposure), then measure antennular flicking response after presentation of a filtered SW extract (control) or 10^{-6} g/L FD extract (low-dose treatment).
- ***Trial 2*** – 20-hr exposure to 3-mT EMF or control (no exposure), then measure antennular flicking response of each crab to a SW extract (control) followed by a 10^{-1} g/L FD extract (high-dose treatment).

4.3.1 Experimental Design

The exposure test system used for Phase 2 experimentation was the same as that used for Phase 1 (Section 4.2.1, Figure 2.7 (left), Figure 2.8) with the exception of the addition of a buret delivery system for adding a SW and/or FD extract (Figure 2.7 right). The day before testing, four naïve crabs were transferred to the testing chambers, the seawater flow rate adjusted, and blinds secured. The Helmholtz coils were either turned on to deliver an approximate 20-hr, 3-mT EMF exposure or left off, providing a background EMF exposure (control).

For Trial 1, crabs were randomly assigned to receive either 20 mL of filtered SW as a control or 10^{-6} g/l food (clam) extract filtrate (FD). The testing order was assigned randomly to each crab, however, active or sleeping crab were passed over initially. For each test, a crab was individually video recorded for 5 min. A randomly assigned SW or FD extract was then introduced into the chamber from a buret and tubing connected to the funnel and inlet manifold (Figure 2.7). Video recording continued for an additional 5 min for a total of 10 min of video recording per crab. The video data for each crab was post-processed for antennular flicking rate and other behavioral responses. Data were evaluated statistically to examine the SW and 10^{-6} g/L FD treatment responses to background and EMF exposures. Ninety-five percent confidence intervals were calculated as the mean $\pm$ $t_{(\alpha,n-1)}$·(standard error of the mean) to describe selected time periods. A non-parametric Kruskal–Wallis test was used to compare treatment combinations (odor stimulant and EMF exposure) for given time periods. A general linear model (GLM) was used to compare the main effects of the odor stimulant and the EMF exposure when the interaction of these effects was not significant.

For Trial 2, crabs were exposed to 20 hr of 3-mT EMF or background exposure as in Trial 1. For response testing, each crab received 20 mL of SW extract followed by 20 mL of a high dose of FD extract (10^{-1}) approximately 40 min later. Video was recorded 5 min prior to the SW treatment, 5 min after SW treatment, 5 min before FD extract treatment, and 5 min after FD treatment, for a total of 20 min of video observations per crab. Ninety-five percent confidence intervals were calculated as in Trial 1. A non-parametric Kruskal–Wallis test was used to compare treatment combinations (odor stimulant and EMF exposure) for given time periods. A GLM was used to compare the main effects of the odor stimulant and the EMF exposure when the interaction of these effects was not significant.

Preparation of Treatment Stimulus Extracts. The FD extract used as an odor stimulant for the crabs was prepared from native littleneck clams (*Protothaca staminea*). Clams were held in outdoor holding tanks long enough to purge sediment. The shucked clam meat and liquid was freeze-dried, powdered, and stored at −80°C. For testing, a stock solution was prepared by mixing a weighed portion of the FD powder with 0.45-μ filtered SW. This solution was mixed for ~2 hr, then filtered through pre-tared glass fiber pre-filters and Whatman GF/C filters. The final FD stock concentration was corrected for loss of material retained on the filters. A stock solution was used for up to 5 days to create the daily stimulus extract used for testing. For each day of testing, the FD stimulus solution was made shortly before testing using fresh-filtered SW from the experimental test source. An aliquot of the test source water was used as the SW control stimulant. Both stimulants were kept in a water bath at ambient temperature until use, when 20 mL was delivered through burets calibrated to deliver at a specified rate. The effective concentration of FD stimulus delivered to the test chambers was 10^{-6} g/L for Trial 1(low dose) and 10^{-1} g/L for Trial 2 (high dose).

4.3.2 Results

Trial 1 – Low-Dose Stimulant Response. During Trial 1, 44 crabs were tested and evaluated through video post-processing of antennular flicking rate, for EMF-exposed or EMF-background crabs that received either a SW or low-dose FD extract stimulant. Five 1-min intervals of each crab were evaluated pre-SW or FD stimulant introduction, and the 1-min interval was evaluated after stimulant introduction. Twenty-one crabs were removed from analysis because some EMF exposure (i.e., minute 1 through 5) and five 1-min intervals were missing (e.g., antennules retracted, not visible to camera).

The range of pre-odor stimulus flicking rates was similar between exposure groups and similar to Phase 1 background flicking responses (Figure 4.3a); EMF off (SW) 15.6 ±9.7 flicks/min, EMF on (SW) 16.0 ±6.7, EMF off (low-dose FD) 16.8 ±4.9, EMF on (low-dose FD) 20.8 ±10.6. There was no statistically significant difference between medians from any of these pre-stimulus treatments (Kruskal–Wallis, $p = 0.46$). The 1-min post-stimulus flicking rates were similar between EMF off-SW (29.6 ± 12.8) and EMF on-SW (28.0 ± 15.5), and between EMF off-FD (49.0 ± 12.4) and EMF on-FD (46 ± 22.8). There was a statistically significant difference in the median 1-min post-stimulus flicking rates (Kruskal–Wallis, $p = 0.034$). The significance was associated with the significantly greater flicking rates from crabs presented with FD compared to those receiving SW (GLM; error d.f. = 14; $p = 0.005$) and not between those exposed to 3-mT EMF or receiving background EMF (GLM; error d.f. = 14; $p = 0.68$).

As before, video recordings were evaluated to determine if other behavioral changes occurred between EMF exposures and food extract treatments, particularly those that are indicative of feeding behaviors (i.e., probing motions of dactyl and chelae, movement of chelae to mouthparts). Although these behaviors have not been analyzed statistically yet, there was strong anecdotal evidence to suggest an increase in the feeding response by crabs after receiving the food extract. This was evident for both EMF-exposed and background EMF-exposed crabs.

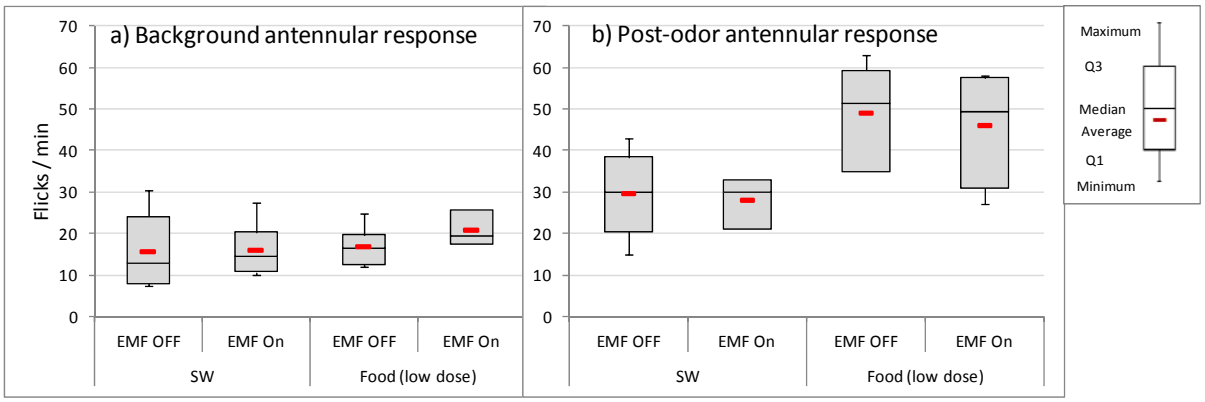


Figure 4.3. Phase 2 – Trial 1 Results of Antennular Flicking Rate a) Averaged 5 min Prior to Odor Stimulant Introduction of Those Exposed to Background EMF (EMF-off) or 3-mT (EMF-on), and b) 1 min After SW or Low Dose FD Stimulant Introduction

Trial 2 – High-Dose Stimulant Response. During Trial 2, the antennular flicking rate of 27 crabs was evaluated; however, a higher dose of FD (10^{-1} g/L) was used. In addition, each crab was tested using SW stimulant initially, then FD stimulant a short time later. Five 1-min intervals of each crab were evaluated pre-SW or FD stimulant introduction and five 1-min intervals evaluated after stimulant introduction. One crab was removed from analysis because some EMF exposure (i.e., min 1 through 5) and five 1-min intervals were missing (e.g., antennules retracted, not visible to camera).

The background antennular response flicking rates were similar to those in Phase 1 and Phase 2 – Trial 1 (Figure 4.4a); EMF-off (SW) 18.5 ± 7.5, EMF-on (SW) 14.9 ± 5.2, EMF-off (high-dose FD) 14.7 ± 7.1 and EMF-on (high-dose FD) 11.9 ± 3.9. There was no statistically significant difference between the background flicking rates for SW and FD (Kruskal–Wallis, p = 0.38, p = 0.45 respectively). The 1-min post-stimulus flicking rates were higher than background flicking rates (Figure 4.4b); EMF-off (SW) 29.7 ± 9.6, EMF-on (SW) 21.1 ± 7.0, EMF-off (high-dose FD) 39.0 ± 18.6, EMF-on (high-dose FD) 32.9 ± 13.2). Although the 1-min post-stimulus flicking rates were not significantly different between the post-SW response for EMF on and off (Kruskal–Wallis, p = 0.14) or the post-FD response for EMF on and off (Kruskal-Wallis, p = 0.30), there was a trend toward lower flicking rates of the EMF-exposed crabs for both stimuli (GLM; error d.f. = 41, p = 0.19). There was a nearly significant difference in the mean flicking rate between the 1-min post-SW and post-FD crabs (GLM; error d.f. = 41; p = 0.06), regardless of whether the EMF was on or not.

Other feeding response behaviors were noted in most of the crabs that received the high dose of the FD such as moving from a resting position to standing and dactyl probing. These responses occurred more often with the crabs receiving the higher dose (10^{-1} g/L) of FD than the lower dose (10^{-6} g/l) of FD. Crabs that received the higher dose as part of Trial 2 exhibited more active behavior in general than those receiving the lower dose as part of Trial 1. Because of the increased activity level in Trial 2, the antennules were out of view of the camera more frequently. Hence, some of the high flicking rate counts could not be included in the analysis, which partially explains why the mean flicking rates for the higher dose in Trial 2 were slightly lower than those in Trial 1. Further analysis of the data is warranted to understand this component.

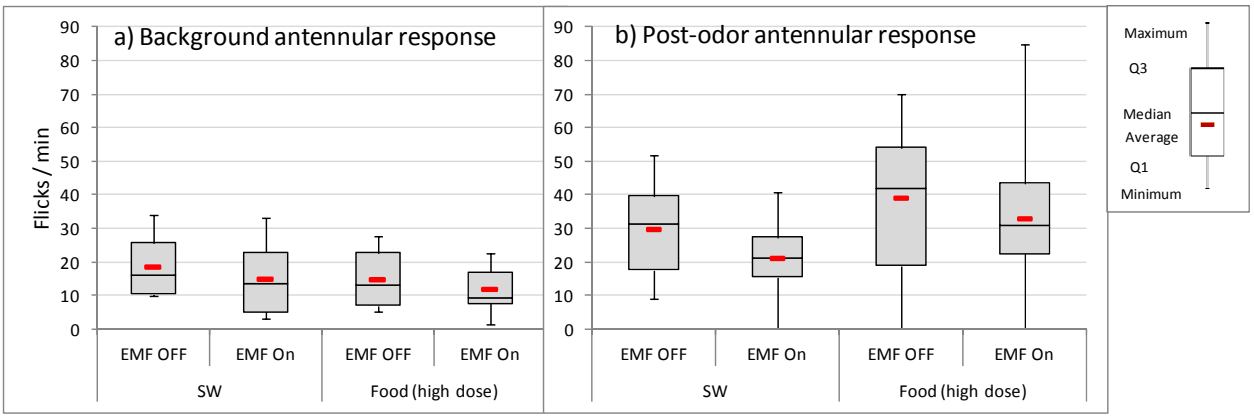


Figure 4.4. Phase 2 – Trial 3 Results of Antennular Flicking Rate a) Averaged 5 min Prior to Odor Stimulant Introduction of Those Exposed to Background EMF (EMF off) or 3 mT (EMF on), and b) 1 min After SW or High-Dose FD Stimulant Introduction

4.4 Avoidance/Attraction to EMF

4.4.1 Experimental Design

To test whether the crabs' choice of habitat or behavior was modified by the EMF, the Helmholtz coil was reconfigured using larger tanks to allow crabs freedom of movement in a spatially varying EMF. Two 1700-L tanks (3.3 × 0.75 × 0.69 m) were used as a control, and the EMF exposure system with the Helmholtz coil was split between the two (Sections 2.1, 2.2.2, Figure 2.8). The Helmholtz coil centered over the exposure tank was energized to provide an average of 1.1mT EMF in the center, decaying approximately an order of magnitude toward each end of the tank (Figure 2.3). Filtered SW [13.9 (±0.5)°C, 30.7 (±0.8) psu, 6.4 (±0.2) mg l^{-1}dissolved oxygen, pH 7.4 (±0.2)] entered the tanks at ~24 L/min. Subdued daylight spectrum lighting (16 ± 3.5 lux) provided illumination on a photoperiod synchronized to civil sunrise and sunset. Clean sand (15-cm depth) in each tank provided adequate depth for crabs to bury at will. Baffles at either end of the tank allowed for a uniform longitudinal flow from one end to the other. The effective surface area of available crab habitat was 2.45 × 0.75 m. Video cameras recorded the crabs' behavior for the duration of the experiment, including during nighttime with infrared lighting supplied.

Prior to the experiment, 10 healthy adult male Dungeness crabs, tagged with unique identifiers on their carapace, were placed in the control and exposure tanks (five per tank). They were partitioned individually at the downstream end of each tank with a PVC enclosure and allowed to acclimate for 30 min with the Helmholtz coil turned on in the exposure tank. At the initiation of the experiment, the structures were removed and observations were video recorded for 72 hr. Live observations were made every 30 min during the day, noting the location and behavior of each crab.

Video Processing and Statistical Analysis. The video data was analyzed statistically using five zone locations delineated in the exposure tank based on the EMF dose. Figure 4.5 shows the zone locations, surface area in each zone, and EMF dose. They include - Zone 1 – low dose (downstream), Zone 2 – mid-dose (downstream), Zone 3 – high dose (center), Zone 4 – mid-dose (upstream), and Zone 5 – low dose (upstream). The amount of surface area in each zone was determined by the measured EMF; hence,

the surface area varied slightly in each zone. For comparison purposes, the zone delineations were replicated in the control tank with background EMF shown in the lower half of Figure 4.5. The behavior of each crab was recorded every 15 min and noted as either buried, resting on the surface of the sediment, (Figure 4.6), or a variety of active behaviors including walking and digging.

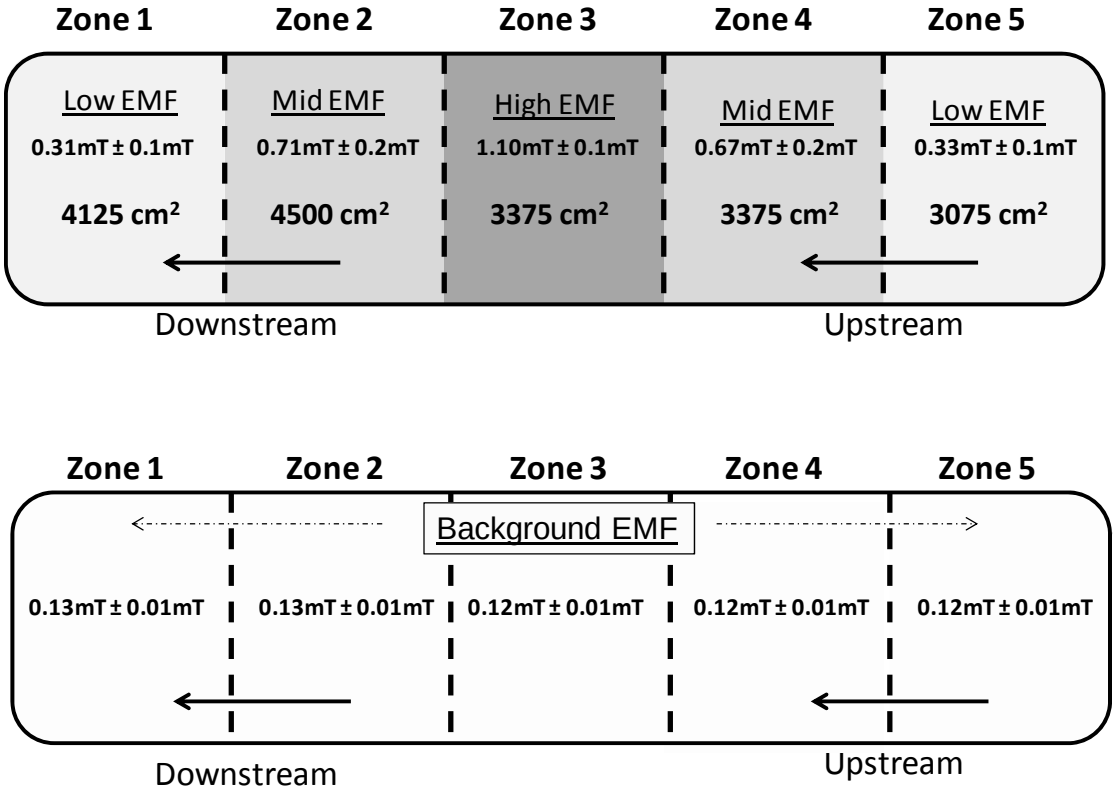


Figure 4.5. Schematic of EMF Zone Delineations and Area in Experimental Tanks for Avoidance Attraction (top, exposure tank; bottom, control tank)

Figure 4.6. Examples of Typical Burial Behavior of Crab in Sediment

The number of observations for which crabs were active or resting were analyzed using a 2 × 3 Chi-square analysis. Successive 15-min intervals were not considered independent if the crab was buried.

However, the length of time a crab remained buried during testing was independent and was calculated for each zone and exposure treatment. The number of times a crab changed behavior (active, resting, or buried) was also calculated. The expected number of minutes a crab would remain buried in any zone was calculated based on the proportional area of that zone multiplied by the total number of minutes crabs were observed buried in each exposure treatment. For each zone, a nonparametric Kruskal–Wallis test was used to compare EMF exposure treatments. Variables included the number of 15-min intervals crabs remained buried and the difference between the observed and expected number of minutes they remained buried.

4.4.2 Results

During the 3-day experiment, 2890 15-min observations were made 10 crabs' locations and activities. Six observations were made while the crabs were moving between EMF zones and were removed from further analysis. Initial analysis of the data focused on two behavioral endpoints (the zone location of the crab in relation to the electromagnetic field (low, moderate, high) and whether or not they were buried or active). Table 4.1 shows the number of crab observations for the duration of the experiment based on zone location and activity level. The proportion of observations of the active and resting crabs in each of the zones was not significantly different between the control and EMF-exposed crabs (Chi-square, $p = 0.32$).

Table 4.1. Number of Observations of Crab Behavior and EMF Zone Location Based on 15-min Intervals for 72 hr

		EMF Zone			
Treatment	Behavior	Low	Moderate	High	Total
Control (5 crabs)	Buried	459	609	44	1112
	Active or Resting	234	78	19	331
	Total	**693**	**687**	**63**	**1443**
EMF exposed (5 crabs)	Buried	141	653	118	912
	Active or Resting	394	102	33	529
	Total	**535**	**755**	**151**	**1441**
Total Observations		**1228**	**1442**	**214**	**2884**

The average number of minutes crabs were buried in each tank was examined in each zone. In the low EMF zone, the control crabs remained buried significantly longer than the EMF exposed crabs (Kruskal–Wallis, $p = 0.016$). The observed EMF number of minutes were significantly different than the expected number of minutes remaining buried in the low zone (Kruskal–Wallis, $p = 0.05$) (Figure 4.7). There was no significant difference in the total time buried or the difference in the observed and expected number of minutes crabs were buried in the moderate or high EMF zones (Kruskal–Wallis, $p > 0.12$); (Figure 4.7). For the high EMF zone, the observed number of minutes was not significantly different from the expected number of minutes remaining buried because of the high variability (Kruskal–Wallis, $p = 0.12$) (Figure 4.7).

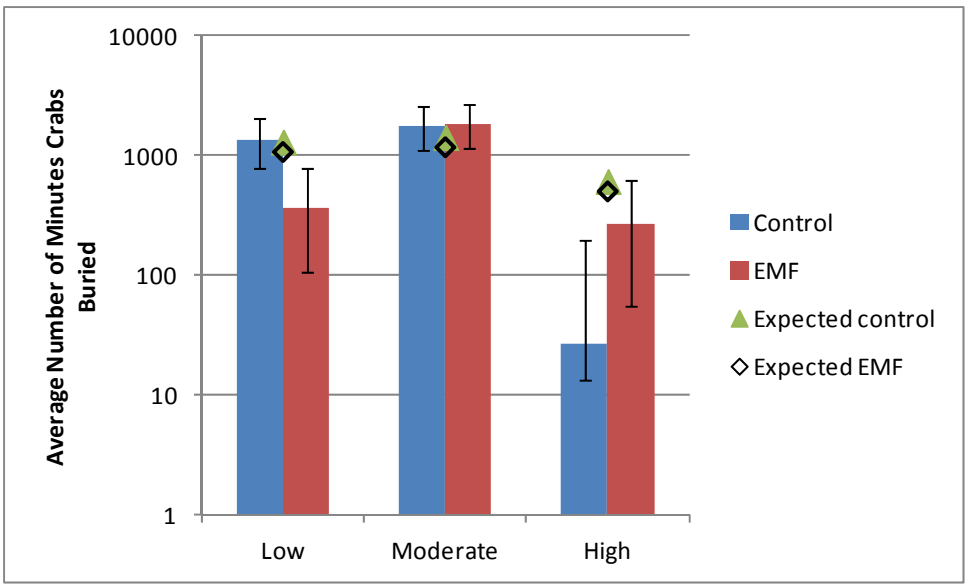


Figure 4.7. The Average Number of Minutes Crabs were Buried in Sediment in the Control and EMF-Exposed Tanks (±95% confidence interval) (n = 5 per tank). The expected number of minutes based on the proportional area of each zone for the control and EMF-exposed tanks is also shown.

In addition to the total time buried, we examined behavioral responses as measured by the number of times specific behaviors changed between buried, resting, and active (e.g., standing, walking, climbing). The number of times crabs changed behavior during the 3-day experiment was greater in the EMF-exposed tank compared to the control tank and nearly statistically significant (Kruskal–Wallis, $p = 0.076$). When the experiment was partitioned into 24-hr periods and the periods evaluated separately, both Day 1 and Day 2 had significantly more changes occurring in the EMF-exposed tank (Kruskal–Wallis, $p < 0.05$) (Figure 4.8). By Day 3, the number of behavioral changes was not significantly different between the control tanks and the EMF-exposure tank (Kruskal–Wallis, $p = 0.35$), although the change in activities by the EMF-exposed crabs was still greater.

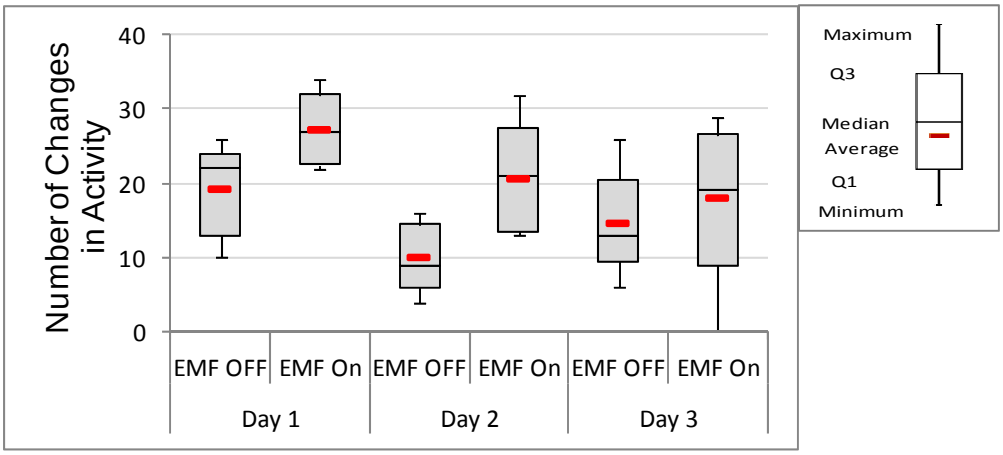


Figure 4.8. The Number of Changes Between Behavioral Responses (buried, resting, active) for the Control (EMF off) and Treatment (EMF on) Tanks On. Responses were evaluated on a 24-hr basis for the 3-day experiment.

Although the differences in the mean activity changes between control and EMF-exposed crabs became less significant by the third day of experimentation, the variability in crab behavior increased significantly during the 3 days in the EMF-exposed tank but not in the control tank (Figure 4.9) (Regression, $p = 0.04$ and $p = 0.22$, respectively). The slopes associated with the percent coefficient of variation of the number of changes in behavior were significantly different between the exposure treatments (Regression, $p = 0.05$).

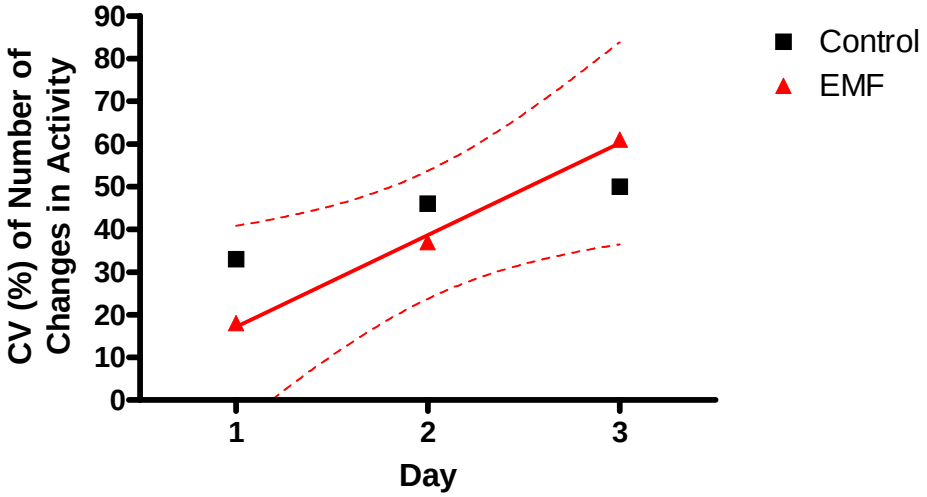


Figure 4.9. Percent Coefficient of Variation (CV %) in Number of Activity Changes Through Time on a Daily Basis

4.5 Summary of FY 2011 Invertebrate Experiments

During FY11 EMF experiments focused on Dungeness crab as an important and representative bottom-dwelling decapod crustacean that could be exposed to MHK devices, particularly transmission cables. The results of these experiments are summarized in Table 4.2 Initial tests measured the crabs' ability to sense EMF fields using the antennular flicking rate as a means of detection. While the antennular ficking rate decreased slightly, it was not significantly different than before EMF exposure. Likewise, the flicking rate response to a food odor decreased slightly after exposure to EMF; however the difference was not statistically significant. A preliminary avoidance/attraction experiment shows some evidence of subtle changes in behavior (e.g. amount of time buried, number of changes and variability in activity through time); however no major behavioral modifications were observed. Additional results from the avoidance/attraction will be analyzed at the beginning of FY12 to assess the influence of tidal stage and diel cycles on response activity. Avoidance/attraction experiments with another crustacean, the American lobster, this coming year will provide additional information on marine crustacean responses to relatively high EMF conditions.

Table 4.2. Summary of EMF Experiments with Crustaceans during FY 2011

Experiment	Species Tested	Testing Endpoint	Result
EMF detection	Dungeness crab *Metacarcinus magister*	Antennular flicking response	No significant detection of EMF observed
Food detection during EMF exposure	Dungeness crab *Metacarcinus magister*	Antennular flicking response	Ability to detect food after 20 hr, 3-mT EMF similar but slightly ↓ in EMF-exposed crab compared to control
		Feeding behaviors	Similar between control and EMF-exposed crab
EMF avoidance/attraction	Dungeness crab *Metacarcinus magister*	Location observed active and resting	No significant difference between control and EMF-exposed
		Time buried in sand	Controls > EMF-exposed for first 48 hr
		Number of activity changes through time	EMF-exposed > control crabs for first 48 hr
		Variability in number of activity changes	EMF-exposed crabs activity variability↑through time

4.6 FY 2012 Activities

During the first part of FY 2012, the Dungeness crab avoidance/attraction experiments and analysis will be completed. We will then conduct mesocosm avoidance/attraction experiments using juvenile stages of American lobster (*Homarus americanus*). A modification of the Helmholtz single-coil configuration will be used (Section 2.2.2) for this work. The experimental design will be based on results from the Dungeness crab exposures and prior knowledge of lobster habitat and behavior.

5.0 Discussion of Potential EMF Effects and FY 2012 Activities

A review of published studies that assessed the potential for adverse effects related to EMF exposure, made it apparent that much of the available information was either not directly related to marine and estuarine settings, or provided inconclusive or contradictory findings (Schultz et al., 2010). In some instances, experimental designs were not robust, and laboratory equipment limitations resulted in EMF exposures that were estimated or inferred. Since published EMF literature often contained uncertainties related to the actual EMF exposure or dose used during experiments, key investments were made at the beginning of this project to acquire a Helmholtz coil system that was robust and could produce a steady, uniform and measurable EMF signal to support laboratory fish and invertebrate testing. As described in Section 2, this system enabled the project team to work with uniform magnetic fields ranging from 0.1 to 3 mT, thus allowing for range-finding experiments that included exposures much higher than might occur in a realistic field setting, but were found in some of the published literature. It essentially provided an upper bounding limit estimate of effects.

To better understand the potential effects of MHK in marine, estuarine, and freshwater environments, the project team chose test species that were considered 1) reasonable surrogates for threatened and endangered species, or those with ecological, commercial or recreational importance, 2) likely to encounter MHK devices or power cables during part or all of their life-cycle, and 3) an integral component of the complex food webs present in coastal environments. This approach enabled us to select reasonable environmental surrogates, and develop tests that reflected likely exposure regimes. As described in Sections 3 and 4, selected fish and invertebrate species meeting these criteria were chosen for environmental effects testing during FY10-FY11.

Based on the available literature, acute effects including death from EMF exposure were not expected to occur, therefore testing focused on sub-lethal endpoints; organism growth and development (e.g. egg development, larval to juvenile metamorphosis and parr to smolt transformation), physiological changes (e.g. stress hormones), and behavioral responses (e.g. detection of EMF, interference with prey detection, avoidance or attraction to EMF). For developmental and physiological tests, species were purposefully acquired from aquaculture or related facilities where organism age, condition and past exposure were known, in order to reduce potential confounding test factors. For behavioral testing of crustaceans, experimental organisms acquired from the field shortly before testing were selected as most representative of a potential native populations' behavioral response to an EMF exposure. These combined endpoints provide a broad picture of potential EMF effects from ocean energy devices that could affect important aquatic species, and provide a body of knowledge that can be used to inform regulatory and stakeholder concerns regarding MHK pilot- or full-scale deployment projects.

EMF experiments with fish included species common to marine, estuarine, and freshwater environments. Juvenile salmonids were tested using a behavioral endpoint to determine if EMF exposure would decrease their ability to successfully evade predators, under the scenario that juvenile fish inhabiting nearshore locations could encounter MHK transmission cables. Tests were patterned after work by NOAA fisheries and others (Stone et al. 1994; Brown and Smith 1997; Scholz et al. 2000; Tierney et al. 2006) that assessed the effect of trace metals in stormwater on the predator response ability. Although the salmonid alarm response endpoint was inconclusive, concurrent exposure marker experiments showed no evidence of stress as measured by levels of cortisol in juvenile salmon. Decreases in melatonin levels, involved in smoltification of salmonids (Gern et al. 1984) were observed, however were not statistically significant.

Developmental experiments using rainbow trout addressed the potential exposure of fertilized eggs to transmission cables in riverine settings. There was no apparent affect on fertilization success rate. Exposure of fertilized trout eggs to EMF for extended periods appeared to influence egg development rate, however it was not statistically significant. This test organism is also being used by researchers at Oak Ridge National Laboratory for EMF exposures using different end points and test mechanisms.

Halibut were chosen as model surrogate flatfish that could be exposed to EMF directly from MHK devices during their pelagic (open water) lifestage, as well as exposed to transmission cables after metamorphosis and settlement on the bottom. Flatfish metamorphosis is a complex and important component of growth and survival that has been well established for many years (Gisbert et al. 2002; Saele et al. 2003).As an example, one of the stages of halibut development involves transitioning between larval forms with eyes on both sides of the body to juvenile forms with eyes on one side of the body (Saele et al. 2003). It was therefore possible to adapt the well-understood life history staging protocols to assess EMF exposure. Experiments with Atlantic halibut suggested that a high EMF exposure may have reduced both growth and development in early life stages, although neither was statistically different than the controls. Experiments with California halibut showed no change in growth or development.

Dungeness crabs were chosen as a representative bottom-dwelling crustacean, inhabiting a wide-ranging coastline from the Gulf of Alaska to California. This commercially and recreationally important specie forages on bivalves, crustaceans and fish in estuarine, nearshore and offshore coastal waters, and may encounter EMF transmission cables. Initial tests were designed to assess whether crab could detect EMF fields by measuring a sensory receptor response, the antennular flicking rate before and during an acute EMF exposure. Similar to tests discussed above, these experiments were based on a previous body of work and developed protocols that examined antennular flicking rate in Dungeness crab as a quantitative measure of chemosensory acuity in the water column for food extracts (Pearson et al. 1979), petroleum hydrocarbons (Pearson et al. 1980; 1981) and salinity (Sugarman et al. 1983). The antennules are primarily involved in recognition of chemical signatures in the water column, however other stimuli such as vibration and sound are known to elicit a flicking response. During EMF exposure, antennular flicking rate decreased slightly but was not significantly different than before the exposure. Likewise, the flicking rate response to a food odor decreased slightly after exposure to EMF, but was not statistically significant. More recent avoidance/attraction experiments have provided some preliminary evidence of subtle changes in behavior (e.g. amount of time buried, number of changes and variability in activity through time), however these results are preliminary and further analysis of the data is needed to understand the meaning of these results.

The body of scientific evidence created in FY10-11 with the selected fish species and Dungeness crab suggests that in a laboratory setting with high EMF and extended exposure circumstances there may be subtle sub-lethal behavioral, developmental or biochemical responses by some of these species (Tables 3.1, 4.2). However these results are varied, and many were not statistically significant. Furthermore, these studies were conducted at the upper bounds of likely EMF exposures in the field. Hence, lower exposure levels and/or duration would be expected to produce a lesser effect. Additional tests or trials using these species may enable a refinement of these test results, however limited budget and time constraints require prioritization of the last FY efforts. Hence, experiments with additional species of interest to regulatory agencies and stakeholder groups (e.g., American lobster, elasmobranch species) will be conducted to augment existing results and provide a body of scientific evidence to assess the potential effects and responses to EMF.

6.0 References

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