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Michelle M. Scanlan for the degree of Master of Science in Fisheries Science, presented on May 27, 2015.

Title: An Evaluation of the Effects of Hatchery and Management Practices on Geomagnetic Orientation Behavior in Juvenile Pacific Steelhead Trout (Oncorhynchus mykiss).

Abstract approved: ______________________________________________________

David L.G. Noakes

Numerous populations of salmon and trout undergo extensive migrations, migrating from freshwater to the sea and throughout ocean basins. Years later they return to their natal streams with remarkable accuracy and precision. The outward migration is accomplished without the benefit of following experienced migrants or prior individual experience. Evidence suggests that Pacific salmon and trout use the earth’s geomagnetic fields when undertaking these migrations. My study evaluated current hatchery and management practices that may influence the ability of Pacific steelhead trout (Oncorhynchus mykiss) to perceive or respond to geomagnetic fields. Magnetized coded wire tags (CWT) were implanted into Alsea river steelhead pre-smolts (juveniles that have not undergone the physiological transition to prepare for oceanic life stage) during the fall of 2013. Test subjects were exposed to simulated magnetic fields at the latitudinal periphery of their oceanic foraging range in the North Pacific. Orientation responses were recorded and compared to non-tagged individuals. However, due to fish illness or other factors, control test subjects did not exhibit the north/south orientation responses observed during previous studies and results were inconclusive. Magnetic susceptibility, total magnetic field intensity, and median destructive field were assessed by analyzing 48 CWTs from five injection units. The total field intensity was consistent among machines and tags within a single machine. The strength of the fields produced by the tags relative
to the known magnetic sensitivity of steelhead trout suggest that they are unlikely to pose major problems for large-scale navigation in fish. Sex differences in geomagnetic orientation response were evaluated based on work that documented early movement downstream by female steelhead. Experiments documented no significant difference in geomagnetic orientation responses based on genetic sex, though these results may also have been confounded by the same problems associated with the CWT orientation experiments. Lastly, effects of rearing juvenile steelhead in concrete raceways (subject to magnetic distortion due to iron rebar) were examined following similar “simulated magnetic displacement” protocols. These juvenile steelhead exhibited random orientation responses. The inability of fish reared in raceways to distinguish between fields at the periphery of their oceanic foraging range could pose potential problems for homing and navigation in hatchery fishes. The duration of these affects is unknown and warrants further study. Based on these findings, there is evidence to suggest that some current hatchery and management practices may influence the ability of juvenile salmon and trout to perceive geomagnetic fields. In turn, this may influence successful navigation to foraging grounds in the Pacific, subsequent return rates, as well as homing and straying behavior.
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An Evaluation of the Effects of Hatchery and Management Practices on Geomagnetic Orientation Behavior in Juvenile Pacific Steelhead Trout (*Oncorhynchus mykiss*)

by

Michelle M. Scanlan

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APPROVED:

Major Professor, representing Fisheries Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Michelle M. Scanlan, Author
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CONTRIBUTION OF AUTHORS

Dr. David Noakes, Dr. Nathan Putman, and Dr. Tom Quinn contributed to the study design and editing all sections of this manuscript. Dr. Nathan Putman also contributed to the construction of the testing apparatus, assisted with data analysis, and provided figures of simulated test fields. Dr. Joseph Stoner provided a magnetometer to calibrate the testing apparatus, and his staff assisted with the magnetic analysis of the coded wire tags.
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AN EVALUATION OF THE EFFECTS OF HATCHERY AND MANAGEMENT PRACTICES ON GEOMAGNETIC ORIENTATION BEHAVIOR IN JUVENILE PACIFIC STEELHEAD TROUT (*Oncorhynchus mykiss*)

CHAPTER 1 - INTRODUCTION
Marine migrants must obtain and utilize food resources under a variety of oceanic conditions. Explaining how navigationally naïve juveniles successfully travel to foraging grounds that are hundreds or thousands of kilometers away poses a considerable challenge to scientists (Gould and Gould 2012; Lohmann et al. 2008; Neave 1964; Quinn 1991). Spawning may occur in proximity to ocean currents that facilitate the out-migration of offspring, but variation in ocean currents makes this method of transport unreliable (Lohmann et al. 2012; Staaterman et al. 2012). Therefore, it is plausible that juveniles take an active role in navigating to foraging grounds.

Evidence suggests that some migratory animals such as birds (*Columba livia domestica*), newts (*Notophthalmus viridescens*), and sea turtles (*Caretta caretta*), use the Earth’s magnetic field to navigate along their migratory routes (Gould 2010; Gould and Gould 2012; Lohmann et al. 2008). We define navigation as the ability of an individual to derive positional information from the Earth’s magnetic field and orient accordingly (Lohmann et al. 2007). In salmon, this hypothesis has been supported by studies of navigationally naïve juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) (Putman et al. 2014a; Putman et al. 2014b). The studies used simulated magnetic field displacements representative of specific geographic coordinates. When exposed to fields at the latitudinal periphery of their oceanic foraging range, juvenile Pacific salmon exhibit an orientation response and effectively course correct, thus leading them back to the foraging grounds. Given that such abilities require an acute sensitivity for detecting magnetic fields and that anthropogenic influences can distort the ambient magnetic field, additional information is needed to evaluate current hatchery and management practices as they may affect the navigational abilities of Pacific salmon and trout (Engels et al. 2014; Fuxjager et al. 2014; Putman et al. 2014a).

Steelhead trout are anadromous members of the family Salmonidae. Their resident counterparts, rainbow trout, remain in freshwater and do not migrate to oceanic foraging grounds. Steelhead are iteroparous, meaning that they have the ability to spawn multiple times. Adults that survive after spawning are referred to as kelts (Seamons and Quinn 2010). Hence, the ability to navigate to marine foraging grounds is paramount. Steelhead native range spans from northwestern Mexico to northwestern Alaska in the
Northeastern Pacific Ocean, and populations also occur in Asia. Populations toward the southern edge of the range tend to be isolated and non-anadromous (Quinn 2005). Anadromous individuals spend 1-3 years in freshwater, and approximately 1-3 years in oceanic environment prior to homing back to their natal streams to spawn (Quinn 2005). Homing refers to the return of adults from an oceanic foraging ground to natal spawning sites (Quinn 2005).

The major component of this project is the examination of the effect of coded wire tags on the response of steelhead to magnetic fields. Coded wire tags (CWT) manufactured and distributed by Northwest Marine Technology, Inc. (Shaw Island, Washington), are magnetized 1mm pieces of stainless steel wire encrypted with binary code. The tags are implanted in the snout of salmonids, which places these magnetized pieces of metal in close proximity to magnetoreceptors (Eder et al. 2012). CWTs were first introduced in the Pacific Northwest during the late 1960s for the identification of salmon stocks (Johnson 2004). Additionally, they are used to calculate return rates of adult fishes. To date, a single study has attempted to examine the effects of coded wire tags on magnetic orientation in salmon (Quinn and Groot 1983). In that study, the orientation of hatchery-reared juvenile chum salmon out-migrating through Nootka Sound on the way to the North Pacific Ocean, were tested in circular arenas. In ambient magnetic conditions, salmon without CWTs oriented westward. When magnetic north was shifted by 90 degrees, fish orientation remained westward. Fish implanted with CWTs behaved similarly and no statistical difference in orientation was detected. However, because shifting the magnetic north did not result in the predicted shift in orientation in control fish, it is not clear that salmon were using the field to orient in this experimental set-up, or whether CWTs disrupt magnetic orientation. Additionally, that experiment was designed to determine effects on the magnetic “compass” of salmon, not the “map” (i.e., their ability to derive positional information from the field, rather than simply directional).

My project expands on the chum salmon study conducted by Quinn and Groot (1983) that examined the effects of CWTs on magnetic compass orientation. Using the apparatus and test fields from Putman et al. (2014), I conducted studies during the day in
a series of small circular testing arenas with no water flow (Putman et al. 2014a; Putman et al. 2014b). Instead of using reservoirs to capture fish, two GoPro® cameras (models Hero and Hero 3) were mounted above the testing arena to document the movements of test subjects throughout the trial. Digital photos were analyzed and headings were recorded by overlaying a digital compass on the photos in Microsoft® Power Point.

Do CWTs disrupt the behavioral responses of trout to the magnetic field? I predicted that if steelhead are using magnetic fields for orientation, tagged individuals would not exhibit the north/south orientation response similar to the control steelhead responses documented in previous studies. In addition to the behavior trials, I examined the magnetic properties of the tags to define field consistency among five injection units and tags magnetized within a single unit.

Recent evidence suggests a sex difference in the response to magnetic fields in first-time migrant birds (Rakhimberdiev et al. 2014). Based on the capture rate in Finland, researchers were able to detect a difference in sex ratios of captured individuals of ruff (*Philomachus pugnax*), a sexually dimorphic species that undertakes extensive seasonal migrations, in response to magnetic distortion. If there is a difference in migratory strategy between sexes in birds, could this also apply to salmonid species? Current research suggests that female steelhead trout are more likely to migrate to the ocean, and they tend to migrate earlier compared to their male counterparts (Ohms et al. 2013; Thompson 2014). This female sex bias has been observed in a wide geographic range throughout the Northeastern Pacific coast. Additionally, researchers have observed differences in brain size and structure, depending on sex and migration strategy (freshwater residents versus anadromous individuals) (Kolm et al. 2009). Given these sexual differences, I hypothesized that females would pay more attention to navigational cues compared to males. Therefore, I predicted that females would exhibit less variation in orientation response compared to males.

Lastly, I examined the differences in the orientation response of steelhead trout that are reared in concrete raceways, compared to the control group housed in ambient field conditions in an outdoor tank yard. There is evidence to suggest that artificial magnetic fields generated by the metal infrastructure in hatcheries disrupt the responses
of juvenile steelhead to magnetic fields typical of their oceanic foraging grounds (Putman et al. 2014a). That study found that steelhead reared in distorted magnetic fields showed a random distribution when presented with two fields representative of locations at the northern and southern periphery of the oceanic foraging range. Since concrete raceways contain iron rebar, fish reared in that environment may have altered magnetic responses. I predicted that raceway reared individuals would not exhibit the north/south orientation responses observed of control fish in previous studies. This would be very important information considering that many production hatcheries rear juvenile salmonids in raceways, and there are considerable questions regarding the failure of hatchery fish to correctly home to natal streams.

My study aims to produce additional information to understand the sensory ecology of salmonid migration in the open ocean. This information could be incorporated into future management practices to produce juvenile fishes with higher fitness and thus help improve the return rates of adult salmonids. Furthermore, my study attempts to expand our knowledge of magnetoreception in migratory fishes and allow us to draw comparisons with other vertebrate species.
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CHAPTER 2 - Effects of Coded Wire Tags on the Geomagnetic Orientation Response of juvenile steelhead trout (*Oncorhynchus mykiss*)
Migratory marine animals obtain and utilize food resources under a variety of oceanic conditions. The ability of migrants to follow spatiotemporal variations in resource abundance is important when traversing ocean basins. Spawning may occur in particular proximity to ocean currents that facilitate the out-migration of offspring, but variation in ocean currents makes this method of transport unreliable (Lohmann et al. 2012; Putman et al. 2012; Staaterman et al. 2012). Therefore, it is plausible that juveniles take an active role in navigating to foraging grounds.

Evidence suggests that some migratory animals such as birds (*Columba livia domestica*), newts (*Notophthalmus viridescens*), and sea turtles (*Caretta caretta*), use the Earth’s magnetic field to navigate along their migratory routes (Gould 2010; Gould and Gould 2012; Lohmann et al. 2008; Quinn 1991). In salmon, this hypothesis has been supported by studies of navigationally naïve juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) (Putman et al. 2014a; Putman et al. 2014b). The studies used simulated magnetic field displacements representative of specific geographic coordinates. Fields at the periphery of juvenile salmon oceanic foraging range elicited an orientation response, resulting in course correction toward the foraging grounds.

Given that successful migration is an essential component of the steelhead life-cycle we explored whether hatchery practices altered orientation behavior in steelhead. Such abilities require an acute sensitivity for detecting magnetic fields, and anthropogenic influences can easily distort the local magnetic field (Engels et al. 2014; Fuxjager et al. 2014; Putman et al. 2014a). Additional information is needed on the sensory ecology of magnetic orientation in salmonids to evaluate hatchery and management practices that may impact the navigational abilities of Pacific salmon and trout.

To evaluate the status of Pacific salmon and steelhead stocks, managers employ multiple methods to estimate the number of returning adults. A widely used method of evaluating return rates is the use of coded wire tags (CWT). CWT were first used for tracking anadromous fishes within the Pacific Northwest during the late 1960s (Johnson 2004). Circa 2010, more than 50 million Pacific salmon and steelhead were tagged by a
variety of state, federal, tribal, and private organizations (Nandor et al. 2010). Total U.S. tagging costs were approximately US $9 million (15 to 20 cents per fish). Approximately 275,000 tags are recovered at a cost of $12-$13 million annually (Nandor et al. 2010). Circa 2004, the Alaska Department of Fish and Game estimated a cost of $20 per fish to dissect and decode the tags, and an additional $18 per fish to make the tag return data available to the public (Nandor et al. 2010). The total cost of marking, recovering tags, interpreting tag data and making those data available for analyses are very considerable. The data obtained from CWT programs are very important to fisheries management programs, and so those costs must be assumed by the various agencies involved.

Prior to the 1970s, fin clipping was a common method for stock identification. When CWTs were introduced, clipped adipose fins indicated tag presence. CWT were implanted in the snout of hatchery fish and scanned with a handheld wand detector, since adipose fins were removed to denote the hatchery stock (Johnson 2004). During the 1990s, adipose fin clips were used to mark hatchery stock, regardless of tag presence. CWT (manufactured by Northwest Marine Technology) (NWT) are 1mm pieces of stainless steel wire that are magnetized for detection and retrieval, and laser etched using a decimal numerical system (Johnson 2004). Tags are implanted in the snout of salmonids, which places these magnetized pieces of metal in close proximity to magnetite containing cells responsible for magnetoreception in trout (Eder et al. 2012). The CWT are placed in the snout to minimize the chance that tags could enter the human food chain when tagged fish are processed. The location in the snout also means that the tags can more easily be detected and removed from the fish without destroying the valuable flesh of the fish.

Since CWT pose a considerable time and monetary investment, there is interest in tag recovery rates and coded wire tag affects. For example, according to the Regional Mark Information System (RMIS) the main database for tag release and recovery information, the Dworshak National Fish Hatchery (Ahsahka, Idaho) injected CWT in the snouts of 17,153 adipose fin clipped steelhead from the 2002 broodstock. The total number of adipose clipped individuals was 142,212. Thus, 12% of the 2002 broodstock from this hatchery were tagged and released. During the 2005 and 2006 seasons, a total
of 61 tagged adults from the 2002 broodstock returned to the hatchery, resulting in a 0.355% return rate for tagged individuals only.

To date, a single study has attempted to examine the effects of coded wire tags on magnetic orientation in salmon (Quinn and Groot 1983). In that study, the orientation of hatchery-reared juvenile chum salmon out-migrating through Nootka Sound on the way to the North Pacific Ocean were tested in circular arenas. In ambient magnetic conditions, salmon without CWTs oriented westward (mean heading = 241°, Rayleigh r = 0.147, p <0.001). When magnetic north was shifted by 90 degrees, fish orientation remained westward (mean heading = 278°, Rayleigh r = 0.125, p <0.001). Fish implanted with CWTs behaved similarly and no statistical difference in orientation was detected. However, because shifting the magnetic north did not result in the predicted shift in orientation in control fish, it is not clear that salmon were using the field to orient in this experimental set-up, or whether CWTs disrupt magnetic orientation. Additionally, that experiment was designed to determine effects on the magnetic “compass” of salmon, not the “map” (i.e., their ability to derive positional information from the field, rather than simply directional).

The goal of my project was to evaluate the orientation response of juvenile steelhead implanted with CWT and compare those orientation responses to non-tagged individuals. Do CWT disrupt the ability of trout to use the magnetic field as a “map” (i.e., derive positional information from the field, rather than simply directional) (Lohmann et al. 2007)? If salmon and steelhead have extremely sensitive orientation responses, would the presence of a small magnetized tag influence the response? If fish were using the magnetic field to assess their position, we predicted that those exposed to a field existing at the northern periphery of their oceanic range would orient southward, to counter the perceived displacement. In contrast, those fish exposed to a field existing at the southern periphery of their range would orient northward. Thus, fish would orient in opposite directions in the two test fields, as previously documented in juvenile Chinook salmon and steelhead trout. (Putman et al. 2014b). Given that field distortions of CWTs measured by Quinn and Groot (1983) might alter the fields perceived by fish by more than 100% (total magnetic field intensity measured 1mm from the tag was 66 µT compared to 55.5
μT for our northern test field), we predicted that steelhead implanted with tags would not orient in the opposing directions (north/south) documented in previous studies.

**Methods**

**Test Site and Subject Description**

We conducted all tests at the Oregon Hatchery Research Center (OHRC), Alsea, Oregon (Noakes and Corrarino 2010). Juvenile steelhead from the North Fork Alsea River stock, were spawned by hand from 60 pairs during 1 of 4 spawning events from December 2012 to February 2013. Young fish were implanted with coded wire tags at the North Fork Alsea Fish Hatchery during the third week of November 2013. Fish were tagged and marked during the fall to coincide with the rains that provided the creek water needed to fill the rearing ponds. All spawning, rearing and tagging procedures were carried out by ODFW hatchery personnel, following standard operating procedures (Matt Frank, personal communication). Test subjects were transported from the Alsea Hatchery to the OHRC by employees of the Oregon Department of Fish and Wildlife (ODFW) on January 14th, 2014. We transported 500 additional steelhead from the same cohort to the OHRC on March 18, 2014 as replacements for fish lost to a mortality event. Juveniles were held in adjacent fiberglass tanks (1.83 m diameter, 0.762 m depth) and fed a commercial pellet diet (Skretting at Alsea North Fork hatchery, Bio-Oregon at the OHRC) to satiation multiple times per day. When tested, subjects ranged from 17 to 24 cm in fork length and 16 to 17 months in age from the time of spawning. Wild juvenile steelhead of that size and age would begin moving downstream to the Pacific Ocean as smolts (Romer et al. 2013).

**Magnetic Orientation Analysis**

**Behavior Analysis**

Test subjects were separated into two treatments: control and coded wire tagged. Prior to the start of the behavioral trials, both the control and CWT fish were held for 2 months in adjacent fiberglass tanks (1.83 m diameter, 0.762 m depth), located in the outdoor tank yard, at least 10 meters away from buildings and the testing apparatus. Water flow within the rearing tanks was approximately 56 - 76 liters per minute (lpm)
and was increased with increasing fish size. The fish remained in the tanks for the duration of the project. Control and CWT fish experienced ambient magnetic field conditions (field intensity = 53.69 µT, inclination angle = 67.2°). Each group consisted of 500 individuals that were divided into two sub-groups (North field and South field) of 250 individuals each (see Appendix I Figure A1.2). The magnetic test fields were 55.54 µT and 73.3° (59°N, 145°W) representative of a location in the Gulf of Alaska (northern field) and 44.40 µT and 56.7° (38°N, 145°W) representative of a location west of southern California (southern field) (Figure 2.1). Treatment testing order was assigned by alternating control and CWT groups each day and alternating North and South testing fields (north day, south day) to avoid bias due to diurnal or weather effects. Testing occurred outdoors between 0730 and 1700 hours, Pacific Daylight Time (PDT). Each fish was tested once. A black mesh cover was placed over the framework of the test apparatus resulting in a 70% reduction of incident light, to minimize stress to the fish. Solar cues were otherwise visible throughout the trials.

Subjects were placed in opaque circular arenas 30.5 cm in diameter filled to a depth of 21.5 cm with water from neighboring East Fall Creek (the same water source as rearing conditions) (Appendix I Figure A1.1). Water was completely emptied from each container after each test and the container was rinsed with running water from the same source to clear debris from the test arenas. Containers were re-filled for the subsequent tests to maintain temperature and dissolved oxygen levels. One fish was placed in each of the 25 circular arenas and allowed to adjust to ambient conditions for 10 minutes. After the initial adjustment period, the test subjects were exposed to a magnetic field that was changed by two orthogonally arranged four-coil systems (outer vertical coil length equals 3.57 m and inner horizontal coil length equals 3.33 m) connected to a DC power supply housed in a nearby building (<10 m distance) (Putman et al. 2014b) (see Appendix I Figure A1.1). Fields were altered by pairing specific combinations of amperage for the horizontal and vertical components of the coil to generate simulated magnetic fields, corresponding to geographic points at the latitudinal periphery of steelhead oceanic foraging range. Test duration consisted of 8 minutes (7 for fish to adjust to altered conditions and 1 minute used for photo analysis). Magnetic fields were determined by the
International Geomagnetic Reference Field (IGRF-11) (Findlay et al. 2010). The ambient magnetic field was measured using a triaxial fluxgate magnetometer (Applied Physics 520A). Field uniformity was better than ± 1%, and no magnetic gradients were detected within the test arenas.

Using two GoPro® cameras mounted above the testing arena, digital photographs were taken of the fish in the arenas at 10 second intervals. Images were analyzed in Microsoft® PowerPoint by overlaying a compass figure and measuring the direction that the head was pointing, relative to magnetic north. Six measurements were averaged for each fish. Measurements were recorded to the nearest 5° by assistants unaware of the experimental conditions. Experiments were conducted between May 5th, 2014 and June 15th, 2014.

At the end of the orientation trials, the parr snouts were scanned with a handheld wand to confirm tag presence. Fish within the CWT group that tested negative for tags were segregated and sacrificed by a lethal dose of MS 222 anesthetic. Individuals were transported to Oregon State University and were assessed with a Faxitron® x-ray MX-20. With the assistance of Dr. Brian Sidlauskas, 25 individuals were evaluated. None of the fish that tested negative for tag presence possessed tags and were thus excluded from the CWT analyses.

**CWT Magnetic Analysis**

The U.S. Fish and Wildlife Service (USFWS) Vancouver, Washington office donated approximately 50 tags from 5 machines (10 tags/machine and 5 machines total) to assess potential differences between tag magnetization among machines and tags within a single machine. Tags were mounted inside plastic 8 cc cubes and secured using color coded mounting media so that tag orientation within the cube was consistent. Tags from the experimental fish were not used because tag extraction from the fish snouts might result in magnetic transference thus, influencing the measurements and rendering any obtained results unreliable.

CWT were assessed using multiple prescreening tests to examine the composition and magnetic properties of the tags. First, tags were mounted inside plastic cubes (8cc).
Since tags needed to be centered within the cube, non-toxic assorted mounting media was used, since it does not contain heavy metals. Tags were examined using a dissecting microscope and were sandwiched between two pieces of Scotch® tape to fix the tag position. The tags were then deposited within a cube half full of mounting media. Once the tags were centered in the cube, the remaining space was filled with additional mounting media. Samples were transferred to the Paleomagnetics lab at Oregon State University to undergo a series of tests. The first test analyzed the susceptibility of the CWT to induced magnetic fields. The empty plastic cubes, cubes with mounting media, and cubes with tags and mounting media were assessed. Susceptibility measures the magnetizability of a given substance. This was evaluated by applying a magnetic field and measuring the response of the substance. Highly magnetized objects change or interact more with the induced magnetic fields, resulting in higher susceptibility. Once susceptibility was assessed, samples were then transferred to an apparatus that creates a magnetic “free” space, essentially canceling out the earth’s magnetic field. The field intensity of the sample was measured using a triaxial fluxgate magnetometer with a probe attached to a non-magnetic wand. Fields were measured in the x, y, and z axes. Changes in the magnetic field of the samples occurred in fields that were 2 to 3 orders of magnitude weaker than the Earth’s magnetic field. Tags were tested in the liquid helium free 2G Enterprises superconducting rock magnetometer (SRM). The total magnetic intensity of the cubes was measured in 1 cm increments and later converted the microTelsa (µT) to make comparisons to behavioral trial data. Additionally, CWT demagnetization was assessed in the SRM by exposing a subset of tags to alternating magnetic fields and measuring the median destructive field (50% reduction in total magnetic field intensity) (Dunlop and Özdemir 2001).

All experiments were performed in accordance with Oregon State University Animal Care and Use Protocol #4394.

**Statistical Analysis**

Statistical analysis was conducted using Oriana 2 (Kovach Computing Services). A Rayleigh uniformity test was used to assess orientation responses in each treatment
A Mardia-Watson Wheeler nonparametric test was conducted to analyze pairwise comparisons to assess differences in orientation response between treatments. A multisample test was conducted to assess differences among all respective treatments, consistent with previous studies (Putman et al. 2014a; Putman et al. 2014b).

Results

Behavior trials
There was no statistically significant difference in orientation responses between the control and CWT treatments. However, the treatments were significantly oriented; North Control (Rayleigh r = 0.304, p-value <0.0001, n = 250), South Control (Rayleigh r = 0.26, p-value <0.0001, n = 240), North CWT (Rayleigh r = 0.294, p-value <0.0001, n = 229), South CWT (Rayleigh r = 0.323, p-value <0.0001, n = 234), but the mean headings indicate an eastward orientation (North Control = 111°, North CWT = 109°, South Control = 86°, South CWT = 101°). This was considerably different compared to the north/south orientation responses observed in control Pacific salmon and trout during previous studies (Putman et al. 2014b). The comparison of control groups (northern vs. southern field) did not differ (Mardia-Watson-Wheeler p = 0.106), the same was true of the CWT treatment (northern vs. southern field) (Mardia-Watson-Wheeler p = 0.472). Comparisons between CWT north vs. control north and CWT south vs. control south yielded no significant differences (Mardia-Watson-Wheeler p > 0.1). For additional results, see Tables 2.1 and 2.2.

CWT Magnetic Analysis
In addition to evaluating the orientation response of the test subjects, the magnetic characteristics of the CWT were also analyzed. There were a total of 48 tags tested from five different machines. There were very slight variations in the total magnetic field intensity of the tags within a single machine and among all of the machines (Table 2.3). The range for total magnetic field intensity across all five machines was 0.0921 µT to 0.1112 µT. The ability of tags to resist demagnetization was also analyzed. Natural remanent magnetism (NRM) is the permanent magnetism of sediment or a substance. A subset of tags were exposed to fields within the magnetometer and the strength of the alternating magnetic field necessary to reduce the total field intensity of the tags by 50%
(median destructive field) was assessed. In order to observe a reduction of this magnitude, tags were exposed to total field intensities of 17,000 µT to 18,000 µT. The magnetic susceptibility of the tags from all five machines ranged from 0.0003 µT to 0.0006 µT. Results for each machine are included in Table 2.4. Very slight interactions between the tags and the applied test field were documented, indicating that the tags are not very magnetizable.

**Discussion**

There were a multitude of factors that may have influenced the behavioral trial results. The first set of my control fish (n=500) perished as a result of a storm event that flushed sediment into the holding tanks. A set of replacement control fish from the same broodstock was transferred to the OHRC and held for 1 month to acclimatize before the start of the behavior trials. However, during this extra month of holding time, all test subjects continued to grow. Therefore, at the time of behavioral testing, test subjects were considerably larger (17-24 cm) than the ideal 7-10 cm fork length for orientation testing. More importantly, all replacement test subjects were originally held in concrete raceways at the North Fork Alsea hatchery for approximately 8 months prior to transport to the OHRC. This may have an impact on magnetoreception, but additional information regarding this subject is detailed in Chapter 4. Regardless, control subjects did not orient in the typical north/south orientations that have been observed in multiple studies using the same testing arena with control Chinook salmon and steelhead trout (Putman et al. 2014a; Putman et al. 2014b). It is interesting to note that the CWT group oriented eastward regardless of test field, and this group of fish was not sick at any point during the trials. However, there were no fish tested in the ambient field, so there currently is no evidence to suggest or refute that these fish were using magnetic fields for orientation. Additional testing of control, CWT (non-magnetized and magnetized), and ambient groups will commence during the summer of 2015, and resulting orientation responses will be evaluated. Although the behavioral analysis did not shed additional light on the effect of coded wire tags on geomagnetic navigation, testing of the magnetic properties of the tags may offer some insight regarding the influence of the tags on navigation abilities.
The pretesting and subsequent magnetic tests have provided some valuable insight regarding the properties of CWT. The susceptibility test indicated that the tags are not strongly magnetic. Unlike the total magnetic field intensities measured in Quinn and Groot (1983) that indicated that tags altered the fields perceived by fish by more than 100%, I observed changes in the magnetic field of the tags that were detectable at field intensities 2 to 3 orders of magnitude less than the Earth’s magnetic field. This difference may be attributed to a change in the magnetization settings of the injection units, changes in the elemental composition of the tags, or a difference in the magnetometers used to measure magnetic fields, although this is purely speculative. This also confirms that the tags possess subtle magnetic properties. However, demagnetization of tags occurs at magnetic intensities that are 3 orders of magnitude greater than the Earth’s magnetic field. It is unlikely that salmon and trout will encounter naturally occurring magnetic fields of this magnitude, but the magnetic properties of tags may be influenced by artificially augmented fields. More information is needed to evaluate anthropogenically modified magnetic fields and the resulting orientation responses of Pacific salmon and trout.
Table 2.1 Magnetic orientation information for CWT and control treatments exposed to simulated magnetic fields in the North Pacific Ocean.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Location</th>
<th>Total Field Intensity (µT)</th>
<th>Inclination Angle (°)</th>
<th>Mean Heading (°)</th>
<th>Rayleigh r</th>
<th>Rayleigh p</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient field</td>
<td>44.404°N, 123.753°W</td>
<td>52.54</td>
<td>66.5</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Control (North)</td>
<td>59°N, 145°W</td>
<td>55.55</td>
<td>73.3</td>
<td>111</td>
<td>0.304</td>
<td>9.90E-11</td>
<td>250</td>
</tr>
<tr>
<td>Control (South)</td>
<td>38°N, 145°W</td>
<td>44.46</td>
<td>56.7</td>
<td>86</td>
<td>0.26</td>
<td>8.99E-08</td>
<td>240</td>
</tr>
<tr>
<td>CWT (North)</td>
<td>59°N, 145°W</td>
<td>55.55</td>
<td>73.3</td>
<td>109</td>
<td>0.294</td>
<td>2.37E-09</td>
<td>229</td>
</tr>
<tr>
<td>CWT (South)</td>
<td>38°N, 145°W</td>
<td>44.46</td>
<td>56.7</td>
<td>101</td>
<td>0.323</td>
<td>2.47E-11</td>
<td>234</td>
</tr>
</tbody>
</table>
Table 2.2 Mardia-Watson Wheeler Pairwise Treatment Comparisons of simulated North Pacific magnetic fields (North = 59°N, 145°W; South = 38°N, 145°W).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>W</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (North vs South)</td>
<td>4.482</td>
<td>0.106</td>
</tr>
<tr>
<td>CWT (North vs South)</td>
<td>1.504</td>
<td>0.472</td>
</tr>
<tr>
<td>Control vs CWT (North)</td>
<td>0.111</td>
<td>0.946</td>
</tr>
<tr>
<td>Control vs CWT (South)</td>
<td>2.714</td>
<td>0.257</td>
</tr>
</tbody>
</table>
Table 2.3 Range of total field intensity (µT) and inclination angles (°) of 48 coded wire tags from five NMT injection units.

<table>
<thead>
<tr>
<th>Injection unit</th>
<th>Inclination (°)</th>
<th>Total Intensity (µT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 182</td>
<td>81.8 - 87.0</td>
<td>0.0941 - 0.1075</td>
</tr>
<tr>
<td>M 211</td>
<td>82.7 - 88.4</td>
<td>0.1112 - 0.1202</td>
</tr>
<tr>
<td>M 214</td>
<td>75.4 - 86.1</td>
<td>0.0921 - 0.1019</td>
</tr>
<tr>
<td>M 549</td>
<td>79.0 - 86.6</td>
<td>0.0982 - 0.1144</td>
</tr>
<tr>
<td>M 953</td>
<td>74.5 - 87.2</td>
<td>0.0962 - 0.1109</td>
</tr>
</tbody>
</table>
Table 2.4 Range of magnetic susceptibilities (µT) of 25 coded wire tags from five NMT injection units.

<table>
<thead>
<tr>
<th>Injection Unit</th>
<th>Magnetic Susceptibility Range (µT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 182</td>
<td>0.0003 - 0.0006</td>
</tr>
<tr>
<td>M 211</td>
<td>0.0004 - 0.0005</td>
</tr>
<tr>
<td>M 214</td>
<td>0.0004 - 0.0006</td>
</tr>
<tr>
<td>M 549</td>
<td>0.0004 - 0.0005</td>
</tr>
<tr>
<td>M 953</td>
<td>0.0003 - 0.0005</td>
</tr>
</tbody>
</table>
Figure 2.1 Mean heading for control treatment (n=250) exposed to northern simulated magnetic field representing a location within the Gulf of Alaska (total magnetic field intensity = 55.54 µT and inclination angle = 73.3°) (59°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 110°, r-value = 0.304, and p-value <0.0001.
Figure 2.2 Mean heading for control treatment (n=240) exposed to southern simulated magnetic field representing a location west of Southern California (total magnetic field intensity = 44.40 µT and inclination angle = 56.7°) (38°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 86°, r-value = 0.26, and p-value <0.001.
Figure 2.3 Mean heading for coded wire tag (CWT) treatment (n=229) exposed to northern simulated magnetic field representing a location within the Gulf of Alaska (total magnetic field intensity = 55.54 µT and inclination angle = 73.3°) (59°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 109°, r-value = 0.294, and p-value <0.0001.
Figure 2.4 Mean heading for coded wire tag (CWT) treatment (n=234) exposed to southern simulated magnetic field representing a location west of Southern California (total magnetic field intensity = 44.40 µT and inclination angle = 56.7°) (38°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 101°, r-value = 0.323, and p-value <0.0001.
References


Putman NF, et al. (2014b) An inherited magnetic map guides ocean navigation in juvenile Pacific salmon. Current Biology 24(4):446-450

Quinn T, Groot C (1983) Orientation of chum salmon (Oncorhynchus keta) after internal and external magnetic field alteration. Canadian Journal of Fisheries and Aquatic Sciences 40(10):1598-1606


Romer JD, et al. (2013) Survival and behavior of juvenile steelhead trout (Oncorhynchus mykiss) in two estuaries in Oregon, USA. Environmental biology of fishes 96(7):849-863

CHAPTER 3 - Comparison of sex differences in geomagnetic orientation responses of juvenile steelhead trout (Oncorhynchus mykiss)
Migratory marine animals locate and obtain food resources in dynamic oceanic conditions. The ability of migrants to follow spatiotemporal variations in resource abundance is important when traversing ocean basins. Spawning may occur in particular proximity to ocean currents that facilitate the out-migration of offspring, but variation in ocean currents makes this method of transport unreliable (Lohmann et al. 2012; Putman et al. 2012; Staaterman et al. 2012). Therefore, it is possible that juveniles take an active role in navigating to foraging grounds.

Evidence suggests that some migratory animals such as birds (Columba livia domestica), newts (Notophthalmus viridescens), and sea turtles (Caretta caretta), use the Earth’s magnetic field to navigate along their migratory routes (Gould 2010; Gould and Gould 2012; Lohmann et al. 2008; Quinn 1991). In salmon, this hypothesis has been supported by studies of navigationally naïve juvenile Chinook salmon (Oncorhynchus tshawytscha) and steelhead trout (Oncorhynchus mykiss) (Putman et al. 2014a; Putman et al. 2014b). The studies used simulated magnetic field displacements representative of specific geographic coordinates. Fields at the periphery of juvenile salmon oceanic foraging range elicited an orientation response, resulting in course correction toward the foraging grounds.

Since evidence suggests that salmonids possess a magnetic “map sense,” we examined potential sex differences in this ability. Steelhead trout are iteroparous, and may spawn multiple times (adults surviving after spawning are referred to as kelts), although most steelhead reproduce only once (Seamons and Quinn 2010). Fecundity is linked to body size in females, so successfully traveling to oceanic foraging grounds confers a major advantage for reproductive success. Females have been documented to spawn multiple times, with a 10% increase in lifetime reproductive success (LRS) during the second spawning event (Seamons and Quinn 2010). Some males also returned to breed twice, producing no detectable adult offspring during the first spawning event, but successfully spawn during the second year (Seamons and Quinn 2010). Since there are multiple life history tactics used by individuals of a given population, perhaps there might be differences in how males and females utilize their map sense.
Recent evidence suggests a sex difference in the response to magnetic fields in first-time migrant birds (Rakhimberdiev et al. 2014). Those authors tested the linear relationship of yearly sex ratios of juvenile ruff (*Philomachus pugnax*) and the intensity of geomagnetic disturbance during the migration period of the same year. Disturbance of magnetic field negatively correlated with the proportion of males in stopover sites in southern Finland during the migration period. Correlations explained 45% of variance in sex ratios of captured individuals of ruff, a sexually dimorphic species that undertakes extensive seasonal migrations. If there is a difference in migratory strategy mediated by magnetic variation between sexes in birds, could this also apply to salmonid species?

Current research suggests that female *O. mykiss* are more likely to migrate to the ocean, and they tend to migrate earlier compared to their male counterparts (60:40 sex bias) (Ohms et al. 2013; Thompson 2014). This female sex bias has been observed in a wide geographic range throughout the Northeastern Pacific coast. Additionally, researchers have observed differences in brain size and structure, depending on sex and migration strategy (freshwater residents versus anadromous individuals) (Kolm et al. 2009). Females have a higher gametic investment (in terms of energetic costs of ovaries compared to testes) compared to males, resulting in stronger selection pressures to successfully navigate to and from oceanic foraging grounds. For example, a study of the migration and spawning energetics of anadromous sea lamprey (*Petromyzon marinus*) found that the total caloric content of testes was 10.2 kcal compared to 435 kcal for the ovaries (Beamish 1979). If that is the case, do females pay more attention to geomagnetic navigational cues compared to males? Based on these studies, I examined this question by coupling genetic sex information with magnetic orientation responses, to determine if females exhibit less variation in orientation response (mean heading) compared to males, when exposed to magnetic cues. If there is a difference in orientation response, what degree of variation is observed between males and females?
Methods

Test Site and Subject Description

We conducted all tests at the Oregon Hatchery Research Center (OHRC), Alsea, Oregon (Noakes and Corrarino 2010). Juvenile steelhead from the North Fork Alsea River stock, were spawned by hand from 60 pairs during 1 of 4 spawning events from December 2012 to February 2013. Young fish were implanted with coded wire tags (CWT) at the North Fork Alsea Fish Hatchery during the third week of November 2013. Fish were tagged and marked during the fall to coincide with the rains that provided the creek water needed to fill the rearing ponds. All spawning, rearing, and marking of the fish was carried out by Oregon Department of Fish and Wildlife (ODFW) personnel, following standard operating procedures (M. Frank, R. Couture, personal communications). Test subjects were transported from the Alsea Hatchery to the OHRC by ODFW employees on January 14th, 2014. We transported 500 additional steelhead from the same cohort to the OHRC on March 18, 2014 as replacements for fish lost to a mortality event. Juveniles were held in adjacent fiberglass tanks (1.83 m diameter, 0.762 m depth) and fed a commercial pellet diet (Skretting at Alsea North Fork hatchery, Bio-Oregon at the OHRC) to satiation multiple times per day. When tested, subjects ranged from 17 to 24 cm in fork length and 16 to 17 months in age from the time of spawning. Wild juvenile steelhead of that size and age would begin moving downstream to the Pacific Ocean as smolts (Romer et al. 2013).

Magnetic Orientation Analysis

Behavior Analysis

Test subjects were separated into two treatments: control and coded wire tagged. Prior to the start of the behavioral trials, both the control and CWT fish were held for 2 months in adjacent fiberglass tanks (1.83 m diameter, 0.762 m depth), located in the outdoor tank yard, at least 10 meters away from buildings and the testing apparatus. Water flow within the tanks was approximately 56 - 76 liters per minute (lpm) and was increased with increasing fish size. The fish remained in the tanks for the duration of the
project. Control and CWT fish experienced ambient magnetic field conditions (field intensity = 53.69 µT, inclination angle = 67.2°). Each group consisted of 500 individuals that were divided into two sub-groups (North field and South field) of 250 individuals each (see Appendix I Figure A1.2). The magnetic test fields were 55.54 µT and 73.3° (59°N, 145°W) representative of a location in the Gulf of Alaska (northern field) and 44.40 µT and 56.7° (38°N, 145°W) representative of a location west of southern California (southern field) (Figure 3.1).

Treatment testing order was assigned by alternating control and CWT groups each day and alternating North and South testing fields (north day, south day) to avoid bias due to diurnal or weather effects. Testing occurred outdoors between 0730 and 1700 hours, Pacific Daylight Time (PDT). Each fish was tested once. A black mesh cover was placed over the framework of the test apparatus resulting in a 70% reduction of incident light, to minimize stress to the fish. Solar cues were otherwise visible throughout the trials.

Subjects were placed in opaque circular arenas 30.5 cm in diameter filled to a depth of 21.5 cm with water from neighboring East Fall Creek (the same water source as rearing conditions) (Appendix I Figure A1.1). Water was completely emptied from each container after each test and the container was rinsed with running water from the same source to clear debris from the test arenas. Containers were re-filled for the subsequent tests to maintain temperature and dissolved oxygen levels. One fish was placed in each of the 25 circular arenas and allowed to adjust to ambient conditions for 10 minutes. After the initial adjustment period, the test subjects were exposed to a magnetic field that was changed by two orthogonally arranged four-coil systems (outer vertical coil length equals 3.57 m and inner horizontal coil length equals 3.33 m) connected to a DC power supply housed in a nearby building (<10 m distance) (Putman et al. 2014b) (see Appendix I Figure A1.1). Fields were altered by pairing specific combinations of amperage for the horizontal and vertical components of the coil to generate simulated magnetic fields, corresponding to geographic points at the latitudinal periphery of steelhead oceanic foraging range. Test duration consisted of 8 minutes (7 for fish to adjust to altered conditions and 1 minute used for photo analysis). Magnetic fields were determined by the
International Geomagnetic Reference Field (IGRF-11) (Findlay et al. 2010). The ambient magnetic field was measured using a triaxial fluxgate magnetometer (Applied Physics 520A). Field uniformity was better than ± 1%, and no magnetic gradients were detected within the test arenas.

Using two GoPro® cameras mounted above the testing arena, digital photographs were taken of the fish in the arenas at 10 second intervals. Images were analyzed in Microsoft® PowerPoint by overlaying a compass figure and measuring the direction that the head was pointing, relative to magnetic north. Six measurements were averaged for each fish. Measurements were recorded to the nearest 5° by assistants unaware of the experimental conditions. Experiments were conducted between May 5th, 2014 and June 15th, 2014.

**Genetic Analysis**

Fin clips (1 cm²) were removed from the caudal fin of 1000 test subjects (500 CWT and 500 control) and placed individually in clear, 1.7mL graduated microtubes. Samples were stored in 95% ethanol. Each sample contained an internal and external identification tag with a unique code. Samples were transported to Oregon State University for genetic testing. Polymerase chain reactions and gel electrophoresis were used to identify a master sex determining gene sdY (sexually dimorphic on the Y-chromosome) as described in (Yano et al. 2013). Using the unique identification code, genetic sex was linked to photos of each test subject and comparisons of orientation responses between males and females were examined.

All experiments were performed in accordance with Oregon State University Animal Care and Use Protocol #4394.

**Statistical Analysis**

Statistical analysis was conducted using Oriana 2 (Kovach Computing Services). A Rayleigh uniformity test was used to assess orientation responses in each treatment group. A Mardia-Watson Wheeler nonparametric test was conducted to analyze pairwise
comparisons to assess differences in orientation response between treatments. A multi-sample test was conducted to assess differences among all respective treatments, consistent with previous studies (Putman et al. 2014a; Putman et al. 2014b).

**Results**

Almost all treatments (control, CWT, male, female, north field, and south field) showed a significant orientation response with Rayleigh uniformity test p-values less than 0.0001. The only exception was the female control group exposed to the northern field (Rayleigh p-value = 0.118). Comparisons of males and females in both the control and CWT treatments demonstrated no significant difference in orientation response between sexes, regardless of test field (p-values > 0.05) (Table 3.2). However, there was a finding of interest. Although there were no differences in orientation response between the control and CWT groups, there was a difference in orientation response within the female control group (north vs south fields) and a difference within the male control group (north vs south). A Mardia-Watson-Wheeler pairwise test detected a difference in orientation response in females (p-value = 0.017) and for males (0.031). However, this difference was not detected within the CWT female and male groups (Mardia-Watson-Wheeler p-values >0.05). The mean headings for control females were 120° in the northern field, and 68° in the southern field. For males, the mean headings for the northern and southern fields were 126° and 70° respectively. There were a total of 192 females and 208 males in the control group. Both the northern and southern field exposures tested 96 females and 104 males. There was an even distribution of males (n=200) and females (n=200) within the CWT treatment. A summary of field information and orientation responses for all treatments is detailed in Table 3.1. Graphical comparisons of orientation responses are located in Figures 3.1-3.8.
Discussion

These results show that both the males and females within the control group responded differently to different magnetic conditions. Surprisingly, this difference is not observed within the CWT treatment. In essence, within the control group, both sexes detect a difference in magnetic fields, but in the CWT group, neither sex does. Given previous studies on a bird species that indicate the possibility of sex differences in magnetic orientation, it was interesting to observe no significant differences between the orientation responses of females and males steelhead in any magnetic condition. If females are preferentially migrating earlier downstream, it is reasonable to predict that they may pay greater attention to geomagnetic cues. If females are leaving sooner, why are they not paying more attention to these cues? Females have a considerably higher energetic gametic investment compared to males, so there is more pressure to successfully navigate to oceanic foraging grounds to maximize that investment. It makes sense that males did not exhibit a strong orientation response, since they have the opportunity to mate with females in a given stream system, and their genetic material is passed on regardless of returns to their natal stream.

This also has interesting implications for comparing geomagnetic orientation responses to other migratory species. It is important to note that although there was no difference between males and females in their orientation responses, this is separate from the decision for fish to move downstream. We can conclude that the sexual difference in proportions of female and male smolts moving downstream is not a consequence of differences in their ability to detect or respond to geomagnetic cues, although these results may be confounded by the orientation responses of the control group. Contrary to steelhead, some sexually dimorphic bird species do exhibit differences in orientation response. Why are selection pressures different among these migratory species? The temptation is to make overarching generalizations about the navigation of migratory species, but it is apparent that the mechanisms differ on some level. Additional research is needed to parse out the intricacies of navigational tools and how they are utilized by different species.
Table 3.1 Magnetic orientation information for CWT and control treatments (males (M) and females (F)).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Location</th>
<th>Total Field Intensity (µT)</th>
<th>Inclination Angle (°)</th>
<th>Mean Heading (°)</th>
<th>Rayleigh r</th>
<th>Rayleigh p</th>
<th>n</th>
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<td>Ambient field</td>
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<td>52.54</td>
<td>66.5</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<td>59°N, 145°W</td>
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<td>73.3</td>
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<td>0.149</td>
<td>1.18E-01</td>
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<td>73.3</td>
<td>130</td>
<td>0.388</td>
<td>1.13E-06</td>
<td>91</td>
</tr>
<tr>
<td>CWT North (F)</td>
<td>59°N, 145°W</td>
<td>55.55</td>
<td>73.3</td>
<td>104</td>
<td>0.307</td>
<td>2.13E-04</td>
<td>90</td>
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<tr>
<td>Control South (M)</td>
<td>38°N, 145°W</td>
<td>44.46</td>
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<td>0.388</td>
<td>1.13E-06</td>
<td>91</td>
</tr>
<tr>
<td>CWT South (F)</td>
<td>38°N, 145°W</td>
<td>44.46</td>
<td>56.7</td>
<td>104</td>
<td>0.307</td>
<td>2.13E-04</td>
<td>90</td>
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</table>
Table 3.2 Mardia-Watson Wheeler Pairwise Comparisons of Orientation differences based on genetic sex.

<table>
<thead>
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<tr>
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</tr>
<tr>
<td>Female N vs S Control</td>
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</tr>
<tr>
<td>Male N vs S Control</td>
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<td>0.031</td>
</tr>
<tr>
<td>Female vs Male CWT (North)</td>
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<td>0.322</td>
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<tr>
<td>Female vs Male CWT (South)</td>
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</tr>
<tr>
<td>Female CWT N vs S</td>
<td>0.743</td>
<td>0.69</td>
</tr>
<tr>
<td>Male CWT N vs S</td>
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</tr>
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</tr>
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<td>Male Control vs CWT (North)</td>
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<td>Male Control vs CWT (South)</td>
<td>2.831</td>
<td>0.243</td>
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</table>
Figure 3.1 Mean headings for females in the control treatment (n=96) exposed to northern simulated magnetic field representing a location within the Gulf of Alaska (total magnetic field intensity = 55.54 µT and inclination angle = 73.3°) (59°N, 145°W). Red bar represents 95% confidence intervals. Mean vector was 120°, r-value = 0.149, and p-value 0.118.
Figure 3.2 Mean headings for males in the control treatment (n=104) exposed to northern simulated magnetic field representing a location within the Gulf of Alaska (total magnetic field intensity = 55.54 µT and inclination angle = 73.3°) (59°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 108°, r-value = 0.4, and p-value <0.0001.
Figure 3.3 Mean headings for females in the coded wire tag (CWT) treatment (n=90) exposed to northern simulated magnetic field representing a location within the Gulf of Alaska (total magnetic field intensity = 55.54 µT and inclination angle = 73.3°) (59°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 104°, r-value = 0.307, and p-value <0.0001.
Figure 3.4 Mean headings for males in the coded wire tag (CWT) treatment (n=91) exposed to northern simulated magnetic field representing a location within the Gulf of Alaska (total magnetic field intensity = 55.54 μT and inclination angle = 73.3°) (59°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 130°, r-value = 0.388, and p-value <0.0001.
Figure 3.5 Mean headings for females in the control treatment (n=92) exposed to southern simulated magnetic field representing a location west of Southern California (total magnetic field intensity = 44.40 µT and inclination angle = 56.7°) (38°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 68°, r-value = 0.35, and p-value <0.0001.
Figure 3.6 Mean headings for males in the control treatment (n=96) exposed to southern simulated magnetic field representing a location west of Southern California (total magnetic field intensity = 44.40 µT and inclination angle = 56.7°) (38°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 70°, r-value = 0.256, and p-value 0.002.
Figure 3.7 Mean headings for females in the coded wire tag (CWT) treatment (n=98) exposed to southern simulated magnetic field representing a location west of Southern California (total magnetic field intensity = 44.40 µT and inclination angle = 56.7°) (38°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 103°, r-value = 0.291, and p-value <0.0001.
Figure 3.8 Mean headings for males in the coded wire tag (CWT) treatment (n=94) exposed to southern simulated magnetic field representing a location west of Southern California (total magnetic field intensity = 44.40 μT and inclination angle = 56.7°) (38°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 97°, r-value = 0.328, and p-value <0.0001.
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Putman NF, et al. (2014b) An inherited magnetic map guides ocean navigation in juvenile Pacific salmon. Current Biology 24(4):446-450


Romer JD, et al. (2013) Survival and behavior of juvenile steelhead trout (*Oncorhynchus mykiss*) in two estuaries in Oregon, USA. Environmental biology of fishes 96(7):849-863


CHAPTER 4 - Hatchery Raceway Rearing Affects Geomagnetic Orientation in juvenile Pacific steelhead trout (*Oncorhynchus mykiss*).
Marine migrants procure and utilize food resources under diverse oceanic conditions. The ability of migrants to follow spatiotemporal variations in resource abundance is important when traversing ocean basins. However, the processes by which juvenile marine migrants navigate to foraging areas are poorly understood. Adults may spawn in proximity to ocean currents that facilitate the out-migration of offspring, but variation in ocean currents makes this method of transport unreliable (Lohmann et al. 2012; Putman et al. 2012; Staaterman et al. 2012). Therefore, it is plausible that juveniles take an active role in navigating to foraging grounds.

Evidence suggests that diverse migratory animals such as birds (*Columba livia domestica*), lobsters (*Panulirus argus*), and sea turtles (*Caretta caretta*) use the Earth’s magnetic field to navigate along their migratory routes (Gould 2010; Gould and Gould 2012; Lohmann et al. 2008; Quinn 1991). In salmonids, this hypothesis has been supported by studies of navigationally naïve juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) (Putman et al. 2014a; Putman et al. 2014b). The studies used simulated magnetic field displacements representative of specific geographic coordinates. Fields at the periphery of juvenile salmon oceanic foraging range elicited an orientation response, resulting in course correction toward the foraging grounds. Given that such abilities require an acute sensitivity for detecting magnetic fields, and anthropogenic influences can easily distort the local magnetic field (Engels et al. 2014; Fuxjager et al. 2014; Putman et al. 2014a), additional information is needed on the sensory ecology of magnetic orientation in salmonids to evaluate hatchery and management practices that may impact the navigational abilities of Pacific salmon and trout.

Pacific steelhead trout hatch in freshwater, but juveniles undertake extensive oceanic migrations to foraging grounds. Steelhead trout are iteroparous, and may spawn multiple times (adults surviving after spawning are referred to as kelts), although most steelhead reproduce only once (Seamons and Quinn 2010). Efficiently transiting between natal site and foraging grounds is energetically important and likely requires a fairly precise navigational system. The reduction in fitness of hatchery salmon compared to wild raises concerns that certain aspects of rearing may be harmful to the biology of these
fish. There are concerns regarding straying rates and the failure of hatchery fish to return to their source hatchery (Westley et al. 2013). Recent research has identified numerous aspects of hatchery operations that might streamline processes, produce higher quality hatchery fishes, and reduce interactions between hatchery and wild stocks (Ford 2011). This includes evaluation of the effects of concrete raceways on smolt to adult survival and return rates (Brignon et al. 2012; Tipping 1994; Vidergar et al. 2003). Comparisons of smolt to adult survival between baffled and standard raceways did not yield significant differences in survival (Brignon et al. 2012). Likewise, there was no significant difference in steelhead survival in raceways cleaned multiple times per week (Tipping 1994). Additionally, there was no statistical difference in smolt to adult survival when salmonids were reared in raceways that simulated natural riverine conditions versus standard raceways (Vidergar et al. 2003).

Concrete raceways are a common rearing method for mass production of hatchery salmon and trout. Status reviews of 178 hatchery programs in the Columbia River Basin evaluated hatchery practices for Pacific salmon and steelhead populations (Hatchery Scientific Review Group 2009). In 2011, an estimated 3 million steelhead were released from hatcheries in the lower Columbia River Evolutionary Significant Unit (ESU) (Ford 2011). Many of these hatcheries hold juvenile salmonids in concrete raceways at some point.

Given that successful migration is an essential component of the steelhead life-cycle we explored whether rearing practices altered orientation behavior in steelhead. We were particularly interested in iron rebar-raceways given recent evidence that fish use the Earth’s magnetic field for orientation and relatively weak distortions to the field can disrupt this ability (Engels et al. 2014; Fuxjager et al. 2014; Putman et al. 2014a). Fish were reared in different ways, with different field uniformity. Orientation was subsequently tested in fields that had previously elicited north/south swimming to determine whether rearing fish in raceways influenced magnetic orientation. Steelhead reared in distorted magnetic fields exhibited a random orientation response when tested with experimental magnetic fields at the northern and southern periphery of their oceanic foraging range. We define orientation as the direction of the test subjects’ heads relative
to magnetic north (Putman et al. 2014b). Distorted fields refer to non-natural deviations in the ambient magnetic field resulting from anthropogenic influences (Putman et al. 2014a). Typical concrete raceways contain iron rebar, so fish reared in that distorted magnetic environment may also have altered geomagnetic orientation responses.

The goal of this study is to determine the effects of typical concrete raceways on the navigation responses of salmonids. Since most hatcheries rear juvenile salmonids in raceways, and there are significant questions regarding the failure of many hatchery fish to correctly return to natal streams (Westley et al. 2013). Does rearing juvenile steelhead in distorted magnetic fields within concrete raceways influence geomagnetic orientation response, thus reducing adult return rates to their hatchery of origin? I hypothesized that when presented with magnetic fields corresponding to locations in the Gulf of Alaska and west of southern California (latitudinal periphery of oceanic foraging range), fish reared in concrete raceways will not demonstrate the north/south orientation response observed during previous studies.

Methods

Test Site and Subject Description

We conducted all tests at the Oregon Hatchery Research Center (OHRC), Alsea, Oregon (Noakes and Corrarino 2010). Juvenile steelhead from the North Fork Alsea River stock, were spawned by hand from 60 pairs during 1 of 4 spawning events from December 2012 to February 2013. Test subjects were transported from the Alsea Hatchery to the OHRC by employees of the Oregon Department of Fish and Wildlife (ODFW) on January 14th, 2014. We transported 500 additional steelhead from the same cohort to the OHRC on March 18, 2014 as replacements for fish lost to a mortality event. Juveniles were fed a commercial pellet diet (Skretting at Alsea North Fork hatchery, Bio-Oregon at the OHRC) to satiation multiple times per day. When tested, subjects ranged from 17 to 24 cm in fork length and 16 to 17 months in age from the time of spawning. Wild juvenile steelhead of that size and age would begin moving downstream to the Pacific Ocean as smolts (Romer et al. 2013).
Magnetic Orientation Analysis

Behavior Analysis

At 14 to 15 months of age from the time of spawning, test subjects were separated into two treatments: control and raceway rearing. Prior to the start of the behavioral trials, both the control and raceway fish were held for two months. Control fish were housed in a fiberglass tank (1.83 m diameter, 0.762 m depth), located in the outdoor tank yard, away from buildings and the testing apparatus. Water flow within the tank was approximately 56 - 76 liters per minute (lpm) and was increased with increasing fish size. The fish remained in the tank for the duration of the project. Raceway reared individuals were housed for two months prior to testing in a nylon mesh rectangular net pen (0.91 m width, 1.83 m length, 0.76 m depth), situated in a concrete raceway (3.6 m width, 24.3 m length, 0.76 m depth) located at the Oregon Hatchery Research Center. Water flow within the raceway was approximately 570 lpm. Total magnetic field intensity within the net pen ranged from 43.32 microTeslas (µT) to 51.62 µT and inclination angles ranged from 58.4° to 67.1°, representative of magnetic field conditions spanning the coast of Oregon to Southern California (Appendix I Figure A1.3). The magnetic test fields were 55.54 µT and 73.3° (59°N, 145°W) representative of a location in the Gulf of Alaska (northern field) and 44.40 µT and 56.7° (38°N, 145°W) representative of a location west of southern California (southern field) (Figure 4.1). This indicates a considerable overlap between the magnetic intensity and inclination angles of the test and rearing fields. Control fish experienced ambient magnetic field conditions (field intensity = 53.69 µT, inclination angle = 67.2°). Test subjects remained in the net pen until individuals were selected for the behavioral trials. Each group consisted of 500 individuals that were divided into two sub-groups (Northern field and Southern field) of 250 individuals each (see Appendix I Figure A1.2).

Treatment testing order was assigned by alternating control and raceway reared groups each day and alternating North and South testing fields (north day, south day) to avoid bias due to diurnal or weather effects. Testing occurred outdoors between 0730 and 1700 hours, Pacific Daylight Time (PDT). Each fish was tested once. Subjects were
placed in opaque circular arenas 30.5 cm in diameter filled to a depth of 21.5 cm with water from neighboring East Fall Creek (the same water source as rearing conditions) (Appendix I Figure A1.1). Water was completely emptied from each container after each test and the container was rinsed with running water from the same source to clear debris from the test arenas. Containers were re-filled for the subsequent tests to maintain temperature and dissolved oxygen levels. One fish was placed in each of the 25 circular arenas and allowed to adjust to ambient conditions for 10 minutes. After the initial adjustment period, the test subjects were exposed to a magnetic field that was changed by two orthogonally arranged four-coil systems (outer vertical coil length equals 3.57 m and inner horizontal coil length equals 3.33 m) connected to a DC power supply housed in a nearby building (<10 m distance) (Putman et al. 2014b) (see Appendix I Figure A1.1). Fields were altered by pairing specific combinations of amperage for the horizontal and vertical components of the coil to generate simulated magnetic fields, corresponding to geographic points at the latitudinal periphery of steelhead oceanic foraging range. Test duration consisted of 8 minutes (7 for fish to adjust to altered conditions and 1 minute used for photo analysis). Magnetic fields were determined by the International Geomagnetic Reference Field (IGRF-11) (Findlay et al. 2010). Magnetic fields were measured using a triaxial fluxgate magnetometer (Applied Physics 520A). Field uniformity was better than ± 1%, and no magnetic gradients were detected within the test arenas. A black mesh cover was placed over the framework of the test apparatus resulting in a 70% reduction of incident light, to minimize stress to the fish. Solar cues were visible throughout the trials.

Using two GoPro® cameras mounted above the testing arena, digital photographs were taken of the fish in the arenas at 10 second intervals. Images were analyzed in Microsoft® PowerPoint by overlaying a compass figure and measuring the direction that the head was pointing, relative to magnetic north. Six measurements were averaged for each fish. Measurements were recorded to the nearest 5° by assistants unaware of the experimental conditions. Experiments were conducted between May 5th, 2014 and June 15th, 2014.
Statistical Analysis

Statistical analysis was conducted using Oriana 2 (Kovach Computing Services). A Rayleigh uniformity test was used to assess orientation responses in each treatment group. A Mardia-Watson Wheeler nonparametric test was conducted to analyze pairwise comparisons to assess differences in orientation response between treatments. A multi-sample test was conducted to assess differences among all respective treatments, consistent with previous studies (Putman et al. 2014a; Putman et al. 2014b).

Results

The control treatment group showed significant orientation responses when exposed to either the northern or southern fields (Rayleigh uniformity test, p-value = <0.0001 for both treatments). The mean headings for the northern and southern fields were 111° (Rayleigh r value = 0.304, n = 250) and 86° (Rayleigh r value = 0.26, n = 240), respectively. Individuals from the raceway treatment group exposed to the northern field showed random orientation responses (Rayleigh uniformity test, p-value = 0.795, Rayleigh r value = 0.038, n = 163) as did those exposed to the southern field (Rayleigh uniformity test, p-value = 0.207, Rayleigh r value = 0.09, n = 196). Mean heading for the northern and southern fields were 95° and 130° respectively, but the orientation of the fish was random in both cases (Mardia-Watson-Wheeler test, p-value = 0.808). Since both of these values fall within the same quadrant of the circular distribution, there are no apparent differences in orientation response within the raceway treatments. The differences between treatments are detailed by field (Mardia-Watson Wheeler pairwise test north p-value = 0.003, south p-value = 0.015). Summary data are presented in Tables 4.1 and 4.2. Circular distributions of mean orientation angles are displayed by treatment in Figures 4.1-4.4.

Discussion

Our data show that rearing juvenile salmonids in concrete raceways affects the orientation response (degree of scatter) of juvenile steelhead. Whether this influenced magnetic orientation could not be determined, as in these experiments, the testing
magnetic fields had no effect on orientation. We observed similar orientation responses in previous studies of steelhead reared inside hatchery tanks (Putman et al. 2014a). The measured intensities and inclinations of magnetic fields within the net pen were very similar to our test fields. There was a complete overlap between the southern test field and the magnetic fields within the pen. The magnetic field characteristics are typical of environmental fields found throughout the Northeastern Pacific Ocean. However, there was no overlap between the northern test field and the raceway fields, although the values were fairly close. Since the test fields were not very different compared to the rearing field conditions, this may explain the random orientation response.

Millions of juvenile salmonids are reared in concrete raceways in California, Oregon and Washington every year. Researchers have attempted to address causes of straying and particularly the failure of hatchery fish to return to their source hatchery, but until now the effects of magnetic distortion within raceways had not been considered or evaluated. The important management questions are, what induces this effect and how long does this effect last? At this time, there is no indication that the magnetic distortion within concrete raceways influences orientation response because there is no indication that the fish were using the magnetic field to orient during our tests. This does not mean that raceway rearing practices do not have an influence on orientation behavior, but specific issues have not been identified. This question is beyond the scope of this project, but merits further study.
Table 4.1 Summary results of orientation responses of control and raceway treatments exposed to northern test field (Gulf of Alaska) and southern test field (west of Southern California). Rayleigh uniformity test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Location</th>
<th>Total Field Intensity (µT)</th>
<th>Inclination Angle (°)</th>
<th>Mean Heading (°)</th>
<th>Rayleigh r</th>
<th>Rayleigh p</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (North)</td>
<td>59°N, 145°W</td>
<td>55.55</td>
<td>73.3</td>
<td>111</td>
<td>0.304</td>
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<td>Control (South)</td>
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<td>0.26</td>
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<tr>
<td>Raceway (South)</td>
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<td>44.46</td>
<td>56.7</td>
<td>130</td>
<td>0.09</td>
<td>2.07E-01</td>
<td>196</td>
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</table>
Table 4.2 Treatment comparisons of orientation response northern versus southern simulated fields (Gulf of Alaska vs west of southern California). Mardia Watson-Wheeler Pairwise test.

<table>
<thead>
<tr>
<th>Treatment</th>
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<tr>
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</tr>
<tr>
<td>Control vs Raceway (South)</td>
<td>8.439</td>
<td>0.015</td>
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</table>
Figure 4.1 Mean heading for control treatment (n=250) exposed to northern simulated magnetic field representing a location within the Gulf of Alaska (total magnetic field intensity = 55.54 µT and inclination angle = 73.3°) (59°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 110°, r-value = 0.304, and p-value <0.0001.
Figure 4.2 Mean heading for control treatment (n=240) exposed to southern simulated magnetic field representing a location west of Southern California (total magnetic field intensity = 44.40 µT and inclination angle = 56.7°) (38°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 86°, r-value = 0.26, and p-value <0.0001.
Figure 4.3 Mean heading for raceway treatment (n=163) exposed to northern simulated magnetic field representing a location within the Gulf of Alaska (total magnetic field intensity = 55.54 µT and inclination angle = 73.3°) (59°N, 145°W). Red bar represents 95% confidence intervals. Mean vector was 95°, r-value = 0.038, and p-value = 0.795.
Figure 4.4 Mean heading for raceway treatment (n=196) exposed to southern simulated magnetic field representing a location west of Southern California (total magnetic field intensity = 44.40 µT and inclination angle = 56.7°) (38°N, 145°W). Black bar represents 95% confidence intervals. Mean vector was 130°, r-value = 0.09, and p-value = 0.207.
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Putman NF, et al. (2014b) An inherited magnetic map guides ocean navigation in juvenile Pacific salmon. Current Biology 24(4):446-450


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CHAPTER 5 – CONCLUSIONS
Analyses of common hatchery and management practices has provided insight into possible effects on the homing and straying of steelhead populations. Use of coded wire tags in mark selective fisheries is a common method for estimating return rates of Pacific salmon and trout. I initially hypothesized that tagged individuals would show disruptions of the orientation responses that have been reported from previous studies. Coded wire tagged and non-tagged animals both oriented eastward, contrary to the results from control steelhead in previous studies. However, responses of control animals during these behavioral trials were probably complicated by health and handling of the fish. The magnetic analysis of the coded wire tags showed that total field intensities were consistent between tagging units and among tags within a single unit. Variations in the magnetic fields of the coded wire tags were 2 to 3 orders of magnitude weaker than the Earth’s magnetic field.

Examination of the potential differences in orientation responses between sexes offers additional understanding regarding homing mechanisms in salmonids. I hypothesized that females would demonstrate less variation in their orientation responses compared to males, since females are significantly more likely to migrate as smolts than are males. Contrary to my prediction, there was no significant sex difference in orientation responses. This is interesting, since female steelhead smolts migrate earlier than males, in higher proportion than males and have a greater gametic benefit from ocean migration. My results indicate that sexual differences in steelhead smolt migration are not a consequence of differences in orientation abilities of females and males.

The raceway rearing effects study was a test of the generality of the disruption of geomagnetic orientation responses by hatchery rearing structures. I predicted that raceway reared individuals would be exposed to magnetic distortions of the ambient fields within a concrete raceway, and they would not orient in the directions observed in control fish. There was a considerable difference in orientation response between the control and raceway rearing treatments. The control group was significantly oriented, while the raceway group showed a random orientation response. This indicated an inability of juvenile salmonids to derive positional information from a magnetic “map sense” when they were reared in distorted magnetic fields. This could result in straying
and reduced return rates of adults to their source hatcheries. This information will add to the current knowledge of magnetoreception in fishes, draw comparisons between vertebrate species, and inform current hatchery and management practices.
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Romer JD, et al. (2013) Survival and behavior of juvenile steelhead trout (*Oncorhynchus mykiss*) in two estuaries in Oregon, USA. Environmental biology of fishes 96(7):849-863


mykiss) differ among species, life history types, and populations. Canadian Journal of Fisheries and Aquatic Sciences 70(5):735-746

APPENDIX I
Figure A 1.1. Experimental testing apparatus used for behavioral trials. Merritt 4-coil system with a platform centered within the coil to provide uniform exposure to simulated magnetic fields. Platform consists of 25 circular testing arenas (5 arenas per row, 5 rows) with two GoPro® cameras to document orientation behavior during the trials.
Figure A 1.2. Northern and Southern magnetic fields simulated by using unique combinations of amperage to produce fields representative of the latitudinal boundaries of steelhead oceanic foraging range. Circles with cross pattern indicate the intersection of inclination angle and total magnetic field intensity. The northern simulated field represents a location in the Gulf of Alaska (59°N, 145°W) and the southern field represents a location west of southern California (38°N, 145°W).

Figure A 1.3. Magnetic field intensities measured using a fluxgate magnetometer within a nylon mesh rectangular net pen (0.91 m width, 1.83 m length, 0.76 m depth), situated in a concrete raceway (3.6 m width, 24.3 m length, 0.76 m depth) located at the Oregon Hatchery Research Center. Total magnetic field intensity within the net pen (yellow arrow) ranged from 43.31 microTesla (µT) to 51.62 µT. The magnetic test fields (blue circle with cross) were 55.54 µT and 73.3° (59°N, 145°W) representative of a location in the Gulf of Alaska (northern field) and 44.40 µT and 56.7° (38°N, 145°W) representative of a location west of southern California (southern field). Ambient magnetic field conditions at the test and rearing site were (field intensity = 53.69 µT, inclination angle = 67.2°) indicated by the yellow star. Note: Map measurements (ex. 55,000) are in nanoTesla. Map courtesy of NOAA and NGDC 2014 (http://ngdc.noaa.gov/geomag/WMM).
APPENDIX II
Materials

Tri-axial fluxgate magnetometer (Applied Physics 520A)

GW Instek PSW 80-13.5 Programmable Multi-Range Switching DC Power Supply, 0-80 Volts, 0-13.5 Amps, 360 Watts

14 AWG THHN Stranded Building Wire (762 m/spool) X 2 Spools

#10 7.6 cm Stainless steel screws (2.27 kg)

1.27 cm – 1.22 m x 2.44 m CDX Plywood – 5Ply = 6 sheets

5 cm x10 cm, 3.56 m boards = 20

20 cm x 20 cm x 40 cm Cement Blocks (Tower supports) = 40 blocks

Assorted power tools (drills, saws, etc.)

Construction Methods

A Merritt 4-Coil system consists of 4 horizontal and 4 vertical coils (rings) wrapped in wire. The outer rings consist of “half sandwiches” as illustrated in Figure A 2.1. They are composed of one intact 5 cm x 10 cm board (base) and one 5 cm x 10 cm board with a subdivided width (5 cm). The subdivided portion of the board is offset on one end by 10 cm and affixed to one side of the base forming an “L” shape. This forms a tongue and groove that will aid in the assembly of each ring. The “L” shape also provides a groove which houses the wire when wrapped around the outer rings. To determine the length of the base of the half sandwich boards we used the equation: (D*1.011) + (2*W) where D is the length of wire along one side of the full sandwich boards and W is the width of the board. A completed outer ring is depicted in Figure A 2.2. The interior rings consist of full sandwich boards that are constructed using 2 full width 5 cm x 10 cm boards and one subdivided board affixed along one edge of the boards as illustrated in Figure A 2.3. Similar to the half sandwich boards, the middle subdivided board is offset to form a tongue and groove construction. The groove length of the full sandwich board is 3.35 m. Once the full sandwich boards are assembled, 4 sandwiches are used to form a ring with the corners secured by stainless steel screws (Figure A 2.4). Once the rings are
constructed, wire is wrapped around each frame starting in the top left corner and wrapping the wire counterclockwise. Mark this corner and the direction the wire is wrapped as this will be important during the coil raising stage. The outermost rings (North, South, Top, and Bottom) are wrapped 26 times while the innermost rings are wrapped 11 times.

Once all of the respective rings (interior and exterior) have been assembled and wrapped with wire, the base for the testing apparatus is constructed. The base consists of a series of concrete cinder blocks that are situated at all four corners and at midpoints on each side to support the rings. The blocks need to be level prior to incorporating the rings. Once this is achieved, the base outer ring is placed onto the center of the blocks. When the base is secured, square, and level, the interior rings are placed inside the base (resting on the cinder blocks) Figure A 2.5. Spacing between the outermost vertical rings and the inner rings is 124 cm. The spacing between the two innermost vertical rings is 87 cm. For the horizontal rings, the spacing between the bottom and top outer rings to the middle rings is 129 cm and the spacing between the two middle rings is 88 cm. Interior rings are affixed using supports until the remaining outer rings are placed on the coil. External supports are added to the exterior rings in order to stabilize the coil (Figure A 2.6). A series of connectors join the wires from each ring so that there are a total of four wires that are connected to the DC power supply (North, South, Top, and Bottom). The completed test apparatus and arena is depicted in Figure A 2.7.

When coil construction is complete, calibration can occur using a tri-axial fluxgate magnetometer (Applied Physics 520A). Multiple measurements are taken within the coil at each corner (Northwest, Northeast, Southwest, Southeast) and at the midpoints between corners. Measurements are also taken in the center of the testing arena, which is approximately the center of the coil. Each measurement is taken in the X, Y, and Z axes and total magnetic field intensity and inclination angle are calculated (Table A 2.1).
Figure A 2.1. Half sandwich boards used to construct the outer rings of the test apparatus.
Figure A 2.2. Completed outer horizontal coil rings with tongue and groove corners.
Figure A 2.3. Full sandwich board with tongue and groove used to construct interior vertical coils. Wire sits within the center groove.
Figure A 2.4. Completed inner vertical coil ring with tongue and groove construction. Wire is wrapped around the center groove.
Figure A 2.5. Vertical ring installation on cinder blocks with additional support to secure the rings during coil construction.
Figure A 2.6. Installation of outer coil rings and extra supports.
Figure A 2.7. Completed test apparatus and testing arena.
Table A 2.1. Merritt 4-Coil calibration measurements using a tri-axial fluxgate magnetometer (Applied Physics 520A). Measurements of the ambient magnetic field were taken at multiple locations within the test coil in the X, Y, and Z axes and field uniformity was better than 1% for magnetic field intensity (µT).

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