THE INFLUENCE OF CONSPECIFIC LARVAL ODOR ON THE RIVERINE AND LACUSTRINE MIGRATION OF SEA LAMPREY.

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Fisheries and Wildlife

2011

ABSTRACT

THE INFLUENCE OF CONSPECIFIC LARVAL ODOR ON THE RIVERINE AND LACUSTRINE MIGRATION OF SEA LAMPREY.

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Migratory phase sea lampreys (*Petromyzon marinus*) use odors released by conspecific larvae on the spawning ground to identify spawning habitat. In the aquatic environment, olfaction has the potential to identify the chemical stimuli associated with distant resources at greater distances than any other known sensory capability. I present two studies that evaluate the importance of putative components of larval odor when sea lampreys encounter them in a river during upstream migration and in the lake during the lacustrine migration. Chapter 1 focuses on how the addition of three putative components of larval odor to a river, affects sea lamprey upstream movement and channel bias in comparison to the full larval odor cue. Chapter 2 characterizes the sea lamprey's near-shore lacustrine migration and evaluates how the two most common synthesized putative components of larval odor may mediate the migration behavior. These two thesis chapters offer support for the continued investigation of the effects of PADS, PSDS, and PZS on the migration of sea lamprey and suggests that specific attention should be given to how these components function at the river mouth.

To my mom for flash cards in the summer,
To my dad for showing me what it means to work hard,
To my wife for her constant patience and support.

AKNOWLEDGEMENTS

I would like to thank my advisor Dr. C. Michael Wagner, and members of my graduate committee Dr. Weiming Li, Dr. Jim Miller, and Dr. Daniel Hayes for intellectual and technical support throughout this research. I thank all personnel at the United States Geological Survey Hammond Bay Biological Station, Millersburg, Michigan, and the United States Fish and Wildlife Service Marquette Biological Station, Marquette, Michigan, for their assistance in animal capture, housing, maintenance, and technical support during these projects. In addition, I would like to thank specific individuals at the station who have worked directly with me on my projects in some capacity including: Dr. Roger Bergstedt, Christopher Holbrook, Erick Larson, and Dr. Nicholas Johnson. I would like to give special thanks to field technicians and colleagues for their hard work, dedication, and patience regarding these projects: Tyler Buchinger, Adam Thomas, Eric Willman, Cory Olaf Brant, Brett Diffin, Amber Masters , and Jeff Yaklin.

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INTRODUCTION TO THE THESIS

Chemoreception entails the ability to perceive chemical stimuli in the environment. Olfactory perception of odors in the environment can initiate changes in reproduction, schooling, defensiveness, feeding, migration and a suite of other behaviors (Kasumyan 2004). Many aquatic organisms rely on olfactory cues in combination with the physical environment to direct migrations (Lohmann et al. 2008; Debose & Nevitt, 2008; Kasumyan 2004). Olfactory signals allow for identification of remote resources at greater distance than any other known search cue (Kasumyan 2004). In long-distance spawning migrations in fishes, encountering an olfactory signal indicates the presence of certain habitat but the physical environment in which the cue is encountered must be used to identify the organism's current orientation to the odor source (Webster et al. 2001, Moore & Crimaldi 2004). The fluid environment of the migration dictates how the odor signal is transported and what sensory capabilities will be useful for the fish to orient to the signal (Vickers 2000; Moore & Crimaldi 2004; Debose & Nevitt 2008).

The sea lamprey (*Petromyzon marinus L.*) relies on pheromones to mediate migration (Teeter 1980; Bjerselius et al. 2000) and reproduction (Li et al 2003; Johnson et al. 2009). The sea lamprey is non-homing and semalparous (Bergstedt & Seelye 1995; Waldman et al. 2008), investing its total lifetime fitness into a single reproductive event that is orchestrated by at least two olfactory cues. Migratory phase sea lamprey in the lake and river use bile acids released by conspecific larvae as a distant cue to identify upstream spawning habitat (Bjserlius et al. 2000, Vreize et al. 2001; Vreize et al. 2011). Once in a spawning river, female lamprey are attracted to a mature male's sex pheromone that functions as a close proximity signal to encourage upstream movement and to bring females to the nest (Li et al. 2003, Johnson et al 2009). Between the two pheromones, four bile acid and bile alcohol derivatives are postulated to be pheromone components of both pheromone systems (Haslewood and Tokes, 1969; Li et al., 1995; 2002, Yun et al., 2003; Sorensen et al., 2005). At this time, three putative components of the larval odor petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), petromyzonal sulfate (PS), have been described (Polkinghorne et al. 2001; Sorensen et al., 2005), synthesized (Sorensent et al. 2005), confirmed via elecrtroolfactogram (EOG) (Sorensen et al. 2005), and found to be as attractive as the full larval odor when tested in the lab (Fine and Sorensen 2008).

The sea lamprey is invasive and damaging to large bodied fishes, altering the food web of the Great Lakes (Madenjian et al. 2008; Smith and Tibbles 1980). Control efforts focus on removing adults from rivers prior to spawning and carefully timed lampricide treatments of rivers to kill larvae prior to their out migration as newly metamorphosed parasites to the lake (Christie and Goddard, 2003). Although effective, there are environmental concerns with prolonged pesticide treatment; additional and novel control approaches that attack vulnerabilities in the sea lamprey's life history are becoming increasingly desirable (Christie 1974; Simberloff et al., 2005). This thesis outlines the potential to use the three identified synthetic components of larval odor to manipulate sea lamprey migration behavior and to develop novel methods for sea lamprey control with pheromones. To accomplish this we evaluated what behaviors these compounds elicit during the lake and river phases of migration.

In Chapter 1, titled, "The behavioral response of migratory phase sea lamprey to three synthesized components of larval odor in a river," we specifically focus on how attractive the synthetic components are in a river compared to the full larval odor cue. In this field test, we tested the effects of six synthesized pheromone treatments, including two concentrations (10^{-12} M, 10^{-13} M) of three combinations of synthesize larval components (PADS, PADS + PSDS, PADS + PSDS +PS) to evaluate the effects of these compounds on upstream movement and channel bias. We aimed to identify the most attractive combination and concentration of components and hypothesized that different combinations and concentrations of components would be more attractive than others, in the same way observed in a laboratory setting (Sorensen et al 2005). We unexpectedly did not observe any of the synthetic larval treatments eliciting attractive behaviors in the river. Either synthesized components do not represent the most attractive components in larval extract, or they do not represent the full compilation of compounds necessary to guide the riverine migration in the same way as the full larval extract.

The finding in Chapter 1 led us to our question in Chapter 2, titled, “A pilot investigation of migratory phase sea lamprey habitat selection behavior at a river mouth using manual acoustic telemetry.” Given that PADS and PSDS are the two most common components produced by larval lamprey (Sorensen et al. 2005), these components could be more important in diffuse environments, such as at the river mouth. There is a potential that the components could independently function during the lacustrine to riverine transition of the migration. In order to accomplish this evaluation, we characterized the behaviors used by sea lamprey to locate river plumes and localize the river mouth in the near shore environment with the use of directional acoustic telemetry. Our findings suggest that there were two dominant search strategies and these supported the migration modes described by Vrieze et al. (2011). The first strategy, coastal search (CS), matches common long distance search strategies observed in other marine fishes (Dusenbery 1989; Debose & Nevitt 2008). The second strategy, river plume investigation (RPI), is a close range river plume search strategy consistent with observations of foraging insects that increase their turn angle after encountering a resource cue to stay in the vicinity of the resource (Bell 1990). CS specifically involved straight movements parallel to the shoreline, roughly perpendicular to the cue field, coupled with vertical and horizontal excursions that allow for efficient sampling of the three dimensional coastal environment. The horizontal sampling was not observed by Vrieze et al. (2011), and would be an effective strategy for locating smaller river plumes, which are dynamic and have the potential to be confined very close to the shoreline (Gordon et al. 2004). RPI specifically involved movement with large angle turning and stopping that maintained sea lamprey position near the river and in contact with the estimated river plume. We cannot confirm an effect of synthesized PADS and PSDS at the river mouth because we observed an insignificant 37 % increase in the likelihood of lamprey performing extended periods of RPI (n=13) at a river mouth. Although not significant, this is a large increase and given the small sample size, we consider these compounds worthy of further investigation. These components in concert with other cues, may maintain the animals in the vicinity of the river mouth, which could increase the likelihood of future river entry, and could make the application of PADS and PSDS a relevant control strategy.

These two chapter offer support for the continued investigation of the effects of PADS, PSDS, and PZS on the migration of sea lamprey and suggest that specific attention should be given to the how these compounds function at the river plume interface. Future studies will require further characterization of the coastal migration and a stronger understanding of how river plume dynamics affect the dispersion of olfactory clues in the coastal environment.

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CHAPTER 1

THE BEHAVIORAL REPSONSE OF MIGRATORY PHASE SEA LAMPREY TO THREE SYNTHESIZED COMPONENTS OF LARVAL ODOR IN A RIVER.

ABSTRACT

During riverine migration, sea lamprey, *Petromyzon marinus*, uses odor released by conspecific larvae from previous generations to identify suitable spawning habitat upstream. Odor is the only cue known to mediate stream selection and channel navigation during the river phase of migration, and presents itself as an ideal system for behavioral manipulation. Three putative components of larval odor (PADS, PSDS, and PS) have been identified, synthesized, confirmed via elecrtroolfactogram (EOG), and found to be as attractive as the whole larval odor to migratory phase sea lamprey in controlled flume environments. In a field test, we did not find any of six synthesized pheromone treatments, including two concentrations of three combinations of PADS, PSDS and PS, to elicit detectable attraction through either stream channel bias or upstream movement tendency in the river. Either the synthesized components do not represent the most attractive components in larval extract, or they do not represent the full compilation of compounds necessary to guide the riverine migration in the same way as the full larval extract.

INTRODUCTION

The sea lamprey (Petromyzon marinus) is an invasive species of the Laurentian Great Lakes believed responsible for a shift in the ecosystem's food web due to heavy predation on the lake trout (Salvelinus namaycush), the dominant native piscivore, and the complete extinction of three endemic cisco species (Madenjian et al. 2008; Smith and Tibbles 1980). Current management focuses on removing adults from rivers prior to spawning and carefully timed lampricide treatments of rivers to kill larvae prior to their out migration as newly metamorphosed parasites to the lake (Christie and Goddard, 2003). Although effective, there are environmental concerns with prolonged lampricide treatment and novel control approaches that attack vulnerabilities in the sea lamprey's life history are becoming increasingly desirable (Christie 1974; Simberloff et al. 2005).

The sea lamprey is semalparous, investing its total lifetime fitness into a single reproductive event that is orchestrated by at least two olfactory cues. It has been shown that sea lamprey migrating in the lake and river cue in on bile acids released by conspecific larvae to identify upstream spawning habitat (Bjserlius et al. 2000; Vreize et al. 2001; Vreize et al. 2011). Once in a spawning river, female lamprey are attracted to a mature male's sex pheromone that functions as a close proximity signal to encourage upstream movement and to bring females to the nest (Li et al. 2003, Johnson et al 2009). Because this critical stage of their life history is reliant on innate responses, the species is vulnerable to control through behavioral manipulation with chemical signals (Sorensen & Vrieze 2003; Li et al. 2007). Behavioral manipulation is defined as the use of stimuli to stimulate or inhibit the expression of a behavior and subsequently alter the function of a behavioral stanza (Gould 1991; Foster & Harris 1997). Olfaction-based manipulation is promising with both odor cues. First, both sexes of migratory phase lamprey are obligatorily reliant on olfaction to identify a spawning river from the river mouth, as was shown by Vrieze et al. (2010) when nasally occluded migrants in Lake Huron were unable to locate a river effectively. The second cue, mature male odor, is highly attractive to female migrants as they locate a mate, and as shown by Johnson et al. (2005), the odor is capable of drawing mature female lamprey into traps. Although temperature and light are important cues in regulating the timing of migratory movements (Binder and McDonald 2007; Binder et al. 2010; Vrieze et al. 2011), only olfactory cues appear responsible for habitat selection and mate finding (Wagner et al. 2009).

The lamprey migration from the lake to the spawning ground is susceptible to manipulation based control because larval odor offers a tool that naturally functions to concentrate both sexes at a time in the lamprey's life cycle when individuals are the least numerous, have stopped feeding and must synchronize maturation with others to successfully reproduce before an imminent death (Fine & Sorensen 2008; Li et al. 2007; Wagner et al. 2009). At this time, three putative components of the larval odor petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), petromyzonal sulfate (PS), have been described (Polkinghorne et al. 2001; Sorensen et al., 2005), synthesized (Sorensen et al. 2005), confirmed via elecrtroolfactogram (EOG) (Sorensen et al. 2005), and found to be as attractive as the full larval odor when tested in the lab (Fine and Sorensen 2008).

Laboratory and field studies designed to disentangle complex behaviors by simplifying and controlling the physical variables often arrive at different findings (Johnson and Li 2010). In the lab elecrtroolfactogram (EOG) results and controlled flume environments have provided a foundation for evaluating odor mediated behavior in sea lamprey (Johnson and Li 2010). Now it is important to evaluate the currently identified components in appropriate environmental context to the questions at hand (Johnson & Li 2010). For example, Hartman and Abrahams (2000) found that fathead minnows only react to alarm odors in a laboratory setting under low light conditions when vision is a less reliable tool for predator avoidance. Contradictory to this study, a field study designed to verify the finding could not find light level to affect the avoidance pattern of fat head minnows under any concentration of fathead minnow alarm cue and they were unable to identify the interacting or overriding variable(s) (Tremaine et al. 2005). This example displays the advantage and disadvantage to field based studies; generally these test offer more environmentally relevant conclusions, but it is difficult to disentangle the environmental complexity in the occurrence of a null finding. Therefore, it is important to take careful steps from the lab to highly controlled field tests and finally towards the most natural settings to identify the function and relevance of an odor and the elicited behavior.

The objective of this study was to evaluate the effect of three synthesized larval components on upstream movement and channel bias of migratory phase sea lamprey. We specifically address; 1) do synthesized components affect upstream migration and are they as attractive as the whole larval odor as was described by Fine et al (2004), in the lab and; 2) in the case that the synthesized components are attractive, what concentration and combination of components are most attractive, and are any of the three components more effective than others as described by Fine and Sorensen (2008). Test treatments included three combinations (PADS, PADS+PSDS, PADS +PSDS+PS) and two concentrations (10^{-12} M, 10^{-13} M) of synthesized components that were applied down one of two sides of a single river channel opposite a methanol control. In 2007, we compared the attractiveness of migratory phase sea lamprey to three synthesized components of larval odor, in order to conclude whether the components affect the behavior of migratory phase lamprey in a natural river, and consequently whether the components elicit any behavior that would suggest that the compounds could be implemented in a management scenario during the river phase of migration.

METHODS AND MATERIALS

Site

In 2007, we investigated if migratory phase sea lamprey were attracted to synthesized pheromone components in a natural stream in order to evaluate their impact on upstream river migration. On 47 nights from the 9th of May to the 5th of July 2007 we tested the channel bias of sexually immature, migratory phase sea lamprey in a bifurcated fourth order Michigan stream. This test was conducted by adding combinations of synthesized larval odor components to one side of the channel per the approach of Wagner et al. (2009). The study site is located on the Ocqueoc River above a sea lamprey barrier (Fig. 1-1); it is a river with good spawning habitat and known to historically recruit large numbers of migrants (Applegate 1950). The study site is useful for studying the response of sea lamprey to olfactory cues because it offers appropriate environmental conditions while remaining devoid of annual runs of sea lamprey and natural larval odor.

Subjects

Adult, migratory-phase sea lampreys used in the study were obtained by the US Fish and Wildlife Service and the Department of Fisheries and Oceans Canada from barrier integrated traps operated during the annual spawning run (April-July 2007) in several rivers discharging to Lake Huron. All procedures involving sea lamprey were approved by the Michigan State University Animal Use and Care Committee under permit number 04-07-033-00. After capture, the lampreys were transported at a maximum density of 0.5 lampreys L^{-1} of lake water in hatchery trucks to the Hammond Bay Biological Station near Millersburg, MI. Upon arrival at the station, the animals were separated by sex and held in flow-through holding tanks receiving fresh Lake Huron water at a rate sufficient to achieve 100% exchange every 30 minutes. Water temperatures in the holding tanks ranged from 5 to 18 (° C) based on ambient temperature of Lake Huron water.

We implanted female sea lampreys with a 23-mm passive integrated transponder (PIT) tag through a 2-3 mm lateral incision in the mid-abdominal region. The incision was sealed with a veterinary adhesive, Vetbond (Minnesota Mining and Manufacturing, St. Paul, Minnesota), immediately after tag insertion. The surgery typically took less than 30 seconds and no anesthesia was deemed necessary due to the short period of discomfort and the potential for anesthesia to affect olfaction and behavior during the study (Lewis et al 1985; Losey & Hugie 1994; Munday & Wilson 1997). After surgery, animals were returned to the holding tanks for up to 24 hours before they were stocked into holding cages in the stream. Mortality was low and no tag loss was observed during this process. Approximately 24 hours prior to release, lamprey were moved from the lab to metal mesh acclimation cages. Lampreys were released in trials at sunset two hours after the start of test article pumping 150 meters upstream.

*T*reatments

The treatments included larval extract, methanol, petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), and petromyzonal sulfate (PS) (Fig. 1-2). Field application of synthesized larval components and extracted larval odor was approved by the Michigan Department of Environmental Quality and United States Environmental Protection Agency through Experimental Use Permit number 75437-EUP-1. We tested a total of eight treatments: 1) PADS at 10^{-12} M vs. methanol control (N=4), 2) PADS at 10^{-13} M vs. methanol control (N=6),

3) PADS + PSDS at 10^{-12} M vs. methanol control (N=4), 4) PADS + PSDS at 10^{-13} M vs. methanol control (N=6), 5) PADS + PSDS + PZS at 10^{-12} M vs. methanol control (N=4), 6) PADS + PSDS + PZS at 10^{-13} M vs. methanol control (N=6), 7) Larval extract at 10^{-12} M vs. methanol control (N=5), 8) Larval extract at 10^{-13} M vs. methanol control (N=5), (Table 1-1). Additionally a methanol vs. methanol control was used to evaluate stream bias unrelated to treatment (N=6).

The synthesized pheromone components were produced by the Bridge Organics Co., Kalamazoo, Michigan in 2007 and its purity was verified at Michigan State University by Dr. Xiaodan Xi via a combination of NMR, MS, GC-MS/MS, HPLC, and TLC. Observed purity was > 95 %. Test articles were stored at or below – 20° C. Larval odor was collected in 2006 from a captive population of approximately 50 000 larval sea lampreys that were held in 500 liter flow through tanks filled with Lake Huron water at Hammond Bay Biological Station. The tanks were filled with sand substrate and the larvae were fed yeast weekly. Larvae odors were concentrated by shutting off the tank flow at night and then the held water was passed through vertical columns containing 500 g of methanol-activated absorbent resin, Amberlite XAD7HP, by the method described by Fine et al. (2006). The final extract was also stored at or below -20° C until use to ensure stability. The half life of PADS, PSDS and PS at 15 ° C in natural light conditions is between 48 and 72 hours (Sorensen & Hoye, 2007; Fine & Sorensen 2010).

The stream discharge was measured using the USGS midsection method as described by Gore (1996) with a flow meter (Flo-Mate Model 2000, Marsh-Mcbriney) (m^3 sec^{-1}), to estimate the amount of synthesized component necessary to reach the desired pheromone concentrations. We individually diluted the components with 1 mL of methanol and applied it to the river by adding the methanol solution's of putative synthesized components into a 10 L carboy of river water and pumping the pheromone spiked water into the river at a rate of 1 L/h via a peristaltic pump (Masterflex 7553-70, Cole-Parmer).

Experimental Procedures

The holding cages at the field study site were located 150 meters downstream of a pheromone application site to allow for monitoring movements through key points of the study site. Two hours prior to lamprey release, we applied two test articles, separately, into one of two assessment traps integrated into a temporary barrier at the upstream end of the study site (Fig. 1-1). To ensure natural movement tendencies, we released lamprey at sunset, two h after the beginning of treatment application, and monitored for the next 5 h for movement past three PIT-tag readers (Oregon RFID); 1) the first located just upstream of the lamprey release to evaluate if animals entered the study site; 2) The second and third were located on the left and right halves of the channel to monitor if animals moved up the control or treatment side of the channel. Prior to each trial, we checked the PIT reader stations to ensure correct operation and to confirm the appropriate sensitivity of all three PIT reader stations, the multiplexer and associated antennas, via a test PIT tag.

Data Analysis

The number of subjects moving upstream and the side chosen by those that moved upstream was recorded. Temperature and a suite of other characteristics including maturity make it difficult to compare upstream movement and animal behaviors over the 47 day study period. Before testing the effects of the treatments on upstream movement, we accounted for the effects of measureable variables such as start temperature and Julian date on upstream movement tendency. We completed this by using a generalized linear model and a Pearson's correlation; in particular we looked for correlations between Julian date, start temperature and upstream movement. To investigate the effect of pheromone concentration and combination on the tendency to swim upstream we compared the mean residual variation by treatment to an expected value of 0 with one-way t-tests (residuals untransformed). To ascertain whether lampreys tended to select the stimulus side of the channel either combinations of synthesized components or larval extract) vs. methanol, we tested preference for the treatment side using one-way t-tests on arc-sin square root transformed proportions with an expected distribution of 50% of the actively migrating lampreys on each side of the channel.

RESULTS

Upstream Movement Tendencies:

1410 PIT-tagged female lampreys were released of which 69.9% (985) moved upstream into the experimental area and reached the upstream antennas. We observed a strong seasonal pattern in lamprey responsiveness with a decline evident after Julian day 160 (9 June 2007, Fig. 1-2a). A quadratic regression fit the movement data reasonably well ($y=ax^2+bx+c$, $r^2=0.56$, $P<0.0001$) and was used to remove the seasonal effect given that the data represents multiple pheromone treatments. The fitted equation parameters were: 1) c =-175.8, P=0.02; 2) b=2.85, P=0.003; 3) a=-0.01, P=0.001. Three Pearson's correlations were considered and all were significant; Between starting temperature and Julian date there was a significant, positive correlation ($r=0.587$ $p < 0.001$), between starting temperature and upstream movement, there was a significant but weak negative correlation ($r= -0.31$, $p < 0.033$); and finally, between Julian date and upstream movement there was a significant and strong negative correlation (-0.669, $p < 0.001$) (Fig. 1-3b). Prior to this test we examined the pattern in the residuals vs. water temperature (Fig. 1-3a) and Julian day (Fig. 1-3b) to ascertain whether there were any apparent patterns or higher order effects of these variables in the original regression. No patterns were detected (Fig 3a).

The results of the one-way t-tests are presented in Figure 1-5. A significant positive deviation was detected only for 1 x 10^{-12} M larval extract ($t_{1,\ 4}$= 5.29, P=0.006) with, on average, six additional lampreys venturing upstream when exposed to the mixture. No mixture of synthesized pheromone components had a positive effect. However, PADS+PSDS at 10^{-12} M did have a weak negative effect and was nearly significant with P=0.06 ($t_{1,3}$= -2.95), with four fewer lamprey venturing upstream per trial. PADS and PADS+PSDS+PS at 10 $^{-12}$ M and all combinations of synthesized components at 10 $^{-13}$ M were neutral and non-significant.

Odor Preference

The results of the one-way tests are presented in figure 1-6. Methanol vs. methanol trials (neutral control) verified no underlying preference for either side of the channel when pheromone odors were not present ($t_{1,5}$= 0.15, P=0.89). A significant preference for the stimulus side was only evident during the larval extract trials at both 10 $^{-12}$ M ($t_{1,4}$= 3.01, P=0.04) and 10 $^{-13}$ M ($t_{1,5}$= 2.38, P=0.05) concentrations. No combination of synthesized components elicited a preference for the treatment side of the channel (all P > 0.1616), (Table 1-2).

DISCUSSION

In previous lab and field tests, sea lampreys were attracted to larval odor and avoided swimming in water that lacked larval odor (Sorensen et al. 2005, Wagner et al. 2006, 2009). The presence of larval odors and temperature change has been observed to increase upstream movement of sea lamprey in previous field studies (Bjerselius et al. 2000; Binder et al. 2010). In the lab, migratory phase sea lamprey appear to be attracted to 10^{-12} M and 10^{-13} M concentrations of PADS, PSDS and PZS (Sorensen et al 2005). Although we observed increases in upstream movement and channel bias towards larval extract, we did not observe attraction of migratory phase sea lamprey to any concentration or combination of synthesized larval components in our field test. The synthesized-pheromone mixtures had no effect on channel bias at any concentration and opposite to our expectations, PADS and PSDS (10^{-12} M) had a nearly significant negative impact on upstream movement tendencies.

There are at least three potential explanations for the failure of the synthesized components to elicit behavioral responses in the field: 1) the experimental design did not address the question; 2) the synthesized components differ from the natural components and do not elicit physiological responses in the lamprey olfactory epithelium; or, 3) these components are do not elicit strong channel bias or upstream movement.

The study site and animals appear to be sufficient given that lamprey displayed side preference to the full larval extract within ranges previously observed in the field (63- 90 %, Wagner et al. 2006, 2009) and did not show channel bias during the methanol control trials. There is no evidence that suggests the components were incorrectly synthesized and subsequent work suggests the components were correctly synthesized and are consistent in formulation with the natural substances (Fine & Sorensen 2008; Li et al. Unpub.). The third explanation is supported by the lack of channel bias or upstream movement to any of the synthetic pheromone treatments. This finding does not discredit the possibility that these components could be important during other portions of the migration, although it does suggest that these components do not elicit channel selection in rivers as was proposed by Fine and Sorensen (2008).

Although larval odor at a 10^{-12} M concentration had a significant positive effect on upstream movement and side preference, the larval odor at 10^{-13} M, unexpectedly had no significant effect on upstream movement and drew a considerably lower 63.5 % of animals to the treatment side. This finding is inconsistent with other studies that have found 10^{-13} M concentration to have a strong effect on upstream movement and channel preference (Sorensen et al. 2005). One explanation for the discrepancy could be the presence of a population of year 2 age-class larvae that were later deemed to be present upstream of the research site and required lampricide treatment in 2009 (Sullivan & Adair, 2009).

Larval odor is a long distance search cue and migratory phase sea lamprey only preferred moving in higher concentrations of larval odor when there was a tenfold contrast across the channel (Wagner et al. 2009). The background odor from the larval population could have made the lower treatment concentration of larval odor less obvious or desirable to subjects then the adjacent areas. The 10^{-12} M treatment of larval odor was ten times more concentrated and could have created enough of a contrast to the background odor to draw a stronger channel bias. The same masking phenomenon by background odor could explain why the addition of larval extract at low concentration (10^{-13} M) had an insignificant effect on upstream movement by animals once it had spread throughout the water column and reached the downstream location.

The effect of low background odor on the effects of PADS, PSDS, and PS on sea lamprey has not been studied. The fact that the synthetic components at high concentration did not affect upstream movement or channel bias in a way comparable to larval odor at low concentration, suggests that these components are either not important during the riverine migration or not as important as other components in larval odor. The synthesized larval odor treatments were not as attractive as larval odor under natural background odor, indicating that unknown components in larval odor are more attractive during the riverine portion of the migration then the known components. Given that PADS, PSDS, and PS are the most common components produced by larval lamprey, with PS being the least common (Sorensen et al. 2005), these components would be the most likely to be masked by natural background odor. Even with this consideration, it appears that there are more important components in larval odor or additional components that when encountered increase the attractiveness of the whole odor. Because the larval odor treatments elicited channel bias at high and low concentration, we can still conclude that these three synthetic components do not elicit similar behaviors as the full larval odor during the sea lamprey riverine migration.

Although it appears that PADS, PSDS, and PS are not as important to the upstream migration as other components in larval odor, it will be still be important to explore the potential for the synthesized larval components to play an independent role during the transition from the lacustrine to riverine phase of migration. Our null finding does not discredit the possibility that PADS, PSDS and PS could affect migration behavior in a river environment; however under this masking scenario, attraction by these compounds would only be possible without the presence of other attractive components in the full larval odor. One reason to continue to investigate these compounds is that the importance of more common components may increase at the river mouth.

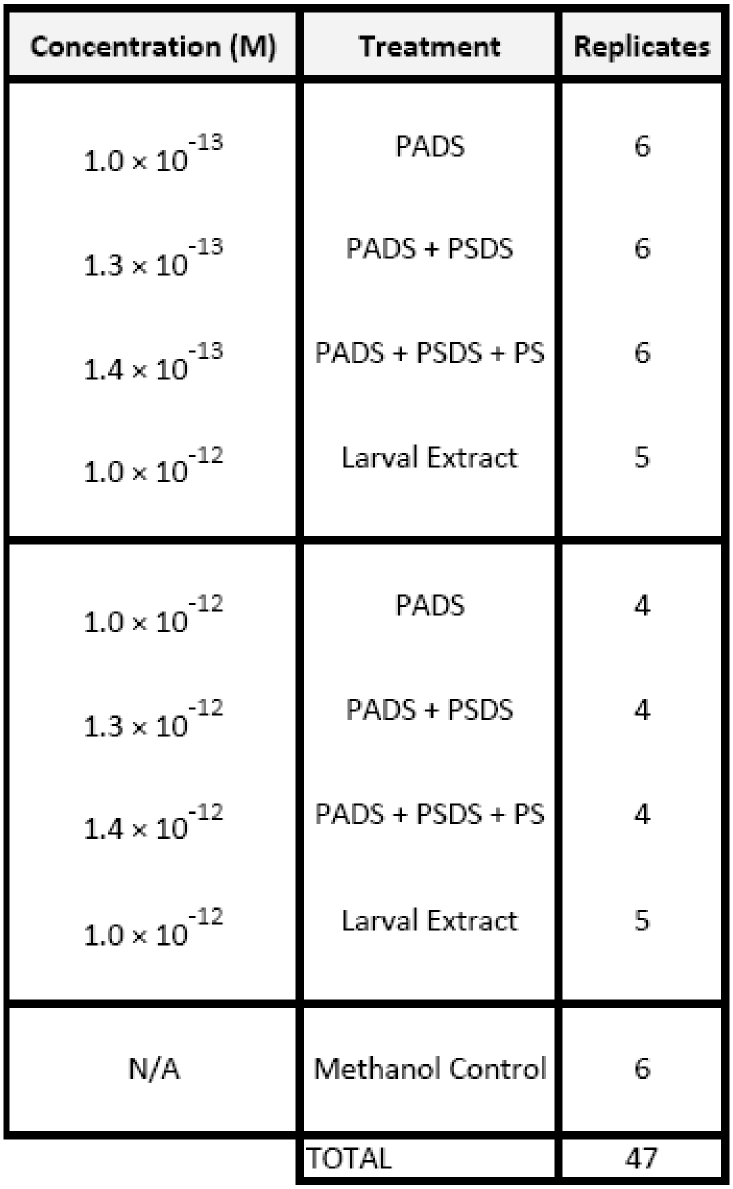
At the river mouth the most common components could be the easiest to encounter and the unknown attractive and potentially less common components could be indiscernible in the lake. In addition to this suggestion, both elecrtroolfactogram (EOG) work and controlled flume environments have shown concrete evidence that these components are physiologically and behaviorally relevant (Sorensen et al. 2005). This validates the need for future evaluation of the behavioral importance of PADS, PSDS, and PS, at the transition from the lacustrine to riverine stage of migration.

APPENDICES

APPENDIX A

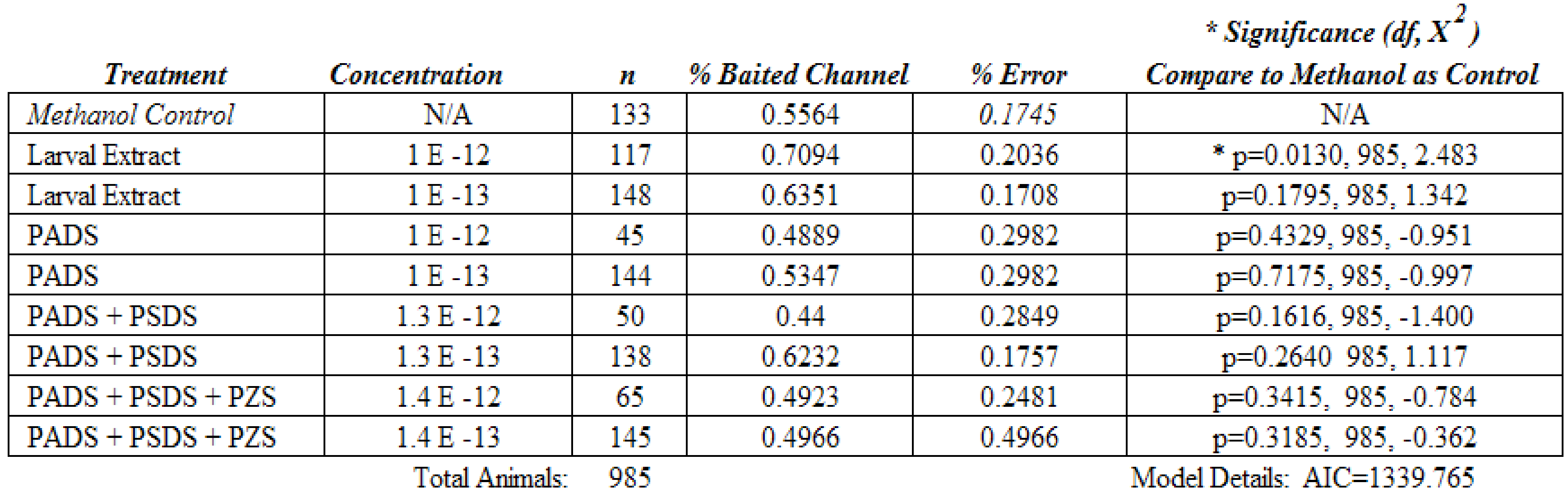
TABLES

Table 1-1. The effects of treatments on upstream movement and channel bias were evaluated in the field. A single study involved the application of s 1 of 8 of the test articles (treatments shown below) down one side of a river channel, and methanol applied down the opposite channel. The treatments included two concentrations of synthesized larval odor (treatment), petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), and petromyzonal sulfate (PS). In addition 6 trials were run with methanol applied down each channel as a negative control to evaluate the natural upstream movement tendency and natural side bias of migratory lamprey in the absence of larval odor in a natural environment.



Concentration (M)	Treatment	Replicates
1.0×10^{-13}	PADS	6
1.3×10^{-13}	PADS + PSDS	6
1.4×10^{-13}	PADS + PSDS + PS	6
1.0×10^{-12}	Larval Extract	5
1.0×10^{-12}	PADS	4
1.3×10^{-12}	PADS + PSDS	4
1.4×10^{-12}	PADS + PSDS + PS	4
1.0×10^{-12}	Larval Extract	5
N/A	Methanol Control	6
	TOTAL	47

Table 1-2. The results of the one-way tests are presented in Fig. 1-5. Methanol vs. methanol trials (neutral control), verified no underlying preference for either side of the channel when pheromone odors were not present (t $_{1,5}$=0.15, P=0.89). A significant preference for the stimulus side was only evident during the larval extract trials at both 10 $^{-12}$ M (t$_{1,4}$=3.01, P=0.04) and 10 $^{-13}$ M (t$_{1,5}$= 2.38, P=0.05) concentrations. No combination of synthesized components elicited a preference for the treatment side of the channel (all, P > 0.1616).



					**** Significance (df, X^2)***
Treatment	***Concentration***	***n***	***% Baited Channel***	***% Error***	***Compare to Methanol as Control***
Methanol Control	N/A	133	0.5564	*0.1745*	N/A
Larval Extract	1 E -12	117	0.7094	0.2036	* p=0.0130, 985, 2.483
Larval Extract	1 E -13	148	0.6351	0.1708	p=0.1795, 985, 1.342
PADS	1 E -12	45	0.4889	0.2982	p=0.4329, 985, -0.951
PADS	1 E -13	144	0.5347	0.2982	p=0.7175, 985, -0.997
PADS + PSDS	1.3 E -12	50	0.44	0.2849	p=0.1616, 985, -1.400
PADS + PSDS	1.3 E -13	138	0.6232	0.1757	p=0.2640 985, 1.117
PADS + PSDS + PZS	1.4 E -12	65	0.4923	0.2481	p=0.3415, 985, -0.784
PADS + PSDS + PZS	1.4 E -13	145	0.4966	0.4966	p=0.3185, 985, -0.362
	Total Animals:	985			Model Details: AIC=1339.765

APPENDIX B

FIGURES

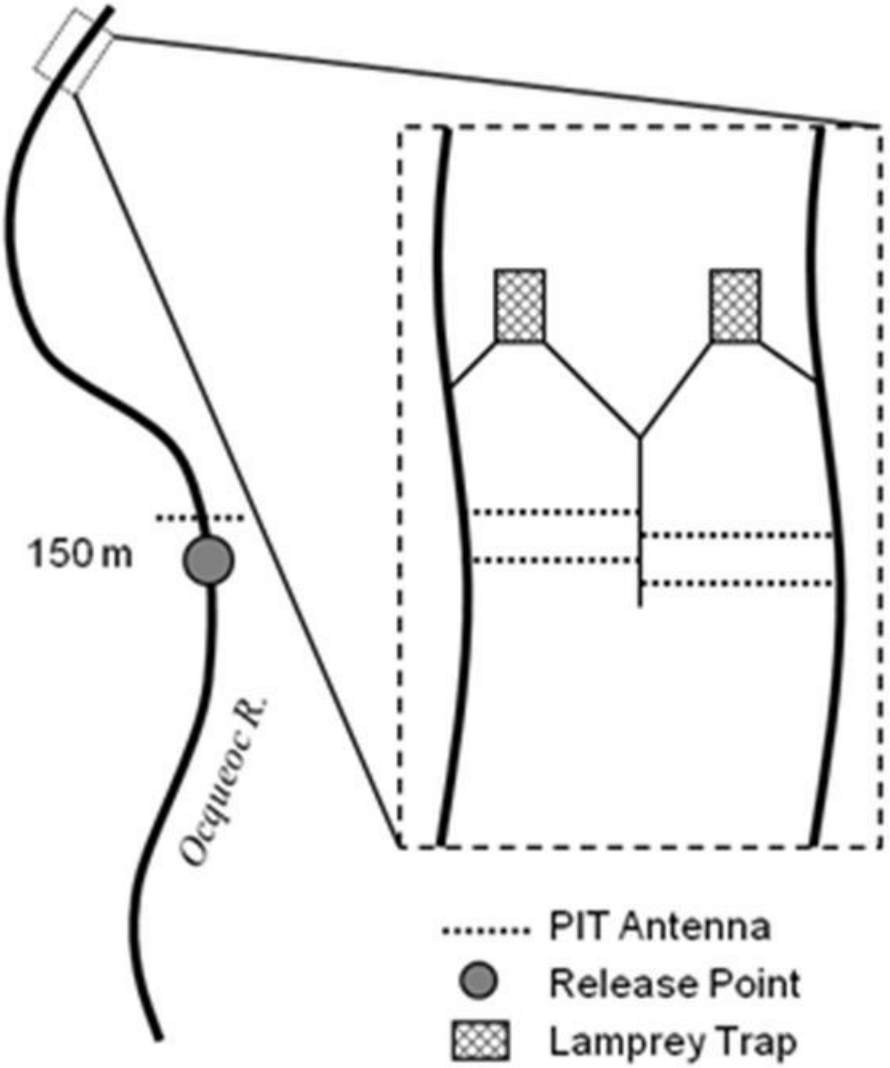
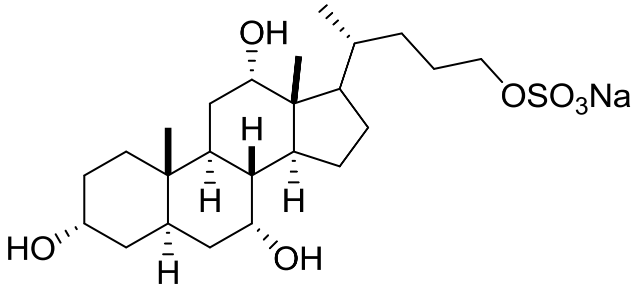


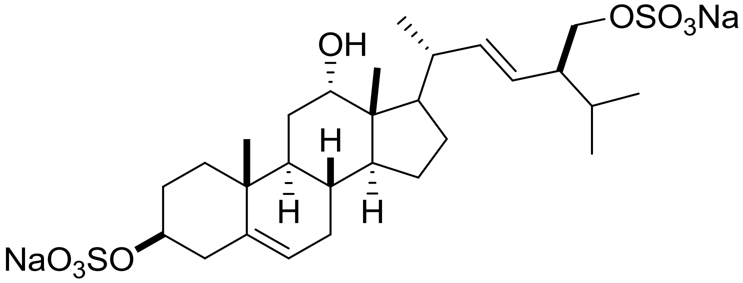
Fig. 1-1. Schematic of the study site on the Ocqueoc River in Millersburg, MI (Maximum Width: 10 m and Maximum depth 2 m).The river flow of the study is from top to bottom of the figure (South to North). The lamprey traps were identical to assessment traps used by the Fish and Wildlife service to trap lamprey at temporary and permanent barrier.

(a)



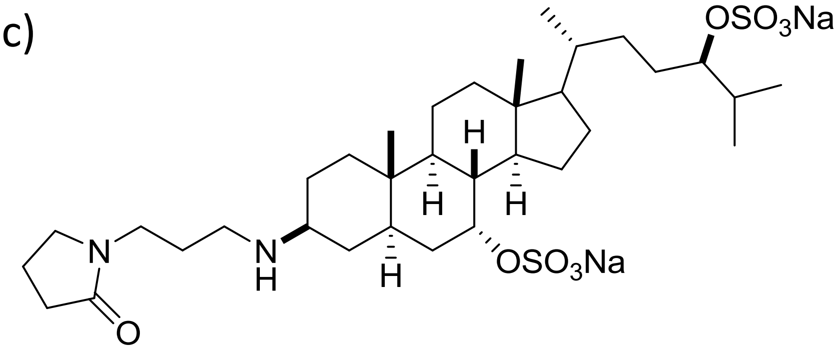
Petromyzonol sulfate (PZS)

(b)



Petromyzosterol disulfate (PSDS)

(c)



Petromyzonamine disulfate (PADS)

Fig. 1-2. Three structure of three biologially active components found in larval odor: (a) petromyzonal sulphate (PZS), (b) peptromyzosterol disulphate (PSDS) and (c) petromyzonamnie disulphate (PADS), as described by Sorensen and Hoye (2007). The above illustrations are complimentary of Dr. Ke Li, Michigan State University.

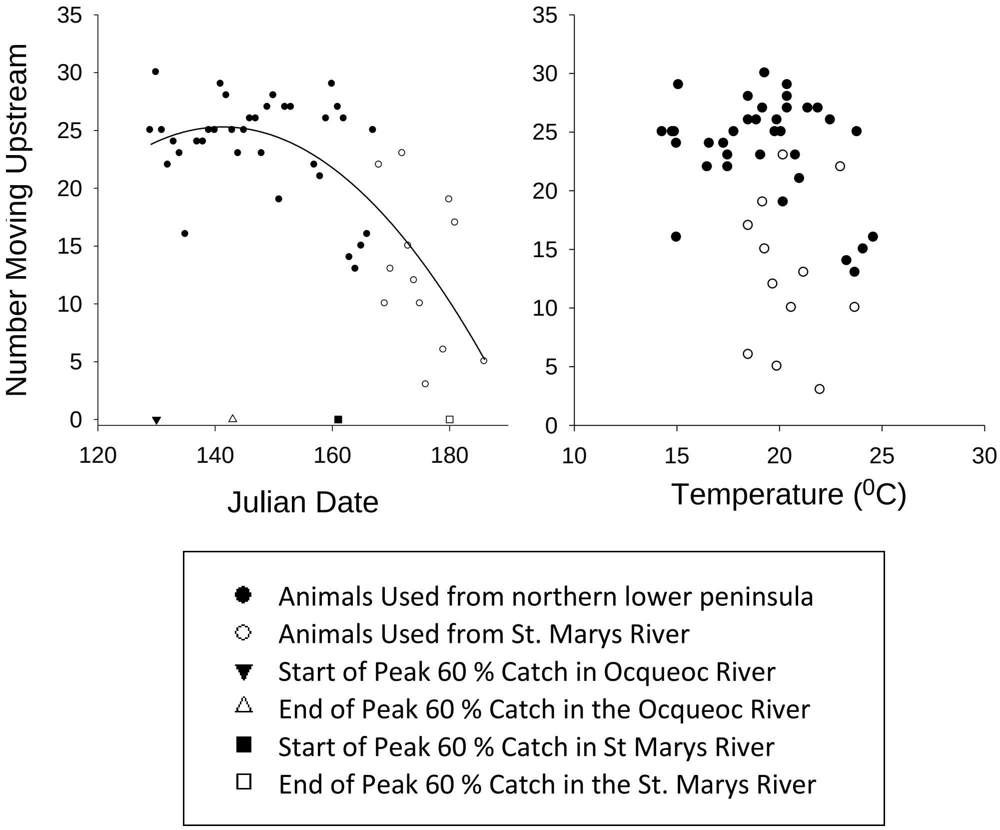


Fig. 1-3. The graphs display the number of lampreys moving upstream during each trial arranged by Julian date (a) and water temperature (b).at the start of the trial. The triangles and squares indicate the peak upstream migration of sea lamprey in the Ocqueoc and St. Marys Rivers as defined from the total catch of sea lamprey at the upstream barrier. The peak period is defined as the period from when 20% of the total lampreys are caught until 80 % of the total catch occurs. Each point represents a single trial, all trials are included. The maximum possible movement value was 30. The relationship to date was fit with a non-linear regression. Pearson correlation is reported for the relationship to water temperature.

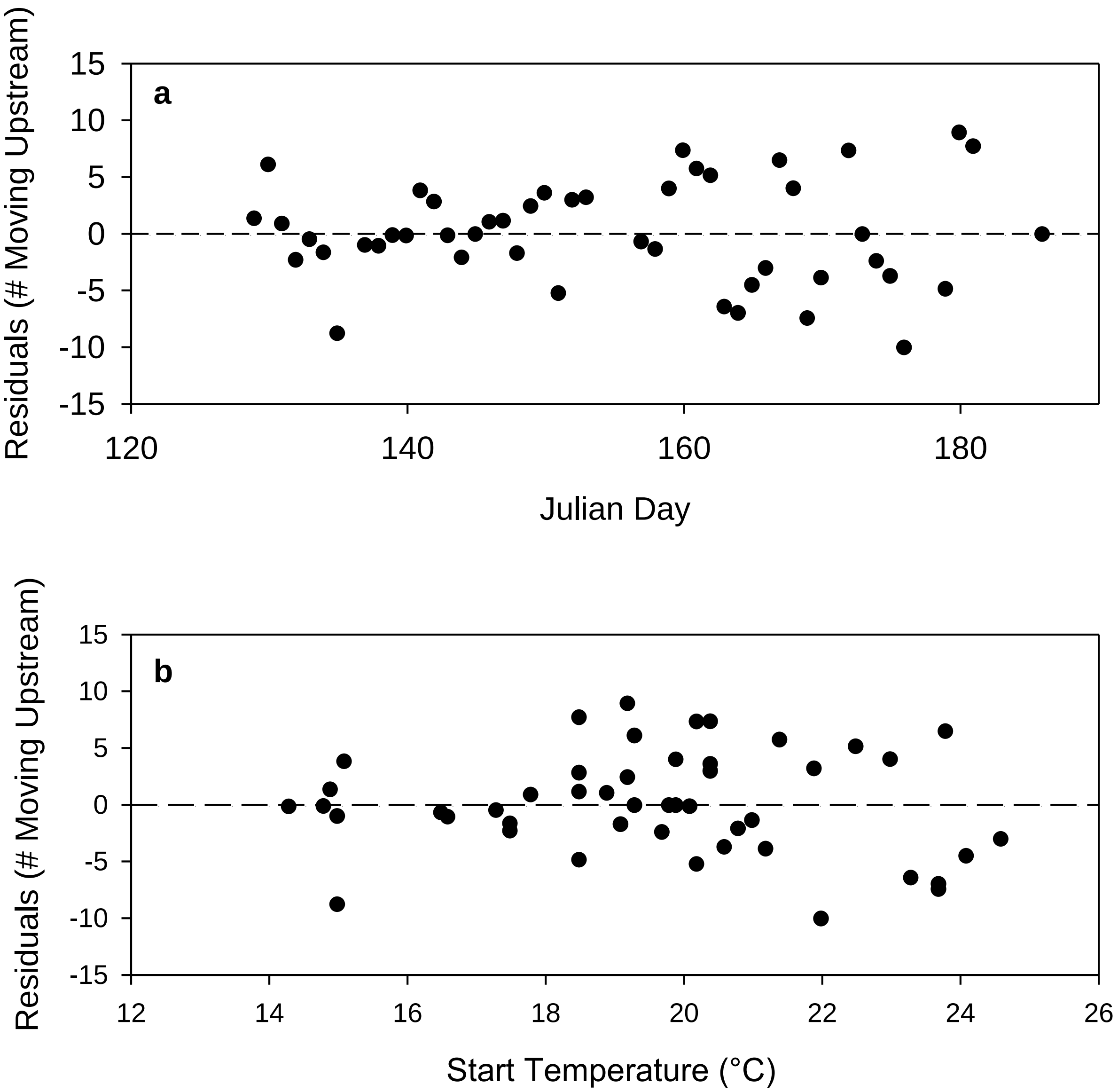


Fig. 1-4. The residual number of lampreys moving upstream was derived from the regression analysis presented in Fig. 2 and is shown organized by Julian Day (a) and water temperature at the start of the trial (b). No obvious patterns arise in the residuals.

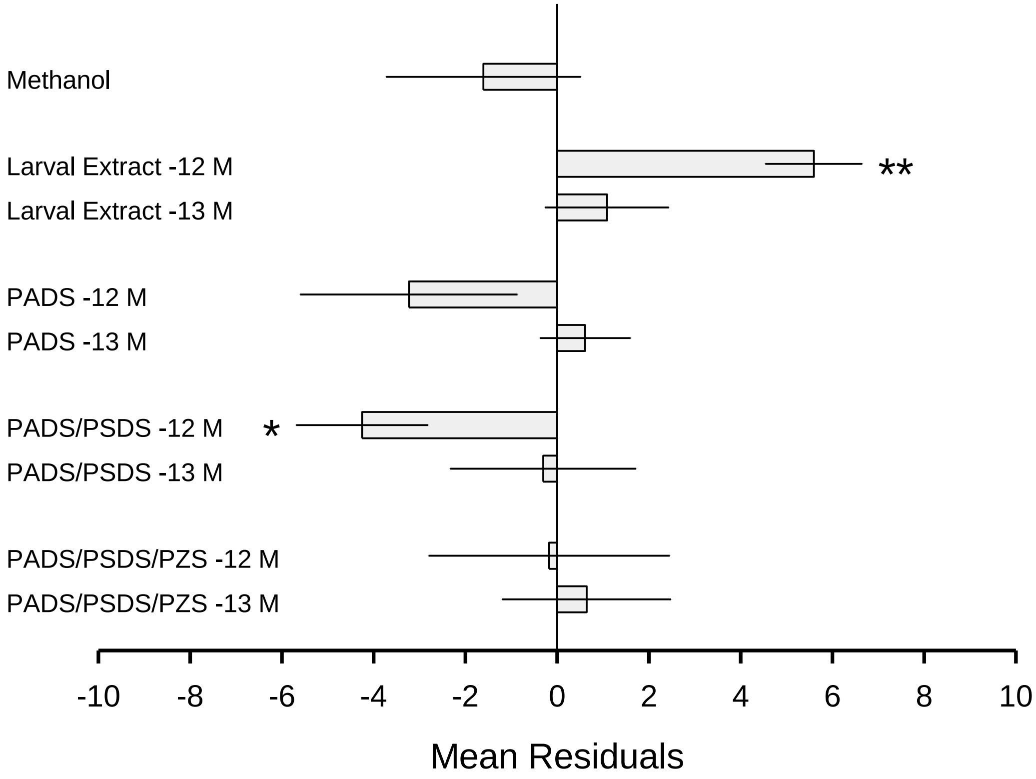


Fig. 1-5. The mean residual number of subjects moving upstream (number of lampreys, ± 1 SE) by treatment, after extraction of the date effect via non-linear regression (reported in Fig. 3), is shown above. A positive residual value indicates the treatment induced greater upstream movement than predicted by the regression model. Statistical significance was determined with a one-way t-test on untransformed residuals with an expected mean of 0 (* $p=0.06$; ** $p<0.01$).

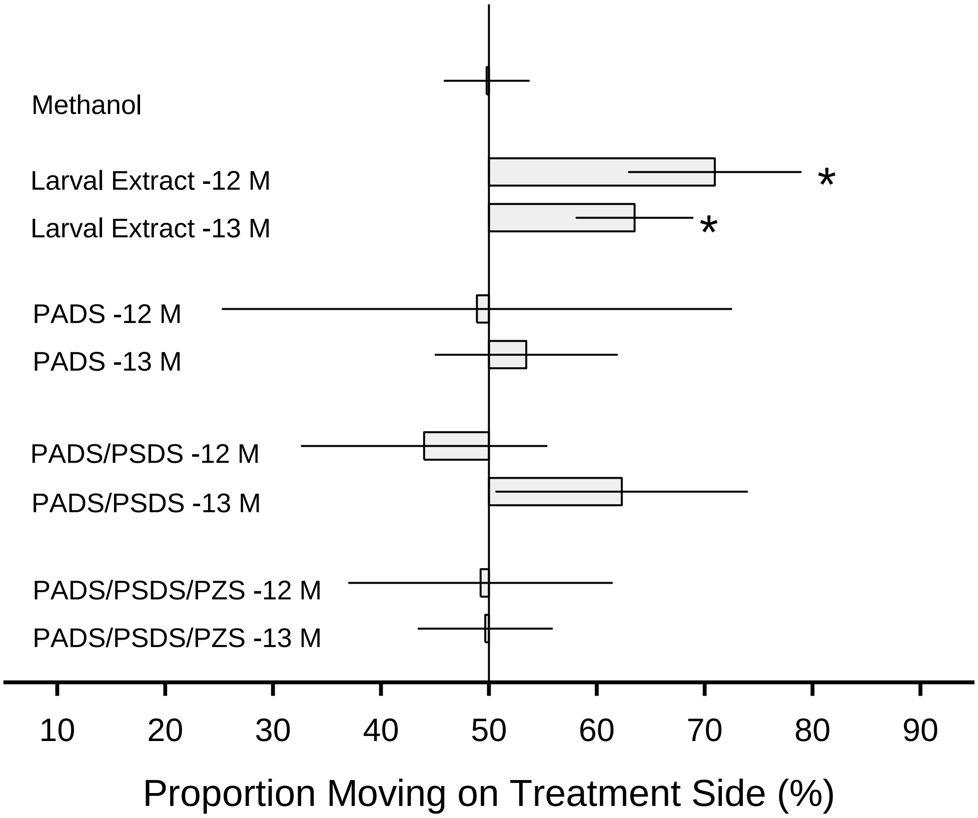


Fig. 1-6. The mean proportion of lampreys selecting the treatment side of the stream (± 1 SE) is shown by treatment. Statistical significance was determined with a one-way t-test on arc-sin sqrt transformed proportions with an expected distribution of 50% (* p=0.06).

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CHAPTER 2

A PILOT INVESTIGATION: SEA LAMPREY HABITAT SELECTION BEHAVIOR AT A RIVER MOUTH.

ABSTRACT

The sea lamprey, *Petromyzon marinus*, is an andromous fish, invasive to the Great Lakes, that uses odor released by conspecific larvae from previous generations to identify suitable spawning habitat. We remotely monitored the movements of individual sea lamprey using directional acoustic telemetry and generated the first continuous whole-night tracks of individual sea lamprey as they moved in the coastal environment in search of spawning habitat in Lake Huron. Our uninterrupted tracks support the previous evidence that two movement strategies are used by migratory phase sea lamprey to navigate through the coastal environment and locate spawning habitat. The first strategy, coastal search, is characteristic of a long distance search strategy that efficiently searches the near-shore environment for river water. The second strategy was observed in close proximity to rivers and is characteristic of a close range search and sampling strategy for an environment with a dynamic and unreliable cue field. This also represents the first effort to manipulate coastal search behavior and river entry through the addition of putative synthesized components of larval odor to the river plume. We observed an insignificant 37 % increase in the number of individuals that primarily displayed coastal search after being released in a river plume with a naturally reduced population of larval lamprey (P= 0.282, n=13).

INTRODUCTION

Long distance odor-mediated search typically incorporates energy efficient strategies that simultaneously maximize the area covered while minimizing costs (risk, inefficiencies) in the absence of relevant search cues (Dusenbery 1989; Zollner & Lima 1999; DØving & Stabell 2003). In olfactory mediated search by fish, odor is often used to indicate if the search path is on track to reach a desired resource, regardless of whether the resource is a food item located tens of meters upstream of a fish in a river or the resource is spawning habitat in a river located 10's to 100's of kilometers away from a fish in the ocean (Carton & Montgomery 2003; DØving & Stabell 2003). Encountering distant odor molecules from a resource does not inherently direct the search towards the resource, but instead it indicates when local physical conditions should be used to orient to the resource (Webster et al. 2001; Vickers 2000; DeBose & Nevitt 2008). The hydrodynamics that disperse the olfactory cue will dictate what makes a successful search strategy by controlling where the cue odor can be encountered, while the orientation cues available in the environment will dictate what comprises a successful orientation strategy (Vickers 2000; Moore & Crimaldi 2004; Lohmann et al. 2008).

The sea lamprey, an invasive species of the Great Lakes, is an ancient anadromous Agnathan that relies on two pheromones to complete its life cycle and is emerging as a model species for evaluating pheromone mediated behavior (Li et al. 2007). Sexually immature, migratory-phase adults use the odor released by stream-resident larvae as a guide to locate riverine spawning grounds during their nocturnal migration from a lake or ocean (Teeter 1980; Vrieze & Sorensen 2001; Vreize et al. 2011). After maturation, a second male-emitted pheromone attracts females onto nests occupied by spawning phase males (Johnson et al. 2009; Li et al. 2003). Because sea lampreys do not home to natal streams (Bergstedt and Seelye 1995;

Waldman 2008), they appear entirely reliant on pheromones to locate appropriate spawning habitat. Nasally occluded lampreys are 90% less likely to find a river mouth and unable to locate the source of male odor vs. unaltered lampreys (Johnson et al. 2006; Vrieze et al. 2010). In addition, four components (three larval, one male) of the pheromones are known to science, which is a major advantage to the use of sea lamprey as a model species (Stacy 2003, Sorensen et al. 1998, Debose & Nevitt 2008; Johsnon & Li, 2010).

Three components of the larval pheromone, petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), petromyzonal sulfate (PS), have been identified (Polkinghorne et al. 2001; Sorensen et al., 2005), synthesized (Sorensen et al. 2005; Hoye et al. 2007), and found to be as attractive as the full larval odor when tested in the laboratory fluvariums (Sorensen et al. 2005; Fine and Sorensen 2008). These small-scale tests were designed to represent conditions at a river mouth and the components remain untested in nature. The capability to observe lamprey movements over large spatial scales in open water with the use of manual acoustic telemetry has been demonstrated for sea lamprey (Vrieze et al. 2011), and makes possible the direct observation of behavioral responses as lampreys migrate. Further, we may now attempt to manipulate those responses via the addition of synthesized larval components in the field.

The information needed to guide a fish through a large body of water, such as a lake or ocean, to a coastal environment and finally into a spawning river depends greatly on the duration of the movement, complexity of the route and the specificity of the goal. The first step in an olfaction mediated migration, is encountering the olfactory cue. When moving without olfactory cues, migratory fish that rely on olfaction generally move at a constant bearing in a direction against the dominant current that increases the likelihood for odor encounter, while vertically sampling the entire water column (Debose & Nevitt 2008; Vickers 2000). Sea lamprey use a very similar search strategy in the coastal environment, as they move with straight movements parallel to the shoreline while vertically sampling the entire water column (Vrieze et al. 2011). However, it is unclear what behaviors sea lamprey employ to locate the coastline. The goal of a migrating fish dictates the type of information needed in the open water, specifically, if the fish only needs a compass, as in the case of an out migrating American eel moving east to locate the Sargasso Sea, or if the fish requires both a map and a compass, as in the case of a Pacific salmon needing to locate a specific natal river (Lohmann et al. 2003, Doving & Stabell 2003). Because sea lampreys only need to locate a coastline and are not homing, they can likely use directional cues to maintain a straight bearing towards shore.

The success of the sea lamprey search behavior along the coast is associated with the hydrodynamics of the coastal environment. The integrity of river water as it spreads into a lake or ocean is affected by buoyancy, wind and tidal forces (Choi & Wilkins 2006; Warrick et al. 2007). The hydrodynamics shape the geometry of the river plume, which affects the area containing odor and the likelihood that a sea lamprey will encounter the olfactory cue (Debose & Nevitt 2008; Wagner et al. 2009).

We tested what behaviors migratory phase sea lamprey use to locate a river from the lake and how larval odor affects this process. We accomplished this by observing movements of 20 female migratory phase sea lamprey for a maximum of 12 h as they approached, entered, or bypassed a river under two conditions. We released 10 of the lampreys offshore of a small river that recently had the larval population greatly reduced via the application of pesticide (3-trifluoromethyl-4-nitrophenol) as part of the normal sea lamprey control operation on Lake Huron. We activated the same river with two synthesized pheromone components (PADS and

PSDS, PS was unavailable at the time of the study) to a concentration of $1X10^{-12}$ Molar and observed the movements of an additional ten lampreys. The objectives of this study were to: 1) complete a full night description of how a sea lamprey locates, evaluates, and selects or rejects a candidate river through remote acoustic observation; and, 2) ascertain whether the addition of synthesized migratory pheromone components altered the sea lampreys search behavior or tendency to enter the river.

METHODS

Site

Each night we released a single acoustically tagged lamprey two hours before sunset within the visible plume of the Black Mallard River, a second order tributary to Lake Huron with a typical spring discharge of 0.18-2.0 ($m^3 \cdot s^{-1}$). The river was treated below a dam that creates the Black Mallard Lake in October 2008 and above the lake on 20 April 2009 to vastly reduce the number of larvae in the system. We anticipate that there were some surviving larvae in the system based on an estimated 95% larval mortality following treatment (Klar & Young 2003).

Subjects

Capture and Preparation of Subjects. We obtained 20 migratory phase female sea lampreys between 20 April and 21 May, 2009 from fyke nets and barrier traps on the Ocqueoc River, MI, USA. The source of sea lamprey has shown no effect on entrance rates or behavior (Vrieze et al. 2010). Subjects (459.0 - 529.8 mm TL, 191.7 - 300.9 g WW) were selected haphazardly from all individuals in apparent excellent condition and held for less than seven days, prior to tag implantation, with a natural light schedule in 150 L flow through tanks that cycled Lake Huron water (5.6 °C - 9.1 °C during study period, 100 % exchange every 1-2 hour) at the Hammond Bay Biological Station (Millersburg, MI, USA). Use of sea lampreys was approved under Michigan State University Institutional Animal Use and Care Committee permit 04/07-033-00.

Tagging procedure

We implanted each lamprey with a single Vemco brand V9P-2L acoustic tag (47 mm L, 3.5 g) that transmitted a depth encoded signal every two seconds (± 0.22 m, precision error) at one of five frequencies (72, 75, 78, 81, 84 hz). These tags were used successfully in previous lamprey tracking studies and our implantation protocol closely followed protocols reported by Close et al (2003). Each tag was tested at 0, 1, 5 meters depth to calibrate the tag and account for possible reading errors of (± 2.5 m, accuracy error). Prior to tag implantation we anaesthetized the subject through direct submersion in a 0.1% solution of clove oil (Eugenol, Spectrum Chemical MFG Corp.) mixed per Munday and Wilson (1997) , until the subject reached stage four of anaesthesia per (Close et al. 2003), which took on average 365 s (258-571 s).

The procedure began with placing the subject into a 2” PVC pipe with a sealed T-end on a foam pad over a catchment tray that drained to a sink. We pumped aerated fresh Lake Huron water via a peristaltic pump into the t-end at a rate sufficient to completely submerge the head and gills (Close et al 2003). We inserted the tag into the peritoneal cavity through a 20 mm incision, made via 3 mm scalpel, approximately 10 mm off the ventral midline ending in line with the anterior insertion of the first dorsal fin. The incision was closed with at least three independent sutures (3-0 Ethicon sterile monocryl monofilament), sanitized via injection of oxytetracycline (Boehringer ingelherm, oxytet 100) and sealed with Vetbond, a veterinary adhesive; (Moser et al. 2002; Vrieze et al. 2011). The surgery took an average of 374 s (275-423 s) to complete. Subjects were monitored during recovery in a covered holding tank. The subject typically regained equilibrium and natural swimming movements within 852 s (240-1805 s). Following the first hour the animal was uncovered and monitored hourly for four hours to confirm a normal recovery. Subjects were held for a minimum of 72 hours prior to release to allow for the metabolizing of accumulated stress components associated with anesthesia and tag implantation (Close et al. 2003).

Experimental Procedures

Tracking Process. Each night we released a single tagged lamprey two hours before sunset within the visible plume of the Black Mallard River (evidenced by tannin-stained water). The tag was monitored from a twelve foot Zodiac boat using a handheld hydrophone and receiver (Vemco VR100 outfitted with a range tested VH110 directional hydrophone receiver). The hydrophone was mounted to the base of a pole and fitted with a fixed compass. An individual lamprey was followed for a maximum of twelve hours or until lost.

A track was assembled by taking individual readings of the direction and signal strength of a lamprey's position with regard to the boat and then subsequently using this information to estimate positions. An individual position was obtained by stopping the boat, ascertaining the compass direction to the transmitter from the boat and simultaneously recording the boats location with a handheld GPS unit (Garmin Oregon, reported ≤ 5 meter accuracy). In addition, the strength of signal and a "gain" was noted. Gains between 00 and 30 were used for positioning. Higher gains (36, 42, and 48) lacked the precision necessary to position animals and were only used for searching for subjects outside of the lower gains detection range (≈ 800-1500 m), with the only goal being to regain a closer proximity to the subject and continue gathering useful positions. Subsequent animal locations were generated by moving the vessel and reacquiring the animal. If a tag was detected on subsequent nights we took a single reading. If a signal was received as an apparent deflection it often would be decoded with an aberrant depth value (vs. the previous depth measurement); these signal strength values were ignored.

Pheromone Preparation. In order to determine whether the addition of synthesized migratory components would induce entry or alter migratory behaviors within the river plume of the Black Mallard River, PADS and PSDS were applied during half of the trial nights (n=10) to activate the discharge to a concentration of $1\text{x}10^{-12}$ M . PADS and PSDS were custom synthesized by Bridge Organics (Vicksburg, Michigan, USA) at a purity greater than 95% (Li Lab, (MSU)) and diluted into a stock of 1 mg/ml with 50% methanol. One batch of 1.0 mg/ml of stock solution of both PADS and PSDS (one each) was used during the entire study.

Pheromone Application. Field Application of synthesized larval components and extracted larval odor was approved by the Michigan Department of Environmental Quality and United States Environmental Protection Agency through experimental use permit number 75437-EUP-1. The stream discharge was measured using the USGS midsection method as described by Gore (1996) with a flow meter (Flo-Mate Model 2000, Marsh-Mcbriney) ($\text{m}^3\ \text{sec}^{-1}$), to estimate the amount of synthesized component to activate the river to a 10^{-12} M concentration. Discharge was measured 0.25 river km south of the mouth of the Black Mallard River (45.534123° -84.124973°). Odors were prepared for experimentation by diluting the necessary amount of both stock solutions into 2 ml of methanol and then applied to the stream by diluting the methanol and pheromone solution in 10 L of river before being pumped into the stream at a rate of 600 ml per hour via a reef-dosing peristaltic pump (Admiral, Reefdosingpumps.com). The pheromone solution was applied from 12 h prior to sunset until 06:00 hours, to the center of the river 0.50 river km north of the mouth of the river.

River Plume Mapping. To evaluate whether there were changes in migratory behaviors as subjects encountered the river plume, we first mapped the location of the river plume.

Temperature-conductivity discontinuities have proven sufficient to generally characterize the introgression of river water into Lake Huron in Hammond Bay, MI (Vrieze et al 2011). In addition to these measureable characteristics, visible hue was a consistent and comparable indicator due to the high levels of tannins in the Black Mallard River. The river plume was defined as the 10% river water isocline with conductivity or temperature indicating that at least ten percent river water was present and this closely matched our third indicator, visible hue. We mapped the position of the river plume using a handheld GPS unit (Garmin Oregon 400T) and an YSI multi-meter (Model 30) fitted with temperature and conductivity probes. We sampled along four longitudinal transects in a radial pattern focused at the river mouth with samples at 50, 100, 500, and 1000 m offshore as shown in Fig. 2-1. Conductivity (units, precision) and temperature (units, precision) were recorded at the surface, a depth of two meters, and at the bottom. In addition we scanned the water column by slowing casting the probe from surface to bottom and recorded the depth of the highest and lowest conductivity and temperature readings. On occasion the plume was visibly pinned to the shoreline, outside of the standard radial of sampling stations. On those days we took additional fixed measurements across the apparent location of the plume to map its location.

Data Processing

Acoustic Equipment Calibration. Manual acoustic tracking equipment is extremely useful for presence absence studies that focus on movement over large areas but is less frequently used for fine-scale positioning (i.e., less than 1000 m accuracy). We developed a model of distance to signal strength by incorporating three analyses of the distance-to-power relationship for our equipment. The effects of tag orientation on signal strength were analyzed with a tag orientation study, the explicit relationship between distance and signal strength was modeled with the fixed range study, and the full technique was evaluated with a blind range test. All three tests were completed in Hammond Bay, offshore of the Black Mallard River (Fig. 2- 2).

Tag Orientation study. The effect of tag orientation (relative to the observer) on observed signal strength was evaluated through direct comparison of signal strength measurements taken from 11 positions at 30 degree intervals surrounding a tag at fixed position and orientation, repeated at two distances (50 and 100 m) in calm conditions (Fig. 2-2b). We attached a 72 hz tag to a 15 gauge steel wire attached to a cement block and positioned 1 m above the substrate at a depth of 6.4 m. Directly next to the cement block we placed a 135 kg anchor used for exact positioning of the boat from the tag and a GPS (Magellan Mobile Mapper CX and Magellan Precision NAP100 antenna) was used to estimate the bearing and confirm the location through 5 post-processed data points (5 second averaged each, 1 point per second). This GPS and method was used in all three tests for positioning tags and confirming position.

There was no apparent effect of tag orientation on signal strength at either 50 or 100 meters from the tag (± 1 db). The small differences in signal strength also had no apparent pattern associated with orientation. Vemco tag specifications indicate a 1 db variation to be expected and no value was outside of this range. There was no measureable effect of tag orientation on signal strength (± 1db) (Fig. 2-3).

Fixed Range Study. The fixed distance test was designed to evaluate how signal strength varied with distance to the transmitter. We recorded signal strength (db) from tags of five frequencies (72, 75, 78, 81, and 84 Hz), across nine gains (0, 6, 12, 18, 24, 30, 36, 42, 48), and in two weather conditions (calm-less than 8 in and rough-12 to 20 in waves) from seven distances (10, 25, 50, 100, 250, 500, and 1000 m) away from a fixed tag (Fig. 2-2c). In an effort to ensure accurate distance to the tag, eight 68.2 kg buoyed reference anchors were deployed at the seven predetermined search distances (10, 25, 50, 100, 250, 500, and 1000 m) and at the tag location.

The depth varied from 5.79 to 6.77 m across the study area. Locations were attained and checked by same the same GPS equipment and techniques as those used in the tag orientation study. All locations 100-1000 m from the tag were determined to be within 3 meters of their predetermined distance and were confirmed on each subsequent day of work. Anchors at 10, 25, and 50 m were each within 2.0 m of the target distance and were measured via direct attachment with a pre-measured rope.

A single regression model of the distance ($\log_{10}$, m) vs. signal strength was generated for all other variables at each gain. The fit was evaluated using the R^2 value and considered with reference to the regression graphs of the models (Table 3). Gains of 00 through 30 were used for positioning because they were expected to be more sensitive to changes in distance up to 500 m (D. Webber, Pers. Comm). Although there was an increase in median error from 30 to 48, only gains 18 and 48 were statistically different in median error (Tukey's all pair-wise comparisons test, ($p< 0.05$, Fig. 2-4). Estimates were only used if they were less than or equal to 500 meters from the boat because measurements at 1000 meters were more affected by weather (wind and wave noise) and during the study period we attempted to be within this distance to the animal.

Blind Range Test. The blind tag search was designed to simulate the process of locating a lamprey, which allowed for the accuracy of the entire positioning method to be evaluated. We searched for two tags (72 and 84 Hz), non-randomly located east of the Black Mallard River (150 and 400 m), attached per Fig. 2-11 and anchored in position from 15 randomized locations surrounding the tags. The R statistical package randomly calculated search locations within at least 700 meters of each tag and at least 5 meters from the Lake Huron shoreline based on the criteria that we would position animals a maximum of 500 meters from the boat (Fig. 2- 2 (Frame C)). The searcher was blind to the location of the tag and only able to view the compass and telemetry device. The blindfold was created from using safety glasses that were blacked out with electrical tape and permanent maker except for where two 5 inch long ¾ inch diameter PVC pipes were attached to the front of the glasses at eye width (one on each side) apart so that the searcher could only see through the pipes.

Data were combined for searches to each tag for the 30 locations. We graphically evaluated the data for the effect of increasing distance from the tag on error. Estimates relied on boat position recorded with the Garmin Oregon and more accurate positions for estimating error were recorded with the Magellan Mobile Mapper CX and Magellan Precision NAP100 antenna. We generated median measurement error at each gain and plotted the information (Fig. 2-3). A one way ANOVA suggested differences in the median values between the gains were greater than would be expected by chance ($p< 0.024$). Although there was an apparent increase in range of error and median error between gains 24 through 48, only gains 18 and 48 were significantly different when compared with an all pairwise multiple comparison Tukey Test ($p < 0.05$).

Data Controls. Controls were developed to avoid incorrect depth readings and poor positions. Lamprey positions were occasionally estimated on shore when a subject was in very shallow water and the measurement was taken within 50 m of shore. When lamprey locations were estimated to be on shore the point was either removed or moved to the midpoint between the boat and the shoreline if the position met the correct criteria. Controls were also developed to remove inaccurate depth readings in an automated method because of over 100,000 total readings. In addition, distant readings with signal strengths of less than 50 db were ignored due to their susceptibility to deflection issues. The second control dropped all extraneously negative numbers after corrections for tag offset were completed. Tags have an accuracy of ± 2.5 meters (0.22 m resolution) and were calibrated to account for the possibility of having an initial offset error. Lastly we ignored values that exceeded a lamprey's ability to realistically move vertically. This was calculated by evaluating large scale movements and looked for the maximum vertical speed and this rarely exceeded a meter per second. All vertical movements were considerably less than 2.5 meters per second and we used this safe criterion for dropping points. The highest horizontal burst speed observed by Vrieze et al. (2011) was 0.84 m/s and we would suspect this to be greater than any vertical movement speed. Since our points are a minimum of two seconds apart, the animal would have needed to travel five to ten meters vertically between consecutive transmissions to violate our assumptions. The total depth of the water column was estimated by looking at fish positions overlaid on a Great Lake Bathymetry layer (NOAA) in arc GIS 9.2. The bathymetry layer had only 5 meter resolution and was supplemented with manual depth measurements recorded with the boat depth sounder (1 foot resolution), during lamprey tracking.

RESULTS

River Plume Dynamics

The river plume was localized considering interpolated conductivity and water temperature, and estimation of visible hue. If the criteria for two of the three recorded variables including temperature, conductivity and visible hue were met, the river plume was considered to be present and measurable. In every case our 100 percent river water condition was warmer and contained a lower conductivity than the 100 percent lake water condition.

During the study, plumes to the south were by far the most common (n=18) (Figa. 2-6, 2-7), followed by central (easterly) plumes (n=6) (Fig. 2-8). Northerly plumes were the least common (n=3) (Fig. 2-9). Our report only includes the plumes that were able to be sampled and underestimates the number of east plumes because wind driven currents from the east often made the lake unsafe to navigate. The common southerly plume was likely the result of the dominant cyclonic flow in Lake Huron (Beletsky et al. 1999) combined with frequent northerly winds during the study (Fig. 2-10). Plumes to the south and north would generally extend the farthest from shore and the longest distance from the river mouth. Plumes to the south often had higher conductivity and more pronounced edges than plumes to the north, which could extend beyond the northern edge of Hammond Bay.

Lamprey Tracking Overview

During the tracking study twenty animals were manually tracked for a combined 170 hours and 46 minutes of track time (Table 2). Out of the twenty animals, five were lost (two pheromone applied, three non-pheromone applied) in less than two hours and 13 of the remaining tracks lasted more than seven hours.

Individual Behaviors – Stopping

Lampreys did not move continuously; stopping was frequently observed and operationally defined as a subject that remained at constant depth, signal strength, and bearing from a fixed vessel location. The most common stopping times were associated with daylight, either in the evening immediately following release and prior to sunset, and/or the following morning between the onset of nautical twilight and sunrise.

We observed three additional circumstances where stopping was not principally motivated by changing light levels. The first case involved a subject stopping in the river plume after previously swimming. For example, Tag# 1072124 settled for a period of just over two hours directly in front of the river mouth (01:30-04:00) (Fig. 2-13). Another example is Tag# 1072131-2, which settled prior to sunset and only moved very slightly after sunset before settling in the river plume and not moving until the following evening (Fig. 2-14).

The second case of settlement was observed when lampreys released on previous days were found during subsequent tracking night(s). In every case these animals moved prior to their tags expected turn off period and were not believed to have died. For example, Tag# 1072129 was found motionless and monitored for 2 hours the night after release in 3 m of water and not likely in the river plume of any river body. Tag# 1072138 was found in the same location of settlement from its previous track night when it had settled in the river plume, although the river plume shifted position during the ensuing day and no longer immersed the lamprey (Fig. 2-15). It remained for 2 days and was monitored from 12:00 to 2:00 on its third night after release. It moved between the hours of 02:00-06:00 on its third night of release. The third case of settlement was stopping in the river channel and was likely associated with finding cover prior to moving upstream. Tag #'s 1072132, 1072131-1, 1072136 all displayed this behavior (Figs. 2-16, 2-17, 2-18).

Individual Behaviors - Movements

The second group of behaviors involved active movement. Based on the error estimation procedures outlined above, we cannot interpret fine-scale two-dimensional movements (X-Y plane) that require less than 100 m displacement to complete. However, the totality of the movements may be interpreted. For example, it would be inappropriate to calculate precise turn angles of an animal executing a large-angle turn, but a sequence of turns can be accurately detected and interpreted as a behavioral activity (large-angle turning). In certain cases two dimensional movements will be discussed on a finer scale but these will be justified on an individual basis. We were able to evaluate vertical movements (Z plane) with much greater precision (0.22 m) based on the tags accuracy.

We observed two basic two-dimensional movements. Straight-line swimming occurred when a lamprey was moving continuously on an approximately constant bearing and did not exhibit course reversals or large-angle turning. The term straight-line swimming is somewhat arbitrary as the sinuosity of the course could not be accurately ascertained. It was common for straight-line swimming to follow the coastline, or a depth contour (e.g., Tag #s 1072121 and 1072123, Figs. 2-19 and 2-11). Alternatively, large-angle turning occurred when course reversals or broad turns were frequent. Although the exact frequency of turns involved with large-angle turning is unclear, the result was retention within an area of the lake near a river mouth and often included repeated movements in and out of the estimated river plume, while making repeated progress towards and away from the river mouth (e.g., Tag #s 1072125, 1072138, Figs. 2-12 and 2-15). Large angle turning often occurred immediately after release or settlement and lasted from as little as 15 minutes as observed with Tag 1072123 to as long as 7.5 hours as observed with Tag 1072138 (Figs. 2-11 and 2-15). Straight line swimming was generally observed after large angle turning or settlement as a result of animals being released in the river plume and the behavior often continued until either the subject closely encountered a river such as Tag 1072125 or sunrise as observed with Tag 1072123 (Figs. 2-12 and 2-11). Straight line swimming was observed to last from as little as 2 hours as observed with Tag 1072125 to as long as 5 hours as observed from Tag 1072123 (Figs. 2-12 and 2-11). The average movement speed across the thirteen interpreted tracks was 0.592 m/s (2.89 BL S^{-1})

We also observed three swimming behaviors relative to vertical position in the water column. The first was surface swimming and was simply the act of moving at or near the surface (arbitrarily defined as moving in the upper 20 percent of a water column in environments shallower than ten meters or in the upper 2 meters if the depth is greater than 10 meters). The second movement was bottom swimming and was more complicated to evaluate due to the inaccuracies in position introducing a greater challenge in pinpointing bottom depth. A very well maintained position near the estimated bottom was considered bottom movement. The final behavior, and the most dynamic, was vertical casting. Vertical casting took place when a lamprey repeatedly dove and surfaced, traversing throughout the water column. This behavior is not just a single dive but a period of repeated dives from the upper few meters of the water column to the bottom. For example Tag 1072130 made at least nine steady dives from the upper few meters of the water column to over ten meters in depth between 00:40-01:50 with the largest of the dives in that interval exceeding 15 m in depth (e.g., Tag # 1072123, time period 5, Fig. 2-11). Vertical casting was observed for as short as ten minute intervals with Tag 1072123 at 04:00

and as long as 3.5 hour intervals with Tag 1072122 as observed from 23:30-02:30 (Figs. 2-11 and 2-20).

Linking Movements and Stopping with the Environment

Here, we briefly discuss the described movements and stopping in association with the timing and physical environment in which those behaviors took place (Table 2).

Stopping was invariably associated with settlement onto the bottom. Therefore the settlement behaviors we discuss above include settlement in the river itself (SR), in the river plume (SRM), and outside of the river plume. Stopping was most frequently observed with changes in light level, which meant the animal typically settled immediately following release (SE) or with the onset of sunrise (astronomical or nautical twilight; SM).

Large angle turning occurred at the surface (STS), on the bottom (STB,) and during vertical casting (STC). It was evaluated as occurring inside and outside of the river plume. Casting was rarely observed in the river plume due to the difficulty of observing vertical movements in less than two meters of water, where the river plume was often found. Straight-line swimming was observed at the surface (SSS), at the bottom (SSB) and with vertical casting (SSC). These behaviors did occur inside and outside of the river plume, although again it was difficult to identify casting in the shallow river plume.

An additional behavior that was apparent, but could not be fully quantified, was course reversal. These movements included straight-line swimming over short distances with frequent 180° turns, either towards or away from shore, or directly up and down plume gradients near the river mouth. It is unclear if the behavior is in part an artifact of the technique used to position the animals; we believe it was a genuine behavior and will refer to it in the figures as movement towards and away from shore or the river mouth (RMT) without reference to a definite degree of curvilinearity (Tag # 1072124,Fig. 2-13).

Behavioral Modes – Coastal Searching and River Plume Investigation

The behavioral modes will be defined by the combination of vertical movements, horizontal movements, and the physical environment in which the movements took place. Each individual lamprey track was unique but two near shore behavioral patterns reoccurred throughout the study and will be identified as coastal searching (CS) and river plume investigation (RPI). In order to ease the discussion of results, two tracks have been chosen as exemplars of the behavioral modes (CS, Tag #1072123, Fig. 2-11; RPI, Tag# 1072125, Fig. 2-13). Coastal searching was typical of animals moving along the coastline, typically in a Northward direction and counter to the averaged cyclonic long shore currents in this region of Lake Huron (Beletsky et al. 1999). Coastal searching was frequently punctuated with protracted periods of vertical casting. There may be two forms of coastal search that hinge on the distance the lamprey is moving from shore while searching and this has different implications on the cues used and effort exerted. The first search behavior entailed movement close to shore in which the animal would move with a northwesterly direction and would repeatedly encounter the coast and correct with a right turn away from shore (Fig. 2-19 and 2-21). The movement away from shore entailed moving along the coast a considerable distance from shore (1.0-1.8 km) and is shown by our exemplars (Fig. 2-11 and Fig. 2-20). The second mode we term river plume investigation (RPI), exemplified by Tag# 1072125 (Fig. 2-12). During RPI, a lamprey typically exhibited frequent course reversals and large-angle turning which resulted in retention near the shore. The depths in these environments were typically low (< 5 m), and lampreys infrequently exhibited vertical casting. Interestingly, these behaviors were only exhibited when a lamprey moved near a river mouth. In Figure 2-12, the exemplar can be seen exhibiting RPI near the mouth of the Black Mallard River before moving southward following the coast. The subject re-engaged in RPI as it approached the discharge from the Ocqueoc River and eventually settled in front of the river mouth in Lake Huron.

Behavioral Modes – The Effect of Synthesized Pheromone Addition

We were able to generate tracks of sufficient duration for evaluation of the pheromone additions in 13 cases (7 nights when the plume was activated with synthesized pheromone, 6 nights without activation) (Table 2, Fig. 2-11 to 2-25). We blindly classified each of the 13 tracks into either CS or RPI movements (blind as to pheromone treatment) based on the X-Y movements and vertical casting (Fig. 2-26). Six of the seven pheromone cases were classified into RPI, the behavioral pattern we associate with attempts to localize a river mouth (86% of the tracked lampreys). Alternatively, lampreys tracked on non-pheromone nights were equally likely to exhibit RPI or CS. When compared with a Fisher’s exact test we could not detect a significant difference in the proportions given the small sample size (P=0.282) (Fig. 2-26). Even if RPI was the classification for all pheromone treatments the result would not be significant due to small sample size (P =0.08, Fisher’s Exact Test). Because we did not know the end fate of the animals and river entry was infrequent during our study (three lampreys), we could not evaluate the effect of pheromone addition on a lamprey’s tendency to enter an activated stream.

DISCUSSION

Based on this proof-of-concept effort, we were able to generate whole-night tracks of individual sea lamprey as they moved in the coastal environment in search of spawning habitat for the first time (Objective 1). This also represents the first effort to manipulate coastal search behaviors and river entry (Objective 2).

Sea Lamprey Migration

The spawning migration of the sea lamprey is broken into three parts based on the general migratory environment encountered by the lamprey, including open water migration, coastal migration, and riverine migration. The lamprey's open water migration, defined from the point that an individual releases from a host in a lacustine or oceanic system with the objective of finding a river, until it reaches the near shore coastal environment. The coastal migration encompasses the animal's search for river plumes (CS) and movements in and around river plumes (RPI), until the lamprey enters complete river conditions. The riverine migration encompasses the remaining portion of the migration in the river. The sea lamprey spawning migration in the Great Lakes displays the versatility of an odor mediated search strategy that evolved in the ocean.

In the marine system, migratory fishes that rely on olfaction to locate spawning habitat use directional cues to locate the coast while moving against the dominant current and sampling throughout the water column for olfactory cues (Vickers 2000; Moore & Crimaldi 2003; Doving & Stabell 2003). The chemical integrity of river water that has entered into an ocean can be maintained near the surface, evenly mixed throughout the water column, or held in a stratified layer of the water column (Doving and Stabell 2003, Warrick et al. 2007). The potential for odor cues to exist throughout various levels of the oceanic or estuarine water column encourages migrating fish to sample the entire water column when searching for olfactory cues (Doving & Stabell 2003; Debose & Nevitt 2008).

In the Great Lakes, the direction and spread of the river plume is primarily a function of wind driven currents and the local geography, however temperature discrepancies between the lake and river create seasonal differences in the location of the river plume in the water column (Churchill 2003; Fong et al. 1997). Early in the spring, river water temperatures are colder than the lake water and result in a submerged river plume. As the river warms it begins spreading through the entire water column before finally becoming warmer than the lake and resulting in a primarily superficial plume (Saylor & Miller 1976; Churchill et al. 2003, Vrieze et al 2011). The dynamic spring river plume in the Great Lakes maintains vertical casting as a relevant search behavior despite evolving in the oceanic environment.

The open water migration is the least understood portion of the migration and our observations do not pertain to this phase of the migration because our subjects have already naturally detached and successfully located and entered a river. We have restarted our subject's migration by rereleasing them into the coastal environment. Although the process by which a lamprey detaches from a host and locates the coast is unknown, the maximum distance a sea lamprey would have to travel in Lake Huron is unlikely to be greater than the migratory effort in their oceanic environment. Lake Huron, from shore to shore, is 332 km, north to south, and the widest point would be 245 kilometers, east to west (Edsall & Charlton (1996)). The entire coastline is approximately 3,300 km (excluding all islands, Fig. 2-27), although a sea lamprey following the shoreline would only have to travel approximately 1,900-2,500 km, if the animals follow the general shoreline without perfectly following the intricacies of the shoreline, to encounter all 118 river plumes (minimum estimate, Sullivan & Adair, 2009) known to harbor larval sea lamprey (Fig. 2-27). This indicates that on average a sea lamprey would have to migrate 21.19 km to encounter a viable river plume during the coastal migration. In a worst case open water migration (332 km) and an average coastal migration a lamprey would travel 353.19 km in the Great Lakes. During an oceanic migration on the east coast sea lamprey were observed completing riverine migrations of over 200 kilometers alone and this was limited by dams and not the lamprey's biological energy limits (Killam & Beamish; Beamish 1979). The sea lamprey's anguilliform swimming style is a major reason that it is able to swim 10's to 100's of kilometers during the spawning migration. Anguilliform swimmers are not as effective in high current as carangiform swimmers and quickly experience exhaustion; however anguilliform swimmers are highly efficient at long distance movements in slower lake or ocean conditions (van Ginneken et al. 2005; Borazjani & Sotiropoulos 2009; Kern & Koumoutsakos 2006). Anguilliform swimming would be effective during both the open water and coastal phases of migration.

When swimming along the coast, migrating lamprey typically displayed coastal searching (CS) behavior in the absence of olfactory cues and river plume investigation (RPI) in the presence of olfactory cues. CS is consistent with a large scale search for river plumes after a migrant has already located the coastal waters, moving alongshore counter to the current. The second coastal migration strategy is river plume investigation and is characterized by large angle turning that allows a subject to maintain contact with characteristics of the river plume. The first behavior would put the lamprey into a position where it would have a high likelihood of encountering a concentrated river plume and the second behavior maintains contact with the larval odor cues that would allow for further assessment or river mouth localization and entry.

Coastal searching maximizes the area of coastline sampled by moving perpendicular to the available search field while minimizing the distance traveled (Dusenbery 1986). A migrant does not only sample in two dimensions but the long shore movements are accompanied by vertical casting, which allows the lamprey to sample for vertical stratification of the water chemistry within the water column. Straight movements with vertical excursions are ideal for covering large areas in an environment with a very patchy and intermittent or absent olfactory signal (Vickers 2000). Migrants are traveling at night, a useful strategy to avoid shoreline predators in marine areas (Applegate et al. 1950; Watanuki 1986; Hedger et al. 2008). Maximizing area sampled without regard to other concerns, such as predation, is rarely observed because search behavior is heavily mediated by avoiding risks while maximizing returns (Dusenbery 1998; Zollner & Lima 1999).

Coastal search always entailed moving approximately parallel to the shoreline, although there were two more specific strategies within coastal search. Some subjects made straight movements that gently curved towards the shoreline to different degrees and then appeared to deflect off the coastline and continue along the coast, such as Tag 1072121 or 1072126 (Figs. 2-19 or 2-21). Other subjects were observed maintaining a distance of a kilometer or more the entire way along the coastline such as Tag 1072123 or 1072122 (Figs. 2-11 and 2-20). The two strategies may represent horizontal sampling strategies of the coastal environment that, when paired, allows the sea lamprey to efficiently sample the entire coastal environment. Similar strategies of directional correction due to shoreline encounter have been suggested as escapist strategies for salmon avoiding entrapment in bays and inlets while moving along the coastline (Pascual & Quinn 1991).

After a migrant encountered a river plume it would transition from CS to RPI. The RPI behavior involved a transition from straight movements to curvilinear movements and stopping. The change in movement arrested the general progress along the coast and kept the migrant near the river. This behavioral approach is consistent with observations of foraging insects that increase their turn angle and decrease speed after encountering a resource cue to stay in the vicinity of the resource (Bell 1990). Because sea lamprey performed RPI with and without the addition of larval odor, river water alone may elicit RPI. It has previously been shown that river water is important to the attractiveness of larval odor per Sorensen & Vrieze (2003), likely because larval odor is naturally associated with characteristics of a river environment and therefore river water alone may affect search behavior during the coastal migration. Because RPI may be elicited without the presence of larval odor, the behavior may be a sampling behavior that would allow a subject to become nearer to the river and increase likelihood for encountering larval odor but not necessarily a river entry attempt.

In many cases we observed migrants engaged in the river plume for hours before they continued up the coast. The observation of individuals continuing up the coast after spending time in the river plume is further evidence that RPI is a sampling strategy but these observations differed with observations by Vrieze et al. (2011), in which they never observed a lamprey leaving the plume after entering it. This could be due to a number of factors: 1) The tracking methodology differed between Vrieze et al. (2011) and our observations and this could explain the difference in observations; 2) the Black Mallard River had a very reduced output of larval odor and lamprey were less likely to enter; 3) The Black Mallard was a small river and had a small, concentrated, and more dynamic river plume than the Ocqueoc river making it more difficult to locate the entry point. The difference in tracking methodology could be responsible because Vrieze et al. (2011) interpreted 26 lamprey tracks consisting of 12 positions on average per subject and while tracking they switched between multiple animals and moved the boat in close proximity to the subject during tracking; on the other hand, we interpreted 13 tracks that consisted of 89 points each, on average, and followed continuously for a maximum of 12 hours while never operating the boat within 50 m of the animal. Although the difference in tracking is possible, factors 2 and 3 are consistent with previous findings that larger rivers and larval populations receive larger numbers of migrants (Moore & Schleen 1980; Teeter et al. 1980).

River entry and spawning activity by many anadromous fishes is associated with biotic variables that relate to success of their young (Webb & McLay 1996). Water temperature is the most important variable to survival of eggs and larval sea lamprey (Plavis 1961; Morman et al. 1980). Although stream discharge is often related to temperature and may make smaller rivers easier to find and navigate in the Great Lakes, river temperature is the dominant biotic cue that initiates the onset of the riverine migration (Killam & Parsons 1986; Binder & McDonald 2008; Binder et al. 2010). Temperature may not work independently because the amount of odor released by larvae, changes with feeding rates, and feeding rates are likely to increase with increasing temperature (Fine & Sorensen 2010). This means that an increase in temperature would make the river water easier to migrate in and an associated pulse of pheromone could work together to draw the lamprey into the river.

The riverine migration has been the most well studied portion of the migration due to its accessibility (Applegate 1950; Almeida et al. 2002; Wagner et al. 2006; Quintella et al. 2009; Wagner et al. 2009). Sea lamprey use two movement patterns associated with the openness and velocity of the channel (Quintella et al 2004). In the slower flowing pool or run sections of the river lamprey moved with constant steady swimming and in fast and turbulent rapids or riffle conditions lamprey moved with burst of high activity followed by pauses to rest. (Almeida et al 2002; Quintella et al. 2004; Quintella et al. 2009). In addition to this two phase movement pattern, there also is a pausing behavior in which sea lamprey did not continue upstream for a period of several weeks (Almeida et al. 2002).

We had few observations from animals moving in the Black Mallard River (n=3) and observed an average ground speed of 0.144 BL s^{-1} (SE, range 0.108-179 BL s^{-1}). This ground speed is consistent with observations of lamprey moving in high flow and complex environments (average 0.19 BL s^{-1}, range 0.05-0.45 BL s^{-1}), (Quintella 2009). We did not observe stopping behavior consistent with movement in fast flow environments however we only monitored the lampreys at low flow sections of river and did not constantly monitor movement. We did observe two of the three lamprey stop just after entry but this was likely an artifact of lamprey being released and moving briefly before settling inside the river mouth prior to sunset. The animal that did not stop after entry was released the prior day and entered after nautical twilight (Fig. 2-14). During all three tracks, the individuals moved through the lower section of the Black Mallard River without an extended period of settlement on the order of multiple days, as was observed by Almeida et al (2002). Diurnal settlement was observed for the two of the three lampreys and occurred at the same location. The individuals settled in a dense snag of large woody debris that extended the length of the stream. The reason for repeated settlement at the same location could have been the result of the dense structure taking time to move past or it may have represented a protected habitat for the day. There were no obstacles of similar structure downstream of this object for reference.

Coastal Migration behavior and Larval Odor

In the lab, migratory-phase female sea lamprey, in a two-choice bifurcated maze, preferred to spend more time in the channel with river water spiked with migratory pheromone components (PADS, PSDS, and PS at 10^{-12} M) than in the channel with just lake water spiked with river water (Sorensen et al. 2005). This indicates that migratory-phase sea lamprey attend to the components, although the specific response remains unclear.

The addition of the migratory components increased the likelihood of a lamprey displaying RPI by 37%, although our sample size was too small to obtain a statistically significant finding with only 13 tracks of sufficient duration. A 37 % increase in response to the presence of pheromone would be on target with some of the strongest pheromone mediated responses observed in sea lamprey (13-40 %, Wagner et al. 2006, 2009). The insignificant statistical response of sea lamprey to the addition of larval odor may be more a factor of small sample size and less indicative of the lamprey's response to the pheromone. PADS and PSDS may not be responsible for fully retaining lamprey at the river mouth or river entry; however the insignificant result does not rule out the fact that they could elicit search behaviors that draw lamprey nearer to the river mouth.

Summary

Our uninterrupted tracks support the previous evidence that two movement strategies are used by migratory phase sea lamprey to navigate through the coastal environment and locate spawning habitat. The first strategy, CS, is a conceptually reasonable search strategy and matches common search strategies observed by other marine fishes (Dusenbery 1989, Debose & Nevitt 2008). The second behavior, RPI, is consistent with successful local search strategies for grouped resources and migration with patchy search cues (Bell 1990; Moore & Crimaldi 2003). CS does not only involve parallel movement along the coast with periods of vertical casting as previously described by Vrieze et al. (2011), but also involves some horizontal sampling of the near-shore environment. The horizontal sampling would be an effective strategy for locating smaller river plumes, which are dynamic and have the potential to be pinned very closely to the shoreline (Gordon et al. 2004).

The effect of synthesized PADS and PSDS remains to be seen, although we had a statistically insignificant finding, the importance of these components will require further evaluation to validate or reject their independent potential for eliciting RPI and drawing lamprey to the river mouth. If the addition of these components has a 37 % increase on the likelihood of lamprey performing RPI at a river mouth, these components when used in addition to other cues that potentially maintain the animals in the vicinity of the river mouth or result in river entry, could render the application of PADS and PSDS a relevant control strategy. If these components do not independently affect lamprey behavior at the river mouth, they will need to be evaluated when applied in conjunction with less common components in larval extract.

APPENDICES

APPENDIX A

TABLES

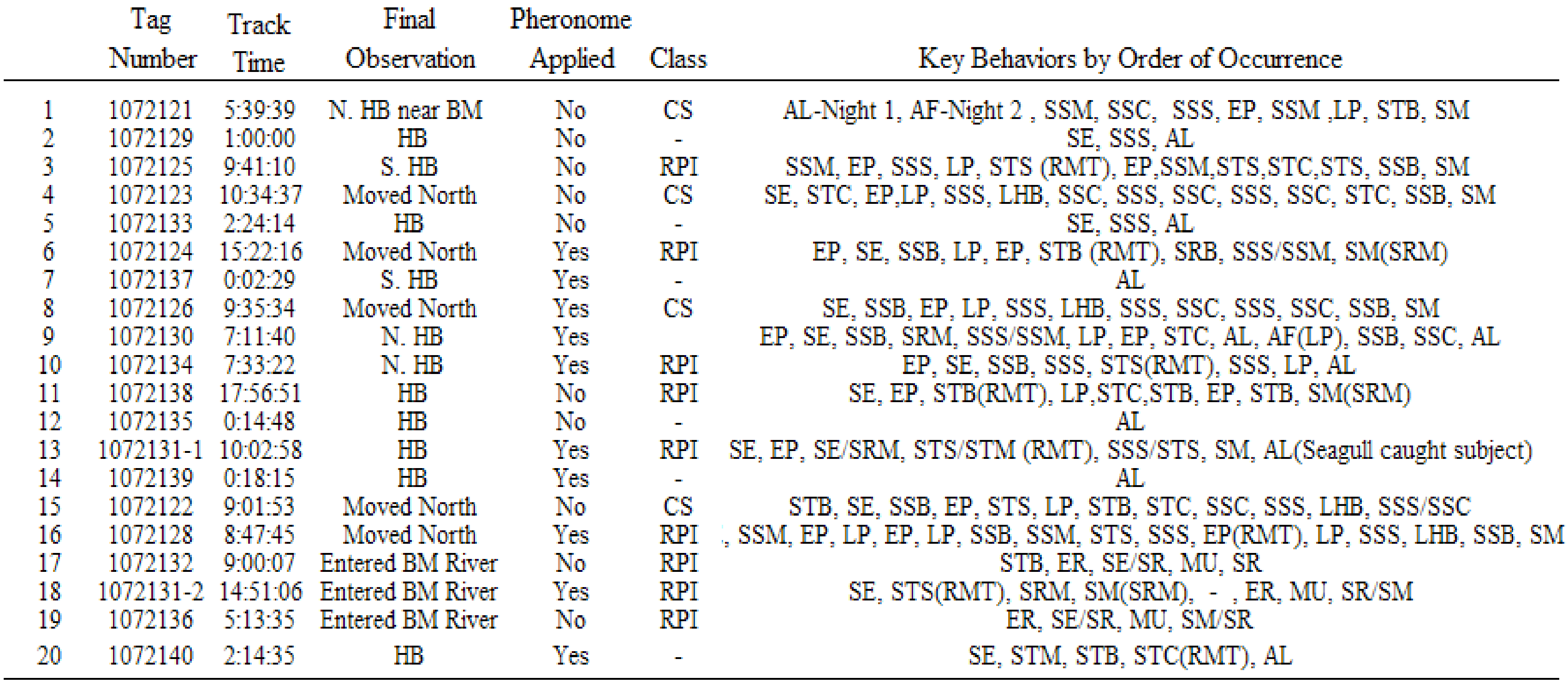
Table 2-1. The relationship between the $\log_{10}$ values of distance to signal strength (S) was modeled for each VR100 gain (7). The models are calculated from data combined across various weather conditions (2) and frequencies (5). Accuracy of positioning can be seen in Figure 2-4. Only Gains 00 through 30 were used for positioning animals based on suggestions per Dale Weber (Pers. Comm.) and due to the increasingly poor fit for the higher gain values. This lack of fit was due to very little sensitivity of higher gains to changes in distance. The higher gains were only used for depth information. In addition only locations within 500 meters of the boat were used because of the increasingly high degree of error for estimates of 1000.

		Relating Distance (D) to Signal Strength (S)						Fit	Error	
VR100 Setting		Variable	Slope	Variable		Intercept		ADJ R^2	Error Slope	Error Intercept
Gain 00	:	Log(D) =	-0.54410	(S)	+	6.492574	:	0.9443	0.002373	0.200371
Gain 06	:	Log(D) =	-0.04855	(S)	+	5.943389	:	0.9010	0.002094	0.175369
Gain 12	:	Log(D) =	-0.05001	(S)	+	6.023571	:	0.8938	0.002261	0.187977
Gain 18	:	Log(D) =	-0.04998	(S)	+	5.986698	:	0.8928	0.002271	0.187302
Gain 24	:	Log(D) =	-0.05488	(S)	+	6.291466	:	0.8501	0.003021	0.243569
Gain 30	:	Log(D) =	-0.06241	(S)	+	6.749284	:	0.7301	0.004967	0.387644
Gian 36	:	Log(D) =	-0.07836	(S)	+	7.739610	:	0.6255	0.007920	0.591580
Gain 42	:	Log(D) =	-0.10280	(S)	+	9.102200	:	0.5146	0.013000	0.911900
Gain 48	:	Log(D) =	-0.15725	(S)	+	12.184850	:	0.3706	0.026520	1.734530

(D) : Refers to Distance in meters

(S) : Refers to a measure of signal strength as read from a VR100 in dB.

Table 2-2. Migratory-phase sea lampreys were monitored as they approached, entered, or bypassed a river mouth under two conditions: 1) with greatly reduced larval odor following larval treatment (n=10); and, 2) condition one combined with the addition of synthesized pheromone components (n=10). The details of the 20 trials include order of animal release (1-20), tag number, length of time animal was followed (hh:mm:ss), the final fate of each animal by abbreviated direction and location (HB: Hammond Bay, N:North, S:South, BM: Black Mallard), whether pheromone was applied (Yes or No) , the type of primary coastal behavior observed (Coastal Search or River Plume investigation), and a list of the three dimensional movements made by each animal.



	Tag Number	Track Time	Final Observation	Pheronome Applied	Class	Key Behaviors by Order of Occurrence
1	1072121	5:39:39	N. HB near BM	No	CS	AL-Night 1, AF-Night 2 , SSM, SSC, SSS, EP, SSM ,LP, STB, SM
2	1072129	1:00:00	HB	No	-	SE, SSS, AL
3	1072125	9:41:10	S. HB	No	RPI	SSM, EP, SSS, LP, STS (RMT), EP,SSM,STS,STC,STS, SSB, SM
4	1072123	10:34:37	Moved North	No	CS	SE, STC, EP,LP, SSS, LHB, SSC, SSS, SSC, SSS, SSC, STC, SSB, SM
5	1072133	2:24:14	HB	No	-	SE, SSS, AL
6	1072124	15:22:16	Moved North	Yes	RPI	EP, SE, SSB, LP, EP, STB (RMT), SRB, SSS/SSM, SM(SRM)
7	1072137	0:02:29	S. HB	Yes	-	AL
8	1072126	9:35:34	Moved North	Yes	CS	SE, SSB, EP, LP, SSS, LHB, SSS, SSC, SSS, SSC, SSB, SM
9	1072130	7:11:40	N. HB	Yes		EP, SE, SSB, SRM, SSS/SSM, LP, EP, STC, AL, AF(LP), SSB, SSC, AL
10	1072134	7:33:22	N. HB	Yes	RPI	EP, SE, SSB, SSS, STS(RMT), SSS, LP, AL
11	1072138	17:56:51	HB	No	RPI	SE, EP, STB(RMT), LP,STC,STB, EP, STB, SM(SRM)
12	1072135	0:14:48	HB	No	-	AL
13	1072131-1	10:02:58	HB	Yes	RPI	SE, EP, SE/SRM, STS/STM (RMT), SSS/STS, SM, AL(Seagull caught subject)
14	1072139	0:18:15	HB	Yes	-	AL
15	1072122	9:01:53	Moved North	No	CS	STB, SE, SSB, EP, STS, LP, STB, STC, SSC, SSS, LHB, SSS/SSC
16	1072128	8:47:45	Moved North	Yes	RPI	, SSM, EP, LP, EP, LP, SSB, SSM, STS, SSS, EP(RMT), LP, SSS, LHB, SSB, SM
17	1072132	9:00:07	Entered BM River	No	RPI	STB, ER, SE/SR, MU, SR
18	1072131-2	14:51:06	Entered BM River	Yes	RPI	SE, STS(RMT), SRM, SM(SRM), - , ER, MU, SR/SM
19	1072136	5:13:35	Entered BM River	No	RPI	ER, SE/SR, MU, SM/SR
20	1072140	2:14:35	HB	Yes	-	SE, STM, STB, STC(RMT), AL

Abbreviations for Key Behaviors: Animal was Found (AF), Animal was Lost (AL), Animal Entered Plume (EP), Animal Entered Black Mallard River (ER), Left Hammond Bay (LHB), The animal left the plume (LP), Move upstream in River (MU),Ocqueoc River (OCQ), RMT-Repeated movements Towards and Away from the River, Animal Settled prior to sunset (SE), Animal Settled in the Morning (SM), Animal Settled in Black Mallard River (SR), Animal Settled at River Mouth (SRM), Animal Swam Straight and Vertically Casted (SSC), Animal is swam straight near the bottom (SSB) or surface (SSM), Animal swam straight at Surface (SSS), subject exhibited large turns and Vertically Casted (STC), The Animal Swam with large Turns in water column (STM), The Animal swam with large Turns and near the Surface (STS).

APPENDIX B

FIGURES

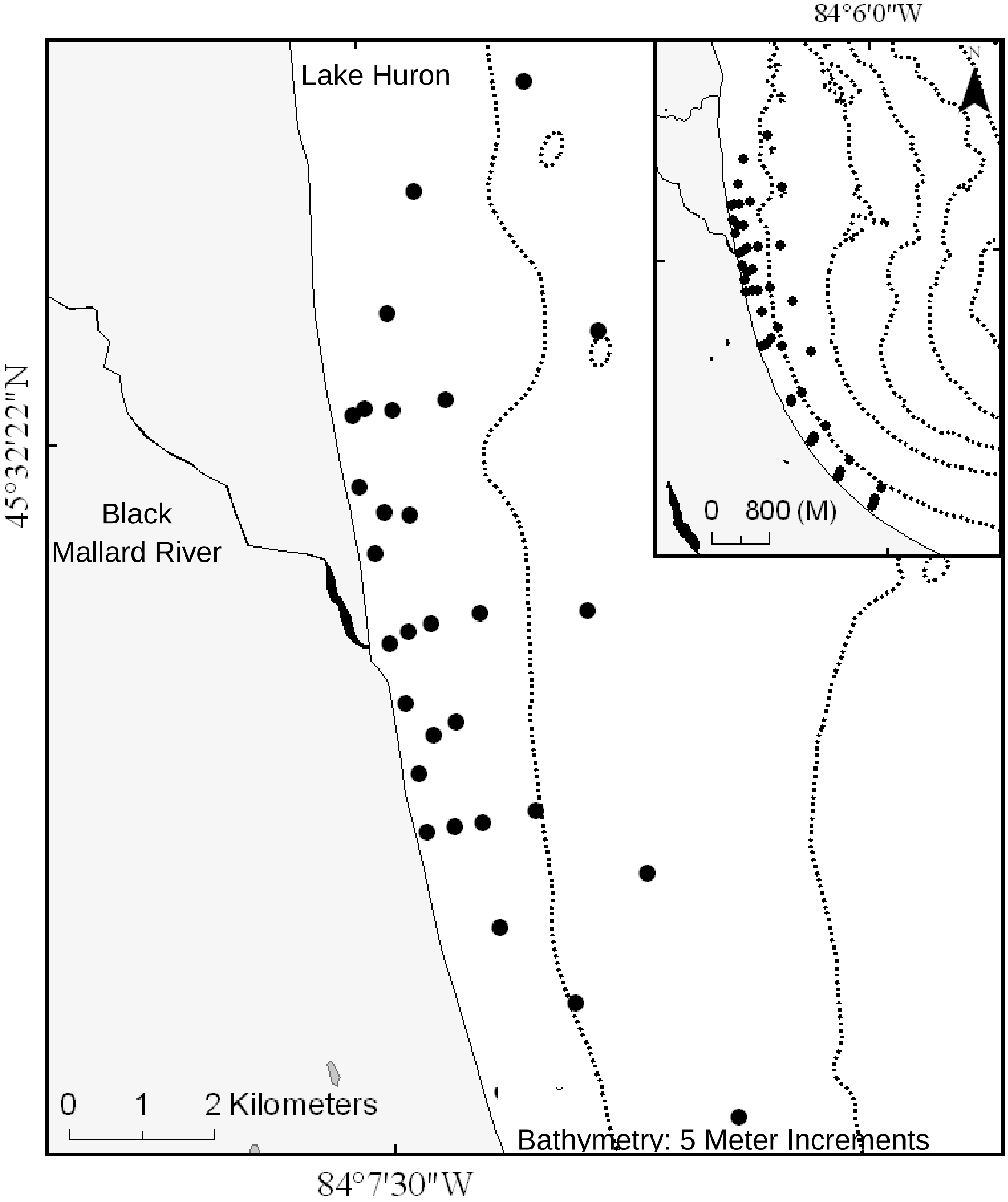


Fig. 2-1. This is a map of the primary study area offshore of the Black Mallard River's drainage to Lake Huron. The dots in the large window represent the approximate location of sampling stations for ascertaining the river plumes location prior to release of a tagged lamprey. The small frame shows the additional sampling locations necessary for interpreting the near shore south reaching river plumes.

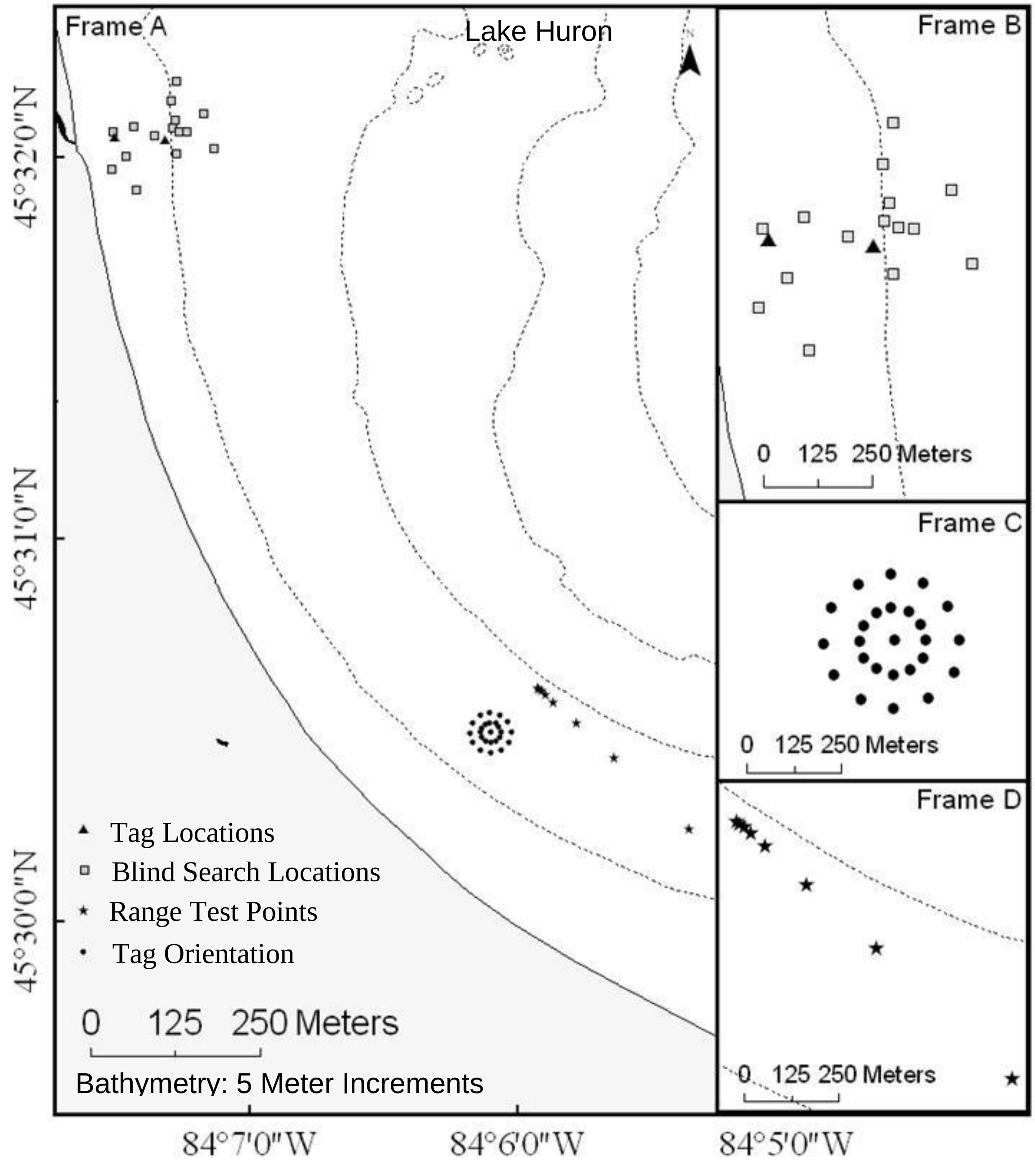


Fig. 2-2. Range testing was required to create estimates of animal positions from manual acoustic measurements. First the effect of tag orientation on signal strength had to be tested (Frame C). The relationship between signal strength and the distance animals were from the boat had to be developed (Frame D) as well as tested for accuracy when used as part of the entire positioning process (Frame B). The full details of these activities are outlined in the methods.

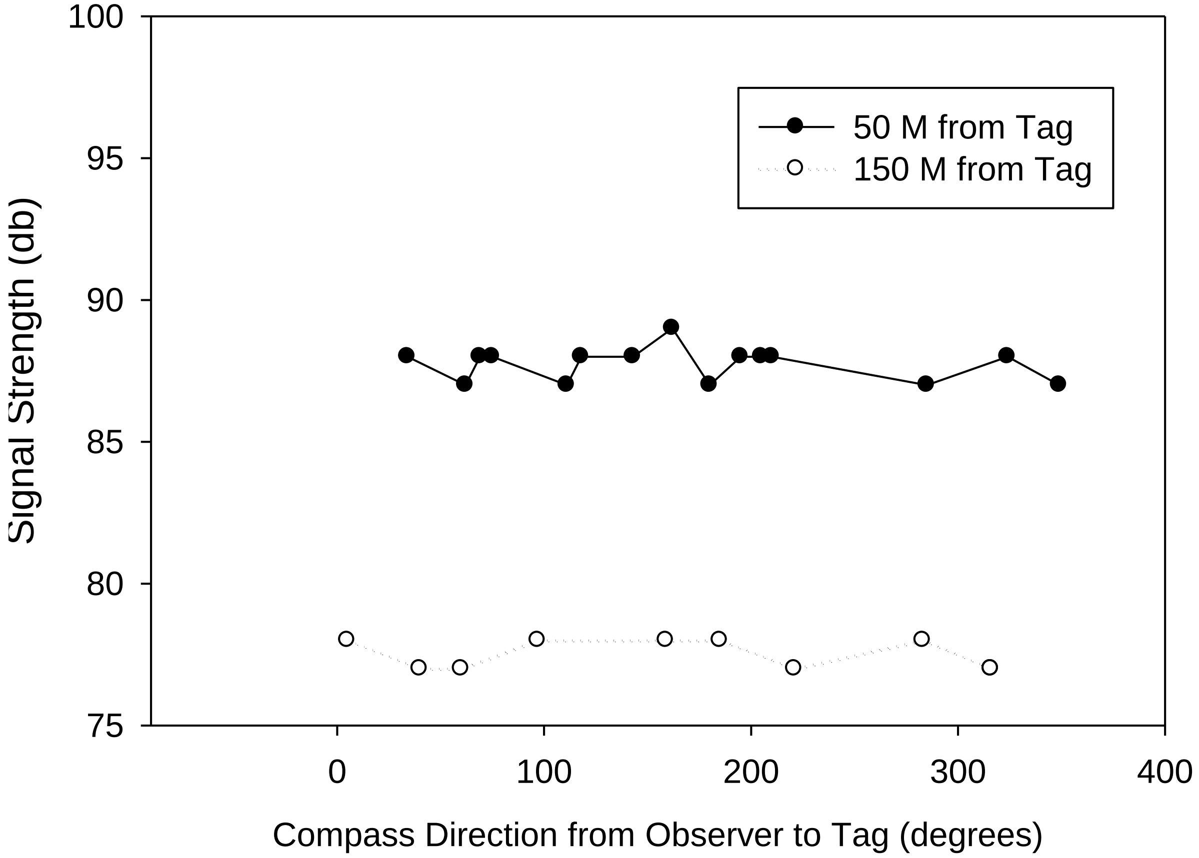


Fig. 2-3. The effect of tag orientation (relative to the observer) on observed signal strength was evaluated through direct comparison of signal strength measurements taken from a present position at approximately 30 degree intervals surrounding a tag at fixed position and orientation at two distances (50 and 100 m) in calm conditions (Fig. 2b). A 72 hz tag was attached to a 15 gauge steel embedded in a cement block and positioned 1 m above the substrate at a depth of 6.4 m. A 135 kg anchor next to the tag was used to position the boat and was confirmed with a GPS (Magellan Mobile Mapper CX and Magellan Precision NAP100 antenna). The exact 30 degree angle was difficult to attain with the exact distance so the exact distance was gained and a relative angle was attempted. This explains why there were more than eleven points for the 50 meter distance and why angels were not exactly at 30 degree intervals. There were less than 11 for the 150 meter distance because the tag turned off due to programming issues.

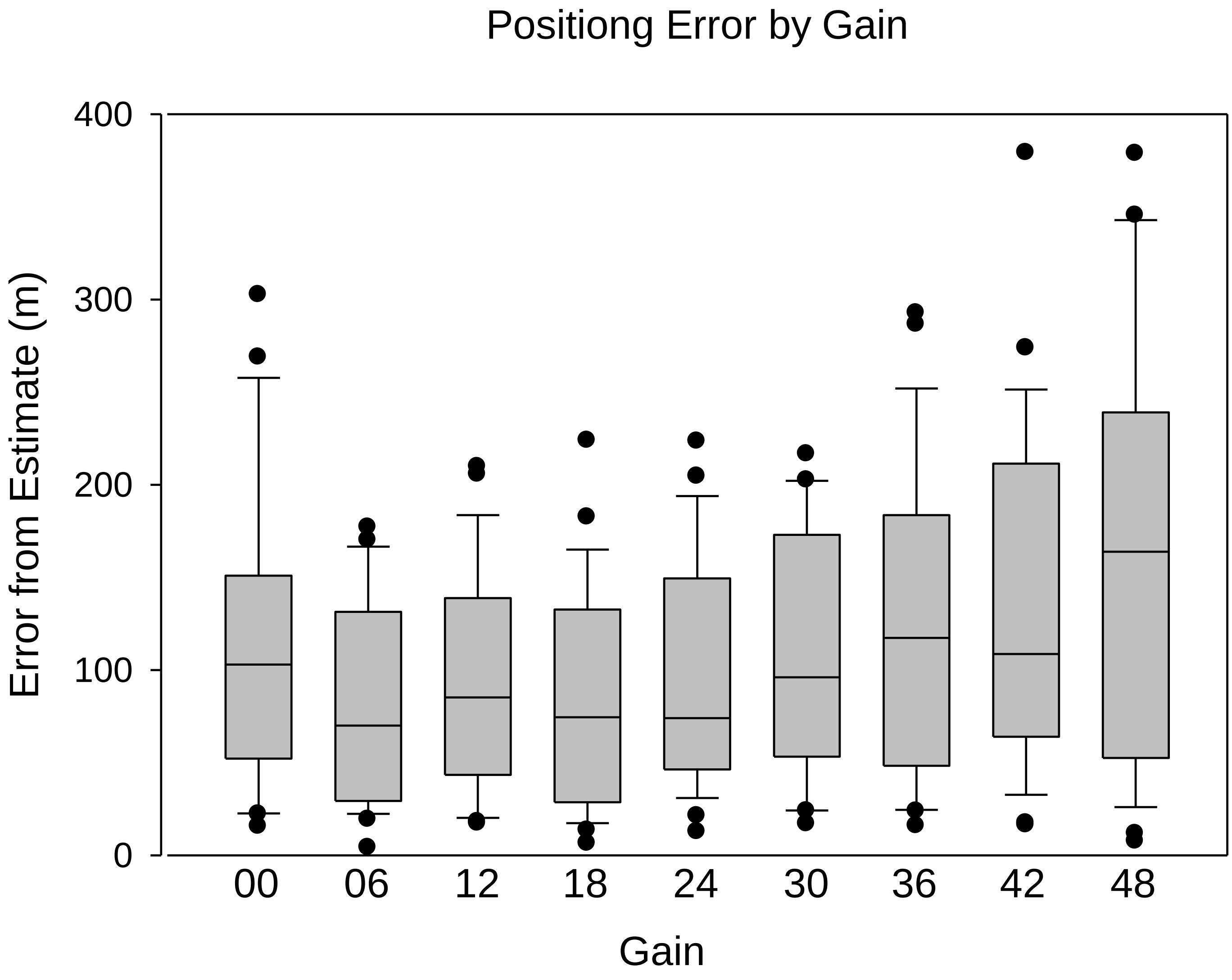


Fig. 2-4. Acoustic tags were positioned by a searcher that was blind to the location of the tags to allow for a realistic estimate of error. Error values were averaged for each gain from 15 sample sites and at two different tag frequencies (72, 84) (n=30). Although there is an apparent increase in error at high gains in both overall range and median value (horizontal line), only the Gain 48 and 18 were statistically different when using a one way ANOVA.

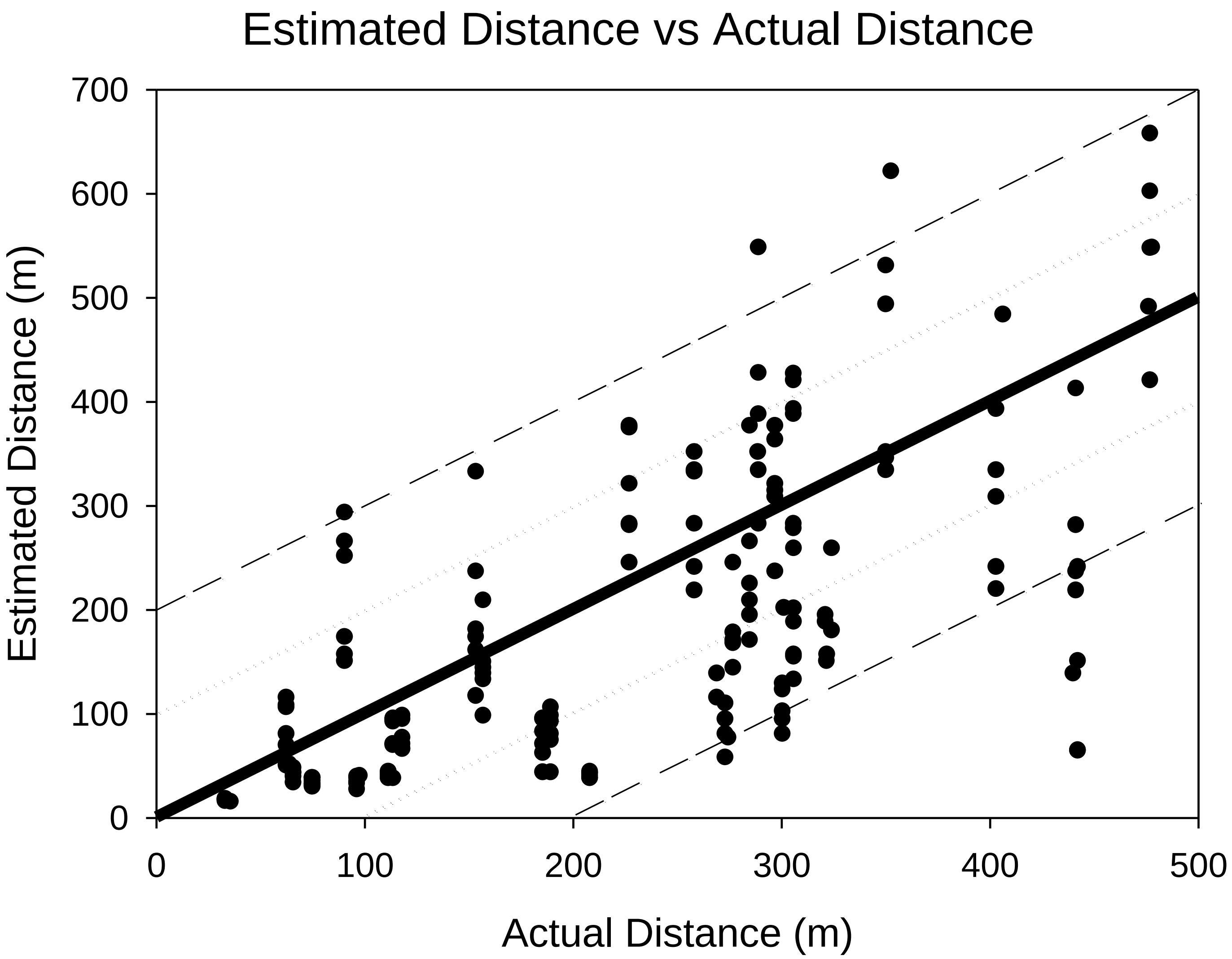


Fig. 2-5. Tags were positioned by a searcher that was blind to the location of the tags to allow for a realistic estimate of error. The data contains estimates across all gains and two tag frequencies (72 and 84 khz). Instead the one to one error to actual distance relationship indicated by the dashed line is a strong indicator of the maximum error observed by a measurement. Across all distances from the tag (0-500 meters) average error was 97.00 meters with a standard deviation of 62.74 meters. Error levels of 100 and 200 meters are indicated by the horizontal dotted lines.

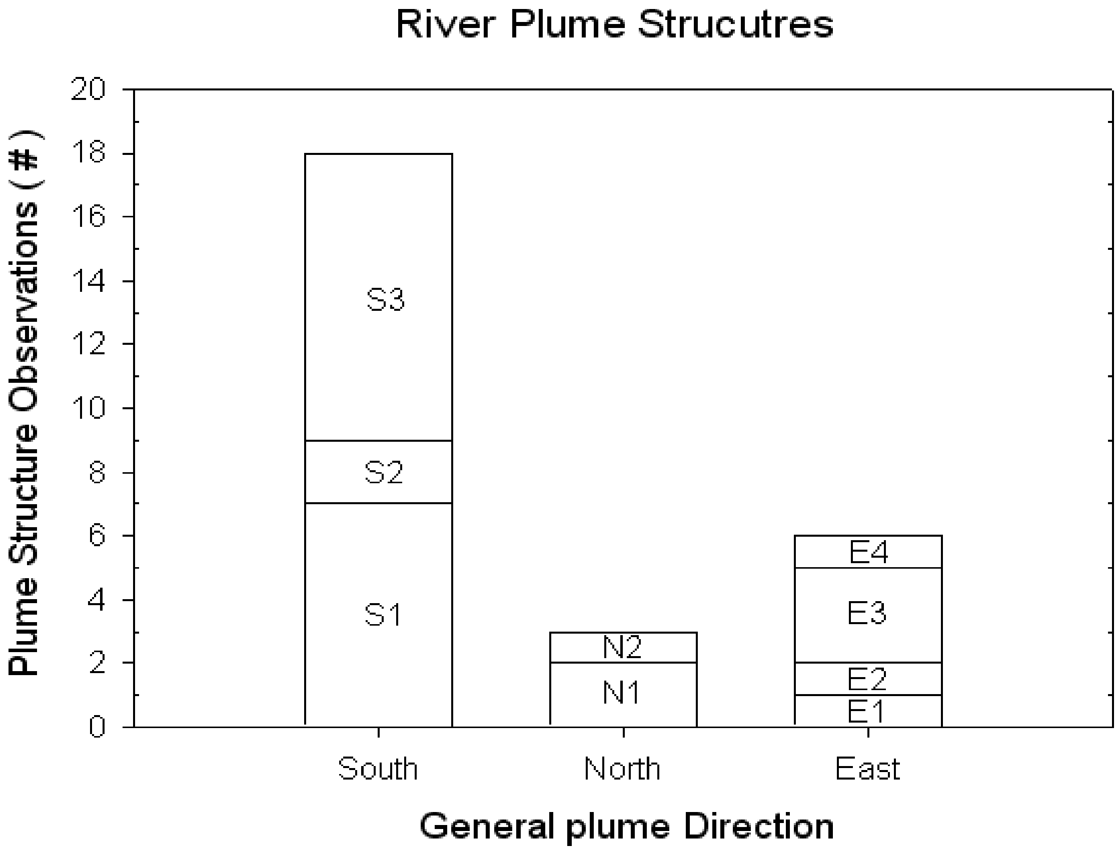


Fig 2-6. During the study, plumes to the south were by far the most common, which was likely the result of the dominant cyclonic flow in Lake Huron combined with frequent northerly winds during the study (Beletsky et al. 1999). Easterly plumes were the second most common and were generally less stable and changed between north and south reaching structures. Northerly plumes were the least common but were generally stable and reached to the northern extent of Hammond Bay. There were multiple specific structures that comprise the three categories of north south and east reaching plumes and are depicted in Figures 5-7.

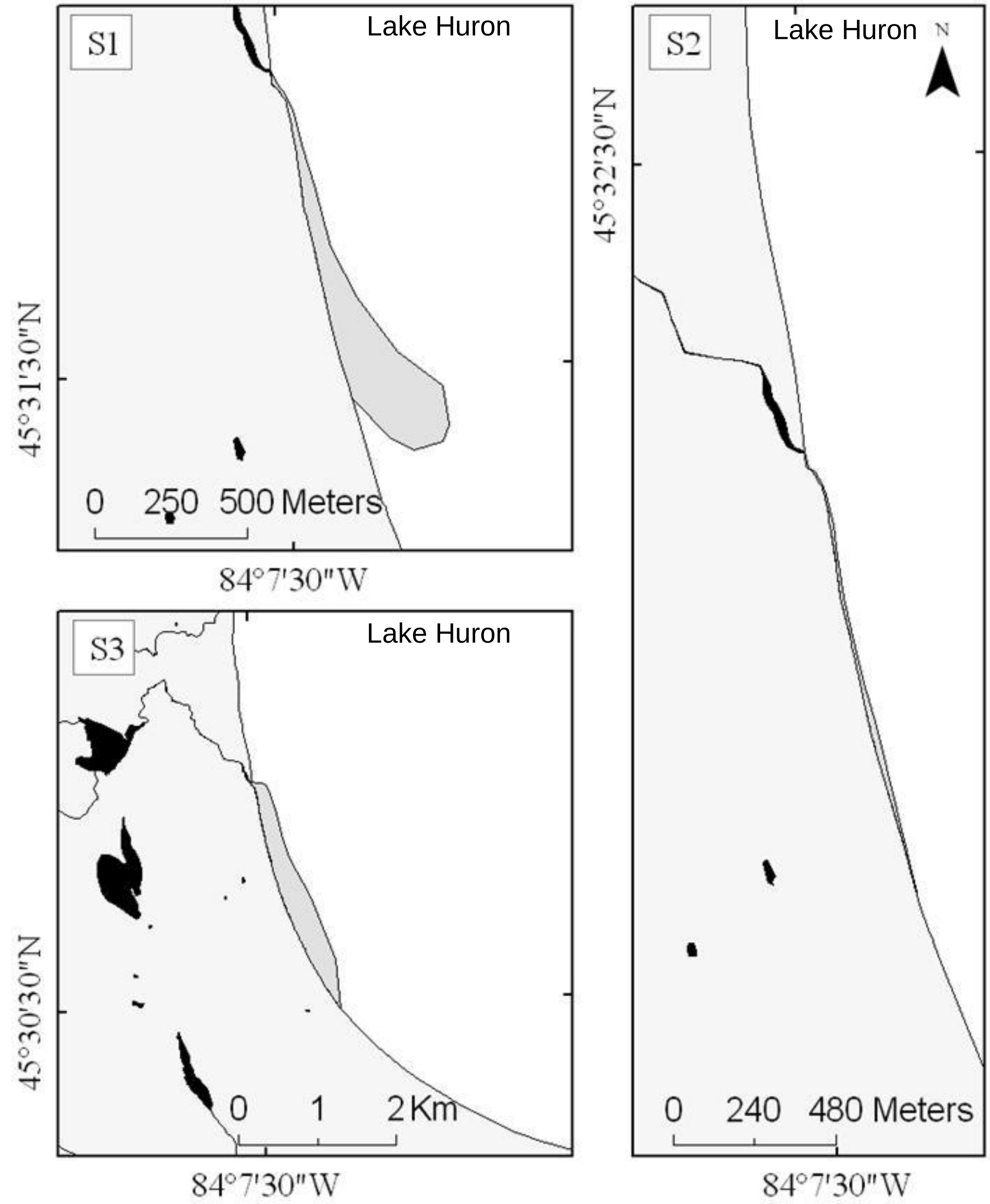


Fig. 2-7. Black Mallard river plumes were generally to the south (70 %) (darker gray area). These three primary forms varied between observations due to minor differences in wind direction or speed. A plume tight to shore that tapered out away from shore (S1) was created by 10-20 mph winds from the W-N-W (n=4) and less than 5 mph winds from the E-N-E (n=3). A plume very tight to shore (< 10 m, S2) and varying distances to the south was created from a weak E wind (<7 mph, n=1), 14-20$^+$ mph W-N-W (n=4), and N and E switching combinations at varying speeds (5-20 mph, n=4). A large plume quickly spreading from shore that extended several kilometers down the coast (S3) was created by a 7 mph E switching to E-S-E wind (n=1) and a 29 mph W wind (n=1).

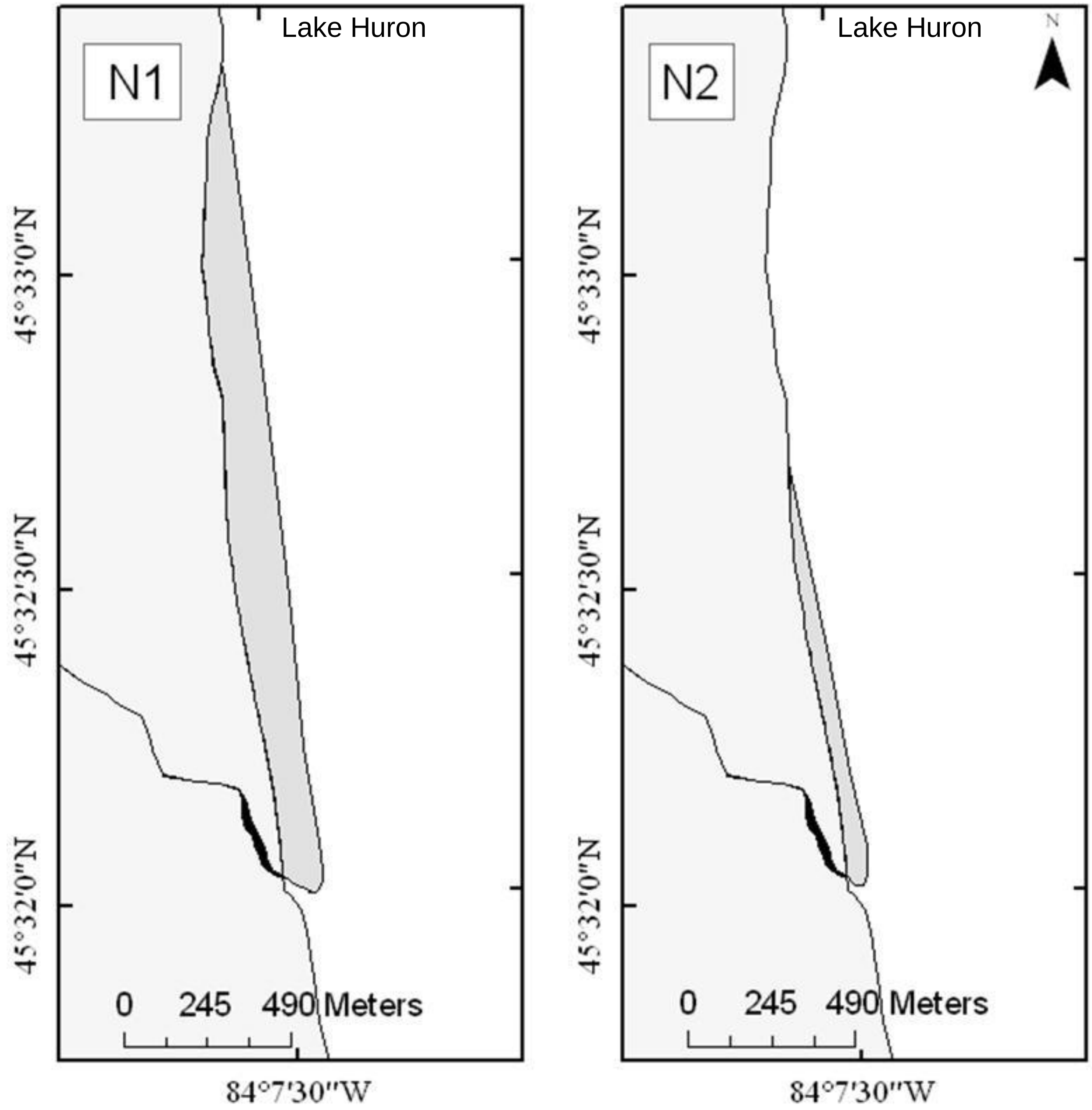


Fig. 2-8. On three occasions the Black Mallard river plume was N of the river mouth (darker gray area). The larger plume structure that extended to the edge of Hammond Bay (N1) and likely extended past the point in low concentration was associated with a 25 mph wind from the W-S-W (n=1) and a 11 mph wind from the S-S-W, changing to the S (n=1). A more reduced version of this plume (N2) was created by an 8 mph wind from the E and changing to the E-S-E (n=1).

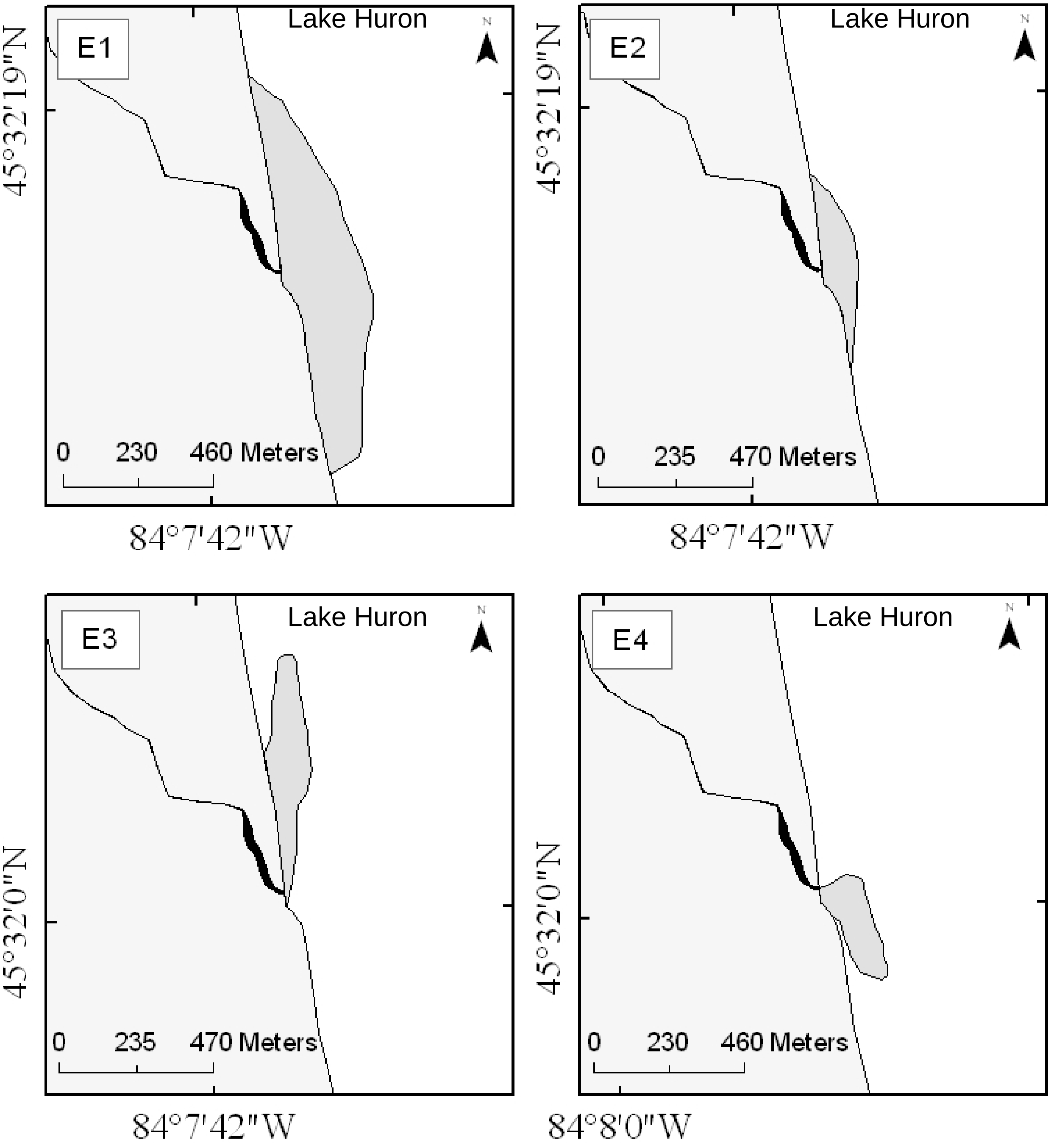


Fig. 2-9. The Black Mallard River plume was occasionally east (darker gray area). The reduced east plumes extended less than 1 km from the river mouth and were often less stable than the long shore plumes that extended to the north or south. The largest central plume structures (E1) were created by an 8.6 mph wind from the E that switched from a 10 mph N-E wind earlier in the day (n=1). A more reduced central plume (E2) was created by a highly fluctuating wind from the E (n=3). A N reaching reduced plume (E3) was created by a 10 mph wind from the W-S-W (n=1). The final reduced plume structure (E4) was to the S and was created by a 2.6 mph wind from the W (n=1). When a strong East wind was observed (> 20 mph), the visible plume was reduced to a few feet from shore directly in front of the river mouth.

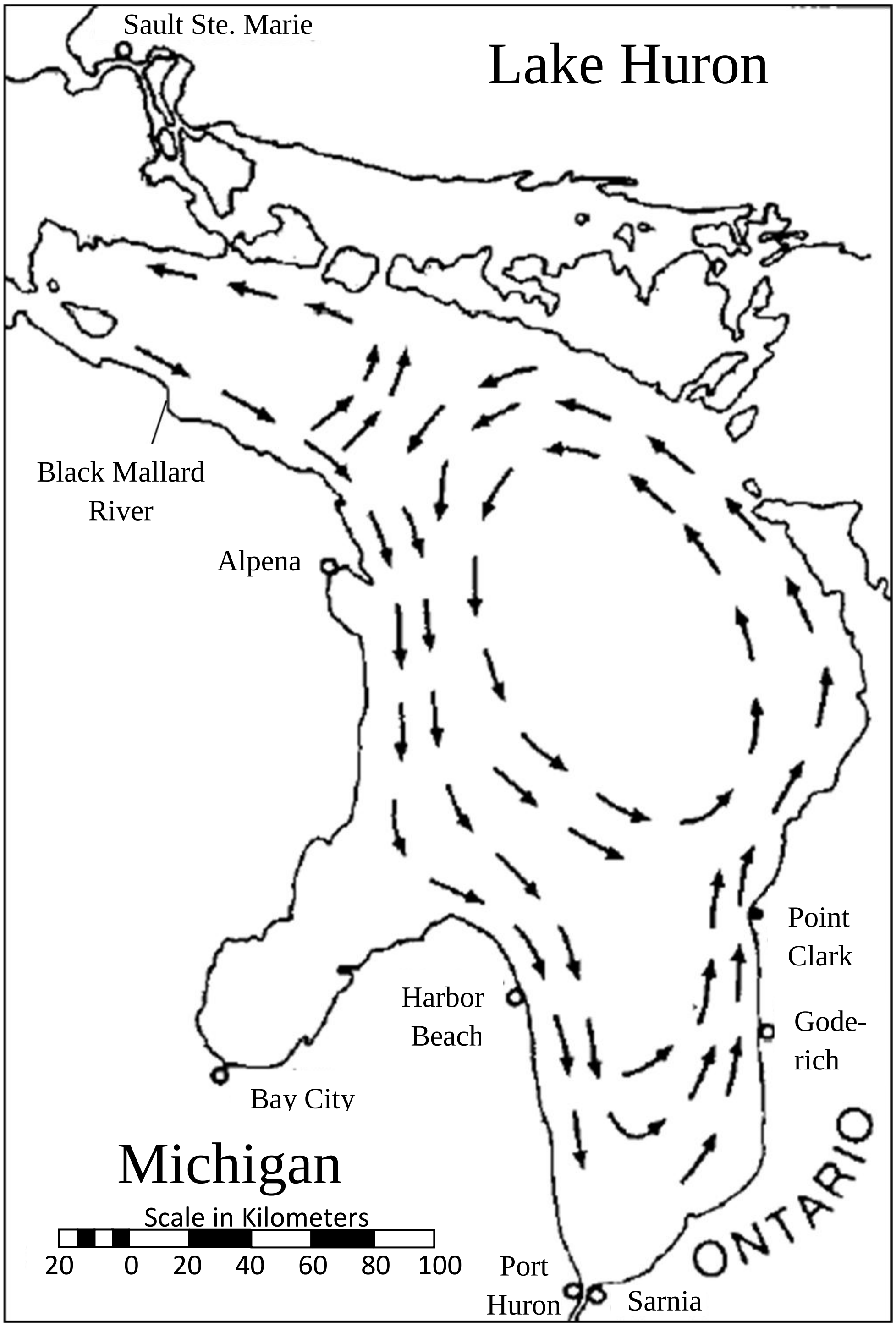


Fig. 2-10. The image depicts the standard currents of Lake Huron in spring and summer and is modified from Saylor & Miller (1976), "Wind Currents in Lake Huron". This standard current creates a southerly long shore current through Hammond Bay. Other river plumes than southerly reaching plumes were observed in Lake Huron, and it appeared that winds moving in other directions were responsible for altering the standard conditions.

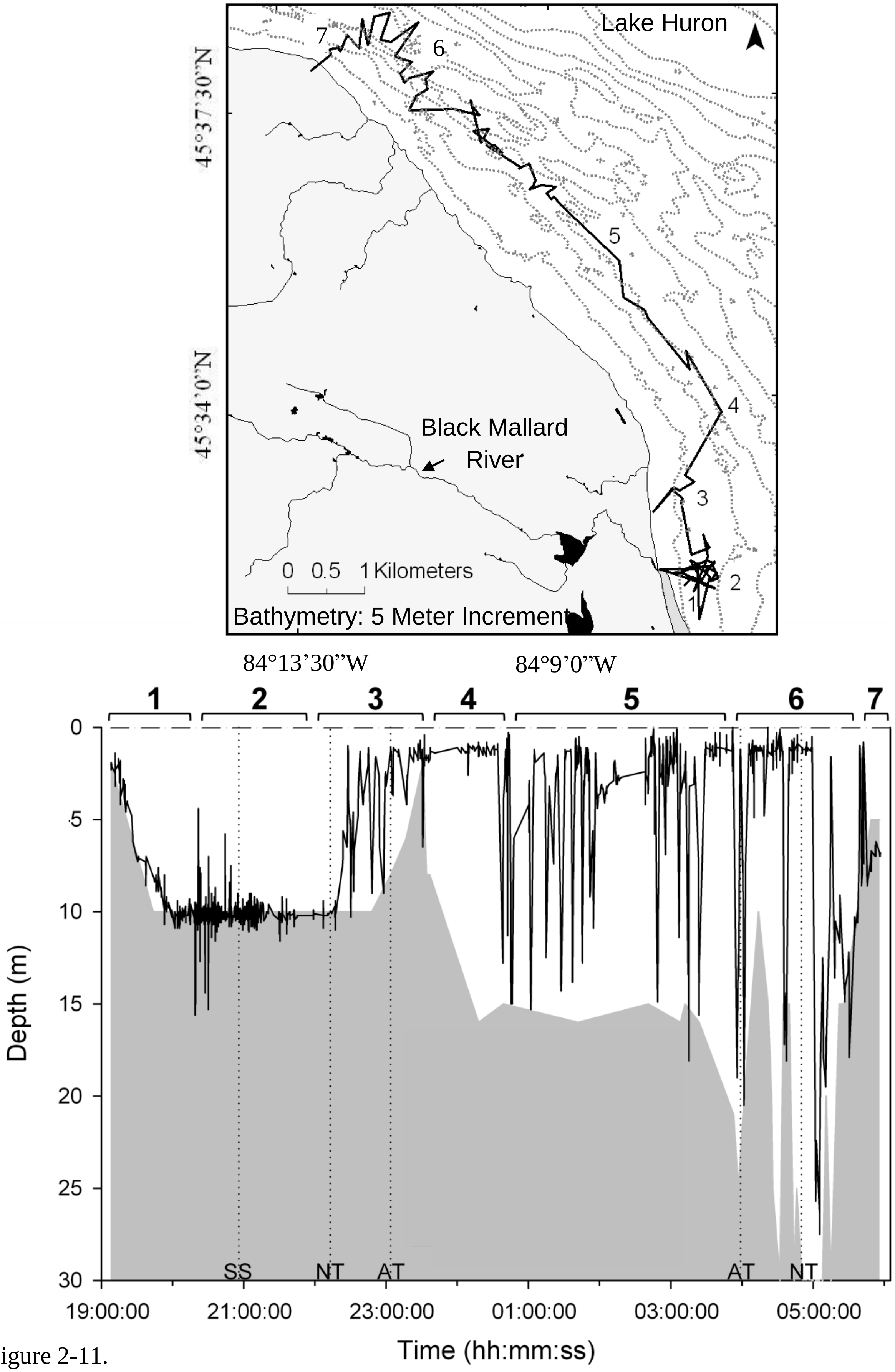

Lake Huron
7
6
5
4
3
2
1
Black Mallard River
0 0.5 1 Kilometers
Bathymetry: 5 Meter Increment
45°37'30"N
45°34'0"N
84°13'30"W
84°9'0"W
1
2
3
4
5
6
7
Depth (m)
0
5
10
15
20
25
30
SS
NT
AT
AT
NT
19:00:00
21:00:00
23:00:00
01:00:00
03:00:00
05:00:00
Time (hh:mm:ss)

Figure 2-11.

Figure 2-11 (cont'd). The lamprey 1072123 was released at 19:02:35 on May 12th, south of the river in a type S3 plume. The lamprey stopped after release (2, 19:30-22:18). The subject moved near the river mouth with wide-angle turning and vertical movements before moving north (3, 22:18-23:00). Next it swam at the surface and left Hammond Bay (4, 23:00-00:15). The subject exhibited many large vertical excursions (> 25m). There is a period of surface swimming for 30 minutes, followed by vertical movements. The subject moved up the coast with primarily straight movements (5, 00:15-04:00). The lamprey made zigzagging movements along and then towards shore on the bottom (6, 04:00-06:00). The lamprey appeared to have stopped but could have been moving when tracking ended.

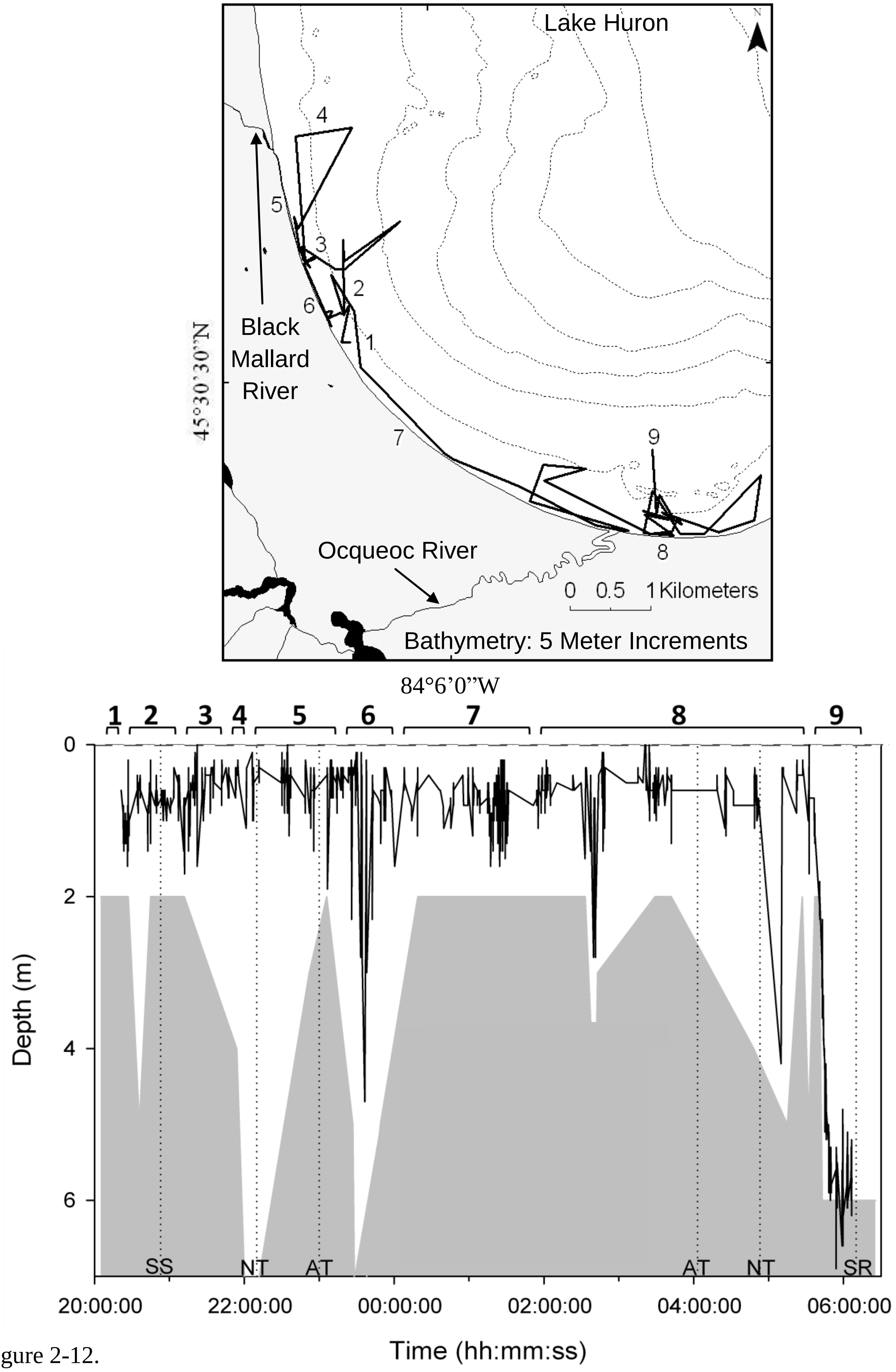

Lake Huron
N
4
5
3
2
6
1
Black Mallard River
45°30'30"N
7
9
8
Ocqueoc River
0 0.5 1 Kilometers
Bathymetry: 5 Meter Increments
84°6'0"W
1
2
3
4
5
6
7
8
9
0
2
4
6
Depth (m)
SS
NT
AT
AT
NT
SR
20:00:00
22:00:00
00:00:00
02:00:00
04:00:00
06:00:00
Time (hh:mm:ss)

Figure 2-12.

Figure 2-12 (cont'd). The lamprey 1072125 was released 20 meters from shore in a type S2 plume on May 10th (1, 20:17). The subject moved with wide-angle turning through the river plume (2, 20:17-21:00) and then further up the coast with straighter movements to the river mouth (3, 21:00-21:50), before passing the river mouth, moving offshore, returning near-shore and continued south (4, 21:50-22:00). The subject re-entered the river plume while wide-angle turning (5, 22:00-23:25), and continued away from shore while diving towards the bottom moving along the coast (6, 23:25-23:59). The subject swam to the mouth of the Ocqueoc River with straight movements (7, 00:00-02:00) before moving with wide angle turning in front of the river (8, 02:00-05:30) and stopping at 06:00 (9).

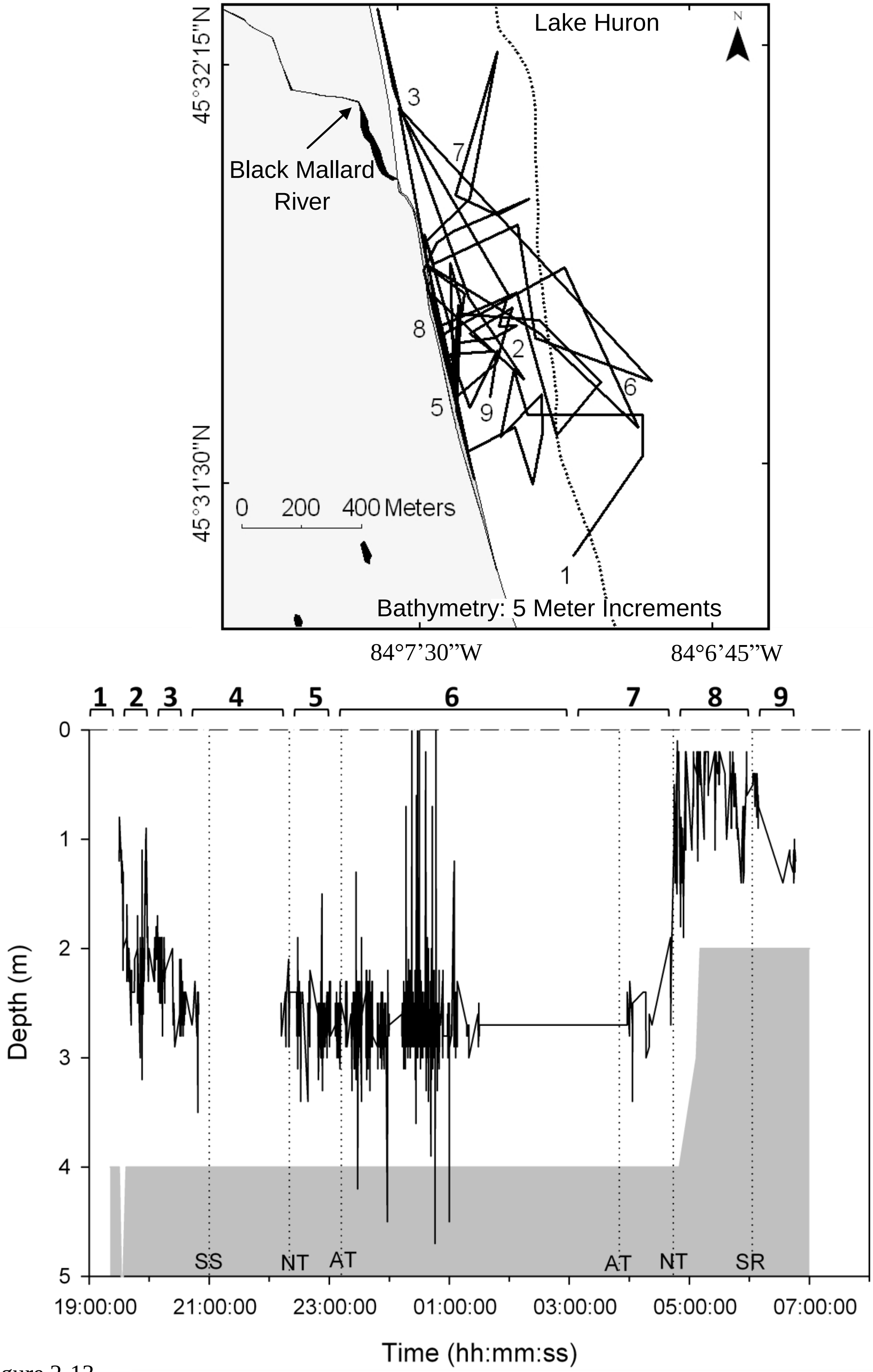

Lake Huron
N
45°32'15"N
45°31'30"N
Black Mallard River
3
7
8
2
6
5
9
1
0 200 400 Meters
Bathymetry: 5 Meter Increments
84°7'30"W
84°6'45"W
1
2
3
4
5
6
7
8
9
Depth (m)
0
1
2
3
4
5
SS
NT
AT
AT
NT
SR
19:00:00
21:00:00
23:00:00
01:00:00
03:00:00
05:00:00
07:00:00
Time (hh:mm:ss)

Figure 2-13.

Figure 2-13 (cont'd). Lamprey 1072124 was released on May 16th at 19:30:53, south of the river in a type S2 plume. The subject swam out of the river plume and settled (1 & 2, 19:37-19:50). The subject passed the river mouth and then returned to the plume. (3, 20:00-20:31). No tracking was performed from 20:31- 22:27 (4). The subject was reacquired south of the river, in the plume (5, 22:27-22:54). We observed several approaches and retreats towards the river mouth, characterized by wide angle turns (6, 22:54-3:00), until it settled in front of the river mouth (7, 3:00-4:50). The subject again began moving south down the coast in shallow water (8, 5:00-6:00).The lamprey finally settled near shore in a deeper section of near-shore water about 800 meters from the river mouth just after 06:00 (9).

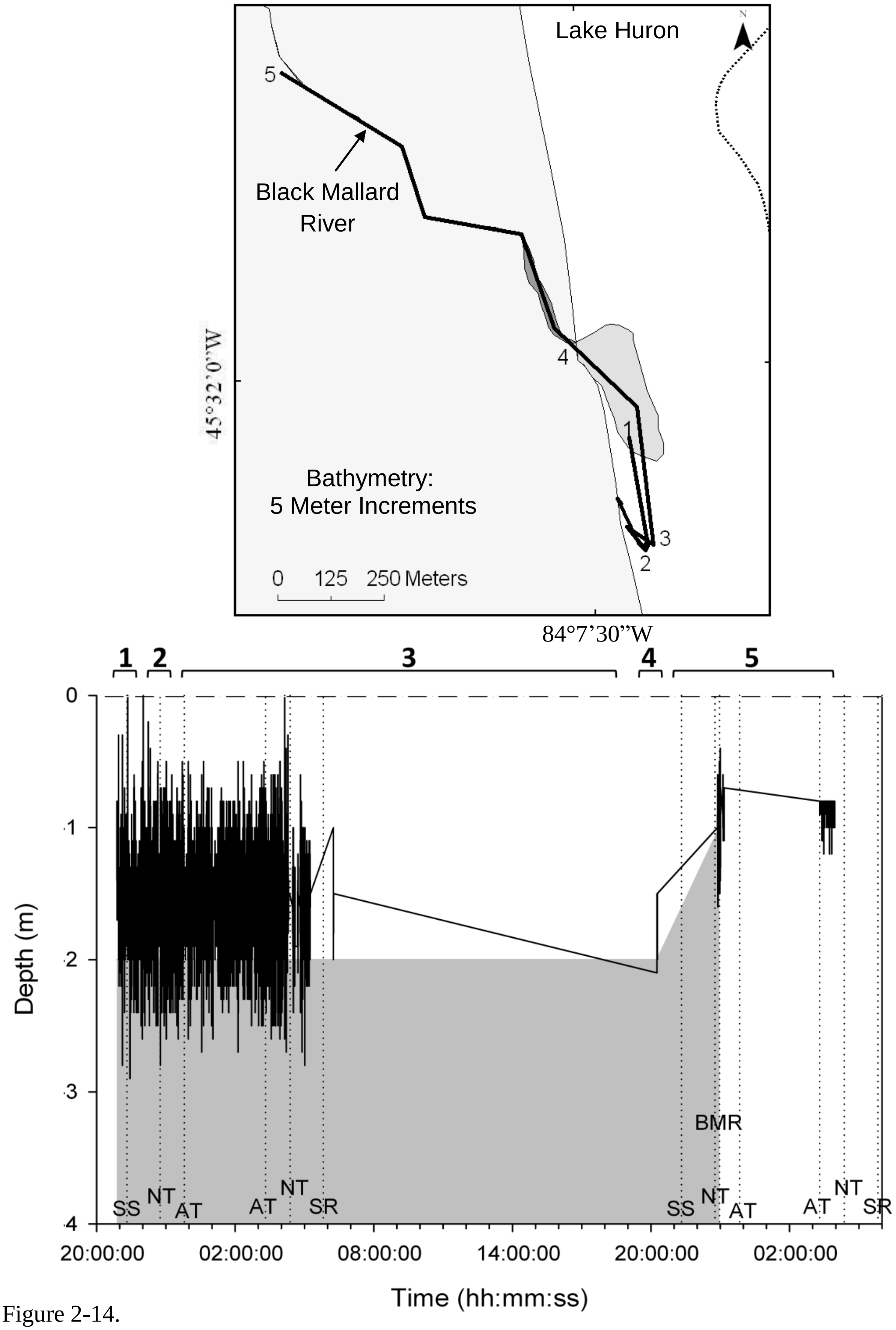

Lake Huron
N
5
Black Mallard
River
45°32'0"W
4
1
3
2
Bathymetry:
5 Meter Increments
0 125 250 Meters
84°7'30"W
1
2
3
4
5
0
-1
-2
-3
-4
Depth (m)
BMR
SS
NT
AT
AT
NT
SR
SS
NT
AT
AT
NT
SR
20:00:00
02:00:00
08:00:00
14:00:00
20:00:00
02:00:00
Time (hh:mm:ss)

Figure 2-14.

Figure 2-14 (cont'd). The lamprey 1072131-2 was released on June 3rd at 20:00, 100 meters south of the river mouth into a type S4 plume and intermittently monitored for 18 hours. The subject settled in a submerged dock (2) and did not move from the dock (3). It eventually entered the river on June 4th at 20:15:35 (4). The subject was found upstream swimming slowly and had its sutures torn (5). It was below the log jam where subjects1072132 and 1072136 settled.

Note: The first night pheromone was applied to the river but the lamprey entered when pheromone was not being applied. It was also a very cold night the first night (Low below 0 ^{0}C air temperature).

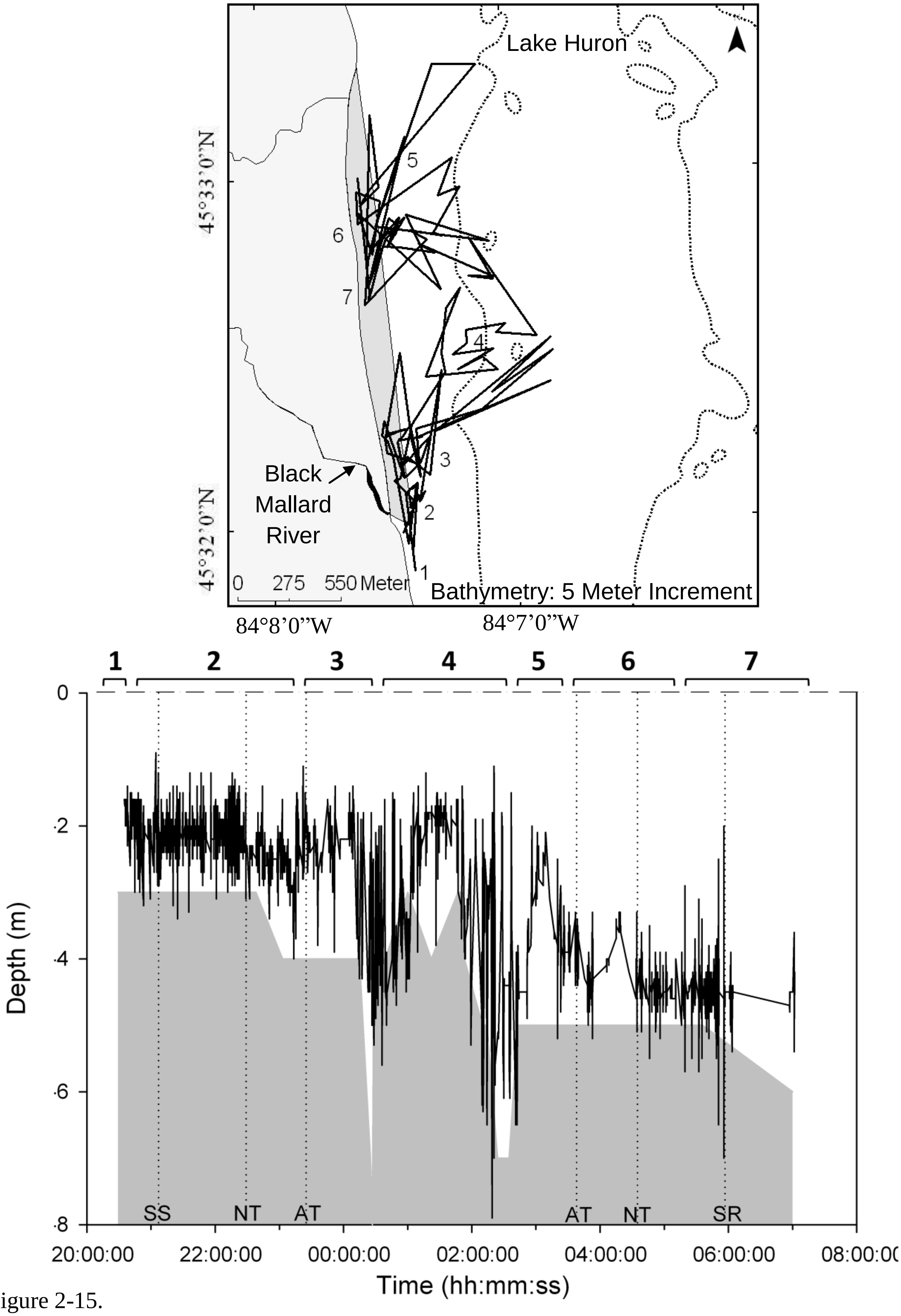

Lake Huron
5
6
7
4
3
2
1
Black
Mallard
River
45°33'0"N
45°32'0"N
0 275 550 Meter
Bathymetry: 5 Meter Increment
84°8'0"W
84°7'0"W
1
2
3
4
5
6
7
0
-2
-4
-6
-8
Depth (m)
SS
NT
AT
AT
NT
SR
20:00:00
22:00:00
00:00:00
02:00:00
04:00:00
06:00:00
08:00:00
Time (hh:mm:ss)

Figure 2-15.

Figure 2-15 (cont'd). The lamprey 1072138 was released on May 22nd at 20:29:57 (1), south of the river in a type E4 plume that switched to a N1 type plume. Upon release, it swam towards the river mouth and stopped (2, 21:50-23:15). The subject moved away from the river mouth, with wide-angle turning and many vertical excursions (3, 23:15-0:30). It then swam away from the river and moved offshore, outside the plume (4, 0:30-2:35). The subject made repeated near-shore and offshore movements with vertical excursions followed by a move to the edge of Hammond Bay before returning to the river plume (5, 2:35-3:30). It moved primarily in the river plume and likely on the bottom with large angle turning (6, 3:30-5:20) before settling in the river plume (7, 5:20-6:00).

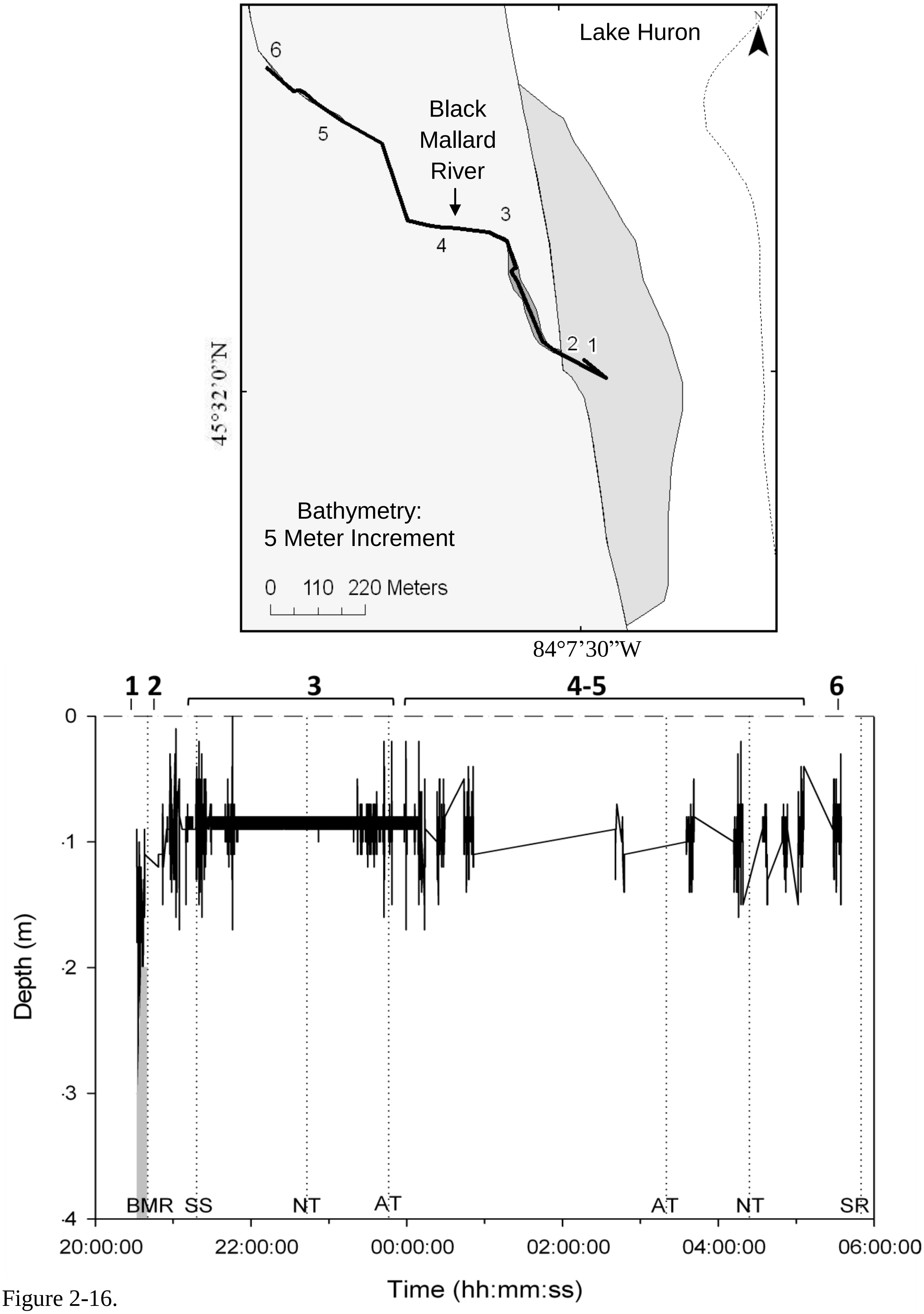

Lake Huron
N
6
5
Black
Mallard
River
3
4
2
1
45°32'0"N
Bathymetry:
5 Meter Increment
0 110 220 Meters
84°7'30"W
1
2
3
4-5
6
Depth (m)
0
-1
-2
-3
-4
BMR
SS
NT
AT
AT
NT
SR
20:00:00
22:00:00
00:00:00
02:00:00
04:00:00
06:00:00
Time (hh:mm:ss)

Figure 2-16.

Figure 2-16 (cont’d). The lamprey 1072132 was released on June 2nd at 20:32, 100 meters south-east of the river mouth into an E1 type plume (1). The subject exhibited large angle turning and then entered the river at 20:40 (2). It immediately stopped at the first bend n the river and began to continue upstream after AT (3)-(21:05-00:00). We were only able to obtain the lamprey position while it passed us (4, 5)-(0:00-5:15). The subject settled in a natural dam obstructing the stream, which was composed of jammed logs, branches and leaves at 05:30 in the morning (6). The natural dam was a very dense structure that covered the width of the stream and the animal made no signs of additional upstream movement by 6:00.

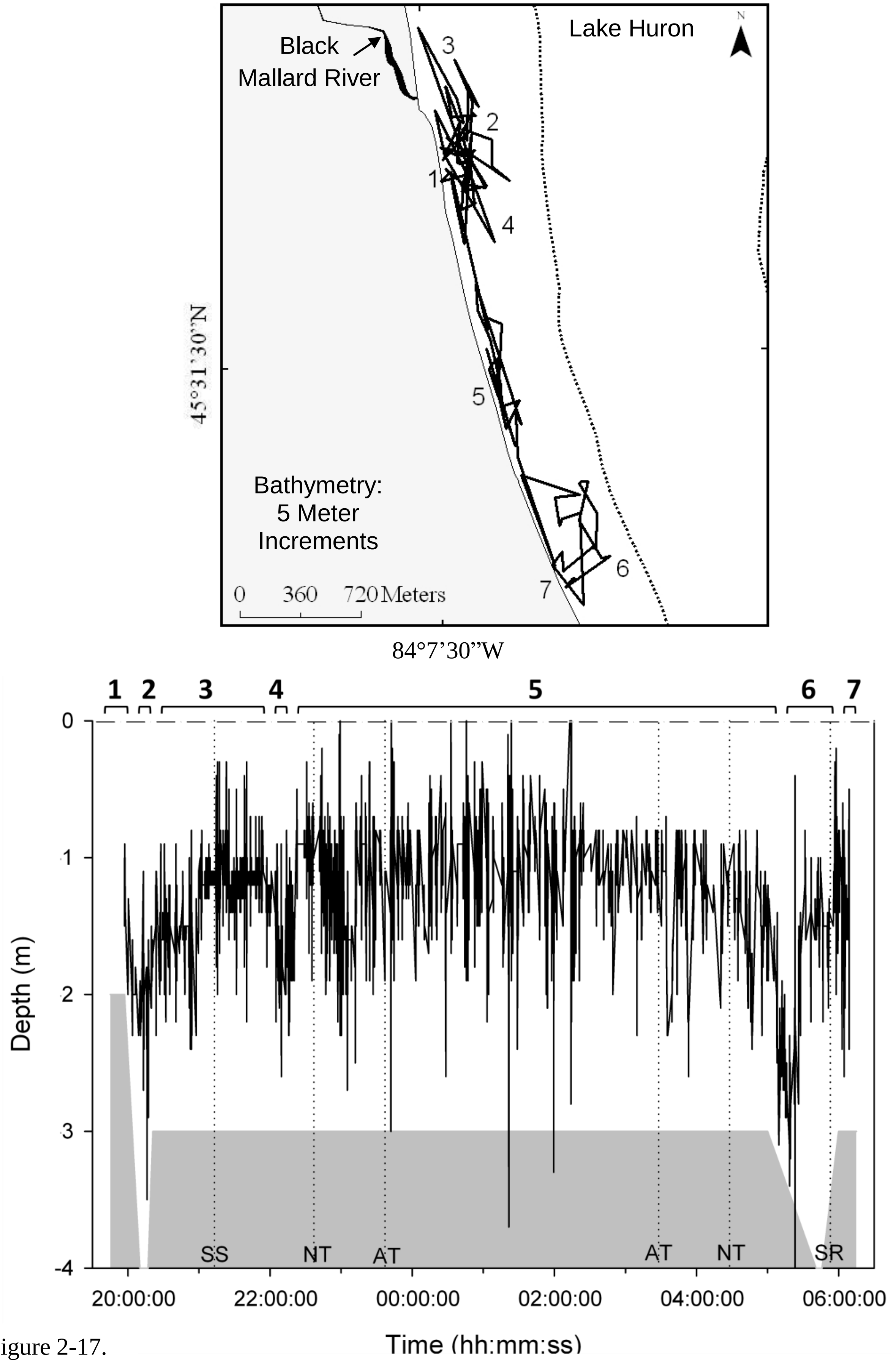

Lake Huron
N
Black Mallard River
3
2
1
4
5
6
7
Bathymetry: 5 Meter Increments
0 360 720 Meters
45°31'30"N
84°7'30"W
1
2
3
4
5
6
7
Depth (m)
0
-1
-2
-3
-4
SS
NT
AT
AT
NT
SR
20:00:00
22:00:00
00:00:00
02:00:00
04:00:00
06:00:00
Time (hh:mm:ss)

Figure 2-17.

Figure 2-17 (cont'd). The lamprey 1072131-1 was released at 19:57 (1) near the river mouth into an E2 type plume that changed to a S? type plume. The subject stopped briefly after release (2, 20:06-20:20), moved near the river mouth (3)-(20:20-22:00) and again briefly stopped (4, 22:00-22:20). Next the subject swam to the south of the river and moved along shore for over 1600 meters from the river mouth with primarily linear movements and intermittent periods of wide-angle turning (5, 22:20-5:12). The animal moved to the bottom and towards shore before it stopped in 2 meters of water (6, 5:12-5:40). The lamprey was disturbed by the boat when the trackers tried to move closer to the subject and the subject was then caught by a seagull (7, 5:50). The lamprey was taken from the bird on shore.

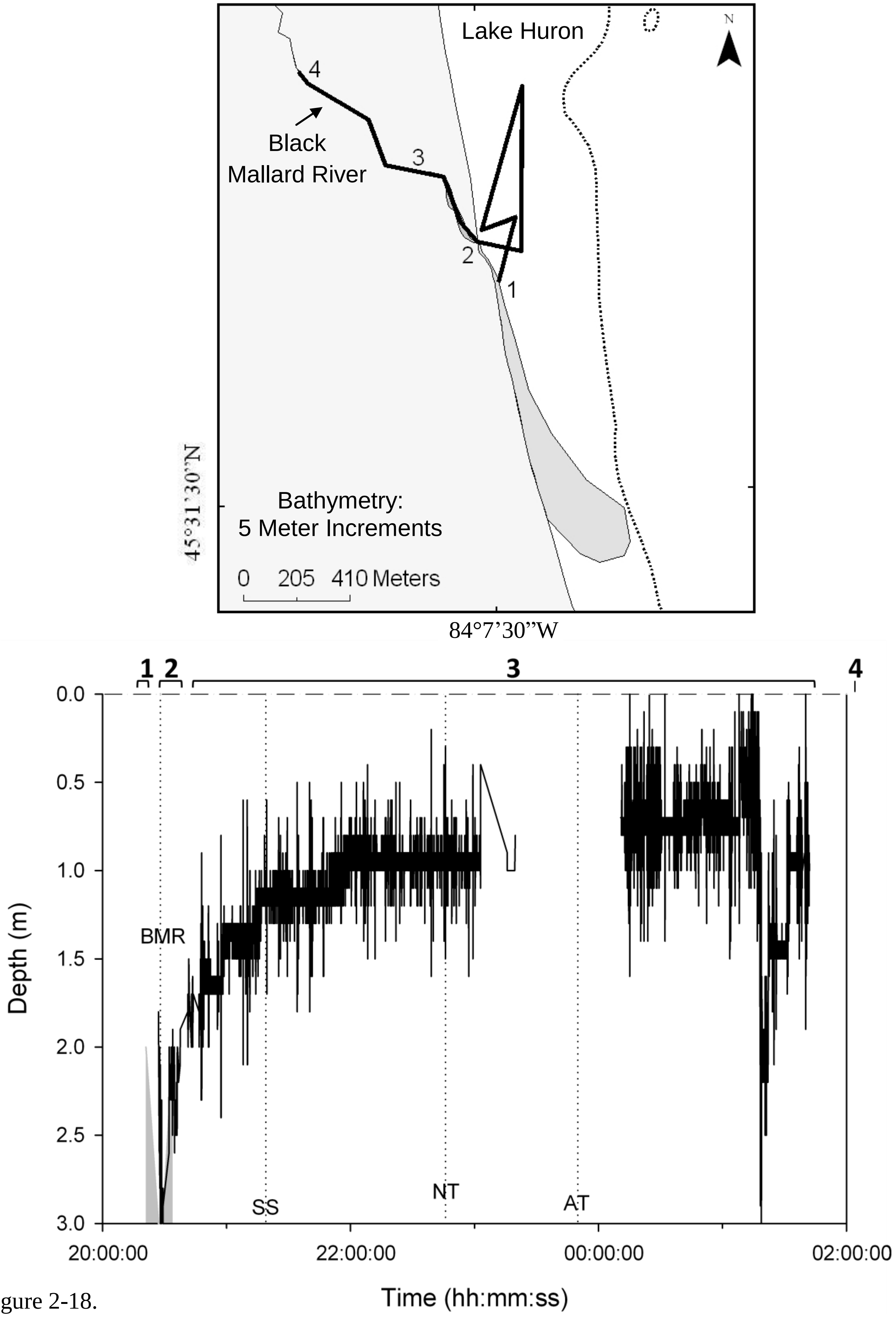

Lake Huron
N
4
Black
Mallard River
3
2
1
Bathymetry:
5 Meter Increments
0 205 410 Meters
45°31'30"N
84°7'30"W
1
2
3
4
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Depth (m)
BMR
SS
NT
AT
20:00:00
22:00:00
00:00:00
02:00:00
Time (hh:mm:ss)

Figure 2-18.

Figure 2-18 (cont’d). The lamprey 1072136 was released on June 4th at 20:21, 200 meters south of the river mouth into S2 type plume (1). The subject swam at the bottom back and forth in front of the river mouth and then entered the river and immediately found refuge in a small bunch of terrestrial weeds that hung into the water from the eroding sandy bank (2)-(20:29-22:28). The subject moved upstream to the first bend and stopped. The lamprey began moving at 01:15 in the area it had settled and moved into a deeper hole before again stopping (3, 22:30-01:42). The animal moved upstream but was not observed. The lamprey was found on June 6th at 3:30 in the same log jam as subject 1072132 (4).

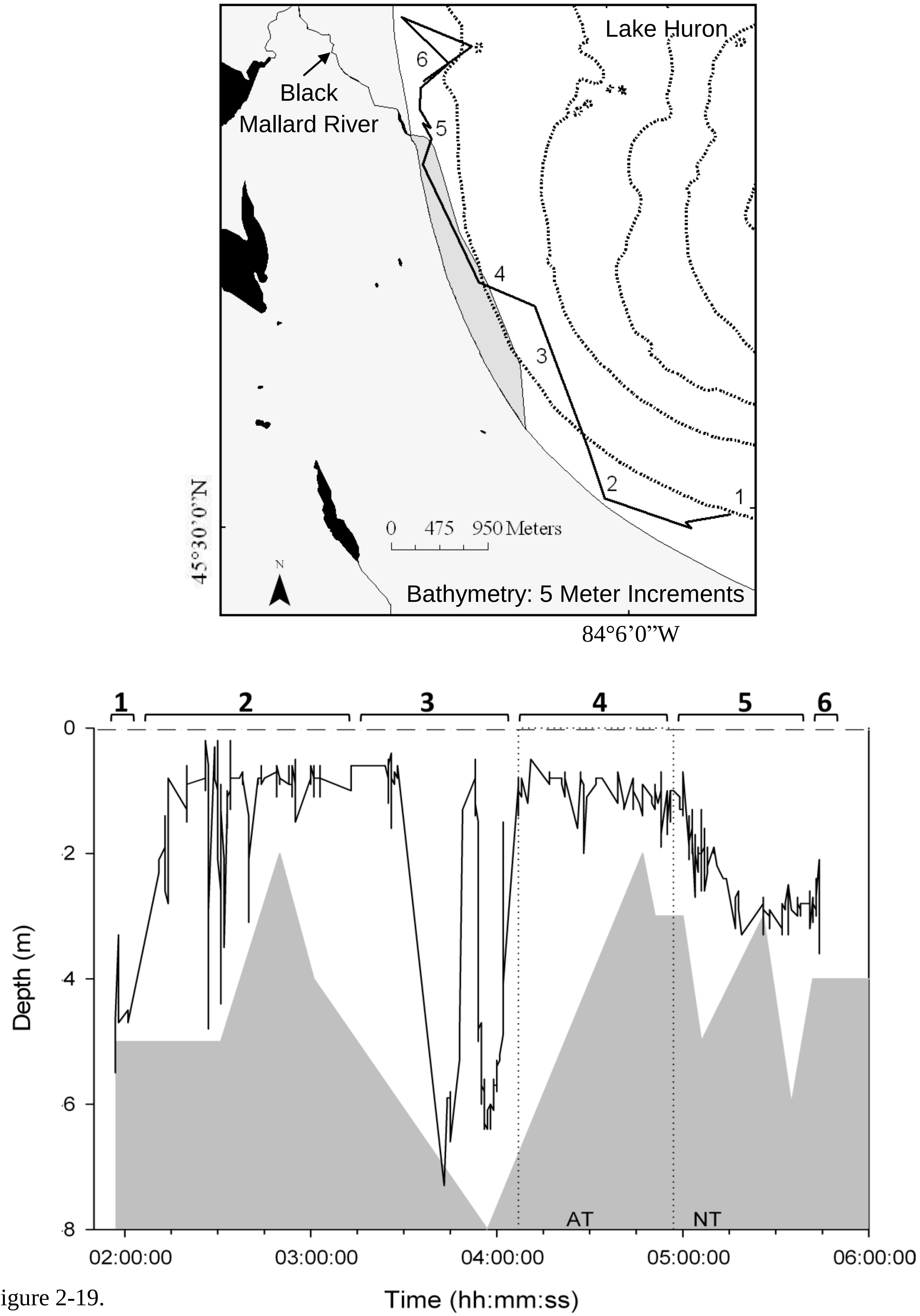

Lake Huron
Black Mallard River
6
5
4
3
2
1
0 475 950 Meters
N
Bathymetry: 5 Meter Increments
45°30'0"N
84°6'0"W
1
2
3
4
5
6
0
2
4
6
8
Depth (m)
AT
NT
02:00:00
03:00:00
04:00:00
05:00:00
06:00:00
Time (hh:mm:ss)

Figure 2-19.

Fig 2-19 (cont'd). The lamprey 1072121: was released on May 7th, 2009 but was not followed until May 9th 2009 due to boat problems. The subject was found straight swimming north towards a type S3 plume and monitored for 4 hours (1, 01:57). The subject initially made only quick vertical excursions while moving north (2, 02:00-03:15), but later engaged in more prolonged vertical excursions (3, 3:15-4:05). As the lamprey moves into the river plume it moved to the surface and begins a period of surface swimming at around (4, 04:05-4:55). After passing the river mouth, at 04:55, the subject appeared to reverse course with wide angle turning (5, 4:55-5:41). The animal stopped north of the river at approximately 05:42 May 9th (6).

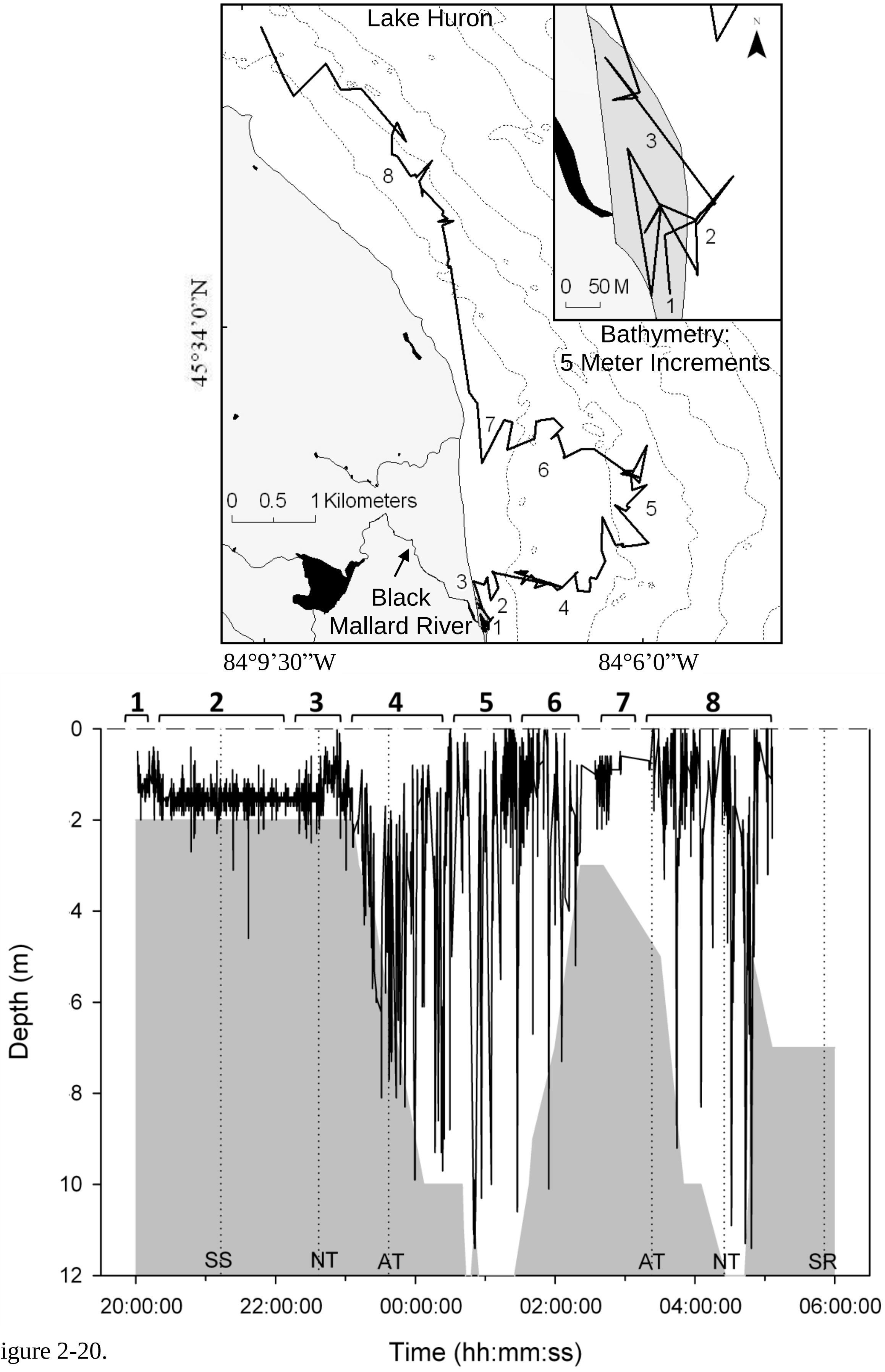
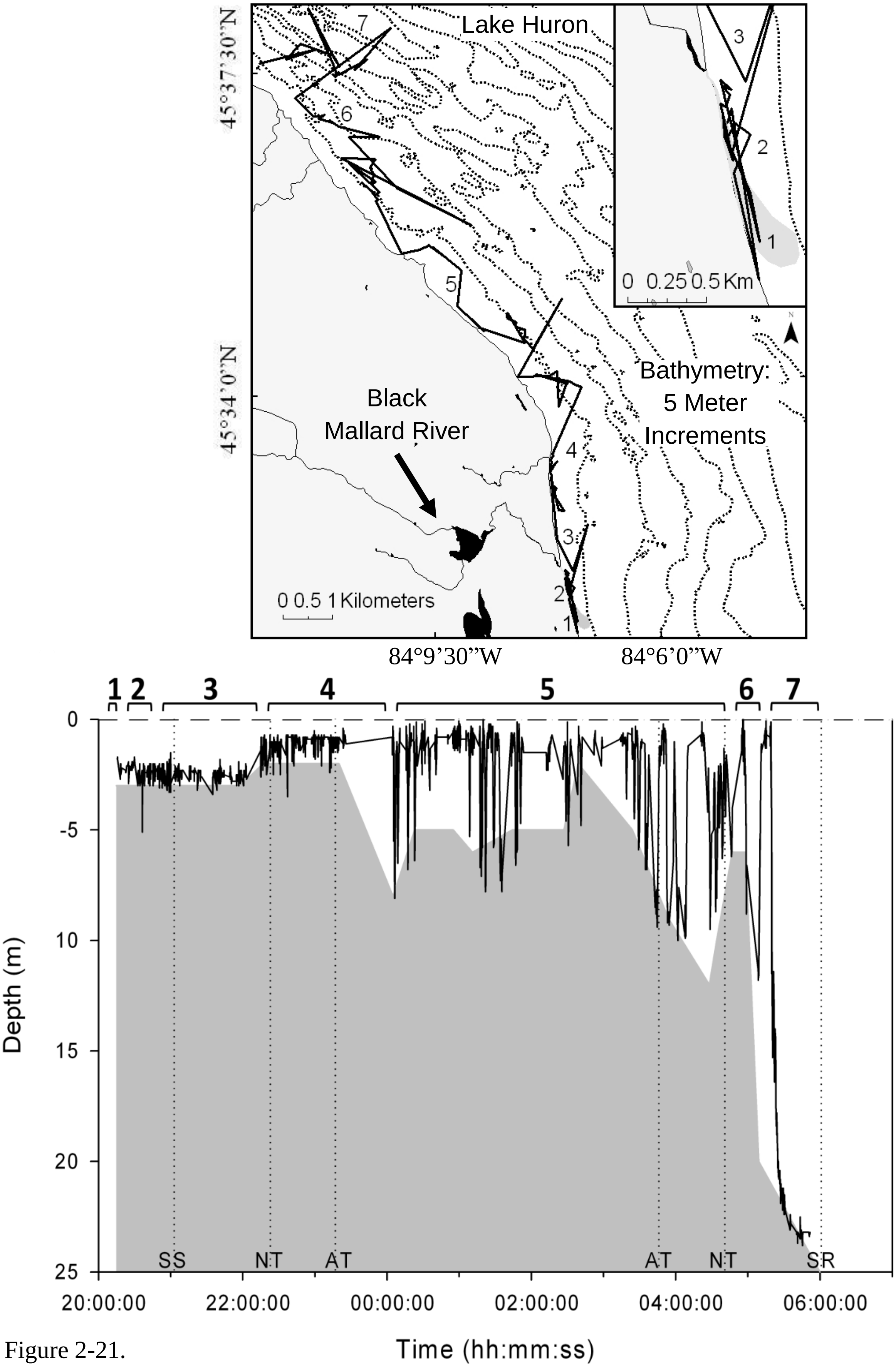

Lake Huron
8
7
6
5
4
3
2
1
3
2
1
0 50 M
Bathymetry:
5 Meter Increments
0 0.5 1 Kilometers
Black
Mallard River
45°34'0"N
84°9'30"W
84°6'0"W
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
Depth (m)
SS
NT
AT
AT
NT
SR
20:00:00
22:00:00
00:00:00
02:00:00
04:00:00
06:00:00
Time (hh:mm:ss)

Figure 2-20.

Figure 2-20 (cont'd). The lamprey 1072122 was released at 20:00, directly in front of the river mouth into an E2 type plume and was tracked for 9 hours. The subject began wide angle turning in the vicinity of the river mouth prior to stopping (2, 20:00-21:18). The subject then swam towards shore and north (3, 21:50-22:50), before finally moving east away from shore (4, 22:50-00:30). The subject swam with many changes in direction near parallel to shore with many vertical excursions (5)-(00:30-01:15). It then moved towards shore (6)-(01:15-02:20) and upon reaching shore it swam north out of Hammond Bay in shallow water (7)-(02:20-02:40). The subject moved up the coast with straight movements and was abandoned an hour early due to weather while the animal was still moving (8)-(02:41-5:06).



Lake Huron
7
6
5
4
3
2
1
Black Mallard River
Bathymetry: 5 Meter Increments
0 0.5 1 Kilometers
0 0.25 0.5 Km
N
45°37'30"N
45°34'0"N
84°9'30"W
84°6'0"W
1
2
3
4
5
6
7
0
-5
10
15
20
25
Depth (m)
SS
NT
AT
AT
NT
SR
20:00:00
22:00:00
00:00:00
02:00:00
04:00:00
06:00:00
Time (hh:mm:ss)

Figure 2-21.

Figure 2-21 (cont'd). Lamprey 1072126 was released on May 18th at 20:15, south of the river in a type S1 river plume (1). The subject stopped after release (2, 20:20-20:50) before repeatedly moving past the river and returning to the plume while wide-angle turning (3, 20:50- 22:15). The subject then swam north out of Hammond Bay and was lost briefly as it moved in shallow water (4, 22:15-23:59). It was reacquired swimming north with straight movements mixed with turns and vertical excursions to over 5 meters as it moved up the coast (5, 5: 0:00-4:50). The subject exhibited many prolonged vertical excursions (5-10 m), as it swam out away from shore to deeper water (6, 4:50-5:30) before finally moving to the bottom of even deeper water (7, 5:30-6:00). The animal likely settled at 6:00.

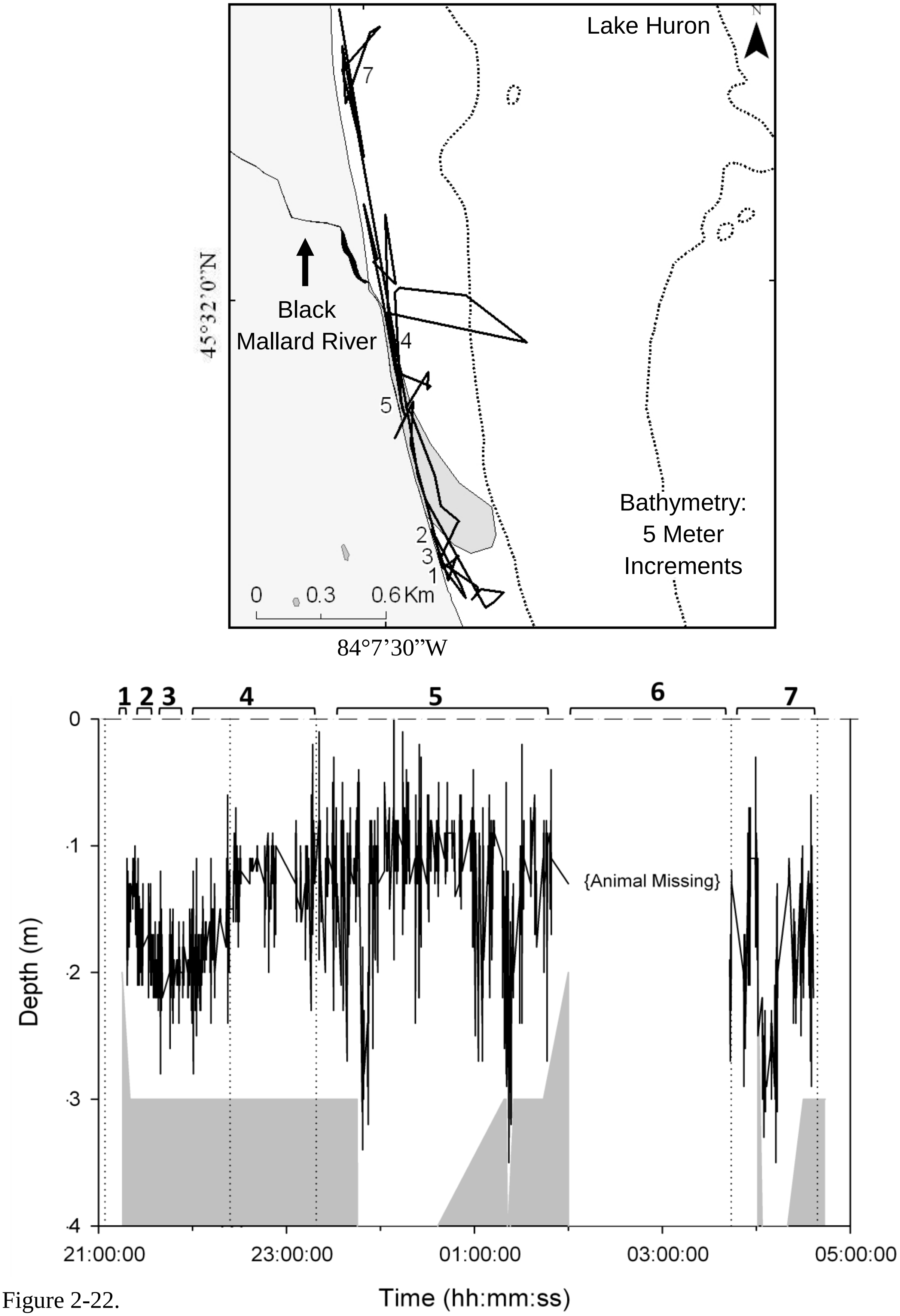

Lake Huron
N
7
Black
Mallard River
4
5
2
3
1
0
0.3
0.6 Km
Bathymetry:
5 Meter
Increments
45°32'0"N
84°7'30"W
1
2
3
4
5
6
7
{Animal Missing}
Depth (m)
0
-1
-2
-3
-4
21:00:00
23:00:00
01:00:00
03:00:00
05:00:00
Time (hh:mm:ss)

Figure 2-22.

Figure 2-22 (cont'd). Lamprey 1072130 was released at 21:15:00 on May 19th south of the river in a type S1 plume with synthesized pheromone and was tracked for 7.5 hours. The subject swam slightly towards the river (1) before it found refuge and settled near the release point (2, 21:19-21:27). It began to move at 21:27 and resettled at 22:35 (3). The subject moved towards the river mouth in shallow water and passed it (4, 22:47-23:32), before returning to the south of the river. It again approached the river (5, 23:32-01:49), however it was lost near shore and was last seen in the plume (6).The subject was briefly reacquired north of the river moving with large turn angles but was again lost (7, 4:00-4:30).

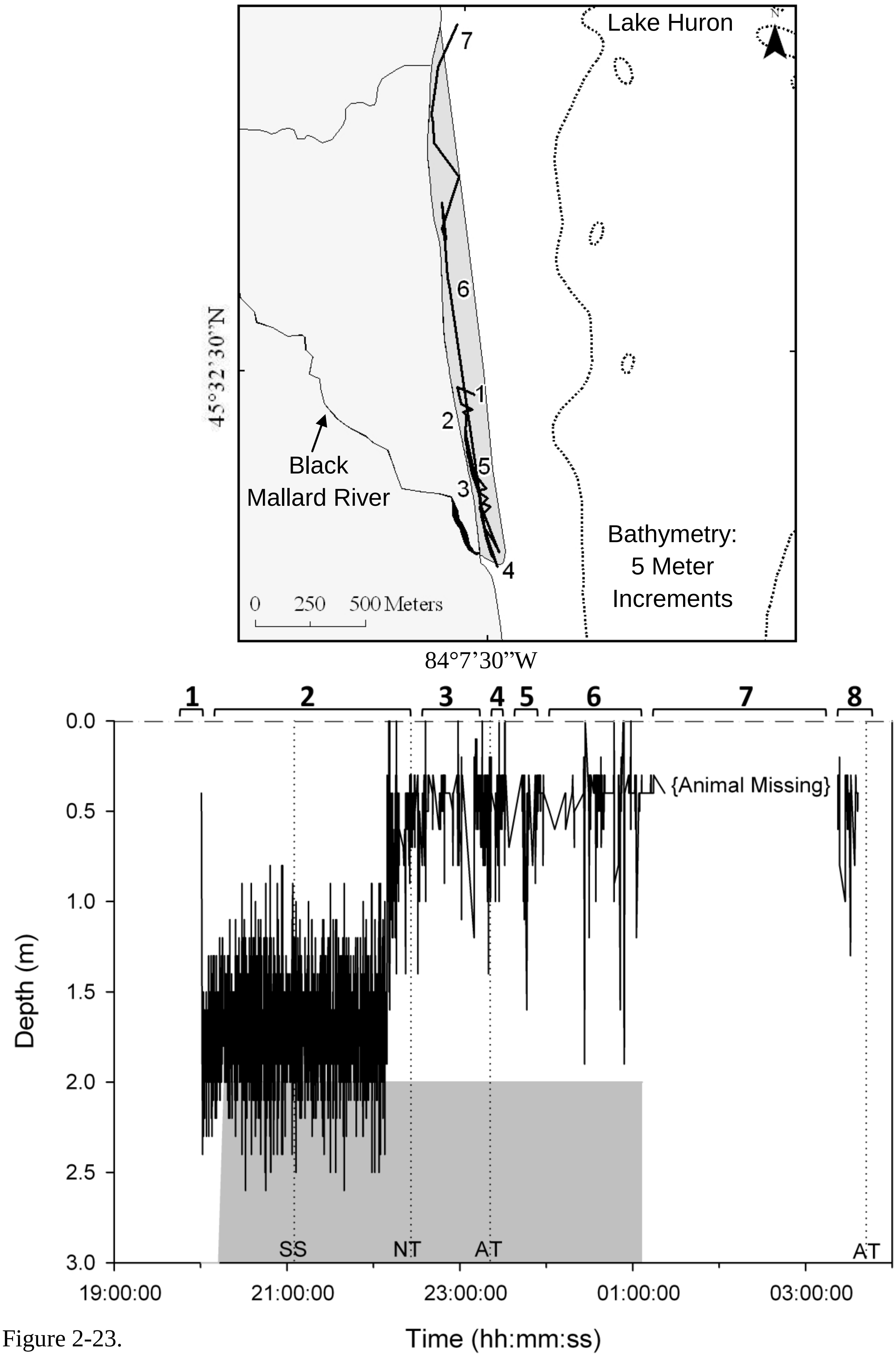

Lake Huron
N
7
6
1
2
5
3
4
Black Mallard River
Bathymetry: 5 Meter Increments
0 250 500 Meters
45°32'30"N
84°7'30"W
1
2
3
4
5
6
7
8
{Animal Missing}
Depth (m)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
SS
NT
AT
AT
19:00:00
21:00:00
23:00:00
01:00:00
03:00:00
Time (hh:mm:ss)

Figure 2-23.

Figure 2-23 (cont'd). The lamprey 1072134 was released on May 21st at 20:00:00 (1), north of the river in a type N1 plume with synthesized pheromone. The subject stopped near the release point (2, 20:00-22:27) before it swam toward the river mouth (3, 22:27-23:12). After reaching the river mouth it passed the river before turning and returning north past the release point (4, 23:12-23:28). Again, the subject swam to the river mouth and spent some time swimming in the vicinity of the mouth (5, 23:28-0:00). The subject then swam north and reached the tip of Hammond Bay at 1:00(6). We lost the animal at this time (7). A faint signal at 3:30 was received from the tag in the direction of Hammond Bay when the boat was positioned at the northern tip of Hammond Bay but an accurate position was never attained (8).

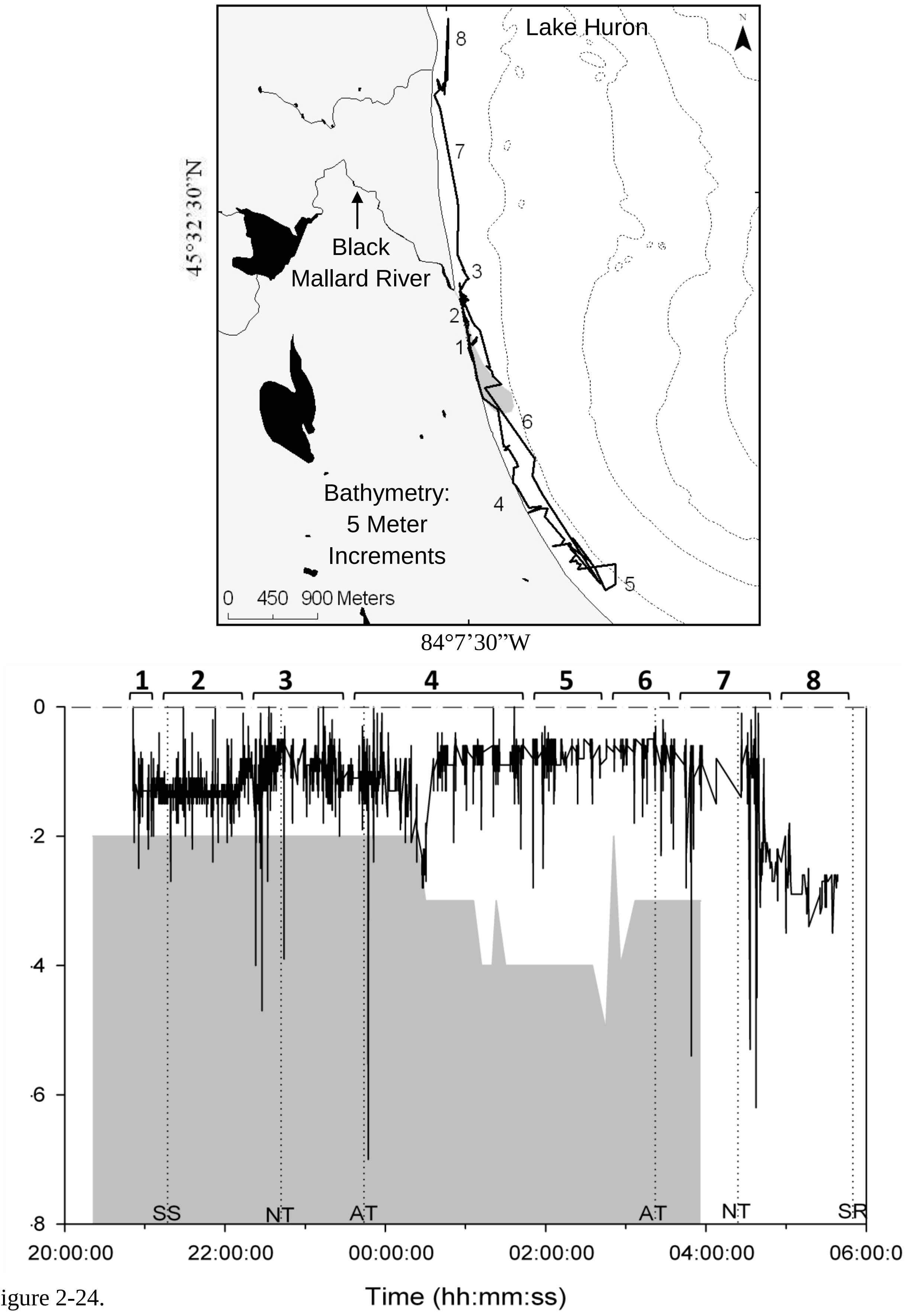

Lake Huron
N
8
7
3
2
1
6
4
5
Black
Mallard River
Bathymetry:
5 Meter
Increments
0 450 900 Meters
45°32’30”N
84°7’30”W
1
2
3
4
5
6
7
8
0
-2
-4
-6
-8
SS
NT
AT
AT
NT
SR
20:00:00
22:00:00
00:00:00
02:00:00
04:00:00
06:00:0
Time (hh:mm:ss)

Figure 2-24.

Figure 2-24 (cont'd). The lamprey 1072128 was released on June 1st at 20:21, 700 meters south of the river mouth into a plume tight to shore and south and was followed for 9 hours (1). The subject stopped (2, 20:30-22:20) before moving towards the river mouth and just past it (3, 22:20-23:35). The subject continued over 3000 meters South-East of the river with mostly straight movements (4, 23:35-01:38). The subject changed directions with large turn angles and then moved back to the north (5, 01:38-02:30) and entered the approximate plume before passing the Black Mallard river mouth (6, 02:30-03:30). The subject continued to the edge of Hammond Bay and passed the submerged peninsula (7, 03:30-04:42). The boat was unable to move past the inlet due to mechanical issues.

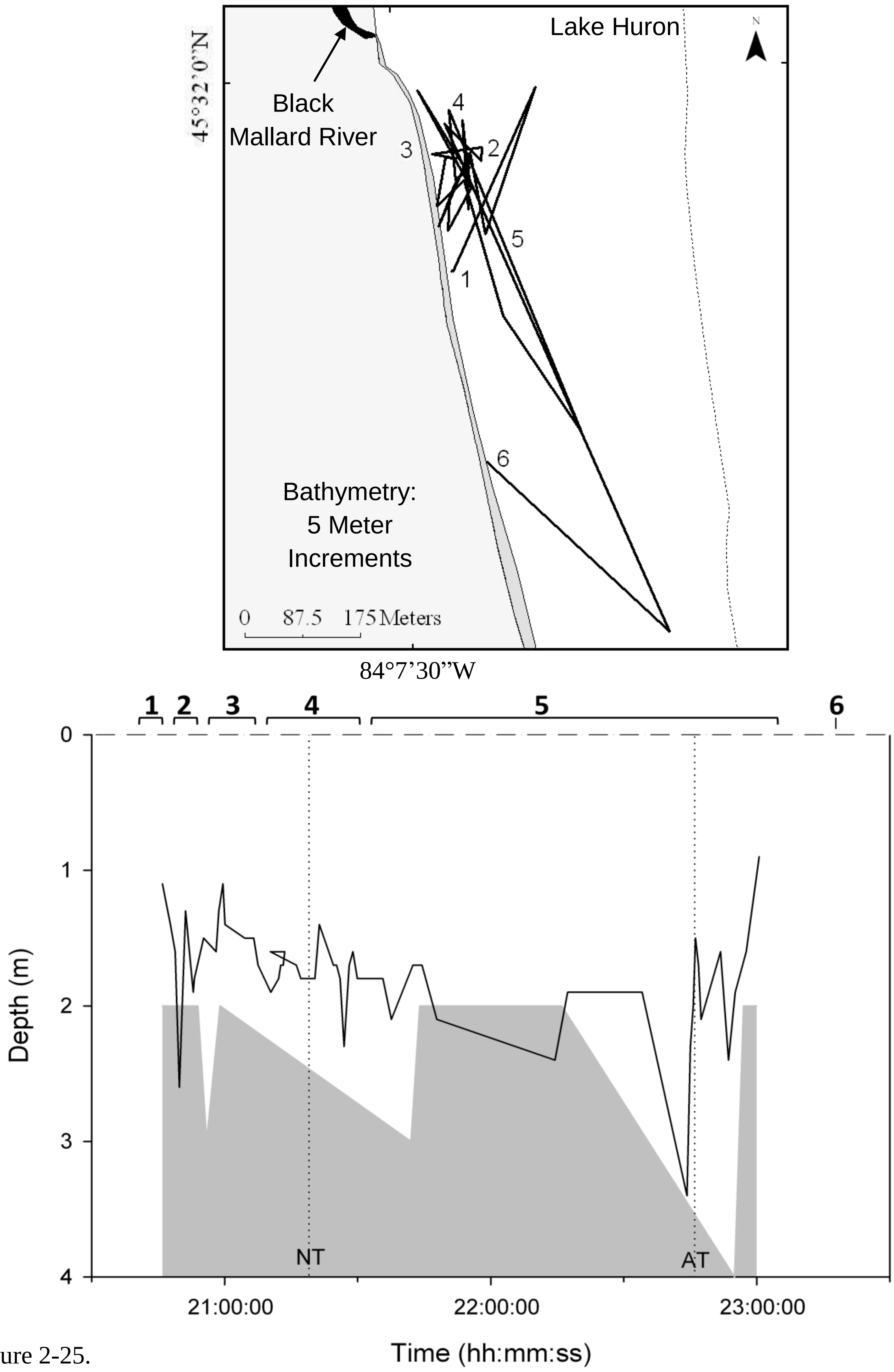

Lake Huron
N
45°32'0"N
Black
Mallard River
1
2
3
4
5
6
Bathymetry:
5 Meter
Increments
0 87.5 175 Meters
84°7'30"W
1
2
3
4
5
6
0
1
2
3
4
Depth (m)
NT
AT
21:00:00
22:00:00
23:00:00
Time (hh:mm:ss)

Figure 2-25.

Figure 2-25 (cont’d). The lamprey 1072140 was released on June 4th at 20:45, 700 meters south of the river mouth into a type S2 plume and was tracked for 2.5 hours (1). The animal settled briefly in front of the river mouth (2, 20:45-20:53). The Lamprey made wide angle turns in front of the river mouth (3, 20:53-21:10) and then moved very slowly in a straight line towards the river (4, 21:10-21:28). The lamprey continued moving near the mouth of the river and made several movements towards and away from the river while it moved throughout the shallow water column (5, 21:28-23:00). We lost the animal as it swam south away from the river mouth near shore (6). The depth positions are only shown for recorded positions because the VR100 did not save positions and we had to rely on the recorded depths at the time of positioning.

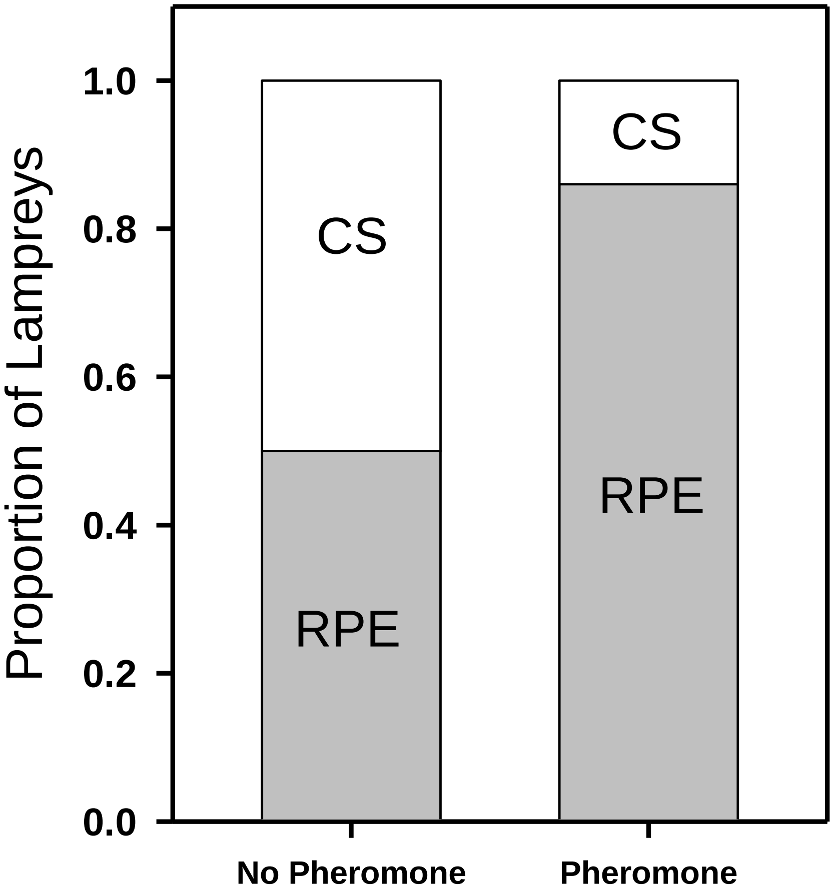


Fig. 2-26. Lamprey in the coastal environment use odor cues to locate a viable river for spawning. Two primary behaviors were identified and evaluated as olfactory mediated coastal search strategies. The first behavior, coastal search, involved primarily straight movements with periods of surface swimming and vertical excursions while moving parallel to the coast. The second behavior, river plume investigation, is a near source search strategy described by movements with wide angle turning. This behavior results in the animal maintaining proximity to a location or an odor source. These two behaviors are important behaviors for sea lamprey to locate spawning ground. Only 13 of the 20 animals fit neatly into one of these two behavior classifications and the classifications are separated based on whether pheromone was applied to the river plume in which they were released. There was no significant difference between behaviors observed and pheromone application (p = 0.282).

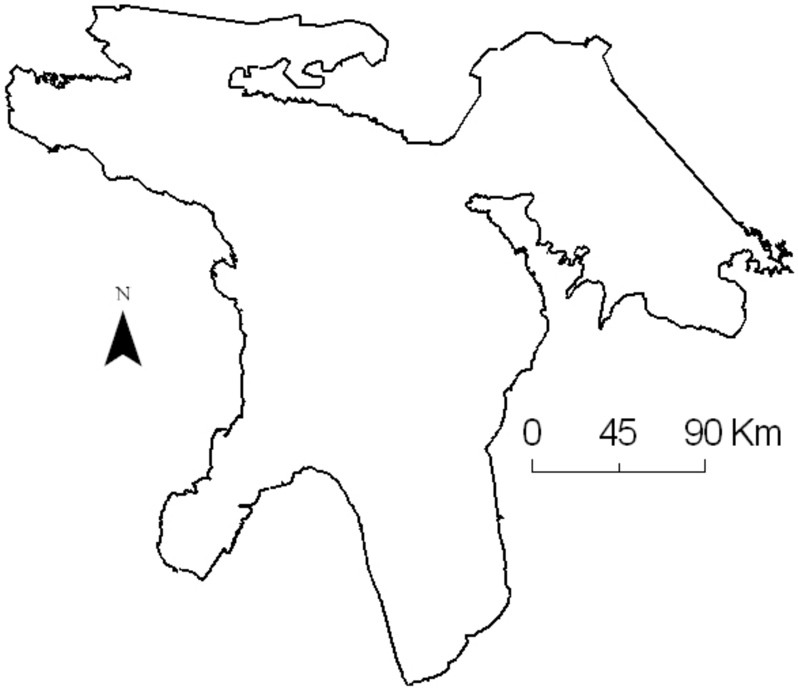


Figure 2-27. The shoreline of Lake Huron is 6,157 km including the perimeter of all shoreline and islands. The figure above shows the outer edge of Lake Huron excluding islands and measures approximately 3300 km. Due to the complex shoreline a migrating lamprey would likely only need to travel approximately 2000-2500 km to traverse the inner edge of the Lake Huron perimeter above, if moving a 100 meters from shore. By moving along the outer edge of the coast a lamprey could encounter the 117 rivers known to be used by lamprey for spawning habitat. The shoreline comes from NOAA for medium resolution digital vector shorelines, digital geography.

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