

THE SEARCH BEHAVIOR OF SEA LAMPREY DURING THEIR NON-HOMING
REPRODUCTIVE MIGRATION IN THE GREAT LAKES

By

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ABSTRACT

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Non-homing organisms are thought to rely on stable geophysical features (e.g., land-slope), and encounter with distinct attributes of the environment (e.g., landmarks) in a predictable sequence to locate reproductive habitat (e.g., rivers always occur on a coastline). However little is known about how non-homing fishes complete a large-scale migration, as most research has focused on homing fishes that rely on geomagnetic cues to return to a natal spawning site. The invasive sea lamprey offers insight into non-homing migration, as individuals complete a single non-homing migration to rivers following translocation by host fishes in the Great Lakes.

In this dissertation, Chapters 2 and 3, are devoted to developing a framework for describing animal behavior from telemetered observations and development of a standardized approach for assessing and filtering VEMCO Positioning System (VPS) data based on an estimate of horizontal position precision (HPE). This methodology was imperative for exploration of the sea lamprey migration with the underwater VPS telemetry technology.

In Chapter 3, we described how sea lamprey orient to a coast when in a lake and hypothesized that sea lamprey navigate to the nearest coast by (1) orienting to the local bathymetric gradient and (2) maintain straight movements counter to the local slope to move towards shallow water. Three-dimensional (3-D) paths of migrating female sea lamprey were obtained by an acoustic array with 3 km² of coverage, centered 3.3 km from the coast in Lake Huron. The findings of this chapter indicate that sea lamprey sampled an area of lake-bottom to

assess absolute hydrostatic pressure and to select a heading towards reducing pressure (shallower water). In contrast to natal homing migrations, the sea lamprey appears consistent with non-homing orientation to a general region with a simple set of rules based on local topography.

Chapter 4 focused on the sea lamprey migration along a coastline and near a river mouth. Upon reaching a coastline, sea lampreys move parallel to shore. Prior studies indicated the presence of larval odor in river water increased the likelihood that a migrant entered a river. However, it was not known whether larval odor played a role in navigation (guiding the migrant to the river mouth) or mediated habitat selection by labeling the suitability of a river for spawning. In a two-year study using a 2 km² acoustic array, the 3-D paths of sea lamprey were documented as they approached, entered, or bypassed the Ocqueoc River in northern Michigan, under one of two conditions: (1) low larval odor; and, (2) higher larval odor, created by increased larval abundance plus the addition of the synthetic larval odor components, petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), and petromyzonol sulfate (PZS) to a 1 x 10⁻¹² M concentration. A coupled hydrodynamic and dye concentration model predicted the hydraulic conditions experienced by each sea lamprey by estimating water conditions (velocity, temperature, etc.) at each fish position and allowed for assignment of whether a position was inside or outside of the Ocqueoc River plume. Encounter with river water appears to trigger localized search, regardless of larval odor content. However, when larval odor was abundant, the migrant was more likely to enter the river. Whether a migrant enters a river, is modulated by the presence of detectable larval odor, manipulation of river selection by invasive sea lamprey for management is viable in rivers with high encounter rates. Finally, Chapter 5 covers the implications for how altering migration routes in the sea lamprey via the application of synthesized pheromones contained in larval odor could be profitable for management.

To my wife for five great years in Michigan...

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CHAPTER 1

AN ADAPTIVE WORKFLOW FOR STUDYING BEHAVIOR WITH TELEMETERED OBSERVATIONS.

ABSTRACT

Ethology is now dominated by remotely sensed observations rather than visual observations. The steps to answer ethological questions with telemetry should include question development, data collection, data processing, and finally analysis, regardless of whether it is an exploratory study of a rarely studied animal or a model organism. Following this structure and reporting the steps clearly is important for ensuring high quality science and for sharing increasingly complex analyses with other practitioners. Herein, we detail an approach for answering proximate and ultimate questions of animal behavior with explanatory, exploratory, and predictive analysis objectives.

INTRODUCTION

Ethology is the study of animal behavior. Mathews and Mathews (2010) defined behavior as the ways in which an animal adjusts to and interacts with its environment (Mathews and Mathews 2010). We extend this definition to define behavior as the regulation of bodily status via spatial adjustments involving neuro-muscular systems operated in response to environmental information to satisfy an internal state (or purpose for acting) (James Miller (MSU Entomology), personal communication). This means that a proper description of a behavior would contain the animal's internal state (A), the local body adjustments (B), the spatial movement pattern (C), and environmental conditions (D). This information makes it possible to sufficiently describe

behavior in terms of the underlying proximate mechanisms (causation and developmental questions), and ultimate mechanisms (functional and evolutionary questions) of the behavior. This does not mean that all four pieces aspects of the behavioral description must be known or are equally important to answer behavioral questions, but it does mean that a complete understanding of behavior includes all four parts. Ethology has transitioned from analyzing visual observations of organisms to reliance on sequential observations of marked individuals, a transition that has usually traded observation precision for the duration of time an individual is observed (Sanderson 1966); but technology has reached a stage where telemetered datasets can provide highly precise data over long time periods, even in aquatic environments (Cooke et al. 2013).

The technological advances have resulted in extensive datasets of spatial and bodily movement. Although, the current proliferation of technology for capturing data and approaches for analyzing data have muddied the water on what is actually being studied. Raising the question of whether ethology should focus on animal displacements or if the focus should remain on the classical study of animal behavior (ethology), with spatial movement representing a single component. Spatial movement is only one aspect of behavior and only becomes interesting when put into the context of the animal's purpose for moving (objective) and the environment in which it moves. Although disagreements occur over the semantics (Bateson 2012, Burghardt 2012), it is usually agreed that observed movements should always be tied to their relevance in behavior.

Studies of the causes, mechanisms, and patterns of spatial movement have formally been coalesced into a heuristic model termed movement ecology (Nathan 2008). Movement ecology focuses on understanding behavior based on the movement signature, and for that reason it fits as an aspect of ethology as Tinbergen (1963) describes ethology, "the biological study of behavior"

(Tinbergen 1963). The movement ecology model encourages consideration of the components of behavior from a fresh perspective, but is not as useful for matching a question to a study design. For this task I prefer to consider the three classifications of study objectives: (1) exploratory, (2) explanatory, and (3) predictive objectives.

An exact and repeatable method to statistically classify movements into behaviors using movement data is unrealistic, as different organism's move at different scales and in different ways and the measures used and data types available to provide the information vary with technological advances (Gurarie et al. 2015, Calenge et al. Unpublished). The general work flow however, is consistent for addressing behavior with movement data and remains independent of the study animal or question, as behavioral questions are hierarchical in nature and can be addressed in the order of exploratory, explanatory and predictive research objectives (Gurarie et al. 2015, Calenge et al. Unpublished). From this hierarchy we can identify what the animal is doing (exploratory), why the animal is doing what it is doing (explanatory), and how an animal will change what it is doing under different conditions (predictive). Identifying the general process of the study and then reporting it in an organized framework can be a powerful approach for sharing and relating methodology (Shamoun-Baranes et al. 2012, Table 1.1).

I recommend a general work flow that leads from question development to analysis selection (Table 1.1). Regardless of whether the objectives fall within any or all exploratory, explanatory, or predictive classifications; a strong plan will prevent failure of a practitioner to capture the necessary information. I also highlight how this organization addresses the types of questions falling under Tinbergen's four pillars of ethology (Table 1.2).

GENERAL WORKFLOW

The study of animal behavior with movement comprises four steps including question development, data collection, data processing, and finally analysis (Table 1.1). Analyses can be structured into three types of objectives including exploratory, explanatory, and predictive analyses (Gurarie et al. 2015). I use this structure to carry out research and I will expand on how these steps should be completed.

Question Development

The way animal behavior has been recorded and defined in ethology has changed; but the questions addressed have remained similar (Table 1.2). Tinbergen (1963) identified four main questions to studying behavior: (1) what is the animal doing (causation), (2) what benefit does what it is doing provide to the animal (functional), (3) how did behavior develop or change over the lifetime of the individual (developmental), and (4) how did the behavior arise over the history of the species (evolutionary) (Tinbergen 1963). These four questions of behavior still resonate as a strong heuristic model with which to segregate types of questions (Bateson and Laland 2013).

Tinbergen's four questions can be answered via an exploratory, explanatory, and predictive analysis hierarchy and this hierarchy informs us of an effective study design.

Causation questions fall within exploratory analysis objectives that use movement in the context of the environment to characterize behavior. Anytime an analysis classifies a behavior based on a movement pattern without an explicit understanding of the environment or animal objective, the analysis is relying on major assumptions, which may be reasonable depending on the baseline understanding of the species (Able 1991). There is no substitute to understanding the ecological needs and general activities of the study animal; when little is known about the animal's habits, it

becomes much harder to characterize patterns (Dodge et al. 2008) and to break behaviors into sub behaviors (e.g. behavior: body care, sub behaviors: preening, washing; Shamoun-Baranes et al. 2012). *Developmental questions* are usually just an extension of causal questions that seek to understand how age and experience influence behavior (Jorge et al. 2011), seeking to understand how the value of different behaviors fits into functional questions.

While relying on effective baseline exploratory evaluations, *functional questions* fall into the explanatory analysis objectives in which the value of a defined behavior is the target. Functional questions are often only answered from sensory manipulations in the field that are able to alter the sensory apparatus of the animal (Barbin et al. 1998, Vrieze et al. 2010) or in the lab where the sensory environment can be controlled instead of monitored (James et al. 2008). Functional questions are difficult to answer absent a strong movement and environmental dataset that can be combined to reveal movement mechanisms that rely on fixed (e.g. depth) and plastic variables (e.g. water current). Lastly *evolutionary questions* fall into the explanatory and predictive objective realm that ranges from simple testing to full model fitting. Evolutionary questions can only be addressed after behavior is defined; the proximate value of the behavior is elicited and can now be considered across other more complex variables (many generations). Thus, explanatory and predictive analyses can only be reached after exploratory analyses have been implemented; motivating our proposed hierarchy (Table 1.2).

Data Collection

Once questions are identified, the data required to answer the questions and collection methods can be determined. Challenges to gathering information depend on the study system and research questions. The scale of the question, size of the organism, type of environment, and

level of precision needed to answer the research questions, limits the types of questions that can be address and the way questions are asked (Espinoza et al. 2011, Cagnacci et al. 2010, Cooke et al. 2013, Meckley et al. 2014b). The greatest risk of advancing technology comes at the risk of misusing it and ignoring major underlying assumptions about the behavior that are not addressed by the collected data (Hebblewhite and Haydon 2010).

Data processing

The data processing stage involves: evaluating the quality of the collected data, filtering data to match data quality with the needs of the research question, merging datasets, and formatting data into a query-able form for use in analyses and summary statistics (Shamoun-Baranes et al. 2012, Meckley et al. 2014a, Dissertation Chapter 2). Remotely sensed environmental and movement data is unlike bench-tested measurement tools (e.g., thermometer), in that data quality varies in space and time, and the tool must be evaluated specifically in each study until its performance is well understood. Data should be filtered if it is necessary to meet the precision needs of the research questions (e.g., Dissertation Chapter 2, Meckley et al. 2014a). After data are evaluated they must be merged into a single database that allows the connection to be made between static attribute information of an individual, to dynamic trajectory data and environmental conditions, a potentially challenging activity. Movement datasets require major formatting efforts and careful annotation of the data is of paramount importance. When building databases, careful consideration should be taken of the current and potential future uses, to ensure data are accessible.

Analyses

Each classification of research objective has entire works dedicated to the objectives of exploratory, (Gurarie et al. 2015), explanatory, and predictive analyses (Smouse et al. 2010) and each category can contain a diverse number of analyses. An explosion in analysis diversity has occurred to match the increasing mountain of diverse data (Cagnacci et al. 2010, Hebblewhite and Haydon 2010, Smouse et al. 2010), though they all perform the task of either revealing patterns in the movement, testing patterns in the data, or making predictions about how the patterns might vary in different circumstances (Figure 1.1).

Exploratory approaches are designed to define behavior and specifically ask what is the animal doing and how is the animal doing it, often including metric based, classification, phenomenological time series, and mechanistic movement modeling (Gurarie et al. 2015). Each approach (data type, collection method, and analysis) has different assumptions (Hebblewhite and Haydon 2010), challenges, and value which must be evaluated prior to selection, though all are designed to separate spatial or bodily movement patterns into distinct phases (Dodge et al. 2008, Gurarie et al. 2015). It is important to be aware that classifications vary in their specificity to behavior depending on the level of understanding of the internal state, bodily movement, and spatial movement (Dodge et al. 2008). For example a pattern may be classified very descriptively based on spatial movement as spatially stopped or spatially active (Meckley et al. 2014a), but if bodily movement is known it could be determined that the animal is swimming or not swimming (Wilson et al. 2006); if the environment is also understood, it could be determined that the animal is swimming with current, if the internal state is understood the pattern could be revealed as selective tidal stream transport (McCleave and Kleckner 1982, Barbin et al. 1998), or if the animal condition is monitored actively, movement could be related to condition in questions of

energetics or respiration (Wilson et al. 2006). When studies describe behavior at the highest level of specificity without all the pieces of information they are making assumptions that could be unsupported or potentially supported from other research. Exploratory analyses separate the bodily and/or spatial movement signatures into patterns, which can be fed into explanatory and predictive analyses to address higher level questions to describe behavior. Exploratory studies also include mechanistic studies of how the behavior is performed in terms of mechanical ability, energetics, and physiology, described by Nathan et al. (2008), as the biomechanical paradigm.

Explanatory analyses address the question of why the animal is doing what it is doing, while predictive questions ask, how I anticipate the system would change if there is a change in a covariate (Bowlin et al. 2010, Gurarie et al. 2015). In a movement context the purpose of explanatory analyses is to understand how a set of behaviors relates to a set of environmental covariates or a measure of success; while, predictive analyses determine how variables interact, and how behaviors might be anticipated to change given a change in one of the covariates. Explanatory and predictive analyses could address both functional and evolutionary questions but they differ in the way they implement analyses and ask questions.

Explanatory analyses include correlative approaches and modeling approaches. Predictive studies require full model fitting and most predictive analysis address questions that take the form of: how will changes in a specific environmental variable influence the ecology (ie. fitness) of a species given the behaviors that comprise the species life cycle as they are currently described (e.g., Bowlin et al. 2010). Approaches are now capable of both explanatory and predictive outputs from the same complex modeling framework (Goodwin et al. 2006). Classical model fitting of variations of a random walk model as a null hypothesis are still used to explain how organisms reach targets (Goodwin et al. 2006; Gurarie and Ovaskainen 2011), referred to as

the “random paradigm” by Nathan et al. (2008). Explanatory and predictive objectives are also often used to understand animal decision making and determine why animals transition between discrete behaviors, described as the cognitive paradigm (Nathan et al. 2008). Predictive models are the most common for describing behavior in light of evolutionary contexts, although studies of this nature are still somewhat rare and fall into the optimality paradigm as Nathan et al. (2008) described it.

APPROACH AND IMPLEMENTATION

This framework is effective for matching data collection, data processing, and analyses to research questions and helps avoid pitfalls of mismatching any of these components to a research question. It also is valuable for sharing a reproducible method as was done by Shamoun-Baranes et al. (2012). The current proliferation of perspectives on behavior are valuable (e.g. psychology, movement ecology, etc.); however, it is important that work done in these realms is tied back into the overarching behavior. Some of the measures used to describe behavior are actually an output of behavior (e.g., speed or tuning), rather than a description of what the animal is doing, where it is doing it, and why it is doing it, an important argument that has been recently presented (Bateson 2012, Burghardt 2012), and should garner additional attention. Below I describe how this framework has been used to answer pertinent fundamental biological questions and management questions regarding the sea lamprey migration in the Great Lakes.

The sea lamprey lake migration in the Great Lakes has only begun to be studied in the last 10 years (i.e., since 2004, e.g. Vrieze et al. 2011), initially involving both mark-recapture studies and trajectory data from individuals monitored by manual underwater acoustic telemetry (Vrieze et al. 2010, Vrieze et al. 2011). The research objectives have focused on how sea lamprey encounter and enter rivers on the coast, and what role larval odor, a known migration

signal, has on the ability of lamprey to find a river or the decision to enter the river for the overarching purpose of improving management of invasive sea lamprey in the upper great lakes

I have applied the research framework found in Table 1.1 to continue to answer important questions detailing the way sea lamprey return from a large lake to enter streams to spawn. Previous research has represented primarily exploratory studies with small field experimental manipulation experiments (Vrieze et al. 2010, Meckley et al. 2014b). The two manual tracking studies reported that sea lamprey transition from extensive movements typified by faster movement and lower turning rates while vertically casting through the water column from surface to the bottom when between rivers and intensive search typified by frequent stopping and turning within the region of the river plume (Vrieze et al. 2011, Meckley et al. 2014b). In addition it was indicated that sea lamprey may rely on the physical edge of the coast as sea lamprey rebounded along the coast horizontally casting and probing the shoreline, proposed as a river mouth localization behavior in the presence of a dynamic river plume that may provide poor directional information (Meckley et al. 2014b). Rebounding is a pattern of movement that includes progress along the coast, while switching between periods of angling towards the coast until reaching very shallow water followed by periods of angling away from the coast. Sea lamprey clearly rely heavily on olfaction as Vrieze et al. (2010) observed few nasally occluded sea lamprey successfully entering a local river and Meckley et al. (2014) observed an increase in the amount of time sea lamprey spent in the presence of a partial larval odor, but no apparent increase in habitat selection (river entry).

Two manual tracking studies have provided a baseline for making predictions about how sea lamprey migration, though they each come with some major complications due to the use of manual telemetry methods. One study did not have a reliable estimate of accuracy as they relied

on the bearing of the transmitter implanted fish from the boat and used the intersection of multiple measurements to estimate position, accuracy in this case is influenced by fish ground speed and their testing method was based on a fixed tag position (Vrieze et al. 2010). Bearing based positioning is best with two boats taking simultaneous measurements (Holland et al. 1992). The second study did have a reportable accuracy but the accuracy was poor and prevented some trajectory type data (e.g., turn angles) to be evaluated (mean error 71.2 m, Meckley et al. 2014). In both cases, motor boats were used to follow the fish and could have influenced behavior of a vertically active fish as has been observed in other fishes (Draštík and Kubečka 2005).

Step 1: Question Development

The exploratory manual tracking studies provided key insights that led to question development. I identified eight overarching questions. The invasive sea lamprey attaches to and parasitizes large fishes that may displace sea lamprey outside of their control, leading us to suspect that they are capable of orientation and migration to the coast following displacement and to ask, (1) how do sea lamprey orient and return to the coast when displaced offshore (Chapter 3)? Once arriving at the coast it is known that sea lamprey move parallel to the coast but I wanted to evaluate this behavior more finely leading us to ask, (2) what aspects of their extensive search influences what rivers they are likely to encounter (Chapter 4)? Once encountering river water I wanted to know, (3) where does the decision to enter the river occur, or maybe more specifically at what point does the search and assessment process for entry become irreversible, (4) is larval sea lamprey odor important to navigation or only the decision to enter a river, and (5) how do sea lamprey navigate within a plume, what senses and what environmental information sources are likely used (Chapter 4)? These questions address both

fundamental biological questions and build to answer invasive sea lamprey management questions such as, (6) why do some rivers garner stable reproduction of sea lamprey requiring regular pesticide treatment, (7) what recommendations would I provide for manipulating sea lamprey with a synthetic larval odor if it were available, and (8) what recommendations do I suggest regarding the current way pesticide treatment is applied, which effectively turns down the larval odor signal (Chapter 5)?

Step 2: Data Collection

I used an underwater acoustic position system from VEMCO, which is a new underwater fine-scale passive monitoring system. The system provides two-dimensional positions derived from raw detection data using proprietary hyperbolic positioning algorithms that estimate positions from the time-difference-of-arrival (TDOA) of a coded signal at three or more receivers in the VPS array (Smith 2013). Each detection record by a receiver consists of a transmission code that is unique to a tag, the time of signal reception, and, depending on the tag configuration, an environmental variable measured by the tag (e.g., pressure, temperature). As with all TDOA-based processes, time synchronization of the autonomous receivers is critical. The VPS system makes use of synchronization transmitters (sync tags) placed at stationary locations throughout the array to account for time drift in individual receiver clocks (Andrews et al. 2011, Smith 2013, Chapter 2) . Like other multi-receiver positioning systems (White and Garrott 1990), the VPS provides a weighted average location from every combination of three receivers that detected each tag transmission (hereafter referred to as a position ‘fix’), and an associated PPE termed horizontal position error (HPE) (Espinoza et al. 2011, Smith 2013).

Step 3: Data Processing

The first data processing step entails putting data into a raw database that can be easily accessed in the future. Animal data should be paired with environmental data at this time if any variables are known. This step was tedious in the case of our sea lamprey data as a hydrodynamic model was developed for several years of data (Chapter 4), so that at each fish position I know the water temperature, velocity fields, and whether the sea lamprey was encountering river water. In addition the animal and environmental datasets had to be matched in MATLAB, the program used to develop the hydrodynamic models, and then transferred for further analysis in a different program, R. At this time all raw data is stored in stable “.robj” files, in which variables such as time are pre-formatted and the risks of storing time data in “.csv” files accessed by Excel, can be avoided.

The second step entails defining data quality objects based on the research question and future analysis methods followed by an assessment of the unfiltered data quality before finally the data should be filtered to remove unacceptably erroneous positions. I developed a four-step filtering approach which incorporates data-filtering principles developed for GPS tracking of terrestrial animals. HPE was evaluated for its effectiveness to remove uncertain fish positions acquired from the VPS system. The use of position precision estimates that reflect the confidence in the positioning process should be considered prior to the use of biological filters that rely on a priori expectations of the subject’s movement capacities and tendencies. Position confidence goals should be determined based upon the needs of the research questions and analysis requirements versus arbitrary selection, in which filters of previous studies are adopted. Data filtering with this approach ensures that data quality is sufficient for the selected analyses and presents the opportunity to adjust or identify a different analysis in the event that the requisite

precision was not attained. Ignoring these steps puts a practitioner at risk of reporting errant findings (Meckley et al. 2014a, Chapter 2). Once data is prepared for a specific analysis it should again be put into its own stable database for question and analysis specific analyses. Annotation of these datasets is very important to avoid improper use by future practitioners.

Step 4: Analyses

At this point our research focused on exploratory and explanatory analyses. First, I use exploratory approaches that characterize patterns in the movement of sea lamprey including changes in sinuosity, heading, and ground speed (Chapter 3, Chapter 4). Analyses focused on explaining patterns before and after changes in behavior with simple tests (Chapter 3) and more complex mixed modeling approaches that take into account multiple observations of the same individual and how environmental features associate with movement patterns (Chapter 4). Through these approaches I can characterize the patterns and began to determine the behaviors. Once different periods of the migration were characterized I moved past the exploratory analyses to characterize what the discrete segments represent in the migration and how they functionally influence how sea lamprey reach key points of the migration. In future work, I can apply the exploratory and explanatory findings to develop predictive models that can predict things like what aspects of rivers make them likely to be encountered and entered by sea lamprey.

CONCLUSION

This hierarchical approach to studying the sea lamprey migration through the monitoring of the movement of sea lamprey and the underlying environmental context under which they were moving has allowed us to progress from inaccurate preliminary studies that revealed very general aspects of the sea lamprey migration to now being able to discuss fine scale behavior and

expand as well as correct our understanding of how sea lamprey migrate in the Great Lakes. I have just scratched the surface of these collected datasets and in the future I expect analyses with a predictive nature to become important.

APPENDIX

Table 1.1: Studies of animal behavior that focus on telemetry data should follow this general framework and report on each component: question development data collection, data processing, and analysis. Using the framework ensures that at the start of the study there is clear consideration of what data is necessary to properly answer the question and describe the behavior in an exploratory, explanatory or predictive context.

Question Development	Data Collection	Data Processing	Exploratory Analysis	Explanatory Analysis	Predictive Analysis
Identify potential questions and determine the data needed.	Collect environmental, body movement, and spatial movement data.	Assess data quality needs and actual data quality.	Classify and merge spatial patterns and/or body movement patterns into behaviors (e.g., decision tree)	Look for patterns between movement and environmental data and/or between body and spatial movement.	If the environment is modified in a particular way, what change in behaviors would we anticipate?
		Merge animal data with environmental data.	Characterize differences in classified phases	Model testing and model fitting	Full Model Fitting
		Build Database	See Gurarie et al. 2015 for complete description	Pattern Testing	
		Filter data.			

Table 1.2: The components of Ethology.

Ethology: The biological study of behavior.
Behavior: The regulation of bodily status via spatial adjustments involving neuro-muscular systems, operated in response to environmental information to satisfy an internal state (or purpose for acting).
Components of behavioral description:
<u>Bodily Movement:</u> The local adjustments of an organism’s neuro-muscular systems that are part of a specific behavior but may or may not result in spatial movement. It represents the mechanical component of behavior.
<u>Spatial movement:</u> The whole body adjustment as the result of bodily movement or environmental propulsion. The spatial position as result of behavior.
<u>Internal State:</u> The underlying reason for the behavior or why the animal is doing what it is doing. The underlying function and internal component of behavior.
<u>Environmental conditions:</u> The external information that the internal state uses to update and subsequently inform the appropriate bodily movement.

Analysis		Aspects of Behavioral Study (Tinbergen’s 4 Questions)
Predictive	Explanatory	Exploratory
		<p>Proximate: Questions that define behaviors.</p> <p><i>Causation</i> – “What is the animal doing,” is often addressed through <u>exploratory analyses</u> and usually requires bodily or spatial movement and at least limited environmental data.</p> <p><i>Developmental</i> – “How does what the animal is doing change with maturity, experience and environmental conditions?” is often addressed with similar data and analyses as causation, though include some explanatory and rarely predictive approaches.</p> <p>Ultimate: Questions that aim to understand the impact of behaviors.</p> <p><i>Functional (adaptive significance)</i> – “What benefit do classified behaviors provide to the organism,” requires an understanding of causation, and usually requires spatial movement, bodily movement and environmental information. These questions are addressed through explanatory and predictive analyses.</p> <p><i>Evolutionary</i> – “How did the behavior form in a species, in light of the environment and under intraspecific and interspecific interaction pressures” is the most complex type of questions that require a strong understanding of causation, developmental, and functional aspects of behavior, and require explanatory and predictive analyses.</p>

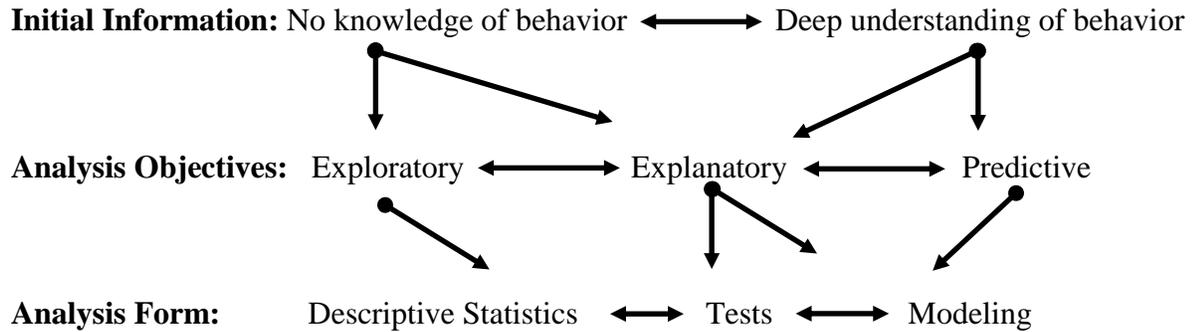


Figure 1.1: Analysis objectives aim to identify what is the animal doing (exploratory), why the animal is doing what it is doing (explanatory), and can we anticipate how it will change what it will do under different conditions (predictive)? The amount known about the animal often plays a role in where researchers aim study objectives initially and we identify the usual process taken, but the best behavioral studies aim to reach explanatory and predictive answers regardless of the initial understanding of behavior.

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CHAPTER 2

A PROCESS FOR FILTERING HYPERBOLICALLY POSITIONED UNDERWATER TELMETRY DATA WITH HORIZONTAL POSITIONING ERROR (HPE)

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ABSTRACT

Background: Telemetry systems that estimate animal positions with hyperbolic positioning algorithms also provide a technology-specific estimate of position precision (e.g., horizontal position error (HPE) for the VEMCO positioning system). Position precision estimates (e.g., dilution of precision for a global positioning system (GPS)) have been used extensively to identify and remove positions with unacceptable measurement error in studies of terrestrial and surfacing aquatic animals such as turtles and seals. Few underwater acoustic telemetry studies report using position precision estimates to filter data in accordance with explicit data quality objectives because the relationship between the precision estimate and measurement error is not understood or not evaluated. A four-step filtering approach which incorporates data-filtering principles developed for GPS tracking of terrestrial animals is demonstrated. HPE was evaluated for its effectiveness to remove uncertain fish positions acquired from a new underwater fine-scale passive acoustic monitoring system. Results: Four filtering objectives were identified based on the need for three sequential future analyses and four data quality criteria were developed for evaluating the performance of individual filters (step 1). The unfiltered, baseline position confidence from known-position test tags was considered to determine if filtering was necessary (step 2). An HPE filter cutoff of 8 was selected to meet the four criteria (step 3), and it was determined that one analysis may need to be adjusted

for use with this dataset. The data quality objectives, criteria, and filter selection rationale were reported (step 4). Conclusions: The use of position precision estimates that reflect the confidence in the positioning process should be considered prior to the use of biological filters that rely on *a priori* expectations of the subject's movement capacities and tendencies. Position confidence goals should be determined based upon the needs of the research questions and analysis requirements versus arbitrary selection, in which filters of previous studies are adopted. Data filtering with this approach ensures that data quality is sufficient for the selected analyses and presents the opportunity to adjust or identify a different analysis in the event that the requisite precision was not attained. Ignoring these steps puts a practitioner at risk of reporting errant findings.

INTRODUCTION

Background

Advances in animal-tracking systems have made high-resolution, high-density position data increasingly available, affording the scientific community a substantial opportunity to finely examine the habits of difficult-to-detect individuals and species [1-4]. Any technology that assigns spatiotemporal positions to an animal produces values that contain varying degrees of measurement error. Determining an appropriate method to detect and remove positions with excessive error from datasets presents a real challenge to researchers as over-filtering (i.e., the omission of 'good positions') is a significant source of error in animal movement studies [5-7]. Each time location data are filtered, researchers must also be cognizant of the tradeoff between increasing confidence in position accuracy and the possibility of introducing systematic biases [8]. A useful technique must therefore discern between measurement error and true variation in animal movement. Two common approaches involve the use of biological filters, which equate

to detecting violations of an animal's expected maximum movement capacity (e.g., maximum observed velocity) or known habits (e.g., observing a fish on land), or a position precision estimate (PPE) in which each measured position has an associated position-specific estimate of measurement error or confidence. We outline a widely applicable approach to use a PPE to remove positions with unacceptable measurement error, and provide an example of a specific application to a telemetry dataset collected from an underwater acoustic positioning system, the VEMCO Positioning System (hereafter VPS; VEMCO Division of AMIRIX Systems; Halifax, Nova Scotia).

The VPS system

VEMCO provides two-dimensional positions derived from raw detection data using proprietary hyperbolic positioning algorithms that estimate positions from the time-difference-of-arrival (TDOA) of a coded signal at three or more receivers in the VPS array [9]. Each detection record in a receiver consists of a transmission code that is unique to a tag, the time of signal reception, and, depending on the tag configuration, an environmental variable measured by the tag (e.g., pressure, temperature, etc.). As with all TDOA-based processes, time synchronization of the autonomous receivers is critical. The VPS system makes use of synchronization transmitters (synch-tags) placed at stationary locations throughout the array to account for time drift in individual receiver clocks [9,10] (Figure 2.1). Like other multi-receiver positioning systems [11], the VPS provides a weighted average location from every combination of three receivers that detected each tag transmission (hereafter referred to as a position 'fix'), and an associated PPE termed horizontal position error (HPE) [9,12].

Filtering spatial data with HPE

HPE is assigned by VEMCO to positions for both synch-tags and animal-implanted tags, but practitioners must perform auxiliary testing to be able to discern when and how HPE can be used to filter data. HPE is a unitless estimate of position precision based on the relationship between theoretical position error sensitivities and observed measurement errors (measured horizontal position error in meters, HPE_M) for synchronization tags [9]. If the relation between HPE and measurement error developed for synch-tags is representative of the relation for animal implanted tags, then the dimensionless HPE value can be used as a confidence estimate for each fish position.

Choosing an appropriate HPE cutoff to remove inaccurate fixes is the primary challenge in filtering VPS data, but few studies have provided an exposition of the filtering approach [13]. Studies either used a conservatively high HPE cutoff to remove the major problematic positions but retain most data (e.g., for an HPE number of 20, 83% retention [14]), or employed a lower HPE cutoff for the study and sacrificed large amounts of data (e.g., for an HPE number of 10, 58% retention [15]). In other cases, HPE was used but the level of data reduction was not reported (for an HPE number of 15 [12]), no filtering occurred and the HPE estimated for synch-tags was assumed to characterize the precision of all animal positions in the array [10], or the authors referenced an example of the accuracy attained from a similarly configured array used in an unrelated study [16]. Despite an extensive literature on PPE estimates [5-8], a consistent framework does not exist for developing and applying an HPE-based filter to positional telemetry data, and many studies using HPE do not clearly report the selection criteria for the cutoff other than reference to prior studies. Confusion exists among practitioners over what

filtering steps are broadly applicable to all studies and what components should be independently derived due to unique research questions.

We propose a generalizable four-step process for filtering VPS spatial datasets via HPE that accommodates the specific data quality objectives of each analysis. The steps include: 1) establishing position confidence goals (maximum tolerable errors) contingent on the scientific question and the chosen data analysis technique; 2) evaluating the unfiltered, baseline position confidence from known-position test tags; 3) selecting a filter cutoff and assessing if data reductions have introduced biases (a-c); and, 4) reporting the data filtering criteria and process in the final manuscript or report. The details that comprise the four steps are not interchangeable for all studies but completing each step will improve the rationale and transparency of data filtering for many telemetry studies. Importantly, the process does not set rigid standards for data inclusion; rather, it requires the assignment of standards that are specific and defensible for each project.

To demonstrate our approach, an extensive data set was derived from a 41-receiver array placed in the waters of Lake Huron (Michigan, USA) near the outlet of the Ocqueoc River (45° 29' 24.53" N, 84° 4' 23.45" W). The array had an internal area of 0.92 km² and a maximum coverage of 8.22 km² in waters that ranged from 0.2 to 20.8 m deep. The dataset included recorded positions taken from stationary tags (9 synch-tags and two additional stationary position tags), three mobile tag tests (submerged tags dragged behind a slow-moving boat with continuous GPS recording), and animal location data recorded for 76 free-swimming adult female sea lampreys (*Petromyzon marinus*) with surgically-implanted tags (V9P-2H, 15 to 45 second transmission rate). With this dataset we demonstrate a framework for filtering VPS data

with HPE, provide a comparison to current filtering approaches used by other VPS studies, and contrast the advantages of PPE based filters and biological filters.

METHODS

A telemetry system (VPS, VEMCO) composed of 41 receivers (VR2W) in diamond formations with between-receiver spacing ranging from 125 m to 250 m covering 2 km² was used to position a stationary tag at two locations, a mobile tag, and 76 tagged sea lamprey. Sea lamprey were implanted with acoustic transmitters (VEMCO model V9P-2H, 69 kHz, 150 db, 47 mm (length), 3.5 g (dry weight), random interval: 15 to 45 s, burst length: 3.54 s or 3.26 s, between 09 April 2010 and 09 July 2010 (Figure 2.1). The depth of the site ranged from 0 to 5.8 m within the array but sea lamprey were positioned at greater depths on the fringes of the array. Nine synchronization transmitters (VEMCO model V16-2H, 69 kHz) were deployed in stationary positions through the array. Compared to fish tags, synch-tags emitted a more powerful signal (160 db) less frequently (500 to 700 s). The performance of the VPS array was tested by comparing the position estimates of one stationary V9P-2H transmitter (stationary testing) at two locations (Location 1: 17 June 2010 19:43:20 to 01 July 2010 14:34:36, Location 2: 01 July 2010 14:52:47 to 08 July 2010 17:03:20) and a slow moving V9P-2H transmitter (mobile testing) inside the body cavity of a sea lamprey that was recently deceased, located 1 m off the bottom and below a floating boat powered by an electric motor with a GPS (Trimble Geo XH) mounted directly above the tag monitoring the tags' true position. Due to a GPS malfunction we did not have GPS measured positions for the stationary tests and were forced to use position averages for comparison. This is not an ideal solution. The GPS and tag were maintained in alignment with a down rigger and there was no visual layback of the tag. The mobile test was comprised of three transects through the array starting near the coast and ending

outside of the internal array area on 06 July 2010 at an average speed of 0.60 ms^{-1} (range: 0.25 to 1.15 ms^{-1}) to mimic sea lamprey ground speed: (i) Boat Path 1, 13:12:59 to 13:57:24 (distance 934 m); (ii) Boat Path 2, from 14:22:19 to 15:10:08 (distance 754 m); and (iii) Boat Path 3, from 15:35:08 to 15:59:42 (distance 309 m) (Figure 2.1; left to right). The GPS positions were post-processed with three local reference stations that have one second reference intervals (CHB5, MIAL, MIMC) and the positions had an estimated accuracy of 0.36 m, 0.29 m, and 1.03 m (mean, min., max.). In our analysis, we assume that these GPS positions represent the true mobile tag position. We were not able to synchronize the acoustic receivers to the GPS clock at the time of tests, so we applied a constant offset to the timestamps of GPS positions until error between the GPS track and mobile tag track (derived from VPS) was minimized. Error was minimized using the Optim function in R (Additional file 1), which used the Broyden–Fletcher–Goldfarb–Shanno method [28]. The test tag was identical to the tag inserted into female sea lamprey (V9P-2H, 69 kHz, 150 db, 47 mm (length), 3.5 g (dry weight)), which were monitored as they approached, entered or bypassed the Ocqueoc River mouth (45.490278° , -84.072931°) in Hammond Bay (Lake Huron), where lake water temperatures ranged from 6.0 to 19.7°C throughout the study (April 9, 2010 to June 7, 2010). The use of sea lampreys was approved by the Michigan State University Institutional Animal Use and Care Committee via animal use permit 02/10-020-00.

Error evaluation based on test data

To evaluate the baseline array accuracy the position yield (number of positions observed/maximum possible number of positions) based on transmission rate, the proportions of data that were below 6 m error and the number of positions with greater than 15 m error were enumerated for stationary and mobile test tags. The position yield was calculated by determining

the total number of locations positioned by the VPS versus the number of acoustic tag signals emitted in the time period (maximum transmission number). The true number of fixes can be readily counted. To estimate position yield, the average time for one complete transmission cycle must be determined by adding the average programmed transmission interval between the start and end of a transmission plus the length of the transmission (burst length). The V9P-2H tag had a pre-set transmission interval that ranged from 15 to 45 seconds (average: 30 seconds) and a burst length that alternated between 3.26 and 3.54 seconds. The burst length was always composed of a series of eight pings, but varied because two different alternating signals were transmitted from these tags as one provided a depth code and a short tag identifier (two digit) and the other provided a longer tag identifier (five digit) that is more robust to individual misidentification. The resulting average length for one transmission cycle was 33.4 seconds. The time between transmissions is readily available; however, the burst length should be obtained prior to performing this calculation. Second, the total transmission period must be divided by the length of one transmission cycle to determine the maximum transmission number. Average error was calculated and the presence of positions with unacceptably large error values (>15 m error) were enumerated for all three tests (two stationary and one mobile).

HPE evaluation with test data

The average error (criteria 1) and number of positions with large error were estimated (>15 m, criteria 2) for all HPE cutoff possibilities (3 to 15) to evaluate the first two criteria (Table 2.1). To evaluate if the HPE value provided for fish tags and calculated by VEMCO using synch-tag detections was a representative estimate of locational error for fish tags, we calculated 2DRMS for each stationary test tag by first calculating the Euclidian distance between each individual test position and the median or best estimate of the 'true' test tag location. The HPE

calculated by VEMCO is scaled to an approximately 1:1 relationship between HPE and measured error in m for synch-tags [9]. 2DRMS can be used to compare the relationship to fish tags and is reported in the base line evaluation of the dataset. The 2DRMS for the median value is actually a measure of precision [8], but it was also the best available estimate of accuracy. The 2DRMS linear model was calculated by first binning all data by one unit HPE increments and an average of the HPE within the bin was calculated to represent the bin. The error in the x direction (X_e) and y direction (Y_e) was estimated for each location within a bin. The 2DRMS was then estimated for the 95% confidence interval using X_e and Y_e within each bin and a line was fit to the 2DRMS data $\left(2\text{DRMS Error} = 2 * \sqrt{\left(\text{SD}(X_e)^2 + \text{SD}(Y_e)^2\right)}\right)$. A linear model fit to those 2DRMS values for each HPE (2DRMS line) of interest (3 to 15) was used to predict the proper HPE filter for 95% confidence about a given target error value. The 2DRMS regression developed for the mobile test was not considered due to the small number of recorded transmissions (138). The 2DRMS model was used to estimate the HPE at which there was 95% confidence that positions had less than 6 m accuracy (criteria 3). A range of HPE cutoffs (3 to 15) for removing unacceptably erroneous positions (>6 m error) while avoiding the loss of acceptable positions (<6 m error) were considered based on the percentage of incorrectly retained (of all retained) and incorrectly rejected positions (percentage of acceptable positions) (Table 2.2). The goal for the incorrectly retained percentage of all positions was >99% and incorrectly rejected of all positions was >95%, as the loss of data was considered more acceptable than allowing the erroneous positions to remain. This was an arbitrary proportion, though the loss of positions when evaluating trajectory data can quickly become problematic and retaining unacceptably erroneous positions could result in incorrect habitat assignment, so we

selected high target proportions. The variation in temporal gaps was not considered but could be very important to a study.

Filtering fish data

We examined all fish data classified by HPE in a schematic to assess any spatial pattern in HPE and to identify areas of poor array coverage. For each candidate HPE cutoff, we calculated the amount of data rejected inside and outside of the array area as defined as a polygon drawn around the outer most receivers. The post-filtering spatial coverage of the array was also tabulated for all candidate HPE values with the expectation that locations outside of the array are likely to have an increased HPE and lower accuracy [12] with increasing distance from the array periphery. Coverage was calculated by drawing a polygon around the outer most fish positions retained after filtering, and calculating the area (km²). Lastly, the evenness of filtered positions, referring to how grouped filtered positions were in the array, was considered for each individual fish to determine if specific subjects were more prone to being rejected. We visually assessed whether these positions were associated with any particular four-receiver diamond or if they were missed throughout the array, occurring in multiple four-receiver diamonds.

RESULTS

We demonstrate our approach by filtering the example dataset according to the data quality objectives of the study.

Step 1: Establishing data quality objectives

We adopted four data quality objectives. Data quality objectives are explicit assignments of acceptable errors in precision that derive from the requirements of the data analysis technique.

Any defensible objective could have been adopted to fulfill this step because the details are study-specific and could be simplified to a single criterion.

Our four objectives included the ability to detect changes in trajectories (e.g., ground speed, turn angle, etc.), perform basic behavioral assignments (e.g., is the animal moving or stationary?), assign fish positions to habitat types (point data), and avoid the loss of acceptably accurate data while ensuring the removal of unacceptable positions if filtering is necessary to perform habitat assignment (Table 2.1). These objectives include both position specific criteria (i.e., <15 m error) and global criteria (i.e., mean error <1.77 m), though in each case a position specific filter (i.e., precision target) must be identified. The filter value should represent the minimum value that must have been met by a filtered dataset in order to achieve each objective. Among candidate filters, we identified the preferred filter as the one with the largest HPE that best met all criteria. The criteria provided a clear framework with which to select the best filter.

Step 2: Estimating the baseline position confidence in the array

Baseline position confidence refers to the quality of the unfiltered position data and is partly a function of array coverage (i.e., low quality positions are likely more abundant in regions of the array with poor coverage). Baseline confidence must be identified to determine if filtering is required. The baseline position confidence was described by mean and median array accuracy; position yield, the proportion of data that was below 6 m error, and the number of positions with greater than 15 m error were enumerated for stationary and mobile test tags that collectively determined if the array met the first three criteria. The fourth criteria dealt with data reduction. The initial evaluation was based on position yield (percentage of positions estimated vs. expected during the time period), average data accuracy, presence of large errors (>15 m), the twice the

distance root mean square (2DRMS) model equations, the proportion of data that was below 6 m error, and array coverage based on actual fish telemetry data.

There were three tests of accuracy and each test varied in length, including 29,355, 16,400, and 138 positions (stationary site one, two, and mobile test) (Figure 2.1). The position yield for the stationary tags were 82.3% (location #1) and 89.4% (location #2), and 94.8% for the mobile test; this indicates that 5.2 to 17.7% of the tag transmissions did not result in a position. The estimated positions of the stationary tags were generally clustered around a central position; each exhibited a ‘tail’ of increasingly erroneous positions offset to the west. Without filtering, the mean error was 11.7 m for stationary test one (median: 2.9 m, range: 2.7 to 29,289.3 m), 4.2 m for stationary test two (median: 2.6 m, range: 2.5 to 1,425.8 m), and 5.81 m for the mobile test (median: 2.95 m, range: 2.50 to 186.40 m). There were 625, 134, and 0 positions with greater than 15 m of error (stationary test one, stationary test two, mobile test). The linear models for the 2DRMS regression line relating HPE and measured error obtained from the stationary-location tags were $y = 0.18x - 0.89$ ($r^2 = 0.92$) for location #1 and $y = 1.1x - 0.57$ ($r^2 = 0.78$) for location #2 (Figure 2.2). The slopes were both near 1 (0.81 and 1.1), indicating a similar relationship of HPE and synchronization tags to HPE and fish tags, with HPE potentially representing a conservative estimate of position error for fish tags (the slope of the 2DRMS line was less than 1). The proportion of test positions with 6 meter accuracy exceeded 90% for all three tests (Figure 2.3). Maximum array coverage was 8.22 km², estimated by forming a polygon around the outer most estimated fish positions.

Step 3a: Evaluating HPE filters consistent with the data quality objectives and guides

The necessary filter cutoff for each criterion to be met must be determined prior to selection of a filter cutoff. Although the highest acceptable HPE value will retain the most data,

the range of HPE values that fulfill each criterion to reach each objective should be evaluated and reported. If no value meets all criteria, either the analysis associated with an objective must be reevaluated, a different analysis should be selected, or the research question will prove difficult to address with the original planned approach.

Criterion 1

Any HPE cutoff from 3 (minimum observed in the test data) to 15 (maximum considerable due to objective 2) was sufficient to meet criterion 1 for stationary tests but not for the mobile test, although the mobile test methodology was susceptible to inherently high average error (Table 2.2).

Criterion 2

The number of positions that violated criterion 2 increased greatly between HPE filters of 6 and 15 (max error: location 1: 10.0 to 26.7 m, location 2: 9.2 to 25.0 m; violating positions: location 1: 0 to 21, location 2: 0 to 10) (Figure 2.4). Only HPE cutoffs less than 7 (stationary location 1) or 8 (stationary location 2) met criteria 2, although no position had greater than 15 m error for the mobile test (Table 2.2).

Criterion 3

The 2DRMS equation for desired 95% confidence in 6 m accuracy (Criterion 3) returned an HPE cutoff of 8.5 for stationary test one and 6.0 for stationary test two (Figure 2.2). There was not enough mobile test data to calculate this metric.

Criterion 4

The incorrectly retained proportion of all positions was less than 1% for HPE cutoffs of 3 to 10 for stationary test one and was 1% for all HPE cutoffs 3 to 15 for stationary test two (Figure 2.5). For the mobile test, only an HPE of 3 reduced the incorrectly retained proportion below though only four positions were incorrectly retained (3% of total; max. error = 7.7 m) when HPE cutoffs ranged from 6 to 10 (Figure 2.6; Table 2.2). Incorrectly rejected positions occurred at less than 5% of all positions for any HPE cutoff greater than 8 for stationary test one, greater than 3 for stationary test two, and greater than 5 for the mobile test, representing a minimal loss of acceptable data. When comparing the relationship between incorrectly retained and incorrectly rejected, acceptable HPE cutoffs of 8 to 10 (stationary test one), 4 to 15 (stationary test two), and no HPE was effective for the mobile test, although 5 to 10 was closest.

Step 3b: Selecting an HPE cutoff

The highest HPE cutoff that met or was the closest to meeting all criteria was an HPE of 8, although from this dataset HPE cutoffs between 6 to 8 all fit the criteria similarly. There were only two positions remaining with error greater than 15 m and an HPE less than 8 (criterion 2) during the combined 45,744 transmissions during the two stationary tests, which may be good enough for first passage time analysis if visual inspection of the remaining data points allows the remaining problematic values to be easily identified or the analysis for this step may need to be adapted. If we selected a lower HPE to meet this criterion perfectly, an HPE cutoff of 6 would have been required. An HPE cutoff of 6 would have incorrectly rejected 7.7% of data from stationary test one. Although a practitioner could make a case for this level of filtering, we preferred the increased coverage as data loss is a major issue with trajectory based analyses (Figure 2.7); 4,124 more positions would have been lost with an HPE of 6 vs. 8 (28.35% lost

outside array and 11.22% lost inside array for HPE 6) and total areal coverage would have reduced from 2.11 km² (HPE 8) to 1.89 km² (HPE 6) (Figure 2.8).

Step 3c: Evaluating the potential for introduced bias through application of HPE cutoff

There was evidence for spatial bias in filtering inside versus outside of the array and potentially behavioral or habitat filter bias. Only three mobile test positions with acceptable accuracy (<6 m) were rejected and all occurred at the array edge, suggesting that HPE may over-filter at the periphery of coverage (Figure 2.6a). In the sea lamprey data set, the HPE cutoff (8) rejected 8.8% of 58,025 total positions inside the array and 23.5% of 53,435 total positions outside of the array, though this comparison does not discriminate between removal of inaccurate and accurate positions (Figure 2.8). There was no evidence for spatial filter bias inside of the array as no region appeared more prone to poor positioning across individuals (Figure 2.8). A behavioral or habitat filter bias was evident, as the majority of positions rejected from inside of the array (3,070 positions, 74.6% of all rejections) were associated with seven lampreys during daylight hours that were likely stationary, as sea lamprey are nocturnal. This observation is consistent with previous observations that sea lamprey may settle in locations that interfere with acoustic tag signal transmission, blocking the line of sight between receivers and tags [19].

DISCUSSION

We developed a straightforward conceptual approach for using a PPE to filter hyperbolically positioned data and demonstrated this approach with a technology (underwater acoustic telemetry) in which users evaluate position accuracy with HPE. The framework included selection of defensible data quality objectives, evaluation of the array's positioning

accuracy with an independent dataset, and determination of the relationship of the selected PPE to measured accuracy and data retention.

Data-filtering with a PPE estimate of position accuracy has certain conceptual advantages over a biological filter if the PPE is properly evaluated. PPE's are calculated for each position obtained from the telemetry apparatus, whereas biological filters ignore the positioning process and only evaluate resultant positions based on an expectation of what is biologically reasonable for the study species. Biological filters can be useful and are frequently used because they are conceptually straightforward, and at times, the only available option. Some habitat filters are quite reasonable (e.g., fish do not swim 500 m onto land); although the rule could introduce filter bias as positions closer to the physical habitat edge are more likely to be rejected. Similarly, as HPE represents a 95% confidence value, HPE becomes large when solutions become less precise, as typically occurs outside the array periphery where the overlapping of parabolas allows for multiple potential solutions [9]. Failure to select a proper filter is more problematic for calculating movement trajectories (e.g., maximum ground speed), and identifying a useful biological filter is especially challenging for aquatic species for which maximum movement capacities are often unknown [22], or poorly estimated. Selection of a biological filter cutoff that is high allows incorrectly retained positions to remain in the dataset, whereas cutoffs that are low near the average speed remove valuable data. Either case will serve to infuse the data with a perceived improvement in accuracy that is not supportable (i.e., the rejected positions may be no less accurate than many retained positions). Unlike biological filters, PPE filters are position specific and rely principally on the assumption that animal-integrated tags match the performance of stationary or towed tags, and that the array is well-constructed to ensure sufficient areal coverage, avoidance of obstructions, etc. [9,23]. If these assumptions are supported, carefully selected PPEs that are based on the position quality should be used prior to

biological filters that are based upon the biological plausibility of resultant positions and not the quality of the positioning process.

PPE filter selection ranges from choosing an arbitrary HPE based on subjective operator preferences to developing a complete algorithm that would output a filter threshold based on a set of inputs (i.e., the model determines both performance and value). The approach we suggest clearly falls in the middle (i.e., performance measurement is objective but value judgment can be subjective) but still represents a significant improvement over the use of arbitrarily selected filter values. The selection of an HPE of 8 fell within the most effective range for the criteria and ensures high confidence in 6 m accuracy (criteria 3). Although we identified three different criteria to complete a complex analysis, a single criterion could be chosen for a single analysis, or, if multiple analyses are contemplated, a different cutoff could be chosen for each, which would make selection and reporting straightforward. In our example, the criteria for objective 2 was not met, as it required no positions to have greater than 15 m of error, clearly representing an ineffective criteria for an objective. At very low numbers of violating positions, only extreme positions remain and improving the filter to remove these positions came at a high cost (increase in positions incorrectly rejected). These extreme outliers may not even represent predictable performance of the system. If the filter is to be useful, the proper response is to adjust the first passage time analysis, which is very easy and would have only required shifting the moving designation by a few meters (17.7 m was the largest remaining error). However, perhaps a better criterion would be to choose a high percentile (e.g., 99.5%) that represents an acceptable level of risk or error in your future analysis. With the VPS system, little effort has been made to defend filtering cutoffs beyond reference to prior use [13], and ambiguous filter criteria are at risk of inadvertently becoming acceptable practice through the accumulation of use. In our case, adoption of an ambiguous filter based on a previous study (HPE 10 to 20 [14-16]), would have

been less useful than the carefully evaluated filter cutoff of 8 and indefensible (Table 2.2).

Telemetry technology represents a very different tool than typical scientific instruments as its design is rarely consistent, is difficult to standardize, and does not generate data points with fixed accuracy and precision. For this reason, we suggest that data filtering should be a flexible process that may progress towards more concrete rules if some level of standardization of use occurs for telemetry equipment as has occurred for the use of positional dilution of precision in certain GPS applications (e.g., [24]).

A VPS system is capable of attaining accurate positions (2DRMS <6 m, <2 m average) with a high position yield (>82%) via autonomous receivers that are capable of covering large areas (>2 km²), although these results were specific to this system and environment. As with all systems, the VPS was susceptible to spatial, temporal, and behavioral or habitat bias in position yield and position precision which could cloak important biological phenomena [6,24]. For example, when an animal occupies a habitat that blocks the line of sight from tags to receivers, as was suspected for the seven stationary sea lamprey that composed 74.6% of our filtered data inside of the array [19], there is a potential habitat and behavioral bias [9]. Observed HPE values also increased with distance outside of the test array, consistent with observations from other studies [9,13]. Confirming a temporal or spatial bias is challenging because, depending on placement, stationary tags may not reveal systematic spatial biases in the array [9,25], and may be sensitive to regular variation in environmental conditions (e.g., louder waves in shallow vs. deep waters) that differentially impact receiver performance. Mobile range testing is recommended for spatial evaluations of filter performance, though results may not be representative of the full range of environmental conditions encountered by tagged animals due to the typically short duration of mobile tests, selection of favorable boating conditions, etc. The

mobile test appeared to be biased towards higher error estimates, though the spatial patterns were consistent between fish tracking and mobile test data.

Both mobile and stationary tests presented unforeseen challenges and unexpected findings. Mobile testing suggested an HPE of 8 was overly conservative outside of the internal array area, though we lacked stationary tests in this region that would have confirmed this conclusion (Figure 2.6). The mobile test presented some challenges to evaluation because the GPS clock was not synchronized with VPS time and only recorded a position every second. To minimize the effects of clock differences on position error estimates, we applied a constant time offset to all mobile test positions that minimized error between the mobile test tag tracks and corresponding GPS tracks (Additional file 1). In addition, we likely over-estimated error by assuming that the GPS track represented the true path of the test tag because we only collected a single post-processed position every second along the track. Collecting positions with sub-meter accuracy usually requires averaging several GPS points at a given location, but point averaging over time is not an option while moving. The tail of positions that were observed in the stationary tests could not be explained with the example dataset but was likely the result of a specific set of receivers with poor geometry that consistently estimated inaccurate positions in one direction. The tails were not troubling to us as these positions were easily filtered with HPE. Many challenges can be avoided in advance by careful project planning. We recommend multiple mobile and stationary tag tests with the animal tag during the same time period that the animals are being monitored, and ideally, at least some fraction of the synch-tags would be the same model as the fish tags. Receivers should be positioned outside the maximum spatial extent of interest, or at a minimum, stationary animal tags should be monitored in any area of interest to ensure the chosen HPE cutoff has met the filter goals.

Although we present a process for selecting a single fixed HPE cutoff and provide the R code necessary for performing this approach (Additional file 1), a dynamic filter in which the HPE cutoff could be either temporally (e.g., [26]), or both temporally and spatially flexible, might prove useful, although it also would require synchronization tags that match fish tags. Regardless of how the HPE selection process is fine-tuned, the key is following a standard process like the one we have described, including reporting the process for others to properly assess the research findings. Even if the necessary accuracy required to perform the most-preferred analysis cannot be attained with the data collected, PPEs can be used to tailor the selection of an analytical tool, or the spatial scale at which the behavior is considered. Either adaptation to the study is preferred over the reporting of errant observations [17,27].

Conclusions

PPE error estimates are frequently available from animal telemetry systems that rely on hyperbolic positioning and can be used to evaluate data quality prior to analysis. When using PPE to filter data, practitioners should undertake (a) *a priori* determination of data accuracy requirements; (b) independent assessment of the telemetry system performance; (c) a determination of how well the PPE represents measured accuracy; (d) selection of a filter cutoff based on the balance between accuracy improvement and data retention; and (e) explicit consideration of spatial, behavioral, and habitat bias associated with the telemetry system and the animal under observation. A carefully constructed PPE filter is more defensible than biological filters that can improve data accuracy but require (1) an interpretation of the data vs. an assessment of its precision, and (2) are only applied to a subset of the data collected (extreme movements). HPE offers the intriguing possibility for direct use in the analysis as an error estimate (vs. a criterion for data retention); akin to bench apparatus precision estimates, though

there is no evidence that this approach has been used in such a manner with other hyperbolic positioning systems. Because data analysis requirements are likely to be as varied as the movement data to which they are applied, complete exposition of the selection process and criteria should be included in the methods section of any subsequent reports or publications. The minimum level of a reporting should include a description of the data quality objectives, criteria, rationale for cutoff selection, and evidence of reaching the criteria, which could come in the form of a paragraph, table, or appendix and does not need to be a substantial component of the report or paper.

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APPENDIX

Table 2.1: Filtering Objectives. The four specific criteria adopted to establish the data quality objectives for the project.

	Objective	Criteria	Rationale
1	Detect changes in trajectory	Mean error ≤ 1.77 m	Many trajectory based analyses are only preserved when the average position error is $<10\%$ of the mean step length [17]. Transmission delay was (33.4 s), sea lamprey ground speed is 0.53 ms^{-1} [18,19], equating to a step length of 17.7 m.
2	Assign behavioral state (moving or stationary) to fish positions	Max error <15 m	Simple behavioral assignment of moving or stationary with first passage time analysis, which is a measure of the time it takes an individual to leave a circle of fixed radius r drawn around each measured location to determine if movement is occurring [20,21]. Based on prior analyses, a displacement of greater than 15 m per transmission at the average tag transmission rate (33.4 s) could result in a false designation of moving when the fish was stationary.
3	Assign habitat to fish positions	2DRMS ≤ 6 m	To ensure accurate assignment of each estimated fish position to habitat types that are defined at the resolution of 18×18 m grid cells we selected a twice the distance root mean square (2DRMS) error of <6 m for our selected HPE values.
4	Balance loss of acceptable data and retention of unacceptable data	Retain 95% of acceptable data, <i>Reject</i> $\geq 99\%$ of unacceptable data	To ensure accurate assignment of each estimated fish position to habitat types while avoiding loss of accurate data. We aimed to keep 95% of acceptable data (≤ 6 m) while retaining $<1\%$ of unacceptable data (>6 m) of all retained positions (unacceptable data remaining/all retained positions $\times 100$).

Table 2.2: Criteria for selection of an HPE filter cutoff

Criteria	Stationary test		Mobile test	Rational for selection of a HPE filter cutoff of 8
	1	2		
Criterion 1 Mean ≤ 1.77 m	3–15	3–15	3	This criterion was met for stationary tests (Figure 2.4), but the mobile test would have required a very low HPE to attain below 1.77 m of average error as the unfiltered accuracy was lower (Figure 2.3). See review of the mobile test methodology in the Discussion.
Criterion 2 Max error < 15 m	< 7	< 8	≤ 15	An HPE of 8 did not meet the criteria for stationary tests, but only 1 of 625 positions (test one) and 1 of 134 positions (test two) remained. The mobile test criteria were met. Only 2 of 45,744 positions were problematic ($< 0.001\%$) for the combined stationary tests (Figure 2.4). The maximum point remaining was 17.7 m error.
Criterion 3 2DRMS ≤ 6 m	8.5	6.0	NA	The criterion was met for stationary test one, which was the longer test that covered more variable weather conditions. The HPE 8 bin only was 95% confident in an HPE of 8.25 meters for test two but the estimate was based on data calculated within each bin and few points had an HPE of > 6 for test two (3% of the data), which results in a less reliable 2DRMS prediction.
Criterion 4 <i>Percentage incorrectly retained vs. percentage incorrectly rejected</i>	8–10	3–15	5–10	None An HPE cutoff of 8 met the criteria for both stationary tests, and although the mobile test did not have a suitable range, the range from 5–10 was equally effective.

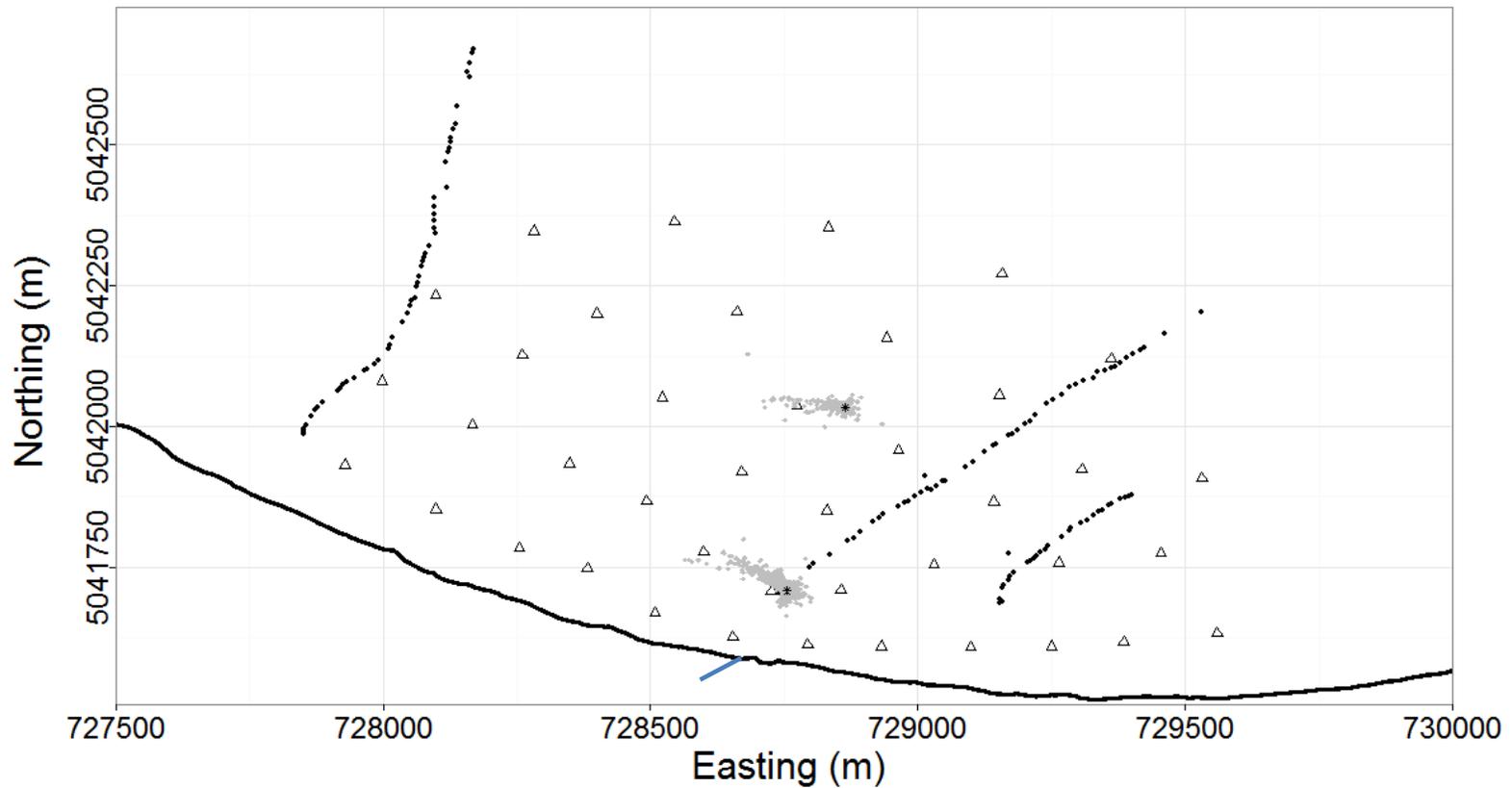


Figure 2.1: Acoustic telemetry activities at the Hammond Bay field site. A schematic of the VPS array that was located in Lake Huron around the mouth of the Ocqueoc River (blue line). Triangles represent receiver (VR2W) positions. VPS array testing in 2010 included two stationary tag tests (Gray dots, with median point as a black dot) and three mobile test transects (black dots forming lines). The schematic is oriented with north up and the black line running from left to right (east to west) represents the coast.

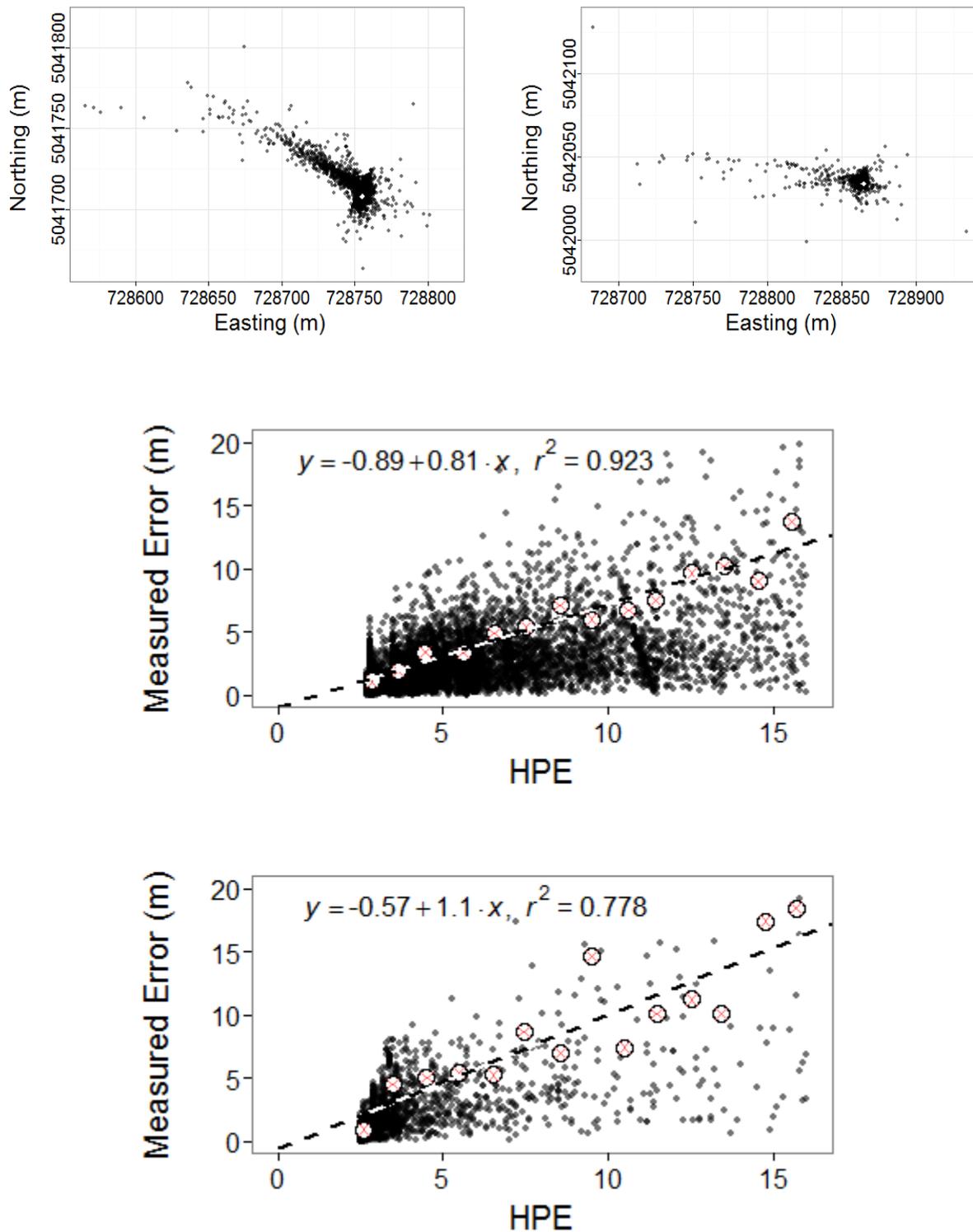


Figure 2.2: The stationary test schematics and 2DRMS plots of all stationary test positions. Two schematics depict all VPS positions during two stationary tag tests, including (a) 29,355

Figure 2.2: (cont'd)

positions between the dates (6/17/2010 to 7/01/2010), and **(b)** 16,400 positions between the dates (7/01/2010 to 7/08/2010), allowing us to evaluate array performance at two locations through an extended period of time. The white dot in the center of the clusters is the median location. The HPE versus measured error to the median point is shown for each estimated position during test one **(c)** and test two **(d)**. The white circles with black outline and red x represent twice the distance root mean square error of x and y components of error within an HPE bin of one; 95% of tag detections have an error less than this point within each bin. Note there is a minimum HPE of 2.7 and 2.5 within the data. The line running between these points represents the 2DRMS and the equation and fit for this line are shown in the top left corner of **(c)** and **(d)**, respectively. Data points above the 15 m bin, which can be seen in **(a)** and **(b)** are not shown in **(c)** or **(d)**, because they are outside of the zone of interest.

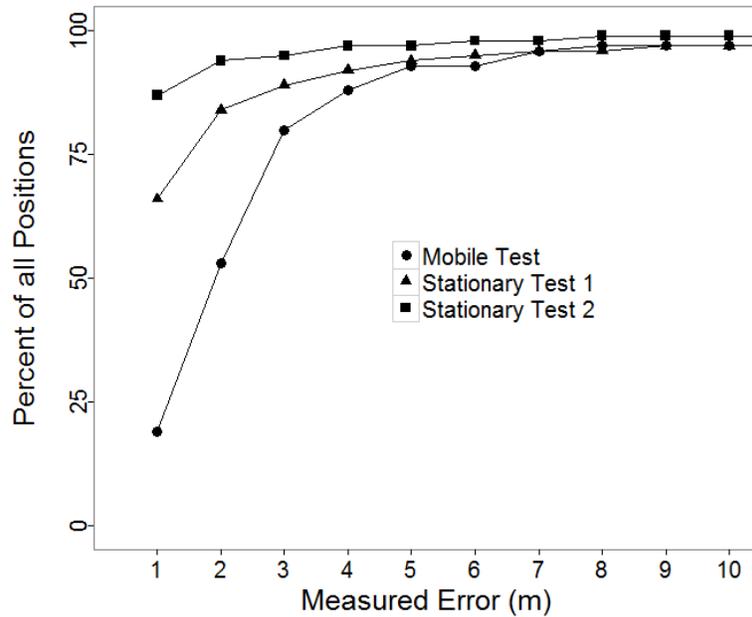


Figure 2.3: Proportion of test positions with measured error from 1 to 10 m. Percent of positions with accuracy equal to or less than each measured value for the mobile tag test (circle), stationary tag at location one (triangle), and the stationary tag at location two (square) depicted an array with most positions having accuracy better than 6 m. The average error of the unfiltered data for the stationary tag at location one (1.98 m), location two (1.11 m), and the mobile test (6.83 m) are marked by representative symbols along the x-axis.

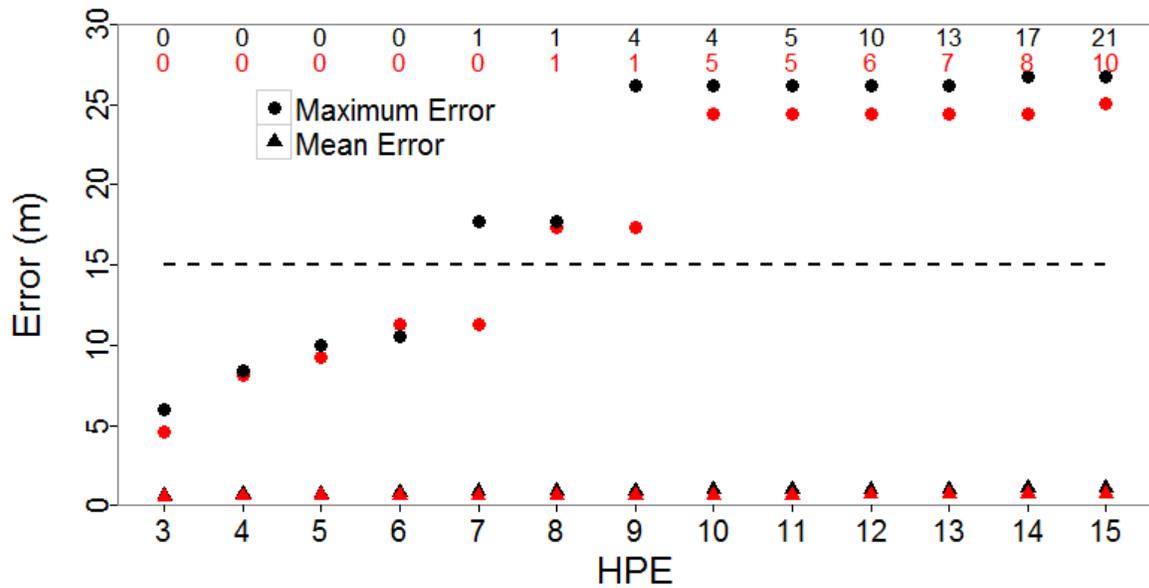


Figure 2.4: Resultant data quality for HPE cutoffs of 3 to 15. The mean and maximum measured error is below 1.77 m for all HPE thresholds, sufficient to meet criteria 1 for both stationary test one (black) and test two (red). The maximum error exceeds 15 m at an HPE of 7 for test one and 8 for test two (violating criteria 2). The number of positions violating each test is located at the top of the figure for HPE cutoffs of 3 to 15.

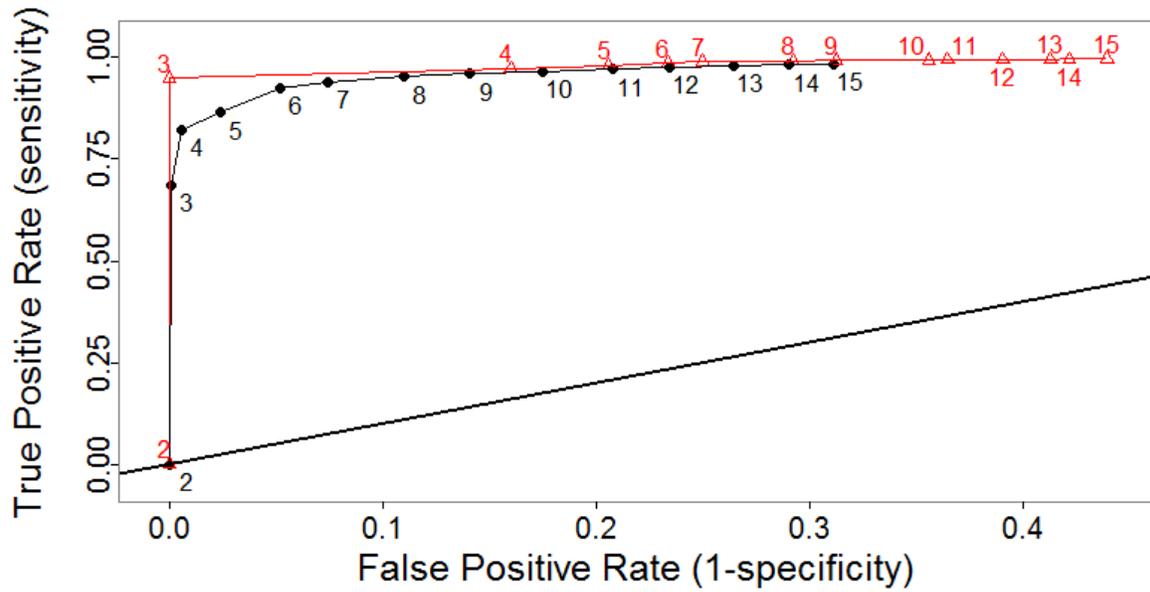


Figure 2.5: Data loss versus error retention for HPE cutoffs of 3 to 15. The relationship of the percent of incorrectly rejected positions of all acceptable positions and percent of incorrectly retained positions of all retained positions suggested that HPE cutoffs of 8 to 10 for stationary test one and 3 to 15 for stationary test two, met criteria 4.

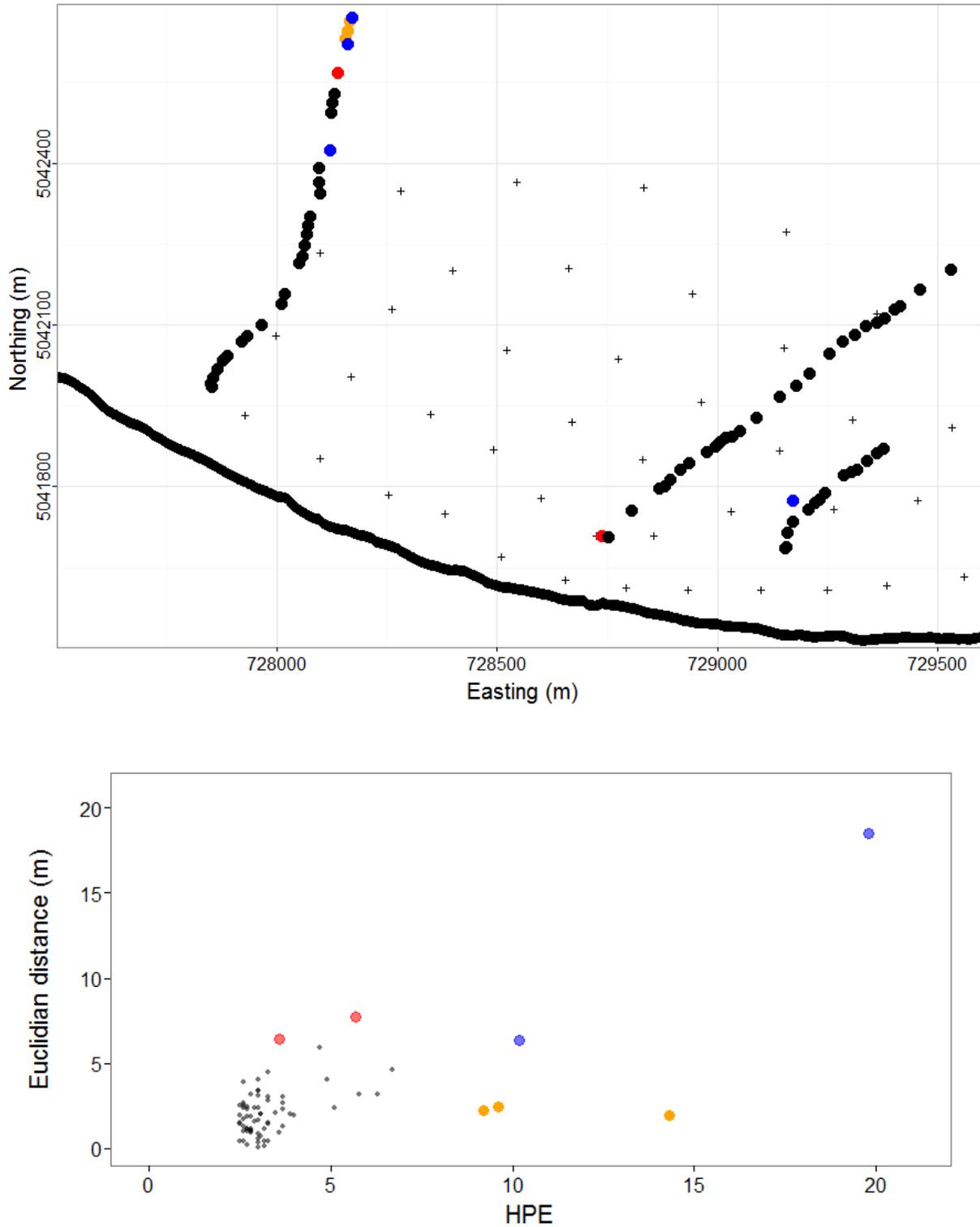


Figure 2.6: An evaluation of the performance of the VPS array with a mobile tag. (a) A schematic depicting receiver positions (+) and the coast (black line) during the 2010 research

Figure 2.6: (cont'd)

season. A mobile tag test was completed on 7/6/2010. The small dots represent the VPS estimated positions during the mobile test. There were a total of 126 correctly retained positions (black, <6 m error, <HPE 8), 5 correctly rejected positions (blue), 3 incorrectly rejected positions (orange), and 5 incorrectly retained positions (red). The incorrectly rejected (n = 3) positions occurred consecutively and were located at the furthest distance from the array in the left transect. **(b)** A graph scaled to cover positions with an HPE between 0 and 20 depicts correctly rejected, correctly retained, incorrectly rejected, and incorrectly retained positions. All values with an HPE greater than 20 were correctly rejected (n = 3, not shown). Euclidean distance is our best estimate of measured error.

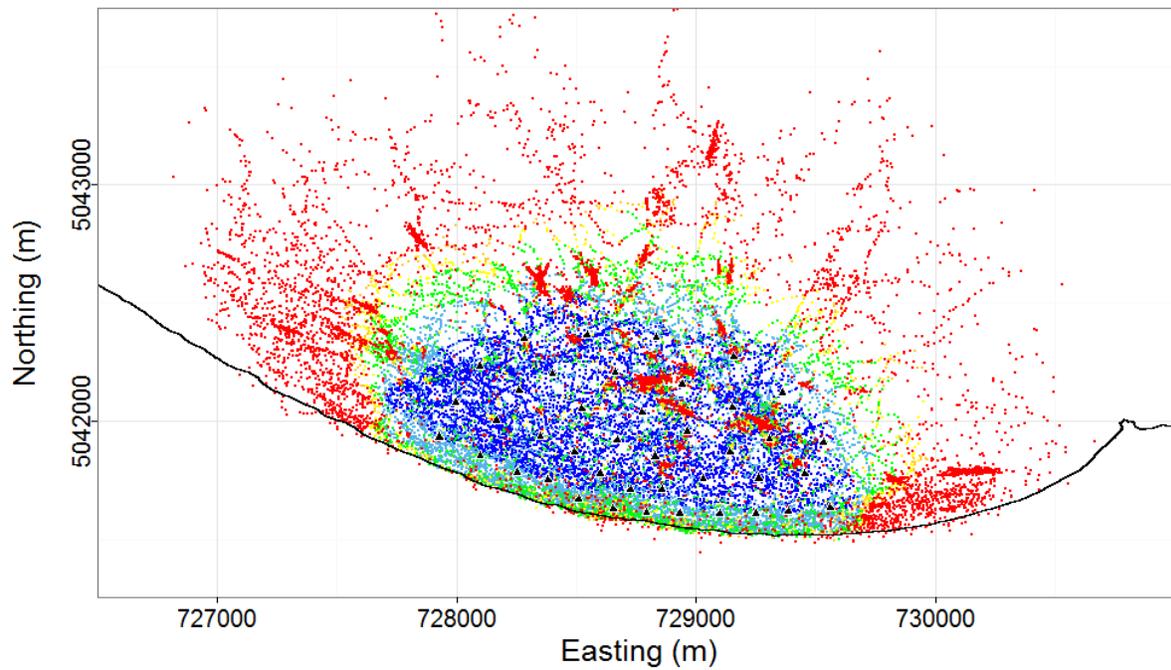


Figure 2.7: HPE values for all fish positions. The schematic depicts the HPE value for each fish position during active monitoring of study subjects. The coast is represented by the black line, receivers are represented by black triangles, and all sea lamprey positions are color coded by their associated HPE estimate and it is clear that HPE greatly increases when the sea lamprey position is outside of the array proximity, other than for a few positions inside the array.

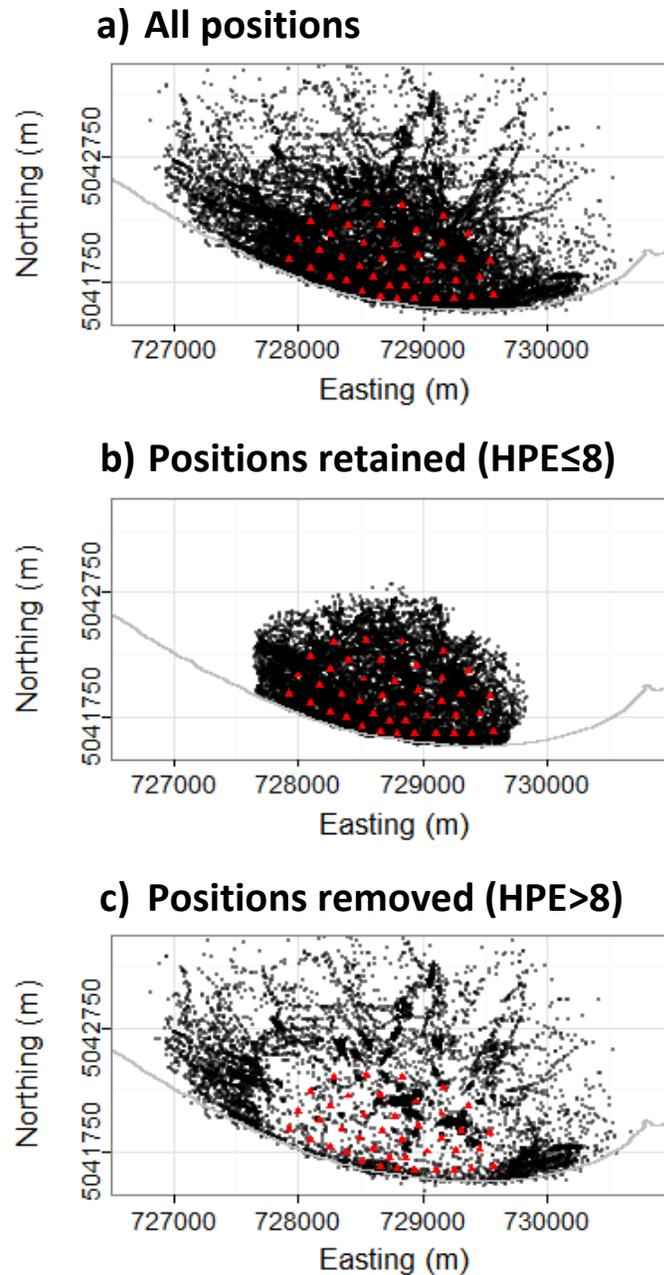


Figure 2.8: Fish positions filter by an HPE cutoff of 8. A depiction of how many of all fish locations in 2010 would be rejected by an HPE filter of 8.0. Fish locations are represented by small black dots, receiver positions are shown by red triangles and the coast is depicted by a gray line. These are cumulative graphs with all sea lamprey tags shown in **(a)**, fish positions with an HPE below or equal to 8.0 are displayed in **(b)**, and fish positions with an HPE greater than 8.0 are shown in **(c)**.

Figure 2.8: (cont'd)

Supplementary Material: R code for filtering VEMCO positioning system data with Horizontal Positioning Error (HPE).

Available at: <http://www.animalbiotelemetry.com/content/2/1/7/additional>

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CHAPTER 3

HOW DO NON-HOMING FISHES FIND THE SHORE? EVIDENCE FOR BATHYMETRIC ORIENTATION IN MIGRATING SEA LAMPREY

ABSTRACT

How non-homing fishes like the sea lamprey (*Petromyzon marinus*) find distant targets is poorly understood. Following translocation by host fishes during parasitic feeding in the Great lakes, sea lamprey must find the coast and enter a river to spawn. Three-dimensional paths of 22 captured and released spawning-phase sea lamprey were quantified from the commencement of movement from a settled state until exiting an acoustic array with 3 km² of coverage, centered 3.3 km from the western coast of Lake Huron in greater than 33 m of water. 81 % of individuals arrived at the nearest coast within 72 hours, suggesting efficient orientation to the coast termed y-axis orientation. Two phases of movement were documented for most individuals: i) in phase 1, sea lamprey moved slowly on the bottom and turned in a consistent direction and made vertical ascents to the surface and returned to the bottom; and, ii) in phase 2, sea lamprey moved more quickly and steered a straight course while making occasional heading corrections and pirouettes and moved primarily at the surface, with occasional descents to the bottom. During phase 2, sea lamprey moved in the direction of the bathymetric slope towards shallower water rather than towards the nearest coast, and no apparent effect was observed due to where the sea lamprey had previously entered a river in Michigan. We postulate that these sea lamprey used the absolute hydrostatic pressure sensed at successive sites along the lake bottom to pick a heading towards shallower water during Phase 1 and used declining hydrostatic pressure at the lake bottom to steer towards shallower water during Phase 2. In contrast to a natal homing migration,

the non-homing sea lamprey migration is consistent with a growing number of observations of organisms that orient to a region of resource availability with a simple set of movement rules based on topographic features of the environment.

INTRODUCTION

Efficient navigation to a distant target requires that a searching animal must first orient itself to environmental features (referents) that reveal the direction to the target, undertake directed movements in accordance with the referent(s) (Able 1991, Åkesson and Hedenström 2007, Luschi 2013), and recognize the target upon arrival (Dittman and Quinn 1996, Walker et al. 2002). Animals can reach resources via: i) non-oriented search (random), ii) oriented search with information that is independent of the resource but related to a region of high target availability, iii) a cue directly associated to the resource, or iv) based on prior knowledge of the target location (Mueller and Fagan 2008). To understand how animals orient during migration, large scale detailed data on the environment, the movement path and an understanding of the internal state of the animal is necessary (Nathan et al. 2008). Studies of this nature in aquatic environments are rare (Lohmann et al. 2008, Cooke et al. 2013), as underwater positioning technologies and methodologies capable of large-scale coverage with high resolution are just becoming available (Jonsen et al. 2003, Espinoza et al. 2011, Biesinger et al. 2013).

The emerging theory for homing migrations is that migrants switch between navigation methods at different scales but rely predominantly on geomagnetic information to arrive near their natal home before relying on other senses, notably olfaction, to move more specifically to a reproduction site (Lohmann et al. 2008, Putman et al. 2014). A major difference in non-homing oriented search is that individuals can rely on topography to locate a distinct geophysical region

with high resource availability (e.g., Pe'er et al. 2004). Orientation to topographic features is common to many species including hilltopping butterflies, *Melitaea trivia*, that move upslope to locate mates, sand hoppers, *Orchestoidea corniculata*, that rely on slope and other features to maintain perpendicular orientation to the shoreline in a preferred tidal zone (Craig 1973, Scapini et al. 1996), or Monarch butterflies, *Danaus plexippus*, that rely on simple geographic barriers to funnel their migration towards Mexican overwintering sites (Mouritsen et al. 2013). Geophysical features that inform an organism of its position are commonplace, though they may be masked by direct cues or not useful at all in a homing migration due to the specificity of their target. Specifically, many aquatic organisms orient themselves perpendicularly to the coast in a special case of orientation termed y-axis orientation (Ferguson 1971). Y-axis orientation has been observed in many species including multiple amphibians that rely on celestial features to orient to shore (Ferguson et al. 1965, Landreth and Ferguson 1967) or green turtles, *Chelonia mydas*, that use windblown odors to orient to a specific beach (Luschi et al. 2001). Over large distances in large bodies of water, moving opposite a gradient such as slope along the lake bottom could theoretically provide directional cues leading to a coast, which could be considered analogous to navigating up a hill.

Orientation in a non-homing species is more difficult to study than in homing species because the animal has greater flexibility to use indirect information to orient that may not be directly associated to a specific target (Benhamou 2006). For this reason, orientation mechanisms have principally been studied in species with prior knowledge of the target, wherein referents can be manipulated to reveal if the change in the referent alters how the animal searches for a specific site (Lohmann et al. 2008, Holland et al. 2010, Luschi 2013). The time required for an animal to orient depends on how long it takes to gather directional information and calibrate an

internal compass. Such a situation often results in an initial period of sinuous exploratory movement (Wendler and Scharstein 1986, Ueda et al. 1998, Pe'er et al. 2004). When searching for distant targets, oriented animals usually move in a straighter fashion. The heading of the straight movement or direction of the final point observed in a study (vanishing point) can be compared with environmental features to determine if there is a consistent pattern and what information the animal is using to orient (Girard et al. 2004, Holland et al. 2010).

The obligation of non-homing adult sea lamprey to efficiently find a coastline and subsequently enter a suitable river to spawn after feeding has terminated makes them an excellent candidate for studying y-axis orientation in fishes. The Great Lakes adult sea lamprey completes a non-homing reproductive migration from lacustrine feeding grounds, where they parasitize large bodied fishes, to riverine spawning habitat (Bergstedt and Seelye 1995, Waldman et al. 2008). The semelparous migration initiates when they release from their final host, which may occur at a location never previously visited, and may represent an uncontrolled displacement (Clemens et al. 2010, Silva et al. 2014). Any coast is a viable target for fishes in a lake (Meckley et al. 2014b), whose target is a river labeled locally by olfactory cues (Waldman et al. 2008, Vrieze et al. 2010). Therefore, sea lamprey could feasibly rely on indirect cues that allow for straight movement in any direction or cues directly associated to the nearest coast. Once arriving at the coast, sea lamprey move parallel to the coastal edge while searching for river water at the lake edge (Vrieze et al. 2011, Meckley et al. 2014b). If sufficient larvae occupy a river, water at the mouth will contain larval odor indicating adequate proven spawning and rearing habitat within the watershed (Teeter 1980, Sorensen et al. 2005, Wagner et al. 2006). Sea lamprey rely heavily on non-visual information during their nocturnal migration limiting potential orientation mechanisms (Binder and McDonald 2007, Keefer et al. 2013). Based on the

life history and sensory capacity of sea lamprey, we postulate that sea lamprey rely on an aspect of the local bathymetric gradient to determine the direction to shore (y-axis orientation).

We investigated the three dimensional path of sea lamprey in Lake Huron by following their release 3.3 km from the nearest coast in 30 m of water to test whether they complete y-axis orientation and return to the coast by following the bathymetric gradient. We specifically evaluated a bundled set of predictions to address our complex hypothesis, (1) sea lamprey will appear at the nearest coastline within 72 hours, indicating that they performed y-axis orientation and approached the nearest coast first; (2) sea lamprey move in a two-phase pattern after displacement consistent with local exploration (high sinuosity) followed by search for a distance target (straight movement), indicating that we observed an orientation process; (3) the cardinal direction of movement during the second phase across individuals is not in a consistent direction, signifying a variable search strategy or use of a locally varying feature; (4) the cardinal direction will be consistent with following the local bathymetric gradient ; and (5) the direction will be based on local information in the array and not on which river the sea lamprey was trapped in Michigan.

METHODS

Ethics Statement

The treatment and acoustic tagging of sea lamprey was approved by the Michigan State University Institutional Animal Use and Care Committee via animal use permit 02/10-020-00.

Study Design

The tracks of 22 sea lamprey were analyzed after their release in the center of an acoustic

positioning array located 3.3 km from the nearest coast (Figure 3.1). The array positioned fish over a region of the coast that had shallower bathymetric contours to the south and west depending on where movement commenced (Figure 3.2). Sea lamprey did not always stop quickly after release so movement could commence at different regions within the array despite release occurring at the center of the array. We bundled predictions to test our hypothesis that sea lamprey rely on an aspect of the bathymetric gradient to determine the direction to shore regardless of the compass direction of the bathymetric slope in front of the river in which they were trapped. To determine if sea lamprey performed y-axis orientation we relied on the first detection of each sea lamprey at acoustic receivers located along the coast (Figure 3.1). To determine if sea lamprey employed a two-phase strategy indicating that we captured the orientation process, we relied on visual inspection and evaluation of track sinuosity. The heading of the sea lamprey from the point it began moving in a straight path until exiting the array was extracted and used to determine if sea lamprey move directly towards the closest coast or follow the local bathymetric contours, and whether there was an effect of past experience, in terms of the river they were caught. Lastly we characterized the vertical movement of sea lamprey to build hypotheses why sea lamprey make excursions through the entire water column and what role it may have in search behavior.

Typically sea lampreys were transported to the field site in aerated coolers and released in the center of the acoustic array at 15:00 when weather permitted (09:00 - 17:30 EST) by being lowered in a release cage at a rate of $6 \text{ m} \cdot \text{min}^{-1}$ that opened at a fixed depth of 30 m when door clamps were triggered by pressure. Three sea lamprey were released at the surface to determine the minimum rate at which lamprey could descend without apparent harm and all reached the

bottom in less than 2 min, equating to a descent rate of faster than $17.5 \text{ m}\cdot\text{min}^{-1}$. The surface-released sea lamprey were not considered in any other test.

Sea lamprey came from six different sources, located in three different scenarios where the contours from deeper to shallower water (bathymetric slope) occurred in three different compass directions (Figure 3.1). If sea lamprey orient to any aspect of the coast and relied on geomagnetic information from previous experience we would expect these conditions to influence their selected headings. Adult female sea lamprey were obtained from five rivers in Michigan via barrier traps (Manistee River: 44.249981, -86.344531 (N=8, trapped 15-May-2012), Cheboygan River: 45.656202, -84.464478 (N=21, trapped 02-May-2012), Manistique River: 45.945189, -86.247733 (N=21, trapped 05-May-2012), Betsie River: 44.630058, -86.252273 (N=3, trapped 21-May-2012), Ocquoec River: 45.490246, -84.072981 (N=4)), and parasites were caught while attached to fish in the lake (Lake Huron, Hammond Bay (N=10, caught January 03,2012 February 28, 2012)) (Figure 3.1). Individuals from the Manistee and Betsie rivers did not move for several days after release and were dropped from the study. These animals were likely more mature and no longer migratory, as they came from rivers that were warmer. As a result, we compared only the Manistique and Cheboygan River sea lamprey to directly evaluate experience, in terms of the river in which they were trapped.

Study Site

The center of the study site (45.527799, -84.044466) was in Lake Huron and located 3.3 km from the nearest shoreline in an area of the lake where bathymetry varied only in two directions (Figure 3.1). A telemetry system (VPS, VEMCO, Halifax, NS) composed of 43 receivers (VR2W) in diamond formations with between receiver spacing of 275 m and an

internal area of 3 km² recorded sea lamprey position (Figure 3.2; see Meckley et al. 2014a). Approval for temporary placement of equipment was provided by the United States Coast Guard (Permit: 16518, Ser. 09-12). Receivers were placed 3 m from the lake bottom in a region of the lake ranging from 30.6 m – 37.2 m deep, although some fish positions were obtained for fish located outside of the array in an area ranging from 21-39 m deep. Nine synchronization transmitters (VEMCO model V16-2H, 69 kHz, 160 db, 500 to 700 s transmission interval) were collocated with receivers to maintain time synchronization of the receiver clocks. In addition to the VPS array, individual receivers provided detection data along multiple locations on the coast and in the Black Mallard, Ocqueoc and, St. Mary's Rivers as part of the Great Lakes Acoustic Telemetry Observation System (GLATOS, Figure 3.1). Detections on these receivers informed us of whether sea lamprey reached the nearest coast prior to other coasts. Solitary receivers were positioned at the Black Mallard River Mouth (BM, 45.532888, -84.120801) and Ocqueoc River Mouth (OCQ, 45.491893, -84.071879). Receiver strings were treated as individual detection points and were located near the Cheboygan River mouth (CHB, 45.67239, -84.429368, N=5), 40 mile point (FMP, Outer receiver: 45.507563, -83.901379, N=3), Presque Isle (PRS, Outer receiver: 45.333842, -83.458343, N=3), and Detour Pass, which leads to the St. Mary's River (SMR, West side of Drummond Island, centered at: 45.984929, -83.891787, N=6).

Detection data revealed sea lamprey presence and was used to determine the fate of sea lamprey on the coast. Data were obtained from shared receivers as part of GLATOS. The detection range of a tag with 150 db of power placed in 5 m of water will vary and is based on line of sight to a receiver, and the environment. A 2-D range test in Hammond Bay under calm conditions revealed a 95% detection efficiency at 155 m and a 23% detection at 1.1 km. Detections exceeding 1.5 km and passing the nearest neighbor filter were unlikely. The detection

range of a receiver placed in < 3 m of water directly in front of the Ocqueoc and Black Mallard rivers, as was done in this study, was likely lower than observed in the range test, especially because the receiver was located in the wave zone. A nearest neighbor filter of 30 minutes was used to remove spurious detections and rare detections near the maximum extent from the GLATOS detection data.

Bathymetry in the detection region of the array was measured along transects spaced 50 m apart and oriented northwest to southeast, and then repeated crisscrossing the first grid at an orientation of southwest to northeast. A Lowrance depth sounder (HDS-8) streamed depth and GPS location to a laptop to record bottom depth during calm conditions and was matched to more accurate post processed GPS positions collected by a Trimble GeoXH and Tornado antennae. Total water column depths at fish positions were interpolated in program R from the georeferenced depth data using inverse distance weighting (function “interp”, package akima) (Akima 1978, Gebhardt et al. 2013).

Tagging Procedure

All sea lamprey were held in 150 L flow-through tanks that cycled ambient Lake Huron water (100% exchange every 2 h) under a natural light cycle. Two types of acoustic transmitters transmitted signals every 15-45 s, though one contained a pressure sensor (n=49, model V9P-2H, Vemco, Halifax, Nova Scotia, Canada 9mm D x 47 mm L, mass: 6.4 g in air, 3.5 g in water, power output 150 dB (re 1 μ Pa at 1 m)) and the other only provided horizontal position (n= 18, model V9-2H, Vemco, Halifax, Nova Scotia, Canada, 9mm diameter, 29 mm length: mass: 3.6 g in air, 2.2 g in water, power output 151 dB (re 1 μ Pa at 1 m)). Sea lamprey were 273-577 mm long (mean 499 mm) and weighed 139-398 g (mean 274 g). Acoustic tagging procedures

followed Meckley et al. (2014a, b). Prior to surgery, sea lamprey were anesthetized by immersion in $0.2 \text{ mL} \cdot \text{L}^{-1}$ clove oil solution. The anesthetic solution was produced through dilution of 2 mL of clove oil (minimum 84%-88% eugenol, Lot No. HB9387, Hilltech Canada Inc. Vankleak Hill. Ontario, Canada) into 18 mL of 70% ethanol and vigorously mixed into 10 L of Lake Huron water. Sea lamprey were removed from the bath upon reaching stage four of anesthesia, determined by individuals that did not respond to handling but retained gill movement (mean time to stage $\pm 1 \text{ SE}$, $559 \pm 14.15 \text{ s}$, maximum 846 s). The surgery was performed in a PVC pipe with continuous water flow that allowed gill irrigation to be maintained by completely submerging the head and gills. We inserted the transmitter into the peritoneal cavity through a 20 mm incision approximately 10 mm off the ventral midline that ended in line with the anterior insertion of the first dorsal fin. The incision was closed with three independent interrupted surgeon knots (3-0 Ethicon sterile monocril monofilament) and each knot was sealed with veterinary adhesive (Vetbond, *n*-butyl cyanoacrylate adhesive). The surgical procedure took an average of $283 \pm 4 \text{ s}$ to complete. Each subject was monitored in a postoperative holding tank until we observed the animal regain equilibrium and begin natural swimming movements (recovery time, mean $\pm 1 \text{ SE}$, $360 \pm 28 \text{ s}$, maximum 1240 s). Transmitter-implanted lamprey were held for 72 h prior to release to ensure metabolism of stress compounds (Close et al. 2003).

Data quality assessment and position filtering

The position accuracy of the VPS array was tested by comparing the VPS position estimates to GPS measured positions (Trimble Geo XH, post processed) of two transmitters at fixed locations (Fixed test 1: June 17, 2014 – June 26, 2014; Fixed test 2: June 17, 2014 – July 01, 2014) and two transmitters pulled through the array (Drag 1: June 13, 2014; Drag 2: June

17,2014) (V9P-2H transmitter). To avoid the effects of positioning error on path sinuosity, a data quality objective was set to attain 95 % confidence that reversals did not erroneously occur in the data. VEMCO positioning systems provide a position precision estimate for each position, horizontal positioning error (HPE), if evaluated the HPE can be used to remove erroneous positions (Smith 2013, Meckley et al. 2014a, Roy et al. 2014). The step length was 22 m in calm wave conditions, which equated to an objective of 95% confidence in an error less than 11 m. We estimated the twice the distance root mean square error (2DRMS) equations for each fixed tag and calculated maximum HPE values that would allow 95 % confidence in 11 m of error. We selected an HPE filter of 15.17 (Appendix 1).

To classify sea lamprey positions as active or stopped, a first passage-time classification method was performed with the program R. The first passage-time tool classified a position as moving if it left a radius of 10 meters in 250 s, had a minimum displacement of 15 m in a 3 position moving average, and at least 3 consecutive moving observations (function “fpt”, package adehabitatLT) (Calenge 2006, Gurarie et al. 2015). The assignment accuracy was verified for each sea lamprey through visual inspection and was robust to imprecision in acoustic positioning largely because we did not observe position error of greater than 10 m for three consecutive positions.

Sea lamprey were assigned to one of 5 categories based on post-release behavior: (A) those that stopped in the array and exited the array on the first night (n=22); (B) those that stopped at the edge of the array and began on the first night but few active points were captured because they were near the edge of the detection range (n=5); (C) those that stopped in the array and exited on the second night or later (n=10); (D) those that immediately abandoned the array on the bottom after release (n=8); and (E) those that immediately abandoned the array after

release but moved throughout the water column (n=22). Only those in the predefined group A, that stopped before leaving the array on the first night were evaluated for orientation behavior and vertical movement patterns although we compared groups A and E in terms of their use of the water column when moving vertically at day and night as there was an apparent daytime avoidance of movement in the upper area of the water column. Sea lamprey are nocturnal and the internal state of the subjects that exited the array during the day immediately after release cannot be reliably interpreted as searching for the coast or searching for cover (Almeida et al. 2002, Vrieze et al. 2011, Meckley et al. 2014b). Only considering fish that followed a progression of settlement and inactivity during the day and activity at night protects our findings from adverse tagging effects commonly seen immediately after release (Frank et al. 2009).

Statistical analyses

(1) Do Sea lamprey perform y-axis orientation?

To determine if sea lamprey approached the nearest southern coastline, we analyzed detection of tags at receivers on the coast within 72 hours of exiting the array. This timeframe ensured that sea lamprey could not have reached a northern coast prior to reaching the nearest southern coast. Lamprey observed on the southern coast, were classified as successfully orienting; the null expectation was that absent an orientation mechanism the probability of success would be 50%.

(2) Are there two phases of movement?

To determine if a transition occurred between two phases of movement and whether trajectory parameters differed between the postulated phases, we evaluated the paths of 22 sea

lamprey from the commencement of movement from a settled state until exiting the region of VPS array coverage. To classify the two phases of: i) apparent initial undirected (sinuous movement), followed by ii) directed movement (extensive straight movement), we analyzed the sinuosity of the path in terms of the progression of the backward beeline distance to total backward path length at each position along the track to the vanishing point for each sea lamprey (Bovet and Benhamou 1988, Girard et al. 2004). A broken stick model was fit to each path and optimized across four parameters including initial slope (β_1), break point (τ), sigma (σ), and final slope (β_2), using maximum likelihood estimation with function “optim” in program R (R Development Core Team 2015). In the model backward path length (x) was the explanatory variable for estimating the response variable backward beeline distance (y). The y intercept (β_0) was fixed at 0 due to the nature of the parameter always returning to (0, 0). Confidence intervals were estimated from the Hessian of the log-likelihood. The model used:

$$y = \hat{y} + \varepsilon; \quad \varepsilon \sim \text{Normal}(0, \sigma^2)$$

$$\hat{y} = \beta_1 x_1 + \beta_2 x_2 + \varepsilon$$

$$x_1 = \begin{cases} \beta(x), & x \leq \tau \\ \tau, & x > \tau \end{cases}$$

$$x_2 = \begin{cases} 0, & x \leq \tau \\ (x - \tau), & x > \tau \end{cases}$$

When $x \leq \tau$ the model is linear with slope β_1 and when $x > \tau$ the model linear with slope β_2 and intercept constrained by continuity to the first part of the stick. In the special case of $\beta_1 = \beta_2$ the model is a simple linear relationship between x and y (White et al. 2008). The initial break point parameter provided was estimated through visual inspection of each of the 22 tracks. Individual pairwise t-tests were used to determine if trajectory parameters that are not directly associated with track sinuosity (e.g. ground speed, turning bias, average depth, and variation in

depth) varied between phase one (before break point) and phase two (after break point). We predict that if a distinct exploratory phase occurs, the initial behavior will be accompanied by slower more sinuous movement followed by straighter faster movement.

(3) Is there evidence for orientation towards a consistent cardinal direction?

We tested if there was a significant sample mean direction with a Raleigh test based on an unspecified mean (function “*raleigh.test*”, R package *circular*) or a significant non-uniform distribution via a Watson’s test (function “*watson.test*”, R package *circular*) for the portion of tracks between the transition point and departure point from the array (Agostinelli and Lund 2013). These tests are similar in nature and answer the same question by either using a z-test statistic (Raleigh test) or goodness of fit to a circular uniform distribution (Watson’s test). If sea lamprey orient to a consistent broadly available feature such as visual recognition of the closest coast, we predicted sea lamprey would have a significantly clustered mean direction and a non-uniform distribution. If sea lamprey were not orienting, oriented to multiple cues at different times, or oriented to a locally varying feature such as bathymetry in our array, we predicted sea lamprey would not have a significant mean heading or a uniform distribution in any compass direction.

(4) Do sea lamprey orient directly to a coastal feature or the bathymetric gradient?

To evaluate if sea lamprey orient to a feature of the closest coast or bathymetry, we evaluated the heading of each individual during phase 2, defined as the portion of tracks between the transition point and departure point from the array. We tested if sea lamprey moved towards the closest coast (coast test) or towards shallower water (bathymetry test). For the bathymetry

test, the bearing of the local bathymetric slope was first calculated from the bearing of the deepest to shallowest depth point on a 1000 point ring of positions in a 100 m radius around the final point to capture the general slope for each sea lamprey leading to the exit. After all bathymetric headings were verified visually on a map, the heading was subtracted from the phase two headings for each individual so that a bathymetry test heading of near 0° (or 360°) indicated that the sea lamprey was moving in the ideal bathymetric direction and 180° represented moving the opposite direction. If sea lamprey oriented in the direction of the local bathymetric slope towards shallower water we predicted the Rayleigh test of the turn angle between fish heading and shallower slope heading with a specified mean turn angle of 0 would be significant and a Raleigh test of the fish heading with a specified mean of 172° for the direction to shallower water would be insignificant.

(5) Did the river the sea lamprey was trapped in influence orientation?

To determine if the lamprey's capture river influenced orientation (experience), the distribution of Phase 2 headings between river sources was compared using a Watson-Wheeler test ("Watson.wheeler.test", R package circular) and the differences in the distance traveled during Phase 1 were considered (two sample t-test) (Agostinelli and Lund 2013). We compared the sea lamprey trapped in the Manistique River and Cheboygan River. If the river they were trapped in had an effect, we would expect sea lamprey migrants from the Cheboygan River to reach the nearest coast after a shorter orientation process, and have a different distribution of directed headings. Although the Watson Wheeler test only tests for a difference between groups, our expectation was that the phase 2 heading would be more southerly towards the local coast,

than the Manistique source subjects. If a sea lamprey relied on local information to orient, we predicted no effect of river source on orientation.

Characterizing the process: Do lamprey show consistent vertical excursions?

To characterize the vertical movements of sea lamprey through the water column beyond average depth and standard deviation of depth, vertical occupancy was characterized for each step (two consecutive points) into one of three classes of vertical movement (ascent, descent, or horizontal phases) based on pitch (descent $< -5^\circ$, horizontal $\geq -5^\circ$ and $\leq 5^\circ$, ascent $> 5^\circ$). Because sea lamprey move from surface to bottom during each phase, we compared vertical movement with the time spent moving horizontally within each sinuous phase, the depth of sea lamprey when moving horizontally in each phase, and the number of casts, defined as the number of times sea lamprey ascended and descended at least 10 m in succession. These variables were used to assess whether the vertical movements represented constant and consistent oscillations or primarily horizontal movements on the surface or bottom with intermittent movements to either the surface or bottom.

Individual pairwise t-tests for each sea lamprey were checked for differences in depth between Phase 1 and 2 during horizontal movements (horizontal $\geq -5^\circ$ and $\leq 5^\circ$). To test for differences in elapsed time spent moving horizontally in the water column during phases 1 and 2 and if a difference existed in the rate of vertical displacement or pitch during ascent and descent, we used a logistic regression analysis. A single response variable including time moving horizontally(s), pitch angle ($^\circ$), or vertical displacement rate (m/s)), respectively. Each was fit using a generalized linear mixed effects modeling framework. For testing for differences in time spent moving horizontally, the movement phase was a fixed effect, individual sea lamprey

(identification number) was a random effect and we weighted the model by the number of observations. For testing displacement rates and pitch during ascent and descent, ascent and descent were fixed effects, while individual sea lamprey was a random effect. The explanatory variables, pitch and vertical movement rate, were considered positive values, regardless of whether the sea lamprey was ascending or descending in the water column. Finally the shallowest depth reached for sea lamprey that showed regular vertical movements during the day versus at night were tested with a simple two way t-test. This was the only test where data for sea lamprey that did not stop immediately after release were used. All analyses were performed in R (R Development Core Team 2015), including the lme4 package (Bates et al. 2014) for fitting the mixed effects model.

RESULTS

(1) Do Sea lamprey perform y-axis orientation?

Evidence suggested that sea lamprey perform y-axis orientation, although only 22 of 67 released sea lamprey stopped in the array after release and left on the first night (Group A). An additional five individuals stopped at the edge of the array where only intermittent positions were attained and nocturnal activity was not recorded (Group B). Prior to stopping at the edge of the array, two fish made vertical excursions of greater than 10 m prior to stopping, and one fish moved laterally on the bottom; the remaining individual did not have depth data. Of 10 fish that did not exit the array on the first night, six were from the eight total Manistee River fish (Group C). Sea lamprey that did not stop included eight (Group D) individuals that exited the array on the bottom and 22 that showed vertical movements of at least 10 m off of the bottom (Group E). Of the 27 sea lamprey (Group A and Group B), 21 (78%) arrived at the nearest coastline within 72 hours and most that were detected at the nearest two receivers were detected on the first night

(12 of 15) (Table 3.1). If considering all tagged lamprey, 43 of the 67 (61%) sea lamprey were detected on the nearest coast (Table 3.1).

(2) Are there two phases of movement?

We observed a post-settlement orientation pattern consistent with initial sinuous movements (Phase 1) followed by straight movements (Phase 2) for 21 of the 22 sea lamprey (Figure 3.3, Figure 3.2). The remaining individual immediately departed in an apparent straight line without an exploratory phase (T26, Figure 3.2). Phase 1 persisted for an average track length of $808 \text{ m} \pm 178 \text{ m}$ (mean \pm 1SE) or an interquartile range of 409-837 m (1st IQR-3rd IQR). Phase 2 lasted $975 \text{ m} \pm 110 \text{ m}$ with an Interquartile range of 625-1311 m. Several aspects of the sea lamprey movement varied between the classified phases, including persistence in turning in one direction, amount of turning, mean depth, variation in depth, and ground speed (Table 3.2, Table 3.3). During Phase 1, 10 of the 21 total individuals turned with a left-bias while four individuals were right-biased and the magnitude of persistence in turning of biased individuals was greater before than after the transition (Table 3.2, Figure 3.4). Sea lamprey turned less during Phase 2 though this observation could be confounded by the fact that the break point was defined in terms of the path sinuosity (Table 3.2). Mean depth was shallower after the break point for 14 of 16 individuals and the standard deviation in depth was significantly greater for five of 16 individuals vs. significantly reduced for two of 16 individuals, who appeared to swim near the surface without surface to bottom casting in Phase 2. Lastly, ground speed was significantly faster for 18 of 21 individuals during phase two when compared to phase one (Table 3.4).

(3) Is there evidence for orientation towards a consistent cardinal direction?

During Phase 2, sea lamprey assumed an average absolute heading of $239^{\circ} \pm 96^{\circ}$ (mean \pm 2STD, 95 % CI: 201-276 deg.; Figure 3.5). Sea lamprey did not show an absolute orientation in any cardinal direction ($r = 0.24$, $p = 0.26$; Rayleigh Test, unspecified mean) and their circular distribution was not significantly different from random (test statistic = 0.091, critical value at a significance of 0.05 = 0.187; Watson's Test) (Figure 3.6).

(4) Do sea lamprey orient directly to a coastal feature or the bathymetric gradient?

No evidence indicated that sea lamprey oriented towards the nearest coast ($r = 0.11$, $p = 0.22$; Rayleigh Test, specified mean: 173 deg.). By contrast, Phase 2 headings (mean: 353 deg., 95% CI: 307-49 deg., were not randomly distributed with respect to bathymetry (Watson's Test: test statistic= 2.244, critical value= 0.187, reject Null) and were significantly oriented to local bathymetry in the region where they exited the array ($r = 0.32$, $p = 0.017$; Rayleigh Test, specified mean: 0°).

(5) Did the river from which the sea lamprey was trapped influence orientation?

The Watson-Wheeler test of homogeneity of angles found no significant difference between sea lamprey trapped in different places, although the strength of this conclusion is limited because of the small sample size ($N < 10$) for each group ($W = 0.76$, $p = 0.69$). No significant difference occurred between the distances traveled prior to the break point for Cheboygan River fish (mean \pm 1SE, 972 m \pm 207 m) or Manistique River fish (mean \pm 1SE, 976 m \pm 244 m) ($p = 0.99$).

Characterizing the process: Do lamprey show consistent vertical excursions?

Sea lamprey moved vertically throughout the entire water column (e.g., Figure 3.7). On average, they made seven ascents or descents of at least 10 meters (6.7 ± 3.2 dives per h Phase 1, 7.3 ± 4.5 dives per h Phase 2). Sea lamprey maintained a straight course (no turns $>15^\circ$) for >500 m during the day, at night, and while moving at the surface, moving on the bottom, and while ascending and descending through the water column. Sea lamprey ascended at a pitch of 15.0 ± 7.8 and vertical speed of $5.1 \pm 2.8 \text{ m}\cdot\text{min}^{-1}$ during Phase 1 and a pitch of $13.0 \pm 6.8^\circ$ and vertical speed of $5.6 \pm 2.5 \text{ m}\cdot\text{min}^{-1}$ during Phase 2. Sea lamprey descended at a pitch of $14.9.0 \pm 8.0^\circ$ and vertical speed of $7.1 \pm 7.9 \text{ m}\cdot\text{min}^{-1}$ during phase one and a pitch of $14.9 \pm 7.7^\circ$ and vertical speed of $8.3 \pm 5.2 \text{ m}\cdot\text{min}^{-1}$ during phase two. During Phase 1, sea lamprey primarily moved on the bottom and made occasional vertical excursions to the surface (Figure 3.8). During Phase 2, sea lamprey made more vertical excursions and spent more time at the surface than the bottom (Table 3.4). Mixed effects logistic regression modeling revealed that sea lamprey spent significantly less time moving horizontally during Phase 2 than Phase 1 (estimate: -0.42 , $p < 0.01$), and more time moving vertically through the water column. Mixed effects logistic regression revealed that no difference occurred in the pitch of sea lamprey during ascent or descent ($p = 0.08$), but a difference did exist in the vertical rate of ascent and descent ($p < 0.001$). A two way t-test revealed that vertical excursions during the day did not extend as close to the surface as at night, rarely entering the upper 10 meters of the water column during the day ($p < 0.01$; mean minimum depth \pm SD; Day: $10.4 \text{ m} \pm 5.4\text{m}$, Night: $2.6 \text{ m} \pm 3.6 \text{ m}$).

DISCUSSION

The offshore movement of sea lamprey was consistent with y-axis orientation to the local bathymetric gradient towards shallower water; we will refer to this class of search as

bathokinesis. We addressed this complex hypothesis through a bundle of predictions. (1) The majority of sea lamprey (78%) arrived at the nearest coast following release. (2) Sea lamprey (21/22) displayed a two-phase pattern that varied in sinuosity consistent with orientation (Phase 1) followed by directed search (Phase 2). During Phase 1, sea lamprey moved slowly on the bottom and turned, often in a consistent direction. These tracks included vertical ascents to the surface and returns to the bottom, building from partial to full excursions through the water column. During Phase 2, sea lamprey moved more quickly and steered a straight course while making occasional heading corrections and small looping turns (pirouettes), and moved primarily at the surface, with occasional descents to the bottom that returned to the surface. (3) Sea lamprey did not orient in a consistent cardinal direction, signifying a variable search strategy or use of a varying local feature. (4) Sea lamprey did move perpendicular to the local bathymetric contours to shallower water. (5) The orientation process and resulting heading was not different for sea lamprey trapped in different rivers. In contrast to a natal homing migration, the non-homing sea lamprey migration joins a growing number of organisms that orient to a region of resource availability with a simple set movement rules.

These findings revealed that sea lamprey oriented consistently with the bathymetric slope, though the findings did not indicate an orientation mechanism or how sea lamprey maintained a straight course. We postulated that these sea lamprey: i) used the absolute hydrostatic pressure sensed at successive sites along the lake bottom to pick a heading towards shallower water during Phase 1, and ii) used declining hydrostatic pressure at the lake bottom to steer towards shallower water during Phase 2. Because sea lamprey moved straight during phase two regardless of their position in the water column, it would be reasonable to infer that sea lamprey rely on additional directional features to maintain course, rather than relying on a

gradient based feature of the environment. We have identified four possible mechanisms for how sea lamprey follow the bathymetric gradient based on the characteristics of the physical water column and the nature of sea lamprey movement.

Sea lamprey could orient to a feature of bathymetry when moving at the bottom such as absolute hydrostatic pressure, a physical aspect of the bottom substrate or bottom pitch, or by moving vertically through the water column and using a vertically collected feature like dynamic hydrostatic pressure or the physical extent (e.g., time or distance) traversed through the water column (Taylor et al. 2010). An internal clock method keeping track of the time or distance between the surface and bottom was not supported as sea lamprey did not maintain pitch and vertical movement speed. Vertical speed was significantly faster when descending and pitch was nearly significant ($p=0.08$) shallower during ascent. Bottom substrate did not contain features like sand waves found in shallower water that would inherently indicate the direction to the coast (Figure 3.9). Fishes have been shown to orient to hydrostatic pressure (Cain 1995, Holbrook and Burt de Perera 2009, Holbrook and Burt de Perera 2013), and can discern differences in absolute or differential hydrostatic pressure using the lateral line, inner ear (labyrinth), and swim bladder (Fraser 2002, Fraser et al. 2008, Bleckmann and Zelick 2009). Sea lamprey lack a swim bladder and have a primitive lateral line and inner ear that lacks a lagena (otolith endorgan); however, sea lamprey possess semicircular canals that are structurally similar to the otolith of other pressure sensitive fishes (Hammond and Whitfield 2006, Fraser et al. 2008, Khorevin 2008), which can detect changes on the order of 0.5-2.0 kPa (5-20 cm) (Blaxter 1980). We postulate that sea lamprey use absolute hydrostatic pressure at the maximum depth to orient towards shallower water through turning and correcting towards shallow water (bathokinesis), and short looping pirouettes during the directed phase to reaffirm their selected heading. The movement

pattern was consistent with kinesis rather than taxis, as initial headings were undirected (appearing random) and turns were made until movement occurred towards shallower water (Miller et al. 2009).

The purpose of excursions to the surface is unclear and introduces two challenges to using hydrostatic pressure to orient; first, sea lamprey must be capable of recalling previous dives and second they must be able to maintain a course when moving off of the bottom. Some fishes are capable of remembering information in three-dimensional space to locate targets (Holbrook and Burt de Perera 2013) and in open-water environments lacking horizontal edges (walls), the vertical component of the water column may be more informative to navigation (Holbrook and Burt de Perera 2009, Holbrook and Burt de Perera 2013). Recalling the maximum hydrostatic pressure would allow for orientation but maintenance of a straight path requires a second directional feature. Two common navigational mechanisms include reliance on geomagnetic fields or current direction to maintain course. Whether sea lamprey are capable of geomagnetic navigation is unknown, though this form of navigation is commonly used by other fishes that make large migrations and should be tested in this basal vertebrate. Water currents in this environment are highly variable vertically and horizontally and usually weak (< 2 cm/s) (Beletsky et al. 1999), making for a potentially poor directional cue in the lake. On the other hand, current is strongest at the surface (Beletsky and Schwab 2001), a potential reason for moving to the surface. Combining this with knowledge that sea lamprey do not vertically cast in deep rivers (16 m depth, Holbrook et al. 2015), but do vertically cast in the presence of river water (i.e., Meckley et al. 2014b), we propose that vertical movement is designed to encounter stronger currents, or navigate absent strong currents.

Animals could make vertical excursions to improve migration performance, to use navigation cues located at the surface and bottom, to search for a missing cue stratified in the water column, or to avoid sensory habituation to a cue (Westerberg 1982, Klimley et al. 2002). We had no evidence for an improvement in migratory performance as the movement increases the total path length and sea lamprey do not spend more time falling than rising as would likely be required to receive an energetic advantage (Carey et al. 1990, Klimley et al. 2002). The occurrence of vertical excursions in a shallow fully mixed water column (Vrieze et al. 2011, Meckley et al. 2014b), suggests against the presence of a thermoregulatory advantage (Katz 2002). Sensory habituation can occur if a receptor is constantly exposed to a stimulus and becomes fatigued resulting in a loss of sensitivity (Ferrari et al. 2010). In theory, vertical excursions in and out of a cue could prevent sensory habituation. Sea lamprey could encounter information that is stratified at the surface or when present would reside at the surface (missing cues). River water can be thermally pinned at the lake surface or bottom of the water column (Masse and Murthy 1990, Churchill et al. 2003), or mixed throughout the water column, which led Vrieze et al. 2011) to conclude that sea lamprey vertically cast to encounter olfactory evidence of a river. This is a plausible explanation, though it is likely that additional navigational benefits are gained by moving vertically (Meckley et al. 2014b), but from our observations we cannot report a dominant purpose.

The sea lamprey migration in the Great Lakes appears to rely on a simple set of rules. Sea lamprey follow the local bathymetry to return to the coast, potentially relying on absolute hydrostatic pressure. Once arriving at the coast, they move parallel to the coast until encountering a river plume, and then transition to deflecting along the local coast around the river mouth to ensure encounter with the river outflow, as they assess or enter the river (Vrieze et

al. 2011, Meckley et al. 2014b). The leading hypothesis is that once encountering a river plume individuals either enter, stage, or search for a different river based on the presence of larval odor (quality) and river temperature (timing)(Binder et al. 2010, Clemens et al. 2010, Meckley et al. 2012). The odor of river water and larvae from previous generations is attractive to sea lamprey and is surmised to inform sea lamprey of the presence of a river mouth and the quality of the spawning and rearing habitat upstream (Moore and Schleen 1980, Bjerselius et al. 2000, Vrieze and Sorensen 2001). Simple orientation rules based on features of the geophysical environment that can reliably lead to a target rich region appear to be a common attribute of non-homing animal search, rather than reliance on direct information associated to a target as is found in homing migrations.

Future Directions

As commonly occurs in novel studies of animal migration, we identified two hypotheses that should be tested further and additional features of the sea lamprey migration worth consideration. First, we postulate that sea lamprey can use gradients of hydrostatic pressure at the bottom of the water column to determine the bathymetric gradient which provides them the direction towards shallower water. Second, we postulate that sea lamprey move to the surface to gather other navigational information and/or to encounter additional cues (e.g. chemical, current) that could be structurally stratified at some point throughout the water column, while also preventing habitation to sensory cues in the environment.

Unexpectedly a larger proportion of sea lamprey in this study did not stop immediately after release and still moved vertically through the water column (33 %), a finding conflicting with two years of study that found in shallow water (<10 m) in both years only 17 % of sea

lamprey didn't stop and the movement was nearly exclusively on the bottom (unpublished data). Though in this study, when sea lamprey did move after release they did not move into the upper 10 meters of the water column during the day but moved all the way to the surface at night. It remains unclear if sea lamprey are more active during daylight hours in deeper water (>10 m). During the open water migration if sea lamprey avoid the upper water column, the strike range of the sea lamprey's most documented predator (avian predators, Close et al. 2002) could be avoided. It is also possible that this observation was just an artifact of being released during the day coupled with an inherent aversion to strong light and less inclination to stop immediately in deeper darker water.

Three other observations of uncertain relevance occurred including the persistent turning that created loops in a consistent direction during phase one, the observation that few fish went east after reaching the coast, and the failure of fish from more southerly rivers to exit the array. The handedness of turning appeared to be dominated by counterclockwise turners, which could be an artifact of our small data set or exist due to an ecological advantage to handedness. Handedness in turning during search has been suggested to improve energetic efficiency (e.g., Kells and Goulson 2001), making the behavior worth additional consideration. Secondly, fish appeared less likely to go east after reaching the coast (Table 3.1). This directionality could be the result of moving opposite the dominant lake current in this region of the lake (Beletsky et al. 1999) or some other bias. The final interesting feature of the study came from the failure of sea lamprey from more southerly rivers to exit the array on the first night. These individuals did leave the array after a period of several days. These individuals were caught farther upstream prior to tagging, and later in the year than the other individuals, and had visibly more developed

gonad. It appeared that these individuals required some type of reset period before they began to migrate out of the array.

Bathokinesis should be examined in ocean-run sea lamprey. Both Pacific lamprey and sea lamprey have been are neither panmictic nor philopatric (Waldman et al. 2008, Spice et al. 2012, Hess et al. 2013), suggesting that some aspect of their life cycle constrains them to a region but also disperses them. Additional evidence of restricted movement exists, as North American and European populations do not mix (Rodriguez-Munoz et al. 2004, Genner et al. 2012), and move broadly throughout the ocean when parasitizing other fishes, in some cases moving over 815 km from the nearest coast (Silva et al. 2014). Additional mechanisms may occur to prevent navigational mistakes that result in the mixing of populations within the Atlantic basin sea lamprey or some control over when a parasite will release from a host to prevent large displacements. If sea lamprey select hosts that migrate back to rivers, it is plausible that sea lamprey could hitch a ride back to rivers in some instances; a behavior termed phoresy, although we suspect this is not the dominant strategy. The only evidence available is consistent with bathokinesis by ocean-run sea lamprey, as parasitic sea lamprey feeding within a general basin were constrained by basin topography during their return migration (Lanca et al. 2014).

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APPENDIX

Table 3.1: Sea lamprey were primarily detected on the nearest coast within 72 hours of release.

This included 78 % of individuals that were observed stopping in the array and moving on the first night (n=22) and 61% of all individuals released (n=67). Note that Time to reach the receiver mean and min/max are only listed for the 22 individuals. Only 4 of 67 sea lamprey's first detections occurred outside of the nocturnal movement period, with 3 of 4 occurring on the first day of release with 2 at Forty Mile point (East), 1 at the Ocquoec river mouth (South) and 1 near Cheboygan river mouth (West).

Receiver/ detection Site	Distance from array (km)	No. of 1 st Detections in 72 hours (N=22, N=67)	Time to reach receiver hours (mean ±SE)	Time to reach receiver hours (min, max)	No. of unique individuals (N=22, N=67)
OCQ	4.3	(9, 17)	(6.9 ± 5.0)	(0.83 - 46.7)	(12, 26)
BM	6.2	(6, 11)	(17.7 ± 10.7)	(1.7 – 69.3)	(9, 17)
CHB	33.8	(2, 9)	(51.8 ± 0.4)	(51.4 - 52.2)	(4, 15)
SMR	51.6	(2, 5)	(141.0 ± 86.3)	(54.7, 227.3)	(2, 5)
FMP	11.1	(0, 4)	-	-	(1, 5)
PRS	48.9	(0, 0)	-	-	(1, 1)

The number of sea lamprey detected on any receiver was 19 of 22 and 46 of 65). Ten of 23 and 19 of 65 sea lamprey entered the Ocquoec River. 1 of 22 and 2 of 65 entered the Black Mallard River. Four of 23 and seven of 67 sea lamprey were trapped in the Ocquoec River and one of 67 was trapped in the Cheboygan River.

Table 3.2: Individual t-tests for each sea lamprey of whether sea lamprey exhibited a persistence in turning (theta; $-\pi$ to π), different from 0, revealed that more sea lamprey had a bias of left (negative) or right (positive) turns during phase 1 (P1), but not during phase 2 (P2). Most persistent turns were counterclockwise. The magnitude is also greater for those that are significant in phase 1 than phase 2. A pairwise t-test of the concentration in turning (Rho) for each individual between phase 1 and phase 2 revealed that individuals tended to go straighter during phase 2. Bold values are significant.

ID	Theta				Concentration of turns (Rho)		
	P1	P1 ρ	P2	P2 ρ	P1	P2	ρ
T02	-0.03	0.488	-0.08	0.04	0.81	0.83	0.61
T04	-0.06	0.494	0.00	1.00	0.70	0.86	0.01
T05	-0.17	0.214	0.01	0.79	0.74	0.86	0.18
T07	-0.81	0.000	-0.21	0.29	0.74	0.65	0.47
T08	0.07	0.689	0.04	0.46	0.48	0.84	<0.01
T12	-0.58	0.000	0.06	0.48	0.61	0.77	0.18
T17	0.14	0.625	-0.10	0.48	-	0.75	0.66
T22	-0.43	0.001	-0.04	0.51	0.54	0.88	<0.01
T25	0.50	0.001	-0.07	0.27	0.59	0.81	0.01
T27	-0.26	0.004	-0.06	0.67	0.64	0.75	0.18
T31	-0.47	0.001	0.14	0.24	0.61	0.73	0.36
T32	0.07	0.792	0.05	0.52	0.63	0.69	0.77
T36	-0.58	0.000	0.01	0.88	0.65	0.75	0.19
T37	0.02	0.878	0.33	0.00	0.67	0.85	0.12
T42	0.14	0.082	-0.14	0.25	0.69	0.78	0.34
T44	-0.28	0.836	0.03	0.61	-0.28	0.79	0.21
T47	0.68	0.028	-0.08	0.30	0.62	0.84	0.14
T54	-0.12	0.107	0.01	0.76	0.72	0.81	0.10
T56	-0.13	0.006	-0.18	0.01	0.69	0.72	0.37
T61	-0.01	0.903	0.06	0.56	0.75	0.73	0.82
T63	-0.20	0.005	-0.07	0.02	0.65	0.86	<0.01
Total ($\rho < 0.05$):		10		4			5
Total Possible:		21		21			21

Table 3.3: Pairwise t-tests to determine if there was a difference in the mean depth, standard deviation in depth, mean depth when

ID	Mean Depth (m)			Standard Deviation Depth (m)			Hz Mean Depth (m)			Ground Speed (m/s)			
	P1	P2	ρ	P1	P2	ρ	P1	P2	ρ	P1	P2	ρ	
T02	32.85	17.98	< 0.01	4.86	9.38	0.00	35.1	14.6	0.05	0.38	0.49	< 0.01	
T04	32.69	15.85	< 0.01	6.60	9.89	0.08	36.8	7.1	< 0.01	0.38	0.48	< 0.01	
T05	29.80	21.59	< 0.01	8.79	11.45	0.38	34.1	20.3	0.16	0.38	0.49	< 0.01	
T07	33.86	23.76	< 0.01	0.15	8.30	0.00	33.9	24.8	0.21	0.33	0.50	< 0.01	
T08	35.07	28.09	< 0.01	0.92	6.84	0.00	35.3	27.5	0.23	0.29	0.51	< 0.01	
T12	21.38	8.04	< 0.01	10.54	6.69	0.14	26.5	4.4	< 0.01	0.33	0.51	< 0.01	
T17	23.52	14.67	0.09	8.42	10.96	0.62	32.0	6.2	< 0.01	0.59	0.69	0.08	
T22	22.72	4.97	< 0.01	12.08	3.62	0.00	24.2	3.8	< 0.01	0.30	0.52	< 0.01	
T25	32.91	5.99	< 0.01	3.90	3.85	0.95	34.4	4.8	< 0.01	0.17	0.30	< 0.01	
T27	23.16	7.51	< 0.01	11.88	6.93	0.03	25.0	3.9	< 0.01	0.35	0.47	< 0.01	
T31	27.31	13.95	< 0.01	6.51	11.37	0.13	25.8	4.7	< 0.01	0.34	0.57	< 0.01	
T32	30.48	11.31	< 0.01	3.44	9.39	0.00	21.5	3.4	0.12	0.19	0.40	< 0.01	
T36	31.98	31.06	0.13	1.79	5.61	0.00	32.1	32.5	0.35	0.36	0.43	< 0.01	
T37	28.93	26.25	0.04	2.90	3.11	0.87	30.2	26.1	0.28	0.40	0.44	0.15	
T42	-	-	-	-	-	-	-	-	-	0.42	0.73	< 0.01	
T44	-	-	-	-	-	-	-	-	-	0.19	0.44	< 0.01	
T47	-	-	-	-	-	-	-	-	-	0.56	0.69	0.08	
T54	-	-	-	-	-	-	-	-	-	0.37	0.60	< 0.01	
T56	-	-	-	-	-	-	-	-	-	0.37	0.52	< 0.01	
T61	15.02	3.84	< 0.01	9.06	2.14	0.06	8.6	2.3	-	0.46	0.51	< 0.01	
T63	26.67	5.94	< 0.01	9.94	7.45	0.28	-	4.5	-	0.27	0.53	< 0.01	
Total Significant ($p < 0.05$):			12				7				7	18	
Total Possible:			16				16				14	21	

Table 3.3: (cont'd)

maintaining vertical depth (Hz Mean Depth), or ground speed during phase 1(P1) and before the transition to phase 2 (P2). The tests support our observations that these two phases are different. Sea lamprey had greater mean depths and less variation during phase 1, and when moving at a particular depth in the water column, sea lamprey spent time on the bottom during phase 1 and closer to the surface during phase 2. The ground speed (GS) was also significantly lower during phase 1 than phase 2. The only individuals with less variation in depth during phase two were those individuals swimming mostly at the surface with occasional vertical excursions. Individuals T42-T56 did not have pressure sensitive tags.

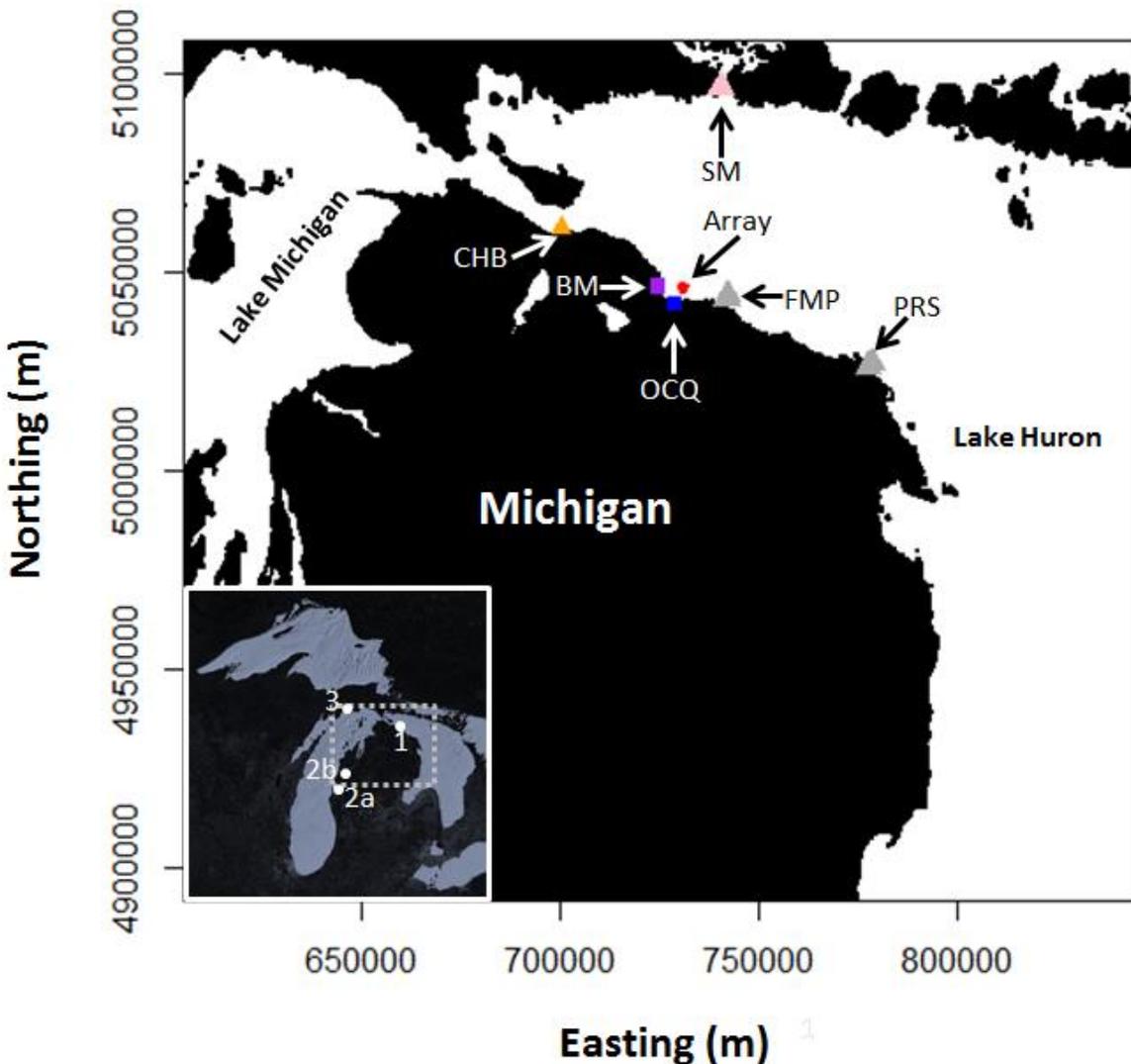


Figure 3.1: Adult female sea lamprey were obtained from 5 rivers via barrier traps (Manistee River (2a), Cheboygan River (1), Manistique River (3), Betsie River (2b), Ocquoec River (“OCQ”), and parasites were caught attached to fish in the lake. A telemetry system (VPS, VEMCO) composed of 41 receivers was used to position sea lamprey (“array”). There were individual receivers that provided detection data along multiple locations on the coast and in front of as well as in the Black Mallard (BM), Ocquoec (OCQ) and, St. Mary’s Rivers (SM). Receiver strings were treated as individual detection points and were located near the Cheboygan River mouth (CHB), 40 mile point (FMP), and Presque Isle (PRS).

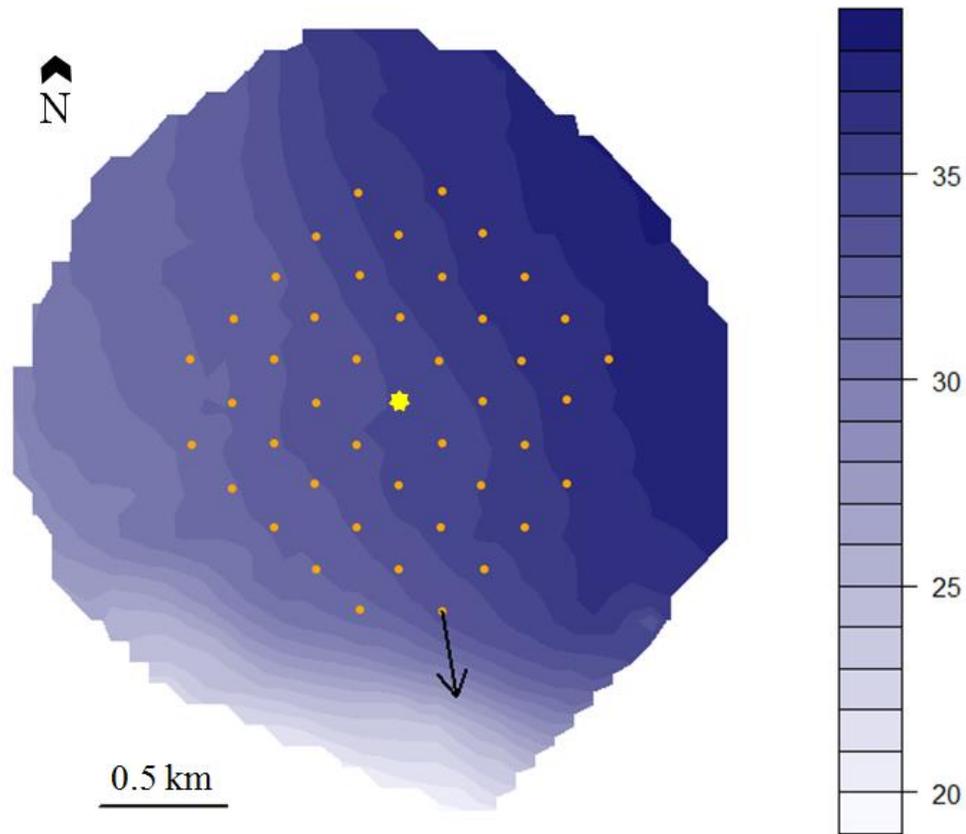


Figure 3.2: Each sea lamprey was released near the bottom in the middle (yellow star) of a 43 receiver array (orange dots) with 3 km² coverage. A receiver in the center of the array at the release point and on the east side of the array were not recovered, resulting in an irregular design. The black arrow demonstrates the direction to the nearest coastline 172° (3 radians).

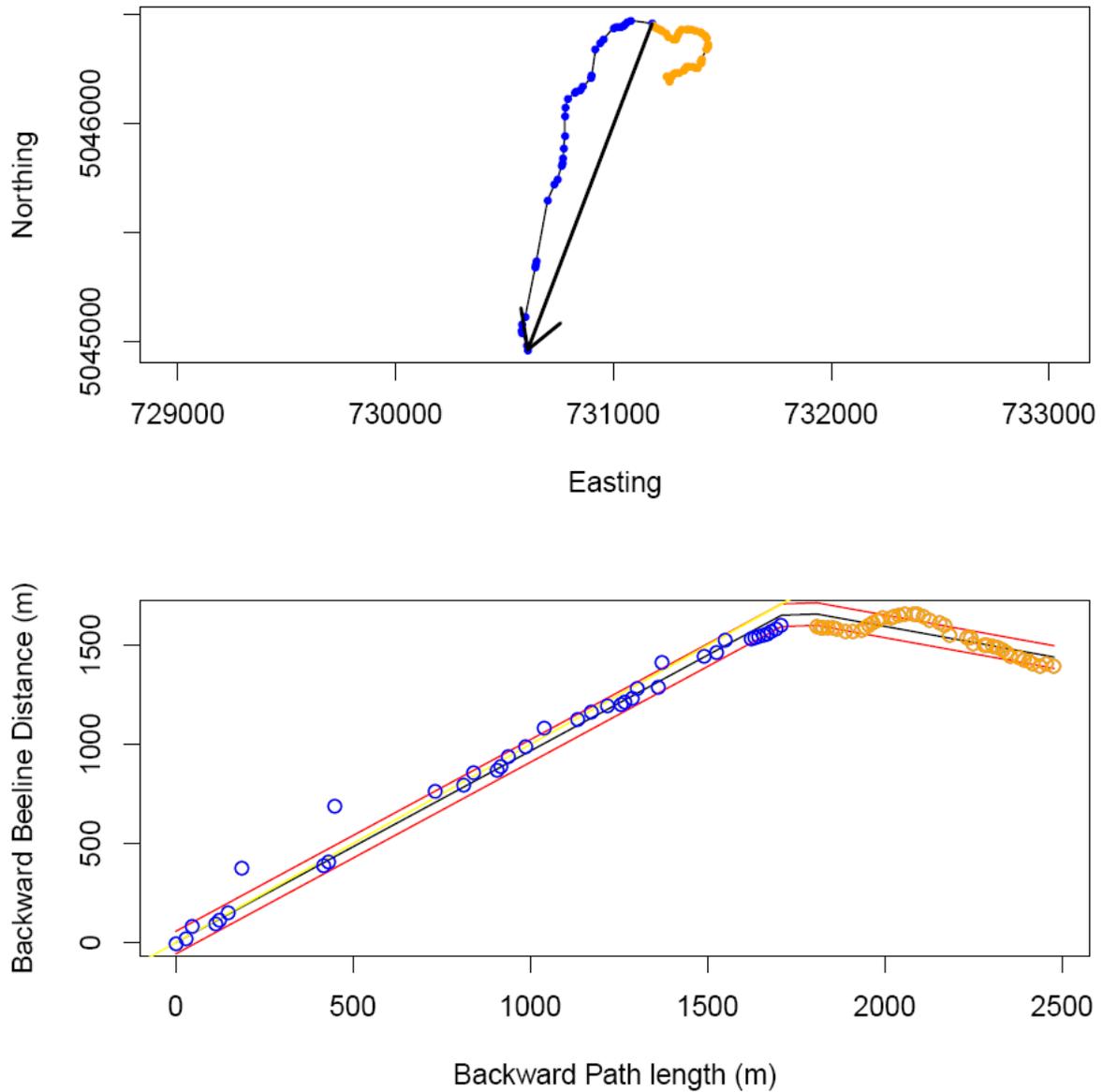


Figure 3.3: The track of sea lamprey T04 is depicted as it exits the array (a), and below is the relationship between backward path length and backward beeline distance of the track (b). An arrow is drawn from the breakpoint to the end of the phase two to demonstrate the direction headed during Phase 2 (a). In the lower graph the yellow line is a one to one relationship from 0. A broken stick model fit is shown with the red lines representing the standard error and the black line is the line fit (b). The break point separates phase one (orange) from phase two (blue). The 22 tracks of Group A are shown in Figure 3.10.

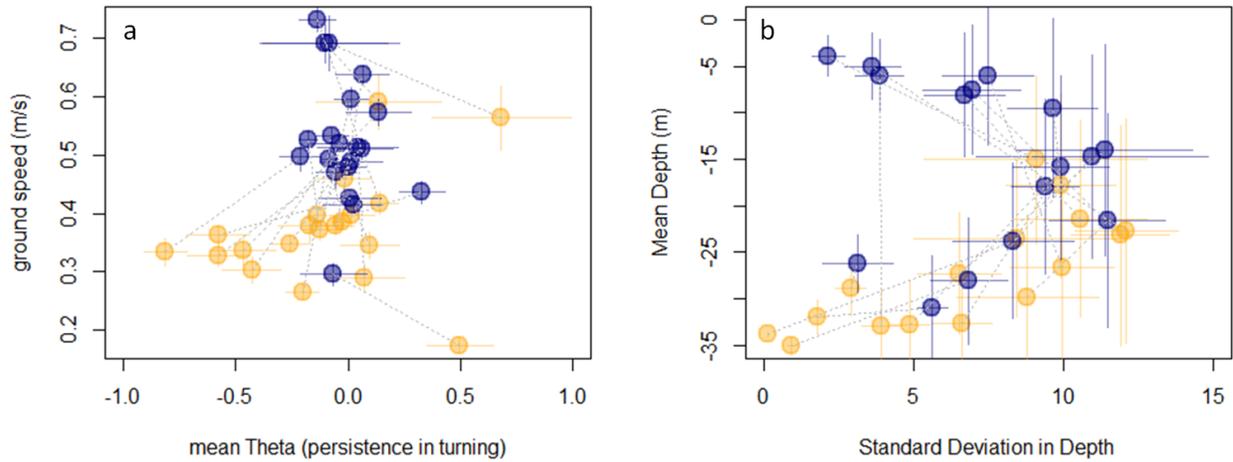


Figure 3.4: Dotted lines connect phase 1 (orange) and phase 2 (blue), data points for each sea lamprey subject, separated by a transition characterized by backward path length versus backward beeline distance sinuosity. Both the mean value is shown (dot) as well as the standard deviation in each axis (+). There is an increase in ground speed and a transition from a turning bias in one direction to no bias in turning direction (a), as turning persistence overlaps 0 during phase 2. Also apparent was a change in the mean depth from mostly movement on the bottom in phase one with brief vertical excursions to the surface followed by movement on the surface during phase two and brief vertical excursions to the bottom (b).

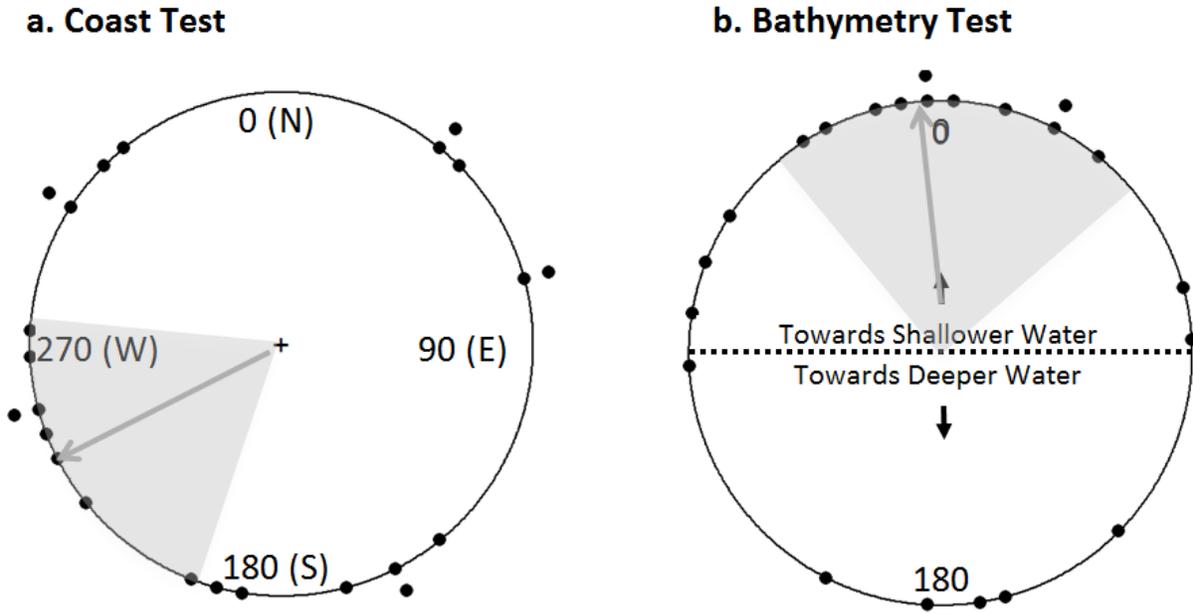


Figure 3.5: The overall heading of 22 sea lamprey during phase two is depicted as a black dot within one of 60 bins separating the circle and shows an average heading of 239° (gray arrow) with a 95% confidence interval of $201\text{-}276^{\circ}$ (shaded area) (a). The heading of sea lamprey with respect to the ideal heading if following local bathymetry at the exit of the array is depicted by the gray arrow with most sea lamprey clustered near 0 (mean= -7 , 95% CI: $307\text{-}49^{\circ}$), representing a movement towards shallower water, while a few individuals were observed moving opposite of the ideal bathymetric contour. The phase two headings were significantly oriented to local bathymetry in the region where they exited the array ($r = 0.32$, $p = 0.017$; Rayleigh Test, specified mean: 0°).

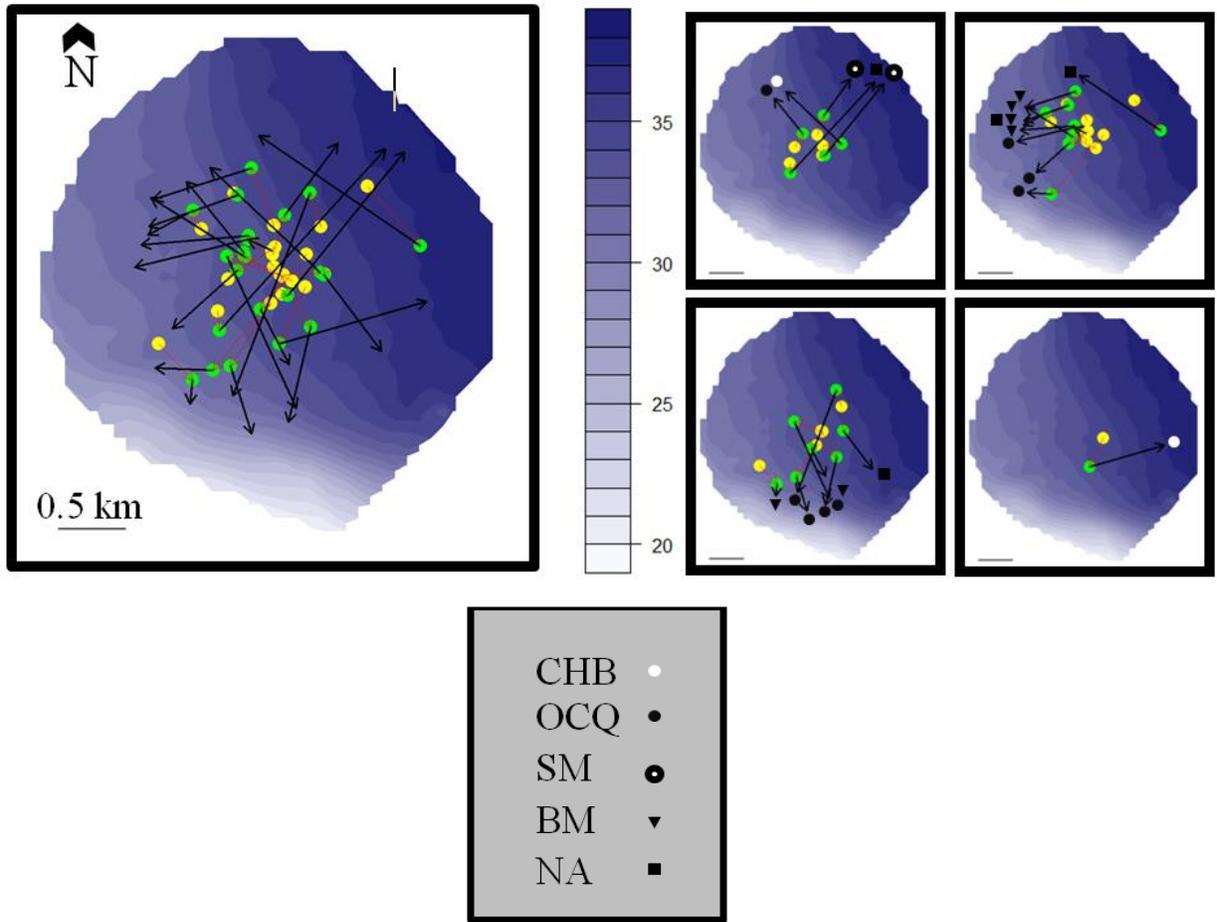


Figure 3.6: Sea lamprey (n=22) settled in the array during the day and began moving at night (yellow), transitioning (green circle) between phase one (red arrow) and phase two (black arrow) before they exited the array. Sea lamprey that moved north (b), east (c), south (d), and west (e) are shown in individual frames with symbols indicating where each individual was first detected on the coast. The array is color coded by depth 20-39(m).

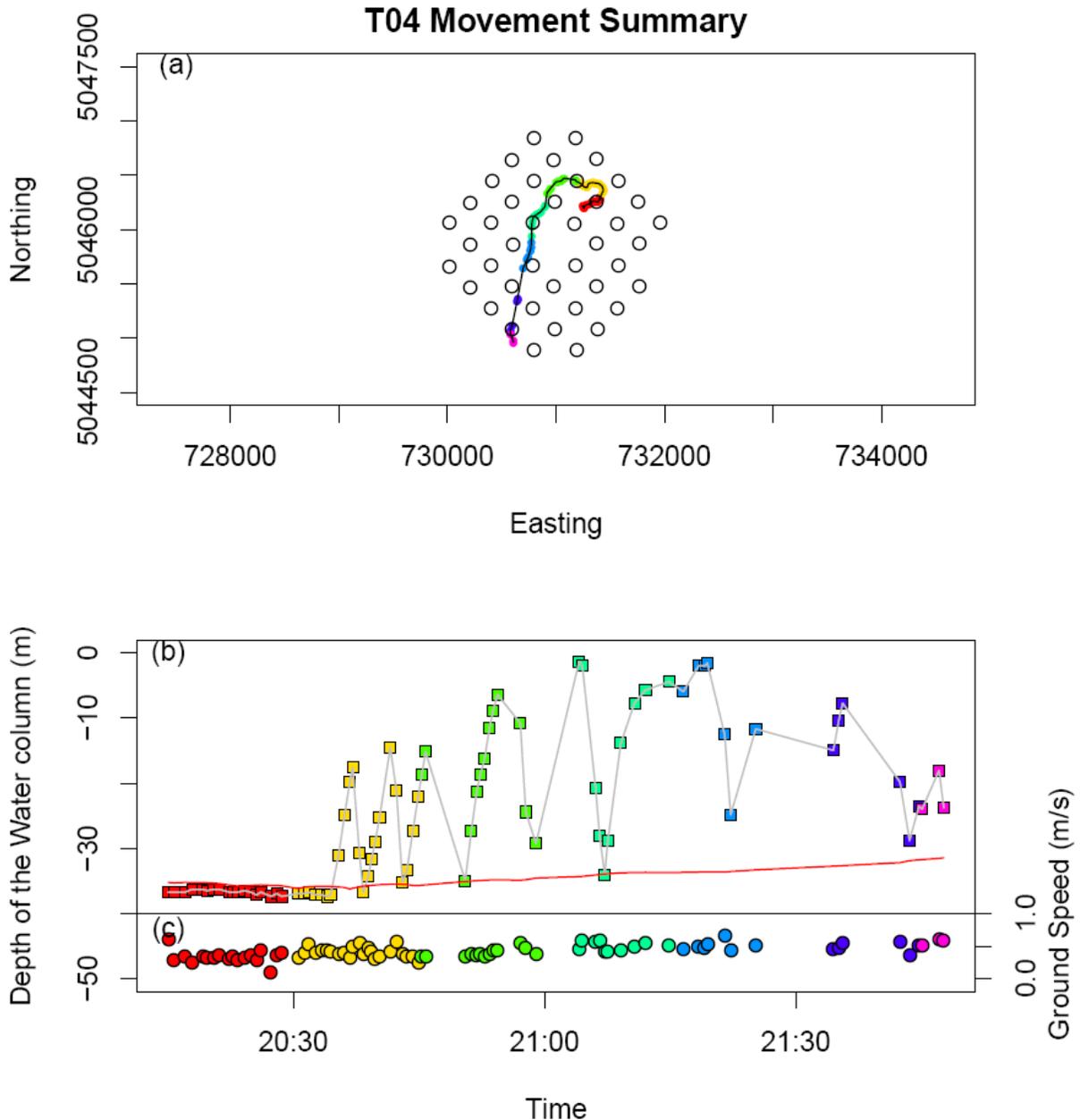


Figure 3.7: The track of sea lamprey T04 is depicted as it exited the array (a), and below is the water column depth (b) and ground speed during the track (c). The graphs are color coded by 15 minute intervals. In inset “a” small circles represent receiver positions and the small colored dots represent fish positions with a line showing the path. In inset “b” the red line represents the total water column depth and the squares show the depth of the fish with respect to the total water column depth through time.. All 22 tracks are shown in Figure 3.10.

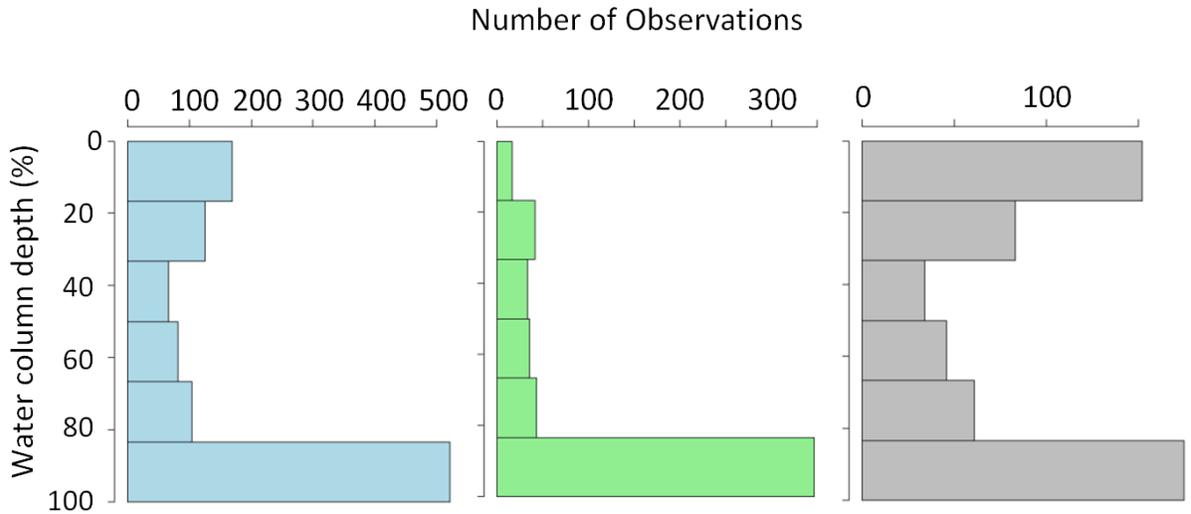


Figure 3.8: Overall most observations occurred near the bottom (All observations; left panel). During phase 1 most sea lamprey moved on the bottom and made occasional excursions to the surface that returned to the bottom (center panel). Phase 2 consisted of more surface movements with occasional excursions to the bottom, although a few individuals did move vertically and only moved on the bottom (right panel).

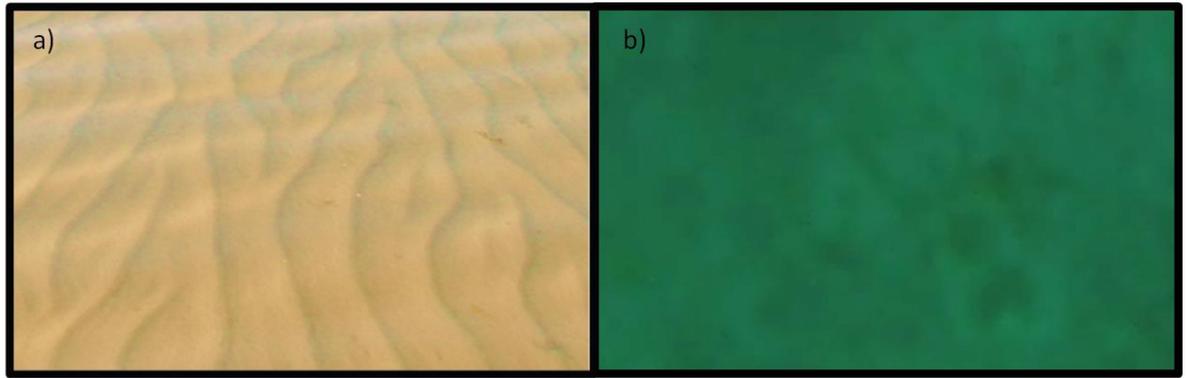


Figure 3.9: Near shore sand waves in shallow water of Lake Huron (a) versus offshore detritus and algae covered bottom without clear features associated to a common direction (b).

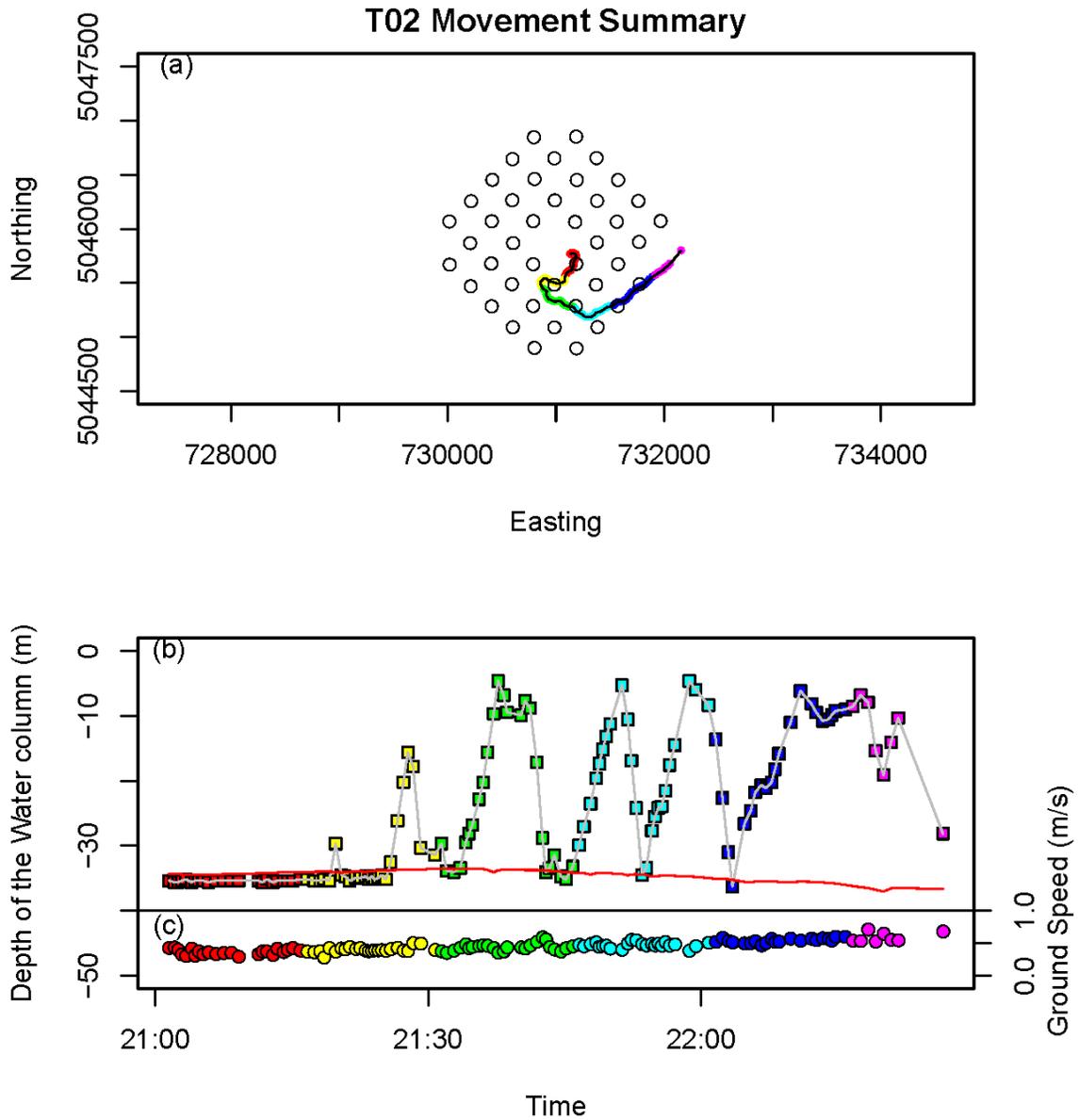


Figure 3.10: There are 2 pages dedicated to each subject and only the data is shown for the points when fish began to move following settlement, with the exception of transmitter 21 that was shown moving immediately after release. The two pages correspond to the pathways of 22 subjects. Page one, “Movement Summary”, depicts the path of each sea lamprey through the array when active (a) and is color coded by 15 minutes intervals corresponding to the lower graph (b,c). Figure 3.10: (cont’d) Time is in Eastern Standard Time (UTC-4) and shown in a 24

Figure 3.10: (cont'd)

hour time scale. The lower graph depicts the vertical movements of sea lamprey for those individuals that had transmitters with pressure sensors corresponding to the left y axis (b) and ground speed corresponding to the right y axis for all individuals (c). The red line is the total water column depth at each fish position. Note only T21 is shown moving during the day as it was notable because it appears to orient during the day and then repeated the process

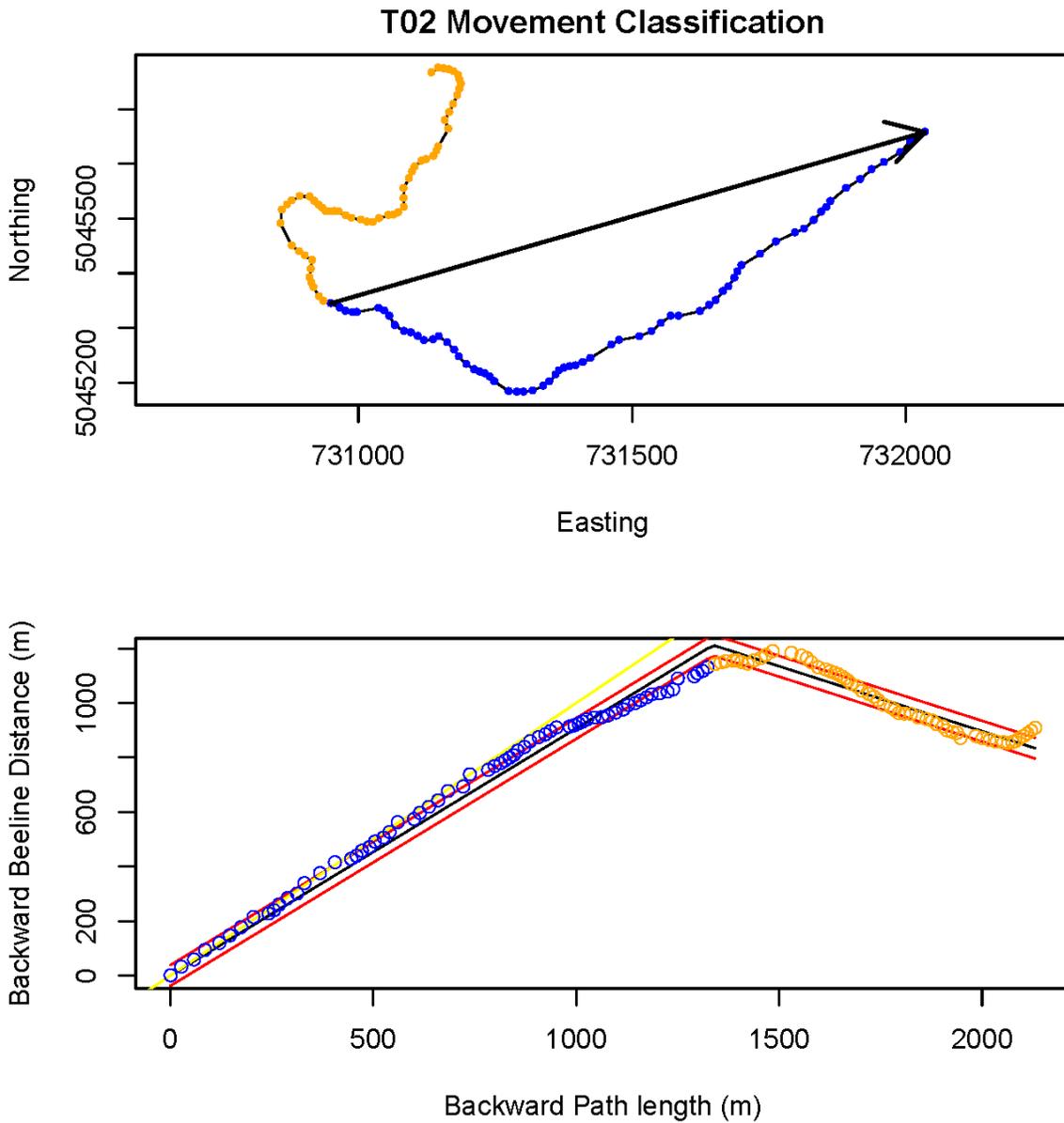


Figure 3.10: (cont'd)

at night but the night time track was too short to consider in the movement classification. This suggests that this is a daily orientation process. [Page 2](#), “Movement Classification”, the lower graph depicts the backward path length versus the backward beeline distance for each track. The point at (0, 0) represents the point that the subject left the array. The yellow line is a one to one

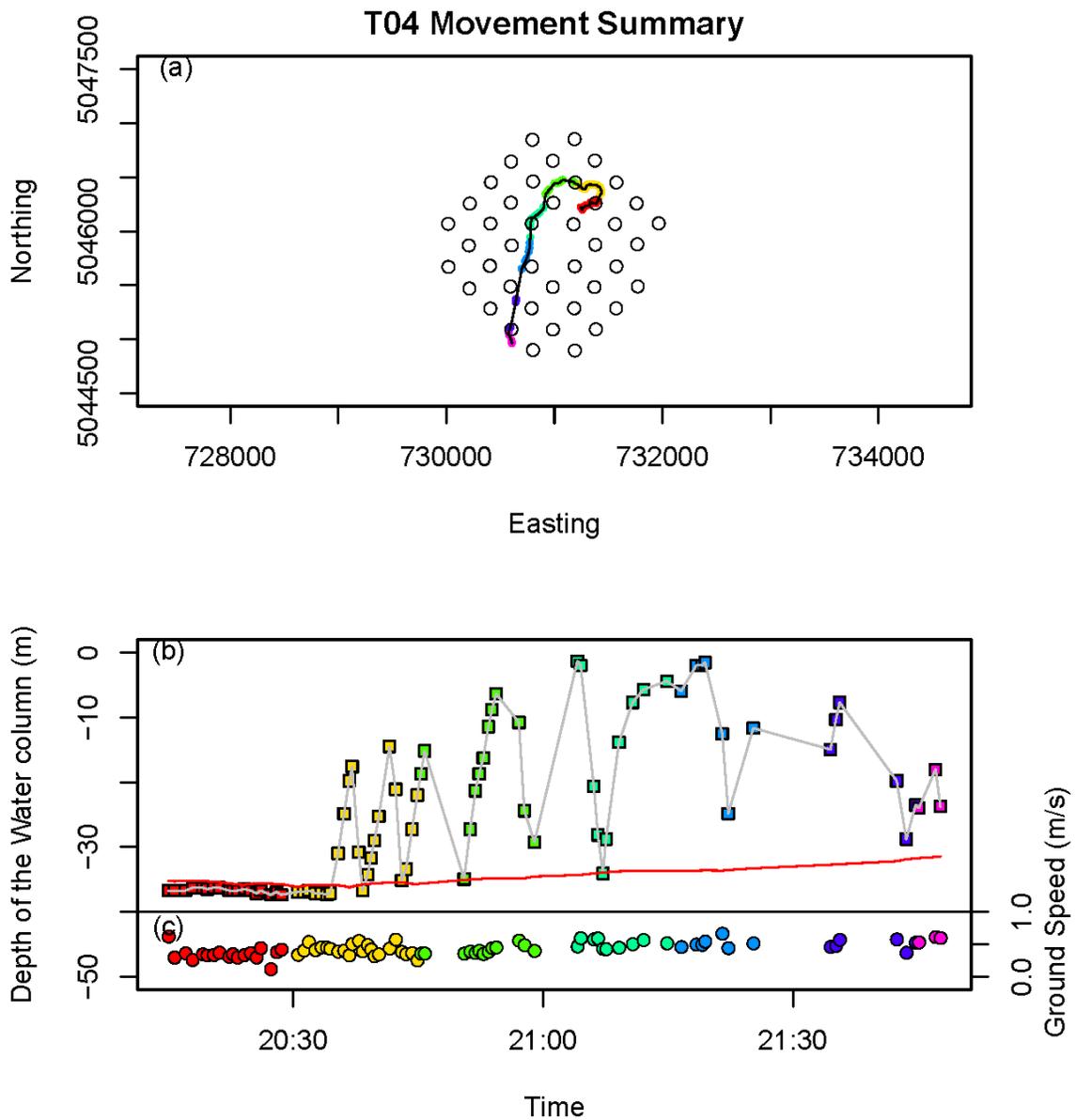


Figure 3.10: (cont'd)

relationship from 0. A perfectly straight path from the exit would move along this yellow line. The broken stick model fit is shown with the black line and the red lines represent the standard error. The break point is the point we use to separate phase one from phase two. Phase one is highlighted in the in orange and phase two is depicted in blue. In the upper graph, the path of each individual is shown and an arrow is drawn from the breakpoint to the end of phase two

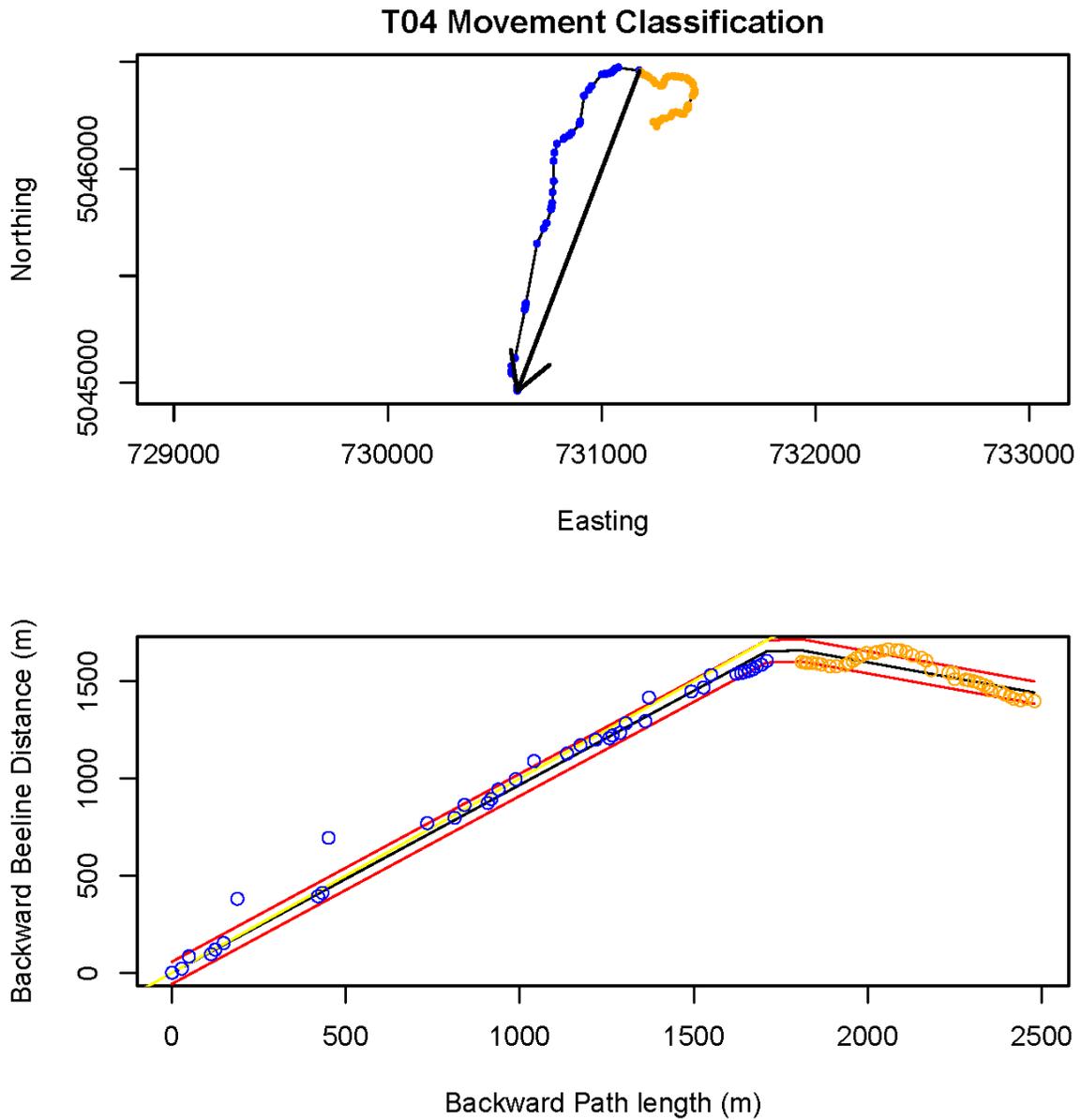


Figure 3.10: (cont'd)

when the fish exits the array coverage to demonstrate the heading of phase two that was used to test against the direction to shallower water and the direction to the nearest coast using Raleigh tests.

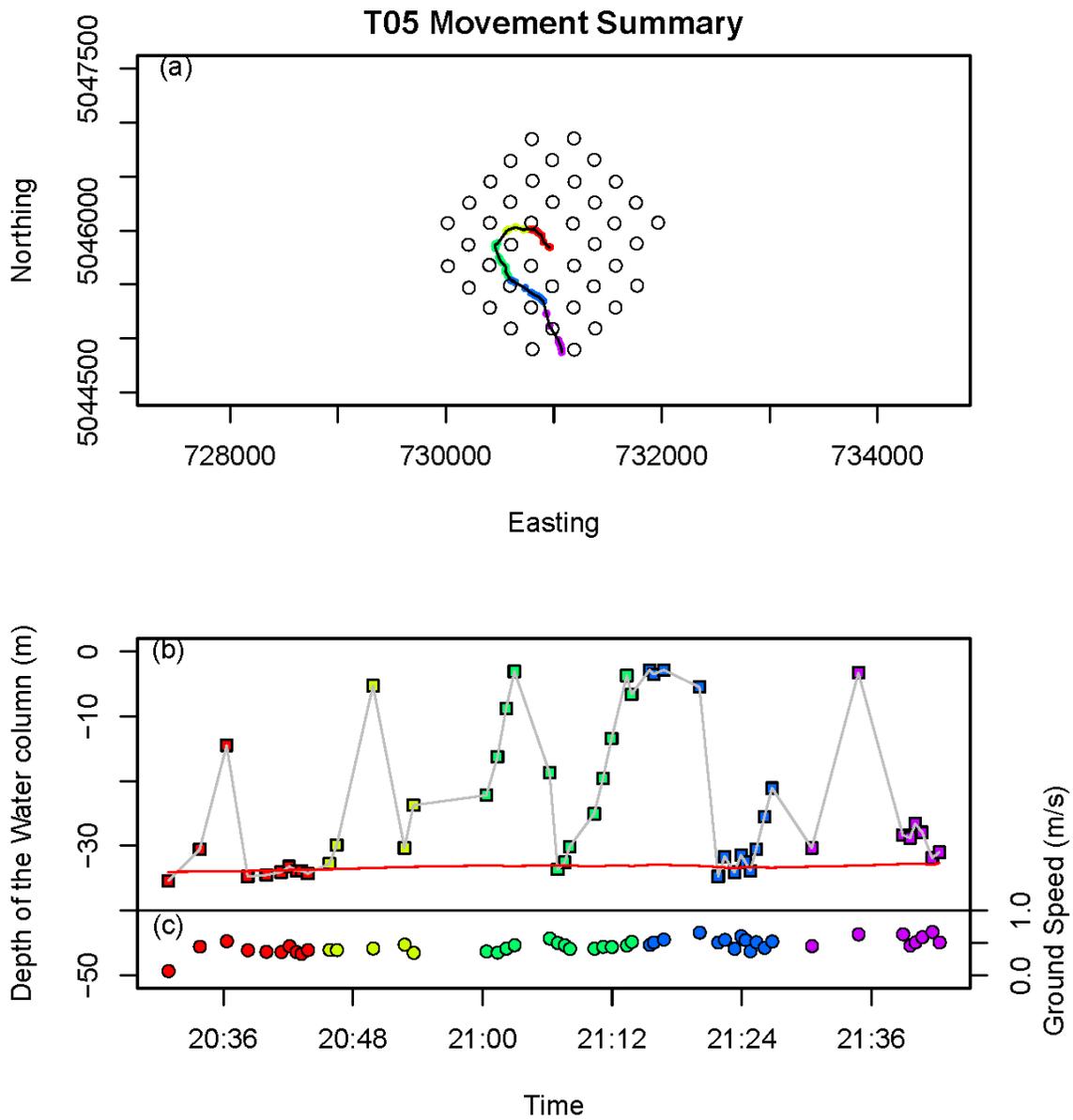


Figure 3.10: (cont'd)

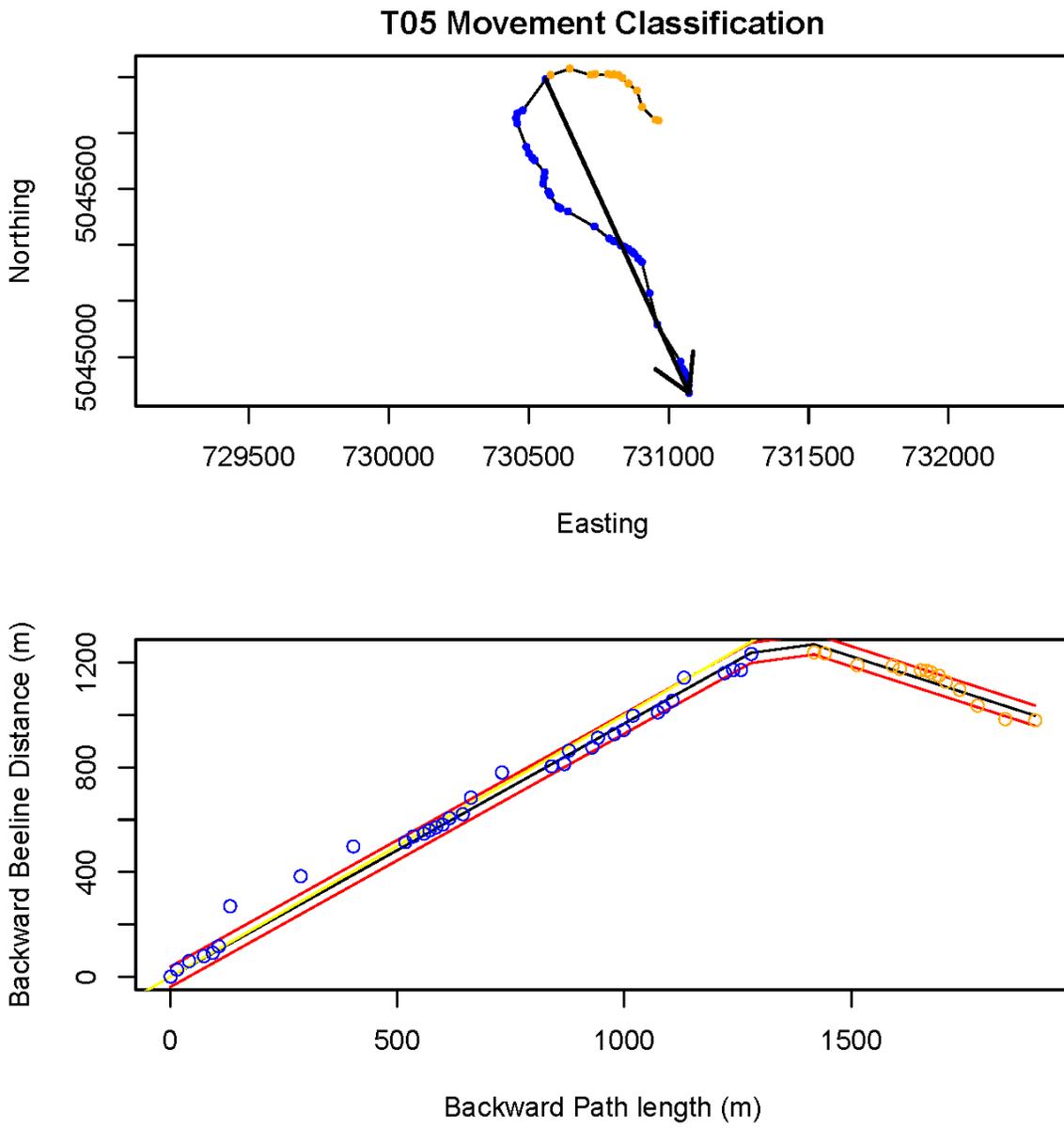


Figure 3.10: (cont'd)

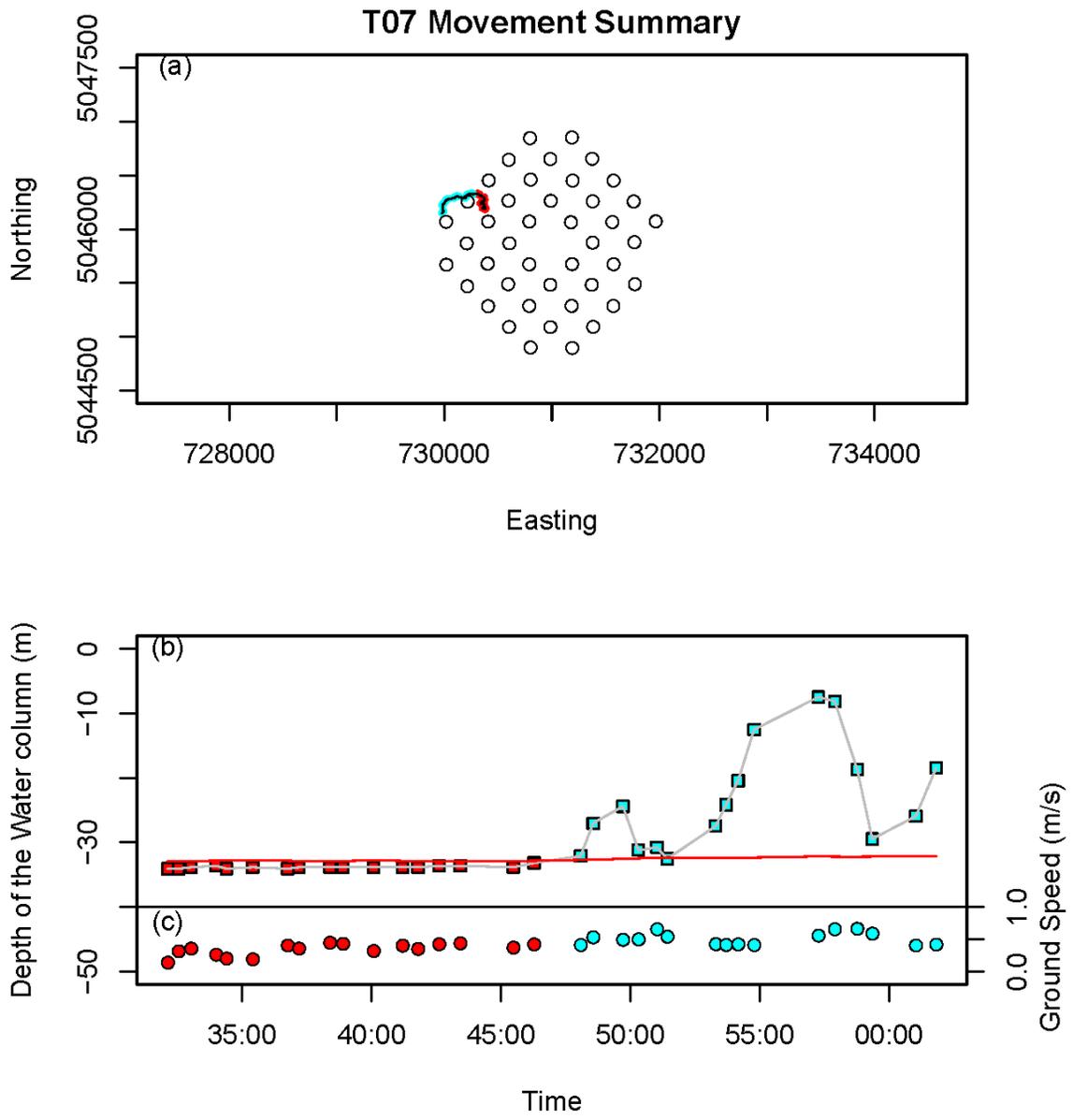


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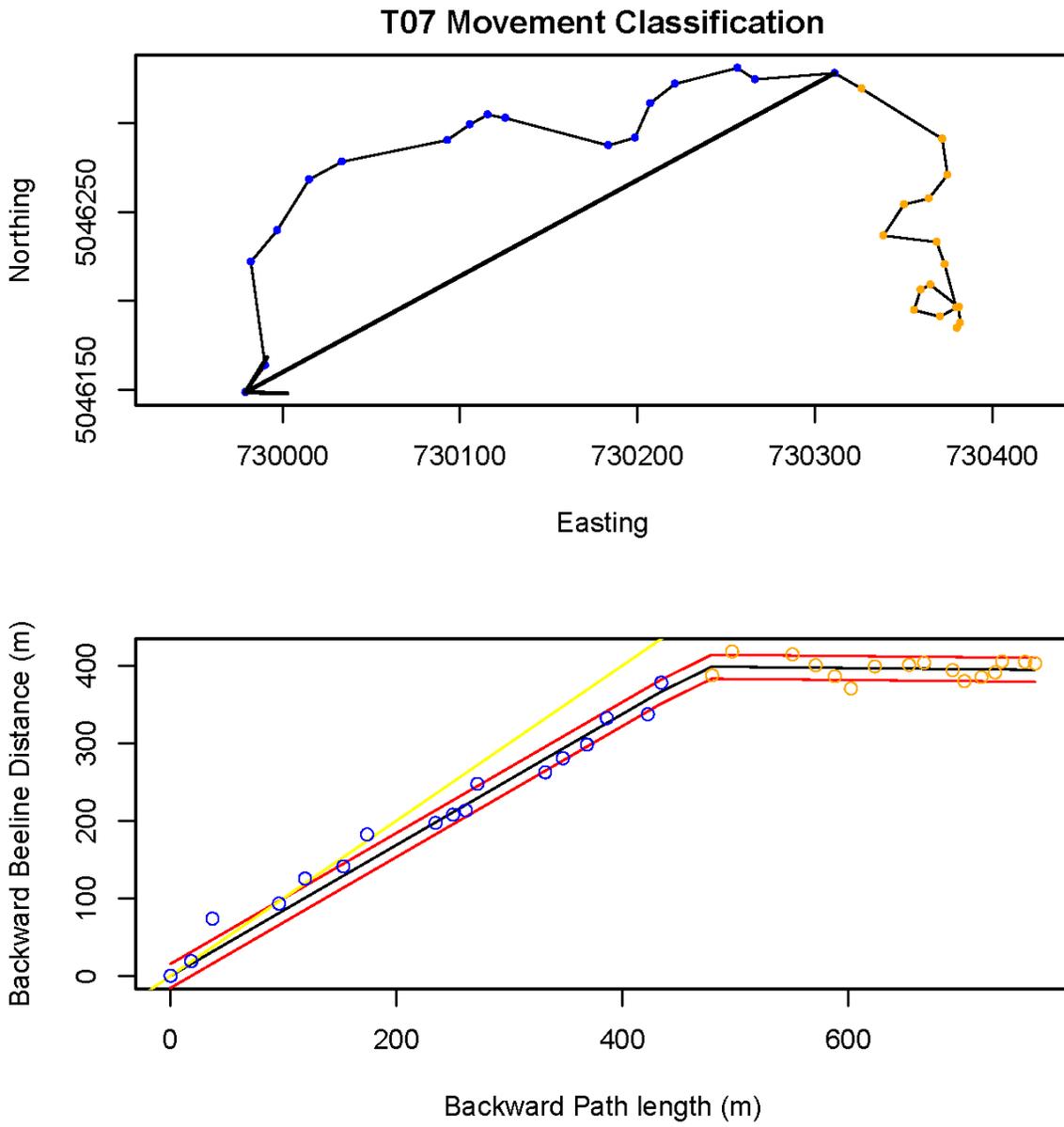


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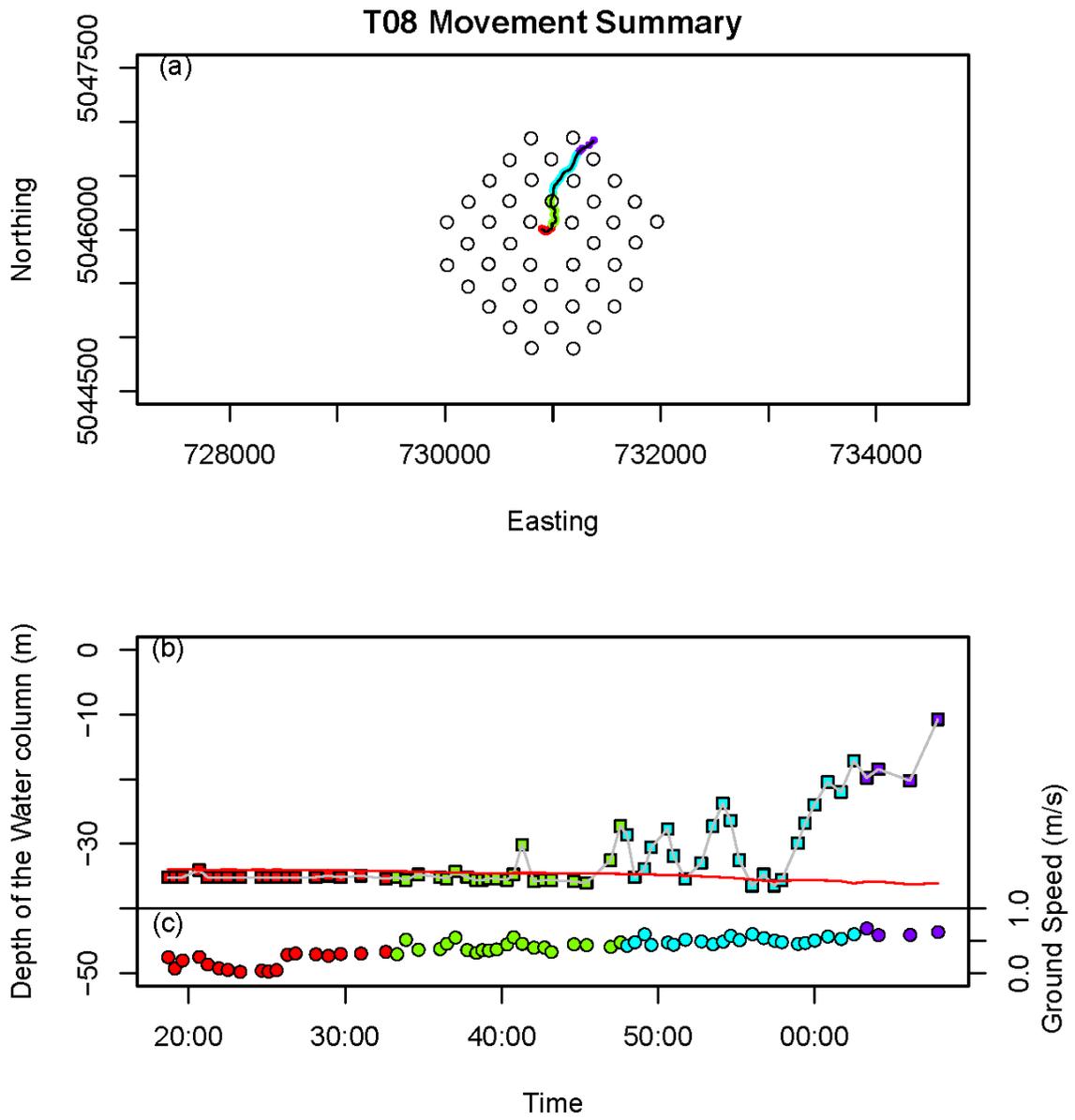


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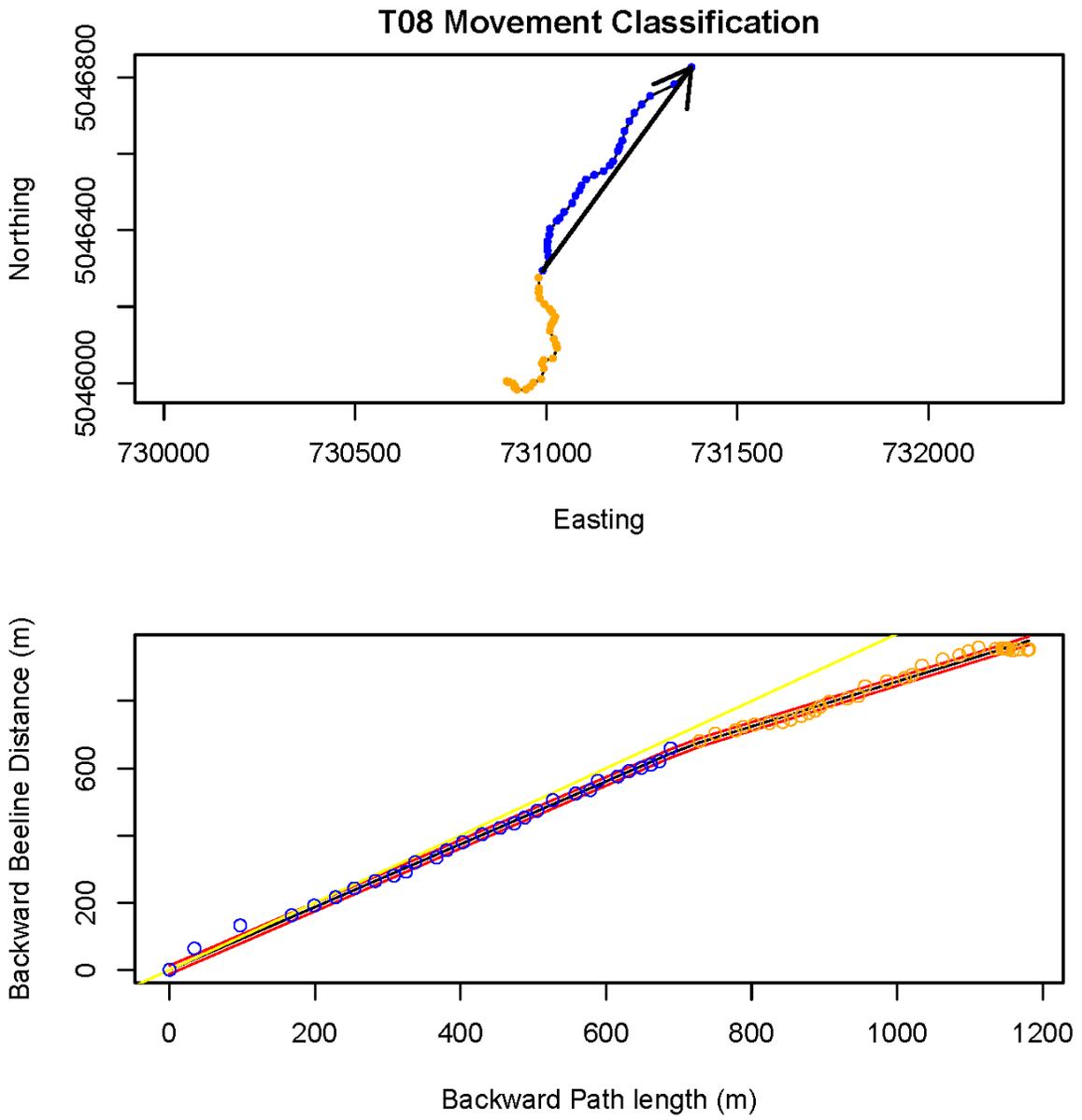


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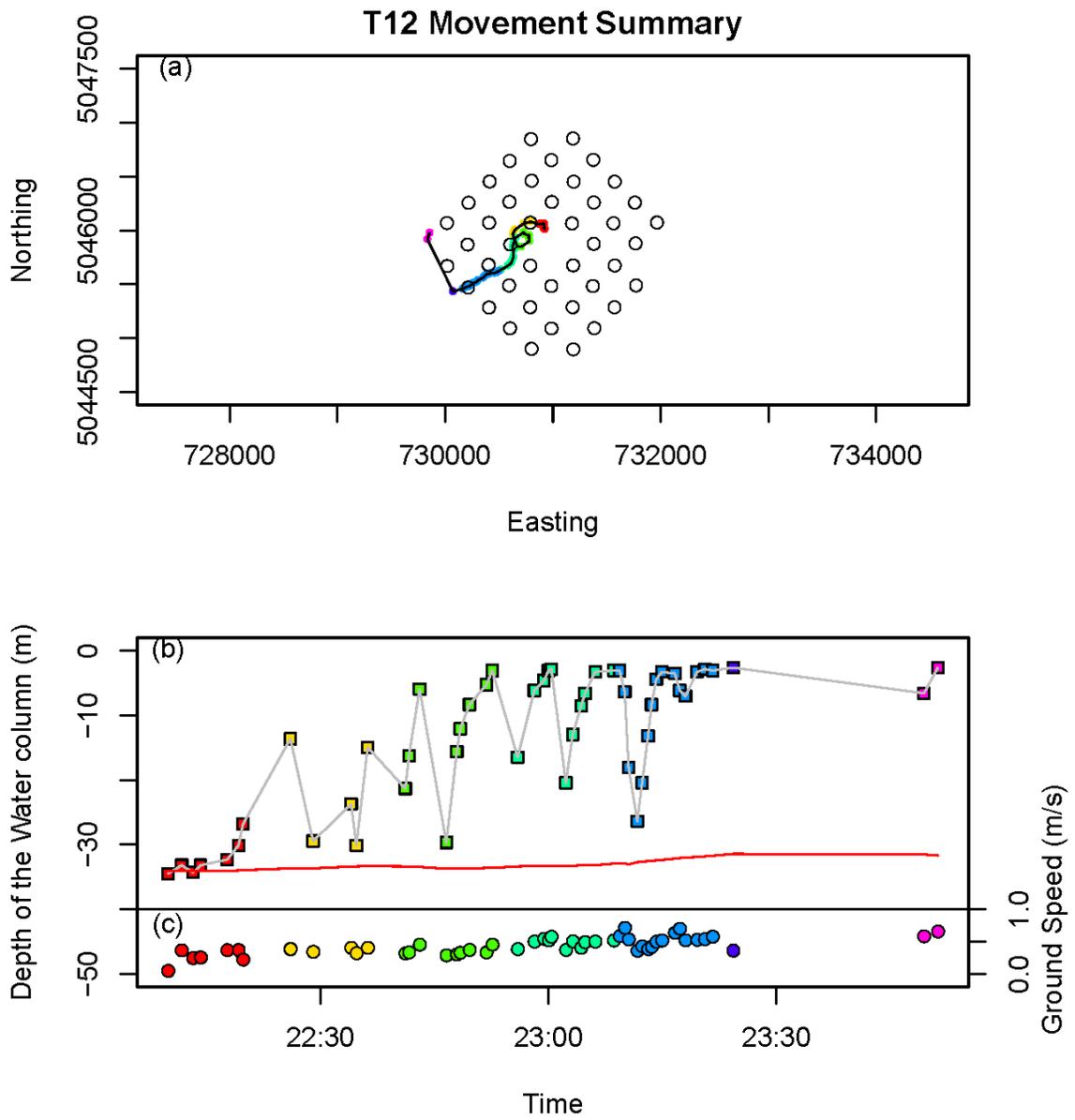


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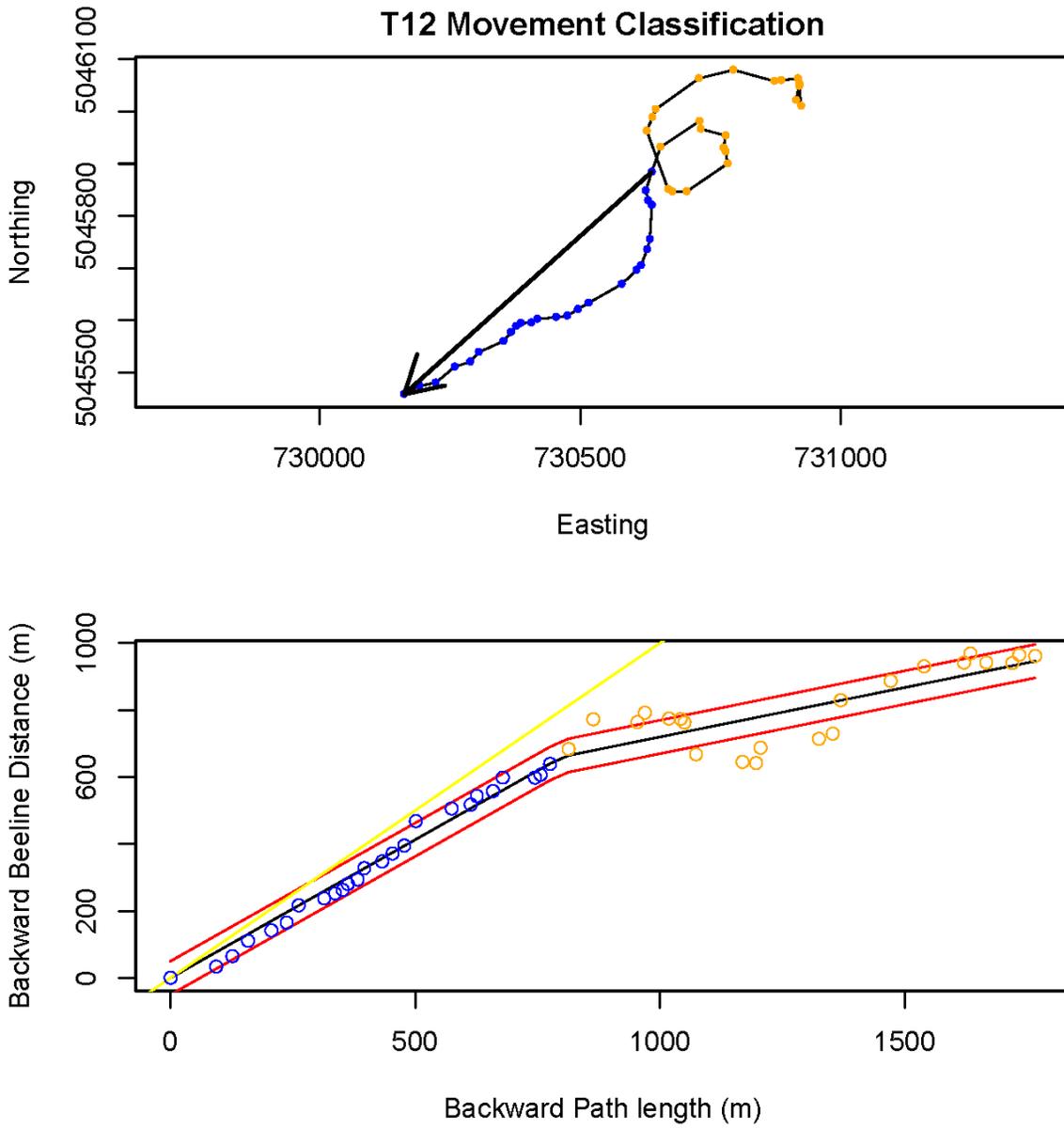


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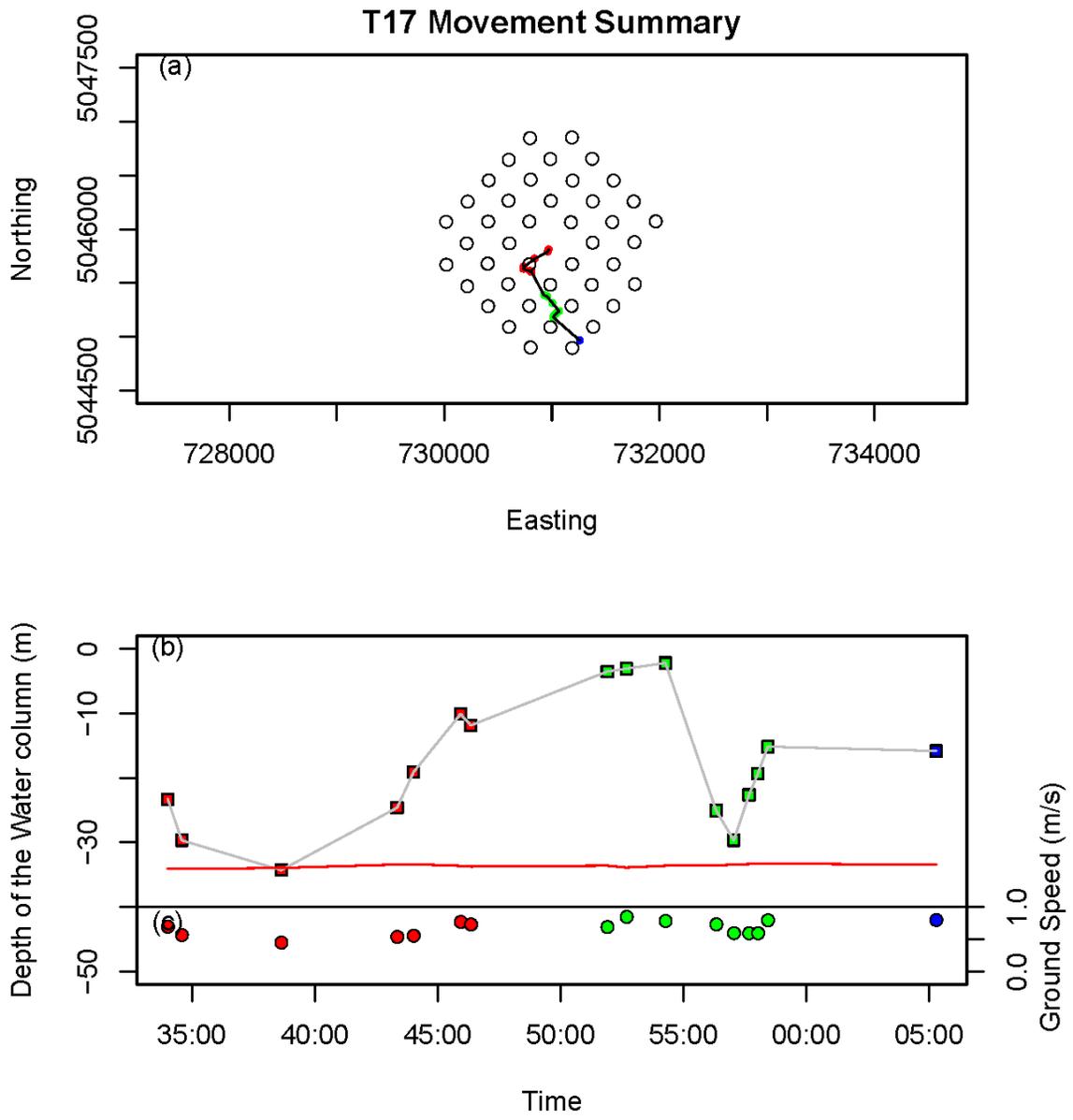


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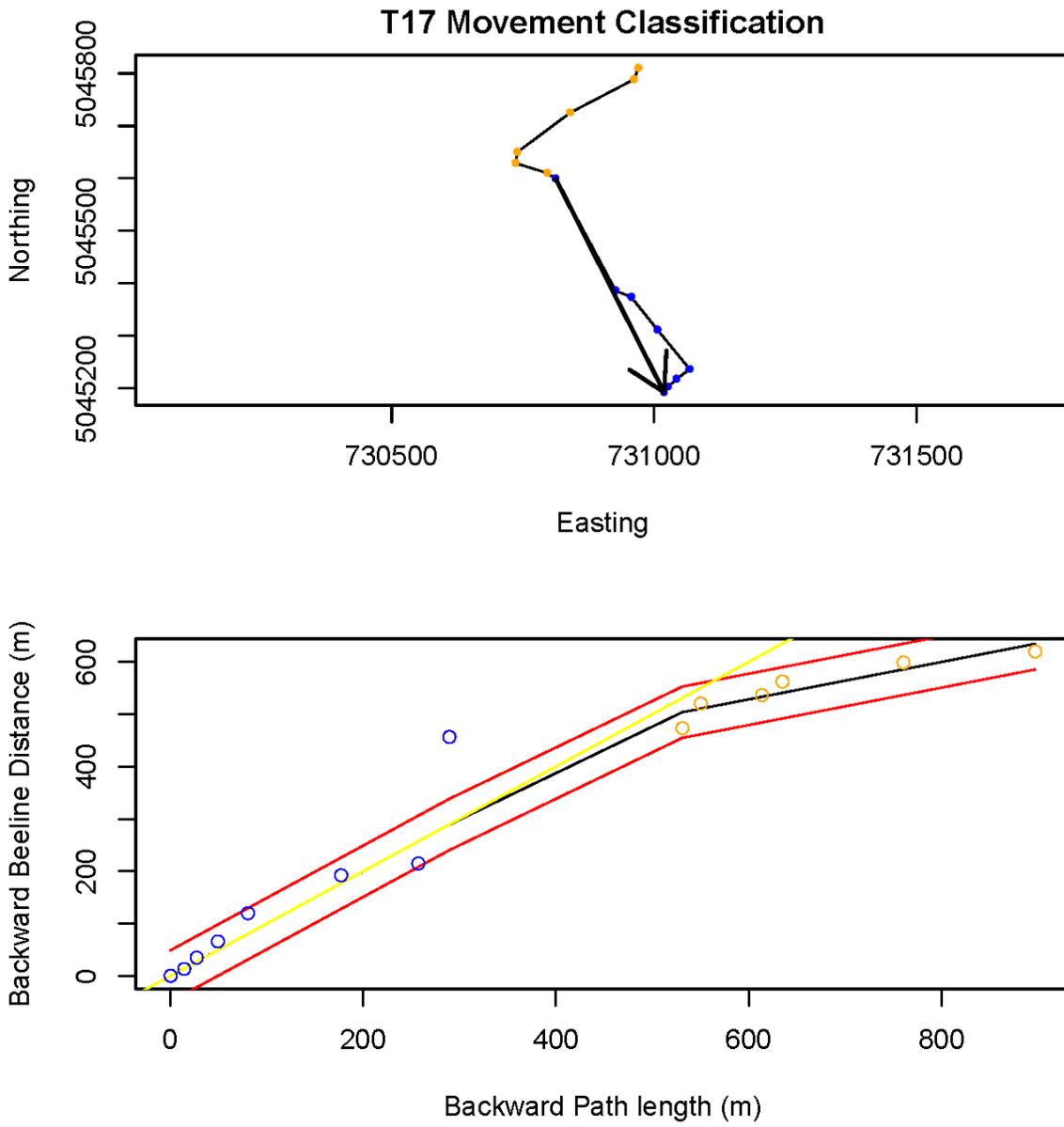


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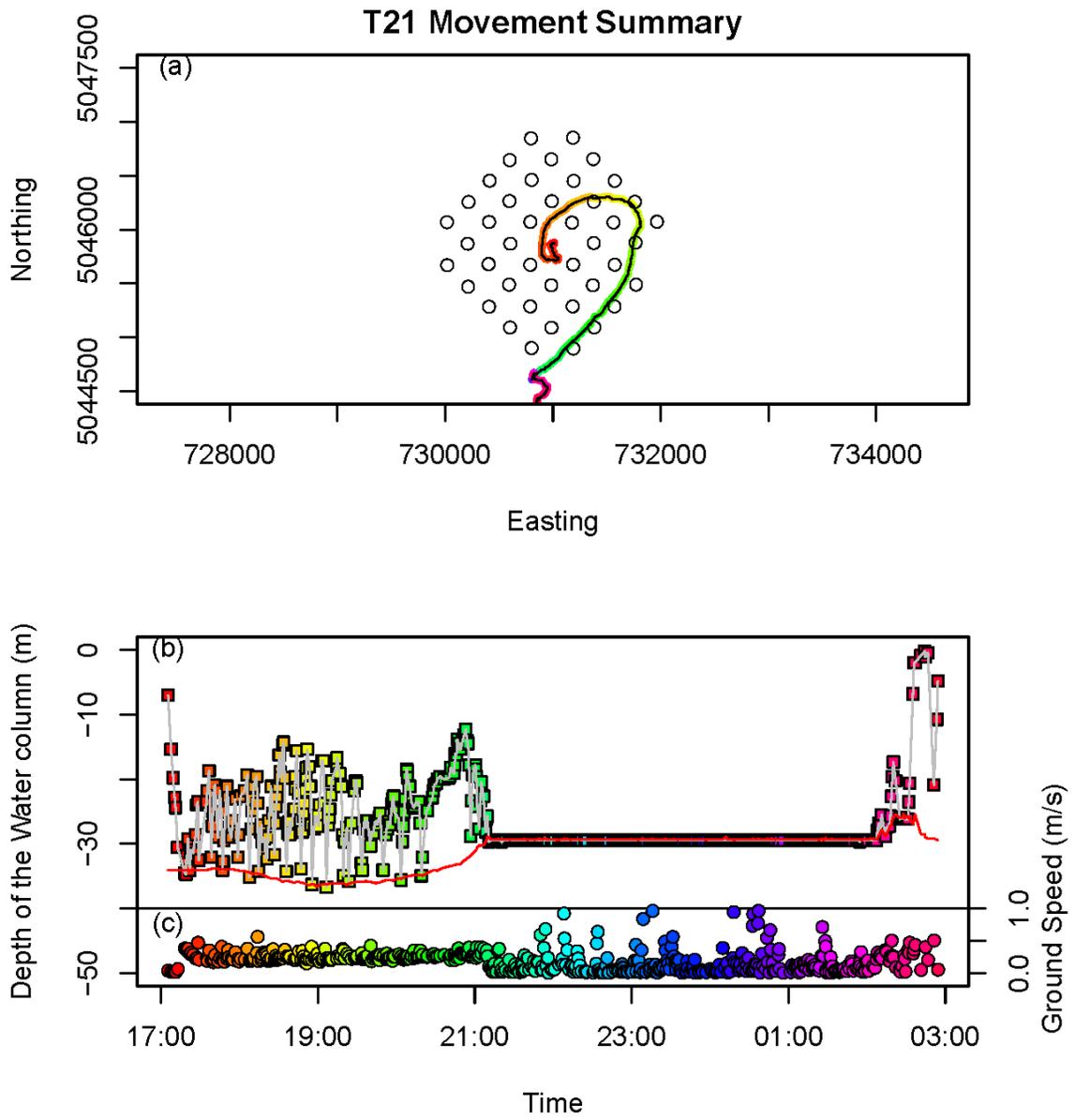


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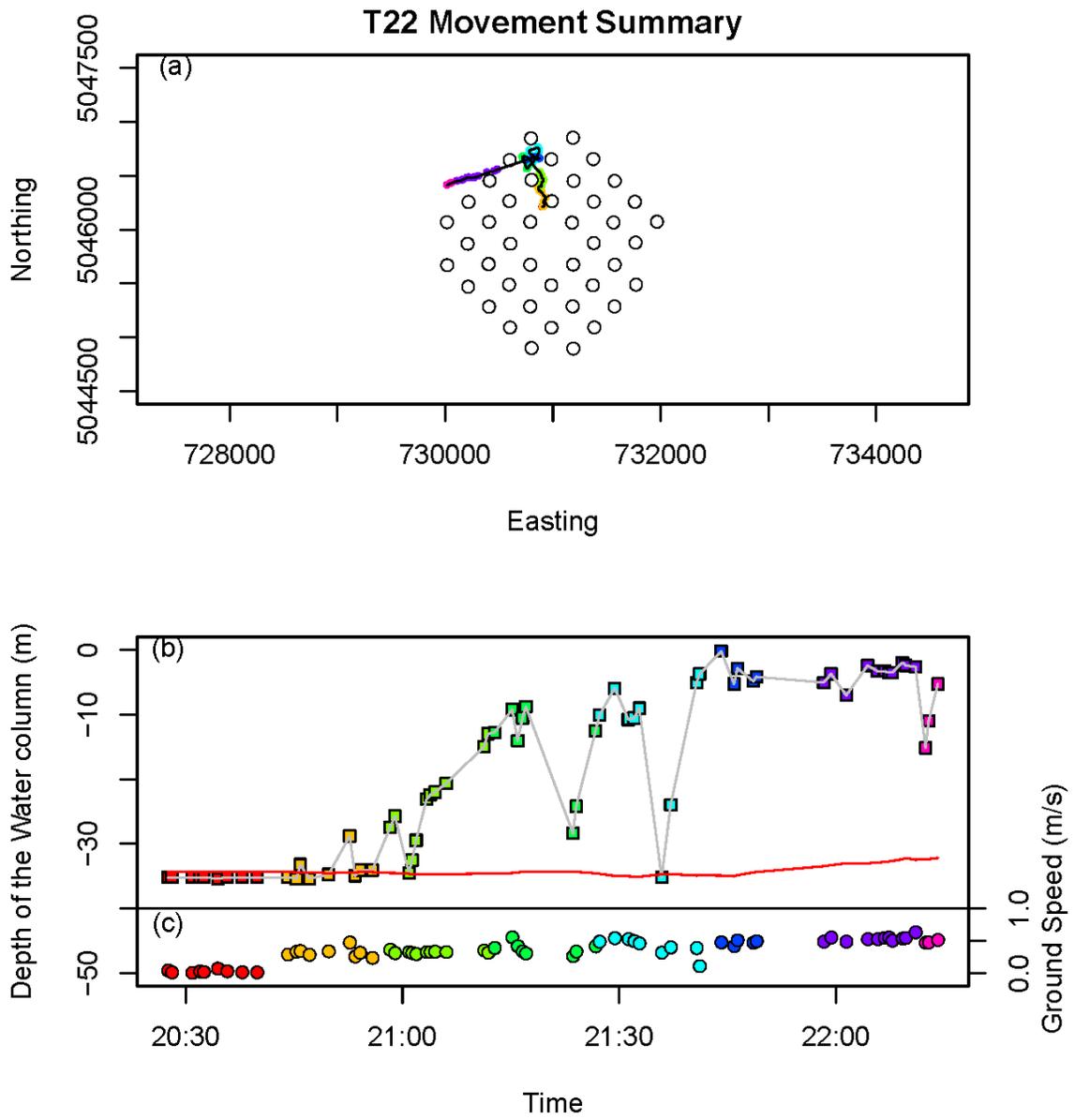


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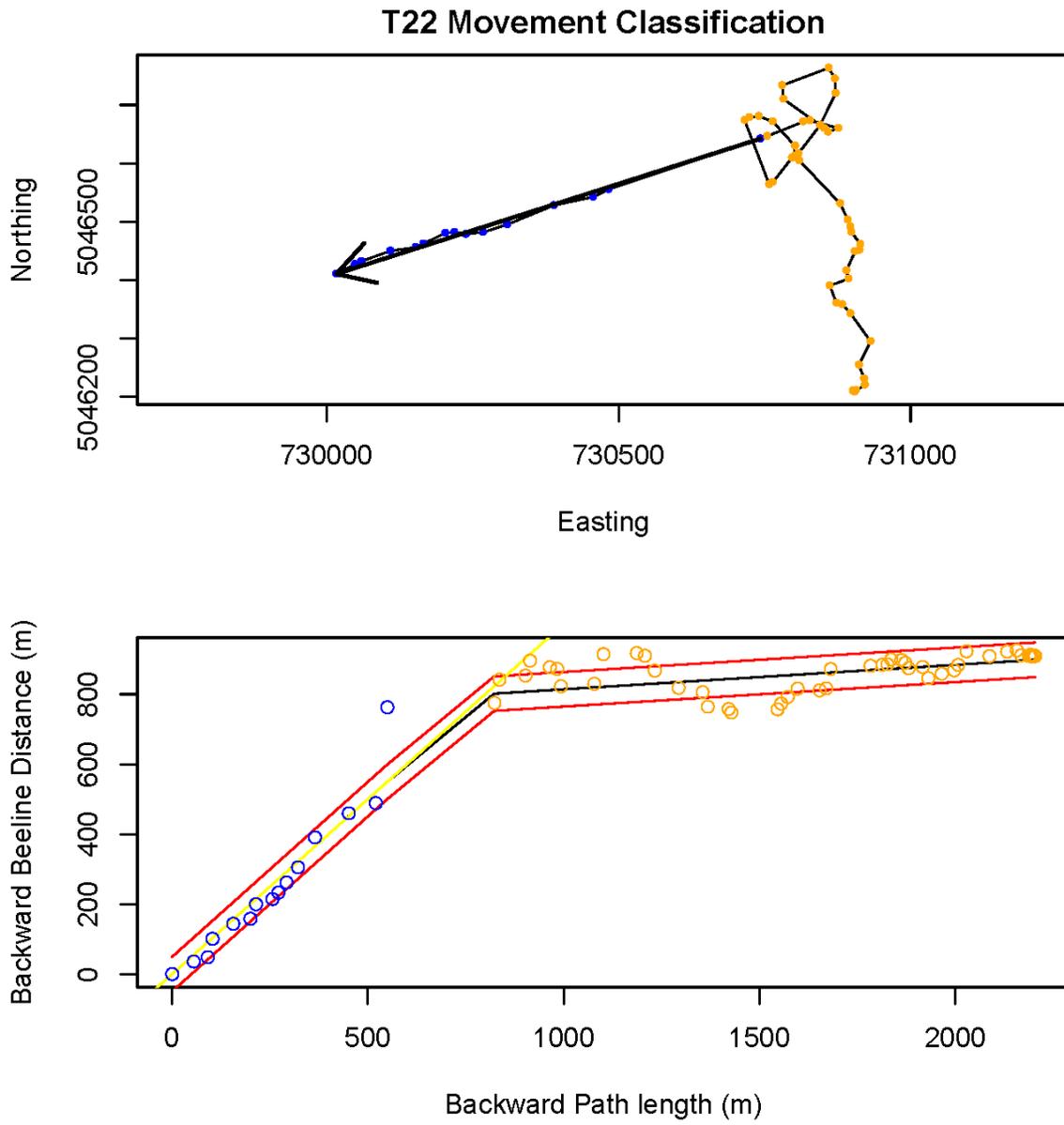


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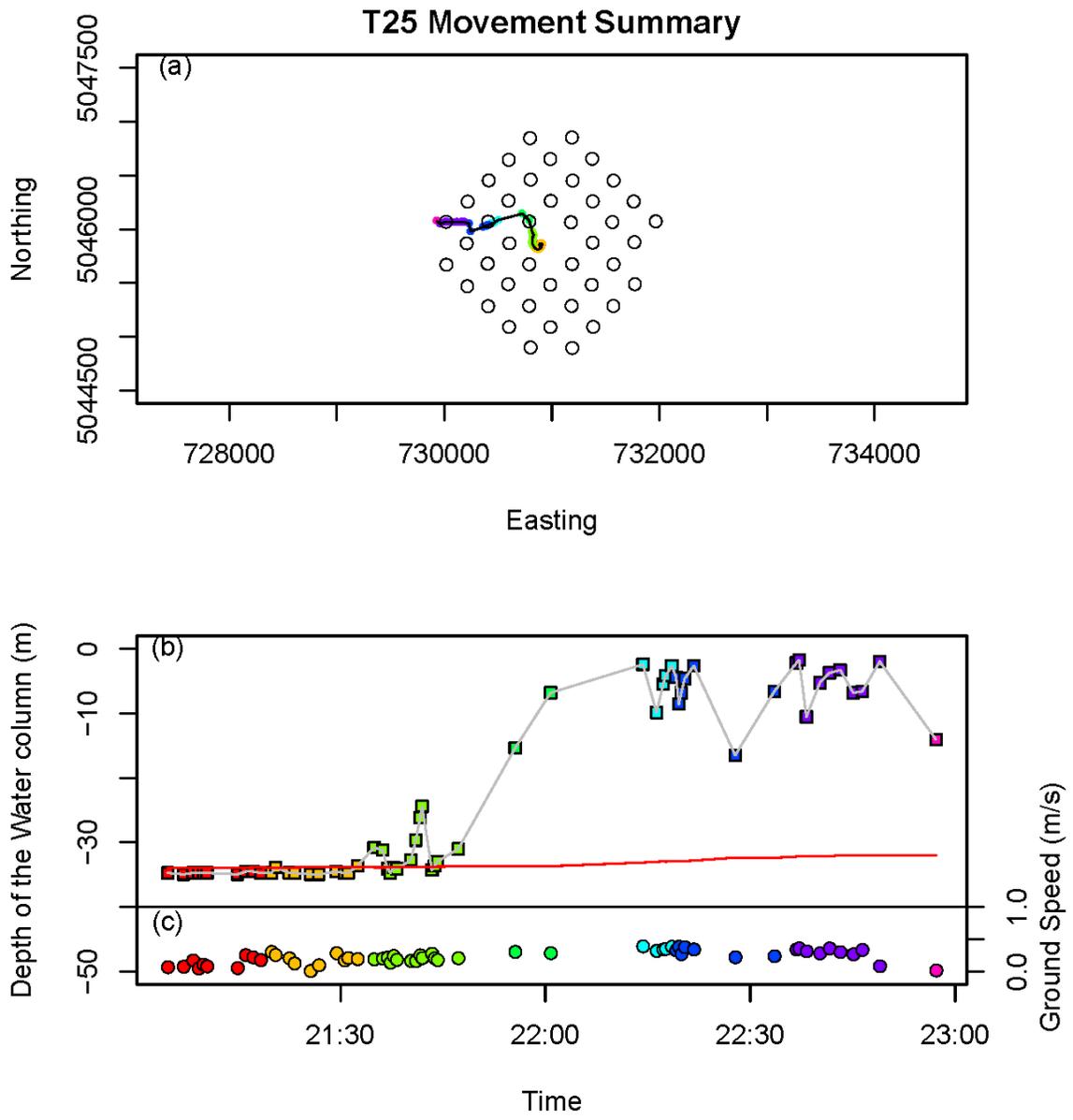


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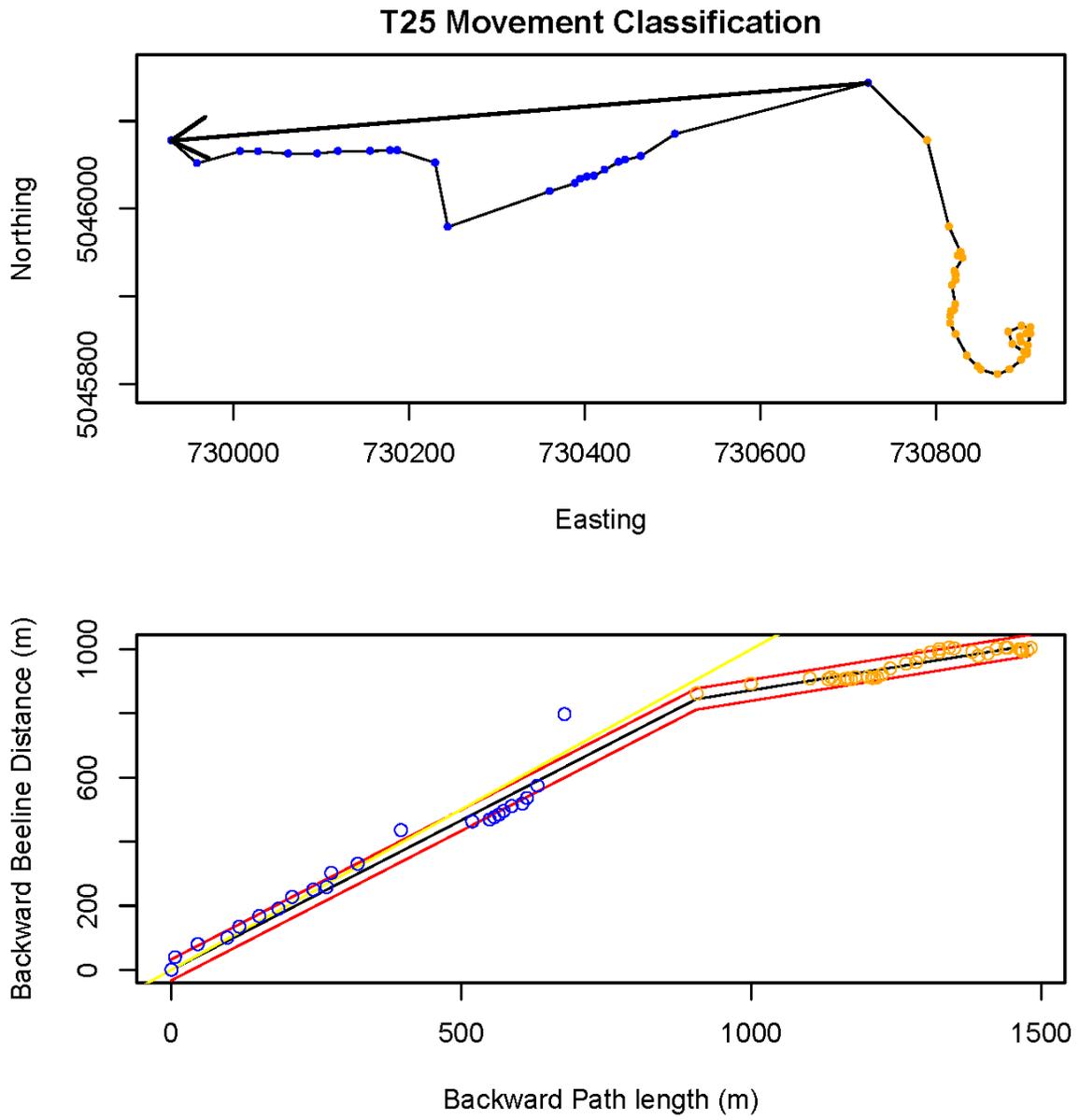


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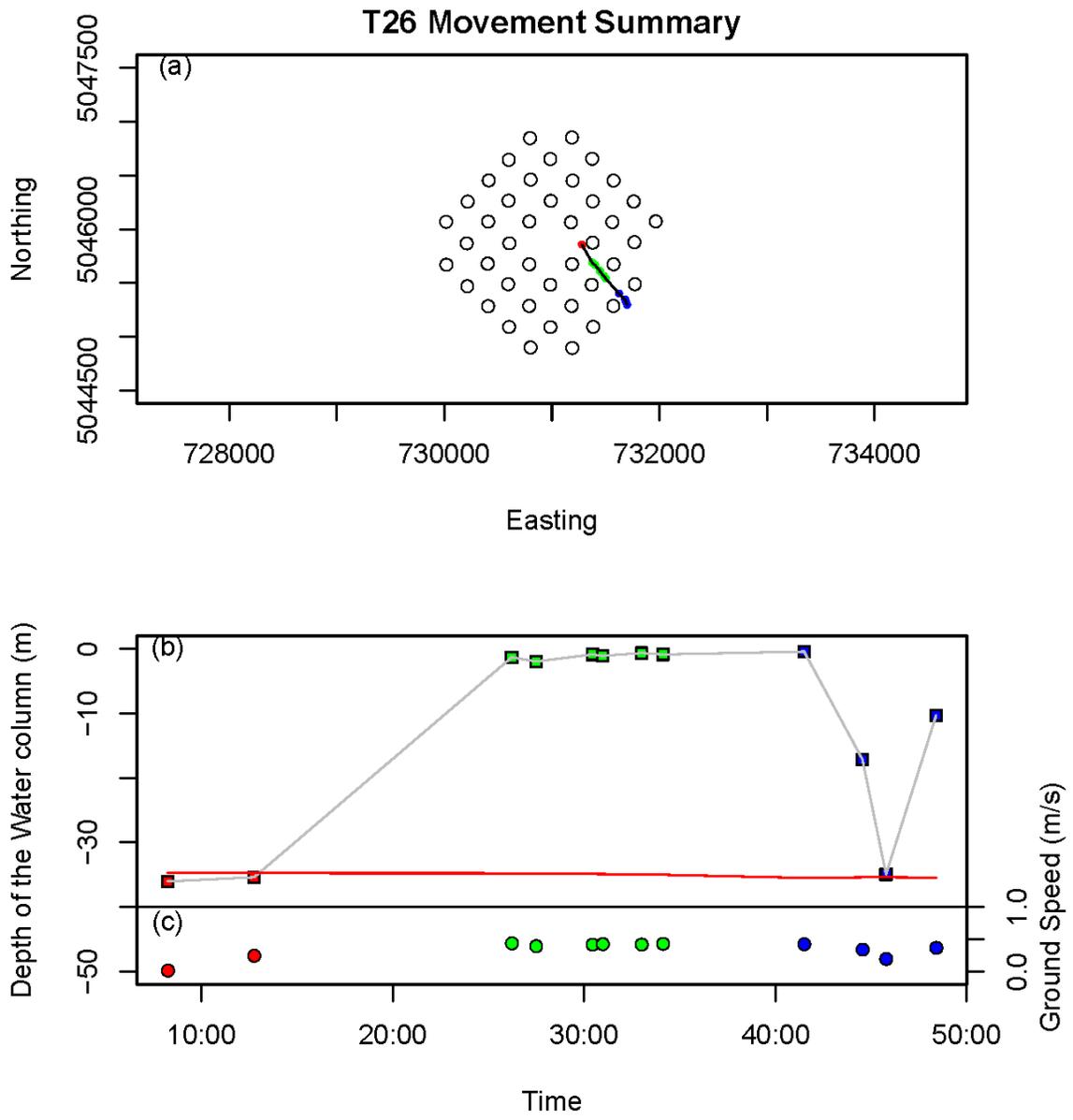


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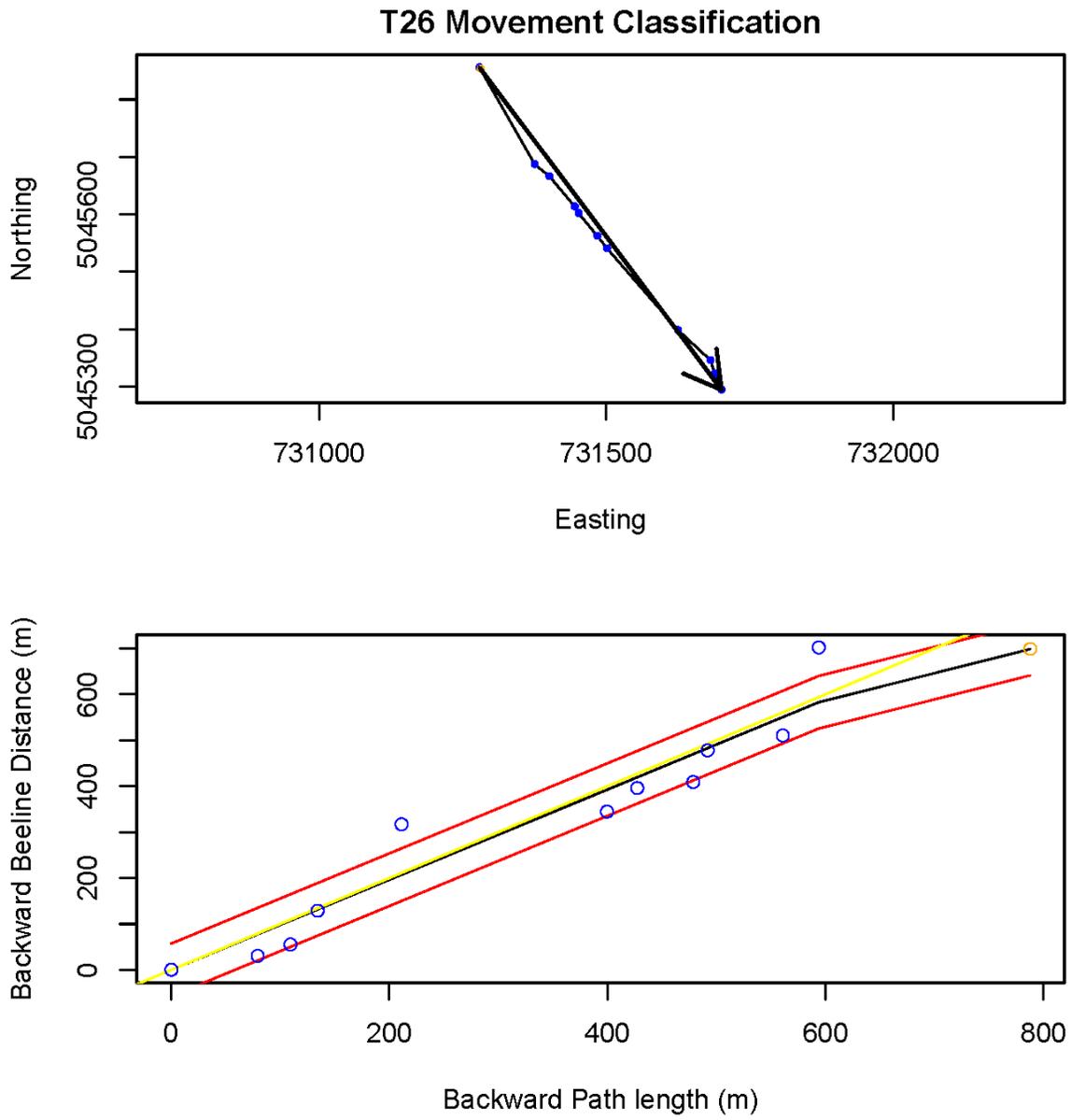


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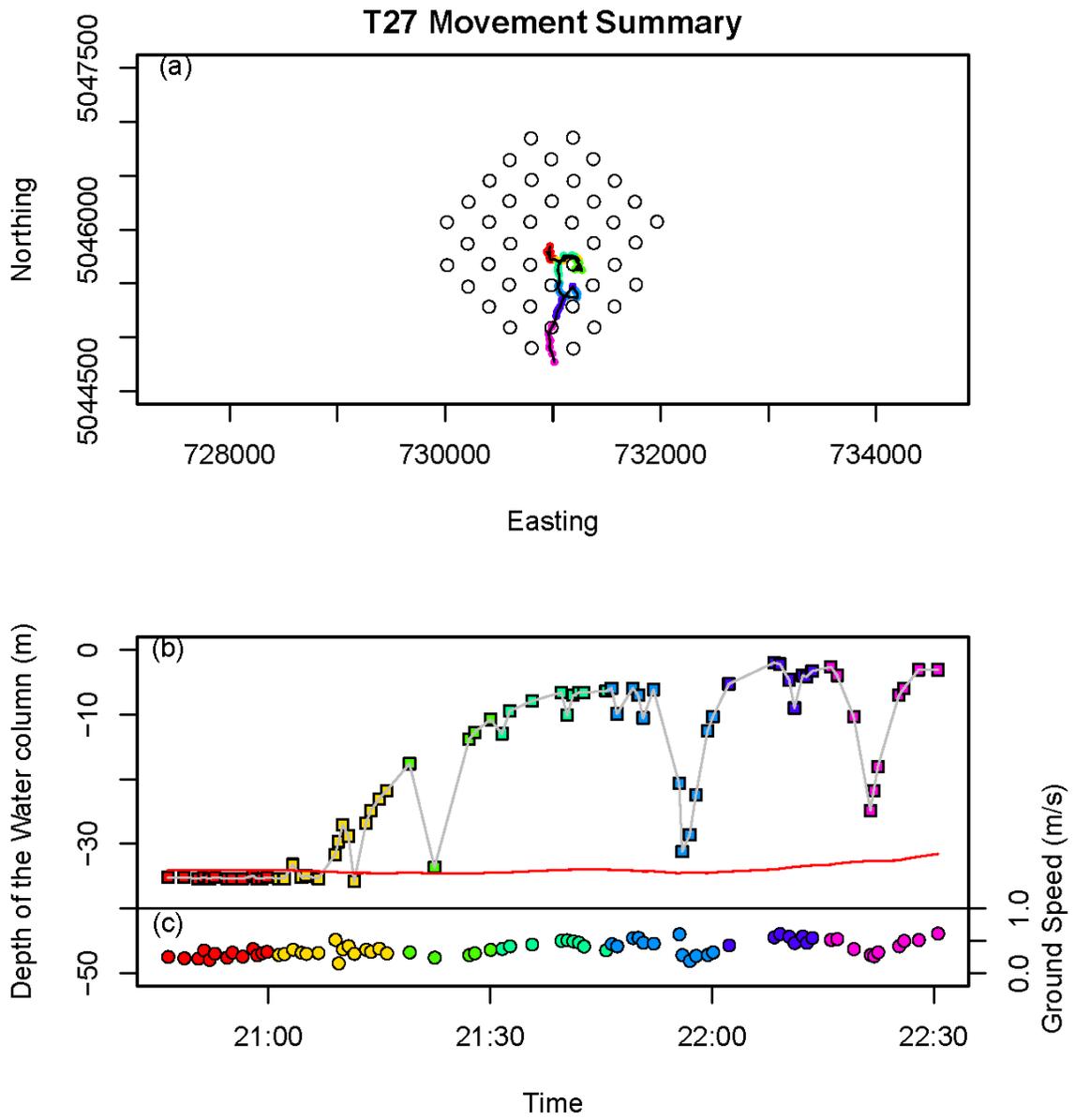


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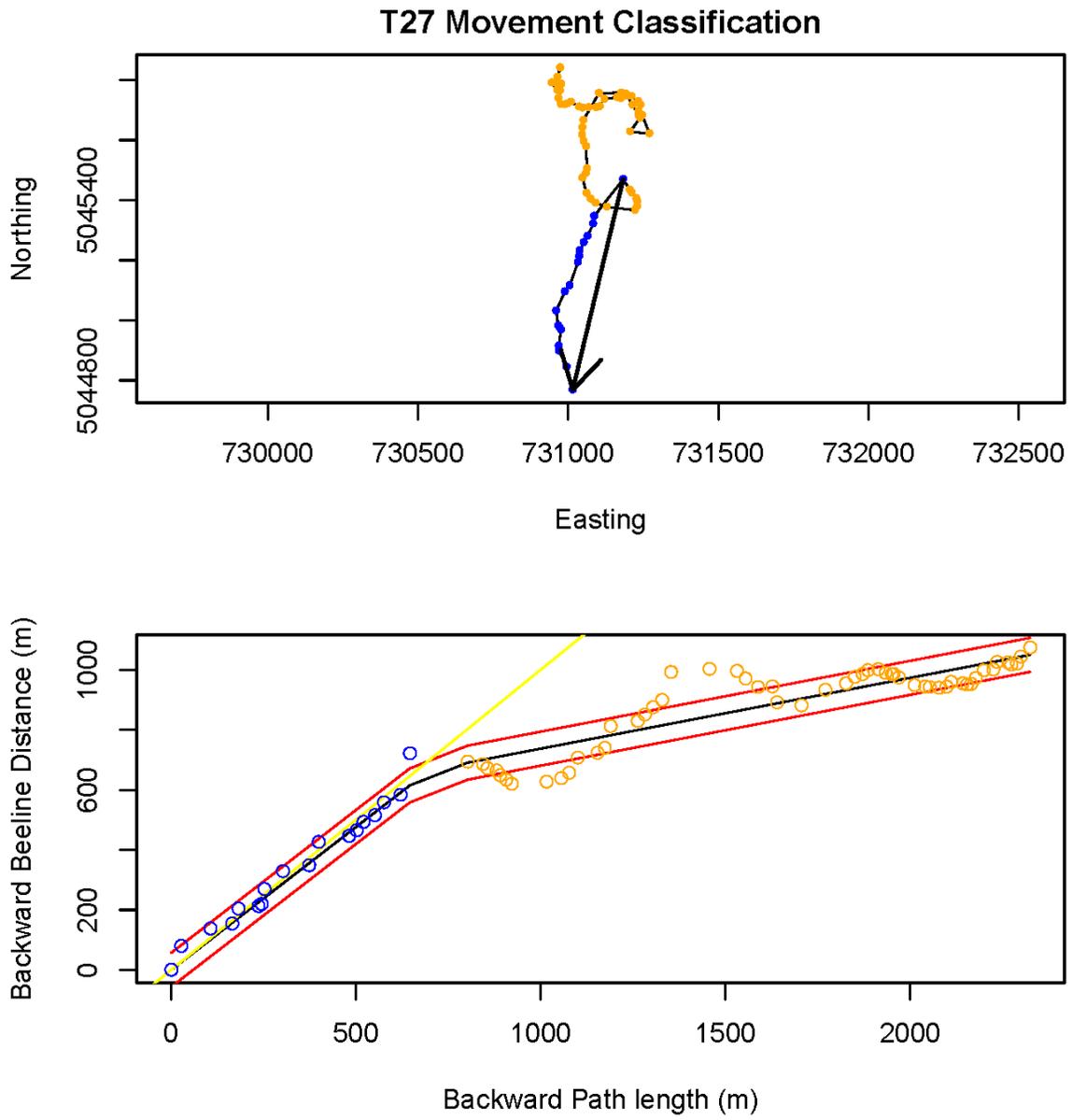


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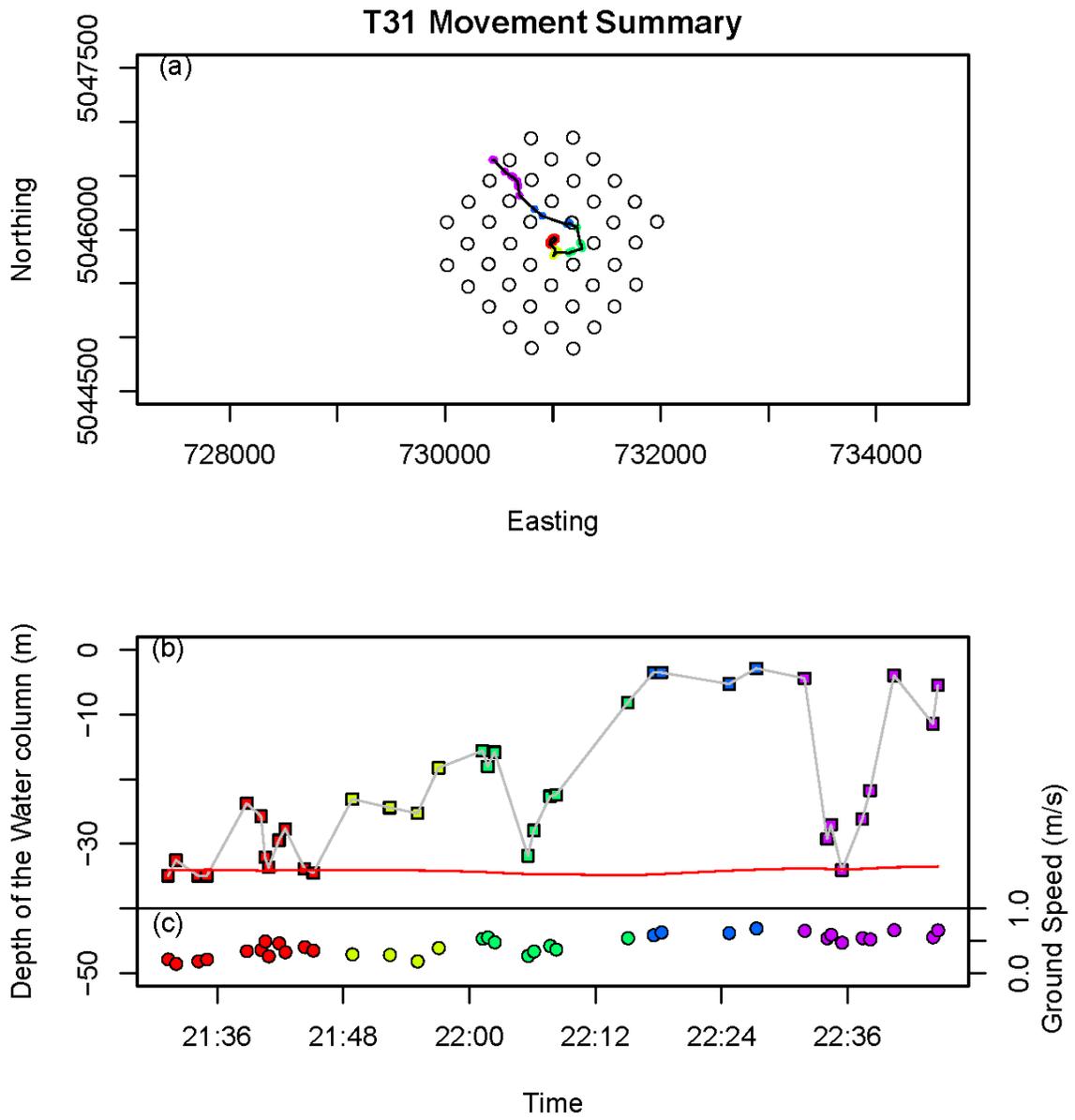


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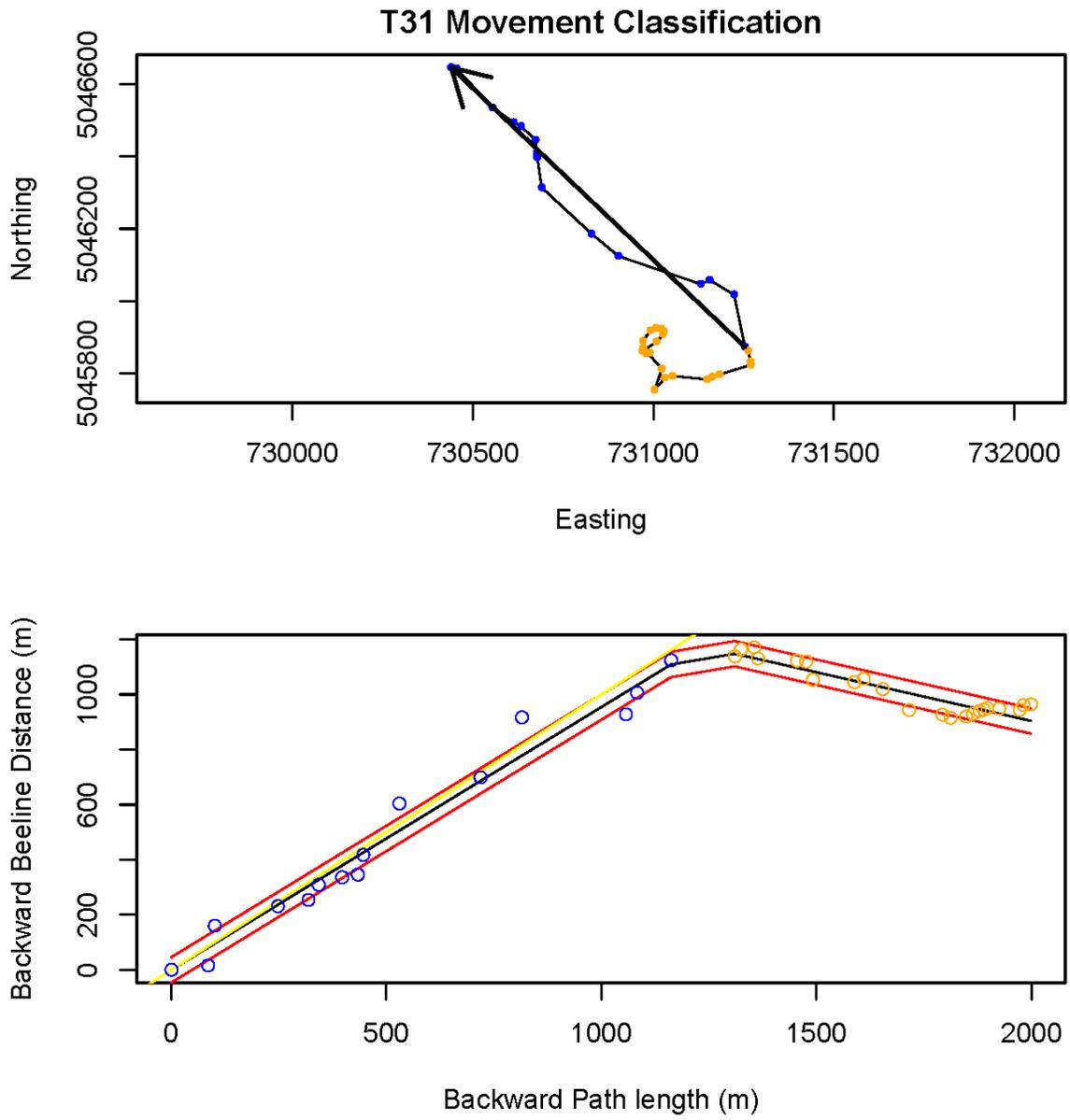


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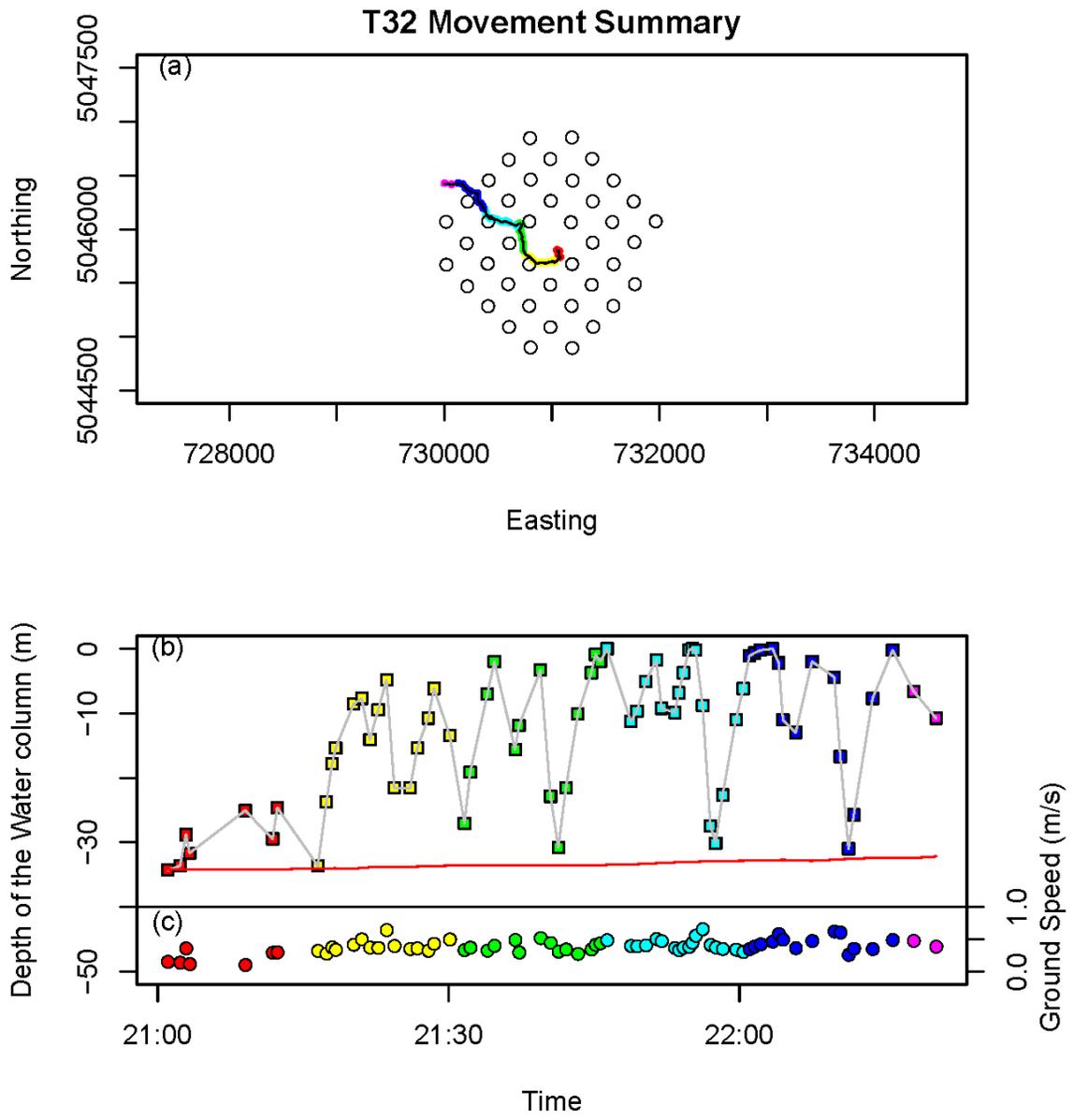


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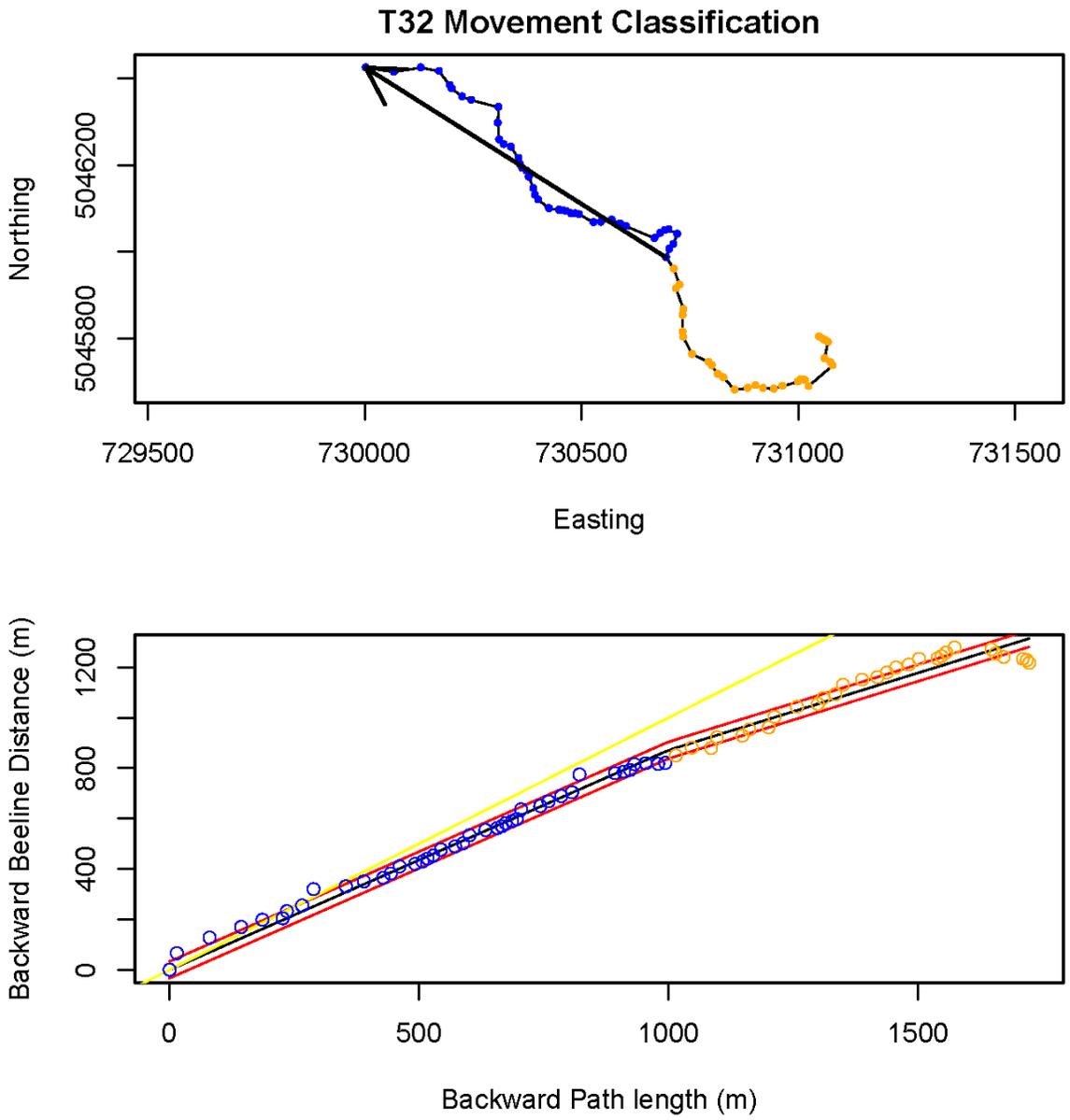


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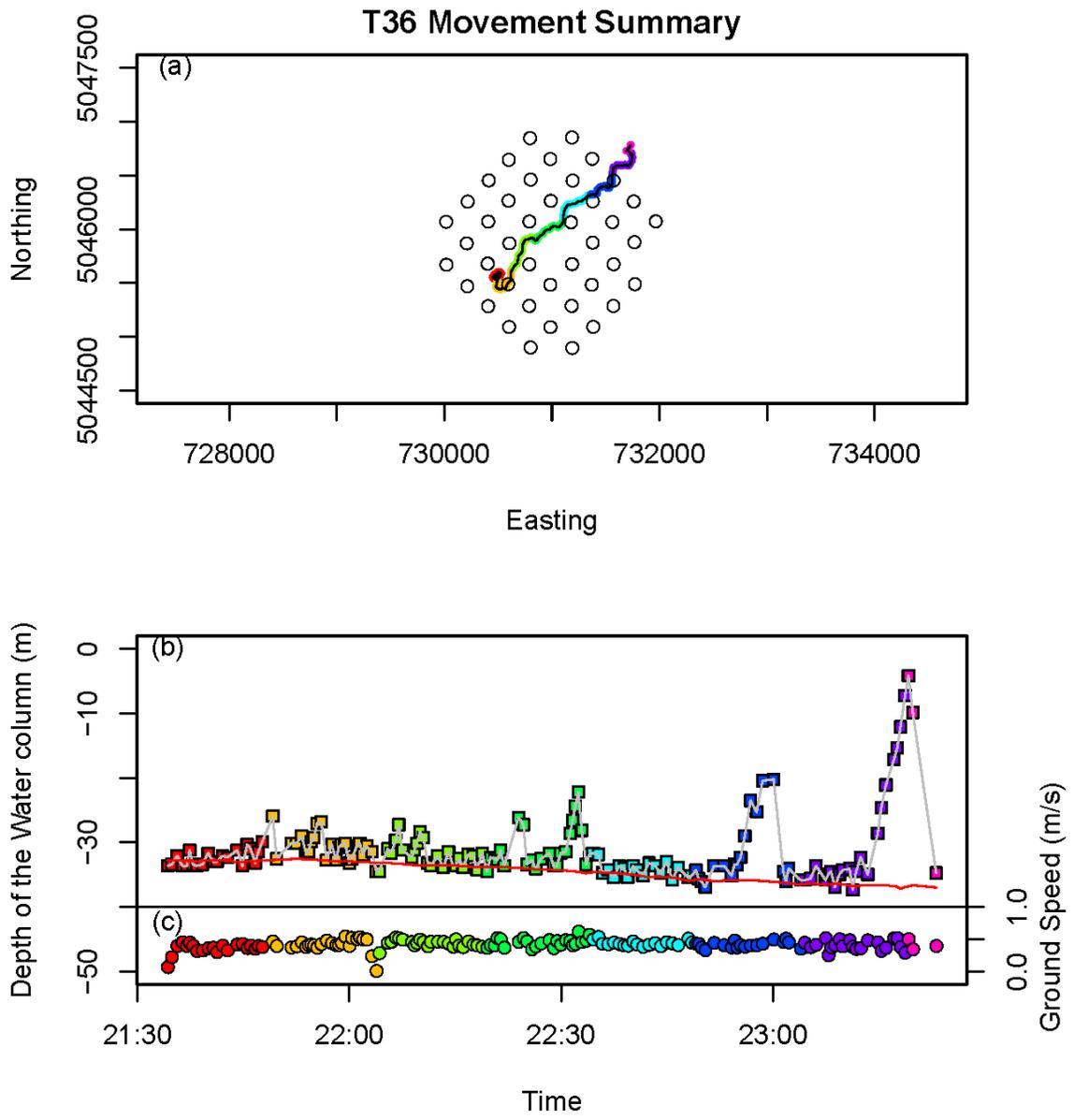


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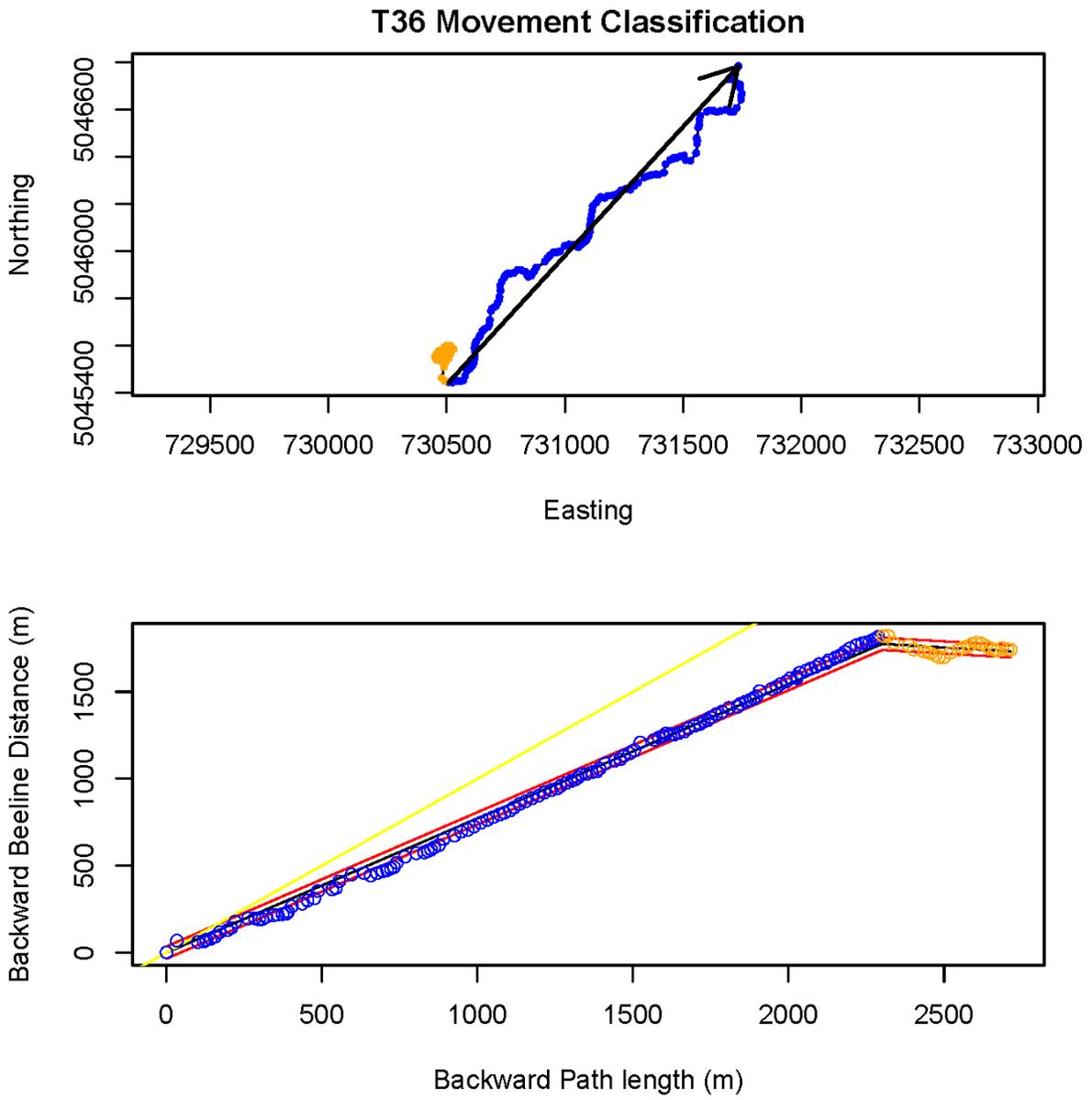


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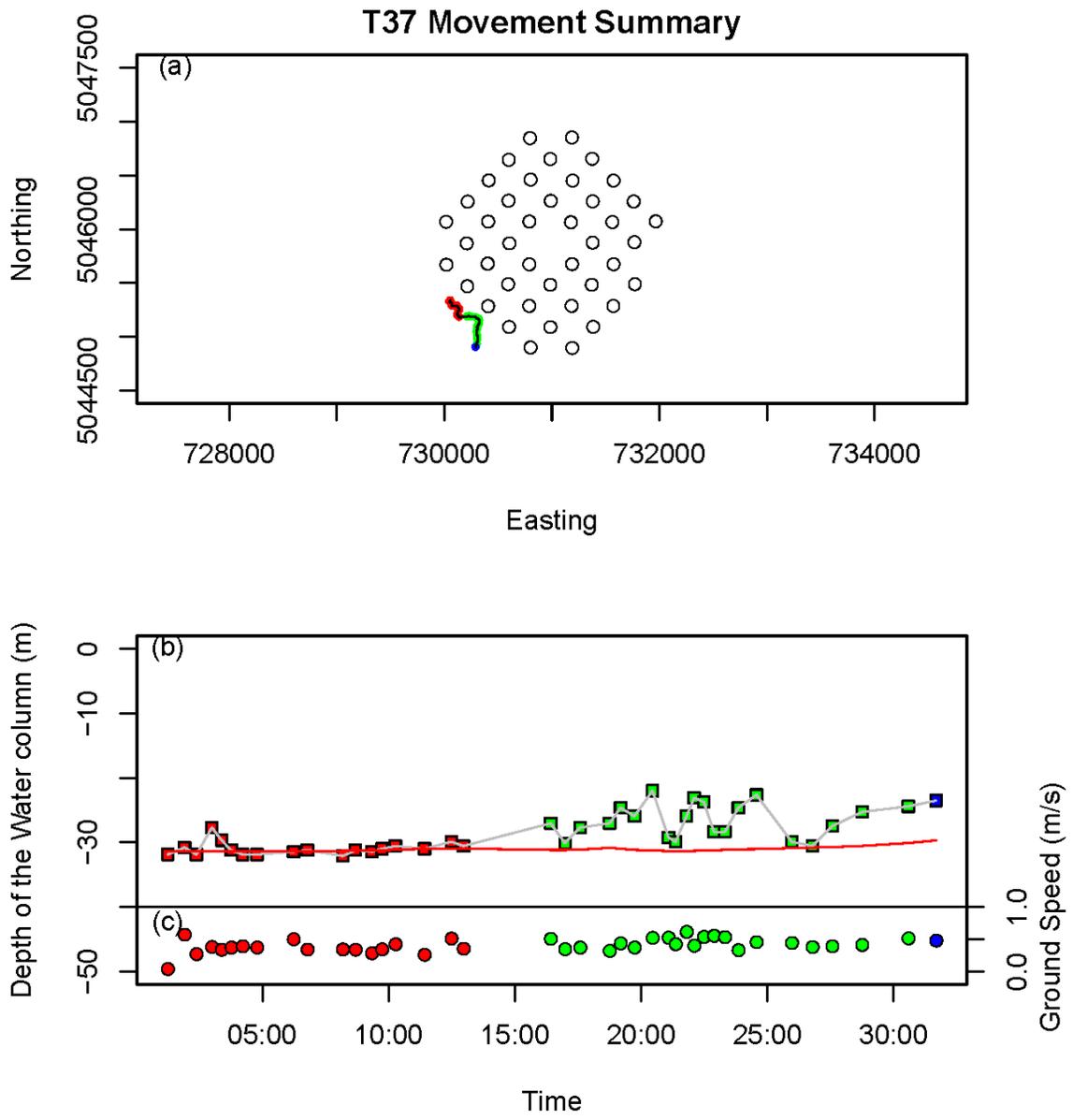


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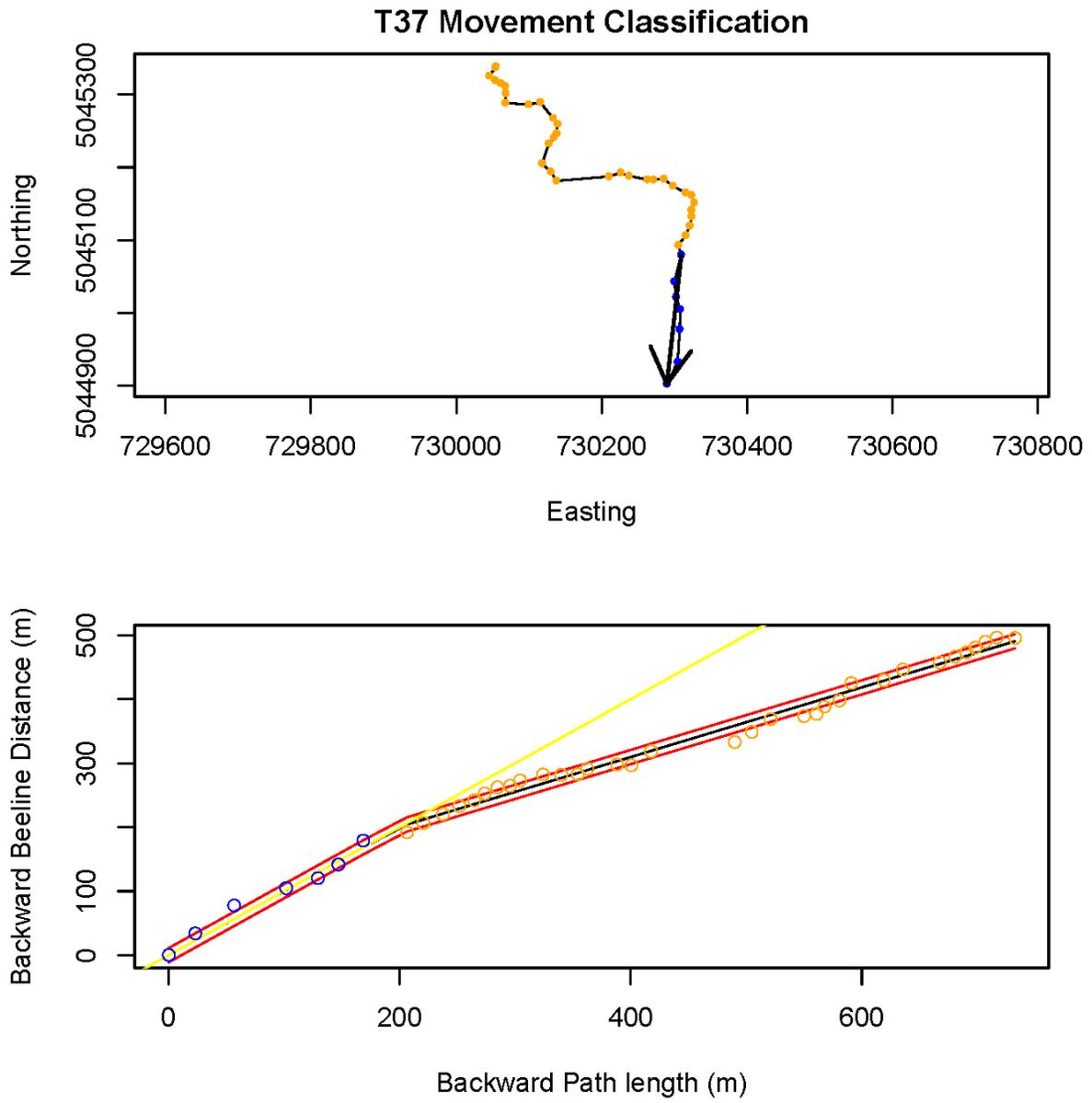


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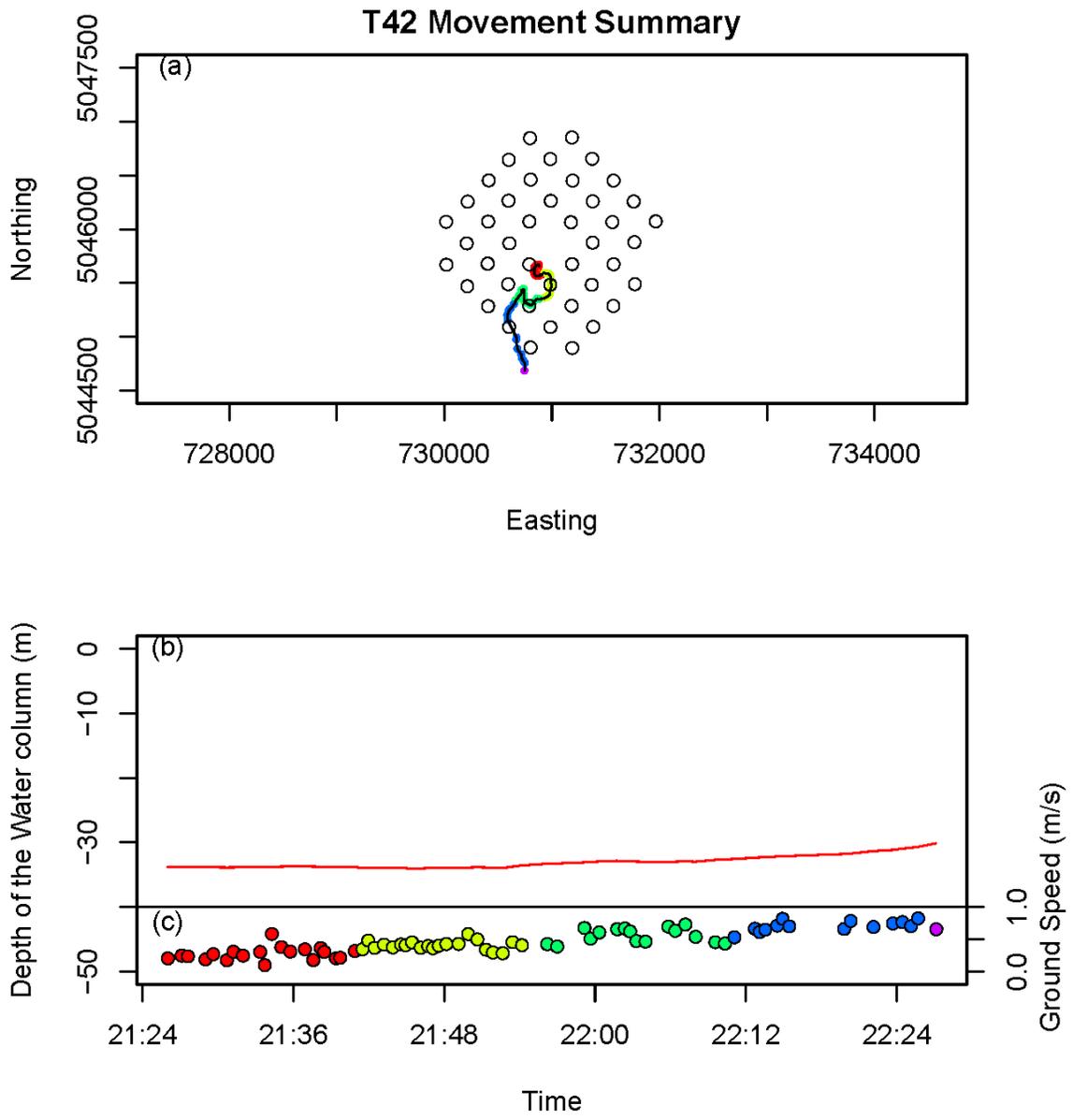


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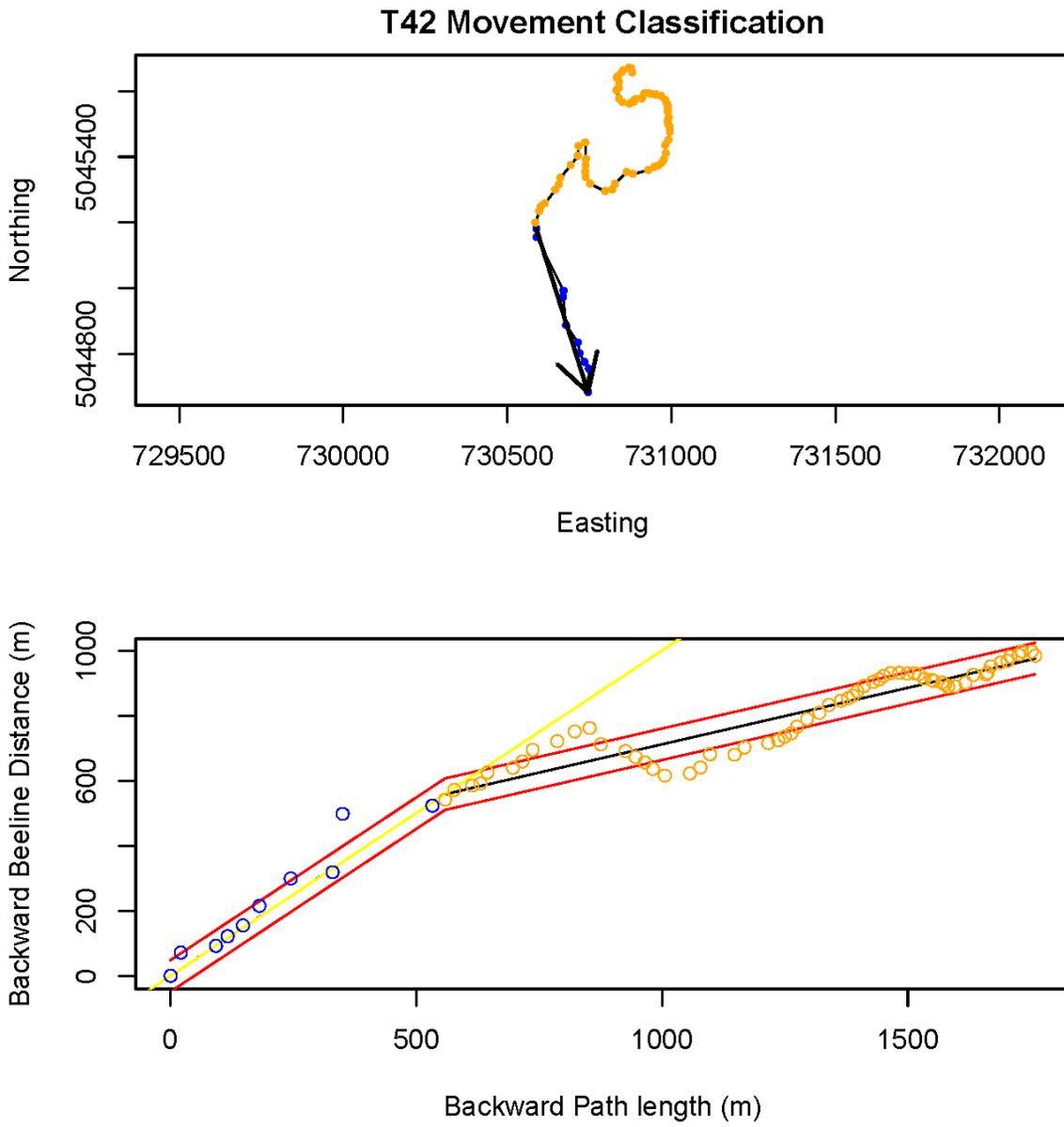


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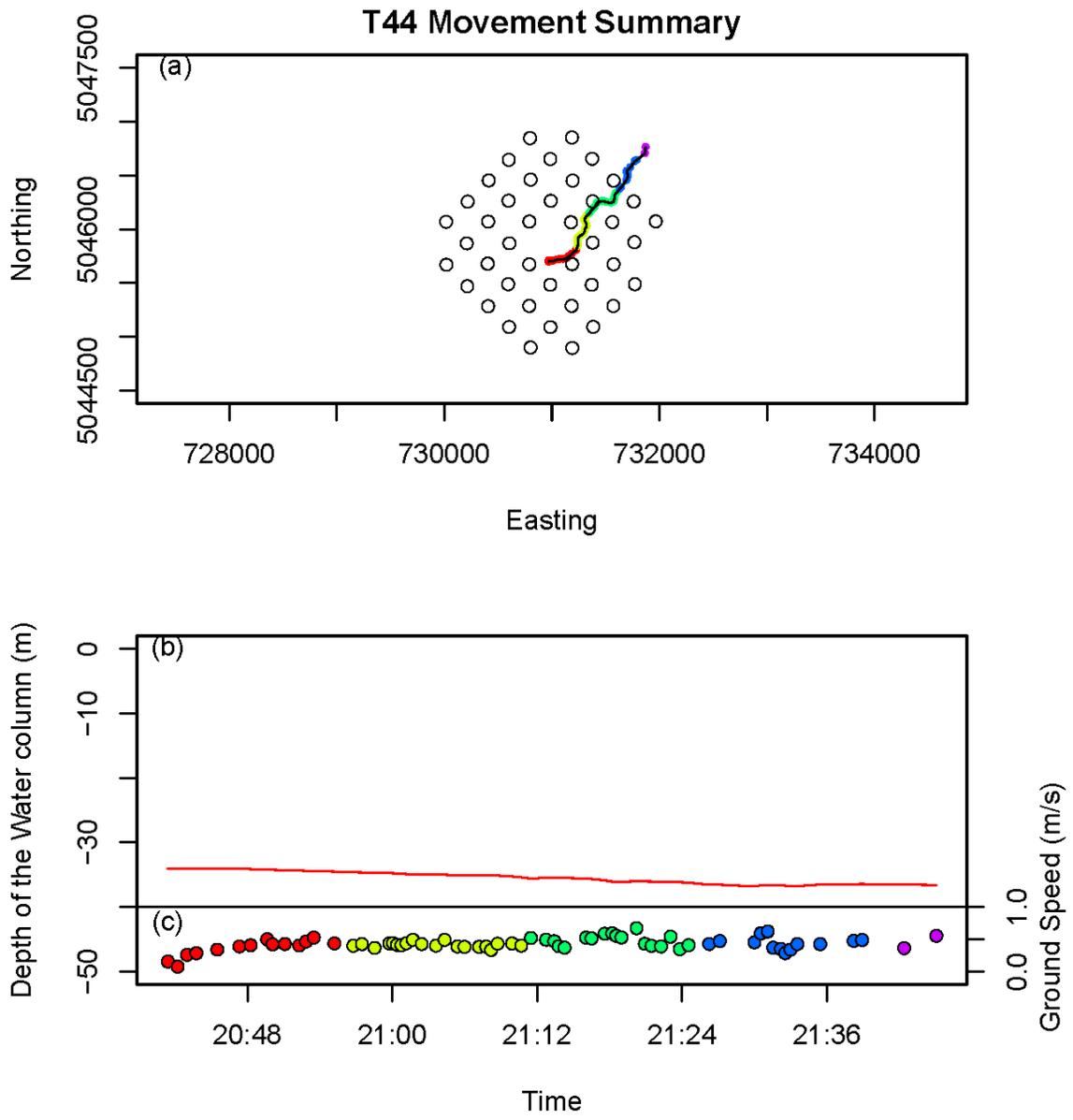


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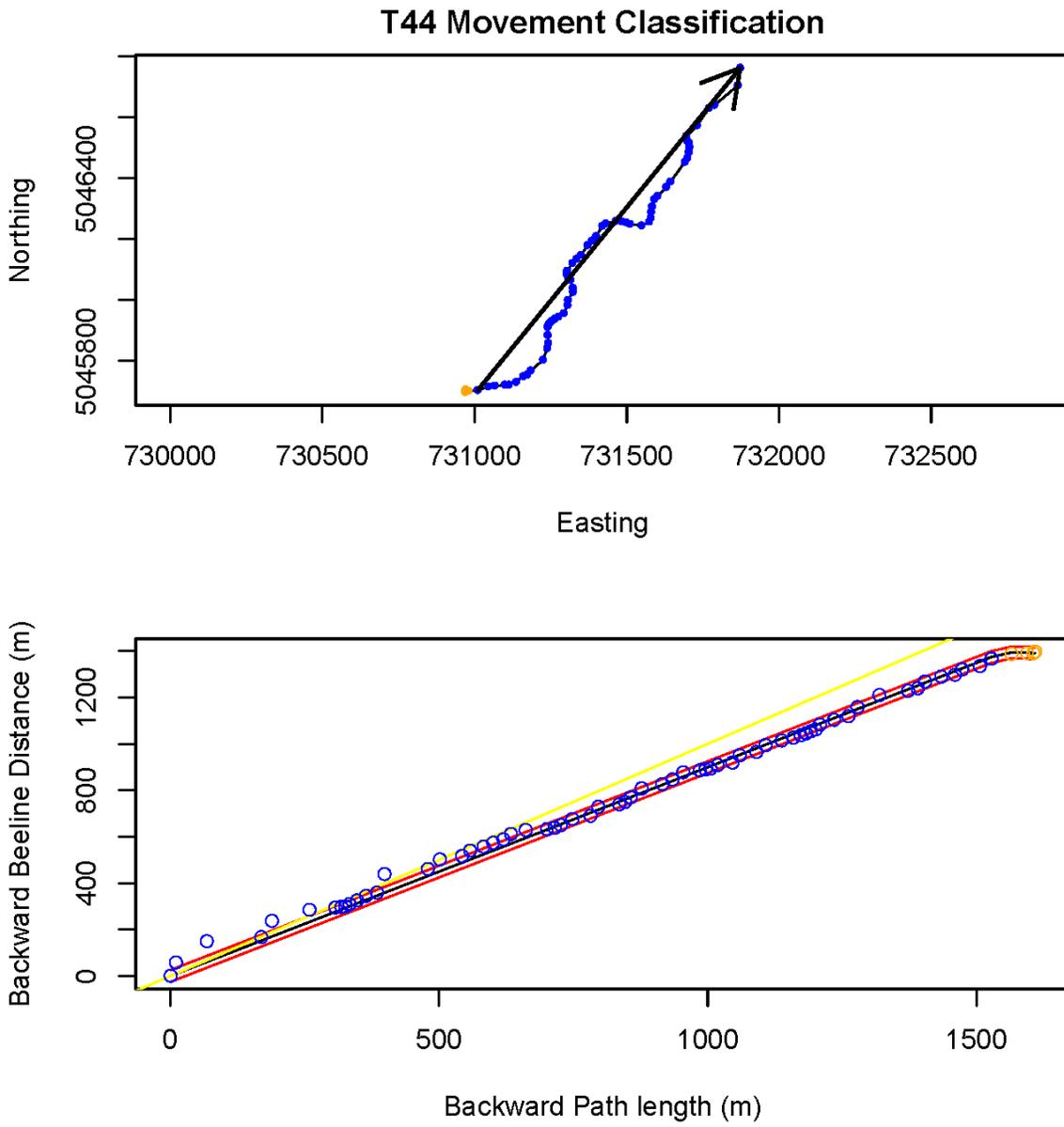


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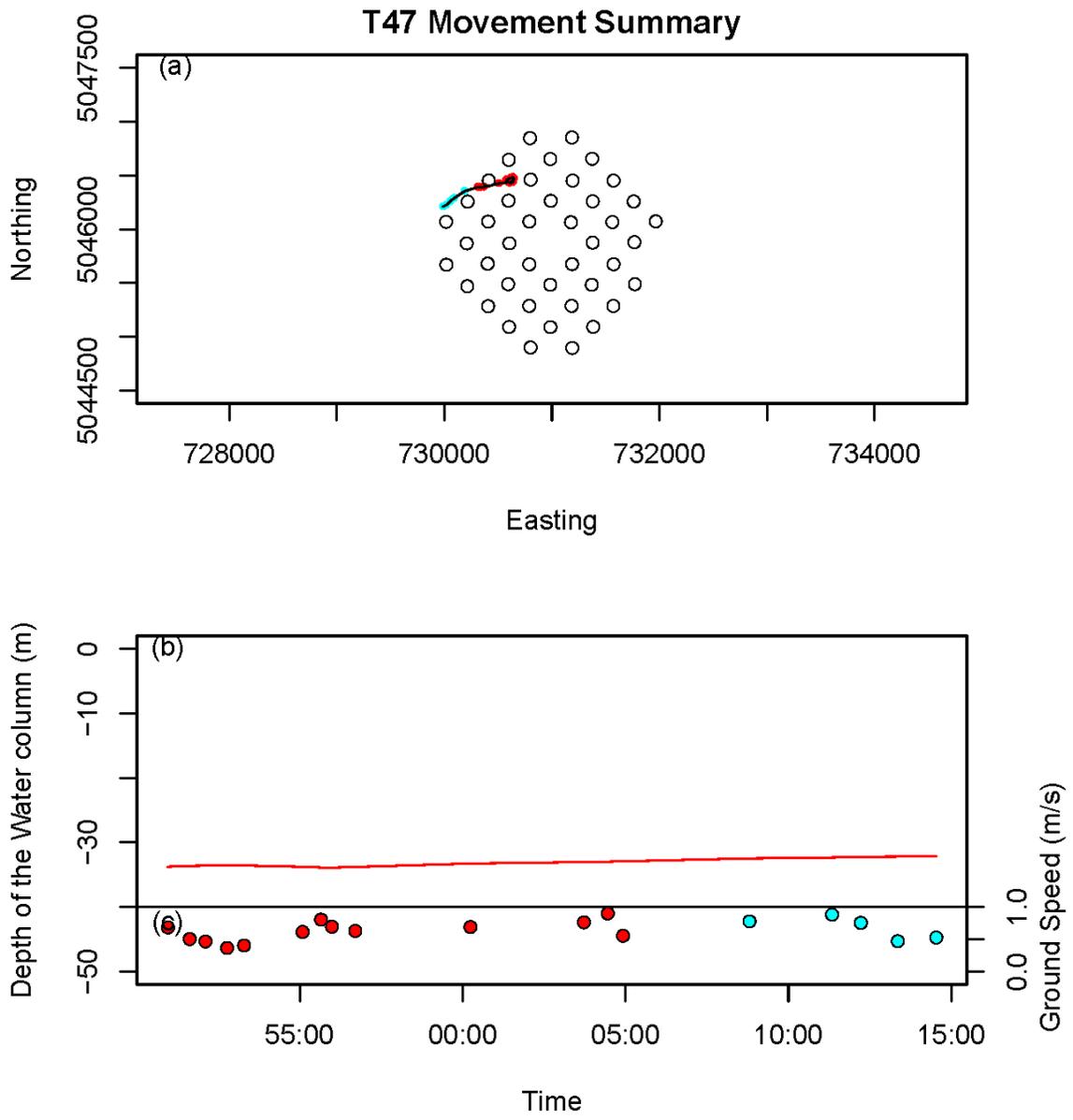


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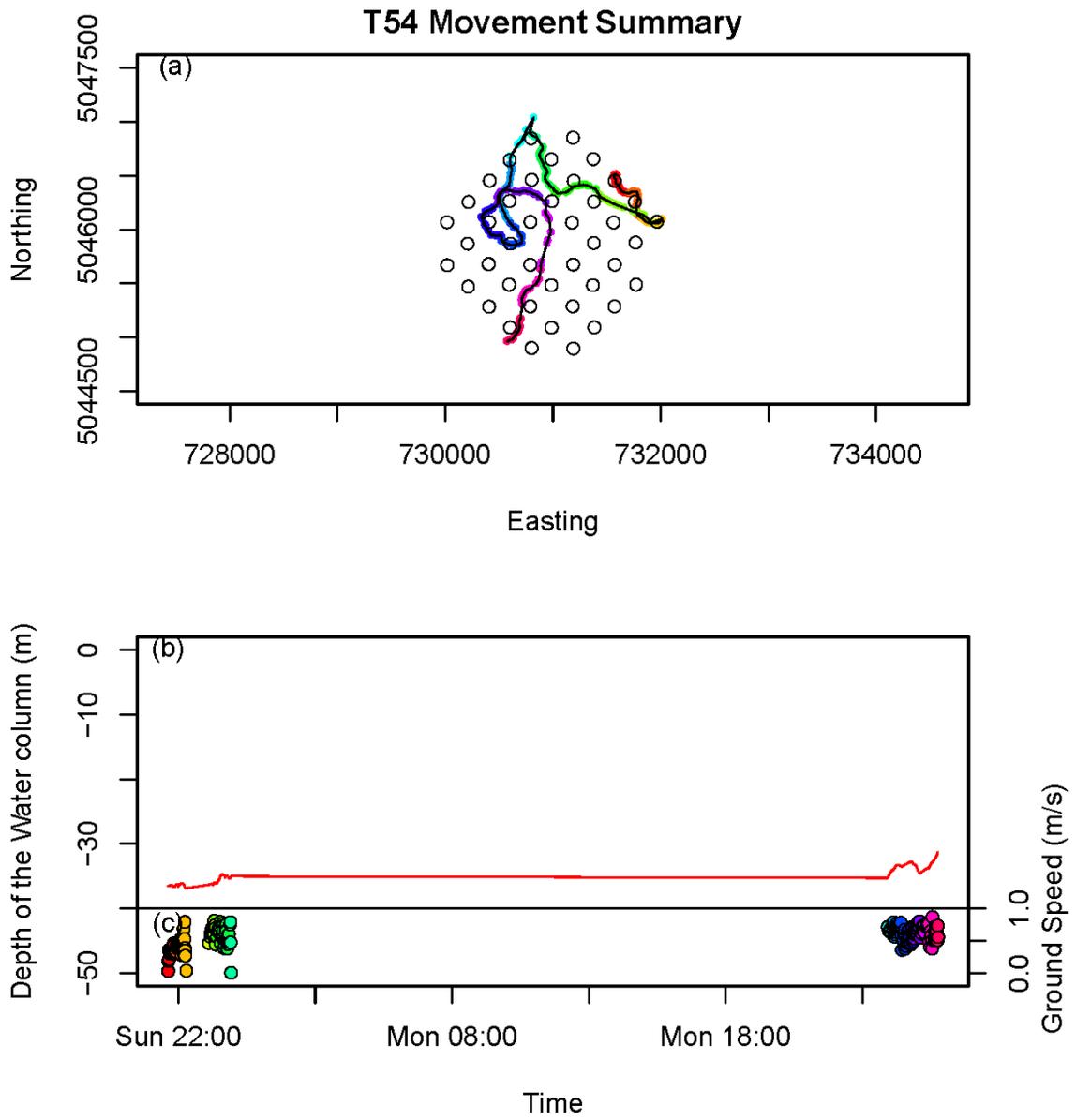


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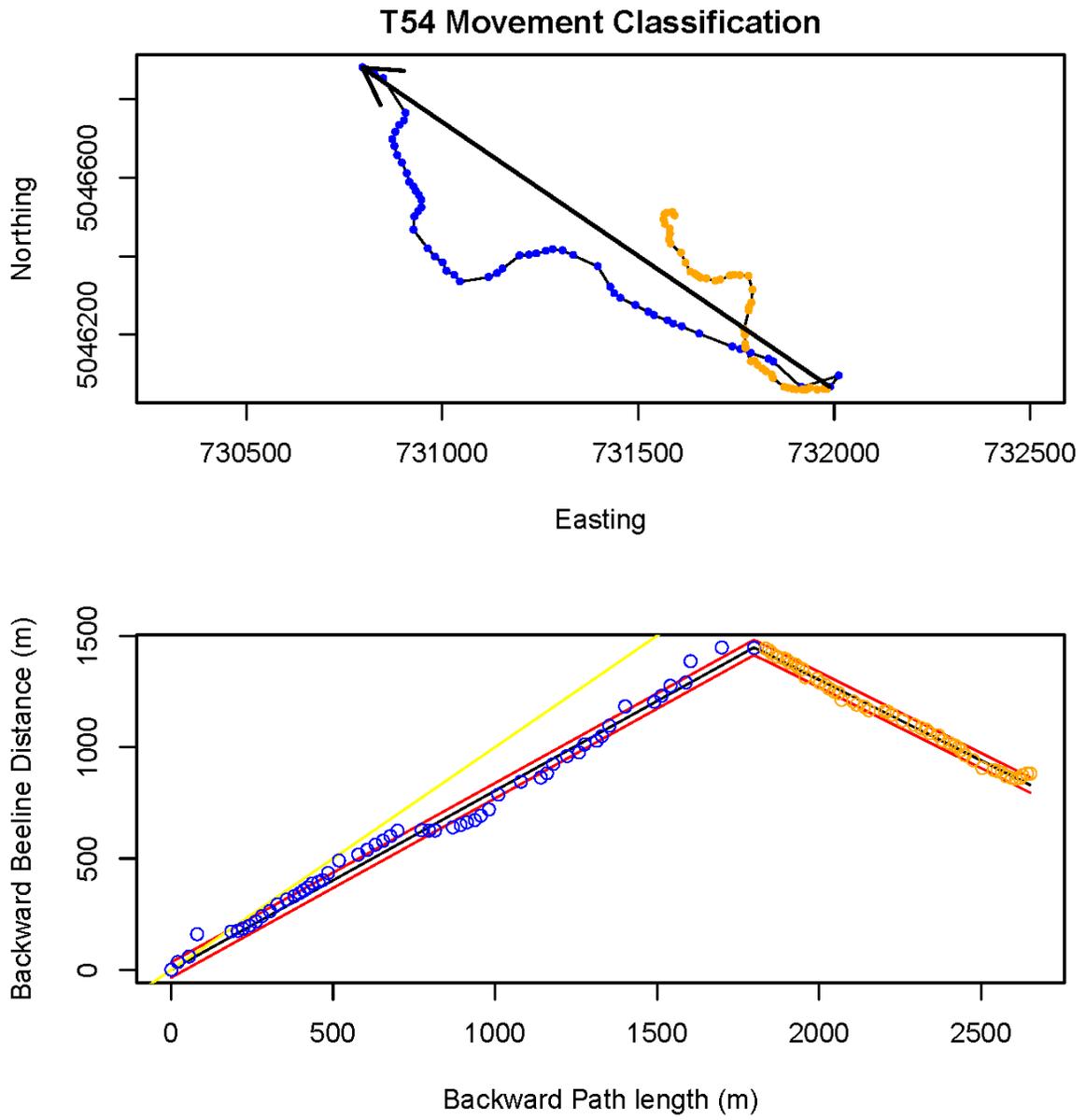


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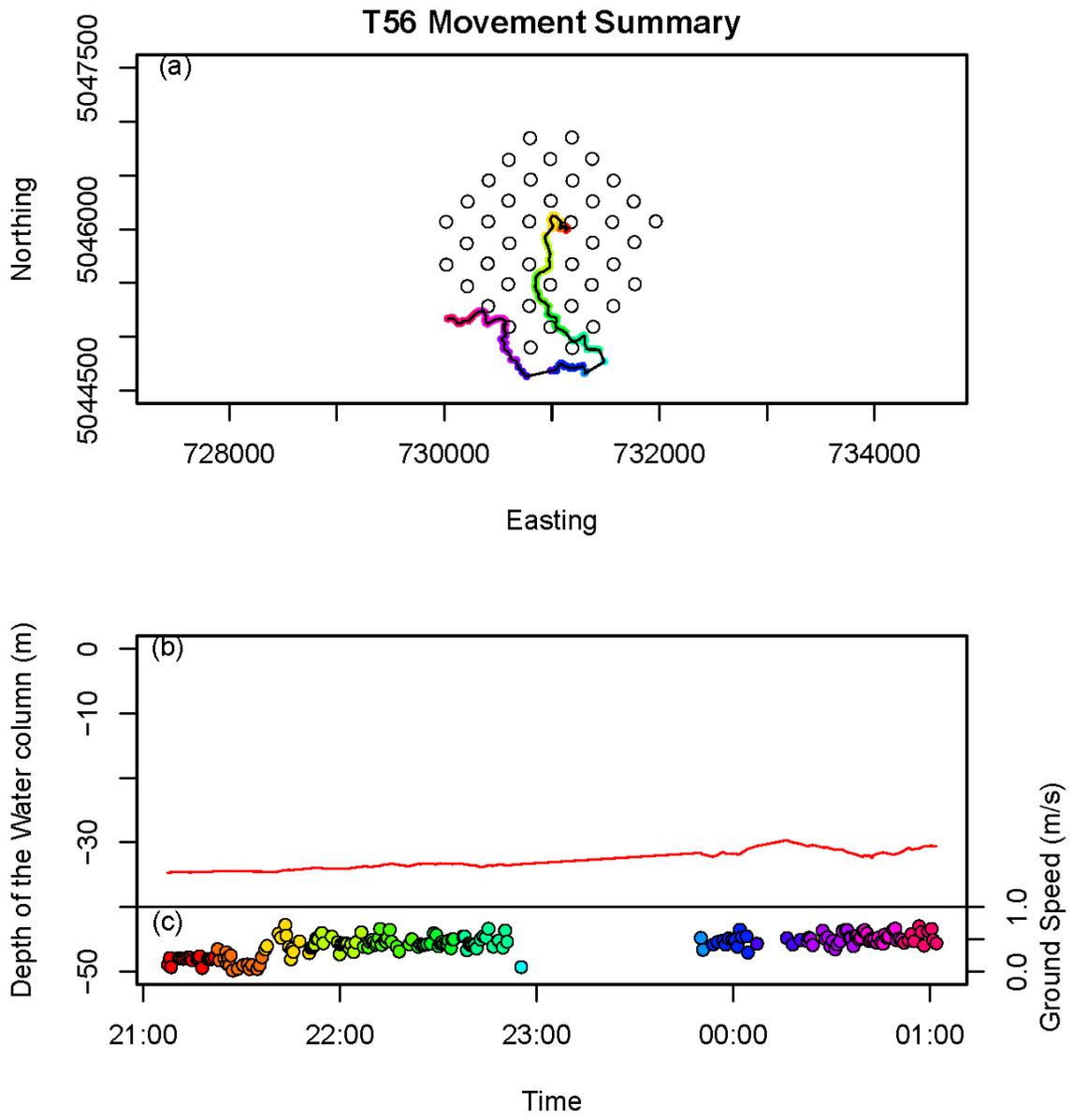


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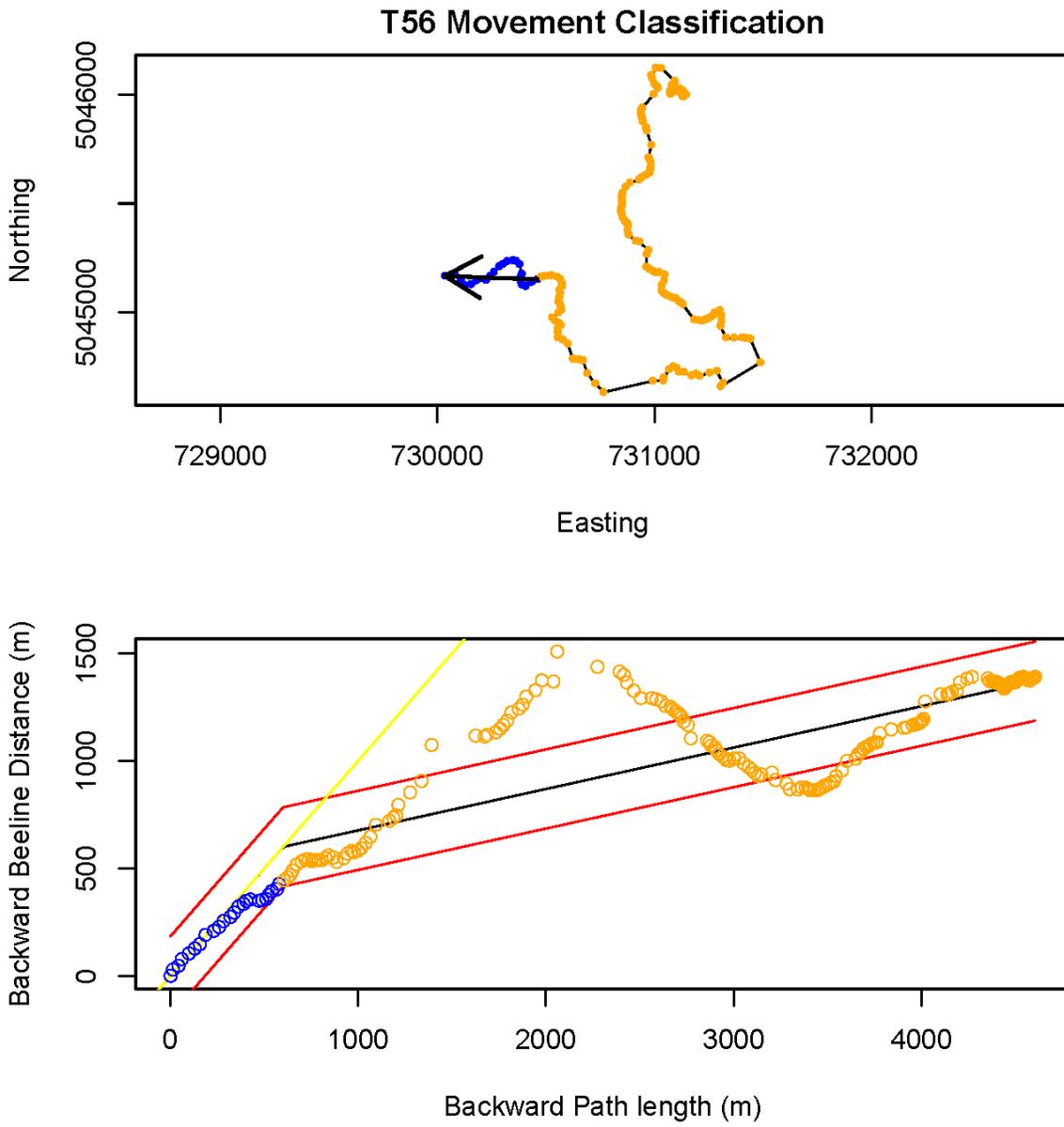


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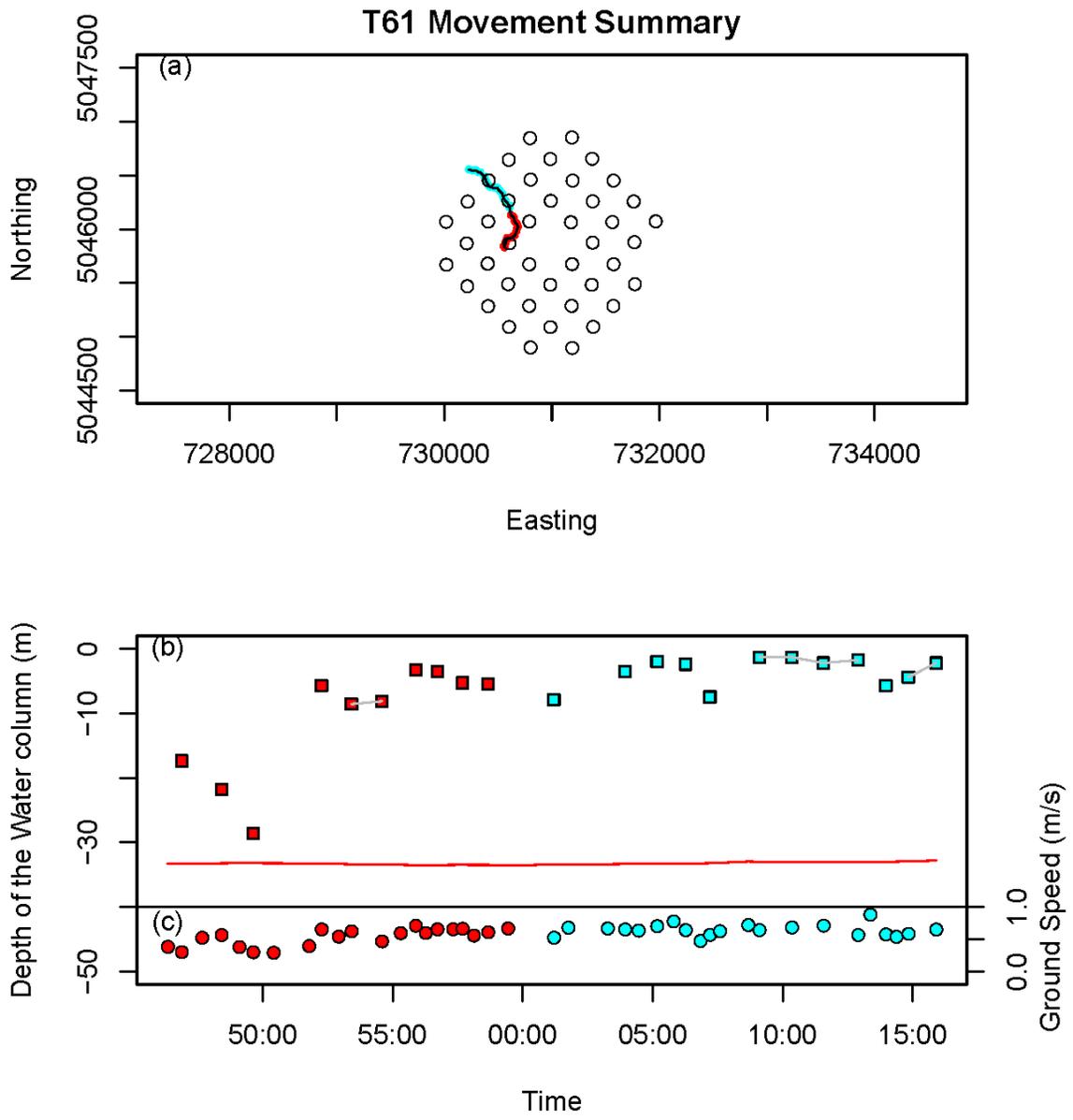


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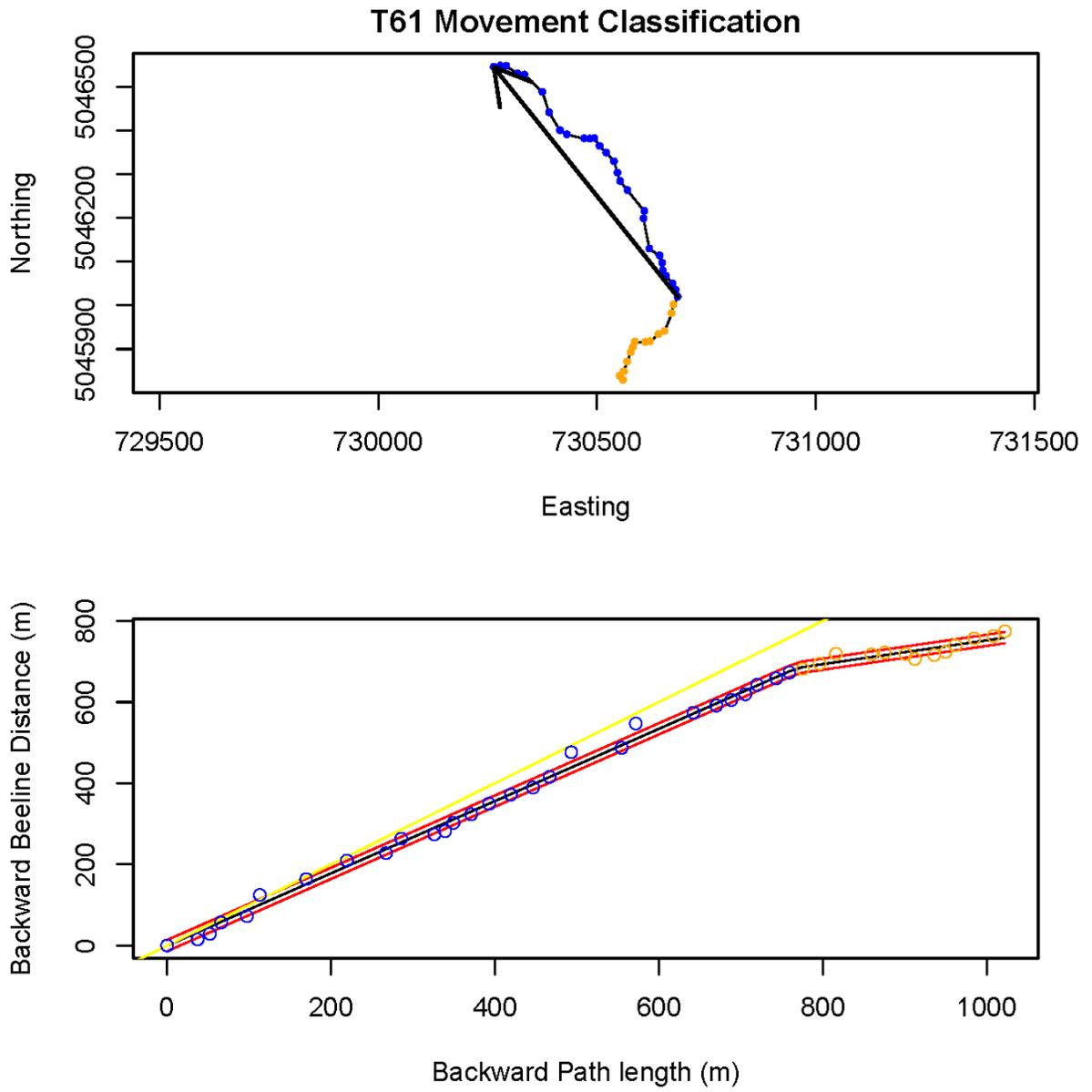


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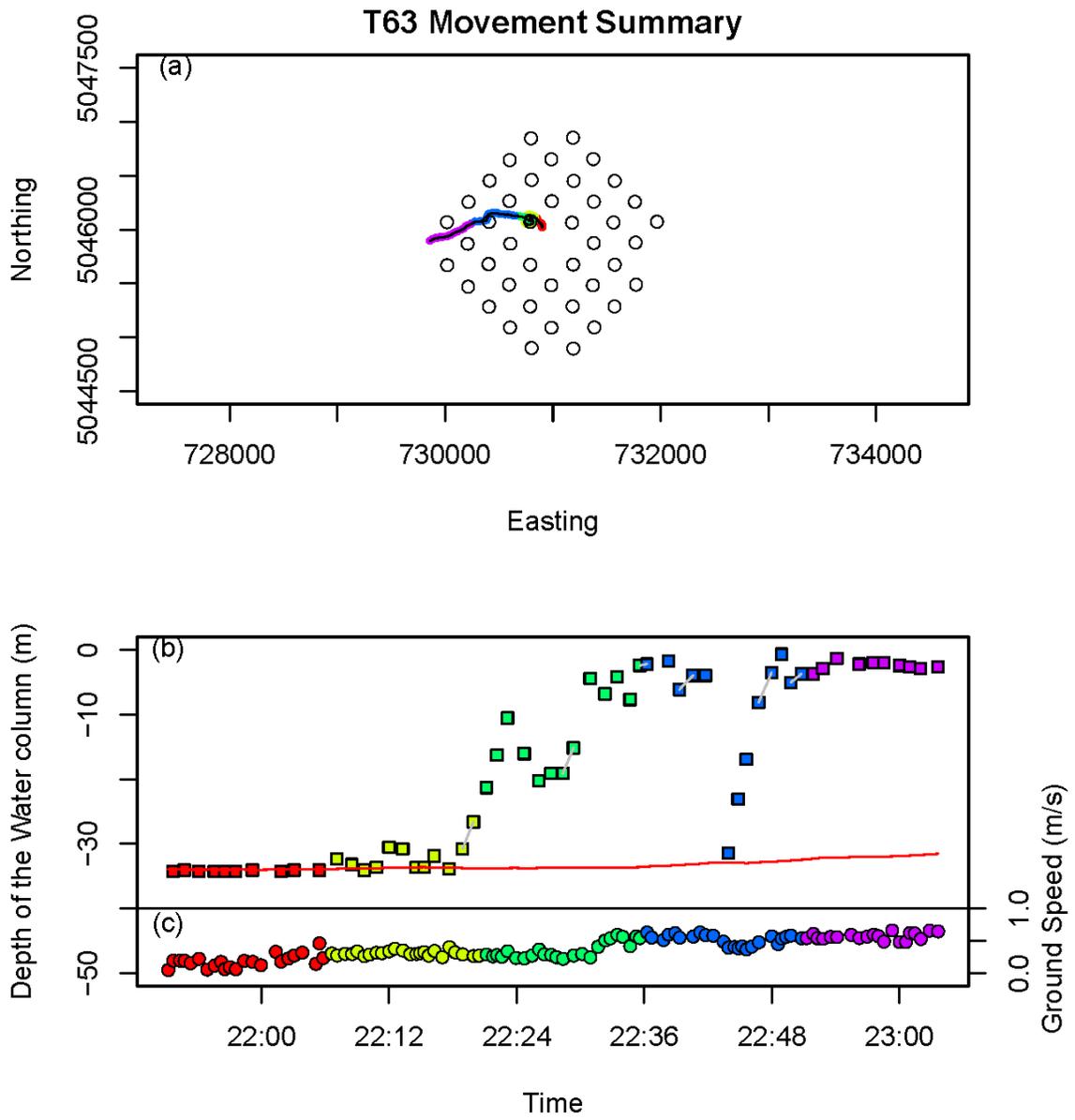
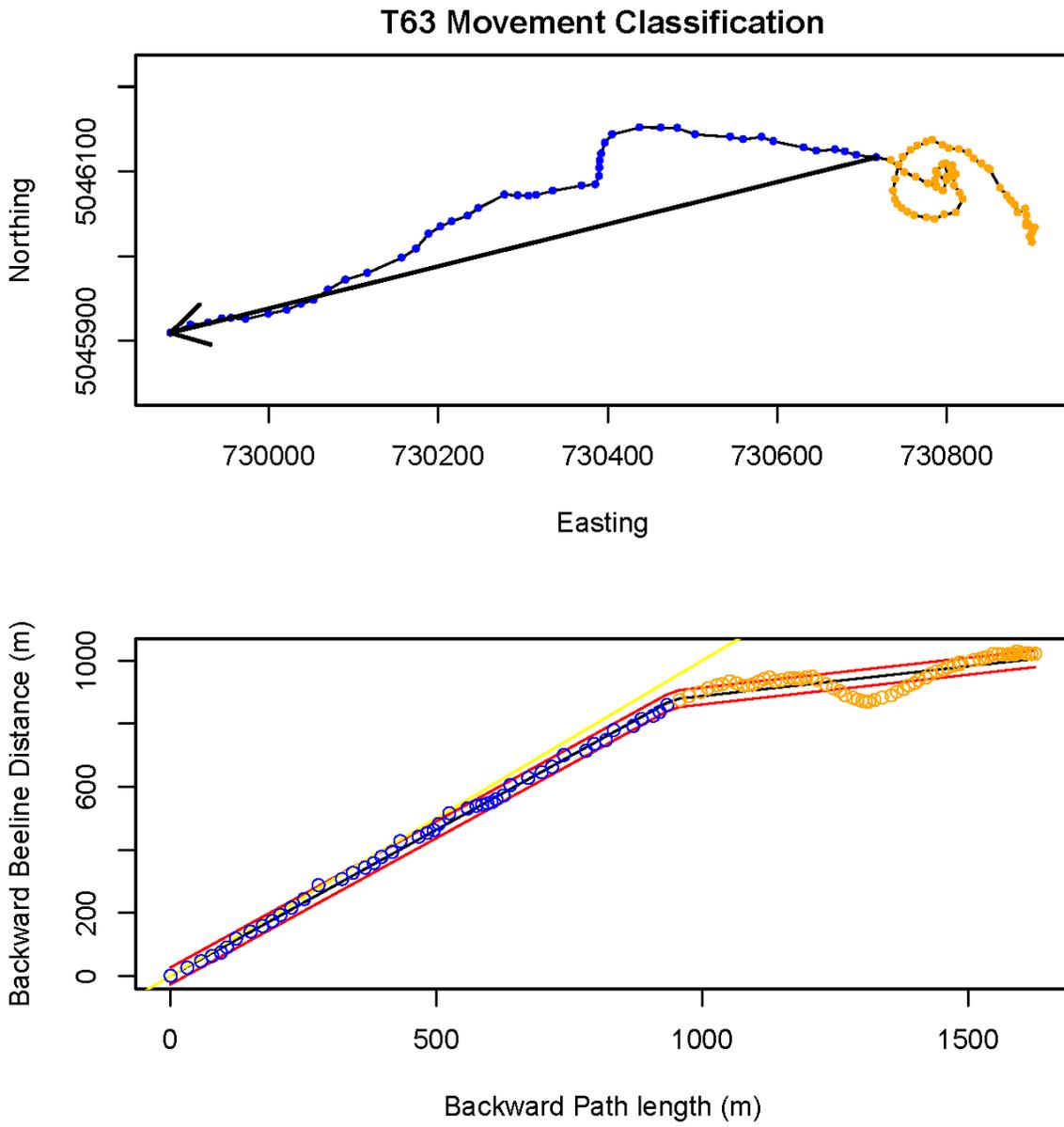


Figure 3.10: (cont'd)



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CHAPTER 4

DOES LARVAL SEA LAMPREY ODOR AID NAVIGATION OR GUIDE HABITAT SELECTION DECISIONS BY ADULTS

ABSTRACT

The ways in which non-homing fishes, such as sea lamprey (*Petromyzon marinus*), locate and select reproductive habitat is poorly understood. Prior studies indicated the presence of larval odor in river water increased the likelihood that a sea lamprey entered a river. However, it was not known whether larval odor played a role in navigation (guiding the migrant to the river mouth) or mediated habitat selection by labeling the suitability of a river for spawning. Fixed acoustic telemetry captured sea lamprey movement and a hydrodynamic model coupled with a dye concentration model provided estimates of the Ocqueoc River plume position through time. This allowed for the reconstruction of the hydrodynamic experience of sea lamprey as the approached the coast, encountered river water, and entered a river in the Great Lakes. Once reaching a coast and encountering river water, sea lamprey deflected along the physical coastal edge within the river water labeled region of the lake and approached the river, consistent with a thigmotactic search mechanism rather than a chemotactic search mechanism, where gradients of odorants are important. These observations compliment other evidence to suggest that encounter with river water absent larval odor is sufficient for migrants to transition to local search, while larval odor guides the river entry decision. The river entry decision may have occurred before entry, though it was never reversed following entry. Three synthesized components found in the full larval odor had no apparent effect on the likelihood of river entry when compared to the entry rate previously observed in the Ocqueoc River between 1 and 2 years after pesticide based

removal of larvae. These known components of larval odor appeared insufficient to bias river entrance; however, the results suggested that the use of the entire larval odor could be a powerful management tool, capable of manipulating the river entry decision.

INTRODUCTION

Animals can use physical and chemical information to find and select reproductive habitat. Olfactory signals associated with a resource can label directional information that leads to a target and remotely inform the searcher of the profitability of the target (Vickers 2000). When studying animal search, determining whether a particular odor is important to navigation to a target or assessment of target quality is difficult. Sea lamprey adults do not home and rely on the odor of larval sea lamprey from previous generations residing in a stream to select a river (Morman et al. 1980, Teeter 1980, Bergstedt and Seelye 1995, Waldman et al. 2008). Once in a river, sea lamprey are hypothesized to search using odor mediated rheotaxis to locate habitat and mates (Bjerselius et al. 2000, Vrieze et al. 2011, Johnson et al. 2012, Meckley et al. 2014b). However, it is unclear how sea lamprey search in a river plume, defined as the dynamic region of a lake where river water mixes with lake water, or how they enter rivers. Lab tests designed to mimic a river plume demonstrated that sea lamprey are attracted to river water lacking larval odor (Vrieze and Sorensen 2001). This opens the question of whether larval odor or river water plays a role in the decision to enter a river or whether larval odor is important to both river selection and navigation in a river plume.

The sea lamprey migration in the lake is just beginning to be described. On the coast in the Great Lakes, sea lamprey display extensive movements parallel to the coast, typified by straight movements while completing vertical excursions from the surface to the bottom of the water column until encountering a river plume (Vrieze et al. 2011, Meckley et al. 2014b), during

their non-homing migration (Bergstedt and Seelye 1995, Waldman et al. 2008). The vertical movement is hypothesized to improve encounter with migration cues found in stratified layers of the water column and may be involved in orientation (Vrieze et al. 2011, Meckley et al. 2014b, Chapter 3). Upon encountering a river plume sea lamprey transitioned to intensive movements typified by horizontal turning and continued vertical excursions, as well as regular deflections off the coast in the area of the river plume, as individuals moved through the river plume and localized the river mouth (Meckley et al. 2014b).

A general characterization exists of how sea lamprey navigate in rivers and move along the physical coastal edge to encounter river plumes (Vrieze et al. 2011, Meckley et al. 2014b), though how sea lamprey move in river plumes and the functional role of larval odor remains unclear due to the limitations of the tracking equipment of earlier studies. Some have suggested sea lamprey search in river plumes through kinesis (e.g., Vrieze et al. 2011), an undirected search absent directional information, while others have suggested a directed search mechanism reliant on physical edges (e.g., Thigmotaxis, Meckley et al. 2014b). Larval odor could increase selection of a river by labeling good habitat that guides decision making or by playing a role in navigation, allowing sea lamprey to localize the river mouth more easily. Larval odor influences the number of sea lamprey to enter a river, demonstrated by the large reduction in sea lamprey trapped in rivers the year after mass removal of lamprey larvae (Moore and Schleen 1980).

Olfaction is thought to be integral to sea lamprey finding and entering rivers in the Great Lakes (Vrieze et al. 2010). Reliance on odor for habitat selection sets up the potential to switch from traditional management (e.g., dams, pesticide) to odor based manipulation of migration routes, guiding sea lamprey into habitat that is easy to manage or is unfit for larval survival (Li et al. 2007). Manipulation of the invasive sea lamprey's habitat selection with the use of larval odor

has been greatly anticipated as an integrated pest management solution, although it requires identification and synthesis of the components in larval odor to be able to activate rivers (Sorensen et al. 2003, Johnson et al. 2006, Hoye et al. 2007). To our knowledge, only three components of the larval odor have been identified and synthesized (Hoye et al. 2007). Field experiments indicate that sea lampreys can detect and respond to incomplete odors to facilitate the search for reproductive opportunities (Fine and Sorensen 2008, Meckley et al. 2014b), but ultimately require encounter with a complete composite of the components that represent a signal prior to selecting spawning habitat (Wagner et al. 2009, Meckley et al. 2012, Meckley et al. 2014a) or a mate (Johnson et al. 2006, Johnson et al. 2009, Luehring et al. 2011). The river mouth would be the most likely location for a partial odor to inform a decision, as the complete odor could be the most dilute rendering the most concentrated components the most reliable indicators of larval presence (Meckley et al. 2012). If a collection of partial odors fails to elicit entry at the river mouth, it would indicate the need for identification and synthesis of additional compounds found in larval odor.

Here, we report a field experiment that evaluated whether larval odor influences the river-entry decision or whether the cue is important to navigation. Secondly we provided a test of whether the addition of synthetic larval odor increases entry beyond what is expected two years after TFM treatment. During a two-year study in 2010 and 2011, we documented the three dimensional movements of acoustically tagged sea lamprey as they approached, entered, or bypassed a river under one of two conditions: (1) low larval odor, following larval removal with a pesticide (TFM), below (2008) and above a barrier (2009) (2010); and, (2) higher larval odor, created by larval recruitment, plus synthetic larval odor components Petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), and petromyzonol sulfate (PZS) to a 1×10^{-12} M

concentration (2011). A coupled hydrodynamic model and dye concentration model provided environmental information including water current direction and speed, water temperature, and the presence of river water at each fish position for the entire study period allowing us to characterize the sea lamprey migration experience with unprecedented precision. We specifically tested three hypotheses. First, sea lamprey will encounter the river plume at the same rate between years, although more will enter in 2011 due to a higher larval odor concentration a year after any TFM treatment in the Ocqueoc River and following natural recruitment of larvae (H1). Second, higher larval odor will not increase how close sea lamprey come to the river mouth but will influence how sea lamprey move in the river plume for those that encounter river water (H2). Third, the addition of the known synthetic odor components of the full larval odor composition will be insufficient to increase entry rate beyond the expected river entry rate (H3). The expected river entry rate was based on the increase historically observed between one and two years after pesticide treatment in the Ocqueoc River (H3).

METHODS

General Methods

Acoustically tagged sea lamprey were released at three staggered locations 700 m north of the Ocqueoc River (45.490278°, -84.072931°) in Hammond Bay (Lake Huron) at the center of a VEMCO positioning array capable of providing 3-D positions every 30 seconds (Figure 4.1). Sea lampreys were released over two different spawning seasons (N=72, 2010; N=79, 2011) under two conditions including an assumed lower (2010) and higher (2011) larval population condition. Prior to the first year of the study (April 2010) both the lower (6 October 2008) and upper (22 August 2009) sections of the Ocqueoc river, separated by a dam, were treated with the pesticide 3-trifluoromethyl-4-nitrophenol (TFM). TFM treatment vastly reduced

the population in the Ocqueoc River, although the Ocqueoc River still receives a reduced but reliable sea lamprey run the year after treatment, as treatment is required every four years prior to larvae transforming to parasites and out migrating from the river to the lake (Aaron Jubar, US Fish and Wildlife Service, personal communication).

Study Site

The Ocqueoc river had an annual spring median discharge during trials of $2.64 \text{ m}^3 \cdot \text{s}^{-1}$ in 2010 and $3.9 \text{ m}^3 \cdot \text{s}^{-1}$ in 2011 (range: $0.6\text{-}9.7 \text{ m}^3 \cdot \text{s}^{-1}$ 2010, $1.8\text{-}8.6 \text{ m}^3 \cdot \text{s}^{-1}$ 2011). A telemetry system (VPS, VEMCO) with a similar configuration of acoustic receivers (VR2W) and coverage in 2010 and 2011 was composed of 41 receivers located in a 0.90 km^2 in 2010 and 37 receivers covering 1.57 km^2 in 2011 and centered on the mouth of the Ocqueoc River (45.490278° , -84.072931°). The receiver configuration was in diamond formations with between receiver spacing ranging from 75 m to 250 m (Figure 4.1). The depth of the site ranged from 0 to 5.8 m within the array but sea lamprey were positioned at greater depths on the fringes of the array. Nine independently moored synchronization transmitters (VEMCO model V16-2H, 69 kHz) were deployed in stationary positions through the array. In addition to the receivers in the acoustic array, receivers were positioned in the Ocqueoc River at six locations monitoring upstream progress in 2010 and 2011.

Experimental Subjects

The ethical treatment and acoustic tagging of sea lamprey was approved by the Michigan State University Institutional Animal Use and Care Committee via animal use permit 02/10-020-00. Adult female sea lamprey were obtained from the Cheboygan River at a barrier integrated trap (2010: N=72, trapped from 15, April to 10, May; 2011: N=79, trapped from 30, April to 15, May). In 2010, Sea lampreys were 417-567 mm in length (mean 495 mm) and weighed 148-409

g (mean 247.7 g). In 2011, Sea lampreys were 458-588 mm in length (mean 506 mm) and weighed 191-389 g (mean 274 g). All Sea lamprey were held in 150 L flow through tanks that cycled ambient Lake Huron water (100% exchange every 2 h) and experienced a natural light cycle. Acoustic transmitters transmitted signals every 15-45 s, though one contained a pressure sensor that transmitted pressure every transmission (2011) and the other transmitted pressure every other transmission (2010) (model V9P-2H, Vemco, Halifax, Nova Scotia, Canada 9mm D x 47 mm L, mass: 6.4 g in air, 3.5 g in water, power output 150 dB (re 1 μ Pa at 1 m)) (model V9-2H, Vemco, Halifax, Nova Scotia, Canada, 9mm diameter, 29 mm length: mass: 3.6 g in air, 2.2 g in water, power output 151 dB (re 1 μ Pa at 1 m)). The model with alternating pressure transmissions was selected in 2010 due to its shorter duration transmission length to ensure transmission collisions did not prevent position acquisition. Acoustic tagging procedures followed methods by Meckley et al. 2014b. Prior to surgery sea lamprey were anesthetized by immersion in 0.2 mL \cdot L⁻¹. The anesthetic solution used in this experiment was composed of 2 mL of clove oil (minimum 84%-88% eugenol, Lot No. HB9387, Hilltech Canada Inc. Vankleak Hill, Ontario, Canada) diluted in 18 mL of 70% ethanol and vigorously mixed into 10 L of Lake Huron water. Sea lamprey took 634 ± 11 s to reach stage four of anesthesia, as denoted by unresponsiveness to touch but retained gill movement, at a temperature of 8.0 ± 0.1 °C (mean \pm 1 SE). The surgical procedure took an average of 319 ± 7 s (mean \pm 1 SE) and equilibrium was regained in 527 ± 32 s (mean \pm 1 SE). Transmitter-implanted subjects were held for 72 h prior to release to ensure metabolism of stress compounds (Close et al. 2003).

Pheromone application

Synthesized PADS (MW: 722 g), PSDS (MW: 625 g), and PZS (MW: 473.4 g) were applied continuously at a ratio of 1:1:1 based on molecular weight, from 4 May to 6 June 2011,

900 m from the river mouth to the middle of the water column in the center of the stream. PADS, PSDS and PZS were synthesized by Bridge Organics, Inc. (Kalamazoo, Michigan, USA). The purity of each product was confirmed at >95% via high-pressure liquid chromatography (HPLC) with a purity evaporative light scattering detector and mass spectrometry by Xiodan Xi at Michigan State University. Pheromone applications were permitted by the US Environmental Protection Agency through experimental use permit 75437-EUP-1 and the Michigan Department of Environmental Quality pursuant to Rule 97. Prior to application, we created 1.0 mg·mL⁻¹ stock solutions for each compound via dissolution into 1:1 v/v methanol:water. A single stock solution for each compound was used during the course of the study. The final odor was prepared by mixing the required amount of each stock solution into a necessary amount of river water before being pumped into the stream at a fixed rate of 600 mL·h⁻¹ via a battery-operated programmable peristaltic pump (Admiral Reef Dosing Pump, Norwich, Connecticut, USA).

VPS Array Performance and data treatment

The performance of the VPS array in terms of position accuracy was tested by comparing the VPS position estimates of V9P-2H transmitter to GPS measured positions (Trimble Geo XH, post processed). Array performance was tested by either attaching a transmitter to an anchored line as described in Meckley et al. 2014a (stationary testing) that provided longer term information (2010: Location 1: 17 June 2010 19:43 01 July 2010 14:34 (N= 29,355 detections), Location 2: 01 July 2010 14:52 to 08 July 2010 17:03 (N= 16,400 detections); 2011: 09 June 2011 016:30 to 24 June 2011 04:07 (N= 26,628 detections)) or by dragging transmitters while inside the body cavity of a sea lamprey that was recently deceased, located 1 m off the bottom and below a floating boat powered by an electric motor with the GPS mounted directly above the tag monitoring the tags' true position (2010: N=138; 2011: N=309). The mean unfiltered

accuracy estimates from stationary tests were 2.0 ± 6.6 m (2010 Test 1), 1.1 ± 5.3 m (2010 Test 2), 1.5 ± 1.4 m (2011 Test 1) (mean \pm SD). The mean unfiltered accuracy from the mobile tests was 3.0 ± 6.2 m (2010) and 5.4 ± 12.5 m (2011) (mean \pm SD).

Three data quality objectives were selected, including the need to ensure that trajectory data (e.g. turn angles) could be considered, designations of when the sea lamprey were stopped or moving were reliable based on first passage time; large losses in data did not occur while achieving the first two objectives that could result in misrepresenting the tracks (Table 4.1). Trajectory based analyses often break down when average position error is > 10 % of the mean step length (Bradshaw et al. 2007). In this case the mean step length was 17.7 m, resulting in a filter objective of less than 1.77 m average error (Meckley et al. 2014 a; Table 4.2). To avoid incorrect behavioral assignment of swimming or stopped we wanted to remove large erroneous positions (e.g. >15 m (citation)). Finally we wanted to maximize the removal of inaccurate data and retention of accurate data to ensure proper assignment of when the fish encountered river water in our dye concentration model, a step that should be possible in our array conditions, this meant retention of 95 % of acceptably accurate (<6 m) data and removal of 99 % of inaccurate data (> 6 m). If a large amount of data was lost, the analysis that required the data quality objective would have been replaced. An HPE filter of 8 was selected in 2010 and an HPE filter of 7 was selected in 2011 based on the three criteria regarding the position precision estimates and data retention (Table 4.1, Table 4.2). The filtered coverage was 8.22 km^2 in 2010 and 10.95 km^2 in 2011. The filter removed 9% of fish positions inside the array in 2010 and 7% of positions inside the array in 2011.

Hydrological Data

Bathymetry in the region of the array was recorded via 50 meter transects oriented

towards and away from shore and then repeated parallel to shore. Bathymetric data were collected with an acoustic Doppler current profiler (ADCP; Workhorse Rio Grande 1200 kHz, Teledyne RD Instruments, Poway, CA). Data from four ADCP beams were combined in real time with geographic locations from a WAAS-enabled GPS (model Geo-XT, Trimble Navigation Ltd., Sunnyvale, CA) using the software program BathMapper (courtesy of A. Blake, U.S. Geological Survey, California Water Science Center). Total water column depths at fish positions were interpolated from the georeferenced depth data using inverse distance weighting. A 2 MHz upward facing Aquadopp Current Profiler (ADCP, Nortek-USA) was deployed on the bottom in 7.9 m of water at two nearby locations asynchronously from the 8 May to 30 May (45.50207737, -84.07552895) and from the 30 May to 17 June, 2011 (45.50207846, -84.07552753). The ADCP recorded water movement in 3 dimensions at twenty slices through the water column and was used to check the hydrodynamic model (see below for more information). Five HOBO temperature loggers were deployed each at three locations in front of the river mouth in the lake (from mouth; left: 300 m, center: 150 m, right: 350 m) farther offshore (700 m) and in the river for the duration that receivers were deployed. We estimated the stream discharge daily at the application site using the midsection transect method with a Doppler flow meter (Flo-Mate Model 2000, Marsh-McBirney)(Gore 2007).

Hydrodynamic Models: Two models describe the circulation of lake water in Hammond Bay of Lake Huron (Numerical Model for 2010 and 2011) and how river water mixes with lake water forming a zone of river influenced lake water, which we refer to as the river plume (dye concentration model). We estimated the water conditions at each fish position and through the region including current speed and direction, water temperature, and dye concentration as an estimate of the presence of river water.

Numerical Model: The three-dimensional unstructured grid numerical model (FVCOM; minimum grid size: 9.15 m²) is used to describe circulation and thermal structure in Hammond Bay, Lake Huron (Nguyen et al. 2014) (Figure 4.2). The model solves the hydrodynamic primitive equations using the hydrostatic assumption in the vertical direction with the Boussinesq simplification for convective flows. The continuity, momentum, and temperature equations are shown in Equation 1 to Equation 5.

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = 0 \quad (1)$$

$$\frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} + w \frac{\partial u}{\partial z} - fv = -\frac{1}{\rho_0} \frac{\partial P}{\partial x} + \frac{\partial}{\partial z} \left(K_M \frac{\partial u}{\partial z} \right) + F_u \quad (2)$$

$$\frac{\partial v}{\partial t} + u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial y} + w \frac{\partial v}{\partial z} + fu = -\frac{1}{\rho_0} \frac{\partial P}{\partial y} + \frac{\partial}{\partial z} \left(K_M \frac{\partial v}{\partial z} \right) + F_v \quad (3)$$

$$\frac{\partial P}{\partial z} = -\rho g \quad (4)$$

$$\frac{\partial T}{\partial t} + u \frac{\partial T}{\partial x} + v \frac{\partial T}{\partial y} + w \frac{\partial T}{\partial z} = \frac{\partial}{\partial z} \left(K_H \frac{\partial T}{\partial z} \right) + F_T \quad (5)$$

Here (u, v, w) are velocity components in horizontal and vertical directions, respectively; ρ is the density; ρ_0 is the reference density; T is the temperature; P is the pressure; f is the Coriolis parameter; g is the acceleration due to gravity; (F_u, F_v, F_T) are the horizontal momentum and thermal diffusion terms. Vertical eddy viscosity and diffusivity (K_M, K_H) are modeled using the Mellor-Yamada 2.5 level turbulence closure scheme (Mellor and Yamada 1982, Galperin et al. 1988). The horizontal diffusion coefficients are calculated using the Smagorinsky turbulence closure model (Smagorinsky 1963). Comparisons between vertically averaged current and temperature from the ADCP observations and simulations provided a good fit suggesting the

models effectively predicted water conditions in Hammon Bay (Nguyen et al. 2014) (Figure 4.3, Figure 4.4).

Dye Concentration Model; the dye concentration model is coupled with the hydrodynamic model and was solved using the following equations:

$$\frac{\partial DC}{\partial t} + \frac{\partial DuC}{\partial x} + \frac{\partial DvC}{\partial y} + \frac{\partial wC}{\partial \sigma} - \frac{1}{D} \frac{\partial}{\partial \sigma} \left(K_h \frac{\partial C}{\partial \sigma} \right) - DF_c = DC_0(x, y, \sigma, t)$$

where C is the concentration of the dye, D is the total depth, u , v , and w are the x , y , and σ components of the water velocity, K_h is the vertical diffusion coefficient, F_c is the horizontal diffusion term, and C_0 is the initial dye concentration (*Chen et al.*, [2012]). The dye concentration is calculated at every grid cell throughout all layers in the water column in which the initial condition of dye concentration is zero at every grid cell of the FVCOM grid. The dye concentration from river was kept at 100ppm throughout the simulation time though the amount of water released is based on river discharge and influences C_0 . The river plume is then determined based on the values of the vertically-integrated concentration at every grid cell. The comparisons for velocity and temperature from hydrodynamic model are acceptable, providing confidence for the dye concentration model as it is coupled with hydrodynamic model. The fact that this model is coupled with the hydrodynamic models and those models have a good fit with observed data, we have confidence in the predictions of the dye concentration model (Figure 4.3, Figure 4.4).

Identifying active sea lamprey

To characterize the periods of sea lamprey activity in the lake, we first had to robustly classify sea lamprey positions as active or stopped. We achieved this with a first passage time classification method performed in R (R Development Core Team 2015). The first passage time

tool classified a position as moving if it left a radius of 10 meters in 250 seconds, had a minimum displacement of 15 m in a 3 position moving average, and at least 4 consecutive moving observations. The characterization method was checked through visual inspection and was robust to imprecision in acoustic positioning after filtering (Gurarie et al. 2015).

Each night of activity that an individual sea lamprey was observed in the array was characterized into a unique bout of activity for that individual. For each step comprised of two consecutive positions that was separated by less than ten minutes was used to calculate trajectory data. The midpoint of each step was used to estimate environmental information experienced during the step and the distance to key features (e.g. nearest coast or river). The trajectory data included heading (Φ ; radians $0-2\pi$), turn angle (Θ , phase angle; radians π (right turn) – 0 (straight) - $-\pi$ (left turn)), and ground speed (m/s). The lake water temperature, lake water current magnitude and direction, whether during a bout a sea lamprey had yet encountered river water, the distance to the nearest coast and the distance to the river were all determined in R for each mid-point.

Characterizing river plume encounter and entry

We separated individuals into two categories including individuals that stopped in the array after release (A) and all individuals released (B), as sea lamprey that stopped in the array after release provided a more complete depiction of the start of the lake migration. Both categories were further separated into sea lamprey that encountered river water on the first night and those that did not encounter river water. To determine if differences occurred in the rate of entry between years on the first night or ever, the rates at which sea lamprey encountered river water and entered the Ocqueoc River for both groups (A and B) were evaluated with a chi square tests of independence (χ^2)(R Development Core Team 2015). To consider if the rate of river

entry changed when individuals encountered the river plume on more than one night, entry of all sea lamprey (B) were tabulated in light of the number of nights they encountered the river plume before entry (rate of all available). Similarly the specific entry rates of individuals that returned and encountered river water on 1, 2, or more than 3 nights were quantified.

To evaluate the effect of a host of variables on river entry, a mixed effects logistic regression with a binary response variable (river entry: 1 or 0) was implemented in R using glmer (lme4 package, Bates et al. 2014). The binary response variable river entry (E) was considered for each night of activity that a sea lamprey encountered river water based on the dye model (dye concentration > 5); where individual (ID) was a random effect since multiple nights of activity could occur for the same sea lamprey. The fixed effects included year (Y), iterative number of nights encountering river water (N), a year by night number interaction(YxN), average nightly discharge of the Ocqueoc River (DC: $\text{m}^3 \cdot \text{S}^{-1}$), average lake temperature encountered (LT: $^{\circ}\text{C}$), average nightly river temperature (RT; $^{\circ}\text{C}$), the difference in experienced lake temperature and river temperature(DFT; $^{\circ}\text{C}$), minimum distanced reached from the river mouth (D: km), and average water velocity experienced in the lake (V) (Table 4.3). We used AIC (Akaike's information criterion) to guide model selection (Burnham and Anderson 1998; Burnham and Anderson 2002). We used AIC without criteria adjusted for sample sizes (AIC_C), as AIC_C requires a known number of independent observations and this is difficult to determine in mixed-effects models (Dodge et al. 2014; Burnham and Anderson 1998). All analyses were performed in R (R Development Core Team 2015), including the lme4 package.

To determine what influenced the way sea lamprey move we quantified movement behavior with ground speed (V_m , $\text{m} \cdot \text{s}^{-1}$) and path straightness. Activity was considered on the first night of activity, for active sea lamprey that stopped in the array following release and

encountered river water on the first night. Each step was associated with a ground speed (V) and all location associated variables were based on the mid-point of the step. Path straightness (S) was estimated by looking at the total path length versus the beeline distance for sets of ten consecutive points. We fit models with a nonlinear mixed-effects model in the formulation described by Lindstrom and Bates (1990), using the function “nmlme” in R (R Development Core Team 2015). This method allowed for incorporation of the random effect of each individual lamprey (ID) and first order auto-correlation in the residuals to be accounted for in the models. The significance of effects within the best model was assessed by evaluating the t-values and p-values; where large t values and p-values less than 0.05 were considered significant.

The closest distance each sea lamprey was observed to the river for all sea lamprey and for those that encountered river water and did not enter was determined and tested for differences between years with a Mann-Whitney U test with an alternative hypothesis that there was no difference between the means. The time it took for entry to occur was also tested between years with a Mann-Whitney U test that sea lamprey would take longer to enter in 2010. The upstream progress of sea lamprey was monitored at 3 pairs (N=6) of acoustic receivers in the river that could capture passage and allow for interpretation of upstream or downstream movement. We used these receivers to determine river entry and the time it took for sea lamprey to reach the lamprey barrier on the Ocqueoc River located 7916 m from the river mouth.

Movement Patterns

Ground speed was evaluated as a continuous response variable with respect to year (Y), water temperature at fish location (T, C°), an interaction between current magnitude and the angle between fish heading and current direction (as a turn angle in radians: $0-\Pi$, where 0 indicated movement into the current and Π away from current) (CxF, radians $0-\Pi$), whether the

fish position was inside or outside of the river plume (dye concentration of > 5) (P), and whether encounter with river water occurred yet in each path (E). The average time difference between points was 49 s with a max of 569 s (<10 min). We included the cumulative distance traveled (D) for each sea lamprey path in each model as previous observations captured increasing ground speed at the start of the night (Chapter 3). Animal identification number (ID) was included as a random effect and first order autocorrelation in the residuals was taken into account. Ten competing mixed effects models were fit (Table 4.4).

Path straightness (S_m) ranges from 0-1 and was evaluated as a continuous response variable with respect to the fixed effects of whether sea lamprey had encountered river water in the path yet (E), whether sea lamprey were in the river plume (P), year (Y), total cumulative path length (D), and additional interactions within these variables. Changes in path straightness are often used to identify local search behavior (Lohmann et al. 2008, Dodge et al. 2014). Animal identification number (ID) was included as a random effect and first order auto correlation in the residuals was taken into account. Ten competing mixed effects models were fit (Table 4.5).

To characterize how sea lamprey migrate outside of the river plume, we summarized path length versus the approach ratio (distance moved towards the coast/total distance moved) to the coast where -1 would indicate progress exactly away from the coast, 0 would be movement parallel to the coast and 1 would represent movement towards the coast. The distance from shore when sea lamprey exited the array while moving parallel to the coast was summarized and the direction chosen with respect to the local current encountered was tabulated. To test if the direction chosen was opposite the local water current encountered while moving in the array, we tested the proportion of sea lamprey to move east on a night when the current was moving from the east or from the west with a chi square test (χ^2).

Effect of Synthetic Odor

The effect of synthetic larval odor is difficult to evaluate in light of the presence of larvae in the system. The only metric for evaluating the influence of the three synthetic larval odor components comes through comparison of the historical entry rate of wild sea lamprey one and two years post TFM treatment and compared to the entry rate of the sea lamprey released in our study. The percentage of sea lamprey to enter the Ocqueoc River of the total available migratory sea lamprey in Lake Huron was achieved by comparing population estimates provided by Jessica Barber of the Fisheries and Wildlife Service. The Ocqueoc River population estimate was based on mark recapture estimates from the Ocqueoc River alone and the Lake Huron estimate was estimated based on extrapolating mark recapture data from a set of reference streams (Mullett et al. 2003). Although there are uncertainties in comparing the magnitudes of estimates through time, the general pattern post pesticide application is robust.

RESULTS

Sea Lamprey Activity

Sea lamprey primarily started moving during the half hour before nautical twilight and stopped in the half hour before sunrise (Figure 4.5A). River entry was occasionally observed prior to nautical twilight (set), although river entry was never observed after nautical twilight (rise) in the morning (Figure 4.5D), despite activity in the lake extending until and occasionally after sunrise (Figure 4.5B). There was some evidence of multiple pulses of river entry, including a pulse of entry after nautical twilight (set), 2.5 hours after nautical twilight (set), and just prior to nautical twilight (rise) (Figure 4.5C, Figure 4.5D). Stopping in the array during nautical twilight was rare and prolonged periods of inactivity consistent with staging were not observed.

Characterizing river plume encounter and river entry

There was no difference in river plume encounter between 2010 and 2011, although all measures suggested a higher rate of entry in 2011, in support of hypothesis 1. The majority of sea lamprey stopped in the array after release in 2010 (53 of 72; 74%) and 2011 (59 of 79; 75%). A low proportion of sea lamprey that exited the array coverage immediately after release were not observed again (N=9, 2010; N=11, 2011). Our observations showed no difference in the likelihood of encountering river water on the first night in 2010 (31/53; 58%) or 2011 (41/59; 69%) for trial animals that stopped in the array after release ($\chi^2 = 1.03$, $df=1$, $p=0.3$; R-core Team 2015). Similarly, there was no significant difference in the likelihood of ever encountering river water from the Ocqueoc River for all released in 2010 (60/72; 83%) and 2011 (61/79; 77%) ($\chi^2 = 0.54$, $df=1$, $p=0.46$); which includes individuals that encountered river water while moving after release, and those that encountered river water after the first night. Of those we observed moving at night, 48 (66%; 2010) and 53 (67%; 2011) encountered river water during search, the eventual river entry rate of these individuals was 0.5 in 2010 and 0.85 in 2011. Sea lamprey entered the Ocqueoc river at a higher rate (1.72) in 2011 (45/79; 57%) than in 2010 (24/72; 33%) ($\chi^2 = 7.55$, $df=1$, $p=0.006$). Sea lamprey that stopped in the array and encountered the Ocqueoc River water on the first night in 2011 were significantly more likely to enter the river on the first night than in 2010 (2010: 7/31, 23%; 2011: 18/36, 50%; $\chi^2 = 0.18$, $df=1$, $p=0.04$). Sea lamprey that ever encountered river water during the year eventually entered the Ocqueoc River at a higher rate (1.85) in 2011 (45/61; 74%) than 2010 (24/60; 40%) ($\chi^2 = 12.732$, $df=1$, $p<0.001$). The pattern of higher entry in 2011 than 2010 was consistent for those that did not enter on the first night and were observed encountering river water on additional nights (Figure 4.6).

The best fitting models for river entry included year and minimum distance reached from the river, and either the lake temperature experienced (Model 10) or the difference in lake temperature experienced and the river temperature (Model 11) (Table 4.3). Only year (year 2011; $\beta=0.89$), and the effect of the minimum river distance was significant (-2.1), where minimum distance observed related to a reduced rate of entry ($p<0.5$) (Table 4.6, Model 11 output). River entry did not appear to be influenced by discharge or absolute river temperature. The number of nights observed in the array did not appear as an important factor as we expected, though the pattern of sea lamprey entering if they returned in 2011, and not entering upon return in 2010 was present in the interaction effect between night and year (Figure 4.6).

The distance sea lamprey were observed from the Ocquoec River mouth did not vary between 2010 and 2011 for all sea lamprey, in support of hypothesis 2 (mean \pm 1 SD: 210 \pm 266 m (2010); 129 \pm 177 m (2011; Mann-Whitney $U = 1065$, $p=0.54$) or for those sea lamprey that didn't enter the river (mean \pm 1 SD: 291 \pm 281 m, 2010; 349 \pm 236 m, 2011) following river water encounter (Mann-Whitney $U = 229$, $p=0.25$) (Function "wilcox.test"; R Development Core Team 2015). Sea lamprey did take significantly longer to enter the Ocqueoc River in 2010 (116.9 \pm 125 hours; mean \pm 1 SD) than in 2011 (86.8 \pm 142 hours) based on a one sided Mann-Whitney U with an alternative hypothesis that sea lamprey would enter more quickly in 2011 ($W=1006$, $p=0.046$) (Function "wilcox.test"; R Development Core Team 2015).

In 2010, 24 of 72 sea lamprey (33%) entered the Ocqueoc River and were detected passing a receiver located 342 m from the river mouth and never reversed course past that point until late in the year once spawning had occurred. Sea lamprey that moved downstream were likely dead or dying, as determined by tags that were stationary in the array and based on recovery of some dead individuals. In addition, two sea lamprey moved close enough to the river

mouth or briefly entered the main channel and were detected by a receiver located 103 m from the river mouth. In 2011, 45 of 79 (57%) sea lamprey entered the Ocqueoc River and were detected passing a receiver located 342 m from the river mouth, and never reversed course and exited the river. One sea lamprey was detected on the lowest receiver and did not continue upstream. Two non-trial subjects (2 of 12) released prior to the start of odor pumping in 2011 in an attempt to capture staging behavior, did enter the river and go within the detection range of the receiver located 342 meters upstream before exiting the river, with one of the two returning to the river and progressing upstream in the same night. These two individuals were released in the visible river plume. In 2010, 19 of 24 sea lamprey reached the upper receiver, including 4 that reached the upper receiver on the first night, equaling an upstream ascent of $0.44 \pm 0.11 \text{ m}\cdot\text{s}^{-1}$ (mean \pm 1 SE) over the 7.9 km stretch of river. In 2011, 41 of 45 sea lamprey reached the upper receiver including 5 on the first night that had a ground speed of $0.38 \pm 0.08 \text{ m}\cdot\text{s}^{-1}$ (mean \pm 1 SE). The average time to reach the upper receivers (5 or 6) after reaching the second receiver was 44.8 ± 14.6 hours (2010) and 35.5 ± 6.8 hours (2011) (mean \pm 1 SE). The time to reach the upper receivers varied from 3.9 – 290.5 hours in 2010 and 4.1 – 187 hours in 2011.

Movement Patterns

Ground speed did not vary between 2010 and 2011, in support of hypothesis 2 (body length/Second; $\text{BL}\cdot\text{S}^{-1}$). The model comparison suggested that Model 9 had the best fit. The best model included temperature (T), if the sea lamprey was in the river plume (P), whether river plume encounter occurred (E), the interaction between lake current magnitude and whether the fish was moving towards it or away from it (CxP), and the interaction effect of cumulative path length and before and after encounter. Year had no apparent effect, as the two worst models included year (Table 4.4). The Model 9 fixed effects parameter estimates revealed that ground

speed increased with warmer water temperature ($\beta=0.02, p < 0.05$), increased following river plume encounter ($\beta=0.36, p < 0.05$), was influenced by the current direction fish movement interaction ($\beta=0.29, p < 0.05$), and prior to river encounter speed increased with cumulative path length ($\beta=0.16, p < 0.05$) but not after encounter ($\beta=-0.001, p=0.66$). Despite being part of the best model the effect of being in the river plume was small ($\beta=0.002$) and insignificant ($p = 0.88$) (Table 4.7, model 9 output).

Similarly, path straightness did not vary between 2010 and 2011, in support of hypothesis 2. The model comparison suggested that Model 9 and Model 6 were the best fit. The best two models included whether the sea lamprey was in the plume (P) and Model 9 included the interaction effect between cumulative distance traveled (D) and whether the river plume had been encountered (E) (Table 4.5). Model 9 fixed effects parameter estimates indicated that paths were more sinuous in the plume ($\beta= -0.0045, p < 0.05$), and that the path became straighter after settlement and before encounter with the plume as the path progressed ($\beta= 0.048, p < 0.05$), and again became straighter after encounter as the path progressed but the effect was weaker ($\beta= 0.018, p < 0.05$) (Table 4.8; model output).

Sea lamprey that did not encounter river water (N=33) in the array on the first night moved in a circuitous path before approaching the coast and then turning and moving parallel to the coast at some distance (Figure 4.7, Figure 4.8). The average distance from the coast at the final point exiting the array when moving parallel to the coast was 303 ± 221 m. More sea lamprey exited the array to the west (N=17) than to the east (N=7), while 9 were last observed moving to the north or south ($\chi^2 = 4.17, df=1, p < 0.05$). Individuals were not more likely to move east when current came from the east rather than the west (0.38 moved east with east current,

N=8; 0.15 moved east with west current, N=13) ($\chi^2 = 0.39$, $df=1$, $p=0.735$, R function “prop.test”).

Effect of synthetic odor

An annual increase in the number of sea lamprey estimated to enter the Ocqueoc River of the entire Lake Huron population is observed. Rates increased on the order of 1.8 (1995-1996), 1.3 (1999-2000), and 3.1 (2003-2004), an average historical rate increase of 2.1 times from one to two years after treatment. The observed rate increase of untagged sea lamprey was 2.1 for 2010 to 2011 for the Ocqueoc River population estimate for our two-year study, matching the historical average. To test if our observed total entry rate of sea lamprey that stop in the array on the first night and encounter river water was higher than the expected 2.1 historical average increase in entry, we ran a chi square test with an alternative hypothesis that the rate observed in 2010 of 0.35 would rise to greater than the expected 0.71 rate in 2011. 41 sea lamprey stopped in the array after release and encountered river water with 28 eventually entering the river. We would expect significantly more than a 29.11 individuals to enter if the entry increased with the average increase between year one and year two. There was no evidence of a greater rate of entry than would be expected two years after TFM treatment, which suggested no effect of synthetic larval odor (hypothesis 3) ($\chi^2 = 0.0$, $df=1$, $p=0.50$, alternative H0: “greater than”; function prop.test).

DISCUSSION

These results support our hypothesis that sea lamprey enter rivers with higher larval sea lamprey odor at a higher rate (H1). The effect of larval odor is consistent with altering the decision of sea lamprey to enter a river but does not have a measurable influence on how sea lamprey navigated in the river plume, as ground speed, path straightness, and the minimum

distance sea lamprey were observed from the river mouth was not influenced by the effect of year (H2). No evidence was obtained supporting an effect of synthetic larval odor on river entry (H3). We characterized the sea lamprey migration and report which aspects of the prior descriptions of sea lamprey migration in the lake are consistent with our observations.

We observed four general patterns of movement. First sea lamprey performed maneuvers consistent with the offshore pattern described as an orientation mechanism (Chapter 3), in which sea lamprey turn in a persistent direction and move slowly before approaching the coast at a faster ground speed and with a relatively straight course (Figure 4.9). Second, if sea lamprey did not encounter river water at some point when approaching the coast (mean 303 m from shore), they turned and moved parallel to the coast (Figure 4.8) more often to the west (Figure 4.7), with straight movements, presumably to encounter a river plume (Vrieze et al. 2011, Meckley et al. 2014b). The dominant current of Lake Huron would come from the west (Beletsky et al. 1999), though the local current experienced by individuals did not relate to the direction individuals exited the array (Figure 4.7). Third, when sea lamprey did encounter river water most individuals made repeated passes up and down the coast in the river plume, which switching between movements towards and away from the coast in an apparent rebounding pattern and counter turning at presumably the river plume edge in a behavior we term, coastal rebounding that was associated with faster ground speed and a more sinuous path. A series of tight rebounds were often observed before sea lamprey entered the river and we postulate with others (e.g., Vrieze et al. 2011, Johnson et al. 2012, Meckley et al. 2014b) that this is the point where sea lamprey transition from edge guided search to odor mediated rheotaxis.

Movement outside of the river plume

After stopping on the bottom in the array for the day most sea lamprey began moving shortly after nautical twilight, those that did not encounter river water approached the coast and then turned and moved parallel to the coast while either making regular vertical movements between the surface and bottom, alternating between periods at the surface and periods at the bottom, or by moving primarily on the bottom. The majority of sea lamprey exited the array towards the west (55% west, 25% north, 21% east), consistent with observations that most sea lamprey released 3.3 km from shore in Hammond Bay went west if they didn't enter the closest river and were observed at least 10 km away from the release point (60% (west), 20% (east), 20% (north) (Chapter 3). No advantage to moving in a consistent direction was identified unless a heading in a particular direction allows for the movement parallel to the coast to be maintained more easily or a direction is energetically more efficient. There was no apparent influence of the local current encountered on the direction sea lamprey moved along the coast (Figure 4.7), although the dominant lake current in this area of the lake would be from the west to the east (Beletsky et al. 1999). It is unclear if more sea lamprey moved west as a result of an inherent bias associated with handedness (Kells and Goulson 2001) or if it is due to a geographic or hydrological feature that is valuable to navigation. Observations of the sea lamprey migration on a coast oriented in a different direction would be valuable, as all observations of the lake migration to our knowledge have occurred in or near Hammond Bay, Michigan. It is important to note that eight of the 33 sea lamprey that did not encounter river water and exited the array on the first night returned and entered the river on a subsequent night, clouding the picture of whether sea lamprey move in a consistent compass direction or what features guide the direction of search along the coast.

Sea lamprey are likely capable of relying on multiple features of the coastal environmental as they traverse the coast in search of river water, as redundancy in search mechanisms is common (Able 1991). The same environmental features including the bathymetric slope that sea lamprey were postulated to use to return to a coast in chapter 3 could allow sea lamprey to move parallel to the coast; including, water column depth, absolute hydrostatic pressure, or water current. In addition to these feature, physical features of the lake bottom such as the coastal wave zone comprised of a comparatively steep series of banks and troughs that run horizontal to the coast and are easily observed on satellite images could be valuable (Figure 4.10). Use of the bottom structure could explain the periods of movement observed on the bottom before or after periods of vertical casting. A thermal bar along the coast also forms in spring when water above and below 4 degrees Celsius mixes, creating a physically distinct water mass along the coast (2-5 km wide, Rao and Schwab 2007), though this zone should extend beyond the distance sea lamprey were observed moving parallel to shore it still could label the coastal zone. Although blinded sea lamprey were capable of completing a river migration (Binder and McDonald 2007), vision may be valuable to the nocturnal migration as sea lamprey's eyes are sensitive to light, although it is unclear if this equates to strong night vision (Morshedien and Fain 2015). Our observations confirm the pattern of extensive search parallel to the coast but do not confirm a mechanism.

River plume encounter

River entry hinges on sea lamprey first encountering river water in the lake, localizing the river mouth, and finally entering the river. The number of sea lamprey to encounter a river plume will depend on both the rate of encounter and the number of sea lamprey to move past the river mouth. Olfaction is postulated as the primary mechanism for detecting river plume encounter;

however, other factors such as temperature or anomalies in river current at the mouth could be useful and could explain how nasally occluded sea lamprey still entered rivers in the Great Lakes (Vrieze et al. 2010). The rate of encounter will vary with the size of the river plume, principally how far river water extends away from the coast and how far from shore sea lamprey traverse the coast in that region. The size of river plumes vary with river discharge, coastal bathymetry, coastline configuration at the river mouth (e.g., embayment trapping water), and wind driven lake current (Churchill et al. 2003, Rao and Schwab 2007). Wind-driven current and the coastal boundary are the dominant features regulating the extent of the river plume in the Great Lakes (Churchill et al. 2003). River plume size is likely a key factor but is not explicitly linked with river discharge a potential explanation to why Moore and Schleen (1980) failed to see a relationship between sea lamprey entry and river discharge for rivers that were known to contain sea lamprey larvae. Sea lamprey traversing the coast at 300 m from shore in search of river water may miss many smaller river plumes, such as the Black Mallard River plume that was observed extending less than 250 m from shore on 80 % of nights in spring (Meckley et al. 2014b). The number of sea lamprey available to encounter the plume will depend on the number of sea lamprey to return to the particular region after the parasitic stage and whether they reach a given river or stop at a previous river. It remains unclear if the start of the migration could bias encounter with a specific region of a lake or if release from a host occurs broadly throughout the lake without major patterns, as virtually nothing is known about the behavior of parasitic sea lamprey beyond wounding rates (Jorgensen and Kitchell 2005, Silva et al. 2014). Additional research evaluating the conditions associated with the number of sea lamprey to enter a river would be valuable.

Movement in the river plume

Once in the river plume sea lamprey regularly moved towards and away from the coast as they made persistent progress along the coast; they reversed direction in shallow water and at consistent distances from the coast, a pattern termed coastal rebounding (Meckley et al. 2014) (Figure 4.11, Figure 4.12). We hypothesize that coastal rebounding relies on thigmotaxis within an odor defined region of the coast, where sea lamprey turn once reaching the edges of the river plume and the physical coastal edge to progress up and down the coastline. The behavior is consistent with localization and assessment, as sea lamprey often approached very close to the river mouth on multiple occasions without entering the river. A river mouth will almost always occur near one end of the river plume although some lake conditions can consistently create a more centrally located river mouth with respect to the river plume (García Berdeal et al. 2002, Churchill et al. 2003), and the river mouth may not be located at the strongest concentration of river water in cases where water is trapped within a basin. Coastal rebounding would allow for localization of the river mouth regardless of the dynamic river plume conditions.

Sea lamprey do not follow the coast in shallow water but instead repeatedly move toward the coast and offshore as progress is made along the coast. There are at least three explanations. First the rebounding nature may enable sea lamprey to avoid entrapment in complex shorelines but second, it could result in avoidance of habituation to olfactory information in the river plume. Sensory habituation can occur if a receptor is constantly exposed to a stimulus and becomes fatigued resulting in a loss of sensitivity (Ferrari et al. 2010). Olfactory habituation can be induced by multiple paradigms, although the type of habituation this type of movement would best avoid is habituation by a change in the function of the olfactory bulb, a process which takes longer than 30 minutes (Chaudhury et al. 2010). For example sea lamprey habituate to the odor

of dead adult sea lamprey after exposure for between two and four hours (Jason Bals, unpublished data). To address whether sea lamprey are reaching the edge of the river plume, a more accurate representation of the river plume than a dye concentration model would be required, which could be provided by a particle diffusion model as a large number of particles provide a more reliable edge structure (Mantha S. Phanikumar, personal communication). Third, the search could be a sampling period and sea lamprey could be collecting other information such as the presence of mates. Given that sea lampreys conceptually need to locate two things, spawning habitat and mates; it seems that recognition of mates could be important.

The role of larval odor

Our leading hypothesis is that once encountering a river plume individuals either enter, stage, or search for a different river based on the quality of the river and river temperature (Binder et al. 2010, Clemens et al. 2010, Vrieze et al. 2011, Meckley et al. 2014b). Odor and in particular larval odor has support from lab and field tests as an important cue that informs a migrant of the quality of a river (Moore and Schleen 1980, Vrieze and Sorensen 2001, Vrieze et al. 2010, Meckley et al. 2014b). In this study sea lamprey entered the Ocqueoc River at a higher rate in 2011 than 2010, two years removed from any TFM treatment. We saw no evidence that an environmental variable was associated with the way sea lamprey moved in the river plume or entry rate, although features like river discharge were higher in 2011 (discharge mean ($\text{m}^3 \cdot \text{S}^{-1}$) (minimum, maximum) 2010: 2.64 (0.6-9.7); 2011: 3.9 (1.8-8.6)) and river temperature varied between years (Figure 4.13). Our observations of reduced entry after treatment is consistent with historical observation in the Ocqueoc River (Figure 4.14), and is a wide spread pattern throughout rivers in the Great Lakes (Moore and Schleen 1980). These observations provide

additional support that river water alone is sufficient to induce local search, while larval odor influences the river entry decision.

Search behavior did not differ between years, consistent with the hypothesis that river water alone induced the transition from extensive search to local search in the river plume and larval odor influenced the river entry decision. This could only have been confirmed if no sea lamprey larvae were in the river in 2010, which was not the case in this study (Personal communication Aaron Jubar of the U.S Fish and Wildlife Service). Previous suggestions that sea lamprey moved faster outside of the river plume, searched the river plume through klinokinesis (Vrieze et al. 2011), and stopped more frequently in the river plume (Meckley et al. 2014b), were unsupported and likely the result of inaccuracies in manual acoustic telemetry and short-term observations. The interpretation that sea lamprey moved slower in the river plume was likely a result of the order of observations as activity in the river plume was often captured at the start of the night and movement outside of the river plume was observed later in the night. This resulted in the slow ramp up in speed we observed after settlement being attributed to movement in the river plume. The in plume search does not appear to be a kinesis as there is clear persistence and reliance on the coastline during search. The persistent exploration up and down the coastline in the river plume is consistent with an absolute olfactory search mechanism where the presence of river water defines the search boundary, rather than a differential mechanism where the cue intensity is important and gradients are used to provide directional information (Benhamou and Bovet 1989). The pattern of movement is inconsistent with reliance on odor gradients for navigation but we are unable to fully rule out chemotaxis as increases in a chemical cue gradient while rebounding could inform the migrant if it is moving towards a river mouth and could be undetected in our study as sea lamprey do make repeated passes up and down the coast. For

example, Blue crabs (*Callinectes sapidus*) use a combined mechanism in which they locate odor sources with rheotaxis but require chemotaxis to adjust to gradients across the current stream (Zimmer-Faust et al. 1995).

River entry

The decision of a sea lamprey to enter a river could occur prior to reaching the river mouth, though in the Ocqueoc River when other migrants were present and spawning and rearing habitat are available, individuals were never observed reversing course after moving past the immediate river mouth. Sea lamprey that reached the immediate river mouth did not always enter. In both years sea lamprey came to the immediate river mouth without entering and encountered river water on multiple nights, though in 2011 sea lamprey that did either task entered at a high rate (Figure 4.6). River entry most often occurred following a series of very tight rebounds along the coast before moving into the river mouth. Sea lamprey transition from rebounding movement to straight upstream movements on the bottom, suggesting that sea lamprey transition from edge guided search to odor mediated rheotaxis as they enter the river (Vrieze et al. 2011, Johnson et al. 2012, Meckley et al. 2014b). Vertical excursions also cease at this point in the migration (Chris Holbrook, personal communication).

Two previously reported aspects of entry were not regularly observed in this study, including staging in front of the Ocqueoc River or repeated entries and exits at the river mouth (Applegate 1950). Sea lamprey rarely remained stationary during nautical twilight and only in three instances of 151, they entered the river mouth and then exited. One of the three individuals reentered the river. This does not support or discredit the occurrence of staging or repeated entry as the sea lamprey in this study had already entered a river and the river temperatures were between 10-20 C° for the majority of the study period, well above the 4.4 C° temperature in

which these behaviors were reported by Applegate (1950) and above the 10 C° thermal threshold for active migration in the river (Binder and McDonald 2008). Given that many individuals were reported staging together, and sea lamprey often spent multiple days moving in the plume in front of the river, there is a possibility that social information is important to the decision to enter a river. Sea lamprey would not have to actively follow a sea lamprey, by maintaining proximity to an individual, to use social information to enter a river. Social information could come in the form of visual observations of other individuals or odor recognition of mates in the area. The odor or visual recognition of mates could be an important piece of information, as it is known that the odor of maturing migrants synchronizes the migration (Chung-Davidson et al. 2013). Although channel bias was not observed in immature female lamprey in response to the male released sex pheromone (Siefkes et al. 2005), migrants are believed to increase upstream migration in the odor's presence (Johnson et al. 2013), suggesting that odor-based mate recognition is possible.

Odor mediated manipulation of sea lamprey habitat selection

In addition to the goal of manipulation of sea lamprey river selection by encouraging sea lamprey to enter streams with poor spawning or rearing habitat as suggested by Li et al. (2007), we suggest that a second potential method would be encouraging sea lamprey to enter a river the year after TFM application, in rivers treated every three years. Ideally recruitment could be raised in the year after treatment to a rate similar to the second or third year entry rates following treatment. It is unclear if the high rate of recruitment in the third year is due to a further increase in entry between the second and third year post TFM treatment or if additional encounter occurs due to TFM treatment of other neighboring rivers on a different pesticide treatment schedule. The only weak comparison for evaluating the effect of the three synthetic compounds found in

larval odor provided no evidence of an effect on river entry in this study. Combined with other field tests it appears that the full larval odor or at minimum additional or different components found in larval odor will be required to manipulate tributary selection or river entry (Meckley et al. 2012, Meckley et al. 2014b).

Summary

The sea lamprey lake migration has now begun to be characterized over a narrow region of Lake Huron in Hammond Bay (Vrieze et al. 2010, Vrieze et al. 2011, Meckley et al. 2014b), suggesting the evaluation of the migration on other coastlines could be a valuable future contribution. All paths of sea lamprey movement observed on the first night that did not encounter river water and did encounter river water can be observed in Figure 4.15 and Figure 4.16, respectively. This study represents the finest scale exploration of in plume search to date, however an improvement would be the development of a hydrodynamic model that depicts the plume edges more finely and captures the exact role of the river plume edge in the search process. Notably, do sea lampreys exit the plume before counterturning back toward the coast or do sea lamprey reverse back towards the coast once reaching a physical feature of the water column? Observing the behavior of sea lamprey near the end of the river plume not near the river mouth could also be informative as our array only offered a window into the river mouth side of plume search. It will be difficult to reveal a chemotactic search mechanism in the river plume even if it exists because sea lamprey often make many passes up and down the coast, providing the impression that the movement is not explicitly in the river plume or performed to localize the river mouth but may be a direct assessment of the river quality (e.g., searching for larval odor or presence of mates). Sea lamprey appear to be a physical edge-guided species, that relies heavily on the surface, bottom, and coastal edges to guide the migration first to the coast

(Chapter 3), and then along the coast in search of rivers. Simple movement rules leading sea lamprey to the coast, along the coast, and within a river plume, appear sufficient to guide sea lamprey to a river mouth in a similar way that simple movement rules guide other species to geographically distinct spawning habitat and to find mates (e.g. Pe'er et al. 2004).

These findings suggest that the currently identified components of larval odor are insufficient to influence river entry, although a more thorough test would be completed in a river lacking any larval odor of any lamprey species. Given the likely lack of coevolution that would bias how sea lamprey larvae release the larval signal, it is possible that components of larval odor that are used by migrants is composed of a more complex number of components than would be common to a sex pheromone and may be harder to replicate. However our observations do suggest that the use of the entire synthetic larval odor could be a powerful management tool, a designation that appears less likely for the male released sex pheromone.

ACKNOWLEDGEMENTS

This research was funded through grants from the Great Lakes Fishery Commission including by way of the Great Lakes Restoration Initiative appropriations (GL-00E23010-3) and was accomplished through considerable assistance from the US Fish and Wildlife Service and the USGS Hammond Bay Biological Station.

APPENDIX

Table 4.1: Three specific criteria were adopted to establish the data quality objectives based on an extensive review of data quality (Meckley et al. 2014a).

	Objective	Criteria	Rationale
1	Detect changes in trajectory	Mean error ≤ 1.77 m	Many trajectory based analyses are only preserved when the average position error is $<10\%$ of the mean step length (Bradshaw et al. 2007). Transmission delay was (33.4 s), sea lamprey ground speed is 0.53 ms^{-1} (Vrieze et al. 2011, Meckley et al. 2014b), equating to a step length of 17.7 m.
2	Assign behavioral state (moving or stationary)	Max error <15 m	Simple behavioral assignment of moving or stationary with first passage time analysis, which is a measure of the time it takes an individual to leave a circle of fixed radius r drawn around each measured location to determine if movement is occurring (Fauchald and Tveraa 2003, Barraquand and Benhamou 2008).
3	Balance loss of acceptable data and retention of unacceptable data	Retain 95% of acceptable data, <i>Reject</i> $\geq 99\%$ of unacceptable data	To ensure representation of fish tracks while avoiding loss of accurate data. We aimed to keep 95% of acceptable data (≤ 6 m) while retaining $<1\%$ of unacceptable data (>6 m) of all retained positions.

Table 4.2: Criteria for selection of an HPE filter cutoff. In the data values listed for each criterion the HPE cutoffs are listed acceptable HPE cutoffs are listed for 2010 above and 2011 below.

Criteria	Stationary test		Mobile test	Rational for selection of a HPE filter cutoff of 8
	1	2		
Criterion 1 Mean ≤ 1.77 m	All	All	3 None	This criterion was met for stationary tests. The mobile test would have required a very low HPE to attain below 1.77 m. Although for a number of reasons stationary tests are more reliable estimators for this metric (Meckley et al. 2014a).
Criterion 2 Max error < 15 m	< 7	< 8 NA	≤ 19 ≤ 1	In 2010: An HPE of 8 did not meet the criteria for mobile tests, but only 1 of 134 positions remained. The stationary test criteria were met. Only 2 of 45,744 positions were problematic ($< 0.001\%$) for the combined stationary tests (Figure 4.4). In 2011 an HPE of 7 removed all except one position for the stationary test, and one position for the mobile test exceeding 15 m error.
Criterion 3 <i>% incorrectly retained vs. % incorrectly rejected</i>	8–10 3-15	3–15 NA	None None	In 2010: An HPE cutoff of 8 met the criteria for both stationary tests, and although the mobile test did not have a suitable range, the range from 5–10 was equally effective. In 2011: An HPE of 7 met criteria for the stationary test as it fell within at least 3-15.

Table 4.3: Akaike’s information criterion (AIC) values for linear mixed effects models using river entry (Em) as a binary response variable to various combinations of fixed effects including year (Y), iterative number of nights the sea lamprey encountered river water (N), a year by night number interaction (YxN), average nightly discharge of the Ocqueoc River (DC: $m^3 \cdot S^{-1}$), average lake temperature encountered (LT: C°), average nightly river temperature (RT; C°), the difference in experienced lake temperature and river temperature (DFT; C°), minimum distanced reached from the river mouth (D: km), and average water velocity experienced in the lake (V).

Model	Fixed Effects	df	V_m AIC (Δ AIC)
0	1	2	209.8 (13.0)
1	Y	3	202.6 (5.8)
2	N	3	211.7 (14.9)
3	Y + YxN	5	205.7 (8.9)
4	DC	3	211.8 (15.0)
5	Y + DC	4	204.5 (7.7)
6	LT + RT	4	207.5 (10.7)
7	Y + LT	4	199.7 (2.9)
8	Y + RT	4	204.3 (7.5)
9	Y + DFT	4	199.6 (2.8)
10	Y + LT + D	5	197.1 (0.3)
11	Y + DFT + D	5	196.8 (0)
12	Y + DFT + LT	5	201.5 (4.7)
13	Y + DC + LT + RT	6	203.5 (6.7)
14	Y + V	6	199.2 (2.4)
15	Y + LT + RT + V + DC + D + YxN	10	205.8 (9)

Table 4.4: Akaike’s information criterion (AIC) values for a nonlinear mixed-effects model in the formulation described by Lindstrom and Bates (1990), using the function “nmlme” in R (R Development Core Team 2015). Ground speed (V_m) as a response variable to various combinations of fixed effects including year (Y), the log of the cumulative distance traveled (D), whether encounter with river water occurred yet in the path (E), or if the point was currently in the plume (P), temperature at fish position (T), and an interaction effect between water current magnitude and direction by fish heading interaction (CxF); and finally the random effect of individuals (animal ID).

Model	Fixed Effects	Df	V_m AIC (Δ AIC)
0	E	5	178.3 (99.2)
1	E + E:D	6	89.2 (10.1)
2	Y + E:D	5	276.2 (197.1)
3	T	5	279.5 (200.4)
4	P + Y	6	290.9 (211.7)
5	T + P + E	7	168.9 (89.4)
6	T + P + Y	7	279.7 (200.6)
7	T + CxF	6	278.1 (199.0)
8	P + CxF + E:D	8	98 (19.7)
9	T + P + CxF + E + E:D	9	79.1 (0)

Table 4.5: Akaike’s information criterion (AIC) values for a nonlinear mixed-effects model in the formulation described by Lindstrom and Bates (1990), using the function “nmlme” in R (R Development Core Team 2015). Path straightness (S_m) as a response variable to various combinations of fixed effects including year (Y), the log of the cumulative distance traveled (D), if whether encounter with river water occurred yet in the path (E), or the point was currently in the plume (P); and finally the random effect of individuals (animal ID). Model 6 and 9 had the lowest AIC scores.

Model	Fixed Effects	Df	V_m AIC (Δ AIC)
0	D + Y	6	-354.6 (7.5)
1	D + E	6	-358.0 (4.1)
2	E + Y	7	-340.4 (21.7)
3	P + Y	7	-348.9 (13.2)
4	P + E	6	-355.2 (6.9)
5	E	5	-353.2 (8.8)
6	P	5	-360.4 (1.7)
7	D + E + P	7	-356.8 (5.3)
8	D + E + P + Y	8	-351.9 (10.2)
9	DxE + P		-362.0 (0)

Table 4.6: To evaluate the effect of a host of variables on river entry (Em), a mixed effects logistic regression with a binary response variable (river entry: 1 or 0) was implemented in R using glmer (lme4 package, Bates et al. 2014). The best fitting models, as determined by AIC,

R code: `m11<-glmer(Em ~ Y+ DFT+ D+ (1|ID), data=entry, binomial)`

Family: binomial (logit)

Formula: `Em ~ Y + DFT + D + (1 | ID)`

Data: entry

AIC	BIC	logLik	deviance	df.resid
196.8	211.9	-93.4	186.8	144

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.1786	-0.7532	-0.5324	0.9475	2.8220

Random effects:

Groups Name	Variance	Std.Dev.
ID (Intercept)	1e-14	1e-07
Number of obs:	149, groups: ID, 98	

Fixed effects:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-0.64956	0.41248	-1.575	0.1153
year2011	0.88611	0.35433	2.501	0.0124 *
DFT	0.02464	0.04996	0.493	0.6219
D	-2.08616	1.05819	-1.971	0.0487 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	yr2011	DFT
year2011	-0.526		
DFT	-0.615	0.017	
M	-0.436	0.050	-0.015

Table 4.6: (cont'd)

for river entry included year and minimum distance reached from the river and either the lake temperature experienced (Model 10) or the difference in lake temperature experienced and the river temperature (Model 11). The fixed effects included year (Y), iterative number of nights encountering river water (N), a year by night number interaction(YxN), average nightly discharge of the Ocqueoc River (DC: $m^3 \cdot S^{-1}$), average lake temperature encountered (LT: C°), average nightly river temperature (RT; C°), the difference in experienced lake temperature and river temperature(DFT; C°), minimum distanced reached from the river mouth (D: km), and average water velocity experienced in the lake (V). Here is Model 11 output.

Table 4.7: Output from the summary of the best fit nonlinear mixed-effects model for the continuous response variable ground speed (body lengths per second), Model 9. The model

Linear mixed-effects model fit by REML

Data: GirardEnc

AIC	BIC	logLik
79.13117	146.8898	-29.56558

Random effects:

Formula: ~1 | ID

(Intercept)	Residual
-------------	----------

StdDev: 0.2078676 0.2983834

Correlation Structure: AR(1)

Formula: ~1 | ID

Parameter estimate(s):

Phi: 0.5945631

Fixed effects: Vm ~ T+ P + C:F + E + I(D/1000): E

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.6803459	0.04771404	6437	14.258818	0.0000
t	0.0198588	0.00355488	6437	5.586343	0.0000
as.factor(P)Y	0.0018479	0.01246258	6437	0.148279	0.8821
as.factor(E)b	0.3591986	0.02492367	6437	14.411945	0.0000
C:F	-0.2506198	0.11486012	6437	-2.181956	0.0291
Ea:I(D)	0.1645548	0.01549279	6437	10.621383	0.0000
Eb:I(D)	-0.0016136	0.00364662	6437	-0.442490	0.6581

Correlation:	(Intr)	t	P	E	C:F	Ea:D
t	-0.606					
PY	0.167	-0.250				
E	-0.297	0.040	-0.273			
C:F	-0.006	-0.163	-0.027	0.042		
Ea:D	-0.238	-0.021	0.007	0.458	0.042	
Eb:D	0.021	-0.046	0.107	-0.482	-0.017	0.073

Standardized Within-Group Residuals:

Min	Q1	Med	Q3	Max
-4.9337612	-0.4086086	0.1300112	0.6036556	8.6886942

Number of Observations: 6483

Number of Groups: 40

Table 4.7: (cont'd)

included the temperature experienced (T), whether encounter with the river plume had occurred in the path (E), whether the sea lamprey was in the river plume (P), and an interaction between water current and if the fish was moving towards or away from the current direction. The correlation statement accounts for first order autocorrelation in the residuals that occurred from analyzing multiple simultaneous steps relating to the same individual. The random effect of animal ID is included.

Table 4.8: Output from the summary of the best fit nonlinear mixed-effects model for path straightness (0-1), Model 9. The model included the effect of the interaction between cumulative

R code: *m9 <- lme(fixed = S ~ I(D/1000):as.factor(E) + as.factor(P),
 random = ~ 1 | ID,
 correlation = corARI(form = ~ 1|ID),
 data = sin)*

Linear mixed-effects model fit by REML

Data: sin

AIC	BIC	logLik
-362.0493	-329.653	188.0247

Random effects:

Formula: ~1 | ID

(Intercept)	Residual
StdDev: 0.06001644	0.1812923

Correlation Structure: AR(1)

Formula: ~1 | ID

Parameter estimate(s): Phi 0.1026954

Fixed effects: sin ~ I(D/1000):as.factor(E) + as.factor(P)

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.7951321	0.01479099	684	53.75785	0.0000
as.factor(P)1	-0.0450035	0.01790223	684	-2.51385	0.0122
I(D/1000):as.factor(E) 0	0.0482397	0.01201762	684	4.01408	0.0001
I(D/1000):as.factor(E)1	0.0179331	0.00434604	684	4.12631	0.0000

Correlation:	(Intr)	as.()1	I(/1000):.()0
as.factor(plume)1	-0.318		
I(stepcum/1000):as.factor(enc)	0	-0.552	0.206
I(stepcum/1000):as.factor(enc)1	-0.415	-0.262	0.324

Standardized Within-Group Residuals:

Min	Q1	Med	Q3	Max
-4.1849828	-0.3403049	0.3338883	0.6488679	1.5624139

Number of Observations: 760

Number of Groups: 73

Table 4.8: (cont'd)

distance traveled (D) and Encounter with the river plume and the fixed effect of being in the river plume. The correlation statement accounts for first order autocorrelation in the residuals that occurred from analyzing multiple simultaneous steps relating to the same individual. The random effect of animal ID is included. The distance is divided by 1000 to put the variable in km rather than m for easier interpretation.

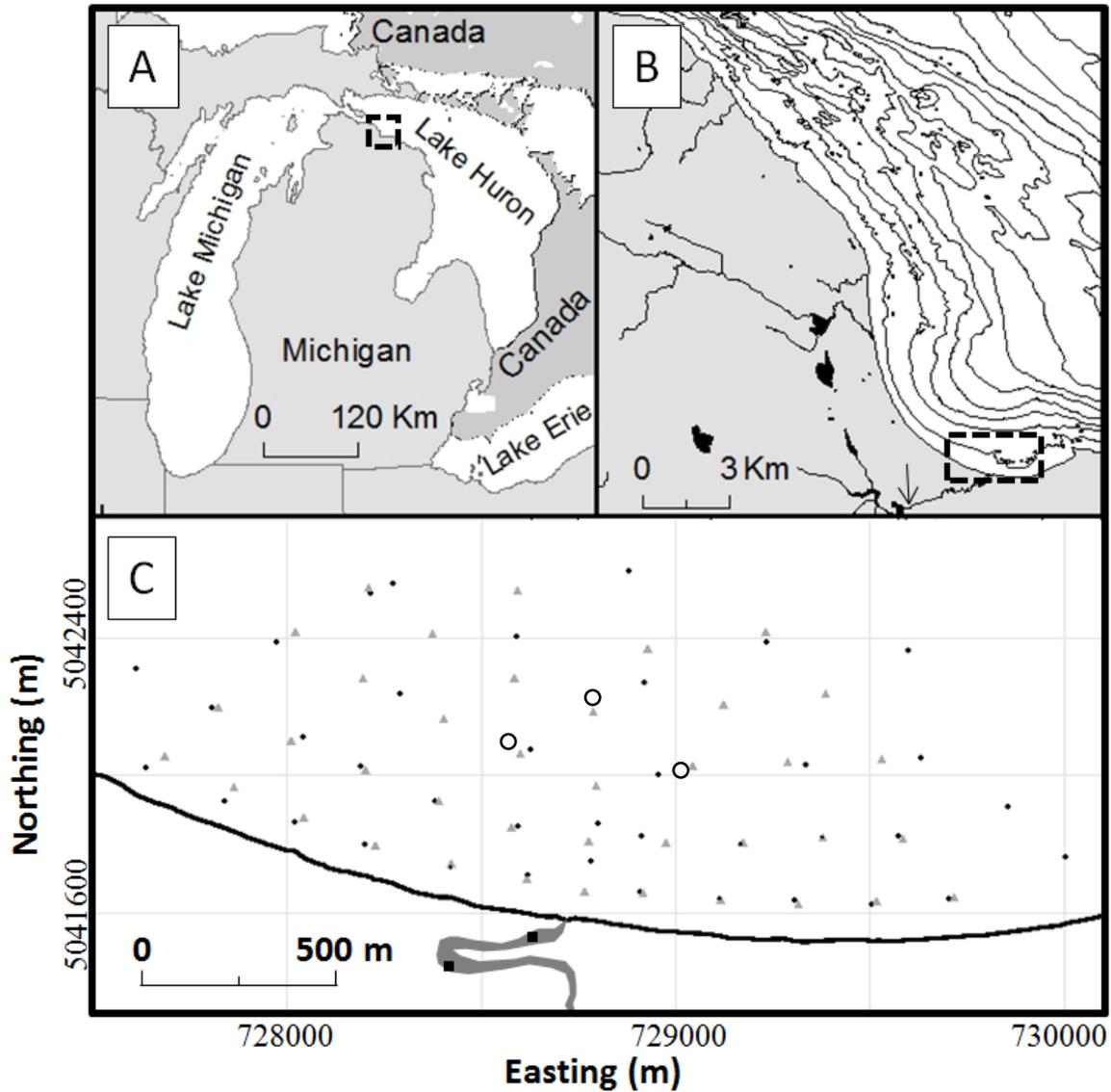


Figure 4.1: The study site was located in Hammond Bay of Lake Huron (A, B). Sea lamprey were released at three locations (open circles). The study site was covered by a two kilometer wide array that reached a kilometer from shore (C). The receiver positions in 2010 are represented by triangles, and in 2011 they are represented by circles. In each year nine sync tags were distributed in two rows across the array. During peak performance a transmitter could be positioned every 15-45 s. Two of the six river receivers used for monitoring passage are represented by black squares. The receivers in the river could not detect tags in the lake.

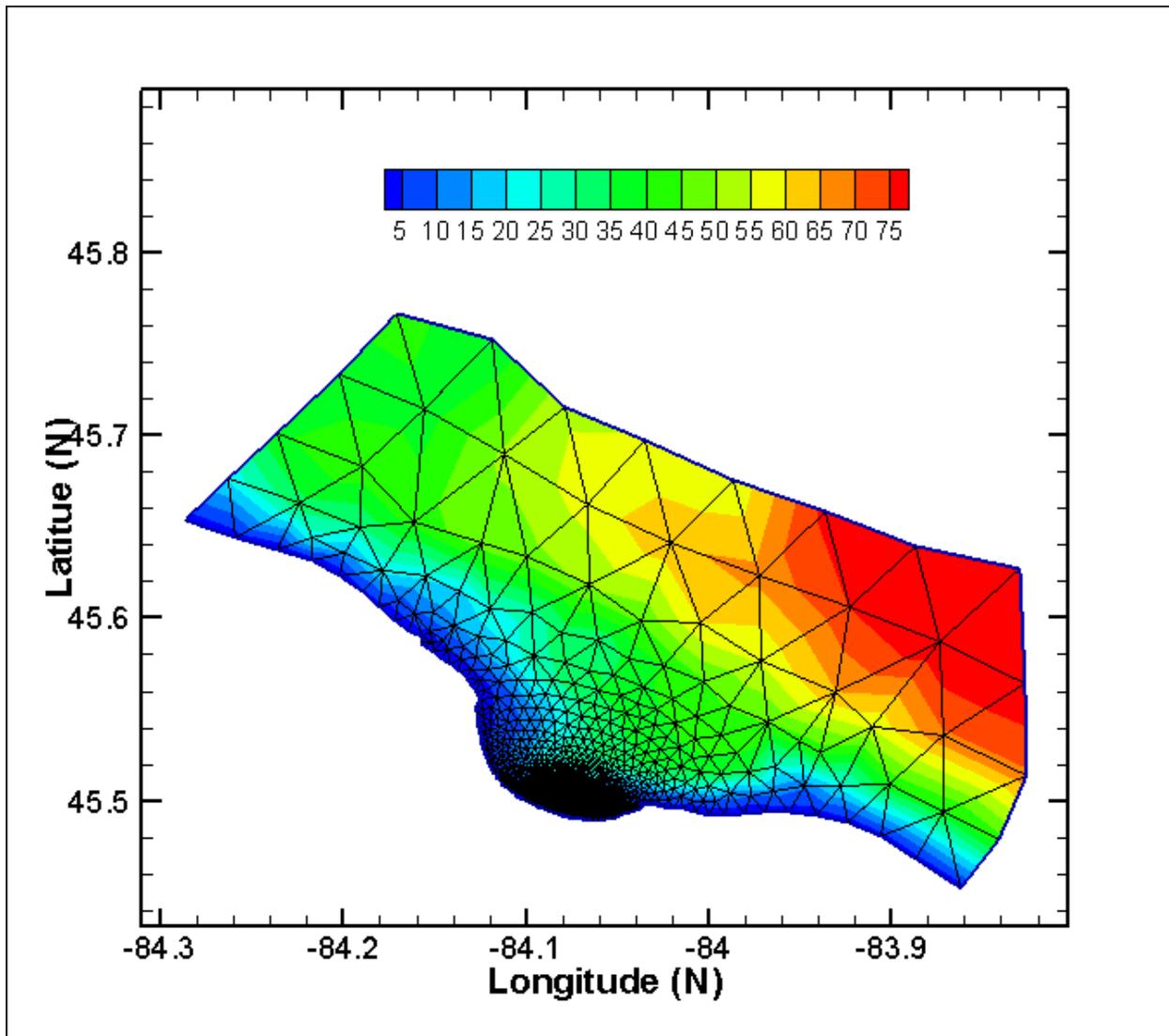


Figure 4.2: The hydrodynamic model makes predictions of the current speed and direction and lake temperature at each node (N=4346) of an unstructured grid fit to Hammond Bay (FVCOM grid). The size of the triangular grid cells decreases near the river mouth to provide a higher precision near our fish tracking array (minimum grid size: 9.15 m²). The hydrodynamic model provides predictions of current speed and direction and water temperature. The model is three dimensional and provides predictions in 2010 and 2011 at 20 slices through the water column water column, independent of depth (m), which is color coded.

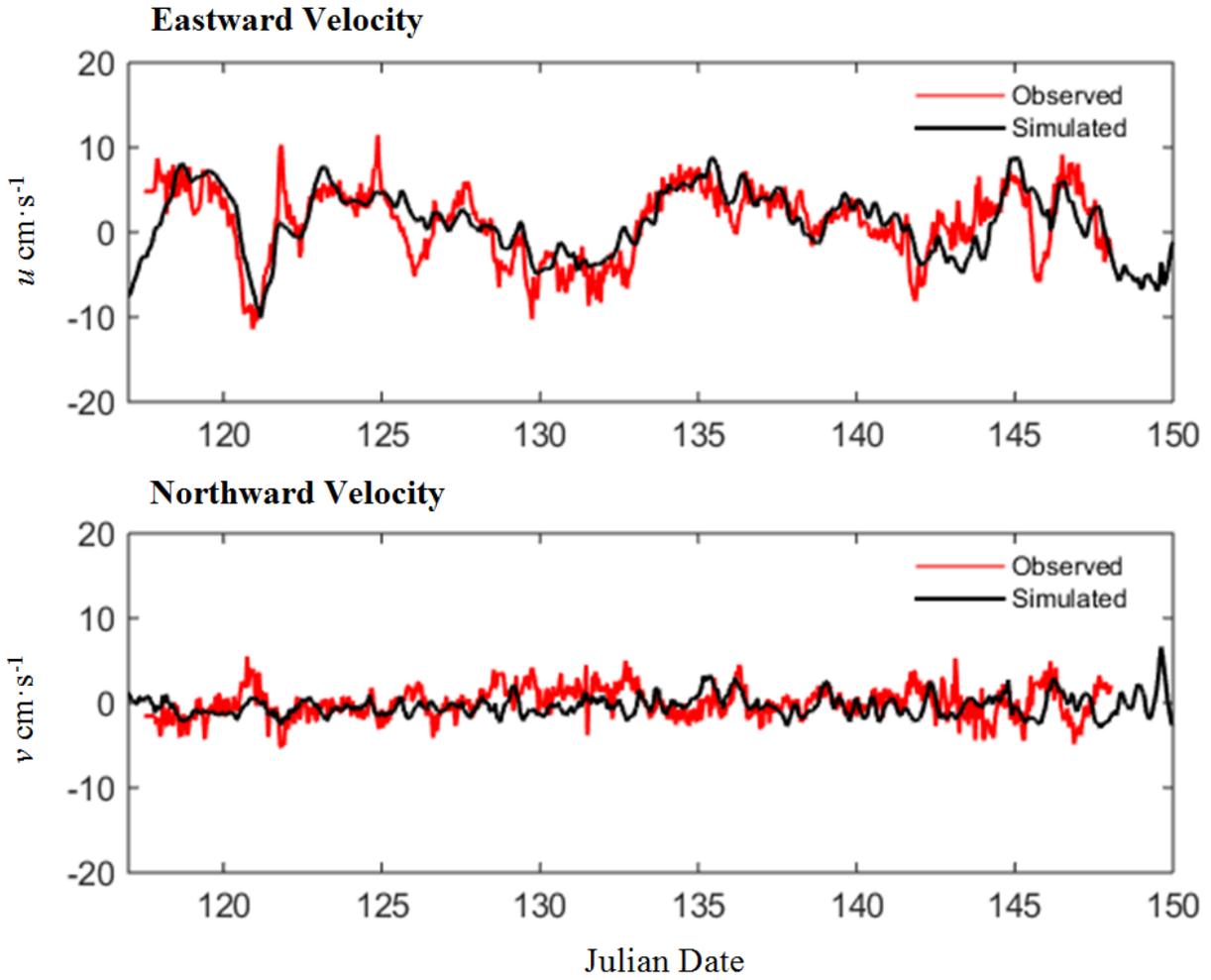


Figure 4.3: Comparisons between vertical averaged currents from ADCP observations and simulations taken in 2011.

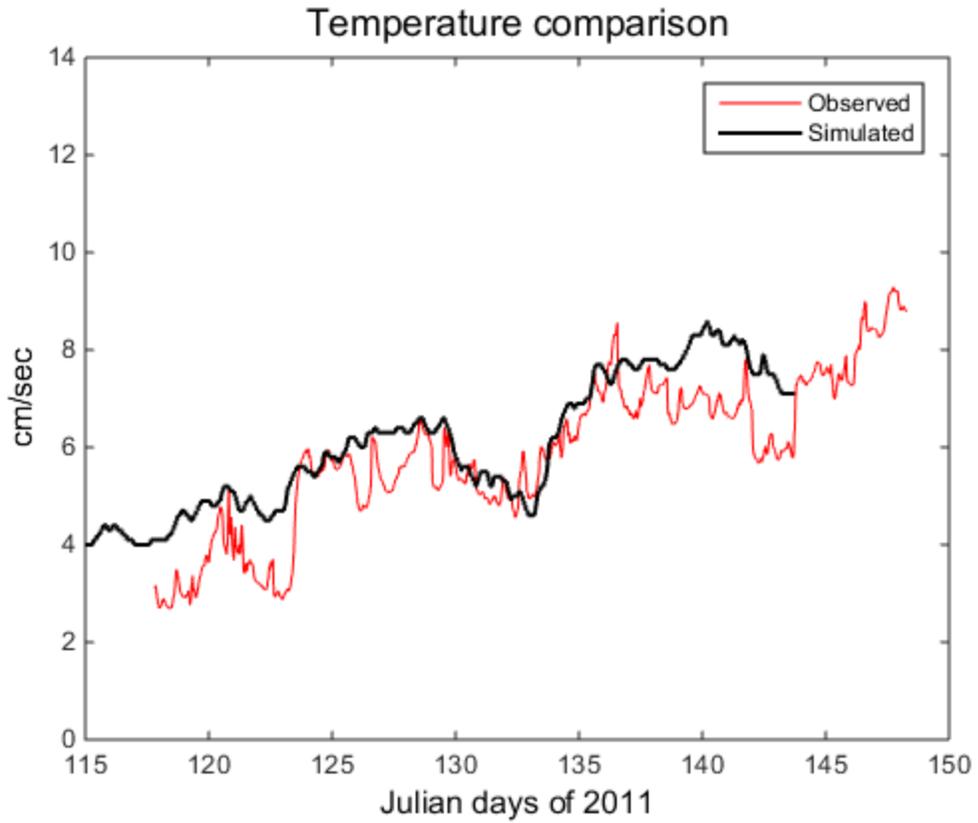


Figure 4.4: Comparisons between observed temperature recorded by the ADCP and hydrodynamic model simulations in 2011.

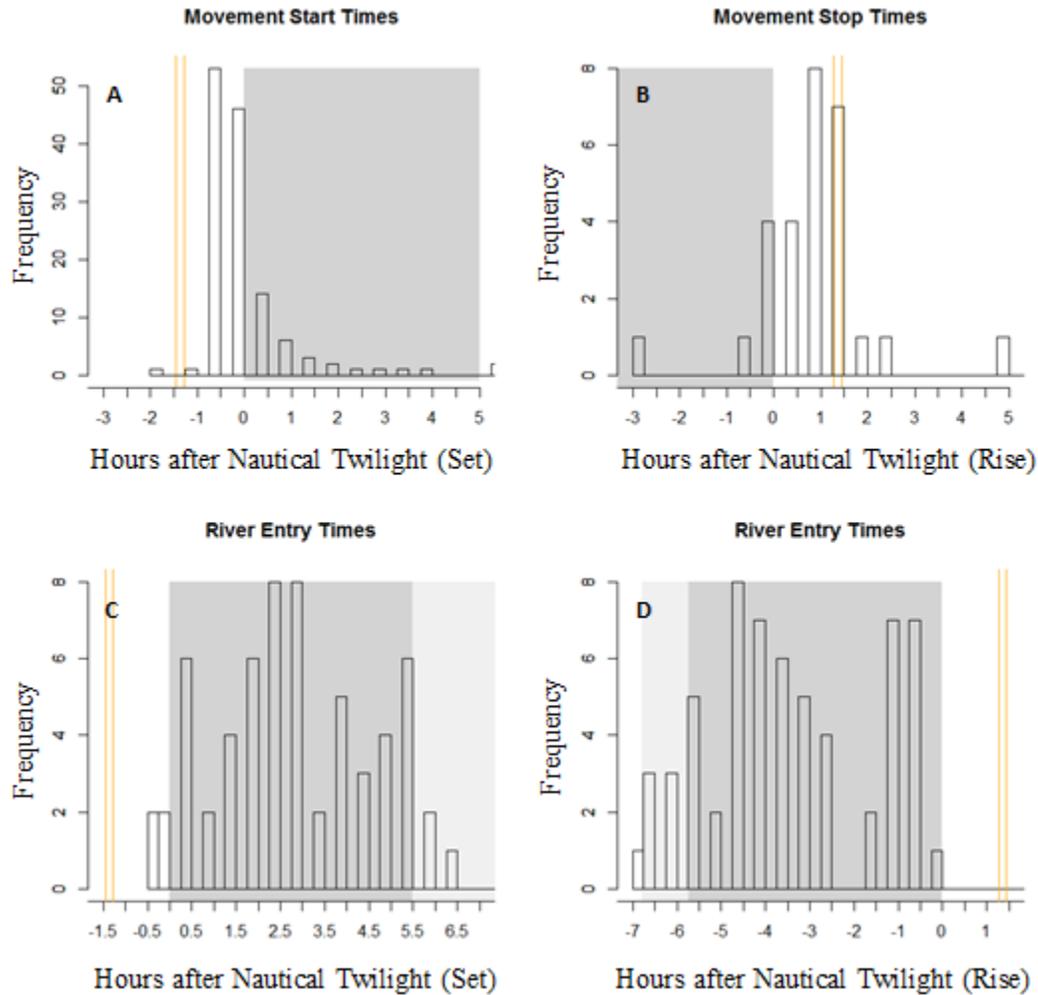


Figure 4.5: Sea lamprey mostly began moving between sunset (orange vertical lines) and nautical twilight NT (gray box) in most cases (A). Activity continued until between NT and sunrise, although activity continued in lighter conditions than under which it commenced (B). The double vertical lines represents sunrise and sunset as the time from NT changes with night length. Sea lamprey entered the river throughout the night but the earliest they were observed entering was just before NT (C) and despite activity being observed at sunrise in the lake, river entry was never observed outside of NT in the lake (D). Two graphs show river entry as night length changes with day length, this explains the dark gray areas (NT, all year), and light gray areas (NT during some parts of the year). Focus on the 0 side of insets C and D.

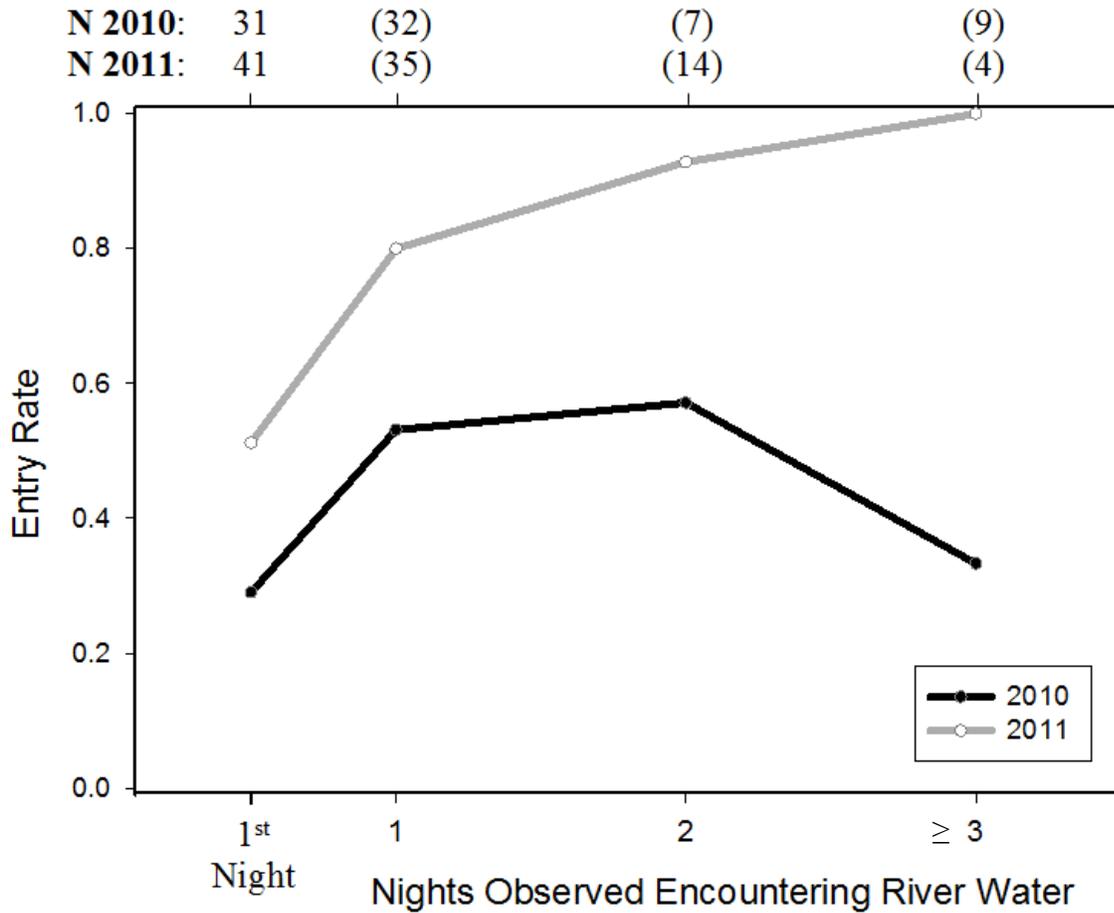


Figure 4.6: The entry rate based on the total number of different nights that sea lamprey were observed encountering river water in 2010 and 2011. The nights do not have to be consecutive and don't have to include the first night after release. The entry rate on the first night "1st night" refers to the entry rate of sea lamprey that encountered river water on the first night of release after settlement. The number of sea lamprey that fall into each category for each year are found at the top of the graph.

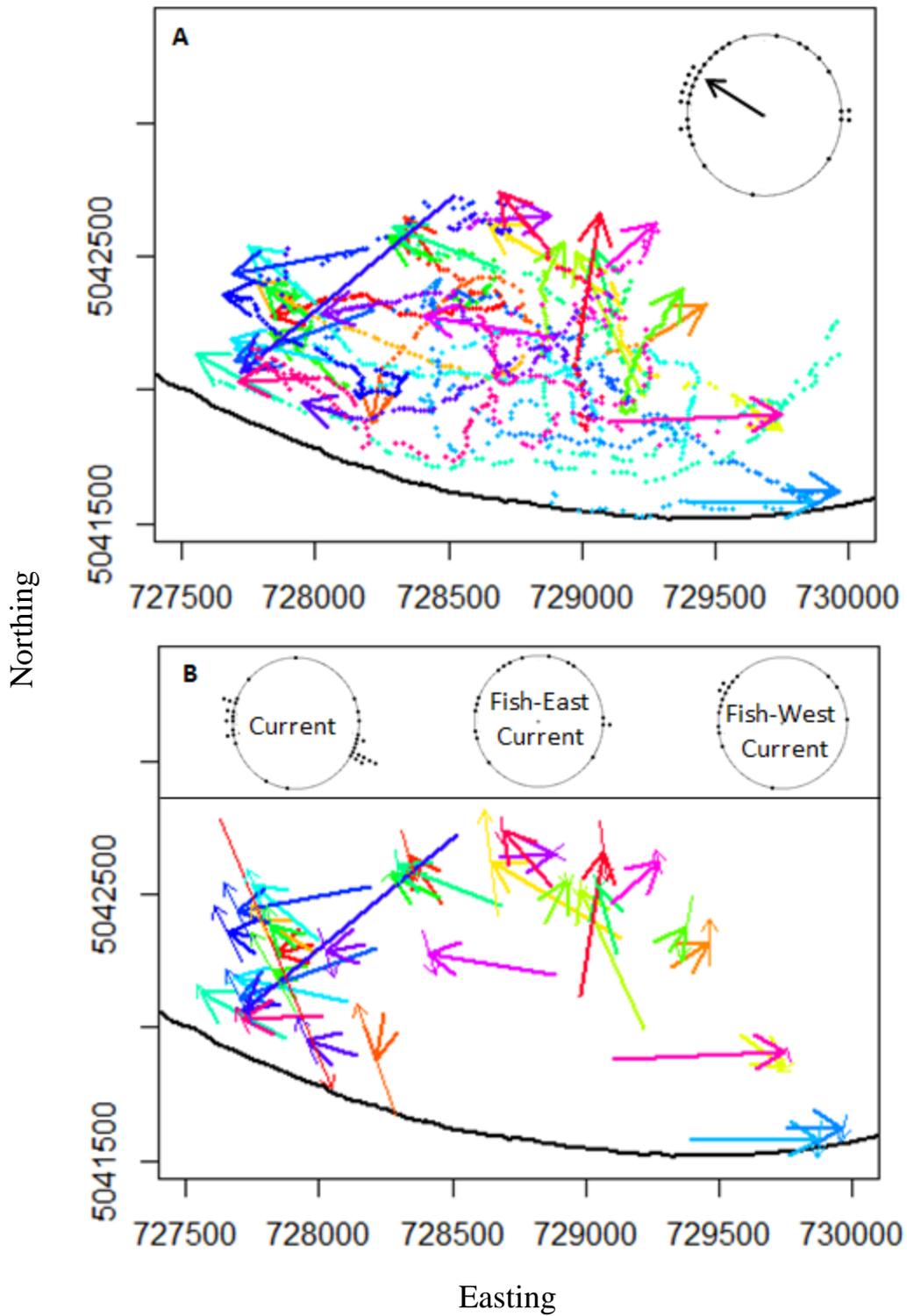


Figure 4.7: The 33 tracks of sea lamprey that did not encounter river water on the first night are color coded and the arrow of the heading from the 10th to last point to final point are shown (A).

Figure 4.7: (cont'd)

The circular plot in A (top) shows that most fish moved west out of the array. The exit arrow (thick arrow) is shown for each sea lamprey and the average current experienced is shown by the thin arrow, where the arrow points the current direction and the length represents current strength (max: $27 \text{ cm}\cdot\text{s}^{-1}$, average: $4.9 \text{ cm}\cdot\text{s}^{-1}$) (B). The circle plots show the current directions observed on the 33 nights (“Current”), the direction sea lamprey moved when the current was from the East (“Fish-East Current”), the direction the sea lamprey moved when the current was from the west (“Fish-West Current”).

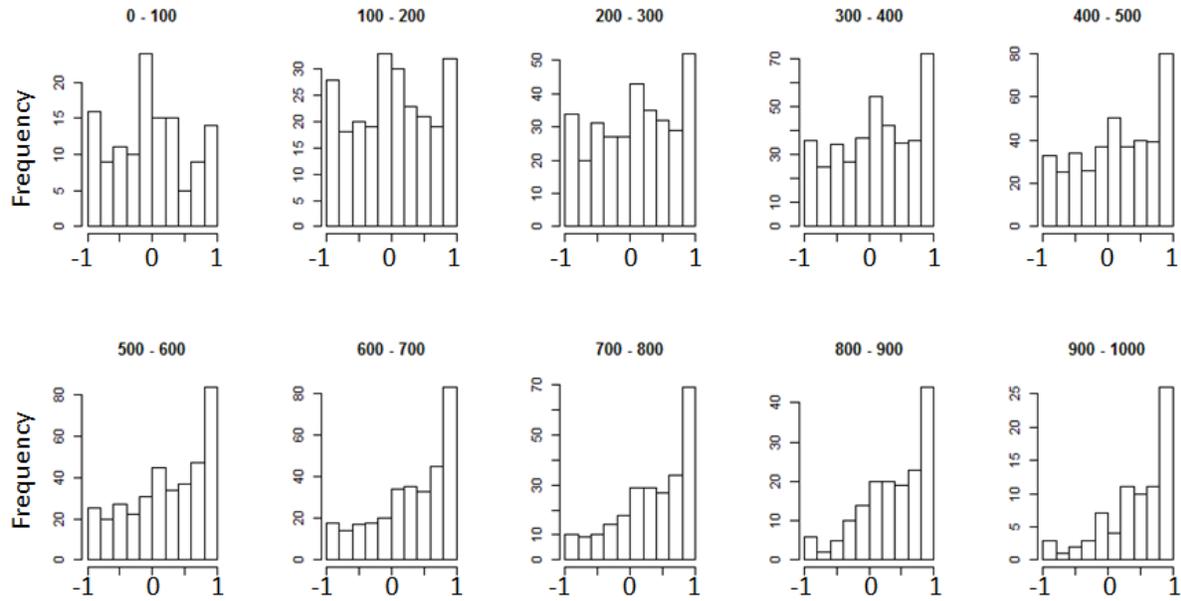


Figure 4.8: Each transect (set of two points) for sea lamprey that never encountered river water were binned by 100 m increments from the coast based on their approach ratio (distance moved towards the coast/total distance traversed). When sea lamprey were 500-1000 m from the coast movement was directed towards the coast (1) before turning and moving parallel to the coast (0-500 m). An increase in movement away from the coast occurs between 100-300 m from the coast resulting in an average parallel movement along the coast of $303 \text{ m} \pm 221 \text{ m}$ when exiting the array (centered at an approach ratio of 0).

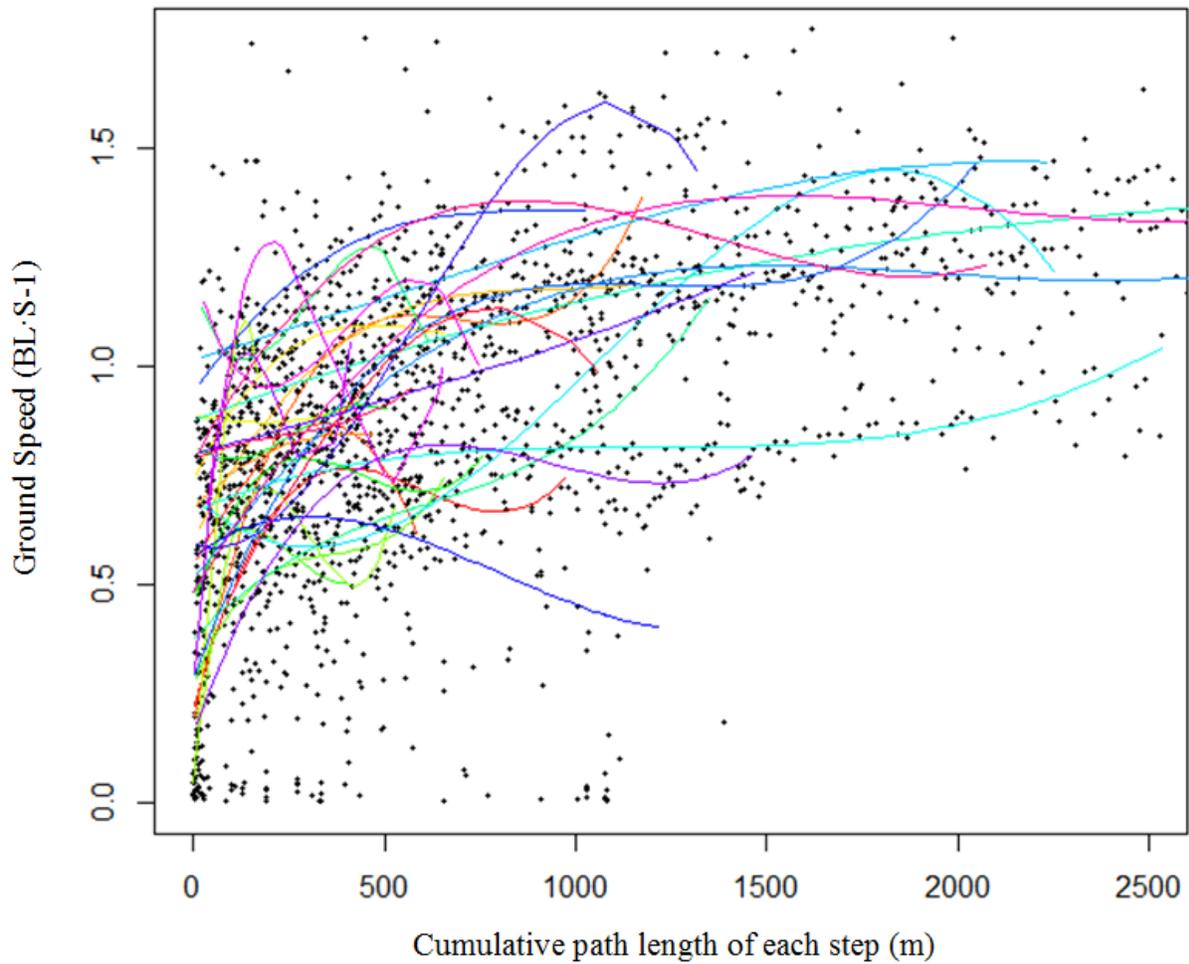


Figure 4.9: Ground speed (Body lengths per second) is shown for each step over the cumulative path length for all sea lamprey (N=33) that did not encounter river water on the first night. Each line represents a different individual. The pattern for before river plume encounter (black circles) and after river plume encounter (red squares) is shown. Observations are restricted to a cumulative path length of 2500 meters. A ramp up in speed to one body length per second is evident in the first 500 meters of activity.

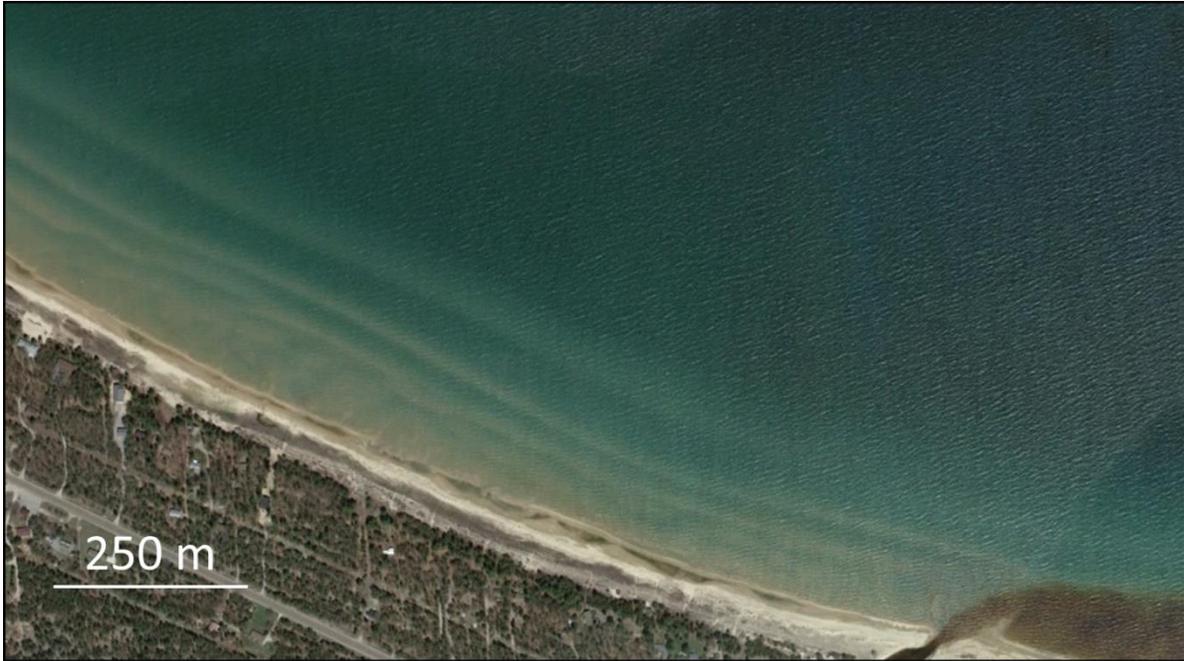


Figure 4.10: The google earth image depicts the distinct nearshore wave zone which includes a comparatively steep series of banks and troughs that run horizontal to the coast compared the shallow bathymetric gradient in Hammond Bay. The image captures the coastline in Hammond Bay in front of the Ocquoec River Mouth, located in the bottom right of the image. The edge of the banked zone extends from 204-308 m from shore in this area of the array.

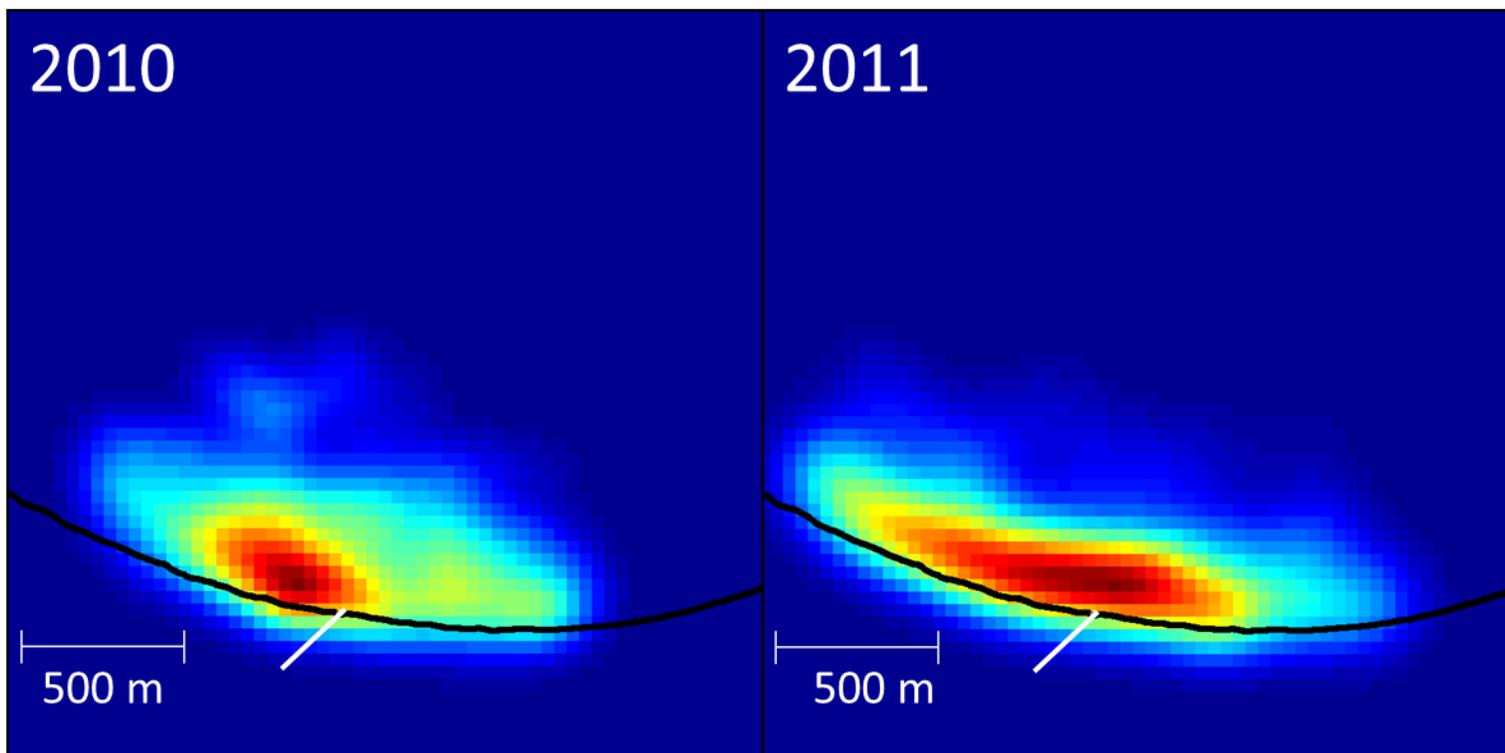


Figure 4.11: A smoothed utilization distribution in 50 m cells of all active sea lamprey points following river plume encounter as defined by encounter with a dye concentration of greater than 5 ppm in 2010 and 2011. The white line represents the Ocquoec River. Movement extended farther east and west along the coast in both cases but was not captured by the array.

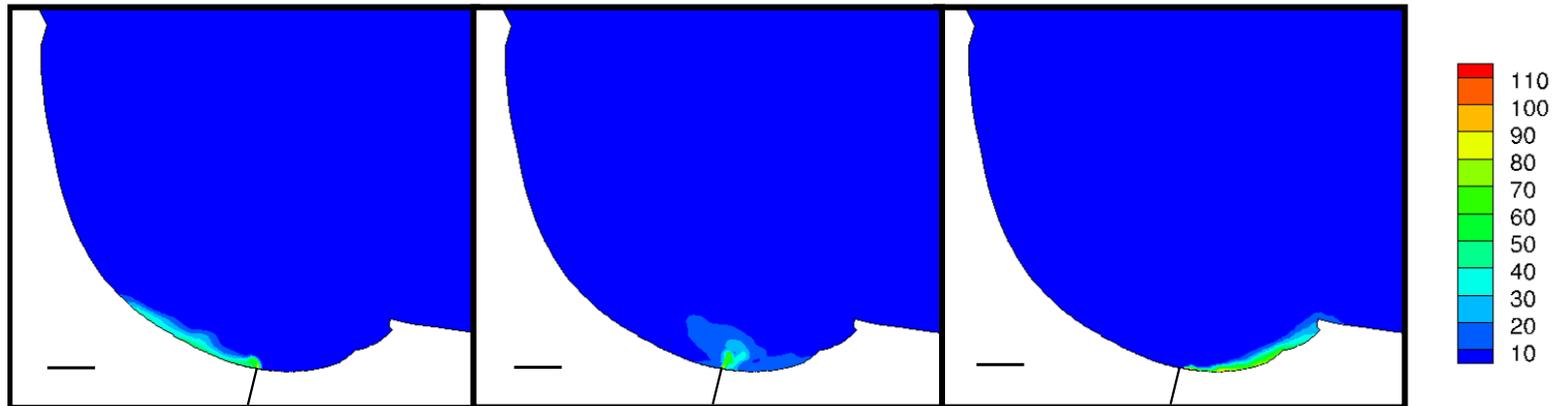


Figure 4.12: The river plume occupied three general forms in Hammond Bay as represented by the output of the dye concentration model. The image to the left represents a less common river plume extending to the west. The central schematic depicts the river plume that occurs when the plume switches from either east to west or occasionally when currents were moving in northerly or southerly directions and pushed the plume out from shore. The most common river plume extended to the east and extended out of Hammond bay. The river water was often trapped to the east and west due to the shape of the coast.

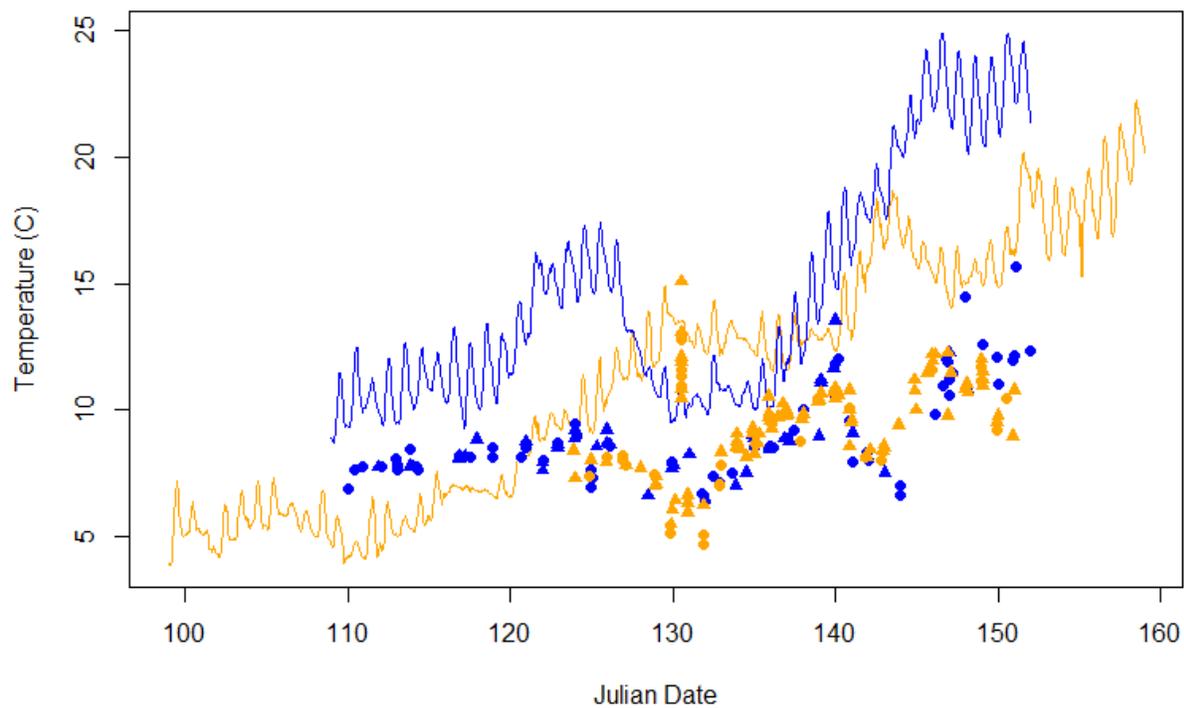


Figure 4.13: The temperature by Julian date for 2010 (Blue Line) and 2011 (Orange Line) is shown. A symbol represents each fish if it was in the array on a given night in 2010 (Blue) and 2011 (Orange) at the maximum temperature encountered by the fish. Triangles represent fish that entered the river on that night and circles representing fish that do not enter that night. An individual fish is represented multiple times if it returns on multiple nights.

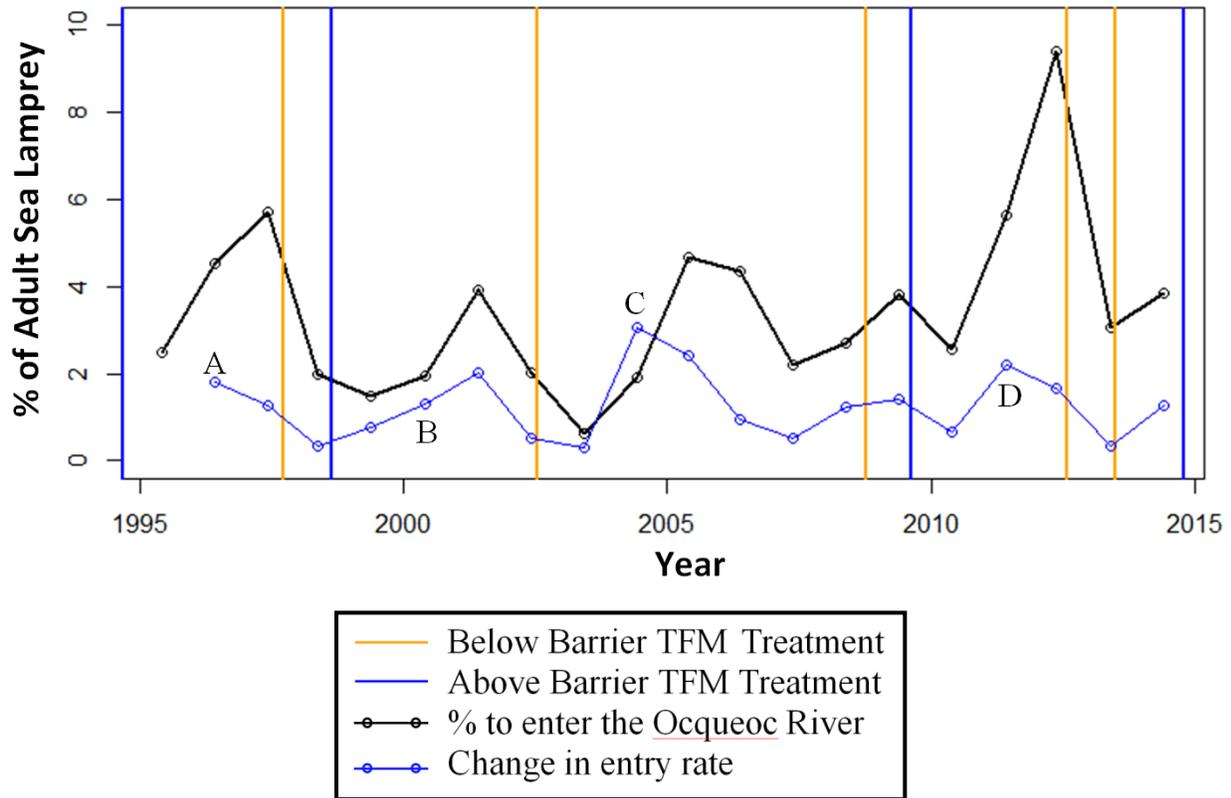


Figure 4.14: The percentage of the entire population of adult sea lamprey in Lake Huron that return to the Ocqueoc River to spawn from 1995 to 2015 based on mark recapture (black circles) and the change in entry between years (blue circles). A change of entry rate of 2 represents a doubling in the rate of entry. Vertical lines represent the times of TFM treatment to the Ocqueoc River. The change in entry rate from one year after TFM treatment to the second year shows that the increase in entry rate between 2010 and 2011 (D) fits within the historical increase in rate (A: 1.8, B: 1.3, C: 3.1, D: 2.1).

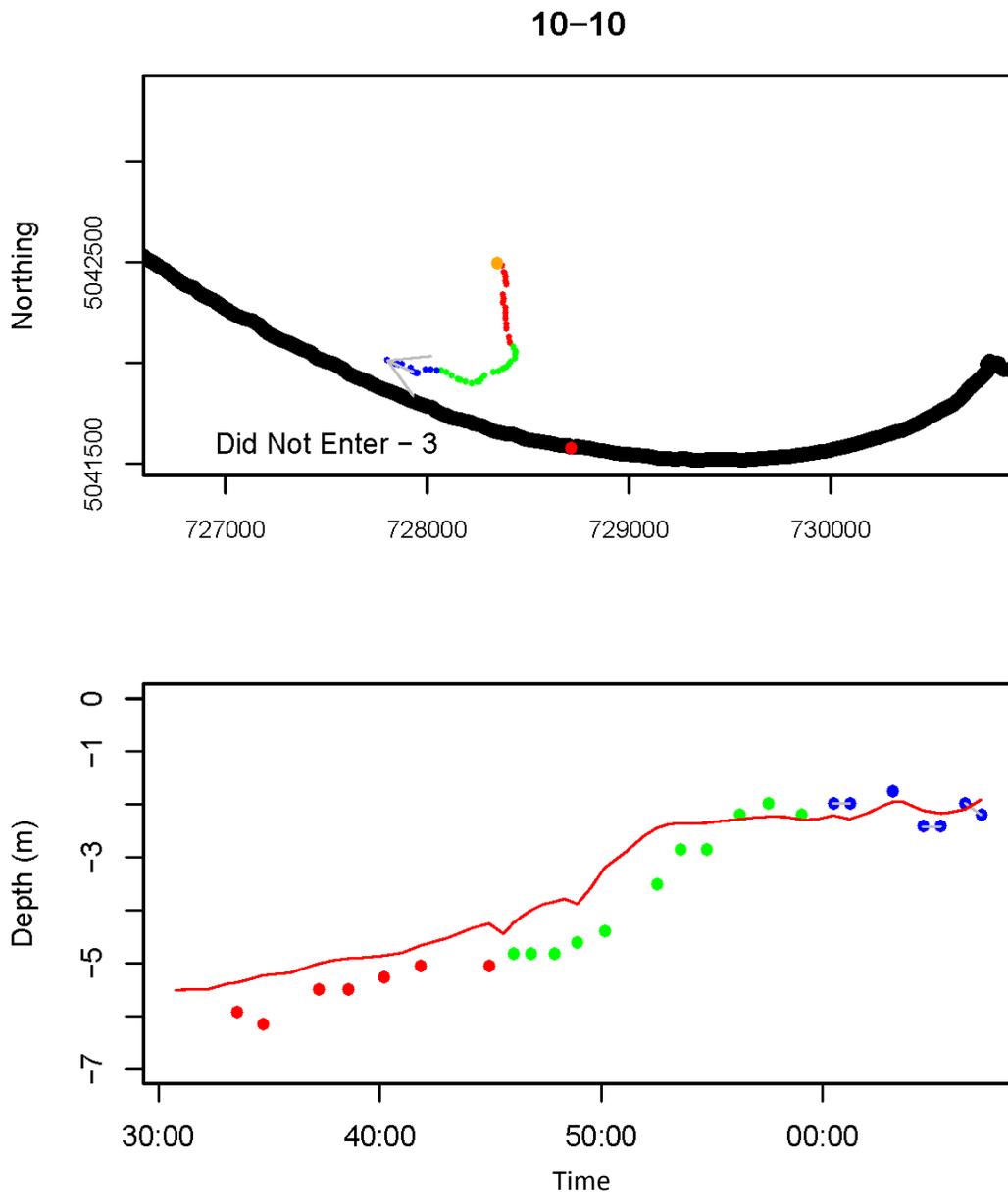


Figure 4.15: The 40 sea lamprey that passed through the river plume based on the estimates of the dye concentration at each fish position are shown in a schematic of Hammond bay, where the black line represents the coast and the red dot represents the river mouth. Each page is labeled by the year (10 or 11) and animal ID. The upper graph shows the sea lamprey movement on the first night from where the sea lamprey settled in the array after release (orange dot). In the schematic of movement, the gray arrow points the heading during the last 5 points of the track and the track

Figure 4.15: (cont'd)

is color coded by 15 minute intervals of activity. The label of “Entered” indicates that sea lamprey eventually entered the Ocqueoc River and the label of “Did Not Enter” indicates that the sea lamprey never entered the Ocqueoc River. The number of bouts follows the label indicating the number of different nights the sea lamprey was observed in the array. If the sea lamprey

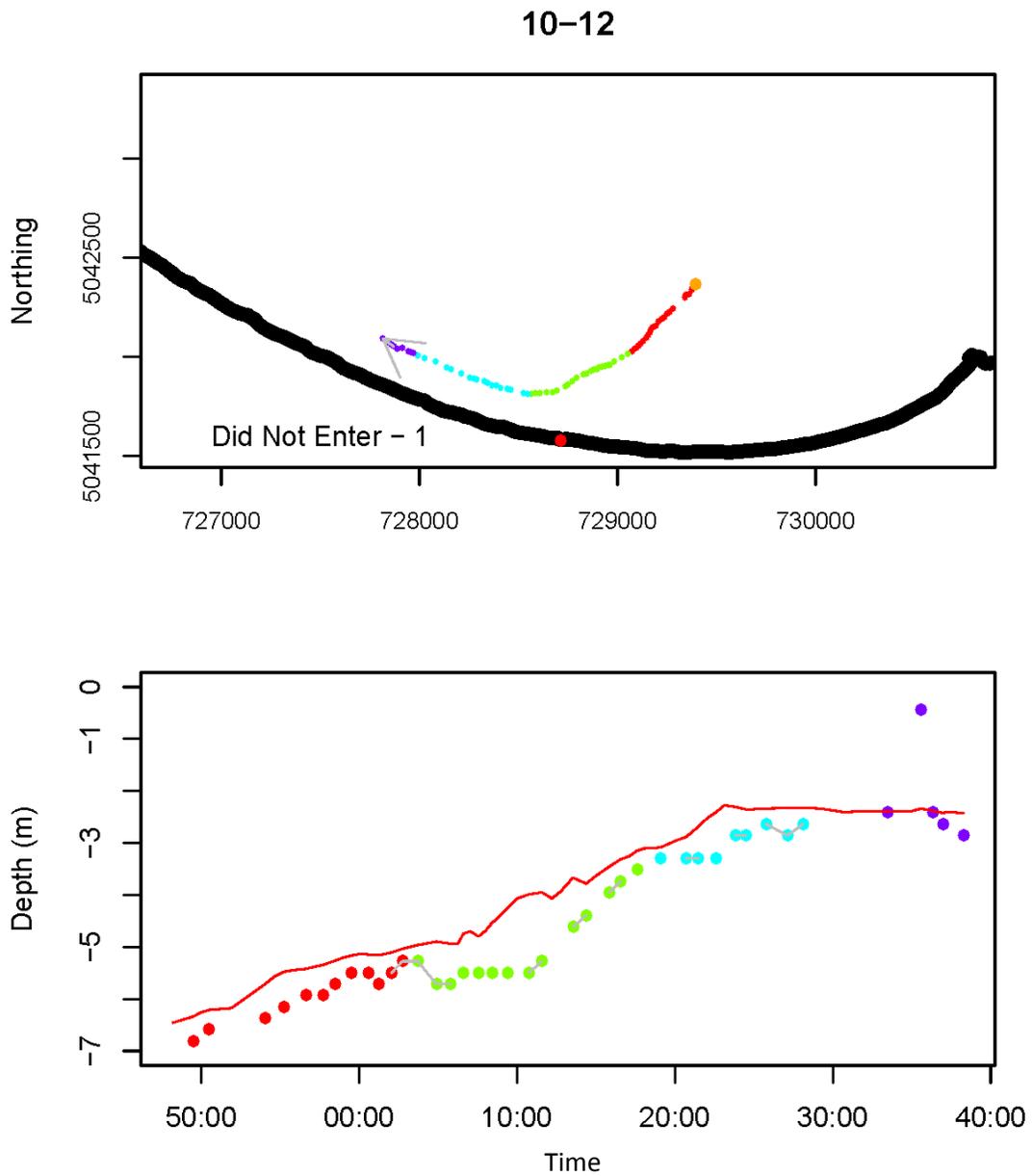


Figure 4.15: (cont'd)

entered and the bout is one, the individual entered at the end of the track shown. In some cases entry is not captured well by the array (e.g. 10-17). The lower graph depicts the depth of the sea lamprey during movement through time and the color of the points corresponds to the schematic in the upper graph. The red line represents the estimated total water column depth. When the fish is below the line it can be assumed the fish is on the bottom.

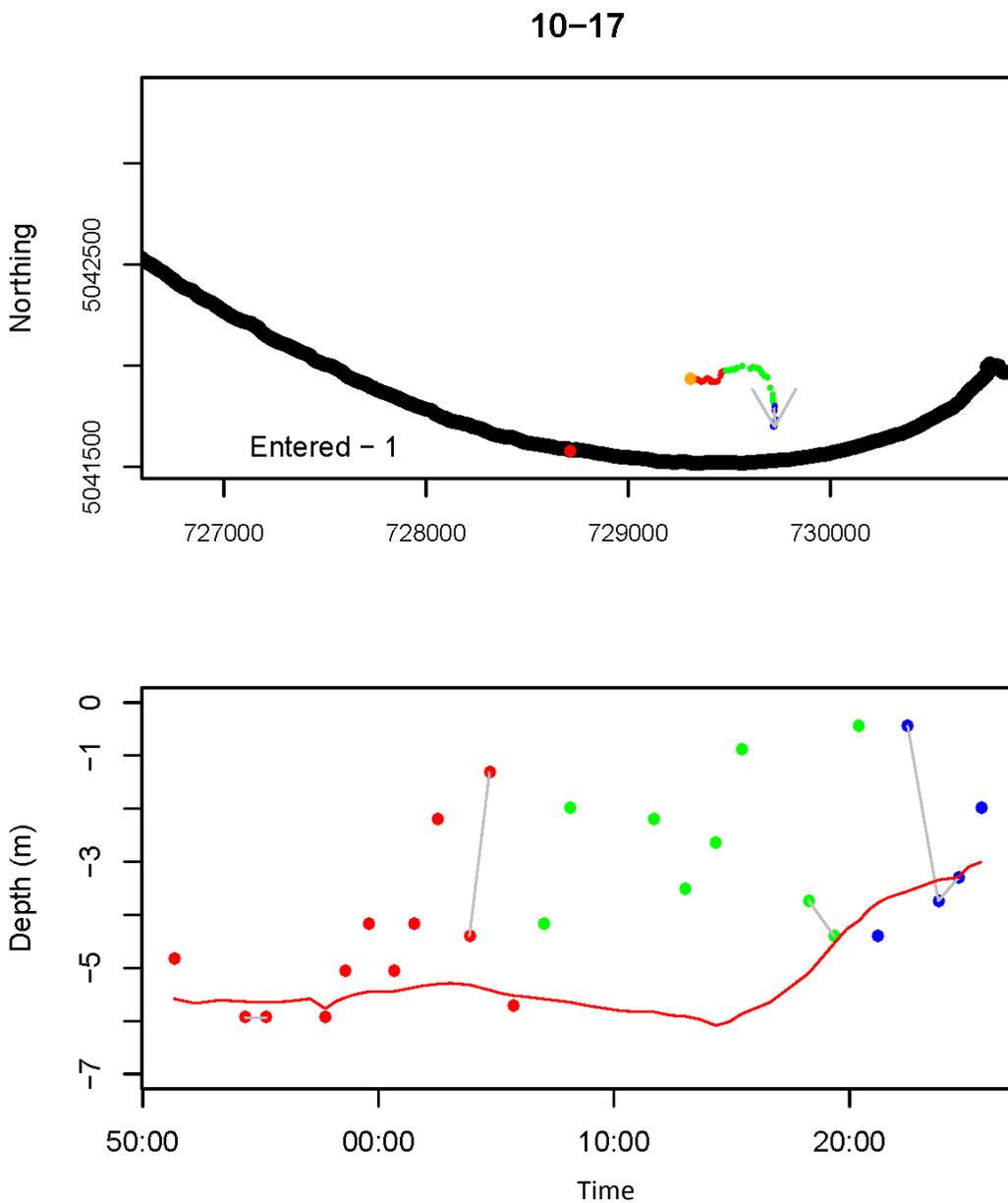


Figure 4.15: (cont'd)

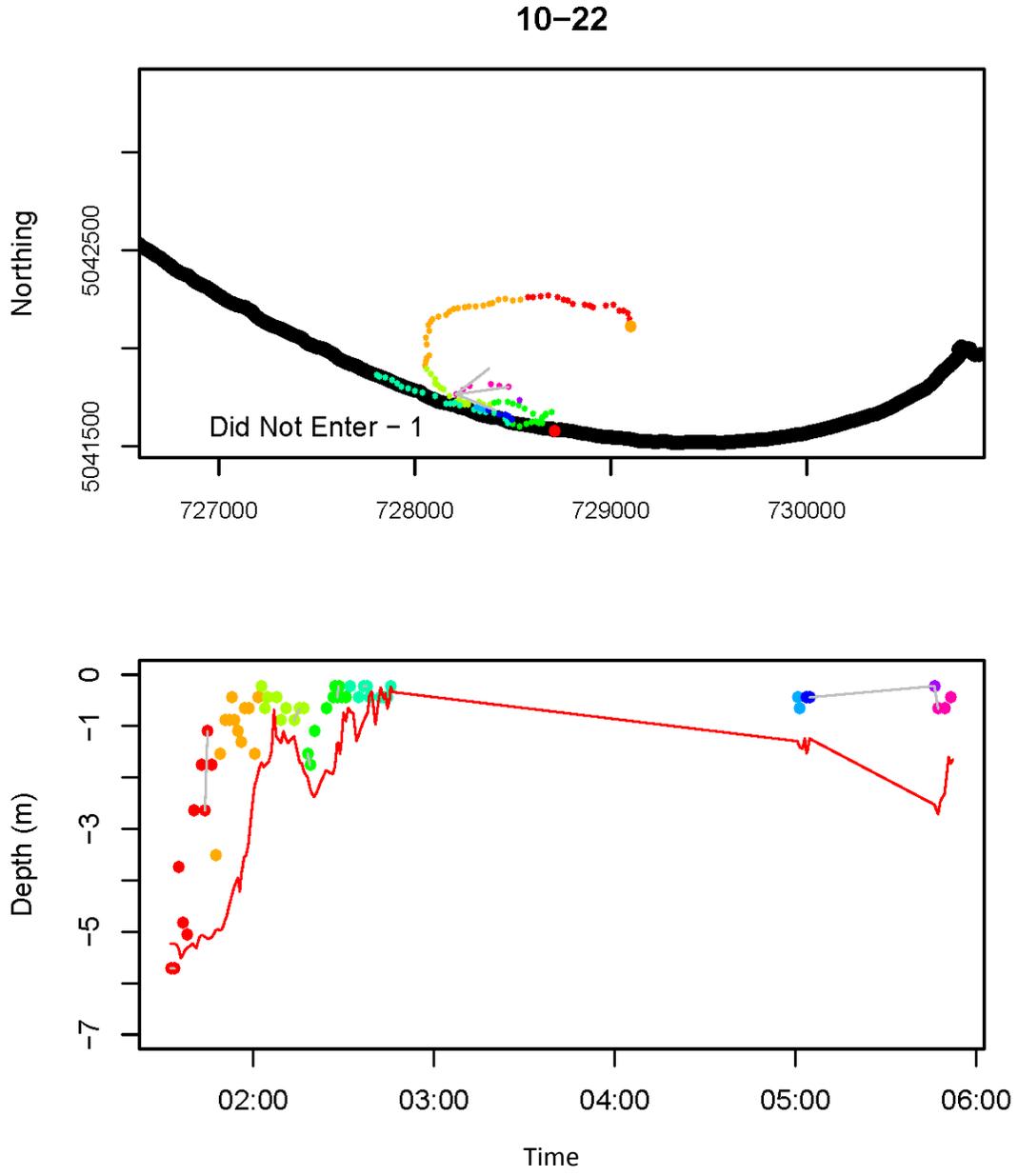


Figure 4.15: (cont'd)

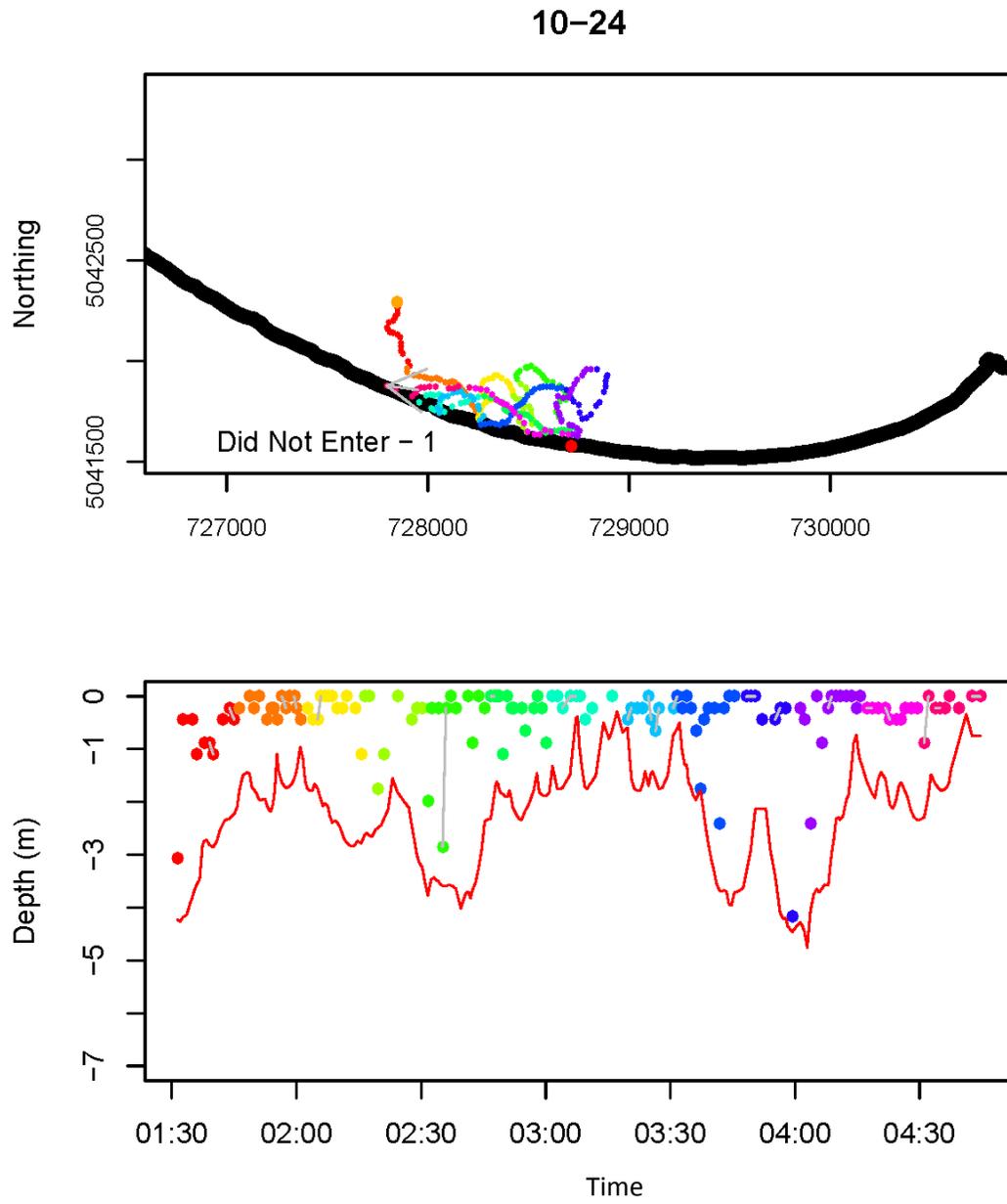


Figure 4.15: (cont'd)

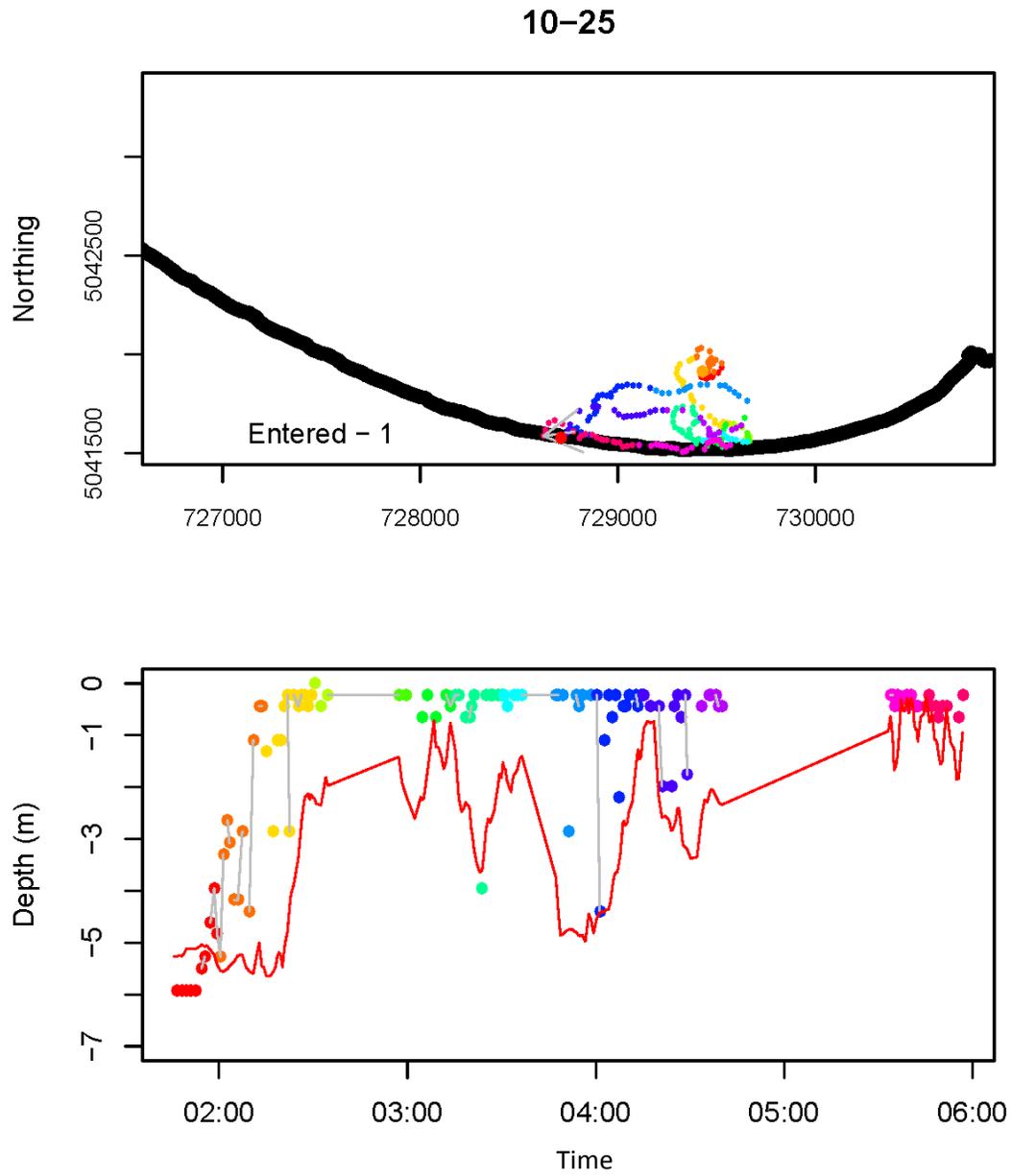


Figure 4.15: (cont'd)

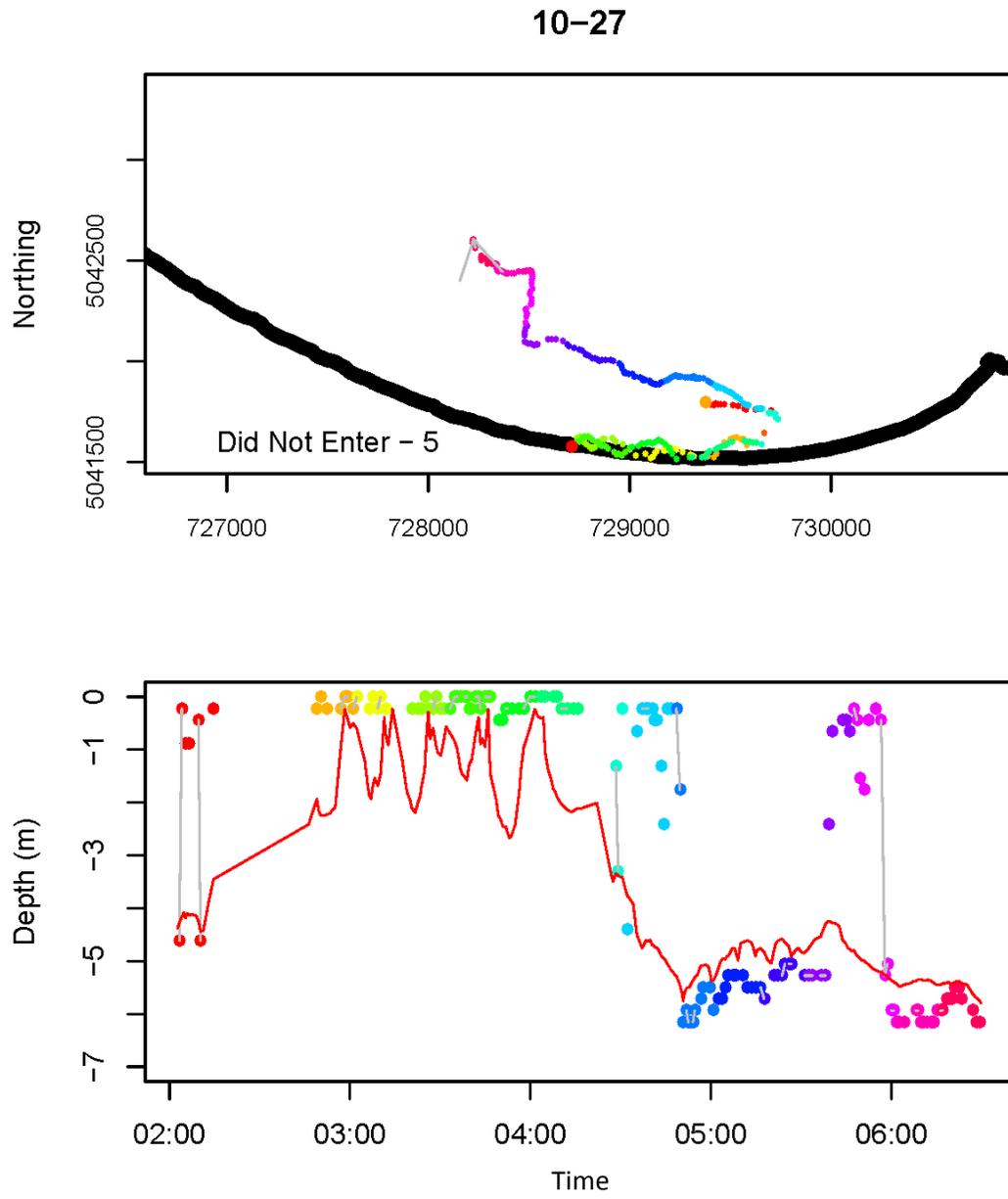


Figure 4.15: (cont'd)

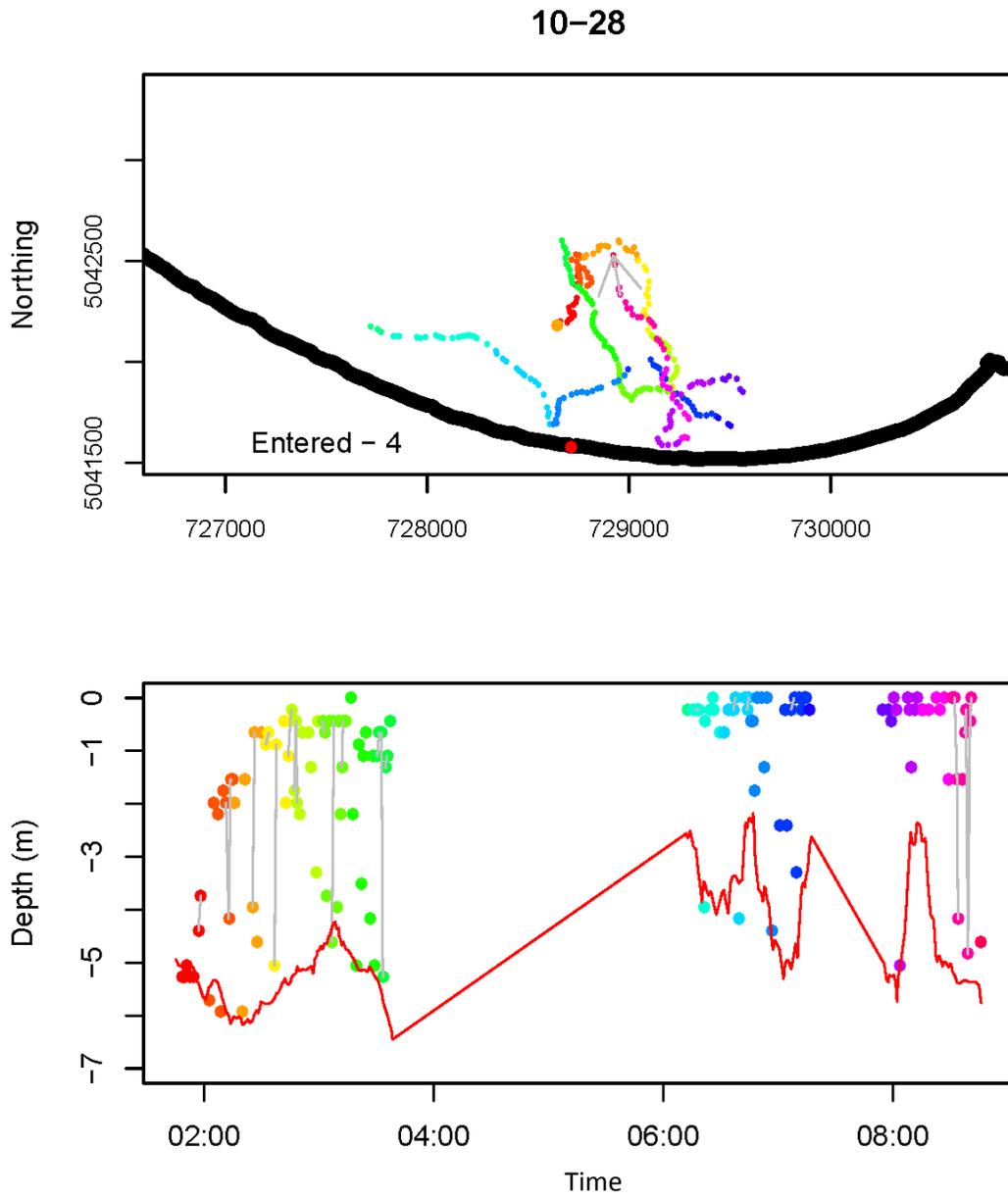


Figure 4.15: (cont'd)

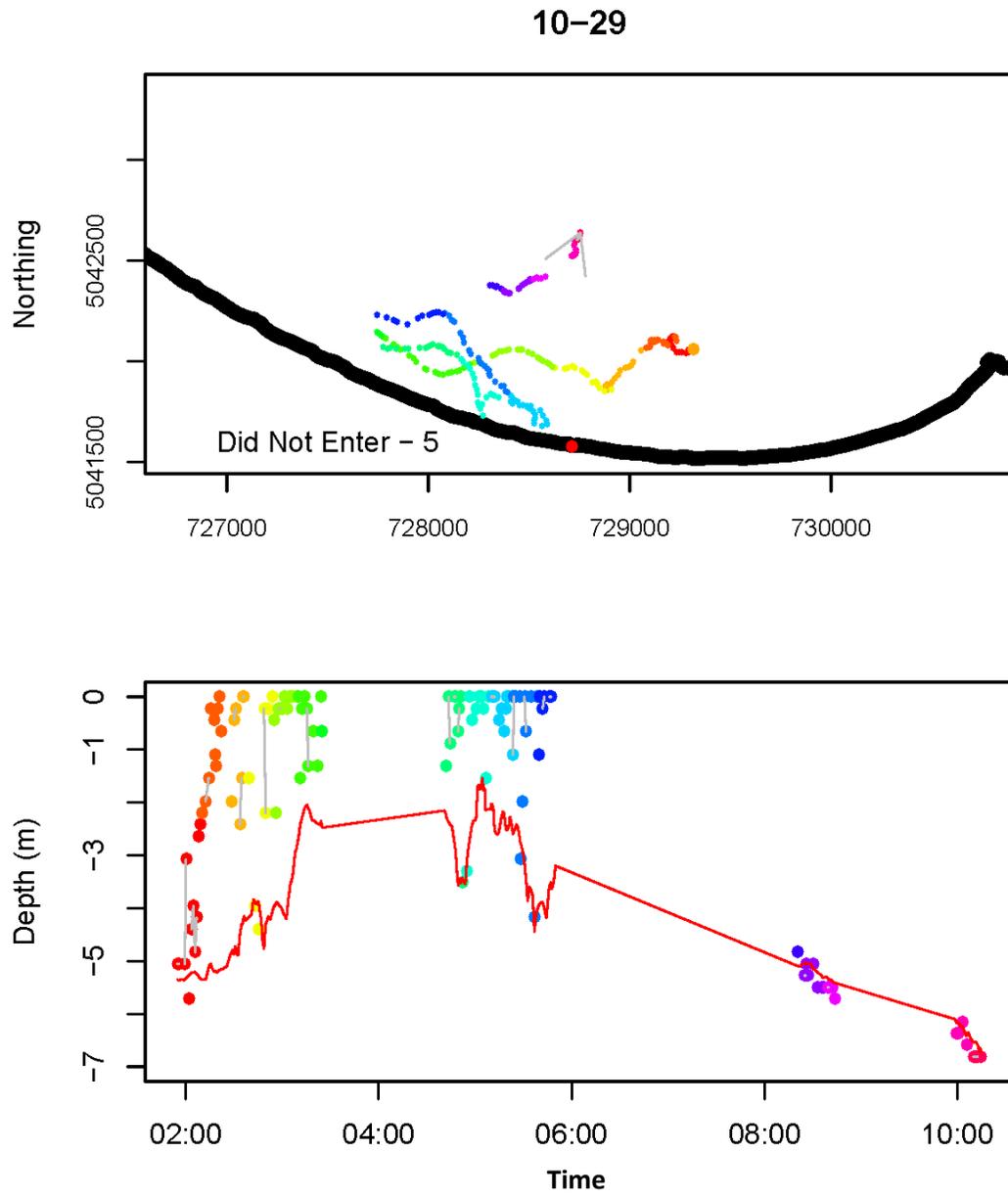


Figure 4.15: (cont'd)

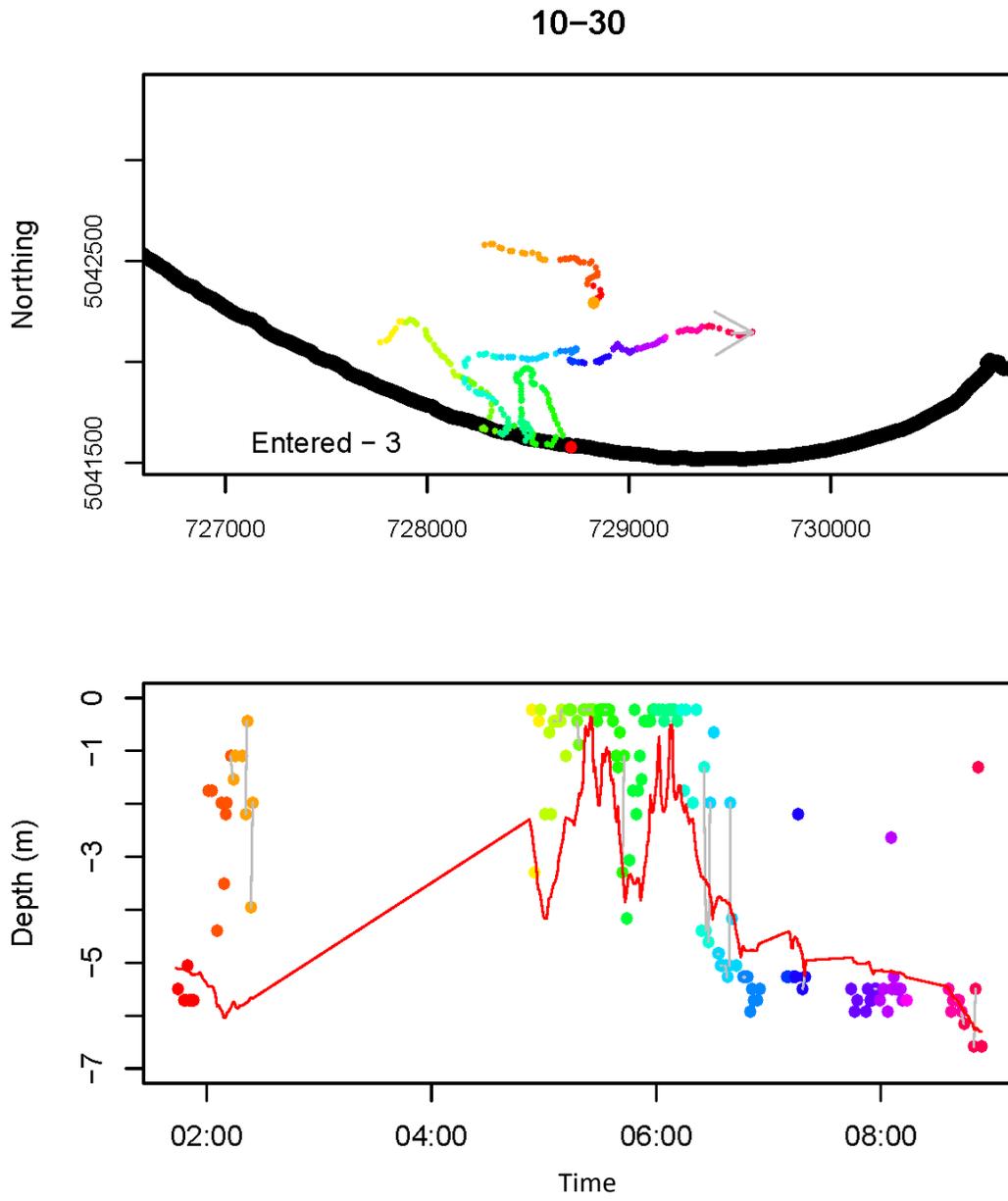


Figure 4.15: (cont'd)

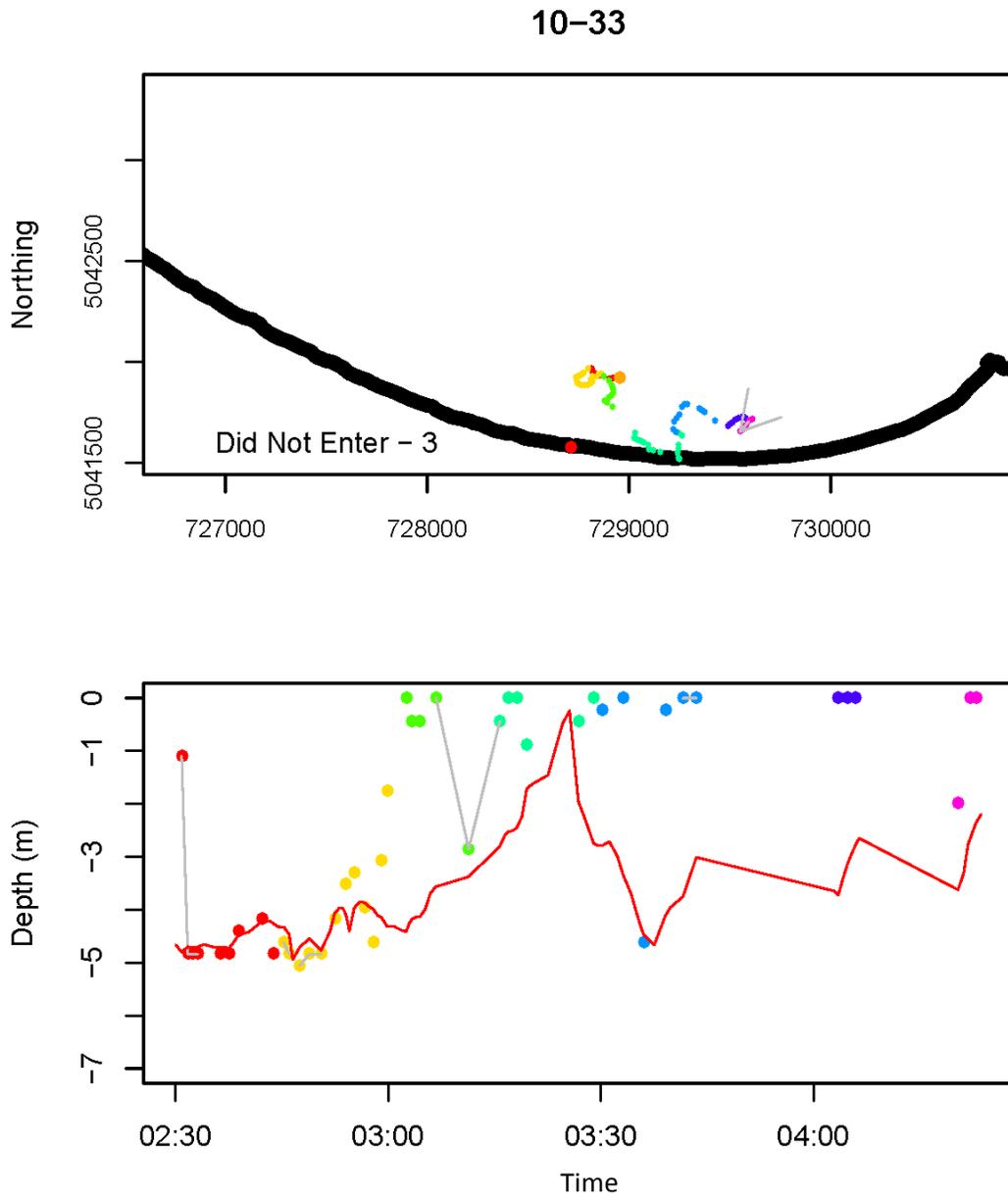


Figure 4.15: (cont'd)

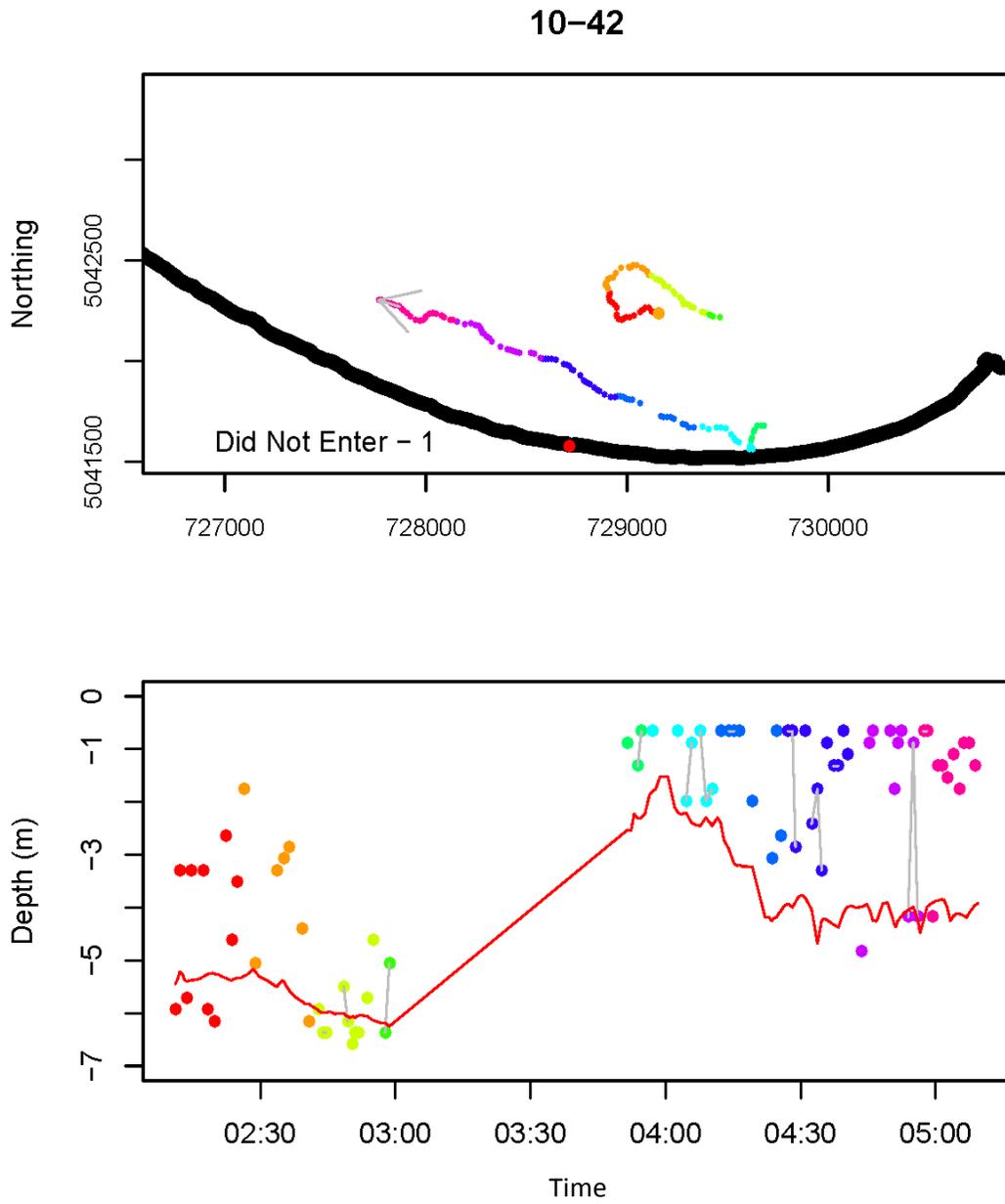


Figure 4.15: (cont'd)

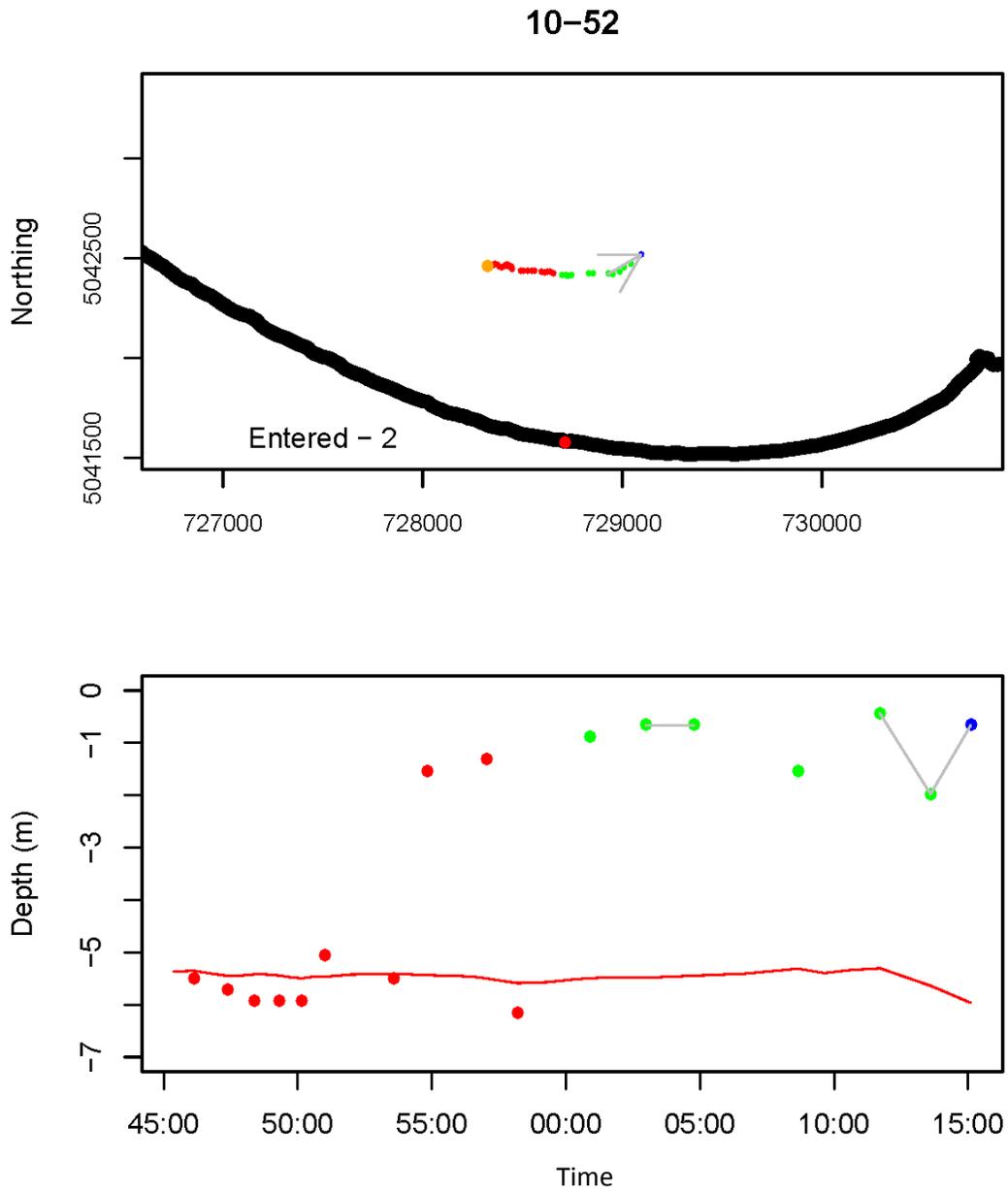


Figure 4.15: (cont'd)

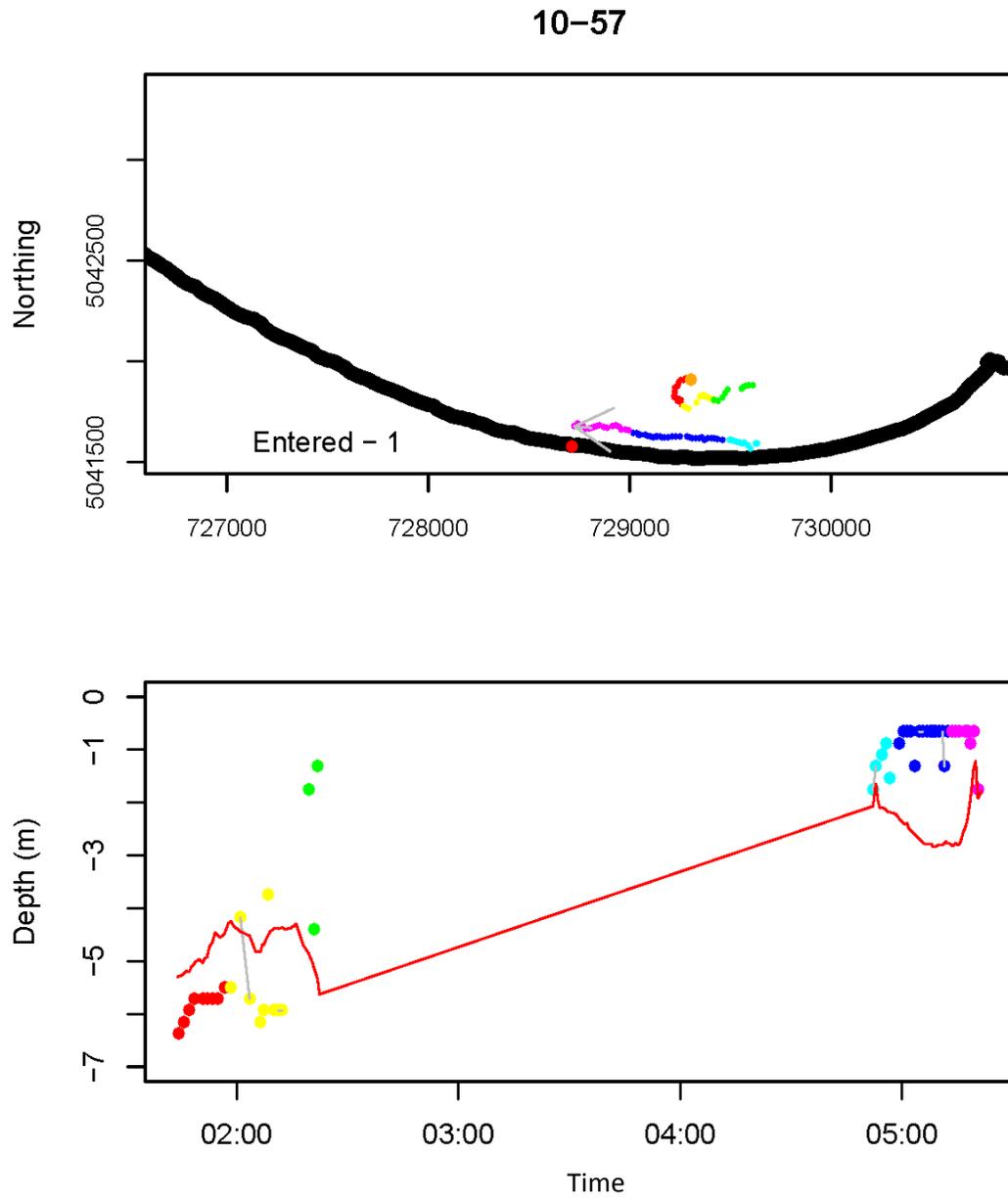


Figure 4.15: (cont'd)

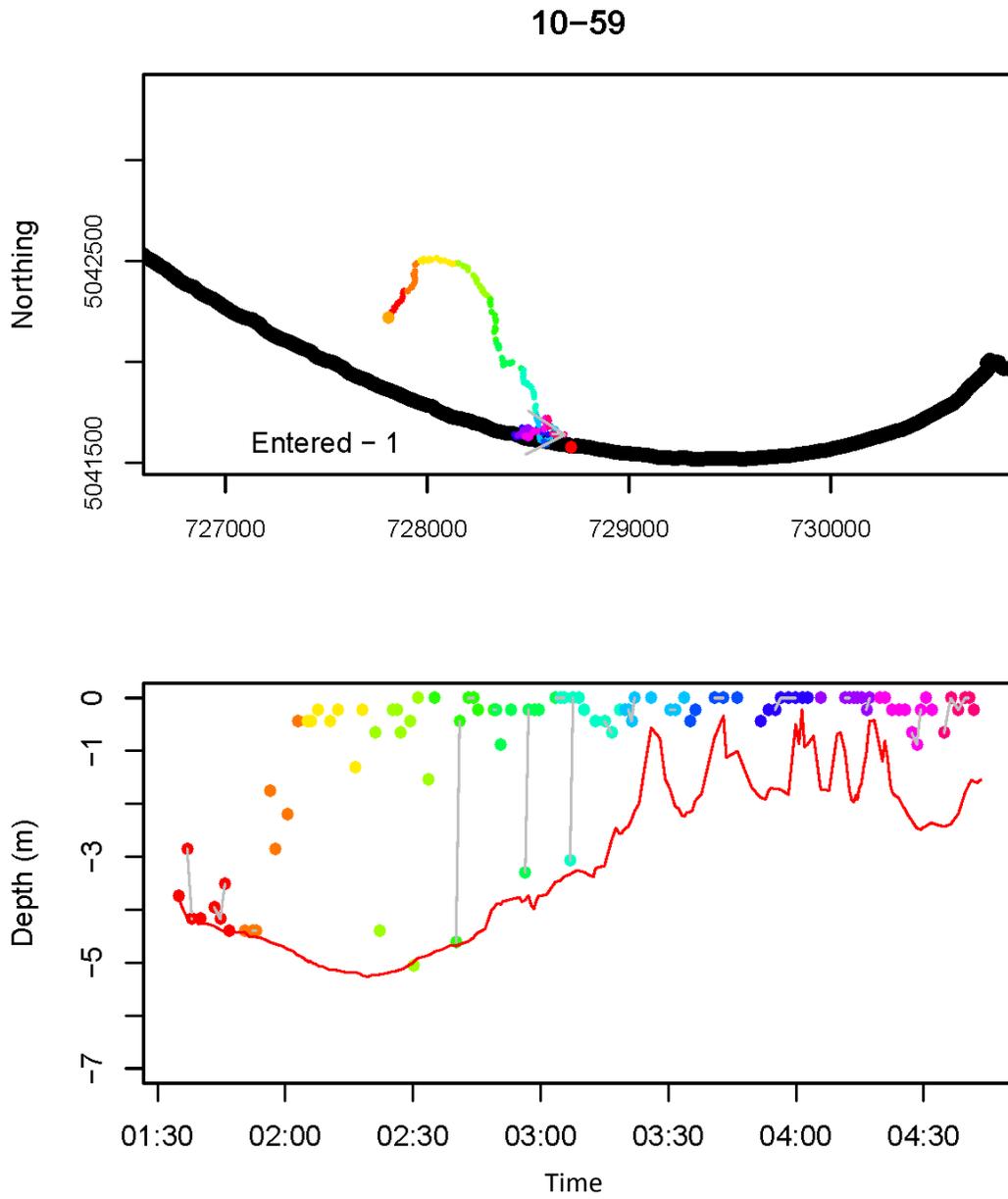


Figure 4.15: (cont'd)

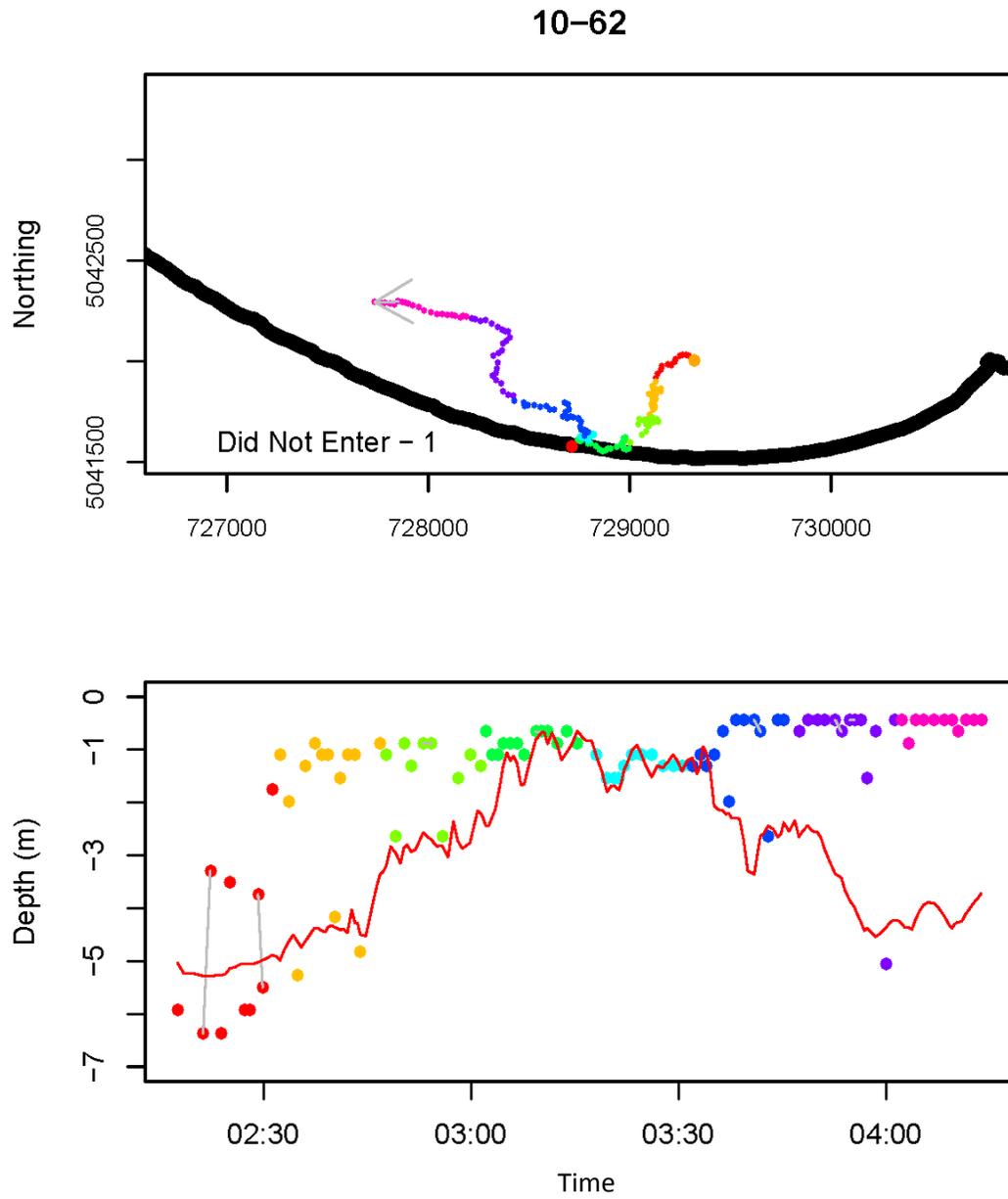


Figure 4.15: (cont'd)

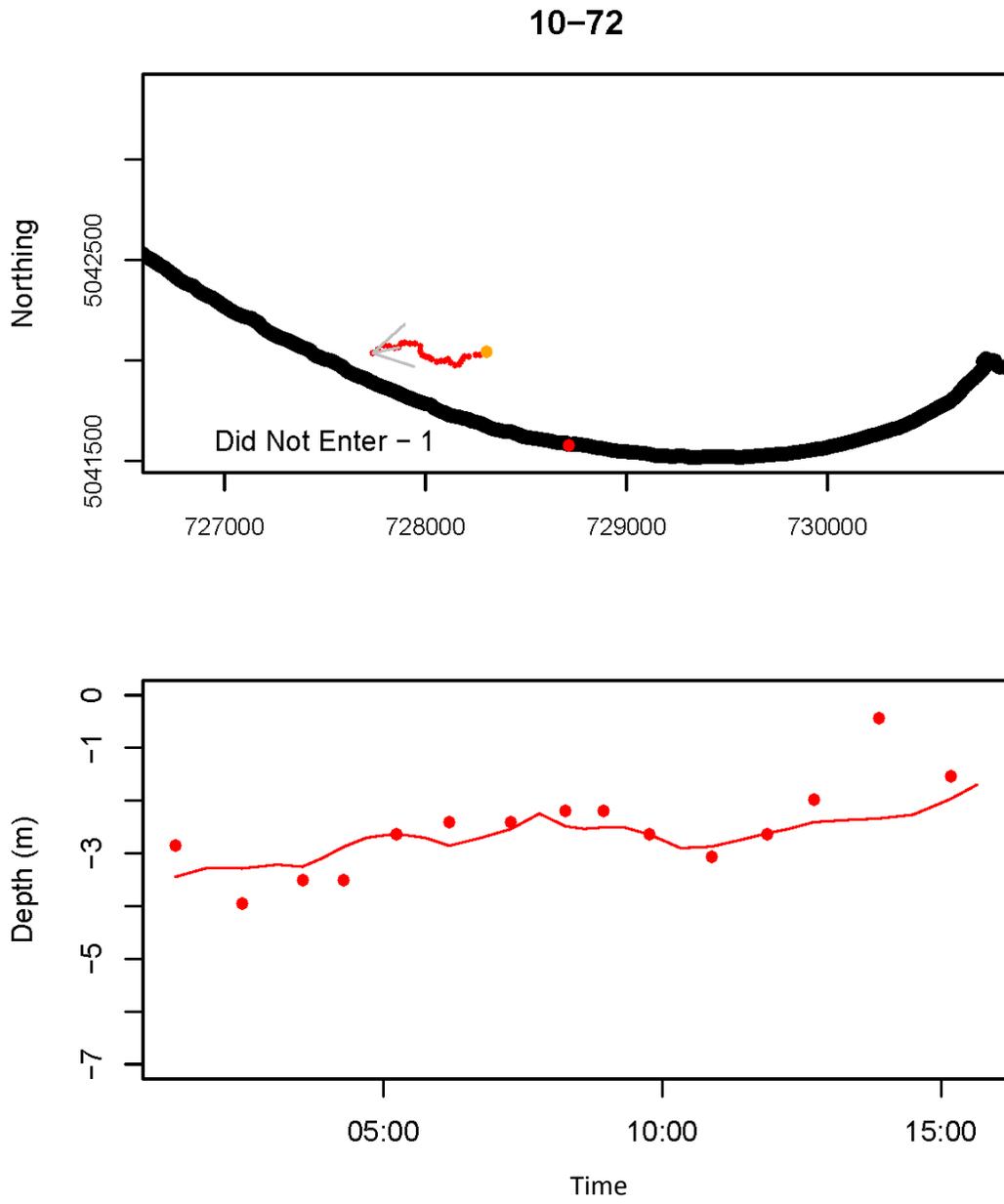


Figure 4.15: (cont'd)

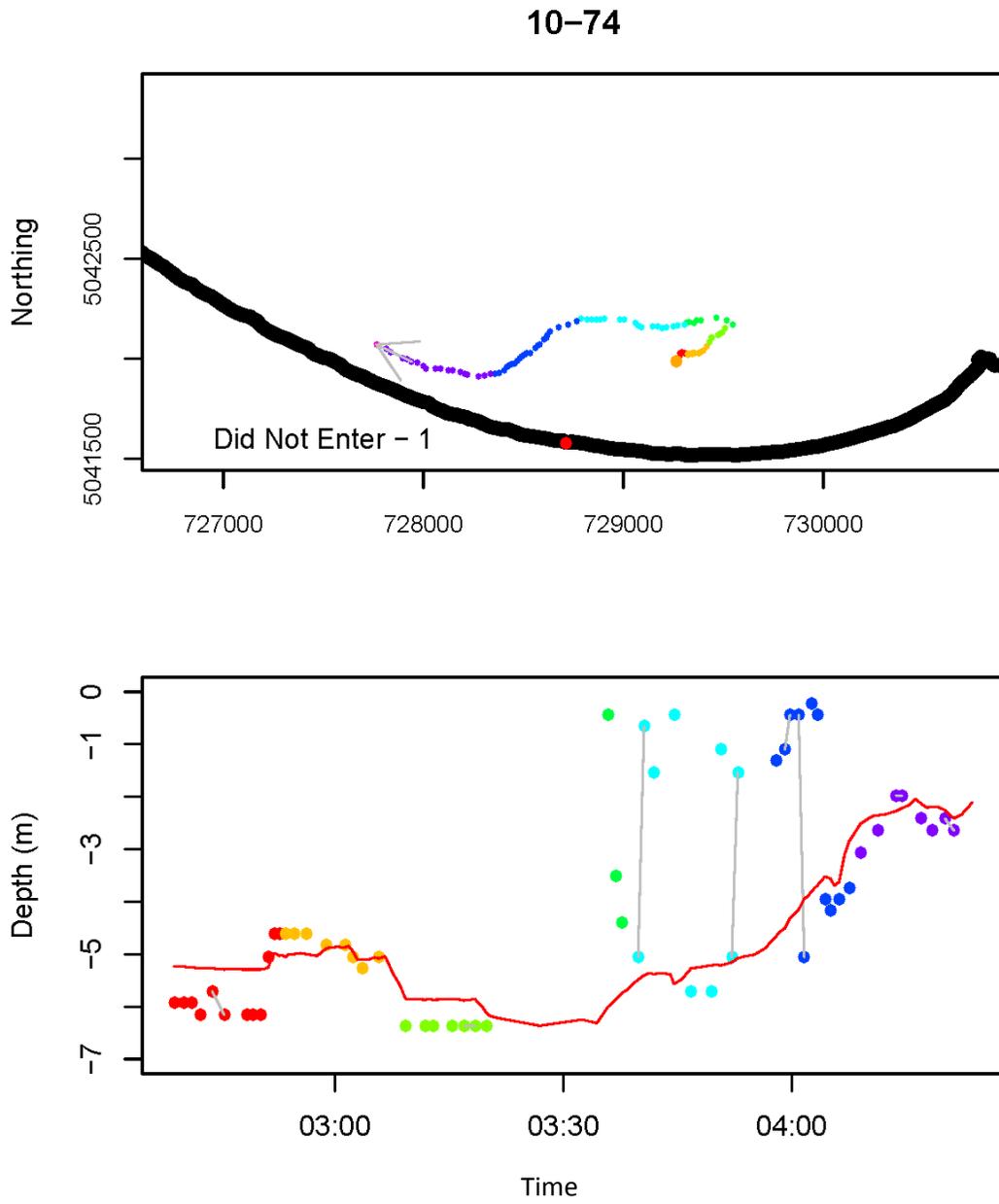


Figure 4.15: (cont'd)

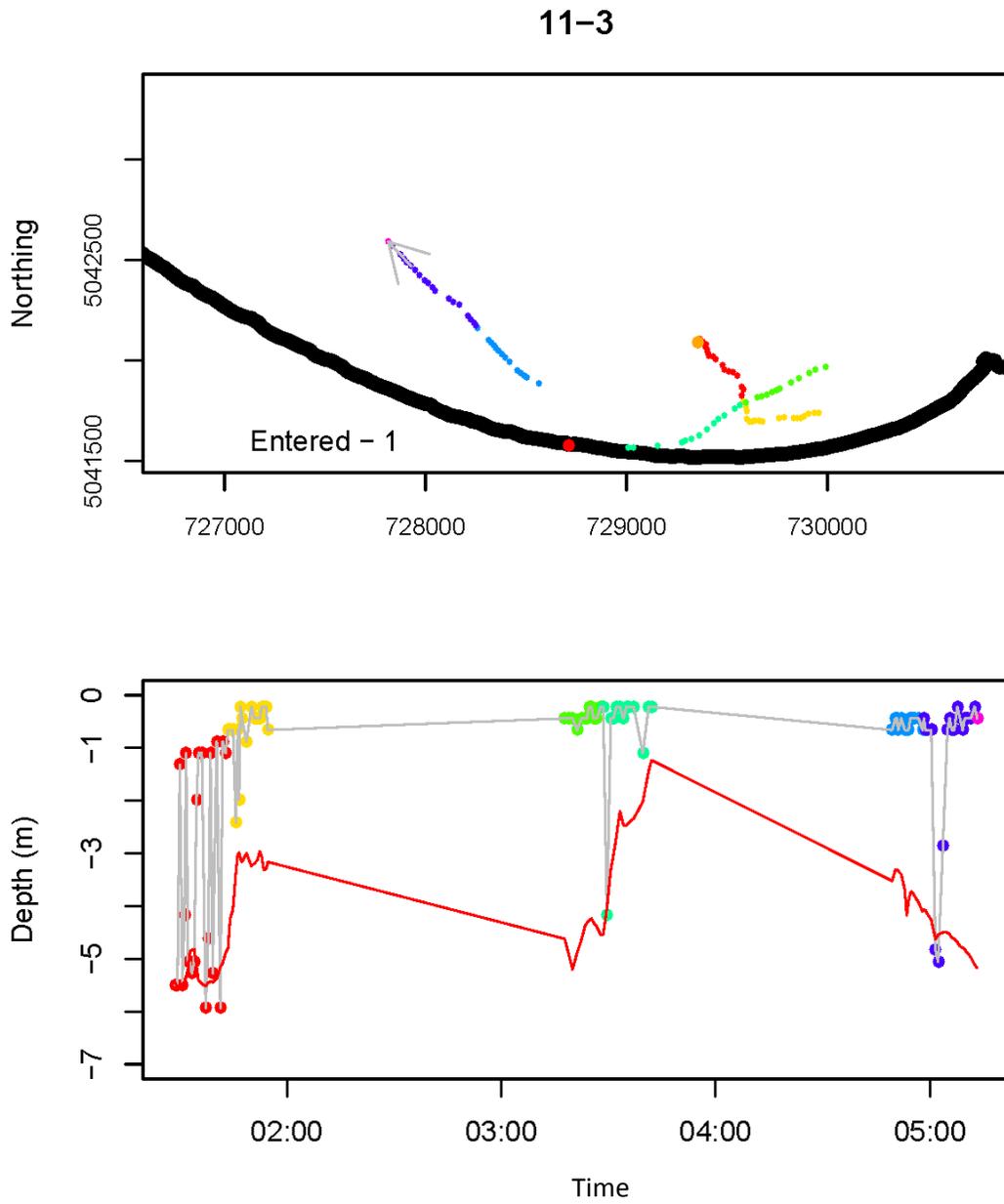


Figure 4.15: (cont'd)

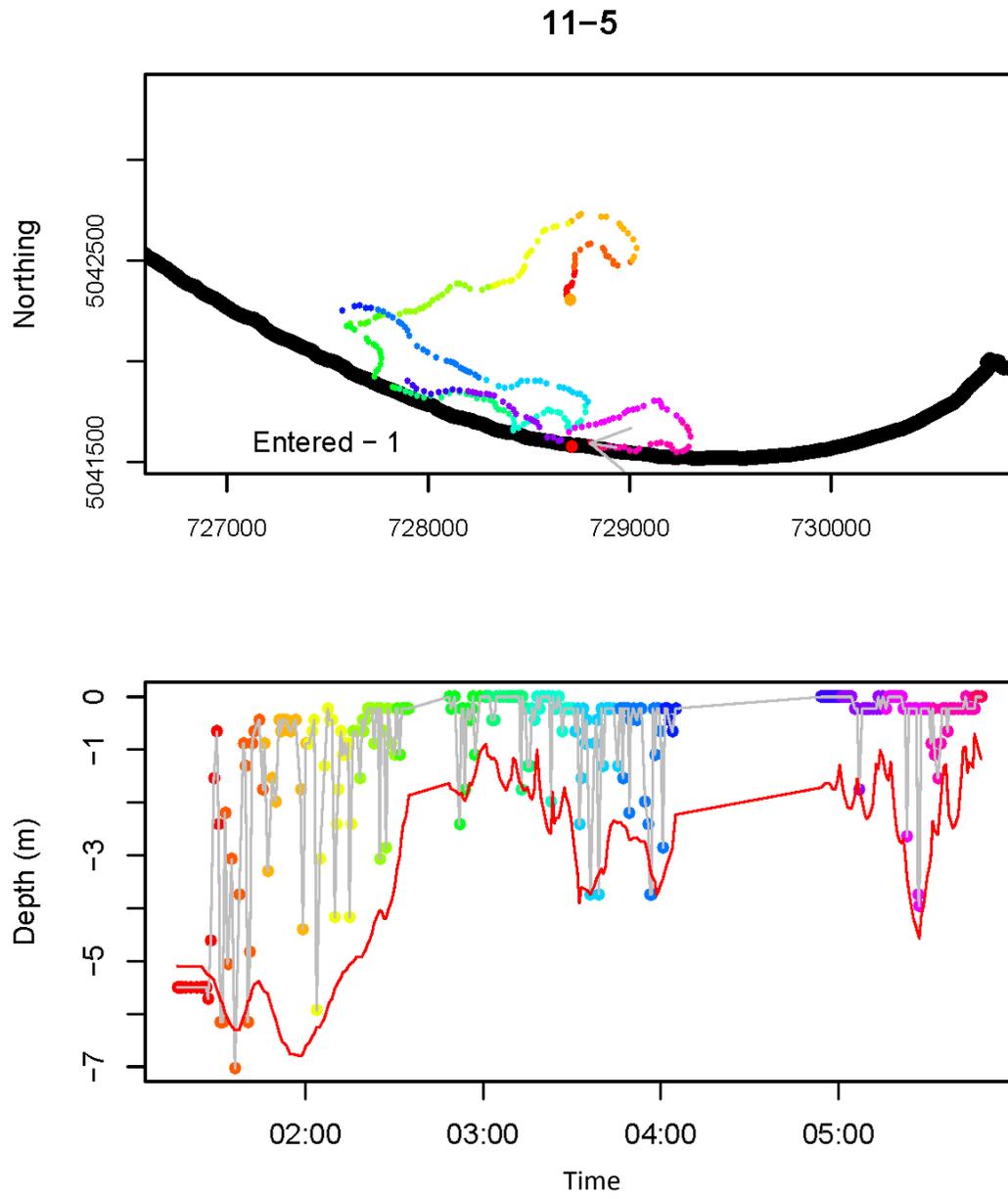


Figure 4.15: (cont'd)

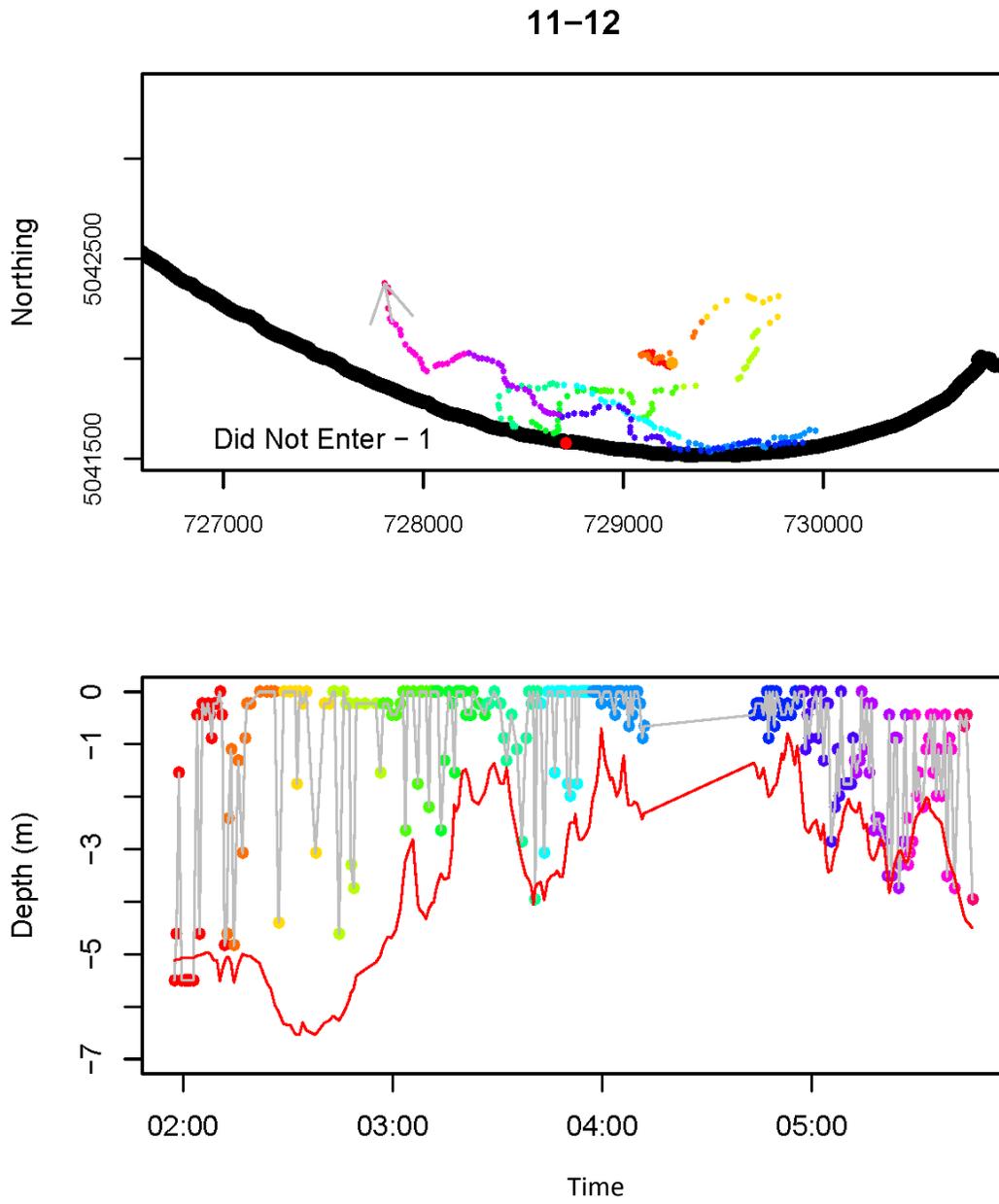


Figure 4.15: (cont'd)

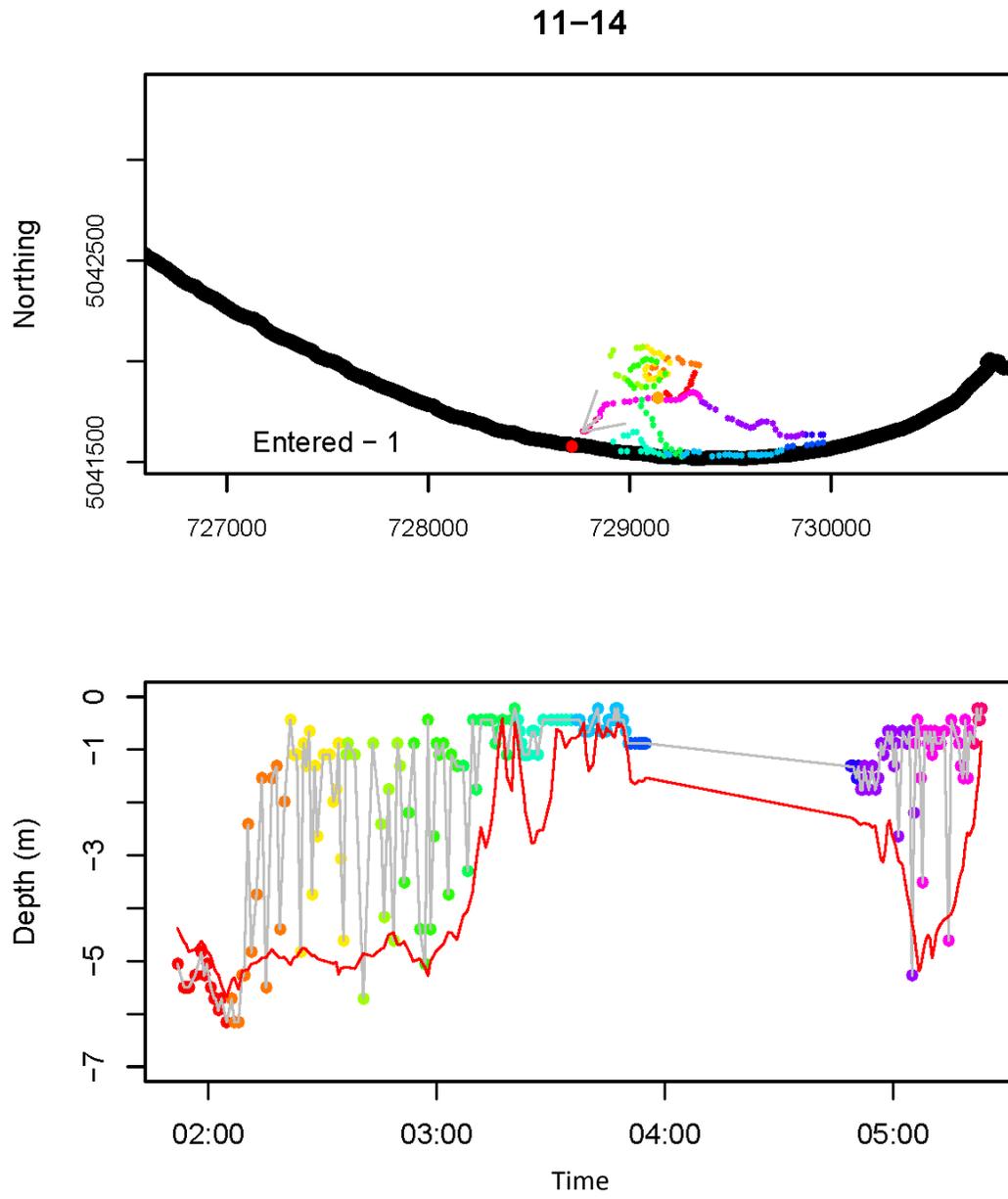


Figure 4.15: (cont'd)

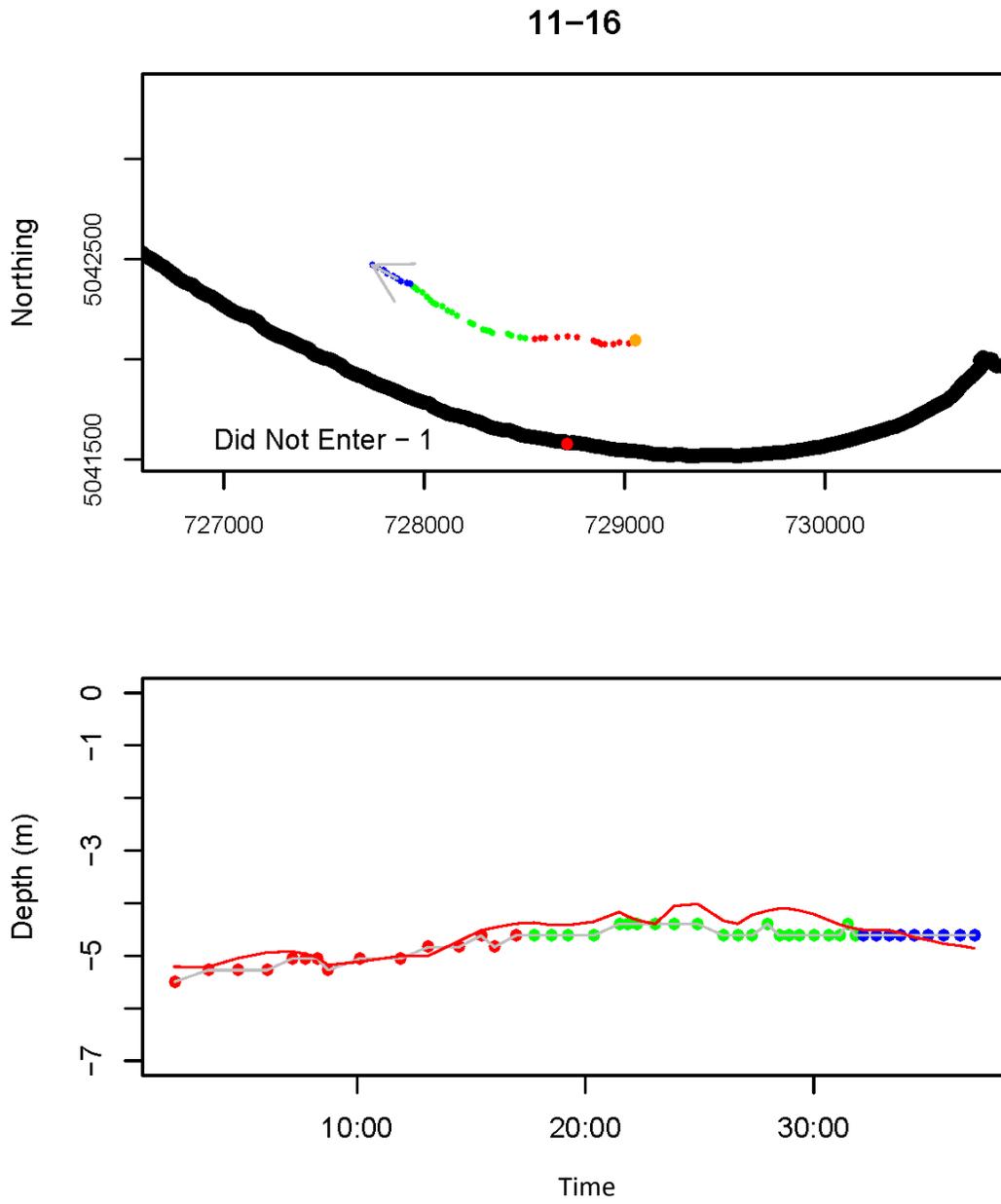


Figure 4.15: (cont'd)

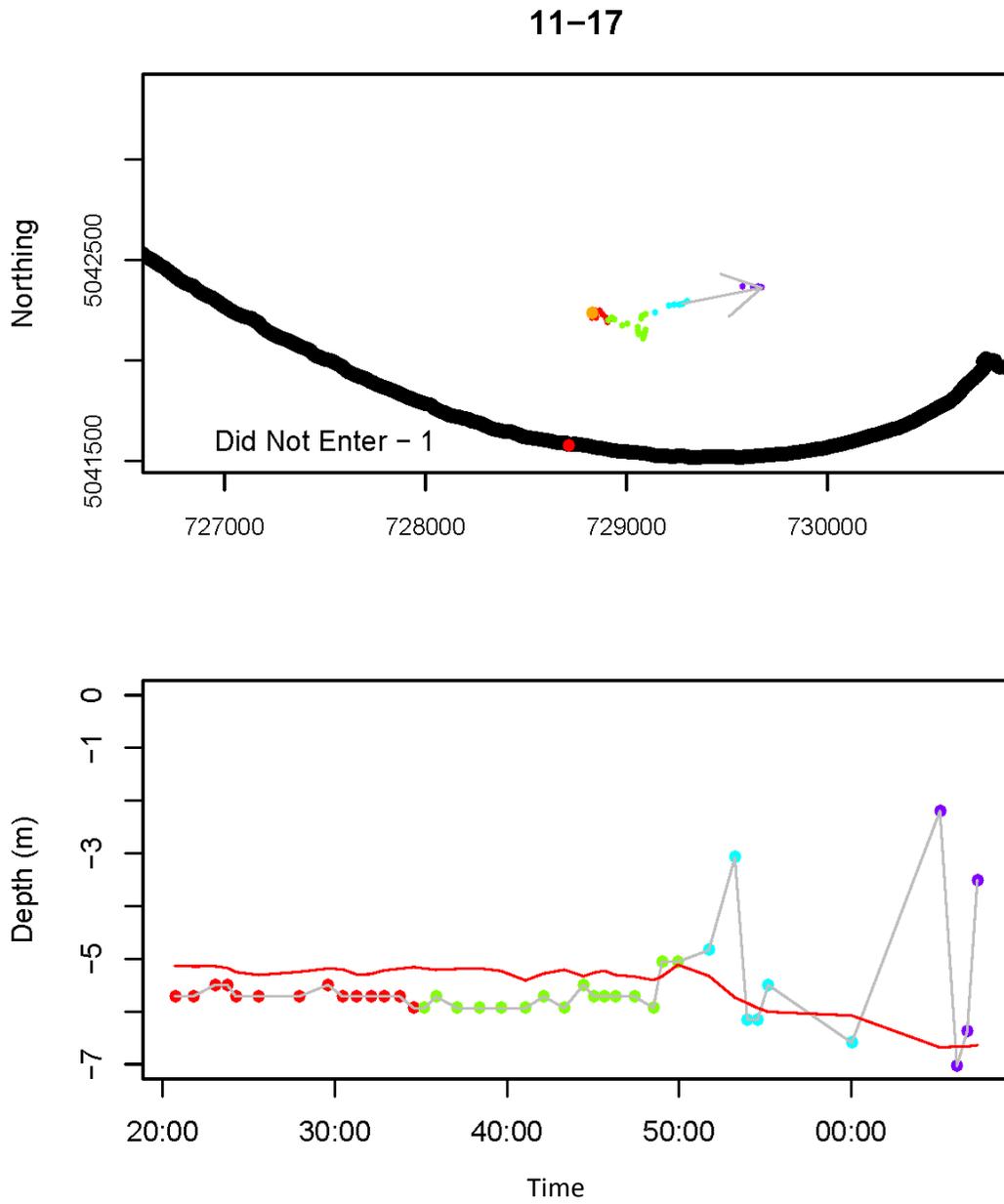


Figure 4.15: (cont'd)

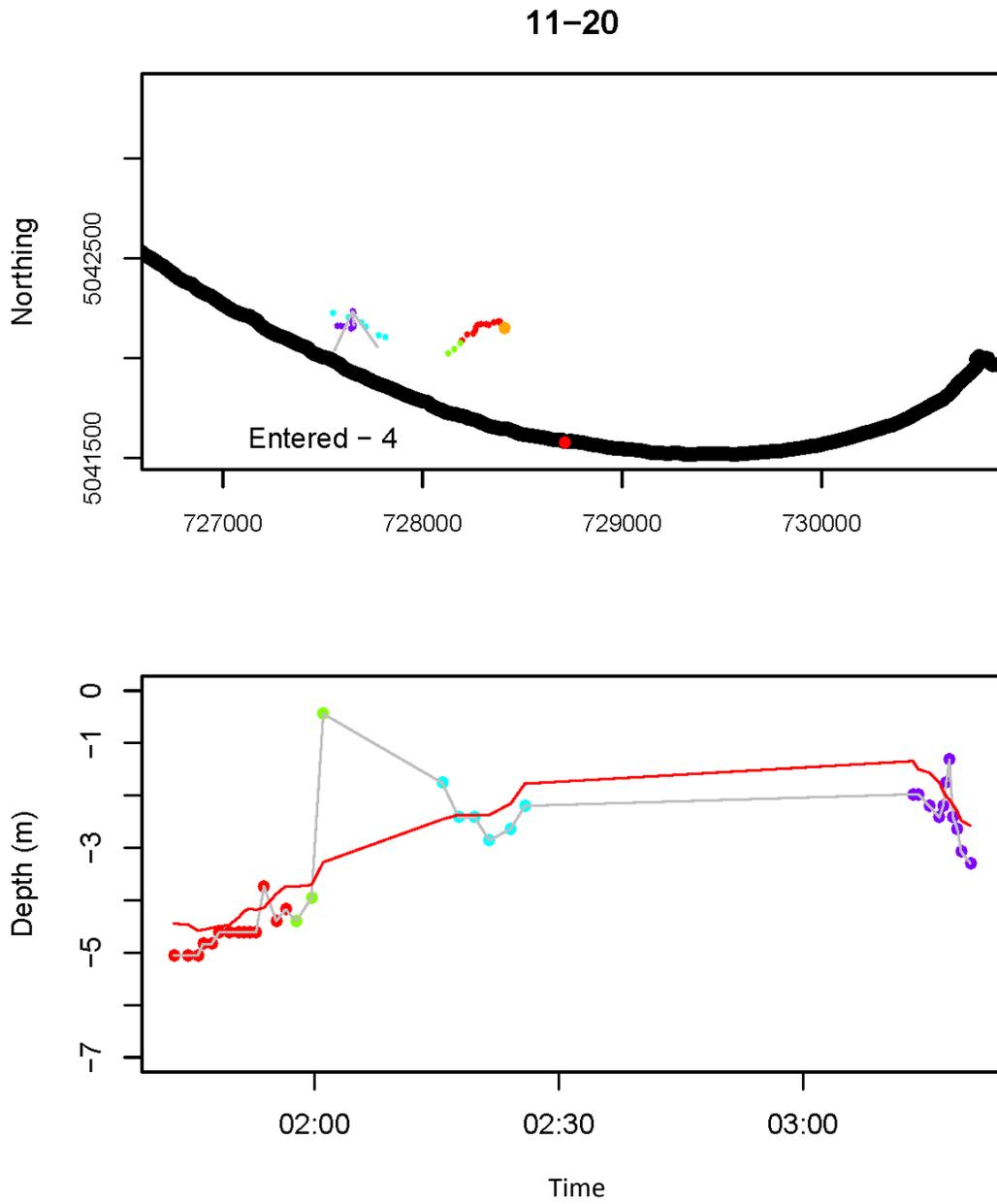


Figure 4.15: (cont'd)

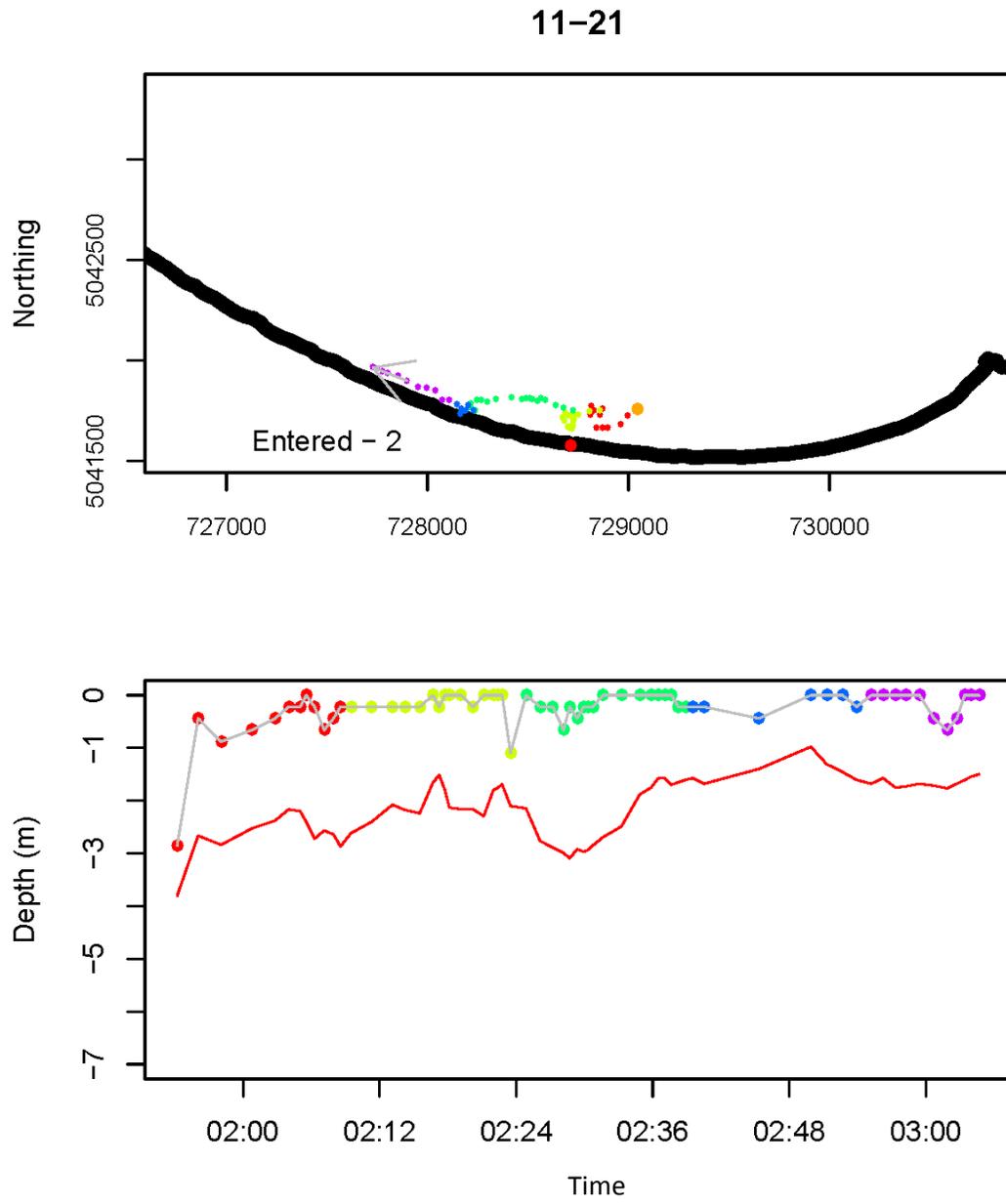


Figure 4.15: (cont'd)

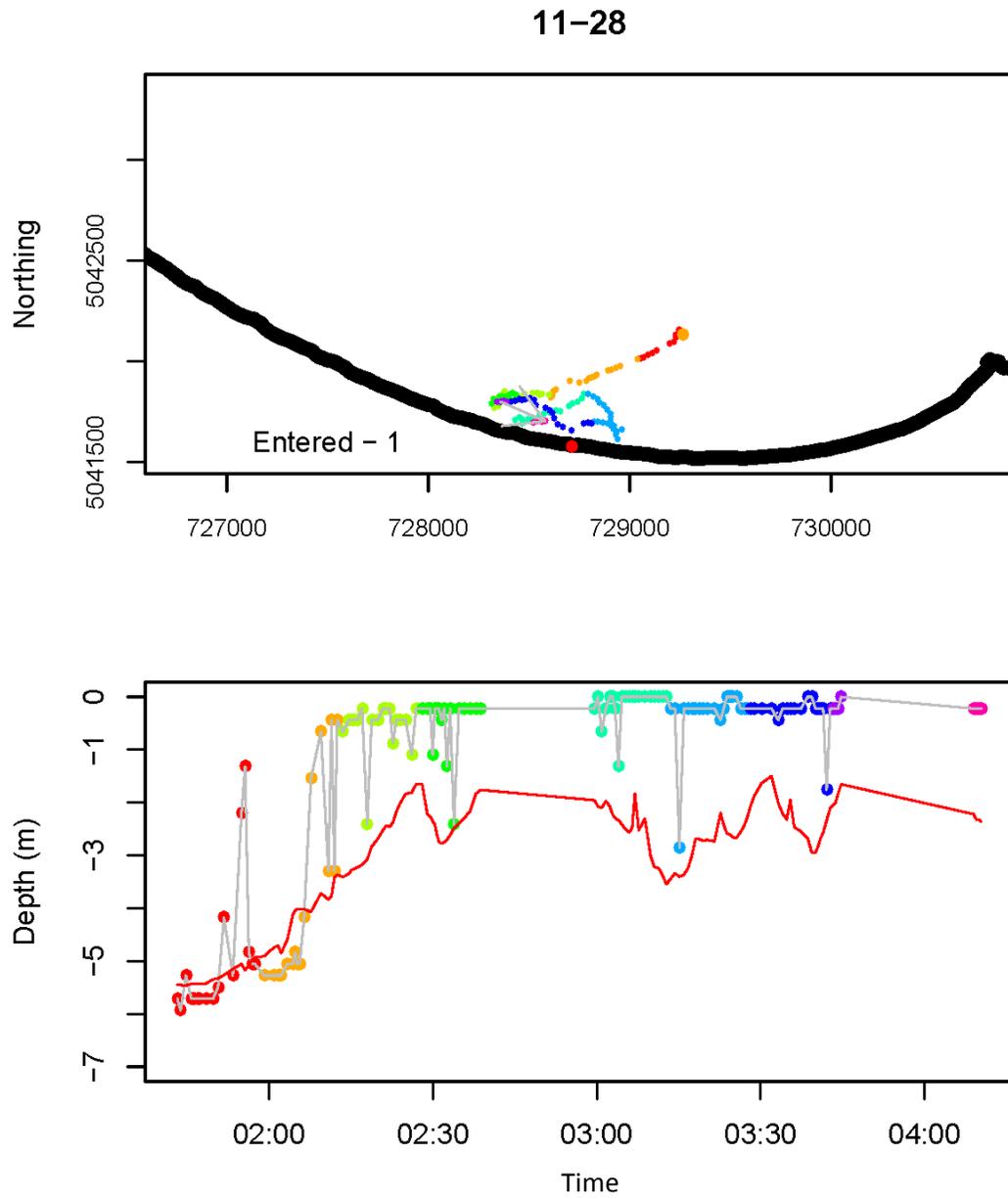


Figure 4.15: (cont'd)

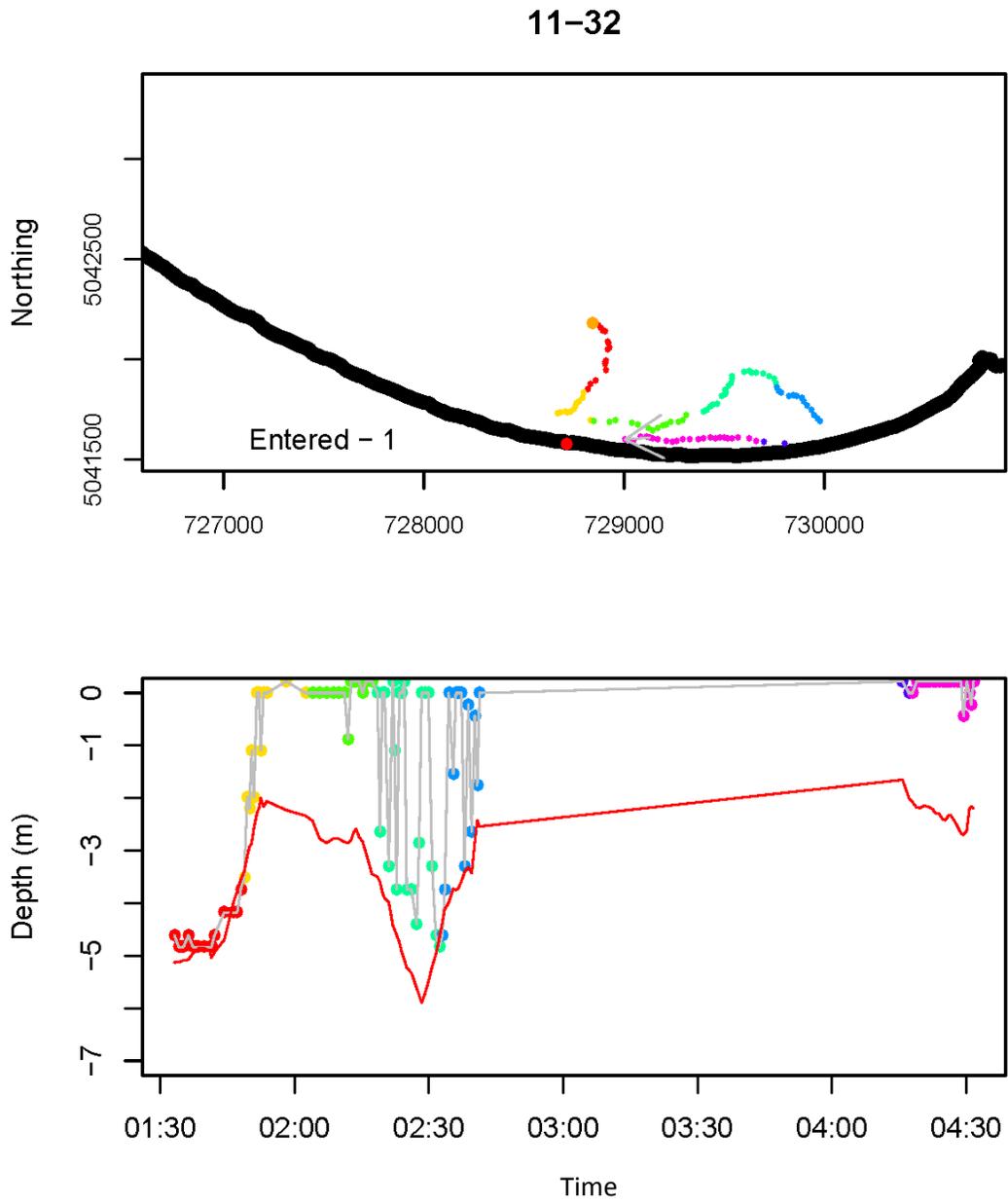


Figure 4.15: (cont'd)

11-37

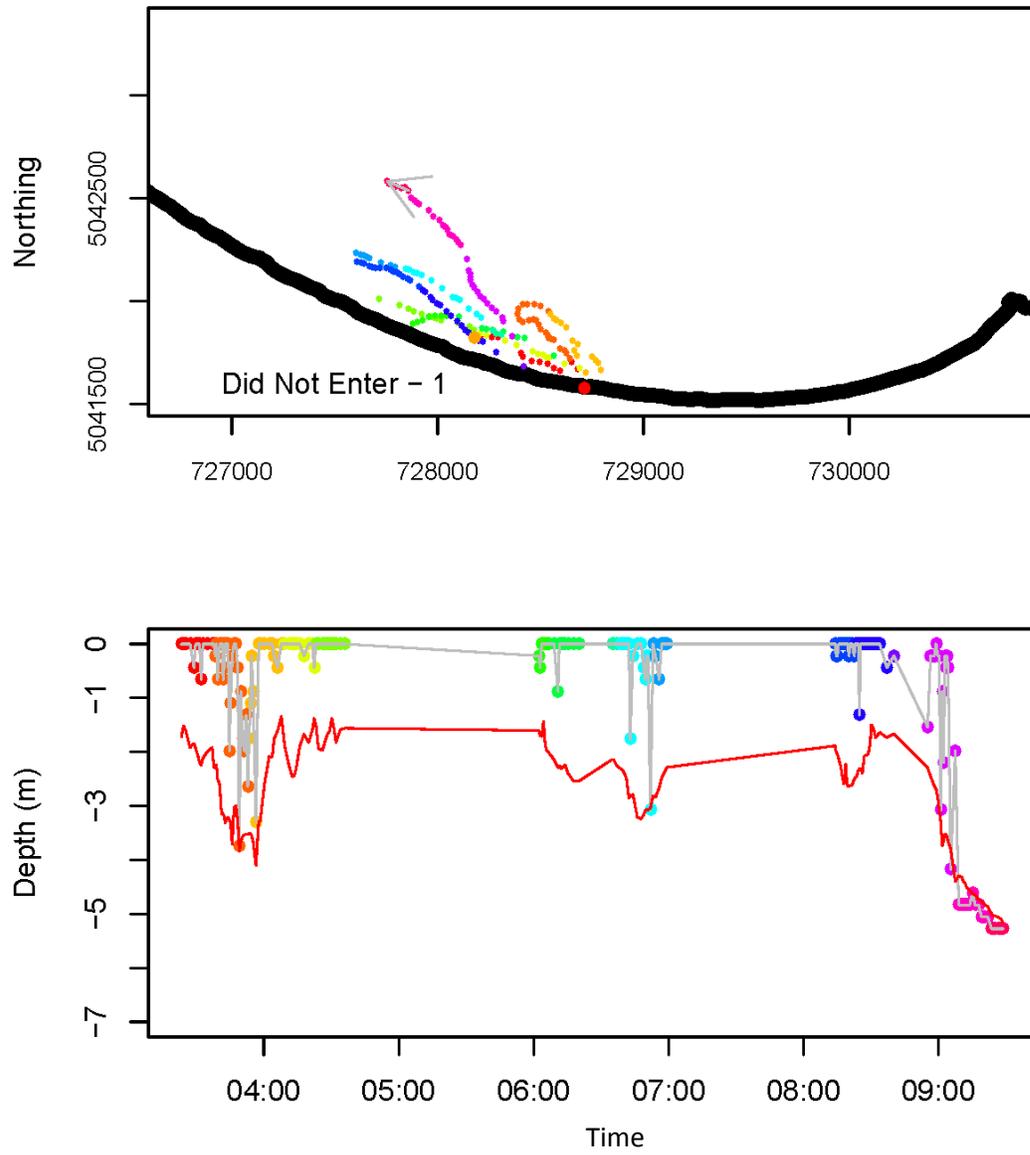


Figure 4.15: (cont'd)

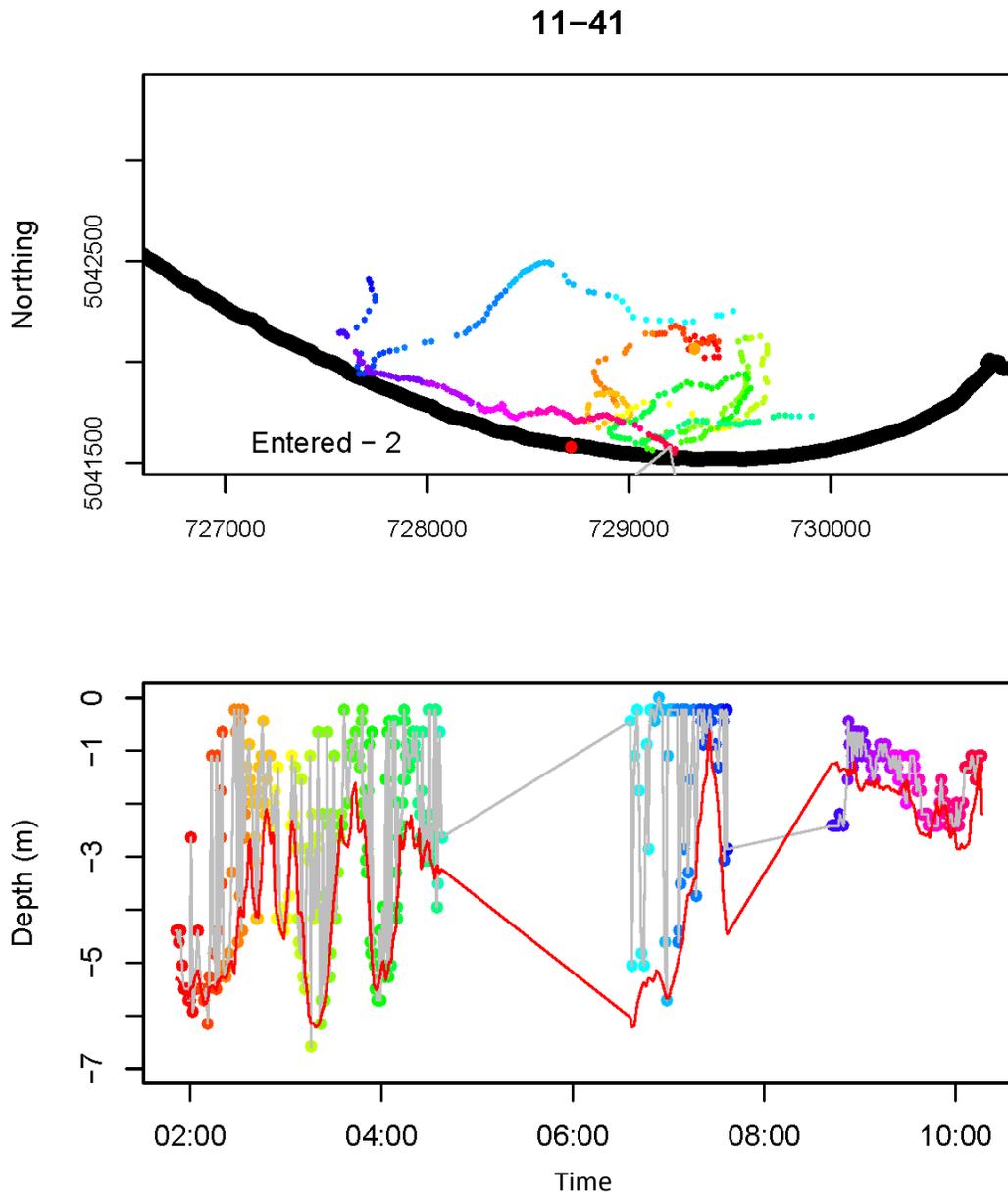


Figure 4.15: (cont'd)

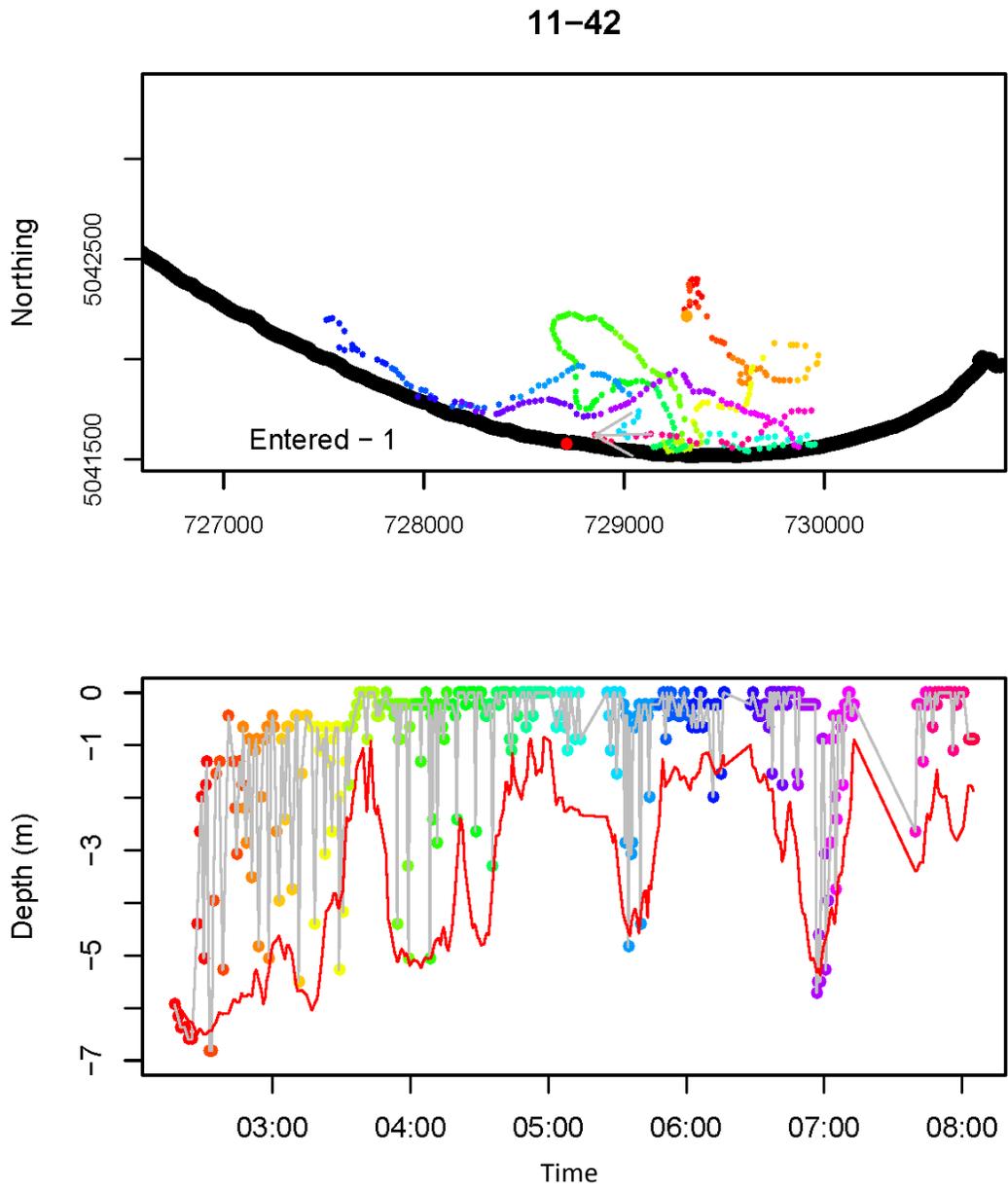


Figure 4.15: (cont'd)

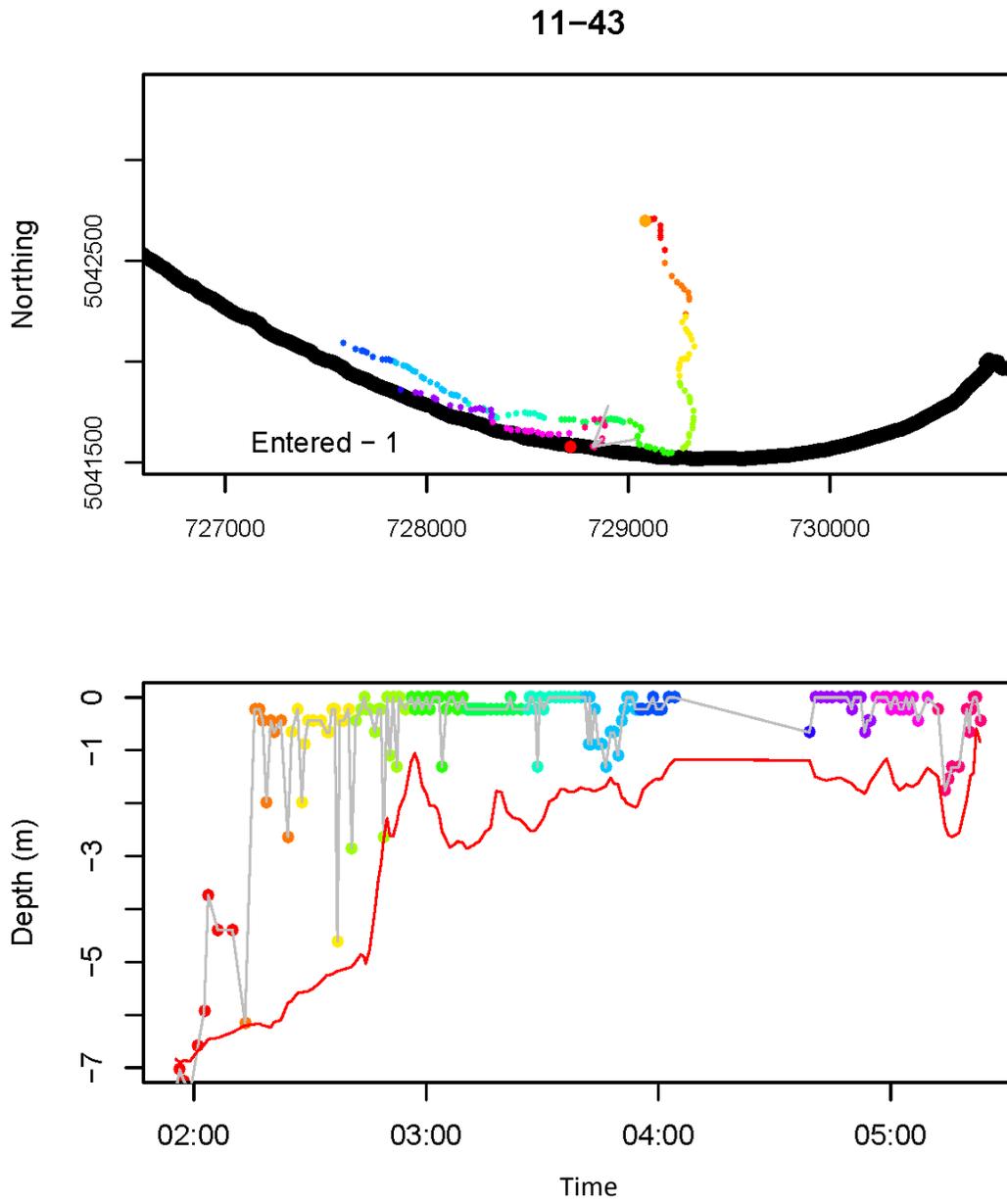


Figure 4.15: (cont'd)

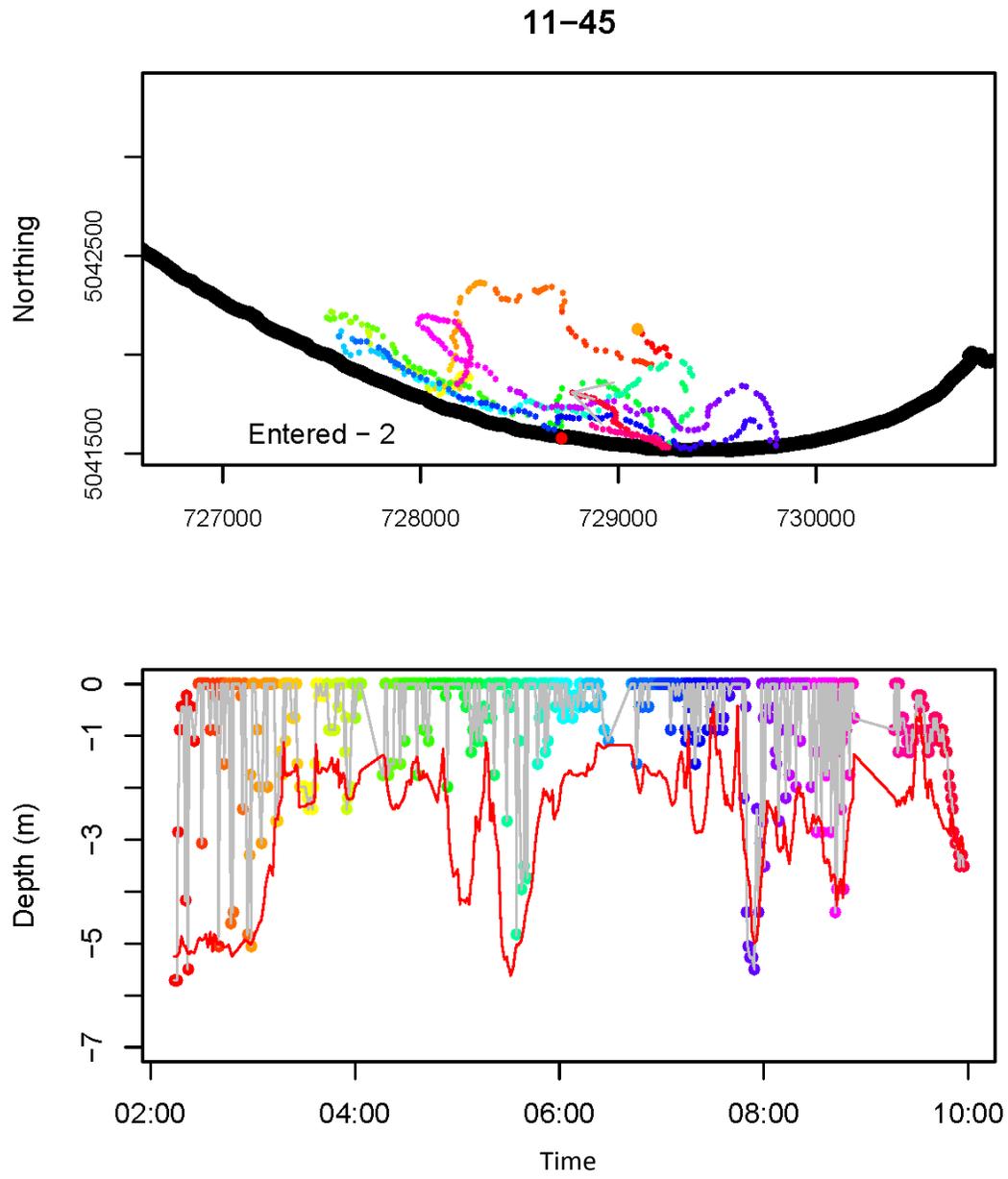


Figure 4.15: (cont'd)

11-46

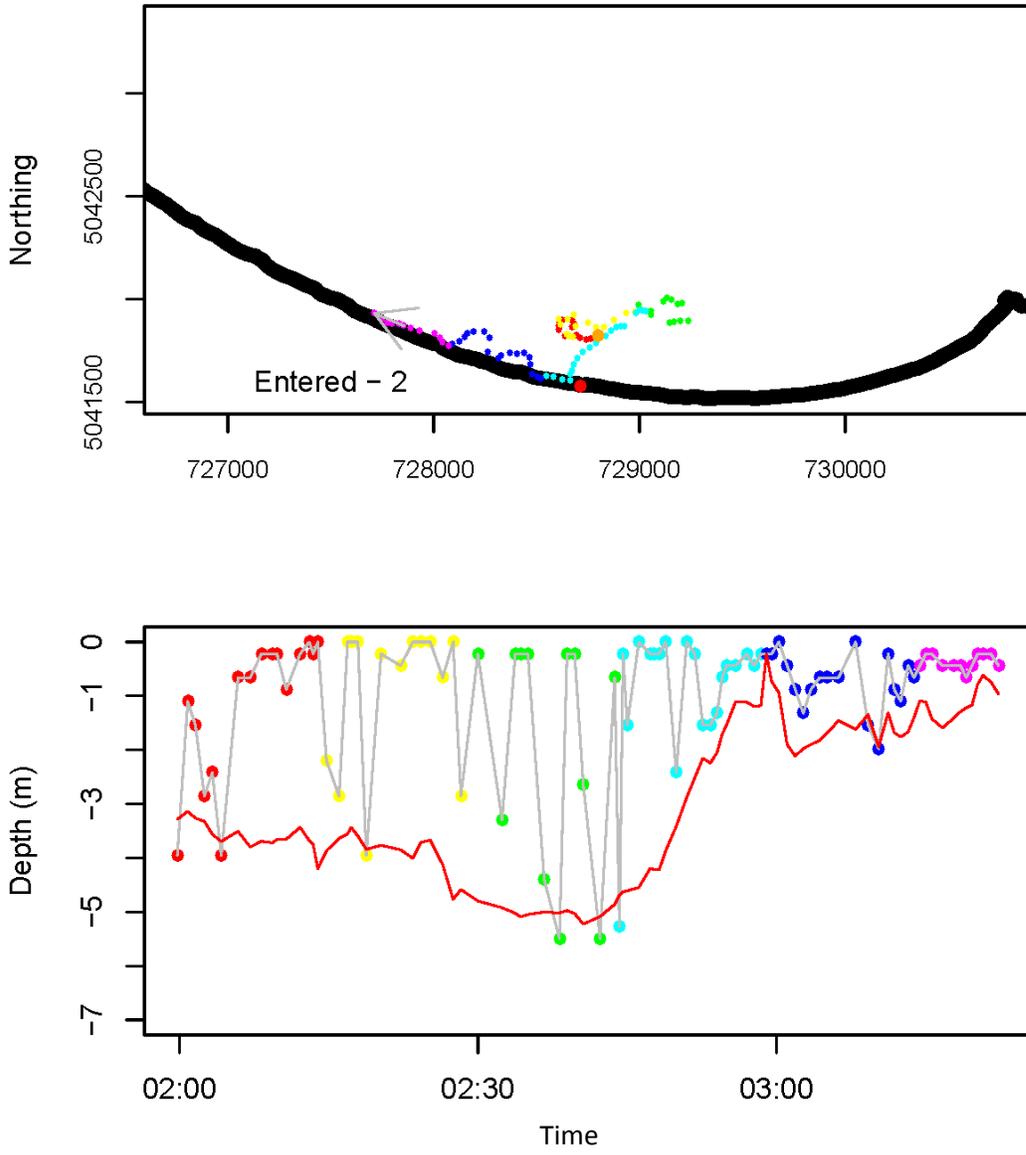


Figure 4.15: (cont'd)

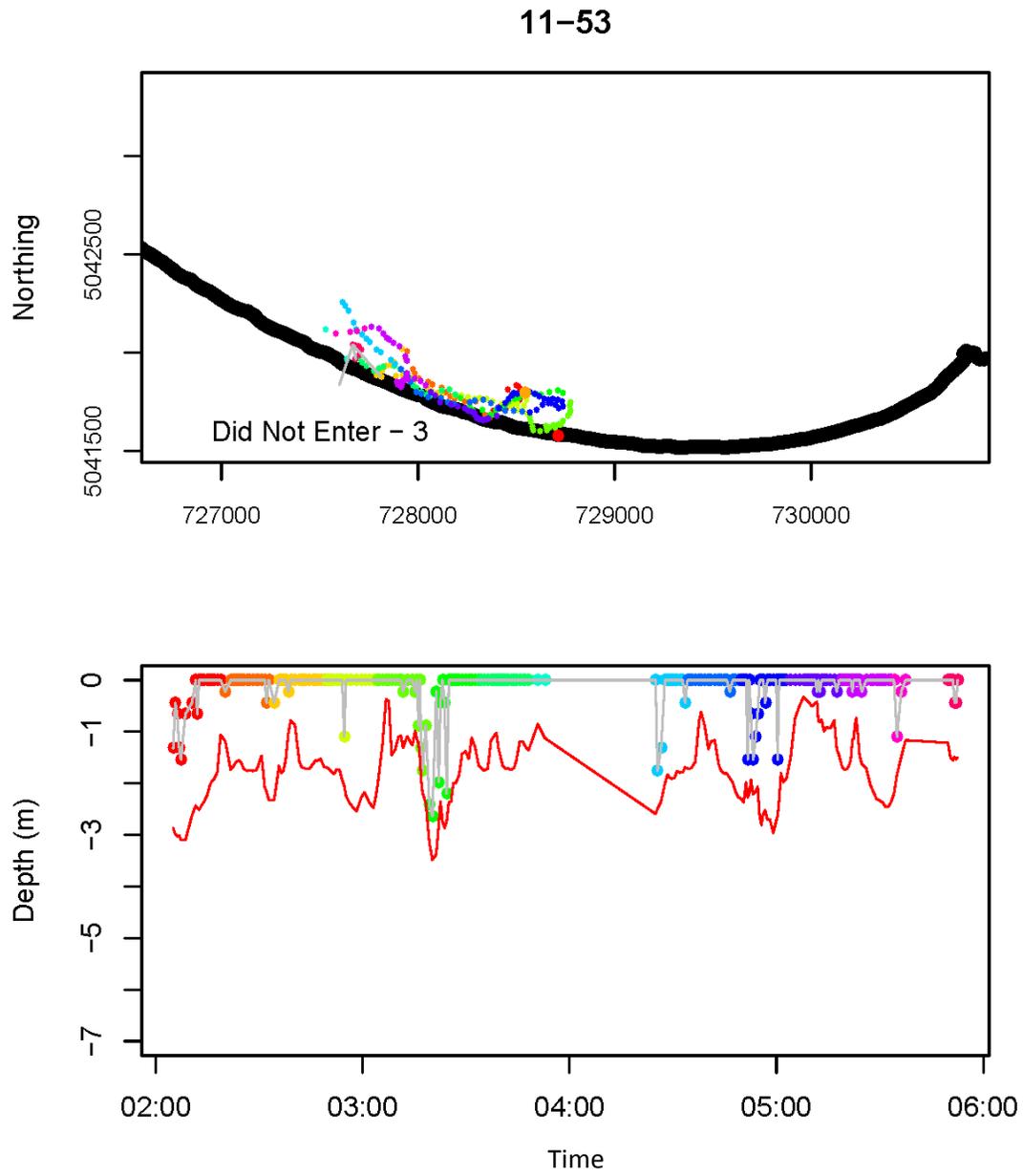


Figure 4.15: (cont'd)

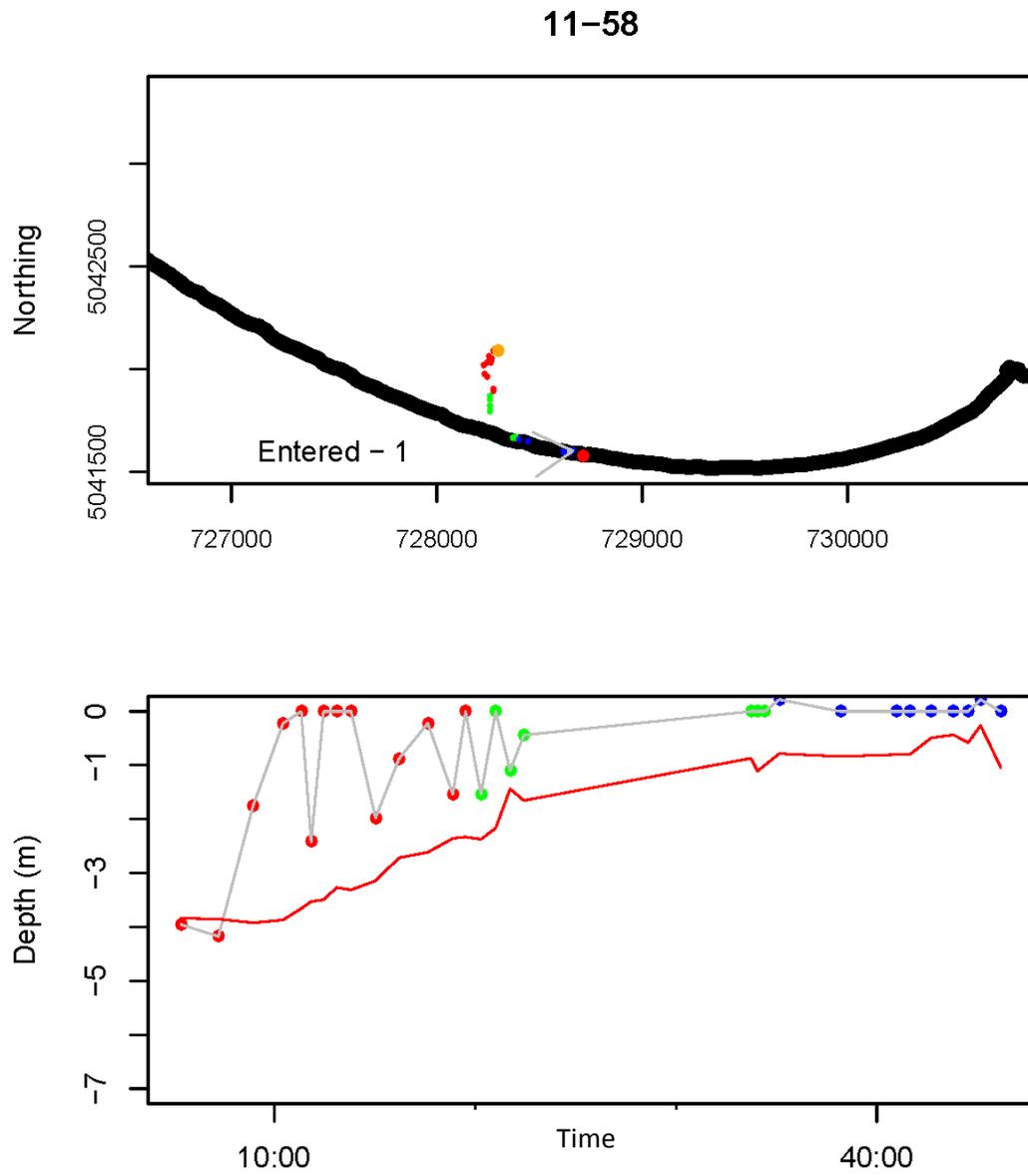


Figure 4.15: (cont'd)

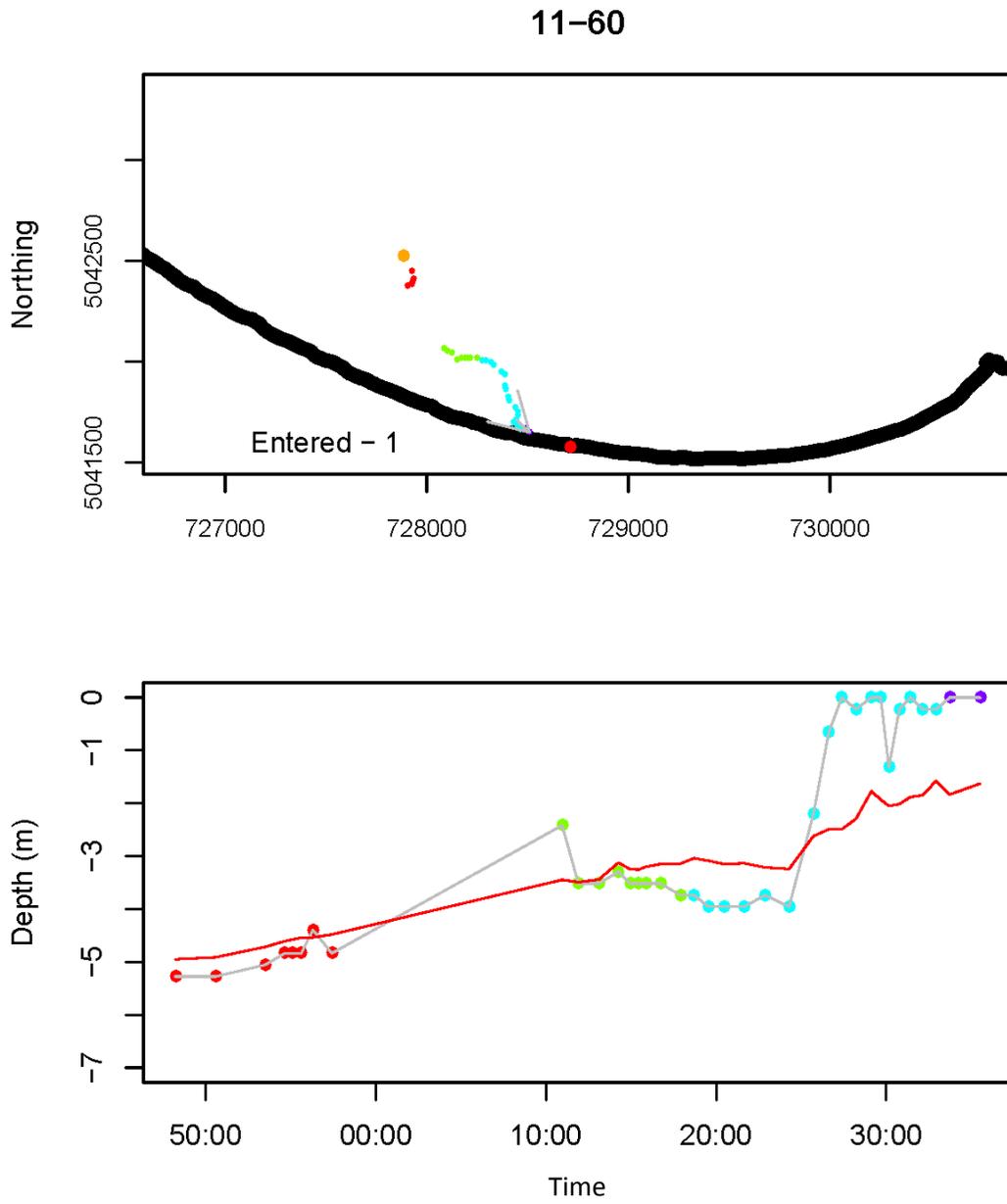


Figure 4.15: (cont'd)

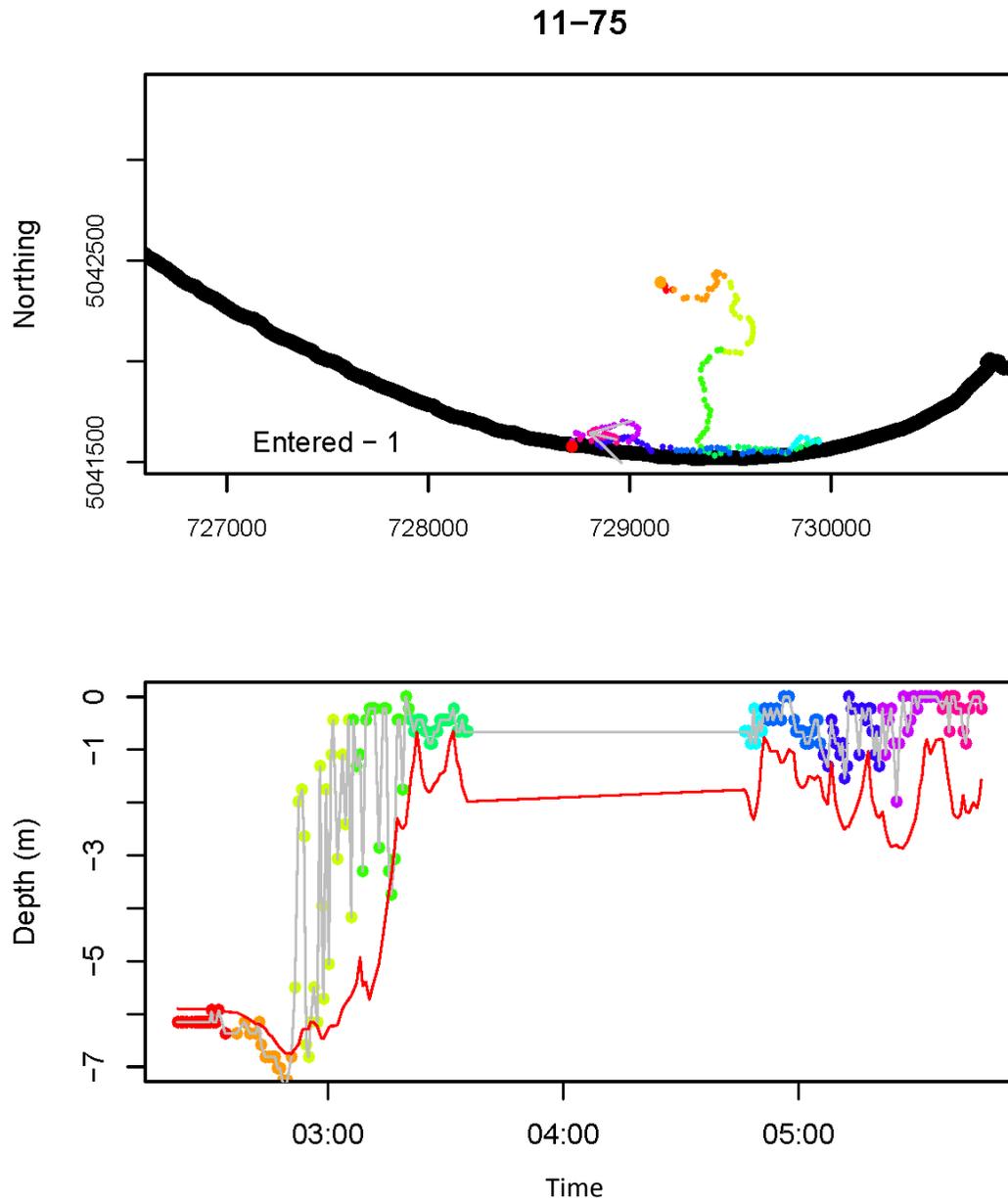


Figure 4.15: (cont'd)

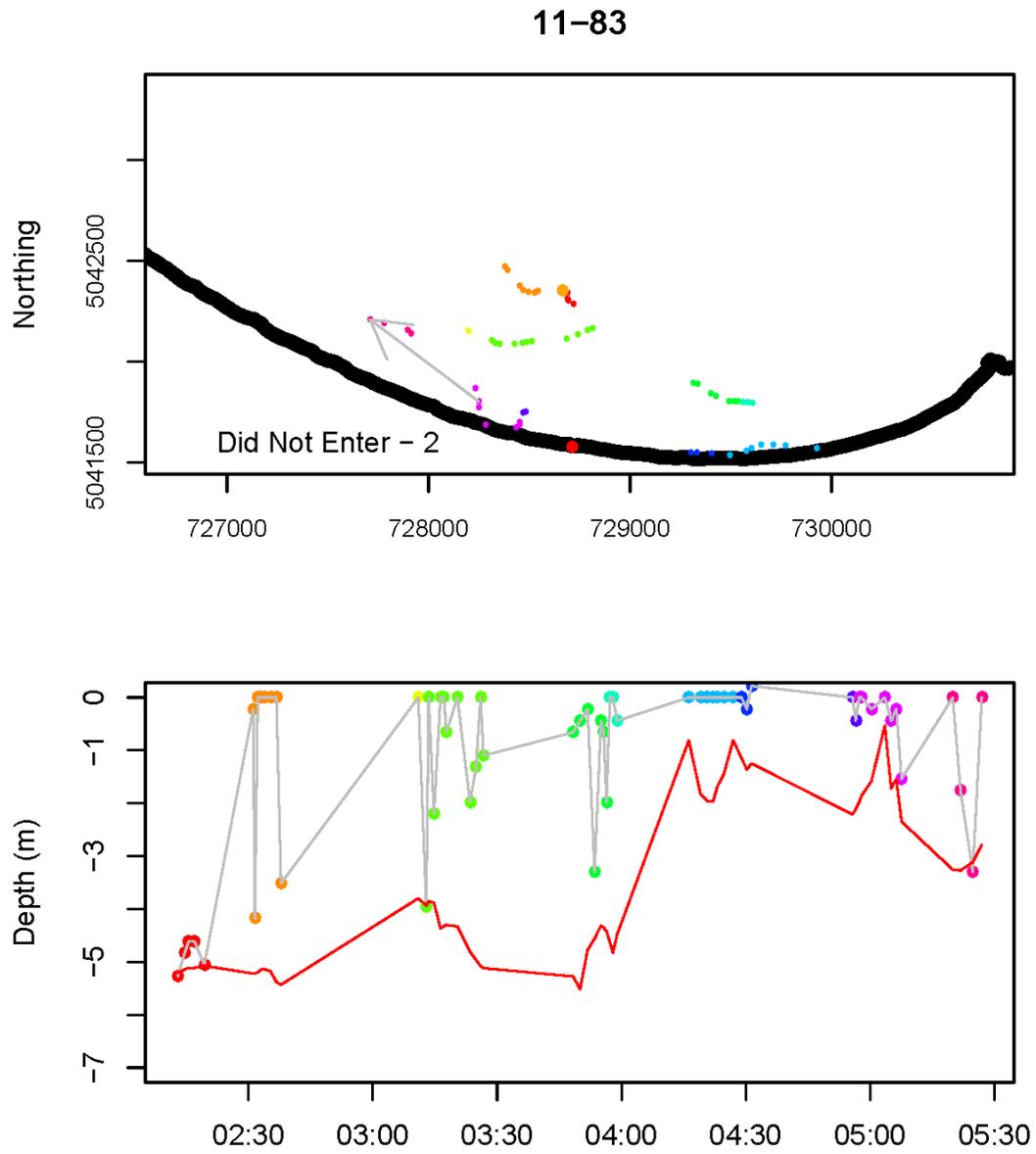
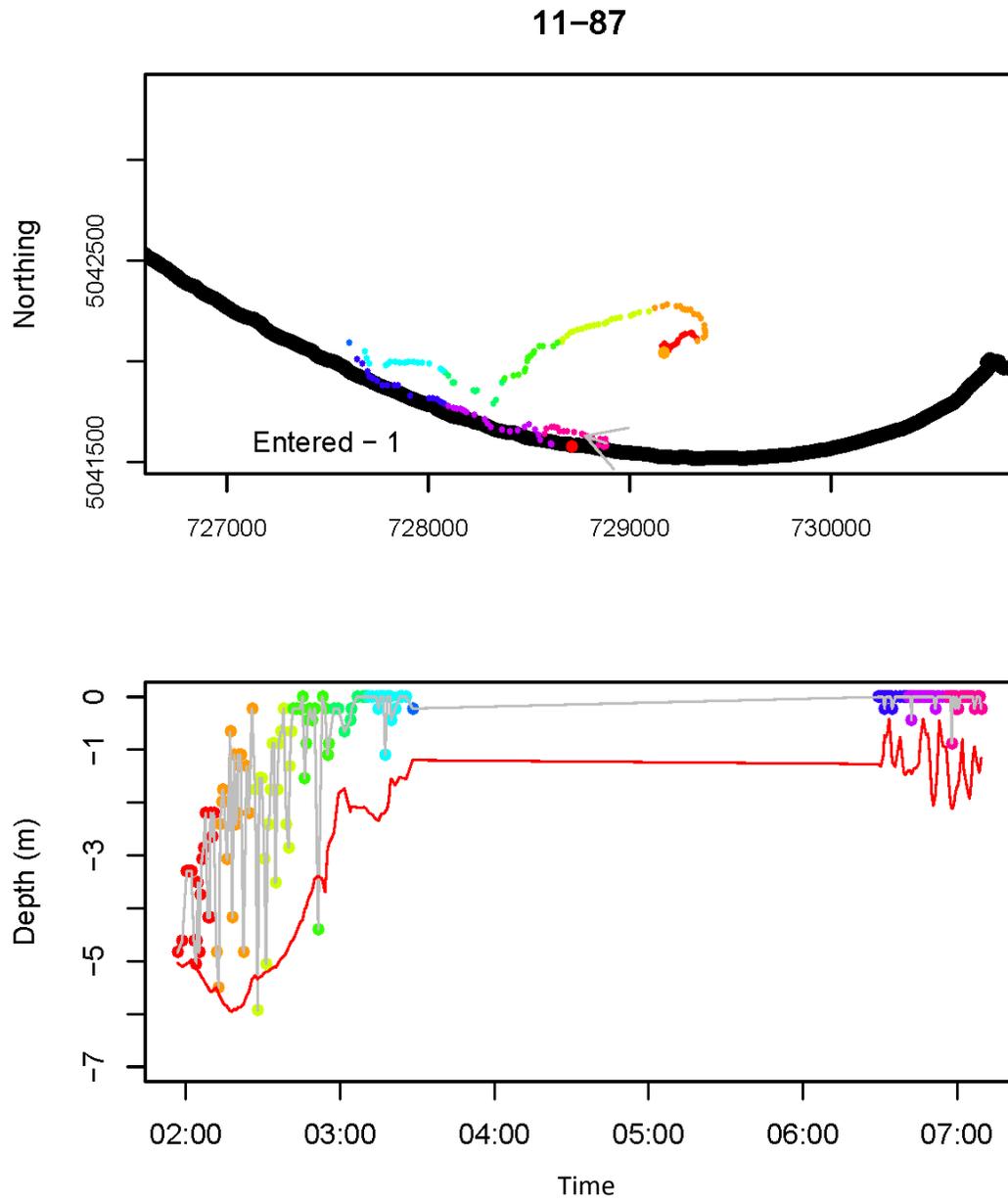


Figure 4.15: (cont'd)



10-14

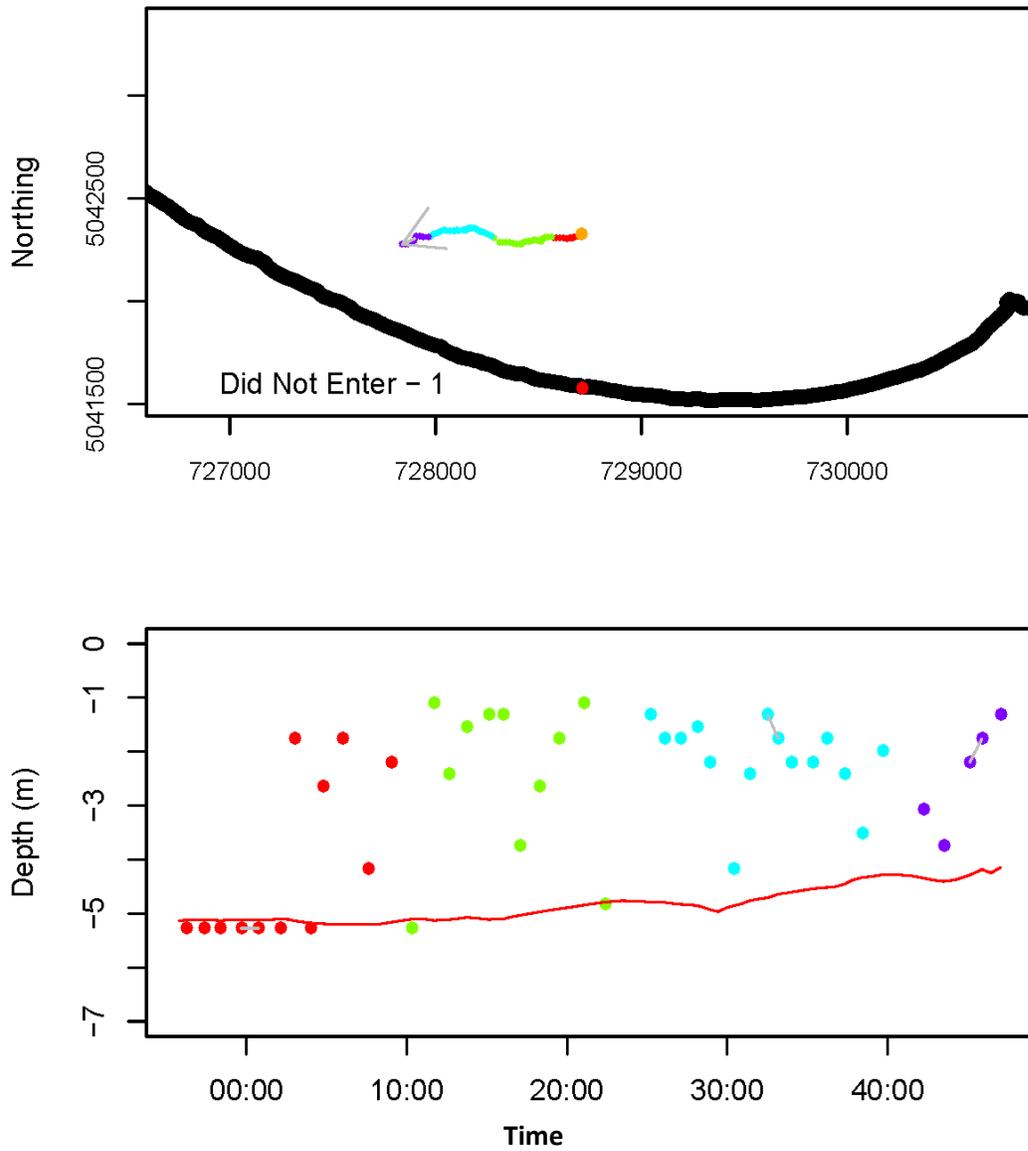


Figure 4.16: The 33 sea lamprey that did not encounter the river plume on the first night based on the estimates of the dye concentration at each fish position are shown in a schematic of Hammond bay, where the black line represents the coast and the red dot represents the river mouth. Each page is labeled by the year (10 or 11) and animal ID. The upper graph shows the sea lamprey movement on the first night from where the sea lamprey settled in the array after release (orange dot). In the schematic of movement, the gray arrow points the heading during the

Figure 4.16: (cont'd)

last 5 points of the track and the track is color coded by 15 minute intervals of activity. The label of “Entered” indicates that sea lamprey eventually entered the Ocqueoc River and the label of “Did Not Enter” indicates that the sea lamprey never entered the Ocqueoc River. The number of bouts follows the label indicating the number of different nights the sea lamprey was observed in

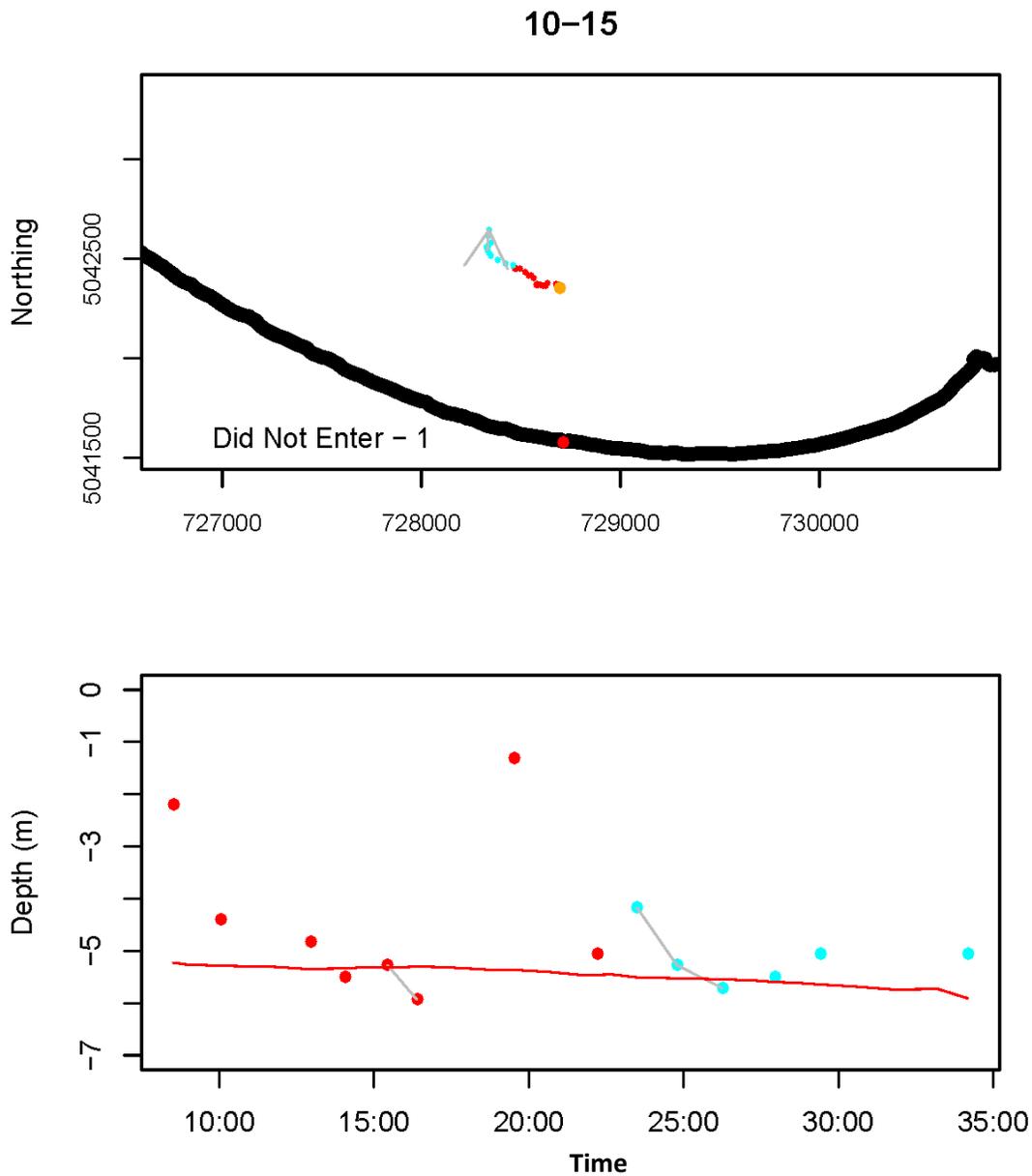


Figure 4.16: (cont'd)

the array. If the sea lamprey entered and the bout is one, the individual entered at the end of the track shown. In some cases entry is not captured well by the array (e.g. 10-17). The lower graph depicts the depth of the sea lamprey during movement through time and the color of the points corresponds to the schematic in the upper graph. The red line represents the estimated total water column depth. When the fish is below the line it can be assumed the fish is on the bottom.

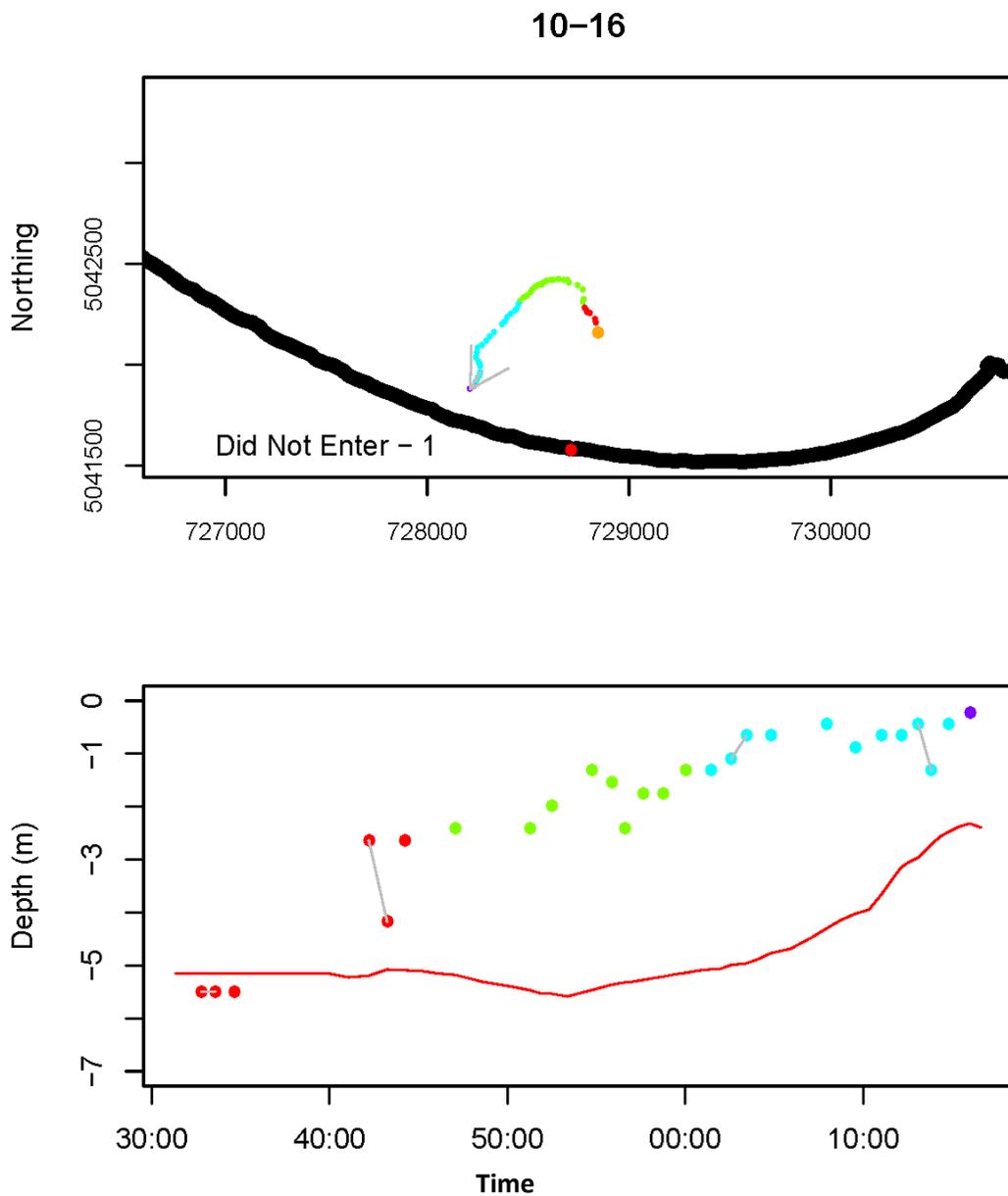


Figure 4.16: (cont'd)

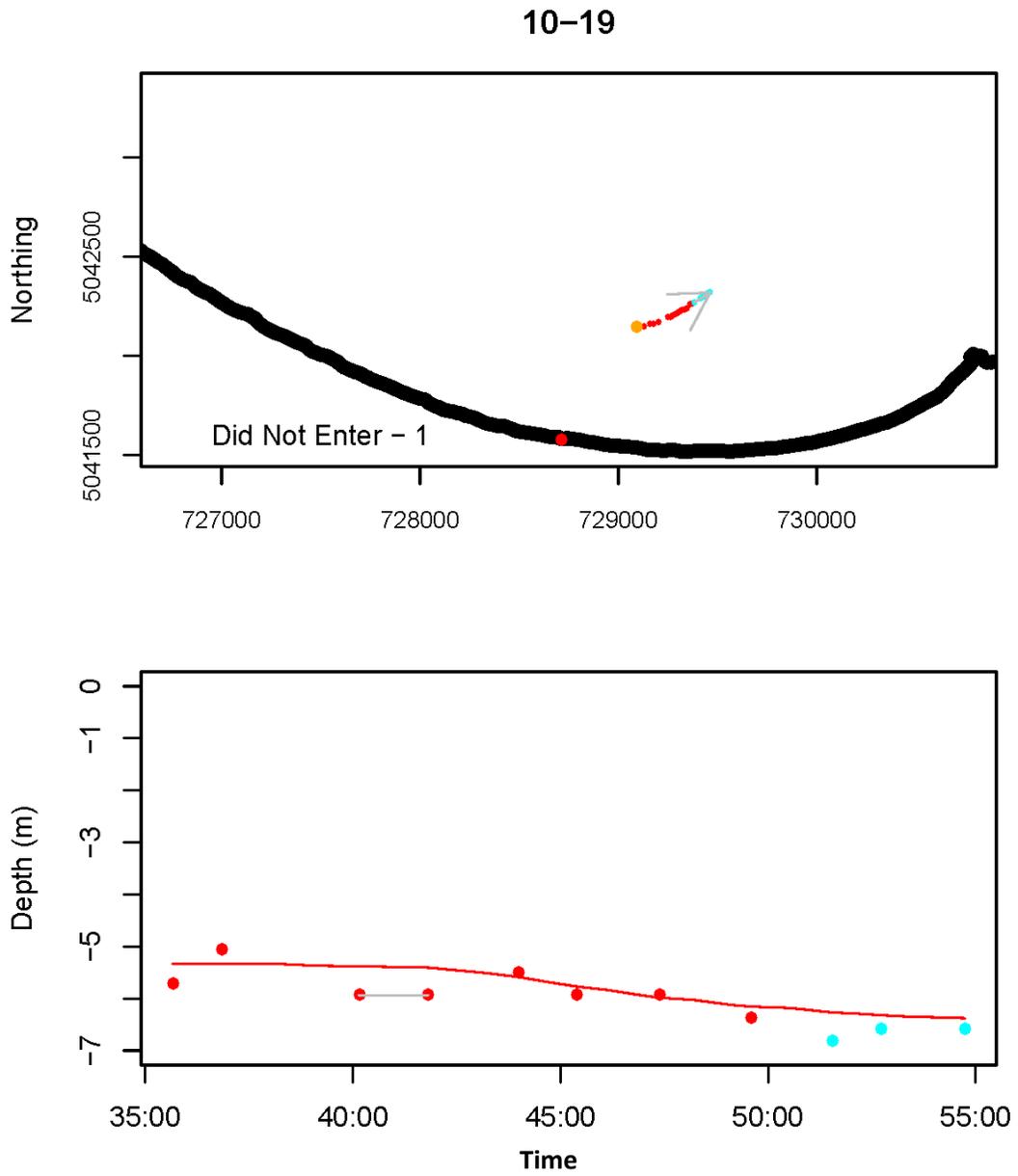


Figure 4.16: (cont'd)

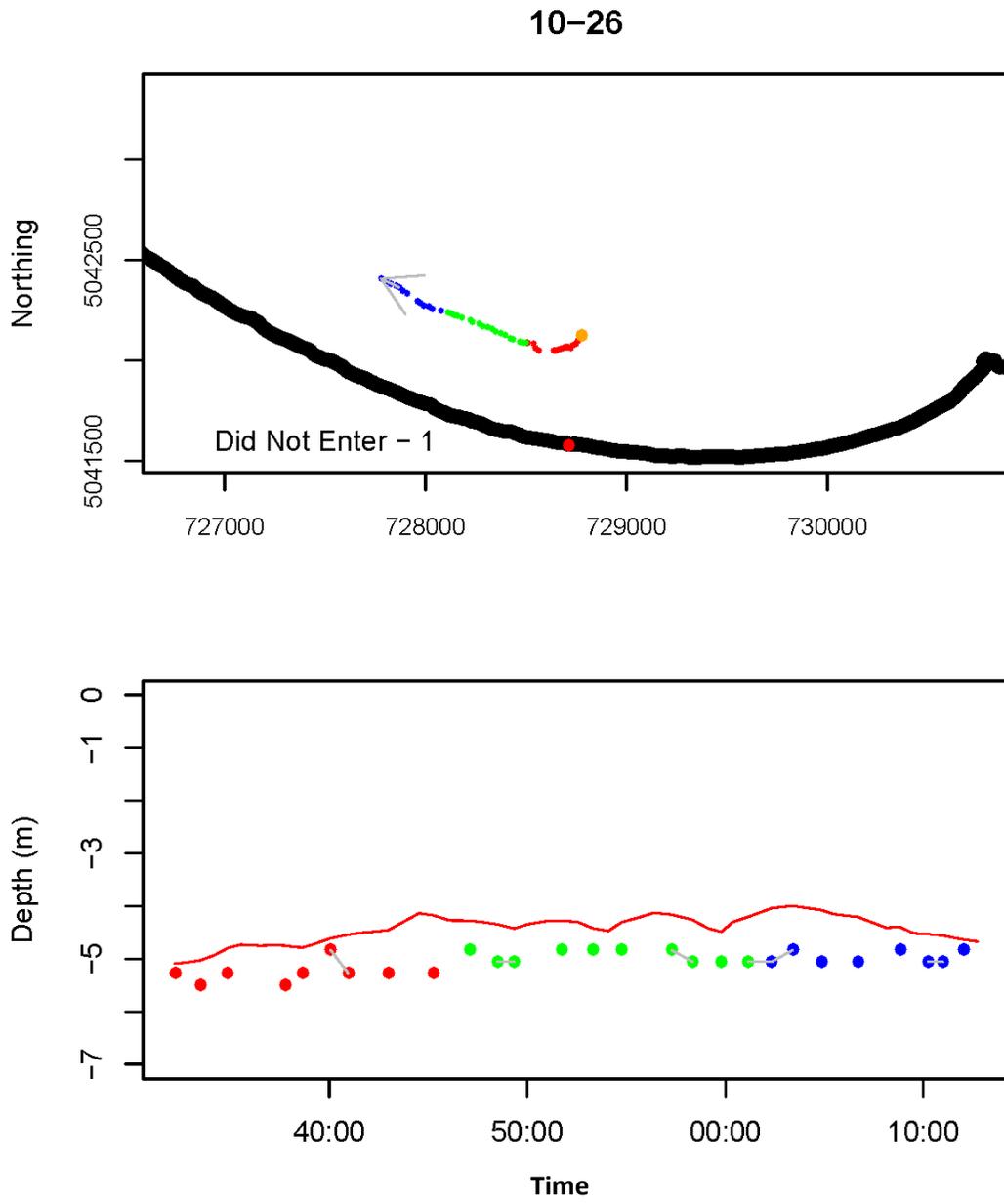


Figure 4.16: (cont'd)

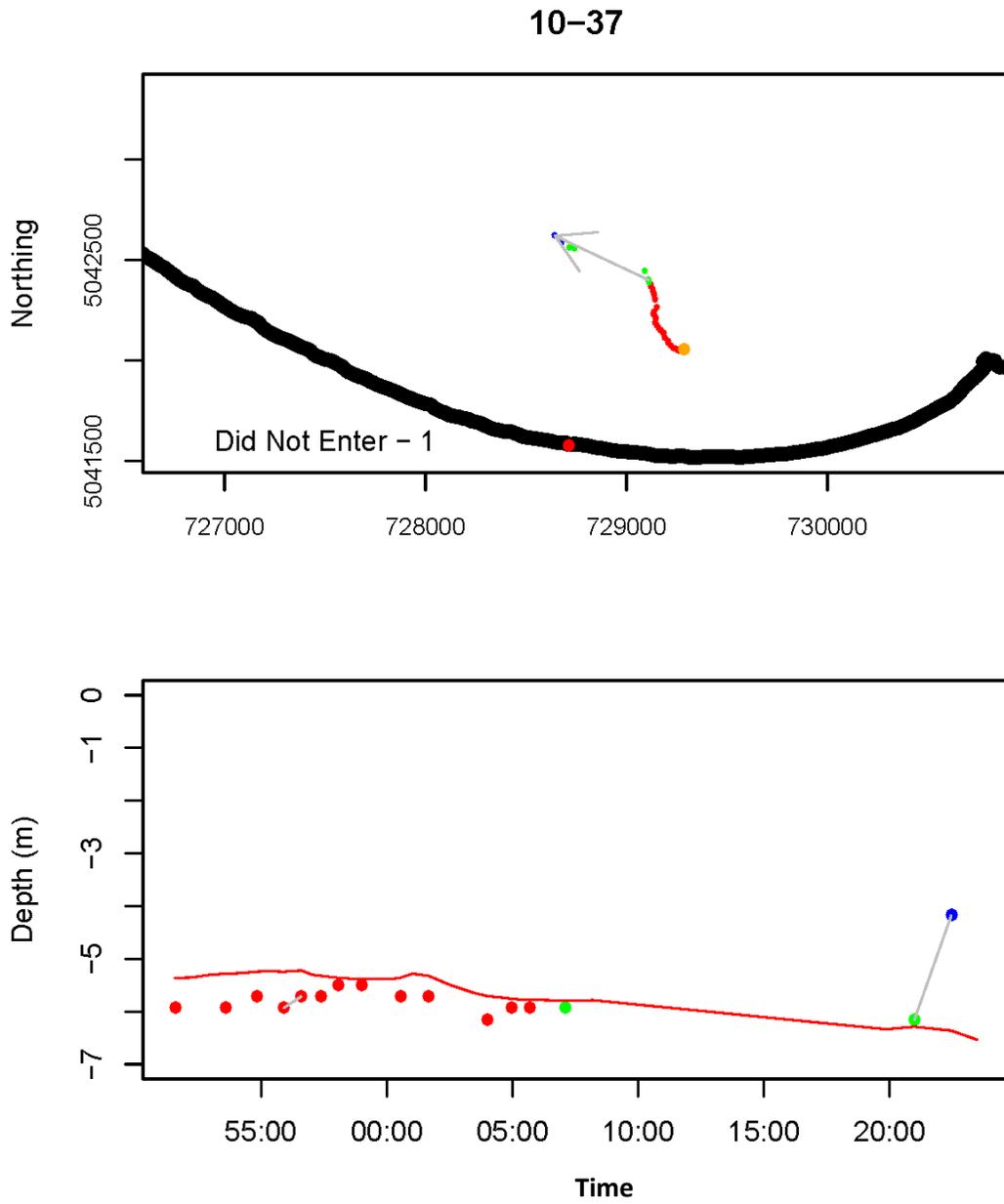


Figure 4.16: (cont'd)

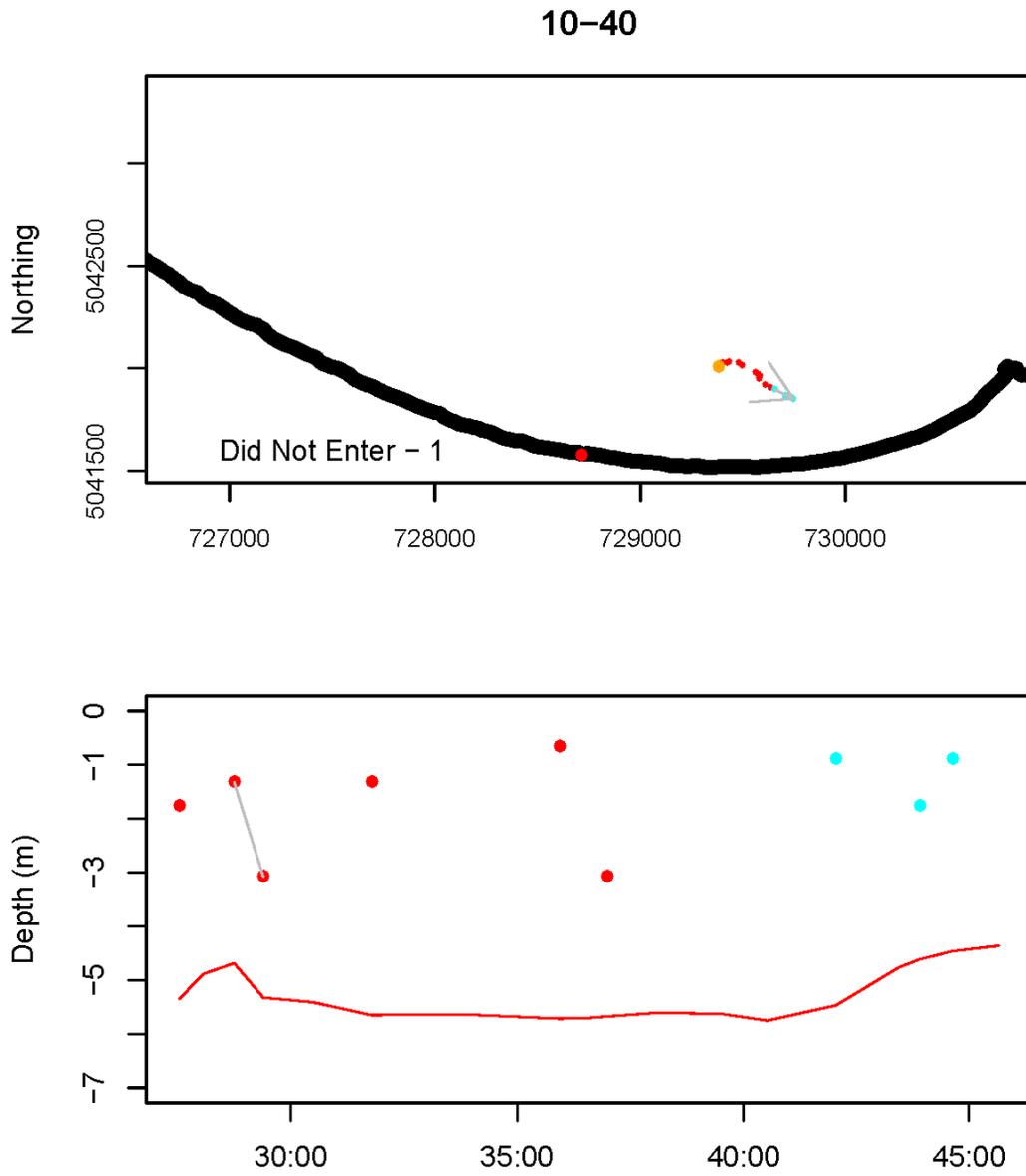


Figure 4.16: (cont'd)

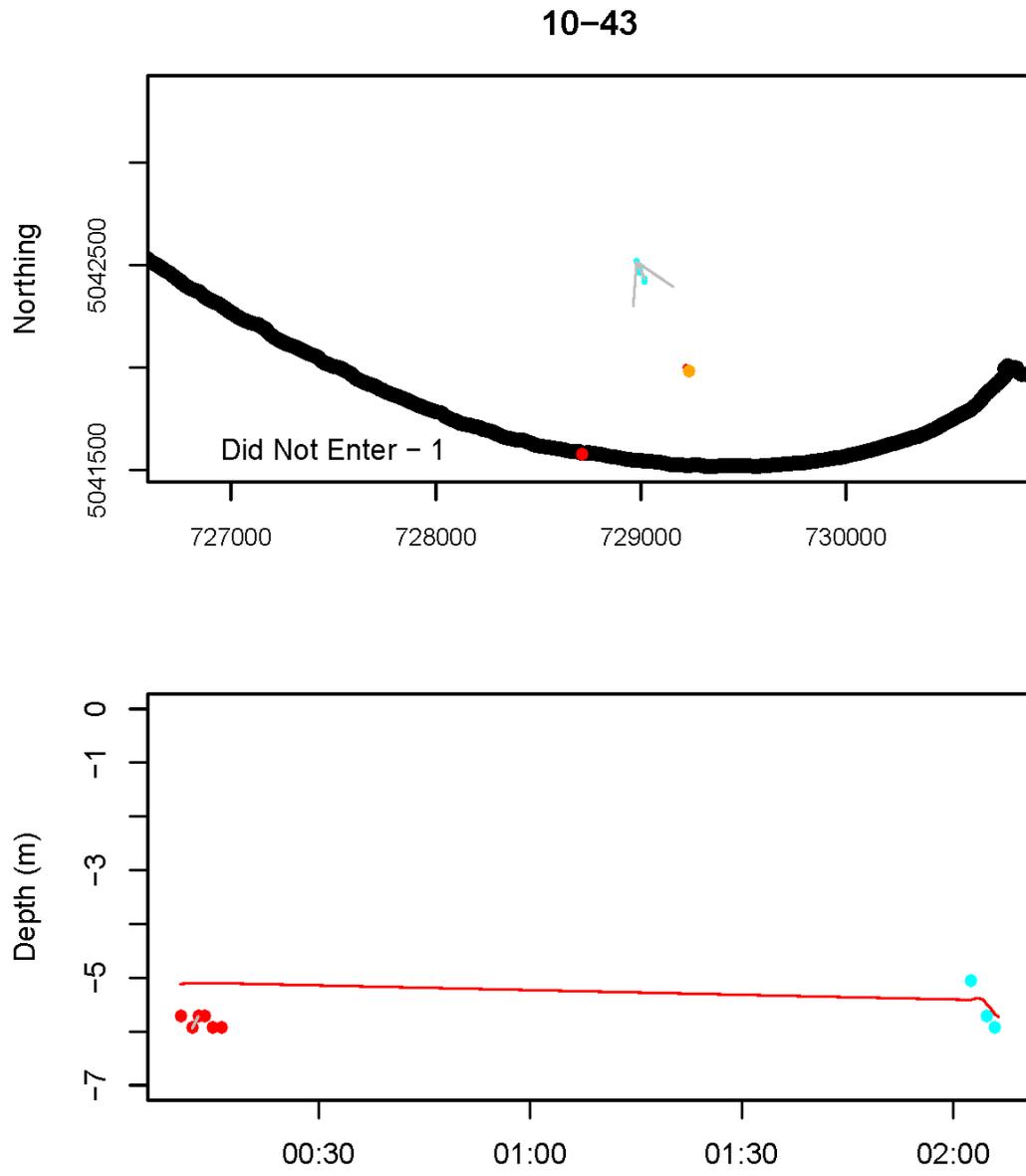


Figure 4.16: (cont'd)

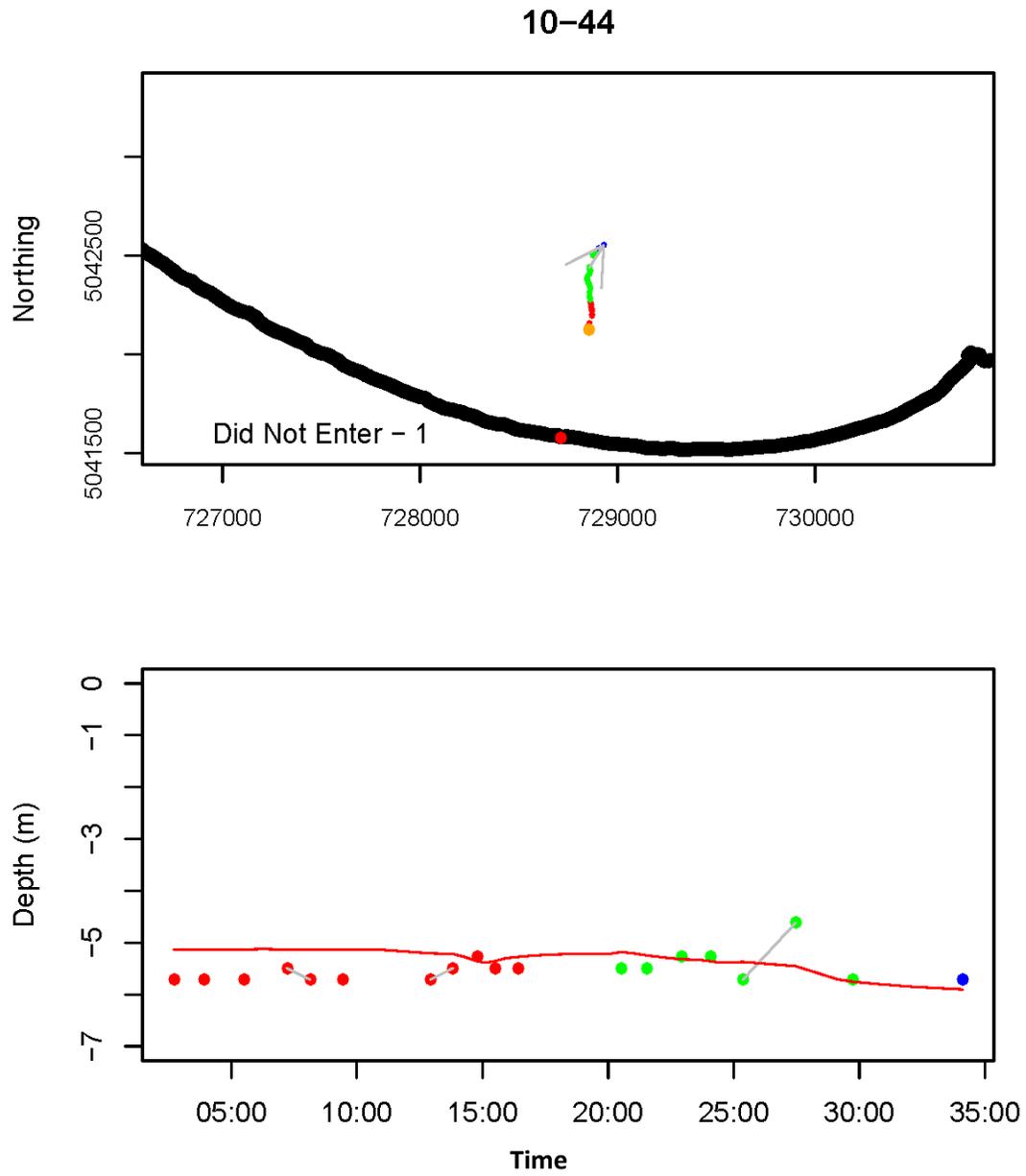


Figure 4.16: (cont'd)

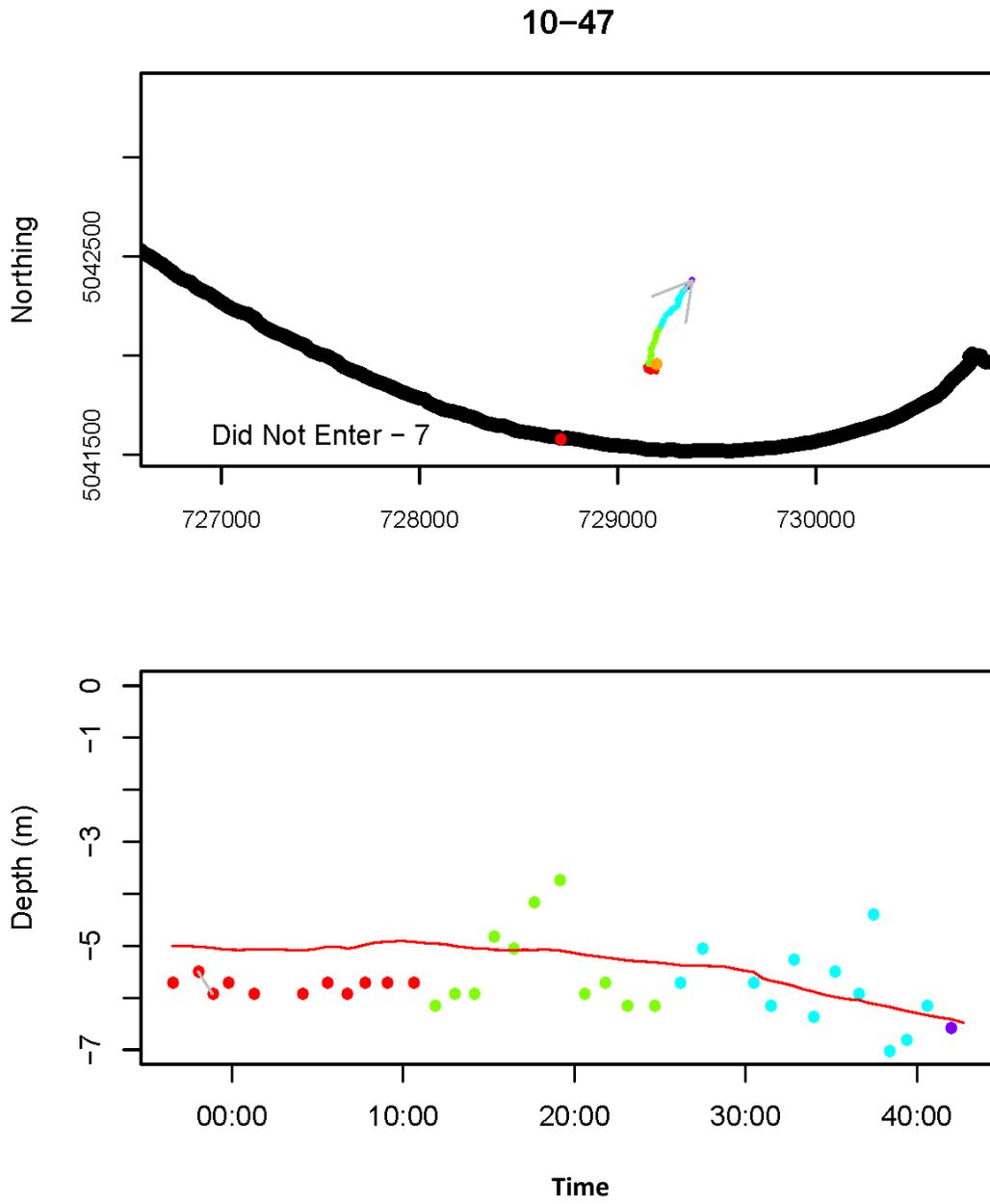


Figure 4.16: (cont'd)

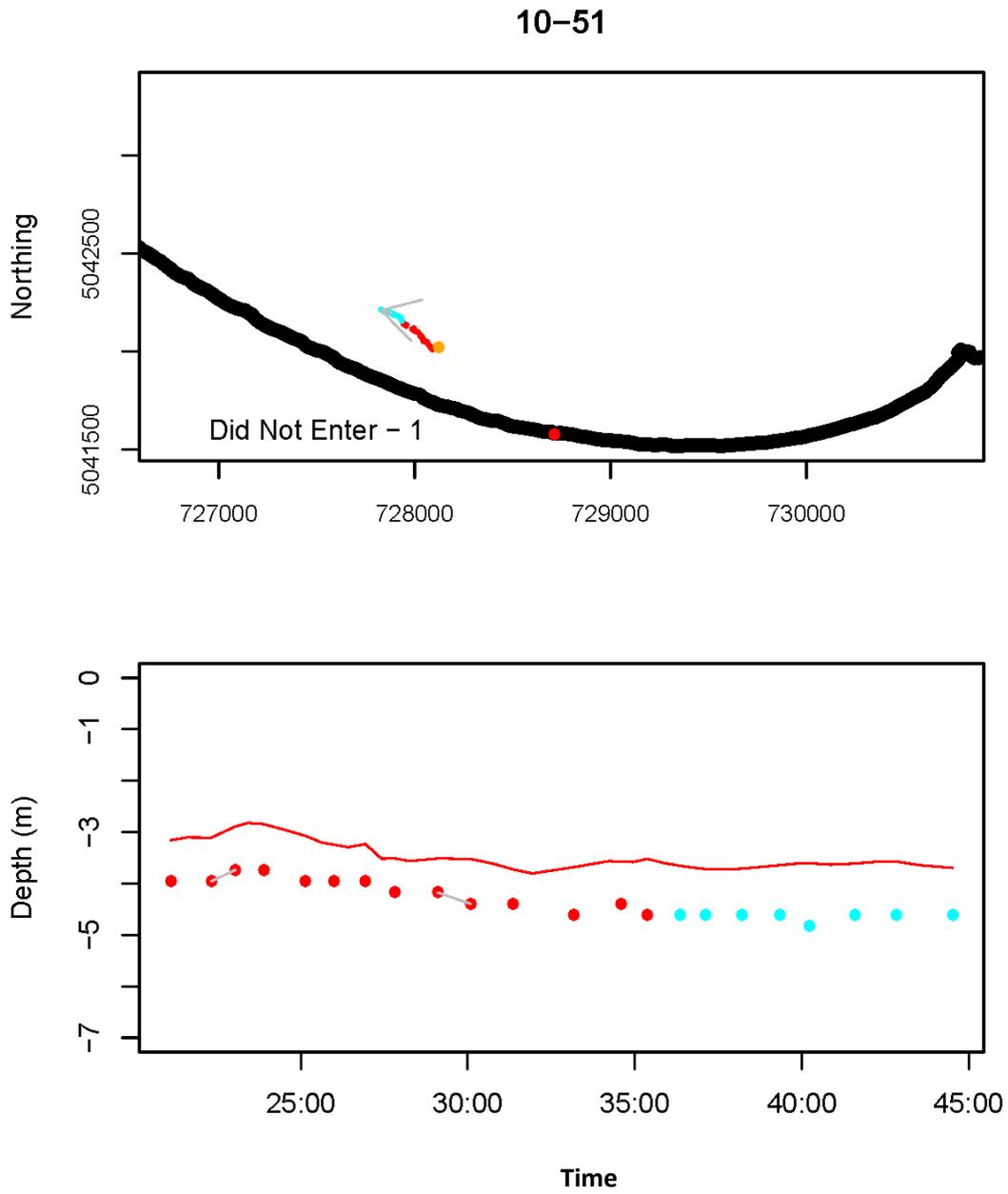


Figure 4.16: (cont'd)

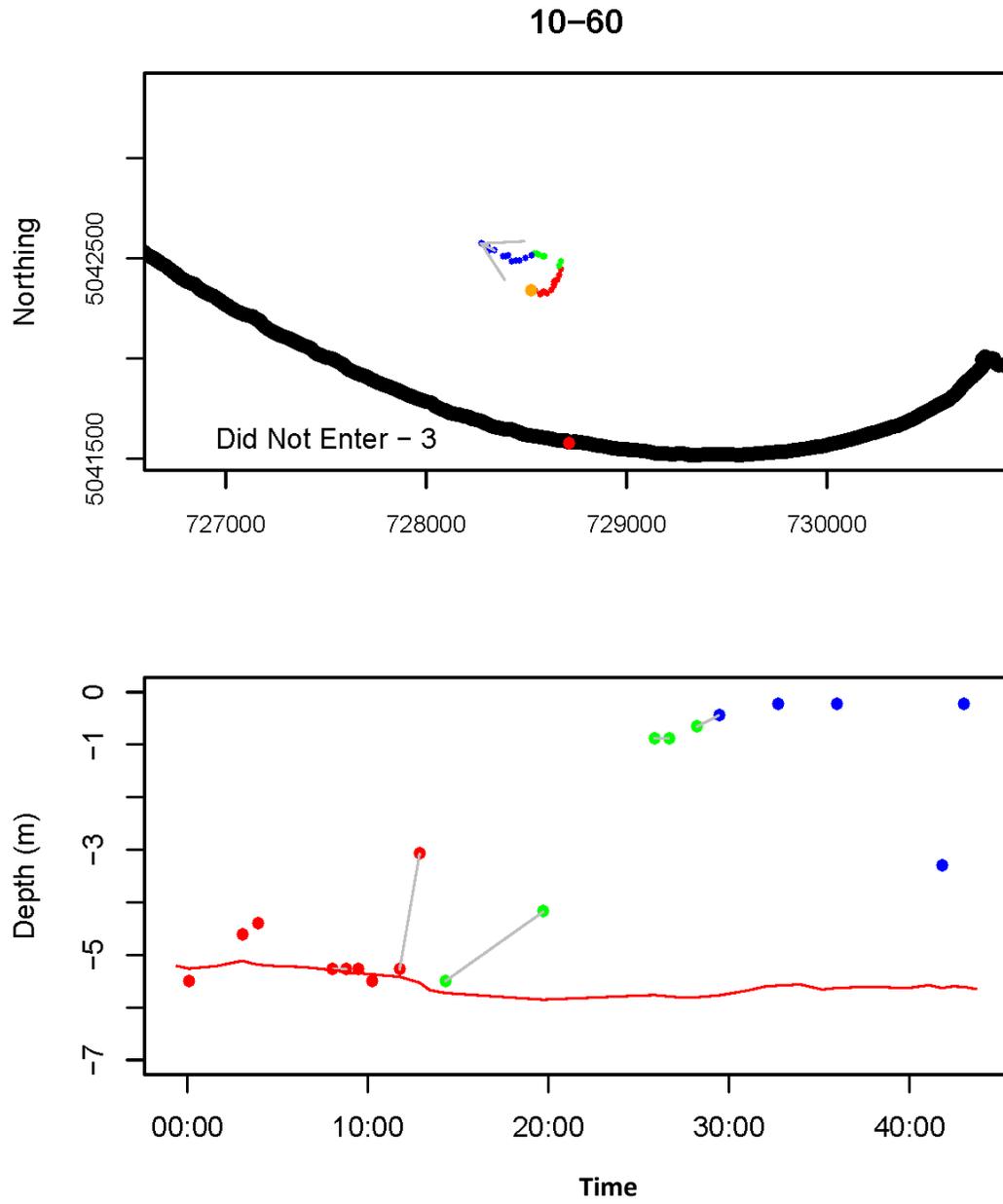


Figure 4.16: (cont'd)

10-64

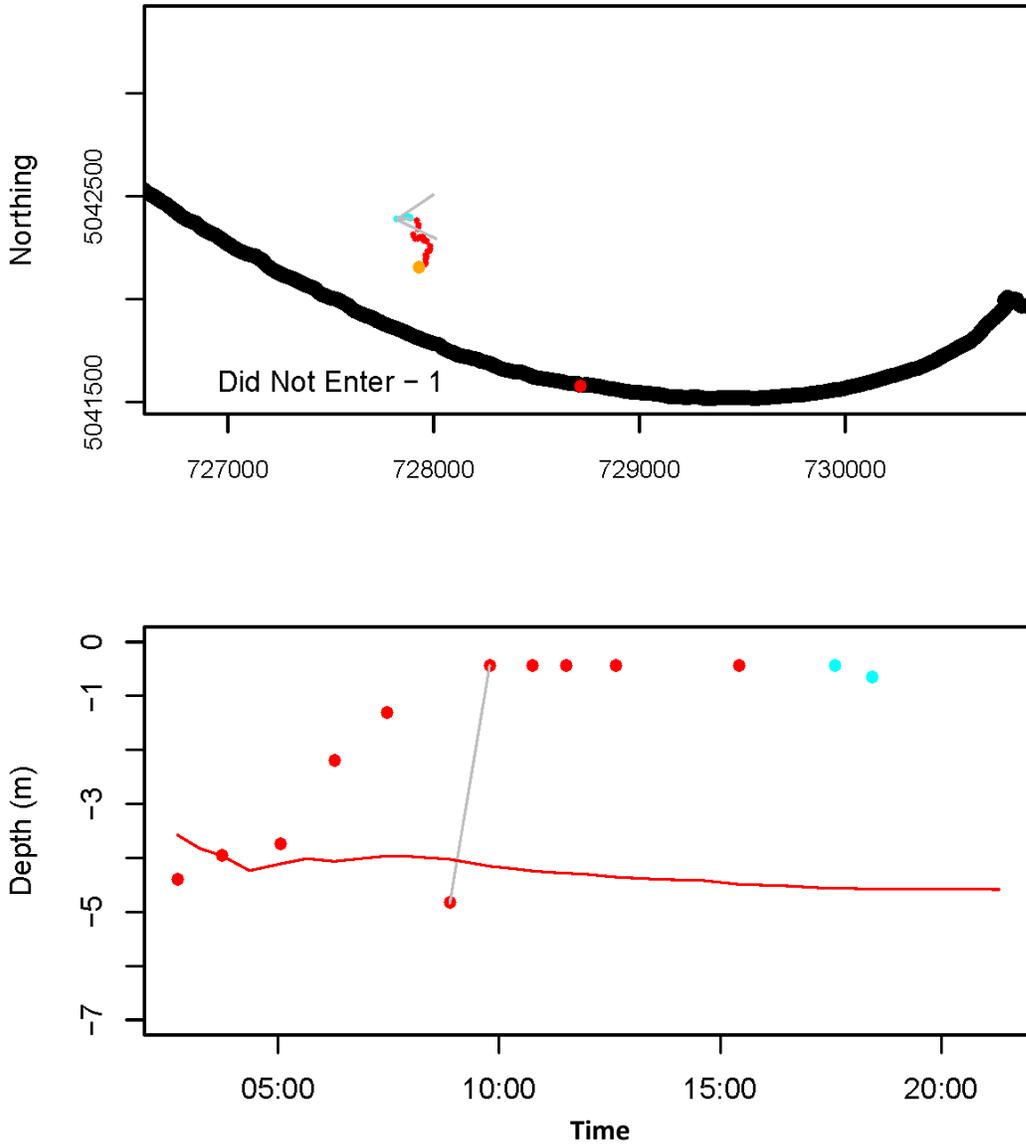


Figure 4.16: (cont'd)

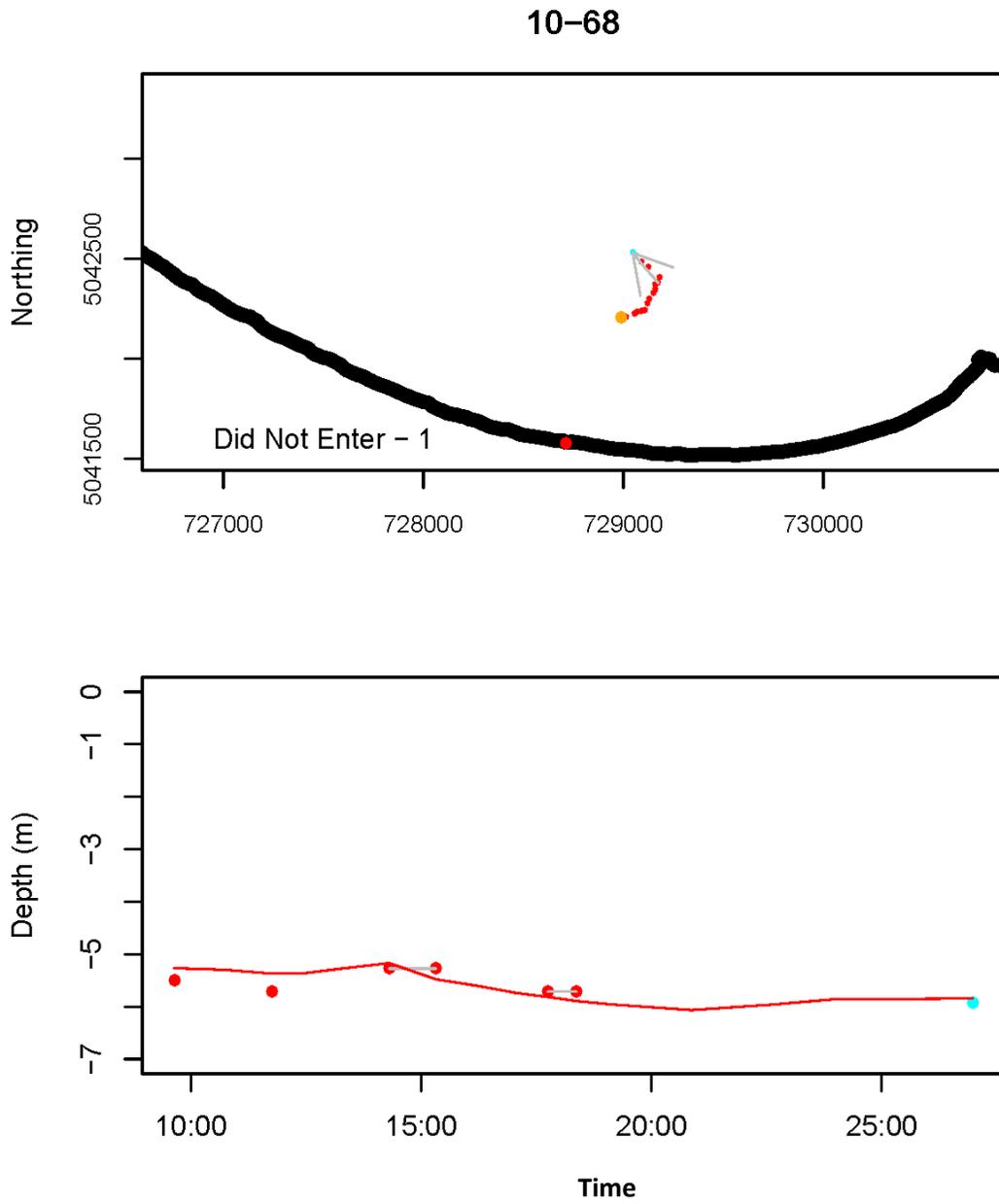


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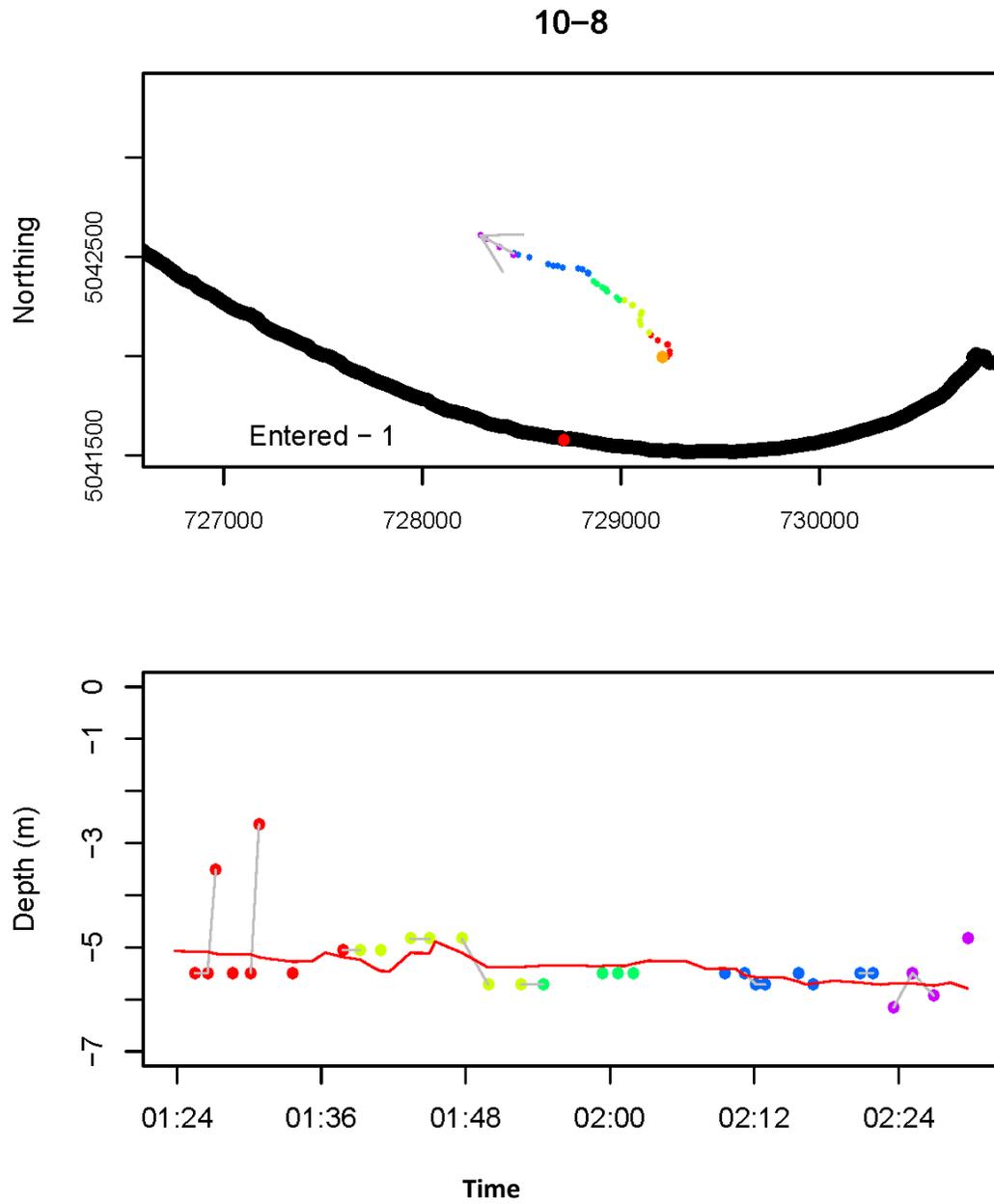


Figure 4.16: (cont'd)

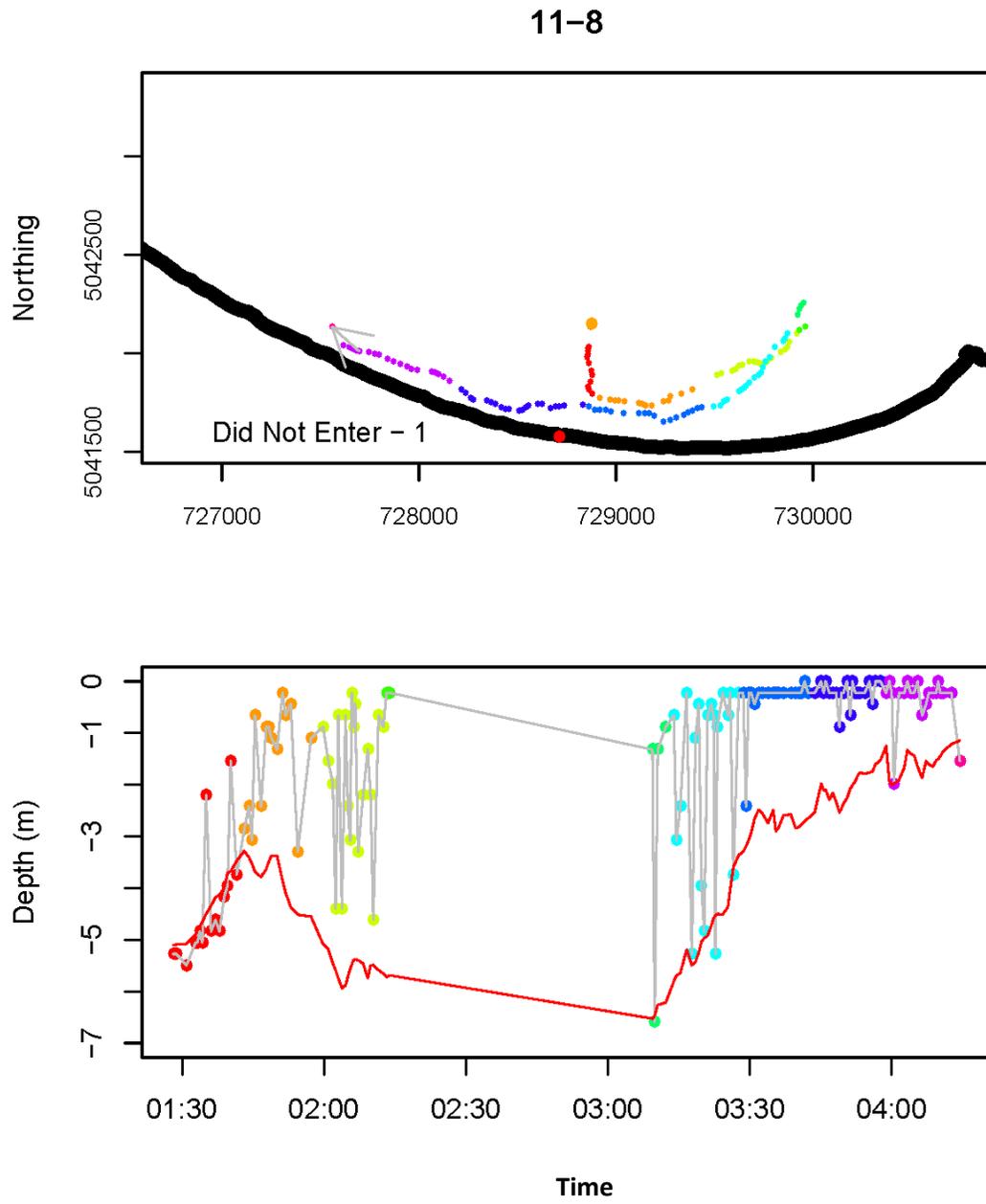


Figure 4.16: (cont'd)

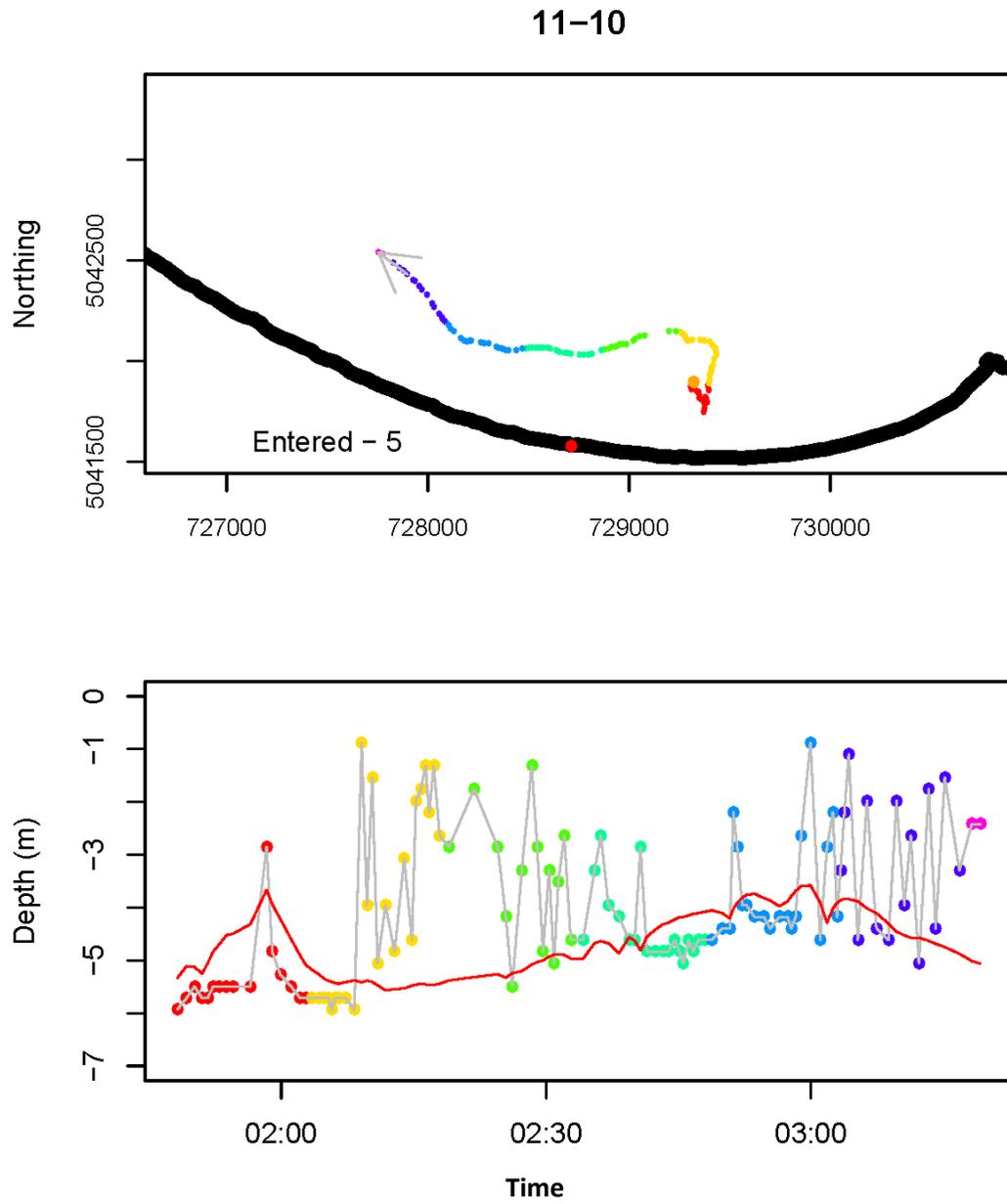


Figure 4.16: (cont'd)

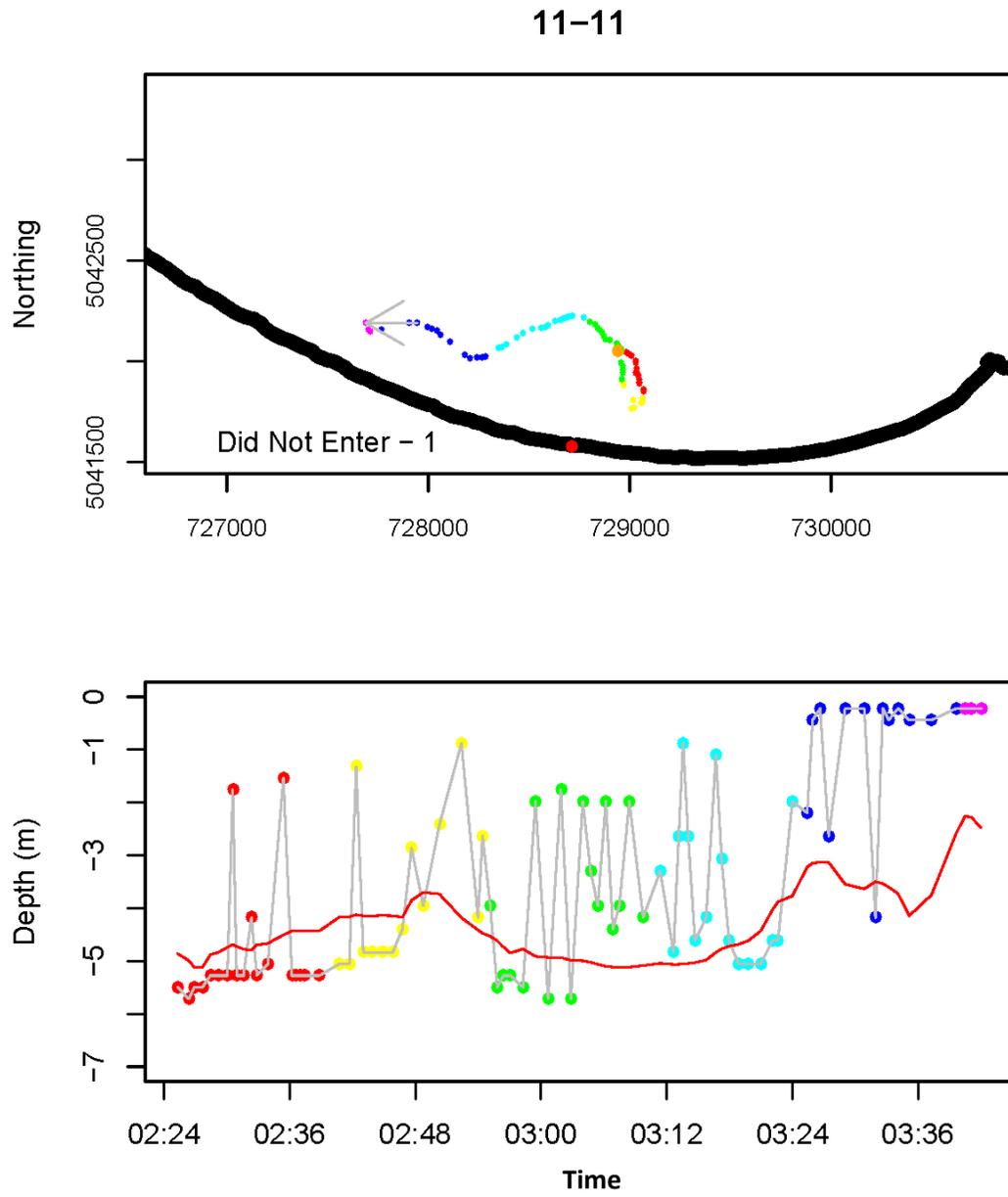


Figure 4.16: (cont'd)

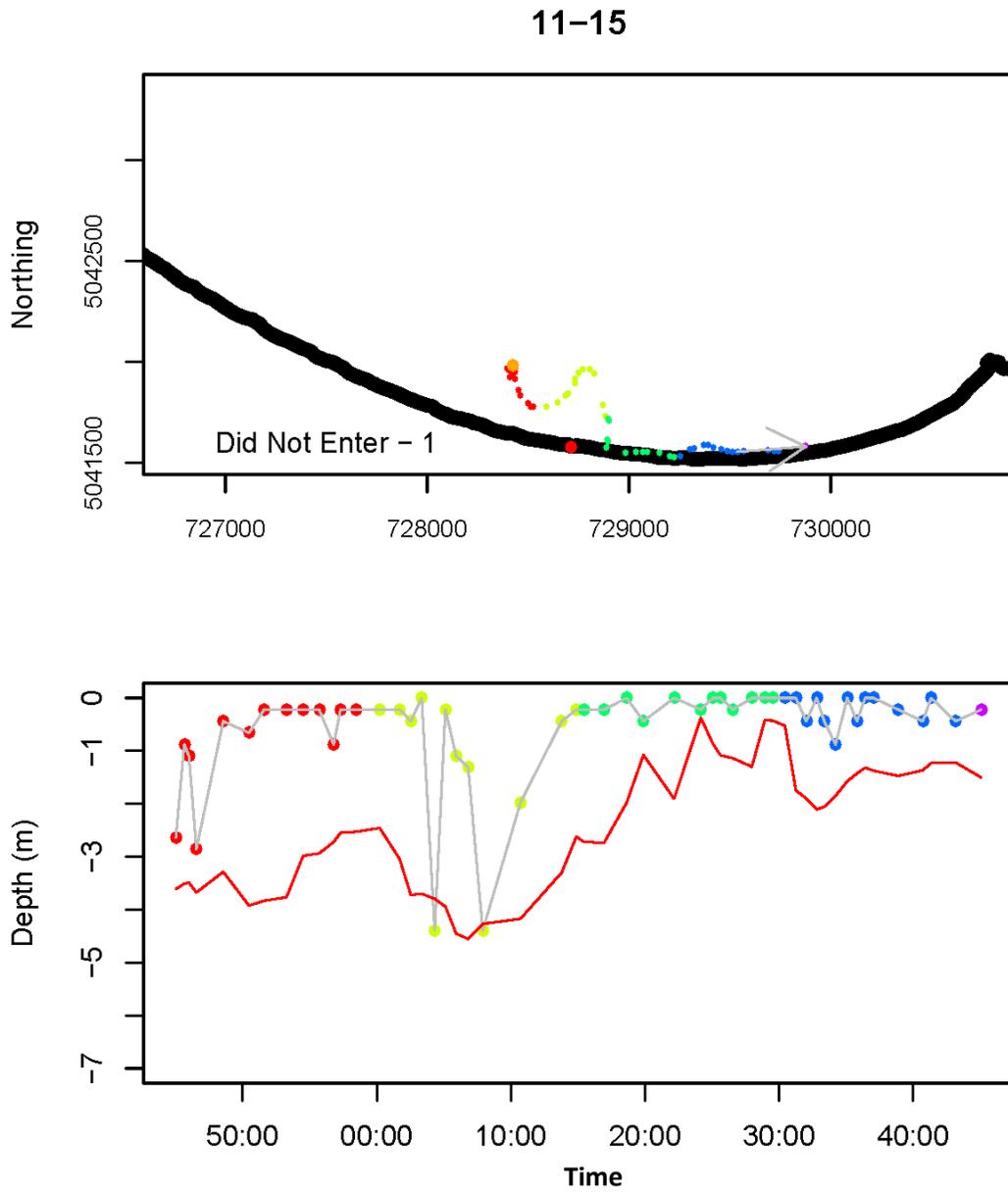


Figure 4.16: (cont'd)

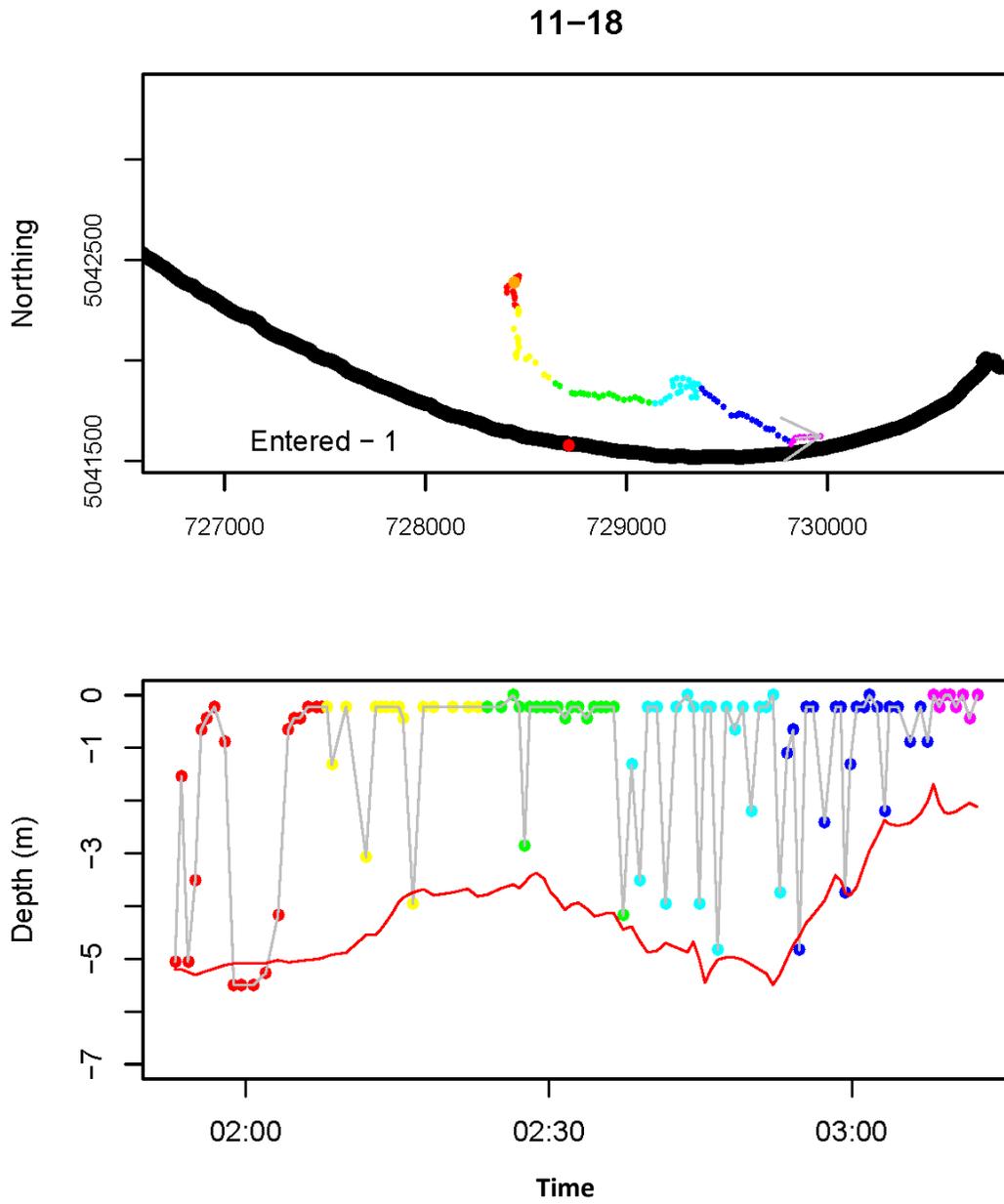


Figure 4.16: (cont'd)

11-23

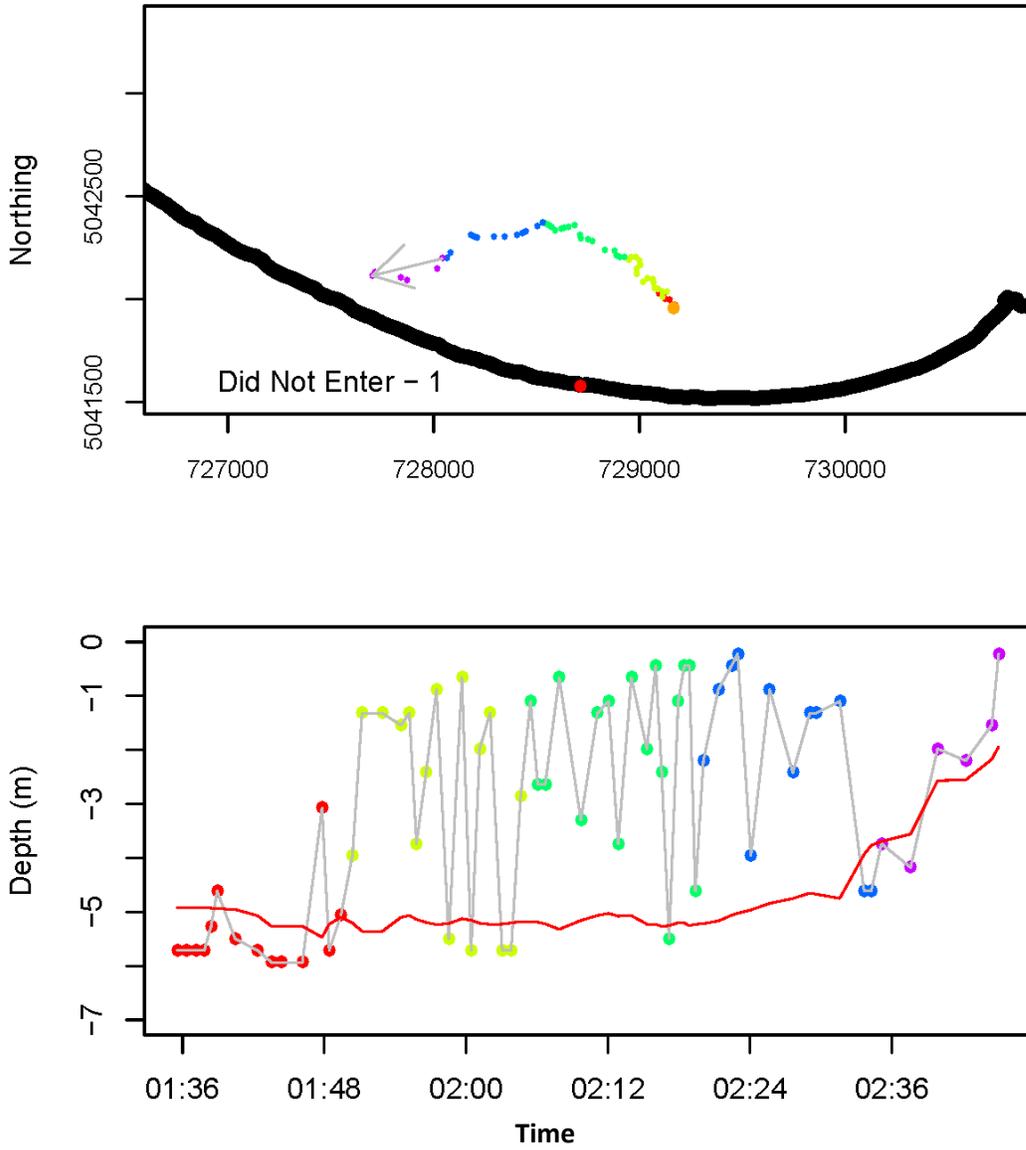


Figure 4.16: (cont'd)

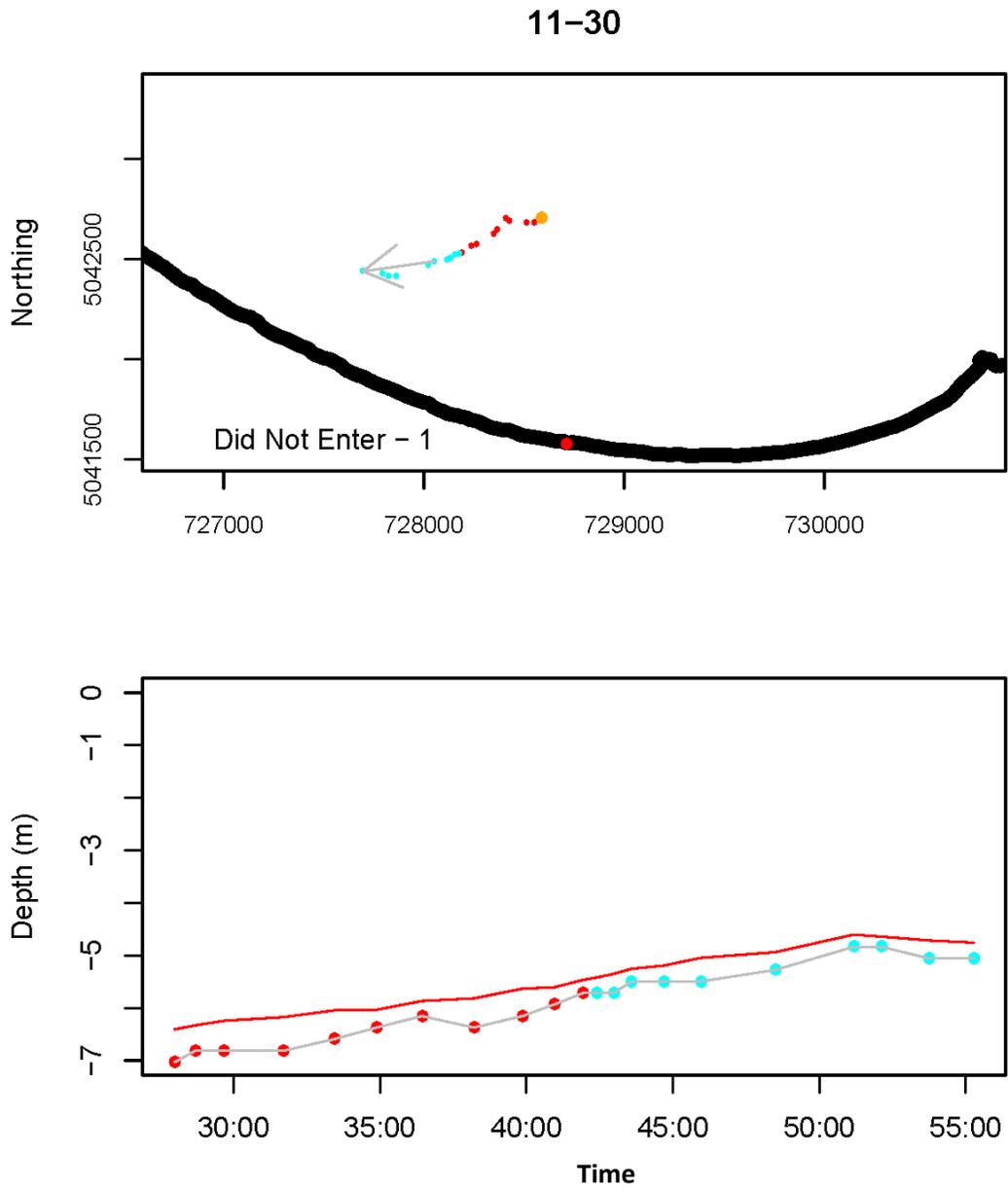


Figure 4.16: (cont'd)

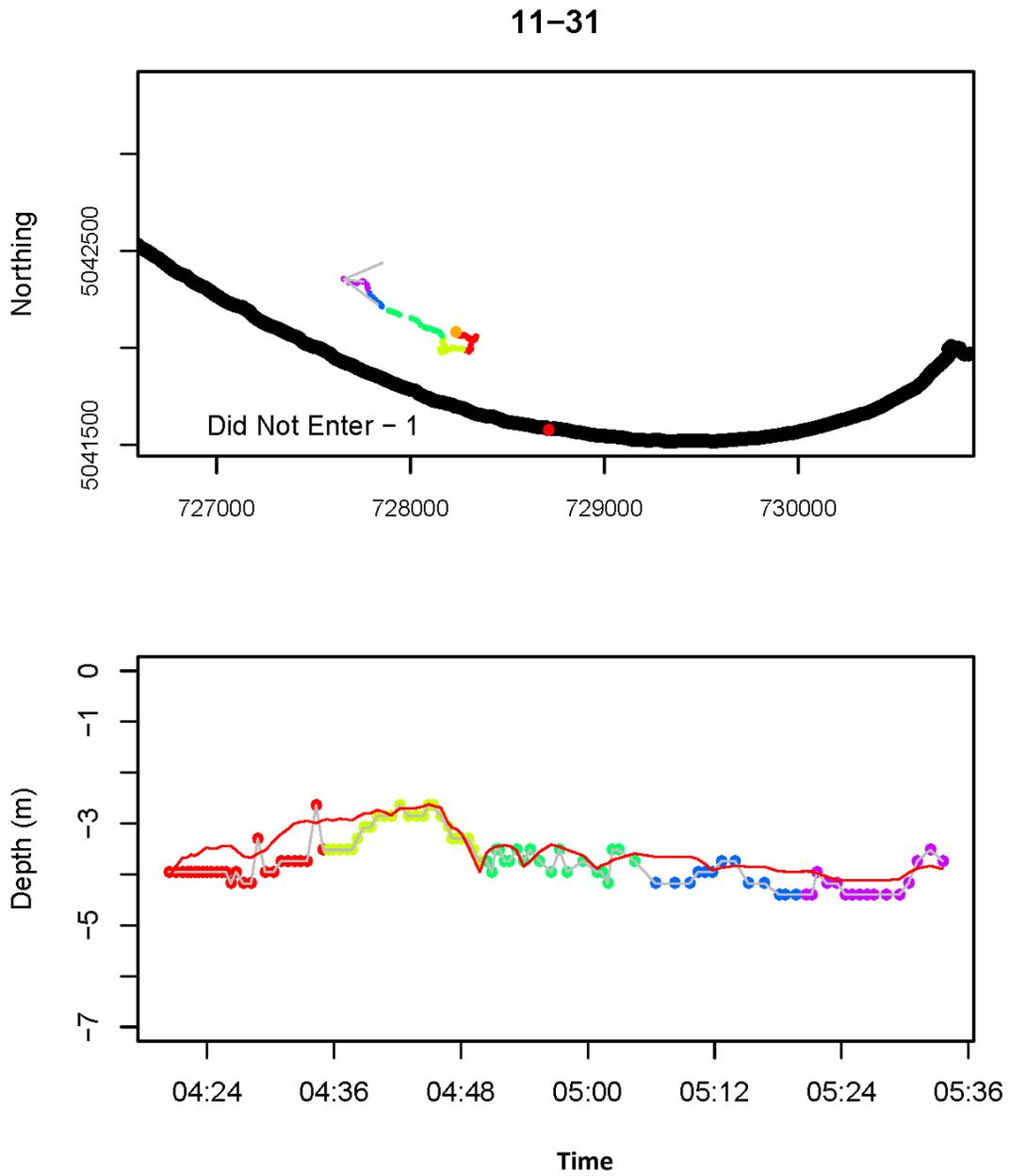


Figure 4.16: (cont'd)

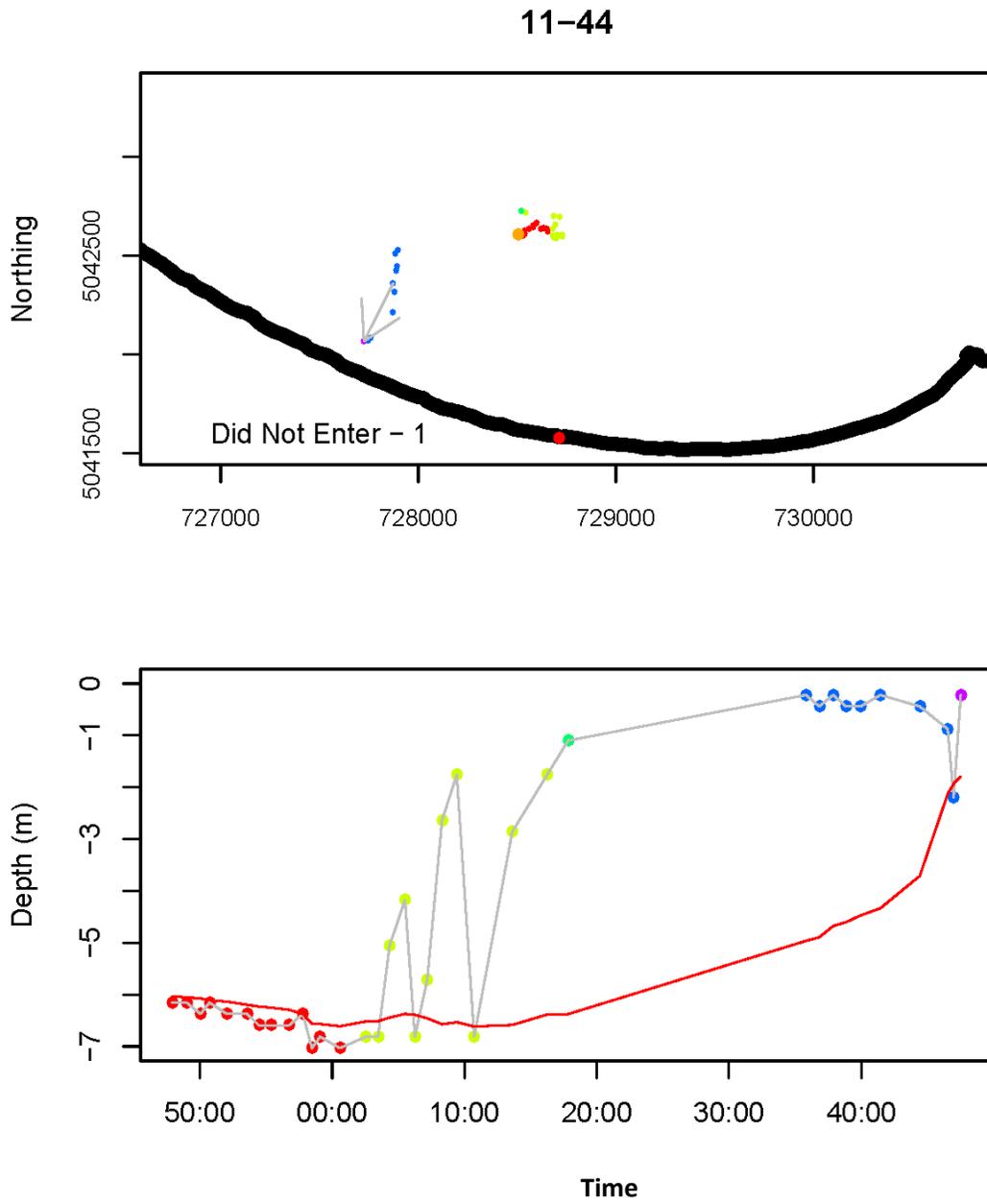


Figure 4.16: (cont'd)

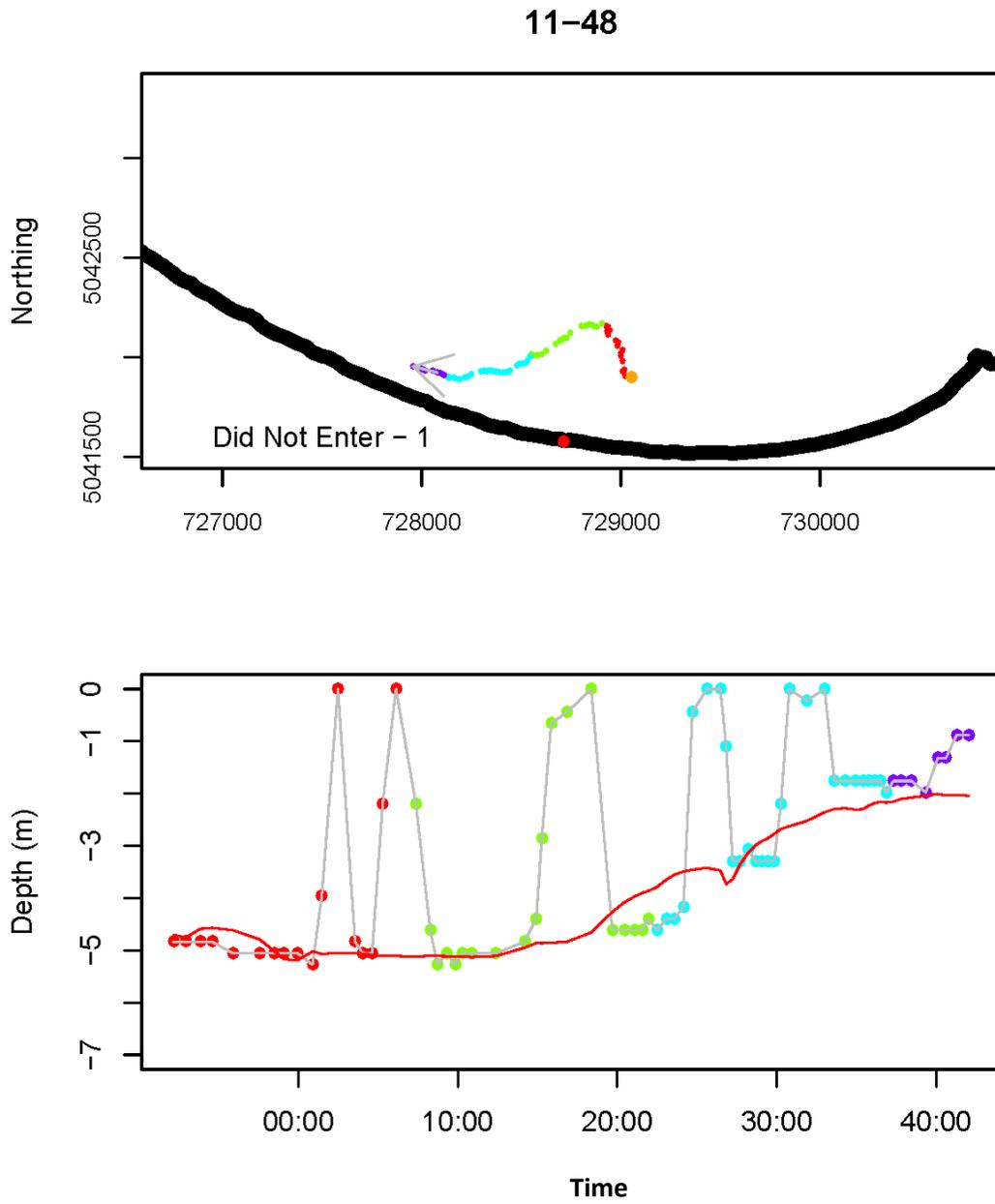


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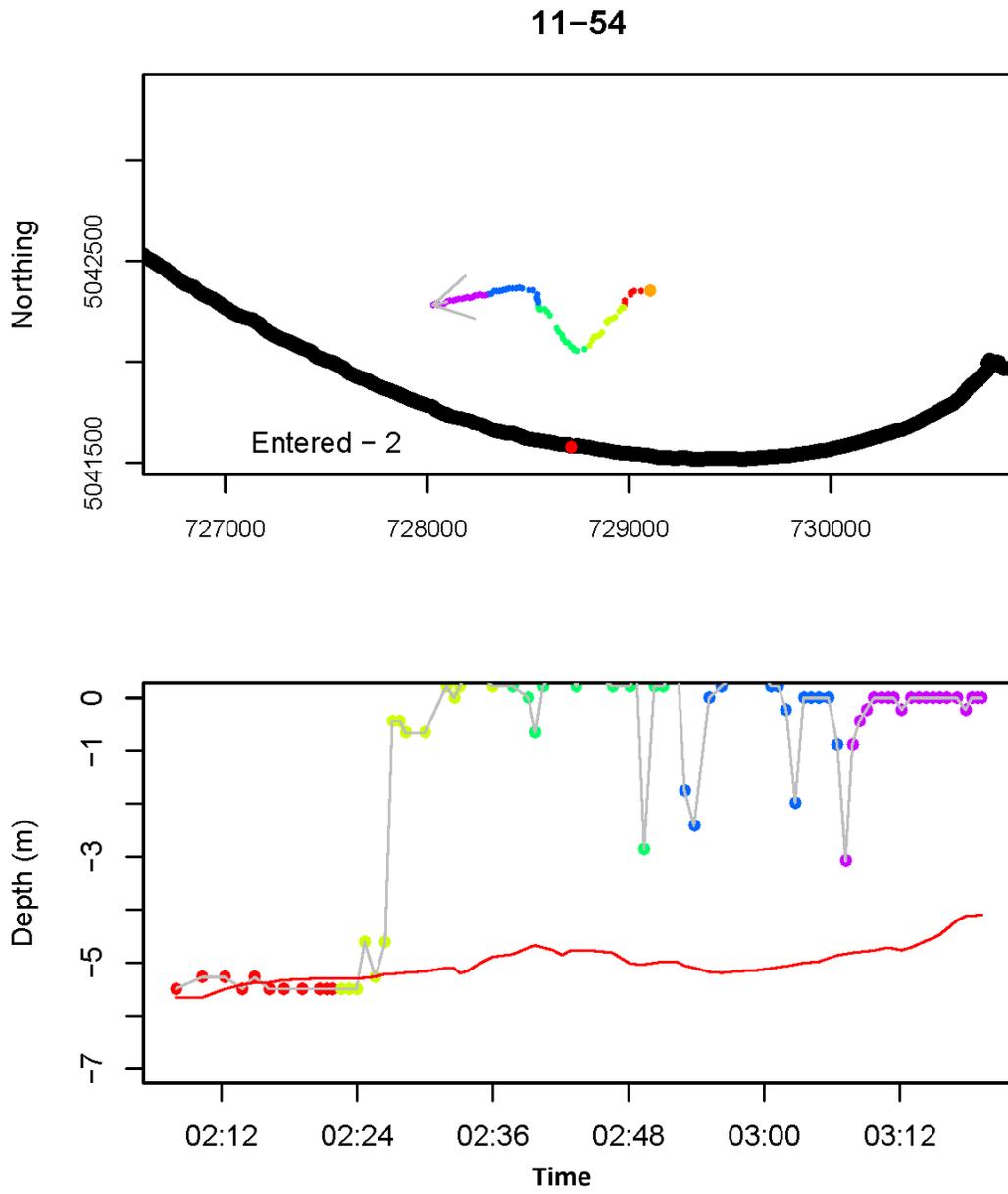


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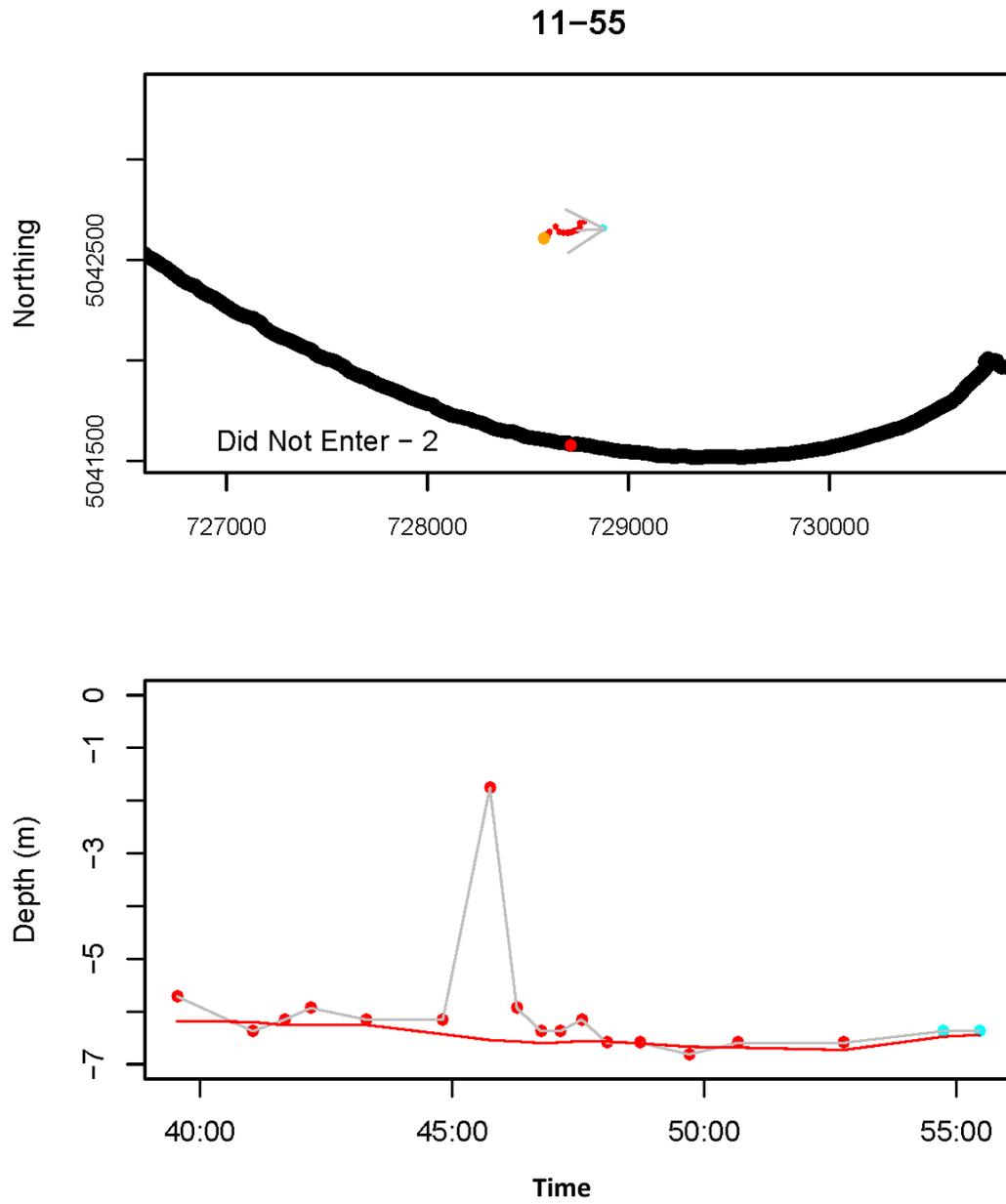


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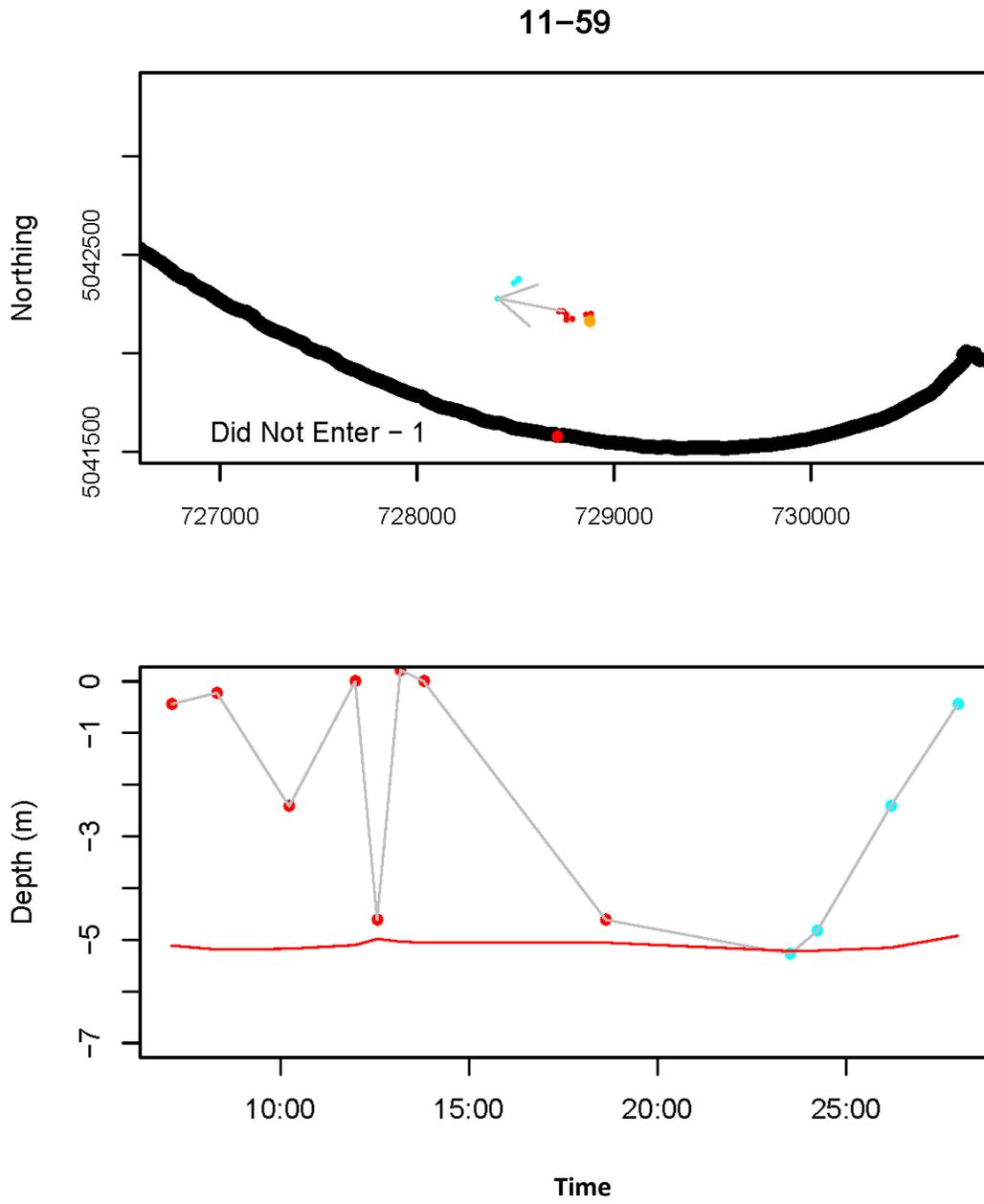


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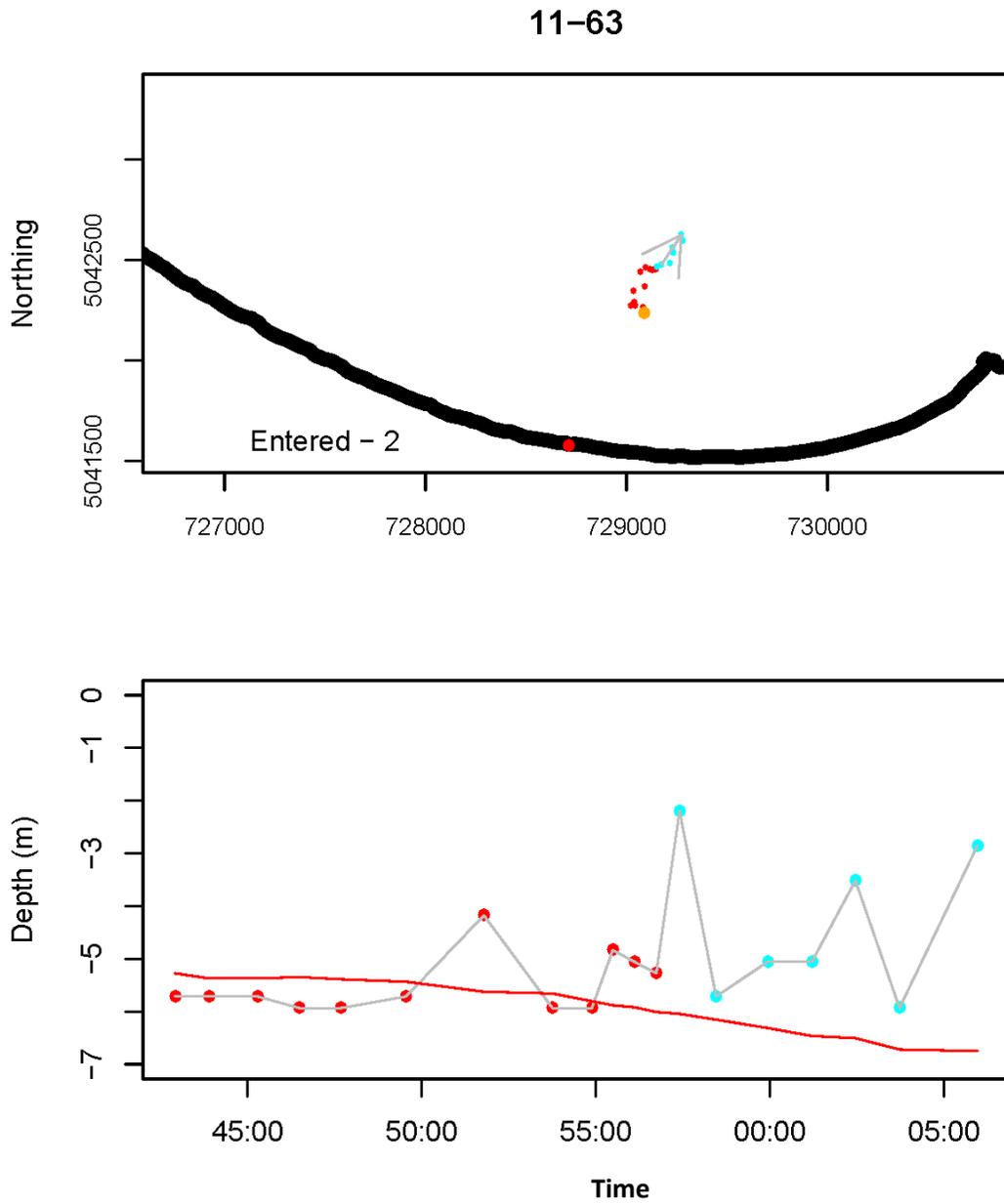


Figure 4.16: (cont'd)

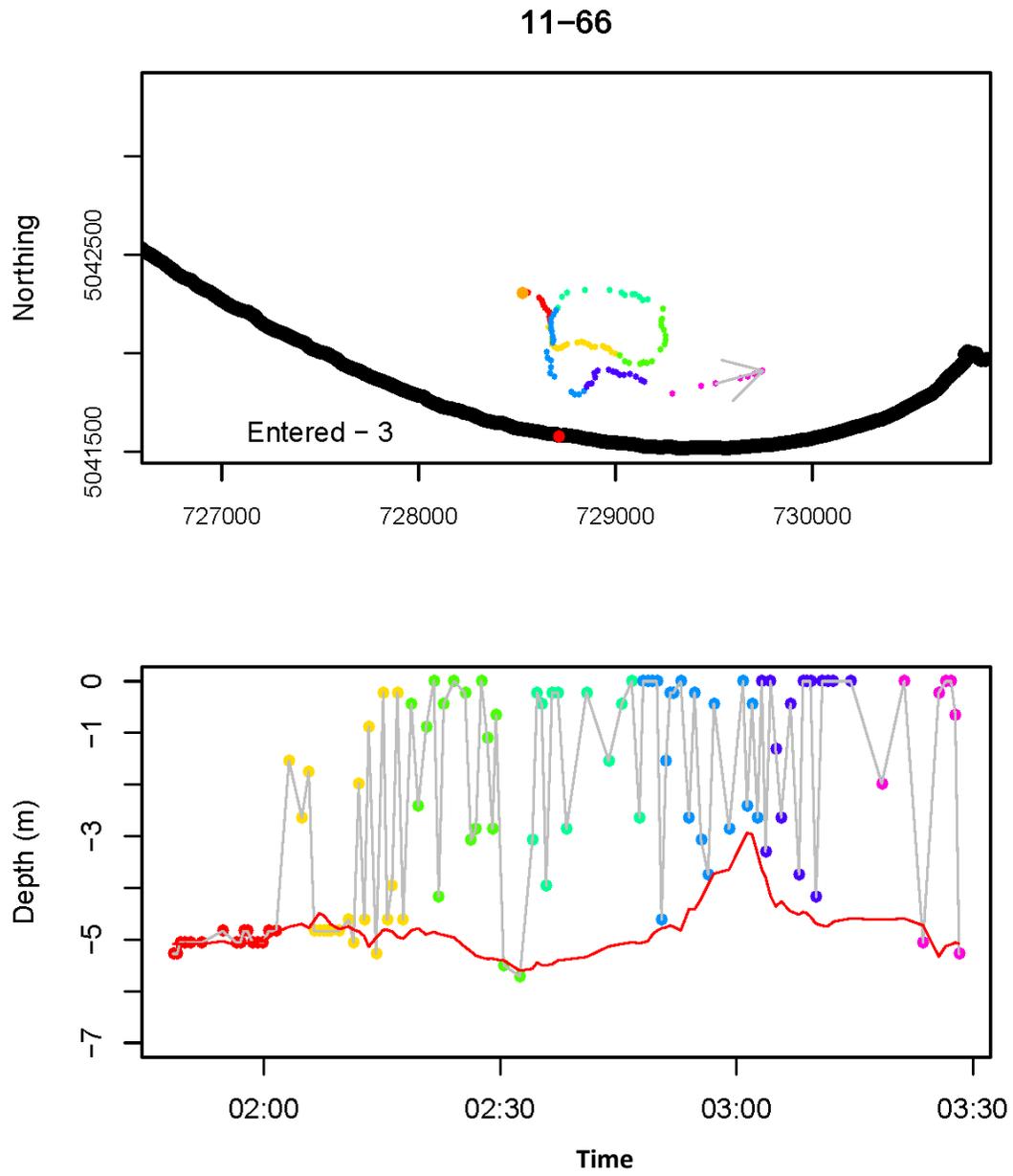


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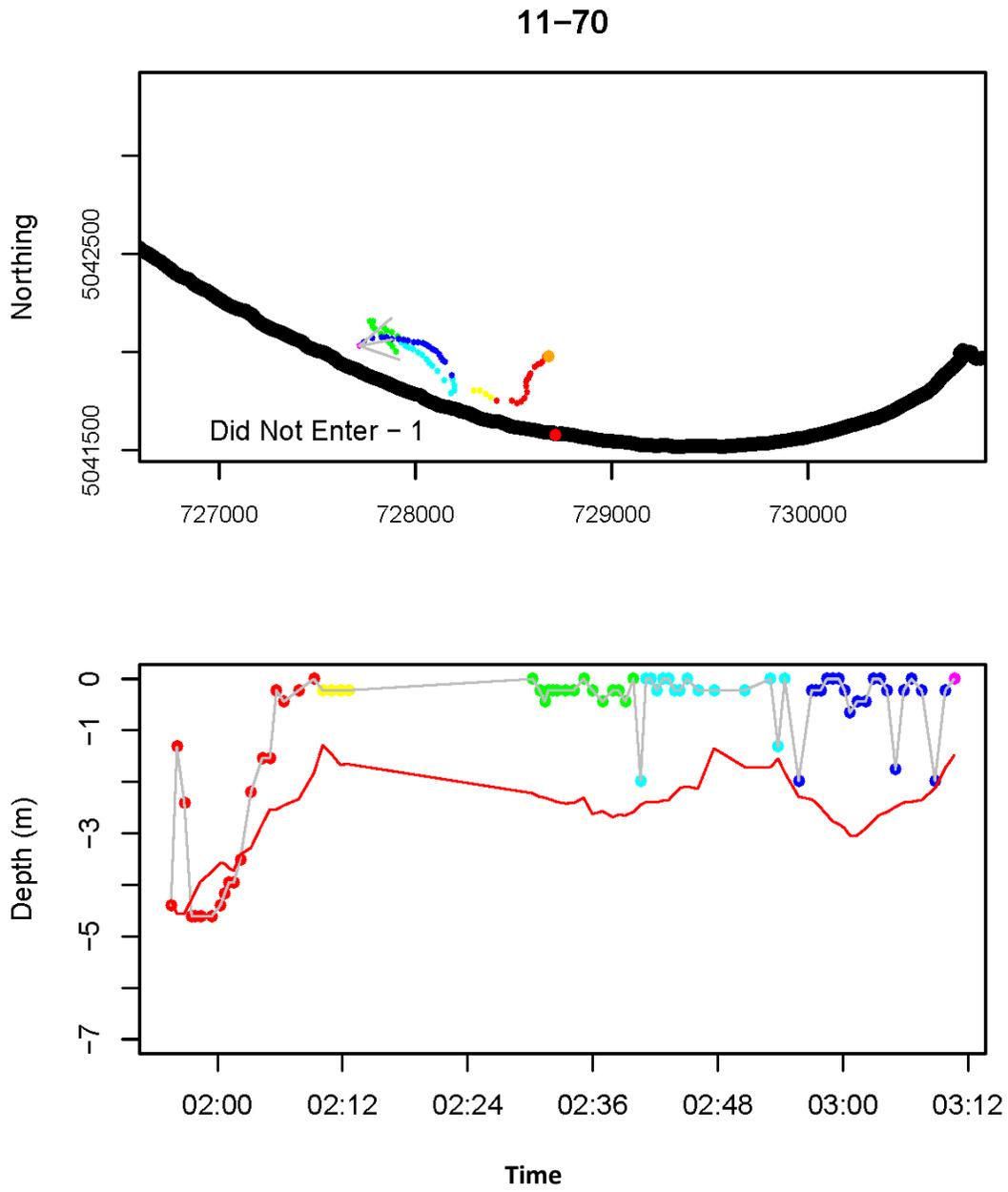


Figure 4.16: (cont'd)

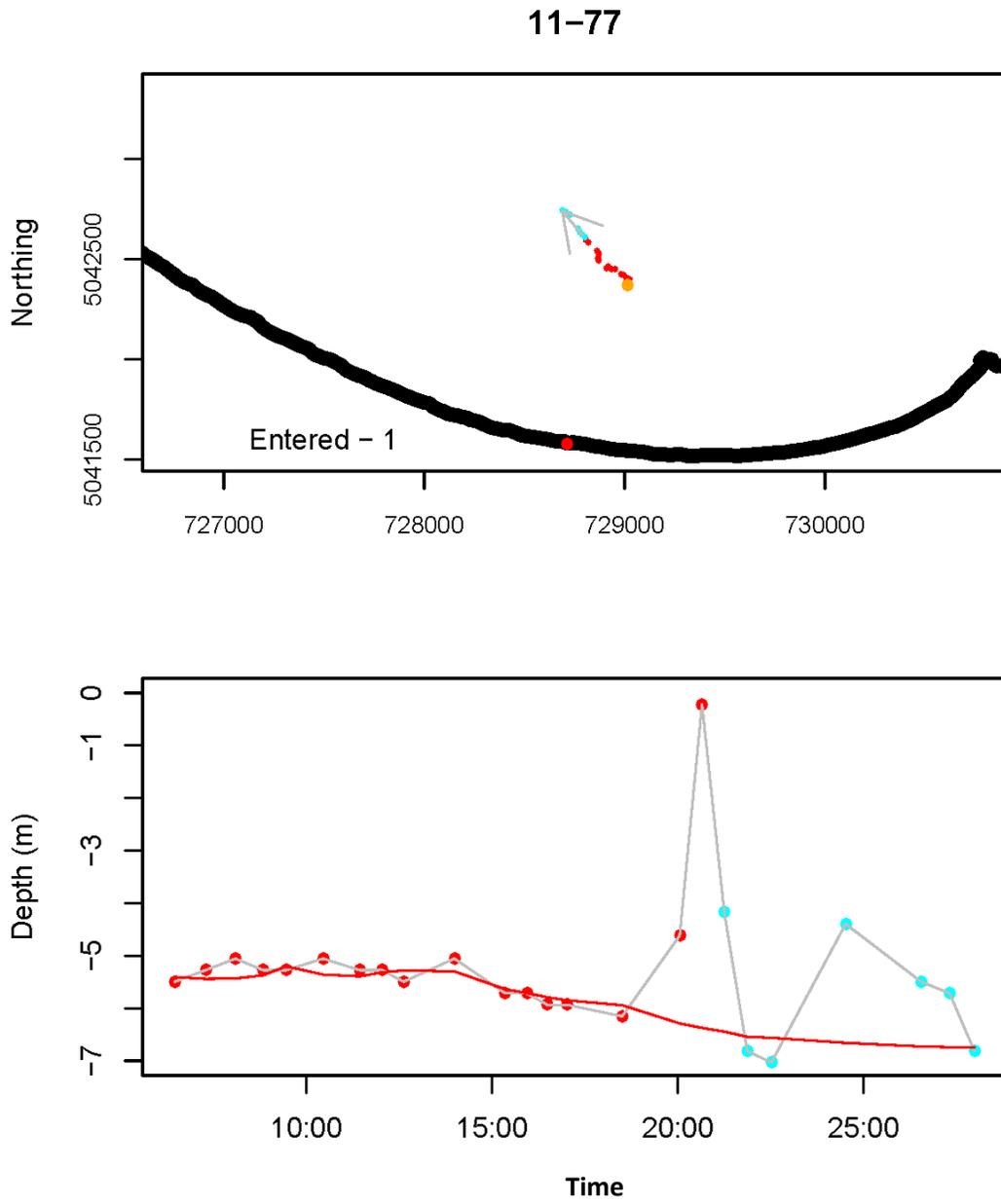
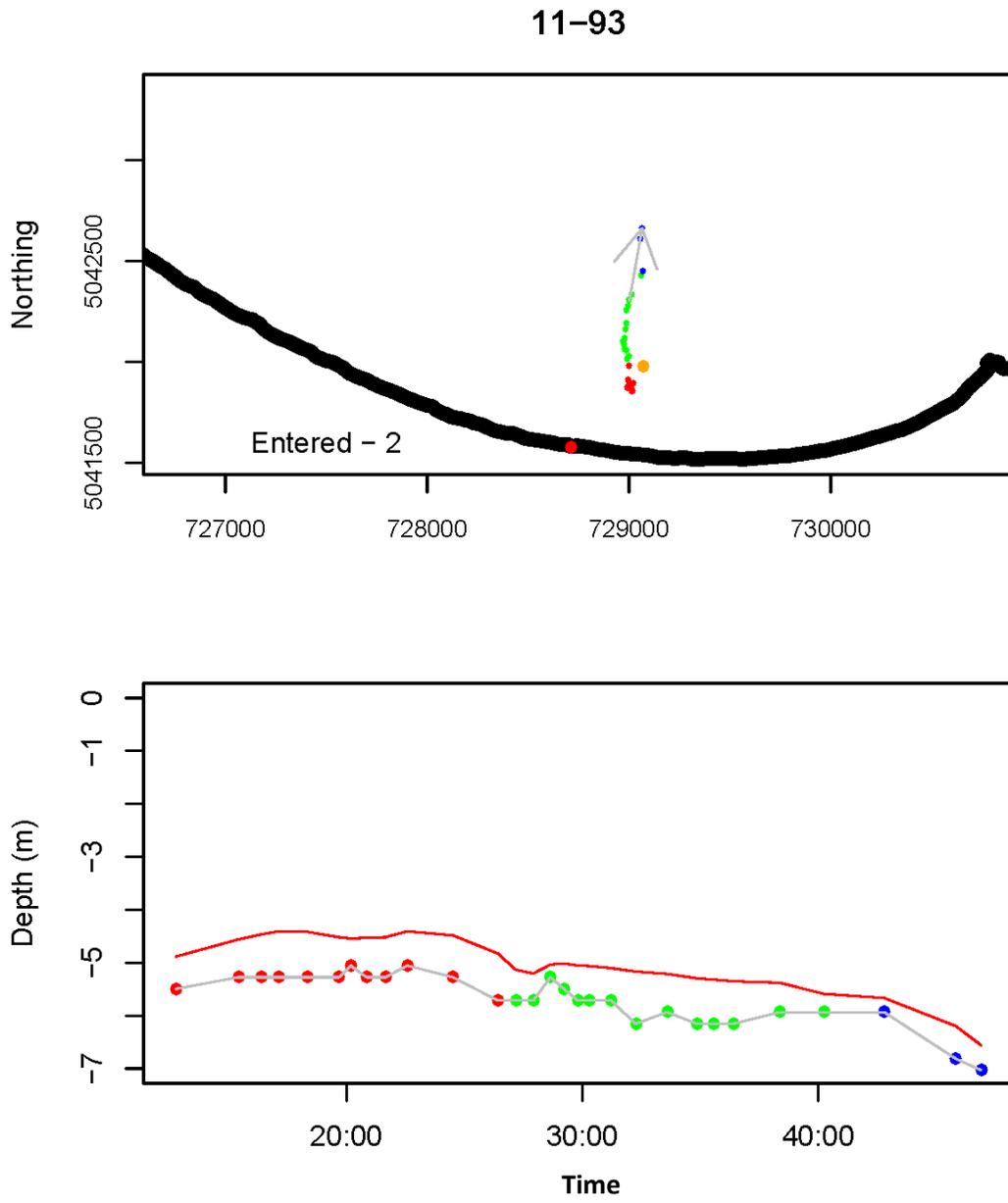


Figure 4.16: (cont'd)



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CHAPTER 5

ISOLATING OPPORTUNITIES FOR MANIPULATING SPAWNING HABITAT SELECTION OF INVASIVE SEA LAMPREY (PETROMYZON MARINUS) IN THE LAURENTIAN GREAT LAKES

ABSTRACT

Recent advances in the understanding of the sea lamprey lake migration have shed light on how sea lamprey encounter, enter, and ascend rivers in the Great Lakes. Herein, a simple framework is provided for discussion of the factors that influence the transition between each step of the migration and how these factors could be manipulated for improved management of the invasive sea lamprey. A profitable alternative management effect either reduces the amount of pesticide applied, increases the effect of the pesticide (reduces cost per kill), or allows for the removal of barriers increasing stream continuity, while maintaining or decreasing the sea lamprey population. Six management scenarios are discussed including what behaviors would be manipulated in each scenario, what aspects of the migration need to be better understood to determine the probability of success, and what scientific advancements are necessary for each manipulation to be performed in nature.

INTRODUCTION

The invasive sea lamprey has been historically controlled by preventing access to habitat with dams and killing larvae with pesticides in the areas of watersheds sea lamprey could reach, driving the population towards, but not to, management targets designed to protect the ecosystem (Christie and Goddard 2003). Alternatives to traditional management options or maximization of current control methods could be valuable if they achieve sea lamprey management objectives or maintain the current efficacy with fewer negative effects. The current methods include

lampricides which kill non-target species (Boogaard et al. 2003) and are expensive (Christie and Goddard 2003), and physical barriers that prevent access to other migratory species. Particularly promising is the potential to use natural odorants that are part of the habitat and mate selection decisions of sea lamprey to manipulate their behavior for control (Li et al. 2007).

Three elements make behavioral manipulation of a pest for protection of a resource possible: a known behavior, a known stimulus that initiates the behavior, and a scenario in which the stimulus can be presented to induce the behavior subsequently resulting in protection of a resource (Foster and Harris 1997). Behavioral manipulation of the potadromous Great Lakes sea lamprey is promising, given their known reliance on odorants to select habitat (Teeter 1980). However, a lack of understanding of much of their life cycle and the challenge of identifying and replicating the odorants has made this alternative control method difficult to achieve.

Sea lamprey are known to alter behavior in response to the odor of larval lamprey, adult sea lamprey, and dead adult or larval sea lamprey as they complete a non-homing return migration from offshore feeding grounds to riverine spawning grounds (Bjerselius et al. 2000, Siefkes 2000, Fine et al. 2004, Waldman et al. 2008, Wagner et al. 2011, Bals 2012). The odorants represent a potential tool for controlling habitat selection, trapping sea lamprey, or disrupting the migration system by masking odorants in the system through pumping synthetic replicates of the odors at high concentrations (Teeter 1980), classic concepts of insect pheromone-based control strategies (Steiner 1952, Carde and Minks 1995). Odorants can act as a releaser (e.g. a male sex pheromone component, 3kPZS, induces upstream search for mates), and (or) as a primer, (e.g., 3kPZS modulates release of gonadotropic releasing hormone, resulting in maturation of immature sea lamprey), helping to synchronize the migration (Brennan and Zufall 2006, Chung-Davidson et al. 2013). We are focused on releaser effects for manipulating

behavior. Though progress has been made in understanding the migration system and identification of some odorants, an understanding of how to best use odor in a control scenario has not materialized. Through a simple migration model that is analogous in some respects to trap catch steps previously developed for sea lamprey (Bravener and McLaughlin 2013), we identify the steps of the migration, although in this case the sea lamprey reaches spawning habitat instead of entering a trap and we identify the steps of the migration, the factors that influence transitions, and how the process can be manipulated.

RIVER ENTRY FRAMEWORK

The framework covers river plume encounter, river entry, and river ascension to the spawning grounds. It allows us to outline what factors influence each step and to discuss the potential to manipulate these steps to better manage sea lamprey in the Great Lakes (Figure 5.1).

River Plume Encounter

The number of sea lamprey to encounter a river plume will depend on the number of individuals that pass the river mouth and how far they are from shore compared to the distance the river plume extends from shore (Figure 5.2). The probability of encounter for each river will vary with the size of the river plume, principally how far river water extends from the coast and how far from shore sea lamprey traverse parallel to the coast in that region (Chapter 4). River plume size varies with river discharge, coastal bathymetry, coastline configuration at the river mouth (e.g., embayment trapping water), and wind driven lake current (Churchill et al. 2003, Rao and Schwab 2007). Wind driven currents and the coastal boundary are the dominant features regulating the extent of the river plume in the Great Lakes (Churchill et al. 2003). The number of sea lamprey available to encounter the plume will depend on the number of sea lamprey to return

to the particular region following the parasitic stage and whether they reach a given river or stop at a previous river.

River water alone is attractive to sea lamprey in two-choice tests and appears to be the primary feature that initiates a transition to local search behavior around the river mouth in the lake (Vrieze and Sorensen 2001, Vrieze et al. 2011, Meckley et al. 2014, Chapter 4). However nose plugged sea lamprey were still able to locate a nearby river, albeit much less efficiently (12.5 %) (Vrieze et al. 2010), indicating that other features such as conspecific attraction or physical features such as substrate, water color (tannins), temperature gradients, or outflow may be sufficient for a migrant to recognize their proximity to a river mouth and enter the river.

River Entrance, Upstream Movement, and Tributary Selection

The timing of river entry and upstream movement is related to river temperature, changes in river temperature, stream flow, and sexual maturation (Almeida et al. 2002, Binder et al. 2010). River and tributary selection appears most reliant on the presence of larval sea lamprey odor, a form of public information that provides evidence of spawning and rearing habitat quality (Bjerselius et al. 2000, Sorensen et al. 2005, Waldman et al. 2008). However, additional odorants influence sea lamprey behavior and may influence the migration. For example, it is unclear how public information such as larval odor of other lamprey species or decaying lamprey larvae modulates river entry.

Unlike specialized sex pheromones that are often a compilation of a few compounds at a specific ratio (Ando et al. 2004), tuned as a result of receiver bias, when an odor releaser is rewarded with more mates because of releasing a more attractive odor (Buchinger et al. 2013); the unspecialized release of larval odor is unlikely to be discernable as a species specific signal by adults and may be a larger number of components (Li et al. 1995). The composition of the

gut microbiome of sea lamprey is just beginning to be explored (Tetlock et al. 2012), but if the larval diet, gut microbiome, and subsequent bile salts released by larvae are similar between different types of larvae, larval odor may be a general lamprey habitat quality cue. This is supported by evidence that different species of lamprey showed attraction to different lamprey species larval odors and larvae of different species release similar components (Fine et al. 2004). Although a blend of active compounds must be present (Fine and Sorensen 2008, Meckley et al. 2012), odors are released to a constrained environment and ratios may not be important (Derby and Sorensen 2008). Little is known about the influence of the odor of dead larvae on the sea lamprey migration, beyond that the odor of dead lamprey larvae is repulsive to migratory sea lamprey in lab tests (Imre et al. 2010, Wagner et al. 2011, Bals and Wagner 2012), and has been hypothesized to cause habitat avoidance where larval sea lamprey have died during the winter. Migratory sea lamprey avoid the odor of dead adults and larvae in the lab, though it is unclear if different compounds are responsible for the avoidance (Bals and Wagner 2012). These odor cues are unlikely to be species specific.

The second type of information widely relied upon by animals but poorly described in sea lamprey is reliance on interactions with mates, known as conspecific attraction (Doligez et al. 2003). The conspecific attraction strategy always coexists with other strategies because it allows individuals to utilize the decisions of other individuals and ensures the availability of mates (Doligez et al. 2003). The presence of mates could be determined visually or through olfaction as sea lamprey migrants can smell the odor of other migrants at different stages of the migration and the odor of dead mates upstream. To date the natural progression of the sea lamprey migration has been poorly observed as studies utilize subjects trapped in a river (Vrieze et al. 2010, Vrieze et al. 2011, Meckley et al. 2014), and activities like staging at a river mouth could

be a major component of the migration (Applegate 1950, Clemens et al. 2010), as it could signal the presence of mates and synchronize the start of the migration. Unlike larval odors these cues are more likely to be species specific migration cues.

The odor of adult conspecifics synchronizes adult maturation in the river (Chung-Davidson et al. 2013) and the presence of a male released sex pheromone may encourage female river entry (Meckley et al. 2014) and tributary selection. However, sex pheromone was not found to be attractive to immature migratory sea lamprey caught in early spring (Siefkes et al. 2005), and may never be available at a river mouth to migrants experiencing a natural migration progression. More progress has been made towards synthetically replicating the sex pheromone (Johnson et al. 2005), though an effective large scale management scenario has not been identified to date (Luehring et al. 2011, Johnson et al. 2013) and as a trap bait where an effect has been shown, the method may only be effective at low sea lamprey densities (El-Sayed et al. 2006, Johnson et al. 2013).

The odor of dead migratory sea lamprey is repulsive to non-mature male and female sea lamprey in lab tests (Imre et al. 2010, Wagner et al. 2011, Bals and Wagner 2012) and may represent predator avoidance or the end of spawning, indicating sea lamprey have already reproduced and senesced. The function of dead odor is further confounded by the finding that migrants become habituated to the odor of dead adults after approximately four hours of exposure (Bals 2012), and initial field tests suggest that the odor of dead sea lamprey is insufficient to prevent river entry and instead increased river entry (Tom Luehring, Personal Communication). The odor functions to bias the side of the stream channel occupied by sea lamprey during upstream movement when the odor can be avoided (Bals 2012), but may increase

upstream movement past the source of the odor when the odor is mixed throughout the entire channel (Tom Luhring, Personal Communication).

River Retention

If a river contains a large larval population, spawning habitat, and conspecific mates, sea lamprey are unlikely to reverse course after river entrance. This is supported by a two-year study that never observed sea lamprey exiting a river after they entered and moved more than 300 m upstream, and some individuals enter the immediate river mouth without ascending the river (Applegate 1950, Meckley et al. 2014, Chapter 4), together leading to the suggestion that the decision to enter occurs at the river mouth (Chapter 4). Alternatively, some sea lamprey return to a river plume over multiple nights without ever entering the river, regularly reversing between movement inside and outside of the river plume, and moving very close to the river mouth, although this occurred less with higher larval odor (Chapter 4). Observations that sea lamprey moved to the river mouth without entering supports that the concept that the decision to enter a river usually occurs at the river mouth.

MANIPULATING THE MIGRATION

Management of an invasive species is difficult because a strong understanding of the species biology and behavior in the new environment is often unavailable. Because eradication of established invasive species is rare (Simberloff 2003), an explicit definition of what constitutes a profitable outcome is required. We define a profitable alternative management effect as one that reduces the amount of pesticide applied, increases the effect of the pesticide (reduces cost per kill), or allows for the removal of barriers, while maintaining or decreasing the sea lamprey population. The results must be measureable to determine whether a strategy has a profitable effect, a difficult proposition given the dynamic pesticide treatment schedule. An

effective odor-based control method must identify and synthesize the active compounds cheaply, a very elusive process (Li et al. 2007), that we will not discuss in detail. We discuss six management scenarios for manipulating the sea lamprey migration for improved management (Table 5.1).

River Plume Encounter

Encounter with a river plume must occur for a sea lamprey to enter the river. River plume encounter could be manipulated by changing the number of individuals to pass the river, physically influencing the distance that sea lamprey move from the coast while passing the river plume, through providing chemical information indicative of a river plume farther from shore, or by physically increasing the distance the river plume extends into the lake. Physically altering the distance sea lamprey travel from shore or the size of the river plume would likely require large construction investments and extensive research. However, there are navigational features that may provide suggestions for how manipulation of the river plume size could be achieved (Figure 5.3). An alternative would be the release of river odor out in front of a river mouth that indicates the presence of a nearby river, inducing local search (Table 5.1, Option 1). The encounter rate may be challenging to alter; thereby changing the number of sea lamprey to pass a river is the most plausible approach if entry rates of nearby rivers can be influenced. River plume encounter is an event not a decision. For this reason it may not have management value unless encounter can be prevented, however if it is combined with some decision manipulation following encounter, the encounter rate becomes important.

Cohort-Size Maintenance

If synthetic larval odor was available, the most immediate value could come in the form of maintaining the number of sea lamprey to enter streams that require pesticide treatment every

four years (Table 5.1, Option 2; Figure 5.2, iii). If the larval odor signal is maintained, migrant dispersion could be abated and could reduce the cohort size of sea lamprey to enter other rivers, potentially including small river plumes that may get irregular sea lamprey recruitment. It is unconfirmed whether the increased river entry rates observed each year after treatment is due only to the increase in larval odor, or if for example an increase in river recruitment of 2.4-6.8% of the total population of Lake Huron (i.e., as observed between 1 and 3 years post treatment in the Ocqueoc River; Figure 5.4), is sufficient for a lake wide management value. A potential downside to the strategy is the need for continued pesticide treatments. There are many smaller streams that have sea lamprey populations that are too small, or are too dispersed to be treated effectively and never or rarely meet the standard to qualify for treatment (Jones et al. 2003). This strategy works under the premise that the constant presence of larval odor in rivers with large river plumes that are regularly treated would reduce the spreading of migrants to other rivers by reducing the coastal search time and associated likelihood of encountering smaller rivers. This strategy is only important if sea lamprey are spread to non-expert treatment streams that do not get treated every four years. If sea lamprey are only being pushed between regularly treated streams, then the cohort-size maintenance approach is not valuable.

Recruitment to habitat deficient for larval survival

Synthetic larval odor could encourage entry into rivers with poor spawning or rearing habitat (Li et al. 2007; Table 5.1, Option 3). We suspect a river with limited habitat, such as a river with a physical barrier to movement low in the river before spawning habitat is reached, is unlikely to retain migrants, as sea lamprey have been observed exiting large rivers with waterfalls low in the river (Teeter 1980). However if a river has ample spawning habitat and poor larval habitat, sea lamprey may stay and spawn. It would need to be confirmed that larvae

do survive poorly in any river site considered for this strategy. Encouraging sea lamprey into a river lacking larvae or with few larvae could be counterproductive if enough larvae survive to warrant pesticide treatment of the river or if it results in an unacceptable number of parasites. It is unclear if there are rivers that fit this strategy as most rivers that do not contain larvae, are intermittent or have very low flow (Teeter 1980).

Chemical barrier and Push-Pull

The potential to create a chemical barrier at a river mouth or at tributaries has begun to be tested (Bals 2012, Bals and Wagner 2012, C. Michael Wagner personal communication; Figure 5.2, iv). Currently only physical barriers are plausible for blocking sea lamprey access to rivers. Dead adult odor does not prevent river entry, though the dead odor of larvae has not been tested in this scenario (Table 5.1, Option 6). Parts of a watershed may be blocked through application of dead migratory sea lamprey odor alone, although the application method will need to avoid extended exposure resulting in odor habituation which was observed after sea lamprey were exposed to the odor for approximately four hours (Bals 2012, Bals and Wagner 2012, Tom Luehring personal communication). The effect may be strengthened with a “pull” comprised by larval odor, as sea lampreys prefer to move in the stronger larval signal (Wagner et al. 2009, Meckley et al. 2012; Table 5.1, Option 4; Figure 5.2, v, vi).

This method of providing a chemical barrier to part of the watershed has promise to limit sea lamprey to a narrow region of the watershed that could reduce the pesticide necessary for treatment. The level of success necessary for a management value would need to be evaluated and the method will fail to manipulate individuals that spawn before encountering the selected stream bifurcation. If sea lamprey are sufficiently repelled to delay the need for pesticide

treatment, or allow for a physical barrier to be removed or placed higher in the watershed, the method would have value.

Larval Odor Removal (TFM)

We only highlight the use of TFM as an alternative control measure because the way it is applied removes the larval signal from a river and has a measurable effect on entry into neighboring rivers (e.g., Teeter 1980; Table 5.1, Option 5). The more effective the removal of lamprey larvae in the river the more effectively the treatment will likely reduce entry the following year, creating a repellent from the absence of a cue. In the circumstance pesticide treatment occurs in many rivers, the reduction in entry by migrants the year after treatment is a negative management effect. If TFM treatment is required every four years before larvae can transform to the parasitic stage, no management advantage is gained from the reduction in entry and instead sea lamprey are spread to other rivers. Although where migrants are dispersed the year after treatment, is poorly understood. In many cases, only a few rivers receive a large proportion of the sea lamprey population estimated in the lake, removing the larval signal from one of these rivers can cause a substantial shift in where migrants go to spawn and the more time spent searching may increase the likelihood of encountering smaller river plumes that are rarely encountered (Meckley et al. 2014).

Based on similar logic, we would recommend against the treatment of multiple large rivers in a region in the same year. Ideally alternating pesticide application between large rivers may result in shifting recruitment between the major rivers rather than encouraging encounter and entry with many smaller rivers, a concept that needs further testing. Similarly we recommend against concepts like treatment of all rivers in back to back years, as this is likely to result in spreading migrants broadly. Encouraging dispersal of a pest is counterproductive to

chemical based treatment. Finally shifting treatments to after the spawning season would be the most valuable as the larval signal that acts to congregate migrants would congregate sea lamprey in rivers that were about to be treated. It is likely infeasible to treat all rivers after spawning occurs although the more the treatments can be shifted to after the spawning season the more effectively the treatment program would be using the natural sea lamprey habitat selection decisions to the control program's advantage.

CONCLUSION

The importance of pesticide application and physical barriers to sea lamprey movement will not be quickly replaced. As knowledge of the sea lamprey migration increases more options become available for how the sea lamprey migration can be manipulated. There are variants of these six potential alternative management strategies, although they cover the primary aspects of the migration that can be manipulated (Figure 5.1; Figure 5.2).

APPENDIX

Table 5.1: Six general strategies for manipulating sea lamprey migration behavior are presented including the stimulus affected, how the stimulus is affected (action), the goal of the manipulation and the type of manipulation. Only option one does not stand alone as a management strategy as it manipulates behavior but not a decision to select habitat.

	Stimulus Altered	Manipulation Action	Intended Result	Type of Manipulation
1	River water	Release river water out in front of a river mouth	River Plume Assessment	<i>Pull/transition</i>
2	Larval Odor	Add larval odor to “expert judgement” stream	River Entry	Pull
3	Larval Odor	Add larval odor to stream with poor larval survival	River Entry	Pull
4	Larval Odor And Dead Odor	Add larval odor and dead lamprey odor	Tributary selection	Push-Pull
5	Larval Odor	Remove larval odor signal via lampricide application (TFM)	River rejection	Push
6	Dead Larval Odor	Add dead lamprey odor to entire river	River avoidance	Push

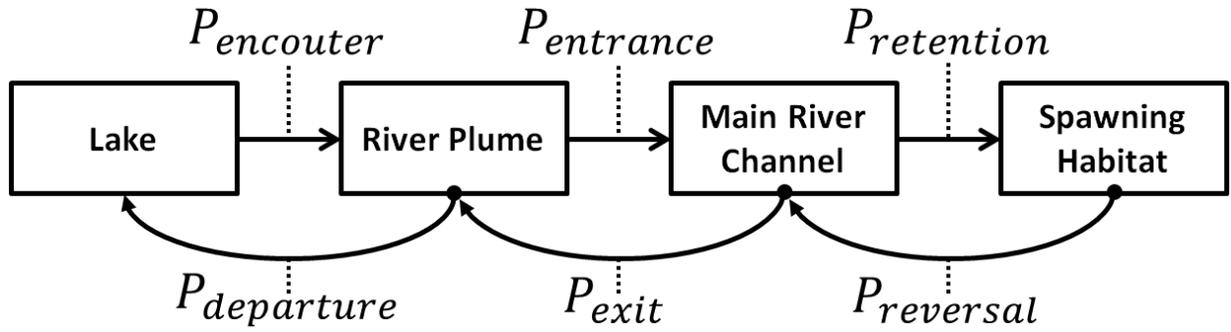


Figure 5.1: The steps of the spawning migration are analogous to the steps involved in trapping sea lamprey (Bravener and McLaughlin 2013), although in this case the river takes the place of the trap and the model depicts the transitions that occur when a sea lamprey enters a river in the great lakes. The arrows refer to the probabilities of progressing from the lake to spawning habitat or reversing through any prior step. The P refers to the probability of the event.

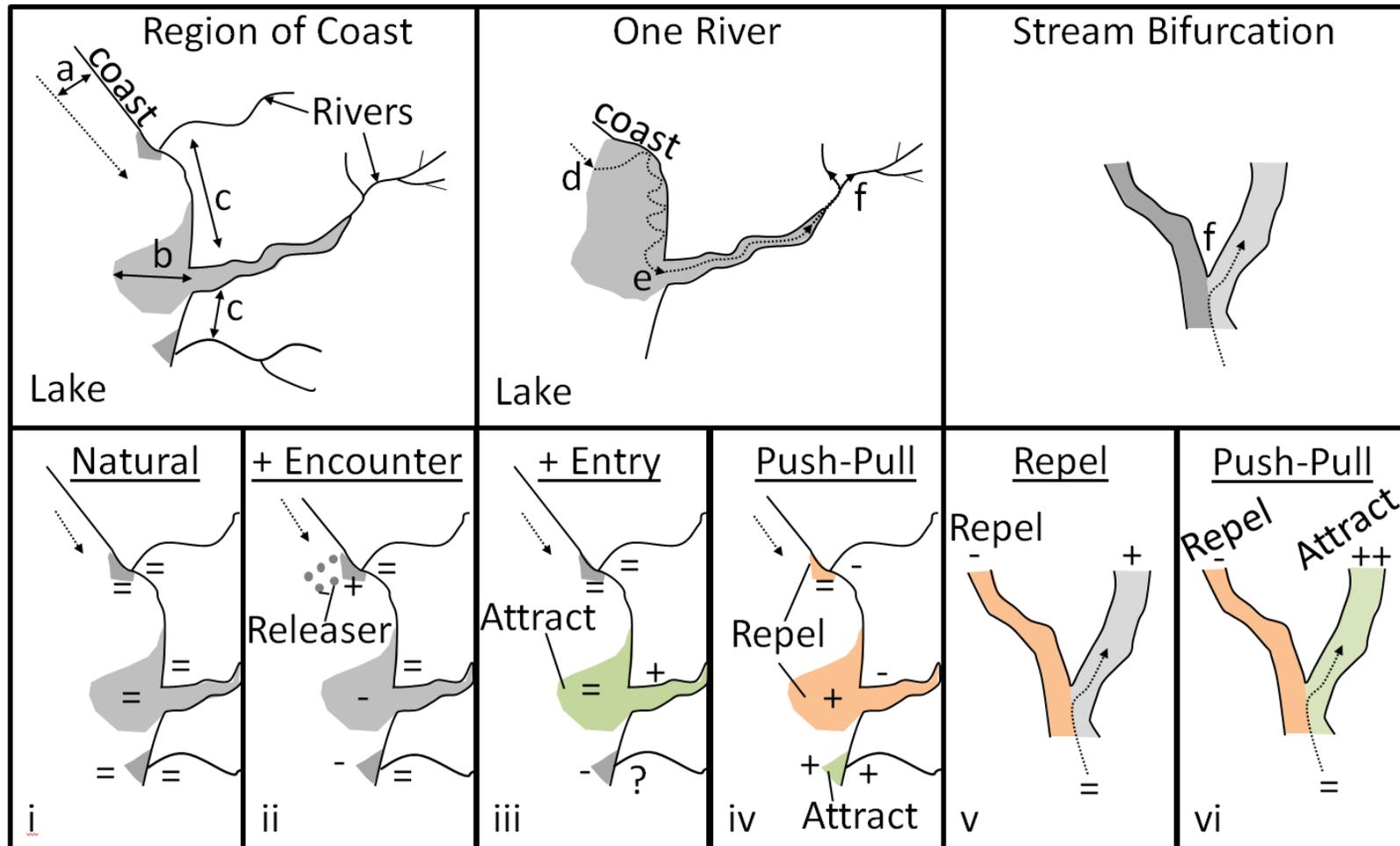


Figure 5.2: The distance sea lamprey travel from shore (a, dotted arrow), the distance the river plume extends from the coast (b), the presence of other rivers on the coast and the number of rivers encountered prior to reaching a river (c), are all important to a sea lamprey encountering a particular river plume (Region of Coast). Following encounter with a river plume (d) sea lamprey become

Figure 5.2: (cont'd)

available for manipulation of their habitat selection decisions including river entry (e), or tributary entry (f). Five general manipulation scenarios are shown including the manipulation of river plume encounter (event) (ii) or habitat selection (decision) (iii-vi). The operator (+, -, =), indicates how the manipulation of adding an attractant (“attract”), a repellent, or removing an attractant (“repel”), influences encounter rates at nearby rivers and entry of those rivers. The manipulation of decisions through the addition of odorants (iii-vi), uses general terms based on the effect desired and not specific odorants that will cause the desired effect. Encounter is shown to change due to the order of sequential river plumes. Encounter at a river can change due to a manipulation at a prior river but not the current river manipulated (decrease, iii; increase, iv). It is unclear how past experience might influence entry of future rivers (iii, “?”).



Figure 5.3: Many river mouths have preexisting structures that may extend the distance the river plume would otherwise reach from shore (i.e., as seen west of river mouths in Ludington, MI (left) and Sagatuck, MI (right)). Structures of this type designed for boat traffic could present unique solutions for influencing the sea lamprey migration and may be the only scenario that would create a river plume extending a greater distance from shore than would otherwise occur for that river. In each image the river plume can be faintly seen due to darker turbidity.

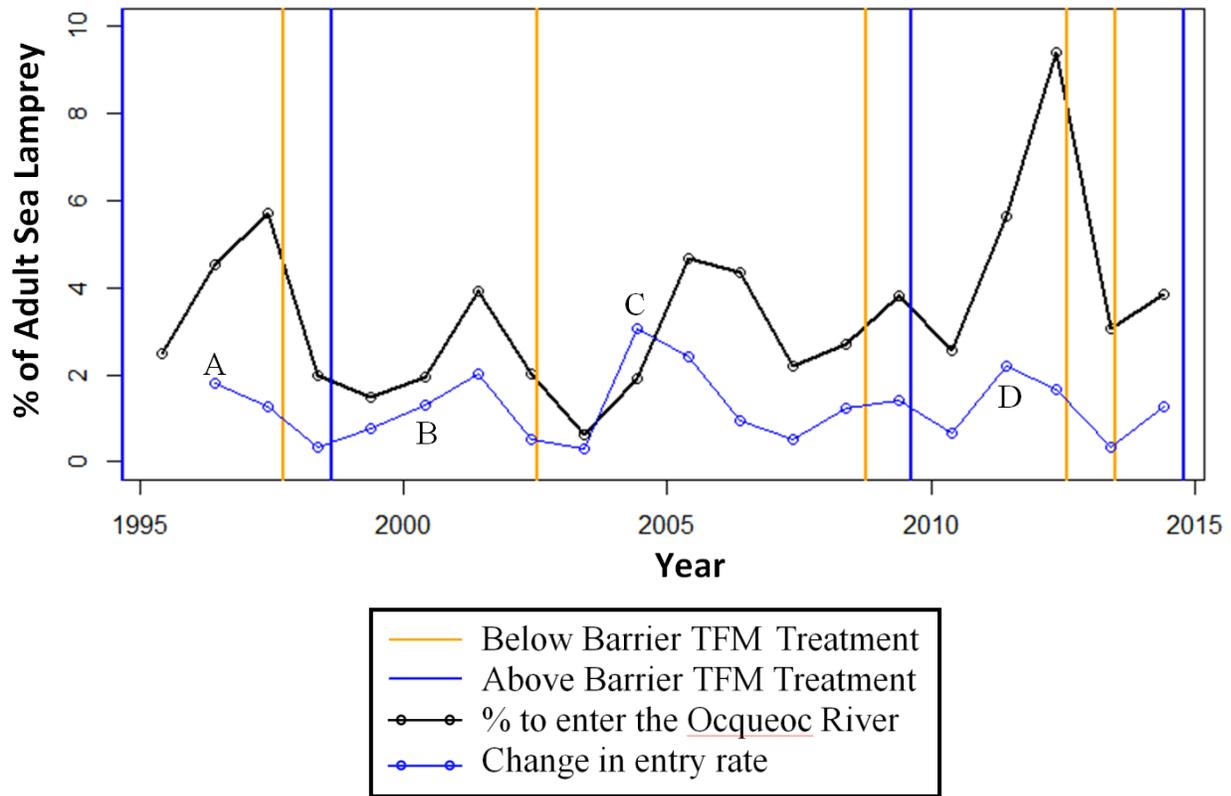


Figure 5.4: The percentage of the entire population of adult sea lamprey in Lake Huron that return to the Ocqueoc River to spawn from 1995 to 2015 based on mark recapture (black circles) and the change in entry between years (blue circles). A change of entry rate of 2 represents a doubling in the rate of entry. Vertical lines represent the times of TFM treatment to the Ocqueoc River. The change in entry rate from one year after TFM treatment to the second year shows that the increase in entry rate between 2010 and 2011 (D) fits within the historical increase in rate (A: 1.8, B: 1.3, C: 3.1, D: 2.1).

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